Amino-acid-induced signalling via the SPS-sensing pathway in yeast

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Abstract

Yeast cells rely on the SPS-sensing pathway to respond to extracellular amino acids. This nutrient-induced signal transduction pathway regulates gene expression by controlling the activity of two redundant transcription factors: Stp1 and Stp2. These factors are synthesized as latent cytoplasmic proteins with N-terminal regulatory domains. Upon induction by extracellular amino acids, the plasma membrane SPS-sensor catalyses an endoproteolytic processing event that cleaves away the regulatory N-terminal domains. The shorter forms of Stp1 and Stp2 efficiently target to the nucleus, where they bind and activate transcription of selected genes encoding a subset of amino acid permeases that function at the plasma membrane to catalyse the transport of amino acids into cells. In the present article, the current understanding of events in the SPS-sensing pathway that enable external amino acids to induce their own uptake are reviewed with a focus on two key issues: (i) the maintenance of Stp1 and Stp2 latency in the absence of amino acid induction; and (ii) the amino-acid-induced SPS-sensor-mediated proteolytic cleavage of Stp1 and Stp2.

Extracellular amino acid sensing

A fundamental problem in biology is how cells respond to discrete environmental signals and make appropriate adjustments in the patterns of gene expression. Yeast and mammalian cells respond to the presence of low levels of extracellular amino acids by pleiotropically enhancing amino acid uptake, thus amino acids induce their own uptake. Although it remains unclear how this is achieved in mammalian cells, approx. 10 years ago, work in three laboratories independently converged, leading to the identification of a nutrient-sensing system in yeast that was responsive to extracellular amino acids [1–3]. In subsequent work, the primary sensor was found to be a plasma-membrane-localized complex comprising three core components, Ssy1, Ptr3 and Ssy5, and accordingly was dubbed the SPS-sensor [4]. The sensor and the downstream components, i.e. the SPS-sensing pathway, comprise a regulatory network that controls the amino-acid-uptake capacity of cells by inducing the transcription of genes encoding a subset of AAPs (amino acid permeases) (reviewed in [5–7]).

The SPS-sensor

Mutations in SSY1, PTR3 and SSY5 were isolated independently in several laboratories in genetic screens aimed at identifying components required for proper peptide [8] and amino acid uptake [1–4,9]. Ssy1 shares significant sequence homology with AAPs. However, Ssy1 is unique, as it has an extended cytoplasmically oriented N-terminal domain that is not present in the other AAP family members and does not catalyse measurable amino acid uptake [1,2,10]. Although Ssy1 has lost the ability to transport amino acids, it is thought that, in analogy to the canonical four-state model of transport, Ssy1 senses extracellular amino acids by undergoing ligand-dependent conformational shifts between an outward-facing (signalling) and an inward-facing (non-signalling) conformation [11].

The N-terminal domain of Ssy1 interacts physically with the other two core SPS-sensor components, the peripheral membrane proteins Ptr3 and Ssy5 [4]. PTR3 was originally identified in a screen for mutations that abolish the amino-acid-induced dipeptide transport mediated by the peptide transporter Ptr2 [12]. Sequence comparisons have identified a putative allosteric site [3] and five WD40 repeats [13] within the C-terminal portion of Ptr3. The significance of these sequence motifs has not been examined rigorously. SSY5 encodes a putative chymotrypsin-like serine protease that exhibits several hallmarks of a protease, including constitutive autoproteolyis of a Pro-domain, and a catalytic triad with a serine residue required for autolysis and transduction of amino-acid-inducing signals [14,15]. SSY1, PTR3 and SSY5 are required for proper compartmentalization of basic amino acids in the vacuole, a finding that first drew attention to these interesting genes [3,4].

Stp1 and Stp2

Stp1 and Stp2, two homologous zinc-finger transcription factors, are the only known downstream effectors of the SPS-sensor [16–18]. These factors exhibit overlapping and partially redundant functions, and both are synthesized as latent cytoplasmic proteins with 10 kDa N-terminal domains crucial for the regulation of their activity [18]. In
the absence of extracellular amino acids, Stp1 and Stp2 are diffusely localized throughout the cell and, importantly, do not accumulate in the nucleus [18–21]. In response to amino acids, these factors are activated by endoproteolytic removal of their N-terminal domains [18]. The shorter forms of Stp1 and Stp2 efficiently target to the nucleus, where they directly bind to specific UASaa (upstream activating sequences) present within promoters of SPS-sensor-regulated genes [16,17,22]. Stp1 and Stp2 target genes include AAP genes AGP1, BAP2, BAP3, DIP5, GNP1, MUP1, TAT1 and TAT2, and the peptide transporter gene PTR2.

Newly translated Stp1 and Stp2 are excluded from gaining access to SPS-sensor-regulated genes by two parallel activities that converge on the N-terminal regulatory domain (amino acids 1–125) [23]. This domain possesses two conserved sequence motifs, Region I and Region II, both of which are required to control the latent behaviour of Stp1. Region I (amino acids 16–35) has a high content of branched-chain amino acids and is required for cytoplasmic retention and promoter exclusion. The deletion of Region I (STP1Δ131), or mutations that replace branched-chain amino acids with alanine (STP1-133), interferes with cytoplasmic retention and results in constitutive derepression of SPS-sensor-regulated genes independently of processing. Region II (amino acids 65–97) is required for Stp1 and Stp2 binding to Ssy5 and is required for amino-acid-induced proteolytic processing. Mutations within Region II that abolish processing abrogate amino-acid-induced signalling. These motifs are modular and function when fused to bacterial DNA-binding protein LexA, conferring full SPS-sensor-controlled expression of lexA operator-controlled reporter genes [23].

The **SPS-sensing pathway**

Our current model for the SPS-sensor signalling pathway is presented schematically in Figure 1. In the absence of inducing amino acids (Figure 1A), the SPS-sensor is in its resting pre-activation conformation (red). The transcription factors Stp1 and Stp2 (DNA-binding motifs, green boxes) are synthesized as inactive precursors that localize to the cytosol due to the presence of N-terminal cytoplasmic retention/promoter exclusion domains (anchor) [23]. In the absence of the inner nuclear membrane Asi complex (Asi1–Asi2–Asi3, discussed below), low levels of full-length Stp1 and Stp2 that escape cytoplasmic retention (broken arrow) are able to derepress AAP gene expression [20,21]. Derepression of AAP gene expression that occurs when the Asi complex is mutationally inactivated requires the co-activator Dal81 [19]. The ability of the Asi complex to prevent transcription (red line) is dependent on the presence of the N-terminal regulatory sequences of Stp1 and Stp2. Together, these regulatory elements ensure low levels of AAP gene expression.

In the presence of inducing extracellular amino acids (Figure 1B), the SPS-sensor is activated (green), which unfetters the intrinsic proteolytic activity of Ssy5 [14,15], leading to the endoproteolytic processing of Stp1 and Stp2 (scissors) [18]. The shorter processed forms of Stp1 and Stp2, lacking the inhibitory N-terminal domains, efficiently target to the nucleus where they bind SPS-sensor-regulated promoters (UASaa) and induce transcription [18–20]. AAPs are cotranslationally inserted into the endoplasmic reticulum membrane, which is contiguous with the outer nuclear membrane. Movement of AAPs to the plasma membrane...
results in a conformational shift of the full complex from a
wild-type alleles of the other two components. These
signalling by a mutant allele depends on the presence of
role of phosphorylation, Ptr3 becomes hyperphosphorylated
SPS-sensor components are involved during the transduction
changes in the ubiquitination and phosphorylation status of
activity of either of the two type I yeast casein kinases Yck1
or Yck2 [14,23,26,27]. These latter findings suggest that
in a casein kinase-dependent manner [28]. Also, inactivation
ASI of the
promoters, in cells carrying inactivating mutations in any
only processed forms of Stp1 and Stp2 bind SPS-regulated
promoter access of unprocessed forms of Stp1 and Stp2
concerted action of three inner nuclear membrane proteins,
Stp1 and Stp2 in the cytoplasm are not absolute [23]. The
unusually large N-terminal Pro-domain and a C-terminal
chymotrypsin-like protease that is synthesized with an
SPS-sensor component Ssy5 [14,15]. Ssy5 is an atypical
signalling mechanisms in that it does not rely on modulating
19,21,31]. Clearly, if given the opportunity, latent forms of Stp1 and Stp2 can efficiently bind promoters and induce transcription [19–21]. These findings demonstrate that negative regulation of Stp1 and Stp2 activity is not limited to controlling cytoplasmic retention, and that cells require the Asi proteins to maintain the repressed state of signalling in the absence of inducing amino acids. Thus two independent mechanisms control the latent properties of Stp1 and Stp2, i.e. cytoplasmic retention that restricts nuclear targeting and Asi-dependent repression that restricts promoter access. Notably, both mechanisms exert their regulatory effects via the first 70 amino acids within the N-terminal regulatory domains of these factors [20].

Dal81 greatly augments the transactivation potential of Stp1 and Stp2 [19], and Dal81 is required for full induction of SPS-sensor-dependent AAP gene expression [2,22,32]. Without Dal81-dependent amplification, negative modulation by Asi1, Asi2 and Asi3 is not required to ensure the ‘off’ state of SPS-sensor-regulated gene expression [19]. In summary, the data regarding Asi protein function reveal a novel role of inner nuclear membrane proteins and illuminate an additional and unanticipated layer of transcriptional control in eukaryotic cells.

RAP (receptor-activated proteolysis)
The proteolytic processing event catalysed by the SPS-sensor has been dubbed receptor-activated proteolysis (RAP) [15]. RAP controls the catalytic activity of an intracellular protease by a plasma-membrane-localized receptor, and is distinguished from previously described protease-dependent signalling mechanisms in that it does not rely on modulating substrate accessibility. The RAP-controlled protease is the SPS-sensor component Ssy5 [14,15]. Ssy5 is an atypical chymotrypsin-like protease that is synthesized with an unusually large N-terminal Pro-domain and a C-terminal Cat- (catalytic) domain. During biogenesis, these domains are cleaved from each other via autolysis [14,15]. Strikingly, although not covalently attached, the Pro- and Cat-domains remain associated forming a catalytically competent but inactive protease [15]. The Pro-domain is required for the unfolding of the Cat-domain; individually expressed Pro- and Cat-domains do not reconstitute a functional protease when co-expressed [15]. The fact that autolysis does not suffice for Ssy5 activation indicates that RAP regulation involves a second and distinct SPS-sensor-dependent event.

Available data suggest that the second and activating event of RAP is the amino-acid-induced dissociation of the Pro-domain from the Cat-domain (Figure 2). This is based on the following observations. First, the manipulation of the Pro-domain by the addition of an epitope at the extreme N-terminus of Ssy5 (Ssy55) constitutively activates Stp1 and Stp2 processing [15]. This suggests that the Pro-domain influences the enzymatic activity of the Cat-domain. It is likely that the HA (haemagglutinin) tag fused to the N-terminus of the Pro-domain impairs its normal function, indicating that

Regulation of transcription factor latency
by the inner nuclear membrane Asi complex
The mechanisms responsible for retaining latent forms of Stp1 and Stp2 in the cytoplasm are not absolute [23]. The concerted action of three inner nuclear membrane proteins, Asi1, Asi2 and Asi3 (Figure 1A), is required to restrict promoter access of unprocessed forms of Stp1 and Stp2 that escape cytoplasmic retention and inappropriately enter the nucleus [20,21]. In contrast with wild-type cells, where only processed forms of Stp1 and Stp2 bind SPS-regulated promoters, in cells carrying inactivating mutations in any of the ASI genes, unprocessed latent forms of Stp1 and Stp2 bind promoters, resulting in constitutive activation of SPS-sensor-regulated genes even in the absence of amino acids or a functional SPS-sensor [20,21,31].

Critical to our understanding of the role of Asi proteins is the observation that Stp1 and Stp2 do not accumulate in the nucleus of asi mutant strains, thus eliminating the possibility that Asi proteins grossly affect cytoplasmic-retention mechanisms [19,20]. Remarkably, the low levels of full-length Stp1 and Stp2 that enter the nucleus of uninduced asi mutants, or asi mutants lacking a functional SPS-sensor, suffice to induce SPS-sensor gene expression at levels indistinguishable from those observed in induced wild-type cells [31].
Receptor-activated proteolysis

The Pro-domain of Ssy5 has an inhibiting activity. The constitutive nature of Ssy5 may simply be the consequence of a reduced affinity for the HA-tagged Pro-domain for the Cat-domain. Secondly, the analysis of the steady state levels of the Pro- and Cat-domains during an SPS-sensor induction time course demonstrated that Stp1 processing positively correlated with the release of the Cat-domain from its inhibitory association with the Pro-domain [15]. Consequently, it is likely that the Pro-domain has an inhibiting activity. The constitutive nature of Ssy5 may simply be the consequence of a reduced affinity for the HA-tagged Pro-domain for the Cat-domain. The catalytically competent Cat-domain (orange) is inhibited by the close association of the Pro-domain (red). The active Cat-domain (green) processes Stp1 and Stp2, releasing N-terminal regulatory domains, enabling the shorter form to efficiently enter the nucleus, bind relevant promoters and induce SPS-sensor regulated gene expression. See the text for a more detailed description.

Additionally, the Pro-domain of Ssy5 is uncharacteristically large (381 compared with 166 amino acids), which may be consistent with a role either in mediating interactions with substrates Stp1 and Stp2, to respond to amino-acid-induced signals, or a combination of these two activities.

The SPS-sensing pathway in the context of TOR (target of rapamycin) and other nutrient-based signals

Overlapping transcriptional regulatory networks contribute to the expression of several SPS-sensor-controlled genes. For example, the SPS-sensing pathway is independent of, but interconnected to, TOR-controlled signalling. Work in the André laboratory has clearly demonstrated that Stp1 binding to UASα motifs in the promoters of SPS-sensor-regulated genes is insensitive to the nitrogen status of the cell and to factors controlled by TOR, i.e. Gln3, Ure2 and Gz3 [22]. Thus TOR signalling does not directly affect SPS-sensor-mediated signalling, but nitrogen source availability does influence TOR activity, which in turn affects the magnitude of the transcriptional response [39]. An excellent example of this is the SPS-sensor and nitrogen-controlled permease AGP1. AGP1 expression is strictly dependent on extracellular amino acid and SPS-sensor induction; however, AGP1 transcript levels are substantially elevated in cells grown under conditions that do not elicit nitrogen catabolite repression [22,39]. Thus, in contrast with other permease genes responsive to Gln3 control (e.g. GAPI, DAL3 and MEP2), Gln3 is not able to transactivate AGP1 expression without the prior binding of processed Stp1 to the UASα element in its promoter.

The analysis of dipeptide uptake in yeast also provides a clear demonstration of how SPS-sensor-induced signals are integrated with other nutritionally regulated activities. Amino-acid-induced and SPS-sensor-mediated transactivation of dipeptide transporter PTR2 expression is a requisite for peptides to enter the cell. However, full induction of peptide uptake requires an additional activation step [40–42]. Peptides containing N-terminal amino acids recognized according to the N-rule as destabilizing [43] allosterically activate Ubr1, an E3 ubiquitin ligase, which in turn accelerates the degradation of the transcriptional repressor Cup9. Decreased levels of Cup9 gives rise to fully derepressed PTR2 expression and dipeptide transport. Although Ubr1 and Cup9 are absolutely required for full induction of PTR2 transcription, they do not affect PAGP1-lacZ [44] or BAP2 [45] expression. The full induction of the amino acid permease BAP2 requires Leu3, a transcription factor responsive to internalized leucine [46].

Finally, the finding that the constitutive nuclear factor Dal81 has an important and synergistic role in amplifying the induced expression of genes in several well-characterized nitrogen source utilization pathways, i.e. urea and allantoine, GABA (γ-aminobutyric acid) and SPS-sensor pathways is significant. In all of these pathways, Dal81 functions together with an inducer-specific transcription factor (Ugs3 [47–49], Dal82 [50] and Stp1 and Stp2 [2,19,22] respectively) to activate...
target genes via inducer-specific sequences. As pointed out by Abdel-Sater et al. [22], sharing of a co-activator raises the distinct possibility that signals derived from these different nitrogen sources become integrated by the hierarchical binding of the specific factors to Dal81. For example, amino-acid-induced targeting of activated forms of Stp1 and Stp2 is likely to preferentially recruit Dal81 to SPS-sensor-regulated promoters [19], enabling cells to take advantage of the availability of amino acids as preferred nitrogen sources.

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References

26 Bernard, F. and André, B. (2001) Ubiquitin and the SCFTIR ubiquitin ligase complex are involved in the signalling pathway activated by external amino acids in Saccharomyces cerevisiae. FEMS Lett. 204, 65-75

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