2020

Trafficking Patterns of KCNQ1 and KCNE1 and Assembly into the Slow Delayed Rectifier (Iks) Channel

Sukhleen Kaur

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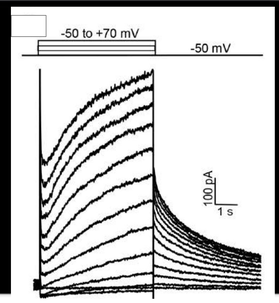

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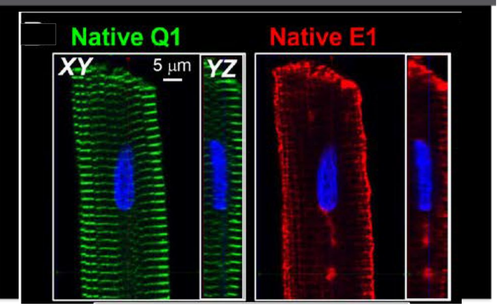

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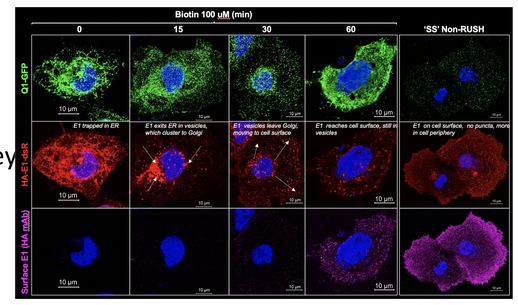
Sukhleen Kaur
Mentor: Dr. Gea-Ny Tseng (1)
(1) - Department of Physiology and Biophysics, Sanger Hall, Virginia Commonwealth University, Richmond, VA

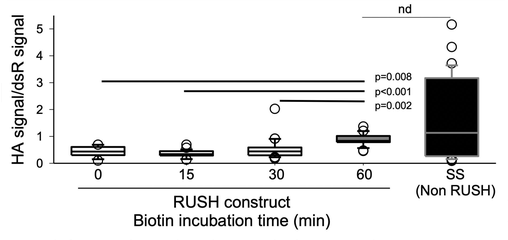
Background and Significance

KCNQ1 and KCNE1 are both proteins that are essential in maintaining cardiac electrical stability. The assembly of the two units forms the slow delayed rectifier channel (I_{ks}) which plays a role in repolarization of the action potential in adult ventricular myocytes, as shown in the figure on the top right.. I_{ks} remains unassembled under basal conditions as KCNQ1 and KCNE1 are segregated. While KCNQ1 is a pore-forming subunit, KCNE1 is a smaller, auxiliary subunit. The assembly of both subunits is essential in forming I_{ks}, for they do not function when they are present as separate, individual units. In other words, the two units are mutually inclusive, yet they both have very different properties, especially in reference to the trafficking phenomenon of each protein following translation. The question being addressed, therefore, was "how do kcnq1 and kcne1 assemble to form the I_{ks} channels? Answering this question provides insight into the molecular mechanisms that cause a long QT syndrome, an abnormal heart condition that results from the loss of function of the Iks channel.

Results (1) - COS-7 RUSH

The purpose of this imaging experiment was to test whether we can observe time-dependent movement along the secretory pathway of HA-E1-dsR after it is released from the endoplasmic reticulum by the addition of biotin. The results showed a time-dependent increase of HA-E1-dsR on the cell surface.

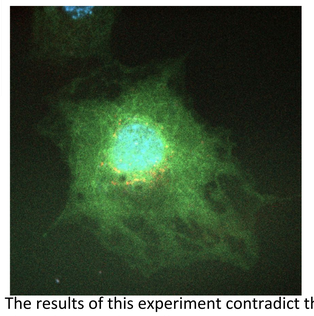




Both figures above show how as the biotin incubation time increases, the cell surface expression of HA-E1-dsR increases as well. This indicates that proteins released from the ER traffic to the plasma membrane.

Results (2) - RUSH live cell imaging

The purpose of this live cell imaging experiment was to test the trafficking pattern of Q1-GFP and E1-DsR as well as if they show a time-dependent release/spread of proteins from being contained in the ER to golgi to the plasma membrane? The results obtained in this experiment contradict those of the primary RUSH experiment with fixed cells, yet these differences can be attributed to the various complications when working with live cells and maintaining physiological conditions when imaging the cells.

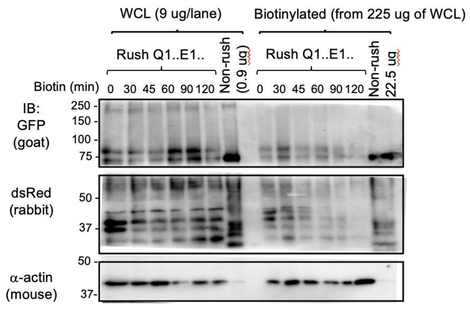


The figure to the left shows a single time frame of a COS-7 cell undergoing live cell imaging. Q1-GFP is seen throughout the ER network and, concentrated in the perinuclear region. No protein movement to the cell surface was observed.

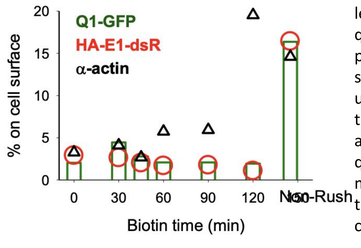
The results of this experiment contradict that of experiment 1. This is because live cell imaging is very challenging to perform as cells must be kept under physiological conditions for accurate phenomena to be observed. Even the slightest variations can result in inaccurate observations of protein movement; as we observed here, no proteins trafficked out of the ER and perinuclear region..

Results (3) - Biotinylation

The purpose of this experiment was to quantify the Q1-GFP and E1-dsR proteins at different time points to track the time-dependent increase/decrease of surface KCNQ1 and KCNE1 proteins.



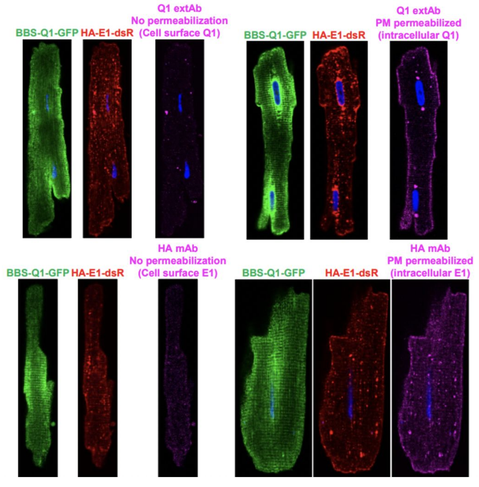
The figure above shows the Western blot results of the biotinylation experiment. The left shows the whole cell lysate while the right shows the biotinylated fraction. A time dependent increase of proteins is evident between times 45-90 minutes. Alpha-actin was used as a control. This helped us determine that the biotin had labelled cytosolic proteins, thus rendering the results inconclusive.



The chart on the left shows a quantification of protein on the cell surface. It shows us the results from the Western blot above but in a quantitative manner, leading to the same conclusion.

Results (4) - Myocytes

The purpose of this experiment was to visualize the distribution pattern of BBS-Q1-GFP and HA-E1-dsR in adenovirus-mediated cardiomyocytes.



The myocytes above were imaged using a Zeiss 710 confocal microscope. They show the distribution patterns of BBS-Q1-GFP and HA-E1-dsR. These results confirm that within the cell, KCNQ1 and KCNE1 are segregated yet on the cell surface, the proteins are colocalized to form ion channels. Some of the limitations in this experiment included low protein expression and complications in the functioning of the Q1 external antibody which labels cell surface KCNQ1.

Methods

Four different types of experiments were performed:

1) The RUSH (retention using selective hooks) construct was used to keep proteins in the ER following translation until the addition of biotin, which allowed release of the target proteins from their respective ER hooks. The proteins (Q1-GFP and HA-E1-dsR) then trafficked to the cell surface. The COS-7 cells were then fixed at different time points, visualized using a Zeiss 710 confocal microscope, then quantified using ImageJ to determine protein expression in the cells.
2) The RUSH construct was once again used, this time with COS-7 and HEK-293. Cells were not fixed this time; live cells were visualized using a spinning disc microscope to analyze the trafficking routes of KCNQ1 and KCNE1.
3) A biotinylation experiment was conducted to quantify cell surface Q1/E1 expression. In this experiment, cell surface proteins were first labelled, cells were lysed, then proteins were purified. A Western blot was then conducted followed by densitometry analysis.
4) In the final experiment, adenovirus-mediated expression of KCNQ1 and KCNE1 was studied in adult ventricular cardiomyocytes. BBS-Q1-GFP and HA-E1-dsR were expressed in the myocytes and visualized using a confocal microscope.

Conclusions

- KCNQ1 and KCNE1 traffic separately, as observed in both COS-7 cells and myocytes
- As both proteins are translated in the ER, over time they continue to traffic to the plasma membrane on different routes
- On the cell surface, both proteins show patterns of colocalization indicating the formation of ion channels

References

1) Boncompain, G., Divox, S., Gareil, N., Forges, H. D., Lescure, A., Latreche, L., . . . Perez, F. (2012). Synchronization of secretory protein traffic in populations of cells. Nature Methods, 9(5), 493-498. doi:10.1038/nmeth.1928
2) Jiang, M., Wang, Y., & Tseng, G. (2017). Adult Ventricular Myocytes Segregate KCNQ1 and KCNE1 to Keep the I Ks Amplitude in Check Until When Larger I Ks Is Needed. Circulation: Arrhythmia and Electrophysiology, 10(6). doi:10.1161/circep.117.005084