

## Regulation of the epithelial Na<sup>+</sup> channel by caveolin

I.-H. Lee,<sup>1</sup> A. Dinudom,<sup>1</sup> C.R. Campbell,<sup>1</sup> S. Kumar<sup>2</sup> and D.I. Cook,<sup>1</sup> <sup>1</sup>Discipline of Physiology, Faculty of Medicine, University of Sydney, NSW 2006, Australia and <sup>2</sup>Hanson Centre of Cancer Research, IMVS, SA 500, Australia.

Function of the epithelial Na<sup>+</sup> channels (ENaC) in the kidney and colon is essential for the regulation of Na<sup>+</sup> and fluid homeostasis, and the control of blood pressure. Membrane expression of ENaC is regulated by Nedd4-2, a ubiquitin protein ligase that induces ubiquitination of ENaC (Snyder *et al.*, 2004). It has been suggested that ubiquitinated-ENaC may undergo internalisation *via* clathrin-dependent endocytosis (Shimkets *et al.*, 1997). This notion is supported by the findings that ENaC subunits are localised in clathrin-coated vesicles (Wang *et al.*, 2006) and that they interact with several clathrin adaptor proteins (Staruschenko *et al.*, 2005; Wang *et al.*, 2006). ENaC subunits were also detected, however, in the detergent-insoluble glycosphingolipids- and cholesterol-enriched, membrane fraction which contains lipid rafts (Hill & Johnson, 2002). It is well established that caveolin is a structural protein that is essential for the formation of lipid rafts. A subsequent finding that ENaC and caveolin are colocalized (Jornot *et al.*, 2005) has prompted speculation that clathrin-independent endocytosis, mediated by caveolin, may also regulate membrane abundance of ENaC. In this study, we investigated the role of caveolin in the regulation of ENaC in Fisher rat thyroid (FRT) cells heterologously expressing ENaC grown on a permeable support. Activity of ENaC was measured as the amiloride-sensitive short-circuit current under open-circuit conditions (Lee *et al.*, 2007). Activity of caveolin in FRT cells was increased by co-transfecting the cells with caveolin constructs. Conversely, caveolin expression was reduced by transfecting the cell with an siRNA specific to caveolin isoform. Our findings suggest that caveolin is an important regulator of ENaC that controls its activity by regulating membrane expression of the channel.

Hill WG, An B & Johnson JP. (2002) *Journal of Biological Chemistry*, **277**: 33541-4.

Jornot L, Rochat T, Caruso A & Lacroix JS. (2005) *Journal of Cell Physiology*, **204**: 859-70.

Shimkets RA, Lifton RP & Canessa CM. (1997) *Journal of Biological Chemistry*, **272**: 25537-41.

Snyder PM, Steines JC & Olson DR. (2004) *Journal of Biological Chemistry*, **279**: 5042-6.

Staruschenko A, Pochynyuk O & Stockand JD. (2005) *Journal of Biological Chemistry*, **280**: 39161-7.

Wang H, Traub LM, Weixel KM, Hawryluk MJ, Shah N, Edinger RS, Perry CJ, Kester L, Butterworth MB, Peters KW, Kleyman TR, Frizzell RA & Johnson JP. (2006) *Journal of Biological Chemistry*, **281**: 14129-35.

Lee IH, Dinudom A, Sanchez-Perez A, Kumar S & Cook DI. (2007) *Journal of Biological Chemistry*, <http://www.jbc.org/cgi/reprint/M701923200v1>