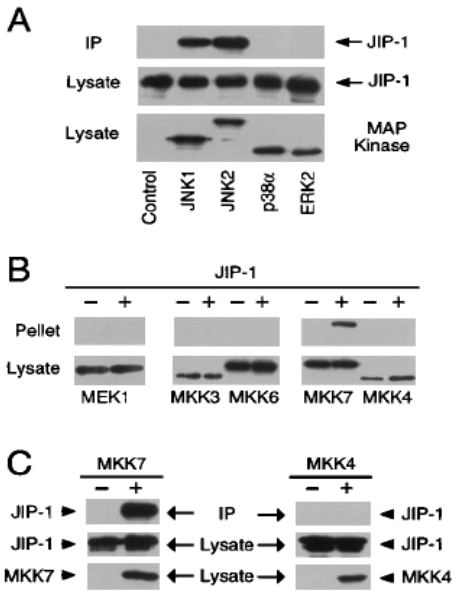


Fig. 1. Selective binding of JIP-1 to the MAP kinase JNK and the MAP kinase kinase MKK7. (**A**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with the HA-tagged MAP kinases ERK2, p38α, JNK1, and JNK2 (*15, 16*). The MAP kinases were immunoprecipitated with an antibody to HA. The presence of JIP-1 in the immunoprecipitates (IP) was detected on immunoblots probed with an antibody to T7-Tag. The amount of JIP-1 and MAP kinases in the cell lysates was examined by protein immunoblot analysis. (**B**) JIP-1 was expressed in cells as a GST fusion protein together with epitope-tagged MEK1, MKK3, MKK4, MKK6, or MKK7 (*15, 16*). JIP-1 was precipitated from cell lysates with glutathione-agarose, and the MAPKKs present in the pellet were detected by protein immunoblot analysis. The amount of the MAPKKs in the cell lysates was examined by protein immunoblot analysis. (**C**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with Flag-tagged MKK4 or MKK7 (*15, 16*). The presence of JIP-1 in Flag IP was detected by protein immunoblot analysis with an antibody to T7-Tag. The amount of the MAPKKs in the cell lysates was examined by protein immunoblot analysis.

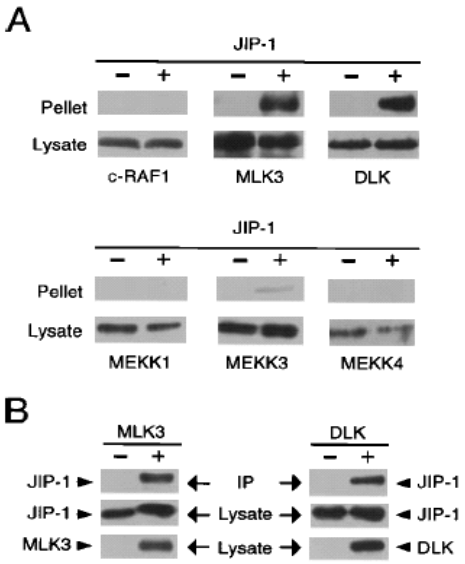
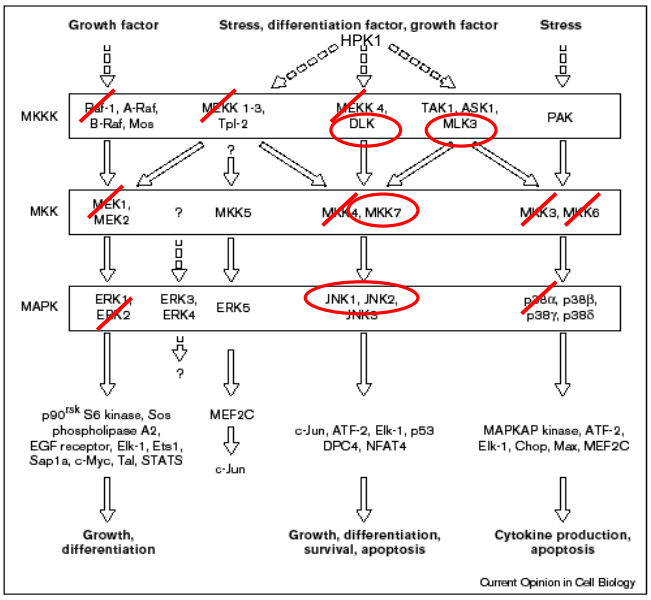
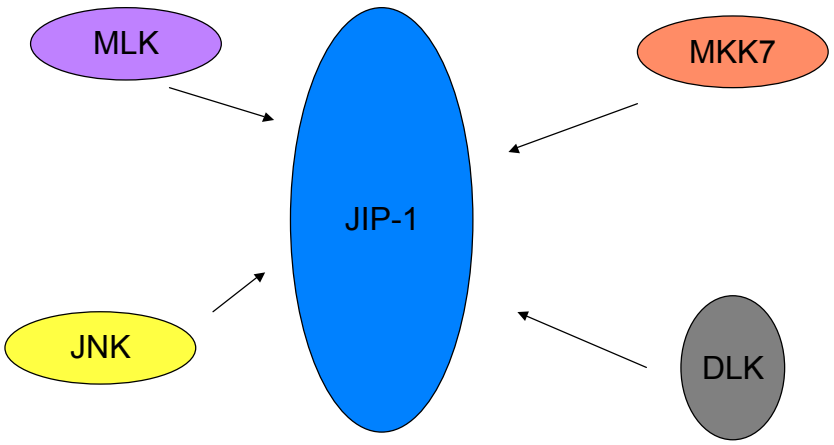


Fig. 2. Selective binding of JIP-1 to the mixed-lineage group of MAPKKKs. (**A**) JIP-1 was expressed in cells as a GST fusion protein together with the epitope-tagged MAPKKKs (*15, 16*). The presence of MAPKKKs in glutathione-agarose precipitates (pellet) was assayed by protein immunoblot analysis. The amount of the MAPKKKs in the cell lysates was examined by protein immunoblot analysis. (**B**) Epitope-tagged JIP-1 was coexpressed in cells with epitope-tagged MLK3 or DLK (*15, 16*). The presence of JIP-1 in the MLK3 and DLK immunoprecipitates (IP) was examined by protein immunoblot analysis. The amount of the MAPKKKs in the cell lysates was examined by protein immunoblot analysis.



Mitogen-activated protein kinase modules. The MAPK module consists of an MKKK, an MKK, and a MAPK. MKKKs respond to a variety of extracellular signals, including growth factors differentiation factors and stress. The activated MKKKs can then activate one or several MKKs. In contrast, the MKKs are relatively specific for their target MAPKs. Once activated, MAPKs can then phosphorylate transcription factors (for example ATF-2, Chop, c-Jun, c-Myc, DPC4, Elk-1, Ets1, Max, MEF2C, NFAT4, Sap1a, STATs, Tal, p53), other kinases (MAPKAP kinase, p90rsk S6 kinase), upstream regulators (EGF receptor, son of sevenless [Sos] Ras exchange factor), and other regulatory enzymes such as phospholipase A2. These downstream targets then control cellular responses including growth, differentiation, and apoptosis [1••,2,3].



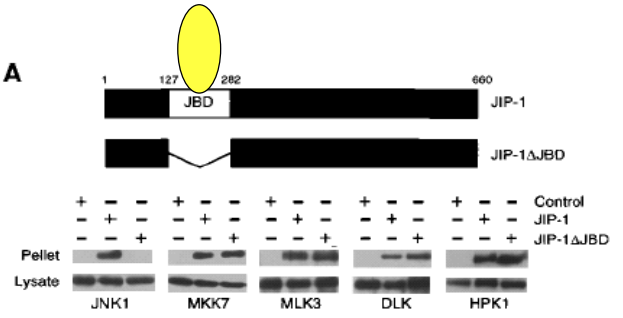
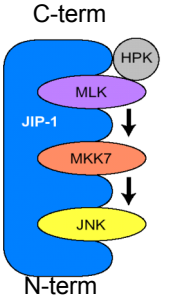
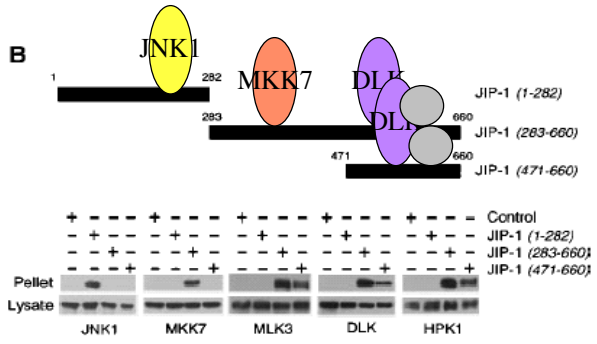


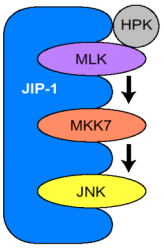
Fig. 3. Binding of JIP-1 to MKK7, MLK, and HPK1 independently of JNK. (**A**) The binding of JIP-1 to HPK1, MLK3, DLK, and MKK7 is independent of JNK. GST (Control), GST-tagged JIP-1, and a GST-tagged JIP-1 mutant with an in-frame deletion of the JNK binding domain (JIP-1ΔJBD) were coexpressed in cells together with HPK1 and epitope-tagged JNK1, MKK7, MLK3, and DLK (*15, 16*). The presence of kinases in the reduced glutathione-agarose precipitates (pellets) and cell lysates was examined by protein immunoblot analysis. (**B**) Deletion analysis of the

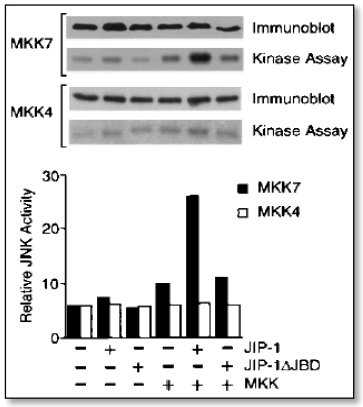




(**B**) Deletion analysis of the binding of JIP-1 to JNK1, MKK7, MLK3, and DLK. JIP-1 was expressed in cells as a GST fusion protein together with HPK1 or epitope-tagged JNK1, MKK7, MLK3, and DLK (*15, 16*). The presence of these kinases in glutathione-agarose precipitates was examined by protein immunoblot analysis. (**C**) The subcellular distribution of MKK7 and MKK4 was examined in

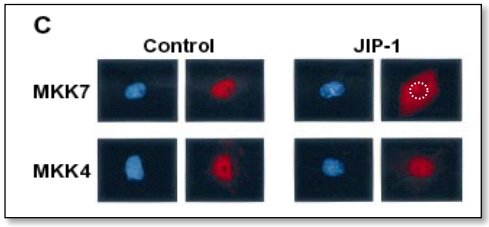
JIP1 enhances the activation of JNK by MKK7 and MLK



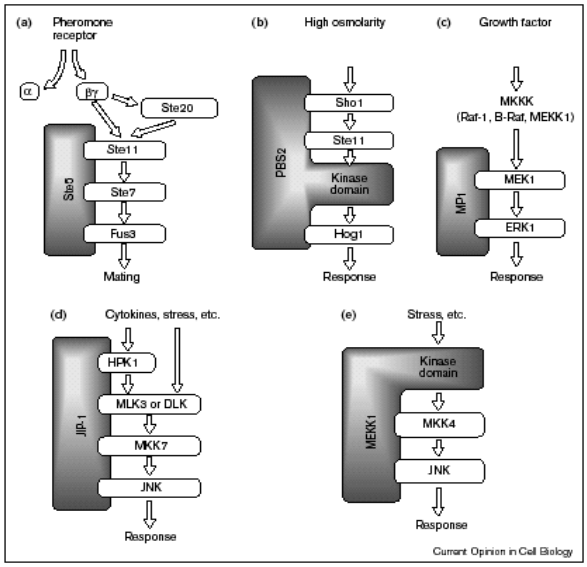


JIP=JNK inhibiting protein, why?

Overexpression of JIP1 inhibits JNK traslocation to the nucleus and in vivo activity



Protein scaffolds. The scaffolding protein for each example is shaded. (a) The yeast signal transduction pathway involved in the mating response uses Ste5 as a scaffolding protein to bind the members of the MAPK module, Ste11 (MKKK), Ste7 (MKK), and Fus3 (MAPK). Ste20 is an MKKKK in this pathway. Activation of Ste11 and Ste20 occurs with pheromone binding to the seven-transmembrane protein pheromone receptor, which then leads to dissociation of the Gα subunit from the Gβγ subunit. The Gβγ subunit then activates Ste11 and Ste20 [10–13]. (b) The high osmolarity response pathway. In this pathway, the same MKKK (Ste11) is used. PBS2 acts as both the MKK and the scaffold protein. Hog1 acts as the MAPK [18•]. (c) MP1 is a recently described scaffolding protein which binds to MEK1 (an MKK) and ERK1 (a MAPK), enhancing the efficiency of MAPK cascades involving these proteins [19••]. (d) JIP-1 binds HPK1 (an MKKKK), MLK3 or DLK (MKKKs), MKK7, and JNK (a MAPK), leading to enhanced JNK activation [22••]. (e) MEKK1, in addition to acting as a kinase for MKK4, is able to bind JNK, allowing it to act as a scaffold to bring together all three components of this MAPK module [26••].



Organization and regulation of mitogen-activated protein kinase signaling pathways

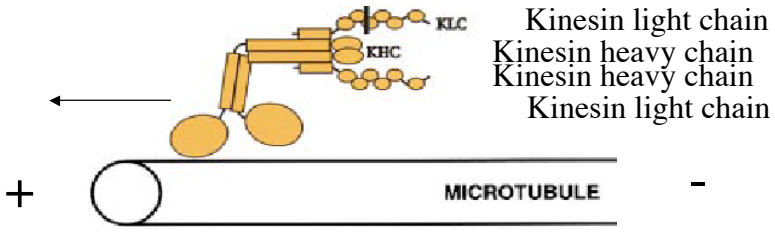
Timothy P Garrington* and Gary L Johnson†

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

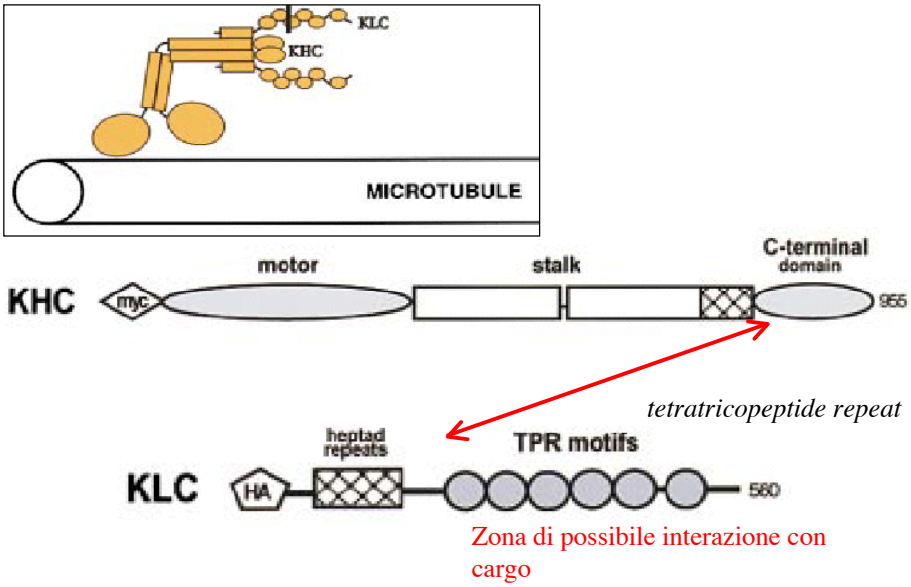
Kristen J. Verhey,* Debra Meyer,§ Reneé Deehan,* John Blenis,‡ Bruce J. Schnapp,‡ Tom A. Rapoport,* and Ben Margolis§

*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

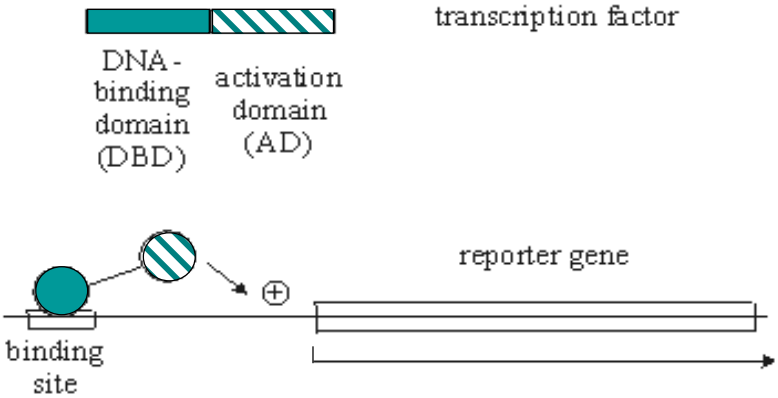
Two-hybrid screen

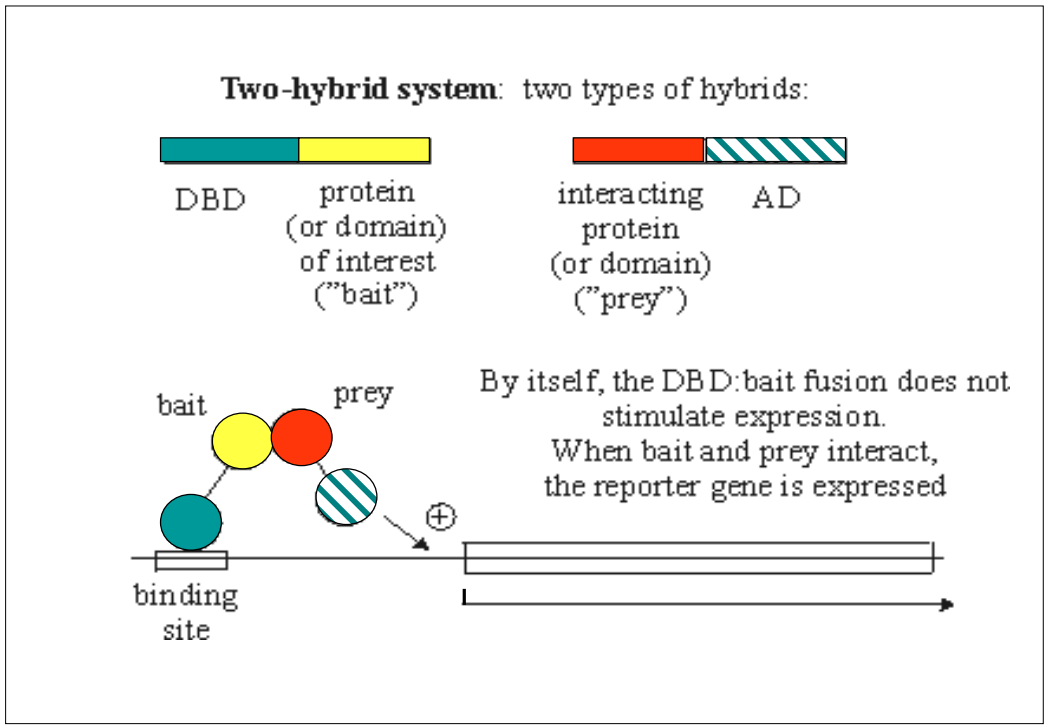
Direct Interaction of KLC and the JIP Proteins

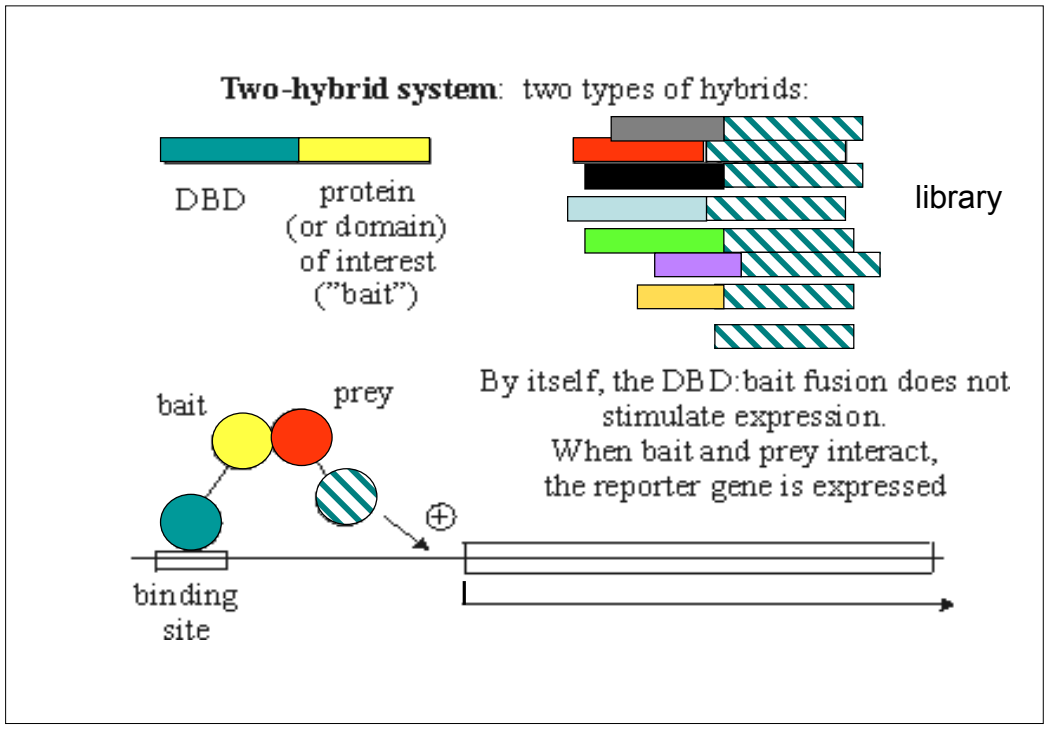
To identify proteins that interact directly with kinesin, we screened a mouse brain cDNA library using the yeast two-hybrid procedure with the TPR motifs of KLC as a bait. Nine of the clones isolated correspond to overlapping fragments of three different cDNAs encoding JIP-1, JIP-2, and JIP-3 (Fig. 1 B). No interaction of these clones was seen with either of two control bait proteins, the GAL4 DNA binding domain alone, or the TPR motifs of PP5 (data not shown).

Clones of cDNA from JIP-1,2,3

Structure-function properties of a typical **transcription factor**:




Two-hybrid system: two types of hybrids:
DBD
protein
(or domain)
of interest
("bait")
interacting
protein
(or domain)
("prey")
AD
bait
prey
By itself, the DBD:bait fusion does not
stimulate expression.
When bait and prey interact,
the reporter gene is expressed
binding
site


Two-hybrid system: two types of hybrids:
DBD
protein
(or domain)
of interest
("bait")
library
bait
prey
By itself, the DBD:bait fusion does not
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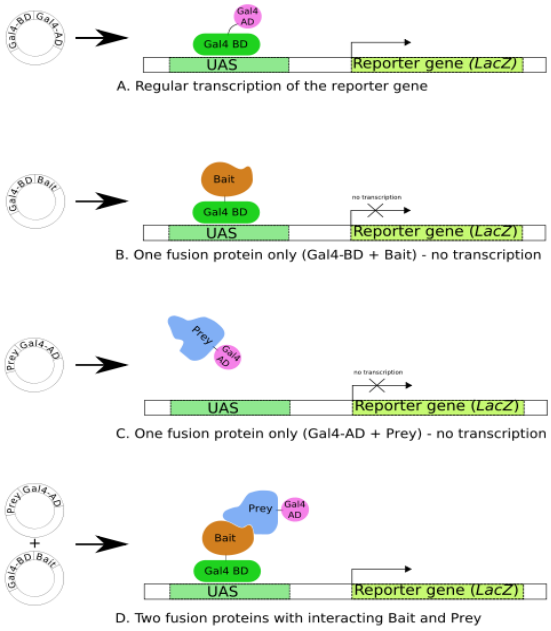
When bait and prey interact,
the reporter gene is expressed
binding
site

Wikipedia



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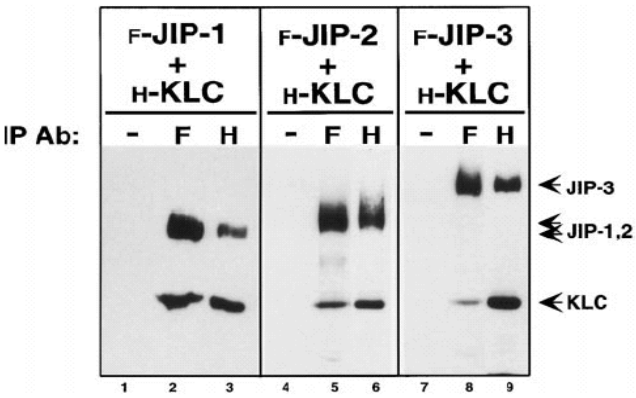


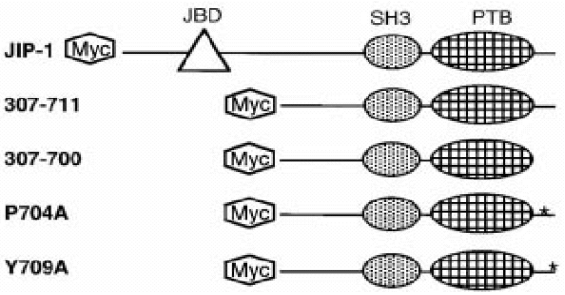
Figure 2. Coimmunoprecipitation of KLC and the JIP proteins. Lysates of COS cells expressing Flag-tagged JIP-1, JIP-2, or JIP-3 together with HA-tagged KLC were immunoprecipitated (IP) with no primary antibody (−), with an anti-Flag mAb (F), or with an anti-HA mAb (H). Precipitates were immunoblotted to detect the expressed proteins using polyclonal antibodies to both epitope tags.

The extreme COOH-term of JIP-1 and JIP-2 are identical and conserved across species

```
                                                  *   *
hJIP-1/IB1  ...EDSTKALAESVGRAFQQFYKQFVEYTCPTEDIYLE
mJIP-1b     ...EDSTKALAESVGRAFQQFYKQFVEYTCPTEDIYLE
hJIP-2      ...QESMRPVAQSVGRAFLEYYQEHLAYACPTEDIYLE
pJIP-2      ...QESMRPVAQSVGRAFLEYYQEHLEYACPTEDIYLE
dJIP/SP512  ...SESTRPVAEAVGRAFQRFYQKFIETAYPIEDIYIE
ceJIP       ...KNTTQPIVEAIGRAFKRSYDEYMAFAHPTEDIYLE
```

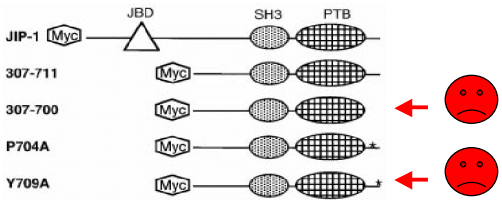
h: human
m: mouse
p: porcine
d: drosophila
ce: C. Elegans

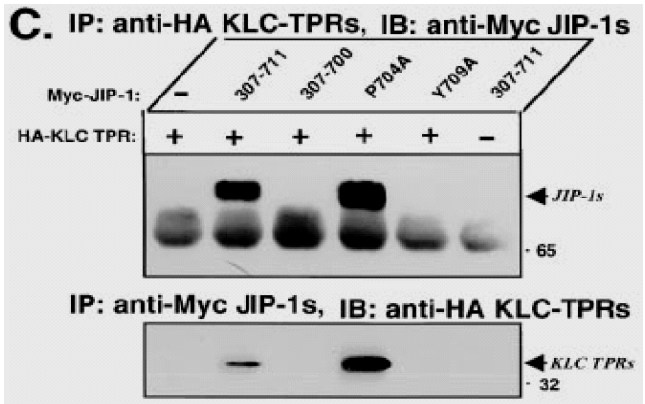
Are JIP-1 COOH-term residues required for binding to KLC TPR?



Site-directed mutagenesis
(*in vitro* mutagenesis)

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





BCII - Perroteau - Trasduzione del segnale e proteine scaffold. Verhey et al. 2001

Kinesin brings JIP-1 to neuronal processes

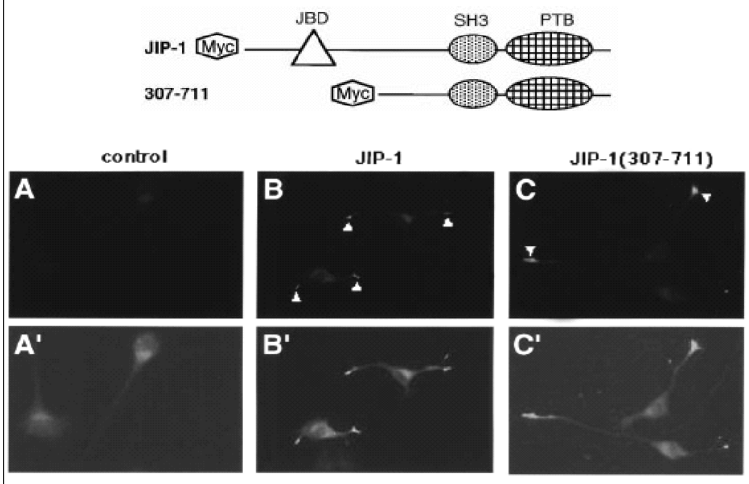
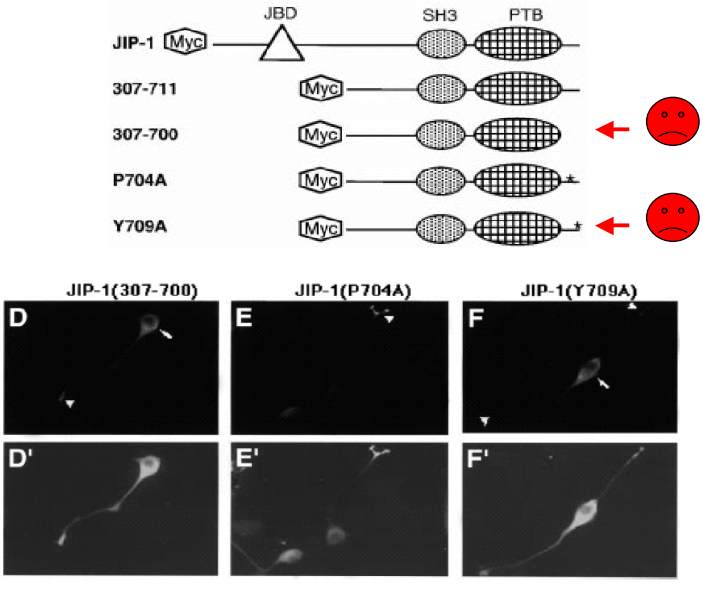


Figure 4. The COOH-terminal residues of JIP-1 are required for proper subcellular localization. NIE 115 cells were transiently transfected with the parental plasmid (control) or with plasmids encoding the indicated JIP-1 variants, differentiated, and the expressed proteins were detected by indirect immunofluorescence 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).



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