Optical nano-control of neuronal Connexin-36 Gap Junctions

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Objectives:

- to understand how neuronal connexins are maintained in the plasma membrane we performed proteomics screened from brain extracts in search for molecules interacting with cytosolic moieties of Connexin 36 and preferentially localized to the plasma membrane.

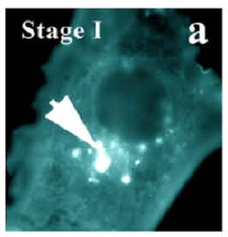
- to test whether found by proteomics molecules have biological relevance we analysed their effects on connexin36 in living cells.

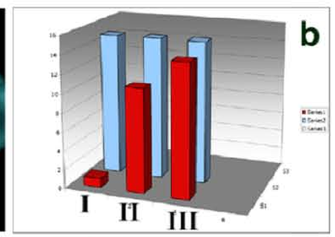
Methods: EM, cryo-EM, biochemistry, Proteomics, nano-Spectroscopy. Live cell imaging: High resolution Spinning Disk Nikon based set-up with CO2, z-PFS, anisiotropy, FCS and dual split FRET measurements devices were set to resolve cellular structures and protein-protein interactions in living cells.

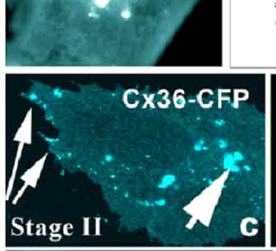
Results: Fig. 1: Cx36-ECFP expressed in non-neuronal cells usually unstable at the PM and shortly after transfection and short appearance at the PM are internalized to be degraded in lysosomes. Here we tested effect of found in proteomics screen Drebrin on the stability of Cx36 at the cell surface. The presence of Cx36 interacting protein Drebrin (found in PSD fractions) strongly increases connexin-containing clusters at the plasma membrane of Vero cells. The phenomena can be observed in both cases: when cells are forming cell-cell contacts and at intact non-contacting membranes, suggesting that drebrin may stabilize Cx36 in non-neuronal cells by linking it to the submembrane cytoskeleton.

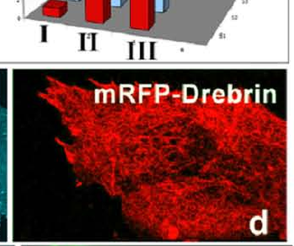
Introduction:

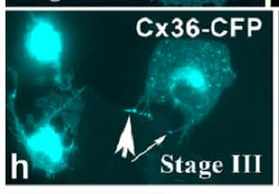
Four lines of evidence support the idea that neuronal Gap Junctions (GJ) are operating in accord with synaptic transmission:
I - First, electron microscopy analyses of brain sections revealed typical double membrane of GJ that are often present close to the synapses, (Fukuda 2007, Fukuda 2009, Fukuda, et al., 2006 and our own data).
II - Second, the amplitude of neural responses in many brain regions found to be modified. This fact would be difficult to explain without appreciation of electrical synapses and Belousov's group showed that NMDA receptors regulate developmental gap junction uncoupling via CREB signaling, (see Arumugam et al., 2005).
III – Cx36-deficient mice demonstrate that transmission through electrical synapses is important for neuron and brain function. Generation and analyses of Connexin 36-GFP expressing mice revealed that electrical synapses are abundant in the mice brain and their function believed to be important for the generation of synchronous oscillations, (Hormuzdi et al., 2001, Blatow et al., 2003; Buhl et a Galarreta et al., 1999, Hestrin, S., Galarreta, M., 2005 Deans et al., 2001). The functional consequences of electrical synapses are still incompletely understood, but recent reports documented abnormal circadian activity, deficits in motor-coordination, motor learning, and impaired memory recall, (Long et al., 2005).
IV – Biochemical analyses (Ciolofan et al., 2007), show that Cx36 present in a complex with the scaffold protein zonula occludens (ZO-1).
Thus, neuronal coupling via gap junctions is extremely important in early development (Arumugam et al., 2005, Spitzer 2006). Studies of gap junction coupling between interneurons in the cortex, amygdala, and hippocampus, shown to be mediated mainly by Cx36, although in some instances electrical synapses may include other connexins as e.g. Cx45, Cx47, Cx57. It still remained to be demonstrated that considerable specificity in connexin distribution in brain play an important role in electrically-coupled neural circuits, (for review see Bennett and Zukin 2004)

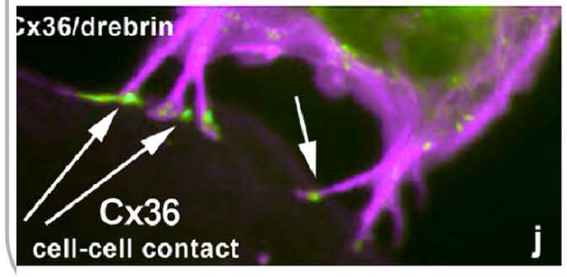


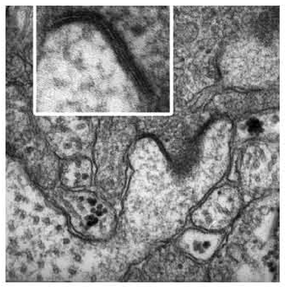












Gap junctions can be often found in CA1 close to synaptic zones

haracterization of the epitop-specific antibodies ected against cytoplasmic loop and the C-terminus of neuronal Connexun-36

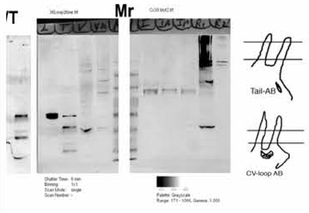


Fig.5: EM and live cell imaging revealed presence of Cx36 in the "folded" structures of the ER membrane close to Exit sites . This structures strongly resemble lamella bodies found in brains of hibernating animals.

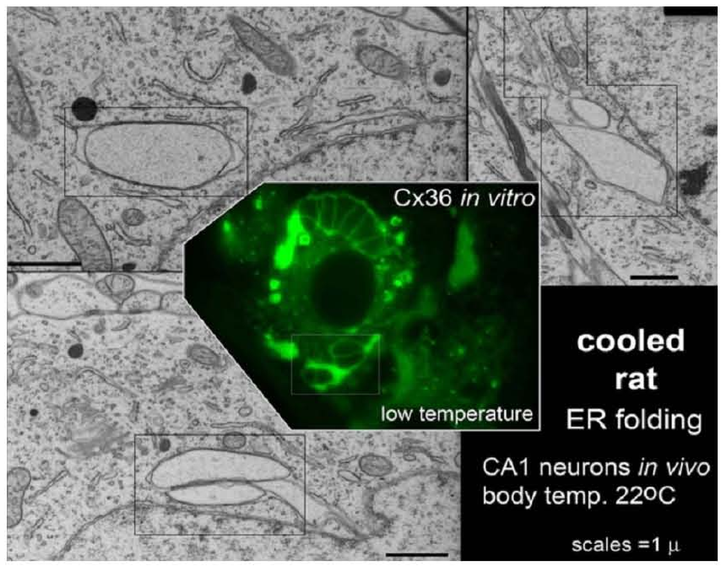
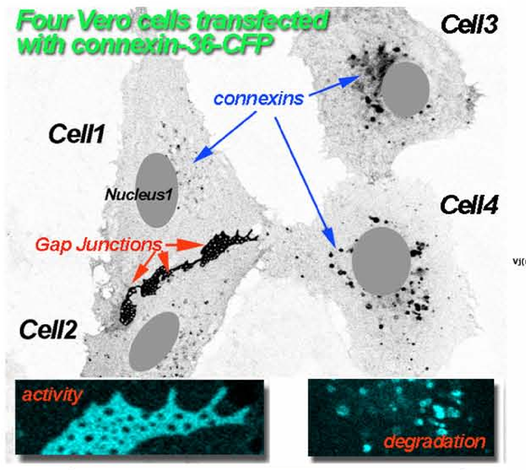
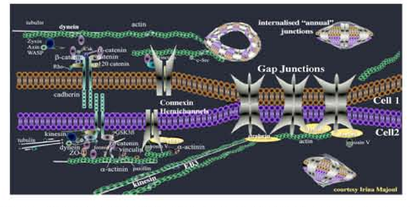
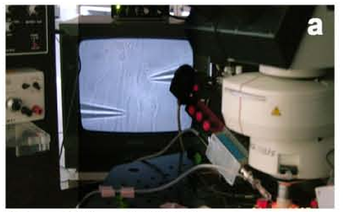


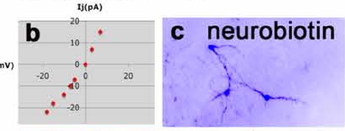
Fig.6:Cell culture reconstructions at cell-cell interface: Drebrin expressing cells Cell1 and Cell 2 able to maintain Cx36 At cell-cell interface. In the absence of Drebrin (Cell3 and Cell 4) Cx36 is degraded in ER and lysosomal structures.

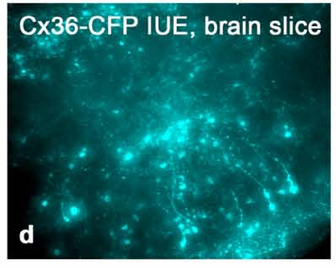






Current-Voltage Cx36-CFP





Conclusions:

We demonstrate here that newly described protein Drebrin, (Developmentally REgulated BRain protelN) may directly interact with Cx36 in living cells and removal of drebrlin may have consequences on the stability and formation of neuronal cell-cell contacts.
Second, we show that cells may store connexins in the ER (Endoplasmic Reticulum) under unfavorable conditions. If the activity-dependent transport of connexins to the PM is delayed, Cx36 may undergo ER associated degradation.
Mapping Cx36 domains and testing them against corresponding domains of Drebrin revealed potential sites in Cx36 cytosolic loop and tail that may have biological relevance for in vivo function and thereby incorporation of Cx36 channels into zones adjacent to electrical synapses.

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