Regulation of the Sodium-Activated Potassium Channel Slick (KCNT2) by Magi-1

Allan Nip, Kerri Pryce, Arin Bhattacharjee

Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, SUNY at Buffalo, Buffalo, NY, 14214, USA

University at Buffalo
The State University of New York

ABSTRACT

The sodium-activated potassium (K_{Na}) channels called Slack and Slick, share 74% amino acid sequence homology, with the last 8 amino acids being identical. This region of the channels is predicted to be a Post-synaptic density-95/**D**iscs large/**Z**onulaoccludens-1 PDZ binding domain and therefore predicted to interact with PDZ containing scaffold proteins. Scaffold proteins stabilize and determine the localization of membrane proteins to various sub-cellular locales. In this study we have identified the PDZ protein Magi-1, as a binding partner that is essential for plasma membrane expression for both Slick and Slack. Using co-immunolocalization and immunoblotting assays, we demonstrated that Magi-1 localized with Slack and Slick in heterologous expression systems. Next using voltage-clamp patch-clamp recordings we demonstrated that Magi-1 co-expression with Slick increases the Slick outward current density. In summary, our preliminary findings suggest that the Slack and Slick binding to the Magi-1 scaffold protein stabilizes their expression at the plasma membrane.

INTRODUCTION

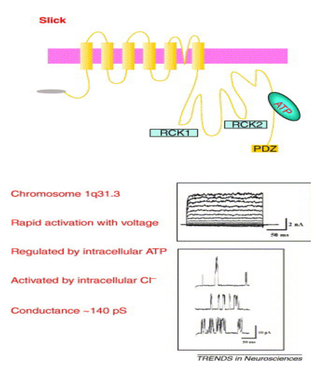
Slick Channel

- Potassium channels are essential for regulating neuronal firing patterns
- The large conductance K_{Na} potassium channel family consist of two members, namely Slick and Slack.
- The two genes is about 74% identical, Slick diverges from Slack with faster gating kinetics, a higher chloride sensitivity, and is inhibited by low intracellular ATP binding.
- Slick is selectively expressed in the nervous system and heart

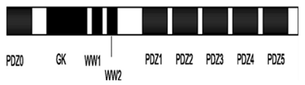
Magi-1

- Magi-1 (Membrane associated Guanylate kinase with inverted orientation 1) is a unique member of the MAGUK family of PDZ domain containing proteins.
- Magi-1 has three unique structural features: the GuK domain at the NH_2 terminus rather than the COOH terminus, the SH3 domain is replaced by two WW domains, the six PDZ domains.
- Magi-1 is known to assembly ion channel and signaling molecules at the cell membrane.

One of the ongoing goals in the lab is to understand the mechanisms that determines the trafficking and localization of Slick and Slack. Here we demonstrated that Magi-1, a member scaffold protein, co- expression with Slick results in an increase Slick outward current density and subsequently Slick protein expression in an Heterologous expression system.

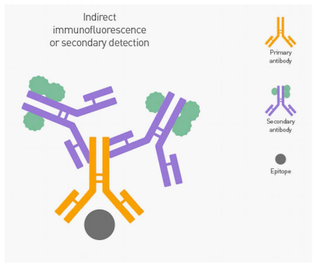


Slick

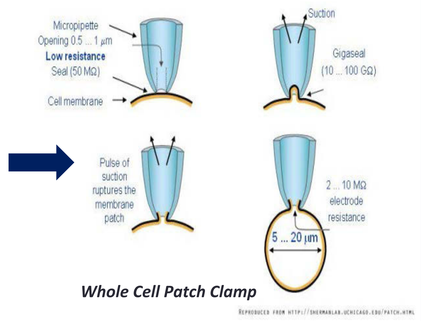


Magi-1

METHODS



Immunolabeling



Whole Cell Patch Clamp

RESULTS

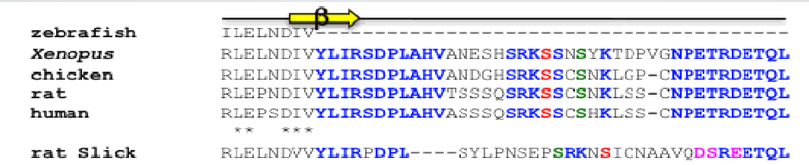


Figure 1: The last four amino acids of Slick and Slack are evolutionary conserved. These conserved four amino acids (ETQL) represent a Type-1 PDZ motif. PDZ domain containing are known to bind these motifs and anchor ion channels at the plasma membrane .

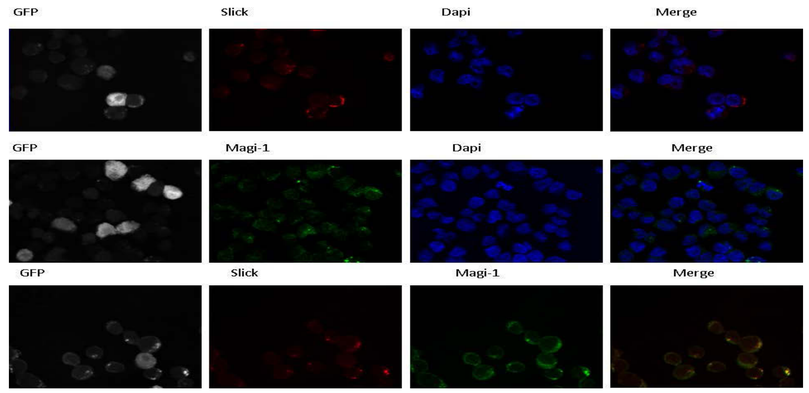


Figure 2. Magi-1 localize with Slick in a Heterologous expression system. CHO cells were transiently transfected with plasmid constructs of Magi-1, Slick or both Slick and Magi-1. Cells were allowed to grow for 48-72 hours to facilitate protein expression. Transfected cells were then washed three times with PBS and fixed with 4%PFA for 10 minutes. Cells were then permeabilzed by incubation in PBS with 0.02% triton X. Cells were incubated for 1hour with primary antibody against Magi-1 (rabbit polyclonal anti-Magi-1) or Slick (Mouse monoclonal anti-Slick). This was followed by secondary antibody incubation (Alexa Flour 546 and 633 respectively).

Magi-1 increase Slick Current Density in Transiently Transfected CHO cells

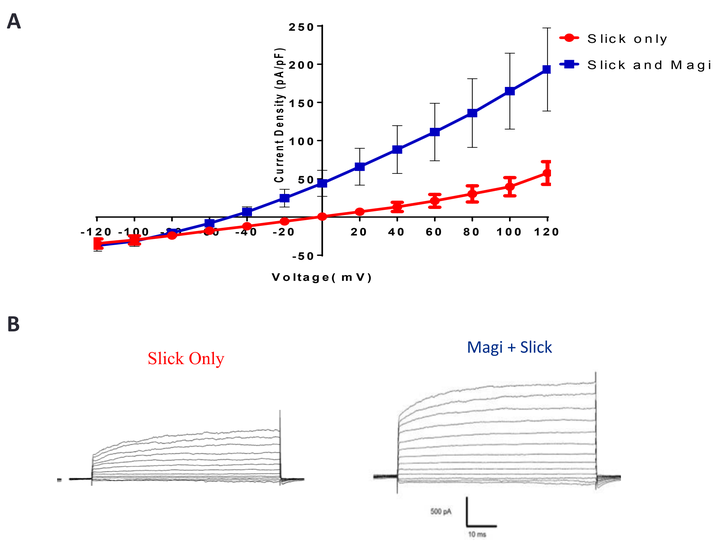


Figure 2. Magi-1 increase Slick current Density in CHO cells . A. Co-expression of Slick with Magi-1 resulted in an increase in Slick outward current density. Representative traces recorded from CHO cells co-transfected with Magi-1 and WT Slick (n=15) or Slick only (n=20). Slick current was recorded using the whole cell patch clamp technique. Voltage steps were applied in increments of 20mV to CHO cells in external bath solution containing 140mM NaCl, 10mM $CaCl_2$, 3mM KCl, 29mM Glucose, 25mM Hepes (pH 7.4). The pipette solution contained 32.5mM KCl, 97.5mM potassium gluconate, 5mM EGTA and 10mM Hepes (pH 7.2).

RESULTS

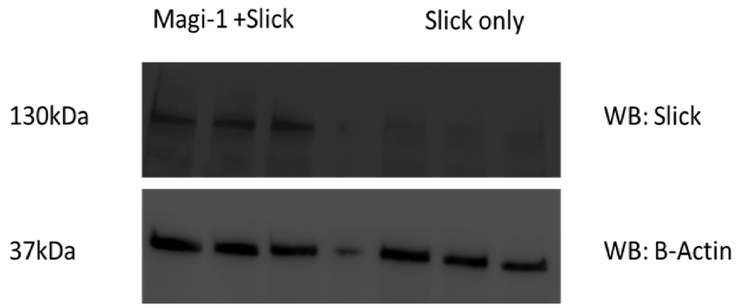
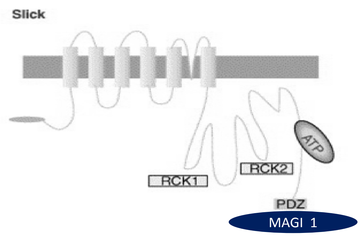


Figure 3. Magi-1 increase Slick Channel expression in CHO cells. Briefly, CHO cells were transiently transfected with either Magi-1 + Slick or Slick + PcDNA. Western blot analysis was conducted using a mouse monoclonal Slick antibody. Results demonstrated an increase in Slick protein expression with co-expression of Magi -1.

SUMMARY

- Magi-1 and Slick show similar subcellular localization in transiently transfected CHO cells.
- Magi-1 stabilizes Slick outward current density in CHO cells
- Co-expression of Magi-1 with Slick increases Slick Protein expression. Results suggest that Magi-1 may be protecting Slick channel from degradation



Proposed Model for Slack binding by Magi 1

FUTURE GOALS

- Identify if there is physical interaction between Magi-1 and Slick.
- Verify if Magi-1 interacts and localize with Slick *in vivo.*
- Characterize the expression of Magi-1 and Slick in different populations of DRG neurons
- Verify why co-expression of Magi-1 increase slick protein. Verify if Magi-1 prevents Slick channel from degradation.

REFERENCES

1. Nuwer M et al. J Neuroscience. 2010; 30(42)14165-72
2. Dobrosotskaya I,. J. Biol. Chem. 1998 **272** (50): 31589–97
3. Ridgway LD et.al American journal of physiology Cell physiology **297**:C55-65
4. Tomasello DL et al J Biol Chem. 2015 Jul 24;290(30):18575-83
5. Kim GE, Cell Neurosci. 2014 Jul 28;8:209. doi: 10.3389/fncel.2014.00209
6. Bhattacharjee A, J Neurosci. 2003 Dec 17;23(37):11681-91.