ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Medicine and Surgery

A.Y. 2025/2026 - Programme Code: 6734

84284-Signaling pathways in health and disease I.C.

Module A – 84285 **Cell signaling** 4 CFU, 34 hrs – BIOS-07/A

+ asynchronous online activities (16 hrs)

Lecture A.04
Biosynthesis and secretion of insulin

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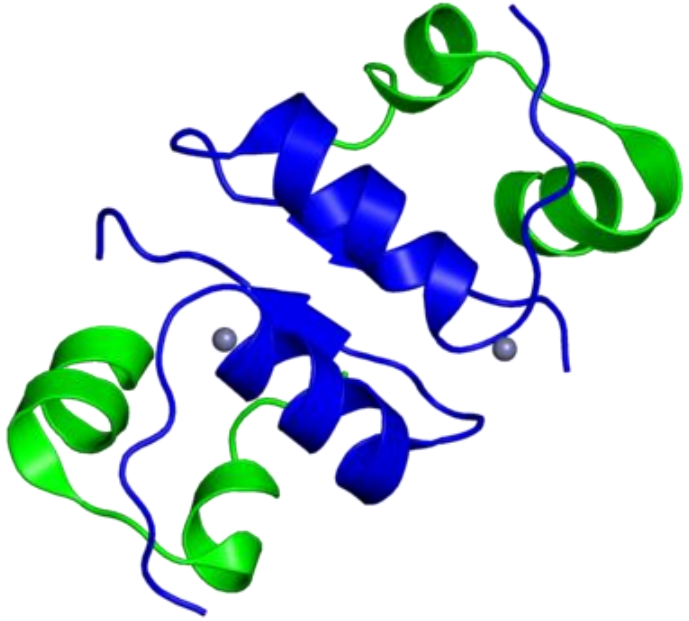
FREDERICK SANGER

The chemistry of insulin

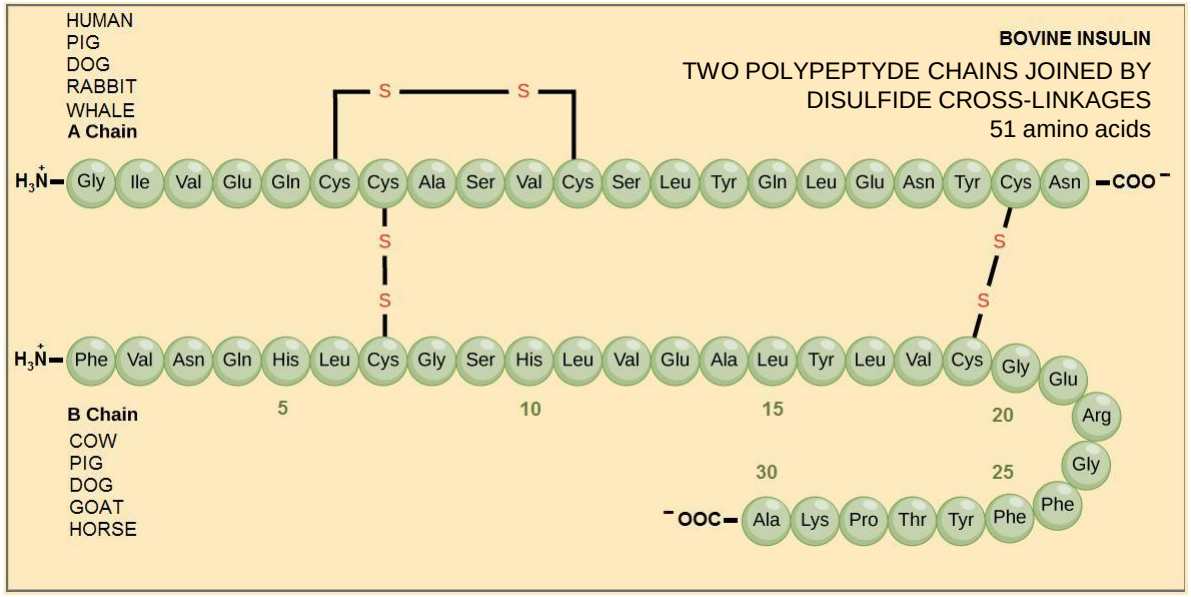
Nobel Lecture, December 11, 1958

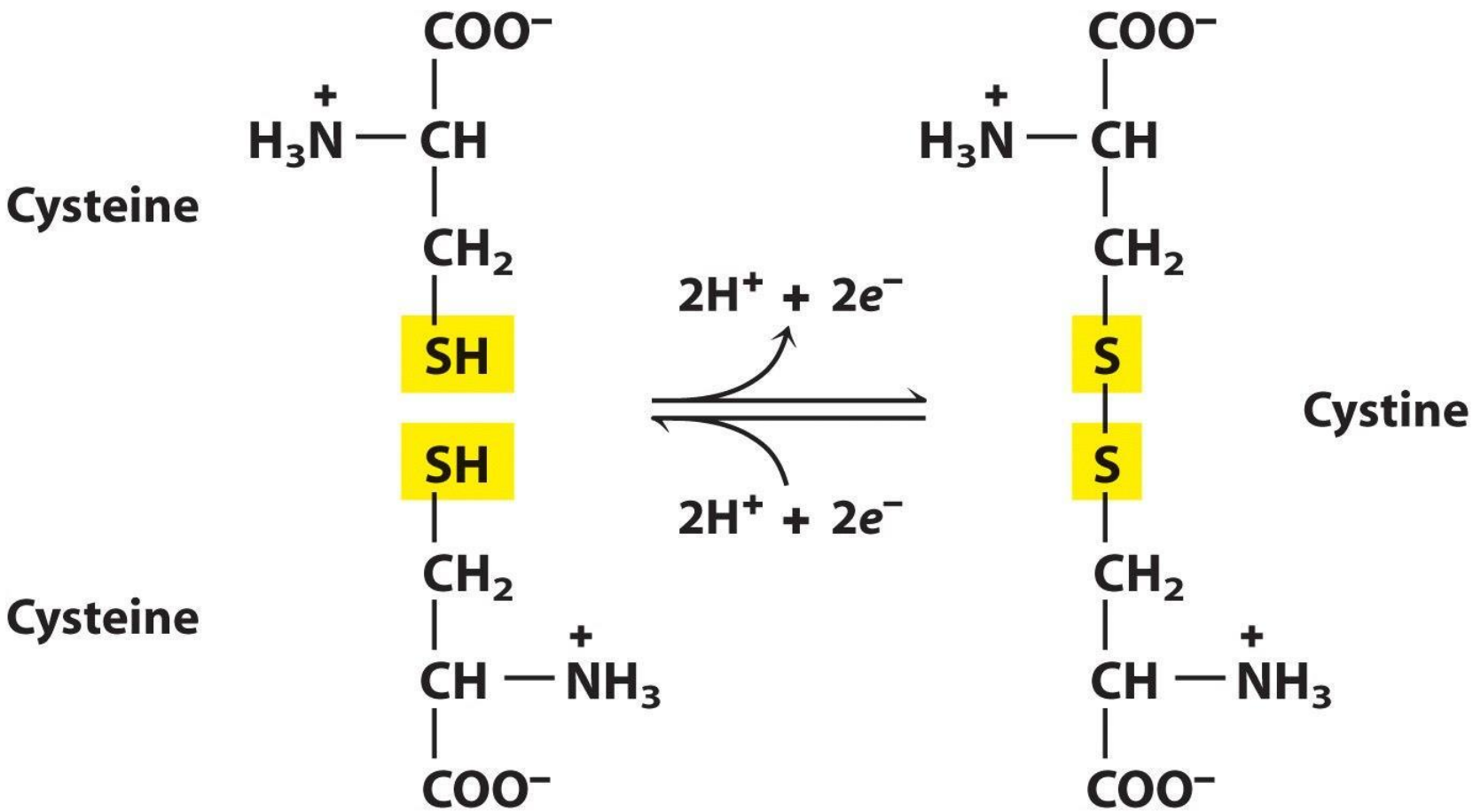
It is great pleasure and privilege for me to give an account of my work on protein structure and I am deeply sensitive of the great honour that has been done to me in recognizing my work in this way. Since the work on insulin has extended over about 12 years it will be necessary to give a somewhat simplified account and to omit most of the work that did not contribute directly to the main problem, the determination of the structure of a protein.

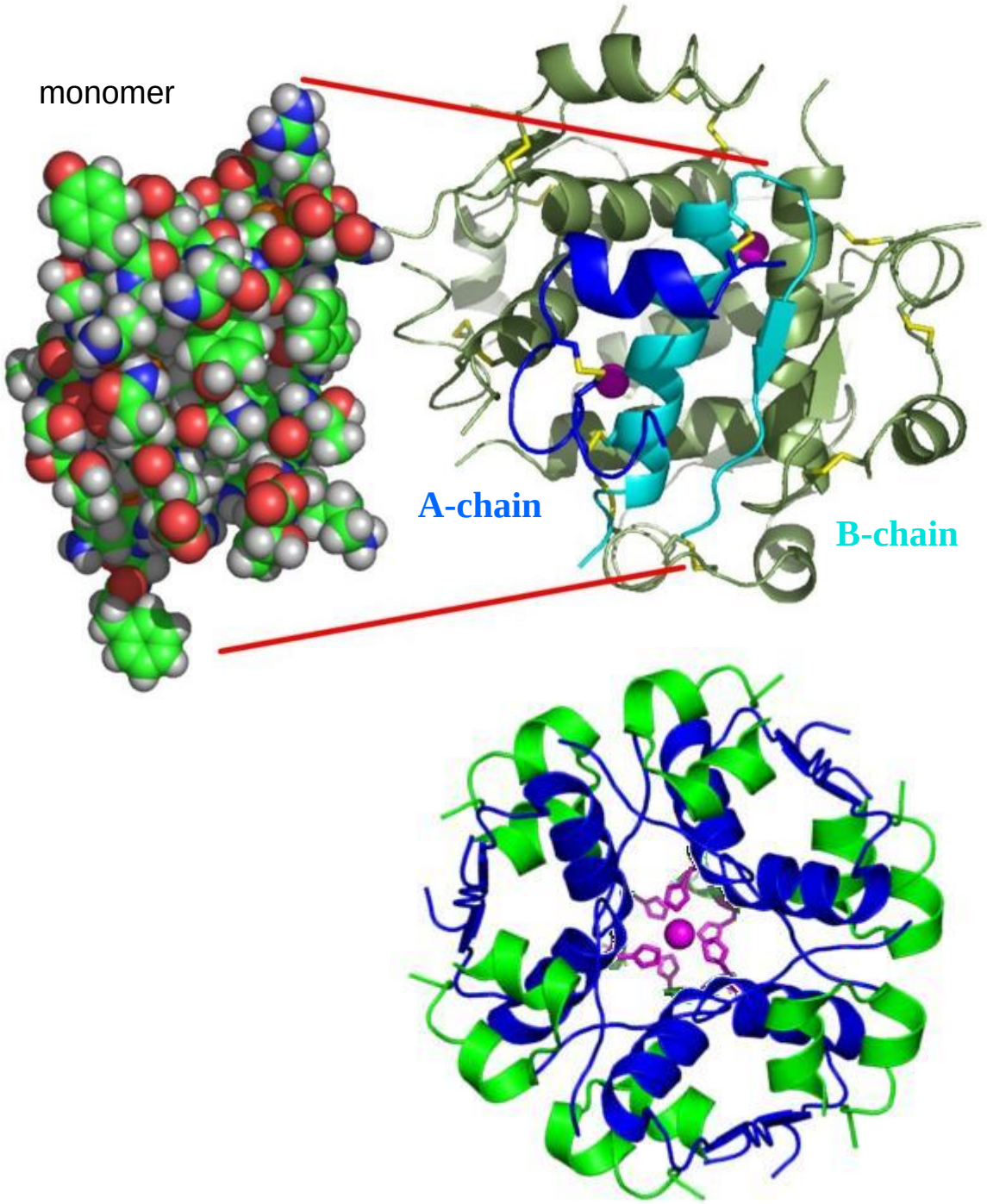


The structure revealed that the longer chain (B chain) forms an α-helix and a β-strand, whereas the A chain consists of two α-helices. These chains are linked by two disulfide bonds.
This is the mature form of the insulin molecule, as biosynthesized by the pancreas.

The two disulfide-linked polypeptides associate with another insulin molecule to form **a homodimer**




Cysteine
COO⁻
+
H₃N — CH
CH₂
SH
SH
CH₂
CH — NH₃
+
COO⁻
Cysteine
2H⁺ + 2e⁻
2H⁺ + 2e⁻
COO⁻
+
H₃N — CH
CH₂
S
S
CH₂
CH — NH₃
+
COO⁻
Cystine

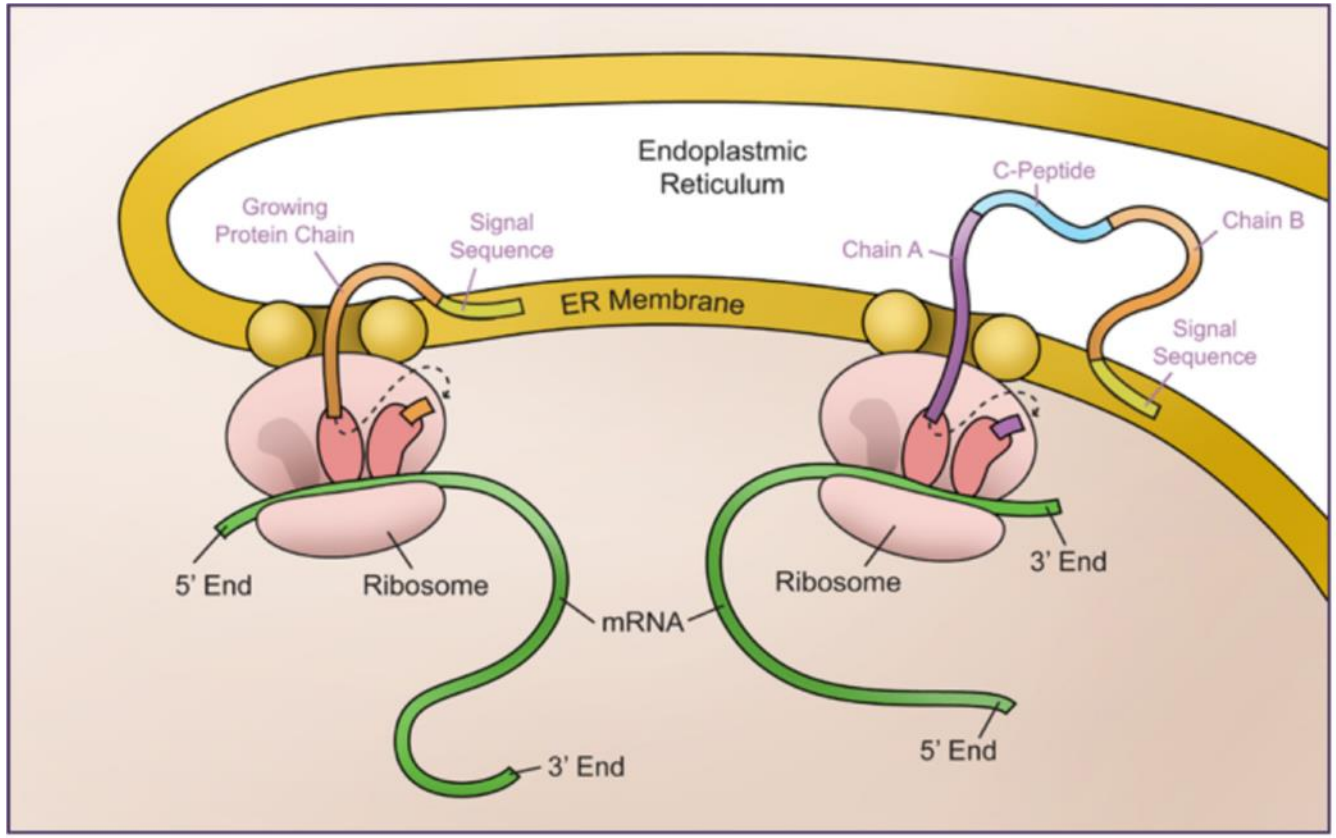


The two disulfide-linked polypeptides associate with another insulin molecule to form **a homodimer**

and the latter aggregates with two additional dimers to form **a hexamer**.

Within the pancreatic beta cells, ***proinsulin*** is stored in such hexameric, inactive and very stable form before release.

A-chain: 21 amino acids
C-peptide: 31(+4) amino acids
B-chain: 30 amino acids
Signal sequence: 23(+1) aa

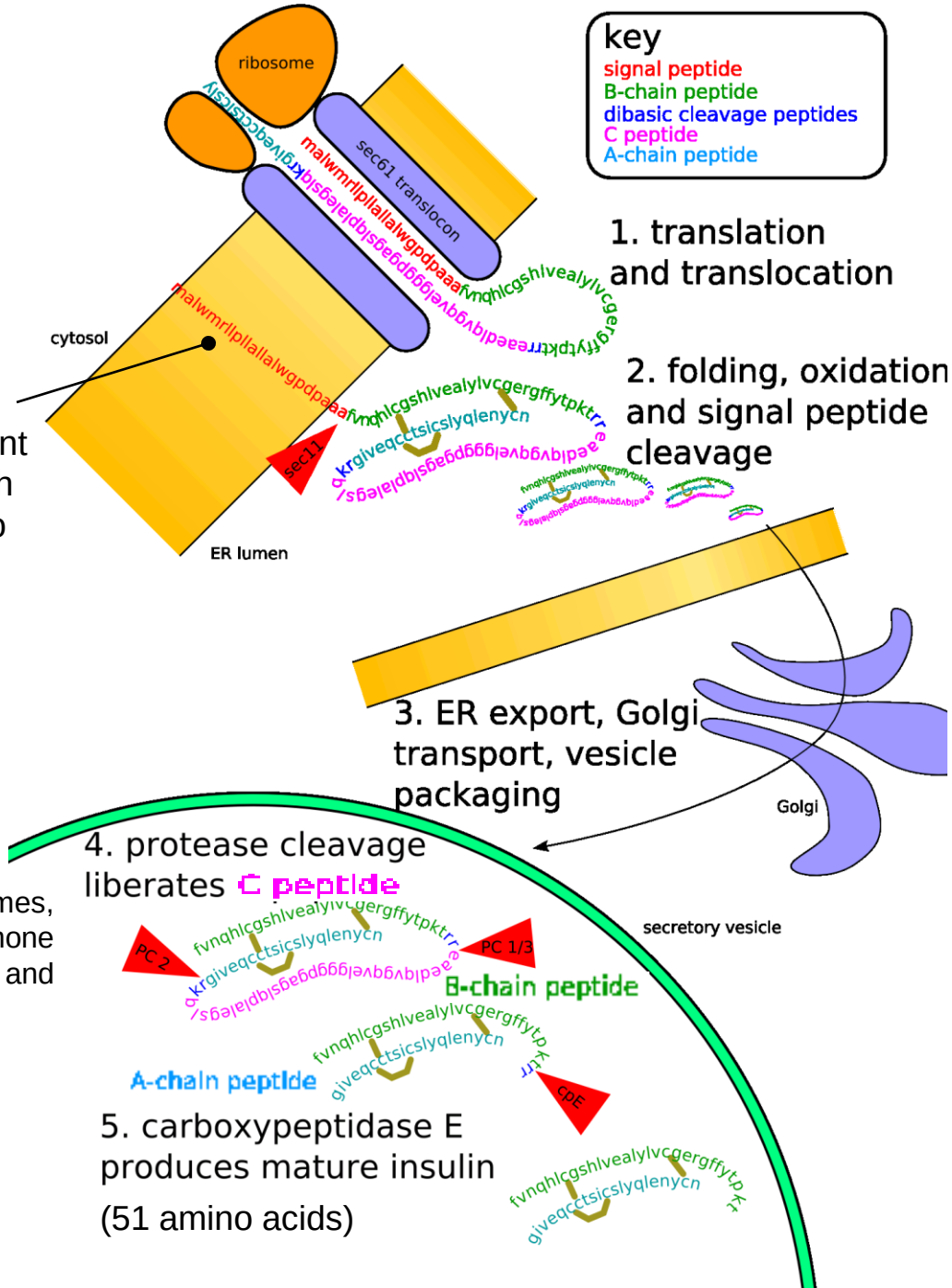


TRANSLATION and TRANSLOCATION OF PRE-PROINSULIN
(primary product of the INS gene**)**

SCAN ME

https://pdb101.rcsb.org/motm/14

SIGNAL SEQUENCE
a 23 amino acid segment at the N-terminus, which directs the passage into secretory vesicles

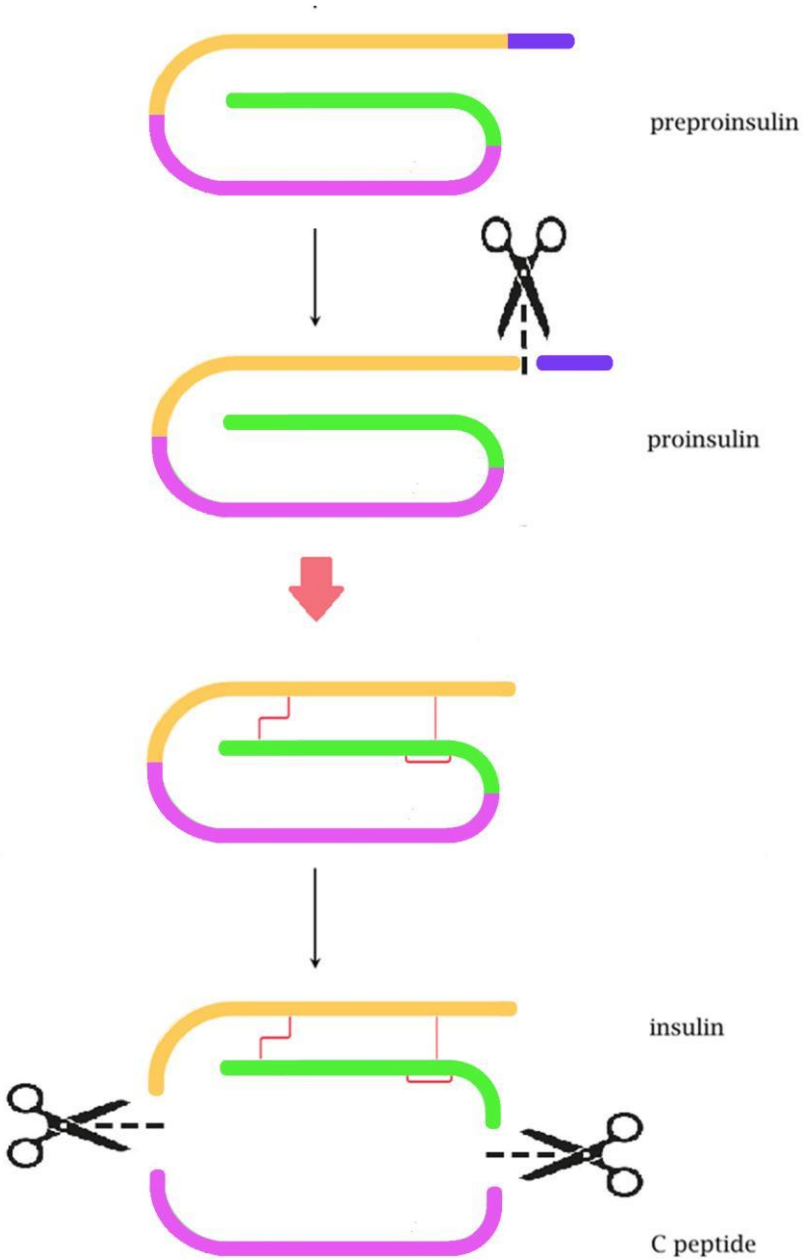


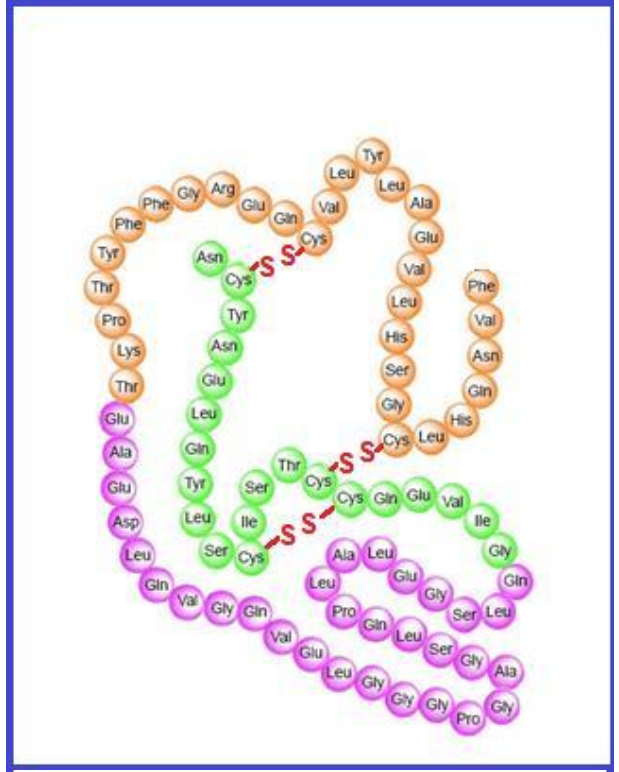
proteolytic enzymes, known as prohormone convertases (PC-1 and PC-2)

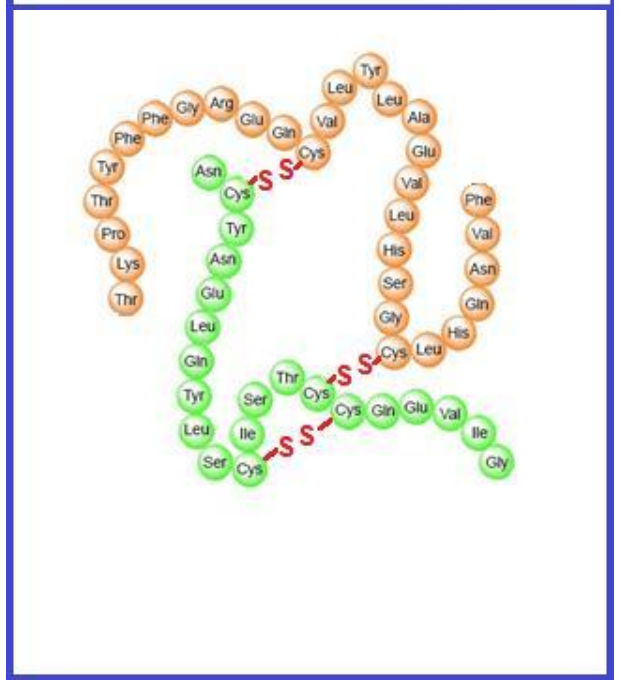
pre-proinsulin (110 amino acids)

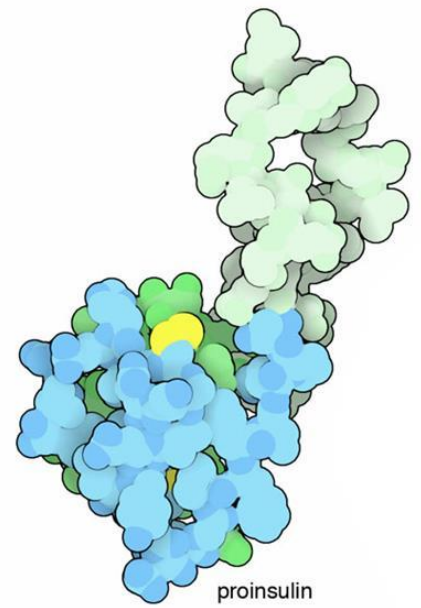
Proteolytic removal (sec11) of the sequence signal and formation of 3 disufide bonds produces ***proinsulin***, which is stored, in hexamer form (not shown), in the secretory granules.

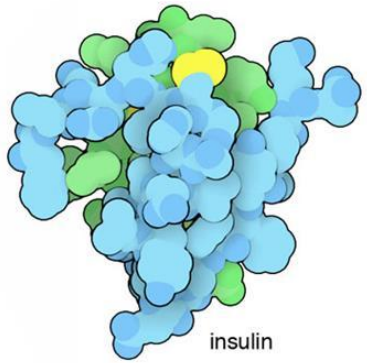
WHEN BLOOD GLUCOSE IS SUFFICIENTLY ELEVATED, proinsulin is converted to ***mature*** *(active)* ***insulin*** by proteases (PC-1, PC-2) and is released into the blood by exocytosis, mixed in equimolar amounts with the ***C-peptide*** (31 amino acids).







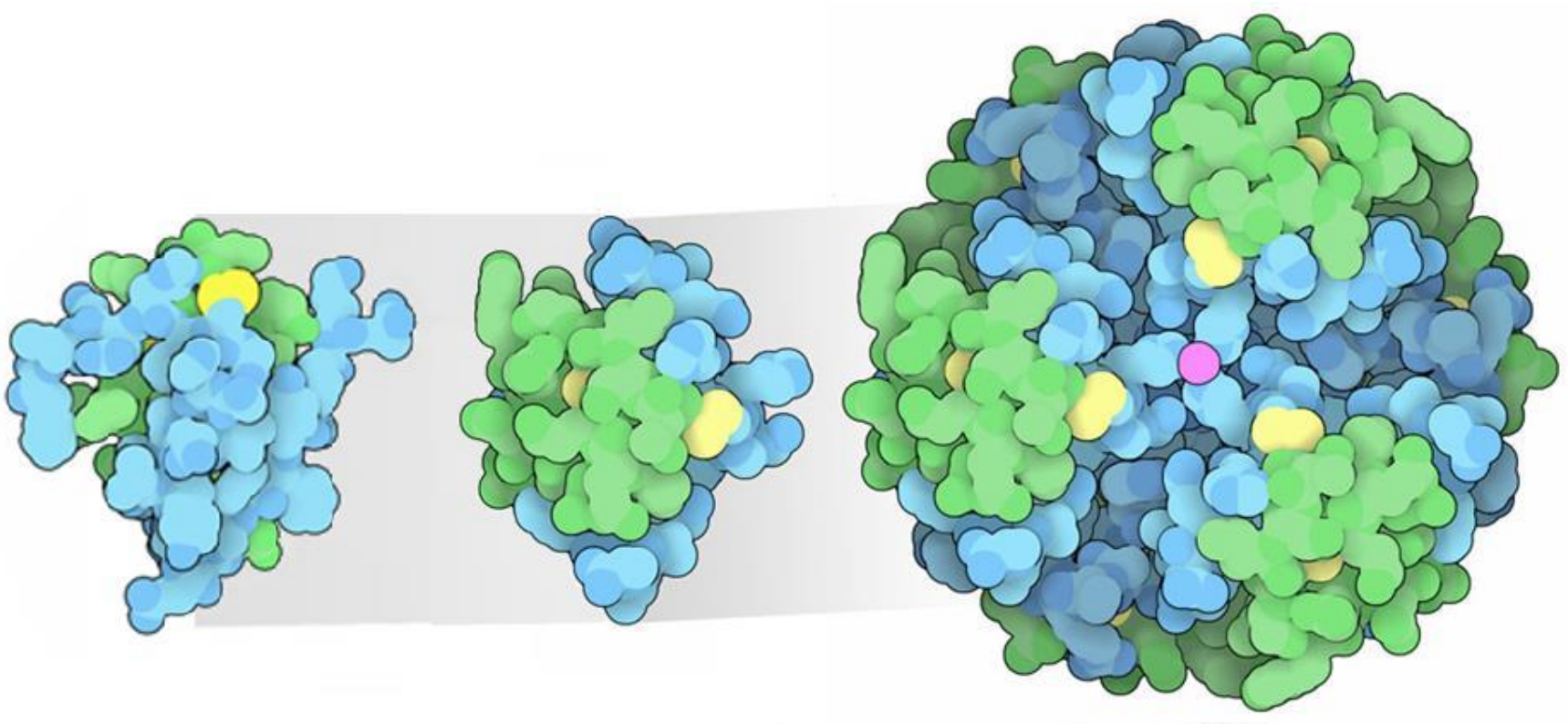


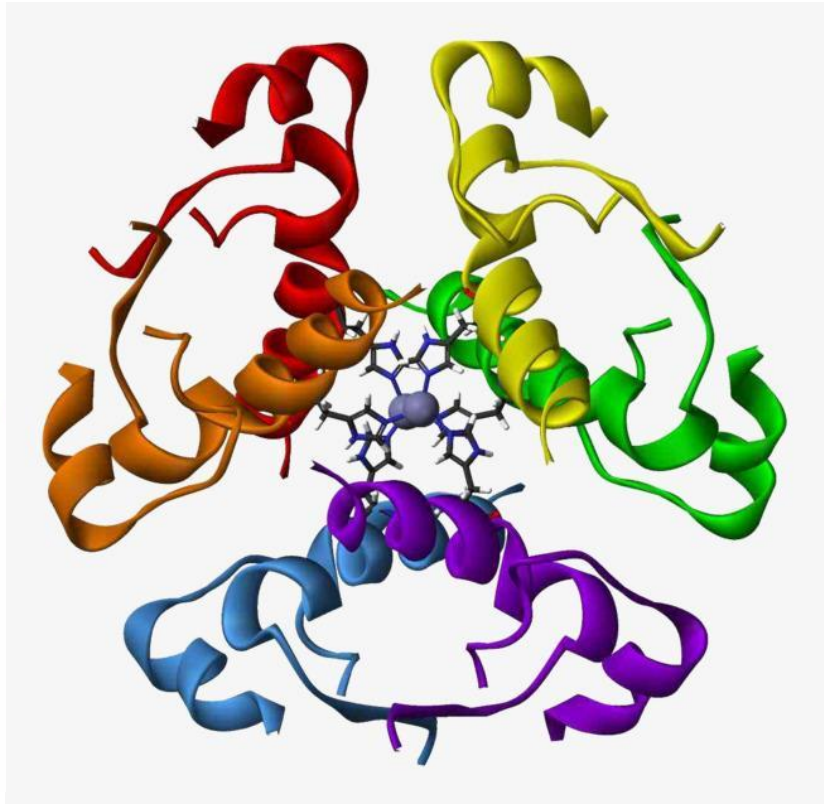


Three-dimensional confromation of the protein (tertiary structure)

A-chain, green; B-chain, cyan; disulfide bonds, yellow

INSULIN
(quaternary structure)





monomer → dimer → hexamer
(secretory vesicles)

Further exploration

More than fifty years after the structure of insulin was first determined, and after four Nobel prizes, there are still new structures of insulin being deposited in the PDB, and amazingly, the structure of insulin bound to its receptor was only published in early 2013

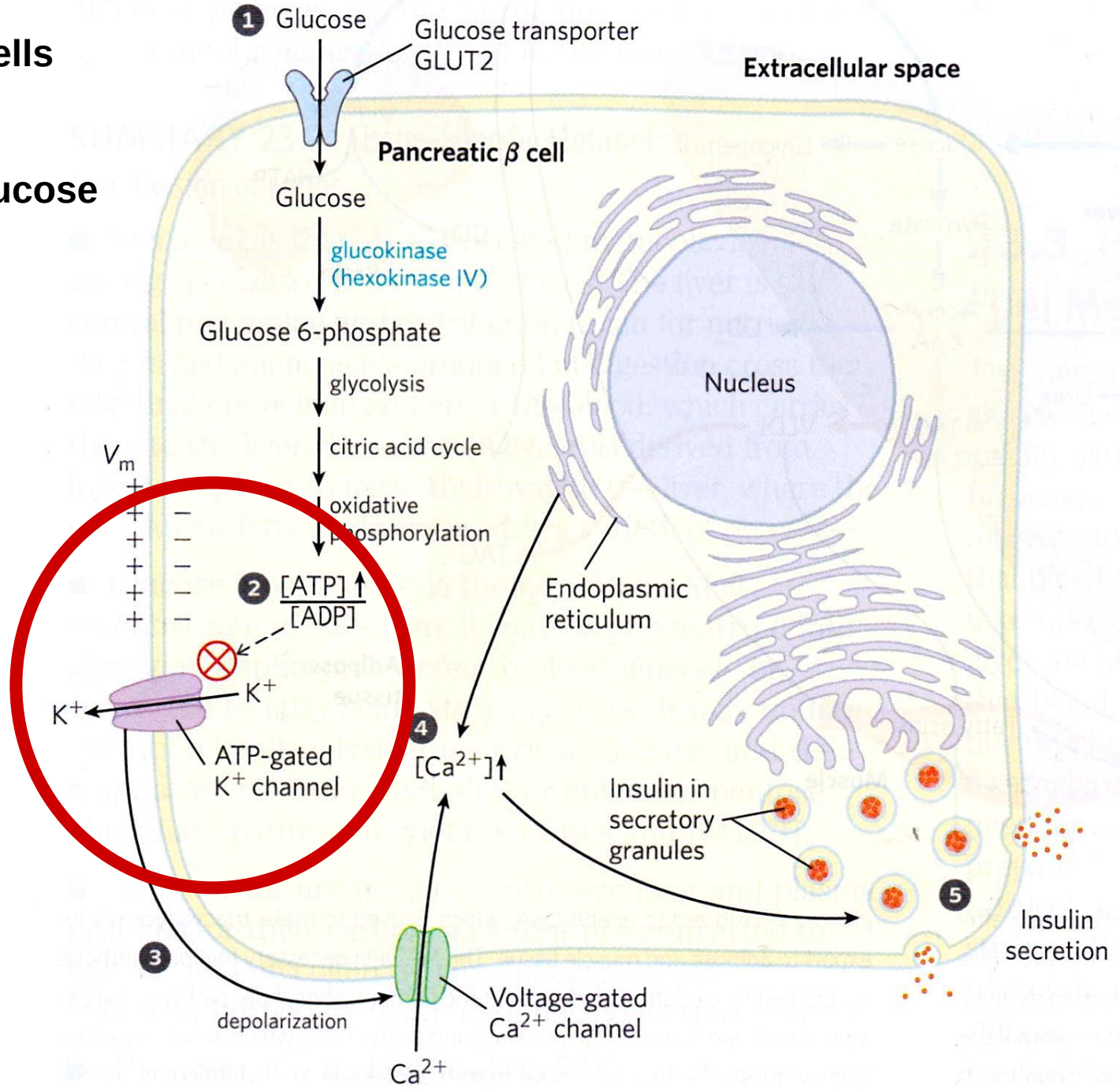
Frederick Sanger
1958 and 1980

Dorothy C. Hodgkin
1964

Frederick G. Banting & John Macleod
1923

https://pdb101.rcsb.org/global-health/diabetes-mellitus/drugs/insulin/insulin

Pancreatic beta-cells secrete insulin in response to changes in blood glucose

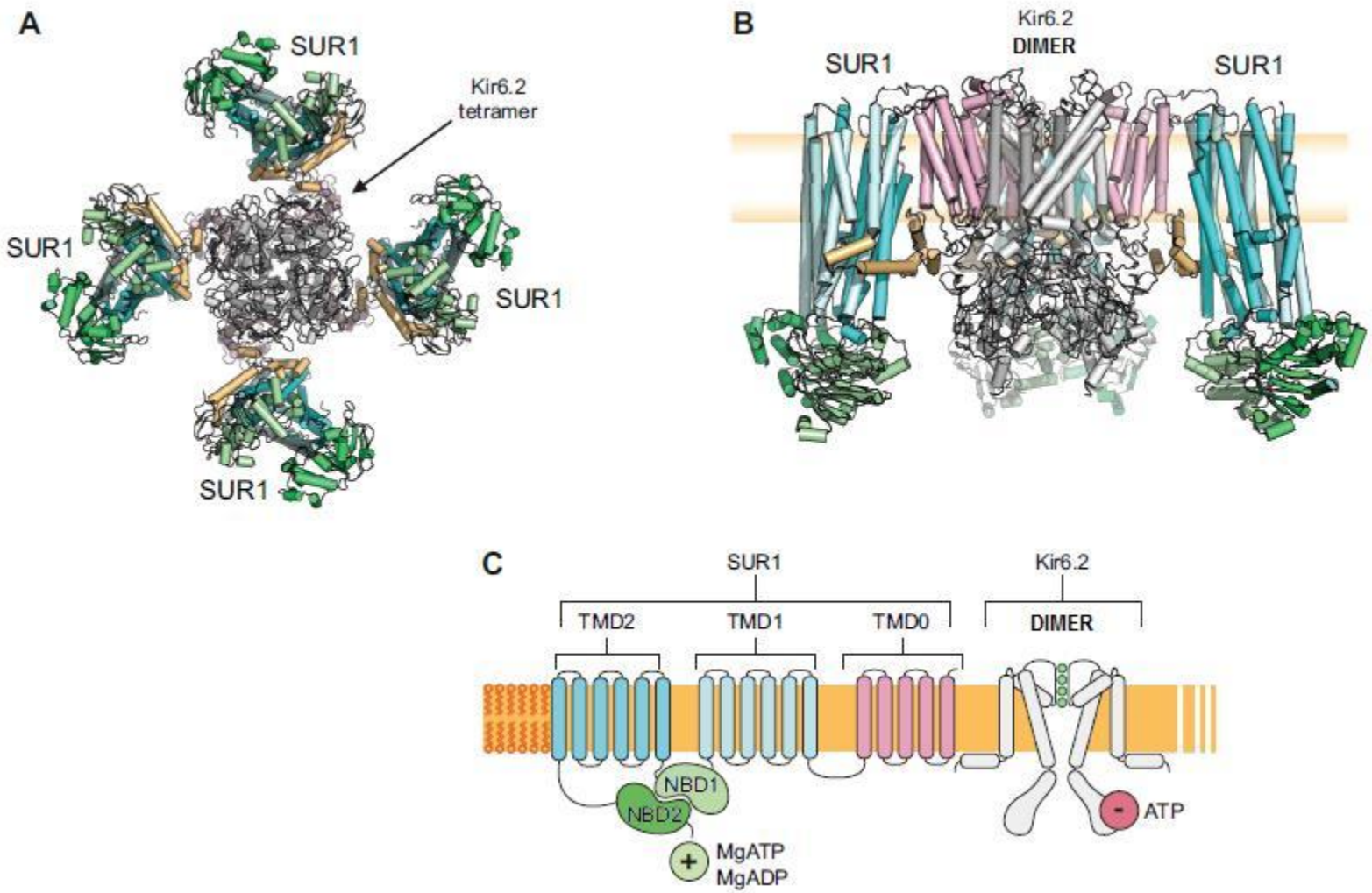


REMINDER

- GLUT transporters
- GLYCOLYSIS
- ATP
- LIGAND-GATED CHANNELS
- VOLTAGE-GATED CHANNELS
- Ca^{++}

Molecular structure of the β-cell ATP-sensitive potassium channel (K_{ATP})

The functional K_{ATP} exists as octamer comprising 4 Kir6.2 subunits and 4 SUR1 subunits.
In B, the front subunit has been removed for clarity.



Membrane topology showing one SUR1 and two Kir6.2 subunits.

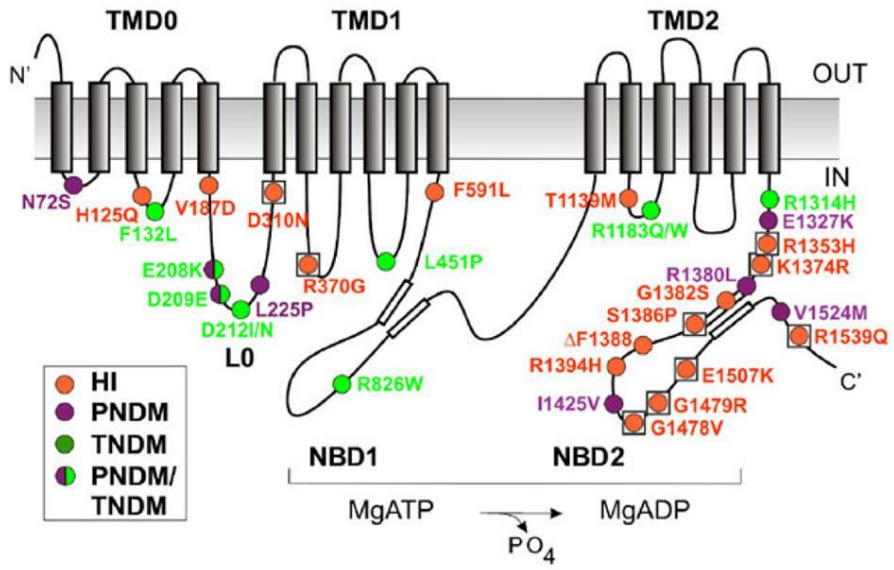
Binding of ATP (in red) to Kir6.2 inhibits channel activity by stabilizing the closed state of the pore. Each Kir6.2 subunit in the tetramer is capable of binding a molecule of ATP, and binding to one subunit is sufficient to cause channel closure → membrane depolarization

Paradoxically, K_{ATP} channels in the pancreatic β-cell are predicted to be largely closed, even at resting glucose concentrations, since ($K_{ATP,1/2}$) in isolated membrane patches is ~10 μM, yet cellular ATP concentrations in the energized cell reside in the low mM range.
However, measured K_{ATP} activity "on-cell" is significantly higher, likely reflecting the net stimulatory input from MgADP that reduces ATP-inhibition in the cellular milieu.
At 5 mM glucose, the conductance (open pores) is 7% of maximal, and this falls to 3% of maximal when glucose is increased to 10mM. Nevertheless, this tiny change in conductance can cause a marked change in membrane potential.

The SUR1 subunit, a member of the ABC superfamily, confers sensitivity of K_{ATP} to stimulatory Mg^{2+}-nucleotides (MgADP and MgATP).
The conformational changes associated with SUR1 MgATP binding → hydrolysis at the NBDs are presumably transduced to the activation gates of Kir6.2, stabilizing the opening state of the channel pore.

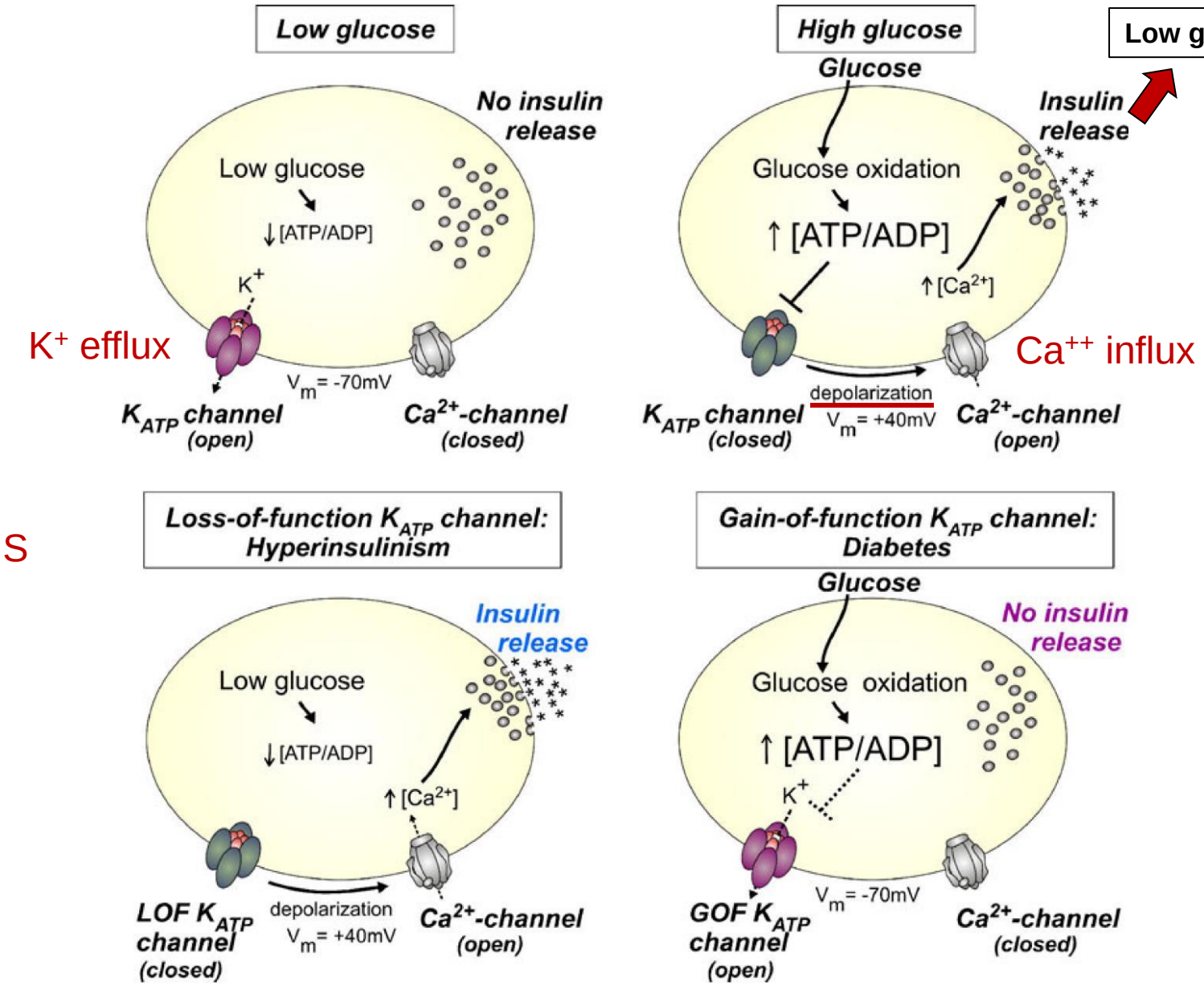
Molecular structure of the β-cell ATP-sensitive potassium channel (K_{ATP})

*Selected **SUR1 loss-of-function** (=closed K_{ATP} pore) mutations in congenital hyperinsulinism (HI) are shown in orange*
*Selected **SUR1 gain-of-function** (=open K_{ATP} pore) mutations in permanent or transient neonatal diabetes mellitus (PNDM and TNDM) are shown in purple or green, respectively*

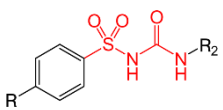


ATP-sensitive potassium channel (K_{ATP})

Pflugers Arch - Eur J Physiol (2010) 460:307–320



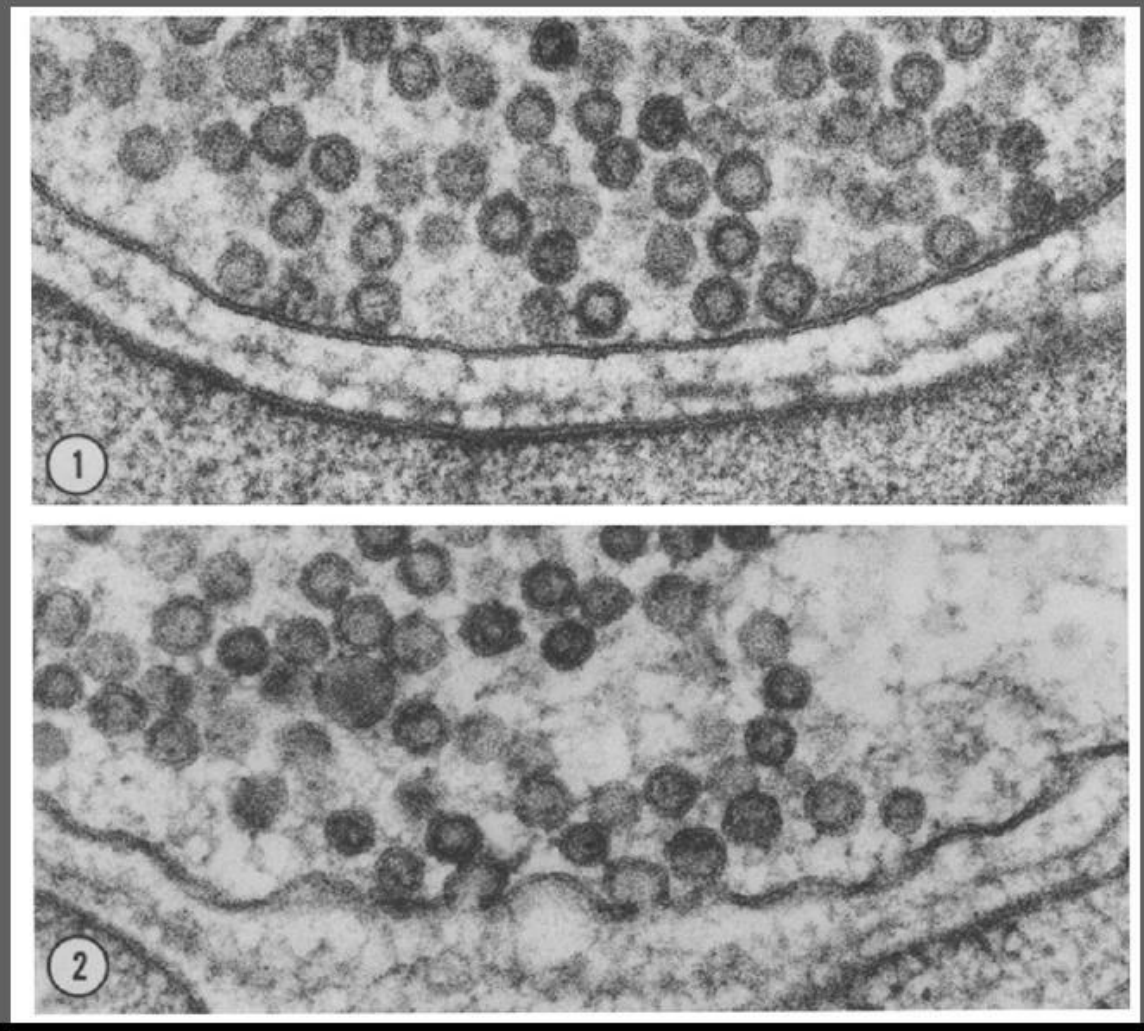
RARE MUTATIONS



Sulfonylurea

can bind and close the K_{ATP} channel

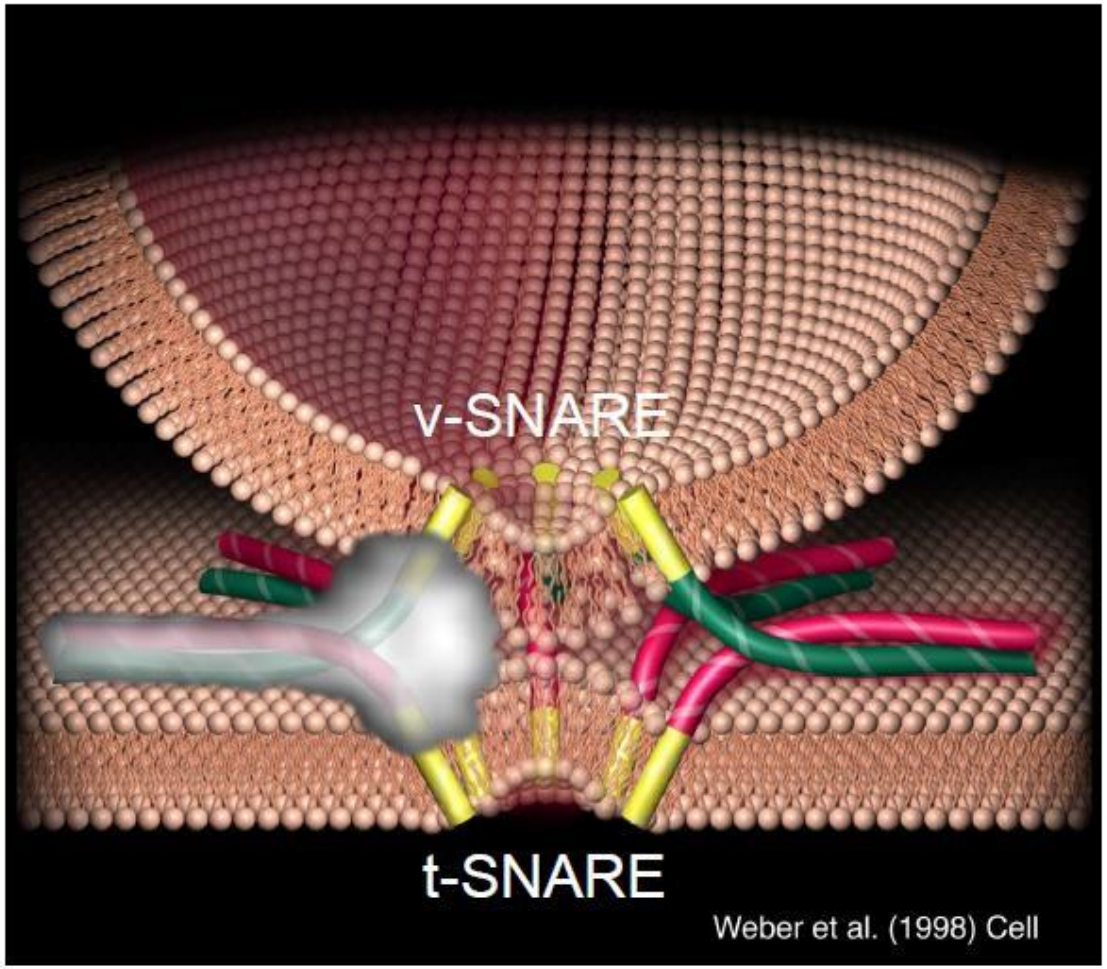
Quantal Release of Neurotransmitters by Fusion of Synaptic Vesicles at Nerve Terminals Triggered by Calcium Ion Entry in < 1 msec – How?



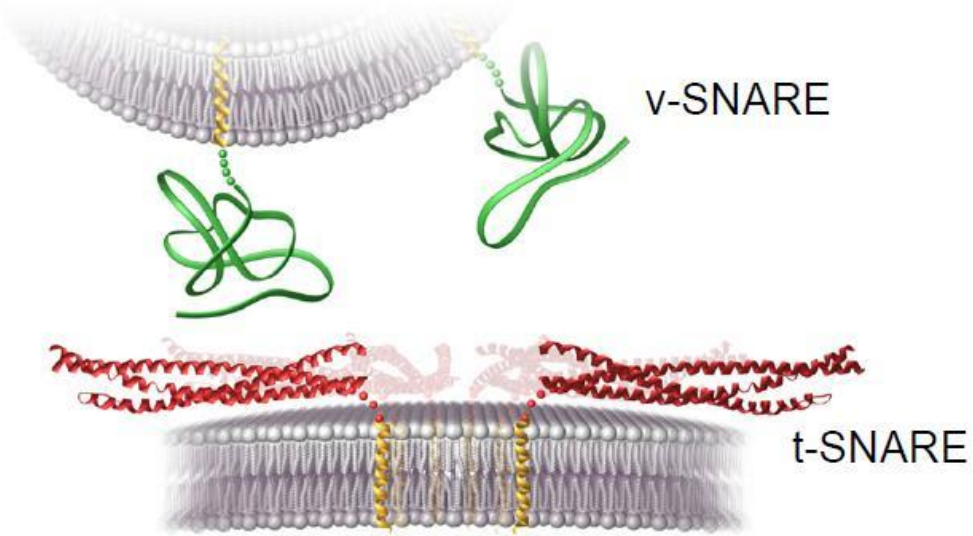
Fixed at rest

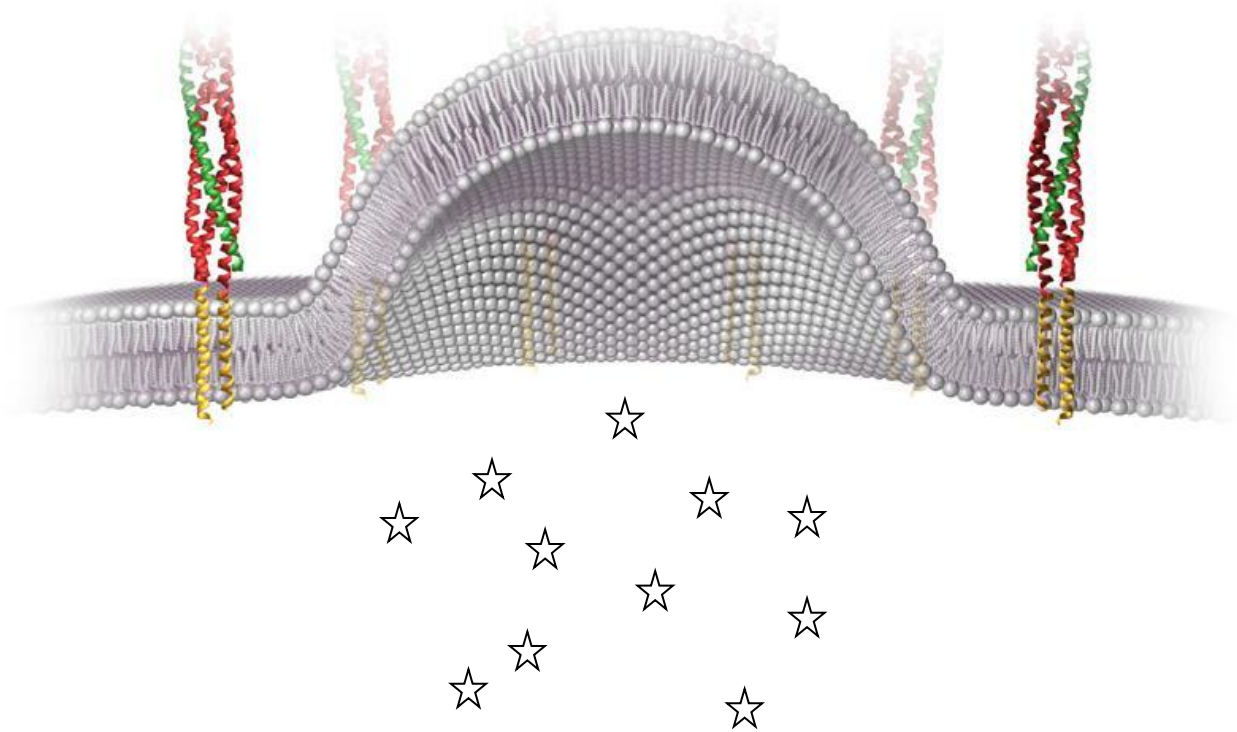
Fixed 5ms after stimulation

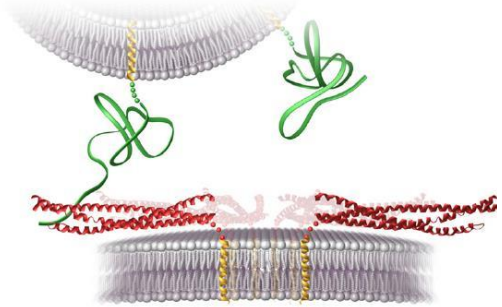
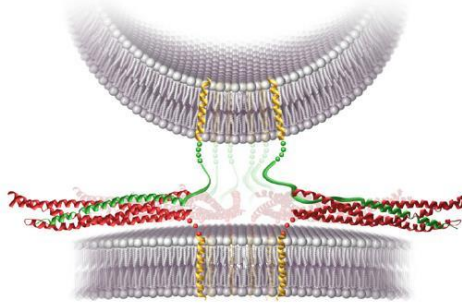
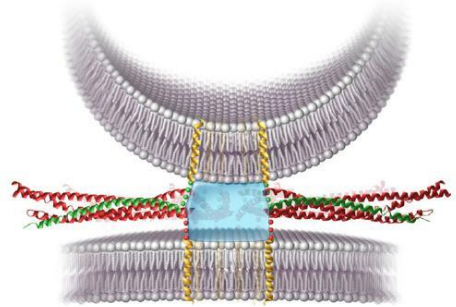
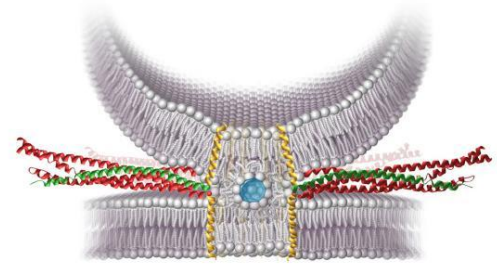
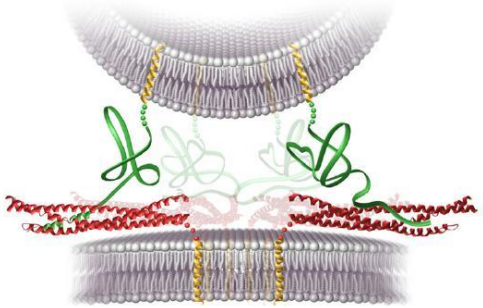
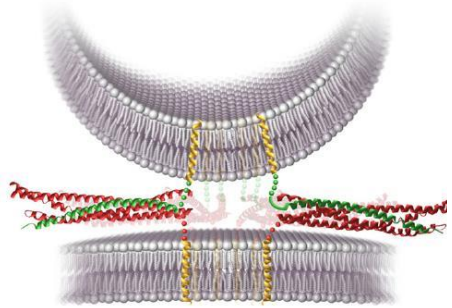
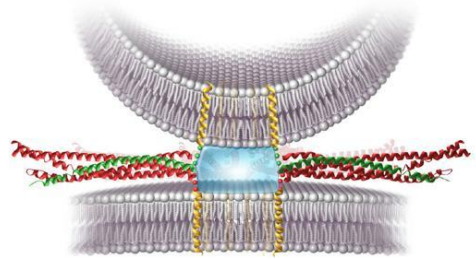
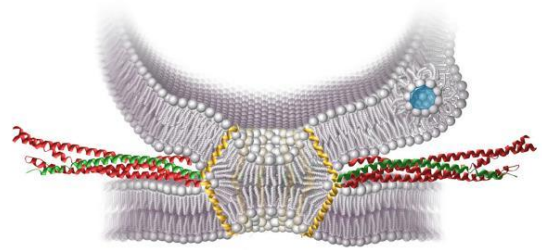
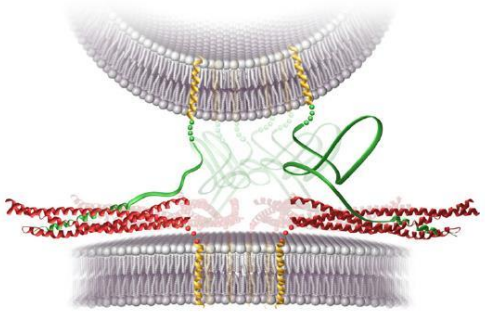
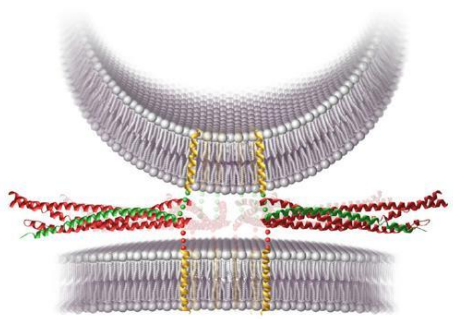
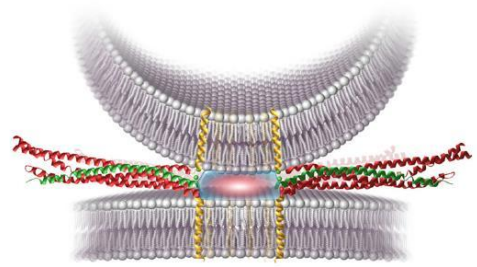
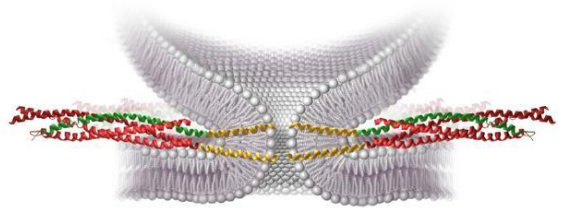
SNAREs – The Core Fusion Machinery



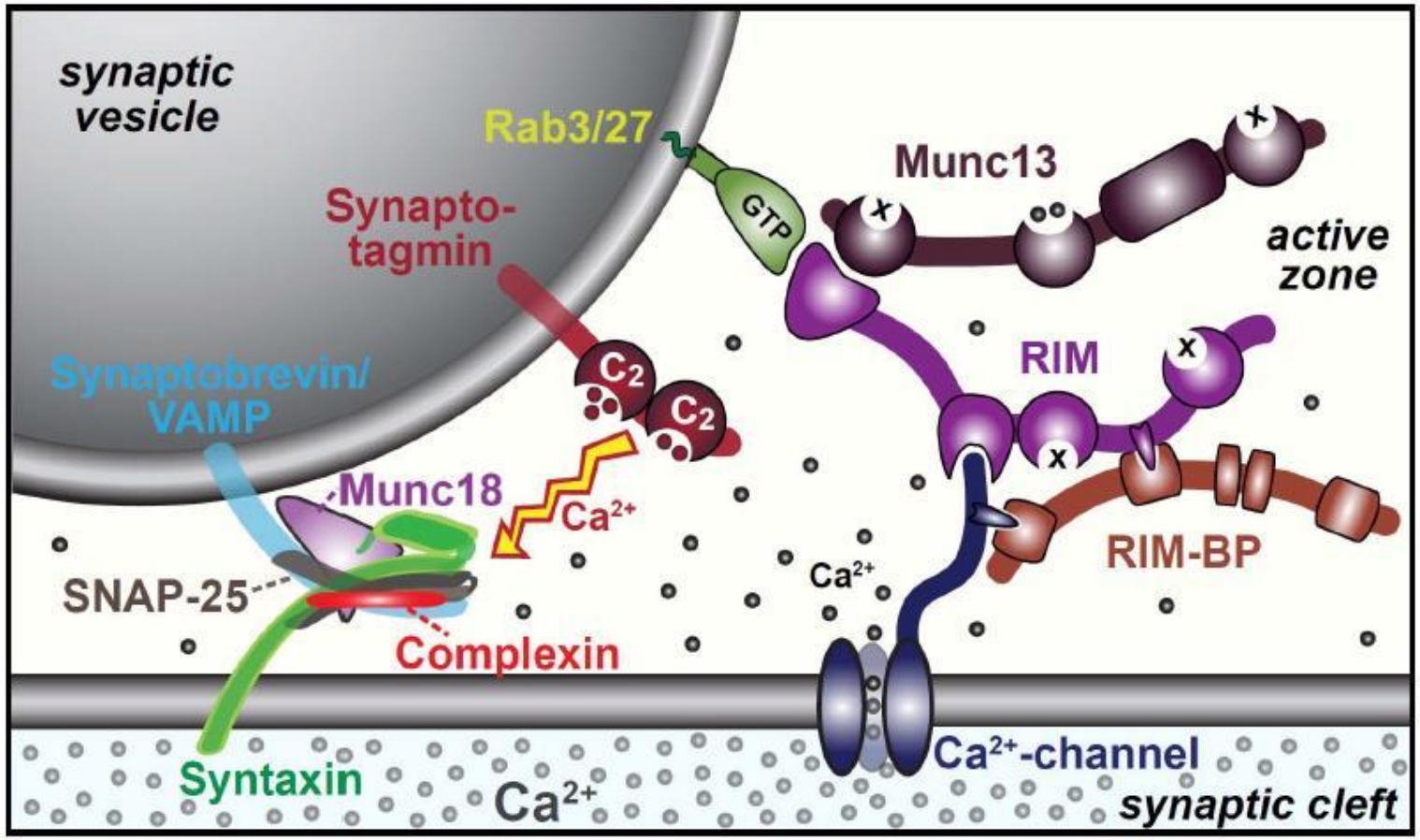
SNARE: SNAP (Soluble N-ethylmaleimide-sensitive factor Attachment Protein) REceptor


v-SNARE
t-SNARE



Synaptotagmin-1 is a Synaptic Vesicle Ca^{2+}-Sensor Essential for Ca^{2+}-Triggered Vesicle Fusion



McMahon et al., Cell 1995; Chen et al., Neuron 2002

The Nobel Prize in Physiology or Medicine 2013

Photo: A. Mahmoud

James E. Rothman

Prize share: 1/3

Photo: A. Mahmoud

Randy W. Schekman

Prize share: 1/3

Photo: A. Mahmoud

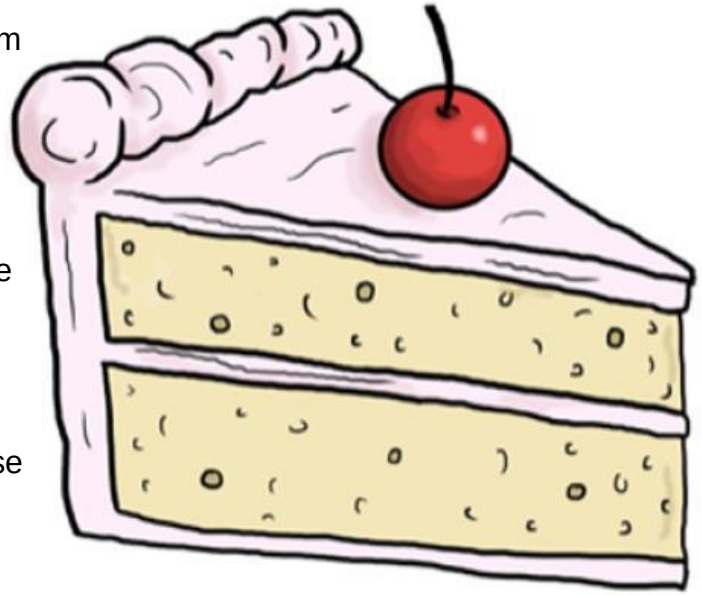
Thomas C. Südhof

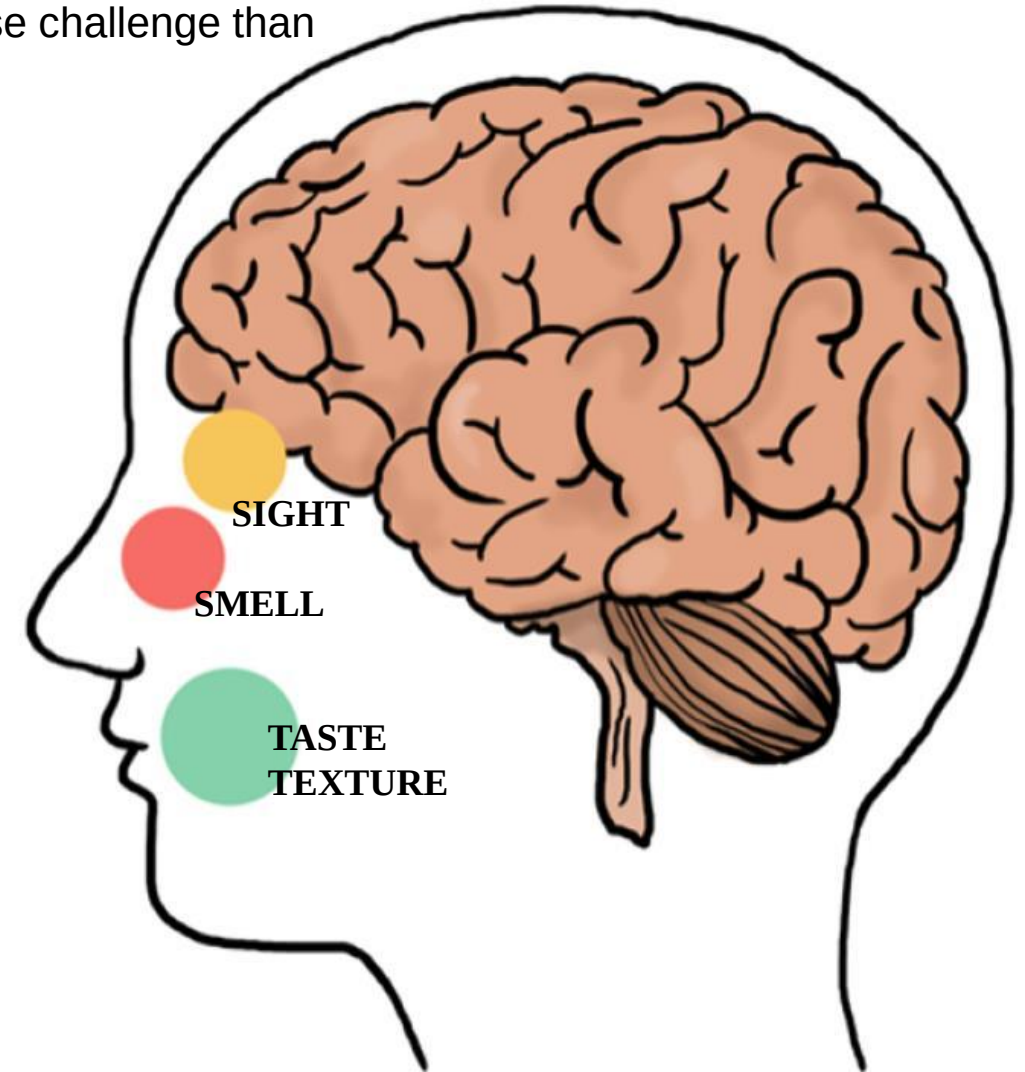
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2013 was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof *"for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells"*.

It is worth noting that *in vivo* the primary signal for insulin secretion is not usually glucose, but neurotransmitters released in response to the sight or smell of food (the cephalic phase of release) or incretins (peptides released from the gut due to the presence of food in the gut lumen).
Together, these mechanisms prepare the body for the subsequent increase in plasma glucose and prevent blood glucose levels from rising too high after a meal.
It also explains why insulin secretion is greater in response to an oral glucose challenge than an intravenous one.

Food-related sensory inputs (e.g., taste bud cells) relayed by cranial nerves
→ sensory processing areas in the brainstem
→ efferent signals are then carried through cholinergic fibers in the vagus nerve
→ In rodents, some of these fibers make direct contacts with beta cells in pancreatic islets.
  → Stimulation of these fibers causes the release of acetylcholine (ACh), which binds to muscarinic receptors on the surface of beta cells.
→ In humans, the neural mechanisms that stimulate beta cells are less clear because the pancreatic islets appear to have sparse innervation





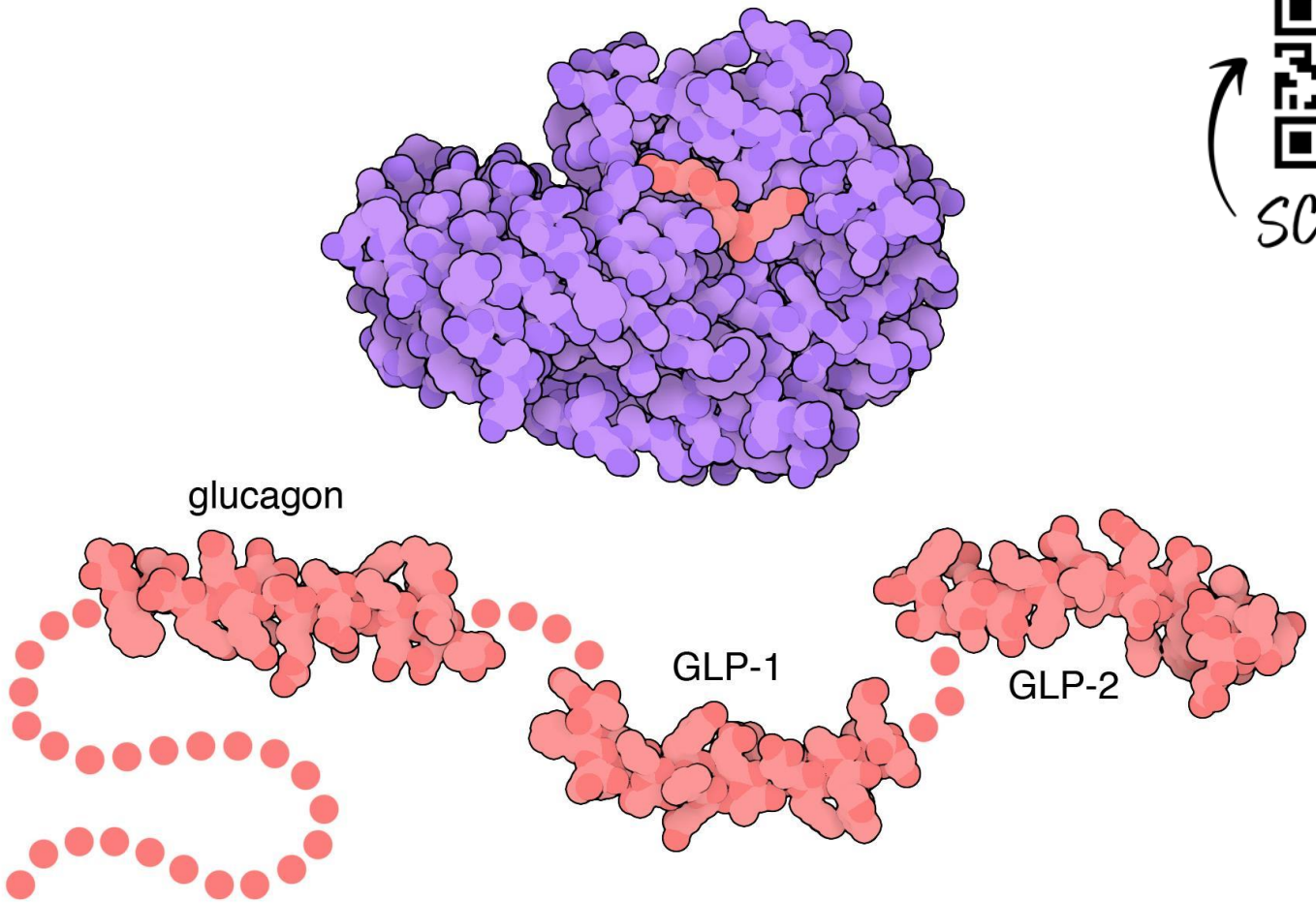
The binding of ACh to muscarinic receptors on beta cells is thought to activate a K_{ATP}-independent depolarizing current

https://pdb101.rcsb.org/motm/184

Pancreatic alpha-cells secrete glucagon in response to changes in blood glucose

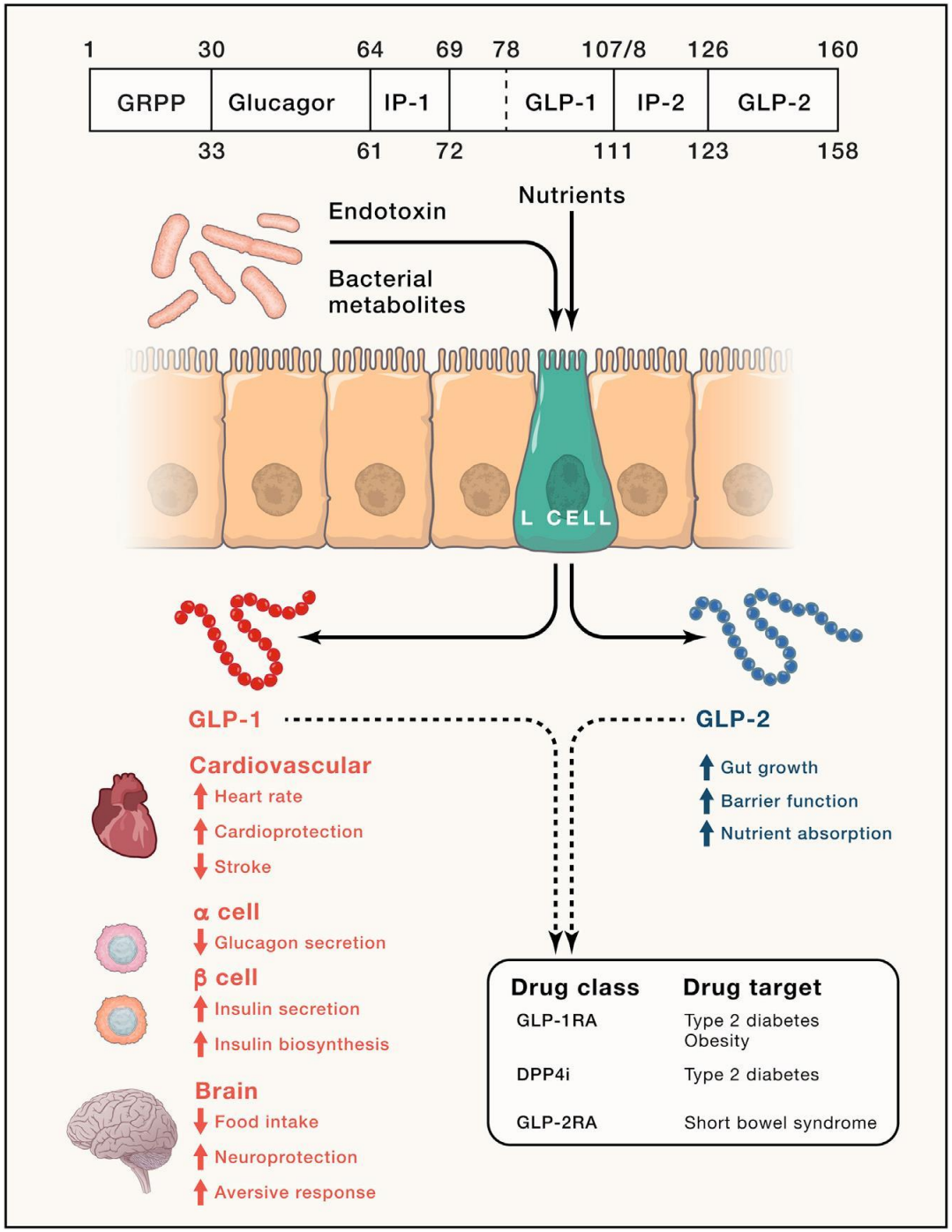
The mature form of glucagon is a single polypeptide chain of 29 amino acids

The discovery that, embedded in the proglucagon sequence, there are at least two additional peptides, which have important roles in physiology and which can have major impact in human disease when manipulated pharmacologically, has brought the proglucagon polypeptide to center stage



Furin (top) and three hormones created from proglucagon (bottom).

Glucagon is one of a collection of similar hormones that help manage metabolism. Several are built together as a longer protein, called proglucagon. Then, enzymes termed prohormone convertases clip it into the functional hormones. Three are shown here: glucagon (PDB entry 1gcn), glucagon-like peptide 1 (PDB entry 3iol), and glucagon-like peptide 2 (PDB entry 2l63). The convertase shown here is furin (PDB entry 1p8j), which is similar to the ones that process proglucagon.



The discovery of Glucagon-like peptide 1 and its role as an incretin

Figure 1. GLP-1 and GLP-2: Peptides encoded the proglucagon precursor with important roles in physiology and therapeutics

Glucagon-like peptide 1 (GLP-1) and 2 (GLP-2) are produced in intestinal entero-endocrine cells as a result of the post-translational processing of proglucagon. The peptides have a wide range of important physiological effects, which have been exploited to create three classes of licensed drugs: GLP-1 receptor agonist (GLP-1-RA) for type 2 diabetes (T2D) and obesity, GLP-2 receptor agonists (GLP-2-RA) for short bowel syndrome (SBS), and dipeptidyl peptidase-4 inhibitors (DPP-4 s), which inhibit the breakdown of GLPs and are used to treat T2D. GRPP, glicentin-related polypeptide; IP-1, intervening peptide 1; IP-2, intervening peptide 2.

O'Rahilly, The islet's bridesmaid becomes the bride: Proglucagon-derived peptides deliver transformative therapies, Cell (2021), https://doi.org/10.1016/j.cell.2021.03.019