Helping daughters succeed: asymmetric distribution of glucose transporter mRNA

Allyson F O'Donnell[1] & Martin C Schmidt[2] (ID)

Rapidly proliferating cells growing by glucose fermentation must first transport glucose into the cell. Both budding yeast and human tumor cells utilize members of a conserved family of glucose transporters. In this issue of *The EMBO Journal*, Stahl *et al* (2019) reveal that budding yeast cells confer a growth advantage to their daughters using a novel mechanism, the asymmetric distribution to the daughter cell of the mRNA for a specific glucose transporter.

Rapidly proliferating cells, including human tumor and budding yeast cells, use a metabolic program that generates energy by glucose fermentation. While complete oxidation of glucose to CO_2 is more efficient at generating ATP, partial oxidation of glucose during fermentation serves an added purpose; fermentation provides the carbon skeletons that feed biosynthetic pathways during rapid proliferation. A limitation of the fermentative lifestyle is the need for high-flux glucose transport. To achieve high rates of glucose uptake, tumor cells increase expression of glucose transporters, like GLUT1. Glucose transporters in human and yeast comprise a conserved family of 12 transmembrane span-containing proteins at the plasma membrane (PM) that import glucose by facilitated diffusion. Yeast differ from tumor cells in that they lack a constant supply of blood glucose and must adapt to great variations in glucose concentration. To adapt to

external glucose changes, yeast express specific glucose transporters (called hexose transporters or HXTs) with distinct affinities and capacities for glucose transport. When glucose abounds, yeast utilize HXTs with a high capacity for glucose transport. When glucose is scarce, yeast use HXTs with a lower capacity for transport but a higher glucose binding affinity. Human cells also express different members of the related GLUT family, which have distinct transport properties tailored to cell type needs. Glucose transporter selection and its regulated expression are critical determinants of growth rates in human and yeast cells.

Initial studies of yeast *HXT* expression focused on transcriptional regulation. Measurement of *HXT*-promoter-lacZ gene fusion expression determined the differential response of *HXT*s to glucose concentrations. Transcription of high-capacity *HXT*s, such as *HXT1* and *HXT3,* is induced at high glucose concentrations while high-affinity *HXT*s, such as *HXT5* and *HXT6,* are induced at low glucose concentrations (Ozcan & Johnston, 1995). Regulation of *HXT* transcription relies on two glucose sensors, Snf3 and Rgt2, that are closely related to the *HXT*s but have important differences: (i) Snf3 and Rgt2 bind and sense external glucose but are not competent for glucose transport, and (ii) they contain long C-terminal extensions, not present in the other *HXT*s, which serve as binding sites for the transcriptional regulators Std1 and Mth1 (Schmidt *et al*, 1999). Glucose-bound Snf3 and Rgt2 recruit Std1 and Mth1 from the nucleus and promote their phosphorylation. Phosphorylated Std1 and Mth1 are degraded or sequestered, allowing the transcriptional activator Rgt1 to

escape inhibition and induce *HXT* expression. A second layer of HXT regulation uncovered more recently is mediated by endocytosis. The PM is a finite and valuable piece of cellular real estate. Transporters that are not needed or that become misfolded are removed from the PM and sent to the vacuole for degradation. This process relies on the ubiquitin ligase Rsp5, a member of the NEDD4 family of ubiquitin ligases. Selecting membrane targets for ubiquitination and endocytosis is controlled in yeast and humans by a family of protein trafficking adaptors called the α-arrestins (O'Donnell & Schmidt, 2019). Yeast encode at least 14 α-arrestins, and identifying the network of α-arrestin–membrane cargo pairs is an area of active investigation. In yeast, the α-arrestins Rod1, Rog3, and Csr2 control the endocytosis of HXTs. The yeast AMP-activated protein kinase Snf1 negatively regulates these α-arrestins to maintain the PM localization of the high-capacity glucose transporters, Hxt1 and Hxt3 (O'Donnell *et al*, 2015).

In this issue, Stahl *et al* (2019) add an exciting new layer to the already exquisite regulation of glucose transporters in yeast. This paper starts with the deceptively simple observation that GFP-tagged Hxt2 is asymmetrically distributed between mother and daughter cells; Hxt2-GFP is at the PM in mother cells, but not in daughter cells of small or medium bud size. Strikingly, Hxt2-GFP fluorescence equilibrates between the mother and daughter cell at a specific stage of the cell cycle. To define the mechanism underlying this selective partitioning of Hxt2-GFP, quantitative fluorescence *in situ* hybridization (FISH) experiments were

1 Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA. E-mail: allyod@pitt.edu
2 Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA, USA. E-mail: mcs2@pitt.edu

performed and show that *HXT2* mRNA is initially restricted to the mother cell but becomes evenly distributed between the mother and daughter cell at the metaphase–anaphase transition. However, the most striking observation is the further enrichment of *HXT2* mRNA in the daughter cells after nutrient-starved cells are provided glucose (Fig 1). This effect is restricted to *HXT2* mRNA and is not observed for *HXT1*, *HXT3*, or *HXT4* mRNAs, hinting that there may be something uniquely beneficial to specifically expressing *HXT2*, and not the other *HXT*s, in daughter cells. How is this selective partitioning of *HXT2* mRNA achieved?

Selective partitioning of mRNAs in dividing cells is a conserved regulatory feature from yeast to man. In multicellular eukaryotes, mRNA partitioning plays an important role in cell polarity and differentiation, as well as organismal development (Martin &

Ephrussi, 2009). Yeast has been an important model system for unraveling the mechanisms of selective mRNA partitioning. First described in yeast in 1997, *ASH1* mRNA selectively partitions to daughter cells (Long *et al*, 1997; Takizawa *et al*, 1997) in a process that requires SWI5-dependent HO expression (She) proteins. The She proteins encode an *ASH1* mRNA binding protein, adaptor proteins that hold the mRNA-protein complex together, a type V myosin motor, and a formin for actin filament nucleation. Together, the She proteins ensure that *ASH1* mRNA moves along actin cables to partition into daughter cells where, once translated, it represses transcription of specific genes in the daughters. Regulation by mRNA partitioning is not limited to *ASH1*; the mRNAs encoding several yeast cell polarity proteins use a similar machinery (Aronov *et al*, 2007), and over 30 transcripts are known to

selectively partition into the daughter cell. However, not all mRNA partitioning uses the same machinery. Notably, some mRNAs use the Scp160 protein rather than the She proteins (Gelin-Licht *et al*, 2012). Further, not all mRNAs partition to sites of polarized growth; the mRNA encoding the actin-binding protein Abp140 is co-translationally targeted to the distal pole in mother cells (Kilchert & Spang, 2011). Unlike other mRNA partitioning, Stahl *et al* show that *HXT2* mRNA asymmetric distribution upon nutrient starvation and refeeding is reliant upon both actin filaments and microtubules. Interestingly, the retention of *HXT*s in the mother cell in the absence of starvation did not rely on the cytoskeleton. Nuclear segregation between mother and daughter cells is required for *HXT2* mRNA targeting to daughter cells. Specifically, the spindle positioning factors Kar9 and Bim1, which are the
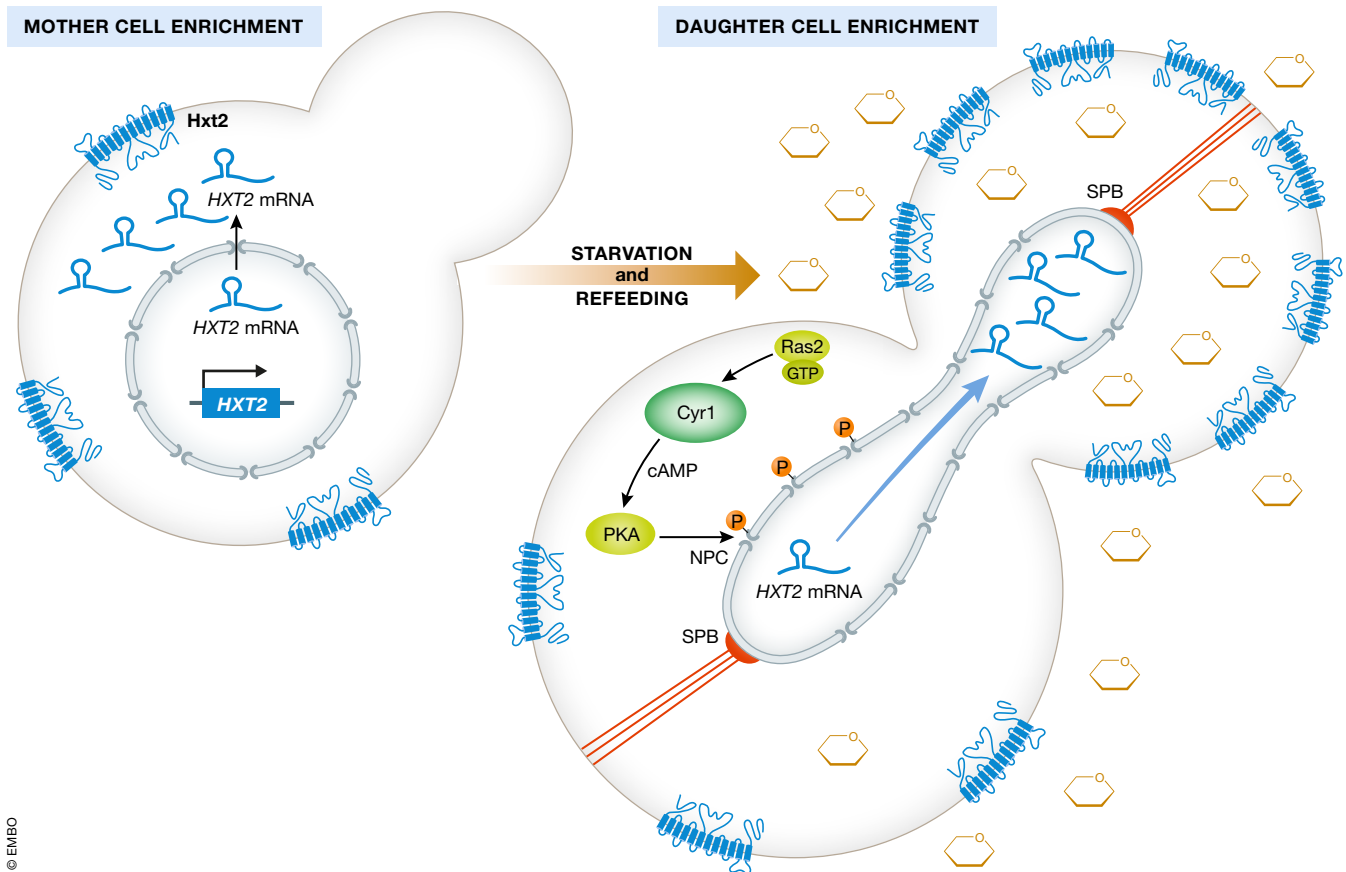

Figure 1. Asymmetric partitioning of *HXT2* mRNA.
In starved cells, glucose transporter mRNA and protein are restricted to the mother cell until the metaphase–anaphase transition. Upon refeeding with glucose, the *HXT2* mRNA and protein are enriched in the daughter cell, conferring a growth advantage to the daughter cells. Appearance of glucose after starvation activates the Ras signaling pathway which then stimulates adenylate cyclase (Cyr1). Cyclic AMP alleviates repression of protein kinase A (PKA), which in turn promotes *HXT2* mRNA partitioning, possibly by phosphorylating components of the nuclear pore complex (NPC). *HXT2* mRNA partitioning to the daughters requires the cytoskeleton, spindle pole body (SPB), and nuclear segregation.

homologues of mammalian APC and EB1, respectively, are required for the enrichment of *HXT2* mRNA into daughter cells. The *HXT2* mRNA inherited by daughter cells is associated with the nucleus, and factors needed for nuclear pore formation in the nuclear envelope are required for regulated partitioning of *HXT2* mRNA.

The asymmetric distribution of *HXT2* mRNA is most apparent in daughter cells when glucose reappears after starvation, and these same conditions activate protein kinase A (PKA). Stahl *et al* show that PKA signaling is required for *HXT2* mRNA localization. A potential target for PKA is Nup2, a nucleoporin and component of the nuclear pore complex, which the authors show is required for efficient distribution of *HXT2* mRNA. With this novel partitioning mechanism and some of the molecular players defined, the authors sought to answer the more challenging question of what is the biological significance underlying partitioning? In competitive growth assays, expression of *HXT2*—but not *HXT*s 1, 3, or 4—confers a growth advantage to daughter cells. Conversely, cells lacking *HXT2* are at a disadvantage upon resumption of growth after nutrient exhaustion. Hxt2 is thought to be a generalist among the HXTs; good under almost all glucose conditions. Perhaps *HXT2*'s selective expression in daughter cells ensures the best chance of growth before fine-tuning of the *HXT* expression profile begins.

In sum, this work provides a new mechanism for mRNA partitioning that relies on PKA signaling, the nuclear envelope, and the cytoskeleton. It further defines a novel mode of glucose transporter regulation in the selective targeting of the Hxt2 transporter to daughter cells. Moving forward, it will be interesting to identify the target of PKA that regulates *HXT2* movement and define factors needed for *HXT2* mRNA to piggy back on the nuclear envelope during partitioning. Conservation of this mRNA partitioning of glucose transporters during cell polarity changes, organismal development, or the transition to disease states like cancer would be exciting areas for future research.

References

Aronov S, Gelin-Licht R, Zipor G, Haim L, Safran E, Gerst JE (2007) mRNAs encoding polarity and exocytosis factors are cotransported with the cortical endoplasmic reticulum to the incipient bud in *Saccharomyces cerevisiae*. *Mol Cell Biol* 27: 3441–3455

Gelin-Licht R, Paliwal S, Conlon P, Levchenko A, Gerst JE (2012) Scp160-dependent mRNA trafficking mediates pheromone gradient sensing and chemotropism in yeast. *Cell Rep* 1: 483–494

Kilchert C, Spang A (2011) Cotranslational transport of ABP140 mRNA to the distal pole of *S. cerevisiae*. *EMBO J* 30: 3567–3580

Long RM, Singer RH, Meng X, Gonzalez I, Nasmyth K, Jansen RP (1997) Mating type switching in yeast controlled by asymmetric localization of ASH1 mRNA. *Science* 277: 383–387

Martin KC, Ephrussi A (2009) mRNA localization: gene expression in the spatial dimension. *Cell* 136: 719–730

O'Donnell AF, McCartney RR, Chandrashekarappa DG, Zhang BB, Thorner J, Schmidt MC (2015) 2-Deoxyglucose impairs *Saccharomyces cerevisiae* growth by stimulating Snf1-regulated and alpha-arrestin-mediated trafficking of hexose transporters 1 and 3. *Mol Cell Biol* 35: 939–955

O'Donnell AF, Schmidt MC (2019) AMPK-mediated regulation of alpha-arrestins and protein trafficking. *Int J Mol Sci* 20: E515

Ozcan S, Johnston M (1995) Three different regulatory mechanisms enable yeast hexose transporter (HXT) genes to be induced by different levels of glucose. *Mol Cell Biol* 15: 1564–1572

Schmidt MC, McCartney RR, Zhang X, Tillman TS, Solimeo H, Wolfl S, Almonte C, Watkins SC (1999) Std1 and Mth1 proteins interact with the glucose sensors to control glucose-regulated gene expression in *Saccharomyces cerevisiae*. *Mol Cell Biol* 19: 4561–4571

Stahl T, Hümmer S, Ehrenfeuchter N, Mittal N, Fucile G, Spang A (2019) Asymmetric distribution of glucose transporter mRNA provides a growth advantage in yeast. *EMBO J* 38: e100373

Takizawa PA, Sil A, Swedlow JR, Herskowitz I, Vale RD (1997) Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast. *Nature* 389: 90–93