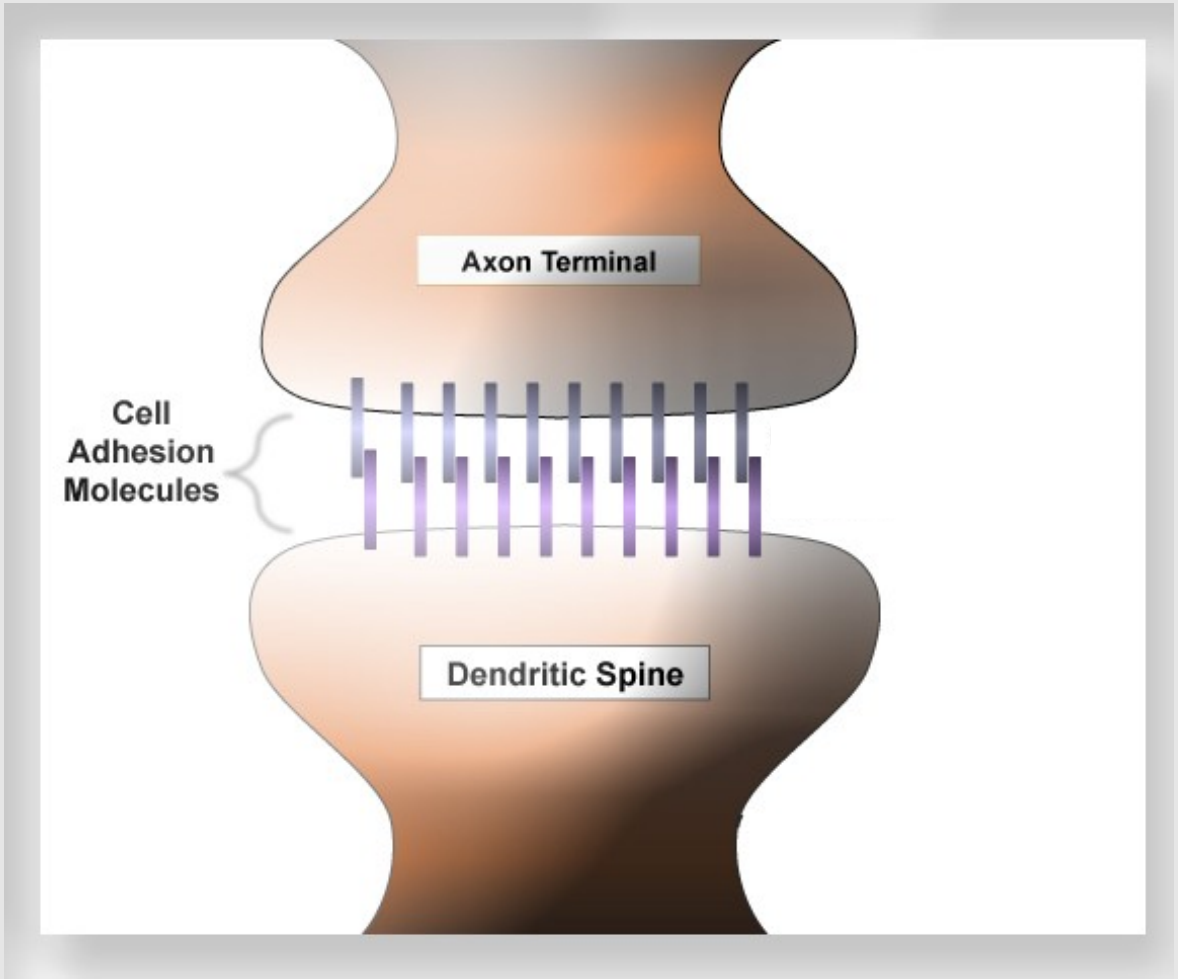
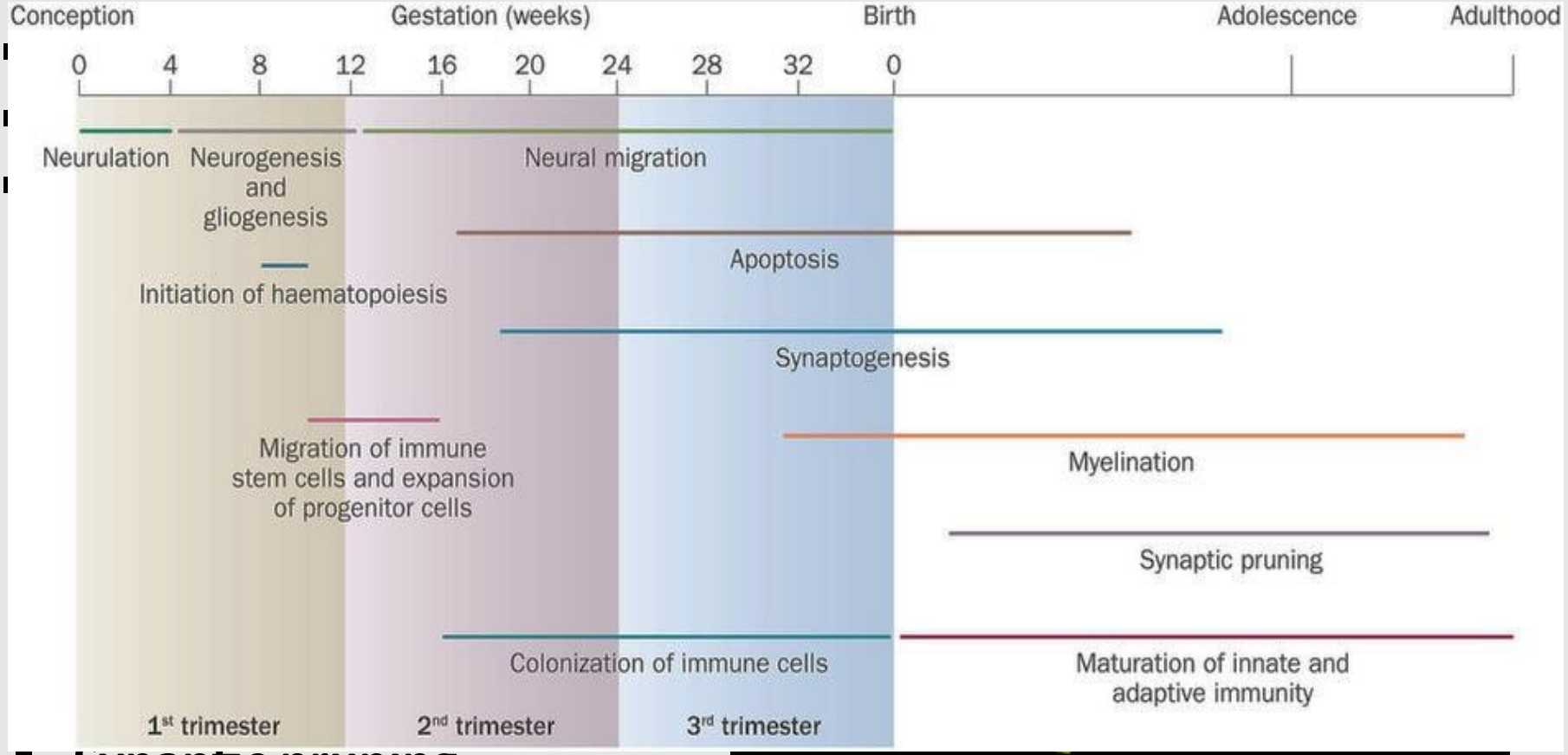
Molecole di adesione alla sinapsi



Lo sviluppo del cervello

Before birth (mostly)



- Synaptic pruning

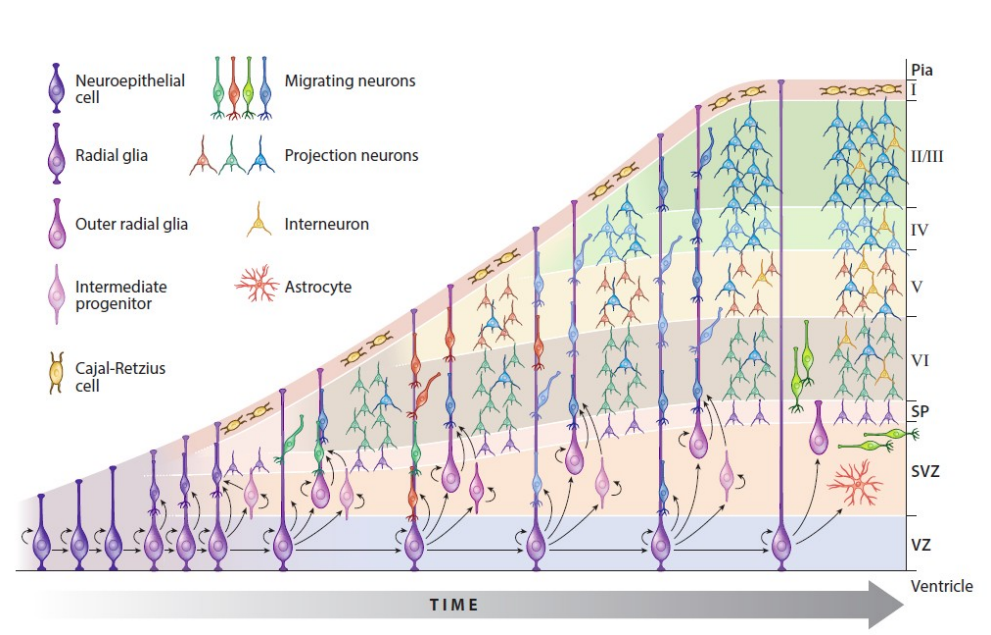
Lo sviluppo del cervello prima della nascita

- **Proliferazione**
- **Migrazione**
- **Differenziamento**

Prevalentemente prima della nascita (in utero)

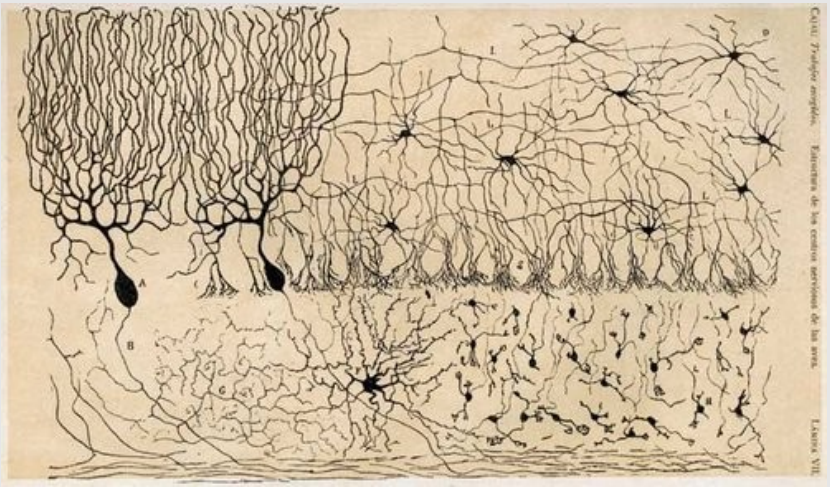
Mutazioni in questo stadio si moltiplicano e si espandono

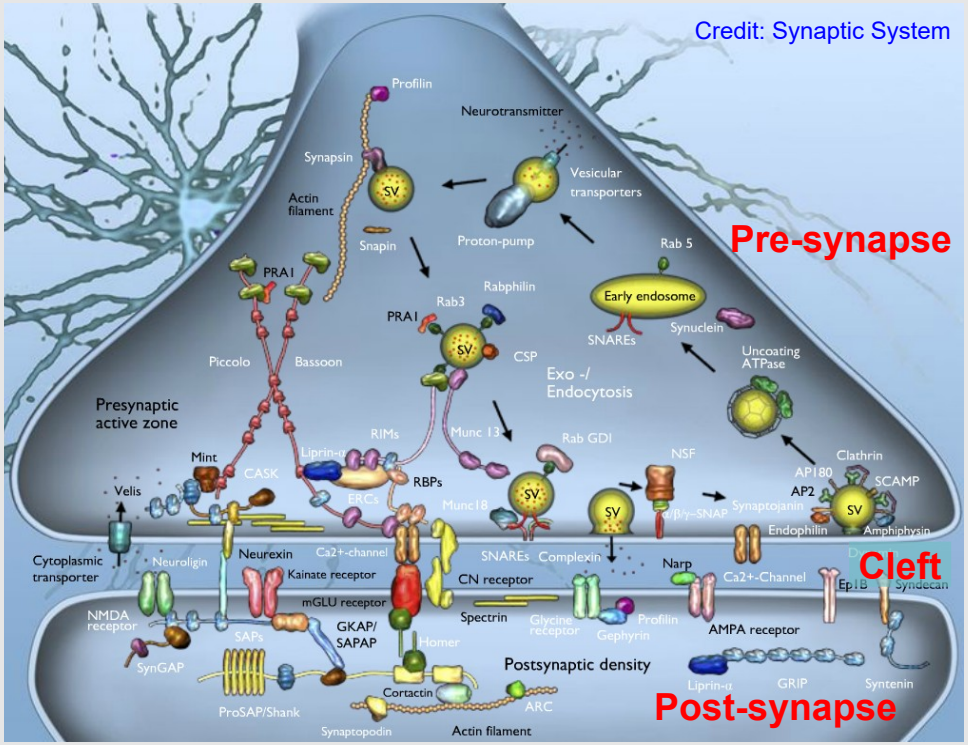
Diagnosi è difficile e intervenire anche.



Lo sviluppo del cervello dopo la nascita

- **Formazione dei circuiti**
- **Formazione della sinapsi**
- **Eliminazione sinaptica**

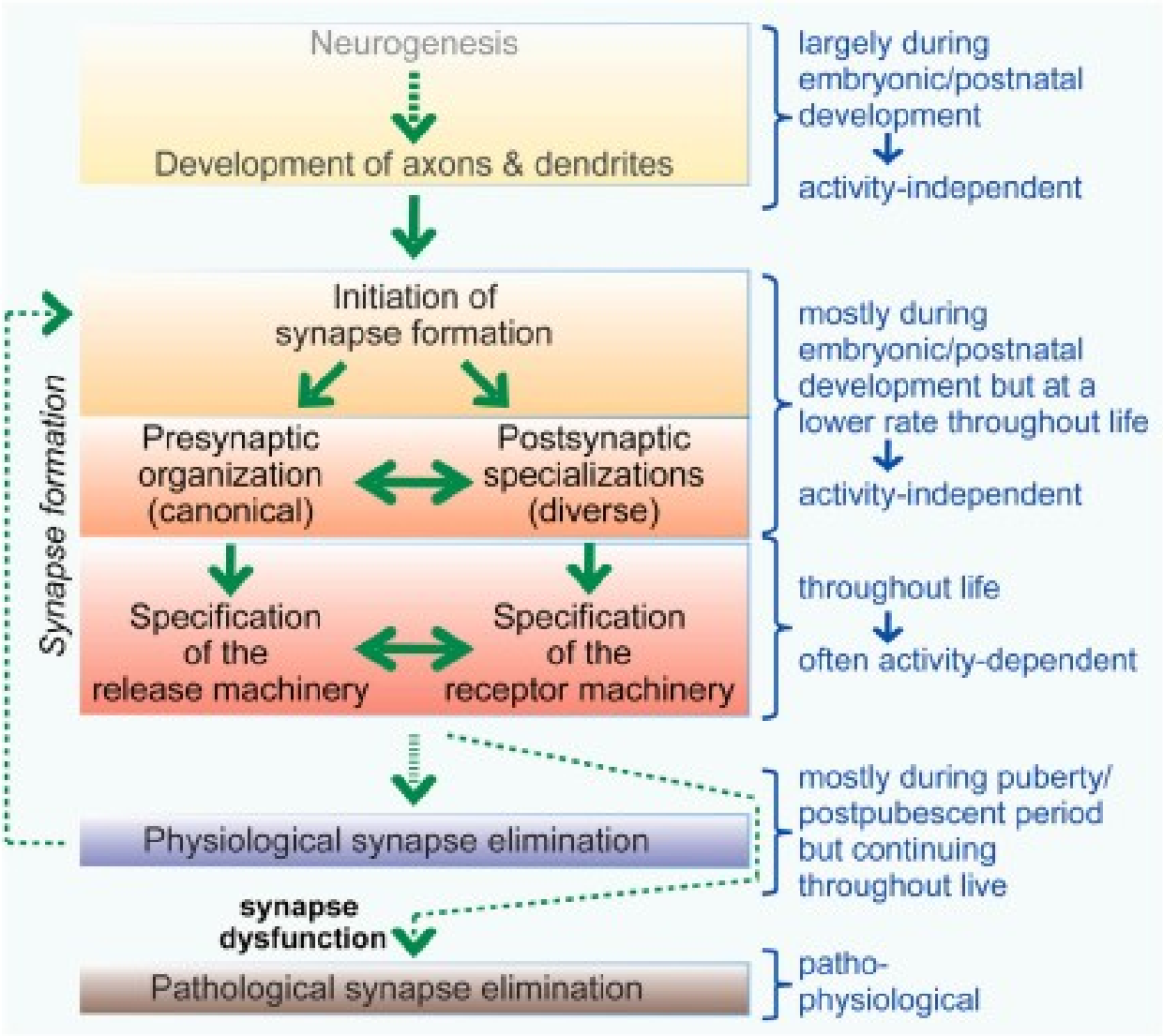




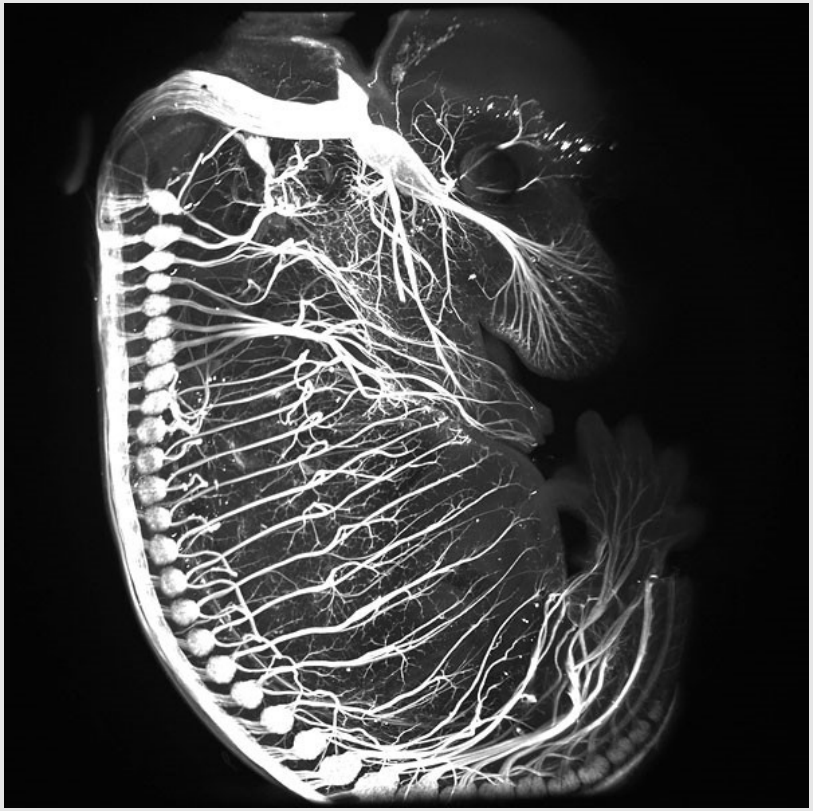
Dopo la nascita

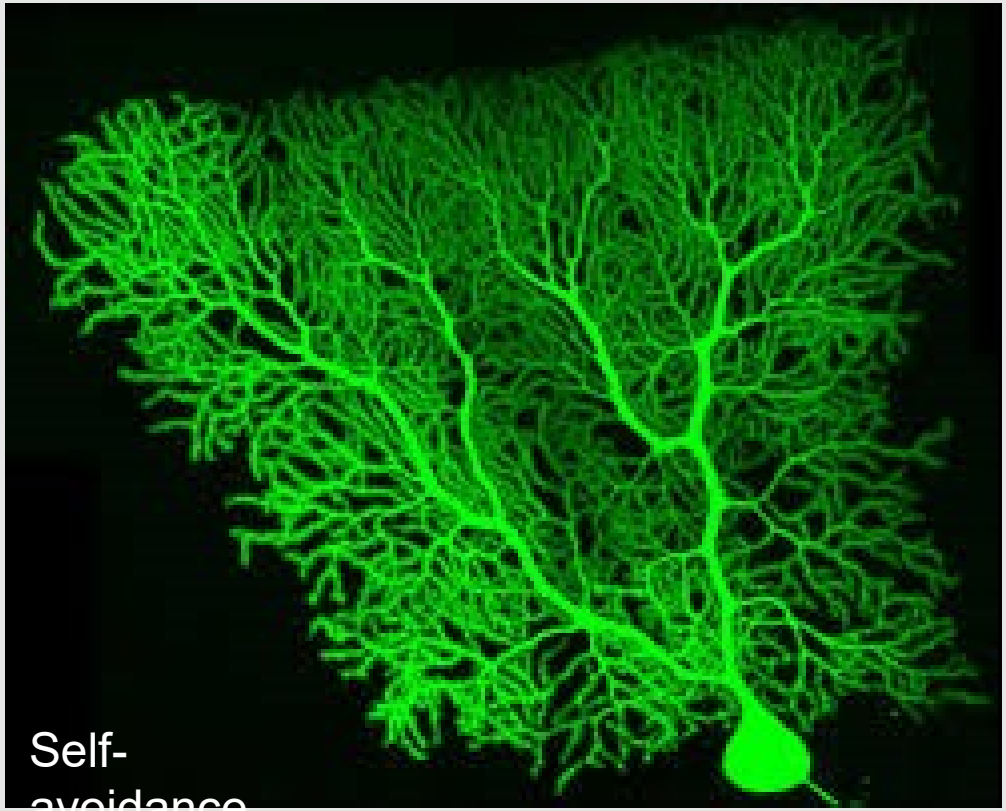
Mutations can avere un effetto su molti di questi processi

Diagnosi ed interventi possono Essere tardivi


Neurogenesis
Development of axons & dendrites
largely during embryonic/postnatal development
activity-independent
Initiation of synapse formation
Presynaptic organization (canonical)
Postsynaptic specializations (diverse)
mostly during embryonic/postnatal development but at a lower rate throughout life
activity-independent
Synapse formation
Specification of the release machinery
Specification of the receptor machinery
throughout life
often activity-dependent
Physiological synapse elimination
mostly during puberty/ postpubescent period but continuing throughout live
synapse dysfunction
Pathological synapse elimination
patho-physiological

Cosa è la connettività sinaptica?



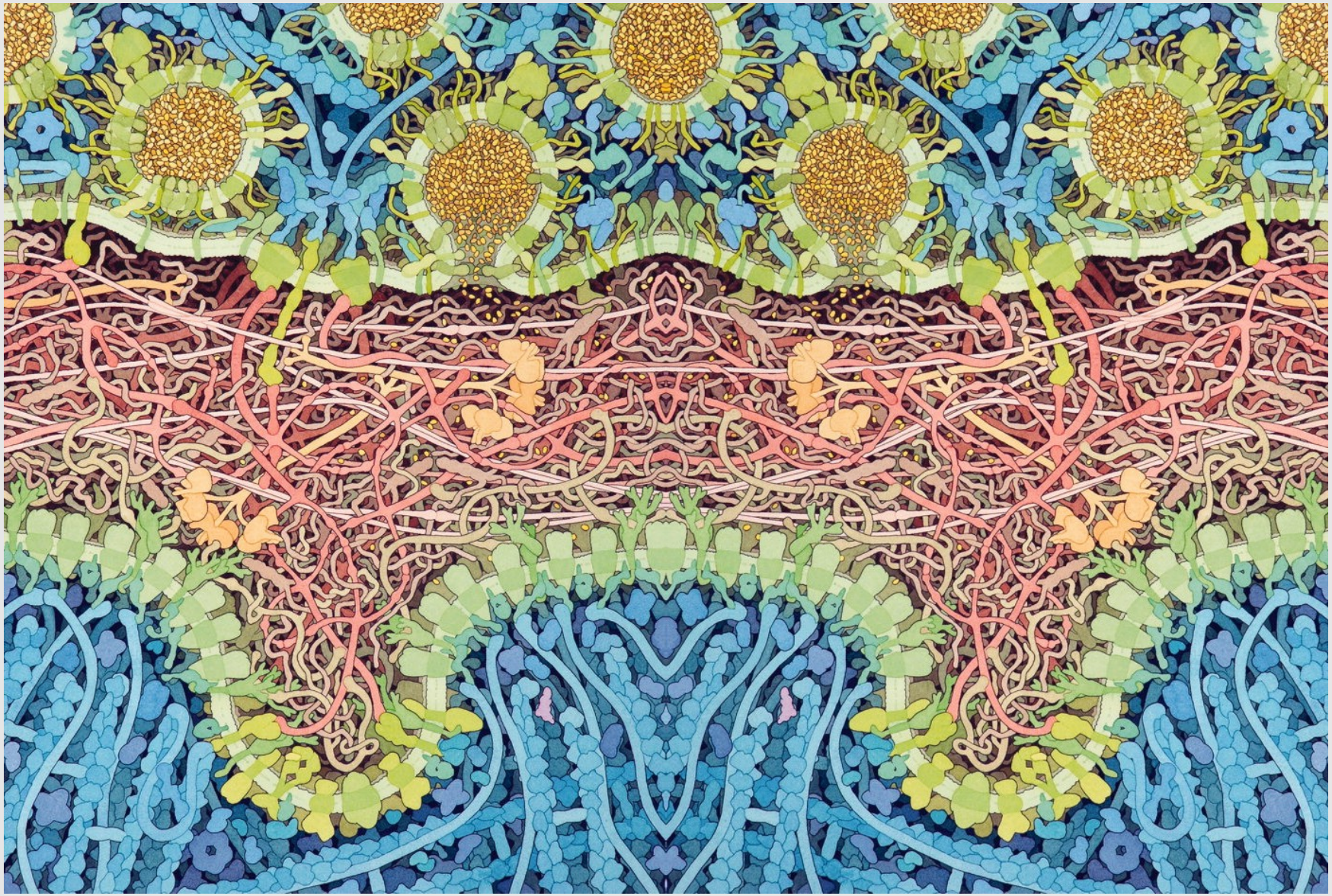


Self-avoidance

A hallmark of CNS organization is the highly **precise pattern of connectivity** between neurons.

The organization of many brain regions into distinct anatomical layers aids wiring specificity → laminar specificity, limits the range of target cells available for synapse formation.

The Synaptic Cleft



David S. Goodsell: Illustrating the Machinery of Life – The Neuromuscular Synapse

Come funziona e cosa è importante

Neuronal functions

Neuronal migration

Axon and dendrite branching

Axon and dendrite guidance
ephrins, semaphorins, netrins, etc.

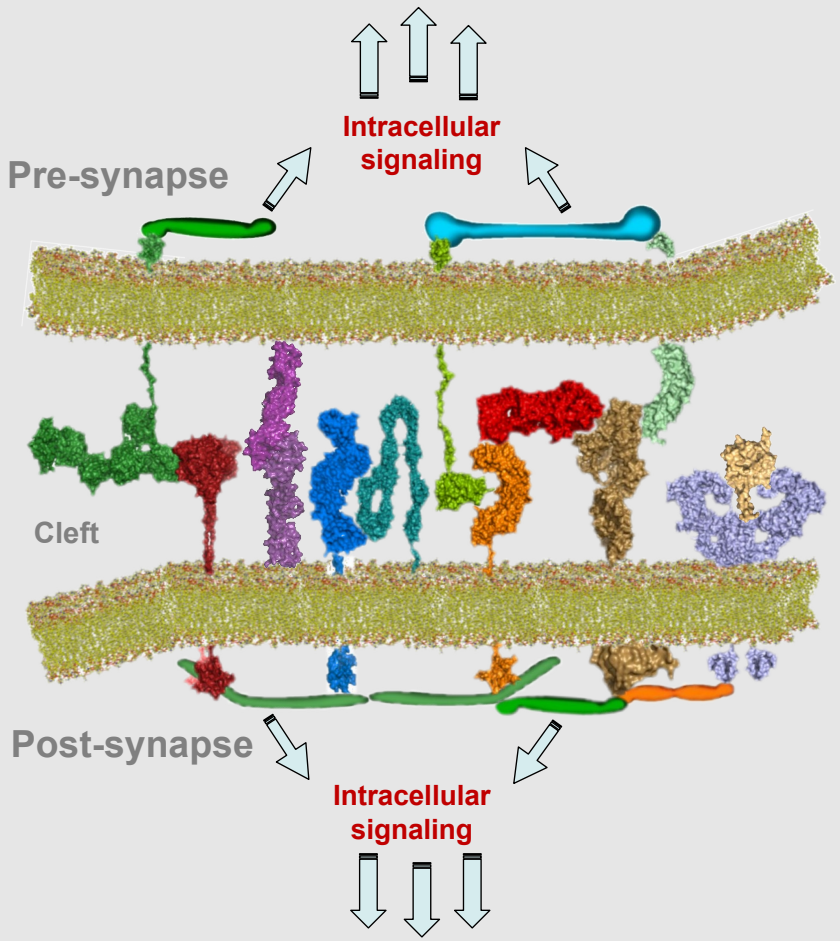
Target recognition
Neurexin, Neuroligins, LRRTMS, etc.

Self-avoidance
Protocadherins

Synaptogenesis
Neurexin, Neuroligins, LRRTMS, SLITRKs, etc.

Axon and dendrite regeneration

Dysfunctions
ASD, schizophrenia, etc



Non-neuronal functions

Vascular patterning

Cell migration

Immune cell differentiation and function

Disease related functions

Autoimmune activity

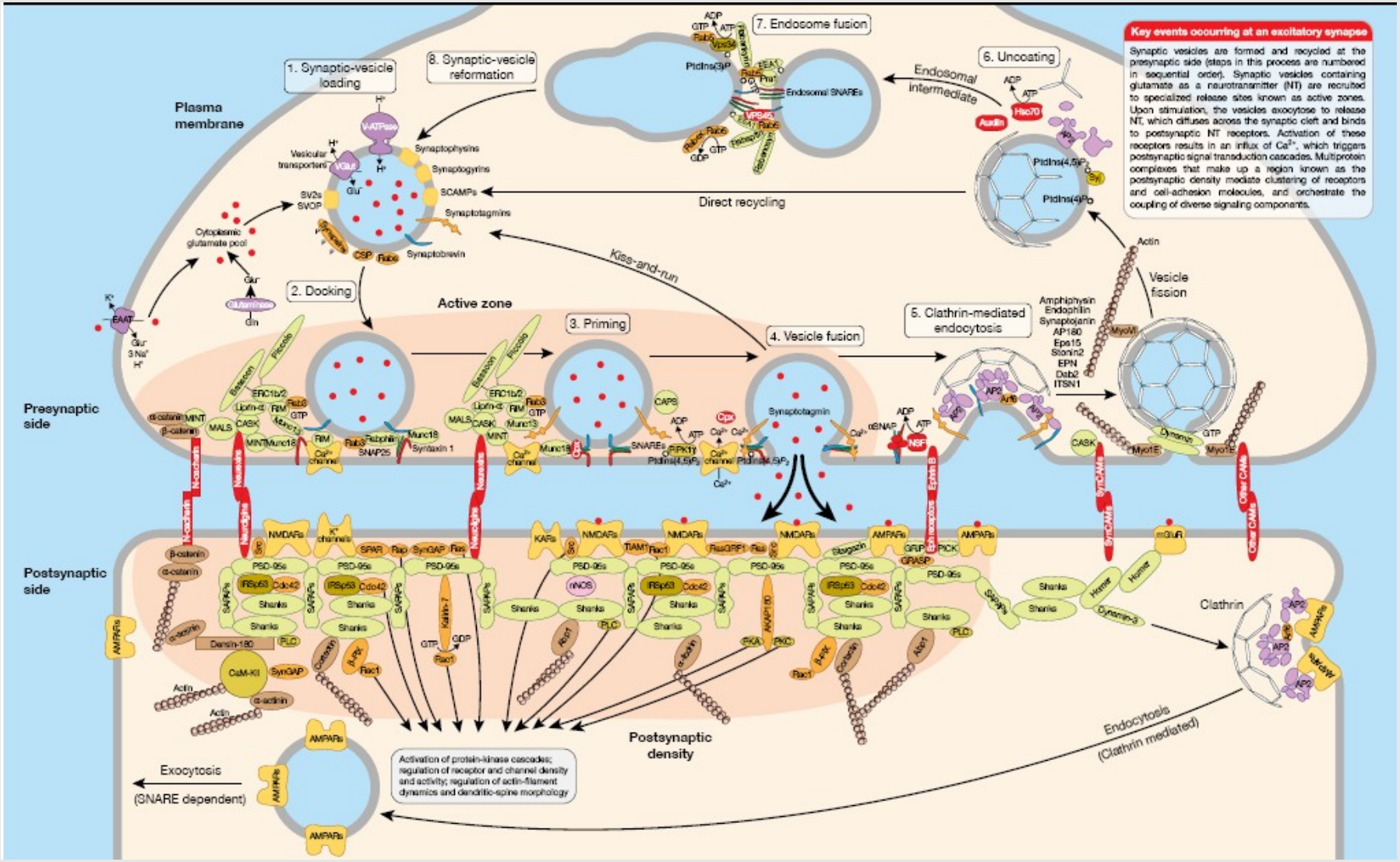
Tumorigenesis & Metastasis

Neurotropic viral receptor

Therapeutic targets

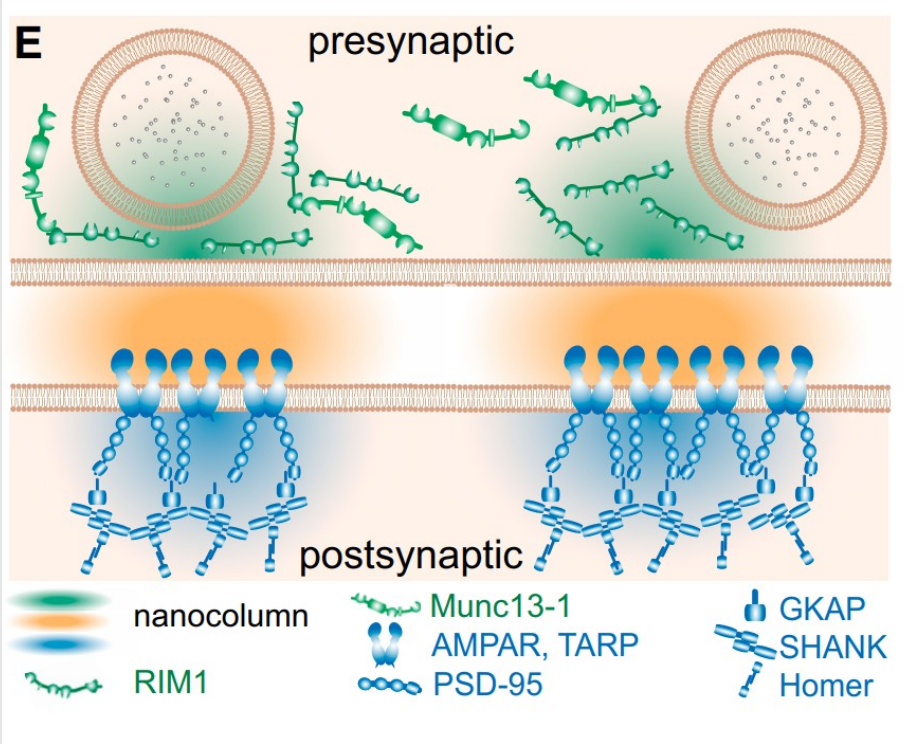
Rituximab (CD20), Herceptin (EGFR2)

LA COMPLESSA ARCHITETTURA DI UNA SINAPSI



Le molecole di adesione tengono insieme le membrane pre- e post-sinaptiche attraverso lo spazio sinaptico, ma in realtà fanno molto di piu'!

La sinapsi non è omogenea



- **Synaptic nanocolumns** are subcellular structures that span across synapses.
- They help **aligning** and organizing key components of the synapse
- Enhance synaptic **efficiency** by aligning receptors directly opposite release sites
- Increase the **speed** of neurotransmission
- Allow for **modulation** of synaptic strength through reorganization
- Nanocolumns are important for **smaller vesicles**, as they help concentrate the released molecules near receptors

Come funziona e perchè è importante

By engaging trans-cellular interactions, SAMs can:

1. nucleate nascent synapses,
2. drive synapse maturation,
3. control the properties of synapses,
4. regulate synapse elimination

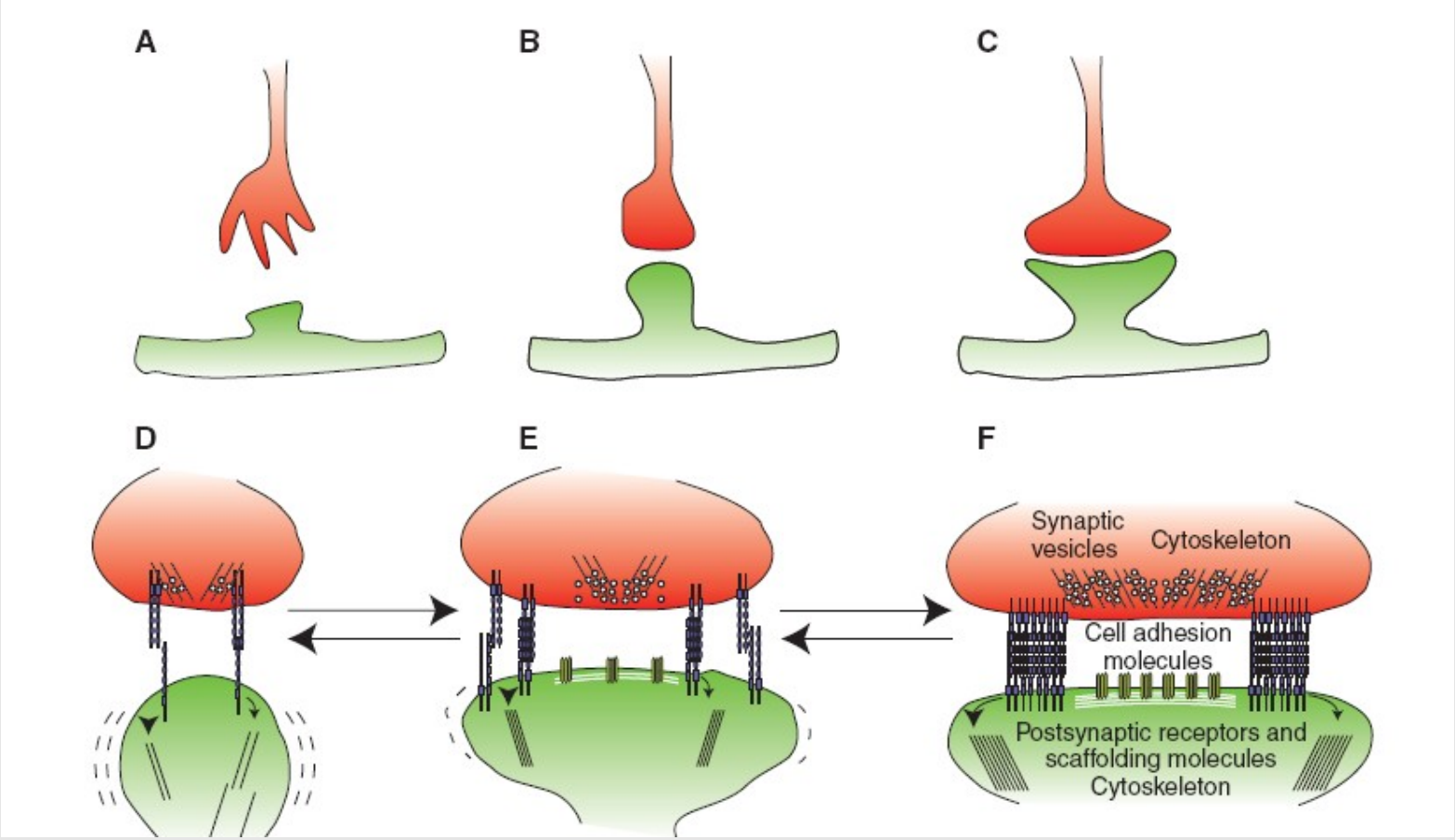
But: **No single "master"** SAM likely controls everything; instead, an **orchestra of SAMs** mediates assembly of diverse synaptic junctions

Presynaptic SAMs are mostly "hub" molecules that are present in excitatory and inhibitory synapses, like Neurexins

Postsynaptic SAMs are more diverse as ligands for these hub molecules and are often specific for excitatory or inhibitory synapses.

Post-synaptic sites have receptors, more diverse than pre-synaptic

…INFATTI PARTECIPANO AI VARI STADI DURANTE LA FORMAZIONI DI UNA SINAPSI



Selezione del bersaglio

Formazione della sinapsi
Reclutamento delle specializzazioni pre e post sinaptiche

Maturazione e stabilizzazione
Plasticità sinaptica

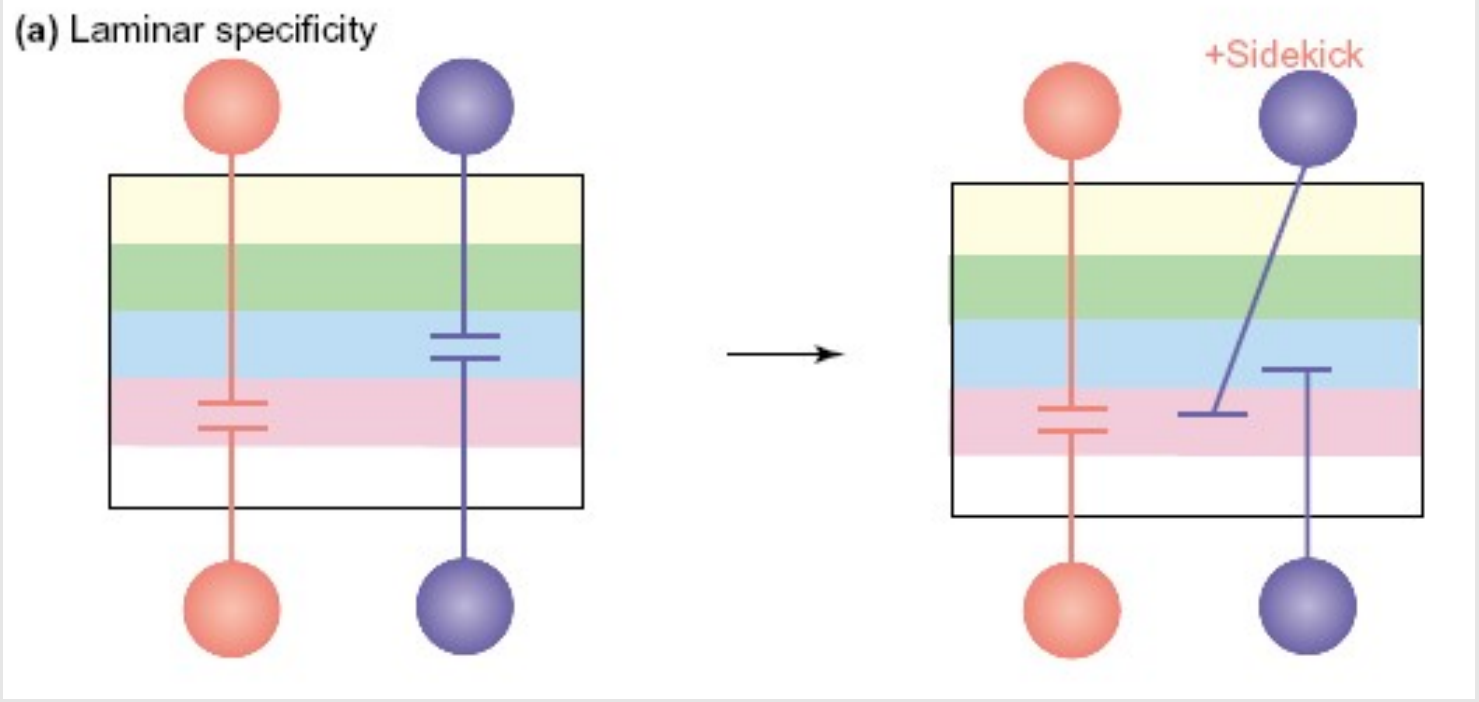
RICONOSCIMENTO DEL TARGET

Il preciso indirizzamento degli assoni dipende dall'espressione di molecole di adesione. Esempi:

Eph/ephrins

NEPH-1/nephrin (SYG-1 Droso)

SIDEKICK (famiglia delle immunoglobuline): formazione di sinapsi selettive in zone stratificate (specificità laminare) nella retina di pollo



La sovraespressione di sidekick altera la connettività

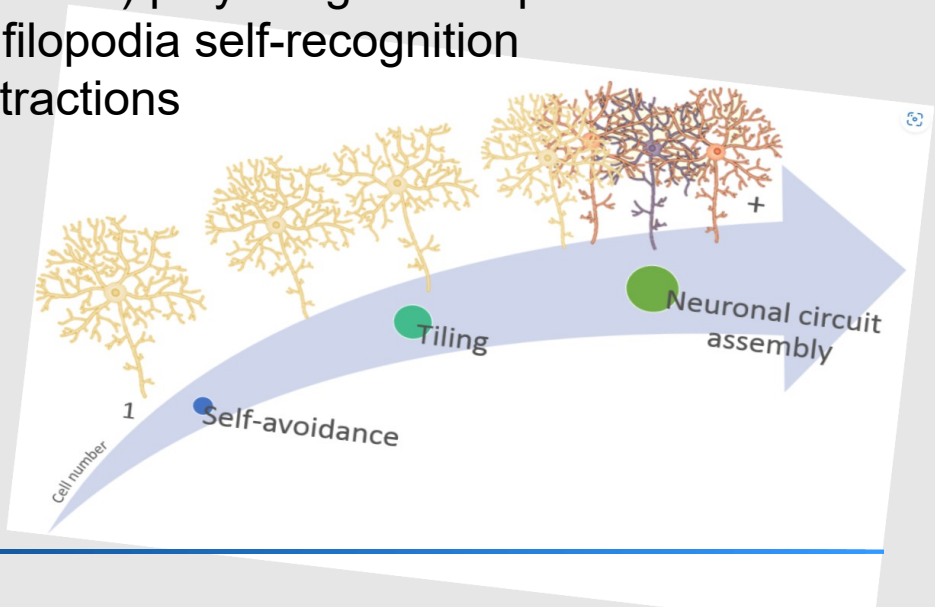
Dendrite Self-Avoidance

Sister branches from the **same neuron** recognize and repel each other → a) preventing overlap and, b) ensuring uniform coverage of the receptive field.

Molecular Mechanisms
In Drosophila, Dscam1 (Down Syndrome Cell Adhesion Molecule 1) provide most of the dendritic self-avoidance
Homotypic repulsion is mediated by **binding of identical Dscam1 isoforms** on opposing sister branches
Dscam1 has huge molecular diversity (thousands of isoforms)

In mammals, Clustered Protocadherins (cPcdhs) play a significant part
Regulate contact-induced retractions upon filopodia self-recognition
Selectively mediate self-contact-induced retractions
Not enough molecular diversity?

Other molecular players are likely involved
Other mechanisms involved?



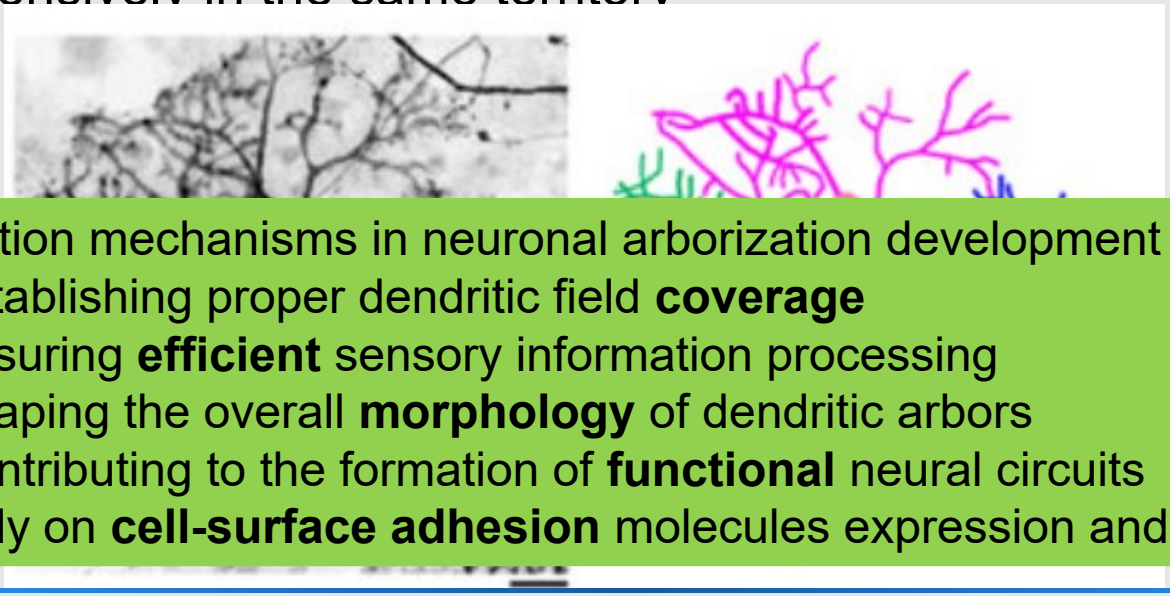
Dendrite tiling

Tiling is where dendrites of neighboring neurons of the same type avoid overlap, ensuring complete but non-redundant coverage of the receptive field.

Complete coverage: Dendrites of neurons belonging to the same type cover their sensory field as completely as possible

Minimal overlap: Dendrites of the same neuronal type have little to no overlap with each other.

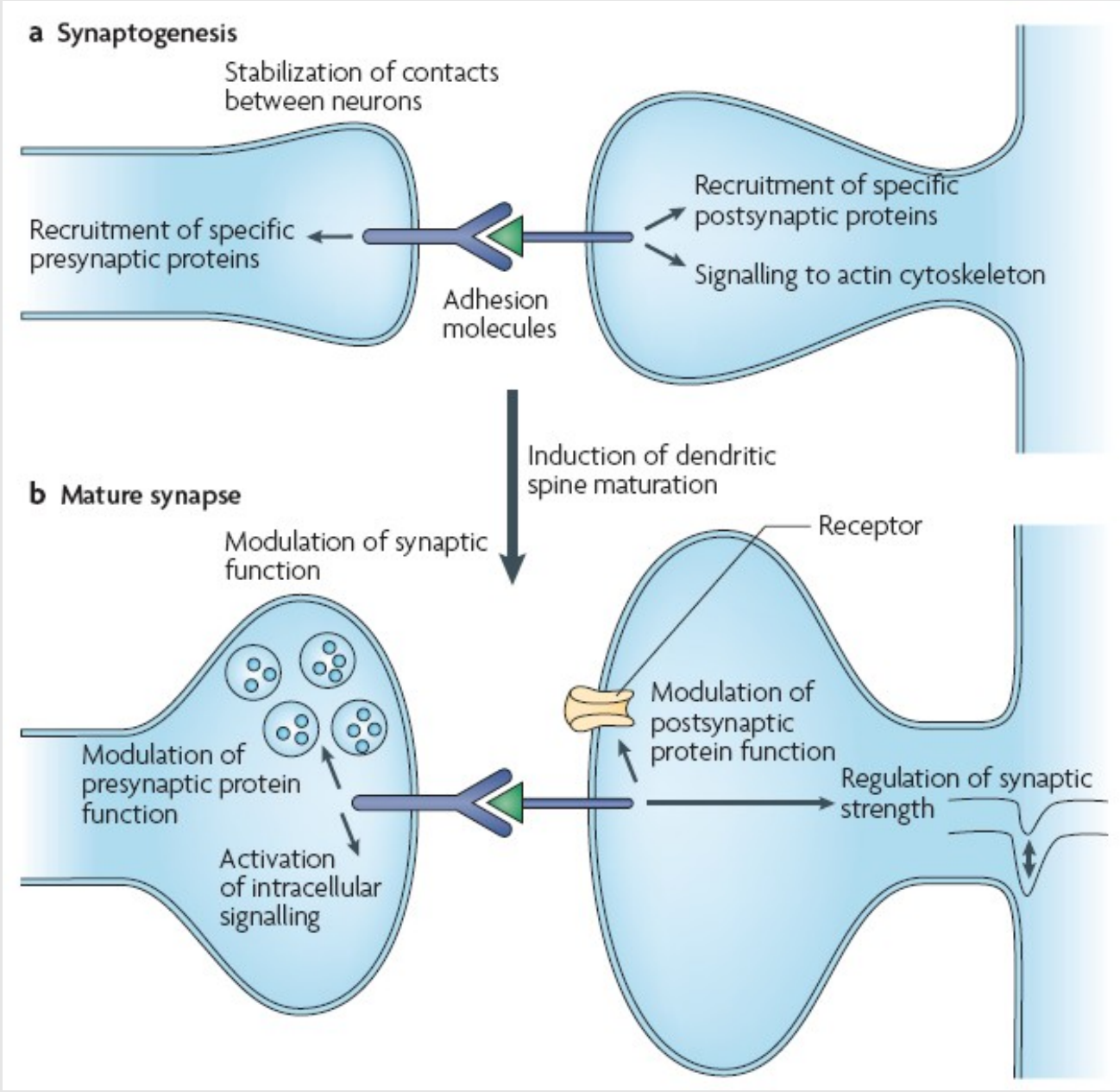
Type-specificity: Dendrites of different neuronal types can coexist and overlap extensively in the same territory



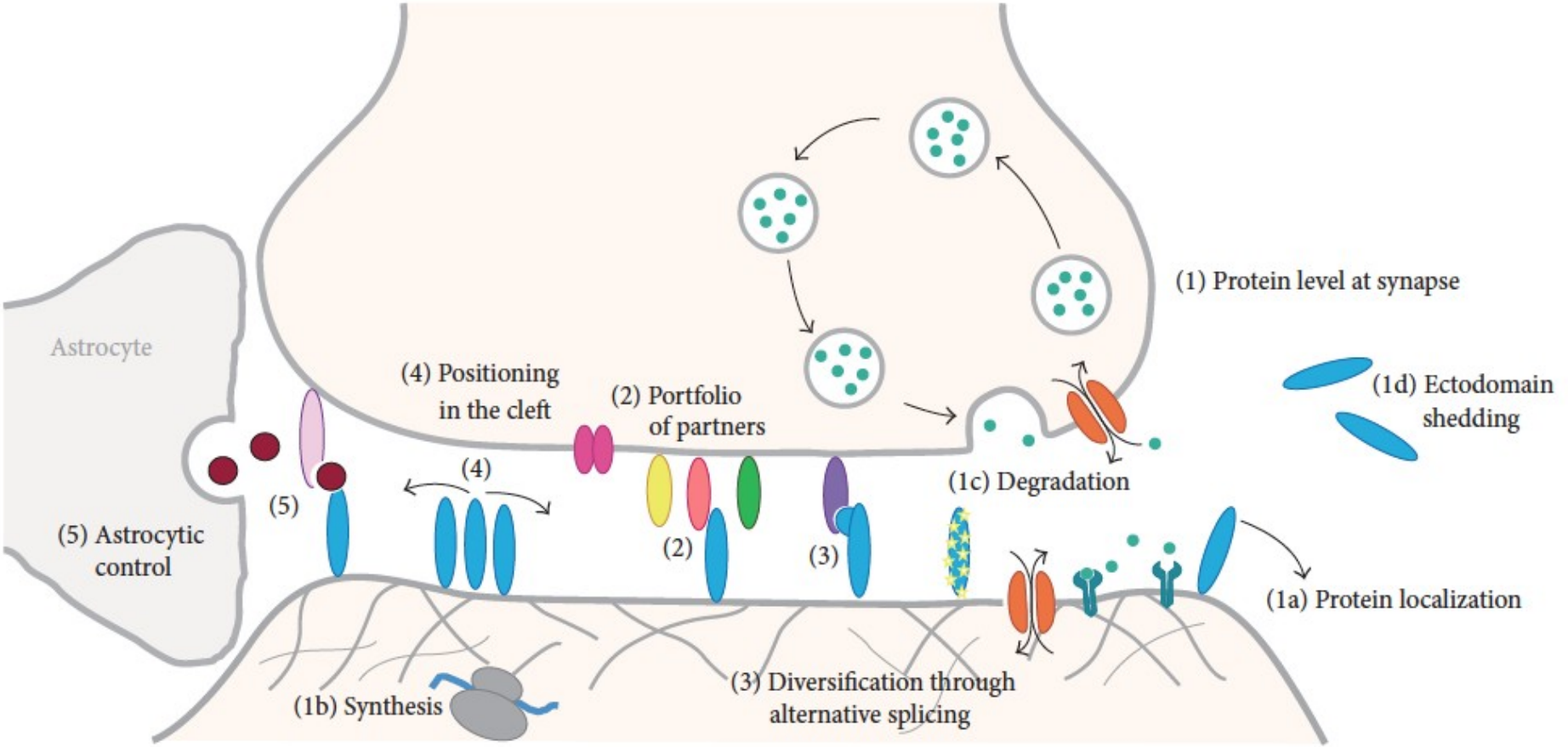
Self-recognition mechanisms in neuronal arborization development are critical for:

- Establishing proper dendritic field **coverage**
- Ensuring **efficient** sensory information processing
- Shaping the overall **morphology** of dendritic arbors
- Contributing to the formation of **functional** neural circuits
- Rely on **cell-surface adhesion** molecules expression and interactions

SINAPTOGENESI E MATURAZIONE SINAPTICA

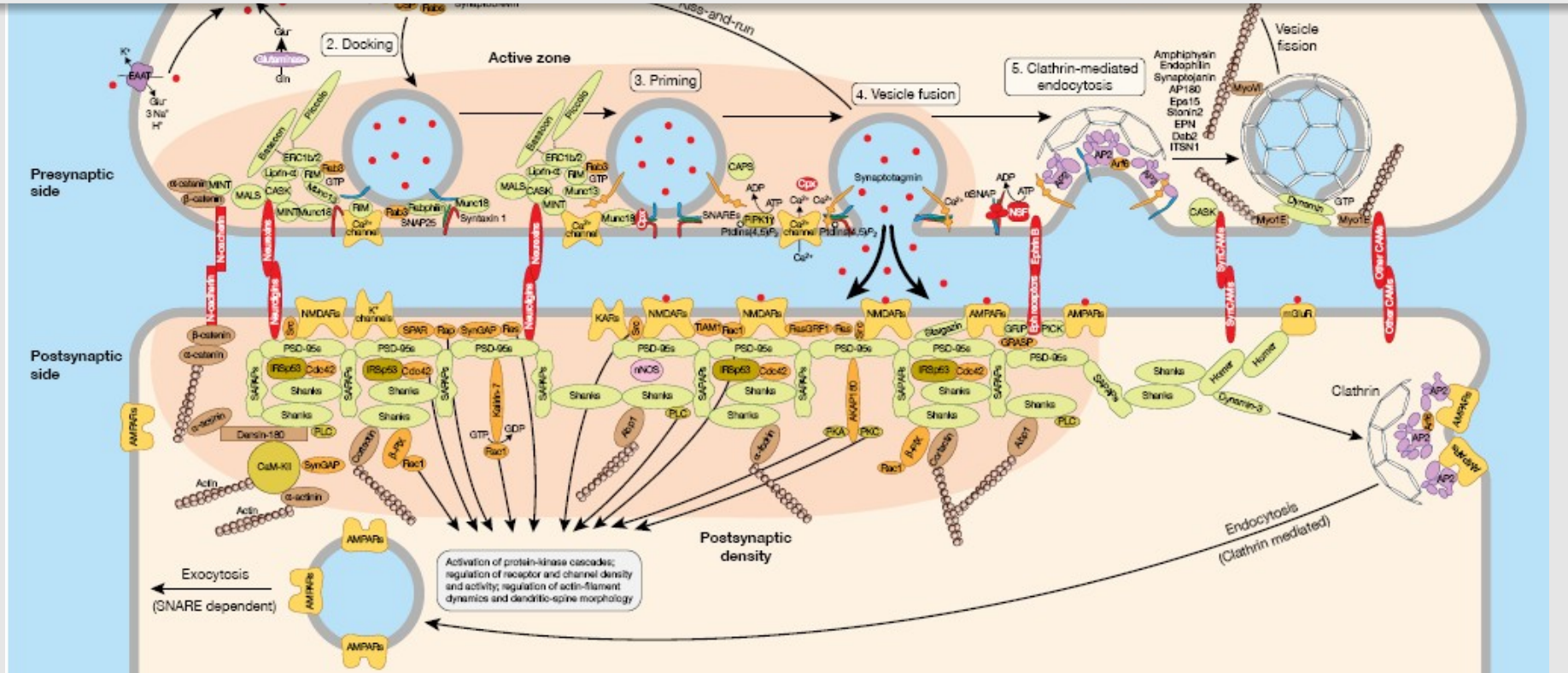


SAMs sono strategicamnte posizionate per contribuire alla plasticità sinaptica, infatti possono modificare la struttura della sinapsi e la sua funzione attraverso la loro capacità di scolpire e regolare la rete delle interazioni proteiche alla sinapsi.

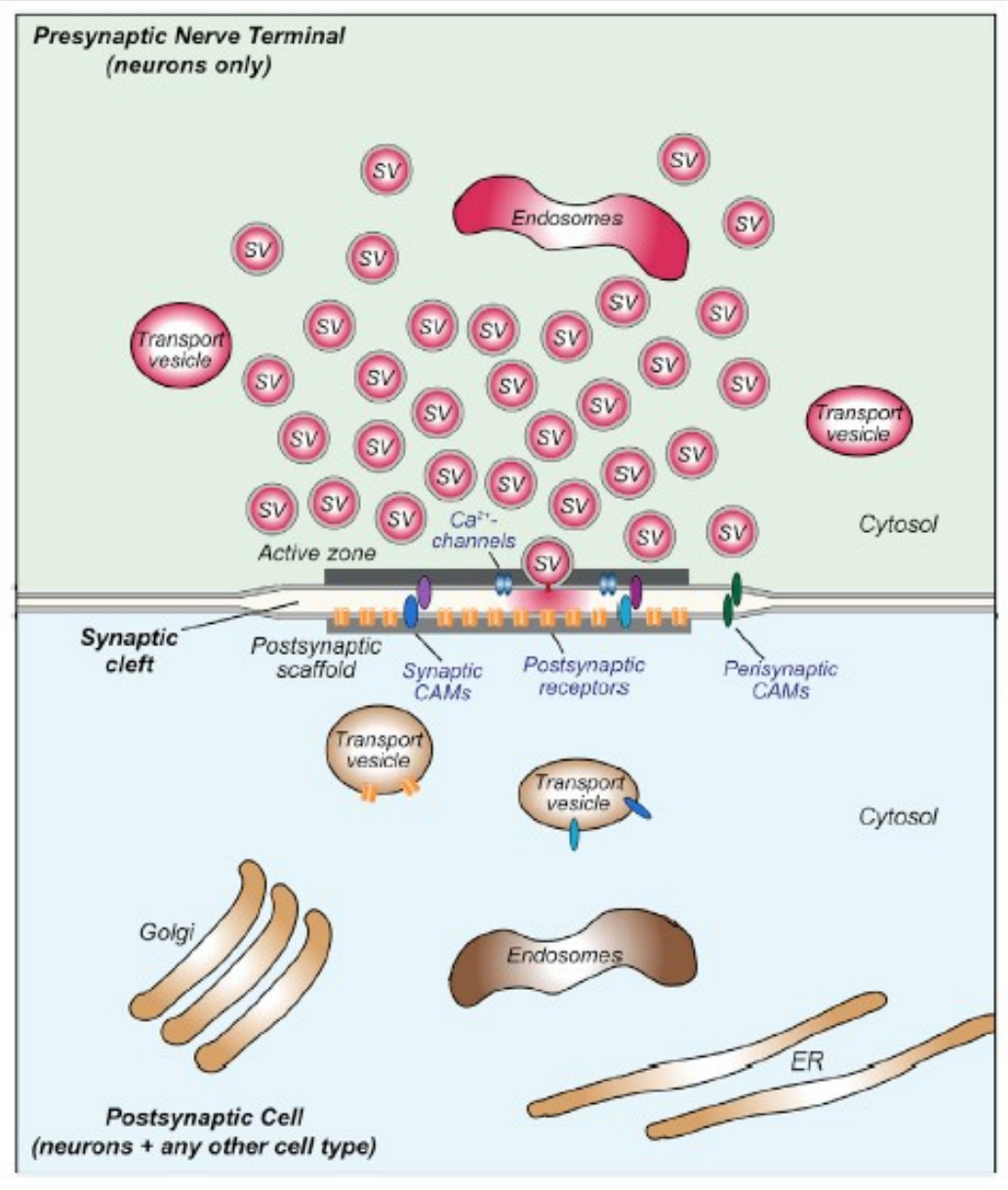


Ancoraggio e segnalazione intracellulare delle molecole di adesione sinaptiche: impalcature multimolecolari

- Interazione fra proteine tramite domini citoplasmatici e/o proteine scaffold
- Caderine: interazione con catenine e col citoscheletro di actina
- Integrine: interazione col citoscheletro di actina tramite vinculina e spectrina
- Segnalazione via attività enzimatica propria o tramite interazione con altre proteine (kinasi, fosfatasi)



RAPPRESENTAZIONE CLASSICA DELLA SINAPSI

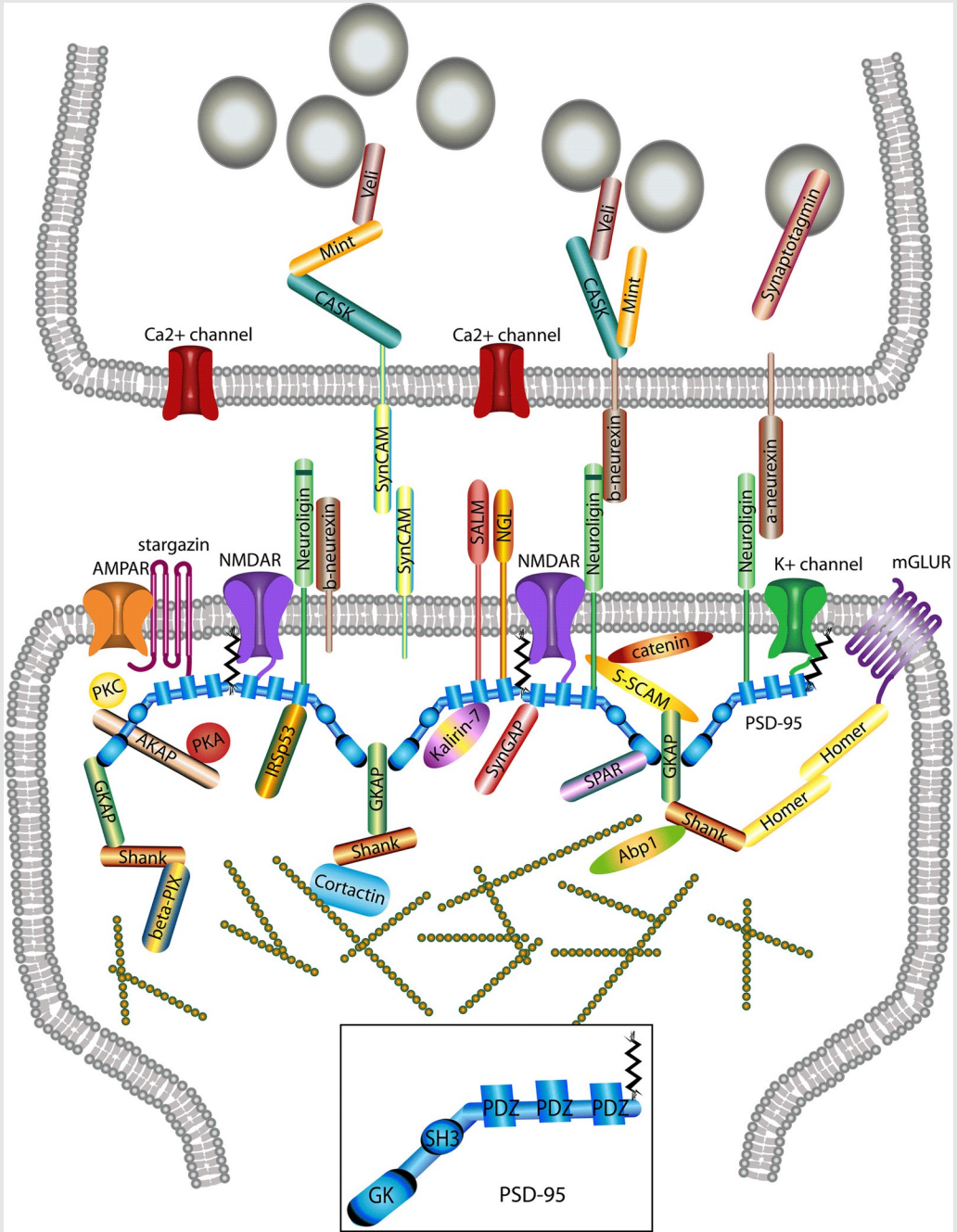


Tutte le sinapsi contengono componenti pre-sinaptici simili independentemente dal tipo cellulare, sebbene isoforme specifiche possono variare.

Mentre la componente post-sinaptica ha meno omologie

Le specializzazioni pre-sinaptiche sono formate da neuroni mentre quelle post-sinaptiche possono essere formate da cellule anche diverse.

DOMINII DI INTERAZIONE INTRACELLULARE: IL DOMINIO PDZ



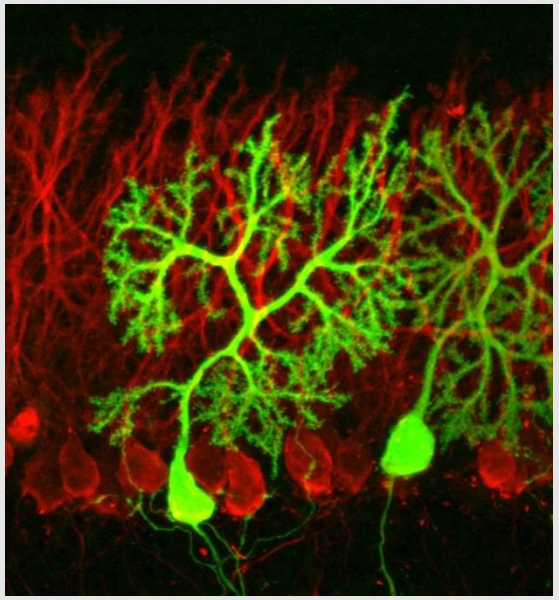
Domini PDZ: lunghi 90 residui, identificati come regioni omologhe in diverse proteine di segnalazione

Il nome PDZ deriva dalle prime 3 proteine in cui il dominio è stato identificato: PSD-95 (proteina di 95 kDa nella densità post-sinaptica; Dlg (the *Drosophila* discs large protein), and ZO1 (proteina della zonula occludente).

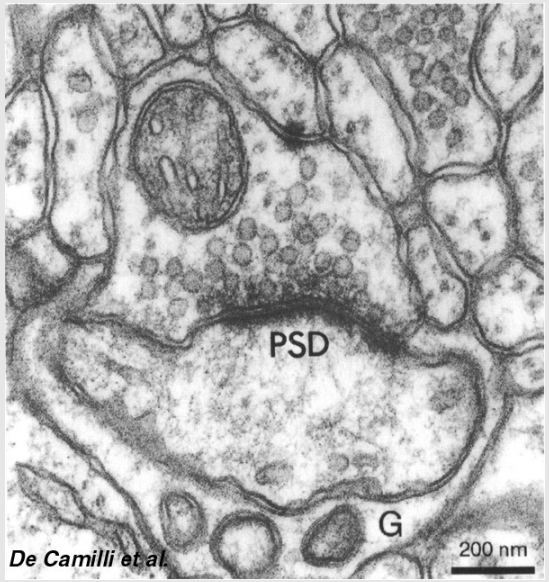
DALLE MOLECOLE DI ADESIONE AI COMPLESSI SINAPTICI

REGOLAZIONE DELLA STRUTTURA E FUNZIONE

- Le molecole di adesione sono i regolatori centrali della polarizzazione cellulare, della migrazione e dell'organizzazione tri-dimensionale della sinapsi

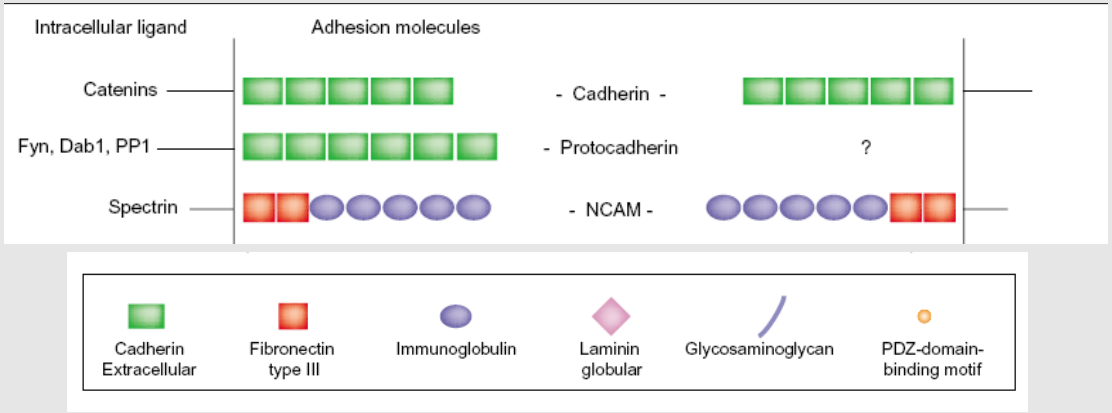


- I complessi sinaptici composti da molecole di adesione cellulare ed altre molecole sinaptiche sono regolati in maniera dinamica per modificare la architettura e la funzione cellulare.



Si stima che nell'uomo ci siano 470 molecole di adesione sebbene non sia noto Quante di queste siano espresse nel cervello e quante siano quelle sinaptiche.

Come è raggiunta la diversità:
-La maggior parte ha una regione extracellulare con un'organizzazione modulare che alterna differenti moduli strutturali.
-In più, la maggior parte delle famiglie di molecole di adesione sinaptica contengono diversi membri che pur presentando strutture conservate hanno un certo grado di diversità nella sequenza aminoacidica.
- possono assumere funzioni diverse durante fasi diverse dello sviluppo mentre le connessioni si stanno formando e nel cervello maturo:
Es. in fasi precoci dello sviluppo: neuroligins and LRRTMs si compensano;
Ma una volta formate le sinapsi, hanno effetti diversi sulla trasmissione di tipo eccitatoria



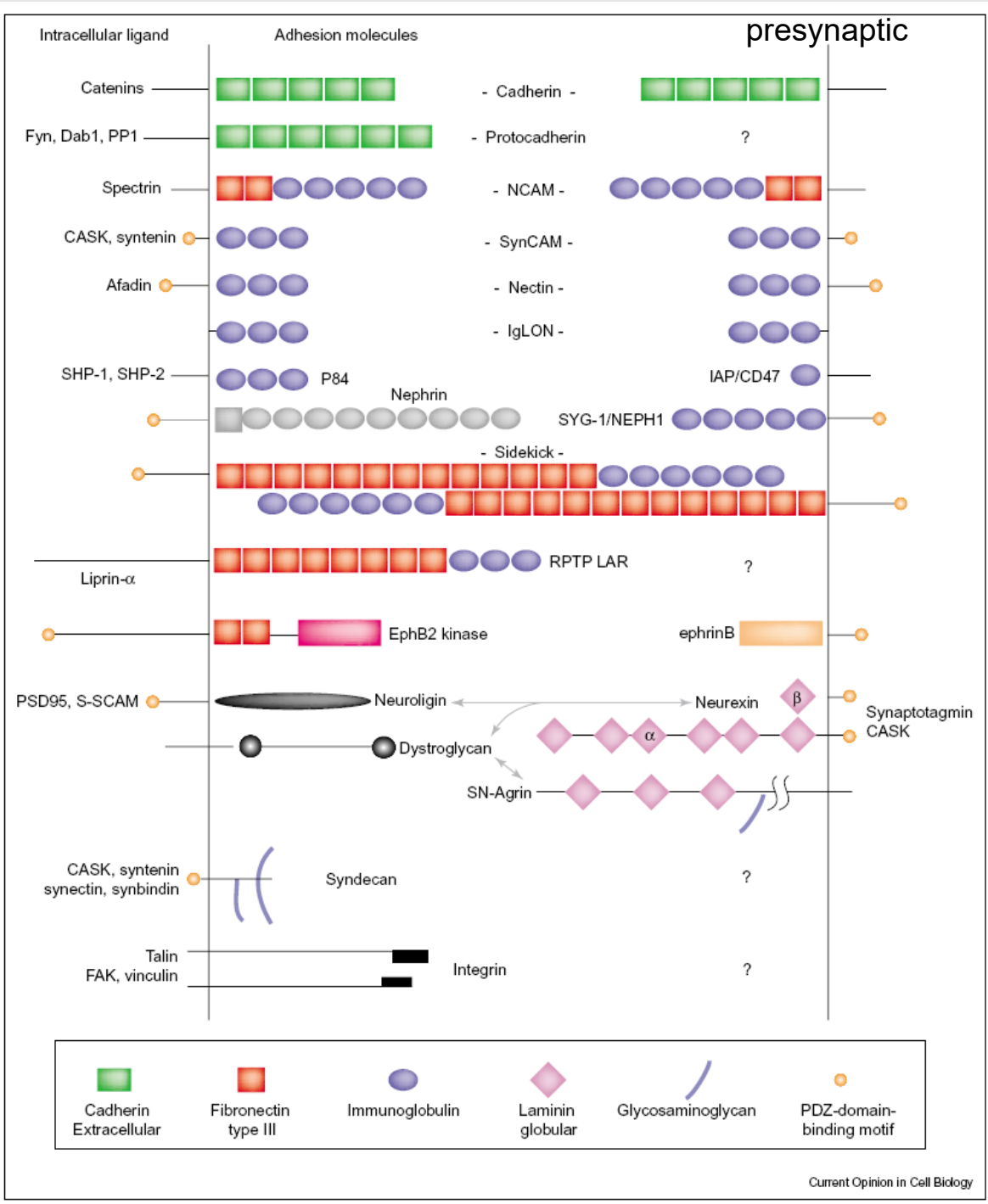
Sebbene ad oggi si conoscano molte molecole di adesione sinaptica, di molte di loro ancora non si conosce l'esatto ruolo funzionale….

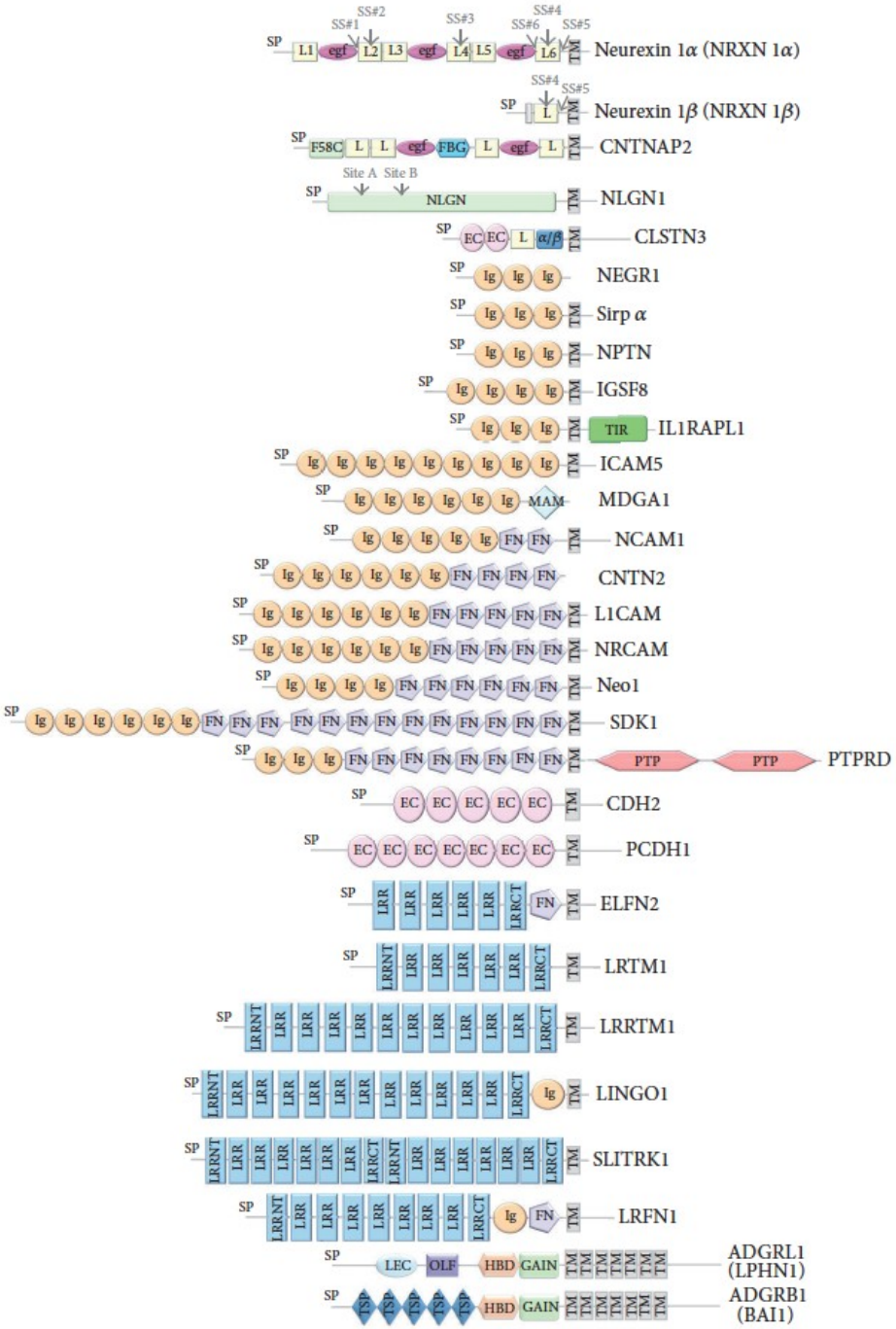
MOLECOLE DI ADESIONE (CAM/SAM) CONCENTRATE ALLE SINAPSI NEURONE-NEURONE

è possibile raggrupparle in base alla loro struttura in sottofamiglie.
Quelle principali sono:

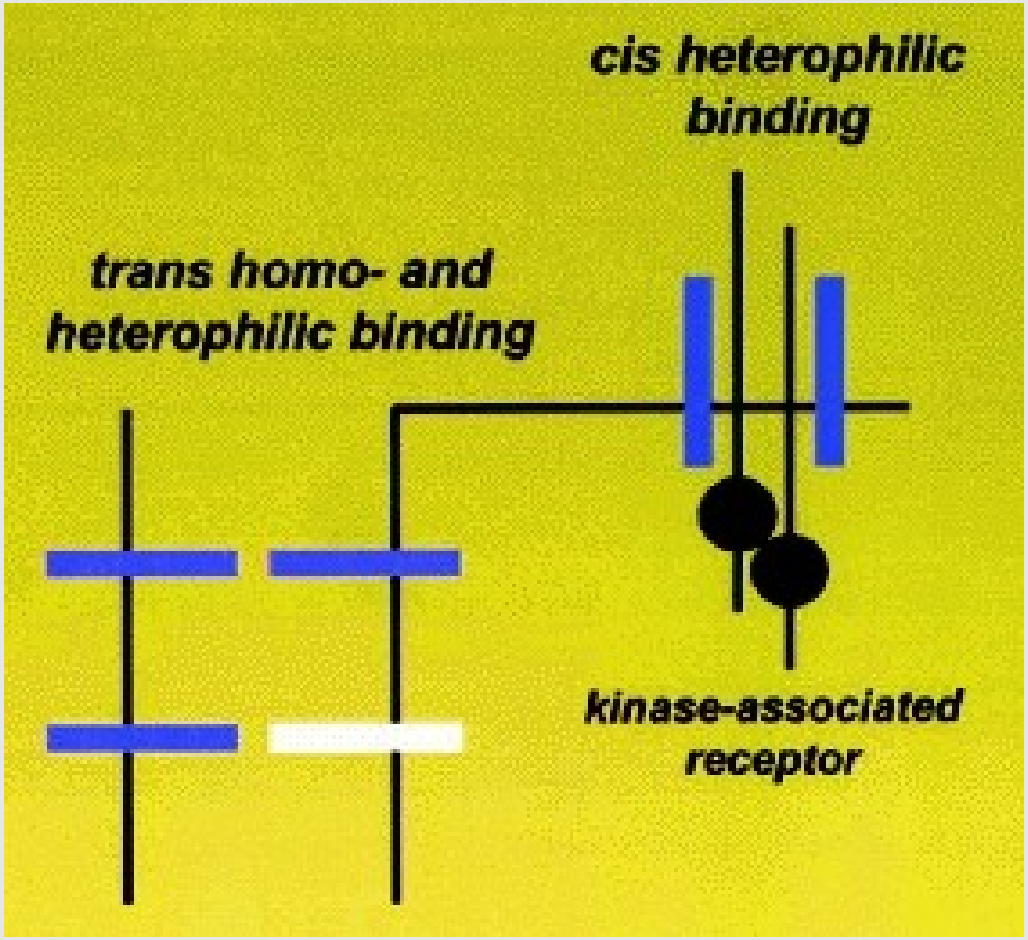
- Caderine
- Immunoglobuline CAM
- Recettori Eph-efrine
- Neurexine-neurolighine

La classifica delle famiglie di molecole di adesione puo' avvenire in base a diversi criteri, uno dei quali e' il tipo di legame che i componenti di ogni famiglia mediano




SS#1
SS#2
SS#3
SS#4
SS#5
SS#6
SP
L1
egf
L2
L3
egf
L4
L5
egf
L6
TM
Neurexin 1α (NRXN 1α)
L
Neurexin 1β (NRXN 1β)
F5/8C
FBG
CNTNAP2
Site A
Site B
NLGN
NLGN1
EC
α/β
CLSTN3
Ig
NEGR1
Sirp α
NPTN
IGSF8
TIR
IL1RAPL1
ICAM5
MAM
MDGA1
FN
NCAM1
CNTN2
L1CAM
NRCAM
Neo1
SDK1
PTP
PTPRD
CDH2
PCDH1
LRR
LRRCT
ELFN2
LRRNT
LRTM1
LRRTM1
LINGO1
SLITRK1
LRFN1
LEC
OLF
HBD
GAIN
ADGRL1
(LPHN1)
TSP
ADGRB1
(BAI1)

LEGAME OMOFILICO ED ETEROFILICO



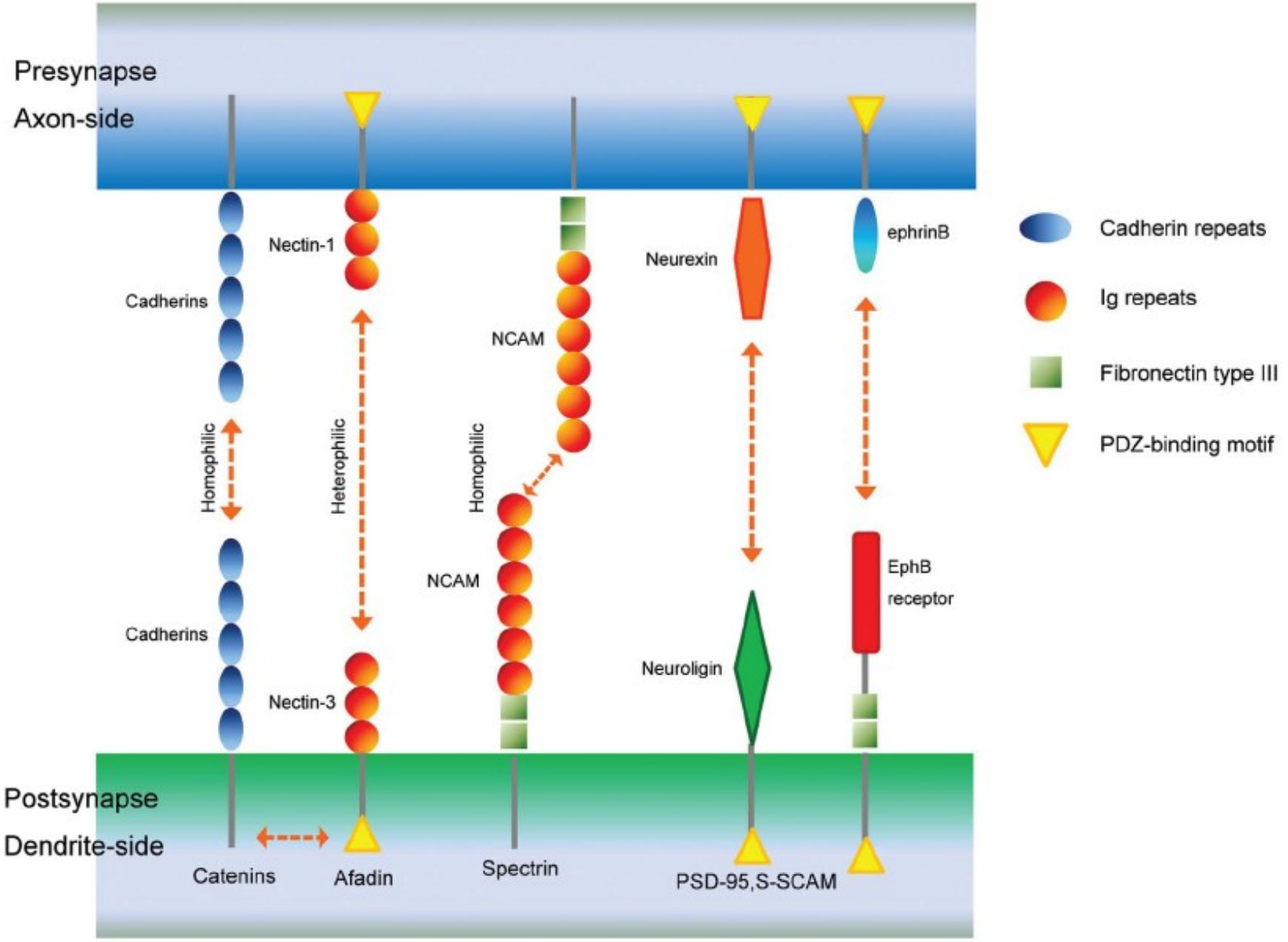
Omofilico: legame con la stessa molecola sulla membrana della cellula adiacente

Eterofilico: legame con una molecola differente sulla membrana della cellula adiacente

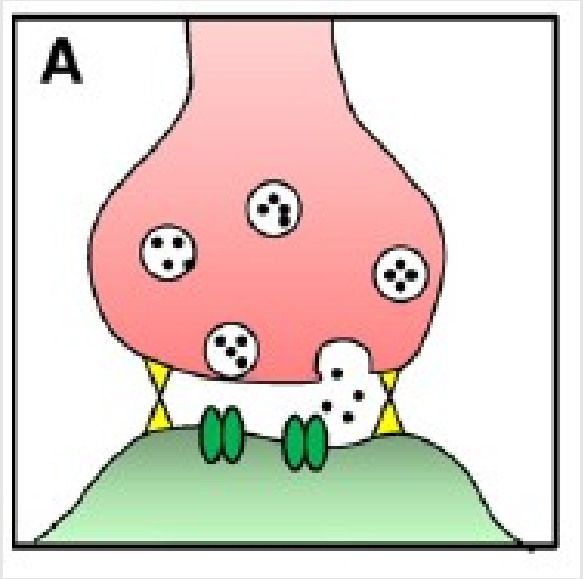
Le molecole di adesione possono interagire in maniera etero- o omofilica tra due cellule diverse (interazione in trans) o sullo stesso piano della membrana di una singola cellula (interazione in cis)

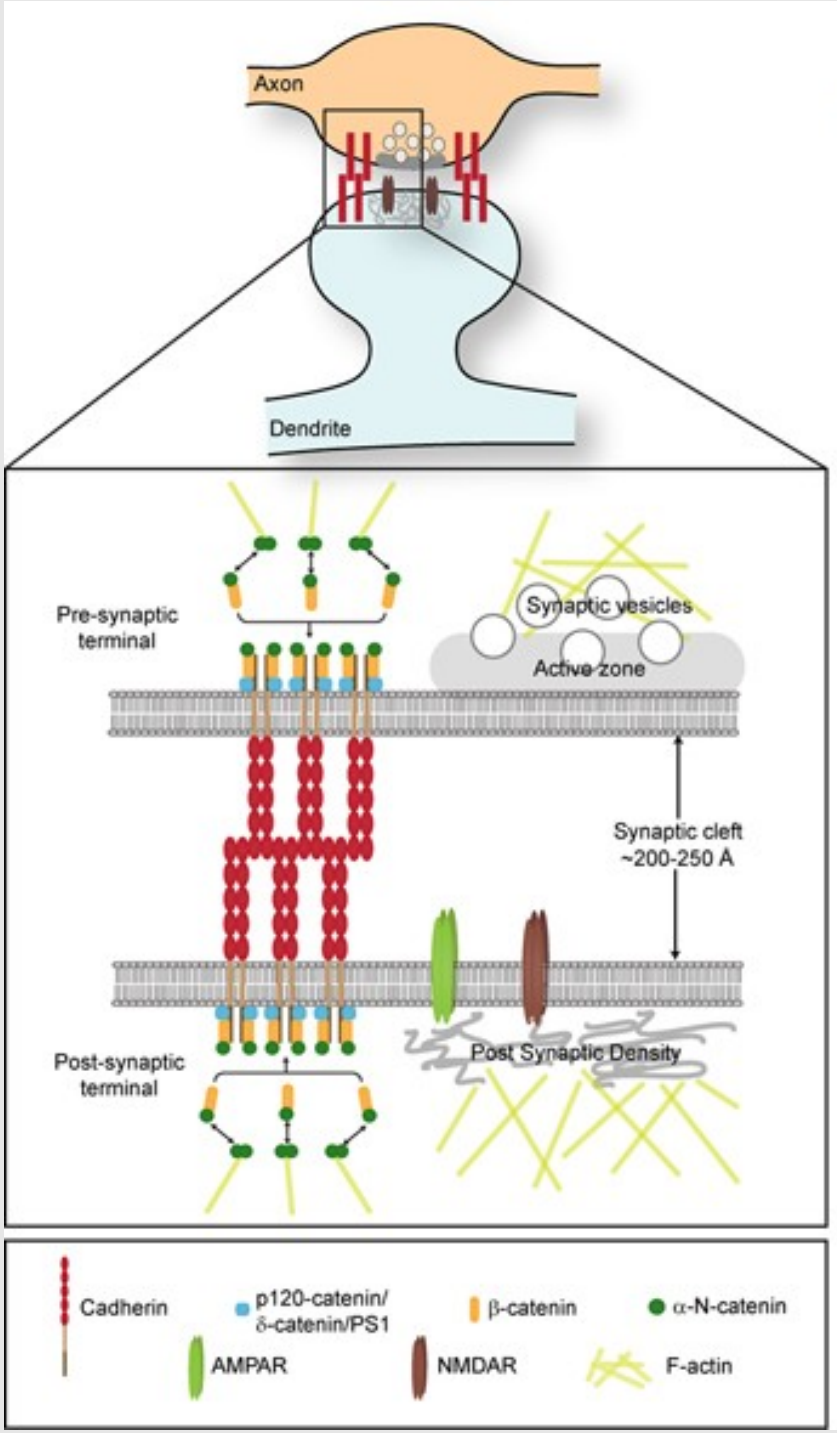
Legame omofilico: caderine, famiglia delle immunoglobuline (NCAM)

Legame eterofilico: recettori Eph/ Efrine, neurolighine/neurexine



LEGAME OMOFILICO





LA SUPERFAMIGLIA DELLE CADERINE

Presenti sia ai terminali presinaptici che post-sinaptici

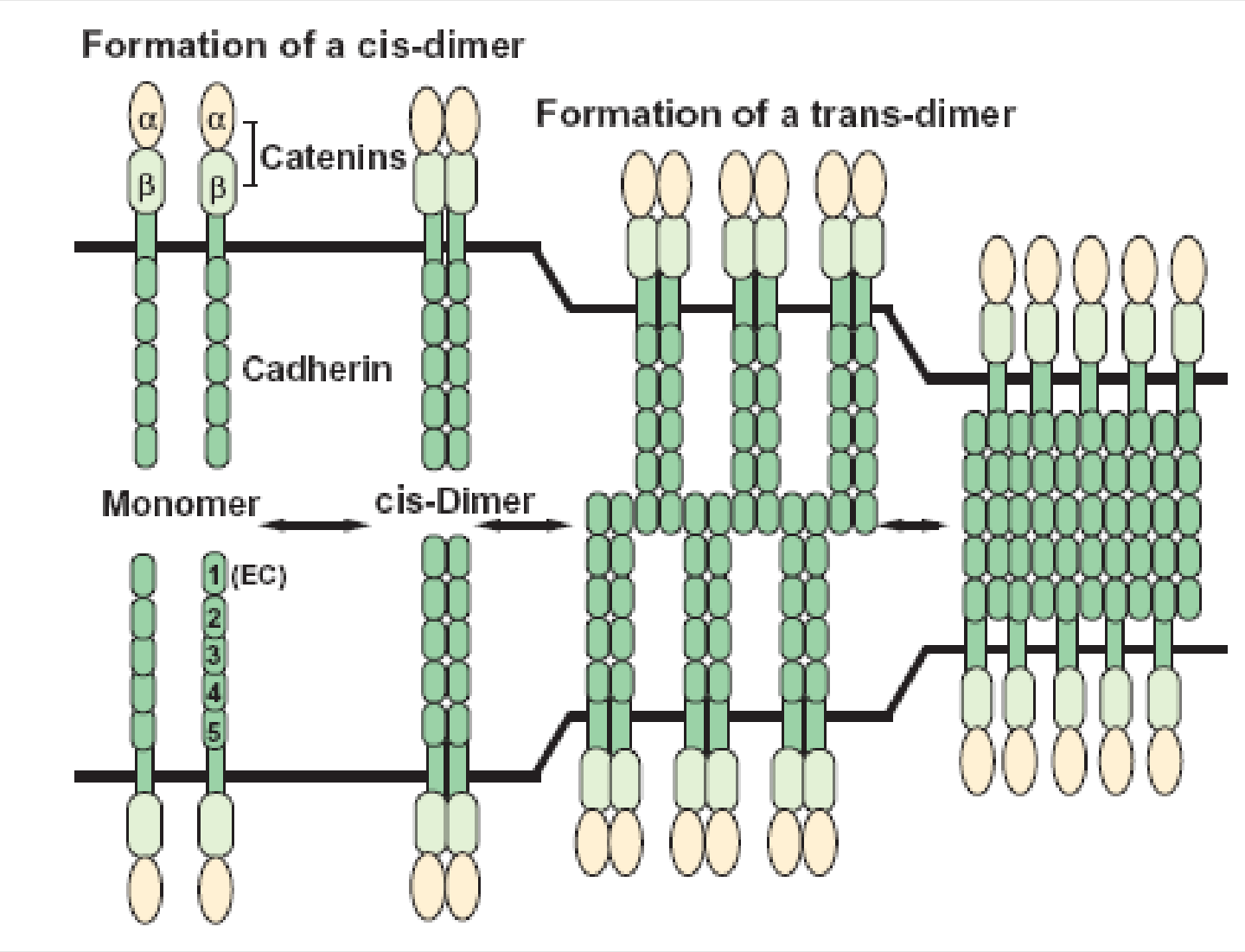
Legano altre Caderine
sia in cis che in trans

Legame calcio dipendente

Dimeri in cis che poi associano con altri dimeri in cis nello spazio sinaptico per formare complessi di adesione in trans (legame a cerniera)

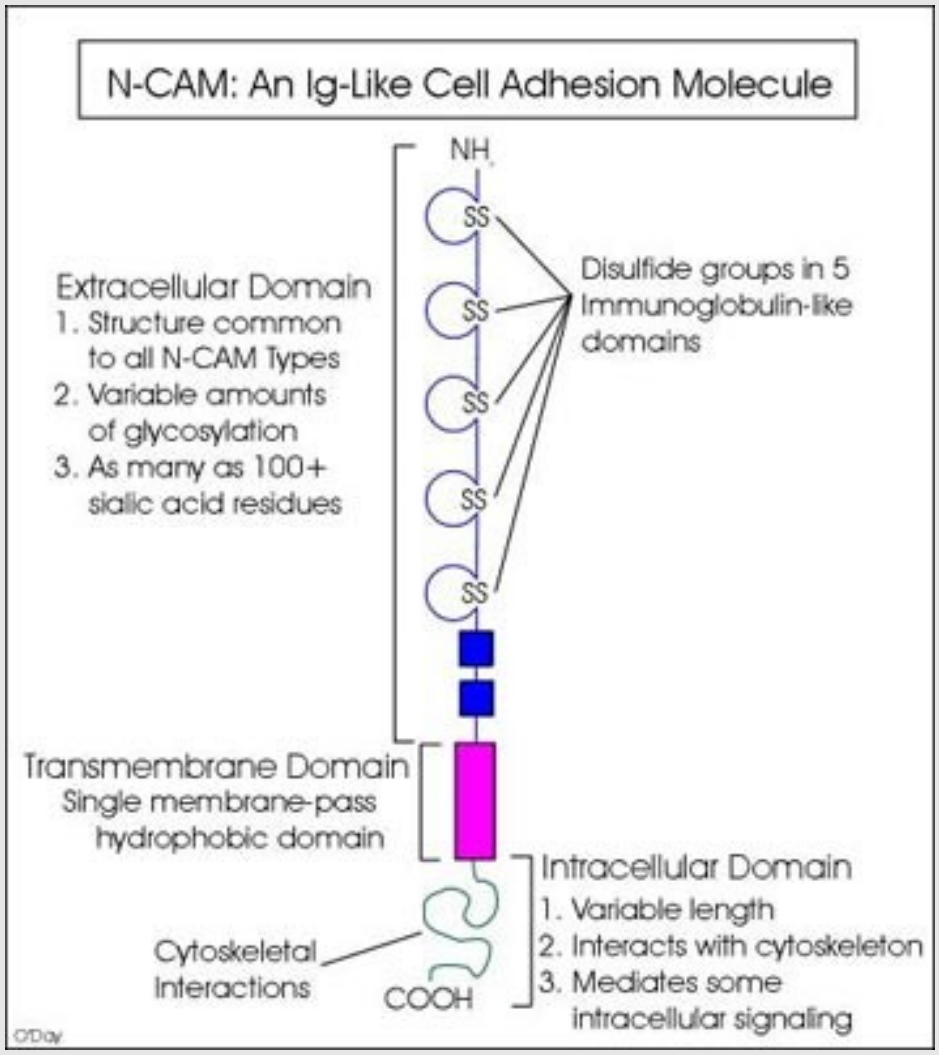
Interazione con il citoscheletro tramite catenine

Si formano dimeri in *cis* che associano con altri dimeri in *trans* nello spazio sinaptico per formare complessi di adesione altamente resistenti



MOLECOLE DI ADESIONE SUPERFAMIGLIA IMMUNOGLOBULINE

Maggiori rappresentanti: NCAM, L1, Sidekick, SynCAM, Nectina



Legami di tipo omofilico sia in trans che in cis

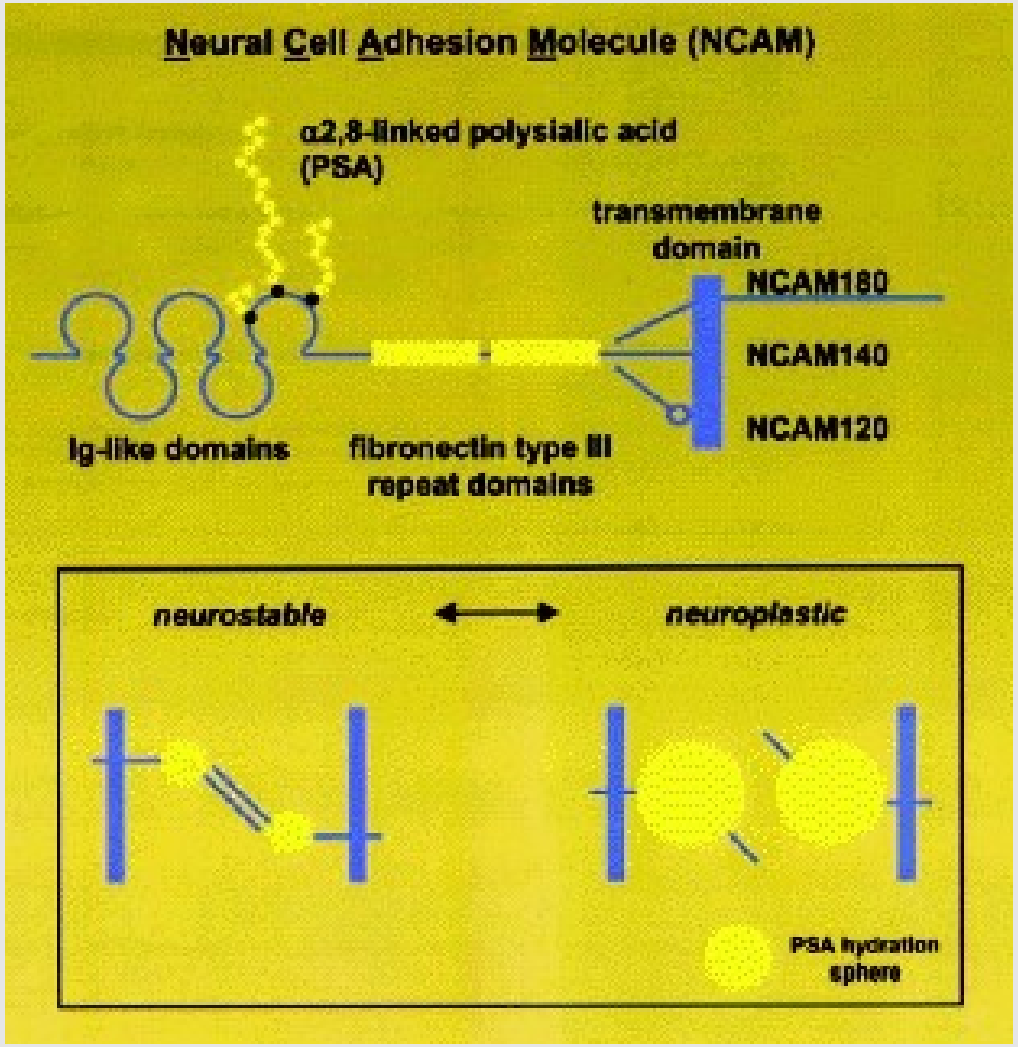
Legame indipendente dal calcio

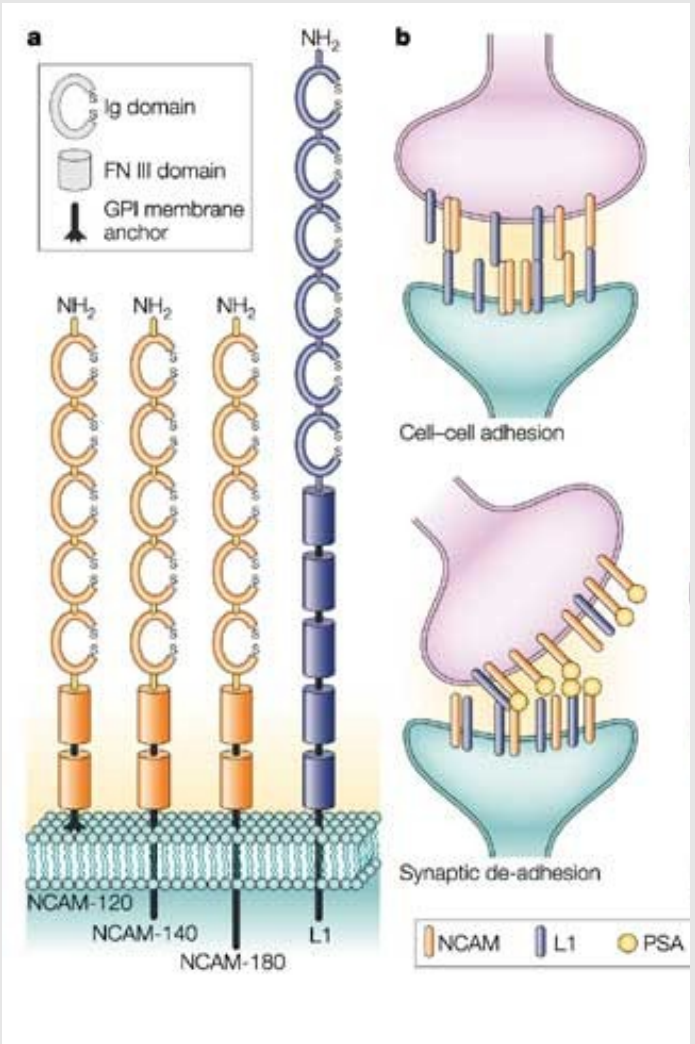
Il dominio extracellulare ha 5 dominii tipici delle immunoglobuline seguiti da dominii tipici della fibronectina (in blu)

Dominio Ig media legame omofilico

L'acido polisialico (PSA) regola il legame omofilico di NCAM

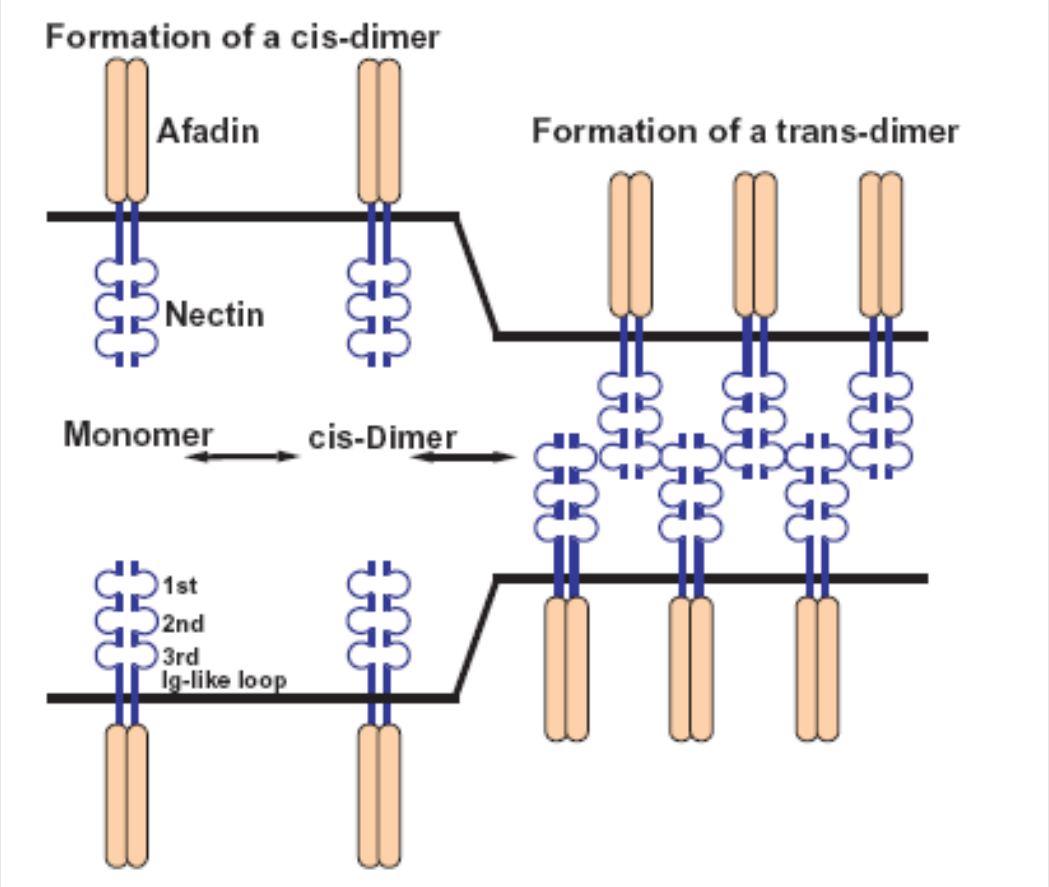
NCAM è glicosilata e viene modificata post-traduzionalmente per aggiunta di acido polisialico al quinto dominio Ig, questo sembra ridurre la sua capacità di adesione in eventi di migrazione cellulare





“Con-nectin axons and dendrites”

Le nectine fanno parte della famiglia delle immunoglobuline e si associano principalmente tramite legami di tipo omofilico che ricordano le interazioni tra le caderine, ma possono anche legarsi in maniera eterofilica con nectine di tipo diverso (principalmente nectina 1 con nectina 3).

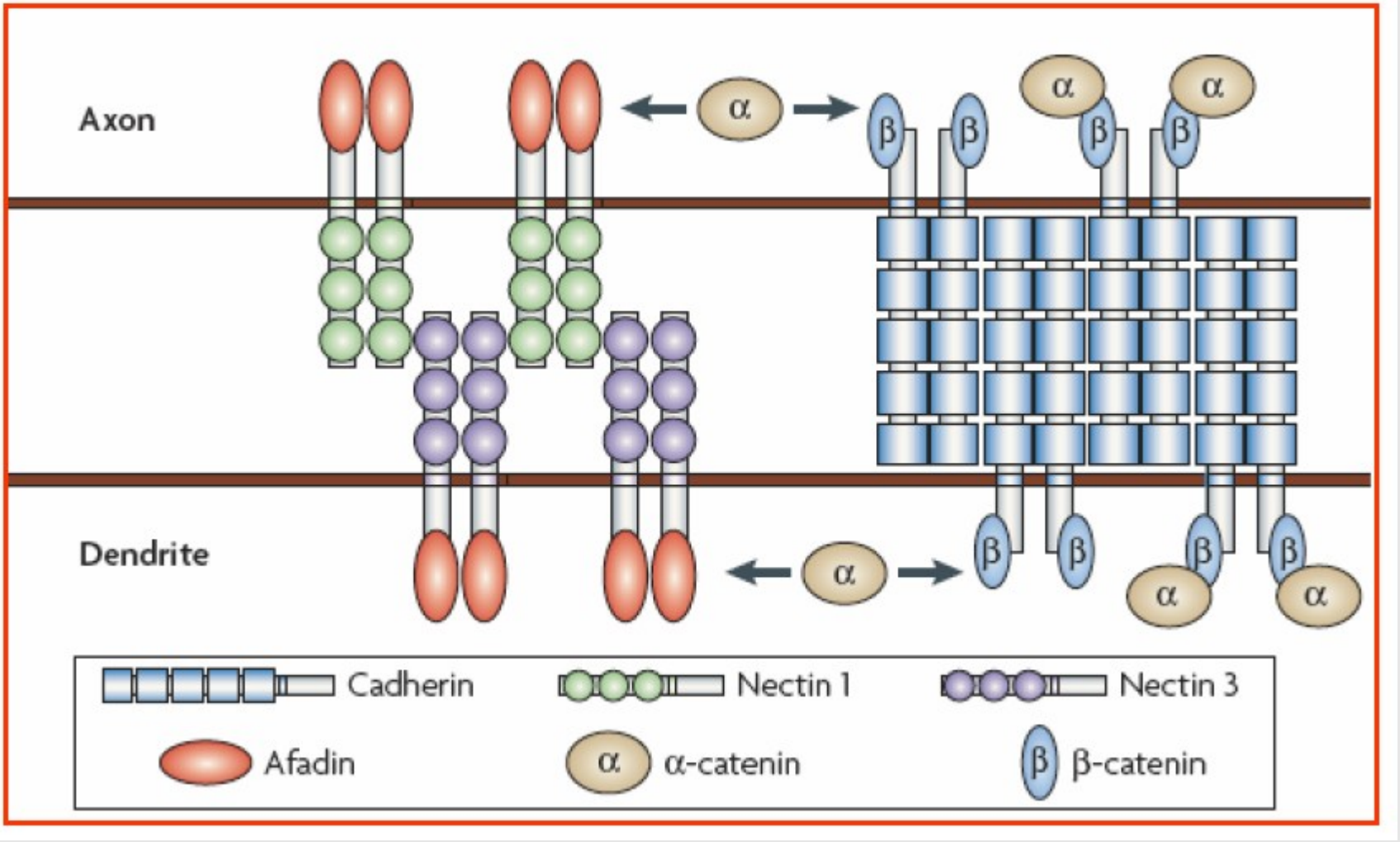


In maniera analoga al sistema caderine/catenine, nectine interagiscono con con il dominio intracitoplasmatico con le afidine

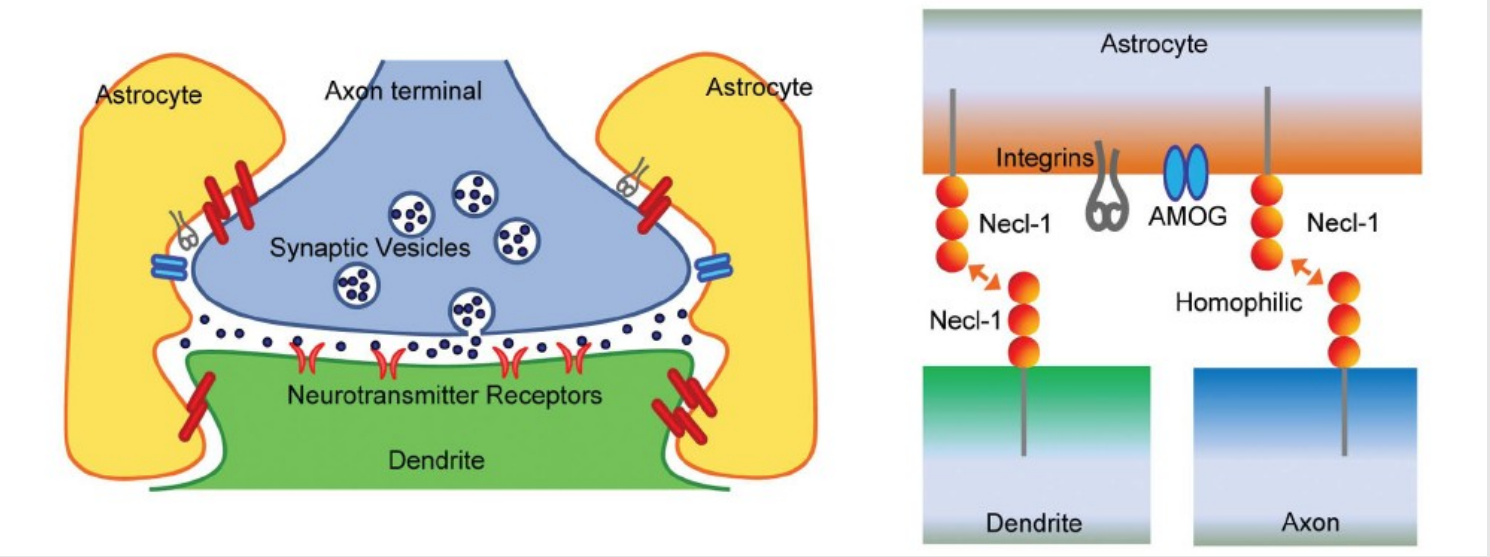
COMPLESSI DI NECTINE E CADERINE SONO CONNESSI DA INTERAZIONI INTRACITOPLASMATICHE

COMPLESSI COMPOSTI DA NECTINE

COMPLESSI COMPOSTI DA CADERINE



MOLECOLE DI ADESIONE SPECIFICHE FRA NEURONE E GLIA

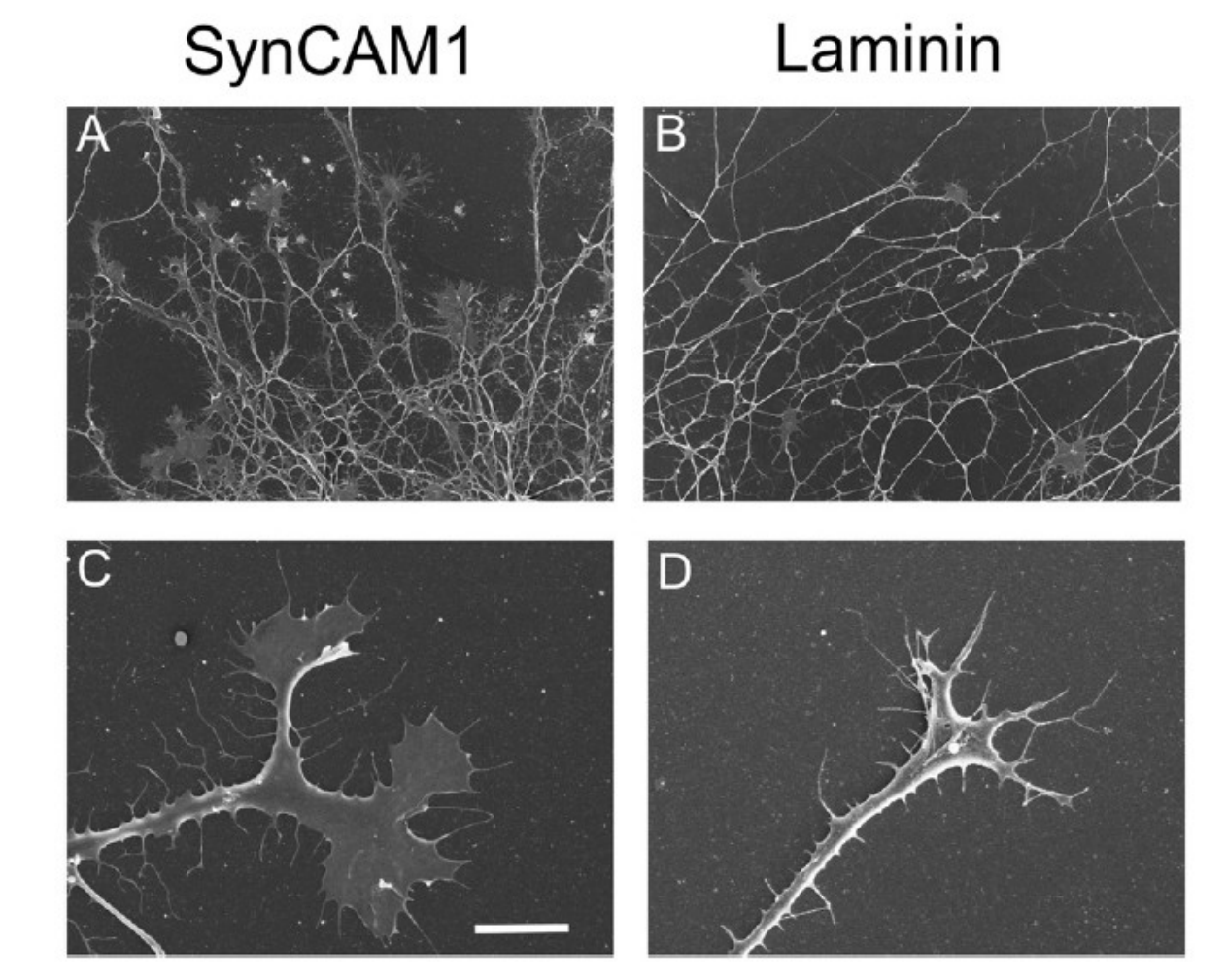


Le proteine Necls (Nectin like) sono molecole di adesione di tipo omofilico con struttura simile alle Nectine, non richiedono calcio per adesione e sono importanti per la interazione neurone-glia

Table 1 **Lists of the neuron-neuron and neuron-glia interactions in the nervous systems**

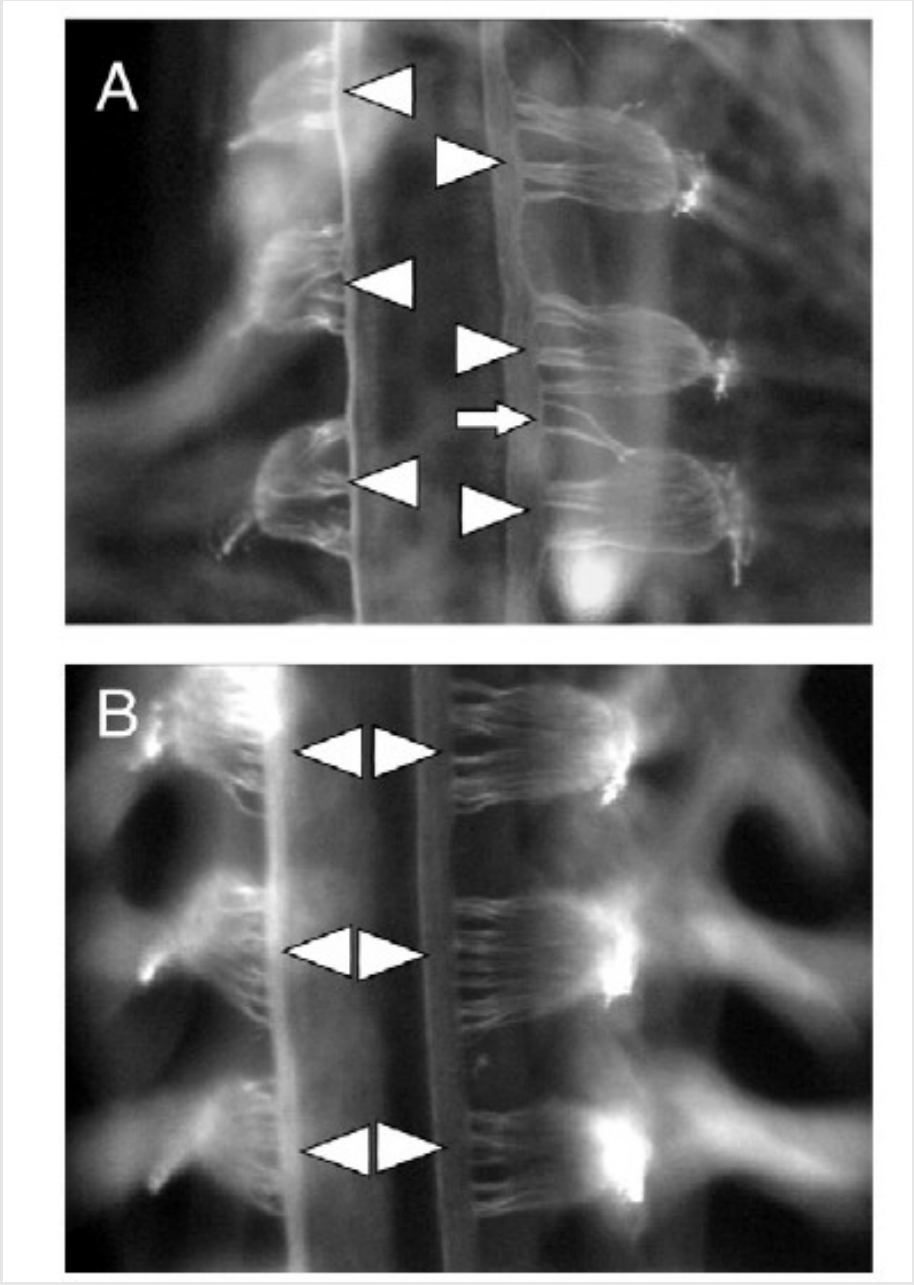
Classification	Adhesion molecules	Localization
Cadherin Super Family	Classic cadherins	Synapse (PAJs), Neuron-Glia
	Proto-cadherins	Synapse (?)
Ig-like Molecules	Nectins	Synapse (PAJs), Neuron-Glia
	Nectin-like molecules (Necls)	Neuron-Glia
	NCAM	Synapse
	Syg-1, Syg-2	Synapse
	Sidekicks	Synapse
	Integrins	Synapse, Neuron-Glia
Others	Neuroligins, neurexins	Synapse (SJs)
	Eph receptors, ephrins	Synapse (SJs)

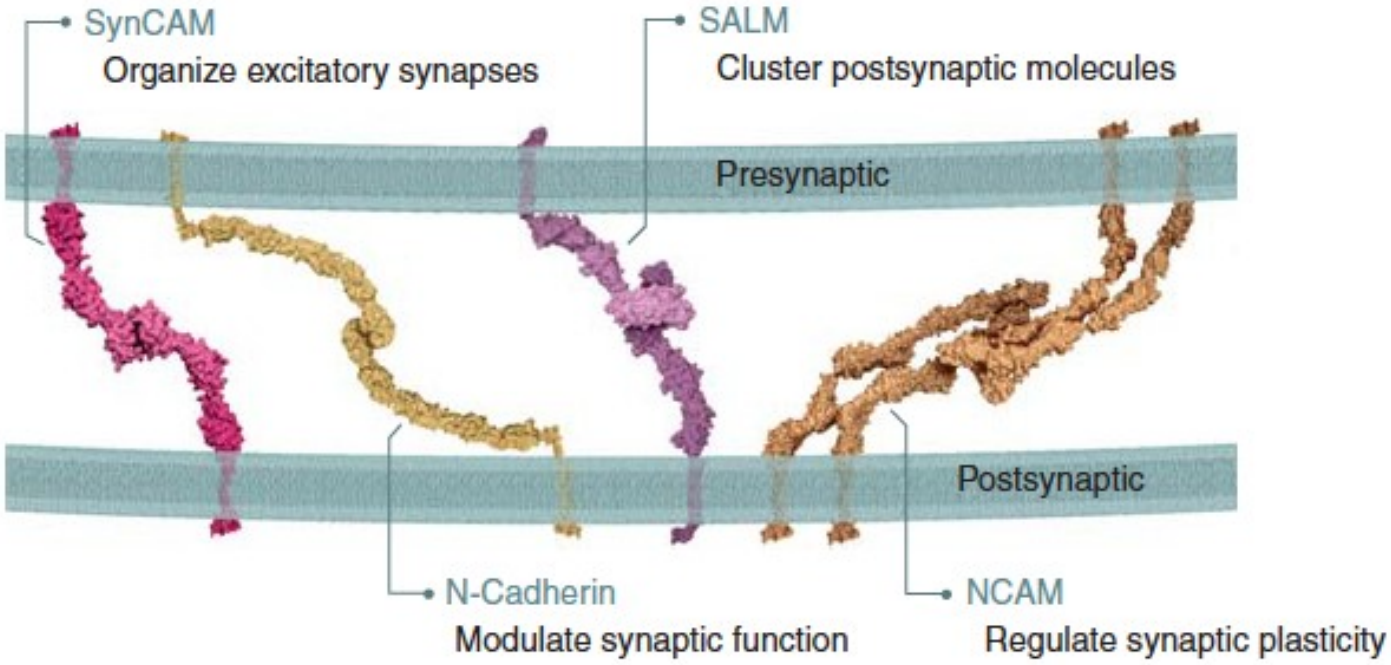
SynCAMs (synaptic cell adhesion molecules)
subfamily of the immunoglobulin superfamily of cell adhesion molecules.
They were first identified as cell adhesion molecules at the synapse which were sufficient to trigger synapse formation.
axon guidance,
synapse formation,
synaptic plasticity.

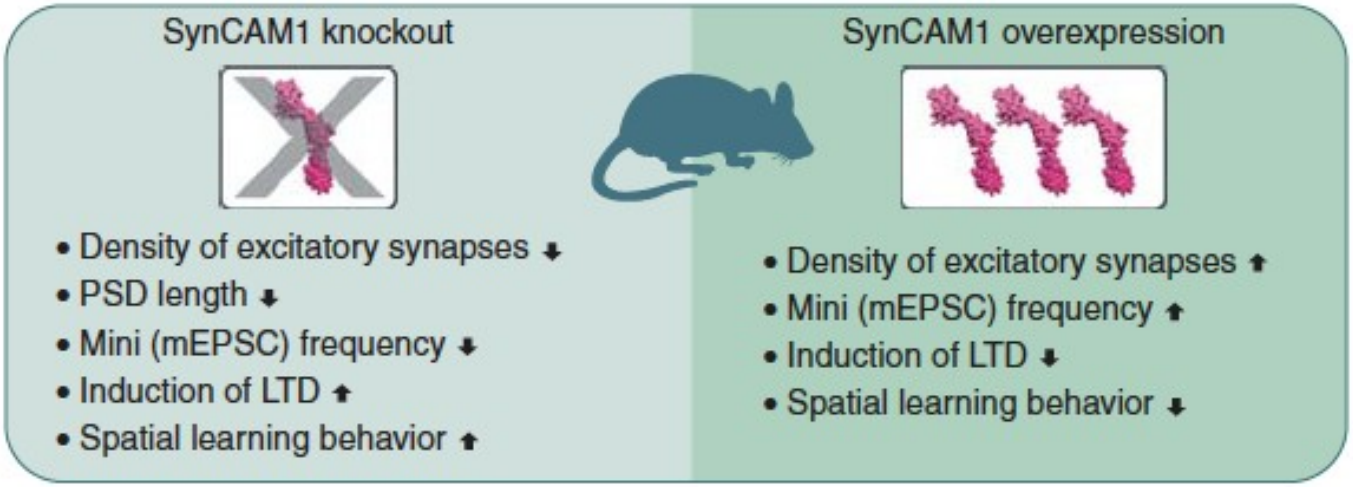


Sensory neurons grown on SynCAM1 substrate exhibit
a striking axonal flattening compared to Laminin.
The growth cones on SynCAM1 are three times larger than those on Laminin.

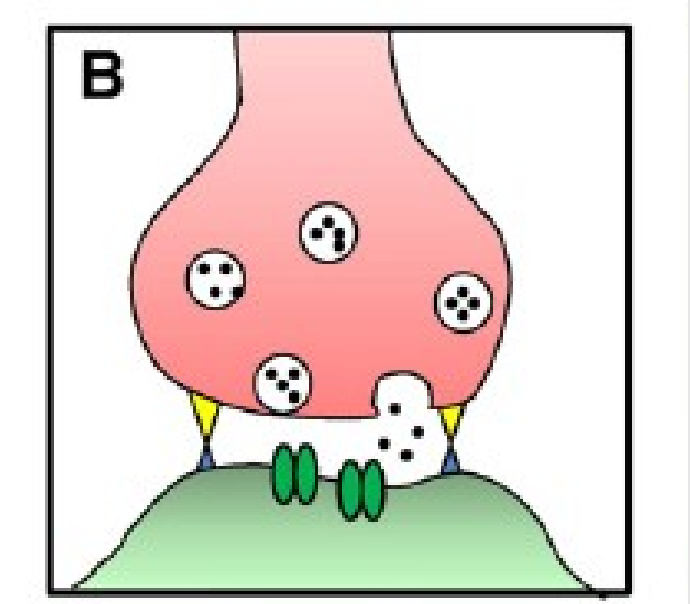
dorsal root ganglia and dorsal roots. Silencing SynCAM3 in neural crest cells by in ovo RNAi resulted in aberrant arrangement of dorsal root ganglia (compare position of arrowheads in the embryo lacking SynCAM3, shown in A, with a control-treated embryo shown in B)




SynCAM
Organize excitatory synapses
SALM
Cluster postsynaptic molecules
Presynaptic
Postsynaptic
N-Cadherin
Modulate synaptic function
NCAM
Regulate synaptic plasticity

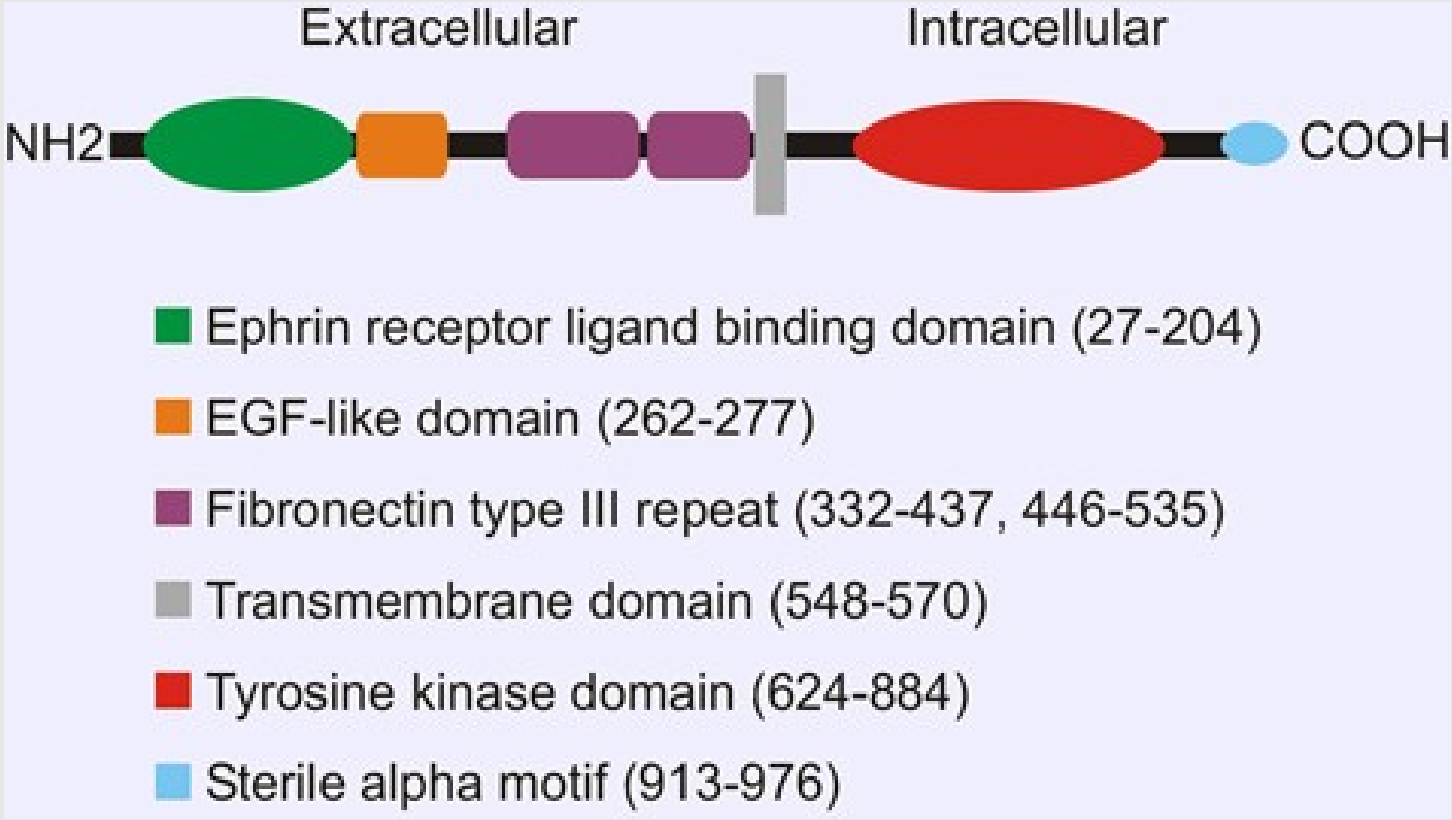

SynCAM1 knockout
• Density of excitatory synapses ↓
• PSD length ↓
• Mini (mEPSC) frequency ↓
• Induction of LTD ↑
• Spatial learning behavior ↑
SynCAM1 overexpression
• Density of excitatory synapses ↑
• Mini (mEPSC) frequency ↑
• Induction of LTD ↓
• Spatial learning behavior ↓

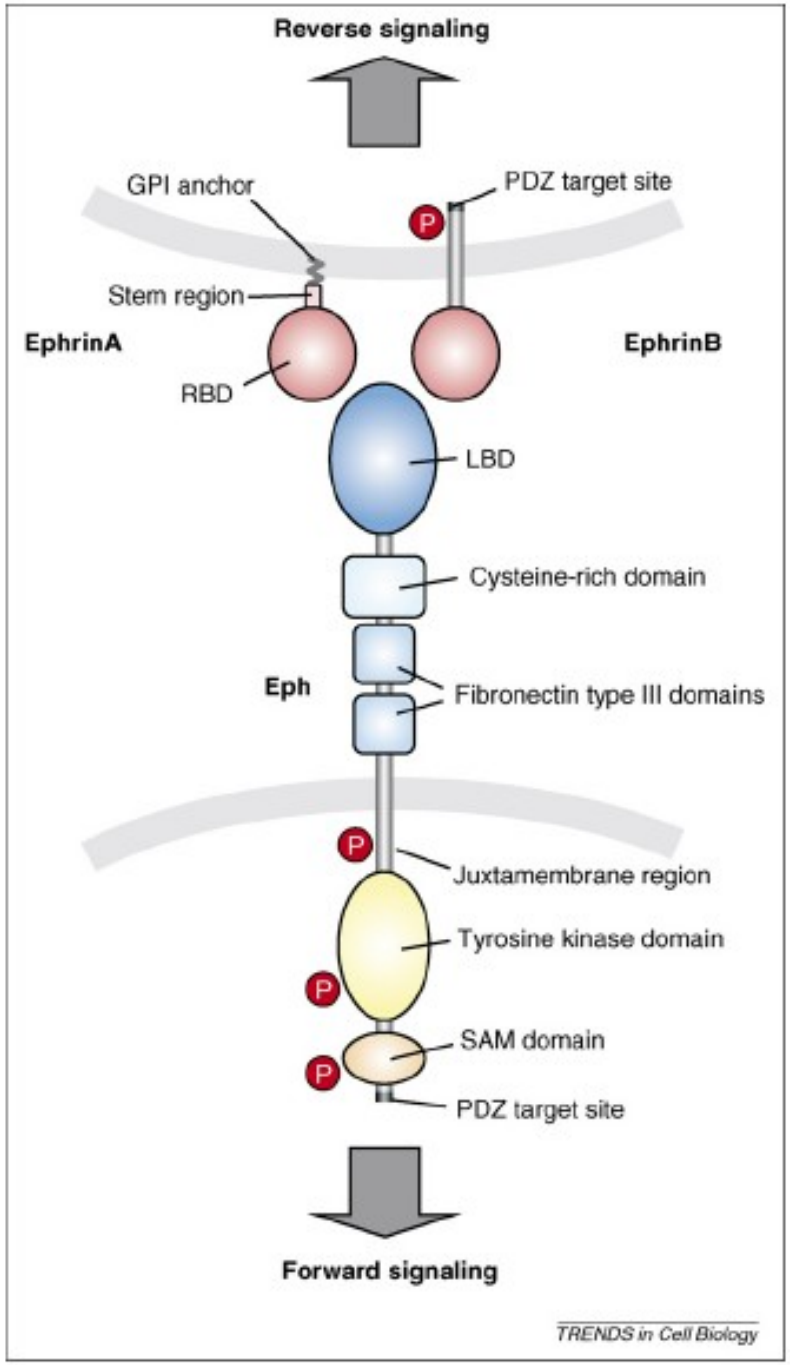
LEGAME ETEROFILICO



I RECETTORI EPH e LE EFRINE

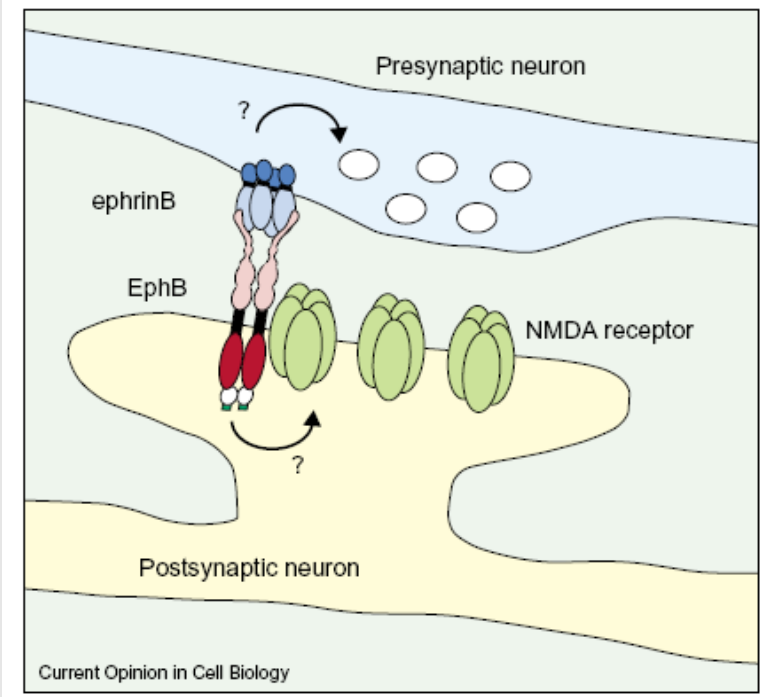
- Recettori tirosin chinasici (EphA, EphB)
- Legano proteine di membrana, le efrine (efrineA, efrineB)



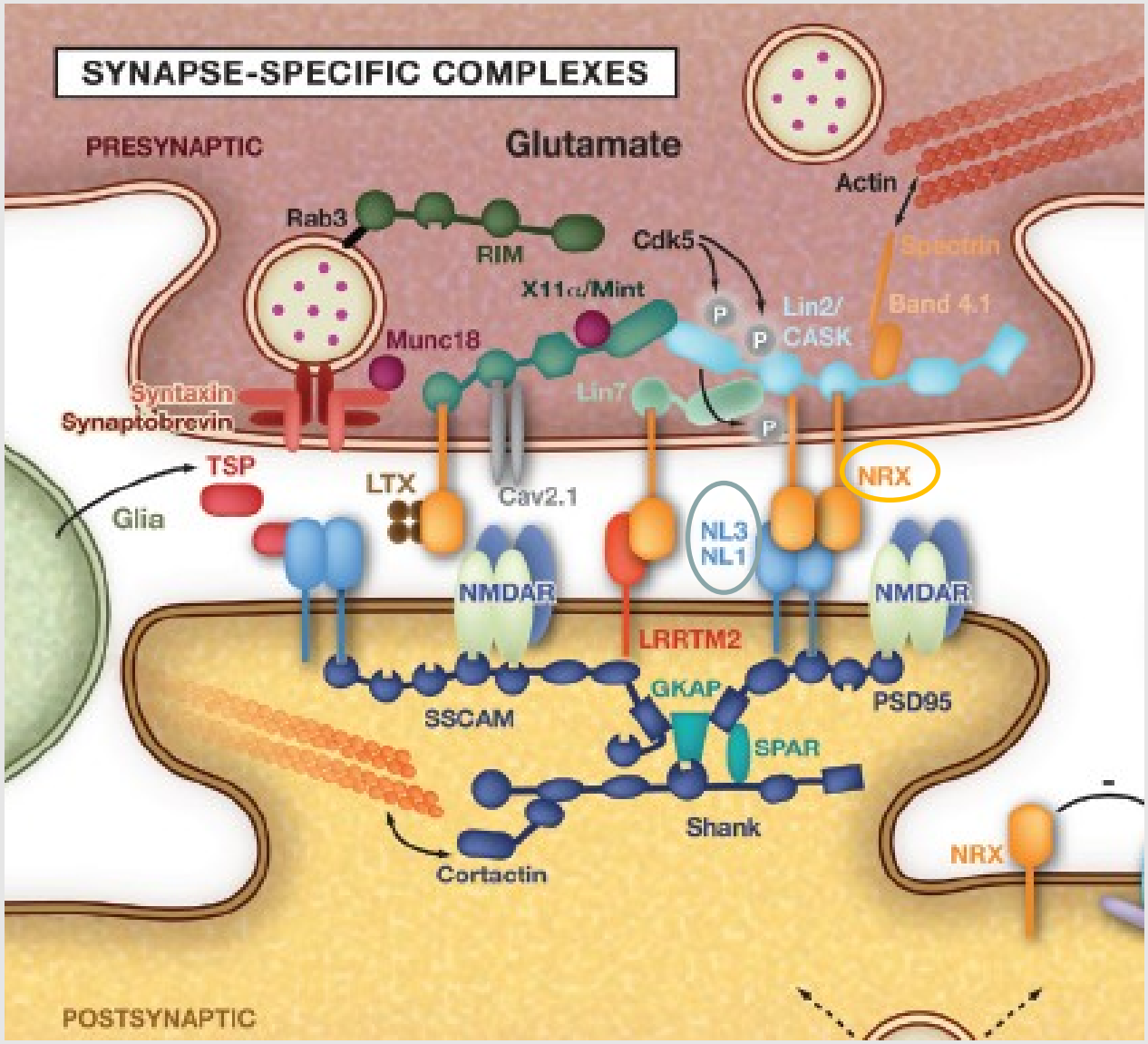


L'INTERAZIONE RECETTORE EPH-EFRINA NON E' UN ESEMPIO CLASSICO DI ADESIONE PERCHE' PUO' MEDIARE ANCHE REPULSIONE

I RECETTORI EphB SONO STATI LOCALIZZATI ALLA SINAPSI ECCITATORIE NELLE DENSITA' POST-SINAPTICHE DOVE LEGANO I RECETTORI DEL GLUTAMMATO DI TIPO NMDA



Neurolighine e Neurexine



Baudouin and Scheiffele, 2010

The Neuroligin Family

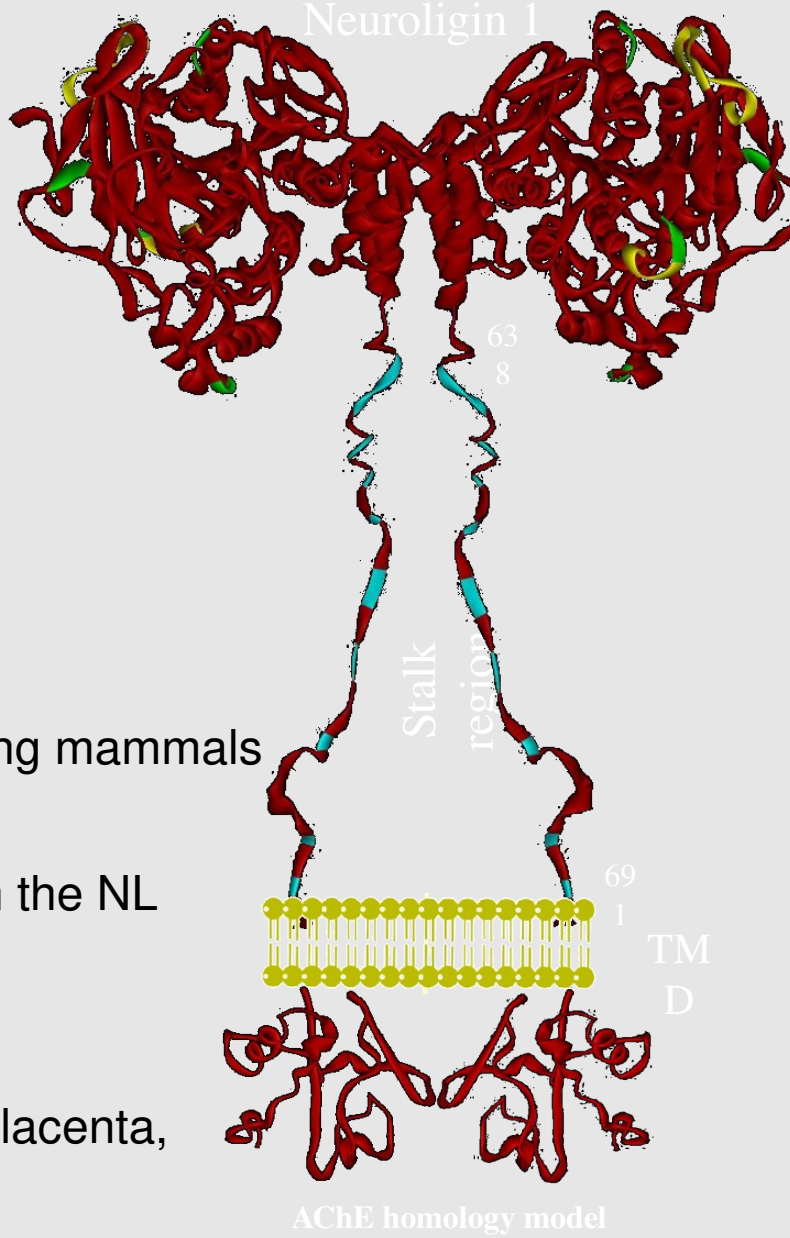
- 4 genes: NL1, NL2, NL3, NL4 :
(NL3 e NL4 map on X chr in humans)

- Form non-covalent dimers

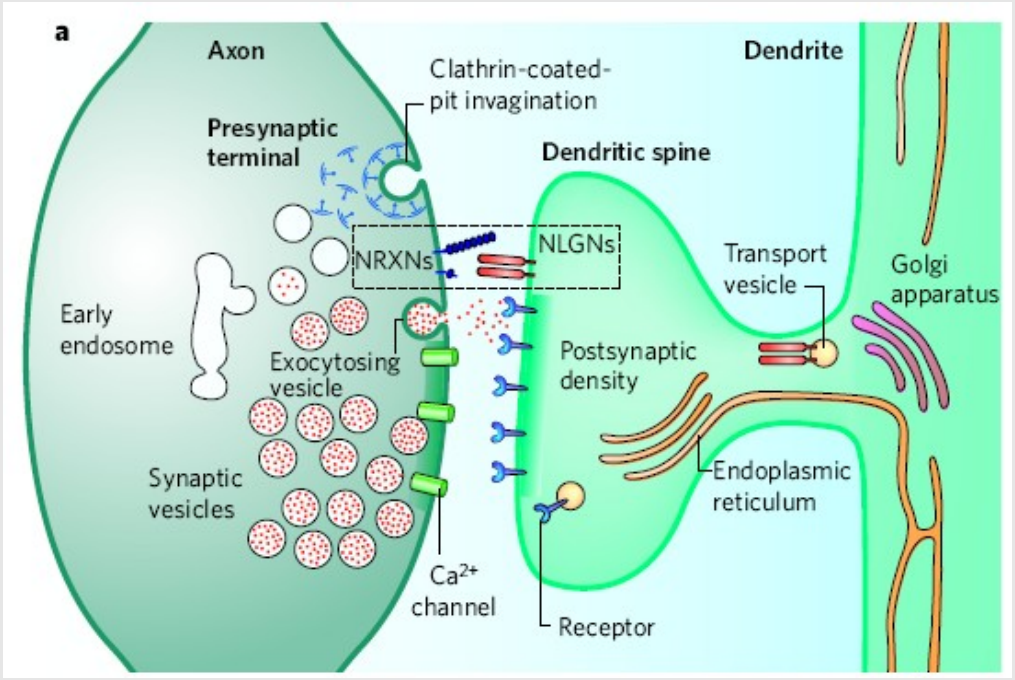
- Extra-cellular cholinesterase-like domain:
  - 2 splice inserts
  - An oligomerization domain
  - Several N- and O-linked glycosylation sites
  - Binds to the Neurexins

- High degree (~99% identity) of conservation among mammals

- High degree (~60% identity) of conservation within the NL family
- NL1 to 4 mainly expressed in the CNS
- NL3 and 4 also in heart, lung, pancreas, muscle, placenta, etc.



NEUREXINE E NEUROLIGINE MEDIANO ADESIONE ETEROFILICA ALLA SINAPSI



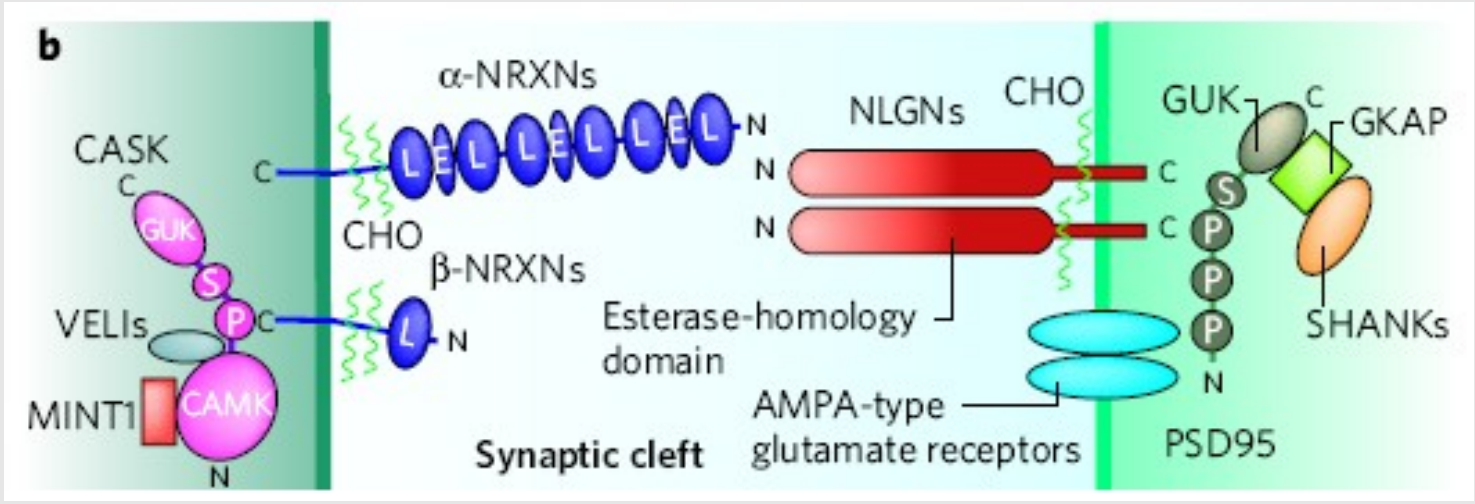
NEUREXINE

- Espresse nel SNC a livello presinaptico
- Esistono neurexine α e β
- Differiscono nel dominio extracellulare

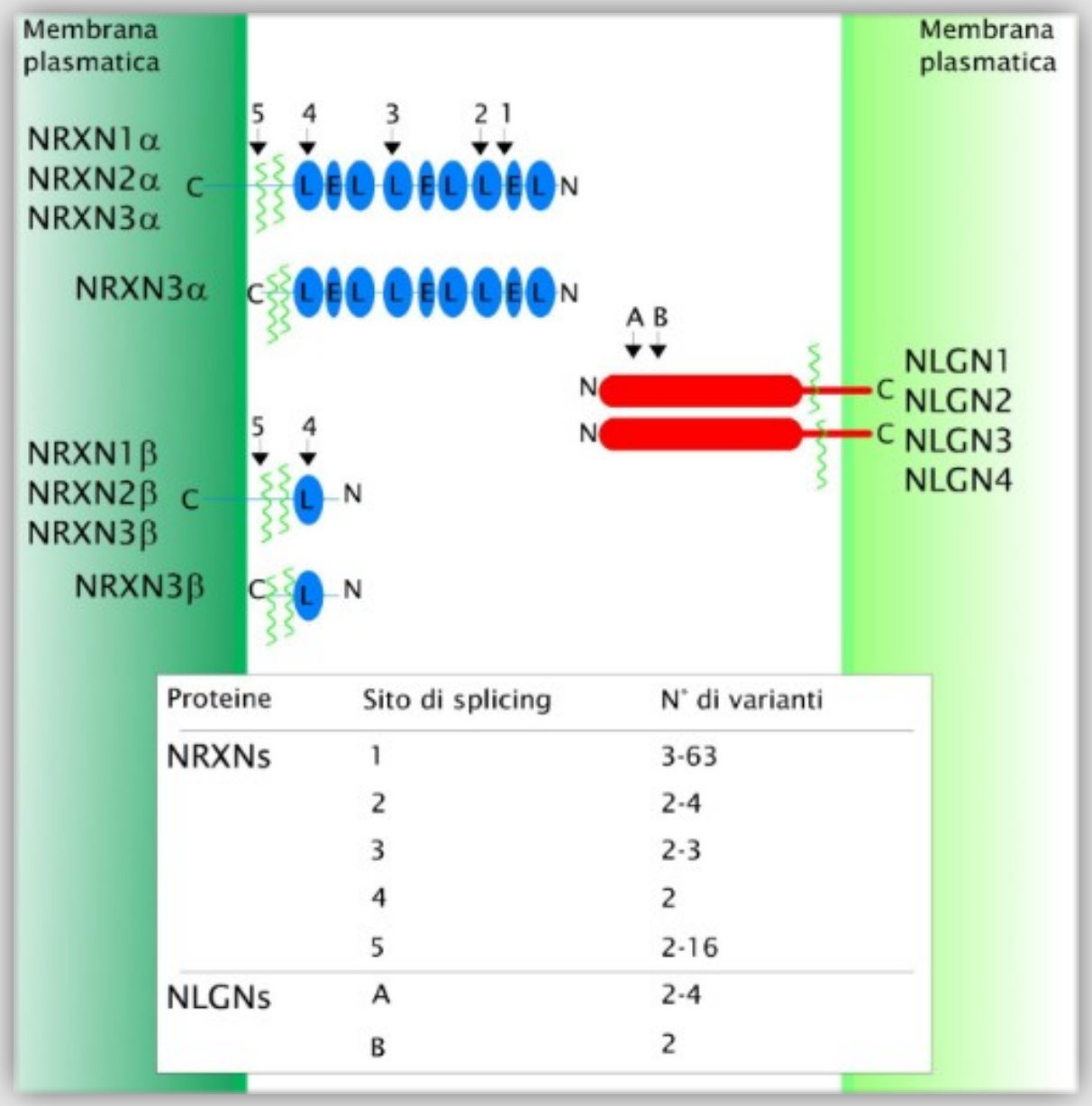
ma hanno identici dominii transmembrana e citoplasmatico.

NEUROLIGINE

- Espresse nel SNC a livello postsinaptico
- Dominio extracellulare e' simile all'acetilcolinesterasi e lega le neurexine



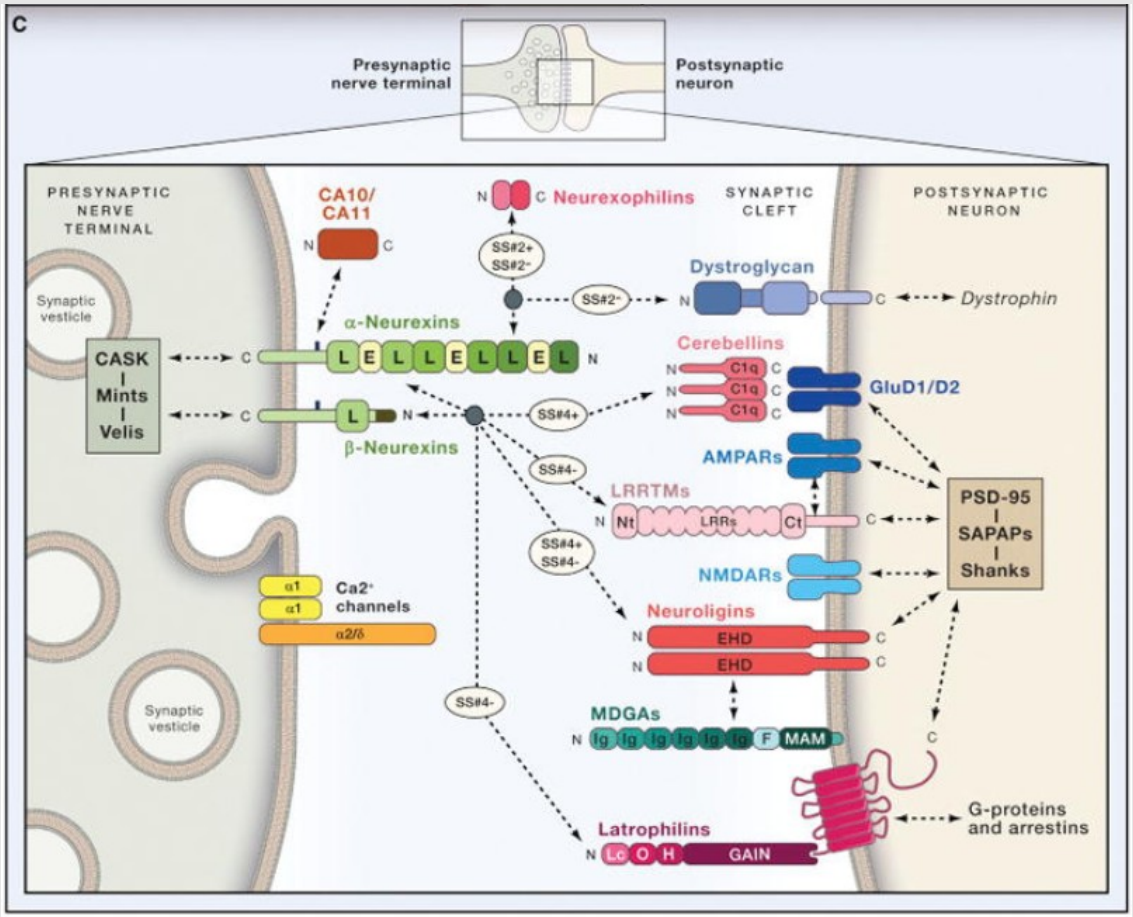
Cosa regola il legame tra Neuroligine e Neurexine?



Proteine	Sito di splicing	N° di varianti
NRXNs	1	3-63
	2	2-4
	3	2-3
	4	2
	5	2-16
NLGNs	A	2-4
	B	2

- Il dominio extracellulare delle α-NRXNs contiene 5 siti di splicing e 2 nelle β-NRXNs
- Questo significa che esistono più di 3000 isoforme delle Neurexine!
- Le Neuroligine contengono solo 2 siti di splicing alternativo

NLGN-NRXN



Neurexins (blue ovals) bind many protein partners tethered to the postsynaptic membrane including neuroligins, LRRTMs, α-dystroglycan, calsyntenins (CLSTN), and the GABAA-receptor, as well as partners that are secreted such as neurexophilins. (

b) NLGN1 and LRRTM2 can both bind neurexins at an overlapping binding site generating two competing trans-interactions.

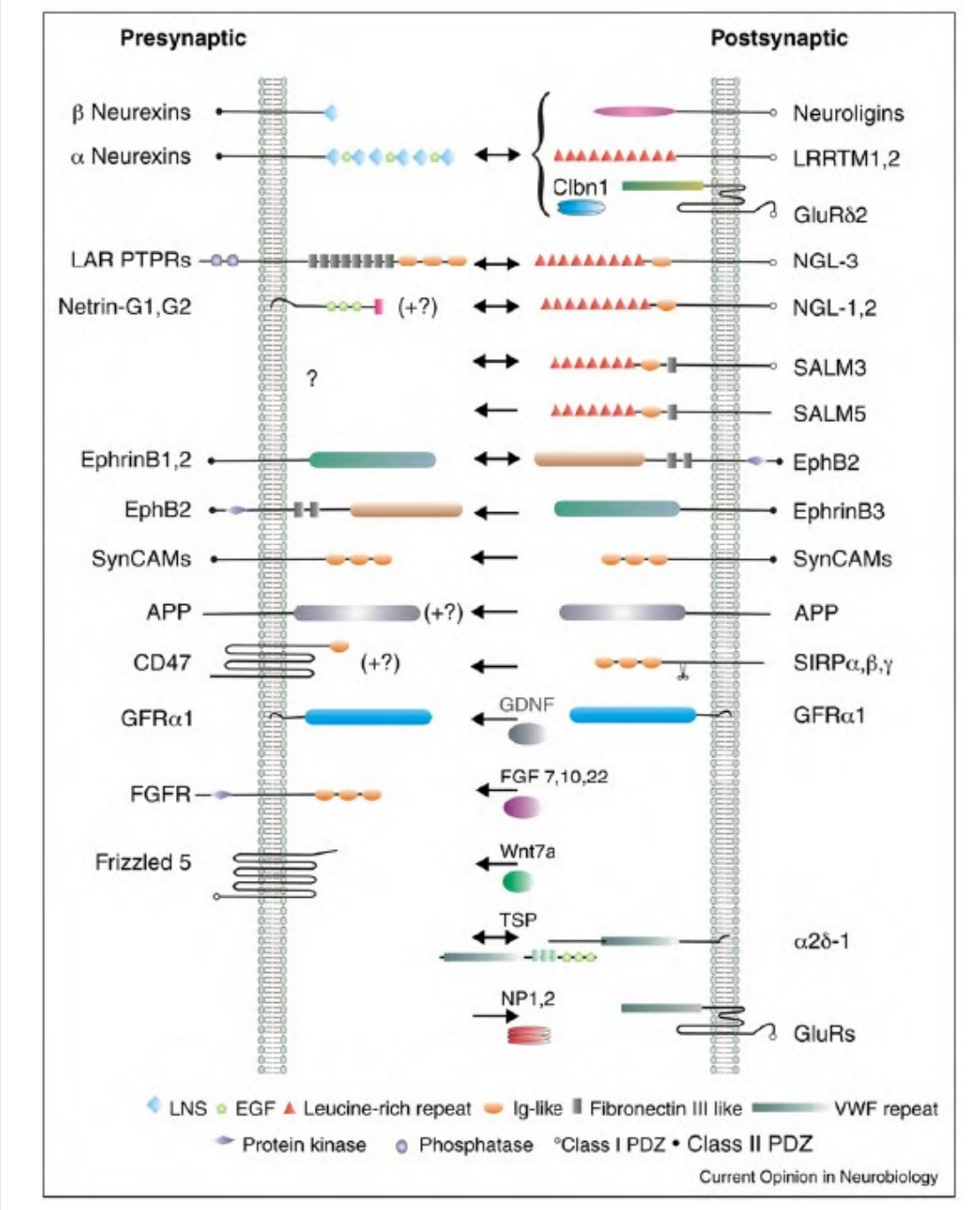
(c) Neurexins and MDGA1 can both bind NLGN2 at an overlapping binding site generating competing cis- and trans-interactions.

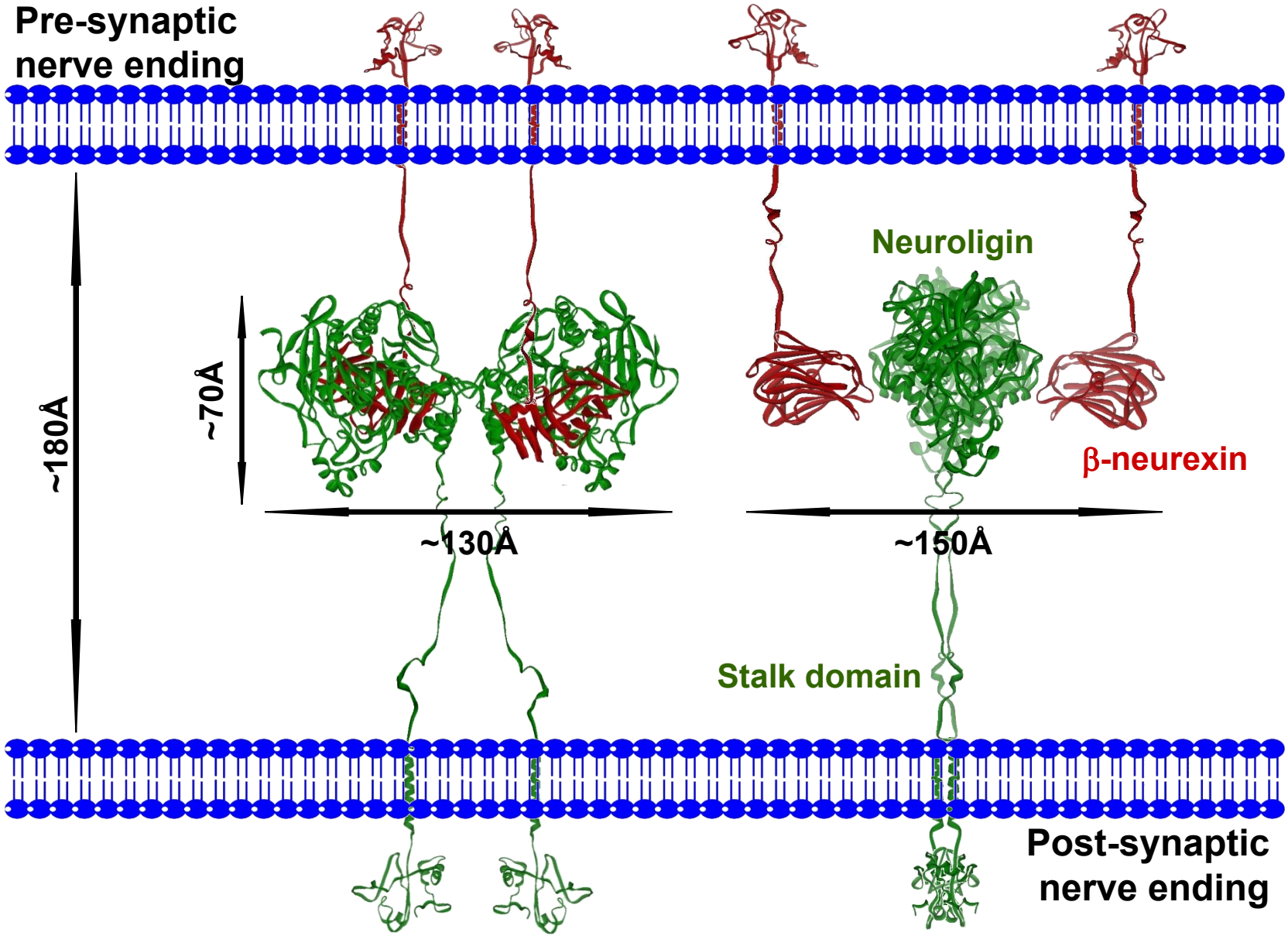
Südhof TCS, 2017

Le Neurexine non legano solo le Neurolighine

leucine-rich repeat (LRR) domains

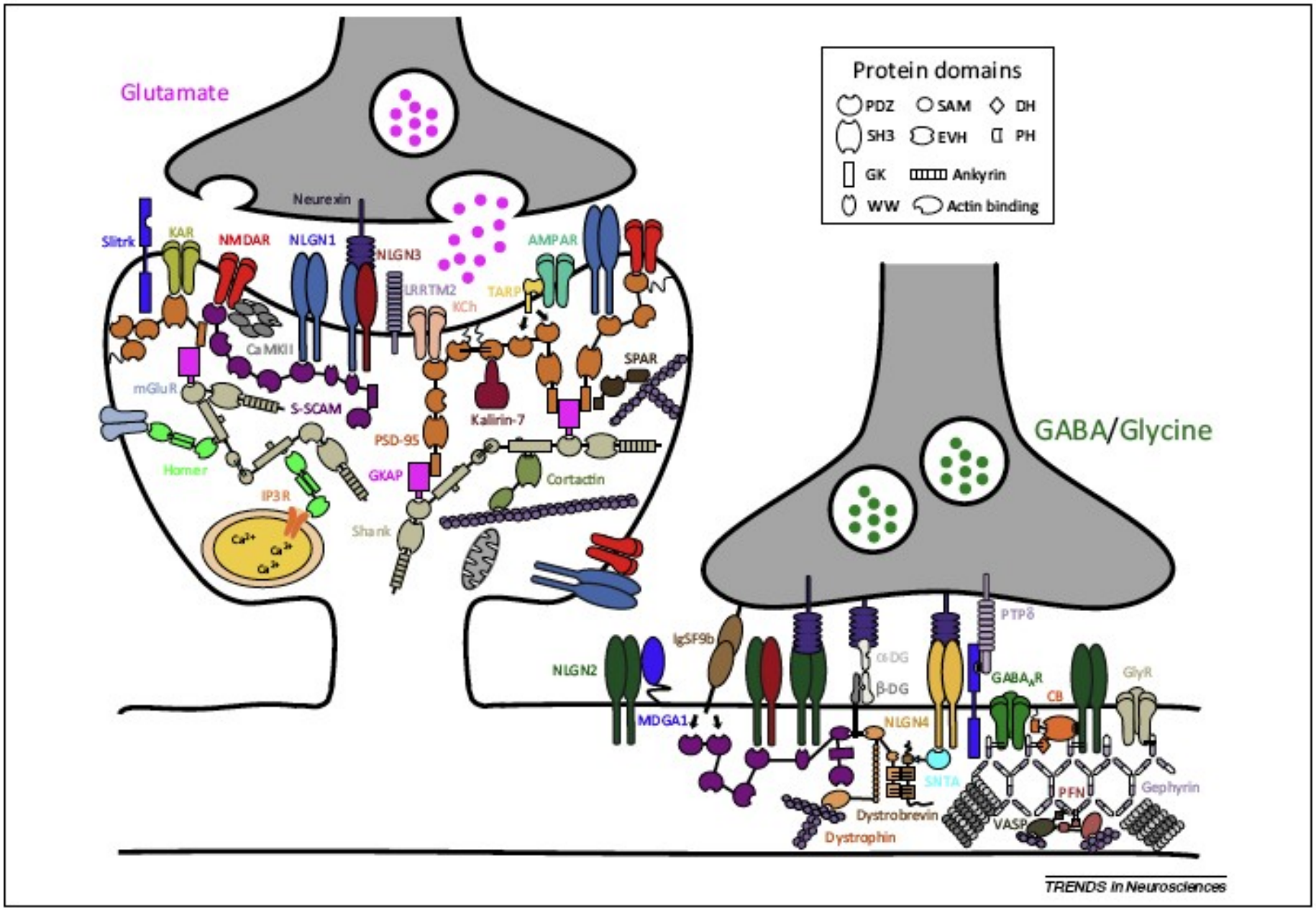
LRRTMs and Slitrks



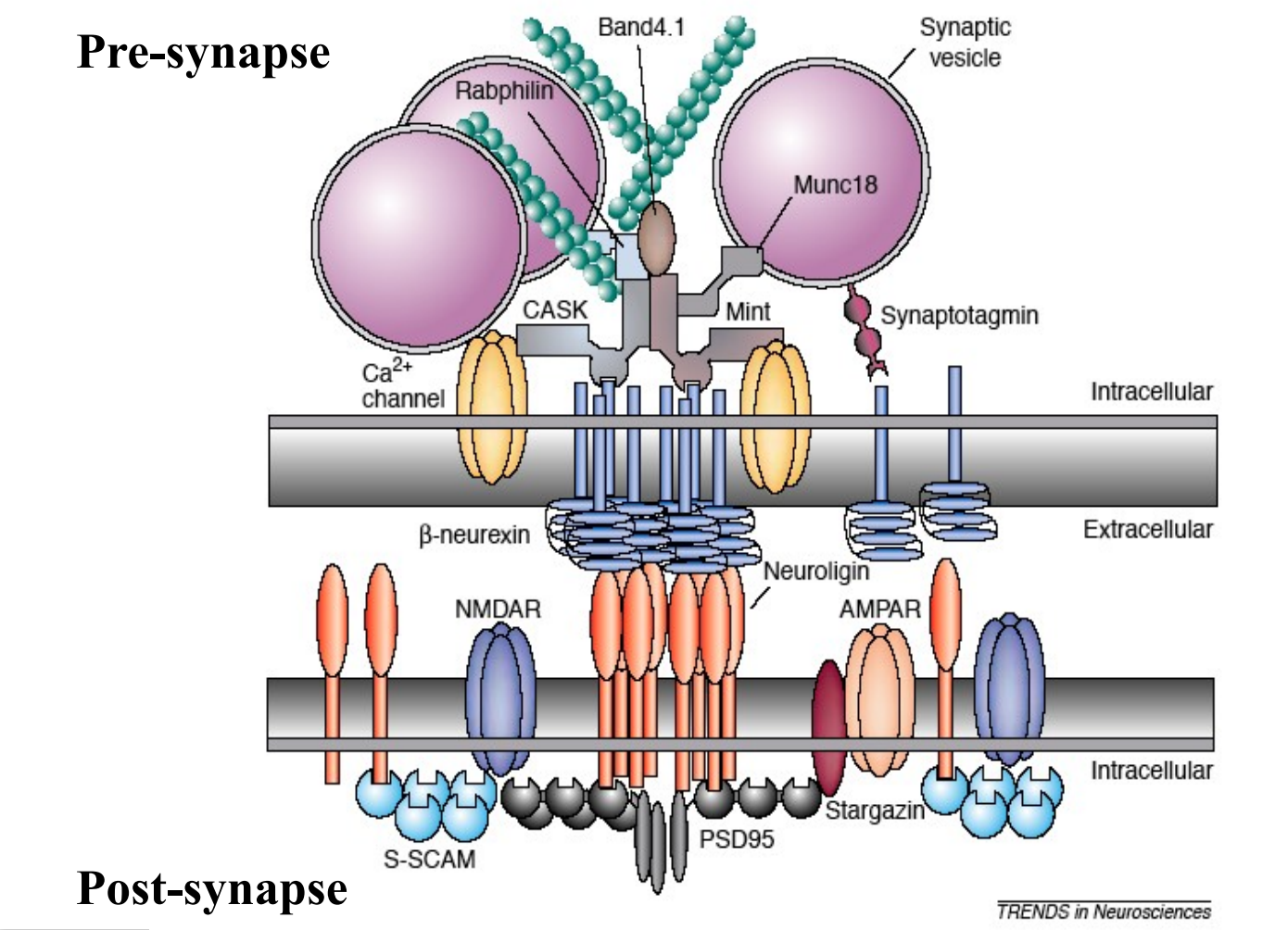


Comoletti et al., 2007

Distribuzione di Neuroligina 1, 2, 3 e 4 alle sinapsi



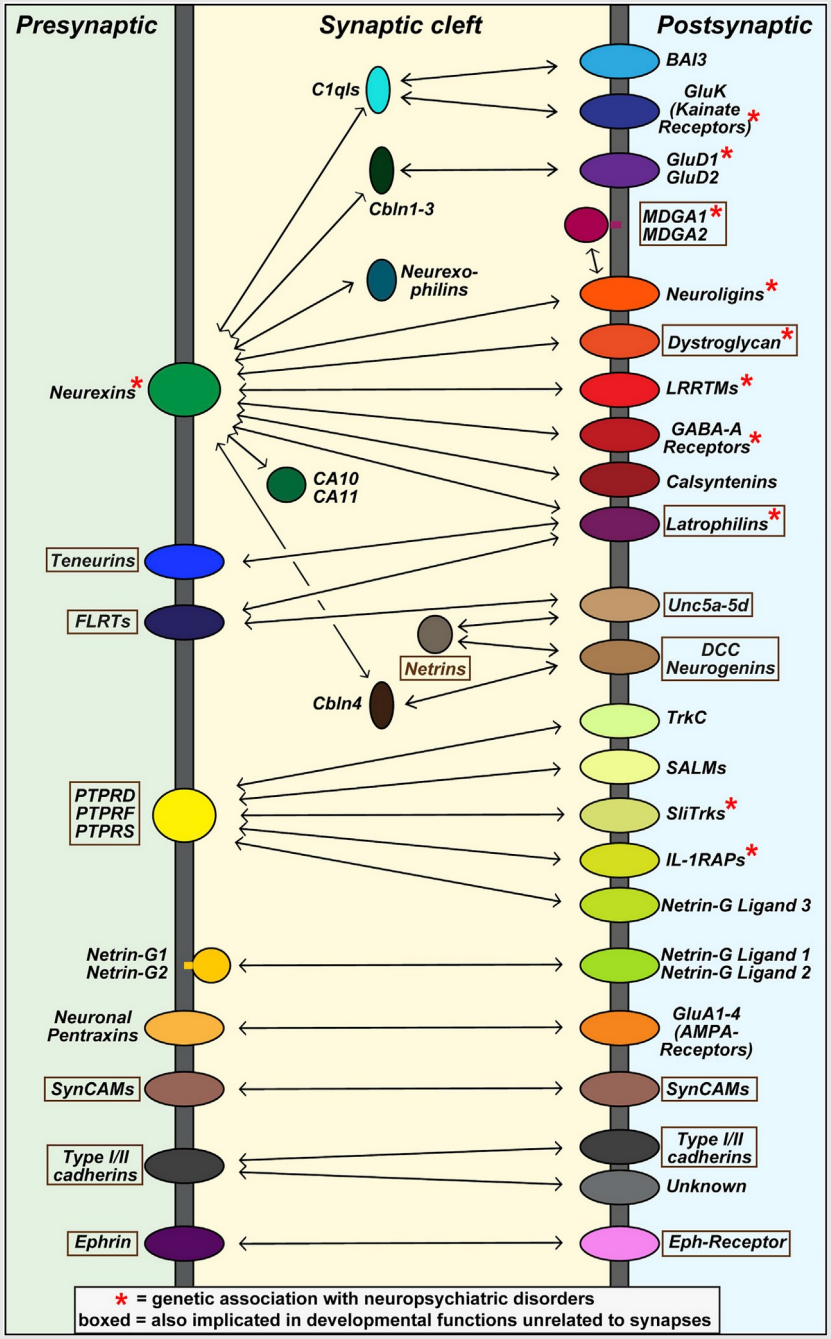
Interazione *in trans:* Neurexina/Neuroligina



NEUROLIGINE E NEUREXINE LEGANO CON LA REGIONE CITOPLASMATICA PROTEINE CHE PRESENTANO DOMINII PDZ, IN PARTICOLARE CASK PER NEUREXINA E PSD95 PER NEUROLIGINA CHE HANNO IL RUOLO DI “ANCORARE” LE MOLECOLE DI ADESIONE ALLA RETE DI PROTEINE SOTTOSTANTI LA MEMBRANA

ALCUNE MOLECOLE CAMs HANNO PIU' INTERATTORI

*GENETIC ASSOCIATION WITH NEUROPSYCHIATRIC DISORDERS



The LRR-containing proteins
mainly localized to postsynaptic sites and bind across the synap-tic cleft
to their presynaptic partners
-leucine-rich-repeat transmembrane neuronal proteins (LRRTMs) (Linhoff et al.,2009),
-netrin-G ligands (NGLs)
-SLIT and NTRK-likeprotein (Slitrks)
-synaptic adhesion like molecules (SALMs)
-fibronectin leucine richtransmembrane proteins (FLRTs)

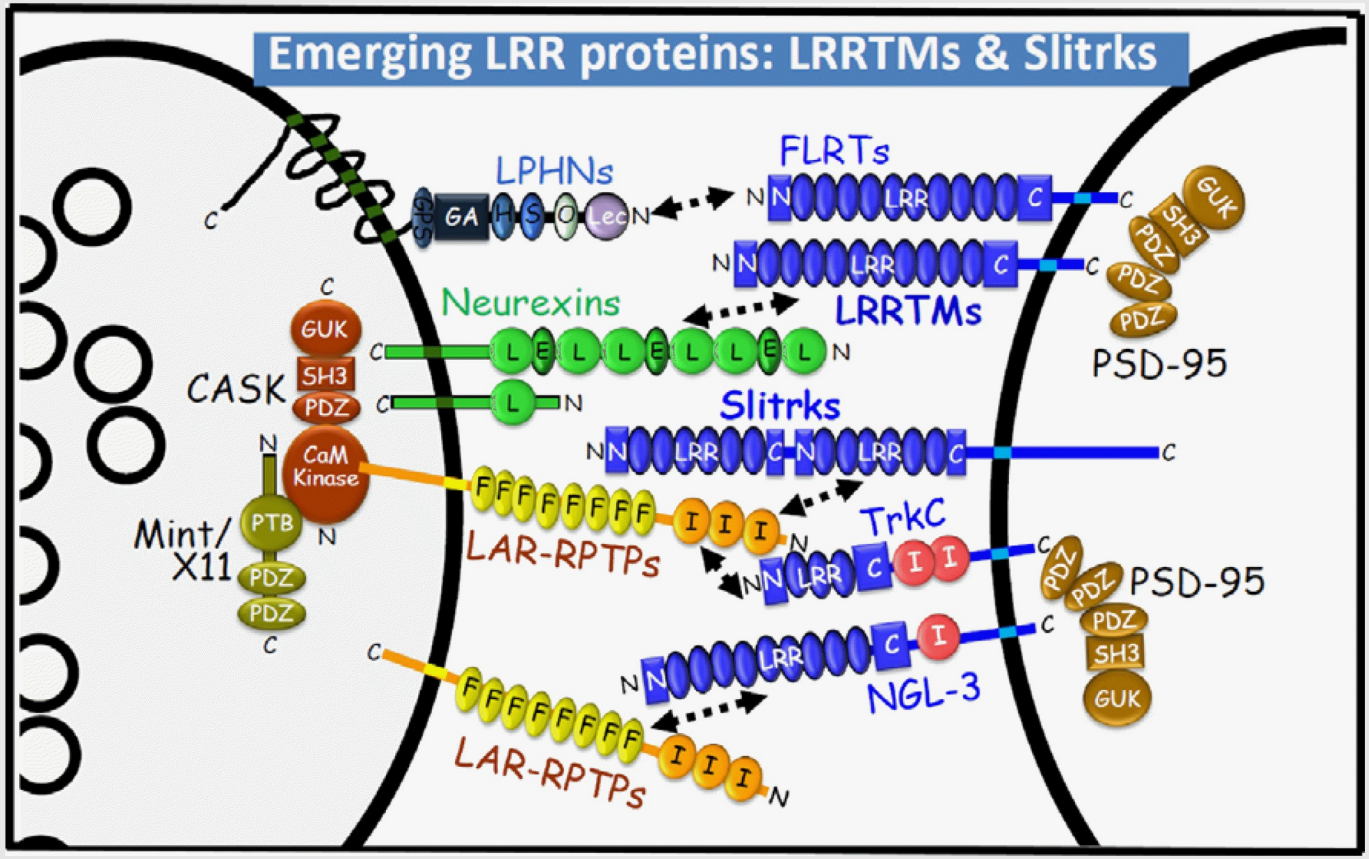


Table 1
Leucine-rich repeat containing synapse organizing proteins and their presynaptic binding partners.

Proteins	Protein domains	Presynaptic partner
LRRTMs		
LRRTM1	LRRNT, LRR1–10, LRRCT, TM, C-terminal	Neurexin 1,2,3 α/β (−S4)
LRRTM2	LRRNT, LRR1–10, LRRCT, TM, C-terminal	Neurexin 1,2,3 α/β (−S4)
LRRTM3	LRRNT, LRR1–10, LRRCT, TM, C-terminal	Neurexin 1β (−S4)
LRRTM4	LRRNT, LRR1–10, LRRCT, TM, C-terminal	Heparan sulphate proteoglycans
Slitrks		
Slitrk1	LRRNT1, LRR1–6, LRRCT1, LRRNT2, LRR7–12, LRRCT2, TM, C-terminal	PTPσ, PTPδ
Slitrk2	LRRNT, LRR1–6, LRRCT1, LRRNT, LRR7–12, LRRCT2, TM, C-terminal	PTPσ, PTPδ
Slitrk3	LRRNT, LRR1–6, LRRCT1, LRRNT, LRR7–12, LRRCT2, TM, C-terminal	PTPδ
Slitrk4	LRRNT, LRR1–6, LRRCT1, LRRNT, LRR7–12, LRRCT2, TM, C-terminal	PTPσ
Slitrk5	LRRNT, LRR1–6, LRRCT1, LRRNT, LRR7–12, LRRCT2, TM, C-terminal	PTPσ, PTPδ
Slitrk6	LRRNT1, LRR1–5, LRRCT1, LRRNT2, LRR6–11, LRRCT2, TM, C-terminal	PTPσ
NGLs		
NGL-1	LRRNT, LRR1–9, LRRCT, Ig, TM, C-terminal	Netrin-G1, LAR
NGL-2	LRRNT, LRR1–9, LRRCT, Ig, TM, C-terminal	Netrin-G2
NGL-3	LRRNT, LRR1–9, LRRCT, Ig, TM, C-terminal	LAR-RPTPs
SALMs		
SALM1	LRRNT, LRR1–7, LRRCT, Ig, FNIII, TM, C-terminal	
SALM2	LRRNT, LRR1–7, LRRCT, Ig, FNIII, TM, C-terminal	
SALM3	LRRNT, LRR1–7, LRRCT, Ig, FNIII, TM, C-terminal	LAR-PTPs
SALM4	LRRNT, LRR1–6, LRRCT, Ig, FNIII	SALM4
SALM5	LRRNT, LRR1–7, LRRCT, Ig, FNIII	SALM5, LAR-RPTPs
Trks		
TrkC	LRRNT, LRR1–3, LRRCT, Ig1, Ig2, TM, C-terminal	PTPσ
FLRT		
FLRT1	LRRNT, LRR1–7, LRRCT, Ig, FNIII, C-terminal	Not known
FLRT2	LRRNT, LRR1–7, LRRCT, Ig, FNIII, C-terminal	LPHN3
FLRT3	LRRNT, LRR1–6, LRRCT, Ig, FNIII, C-terminal	LPHN3, Unc5

Tecniche di studio per le molecole di adesione sinaptica

Tecniche di guadagno di funzione (gain of function)

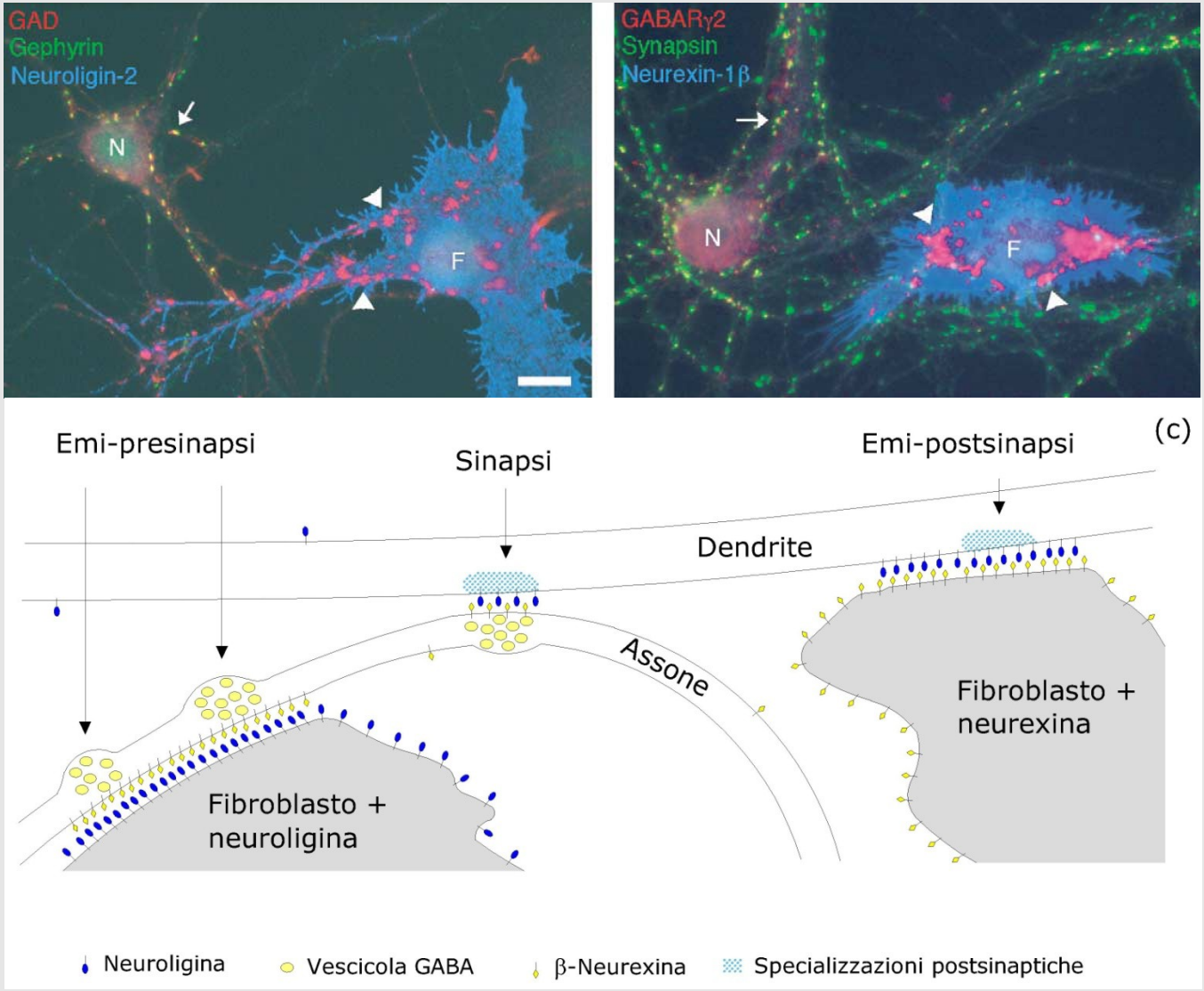
- Saggi di adesione cellulare (sopra-espressione della molecola in studio in cellule non-neuronali in coltura)
- Saggio di formazione di sinapsi artificiali (co-coltura di neuroni e cellule non-neuronali trasfettate)
- Saggio di trasfezione di neuroni

Tecniche di perdita di funzione (loss of function)

- RNA interference. Limitazione: knockdown inefficiente; redundancy; reazioni compensatorie
- Manipolazione genetica (animali transgenici), costitutiva o condizionale. Limitazioni per quella costitutiva: fenotipo di malformazione o morte embrionale
- Inibizione farmacologica. Limitazioni: mancanza d'inibitori per tutte le molecole sotto studio

ESPRESSIONE ESOGENA DI NLGNs E NRXNs

“Saggio di formazione di sinapsi artificiali”



- Esperimenti di RNAi knockdown delle NLGNs correlano un decremento di NLGNs con un decremento nel numero di sinapsi (Chih et al., 2004).

- Overespressione di NLGN1 in neuroni in coltura aumenta il numero delle sinapsi eccitatorie mentre NLGN2 aumenta il numero delle sinapsi inibitorie (Chih et al., 2005).

- Overespressione di NLGN1 in neuroni in coltura aumenta il numero delle sinapsi eccitatorie e queste nuove sinapsi sono funzionali (capaci di evocare neurotrasmissione). NLGN1 e' selettiva per le sinapsi di tipo eccitatorio. Mentre NLGN2 incrementa il numero delle sinapsi inibitorie (Chubykin et al., 2007).

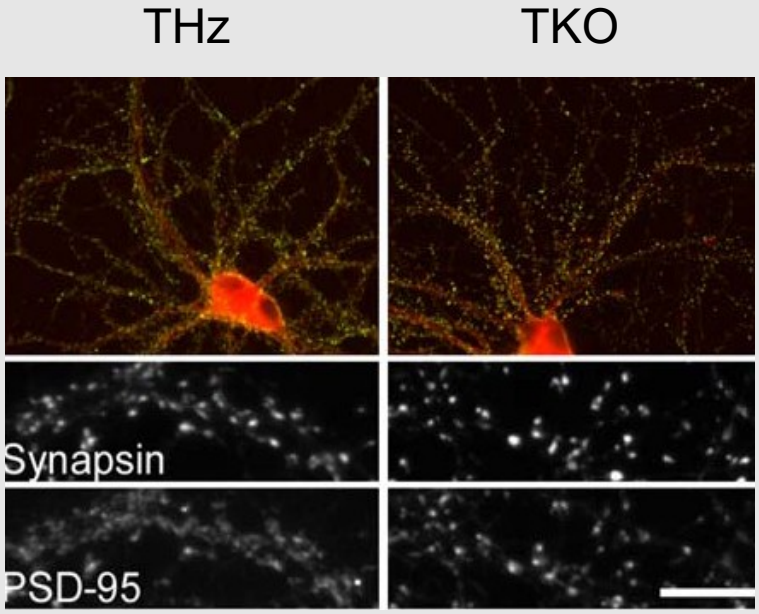
MOLECOLE DI ADESIONE E SINAPTOGENESI

Table 1 | ***In vitro* evidence for trans-synaptic signalling in synaptogenesis**

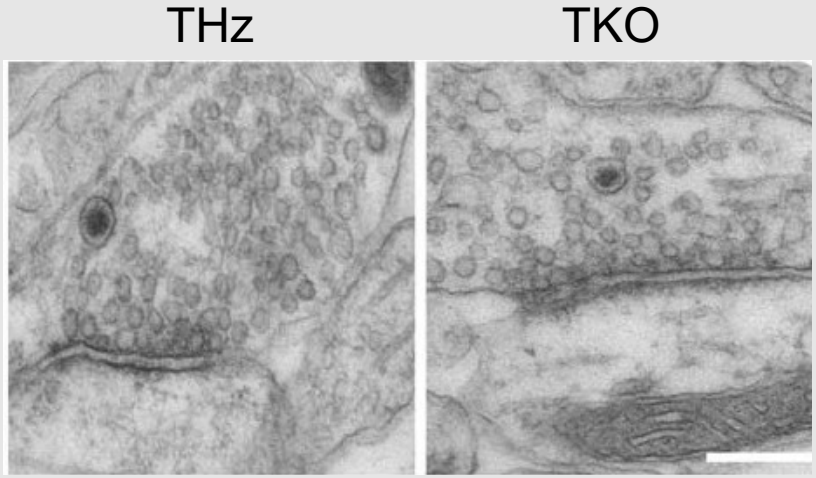
SAM	Excitatory postsynaptic differentiation	Inhibitory postsynaptic differentiation	Presynaptic differentiation	Dendritic spine formation
Neurexins/ neuroligins	Direct association with PSD-95 via PDZ binding domain interactions[14]; NMDARs recruited by neuroligin[17,20], but mechanism unclear; AMPARs recruited in co-culture only in the presence of glutamate[22]	Recruitment of GABAR and gephyrin in co-culture[20]; fewer inhibitory synapses with neuroligin knockdown[17]	Induced in co-culture[21]; neurexin associates with CASK/MINT[15,16,25]	Induced with overexpression of neuroligins[17]; mechanism unknown
EphBs/ ephrin-Bs	Direct extracellular domain interaction of EphB2 with NMDARs[43]; EphB2 also associates with AMPARs via PDZ binding domain interactions[46,50]	ND	Induced in co-culture and requires ephrin binding domain[50]; mechanism unknown	Induced by EphBs/ ephrin-Bs via multiple signalling pathways (Rho GEFs, syndecan, FAK)[44,49,51,53,54]. Fewer spines form with expression of EphB2 kinase inactive mutant[51]
SynCAM	Direct association with PSD-95 via PDZ binding domain interactions[57]	ND	Induced in co-culture[57]; SynCAM binds CASK/ MINT[57]	ND
SALM2	Direct association with PSD-95 via PDZ binding domain interactions[59]; AMPARs recruited in co-culture, but NMDARs less so[59]	ND	No induction in co-culture[59]; presynaptic ligand is unknown	Induced with overexpression[59]; mechanism unknown
NGL2	Direct association with PSD-95 via PDZ binding domain interactions[60]; NMDARs recruited in co-culture, but not AMPARs[60]	ND	Induced in co-culture and requires NGL2 extracellular domain[60]; mechanism unknown	Induced with overexpression of NGL2 via PSD-95-dependent and -independent mechanisms[60]
N-cadherin	Fewer clusters of PSD-95 with inhibition of cadherin signalling[76]; evidence for interaction with AMPARs[78]	ND	No induction in co-culture[80] (but see *in vivo* evidence in TABLE 2)	Fewer spines with N-cadherin dominant-negative[76]; α-catenin stabilizes spines[82]; p120 required for spine formation and maturation[81]

FUNZIONE: PROVE *IN VIVO*

THz: neuroni di controllo (tripli eterozigoti)
TKO: neuroni con triplo knockout per le NLGNs 1-3



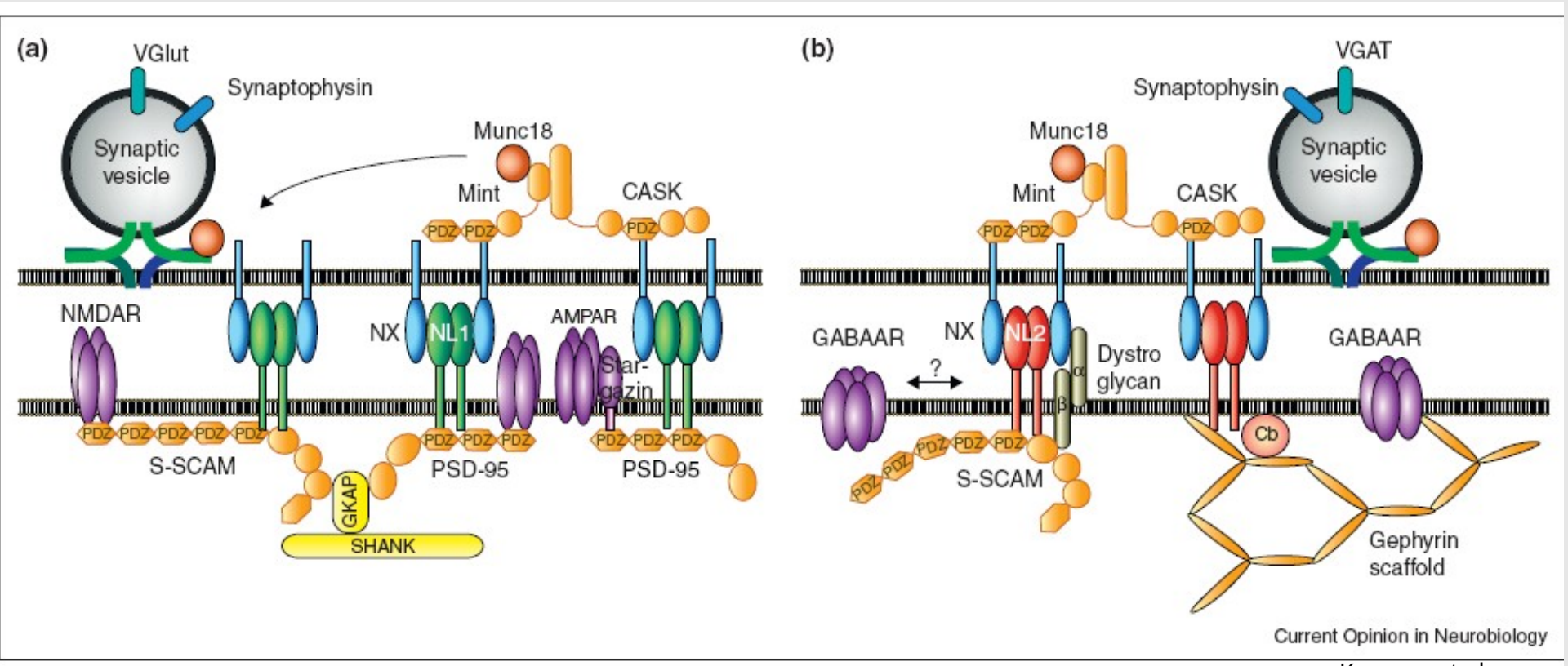
Sinapsina: marker presinaptico
PSD-95: marker postsinaptico



Espianti corticali
dopo 2 settimane in coltura

Topi KO per Neuroligine 1-3 muoiono per insufficienza respiratoria, ma il numero delle sinapsi e' normale ed anche la ultra struttura al microscopio elettronico

Proposed roles for the Neuroligins proteins



Krueger et al., 2012

***In vitro* studies:**

Neuroligins and Neurexins may induce synapse formation

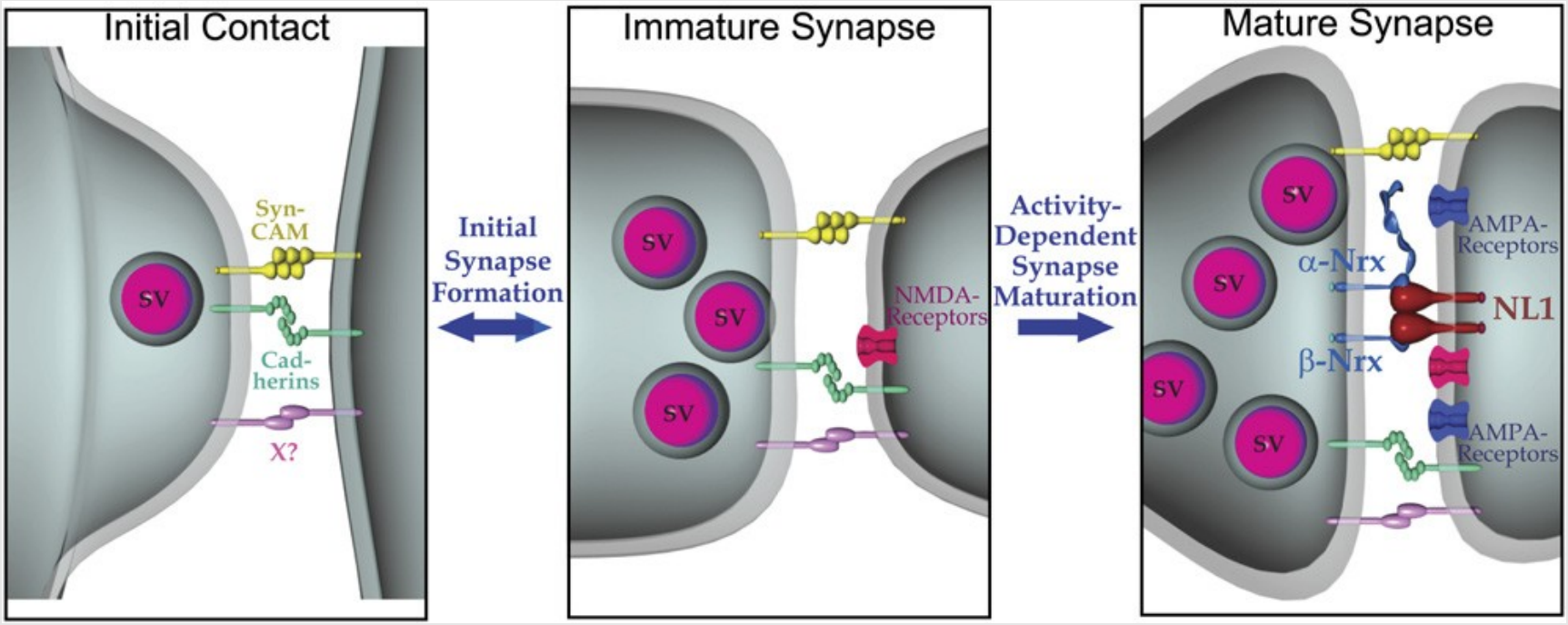
(Artificial synapse formation assay, Neuronal transfection assay)

Gene Knock out studies:

Neuroligins and Neurexins are essential for **synapse function**

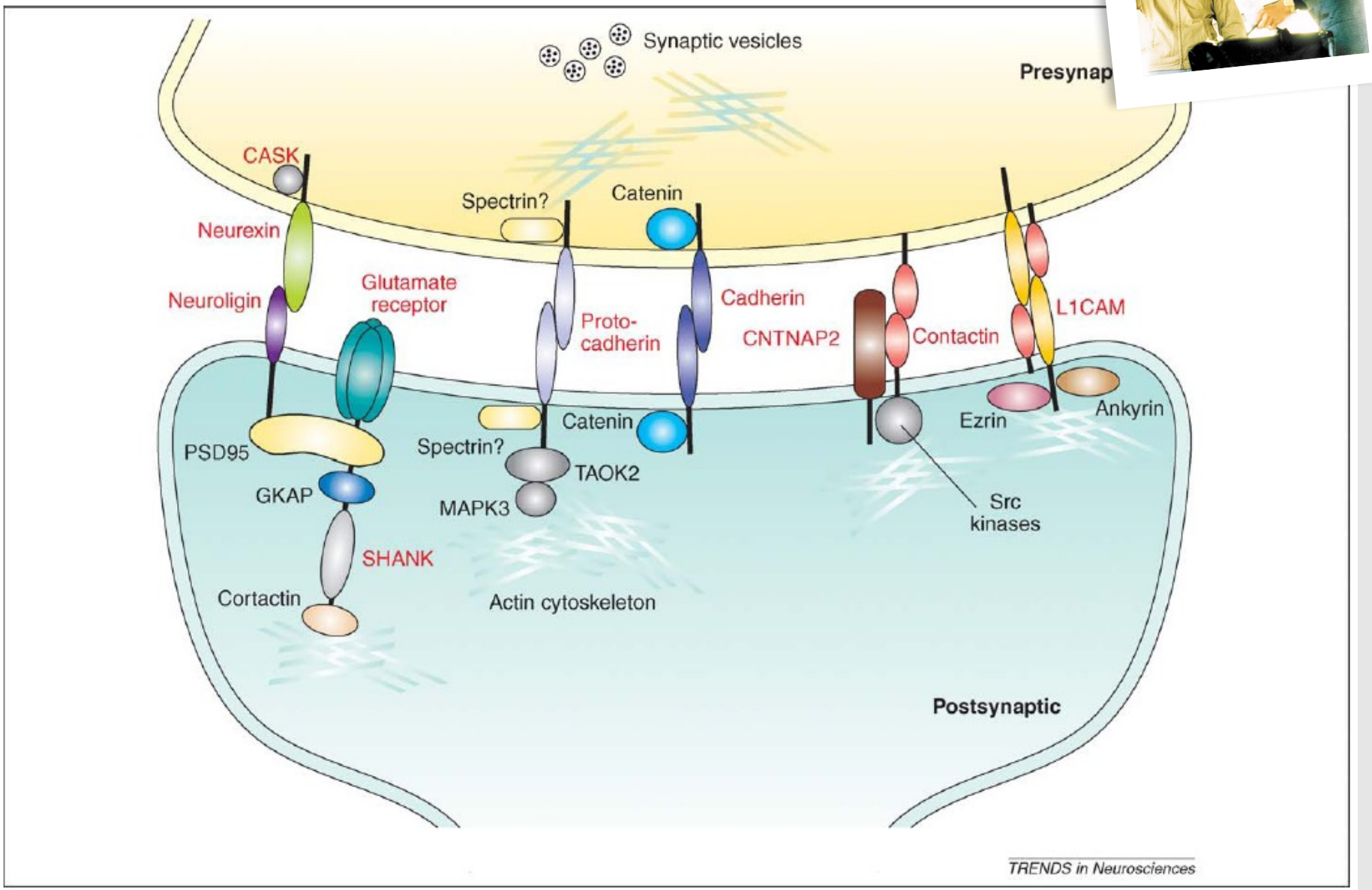
Role in the specification and identity of synaptic connections

LA TEORIA CORRENTE



Contatti sinaptici iniziali coivolgono diverse molecole di adesione che conferiscono specificità. La sinapsi immatura è funzionale ma ha bisogno di acquisire proprietà specifiche attraverso processi dipendenti dall'attività. Le neuroligine possono mediare la stabilizzazione dipendente dall'attività dei contatti sinaptici transienti. Neuroligine e neurexine non sono fondamentali per la formazione della sinapsi ma sono importanti per formazione di complessi sinaptici funzionali.

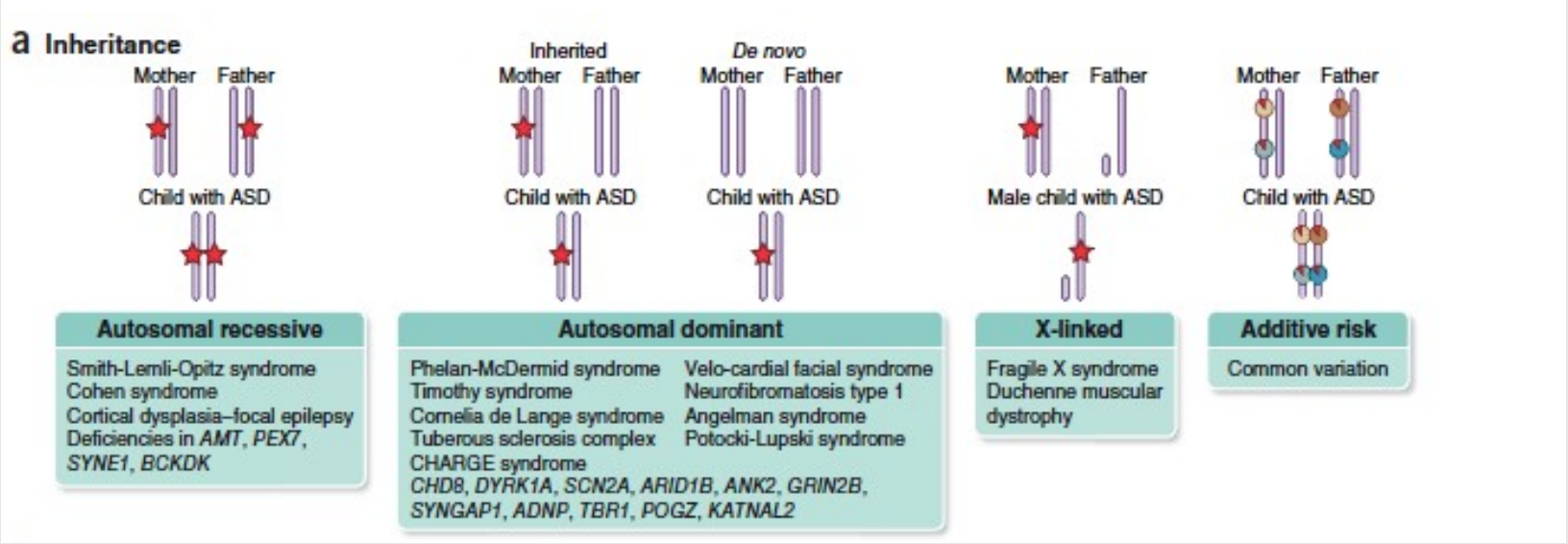
MOLECOLE SINAPTICHE E DISTURBI DELLO SPETTRO AUTISTICO

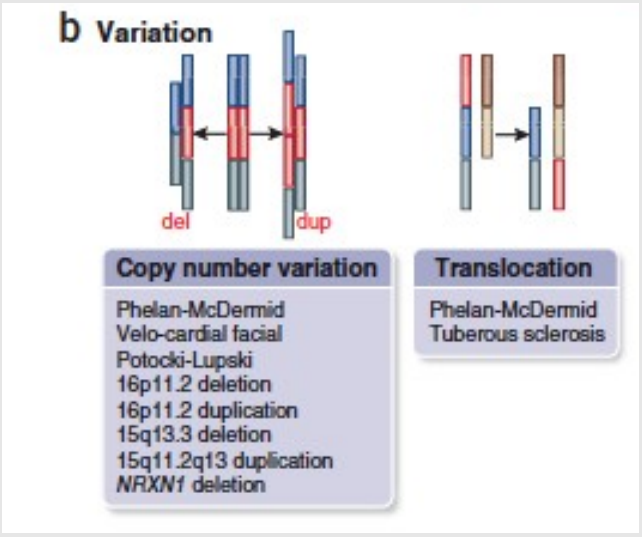


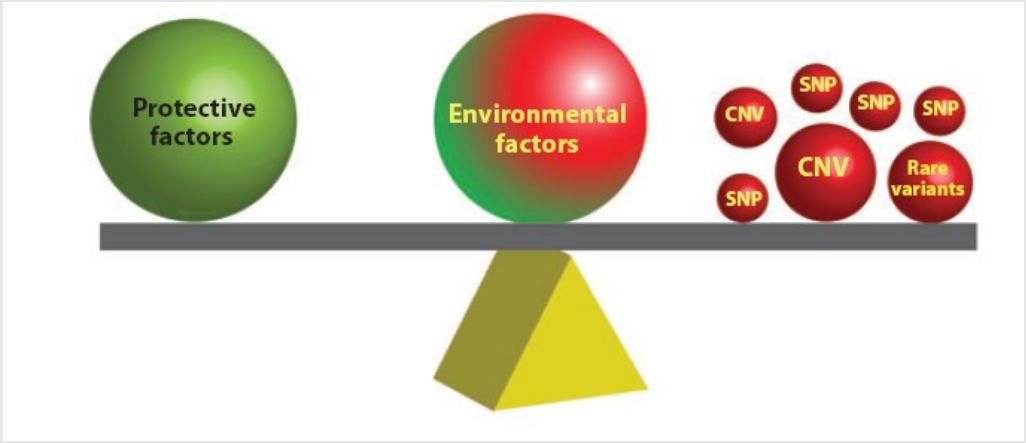
Autism Spectrum Disorders

- Onset by the age of 3
- Male to female ratio: 4 to 1
- Prevalence: once 1/1,000; recently up to 1/68 (USA)
- Diagnosis is based on behavioral criteria
- Strong genetic background interlinked with unknown environmental influences

The genetics of autism spectrum disorders



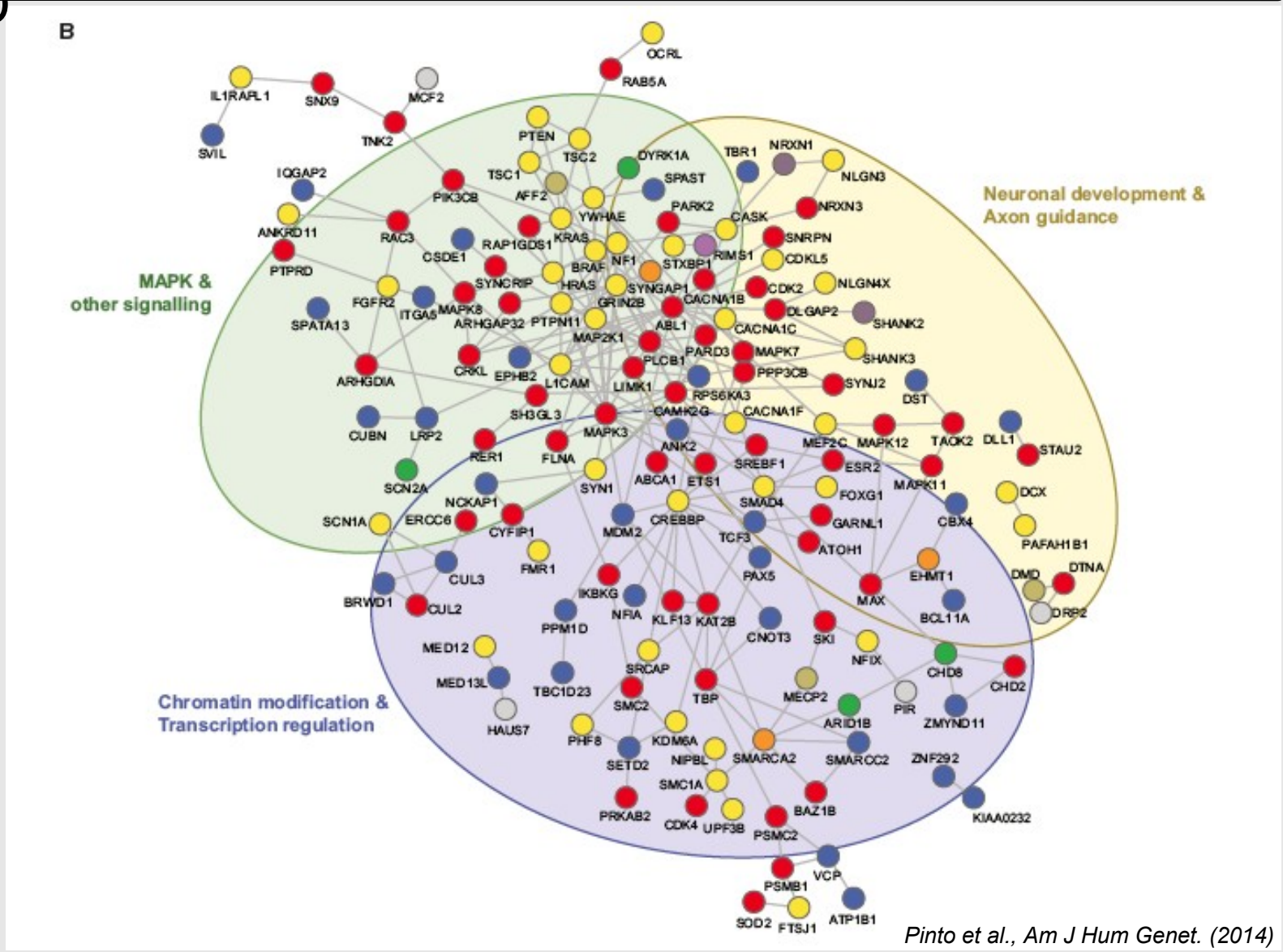




De la Torre-Ubieta et al., 2016

unique and recurrent CNV as significant contributors to

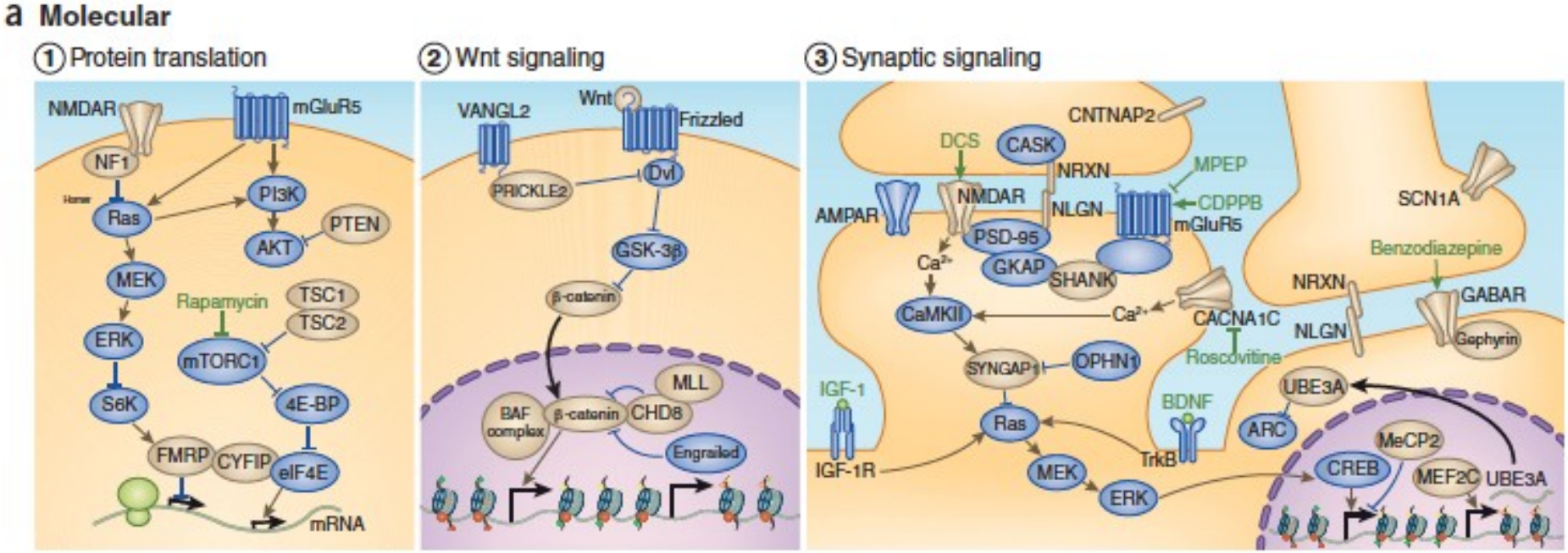
Genes and cellular pathways dysregulated in ASD



Pinto et al., Am J Hum Genet. (2014)

No single genetic mutation accounts for more than 1% of ASD

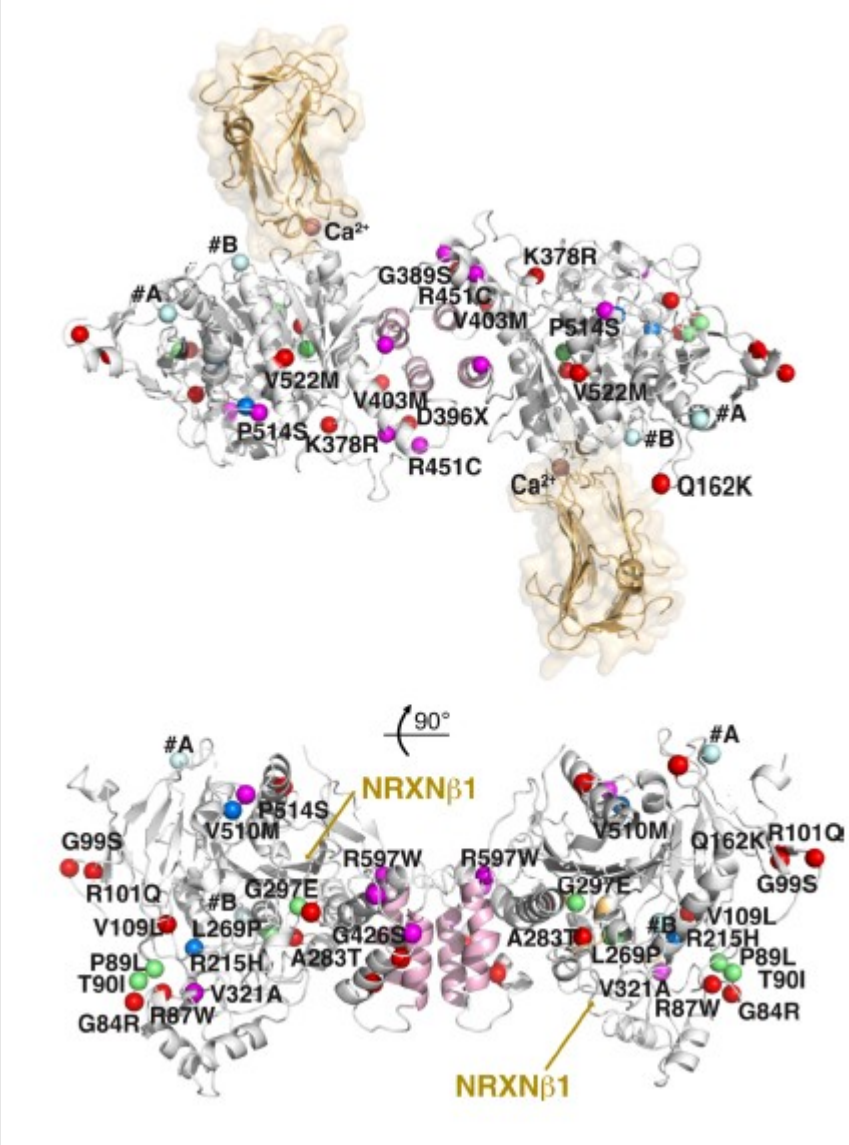
Molecular Signaling involved in ASDs



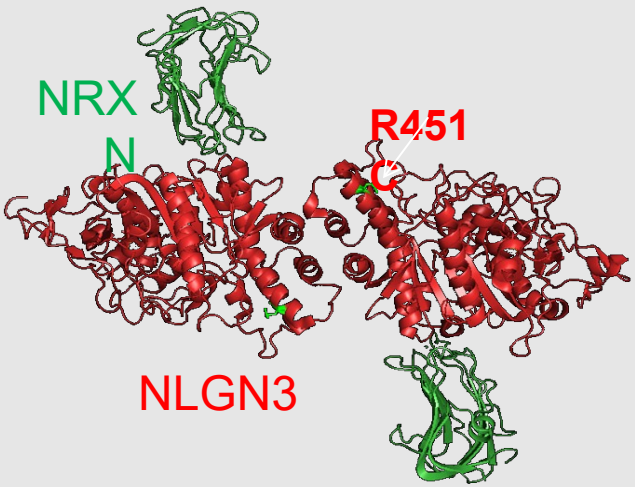
1. Activity dependent transcriptional and traslational regulators are associated with ASDs.
2. Wnt signaling is known to regulate neurogenesis
3. Dysregulation in synaptogenesis and synaptic trasmission.

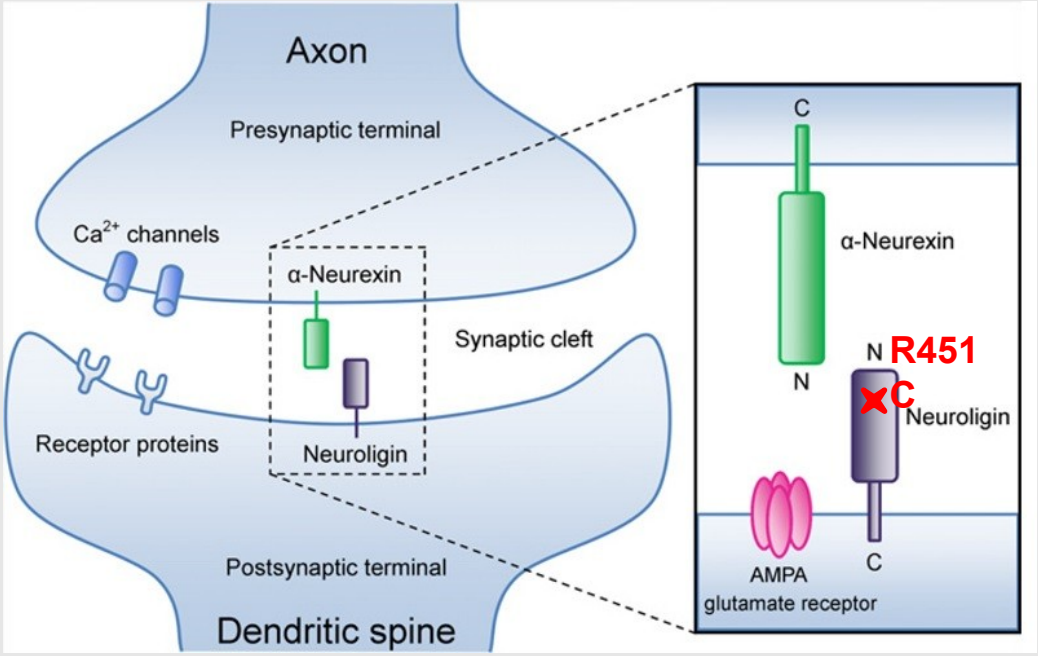
De la Torre-Ubieta et al., 2016

MUTAZIONI RISCONTRATE NEL DOMINIO EXTRACELLULARE DELLE PROTEINE DELLA FAMIGLIA DELLE NEUROLIGHINE



The R451C mutation maps in the extracellular domain of Neuroligin3

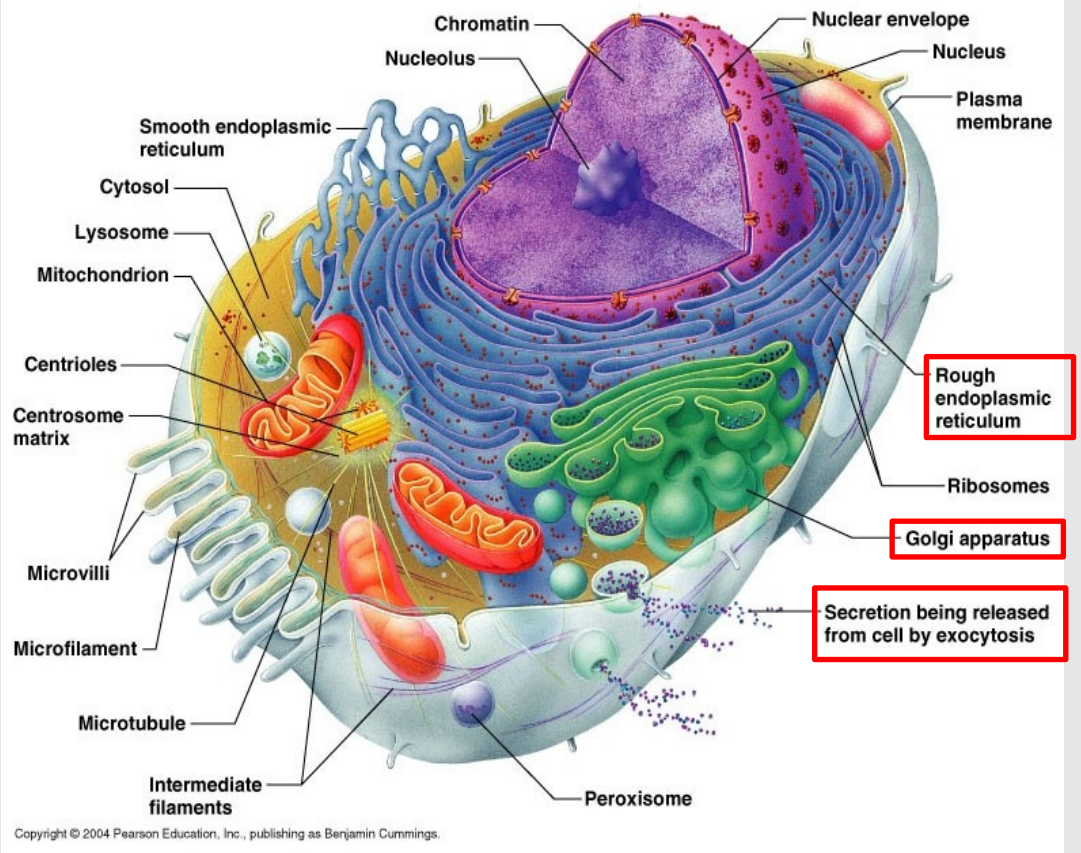


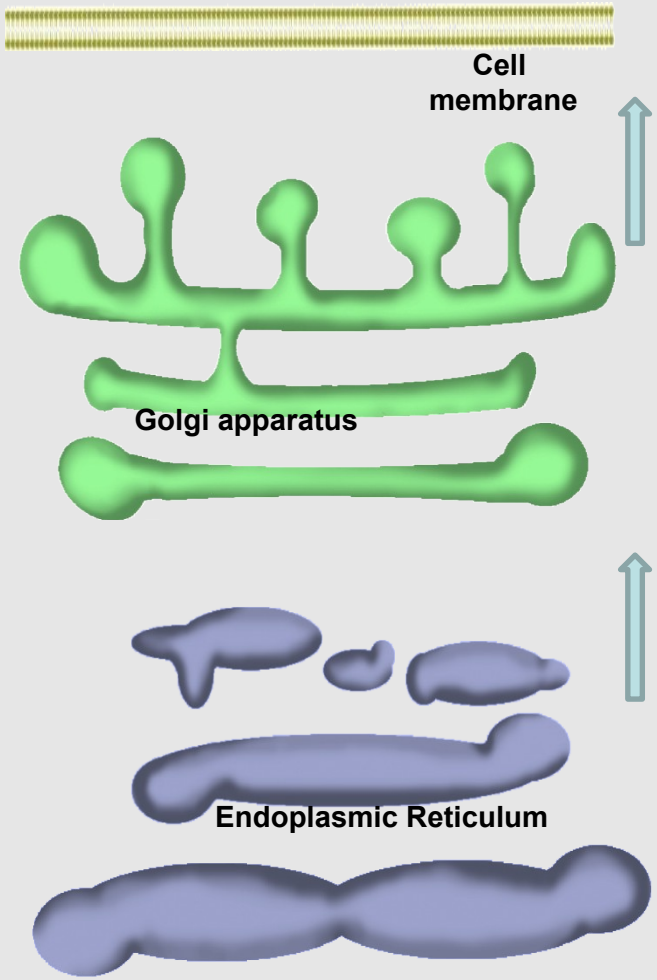


More than 30 genetic alterations have been associated with autism including a Neuroligin3 point mutation and a deletion

Jamain et al., 2003

Structure of a cell and trafficking principles

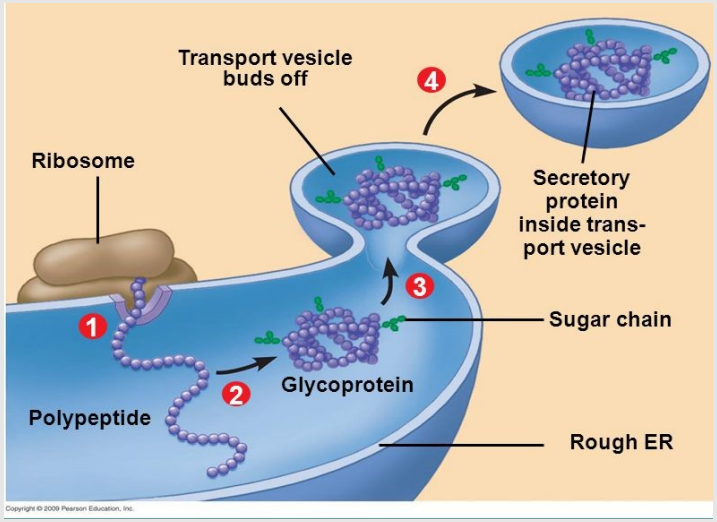




Extracellular proteins needs to be **stably folded** to maximize their chance to **recognize** (or be recognized) by other molecules and not be subjected to random **proteolysis** that would occur if folding was partial and unstable.

Posttranslational modifications (PTM)

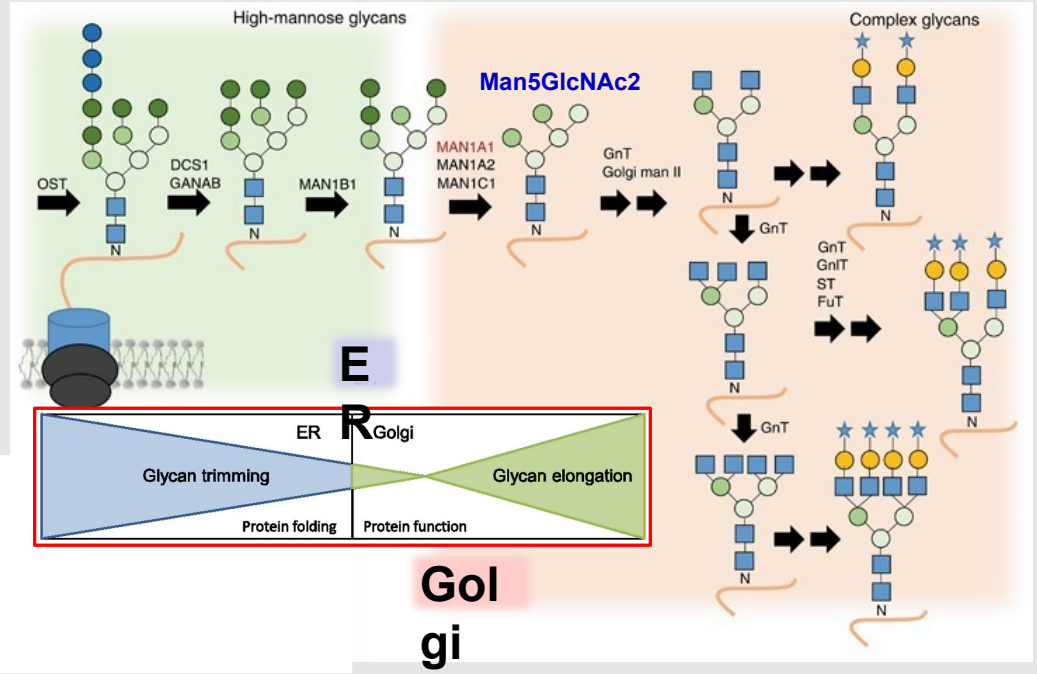
1) **Removal of leader peptide** – necessary to enter the ER, not needed afterward
2) **Formation of disulfide bonds (Cys isomerases) -** Maintain **folding** and **stability** by holding two segments of the protein together, biasing the protein towards the folded topology
3) **Addition and processing of N- and O-linked carbohydrates -** Primarily involved in the **folding** and the maintenance of protein **conformation & solubility.** Protect against premature protein **degradation** and can be **receptors** for other proteins or pathogens



4) **Assembly into multimeric proteins** - channels, pores, etc.
5) **Formation of GPI linkage -** if GPI anchor
6) **Controlled proteolytic cleavages** – pro-peptide activation, ectodomain **shedding** and other hydrolytic cleavages, e.g. Furin, ADAMS, etc.)
7) **Other PTM** include phosphorylation, hydroxylation in collagen, sulfation - uncommon.

N- linked Glycosylation processing in the GOLGI

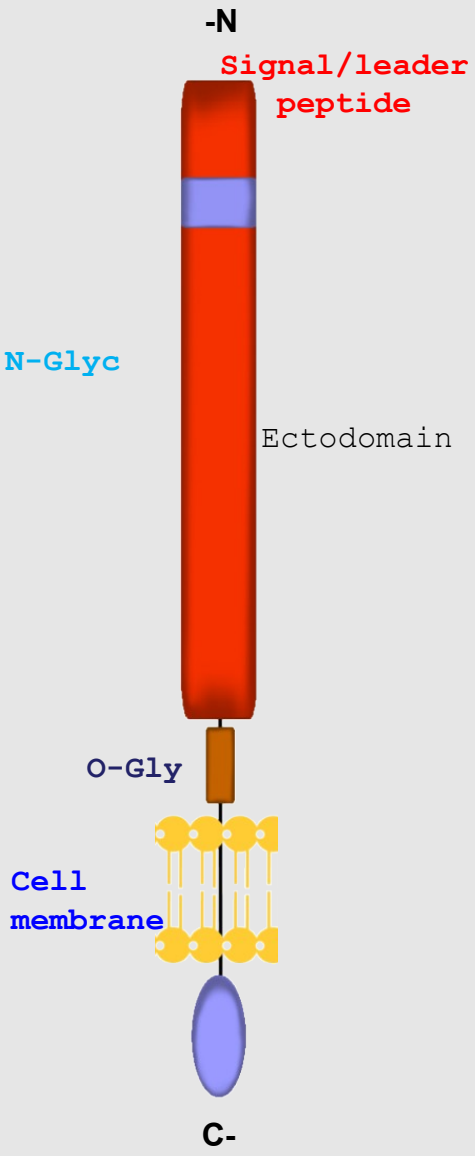
Mannose-trimming enzymes in the **ER** prepare the **$Glc_3Man_9GlcNAc_2$** precursor for subsequent processing in the **Golgi**
→ generate the **Man5GlcNAc2-**Asn intermediate



Glycoproteins, acquire their **final sugars** in the **Golgi**.
In mammals, there are >**250 glycosyltransferases** in the **Golgi** catalyzing the transfer of one sugar to another on a glycan chain.

Whereas the **ER** contains many soluble proteins, all the **Golgi-resident** protein (**glycosydases** and **glycosyltransfereases**) are Type I proteins

Basic architecture of cell surface molecules



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GGGLLRSKI

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GGGLLRSKI

MAWLGVVIFALVIALFPSAPVLVTVRHLKANSAVVSWDV**C**LKF**C**SGRGQ**C**TRHG**C**K**C**DPGFS
GPA**C**IQEV**NAS**TTRSCALWDLEEDTEYIVHVQAI**NDT**GQSPASEPVLFKTPREAEKMASKNK
DE**PTMSTTPTNSSLRTGEVLIIVVVLFMWAGVIALF**C**R**Q**K**D**R**I**K**DNEPNNNKEKTKSASETS
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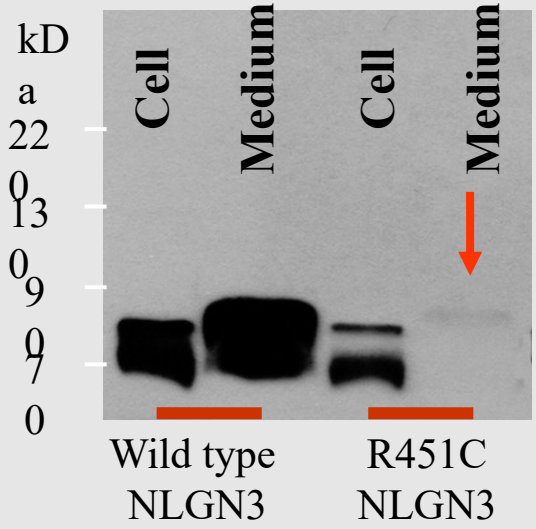
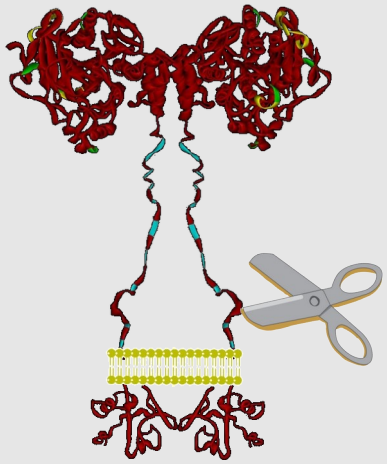
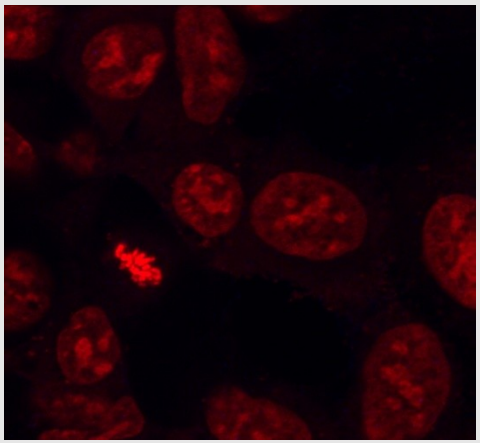
Intracellular retention of R451C mutant soluble Neuroligin3

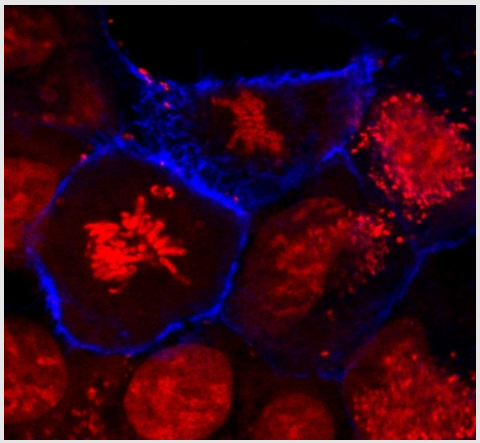




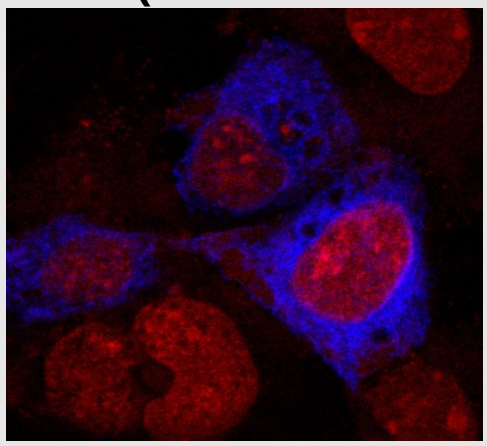
HEK-293 mock

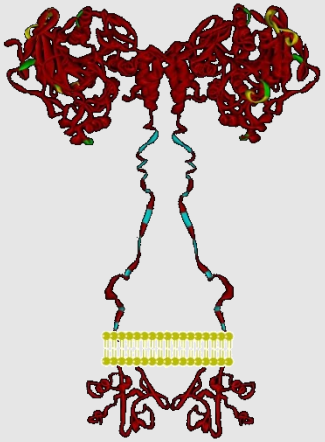


NLGN3-wild

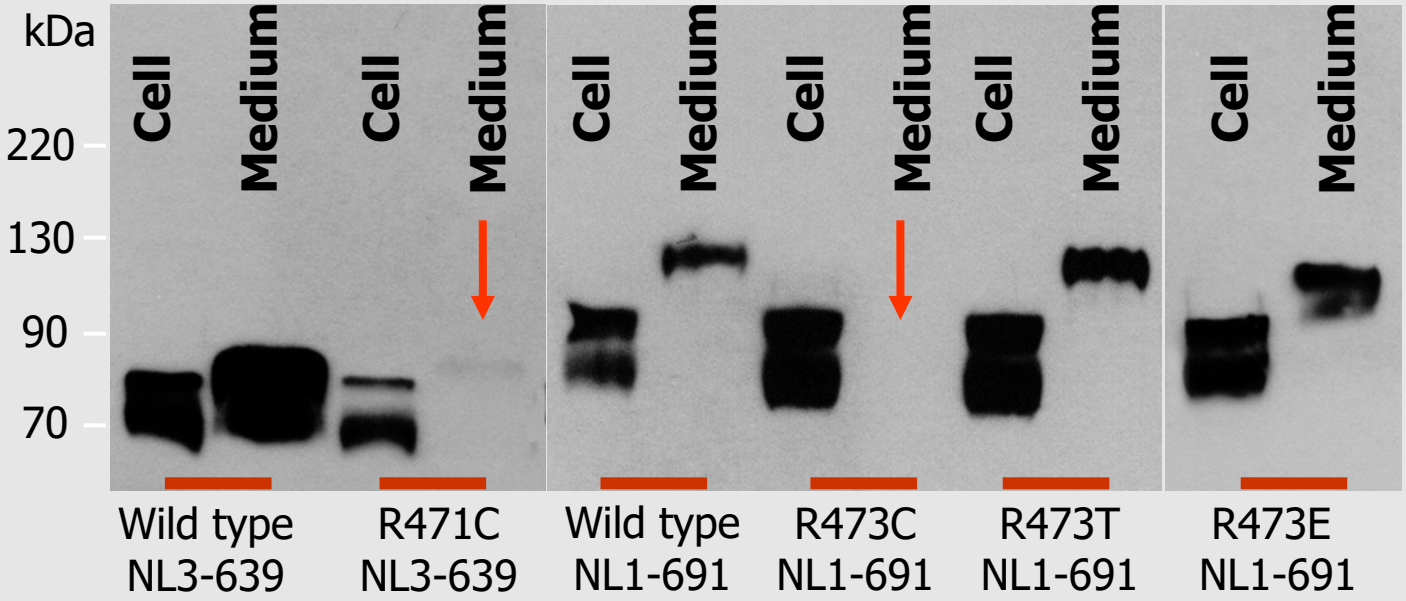


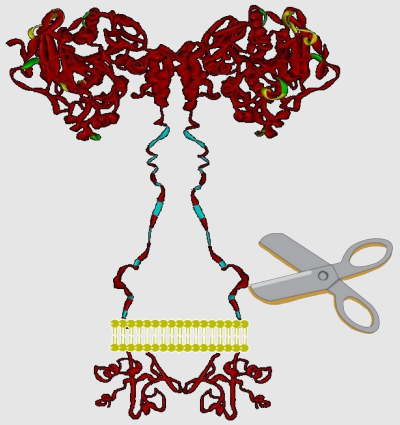
NLGN3-R451C (Autism





Cellular secretion of wild-type and mutant NLGN1 and NLGN3

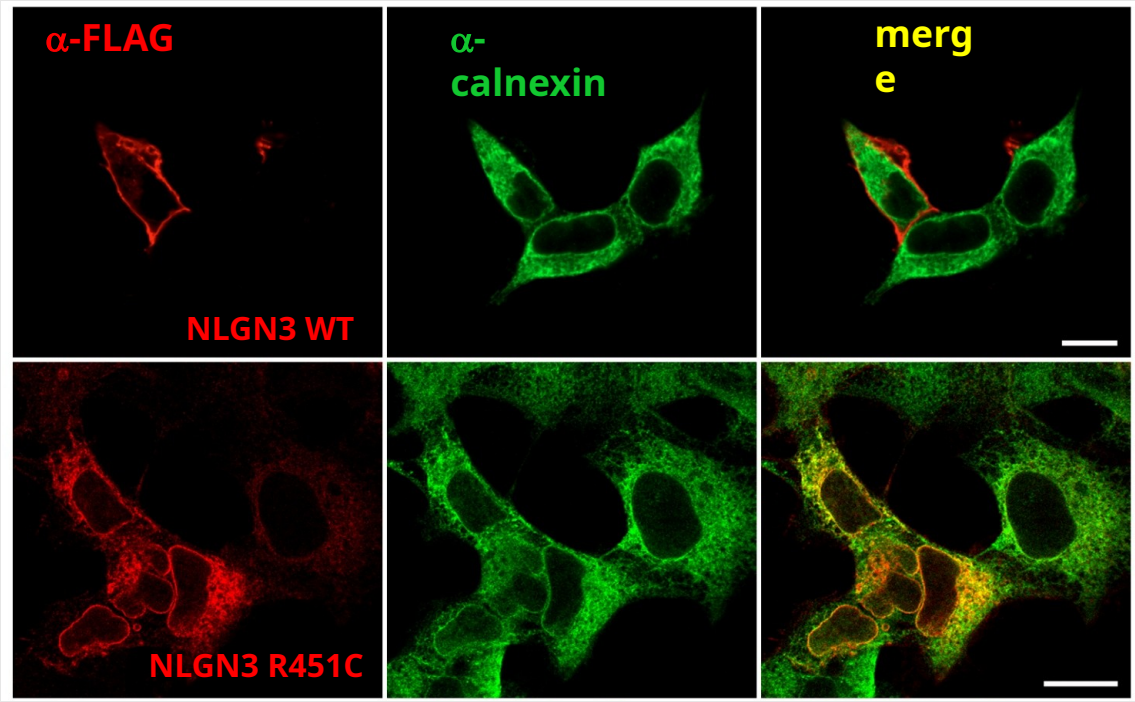


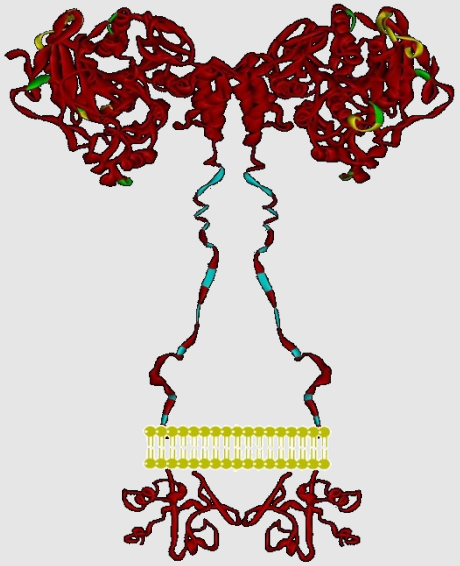


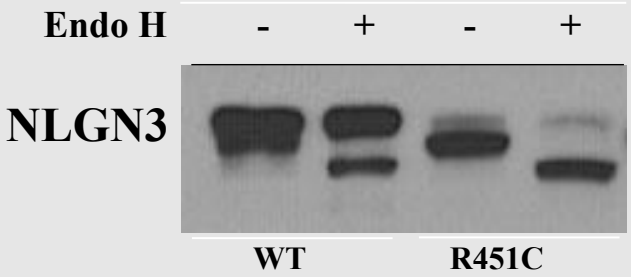
- Arg451 to Cys mutation in NL3 and Arg453 in NL1 compromises the export of NL1 and NL3. When Arg451 is mutated to Thr or Glu the protein is normally expressed.

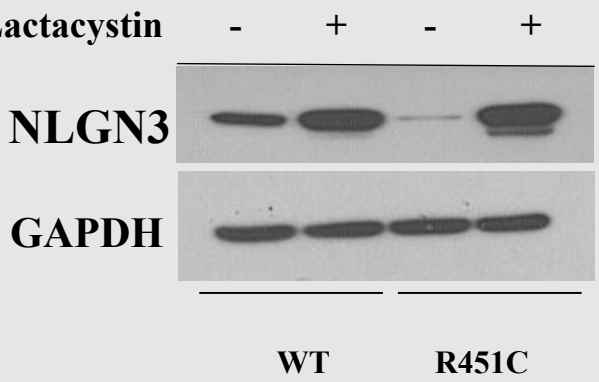
Comoletti et al. J.Neurosci., 2004

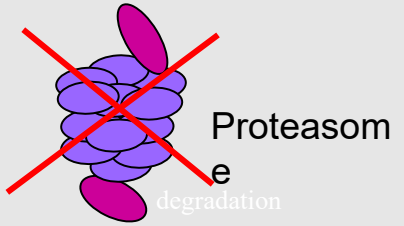
Co-localization staining of Neuroligin3 and calnexin







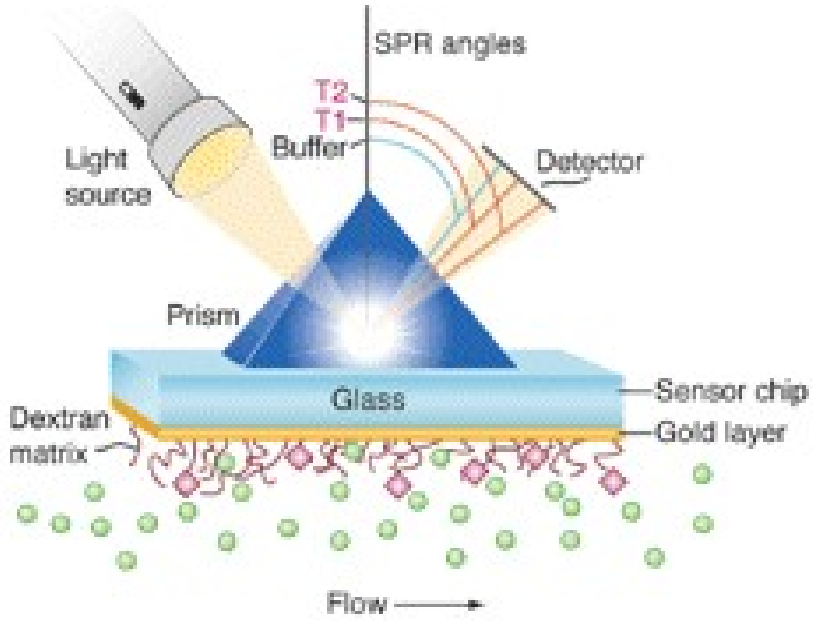


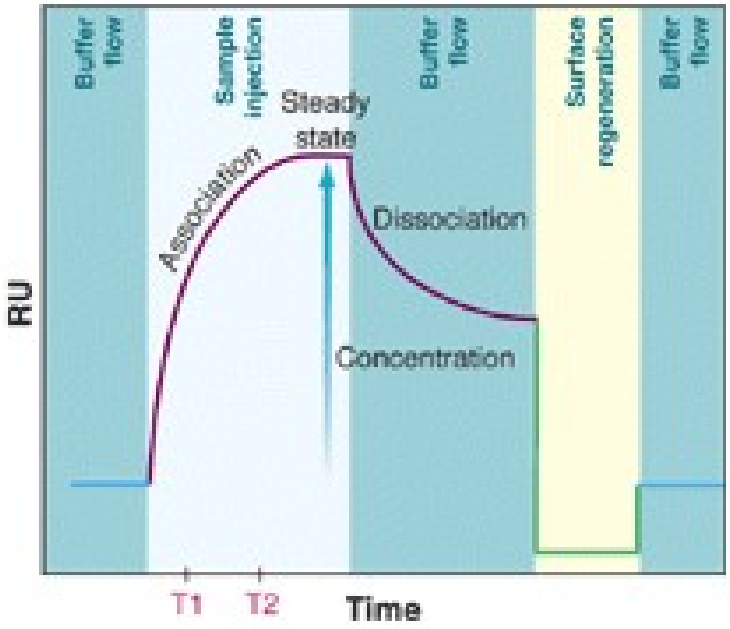


Neuroligin3 R451C is in the Endoplasmic Reticulum

De Jaco et al., 2006

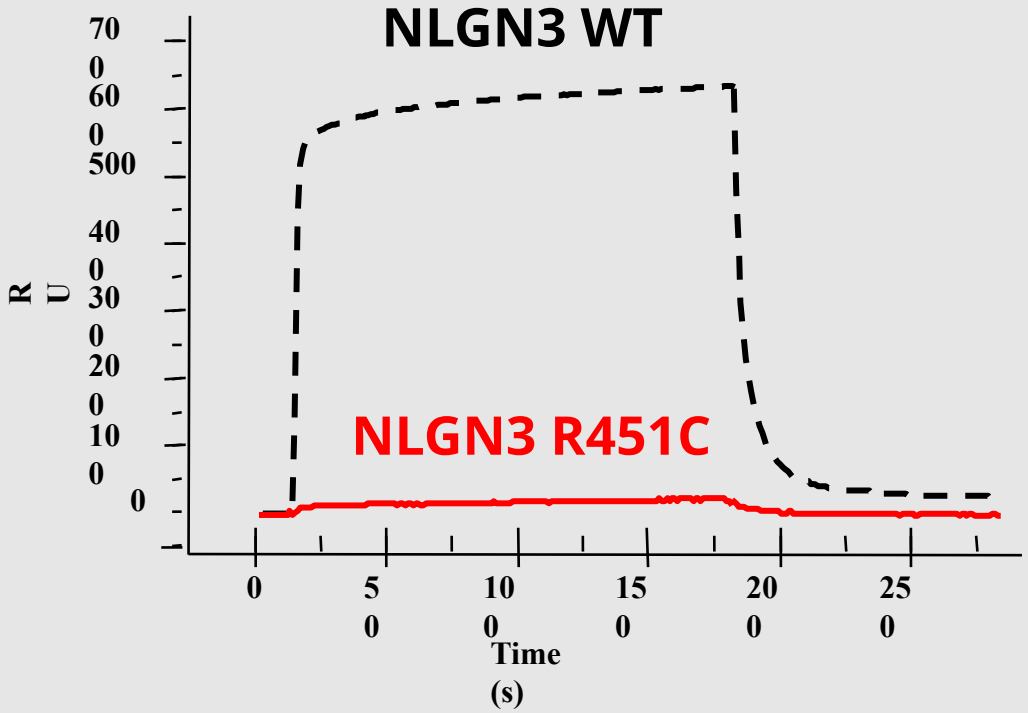
SURFACE PLASMON RESONANCE





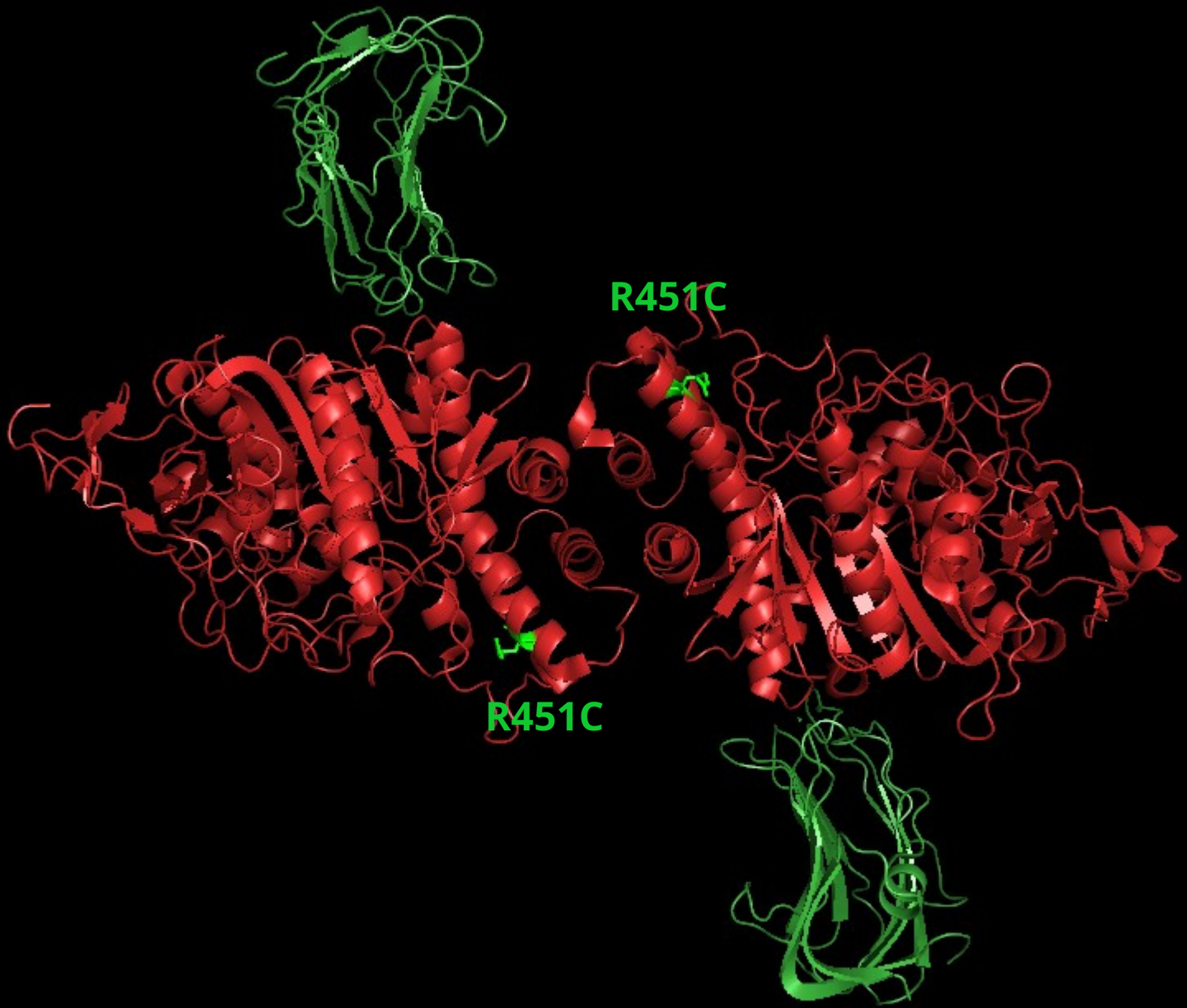
La variazione dell'angolo di risonanza e' direttamente proporzionale alla quantita' di Ligando legato alla macromolecola immobilizzata

The mutation is affecting Neuroligin3 function

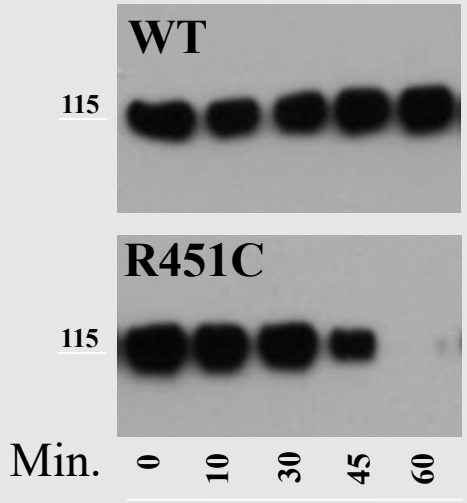


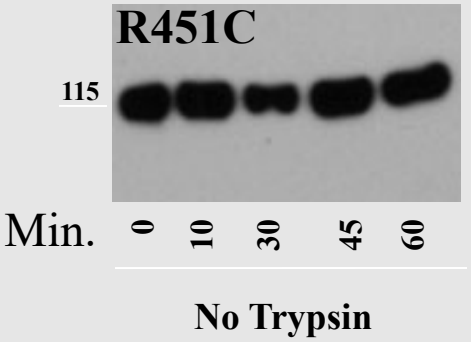
Reduced binding activity
of purified R451C Neuroligin3
in comparison to WT protein

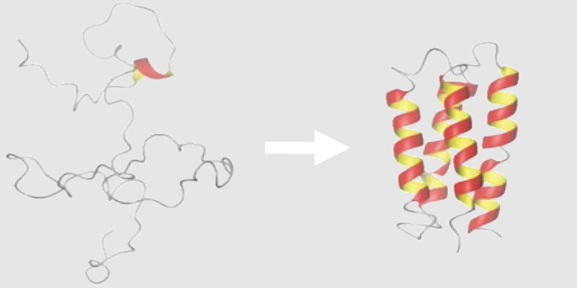
Comoletti et al., 2004


R451C
R451C

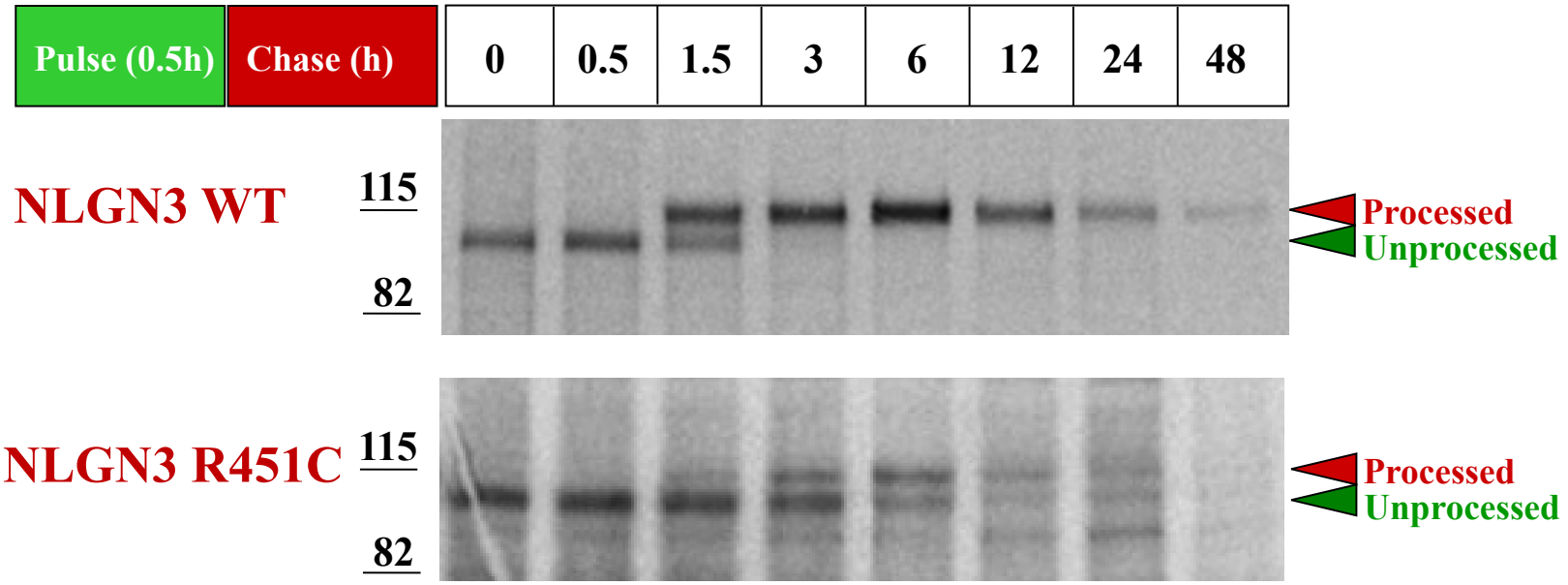
Misfolding of NLGN3 R451C mutant protein: protease sensitivity







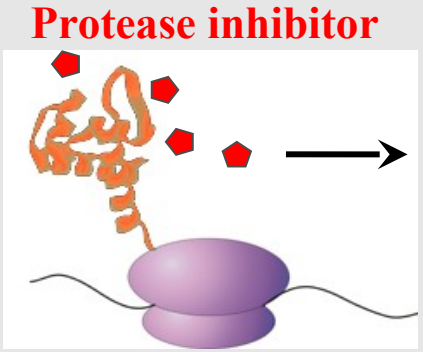
Processing of NLGN3 in HEK-293 cells

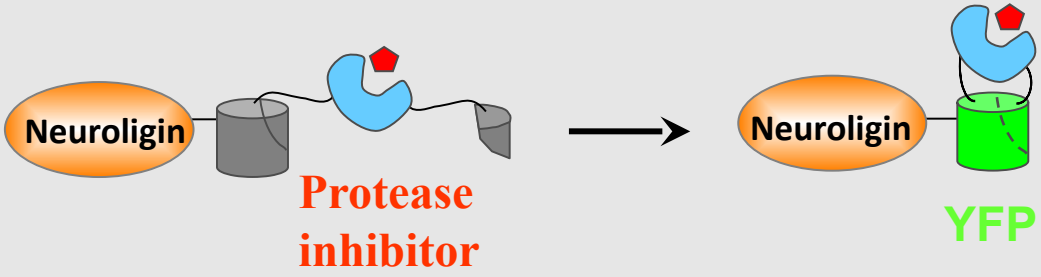


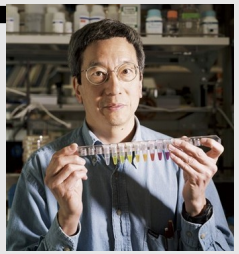
R451C Mutation in NLGN3 affects glycosilation processing of the protein in the ER

De Jaco et al., 2010

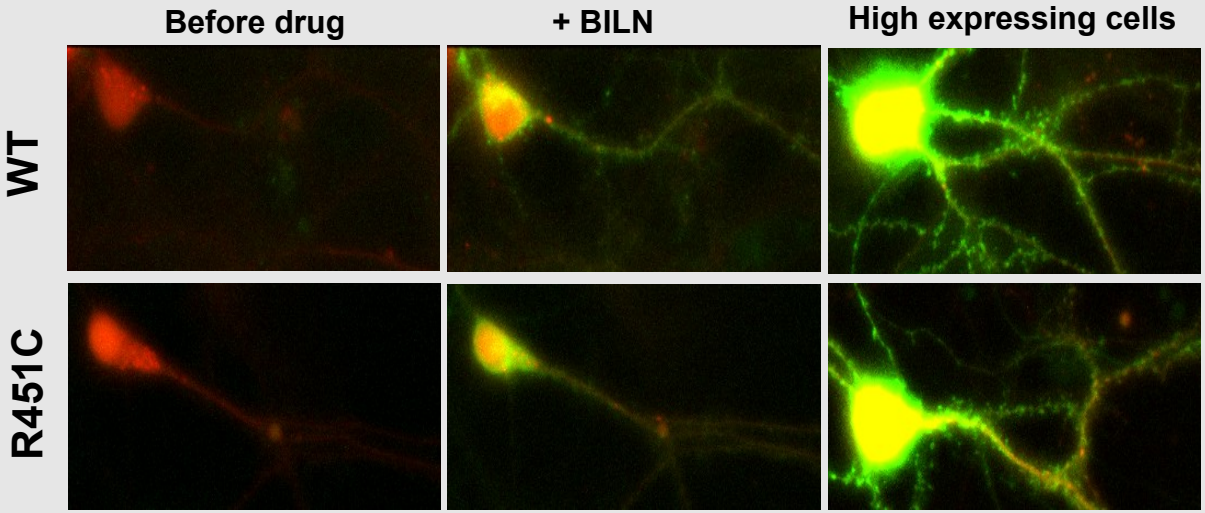
Trafficking of fluorescent Neuroligin3 in hippocampal neurons

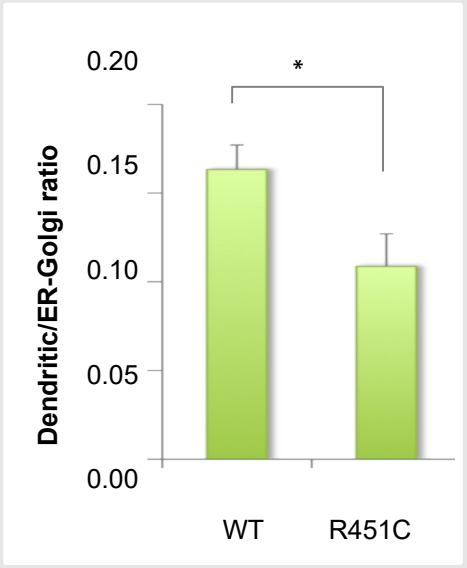






Dr. Roger Tsien





- Neuroligin3 protein is represented by the green dots.
- Protein translocation from the soma to dendrites was quantified by the ratio of the measured fluorescent signal!

De Jaco et al., 2010

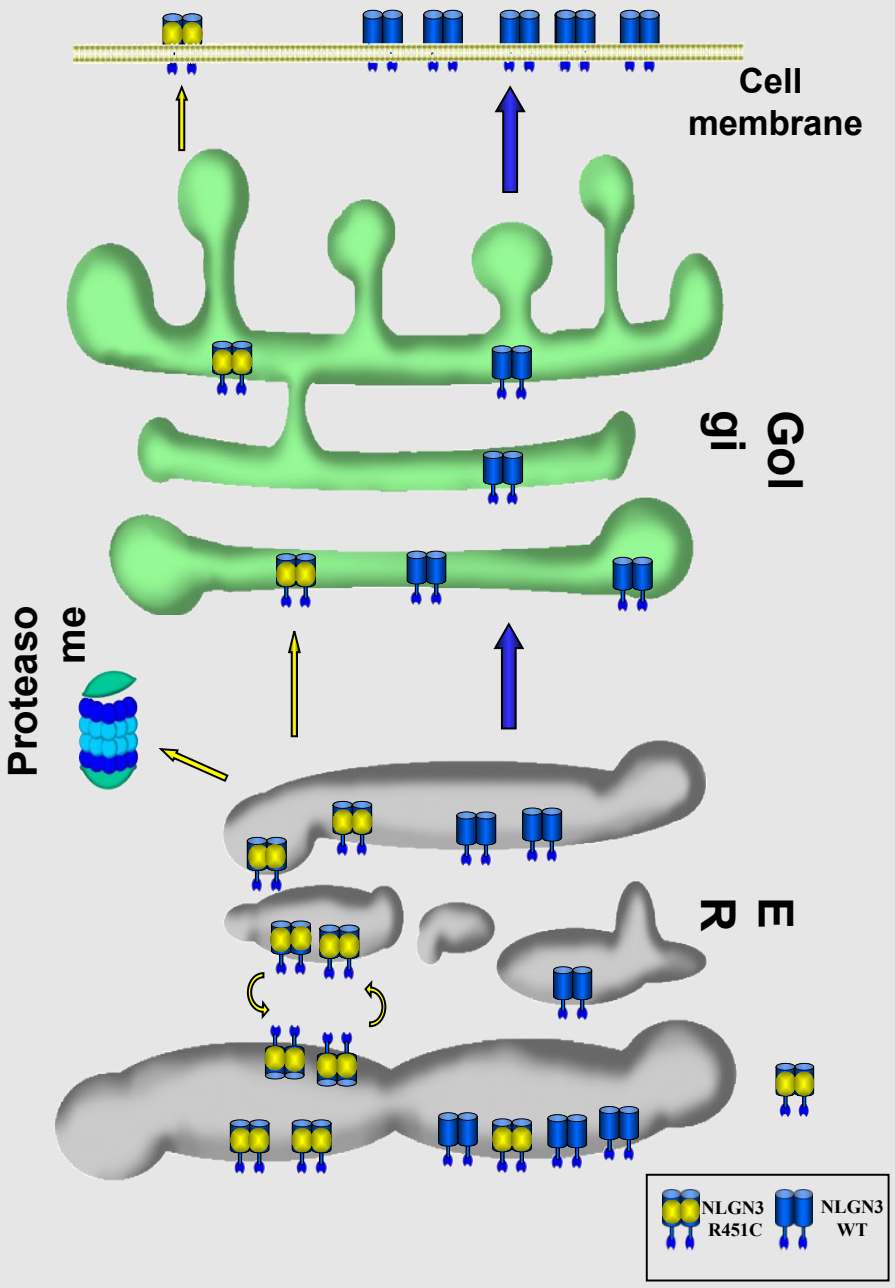
R451C mutation in Neuroligin3 causes misfolding and ER retention

R451C NLGN3 is mainly retained in the Endoplasmic Reticulum (ER).

R451C mutation causes a local misfolding of NLGN3.

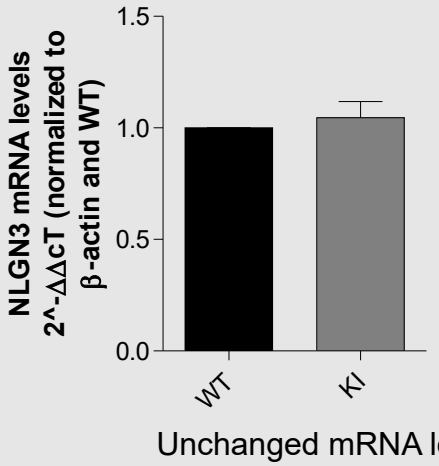
The majority of the protein is degraded by the proteasome

A small protein fraction reaches the cell surface to incorporate into synapses.

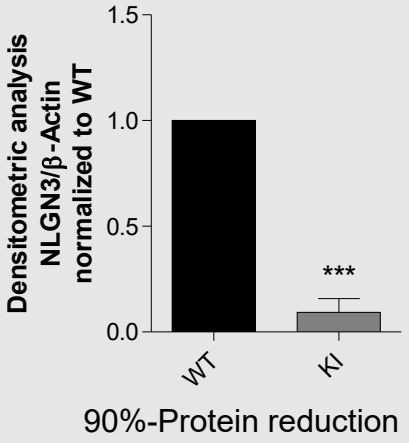


Comoletti et al., 2004, De Jaco et al., 2010

Neuroligin3 R451C *Knock In* mouse model of autism



Unchanged mRNA levels



90%-Protein reduction

Behavioural and electrophysiological alterations of R451C Neuroligin3 KI mouse:

- 90% decrease in Neuroligin3 protein levels (adult)
- Autism-like behaviors (impaired sociality, enhanced spatial learning)
- Imbalance excitatory/inhibitory neurotransmission (hippocampus, cerebral cortex)
- “Gain of function” phenotype compared to Neuroligin3 *Knock Out* mice

Molecular mechanisms by which NLGN3 R451C may give rise to ASDs are not completely known

Tabuchi et al., 2007; Etherton et al., 2011; Pizzarelli and Cherubini, 2013; Favaloro et al. In preparation

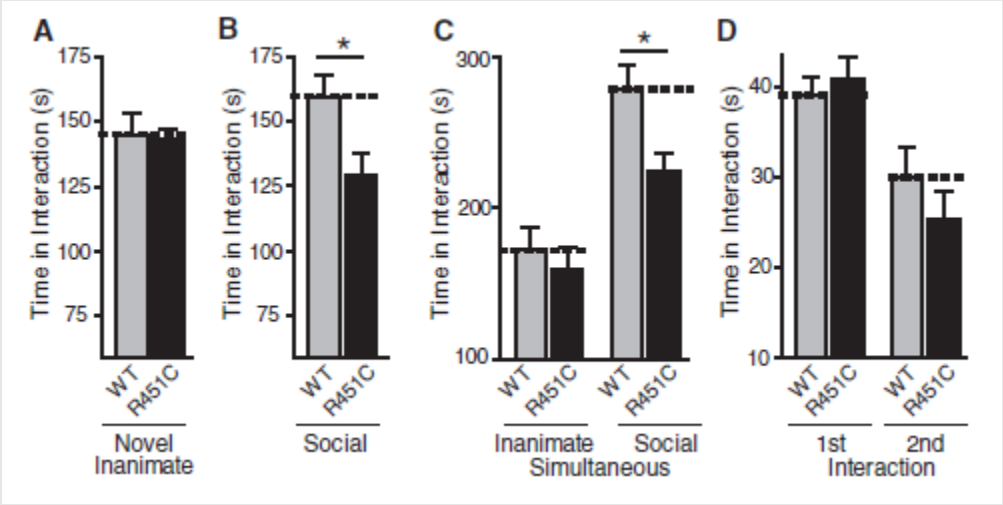
Table 1 Mouse models of ASD

Molecular function	Mouse model	Social interaction	Social communication	Repetitive behavior	Other symptoms	Molecular, cellular and circuit phenotypes	Treatment
Multiple	dup 15q11-q13 (refs. 267,268)	Impaired	↓Calls	Behavioral inflexibility	NA	Altered serotonergic signaling, ↑spine dynamics	NA
Transcriptional regulator	*Tbr1* HT	Impaired	Impaired STFP	Behavioral inflexibility	CTA defects, learning deficits	Axonal projection defects in amygdala ↓NMDAR function	DCS (adult)[170] clioquinol (A)[269]
Translational regulator	*Fmr1* KO[255]	Impaired	↓Calls	Hand flapping	**PPI of startle, audiogenic seizure, learning deficits**	**↑mGluR function, immature protrusion**	MPEP (A)[233,237]
					Learning impairment	PI3K signaling, **↑spine density, impaired AMPAR-mediated synaptic plasticity**	5-HT and DA compound (A)[270,271]
					Audiogenic seizure	Hypersensitivity to ERK1/2 pathway activation, **↑protein synthesis**	SL327 (A)[272]
			↓Calls		NA	**↑Fetal or early postnatal GABA and Cl^-, abnormal EEG**	Bumetanide, oxytocin (P13-15)[251]
	Tsc1 HT, *Tsc1*Cb KO	**Impaired**	↑Calls	Grooming, **behavioral inflexibility**	Ataxia	**Cerebellar deficits**	Rapamycin (P7)[183]
	Tsc2 HT	**Impaired**	↑Calls	Increased marble burying	**Lethality, learning deficits**	**Brain enlargement**, hyperactive mTOR signaling, autophagy deficiency	Rapamycin (A)[144,240]
	Pten cKO	**Impaired**	NA	NA	**Learning deficits, seizure, anxiety**	**Macrocephaly, cellular hypertrophy**, PI3K pathway hyperactivation	Rapamycin (4–6 weeks)[114,184]
Neuron-glia interaction, K^+ channel clustering	*Cntnap2* KO	*Impaired*	↓Calls	**Grooming**	Seizure, **hyperactivity**	↓Interneuron number, abnormal neuronal migration	Risperidone (A)[109]
		Impaired		*Grooming*	*Hyperactivity*	**↓Oxytocin neurons**	Oxytocin (P7–P21; A)[249]
Na^+ channel	*Scn1a* KO	**Impaired**	NA	Grooming	Seizure, **learning impairment**	**↓GABAergic interneuron firing**	Clonazepam (A)[239]
Synaptic adhesion molecule	*Nrxn1a* KO[273,274]	Impaired, aggression in males	NA	Grooming	Anxiety, sensory-gating deficits, motor learning	↓Glutamatergic trans. and synaptic density	NA
	Nlgn3 R451C KI[275,276]	Impaired	↑Calls	NA	Enhanced learning	Context-dependent impaired glutamatergic and GABAergic trans	NA
	Nlgn3 KO[277]	Impaired	↓Calls	Normal behavioral flexibility, **Stereotyped motor routine**	Hyperactivity	↓Brain volume, cerebellar deficit	NA
						↓GABAergic trans. in D1-MSN in NAc	NLGN3 expression in D1-MSN[211]
	Nlgn4 KO[278]	↓Impaired, aggression	↓Calls	Normal	NA	↓Brain volume	NA
Synaptic scaffolding molecule	*Shank2* exon7 KO[279]	Impaired	↓Calls, pattern change	Grooming	Hyperactivity, anxiety	↑NMDAR function, ↑LTP	NA
	Shank2 exons 6–7 KO	**Impaired**	↓Calls	*Jumping*	*Hyperactivity, anxiety*	**↓NMDAR function, ↓LTP and LTD**	CDPPB, DCS (A)[232] clioquinol (A)[269]
	Shank3B KO[210]	Impaired	NA	Grooming	Anxiety	Striatal dysfunction	NA
	Shank3 exons 4–9 KO[280]	Impaired	Pattern change	Grooming	Learning deficits	↓Activity-dependent AMPAR distribution and LTP	NA
	Shank3 HT[281]	Impaired	↓Calls	NA	**Motor coordination**	**↓Glutamatergic trans. by presynaptic mechanism**, ↓LTP	IGF1 (P13–P28)[177]
	Shank3$^{+/\Delta C}$ (ref. 282)	**Impaired**	NA	**Grooming**	NA	**↓NMDAR function, Rac1, PAK, cofilin signaling defects,** F-actin dysregulation in PFC	TAT–p-cofilin peptide (A)
		Impaired		Grooming			CA-Rac1 (A)

Neuroligin 3 R451C Knock In mice

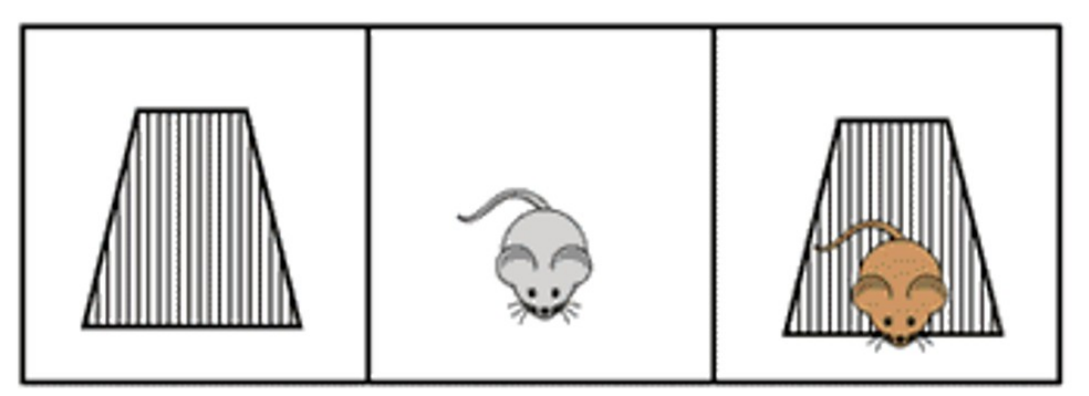
Animal model to study a monogenic form of ASD

- Decrease of Neuroligin3 protein levels
- Autism-like behaviors (Impared social interactions)
- Imbalance of excitatory - inhibitory neurotransmission
- Gain of function phenotype compared to KO



Tabuchi et al., 2007

Il test delle TRE CAMERE per studiare il comportamento sociale



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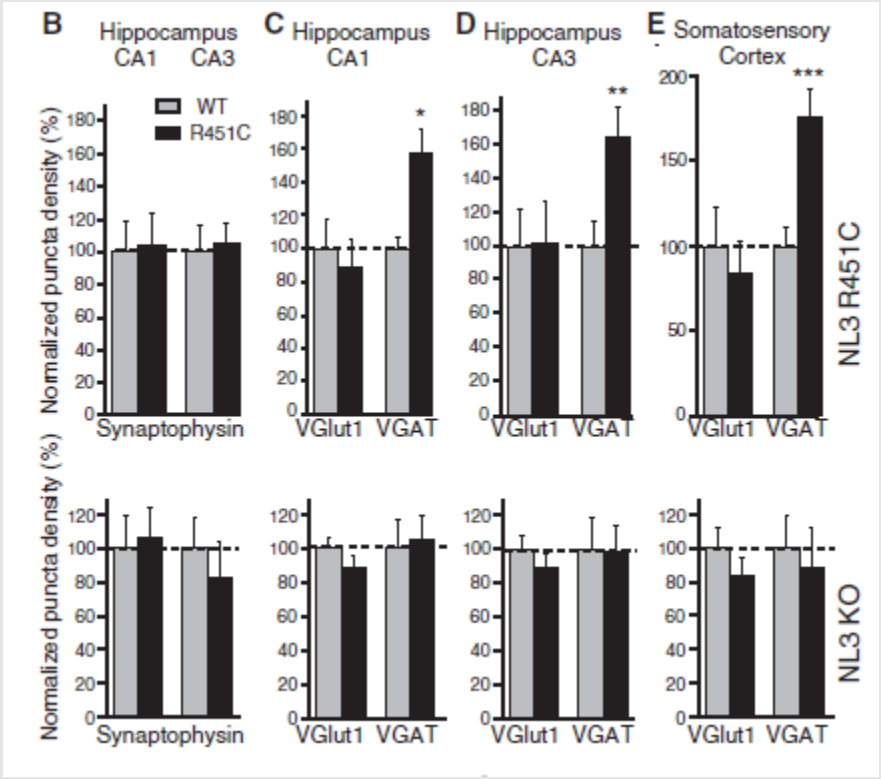
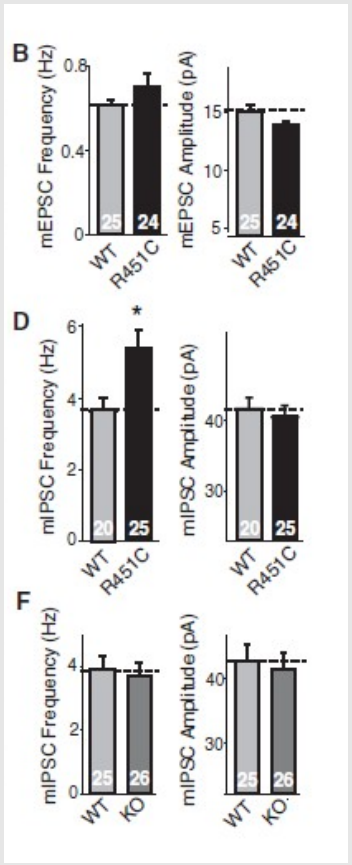


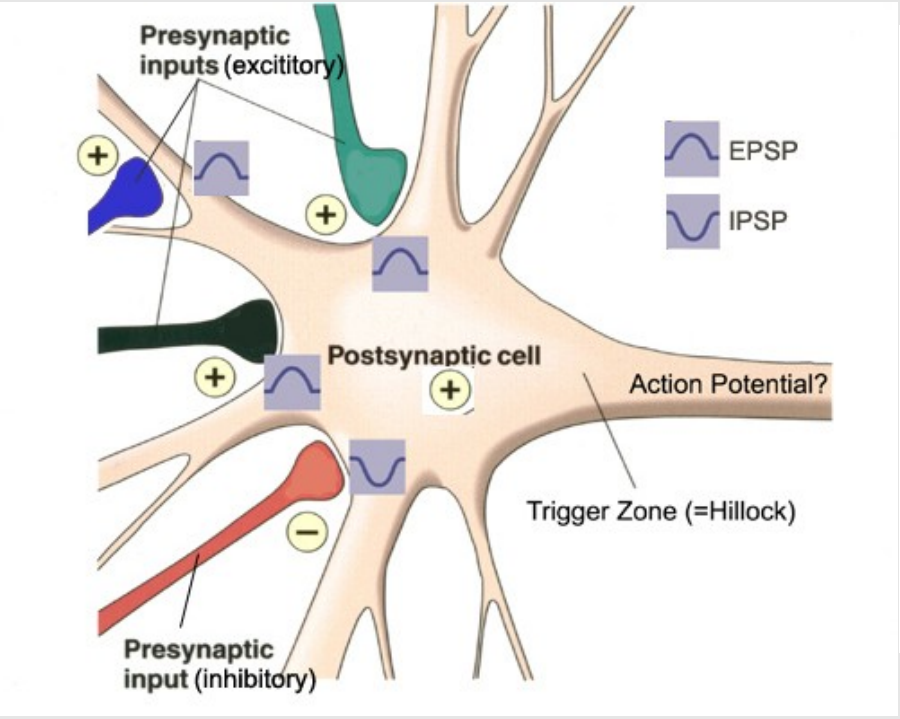


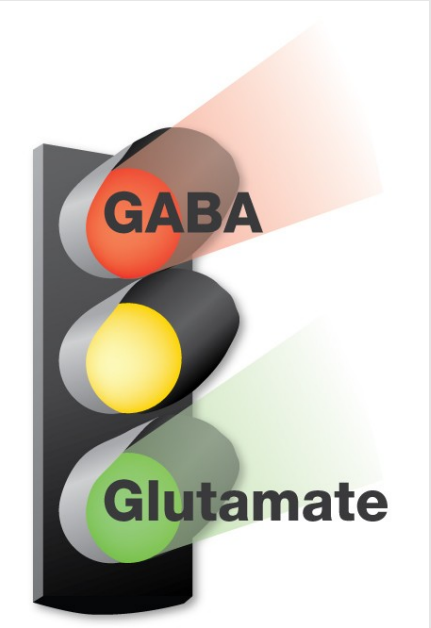
Vesicular GABA transporter **VGAT**

Vesicular glutamate transporter 1 **VGLUT** 1,

Tabuchi et al., 2007
Etherton et al., 2011

The excitatory/Inhibitory balance is altered in ASDs





Neuronal excitability relies on the summation of excitatory and inhibitory signals a process regulated by the number of excitatory vs inhibitory (E/I) contacts received by a single neuron.

Impaired glutamatergic and GABAergic transmission, as has been reported in several mouse models of ASD, can result in ASD-like behaviors that can be alleviated by modulators of AMPA receptor (AMPAR), NMDA receptor (NMDAR) and GABAAR.

IN UNA SINAPSI ECCITATORIA MOLECOLE DI ADESIONE DIVERSE COESISTONO E PARTECIPANO ALLA FORMAZIONE DI COMPLESSI SINAPTICI CHE MANTENGONO LA SINAPSI ALTAMENTE ORGANIZZATA