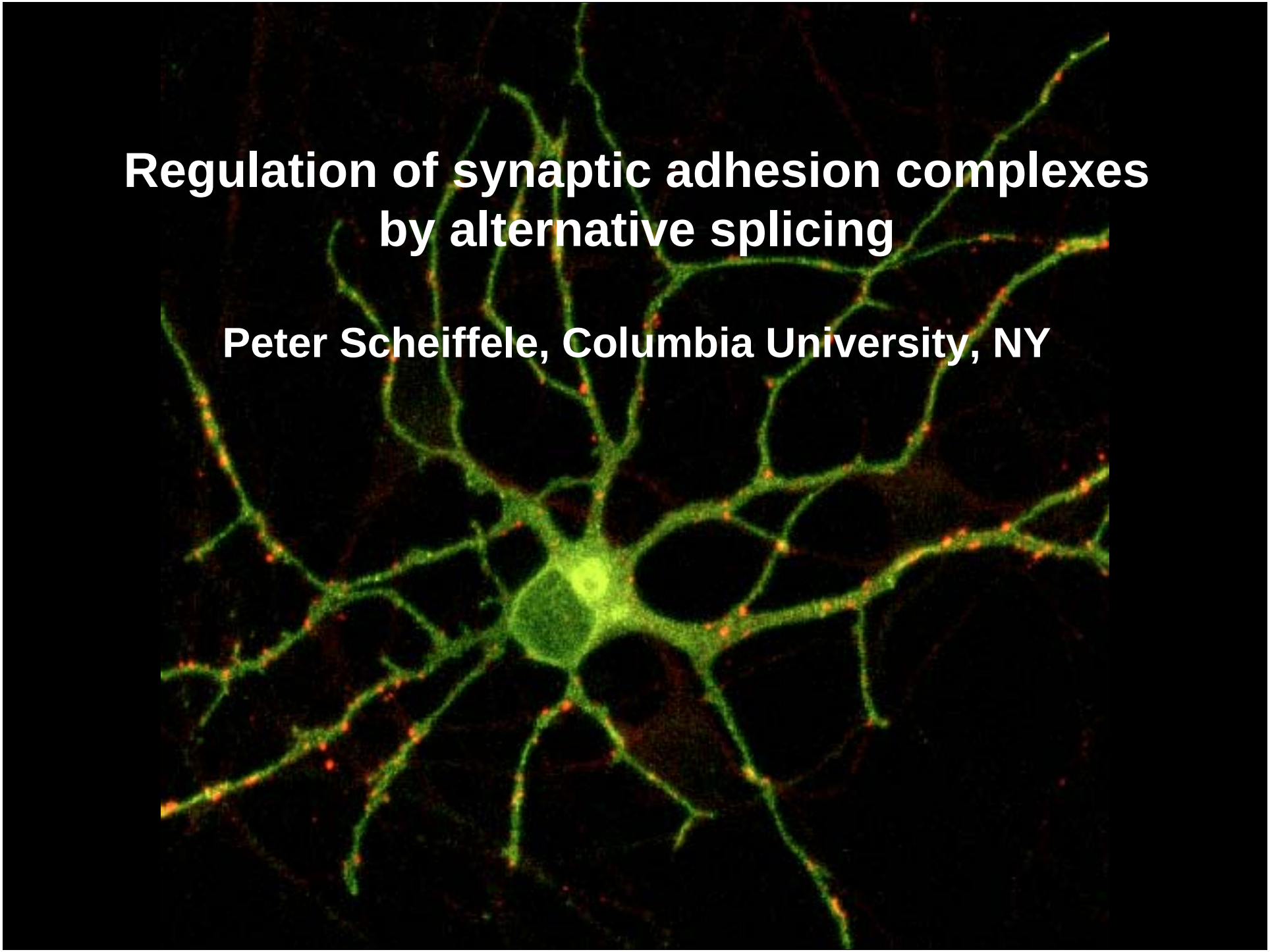
Regulation of synaptic adhesion complexes by alternative splicing

Peter Scheiffele, Columbia University, NY

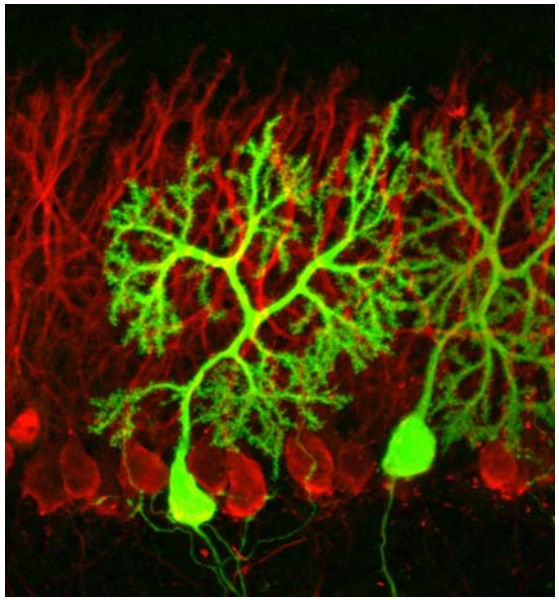


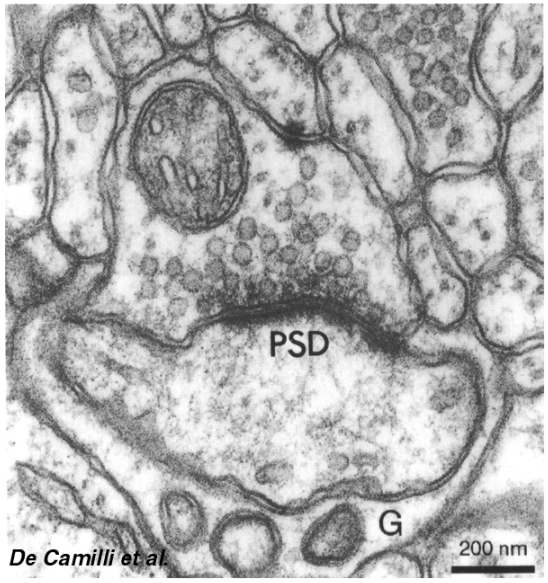
Adhesion complexes at CNS synapses

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

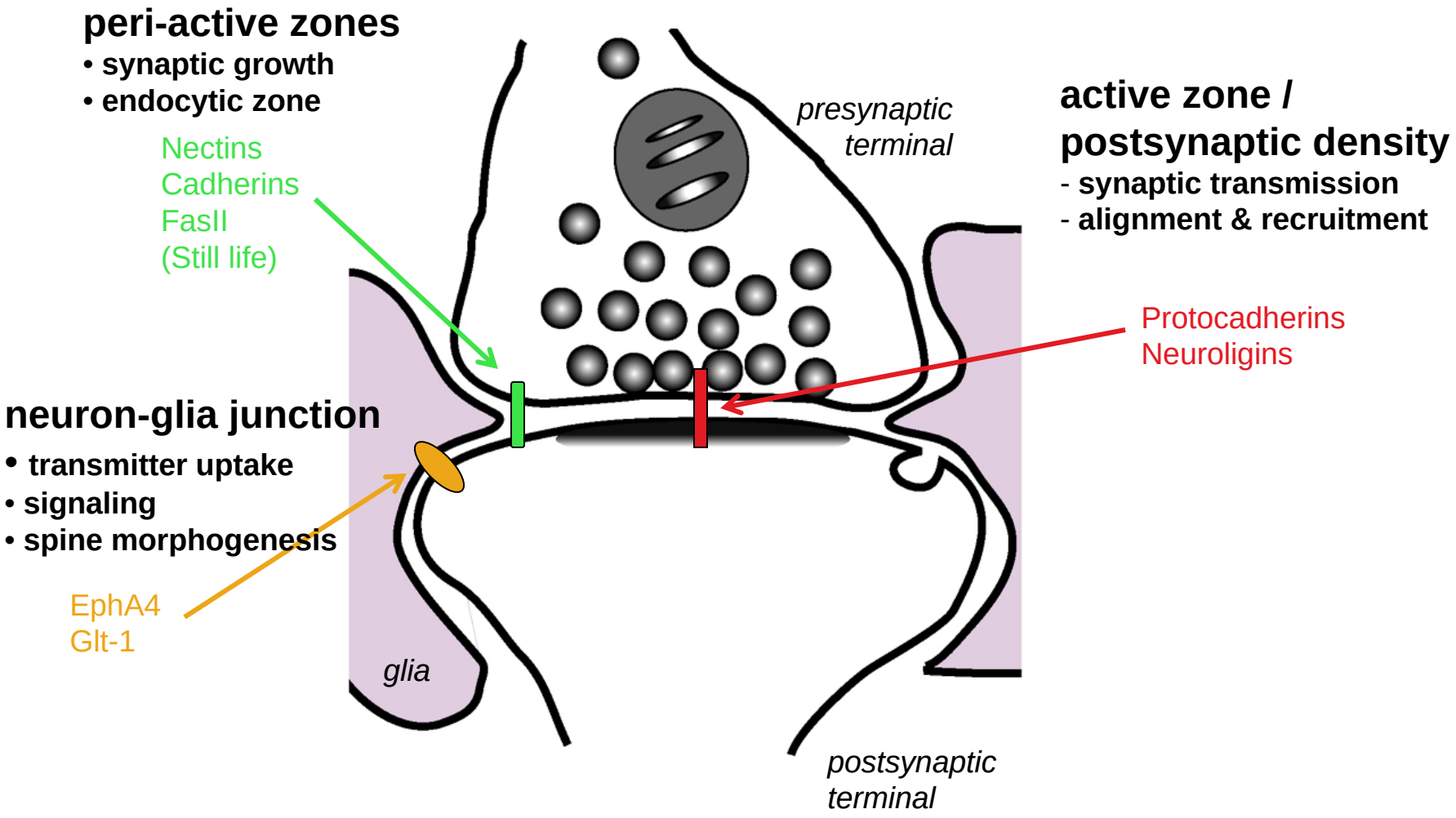
- central regulators for cell polarization, migration, and for three-dimensional organization

- cell adhesion complexes are dynamically regulated to alter cellular architecture and function



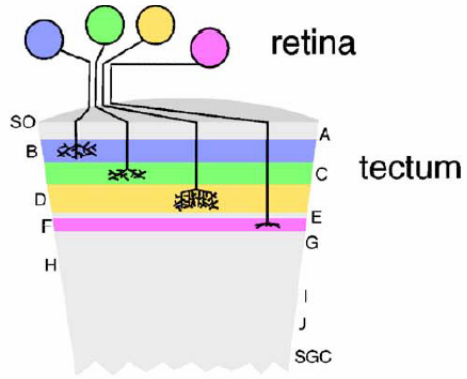


Adhesion complexes mediate cell-cell interactions at multiple synaptic sub-domains

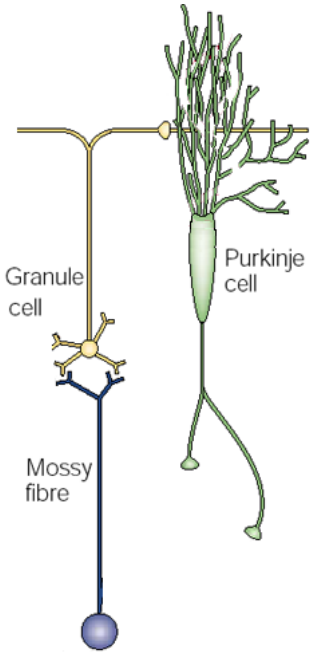


Potential roles for adhesion molecules in local regulation of connectivity

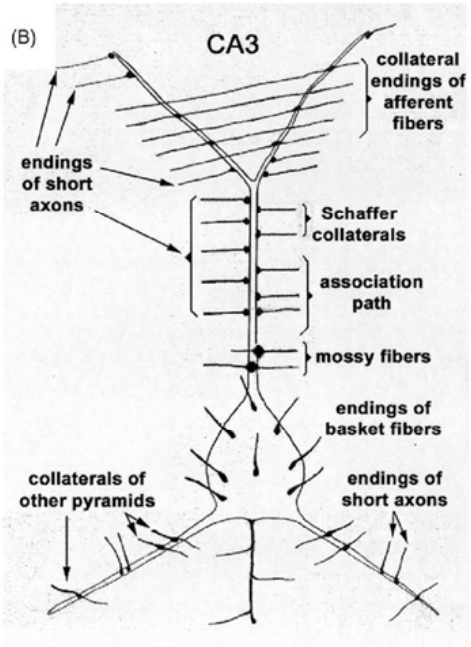
Laminar specificity



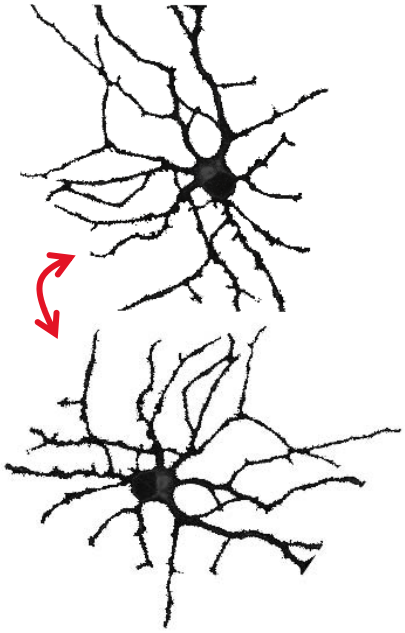
Cell type specificity



Subcellular specificity

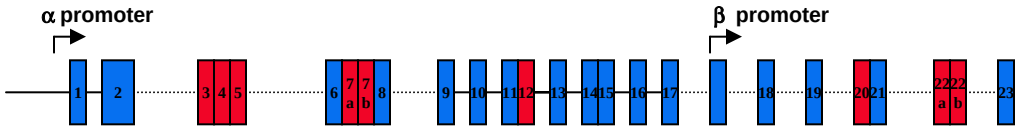


Branching, tiling (repulsive self-recognition)

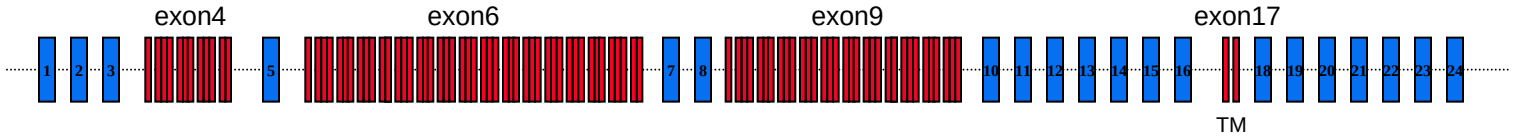


Alternative splicing is one of the key processes that increases the diversity of cell adhesion molecules

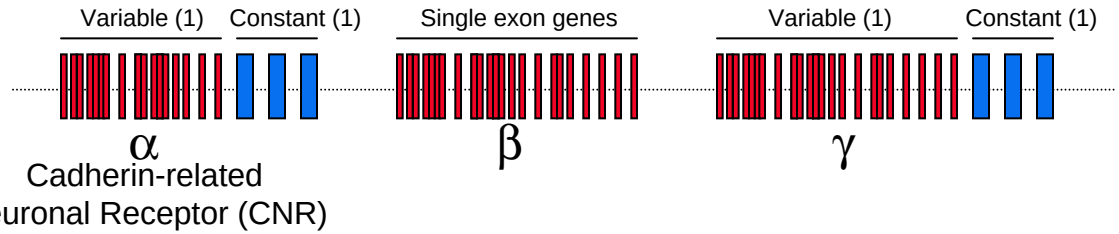
Neurexin-1 genomic locus



DSCAM genomic locus

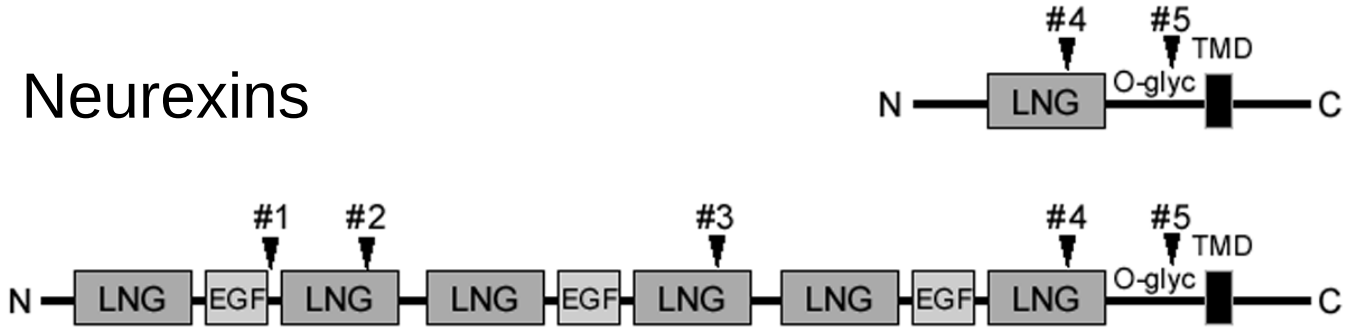


Protocadherin genomic locus



Families of surface molecules encoded by multiple genes:
olfactory receptors, classical cadherins, immunoglobulin-domain proteins, leucine-rich repeat proteins

Neurexins

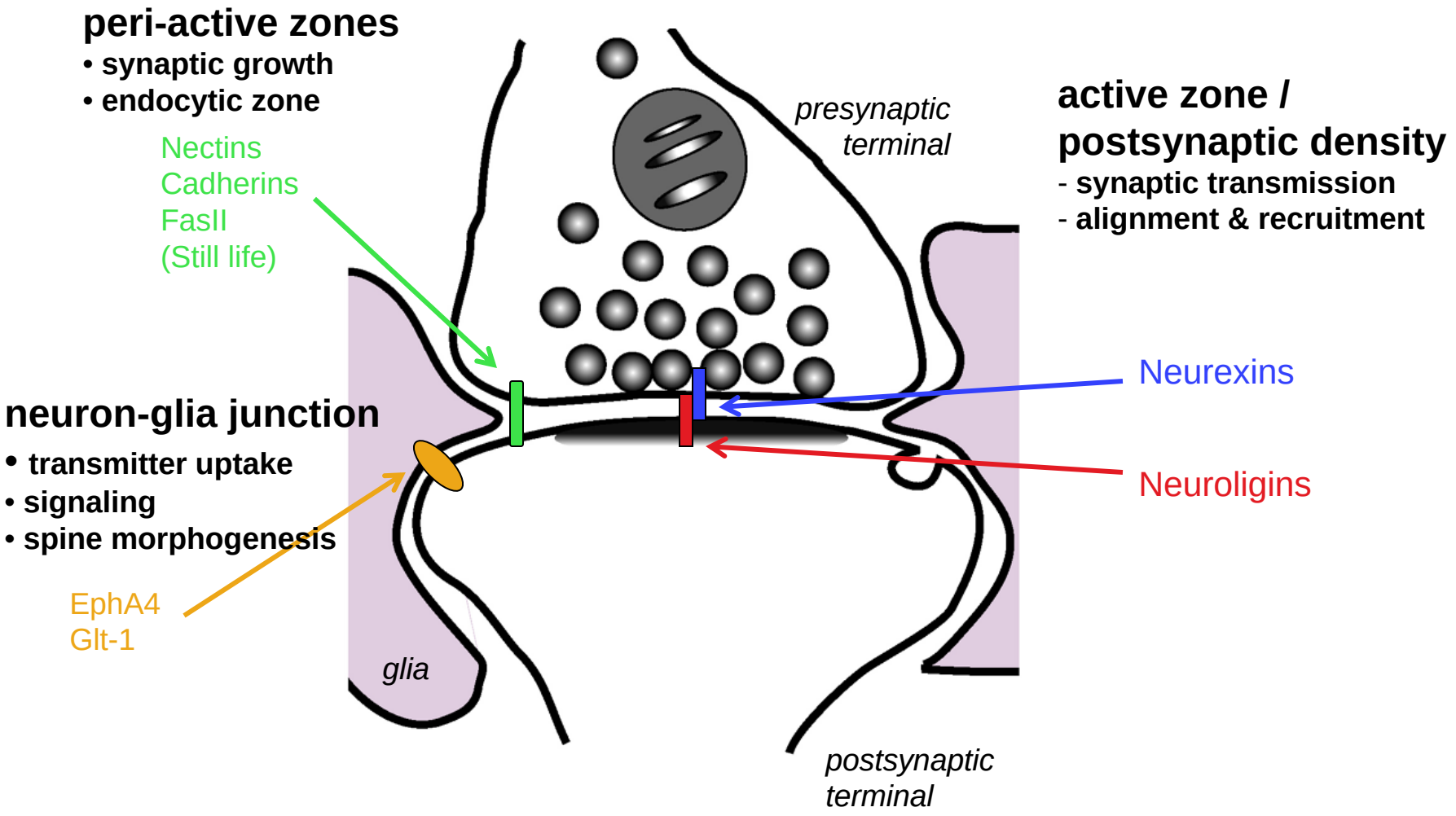


- three neurexin genes in mice (NRX1,2,3)
- alternative promoter choice generates 2 transcripts per gene (α and β NRX)
- alternative splicing at 5 sites generates more than 1,000 variants (Ushkaryov et al., 1992)

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)

Adhesion complexes mediate cell-cell interactions at multiple synaptic sub-domains



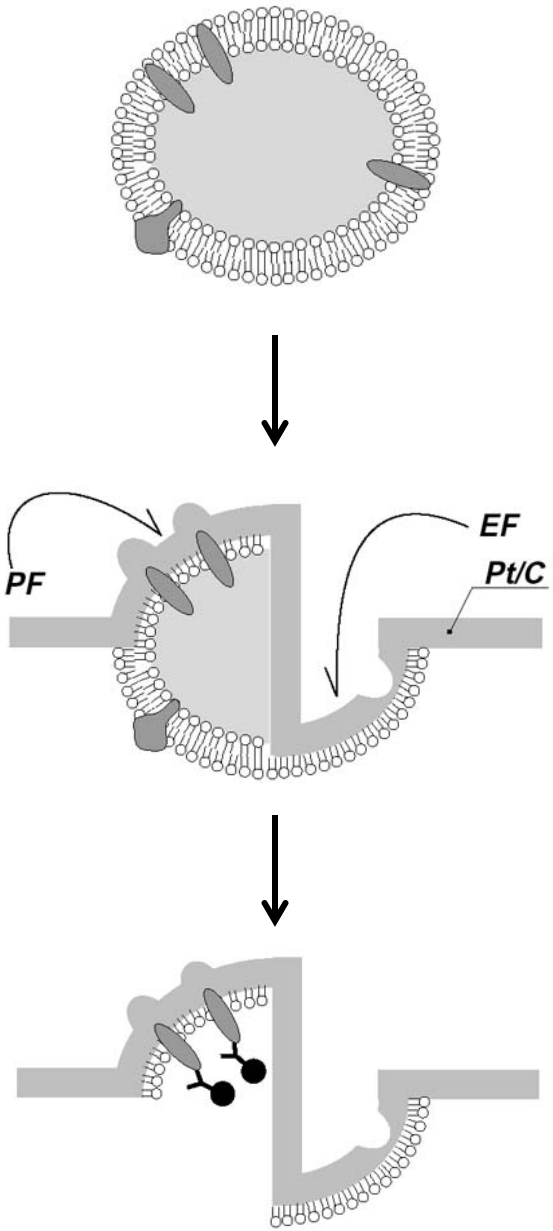
Outline

1. Subcellular localization of neuroligins and neurexins at hippocampal synapses

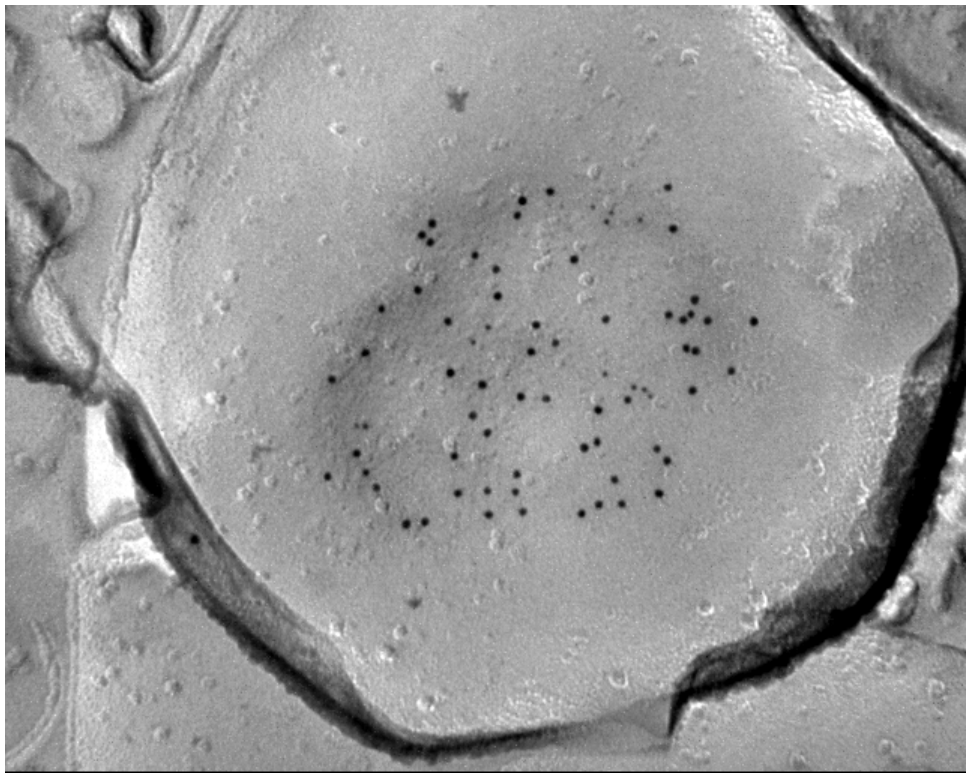
2. Analysis of splice isoforms-specific functions

3. Mechanisms that control alternative splicing

Freeze-fracture replica immuno-EM



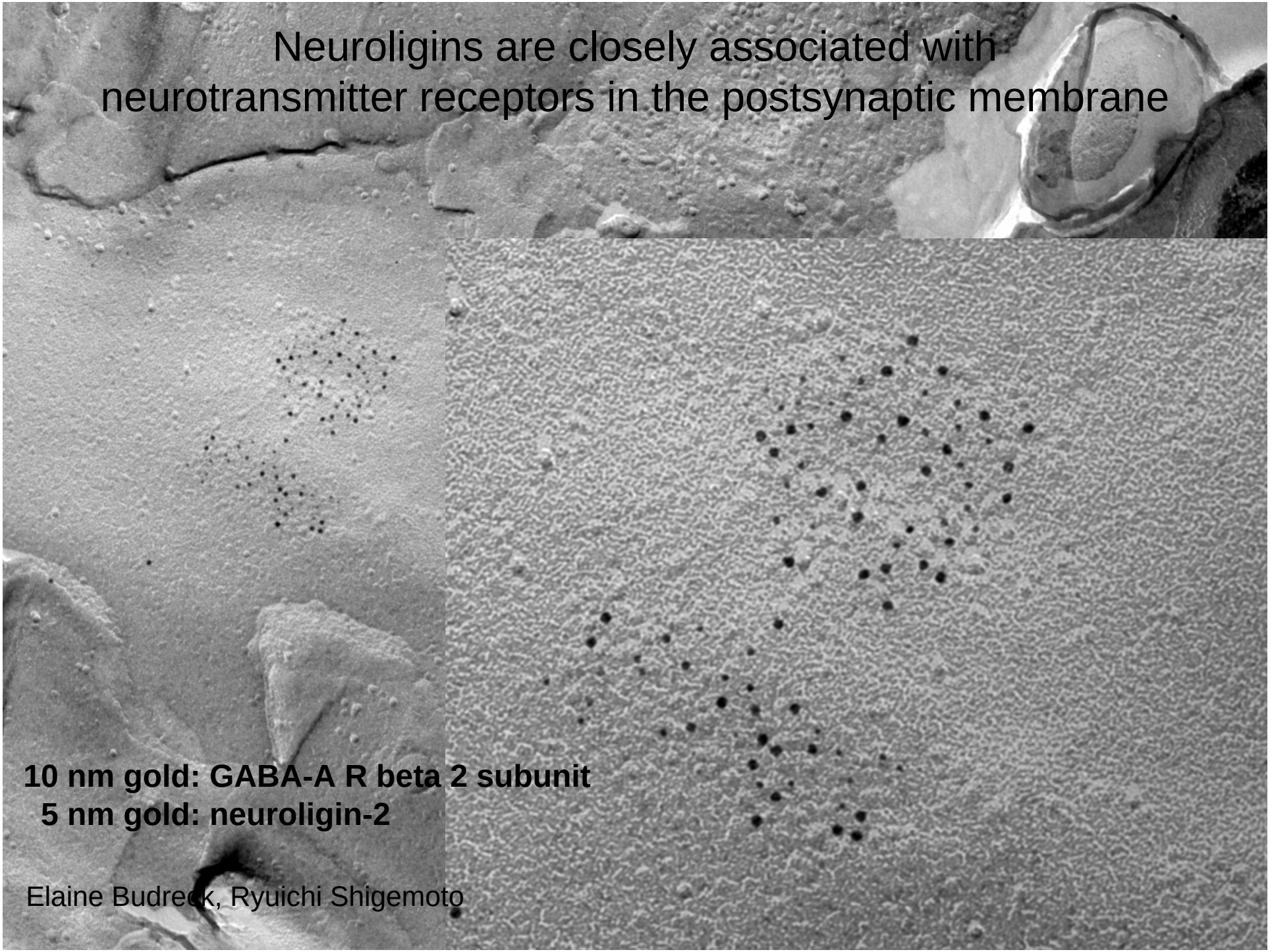
Elaine Budreck
Ryuichi Shigemoto



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

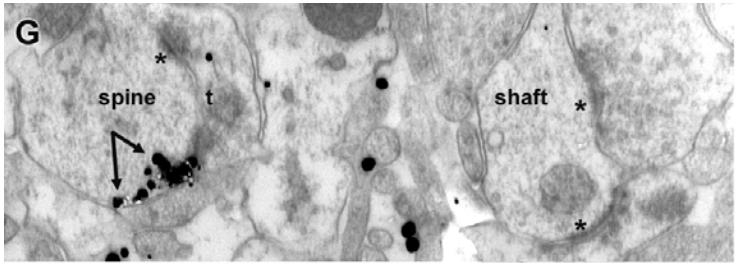
Kazushi Fujimoto, J. Cell Sci., 108:3443-3449, 1995

Neuroligins are closely associated with neurotransmitter receptors in the postsynaptic membrane

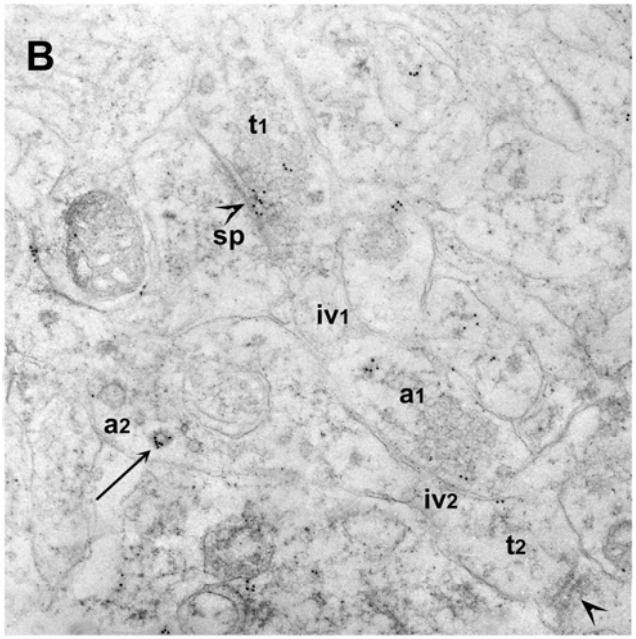


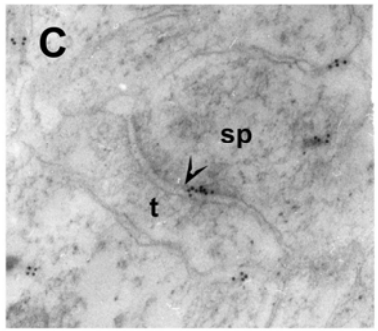
10 nm gold: GABA-A R beta 2 subunit
5 nm gold: neuroligin-2

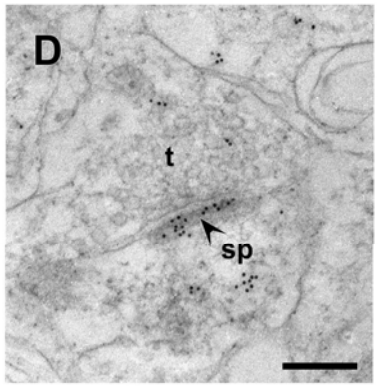
Elaine Budreck, Ryuichi Shigemoto

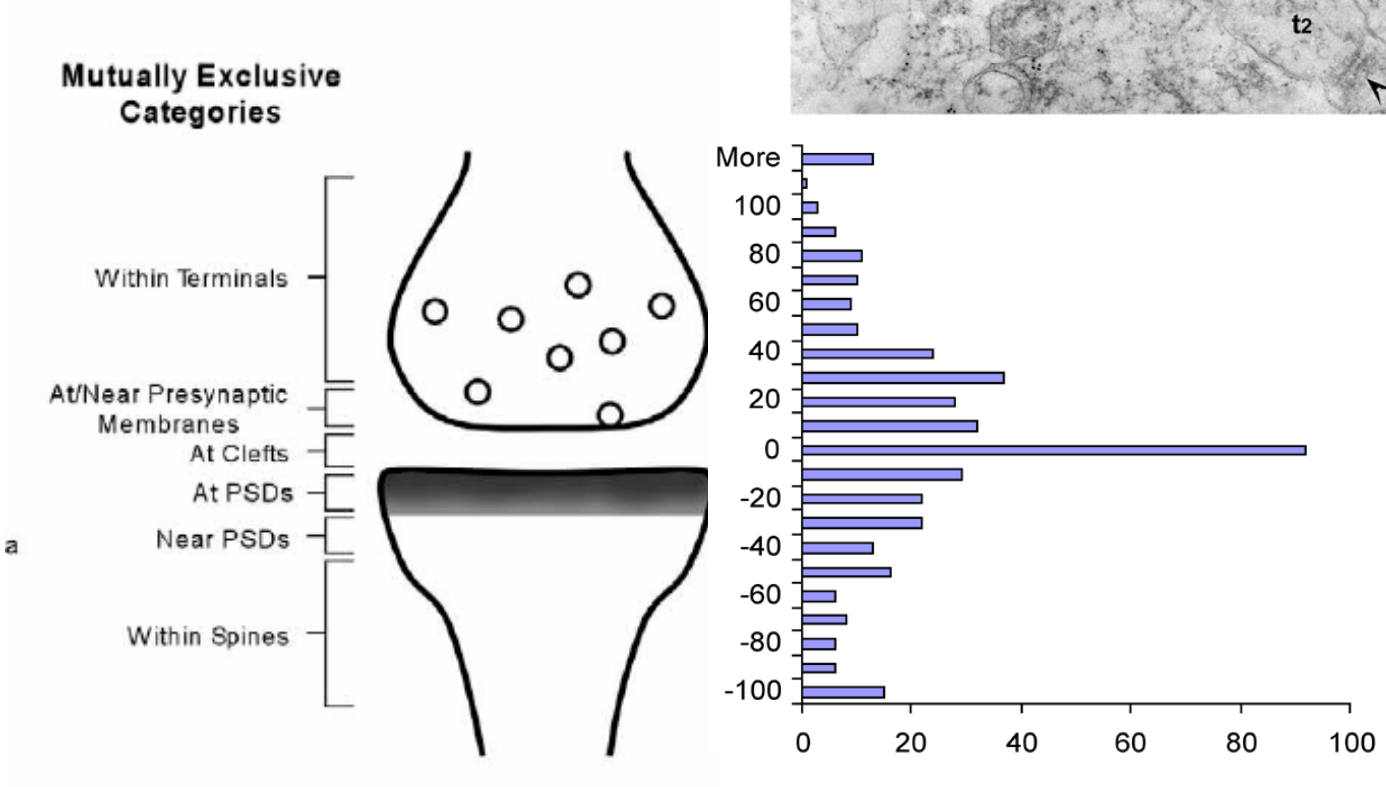


GFP-beta-Neurexin-1 mice





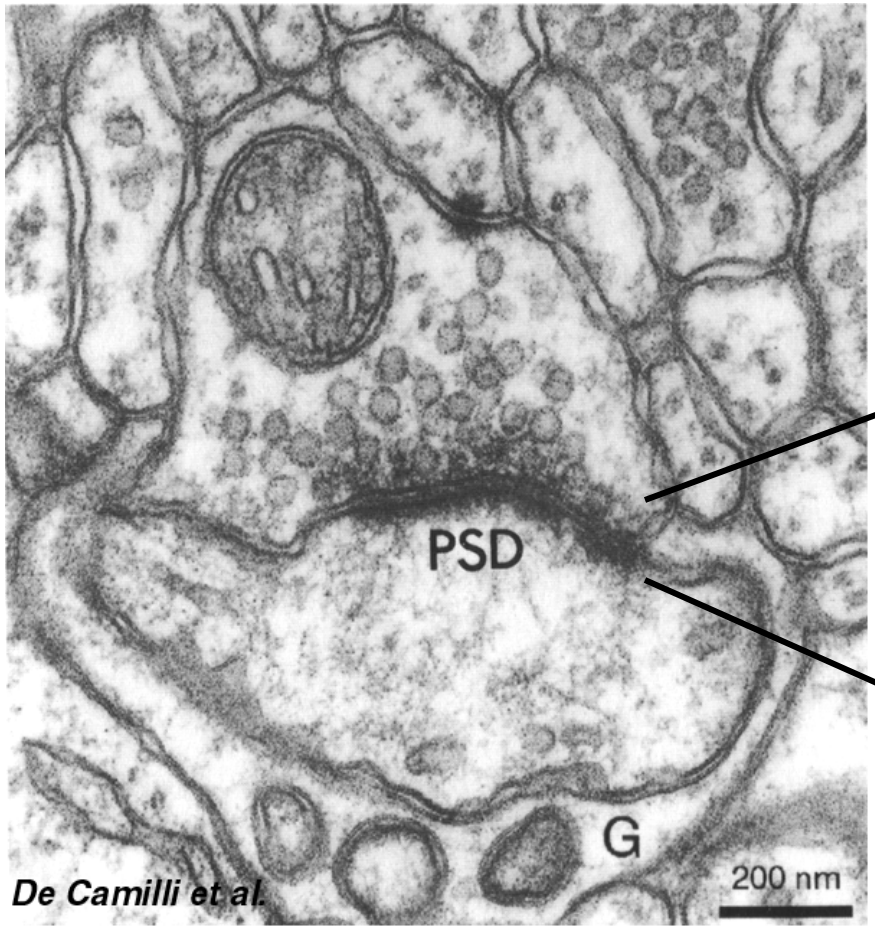


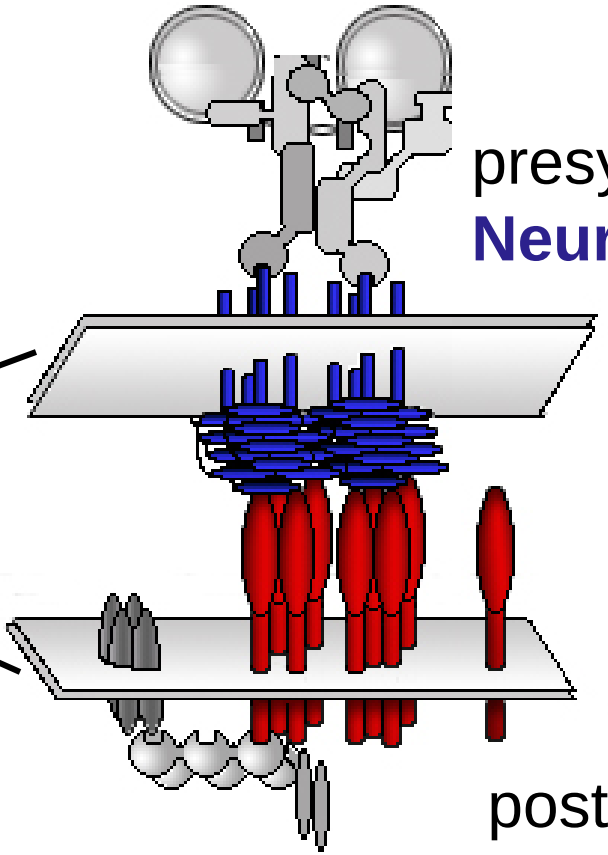


pan-neurexin antibodies

Chiye Aoki
Francisco G. Scholl

Model of the synaptic Neuroligin-Neurexin adhesion complex

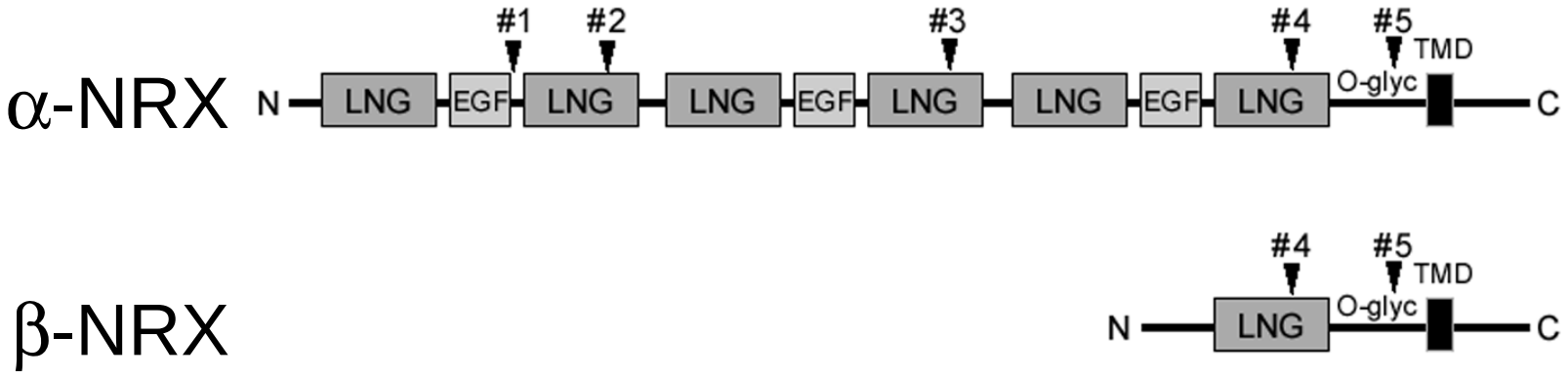


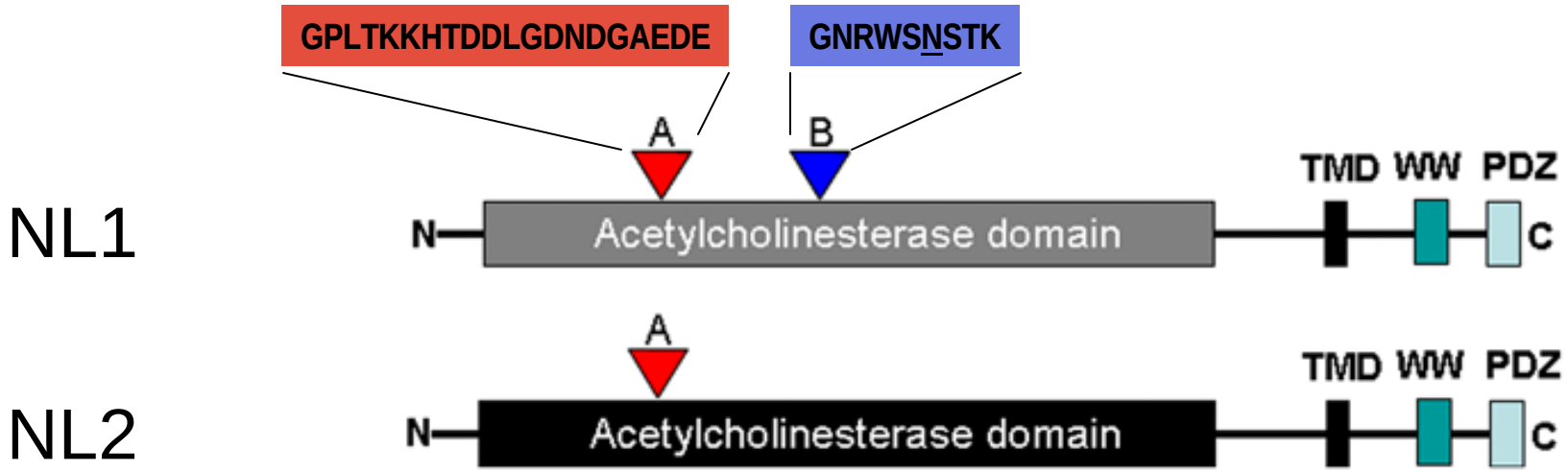


presynaptic:
Neurexin

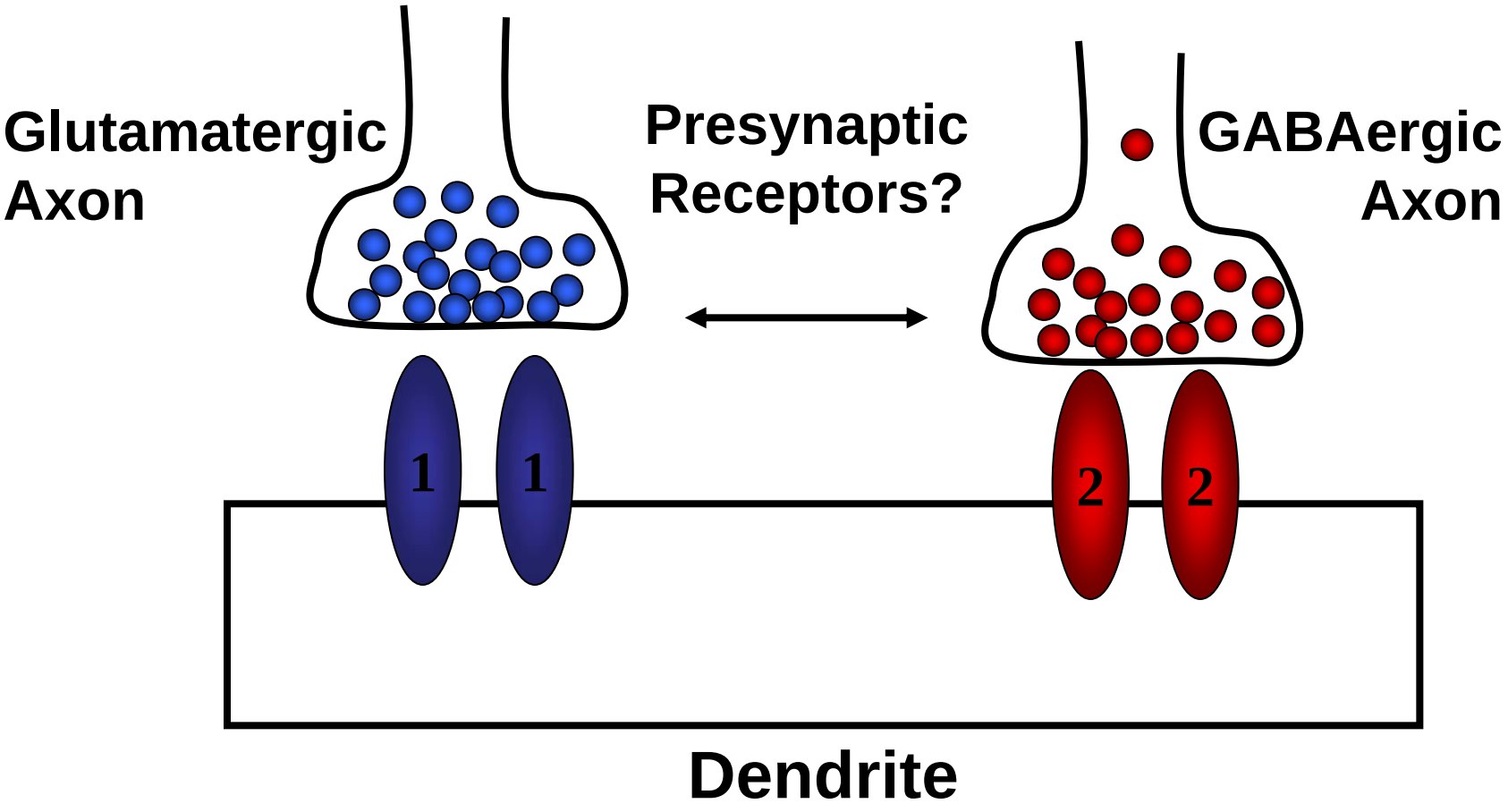
postsynaptic:
Neuroligin

Neurexin and neuroligin genes encode large numbers of splice variants



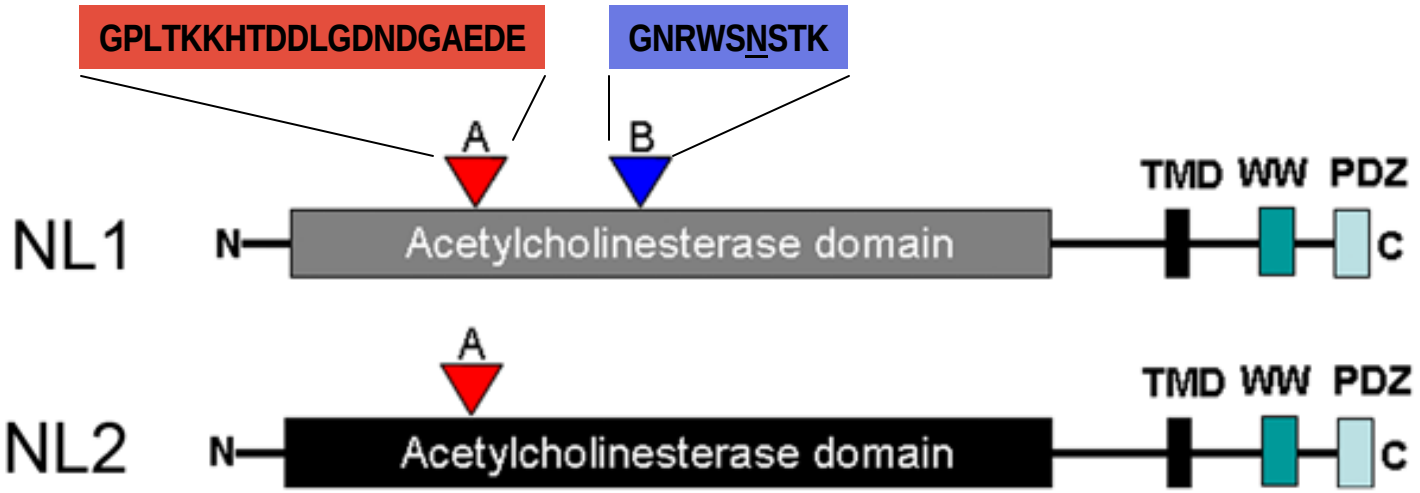


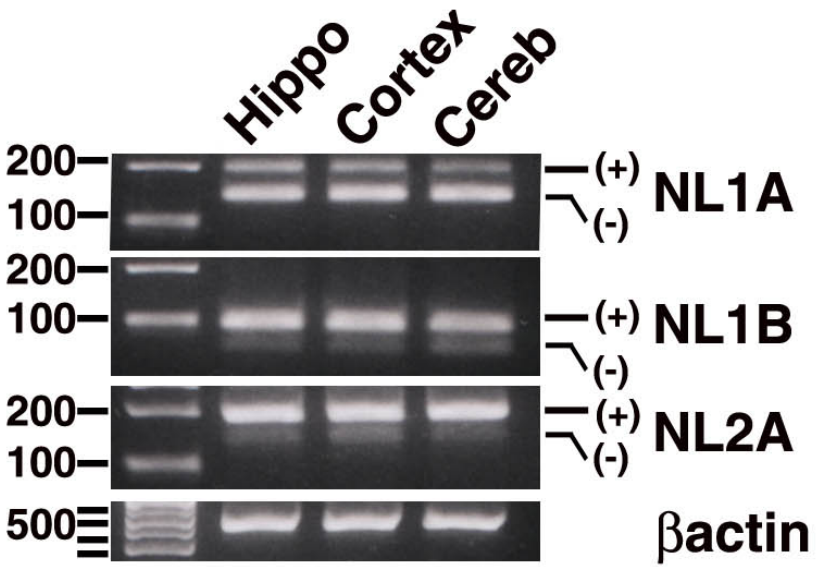
Selectivity of neuroligins for glutamatergic and GABAergic synapses



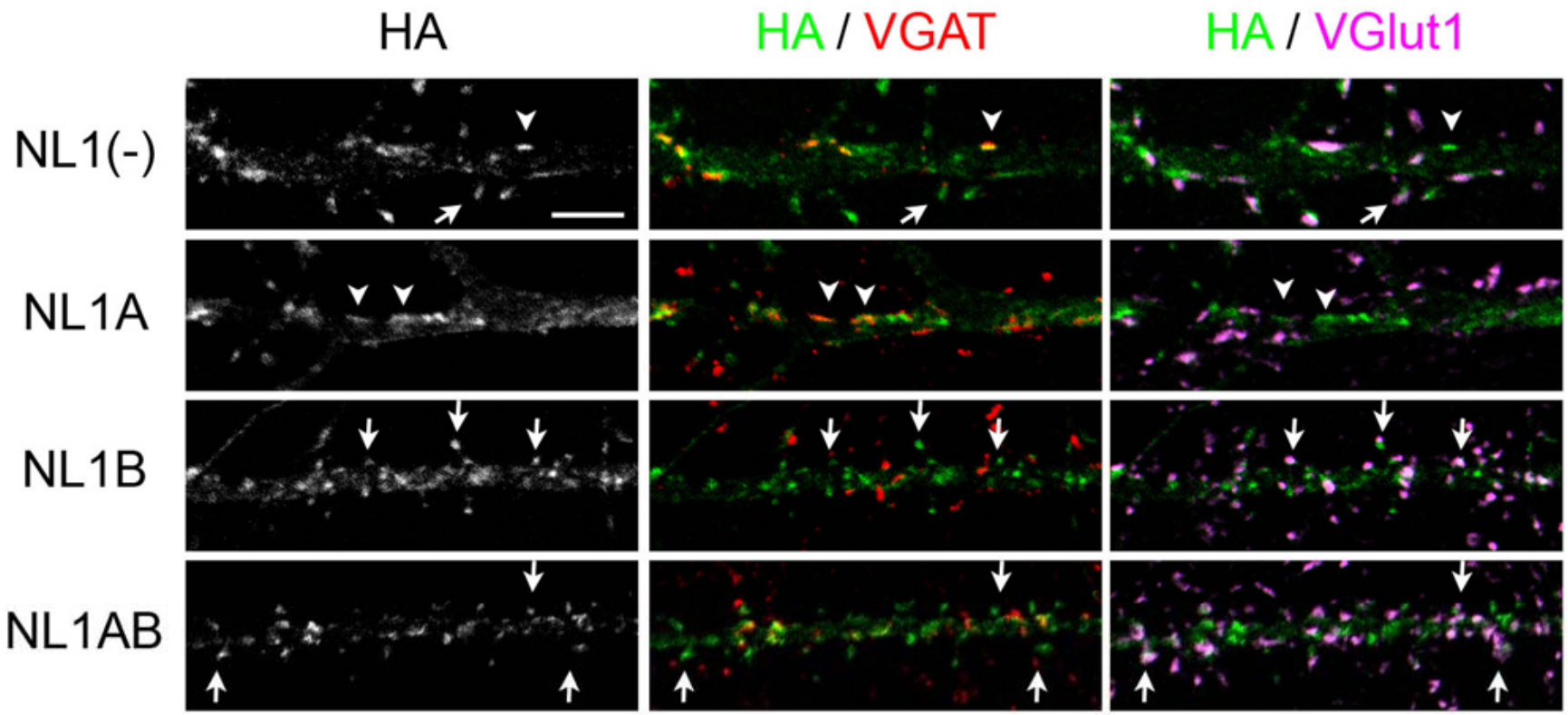
(Song et al. 1999; Prange et al, 2004; Varoqueaux et al., 2004, Graf et al. 2004)

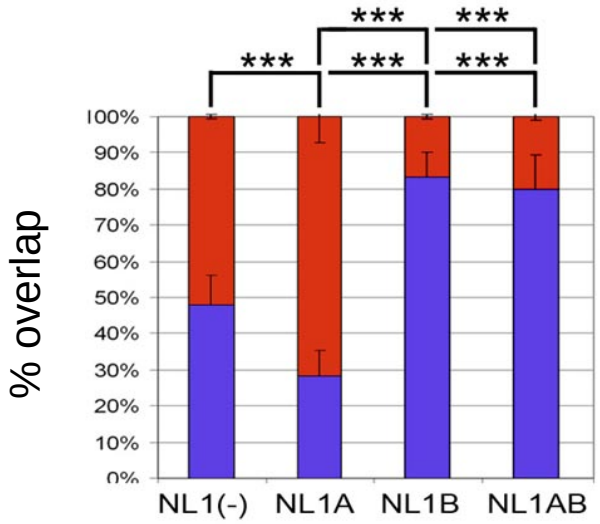
Neuroligin variants generated by alternative splicing

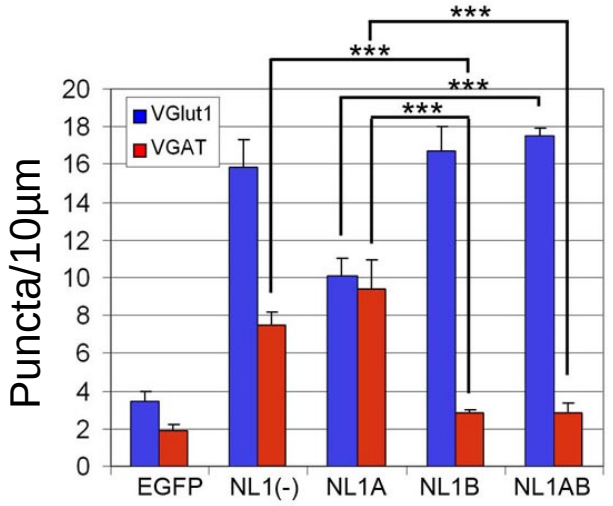




Leora Gollan



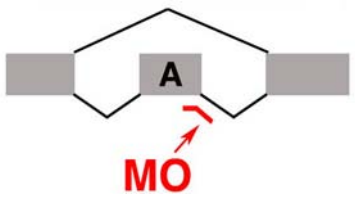


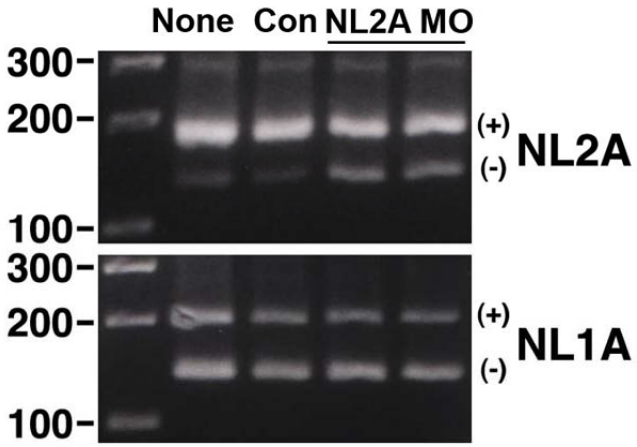


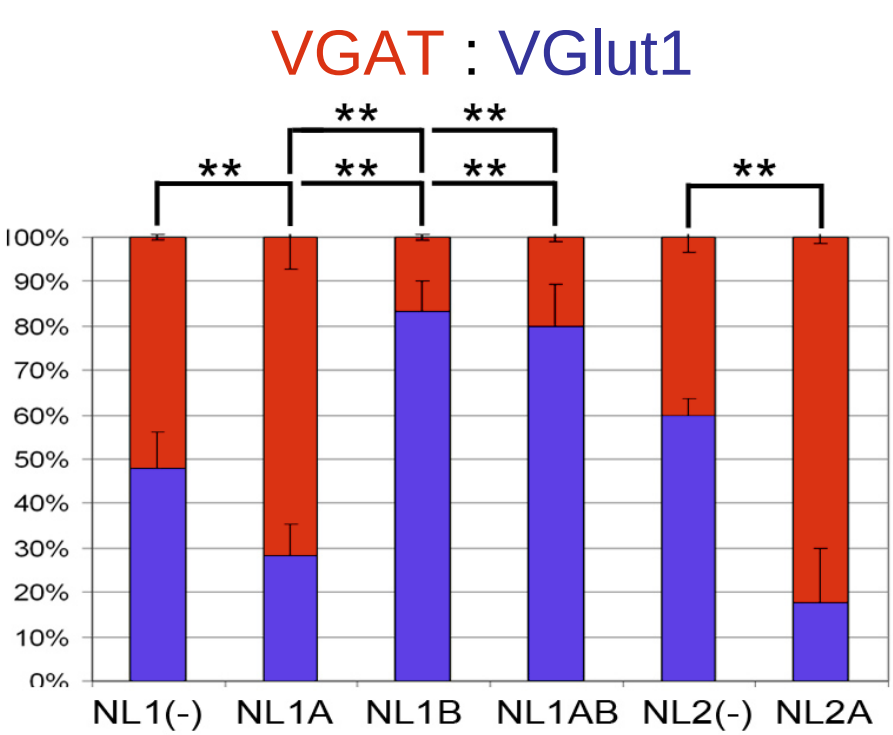
VGAT : VGlut1

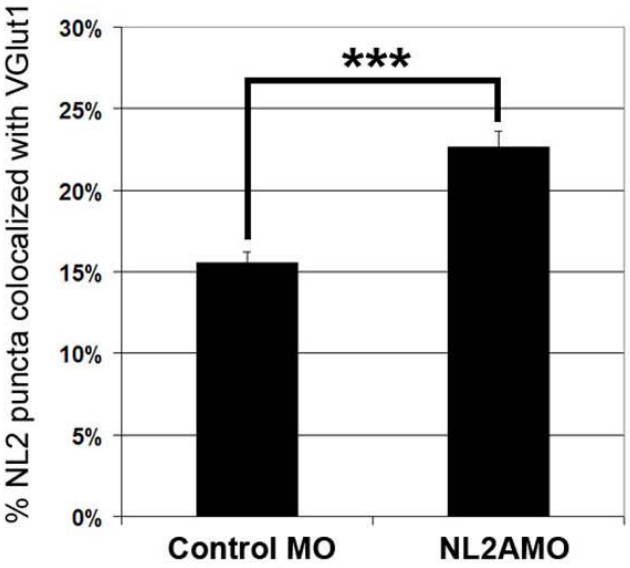
Ben Chih

Alternative splicing controls localization of neuroligin-2



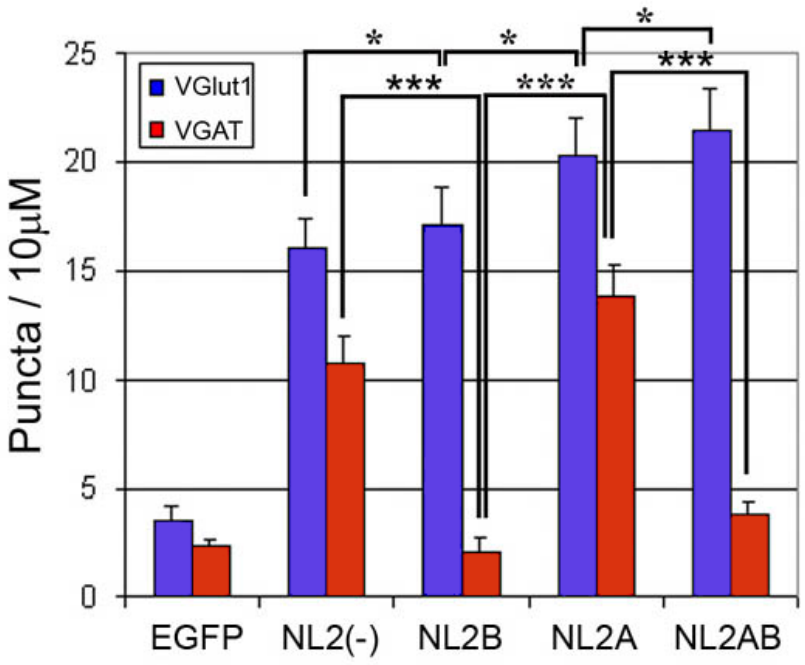






Ben Chih, Leora Gollan

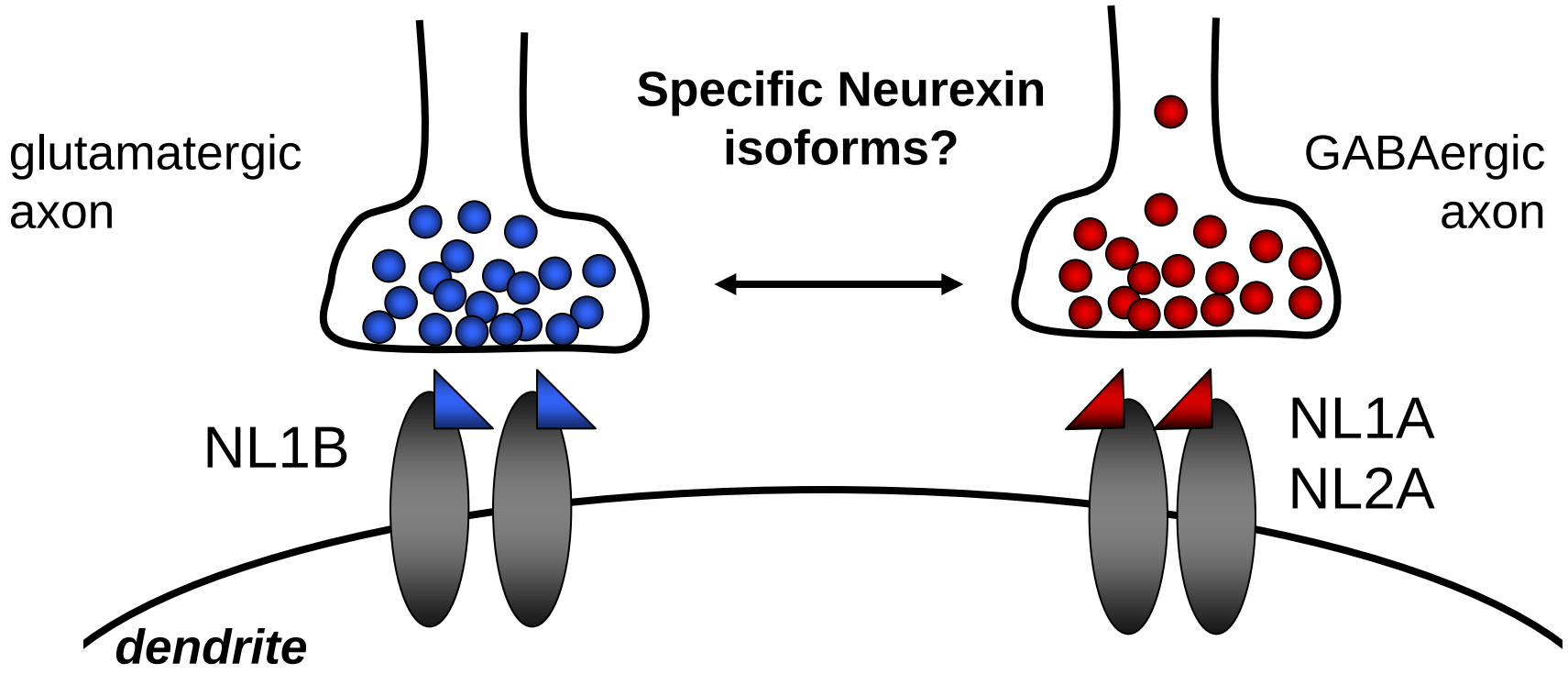
Presence of the B insertion is sufficient to prevent activity of neuroligin-2 towards GABAergic axons



VGlut1 : VGAT

Ben Chih

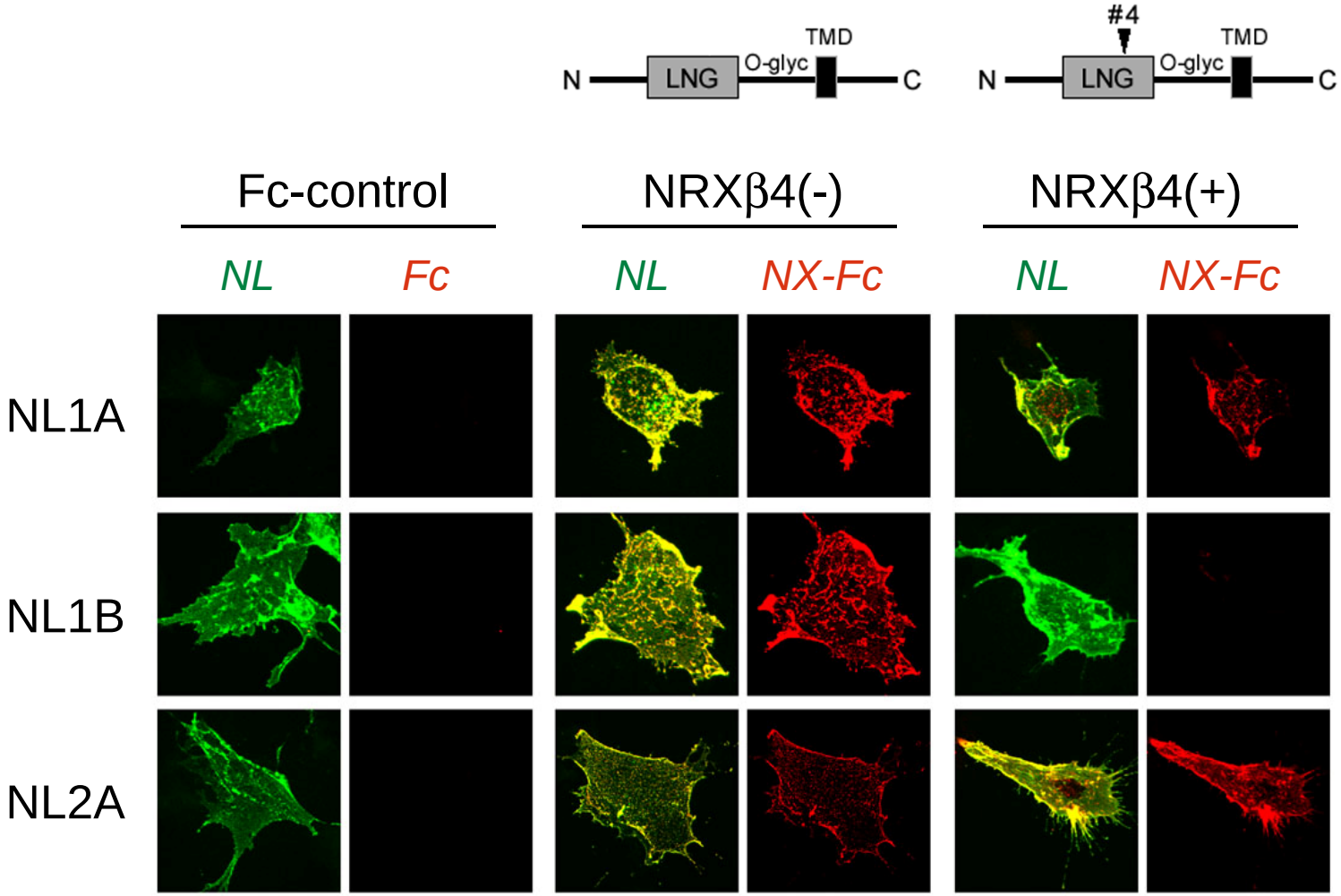
Synaptic selectivity of NL splice variants



insertion A: localization and function at GABAergic contacts

insertion B: localization and function at glutamatergic contacts
B insertion is dominant

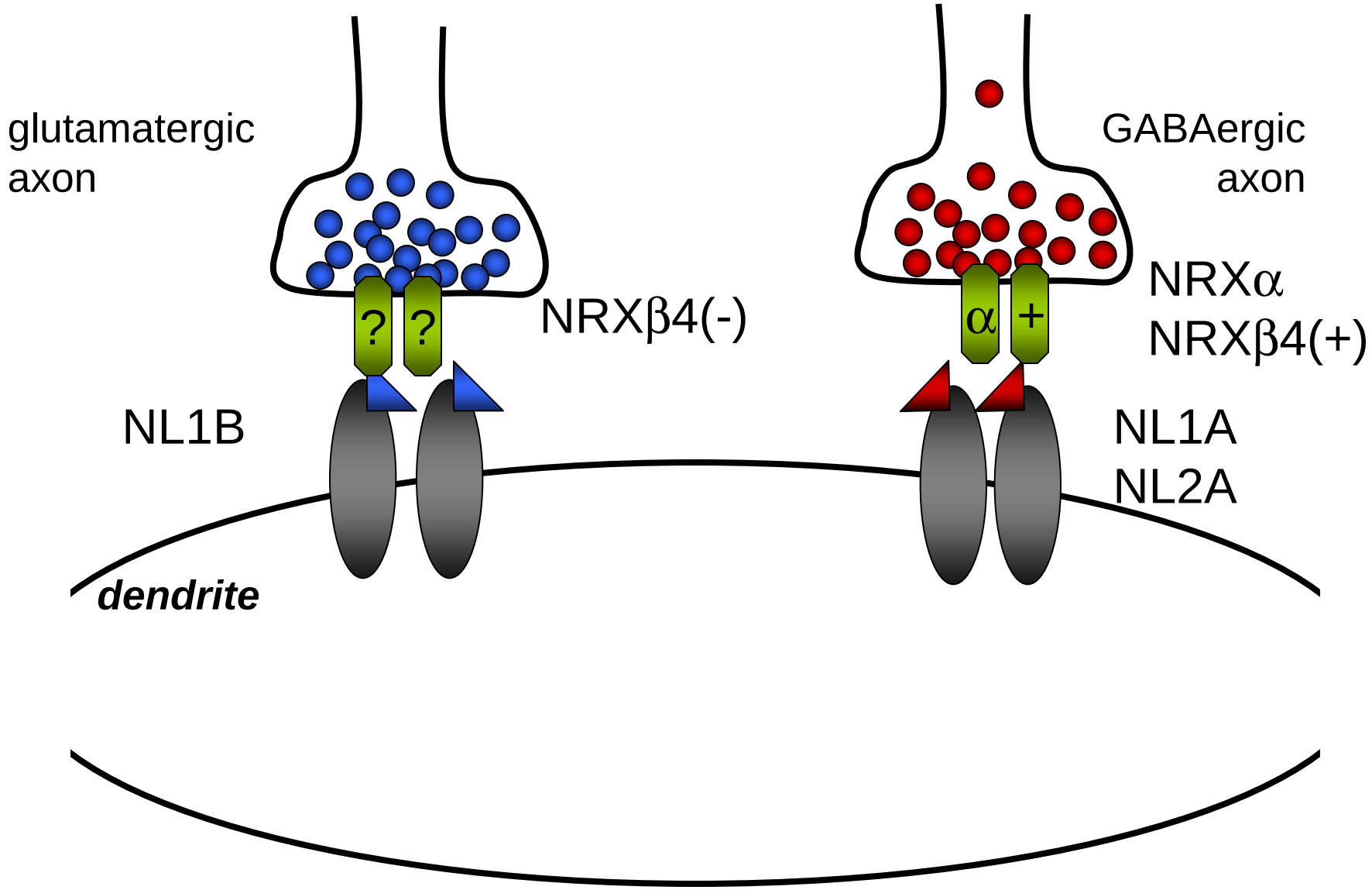
Neuroligin-1 splice variants differ in their interactions with neurexin-1β isoforms



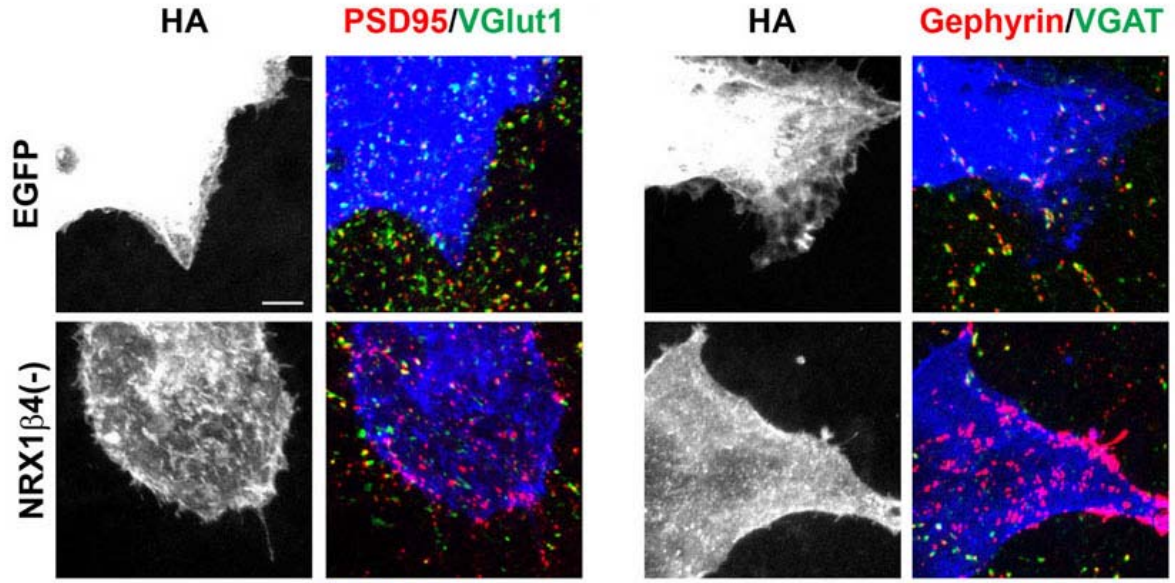
similar findings: Boucard et al., 2005; Graf et al. 2006

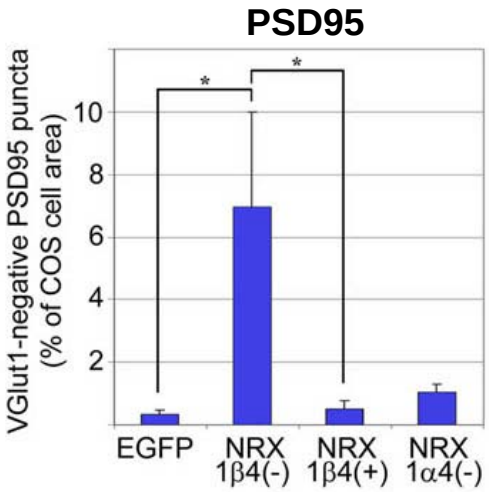
Ben Chih

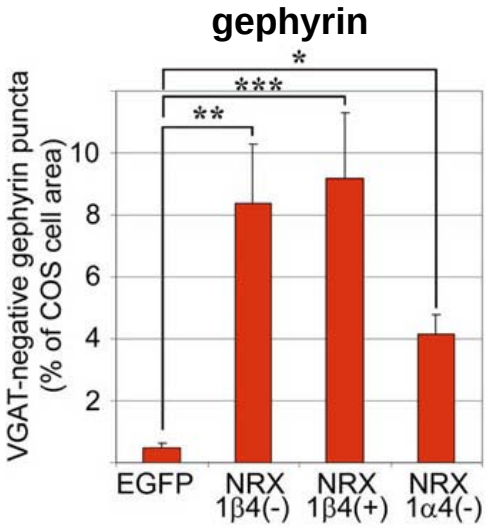
Candidate neurexins for interactions at GABAergic synapses



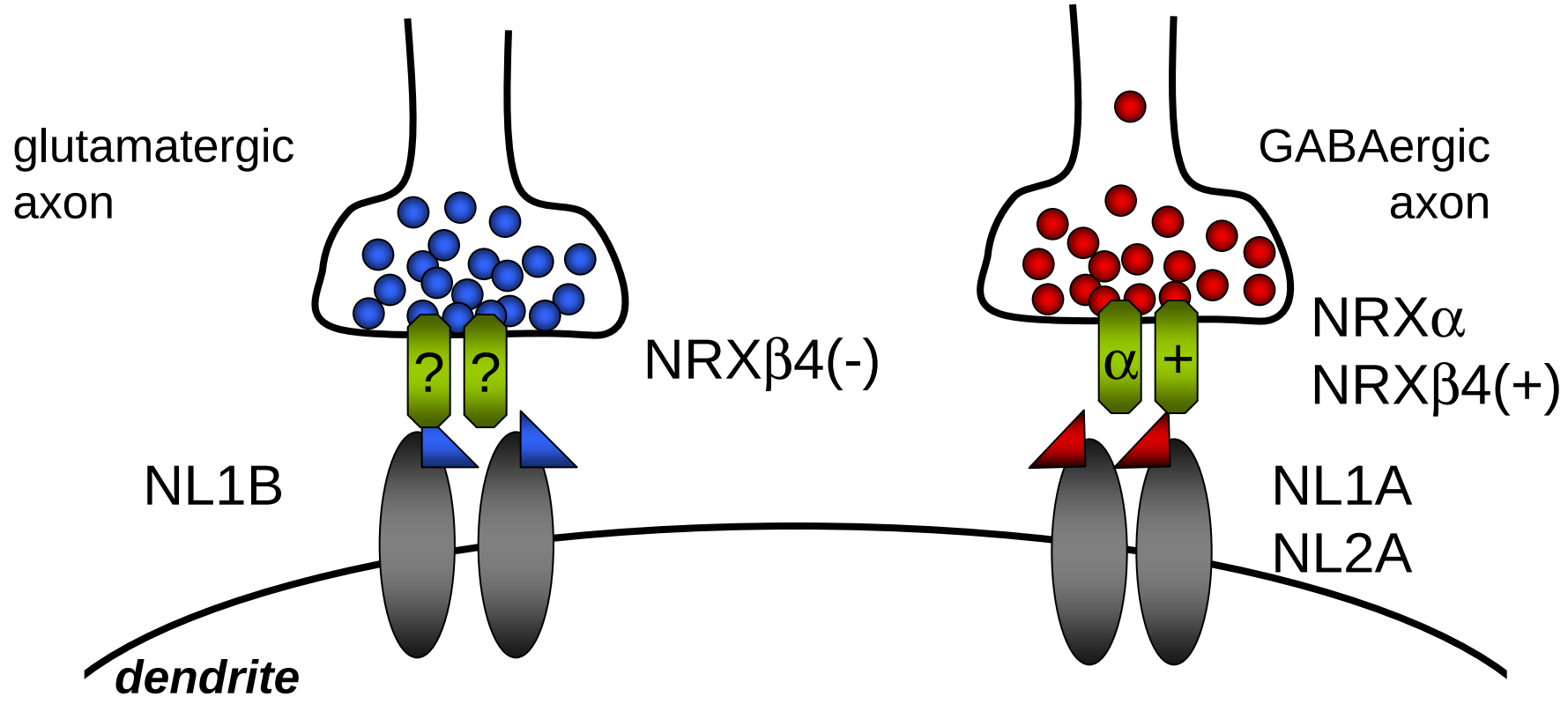
Selective function for neurexin variants in GABAergic postsynaptic differentiation

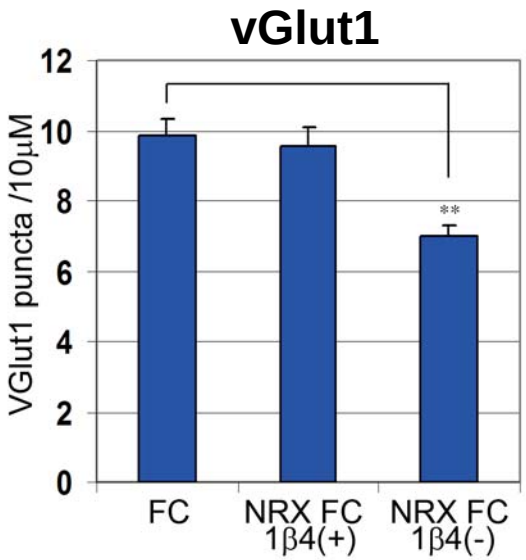


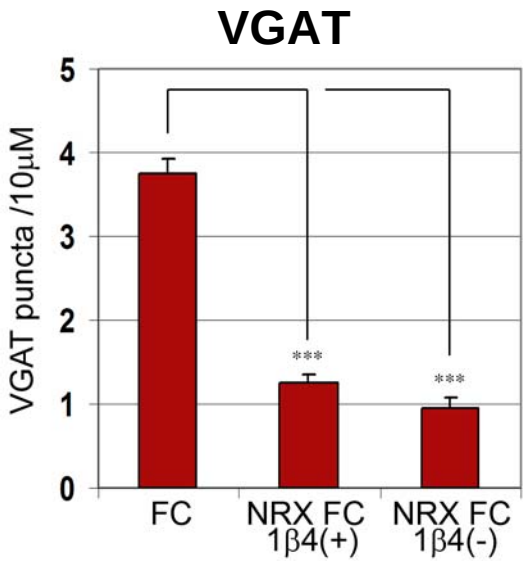




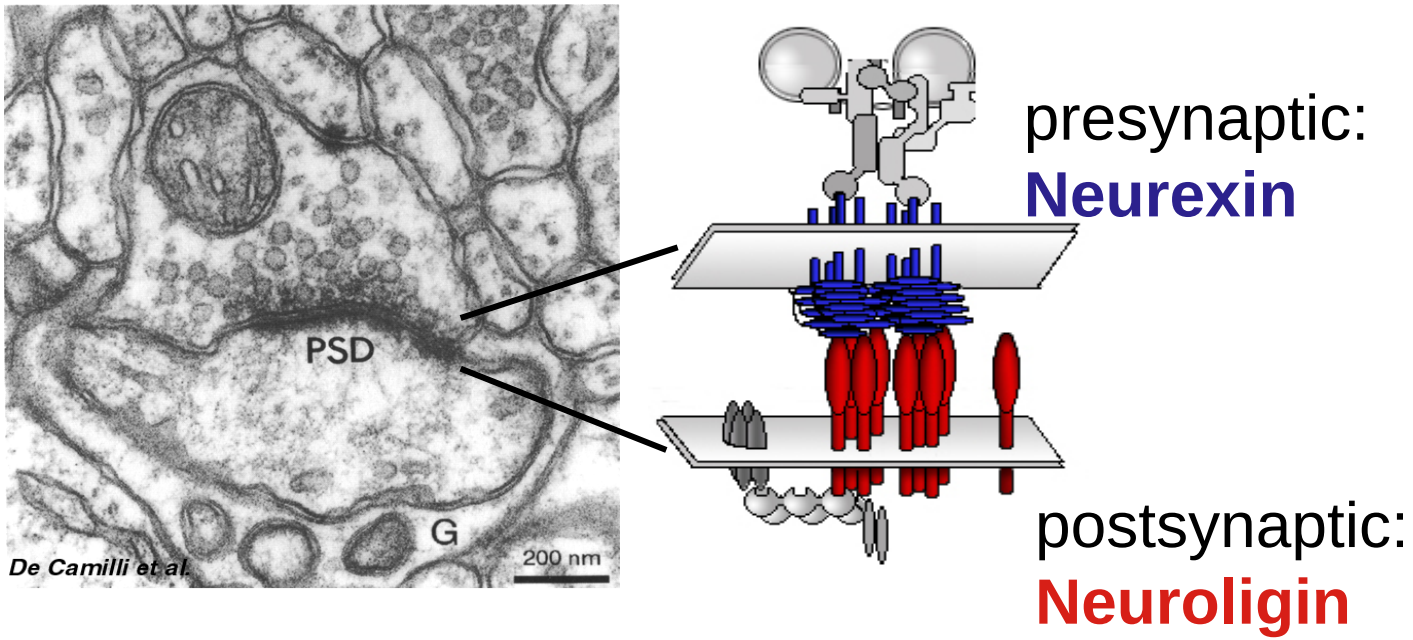
Ben Chih


glutamatergic
axon
GABAergic
axon
NRXα
NRXβ4(+)
NRXβ4(-)
? ?
α +
NL1B
NL1A
NL2A
dendrite


vGlut1
VGlut1 puncta /10μM
12
10
8
6
4
2
0
**
FC
NRX FC 1β4(+)
NRX FC 1β4(-)


VGAT
VGAT puncta /10μM
5
4
3
2
1
0
***
***
FC
NRX FC 1β4(+)
NRX FC 1β4(-)

Ben Chih

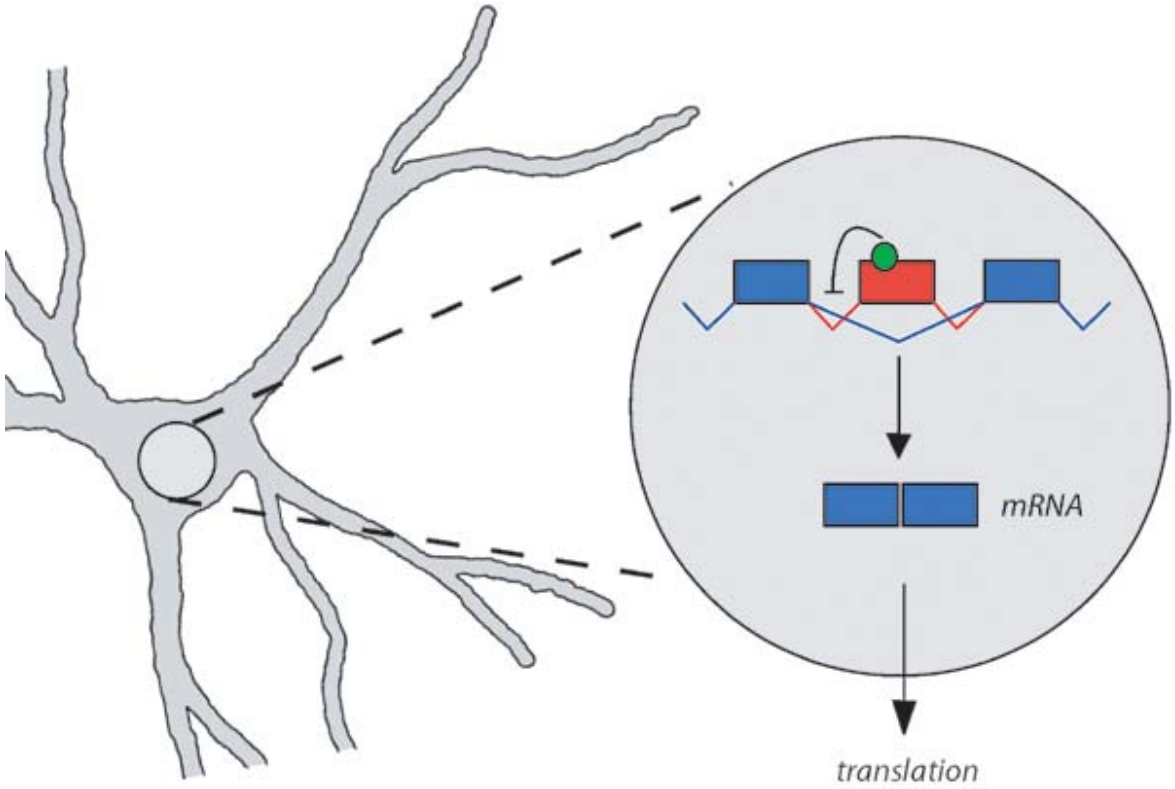


- alternative splicing of neuroligin-1 and -2 regulates localization and function at GABAergic *vs.* glutamatergic contacts

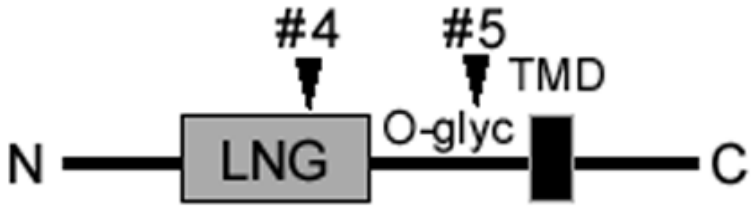
- neurexin splice variants that interact selectively with the GABAergic neuroligin variants selectively induce GABAergic postsynaptic differentiation

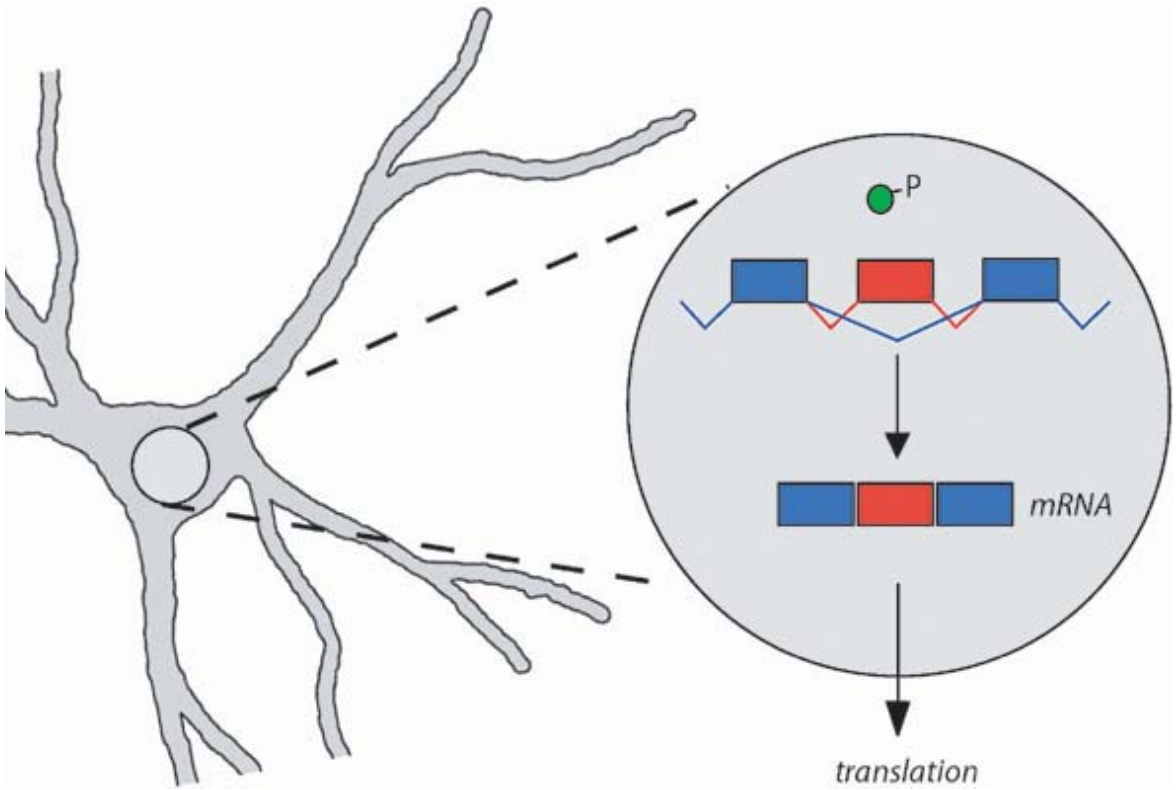
- alternative splicing underlies selective trans-synaptic interactions of the neuroligin - neurexin complex

What is the spatial and temporal regulation of Neurexin splicing?



What is the spatial and temporal regulation of Neurexin splicing?

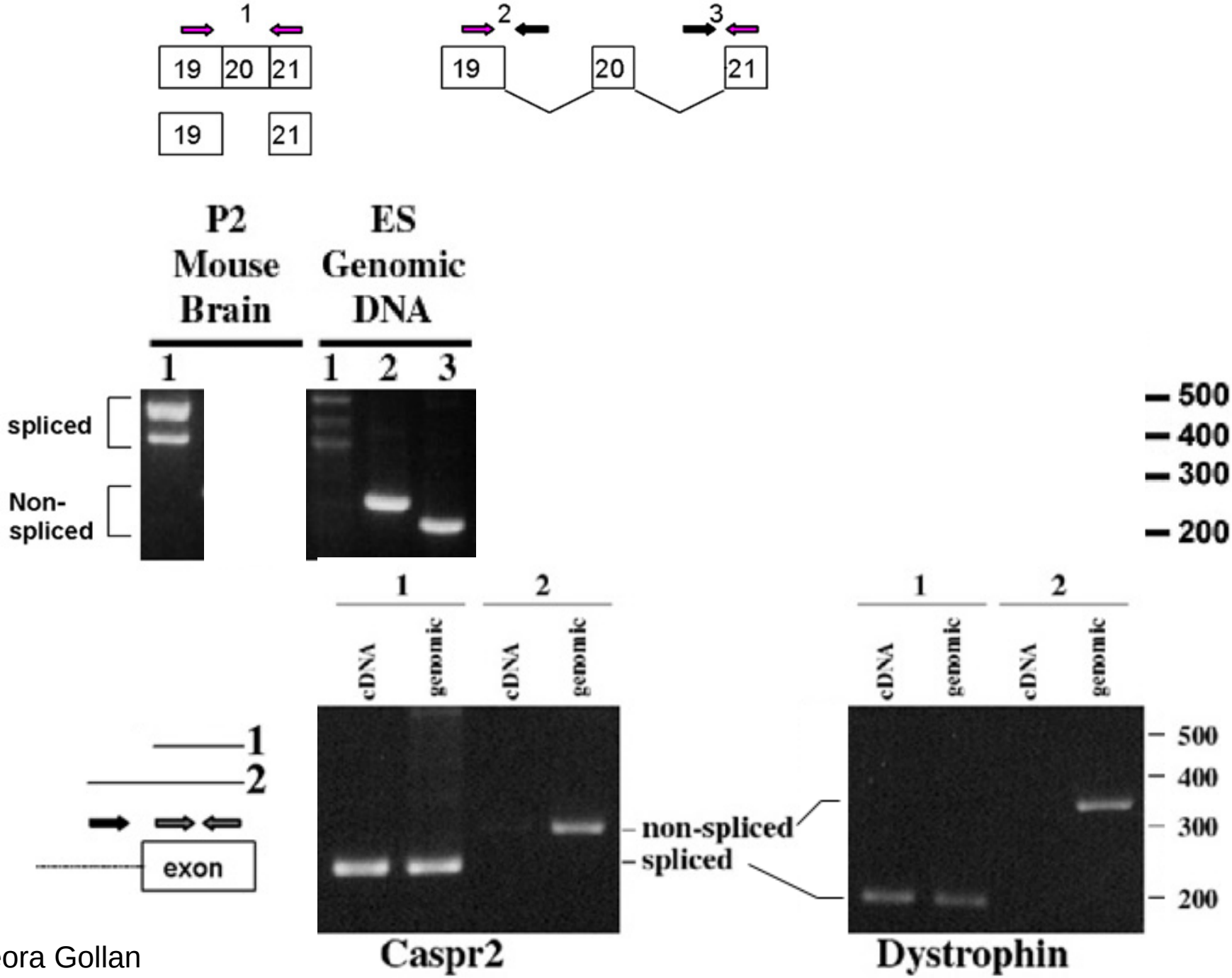




a) Cell-type specific expression of splicing factors

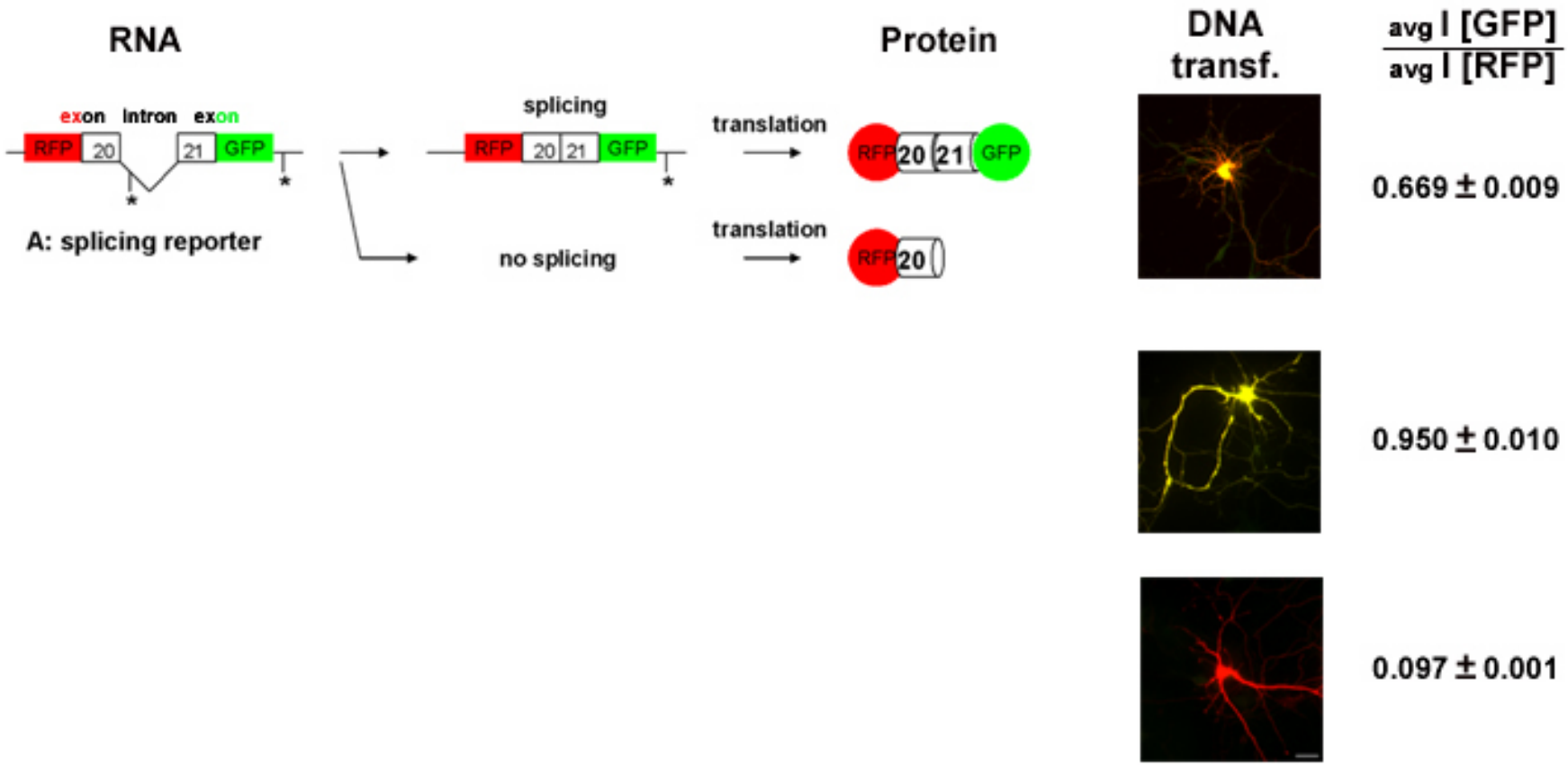
b) Dynamic regulation by post-translational modifications, e.g. phosphorylation

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)



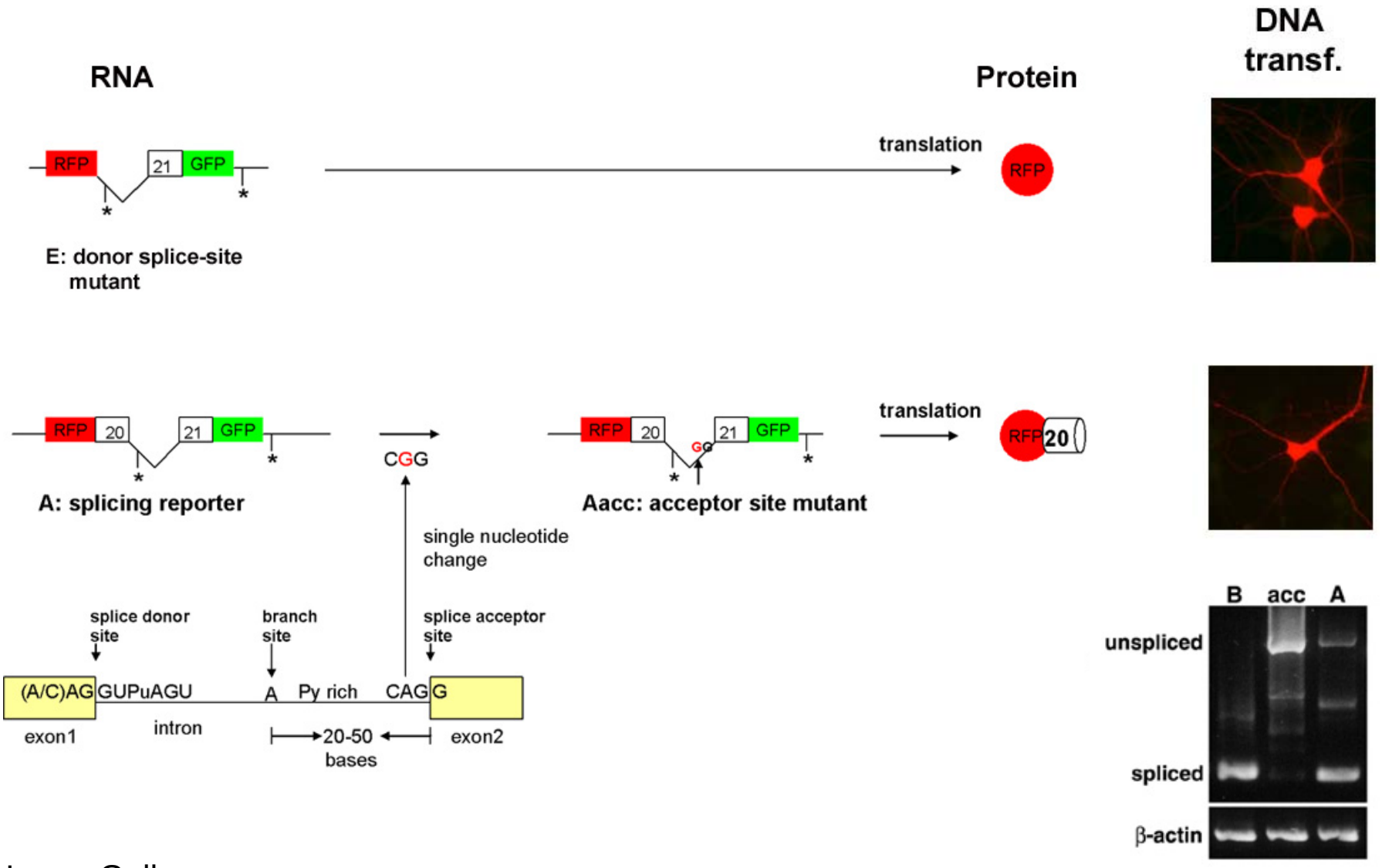
Leora Gollan

A quantitative assay for neurexin splicing



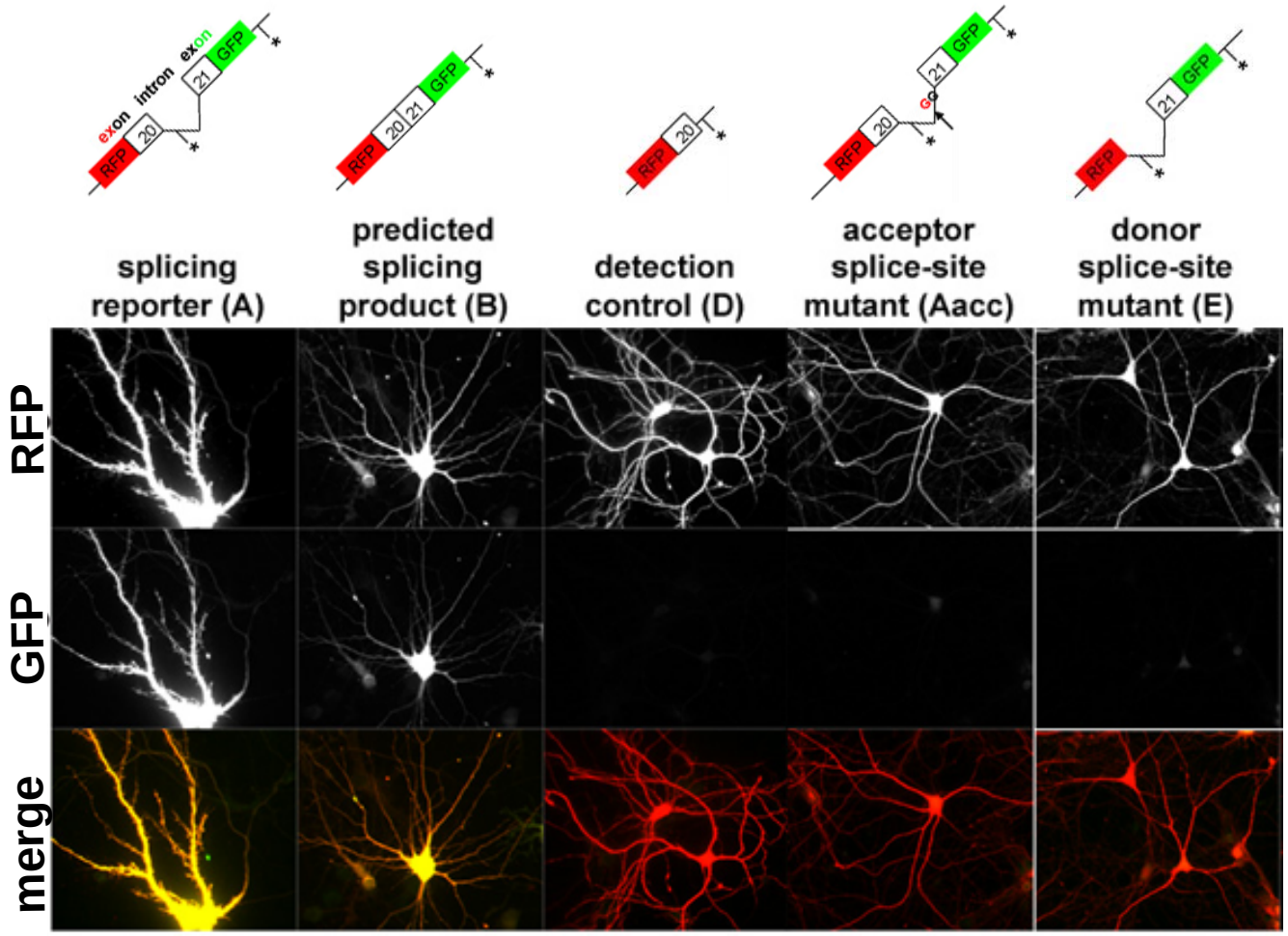
Leora Gollan

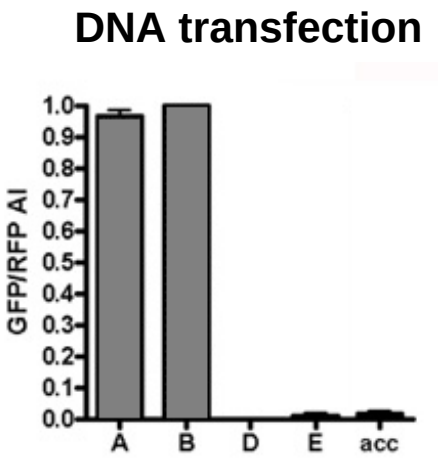
Splice reporters with inactivated donor and acceptor sites

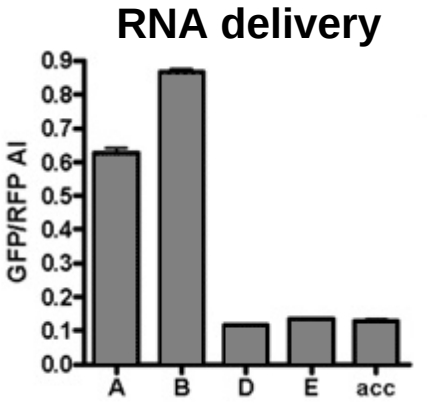


Leora Gollan

Processing of splice reporter RNAs in hippocampal neurons







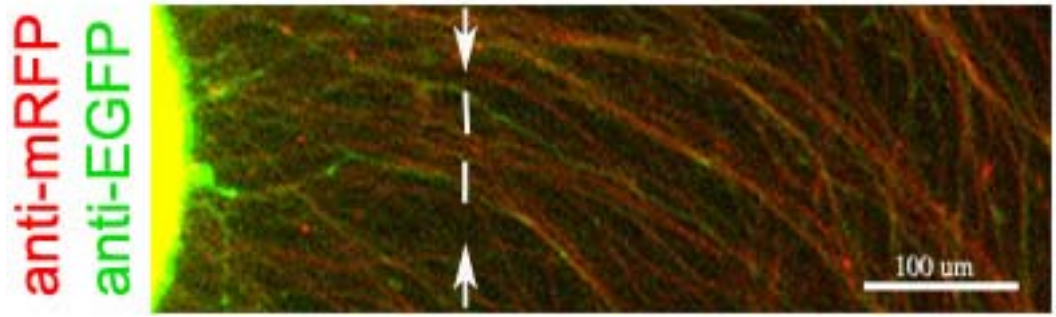
Leora Gollan

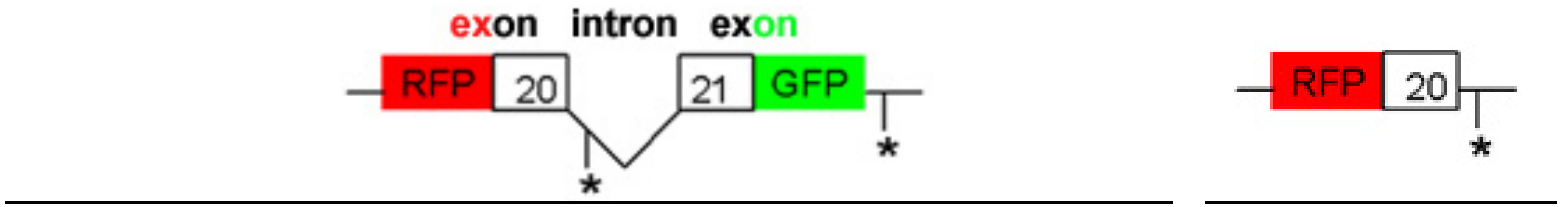
1. specific introns are retained in cytoplasmic neurexin-1 mRNA

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

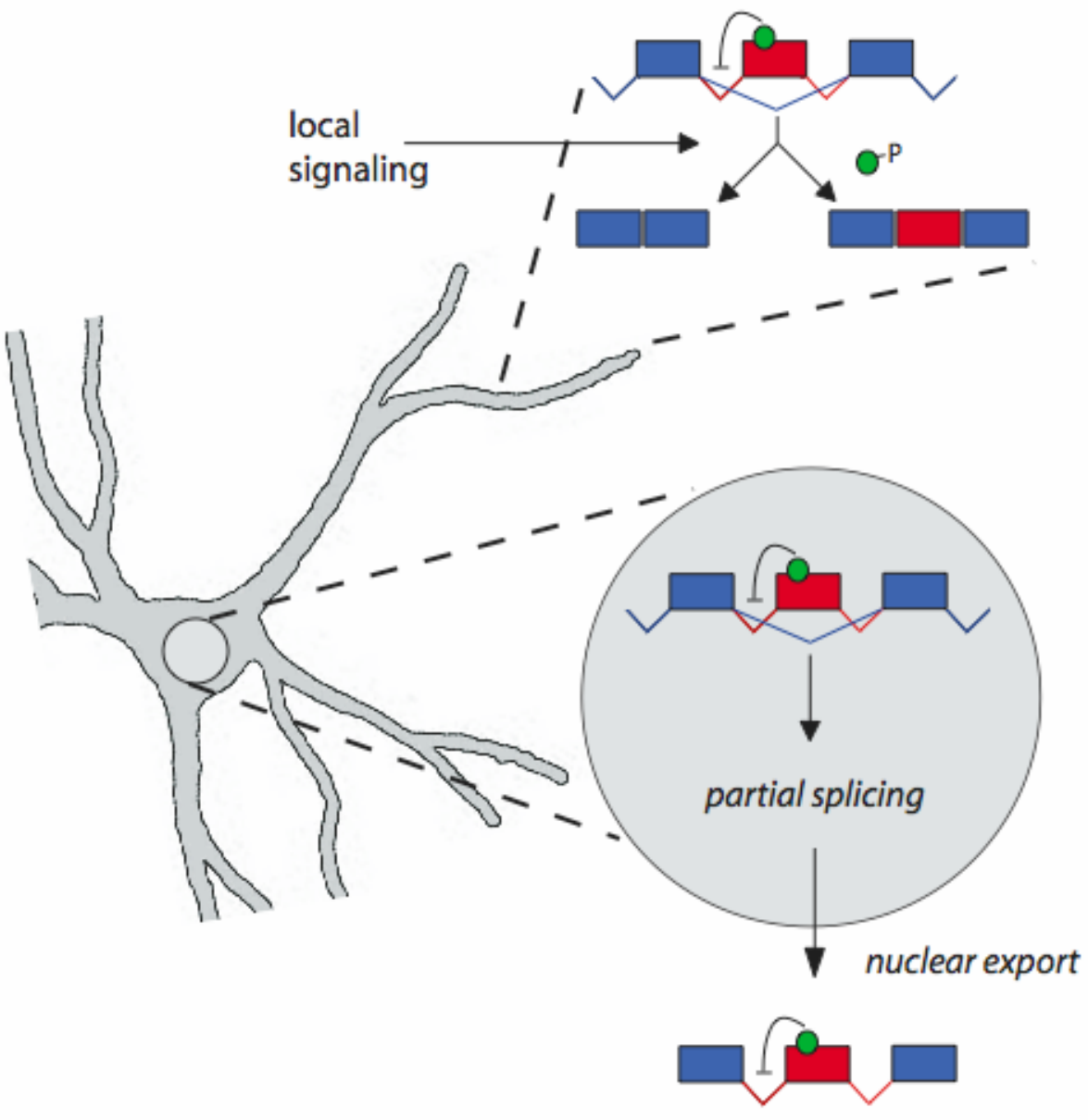
→ *is the neurexin-1 mRNA processed through a cytoplasmic splicing mechanism?*

RNA processing in mechanically isolated axons





Leora Gollan



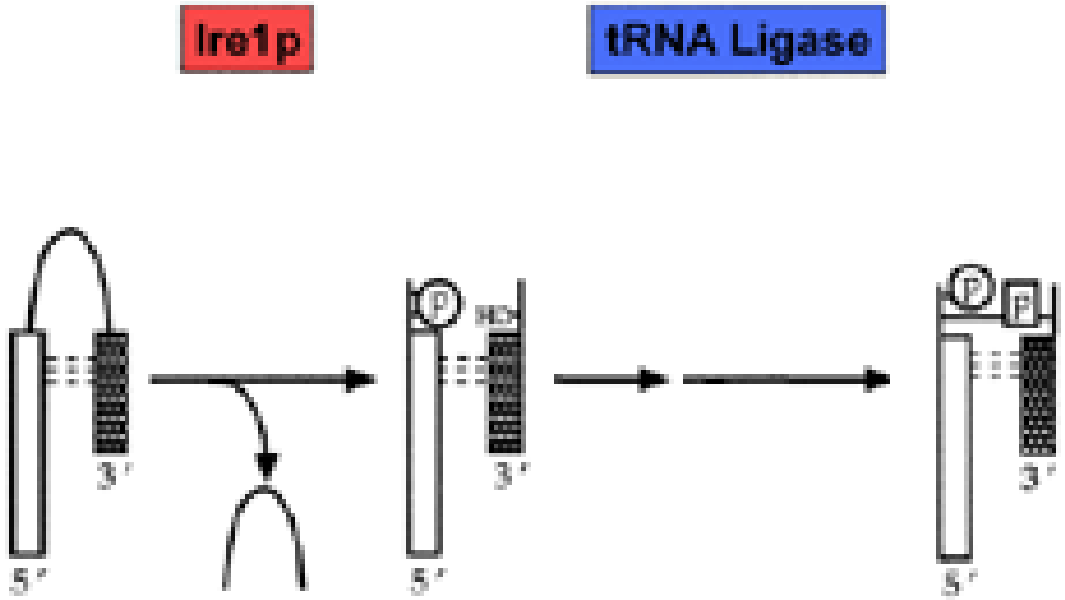
Cytoplasmic splicing of synaptic proteins would provide a novel mechanism for local modifications of cell function

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

Acknowledgements

Ben Chih (Genentech)
Leora Gollan (POSTER: Wednesday 715.13/B25)
Elaine Budreck
Francisco Gomez-Scholl (Univ. Seville)

Collaborators:
Ryuichi Shigemoto (NIPS, Okazaki)
Chiye Aoki (NYU)

Support: ***NIDA***
NINDS
Irma T. Hirschl Fund
The John Merck Fund
The Simons Foundation
Searle Scholar Program
Alfred P. Sloan Foundation
Joseph and Esther Klingenstein Fund
National Alliance for Autism Research