Sucrose Utilization in Budding Yeast as a Model for the Origin of Undifferentiated Multicellularity

John H. Koschwanez, Kevin R. Foster, Andrew W. Murray

PLoS Biol 9(8): e1001122

Supporting information

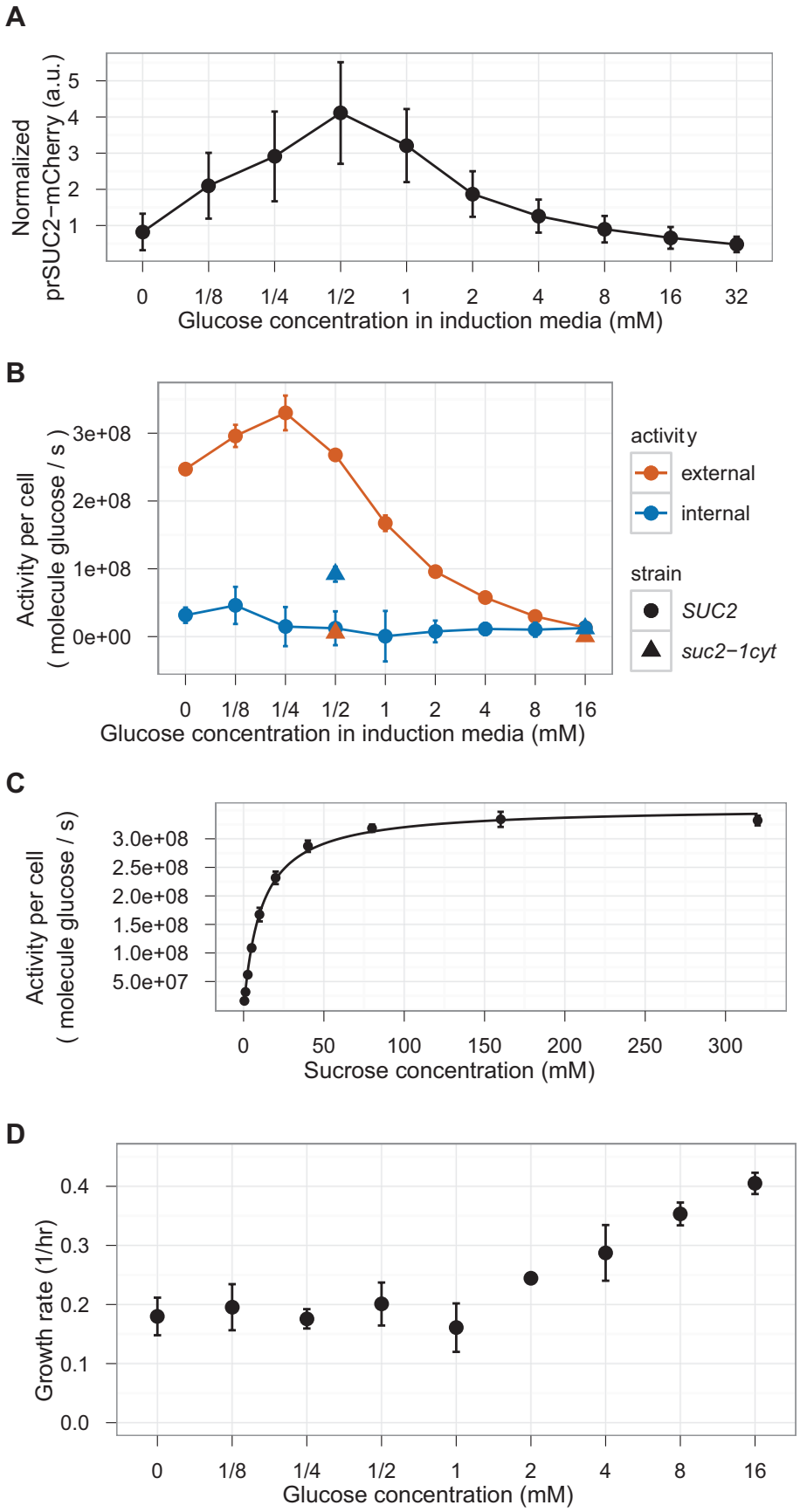
A
Normalized
prSUC2−mCherry (a.u.)
5
4
3
2
1
0
1/8
1/4
1/2
1
2
4
8
16
32
Glucose concentration in induction media (mM)
B
Activity per cell
(molecule glucose / s)
3e+08
2e+08
1e+08
0e+00
0
1/8
1/4
1/2
1
2
4
8
16
Glucose concentration in induction media (mM)
activity
external
internal
strain
SUC2
suc2−1cyt
C
Activity per cell
(molecule glucose / s)
3.0e+08
2.5e+08
2.0e+08
1.5e+08
1.0e+08
5.0e+07
0
50
100
150
200
250
300
Sucrose concentration (mM)
D
Growth rate (1/hr)
0.4
0.3
0.2
0.1
0.0
0
1/8
1/4
1/2
1
2
4
8
16
Glucose concentration (mM)

Figure S1

Invertase expression and activity. (A) Activity of the SUC2 promoter as a function of extracellular glucose concentration for cells grown in minimal synthetic media. FACS was used to measure the fluorescent intensity of mCherry driven by the SUC2 promoter, which was normalized using a constitutively expressed mCitrine driven by the ACT1 promoter (strain yJHK383). The length of the error bar corresponds to one standard deviation. Cells were grown in the given concentration of glucose in exponential phase for 12 h before measurement. (B) Internal and external enzyme activities of the prototrophic SUC2 strain yJHK222 and prototrophic suc2-1cyt strain yJHK290, measured as the number of molecules of glucose liberated per second in 128 mM sucrose in pH = 4.5 tartrate buffer. Cultures were washed and inoculated from an exponentially growing culture into the specified concentration of glucose plus YEP at ≈1E5 cell/ml and grown for 6 h. The cultures were then washed and resuspended in 1 mM potassium phosphate, pH = 7.5, at 1.5E7 cell/ml. The cultures were split into two: one for intact cell invertase activity, and one for lysed cell invertase activity. 0.5% Zymolyase (Zymo Research Corp, http://www.zymoresearch.com/) was added to each of the lysed cell cultures. The cells were incubated at 30°C for 45 min to allow lysis to occur. 1.5E5 (10 μl) cells or cell equivalent were then added to prewarmed 390 ml 5 mM tartrate buffer (pH = 4.5). 100 μl of prewarmed 640 mM sucrose was added and sucrose hydrolysis was allowed to occur at 30°C for 35 min. Samples were then diluted 10:1 in 50 mM sodium phosphate (pH = 7.5) plus 0.25 mM N-ethylmaleimide[18]. The amount of glucose in each sample was then measured using an Amplex Red Glucose Assay Kit (Invitrogen, http://www.invitrogen.com/). The external invertase activity data points correspond to the mean intact cell measurements and the internal activity data points correspond to the mean lysed cell measurements minus the mean intact cell measurements. Three technical replicates were performed per sample. The error bars in the external activity measurements refer to the 95% confidence interval calculated using the one-sample Student's t-test of the three replicates, and the error bars in the internal activity measurements refer to the 95% confidence interval calculated using the two-sample Student's ttest of the three replicates (external and lysed activity). suc2Δ strain yJHK302 was also measured in parallel and used as a zero reference. suc2-1cyt strain yJHK290 was measured at ½ mM glucose and 16 mM glucose only. (C) Michaelis-Menten curve of invertase activity for the prototrophic SUC2 strain yJHK222. Cells were pregrown in 0.5 mM glucose and inoculated into various levels of sucrose and incubated as described above (without the cell lysis step) for 28 min to determine the rate of sucrose hydrolysis by invertase. Four samples were used per data point; error bars refer to the one-sample Student's t test. The R function nls (nonlinear least squares) was used to fit the shown Michaelis-Menten curve to the data set and to obtain the following values: Km = 11 mM sucrose, Vmax = 3.6E8 molecule glucose s^{-1} $cell^{-1}$. suc2Δ strain yJHK302 was also measured in parallel and used as a zero reference. (D) Growth rate in YEP plus various concentrations of glucose of the prototrophic strain yJHK222. Cultures were inoculated from an exponentially growing culture into the specified concentration of glucose plus YEP at 2,000 cell/ml. Cultures were first grown for 8 h, and then samples were taken at four time points over the next 6 h. Samples were briefly sonicated and then measured using a Coulter Counter (Beckman Coulter,http://www.beckmancoulter.com/). Three replicates were measured in parallel for each glucose concentration. The R function nls (nonlinear least squares) was used to find an exponential growth rate for each set of four time points. The error bar for each data point on the plot refers to 95% confidence interval for the three replicates.

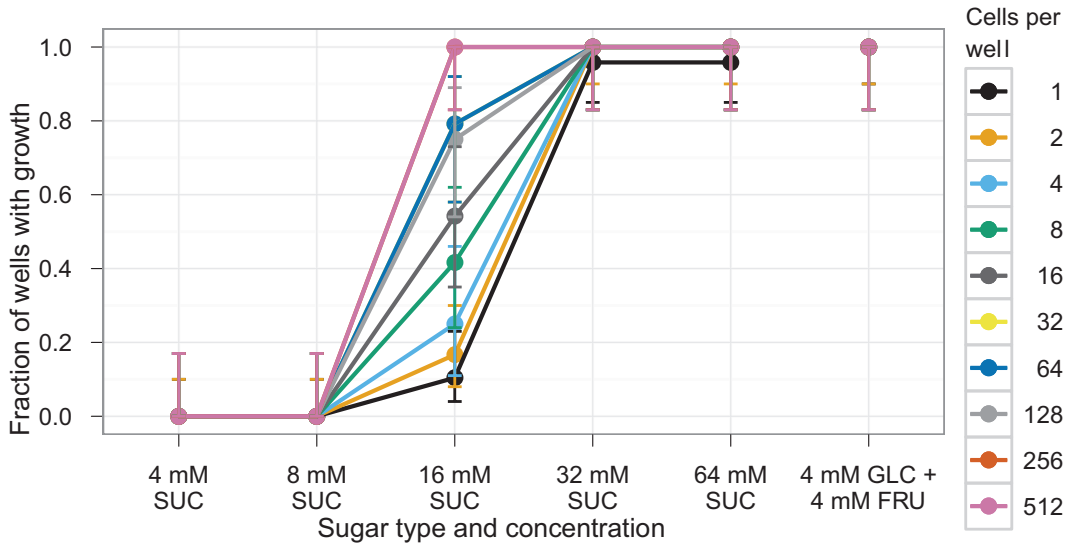

Figure S2

Lab yeast strains cannot grow at low sucrose concentrations. Cells were inoculated by FACS into 150 µl wells at the given cell density and sugar concentration and allowed to grow for 85 h without shaking at 30°C. The fraction of wells with growth using S288C background strain yJHK361 is shown (this figure is similar to Figure 1 except the strain is S288C background instead of W303 background). Results shown are totals of three experiments; each experiment used one plate for each sugar concentration / strain combination. Error bars refer to 95% binomial confidence interval using the adjusted Wald method.

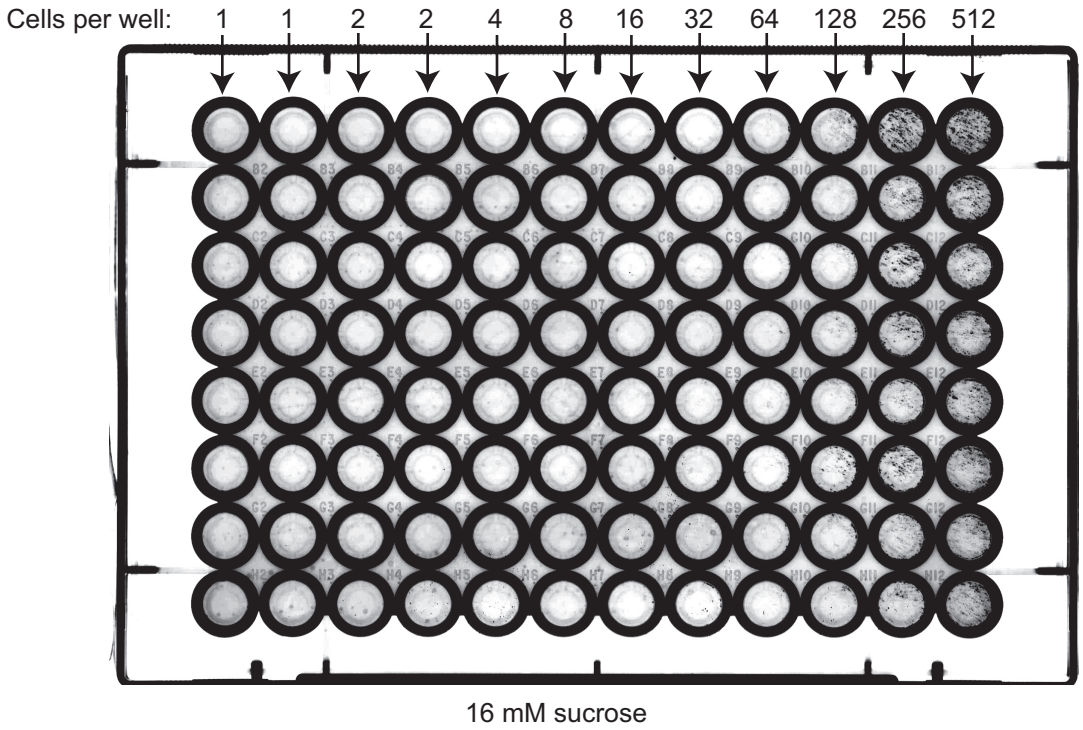

Figure S3.

Typical fluorescent scan of a plate containing 16 mM sucrose inoculated with the indicated number of cells. Note the faint and uniform growth in the wells containing 256 and 512 cells. If only a small fraction of cells were capable of growing in low concentrations of sucrose, we would expect to see a few discrete colonies at the highest cell numbers, rather than the nearly uniform growth that we observe. The contrast of this image was increased to improve visibility.

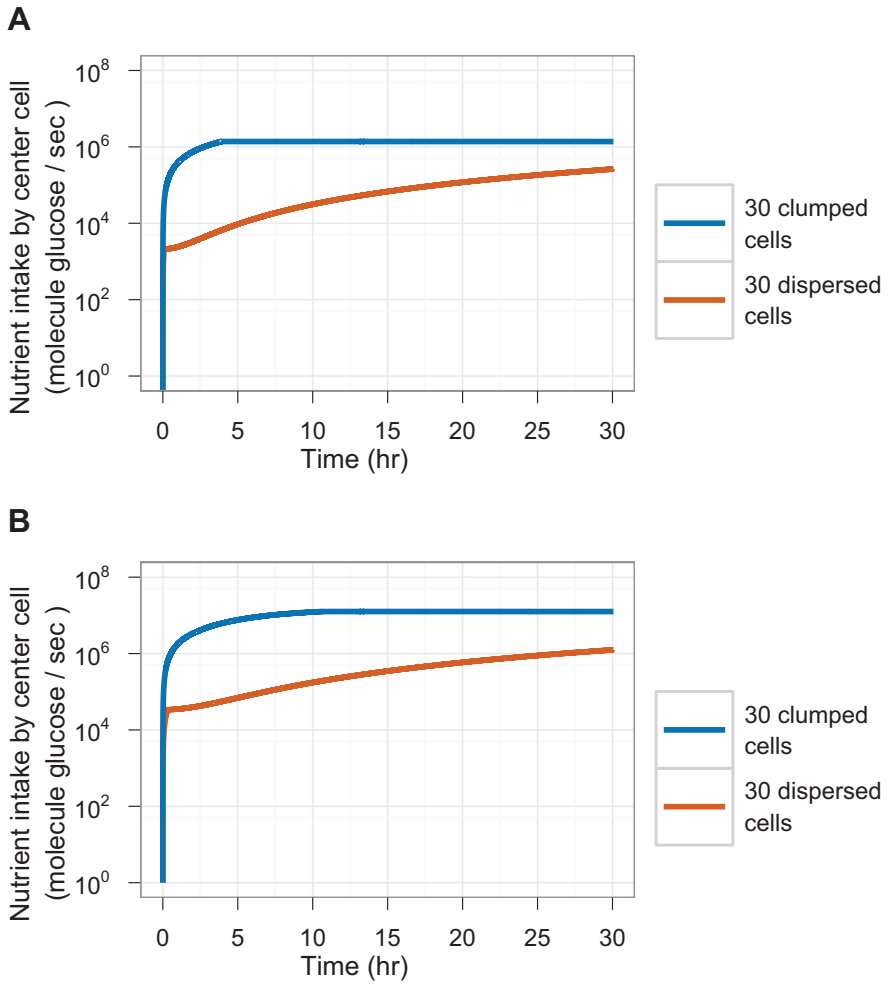

Figure S4.

Simulation of glucose uptake in isolated cells and a cell clump. The simulated local glucose concentration and glucose uptake of a cell inoculated at the center of a 150 µl sphere in two environments: at the center of a clump of 30 cells and at the center of a total of 30 cells uniformly dispersed throughout the volume. (A) 2 mM sucrose: glucose intake rate of the cell as a function of time after inoculation. (B) 32 mM sucrose: glucose intake rate of the cell as a function of time after inoculation. Note the logarithmic scale on the y-axis. See Supporting Information for details of code and parameters.

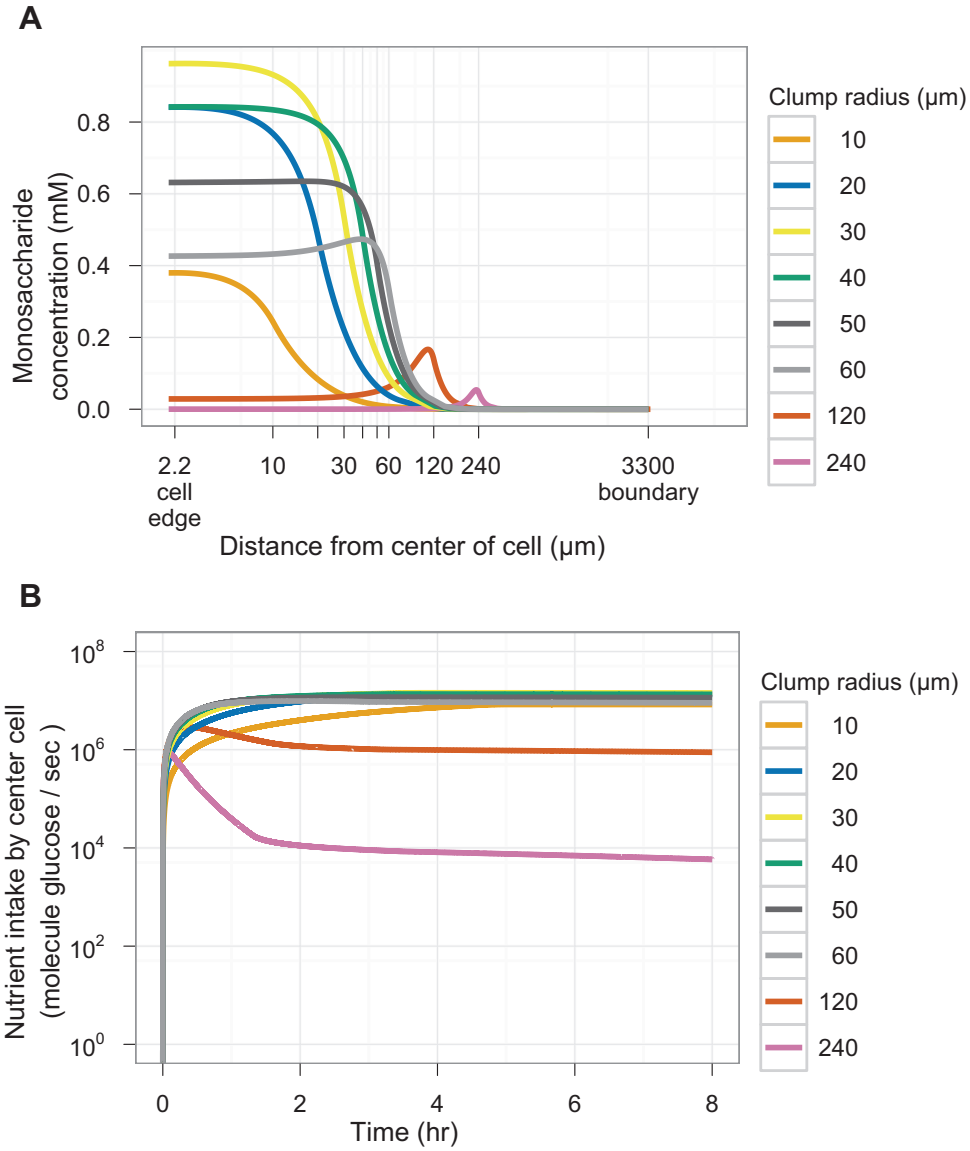

Figure S5.

Simulation of glucose uptake at the center of different sizes of cell clump. The simulated local glucose concentration and glucose uptake of a cell inoculated at the center of a 150 μl sphere. (A) Glucose concentration as a function of radial distance from the center of the cell after 8 h of incubation. Note the logarithmic scale on the x-axis. (B) Glucose intake rate of the cell as a function of time after inoculation. Note the logarithmic scale on the y-axis. Cells continue to consume low levels of glucose at large clump size because sucrose diffuses into the clump and is available for immediate hydrolysis and consumption. See Supporting Information for details of code and parameters.

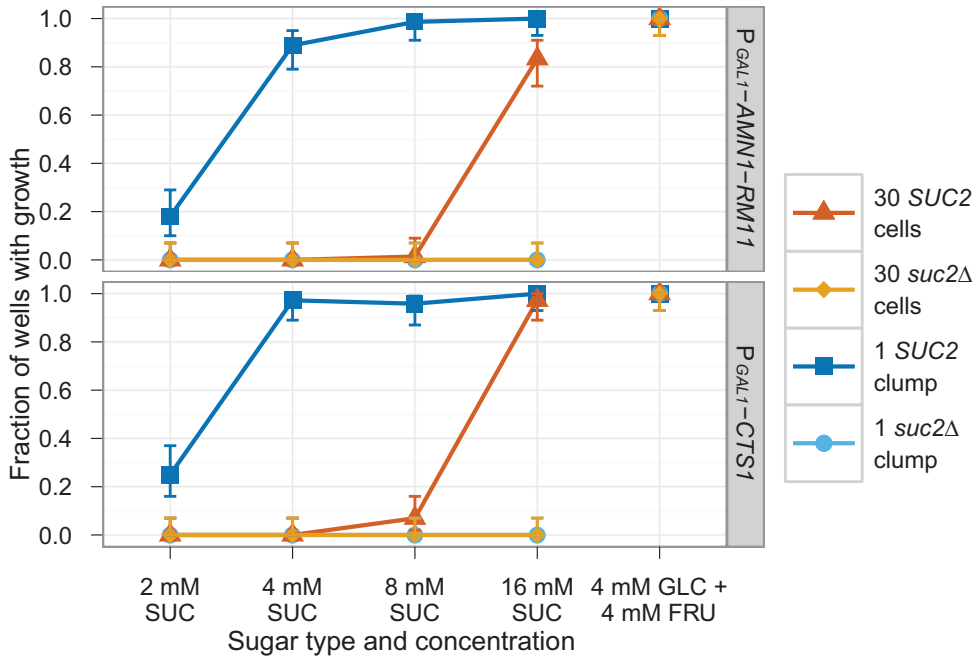

Figure S6.

Clumps of cells produced by a variety of methods have a growth advantage over an equal number of single cells in low sucrose concentrations. Cells were inoculated by FACS as described in Figure 4. (Top) Galactose-induced AMN1-RM11 strains yJHK226 (SUC2) and yJHK227 (suc2Δ). Cells were pregrown without galactose to produce single cells or with galactose to produce clumps. The assay medium contained sucrose but lacked galactose. (Bottom) Galactose-induced CTS1 (chitinase) strains yJHK228 (SUC2) and yJHK229 (suc2Δ). Cells were pregrown with galactose to produce single cells or without galactose to produce clumps. The assay medium contained sucrose but lacked galactose. Results shown are totals of three experiments; each experiment used one plate for each sugar concentration / clumpiness-induction-method combination, and each plate represents 24 wells for each combination of genotype and clumpiness. Error bars refer to 95% binomial confidence interval using adjusted Wald method.

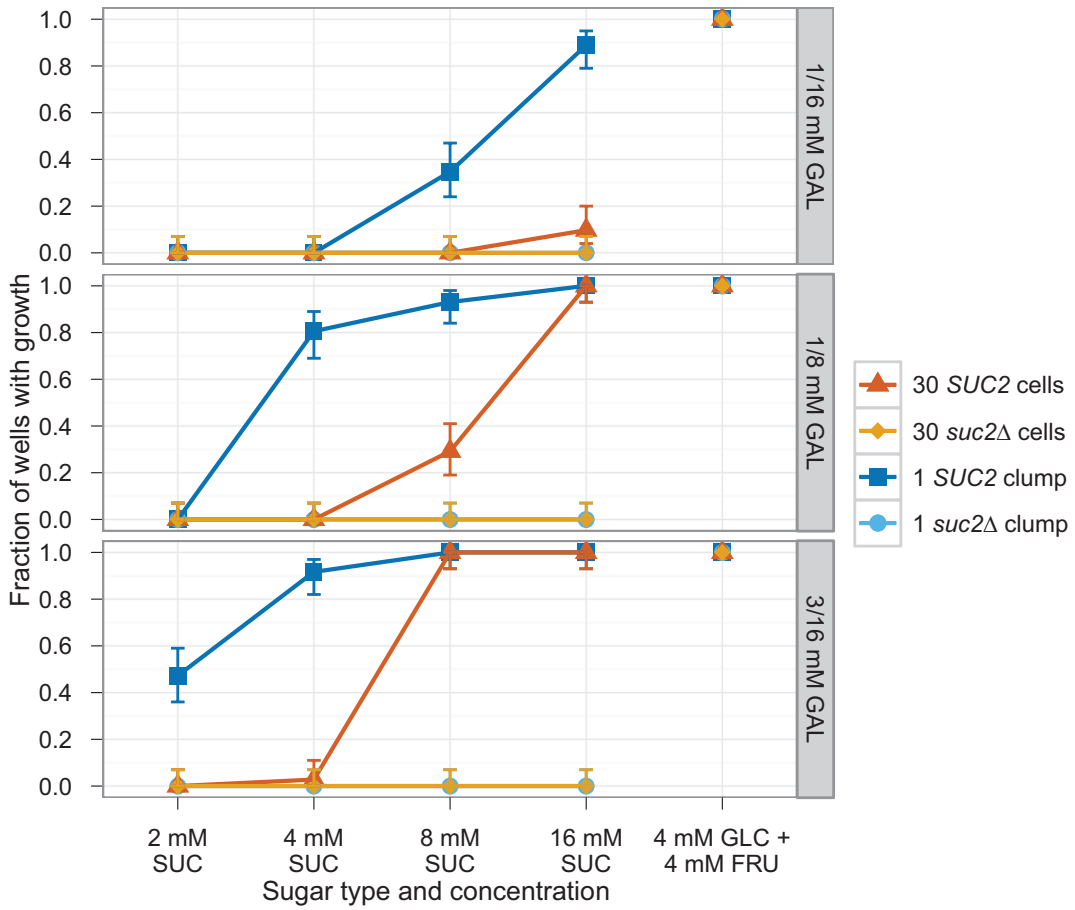

Figure S7.

Clumps of cells have a growth advantage over an equal number of single cells when SUC2 is expressed constitutively. 30 cells or a single 15–30 cell clump were inoculated by FACS into 150 µl wells at the given sugar and galactose concentration and grown for 85 h at 30°C without shaking. In the invertase-producing, AMN1-RM11 strain yJHK315, SUC2 is driven by the GAL1 promoter (PGAL1-SUC2). SUC2 is deleted in the suc2Δ strain yJHK317. Galactokinase (GAL1) is deleted from both strains so that galactose acts as an inducer and not as a carbon source. Results shown are totals of three experiments; each experiment used one plate for each sugar concentration / induction-level combination, and each plate represents 24 wells for each combination of genotype and clumpiness. Error bars refer to 95% binomial confidence interval using adjusted Wald method. Galactose was added to all wells in the following concentrations: (Top) 1/16 mM, (Middle) 1/8 mM, and (Bottom) 3/16 mM.

Table S1: Parameters used for software simulation and description of algorithm

Parameter	Value	Reference
Glucose diffusion coefficient in water (and cell wall and cell clump)	670 $\mu m^2/s$	[1]
Sucrose diffusion coefficient in water (and cell wall and cell clump)	520 $\mu m^2/s$	[1]
Yeast cell volume	42 μm^3	[2]
Yeast cell radius	2.2 μm	derived from cell volume
Cell wall thickness	0.2 μm	[3]
Outer boundary	3.3 mm	derived from sphere of 150 μl (inoculation volume)
Glucose transport K_m	0.8 mM (high affinity) 21 mM (low affinity)	[4]
Glucose transport V_{max}	167 nmol min^{-1} mg^{-1} (high affinity) 104 nmol min^{-1} mg^{-1} (low affinity)	[4]
Cell dry weight	15E-12 g	[5]
Activity of purified invertase octamer	3E4 molecule glucose s^{-1}	[6]
Invertase K_m	11 mM sucrose	Figure S1C
Invertase production per cell in equivalent purified octamers per second	Glucose concentration / Equivalent purified invertase production (molecule/s) 0 / 0.46 0.125 / 0.55 0.25 / 0.61 0.50 / 0.50 1.0 / 0.31 2.0 / 0.21 4.0 / 0.15 8.0 / 0.10 >16 / 0	Figures S1B, S1D, and activity of purified invertase octamer

The diffusion simulation was written in the Python programming language (http://www.python.org/). The basic algorithm is as follows:

1. Set up a one-dimensional radial grid with the central cell at the center of the grid and with the wall boundary at the outer boundary of the grid. The innermost grid point is the cell membrane. Four different sub-grids of composite media are used: the cell wall, the clump, a fine-grained region of media and a course-grained region of media.
2. Set up a diffusion matrix for three components: glucose, sucrose, and invertase. Glucose and invertase have flux boundaries at the cell membrane, and sucrose has a reflective boundary at the cell membrane (flux = 0). All three components have reflective boundaries at the outer wall. Each component's flux across each internal boundary between composite media is continuous. The initial glucose concentration and invertase concentrations are zero, and the initial sucrose concentration is set to the same value at all points.
3. Perform the following actions at each one-second time point:
    a. From the glucose concentration at the cell membrane, set the outward flux of invertase and the inward flux of glucose at the inner boundary (cell membrane). Set the glucose consumed and the invertase produced by the clump. It is assumed that there is no time delay between invertase production and secretion to the cell wall.
    b. Allow the sucrose, glucose, and invertase to diffuse.
    c. Record the concentrations of each diffusing molecule and the glucose flux of the center membrane.

Diffusion is solved using the Crank-Nicholson method for solving partial differential equations [7]. The `linsolve` function from the SciPy (http://www.scipy.org/) library was used to solve the matrices. Python and SciPy were both used as part of the Enthought Python Distribution (http://www.enthought.com).

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Table S1 References

1. Lide DR (2008) CRC Handbook of Chemistry and Physics: CRC Press.
2. Jorgensen P, Edgington NP, Schneider BL, Rupes I, Tyers M, et al. (2007) The size of the nucleus increases as yeast cells grow. Mol Biol Cell 18: 3523-3532.
3. Schmidt M, Strenk ME, Boyer MP, Fritsch BJ (2005) Importance of cell wall mannoproteins for septum formation in Saccharomyces cerevisiae. Yeast 22: 715-723.
4. Reifenberger E, Boles E, Ciriacy M (1997) Kinetic characterization of individual hexose transporters of Saccharomyces cerevisiae and their relation to the triggering mechanisms of glucose repression. Eur J Biochem 245: 324-333.
5. Sherman F (2002) Getting started with yeast. Meth Enzymol 350: 3-41.
6. Esmon PC, Esmon BE, Schauer IE, Taylor A, Schekman R (1987) Structure, assembly, and secretion of octameric invertase. J Biol Chem 262: 4387-4394.
7. Crank J (1980) The Mathematics of Diffusion: Oxford University Press, USA. 424 p.

Table S2. Yeast nitrogen base recipe

Chemical	1X concentration (mg/l)
Ammonium sulfate	5000
Magnesium sulfate	500
Sodium chloride	100
Calcium chloride	100
Potassium phosphate monobasic	1000
Boric acid	0.5
Copper (II) sulfate	0.04
Potassium iodide	0.1
Iron (III) chloride	0.2
Manganese sulfate	0.4
Sodium molybdate	0.2
Zinc sulfate	0.4
Biotin	0.002
Calcium pantothenate	0.4
niacin	0.4
PABA	0.2
Pyridoxine HCl	0.4
Thiamine hydrochloride	0.4

Table S3 Fitness cost of endogenous invertase expression for exponentially growing cells.

Competition	s: 80 mM glucose:	s: 1 mM glucose:	**change in s from 80 mM to 1 mM glucose**
*suc2*Δ mCitrine (1) *suc2*Δ mCherry (2)	-0.00056 (-0.0020, 0.00091)	0.00006 (-0.0032, 0.0033)	**0.00062** (-0.0021, 0.0033)
SUC2 mCitrine (1) *suc2*Δ mCherry (2)	-0.0029 (-0.0047, -0.0011)	-0.0066 (-0.0088 ,-0.0044)	**-0.0037** (-0.0056, -0.0019)
SUC2 mCherry (1) *suc2*Δ mCitrine (2)	-0.0019 (-0.0027, -0.0011)	-0.0052 (-0.0075, -0.0030)	**-0.0033** (-0.0053, -0.0011)

To measure fitness cost of endogenous invertase, each strain was individually inoculated into liquid YPD directly from frozen glycerol stock. The strains were grown to saturation and then diluted into the test media and grown for an additional 12 hours, not allowing cells to reach saturation. At least 100,000 cells of each strain were then mixed in 30 ml of the test media: YEP plus the given concentrations of glucose. The 30 ml culture was then split between 3 glass tubes for the 3 technical replicates. These tubes were then grown at 30 ºC in a rotating drum for 12 hours, not allowing cells to reach saturation, in preparation for time point "t0". Every 12 hours for four total time points, cell concentration of one replicate in each competition was measured using a Coulter Counter (Beckman Coulter, http://www.beckmancoulter.com/). 200,000 cells from each replicate were then diluted into a prewarmed glass tube containing identical media and placed back into the rotating drum at 30 ºC. Cultures were maintained at a concentration below saturation (<1E7 cells/ml in 80 mM glucose and <1.5E6 cells/ml in 1 mM glucose). At least 150,000 cells from each replicate were also analyzed on a MoFlo FACS (Beckman Coulter, http://www.beckmancoulter.com/) in order to measure the population ratio.

The FACS files were analyzed using FlowJo Flow Cytometry Analysis Software (FlowJo, http://www.flowjo.com/) in order to find the number of cells in each population. The fitness value was obtained by analyzing the populations using custom-written software in the R programming language. The analysis steps were as follows:

1. Find the log ratio of the populations for each replicate at each time point.
2. Find the number of generations at each time point for one of the two strains.
3. Fit a line to log ratio vs. time in generations. The slope of this line is the value of *s*.
4. Find the mean value of s and the 95% confidence interval (using the 1-sample t-test) among the three technical replicates.
5. In comparing two media conditions, find the mean difference and the 95% confidence interval using the 2-sample t-test.

All values of s refer to the selective advantage of strain 1 (listed first with a (1)) over strain 2 (listed second with a (2)). The values of s listed in parentheses refer to the 95% confidence interval

calculated using 1- or 2-sample Student's t-test, as appropriate for the three replicates in each competition.

Strains (all are prototrophic and express identical drug markers):
SUC2 mCitrine: yJHK401
SUC2 mCherry: yJHK410
*suc2*Δ mCitrine: yJHK302
*suc2*Δ mCherry: yJHK437

Table S4: Yeast strains

Strain	Background strain	*AMN1* allele	Constitutive color marker	*SUC2* changes	*MAL* changes	Additional changes
yJHK111	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*			
yJHK112	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCherry-*tADH1-His3MX6*			
yJHK222	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*		*mal11/12*Δ::*hphMX4*	
yJHK223	W303 *BUD4* ; MATa ; *can1-100*	*AMN1-RM11*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*		*mal11/12*Δ::*hphMX4*	
yJHK224	W303 *BUD4* ; MATa ; *can1-100*	*AMN1-RM11*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*	*suc2*Δ::*natMX4*	*mal11/12*Δ::*hphMX4*	
yJHK226	W303 *BUD4* ; MATa ; *can1-100 ; his3-11,15*	*AMN1-RM11*	*ura3*Δ::P_{ACT1}-ymCitrine-*tADH1-URA3*		*mal11/12*Δ::*ble*	*gal1/10*Δ::*hphMX4;* P_{GAL3}Δ::*His3MX6-* P_{ACT1}*-GAL3;* P_{AMN1}Δ::*kanMX6-* P_{GAL1}*-AMN1-RM11*
yJHK227	W303 *BUD4* ; MATa ; *can1-100 ; his3-11,15*	*AMN1-RM11*	*ura3*Δ::P_{ACT1}-ymCitrine-*tADH1-URA3*	*suc2*Δ::*natMX4*	*mal11/12*Δ::*ble*	*gal1/10*Δ::*hphMX4;* P_{GAL3}Δ::*His3MX6-* P_{ACT1}*-GAL3;* P_{AMN1}Δ::*kanMX6-* P_{GAL1}*-AMN1-RM11*
yJHK228	W303 *BUD4* ; MATa ; *can1-100 ; his3-11,15*	*AMN1-RM11*	*ura3*Δ::P_{ACT1}-ymCitrine-*tADH1-URA3*		*mal11/12*Δ::*ble*	*gal1/10*Δ::*hphMX4;* P_{GAL3}Δ::*His3MX6-* P_{ACT1}*-GAL3;* P_{CTS1}Δ::*kanMX6-* P_{GAL1}*-CTS1*
yJHK229	W303 *BUD4* ; MATa ; *can1-100 ; his3-11,15*	*AMN1-RM11*	*ura3*Δ:: P_{ACT1}-ymCitrine-*tADH1-URA3*	*suc2*Δ::*natMX4*	*mal11/12*Δ::*ble*	*gal1/10*Δ::*hphMX4;* P_{GAL3}Δ::*His3MX6-* P_{ACT1}*-GAL3;* P_{CTS1}Δ::*kanMX6-* P_{GAL1}*-CTS1*
yJHK259	W303 *BUD4* ; MATa ; *can1-100*	*AMN1-RM11*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*	*suc2-1cyt*	*mal11/12*Δ::*hphMX4*	
yJHK290	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*	*suc2-1cyt*	*mal11/12*Δ::*hphMX4*	

yJHK302	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*	*suc2*Δ::*kanMX6*	*mal11/12*Δ::*hphMX4*	
yJHK315	W303 *BUD4* ; MATa ; *can1-100 ; his3-11,15*	*AMN1-RM11*	*ura3*Δ::P_{ACT1}-ymCitrine-*tADH1-URA3*	P_{SUC2}Δ::*kanMX6*- P_{GAL1}-*SUC2*	*mal11/12*Δ::*hphMX4*	*gal1/10*Δ::*LEU2*; P_{GAL3}Δ::*His3MX6*- P_{ACT1}-*GAL3*
yJHK317	W303 *BUD4* ; MATa ; *can1-100 ; his3-11,15*	*AMN1-RM11*	*ura3*Δ::P_{ACT1}-ymCitrine-*tADH1-URA3*	*suc2*Δ::*kanMX6*	*mal11/12*Δ::*hphMX4*	*gal1/10*Δ::*LEU2*; P_{GAL3}Δ::*His3MX6*- P_{ACT1}-*GAL3*
yJHK361	S288C (BY4714)	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*			
yJHK383	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*	P_{SUC2}-*ymCherry-tSUC2-NatMX4*-P_{SUC2}-*SUC2*	*mal11/12*Δ::*hphMX4*	
yJHK390	W303 *BUD4* ; MATa ; *can1-100*	*AMN1-RM11*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*		*mal11/12*Δ::*hphMX4*	*ho*Δ::*kanMX4*
yJHK391	W303 *BUD4* ; MATa ; *can1-100*	*AMN1-RM11*	*his3*Δ::P_{ACT1}-ymCherry-*tADH1-His3MX6*		*mal11/12*Δ::*hphMX4*	*ho*Δ::*kanMX4*
yJHK401	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*		*mal11/12*Δ::*hphMX4*	*ho*Δ::*kanMX4*
yJHK410	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCherry-*tADH1-His3MX6*		*mal11/12*Δ::*hphMX4*	*ho*Δ::*kanMX4*
yJHK433	W303 *BUD4* ; MATa ; *can1-100*	*AMN1-RM11*	*his3*Δ::P_{ACT1}-ymCitrine-*tADH1-His3MX6*	*suc2*Δ::*kanMX6*	*mal11/12*Δ::*hphMX4*	
yJHK435	W303 *BUD4* ; MATa ; *can1-100*	*AMN1-RM11*	*his3*Δ::P_{ACT1}-ymCherry-*tADH1-His3MX6*	*suc2*Δ::*kanMX6*	*mal11/12*Δ::*hphMX4*	
yJHK437	W303 *BUD4* ; MATa ; *can1-100*	*amn1-W303*	*his3*Δ::P_{ACT1}-ymCherry-*tADH1-His3MX6*	*suc2*Δ::*kanMX6*	*mal11/12*Δ::*hphMX4*	

Strain notes:

1. All strains are *MATa* and prototrophic, and were created for this project. All strains except for yJHK361 are from a W303 background and contain the S288C (corrected) allele of *BUD4* (standard W303 strains have a mutation in *BUD4* [1].) *BUD4* was corrected by the authors by using plasmid pJHK047.

2. Strain yJHK361 is derived from BY4714 (S288C background), which was a generous gift from the Boeke lab [2].

3. ymCherry is a yeast optimized version of mCherry [3] and was generously provided by Nicolas Ingolia of the Weissman Lab. ymCitrine is a yeast optimized version of mCitrine [4].

4. In the galactose-induction strains, *GAL3* was placed on the *ACT1* promoter in order to achieve a graded response from galactose [5].

5. *HO* was deleted by *kanMX4* (*ho*Δ*::kanMX4*) in some strains in order to match drug markers with other strains used in the same experiment. All strains used in this study are heterothallic.

6. *amn1-W303* was replaced by *AMN1-RM11* by using plasmid pEF607, which was a generous gift from the Kruglyak Lab [6].

7. *His3MX6*, *kanMX6* (G418 resistance), *natMX4* (Clonnat resistance), and *hphMX4* (hygromycin resistance) come from pFA6a-series plasmids [7,8].

8. *ble* (phleomycin resistance) comes from plasmid pUG66 [9].

9. *MAL11* and *MAL12* are not active in lab yeast strains [10] and were deleted in all but two strains to match strains used in other ongoing and unpublished research by the authors.

10. We produced a form of the *SUC2* gene that could only produce cytoplasmic invertase (see figure S1B). We created the *suc2-cyt1* allele by deleting the two ATG codons that precede the transmembrane domain of the secreted form of Suc2. This allele is similar to other alleles that have been shown to only produce cytoplasmic invertase [11,12].

11. To request strains or plasmids, please see instructions on the Murray Lab web site (http://www.mcb.harvard.edu/murray/contact.html).

Table S4 references:

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