Introduction to Peptides

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Disclosure

The following potential conflict of interest relationships are germane to my presentation.

Equipment: N/A

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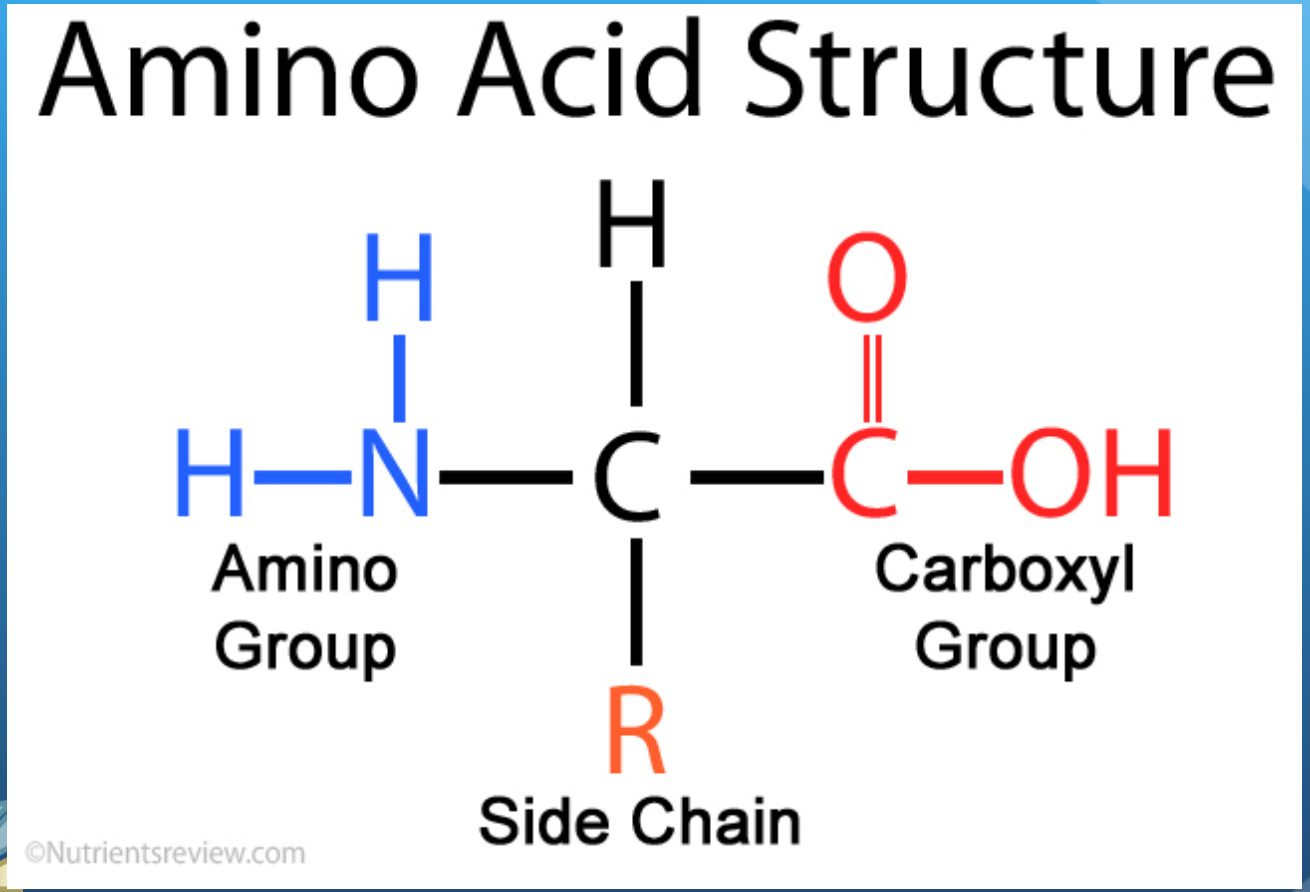
What Are Peptides?

Peptides are naturally-occurring substances that form when two or more single amino acids join together via peptide bonds to form a short chain.

In human we have 20 Amino acids



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9 ESSENTIAL AMINO ACIDS

1. LYSINE
2. METHIONINE
3. CYSTINE
4. TRYPHOTOPHAN
5. VALINE
6. ISOLEUCINE
7. HISTIDINE
8. PHENYLALANIE
9. THREONINE

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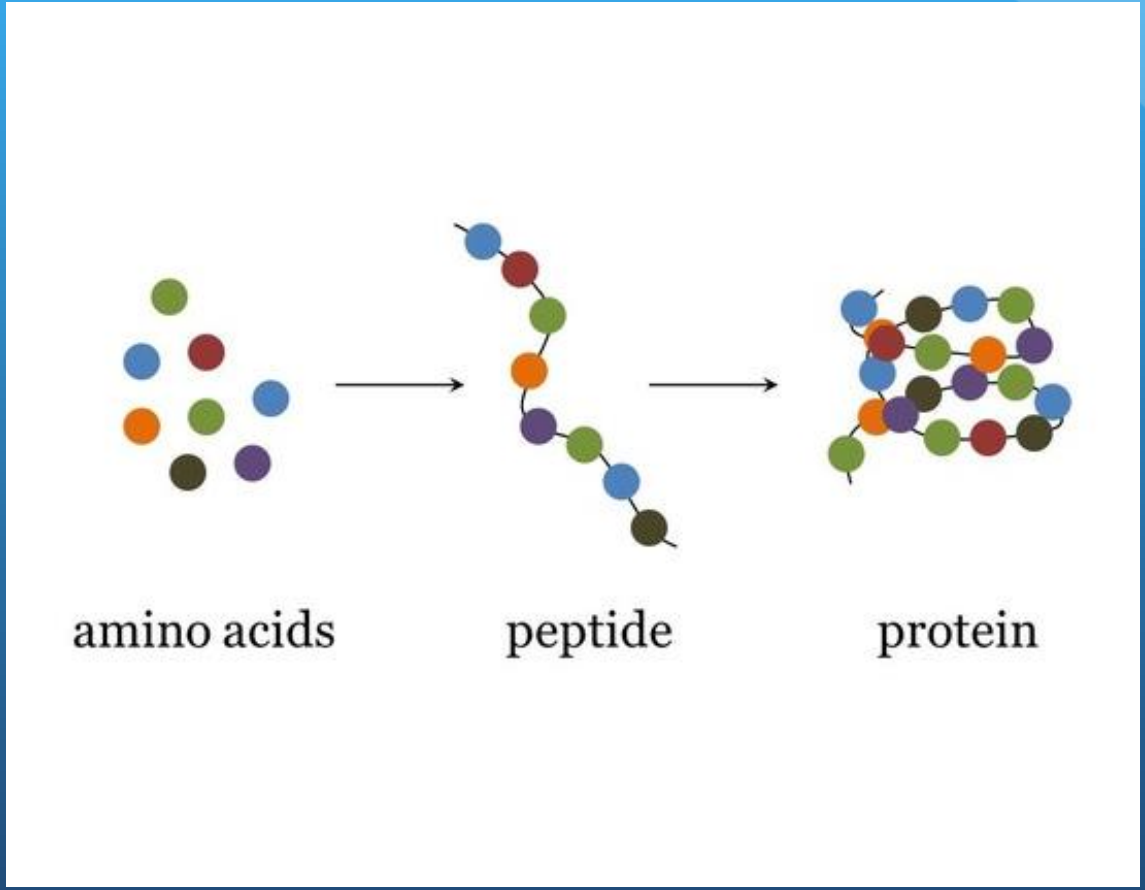
Peptide



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Peptide vs Protein

- Peptides are made from 2 to 50-70 amino acids
- Proteins are made up of 100 or more amino acids

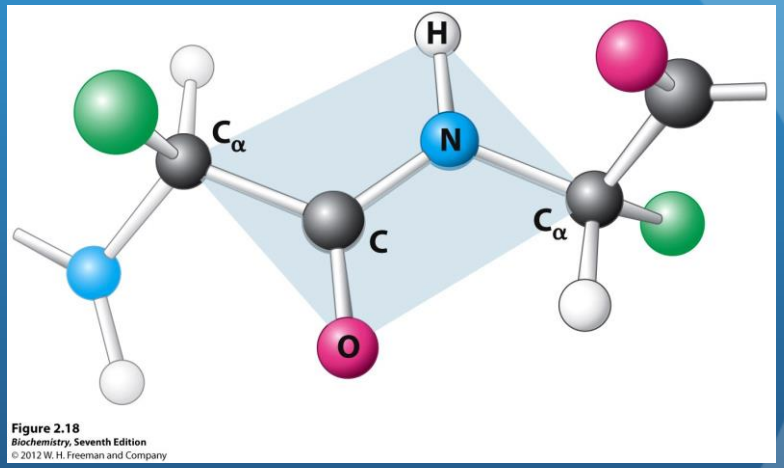

Figure 2.18
Biochemistry, Seventh Edition

- www.britannica.com/demystified/what-is-the-difference-between-a-peptide-and-a-protein
- Pennington M, et al. Bioorganic & Medicinal Chemistry 26 (2018) 2738–2758

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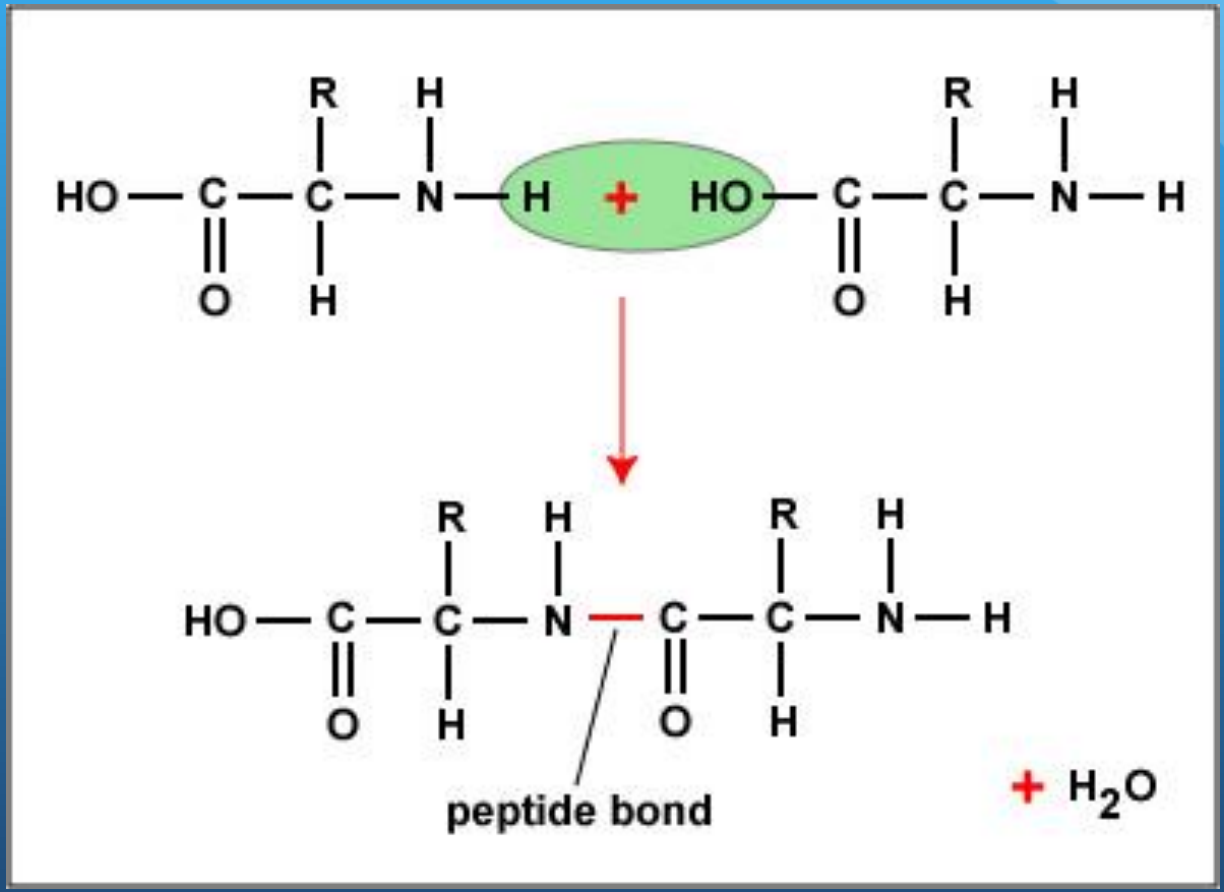
Peptide bond



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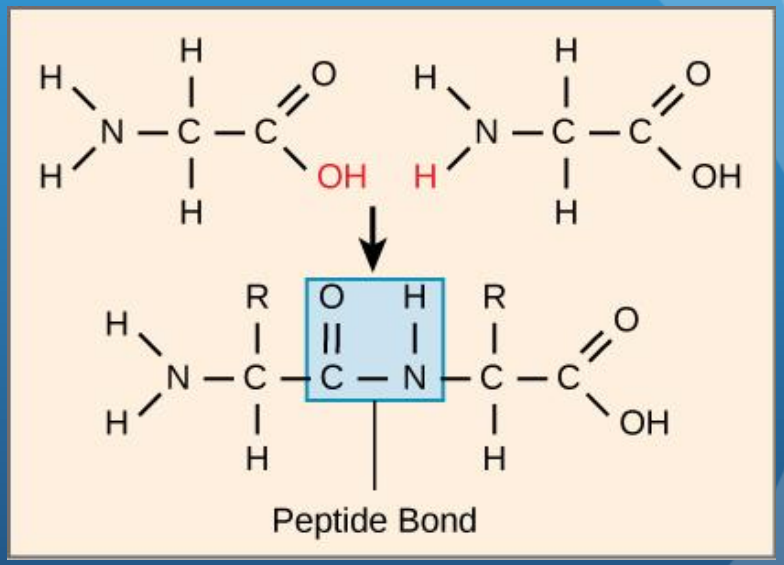
Peptide bond- dehydration reaction

- A peptide bond is a covalent bond formed between two amino acids
- a peptide bond is formed through a *dehydration reaction*
- two amino acids are able to bond together with the loss of water



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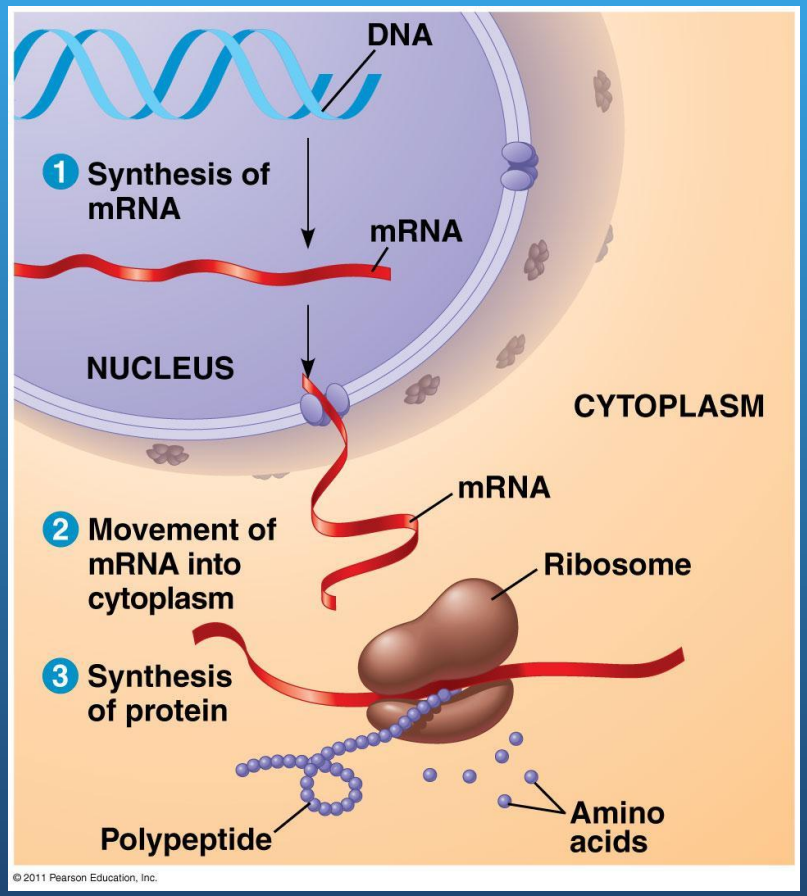
Peptide Synthesis - translation



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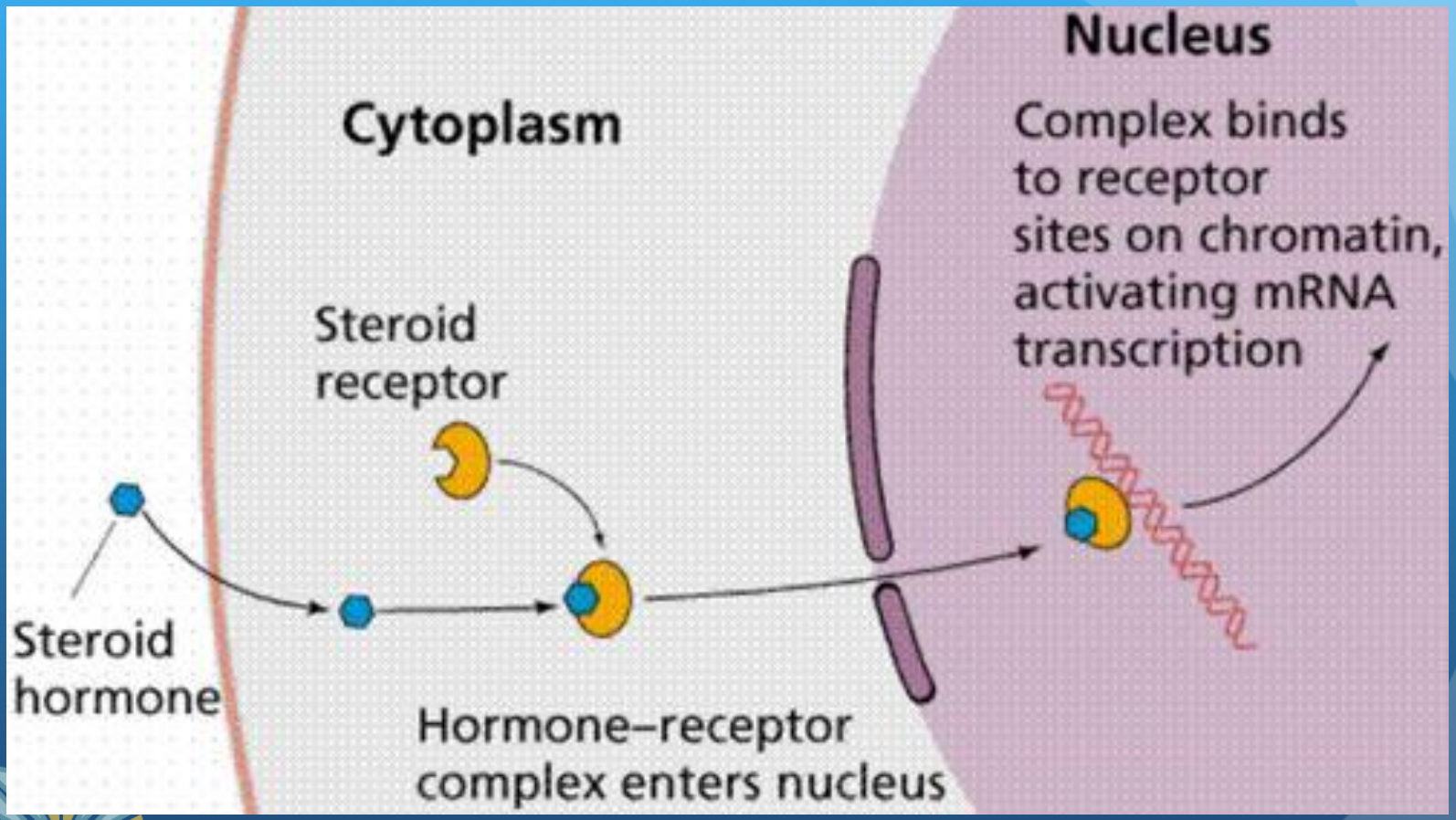
How many proteins are in us?

- According to the Human Proteome Map
- With 17,294 genes identified
- Derived from about 85% of the protein coding genes
- 30,057 proteins identified
  - Kim *et al*. A draft map of the human proteome. 2014. *Nature*. 509, 575-581

How many peptides are in us?

- According to the Human Proteome Map
- There is 293,700 peptides (non redundant)
- Derived from about 85% of the protein coding genes
  - Kim *et al*. A draft map of the human proteome. 2014. *Nature*. 509, 575-581

Steroid hormone action



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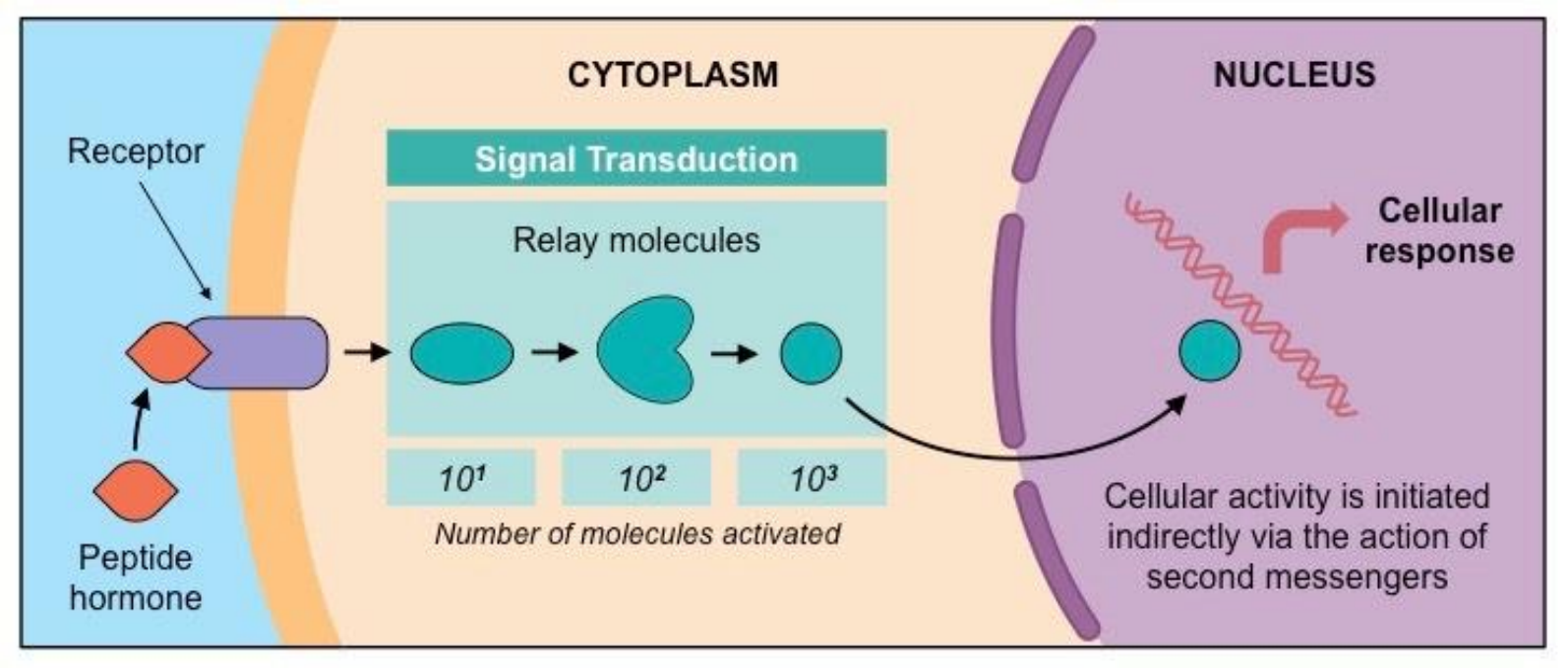
Peptide Action



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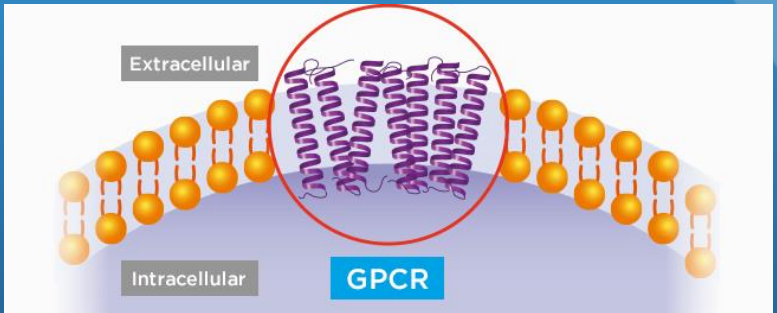
Peptide Receptors

Many are G Protein-Coupled Receptors

- Peptide receptors constitute a large group of G protein coupled receptor (GPCRs)
- GPCR are Membrane receptors
- Over 1000 different types of GPCR
- 2012 Nobel Prize in chemistry in GPCR



GPCR distribution through the surface of the cell membrane, the cytoplasm, and the nucleus. Extracellular stimuli are transmitted into the cell

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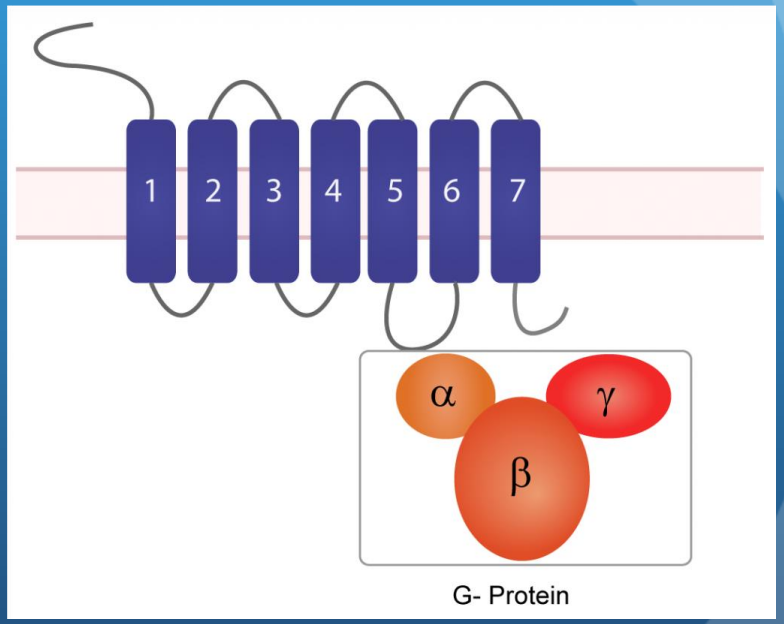
G protein coupled receptor

- GPCRs are the largest family of cell surface receptor
- Important characteristic is that they have 7 transmembrane alpha helices
- Coupled with a G protein with 3 subunits Alpha, Beta and Gamma



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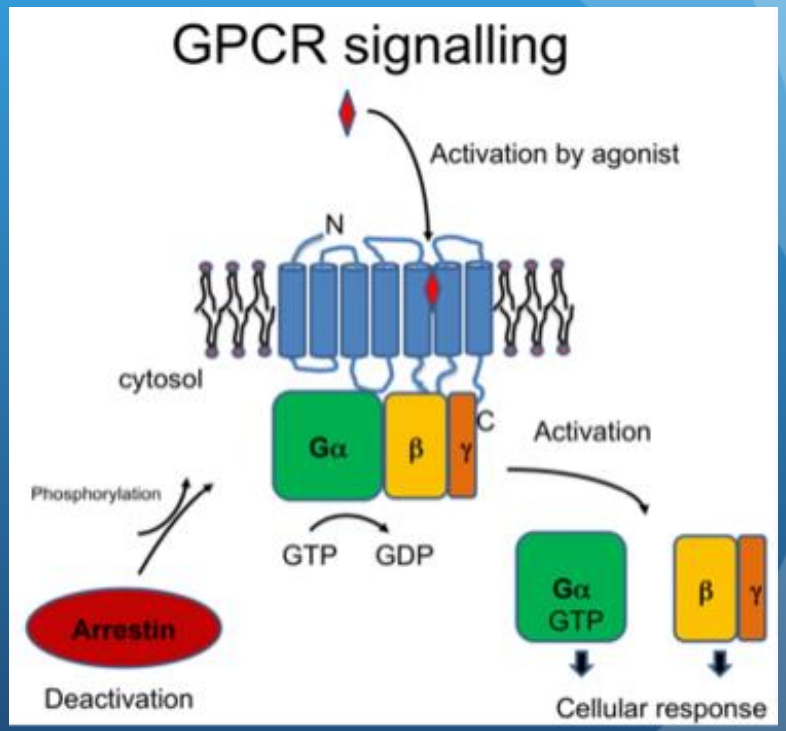
G protein coupled receptor

- When a peptide hormone binds to a receptor this activates the G protein
  - dissociates into a G-alpha and Beta Gamma complex (second messenger)
- Which triggers a cellular response



- Wu F, et al. JMB Volume 429, Issue 17, 18 August 2017, Pages 2726-2745

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Peptide action

- Critical in many biological functions
- Hormones
- Neurotransmitters
- Signaling molecules
- Expressed locally
- Antimicrobial
- Short half life

- Radar A, et al. Bioorganic & Medicinal Chemistry 26 (2018) 2766–2773

History of Peptides

- For 1000 years venom has been used for treating a variety of ailments such as arthritis, cancers, and gastrointestinal issues

History of Peptides

- Venom are composed of inorganic salts, low molecular weight organic molecules, enzymes, proteins and peptides

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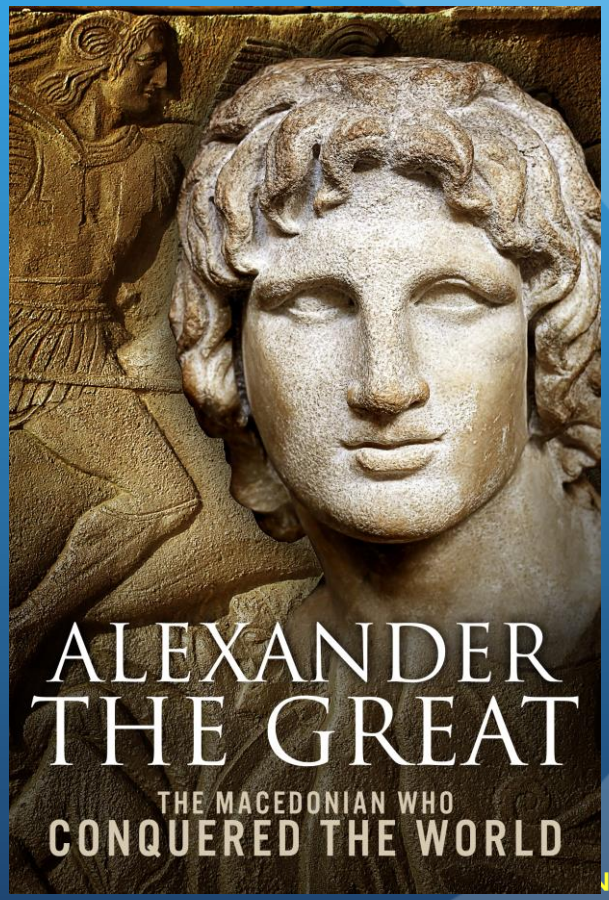
326 BC – Battle in India

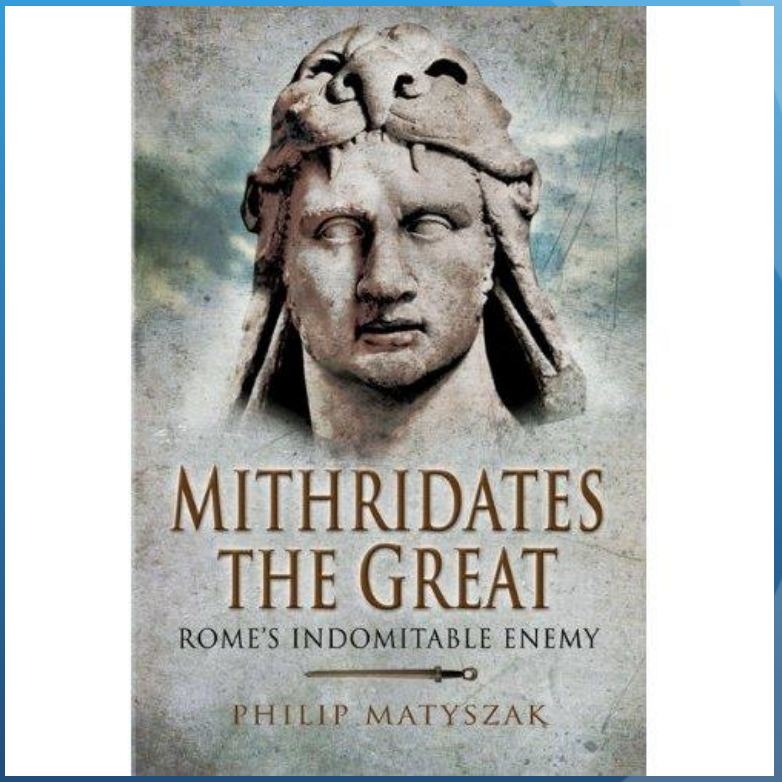
- In 326 B.C.E., Alexander the Great's army encountered arrows dipped in Russel's viper (Daboia russelii) venom in India, as evidenced by the symptoms that were recorded of his dying soldiers



- Mayor A. Greek Fire, Poison Arrows & Scorpion Bombs: Biological and Chemical Warfare in the Ancient World, New York. New York: Overlook Books; 2003.

37 BC -First medical use of venom

- King Mithradates VI of Pontus suffered a deep sword wound to his thigh with profuse bleeding
- As he was near death, his doctor used a small amount of steppe viper venom (Vipera ursinii) to stop the profuse bleeding and saved his life



- Bhattacharjee P, Bhattacharyya D. Therapeutic use of snake venomcomponents: a voyage from ancient to modern India. Mini-Rev Org Chem. 2014;11:45-54.

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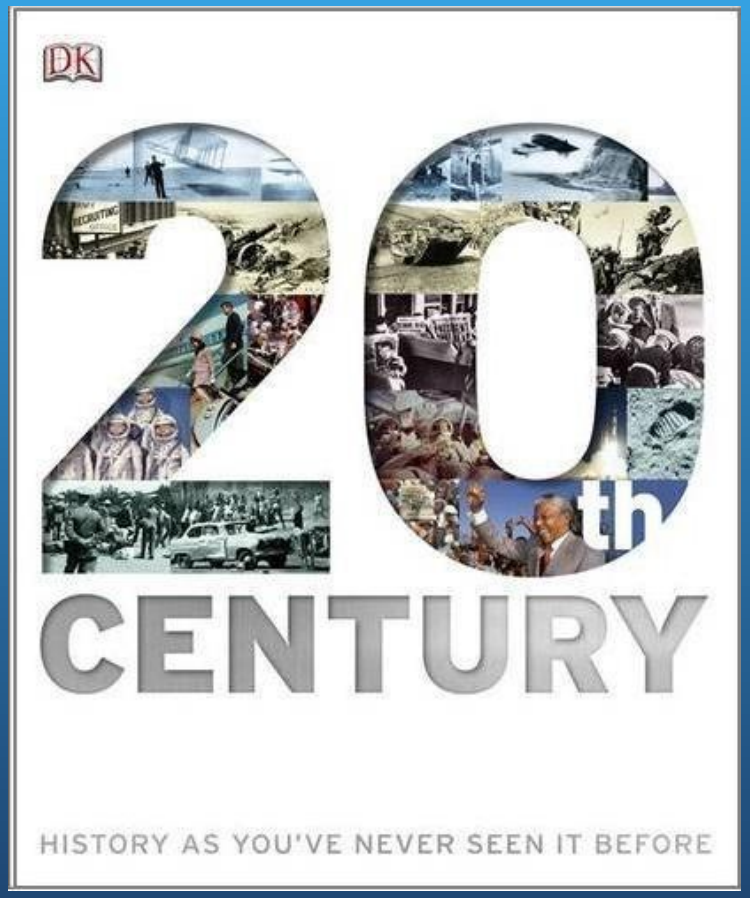
FAST FORWARD



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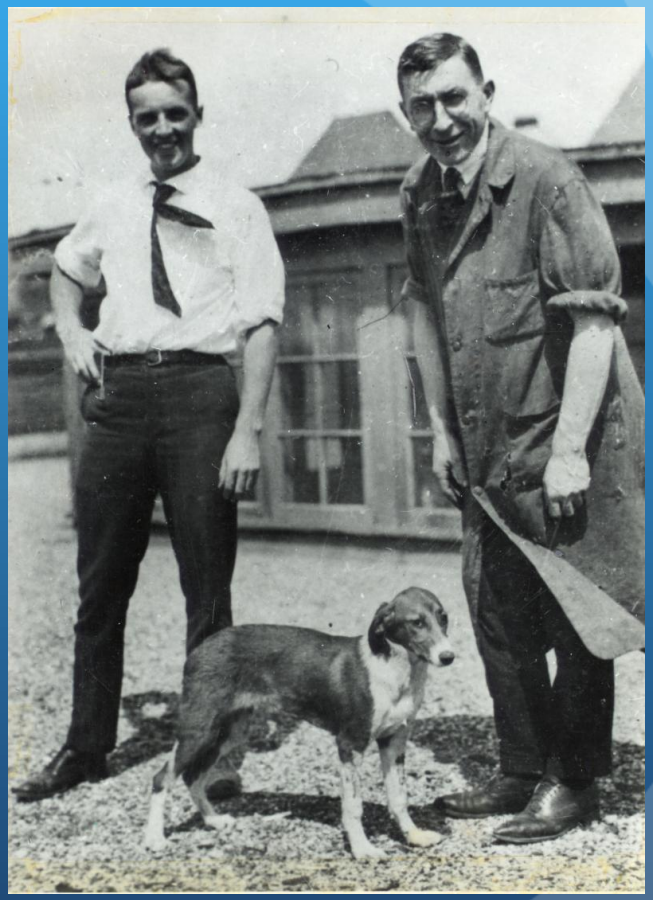
What is the most widely recognized peptide hormone?

Discovery of Insulin- 1921

- Before Insulin was discovered everyone with type 1 diabetes died within weeks to years of its onset.
- Insulin discovered in 1921



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Insulin- 1922

- The first medical success was with a boy with type 1 diabetes - 14-year-old Leonard Thompson - who was successfully treated in 1922. Close to death before treatment, Leonard bounced back to life with the insulin.

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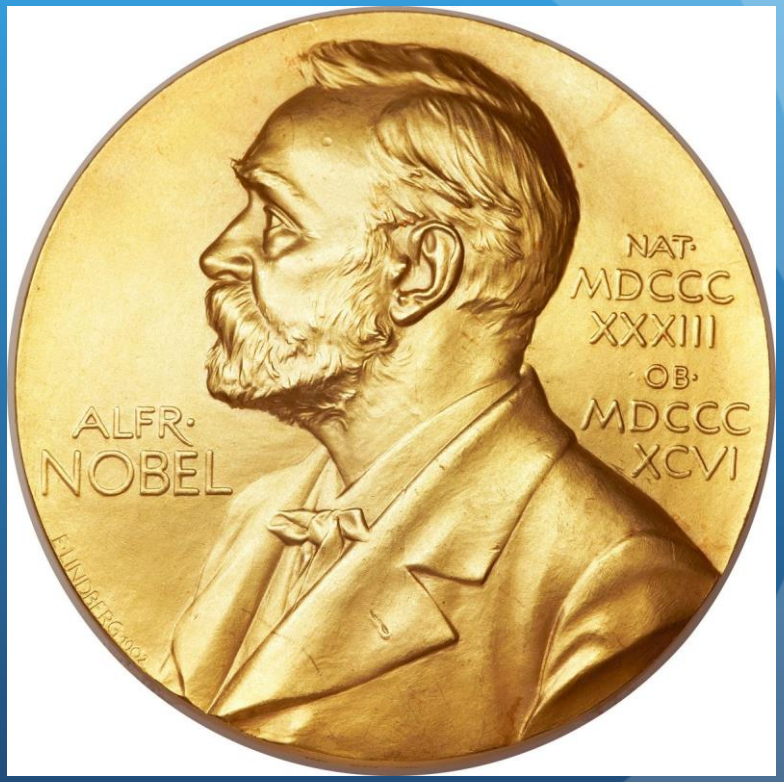
Nobel Prize 1923

- In 1923, the **Nobel Prize** Committee honoured **Banting and** J. J. R. Macleod with the **Nobel Prize** in Medicine for the discovery of insulin, ignoring **Best and** Collip. **Banting** chose to share half of the **prize** money with **Best**



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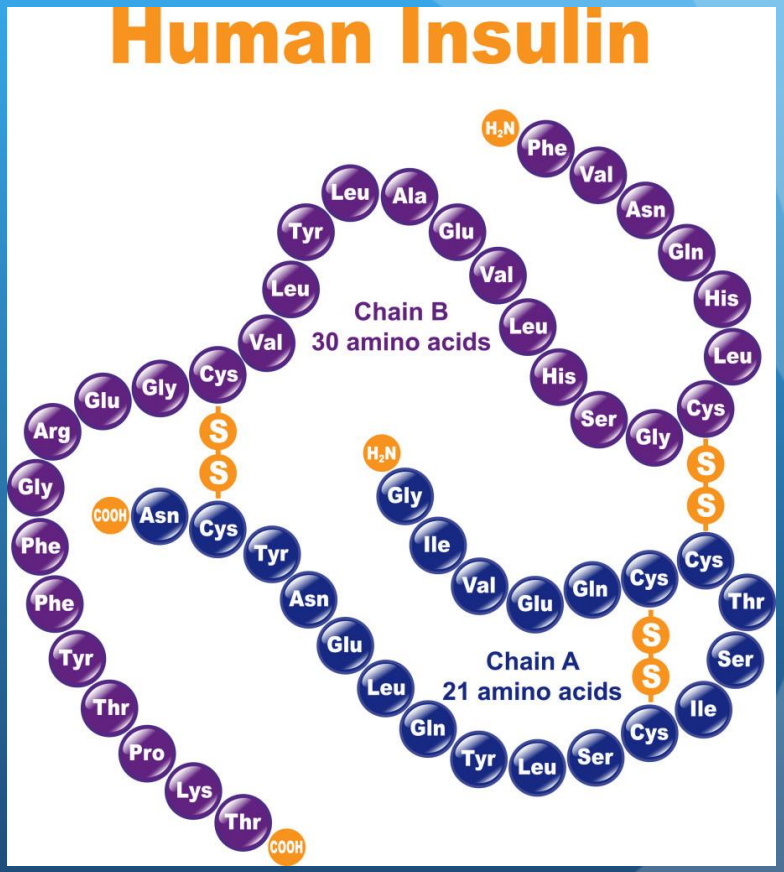
Insulin- 1922

- Insulin is considered a peptide hormone with 51 amino acids
- Alpha chain – 21 amino acids
- Beta chain – 30 amino acids



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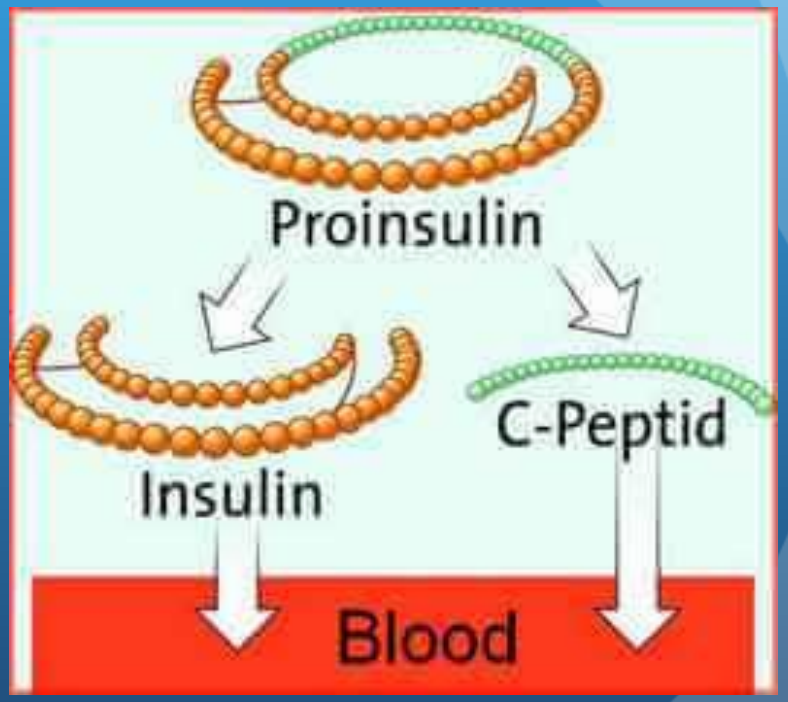
C- Peptide

- C- Peptide is used in the workup of hypoglycemia
- To differentiate between type 1 and type 2 Diabetes
- Good indicator of Beta cell function



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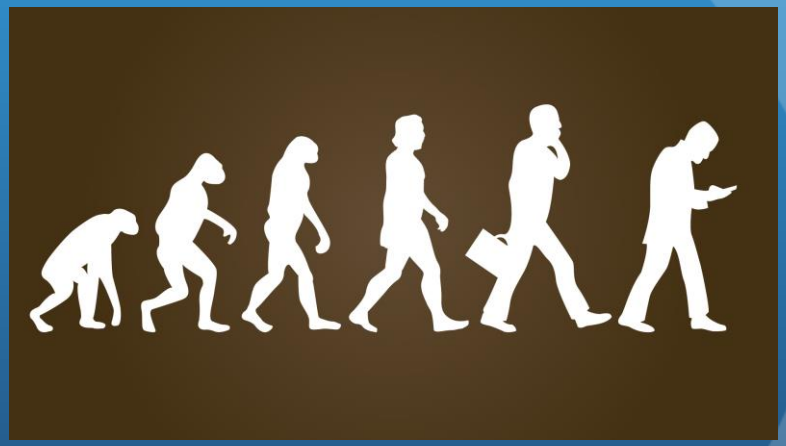
Peptide Hormones

- *ACTH*- 39 Amino acids
- *Calcitonin* – 32 Amino acids
- *GHRH* – 44 Amino acids
- *Parathyroid Hormone* – 34 Amino acids
- *Atrial Natriuretic Peptide* (ANH) – 28 Amino acids
- *Vasopressin* – 9 Amino acids
- *Oxytocin* – 9 Amino acids

Evolution of Peptides- Natural

- First half of 20th century
  - Peptides isolated from natural sources –
  - Insulin – Isolated from dog and cow pancreas
  - ACTH- Isolated from cow and pig pituitary glands
  - Calcitonin – Isolated from salmon



Evolution of Peptides - synthetic

- 1960s synthetic peptides
  - Oxytocin – 9 AA
  - Vasopressin – 9 AA
  - Lau J, et al. Bioorganic & Medicinal Chemistry 26 (2018) 2700–2707

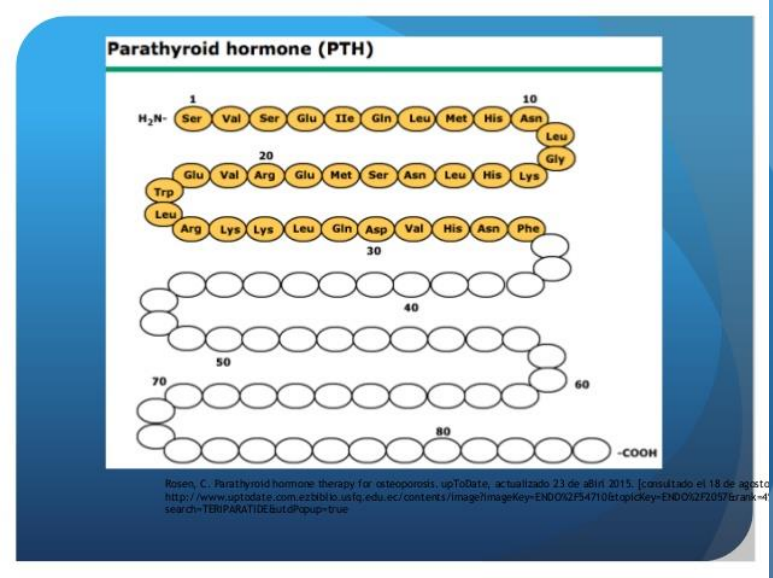
Evolution of Peptides- synthetic and altered

- 1980s
  - Octreotide – Analog of somatostatin
    - 8 Amino Acids
    - Longer half life compared to somatostatin -90 minutes compared to 2 minutes
    - Katz MD, Erstad BL, Clin Pharm. 1989 Apr;8(4):255-73.

- Leuprorelin – Analog of gonadorelin (GnRH)
  - 10 Amino Acids
  - More potent and also greater affinity to the receptor
  - Conn PM, Crowley WF Jr. Gonadotropin-releasing hormone and its analogues. N Engl J Med 1991; 324:93-103.

Evolution of Peptides- synthetic and altered

- Parathyroid hormone (PTH) is 88 Amino acid
- The bioactive active part of PTH is the 34-N-terminal amino acids
- Teriparatide (Forteo) is a recombinant peptide of PTH consisting first 34 N terminus amino acids
- FDA approved in 2002



Rosen, C. Parathyroid hormone therapy for osteoporosis. upToDate; actualizado 23 de abril 2015. [consultado el 18 de agosto
http://www.uptodate.com.ezbtblio.usfq.edu.ec/contents/image?imageKey=END0%2F54710&topicKey=END0%2F2057&trank=4
search=TERIPARATIDE&utdPopup=true

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Evolution of Peptides –Venoms/ Exotic source

- From the late 20th century modern medicine adopted a more systematic and rigorous approach to utilizing venoms as therapeutic agents
- “This is an absolute pharmacological goldmine that nobody’s really looked at. Clearly hundreds and hundreds of different peptides.” Dr. Glenn King at the University of Queensland in Brisbane, Australia

Evolution of Peptides –Venoms/ Exotic source

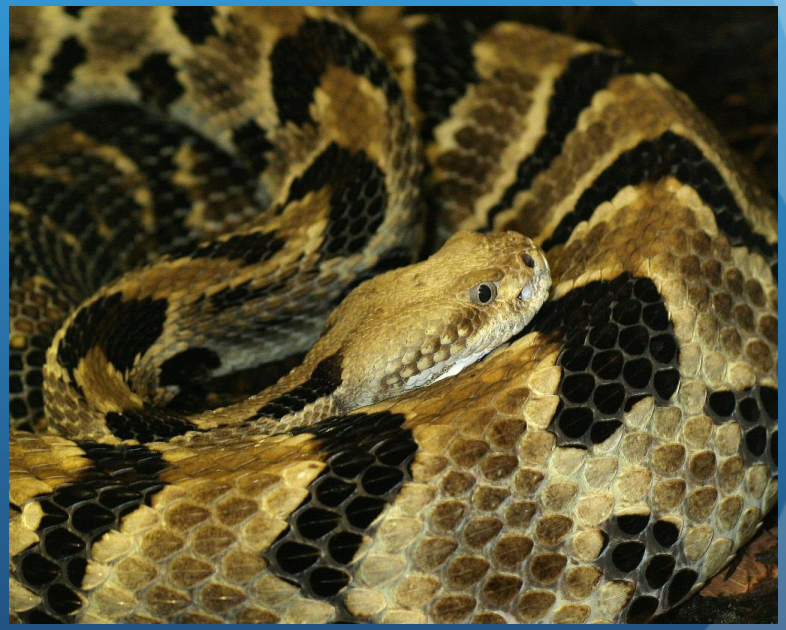
- After 1000 years of use the FDA approves the use of leeches in 2004
- Leechs help heal wounds and restore circulation in blocked blood veins
- Also used to enhance circulation in skin grafts or reattachment surgeries

Evolution of Peptides –Venoms/ Exotic source

- More than 60 compounds in their venom that have anaesthetic, anti-coagulant, vasodilatory and anti-inflammatory properties
- Secrete a peptide Hirudin (65 AA) to prevent the blood from clotting
- In 2000 FDA approval of Hirudin (Angiomax)

Evolution of Peptides –Venoms/ Exotic source

- Pit Vipers
- Includes Asian pit vipers, rattlesnakes, & lanceheads
- Hemotoxic venom
- Neurotoxic venom
- Cytotoxic venom has a localised effect on the bite area.



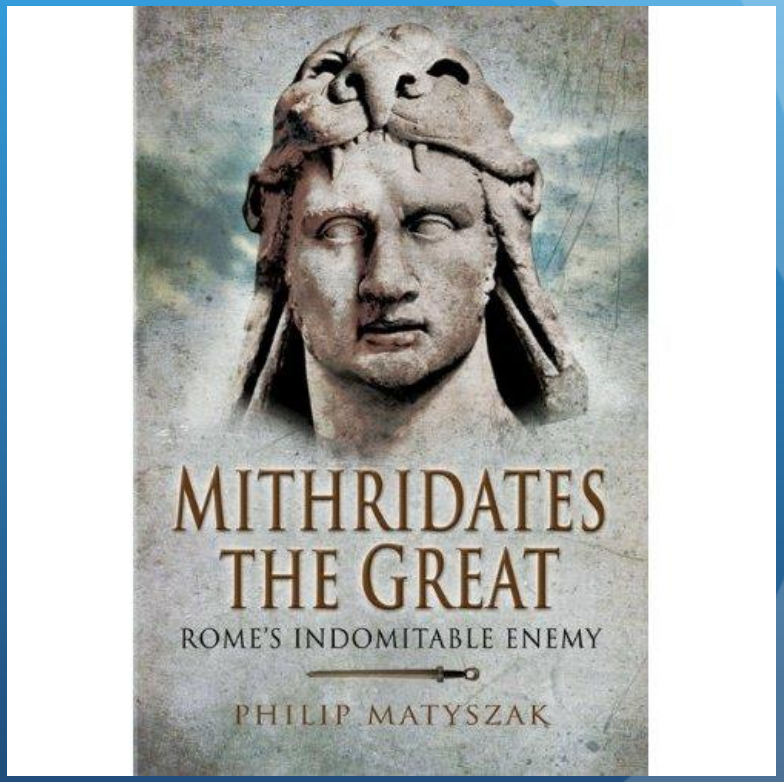
Evolution of Peptides –Venoms/ Exotic source

YouTube Snake Venom turns human blood into Jelly

- Batroxobin -231 AA is a coagulant and closely resembles thrombin.
- Batroxobin causes blood to clot
- Patented as Reptilase but not FDA approved in the US

37 BC -First medical use of venom

- King Mithradates VI of Pontus suffered a deep sword wound to his thigh with profuse bleeding
- His doctor used a small amount of steppe viper venom (Vipera ursinii) to stop the profuse bleeding and saved his life.
- Unknown if batroxobin is in the venom of steppe viper



- Battacharjee P, Bhattacharyya D. Therapeutic use of snake venomcomponents: a voyage from ancient to modern India. Mini-Rev Org Chem. 2014;11:45–54.
- Georgieva D, et al. Journal of Proteome Research 2008, 7, 866–886

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Evolution of Peptides –Venoms/ Exotic source – Gila Monster

- Glucagon Like Peptide-1 (GLP-1) is a 30 amino acid peptide hormone/incretin
- Secreted from the Intestinal L cells
- FDA approval of Exenatide (Byetta- 39 AA) in 2005 for the Rx of Type 2 Diabetes
- Exenatide has a half life of 2.4 hours versus 2 minutes for GLP-1

Evolution of Peptides –Venoms/ Exotic source

- Derived from the spit of the Gila Monster
- 8 FDA approved drugs – GLP-1 analogs
- (Peptides hormones)

- Exenatide (Byetta/Bydureon)
- Liraglutide (Victoza, Saxenda)
- Lixisenatide (Lyxumia)
- Albiglutide (Tanzeum)
- Dulaglutide (Trulicity)
- Semaglutide (Ozempic)

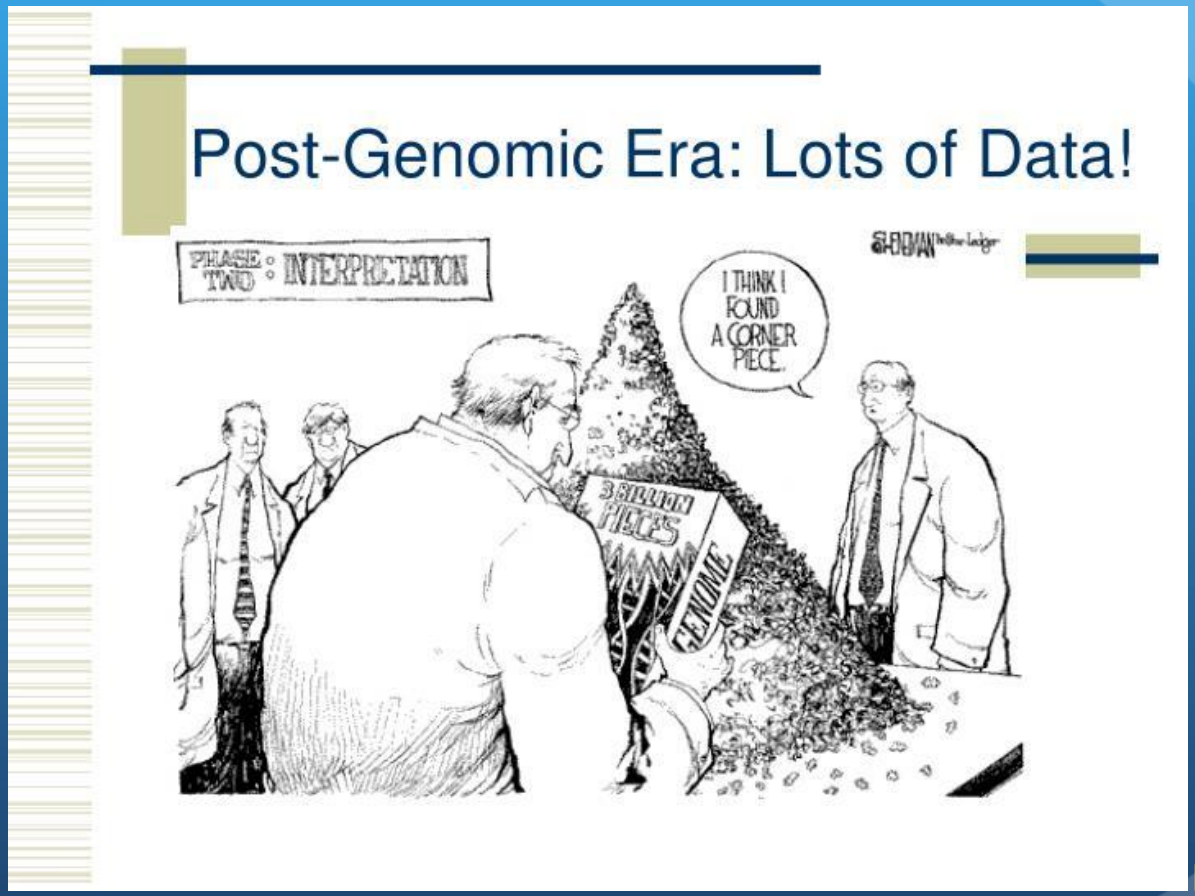
Evolution of Peptides –Venoms/ Exotic source Death stalker scorpion

- Chlorotoxin – 36 AA
- Specifically Binds to Glioma cells
- Under investigation for the treatment and diagnosis of several types of cancer

- Deshane J, et al *J. Biol. Chem.* ***278*** *(6): 4135-44.*

Evolution of Peptides Current Genomic era

Discovery new receptors with peptide ligands of the receptors



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Evolution of Peptides –Genomic era

- Vasopressin (ADH) 9 AA discovered in 1953
- 3 Vasopressin receptors discovered in 1992
  - Morel A, O'Carroll A-M, Brownstein MJ, Lolait SJ 1992 Nature 356{6369):523-526
  - Turner RA, Pierce JG, Du Vigneaud V 1951 The purification and the amino acid content of vasopressin preparations. J Biol Chem 191(1):21-28
- All 3 vasopressin receptors are G protein coupled receptors
- Activation of V1 and V3 stimulates phospholipase C
- Activation of V2 stimulates cyclic AMP (cAMP)

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Evolution of Peptides –Genomic era

- Half of the marketed drugs and most of the drugs in clinical trials that interact with peptide GPCRs are small molecules with a wide range of binding modes distinct from those of large peptide ligands

- Vasopressin receptor antagonists (Vaptans) are a new group of nonpeptide drug

- Treat hyponatremia
  - Action on vasopressin type 2 (V2R) receptors in the collecting duct and thus increase solute free water excretion
  - Narayen G, et al. Indian J Endocrinol Metab. 2012 Mar-Apr; 16(2): 183-191

Evolution of Peptides –Genomic era

- Autosomal Dominant Polycystic Kidney Disease
- most prevalent, potentially lethal, genetic human disorder
- estimated to affect at least 1 in every 1000
- Hanaoka K, et al. J Am Soc Nephrol. 2000 Jul;11(7):1179-87

- Increase in cAMP stimulate cyst enlargement and fluid secretion in ADPKD cells
- Two genes identified: PKD1 (chromosome region 16p13.3; around 85% cases) and PKD2 (4q21; around 15% cases)

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Evolution of Peptides –Genomic era

Autosomonal Dominant Polycystic Kidney Disease (ADPKD)

- In 2014, Japan was the first country in the world to approve a pharmacological treatment for ADPKD
- In 2018 the US FDA approved Tolvaptan (V2 receptor antagonist) – reduces the cAMP and total kidney volume
- Torres V, et al. Dec 2012 N Engl J Med 2012; 367:2407-2418

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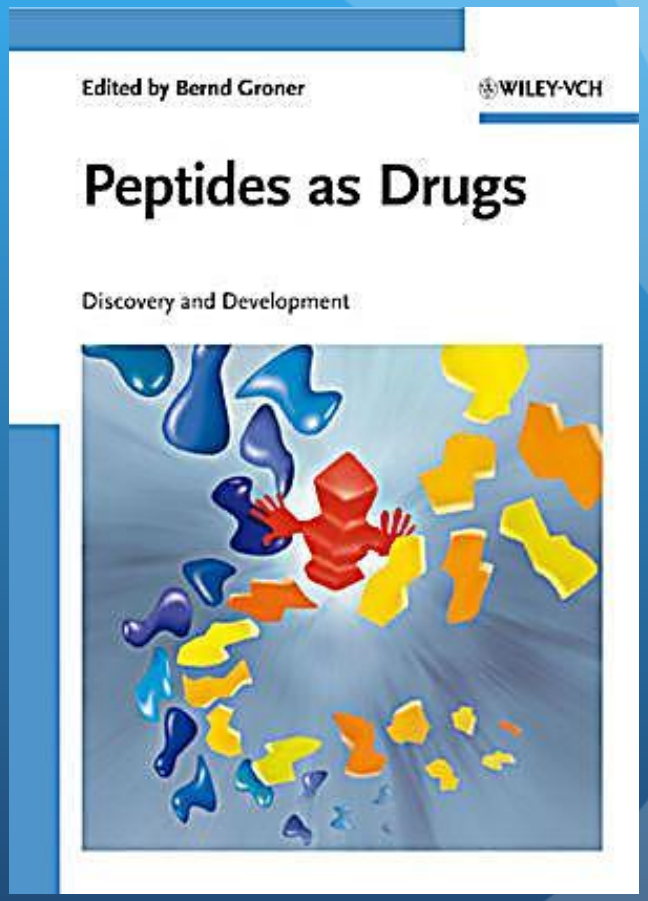
Peptide drugs

- As of 2018 there are 60 peptide drugs have been approved in the United States, Europe, and Japan
- Over 150 are in active clinical development
- 260 have been tested in human clinical trials
  - Lau J and Dunn M. Bioorganic & Medicinal Chemistry 26 (2018) 2700-2707



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Conclusion

- Peptides are small protein of 2 to 50 - 70 Amino Acids
- Most Peptides bind to G protein coupled receptors that cause a signaling for a cellular response
- Peptides are one the most important bio molecules

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