Inorganic Phosphate Export by the Retrovirus Receptor XPR1 in Metazoans
D. Giovannini, J. Touhami, Pierre Charnet, Marc Sitbon, J. L. Battini*

To cite this version:
D. Giovannini, J. Touhami, Pierre Charnet, Marc Sitbon, J. L. Battini*. Inorganic Phosphate Export by the Retrovirus Receptor XPR1 in Metazoans. Cell Reports, Elsevier Inc, 2013, 3 (6), pp.1866–73. 10.1016/j.celrep.2013.05.035 . hal-02191611
Inorganic Phosphate Export by the Retrovirus Receptor XPR1 in Metazoans

Donatella Giovannini,1,3,4 Jawida Touhami,1,3,4 Pierre Charnet,2,3,4 Marc Sitbon,1,3,4,5,* and Jean-Luc Battini1,3,4,5,*

1Institut de Génétique Moléculaire de Montpellier UMR 5535 CNRS, 1919 route de Mende, 34293 Montpellier cedex 5, France
2Centre de Recherche de Biochimie Moléculaire, UMR 5237 CNRS, 1919 route de Mende, 34293 Montpellier cedex 5, France
3Université Montpellier 1, Place Eugène Bataillon, 34085 Montpellier cedex 5, France
4Université Montpellier 1, 5 Bd Henry IV, 34067 Montpellier cedex 2, France
5These authors contributed equally to this work
*Correspondence: marc.sitbon@igmm.cnrs.fr (M.S.), jean-luc.battini@igmm.cnrs.fr (J.-L.B.)

http://dx.doi.org/10.1016/j.celrep.2013.05.035

SUMMARY

Inorganic phosphate uptake is a universal function accomplished by transporters that are present across the living world. In contrast, no phosphate exporter has ever been identified in metazoans. Here, we show that depletion of XPR1, a multipass membrane molecule initially identified as the cell-surface receptor for xenotropic and polytropic murine leukemia retroviruses (X- and P-MLV), induced a decrease in phosphate export and that reintroduction of various XPR1 proteins, from fruit fly to human, rescued this defect. Inhibition of phosphate export was also obtained with a soluble ligand generated from the envelope-receptor-binding domain of X-MLV in all human cell lines tested, as well as in diverse stem cells and epithelial cells derived from renal proximal tubules, the main site of phosphate homeostasis regulation. These results provide new insights on phosphate export in metazoans and the role of Xpr1 in this function.

INTRODUCTION

Phosphate is one of the most abundant minerals in the body. Maintenance of constant serum phosphate by hormonal regulation is critical as phosphate imbalance can lead to major disorders (see, for instance, Prié and Friedlander, 2010). Phosphate plays various key functions; it is a major component of membranes, nucleic acids, bones, and teeth, it provides the high-energy bonds in ATP and creatine-phosphate, and it regulates signaling and enzymatic activities via kinase and phosphatase-mediated processes.

The bulk of dietary phosphate is imported at the brush-border membrane of small-intestine enterocytes by the type Iib sodium-dependent phosphate transporter, NPT2b/SLC34A2 (Hilfiker et al., 1998), whereas phosphate in glomerular filtrates is reabsorbed by proximal tubule epithelial cells, mainly by the apical NPT2a/SLC34A1 phosphate transporter and to a lesser extent by NPT2c/SLC34A3 (Murer et al., 2000). However, how phosphate taken up at the apical site of epithelial cells exits at the basal site remains unknown. Two other sodium-dependent phosphate importers have been identified: PiT1/SLC20A1 and PiT2/SLC20A2 (Kavanaugh et al., 1994; Olah et al., 1994). They are ubiquitously expressed (Kavanaugh and Kabat, 1996) and described as phosphate suppliers for cell metabolism (Collins et al., 2004) and bone mineralization (Suzuki et al., 2010; Yoshiko et al., 2007). PiT1 and PiT2 are members of a family of cell-surface nutrient transporters that are used as retroviral receptors (Manel et al., 2005; Overbaugh et al., 2001), with PiT1 as the receptor of the gibbon ape leukemia virus (GaLV) and koala endogenous retrovirus (KoRV) (O’Hara et al., 1990; Oliveira et al., 2006), and PiT2 as the receptor of the amphotrophic murine leukemia virus (A-MLV) (Kozak et al., 1995; van Zeijl et al., 1994).

XPR1 is another retroviral receptor, used by xenotropic and polytropic MLV (X-MLV and P-MLV) (Battini et al., 1999; Tailor et al., 1999; Yang et al., 1999), two gammaretroviruses that can infect human cells (for a review, see Kozak, 2010). XPR1, whose function has remained elusive, is a cell-surface multipass membrane protein that contains a 180-residue-long amino-terminal SPX domain (named after SYG1, Pho81, and XPR1) identified in several yeast and plant proteins that play a role at many levels of phosphate regulation, from transport to transcriptional modulation (Secco et al., 2012a, 2012b). Here, we identify XPR1 as a phosphate exporter in metazoans, a function that does not require the SPX domain. Furthermore, we describe a soluble and potent inhibitor of phosphate export derived from the X-MLV envelope glycoprotein.

RESULTS

XPR1 Is Widely Expressed in Human Cells and Is Not Modulated by Phosphate

XPR1 is the entry receptor of X-MLV, a murine gammaretrovirus that can infect human cells. Entry requires specific binding between receptors and the amino-terminal receptor-binding domain (RBD) of the viral envelope glycoproteins (Env) (Battini et al., 1995; Fass et al., 1997). In the absence of reliable exofacial antibodies (data not shown; Vaughan et al., 2012), we monitored cell-surface expression of XPR1 with a soluble ligand derived from X-MLV Env RBD (XRBD) (Figure 1A). XRBD binding to human HEK293T cells was specifically abolished upon
introduction of a small interfering RNA (siRNA) targeting human XPR1 (siXPR1) (p value ≤ 0.001) (Figure 1B). XPR1 was expressed in all human cell lines we tested, albeit at various levels, and absent from human red blood cells (RBC) (Figure 1C). No binding was detected on Chinese hamster ovary (CHO) and NIH 3T3 cell lines (Figure 1C) due to XPR1 species polymorphism, known to prevent X-MLV Env-mediated binding and infection in hamster and mouse cells (Marin et al., 1999; Van Hoeven and Miller, 2005; Yang et al., 1999).

Given the presence of a SPX domain in XPR1 and the association of this domain to phosphate metabolism in fungal and plant proteins (Secco et al., 2012a, 2012b), we tested whether XPR1 varied with phosphate concentration (Figure 1D). Cell-surface XPR1 levels remained unchanged, whereas PIT1 and PIT2 levels increased in 0 mM phosphate (Figure 1D). Furthermore, introduction of human XPR1 in CHO cells did not alter phosphate uptake, whereas that of PIT1 or PIT2 led to significantly increased uptake (data not shown). These data and the fact that siXPR1 did not alter phosphate uptake (Figure S1A) argued against a role of XPR1 in phosphate import.

XPR1 Mediates Phosphate Export in Vertebrates

We next monitored phosphate content in culture medium of XPR1-depleted cells. Following a 1 hr incubation in phosphate-free medium, XPR1 knockdown with siXPR1 resulted in significantly lower phosphate accumulation in the medium (Figure 2A, left panel), with no effect on PIT1 and PIT2 (Figure S1B). This was not due to a general alteration of the export machinery because accumulation of lactate, the main exported glycolytic product, remained unchanged (Figure 2A, right panel).

Interestingly, we found that efflux of radiolabeled phosphate was influenced by extracellular phosphate concentration, reaching 38% at 10 mM phosphate as compared to 25% and 17% at 1 or 0 mM phosphate, respectively (Figure 2B). Notably, phosphate release was markedly reduced in cells wherein siXPR1 was introduced (Figure 2C). Efflux of bona fide phosphate was assessed by one-dimensional thin-layer chromatography (Figure 2D), and a marked decrease in phosphate efflux upon introduction of siXPR1 was also observed in HeLa and U2OS human epithelial cell lines (Figure 2E). Again, this decrease was specific, as efflux of radiolabeled sulfate, another divalent anion, was not altered (Figure 2F). Altogether, these results provide evidence that XPR1 is a phosphate exporter in vertebrates.

(siLUC), XPR1 (siXPR1), or PIT1 (siPIT1). Nonspecific staining due to the secondary Alexa-Fluor-488-conjugated anti-mouse IgG Ab (filled histograms) and specific binding (solid line histograms) are represented. Numbers indicate specific mean fluorescence intensities (ΔMFI) in one representative experiment (n ≥ 3).

(C) XPR1 cell-surface expression measured as a function of XRBD binding. Binding was tested on various human cell types, including RBC and negative control hamster (CHO) and mouse (NIH 3T3) established cell lines. ΔMFI (±SEM), as assessed by flow cytometry, is shown for each cell type (n ≥ 3).

(D) XRBD, PIT1, and PIT2 cell-surface expression was monitored on HEK293T with XRBD, KoRBD, and ARBD ligands, respectively. Binding was measured after 24 hr culture in the presence of 1 mM (upper panels), 0 mM (central panels), or 10 mM phosphate (lower panels). Numbers represent the ΔMFI in one representative experiment (n ≥ 3). See also Figure S1.
The SPX Domain of XPR1 Is Dispensable for Phosphate Efflux

We further assessed the role of XPR1 in phosphate efflux after introducing a siRNA matching the XPR1 3' UTR. This siXPR1 specifically downmodulated expression of endogenous XPR1 while allowing expression of a transfected engineered XPR1. Both XPR1 cell-surface expression and phosphate efflux were decreased by the siXPR1, and both were restored after reintroduction of a hemagglutinin (HA)-tagged human XPR1 (Figure 3A). XPR1 complementation was specific because PAR2, the receptor of the porcine endogenous retrovirus type A (PERV-A) and a riboflavin transporter (Yonezawa et al., 2008), did not restore phosphate efflux despite increased cell-surface expression as assessed by the binding of PERV-A Env RBD (Figure 3A). Interestingly, phosphate efflux was also restored by XPR1 isolated from various metazoans, including phylogenetically distant vertebrates and invertebrates (Figures 3B and 3C). Thus, XPR1 from the zebrafish (79% amino acid identity with human XPR1) and that from the fruit fly (54% identity) complemented efficiently phosphate efflux in XPR1-depleted human cells. Proteins harboring an SPX domain play key roles in phosphate regulation in yeast and plants (Secco et al., 2012a, 2012b). Moreover, the SPX domain of XPR1 has been shown to interact with the β subunit of the G protein heterotrimers (G8) and modulate cyclic AMP levels (Vaughan et al., 2012). To address the potential role of SPX in phosphate efflux, we performed complementation assays with a human XPR1 construct recognized by XRBD, lacking the entire SPX domain (hΔSPX) and properly expressed at the plasma membrane (Figure 3D). The hΔSPX mutant was an efficient X-MLV receptor, mediating infection in otherwise nonpermissive cells (Table S1) and fully restored phosphate efflux in siXPR1-transfected cells (Figure 3D). Therefore, XPR1 appeared to be directly responsible for phosphate efflux without requiring SPX-mediated G8 protein recruitment.

XRBD Is an Efficient Inhibitor of XPR1-Mediated Phosphate Efflux

Interaction between retroviral Env and their cognate receptors often leads to altered transporter activities (Kavanaugh et al., 1994; Manel et al., 2003; Rasko et al., 1999). We found that intracellular X-MLV Env expression dramatically reduced phosphate efflux, whereas a control PERV-A Env had no impact on phosphate efflux (Figure 4A). In contrast, PERV-A Env efficiently
downmodulated PAR2, its cognate receptor (Figure S2). To further assess the direct interaction between X-MLV Env and XPR1 in phosphate efflux inhibition, we coexpressed X-MLV Env with human or rodent XPR1, as X-MLV Env does not bind hamster and mouse (Mus musculus) XPR1 (Figure 1C; Marin et al., 1999; Van Hoeven and Miller, 2005; Yang et al., 1999; Kozak, 2010). X-MLV Env inhibited phosphate efflux following transfection with human XPR1, whereas it did not impairefflux in cells transfected with either hamster XPR1 (Figure 4B) or mouse XPR1 (data not shown). The fact that rodent XPR1, and not human XPR1, can compensate for defective phosphate efflux induced by X-MLV Env was strongly in favor of a direct interaction between X-MLV Env and human XPR1 in phosphate efflux inhibition.

We further showed that intracellular expression of a soluble XRBD module that lacked the Env remainder (Figure 1A) efficiently inhibited phosphate efflux mediated by XPR1 (Figure 4C), whereas an XRBD-binding-defective mutant (M1RBD) did not (Figure S3).

**XPR1 Mediates Phosphate Efflux in Various Primary and Stem Cells**

Finally, we tested whether soluble XRBD maintained XPR1-inhibiting properties when added in culture medium. XPR1-binding sites on human HEK293T cells were saturated in a concentration-dependent manner with ectopically added XRBD (Figure 4D), whereas no significant binding was achieved on mouse cells (Figure 4D). Specific and efficient inhibition of
phosphate efflux could also be achieved in a dose-dependent manner with purified XRBD added to the culture medium (Figure 4E). Phosphate efflux was inhibited by XRBD in all other human cell lines tested (data not shown).

Because primary cells are not readily transfectable, we tested the anti-XPR1 properties of soluble XRBD directly in the culture medium of various primary cell types, after verifying the presence of XPR1 at the cell surface (Figure S4). Interestingly, higher levels of XPR1 at the cell surface of neuronal stem cells (NSC) were not accompanied by increased phosphate efflux (Figures S4 and 4F), suggesting that cofactors might be required for phosphate efflux. Alternatively, XPR1 may exert an additional function. The concomitant transport of different substrates has thus been described for GLUT1, the main glucose transporter, in the context of human red blood cells (Montel-Hagen et al., 2008). The antagonist effect of XRBD on phosphate efflux was readily detected on human umbilical vein endothelial cells (HUVEC) and renal proximal tubule epithelial cells (RPTEC), as well as on various human stem cells, including mesenchymal (MSC), NSC, skin (SSC), and umbilical cord CD34+ hematopoietic stem cells (Figure 4F). The significant decreases in phosphate efflux observed in these different cell types unveiled the highly conserved role mediated by XPR1.

**DISCUSSION**

Although evidence for phosphate efflux has accumulated in diverse tissues, factors mediating phosphate export have remained elusive. Here, we show that inorganic phosphate efflux in human cells is accomplished by XPR1, the X-MLV receptor (Battini et al., 1999; Tailor et al., 1999; Yang et al., 1999). Moreover, we were able to impair phosphate efflux by blocking XPR1 with XRBD, its X-MLV Env-derived ligand. Although alternative pathways for phosphate export may exist, the severity of the inhibition observed with XRBD indicated that, in the models tested, XPR1 is the main, if not the sole, effector of this activity.
In agreement with our findings, two reports have separately indicated that activation of the receptor-activated NF-κB-ligand-receptor-activated NF-κB signaling pathway in murine osteoclasts leads to higher levels of phosphate efflux (Ito et al., 2007) and to XPR1 upregulation (Sharma et al., 2010). Although a direct link between the two studies is missing, we postulate that phosphate export driven by XPR1 is likely to play a role during bone resorption. Also, cellular efflux is important in phosphate homeostasis as an obligatory step of intestinal absorption and renal reabsorption. Thus, it has been proposed that a Na+-dependent electroneutral anion exchanger is at least partially responsible for phosphate exit in the proximal tubule (Barac-Nieto et al., 2002). Our finding that XRBD ligand blocked phosphate exit in human RPTEC indicates that XPR1 is likely to represent the “missing player” in trans-epithelial phosphate transport to bloodstream.

The lack of specific antibodies directed against exofacial determinants of multispot nutrient transporters is notorious (see, for instance, Kinet et al., 2007; Yonezawa et al., 2008). This is also the case for XPR1 (Vaughan et al., 2012). Therefore, siRNA-mediated downmodulation of cell-surface XPR1 could not be easily monitored. In contrast, XRBD, our XPR1 ligand, allows the simple monitoring of XPR1 surface expression in various human cell types. XPR1 appeared ubiquitous, in agreement with EST databases and previous studies (Battini et al., 1999; Tailor et al., 1999) wherein XPR1 mRNA is found in many tissues and as early as the 2-cell stage embryos. Moreover, XPR1 also acts as phosphate exporter in mammals as well as in more distant species, because phosphate efflux defect could be efficiently restored by ectopic expression of various XPR1, from fruit fly to human. Given the high conservation of XPR1 sequences (over 90% amino acid identity in mammals and 79% and 54% in zebrafish and fruit fly, respectively), it is likely that XPR1-mediated phosphate export is a universal feature in metazoans.

Phosphate exporters have been recently described in plants and yeasts. Specifically, the Arabidopsis thaliana PHO1 has been shown to play a crucial role in root phosphate efflux (Stefanovic et al., 2011). On the other hand, the yeast PHO91 has been shown to function intracellularly, delivering vacuolar hydrolyzed polyphosphate to the cytosol (Hürlimann et al., 2007). The plant PHO1 and yeast PHO91 share 21% identity with human XPR1 and also harbor an amino-terminal SPX domain. Our finding that SPX was dispensable for phosphate export by XPR1 suggested a regulatory role rather than a role in transport activity per se, as previously reported for the PHO87 and PHO90 yeast proteins (Ghilbert et al., 2011; Hürlimann et al., 2009). Moreover, the SPX domain of SYG1, the closest yeast homolog of XPR1 (23% identity), with no identified function, binds Gβ proteins (Spain et al., 1995). XPR1 has also been shown to interact with Gβ in human cells (Vaughan et al., 2012), presumably via its SPX domain. Whether this interaction influences phosphate export remains to be established.

Loss of phosphate homeostasis regulation can lead to hypophosphatemia, with potentially severe clinical consequences (Prie and Friedlander, 2010). Associated genetic alterations have been found in genes involved in renal phosphate homeostasis regulation. However, it is believed that other genes involved in genetic phosphate disorders have yet to be discovered. In this regard, XPR1 represents a candidate to be taken into account when stable phosphate homeostasis is compromised.

EXPERIMENTAL PROCEDURES

Cell origins and culture conditions, plasmids, expression vectors, molecular constructions, siRNA, RBD preparations, binding assays, and efflux procedures are detailed in the Extended Experimental Procedures.

Cell Culture, Plasmids, Expression Vectors, and siRNA

Cell cultures included human kidney HEK293T, lung A549, colon CaCo2, cervical HeLa, hepatocyte HuH-7, breast MDA-MB436, pancreas PANC-1, prostate PNT2, muscle TE671, bone U2OS, and Jurkat T cells; murine embryonic fibroblast NIH-3T3 and hamster ovary CHO established cell lines; and primary cells comprising HUVEC, RPTEC, CD34+ hematopoietic stem cells and mesenchymal, neuronal, and skin stem cells (kindly provided by X. Nissan and M. Peschanski, I-Stem, Evry).

Retroviral Env-Derived Ligands and Binding Assays

The KoRV, A-MLV, X-MLV (N2B-IL-6 strain), and PERV-A RBD were C-terminally fused to a mouse immunoglobulin (lg) G1 Fc fragment and produced on HEK293T cells. Binding assays were carried as previously described (Lavanya et al., 2008; Manel et al., 2003) or as detailed in the Extended Experimental Procedures.

Colorimetric and Phosphate Efflux Assays

Inorganic phosphate and lactate concentrations in the culture medium were measured using the Malachite Green Phosphate Detection Kit (R&D Systems) and ABX Pentra Lactic Acid Kit (ABX Diagnostic), respectively. Phosphate efflux was measured from cell monolayers or suspension cell culture medium as the ratio of released $[^{33}P]$phosphate to total cellular $[^{33}P]$phosphate. $[^{35}S]$sulfate efflux was measured in similar conditions.

Thin-Layer Chromatography

Samples (1.5 μl) were loaded on polyethyleneimine-cellulose plates and run vertically in closed chambers with 0.5–0.8 cm of solvent (KH$_2$PO$_4$ 0.85 M adjusted to pH 3.4 with H$_3$PO$_4$), as recently described for quantification of intracellular nucleoside triphosphates (Jendresen et al., 2011). Dried plates were exposed for $[^{35}S]$radioactivity in a storage phosphor screen overnight and scanned with a Typhoon 9200 Imager (GE Healthcare).

Statistical Analysis

Data are expressed as means ± SEM. All p values were calculated using Student’s t test in GraphPad Prism 5 software, and the following convention was used throughout the paper: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001.

ACCESSION NUMBERS

The GenBank accession number for the opossum XPR1 sequence reported in this paper is KF007921.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.05.035.

LICENSING INFORMATION

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.
ACKNOWLEDGMENTS

This work was supported by grants from the Association pour la Recherche sur le Cancer and Fondation pour la Recherche Médicale (to M.S.); Association Française contre les Myopathies, Ligue Nationale contre le Cancer (Comité de l’Hérault) and Agence Nationale de la Recherche JCJC (to J.-L.B.); ANR Blanc and INCa (to M.S. in copartnership with N. Taylor); and INCa-DOGS (to M.S. in copartnership with D. Decaudin). D.G. was supported by FRM and INCa; J.T. was supported by CNRS and ANR. J.-L.B. and M.S. were supported by INSERM. We are indebted to G. Friedlander, A.D. Miller, R.K. Na- viaux, and N. Taylor for insightful and helpful discussion and N. Taylor for critical reading of the manuscript; we are grateful to Y. Poirier for sharing unpublished results; to J. Donner for the kind gift of the KoRV envelope plasmid; to J. Stoye for the PERV-A envelope plasmid; to X. Nissam, M. Pe- schanski, and the I-Staff for kindly providing various stem cells; to D. Ma- thieu for the HUVECs; to M. Rousset for his help with oocyte experiments; and to the NTL lab for blood hematopoietic stem cells and generous support. We thank R. Rua, S. Weiss, M. Lavanya, and A. Du-Thanh for their initial work; E. Antoine for metabolic measurements; J. Feuillard and the Production of Re- combinant Proteins facility for their invaluable help in lipid purification; and present and past members of our laboratory for constant support and stimu- lating discussions.

Received: December 20, 2012
Revised: April 5, 2013
Accepted: May 22, 2013
Published: June 20, 2013

REFERENCES


