Cell

Presenilin-Dependent ErbB4 Nuclear Signaling Regulates the Timing of Astrogenesis in the Developing Brain

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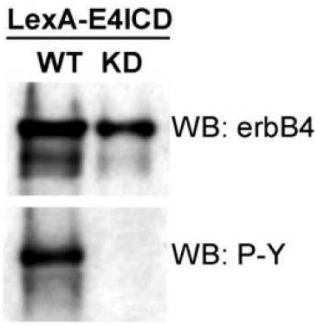
Identification of Proteins that Interact with Activated E4ICD

To identify ErbB4-binding proteins that are involved in transcriptional regulation they used a yeast two-hybrid system that facilitates the isolation of proteins that interact with activated RTKs

Screening of a cDNA expression library from rat embryonic day 14 (E14) spinal cord and dorsal root ganglia with a bait containing the entire E4ICD in an activated state led to isolation of several putative E4ICD-interacting proteins.



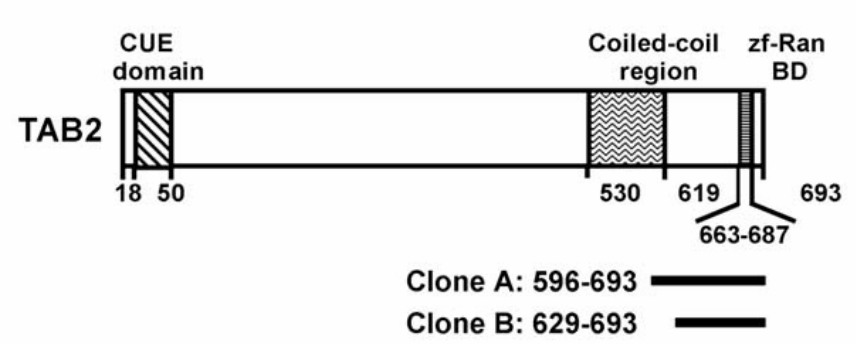
LexA-E4ICD fusion, when expressed in mammalian cells, dimerizes and becomes autophosphorylated.

Yeast expressing wild-type (WT) and kinase-dead (KD) LexA-E4ICD fusion proteins were lysed and immunoblotted with ErbB4 or phosphotyrosine (P-Y) antibodies, showing that both proteins are expressed (top panel) but only the WT is tyrosine phosphorylated.

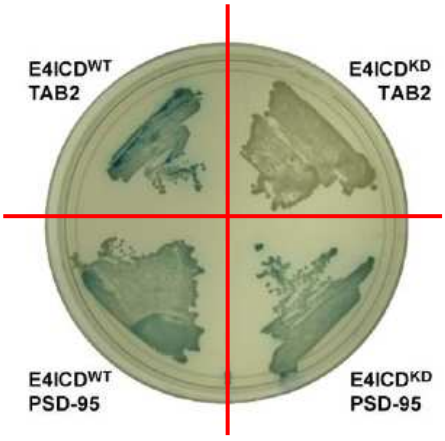
Two clones contained cDNAs encoding the C-terminal region of TAB2 (TAK1 binding protein 2), a protein first identified as an adaptor for TAK1 (transforming growth factor β-activated kinase 1)

Schematic diagram of TAB2

Regions of TAB2 included in two clones identified in the screen are indicated.
zf-Ran BD: zinc finger Ran-binding domain.



Wild-type E4ICD also interacted with full-length TAB2 in yeast, and this association was abolished when the tyrosine kinase activity of E4ICD was eliminated by a mutation in the ATP-binding site (E4ICDKD).

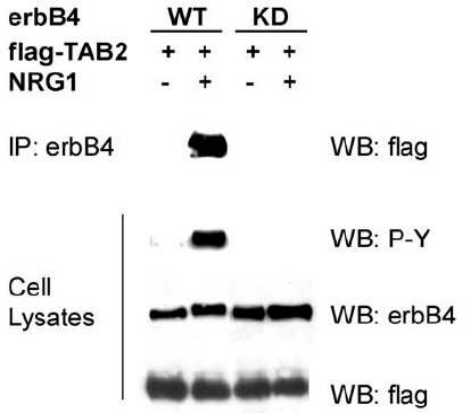


Tyrosine kinase activity of E4ICD is necessary for interaction with TAB2 in yeast. WT or KD LexA-E4ICD was coexpressed in yeast with full-length TAB2 or PDZ domains 1 and 2 of PSD-95 as fusion proteins with the activation domain.
TAB2 only interacts with phosphorylated E4ICD (blue color), whereas PSD-95 interacts with both baits.

→ binding of TAB2 to ErbB4 in yeast appears to occur only when the receptor is activated.

Does TAB2 binds to ErbB4 in mammalian cells?
Does this interaction depends on receptor activation by NRG1?

Cells were cotransfected with FLAG-TAB2 and full-length ErbB4 expression constructs and then subjected to immunoprecipitation with ErbB4 antibodies.



Cosa manca a questa IP?

→ TAB2 coprecipitated with ErbB4 only after NRG1 treatment

Presenilin-Dependent Cleavage of ErbB4 does not induce TAB2 phosphorylation

The dependence of ErbB4/TAB2 interaction on NRG1 suggested that ErbB4 activation could alter TAB2, particularly its state of phosphorylation or its cellular localization.

Phosphotyrosine western blot and ^{32}P incorporation assays in cells expressing ErbB4 did not demonstrate induction of TAB2 phosphorylation by NRG1 (data not shown).

→ It appears that ErbB4 activation does not induce TAB2 phosphorylation.

Presenilin-Dependent Cleavage of ErbB4 promotes TAB2 Nuclear Translocation

NRG1-ErbB4 signaling had dramatic effects on the cellular distribution of TAB2:
- in quiescent NIH 3T3 cells, TAB2 was excluded from the nuclei independently of whether the cells expressed ErbB4 or not.
- upon treatment with NRG1 for 2 hours, TAB2 translocated to the nucleus in cells expressing ErbB4-JMa.