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CaRCHI基因同源,因此把它命名为ScRCHI,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HXTI,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Ca²⁺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Ca2+-ATPase的PMCI和编码内质 网/高尔基体(ER/Golgi)Ca²⁺-ATPase的PMR1。Pmc1 和Pmr1分别将胞质内过多的Ca²⁺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CaRCHI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 转运到最后目的地一细胞质膜。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; CaCl₂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BamH 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(2087 bp), 然后将这个片段克隆到pGFP33(Michael N. Hall赠 送)的BamH I和Sph I位点, 重组子分别经过Hind III 和BamH 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°C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pG-FP33-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 CaCl₂,继续培养2 h。从 CaCl₂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BamH I/Sph I双酶切(图 1B),连接后得到重组子。重组子分别经过Hind III 和BamH 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(Hind III/BamH 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Hind III酶切,第6~8泳道为BamH 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 *Hind* 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 CaCl₂ 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

系统名	标准名	系统名	标准名	系统名	标准名	系统名	标准名	系统名	标准名
Systerm	Standard	Systerm	Standard	Systerm	Standard	Systerm	Standard	Systerm	Standard
Genes encod	ling endosom	e membrane pr	oteins (148)	name	name	name	name	name	
YAL018c	LDS1	YOR307c	SLY41	YHL035c	VMR1	YPR192w	AOYI	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	AUSI	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 encod	ling plasma n	iembrane prote	eins (143)						
YLR120c	YPSI	YDR122w	KINI	YCR011c	ADPI	YHR135c	YCKI	YFL050c	ALR2
YMR243c	ZRCI	YMR008c	PLBI	YCL027w	FUSI	YOR101w	RASI	YOL109w	ZEOI
YDR077w	SEDI	YPL092w	SSUI	YDR420w	HKRI	YGR241c	YAP1802	YIL121w	QDR2
YAL022c	FUN26	YLL010c	PSRI	YLR138w	NHAI	YNL154c	YCK2	YML116w	AIRI
YGK121c	MEP1	YNL2/5W	BUKI DUT4	YHLUI9C	APM2	YNL1/30	MDGI	YIL 170	AAL2
YOR008C	SLGI	Y OK 3480	PU14	YLK242C	ARVI	YNKU/2W	HXII/	YIL1/0W	HX112 TOV1
1 FL2/4W	SAM3 UVT14	Y NL2/UC	ALP1	1 DK3840	AIUS	YU 012-	DUNI	1 JL0930	IUNI SPO7
INLJISC	ПАТТ4 КІМ2	1 FL200W	GALZ	I ELUODW	SITT ECV2	VCL077a		IFKU32W	SKU/
I LKU96W	NINZ DI D 2	ILKUðIW	GALZ	1 EKU300		1 GLU//C		1 DKU090	IALI MAL21
VDI 174	TDE1	1 LK0200	IEEZ	I ELUI /C-a	FNIF2 DCD1	I INL239W	LAP'S	1 DK2980	MALSI RCT2
YPL1/6C	IKEI	YLK121c	11955	YGK1520	KSK1	Y CK098c	GIII	YDL138W	KG12

	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 4	02 nonessential yeast genes encoding membrane and liposome proteins

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Integration Integration <thintegration< th=""> <thintegration< th=""></thintegration<></thintegration<>	VAR031w	PRMQ	VOL 020w	TAT2	VI R21/w	ERE1	VGR224w	47R1	VDR046c	RAP3
LEDOW DATE LENOS LENOS <thl< td=""><td>VII 016w</td><td>SDC25</td><td>VCR021c</td><td>HSP30</td><td>VCR03/w</td><td>FENI</td><td>VOI 103w</td><td>ITR?</td><td>VKR093w</td><td>PTR?</td></thl<>	VII 016w	SDC25	VCR021c	HSP30	VCR03/w	FENI	VOI 103w	ITR?	VKR093w	PTR?
TAM.Y2N OLD THENDAW HOLD THENDAW HOLD THENDAW SUL1 YMR307w GALJ YDRA364 HAT3 YUL047c SYGI YMR319c EE74 YBR039w GAP1 YDR932w DNF2 YHR096c HXT3 YUL042c SYGI YMR319c EE74 YBR039w GAP1 YDR010w - YHR096c HXT4 YPL036w PMP2 YUL122c SMF1 YKR039w GAP1 YDL019w - YHR092c HXT4 YPL036w PMA2 YKR050w TRK2 YNL042w ME71 YMR138c SSO2 YCL025c AGP1 YBR038w CH32 YPR201w ARR3 YER166w DNF1 YMR011w HXD35c GRF1 YOR04c SG2 YDR36w STZ1 YDL128w TCK3 YKL200c FKS1 YER123w TCK3 YKL200c FR62 YDL035c GRF1 YGR16Sw HZ73 YDL047c TPA1 YMR058w <td< td=""><td>VMP102w</td><td>GVL1</td><td>VHI 036w</td><td>MUP3</td><td>VCP028c</td><td>FEN2</td><td>VGP014w</td><td>MSR2</td><td>VID040w</td><td>GEF1</td></td<>	VMP102w	GVL1	VHI 036w	MUP3	VCP028c	FEN2	VGP014w	MSR2	VID040w	GEF1
TABLOW GASJ TEDER-2 TATA TEDER-2 TATA TEDER-2 CALA YDR093w DNF2 YHR096 HXT3 YCR024c-a PMP1 YOL122c SMF1 YKR039w GAP1 YOR328w PDR10 YHR161c YLP1801 SNQ2 YDR276c PMP3 YNL098c RAS2 YOL199w - YHR094c HXT1 YLL00w QDR1 YBL042c FU11 YJL29c TRK1 YMR18a SS02 YCL25c AGP1 YBR038w CR52 YDR36w FU11 YDL129c TRK1 YMR011w HZ2 YHL016c DUR3 YLR047c STL1 YDL194w SNF1 YDL194w SNF1 YKR011w HA22 YHL035w DAL5 YLR34w FKS1 YER123w YCX3 YKR190w F771 YDR033w MRH1 YIR15w DAL5 YLR34w FKS1 YER123w YCX3 YKL208v F472 YLR035c SL11 YDL047c <td< td=""><td>VMD 207m</td><td>GASI</td><td>VDP245a</td><td>HYT3</td><td>VII 047a</td><td>SVG1</td><td>VMP210a</td><td>FFTA</td><td>VDD022</td><td>CHS3</td></td<>	VMD 207m	GASI	VDP245a	HYT3	VII 047a	SVG1	VMP210a	FFTA	VDD022	CHS3
TOKOSSW DAT2 THN096 DAT3 TOKN24-4 FAIL TOK1226 SUT1 TEKN39W CAT1 YOR328-W PDR10 YHR161c X4P1801 YDR011W SNQ2 YDR276c PMF2 YNL098c RAS2 YDL019W - YHR092c HXT4 YDR011W SNQ2 YKR050W TRK2 YNL018c YNL12b TRK1 YMR183c SSO2 YCL025c AGP1 YBR038W CHS2 YPR201W ARB3 YER166W DNF1 YMR011W HXT2 YHL036C DLB3 YLR452c SS72 YDR33W STL1 YDL124W STL1 YDL124W SNF3 YLR037c CSF1 YOR153W PDR5 YIR04c SG71 YDL32W CK3 YKL12W CK3 YKL20c FRE2 YDL035c GPR1 YGR055W MUP1 YPL24W CTR1 YNL47C SLA2 YBR38W SU11 YGR052W GSC2 YLL05C SLM1 YNL26W L7K3 YMR058W FE73 YDR497c ITR1 YDR128W MTC5 YD	VDD002m	DNE2	VUD006	IIXI J	VCD024a a		VOL 122a	SME1	VKD020	CAP1
YOR328wPDR10YHR161cYAP180YDR011wSNQ2YDR27cePMP3YNL088cRAS2YOL019w-YHR092cHX74YPL036wPMA2YKR050wTRK2YRL142wMEP2YPL203wTPK2YHR094cHX71Y1L120wQDR1YBL042cFU1YJL120wTRK1YMR183cSCS2YCL025cAGR1YBR03wClS2YPR01bwARR3YER160wDNF1YMR011wIJX7YHL016cDJR3YLR452cSS72YDR35cwSTL1YDL194wSNF3YLR087cCSF1YDR033wMR11YIR152wDAL5YLR432wFK51YER18cSJAOYRL220cFR71YDR033wMR11YIR152wDAL5YLR442wFK51YRL123wTCK3YRL220cFF73YDR497cITR1YML123cPHO84TTP4YNL047cSJAOYRL230wSGC2YLI05cSJAUYNL24wCTR1YNL047cSJAOYRL520wFF73YDR497cITR1YDL128wPHO84TTP4YLR058wFF73YDR497cITR1YDL128wPHO84YNL048wPHO84YRL51wFF73YDR497cITR1YDR128wPHO84YNL018wITP4YBL054wFF73YDL918wITF1YDR128wYDR148wPHO84YDR148wYBL054wFF73YDL128wITF1YDL128wITF1YDR188wPHO84YLL054wMA73YDL189wITF1YDL128wIT	I DK095W	DWP2	111K090C	IIAIJ	1 CK024C-a	1 1/11 1	TOL122C	511111	1 KK039W	UAL I
YOL019w-YHR092cHX74YPL036wPMA2YKR050wTRK2YNL142wMEP2YPL20awTRK2YHR094cHX71Y1L120wQR1YBL042cFU1Y1L120eTRK1YMR18acSZ02YCL025cAGP1YBR038wCHS2YPR21wARR3YER166wDNF1YMR011wI/X72YHL016cDUB3YLR452cSX72YDR53wSTL1YDL194wSNF3YLR087cCSF1YOR153wPDR5YJR04cSAG1YLR44wFKS1YER18cSHO1YER145cFTR1YDR033wMR11YJR152wDAL5YLR44wFKS1YER123wICK3YRL20cFR62YDL032wGSC2YL105cSLM1YNL68wL7P1YNL07cSLM2YRR58wFE73YDR49cITR1YML12cPH084YN171WYBL047cSLM2YRL058wFE73YDR49cITR1YML12cPH084YP109YD0YEL051wFH44YPL09kITZ3YDR68wITC5YDR118wIFD6YD0723cTP04YEL051wF443YPL09kITZ3YDR68wITC5YD119wIFB6YD0723cIFD6YR1068wIF14YDL12wITP1YDL12wITP4YD128wIFA6YD70YEL051wIF448YPL04kcIF37YDR18wIAA1YD182wIFA6YD70YR1060wIAA3YDL19wIFG7YDR68wIF77YDR35cYCF1YDR48cIF26	YOR328w	PDR10	YHR161c	YAP1801	YDR011w	SNQ2	YDR276c	PMP3	YNL098c	RAS2
YPL203wTPK2YHR094cHXT1YHL120wQDR1YBL042cFU11YJL129cTRK1YMR183cSSO2YCL025cACP1YBR038wCH52YPR201wARR3YER160wDNF1YMR01wHXT2YHL016cDUR3YLR452cSST2YDR536wST11YDL194wSNF3YLR087cCSF1YOR153wPAD5YIR04bcSAG1YLR41wFET3YER18cSH01YER145cFRE2YDL035cGPR1YGR055wMUP1YPR124wCTR1YNL047cSLM2YBR294wSUL1YGR032wGSC2YIL105cSLM1YNL268wLTP1YOL081wIR.2YMR058wFET3YDA97cITR1YML123cPH084TP21YDL047cTP04YHL178cATG27YLL048cFZ11YDR123wMTC5YDR119wIBA4YOR27acTP04YEL031wIAA8YPL048cFM74YLL05wBP71YDL162c-YBR12mIMA2YEL051wIAA8YPL048cEMP47YLL05wBP71YDL62c-YBR19wEAB1YKL146wIAA3YDL18wI-YDR36wNCR1YDR13cYDR19wFB11YDL62cYR019wFB11YKL146wIAA3YDL18wI-YDR36wNCR1YDR35cYE11YB162cYCR04cFE73YKL146wIAA3YDL18wI-YDR35cYDR19wFTH1YDR46cIP530YKL146wIAA3YDL18w<	YOL019w	-	YHR092c	HXT4	YPL036w	PMA2	YKR050w	TRK2	YNL142w	MEP2
YMR183cSSO2YCL025cAGP1YBR038wCHS2YPR201wARR3YER166wDNF1YMR011wHX72YHL016cDUR3Y1.R452cSST2YDR556wSTL1YDL194wSNF3YLR087cCSF1YDR033wMRH1YJR052wSAG1YFL041wFET5YER18cSHO1YER145cFTR1YDR033wMRH1YJR152wDAL5YLR342wFES1YER13wYCK3YKL220cFRE2YDR035wMCP1YRL94wCTR1YNL047cSLM2YBR294wSUL1YGR032wGSC2YIL105cSLM1YNL268wLYP1Y0.0481wIRA2YMR058wFET3YDR497cITR1YML123cPH084FET6YDP4YTP1YD115wFEA4YOR273cTP04YLL051wATG27YLL048cYBT1YDR128wYDR19wVB119wYBA4YOR273cTP04YEL051wFAG8YPL045wIF23YDR19wVB119wYB10YB126YDR17cYBR17cFM42YCL064wIAA3YOL129wIF268YPL065wNCR1YDR35cYCF1YFR019wFEB1YLL146wAT73YDL185wIAAYDR23bwNAAYDR23bwPHA1YDR05cTMS1YLR48wFF56YGR164wIF23YNL054wIACYMR08cIF71YBR24bcYLA1YBR05cIF56YF56YGL154wIFA3YDL18bwIACYMR08cIFA1YDR05cTMS1YLR48w <td>YPL203w</td> <td>TPK2</td> <td>YHR094c</td> <td>HXT1</td> <td>YIL120w</td> <td>QDR1</td> <td>YBL042c</td> <td>FUII</td> <td>YJL129c</td> <td>TRK1</td>	YPL203w	TPK2	YHR094c	HXT1	YIL120w	QDR1	YBL042c	FUII	YJL129c	TRK1
YMR011wHXT2YHL016eDUR3YLR452cST72YDR35wSTL1YDL194wSNF3YLR087cCSF1YOR153wPDR5YJR004cSAG1YFL041wFET5YER118cSH01YER145cFTR1YDR033wMRH1YJR152wDAL5YLR342wFES1YER123wYCAYRL20ceFRE2YDL035cGPR1YGR055wMUP1YPR124wCTR1YNL047cSLM2YBR294wSU11YGR032wGSC2YIL105cSLM1YNL26wLYP1YOL01wIM2YMR058wFET3YDR497cITR1YML12acPH084FET3YOR273cTPO4YL178cATG27YLL048cFB71YDR128wMTC5YDR119wVBA1YOR273cTPO4YEL013wVMA8YPL049cFTG3YDR080wVFS41YHL162c-YDR481cPH08YEL013wVMA8YPL048cEMP47YLL015wBP71YPL162c-YBR17cVMA2YCR16wVAA3YOL19wVFS68YPL06wNCR1YDR35cYCF1YER019wFAB1YLL146wAV73YDL185w1-YDR284cDPP1YOR36wPEP12YCR04cPER1YLL146wAV73YDL185wVAA1YDR28wMTG1YBR207wFTH1YDR486cPF560YGR16awG7R2YDL185wVAA1YDR35cATA1YDR35cTAA1YLR48wPE73YLL146wPF33YNL54wKAC7YMR08	YMR183c	SSO2	YCL025c	AGP1	YBR038w	CHS2	YPR201w	ARR3	YER166w	DNF1
YLR087cCSF/IYOR153wPDR5YJR04ceSAGIYFL041wFET5YER18ceSHOIYER145cFTRIYDR033wMRHIYJR152wDAL5YLR342wFKSIYER123wYCK3YKL20ceFRE2YDL035cGPR1YGR055wMUP1YPR124wCTRIYNL047cSLM2YBR294wSUL1YGR032wGSC2YIL105cSLM1YNL268wLPIYOL081wIRA2YMR058wFET3YDR497cITAYML123cPH084TPO4YLL178cATG27YLL048cYBT1YDR128wMTC5YDR119wPBA4YOR273cTPO4YEL051wFMA8YPL019cFTC3YDR080wPFS41YML018c-YDR451cFH08YEL013wFAC8YFL048cEMP47YLL015wBPT1YDL162c-YBR17cFH04YEL013wFAC8YFL048cEMP47YLL015wBPT1YDL62c-YBR17cFAB1YCL060wFA33YOL19wFJS68YDR080cVCR1YDR35cYCF1YBR017cFAB1YLL164cFA33YDL185wFM11YIL08cAT77YBR207cFTH1YDL48cFPS60YGR163wGTR2YDL18bwFM14YIL08cAT77YBR21c-YLL045wFPS61YLL164cFP335YNL054wFM21YMR08cFB11YDR486cFPS60YS36YGR163wGTR2YDL18bwFM21YMR08cFM14YDR15c<	YMR011w	HXT2	YHL016c	DUR3	YLR452c	SST2	YDR536w	STL1	YDL194w	SNF3
YER145cFTR1YDR033wMRH1YJR152wDAL5YLR342wFK31YER123wYCK3YKL220cFRE2YDL035cGPR1YGR055wMUP1YPR124wCTR1YNL04rcSLM2YBR294wSUL1YGR032wGSC2YIL105cSLM1YNL268wLYP1YOL081wIR42YMR087wFET3YDR49rcITR1YDL128cPH084LYP1YOL081wIR42YMR087wFET3YDL94wFBT1YDL128cPH084FYDR19mYBA47YDR19mYBA47YL178cATG27YL1048cFBT1YDR128wMTC5YDR119wVBA4YOR273cTPO4YEL051wVMA8YPL04eFBT1YDR128wMTC5YDR19mVBA4YOR273cTPO4YCL060wVAA8YPL04eFBT1YDR128wMTC1YDR126c-YBR127cVMA2YCL164wVAA3YOL129wVPS66YPL06cwNCR1YDR13cwYCF1YBR04ccPER1YCL164wVBA3YDL185wIM11YIL08cAV71YBR241c-YER04ccPER3YCL164wVBA3YDL185wIM11YIL08cAV71YBR241c-YP1045wPF36YGL164wVB135wVM10YGL70YBR19wAFA1YBR37wYBR19wYB164wPF36YGL164wIPA1YIL08cwAV71YBR241c-YP1045wPF36YB164wYGL164wIPA1YIL08cwIPA1YBR37w	YLR087c	CSF1	YOR153w	PDR5	YJR004c	SAG1	YFL041w	FET5	YER118c	SHO1
YKL220e <i>FRE2</i> YDL035e <i>GPR1</i> YGR055w <i>MUP1</i> YPR124w <i>CTR1</i> YNL047e <i>SLM2</i> YBR294w <i>SUL1</i> YGR032w <i>GSC2</i> YIL105e <i>SLM1</i> YNL268w <i>LYP1</i> YOL081w <i>IR42</i> YMR058w <i>FE13</i> YDR47c <i>ITR1</i> YML123e <i>PH084</i> </td <td>YER145c</td> <td>FTR1</td> <td>YDR033w</td> <td>MRH1</td> <td>YJR152w</td> <td>DAL5</td> <td>YLR342w</td> <td>FKS1</td> <td>YER123w</td> <td>YCK3</td>	YER145c	FTR1	YDR033w	MRH1	YJR152w	DAL5	YLR342w	FKS1	YER123w	YCK3
YBR294wSUL1YGR032wGSC2YIL105cSLM1YNL268wLYP1YOL081wIRA2YMR058wFE73YDR497cITR1YML123cPH084FUFUFUGenes encodication of the phane state stat	YKL220c	FRE2	YDL035c	GPR1	YGR055w	MUP1	YPR124w	CTR1	YNL047c	SLM2
YMR058wFE73YDR497cJTR1YML123cPH084Concest structureYMR058wFE73YL048cJBT1YDR128wMTC5YDR119wVB.44YOR273cTP04YL178cATG27YL108cVBT10YDR180wPF341YML018c-YDR481cPH08YEL051wFM48YPL019cTTG3YDR080wPF341YML018c-YDR481cPH08YEL013wIA48YPL019cIP13YDL132cCF1YBR127cIP442YGR104wIA43YDL18ywIP173YDL162c-YBR127cIP442YCL060wIF43YDL18ymIP17YDR326cIP171YDR35cYCF1YPR019wFF361YCL063wIF43YDL18ymIT41YIL08kcIP171YBR217cYRA46cIP561YL154cIF43YDL18ymIF41YIL08kcIP171YBR341c-YER37cYRA56cIP533YDR352wIP20YDL123wSN44YDR229wIP171YBR34cIP13YL184wPE93YBR07xYB23YL144ymYBL36cIP63YOL060cMAM3YGL15wAM51YBR07xYR17YBL36cIP64YMR16w-YE135cIP171YMR16w-YBR07xYR17YBL36cIP63YGL060cMAM3YGL15wMA51YM171YMR16wZG15wYBR07xYR17YBR04wYBL36cIP63YGL07wYBR16wYM171Y	YBR294w	SUL1	YGR032w	GSC2	YIL105c	SLM1	YNL268w	LYP1	YOL081w	IRA2
Genes encode service servi	YMR058w	FET3	YDR497c	ITR1	YML123c	PHO84				
YJL178cATG27YLL048cYB71YDR128wMTC5YDR119wVBA4YOR273cTPO4YEL051wVMA8YPL019cVTC3YDR080wVPS41YML018c-YDR481cPH08YEL013wVAC8YFL048cEMP47YLL015wBPT1YPL162c-YBR127cVMA2YOR106wVAM3YOL129wVP568YPL006wNCR1YDR135cYCF1YFR019wF4B1YKL146wA'T3YDL185w1-YDR284cDP11YOR036wPE12YCR044cPER1YCL069wVBA3YDL149wATG9YGR020cVMA7YBR207wFTH1YDR486cVP560YGR163wGTR2YDL185wVMA1YIL088cAVT7YBR21c-YPL045wVPS16YJL178cVP535YNL04wVAC7YMR088cVBA1YD105cTMS1YLR396cVPS33YDR352wYPQ2YDL13wSNA4YDR229wIV11YNL326cPE43YKL080wVMA51YHR028cDAP2YLR447cI/MA6YR070wMEH1YPL36cENV7YLR48wPEP3YHR028cDAP2YLR47cI/MA6YR070wMEH1YPL36cENV7YLR48wPEP3YHR028cDAP2YGL02acPIB2YGL12wVAM7YDL36cENV7YLR48wPEP3YEL07wVAG1YBR03cFMG1YMR07cVPS20YPL149wATG5YOR270cVPH1YCL063wVAC17YPR036w <t< td=""><td>Genes encod</td><td>ing vacuolar</td><td>membrane prot</td><td>eins (87)</td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	Genes encod	ing vacuolar	membrane prot	eins (87)						
YEL051wVMA8YPL019cVTC3YDR080wVPS41YML018c-YDR481cPH08YEL013wVAC8YFL048cEMP47YLL015wBPT1YPL162c-YBR127cVMA2YOR106wVAM3YOL129wVPS68YPL006wNCR1YDR135cYCF1YFR019wFAB1YKL146wAVT3YDL185w1-YDR284cDPP1YOR036wPEP12YCR044cPER1YCL069wVBA3YDL149wATG9YGR020cVMA7YBR207wFTH1YDR486cVPS60YGR163wGTR2YDL185wVMA1YIL088cAVT7YBR21c-YPL045wVPS16YJL154cVPS35YNL054wVAC7YMR088cVBA1YDR13ccTAS1YLR396cVPS33YDR352wYPQ2YDL123wSNA4YDR229wIVY1YNL326cPF43YKL080wVMA5YHR028cDAP2YLR447cVMA6YKR07wMEH1YPL236cENV7YLR48wPEP3YEL027wVMA3YBR07cSLM4YPR156cTP03YOL060cMAM3YGL156wAMS1YER072wVTC1YJL012cVTC4YGL006wPMC1YOR087wYUC1YMR160w-YGR138cTP02YGL023cPIB2YGL121wVAM7YPL180wTC089YDL054cMCH1YCL063wFAC17YPR36wVMA13YMR07cVPS20YPL149wATG5YOR270cVPH1YOR316cCO71YML01w<	YJL178c	ATG27	YLL048c	YBT1	YDR128w	MTC5	YDR119w	VBA4	YOR273c	TPO4
YEL013w <i>VAC8</i> YFL048c <i>EMP47</i> YLL015w <i>BP71</i> YPL162c-YBR127c <i>VMA2</i> YOR106w <i>VAM3</i> YOL129w <i>VPS68</i> YPL006w <i>NCR1</i> YDR135c <i>YCF1</i> YFR019w <i>EAB1</i> YKL146w <i>AV73</i> YDL185w1-YDR284c <i>DPP1</i> YOR036w <i>PEP12</i> YCR044c <i>PER1</i> YCL069w <i>VBA3</i> YDL149w <i>ATG9</i> YGR020c <i>VMA7</i> YBR207w <i>FTH1</i> YDR486c <i>VPS60</i> YGR163w <i>GTR2</i> YDL185w <i>VMA1</i> YIL088c <i>AVT7</i> YBR241c-YPL045w <i>VPS16</i> YJL154c <i>VPS35</i> YNL054w <i>VAC7</i> YMR088c <i>VBA1</i> YDR105c <i>TMS1</i> YLR396c <i>VPS33</i> YDR352w <i>YPQ2</i> YDL123w <i>SNA4</i> YDR229w <i>IVY1</i> YNL326c <i>PFA3</i> YKL080w <i>VMA5</i> YHR028c <i>DAP2</i> YLR447c <i>VMA6</i> YKR007w <i>MEH1</i> YPL236c <i>ENV7</i> YLR148w <i>PEP3</i> YEL027w <i>IMA3</i> YBR077c <i>SLM4</i> YPR156c <i>TPO3</i> YOL060c <i>MAM3</i> YGL156w <i>AMS1</i> YER072w <i>ITC1</i> YJL012c <i>ITC4</i> YGL006w <i>PMC1</i> YOR087w <i>YIC1</i> YMR160w-YGR138c <i>TPO2</i> YGR02c <i>PIB2</i> YGL212w <i>VAM7</i> YPL180w <i>TC089</i> YDL054c <i>MCH1</i> YCL063w <i>IAC17</i> YPR36w <i>IMA13</i> YMR077c <i>VPS20</i> YPL149w <i>ATG5</i> YOR270c <i>VPH1</i> YOR316c <i>C071</i> YML121w <i>GTR1</i> YHR39c-a </td <td>YEL051w</td> <td>VMA8</td> <td>YPL019c</td> <td>VTC3</td> <td>YDR080w</td> <td>VPS41</td> <td>YML018c</td> <td>-</td> <td>YDR481c</td> <td>PHO8</td>	YEL051w	VMA8	YPL019c	VTC3	YDR080w	VPS41	YML018c	-	YDR481c	PHO8
YOR106w <i>IAM3</i> YOL129w <i>IPS68</i> YPL006w <i>NCR1</i> YDR135c <i>YCF1</i> YFR019w <i>FAB1</i> YKL146w <i>AV73</i> YDL185w1-YDR284c <i>DPP1</i> YOR036w <i>PEP12</i> YCR044c <i>PER1</i> YCL069w <i>IBA3</i> YDL149w <i>ATG9</i> YGR020c <i>IMA7</i> YBR207w <i>FTH1</i> YDR486c <i>IPS60</i> YGR163w <i>GTR2</i> YDL185w <i>IMA1</i> YIL088c <i>AV77</i> YBR241c-YPL045w <i>IPS60</i> YJL154c <i>IPS35</i> YNL054w <i>IAC7</i> YMR088c <i>IBA1</i> YDR105c <i>TMS1</i> YLR396c <i>IPS33</i> YDR352w <i>IPQ2</i> YDL123w <i>SNA4</i> YDR229w <i>IIV1</i> YNL326c <i>PFA3</i> YKL080w <i>IMA5</i> YHR028c <i>DAP2</i> YLR447c <i>IMA6</i> YKR007w <i>MEH1</i> YPL236c <i>ENV7</i> YLR48w <i>PEP3</i> YEL027w <i>IMA3</i> YBR077c <i>SLM4</i> YPR156c <i>TPO3</i> YOL060c <i>MAM3</i> YGL156w <i>AMS1</i> YER072w <i>ITC1</i> YJL012c <i>ITC4</i> YGL006w <i>PMC1</i> YOR087w <i>YIC1</i> YMR160w-YGR138c <i>TPO2</i> YGR036w <i>IMA13</i> YMR077c <i>VPS20</i> YPL149w <i>ATG5</i> YOR270c <i>IPH1</i> YGR37c <i>SNX3</i> YJL019w <i>ATG1</i> YMR39c-a <i>IMA10</i> YPL234c <i>YMA11</i> YAR002c-a <i>ERP1</i> YGR37c <i>SNX3</i> YJL059w <i>YHC3</i> YMR195w <i>ICY1</i> YDL077c <i>YAM6</i> YOR32w <i>MA4</i> YOR081c <i>GL5</i> YMR246w <i>FA44</i> YMR313c	YEL013w	VAC8	YFL048c	EMP47	YLL015w	BPT1	YPL162c	-	YBR127c	VMA2
YKL146wAVT3YDL185w1-YDR284cDPP1YOR036wPEP12YCR044cPER1YCL069wVBA3YDL149wATG9YGR020cVMA7YBR207wFTH1YDR486cVPS60YGR163wGTR2YDL185wVMA1YIL088cAVT7YBR21tc-YPL045wVPS16YJL154cVPS35YNL054wVAC7YMR088cVBA1YDR105cTMS1YLR396cVPS33YDR352wYPQ2YDL123wSNA4YDR229wI/Y1YNL326cPFA3YKL080wVMA5YHR028cDAP2YLR447cVMA6YKR007wMEH1YPL36ccENV7YLR48wPEP3YEL027wVMA3YBR077cSLM4YPR156cTPO3YOL060cMAM3YGL156wAMS1YER072wVTC1YJL012cVTC4YGL006wPMC1YOR087wYVC1YMR160w-YGR138cTPO2YGL023cPIB2YGL212wVAM7YPL80wTC089YDL54cMCH1YCR0316cCO71YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR01wAVT1YBR217wATG12YDL128wVCX1YNL101wAVT4YOR0357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOR081cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFDJYMR148wOSW5YML008cERG6 <td< td=""><td>YOR106w</td><td>VAM3</td><td>YOL129w</td><td>VPS68</td><td>YPL006w</td><td>NCR1</td><td>YDR135c</td><td>YCF1</td><td>YFR019w</td><td>FAB1</td></td<>	YOR106w	VAM3	YOL129w	VPS68	YPL006w	NCR1	YDR135c	YCF1	YFR019w	FAB1
YCL069wVBA3YDL149wATG9YGR020cVMA7YBR207wFTH1YDR486cVPS06YGR163wGTR2YDL185wVMA1YIL088cAV77YBR241c-YPL045wVPS16YJL154cVPS35YNL054wVAC7YMR088cVBA1YDR15cTMS1YLR396cVPS33YDR352wYPQ2YDL123wSNA4YDR229wIVY1YNL326cPFA3YKL080wVMA5YHR028cDAP2YLR447cVMA6YKR007wMEH1YPL236cENV7YLR148wPEP3YEL027wVMA3YBR077cSLM4YPR156cTPO3YOL060cMAM3YGL156wAMS1YER072wVTC1YJL012cVTC4YGL006wPMC1YOR087wYVC1YMR160w-YGR138cTPO2YGL023cPIB2YGL212wVAA7YPL180wTCO89YDL054cMCH1YCL063wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCO71YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR010wAV71YBR217wATG12YDL128wVCX1YNL101wAV74YOR357cSNX3YJL059wYHC3YMR195wICY1YDL07cVAM6YOR322wVMA4YOL082wATG19YGR166eFA44YMR313cTGL3YMR110cHFD1YMR48wOSW5YML008cERG6 <td>YKL146w</td> <td>AVT3</td> <td>YDL185w1</td> <td>-</td> <td>YDR284c</td> <td>DPP1</td> <td>YOR036w</td> <td>PEP12</td> <td>YCR044c</td> <td>PER1</td>	YKL146w	AVT3	YDL185w1	-	YDR284c	DPP1	YOR036w	PEP12	YCR044c	PER1
YGR163wGTR2YDL185wVMA1YIL088cAVT7YBR241c-YPL045wVPS16YJL154cVPS35YNL054wVAC7YMR088cVBA1YDR105cTMS1YLR396cVPS33YDR352wVPQ2YDL123wSNA4YDR229wIVY1YNL326cPEA3YKL080wVMA5YHR028cDAP2YLR447cVMA6YKR007wMEH1YPL236cENV7YLR148wPEP3YEL027wVMA3YBR077cSLM4YPR156cTPO3YOL060cMAM3YGL156wAMS1YER072wVTC1YJL012cVTC4YGL006wPMC1YOR087wYVC1YMR160w-YGR138cTPO2YGL023cPIB2YGL212wVAM7YPL180wTCO89YDL054cMCH1YCL063wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCOT1YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR001wAVT1YBR217wATG12YDL128wVCX1YNL101wAVT4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR32awVMA4YOL082wATG19YGR16eFA44YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR24cLDH1 <td>YCL069w</td> <td>VBA3</td> <td>YDL149w</td> <td>ATG9</td> <td>YGR020c</td> <td>VMA7</td> <td>YBR207w</td> <td>FTH1</td> <td>YDR486c</td> <td>VPS60</td>	YCL069w	VBA3	YDL149w	ATG9	YGR020c	VMA7	YBR207w	FTH1	YDR486c	VPS60
YJL154cVPS35YNL054wVAC7YMR088cVBA1YDR105cTMS1YLR396cVPS33YDR352wYPQ2YDL123wSNA4YDR229wIVY1YNL326cPFA3YKL080wVMA5YHR028cDAP2YLR447cVMA6YKR007wMEH1YPL23ccENV7YLR148wPEP3YEL027wVMA3YBR077cSLM4YPR156cTPO3YOL060cMAM3YGL156wAMS1YER072wVTC1YJL012cVTC4YGL006wPMC1YOR087wYVC1YMR160w-YGR138cTPO2YGL023cPIB2YGL212wVAM7YPL180wTCO89YDL054cMCH1YCL63wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCO71YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR001wAVT1YBR217wATG12YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1VVVMA4YOL082wYMR148wOSW5YML008cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRT8YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1 <t< td=""><td>YGR163w</td><td>GTR2</td><td>YDL185w</td><td>VMA1</td><td>YIL088c</td><td>AVT7</td><td>YBR241c</td><td>-</td><td>YPL045w</td><td>VPS16</td></t<>	YGR163w	GTR2	YDL185w	VMA1	YIL088c	AVT7	YBR241c	-	YPL045w	VPS16
YDR352wYPQ2YDL123wSNA4YDR229wIVY1YNL326cPFA3YKL080wVMA5YHR028cDAP2YLR447cVMA6YKR007wMEH1YPL236cENV7YLR148wPEP3YEL027wVMA3YBR077cSLM4YPR156cTPO3YOL060cMAM3YGL156wAMS1YER072wVTC1YIL012cVTC4YGL006wPMC1YOR087wYVC1YMR160w-YGR138cTPO2YGL023cPIB2YGL212wVAM7YPL180wTCO89YDL054cMCH1YCL063wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCO71YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YIR001wAVT1YBR217wATG12YDL128wVCX1YNL101wAVT4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL055wLDB16YBR17cEHT1YLL232wSS01YDR425wSNX41 <td< td=""><td>YJL154c</td><td>VPS35</td><td>YNL054w</td><td>VAC7</td><td>YMR088c</td><td>VBA1</td><td>YDR105c</td><td>TMS1</td><td>YLR396c</td><td>VPS33</td></td<>	YJL154c	VPS35	YNL054w	VAC7	YMR088c	VBA1	YDR105c	TMS1	YLR396c	VPS33
YHR02&cDAP2YLR447cVMA6YKR007wMEH1YPL236cENV7YLR148wPEP3YEL027wVMA3YBR077cSLM4YPR156cTPO3YOL060cMAM3YGL156wAMS1YER072wVTC1YJL012cVTC4YGL006wPMC1YOR087wYVC1YMR160w-YGR13&cTPO2YGL023cPIB2YGL212wVAM7YPL180wTCO89YDL054cMCH1YCL063wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCO71YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR001wAVT1YBR217wATG12YDL128wVCX1YNL101wAVT4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR17cEHT1YLL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2YML10	YDR352w	YPQ2	YDL123w	SNA4	YDR229w	IVY1	YNL326c	PFA3	YKL080w	VMA5
YEL027wVMA3YBR077cSLM4YPR156cTPO3YOL060cMAM3YGL156wAMS1YER072wVTC1YJL012cVTC4YGL006wPMC1YOR087wYVC1YMR160w-YGR138cTPO2YGL023cPIB2YGL212wVAM7YPL180wTCO89YDL054cMCH1YCL063wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCO71YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR001wAVT1YBR217wATG12YDL07rcVAM6YOR332wVMA4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL07rcVAM6YOR322wVM44YOL082wATG19YGR106cVOA1YOR081cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR17rcEHT1YPL332wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2YUN	YHR028c	DAP2	YLR447c	VMA6	YKR007w	MEH1	YPL236c	ENV7	YLR148w	PEP3
YER072wVTC1YJL012cVTC4YGL006wPMC1YOR087wYVC1YMR160w-YGR138cTPO2YGL023cPIB2YGL212wVAM7YPL180wTCO89YDL054cMCH1YCL063wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCOT1YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR001wAVT1YBR217wATG12YDL077cVAM6YOR332wVMA4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1YOR081cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR17cEHT1YPL332wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2V	YEL027w	VMA3	YBR077c	SLM4	YPR156c	TPO3	YOL060c	MAM3	YGL156w	AMS1
YGR138cTPO2YGL023cPIB2YGL212wVAM7YPL180wTCO89YDL054cMCH1YCL063wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCOT1YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR001wAVT1YBR217wATG12YDL128wVCX1YNL101wAVT4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1YOR081cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR177cEHT1YPL322wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2VU	YER072w	VTC1	YJL012c	VTC4	YGL006w	PMC1	YOR087w	YVC1	YMR160w	-
YCL063wVAC17YPR036wVMA13YMR077cVPS20YPL149wATG5YOR270cVPH1YOR316cCOT1YML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR001wAVT1YBR217wATG12YDL128wVCX1YNL101wAVT4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1YOR081cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRRT8YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YCR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR17cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2-	YGR138c	TPO2	YGL023c	PIB2	YGL212w	VAM7	YPL180w	TCO89	YDL054c	MCH1
YOR316cCOTIYML121wGTR1YHR039c-aVMA10YPL234cVMA11YAR002c-aERP1YLR001c-YJR001wAVT1YBR217wATG12YDL128wVCX1YNL101wAVT4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1Genes encodirIpid protectorVOA1YOR081cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR17cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2V	YCL063w	VAC17	YPR036w	VMA13	YMR077c	VPS20	YPL149w	ATG5	YOR270c	VPH1
YLR001c-YJR001wAVT1YBR217wATG12YDL128wVCX1YNL101wAVT4YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1Genes encodir lipid protocolsVOA1YOR081cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR177cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2-	YOR316c	COTI	YML121w	GTR1	YHR039c-a	VMA10	YPL234c	VMA11	YAR002c-a	ERP1
YOR357cSNX3YJL059wYHC3YMR195wICY1YDL077cVAM6YOR332wVMA4YOL082wATG19YGR106cVOA1<	YLR001c	-	YJR001w	AVT1	YBR217w	ATG12	YDL128w	VCXI	YNL101w	AVT4
YOL082wATG19YGR106cVOA1Genes encodirYOR081cTGL5YMR246wFAA4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRRT8YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR17cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2	YOR357c	SNX3	YJL059w	YHC3	YMR195w	ICY1	YDL077c	VAM6	YOR332w	VMA4
Genes encodispit protectedYOR081cTGL5YMR246wFA.4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRR78YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR177cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2VC	YOL082w	ATG19	YGR106c	VOA1						
YOR081cTGL5YMR246wF4A4YMR313cTGL3YMR110cHFD1YMR148wOSW5YML008cERG6YOL048cRRT8YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR177cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2SSC1	Genes encoding lipid proteins (24)									
YML008cERG6YOL048cRRT8YNL231cPDR16YDL052cSLC1YKL140wTGL1YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR177cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2V	YOR081c	TGL5	YMR246w	FAA4	YMR313c	TGL3	YMR110c	HFD1	YMR148w	OSW5
YBR204cLDH1YOR246cENV9YDR525w-aSNA2YKR046cPET10YBR041wFAT1YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR177cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2	YML008c	ERG6	YOL048c	RRT8	YNL231c	PDR16	YDL052c	SLC1	YKL140w	TGL1
YLL012wYEH1YKL179cCOY1YKR089cTGL4YCL005wLDB16YBR177cEHT1YPL232wSSO1YDR425wSNX41YBR042cCST26YDR275wBSC2	YBR204c	LDH1	YOR246c	ENV9	YDR525w-a	SNA2	YKR046c	PET10	YBR041w	FAT1
YPL232w SSO1 YDR425w SNX41 YBR042c CST26 YDR275w BSC2	YLL012w	YEH1	YKL179c	COYI	YKR089c	TGL4	YCL005w	LDB16	YBR177c	EHT1
	YPL232w	SSO1	YDR425w	SNX41	YBR042c	CST26	YDR275w	BSC2		

系统名	标准名	功能
Systerm name	Standard name	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. cer-
		evisiae 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)

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



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 CaCl₂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 CaCl₂, and DIC and GFP pictures on the right are from cells treated with 0.2 mol/L CaCl₂.

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 CaCl₂处理的细胞。显微镜放大倍数为1 000倍; B:左侧为A图中CaCl₂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CaCl₂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 CaCl₂, and DIC and GFP pictures on the right are from cells treated with 0.2 mol/L CaCl₂. Amplification scale is 1 000×; B: close-up DIC and GFP pictures of *snq2/snq2* and *ydr417c/ydr417c* cells treated with CaCl₂ from the red frame field in Fig.2A; C: close-up DIC and GFP pictures of *ybr219c/ybr219c* and ybr224w/ybr224w cells treated with CaCl₂ 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 CaCl₂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 CaCl₂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HXTI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, HXTI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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