

**CAENORHABDITIS ELEGANS IClns: FUNCTIONAL AND MOLECULAR CHARACTERIZATION IN EMBRYONIC CELLS**

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The ICln protein is essential for cell volume regulation and bilayer reconstitution experiments suggested that it could be the regulatory volume decrease channel (RVDC) or a part of it. In *C. elegans* we found two ICln splice variants (IClnN1 and IClnN2). The IClnN1 (GenBank™/EBI Data Bank accession #AF202931) aminoacid sequence is homologous to ICln proteins identified in other species, while the IClnN2 (accession #AF202932) one possesses 20 additional aminoacids (aa, encoded by exon 2a) close to the inner mouth of the putative channel pore and important for the channel inactivation [1]. To investigate the role of the two splice variants of ICln in native cells, patch-clamp whole-cell and cell-attached experiments in native cells have been performed. In parallel, Western blot with an affinity purified anti-IClnN2 antibody on total protein extract from *C. elegans* and in-situ hybridization (ISH) experiments on embryos in different stages of embryogenesis have been done. Single-cell RT-PCR confirmed the presence of both transcripts in embryo cells, where an inwardly rectifying, non inactivating, DIDS-sensitive anion current was elicited upon cell swelling. Single-channel experiments showed the presence in hypotonic extracellular solution of an inwardly rectifying anion channel with a conductance of about 180 pS at -100 mV. ISH experiments were performed with digoxigenin (DIG) labeled probes and the signal was detected by means of a fluorescent antibody anti-DIG. Confocal microscopy analysis revealed a high expression level of the IClnN1 transcript in *C. elegans* embryos since the earlier stages of embryogenesis (i.e. 4 cells), in accordance with the need of the protein for cell viability. On the other hand, the mRNA abundance and the expression level of IClnN2 protein is very low in *C. elegans* embryo cells. Moreover, the RVD current recorded seem not directly due to IClnN1 expression, suggesting, in this particular cell type, other roles for ICln proteins, probably not related to the volume regulation.

[1] J. Furst, M. Ritter, J. Rudzki, J. Danzl, M. Gschwentner, E. Scandella, M. Jakab, M. Konig, B. Oehl, F. Lang, P. Deetjen, M. Paulmichl, "ICln ion channel splice variants in *Caenorhabditis elegans*: voltage dependence and interaction with an operon partner protein", *J. Biol. Chem.*, **277**(6): 4435-45 (2002).