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REVIEW ARTICLE

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Sensors of extracellular nutrients in *Saccharomyces cerevisiae*

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Abstract It has been known for a long time that yeast are capable of making rapid metabolic adjustments in response to changing extracellular nutrient conditions. Until recently it was thought that yeast, in contrast to mammalian cells, primarily monitored nutrient availability through the activity of intracellular sensors. Recent advances in our understanding of nutrient sensing indicate that yeast cells possess several nutrient-sensing systems localized in the plasma membrane that transduce information regarding the presence of extracellular amino acids, ammonium, and glucose. Strikingly, the transmembrane components of several of these sensors, Ssy1p, Mep2p, Snf3p, and Rgt2p, are unique members of nutrient-transport protein families. Perhaps with the exception of Mep2p, the ability of these transporter homologues to transduce nutrient-(ligand)-induced signals across the plasma membrane appears to be independent of nutrient uptake; and thus these sensor components may function analogously to traditional ligand-dependent receptors. Additionally, the G protein-coupled receptor Gpr1p has been shown to exhibit properties consistent with it being a sensor. These recent advances indicate that yeast cells obtain information regarding their growth environments using sensing systems that are more similar to those present in mammalian cells than previously thought. The fact that yeast plasma membrane nutrient sensors have only recently been discovered reveals how little is understood regarding the molecular signals that enable eukaryotic cells to adapt to changing environments.

Keywords Signal transduction · Nutrient sensing · Metabolite-transport proteins · Fungal proteins

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Introduction

Eukaryotic cell proliferation and growth is tightly regulated. Cell division at inappropriate moments can have deleterious effects. For example, erroneous uncontrolled cell division in multicellular organisms is the cause of cancer. Cell proliferation and development in mammalian systems is generally assumed to be regulated primarily by signals derived from receptors in the plasma membrane (PM) that respond to specific ligands, e.g., growth factors. In contrast and until recently, it was thought that the decisions affecting developmental outcomes in the yeast *Saccharomyces cerevisiae* were made in response to intracellularly derived nutritional signals. In the past 5 years it has become clear that yeast cells also possess and use PM-localized sensors to obtain information regarding concentrations of nutrients in the extracellular environment, including the availability of amino acids, ammonium, and glucose.

Recent work in this and other laboratories has significantly advanced our understanding of the molecular mechanisms that enable yeast cells to sense extracellular amino acids. The prime sensing components and several downstream targets that respond to amino acid induced signals have been identified. The primary amino acid sensor is a multimeric complex localized to the PM. One of the PM components, Ssy1p, closely resembles amino acid permeases, a family of proteins that normally catalyze the transport of amino acids into the cell. There are other nutrient-sensing systems known in yeast where transport protein homologues have important roles as nutrient sensors, i.e., Snf3p/Rgt2p function as glucose sensors and Mep2p functions as an ammonium sensor. In the bacterial world, transporter-like nutrient sensors also participate in nutrient sensing. It is thus likely that in response to selective pressure, diverse nutrient sensors have evolved from pre-existing proteins with defined substrate-binding sites.

Interestingly, the ability of the yeast transporter homologues to transduce nutrient-(ligand)-induced signals

across the PM appears to be independent of nutrient uptake; and thus these sensor components may function analogously to traditional ligand-dependent receptors. Recently, the G protein-coupled receptor, Gpr1p, has been implicated as a sensor of high concentrations of glucose. These novel environmental sensors operate in parallel with known intracellular metabolite-sensing systems, indicating that yeast cells have the capacity to integrate nutrient-based metabolic signals from spatially separated sensing systems to properly regulate gene expression. Together, these recent advances in understanding nutrient-sensing in yeast indicate that yeast cells obtain information regarding their growth environments using mechanisms that are more similar to mammalian cells than previously thought. The purpose of this review is to give an overview of what is currently known about yeast sensors of extracellular nutrients and their role in yeast growth and development. Additionally, examples of similar systems in other organisms provide comparative information that is included as a basis for further understanding nutritional sensing in eukaryotic cells.

Nutrients are yeast growth factors

Once every cell cycle, at a particular point in G_1, commonly referred to as START (Pringle and Hartwell 1981), yeast are forced to make developmental decisions that engage distinct programs of gene expression (Wittenberg and Reed 1996). Except for the process of mating, the choice of which developmental pathway yeast cells enter is dependent on nutrient availability (Fig. 1). The availability and quality of nitrogen and carbon sources are perhaps the best documented factors known to affect yeast growth and development. However, the molecular mechanisms for sensing and transducing signals regarding these essential nutrients, and the manner in which yeast cells integrate nutritional signals from a diverse set of nutrient-sensing systems remain largely obscure.

When sufficient amounts of nitrogen and carbon sources are present in the growth environment, both haploid and diploid yeast grow vegetatively, initiating successive rounds of the cell cycle (for review, see Lew et al. 1997). In the absence of either a nitrogen or carbon source, both haploids and diploids exit the cell cycle and enter a developmental pathway leading to G_0 arrest, a stationary phase. When diploid cells are starved of both nitrogen and carbon, they enter a meiotic pathway leading to the formation of four haploid spores (Kupiec et al. 1997). Spores and G_0 arrested cells are characteristically resistant to various environmental stresses, including heat and prolonged nutrient deprivation, and are considerably more resistant to desiccation. Thus, these physiological states enable yeast to better survive adverse environmental conditions. When spores are placed on media containing appropriate nitrogen and carbon sources, they germinate and the resulting cells re-enter the cell cycle. Similarly, G_0 arrested cells can re-enter the cell cycle when nutrients become available.

Under certain conditions, yeast initiate filamentous modes of growth (for review, see Mösch 2000). When diploids are grown on limiting nitrogen sources, cells become elongated, bud in a polar manner, and grow in branched filaments (Gimeno et al. 1992). This phenomenon, referred to as pseudohyphal growth (PHG), is induced when cells are grown on media containing low concentrations of ammonium or amino acids (e.g., proline or glutamine). This mode of growth may enable immobile yeast colonies to better exploit nutrient-poor environments by enabling cells to forage for nutrients (Gimeno et al. 1992). In contrast, haploid cells exhibit filamentous invasive growth on rich media under conditions of nutrient excess (Roberts and Fink 1994). Haploid invasive growth is similar to PHG, cells become elongated, bud in a polar manner, and grow in branched chains. Although compared to PHG, cell elongation and chain formation are not as pronounced and, instead of

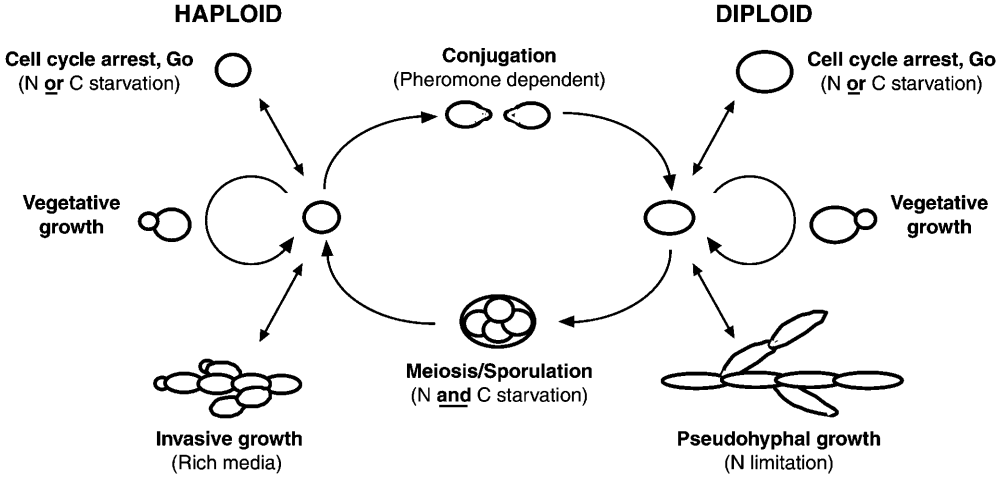

Fig. 1 Yeast growth and development. Once every cell cycle, haploid and diploid yeast cells must choose to enter into one of four developmental pathways. All of the developmental outcomes, with the exception of the mating pathway, are nutritionally regulated. See text for details

growing laterally away from colonies, filaments of invasive haploid cells are almost exclusively seen beneath developing colonies. Glucose limitation has been shown to affect haploid invasive growth (Cullen and Sprague 2000), although the benefit of this mode of growth remains obscure. The ability to undergo dimorphic transitions leading to filamentous growth is required for pathogenic fungi to invade hosts and become infectious (Gimeno et al. 1992; Xu and Hamer 1996; Beckerman et al. 1997; Lo et al. 1997). Therefore, further characterization of how these transitions are regulated is of interest in developing strategies for controlling fungal infections in man and in agriculturally important products.

Sensors of extracellular nutrients in yeast

Clearly, yeast cells make assessments of nutrient availability. Until recently, it was thought that yeast, in contrast to mammalian cells, primarily monitored nutrient availability through the activity of intracellular sensors. This view was based on several considerations. First, the finding that cell division cycle (*cdc*) mutants that arrest cell cycle progression at post-START phases of the cell cycle are relatively insensitive to nutrient deprivation (Pringle and Hartwell 1981). Such mutants, typically arrested at non-permissive temperatures, can complete the cell cycle and arrest normally in G_1 even in the absence of nutrients when permissive temperatures are restored. These findings suggested that cells monitor intracellular nutrient pools prior to committing to each successive round of the cell cycle; and only cells that have accumulated sufficient intracellular pools to complete a full cycle actually proceed to initiate another round of division. Secondly, there has been a lack of experimental evidence for the existence of growth factor-like receptors or PM-localized nutrient sensors. Finally, several intracellular sensors have been identified. These include Gcn2p, the well characterized primary sensor of the general control of amino acid biosynthesis (Hinnebusch 1988, 1994). The phenomena of glucose regulation (Gancedo 1998; Carlson 1999; Rolland et al. 2001b) and nitrogen regulation (Magasanik 1992; ter Schure et al. 2000) are well documented and many of the major effector molecules are known to be internalized metabolites.

The view that yeast cells only assess intracellular nutrients prior to making metabolic adjustments requires modification. It has been known for a long time that yeast are capable of making rapid metabolic adjustments in response to changing extracellular nutrient conditions. The speed at which yeast cells implement adjustments, and the fact that adjustments are often made independently of the metabolic processing of the initiating substance, suggested that yeast possess nutrient-sensing systems that function independently of uptake. For example, amino acids are mobilized from vacuolar pools within minutes after cells are removed from amino acid-containing media (Wiemken and Durr 1974). Arginase (*CAR1*) expression is rapidly induced in cells in the presence of micromolar concentrations of a variety of amino acids (Dubois and Wiame 1976). The non-specific induction of *CAR1* is independent of the induction by cytoplasmic arginine (Dubois and Messenguy 1997). Similarly, the rates of dipeptide and leucine uptake are non-specifically stimulated by micromolar amounts of amino acids (Island et al. 1987; Didion et al. 1996). The rediscovery that *S. cerevisiae* cells are capable of filamentous growth (Gimeno et al. 1992), made using homozygous *shr3* diploid cells that have severely reduced capacity to take up amino acids, strongly suggests that yeast cells possess sensors of extracellular nutrients (Ljungdahl et al. 1992).

In the past 5 years, several components of PM-localized nutrient-sensing systems have been identified and characterized. These include two sensors of extracellular nitrogenous compounds: (1) the amino acid SPS sensor, a multimeric sensing complex minimally comprised of Ssy1p, Ptr3p, and Ssy5p, and (2) a sensor of extracellular ammonium, Mep2p. Additionally, the physiological roles of previously identified putative glucose-transport proteins, Snf3p and Rgt2p, have been re-evaluated. It is now clear that these proteins function as primary glucose sensors that are active at low and high extracellular glucose concentrations, respectively. Strikingly, the transmembrane components of these sensors, Ssy1p, Mep2p, Snf3p, and Rgt2p, are unique members of nutrient-transport protein families (Fig. 2). Finally, the G protein-coupled receptor, Gpr1p, has been shown to

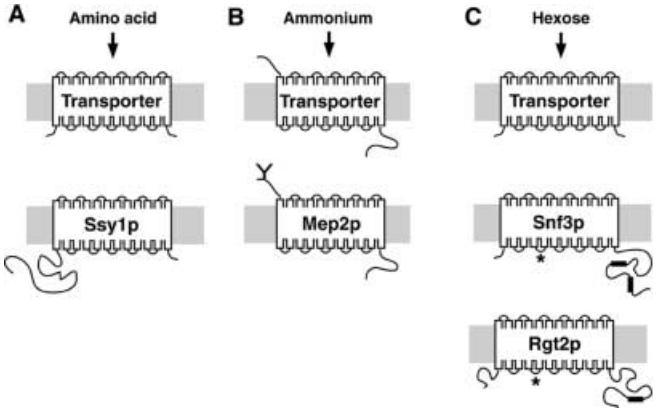

Fig. 2A–C Several yeast nutrient sensors are unique members of members of nutrient transporters of proteins. **A** The amino acid sensor Ssy1p is one of 18 core members of the amino acid permease family. Ssy1p has a N-terminal extension that is not present in the other 17 permeases. **B** The ammonium sensor Mep2p is one of three ammonium transporters. Mep2p is glycosylated (Y) within its extracellular N-terminal domain. The other two ammonium permeases are not glycosylated. **C** The glucose sensors Snf3p and Rgt2p are members of the hexose transporter family. These two proteins carry extended C-terminal domains that are not present in the other 18 hexose-transport family members. The positions of the dominant positive arginine to lysine substitution (*) and the conserved 17 aa sequence motifs (*black boxes*) in the C-termini of Snf3p and Rgt2p are indicated. See text for details

function as a sensor of extracellular glucose in cells that are starved of glucose.

Sensors of extracellular nitrogen sources

The Ssy1p/Ptr3p/Ssy5p (SPS) sensor of extracellular amino acids

Apart from being metabolites for protein synthesis, amino acids have an important role in nitrogen homeostasis in yeast; and although yeast cells are able to synthesize all amino acids, yeast has developed a sophisticated system to efficiently import amino acids from their external environment. Amino acids are transported into the cell by general and specific transport systems. These polytopic transport proteins are members of the conserved amino acid permease (AAP) family that includes 18 core members (André 1995; Nelissen et al. 1997; see Table 1 for names and presumed substrate specificities). Each of the AAP family members have defined substrate specificities and transport capacities. The genes encoding AAPs are differently regulated. Available data are generally consistent with the idea that high-capacity permeases are expressed under conditions where amino acids are needed to support growth. The high-capacity permeases, *AGP1*, *GAP1*, and *PUT4*, are nitrogen-regulated and become down-regulated at the transcriptional, as well as the post-translational level, in response to high quality nitrogen sources like ammonium (Courchesne and Magasanik 1983; Jauniaux et al. 1987; Jauniaux and Grenson 1990; Grenson 1992; Magasanik 1992; Daugherty et al. 1993; Stanbrough and Magasanik 1995; Schreve et al. 1998; Soussi-Boudekou and André

Table 1 The amino acid permease family

Gene[a]	Name	Gene product (substrate specificity)	aa induced	SPS[b] dependent	Reference
Cluster I					
YCL025c	*AGP1*	Broad substrate range, medium-capacity permease (val, ile, phe, met, ser, leu, thr, cys, asn, tyr, ala, gly, gln)[c]	Yes	Induced	Schreve et al. (1998); Iraqui et al. (1999)
YBR068c	*BAP2*	Branched-chain amino acid permease (val, ile, phe, tyr, leu, trp, met, cys, ala)[c]	Yes	Induced	Grauslund et al. (1995)
YDR046c	*BAP3*	Branched-chain amino acid permease (val, ile, phe, tyr, trp, leu, met,cys, thr, ala)[c]	Yes	Induced	Didion et al. (1998)
YDR508c	*GNP1*	High-affinity glutamine permease (thr, gln, ser, cys, leu, met, asn)[c]	Yes	Induced	Zhu et al. (1996)
YBR069c	*TAT1*	Tyrosine and tryptophan permease (val, thr)[c]	Yes	Induced	Schmidt et al. (1994)
YOL020w	*TAT2*	High-affinity tryptophan permease (phe, tyr, trp, gly, ala)[c]	Yes	Induced	Schmidt et al. (1994)
Cluster II					
YKR039w	*GAP1*	General, high-capacity, amino acid permease		Repressed	Jauniaux and Grenson (1990)
YGR191w	*HIP1*	Histidine permease			Tanaka and Fink (1985)
YLL061w	*MMP1*	High-affinity S-methyl methionine permease			Rouillon et al. (1999)
YPL274w	*SAM3*	High-affinity S-adenosyl methionine permease			Rouillon et al. (1999)
Cluster III					
YNL270c	*APL1*	Arginine permease			Sychrova and Chevallier (1994)
YEL063c	*CAN1*	Arginine permease			Hoffmann (1985)
YNL268w	*LYP1*	Lysine permease (lys, met)[c]			Sychrova and Chevallier (1993)
Unclustered					
YBR132c	*AGP2*	Carnitine transporter			Van Roermund et al. (1999)
YFL055w	*AGP3*	Broad substrate specificity amino acid permease (ser, asp, glu)[c]			Regenberg et al. (1999)
YPL265w	*DIP5*	Dicarboxylic amino acid permease (ser, ala, asn, asp, gln, gly, glu)[c]			Regenberg et al. (1998)
YOR348c	*PUT4*	High-affinity proline permease (val, ala, pro)[c]			Jauniaux et al. (1987)
YDR160w	*SSY1*	Amino acid sensor			(see text)

[a] Genes are clustered according to Nelissen et al. (1997)
[b] Amino acid induced and SPS dependence are according to Didion et al. 1998; Iraqui et al. 1999; Klasson et al. 1999; Forsberg and Ljungdahl 2001
[c] Substrate specificity as reported by Regenberg et al. (1999)

1999). The more specific permeases, with lower transport capacities, are thought not to be able to support growth when amino acids are the sole nitrogen source; and apparently these AAPs function to supply cells with amino acids for protein synthesis.

The *AGP1*, *GNP1*, *BAP2*, *BAP3*, *TAT1*, and *TAT2* permeases comprise a subset of more related AAPs (Table 1, Cluster I; Nelissen et al. 1997). It has been demonstrated that the expression of genes encoding the Cluster I permeases is induced by the presence of amino acids in the growth media (Didion et al. 1996, 1998; De Boer et al. 1998, 2000; Iraqui et al. 1999; Klasson et al. 1999). Amino acid induction of more distantly related AAP genes, i.e., *HIP1*, *GAP1* (Cluster II), *CAN1*, *ALP1*, *LYP1* (Cluster III), *DIP5*, and *PUT4* (Unclustered), has not been demonstrated. The induced expression of the cluster I permeases in response to the presence of extracellular amino acids requires the *SSY1*, *PTR3*, and *SSY5* gene products.

Mutations in *SSY1*, *PTR3*, and *SSY5* have been independently isolated in several laboratories, in genetic screens aimed at identifying components required for proper amino acid uptake (Barnes et al. 1998; Didion et al. 1998; Jørgensen et al. 1998; Iraqui et al. 1999; Klasson et al. 1999; Forsberg and Ljungdahl 2001). These genes have also been shown to be required for proper compartmentalization of basic amino acids in the vacuole (Klasson et al. 1999; Forsberg and Ljungdahl 2001). Additionally, *SSY1* and *PTR3* have been shown to be required to fully repress the expression of *GAP1* in cells grown on rich complex media, or in synthetic ammonium-based media in the presence of amino acids (Klasson et al. 1999). *PTR3* was earlier identified in a screen for mutations that abolish amino acid-induced stimulation of dipeptide transport that is mediated by the peptide transporter Ptr2p (Island et al. 1991). The role of Ptr3p in regulating peptide uptake in yeast has recently been reviewed (Hauser et al. 2001).

Ssy1p

Ssy1p is a unique member of the AAP family (Fig. 2A; Jørgensen et al. 1998). Ssy1p is comprised of 852 amino acids (aa); and the other 17 members of this family are comprised of 558–663 aa. The sequence of Ssy1p is 22–28% identical to the other members of the AAP family. The homology between Ssy1p and the other AAPs begins with aa 278 of Ssy1p and stretches throughout the remaining 574 aa. The extended N-terminus of Ssy1p contains three entirely unique regions, a total of 140 aa, that are not present in the other members of the permease gene family; and these regions are interspersed between three regions of weak homology. Additionally, the Ssy1p sequence contains an extra 25 aa and 33 aa in the hydrophilic loops between hydrophobic domains V–VI and VII–VIII, respectively. None of the extra domains present in Ssy1p share homology with other sequences in the databases. Within the region of homology (aa 278–852) there are 12 hydrophobic segments that may function as membrane-spanning segments. The in vivo topology of Gap1p has recently been determined: each of the 12 hydrophobic segments of Gap1p span the membrane and both the N- and C-terminal domains are cytoplasmically oriented (Gilstring and Ljungdahl 2000). Given the extensive sequence homology and similarity of hydrophobic profiles, the other members of the AAP family, including Ssy1p, are likely to have similar membrane topologies. Thus, the extended N-terminus of Ssy1p is expected to be oriented towards the cytoplasm.

The N-terminal extension of Ssy1p is essential for sensor function. Several small, in-frame mutations within the N-terminal domain, that do not significantly reduce levels of protein expression, abolish Ssy1p function (Klasson et al. 1999). Overproduction of the N-terminal domain interferes with Ssy1p function in a dominant, negative manner (Bernard and André 2001a; Forsberg and Ljungdahl 2001). This result suggests that the overproduced N-terminal domain competes with full-length Ssy1p for binding of an essential component required for signal transduction. Finally, a plasmid containing sequences encoding the N-terminal domain of Ssy1p under the control of the inducible *GAL1* promoter (pGAL-SSY1(1–276), is able to partially complement growth on selective galactose-containing media in an *AGP1*-dependent manner (Bernard and André 2001a). This latter finding suggests that the N-terminal domain of Ssy1p possesses the intrinsic ability to interact with other signaling components and propagate amino acid-induced signals.

SSY1 is very weakly expressed in comparison to other members of the AAP family (Didion et al. 1998; Iraqui et al. 1999; Klasson et al. 1999). The low levels of Ssy1p observed in cells is consistent with its low codon bias index (CBI) of 0.013, a value that is considerably lower than the CBIs of the other 17 permeases (average 0.208 ± 0.102, range 0.093–0.434; André 1995). Like the other characterized AAP family members, Ssy1p is situated in the PM and requires the action of the AAP-specific packaging chaperone Shr3p (Gilstring et al. 1999) to exit the ER (Klasson et al. 1999).

Ssy1p transduces signals regarding extracellular amino acids across the PM independently of amino acid uptake. This conclusion is based on three separate experiments. First, *SSY1*-dependent transcription was shown to be induced by D-leucine in a *gap1* null mutant strain that lacked measurable rates of D-amino acid uptake (Didion et al. 1998). Secondly, *ssy1* null mutant strains do not exhibit amino acid-induced transcription of an *AGP1-lacZ* reporter construct, despite the demonstrated ability to take up amino acids across the PM in vitro transport assays (Iraqui et al. 1999). The lack of transcriptional induction in *ssy1*-deleted strains is thus unlikely due to inducer exclusion. Finally, a mutation in *TRP2* ($trp2^{fbr}$) that causes accumulation of tryptophan in cells by abolishing feed-back inhibition of tryptophan synthesis, does not induce *AGP1-lacZ* transcription (Iraqui et al. 1999). Under these conditions, intracellular

tryptophan-sensitive *ARO80*-dependent transcription of *ARO9-lacZ* was greatly induced, even in the absence of exogenously added amino acids.

Ssy1p exhibits marked substrate (ligand) preferences (Iraqui et al. 1999). With the exception of arginine and proline, all amino acids can induce the expression of a *AGP1-lacZ* reporter construct in a Ssy1p-dependent manner, although the induction is variable. Non-polar amino acids (leucine, isoleucine, methionine, phenylalanine, tryptophan) and polar uncharged amino acids (tyrosine, threonine) are among the strongest inducers of *AGP1-lacZ* expression. Valine, cysteine, alanine, serine, and even citrulline induce intermediate levels of *AGP1-lacZ*. Recently, a mutant allele of *SSY1* (*ssy1-23*) was isolated that exhibits an altered response to amino acids (Bernard and André 2001a). Strains carrying the *ssy1-23* allele are no longer able to respond to phenylalanine, isoleucine, or methionine, but exhibit 50% of the wild-type leucine-inducible expression of *AGP1-lacZ*. The altered spectrum of induction suggests that Ssy1p indeed functions as a primary sensing component.

Ptr3p

The *PTR3* open reading frame encodes a protein of 678 aa, with a calculated molecular mass of 76.4 kDa (Barnes et al. 1998). Although Ptr3p is predicted to be a hydrophilic protein, it fractionates as a PM protein and localizes to the cytoplasmic face of the PM independently of Ssy1p (Klasson et al. 1999). A small region within the C-terminal portion of Ptr3p (aa 511–575) shares limited sequence homology with the transcription factor Gcn4p and AAPs (Klasson et al. 1999). The homologous region in AAPs begins with amino acids predicted to be at the end of transmembrane segment VII and terminates with amino acids at the end of transmembrane segment VIII. This region within AAPs includes the small hydrophilic loop between membrane spanning segments VII and VIII that is predicted to be exposed to the external extracellular environment.

Although the functional significance of the Gcn4p/Ptr3p/AAP homology is not clear, it is tempting to speculate that this region defines a substrate-binding site that allosterically affects regulatory events. Indirect evidence supporting this idea has been obtained in several laboratories. Mutations within the homologous region of the arginine specific AAP (*CAN1*) have been isolated that enable Can1p to transport citrulline (Regenberg 1999). Similarly, the mutation in the *ssy1-23* allele, that affects the capacity of Ssy1p to respond to amino acids, results in a Thr to Ile substitution at position 639 in Ssy1p (Bernard and André 2001a). Amino acid residue 639 is predicted to be located within the hydrophobic eighth membrane-spanning segment. Together, these findings strongly suggest this region affects amino acid recognition. Gcn4p is a transcription factor responsible for the transcription of amino acid metabolic genes in response to amino acid starvation (Hinnebusch 1988). Interestingly, the homologous region within Gcn4p has been shown to be a regulatory domain (Kornitzer et al. 1994; Meimoun et al. 2000). The stability of Gcn4p, like AAPs, is regulated by phosphorylation. In the presence of amino acids, Gcn4p is phosphorylated by the Pho85p protein kinase and is rapidly degraded. Mutational analysis has shown that Thr165, which is located within the conserved region and is one of the amino acids comprising a Pho85p target consensus site (serine or threonine followed by a proline), is required for proper degradation. Perhaps this region functions as an allosteric site that affects the ability of Pho85p to phosphorylate Gcn4p.

Ssy5p

SSY5 encodes a 76-kDa protein comprised of 687 aa that does not share significant sequence homology with other known proteins (Jørgensen et al. 1998). Similar to Ptr3p, Ssy5p is predicted to be a hydrophilic protein that lacks identifiable transmembrane domains and N-terminal ER-targeting signal sequences. However, Ssy5p fractionates as a peripherally associated membrane protein. Using the SOS membrane recruitment system, it has been shown that Ssy5p associates with the PM (Forsberg and Ljungdahl 2001). This experimental assay system exploits the ability of the human Cdc25p homologue, h-SOSp, to suppress the temperature-sensitive *cdc25-2* mutation in strain cdc25H (Petitjean et al. 1990). Fusion proteins that direct h-SOSp to the cytosolic face of the PM enable cdc25H cells to grow at 37 °C (Aronheim et al. 1994).

SPS sensor complex

Genetic and biochemical data suggest that Ssy1p, Ptr3p and Ssy5p are components of a sensor complex within the PM (Fig. 3). This sensing system has been called the SPS sensor after the names of the known components. Genetic and biochemical analysis indicates that cells require the presence of each component to form a functional sensor (Forsberg and Ljungdahl 2001). Four lines of evidence supporting the model of a SPS sensor complex are summarized in the next two paragraphs.

First, genetic analysis indicates that mutations in *SSY1*, *PTR3*, and *SSY5* belong to the same epistasis group. Null mutations in *PTR3* and *SSY5* give rise to identical phenotypes as null mutations in *SSY1*, including the inability of inducing AAP and *PTR2* transcription in response to amino acids (Barnes et al. 1998; Jørgensen et al. 1998; Iraqui et al. 1999; Klasson et al. 1999; Forsberg and Ljungdahl 2001). Moreover, combinations of *ssy1*, *ptr3*, and *ssy5* deletions (i.e., *ssy1*Δ *ptr3*Δ, *ssy1*Δ *ssy5*Δ, *ptr3*Δ *ssy5*Δ) exhibit identical growth phenotypes as individual single mutants (Forsberg and Ljungdahl 2001). Second, the overexpression of the N-terminal domain, or full-length Ssy1p, exerts a dominant, negative effect on sensor function, suggesting that

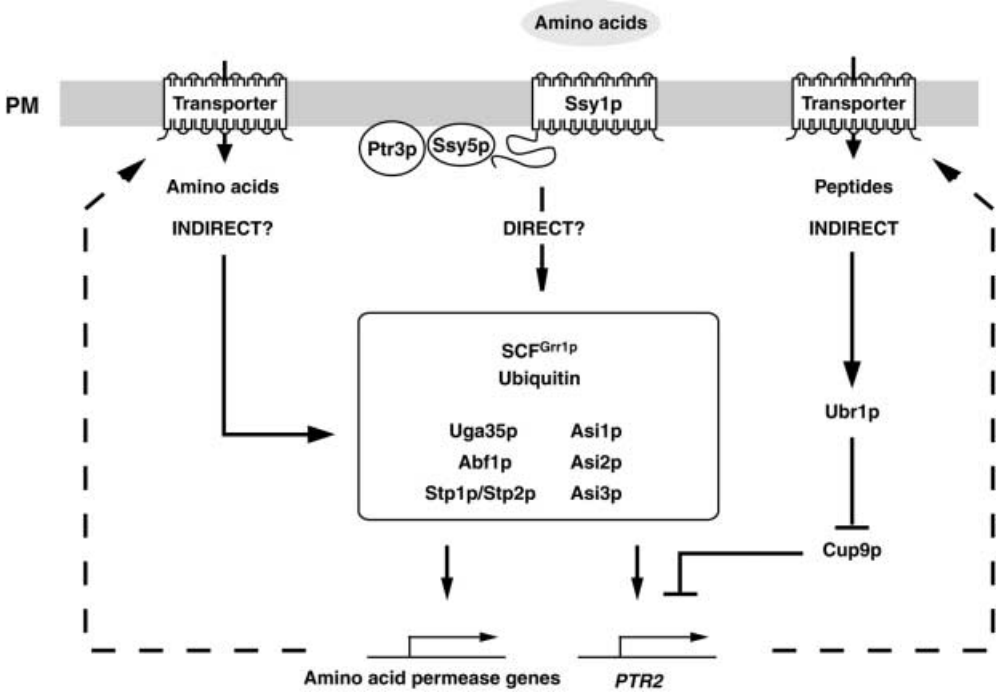

Fig. 3 Schematic model of SPS sensor- (Ssy1p/Ptr3p/Ssy5p)-dependent amino acid-induced signaling. The SPS sensor is localized to the plasma membrane (*PM*). *Solid arrows* represent signal transduction pathways (and actual amino acid or dipeptide uptake through transport systems) and *dashed arrows* reflect the post-transcriptional events leading to the functional expression of amino acid permeases and the peptide transporter (Ptr2p) in the PM. The terms *DIRECT* and *INDIRECT* refer to whether signals influencing the activity of downstream components are derived from the SPS sensor or from transported substrates, respectively. See text for further details

stoichiometric relationships are important (Forsberg and Ljungdahl 2001; Bernard and André 2001a). It is likely that overproduction of either the N-terminal domain or full-length Ssy1p leads to the formation of incomplete complexes with proteins that normally interact with Ssy1p. Such non-productive interactions would effectively decrease the availability of a limiting component to form functional sensor complexes.

Third, biochemical analysis indicates that all three components display distinct electrophoretic properties that are dependent upon both the presence of the other two components and the availability of amino acids (Forsberg and Ljungdahl 2001). Both Ssy1p and Ptr3p acquire distinct modifications in cells grown in the presence of amino acids, although the precise nature of the modifications have not been determined. In wild-type cells grown in the absence of amino acids, Ssy1p migrates as a high molecular mass, slower migrating form. In contrast, a faster migrating form of Ssy1p is observed in cells grown in the presence of amino acids. In mutant cells lacking either *PTR3* or *SSY5*, Ssy1p exhibits characteristics that mimic those of Ssy1p isolated from wild-type cells grown in the presence of amino acids. Similarly, Ptr3p migrates as several bands that exhibit significant differences in electrophoretic mobility. The appearance of a slower migrating Ptr3p species in amino acid-containing media is dependent on *SSY1* but not *SSY5*. The slower migrating forms of Ptr3p are observed in lysates isolated from *ssy5* null mutant cells grown in amino acid-free media. Thus, in these cells, Ptr3p exhibits characteristics identical to those of wild-type cells grown in the presence of amino acids. In wild-type cells, Ssy5p is proteolytically processed in a *PTR3*-dependent manner. In *ssy1* null mutant cells, N-terminally tagged Ssy5p is present at levels too low to detect. These latter two findings suggest that a Ptr3p-dependent processing event occurs within the C-terminal portion of Ssy5p and that, in the absence of Ssy1p, Ssy5p is subject to a proteolytic cleavage event that minimally removes its N-terminus. Finally, two-hybrid analysis demonstrates that the extended N-terminus of Ssy1p is capable of interacting with both Ssy5p (Madsen .L, Forsberg H, Kielland-Brandt M, Ljungdahl PO, unpublished data) and Ptr3p (unpublished data described in Hauser et al. 2001). Additionally, Ptr3p interacts with itself and Ssy5p (Bernard and André 2001a).

Amino acid sensing is a dynamic process

When leucine is added to wild-type cells grown in media without supplementary amino acids, the transcription of *PTR2* and *CAR1* is transiently induced (Forsberg and Ljungdahl 2001). The response is quite rapid and within 10 min there is a 15-fold induction of *PTR2* and a 2-fold induction of *CAR1*. After reaching maximum levels (*PTR2* within 30 min, *CAR1* within 10 min), their transcript levels slowly adjust back to basal levels. Similar patterns of induction have been reported for the branched-chain amino acid permeases (*BAP2*, *BAP3*; De Boer et al. 2000). Concurrent with its effect on transcription, leucine stimulates each of the components of the SPS sensor to become physically modified and causes the levels of each sensor component to diminish (Forsberg and Ljungdahl 2001). Levels of Ssy5p diminish very rapidly, within minutes after amino acid addition, while Ssy1p and Ptr3p levels decrease more slowly. The rapid physical alterations and reduced levels of sensor components are consistent with their being

down-regulated in response to amino acid availability. It is important to note that, in cells grown in media supplemented with amino acids, the down-regulated sensor components are necessary to maintain the steady-state transcript levels of *AGP1* and *PTR2* (Forsberg and Ljungdahl 2001) and *GNP1* (Klasson et al. 1999).

Based on the properties of the SPS components observed in the absence of amino acids and the changes in electrophoretic properties and levels of immunodetectable SPS components that occur immediately following amino acid induction, three sensor states have been defined (Forsberg and Ljungdahl 2001). The state I sensor is the complex present in cells grown in the absence of amino acids, where each of the SPS components is present at a high level. In analogy to the G protein-coupled α-factor receptor complex in *MATa* cells (Dosil et al. 2000), the state I conformation may represent a pre-activation complex. State II is a series of transient complexes that are rapidly formed when cells grown in the absence of amino acids are induced by amino acids. The components in the transient state II sensor undergo dynamic changes in expression level. The state III conformation corresponds to a down-regulated complex, the components in this form being indistinguishable from those found in the complex of cells grown in media containing amino acids.

Downstream components of the SPS sensor

A complex network of regulatory processes is active to modify the expression of SPS target genes in a co-ordinated way (Fig. 3). *STP1* and *STP2* (Wang and Hopper 1988) and the global transcription factor *ABF1* (Della Seta et al. 1990) are required for amino acid induction of *BAP2* and *BAP3* (De Boer et al. 1998, 2000) and *AGP1* (Iraqui et al. 1999), respectively. Stp1p/Stp2p and Abf1p are thought to bind directly to the UASaa found in the promoter of *BAP3* (De Boer et al. 2000; Nielsen et al. 2001). The non-specific factor Uga35p is also required for *SSY1*-induced transcription of an *AGP1-lacZ* fusion (Iraqui et al. 1999). *UGA35* is involved in transcription of GABA and allophonate-induced genes (Iraqui et al. 1999). However, neither GABA nor allophonate are potent inducers of *SSY1*-dependent transcription; and therefore it is likely that *UGA35* is a common factor that is required for transcription induced by several pathways. Ubiquitin and components of the SCF-Grr1p complex are required for transcriptional induction of *AGP1-lacZ* and *PTR2-lacZ* constructs, in response to amino acids (Iraqui et al. 1999; Bernard and André 2001b). SCF (Skp1-Cdc53p/Cullin-F-box) complexes act as E3 ligases that link ubiquitin to various substrates (reviewed in Willems et al. 1999). This indicates that ubiquitin-mediated degradation or modification of proteins is required to transmit *SSY1*-derived signals to regulate transcription.

In a genetic selection for amino acid sensor-independent (*ASI*) genes, mutations in three novel genes were isolated that constitutively derepress the transcription of several SPS-controlled genes (Forsberg et al. 2001b). Null mutations in *ASI1*, *ASI2*, and *ASI3* give rise to identical phenotypes. *ASI1*, *ASI2*, and *ASI3* encode novel polytopic membrane proteins that reside in the ER (Zargari A, Ljungdahl PO, unpublished data). Asi1p and Asi3p are homologous proteins with five membrane-spanning segments and conserved ubiquitin ligase-like RING domains at their extreme C-termini. The RING domains are cytoplasmically oriented and are required for function. Asi2p contains three hydrophobic domains and a long, hydrophilic, lumenally oriented N-terminal domain. The finding that cells lacking Asi1p, Asi2p, or Asi3p exhibit constitutively derepressed expression of SPS target genes suggests that these proteins, likely in concert with one another, regulate the activity of a transcription factor, presumably via ubiquitin-mediated processing.

An additional ubiquitin-dependent system has been shown to be required for amino acid-induced expression of *PTR2*. Mutations in *PTR3* were isolated in a selection for mutants deficient in peptide transport and were characterized by their ability to confer resistance to toxic peptides (Island et al. 1991). Studies have shown that *PTR3*-mediated induction of *PTR2* requires Ubr1p-(Ptr1p)-mediated degradation of the transcriptional repressor Cup9p (Barnes et al. 1998; Byrd et al. 1998; Turner et al. 2000). Ubr1p is an E3 ubiquitin ligase that functions in the N-end rule pathway, a pathway that targets proteins containing a destabilizing amino acid at their N-termini for degradation (Bachmair et al. 1986; Varshavsky 1996). The action of Ubr1p/Cup9p, in collaboration with the ubiquitin-conjugating enzymes Ubc2 and Ubc4, provides a positive feed-back loop in the regulation of peptide uptake (Turner et al. 2000). When *PTR2* is expressed, short peptides (di- and tripeptides) are transported into the cell. Peptides containing N-terminal amino acids that are recognized according to the N-rule as destabilizing allosterically activate Ubr1p, which in turn is responsible for increased the degradation of Cup9p. Decreased amounts of Cup9p, which acts as a repressor of *PTR2* (Varshavsky 1996; Byrd et al. 1998; Turner et al. 2000), gives rise to increased peptide transport activity. This is the first clear physiological function of the N-end rule pathway in yeast and is the first reported example of a direct link between the activity of an E3 enzyme (Ubr1p) and environmental signals.

Based on the analysis of di-peptide uptake, it is clear that SPS sensor-induced signals become integrated with other nutritionally derived signals to regulate transcription. For example, although Ubr1p and Cup9p are absolutely required for *PTR2* transcription, they have little effect on the expression of *BAP2* (Alagramam et al. 1995) or *AGP1-lacZ* (Bernard and André 2001a). These results suggest that SPS induction of Ptr2p overcomes the repressing activity of Cup1p to provide an initial stimulation of di-peptide uptake. Once di-peptides are taken up and gain access to the allosteric-binding sites

present in Ubr1p, Cup1p is degraded, leading to high levels of *PTR2* expression.

SPS sensor-derived signals affect the transcription of many non-AAP genes

The SPS sensor affects the transcription of a number of genes in addition to genes encoding AAPs. The non-specific induction of *CAR1* was the first example of a non-transporter gene regulated by the SPS sensor (Klasson et al. 1999). Subsequently, using genome-wide transcriptional analysis that compared the patterns of gene expression in prototrophic wild-type and *ssy1* null mutant strains, it has been shown that the previously identified genes (e.g., *AGP1*, *BAP3*, *GNP1*, *TAT2*, *PTR2*) represent only a subset of the full spectrum of SPS-dependent genes (Forsberg et al. 2001a). Several genes encoding enzymes in amino acid biosynthetic pathways were found to be modulated by the addition of amino acids in a SPS sensor-dependent manner. The pathways affected include the branched-chain, lysine and arginine, and the sulfur amino acid biosynthetic pathways. Additionally, the proper transcription of particular nitrogen-regulated genes, including *GDH2* and several *DAL* genes, and the well characterized GATA transcription factors *NIL1* and *DAL80*, was shown to be dependent upon SPS sensor function. Although the existing transcriptional profile data does not discriminate between primary and secondary effects of amino acid signaling, it clearly demonstrates that yeast cells require the SPS amino acid sensor to properly adjust diverse cellular metabolic processes and it underscores the importance of PM nutritional sensors. An upstream activating sequence (UASaa) in the promoter of *BAP3* that is required for amino acid-induced transcription has been defined (De Boer et al. 1998, 2000). Further analysis of promoter sequences of Ssy1p controlled genes may provide a consensus for amino acid-induced transcription and allow mapping of new putative *SSY1* targets.

Significantly, transcriptional profile analysis revealed that the expression of numerous genes requires SPS sensor function independently of amino acid addition. For example, the expression of the nitrogen-regulated amino acid permeases *CAN1* and *DIP5* in amino acid-free media requires the presence of *SSY1*. Additionally, *ssy1*-null mutant cells do not properly express various enzymes involved in lysine and methionine biosynthesis, in amino acid-free media. The amino acid-independent effects may be explained by two models. In the first model, one or more of the SPS sensor components, or down-stream signal transducing components, interact with other cellular signaling pathways. In this cross-talk model, the mere disruption of the sensor complex could lead to an abnormal distribution/binding of signaling molecules to other pathway components, thereby inducing abnormal transcriptional responses. Alternatively, Ssy1p recognizes and is able to discriminate other compounds present in minimal media, perhaps secondary nitrogenous metabolites excreted during growth. According to this second model, the SPS sensor may enable cells to autoregulate their metabolism. The ability of yeast to autoregulate metabolism in response to carbon utilization during alcoholic fermentations is a well documented phenomenon.

SPS sensor components are conserved in other fungi

Homology searches in available databases reveal several homologues of the *S. cerevisiae* SPS sensor components encoded in the genomes of other fungi (Souciet et al. 2000). Ssy1p homologues are found in *S. bayanus* (72% identity) and *Zygosaccharomyces rouxi* (67% identity); and, importantly, the N-terminal regions of these homologues share a high degree of sequence conservation. *S. bayanus* also has a Ssy5p homologue (80% identity) and *Z. rouxi* has a Ptr3p homologue (63% identity). Additionally, BLAST searches have identified homologues of the SPS sensor components in *Candida albicans*. The Ptr3p and Ssy5p homologues in *C. albicans* exhibit 33% and 40% identity to Ptr3p and Ssy5p, respectively, indicating that a SPS-like sensing system exists in this pathogenic fungi. Since the SPS-system affects filamentous growth of haploids (Klasson et al. 1999), it will be of value to examine whether these components have a role during invasion by *C. albicans* in pathogenesis. Mutant strains of *C. albicans* that are unable to form filaments are avirulent (Lo et al. 1997).

Mep2p, a sensor of extracellular ammonium

Uptake of ammonium ions (NH_4^+) is mediated by the *MEP* gene products (Dubois and Grenson 1979). The *MEP* gene family consists of three members; *MEP1*, *MEP2*, and *MEP3* (Table 2). Mep1p and Mep3p are highly homologous ammonium transporters with 11 predicted membrane-spanning domains (Marini et al.

Table 2 The ammonium permease family

Gene	Name	Gene product	Reference
YGR121C	*MEP1*	Medium-affinity, high-capacity ammonium permease	Marini et al. (1994)
YNL142 W	*MEP2*	High-affinity, low-capacity ammonium permease, ammonium sensor	Marini et al. (1997); Lorenz and Heitman (1998)
YPR138C	*MEP3*	Low-affinity, high-capacity ammonium permease	Marini et al. (1997)

1994, 1997; Marini and André 2000). The yeast Mep proteins are related to the ammonium transporter RhAG, a Rhesus blood-group antigen, expressed on human erythroids (Marini and André 2000). Mep1p is considered a medium-affinity ammonium permease, while Mep3p has a lower affinity for ammonium, but a high transport capacity (Marini et al. 1994). The most divergent member of the *MEP* gene family, *MEP2*, encodes a high-affinity ammonium permease (Marini et al. 1997). Interestingly, Mep2 appears to be N-terminally glycosylated, while Mep1p and Mep3p are not (Marini and André 2000; Fig. 2B). Based upon the relatively high CBI value associated with the Mep2p sequence (0.281), it is predicted that Mep2p is expressed at higher levels than either Mep1p (CBI = 0.081) or Mep3p (CBI = 0.145).

In addition to its role in ammonium uptake, Mep2p has been suggested to act as an ammonium sensor that generates signals for the induction of diploid pseudohyphal growth on limited ammonium sources (Lorenz and Heitman 1998). It was shown that colonies of *mep2*Δ/*mep2*Δ diploid cells are unable to form filaments under nitrogen-limited conditions, whereas *mep1*Δ/*mep1*Δ and *mep3*Δ/*mep3*Δ strains do. Mep2p is thought to regulate the formation of filaments by acting upstream of *GPA2* and *RAS2* (Kübler et al. 1997; Lorenz and Heitman 1997, 1998). *GPA2* and *RAS2* are thought to modulate levels of cAMP to induce pseudohyphal differentiation upon *MEP2* activation. (For a more detailed discussion on the role of *GPA2*, see the following section on the G protein-coupled receptor, Gpr1p.) The mechanism by which Mep2p-derived signals are transmitted to down-stream components has not been elucidated. However, Lorenz and Heitman (1998) introduced the first intracellular loop of Mep2p into Mep1p; and this hybrid protein partially restored the ability of *mep2* null mutants to undergo dimorphic transitions, leading to pseudohyphal growth. This result implies this sequence is important for signal transduction.

Sensors of extracellular carbon sources

Snf3p and Rgt2p are sensors of extracellular glucose

Glucose serves as a high-energy carbon source for yeast cells. Glucose, like other hexoses, is transported into cells by sugar transporters situated in the PM. The hexose transporter family comprises a sub-group of the sugar transporter-related genes (Kruckeberg 1996). The hexose-transporter genes include *HXT1–HXT17*, *GAL2*, *SNF3*, and *RGT2* (Table 3). *HXT1–HXT17* encode highly homologous hexose-transporter proteins with 12 putative transmembrane domains. Characterized hexose transporters display different affinities for sugar and different expression patterns in response to different glucose levels (for a review, see Boles and Hollenberg 1997). The existence of multiple high- and low-affinity hexose transporters enables cells to adjust glucose uptake in response to prevailing conditions to optimize cell growth and metabolism.

SNF3 and *RGT2* encode unique members of the hexose-transporter family (Celenza et al. 1988; Özcan et al. 1996a; Fig. 2C). Snf3p and Rgt2p differ from the other members of the hexose-transporter family in that they carry significant extensions in their C-termini: 303 aa in Snf3p and 218 aa in Rgt2p. Rgt2p also has a somewhat extended N-terminal domain. Based on structural analysis of the related mammalian transporter Glut1p, both the N- and C-termini of hexose transporters, as well as Snf3p and Rgt2p, are thought to face the cytoplasmic side of the PM (Özcan et al. 1996a; Mueckler and Makepeace 1999). Mutations in *snf3* were originally isolated from sucrose non-fermenting strains (Neigeborn and Carlson 1984). Preliminary observations indicated that Snf3p was a high-affinity glucose transporter (Bisson et al. 1987) but, according to kinetic data (Coons et al. 1995), the effects of *SNF3* on glucose transport could not be explained by the contribution of only one functional transporter, but rather several high- and low-affinity uptake systems. Dominant mutations in *RGT2* were recovered as suppressors of *snf3* deficiency (Marshall-Carlson et al. 1991). Accumulating data support the notion that rather than being transporters, Snf3p and Rgt2p act as a sensors of extra-cellular glucose to regulate expression of *HXT* genes. The ability of Snf3p and Rgt2p to act as glucose sensors have been the subject of several recent reviews (see Boles and Hollenberg 1997; Kruckeberg et al. 1998; Johnston 1999; Özcan and Johnston 1999; Rolland et al. 2001b) and we have therefore chosen to restrict our discussion to the mechanisms connected with signal transduction.

The assumption that Snf3p and Rgt2p act as glucose sensors is based on the following observations. First, *SNF3* and *RGT2* are very weakly expressed, compared to other genes in the *HXT* family (Neigeborn et al. 1986; Özcan et al. 1996a). Similar to Ssy1p, the observed low expression levels of Snf3p and Rgt2p correlate well with their lower CBI values, as compared with the other 18 hexose transporters (Iraqui et al. 1999). *RGT2* is constitutively expressed at low levels, whereas *SNF3* is repressed in response to increasing levels of glucose. Second, *SNF3* and *RGT2* are required for the proper expression of other members of the *HXT* gene family. While *SNF3* is required for the expression of a number of *HXT* genes in the presence of low levels of glucose, *RGT2* has only been shown to be needed for the induction of *HXT1* on high levels of glucose (Liang and Gaber 1996; Özcan et al. 1996a; Coons et al. 1997; Table 3). *SNF3* is also required for glucose repression of the high affinity transporters *HXT2*, *HXT6*, and *HXT7* in the presence of high concentrations of glucose (Liang and Gaber 1996; Vagnoli and Bisson 1998). A third finding that strongly supports the notion that Snf3p and Rgt2p act as sensors was the isolation of the dominant active *RGT2-1* allele (Marshall-Carlson et al. 1991). This dominant mutation in *RGT2* (*RGT2-1*), or the same mutation introduced into *SNF3* (*SNF3-1*), leads to

Table 3 The hexose transporter family

Gene	Name	Gene product	Sensor-dependent glucose induction	References
YHR094c	*HXT1*	Low-affinity glucose transporter	*RGT2* (high glucose)	Lewis and Bisson (1991); Özcan and Johnston (1995); Özcan et al. (1996a); Reifenberger et al. (1997)
YMR011w	*HXT2*	High-affinity glucose transporter	*SNF3* (low glucose)[a]	Kruckeberg and Bisson (1990); Özcan and Johnston (1995); Reifenberger et al. (1997)
YDR345c	*HXT3*	Low-affinity glucose transporter	*SNF3* (low glucose)	Özcan and Johnston (1995); Reifenberger et al. (1997)
YHR092c	*HXT4*	Moderate-affinity glucose transporter	*SNF3* (low glucose)	Theodoris et al. (1994); Özcan and Johnston (1995); Reifenberger et al. (1997)
YHR096c	*HXT5*	n.d.		Reifenberger et al. (1995)
YDR343c	*HXT6*	High-affinity glucose transporter	*SNF3* (low glucose)[a]	Reifenberger et al. (1995, 1997); Liang and Gaber 1996
YDR342c	*HXT7*	High-affinity glucose transporter	*SNF3* (low glucose)[a]	Reifenberger et al. (1995, 1997)
YJL214w	*HXT8*	n.d.[b]		
YJL219w	*HXT9*	n.d.		
YFL011w	*HXT10*	n.d.		
YOL156w	*HXT11*	Complements low-affinity uptake defect of *rag1* mutations in *Kluyveromyces lactis*		Nourani et al. (1997)
YIL170/1w	*HXT12*	Pseudogene?		Kruckeberg (1996)
YEL069c	*HXT13*	n.d.		
YNL318c	*HXT14*	n.d.		
YDL245c	*HXT15*	n.d.		
YJR158w	*HXT16*	n.d.		
YNR072w	*HXT17*	n.d.		
YLL081w	*GAL2*	Galactose transporter		Tschopp et al. (1986); Ramos et al. (1989)
YDL194w	*SNF3*	Sensor of low glucose		(See text)
YDL138w	*RGT2*	Sensor of high glucose		(See text)

[a] *HXT2*, *6* and *7* require *SNF3* to be glucose-repressed on high levels of glucose (Liang and Gaber 1996; Vagnoli and Bisson 1998)
[b] *n.d.* Not determined

constitutive expression of *HXT* genes (Özcan et al. 1996a) and the constitutive proteolysis of maltose permease (Jiang et al. 1997), even in the absence of glucose. This mutation results in a single arginine to lysine substitution within the hydrophilic, cytoplasmically oriented loop between hydrophobic regions IV and V of Rgt2p and Snf3p (Özcan et al. 1996a). It has been suggested that the arginine to lysine substitution induces these the sensors to attain their "glucose-bound" conformation. Importantly, these results show that signals may be induced by mutant Snf3p and Rgt2p without glucose entering the cell, thus establishing these proteins as actual sensors rather than importers of an inducer. Consistent with this interpretation, Snf3p does not transport sufficient amounts of glucose to support growth of glucose transport-deficient strains carrying null alleles of seven *HXTs* (*hxt1–hxt7*; Reifenberger et al. 1995), even when *SNF3* is overexpressed (Liang and Gaber 1996).

The C-termini of Snf3p and Rgt2p do not share significant homology to other yeast proteins and are quite dissimilar, except for a stretch of 17 aa (15/17 aa identical). This amino acid sequence occurs once in Rgt2p and twice in Snf3p and contains putative sites for casein kinase II, as well as a nucleotide-binding site (Özcan et al. 1996a). The actual significance and function of this sequence has not been established. However, the overexpression of the extended C-terminus of Snf3p, lacking any hydrophobic domains, is sufficient to partly suppress *snf3*Δ-related growth phenotypes (Coons et al. 1997). Chimeric proteins with the C-terminus of Snf3p fused to the hexose transporters Hxt1p or Hxt2p have been shown to partially restore signaling and induce *HXT* expression in *snf3 rgt2* double-mutant cells (Özcan et al. 1998), although in parallel experiments the overexpression of either *HXT1* or *HXT2* did not exert transcriptional effects. Additionally, removal of the C-terminal extensions of the constitutive *SNF3-1*- and *RGT2-1*-activated alleles diminishes signaling. Together, these results indicate the unique C-terminal domains of Sf3p and Rgt2p play important roles in glucose sensing.

Downstream components of Snf3p and Rgt2p

Several components required to transduce Snf3p- and Rgt2p-initiated signals have been identified (Fig. 4). *STD1* and *MTH1* influence the expression of glucose-

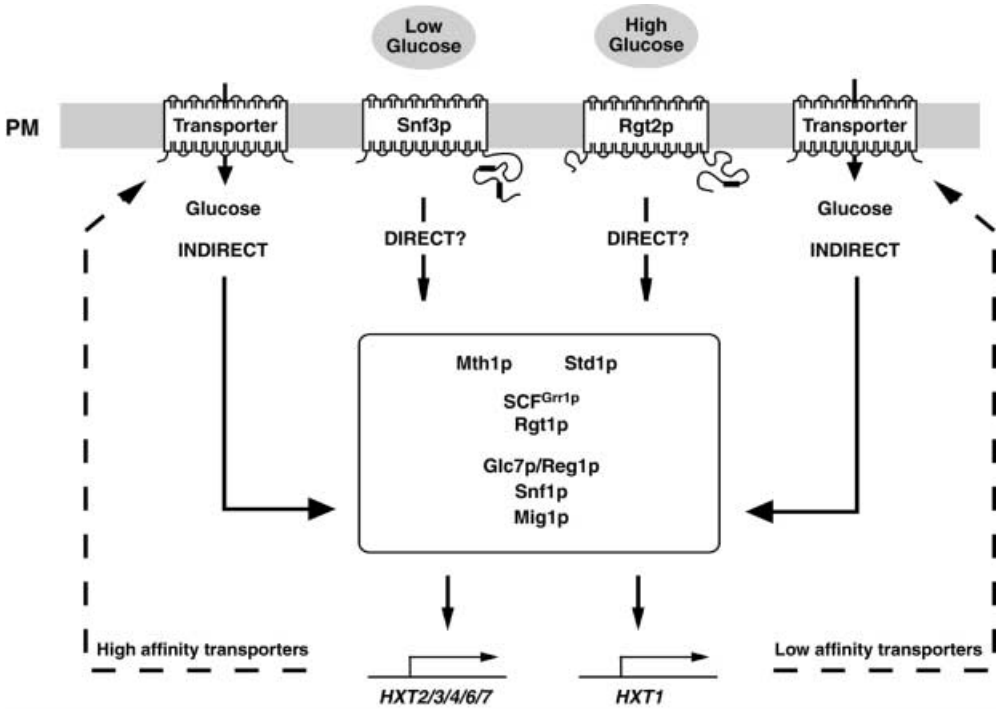

Fig. 4 Schematic model of Snf3p/Rgt2p-dependent glucose-induced signaling. The glucose sensor are localized to the plasma membrane (*PM*). *Solid arrows* represent signal transduction pathways (and actual glucose uptake through transport systems) and dashed arrows reflect the post-transcriptional events leading to the functional expression of high- and low-affinity hexose transporter in the PM. The terms *DIRECT* and *INDIRECT* refer to whether signals influencing the activity of downstream components are derived from the Snf3p and Rgt2p sensors, or from internalized glucose, respectively. See text for further details

sensitive genes (Ganster et al. 1993; Özcan et al. 1993; Gamo et al. 1994; Hubbard et al. 1994; Blazquez et al. 1995) and encode homologous proteins that share 61% amino acid identity. Two-hybrid approaches have shown that Std1p and Mth1p are able to interact with the C-termini of both Snf3p and Rgt2p (Schmidt et al. 1999; Lafuente et al. 2000). Although it is unknown how Std1p and Mth1p affect Snf3p- and Rgt2p-mediated signaling, genetic data suggest these proteins exert antagonistic effects. Mutations in *STD1* lead to an increased expression of glucose transporters, primarily on low glucose, indicating that Std1p may act as a repressor of *HXT* expression. Additionally, Std1p has been shown to be able to interact with the Snf1p protein kinase in two-hybrid experiments; and thus Std1p may affect the activity of Snf1p in the repression of *HXT*s (*HXT1*; Hubbard et al. 1994; Schmidt et al. 1999). Similarly, mutations in *MTH1* result in increased expression of *HXT*s. Additionally, dominant mutations in *MTH1* have been isolated that block Snf3p/Rgt2p-dependent induction of *HXT* transcription, leading to decreased glucose uptake (Özcan et al. 1993; Gamo et al. 1994; Blazquez et al. 1995; Lafuente et al. 2000; Schulte et al. 2000). *MTH1* repression is thought to be independent of Snf1p function (Schmidt et al. 1999). Thus, Std1p and Mth1p seem to have over-lapping, yet distinct functions in modulating the expression of glucose-regulated genes.

The expression patterns of *STD1* and *MTH1* resemble that of *SNF3* and *RGT2*, respectively, in that *STD1* becomes repressed by glucose and *MTH1* is constantly expressed, irrespective of glucose concentration. According to a model presented in Schmidt et al. (1999), *STD1* may act downstream of *RGT2* in modulating transcription by activating the Snf1p kinase under conditions of no or low concentrations of glucose, while *MTH1* modulates the transcription of down-stream targets of *SNF3* under non-signaling conditions. Std1p has been localized both to the cell periphery and to the nucleus (Schmidt et al. 1999), suggesting that it could be involved in transmitting information from the cell surface to the site of transcription. Interestingly, *STD1* (also known as *MSN3*) was isolated as a high-copy suppressor of a deletion in the TATA-binding protein (TBP). TBP and Std1p have been shown to physically interact using two-hybrid and in vitro binding approaches (Tillman et al. 1995). These results suggest that Std1p modulates transcription by directly binding TBP in the nucleus.

Similar to the SPS sensor-initiated signals, the induction of *HXT*s by Snf3p and Rgt2p requires the action of the SCF-F box protein Grr1p (Özcan and Johnston 1995; Özcan et al. 1996a; Jiang et al. 1997). Thus, ubiquitin-mediated processing, presumably mediating the degradation of a negative regulatory factor(s), is required for the transduction of Snf3p- and Rgt2p-initiated signals. The transcription factor Rgt1p is a likely candidate substrate for SCF^{Grr1p} modification. Rgt1p functions as a repressor by recruiting the general Ssn6p/Tup1p co-repressor complex to certain *HXT* promoter sequences (Özcan et al. 1996b). It has been postulated that in response to extracellular glucose, Snf3p- and Rgt2p-initiated signals facilitate the association of Rgt1p with SCF^{Grr1p} complexes, leading to the degradation of Rgt1p and thereby enabling *HXT* expression (Johnston 1999). Additionally, the inducing signals initiated by the Snf3p and Rgt2p sensors ultimately must overcome the well studied glucose-repressing mechanisms, including Glc7p/Reg1p, a putative phosphatase, the kinase Snf1p, and the

Mig1p repressor (for reviews on glucose repression see Gancedo 1998; Carlson 1999).

Snf3p and Rgt2p sensing components are conserved in other fungi

Homologues of Snf3p and Rgt2p exist in other fungi. *RAG4* encodes a Snf3p/Rgt2p homologue in *Kluveromyces lactis*. Interestingly, the C-terminus of Rag4p contains a single conserved Snf3p/Rgt2p C-terminal casein kinase/nucleotide-binding motif. Based on the degree of conservation, it is likely that Rag4p functions as a glucose sensor in *K. lactis*. Additionally, the *RCO3* gene of *Neurospora crassa* has been suggested to encode a sensor of high levels of glucose (Madi et al. 1994, 1997). Rco3p has 12 predicted membrane-spanning domains and a hydrophilic C-terminus comprised of approximately 120 aa. Rco3p is homologous to *S. cerevisiae* sugar transporters, exhibiting the highest degree of similarity with Snf3p and Rgt2p (Madi et al. 1997). Rco3p is 37% identical and 58% similar to Snf3p. The homology is limited to the major portion of the protein that includes the predicted membrane-spanning segments, the C-terminus is not homologous to Snf3p or other fungal transport proteins. *RCO3* is required for proper conidiation. Conidiation, the production of macronucleate spores, follows an initial rapid growth phase of *N. crassa* colonies occurring on minimal medium. Conidiation is accompanied by the onset of conidiation-specific genes and is repressed by rich nutrient sources. Mutations in *RCO3* interfere with both high- and low-affinity uptake of glucose, block the inhibition of conidiation by certain sugars and alter the expression of conidiation-specific genes. The assumption that Rco3p acts as a sensor, rather than a transporter of glucose, is based on the pleiotropic phenotypes displayed by a *rco3* mutant and on its homology to the glucose sensors of *S. cerevisiae*.

Sensing of fermentable carbon sources by the G protein-coupled receptor Gpr1p

GTP-binding protein-coupled receptors (GPCRs) in mammalian cells are known to mediate several cellular responses to ligands, including the neurotransmitters, epinephrine and dopamine (Peroutka 1994). GPCRs are situated in the PM and have the characteristic of seven transmembrane-spanning domains. In yeast, three receptors of this type have been characterized: Ste2p, Ste3p, and Gpr1p. Ste2p and Ste3p are pheromone-binding receptors responsible for inducing the expression of mating-specific genes in haploid cells in response to a- or α-factor, respectively. Upon ligand binding, GPCRs transduce signals via interactions with trimeric GTP-binding proteins, comprised of Gα, Gβ, and Gγ subunits. Gpr1p was recently isolated in several laboratories, based on its ability to bind the G-protein α-subunit Gpa2p in two-hybrid approaches (Xue et al. 1998; Yun et al. 1998; Kraakman et al. 1999). Gpa2p and the pheromone receptor-interacting protein, Gpa1p, are the only two α-subunits encoded by the yeast genome.

When high concentrations of glucose or sucrose (>100 mM) are added to cells growing on a non-fermentable carbon source, or to cells in stationary phase, a short burst of intra-cellular, cyclic AMP (cAMP) is produced. This transient rise in cAMP levels triggers a signal cascade that affects a number of physiological events, including trehalose and glycogen metabolism (Thevelein and de Winde 1999). Surprisingly, cells do not respond similarly to the presence of other fermentable sugars, e.g., fructose and mannose (Rolland et al. 2000). The glucose-stimulated increase in cAMP levels is independent of the presence of the glucose sensors Snf3p and Rgt2p (Rolland et al. 2000).

Studies have shown that the cellular response to high glucose levels requires Gpr1p and Gpa2p (Yun et al. 1998; Kraakman et al. 1999; Lorenz et al. 2000; Rolland et al. 2000). In addition to Gpr1p and Gpa2p, an as yet to be identified intracellular glucose sensor (that depends upon glucose uptake and phosphorylation) is an essential component of this response (Rolland et al. 2001a). The requirement for glucose uptake can be fulfilled by any of the hexose transporters, including Gal2p, or the intracellular hydrolysis of maltose. Based on a complex set of experimental observations, it has been proposed that the catalytic action of hexose kinase (Hxk2p) provides the intracellular signal that enables cAMP levels to rise in response to glucose stimulation (Rolland et al. 2001a). Rgs2p, a membrane-associated protein, functions as a negative regulator of the Gpr1p–Gpa2p signaling pathway (Versele et al. 1999). Rgs2p stimulates the intrinsic GTPase activity of Gpa2p in vitro, suggesting that Rgs2p functions as a GTPase-activating protein. In summary, despite the fact that the cognate Gβ and Gγ subunits have not been identified, available data suggest that Gpr1p and Gpa2p are part of a G protein-coupled sensing system that activates cAMP-regulated pathways in response to levels of extracellular glucose (for a recent review see Rolland et al. 2001b).

Interestingly, Gpr1p and Gpa2p are required for the induction of diploid and haploid filamentous growth (Yun et al. 1998; Ansari et al. 1999; Lorenz et al. 2000; Tamaki et al. 2000). Diploid PHG in response to nitrogen or amino acid limitation requires the presence of a high level of a fermentable carbon source (Lorenz et al. 2000). The expression of *GPR1* is elevated under conditions that favor PHG, i.e., media with limiting amounts of ammonium or amino acids (Xue et al. 1998). Both Mep2p and Gpr1p are required for accurate induction of PHG, although a clear relationship for filamentous control by these nutrient sensors remains to be established.

Gpr1p–Gpa2p related sensors may exist in other fungi. *GIT3* encodes a G protein-coupled receptor in *Schizosaccharomyces pombe* that is homologous to Gpr1p (Hoffman and Winston 1990; Welton and

Hoffman 2000). Git3p is required for glucose-triggered cAMP production and presumably functions via interactions with the *S. pombe* homologue of Gpa2p. Orthologues of Gpa2p have also been found in other fungi (e.g., *K. lactis*; see Welton and Hoffman 2000).

Nutritional sensors in other species

Although to date no precise homologues of the *Saccharomyces cerevisiae* nutritional sensors have been identified in bacteria, plants, or mammalian cells, several examples of nutritional sensors in these species have been reported.

Bacterial sensors

Bacteria contain transporter-related sensors of extracellular nutrients, e.g., the *bgl*-related sensors. BglF is a sugar permease in *Escherichia coli* that is member of the phosphoenolpyruvate-dependent phosphotransferase systems (Chen et al. 1997). BglF catalyzes the import and phosphorylation of β-glucosides. It has been shown that apart from β-glucosides, BglF has the ability to control the phosphorylation status of the transcriptional regulator BglG in response to β-glucoside availability. BglG is a transcriptional anti-terminator that binds RNA and allows transcription of the *bgl* operon in the presence of β-glucosides, resulting in the production of proteins required for β-glucoside utilization. Notably, BglF uses the same phosphoryl-donor site in phosphorylating the β-glucosides and BglG, two substrates that vary greatly in structure (Chen et al. 1997). Other putative BglF/BglG-related sensing systems are the SacX/SacY sucrose sensor of *E. coli* and the BglP/LicT β-glucoside sensor of *Bacillus subtilis* (discussed in Chen et al. 1997; Idelson and Amster-Choder 1998). The dual roles of these *bglF*/*bglG*-related sensors demonstrate that the sensing and transport are not mutually exclusive functions. In this respect, these bacterial sensors are similar to the Mep2p sensor, which catalyzes ammonium uptake into yeast cells and initiates nutritional signals that regulate filamentous growth.

Bacteria have a variety of two-component sensing systems that regulate bacterial gene expression in response to environmental changes (for a review see Hoch 2000). Two-component systems are composed of a sensor kinase, with a specific signal-recognition domain fused to an autokinase domain and a response element that activates transcription (or other possible enzymatic activities). The genomes of *E. coli* and *B. subtilis* encode several dozen two-component sensory systems. The sensor kinases display a wide diversity in size, structure, and ligand specificities (Fabret et al. 1999). The majority of sensor components carry two transmembrane domains with an intervening extracellular loop involved in substrate binding. The cytoplasmically oriented C-terminal domains carry the kinase moiety that interacts with down-stream elements. The majority of characterized two-component systems in bacteria are not essential; and null alleles of sensor components do not exhibit severe effects on growth (Fabret and Hoch 1998). Thus, these systems are likely to provide a growth advantage in relation to other organisms by enabling cells possessing them to rapidly alter gene expression in response to changes in the environment (Fabret et al. 1999). Several examples of two-component systems exist in plants; and a few exist in lower eukaryotes (Hoch 2000).

The glucose-6-phosphate-responsive *uhp* (upper hexose phosphate) system in *E. coli* is an atypical example of a two-component system that provides an additional example of a transporter-like sensor in bacteria (Wright et al. 2000). The *uhp* signaling system may provide important clues for understanding the SPS and Snf3p/Rgt2p sensing systems in yeast. The transmembrane histidine kinase component of the *uhp* system, UhpB, requires the presence of the sugar-6-phosphate transporter homologue UhpC, for transcriptional induction of the functional UhpT sugar-6-phosphate transporter. UhpC is thought to act as the signal receptor that is required for inducer responsiveness. Genetic data suggests that UhpB and UhpC proteins form a complex within the cell membrane. A fusion protein comprised of the cytoplasmic C-terminal domain of UhpB fused to glutathione S-transferase interferes with Uhp signaling by sequestering UhpA and inhibiting its ability to activate transcription. Together, these observations provide the basis for a model of *uhp* sensor function. In the absence of an inducing phospho-sugar, UhpC, UhpB, and UhpA are present at the cell membrane. Upon induction, UphC stimulates the UhpB kinase, leading to the phosphorylation of UhpA. The phosphorylated form of UhpA is ineffectively sequestered at the membrane and is thus able to initiate transcription by binding to the promoter of *uhpT*.

A cDNA clone of a mammalian homologue of UhpC/UhpT has been isolated, and it encodes the P46 protein that is mutated in glycogen storage disease type-1b (Gerin et al. 1997). Sequence analysis of conserved amino acid residues found using multiple sequence alignments suggests that the P46 protein may have roles in both transport and sensing of glucose-6-phosphate in mammalian cells (Mechin and van de Werve 2000). P46, along with the other components of the glucose-6-phosphate system that control the production of glucose from glucose-6-phosphate in gluconeogenesis, is expressed mostly in liver and kidney cells (Gerin et al. 1997).

Transporter-like sensors in multi-cellular organisms

LeSUT2 is a member of the plant sucrose transporter (SUT) family. Based on its similarity to the yeast glucose sensors Snf3p and Rgt2p, LeSUT2 has been suggested to function as a sensor of sucrose in the sieve elements of tomato leaves (Barker et al. 2000). LeSUT2 and its homologue in *Arabidopsis thaliana*, AtSUT2, are predicted

to be weakly expressed. LeSUT2 and AtSUT2 have extended hydrophilic loops of approximately 50 aa located between predicted transmembrane domains 6 and 7 (out of 12 predicted transmembrane regions). These loops are predicted to face the cytoplasm and contain two conserved boxes (CCB1, CCB2) that are conserved only in these two SUT2 homologues, and not in other members of the SUT family. In addition, LeSUT2 and AtSUT2 have extended N-terminal domains (approximately 30 aa, including a unique predicted helical region in the first 15 aa). AtSUT2 has been shown to be a low-affinity sucrose transporter and the N-terminal domain affects its affinity for sucrose (Schulze et al. 2000). Although it remains to be proven that SUT2 proteins participate in sensor functions that mediate regulatory signals in response to sucrose, it is interesting to note that the SUT2 gene in potato is tightly linked to the major quantitative trait (QTL) locus for tuber starch and yield located on chromosome V (Barker et al. 2000). This QTL has a pleiotropic effect on the control of tuber yield and starch content in potato, functions that are closely coupled to sucrose transport in plants.

It has been recently been suggested that GLUT2 transduces signals from the PM to the nucleus, to trigger glucose-regulated gene expression in man (Guillemain et al. 2000). GLUT2 mediates glucose transport in hepatocytes and pancreatic β-cells (Thorens et al. 1988). It is known that these cells stimulate gene expression in response to high levels of glucose (Guillemain et al. 2000). Members of the GLUT family are related to yeast glucose transporters in that they carry 12 predicted transmembrane domains. Of the *S. cerevisiae* hexose transporter family members, Snf3p is most similar to GLUT2. When over-expressed in the presence of endogenous GLUT2, a GFP-fusion construct containing the large hydrophilic loop between transmembrane regions VI and VII of GLUT2 modified glucose-regulated gene expression in a dominant negative manner (Guillemain et al. 2000). This hydrophilic loop, predicted to be cytoplasmically oriented, is not conserved in the other GLUT family members. The dominant negative effects on glucose-induced gene expression occurs independently of glucose metabolism into glycogen. Additionally, it was shown that approximately 50% of the expressed GFP–GLUT2 loop fusion protein rapidly translocated from the cytoplasm to the nucleus in response to high glucose levels and moved in the opposite direction during glucose deprivation. These observations suggest that the extended intracellular loop of GLUT2 may compete for the binding of protein(s) involved in a signaling pathway that regulates gene expression in response to glucose availability in hepatic and other cells.

Concluding remarks

In the wild, single-cell organisms like yeast and bacteria obviously depend on their ability to rapidly respond to constantly changing environments. This necessity is apparently reflected by the multitude of nutritional and other PM-localized sensors found in these organisms. It seems likely that sensors of extracellular nutrients provide the means for cells to monitor changes, both increases and decreases, in the concentration of certain key nutrients, so that they can adapt and achieve a competitive advantage. Indeed, in yeast, existing data support the notion that sensors of extracellular nutrients have important roles in adaptation. Amino acid-dependent transcriptional effects induced by the SPS sensor are more or less transient; and each of the sensor components becomes down-regulated shortly after induction by amino acids. In contrast to wild-type cells, *snf3* mutants adapt much more slowly to growing on media containing low levels of glucose and the respiration-inhibitor antimycin (Coons et al. 1997). However, *snf3* mutants grow well once they have adapted to these conditions and have appropriately modulated the expression of *HXT* genes. These results suggest that a predominant role for Snf3p may be to enable cells to rapidly adapt and adjust gene expression to immediate changes in external glucose concentrations. Gpr1p has been suggested to function in reprogramming cells for the transition from a respiratory to a fermenting mode of growth (discussed in Rolland et al. 2000). The transient, rather than long-lasting, effect on cAMP levels (Kraakman et al. 1999) also implies that the main function of Gpr1p is to enable cells to more efficiently adapt to new environmental conditions, i.e., high levels of glucose. Once cells have adapted, gene expression is likely to be controlled by sensors that monitor the intracellular status of metabolites. However, it should be noted that the extracellular nutrient sensors have been observed to affect transcription of genes during more constant nutritional states, so the role of these sensors in adaptation does not fully describe their function.

Although environmental nutrient sensors play similar roles, i.e., to transduce signals that reflect the existence of a nutrients in the extracellular environment, different organisms have evolved alternative solutions to transduce signals across the PM. The complex evolution of sensors is exemplified by the large and heterogeneous family of two-component systems in bacteria. Despite the obvious complexity, several common themes can be discerned. First, existing nutrient-binding moieties, e.g., nutrient-transport proteins, are found that contain extra domains that confer signal-transducing properties. The yeast sensing components Ssy1p, Snf3p/Rgt2p, and Mep2 belong to such transporter-like nutritional sensors and are likely to have evolved via the fortuitous gain of function mutations. Alternatively, diversity of nutrient sensors appears to have resulted from mutations that modify pre-existing sensing systems. The G protein-coupled receptor, Gpr1p, may be an example of such an altered function mutation.

The similarity between Ssy1p and Snf3p/Rgt2p is striking. These components are unique members of large families of metabolite-transporter proteins. All three components are weakly expressed and carry rather large

extensions (at the N-terminus of Ssy1p and at the C-termini of both Snf3p and Rgt2p) that are not present in the other members of their respective transporter families. The unique extensions are required for proper function and are likely to actively participate in transducing nutrient-based signals. These extended regions do not exhibit homology with characterized regulatory or signaling domains. The lack of homology suggests that signaling occurs by previously uncharacterized mechanisms, although common events, such as phosphorylation and ubiquitylation are likely to be involved. Although these sensing components exhibit similarities, the mechanisms that transduce nutrient-derived signals to the interior of the cell are apparently not conserved. Thus, further experimental analysis will be required to determine the precise nature of each of these novel signal-transduction pathways.

The fact that yeast PM nutrient sensors have only recently been discovered reveals how little is understood regarding the molecular signals that enable eukaryotic cells to adapt to changing environments; and many more novel discoveries can be expected. Although cells within multicellular organisms may not normally compete for nutrients, nutrient-sensing systems have been isolated in a variety of species, including man. In disease states, e.g., uncontrolled growth of cancer cells, competition for nutrients may actually affect the survival of cells in disease-free tissues. The knowledge gained from analyzing novel sensing systems in unicellular organisms may provide the means to better understand similar mechanisms controlling growth in metazoan cells.

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