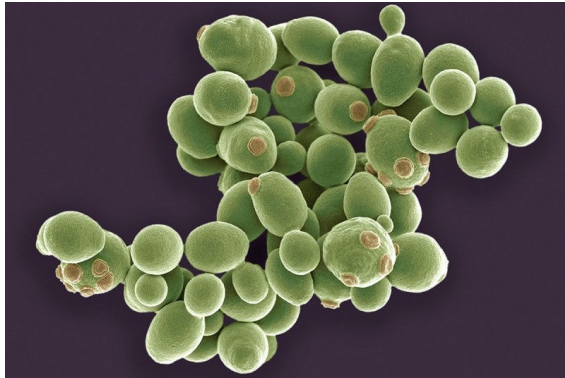




Saccharomyces cerevisiae

Transport and signaling in yeast

Bruno André



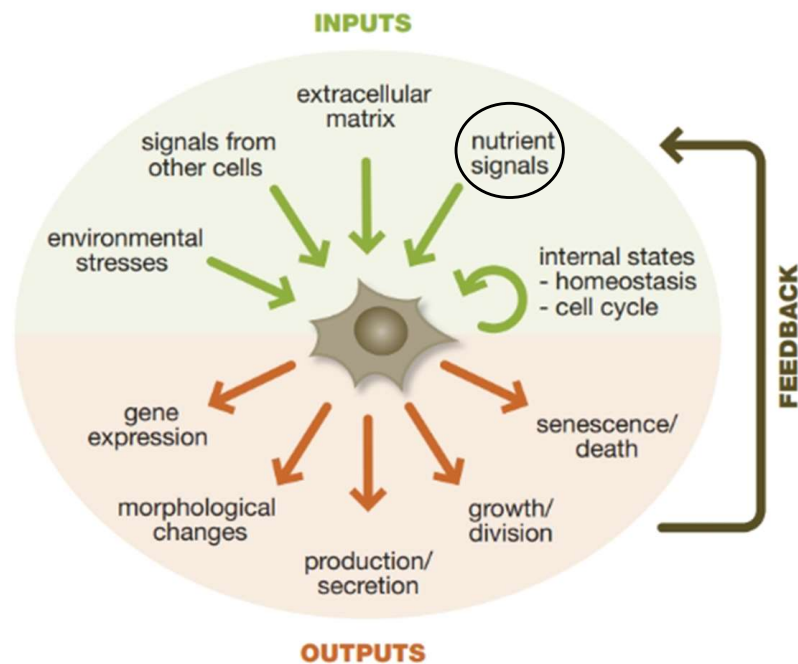
Université Libre de Bruxelles
(ULB)

Molecular Cell Physiology lab

Institute of Molecular Biology and
Medicine (IBMM)

Biopark Gosselies Belgium

ULB, 8 October 2026

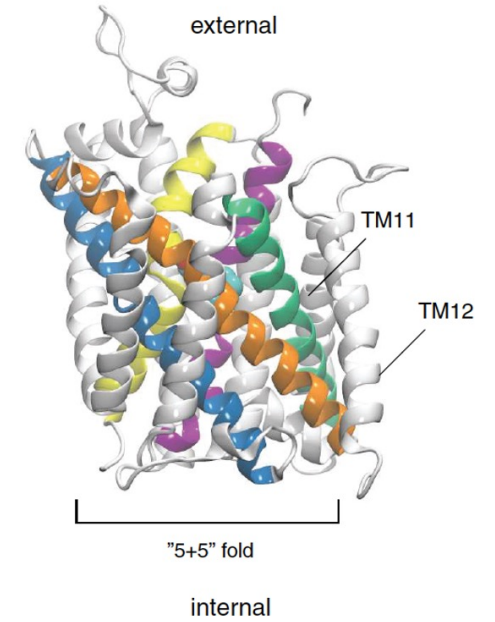
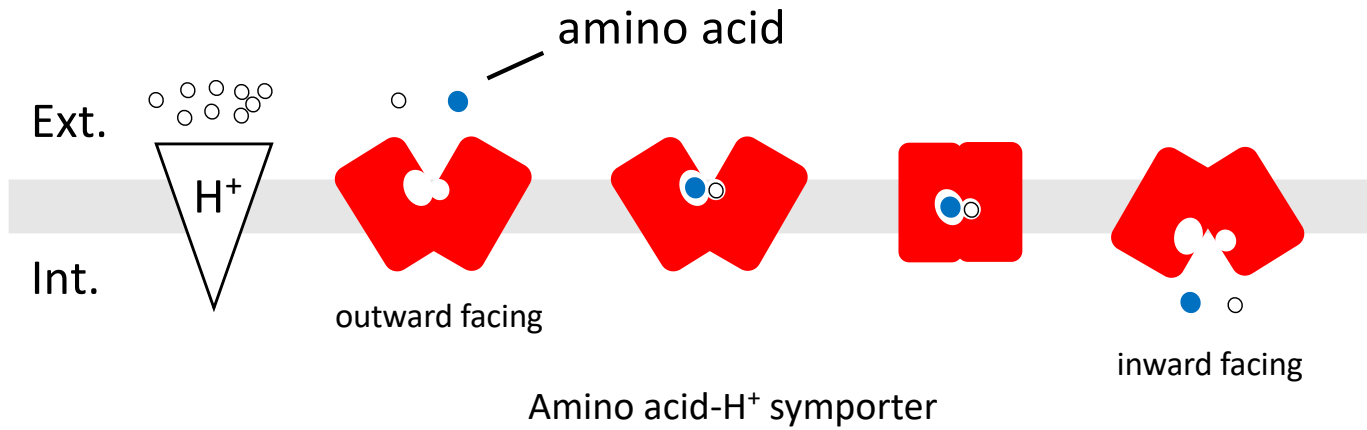


Membrane transporters are likely the most ancient proteins interconnecting the extra- and intracellular environments.

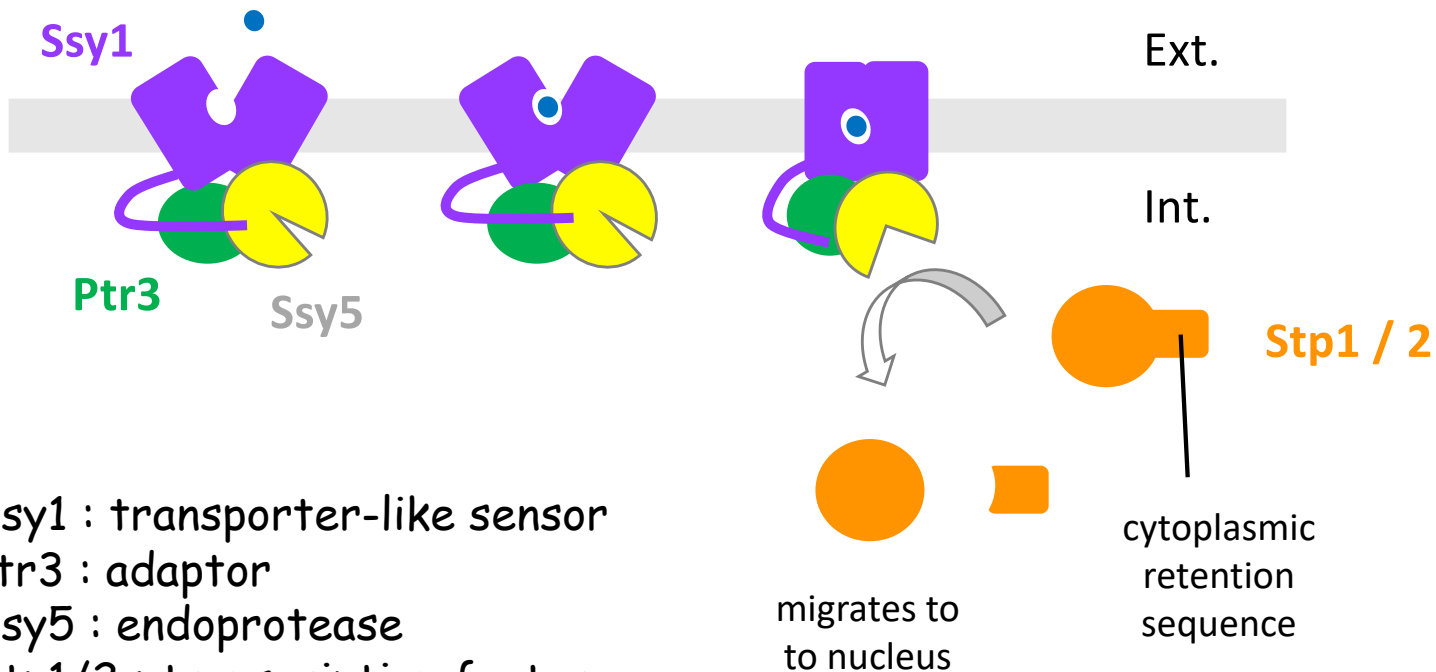
Importantly, it is now well established that certain transporters activate specific *signaling pathways* upon transport of their own substrates. This signaling is induced by the transport-coupled conformational changes of the transporter, not by internalization of its substrates. Such proteins are often referred to as *transceptors* (transporters-receptors).

Specific transceptors appear to have lost their transport activity while retaining their signaling function. The yeast amino-acid sensor Ssy1 is an example of such a protein.

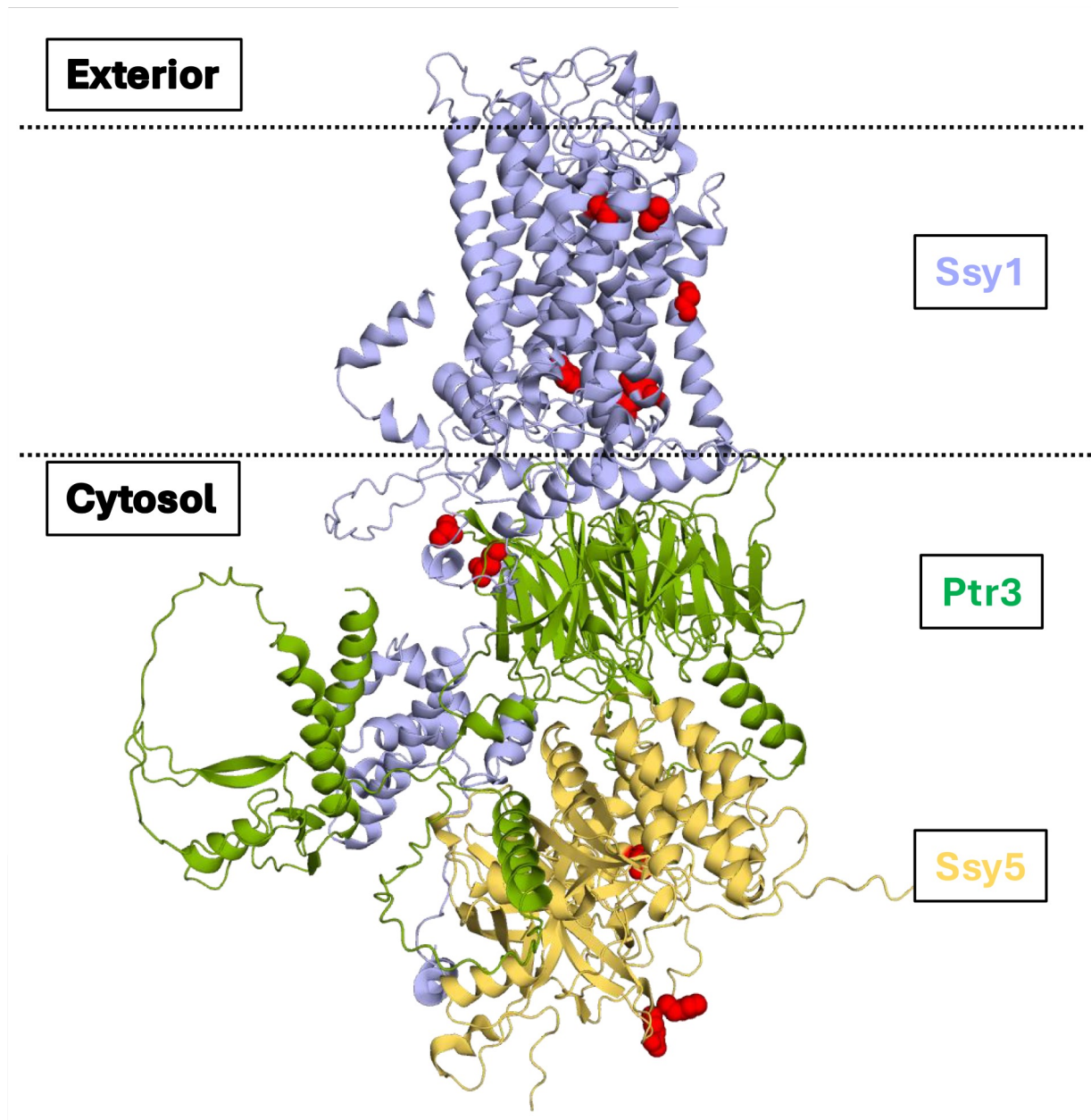
Rappel



Gap1, general aa permease



Ssy1 : transporter-like sensor
 Ptr3 : adaptor
 Ssy5 : endoprotease
 Stp1/2 : transcription factor



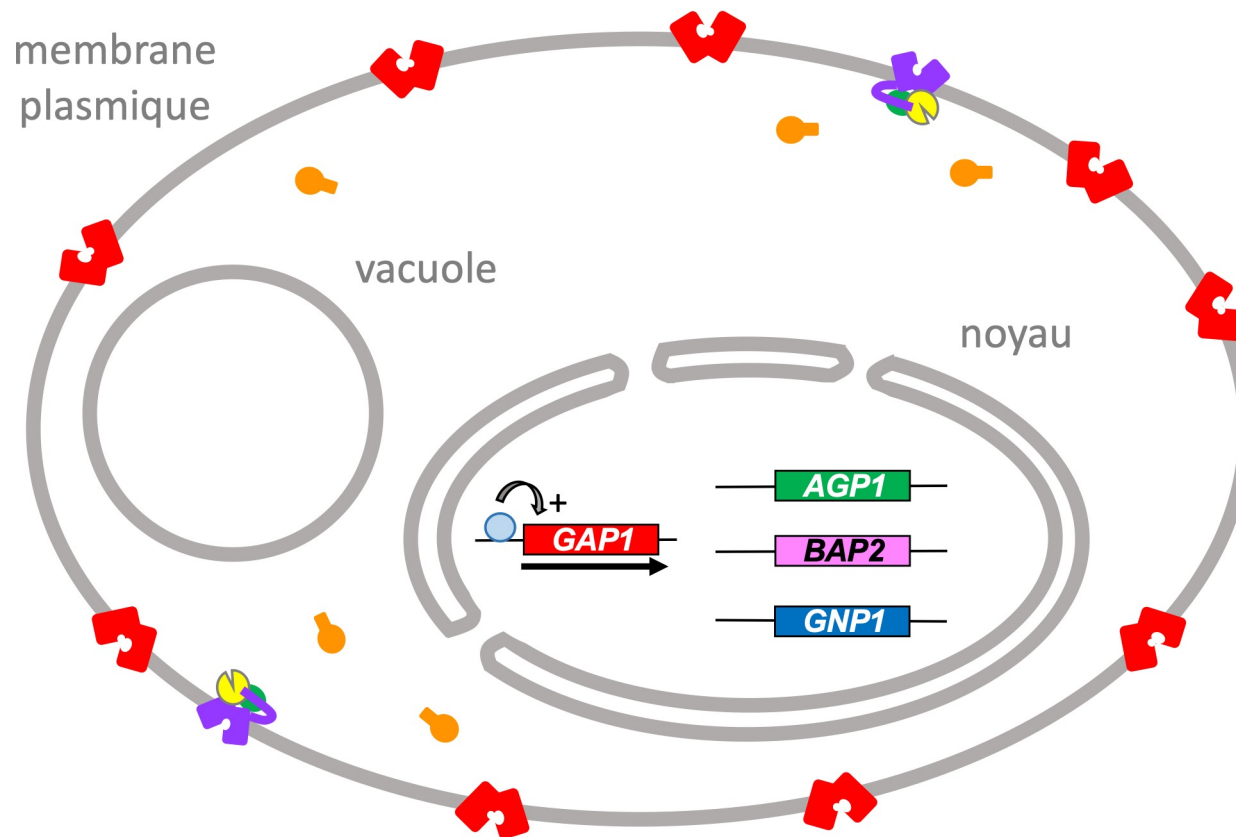
A recent study reported a 3D structural model of the Ssy1-Ptr3-Ssy5 complex.



P. Ljungdahl

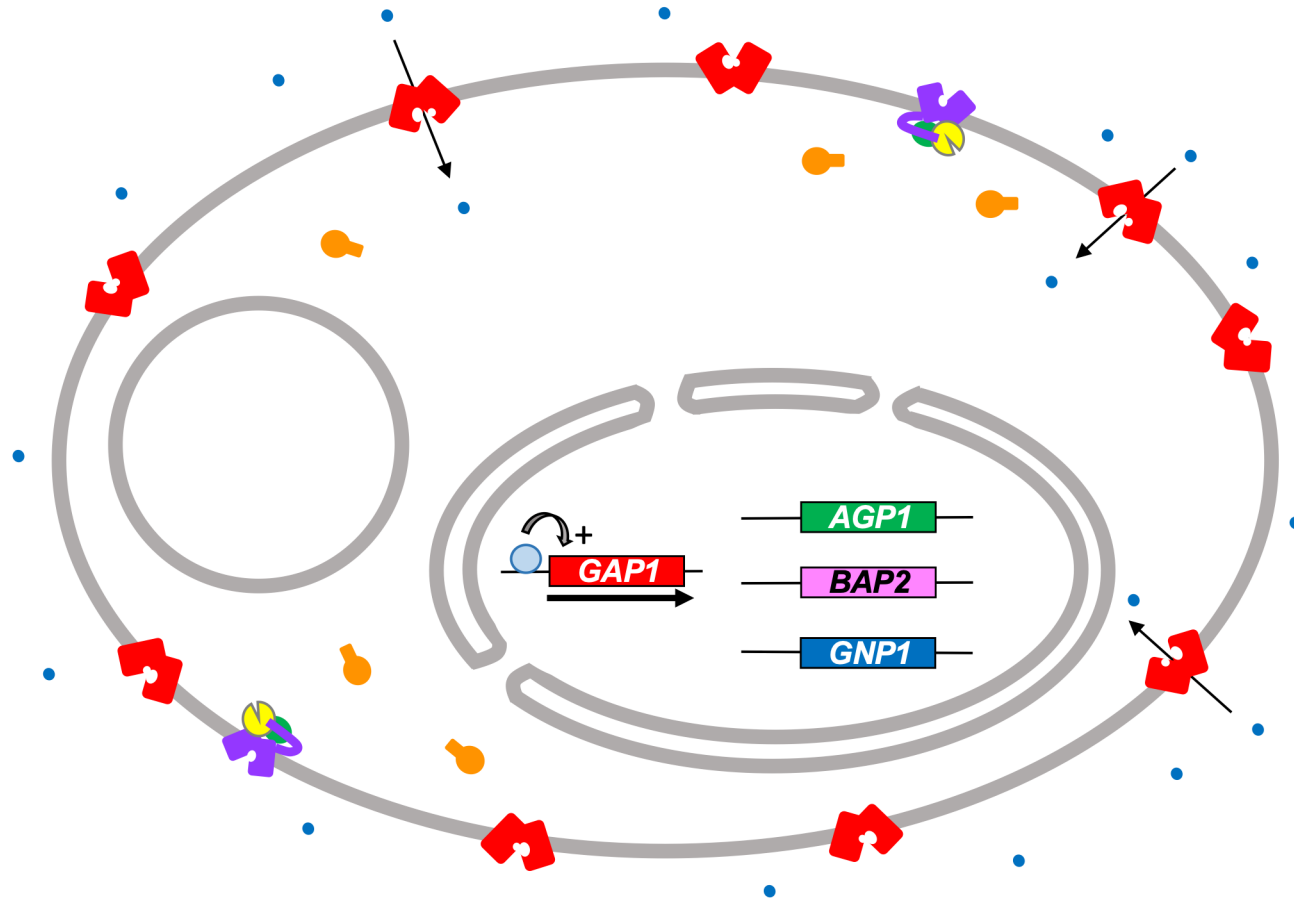
M. Kielland-Brandt

Rappel



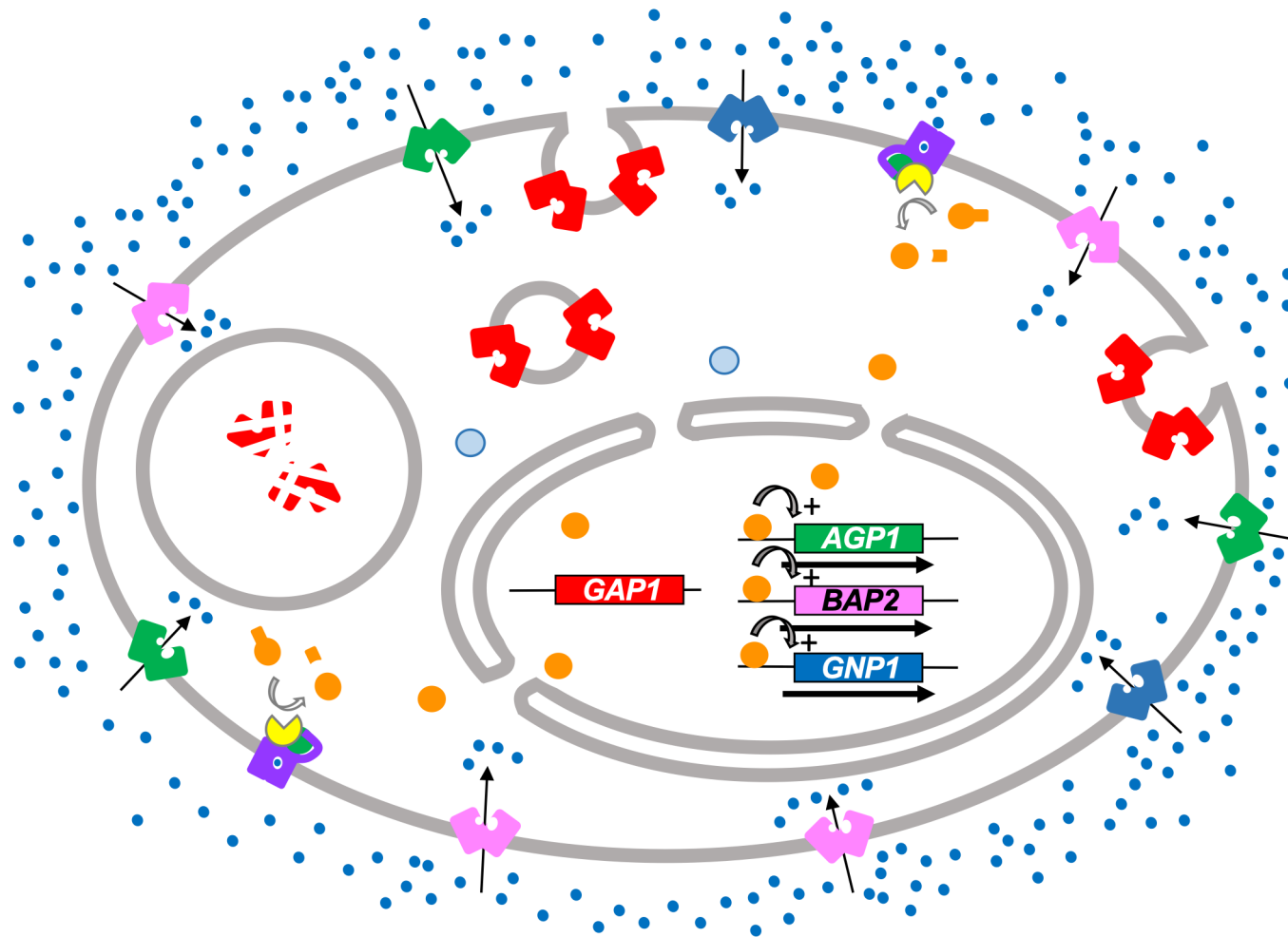
General context: In yeast cells starved for nitrogen or growing on a poor nitrogen source (like urea or proline), the *GAP1* gene encoding the general amino-acid permease is highly expressed and Gap1 proteins accumulate at the plasma membrane.

Rappel



When traces of any amino acid become available in the external medium, Gap1 – a very high-affinity permease - catalyzes their efficient uptake (H^+ symport reaction).

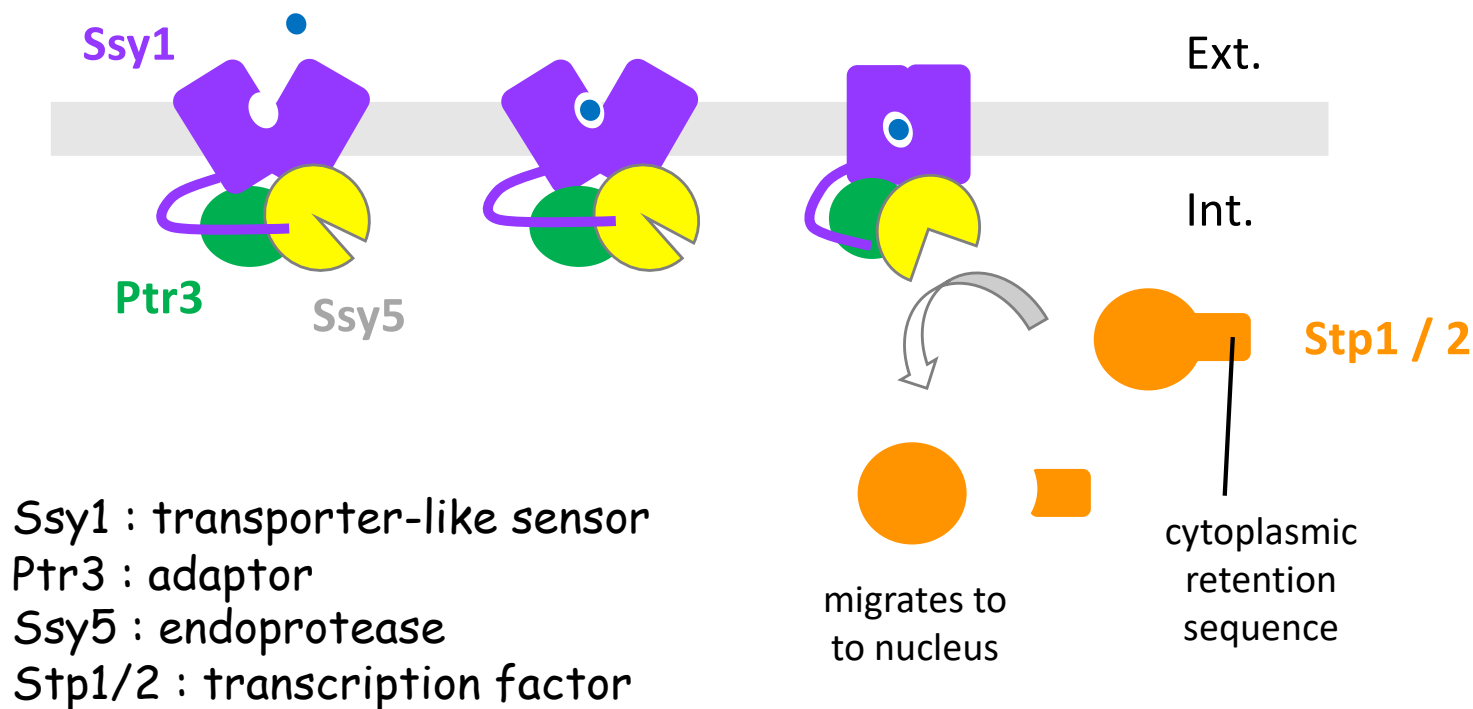
Rappel



If external amino acids become more abundant, Gap1 undergoes endocytosis and is degraded in the lysosome (vacuole). In parallel, the Ssy1/Ptr3/Ssy5 sensing system is stimulated, resulting in cleavage of the cytoplasmic precursor forms of Stp1 and Stp2 transcription factors. The processed Stp proteins then migrate into the nucleus, where they bind to the upstream regions of several target genes (*AGP1*, *BAP2*, *GNP1*) encoding lower-affinity amino acid transporters. These proteins are thus synthesized, taking over Gap1 to mediate uptake of amino acids into the cells.

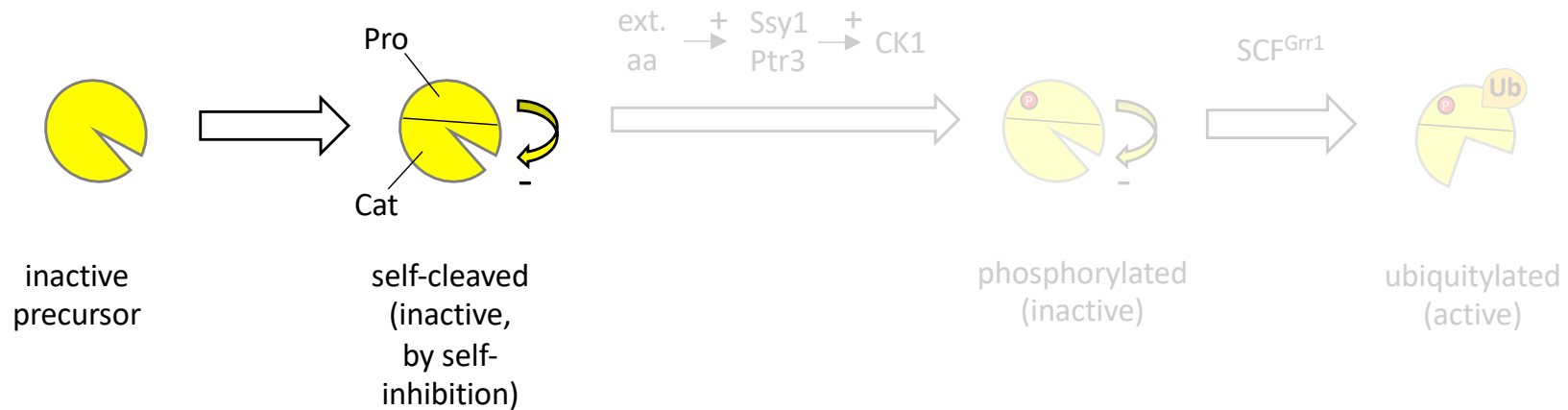
How is the Ssy5 endoprotease activated upon detection by Ssy1 of external amino acids?

Answer: via a cascade of phosphorylation & ubiquitylation events

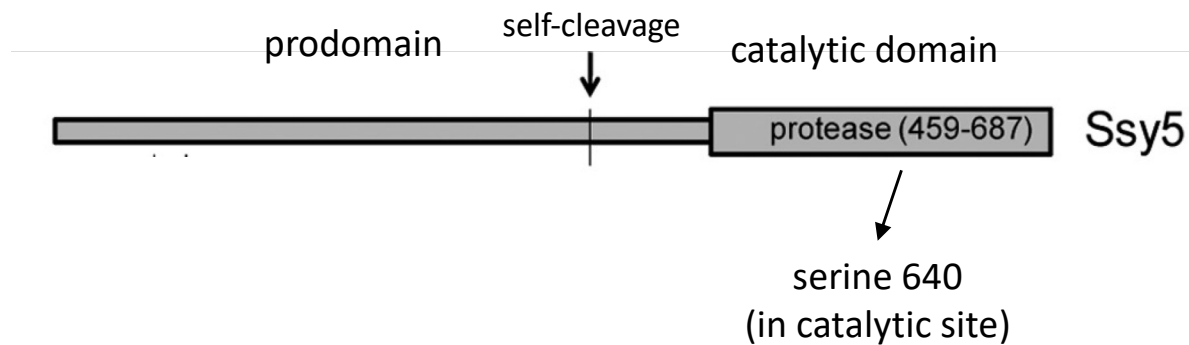


Référence : Abdel-Sater et al. & André (2011) *J Biol Chem* vol 286, 12006-12015.

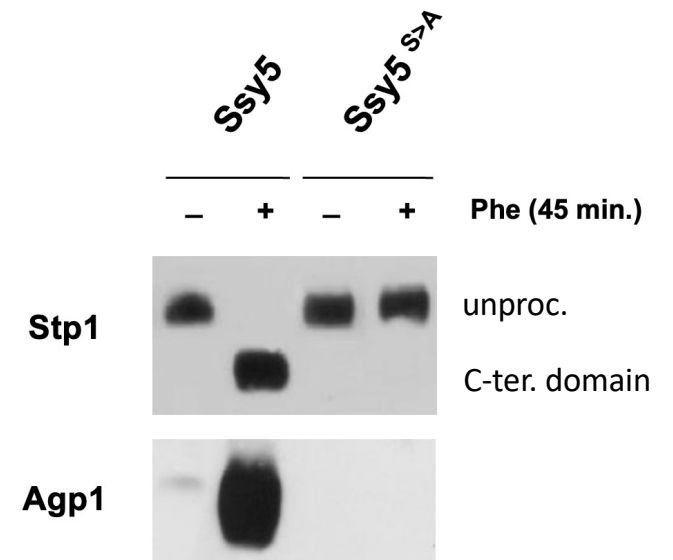
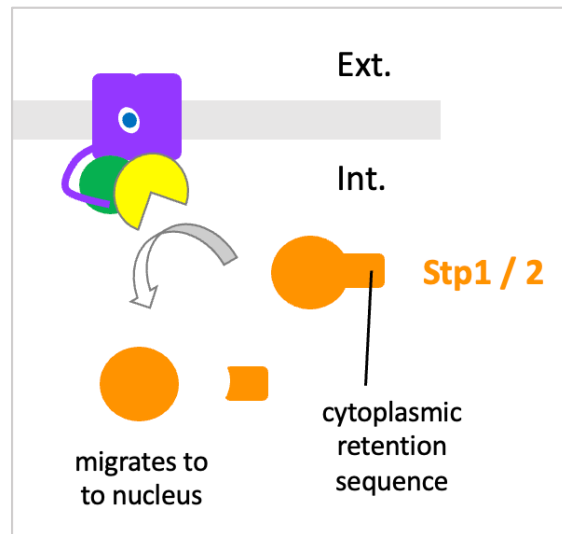
Domain organization and self-cleavage of the Ssy5 endoprotease



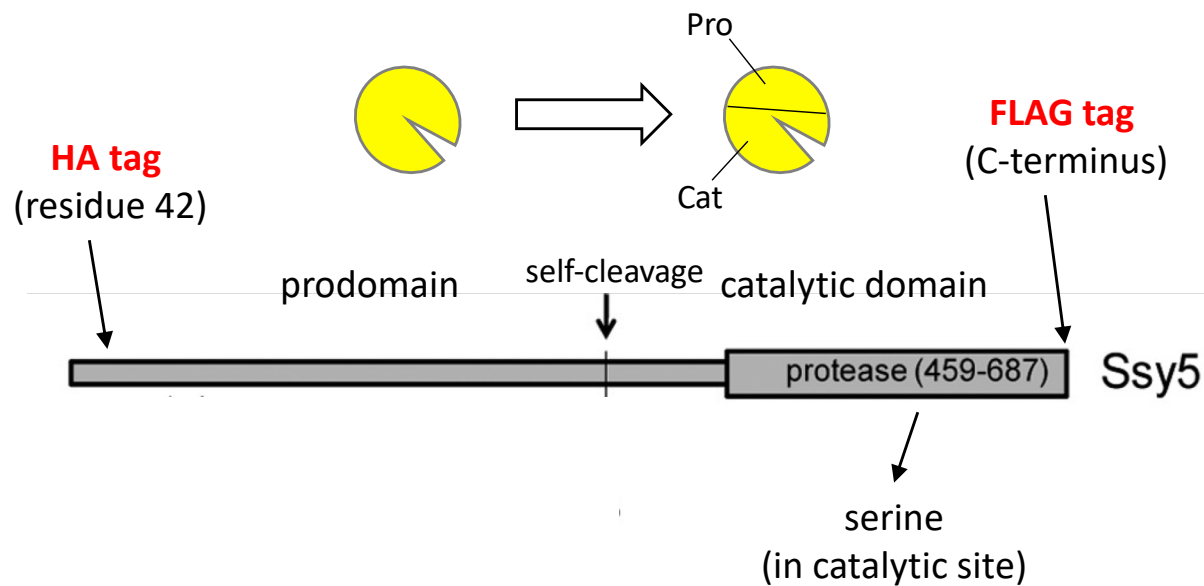
Mutations in the Ssy5 prodomain causes constitutive activation of the catalytic domain (not illustrated)



Schematic representation of the Ssy5 endoprotease

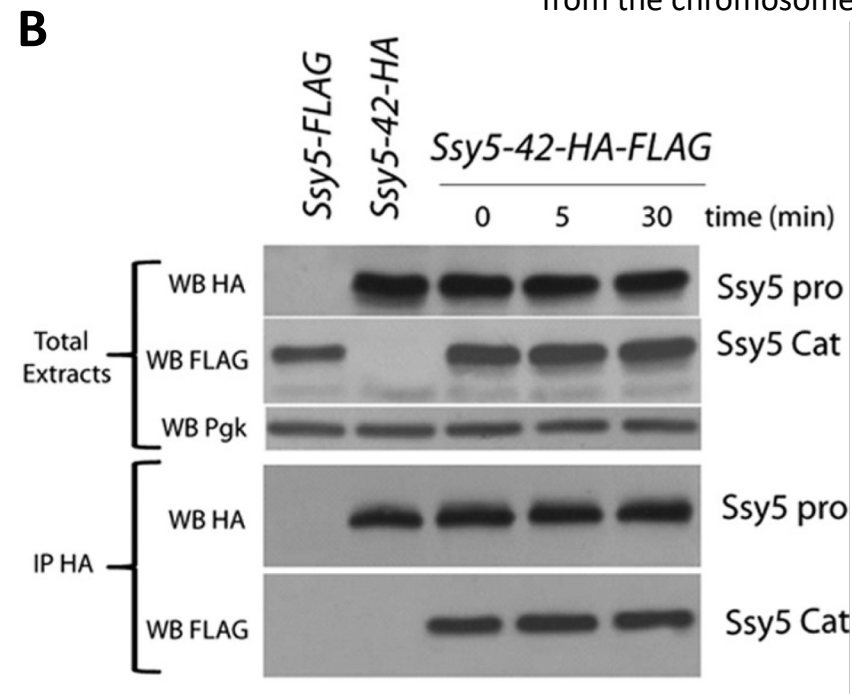
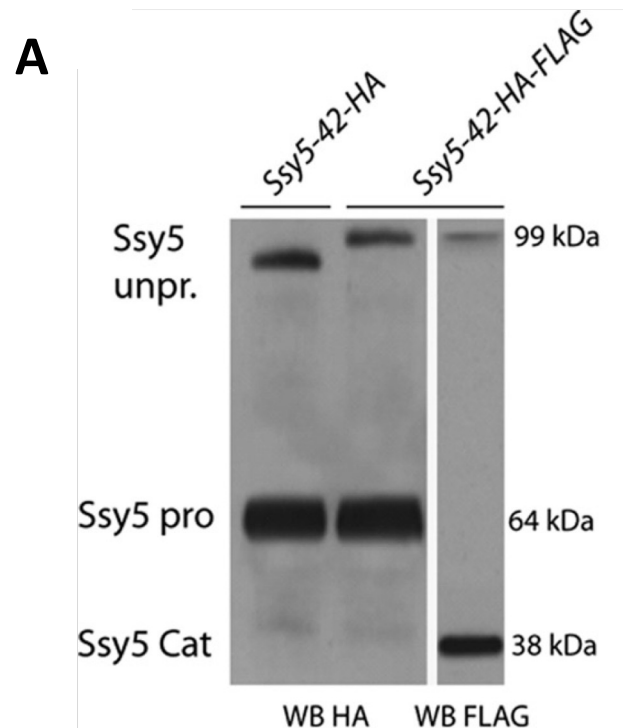


Immunoblot illustrating the phenylalanine (Phe)-induced processing (cleavage) of Stp1-HA (expressed from a plasmid) and synthesis of Agp1, both being defective if the serine in the catalytic site of Ssy5 is replaced with an alanine (S>A). (unpr. : unprocessed).

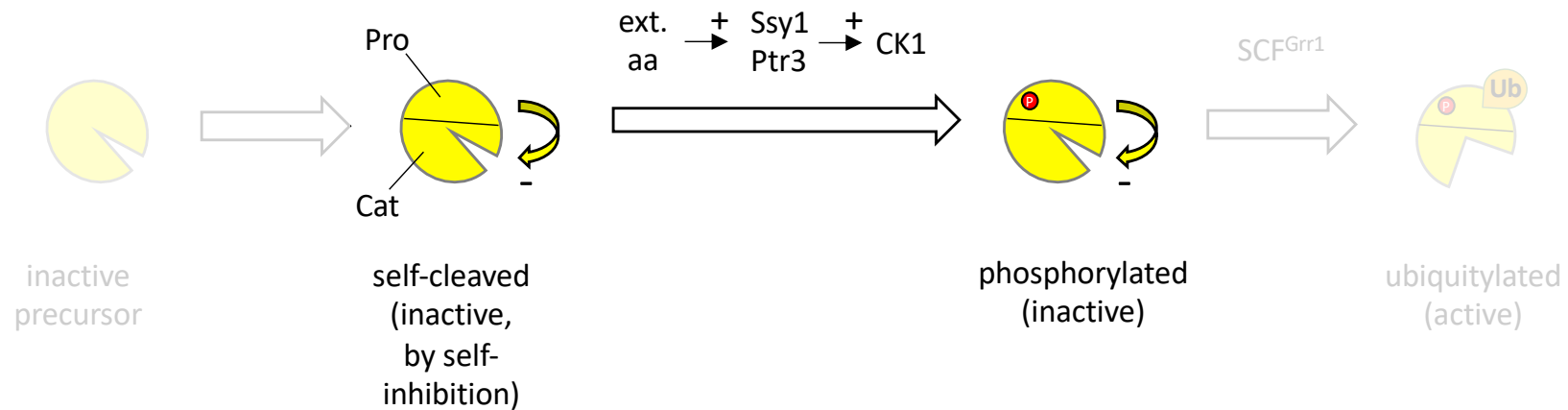


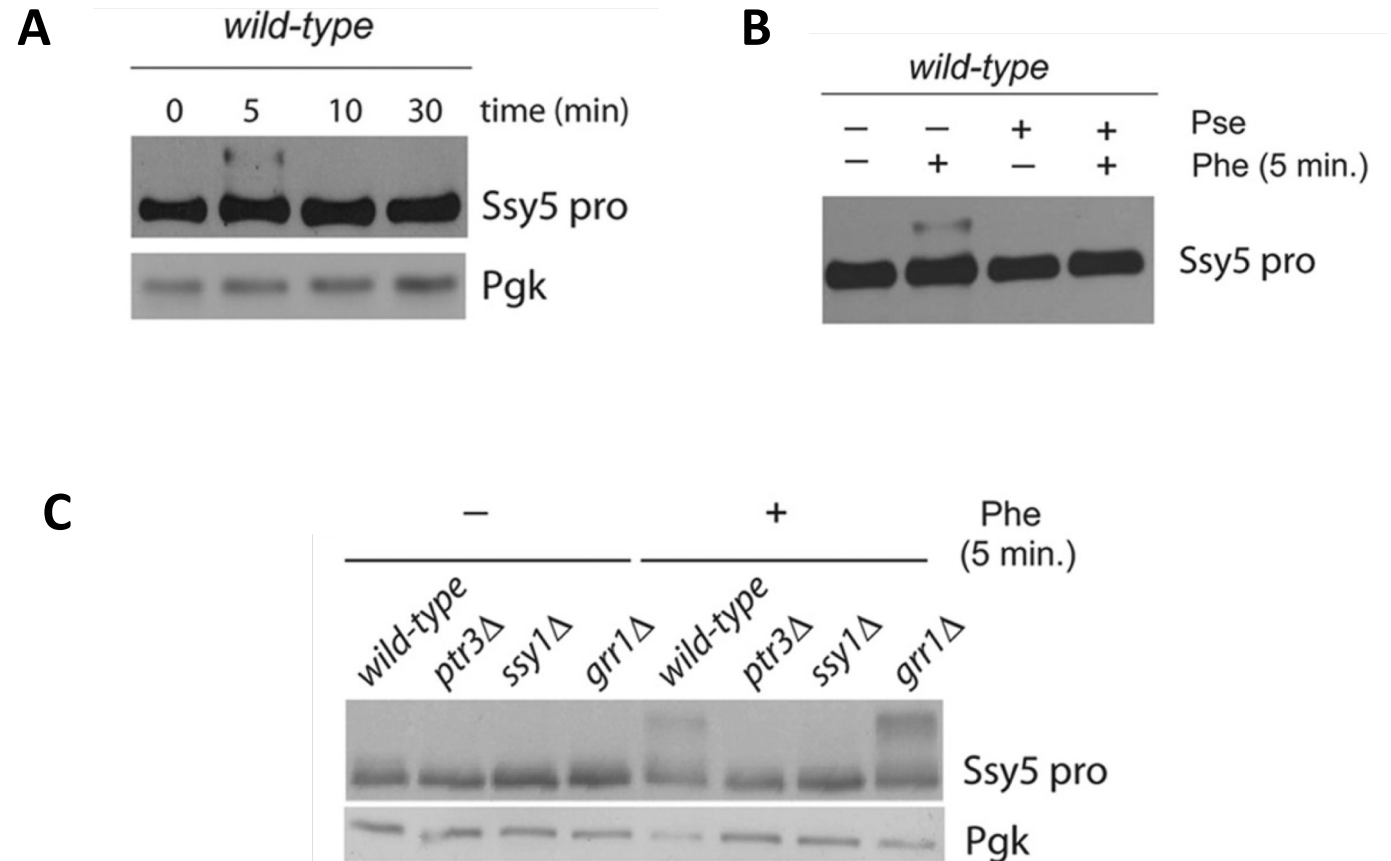
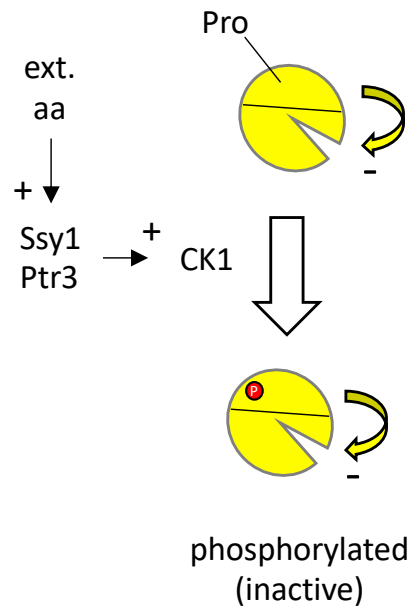
A. Immunoblot illustrating the cleavage of Ssy5 detected in extracts of cells expressing the singly tagged Ssy5-42-HA or doubly tagged Ssy5-42-HA-FLAG from the chromosome (unpr. : unprocessed).

B. Immunoprecipitation (IP) followed by immunoblot (WB, Western blot) illustrating that the pro- and catalytic domains of Ssy5 are bound each other, whether amino acids are absent (time 0) or present (times 5 and 30 min.). Phosphoglycerate kinase (Pgk) was used as a loading control. Each tagged Ssy5 form was expressed from the chromosome.

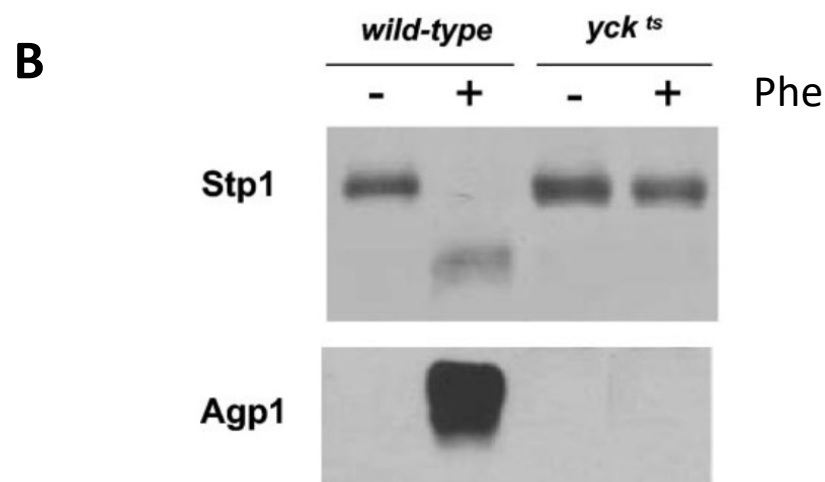
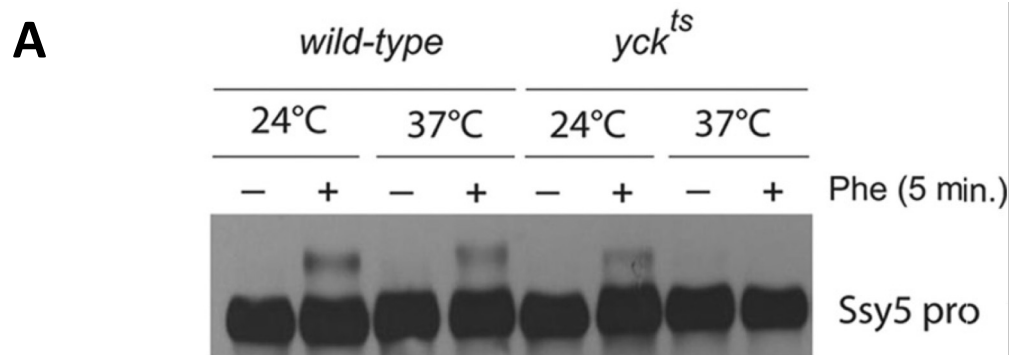
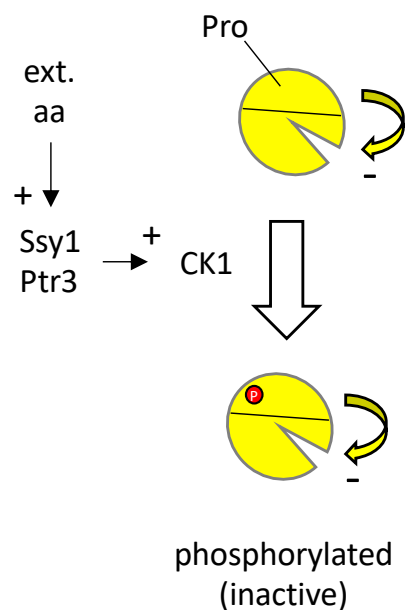


Phosphorylation via CK1 kinase of the Ssy5 prodomain upon detection of external amino acids



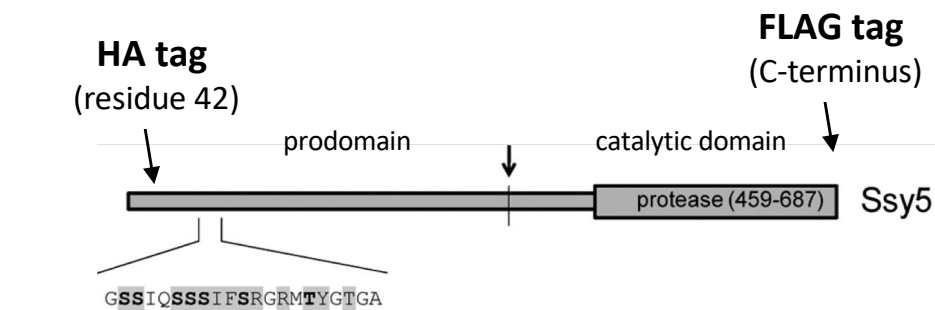


Immunoblots illustrating the amino acid-induced phosphorylation of the Ssy5 prodomain (42-HA version, expressed from the genome) and its dependence on Ssy1 and Ptr3. **A.** An upper band is detected above the prodomain signal five minutes upon addition of phenylalanine (Phe). **B.** Same experiment as in A except that cellular extracts have been treated (+) or not (-) with a phosphatase (Pse) before immunoblotting. **C.** Same as in A except that several mutants have been analyzed in addition to the *wild-type*. Phosphoglycerate kinase (Pgk) was used as a loading control.

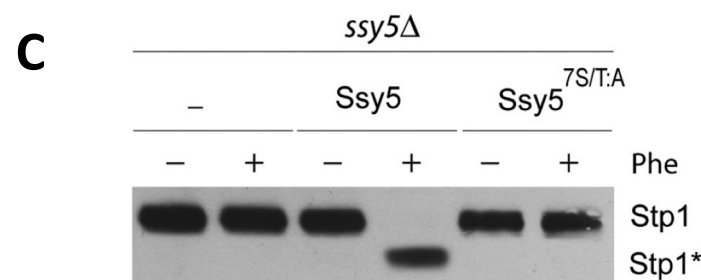
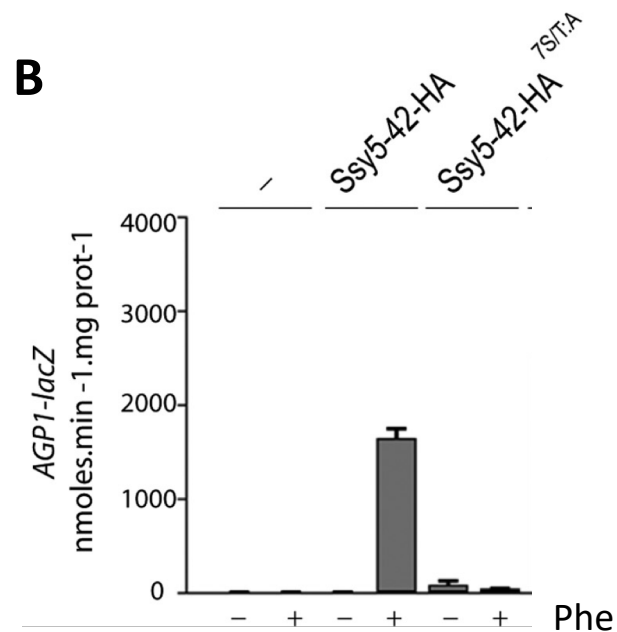
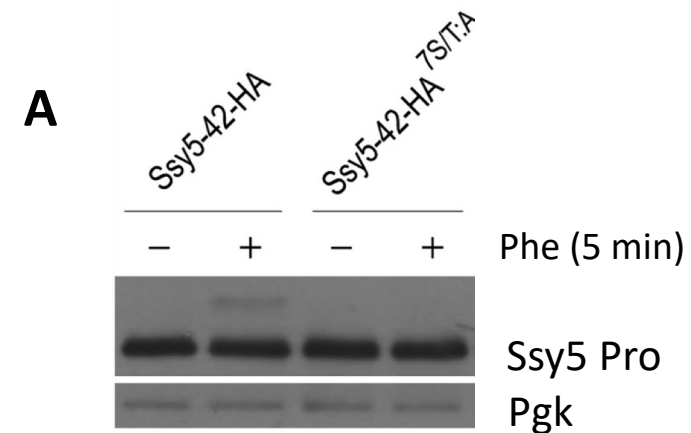


A. Immunoblot illustrating the essential role of casein kinase 1 (CK1) in amino acid-induced phosphorylation of the Ssy5 prodomain. The wild-type and the *yck1Δ yck2^{ts}* thermosensitive mutant (*yck^{ts}*) transformed with a plasmid expressing Ssy5-42-HA were initially grown at the permissive temperature of 24°C. Half of the cultures was then transferred to 37°C for 20 min. Phenylalanine was then added (+) or not (-) to the cultures, for 5 minutes. Crude extracts were then prepared and immunoblotted with anti-HA antibodies.

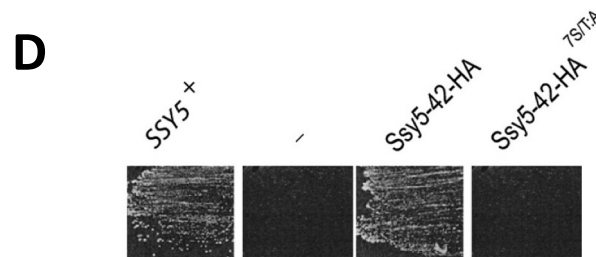
B. Immunoblot illustrating the essential role of CK1 in amino acid-induced Stp1 cleavage and Agp1 induction. Strains and conditions as in A, except that cells expressed Stp1-HA from a plasmid. Cells were incubated for 2 hours with Phe and crude extracts were also immunoblotted with anti-Agp1 antibodies.



highly conserved among Ssy5 orthologs



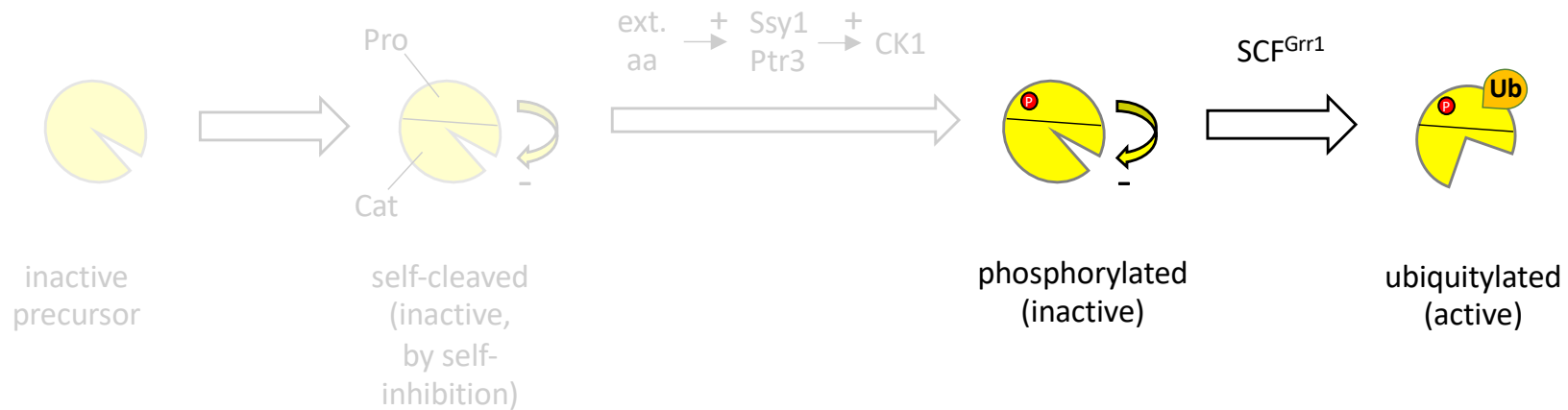
C. Immunoblot illustrating that phenylalanine (Phe)-induced cleavage of the Stp1-HA (expressed from a plasmid) is impaired if Ssy5 contains the 7S/T:A substitutions.



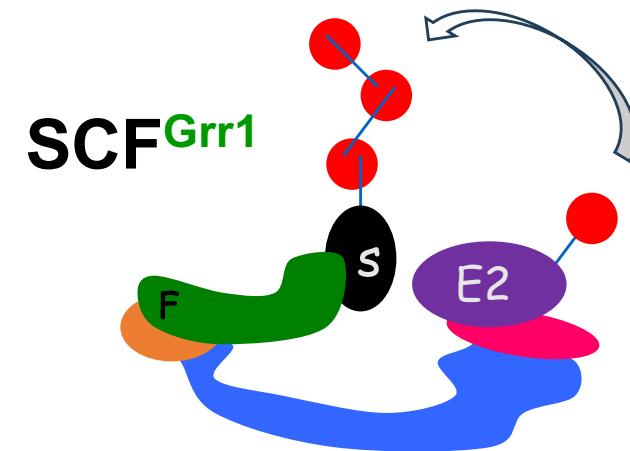
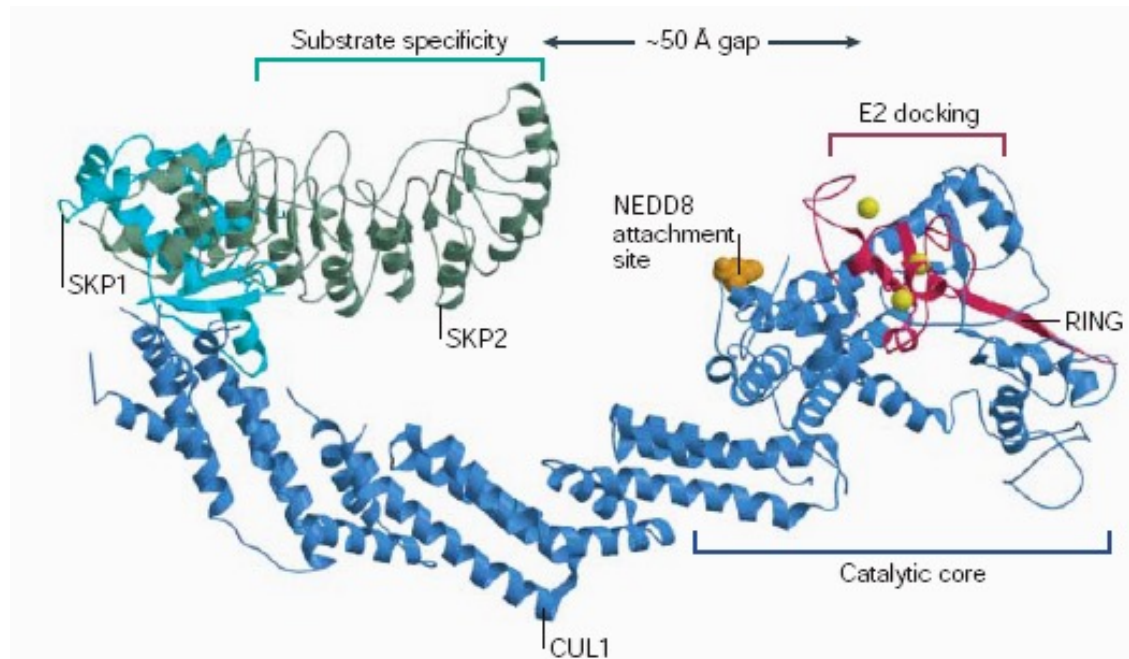
D. Growth test on phenylalanine as sole nitrogen source of a *gap1Δ ssy5Δ* strain expressing (from a plasmid) the wild-type SSY5, or SSY5-42-HA with or without the 7S/T:A substitutions.

A. Immunoblot illustrating that phosphorylation of the Ssy5 prodomain after phenylalanine (Phe) addition (+) is not detected anymore when seven evolutionary-conserved serine/threonine in the N-terminal tail of Ssy5 are replaced with alanine (7S/T:A). **B.** The beta-galactosidase (*lacZ*) reporter gene under the control of the promoter and upstream regulatory sequences of *AGP1* is transcriptionally induced by phenylalanine (Phe) addition to the *ssy5Δ* mutant expressing (from a plasmid) the functional Ssy5-42-HA form, but this induction is defective if Ssy5 contains the 7S/T:A substitutions.

Ubiquitylation the Ssy5 prodomain upon detection via Ssy1 of external amino acids

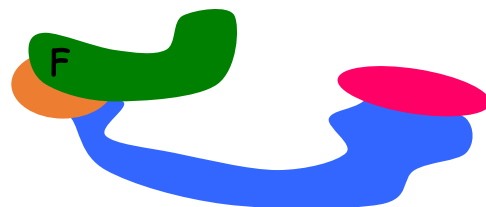


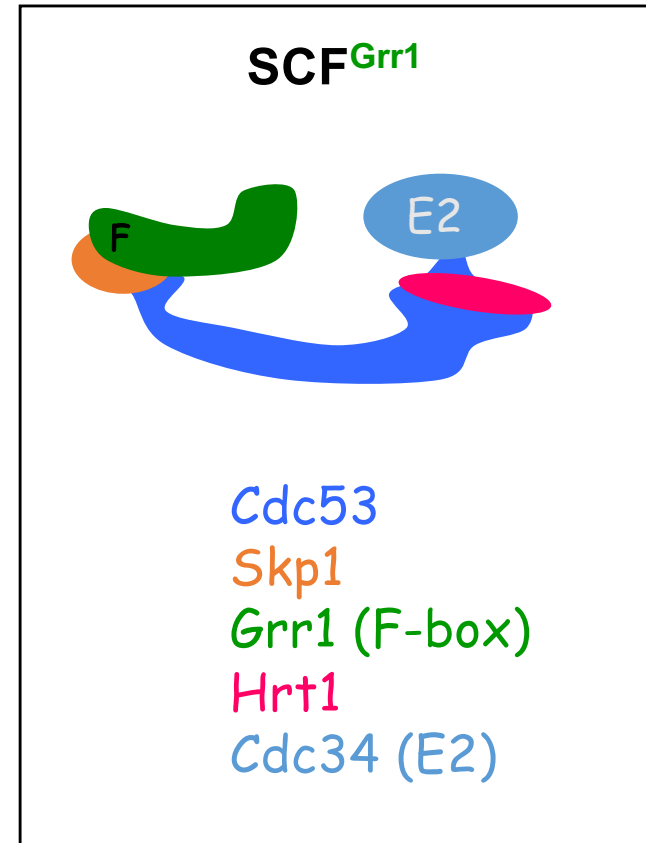
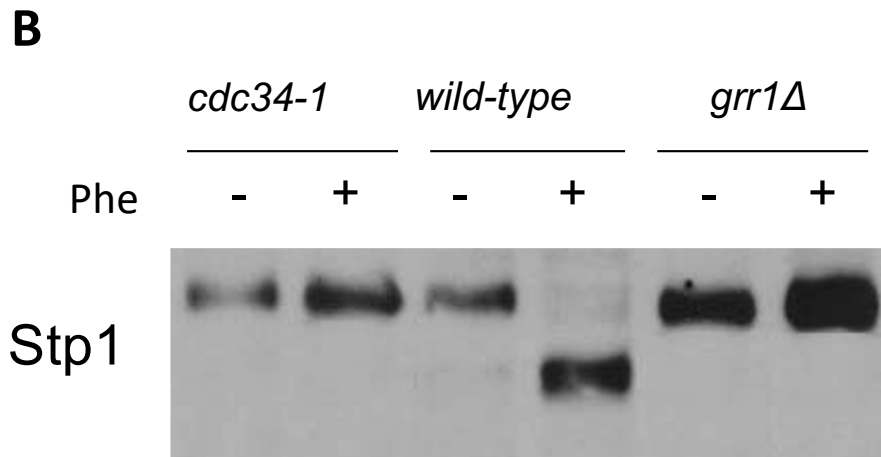
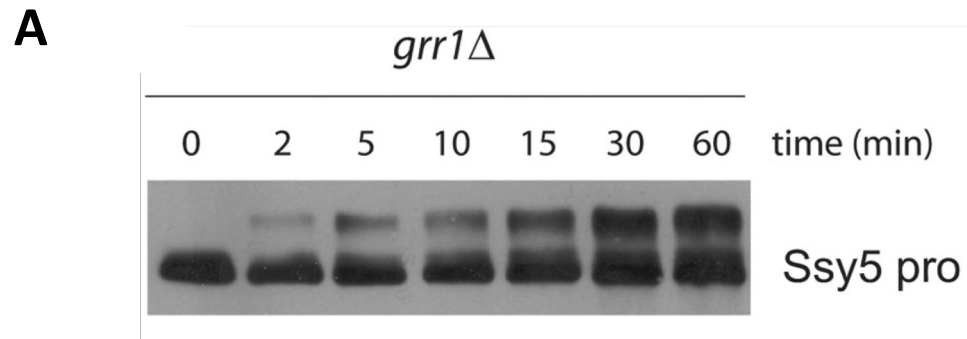
The SCF^{Grr1} ubiquitin ligase (E3) enzyme



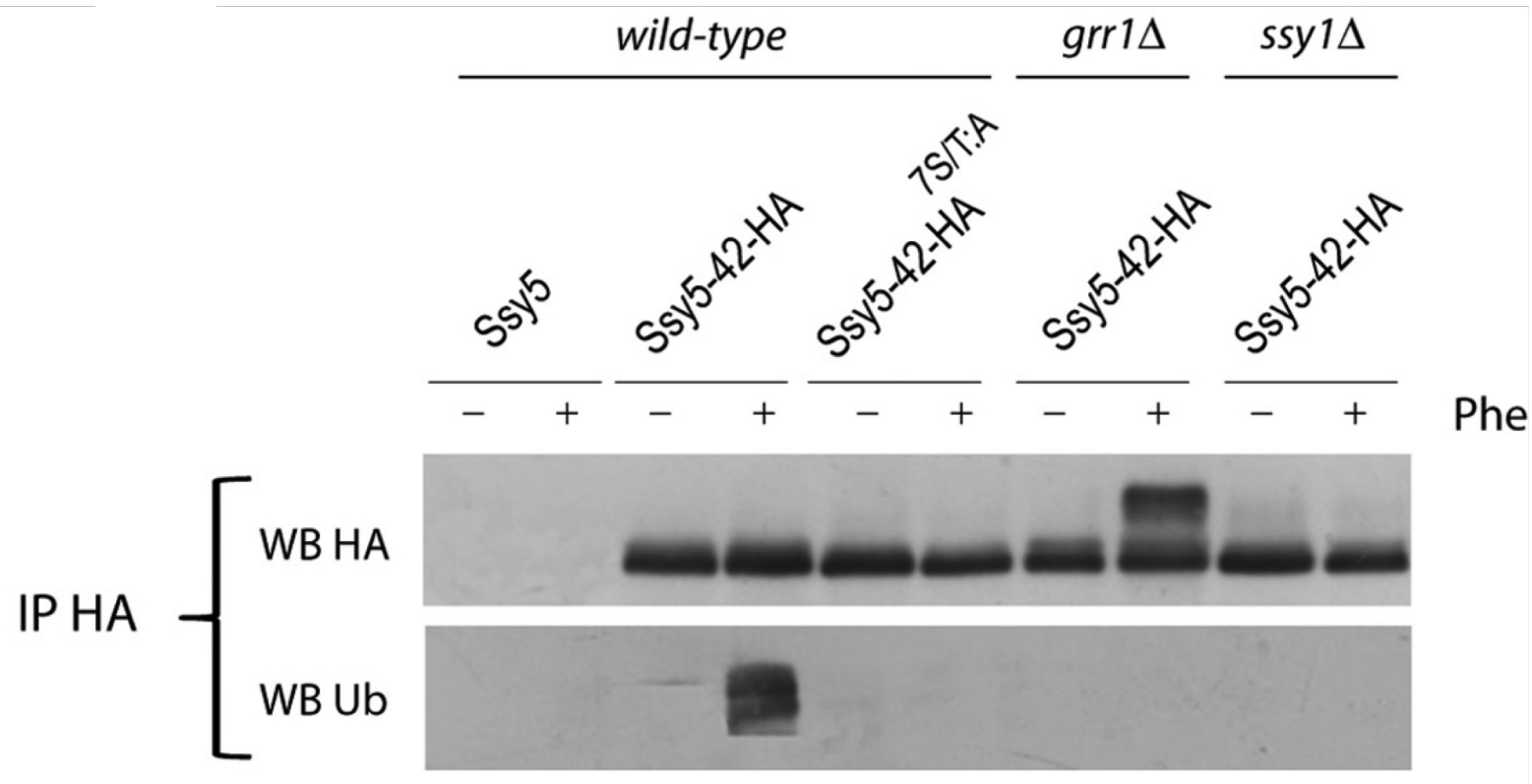
Protein substrates of SCF Ub complexes generally need to be phosphorylated for being recognized by the F-box domain of the SCF

Cdc53
 Skp1
 Grr1 (F-box)
 Hrt1 (RING)
 Cdc34 (E2)



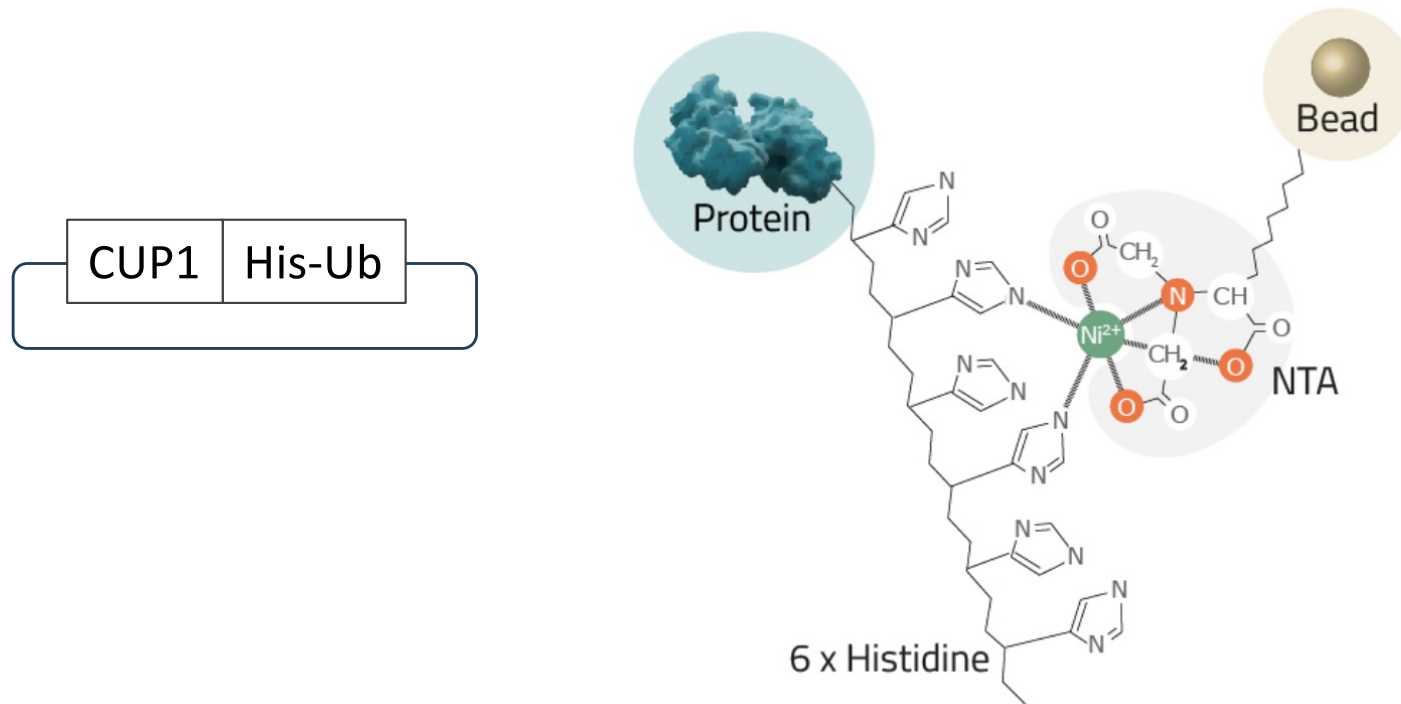


A. Immunoblot illustrating that the phosphorylated form of the Ssy5 prodomain (Ssy5-42-HA form, expressed from the chromosome) tends to accumulate upon phenylalanine addition to the *grr1Δ* mutant. **B.** Immunoblot illustrating that Stp1-HA (expressed from a plasmid) cleavage upon phenylalanine addition is defective in the *grr1Δ* and *cdc34-1* mutants. In these mutants, AGP1-lacZ is not transcriptionally induced by amino acids.



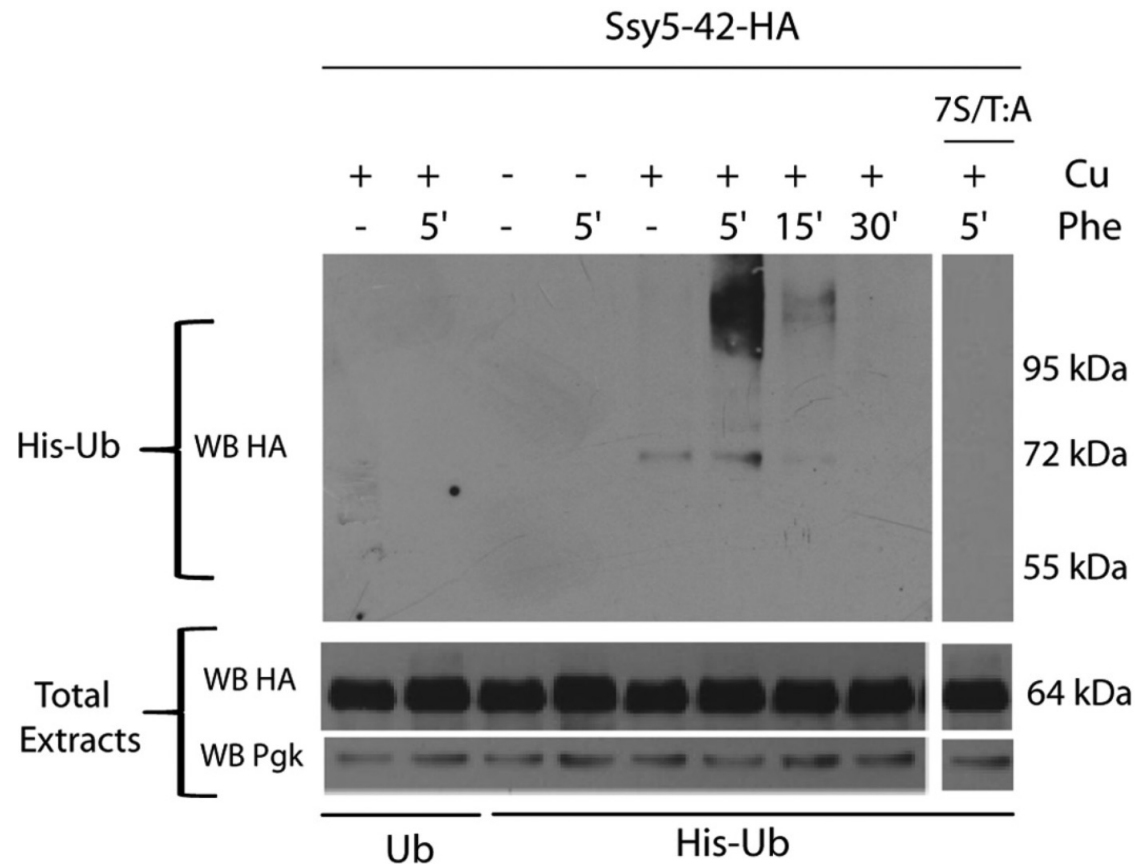
Immunoprecipitation (IP) experiment illustrating that in cells expressing Ssy5-42-HA from a plasmid, the prodomain of Ssy5 is ubiquitylated 30 min after phenylalanine (Phe) addition (+) and that this modification does not occur if Ssy5 contains the 7S/T:A substitutions, or if Ssy1 or Grr1 are defective. The immunoblot also illustrates the high accumulation of the phosphorylated form of Ssy5 prodomain when Grr1 is lacking.

Ubiquitylation of the Ssy5 prodomain may also be illustrated using cells that express ubiquitin fused to a poly-histidine tag



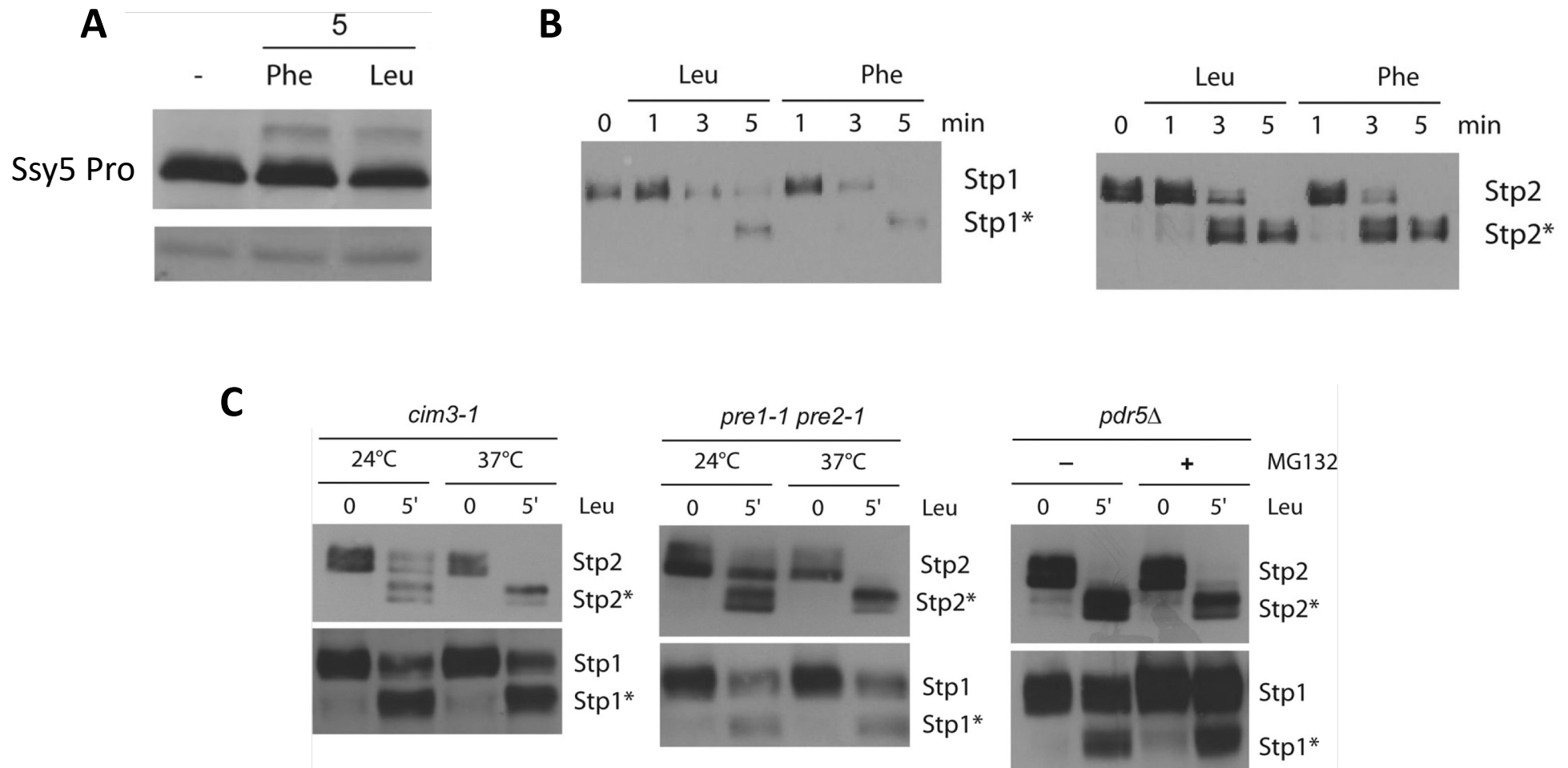
A. Schematic representation of the plasmid expressing the gene for ubiquitin (Ub) tagged with 6 histidines (His) under the control of the copper-inducible *CUP1* promoter. **B.** Schematic representation of the purification of a polyHis-tagged protein using agarose beads coated with nickel-nitrilotriacetic (NTA).

The prodomain of Ssy5 is indeed detectable among ubiquitylated proteins, unless it fails to be phosphorylated



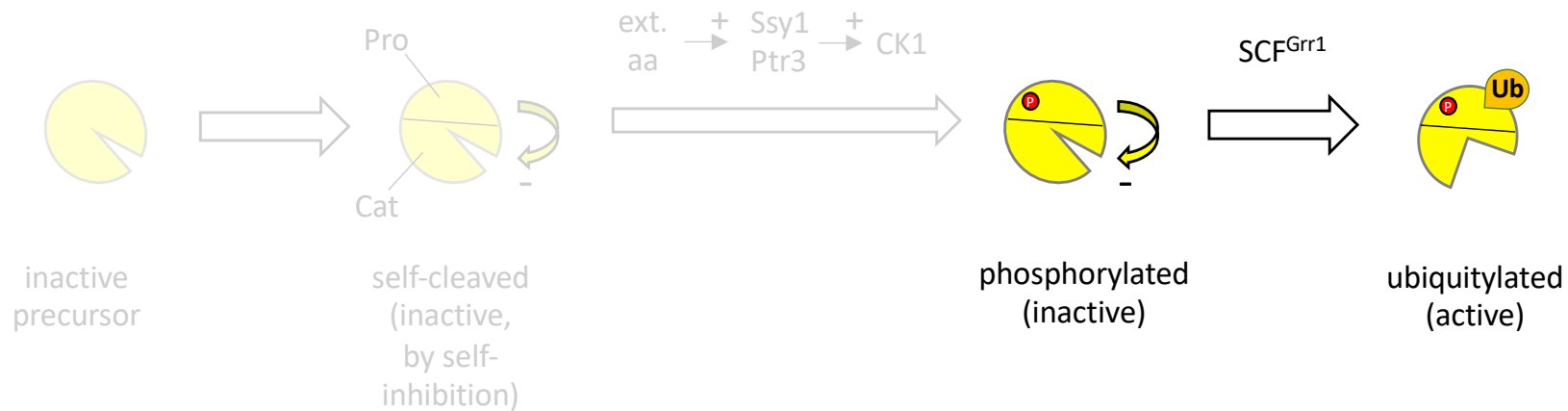
A yeast mutant strain with reduced expression of ubiquitin (Ub) and expressing Ssy5-42-HA from a plasmid was transformed with a second plasmid expressing either Ub or His-Ub (Ub fused to a poly-histidine tag) under the control of the copper-inducible *CUP1* promoter. CuSO₄ (0,1 mM) (Cu) was added (+) or not (-) to the cultures for 3 hours to induce expression of Ub or His-Ub. Phenylalanine (Phe) was then added for 5, 15 or 30 min or not (-). Crude cell extracts were prepared and incubated in the presence of nickel-NTA-Sepharose beads. His6 -tagged (ubiquitylated) proteins retained on the beads were then eluted, and immunoblotting was carried out with anti-HA antibodies. Crude extracts were also immunoblotted with anti-HA and anti-Pgk antibodies.

The prodomain of Ssy5 is not degraded in response to amino acid detection



A. Immunoblot illustrating that the Ssy5 prodomain remains surprisingly stable after phenylalanine addition (upper bands correspond to phosphorylated form). **B.** Immunoblot illustrating that Ha-tagged Stp1 and Stp2 are very rapidly cleaved after phenylalanine addition. **C.** Immunoblot illustrating that in two proteasome thermosensitive mutants transferred to the non-permissive temperature of 37°C, cleavage of Stp1 and Stp2 occurs normally after phenylalanine addition. The same result is observed if the proteasome is inhibited by the MG132 peptide (whose natural excretion by yeast is here prevented by use of *pdr5Δ* mutant lacking an ABC transporter exporting MG132).

Ubiquitylation the Ssy5 prodomain does not induce to its degradation : a case of allosteric control by ubiquitylation?



Of note, if the prodomain of Ssy5 would be degraded, its catalytic domain would be irreversibly active. In contrast, if it is not degraded, active Ssy5 may be subsequently de-activated by de-phosphorylation (see p 13) and de-ubiquitylation (see p 21).

Conclusions and more conceptual lessons

- Ssy1 is an example of a transporter-like protein functioning as sensor (or receptor) of extracellular compounds – model : Ssy1 evolutionarily derives from an amino acid transporter (or transceptor)
- Amino-acid detection via Ssy1 triggers CK1-dependent phosphorylation of the Ssy5 self-inhibitory prodomain – model : a CK1-binding site in Ssy5 prodomain ⁽¹⁾ is unmasked following an allosteric change of Ssy5 conformation ?
- Phosphorylation of the Ssy5 prodomain promotes its ubiquitylation (a common scheme in signaling) and this modification is crucial for Ssy5 activation
- Ssy5 prodomain ubiquitylation is particular in that the modification results in Ssy5 activation without apparent intervention of proteasome-mediated degradation of the prodomain – model : a case of allosteric control by ubiquitylation, likely advantageous because of its reversibility (by de-ubiquitylation)?
- both phosphorylation and ubiquitylation of Ssy5 prodomain are transient – model : existence of a negative feedback control of Ssy5 activation, via dephosphorylation and de-ubiquitylation?

⁽¹⁾ and in Ptr3 as well, as Ptr3 is phosphorylated too (not illustrated)

Acknowledgments



Fadi ABDEL SATER