Regulation of synaptic adhesion complexes by alternative splicing

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Adhesion complexes at CNS synapses

- adhesion molecules represent one of the most important morphogenic determinants in all tissues

- central regulators for cell polarization, migration, and for three-dimensional organization

- cell adhesion complexes are dynamically regulated to alter cellular architecture and function
Adhesion complexes mediate cell-cell interactions at multiple synaptic sub-domains

**peri-active zones**
- synaptic growth
- endocytic zone

Nectins
Cadherins
FasII (Still life)

**active zone / postsynaptic density**
- synaptic transmission
- alignment & recruitment

**neuron-glia junction**
- transmitter uptake
- signaling
- spine morphogenesis

EphA4
Glt-1

Protocadherins
Neuroligins
Potential roles for adhesion molecules in local regulation of connectivity

Laminar specificity

Cell type specificity

Subcellular specificity

Branching, tiling (repulsive self-recognition)
Alternative splicing is one of the key processes that increases the diversity of cell adhesion molecules.

Families of surface molecules encoded by multiple genes: olfactory receptors, classical cadherins, immunoglobulin-domain proteins, leucine-rich repeat proteins.
• three neurexin genes in mice (NRX1,2,3)
• alternative promoter choice generates 2 transcripts per gene (α and β NRX)
• alternative splicing at 5 sites generates more than 1,000 variants (Ushkaryov et al., 1992)

Neuroligins

• four genes in mouse, five in human
• further isoforms are generated by alternative splicing at two sites in the extracellular domain (Ichtchenko et al. 1995, 1996)
• neuroligins are postsynaptic adhesion molecules, interact with postsynaptic scaffolding molecules (Irie et al., 1997, Song et al 1999)
• inactivating mutations in NL3 and NL4 are associated with autism-spectrum disorders and mental retardation (Jaimain et al. 2003, Laumonnier et al 2004)
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EphA4
Glt-1
Neurexins
Neuroligins

presynaptic terminal
postsynaptic terminal

glia
Outline

1. Subcellular localization of neuroligins and neurexins at hippocampal synapses

2. Analysis of splice isoforms-specific functions

3. Mechanisms that control alternative splicing
Freeze-fracture replica immuno-EM

5 nm gold: pan-neuroligin
10 nm gold: PSD95

Neuroligins are closely associated with neurotransmitter receptors in the postsynaptic membrane

10 nm gold: GABA-A R beta 2 subunit
5 nm gold: neuroligin-2

Elaine Budreck, Ryuichi Shigemoto
GFP-beta-Neurexin-1 mice

Mutually Exclusive Categories

Within Terminals
At/Near Presynaptic Membranes
At Clefts
At PSDs
Near PSDs
Within Spines

pan-neurexin antibodies

Chiye Aoki
Francisco G. Scholl
Model of the synaptic Neuroligin-Neurexin adhesion complex

presynaptic: Neurexin

postsynaptic: Neuroligin

De Camilli et al.
Neurexin and neuroligin genes encode large numbers of splice variants

α-NRX

β-NRX

NL1

NL2

A

B

GPTKKTDDLDGNDGAED

GNRWSNSTK

Acetylcholinesterase domain
Selectivity of neuroligins for glutamatergic and GABAergic synapses

(Song et al. 1999; Prange et al, 2004; Varoqueaux et al., 2004, Graf et al. 2004)
Neuroligin variants generated by alternative splicing

NL1

NL2

Acetylcholinesterase domain

TMD WW PDZ

TMD WW PDZ

Hippo Cortex Cereb

NL1A

NL1B

NL2A

βactin

Leora Gollan
Alternative splicing controls localization of neuroligin-2

Ben Chih, Leora Gollan
Presence of the B insertion is sufficient to prevent activity of neuroligin-2 towards GABAergic axons.
Synaptic selectivity of NL splice variants

 insertion A: localization and function at GABAergic contacts

 insertion B: localization and function at glutamatergic contacts

 B insertion is dominant
Neuroligin-1 splice variants differ in their interactions with neurexin-1β isoforms

similar findings: Boucard et al., 2005; Graf et al. 2006
Candidate neurexins for interactions at GABAergic synapses

- Glutamatergic axon
- GABAergic axon

- NRXα
- NRXβ
- NRXβ4
- NL1B
- NL1A
- NL2A
- dendrite
Selective function for neurexin variants in GABAergic postsynaptic differentiation

![Image showing immunofluorescence for PSD95/VGlut1 and gephyrin/VGAT with bar graphs for PSD95 and gephyrin localization](image)

**PSD95**

- EGFP
- NRX1β4(-)
- NRX1β4(+)
- NRX1α4(-)

**VGlut1-negative PSD95 puncta (% of COS cell area)**

- EGFP
- NRX1β4(-)
- NRX1β4(+)
- NRX1α4(-)

**Gephyrin**

- EGFP
- NRX1β4(-)
- NRX1β4(+)
- NRX1α4(-)

**VGAT-negative gephyrin puncta (% of COS cell area)**

- EGFP
- NRX1β4(-)
- NRX1β4(+)
- NRX1α4(-)

Ben Chih
GABAergic axon

glutamatergic axon

NRXβ4(-)

NRXα

NRXβ4(+)

NL1B

NL1A

NL2A

dendrite

vGlut1

VGAT

FC

NRX FC

NRX FC

Ben Chih
• alternative splicing of neuroligin-1 and -2 regulates localization and function at GABAergic vs. glutamatergic contacts

• neurexin splice variants that interact selectively with the GABAergic neuroligin variants selectively induce GABAergic postsynaptic differentiation

• alternative splicing underlies selective trans-synaptic interactions of the neuroligin - neurexin complex
What is the spatial and temporal regulation of Neurexin splicing?
Dynamic alterations in neurexin splicing have been reported, e.g. in response to growth factor signaling, seizure or ischemia (Patzke and Ernsberger, 2000; Gorecki et al. 1999, Sun et al. 2000)
A quantitative assay for neurexin splicing

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Splice reporters with inactivated donor and acceptor sites

RNA

E: donor splice-site mutant

A: splicing reporter

Aacc: acceptor site mutant

Protein

translation

RFP

DNA transf.

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Processing of splice reporter RNAs in hippocampal neurons

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1. specific introns are retained in cytoplasmic neurexin-1 mRNA

2. unspliced RNA delivered into the cytoplasm of hippocampal neurons can be processed by the cellular machinery

→ is the neurexin-1 mRNA processed through a cytoplasmic splicing mechanism?
RNA processing in mechanically isolated axons

Leora Gollan
Cytoplasmic splicing of synaptic proteins would provide a novel mechanism for local modifications of cell function.

In the neuroligin-neurexin complex, alternative splicing regulates selective adhesive interactions.
Candidate mechanisms for cytoplasmic neurexin mRNA processing

**Non-conventional cytoplasmic mechanism:**
-Ire1p - cleavage
-tRNA ligase - exon joining

Sidrauski, Walter and colleagues

**Cytoplasmic mechanism using conventional machinery:**
Glanzer et al. PNAS 102(46):16859-64 suggested splicing-like mRNA processing in dendrites
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