

## The canonical transient receptor potential channel 1 is an essential structural component of the mechanosensitive calcium permeable channel in vertebrate cells

O.P. Hamill<sup>1</sup>, R. Maroto<sup>1</sup>, A. Kurosky<sup>2</sup>, T.G. Wood<sup>2</sup>, A. Raso<sup>3</sup> and B. Martinac<sup>3</sup>, <sup>1</sup>Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, Texas, U.S.A., <sup>2</sup>Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas, U.S.A. and <sup>3</sup>Department of Pharmacology, University of Western Australia, Crawley, WA, Australia.

The mechanosensitive cation channel (MscCa) transduces membrane stretch into cation (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>) flux across the cell membrane, and is implicated in cell volume regulation, cell locomotion, muscle dystrophy and cardiac arrhythmias (Hamill & Martinac, 2001). However, the membrane protein(s) forming the MscCa in vertebrates remains unknown. Here we use an identification strategy based on detergent-solubilizing of frog oocyte membrane proteins followed by liposome reconstitution and evaluation by patch-clamp (Sukharev *et al.*, 1993; Maroto *et al.*, 2005). The oocyte was chosen because it expresses the prototypical MscCa ( $\geq 10^7$  MscCa/oocyte) that is preserved in cytoskeleton-deficient membrane vesicles (Zhang *et al.*, 2000). We identified a membrane protein fraction that reconstituted high MscCa activity and showed an abundance of an 80 kDa protein identified immunologically as the canonical transient receptor potential channel 1 (TRPC1) (Wes *et al.*, 1995; Brereton *et al.*, 2000). Heterologous expression of the human TRPC1 resulted in a > 1000% increase in MscCa patch density, whereas injection of a TRPC1-specific antisense RNA abolished endogenous MscCa activity. hTRPC1 transfection of CHO-K1 cells also significantly increased MscCa expression. These observations indicate that TRPC1 is a component of the vertebrate MscCa, which like various prokaryotic Msc channels (Martinac & Kloda, 2004), is gated by tension developed in the lipid bilayer.

Brereton, H.M., Harland, M.L., Auld, A.M. & Barritt, G.J. (2000) *Molecular and Cellular Biochemistry* **214**, 63-74 (2000).

Hamill, O.P. & Martinac, B. (2001) *Physiological Reviews* **81**, 685-740.

Maroto, R., Raso, A., Wood, T.G., Kurosky, A., Martinac, B. & Hamill, O.P. (2005) *Nature Cell Biology* **7**, 1443-1446.

Martinac, B. & Kloda, A. (2003) *Progress in Biophysics and Molecular Biology* **82**, 11-24.

Sukharev, S.I., Martinac, B., Arshavsky, V.Y. & Kung, C. (1993) *Biophysical Journal* **65**, 177-183.

Wes, P.D., Chesvesich, J., Jeromin, A., Rosenberg, C., Stetten, G., & Montell, C. (1995) *Proceedings of the National Academy of Sciences of the United States of America* **92**, 9652-9656.

Zhang, Y., Gao, F., Popov, V.L., Wen, J.W. & Hamill, O.P. (2000) *Journal Physiology* **523**, 117-130.