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Symposium 4: Regulation of ion transport

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Chair: Peter Barry and Margot Day

Regulation of the epithelial sodium channels

A. Dinudom, Department of Physiology, University of Sydney, NSW 2006, Australia.

The epithelial Na^+ channel (ENaC) plays an important role in the regulation of extracellular fluid volume and blood pressure and in the regulation of the volume of the fluid bathing the apical surfaces of epithelia such as the respiratory and reproductive epithelia (Voilley *et al.*, 2002). Given that ENaC represents a passive pathway for Na^+ to diffuse from the external environment into the cells, it also represents a significant mechanism by which changes in the sodium composition of the external environment, such as in the lumen of the kidney distal collecting tubule or in the lumen of salivary ducts, influences the cytosolic composition and volume of epithelial cells.

ENaC is subject to a wide range of regulatory mechanisms. These regulatory systems include the mineralocorticoid hormone aldosterone, growth factors such as insulin and IGF-I and cytokines such as TNF-α as well as the feedback mechanisms that regulate the activity of the channels in response to changes in intracellular Na^+ and intracellular Cl^- (Dinudom *et al.*, 2002). In addition to these systems, the activity of ENaC is also known to be modulated by the Cl^- channel, cystic fibrosis transmembrane conductance regulator (Stutts *et al.*, 2002). Some of the systems that modulate the activity of ENaC have intracellular pathways in common. A good example of this is the relationship between aldosterone regulation and the feedback regulation of ENaC. It has been suggested that the early effects of aldosterone, on the activity of ENaC are mediated by suppression of the Na^+ feedback regulatory system. This is proposed to operate by aldosterone increasing the expression of the serum and glucocorticoid-dependent protein kinase, Sgk, which phosphorylates the ubiquitin protein-ligase Nedd4-2, a principal mediator of the Na^+ feedback system, so as to render it unable to interact with ENaC (Snyder *et al.*, 2002). Although the effect of Sgk on ENaC activity has been demonstrated for ENaC expressed in *Xenopus* oocytes, this phenomenon has not been observed in either isolated mouse mandibular duct cells or M1 mouse collecting duct epithelia. Interestingly, we have found that the activity of cytosolic kinases other than Sgk is essential for the maintenance of the basal activity of ENaC and that when activated, these kinases inhibit the Na^+ feedback regulatory system. It is conceivable that these protein kinases may also be involved in the mechanism by which aldosterone upregulates ENaC activity.

Dinudom, A., Komwatana, P., Young, J.A. & Cook, D.I. (1995) *Journal of Physiology,* 487, 549-555.
Snyder, P.M., Olson, D.R. & Thomas, B.C. (2002) *Journal of Biological Chemistry,* 277, 5-8.
Stutts, M.J., Canessa, C.M., Olsen, J.C., Hamrick, M., Cohn, J.A., Rossier, B.C. & Boucher, R.C. (1995) *Science,* 269, 847-850.
Voilley, N., Galibert, A., Bassilana, F., Renard, S., Lingueglia, E., Coscoy, S., Champigny, G., Hofman, P., Lazdunski, M. & Barbry, P. (1997) *Comparative Biochemistry and Physiology,* 11, 193-200.

Links between cell proliferation and K channel activity

M.L. Day, Department of Physiology, University of Sydney, NSW 2006, Australia.

Changes in the activity of potassium channels are required for proliferation of a wide variety of cell types. Pharmacological inhibition of K^+ channel activity can cause cell cycle arrest. Our studies on the regulation of ion channels during pre-implantation development of the mouse embryo have provided some insight into the mechanisms linking channel activity to the cell cycle. Using the patch-clamp technique, we have shown that the activity of a large-conductance K^+ channel in the early mouse embryo is regulated by the cell cycle (Day *et al.*, 1993). This K^+ channel is active during M and G1 phases and inactive during S and G2 phases. In parallel with the changes in K^+ channel activity there are changes in cell membrane potential such that the membrane potential is hyperpolarised when the channel is active.

The activation of this K^+ channel at the G2/M transition of early embryonic cell cycles does not depend on the activation of the mitotic kinase, Cdk1, and does not require the presence of the nucleus (Day *et al.*, 1998a). Thus it appears that a cytoplasmic cell cycle is functional in the early mouse embryo to regulate K^+ channel activity. This cytoplasmic clock is, however, not completely uninfluenced by the nuclear cell cycle clock since inactivation of the channel as the cell cycle exits M phase is affected by Cyclin B/Cdk1 activity, and inhibition of DNA synthesis prevents the decrease in channel activity that normally occurs at the G1-S transition. Thus, the K^+ channel in the early mouse embryo is controlled both by nuclear and cytoplasmic clocks.

Several roles for K^+ channels in cell proliferation have been proposed. For example, a change in K^+ channel activity can cause a change in cell membrane potential that can then alter the activity of other voltage-gated ion channels, such as Ca^{2+} channels. In the case of the K^+ channel in the embryo, this role is possible since we have observed not only parallel variations in membrane potential but also cell cycle-dependent changes in the amplitude of a T-type Ca^{2+} current (Day *et al.*, 1998b). A second, possible role for the K^+ channel in the embryo is in cell volume homeostasis. There is some evidence for this possibility since a cell swelling-induced Cl current is regulated by the cell cycle in mouse embryos being inactive during metaphase of mitosis in the 2-cell embryo at a time when the K^+ channel is also active (Kolajova *et al.*, 2001).

Day, M.L., Pickering, S.J., Johnson, M.H. & Cook, D.I. (1993) *Nature,* **365**: 560-562.

Day, M.L., Johnson, M.H. & Cook D.I. (1998a) *EMBO Journal,* **17**: 1952-1960.

Day, M.L., Johnson, M.H. & Cook D.I. (1998b) *Pflügers Archiv European Journal of Physiology,* **436**: 834-842.

Kolajova, M., Hammer, M., Collins, J. & Baltz, J. (2001) *Development,* **128**: 3427-3434.

Regulation of the glutamine transporter SN1 (SNAT3)

S. Bröer, School of Biochemistry & Molecular Biology, Australian National University, Linnaeus way 41, Canberra, ACT 0200, Australia. (Introduced by David Cook)

The glutamine transporter SN1 is mainly expressed in hepatocytes and in brain astrocytes. It is involved in both uptake and efflux of glutamine and its activity is tightly regulated. Transport of glutamine via SN1 is coupled to the cotransport of 1 Na^+ and the antiport $1H^+$ (Chaudhry *et al.*, 1999). As a result glutamine transport is electroneutral and its preferred direction is governed by extracellular pH and intracellular Na^+ concentration. In addition, SN1 is allosterically regulated, becoming inactive at acidic pH (Broer *et al.*, 2002).

In the brain SN1 mediates the release of glutamine from astrocytes, which is used as a precursor for neurotransmitter glutamate biosynthesis in neurons. Increased extracellular glutamate concentrations induced a rapid increase of SN1 activity in astrocytes. The upregulation was not caused by activation of ionotropic or metabotropic glutamate receptors but required uptake of glutamate into astrocytes. Experiments in *Xenopus* oocytes* suggest that glutamate may act as a direct regulator of SN1 activity.

Severalfold evidence suggests that protein trafficking is a major mechanism by which SN1 activity is regulated. In *Xenopus* oocytes SN1 activity rapidly decreased after treatment of oocytes with phorbolester. Confocal microscopy of oocytes expressing a GFP-SN1 construct revealed that loss of activity was accompanied by a retrieval of the transporter from the plasma membrane. Retrieval of SN1 was specific but did not involve phosphorylation of the transporter. A similar downregulation by incubation with phorbolester was observed in cultured HepG2 cells but not in primary cultures of brain astrocytes.

A possible mechanism for the retrieval of transporter may involve ubiquitination followed by degradation of the transport protein. Coexpression of SN1 with the ubiquitin ligase Nedd4-2 reduced the transport activity of SN1, a downregulation that was abrogated by coexpression of protein kinase sgk1 or protein kinase B (PKB) (Boehmer *et al.*, 2003). Coexpression of sgk1 or PKB together with SN1 resulted in an increase of the transport activity.

Taken together these data provide evidence for a regulation of SN1 by plasma membrane trafficking. The actual components of the signal transduction pathways, however, are likely to differ between cell types.

Boehmer, C., Okur, F., Setiawan, I., Broer, S. & Lang, F. (2003) *Biochemical Biophysical Research Communication*, 306, 156-162.

Broer, A., Albers, A., Setiawan, I., Edwards, R. H., Chaudhry, F. A., Lang, F., Wagner, C. A. & Broer, S. (2002) *Journal of Physiology*, 539, 3-14.

Chaudhry, F. A., Reimer, R. J., Krizaj, D., Barber, D., Storm-Mathisen, J., Copenhagen, D. R. & Edwards, R. H. (1999) *Cell*, 99, 769-780.

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*Animal experimentation protocols were approved by the Australian National University.

The familial intrahepatic cholestasis type 1 protein: a P-type ATPase influencing bile acid transporters

M.J. Harris[1,2] and I.M. Arias[2], [1]ANZAC Research Institute, University of Sydney, Concord RG Hospital, Concord, NSW 2139 and [2]Tufts University School of Medicine, Boston MA, USA.
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Progressive familial intrahepatic cholestasis type 1 (PFIC1) and benign recurrent intrahepatic cholestasis (BRIC) result from mutations in the familial intrahepatic cholestasis gene (FIC1). FIC1 is a member of the type IV P-type ATPase subfamily, which function as aminophospholipid translocases. Since the phenotype of these diseases manifests as impaired bile flow and considering that FIC1 is localised to the canalicular membrane in hepatocytes, we investigated whether FIC1 could transport bile acids and/or influence the apical bile acid transporters, either the bile salt export pump (BSEP) or the intestinal apical sodium dependent bile acid transporter (ASBT).

Method: Apical efflux assay: MDCK II cells which stably express Na^+/taurocholate co-transporting polypeptide (NTCP) formed polarised monolayers when grown on Transwell filters and were transfected with FIC1 and/or BSEP. Two days after transfection, the basal medium was replaced with uptake buffer containing ^{3}H-taurocholate (TC) and the cells were incubated at 37°C for 1 hour. TC efflux was determined by measurement of radioactivity in the apical medium. Apical uptake assays: MDCK II cells were transfected with FIC1, FIC1 mutants and/or ASBT. Two days after transfection, the apical media was replaced with uptake buffer containing ^{3}H-TC and incubated at 37°C for 1 hour. Uptake of ^{3}H-TC by ASBT was determined by measurement of intracellular radioactivity. In all studies, transfection with β-gal was used as a control and western blotting of membrane preparations confirmed expression of each relevant protein.

Results: Apical efflux: ^{3}H-TC apical efflux in BSEP transfected cells was 2 fold higher than in non-transfected MDCKII-NTCP cells ($p<0.05$) and was unaffected by co-transfection of FIC1. In addition, FIC1 expression had no effect on ^{3}H-TC efflux in cells which were not transfected with BSEP. Apical uptake: ^{3}H-TC uptake in ASBT expressing cells was 10 fold higher than in β-gal or FIC1 transfected cells, and was unaffected by transfection of cells with both ASBT and FIC1, or ASBT and FIC1 mutants.

Summary: FIC1 did not transport taurocholate across the apical membrane of MDCK II cells. Expression of FIC1 or FIC1 mutants did not affect BSEP or ASBT function. These results suggest that FIC1 affects hepatic bile secretion and/or intestinal bile acid absorption by indirect mechanisms that are currently unknown. An alternative hypothesis is that FIC1 effects the trafficking of bile acid transporters to the apical membrane via its aminophospholipid translocase activity. This "flippase" function is required for the budding of vesicles from organelles such as the golgi, endosomes and the plasma membrane. These mechanisms are being investigated by siRNA knockout experiments.