Nutrient Homeostatic Networks

Outline

1. Background and mechanistic information
2. Systems level - bistability & buffering
Nutrient and Ion Homeostasis

**ENVIRONMENT**
- nutrients, ions
- dynamic, unpredictable

**INTRACELLULAR**
- nutrients, ions
- ~constant

**Nutrients, ions:**
- essential for cellular processes
- toxic in excess

- uptake
- usage
- storage

budding yeast cell

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**Inorganic Phosphate**

\[
P_i^{\text{\textoplus}}\]

- Important for synthesis of ATP and other nucleotides, phospholipids
- Want to maintain constant levels inside cell
- Cannot be made - must be obtained from environment
The Pho Pathway

- **PHO84**: inorganic phosphate transporter
- **PHO81**: CDK inhibitor
- **PHO80, PHO85**: cyclin-CDK complex (protein kinase)
- **PHO4, PHO2**: transcription factors
- **PHO5**: secreted phosphatase

**Increase phosphate uptake**
- Mobilize internal stores of $P_i$
- Sense & transduce
- Change gene expression
- Nucleus
- Secrete phosphatases

**Phosphate starvation**
- $X-P_i$
- Phosphatase
- $P_i$
The Phosphate-Responsive Signaling Pathway

**high phosphate**
- kinase active
- Pho80
- Pho85
- gene expression OFF

**low phosphate**
- kinase inactive
- Pho80
- Pho85
- gene expression ON

Pho4 Localization is Regulated by Phosphorylation

**high phosphate**
- Pho4
- phosphorylated (P)
- Pho4
- GFP

**low phosphate**
- Pho4
- unphosphorylated
- Pho4
- GFP

Images show the localization of Pho4 and GFP under high and low phosphate conditions.
Phosphorylation Regulates Pho4 in Two Ways

1) Location within the cell (subcellular localization)

2) Binding to promoters
Pho4 is Multiply Phosphorylated

In vitro Studies Predict Accumulation of Pho4 Phosphorylated on Site 6
The Kinetic Properties of Pho80-Pho85 Help to Generate Two Thresholds in the PHO Pathway

**Transcription**
- 250 μM: no transcription
- <1 μM: PHO84 on, PHO5 on

**Pho4**
- cytoplasmic
- nuclear
- site 6 phos
- site 6 unphos

**Yeast Cells Tailor Their Response to Environmental Conditions**
- No Phosphate
- Intermediate Phosphate
- High Phosphate

[Phosphate]
Nutrient-Sensitive Regulatory Networks

Copper, Magnesium, Sulfate, Zinc, Phosphate, Inositol

Additional Complexity in Homeostatic Networks

External Nutrient -> Nutrient -> Transporter Synthesis

Post-Translational Regulation

Transcriptional Regulation
<table>
<thead>
<tr>
<th>Questions</th>
<th>Strategy</th>
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<tbody>
<tr>
<td>How does this network structure give rise to homeostasis?</td>
<td>Use single cell reporters to characterize dynamics, response of system</td>
</tr>
<tr>
<td>Is this network structure sufficient to give rise to homeostasis?</td>
<td>Develop computational model based on network structure, experimental measurements</td>
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<tr>
<td>Which aspects of the design are critical for this property?</td>
<td>2 properties: bistability, buffering</td>
</tr>
<tr>
<td>Is the same design/architecture used for many nutrient homeostasis systems?</td>
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Bistability

The Pho Pathway

\[ \text{PHO4} \quad \text{high affinity phosphate transporter} \]

\[ \text{PHO81} \quad \text{CDK inhibitor} \]

\[ \text{PHO80, PHO85} \quad \text{cyclin-CDK complex} \]

\[ \text{PHO4, PHO2} \quad \text{transcription factors} \]

\[ \downarrow \text{PHO5} \quad \text{secreted acid phosphatase} \]

(and other phosphate-responsive genes – e.g. PHO84)

Oshima et al.
Why does deletion of high affinity transporter turn the Pho pathway ON?

Cells Sense Phosphate Internally and Have Two Uptake Systems

- High affinity $P_i$ transport
- Low affinity $P_i$ transport

$[P_i]_{\text{intracellular}}$

$[P_i]_{\text{extracellular}}$
Bistability

either

low affinity transport
ON

or

high affinity transport
ON

Arsenate Sensitivity

- arsenate enters cells through phosphate transporters
- proxy for phosphate uptake

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>pho84Δ</th>
<th>pho84Δ pho81Δ</th>
<th>pho84Δ pho4Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ars^S</td>
<td>ars^R</td>
<td>ars^S</td>
<td>ars^S</td>
</tr>
<tr>
<td>Pi uptake defect</td>
<td>P_i uptake defect</td>
<td>no P_i uptake defect</td>
<td></td>
<td></td>
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</tbody>
</table>

Inhibition of low affinity transport by Pho pathway requires Pho4
Model for Source of Bistability

\[
\text{low } [P_i]_{\text{intra}} \quad \text{Pho pathway ON} \\
\rightarrow \quad \text{high affinity transport ON} \\
\quad \rightarrow \quad \text{low affinity transport OFF}
\]

\[
\text{high } [P_i]_{\text{intra}} \quad \text{Pho pathway OFF} \\
\rightarrow \quad \text{high affinity transport OFF} \\
\quad \rightarrow \quad \text{low affinity transport ON}
\]

Deletion of low affinity transporters \(\rightarrow\) high affinity transport ON (Harashima 2003)

Why does deletion of high affinity transporter turn the Pho pathway ON?

MODEL:

\[
\text{Pho pathway ON} \\
\downarrow \\
\text{low affinity transport} \\
\downarrow \\
\text{decrease in } [P_i]_{\text{intra}}
\]
If inhibition of low affinity transport by Pho pathway requires Pho4...

**PREDICT:**

- \( \text{pho}^{\Delta} \)
- \( \text{pho}^{\Delta} \text{pho}^{\Delta} \)

Pho pathway **ON**

Pho pathway **OFF**

- Pho4 nuclear

- Pho4 cytoplasmic

\( \text{pho}^{\Delta} \)

Monitor Pho\(4^{\Delta}\)DBD-GFP – cannot dimerize or activate transcription but localization regulated appropriately
If low affinity transport is not repressed, \( \text{pho}84\Delta \) strain is not starving for phosphate and does not turn on Pho pathway

Implies Pho84 does not make a significant contribution to phosphate uptake in high phosphate

Why does \( \text{pho}84\Delta \) turn on the Pho pathway?

\[
\begin{align*}
\text{Pho pathway ON} & \\
\downarrow & \\
\text{low affinity transport} & \\
\downarrow & \\
\text{decrease in } [P]_{\text{intra}} &
\end{align*}
\]

MODEL: \( \text{pho}84\Delta \) was transiently starved for phosphate

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**PREDICTIONS:**

(1) \( \text{pho}84\Delta \) should be able to exist with Pho pathway OFF

(2) Pho84 is important for buffering against transient decreases in extracellular phosphate

(3) Phosphate uptake defect in \( \text{pho}84\Delta \) strain should be dependent on growth conditions

**EXPERIMENTS:**

Grow \( \text{pho}84\Delta \) cells containing Pho4-GFP for prolonged period of time in high phosphate – predict Pho4 will be cytoplasmic

Transiently deprive \( \text{pho}84\Delta \) cells of phosphate, add back phosphate – predict that cells will still have Pho4 in nucleus

Measure phosphate uptake of \( \text{pho}84\Delta \) cells grown in hi or no phosphate – predict that phosphate uptake rate will be Pho4-dependent and growth-condition dependent
Phosphate Uptake in \textit{pho84\Delta} Strain

![Graph showing phosphate uptake in different strains](image)

- \textit{PHO84\ pho4\Delta}
- \textit{pho84\Delta\ pho4\Delta}
- \textit{pho80\Delta\ pho4\Delta}

**Graph Details:**
- X-axis: [phosphate] (micromolar)
- Y-axis: Velocity (moles/(min\*OD))
- Lines represent different conditions:
  - \textit{pho84\Delta\ pho4\Delta: Hi P}_i
  - \textit{pho84\Delta: Hi P}_i
  - \textit{pho84\Delta\ pho4\Delta: No P}_i
  - \textit{pho84\Delta: No P}_i
Conclusions...

\( pho84 \Delta \) strain exhibits hysteresis – becomes “stuck” in Pho pathway ON state if transiently starved

Implies Pho84 is important for getting out of state where Pho pathway is ON

![Diagram showing low affinity transport OFF, active Pho4, high affinity transport ON (Pho84)]

- high \([P_i]_{\text{intra}}\), Pho pathway OFF
  - high affinity transport OFF
  - low affinity transport ON

As \([P_i]_{\text{extra}}\) decreases, low affinity transporters can no longer keep up with cellular needs

- \([P_i]_{\text{intra}}\) decreases
- Pho pathway ON
  - high affinity transport ON
  - low affinity transport OFF

Predicts that \( PHO84 \) transcription might be bistable
**PHO84 Transcription is Bistable**

FACS analysis of PHO5::GFP and PHO84pr::GFP induction as a function of Pi concentration

**Slow Interconversion Between Two States**

Grow cells 3 hrs in intermediate P_{i}

**PHO84 OFF**
LAT ON

**PHO84 ON**
LAT OFF

No Pi
Intermediate Pi
High Pi

Pho84 Expression (GFP Fluorescence)
Pho84 Expression (GFP Fluorescence)

Grown 20 hrs post-sort
Properties of Two Populations of Cells in Intermediate Phosphate

**PHO84 ON**
- Pho4 localized to nucleus
- *High affinity transport?*

**PHO84 OFF**
- Pho4 localized to cytoplasm
- *Low affinity transport?*

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**Summary**

- low affinity transport OFF
- active Pho4
- high affinity transport ON (Pho84)

- low phosphate
  - low affinity transport OFF
  - high affinity transport ON

- high phosphate
  - low affinity transport ON
  - high affinity transport OFF

Feedback from repression of low affinity transport by the Pho pathway probably contributes to bistability of *PHO84* induction - test by interfering with feedback and examining bistability
Is this bistability advantageous?

Two populations may have different growth rates or fitness when grown in hi or no phosphate

Test by sorting cells, measuring growth rate in different [phosphate], arsenate resistance

What generates split in population?

Role of stochasticity?

Buffering
**P_i metabolism and signaling**

NUTRIENT

low affinity transporters

high affinity transporter

Pho4

Pho84

Pho90

Phm1

Phm3

Phm5

polyP_i

nucleus

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**PHO84 and PHO5 Exhibit Very Different Kinetics of Induction in No Phosphate**

![Graph](image)

**Kinetics of polyP usage mirrors kinetics of PHO5 induction**
**PREDICTION:**

If polyP is functioning as a buffer that is mobilized to slow PHO5 induction, cells lacking polyP should induce PHO5 rapidly.
Loss of Polyphosphate Does Not Affect Threshold of Phosphate Required for PHO5 Induction

Polyphosphate Mobilization Buffers Cells From Transient Fluctuations in Phosphate
Experimental design

- For the pRS-Pho4-ΔDBD-GFP experiments, the strains were grown O/N in SD, starved for 45', then either fed with 11 mM Pi for 15' (labeled ‘high Pi’) or continued to starve (labeled ‘no Pi’). This starve/feed was done to produce uniformly nuclear signal in the pho84 delete, and mimics our traditional practice of diluting from an overnight saturated culture.

- For the YCp50-Pho4-GFP experiments, the strains were grown in log phase in SD complete for ∼20 hours (1st picture), then starved for 30’ in no Pi (2nd picture), then fed with 7 mM Pi, equivalent to SD, for 20 minutes (3rd picture) and 40 minutes (4th picture). The only source of Pho4 in these strains is the plasmid.

- The TIF files are labeled logically; if you open them with Photoshop, you have to adjust the contrast/brightness (they will look blank until you do so). The JPEG’s for import into Powerpoint are called ‘Pho4feed’ and ‘Pho4DBDGGFP.’

Nutrient-Sensitive Regulatory Networks

Copper
Magnesium
Sulfate
Zinc
Phosphate
Inositol
pho and zinc homeostasis projects

- experimental measurements
  transcription/transporter abundance
  steady-state and dynamic behaviors
  perturbation analysis (mutation and conditions)
  physiological assays

- computational modeling and control theory
  analysis
  fit and predict experimental data
  model ideal behavior and control

**PHO84 Transcription is Bistable**

FACS analysis of \( PHO5::GFP \) and \( PHO84pr::GFP \) induction as a function of Pi concentration