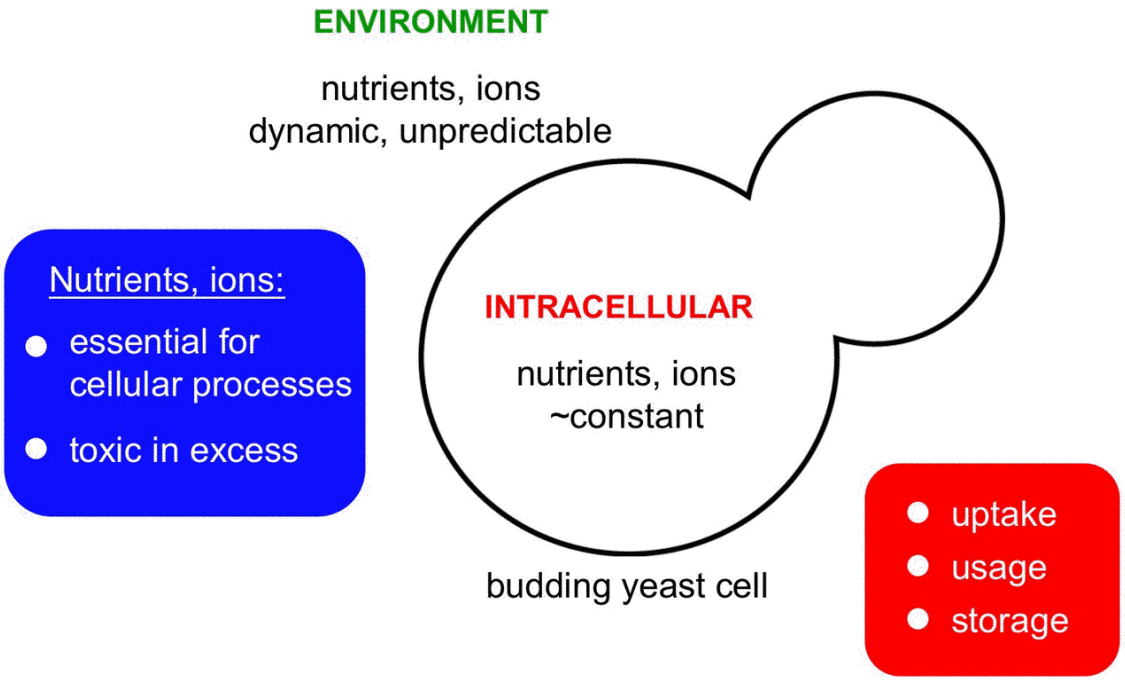
Nutrient Homeostatic Networks

Outline

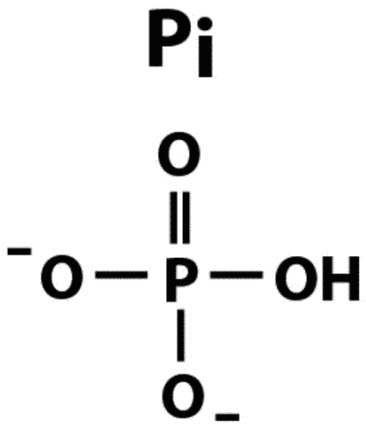
1. Background and mechanistic information

2. Systems level - bistability & buffering

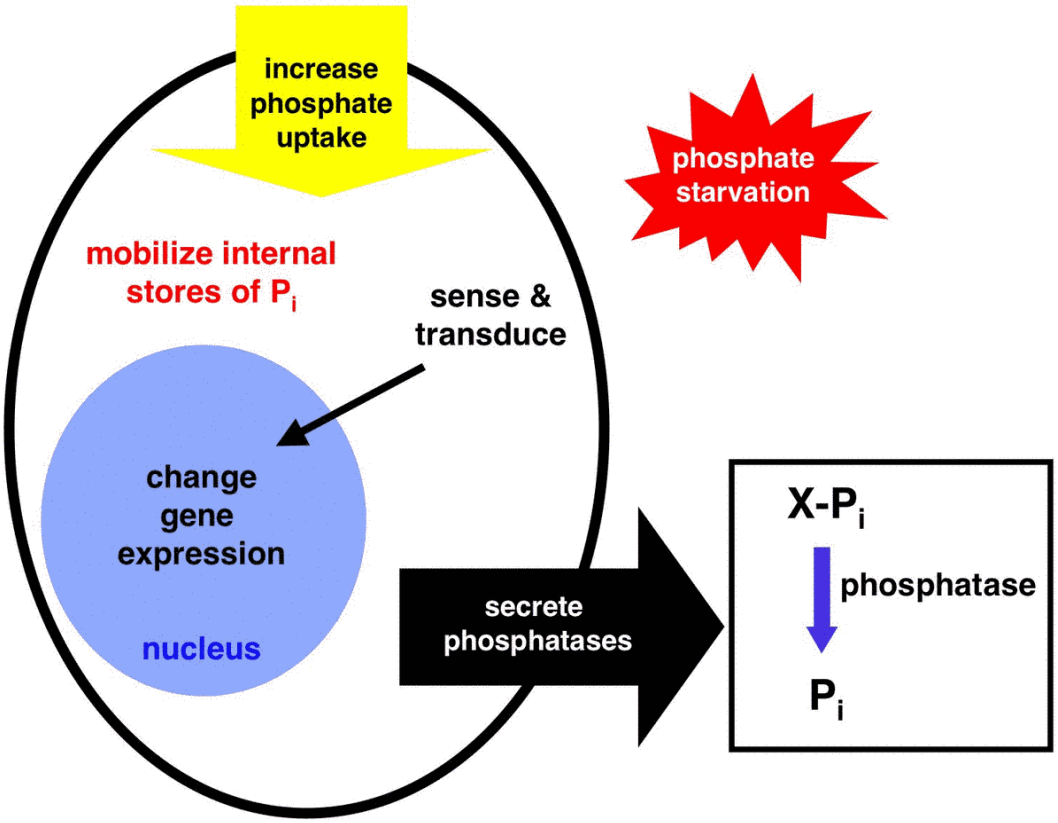
Nutrient and Ion Homeostasis



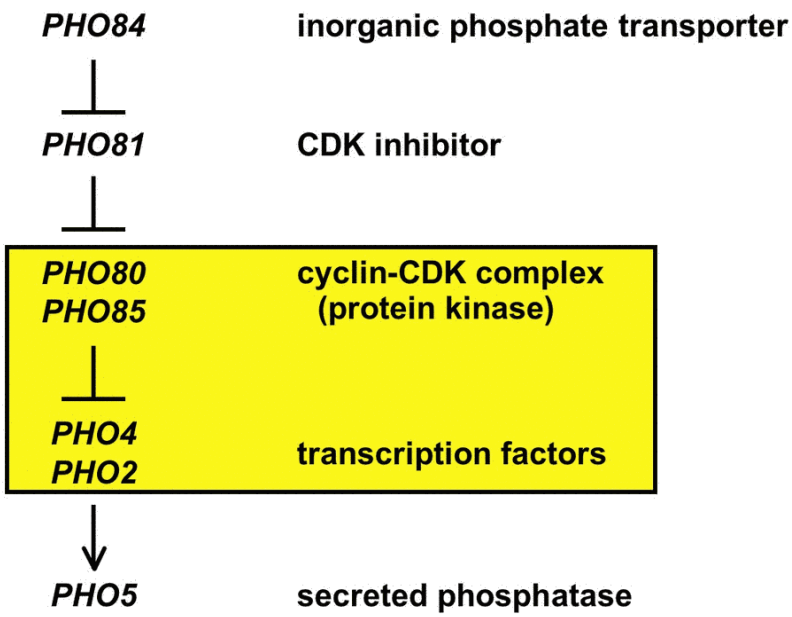
Inorganic Phosphate



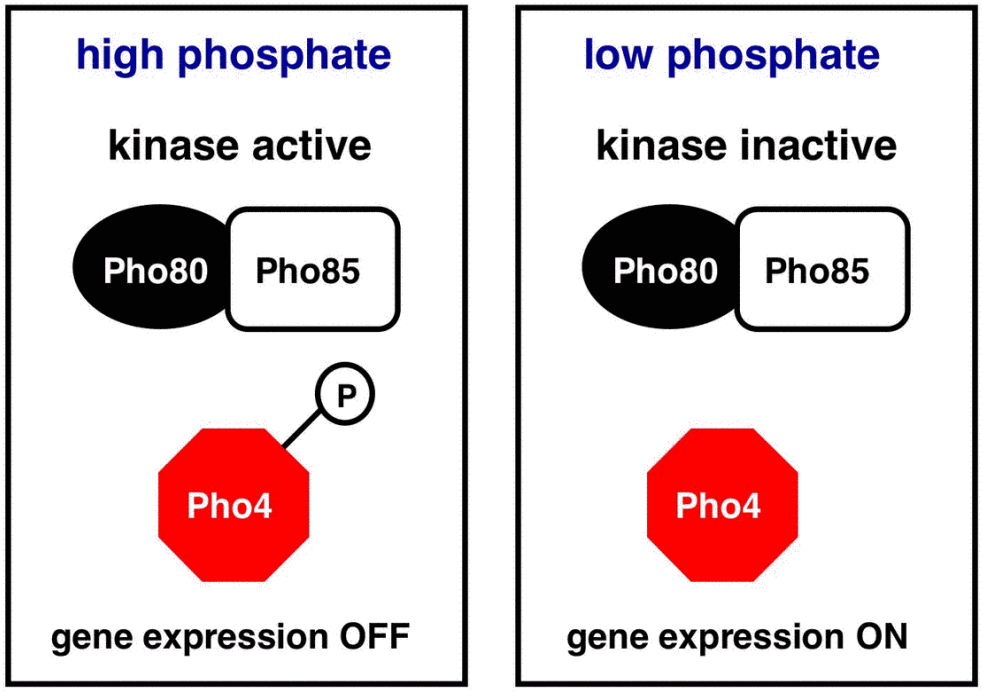
- **Important for synthesis of ATP and other nucleotides, phospholipids**
- **Want to maintain constant levels inside cell**
- **Cannot be made - must be obtained from environment**



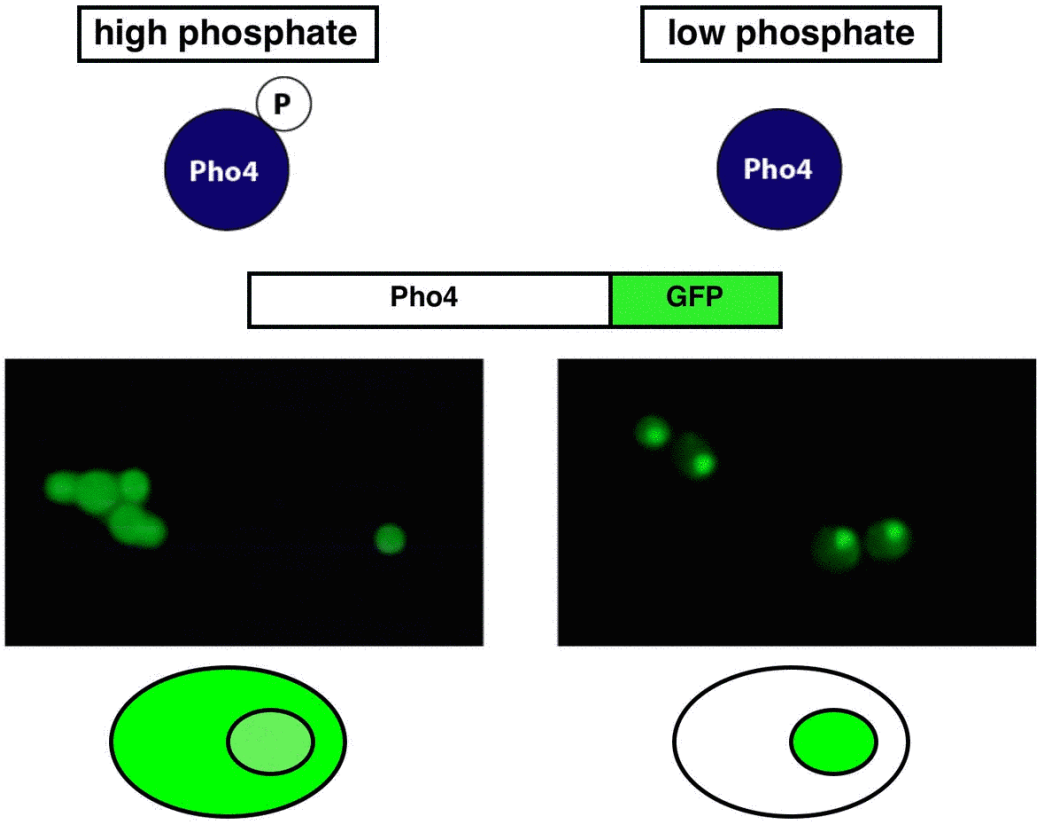
The Pho Pathway

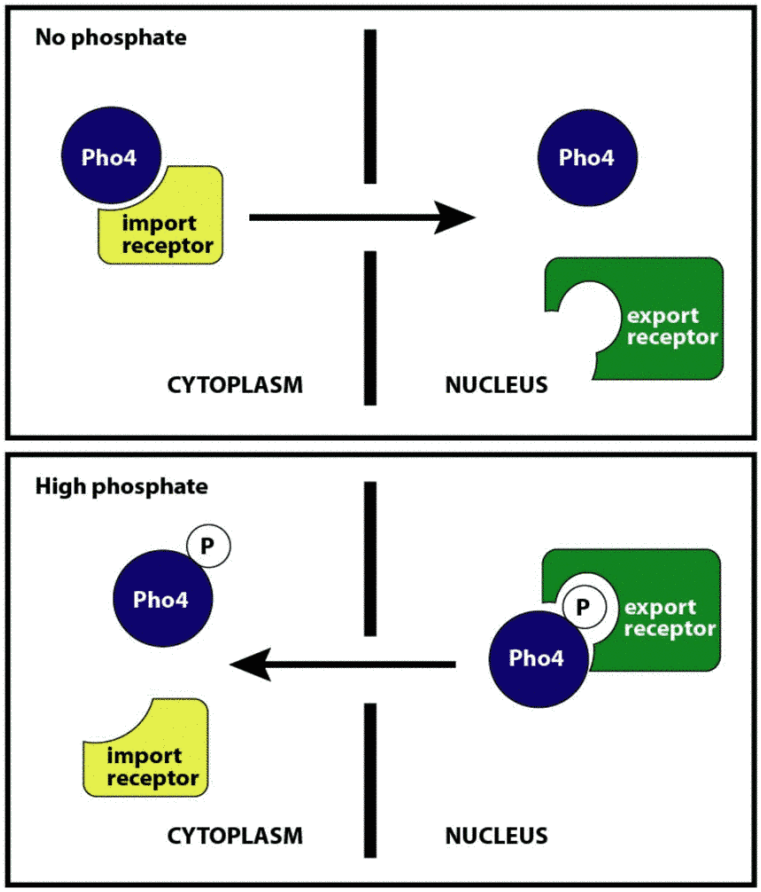


The Phosphate-Responsive Signaling Pathway



Pho4 Localization is Regulated by Phosphorylation

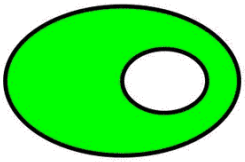




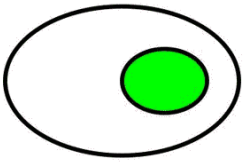
Pho4 localization

Phosphorylation Regulates Pho4 In Two Ways

1) Location within the cell (subcellular localization)

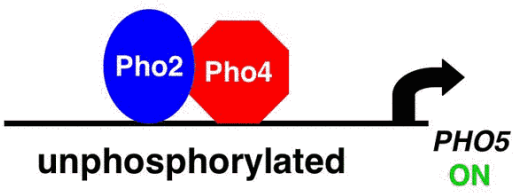


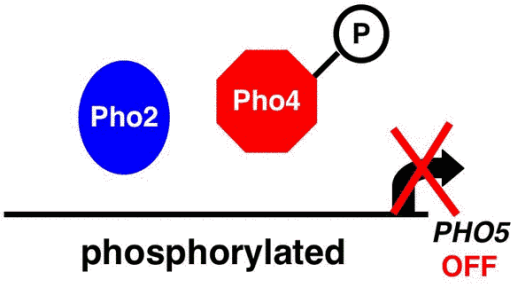
phosphorylated



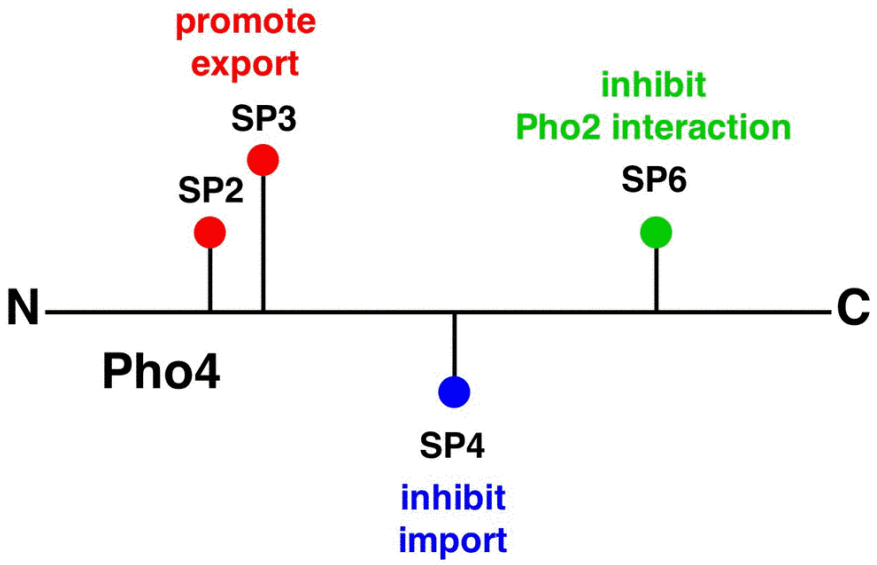
unphosphorylated

2) Binding to promoters

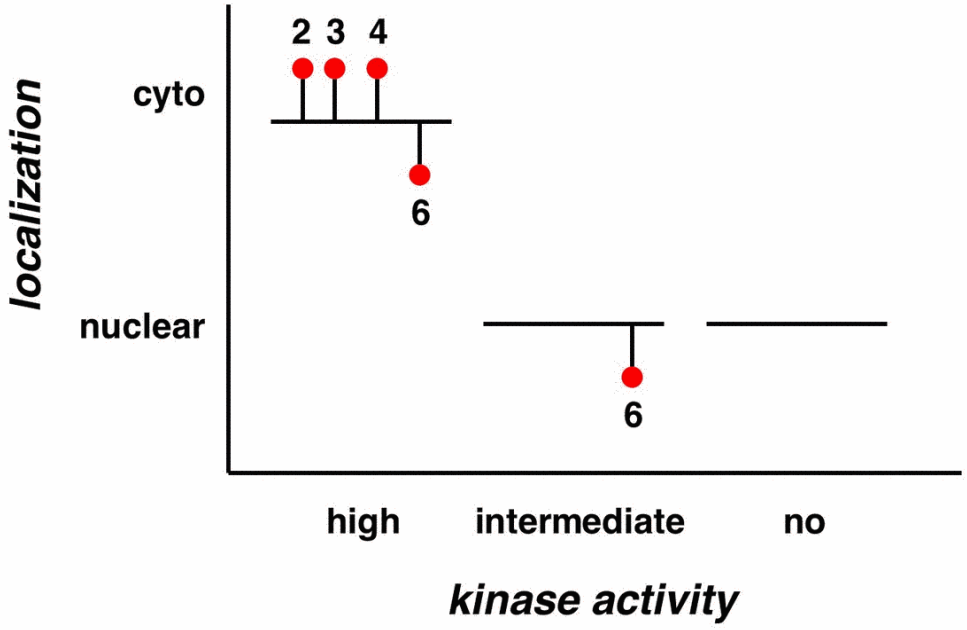




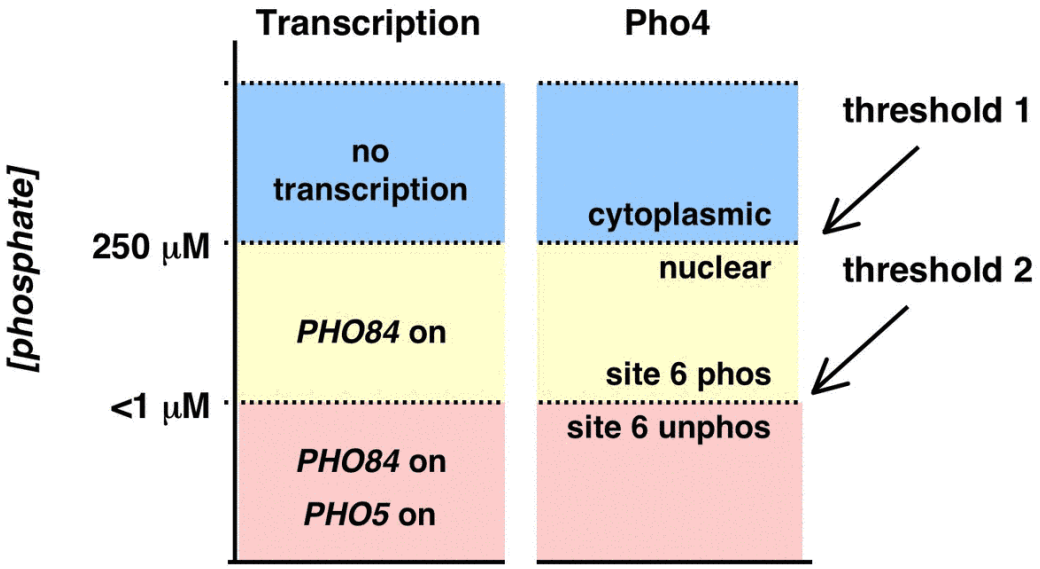
Pho4 is Multiply Phosphorylated



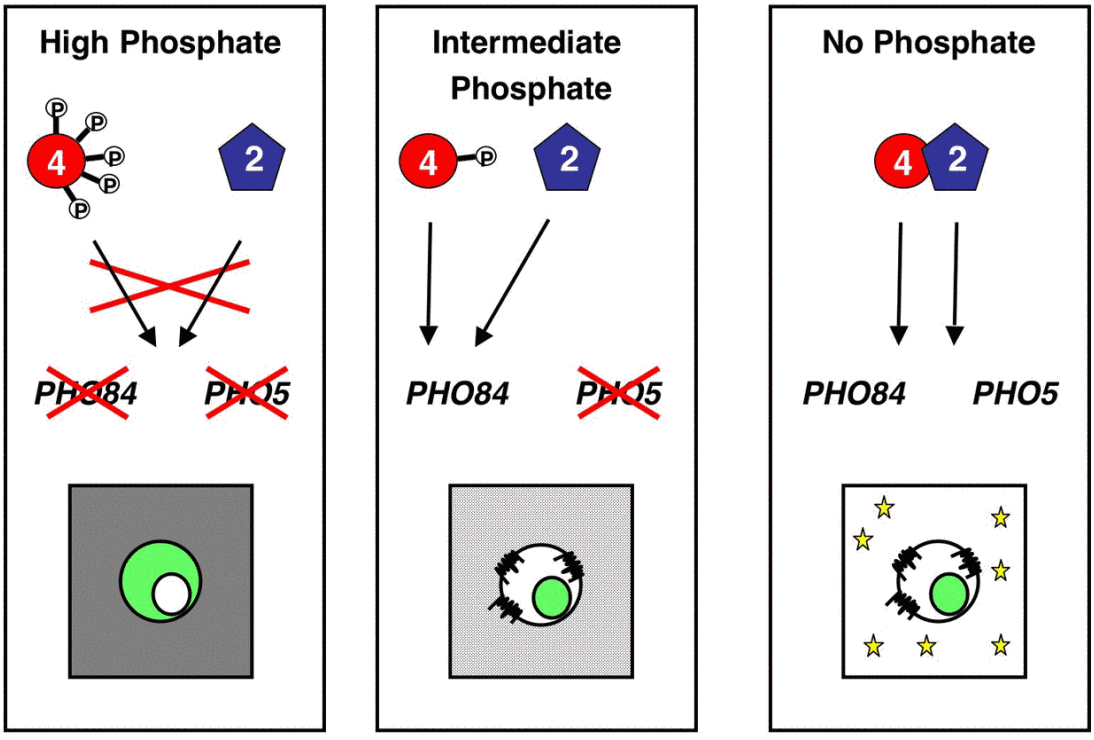
In vitro Studies Predict Accumulation of Pho4 Phosphorylated on Site 6



The Kinetic Properties of Pho80-Pho85 Help to Generate Two Thresholds in the *PHO* Pathway

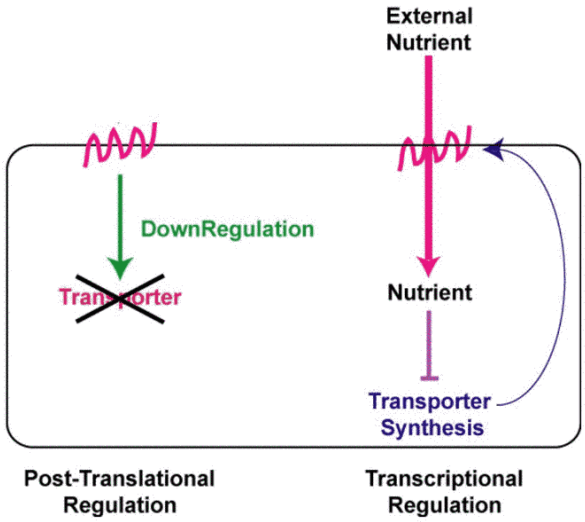


Yeast Cells Tailor Their Response to Environmental Conditions

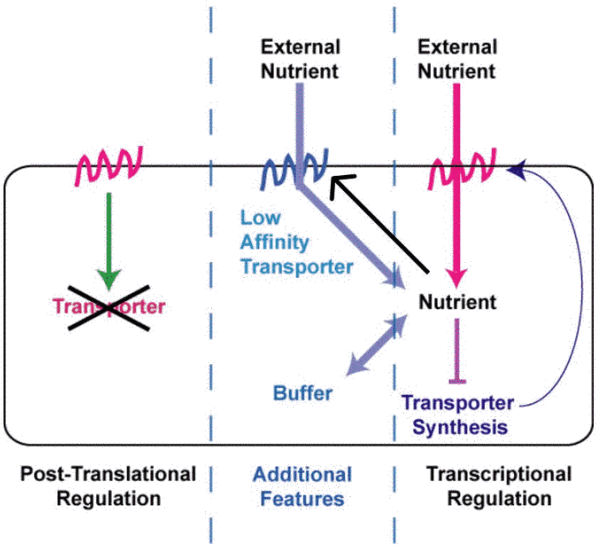


Nutrient-Sensitive Regulatory Networks

Copper
Magnesium
Sulfate
Zinc
Phosphate
Inositol



Additional Complexity in Homeostatic Networks



Questions

How does this network structure give rise to homeostasis?

Is this network structure sufficient to give rise to homeostasis?

Which aspects of the design are critical for this property?

Is the same design/architecture used for many nutrient homeostasis systems?

Strategy

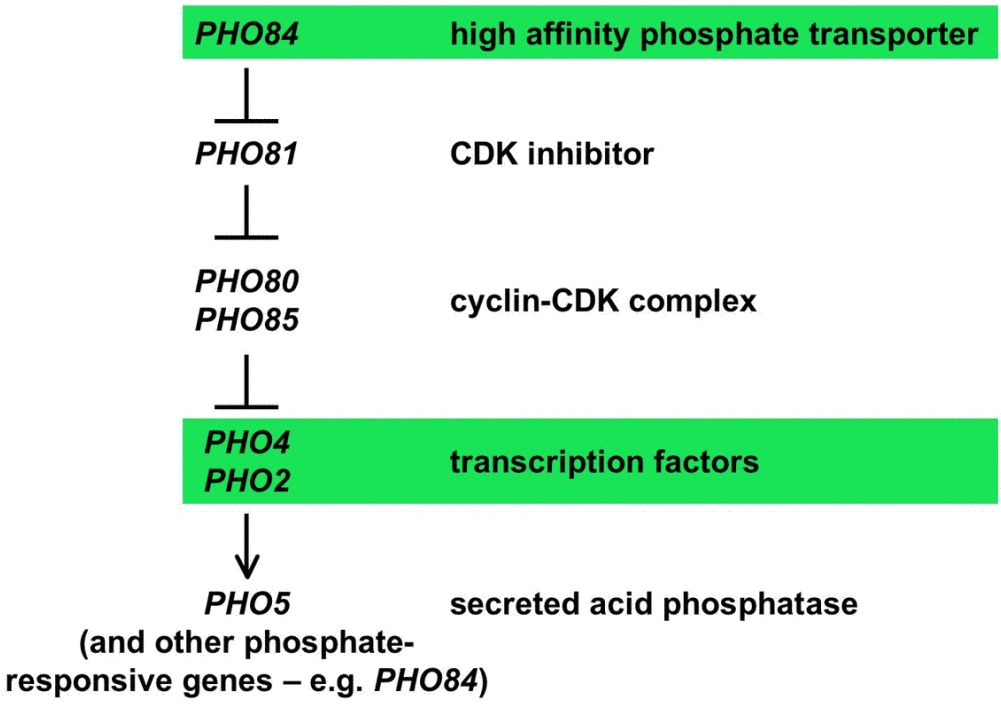
Use single cell reporters to characterize dynamics, response of system

Develop computational model based on network structure, experimental measurements

2 properties: bistability, buffering

Bistability

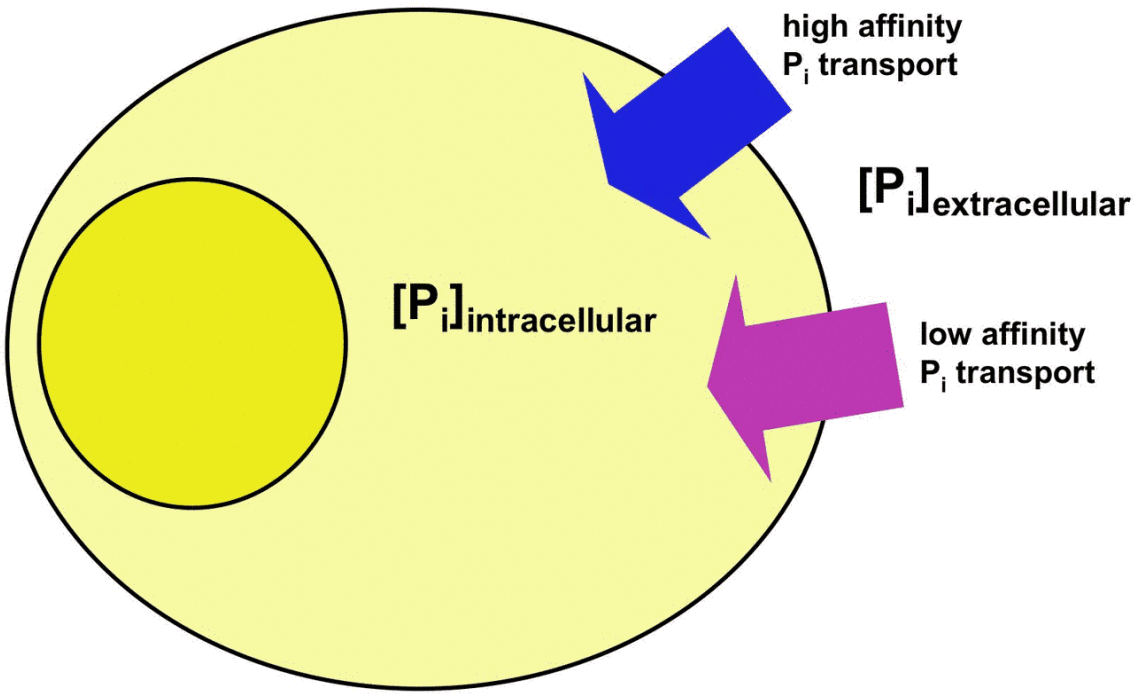
The Pho Pathway



Oshima et al.

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

Cells Sense Phosphate Internally and Have Two Uptake Systems



Bistability

either

low affinity transport
ON

or

high affinity transport
ON

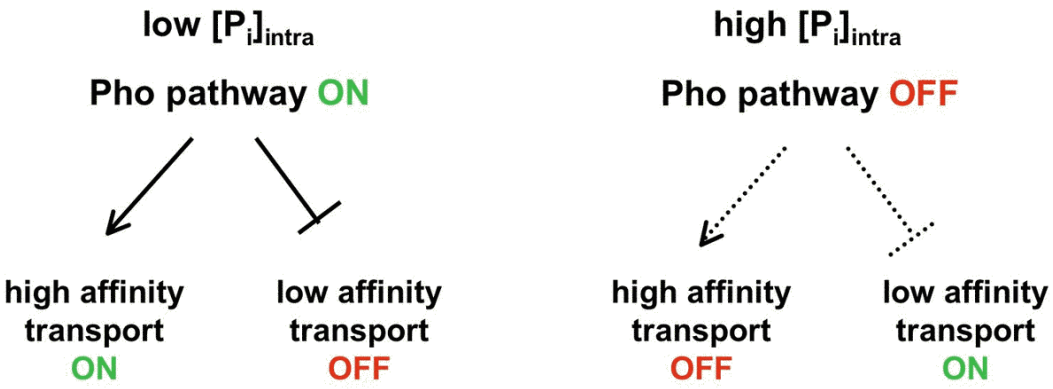
Arsenate Sensitivity

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

WT	ars^S	
pho84Δ	ars^R	P_i uptake defect
pho84Δ pho81Δ	ars^S	no P_i uptake defect
pho84Δ pho4Δ	ars^S	

Inhibition of low affinity transport by Pho pathway requires Pho4

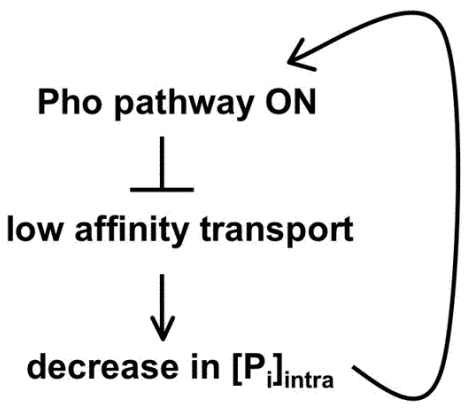
Model for Source of Bistability



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

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

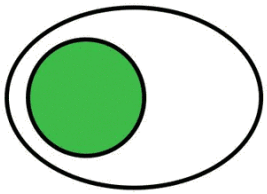
MODEL:



If inhibition of low affinity transport by Pho pathway requires Pho4…

PREDICT: *pho84Δ* — Pho pathway ON
pho84Δ pho4Δ — Pho pathway OFF

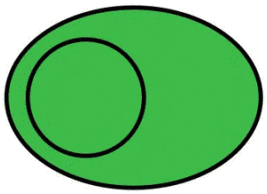
Pho pathway ON



Pho4 nuclear

pho84Δ

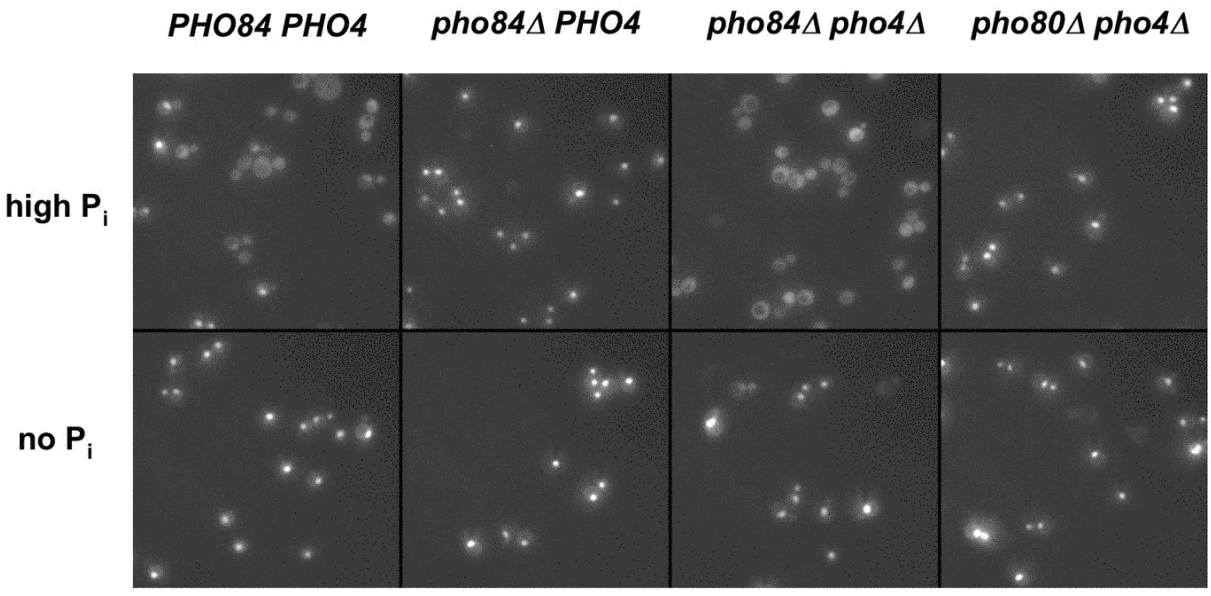
Pho pathway OFF



Pho4 cytoplasmic

pho84Δ pho4Δ

Monitor Pho4ΔDBD-GFP – cannot dimerize or activate transcription but localization regulated appropriately

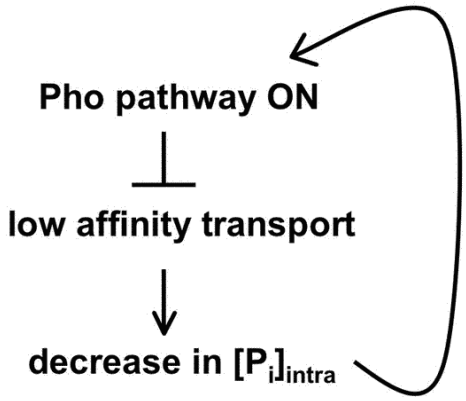


pRS-Pho4ΔDBD-GFP

If low affinity transport is not repressed, *pho84Δ* 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 *pho84Δ* turn on the Pho pathway?



MODEL: *pho84Δ* was transiently starved for phosphate

PREDICTIONS:

(1) *pho84Δ* should be able to exist with Pho pathway OFF

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

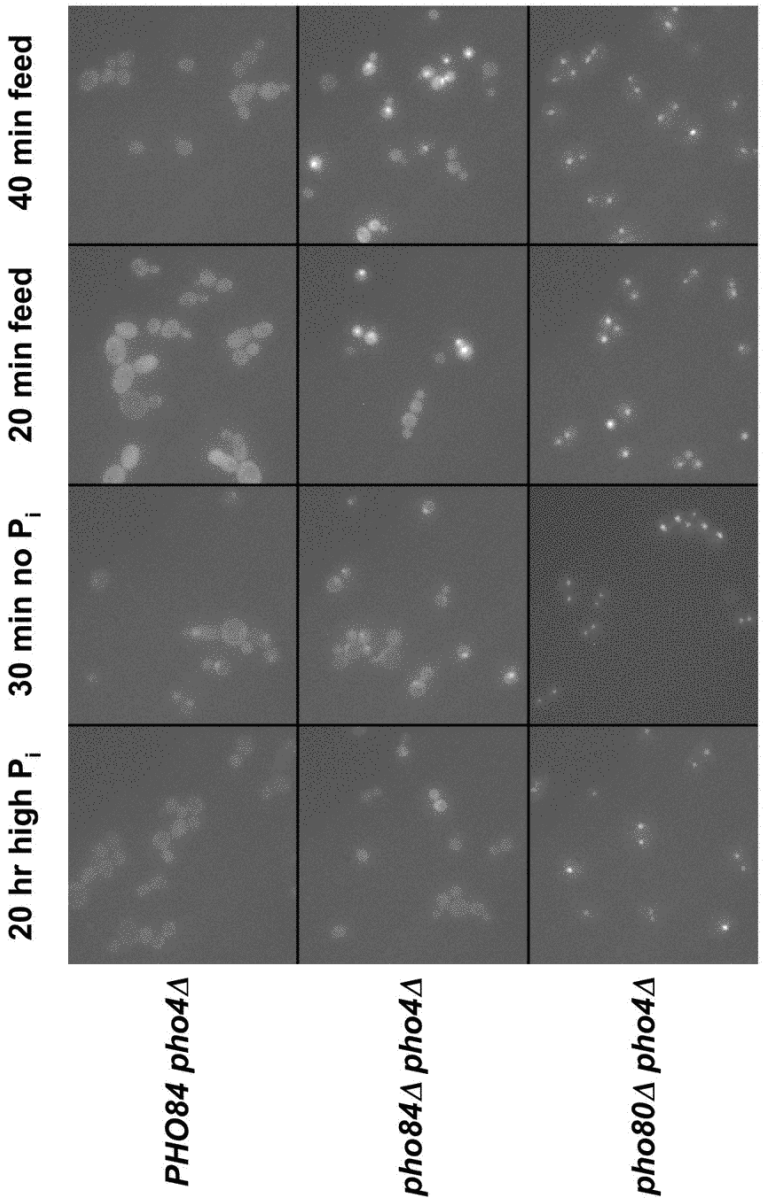
(3) Phosphate uptake defect in *pho84Δ* strain should be dependent on growth conditions

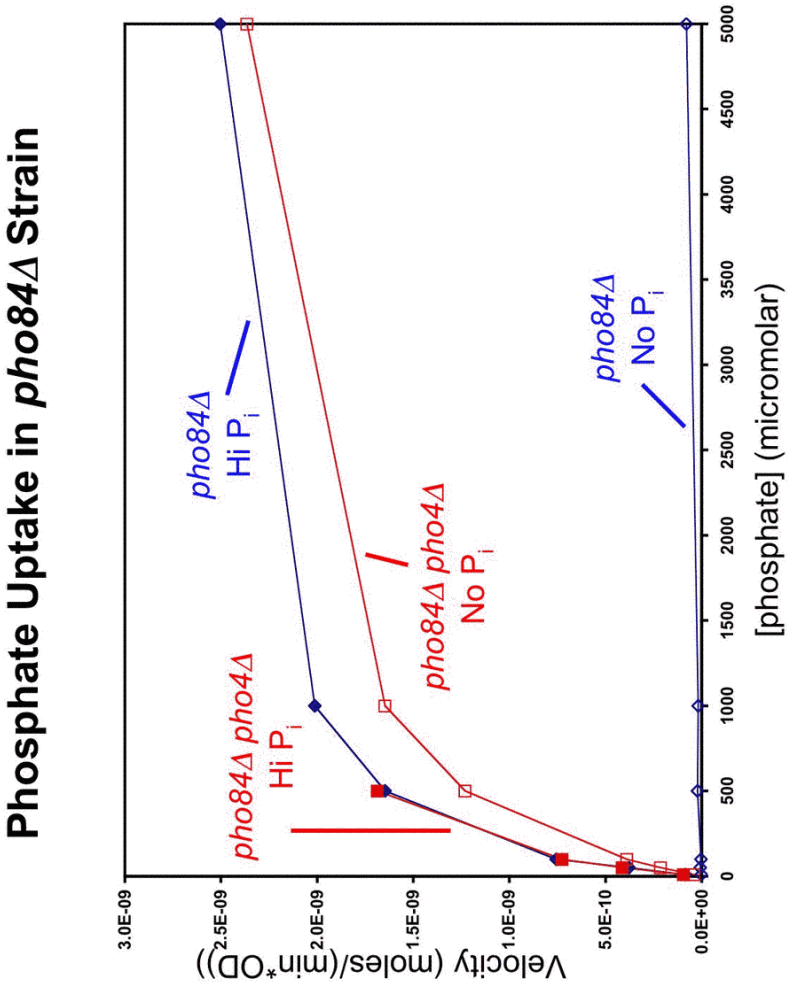
EXPERIMENTS:

Grow *pho84Δ* cells containing Pho4-GFP for prolonged period of time in high phosphate – predict Pho4 will be cytoplasmic

Transiently deprive *pho84Δ* cells of phosphate, add back phosphate – predict that cells will still have Pho4 in nucleus

Measure phosphate uptake of *pho84Δ* cells grown in hi or no phosphate – predict that phosphate uptake rate will be Pho4-dependent and growth-condition dependent

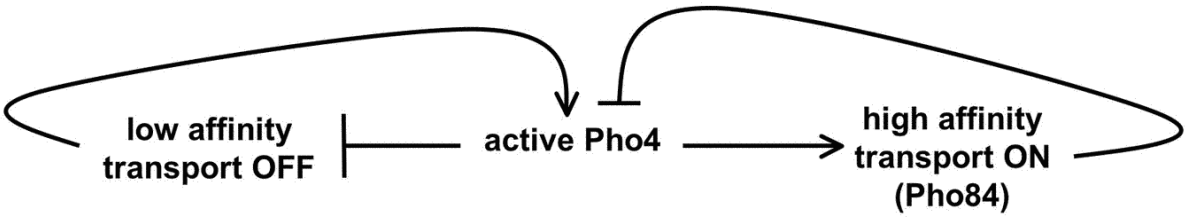

20 hr high P_i
30 min no P_i
20 min feed
40 min feed
PHO84 pho4Δ
pho84Δ pho4Δ
pho80Δ pho4Δ
YCp50-Pho4-GFP


Phosphate Uptake in pho84Δ Strain
Velocity (moles/(min*OD))
[phosphate] (micromolar)
pho84Δ Hi P_i
pho84Δ pho4Δ Hi P_i
pho84Δ pho4Δ No P_i
pho84Δ No P_i
3.0E-09
2.5E-09
2.0E-09
1.5E-09
1.0E-09
5.0E-10
0.0E+00
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000

Conclusions…

pho84Δ **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



high $[P_i]_{intra}$

Pho pathway OFF

high affinity transport OFF

low affinity transport ON

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

$[P_i]_{intra}$ decreases

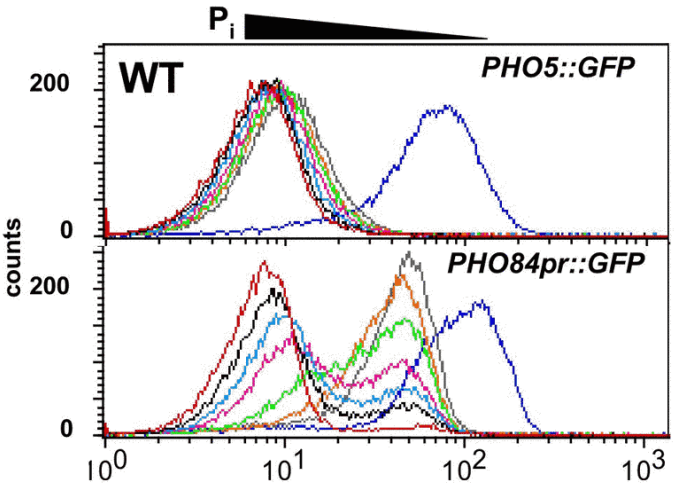
Pho pathway ON

high affinity transport ON

low affinity transport OFF

Predicts that *PHO84* transcription might be bistable

PHO84 Transcription is Bistable

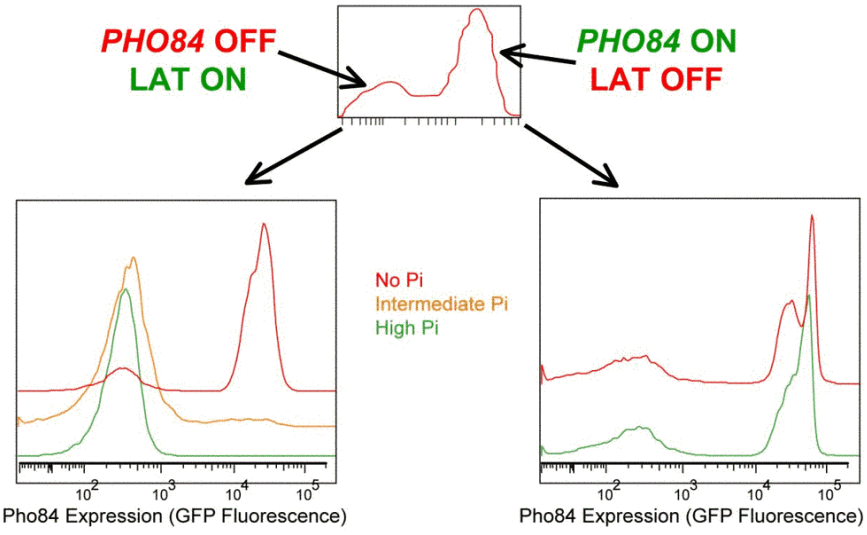


[PO$_4$] (μM)
no (blue)
10 (gray)
50 (orange)
100 (green)
150 (pink)
200 (cyan)
250 (black)
1000 (red)

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$_i$



Grown 20 hrs post-sort

Properties of Two Populations of Cells in Intermediate Phosphate

***PHO84* ON**

Pho4 localized to nucleus

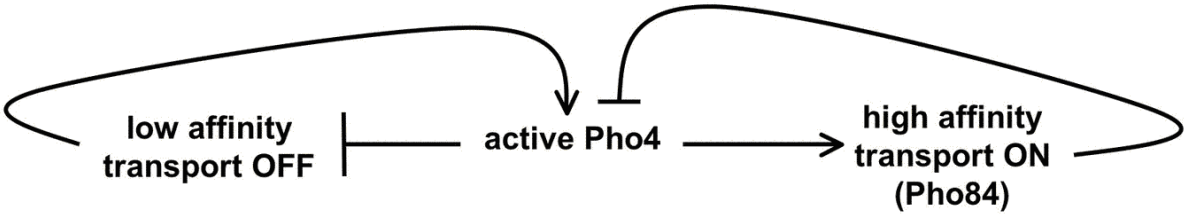
High affinity transport?

***PHO84* OFF**

Pho4 localized to cytoplasm

Low affinity transport?

Summary



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

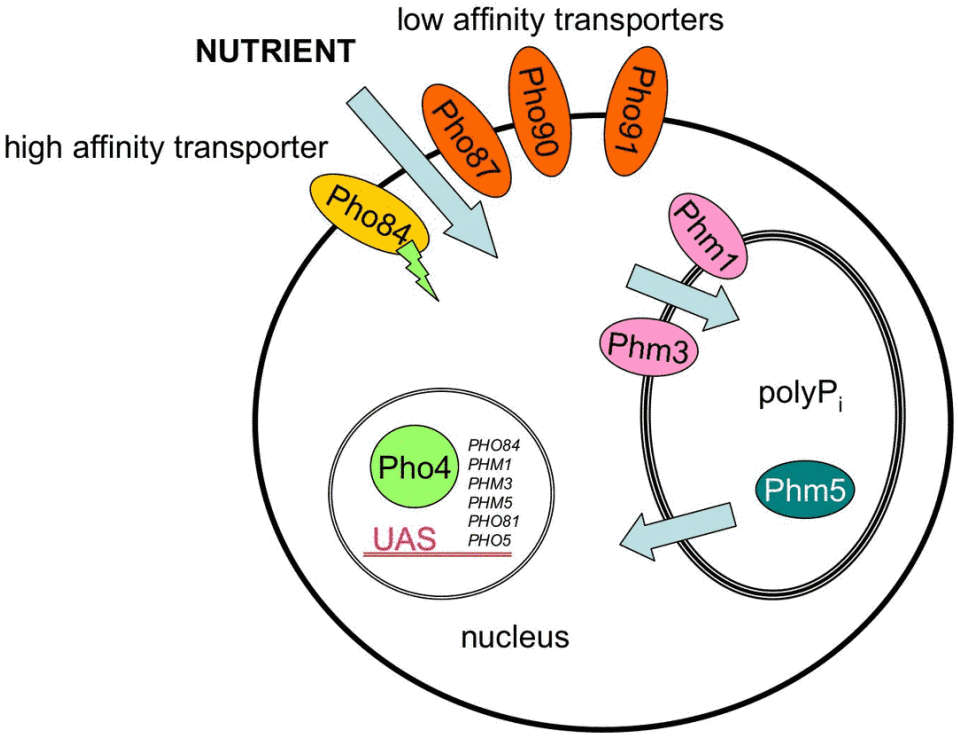
Test by sorting cells, measuring growth rate in different [phosphate], arsenate resistance

What generates split in population?

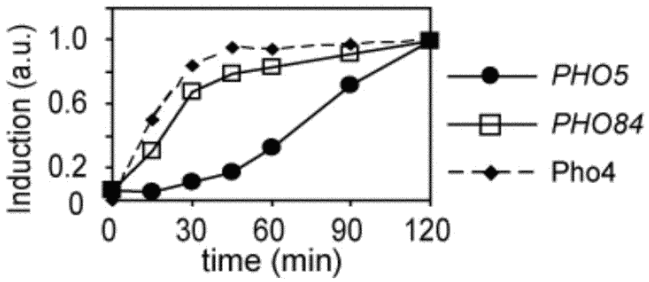
Role of stochasticity?

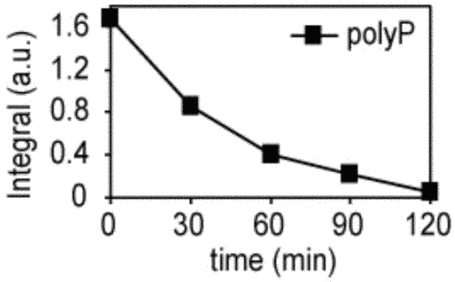
Buffering

P_i metabolism and signaling



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



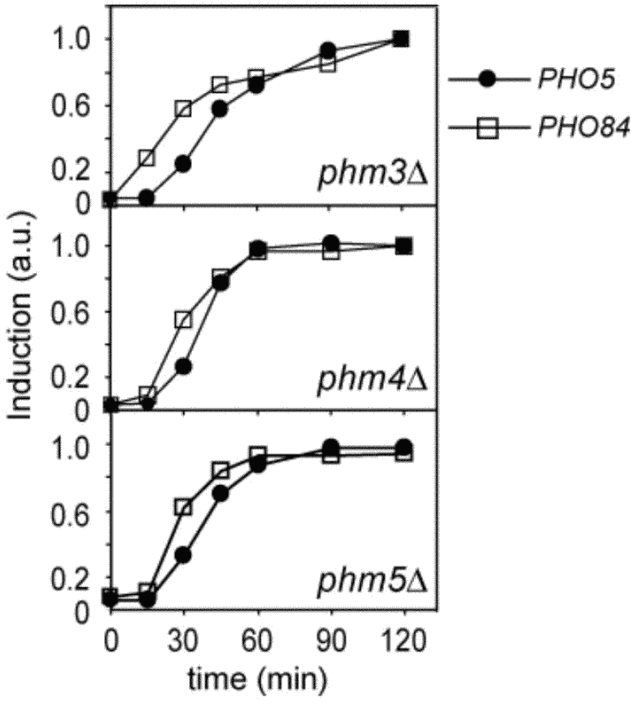


Kinetics of polyP usage mirrors kinetics of *PHO5* induction

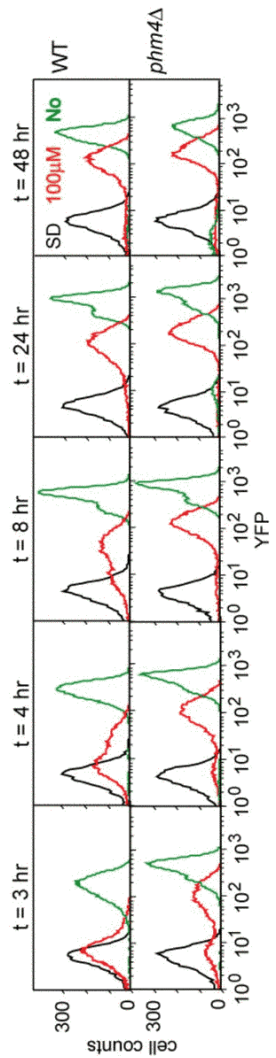
PREDICTION:

If polyP is functioning as a buffer that is mobilized to slow *PHO5* induction, cells lacking polyP should induce *PHO5* rapidly

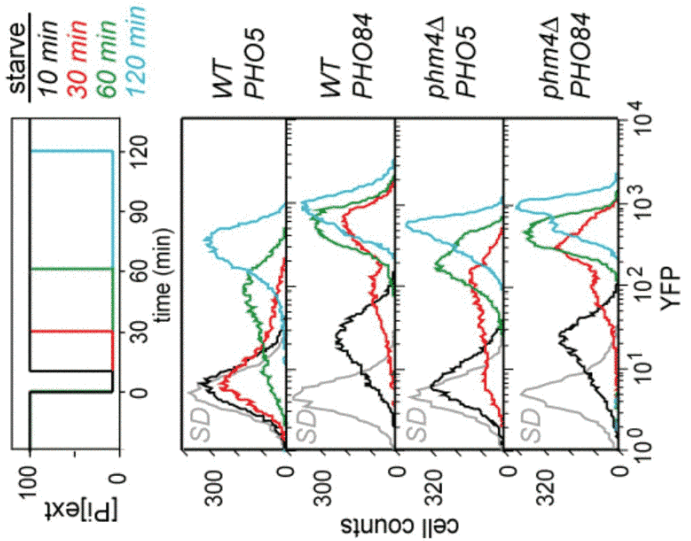
No Kinetic Delay in *PHO5* Induction in Cells With Altered Polyphosphate Metabolism



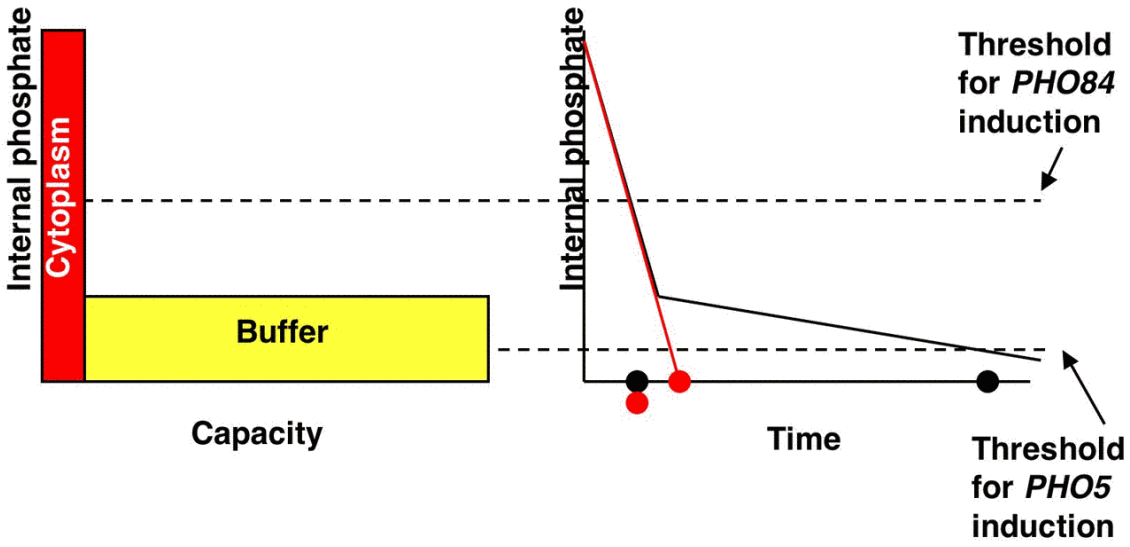
Loss of Polyphosphate Does Not Affect Threshold of Phosphate Required for *PHO5* Induction



Polyphosphate Mobilization Buffers Cells From Transient Fluctuations in Phosphate



Buffering and Sensitivity



Jonathan Raser
Dennis Wykoff
Brian Margolin

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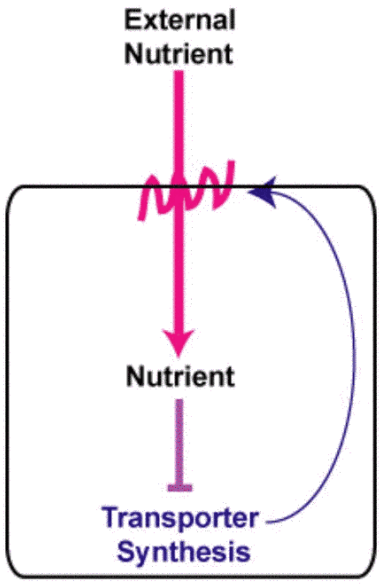
Mike Springer

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 'Pho4DBDGFP.'

Nutrient-Sensitive Regulatory Networks

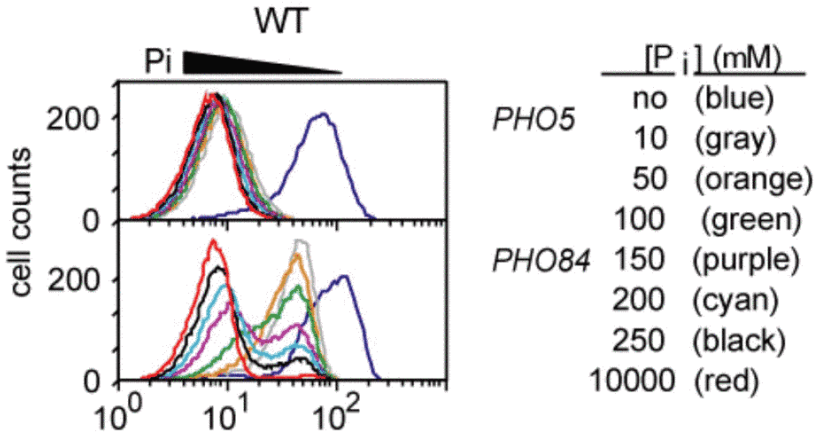
Copper
Magnesium
Sulfate
Zinc
Phosphate
Inositol



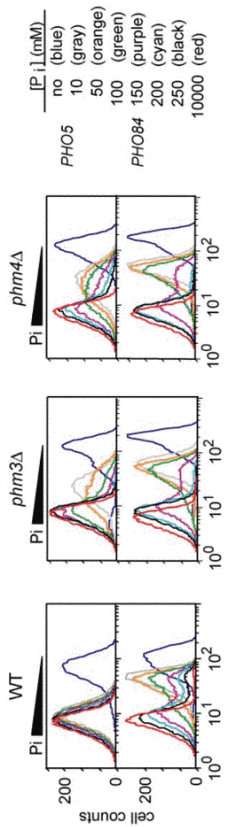
pho and zinc homeostasis projects

- 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


WT
phm3Δ
phm4Δ
Pi
cell counts
200
0
10^0
10^1
10^2
[P i] (mM)
PHO5
no (blue)
10 (gray)
50 (orange)
100 (green)
PHO84
150 (purple)
200 (cyan)
250 (black)
10000 (red)