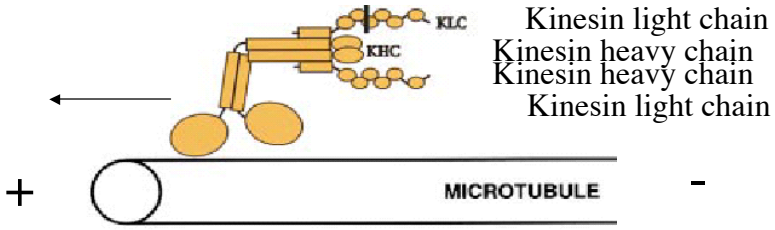
Cargo of Kinesin Identified as JIP Scaffolding Proteins and Associated Signaling Molecules

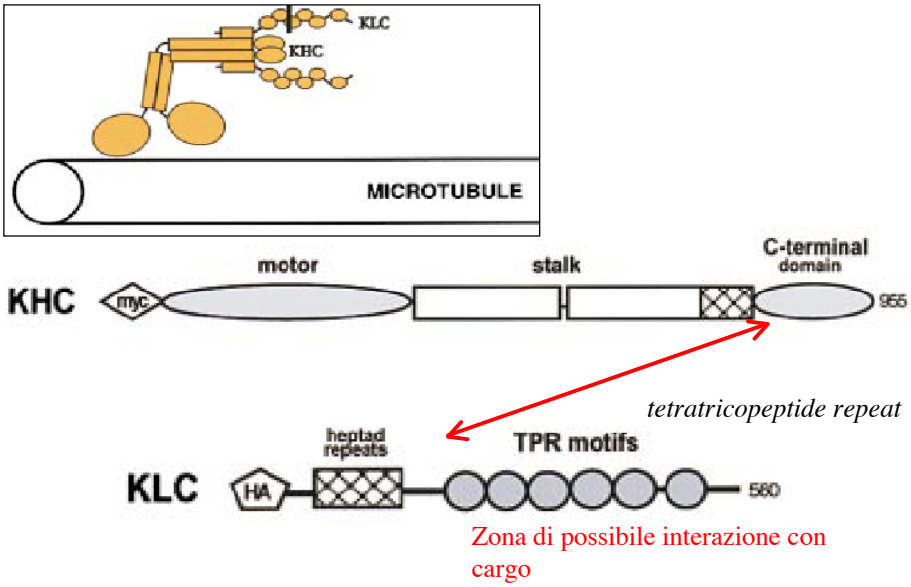
Kristen J. Verhey,* Debra Meyer,§ Reneé Deehan,* John Blenis,‡ Bruce J. Schnapp,‡ Tom A. Rapoport,* and Ben Margolis§

*Howard Hughes Medical Institute and ‡Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115; and §Howard Hughes Medical Institute, Department of Internal Medicine, and Department of Biological Chemistry, University of Michigan Medical Center, Ann Arbor, Michigan 48109

The Journal of Cell Biology, Volume 152, 2001 p.959-970

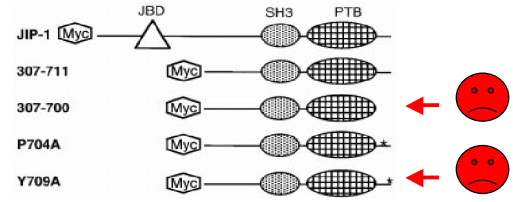


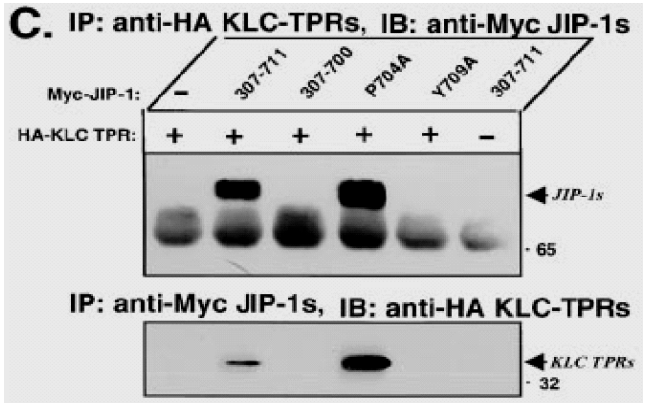
Kinesin light chain
Kinesin heavy chain
Kinesin heavy chain
Kinesin light chain

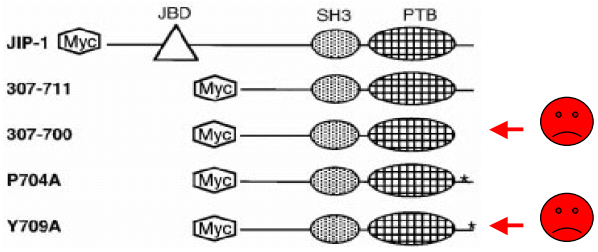


Zona di possibile interazione con cargo

JIP-1 COOH-term residues are required for binding to KLC TPR







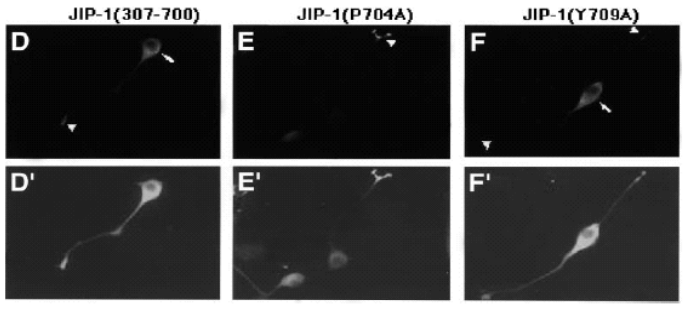
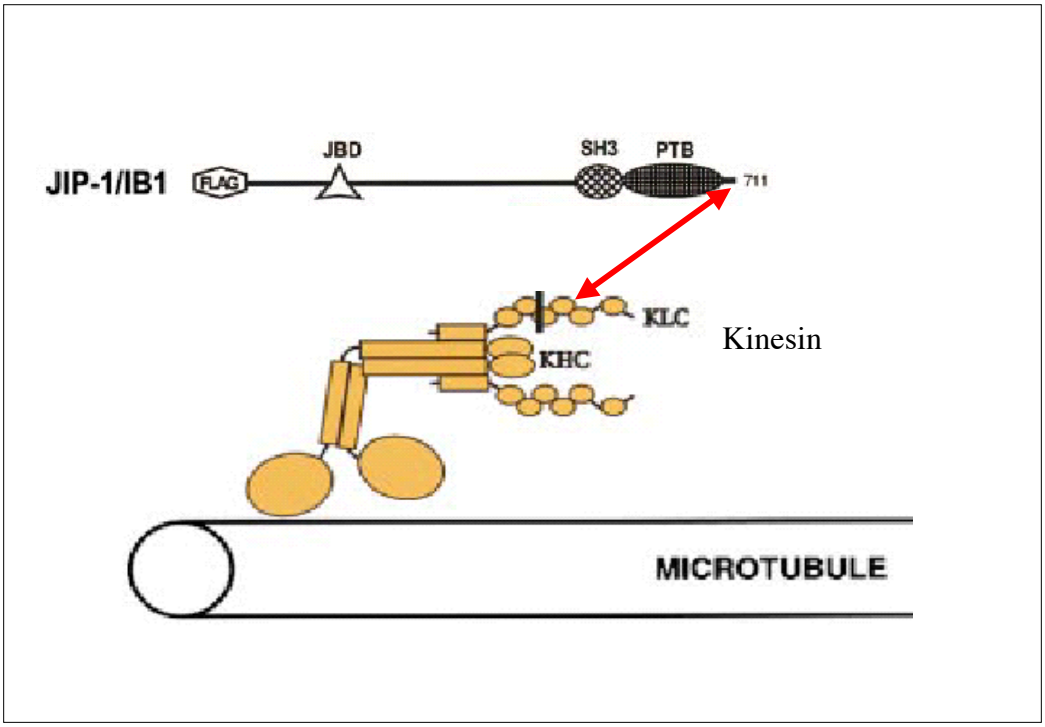
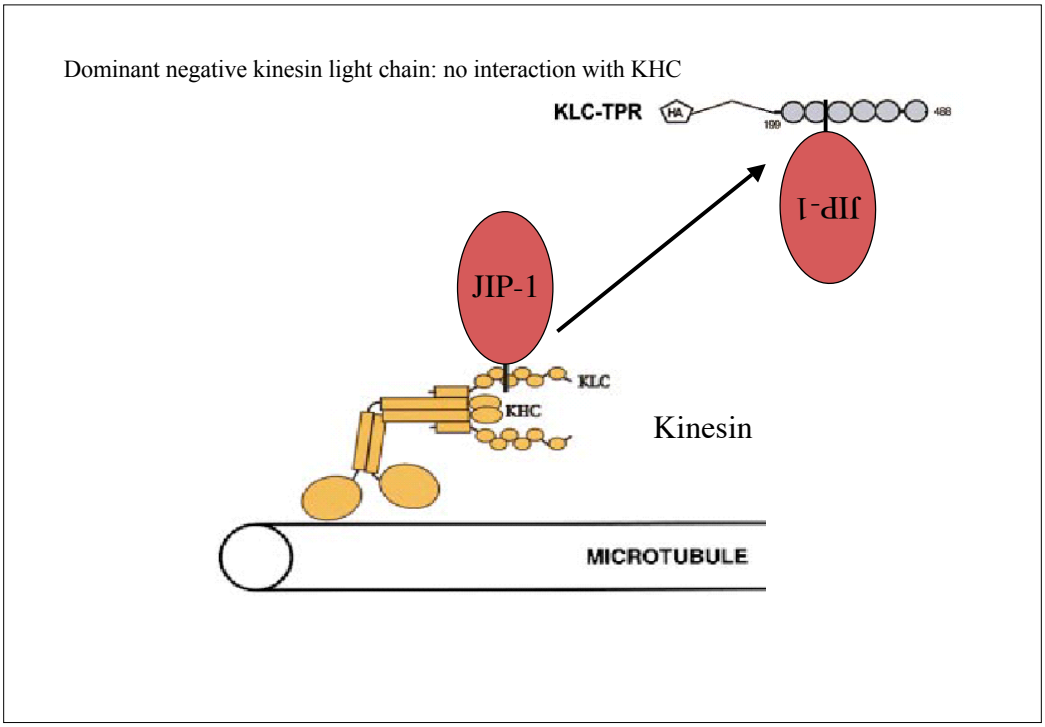


Figure 4. The COOH-terminal residues of JIP-1 are required for proper subcellular localization. NIE 115 cells were transiently transfected with the parental plasmid (control) or with plasmids encoding the indicated JIP-1 variants, differentiated, and the expressed proteins were detected by indirect immunofluorescence microscopy using an anti-Myc mAb. Nonspecific background staining is visible in the control cells and is enhanced in A′–F′ to aid in visualization of the cells. Myc–JIP-1 variants were scored as positive for correct cellular localization (JIP-1, JIP-1 [307–711], and JIP-1 [P704A]) if fluorescence was more pronounced at the neurite tips (arrowheads), whereas transfected proteins were considered negative for localization (JIP-1 [307–700] and JIP-1 [Y709A]) if fluorescence was observed to be more prominent in the cell body (arrows).


JIP-1/IB1
FLAG
JBD
SH3
PTB
711
KLC
KHC
Kinesin
MICROTUBULE


Dominant negative kinesin light chain: no interaction with KHC
KLC-TPR
HA
JIP-1
JIP-1
KLC
KHC
Kinesin
MICROTUBULE

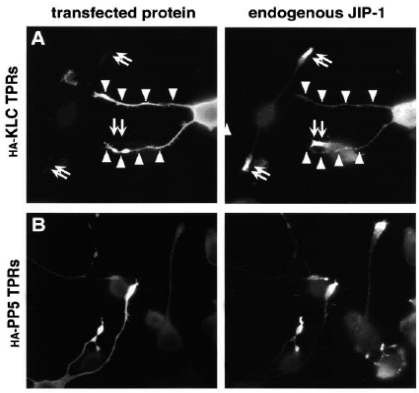
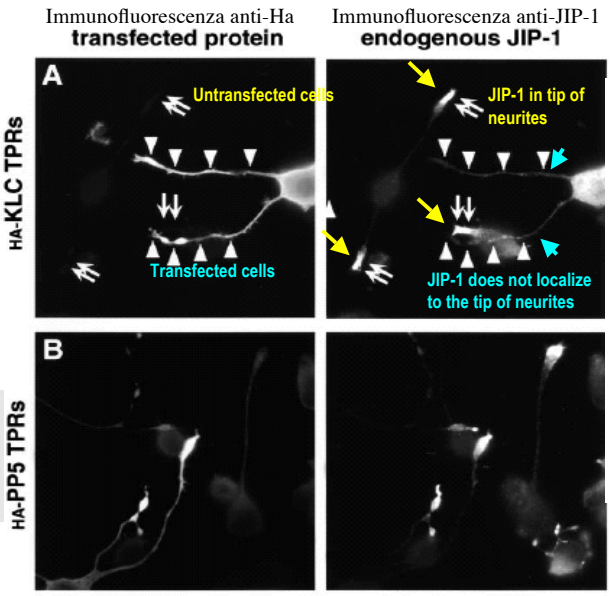


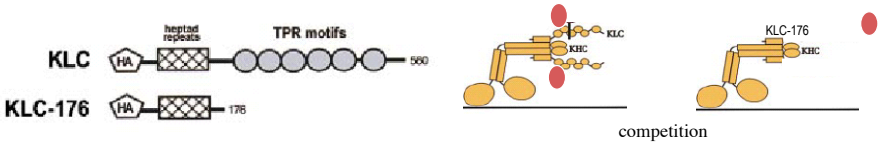
Figure 5. Expression of kinesin dominant negative constructs causes mislocalization of endogenous JIP-1 protein. CAD cells were transiently transfected with plasmids encoding the HA-tagged KLC TPRs (A), HA-tagged PP5 TPRs (B), HA-tagged KLC truncation KLC-176 (C), or Myc-tagged KHC truncation KHC-891 (D). After differentiation, the expressed proteins were detected by indirect immunofluorescence microscopy using mAbs to the epitope tags (left). Endogenous JIP-1 protein was detected with an affinity-purified polyclonal antibody (right). Note that the background fluorescence has been enhanced to show the entire neuronal cell. Arrowheads denote tips of transfected cells; arrows denote tips of untransfected cells.

Mislocalization of endogenous JIP-1



Negative control *TPR of PP5 does not recognize JIP-1*

Figure 5. Expression of kinesin dominant negative constructs causes mislocalization of endogenous JIP-1 protein. CAD cells were transiently transfected with plasmids encoding the HA-tagged KLC TPRs (A), HA-tagged PP5 TPRs (B), HA-tagged KLC truncation KLC-176 (C), or Myc-tagged KHC truncation KHC-891 (D). After differentiation, the expressed proteins were detected by indirect immunofluorescence microscopy using mAbs to the epitope tags (left). Endogenous JIP-1 protein was detected with an affinity-purified polyclonal antibody (right). Note that the background fluorescence has been enhanced to show the entire neuronal cell. Arrowheads denote tips of transfected cells; arrows denote tips of untransfected cells.



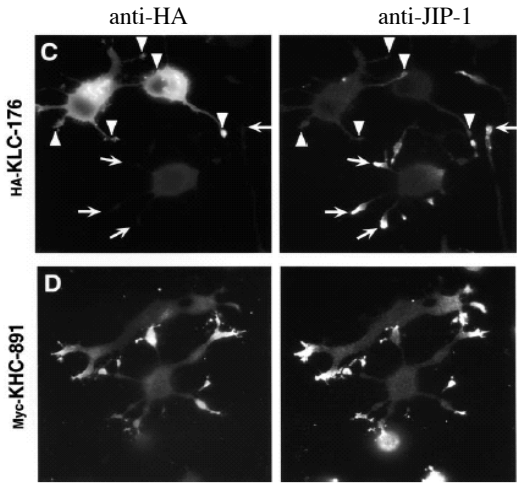
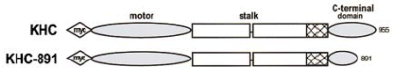
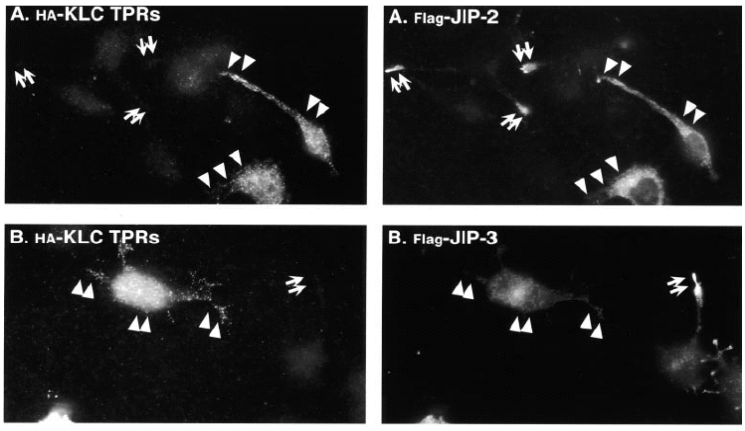
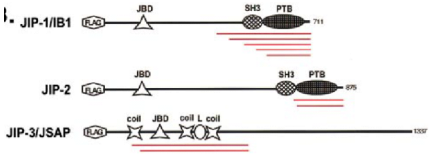


Figure 5. Expression of kinesin dominant negative constructs causes mislocalization of endogenous JIP-1 protein. CAD cells were transiently transfected with plasmids encoding the HA-tagged KLC TPRs (A), HA-tagged PP5 TPRs (B), HA-tagged KLC truncation KLC-176 (C), or Myc-tagged KHC truncation KHC-891 (D). After differentiation, the expressed proteins were detected by indirect immunofluorescence microscopy using mAbs to the epitope tags (left). Endogenous JIP-1 protein was detected with an affinity-purified polyclonal antibody (right). Note that the background fluorescence has been enhanced to show the entire neuronal cell. Arrowheads denote tips of transfected cells; arrows denote tips of untransfected cells.



Kinesin dominant negative induces mislocalization of JIP-2 and JIP-3 as well

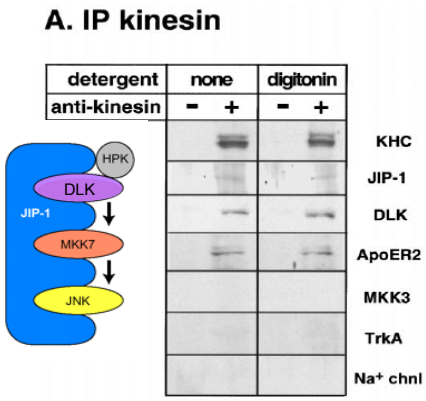




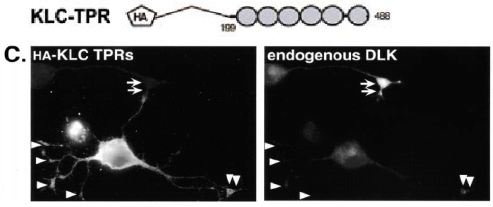
(A and B) CAD cells were transiently transfected with plasmids encoding the HA-tagged KLC TPRs together with Flagtagged JIP-2 (A) or Flagtagged JIP-3 (B). After differentiation, the expressed proteins were detected by indirect immunofluorescence microscopy using a mAb to the HA tag (left) and a polyclonal antibody to the Flag tag (right). Arrowheads denote tips of transfected cells; arrows denote tips of untransfected cells.

Kinesin associates with proteins assembled on the JIP scaffold

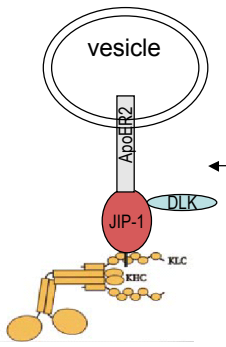
High speed supernatant fraction from rat brain used for IP with mAb to KHC



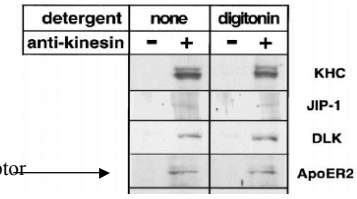
Kinesin dominant negative induces mislocalization of DLK



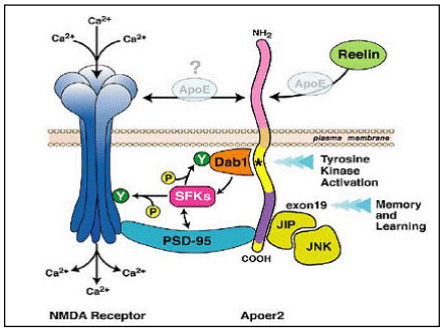
Kinesin associates with proteins assembled on the JIP scaffold and with ApoER2 (transmembrane receptor for Reelin), thus with vesicles.



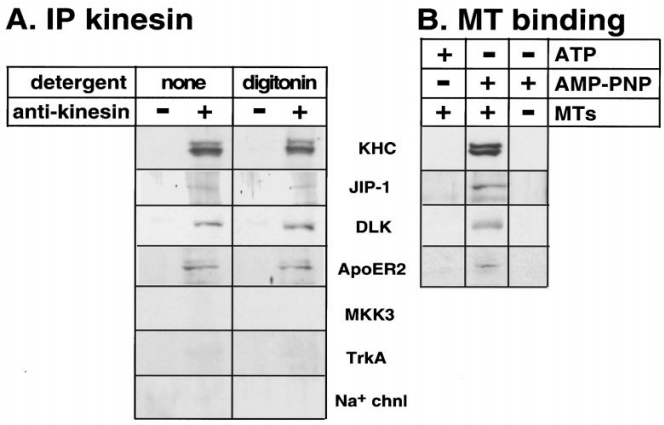
Co-IP of the transmembrane receptor for Reelin



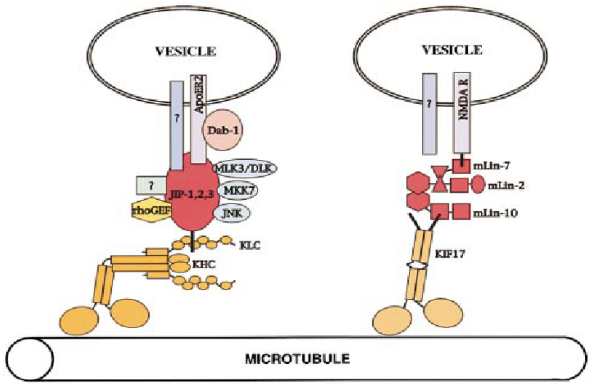
JIP1 is a linker between kinesin-1 and certain vesicle membrane proteins, such as Alzheimer's APP protein and a reelin receptor ApoER2



Kinesin associates with proteins assembled on the JIP scaffold and with microtubules



(B) Rat brain high speed supernatant was subjected to an MT binding assay in the presence of Triton X-100 by adding ATP, AMPPNP (non hydrolysable ATP), and/or MTs as indicated. MTs and bound proteins were sedimented through a sucrose cushion, and the presence of the indicated proteins in the MT pellets was detected by immunoblotting.

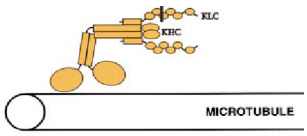


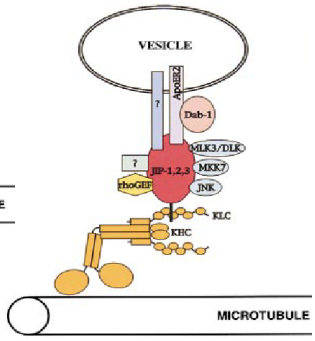
Together results suggest that JIP scaffolding complex, which include DLK, is preassembled before being transportated by kinesin, rather than formed by diffusion of its individual components at the site of action

Model for the transport of cargo by kinesins. JIP proteins form a scaffold, on which cytoplasmic as well as plasma membrane proteins are assembled. The entire complex is transported down an axonal process by conventional kinesin. Note that the JIP proteins are known to form homodimers and heterodimers, although only one polypeptide is drawn for clarity. Similarly, LIN-2, -7, and -10 form a scaffold, on which cytoplasmic and transmembrane proteins assemble. The entire complex is transported down a dendritic process by the kinesin superfamily member KIF17.

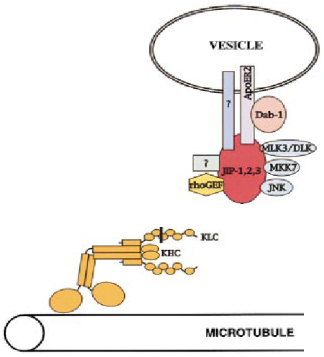
Signal Transduction Pathways May Regulate the Activity of Their Motors?

Without cargoes, kinesins are running along microtubules?





Co-operative or competitive transport of different cargoes?



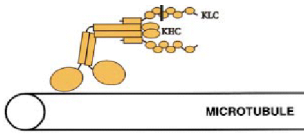
Signal for cargoes to detach?

Two binding partners cooperate to activate the molecular motor Kinesin-1

T. Lynne Blasius,[1] Dawen Cai,[1,2] Gloria T. Jih,[1] Christopher P. Toret,[3] and Kristen J. Verhey[1]

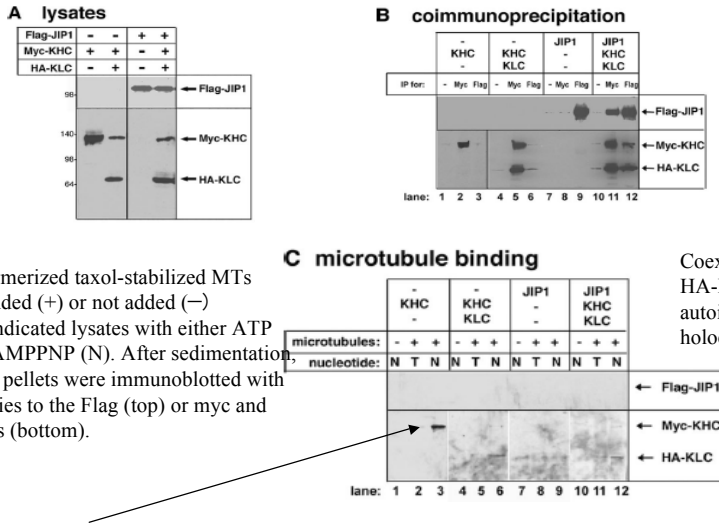
[1]Department of Cell Biology and [2]Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109
[3]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720

The Journal of Cell Biology, Vol. 176, No. 1, January 1, 2007 11–17



In the absence of cargo, Kinesin-1 is thought to be inactive as a result of a folded conformation that enables autoinhibition of the N-terminal motor domain by C-terminal tail domains. Autoinhibition leads to a simple prediction for how Kinesin-1 is activated: cargo binding to the Kinesin-1 tail frees the motor domains for ATP-driven motility. Alternatively, cargo binding may not be suffi cient to activate Kinesin-1, and subsequent events may be required.

Binding of the JIP1 cargo protein is not sufficient to activate Kinesin-1



Prepolymerized taxol-stabilized MTs were added (+) or not added (–) to the indicated lysates with either ATP (T) or AMPPNP (N). After sedimentation, the MT pellets were immunoblotted with antibodies to the Flag (top) or myc and HA tags (bottom).

Coexpression of myc-KHC + HA-KLC recreates the autoinhibited Kinesin-1 holoenzyme

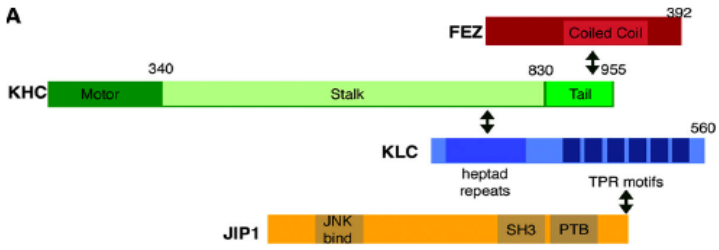
Myc-KHC expressed alone is not autoinhibited and can be cosedimented with MTs in the presence of 5'-adenylylimidodiphosphate (AMPPNP), a nonhydrolyzable analogue of ATP

These results suggest that an additional event is required to activate Kinesin-1.

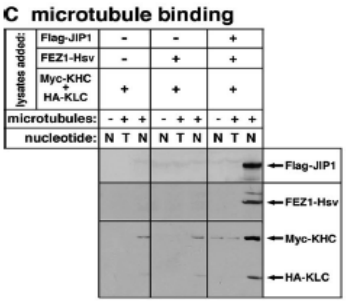
As the complete autoinhibition of Kinesin-1 requires both the KHC inhibitory tail and the KLC subunit, the hypothesis is that the autoinhibitory effects of both of these regions must be relieved for activation.

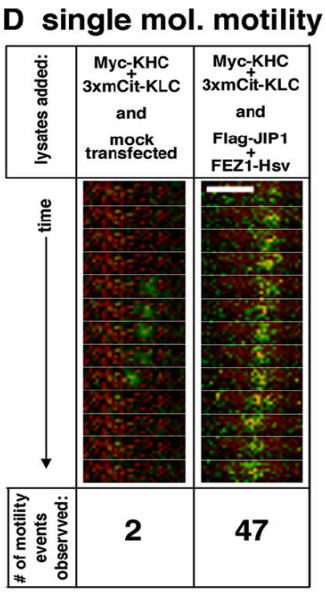
To identify potential cargoes and/or regulators of the KHC tail, a two-hybrid screen of a human brain library using the stalk/tail regions of rat KHC (750–955) as a bait, has been performed.

27 of the positive clones contained sequences encoding either FEZ1 and FEZ2 (Fasciculation and elongation protein)



FEZ1 and JIP1 cooperate to activate Kinesin-1 in vitro.

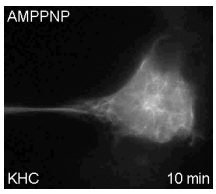


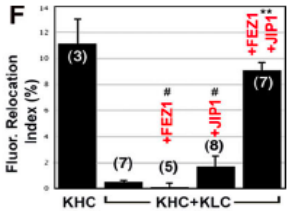


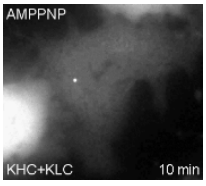
(D) Myc-KHC + 3xmCit-KLC lysates were mixed with lysates of mock-transfected cells (left) or cells expressing Flag-JIP1 and FEZ1-hsv (right). Representative motile events along Cy5-labeled MTs are shown in the kymographs (13 frames; 100-ms intervals). Bar, 1.0 µm.

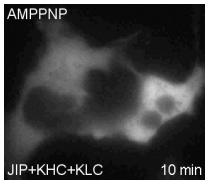
FEZ1 and JIP1 cooperate to activate Kinesin-1 in live cells.

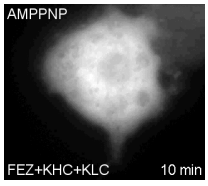
COS cells expressing KHC-mCit were transiently permeabilized with streptolysin O and washed, and then AMPPNP was added. When expressed alone, KHC-mCit is active and becomes locked on the MTs.

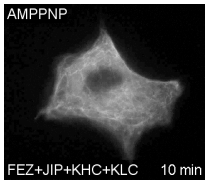












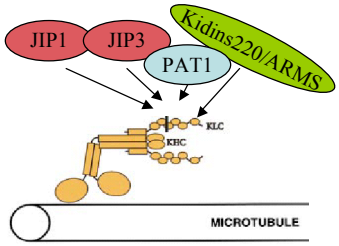
Traffic 2008; 9: 725–741
Blackwell Munksgaard

doi: 10.1111/j.1600-0854.2008.00722.x

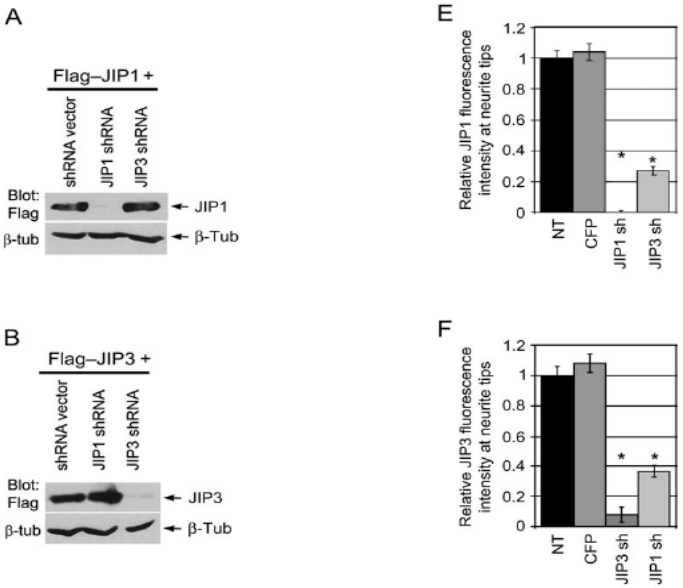
Co-operative Versus Independent Transport of Different Cargoes by Kinesin-1

Jennetta W. Hammond[1], Kelly Griffin[1], Gloria T. Jih[1], Jeanne Stuckey[2] and Kristen J. Verhey[1,*]

Co-operative or competitive transport of different cargoes?



Knockdown of JIP1 abrogates JIP3 transport and vice versa.



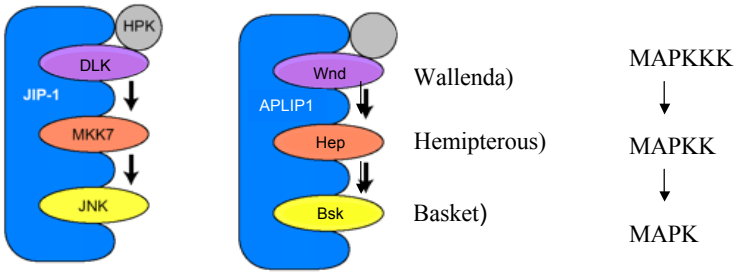
Current Biology 17, 1313–1317, August 7, 2007

Control of a Kinesin-Cargo Linkage Mechanism by JNK Pathway Kinases

Dai Horiuchi,[1,3] Catherine A. Collins,[2,3] Pavan Bhat,[2] Rosemarie V. Barkus,[1] Aaron DiAntonio,[2] and William M. Saxton[1,*]

Drosophila genetic screen for factors that control kinesin-JIP linkage during axonal transport

Drosophila homologs



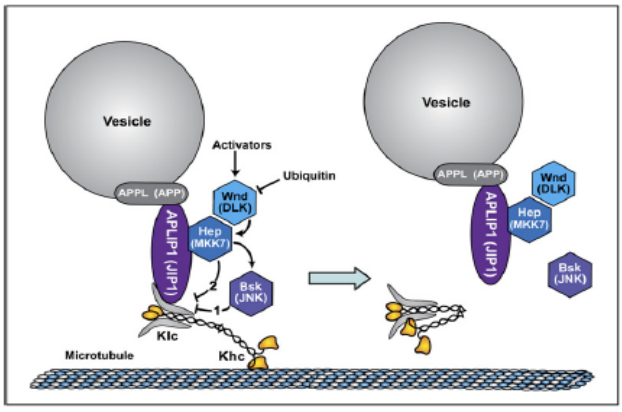


Figure 4. A Model for Wnd/MAPKKK Pathway Control of APLIP1/JIP1-Linked Kinesin-1 Cargo Transport

Components are labeled with *Drosophila* names and parenthetically with names of vertebrate homologs. Lines with arrowheads indicate activation influences, and lines with crossbars indicate inhibition influences. Wnd (MAPKKK), whose levels can be modulated by ubiquitination, is activated by unknown upstream signals. Wnd activates Hep (MAPKK) by phosphorylation, and activated Hep (MAPKK) then causes dissociation of APLIP1 (JIP1) from Klc, probably by phosphoactivation of Bsk (JNK), which then directly or indirectly modifies the linkage complex (pathway 1). It is also possible that phosphorylation of Hep (MAPKK) causes in the linkage complex a conformational change that inhibits APLIP1 (JIP1)-Klc binding independently of Bsk (pathway 2). Disruption of the APLIP1 (JIP1)-Klc linkage may allow kinesin to adopt an inactive, folded conformation that does not bind to microtubules [29, 30].