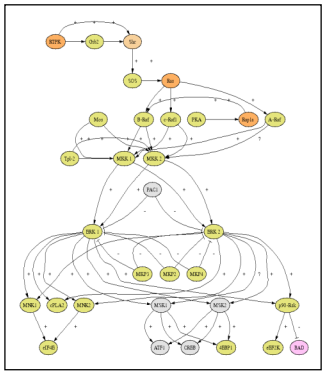
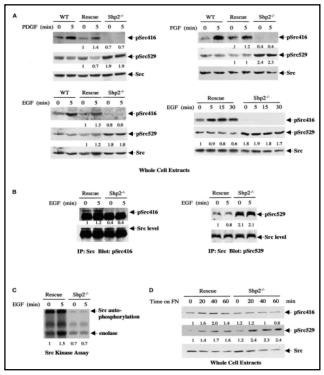
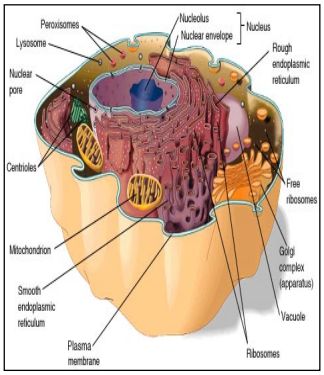
SCAFFOLDING OF TRANSDUCTION MOLECULES

1

Signal Transduction



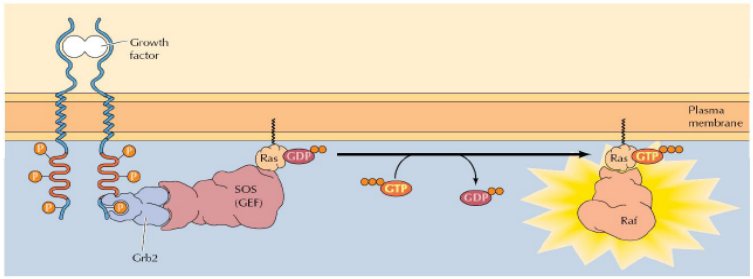




What? **When?** **Where?**

2

Ras activation downstream of receptor protein-tyrosine kinases



A complex of Grb2 and the guanine nucleotide exchange factor Sos binds to a phosphotyrosine-containing sequence in the activated receptor via the Grb2-SH2 domain. This interaction recruits Sos to the plasma membrane, where it can stimulate Ras GDP/GTP exchange. The activated Ras-GTP complex then binds to the Raf protein kinase.

From G.M. Cooper, "The Cell: A molecular approach", 2004, third edition, ASM Press, Sinauer Associates

3

ASSEMBLY OF SIGNALING COMPLEX ON AN ACTIVATED RECEPTOR

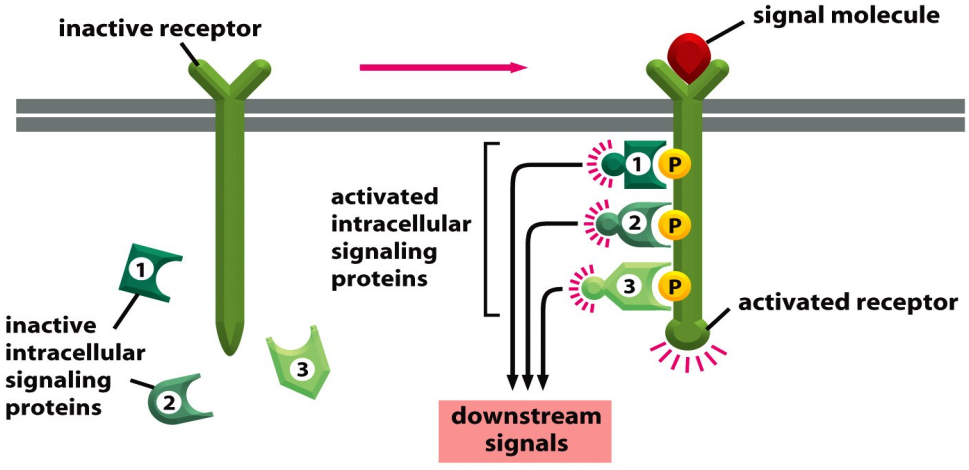


Figure 15-21b Molecular Biology of the Cell 5/e (© Garland Science 2008)

4

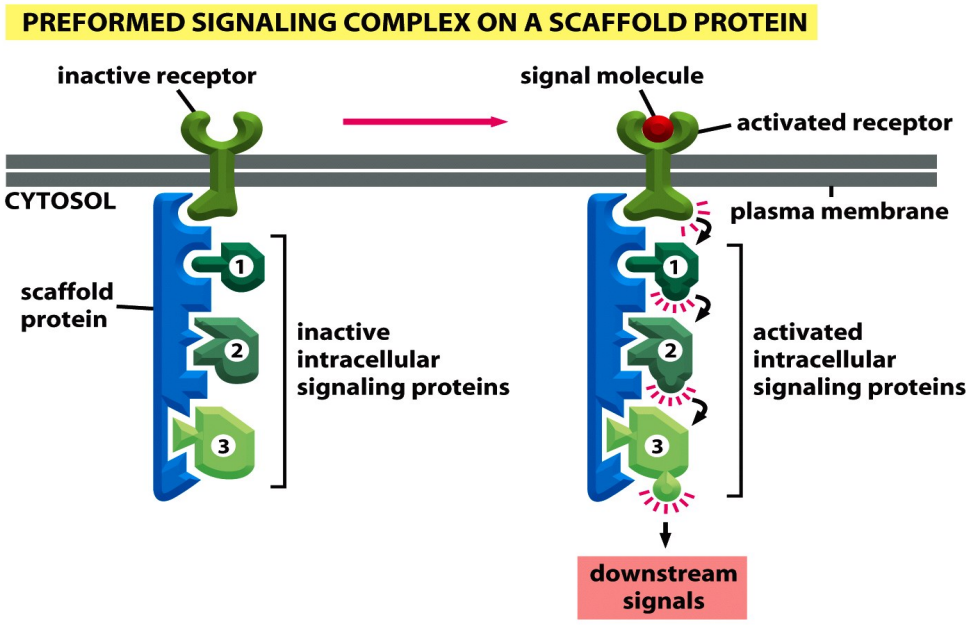
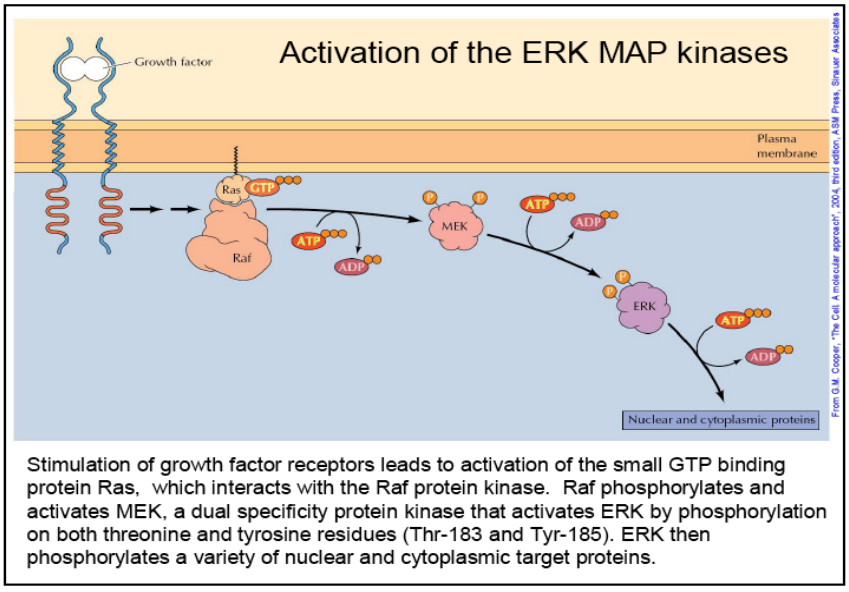
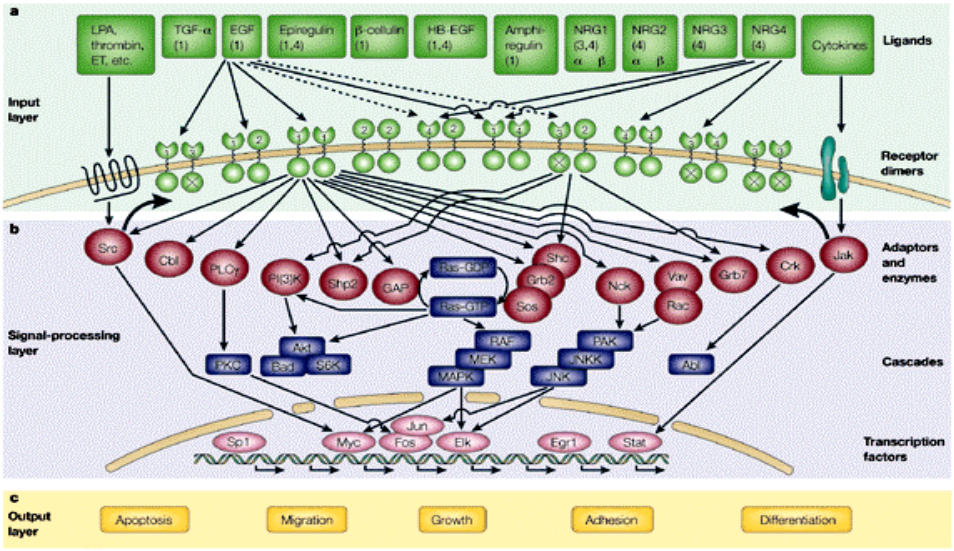


Figure 15-21a Molecular Biology of the Cell 5/e (© Garland Science 2008)



Stimulation of growth factor receptors leads to activation of the small GTP binding protein Ras, which interacts with the Raf protein kinase. Raf phosphorylates and activates MEK, a dual specificity protein kinase that activates ERK by phosphorylation on both threonine and tyrosine residues (Thr-183 and Tyr-185). ERK then phosphorylates a variety of nuclear and cytoplasmic target proteins.

6



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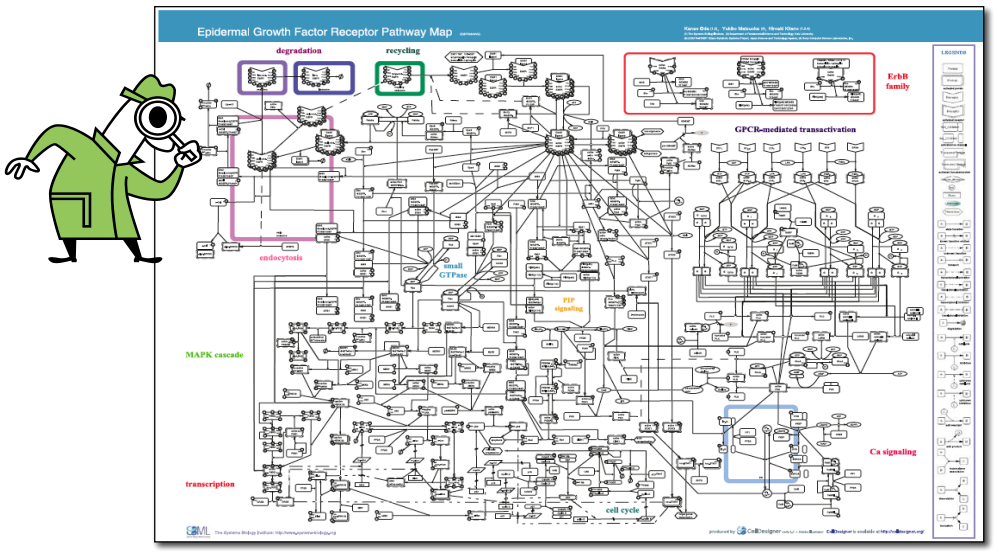

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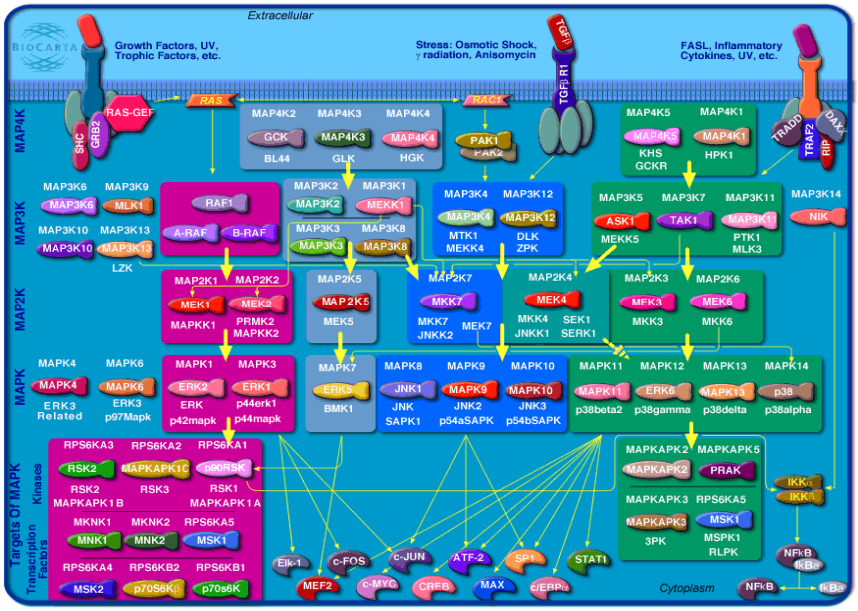

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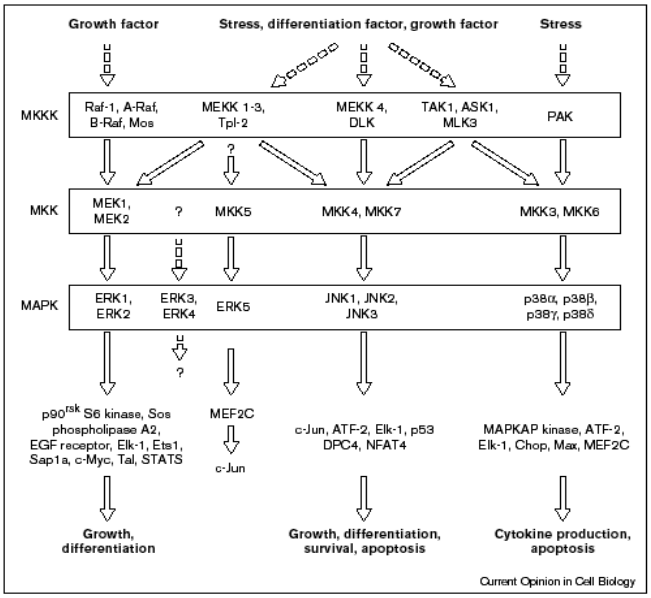


Mitogen-activated protein kinase modules. The MAPK module consists of an MKKK, an MKK, and a MAPK. MKKKs respond to a variety of extracellular signals, including growth factors differentiation factors and stress. The activated MKKKs can then activate one or several MKKs. In contrast, the MKKs are relatively 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].

10

SCIENCE • VOL. 277 • 1 AUGUST 1997

A Cytoplasmic Inhibitor of the JNK Signal Transduction Pathway

Martin Dickens, Jeffrey S. Rogers, Julie Cavanagh, Art Raitano, Zhengui Xia, Jocelyn R. Halpern, Michael E. Greenberg, Charles L. Sawyers, Roger J. Davis*

The c-Jun amino-terminal kinase (JNK) is a member of the stress-activated group of mitogen-activated protein (MAP) kinases that are implicated in the control of cell growth. A murine cytoplasmic protein that binds specifically to JNK [the JNK interacting protein-1 (JIP-1)] was characterized and cloned. JIP-1 caused cytoplasmic retention of JNK and inhibition of JNK-regulated gene expression. In addition, JIP-1 suppressed the effects of the JNK signaling pathway on cellular proliferation, including transformation by the *Bcr-Abl* oncogene. This analysis identifies JIP-1 as a specific inhibitor of the JNK signal transduction pathway and establishes protein targeting as a mechanism that regulates signaling by stress-activated MAP kinases.

11

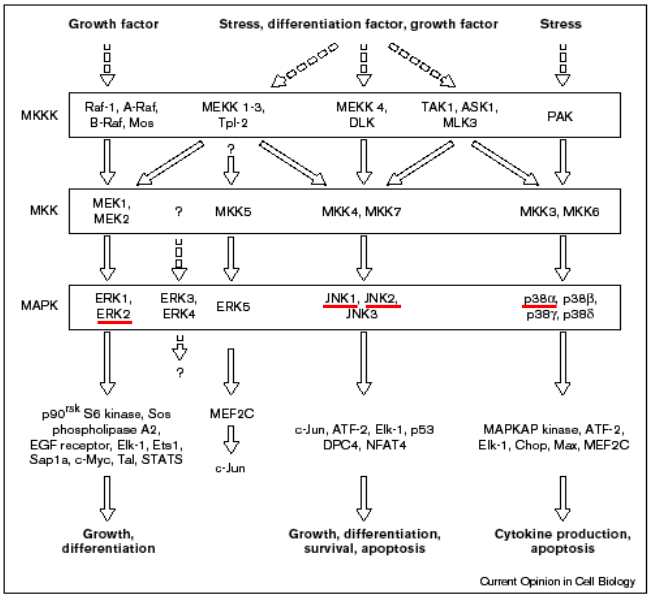
A Mammalian Scaffold Complex That Selectively Mediates MAP Kinase Activation

Alan J. Whitmarsh, Julie Cavanagh, Cathy Tournier, Jun Yasuda, Roger J. Davis*

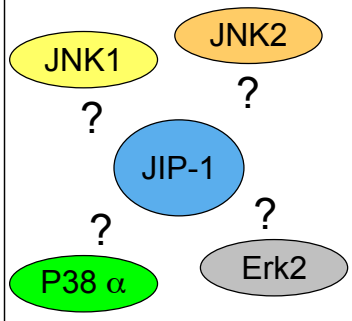
The c-Jun NH_2-terminal kinase (JNK) group of mitogen-activated protein (MAP) kinases is activated by the exposure of cells to multiple forms of stress. A putative scaffold protein was identified that interacts with multiple components of the JNK signaling pathway, including the mixed-lineage group of MAP kinase kinase kinases (MLK), the MAP kinase kinase MKK7, and the MAP kinase JNK. This scaffold protein selectively enhanced JNK activation by the MLK signaling pathway. These data establish that a mammalian scaffold protein can mediate activation of a MAP kinase signaling pathway.

Science. 1998 Sep 11;281(5383):1671-4.

12



Which MAPK interact with JIP-1?



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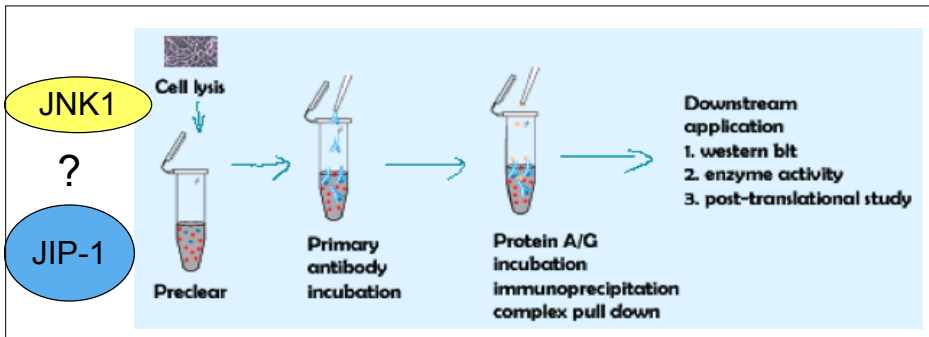
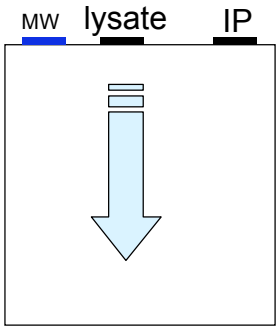


IP anti JNK1



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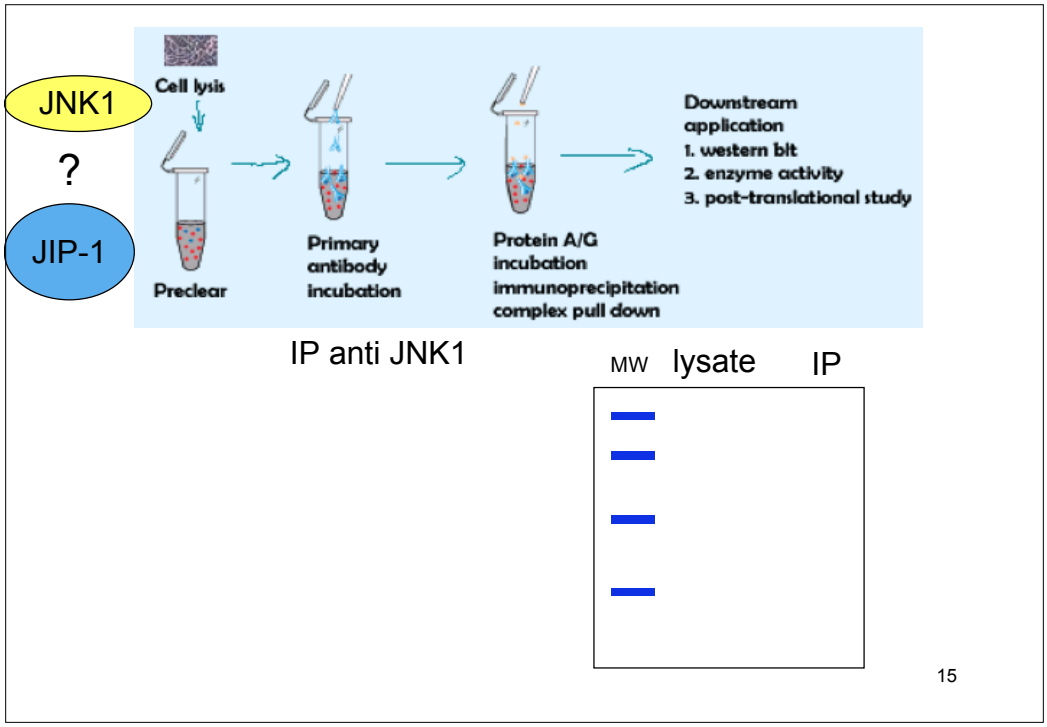
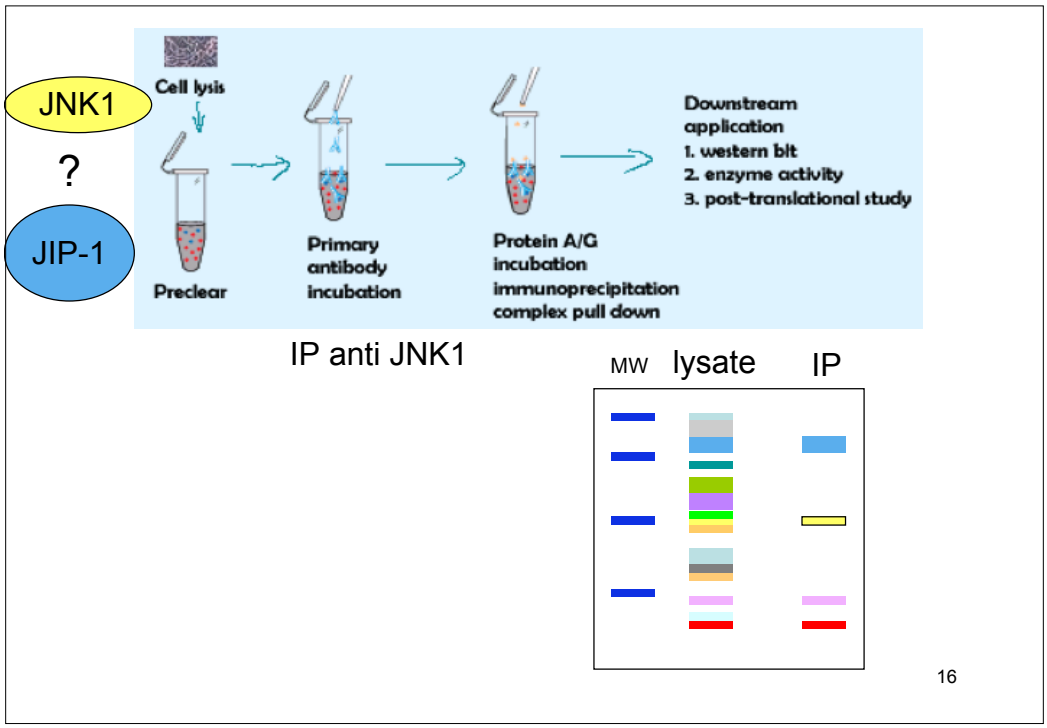
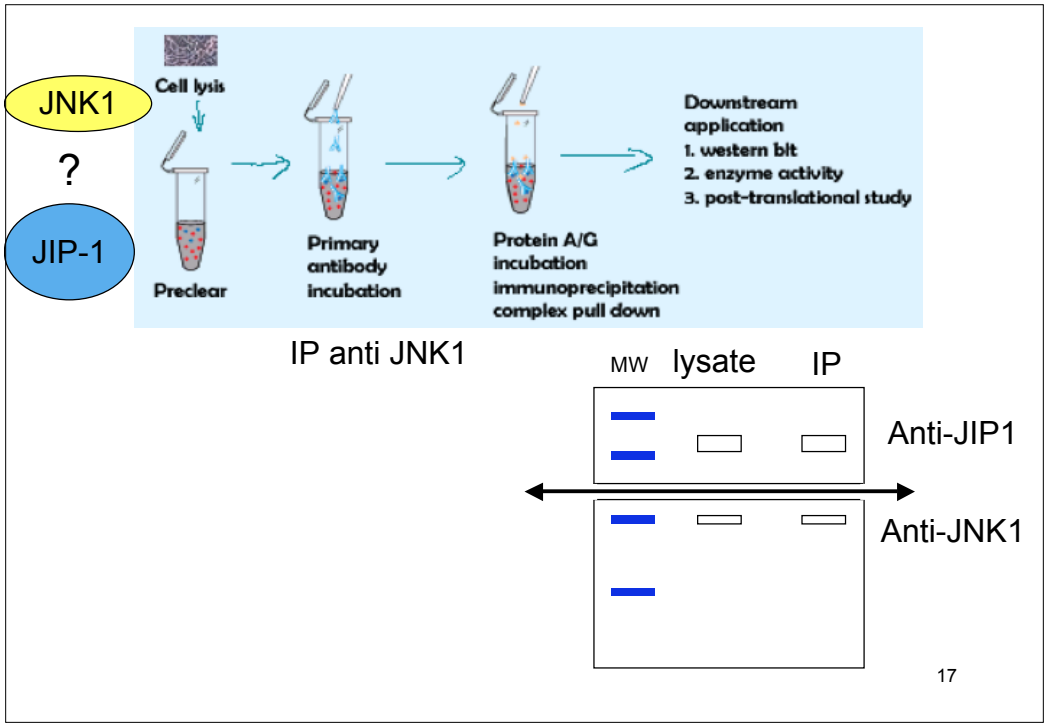
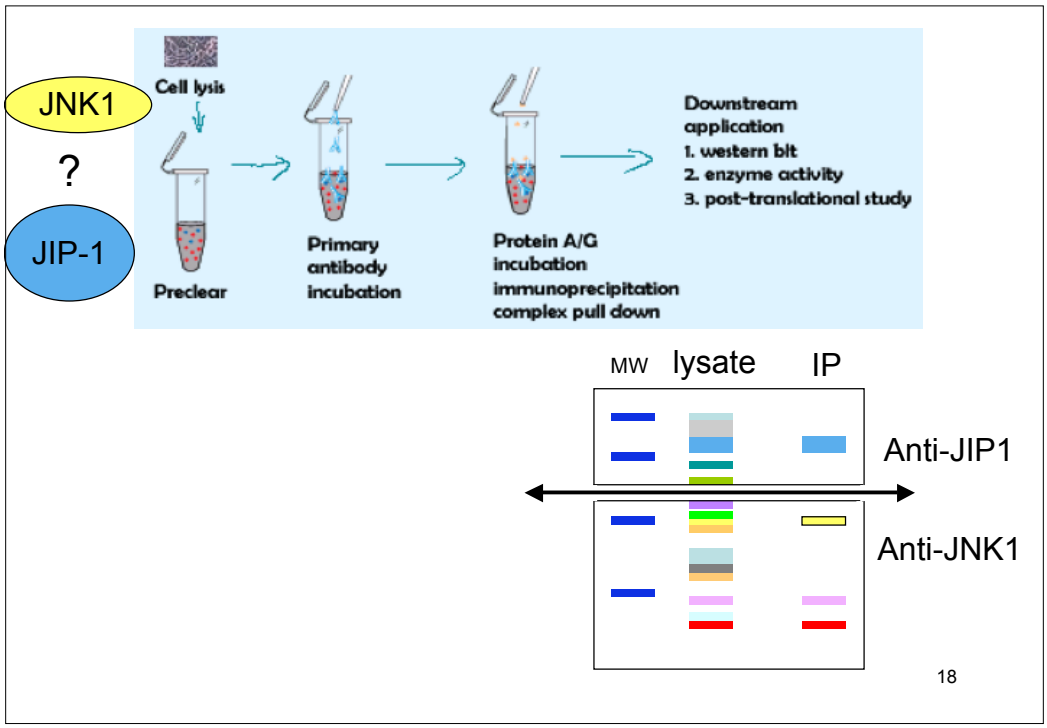
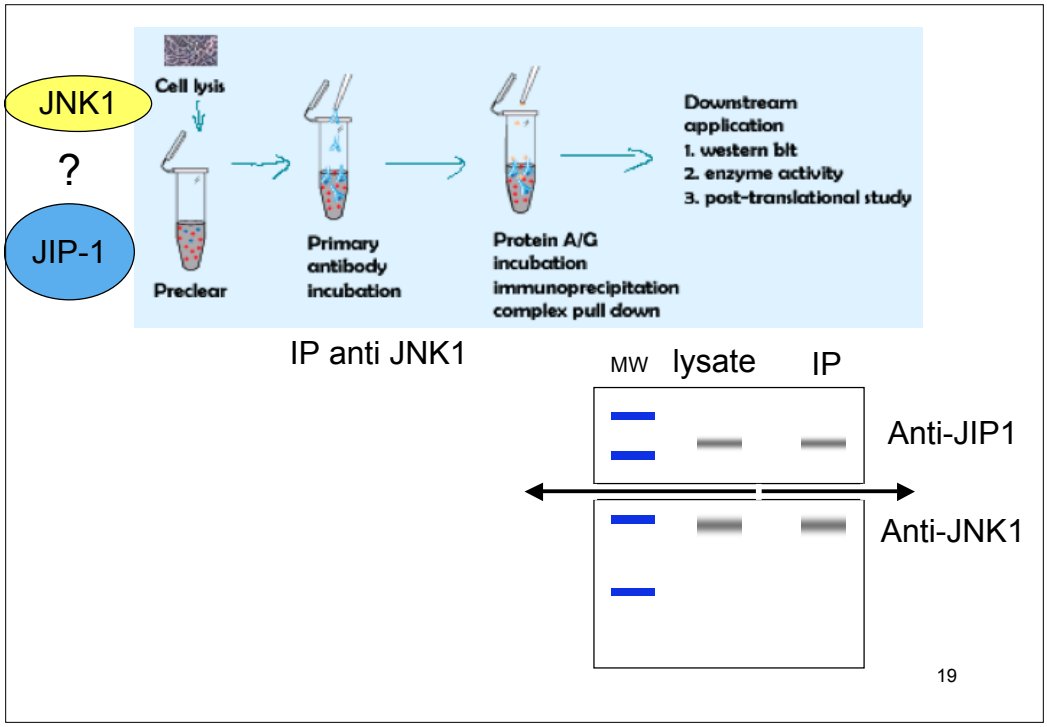
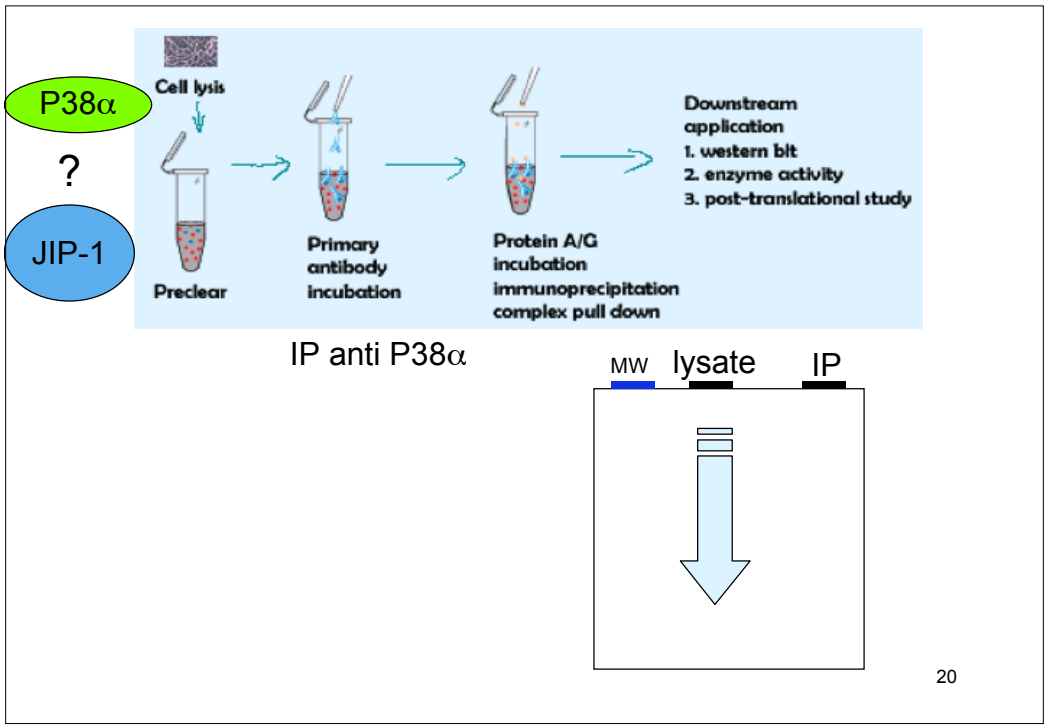


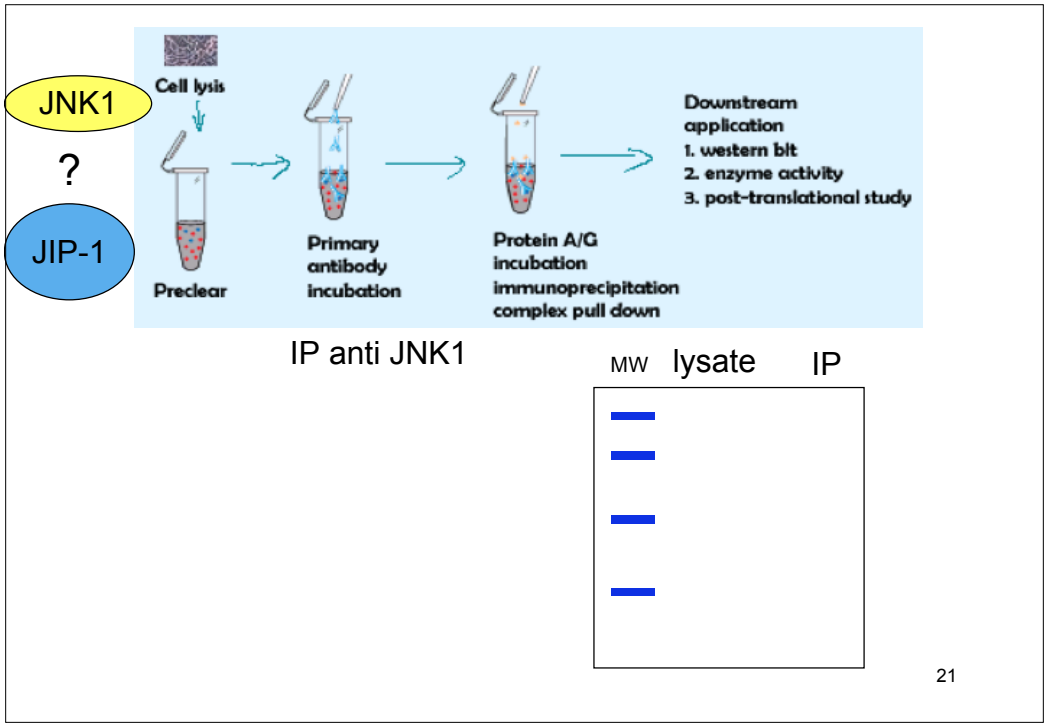


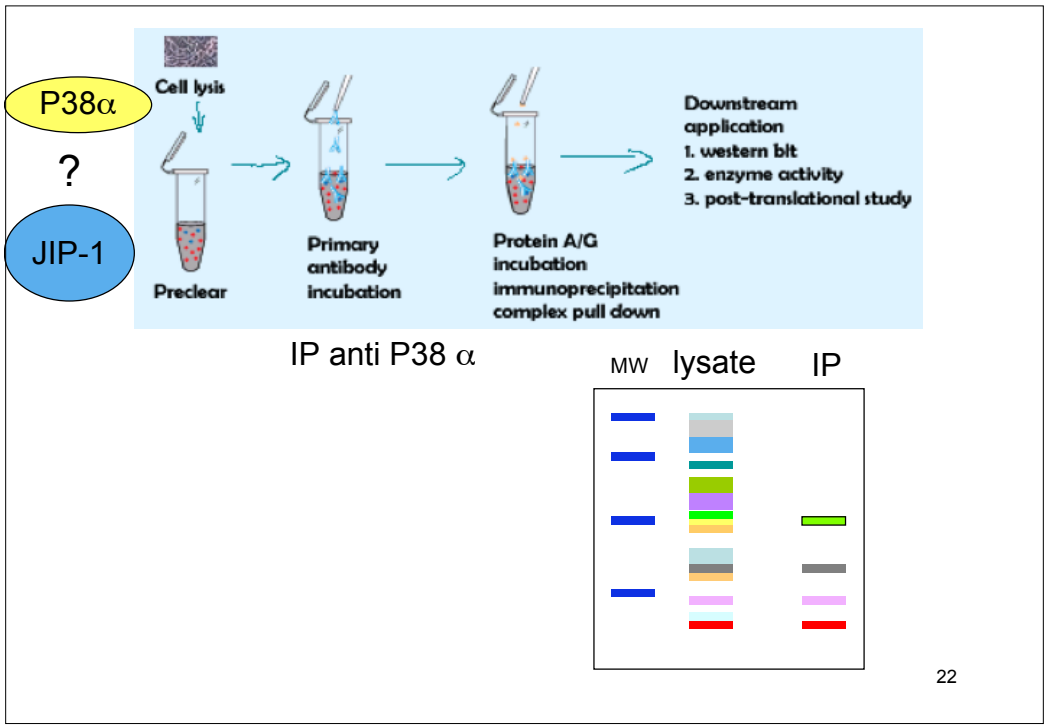


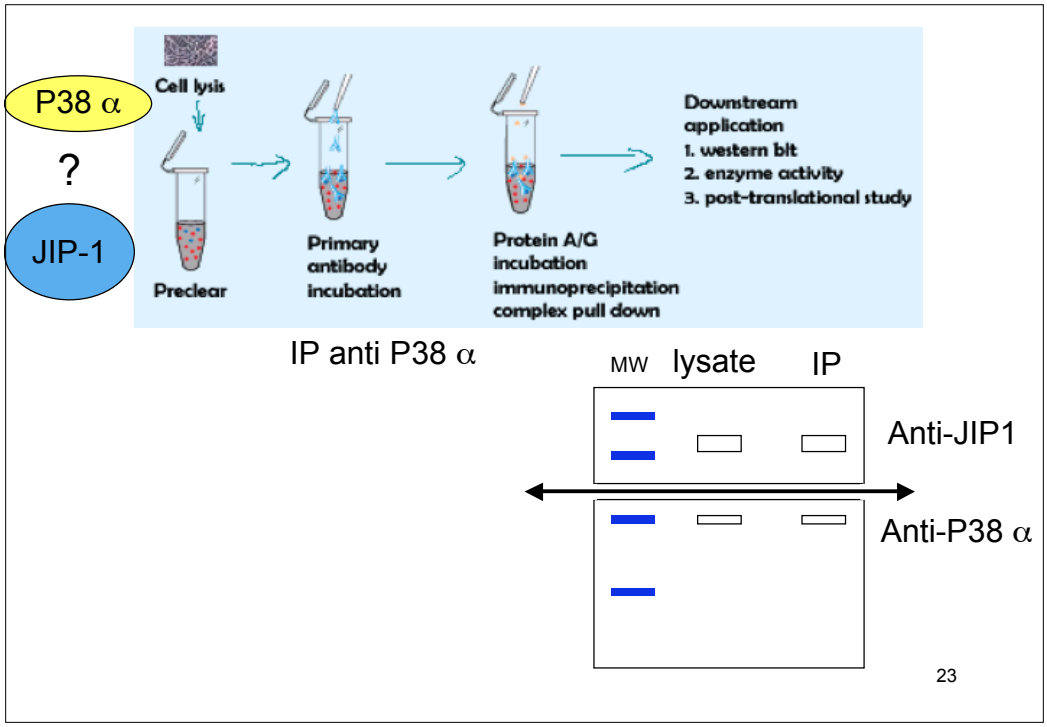


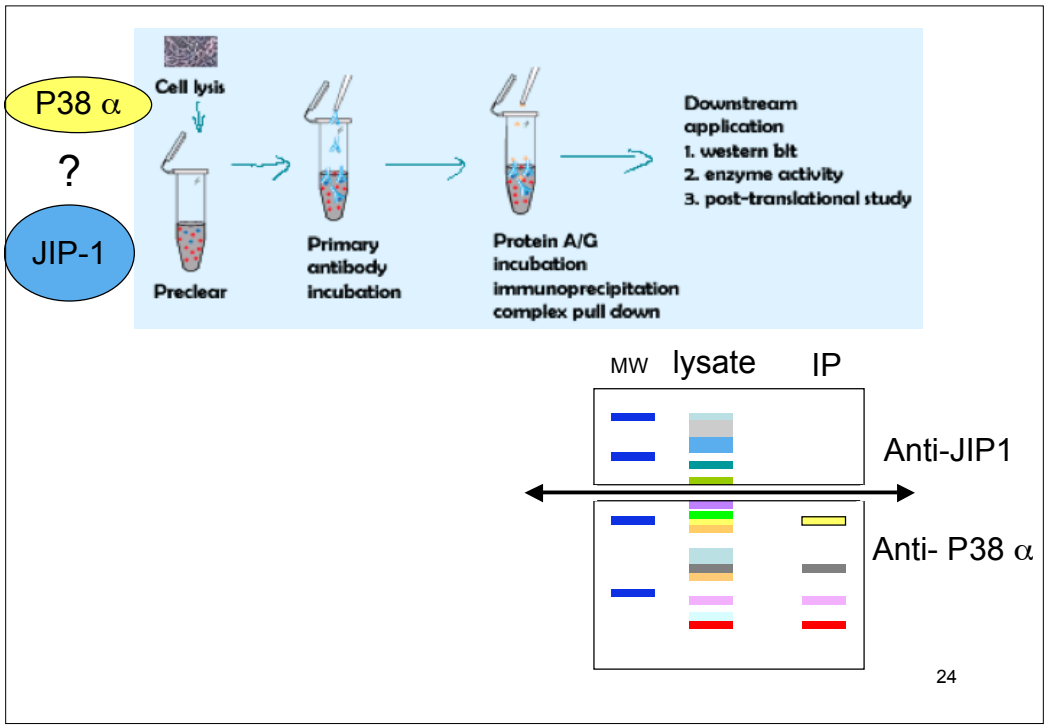


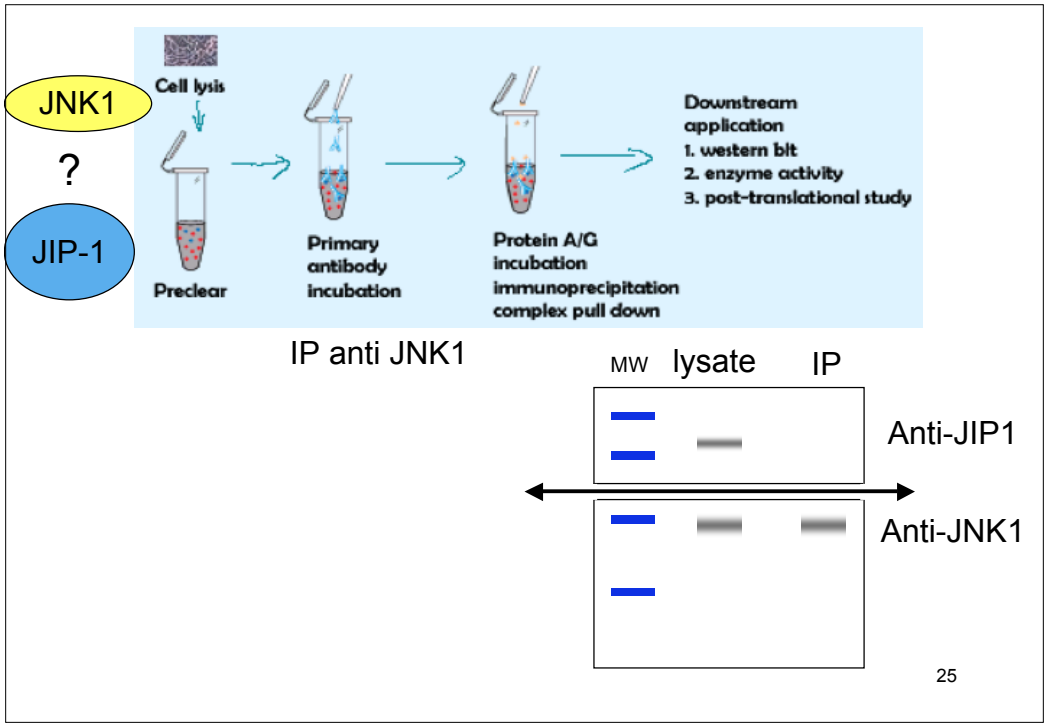












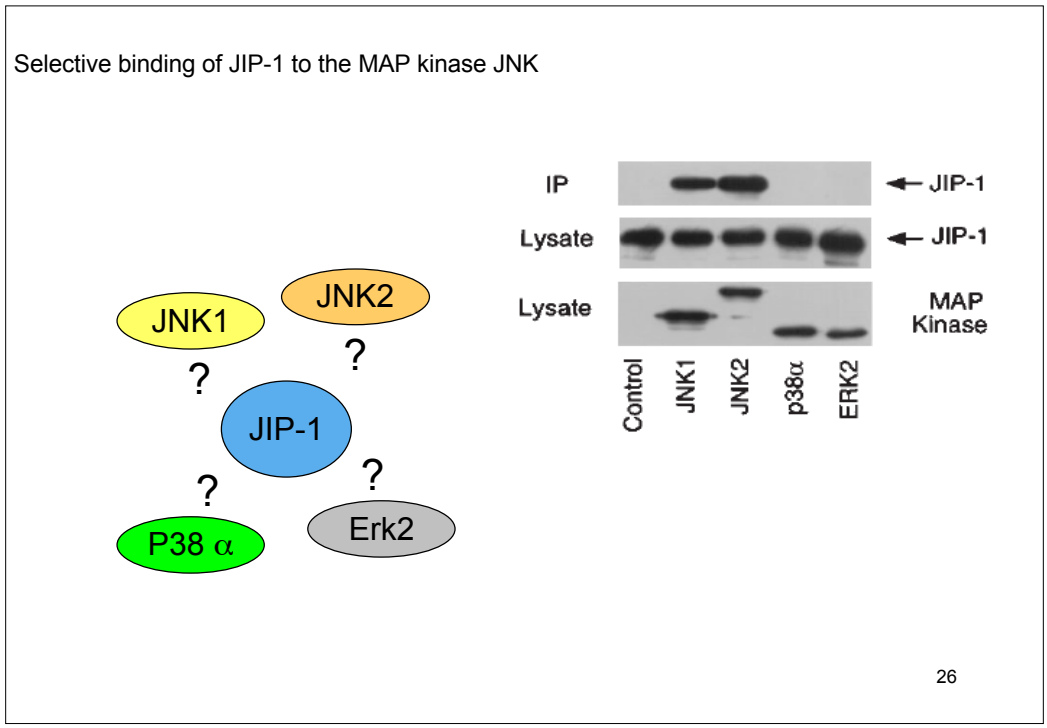
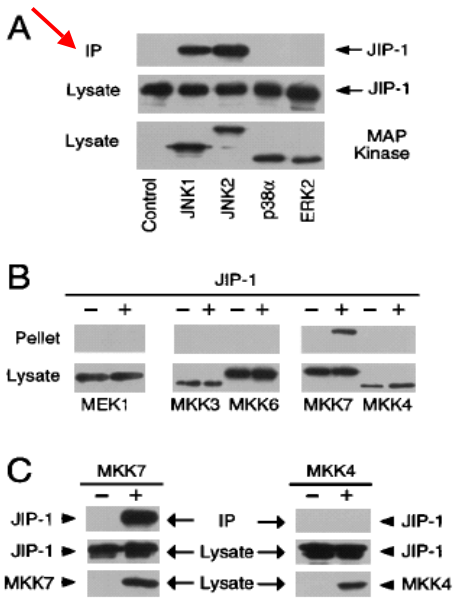


Fig. 1. Selective binding of JIP-1 to the MAP kinase JNK and the MAP kinase kinase MKK7. (**A**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with the HA-tagged MAP kinases ERK2, p38α, JNK1, and JNK2 (*15, 16*). The MAP kinases were immunoprecipitated with an antibody to HA. The presence of JIP-1 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.



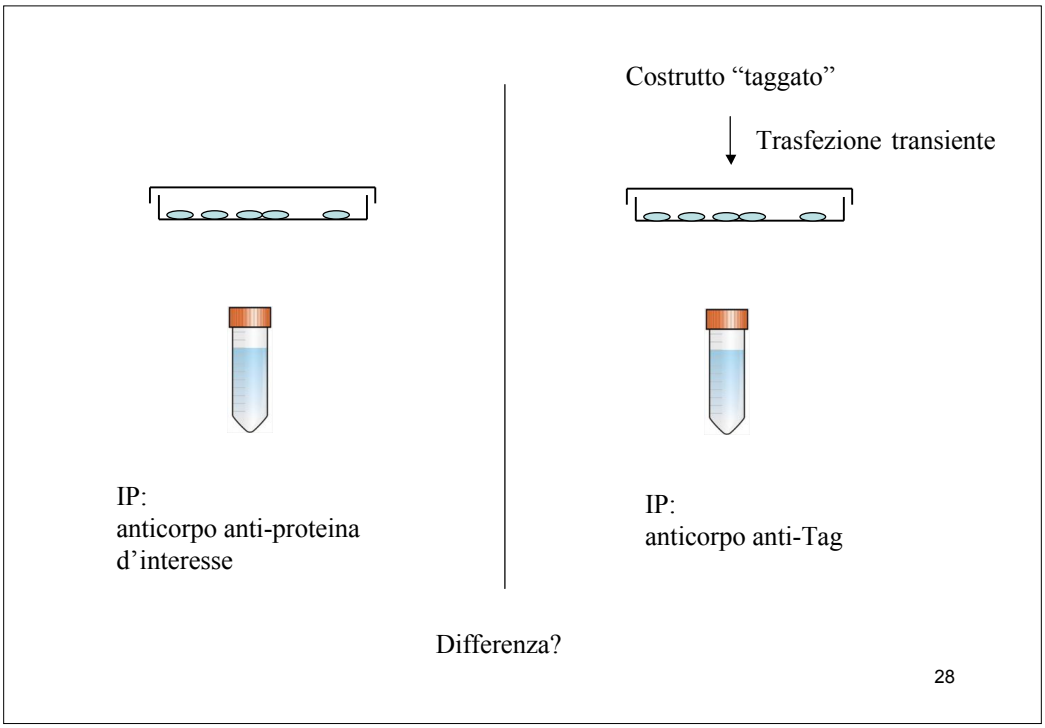
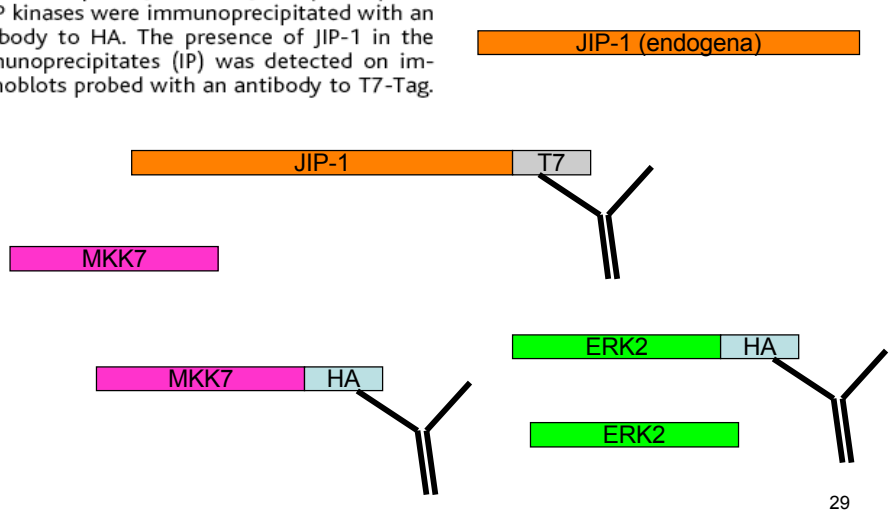


Fig. 1. Selective binding of JIP-1 to the MAP kinase JNK and the MAP kinase kinase MKK7. (**A**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with the HA-tagged MAP kinases ERK2, p38α, JNK1, and JNK2 (*15*, *16*). The MAP kinases were immunoprecipitated with an antibody to HA. The presence of JIP-1 in the immunoprecipitates (IP) was detected on immunoblots probed with an antibody to T7-Tag.



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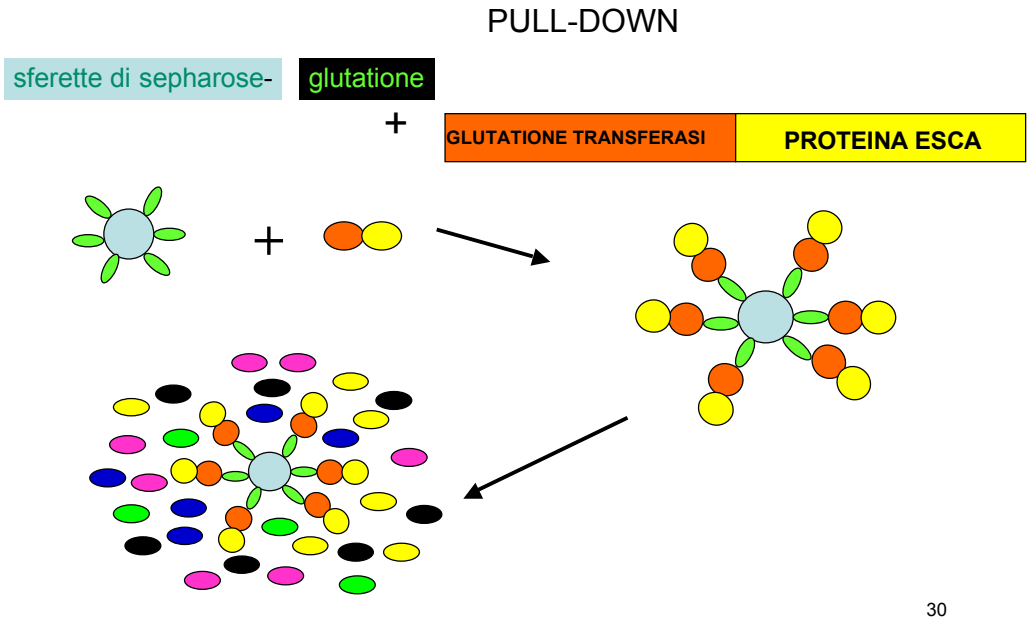
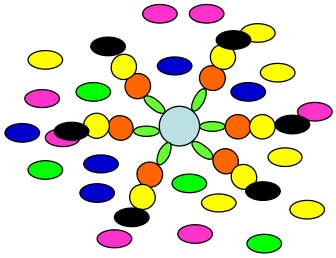
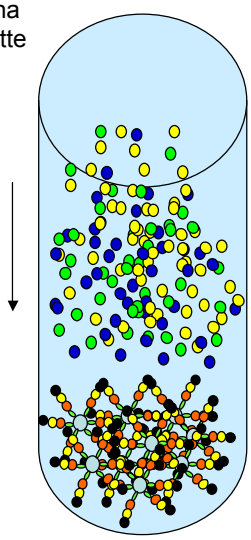


30

• metto in agitazione (rotazione) a 4°C 2h o O/N affinché la proteina "esca", (fusa con la glutatione transferasi) vada in contatto con tutte le proteine presenti nell'estratto





• centrifugo a bassa velocità (3000 rpm, 1 min, 4°C). Sul fondo della provetta si depositano:sferette di sepharose-glutatione--glutationetransferasi-proteina esca; in soluzione restano:proteine non legate alla proteina "esca"

•procedo come per l'immunoprecipitazione

31