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Review

Inorganic phosphate uptake in unicellular eukaryotes ☆

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ABSTRACT

Background: Inorganic phosphate (P_i) is an essential nutrient for all organisms. The route of P_i utilization begins with P_i transport across the plasma membrane.
Scope of review: Here, we analyzed the gene sequences and compared the biochemical profiles, including kinetic and modulator parameters, of P_i transporters in unicellular eukaryotes. The objective of this review is to evaluate the recent findings regarding P_i uptake mechanisms in microorganisms, such as the fungi *Neurospora crassa* and *Saccharomyces cerevisiae* and the parasite protozoans *Trypanosoma cruzi*, *Trypanosoma rangeli*, *Leishmania infantum* and *Plasmodium falciparum*.
Major conclusion: P_i uptake is the key step of P_i homeostasis and in the subsequent signaling event in eukaryotic microorganisms.
General significance: Biochemical and structural studies are important for clarifying mechanisms of P_i homeostasis, as well as P_i sensor and downstream pathways, and raise possibilities for future studies in this field.

1. Introduction

One important role for cellular transport systems is to allow the entry of all essential nutrients into the cytoplasmic compartment and, subsequently, into organelles, allowing for the metabolism of exogenous sources of carbon, nitrogen, sulfur, and phosphorus [1]. Monitoring the external environment is necessary for all living cells, particularly microorganisms [2]. The extra- or intracellular environment of unicellular parasites must adapt to changes. The transport of ions and organic solutes is an important modification that allows the parasite to react to modifications in the external conditions, such as the acquisition of nutrients, the elimination of metabolic waste products, and the regulation of cell volume [3].

Inorganic phosphate (P_i) is an essential nutrient required for a large number of cellular functions. In addition, P_i is involved in many biochemical reactions related to the transfer of phosphoryl groups [4–6]. Low availability of P_i in the environment is a limiting compound for the growth of several organisms [7].

Active P_i uptake by the plasma membrane is central for the maintenance of P_i homeostasis and is the initial point for the utilization of this anion [8,9]. Because of the negative electrochemical potential across the cell membrane, anionic P_i cannot accumulate in the cytosol by simple diffusion. Therefore, P_i uptake must be coupled to an inwardly directed Na^+ or H^+ gradient to facilitate the transport of P_i against the gradient [10].

Two important P_i-responsive P_i transporter families in unicellular eukaryotes are (i) the inorganic phosphate transporter (PiT) family (TCDB# 2.A.20) and (ii) the H^+ symporter (PHS) family (TCDB# 2.A.1.9). The PiT family consists of functionally characterized P_i transporters that catalyze P_i either by H^+ or by Na^+ symport. Members of the PiT family have 354 to 681 amino acid residues and 10 to 12 transmembrane domains. The PHS family is part of the major facilitator superfamily (MFS, TCDB# 2.A.1) and comprises P_i:H^+ symporters of 400–600 amino acid residues and 12 to 14 transmembrane domains [1].

This review provides a critical overview of the recent findings regarding P_i-responsive P_i uptake in eukaryotic microorganisms.

2. *Neurospora crassa*

The filamentous fungus *N. crassa*, which plays a crucial role in modern genetics, was the first microorganism in which the P_i uptake mechanism was identified. It possesses two non-homologous, high-affinity phosphate permeases, PHO-4 and PHO-5 [11]. Similar to other transport systems that have been studied in *Neurospora*, the phosphate transport system appears to depend on metabolic energy [12].

In cells previously grown in P_i-free medium, P_i transport increased significantly, with an 8-fold increase over the original rate [13]. When

☆ This work is dedicated to Adalberto Vieyra on his 70th birthday.

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N. crassa is grown with an adequate supply of phosphorus, phosphate ions are brought into the cells by the phosphate transport system I (PTSI — cyclohexamide-insensitive). This constitutive system has a low affinity for phosphate. The affinity decreases further with increasing pH, and the system barely functions above pH 7 [12]. In contrast, when the organism is grown under conditions of limiting phosphorus, a number of derepressible proteins are synthesized that are necessary for scavenging phosphorus from the environment. These proteins include a high-affinity phosphate permease, which is part of a functional entity called the phosphate transport system II (PTSII — cyclohexamide-sensitive) [13].

For the PTSII, Mann et al. [14] identified an ORF of *PHO-4* that encodes a membrane carrier protein. PHO-4p is a large hydrophobic polypeptide of 590 aa, with 12 transmembrane domains, and a K_m for P_i of 2.56 ± 0.19 μM [11]. In addition, *PHO-5* encodes a high-affinity phosphate permease, with a K_m value of 37.4 ± 2.75 μM [11]. This permease is 569 aa in length with 12 transmembrane domains [15]; however, there is no similarity between *PHO-4* and *PHO-5* [11]. Strains of *N. crassa* containing null alleles of both Δ*pho-4* and Δ*pho-5* are unable to grow under restrictive conditions, indicating that both comprise the high-affinity phosphate transport system (PTSII) of *N. crassa* [15]. PHO-5 is active at neutral pH, whereas PHO-4 is active in alkaline pH, providing P_i during alkaline stress. Moreover, PHO-4 P_i uptake is coupled to the Na^+-gradient, whereas PHO-5 is a H^+-phosphate symporter, suggesting that PHO-4 and PHO-5 may use different mechanisms for phosphate transport [16,11].

Activation of PTSII is regulated by the phosphorus acquisition system, which includes four regulatory genes, *NUC-2*, *PREG*, *PGOV* and *NUC-1* [17]. NUC-1 is a transcription factor that requires nuclear localization to activate the transcription of structural genes related to P_i acquisition [18]. Under high phosphate conditions, a cytoplasmic interaction occurs between PREG and PGOV (a cyclin-like protein and a mitogen-activated protein kinase, respectively), and the PREG–PGOV complex scavenges NUC-1 in the cytosol [19]. However, under low phosphate conditions, an ankyrin repeat protein, NUC-2, inhibits the PREG–PGOV complex and NUC-1 is translocated to the nucleus, thereby activating the transcription of P_i responsive genes [20].

Recently, it was shown that a MAPK activation cascade, the MAK-2 signaling pathway, is also related to the activation of P_i-repressible genes. In *N. crassa*, the MAK-2 signaling pathway includes at least two other MAPKs, NRC1 and MEK-2, which are involved in the hierarchical activation of MAK-2 [21]. During P_i shortage, the two MAPKs are functional and inactive under abundant P_i conditions. Moreover, the nuc-2 mutant strain of *N. crassa* presents similar levels of MAPK transcription, which suggests that the MAPK transcription rate is dependent on NUC-2 activity and indicates the complexity of the metabolic phosphorus-sensing network [22].

3. *Saccharomyces cerevisiae*

Because unicellular eukaryotes, such as yeast, interact directly with the environment, the regulation of P_i transport is maintained solely by the transduction of nutrient signals across the plasma membrane [9]. The phosphate signal transduction pathway (PHO pathway) is responsible for regulating the expression of several genes responsive to P_i that are involved in the scavenging and specific uptake of P_i from extracellular sources in yeast [5]. P_i response, mediated by the PHO pathway, is determined by the activity and localization of the transcription factor PHO4, a homolog of NUC-1 in *N. crassa*. Its localization is controlled by the phosphorylation activity of the cyclin and cyclin dependent kinase (CDK) PHO80–PHO85 complex, which is homologous to PGOV–PREG in *N. crassa*. During P_i starvation, the CDK inhibitor PHO81 acts on the PHO80–PHO85 complex to inactivate it. This allows dephosphorylated PHO4 to localize to the nucleus and associate with other transcription factor, such as PHO2, thus inducing the expression of P_i responsive genes [23,24]. P_i responsive genes codify high affinity transporters (PHO84 and PHO89), secreted acid phosphatases (PHO5, PHO11, and PHO12) and other proteins related to P_i metabolism [25].

Two major types of transporters are responsible for P_i incorporation in *S. cerevisiae*, a high-affinity and a low-affinity transporter system. The low-affinity transporter system comprises PHO87, PHO88 and PHO90, and has an apparent K_m for external phosphate of approximately 1 mM. It has also been proposed that this system is constitutively expressed system due to its insensitivity to P_i starvation conditions [26,27].

The high-affinity transporter system consists of two P_i transporters, PHO-84 and PHO-89. PHO-84 has the highest affinity (K_m = 8.2 μM), whereas PHO-89 has a low K_m value (K_m = 770 μM) [28]. PHO-84 is a H^+:P_i symporter with high activity under acidic conditions [29]. PHO-89 is a Na^+:P_i symporter that is active under alkaline conditions, having a strong preference for Na^+ [30]. *PHO-84* expression and *PHO-89* expression are regulated by PHO system activation during P_i starvation [31].

PHO-84 belongs to the phosphate:H^+ symporter (PHS) family (TC No. 2.A.1.9.1). The protein encoded by *PHO-84* ORF contains 596 amino acid residues and has a molecular size of 65 kDa, homologous to *PHO-5* in *N. crassa* [29]. This symporter is responsible for the largest amount of P_i uptake and is very sensitive to phosphate starvation conditions [32]. Wykoff et al. [33] showed that deletion of *PHO-84* causes the loss of almost all phosphate transport, indicating that low-affinity transport is down regulated in response to phosphate limitation. PKA activation is essential for down-regulation and PHO-84 degradation. Moreover, inhibition of PKA decreases PHO-84 clearance from the plasma membrane in response to exogenous P_i increases [34]. PHO-84 is part of the P_i sensor machinery and involved in the cellular responses to the exogenous P_i concentration [25]. In addition, PHO-84 acts as a transceptor, suggesting that it also has a nutrient sensor function. However, PHO-84 uses the same phosphate-binding site for transport and signaling. Using a nontransported P_i agonist, it was possible to determine that signaling requires a specific conformational change that may be part of the conformational changes that occur during transport but does not require the complete transport cycle [35].

Moreover, Δpho84 cells under high-P_i conditions overexpress PHO87, PHO90, or PHO91, thereby increasing the P_i-uptake ability and suppressing constitutive PHO5 phosphatase expression [33]. This is a major distinction from the *N. crassa* homolog PHO-5, which presents no compensatory effects in Δpho-5 cells [15].

Table 1
Kinetic parameters of P_i transport in trypanosomatid parasites.

	T. rangeli		*T. cruzi*		*L. infantum*	
	H^+:P_i	Na^+:P_i	H^+:P_i	Na^+:P_i	H^+:P_i	Na^+:P_i
$K_{0.5,\ Na}$ (mM)	–	1.2	–	4.5	–	nd
$K_{0.5,\ Pi}$ (μM)	45	58	73	9	16	nd
V_{max} (*)	7	17	13	13	9.4	nd
P_i	$H_2PO_4^-$	$H_2PO_4^-$	$H_2PO_4^-$	$H_2PO_4^-$/HPO_4^{-2}	$H_2PO_4^-$/HPO_4^{-2}	nd
ATPase	H^+-ATPase	Na^+-ATPase	(H^+ + K^+) ATPase	Na^+-ATPase	(H^+ + K^+) ATPase	nd

*pmol $P_i \times min^{-1} \times (10^7\ cells)^{-1}$.
nd — not detected.