Activation of the SPS Amino Acid-Sensing Pathway in *Saccharomyces cerevisiae* Correlates with the Phosphorylation State of a Sensor Component, Ptr3

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Well-studied nutrient-sensing pathways in the budding yeast

- Glucose-sensing pathway

Take a closer look of **Amino acid-sensing pathway**

**Ssy1. Ptr3. Ssy5→ SPS**-sensing pathway

Strategy of the research

We have known several components of SPS-sensing pathway, however the regulators of the pathways are still unknown.

Using known glucose-sensing pathway regulators to suggest possible regulators of the SPS-sensing pathway.

Purpose a more complete SPS-sensing pathway including positive and negative regulators.
Ssy1 is important in SPS-sensing pathway

Ssy1 defection → abolish Ptr3 hyperphosphorylated forms not hypophosphorylated form
Interactions between the N-terminal cytoplasmic extension of Ssy1 and other proteins

Using yeast two-hybrid assay, we can know that the N-terminus of Ssy1 can interact with Yck1, Ssy5, and Ptr3.
Brief conclusion about Ssy1

- N-terminus of Ssy1 can interact with Yck1, Ssy5, and Ptr3.

- Ssy1 defection → abolish Ptr3 hyperphosphorylation.
**Yck1/2 mutations could block **

Ptr3 hyperphosphorylation

**Yck1/2 play a redundant role in regulating the hyperphosphorylation of**

Ptr3
Double mutation of Yck1 and Yck2 blocks the processing of Stp1

Under double mutant condition

- In 26°C, the processing of Stp1 is compromised
- In 37°C, the processing of Stp1 is blocked
Brief conclusion about Yck1/2

- Yck1/2 play a redundant roles in regulating the hyperphosphorylation of Ptr3
- Double mutation of Yck1 and Yck2 blocks the processing of Stp1
What may be the negative regulator of the SPS-sensing pathway?

- Based on mutant library, mutants of Rts1 may keep SPS-mediated pathway activated

- CKI and PP2A are known to act on the same substrate in Xenopus embryos
Rts1/ PP2A phosphatase complex is important in SPS-sensing pathway, not in glucose signal transduction pathway

**HXT1**
- Low-affinity glucose transporter of the major facilitator superfamily,
- Expression is induced by Hxk2p in the presence of glucose and repressed by Rgt1p when glucose is limited

**AGP1**
- Low-affinity amino acid permease with broad substrate range
- Involved in uptake of asparagine, glutamine, and other amino acids
- Expression is regulated by the SPS plasma membrane amino acid sensor

http://db.yeastgenome.org/cgi-bin/locus.pl?dbid=S000001136
Rts1/ PP2A phosphatase complex is important in SPS-sensing pathway, not in glucose signal transduction pathway.

Leucine induces activity significantly in WT

Significantly activate the expression of reporter gene

Mutation doesn’t affect reporter gene expression
Rst1/PP2A phosphatase complex antagonizes Yck1/2 in the regulation of the phosphorylation of Ptr3.

An Rts mutation partially rescues the cell growth defect in Yck1/2 double mutant cells in 34°C.
Brief conclusion of Rts1/PP2A phosphatase complex

- Rts1/PP2A phosphatase complex is important in SPS-sensing pathway, not in glucose signal transduction pathway.

- Rts1/PP2A phosphatase complex antagonizes Yck1/2 in the regulation of the phosphorylation of Ptr3.

- Rts1 may keep SPS-mediated pathway down-regulated in the absence of extracellular amino acids by dephosphorylating a component of the pathway.
**Ptr3 is a phosphoprotein**

- **Ptr3 exists as 4 bands**

- **Treat λ protein phosphatase** → 3 slower-mobility forms ↓ fastest-mobility form ↑

- **Treat λ protein phosphatase+ phosphatase inhibitor** → above effects are abolished

- **Ptr3 phosphorylation** → slower-mobility forms generation
Why mutating **S321** and **T635** and **T525** of **Ptr3**?

- CKI proteins often target the consensus site D/ExxS/T
- **Ptr3** has 5 DxxS/T sites and 10 Exxs/T sites
- Using sequence alignment of **Ptr3s** from 12 fungal species → 2 highly conserved sites (EIYS,S321; ESAT, T635) and 1 completely conserved site (EGIT, T525)
Threonine 525 is critical for Ptr3 function

Mutations in five CKI consensus sites of PTR3 do not significantly affect AGP1-lacZ reporter gene expression.
**T525 mutants are fail to be hyperphosphorylated and activate target gene expression**

No tag con.  
Ptr3 • myc  
Ptr3(S321A) • myc  
Ptr3(T635A) • myc  
Ptr3(T525A) • myc  
Ptr3(T525D) • myc  
Ptr3(T525E) • myc

Remove an N-terminal inhibitory sequence
Introduce a reported constitutively active Ptr3 mutant

The constitutive PTR3 mutants exhibit gain of function

EUKARYOTIC CELL, June 2005, p. 1116–1124
A constitutively active Ptr3 mutant is hyperphosphorylated

Ptr3 mutation causes constitutively activation of a reporter gene

Compared to WT, the mutant revealed a 4-5 fold increase in hyperphosphorylation/hypo phosphorylation without leucine treatment
Hyperphosphorylation of Ptr3 requires Ssy1

- Mutation of Rts1 increases amount of hyperphosphorylation
- Mutation of Ssy1 abolishes Ptr3 hyperphosphorylation
- \textbf{Rts1} mutant effect on the hyperphosphorylation of Ptr3 also requires Ssy1
Self-interaction of Ptr3 is independent of Ssy1, Ssy5, and its phosphorylation status

- Ptr3 interact with itself in a 2-hybrid assay.

- No significant differences between 3 mutants → Self interaction of Ptr3 is independent of Ssy1, Ssy5, its phosphorylation status
Interaction between Ptr3 and Ssy5 is independent of Ptr3 hyperphosphorylation and of Ssy1

- Ptr3 interact with Ssy5 in a 2-hybrid assay.
- Ssy5 is processed into a C-terminal fragment, Ssy5-C.

- Ssy1 mutation → Ptr3 couldn’t be phosphorylated → Ssy5 still process into Ssy5-C
**Ssy5 is constitutively processed**

- **Ssy5** contains a C-terminal chymotrypsin-like serine protease.
- The presence of leucine could not affect the processing of Ssy5

1. \(\text{ssy5}^{\Delta}\)
2. \(\text{ssy1}^{\Delta}\text{ssy5}^{\Delta}\)
3. \(\text{ptr3}^{\Delta}\text{ssy5}^{\Delta}\)
4. \(\text{ssy1}^{\Delta}\text{ptr3}^{\Delta}\text{ssy5}^{\Delta}\)
5. WT

- The existence of ssy1, Ptr3 could not affect the processing of Ssy5
Brief conclusion of **Ptr3**

- **Ptr3** is a phosphoprotein
- **T525** mutants are fail to be hyperphosphorylated and activate target gene expression
- A constitutively active **Ptr3** mutant is hyperphosphorylated
- Hyperphosphorylation of **Ptr3** requires **Ssy1**
Interaction of **Ptr3** with itself and with **Ssy5**

- Self-interaction of **Ptr3** is independent of **Ssy1**, **Ssy5**, and its phosphorylation status.

- Interaction between **Ptr3** and **Ssy5** is independent of **Ptr3** hyperphosphorylation and of **Ssy1**.

- **Ssy5** is constitutively processed.
Protein interactions in the amino acid-sensing pathway

Without amino acids

Ssy1 would recruit Ptr3 and Yck1, and the Yck1 could phosphorylate Ptr3

With amino acids

Ssy1

Amino acids

Dephosphorylation

Yck1/2

Rts1/PP2A

Ssy5-N

Ssy5-C

Ptr3

Stp1/2

Nucleus

AAP

Stp1/2

Nucleus

AAP
Inferring steps of the research

How cells sense nutrients?

Known glucose-sensing pathway regulators

Regulators of SPS-sensing pathway are unknown.

A more complete SPS-sensing pathway

- The hyperphosphorylation of Ptr3 is the key activation step of the SPS amino-acid sensing pathway

http://www.ebi.ac.uk/microarray/biology_intro.html
Thank you for your attention!
Yeast two-hybrid assay

Normal Transcription

Yeast two-hybrid transcription

http://www.scq.ubc.ca
Ptr3 can interact with itself.

Molecular Microbiology (2001) 41(2), 489–502
Plasma membrane proteins used for nutrient sensing in eukaryotic cells
Why growing yeasts under different temperatures?

- **Permissive**: cell don’t show a growth defect
- **Semipermissive**: cells can grow but with some growth defects
- **Restrictive**: cells don’t grow at all

- **Different mutations may vary** in the range of semi-permissive temperatures
Why mutating amino acids into A and D and E?

- A is considered to be a relatively conservative change.
  - it eliminates the side chain beyond the beta-carbon
  - it does not alter the main chain conformation (as glycine or proline would)
  - it does not impose electrostatic or steric effects

- D and E: to introduce a phospho-mimicry mutant
Why adding **leucine** to induce the SPS-sensing pathway?

**Leucine** is most potent among amino acids

EUKARYOTIC CELL, Oct. 2003, p. 922–929
Hemagglutinin

- The receptor-binding and membrane fusion glycoprotein of influenza virus
- The target for infectivity-neutralizing antibodies

http://www.pdb.org/pdb/explore.do?structureId=1ruz
c-myc

- The myc family of oncogenes (c-myc, N-myc, and L-myc) function in the control of cell proliferation, differentiation, and tumorigenesis.

The reporter gene activity

Miller unit values: (optical density at 420 nm *1,000)/(cell volume [ml] reaction time [min] optical density at 600 nm at harvest)

EUKARYOTIC CELL, Jan. 2006, p. 174–179
Glucose induction

(a) No glucose
(b) Low glucose concentration
(c) High glucose concentration

Repression of HXT genes
Induction of HXT genes
Induction of HXT1

TRENDS in Biochemical Sciences Vol.29 No.10 October 2004
Glucose induction