Supplemetary Table 1. Yeast strains

Strain	Genotype	Source/reference
H304	*MAT***a** *ura3-52 leu2-3,112*	P. Novick(NY179)
H973	*MATα ura3-52 his4-619*	P. Novick(NY15)
H956	*MAT***a** *ura3-52 leu2-3,112 ade2 his4 sec61-2*	S. Ferro-Novick(RSY524)
H1107	*MATα ura3-52 leu2-3,112 sec63-1 pep4-3*	S. Ferro-Novick(RSY151)
H1109	*MATα ura3-52 leu2-3,112 his4-619 sec62-1*	S. Ferro-Novick(RSY529)
H3227	*MAT***a** *ura3-52 leu2-3,112 sbh1::HphMX*	This study
H3224	*MAT***a** *ura3-52 leu2-3,112 sbh2::KanMX4*	This study
H3232	*MAT***a** *sbh1::KanMX sbh2::HphMX leu2-3,112 ura3-52*	This study
H3235	*MATα sbh1::KanMX sbh2::HphMX his4-619 ura3-52*	This study
H3384	*MAT***a** *ura3-52 leu2-3,112::α-amylase-LEU2*	This study
H3386	*MATα ura3-52 leu2-3,112 sbh1::KanMX4 leu2-3,112::α-amylase-LEU2*	This study
H3387	*MATα ura3-52 leu2-3,112 sbh2::HphMX leu2-3,112::α-amylase-LEU2*	This study
H3388	*MAT***a** *ura3-52 leu2-3,112 sbh1::KanMX4 sbh2::HphMX leu2-3,112::α-amylase-LEU2*	This study
H3392	*MAT***a** *leu2-3,112 ura3-52 [SEC61-LEU2, 2μ]*	This study
H3393	*MAT***a** *leu2-3,112 ura3-52 [SSS1-URA3, 2μ]*	This study
H3429	*MAT***a** *RTN1:3HA-KanMX sbh1::hphMX leu2-3,112 ura3-52*	This study
H3431	*MAT***a** *RTN1::3HA-KanMX leu2-3,112 ura3-52*	This study
H3543	*MAT***a** *sbh1::KanMX sbh2::HphMX leu2-3,112 ura3-52 trp1::natNT2*	This study
WCG4a	*MAT***a** *leu2-3,112 ura3 his3-11,-15*	(46)

Supplementary Table 2. Yeast expression vectors used in this study

Plasmid name	Type	Promoter	Insert	Marker	Source
pVT102U	2μ	*ADH1*	-	*URA3*	(25)
YEpSBH1(1-82)	2μ	*ADH1*	*SBH1*	*URA3*	This study
YEpSBH1(1-54)	2μ	*ADH1*	*sbh1(1-54)*	*URA3*	This study
YEpSBH1(1-75)	2μ	*ADH1*	*sbh1(1-75)*	*URA3*	This study
YEpSBH1(34-82)	2μ	*ADH1*	*sbh1(34-82)*	*URA3*	This study
YEpSBH1(34-75)	2μ	*ADH1*	*sbh1(34-75)*	*URA3*	This study
YEpSBH1(50-82)	2μ	*ADH1*	*sbh1(50-82)*	*URA3*	This study
YEpSBH1(50-75)	2μ	*ADH1*	*sbh1(50-75)*	*URA3*	This study
YEpSBH1(1-82)L	2μ	*ADH1*	*SBH1*	*LEU2*	This study
YEpSBH1(50-75)L	2μ	*ADH1*	*sbh1(1-75)*	*LEU2*	This study
YEpSBH2(1-88)L	2μ	*ADH1*	*sbh2(1-88)*	*LEU2*	This study
p425ADH	2μ	*ADH1*	-	*LEU2*	(26)
YEpSBH2(1-88)	2μ	*ADH1*	*SBH2*	*URA3*	This study
YEpSBH2(57-82)	2μ	*ADH1*	*sbh2(57-82)*	*URA3*	This study
YEpSss1	2μ	*ADH1*	*SSS1*	*URA3*	This study
YEpSss1TM	2μ	*ADH1*	*sss1(42-75)*	*URA3*	This study
p426ADH	2μ	*ADH1*	-	*URA3*	(26)
YEpBIO-SBH1	2μ	*ADH1*	*BIO-SBH1*	*URA3*	This study
YEpBIO-SBH1TM	2μ	*ADH1*	*BIO-sbh1(50-75)*	*URA3*	This study
YEpαa6	2μ	*ADH1*	α-amylase	*LEU2*	(27)
YIpαa-L	-	*ADH1*	α-amylase	*LEU2*	This study

Supplementary Materials and Methods

Cell fraction

Strains H3384 and H3388 were grown in 200 ml of YPD at 30°C overnight to OD600 1.5. Cells were harvested, wash once with cold water and once with spheroplasting buffer (1.4M sorbitol, 20mM TEA, 40mM β-mercaptoethanol pH 7.4). Cells were resuspended in spheroplasting buffer with 150 µg /ml zymolyase 100T and digested for 15 min at 37 °C. Spheroplasts were collected and washed once with spheroplasting buffer followed by resuspension in lysis buffer (20mM HEPES, 250mM sucrose, 1mM EGTA, 2mM MgCl2, 4mM ABESF) with Complete protein inhibitor coctail and lysed by pipetting. Lysates were centrifuged at 1000g for 10 min at 4°C, the supernantant taken to new tubes and centrifuged at 10 000g for 20 min at 4°C. This supernatant was centrifuge at 100 000g for 30 min at 4°C . Samples from different stages were subjected to SDS PAGE gel and Western blotting with anti-α-amylase antibodies.

Quantitative immunoblotting

The indicated amount of membranes was resolved by SDS-PAGE, transferred to nitrocellulose membranes, and membranes probed with anti-Sec61p polyclonal antiserum at 1:1000 (our lab), and anti-Mns1p at 1:500 (Annette Herscovics), followed by detection with 125I-Protein A (Amersham) and autoradiography and quantitation using a phosphorimager (BioRad).

Supplementary Figure legends

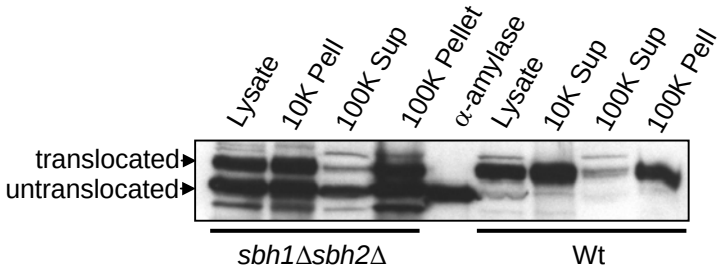
Supplementary Figure 1

Fast migrating form of alfa amylase in Sbh1 Sbh2 deleted cells is cytosolic. H3232 cells were lysed and subjected to differential centrifugation analysis as described in supplementary materials and methods. Samples of each fraction were analysed by SDS-PAGE and Western blotting with anti α-amylase antibodies.

Supplementary Figure 2

Mns1p levels are decreased in *sbh1Δ sbh2Δ* cells. (A) Indicated amounts of membranes prepared from H304 or H3232 cells were subjected to SDS PAGE and Western blotting with anti Mns1p or Sec61p antibodies. (B) Quantitation of the proportion of Mns1p to Sec61p in membranes analysed in (A).

Supplemental Figure 1



Supplemental Figure 2