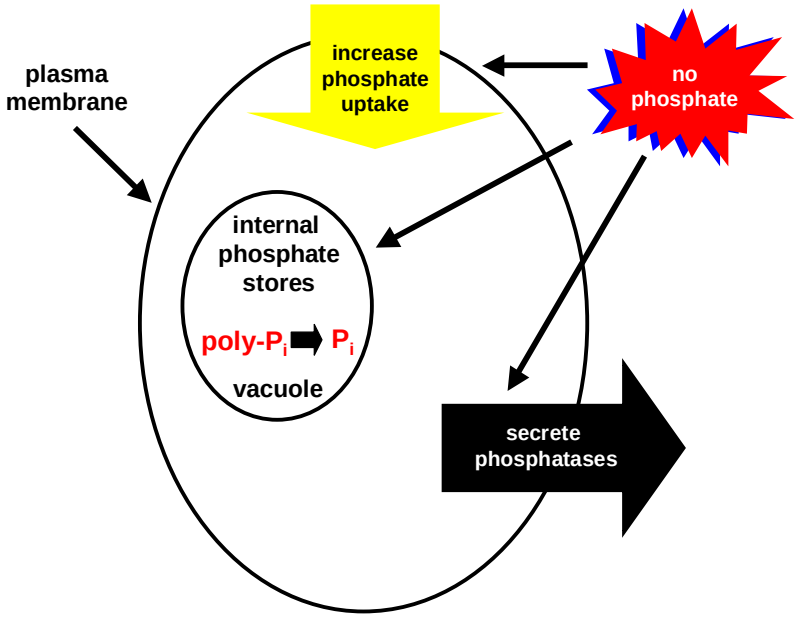
How do cells monitor their environment and respond appropriately?

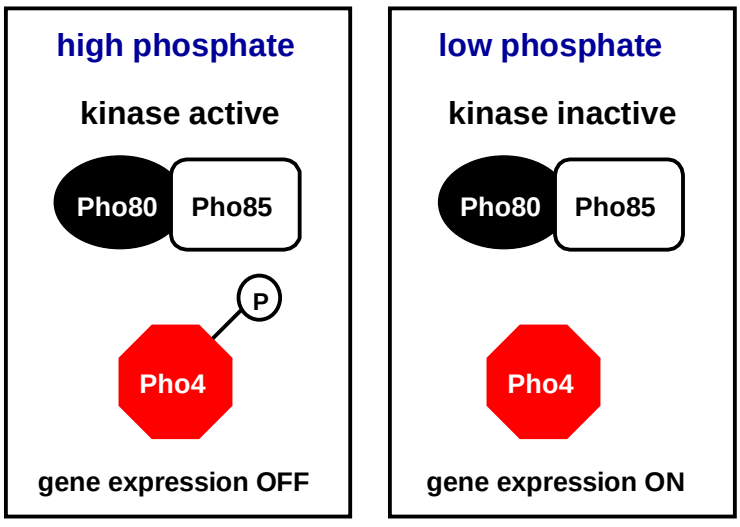
Cell's Response to Phosphate Limitation

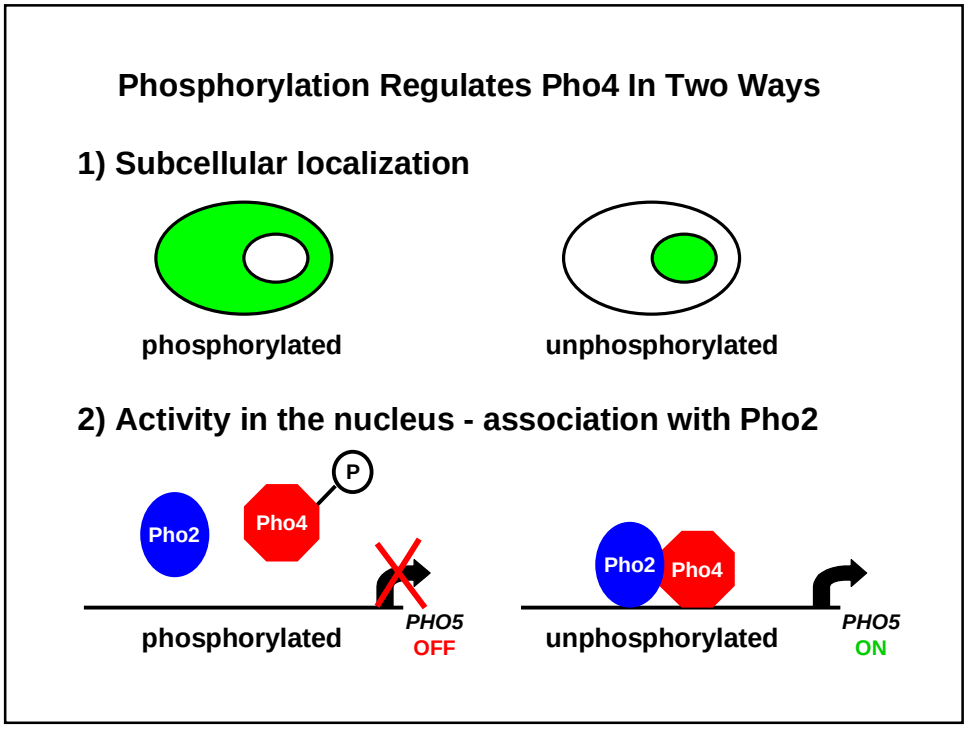


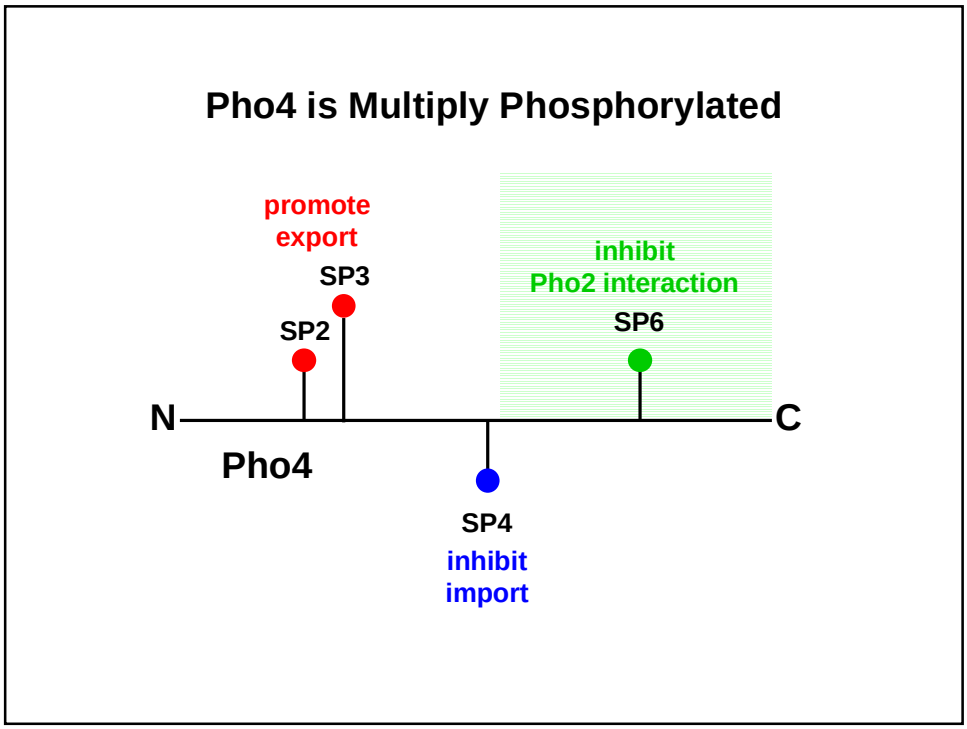
How do cells make different responses to different phosphate levels?

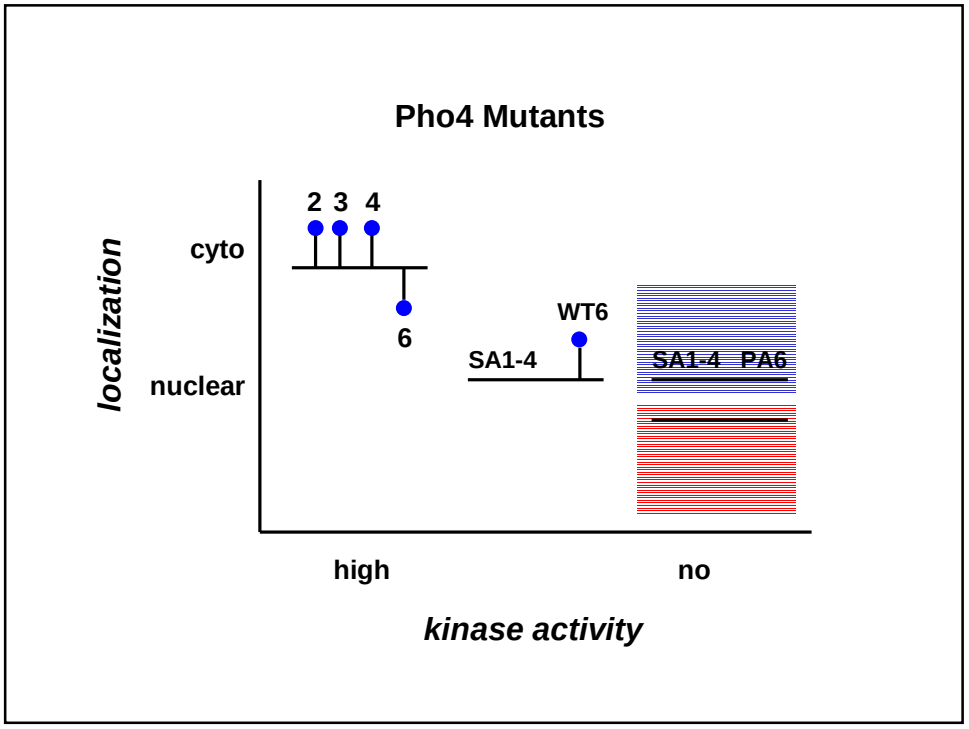
How are phosphate levels sensed?

The Phosphate-Responsive Signaling Pathway




Phosphorylation Regulates Pho4 In Two Ways
1) Subcellular localization
phosphorylated
unphosphorylated
2) Activity in the nucleus - association with Pho2
Pho2
Pho4
P
phosphorylated
PHO5
OFF
Pho2
Pho4
unphosphorylated
PHO5
ON


Pho4 is Multiply Phosphorylated
promote
export
SP3
SP2
inhibit
Pho2 interaction
SP6
N
C
Pho4
SP4
inhibit
import



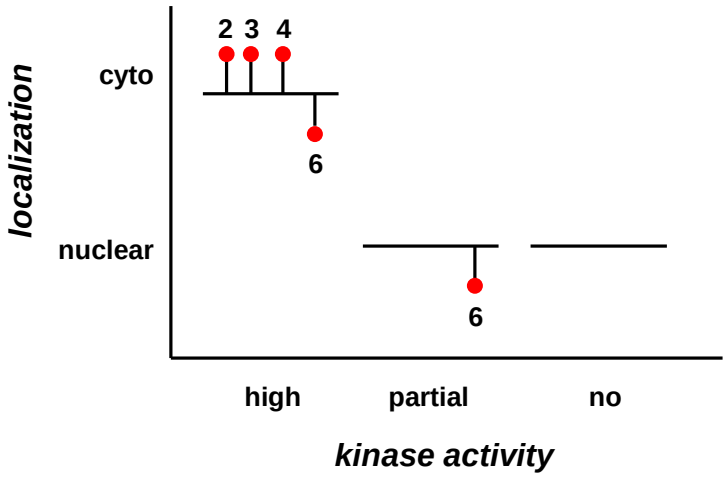
Gene Expression Induced by Pho4 Mutants

Pho4SA1-4PA6		Pho4SA1-4WT6	
100%		>65%	<20%
PHM8	*PHO5*	*PHM8*	*PHO5*
AUT4	*PHO89*	*AUT4*	*PHO89*
CTF19	*PHO11*	*CTF19*	*PHO11*
SDT1	*PHO12*	*SDT1*	*PHO12*
PHM1	*PHM6*	*PHM1*	*PHM6*
PHO8		*PHO8*	
YPL017c		*YPL017c*	
PHM3		*PHM3*	
PHM2		*PHM2*	
PHO86		*PHO86*	
NCP1		*NCP1*	
PHM4		*PHM4*	
GIT1		*GIT1*	
PHO81		*PHO81*	
PHO84		*PHO84*	

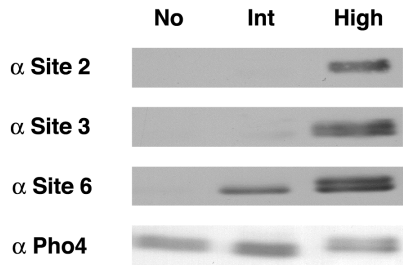
In vitro Phosphorylation of Pho4

- **Reaction is partially distributive: ~2 phosphorylation events per binding**
- **Kinase has site preference: phosphorylates site 6 before sites 2 & 3 90% of the time**

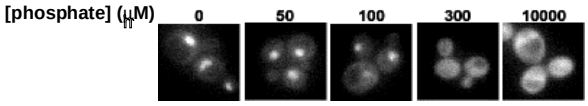
In vitro Studies Predict Accumulation of Pho4 Phosphorylated on Site 6

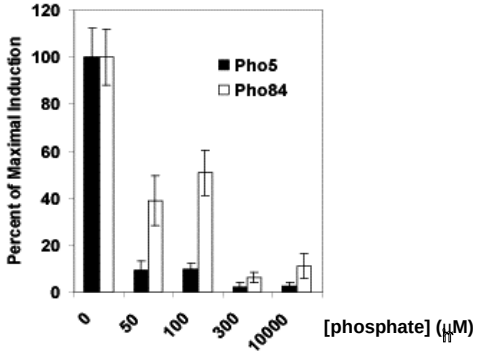


A Partially Phosphorylated Form of Pho4 Accumulates in Intermediate Phosphate Conditions



PHO84 Is Induced in Intermediate Phosphate But Not *PHO5*

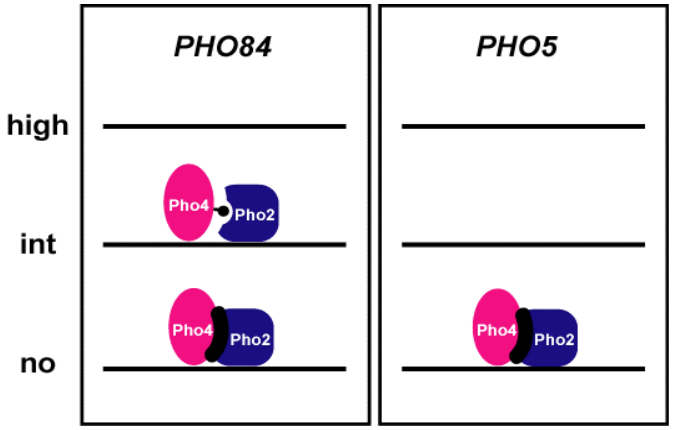


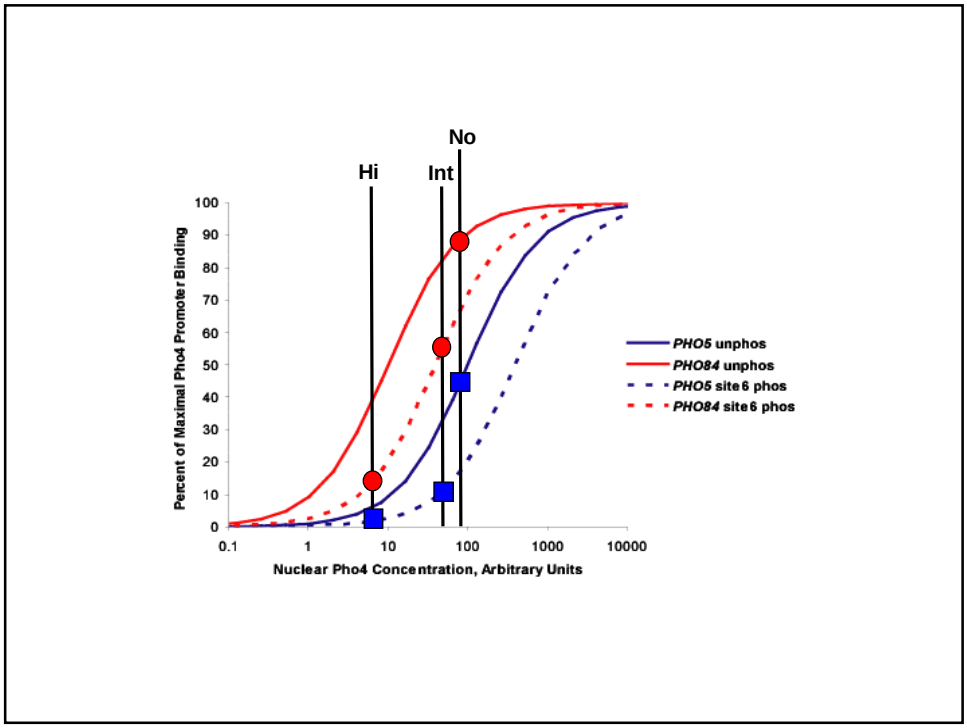


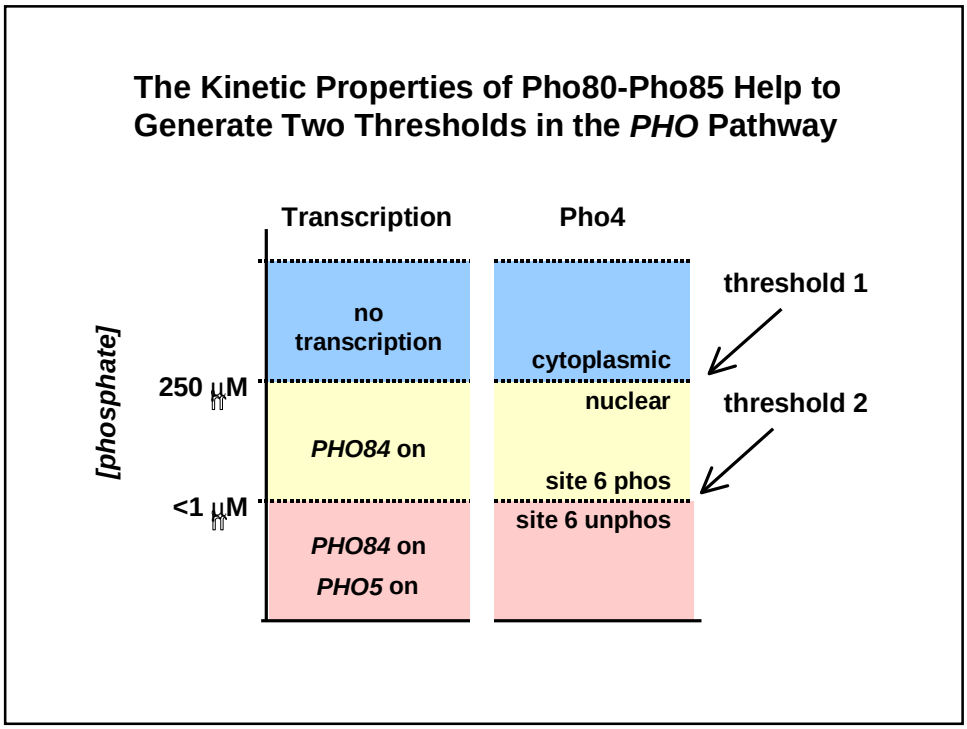
Gene Expression Induced by Intermediate Phosphate

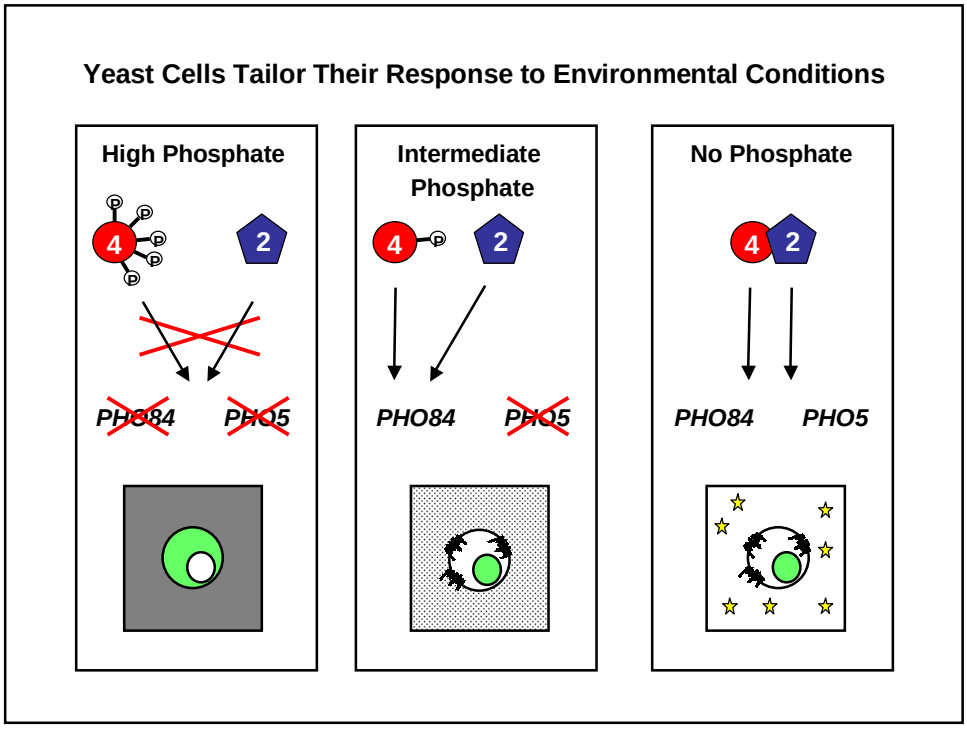
No phosphate		Int phosphate	
100%		>50%	<25%
PHM8	*PHO5*	*PHM8*	*PHO5*
AUT4	*PHO89*	*AUT4*	*PHO89*
CTF19	*PHO11*	*CTF19*	*PHO11*
SDT1	*PHO12*	*SDT1*	*PHO12*
PHM1	*PHM6*	*PHM1*	*PHM6*
PHO8		*PHO8*	
YPL017c		*YPL017c*	
PHM3		*PHM3*	
PHM2		*PHM2*	
PHO86		*PHO86*	
NCP1		*NCP1*	
PHM4		*PHM4*	
GIT1		*GIT1*	
PHO81		*PHO81*	
PHO84		*PHO84*	

Summary of *in vivo* DNA Binding Data




No
Hi
Int
Percent of Maximal Pho4 Promoter Binding
100
90
80
70
60
50
40
30
20
10
0
0.1
1
10
100
1000
10000
Nuclear Pho4 Concentration, Arbitrary Units
PHO5 unphos
PHO84 unphos
PHO5 site 6 phos
PHO84 site 6 phos


The Kinetic Properties of Pho80-Pho85 Help to Generate Two Thresholds in the PHO Pathway
Transcription
Pho4
[phosphate]
250 μM
<1 μM
no transcription
PHO84 on
PHO84 on
PHO5 on
cytoplasmic
nuclear
site 6 phos
site 6 unphos
threshold 1
threshold 2



How are phosphate levels sensed?

External vs. Internal Sensor

External sensor

- *Function[external phosphate]*
- Sensitive to absolute concentration of ligand
- Insensitive to internal concentration of ligand
- Can respond quickly to external change

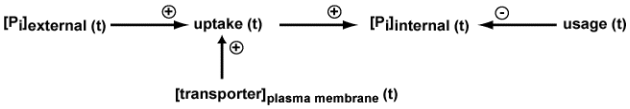
Good for detecting morphogens and growth factors

Internal Sensor

- *Function[internal phosphate]*
- Insensitive to absolute concentration of ligand
- Sensitive to small changes in uptake or usage of ligand
- Uptake rate can have profound effect on time-scale of response

Good for responding to nutrients

Internal Sensor



Internal sensor = Function$[P_i]_{internal}$

$d[P_i]_{internal}/dt$=Uptake-Usage

dUsage/dt=dGrowth/dt

dUptake/dt=Function{transporter,$[P_i]_{external}$,$[P_i]_{internal}$}

If uptake = 100/s and usage =100/s — $[P_i]_{internal}$ is constant

If uptake = 0 and usage = 100/s — $[P_i]_{internal}$ will drop until = 0

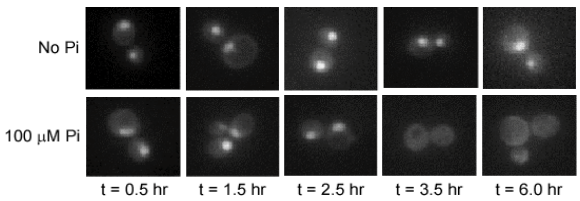
If uptake = 99/s and usage =100/s — $[P_i]_{internal}$ will drop until = 0
but will take 100 times longer to reach 0

Does the Pho Pathway have an Internal Sensor?

- **All transmembrane proteins known to be involved in the Pho pathway are involved in phosphate uptake**
- **Deletion of the high affinity phosphate transporter results in a signaling defect but this can be overcome by overexpression of unrelated transporters**

Feedback in the Pho Pathway

Pho4-GFP protein fusion



Constant external phosphate ⇨ Changes in signaling

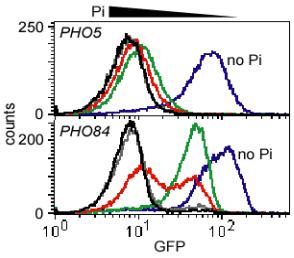
Implies sensor is internal

Where is the feedback coming from?

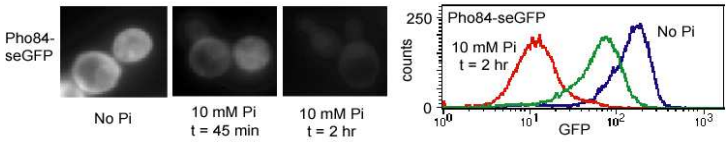
Regulation of *PHO84* transcription

***PHO5*pr::GFP**

***PHO84*pr::GFP**



Regulation of Pho84 endocytosis



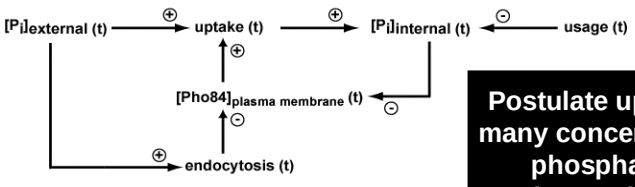
Where is the feedback coming from?

When phosphate levels drop…

- **Phosphate uptake decreases**
- **Pho84 is induced**
- **Pho84 endocytosis is decreased**
- **Phosphate uptake increases back to acceptable levels**

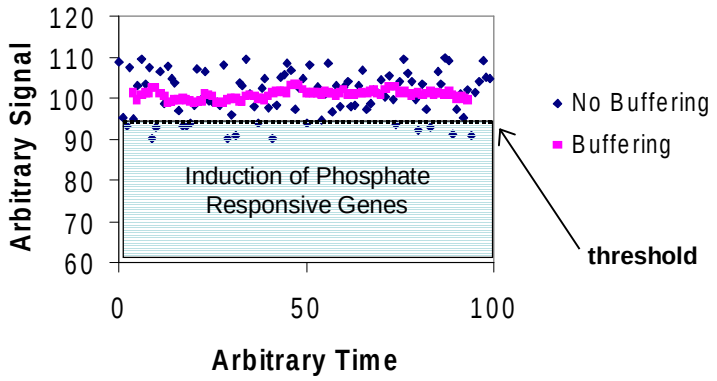
When phosphate levels rise…

- **Phosphate uptake increases**
- **Pho84 is repressed**
- **Pho84 endocytosis is increased**
- **Phosphate uptake decreases back to acceptable levels**



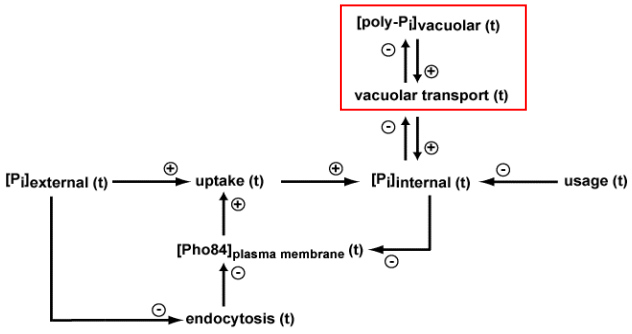
Postulate uptake is stable over many concentrations of external phosphate by balancing endocytosis and transcription

Problems Generated by Sensitivity of Internal Sensor



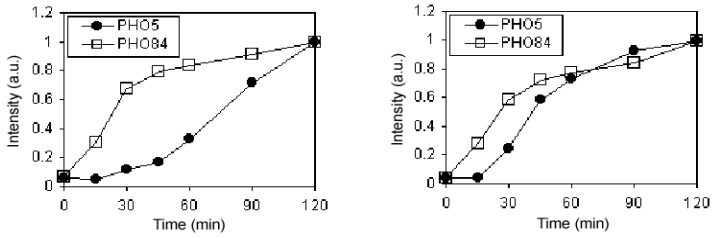
How can the system deal with noise?

Buffer - yeast store polymers of phosphate in the vacuole In the form of polyphosphate



Eliminate putative buffer and compare the kinetics of activation of the pathway to wild-type

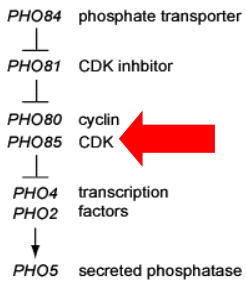
Loss of Polyphosphate Affects the Kinetics Of Pathway Activation



wild-type ***phm3*Δ**

Also predict that in *phm3*Δ threshold for *PHO5* induction will be raised – this is true

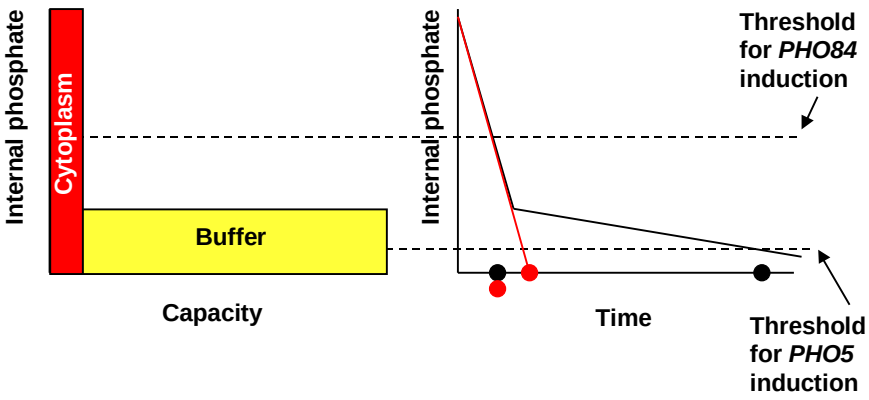
Rapid Inactivation of Pho80-Pho85 Causes Rapid Induction of *PHO5* and *PHO84*



Chemically inactivate kinase (<5 minutes)

***PHO5* and *PHO84* are both induced Quickly ($t_{1/2}$ ~ 5-10 minutes)**

Buffering and Sensitivity



Pho4 Regulation

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