10th French-Belgian ABC meeting ; Brussels, Belgium, October 19-20, 2012

Abstracts

Chronic oral treatment with ivermectin increases expression of genes involved in drug disposition in mice
Mélanie Albérich, Cécile Menez, Anne Lespine
INRA – UMR 1331, Université de Toulouse, INP, TOXALIM, F-31027 Toulouse, France

Heterologous and homologous expression of two plant pleiotropic drug resistance transporters as a tool for their functional characterization and their purification
Amandine Baijot, Justine Fourmeau and Marc Boutry
Institut des Sciences de la Vie , University of Louvain, 1348 Louvain-la-Neuve, Belgium

A Pleiotropic Drug Resistance transporter in *Nicotiana tabacum* is involved in defense against the herbivore *Manduca sexta*
Manuela Désirée Bienert[1], Stephanie Elise Gerlitz Siegmund[2], Anna Drozak[1], Tomasz Trombik[1], Alain Bultreys[3], Ian Thomas Baldwin[2], and Marc Boutry[1]
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[3]Département Sciences du vivant, Centre Wallon de Recherches Agronomiques, Chaussée de Charleroi 234, 5030 Gembloux, Belgium.

microRNA33 targets the ABCA1 pump and regulates cholesterol metabolism
Laure-Alix Clerbaux, Isabelle Gérin, Guido T. Bommer.
Laboratory for chelical physiology, de Duve Institute, Brussels, Belgium

ABCB1 1199G>A genetic polymorphism influences tacrolimus intracellular accumulation in HEK293 and K562 recombinant cell lines.
Géraldine Dessilly[1], Laure Elens[1], Nadtha Panin[1], Arnaud Capron[2], Jean-Baptiste Demoulin[3], Vincent Haufroid[1, 2]
[1]Louvain centre for Toxicology and Applied pharmacology, Institut de recherche expérimentale et clinique, Université catholique de Louvain, Brussels, Belgium ;
[2]Department of Clinical Chemistry, Cliniques Universitaires Saint-Luc, Université catholique de Louvain
[3]De Duve Institute Brussels, Université catholique de Louvain, Belgium.

Influence of the basal cellular level of glutathione in triggering MRP1-cells death
Lauriane Dury, Attilio Di Pietro and Hélène Cortay
IBCP, UMR 5086, BMSSI, 7 passage du Vercors 69007 Lyon, France.

Role of P-glycoproteins in the installation of parasite nematodes in the host.
Issouf M, Guegnard F, Koch C, Sauvé C, Neveu C and Kerboeuf D
INRA, UR1282 Infectiologie Animale et Santé Publique, F-37380 Nouzilly, France

Identification of substrates of a plant pleiotropic drug resistance transporter, NtPDR1 from *Nicotiana tabacum*
Julien Roland, Emmanuel Peeters, Joseph Nader and Marc Boutry
Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud, 4-15, 1348 Louvain-la-Neuve, Belgium.

Over-expression of plant pleiotropic drug resistance transporters for their enzymatic and structural characterization
Frédéric Toussaint, Amandine Baijot and Marc Boutry
Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud, 4-15, 1348 Louvain-la-Neuve, Belgium.

Functional investigation of a multidrug transporter of *Pseudomonas aeruginosa*
Alice Verchère, Isabelle Broutin & Martin Picard
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Transfer of the CFTR chloride channel by membrane - derived microparticles as a therapeutic approach for cystic fibrosis.
C. Vituret [1,2], G. Gonzalez [1], A. Di Pietro [2], M. Chanson [3], P. Boulanger [1], & S.S. Hong [1]
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Further information for registration and abstract submission (http://www.facm.ucl.ac.be/ABC).

8 oral presentations by junior scientists will be selected from submitted abstracts.
Selected speakers will receive a grant.

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Attilio di Pietro : a.dipietro@ibcp.fr / +33-4-72.72.26.29

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Session chosen :

☒	physiology	☐	pathology	☐	mechanism/structure	☐	non mammalian

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Title: Chronic oral treatment with ivermectin increases expression of genes involved in drug disposition in mice

Authors and affiliations: Mélanie Albérich[1], Cécile Menez[1], Anne Lespine[1]
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Abstract:

Background and Objectives:

Macrocyclic lactones (MLs) are antiparasitic drugs widely used in human and veterinary medicines. The behavior of these molecules in host and parasite is decisive for their efficacy and strongly depends on the efflux by ATP Binding Cassette (ABC) transporters and on their biotransformation by cytochromes P450. Moreover, it has been shown that ivermectin increases the P-glycoprotein (Pgp, ABCB1) gene expression *in vitro* in mice hepatocytes (Menez 2011). We have studied the influence of ivermectin on the expression of genes involved in xenobiotic metabolism in cells and *in vivo* in mice. We have assessed putative consequences on drug metabolism and concentrations distribution in mice.

Methods:

Hepatocytes and intestinal mice cells were exposed to ivermectin for 24h. Ivermectin was administered orally by gavage in mice with a single dose (0.2 mg/kg) of ivermectin or chronically by repeated doses (twice a week) during 5 weeks. The expression of ABC transporters and cytochromes genes of interest was assessed by qRT-PCR in cell lines, intestine and liver. In parallel, ivermectin and its main metabolite were quantified by HPLC in mice plasma, intestine, liver and brain.

Results:

Ivermectin induced Pgp and cytochromes gene expression in both type of cells in dose dependant manner and in mice intestine after a chronic exposure. No modulation of gene expression was observed in the liver. Chronic administration of ivermectin resulted in a significant reduction of ivermectin concentration in plasma (40%), intestine (60%) and liver (70%) compared with a single administration. Moreover, chronic administration induced a 6.5 fold increase of the metabolite-to-ivermectin concentration ratio ($p<0.001$).

Conclusions:

In conclusion, chronic exposure to ivermectin modulates the expression of genes involved in the metabolism of xenobiotics and affects drug concentration in mice. This may have impact on drug efficacy in the context of repeated administration or long acting formulations.

MENEZ C, MSELLI-LAKHAL L, FOUCAUD-VIGNAULT M, BALAGUER P, ALVINERIE M, LESPINE A. Ivermectin induces P-glycoprotein expression and function trough mRNA stabilization in murine hepatocyte cell line, 2012, *Biochemical pharmacology*, 83(2):269-78.

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Session chosen :

☐	physiology	☐	pathology	☐	mechanism/structure	X	non mammalian

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Heterologous and homologous expression of two plant pleiotropic drug resistance transporters as a tool for their functional characterization and their purification

Amandine Baijot, Justine Fourmeau and Marc Boutry
University of Louvain, 1348 Louvain-la-Neuve, Belgium

ABC transporters mediate ATP-dependent translocation of a wide range of structurally and functionally unrelated compounds across biological membranes. In our laboratory, we have identified plasma membrane ABC transporters belonging to the pleiotropic drug resistance (PDR) subfamily in the plant species *Nicotiana plumbaginifolia* and *Nicotiana tabacum*. Analysis of transgenic plants silenced for NpPDR1 and NtPDR3 expression indicated that they are involved in the plant response to fungal attack and to iron deficiency, respectively. However, identification of their substrates is required to fully understand their physiological roles. For that purpose, this work aimed at setting up a convenient expression system for plant PDR transporters. First, NpPDR1 and NtPDR3 were expressed in a heterologous system, the yeast *Saccharomyces cerevisiae*. Even though they did not properly localize to the plasma membrane, phenotypic characterization indicated that these plant PDR transporters are functional in yeast. Growth tests in the presence of toxic concentrations of putative substrates showed that NpPDR1 probably transports antifungal diterpenes while NtPDR3 seems to transport molecules structurally related to phenolic compounds that help to solubilize iron in the soil. Second, NtPDR3 expression in *N. tabacum* BY2 suspension cells resulted in its localization to the plasma membrane. Growth tests and direct transport assays confirmed phenolic compounds as substrates.
Finally, expression of a 10-His tagged NtPDR3 led to its solubilization and partial purification by Ni-chromatography.
Altogether, these data allowed us to identity NtPDR3 substrates and indicate that *N. tabacum* BY2 suspension cells are an interesting expression system for transport assays of plant PDR and possibly other ABC transporters.

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☐	physiology	☐	pathology	☐	mechanism/structure	x	non mammalian

Character count (max 2000 with space [excluding illustration])

Title:

A Pleiotropic Drug Resistance transporter in *Nicotiana tabacum* is involved in defense against the herbivore *Manduca sexta*

Authors and affiliations:

Manuela Désirée Bienert[1], Stephanie Elise Gerlitz Siegmund[2], Anna Drozak[1], Tomasz Trombik[1], Alain Bultreys[3], Ian Thomas Baldwin[2], and Marc Boutry[1]

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[2]Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, 07745 Jena, Germany.

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Abstract:

Pleiotropic Drug Resistance (PDR) transporters form a group of membrane proteins belonging to the ABCG subfamily of ATP binding cassette (ABC) transporters. There is clear evidence for the involvement of plant ABC transporters in resistance to fungal and bacterial pathogens, but not in the biotic stress response to insect or herbivore attack. Here, we describe a PDR transporter, ABCG5/PDR5, from *Nicotiana tabacum*. GFP fusion and subcellular fractionation studies revealed that ABCG5/PDR5 is localized to the plasma membrane. Transgenic plants expressing the GUS reporter gene under the control of the *ABCG5/PDR5* transcription promoter and immunoblotting showed that, under standard growth conditions, ABCG5/PDR5 is highly expressed in roots, stems, and flowers, but is only expressed at marginal levels in leaves. Interestingly, ABCG5/PDR5 expression is induced in leaves by methyl jasmonate, wounding, pathogen infiltration, or herbivory by *Manduca sexta*. To address the physiological

role of *ABCG5/PDR5*, *N. tabacum* plants silenced for the expression of *ABCG5/PDR5* were obtained. No phenotypic modification was observed under standard conditions. However, a small increase of susceptibility to the fungus *Fusarium oxysporum* was observed. A stronger effect was observed in relation to herbivory: the silenced plants allowed better growth and faster development of *M. sexta* larvae than wild type plants, indicating an involvement of this PDR transporter in resistance to *M. sexta* herbivory.

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Session chosen :

☐ physiology			

Character count (max 2000 with space [excluding illustration])

Title: microRNA33 targets the ABCA1 pump and regulates cholesterol metabolism.

Authors and affiliations: Laure-Alix Clerbaux, Isabelle Gérin, Guido T. Bommer.
Laboratory for chelical physiology, de Duve Institute, Brussels, Belgium

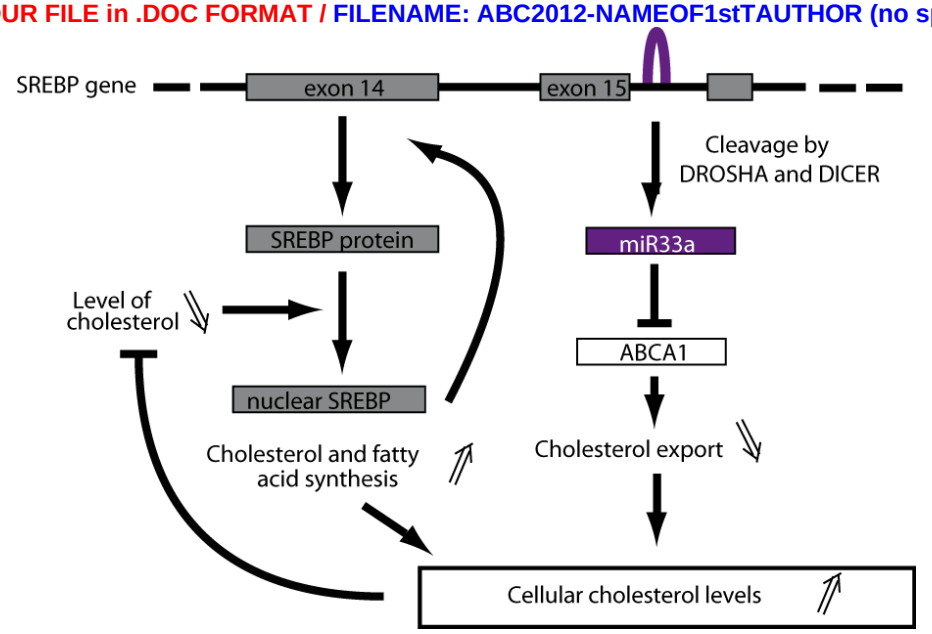
Abstract: The sterol regulatory element binding protein (SREBP) is activated when cellular cholesterol level is low and induce the transcription of many genes involved in the synthesis of cholesterol and fatty acids. In this study, we provide evidence that the primary transcript of SREBP2 contains an intronic miRNA (miR33) that reduces cellular cholesterol export via inhibition of translation of the cholesterol export pump ABCA1.

Background and Objectives: The regulation of synthesis, degradation, and distribution of lipids is crucial for homeostasis of organisms and cells. The objective of my work is to study the role of a particular family of microRNAs, the miR33 family in lipid and cholesterol metabolism. The miR33 family that we are studying is generated from precursor transcripts that in parallel encode the SREBP transcription factor family.

Methods and Results: The protein ABCA1 is a pump that exports cellular cholesterol out of the cell to the HDL (high density lipoprotein) particles. The mRNA transcript of ABCA1 contains a putative and highly conserved binding site for miR33. Using a reporter assay experiment, we have demonstrated that miR33 can directly target this transcript. Moreover we have shown that miR33 inhibits endogenous ABCA1 protein levels and therefore reduces cellular cholesterol export. Notably, overexpression of competitive antagonists of miR33 action in cells led to an upregulation of ABCA1 protein levels, which was accompanied by a significant increase in cellular cholesterol export.

Conclusions: We show evidence that the genetic locus of SREBP2 not only encodes a sterol sensing transcription factor, but also contains a highly conserved miRNA that regulates cholesterol export. miR33 therefore cooperates with its embedding gene SREBP2 to maintain normal cellular cholesterol levels.

Illustration



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Session chosen:

☒	physiology	☐	pathology	☐	mechanism/structure	☐	non mammalian

Character count (max 2000 with space [excluding illustration])

Title:

ABCB1 1199G>A genetic polymorphism influences tacrolimus intracellular accumulation in HEK293 and K562 recombinant cell lines.

Authors and affiliations:

Géraldine Dessilly[1*], MSc, PhD student
Laure Elens[1*], MSc, PhD
Nadtha Panin[1]
Arnaud Capron[2], PhD
Jean-Baptiste Demoulin[3], PharmD, PhD
Vincent Haufroid[1, 2], PharmD, PhD

[1]Louvain centre for Toxicology and Applied pharmacology, Institut de recherche expérimentale et clinique, Université catholique de Louvain, Brussels, Belgium ; [2]Department of Clinical Chemistry, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium, [3]De Duve Institute Brussels, Université catholique de Louvain, Belgium.

* Both authors contributed equally.

Abstract:

Background and Objectives:

ATP-binding cassette, subfamily B, member 1 (ABCB1) transporter, or Pglycoprotein, is an efflux protein implicated in the absorption and the distribution of various compounds, including tacrolimus. In vivo studies suggest an association between ABCB1 1199G>A single nucleotide polymorphism (SNP) and tacrolimus intracellular accumulation. The aim of the present experimental study was to clarify in vitro the impact of the coding ABCB1 1199G>A SNP on ABCB1 transport activity towards tacrolimus.

Methods:

Two recombinant cell lines, i.e. Human Embryonic Kidney (HEK293) and Human Myelogenous Leukemia (K562) cells, overexpressing ABCB1 carrying either the wild-type allele (1199G) or its mutated counterpart (1199A), were generated. The impact of the 1199G>A SNP on ABCB1 activity towards tacrolimus was assessed by accumulation experiments.

Results:

Tacrolimus accumulation was strongly decreased in cells overexpressing the wild-type protein (1199G) compared to control cells, confirming the ability of ABCB1 to transport tacrolimus. By contrast, overexpression of the variant protein (1199A) had nearly no effect on tacrolimus accumulation whatever the model used ($p>0.05$). This observation suggests that tacrolimus ABCB1-mediated transport is blunted by the ABCB1 1199G>A SNP.

Conclusions:

ABCB1 encoded by the 1199G wild-type allele is more efficient to transport tacrolimus in comparison to the variant protein. This observation highlights that the corresponding amino-acid substitution (Ser400Asn) encoded by the 1199A allele decreases drastically the ability of ABCB1 to drive the efflux of tacrolimus. Our study emphasizes the importance of the ABCB1 1199G>A polymorphism for ABCB1 activity and its potential to explain differences in drug response, especially for drugs having intracellular activity and/or toxicity.

Illustration (optional)

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Session chosen :

☐ physiology	☒ pathology	☐ mechanism/structure	☐ non mammalian

Character count (max 2000 with space [excluding illustration])

Title: Influence of the basal cellular level of glutathione in triggering MRP1-cells death

Authors and affiliations: Lauriane Dury, Attilio Di Pietro and Hélène Cortay
IBCP, UMR 5086, BMSSI, 7 passage du Vercors 69007 Lyon, France.

Background and Objectives: MRP1 is implicated in chemoresistance of cancer cells. It transports negatively charged substrates and many drugs in co-transport with glutathione (GSH). We previously found that verapamil induced a very fast and huge GSH extrusion out of MRP1-overexpressing cells (MRP1-cells) which led them to apoptosis, while parental cells were not affected. When screening other compounds, we observed that glutathione extrusion did not always lead to cell death: for example, the flavonoid galangine triggered 84% glutathione depletion but failed to kill MRP1-cells, while another compound led the cells to death with only moderate glutathione depletion. Studying the effect of BSO (an inhibitor of GSH synthesis), in combination with these molecules, we investigated the involvement of basal glutathione levels in triggering MRP1-cells death.

Methods: **Cell proliferation analysis**. The MTT colorimetric assay was used to assess the sensitivity of NCI-H69 (parental cells) and H69AR (MRP1-cells) to compounds by determination of the IC50: concentration of compound where 50% of growth inhibition were observed.
Total cellular glutathione determination. After incubation with compounds, total cellular glutathione content was measured in BHK21 and BHK21-MRP1 cells using the enzymatic method described by Tiezte, modified by Anderson.

Results: We showed that galangine is able to prevent cell death triggered by verapamil or BSO. Moreover, when the GSH basal level in NCI-H69 was brought to the same level as that of H69AR thanks to BSO, the effect of verapamil was not equivalent in the two cell lines.

Conclusions: Low basal glutathione level might be important for triggering death of H69AR, but the depletion of GSH has to be actively mediated by MRP1.

Rôle des P-glycoprotéines dans l'installation des nématodes parasites chez l'hôte

Issouf M, Guegnard F, Koch C, Sauvé C, Neveu C et Kerboeuf D

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Session 4: ABC transporters in non mammalian cells

Le parasitisme est un des principaux problèmes dans les élevages. Les nématodes parasites du tractus digestif des ovins et caprins sont responsable d'importantes baisses de rendement dans la production de lait, de laine, et de viande. La maîtrise de ces parasitoses a été longtemps basée sur l'utilisation de molécules anthelminthiques. Cependant, l'efficacité des traitements est fréquemment remise en cause par l'émergence d'isolats résistants à une ou plusieurs de ces molécules. Dans ce contexte, il est donc nécessaire de développer des nouvelles stratégies de lutte.

Une meilleure connaissance des mécanismes impliqués dans l'installation et la survie des parasites dans leur hôte est essentielle pour le développement de méthodes de lutte efficaces. Pour survivre aux produits de dégranulation des éosinophiles, nous avons envisagé l'implication des mécanismes de détoxication impliquant des transporteurs tels que les P-glycoprotéines (Pgps). Les Pgps sont des pompes membranaires de la superfamille des transporteurs ABC. Les molécules transportées sont très variées, elles ont en commun leur caractère hydrophobe. On peut donc se poser la question de leur éventuel rôle chez les nématodes dans la capture des produits toxiques issus des cellules de l'hôte.

Les travaux réalisés ont permis d'identifier des séquences partielles ou complètes d'ADNc de 12 Pgps du nématode parasite *Haemonchus contortus*. Une forte activation des Pgps des nématodes en présence des produits de dégranulation des éosinophiles de l'hôte a été observée, démontrant ainsi l'interaction entre les Pgps des nématodes et les produits issus de l'hôte. De plus, l'exposition *in vitro* des nématodes parasites aux produits de l'hôte montrent après analyse par PCR quantitative une induction significative de l'expression de cinq Pgps (*hco-pgp-3*, et *hco-pgp-16*). Ce travail constitue la première mise en évidence de l'interaction entre les Pgps des nématodes parasites et des produits issus de leur hôte. Dans le but d'identifier les molécules responsables de cette interaction ces produits seront séparés et analysés. D'autres parts, des Pgps recombinantes seront produites afin d'étudier de manière plus fine ces protéines. Ces résultats pourraient constituer une base solide pour le développement d'une méthode efficace permettant de bloquer ces transporteurs et d'éliminer les nématodes parasites.

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Session chosen :

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Identification of substrates of a plant pleiotropic drug resistance transporter, NtPDR1 from *Nicotiana tabacum*

Julien Roland, Emmanuel Peeters, Joseph Nader and Marc Boutry

Leaf trichomes are particularly rich in the production of secondary metabolites that are involved in the plant protection against pathogens. Some of these metabolites are also toxic for the cells that produce them and have therefore to be actively transported out of the cell. Very little is known concerning their transport although in some cases huge concentration differences are observed between cellular compartments.

We have identified several Pleiotropic Drug Resistance (PDR)-type ABC transporters expressed in trichomes and we hypothesized that they extrude metabolites involved in the plant response to (a)biotic stress. One of them, NtPDR1, is expressed in *Nicotiana tabacum* glandular trichomes and is probably involved in the plant defense by transporting antimicrobial diterpenes. However, direct transport has not been demonstrated yet. The aim of the project was to identify its substrates.

NtPDR1 was expressed in *Nicotiana tabacum* BY2 suspension cells. *In situ* immunolocalization showed plasma membrane localization of this PDR as in the plant. Toxicity assays with putative substrates showed that NtPDR1-expressing lines were more resistant to the diterpenes sclareol, manool, abietic acid and dehydroabietic acid, suggesting their export out of the cell. Transport assays confirmed that these diterpenes are NtPDR1 substrates. However, other molecules such as mono- and sesquiterpenes were not transported, indicating the relative specificity of this transporter.

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Session chosen :

☐	physiology	☐	pathology	☐	mechanism/structure	X	non mammalian

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Over-expression of plant pleiotropic drug resistance transporters for their enzymatic and structural characterization

Frédéric Toussaint, Amandine Baijot and Marc Boutry
University of Louvain, 1348 Louvain-la-Neuve, Belgium

Our team is interested in the biochemical characterization of plant plasma membrane ABC transporters belonging to the Pleiotorpic Drug Resistant (PDR) family. We therefore sought to find a convenient expression system. We first tested a heterologous expression host, *Saccharomyces cerevisiae*, using the inducible promoter, MET25. Although their expression was detected, the PDR transporters mainly localized to internal membranes instead of the plasma membrane. In addition, their solubilization with various detergents was little efficient, indicating that these proteins might aggregate.
We then tested a plant expression system, *Nicotiana tabacum* BY2 suspension cells, using either a constitutive or a heath-inducible promoter. Four PDR were successfully expressed and localized to the plasma membrane: NtPDR1, MpPDR1, NtPDR3, NtPDR5. They were readily solubilized by 0.5% dodecyl maltoside. Providing these transporters with a 6-His tag at the N-terminus did not allow efficient purification by Ni-chromatography. However, extending the tag to 10 His residues and adding a Gly linker strongly improved the purification yield. Further purification is now being tested using a StrepII tag.

Functional investigation of a multidrug transporter of *Pseudomonas aeruginosa*

Alice Verchère, Isabelle Broutin & Martin Picard

Laboratoire de Cristallographie et RMN Biologiques (CNRS UMR8015),
Faculté de Pharmacie, Université Paris Descartes
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Session chosen: ABC transporters: structure and mechanisms

Background and Objectives

Among the various mechanisms developed by the bacteria to counter to the effect of antibiotics, active efflux is on the front line. In *Pseudomonas aeruginosa*, a Gram-negative bacteria, efflux transporters are organized as multicomponent systems where MexB, the pump located in the inner membrane, works in conjunction with MexA, a periplasmic protein, and OprM, an outer membrane protein. MexB acts as a proton motive force-dependent pump with broad substrate specificity.

Methods

We describe an original activity assay for MexB [1]. This pump is co-reconstituted into proteoliposomes together with bacteriorhodopsin (BR), a light-activated proton pump. MexB activity is monitored by fluorescence. Indeed pyranine, a pH fluorescent probe is encapsulated in proteoliposomes allowing us to measure pH in the vesicles.

Results

In this system, upon illumination with visible light, the photo-induced proton gradient created by the BR is shown to be coupled to the active transport of substrates through the pump. This test makes the investigation of the pump possible. In the absence of MexA, MexB has a basal activity which is not substrate-dependent. Once MexB is reconstituted together with MexA, its activity is specific and substrate dependent.

Conclusions and Perspectives

Currently we are working on the reconstitution of the whole pump. To that end, we use an innovative system consisting of a biochip and a micromanipulation device. Such a system should provide a relevant environment for scaffolding the complex and will make it possible to study transport.

[1] Verchère, Broutin & Picard, *Scientific reports*, 2012

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Session chosen :

☐ physiology	☒ pathology	☐ mechanism/structure	☐ non mammalian

Character count (max 2000 with space [excluding illustration])

Title: Transfer of the CFTR chloride channel by membrane-derived microparticles as a therapeutic approach for cystic fibrosis.

Authors and affiliations: C. Vituret [1,2], G. Gonzalez [1], A. Di Pietro [2], M. Chanson [3], P. Boulanger [1], & S.S. Hong [1]

[1] Université Lyon I & INRA UMR-754, 50, av. Tony Garnier, Lyon [2] Unité BM2SI, Université Lyon 1- CNRS UMR 5086, IBCP, 7, Passage du Vercors, Lyon [3] Laboratory of Clinical Investigation III, Faculty of Medicine, University of Geneva, FMR, 64, av. de la Roseraie, 1211 Geneva 4, Switzerland

Abstract:

Background and Objectives: Membrane microparticles (MP) released in the extracellular milieu, spontaneously or in response to stress, can embark membrane and cytosolic components which are specific of their cellular origin. Taking advantage of this property, we tested the possibility of MP-mediated delivery of human CFTR, a dodecaspanin glycoprotein which functions as a chloride channel and is deficient in cystic fibrosis, was then explored.

Methods: Donor cells were CHO cells constitutively expressing the GFP-tagged CFTR construct (1) from an episomal plasmid. MP were isolated from the extracellular medium of nonstressed cells, and fractionated by ultracentrifugation in different subclasses differing in size and density.

Results: The proportion of GFP-CFTR-positive MP varied from 1.5 to 15% between the different MP subclasses, and all subclasses were found to be capable of transferring mature, membrane-inserted GFP-CFTR glyco-protein to recipient CHO cells at early times pt (2-6 h pt). However, this early transfer occurred with a rather low efficiency (5-6% positive cells). Likewise, all MP subclasses delivered GFP-CFTR-encoding mRNA to recipient cells, resulting in the neosynthesis of GFP-CFTR glycoprotein and a delayed, prolonged expression of GFP-CFTR (≥ 5D). Both types of CFTR molecules, transferred as mature glycoproteins or neosynthesized from exogenous mRNA, were detected at the plasma membrane of recipient cells and adopted a correct folding and membrane insertion. However, chloride channel activity was only detectable at late times pt, implying that this newly acquired function was due to neosynthesized GFP-CFTR from transferred mRNA.

Conclusions: Cell-derived MP therefore constitute promising vectors for the delivery of exogenous mRNA exerting the desired therapeutic effect via *de novo* protein synthesis.

(1) Granio et al. (2007) AJRCMB 37,63L-639, Granio ef a/. (2010) Hum. Gene Ther. 21, 2st-269

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