Phosphorus Assimilation

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Phosphorus Assimilation

- Phosphorus is found in nucleic acids, proteins, phospholipids, ATP, and coenzymes like NADP.
- The most common phosphorus sources are inorganic phosphate and organic phosphate esters.
- Inorganic phosphate is incorporated through the formation of ATP in one of three ways:
  - photophosphorylation ,
  - oxidative phosphorylation
  - Substrate level phosphorylation
- Glycolysis provides an example of the latter process.
- Phosphate is joined with glyceraldehyde 3-phosphate to give 1,3-bisphosphoglycerate, which is next used in ATP synthesis.

$$\text{Glyceraldehyde 3-P} + P_i + NAD^+ \longrightarrow \text{1,3-bisphosphoglycerate} + NADH + H^+$$

$$\text{1,3-bisphosphoglycerate} + ADP \longrightarrow \text{3-phosphoglycerate} + ATP$$

...Phosphorus Assimilation

- Microorganisms may obtain organic phosphates from their surroundings in dissolved or particulate form.
- Phosphatases very often hydrolyze organic phosphate esters to release inorganic phosphate.
- Gram-negative bacteria have phosphatases in the periplasmic space between their cell wall and the plasma membrane, which allows phosphate to be taken up immediately after release.
- On the other hand, protozoa can directly use organic phosphates after ingestion or hydrolyze them in lysosomes and incorporate the phosphate.

Pho Regulon

- However, the inorganic phosphate (Pi) normally found in low concentrations in nature.
- Bacteria, as well as other organisms, have developed several systems to cope for the scarcity of this nutrient.
- To date, the unique mechanism responding to Pi starvation known in detail is the Pho regulon, which is normally controlled by a two component system in bacteria.
- The Phosphate (Pho) regulon is a global regulatory mechanism involved in bacterial Pi management that was first characterized in *Escherichia coli*, and later in many other bacterial species.
- The most common members activated by the Pho regulon are:
  - extracellular enzymes capable of obtaining Pi from organic phosphates,
  - Pi-specific transporters, and
  - enzymes involved in storage and saving of the nutrient
- The biosynthetic genes are repressed and activated, respectively, by the Pho regulon.

...Pho Regulon

Transporters

- The Pst Pi-specific transporter is the most conserved member of the Pho regulon in all bacteria.
- Other Pi transporters commonly found in bacteria, such as the low affinity Pi transporter Pit, are regulated in a variable fashion manner in the different species.
- *E. coli* activates additional transporters for phosphorous-containing compounds, such as Ugp (i.e., glycerophosphodiesters uptake) and Phn (i.e., phosphonates uptake).

Enzymes

- Among the Pi scavengers, the alkaline phosphatases (PhoA), phospholipases (PhoD), glycerophosphodiester phosphodiesterases (GlpQ and UgpQ), phytases (PhyC) and 5′-nucleotidases (UshA) are the most common enzymes induced in response to Pi starvation in bacteria.

Storage of Pi

- For the storage of Pi, most bacteria induce the expression of PpK, which is able to accumulate polyphosphate as a Pi reservoir and, when needed, reuse it .
- For saving nutrients, some bacteria are able to replaced teichoic acids (Pi-rich polymers found in the cell wall of Gram-positive bacteria) by teichuronic acids (Pi-free polymers).

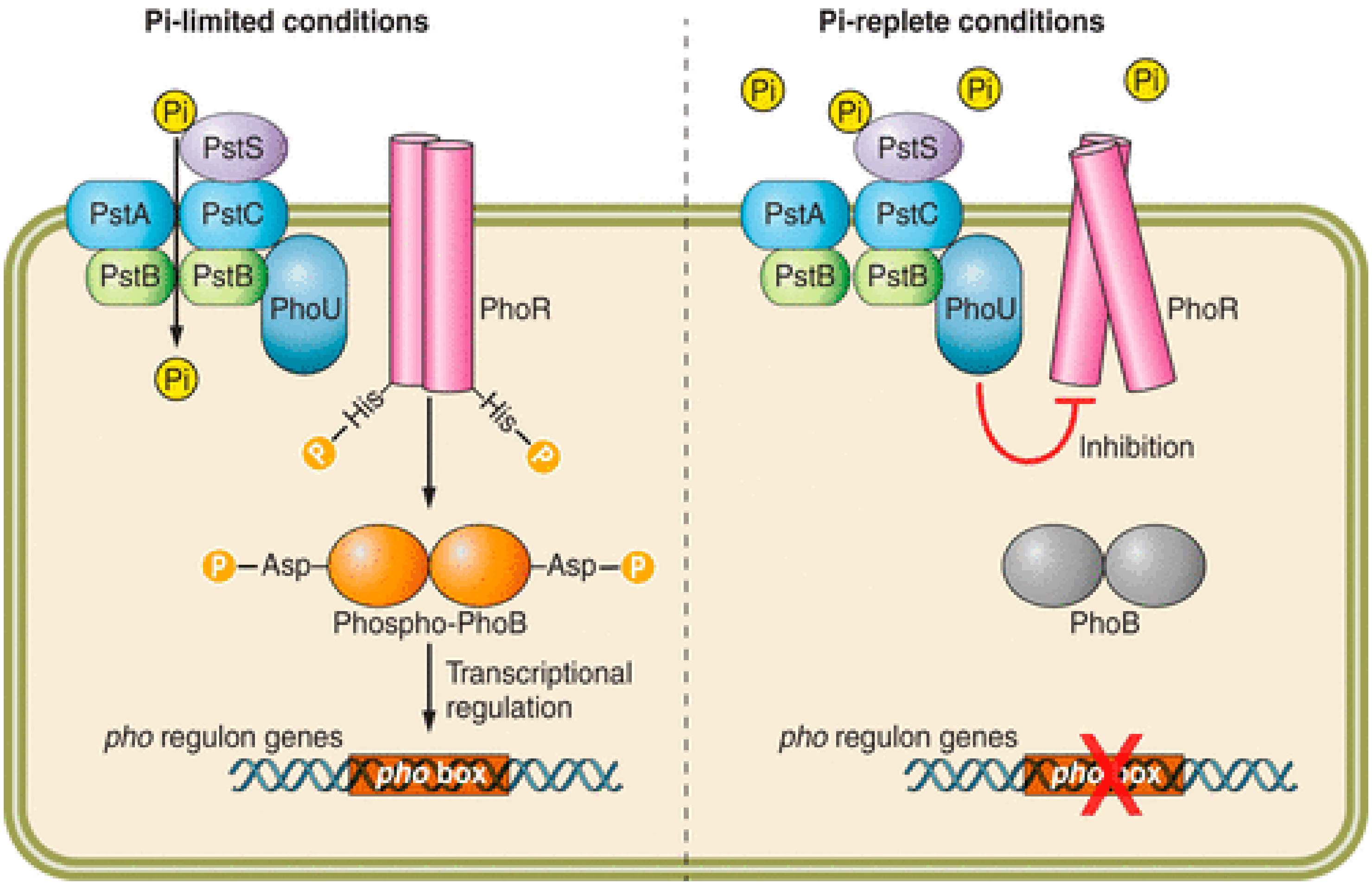
...Pho Regulon

- The Pho regulon is controlled by a two-component regulatory system which comprises an inner-membrane histidine kinase sensor protein and a cytoplasmic transcriptional response regulator.
- These proteins have received different names in some bacteria, such as: PhoR–PhoB in *E. coli.*
- In all cases, upon Pi scarcity, the response regulator (eg. PhoB) is phosphorylated on an aspartic residue by the sensor kinase (eg. PhoR).
- The phosphorylated response regulator is able to bind to specific sequences (known as PHO boxes, were first characterized in *E. coli*) on the DNA and activate or repress the transcription of genes.

E. coli Pho Regulon

- In *E. coli*, the Pi-sensing pathway requires, apart from the two-component system PhoR–PhoB, five additional proteins.
- Four of those are components of the Pi-specific transporter Pst (PstSCAB) and the other is the metal binding protein, PhoU.
- Under Pi limitation, PhoB is activated by PhoR acting as a kinase, but under Pi-replete conditions, PhoB activation is interrupted by PhoR acting as a phosphatase.
- PhoU is required for PhoB dephosphorylation under Pi-rich conditions in an as yet unknown manner.
- PhoU is involved not only in control of the autokinase activity of PhoR, but also in the control of the Pst system in order to avoid an uncontrolled Pi uptake that could be toxic for the cell.
- Whether it is PstSCAB, PhoR, PhoU, or another player the component that senses the Pi scarcity is still unknown.

E. coli Pho Regulon



Involvement of Pho Regulan to other functions

- **Involvement of Pi Regulation in Secondary Metabolite Production**
- The synthesis of many classes of secondary metabolites (i.e., macrolides, tetracyclines, aminoglycosides, etc.) is negatively regulated by high Pi concentrations in the culture media.
- There are some studies reporting the involvement of the PhoR–PhoP system in the control of secondary metabolite production.
- **Pi starvation and general stress**
- Interaction of the Pho regulon with other stresses such as the oxidative, osmotic, acid, and cell wall stresses has been also documented.
- For example, several studies in distinct bacteria have reported the Pho-dependent up-regulation of genes coding for catalases that protect bacteria from the oxidative stress by degrading hydrogen peroxide.
- Alternatively, different works have brought to light links between the Pho regulon and the synthesis of polyphosphate.
- Polyphosphate is a known stress response molecule which is very important for the stationary-phase survival of bacteria and is synthesized in a Pho-dependent manner when environmental stresses occur.

Involvement of Pho Regulan to other functions

- **The Pho Regulon is Involved in Pathogenesis**
- Although the main function of the Pho regulon is the control of the Pi homeostasis, this system also plays a role in pathogenesis.
- There are several strategies accounting for virulence in which the Pho regulon has been shown to be involved. The main ones are:

1. Tolerance to acidity:
   - In some bacteria, the Pho system is able to respond to external acidity by controlling the transcription of genes important for acid shock resistance.
2. Toxin production: One of the clearest examples of the involvement of the Pho regulon on toxin production is reported in *V. cholera*.
   - In this bacterium, the regulatory cascade leading to the production of TCP and CT toxins is repressed by PhoB under Pi-limiting conditions.
   - However, under the normal high Pi conditions of the gut, the regulatory cascade is not repressed, being able to activate TCP and CT productions.

The Pho Regulon is Involved in Pathogenesis

3. Biofilm formation:
   - Biofilms are mixed microbial populations typically embedded in a matrix of extracellular polymers.
   - A link between Pi homeostasis and biofilm formation has been reported in different bacteria.
   - For example, in the plant pathogen *A. tumefaciens* biofilm formation is enhanced in low Pi conditions by PhoB.
   - Influence of the Pho regulon on biofilm formation has been also described in *Pseudomonas*, *Proteus mirabilis*, *E. coli,* and *V. cholerae .*
4. Resistance to antimicrobial compounds
   - The lower susceptibility of biofilm-grown bacteria to antimicrobial agents, such as antibiotics and biocides, has been widely investigated .
   - However, involvement of Pi regulation in antibiotic resistance by distinct mechanisms to those related with biofilms has been also reported.
   - For example, Pi concentration has been shown to regulate to a high extent vancomycin resistance in two different streptomycetes: *S. coelicolor* and *S. lividans*