

Publications de l'U 845 – Equipe Edelman (Inserm) (depuis 1999)

2007

**BENSALEM N., MASSCHELEYN S., MOZO J., VALLEE B., BROUILLARD F.,
TRUDEL S., RICQUIER D., EDELMAN A., GUERRERA I.C., MIROUX B.**

High Sensitivity Identification of Membrane Proteins by MALDI TOF-MASS Spectrometry Using Polystyrene Beads.

J. Proteome Res., 6 (4), 1595-1602, 2007 ; (Facteur d'Impact 2006 : **5,151**)

(Services cités : IRNEM, U845 (AE), UPR 9078)

Membrane proteins play a large variety of functions in life and represent 30% of all genomes sequenced. Due to their hydrophobic nature, they are tightly bound to their biological membrane, and detergents are always required to extract and isolate them before identification by mass spectrometry (MS). The latter, however remains difficult. Peptide mass fingerprinting methods using techniques such as MALDI-TOF MS, for example, have become an important analytical tool in the identification of proteins. However, PMF of membrane proteins is a real challenge for at least three reasons. First, membrane proteins are naturally present at low levels; second, most of the detergents strongly inhibit proteases and have deleterious effects on MALDI spectra; and third, despite the presence of detergent, membrane proteins are unstable and often aggregate. We took the mitochondrial uncoupling protein 1 (UCP1) as a model and showed that differential acetonitrile extraction of tryptic peptides combined with the use of polystyrene Bio-Beads triggered high resolution of the MALDI-TOF identification of mitochondrial membrane proteins solubilized either with Triton-X100 or CHAPS detergents. Keywords: mass spectrometry * detergent * membrane protein identification * peptide mass fingerprinting.

**DOS SANTOS A., THIERS V., SAR S., DERIAN N., BENSALEM N., YILMAZ F.,
BRALET M.P., DUCOT B., BRECHOT C., DEMAUGRE F.**

Contribution of laser microdissection-based technology to proteomic analysis in hepatocellular carcinoma developing on cirrhosis.

Proteom. - Clin. Appl., 1 (6), 545-554, 2007 ; (Facteur d'Impact 2006 : **X**)

(Services cités : U845 (AE))

Hepatocellular carcinoma (HCC) is a major cause of cancer worldwide. Proteomic studies provide opportunities to uncover targets for the diagnosis and treatment of this disease. However, in HCC developing in a setting of cirrhosis, the detection of proteome alterations may be hampered by the increased cellular heterogeneity of tissue when analysing global liver homogenates. The aim of this study was to evaluate whether the identification of proteome alterations in these HCC cases was improved when the differential protein profile between tumour and non-tumour areas of liver was determined using hepatocytes isolated by laser microdissection (LM). Differential profiles established with LM-hepatocytes and liver section homogenates using 2-DE and MS exhibited noticeable differences: 30% of the protein spots with deregulated expression in tumorous LM-samples did not display any modification in homogenates; conversely 15% of proteins altered in tumorous homogenates were not impaired in LM-hepatocytes. These alterations resulted from the presence in cirrhotic liver of fibrotic stroma which displayed a protein pattern different from that determined in LM-hepatocytes. In conclusion, our data demonstrate the interest of LM in distinguishing between fibrotic and

hepatocyte proteome alterations and thus the benefit of LM to proteome studies of HCC developing in a context of cirrhosis.

EDELMAN A., SERMET-GAUDELUS I., ROUSSET J.P.

Genetic testing to provide targeted treatment for cystic fibrosis patients.

Pharmacogenomics, 8 (9), 1101-1104, 2007 ; (Facteur d'Impact 2006 : **3,603**)

(Services cités : Pédiatrie Générale, U845 (AE))

HINZPETER A., FRITSCH J., BOROT F., TRUDEL S., VIEU D.L., BROUILLARD F., BAUDOIN-LEGROS M., CLAIN J., EDELMAN A., OLLERO M.

Membrane cholesterol content modulates CIC-2 gating and sensitivity to oxidative stress.

J. Biol. Chem., 282 (4), 2423-2432, 2007 ; (Facteur d'Impact 2006 : **5,808**)

(Services cités : U845 (AE))

CIC-2 is a broadly expressed member of the voltage-gated CIC chloride channel family. In this study, we aimed to evaluate the role of the membrane lipid environment in CIC-2 function, and in particular the effect of cholesterol and CIC-2 distribution in membrane microdomains. Detergent-resistant and detergent-soluble microdomains (DSM) were isolated from stably transfected HEK293 cells by a discontinuous OptiPrep gradient. CIC-2 was found concentrated in detergent-insoluble membranes in basal conditions and relocalized to DSM upon cholesterol depletion by methyl-beta-cyclodextrin. As assessed by patch clamp recordings, relocalization was accompanied by acceleration of the activation kinetics of the channel. A similar distribution and activation pattern were obtained when cells were treated with the oxidant tert-butyl hydroperoxide and after ATP depletion. In both cases activation was prevented by cholesterol enrichment of cells. We conclude that the cholesterol environment regulates CIC-2 activity, and we provide evidence that the increase in CIC-2 activity in response to acute oxidative or metabolic stress involves relocalization of this channel to DSM.

PLANELLES G.

Ammonium homeostasis and human Rhesus glycoproteins.

Nephron Physiol., 105 (1), 11-17, 2007 ; (Facteur d'Impact 2006 : **X**)

(Services cités : U845 (AE))

The brain ammonium production is detoxified by astrocytes, the gut ammonium production is detoxified by hepatic cells, and the renal ammonium production plays a major role in renal acid excretion. As a result of ammonium handling in these organs, the ammonium and pH values are strictly regulated in plasma. Up until recently, it was accepted that mammalian cell transmembrane ammonium transport was due to NH₄⁽⁺⁾ transport by non-specific transporting systems, and to non-ionic NH₃ diffusion, whereas lower organisms (such as bacteria, yeasts and plants) were endowed with specific ammonium transporters (Amts). Sequence homologies between Amts and human Rhesus (Rh) glycoproteins (RhAG, from erythroid cells, and RhBG and RhCG from epithelial cells) raised the hypothesis that Rh glycoproteins act as specific ammonium transporters, further sustained by the polarized distribution of RhBG and RhCG in gut, kidney and liver. Results from functional studies agree that Rh glycoproteins are the first ammonium transporters reported in mammals. However, the nature of the transported specie(s) is

much debated: in particular, it is proposed that Rh glycoproteins mediate a direct NH₃ transport, or that they mediate an indirect NH₃ transport (resulting from NH₄⁽⁺⁾ for H⁽⁺⁾ exchange). Direct NH₃ transport (associated or not with NH₄⁽⁺⁾ transport) raises the exciting hypothesis that Rh glycoproteins may also transport other gases than NH₃ (namely, CO₂).

SERMET-GAUDELUS I., GIRODON E., HUET F., ABOUTAAM R., BUI S., DENEUVILLE E., GUILLOT M., VRIELYNCK S., LENOIR G., EDELMAN A.

Nasal potential difference in cystic fibrosis diagnosis of very young children.

J. Pediat., 150 (3), e34-e35, 2007 ; (Facteur d'Impact 2006 : 3,991)

(Services cités : Pédiatrie Générale, U845 (AE))

SERMET-GAUDELUS I., RENOUIL M., FAJAC A., BIDOU L., PARBAILLE B., PIERROT S., DAVY N., BISMUTH E., REINERT P., LENOIR G., LESURE J.F., ROUSSET J.P., EDELMAN A.

In vitro prediction of stop-codon suppression by intravenous gentamycin in patients with cystic fibrosis; a pilot study.

BMC Med., 5 (1), 5, 2007 ; (Facteur d'Impact 2006 : X)

(Services cités : U845 (AE), ORL & Chirurgie Cervico-Faciale, Pédiatrie Générale)

ABSTRACT: BACKGROUND: Cystic fibrosis (CF) is caused by mutations in the gene encoding for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, which acts as a chloride channel activated by cyclic AMP (cAMP). The most frequent mutation found in 70% of CF patients is F508del, while premature stop mutations are found in about 10% of patients. In vitro aminoglycoside antibiotics (e.g. gentamycin) suppress nonsense mutations located in CFTR permitting translation to continue to the normal termination of the transcript. Pharmacologic suppression of stop mutations within the CFTR may be of benefit to a significant number of patients. Our pilot study was conducted to determine whether intravenous gentamycin suppresses stop codons in CF patients and whether it has clinical benefits. **METHODS:** A dual gene reporter system was used to determine the gentamycin-induced readthrough level of the most frequent stop mutations within the CFTR in the French population. We investigated readthrough efficiency in response to 10 mg/kg once daily intravenous gentamycin perfusions in patients with and without stop mutations. Respiratory function, sweat chloride concentration, nasal potential difference (NPD) and CFTR expression in nasal epithelial cells were measured at baseline and after 15 days of treatment. **RESULTS:** After in vitro gentamycin incubation, the readthrough efficiency for the Y122X mutation was at least 5 times higher than that for G542X, R1162X, and W1282X. In 6 of the 9 patients with the Y122X mutation, CFTR immunodetection showed protein at the membrane of the nasal epithelial cells and the CFTR-dependent Cl⁻ secretion in NPD measurements increased significantly. Respiratory status also improved in these patients, irrespective of the gentamycin sensitivity of the bacteria present in the sputum. Mean sweat chloride concentration decreased significantly and normalized in 2 patients. Clinical status, NPD and sweat Cl⁻ values did not change in the Y122X patients with no protein expression, in patients with the other stop mutations investigated in vitro and those without stop mutations. **CONCLUSION:** Suppression of stop mutations in the CFTR gene with parenteral gentamycin can be predicted in vitro and is associated with clinical benefit and significant modification of the CFTR-mediated Cl⁻ transport in nasal and sweat gland epithelium. Trial registration:

ClinicalTrial NCT00376428.

SERMET-GAUDELUS I., SOUBERBIELLE J.C., ELADARI D., RUIZ J.C., MALLET E.

Pediatric bone health: Starting at the beginning.

Pediat. Pulm., 42 (Suppl.30), 187-188, 2007 ; (Facteur d'Impact 2006 : **1,965**)

(Services cités : Pédiatrie Générale, U845 (AE))

2006

BAKOUH N., BENJELLOUN F., CHERIF-ZAHAR B., PLANELLES G.

The challenge of understanding ammonium homeostasis and the role of the Rh glycoproteins.
Transfus. Clin. Biol., 13 (1-2), 139-146, 2006

(Services cités : U806)

Rh glycoproteins belong to the superfamily of ammonium transporters, but until recent functional studies their functional role was unknown. This review focuses on the functional results obtained in our laboratory after the heterologous expression of RhAG (the erythroid Rh glycoprotein) and RhCG (an epithelial Rh glycoprotein). RhAG and RhCG were expressed in two different expression systems (HeLa cells and *Xenopus laevis* oocytes) that differed in their endogenous membrane permeabilities for NH₃ and NH₄⁽⁺⁾. To check if RhAG and RhCG are ammonium transporters, we measured intracellular pH changes in cells exposed to an ammonium-containing solution, and analyzed the ammonium-induced NH₃ and NH₄⁽⁺⁾ transmembrane fluxes in control versus transfected cells. We observed that RhAG and RhCG expression induced an enhancement of the ammonium-induced initial alkalinization (related to NH₃ influx into the cell) and secondary acidification (related to NH₄⁽⁺⁾ influx into the cell). Moreover, sub-millimolar ammonium concentrations induced inward currents in voltage-clamped RhAG- and RhCG-expressing oocytes. Taken together, these results show not only that RhAG and RhCG are ammonium transporters, but also that they are promoting the transmembrane transport of NH₃ and of NH₄⁽⁺⁾. Data from our laboratory and from other groups raise several questions that are discussed.

HINZPETER A., LIPECKA J., BROUILLARD F., BAUDOIN-LEGROS M., DADLEZ M., EDELMAN A., FRITSCH J.

Association between Hsp90 and the ClC-2 chloride channel upregulates channel function.
Amer. J. Physiol. - Cell Physiol., 290 (1), C45-C56, 2006

(Services cités : IRNEM, U806)

The voltage-dependent ClC-2 chloride channel has been implicated in a variety of physiological functions, including fluid transport across specific epithelia. ClC-2 is activated by hyperpolarization, weakly acidic external pH, intracellular Cl⁽⁻⁾, and cell swelling. To add more insight into the mechanisms involved in ClC-2 regulation, we searched for associated proteins that may influence ClC-2 activity. With the use of immunoprecipitation of ClC-2 from human embryonic kidney-293 cells stably expressing the channel, followed by electrophoretic separation of coimmunoprecipitated proteins and mass spectrometry identification, Hsp70 and Hsp90 were unmasked as possible ClC-2 interacting partners. Association of Hsp90 with ClC-2 was confirmed in mouse brain. Inhibition of Hsp90 by two specific inhibitors, geldanamycin or radicicol, did not affect total amounts of ClC-2 but did reduce plasma membrane channel abundance. Functional experiments using the whole cell configuration of the patch-clamp technique showed that inhibition of Hsp90 reduced ClC-2 current amplitude and impaired the intracellular Cl⁽⁻⁾ concentration [Cl⁽⁻⁾]-dependent rightward shift of the fractional conductance. Geldanamycin and radicicol increased both the slow and fast activation time constants in a chloride-dependent manner. Heat shock treatment had the opposite effect. These results indicate

that association of Hsp90 with ClC-2 results in greater channel activity due to increased cell surface channel expression, facilitation of channel opening, and enhanced channel sensitivity to intracellular [Cl(-)]. This association may have important pathophysiological consequences, enabling increased ClC-2 activity in response to cellular stresses such as elevated temperature, ischemia, or oxidative reagents.

JUNGERS P., JOLY D., NGUYEN-KHOA T., MOTHU N., BASSILIOS N., GRUNFELD J.P.

Continued late referral of patients with chronic kidney disease. Causes, consequences, and approaches to improvement.

Presse Médicale, 35 (1 Pt 1), 17-22, 2006

(Services cités : Néphrologie Pédiatrique, U507, U806, Biochimie Générale)

OBJECTIVES: Efforts in recent years have aimed at increasing physicians' awareness of the frequent and harmful consequences of late referral to nephrologists of patients with chronic kidney disease (CKD), shown in repeated concordant studies. We sought to determine whether these efforts have led to improved predialysis care of these patients. **METHODS:** This study included all 1391 consecutive patients who began maintenance dialysis at Necker Hospital between January 1989 and December 2000. We examined baseline data and outcomes and determined for four three-year periods the percentage of patients who received early specialized care (at least 6 months before onset of dialysis). **RESULTS:** Late referral (<6 months before dialysis) did not change significantly over the four periods, remaining around 30%, even during the most recent period (1998-2000). Clinical condition and laboratory indicators of patients referred early but not those referred late improved in the latest period, compared with the initial period (1989-1991). Overall, prevalence of major cardiovascular events (myocardial or cerebral infarction, peripheral arteriopathy, or heart failure) was more than twice as high in patients who received nephrologic care for less than 6 months and nearly twice as high even in those followed 6-35 months than in patients followed for at least 36 months before beginning dialysis. Subsequent mortality after maintenance dialysis was also significantly higher in patients with late referral than in those followed at least 3 years before dialysis. Multivariate Cox proportional model analysis identified graded duration of predialysis nephrologic care as a significant independent factor predictive of risk of mortality while on dialysis. **CONCLUSION:** Late referral of CKD patients for specialist care remains frequent, around 30%, although it is most often unjustified. Late referral deprives the patient of early implementation of a reno- and cardioprotective therapeutic strategy that reduces cardiovascular comorbidity and mortality. Better coordinated cooperation between family doctors and nephrologists, through the implementation of regional healthcare networks, now appears as the most effective way to improve the care of CKD patients.

LIPECKA J., NOREZ C., BENSALÉM N., BAUDOUIN-LEGROS M., PLANELLES G., BECQ F., EDELMAN A., DAVEZAC N.

Rescue of DeltaF508-CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) by Curcumin: Involvement of the Keratin 18 Network.

J. Pharmacol. Exp. Ther., (2), 500-505, 2006

(Services cités : U806)

The most common mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, DeltaF508, causes retention of DeltaF508-CFTR in the endoplasmic reticulum and leads to the absence of CFTR Cl(-) channels in the plasma membrane. DeltaF508-CFTR retains some Cl(-)

) channel activity so increased expression of DeltaF508-CFTR in the plasma membrane can restore Cl(-) secretion deficiency. Recently, curcumin was shown to rescue DeltaF508-CFTR localization and function. In our previous work, the keratin 18 (K18) network was implicated in DeltaF508-CFTR trafficking. Here, we hypothesized that curcumin could restore a functional DeltaF508-CFTR to the plasma membrane acting via the K18 network. First, we analyzed the effects of curcumin on the localization of DeltaF508-CFTR in different cell lines (HeLa cells stably transfected with wild-type CFTR or DeltaF508-CFTR, CALU-3 cells, or cystic fibrosis pancreatic epithelial cells CFPAC-1) and found that it was significantly delocalized toward the plasma membrane in DeltaF508-CFTR-expressing cells. We also performed a functional assay for the CFTR chloride channel in CFPAC-1 cells treated or not with curcumin and detected an increase in a cAMP-dependent chloride efflux in treated DeltaF508-CFTR-expressing cells. The K18 network then was analyzed by immunocytochemistry and immunoblot exclusively in curcumin-treated or untreated CFPAC-1 cells because of their endogenous DeltaF508-CFTR expression. After curcumin treatment, we observed a remodeling of the K18 network and a significant increase in K18 Ser52 phosphorylation, a site directly implicated in the reorganization of intermediate filaments. With these results, we propose that K18 as a new therapeutic target and curcumin, and/or its analogs, might be considered as potential therapeutic agents for cystic fibrosis.

MARINI A.M., BOECKSTAENS M., BENJELLOUN F., CHERIF-ZAHAR B., ANDRE B.
Structural involvement in substrate recognition of an essential aspartate residue conserved in Mep/Amt and Rh-type ammonium transporters.

Curr. Genet., 49 (6), 364-374, 2006

(Services cités : [U806](#))

Ammonium transport proteins belonging to the Mep/Amt/Rh family are spread throughout all domains of life. A conserved aspartate residue plays a key role in the function of *Escherichia coli* AmtB. Here, we show that the analogous aspartate residue is critical for the transport function of eukaryotic family members as distant as the yeast transporter/sensor Mep2 and the human RhAG and RhCG proteins. In yeast Mep2, replacement of aspartate(186) with asparagine produced an inactive transporter localized at the cell surface, whilst replacement with alanine was accompanied by stacking of the protein in the endoplasmic reticulum. Introduction of an acidic residue, glutamate, produced a partially active protein. A carboxyl group at position 186 of Mep2 therefore appears mandatory for function. Kinetic analysis shows the Mep2(D186E) variant to be particularly affected at the level of substrate affinity, suggesting an involvement of aspartate(186) in ammonium recognition. Our data also put forward that ammonium recognition and/or transport by Mep2 is required for the sensor role played in the development of pseudohyphal growth. Finally, replacement of the conserved aspartate with asparagine in human RhAG and RhCG proteins resulted in the loss of bi-directional transport function. Hence, this aspartate residue might play a preserved functional role in Mep/Amt/Rh proteins.

OLLERO M., BROUILLARD F., EDELMAN A.

Cystic fibrosis enters the proteomics scene: New answers to old questions.

Proteomics, 6 (14), 4084-4099, 2006

(Services cités : [IRNEM](#), [U806](#))

The discovery in 1989 of the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) and its mutation as the primary cause of cystic fibrosis (CF), generated an optimistic reaction with respect to the development of potential therapies. This extraordinary

milestone, however, represented only the initial key step in a long path. Many of the mechanisms that govern the pathogenesis of CF, the most commonly inherited lethal pulmonary disorder in Caucasians, remain even today unknown. As a continuation to genomic research, proteomics now offers the unique advantage to examine global alterations in the protein expression patterns of CF cells and tissues. The systematic use of this approach will probably provide new insights into the cellular mechanisms involved in CF dysfunctions, and should ultimately result in the finding of new prognostic markers, and in the generation of new therapies. In this article we review the current status of proteomic research applied to the study of CF, including CFTR-related interactomics, and evaluate the potential of these technologies for future investigations.

SERMET-GAUDELUS I., ROUSSEL D., BUI S., DENEUVILLE E., HUET F., REIX P., BELLON G., LENOIR G., EDELMAN A.

The CF-CIRC study: a French collaborative study to assess the accuracy of Cystic Fibrosis diagnosis in neonatal screening.

BMC Ped., 6 25, 2006

(Services cités : Pédiatrie Générale, U806)

BACKGROUND: Cystic fibrosis (CF) is caused by mutations in the gene encoding for the CF transmembrane conductance regulator (CFTR) protein, which acts as a chloride channel after activation by cyclic AMP (cAMP). Newborn screening programs for CF usually consist of an immunoreactive trypsinogen (IRT) assay, followed when IRT is elevated by testing for a panel of CF-causing mutations. Some children, however, may have persistent hypertrypsinogenemia, only one or no identified CFTR gene mutation, and sweat chloride concentrations close to normal values. In vivo demonstration of abnormal CFTR protein function would be an important diagnostic aid in this situation. Measurements of transepithelial nasal potential differences (NPD) in adults accurately characterize CFTR-related ion transport. The aim of the present study is to establish reference values for NPD measurements for healthy children and those with CF aged 3 months to 3 years, the age range of most difficult-to-diagnose patients with suspected CF. The ultimate goal of our study is to validate NPD testing as a diagnostic tool for children with borderline results in neonatal screening. **METHODS/DESIGN:** We adapted the standard NPD protocol for young children, designed a special catheter for them, used a slower perfusion rate, and shortened the protocol to include only measurement of basal PD, transepithelial sodium (Na⁺) transport in response to the Na⁺ channel inhibitor amiloride, and CFTR-mediated chloride (Cl⁻) secretion in response to isoproterenol, a beta-agonist in a Cl⁻ free solution. The study will include 20 children with CF and 20 healthy control children. CF children will be included only if they carry 2 CF-causing mutations in the CFTR gene or have sweat chloride concentrations > 60 mEq/L or both. The healthy children will be recruited among the siblings of the CF patients, after verification that they do not carry the familial mutation. **DISCUSSION:** A preliminary study of 3 adult control subjects and 4 children older than 12 years with CF verified that the new protocol was well tolerated and produced NPD measurements that did not differ significantly from those obtained with the standard protocol. This preliminary study will provide a basis for interpreting NPD measurements in patients with suspected CF after neonatal screening. Earlier definitive diagnosis should alleviate parental distress and allow earlier therapeutic intervention and genetic counseling.

BAKOUH N., BENJELLOUN F., CHERIF-ZAHAR B., PLANELLES G.

The challenge of understanding ammonium homeostasis and the role of the Rh glycoproteins.

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Rh glycoproteins belong to the superfamily of ammonium transporters, but until recent functional studies their functional role was unknown. This review focuses on the functional results obtained in our laboratory after the heterologous expression of RhAG (the erythroid Rh glycoprotein) and RhCG (an epithelial Rh glycoprotein). RhAG and RhCG were expressed in two different expression systems (HeLa cells and *Xenopus laevis* oocytes) that differed in their endogenous membrane permeabilities for NH₃ and NH₄⁽⁺⁾. To check if RhAG and RhCG are ammonium transporters, we measured intracellular pH changes in cells exposed to an ammonium-containing solution, and analyzed the ammonium-induced NH₃ and NH₄⁽⁺⁾ transmembrane fluxes in control versus transfected cells. We observed that RhAG and RhCG expression induced an enhancement of the ammonium-induced initial alkalinization (related to NH₃ influx into the cell) and secondary acidification (related to NH₄⁽⁺⁾ influx into the cell). Moreover, sub-millimolar ammonium concentrations induced inward currents in voltage-clamped RhAG- and RhCG-expressing oocytes. Taken together, these results show not only that RhAG and RhCG are ammonium transporters, but also that they are promoting the transmembrane transport of NH₃ and of NH₄⁽⁺⁾. Data from our laboratory and from other groups raise several questions that are discussed.

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Proteomics, 6 (14), 4084-4099, 2006

(Services cités : IRNEM, U806)

The discovery in 1989 of the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) and its mutation as the primary cause of cystic fibrosis (CF), generated an optimistic reaction with respect to the development of potential therapies. This extraordinary milestone, however, represented only the initial key step in a long path. Many of the mechanisms that govern the pathogenesis of CF, the most commonly inherited lethal pulmonary disorder in Caucasians, remain even today unknown. As a continuation to genomic research, proteomics now offers the unique advantage to examine global alterations in the protein expression patterns of CF cells and tissues. The systematic use of this approach will probably provide new insights into the cellular mechanisms involved in CF dysfunctions, and should ultimately result in the finding of new prognostic markers, and in the generation of new therapies. In this article we review the current status of proteomic research applied to the study of CF, including CFTR-related interactomics, and evaluate the potential of these technologies for future investigations.

SERMET-GAUDELUS I., ROUSSEL D., BUI S., DENEUVILLE E., HUET F., REIX P., BELLON G., LENOIR G., EDELMAN A.

The CF-CIRC study: a French collaborative study to assess the accuracy of Cystic Fibrosis diagnosis in neonatal screening.

BMC Ped., 6 25, 2006

(Services cités : Pédiatrie Générale, U806)

BACKGROUND: Cystic fibrosis (CF) is caused by mutations in the gene encoding for the CF transmembrane conductance regulator (CFTR) protein, which acts as a chloride channel after activation by cyclic AMP (cAMP). Newborn screening programs for CF usually consist of an immunoreactive trypsinogen (IRT) assay, followed when IRT is elevated by testing for a panel of CF-causing mutations. Some children, however, may have persistent hypertrypsinogenemia, only one or no identified CFTR gene mutation, and sweat chloride concentrations close to normal values. In vivo demonstration of abnormal CFTR protein function would be an important diagnostic aid in this situation. Measurements of transepithelial nasal potential differences (NPD) in adults accurately characterize CFTR-related ion transport. The aim of the present study is to establish reference values for NPD measurements for healthy children and those with CF aged 3 months to 3 years, the age range of most difficult-to-diagnose patients with suspected CF. The ultimate goal of our study is to validate NPD testing as a diagnostic tool for children with borderline results in neonatal screening. **METHODS/DESIGN:** We adapted the standard NPD protocol for young children, designed a special catheter for them, used a slower perfusion rate, and shortened the protocol to include only measurement of basal PD, transepithelial sodium (Na⁺) transport in response to the Na⁺ channel inhibitor amiloride, and CFTR-mediated chloride (Cl⁻) secretion in response to isoproterenol, a beta-agonist in a Cl⁻ free solution. The study will include 20 children with CF and 20 healthy control children. CF children will be included only if they carry 2 CF-causing mutations in the CFTR gene or have sweat chloride concentrations > 60 mEq/L or both. The healthy children will be recruited among the siblings of the CF patients, after verification that they do not carry the familial mutation. **DISCUSSION:** A preliminary study of 3 adult control subjects and 4 children older than 12 years with CF verified that the new protocol was well tolerated and produced NPD measurements that did not differ significantly from those obtained with the standard protocol. This preliminary study will provide a basis for interpreting NPD measurements in patients with suspected CF after neonatal screening. Earlier definitive diagnosis should alleviate parental distress and allow earlier therapeutic intervention and genetic counseling.

2005

BAUDOIN-LEGROS M., HINZPETER A., JAULMES A., BROUILLARD F., COSTES B., FANEN P., EDELMAN A.

Cell-specific posttranscriptional regulation of CFTR gene expression via influence of MAPK cascades on 3'UTR part of transcripts.

Amer. J. Physiol. - Cell Physiol., 289 (5), C1240-C1250, 2005

(Services cités : U467)

Expression of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene, which contains the mutations responsible for CF, is regulated by cytokines (TNF-alpha and IL-1beta) in a cell-specific manner. TNF-alpha decreases CFTR mRNA in human colon cell lines (HT-29), but not in pulmonary cell lines (Calu-3), and IL-1beta increases it only in Calu-3 cells. We looked for the cytokine-induced posttranscriptional regulation of CFTR gene expression and

studied the modulation of CFTR mRNA stability linked to its 3' untranslated sequence (3'UTR) in HT-29 and Calu-3 cells. The stability of CFTR mRNA was analyzed by Northern blot after in vitro incubation of total RNAs from CFTR-expressing cells with cytosolic proteins extracted from control or cytokine-treated HT-29 and Calu-3 cells. CFTR mRNA was degraded only by extracts of TNF-alpha-treated HT-29 cells and not by cytosolic proteins from untreated or IL-1beta-treated HT-29 cells. In contrast, extracts of untreated Calu-3 cells enhanced CFTR mRNA degradation, and IL-1beta treatment inhibited this; TNF-alpha had no significant effect. The 3'UTR part of CFTR mRNA was found to be required for this posttranscriptional regulation. The 5' part of the 3'UTR (the 217 first bases), which contains two AUUUA sequences, was implicated in CFTR mRNA destabilization and the following 136 bases, containing several C-repeats in U-rich environment, in its protection. The proteins, which reacted with the U- and C-repeats of CFTR mRNA 3'UTR, were mainly controlled by stimulation of the p42/p44 and p38 MAP kinase cascades with interaction between these pathways. This posttranscriptional control of gene expression is a common feature of CFTR and many proteins of inflammation.

BENJELLOUN F., BAKOUH N., FRITSCH J., HULIN P., LIPECKA J., EDELMAN A., PLANELLES G., THOMAS S.R., CHERIF-ZAHAR B.

Expression of the human erythroid Rh glycoprotein (RhAG) enhances both NH₃ and NH₄⁺ transport in HeLa cells.

Pflugers Arch. - Eur. J. Physiol., 450 (3), 155-167, 2005

(Services cités : U467)

The erythroid Rh-associated glycoprotein (RhAG) is strictly required for the expression of the Rh blood group antigens carried by Rh (D,CE) proteins. A biological function for RhAG in ammonium transport has been suggested by its ability to improve survival of an ammonium-uptake-deficient yeast. We investigated the function of RhAG by studying the entry of NH₃/NH₄⁺ in HeLa cells transiently expressing the green fluorescent protein (GFP)-RhAG fusion protein and using a fluorescent proton probe to measure intracellular pH (pH_i). Under experimental conditions that reduce the intrinsic Na/H exchanger activity, exposure of control cells to a 10 mM NH₄Cl-containing solution induces the classic pH_i response profile of cells having a high permeability to NH₃ (P(NH₃)) but relatively low permeability to NH₄⁺ (P(NH₄⁺)). In contrast, under the same conditions, the pH_i profile of cells expressing RhAG clearly indicated an increased P(NH₄⁺), as evidenced by secondary reacidification during NH₄Cl exposure and a pH_i undershoot below the initial resting value upon its removal. Measurements of pH_i during methylammonium exposure showed that RhAG expression enhances the influx of both the unprotonated and ionic forms of methylammonium. Using a mathematical model to adjust passive permeabilities for a fit to the pH_i profiles, we found that RhAG expression resulted in a threefold increase of P(NH₄⁺) and a twofold increase of P(NH₃). Our results are the first evidence that the human erythroid RhAG increases the transport of both NH₃ and NH₄⁺.

BENSALEM N., VENTURA A.P., VALLEE B., LIPECKA J., TONDELIER D., DAVEZAC N., DOS SANTOS A., PERRETTI M., FAJAC A., SERMET-GAUDELUS I., RENOUIL M., LESURE J.F., HALGAND F., LAPREVOTE O., EDELMAN A.

Down-regulation of the Anti-inflammatory Protein Annexin A1 in Cystic Fibrosis Knock-out Mice and Patients.

Mol. Cell. Proteomics, 4 (10), 1591-1601, 2005

(Services cités : U467, Département de Pédiatrie, U370)

Cystic fibrosis is a fatal human genetic disease caused by mutations in the CFTR gene encoding a cAMP-activated chloride channel. It is characterized by abnormal fluid transport across secretory epithelia and chronic inflammation in lung, pancreas, and intestine. Because cystic fibrosis (CF) pathophysiology cannot be explained solely by dysfunction of cystic fibrosis transmembrane conductance regulator (CFTR), we applied a proteomic approach (bidimensional electrophoresis and mass spectrometry) to search for differentially expressed proteins between mice lacking *cftr* (*cftr*(tm1Unc), *cftr*(-/-)) and controls using colonic crypts from young animals, i.e. prior to the development of intestinal inflammation. By analyzing total proteins separated in the range of pH 6-11, we detected 24 differentially expressed proteins (>2-fold). In this work, we focused on one of these proteins that was absent in two-dimensional gels from *cftr*(-/-) mice. This protein spot (molecular mass, 37 kDa; pI 7) was identified by mass spectrometry as annexin A1, an anti-inflammatory protein. Interestingly, annexin A1 was also undetectable in lungs and pancreas of *cftr*(-/-) mice, tissues known to express CFTR. Absence of this inhibitory mediator of the host inflammatory response was associated with colonic up-regulation of the proinflammatory cytosolic phospholipase A(2). More importantly, annexin A1 was down-regulated in nasal epithelial cells from CF patients bearing homozygous nonsense mutations in the CFTR gene (Y122X, 489delC) and differentially expressed in F508del patients. These results suggest that annexin A1 may be a key protein involved in CF pathogenesis especially in relation to the not well defined field of inflammation in CF. We suggest that decreased expression of annexin A1 contributes to the worsening of the CF phenotype.

BROUILLARD F., BENSALÉM N., HINZPETER A., TONDELIER D., TRUDEL S., GRUBER A.D., OLLERO M., EDELMAN A.

Blue Native/SDS-PAGE Analysis Reveals Reduced Expression of the mCICA3 Protein in Cystic Fibrosis Knock-out Mice.

Mol. Cell. Proteomics, 4 (11), 1762-1775, 2005

(Services cités : U467)

Cystic fibrosis (CF) is a frequent autosomal recessive disorder caused by mutation of a gene encoding a multifunctional transmembrane protein, the cystic fibrosis transmembrane conductance regulator (CFTR), located in the apical membrane of epithelial cells lining exocrine glands. In an attempt to get a more complete picture of the pleiotropic effects of the CFTR defect on epithelial cells and particularly on the membrane compartment, a bidimensional blue native (BN)/SDS-PAGE-based proteomic approach was used on colonic crypt samples from control and CFTR knock-out mice (*cftr*(-/-)). This approach overcomes the difficulties of membrane protein analysis by conventional two-dimensional PAGE and is able to resolve multiprotein complexes. Used here for the first time on crude membrane proteins that were extracted from murine colonic crypts, BN/SDS-PAGE allows effective separation of protein species and complexes of various origins, including mitochondria, plasma membrane, and intracellular compartments. The major statistically significant difference in protein maps obtained with samples from control and *cftr*(-/-) mice was unambiguously identified as mCICA3, a member of a family of calcium-activated chloride channels considered to be key molecules in mucus secretion by goblet cells. On the basis of this finding, we evaluated the overall expression and localization of mCICA3 in the colonic epithelium and in the lung of mice by immunoblot analysis and immunohistochemistry. We found that mCICA3 expression was significantly decreased in the colon and lung of the *cftr*(-/-) mice. In an ex vivo assay, we found that the Ca(2+)-dependent (carbachol-stimulated) glycoprotein secretion strongly inhibited by the calcium-activated chloride channel blocker niflumic acid (100 μ m) was impaired in the distal colon of *cftr*(-/-) mice. These results support

the conclusion that a ClCA-related function in the CF colon depends on CFTR expression and may be correlated with the impaired expression of mClCA3.

CLAIN J., LEHMANN-CHE J., GIRODON E., LIPECKA J., EDELMAN A., GOOSSENS M., FANEN P.

A neutral variant involved in a complex CFTR allele contributes to a severe cystic fibrosis phenotype.

Hum. Genet., 116 (6), 454-460, 2005

(Services cités : U467)

In order to further elucidate the contribution of complex alleles to the wide phenotypic variability of cystic fibrosis (CF), we investigated the structure-function relationships of a severe CF-associated complex allele [p.S912L;p.G1244V]. To evaluate the contribution of each mutation to the phenotype, cystic fibrosis transmembrane conductance regulator (CFTR) mutants were expressed in HeLa cells and analysed for protein processing and Cl(-) channel activity. Both p.G1244V and [p.S912L;p.G1244V] mutants had normal protein processing but markedly decreased Cl(-) channel activity compared with wild-type. Notably, the double mutant displayed a dramatic decrease in Cl(-) channel activity compared with p.G1244V (P<0.001). p.S912L had normal protein processing and no detectable impact on CFTR function. In other respects, the p.S912L variation was identified in compound heterozygosity with p.R709X in a healthy fertile man. Together, these data strongly support the view that p.S912L in isolation should be considered as a neutral variant but one that might significantly impair CFTR function when inherited in cis with another CFTR mutation. Our data also further document the contribution of complex alleles to the wide phenotypic variability of CF. The results of functional studies of such complex alleles in other genetic diseases are discussed.

CLAIN J., LEHMANN-CHE J., DUGUEPEROUX I., AROUS N., GIRODON E., LEGENDRE M., GOOSSENS M., EDELMAN A., de BRAEKELEER M., TEULON J., FANEN P.

Misprocessing of the CFTR protein leads to mild cystic fibrosis phenotype.

Hum. Mutat., 25 (4), 360-371, 2005

(Services cités : U467)

Cystic fibrosis (CF) is mainly caused by mutations that interfere with the biosynthetic folding of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. The aim of this study was to determine the mechanism of dysfunction of a disease-causing mutation associated with variable phenotypes. In order to attain these objectives, we studied the effect of the p.L206W mutation on CFTR protein production and function, and we examined the genotype-phenotype correlation of [p.L206W]+[p.F508del] patients. We showed that p.L206W is a processing (class II) mutation since the CFTR biosynthetic pathway was severely impaired, whereas single-channel measurements indicated ion conductance similar to the wild-type protein. These data raise the larger question of the phenotypic variability of class II mutants, including p.F508del. Since multiple potential partners could modify the processing of the CFTR protein during its course to the cell surface, environmental and other genetic factors might contribute to this variability.

DOS SANTOS A., BENSALÉM N., SAR S., BROUILLARD F., BRALET M.P., DEMAUGRE F., EDELMAN A., THIERS V., BRECHOT C.

Contribution Of Laser Capture Microdissection To Proteomic Analysis Of Hepatitis C Virus

(HCV)-Associated Hepatocellular Carcinoma (HCC).
J. Hepatol., 42 (Suppl.2), 93, 2005
(Services cités : U370, U467)

EDELMAN A.

Pathophysiology in the era of contemporary proteomics.
M S-Méd. Sci., 21 (8-9), 675-678, 2005
(Services cités : U467)

FLIS K., HINZPETER A., EDELMAN A., KURLANDZKA A.

The functioning of mammalian CIC-2 chloride channel in *Saccharomyces cerevisiae* cells requires an increased level of Kha1p.
Biochem. J., 390 (Pt 3), 655-664, 2005
(Services cités : U467)

The mammalian chloride channel CIC-2 is a member of the CLC voltage-gated chloride channels family. This broadly expressed protein shows diverse cellular locations and despite numerous studies, its precise function is poorly understood. Disruption of CIC-2-encoding gene in mouse leads to retinal and testicular degeneration and mutations in CLC2 (gene encoding the CIC-2 channel) are associated with idiopathic generalized epilepsies. CIC-2 may also be responsible for Cl⁻ transport in mouse salivary glands. The only CLC homologue of the yeast *Saccharomyces cerevisiae*, Gef1p, exhibits CLC activity. We expressed the mammalian CIC-2 protein in *S. cerevisiae* devoid of Gef1p in an attempt to identify yeast proteins influencing the functioning of CIC-2. The presence of such proteins in yeast could indicate the existence of their homologues in mammalian cells and would greatly aid their identification. Expression of CIC-2 in yeast required optimization of the sequence context of the AUG translation initiation codon. After obtaining an efficient translation, we found that rat CIC-2 cannot directly substitute for yeast Gef1p. Functional substitution for Gef1p was, however, achieved in the presence of an increased level of intact or C-terminally truncated yeast Kha1 protein. Based on the deduced amino acid sequence, the Kha1 protein can be classified as a Na⁺/H⁺ transporter since it has a large N-terminal domain similar to the family of NHEs (Na⁺/H⁺ exchangers). This suggests that the Kha1p may take part in the regulation of intracellular cation homeostasis and pH control. We have established that Kha1p is localized in the same cellular compartment as Gef1p and yeast-expressed CIC-2: the Golgi apparatus. We propose that Kha1p may aid CIC-2-dependent suppression of the Deltagef1-associated growth defects by keeping the Golgi apparatus pH in a range suitable for CIC-2 activity. The approach employed in the present study may be of general applicability to the characterization of poorly understood proteins by their functional expression in yeast.

PLAETZER K., THOMAS S.R., FALKNER R., FALKNER G.

The microbial experience of environmental phosphate fluctuations. An essay on the possibility of putting intentions into cell biochemistry.
J. Theor. Biol., 235 (4), 540-544, 2005
(Services cités : U467)

We present a model of microbial information processing that contains characteristic features of the phenomenon of physiological adaptation. The backbone of the model is the "adaptive event" in which energy-converting subsystems of the cell interact with the changing environment. In this process, the subsystems pass, via an adaptive operation mode, from one adapted state to the next. An adaptive operation mode takes place when an adapted state is disturbed by an environmental

alteration. These two manifestations of an adaptive event were differently treated in the simulation, based on an application of linear irreversible thermodynamics to the energy transduction of adaptive subsystems. In adapted states, the conductivity coefficients of the flow-force relationships employed remained constant, whereas during an adaptive operation mode, these coefficients were altered in a directional manner during the simulation. An example dealing with the complex relationship between phosphate uptake and cyanobacterial growth is given. In this example, the simulation of adapted states of two subsystems of the incorporating machinery, namely the phosphate carrier in the cell membrane and the F-ATPase in the thylakoid membrane, was in accordance with the measured uptake kinetics, and when fixed, predetermined conductivity coefficients were used. In the adaptive operation mode, however, the simulated behavior was in agreement with experimental observations when the program was able to "interpret" its own performance in the light of environmental phosphate fluctuations, experienced by the cell in the past, and to reconstruct the two subsystems according to this interpretation. Via transitions between adapted states and adaptive modes, information is transferred from one adaptive event to the next: the latter "inherits" the results of former interpretations. By appropriating them selectively, it is entering into a future in which its own interpretation is passed on to the following adaptive event. The model is discussed with respect to the concept of autopoiesis.

PRULIERE-ESCABASSE V., FANEN P., DAZY A.C., LECHAPT-ZALCMAN E., RIDEAU D., EDELMAN A., ESCUDIER E., COSTE A.

TGF-beta1 downregulates CFTR expression and function in nasal polyps of non-CF patients.

Amer. J. Physiol. - Lung Cell. Mol. Physiol., 288 (1), L77-L83, 2005

(Services cités : U467)

Nasal polyposis is a chronic inflammatory disease of the upper airways. It has been suggested that ion transports and CFTR expression could be modified in epithelial cells from nasal polyps of non-cystic fibrosis patients. We compared human nasal epithelial cells from nasal polyps (NP) with control nasal mucosa (CM). The level of CFTR mRNA was studied by Northern blot analysis and protein expression was studied by immunoprecipitation both ex vivo and in vitro in primary cultures of human nasal epithelial cells at the air-liquid interface. Ion transports were evaluated by short-circuit measurements in vitro. CFTR gene and protein expressions were significantly decreased in NP native tissues and in culture on day 4, when a global defect of ion transports was observed in NP cultures, but not in CM. We evaluated the effect of transforming growth factor (TGF)-beta1 on CFTR expression and function in NP cultures on day 14 and showed, for the first time, that TGF-beta1 was able to significantly downregulate the level of CFTR mRNA and cAMP-dependent current in NP cultures. Finally, we showed that the effects of TGF-beta1 on ion transports could be reversed after 48-h removal of TGF-beta1 in NP cultures. In conclusion, our data strongly suggest that chronic inflammation in nasal polyposis downregulates CFTR gene and protein expression.

SERMET-GAUDELUS I., DECHAUX M., VALLEE B., FAJAC A., GIRODON E., NGUYEN-KHOA T., MARIANOVSKI R., HURBAIN I., BRESSON J.L., LENOIR G., EDELMAN A.

Chloride transport in nasal ciliated cells of cystic fibrosis heterozygotes.

Amer. J. Respir. Crit. Care Med., 171 (9), 1026-1031, 2005

(Services cités : CIC 9303, Département de Pédiatrie, U467)

Studying subjects heterozygous for mutations of the cystic fibrosis (CF) gene may help clarify

the impact on disease onset of CF transmembrane conductance regulator protein (CFTR)-dependent chloride secretion. CFTR-mediated chloride transport was evaluated in 52 heterozygous subjects, 32 healthy control subjects, and 77 patients with CF with class I or II mutations. We measured the change in nasal potential difference in response to chloride-free isoproterenol solution for each subject and used a video-imaging fluorescent dye assay to assess the percentage of nasal ciliated cells with cAMP-dependent anion conductance. Our findings did not confirm the standard assumption that heterozygosity implies 50% of normal CFTR function. Half the heterozygous subjects had CFTR-mediated chloride transport levels below 50% of the normal range, and one-third had levels similar to those of the patients with CF. This reduced CFTR function was not associated with an elevated prevalence of CF-like symptoms in heterozygous subjects but was highly related to respiratory status in the patients with CF. These data suggest that CFTR-dependent chloride conductance does not directly modulate disease severity but may be part of a more global defect in patients with CF involving other CFTR functions or currently unknown modulatory factors.

2004

BAKOUH N., BENJELLOUN F., HULIN P., BROUILLARD F., EDELMAN A., CHERIF-ZAHAR B., PLANELLES G.

NH₃ Is Involved in the NH₄ Transport Induced by the Functional Expression of the Human Rh C Glycoprotein.

J. Biol. Chem., 279 (16), 15975-15983, 2004

(Services cités : U467)

Renal ammonium (NH₃ + NH₄⁽⁺⁾) transport is a key process for body acid-base balance. It is well known that several ionic transport systems allow NH₄⁽⁺⁾ transmembrane translocation without high specificity NH₄⁽⁺⁾, but it is still debated whether NH₃, and more generally, gas, may be transported by transmembrane proteins. The human Rh glycoproteins have been proposed to mediate ammonium transport. Transport of NH₄⁽⁺⁾ and/or NH₃ by the epithelial Rh C glycoprotein (RhCG) may be of physiological importance in renal ammonium excretion because RhCG is mainly expressed in the distal nephron. However, RhCG function is not yet established. In the present study, we search for ammonium transport by RhCG. RhCG function was investigated by electrophysiological approaches in RhCG-expressing *Xenopus laevis* oocytes. In the submillimolar concentration range, NH₄Cl exposure induced inward currents (I_(AM)) in voltage-clamped RhCG-expressing cells, but not in control cells. At physiological extracellular pH (pH_(o)) = 7.5, the amplitude of I_(AM) increased with NH₄Cl concentration and membrane hyperpolarization. The amplitude of I_(AM) was independent of external Na⁽⁺⁾ or K⁽⁺⁾ concentrations but was enhanced by alkaline pH_(o) and decreased by acid pH_(o). The apparent affinity of RhCG for NH₄⁽⁺⁾ was affected by NH₃ concentration and by changing pH_(o), whereas the apparent affinity for NH₃ was unchanged by pH_(o), consistent with direct NH₃ involvement in RhCG function. The enhancement of methylammonium-induced current by NH₃ further supported this conclusion. Exposure to 500 microm NH₄Cl induced a biphasic intracellular pH change in RhCG-expressing oocytes, consistent with both NH₃ and NH₄⁽⁺⁾ enhanced influx. Our results support the hypothesis of a specific role for RhCG in NH₃ and NH₄⁽⁺⁾ transport.

DAVEZAC N., TONDELIER D., LIPECKA J., FANEN P., DEMAUGRE F., DEBSKI J., DADLEZ M., SCHRATTENHOLZ A., CAHILL M.A., EDELMAN A.

Global proteomic approach unmasks involvement of keratins 8 and 18 in the delivery of cystic

fibrosis transmembrane conductance regulator (CFTR)/DeltaF508-CFTR to the plasma membrane.

Proteomics, 4 (12), 3833-3844, 2004

(Services cités : U467, IRNEM, U370)

Cystic fibrosis (CF) is a genetic disease caused by mutations in the CF gene (*cftr*).

Physiologically, CF is characterized by an abnormal chloride secretion in epithelia due to a dysfunction of a mutated cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is a cAMP-dependent chloride channel whose most frequent mutation, DeltaF508, leads to an aberrantly folded protein which causes a dysfunction of the channel. However, a growing number of reports suggest that modifier genes and environmental factors are involved in the physiology of CF. To identify proteins whose expression depends on wild-type WT-CFTR or DeltaF508-CFTR, we chose a global proteomic approach based on the use of two-dimensional gel electrophoresis (2-DE) and mass spectrometry. The experiments were carried out with HeLa cells stably transfected with WT-CFTR (pTCFWT) or DeltaF508-CFTR (pTCFDeltaF508). These experiments unmasked keratin 8 (K8) and 18 (K18) which were differentially expressed in pTCFWT vs. pTCFDeltaF508. An immunoblot of K18 confirmed the 2-DE results. Intracellular localization experiments of WT-CFTR, DeltaF508-CFTR, K8, and K18 suggest that the expression of these proteins are linked, and that the concentrations of K8 and K18 and/or their distribution may be involved in the traffic of WT-CFTR/DeltaF508-CFTR. A functional assay for CFTR revealed that specifically lowering K18 expression or changing its distribution leads to the delivery of functional DeltaF508-CFTR to the plasma membrane. This work suggests a novel function of K18 in CF.

DZODIC V., HERVY S., FRITSCH D., KHALFALLAH H., THEREAU M., THOMAS S.R.

Web-based tools for quantitative renal physiology.

Cell. Mol. Biol., 50 (7), 795-800, 2004

(Services cités : U467)

We present the development strategy and present state of progress on an interactive website project for quantitative renal physiology: a) a quantitative kidney database (QKDB), and b) an interactive website presenting mathematical models covering the major aspects of renal physiology. QKDB will house data for quantitative evaluation of hypotheses of renal function, from the cellular, through the epithelial and tubular, to whole organ levels. It will thus facilitate comparisons among different species and under various experimental conditions. It will include especially: transport parameters, tubular concentrations and flow rates along the various nephron segments, and anatomical details, in human kidneys, in experimentally studied species, and in model epithelia, such as cultured cells and amphibian skin and urinary bladder. The modeling resource will provide an interactive user interface to a collection of published models at all levels of renal physiology, enabling non-modelers to exploit the models, altering key parameters according to hypotheses of their own and visualizing the simulation results, thus permitting quantitative exploration of new hypotheses. Implementation will be facilitated by translation of the models into a common markup language such as CellML (cell markup language) and SBML (systems biology markup language). There will thus be a modular separation of model descriptions from their numerical solution methods.

EDELMAN A., AMARAL M.D.

General introduction to section C: Biochemistry and biophysics of CFTR.

J. Cyst. Fibrosis, 3 (sup.2), 69-72, 2004
(Services cités : U467)

EDELMAN A., CHANSON M., HUG M.J.

Microelectrodes and their use to assess ion channel function.

J. Cyst. Fibrosis, 3 (sup.2), 113-117, 2004
(Services cités : U467)

MENDES F., DOUCET L., HINZPETER A., FEREC C., LIPECKA J., FRITSCH J., EDELMAN A., JORNA H., WILLEMSSEN R., BOT A.G.M., de JONGE H.R., HINNRSKY J., CASTILLON N., TAOUIL K., PUCHELLE E., PENQUE E.D., AMARAL M.D.

Immunohistochemistry of CFTR in native tissues and primary epithelial cell cultures.

J. Cyst. Fibrosis, 3 (sup.2), 37-41, 2004
(Services cités : IRNEM, U467)

MENDES F., FARINHA C.M., ROXO-ROSA M., FANEN P., EDELMAN A., DORMER R., MCPHERSON M., DAVIDSON H., PUCHELLE E., de JONGE H., HEDA G.D., GENTZSCH M., LUKACS G., PENQUE D., AMARAL M.D.

Antibodies for CFTR studies.

J. Cyst. Fibrosis, 3 (sup.2), 69-72, 2004
(Services cités : U467)

MUNKONGE F., ALTON E., ANDERSSON C., DAVIDSON H., DRAGOMIR A., EDELMAN A., FARLEY R., HJELTE L., MCLAGHLAN G., STERNA M., ROOMANS G.M.

Measurement of halide efflux from cultured and primary airway epithelial cells using fluorescence indicators.

J. Cyst. Fibrosis, 3 (sup.2), 171-176, 2004
(Services cités : U467)

NESSLER S., FRIEDRICH O., BAKOUH N., FINK R.H., SANCHEZ C.P., PLANELLES G., LANZER M.

Evidence for activation of endogenous transporters in *Xenopus laevis* oocytes expressing the *Plasmodium falciparum* chloroquine resistance transporter, PfCRT.

J. Biol. Chem., 279 (38), 39438-39446, 2004
(Services cités : U467)

A large body of genetic, reverse genetic, and epidemiological data has linked chloroquine-resistant malaria to polymorphisms within a gene termed *pfcr*t in the human malarial parasite *Plasmodium falciparum*. To investigate the biological function of the chloroquine resistance transporter, PfCRT, as well as its role in chloroquine resistance, we functionally expressed this protein in *Xenopus laevis* oocytes. Our data show that PfCRT-expressing oocytes exhibit a depolarized resting membrane potential and a higher intracellular pH compared with control oocytes. Pharmacological and electrophysiological studies link the higher intracellular pH to an enhanced amiloride-sensitive H(+) extrusion and the low membrane potential to an activated nonselective cation conductance. The finding that both properties are independent of each other, together with the fact that they are endogenously present in *X. laevis* oocytes, supports a model in

which PfCRT activates transport systems. Our data suggest that PfCRT plays a role as a direct or indirect activator or modulator of other transporters.

OLLERO M.

Methods for the study of lipid metabolites in cystic fibrosis.

J. Cyst. Fibrosis, 3 (sup.2), 97-98, 2004

(Services cités : U467)

PLANELLES G.

Chloride transport in the renal proximal tubule.

Pflugers Arch. - Eur. J. Physiol., 448 561-570, 2004

(Services cités : U467)

The renal proximal tubule is responsible for most of the renal sodium, chloride, and bicarbonate reabsorption. Micropuncture studies and electrophysiological techniques have furnished the bulk of our knowledge about the physiology of this tubular segment. As a consequence of the leakiness of this epithelium, paracellular ionic transport-in particular that of Cl(-)-is of considerable importance in this first part of the nephron. It was long accepted that proximal Cl(-) reabsorption proceeds solely paracellularly, but it is now known that transcellular Cl(-) transport also exists. Cl(-) channels and Cl(-)-coupled transporters are involved in transcellular Cl(-) transport. In the apical membrane, Cl(-)/anion (formate, oxalate and bicarbonate) exchangers represent the first step in transcellular Cl(-) reabsorption. A basolateral Cl(-)/HCO(3)(-) exchanger, involved in HCO(3)(-) reclamation, participates in the rise of intracellular Cl(-) activity above its equilibrium value, and thus also contributes to the creation of an outwardly directed electrochemical Cl(-) gradient across the cell membranes. This driving force favours Cl(-) diffusion from the cell to the lumen and to the interstitium. In the basolateral membrane, the main mechanism for transcellular Cl(-) reabsorption is a Cl(-) conductance, but a Na(+)-driven Cl(-)/HCO(3)(-) exchanger may also participate in Cl(-) reabsorption.

ROXO-ROSA M., DAVEZAC N., BENSALÉM N., MAJUMDER M., HEDA G.D., SIMAS A., PENQUE D., AMARAL M.D., LUKAS G.L., EDELMAN A.

Proteomics techniques for cystic fibrosis research.

J. Cyst. Fibrosis, 3 (sup.2), 85-89, 2004

(Services cités : U467)

SCHULER D., SERMET-GAUDELUS I., WILSCHANSKI M., BALLMANN M., DECHAUX M., EDELMAN A., HUG M., LEAL T., LEBACQ J., LEBECQUE P., LENOIR G., STANKE F., WALLEMACQ P., TUMMLER B., KNOWLES M.R.

Basic protocol for transepithelial nasal potential difference measurements.

J. Cyst. Fibrosis, 3 (sup.2), 151-155, 2004

(Services cités : Département de Pédiatrie, U467)

2003

BAUDOIN-LEGROS M., BROUILLARD F., TONDELIER D., HINZPETER A., EDELMAN A.

Effect of ouabain on CFTR gene expression in human Calu-3 cells.

Amer. J. Physiol. - Cell Physiol., 284 (3), C620-C626, 2003

(Services cités : U467)

We have previously shown that ouabain, which changes the electrochemical properties of cell membranes by inhibiting Na(+),K(+)-ATPase, induces the expression of multidrug resistance (MDR-1) gene in several human cell lines. Because the expressions of the MDR-1 and CFTR (which encodes the cAMP-activated Cl(-) channel associated with cystic fibrosis) genes are physiologically regulated in opposing directions, we wanted to determine whether ouabain also decreases CFTR transcripts and subsequently to analyze its mechanism of action. We found that the submicromolar concentrations of ouabain that increase MDR-1 mRNAs decrease the CFTR transcripts with analogous time-dependency in human pulmonary Calu-3 cells. By altering or reproducing the ouabain-induced changes in intracellular ionic activities (decreasing in external Na(+) or K(+) or using Na(+) ionophore), we show that the ouabain-induced regulations of both CFTR and MDR-1 transcripts depend on the Na(+)/K(+) pump inhibition but that the decrease in CFTR mRNAs also proceeds from cytoplasm reactions simultaneously activated by ouabain. These data, which emphasize the complex mechanism of action of ouabain, suggest that changes in intracellular ionic activities modulate CFTR/MDR-1 gene expressions.

HERVY S., THOMAS S.R.

Inner medullary lactate production and urine-concentrating mechanism: a flat medullary model. *Amer. J. Physiol. Renal Physiol.*, 284 (1), F65-F81, 2003
(Services cités : U467)

We used a mathematical model to explore the possibility that metabolic production of net osmoles in the renal inner medulla (IM) may participate in the urine-concentrating mechanism. Anaerobic glycolysis (AG) is an important source of energy for cells of the IM, because this region of the kidney is hypoxic. AG is also a source of net osmoles, because it splits each glucose into two lactate molecules, which are not metabolized within the IM. Furthermore, these sugars exert their full osmotic effect across the epithelia of the thin descending limb of Henle's loop and the collecting duct, so they are apt to fulfill the external osmole role previously attributed to interstitial urea (whose role is compromised by the high urea permeability of long descending limbs). The present simulations show that physiological levels of IM glycolytic lactate production could suffice to significantly amplify the IM accumulation of NaCl. The model predicts that for this to be effective, IM lactate recycling must be efficient, which requires high lactate permeability of descending vasa recta and reduced IM blood flow during antidiuresis, two conditions that are probably fulfilled under normal circumstances. The simulations also suggest that the resulting IM osmotic gradient is virtually insensitive to the urea permeability of long descending limbs, thus lifting a longstanding paradox, and that this high urea permeability may serve for independent regulation of urea balance.

HURBAIN I., SERMET-GAUDELUS I., VALLEE B., FEUILLET M.N., LENOIR G., BERNAUDIN J.F., EDELMAN A., FAJAC A.

Evaluation of MRP1-5 gene expression in cystic fibrosis patients homozygous for the delta F508 mutation.

Pediat. Res., 54 (5), 627-634, 2003

(Services cités : U467, Département de Pédiatrie, Biochimie Générale)

Cystic fibrosis (CF), due to mutations of the cystic fibrosis transmembrane conductance regulator (CFTR), exhibits a wide range of disease severity, even among deltaF508 homozygous patients, and the mechanisms of this variability have yet to be elucidated. In view of the close structural homology and possible functional overlap between CFTR and Multidrug Resistance-associated Proteins (MRPs), MRPs were investigated as potentially relevant factors in CF pathophysiology.

MRP1-5 gene expression was analyzed in nasal respiratory epithelial cells from deltaF508 homozygous patients (n = 19) and control subjects (n = 20) using semiquantitative RT-PCR. Significantly lower MRP1 and MRP5 transcript levels were found in CF patients than in control subjects. MRP1 and MRP5 transcript levels were strongly correlated (r = 0.71). In CF patients, low MRP1 transcript levels were associated with more severe disease as assessed by the Shwachman score. A relation was also observed between MRP1 levels and presence of a cAMP-independent chloride conductive pathway, as determined by a halide-sensitive fluorescent assay. These results suggest that MRPs, especially MRP1, might play a role in CF phenotype and might therefore constitute a target for a novel pharmacotherapy of CF.

2002

COUGNON M., BENAMMOU S., BROUILLARD F., HULIN P., PLANELLES G.

Effect of reactive oxygen species on NH permeation in *Xenopus laevis* oocytes.

Amer. J. Physiol. Cell Physiol., 282 (6), C1445-C1453, 2002

(Services cités : U467)

To investigate the effects of reactive oxygen species (ROS) on NH permeation in *Xenopus laevis* oocytes, we used intracellular double-barreled microelectrodes to monitor the changes in membrane potential (V(m)) and intracellular pH (pH(i)) induced by a 20 mM NH₄Cl-containing solution. Under control conditions, NH₄Cl exposure induced a large membrane depolarization (to V(m) = 4.0 +/- 1.5 mV; n = 21) and intracellular acidification [reaching a change in pH(i) (DeltapH(i)) of 0.59 +/- 0.06 pH units in 12 min]; the initial rate of cell acidification (dpH(i)/dt) was 0.06 +/- 0.01 pH units/min. Incubation of the oocytes in the presence of H₂O₂ or beta-amyloid protein had no marked effect on the NH₄Cl-induced DeltapH(i). By contrast, in the presence of photoactivated rose bengal (RB), tert-butylhydroxyperoxide (t-BHP), or xanthine/xanthine oxidase (X/XO), the same experimental maneuver induced significantly greater DeltapH(i) and dpH(i)/dt. These increases in DeltapH(i) and dpH(i)/dt were prevented by the ROS scavengers histidine and desferrioxamine, suggesting involvement of the reactive species (1)DeltagO₂ and .OH. Using the voltage-clamp technique to identify the mechanism underlying the ROS-measured effects, we found that RB induced a large increase in the oocyte membrane conductance (G(m)). This RB-induced G(m) increase was prevented by 1 mM diphenylamine-2-carboxylate (DPC) and by a low Na⁺ concentration in the bath. We conclude that RB, t-BHP, and X/XO enhance NH influx into the oocyte via activation of a DPC-sensitive nonselective cation conductance pathway.

DELLIS O., GANGLOFF S.C., PAULAIS M., TONDELIER D., RONA J.P., BROUILLARD F., BOUTEAU F., GUENOUNOU M., TEULON J.

Inhibition of the Calcium Release-activated Calcium (CRAC) Current in Jurkat T Cells by the HIV-1 Envelope Protein gp160.

J. Biol. Chem., 277 (8), 6044-6050, 2002

(Services cités : U467)

The HIV-1 envelope glycoprotein gp120/160 has pleiotropic effects on T cell function. We investigated whether Ca²⁺ signaling, a crucial step for T cell activation, was altered by prolonged exposure of Jurkat T cells to gp160. Microfluorometric measurements showed that Jurkat cells incubated with gp160 had smaller ([approximate]40%) increases in [Ca²⁺]_i in response to phytohemagglutinin and had a reduced Ca²⁺ influx ([approximate]25%). gp160 had similar effects on Jurkat cells challenged with thapsigargin. We used the patch clamp technique to record the Ca²⁺ current, which is responsible for Ca²⁺ influx and has properties of the

calcium release-activated Ca(2+) current (I(CRAC)). gp160 reduced I(CRAC) by [approximate]40%. The inhibitory effects of gp160 were antagonized by staurosporine (0.1 [mu]m), an inhibitor of protein-tyrosine kinases and protein kinase Cs (PKCs), and by Go 6976 (5 [mu]m), an inhibitor acting especially on PKC[alpha] and PKC[beta]I. 12-O-Tetradecanoyl phorbol 13-acetate (16 nm), a PKC activator, reproduced the effects of gp160 in untreated cells. A Western blotting analysis of PKC isoforms [alpha], [beta]I, [delta], and [zeta] showed that only the cellular distribution of PKC[alpha] and -[beta]I were significantly modified by gp160. In addition, gp160 was able to modify the subcellular distribution of PKC[alpha] and PKC[beta]I caused by phytohemagglutinin. Therefore the reduction in I(CRAC) caused by prolonged incubation with gp160 is probably mediated by PKC[alpha] or -[beta]I.

ELADARI D., CHEVAL L., QUENTIN F., BERTRAND O., MOURO I., CHERIF-ZAHAR B., CARTRON J.P., PAILLARD M., DOUCET A., CHAMBREY R.

Expression of RhCG, a New Putative NH(3)/NH(4)(+) Transporter, along the Rat Nephron.

J. Amer. Soc. Nephrol., 13 (8), 1999-2008, 2002

(Services cités : U467)

ABSTRACT. Two non-erythroid members of the erythrocyte Rhesus (Rh) protein family, RhBG and RhCG, have been recently cloned in the kidney. These proteins share homologies with specific NH(3)/NH(4)(+) transporters (Mep/Amt) in primitive organisms and plants. When expressed in a Mep-deficient yeast, RhCG can function as a bidirectional NH(3)/NH(4)(+) transporter. The aim of this study was to determine the intrarenal and intracellular location of RhCG in rat kidney. RT-PCR on microdissected rat nephron segments demonstrated expression of mRNAs encoding RhCG in distal convoluted tubules, connecting ducts, and cortical and outer medullary collecting ducts but not in proximal tubules and thick ascending limbs of Henle's loop. Immunolocalization studies performed on rat kidney sections with rabbit anti-human RhCG 31 to 48 antibody showed labeling of the apical pole of tubular cells within the cortex, the outer medulla, and the upper portion of the inner medulla. All cells within connecting tubules had identical apical staining. In cortical collecting ducts, a subpopulation of cells that has either apical staining (alpha-intercalated cells) or diffuse staining (beta-intercalated cells) for the beta1 subunit of the H(+)-ATPase, was heavily stained at their apical pole with the RhCG antibody while principal cells identified as H(+)-ATPase negative cells showed a faint apical staining for RhCG that was much less intense than in adjacent intercalated cells. In the outer medulla and the upper portion of the inner medulla, RhCG labeling was restricted to a subpopulation of cells within the collecting duct that apically express the beta1 subunit of the H(+)-ATPase, indicating that RhCG expression in medullary collecting ducts is restricted to intercalated cells. No labeling was seen in glomeruli, proximal tubules, and limbs of Henle's loop. Immunoblotting of apical membrane fractions from rat kidney cortex with the rabbit anti-human RhCG 31 to 48 antibody revealed a doublet band at approximately 65 kD.

FERRONI A., SERMET-GAUDELUS I., ABACHIN E., QUESNE G., LENOIR G., BERCHE P., GAILLARD J.L.

Use of 16S rRNA Gene Sequencing for Identification of Nonfermenting Gram-Negative Bacilli Recovered from Patients Attending a Single Cystic Fibrosis Center.

J. Clin. Microbiol., 40 (10), 3793-377., 2002

(Services cités : Département de Pédiatrie, Laboratoire de Microbiologie, U467)

During 1999, we used partial 16S rRNA gene sequencing for the prospective identification of atypical nonfermenting gram-negative bacilli isolated from patients attending our cystic fibrosis

center. Of 1,093 isolates of nonfermenting gram-negative bacilli recovered from 148 patients, 46 (4.2%) gave problematic results with conventional phenotypic tests. These 46 isolates were genotypically identified as *Pseudomonas aeruginosa* (19 isolates, 12 patients), *Achromobacter xylosoxidans* (10 isolates, 8 patients), *Stenotrophomonas maltophilia* (9 isolates, 9 patients), *Burkholderia cepacia* genomovar I/III (3 isolates, 3 patients), *Burkholderia vietnamiensis* (1 isolate), *Burkholderia gladioli* (1 isolate), and *Ralstonia mannitolilytica* (3 isolates, 2 patients), a recently recognized species.

FLIS K., BEDNARCZYK P., HORDEJUK R., SZEWCZYK A., BEREST V., DOLOWY K., EDELMAN A., KURLANDZKA A.

The Gef1 protein of *Saccharomyces cerevisiae* is associated with chloride channel activity.

Biochem. Biophys. Res. Commun., 294 (5), 1144-1150, 2002

(Services cités : U467)

The Gef1 protein of the yeast *Saccharomyces cerevisiae* (Gef1p) has amino acid homology to the voltage-gated CLC chloride channel family. It has been postulated that it provides the compensatory transport of Cl⁻ anions to the lumen of the Golgi thereby regulating the pH of this compartment. Using GEF1 fusion with heterologous promoter we obtained a yeast strain highly overproducing Gef1p. The electrophysiological properties of the microsomal fraction obtained from this strain were measured using lipid bilayer system. Our data indicate that Gef1p is associated with the chloride channel activity. This anion-selective channel has a unitary conductance of 42 pS when measured in symmetrical 600/600 mM TEA-Cl solutions, is voltage-dependent, and closes at high negative voltages. (C) 2002 Elsevier Science (USA). All rights reserved.

LIPECKA J., BALI M., THOMAS A., FANEN P., EDELMAN A., FRITSCH J.

Distribution of ClC-2 chloride channel in rat and human epithelial tissues.

Amer. J. Physiol. Cell Physiol., 282 (4), C805-C816, 2002

(Services cités : U467)

The ubiquitous ClC-2 Cl⁻(minus sign) channel is thought to contribute to epithelial Cl⁻(minus sign) secretion, but the distribution of the ClC-2 protein in human epithelia has not been investigated. We have studied the distribution of ClC-2 in adult human and rat intestine and airways by immunoblotting and confocal microscopy. In the rat, ClC-2 was present in the lateral membranes of villus enterocytes and was predominant at the basolateral membranes of luminal colon enterocytes. The expression pattern of ClC-2 in the human intestine differed significantly, because ClC-2 was mainly detected in a supranuclear compartment of colon cells. We found significant expression of ClC-2 at the apex of ciliated cells in both rat and human airways. These results show that the distribution of ClC-2 in airways is consistent with participation of ClC-2 channels in Cl⁻(minus sign) secretion and indicate that extrapolation of results from studies of ClC-2 function in rat intestine to human intestine is not straightforward.

NGUYEN T.M., ADICEAM P., KOTTLER M.L., GUILLOZO H., RIZK-RABIN M., BROUILLARD F., LAGIER P., PALIX C., GARNIER J.M., GARABEDIAN M.

Tryptophan missense mutation in the ligand-binding domain of the vitamin D receptor causes severe resistance to 1,25-dihydroxyvitamin D.

J. Bone Miner. Res., 17 (9), 1728-1737, 2002

(Services cités : U467)

In this study, two related young children, brother and sister, exhibited severe vitamin D-resistant

rickets without alopecia. Sequence analysis of the total vitamin D receptor (VDR) cDNA from skin fibroblasts revealed a substitution of the unique tryptophan of the VDR by arginine at amino acid 286 (W286R). Cultured skin fibroblasts of the two patients expressed normal-size VDR protein (immunocytochemistry and Western blotting) and normal length VDR mRNA (Northern blotting). But, these fibroblasts, as well as COS-7 cells transfected with the W286R mutant, failed to bind 3H 1,25-dihydroxyvitamin D3 [1,25(OH)2D3]. The tryptophan substitution did not affect VDR trafficking toward the nucleus but abolished the 24-hydroxylase gene response to 1,25(OH)2D3, even at 10(-6) M concentrations. In conclusion, this case report of a new family with hereditary vitamin D-resistant rickets (HVDRR) emphasizes the crucial role of the VDR tryptophan for ligand binding and for transactivation of 1,25(OH)2D3 target genes. It clearly shows the clinical significance of this VDR amino acid for calcium homeostasis and bone mineralization. This observation suggests further that the presence of a stable VDR-bound ligand may not be obligatory for normal hair follicle development.

PRIE D., HUART V., BAKOUH N., PLANELLES G., DELLIS O., GERARD B., HULIN P., BENQUE-BLANCHET F., SILVE C., GRANDCHAMP B., FRIEDLANDER G.

Nephrolithiasis and osteoporosis associated with hypophosphatemia caused by mutations in the type 2a sodium-phosphate cotransporter.

N. Engl. J. Med., 347 (13), 983-991, 2002

(Services cités : U467)

BACKGROUND: Epidemiologic studies suggest that genetic factors confer a predisposition to the formation of renal calcium stones or bone demineralization. Low serum phosphate concentrations due to a decrease in renal phosphate reabsorption have been reported in some patients with these conditions, suggesting that genetic factors leading to a decrease in renal phosphate reabsorption may contribute to them. We hypothesized that mutations in the gene coding for the main renal sodium-phosphate cotransporter (NPT2a) may be present in patients with these disorders. **METHODS:** We studied 20 patients with urolithiasis or bone demineralization and persistent idiopathic hypophosphatemia associated with a decrease in maximal renal phosphate reabsorption. The coding region of the gene for NPT2a was sequenced in all patients. The functional consequences of the mutations identified were analyzed by expressing the mutated RNA in *Xenopus laevis* oocytes. **RESULTS:** Two patients, one with recurrent urolithiasis and one with bone demineralization, were heterozygous for two distinct mutations. One mutation resulted in the substitution of phenylalanine for alanine at position 48, and the other in a substitution of methionine for valine at position 147. Phosphate-induced current and sodium-dependent phosphate uptake were impaired in oocytes expressing the mutant NPT2a. Coinjection of oocytes with wild-type and mutant RNA indicated that the mutant protein had altered function. **CONCLUSIONS:** Heterozygous mutations in the NPT2a gene may be responsible for hypophosphatemia and urinary phosphate loss in persons with urolithiasis or bone demineralization.

SERMET-GAUDELUS I., VALLEE B., URBIN I., TOROSSO T., MARIANOVSKI R., FAJAC A., FEUILLET M.N., BRESSON J.L., LENOIR G., BERNAUDIN J.F., EDELMAN A.

Normal Function of the Cystic Fibrosis Conductance Regulator Protein Can Be Associated with Homozygous DeltaF508 Mutation.

Pediat. Res., 52 (5), 628-635, 2002

(Services cités : CIC 9303, Département de Pédiatrie, U467)

Cystic fibrosis (CF) is caused by mutations of the gene encoding for the CFTR (CF transmembrane conductance regulator) protein. The most frequent mutation, the DeltaF508 mutation, results in a defective cAMP-regulated chloride transport in the epithelial cells. The spectrum of clinical manifestations in patients bearing homozygous DeltaF508 mutations can vary considerably, suggesting that, in the patients with a mild disease, CFTR could be partly functional. To test this hypothesis, we explored in nasal ciliated epithelial cells (NCC) of 9 control subjects and 23 DeltaF508 homozygous patients the anion conductive pathway by a halide sensitive fluorescent dye assay SPQ (6-methoxy-N-3'-sulfopropylquinolinium) and the CFTR transcript levels by RT-PCR. As 50% represented the lowest fraction of the control subjects NCC demonstrating a cAMP-dependent conductance, a CF patient was considered as "cAMP responder" if at least 50% of the NCC tested displayed a cAMP-dependent conductive pathway. According to these criteria, 8 of the 23 patients were considered as cAMP responders. They had a significantly less severe disease considering the respiratory function and infectious status. The amount of CFTR mRNA did not differ between the control subjects and the patients. No statistical correlation could be found between the transcript level and the expression of a cAMP conductive pathway. This cAMP-dependent Cl(-) conductance detected in homozygous NCC could be due to a residual CFTR activity and may explain the mild phenotypes observed in some DeltaF508 homozygous patients.

VARGAS-POUSSOU R., HUANG C., HULIN P., HOULLIER P., JEUNEMAITRE X., PAILLARD M., PLANELLES G., DECHAUX M., MILLER R.T., ANTIGNAC C.

Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a bartter-like syndrome.

J. Amer. Soc. Nephrol., 13 (9), 2259-2266, 2002

(Services cités : U423, U467)

ABSTRACT. The extracellular Ca(2+)-sensing receptor (CaSR) plays an essential role in extracellular Ca(2+) homeostasis by regulating the rate of parathyroid hormone (PTH) secretion and the rate of calcium reabsorption by the kidney. Activation of the renal CaSR is thought to inhibit paracellular divalent cation reabsorption in the cortical ascending limb (cTAL) both directly and indirectly via a decrease in NaCl transport. However, in patients with autosomal dominant hypocalcemia (ADH), caused by CaSR gain-of-function mutations, a defect in tubular NaCl reabsorption with renal loss of NaCl has not been described so far. This article describes a patient with ADH due to a gain-of-function mutation in the CaSR, L125P, associated with a Bartter-like syndrome that is characterized by a decrease in distal tubular fractional chloride reabsorption rate and negative NaCl balance with secondary hyperaldosteronism and hypokalemia. The kinetics of activation of the L125P mutant receptor expressed in HEK-293 cells, assessed by measuring CaSR-stimulated changes in intracellular Ca(2+) and ERK activity, showed a dramatic reduction in the EC(50) for extracellular Ca(2+) compared with the wild-type and a loss-of-function mutant CaSR (I40F). This study describes the first case of ADH associated with a Bartter-like syndrome. It is herein proposed that the L125P mutation of the CaSR, which represents the most potent gain-of-function mutation reported so far, may reduce NaCl reabsorption in the cTAL sufficiently to result in renal loss of NaCl with secondary hyperaldosteronism and hypokalemia.

2001

BALI M., LIPECKA J., EDELMAN A., FRITSCH J.

Regulation of clc-2 chloride channels in t84 cells by tgf-alpha.

Amer. J. Physiol. - Cell Physiol., 280 (6), C1588-C1598, 2001

(Services cités : U467)

The almost ubiquitously expressed CIC-2 chloride channel is activated by hyperpolarization and osmotic cell swelling. Osmotic swelling also activates a different class of outwardly rectifying chloride channels, and several reports point to a link between protein tyrosine phosphorylation and activation of these channels. This study examines the possibility that transforming growth factor-alpha (TGF-alpha) modulates CIC-2 activity in human colonic epithelial (T84) cells. TGF-alpha (0.17 nM) irreversibly inhibited CIC-2 current in nystatin-perforated whole cell patch-clamp experiments, whereas a superimposed reversible activation of the current was observed at 8.3 nM TGF-alpha. Both effects required activation of the intrinsic epidermal growth factor receptor (EGFR) tyrosine kinase activity, of phosphoinositide 3-kinase, and of protein kinase C. With microspectrofluorimetry of the pH-sensitive fluorescent dye 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein, TGF-alpha was shown to reversibly alkalinize T84 cells at 8.3 nM but not at 0.17 nM, suggesting that 8.3 nM TGF-alpha -induced alkalization activates CIC-2 current. This study indicates that CIC-2 channels are targets for EGFR signaling in epithelial cells.

[References: 64]

BALI M., THOMAS S.

A modelling study of feedforward activation in human erythrocyte glycolysis.

C. R. Acad. Sci. Sér.III Sci. Vie, 324 (3), 185-199, 2001

(Services cités : U467)

Though feedforward activation (FA) is a little known principle of control in metabolic networks, there is one well-known example; namely, the activation of pyruvate kinase (PK) by fructose-1,6-biphosphate (FBP) in glycolysis. The effects of this activation on the enzyme's kinetics are well characterised, but its possible role in glycolytic control has not been determined, and, experimentally, there is as yet no direct way of modifying the enzyme to remove just the FBP activation without affecting other aspects of the enzyme's kinetics. Given this limitation, we used a detailed numerical simulation of human erythrocyte glycolysis to simulate the effects of selective removal of the activation of PK by FBP on steady-state metabolite concentrations and on the dynamic response of glycolytic flux to a sudden increase of the cell's demand for ATP. Our modelling results predict that in the absence of FA steady-state levels of metabolites within the activation loop, i.e. from FBP to phosphoenol pyruvate, would be four- to thirteen-fold higher than normal, whereas levels of ATP and metabolites outside the loop, i.e. glucose-6-phosphate, fructose-6-phosphate and pyruvate, would be lower than normal. Existing clinical evidence in a patient with haemolytic anaemia, correlated with a lack of activation of PK by FBP (Paglia D.E., Valentine W.N., Holbrook C.T., Brockway R., *Blood* (1983) 62 972-979), is consistent with this prediction. In response to changing demand for ATP, the model predicts that the corresponding change of glycolytic flux would entail changes of metabolite concentrations in the absence of FA, but that in its presence the levels of metabolites within the activation loop remain essentially unperturbed. Thus, our results suggest that by stabilising metabolite pools in the face of variable glycolytic flux, FA may serve to avoid perturbations of the oxygen affinity of haemoglobin (sensitive to the levels of 2,3-phosphoglycerate) and of cell osmolality that would otherwise occur during variations in the cell's demand for ATP. In addition, by significantly raising the steady-state setpoint of intermediate metabolite pools, the productivity index (ratio of glycolytic flux to total metabolites in the pathway) of glycolysis would fail almost four-fold in the absence of forward activation. (C) 2001 Academie des sciences/Editions scientifiques et medicales Elsevier SAS. [References: 43]

BLANCHARD A., JEUNEMAITRE X., COUDOL P., DECHAUX M., FROISSART M., MAY A., DEMONTIS R., FOURNIER A., PAILLARD M., HOULLIER P.

Paracellin-1 is critical for magnesium and calcium reabsorption in the human thick ascending limb of henle.

Kidney Int., 59 (6), 2206-2215, 2001

(Services cités : U467)

Background. A new protein, named paracellin 1 (PCLN-1), expressed in human thick ascending limb (TAL) tight junctions, possibly plays a critical role in the control of magnesium and calcium reabsorption, since mutations of PCLN-1 are present in the hypomagnesemia hypercalciuria syndrome (HHS). However, no functional experiments have demonstrated that TAL magnesium and calcium reabsorption were actually impaired in patients with HHS. Methods. Genetic studies were performed in the kindred of two unrelated patients with HHS. Renal magnesium and calcium reabsorption in TAL were analyzed in one homozygous affected patient of each family, one patient with extrarenal hypomagnesemia (ERH), and two control subjects (CSs). Results. We found two yet undescribed mutations of PCLN-1 (Gly 162 Val, Ala 139 Val). In patients with HHS, renal magnesium and calcium reabsorptions were impaired as expected; NaCl renal conservation during NaCl deprivation and NaCl tubular reabsorption in diluting segment were intact. Furosemide infusion in CS markedly increased NaCl, Mg, and Ca urinary excretion rates. In HHS patients, furosemide similarly increased NaCl excretion, but failed to increase Mg and Ca excretion. Acute MgCl₂ infusion in CS and ERH patient provoked a dramatic increase in urinary calcium excretion without change in NaCl excretion. When combined with MgCl₂ infusion, furosemide infusion remained able to induce normal natriuretic response, but was unable to increase urinary magnesium and calcium excretion further. In HHS patients, calciuric response to MgCl₂ infusion was blunted. Conclusion. This study is the first to our knowledge to demonstrate that homozygous mutations of PCLN-1 result in a selective defect in paracellular Mg and Ca reabsorption in the TAL, with intact NaCl reabsorption ability at this site. In addition, the study supports a selective physiological effect of basolateral Mg²⁺ and Ca²⁺ concentration on TAL divalent cation paracellular permeability, that is, PCLN-1 activity. [References: 24]

BROUILLARD F., TONDELIER D., EDELMAN A., BAUDOUIN-LEGROS M.

Drug resistance induced by ouabain via the stimulation of mdr1 gene expression in human carcinomatous pulmonary cells.

Cancer Res., 61 (4), 1693-1698, 2001

(Services cités : U467)

The inhibition of the Na⁺M⁺-ATPase by cardiotonic drugs like ouabain deeply perturbs both the properties of the cell membrane and the ionic composition of the cytoplasm and hence alters fundamental cell reactions. These three types of reactions may be involved in the stimulation of multidrug resistance 1 (MDR-1) gene expression and the synthesis of permeability glycoprotein [P-glycoprotein (P-gp)]. We have determined whether ouabain, which binds to an extracellular motif of the Na⁺/K⁺ ATPase, stimulates MDR-1 gene expression by measuring both mRNA and protein and whether the resulting P-gp extrudes hydrophobic compounds and causes resistance to antimitotic agents. The experiments were performed on Calu-3 cells, a human cell line from a pulmonary carcinoma. Northern blotting showed that treating the cells with submicromolar concentrations of ouabain stimulated MDR-1 gene expression within 24 h. The ouabain-induced stimulation of MDR-1 expression was not restricted to Calu-3 cells but also occurred in human carcinomatous colon (T-84 and HT-29) and hepatic (H7V3) cells. However, it

is not ubiquitous because it was not found in HeLa cells. The stimulation was reproduced by other Na⁺/K⁺-ATPase inhibitors and occurred via enhanced gene transcription, apparently due to the increased cytosolic calcium concentration. Ouabain also increased the membrane content of P-gp, as detected by immunoblotting and immunohistology. We have developed a microvideo assay based on the properties of acetoxymethyl ester calcein and calcein to show that this P-gp extruded the hydrophobic acetoxymethyl ester calcein. Ouabain also caused the Calu-3 cells to become resistant to doxorubicin and vinblastine. Thus, although ouabain acts extracellularly, it may stimulate MDR-1 gene expression and P-gp synthesis and make cells resistant to hydrophobic cytotoxic compounds. [References: 24]

BROUILLARD F., BOUTHIER M., LECLERC T., CLEMENT A., BAUDOUIN-LEGROS M., EDELMAN A.

Nf-kappa b mediates up-regulation of cftr gene expression in calu-3 cells by interleukin-1 beta. *J. Biol. Chem.*, 276 (12), 9486-9491, 2001

(Services cités : U467)

Inflammation of the airways is a major feature of the inherited disease cystic fibrosis. Previous studies have shown that the pro-inflammatory cytokines tumor necrosis factor alpha and interferon gamma reduce the expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene (CFTR) in HT-29 and T84 cells by acting post-transcriptionally. We have investigated the effect of the pro-inflammatory peptide interleukin 1 beta (IL-1 beta) on the expression of the CFTR in Calu-3 cells, IL-1 beta increased the production of CFTR mRNA in a dose- and time-dependent manner. Its action was inhibited by inhibitors of the NF-kappaB pathway, including N-acetyl-L-cysteine, pyrrolidine dithiocarbamate, and a synthetic cell-permeable peptide containing the NF-kappaB nuclear localization signal sequence. Gel shift analysis showed that IL-1 beta activated NF-kappaB in Calu-3 cells, and transfection experiments using p50 and RelA expressing vectors showed that exogenous transfected NF-kappaB subunits increased the concentration of CFTR mRNA. Gel shift analysis with antibody supershifting also showed that IL-1 beta caused the binding of NF-kappaB to a kappaB-like response element at position -1103 to -1093 in the CFTR 5'-flanking region. Transfection experiments using -2150 to +52 CFTR reporter gene constructs showed that the activity of the CFTR promoter is enhanced by exogenous transfected NF-kappaB and IL-1 beta and that this enhancement is due, at least in part, to the -1103 to -1093 kappaB site. We conclude that the intracellular signaling that leads to increased CFTR mRNA in response to IL-1 beta in Calu-3 cells includes the binding of NF-kappaB to the -1103 kappaB element and a subsequent increase in CFTR promoter activity. [References: 27]

CLAIN J., FRITSCH J., LEHMANN-CHE J., BALI M., AROUS N., GOOSSENS M., EDELMAN A., FANEN P.

Two mild cystic fibrosis-associated mutations result in severe cystic fibrosis when combined in cis and reveal a residue important for cystic fibrosis transmembrane conductance regulator processing and function.

J. Biol. Chem., 276 (12), 9045-9049, 2001

(Services cités : U467)

The number of complex cystic fibrosis transmembrane conductance regulator (CFTR) genotypes identified as having double-mutant alleles with two mutations inherited in cis has been growing. We investigated the structure-function relationships of a severe cystic fibrosis (CF)-associated double mutant (R347H-D979A) to evaluate the contribution of each mild mutation to the

phenotype, CFTR mutants expressed in HeLa cells were analyzed for protein biosynthesis and Cl⁻ channel activity. Our data show that R347H is associated with mild defective Cl⁻ channel activity and that the D979A defect leads to misprocessing. The mutant R347H-D979A combines both defects for a dramatic decrease in Cl⁻ current. To decipher the molecular mechanism of this phenotype, single and double mutants with different charge combinations at residues 347 and 979 were constructed as charged residues were involved in this complex genotype. These studies revealed that residue 979, located in the third cytoplasmic loop, is critical for CFTR processing and Cl⁻ channel activity highlighting the role of charged residues. These results have also important implications for CF, as they show that two mutations in cis can act in concert to alter dramatically CFTR function contributing to the wide phenotypic variability of CF disease. [References: 28]

PLANELLES G.

H,K-ATPases.

in: *Wiley Encyclopedia of Molecular Medicine*. (Wiley & S.O.N.S. eds.)
Wiley & Sons (New-York, USA), 2001, pp.1537-1540.

(Services cités : [U467](#))

PLANELLES G.

Takis anagnostopoulos - in memoriam.

Néphrologie, 22 (2), 59, 2001

(Services cités : [U467](#))

THOMAS S.R.

A brief history of theories concerning the mammalian urine concentrating mechanism.

Acta Biotheor, 49 (4), 327-340, 2001

(Services cités : [U467](#))

The mechanism by which the mammalian kidney creates the osmotic gradient necessary for urine concentration remains an open question. We present a brief survey of the give-and-take between theory and experiment on this question over the last half century. We start with the introduction of the countercurrent multiplier paradigm in 1951. We finish with a description of a recent suggestion that the explanation for the enigmatic inner medullary osmotic gradient may reside in the very metabolism of the inner medullary cells, which are required by the region's hypoxia to obtain their ATP largely from anaerobic glycolysis and which thus, by the same token, furnish net osmoles to the medullary interstitium by converting glucose to lactate. [References: 61]

2000

ALLO J.C., MIDOUX P., MERTEN M., SOUIL E., LIPECKA J., FIGARELLA C., MONSIGNY M., BRIAND P., FAJAC I.

Efficient Gene Transfer into Human Normal and Cystic Fibrosis Tracheal Gland Serous Cells with Synthetic Vectors.

Amer. J. Respir. Cell Molec. Biol., 22 (2), 166-175, 2000

(Services cités : [U467](#))

Submucosal gland serous cells are believed to play a major role in the physiopathology of cystic fibrosis (CF) and may represent an important target for CF gene therapy. We have studied the efficiency of re porter gene transfer into immortalized normal (MM-39) and CF (CF-KM I) human airway epithelial gland serous cells using various synthetic vectors: glycosylated

polylysines (glycofectins), polyethylenimine (PEI) (25 and 800 kD), lipofectin, and lipofectAMINE. In both cell lines, a high luciferase activity was achieved with various glycofectins, with PEI 25 kD, and with lipofectAMINE. After three transfections applied daily using alpha-glycosylated polylysine, 20% of the cells were transfected. At 24 h after CF transmembrane conductance regulator (CFTR) gene transfer into CF-KM4 cells using alpha-glycosylated poly lysine, the immunolocalization of CFTR was analyzed by laser scanning confocal microscopy and the transgenic CFTR was detected by an intense labeling of the plasma membrane. The presence of membrane lectins, i.e., cell surface receptors binding oligosaccharides, was also examined on MM-39 and CF-KM4 cells by assessing the binding and uptake of fluorescein-labeled neoglycoproteins and fluorescein-labeled glycoplexes (glycofectins complexed to plasmid DNA). Among all the neoglycoproteins and glycoplexes tested, those bearing alpha-mannosylated derivatives were most efficiently taken up by both normal and CF gland serous cells. However, alpha-mannosylated polylysine was quite inefficient for gene transfer, indicating that the efficiency of gene transfer is determined both by the uptake of the complexes and also by their intracellular trafficking. Moreover, our results show that an efficient in vitro gene transfer was achieved in human airway gland serous cells with the same synthetic vectors described to efficiently transfect human airway surface epithelial cells. [References: 34]

BAUDOIN-LEGROS M., BROUILLARD F., COUGNON M., TONDELIER D., LECLERC T., EDELMAN A.

Modulation of CFTR gene expression in HT-29 cells by extracellular hyperosmolarity.

Amer. J. Physiol., 278 (1), C49-C56, 2000

(Services cités : U467)

Hypertonicity has pleiotropic effects on cell function, including activation of transporters and regulation of gene expression. It is important to investigate the action of hypertonicity on cystic fibrosis gene expression because cystic fibrosis transmembrane conductance regulator (CFTR), the cAMP-regulated Cl⁻ channel, regulates ion transport across the secretory epithelia, which are often in a hypertonic environment. We found that adding >150 mosmol/l NaCl, urea, or mannitol to the culture medium reduced the amount of CFTR mRNA in colon-derived HT-29 cells in a time-dependent manner. Studies with inhibitors of various kinases [H-89 (protein kinase A inhibitor), bisindolylmaleimide (protein kinase C inhibitor), staurosporine (serine/threonine kinase inhibitor) and herbimycin A (tyrosine kinase inhibitor), SB-203580 and PD-098059 (mitogen-activated protein kinase inhibitors)] showed that CFTR gene expression and its decrease by added NaCl required p38 kinase cascade activity. The CFTR gene activity is regulated at the transcriptional level, since adding NaCl diminished the luciferase activity of HeLa cells transiently transfected with the CFTR promoter. This regulation requires protein synthesis. The complexity of the reactions involved in blocking CFTR gene transcription by NaCl strongly suggests that the decrease in CFTR mRNA is part of a general cell response to hyperosmolar stress.

BOUCHOUCHA M., THOMAS S.R.

Error analysis of classic colonic transit time estimates.

Amer. J. Physiol. - Gastrointest. Liver Physiol., 279 (3), G520-G527, 2000

(Services cités : U467)

Estimates of colonic transit times (CTT) through the three colonic segments, right colon, left colon, and rectosigmoid, are commonly based on radiopaque markers. For a given segment, CTT is usually calculated from just the number of markers visible in that segment on abdominal X-

rays. This procedure is only strictly valid for the theoretical, but unrealistic, case of continuous marker ingestion (i.e., not for a single or once-daily ingestion). CTT was analyzed using the usual estimate of the mean CTT of one marker and also using a new, more realistic estimate based on the kinetic coefficients of a three-compartment colonic model. We directly compared our compartmental approach to classic CTT estimates by double-marker studies in six patients. We also retrospectively studied CTT in 148 healthy control subjects (83 males, 65 females) and 1,309 subjects with functional bowel disorders (irritable bowel syndrome or constipation). Compared with the compartmental estimates, the classic approach systematically underestimates CTT in both populations, i.e., in patients and in healthy control subjects. The relative error could easily reach 100% independent of the site of colonic transit delay. The normal values of total CTT are then 44.3 +/- 29.3 instead of 30.1 +/- 23.6 h for males and 68.2 +/- 54.4 instead of 47.1 +/- 28.2 h for females. [References: 12]

COULOIGNER V., TEIXEIRA M., HULIN P., STERKERS O., BICHARA M., ESCOUBET B., PLANELLES G., FERRARY E.

Effect of locally applied drugs on the pH of luminal fluid in the endolymphatic sac of guinea pig. *Amer. J. Physiol. - Regul. Integr. Comp. Physiol.*, 279 (5), R1695-R1700, 2000
(Services cités : U467)

The aim of the present work was to assess the effect of various drugs applied locally on the pH of the luminal fluid (pH(lum)) in guinea pig endolymphatic sac. pH(lum) and transepithelial potential, when measured in vivo by means of double-barrelled pH-sensitive microelectrodes, were 7.06 +/- 0.08 and +6.1 +/- 0.34 mV (mean +/- SE; n = 84), respectively, which is consistent with a net acid secretion in the luminal fluid of the endolymphatic sac. Bafilomycin and acetazolamide increased and decreased, respectively, pH(lum). Amiloride, ethylisopropylamiloride, ouabain, and Schering 28080 had no effect on pH(lum). Results obtained with inhibitors of anionic transport systems were inconclusive; e.g., DIDS reduced pH(lum), whereas neither SITS nor triflocin had any effect. We conclude that bafilomycin-sensitive H⁺-ATPase activity accounts for the transepithelial acid gradient measured in the endolymphatic sac and that intracellular and membrane-bound carbonic anhydrase probably participates in regulating endolymphatic sac pH(lum). The relationship between acid pH, endolymph volume, and Meniere's disease remains to be further investigated. [References: 34]

DE BEAUREGARD M.A.C., EDELMAN A., CHESNOY-MARCHAIS D., TONDELIER D., LAPILLONNE A., EL MARJOU F., ROBINE S., LOUWARD D.

Functional cystic fibrosis transmembrane conductance regulator tagged with an epitope of the vesicular stomatitis virus glycoprotein can be addressed to the apical domain of polarized cells. *Eur. J. Cell Biol.*, 79 (11), 795-802, 2000
(Services cités : U467)

The cystic fibrosis transmembrane conductance regulator (CFTR) is a phosphorylation-activated chloride channel apically localized in epithelial cells. In cystic fibrosis patients, the gene encoding this N-linked glycoprotein is mutated. About 70% of CF patients express a mutated form of CFTR, deleted at the phenylalanine residue at position 508 (Delta F508), CFTR-Delta F508 fails to exit the endoplasmic reticulum; it remains incompletely glycosylated and is rapidly degraded. To optimize CFTR detection for membrane localization studies and biochemical studies, we tagged wild-type and Delta F508 CFTR with the VSV-G epitope at their carboxyterminal ends. We have generated pig kidney epithelial cell clones (LLCPK1) expressing VSV-G-tagged human wild-type and Delta F508-CFTR. In CFTR-expressing cells, the

transfected protein is matured and transported to the apical membrane where it is concentrated. The cells exhibit a strong anion channel activity after stimulation by cAMP, as demonstrated by a halide sensitive fluorescent dye assay (6-methoxy-N-ethylquinolinium, SPQ), and whole-cell patch-clamp approach. This activity of CFTR-VSV-G is indistinguishable from the wildtype CFTR. In contrast, in cells expressing tagged Delta F508-CFTR or in non-transfected cells, no anion channel activity could be detected after stimulation by cAMP. In Delta F508-CFTR-VSV-G-expressing cells, the mutated CFTR remained in the incompletely glycosylated form and was localized in the endoplasmic reticulum. These cell lines reproduce the cellular fate of wild-type and mutated CFTR-Delta F508. To our knowledge, they are the first differentiated epithelial cell lines stably expressing tagged CFTR and CFTR-Delta F508 in which cellular processing and functional activity of these two proteins are reproduced. Thus the addition of the VSV-G epitope does not impair the localization and function of CFTR, and these cell lines can be used to examine CFTR function in vitro. [References: 36]

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E.D.K. (Paris), 2000, pp.69-79.

(Services cités : U467)

FEUILLET-FIEUX M.N., SERMET I., EDELMAN A., TOROSSO T., FERREC M., GUILLOT M., LENOIR G., BONNEFONT J.P., THUILLIER L.

Identification of a novel mutation, 1087delT, in exon 7 of the CFTR gene in a patient with cystic fibrosis.

Hum. Mutat., 16 (1), 95, 2000

(Services cités : U467, Département de Pédiatrie, Biochimie Médicale)

PLANELLES G.

Cloning and functional expression of the major membrane transport system of the renal bicarbonate absorption, the na-hco₃ cotransporter.

Néphrologie, 21 (4), 199-200, 2000

(Services cités : U467)

SERMET GAUDELUS I., BONNEFONT J.P., NGUYEN-KHOA A.T., LENOIR G.

Normal sweat test does not exclude the diagnosis of cystic fibrosis.

Archives Pédiatrie, 7 (6), 594-596, 2000

(Services cités : U467, Génétique Médicale Pédiatrique, Département de Pédiatrie)

THOMAS S.R.

Inner medullary lactate production and accumulation: a vasa recta model.

Amer. J. Physiol. - Renal Fluid Electrol. Physiol., 279 (3), F468-F481, 2000

(Services cités : U467)

1999

COUGNON M., BOUYER P., JAISSER F., EDELMAN A., PLANELLES G.

Ammonium transport by the colonic H(+)-K(+)-ATPase expressed in *Xenopus* oocytes.

Amer. J. Physiol., 277 (2 Pt 1), C280-C287, 1999

(Services cités : U467)

Functional expression of the rat colonic H(+)-K(+)-ATPase was obtained by coexpressing its catalytic alpha-subunit and the beta(1)-subunit of the Na(+)-K(+)-ATPase in *Xenopus laevis* oocytes. We observed that, in oocytes expressing the rat colonic H(+)-K(+)-ATPase but not in control oocytes (expressing beta(1) alone), NH(4)Cl induced a decrease in (86)Rb uptake and the initial rate of intracellular acidification induced by extracellular NH(4)Cl was enhanced, consistent with NH(+)(4) influx via the colonic H(+)-K(+)-ATPase. In the absence of extracellular K(+), only oocytes expressing the colonic H(+)-K(+)-ATPase were able to acidify an extracellular medium supplemented with NH(4)Cl. In the absence of extracellular K(+) and in the presence of extracellular NH(+)(4), intracellular Na(+) activity in oocytes expressing the colonic H(+)-K(+)-ATPase was lower than that in control oocytes. A kinetic analysis of (86)Rb uptake suggests that NH(+)(4) acts as a competitive inhibitor of the pump. Taken together, these results are consistent with NH(+)(4) competition for K(+) on the external site of the colonic H(+)-K(+)-ATPase and with NH(+)(4) transport mediated by this pump.

FANEN P., CLAIN J., LABARTHE R., HULIN P., GIRODON E., PAGESY P., GOOSSENS M., EDELMAN A.

Structure-function analysis of a double-mutant cystic fibrosis transmembrane conductance regulator protein occurring in disorders related to cystic fibrosis.

FEBS Lett., 452 (3), 371-374, 1999

(Services cités : U467)

A number of disorders related to cystic fibrosis have been described since the cloning of the cystic fibrosis gene, including infertility due to the congenital bilateral absence of the vas deferens. We have identified, in several patients, complex cystic fibrosis transmembrane conductance regulator genotypes like double-mutant alleles. We have now analyzed the structure function relationships of one of these mutants, R74W-D1270N cystic fibrosis transmembrane conductance regulator, expressed in HeLa cells, to evaluate the contribution of each mutation in the phenotype. We found that R74W cystic fibrosis transmembrane conductance regulator appears to be a polymorphism, while D1270N cystic fibrosis transmembrane conductance regulator could be responsible for the congenital bilateral absence of the vas deferens phenotype. The combination of the two produced a more severe effect on the chloride conductance pathway as well as on the phenotype. (C) 1999 Federation of European Biochemical Societies.

[References: 18]

TEIXEIRA M., COULOIGNER V., LOISEAU A., HULIN P., STERKERS O., PLANELLES G., FERRARY E.

Evidence for apical K conductance and Na-K-2Cl cotransport in the endolymphatic sac of guinea pig.

Hearing Res., 128 (1-2), 45-50, 1999

(Services cités : U467)

The transepithelial potential in the endolymphatic sac (ESP) was recorded up to 60 min after apical injection of ouabain, bumetanide, quinine, barium, tetraethylammonium, and 4-aminopyridine. After control injection, ESP decreased by 74% and completely recovered at 30 min. After ouabain, barium: or quinine injection, the ESP time course was similar to that in the control group. After bumetanide, tetraethylammonium, or 4-aminopyridine injection, complete recovery was only observed at 60 min. These results suggest that apical K⁺ conductance and Na-

K-2Cl cotransporter could be involved in the genesis of ESP. (C) 1999 Published by Elsevier Science B.V. All rights reserved. [References: 22]

TONDELIER D., BROUILLARD F., LIPECKA J., LABARTHE R., BALI M., de BEAUREGARD M.A.C., TOROSSO T., COUGNON M., EDELMAN A., BAUDOUIN LEGROS M.

Aspirin and some other nonsteroidal anti-inflammatory drugs inhibit cystic fibrosis transmembrane conductance regulator protein gene expression in T-84 cells.

Mediat. Inflamm., 8 (4-5), 219-227, 1999

(Services cités : U467)

CYSTIC fibrosis (CF) is caused by mutations in the CF gene, which encodes CF transmembrane conductance regulator protein (CFTR), a transmembrane protein that acts as a cAMP-regulated chloride channel. The disease is characterized by inflammation but the relationship between inflammation, abnormal transepithelial ion transport, and the clinical manifestations of CF are uncertain. The present study was undertaken to determine whether three nonsteroidal anti-inflammatory drugs (NSAIDs) (aspirin, ibuprofen, and indomethacin) modulate CFTR gene expression in T-84 cells. Treatment with NSAIDs reduced CFTR transcripts, and decreased cAMP-stimulated anion fluxes, an index of CFTR function. However, the two phenomena occurred at different concentrations of both drugs. The results indicate that NSAIDs can regulate both CFTR gene expression and the function of CFTR-related chloride transport, and suggest that NSAIDs act via multiple transduction pathways. [References: 25]