酿酒酵母中与ScRch1定位相关的膜蛋白和脂质体蛋白的筛选

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摘要 酿酒酵母*YMR034c*基因与我们之前发现和鉴定的白念珠菌的*CaRCH1*基因同源, 因此把它命名为*ScRCH1*, 它编码的蛋白ScRch1在胞外高钙离子条件下定位于细胞质膜。为了研究酿酒酵母细胞对ScRch1质膜定位的调控机理, 在编码细胞质膜和液泡膜等膜蛋白以及与脂质颗粒相关蛋白的402个基因的缺失株中, 我们通过荧光显微镜技术检测了融合蛋白ScRch1-GFP的细胞膜定位情况。结果发现, ScRch1在其中10个基因的缺失株细胞里不能定位于质膜。这些基因包括两个编码细胞质膜运输蛋白的基因*SNQ2*和*HXT1*, 一个编码液泡膜运输蛋白的基因*AVT4*, 一个与液泡连接/融合相关的液泡膜蛋白基因*PEP3*, 一个与细胞分化有关的内体膜蛋白基因*DFG10*, 两个编码脂质体蛋白的基因*EHT1*和*LDH1*, 以及三个功能未知的内体膜蛋白基因*YBR219c*、*YBR224w*和*YDR417c*。因此, ScRch1的细胞膜定位可能受到以上多个细胞过程的影响, 这些研究结果为进一步阐明ScRch1的细胞质膜定位机制奠定了基础。

关键词 钙离子; *RCH1*; 细胞膜定位; 基因筛选; 膜蛋白

Screen for Membrane and Lipid Proteins Required for the Subcellular Localization of ScRch1 in Budding Yeast

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Abstract *Saccharomyces cerevisiae YMR034c* is a sequence homolog for the *Candida albicans CaRCH1* gene, named as *ScRCH1*, and we show that ScRch1 localizes to the plasma membrane in response to high levels of extracellular calcium. To find out membrane and lipid proteins related to the subcellular localization of ScRch1, we screened 402 yeast single-gene deletion mutants for genes encoding membrane and lipid proteins through a fluorescence microscope approach. We have identified 10 genes, whose deletion renders ScRch1-GFP failed to localize to the plasma membrane. These genes include *SNQ2* and *HXT1* encoding plasma transport proteins, *AVT4* encoding vacuolar transport proteins, *PEP3* encoding docking/fusion tonoplast protein, *DFG10* related to cell differentiation, *EHT1* and *LDH1* encoding liposome proteins, as well as *YBR219c*, *YBR224w* and *YDR417c* with unknown functions. These data provide a basis for our understanding the regulatory mechanisms of ScRch1 subcellular localization.

Key words calcium; *RCH1*; subcellular localization; membrane

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在外界不同Ca^{2+}环境下, 酿酒酵母细胞质中的钙离子浓度一般都可维持在50~200 nmol/L的浓度范围[1-2]。当细胞外的刺激物引起细胞质内钙离子浓度的突然升高时, 会激活细胞内一系列生理变化, 包括激活钙调磷酸酯酶(Calcineurin), 以应对环境条件的变化[3-5]。但是, 如果胞质内钙离子浓度一直保持高浓度的话, 对细胞是有害的。激活的钙调磷酸酯酶在细胞质内去磷酸化转录因子Crz1, 导致Crz1进入细胞核与目标基因启动子上的钙调磷酸酯酶依赖型应答元件(calcineurin-dependent response element, CDRE)结合[6], 进而激活目标基因的表达, 这些目标基因包括编码液泡Ca^{2+}-ATPase的*PMC1*和编码内质网/高尔基体(ER/Golgi)Ca^{2+}-ATPase的*PMR1*。Pmc1和Pmr1分别将胞质内过多的Ca^{2+}泵到液泡和内质网/高尔基体分泌途径中去, 以把胞质内的钙离子浓度降低到正常的生理状态。因此, 胞内钙离子稳态是受到严格调控的。

溶质转运蛋白SLC10家族有7个成员, 依次为SLC10A1-SLC10A7[7-9], 前6个成员(SLC10A1-SLC10A6)仅存在于脊椎动物中, 而在细菌、酵母菌和植物中有很多*SLC10A7*的同源序列[9]。*SLC10A1*, *SLC10A2*和*SLC10A6*与胆汁酸和硫酸化的固醇类荷尔蒙的运输有关[10-11]。*SLC10A3*、*SLC10A4*、*SLC10A5*和*SLC10A7*的功能还不清楚[12]。CaRch1是白念珠菌中与SLC10A7同源的唯一蛋白, 它与白念珠菌对钙离子、锂离子和硝唑类药物的敏感性有关, 并可以负调节其胞质内钙离子稳态[13]。因此, CaRch1是一个新的胞内钙离子调控因子。酿酒酵母中也存在一个与SLC10A7同源的基因*YMR034c*[9]。之前的转座子突变筛选研究认为*YMR034c*与唑类药物的抗性有关[14]。然而, 目前为止未见有对*YMR034c*其他功能的报道。我们最近的工作发现, *YMR034c*是白念珠菌*CaRCH1*的功能同源基因, 因此我们把它命名为*ScRCH1*。应对外界高钙离子浓度, ScRch1也定位于细胞质膜上。细胞质膜蛋白在内质网上合成后, 通过高尔基体修饰, 有些要在脂质体的帮助下才能被转运到最后目的地—细胞质膜。此外, ScRch1在细胞质膜上的稳定性, 还可能与它在细胞质膜上的互作蛋白和锚定蛋白相关。调控蛋白完成其功能后, 在细胞不再需要的情况下, 通常被运输到液泡里降解。为了发现影响ScRch1细胞质膜定位的因子及其调控机理, 我们首先对酿酒酵母基因组中编码细胞质膜、液泡膜及脂质颗粒上的蛋白的402个基因进行系统筛选, 发现了10个基因的缺失导致ScRch1不能定位于细胞质膜上。

1　材料与方法

1.1　材料

1.1.1　菌株和质粒　本论文所用到的酿酒酵母菌株: 野生型BY4743(*MATa/α ura3Δ0/ura3Δ0*; *his3Δ1/his3Δ1*; *leu2Δ0/leu2Δ0*; *lys2Δ0/LYS2*; *MET15/met15Δ0*)和BY4743为背景的酿酒酵母双倍体单基因缺失株文库(购自美国Invitrogen公司)。表达ScRch1-GFP融合蛋白的质粒为pGFP33-ScRCH1, 由本实验室构建。

1.1.2　培养基　LB培养基: 含1%蛋白胨, 0.5%酵母提取物和1% NaCl。用1 mol/L NaOH调pH至7.0, 定容, 0.1 MPa压力下灭菌20 min。配置固体培养基时加1.5%(w/v)的琼脂粉。YPD培养基: 含2%蛋白胨, 2%葡萄糖和1%酵母提取物, 定容后0.1 MPa压力下灭菌20 min。配置固体培养基时加入2%(w/v)的琼脂粉。SD-Ura培养基: 含0.67%酵母氮源和2%葡萄糖, 0.1 MPa压力下灭菌后加入过滤灭菌10×必需氨基酸混合母液(不含尿氨酸)。

1.1.3　试剂　10 mg/mL ssDNA(南京生兴生物技术有限公司)、1 mol/L LiAc溶液(上海生工生物有限公司)和50%(w/v) PEG(分子量3 350; Sigma公司); 基因克隆相关用限制性内切酶和T4连接酶等试剂购自NEB公司; Taq DNA聚合酶和大肠杆菌感受态细胞购自北京全式金公司; $CaCl_2$等其他试剂购自国药集团。

1.1.4　实验仪器　PCR反应仪(德国艾本德公司)、全温摇瓶柜(太仓强乐实验设备厂)、台式冷冻离心机(日本日立公司)、立式压力蒸汽灭菌锅(上海博迅实业有限公司医疗设备厂)、凝胶成像系统(Bio-Rad公司)、荧光显微镜(Nikon 80i)、数显恒温水浴锅(金坛市医疗仪器厂)。

1.2　方法

1.2.1　pGFP33-ScRch1质粒的构建　用引物ScRCH1-F(CGG GAT CCT TCG ACC CAT ATG TGT CC, 下划线为*Bam*H I位点)和ScRCH1-TAG(ACA TGC ATG CCC TTG GTT GTG TAT ATG G, 下划线为*Sph* I位点)扩增含有785 bp启动子序列而没有终止密码子的*ScRCH1*的ORF的一段DNA片段(2 087 bp), 然后将这个片段克隆到pGFP33(Michael N. Hall赠

送)的*Bam*H I和*Sph* I位点, 重组子分别经过*Hin*d III和*Bam*H I酶切验证, 最后对正确的重组子中的插入片段进行DNA测序, 验证序列没有发生突变, 得到由自己的启动子序列控制的表达ScRch1-GFP的质粒pGFP33-ScRch1。

1.2.2 pGFP33-ScRch1质粒转化酵母细胞 挑取活化的酵母单菌落, 接到含有3 mL YPD液体培养基的试管中, 30 °C过夜培养至饱和。取过夜培养物1.5 mL, 3 000 r/min离心1 min收集菌体, 用无菌水洗细胞一次, 离心弃去上清。向沉淀物依次加入50 μL 1 mol/L LiAc, 20 μL 1 mol/L DTT, 6.75 μL 10 mg/mL ssDNA(ssDNA使用前沸水浴处理5 min, 立即置于冰上备用), 质粒DNA(200 ng至1 μg), 160 μL 50% PEG。充分混匀后置于42 ℃水浴, 热激30 min, 然后3 000 r/min离心收集菌体。用1 mL无菌水洗细胞一次, 然后菌体重悬于200 μL无菌水中, 涂布到SD-Ura固体平板上。30 °C培养3天, 挑取酵母转化子, 在SD-Ura固体培养基上纯化。

1.2.3 细胞处理和荧光显微镜观察 把带有pGFP33-ScRch1质粒的酵母转化子, 接种到含有3 mL SD-Ura液体培养基的试管中, 30 °C过夜培养。分别取100 μL过夜培养液, 加入到两份含有900 μL液体YPD的EP管中, 30 °C培养2 h, 然后向其中一份培养物加入50 μL的4 mol/L $CaCl_2$, 继续培养2 h。从$CaCl_2$处理和未处理的两个EP管中取少许菌液, 分别在荧光显微镜下观察ScRch1-GFP融合蛋白的亚细胞定位。每个样品观察大约100~200个细胞。每个菌株随机选取2个独立的转化子做荧光定位实验。

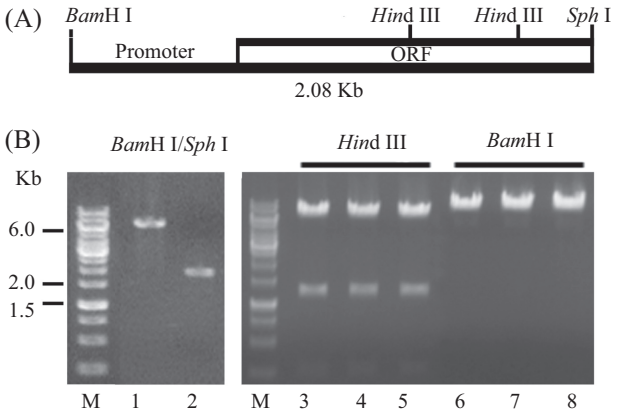
2 结果

2.1 pGFP33-ScRch1质粒的构建

把PCR扩增得到的DNA片段(图1A)和载体pGFP33的质粒DNA, 通过*Bam*H I/*Sph* I双酶切(图1B), 连接后得到重组子。重组子分别经过*Hin*d III和*Bam*H I单酶切验证其插入片段的大小, 并通过DNA测序对正确的重组子中的插入片段的核苷酸序列进行验证, 没有发生突变。从而得到由自身的启动子序列控制的表达ScRch1-GFP的质粒pGFP33-ScRch1(图1)。

2.2 酿酒酵母基因组中编码膜蛋白和脂质体蛋白基因的生物信息学分析

酿酒酵母双倍体单基因缺失株文库包括大约



A: *ScRCH1*基因PCR扩增区域的酶切图谱; B: 左图为载体pGFP33(第1泳道)和*ScRCH1*基因的PCR产物(第2泳道)的双酶切(*Hin*d III/*Bam*H I)产物电泳。右图为三个独立的pGFP33-ScRch1重组转化子质粒的单酶切鉴定。第3~5泳道为*Hin*d III酶切, 第6~8泳道为*Bam*H I酶切。左侧为DNA maker。

A: restriction map of the PCR amplified region of *ScRCH1* gene; B: left: the gel image of restriction digestion products of the pGFP33 vector (lane 1) and the PCR product for *ScRCH1* (lane 2) by both *Bam*H I and *Sph* I. Right: the gel image of restriction digestion of three independent recombinant pGFP33-ScRch1 plasmids with *Hin*d III (lane3~5) and *Bam*H I (lane 6~8), respectively. DNA marker sizes are indicated on the left.

图1 pGFP33-ScRch1的克隆和鉴定

Fig.1 Cloning and restriction digestion of pGFP33-ScRch1

4 757个非必需基因的缺失株[15]。通过the Munich Information Center for Protein Sequences (MIPS) (http://mips.helmholtz-muenchen.de/proj/funcatDB/)生物信息学分析, 我们从所有这些基因中发现共402个基因编码膜蛋白和脂质体蛋白, 它们与细胞中的蛋白运输和分泌相关(表1)。

2.3 ScRch1-GFP在编码膜蛋白和脂质蛋白基因的缺失株细胞中的亚细胞定位观察

为了研究以上402个基因和ScRch1蛋白的亚细胞定位的关系, 我们构建了表达ScRch1-GFP融合蛋白的质粒pGFP33-ScRch1(图1)。我们向这402个基因的缺失株细胞和野生型BY4743细胞中分别导入pGFP33-ScRch1质粒, 然后观察每个菌株中ScRch1-GFP的亚细胞定位。荧光显微镜观察结果表明, 在不外加钙离子的YPD培养基中, 野生型酵母细胞中没有观察到ScRch1-GFP定位信号, 但是在经0.2 mol/L $CaCl_2$处理的野生型酵母细胞中ScRch1-GFP定位于细胞质膜上(图2)。这个结果表明, ScRch1是受外界钙离子胁迫诱导的蛋白。和白念珠菌的CaRch1一样[13], ScRch1是一个细胞质膜蛋白。但是不同之处在于, ScRch1均匀分布于细胞质膜上(图2), 而CaRch1则主要集中

表1　编码酵母细胞膜蛋白和脂质蛋白的402个非必需基因

Table 1　List of the 402 nonessential yeast genes encoding membrane and liposome proteins

系统名 Systerm name	标准名 Standard name	系统名 Systerm name	标准名 Standard name	系统名 Systerm name	标准名 Standard name	系统名 Systerm name	标准名 Standard name	系统名 Systerm name	标准名 Standard name
Genes encoding endosome membrane proteins (148)									
YAL018c	*LDS1*	YOR307c	*SLY41*	YHL035c	*VMR1*	YPR192w	*AQY1*	YKL217w	*JEN1*
YBR205w	*KTR3*	YLR046c	-	YLR178c	*TFS1*	YKR065c	*PAM17*	YBL088c	*TEL1*
YBR219C	-	YBR199w	*KTR4*	YHL003c	*LAG1*	YKL221w	*MCH2*	YGR227w	*DIE2*
YOR002w	*ALG6*	YNL283c	*WSC2*	YHR105w	*YPT35*	YIL049w	*DFG10*	YPR194c	*OPT2*
YOR011w	*AUS1*	YOL079w	-	YEL031w	*SPF1*	YHR140w	-	YFL055w	*AGP3*
YBR210w	*ERV15*	YAL053w	*FLC2*	YCR010c	*ADY2*	YBL089w	*AVT5*	YPR198w	*SGE1*
YOL002c	*IZH2*	YLL052c	*AQY2*	YCR017c	*CWH43*	YOL092w	*YPQ1*	YDR264c	*AKR1*
YOL075c	-	YAL067c	*SEO1*	YKL034w	*TUL1*	YNL212w	*VID27*	YGL012w	*ERG4*
YOR034c	*AKR2*	YDR126w	*SWF1*	YKL187c	*FAT3*	YNL194c	-	YBR054w	*YRO2*
YLL043w	*FPS1*	YLR311c	-	YGR213c	*RTA1*	YNL237w	*YTP1*	YCR087w	-
YMR040w	*YET2*	YKL174c	*TPO5*	YEL059w	*HHY1*	YGL010w	-	YJL051w	*IRC8*
YAL007c	*ERP2*	YCR023c	-	YER060w	*FCY21*	YGL255w	*ZRT1*	YLR443w	*ECM7*
YBR183w	*YPC1*	YDR406w	*PDR15*	YDR417w	-	YGR290w	-	YBR008c	*FLR1*
YBR187w	*GDT1*	YEL004w	*YEA4*	YKL119c	*VPH2*	YKR088c	*TVP38*	YJL117w	*PHO86*
YLL028w	*TPO1*	YKL100c	*YPF1*	YKL046c	*DCW1*	YKR105c	*VBA5*	YML072c	*TCB3*
YMR159c	*ATG16*	YGR149w	-	YGR217w	*CCH1*	YDL113c	*ATG20*	YBR296c	*PHO89*
YNL327w	*EGT2*	YKR067w	*GPT2*	YER060w-a	*FCY22*	YPR071w	-	YNR019w	*ARE2*
YDR090c	-	YPL058c	*PDR12*	YKL051w	*SFK1*	YNR013c	*PHO91*	YNR062c	-
YBR224w	-	YER064c	*VHR2*	YJL170c	*ASG7*	YNR002c	*ATO2*	YOL158c	*ENB1*
YLR023c	*IZH3*	YNL159c	*ASI2*	YKL039w	*PTM1*	YNR048w	*CRF1*	YBR295w	*PCA1*
YOL084w	*PHM7*	YGL198w	*YIP4*	YLR404w	*FLD1*	YKR103w	*NFT1*	YCR037c	*PHO87*
YOR376w	-	YOL101c	*IZH4*	YGR197c	*SNG1*	YNR039c	*ZRG17*	YGR105w	*VMA21*
YLL061w	*MMP1*	YKR044w	*UIP5*	YJL193w	-	YBR040w	*FIG1*	YBR293w	*VBA2*
YPL264c	-	YNL219c	*ALG9*	YLR332w	*MID2*	YCL038c	*ATG22*	YDL199c	-
YBR180w	*DTR1*	YGL084c	*GUP1*	YKR051w	-	YPL189w	*GUP2*	YHR026w	*VMA16*
YNL087w	*TCB2*	YCR061w	-	YKR053c	*YSR3*	YER119c	*AVT6*	YCR048w	*ARE1*
YML038c	*YMD8*	YIL030c	*SSM4*	YKR040c	-	YLR237w	*THI7*	YDR205w	*MSC2*
YDR508c	*GNP1*	YDL012c	-	YKR106w	*GEX2*	YGL161c	*YIP5*	YDL093w	*PMT5*
YNL280c	*ERG24*	YJL094c	*KHA1*	YDR438w	*THI74*	YNR055c	*HOL1*	YGR289c	*MAL11*
YER185w	*PUG1*	YER140w	*EMP65*	YER181c	-				
Genes encoding plasma membrane proteins (143)									
YLR120c	*YPS1*	YDR122w	*KIN1*	YCR011c	*ADP1*	YHR135c	*YCK1*	YFL050c	*ALR2*
YMR243c	*ZRC1*	YMR008c	*PLB1*	YCL027w	*FUS1*	YOR101w	*RAS1*	YOL109w	*ZEO1*
YDR077w	*SED1*	YPL092w	*SSU1*	YDR420w	*HKR1*	YGR241c	*YAP1802*	YIL121w	*QDR2*
YAL022c	*FUN26*	YLL010c	*PSR1*	YLR138w	*NHA1*	YNL154c	*YCK2*	YML116w	*ATR1*
YGR121c	*MEP1*	YNL275w	*BOR1*	YHL019c	*APM2*	YNL173c	*MDG1*	YIL140w	*AXL2*
YOR008c	*SLG1*	YOR348c	*PUT4*	YLR242c	*ARV1*	YNR072w	*HXT17*	YIL170w	*HXT12*
YPL274w	*SAM3*	YNL270c	*ALP1*	YDR384c	*ATO3*	YDR273w	*DON1*	YJL093c	*TOK1*
YNL318c	*HXT14*	YPL265w	*DIP5*	YEL065w	*SIT1*	YIL013c	*PDR11*	YPR032w	*SRO7*
YLR096w	*KIN2*	YLR081w	*GAL2*	YER056c	*FCY2*	YGL077c	*HNM1*	YBR069c	*TAT1*
YOL011w	*PLB3*	YLR020c	*YEH2*	YEL017c-a	*PMP2*	YNL239w	*LAP3*	YBR298c	*MAL31*
YPL176c	*TRE1*	YLR121c	*YPS3*	YGR152c	*RSR1*	YCR098c	*GIT1*	YDL138w	*RGT2*

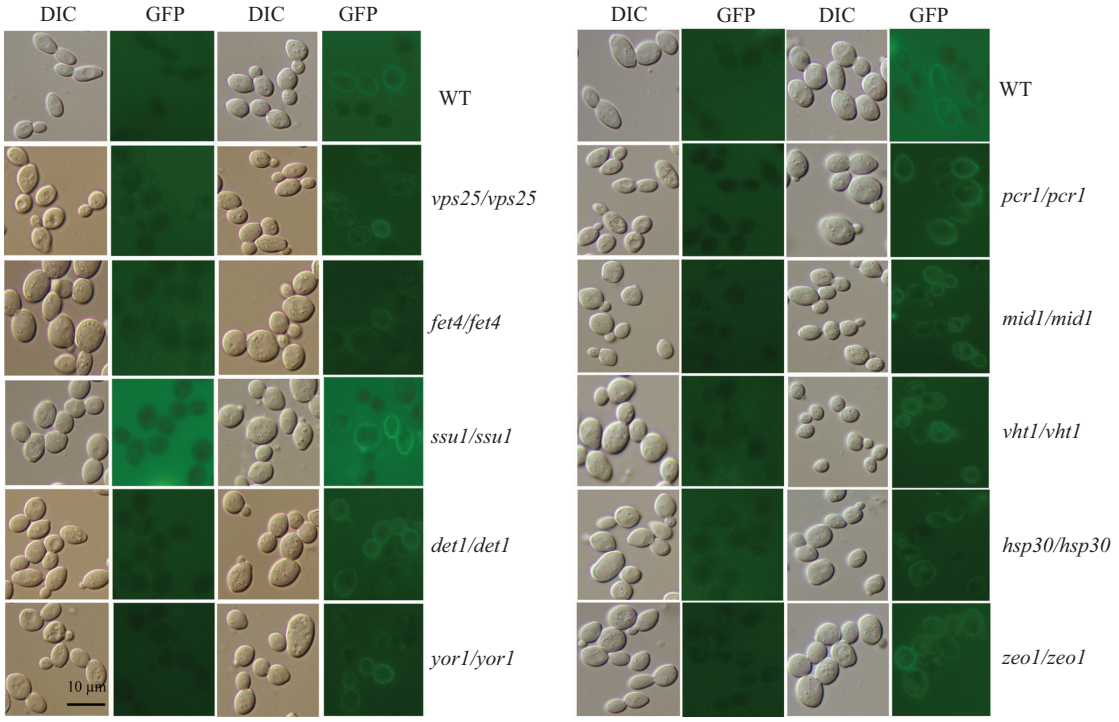
（续表 1）

系统名 Systerm name	标准名 Standard name	系统名 Systerm name	标准名 Standard name	系统名 Systerm name	标准名 Standard name	系统名 Systerm name	标准名 Standard name	系统名 Systerm name	标准名 Standard name
YDR055w	*PST1*	YNL322c	*KRE1*	YLR373c	*VID22*	YFL011w	*HXT10*	YBR043c	*QDR3*
YNL291c	*MID1*	YMR006c	*PLB2*	YJL214w	*HXT8*	YGR281w	*YOR1*	YBR068c	*BAP2*
YPL249c	*GYP5*	YDR144c	*MKC7*	YEL063c	*CAN1*	YIR028w	*DAL4*	YDL210w	*UGA4*
YAR031w	*PRM9*	YOL020w	*TAT2*	YLR214w	*FRE1*	YGR224w	*AZR1*	YDR046c	*BAP3*
YLL016w	*SDC25*	YCR021c	*HSP30*	YCR034w	*FEN1*	YOL103w	*ITR2*	YKR093w	*PTR2*
YMR192w	*GYL1*	YHL036w	*MUP3*	YCR028c	*FEN2*	YGR014w	*MSB2*	YJR040w	*GEF1*
YMR307w	*GAS1*	YDR345c	*HXT3*	YIL047c	*SYG1*	YMR319c	*FET4*	YBR023c	*CHS3*
YDR093w	*DNF2*	YHR096c	*HXT5*	YCR024c-a	*PMP1*	YOL122c	*SMF1*	YKR039w	*GAP1*
YOR328w	*PDR10*	YHR161c	*YAP1801*	YDR011w	*SNQ2*	YDR276c	*PMP3*	YNL098c	*RAS2*
YOL019w	-	YHR092c	*HXT4*	YPL036w	*PMA2*	YKR050w	*TRK2*	YNL142w	*MEP2*
YPL203w	*TPK2*	YHR094c	*HXT1*	YIL120w	*QDR1*	YBL042c	*FUI1*	YJL129c	*TRK1*
YMR183c	*SSO2*	YCL025c	*AGP1*	YBR038w	*CHS2*	YPR201w	*ARR3*	YER166w	*DNF1*
YMR011w	*HXT2*	YHL016c	*DUR3*	YLR452c	*SST2*	YDR536w	*STL1*	YDL194w	*SNF3*
YLR087c	*CSF1*	YOR153w	*PDR5*	YJR004c	*SAG1*	YFL041w	*FET5*	YER118c	*SHO1*
YER145c	*FTR1*	YDR033w	*MRH1*	YJR152w	*DAL5*	YLR342w	*FKS1*	YER123w	*YCK3*
YKL220c	*FRE2*	YDL035c	*GPR1*	YGR055w	*MUP1*	YPR124w	*CTR1*	YNL047c	*SLM2*
YBR294w	*SUL1*	YGR032w	*GSC2*	YIL105c	*SLM1*	YNL268w	*LYP1*	YOL081w	*IRA2*
YMR058w	*FET3*	YDR497c	*ITR1*	YML123c	*PHO84*				
Genes encoding vacuolar membrane proteins (87)									
YJL178c	*ATG27*	YLL048c	*YBT1*	YDR128w	*MTC5*	YDR119w	*VBA4*	YOR273c	*TPO4*
YEL051w	*VMA8*	YPL019c	*VTC3*	YDR080w	*VPS41*	YML018c	-	YDR481c	*PHO8*
YEL013w	*VAC8*	YFL048c	*EMP47*	YLL015w	*BPT1*	YPL162c	-	YBR127c	*VMA2*
YOR106w	*VAM3*	YOL129w	*VPS68*	YPL006w	*NCR1*	YDR135c	*YCF1*	YFR019w	*FAB1*
YKL146w	*AVT3*	YDL185w1	-	YDR284c	*DPP1*	YOR036w	*PEP12*	YCR044c	*PER1*
YCL069w	*VBA3*	YDL149w	*ATG9*	YGR020c	*VMA7*	YBR207w	*FTH1*	YDR486c	*VPS60*
YGR163w	*GTR2*	YDL185w	*VMA1*	YIL088c	*AVT7*	YBR241c	-	YPL045w	*VPS16*
YJL154c	*VPS35*	YNL054w	*VAC7*	YMR088c	*VBA1*	YDR105c	*TMS1*	YLR396c	*VPS33*
YDR352w	*YPQ2*	YDL123w	*SNA4*	YDR229w	*IVY1*	YNL326c	*PFA3*	YKL080w	*VMA5*
YHR028c	*DAP2*	YLR447c	*VMA6*	YKR007w	*MEH1*	YPL236c	*ENV7*	YLR148w	*PEP3*
YEL027w	*VMA3*	YBR077c	*SLM4*	YPR156c	*TPO3*	YOL060c	*MAM3*	YGL156w	*AMS1*
YER072w	*VTC1*	YJL012c	*VTC4*	YGL006w	*PMC1*	YOR087w	*YVC1*	YMR160w	-
YGR138c	*TPO2*	YGL023c	*PIB2*	YGL212w	*VAM7*	YPL180w	*TCO89*	YDL054c	*MCH1*
YCL063w	*VAC17*	YPR036w	*VMA13*	YMR077c	*VPS20*	YPL149w	*ATG5*	YOR270c	*VPH1*
YOR316c	*COT1*	YML121w	*GTR1*	YHR039c-a	*VMA10*	YPL234c	*VMA11*	YAR002c-a	*ERP1*
YLR001c	-	YJR001w	*AVT1*	YBR217w	*ATG12*	YDL128w	*VCX1*	YNL101w	*AVT4*
YOR357c	*SNX3*	YJL059w	*YHC3*	YMR195w	*ICY1*	YDL077c	*VAM6*	YOR332w	*VMA4*
YOL082w	*ATG19*	YGR106c	*VOA1*						
Genes encoding lipid proteins (24)									
YOR081c	*TGL5*	YMR246w	*FAA4*	YMR313c	*TGL3*	YMR110c	*HFD1*	YMR148w	*OSW5*
YML008c	*ERG6*	YOL048c	*RRT8*	YNL231c	*PDR16*	YDL052c	*SLC1*	YKL140w	*TGL1*
YBR204c	*LDH1*	YOR246c	*ENV9*	YDR525w-a	*SNA2*	YKR046c	*PET10*	YBR041w	*FAT1*
YLL012w	*YEH1*	YKL179c	*COY1*	YKR089c	*TGL4*	YCL005w	*LDB16*	YBR177c	*EHT1*
YPL232w	*SSO1*	YDR425w	*SNX41*	YBR042c	*CST26*	YDR275w	*BSC2*		

表2 与ScRch1亚细胞膜定位有关的10个膜蛋白基因

Table 2 List of 10 membrane-encoding genes involved in ScRch1 subcellular localization

系统名 Systerm name	标准名 Standard name	功能 Function
YBR219C	-	Putative protein of unknown function
YBR224W	-	Putative protein of unknown function
YDR417C	-	Dubious open reading frame unlikely to encode a protein, based on available experimental and comparative sequence data; partially overlaps the verified ORF RPL12B/YDR418W
YIL049W	*DFG10*	Probable polyprenol reductase that catalyzes conversion of polyprenol to dolichol, the precursor for N-glycosylation; involved in filamentous growth; mutations in human ortholog SRD5A3 confer CDG (Congenital Disorders of Glycosylation)
YDR011W	*SNQ2*	Plasma membrane ATP-binding cassette (ABC) transporter, multidrug transporter involved in multidrug resistance and resistance to singlet oxygen species
YHR094C	*HXT1*	Low-affinity glucose transporter of the major facilitator superfamily, expression is induced by Hxk2p in the presence of glucose and repressed by Rgt1p when glucose is limiting
YNL101W	*AVT4*	Vacuolar transporter, exports large neutral amino acids from the vacuole; member of a family of seven *S. cerevisiae* genes (AVT1-7) related to vesicular GABA-glycine transporters
YLR148W	*PEP3*	Component of CORVET tethering complex; vacuolar peripheral membrane protein that promotes vesicular docking/fusion reactions in conjunction with SNARE proteins, required for vacuolar biogenesis
YBR177C	*EHT1*	Acyl-coenzymeA: ethanol O-acyltransferase that plays a minor role in medium-chain fatty acid ethyl ester biosynthesis; possesses short-chain esterase activity; localizes to lipid particles and the mitochondrial outer membrane
YBR204C	*LDH1*	Serine hydrolase; exhibits active esterase plus weak triacylglycerol lipase activities; proposed role in lipid homeostasis, regulating phospholipid and non-polar lipid levels and required for mobilization of LD-stored lipids; localizes to the lipid droplet (LD) surface; contains a classical serine containing catalytic triad (GxSxG motif)

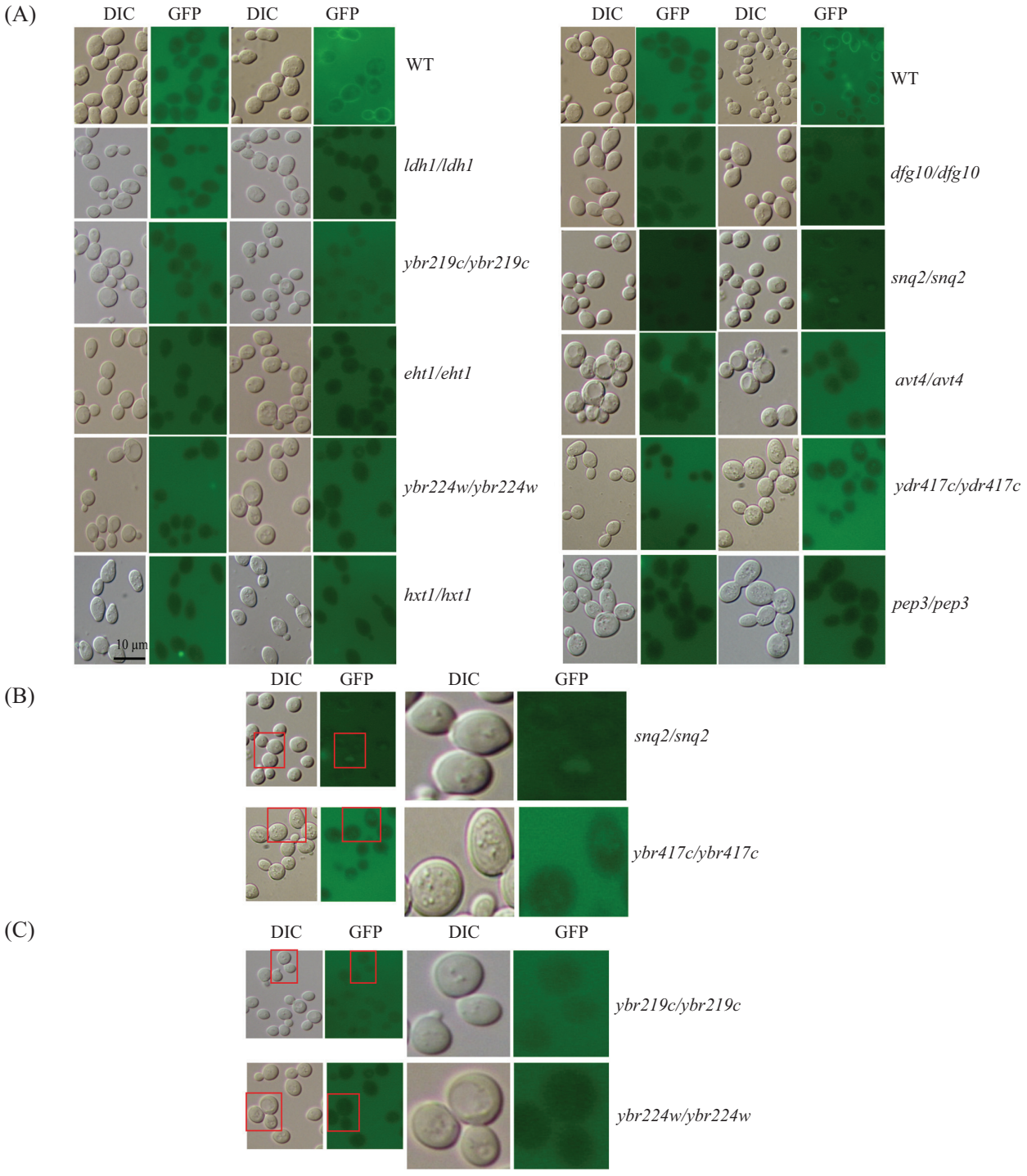


每个菌株的左侧DIC(微分干涉相衬)和GFP(绿色荧光蛋白)图来自未经钙离子处理的细胞, 右侧DIC和GFP图来自经0.2 mol/L $CaCl_2$处理的细胞。

DIC (differential interference contrast) and GFP (green fluorescent protein) pictures on the left of each strain are from cells not treated with $CaCl_2$, and DIC and GFP pictures on the right are from cells treated with 0.2 mol/L $CaCl_2$.

图2 ScRch1-GFP在野生型(WT)菌株和10个双倍体基因缺失菌株中的亚细胞定位

Fig.2 Subcellular localization of ScRch1-GFP in the wild type and 10 mutant strains



A: 每个菌株的左侧DIC和GFP图来自未经钙离子处理的细胞, 右侧DIC和GFP图来自经0.2 mol/L $CaCl_2$处理的细胞。显微镜放大倍数为1 000倍; B: 左侧为A图中$CaCl_2$处理的菌株*snq2/snq2*和*ydr417c/ydr417c*图片, 右侧取自红框标出位置的放大图; C: 左侧为A图中$CaCl_2$处理的菌株*ybr219c/ybr219c*和*ybr224w/ybr224w*图片, 右侧取自红框标出位置的放大图。

A: DIC and GFP pictures on the left of each strain are from cells not treated with $CaCl_2$, and DIC and GFP pictures on the right are from cells treated with 0.2 mol/L $CaCl_2$. Amplification scale is 1 000×; B: close-up DIC and GFP pictures of *snq2/snq2* and *ydr417c/ydr417c* cells treated with $CaCl_2$ from the red frame field in Fig.2A; C: close-up DIC and GFP pictures of *ybr219c/ybr219c* and ybr*224w/ybr224w* cells treated with $CaCl_2$ from the red frame field in Fig.2A.

图3 ScRch1-GFP在野生型(WT)菌株和影响ScRch1定位的10个基因缺失菌株中的亚细胞定位

Fig.3 Subcellular localization of ScRch1-GFP in the wild type strain and the 10 mutant strains for genes affecting ScRch1 localization

于白念珠菌的酵母态细胞的芽颈部位[13]。

在不外加钙离子的YPD培养基中, 和野生型酵母细胞中一样, 所有402个基因缺失株中均没有观察到ScRch1-GFP定位信号(图2和图3; 其他结果未显示)。在外加0.2 mol/L $CaCl_2$的YPD培养基中, 和野生型酵母细胞中一样, 392个基因缺失株中ScRch1-GFP能够正确定位于细胞质膜上(图2; 其他结果未显示), 这表明这392个基因的缺失不影响ScRch1-GFP的正确定位。但是, 在其他10个基因缺失株细胞中, 经过0.2 mol/L $CaCl_2$处理后都没有观察到ScRch1-GFP的细胞质膜定位信号(图3A、表2), 表明这10个基因的缺失导致ScRch1-GFP不能正确定位到细胞质膜上。对这10个菌株细胞中的荧光定位信号进一步放大观察发现, *ybr219c/ybr219c*和*ybr224w/ybr224w*两个缺失菌株经钙离子处理后, 细胞质内没有任何荧光信号(图3C), 而其他8个缺失菌株在钙离子处理后, 虽然细胞质膜上没有荧光信号, 但是在它们的细胞质中有荧光信号(图3B, 随机选出*snq2/snq2*和*ydr417c/ydr417c*; 其他结果没有显示)。

3　讨论

在我们筛选到的10个影响ScRch1亚细胞定位的基因中, *SNQ2*和*HXT1*编码的蛋白都定位于细胞质膜上。Snq2是一种包含ATP结合盒(ABC)的运输蛋白, 在酿酒酵母细胞内介导类固醇或者相关膜脂的运输, 与酿酒酵母对多种药物的抗性有关[16]。此外, Snq2还是一种高亲和力输出铜离子的透性酶[17]。*SNQ2*基因的缺失导致ScRch1不能定位到细胞质膜上, 可能与Snq2的类固醇或膜脂运输功能相关。Hxt1是定位在细胞质膜上的葡萄糖运输蛋白, 它是酿酒酵母细胞中己糖运输蛋白家族中的重要成员, *HXT1*基因的缺失使细胞对葡萄糖的吸收利用能力降低, 影响细胞的生长和乙醇生成[18]。它与ScRch1亚细胞定位的关系有待进一步研究。

液泡是酿酒酵母细胞调节内环境的重要细胞器, 液泡膜上存在大量的运输蛋白。在编码液泡膜蛋白的基因中我们筛选到*AVT4*和*PEP3(VPS18)*两个基因(表2)。Avt(1-7)家族是酿酒酵母细胞中介导液泡双向氨基酸运输的运输蛋白家族, Avt4利用ATP介导酪氨酸、谷氨酰胺、天冬酰胺、亮氨酸和异亮氨酸的运输[19]。在酿酒酵母细胞中, *PEP3*是液泡生物形成必需的基因[20]。*PEP3*是CORVET复合物的组成成分, CORVET复合物是一个拴膜因子, 可以促进蛋白与膜的融合, CORVET复合物还可以与SNAREs结合, 而脂锚定的SNARE介导胞内蛋白的定向运输, 也促进蛋白质与膜的融合[21-22]。因此, 细胞缺失了*PEP3*后, CORVET复合物的功能受到影响, 从而可能导致SNARE胞内蛋白的定向运输功能受到影响, 这可能是造成ScRch1不能运输到细胞质膜上的原因。

我们筛选到两个编码脂质蛋白的基因*LDH1*和*EHT1*。Ldh1定位于脂滴上, 具有酯酶和脂酶的活性, 与细胞中脂质稳态的调节有关[23-24]。*EHT1*编码中链脂肪酸乙酯合成有关的酶, Eht1蛋白具有水解酶活性[25]。脂质体不单单是一种存储体, 越来越多的研究发现, 脂质体不仅可以为细胞提供能量、结构物质和信号分子, 还参与蛋白质的分选定位[26]。在细胞生长过程中, 脂滴与内质网膜保持紧密的联系[27]。这表明ScRch1的亚细胞定位过程中, 可能受到脂质体的调控。

ESCRT复合物负责将蛋白质分选到多囊体(MVB)的管腔囊体上[15]。但是, 本研究没有筛选到影响ScRch1亚细胞定位的任何ESCRT复合物基因。然而, 我们筛到3个(*SNQ2*、*HXT1*和*AVT4*)与细胞运输有关的基因, 其中*SNQ2*和*HXT1*编码细胞质膜上的转运蛋白, *AVT4*编码液泡膜上的转运蛋白。我们发现, *YBR219c*和*YBR224w*两个基因的缺失导致细胞内无任何荧光信号(图3A), 这说明这两个基因的缺失可能影响了*RCH1*基因的表达或者导致ScRch1表达后完全降解。相比而言, 其他8个基因的缺失只是导致细胞质膜上没有荧光信号, 而在细胞质中仍有荧光信号存在, 这说明这8个基因的功能, 可能不影响ScRch1蛋白的表达和合成, 只是协助合成的ScRch1蛋白从合成的位点运输到细胞质膜上。

总之, 本研究通过观察ScRch1-GFP融合蛋白在编码膜蛋白和脂质蛋白的402个酿酒酵母基因缺失株细胞中的定位情况, 发现只有10个基因的缺失导致ScRch1-GFP不能在细胞质膜上正确定位。这些基因的功能涉及的细胞过程包括细胞代谢、细胞转运、细胞类型分化和细胞过程的辅因子。因此, 应对胞外的高浓度钙离子胁迫, ScRch1在酵母细胞内经诱导表达和合成之后, 要经过特定蛋白的协助, 最后才定位于细胞质膜上去行使功能。对这10个基因的进一步研究, 将有助于我们深入了解钙离子调控蛋白ScRch1的亚细胞定位调控机理。

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