Steering kinesin: Getting a grip on GRIP1

Neville Sanjana
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Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites

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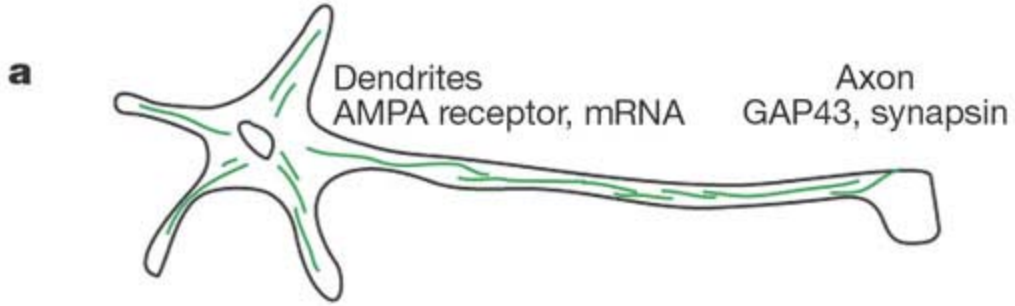
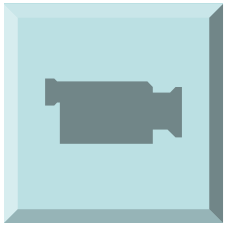
Some motivating questions

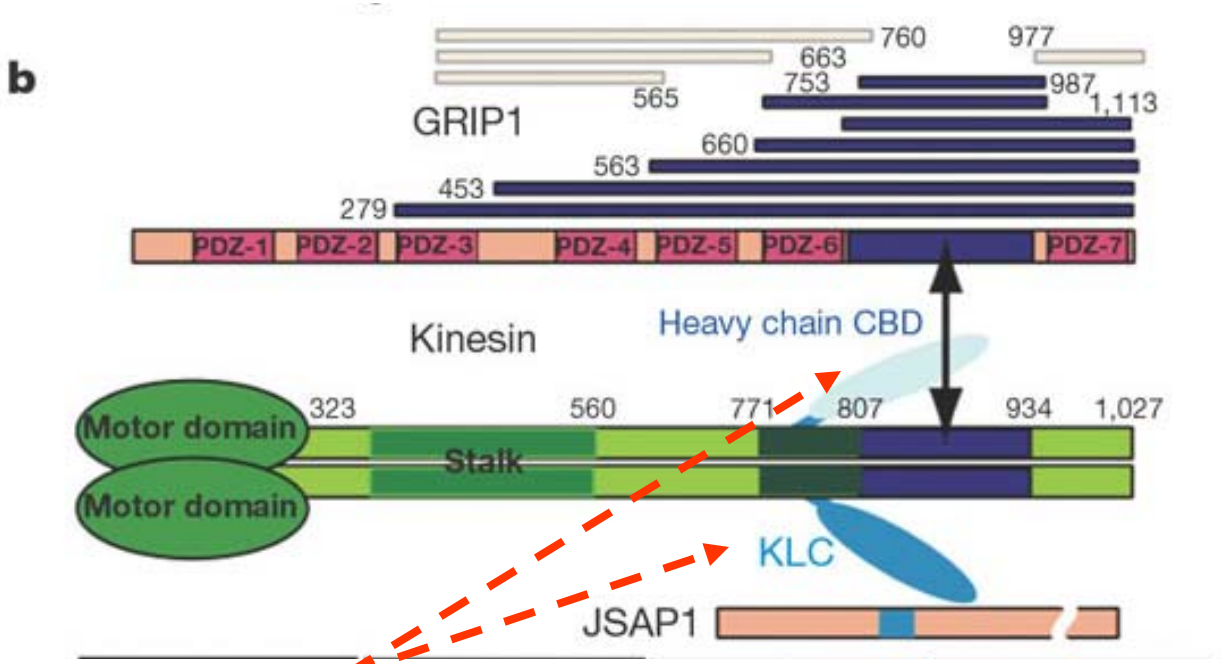
- How are proteins localized to appropriate neuronal domains?
- How can the *same* motor protein transport other proteins to functionally distinct domains?

One model system where we might look for an answer: kinesin





Kinesin movie (Vale Lab, UCSF)



Binding domains: 2 heavy chains, 2 light chains

What is GRIP?

- GRIP = AMPA subunit GluR-interacting protein

- In this study, we're looking only at GRIP1.

What does GRIP do?

- Let's ask Morgan what it does:

"The mechanisms by which glutamate receptors are concentrated in brain excitatory synapses are believed to involve interactions between receptor subunits and postsynaptic anchoring or scaffolding proteins. Recently **GRIP, a protein containing seven PDZ domains**, was identified as an AMPA receptor binding protein and **implicated in the synaptic targeting of AMPA receptors**. Here we show that GRIP mRNA is also expressed in some tissues outside of the brain, including testis and kidney. Specific antibodies were raised to study GRIP protein. On Western blots, GRIP protein appears as a heterogeneous band (**approximately 130 kilodaltons**) which is expressed in widespread brain regions and throughout postnatal development. Biochemical studies reveal that GRIP is largely membrane associated and enriched in the postsynaptic density (PSD), though not as highly concentrated in the PSD as is PSD-95. By immunohistochemistry, **GRIP is distributed in a somatodendritic pattern in neurons of adult rat brain, with especially prominent expression in a subset of interneurons**."

(Wyszynski M, Kim E, Yang FC, Sheng M 1998)

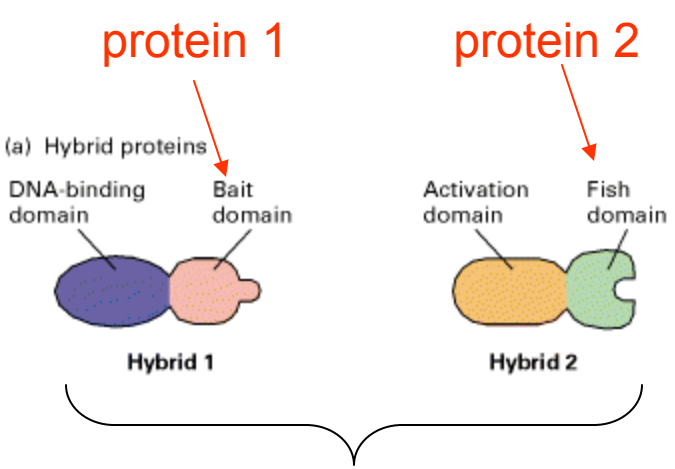
- So why is GRIP important if we want to study molecular transport and kinesin?

- GRIP is the strongest binding partner of the kinesin heavy chain, as identified by a yeast two-hybrid screen.

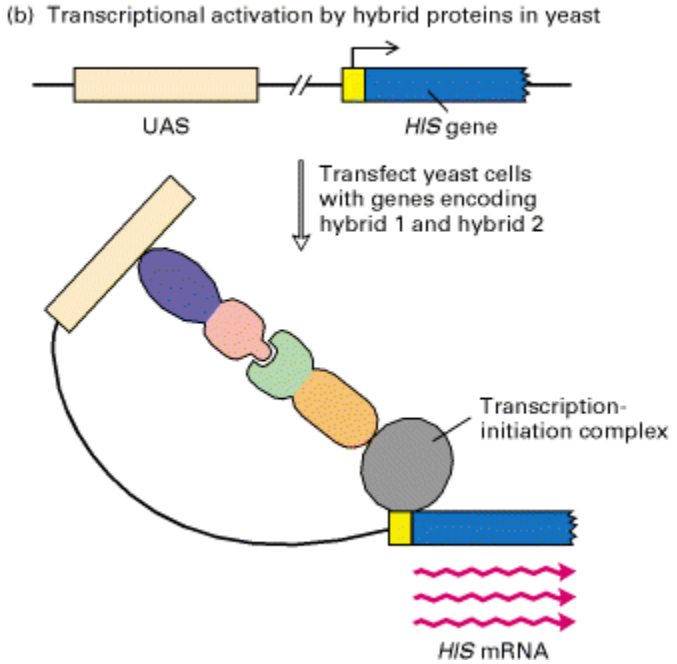
- Everyone knows what a yeast two-hybrid screen is, right?

Well, I didn't

- Goal: Detect if two proteins interact.
- Procedure: Use their interaction/binding in yeast to drive a promoter for a reporter gene.

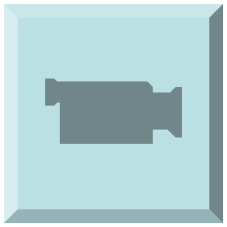


Introduced by transfecting yeast with appropriate cDNA plasmids



Lodish et al. *Molecular Cell Biology* 2000

Yeast two-hybrid



Campbell and Heyer,
Genomics, Proteomics, and Bioinformatics
2002

- Back to our experiment: GRIP1 “fish” produced the highest reporter expression given KIF5, the kinesin heavy binding domain “bait.”

Finding the binding specificity

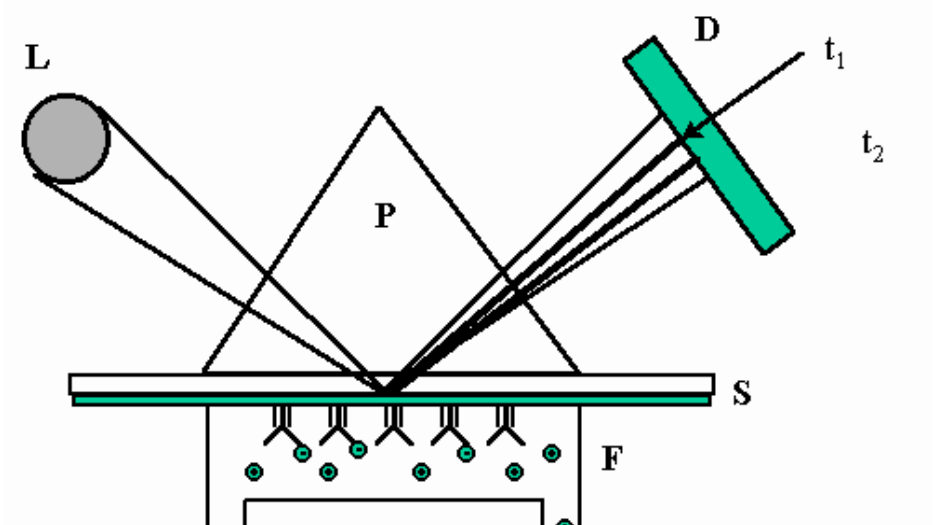
- What's the relevant quantity here?

Binding constant:

$$K_d = \frac{[\text{unbound}]}{[\text{bound}]}$$

How can we measure the K_d?

- One way: Surface plasmon resonance



http://www.astbury.leeds.ac.uk/Facil/spr.htm

Ligand binding to immobilized receptor

Refractive index change in thin metal film

- High K_d (~ nM) matches previous reports of kinesin heavy chain (KHC) binding.

- Conclusion: It is likely that GRIP1 binds directly to KHC.

It binds but does it get transported?

- Question: If we knock-out kinesin, does GRIP1 still get transported to the right places?

- Techniques: Genetic knock-outs from gene targeting and immunostaining for GRIP1.

Kinesin null mutants

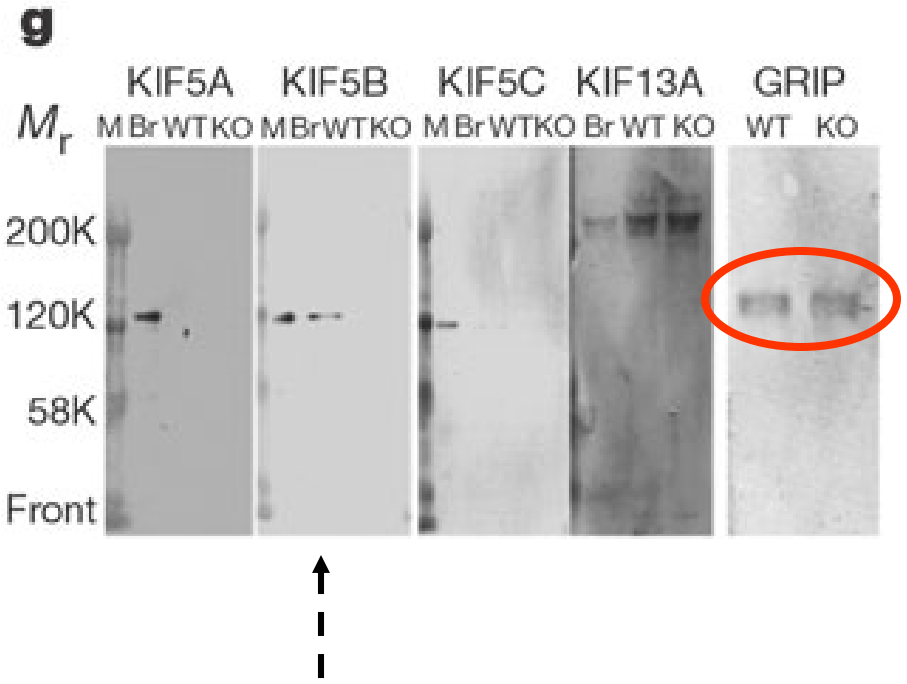
- Extra-embryonic cells from “kinesin-null” phenotype mice (actual genotype: kinesin heavy chain knockout KIF5b mice)

- Translation: Mice had (in essence) no functional kinesin for binding GRIP. This is because one of the heavy chain binding domain *isoforms* (KIF5b) has been deleted.

- Do you see any problems here? (I see two!)

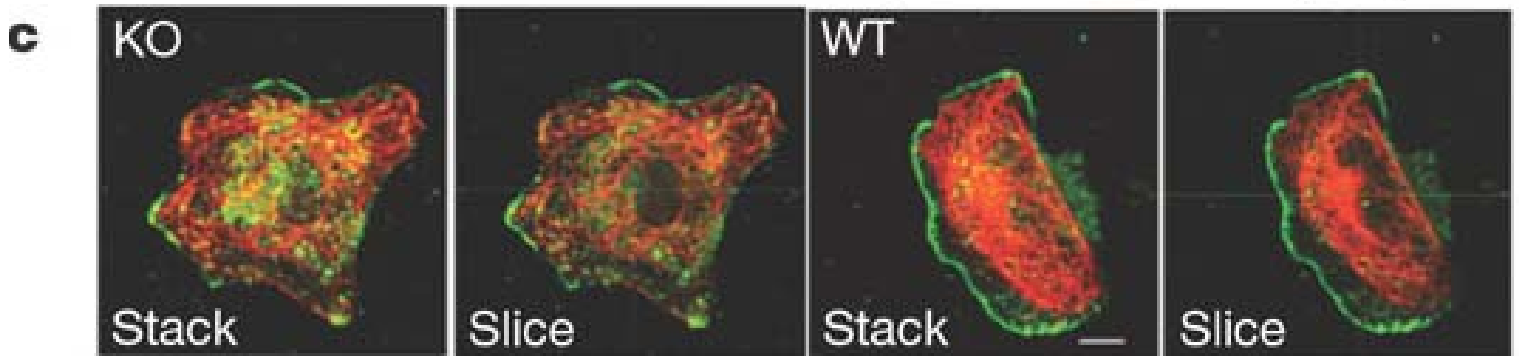
Control: GRIP expression

- GRIP expression is the same in WT and KO



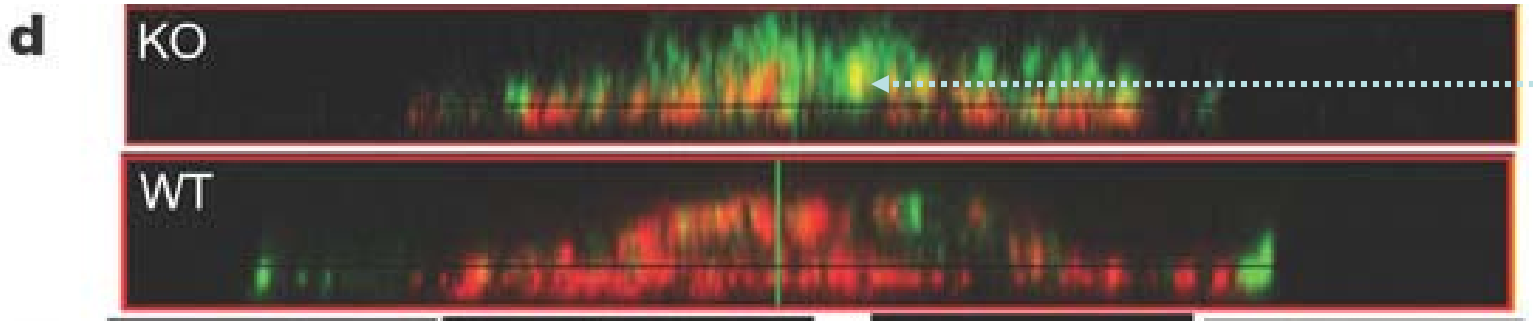
M Marker
Br Brain
WT Wild-type
KO Knock-out

KIF5b KO has strange GRIP1 localization



stack = confocal *z*-stack overlay
slice = one *x-y* image from the stack

GRIP1
Microtubules

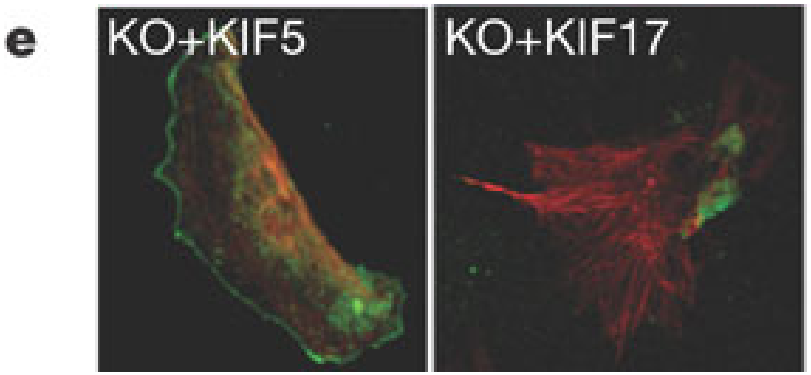


More GRIP at center of soma in KO

Slice in the *x-z* plane

How specific is the KO?

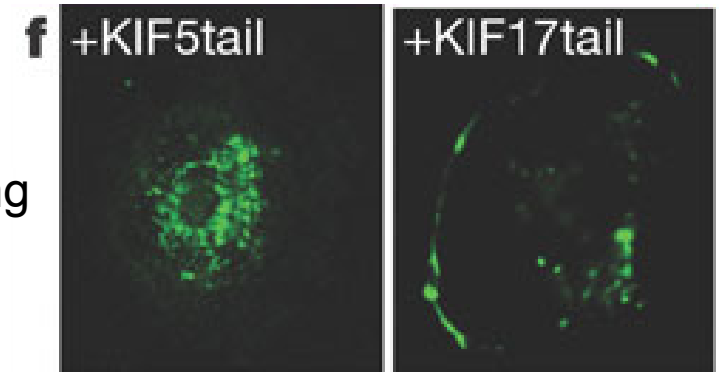
- Rescue with KIF5b but not with KIF17



More abnormal clustering

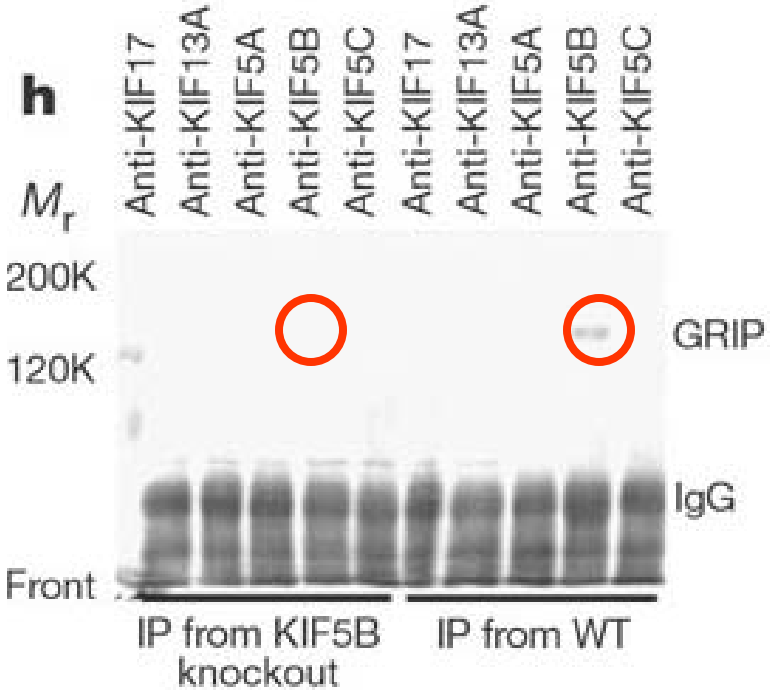
- Express KIF5b & KIF17 binding domain exogenously (i.e. independently from kinesin) in wild-type mice

abnormal
GRIP clustering



normal
GRIP clustering

- KIF5b immunoprecipitates GRIP in WT but not in KO mice

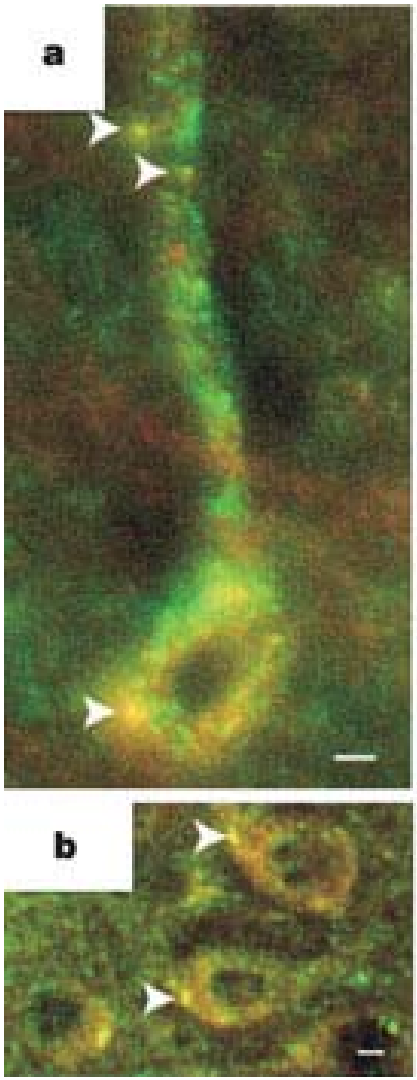


Exactly what we expect to see, since we KO'd KIF5B!

- Now that we know GRIP and kinesin are associated, what is the function of that complex *in vivo*?

- Hypothesis: GRIP with GluR2 attached is binding to kinesin.

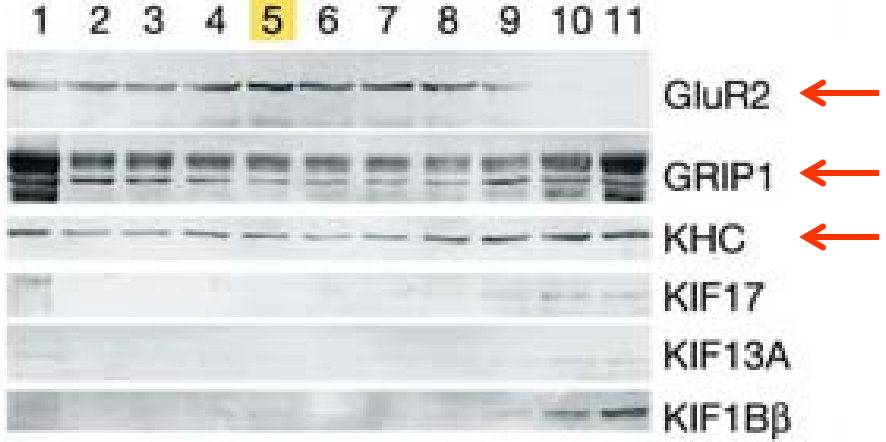
GRIP & Kinesin co-localize in somatodendritic compartments



Kinesin (KIF5a)
GRIP
Co-localization

- Antibodies for KIF5b also immunoprecipiate GRIP

- IP fractions enriched in GluR2 and GRIP also include kinesin's KHC binding domain



- Even if GRIP-GluR2 binds with kinesin, we cannot be certain that GluR2 also comes along *for the ride* (versus associating with the complex after transport).

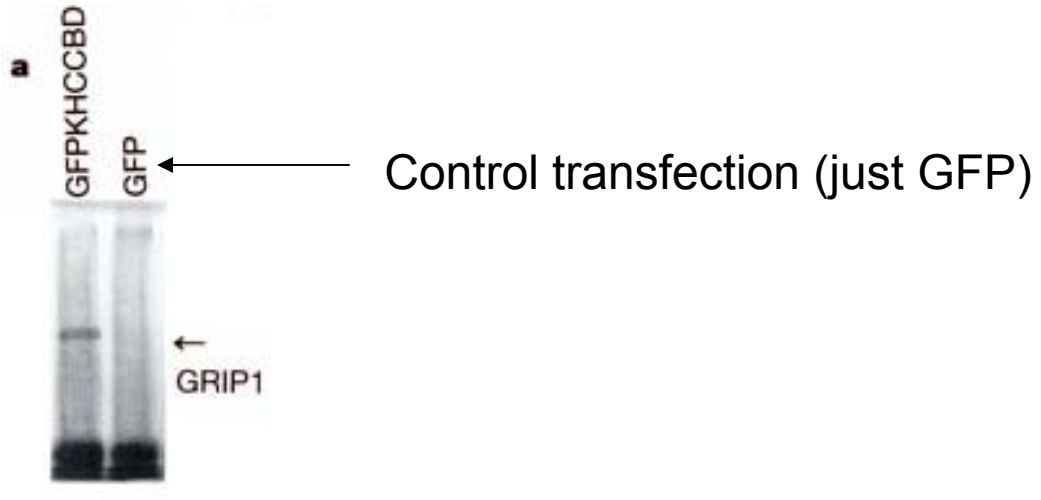
- Technique: Use a KHC dominant negative construct and immunostaining to ascertain GluR2 transport by kinesin.

Dominant negative mutant

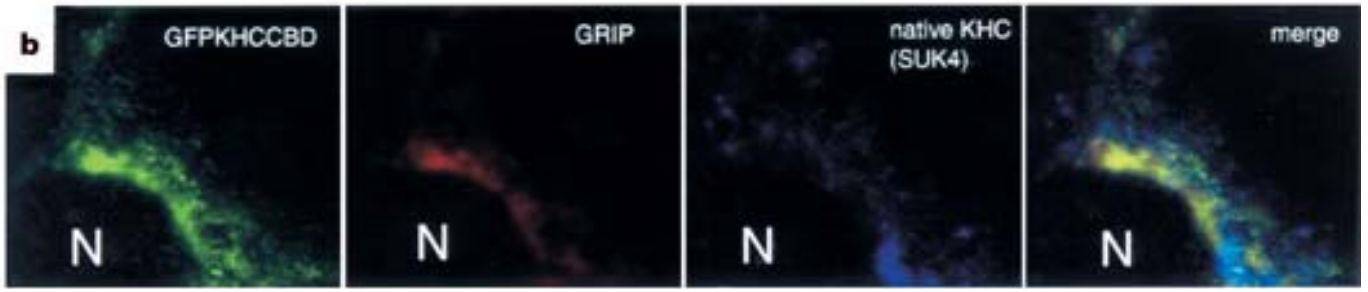
- Mutation of one allele that causes loss of function of wild-type protein encoded by other allele.

- In our case, the kinesin light chain (ie. *not* the one that binds GRIP) is mutated to make the KIF5 DN mutant.

- These DN mutants have GFP tagged to them.



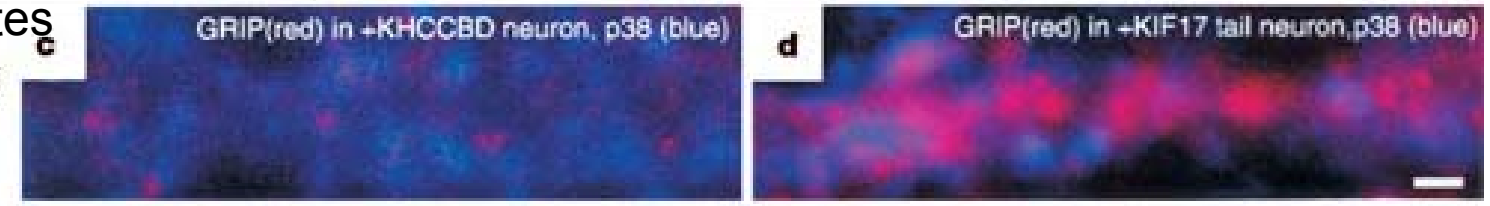
- GRIP and DN KIF5 mutant co-localize.



Where do the co-localize? Why?

DN KIF5 mutant has reduced GRIP in distal dendrites and at synapses

Dendrites

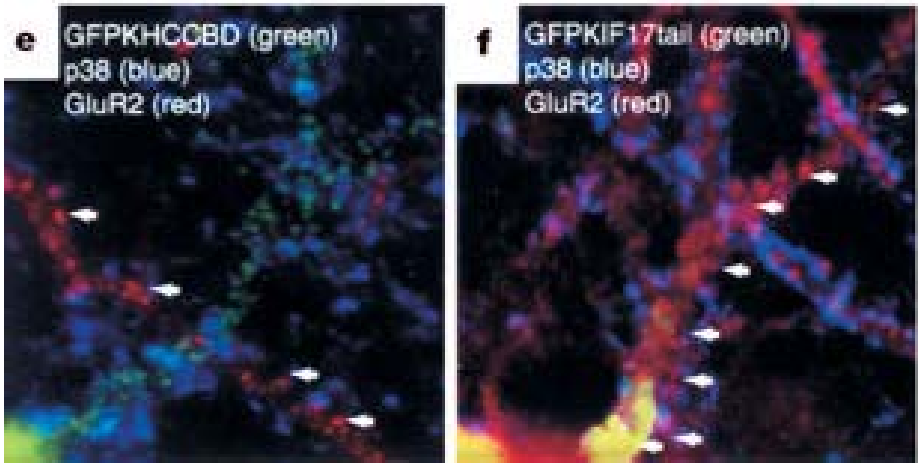


DN KIF5 mutant

DN KIF17 mutant

GRIP
synaptophysin

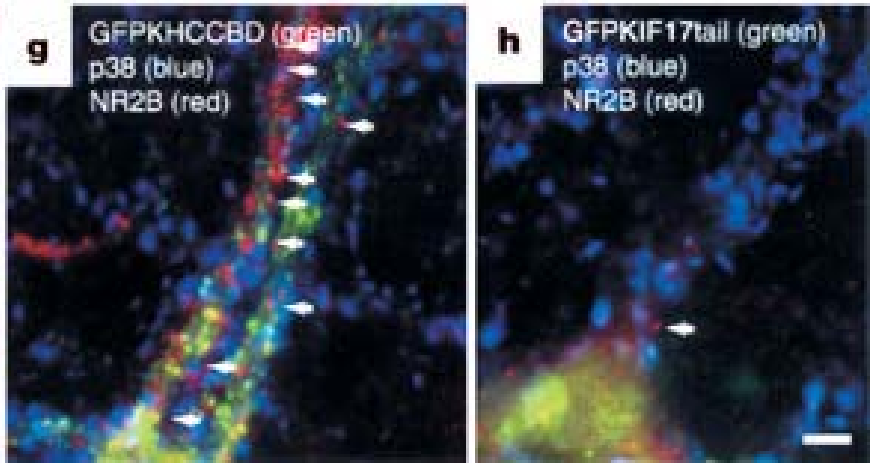
Synapses



Kinesin mutant
GluR2
synaptophysin

KIF5 DN mutants have normal NR2B

- Recall that NR2B is a subunit of the NMDA receptor



KIF5 DN Mutant
Normal NR2B

KIF17 DN Mutant
Abnormal NR2B

Kinesin mutant
NR2B
synaptophysin

- GluR2 but not NR2B is a passenger on the kinesin-GRIP1 transport.

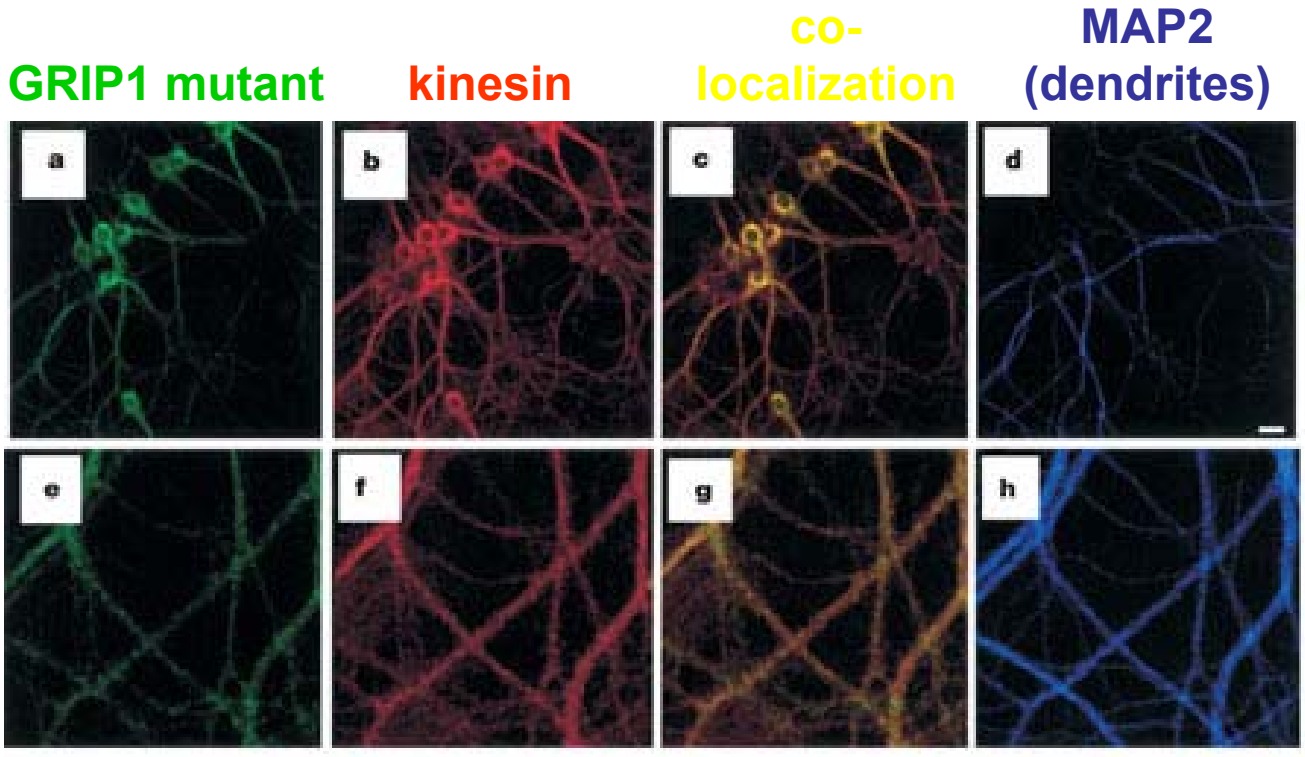
- So, GRIP-GluR2 is transported by KIF5-kinesin. But, if kinesin is also the major motor for molecular transport in *axons*, why does GRIP-GluR2 end up only in the *somatodendritic* compartments?

- Technique: Make *minimal* mutants of proteins that bind kinesin and assay where kinesin ends up.

- Why is it important to make *minimal* mutants?

More triple immunostaining

- GRIP1 mutant with only *minimal KHC binding domain*. Cannot bind GluR2, multimerize, or bind usual regulators like Ras.

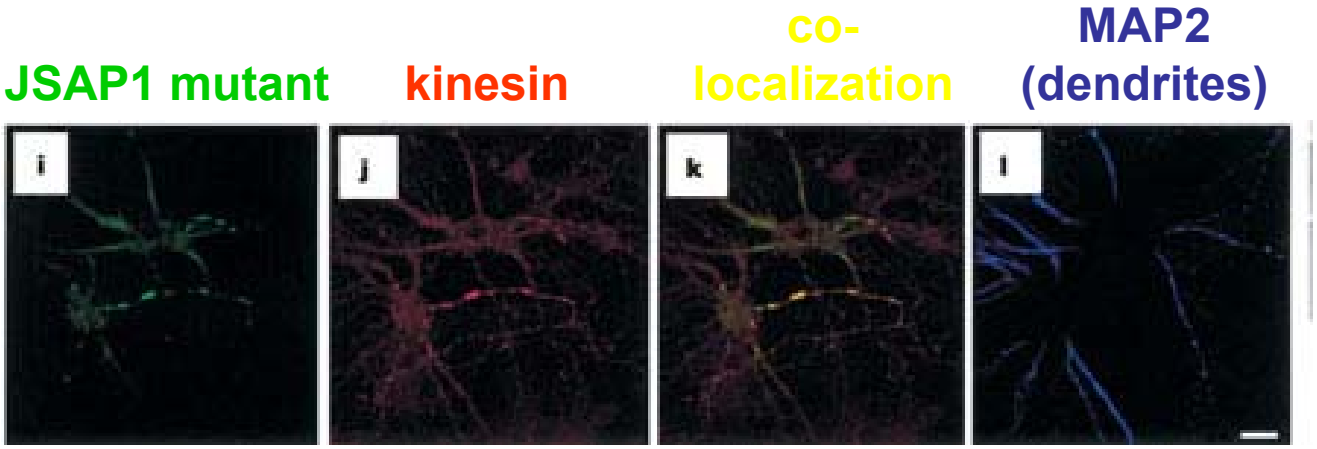


Magnified View

Over-expressed GRIP1 mutant with kinesin is found in soma and dendrites.

More triple immunostaining

- JSAP1 mutant with only *minimal KLC binding domain*.



Over-expressed JSAP1 mutant with kinesin is found in soma and *axons*. Not in dendrites.

Conclusions

- Kinesins may be “smart motors” that can recognize the difference between axonal (via KLC) and dendritic (via KHC) cargoes.
- Most previous work seems to follow the pattern:

	KHC	KLC
Axonal		JSAP APP
Dendritic	kinectin MyoVA	