Primary neuronal cultures from rodent brain for live-cell imaging of protein trafficking (and localization).

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Visualization of protein trafficking using fluorescent fusion proteins

- Classic work from the Lippincott-Schwartz lab
  - A temperature sensitive protein which is trapped in the ER
  - Release of trapped protein reveals trafficking dynamics
Ion channel trafficking and localization

- Tamkun Lab
  - Kv2.1
  - Nav1.6
  - Trafficking vs localization?
Visualizing ion channel localization and trafficking

- **Fluorescent Protein Tags**
  - Traditional
  - Photo-activatable/convertible
  - Reporters

- **Epitope/Biotin tags**
  - Flag, HA, etc.
  - Biotin Acceptor Domain (BAD)/Biotin

Diagram of Kv2.1 α-subunit

- HA or FLAG
- Biotin acceptor peptide
- GFP
Kv2.1 localizes to cell-surface clusters in transfected HEK cells and primary hippocampal neurons.

Kv2.1 with the cluster is mobile as demonstrated by FRAP.

Non-clustered Kv2.1 readily diffuses across the cluster perimeter

D = 0.09 µm²/second
Finding vesicular traffic

- Mature protein accumulation often obscures vesicular traffic
- Photobleaching can uncover the vesicle
Direct vesicular trafficking of Kv2.1 to Kv2.1 clusters
Investigating ion channel trafficking with an extracellular biotin tag

StreptAvadin-Qdot605
GFP-Kv2.1-LoopBAD

Kv2.1 channels are delivered to the perimeter of Kv2.1 clusters in hippocampal neurons

Recycling Kv2.1 channels traffic at Kv2.1 surface clusters in neurons

Summary of the insertion site experiments

Endocytosis of Kv2.1 also occurs at the cluster perimeter.
Investigating Transferrin Receptor trafficking with a pH sensitive fluorescent protein

Hippocampal neuron

- TfR exocytosis preferentially occurs at the perimeter of Kv2.1 clusters
  - 66±11% at Kv2.1 clusters
Kv2.1 clusters are shaped by the cortical cytoskeleton

- Kv2.1 clusters form in areas depleted of cortical actin
- Kv2.1 clusters are frequently bounded by microtubules
Kv2.1-induced remodeling of the cER

A

B

DsRed-ER
GFP-Kv2.1

10µm

C Endogenous cER in TIRF

D Kv2.1-remodeled cER

5 µm

cER structure in cultured rat hippocampal neurons

- cER is tubular in young neurons
- GFP-Kv2.1 expression drastically remodels cER
- Kv2.1 clusters don’t form until after day in vitro 7
Tranferrin-receptor (TfR) fusion near the cER

- HEK cells
  - 82% adjacent to the cER
  - cER = 28% of PM

- In neurons
  - 70% adjacent to cER
  - cER = 34% of PM
Clathrin-mediated Endocytosis at cER perimeter

- TfR localizes to puncta, which are clathrin coated pits (CCPs)
- In HEK cells:
  - 88.5% adjacent to cER
  - cER = 29% of the PM
- In Neurons:
  - 83% adjacent to cER
  - cER = 32% of the PM
Summary of Kv2.1 trafficking

- Kv2.1 clusters are cell-surface trafficking hubs for membrane proteins
- Kv2.1 clusters are formed due to an interaction with the cortical ER
Imaging local protein synthesis in neurites

- Nascent Chain Tracking
  - Uses MS2 stem-loops in the 3’UTR to label mRNA
  - 10x epitope tags on the N-terminus allow for near instantaneous visualization of nascent chains
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Photo-convertable fluorophores for visualizing AMPA receptor trafficking