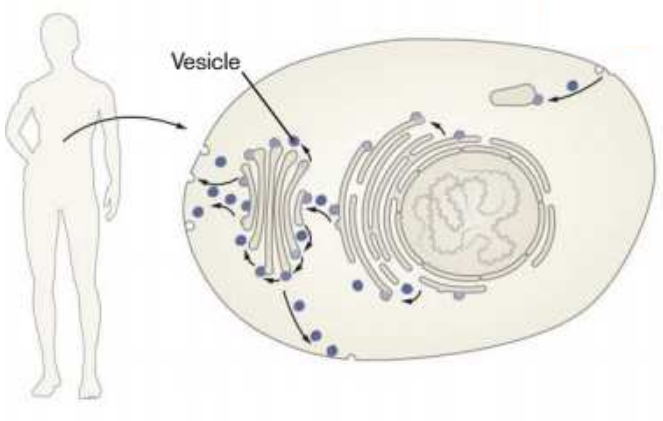
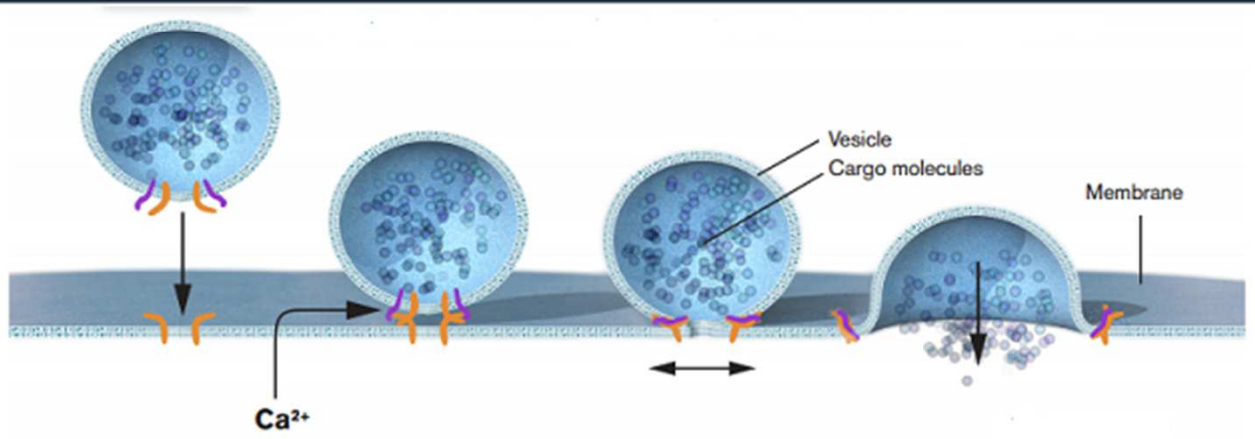
DISCOVERIES OF MACHINERY REGULATING VESICLE TRAFFIC, A MAJOR TRANSPORT SYSTEM IN OUR CELLS

Scientific Background on the Nobel Prize in Medicine 2013



Daniela Scalet 6/12/2013



The Nobel Prize in Medicine 2013 is awarded to J.E. Rothman, R.W. Schekman, T.C. Sudhof for studies of machinery regulating vesicle traffic, a major transport system in our cells

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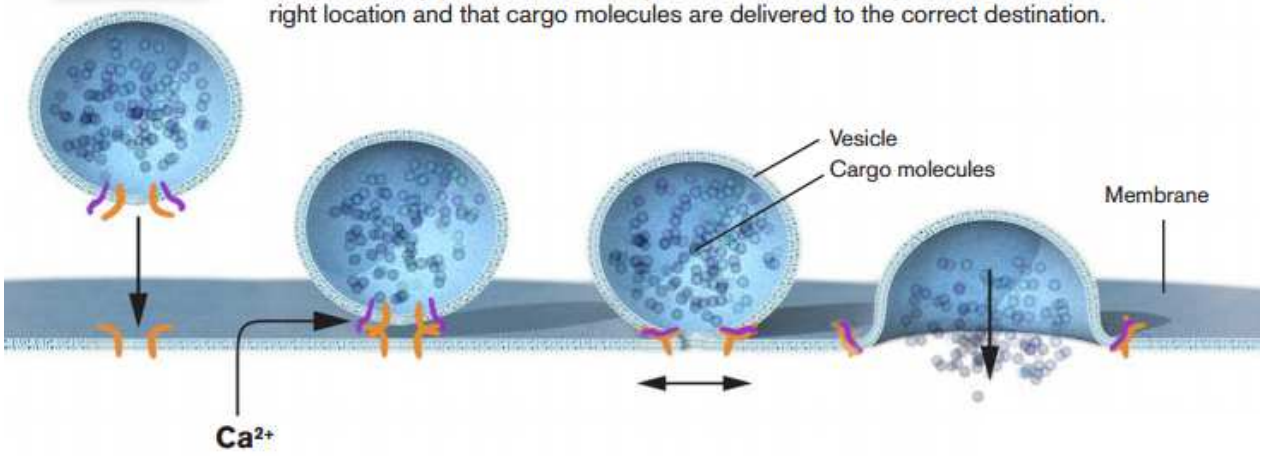
http://www.youtube.com/watch?v=X9qKTXnLzME

James E. Rothman

James E. Rothman discovered that a protein complex (pictured in orange) enables vesicles to fuse with their target membranes. Proteins on the vesicle bind to specific complementary proteins on the target membrane, ensuring that the vesicle fuses at the right location and that cargo molecules are delivered to the correct destination.



Randy W. Schekman

Randy W. Schekman discovered genes encoding proteins that are key regulators of vesicle traffic. Comparing normal (left) with genetically mutated yeast cells (right) in which vesicle traffic was disturbed, he identified genes that control transport to different compartments and to the cell surface.

Thomas C. Südhof

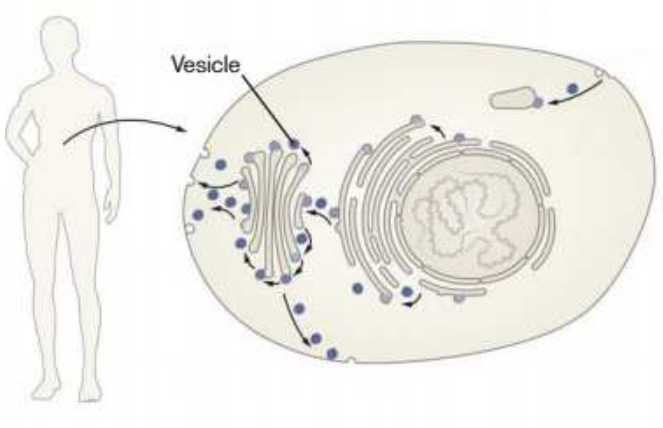
Thomas C. Südhof studied how signals are transmitted from one nerve cell to another in the brain, and how calcium controls this process. He identified molecular machinery (pictured in purple) that senses calcium ions (Ca^{2+}) and triggers vesicle fusion, thereby explaining how temporal precision is achieved and how signaling substances can be released from the vesicles on command.

Introduction

Each cell in the body has complex organization where specific cellular functions are separated into different compartments called organelles.

Molecules produced in the cell are packaged in vesicles and transported with special and temporal precision to the correct locations within and outside the cell.

Mysteries of cellular compartmentalization have long intrigued scientists



How are molecules, including hormonestransport proteins, and neurotrasmitters, correctly routed to their appropiate destination?

IDENTIFICATION OF GENES FOR VESICLE FUSION USING YEAST GENETICS

Randy W. Schekman

- Use yeast genetics to dissect the mechanism involved in membrane and vesicle trafficking

- Baker's yeast (*Saccharomyces cerevisiae*) secrets glycoproteins and that could be use to study vesicle transport and fusion

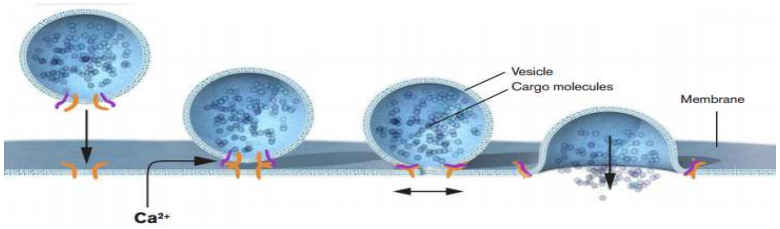
- Divised a genetic screen to identify genes regulating intracellular transport

- Some of the mutations may be lethal, and to bypass the lethality problem, he used temperature-sensitive mutants and screened for genes affecting the intracellular accumulation of secretory enzymes

Results: initially he identified two genes ***sec1*** and ***sec2.*** All genes are 23 divided into three classes based on the accumulation (ER, Golgi complex or cell surface).
Sec 17 and ***sec18*** mutants accumulated small vesicles implicating a role in vesicle fusion.

A BIOCHEMICAL JOURNEY TO IDENTIFY KEY PROTEINS IN THE FUSION PROCESS

James E. Rothman



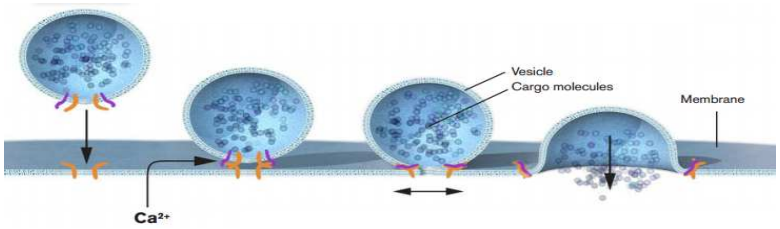
- He developed an in vitro reconstituition assay to dissect events involved in intracellular vesicle transport. He purified essential components of the vesicle fusion process

- System based on vesicular stomatitis virus (VSV) that produced large amounts of viral protein (VSV-G protein) in infected cells
- VSV-G protein is marked by sugar modification when it reaches Golgi -> identify when it reaches its destination
- Use this assay to study both vesicle budding and fusion and purified proteins from cytoplasm that were required for transport:

- **NSF** (N-ethylmaleimide-sensitive factor)
- **SNAPs** that bind to membranes and assist in the recruitment of NSF

A BIOCHEMICAL JOURNEY TO IDENTIFY KEY PROTEINS IN THE FUSION PROCESS

James E. Rothman



Rothman's and Schekman's work:

- *Sec18* corresponded to NSF
- *Sec17* was cloned and provided evidence of its functional equivalence to SNAP

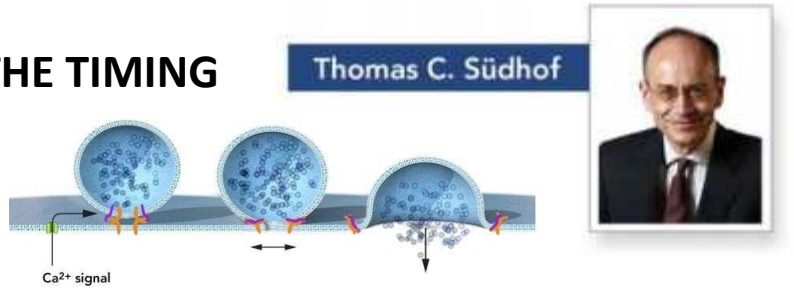
From brian he purified **SNAREs** (soluble NSF-attachment protein receptor):

-VAMP/Synaptobrevin
-SNAP-25
-sintaxin

} the same stechiometric amounts. They functioned toghether in vescicle and target membrane

SNARE hypothesis: target and vesicle SNAREs (t-SNARE and v-SNARE) were critical for vesicle fusion

IDENTIFICATION OF GENES CONTROLLING THE TIMING OF VESICLE FUSION



- He set out to study how synaptic vesicle fusion was controlled.
- He was intrigued by the rapid exocytosis of synaptic vesicles, which is under tight temporal control and regulated by the changes in the cytoplasmic free calcium concentration

SÜDOF ELUCIDATE HOW CALCIUM REGULATES NEUROTRASMITTER RELEASE IN NEURONS AND DISCOVERED THAT **SYNAPTOTAGMIN** IS A CRITICAL PROTEIN IN CALCIUM MEDIATED VESICLE FUSION

Synaptotagmin-1: coupled calcium to neurotrasmitter release; interacts with phospholipids in a calcium-dependent manner. Role: calcium sensor for rapid synaptic fusion

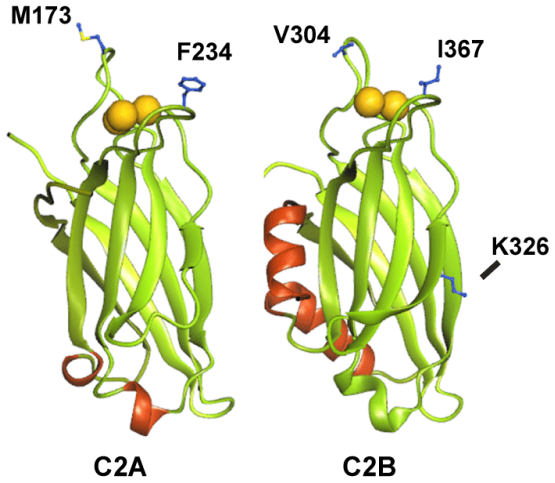
Munc18-1 (sec1 Schekman): SM protein are known to be an integral part of the vesicle fusion protein complex, along with the SNARE. Deletion of Munc18-1 leads to a complete loss of neurotrasmitter secretion from synaptic vesicles.

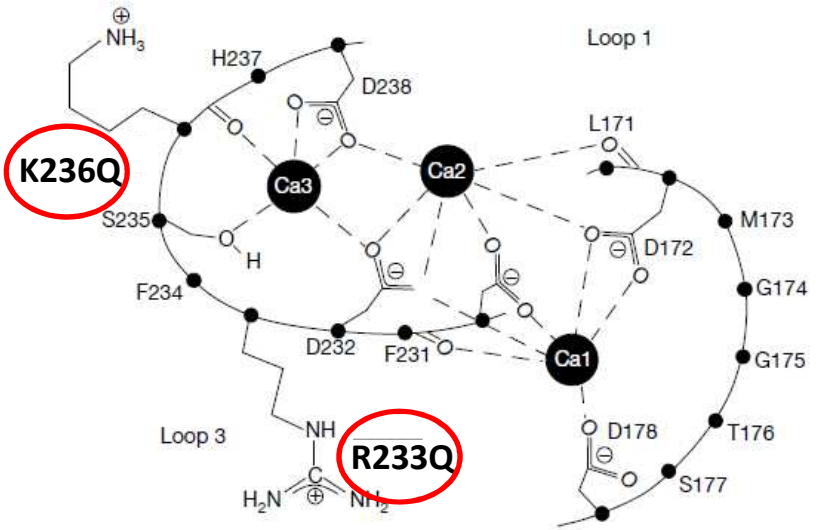
HOW VESICLE FUSION IS TEMPORALLY CONTROLLED AND HOW CALCIUM LEVELS REGULATED NEUROTRASMITTER RELEASE

Synaptotagmin I functions as a calcium regulator of release probability

Rafael Fernández-Chacón*†‡, Andreas Königstorfer‡§, Stefan H. Gerber*, Jesús García‖, Maria F. Matos*, Charles F. Stevens¶, Nils Brose§, Josep Rizo‖, Christian Rosenmund† & Thomas C. Südhof*

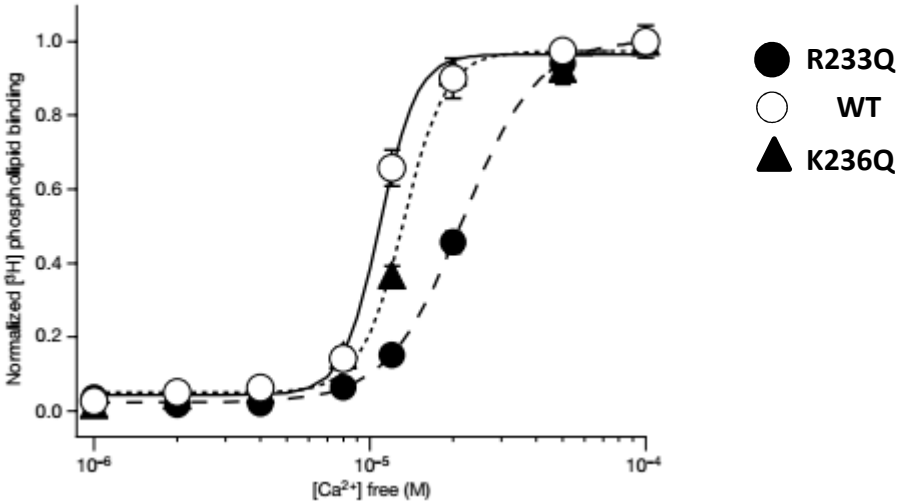
Double C2 domains of Synaptotagmin-1



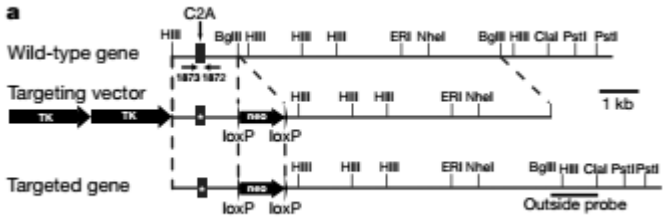


Two point mutations in synaptotagmin I that changes the Ca^{2+} - binding affinity of synaptotagmin I without affecting its three-dimensional structure

R233Q decreases the overall Ca^{2+} affinity of the C2A domain



Generation of mutant mice

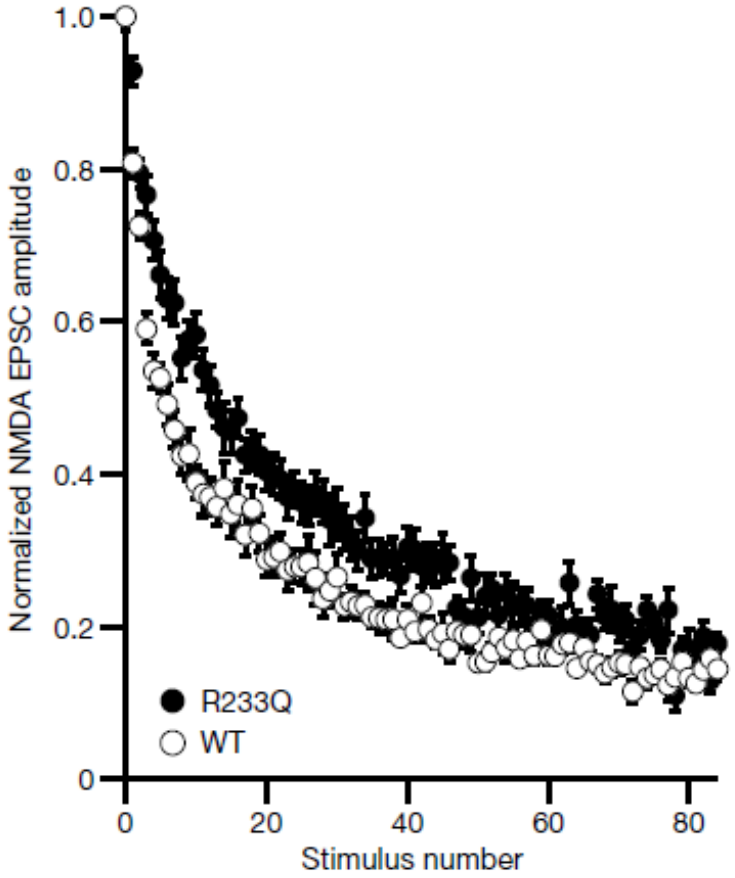


R233Q selectively impairs nerotransmitter release

EPSC= excitatory postsynaptic currents

Measure of the rate of inhibition of NMDA-receptor-dependent EPSC by MK-801

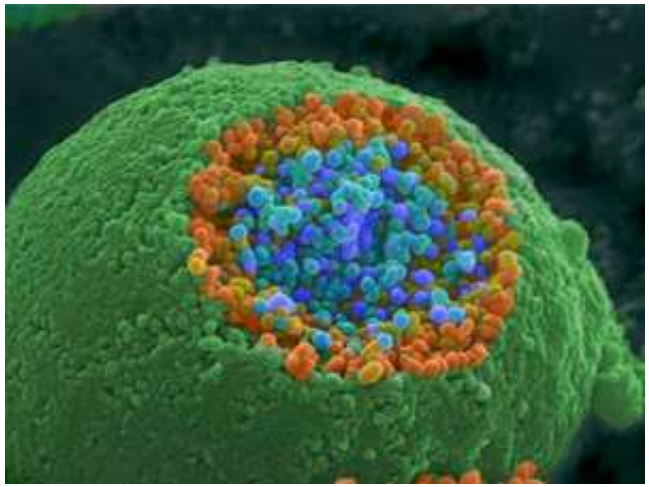
MK-801 caused a multi exponential decay in the EPSC amplitude



The decay was significantly lower in R233Q mutant neurons

The results show that a mutation in synaptotagmin I that selectively decreases its apparent Ca^{2+} affinity corresponding decrease in the Ca2+ sensitivity of neurotransmitter release, suggesting that Ca2+ binding to Synaptotagmin I directly determines Ca^{2+} triggering of exocytosis.

VESICLE FUSION AND ITS IMPORTANCE FOR MEDICINE



These discoveries have had a major impact on our understanding of how molecules are correctly sorted to precise location in cell;

Defects at any any number of steps are associated with disease:
-type 2 diabetes
-mutations in gene encoding the protein in the vesicle fusion machinery like MUNC-18-1 in epilepsy
-In *Clostridoum botulinum* the majority of the toxin types cleave SNAP-25, VAMP/Synaptobrevin and Syntaxin
-The tetanus neurotoxin targets VAMP/ Synaptobrevin in inhibitory interneurons and blocks release of GABA

CONCLUSIONS

They discoveries have had a major impact on our understanding of how cellular comunication occours to sort molecules to precise locations within and outside the cell