Ion channels, membrane potential and synaptic transmission: presynaptic mechanism II

离子通道 膜电势 突触传递:突触前机制 II

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2015.10.29

Outlines

- 1. review: AP generation-> transmitter release -> postsynaptic integration (AP: frequency coding)
- 2. synaptic transmission – presynaptic mechanism:

A) Calcium triggered transmitter release

Block it → Measure it → See it → Move it

B) Molecular mechanism of transmitter release

1. 回顾:动作电位的产生-> 递质的释放-> 突触后整合（动作电位:频率编码）
2. 突触传递-突触前机制:

A) 钙离子激活神经递质的释放
封闭→测量→观察→清除

B) 神经递质释放的分子机制

Neurotransmission

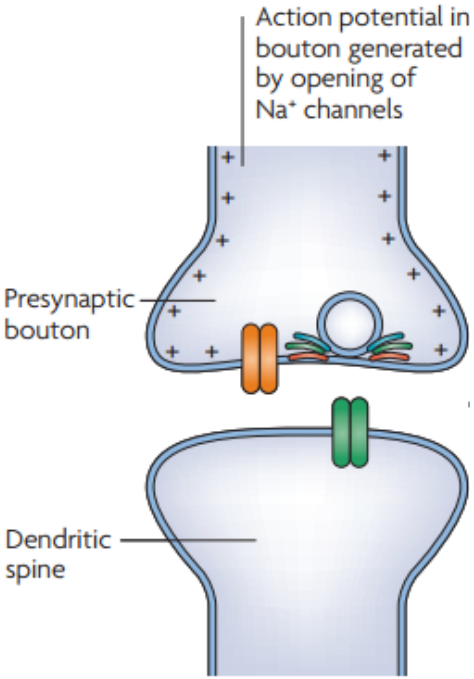
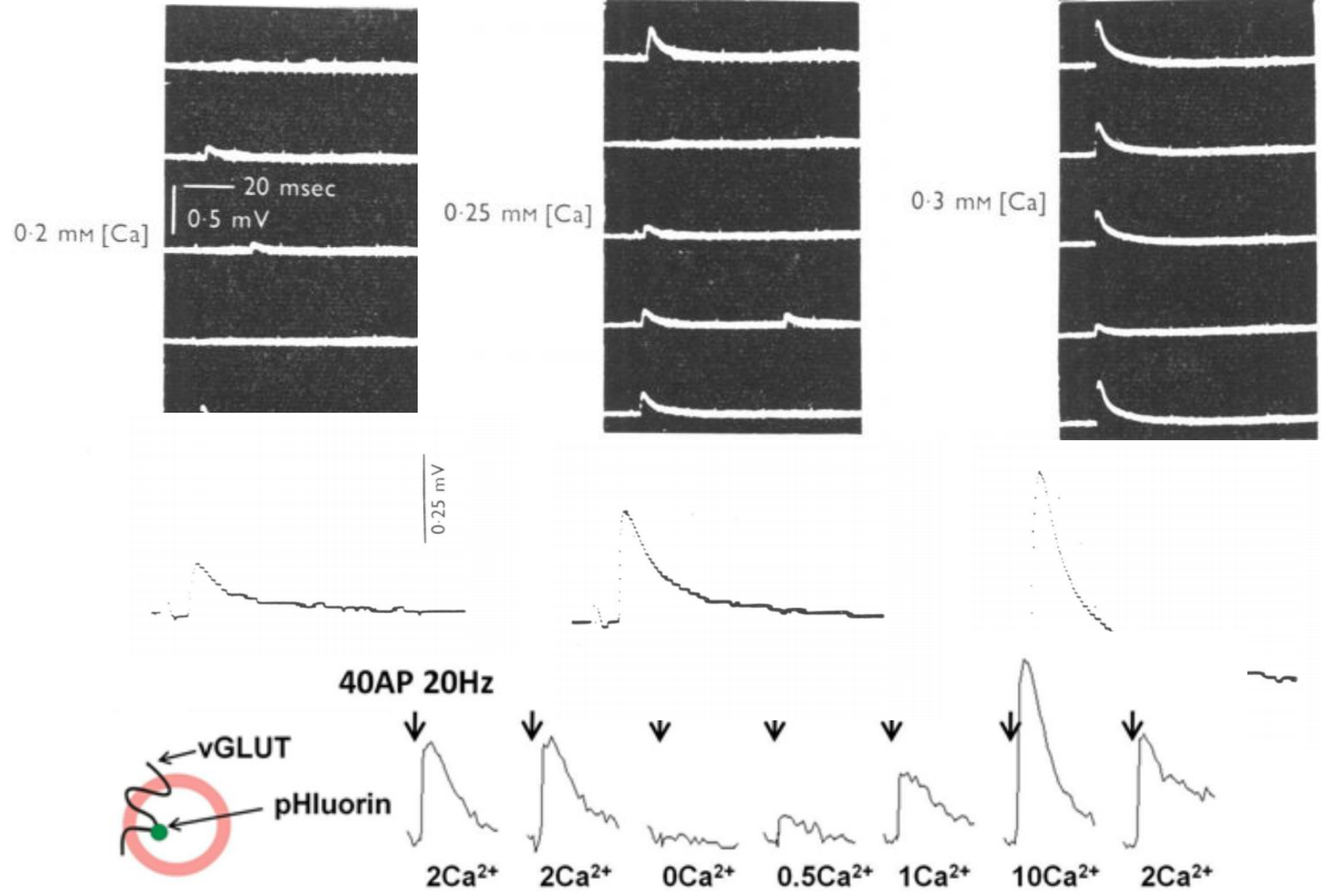
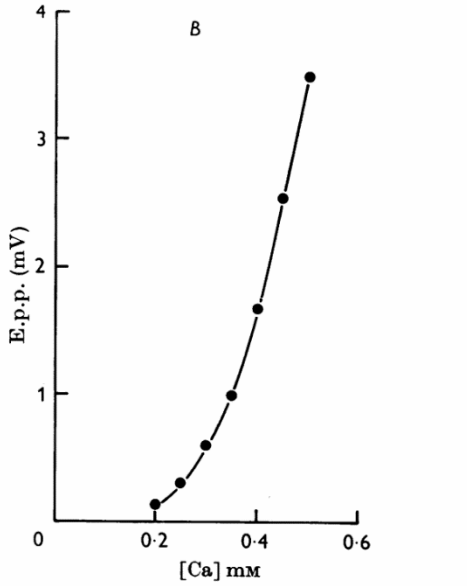


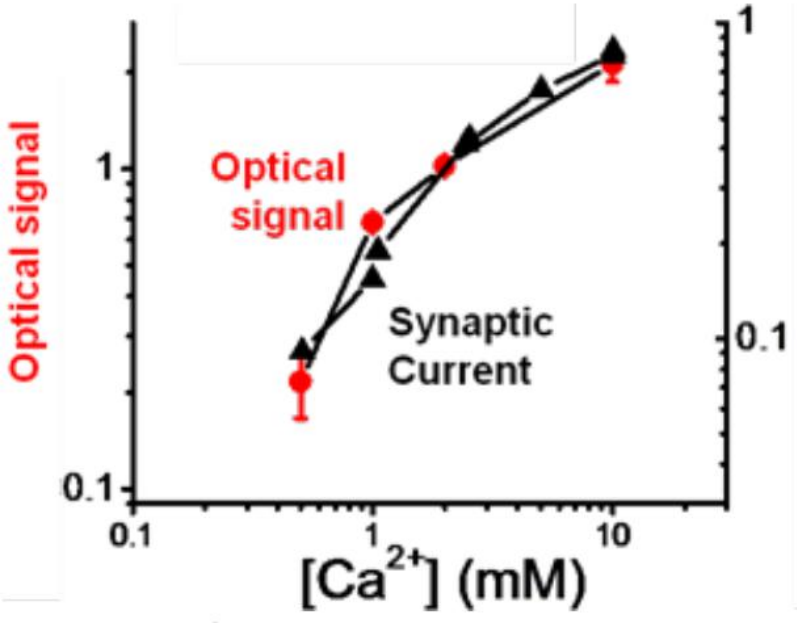
Figure 1 | **Steps in the process of chemical synaptic transmission.** These steps occur in both vertebrates and invertebrates, at the neuromuscular junction and central synapses. Cartoons based on a drawing by J. A. Ernst and A. Brunger.

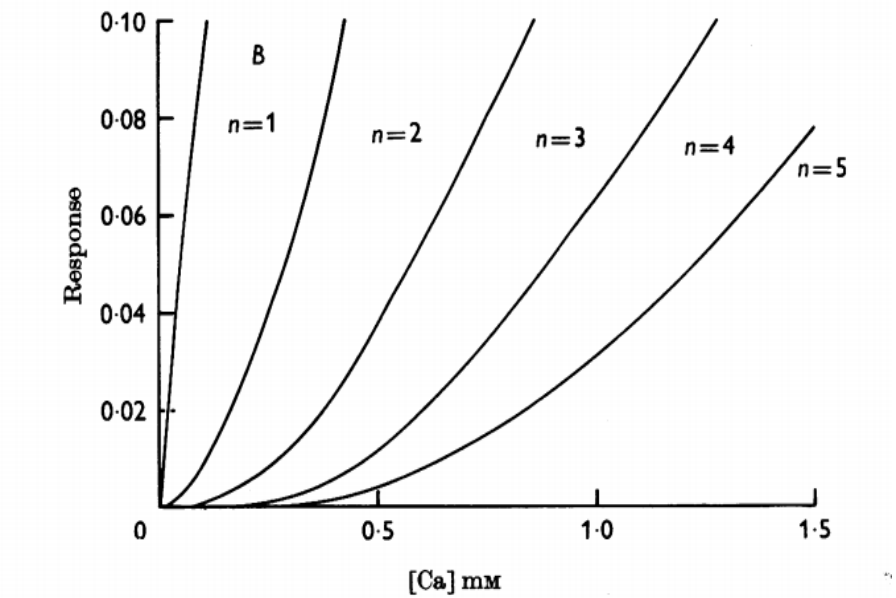
The sequence of events that underlie quantal transmission at central glutamatergic synapses. Nat Rev Neurosci. 2007 Aug



Dodge, F A, & Rahamimoff, R. Physiol. (Lond.) 1967







Dodge, F A, FA & Rahamimoff, R, R. Physiol. (Lond.) 1967

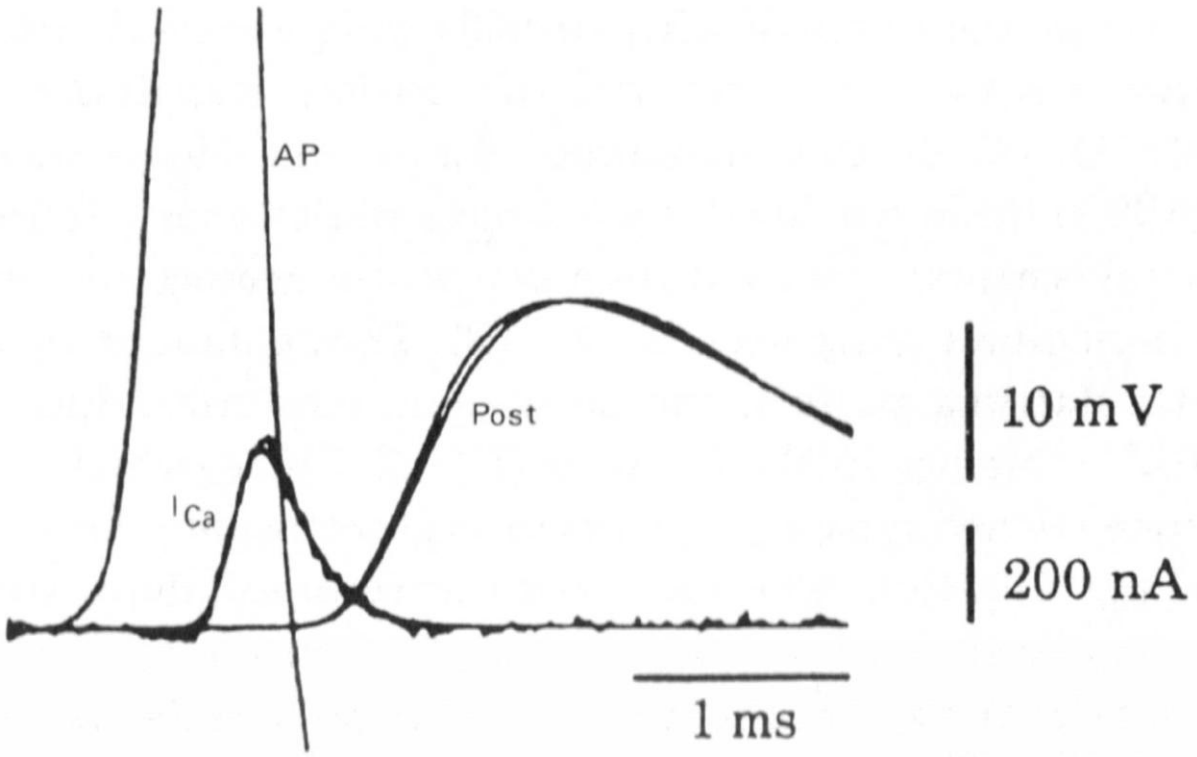


Fig. 2.10. Calcium currents (I_{Ca}) generated in a presynaptic terminal of the squid giant synapse by a replicated action potential. The presynaptic terminal was voltage-clamped with a waveform identical to the normal presynaptic action potential (AP). Post, synaptic potential simultaneously recorded from the postsynaptic axon. (From Llinás *et al.* 1982.)

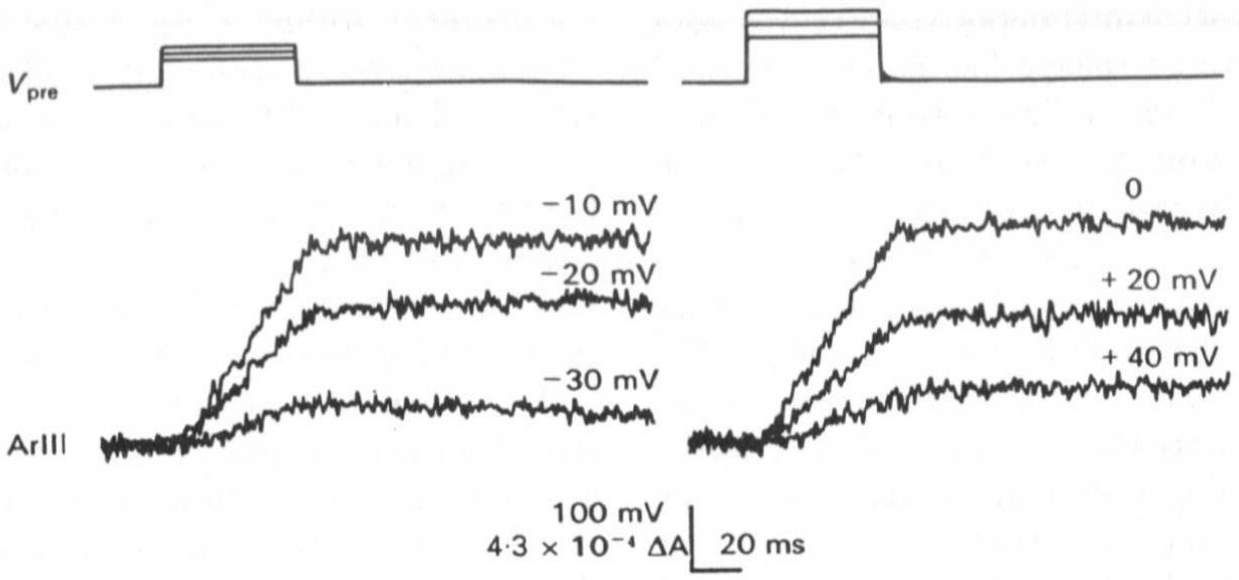
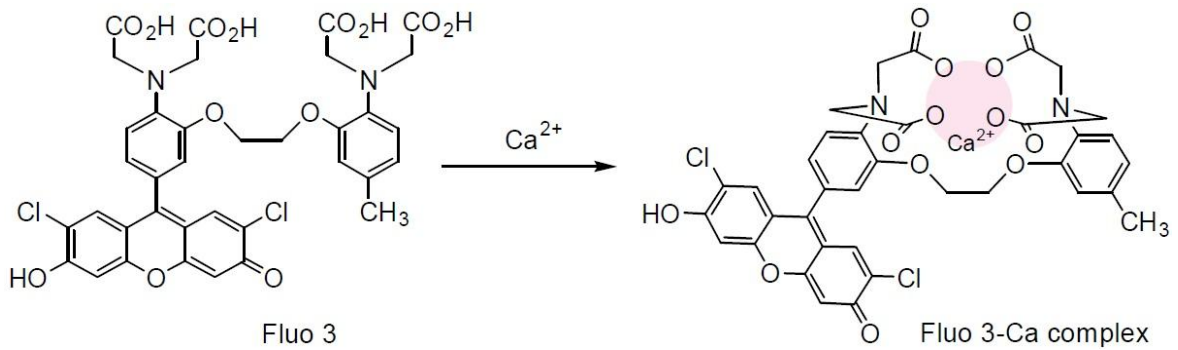
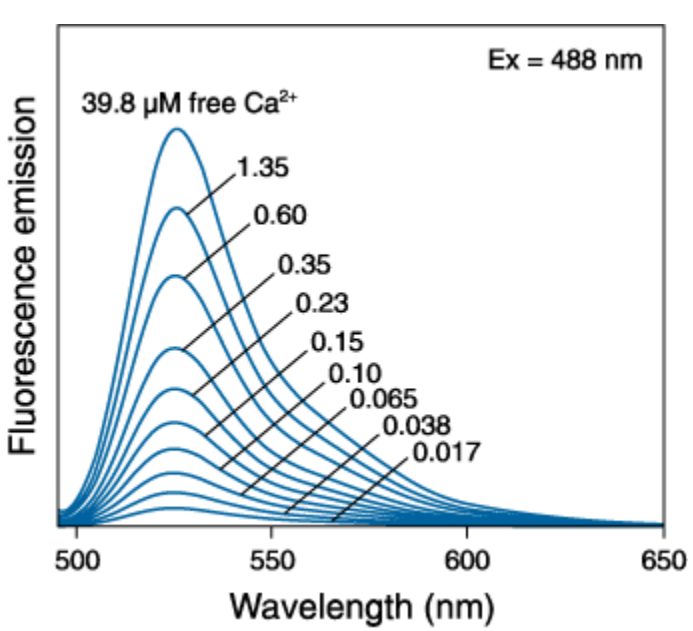


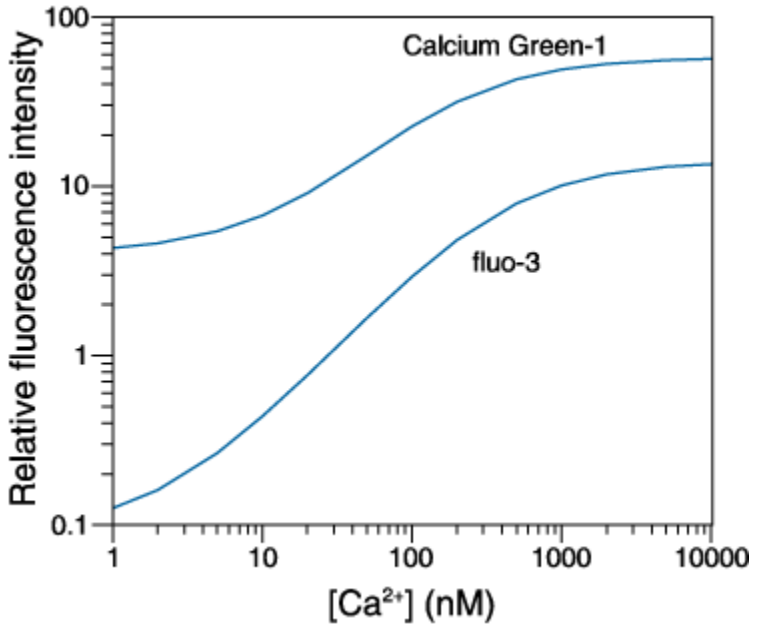
Fig. 2.4. Signals of arsenazo III (ArIII) detected in a presynaptic terminal of the squid giant synapse on depolarization of the presynaptic terminal. The presynaptic terminal was depolarized from a holding potential of −70 mV to varying levels indicated on each record under voltage clamp (V_{pre}). Note progressive increases in arsenazo III transients with stepwise depolarization to 0 mV, whereas the signals were reduced by further depolarization beyond 0 mV. (From Augustine *et al.* 1985.)

Typical Ca2+ dyes with intensity shift

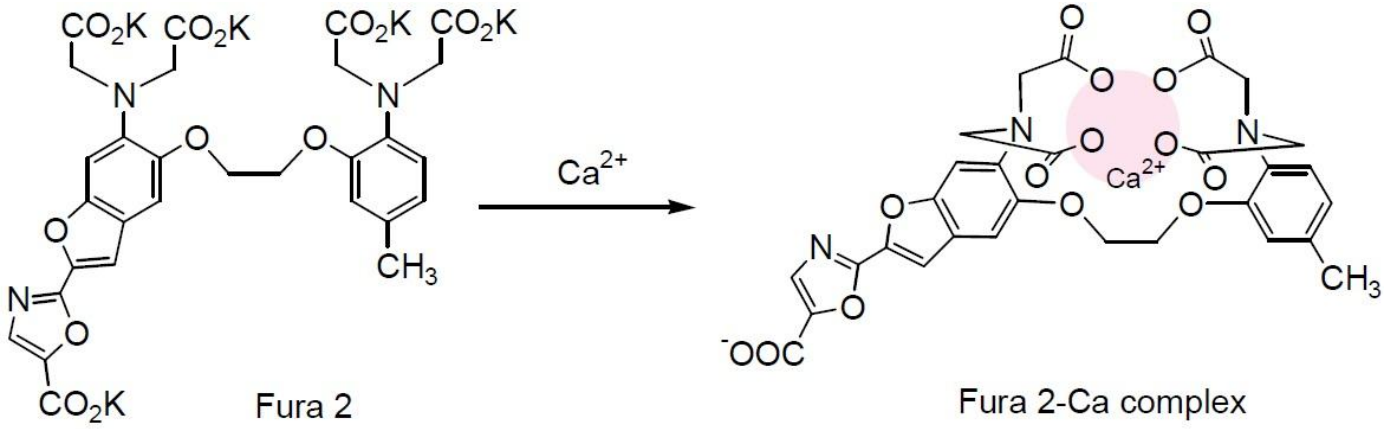
- Fluo-3, fluo-4





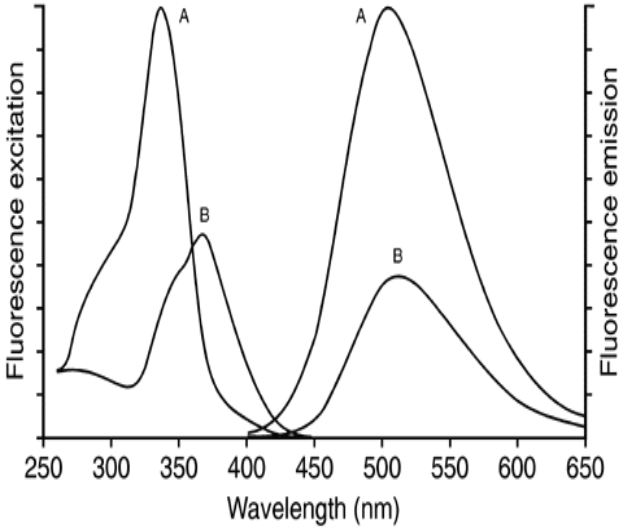


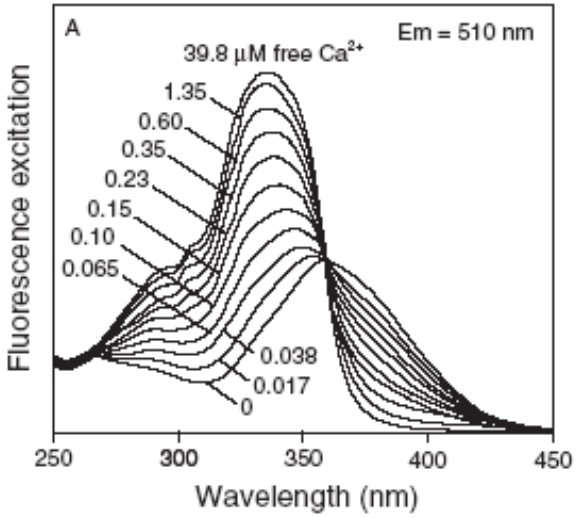
Fura-2



Fura 2

Fura 2-Ca complex





A: Ca2+ saturated B: Ca2+ free

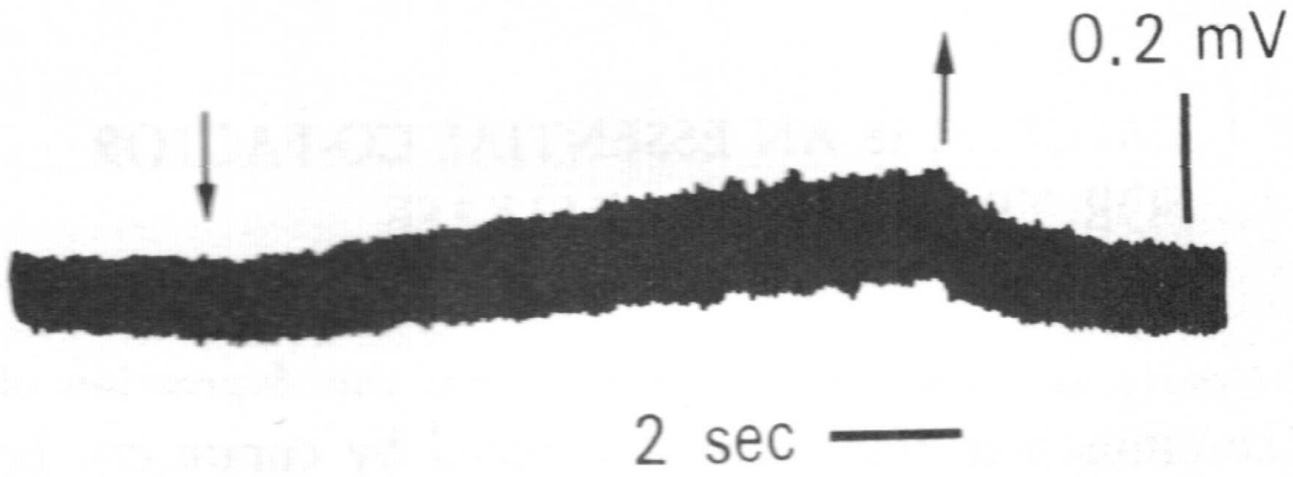
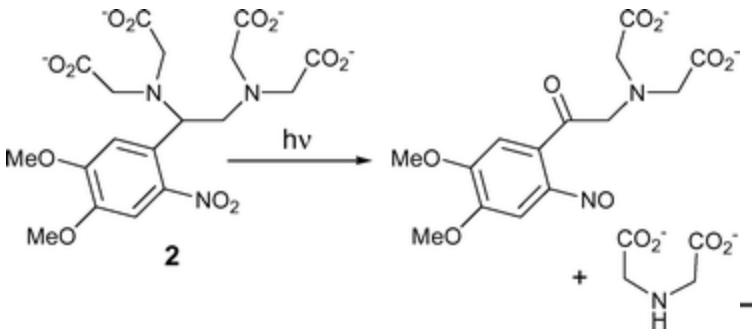
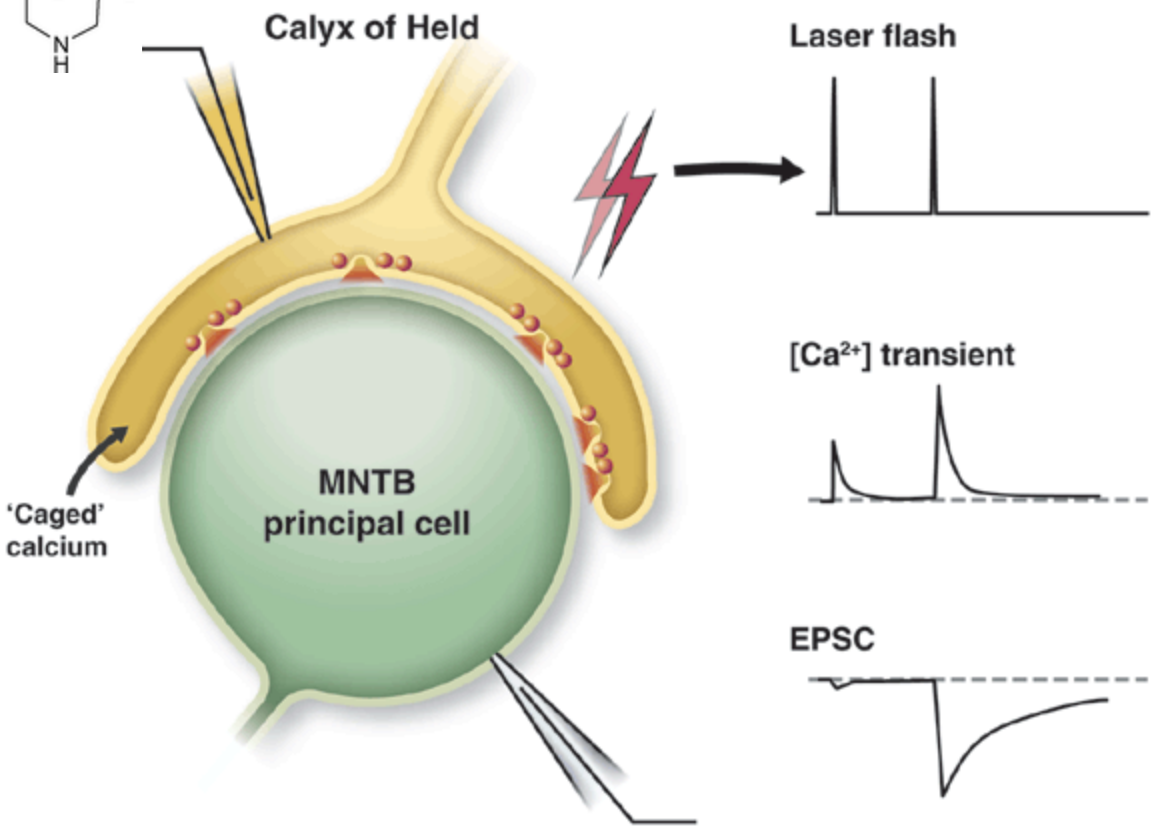


Fig. 2.3. A depolarizing response produced in the postsynaptic axon by injecting Ca^{2+} into the presynaptic axon in a squid giant synapse. The experimental procedure was similar to that illustrated in Fig. 2.2A, but the Ca^{2+}-filled pipette was inserted into the presynaptic axon, while recording the postsynaptic intracellular potentials with another electrode. The preparation was superfused with sea water containing tetrodotoxin. The bias current preventing Ca^{2+} efflux from the pipette was switched off during the period indicated by two arrows. (Adapted from Miledi 1973.)

Photolysis of Caged Calcium

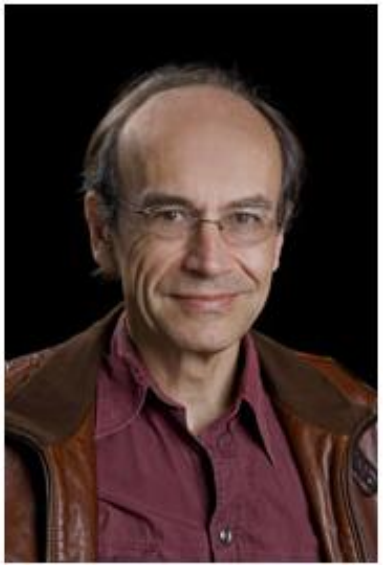




Cesare Montecucco

James E. Rothman

Randy W. Schekman



Thomas Sudhof

Richard Scheller

Reinhard Jahn

Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin

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NATURE · VOL 359 · 29 OCTOBER 1992

Cesare Montecucco

mechanisms of action of toxins as well as bacterial diseases, including tetanus, botulism, anthrax and Helicobacter pylori-associated diseases.

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- **Education:**
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  - Institut Pasteur in Paris, France
  - European Molecular Biology Laboratory in Heidelberg, Germany
  - University of Costa Rica.
- **Awards:**
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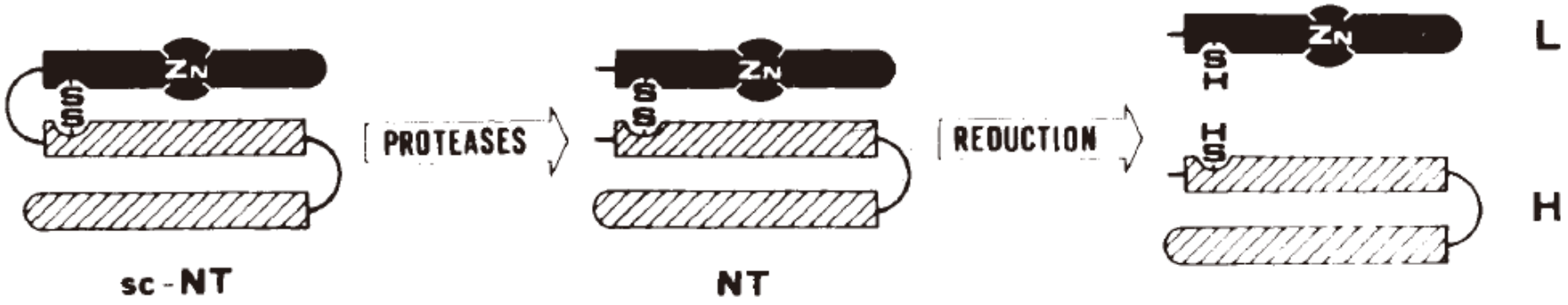
  - Prize of the Italian Consortium for Biotechnology in 1998
  - Prize of the Deutsche Gesellschaft für Hygiene und Mikrobiologie in 2000
  - Prize of the Masi Foundation for Venetian Civilization in 2003
  - Feltrinelli Prize for Medicine in 2004 and the Redi
  - Award of the International Society on Toxinology in 2009
  - Paul Ehrlich and Ludwig Damstaedter in 2011

Botox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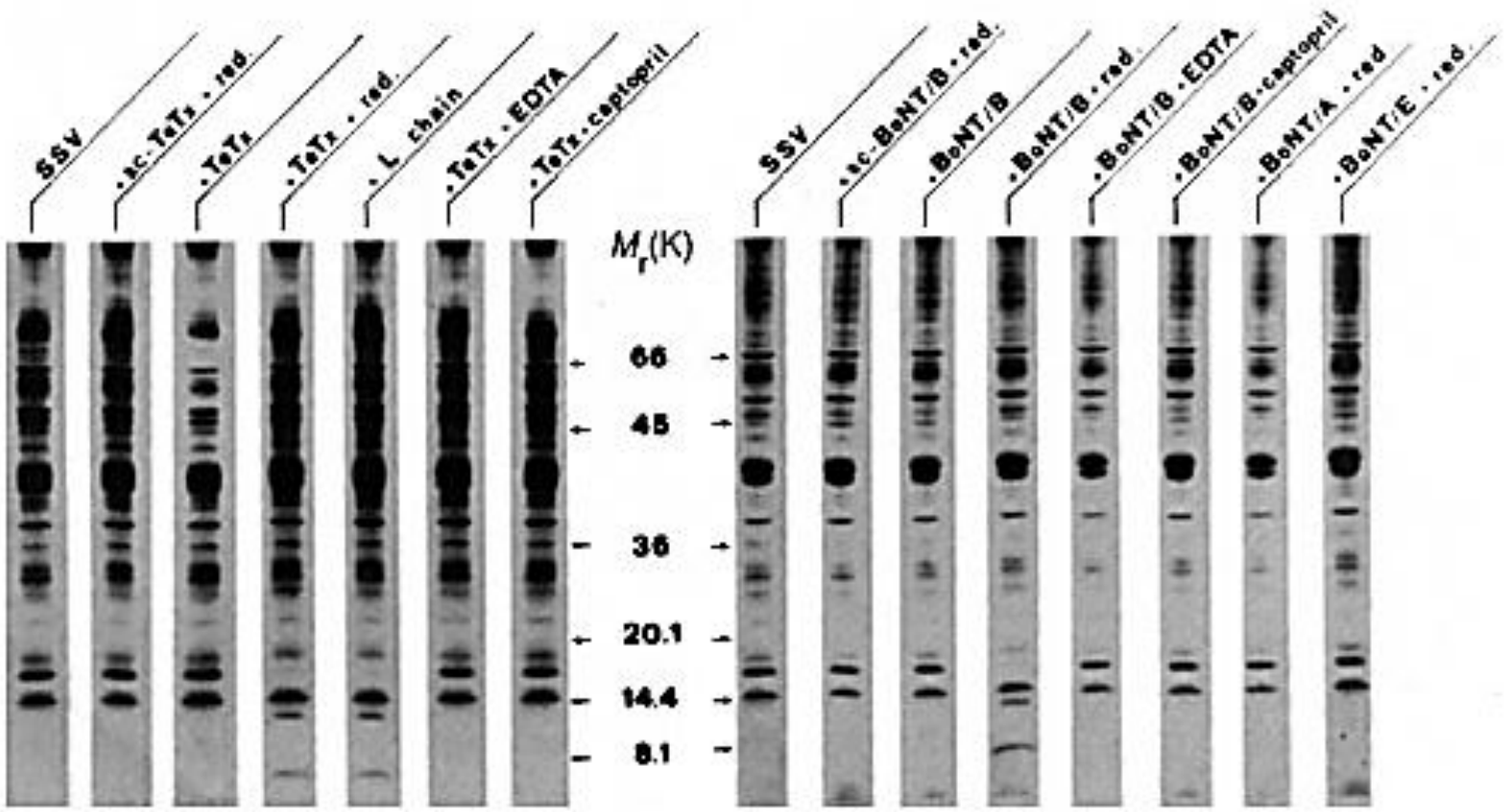
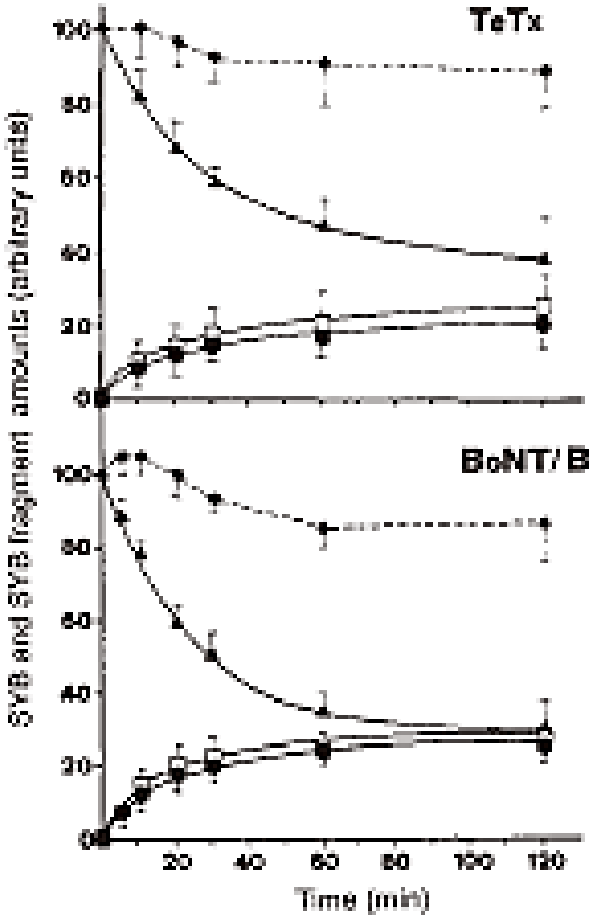


FIG. 1 Structure and proteolytic activity of tetanus and botulinum B neurotoxins on small synaptic vesicles. Upper panel, tetanus and botulinum neurotoxins are produced by bacteria as single chains and cleaved by different proteases at an exposed loop to generate the 2-chain toxin. The two chains can be separated by reduction of the single interchain disulphide bond[31]. H, heavy chain; L, light chain; sc-NT, single-chain neurotoxin; NT, neurotoxin. Lower left panel, 30 μg small synaptic vesicles (SSV) in 5 mM HEPES-Na, 0.3 M glycine, 0.3 M NaCl, 0.02% NaN_3, pH 7.4 (buffer A) were treated for 60 min at 37 °C with buffer (lane labelled SSV); with 200 ng single-chain TeTx reduced by incubation with 10 mM dithiothreitol (DTT) for 30 min at 37 °C (lane labelled sc-TeTx + red.); with 200 ng 2-chain TeTx (TeTx); with 200 ng 2-chain TeTx reduced by incubation with 10 mM DTT for 30 min at 37 °C (TeTx + red.); with 200 ng TeTx L chain (L chain); with the same amount of reduced 2-chain TeTx in the presence of 10 mM EDTA (TeTx + EDTA) or of 1.4 mM captopril ((2S) − 1 − [3-mercapto-2-methyl-propionyl]-L-proline) (TeTx + captopril). Lower right panel shows results of similar treatment with BoNT/B of 30 μg of SSV in buffer A, and in the last two right-hand lanes, of treatments with 200 ng reduced 2-chain BoNT/A (BoNT/A + red.), and 200 ng reduced di-chain BoNT/E (BoNT/E + red.). Samples were made 2% in SDS, 5 mM EDTA, boiled for 3 min, electrophoresed in a 12-cm-long 12–18% polyacrylamide gradient gel and stained with Coomassie blue[21]. Migration of M_r standards is shown in the centre.

FIG. 2 Time course of the proteolysis of the 19K protein (SYB) of small synaptic vesicles (SSV) induced by tetanus and botulinum B neurotoxins. SSV were incubated in buffer A as for Fig. 1 with 15 nM (final concentration) reduced 2-chain TeTx or 22 nM reduced 2-chain BoNT/B at 37 °C. At the times indicated, 30 μg SSV protein aliquots were withdrawn, quenched by addition of SDS–PAGE sample buffer, electrophoresed and stained as for Fig. 1. Gels were scanned with a dual-wavelength Shimadzu CS-930 densitometer and values of the integrated peaks at 19K (SYB, ▲); 12K (P12, ●) and 7K (P7, □) and of their sums (+) at various incubation times are shown. Bars represent s.d. of three different experiments.



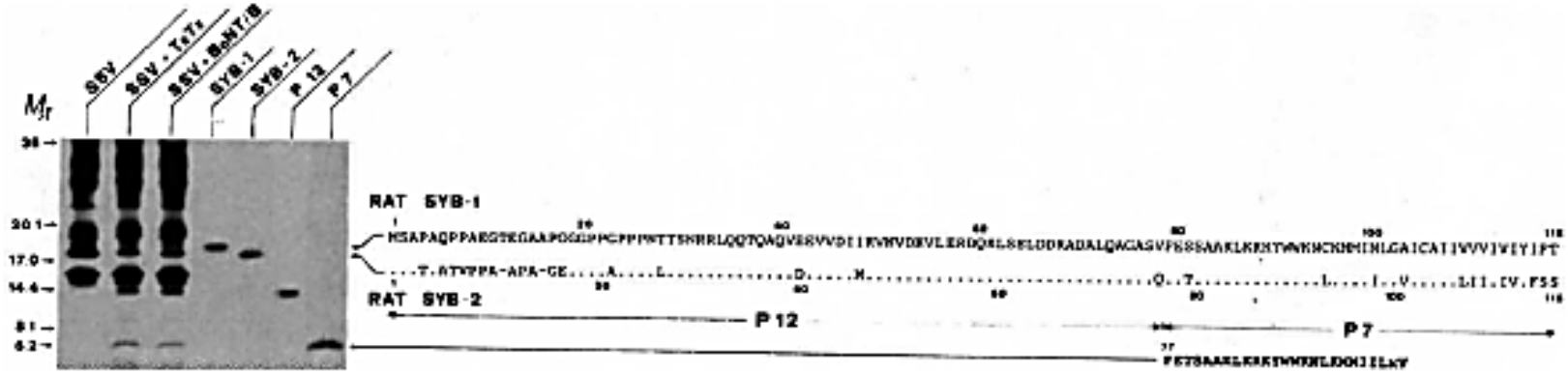


FIG. 3 Synaptobrevin-2 is specifically proteolysed by tetanus and botulinum B neurotoxins at peptide bond Gln 76-Phe 77. SSV protein (8 μg) was incubated as for Fig. 1 with buffer (lane SSV), with reduced 2-chain TeTx (50 ng; lane SSV + TeTx) or with BoNT/B (50 ng; SSV + BoNT/B) for 120 min at 37 °C, then electrophoresed in a 20-cm 12–18% polyacrylamide gradient gel and silver-stained. Loading of small amounts of SSV proteins was necessary to resolve the 19K band of Fig. 1 into a doublet. In the other gel lanes the doublet at 19K and the 12K and 7K fragments were sliced and the individual bands electroeluted. The eluted proteins proved to be single components after electrophoresis and silver staining (lanes SYB-1, SYB-2, P 12 and P 7). Proteins were blotted onto Pro-Blot and sequenced in a pulsed liquid Applied Biosystem Mod.477A protein sequencer. The two 19K and the 12K proteins did not give any signal, whereas the 7K fragment generated both by TeTx and BoNT/B gave the sequence FETSAAKLKRKYWWKNLKMMILLKxV, which corresponds to SYB-2. A single cleavage site is also indicated by comparison of the SYB sequences, because the central region of the two SYBs is identical and additional cleavages must occur in this region to account for the size of the proteolytic products. Each sequencing cycle gave a single residue. From the ratio of the Thr and Ser peaks at the third cycle, it could be estimated that the extent of SYB-1 cleavage was always <4%.

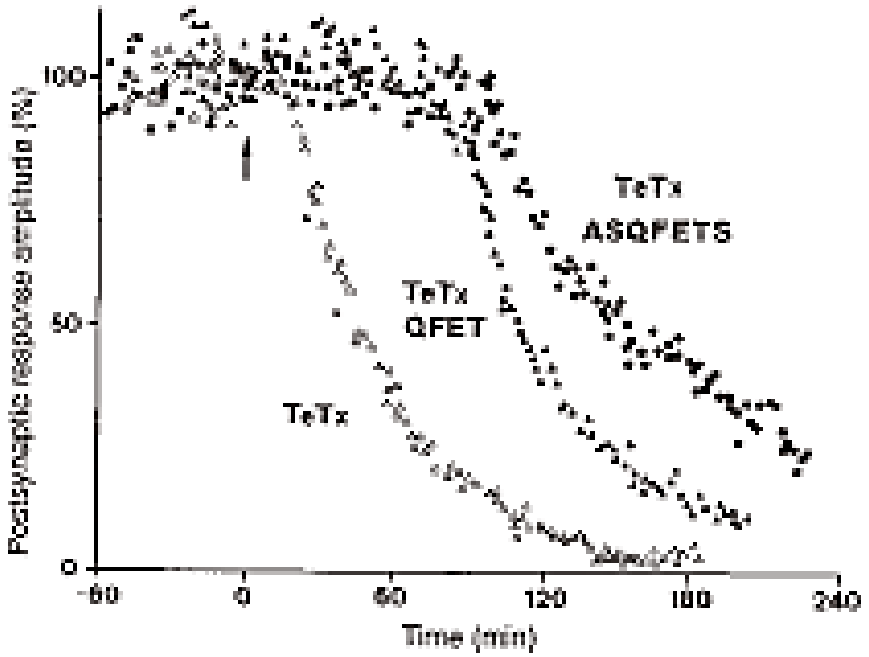
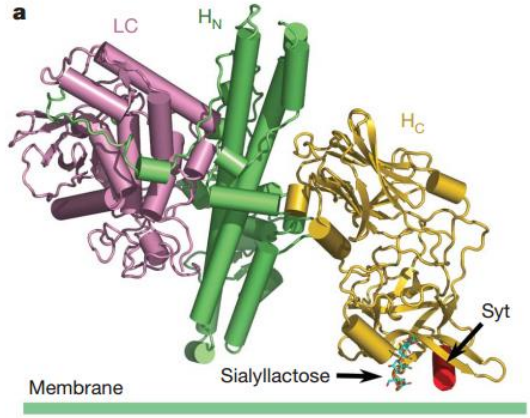
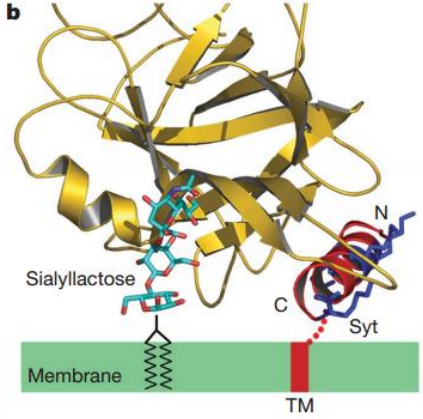


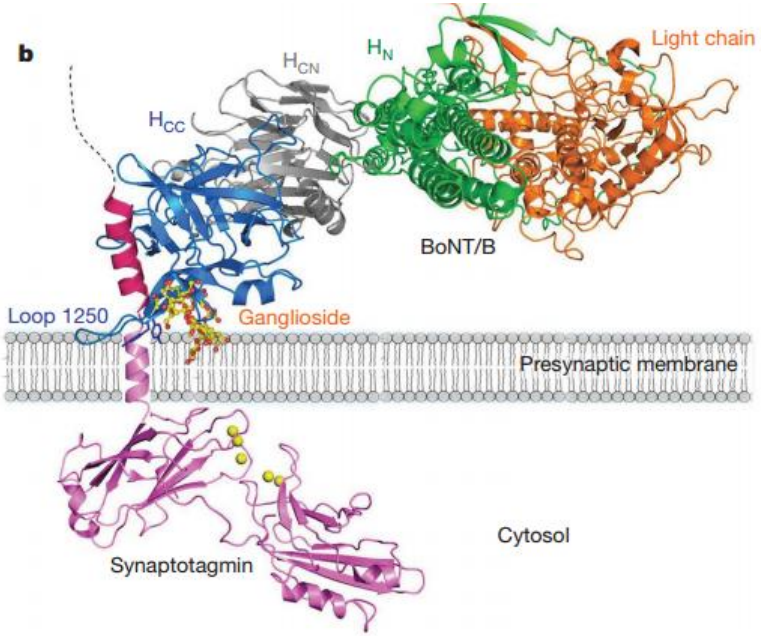
FIG. 4 The inhibition of neurotransmitter release in *Aplysia* neurons injected with tetanus toxin is delayed by SYB-2 peptides ASQFETS and QFET. Release of acetylcholine at identified cholinergic couples of neurons of the buccal ganglion of *Aplysia californica* was evoked by a presynaptic action potential, and the postsynaptic response was recorded under voltage-clamp[8,9]. After control recordings were made, presynaptic neurons were injected (arrow) with TeTx (intracellular concentration 8 nM) or with TeTx preincubated with ASQFETS or QFET (intracellular concentration 80 μM). Injection of buffer or peptides had no effect on acetylcholine release (not shown).

Model of BoNT/B binding to its receptor on a neuron

BoNT/B与神经元上特定受体结合的模型





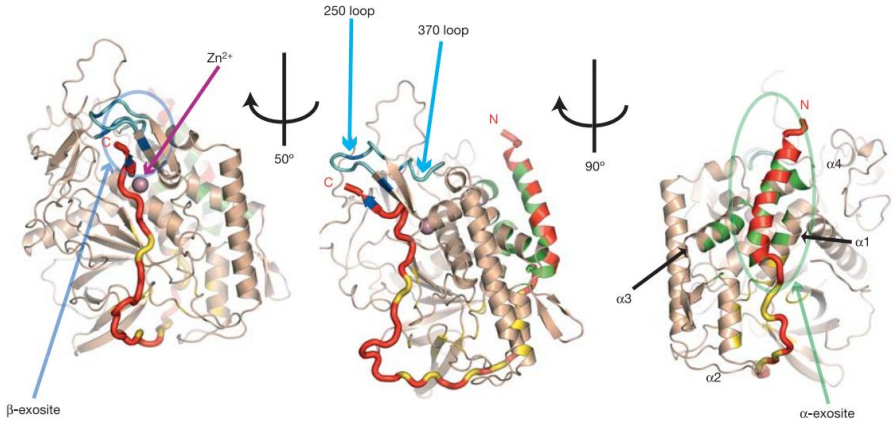


Jin, Rongsheng, et al. "Botulinum neurotoxin B recognizes its protein receptor with high affinity and specificity." Nature 444.7122 (2006): 1092-1095.

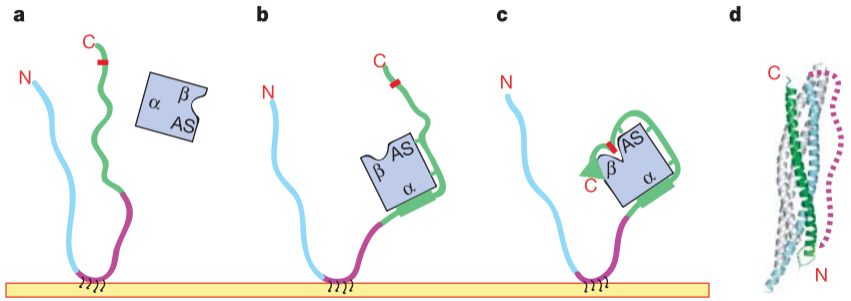
Chai Q, Arndt JW, Dong M, Tepp WH, Johnson EA, Chapman ER, Stevens RC. Structural basis of cell surface receptor recognition by botulinum neurotoxin B. Nature. 2006 Dec 21;444(7122):1096-100.

Substrate Recognition strategy for BoNT/A

BoNT/A 识别底物的策略



Interface between sn2 and BoNT/A



Model of BoNT/A substrate recognition

Breidenbach, Mark A., and Axel T. Brunger. "Substrate recognition strategy for botulinum neurotoxin serotype A." Nature 432.7019 (2004): 925-929.

SNAP receptors implicated in vesicle targeting and fusion

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James E. Rothman

vesicular transport within cells and the secretion of proteins

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Professor of Chemistry
Executive Director, Yale Center for High Throughput Cell Biology
Member of Yale faculty since 2008

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  Andrew W. Mellon Fellow, 1979 82
  Dreyfus Fndn. Teacher Scholar, 1981 86
  The Eli Lilly Award for Fundamental Res. in Biol. Chem., 1986
  The Passano Young Scientist Award, 1986
  The Alexander Von Humboldt Award, 1989
  The Heinrich Wieland Prize, 1990
  Member, Natl. Acad. of Sciences, 1993
  The Rosenstiel Award in Biomedical Sciences, 1994
  Fellow, Amer. Acad. Arts & Sciences, 1994
  The V.D. Mattia Award, 1994
  The Fritz Lipmann Award, 1995
  Member, Institute of Med., Natl. Acad. of Sciences, 1995
  Dr. h.c., Univ. of Regensburg, 1995
  Foreign Assoc., European Molec. Biol. Org'n., 1995
  Mayor's Award For Excellence in Sci. & Tech., 1995
  Gairdner Fndn. Internat'l. Award, 1996
  King Faisal Internat'l. Prize in Science, 1996
  The Harden Medal, 1997
  The Lounsbery Award, Natl. Acad. of Sciences, 1997
  The Feodor Lynen Award, 1997
  Dr. h.c., Univ. of Geneva, 1997
  The Heineken Prize, 2000
  The Otto-Warburg Medal, 2001
  The Lasker Award – Basic Medical Research, 2002
  The Louisa Gross Horwitz Prize, 2002

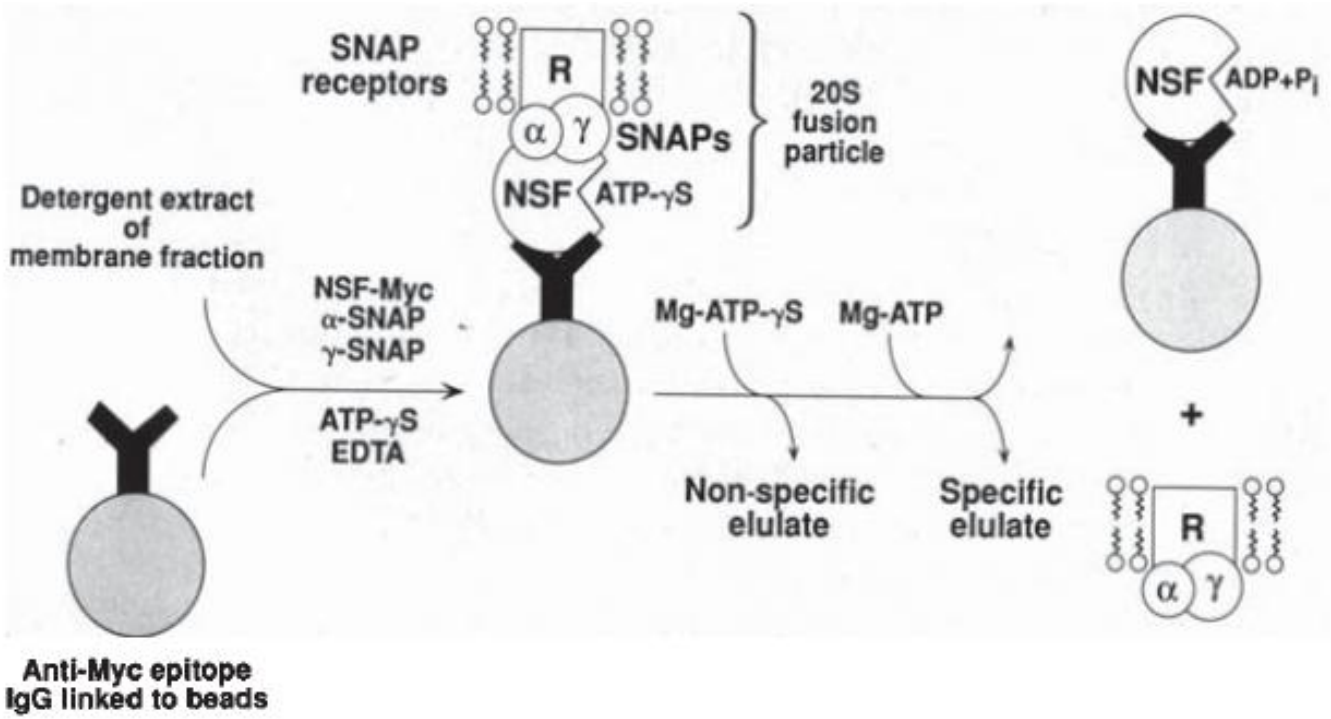

FIG. 1 Procedure used to purify SNAP receptors (SNAREs). Recombinant NSF, α-SNAP, and γ-SNAP are assembled into 20S particles by SNAREs present in a crude detergent extract of membranes. The SNAREs are incorporated stoichiometrically into the particles, which are then bound to beads by means of NSF. For this purpose, the NSF is epitope-tagged with Myc, and an anti-Myc monoclonal IgG is linked to the beads. The beads are washed and then eluted first with Mg–ATP-γS (nonspecific eluate) and then with Mg–ATP (specific eluate). The bound 20S particles disassemble in the presence of Mg–ATP (but not Mg–ATP-γS), releasing stoichiometric amounts of SNAPs and SHAREs. NSF remains bound to the beads. Experimental details are given in Fig. 2 legend.

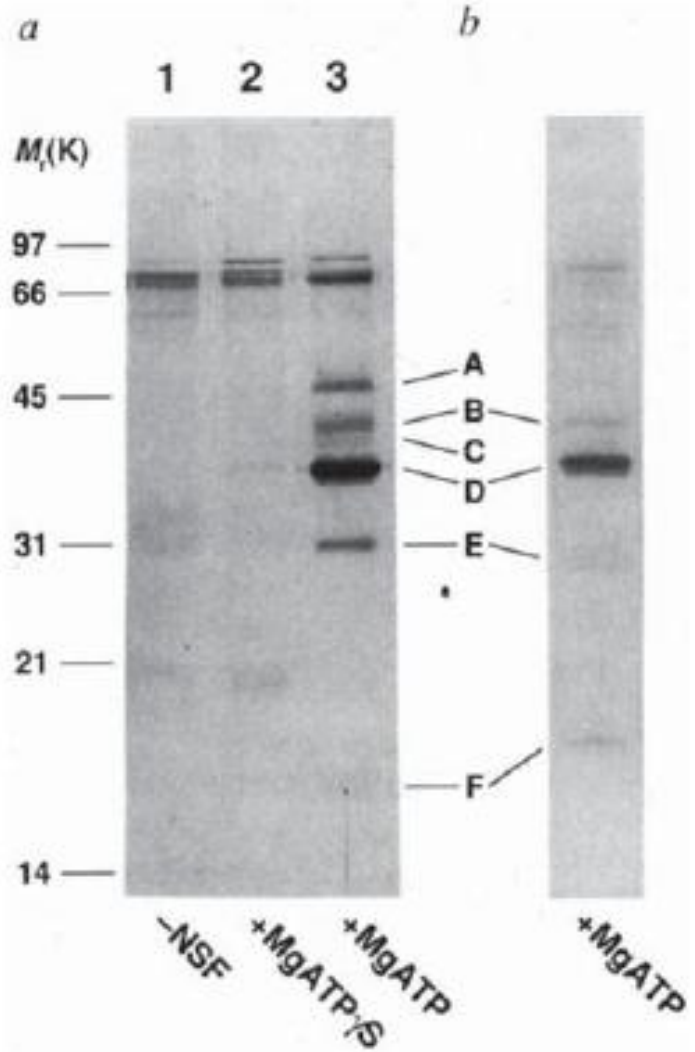

FIG. 2 Identification of proteins released from NSF after ATP hydrolysis. *a*, Polyacrylamide gel stained with Coomassie blue. Lane 1, control, Mg–ATP eluate of control binding reaction in the absence of NSF; lane 2, 'nonspecific' eluate from complete binding reaction with NSF–Myc and Mg–ATP-γS; lane 3, 'specific' eluate of the same column as for lane 2 following the exchange of ATP for ATP–γS (in the presence of EDTA) and addition of Mg^{2+} to allow ATP hydrolysis (Fig. 1). *b*, Silver-stained Laemmli gel of the specific (Mg–ATP) eluate.

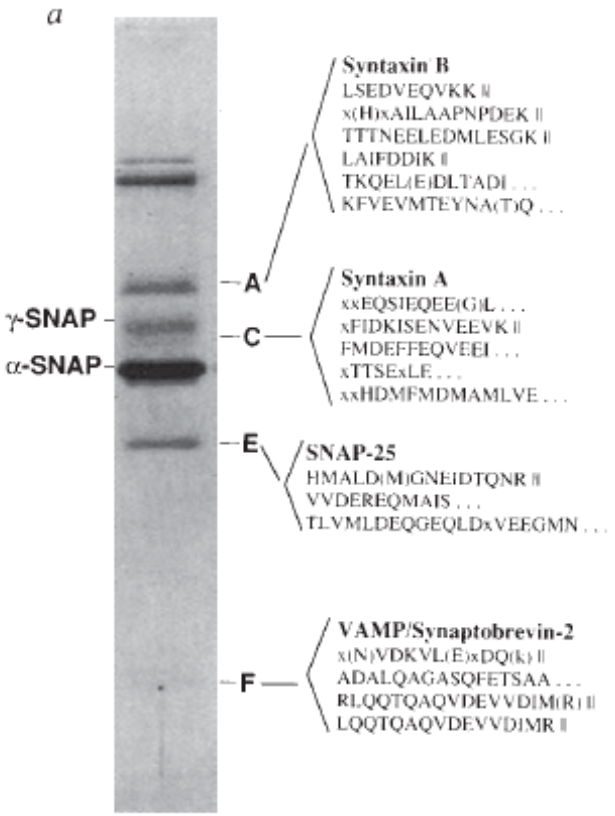

FIG. 3 Identification of SNAP receptors by amino-acid sequencing. Each of bands A–F was excised from an electroblot onto a nitrocellulose membrane after staining with Ponceau S, digested with trypsin, and the fragments separated by reverse-phase HPLC. *a*, Peptide sequences from each of the indicated bands are shown on the right (one-letter code). An 'X' means that no residue could be identified at this position. A capital letter in parentheses means that the residue was identified with a lower degree of confidence; a lower-case letter in parentheses means that the residue was present but in very small amounts. The symbol ‖ indicates that the C terminus of the peptide was known. A dotted line means no more interpretable signals were obtained, but that this was unlikely to be the C terminus. The identify of bands B and D (Fig. 2) as γ-SNAP and α-SNAP, respectively, was confirmed by peptide sequencing. In the case of the syntaxin A peptide XTTSExLE . . . (from band C), only this limited sequence was obtained because of instrument failure. However, mass analysis of this peptide (on 1/25th of the sample) gave a value of $m/z = 3{,}274.8$; this is in good agreement with the value ($MH^+ = 3{,}276.67$) calculated for the predicted tryptic peptide (residues 156–186 of syntaxin A) which contains the limited sequence, so confirming the identify of the entire peptide. Mass analysis was also critical in the identification of band F as VAMP/synaptobrevin-2, confirming the presence of an *N*-acetylated peptide corresponding to residues 2–31 in the digest of band F (see text for details). *b*, Amino-acid sequences of syntaxins A (top) and B (below) from rat brain[23]. Shown above syntaxin A are the peptide sequences obtained from band C, and below syntaxin B are the peptide sequences obtained from band A. *c*, Complete amino-acid sequence of synaptosome-associated protein 25 (SNAP-25) from mouse brain[24]. Above are the peptide sequences obtained from band E. Underlined sequences in *d* and *b* are peptides identified by mass analysis.

b

```
                                        FM DEFFEQVEEI   FIDKISEN
Rat Syntaxin A ---RTQELRT AKDSDDDDDV TVTVDRDRFM DEFFEQVEEI RGFIDKIAEN  47
Rat Syntaxin B MKDRTQELRS AKDSDDEEEV -VHVDRDHFM DEFFEQVEEI RQCIEKLSED  49
                                                                 LSED

          VEEVK                                                  EQSI
          VEEVKRKHSA ILASPNPDEK TKEELEELMS DIKKTANKVR SKLKSIEQSI      97
          VEQVKKQHSA ILAAPNPDEK TKQELEDLIA DIKKTANKVR SKLKAIEQSI      99
          VEQVKKxHxA ILAAPNPDEK TKQELEDLIA DI

          EQEEGL
          EQEEGLNRSS ADLRIRKTQH STLSRKFVEV MSEYNATQSD YRERCKGRIQ      147
          EQEEGLNRSS ADLRIRKTQH STLSRKFVEV MTEYNATQSK YRDRCKDRIQ      149
                                    KFVEV MTEYNATQ

                T TSExLE
          RQLEITGRTT TSEELEDMLE SQNPAIFASG IIMDSSISKQ ALSEIETRHS      197
          RQLEITGRTT TNEELEDMLE SGKLAIFTDD IKMDSQMTKQ ALNEIETRHN      199
                TT TNEELEDMLE SGKLAIFTDD IK

                   HDMFMDMA MLVE
          EIIKLENSIR ELHDMFMDMA MLVESQGEMI DRIEYNVEHA VDYVERAVSD      247
          EIIKLETSIR ELHDMFVDMA MLVESQGEMI DRIEYNVEHS VDYVERAVSD      249


          TKKAVKYQSK ARKKKIMIII CCVILGIIIA STIGGIFG-       285
          TKKAVKYQSK ARKKKIMIII CCVVLGVVLA SSIGGTLGL       288
```

c

```
Mouse SNAP-25
                                                    TLVML DEQGEQLDxV
          MAEDADMRNE LEEMQRRADQ LADESLESTR RMLQLVEESK DAGIRTLVML DEQGEQLERI  60

          EEGMN                                                       V
          EEGMDQINKD MKEAEKNLTD LGKFCGLCVC PCNKLKSSDA YKKAWGNNQD GVVASQPARV  120

          VDEREQMAIS                                 HMALDMGNE IDTQNR
          VDEREQMAIS GQFIRRVTND ARENEMDENL EQVSGIIGNL RHMALDMGNE IDTQNRQIDR  180


          IMEKADSNKT RIDEANQRAT KMLGSG  206
```

d

```
Bovine
VAMP/Synaptobrevin 2
                                  RLQQTQAQVD EVVDIMR NV DKVLExDQk
          MSATAATAPP AAPAGEGGPP APPPNLTSNR RLQQTQAQVD EVVDIMRVNV DKVLERDQKL  60

               ADAL QAGASQFET SAA
          SELDDRADAL QAGASQFET SAAKLKRKYW WKNLKMMIIL GVICAIILII IIVYFSS      117
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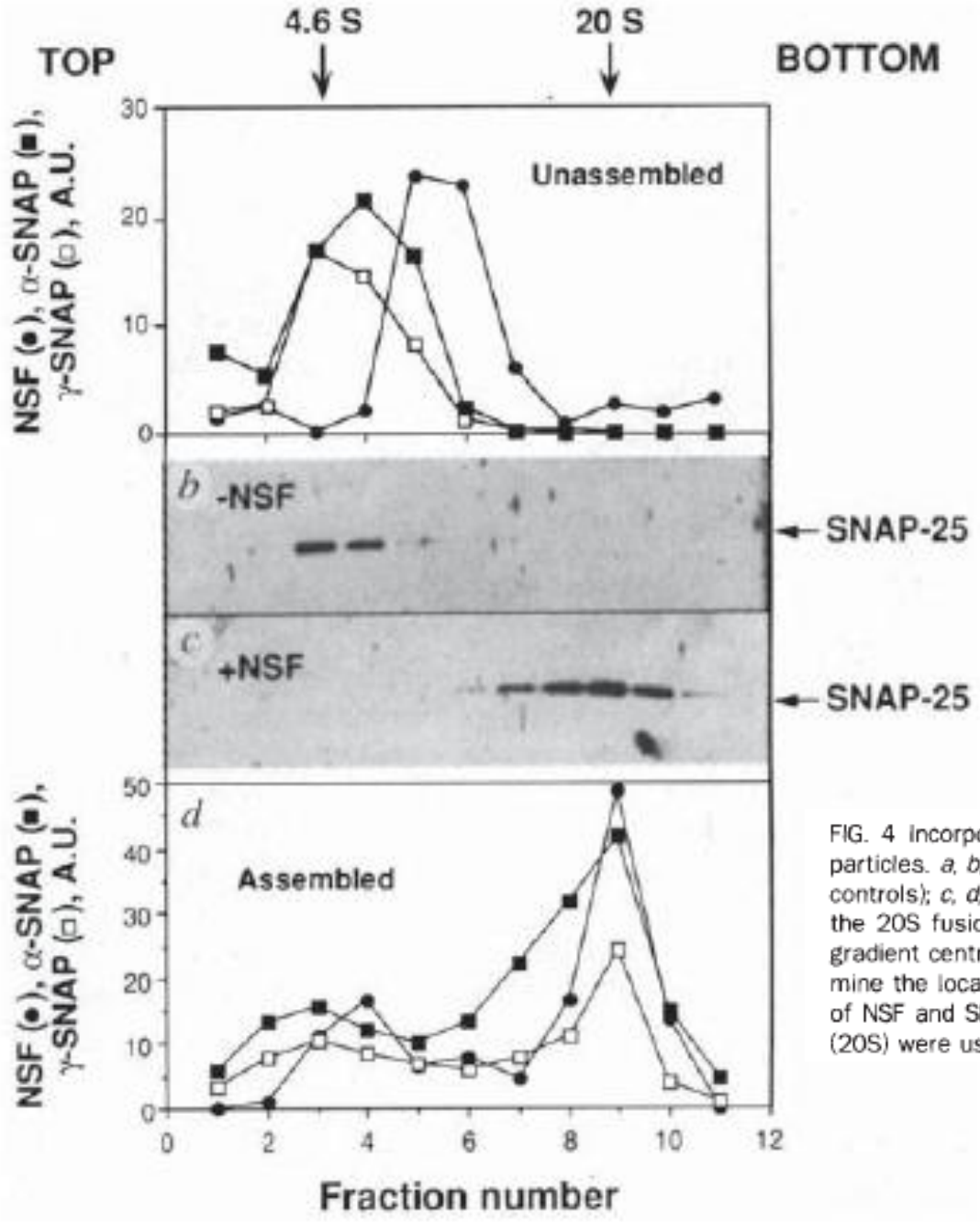

FIG. 4 Incorporation of SNAP-25 and other components into 20S fusion particles. *a*, *b*, Conditions in which 20S particles do not form (for example, controls); *c*, *d*, conditions in which 20S particles assemble. Components of the 20S fusion particle were incubated and then fractionated by glycerol gradient centrifugation, and the fractions analysed by SDS–PAGE to determine the location of α-SNAP and γ-SNAP, and to determine the locations of NSF and SNAP-25. Bovine serum albumin (4.6S) and α_2-macroglobulin (20S) were used as standards.

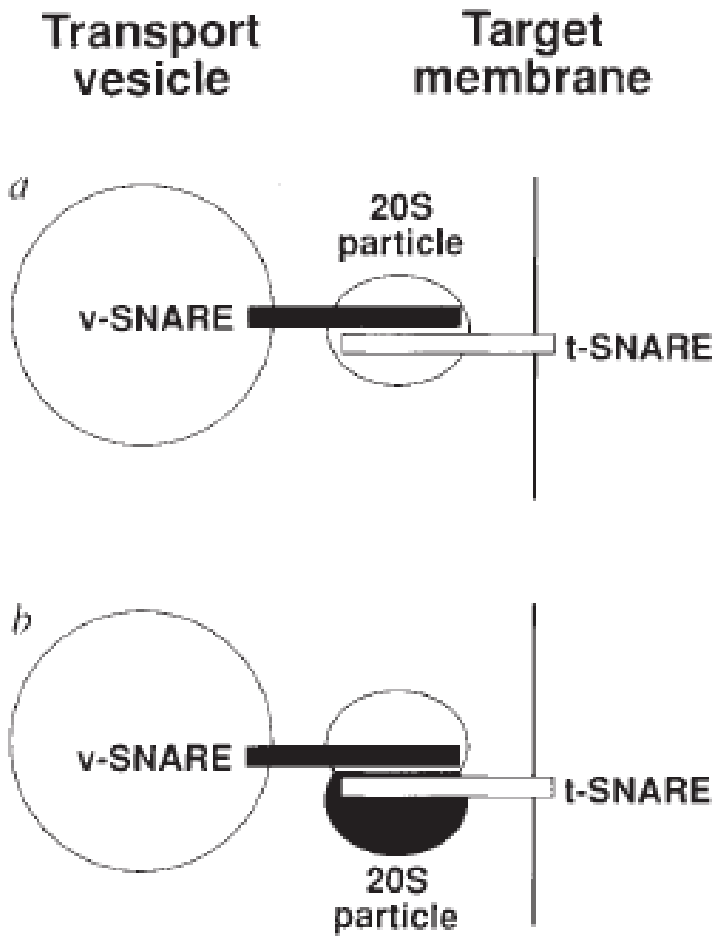

FIG. 5 Models to explain vesicle targeting based on the finding that SNAREs isolated in 20S fusion particles can originate from either the transport vesicle (v-SNAREs) or from the target membrane (t-SNAREs). A 20S particle (containing NSF and SNAPs) that simultaneously binds a v-SNARE and a t-SNARE (*a*) would attach a vesicle to its target. Alternatively (*b*), 20S particles, each capable of binding only one SNARE at a time, could interact to attach vesicle to target, a process that perhaps requires other proteins to assemble together.

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SNAREpins: Minimal Machinery for Membrane Fusion

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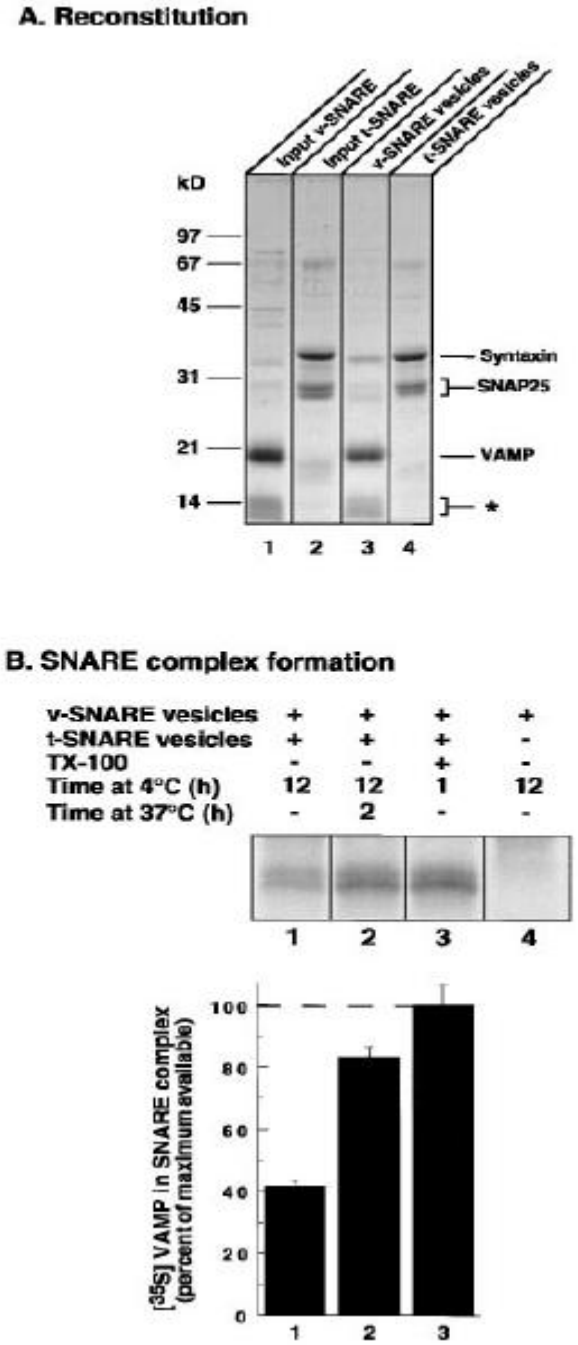

Figure 1. Functional Reconstitution of SNAREs into Vesicles

(A) Purified SNARE proteins can be reconstituted into vesicles. The purified v-SNARE VAMP (lane 1) and the t-SNARE complex, consisting of syntaxin 1 and SNAP25 (lane 2), were separated by SDS-PAGE and stained with Coomassie blue-R250. VAMP and t-SNARE complex that copurified with vesicles after reconstitution are shown in lanes 3 and 4, respectively. The lower band of the SNAP25 doublet is a C-terminal degradation product. Bands labeled by the asterisk are N-terminal degradation products of VAMP. Removal of these degradation products by Mono-S Sepharose chromatography prior to reconstitution (see Experimental Procedures) did not affect experimental results. The band in lane 3, migrating at a position close to that of syntaxin in lane 4, is a dimer of VAMP that becomes apparent after reconstitution.

(B) Reconstituted SNAREs form complexes with their cognate SNARE partner. Fluorescent donor vesicles containing [^{35}S]-labeled VAMP were incubated in the presence or absence of a 3-fold molar excess of t-SNAREs in vesicles. Botulinum neurotoxin D (BoNT D) was used to monitor SNARE complex formation as described in the Experimental Procedures. Fusion of these vesicles, monitored in parallel (not shown) using the lipid mixing assay, was indistinguishable from reactions of vesicles with nonradioactive VAMP. Following BoNT D digestion, the samples were subjected to SDS-PAGE electrophoresis and analyzed by autoradiography (above). Quantitative analysis by phosphorimaging represents the average of two independent experiments (below). (Lane 1) v-SNARE and t-SNARE vesicles incubated for 12 hr at 4°C before toxin treatment led to SNARE complex formation but did not result in fusion (see Figure 2A, zero time point). (Lane 2) Incubation for 2 hr at 37°C following 12 hr at 4°C increased the extent of [^{35}S]-labeled VAMP complexed to t-SNAREs and resulted in fusion (see Figure 2A, 2 hr). (Lane 3) Triton X-100 was used to dissolve a mixture of [^{35}S]-labeled VAMP donor vesicles and t-SNARE acceptor vesicles to determine the maximum fraction of [^{35}S]-labeled VAMP that could partner with excess t-SNAREs under the conditions used (30 ± 2% of input). The data shown in the bar graph are percentages relative to this maximum. (Lane 4) Toxin digestion of free VAMP was complete, since no [^{35}S]-labeled VAMP could be detected when donor vesicles were digested with botulinum neurotoxin D in the absence of t-SNARE vesicles. This resulting trace background was subtracted from every sample before normalization to lane 3.

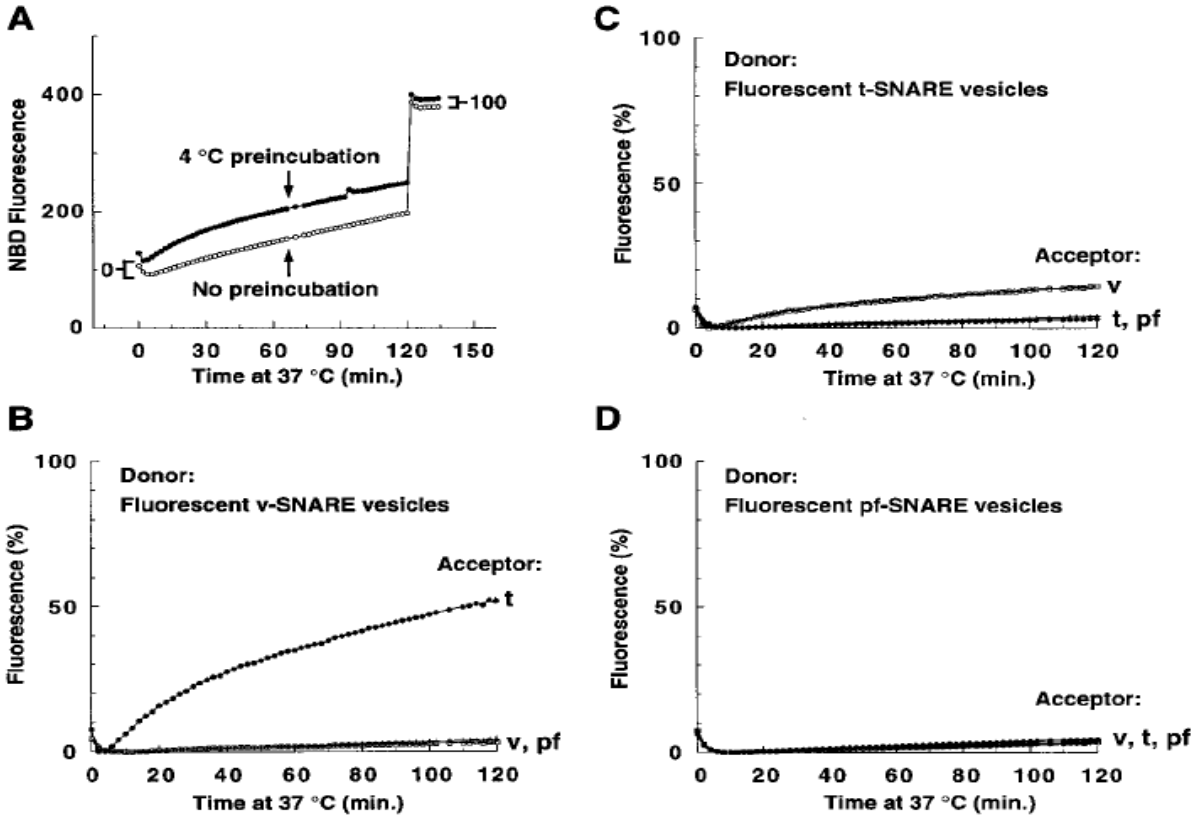

Figure 2. Fusion between Vesicles Containing v- and t-SNAREs

(A) Donor vesicles containing the v-SNARE VAMP were mixed with an excess (15-fold on a lipid basis) of acceptor vesicles containing a 1.5-fold molar excess of the t-SNAREs complex of syntaxin 1 and SNAP25, and NBD fluorescence was monitored at 37°C as described in the Experimental Procedures. One sample (closed circles) had been preincubated at 4°C for 15 hr prior to the temperature shift to 37°C whereas the other (open circles) was not preincubated. Note that preincubation resulted in only a small increase in NBD fluorescence (zero time point), indicating that essentially no fusion had occured after 15 hr at 4°C. After 2 hr at 37°C, Triton X-100 was added to mix the lipids completely and dequench the fluorescence of NBD. To normalize the experiments, in (B)–(D) and in all other figures (unless otherwise stated), the lowest NBD fluorescence signals were set to 0%, and the maximal signals reached after detergent addition were set to 100% fluorescence (as marked).

(B) Donor vesicles containing the v-SNARE VAMP were preincubated with acceptor vesicles containing either no protein (pf, protein free, crosses), the t-SNARE complex of syntaxin 1 and SNAP25, (t, closed circles), or the v-SNARE VAMP (v, open squares) at 4°C for 15 hr. After shifting the temperature to 37°C, NBD fluorescence was monitored as described in the Experimental Procedures.

(C) Donor vesicles containing t-SNAREs were incubated with different acceptor vesicles containing either v-SNAREs (open squares), or t-SNARE complexes (closed circles), or no proteins (crosses) as described in the Experimental Procedures.

(D) Protein-free donor vesicles were incubated with different acceptor vesicles (symbols as in [B] and [C]) as described in the Experimental Procedures.

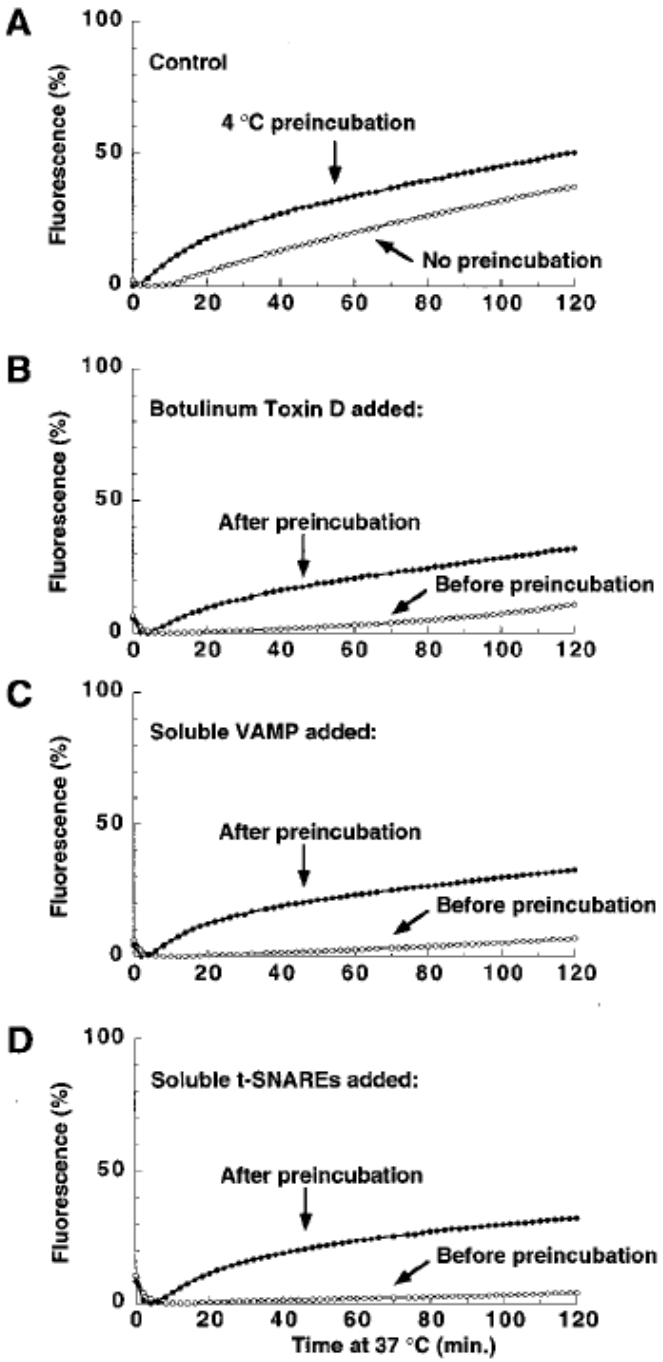

Figure 4. Assembly of SNARE Complexes between Vesicles Is Required for and Precedes Fusion

(A) Standard amounts of donor vesicles containing v-SNAREs and acceptor vesicles containing t-SNAREs were preincubated at 4°C (closed circles) and then incubated at 37°C, as described in Figure 2A, or combined and incubated immediately at 37°C (open circles). Fusion was monitored by the increase in NBD fluorescence, and signals were normalized as described in Figure 2A. Only the time course of the 37° incubation is shown. Zero time marks the start of the 37°C incubation in all cases.

(B) Standard amounts of donor vesicles containing v-SNAREs (see Experimental Procedures) were incubated at 37°C for 1 hr either in presence (open circles) or in absence (closed circles) of recombinant botulinum neurotoxin D light chain (2.1 μg of protein). Samples were chilled on ice, acceptor vesicles containing t-SNAREs were added, and the reactions were incubated at 4°C for 15 hr (the "preincubation"). Botulinum neurotoxin D was then added to the sample that had not been pretreated with this toxin. Next, both samples were warmed up to 37°C and vesicle fusion was followed by NBD fluorescence. Normalized results are shown.

(C) Standard amounts of acceptor vesicles containing t-SNAREs were incubated either in absence (closed circles) or in presence (open circles) of the cytoplasmic domain of VAMP (amino acids 1–94, added in approximately equimolar amounts to t-SNAREs) on ice for 1 hr. After addition of the donor vesicles containing v-SNAREs to each reaction, the samples were incubated for 15 hr at 4°C (the preincubation). Cytoplasmic VAMP was then added to the sample that had not been pretreated with this protein domain. Samples were warmed up to 37°C and vesicle fusion was followed by NBD fluorescence. Normalized results are shown.

(D) Standard amounts of donor vesicles containing the v-SNARE VAMP were incubated with soluble t-SNAREs (amino acids 1–265, approximately 7-fold molar excess over VAMP) on ice for 1 hr. After addition of the acceptor vesicles containing t-SNAREs, the samples were preincubated for 15 hr at 4°C. Cytoplasmic t-SNAREs were added to the sample that had not been pretreated with this protein complex and reactions were further processed as in (C).

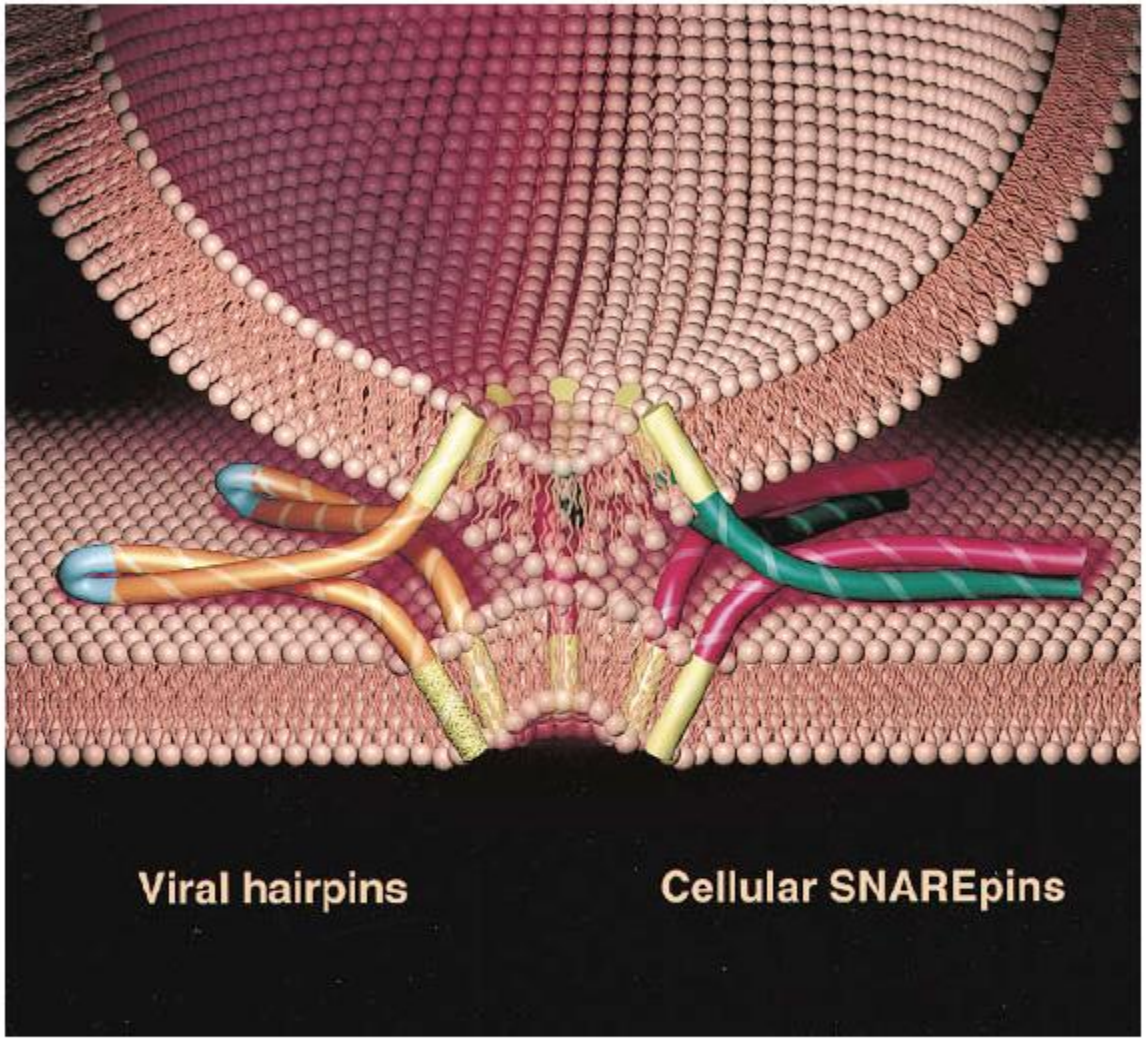

Figure 5. Cellular SNAREpins and Viral Hairpins

v-t SNAREpins (at right) are complexes of cognate v-SNAREs (in green, in the transport vesicle above) and t-SNAREs (in red, in the planar target membrane below) bridging two membranes. Analogous t-t SNAREpins may also be possible. Membrane anchors are highlighted in yellow. The core of certain viral fusion proteins (at left, in orange) is diagrammed in a simplified fashion. The membrane anchor of the fusion protein (in yellow) is inserted into the viral membrane, represented by the spherical lipid bilayer. The fusion peptide (textured yellow) is inserted into the planar target membrane below. Viral fusion proteins generally consist of continuous polypeptides (indicated conceptually by the blue polypeptide loop), within which oppositely oriented (i.e., antiparallel) helical hairpin-like structures assemble in a helical bundle and are proposed to link up the two membranes for fusion (Lu et al., 1995; Chan et al., 1997; Weissenhorn et al., 1997). A SNAREpin (whose precise internal structure is not yet known) consists of a 13–14 nm long helix-rich core rod of 2 nm width, which most likely contains the membrane-proximal helices of VAMP and syntaxin oriented parallel to each other (Hanson et al., 1997; Lin and Scheller, 1997). In contrast to viral hairpins, cellular SNAREpins are formed from separate polypeptides that reside in different membranes before fusion. It is likely that multiple copies of viral hairpins or SNAREpins are needed to trigger fusion, and these are likely arranged in a ring-like structure at a contact point along the lines illustrated. The striking similarity between SNAREpins and viral hairpins suggests that extracellular and lumenally oriented viral fusion proteins, as well as intracellular membrane fusion proteins, all employ a fundamentally similar mechanism to coalesce lipid bilayers.

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Identification of 23 Complementation Groups Required for Post-translational Events in the Yeast Secretory Pathway

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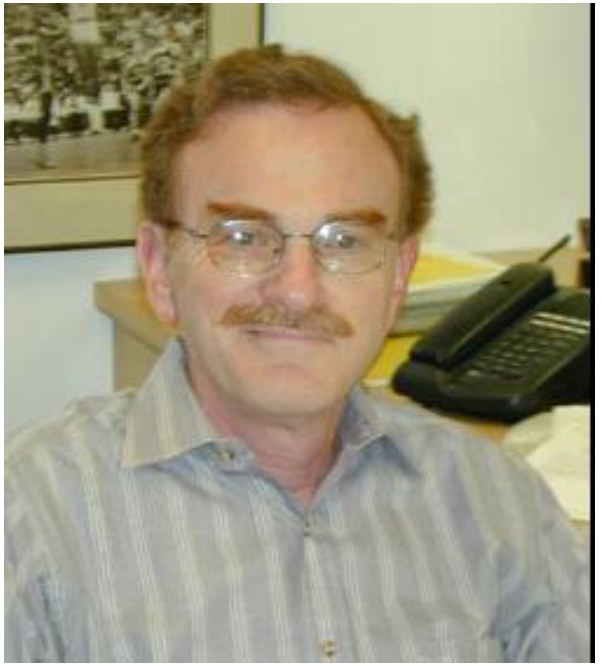
The process of membrane assembly, vesicular transport, and membrane fusion among organelles of the secretory pathway



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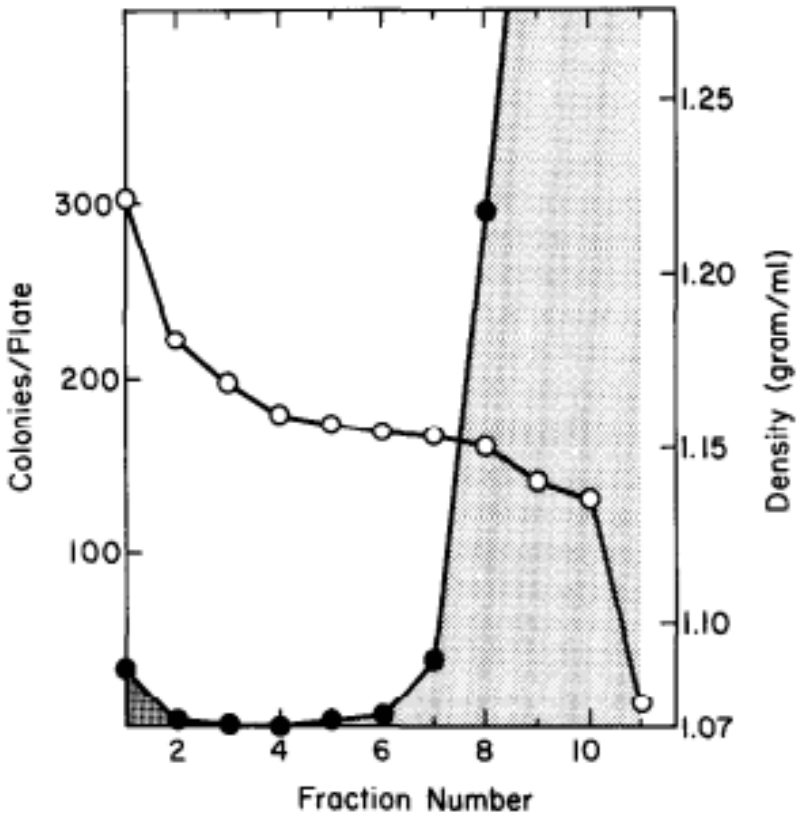

Figure 1. Density Gradient Separation of *sec*1-1 and X2180 Cells

SF150-5C and X2180-1A cells were grown in YPD medium at 25°C. After 3 hr at 37°C, the cells were sedimented, washed and resuspended in 1 ml of water. Cell aliquots were mixed (49.5 A_{600} units of X2180 and 0.5 A_{600} unit of SF150-5C) and sedimented on a Ludox gradient. Fractions were collected and diluted 10^4 fold, and 0.1 ml portions were spread on minimal medium (7 mM phosphate) agar plates. Colonies formed after 2 days at room temperature were stained for acid phosphatase activity (Hansche et al., 1978). Heavy stippling represents the phosphatase-constitutive (*pho*80) *sec*1 colonies (SF150-5C); light stippling represents phosphatase-repressed colonies (X2180).

Table 1. Comparison of Screening Procedure with and without Density Enrichment

Screening Stage	Without Enrichment		With Enrichment	
	Colonies	%	Colonies	%
(1) Colonies tested	5,600	100	18,500	100
(2) TS mutants	291	5.2	2,830	15
(3) TS phosphatase secretion	63	1.1	980	5
(4) TS invertase secretion	16	.29	485	2.6
(5) TS invertase accumulation	2	.04	188	1.0

Table 2. Distribution of Mutants in the *secA* Complementation Groups: EMS versus Nitrous Acid

	EMS		Nitrous Acid	
sec	Isolates	%	Isolates	%
1	8	11	4	3
2	28	39	41	35
3	3	4	0	0
4	7	10	2	2
5	10	14	16	14
6	3	4	3	3
7	1	1	3	3
8	6	8	4	3
9	3	4	4	3
10	1	1	2	2
11	1	1	11	9
12	1	1	3	3
13			4	3
14			4	3
15			2	2
16			2	2
17			1	1
18			2	2
19			1	1
20			1	1
21			1	1
22			4	3
23			1	1

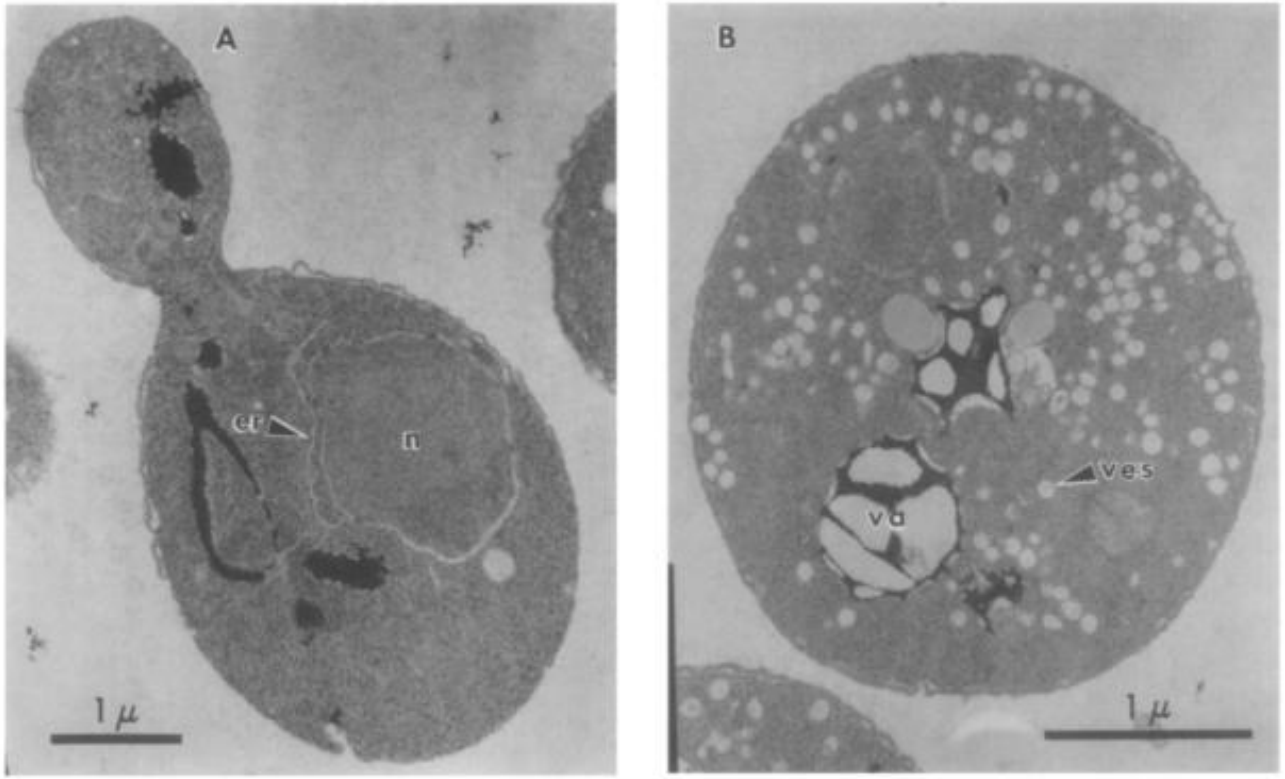

Figure 2. Thin Section Electron Micrographs of Cells Grown in YPD Medium

(A) HMSF 13 (*sec*4-2) grown at 25°C; (B) HMSF 171 (*sec*15-1) incubated at 37°C for 2 hr. Symbols: (n) nucleus; (va) vacuole; (er) endoplasmic reticulum; (ves) vesicles.

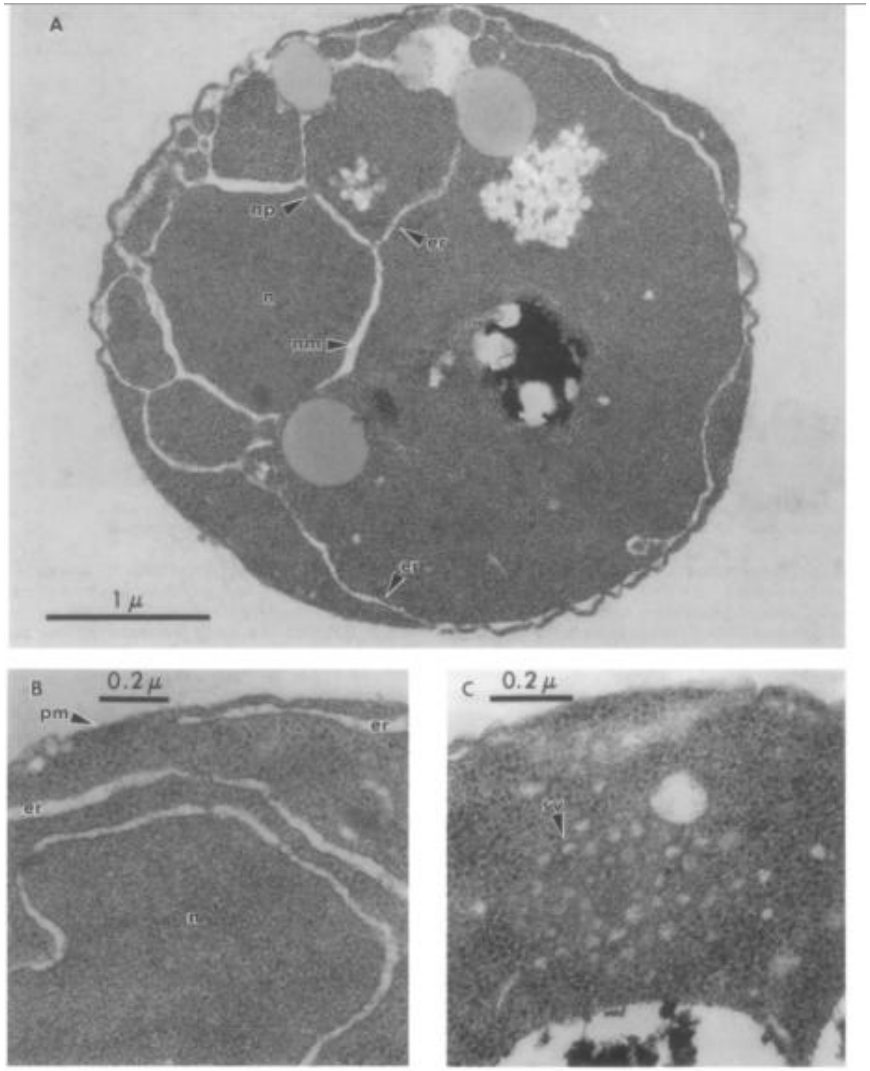

Figure 3. Thin Section Electron Micrographs of Cells Grown in YPD Medium at 25°C, Then Shifted to 37°C for 2 Hr

(A) HMSF 174 (sec16-2); (B) HMSF 190 (sec23-1); (C) HMSF 175 (sec17-1). Symbols are as in Figure 2 and (np) nuclear pore; (sv) small vesicle; (pm) plasma membrane; (nm) nuclear membrane.

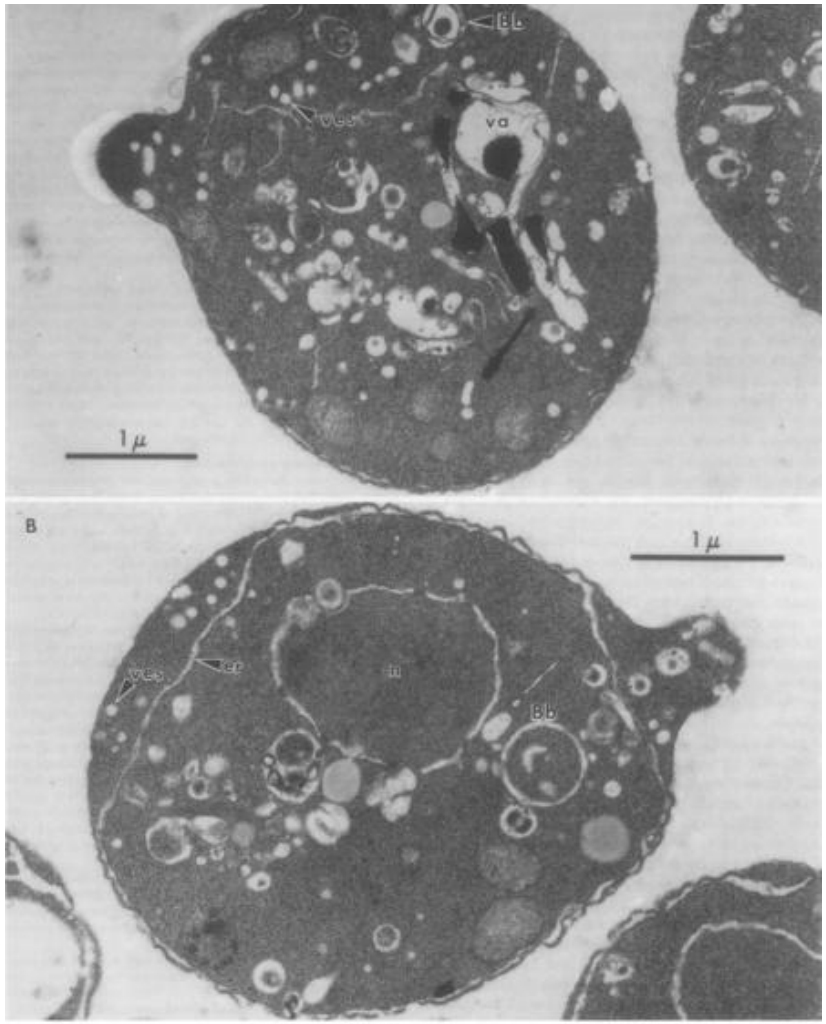

Figure 5. Thin Section Electron Micrographs of Cells Grown in YPD Medium at 25°C, Then Shifted to 37°C for 2 Hr

(A) HMSF 169 (sec14-3); (B) HMSF 178 (sec19-1). Symbols are as in Figures 2–4.

Table 5. Organelles Accumulated in the *sec* Strains

Strain (IIMSF)	*sec*	Structure(s)
1	1-1	vesicles, Berkeley bodies
47	2-7	vesicles
3	3-1	vesicles
13	4-2	vesicles
81	5-8	vesicles
12	6-1	vesicles
6	7-1, -2	Berkeley bodies
93	8-4	vesicles
89	9-3	vesicles, Berkeley bodies
147	10-2	vesicles
154	11-7	
162	12-4	ER
163	13-1	ER
169	14-3	Berkeley bodies, vesicles
171	15-1	vesicles
174	16-2	ER
175	17-1	ER, small vesicles
176	18-1	ER, small vesicles
178	19-1	vesicles, Berkeley bodies, ER
179	20-1	ER
180	21-1	ER
183	22-3	ER, small vesicles
190	23-1	ER

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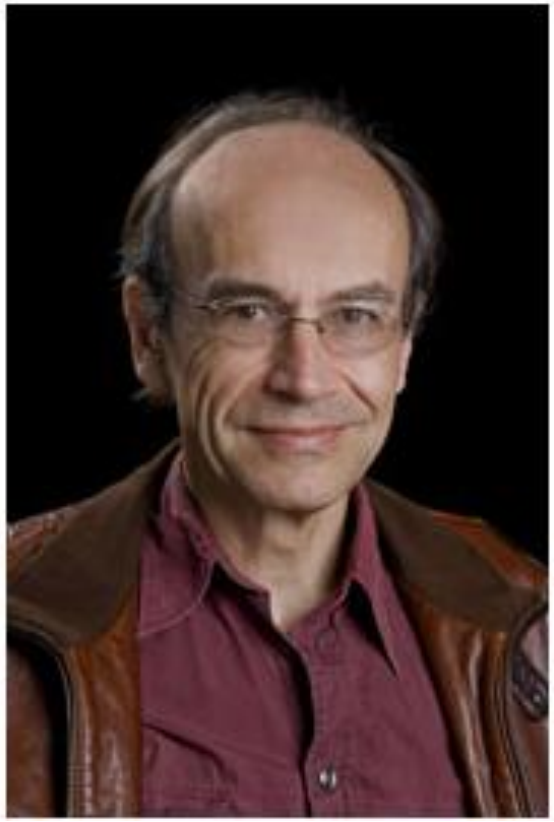

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  - 1982 Stanford University, Department of Biological Sciences, Assistant Professor
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  - 2009 Head Genentech Research and Early Development

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- the W. Alden Spencer Award in 1993
- the NAS Award in Molecular Biology in 1997
- the 2010 Kavli Prize in Neuroscience with Thomas C. Südhof andJames E. Rothman
- the Life Sciences Distinguished Alumni Award from University of Wisconsin–Madison
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