CALCIUM
freiner ou pousser?

Plonk et Replonk

CONTENU

Calcium – propriété, mesures, distribution et concentrations

Dépendance calcique des voies de transport

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Senseurs calciques – synaptotagmine

propriété biochimique et interactions

structure

interaction avec membranes

fonction

CALCIUM
le signal ubiquitaire?

Calcium:

petite, divalente

multiples sites de liaison « peu spécifique » (buffering)

distinguer calcium total et calcium libre

Concentration extracellulaire: ca 1 mM

Concentration intracellulaire au repos: 100 nM

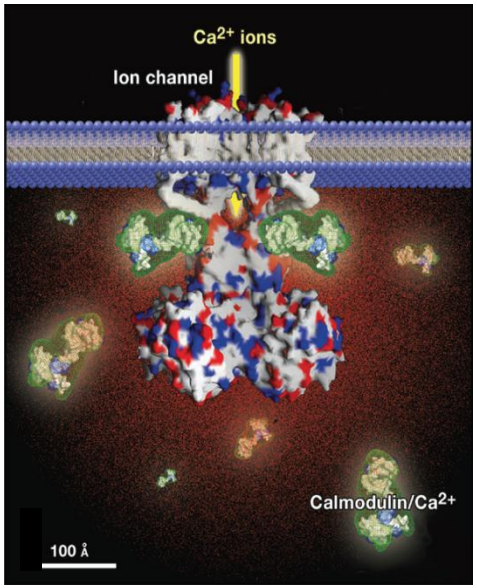
Concentration intracellulaire lors d'une stimulation:

>100 nM et < 1 mM (théoriquement)

Avantage: petit, divalente, tamponnage, pas d'altération chimique; changement charge surface (comme phosphorylation)

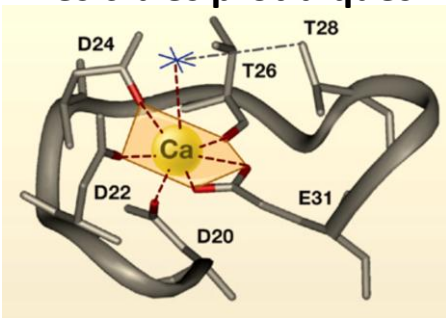
Désavantage: lie H_2O moins fort que Mg^{2+}; précipite phosphore

Gradient: le Ca^{2+} est inerte, donc soit chélation soit compartiméntalisation; coûteux en énergie (une bonne partie du métabolisme)



40 µm in 1 s (40 nm/ms)
en solution saline (Einstein, 1905)
mais…

Les cibles protidiques



4-12 oxygène chèlatent le calcium

CALCIUM
le signal ubiquitaire?

Les cellules excitables ….

a cell that can generate an action potential at its membrane in response to depolarization and may transmit an impulse along the membrane.

Neurones
Muscle
Coeur
Cellules secretrices
- Hypophyse
- Pancréas endocrine
- Cellule endocrine intestine
- Surrenales… (medulla)

CALCIUM
les mésures

Sondes fluorescentes (EGTA; Stoke's shift; ratio)

Protéines recombinantes

Résolution spatiale

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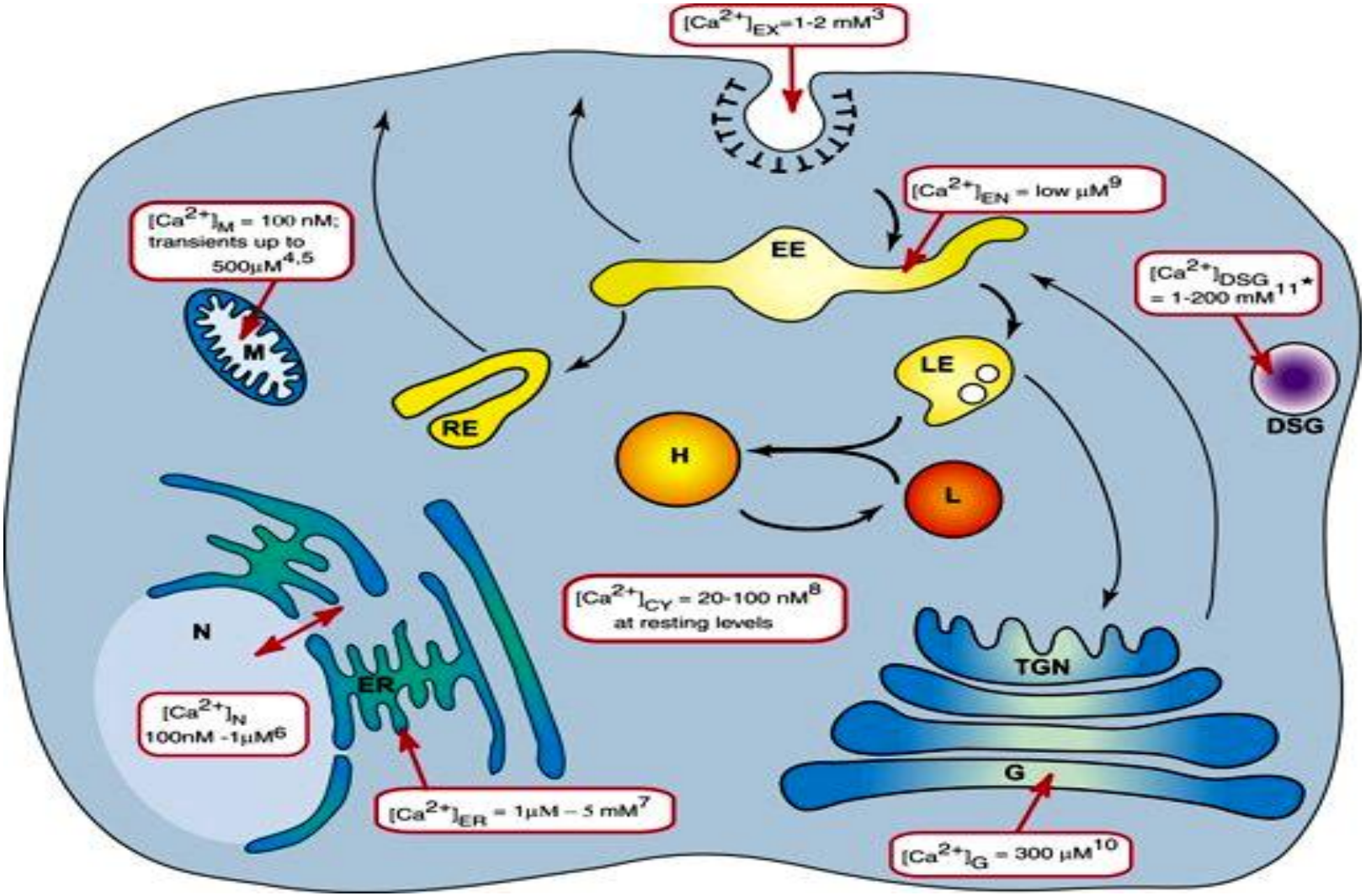
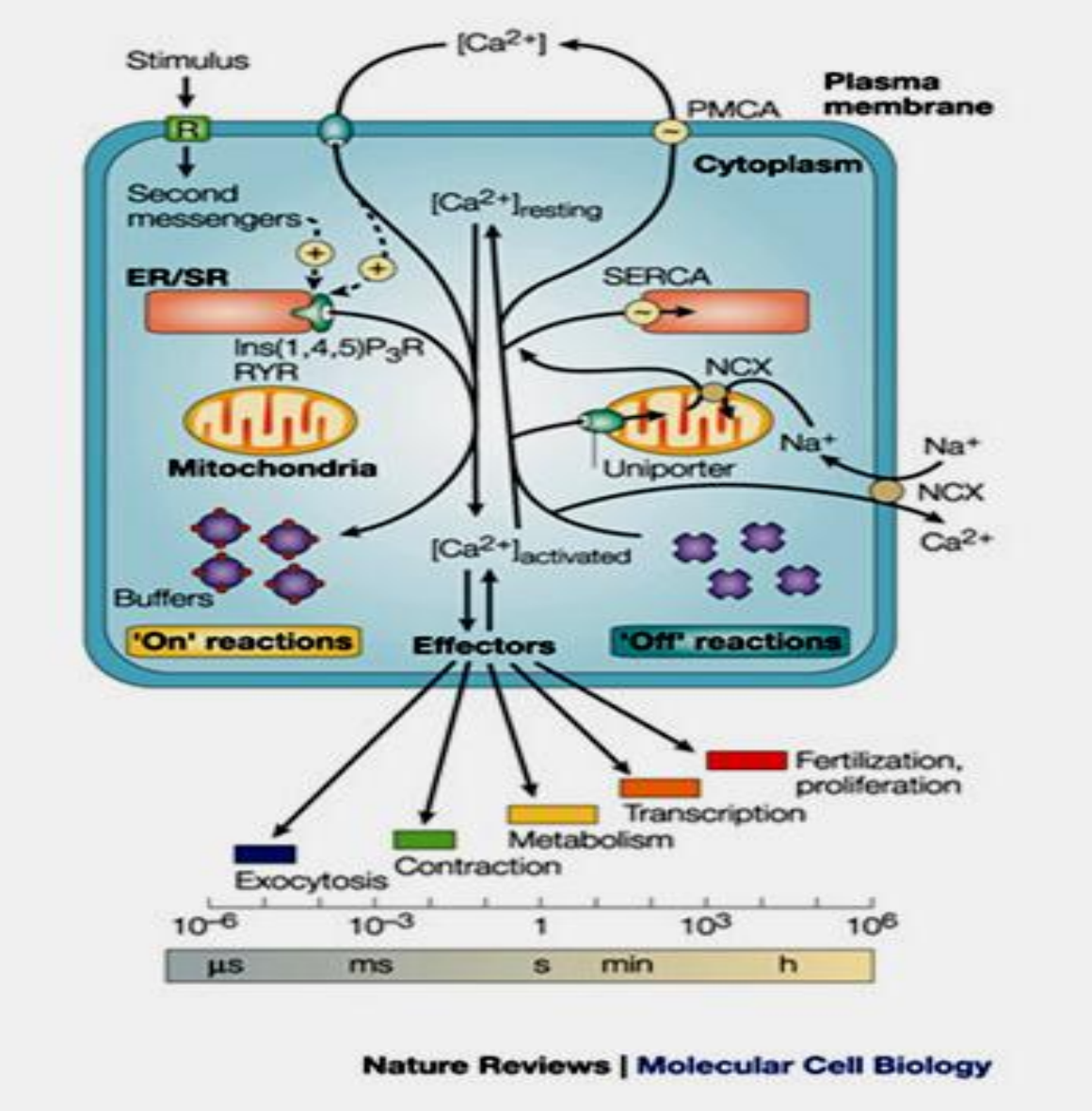
CALCIUM

concentrations intracellulaires



CALCIUM
régulation intracellulaire



CALCIUM

des concentrations fluctuantes…

EC_{50} 1 µM, 1980

Baker PF, Knight DE. J Physiol (Paris) 1980, 76:497-504. *Gaining access to the site of exocytosis in bovine adrenal medullary cells. LDCV*

EC_{50} 27 µM, 1993

Thomas et al. Neuron 1993;11:93-104. *A low affinity Ca^{2+} receptor controls the final steps in peptide secretion from pituitary melanotrophs.* LDCV

EC_{50} 200µM, 1994

Heidelberger R, et al.. Nature 1994; 371:513-5. *Calcium dependence of the rate of exocytosis in a synaptic terminal SV*

1997

Marsault R, Murgia M, Pozzan T, Rizzuto R. EMBO J 1997;16:1575-81. *Domains of high Ca^{2+} beneath the plasma membrane of living A7r5 cells.*

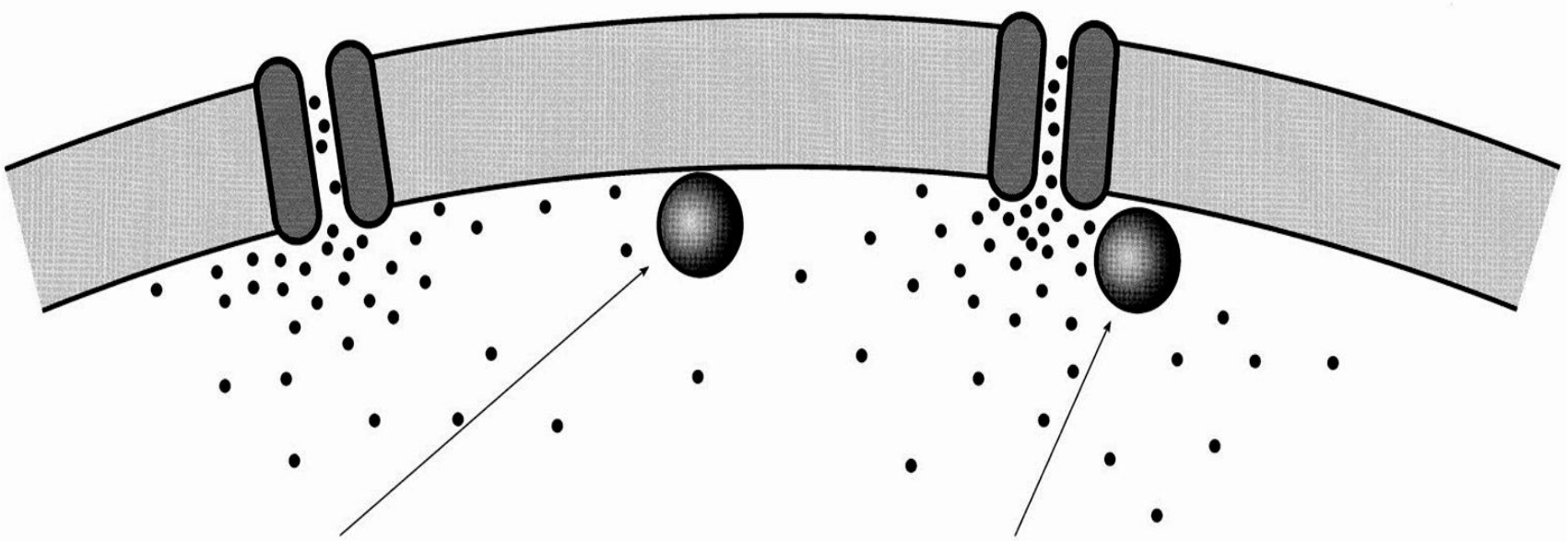
Klingauf J, Neher E. Biophys J 1997 72:674 *Modeling buffered Ca^{2+} diffusion near the membrane: implications for secretion in neuroendocrine cells.*

2000

Schneggenburger R, Neher E. Nature 2000;406:889-93. *Intracellular calcium dependence of transmitter release rates at a fast central synapse.*

CALCIUM

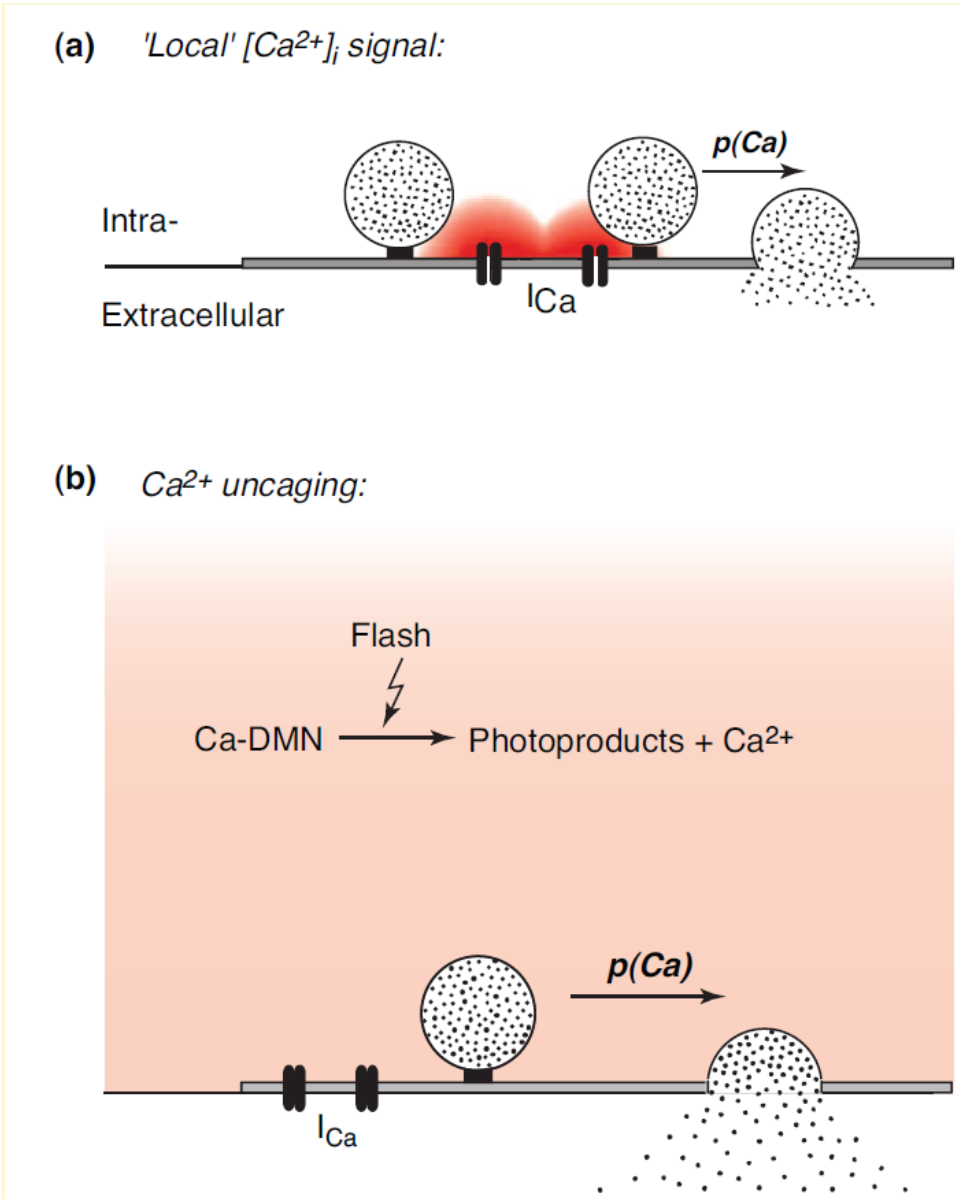
concentrations sous-membranaires et vitesses



At 200 nm distance:	At 20 nm distance:
1.) $[Ca^{++}] \approx$ 5-10 µM	1.) $[Ca^{++}] \approx$ 100 µM
2.) Rises and falls in ≈ 10 msec	2.) Rises and falls within µsec
3.) Is at equilibrium with mobile buffers	3.) Is not at equilibrium with mobile buffers
4.) Strongly dependent on buffers; EGTA as effective as BAPTA	4.) Almost independent of Ca-buffers; EGTA totally ineffective
5.) $[Ca^{++}]$ determined by mean activity of several neighbouring channels	5.) $[Ca^{++}]$ predominantly determined by the <u>local</u> channel

CALCIUM

des concentrations fluctuantes…selon l'activation



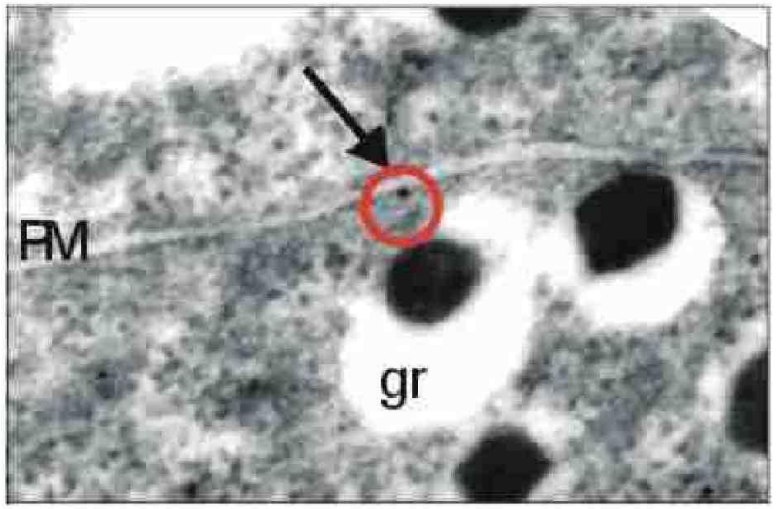
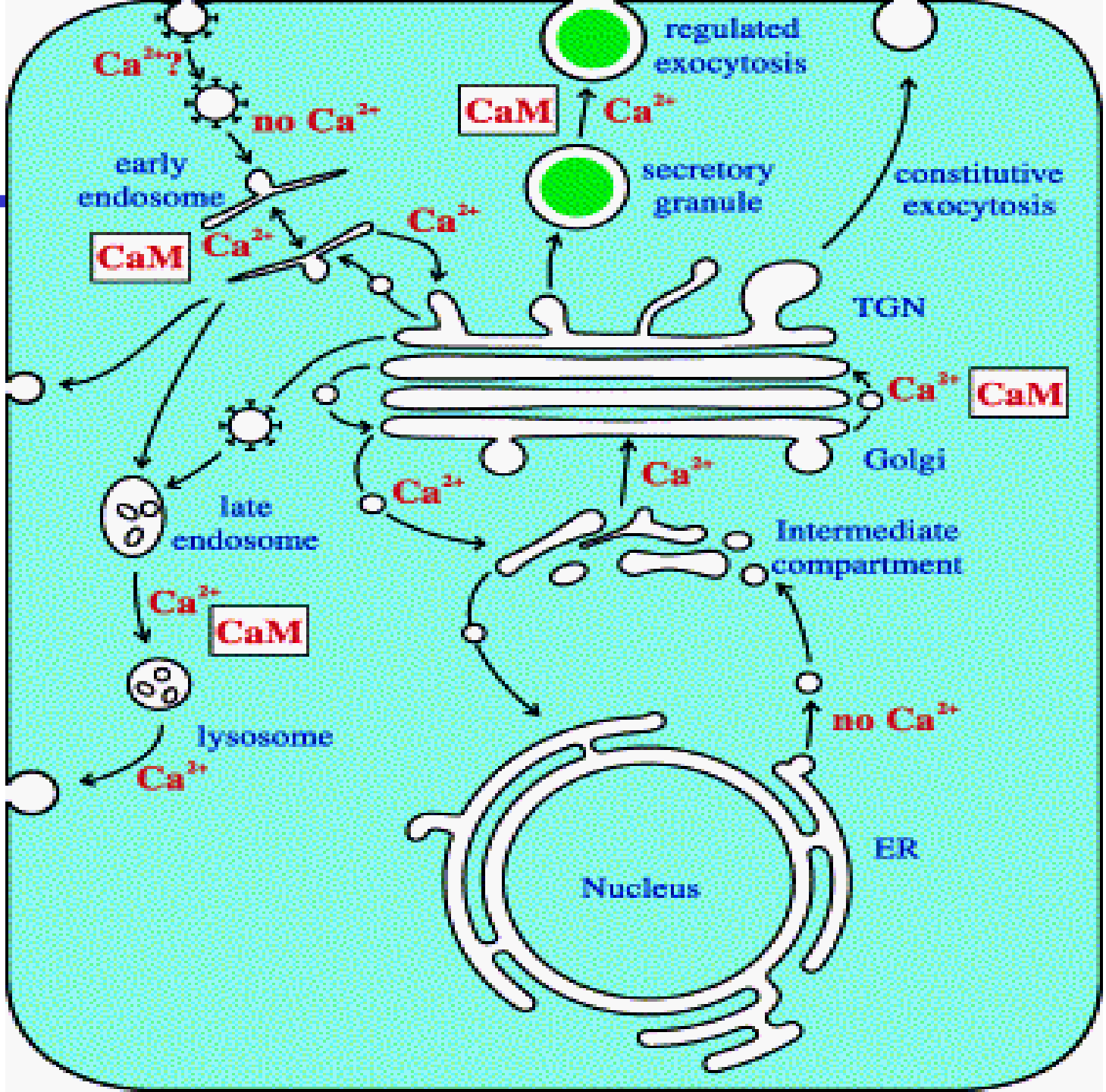


Fig. 2. Secretory granules docked to the plasma membrane and within 30 nm from a Ca2+ channel (gold-dot at the point of the arrow inside red ring).

CALCIUM
le signal ubiquitaire?



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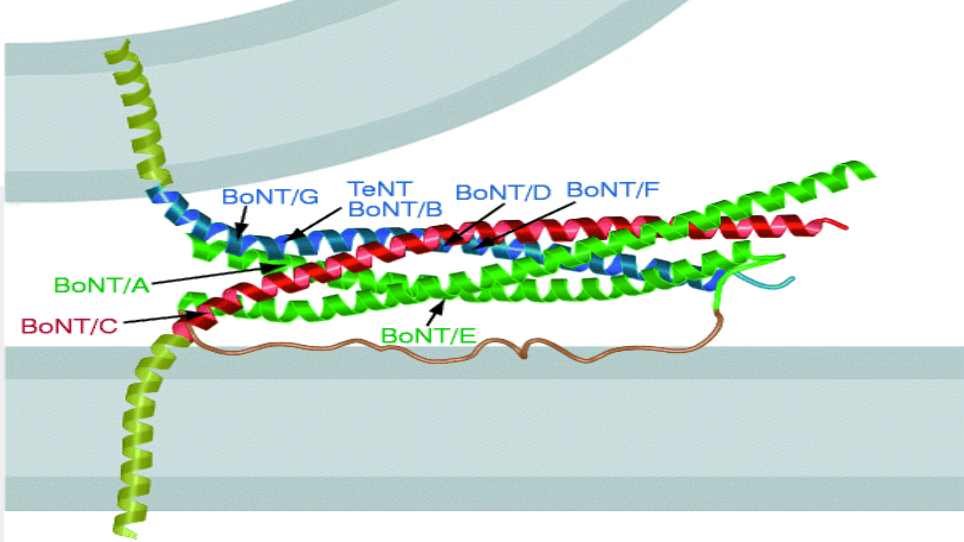
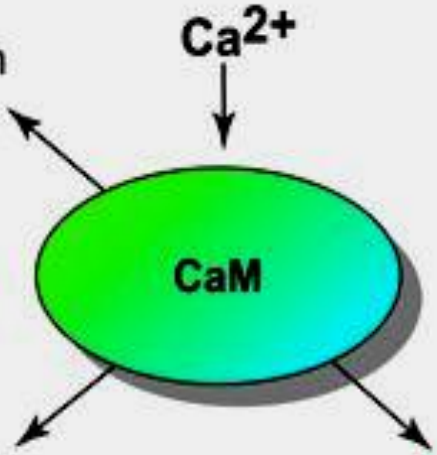
CALCIUM
rôles du calmoduline



Calmodulin in Membrane Traffic

Vesicle transport / anchoring

- Control of traffic in the endocytic pathway
- Binding of proteins involved in membrane traffic (EEA1; Phosphatidylinositol 3-kinase)
- Binding to myosins
- Remodelling of the cytoskeleton



Membrane Fusion

- Control of SNARE complex assembly/function
- Yeast homotypic vacuole-vacuole fusion; Heterotypic late endosome-lysosome fusion

Other calmodulin regulated events[a]

- Phosphorylation/ dephosphorylation events
- Cyclic nucleotide metabolism
- Ca^{2+} transport

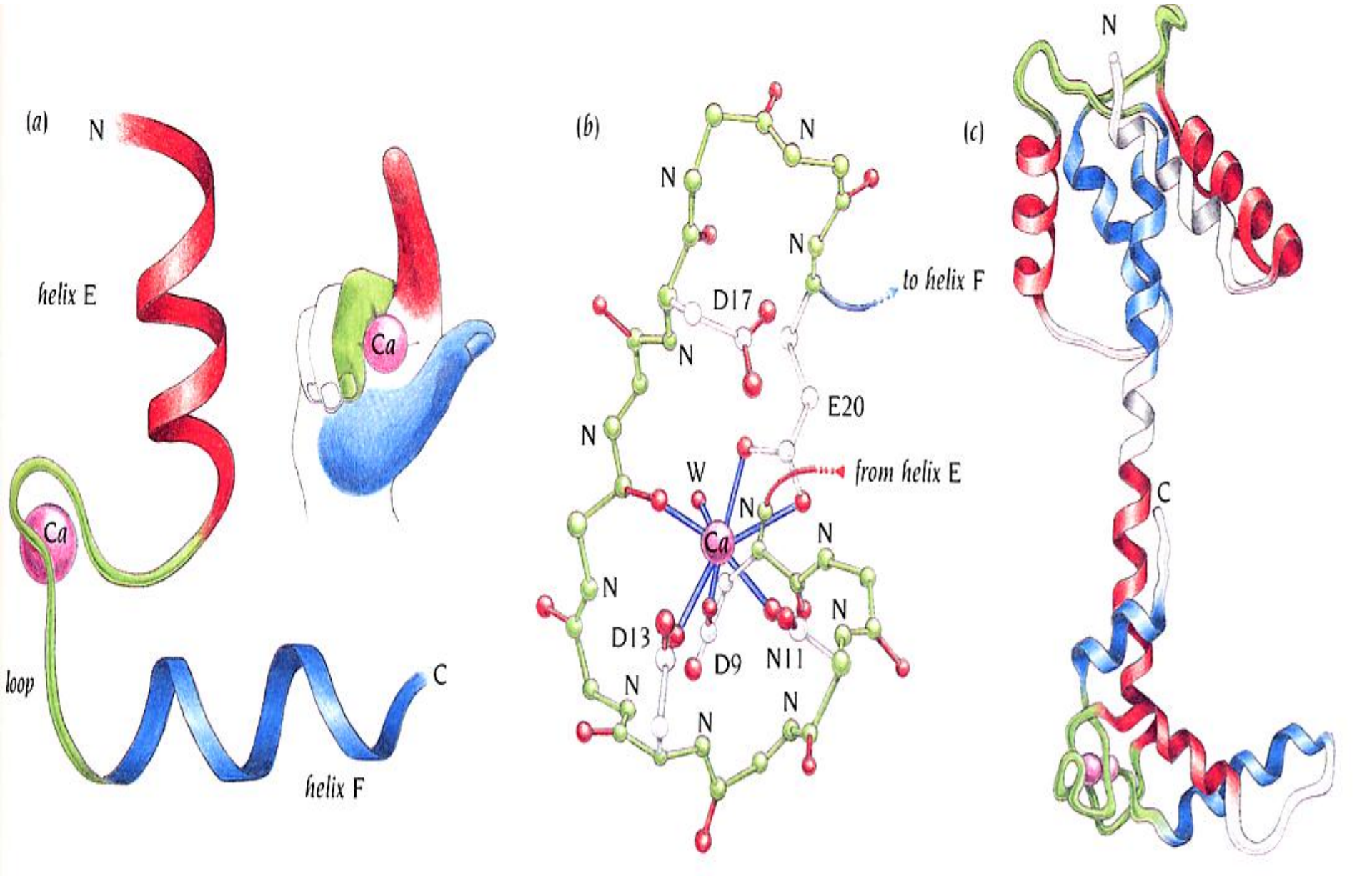
CALCIUM
La main EF

Table 2.2 Amino acid sequences of calcium-binding EF motifs in three different proteins

Parvalbumin	V	K	K	A	F	A	I	I	D	Q	D	K	S	G	F	I	E	E	D	E	L	K	L	F	L	Q	N	F
Calmodulin	F	K	E	A	F	S	L	F	D	K	D	G	D	G	T	I	T	T	K	E	L	G	T	V	M	R	S	L
Troponin-C	L	A	D	C	F	R	I	F	D	K	N	A	D	G	F	I	D	I	E	E	L	G	E	I	L	R	A	T

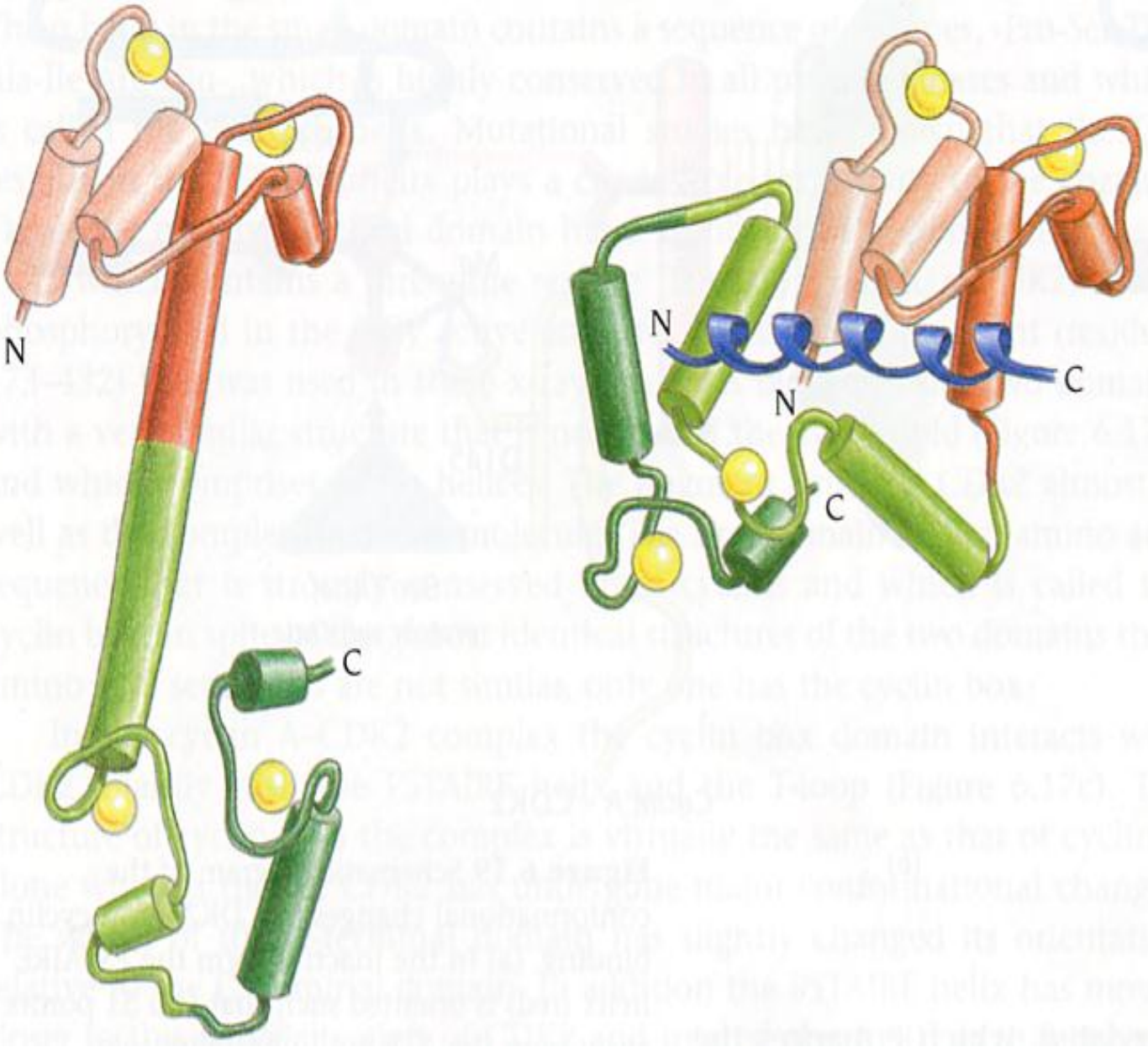
Calcium-binding residues are orange, and residues that form the hydrophobic core of the motif are light green. The helix-loop-helix region shown underneath is colored as in Figure 2.13.

CALCIUM
la main EF - II



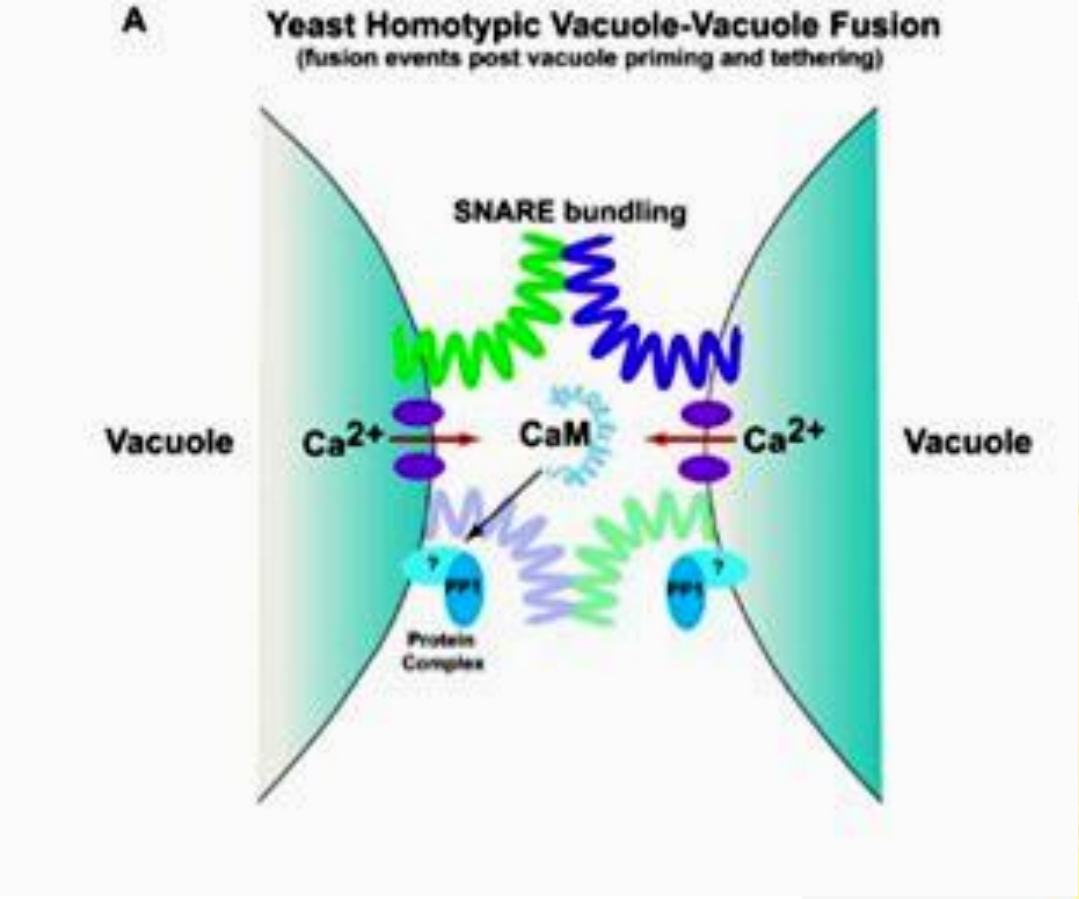
CALCIUM

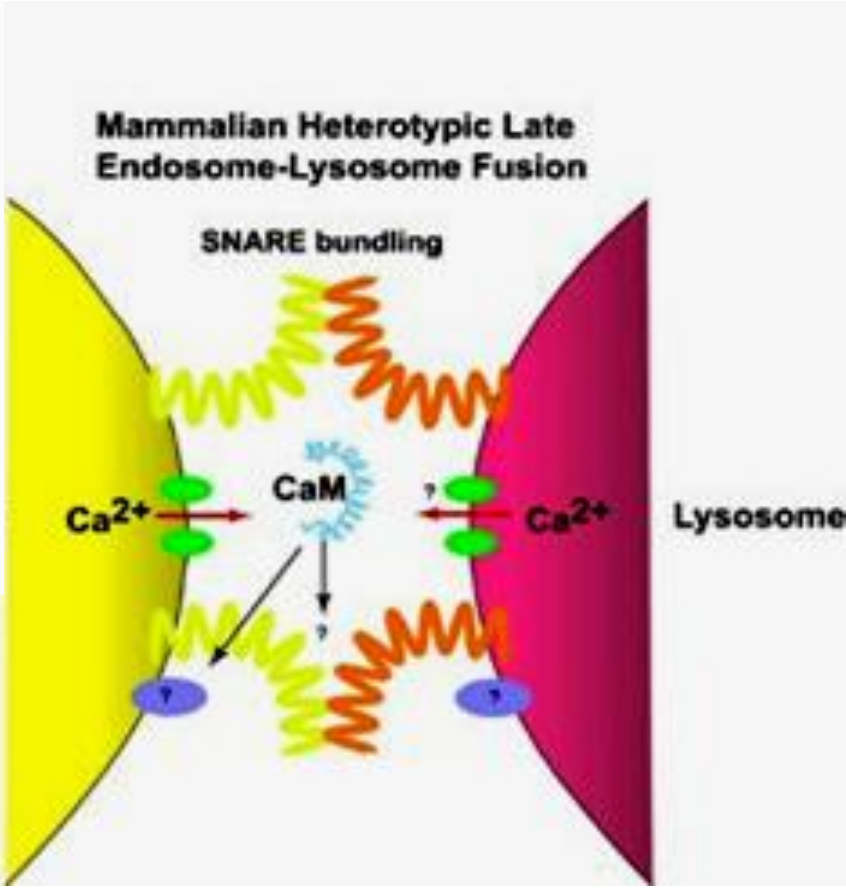
calmoduline



CALCIUM

calmoduline - mécanismes potentiels





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CALCIUM

structure générale des synaptotagmines et isoformes

Nature **353**, 65-68 (5 September 1991)

Binding of synaptotagmin to the α -latrotoxin receptor implicates both in synaptic vesicle exocytosis

Alexander G. Petrenko[*†], Mark S. Perin[*], Bazbek A. Davletov[†], Yuri A. Ushkaryov[*], Martin Geppert[*] & Thomas C. Südhof[*‡]

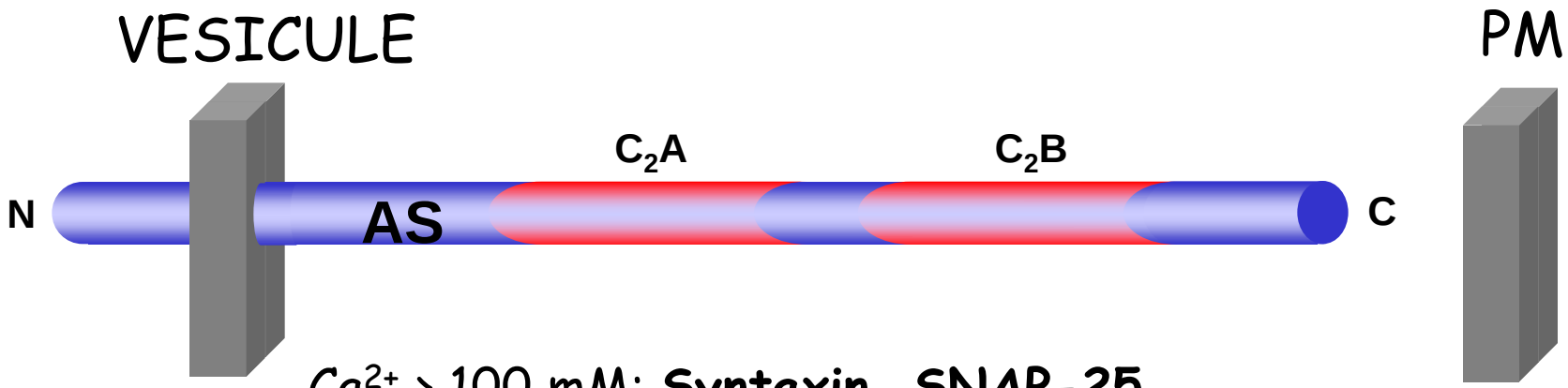
1. [*]Howard Hughes Medical Institute and Department of Molecular Genetics,University of Texas Southwestern Medical Center, Dallas, Texas 75235,USA
2. [†]Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow 117871, USSR
3. [‡]To whom correspondence should be addressed

Thomas Südhof

p65
Veuve noire
Latrotoxine (1978)
Latrophilin/Neurexin/neuroligins

CALCIUM

synaptotagmine – structure générale et interactions



Ca^{2+} > 100 mM: **Syntaxin, SNAP-25**
Ca^{2+} 1 - 10 mM: **Phospholipids** (V or PM?)
Homo/heterodimers (on V or V/PM ?)

Ko mice, drosophila, C. elegans: fast release
Interaction avec canaux (L; P/Q)

•Dogme de Dodge et Rahmaninoff (>3 Ca^{2+} ions)

CALCIUM
synaptotagmine – structure générale et interactions

- Dogme de Dodge et Rahmaninoff (>3 Ca^{2+} ions)

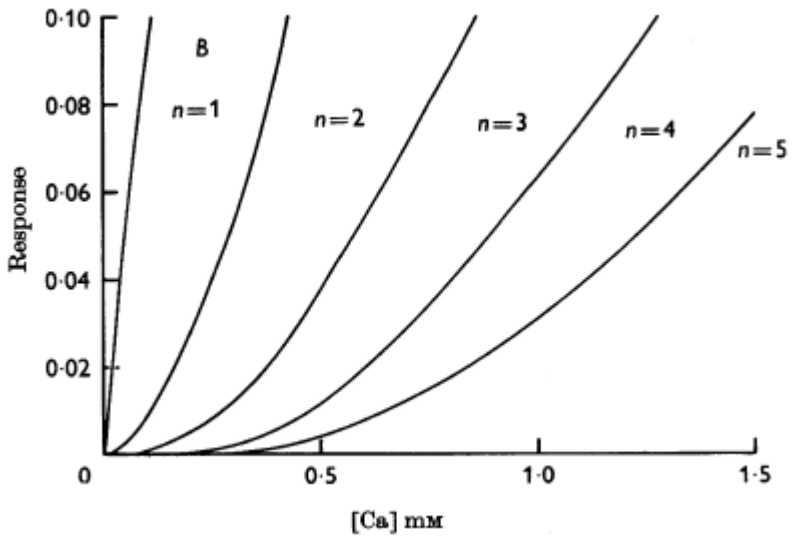
J. Physiol. (1967), **193**, *pp.* 419–432
With 6 *text-figures*
Printed in Great Britain

CO-OPERATIVE ACTION OF CALCIUM IONS IN TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION

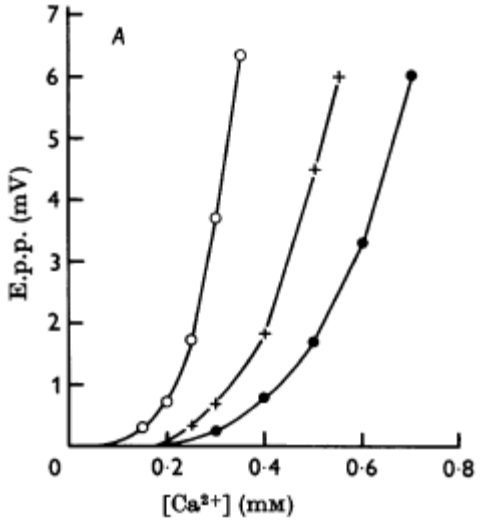
By F. A. DODGE Jr.* AND R. RAHAMIMOFF†
From the Department of Biophysics, University College London

(*Received* 6 *June* 1967)

prédiction



résultat



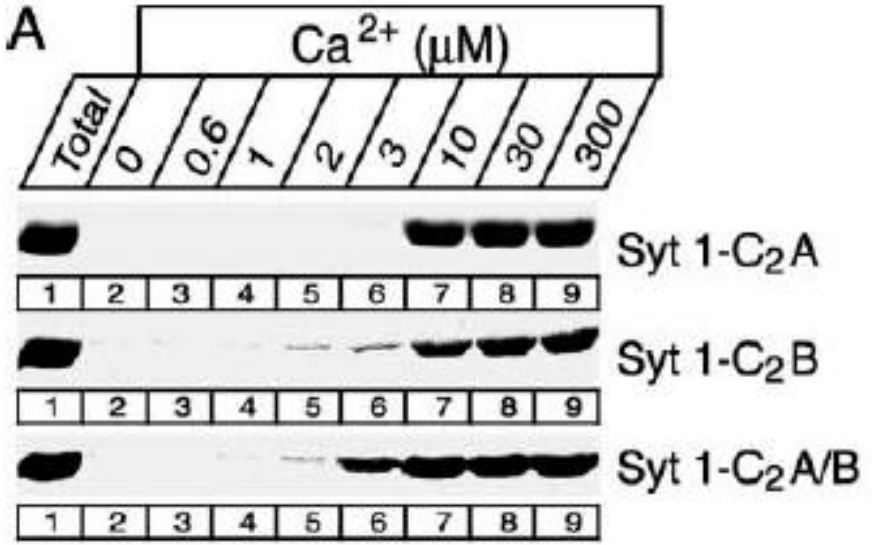
Plaque motrice; importance générale

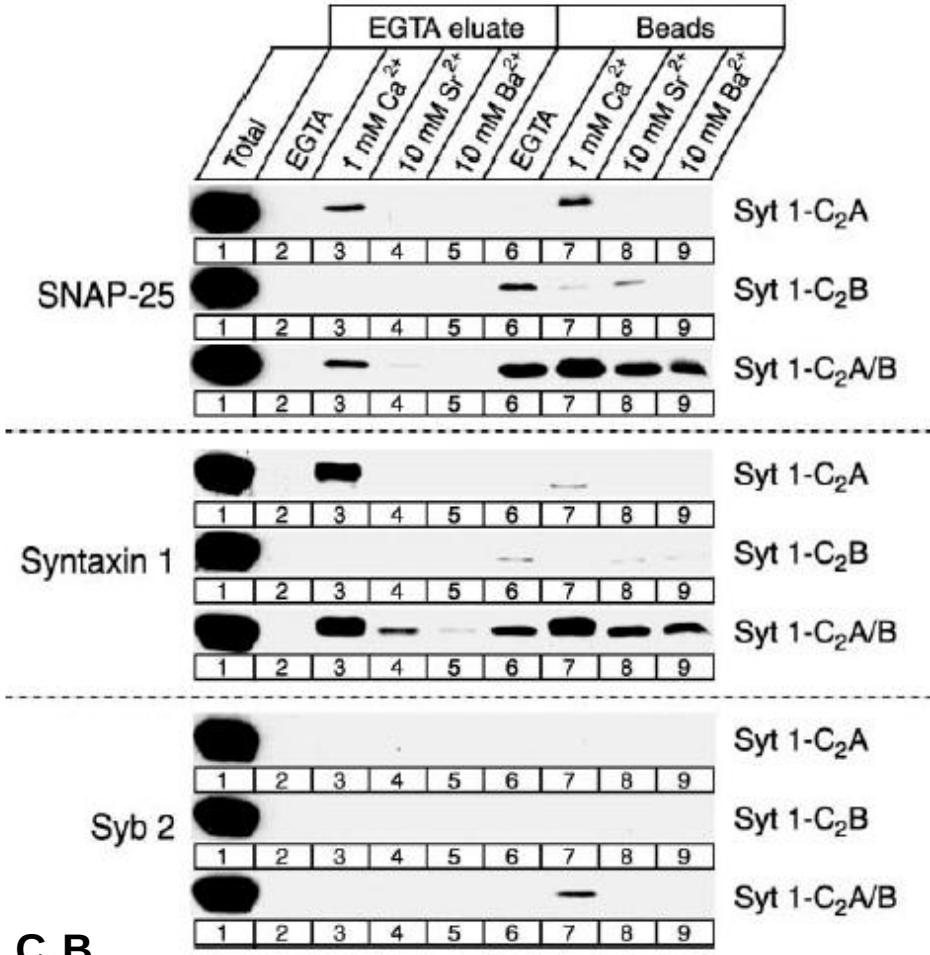
CALCIUM

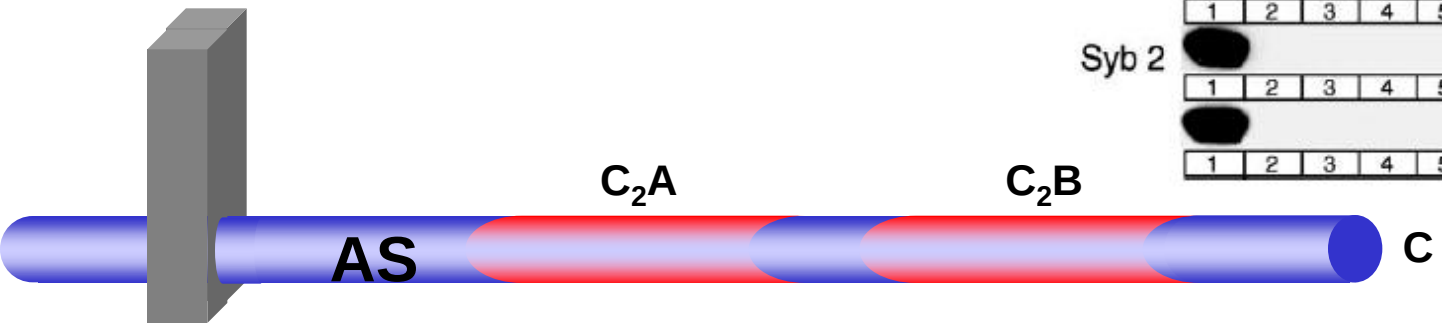
synaptotagmine – liaisons et coopérativité

phospholipides

SNARE







CALCIUM

structure générale des synaptotagmines et isoformes

Properties of synaptotagmins.

Protein	Class	Localization	Ca^{2+}-binding	Special properties	Function
Syt 1	A	Synaptic vesicles LDCVs	Yes	N-terminal *N*-glycosylation Phospholipids and SNARE binding	Ca^{2+}-sensor for fast exocytosis
Syt2	A	Synaptic vesicles LDCVs?	Yes	N-terminal *N*-glycosylation Phospholipid and SNARE binding	Ca^{2+}-sensor for fast exocytosis
Syt3	C	Plasma membrane	Yes	Disulfide bonds at N-terminus Phospholipid binding	Ca^{2+}-sensor for exocytosis?
Syt4	D	Postsynaptic?	No*	Aspartate to Serine substitution in C2A domain loops	Unknown*; not essential for survival
Syt5	C	Plasma membrane	Yes	Disulfide bonds at N-terminus Phospholipid bindings	Ca^{2+}-sensor for exocytosis?
Syt6	C	Plasma membrane	Yes	Disulfide bonds at N-terminus Phospholipid binding	Ca^{2+}-sensor for exocytosis?
Syt7	B	LDCVs Synapses?	Yes	Multiple splicing isoforms Phospholipid and SNARE binding	Ca^{2+}-sensor for LDCV exocytosis
Syt8	F	Primarily GIT tract	No	Only Syt isoform that is expressed at highest levels outside of brain	Unknown
Syt9	A	Synaptic vesicles	Yes	Phospholipid and SNARE binding	Ca^{2+}-sensor for fast exocytosis
Syt10	C	Plasma membrane	Yes	Disulfide bonds at N-terminus Phospholipid binding	Ca^{2+}-sensor for exocytosis?
Syt11	D	Unknown	No	Similar to Syt4, but more abundant	Unknown
Syt12	F	Synaptic vesicles	No	Phosphorylated by PKA	May regulate miniature release
Syt13	F	Unknown	No		Unknown
Syt14	E	Unknown	No	Syt14-16 form a group of related and evolutionarily conserved isoforms	Unknown
Syt15	E	Unknown	No	Syt14-16 form a group of related and evolutionarily conserved isoforms	Unknown
Syt16	E	Unknown	No	Syt14-16 form a group of related and evolutionarily conserved isoforms	Unknown

Impliqué en sécrétion

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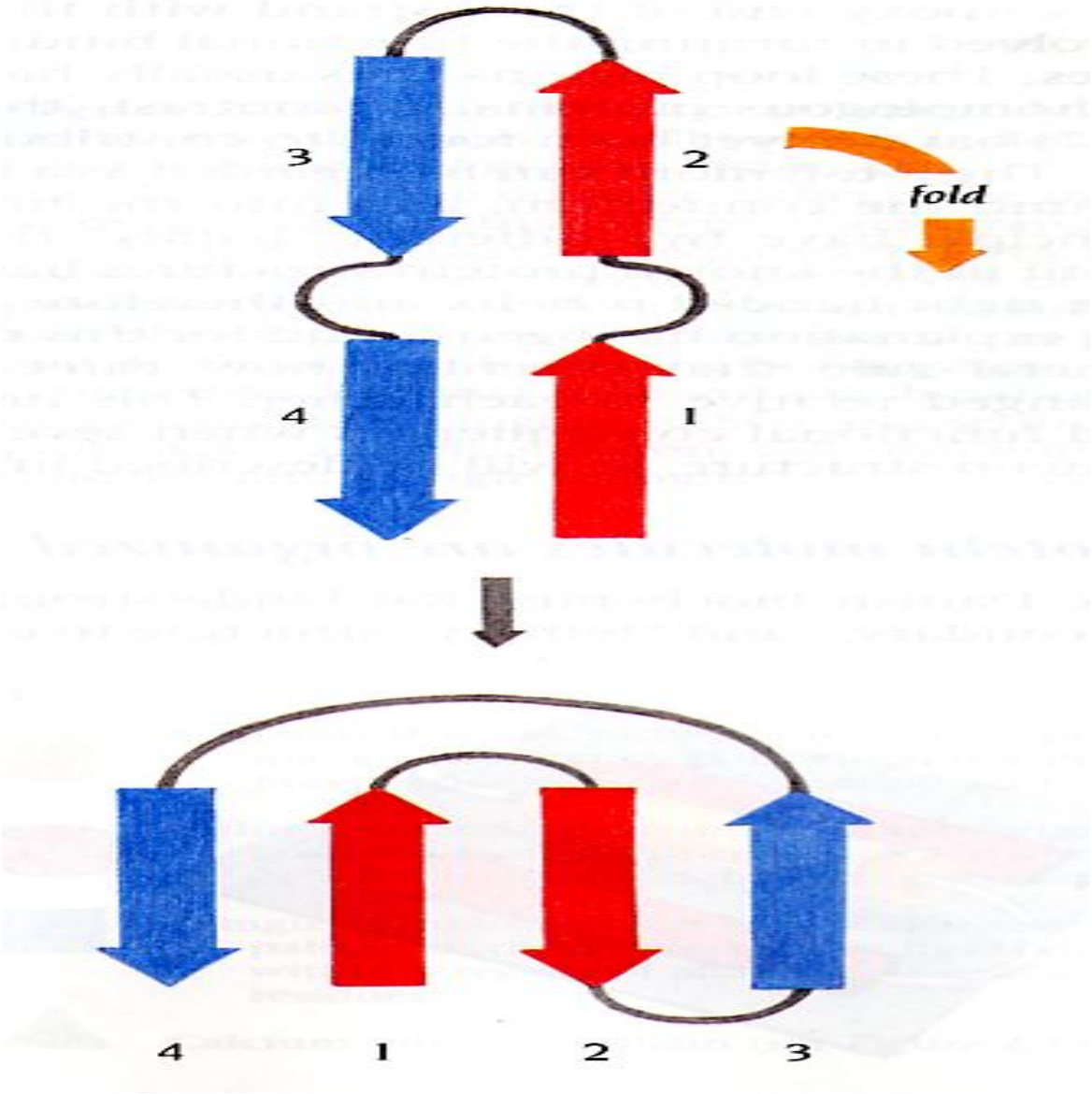
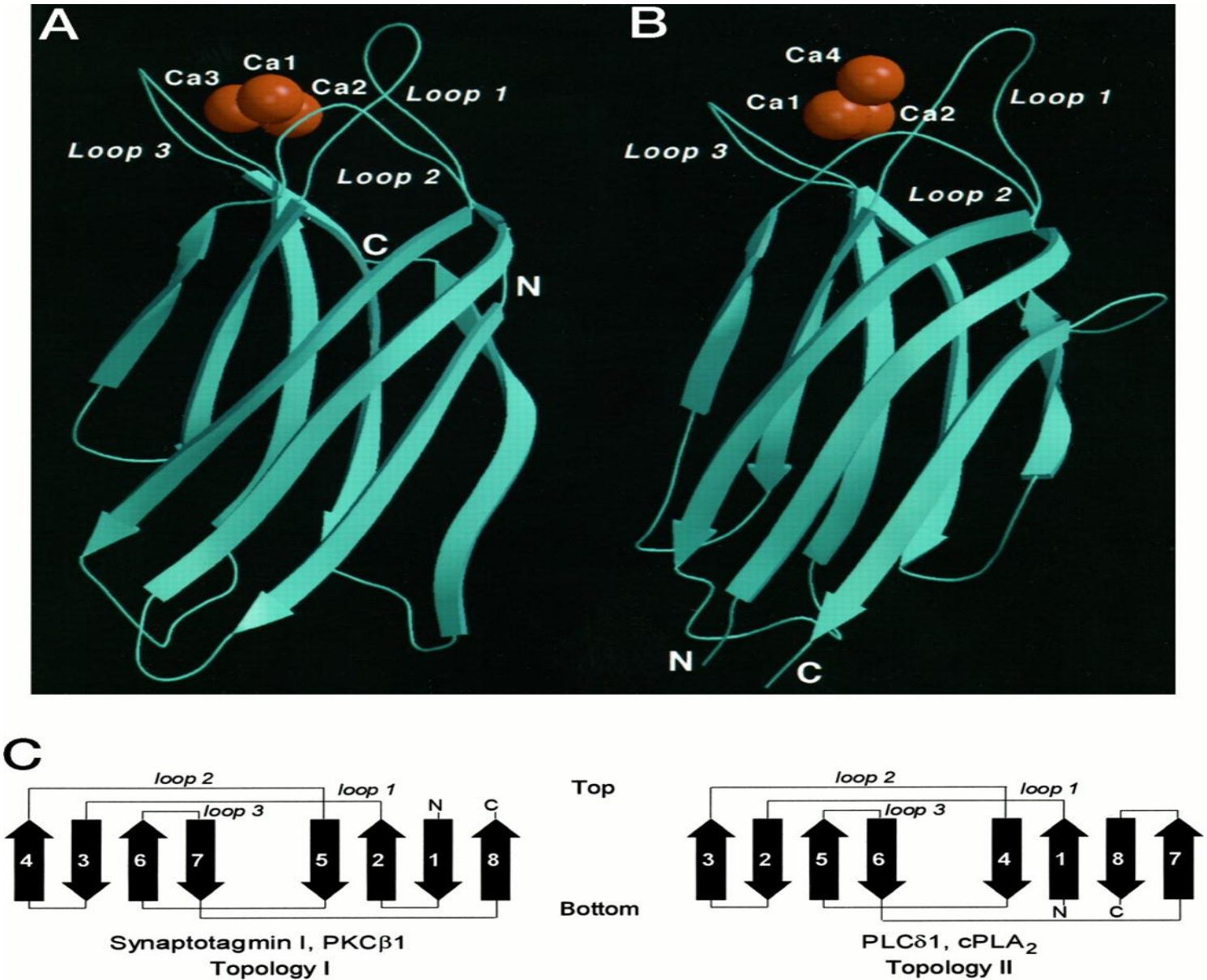
CALCIUM

synaptotagmine structure- la clé grecque



CALCIUM

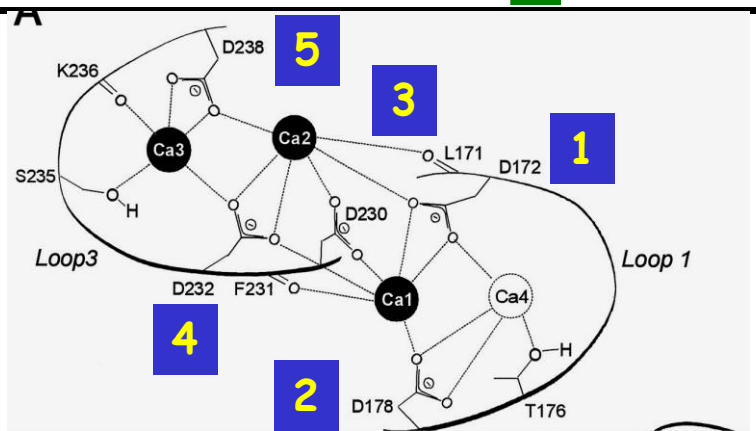
synaptotagmine – les feuillets en sandwiche des C2



CALCIUM

synaptotagmines – coordination calcium et interactions

	C2A					C2B						CaPL	SYX
	1	2	3	4	5		1	2	3	4	5			
I	D	D	D	D	D	I	D	D	D	D	D	I	Y	Y
II	D	D	D	D	D	II	D	D	D	D	E	II	Y	Y
III	D	D	D	D	D	III	D	D	D	D	E	III	Y	*Y*
IV	D	D	S	D	D	IV	D	D	D	E	E	IV	N/Y	N
V	D	D	D	D	D	V	D	D	D	D	E	V	Y	Y
VI	D	D	D	D	D	VI	D	D	D	D	E	VI	N	N
VII	D	D	D	K	D	VII	D	D	D	D	D	VII	Y	*Y*
VIII	L	D	D	D	E	VIII		E	A	G	E	VIII	N	N
IX	D	D	D	D	D	IX	D	D	D	D	E	IX	ND	ND
X	D	D	D	D	D	X	D	D	D	D	E	X	ND	ND
XI	D	D	D	S	D	XI	D	N	D	D	E	XI	N	ND
srg/XII	E	S	D	D	V	srg/XII	D	D	E	S	D	srg/XII	ND	ND
XIII	D	D	D	T	S	XIII	K	D	G	G	E	XIII	ND	ND



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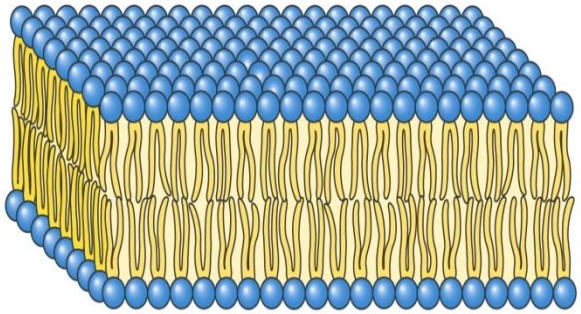
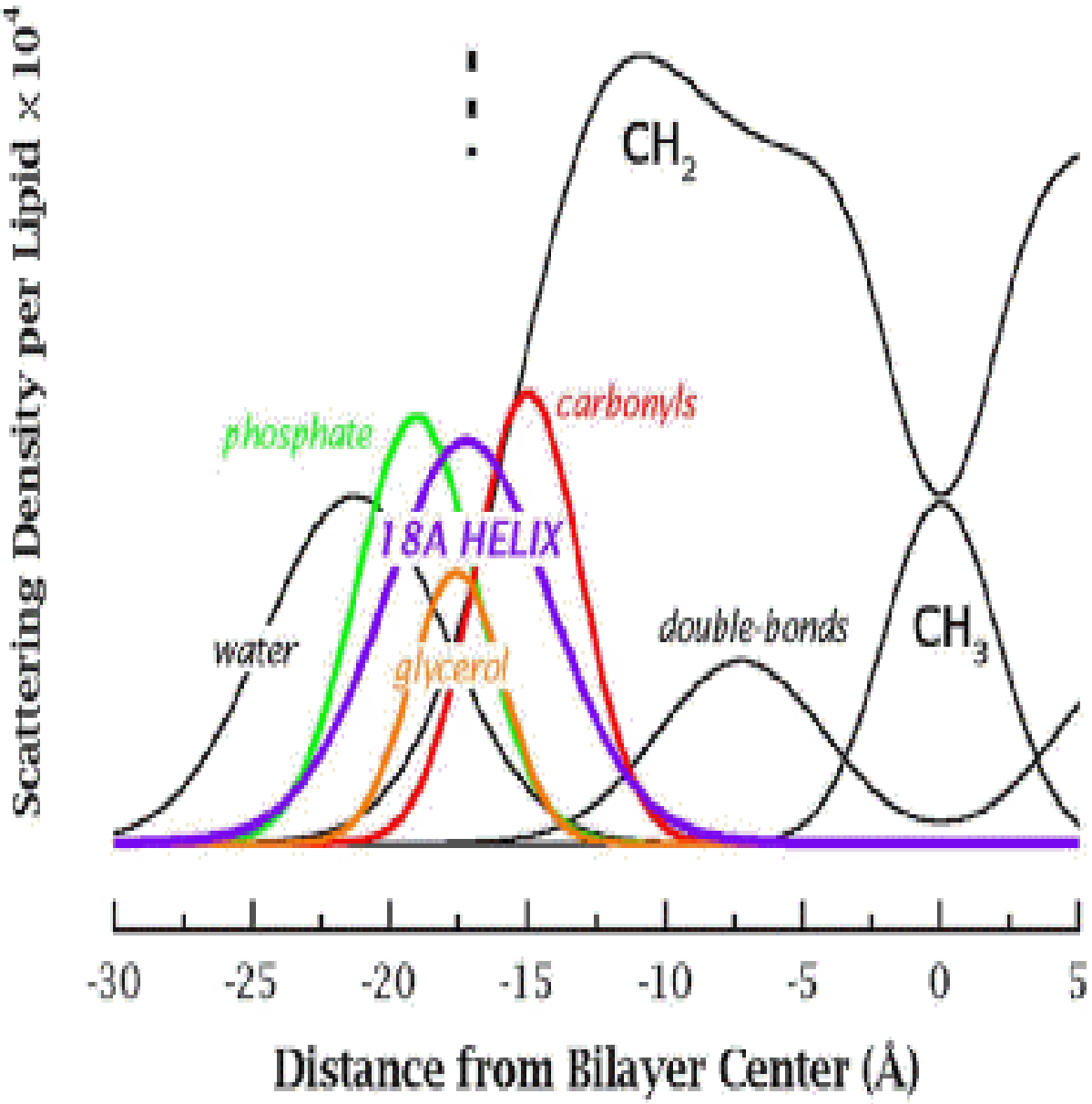
CALCIUM

la bicouche lipidique I – l'interface



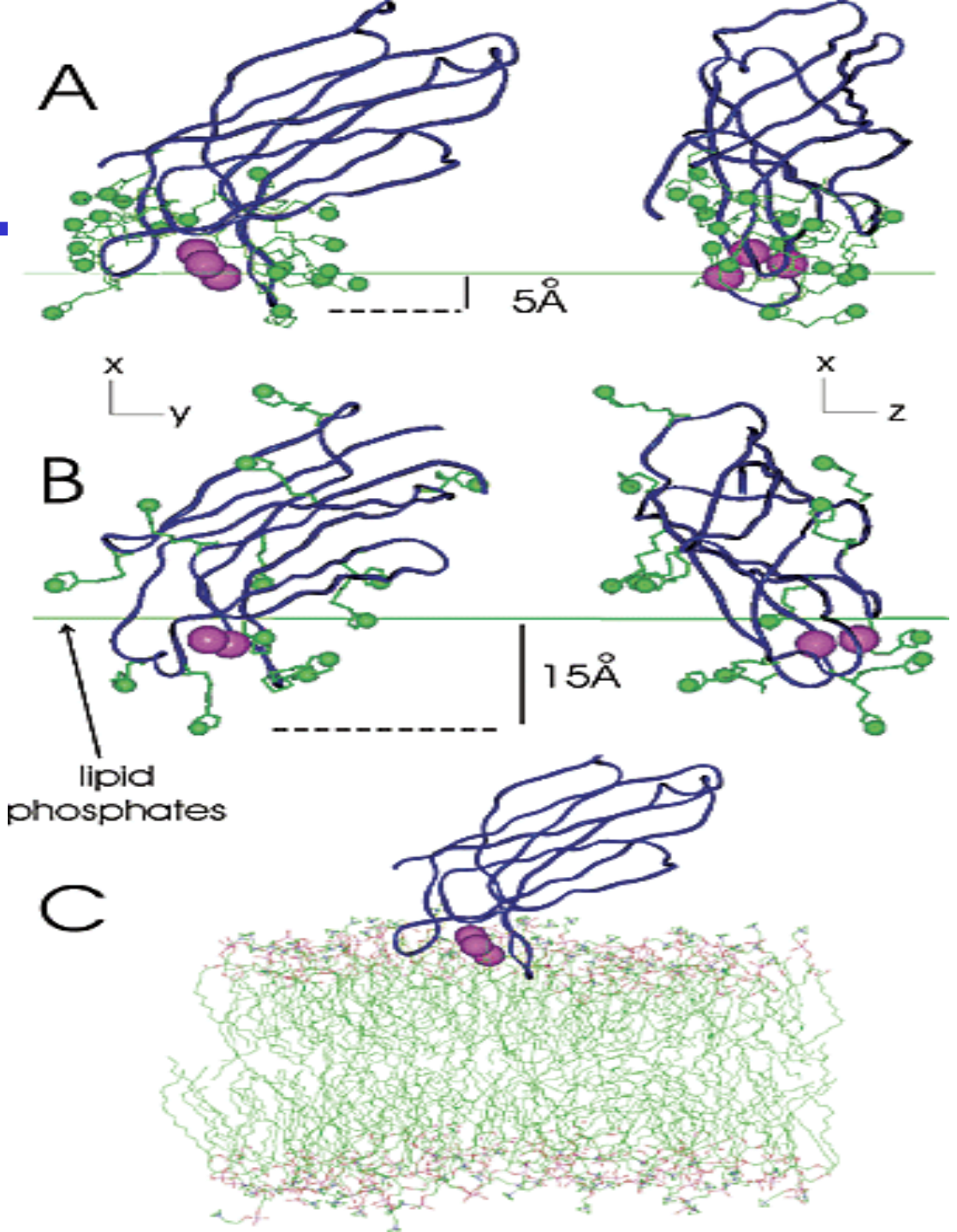


CALCIUM
synaptotagmine – membranes lipidiques

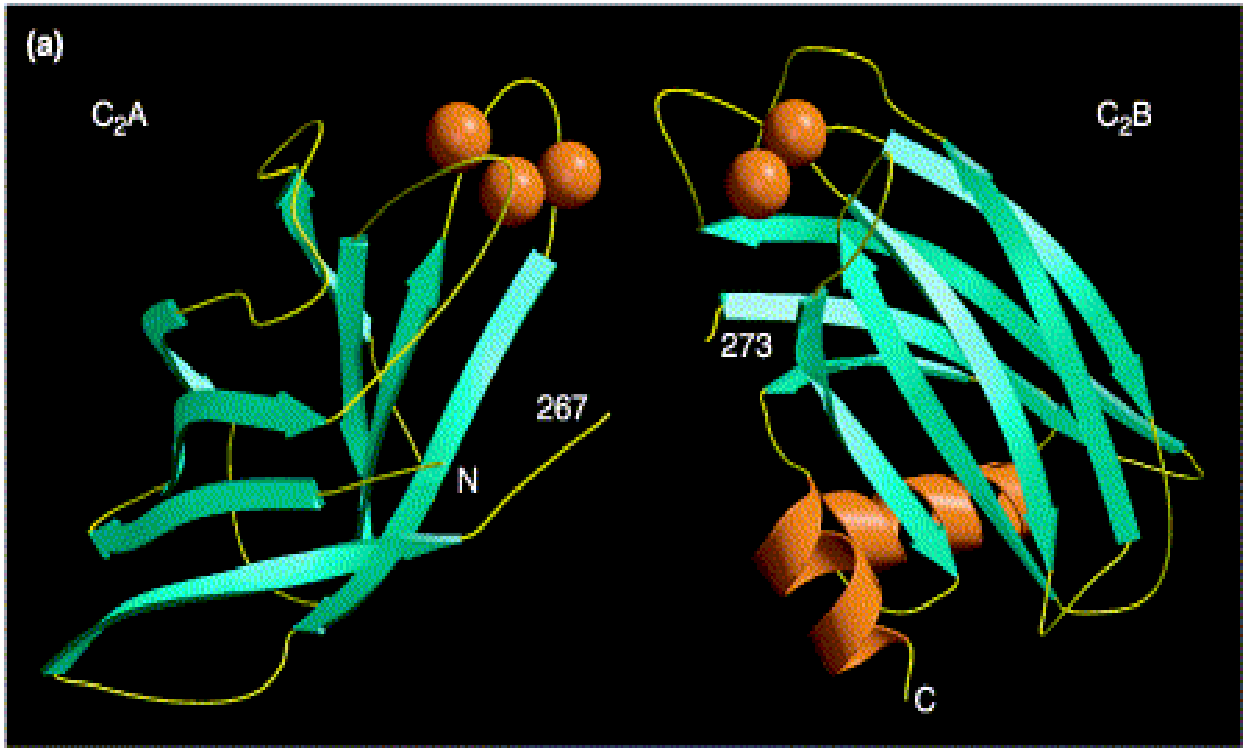
Insertion dans l'interface

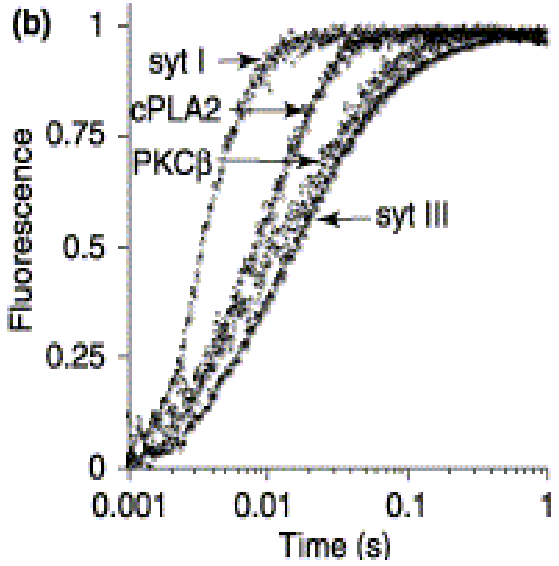
Interaction avec PL

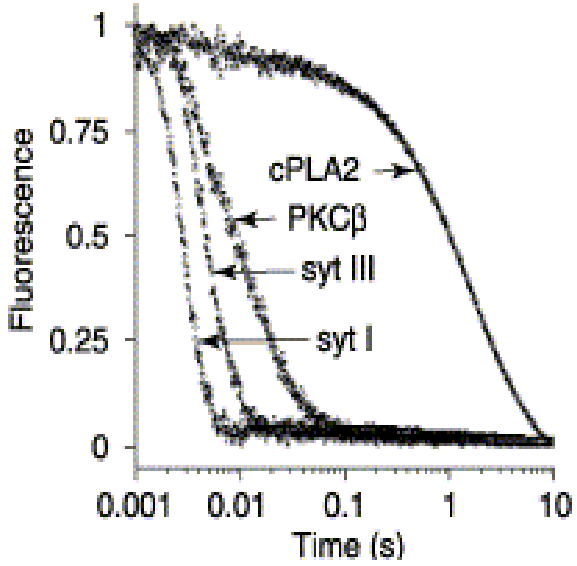
Déstabilisation?
Accrochage?



Vitesse d'association





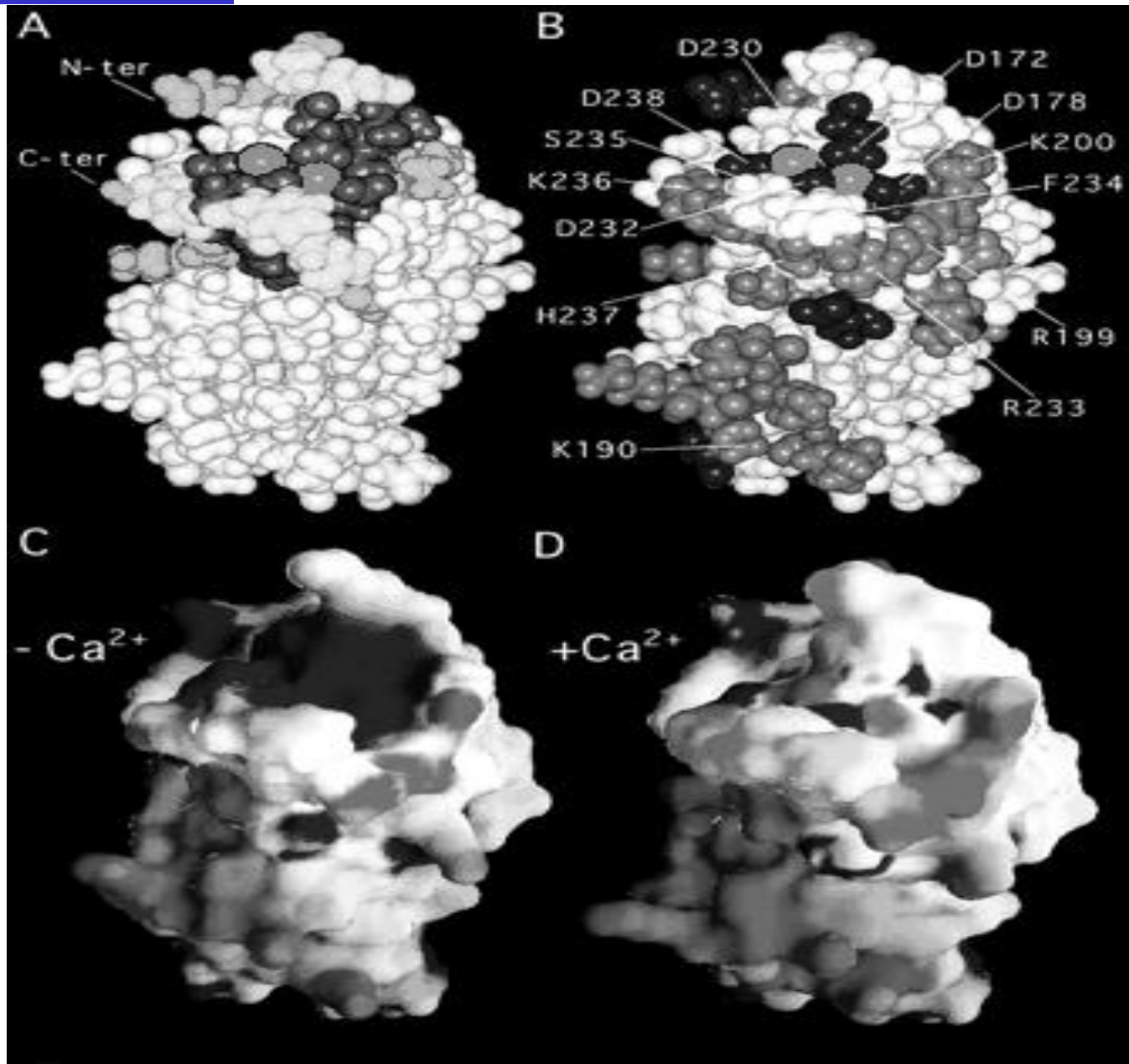


CALCIUM

synaptotagmine – potentiel de surface

Changement Conformation? Vitesse?

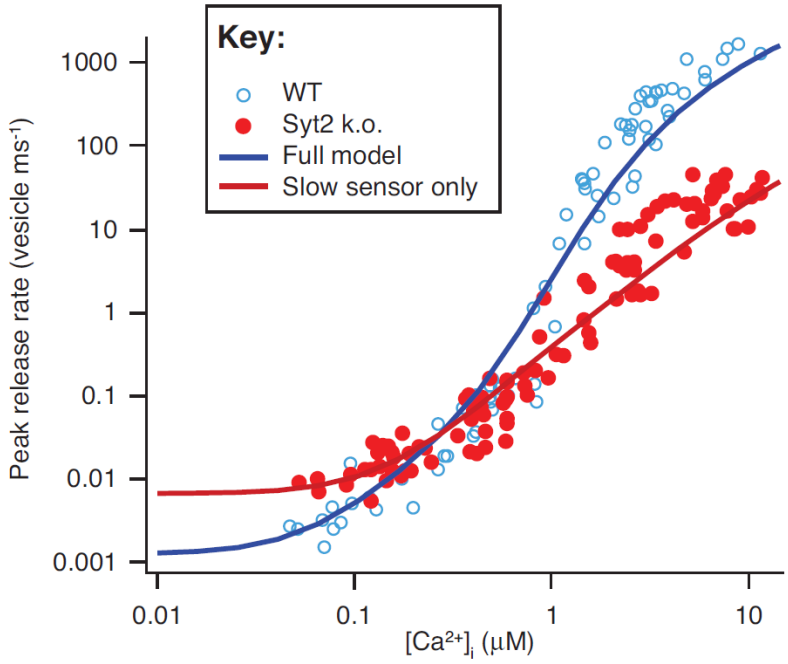
Changement Charges surface



CALCIUM

synaptotagmine – pas le seul senseur calcique

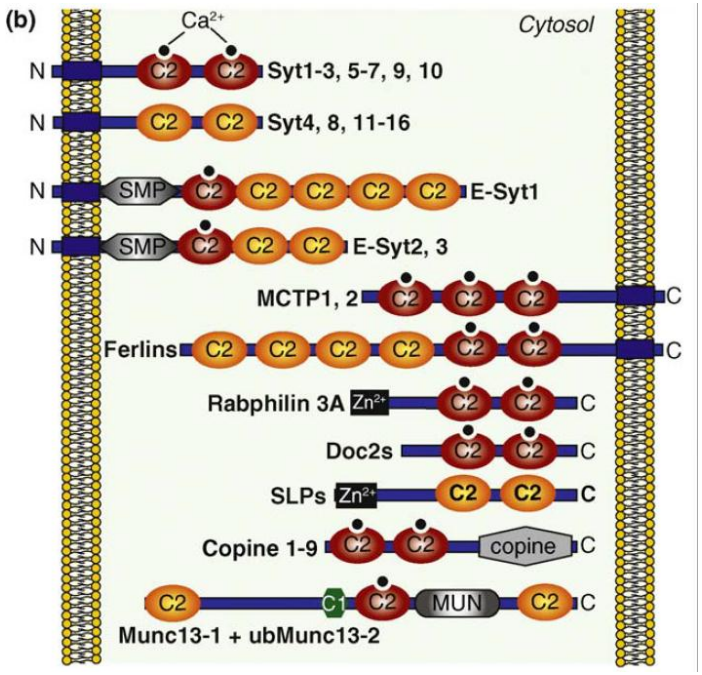
Exemple neurotransmission



Control of exocytosis by synaptotagmins and otoferlin in auditory hair cells
Beurg M, Michalski N, Safieddine S, Bouleau Y, Schneggenburger R, Chapman ER, Petit C, Dulon D.
J Neurosci. 2010;30(40):13281-90.

Otoferlin is a calcium sensor that directly regulates SNARE-mediated membrane fusion.
Johnson CP, Chapman ER.
J Cell Biol. 2010;191(1):187-97.

Doc2 is a Ca^{2+} sensor required for asynchronous neurotransmitter release.
Yao J, Gaffaney JD, Kwon SE, Chapman ER.
Cell. 2011;147(3):666-77.



Ferlins:
6 C2-domains, C-terminal transmembrane region;
canonical Ca^{2+}-binding sequences.
Ferlin sperm exocytosis in C. elegans
Dysferlin Ca^{2+}-dependent exocytosis of repair vesicles in muscle
Otoferlin required for Ca^{2+}-dependent exocytosis in hair cells

MCTPs (multiple C2-domain transmembrane proteins)
E-Syts (extended synaptotagmins)
Synaptotagmin-like proteins (SLPs) (DOC2)
Copines are soluble double C2-domain proteins

CONTENU

Calcium – propriété, mesures, distribution et concentrations

Dépendance calcique des voies de transport

Senseurs calciques – calmoduline

Senseurs calciques – synaptotagmine

propriété biochimique et interactions

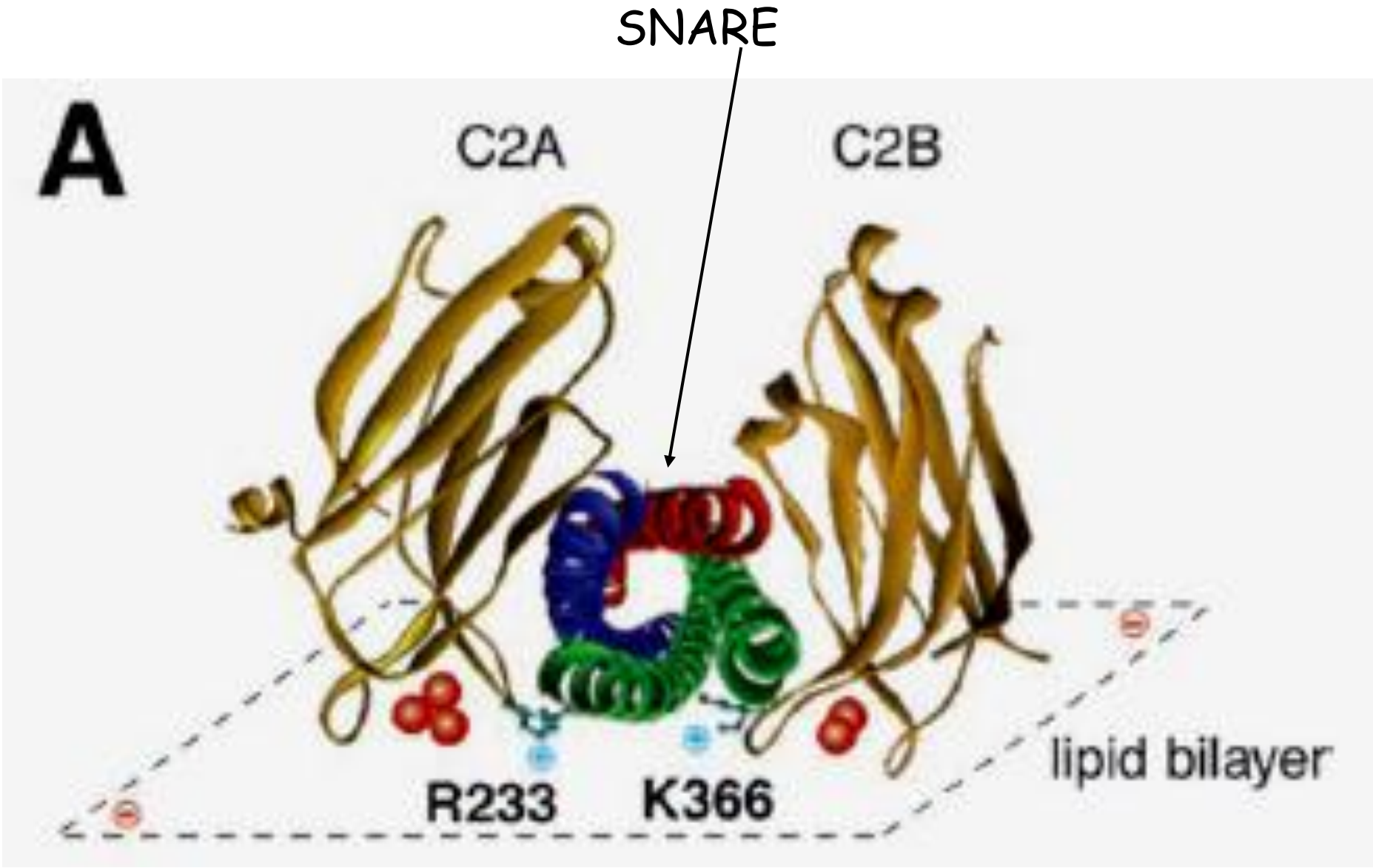
structure

interaction avec membranes

<u>fonction</u>

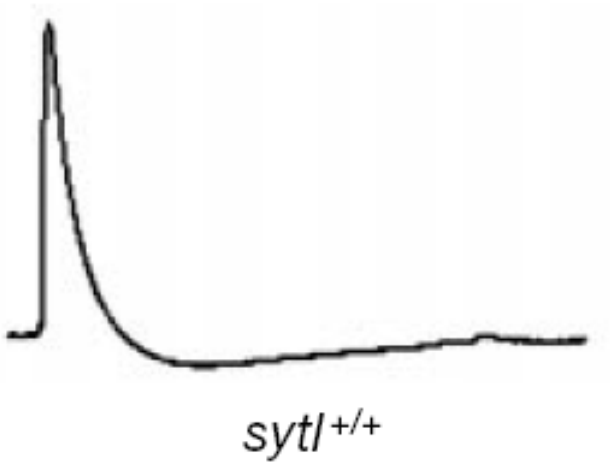
CALCIUM

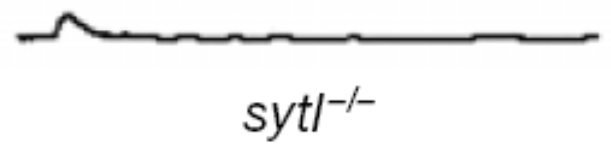
synaptotagmine – potentiel de surface



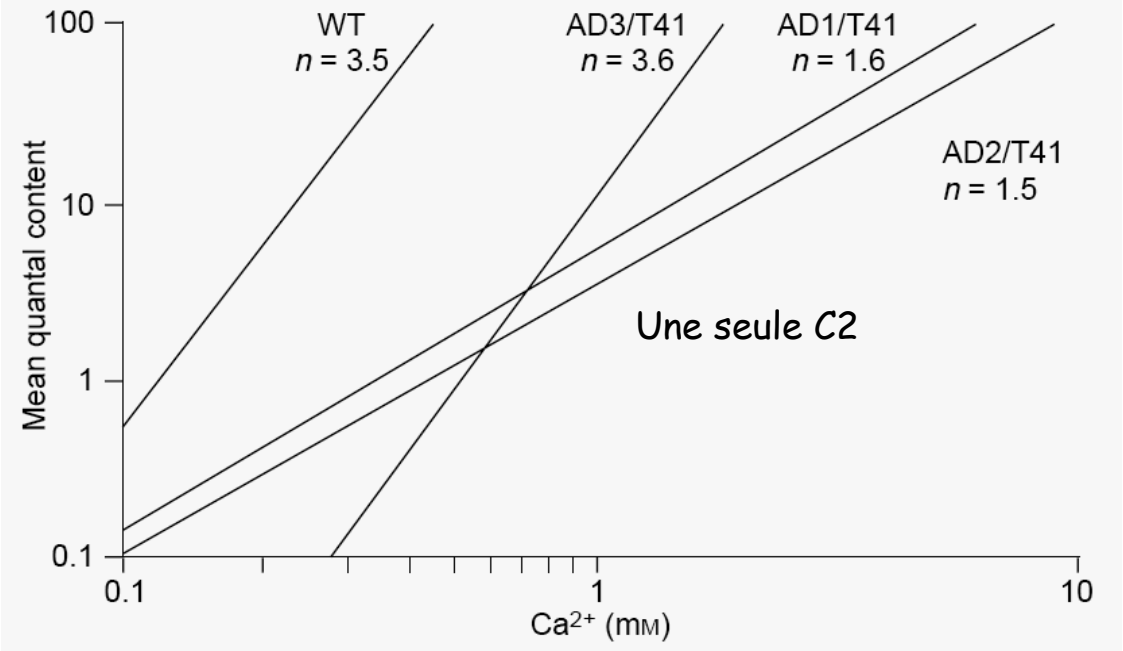
CALCIUM *synaptotagmine – les arguments clés*

Syt nécessaire à l'exocytose



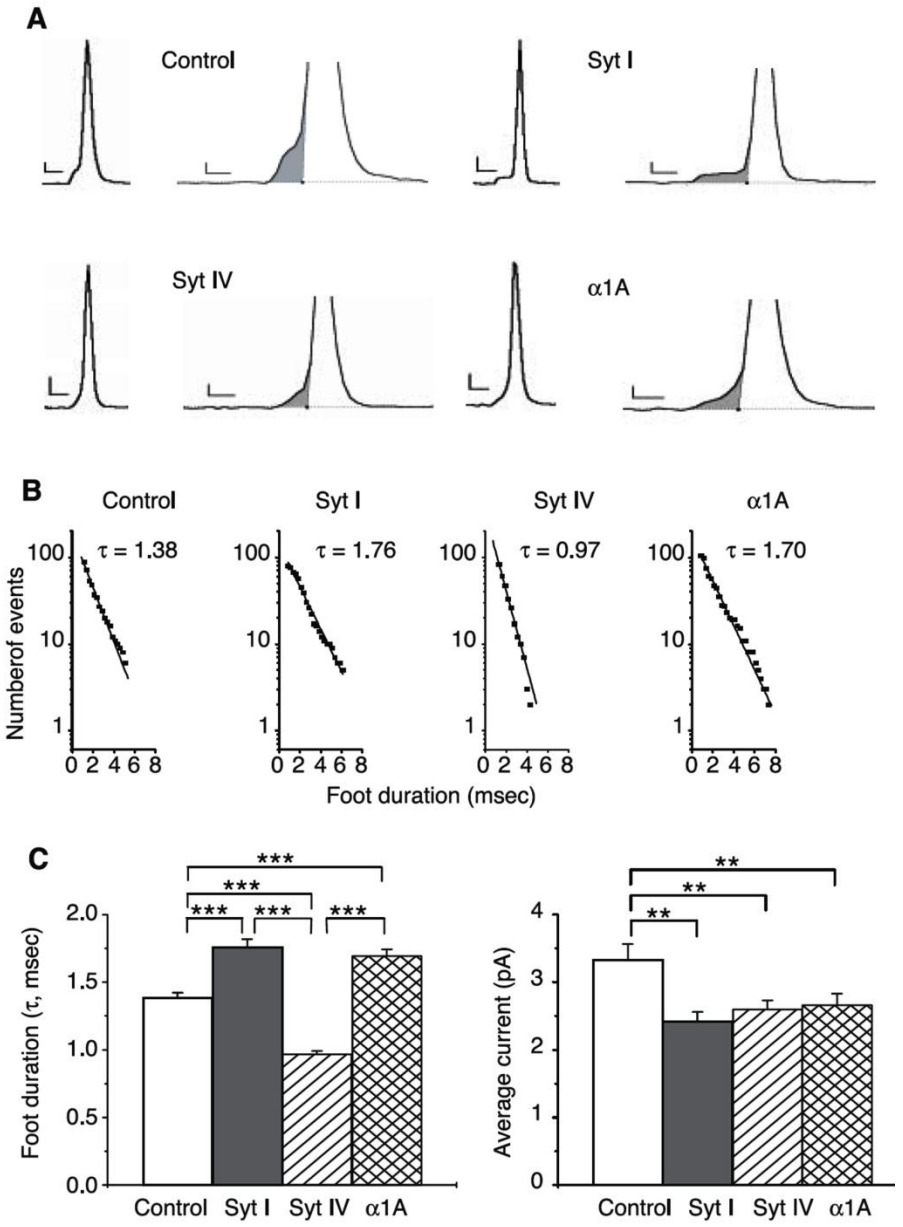


Les mutations changent la dépendance calcique

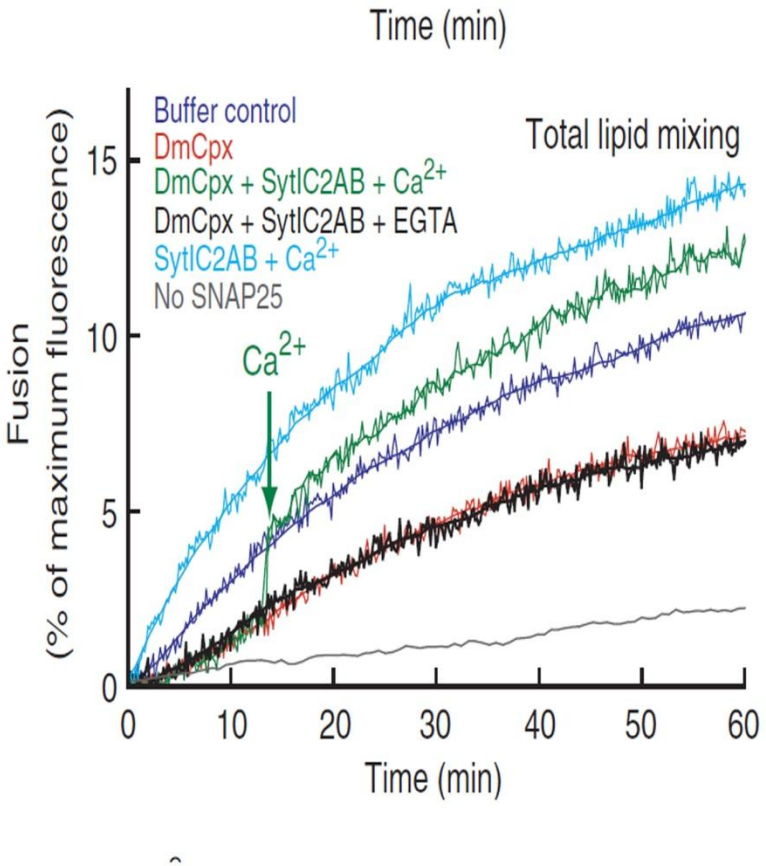


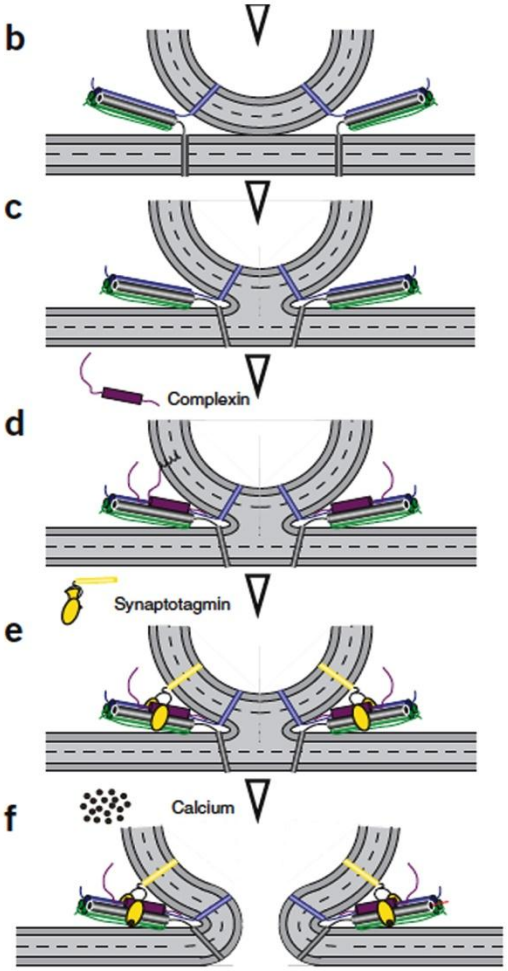
CALCIUM *synaptotagmine – pore de fusion?*

La surexpression de syts change
La durée du pore de fusion



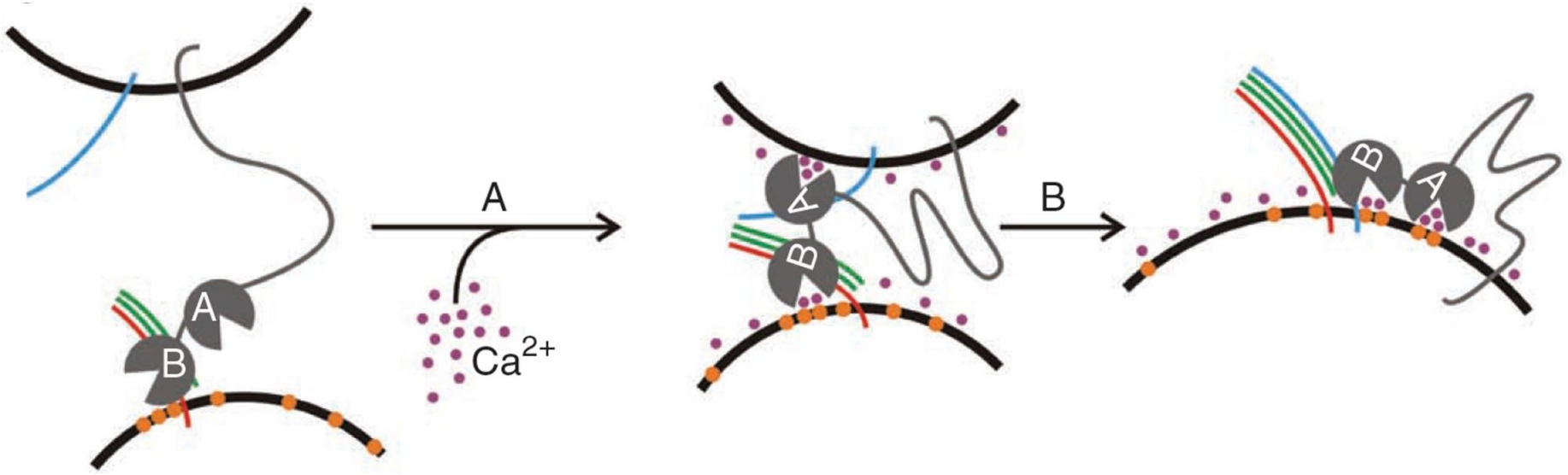
CALCIUM *synaptotagmine – hemifusion?*





Synaptotagmin: intervient après hemifusion
Enlève « clamp » compléxine

CALCIUM *synaptotagmine – mesure de distance?*



CALCIUM conclusion

Calcium nécessaire pour plusieurs étapes de sécrétion/transport (*neurones, cœur, muscle, endocrine, neutrophiles etc*)

Calcium agit aux niveau de protéines

Plusieurs senseurs calciques
Actuellement que Syt remplit le dogme de Dodge et Rahmaninoff

Fonction:
- Accélère fusion?
- Accélère fusion au delà de hémifusion?
- Mètre de distance?
- Ancrage de vésicule?
- Dérangement de bicouche?

Typiquement protéines « multi-domaines »