Published in final edited form as:

Science. 2011 December 9; 334(6061): 1408–1412. doi:10.1126/science.1207154.

The competitive advantage of a dual-transporter system

Sagi Levy*, Moshe Kafri*, Miri Carmi, and Naama Barkai

Department of Molecular Genetics Weizmann Institute of Science Rehovot, Israel

Abstract

Cells use transporters of different affinities to regulate nutrient influx. When nutrients are depleted, low-affinity transporters are replaced by high affinity ones. High-affinity transporters are helpful when concentrations of nutrients are low, but the advantage of reducing their abundance when nutrients are abundant is less intuitive. When we eliminated such reduced production of the *S. cerevisiae* high affinity transporters for phosphate and zinc, the elapsed time from the initiation of the starvation program until the lack of nutrients actually limited growth was shortened, and recovery from starvation was delayed. The later phenotype was rescued by constitutive activation of the starvation program. Dual-transporter systems appear to prolong preparation for starvation and to facilitate subsequent recovery, which may optimize sensing of nutrient depletion by integrating internal and external information about nutrient availability.

Maintaining nutrient homeostasis is critical to all cells and in particular to microorganisms whose environment fluctuates in unpredictable ways. A recurrent design in systems that maintain nutrient homeostasis is the switching between transporters of different affinities. High affinity transporters are used in limiting conditions, but their abundance is decreased in cells growing in conditions where nutrients are abundant (1-14). Under these conditions, nutrients are transported by low-affinity transporters, although, in principle, high affinity transporters could function equally well. One possible advantage of this switching is a reduction in the load of protein production. However, we found that mass production of high affinity transporters in yeast had a marginal effect on fitness in rich media (Figure S1). We therefore explored whether this motif has an additional, perhaps regulatory role in maintaining nutrient homeostasis.

Cells can sense nutrient availability with transmembrane receptors, in which case the external nutrient concentration is monitored. This strategy provides indirect information about the internal pools, which are also influenced by growth rate or availability of other nutrients (15). Alternatively, cells can directly monitor the internal nutrient concentration and activate the starvation response only when internal pools are depleted (13, 16-19). In the latter case, the dual transporter motif may play a role in signaling nutrient starvation and specifically in prolonging the time-window between the initiation of the starvation response and the onset of growth limitation (the ‘preparation phase’).

Correspondence: naama.barkai@weizmann.ac.il.
*co-first authors

To see this, consider first a cell that uses a single transporter type and assume that nutrient is gradually depleted from the medium, either through consumption by the cells or loss by diffusion (Fig. 1A and B). Initially, a decrease in external nutrient concentration does not affect the internal pools, because the transporters function at maximal velocity. Internal nutrient abundance begins to decrease only when the external concentrations are close to the dissociation constant of the transporters. Notably, this reduction activates the starvation response, and, shortly after, lack of nutrients begins to limit growth. Therefore, in a system that relies on a single transporter type, the preparation phase is rather short.

A dual-transporter system prolongs the time-window between the initiation of the starvation response and the onset of limitation (Fig. 1C): the starvation response is still induced when the concentration of external nutrient is comparable to the dissociation constant of the low-affinity transporters. However, because the high-affinity transporters are produced as part of the starvation response, growth limitation ensues only when nutrient decreases further to approximately the dissociation constant of the high-affinity transporters. The dual-transporter motif thus provides the cells with a prolonged time-window in which the starvation response has been activated, but the intracellular-nutrient pools are still sufficient for optimal growth. We hypothesized that this advanced preparation may be beneficial when nutrient levels fluctuate, as foreseeing future conditions may facilitates cellular adaptation (20, 21).

We tested these ideas in two well-studied models: phosphate and zinc homeostasis in budding yeast (3, 9, 13, 16, 22). In both systems, production of high affinity transporters (Pho84 and Zrt1, respectively) is low in rich media but accumulate more than one hundred fold as part of the starvation response (Fig. 2A). Cells directly monitor the internal concentrations of phosphate and zinc: Zap1, the transcription factor activating the zinc-starvation response is directly inhibited by zinc (17, 19), whereas Pho4, the transcription factor activating the phosphate-starvation response is regulated by internal phosphate concentration (13, 16, 18). We predicted that preventing the repression of PHO84 and ZRT1 in rich medium would shorten the respective preparation phases.

We replaced the endogenous promoters of the high-affinity transporters for phosphate and zinc by the promoters of TDH3 or TEF1, two genes expressed at high levels in both rich and poor media. The resulting constitutive strains, PHO84^C and ZRT1^C express the high-affinity transporters in amounts comparable to the amount of the endogenous proteins in starved cells (Fig. 2A). We incubated wild-type and constitutive cells in media with intermediate nutrient concentrations and monitored the temporal induction of the starvation response with fluorescence reporters: YFP fused to the PHO84 promoter was used to monitor the phosphate starvation response, while mCherry fused to the ZRT1 promoter was used to monitor the zinc starvation program (Fig. 2B and C). The phosphate starvation program was further monitored by profiling gene expression (Fig. 2D). Wild-type cells induced the starvation response over 5.5h (phosphate) or 2h (zinc) before the cells constitutively expressing the high affinity transporters did so. During this period, both the wild-type and the modified cells grew rapidly at practically the same rate as they did in rich medium (Fig. S3C and D). For the phosphate system, we characterized the preparation phase also in continuous cultures (Fig. 2E). Wild-type cells fully induced the PHO84-YFP reporter when

phosphate concentration was below $\sim 400\mu\text{M}$, a concentration comparable to the dissociation constant of the low affinity transporter ($\sim 220\mu\text{M}$ (13)), whereas cells constitutively expressing the high-affinity transporters fully induced the reporter only at $\sim 60\mu\text{M}$. In both strains, growth limitation was observed only when phosphate concentration was below $\sim 5\mu\text{M}$ (our detection limit), a concentration comparable to the dissociation constant of the high affinity transporter ($\sim 9\mu\text{M}$ (13); Fig. 2F and G, Fig. S2). We conclude that preventing the expression of high-affinity transporters in rich media enables early induction of the starvation response.

Early induction of the starvation response could enhance the fitness of cells in two complementary ways. First, it could prolong growth in the limiting condition. Second, a regulated entry into starvation could facilitate the recovery from starvation once nutrient is replenished. Rapid resumption of growth once nutrient becomes available would provide yeast cells with a strong selection advantage. We therefore compared the recovery of wild-type cells and cells that constitutively express the high affinity transporters from a prolonged starvation to phosphate or zinc. Constitutive expression of Pho84p prolonged the recovery from phosphate starvation, while constitutive expression of Zrt1p prolonged the recovery from zinc starvation (Fig. 3A, S3A). The effect was specific: constitutive expression of Zrt1 did not prolong the recovery from phosphate starvation, and constitutive expression of Pho84 did not prolong (but, in fact, accelerated) the recovery from zinc starvation. Neither strain was impaired in recovery from starvation to glucose (Fig. 3A, S3A).

To better quantify the recovery of the two strains, we used a sensitive competition assay. Wild-type and constitutive cells were differentially labeled using GFP or mCherry markers driven by the constitutive promoter of the TEF2 gene. We grew the wild type and modified cells together in the same tube and measured the relative fraction of each strain in the population at different time points using flow cytometry (Fig. 3B and C, S3B-D). The cells were transferred into intermediate nutrient conditions where they consumed the nutrients, reached growth limitation, and were transferred back to rich medium or to medium with intermediate concentration of nutrient. Consistent with their delayed recovery, the strains constitutively expressing the high affinity transporters were outcompeted during recovery. Their number in the population rapidly decayed after the transfer to medium rich in nutrient (Figure 3B-C, S3B-D). The effect was specific; in fact, the PHO84^C cells, which were rapidly outcompeted by wild-type cells upon recovery from phosphate limitation, had enhanced recovery from zinc limitation, and the analogous behavior was observed for ZRT1^C. Thus, the constitutive expression of high affinity transporters appears to limit the recovery from starvation to the respective nutrients.

To control for possible effects of toxicity, we repeated the experiments in cells that constitutively expressed the low-affinity transporters for phosphate (Pho90) or zinc (Zrt2). The per-transporter flux is higher for the low-affinity transporters than for the high-affinity ones in both the zinc and phosphate systems (see supporting online information), so we expected that this constitutive expression would exacerbate any toxic effect (see e.g. Figure S4). However, during entry or exit from starvation, the PHO90^C and ZRT2^C cells grew as well as wild-type (Fig. 3B and C, S3B-D). We conclude that the impaired recovery of the high-affinity constitutive strains, PHO84^C and ZRT1^C is not likely to be caused by toxicity.

In addition to the delayed recovery from starvation, ZRT1^C cells were outcompeted, although to a lesser extent, while growing in medium with intermediate concentration of zinc until concentration that caused growth limitation (Fig. 3B and S3C). A similar phenotype was observed for PHO84^C, but only when the cells were inoculated directly into a medium containing low concentration of phosphate (0.1mM, Fig.S3C). PHO84^C grew as well as wild-type when incubated in media containing intermediate concentration of phosphate (0.2mM or 0.5mM, Fig. 3B and S3C), although they depleted phosphate from the media as well (to below our 5μM detection limit). This suggests that depletion of high-affinity transporters from cells in rich media benefits cells more during recovery from, rather than entry to starvation.

We attributed the delayed recovery of the cells constitutively expressing the high affinity transporters to their shortened preparation phase. We therefore predicted that this phenotype would be rescued by constitutively activating the early starvation program, rendering the cells constantly prepared. The phosphate starvation response is induced in two stages: first during intermediate limitations and second in deep starvation (23). We reasoned that the first stage may constitute preparation and used a previously described Pho4 allele (Pho4-SA1234, (23)) to constitutively activate this response in both the wild-type and the PHO84^C cells (Methods). In these cells, Pho4 is present in the nucleus even in conditions in which phosphate is abundant (Fig. 4A and B). As predicted, this allele fully rescued the delayed recovery of the constitutive strains (Fig. 4C and D, S5) strongly supporting our hypothesis that the delayed recovery of constitutive strains results from the shortened preparation of these cells.

Our results indicate that the dual-transporters motif enables cells to prepare in advance for nutrient depletion and by this improves their recovery once nutrients are replenished. How could early activation of the starvation program facilitate recovery? Measuring the gene expression profiles of cells as they depleted phosphate, defined a large number of genes whose expression is induced in wild-type cells during the early starvation program but is delayed in cells that constitutively express the high affinity transporters (c.f. Fig. 2D). Those genes are candidates for improving recovery. Of particular note are the PHM1-4 genes required for the storage of phosphate in the vacuoles, as this storage plays a role during nutrient resupply (24).

Our work suggests that the low-affinity transporters function as signaling entities, allowing cells to sense a reduction in nutrient level before this reduction becomes limiting for growth. In principle, this function could be provided by extracellular receptors, but this will negate the advantages of directly monitoring intracellular nutrients (15, 25-27). The dual-transporter system enables cells to combine internal and external sensing, facilitating the advantage of both strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Sandy Fialkov and Shlomit Meizel for the technical help and Danny Ben-Zvi, Ilya Soifer and our lab members for helpful discussions. We thank Erin O'shea for the PHO4-SA1234 plasmid. Microarrays data have been deposited in GEO (accession number GSE32067). This work was supported by the ERC, the NIH (P50GM068763) and the Hellen and Martin Kimmel award for innovative investigations.

References

1. Daram P, et al. *Plant Cell*. Nov.1999 11:2153. [PubMed: 10559441]
2. Eide DJ. *Annu Rev Nutr*. 1998; 18:441. [PubMed: 9706232]
3. Eide DJ. *J Biol Chem*. Jul 10.2009 284:18565. [PubMed: 19363031]
4. Harris RM, Webb DC, Howitt SM, Cox GB. *J Bacteriol*. Sep.2001 183:5008. [PubMed: 11489853]
5. Lasko PF, Brandriss MC. *J Bacteriol*. Oct.1981 148:241. [PubMed: 7026531]
6. Liu KH, Tsay YF. *EMBO J*. Mar 3.2003 22:1005. [PubMed: 12606566]
7. Marini AM, Soussi-Boudekou S, Vissers S, Andre B. *Mol Cell Biol*. Aug.1997 17:4282. [PubMed: 9234685]
8. Muchhal US, Pardo JM, Raghothama KG. *Proc Natl Acad Sci U S A*. Sep 17.1996 93:10519. [PubMed: 8927627]
9. Persson BL, et al. *Curr Genet*. Jul.2003 43:225. [PubMed: 12740714]
10. Santos-Beneit F, Rodriguez-Garcia A, Franco-Dominguez E, Martin JF. *Microbiology*. Aug.2008 154:2356. [PubMed: 18667568]
11. Seymour RL, Ganapathy V, Mellor AL, Munn DH. *J Leukoc Biol*. Dec.2006 80:1320. [PubMed: 16997853]
12. Townsend DE, Wilkinson BJ. *J Bacteriol*. Apr.1992 174:2702. [PubMed: 1556088]
13. Wykoff DD, O'Shea EK. *Genetics*. Dec.2001 159:1491. [PubMed: 11779791]
14. Yamamura HI, Snyder SH. *J Neurochem*. Dec.1973 21:1355. [PubMed: 4771436]
15. Levy S, Barkai N. *FEBS Lett*. Dec 17.2009 583:3974. [PubMed: 19878679]
16. Auesukaree C, et al. *J Biol Chem*. Apr 23.2004 279:17289. [PubMed: 14966138]
17. Bird AJ, et al. *EMBO J*. Oct 1.2003 22:5137. [PubMed: 14517251]
18. Lee YS, Mulugu S, York JD, O'Shea EK. *Science*. Apr 6.2007 316:109. [PubMed: 17412959]
19. Qiao W, Mooney M, Bird AJ, Winge DR, Eide DJ. *Proc Natl Acad Sci U S A*. Jun 6.2006 103:8674. [PubMed: 16720702]
20. Mitchell A, et al. *Nature*. Jul 9.2009 460:220. [PubMed: 19536156]
21. Tagkopoulos I, Liu YC, Tavazoie S. *Science*. Jun 6.2008 320:1313. [PubMed: 18467556]
22. Wykoff DD, Rizvi AH, Raser JM, Margolin B, O'Shea EK. *Mol Cell*. Sep 21.2007 27:1005. [PubMed: 17889672]
23. Springer M, Wykoff DD, Miller N, O'Shea EK. *PLoS Biol*. Nov.2003 1:E28. [PubMed: 14624238]
24. Ogawa N, DeRisi J, Brown PO. *Mol Biol Cell*. Dec.2000 11:4309. [PubMed: 11102525]
25. Bollenbach T, Quan S, Chait R, Kishony R. *Cell*. Nov 13.2009 139:707. [PubMed: 19914165]
26. Levy S, et al. *PLoS One*. 2007; 2:e250. [PubMed: 17327914]
27. Zaman S, Lippman SI, Schnepfer L, Slonim N, Broach JR. *Mol Syst Biol*. 2009; 5:245. [PubMed: 19225458]

Fig. 1. A dual-transporter system enables advanced preparation to nutrient depletion

(A) Nutrient uptake by high affinity transporters: The flux of incoming nutrient is shown as a function of the external nutrient concentration. The internal nutrient pools follow this flux and are therefore depleted only when external nutrient concentration decreases to below $K_d^{High\ affinity}$. Activation of the starvation response occurs at this range, as does growth limitation.

(B) Nutrient uptake rate by low affinity transporters: Same as (A), except with a higher dissociation constant.

(C) A dual-transporter system prolongs preparation: In a dual transporter system, activation of the starvation response occurs at $K_d^{Low\ affinity}$ (red arrows), whereas growth limitation is reached only when external nutrient is further reduced to $K_d^{High\ affinity}$.

Fig. 2. Constitutive expression of high affinity transporters shortens preparation

(A) Constitutive expression: The constitutive promoters (TDH3 and TEF1) were compared to the endogenous ones (PHO84 or ZRT1) using a fluorescence reporter. Shown are the mean fluorescence during logarithmic growth in rich medium (SC), poor medium (initial levels of 0.5mM phosphate or 10μM of zinc) or prolonged starvation (three days in no-phosphate or no-zinc media), as indicated. Error-bars denote variation within the cell population. The increase in protein abundance and their sub-cellular localization were verified by constitutively expressing YFP-fused transporters (Fig. S6 and S7). This fusion may reduce protein activity, as monitored by slow growth of ZRT1-YFP cells at low zinc, therefore all experiments were performed with native zinc and phosphate transporters.

(B-C) Delayed starvation response in cells with abundant high-affinity transporters: Wild-type and constitutive cells (PHO84^C or ZRT1^C) were transferred to medium containing low concentrations of phosphate (180μM, left) or zinc (10μM, right), respectively. Activation of the starvation program was quantified by following the fluorescence of two reporters: YFP driven by the PHO84 promoter for monitoring the phosphate starvation response or mCherry driven by the ZRT1 promoter for monitoring the zinc starvation response. The single-cell distribution of reporter activation is shown in (B) with the time course summarized in C for two biological repeats (cutoff value = 1500). Note that wild-type and constitutive cells grow at the same rate (Figure S3C-D, “Entry”, left black bars).

(D) Delayed activation of the battery of phosphate-responsive genes: The experiment in (B-C) was repeated, with cells transferred to a media containing 0.5mM. The activation of starvation response was measured using microarrays for wild-type and PHO84^C cells. Values are log₂ ratios, the reference being cells grown in rich media.

(E-G) Response to phosphate depletion in continuous cultures: Cells were grown in a chemostat with different concentrations of phosphate in the feeding vessel. The steady-state level of phosphate in the chemostat was quantified using standard assays (Methods), and the

activation of the phosphate starvation response was monitored by flow-cytometry analysis of the PHO84-YFP reporter. Experiments were repeated for wild-type cells, PHO84^C constitutive strains, and cells expressing only low affinity transporters (*pho84 spl2*).

(F) Activation of the starvation response: The fraction of cells activating the starvation response is shown as a function of the phosphate level in the chemostat. See also Figure S2 showing the bi-stable activation pattern. Lines were added to guide the eye (Methods).

(G) Effect of phosphate concentration on cell density: Normalized cell density is shown as a function of phosphate concentration in the chemostat. The shaded regions correspond to phosphate concentrations that are below our detection limit of 5 μ M. The drop in cell density in low phosphate levels correlates with the reduction of the concentration of phosphate at the feeding vessel of the chemostat (Shaded regions a,b, and c correspond to feeding levels of 0.4mM, 0.3mM and 0.2mM, respectively). Error-bars are standard errors between biological repeats, when available. Lines were added to guide the eye (Methods, see also Figure S2). Constitutive strains in Figure 2B-G were driven by Tdh3 promoter.

Figure 3. Impaired recovery from nutrient limitation in cells constitutively expressing high-affinity transporters

(A) Recovery from starvation: Cells were deprived of the indicated nutrient and then transferred back to rich medium. The increase in cell density after the transfer is shown. *Left:* wild-type (dashed) and PHO84^C (solid) cells during recovery from starvation to phosphate (red) or zinc (grey). *Middle:* Lag time of constitutive and wild-type strains. Each point represents a single experiment, differing by the number of days the cells were starved before recovery. Starvation to the relevant nutrient is shown in red (circles- PHO84^C in phosphate; squares- ZRT1^C in zinc), and to non-relevant nutrients in grey (circles- PHO84^C in zinc, squares- ZRT1^C in phosphate, triangles- PHO84^C or ZRT1^C in glucose). *Right:* Lag-time difference for the conditions and strains indicated. Data are mean $\pm$ SEM. See Figure S3A for additional controls and detailed results for different constitutive promoters. See Table S3 for number of repeats.

(B) Competition in fluctuating nutrient conditions: The fraction of wild-type to constitutive cells during competitive growth in the conditions shown. The following conditions were used: *Entry and Exit*- initial intermediate levels of 0.5mM P_i or 10μM Z_n, *Fluctuations* - initial intermediate levels of 0.5mM P_i or 25μM Z_n. *Constant* - rich levels of 20mM P_i or 1500μM Z_n, and intermediate levels (with cells kept in logarithmic phase) of 0.5mM P_i or 300μM Z_n. The constitutive strains presented here are driven by Tdh3 promoter except of zinc entry-exit experiment shown for Tef1 strains. See additional examples in supplementary Figure S3.

(C) Fold change difference between wild-type and constitutive strains upon exit from starvation: Fraction of wild-type (WT) vs. constitutive cells following recovery from starvation, as indicated. Data are mean $\pm$ SEM. See Table S2 for number of repeats.

Figure 4. Prolonged recovery of the PHO84^C cells is rescued by constitutive activation of the intermediate-starvation program

(A-B) Nuclear localization of the PHO4-SA1234.

(C-D) Rescue of PHO84^C by PHO4-SA1234: Competition assays as in Figure 3B-C above with the PHO84^C cells expressing also the PHO4-SA1234 allele. Data are mean $\pm$ SEM. See Table S2 for number of repeats. The entry phenotype was not rescued by the Pho4-SA1234, suggesting that it does not result from prolonged preparation (Figure S5).

Analogous rescue experiments in the zinc system were not possible since cells expressing an activated Zap1 allele are very sick both in high and low zinc (not shown).