The Endocrine System

☑ Sheet ☐ Slide ☐ Handout

BIOCHEMISTRY

Number:	5
Subject:	**Calcium Binding Proteins / Tyrosine Kinase Receptors/ Eicosanoids.**
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Date:	
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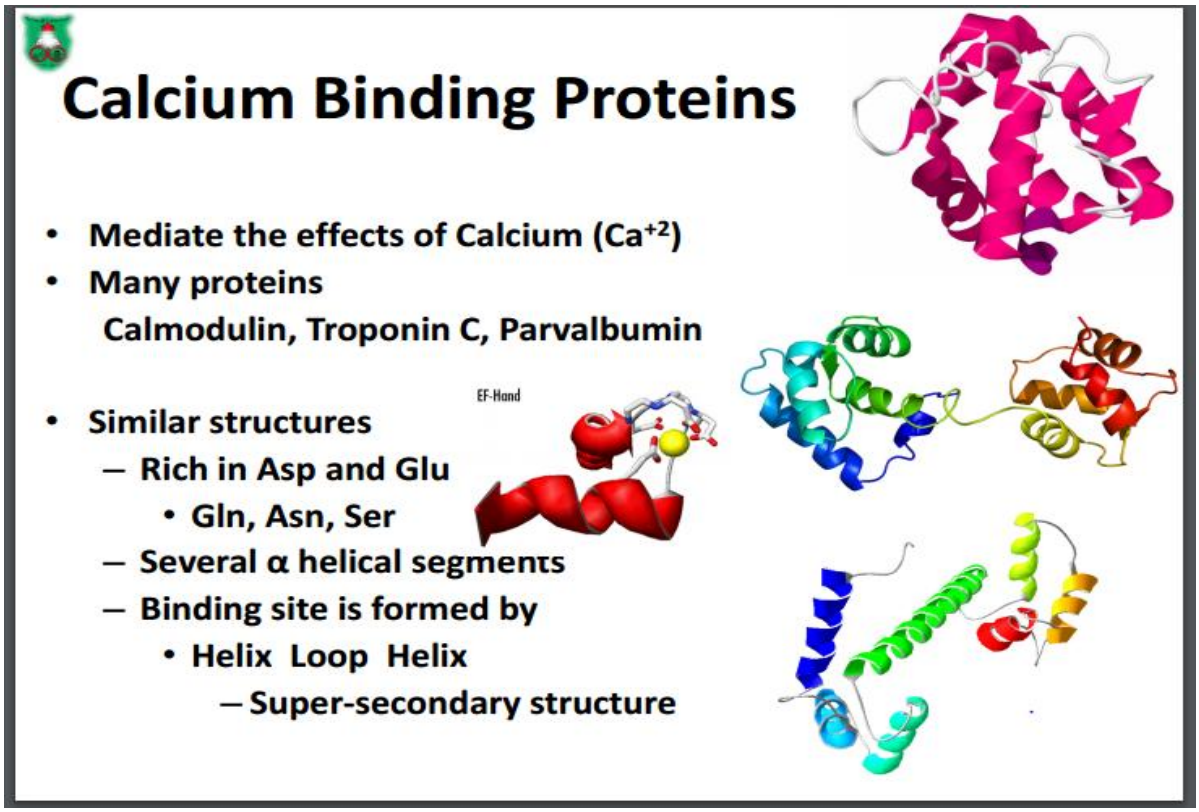
Calcium Binding Proteins.



[The calcium binds to proteins called the calcium binding proteins]

What are the characteristics of the proteins that will bind calcium?

Since calcium is a positively charged ion it can ligate [bind] with both *negatively* charged and *polar* amino acids.

[1] They are associated with a high content negatively charged

amino acids, both the Aspartic Acid [Asp] and the Glutamic Acid [Glu]

[2] High content of polar amino acids [both charged and uncharged]

Serine [Ser] , Threonine [Thr], Glutamine [Gln], Asparagine [Asn]

Calcium is a bulky molecule and in order for it to bind, some areas of the calcium binding protein must have the ability to move. Those areas are called the loops.

So as we can see that the calcium binding proteins will contain

Alpha Helix – **Loop-** Alpha Helix.

But the calcium binding proteins' will never have this domain

Alpha Helix – *Turn* – Alpha Helix

Why?

The turn is made up of only 4 amino acids and in order for those amino acids to stick to each other they are nonpolar [hydrophobic] amino acids {usually rich with the hydrophobic glycine}.

[Alpha helix –*Turn* – alpha helix] domains are better known as the DNA binding domains.

In other words, they did not meet the binding requirements for calcium

[1] Calcium needs both negatively charged and polar amino acids [Not found in the turn]

[2] Calcium needs a large,[freely moving] binding domain [The turn is made up of only 4 amino acids]

Calcium Binding Proteins

[1] Calmodulin

[2] Troponin C

[3] Parvalbumin

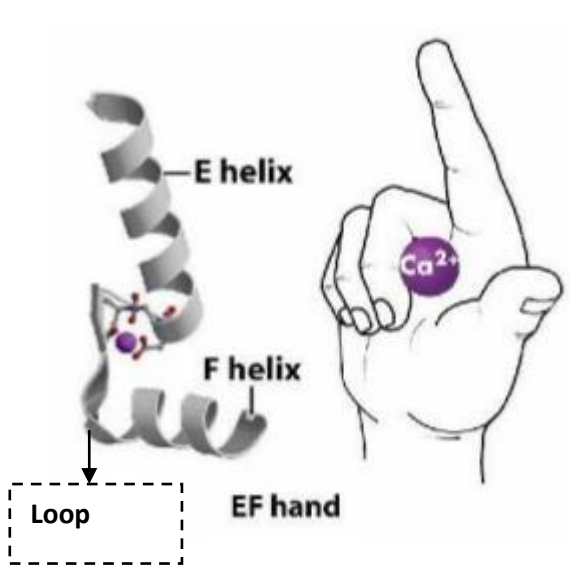
The parvalbumin is the first calcium binding protein with a crystal-like structure, having the protein in the shape of a crystal in order to identify the amino acids.

Parvalbumin has 6 alpha helices→[A,B,C,D,E,F].

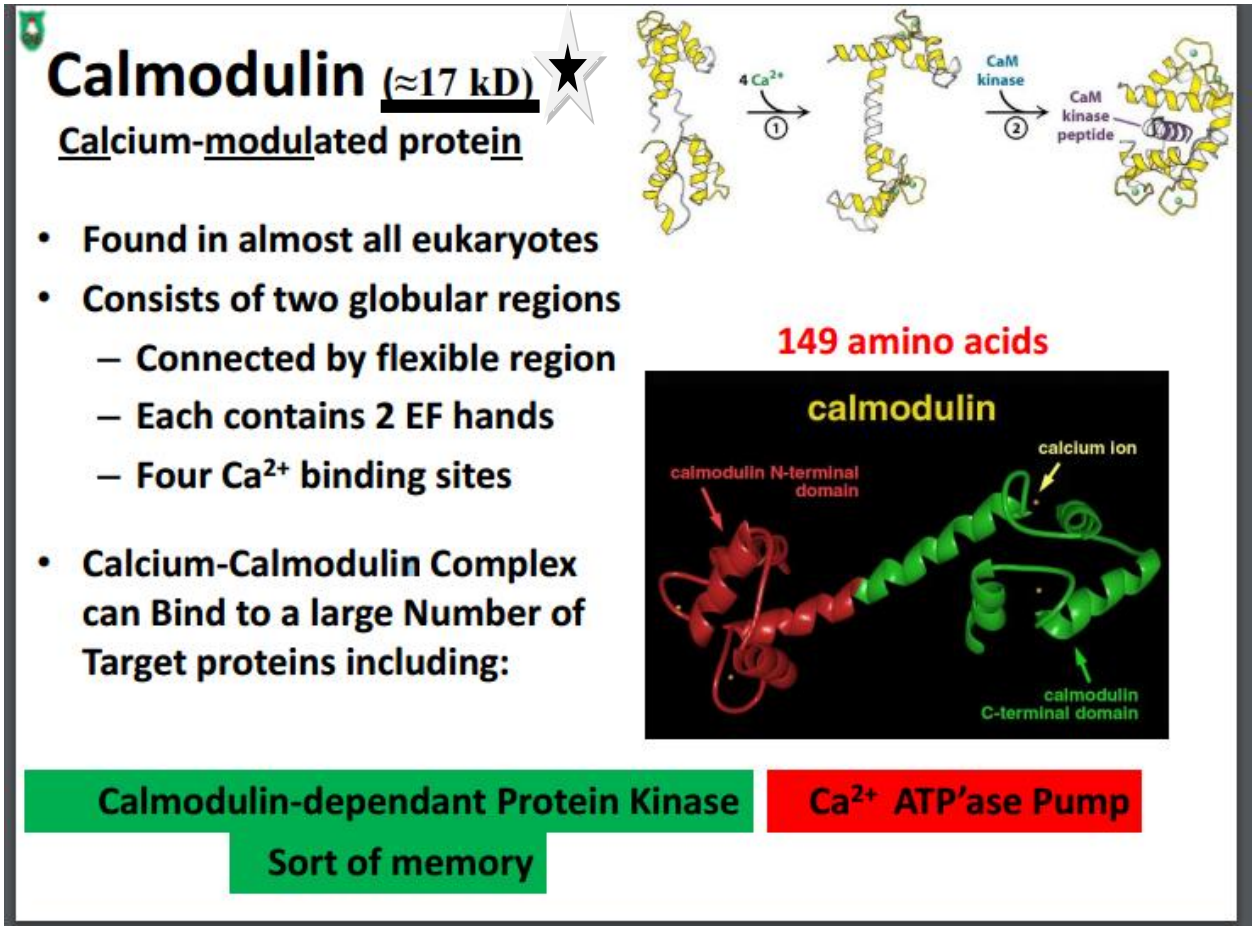
Calcium fits in between the E & F alpha helices.

[E Alpha Helix – **Loop –** F Alpha Helix] . Better known as the EF domain. As the EF domain has the same shape as the thumb and index fingers this domain is also called the EF hand.

Not only does parvalbumin have the EF hand, but also Calmodulin and Troponin C.



Calmodulin



Calmodulin is made up of <u>two</u> large globular regions connected together by a loop.

<u>Each</u> globular region has 2 EF hands, and since each hand will ligate 1 calcium ion, this will be a total of 4 calcium ions bound to calmodulin.

As calcium binds calmodulin, <u>conformational changes</u> will occur :

[1] Unmasking of the hydrophobic patches found in the protein, why ?

In order to bind to other proteins on its hydrophobic region.

[2] The two connected helices, coming from each globular region, will undergo a conformational change

[3] The whole amino acid sequence will change by changing one amino acid only !

Calmodulin will become active when calcium binds to it. Calmodulin will therefore go and bind to other proteins. It is a cascade of actions

It will bind

[1] Calmodulin-Dependant Protein Kinase

Important mediator of learning and memory.

[2] Calcium – ATPase Pump

As calcium binds calmodulin, it will immediately go and bind the Calcium-ATPase pump. This is an active transport pump.

When do we use it ?

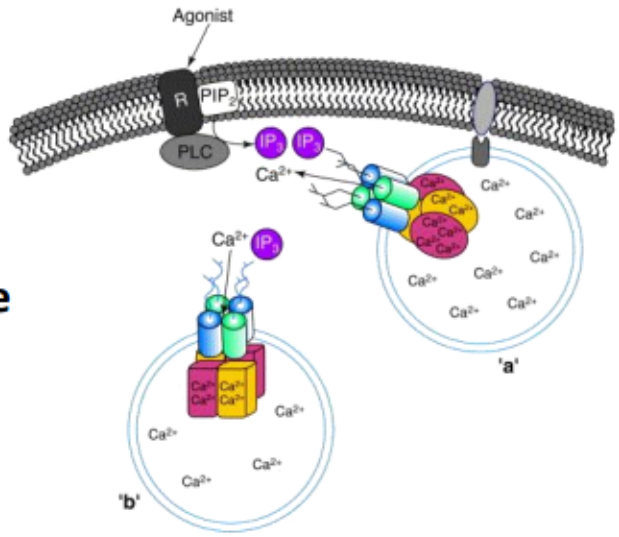
In the beginning of this lecture, we said after calcium's job is done inside the cytoplasm it must return back to original state, inside the sacroplasmic reticulum. It goes against concentration gradient, so energy must be used through this Calcium-ATPase pump. [1 ATP molecule will be used for returning back of 2 Calcium ions]

Quick comparison:

Calcium Movement	**How ?**	**Energy used ?**
ER → Cytoplasm	IP3 Second Messenger	No
Cytoplasm → ER	Calcium-ATPase Pump	Yes

Ca^{2+} Transporter

- In sarcoplasmic reticulum
  - 80% of the membrane proteins
  - 10 membrane spanning helices
  - Ca^{2+} move against a large concentration gradient
  - 2 Ca^{2+} / ATP (high)
    - Depletion of ATP leads to tetany, Rigor mortis



80% of the membrane proteins in the endoplasmic reticulum work on calcium, either transporting it outside or internalizing it back to the ER.

Rigor Mortis [stiffness in the case of death] and tetany, [involuntary contraction of the muscles] are both the result of ATP depletion.

So if there is no ATP, the Calcium-ATPase pump will not function and therefore calcium will not return back to the ER.

Let's remember, what is the function of calcium in the cytoplasm ? Change in metabolism and function, and one of these functions is contraction of muscles.

When the calcium stays in the cytoplasm, the muscle will stay contracted.

No ATP → No Calcium-ATPase Pump → Calcium stays in the cytoplasm → Muscle stays contracted → Rigor Mortis/ Tetany.

There are other types of receptors which hormones bind to, called the tyrosine kinase receptor. As soon as the hormone binds to it, receptor tyrosine kinase will become active, producing lots of signals inside the cell.

There are types of this receptor:

[1] Single Transmembrane Helix [Having the shape of immunoglobulin domains connected by disulphide bridges]

[2] Dimer

In order for the tyrosine kinase receptors to work, they have to be in dimers.

As the hormone binds to the extracellular portion, immediately another receptor will come and bind to it, forming a **dimer**, becoming partially active. This will induce the phosphorylation process→ activation of the kinase, becoming now fully active.

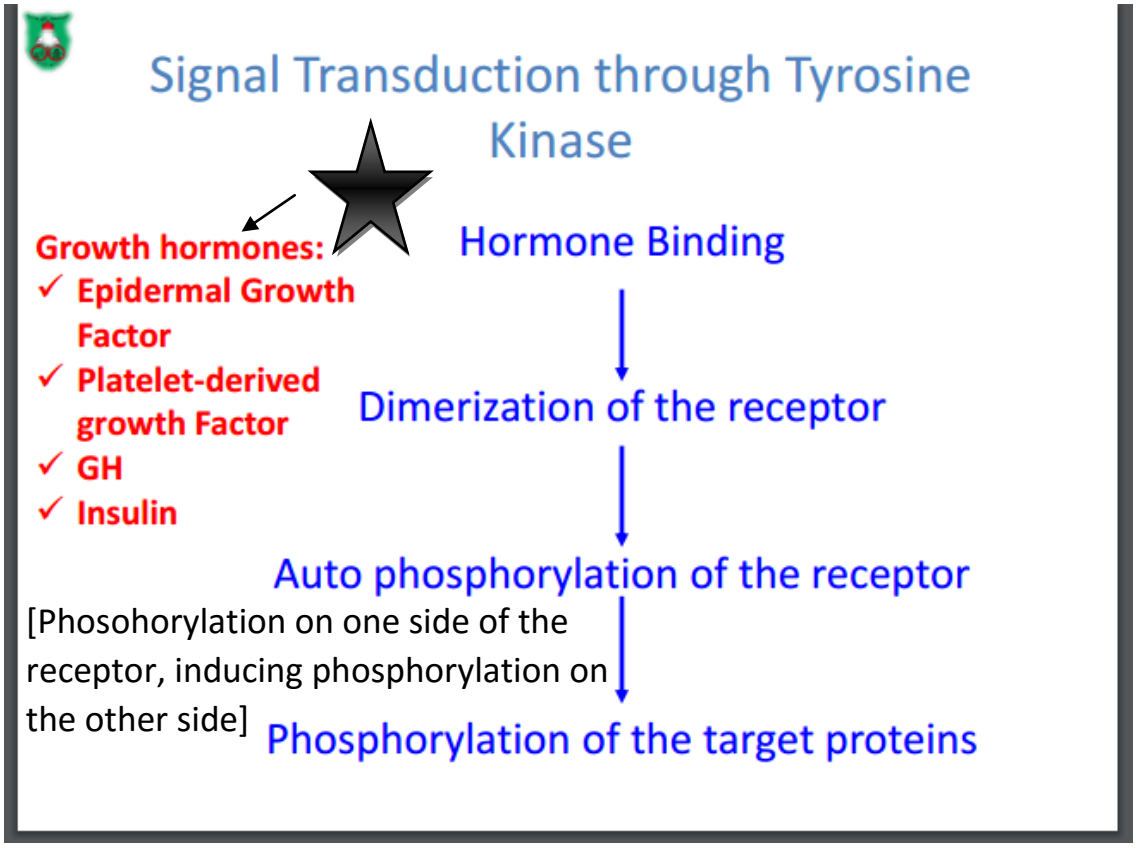
Tyrosine Kinase receptors never function as monomers.

Insulin receptors, a class II tyrosine kinase receptor, is already in a dimer form.

When a hormone binds to it, it will cause conformational change, inducing activation of kinase, and phosphorylating other proteins.

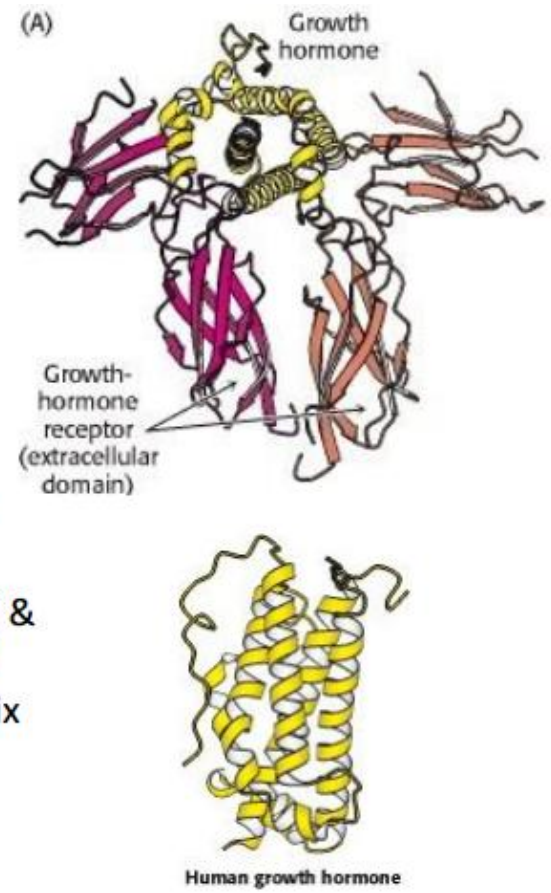
A monomer tyrosine kinase is partially activated by dimerization, and fully activated by both dimerization and phosphorylation {kinase}

A dimer tyrosine kinase receptor is fully activated by hormone binding



Growth Hormone & GH receptor

- GH:
  - Monomeric Protein
  - 217 Amino Acids
  - Compact Four-helix Bundle
- GH receptor (cooperative binding)
  - 638 A.Acid
  - Extracellular Domain (≈250 A.A) & Intracellular Domain (≈350 A.A)
  - Single Membrane-Spanning Helix
  - Monomeric (free) vs. Dimeric (bound)



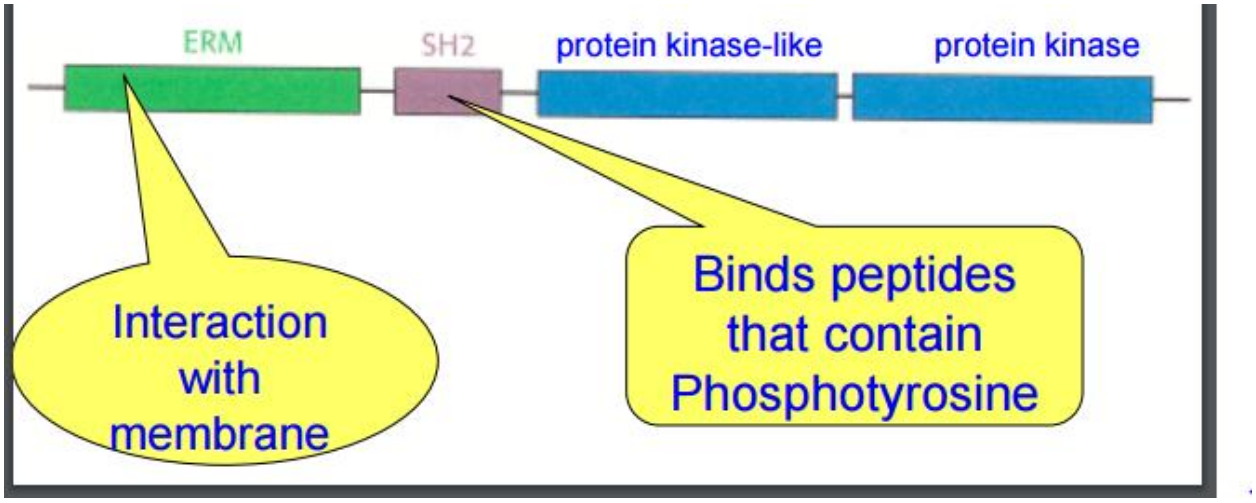
Binding of the GH [Growth Hormone] to its monomer, a tyrosine kinase receptor. The dimerization process will occur fast.

The size of the receptor of the growth hormone is 3 times the size of the growth hormone itself.

Janus Kinase – An enzymatic protein which is usually bound to the growth hormone receptor.

When the dimerization process occurs for the tyrosine kinase receptor, the Janus Kinase will come close to each other → Auto-Phosphorylation will occur → Active Receptor → Phosphorylation in Janus Kinase itself

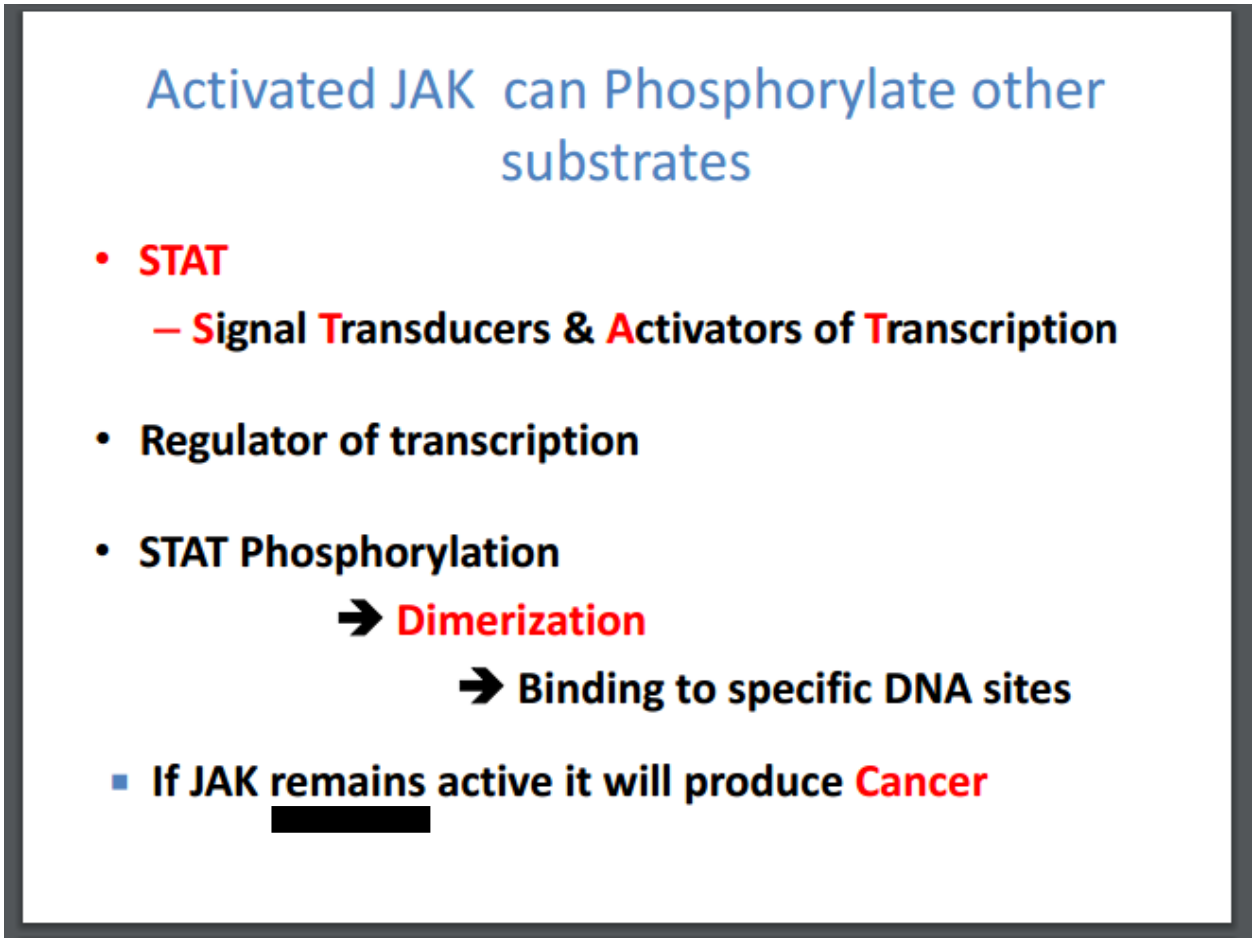
The active Janus Kinase will lead to a phosphorylation cascade.



The SH2 Domain

Its structure consists of B-Sheets, having 2 alpha helices in between them.

When you find a Janus Kinase having SH2 domain, so the Janus Kinase will bind to a phosphorylated tyrosine [Phosphotryrosine]



What is the main target of Janus kinase?

It induces STAT, which regulates transcription.

So the main target at the end will be DNA, activating transcription→ increasing the content of mRNA → Increasing Protein Concentration [Differentiation & Anabolism]

STATs also work in dimers, and are activated by phosphorylation.

If the Janus Kinase Enzyme remains active → STAT remains active → More Transcription → More Protein Concentration → Overgrowth → Cancer

A defect in STAT or Janus Kinase may lead to cancer ⇧

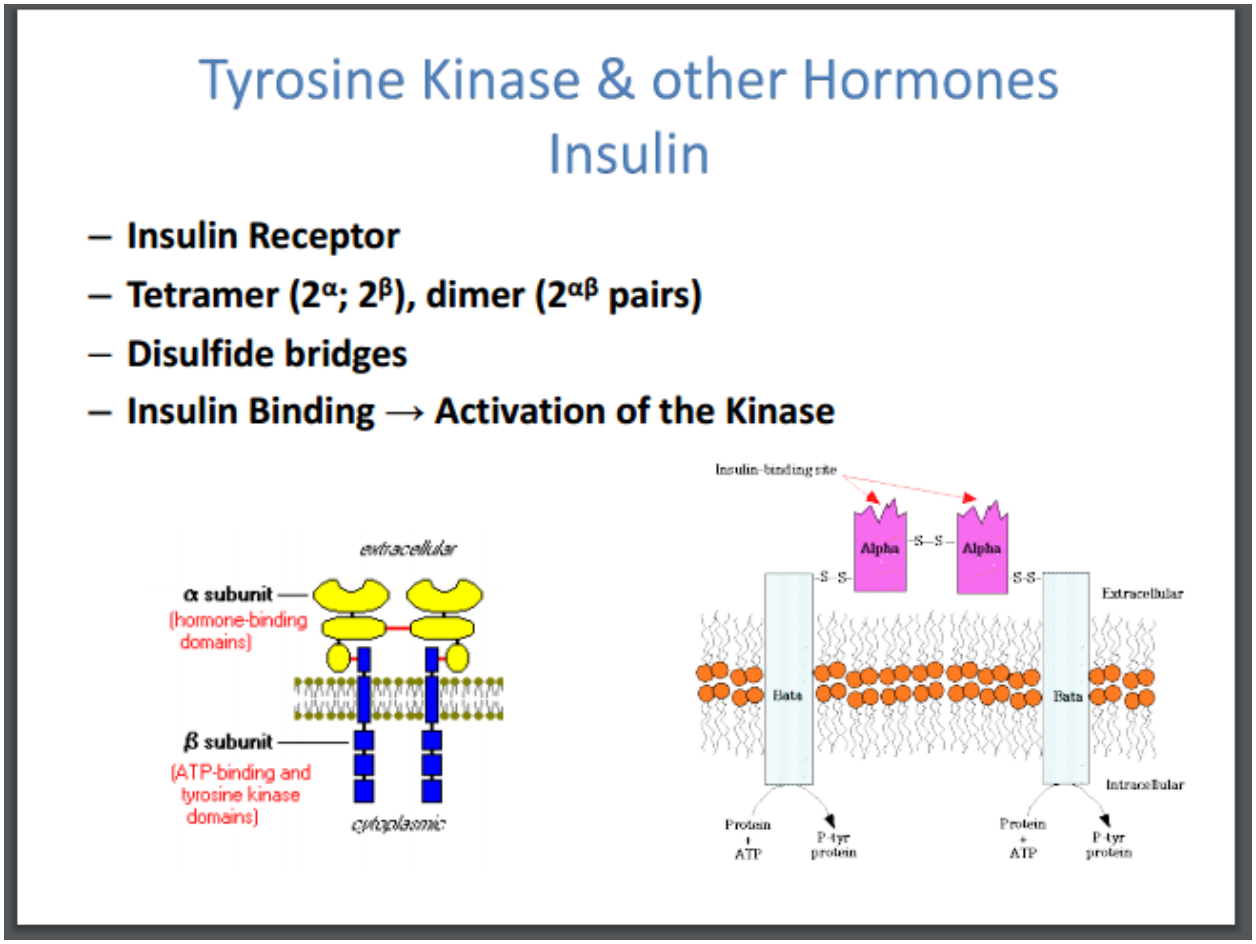
Epidermal Growth Factor Receptor :

It also works in the same mechanism

Dimer Formation → Auto-Phosphorylation → Phosphorylation

The difference is that each monomer binds an epidermal growth factor. So a total of 2 Epidermal Growth factors are needed to activate it while a total 1 Growth Hormone is needed to activate the Growth Hormone Receptor.

Insulin Receptor

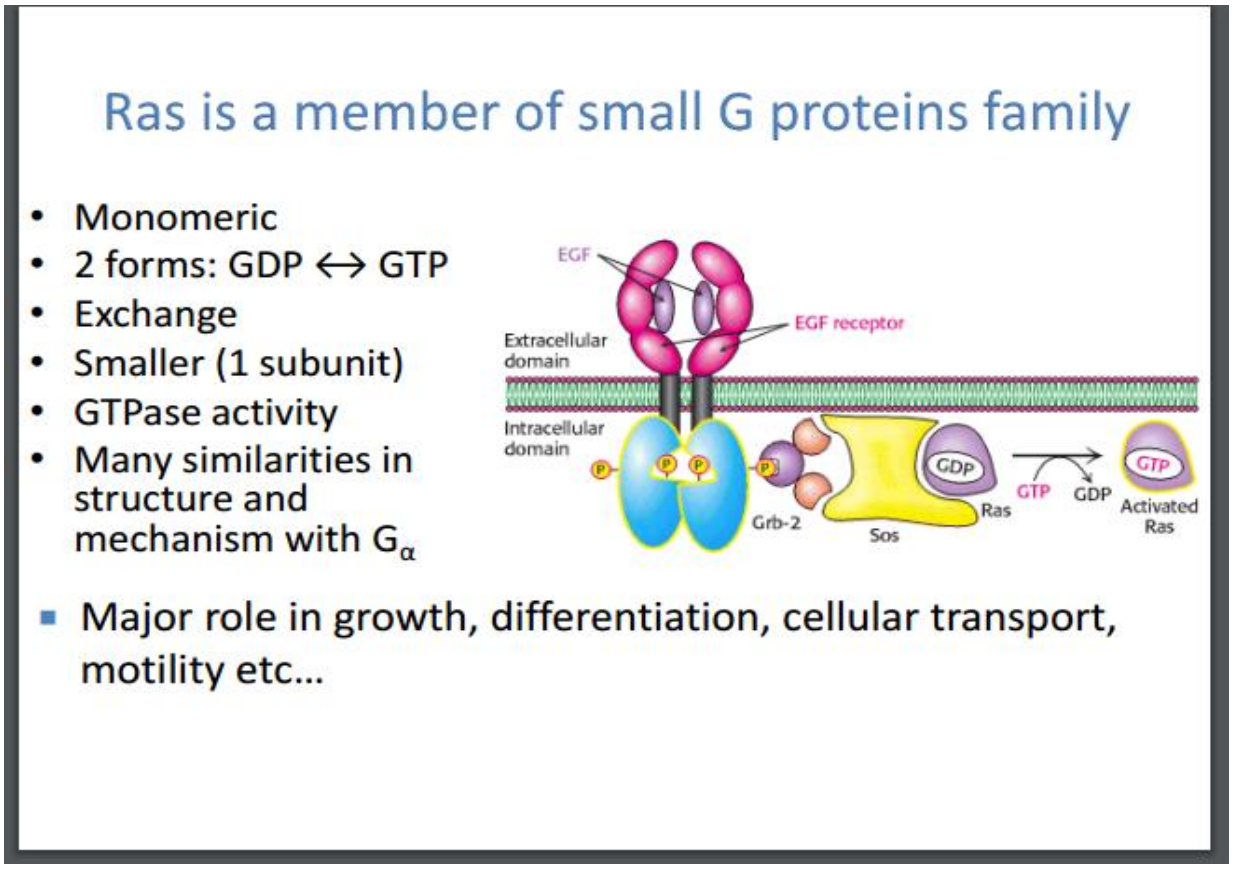


Class II Tyrosine Kinase Receptor → Already in a dimer formation.

The doctor didn't discuss its structure, but you better read it since it's written in this slide.

RAS Molecule

+



It is a growth molecule & one of the downstream effector enzymes and proteins of tyrosine receptors. It is a type of G-Protein

There are 3 different types of RAS proteins .[No need to differentiate between them]

In order to become active, the GDP will be exchanged with a GTP when the hormone binds to its extracellular domain, converting it into its "active" form.

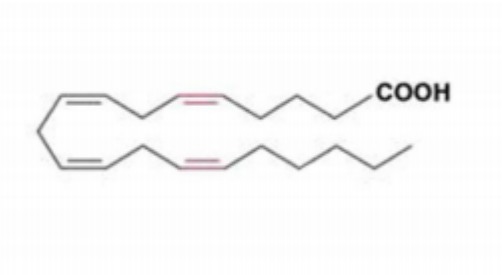
If RAS had a mutation, it will lose the ability to hydrolyze GTP back to GDP, staying active all the time, and the cell will keep on proliferating leading to cancer.

Eicosanoids:

[You must be able to differentiate between structures]

Produced in small concentrations, having a great effect on metabolism. [The same manner of hormones] .**Very Potent** Molecules. Each one of the eicosanoids has **20 carbon** molecules within it.

The main eicosanoid that we know from previous courses is the "**Arachidonic Acid"** found in the cell membrane. It is extracted from the cell membrane from the enzyme **P**hospho**L**ipase(PL).

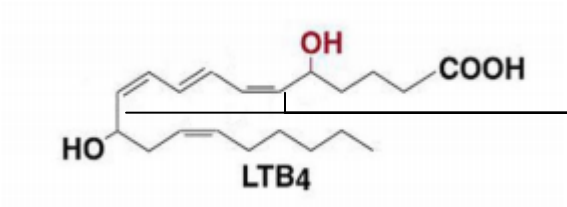


Here is its structure, also know as the ***eicosatetraenoic*** acid. It has 4 double bonds

Eicosatetraenoic [Arachidonic] Acid can be converted to:

Thromboxanes, Prostaglandins, & Leukotrienes.

Leukotrienes. [Derivative of Arachidonic Acid]

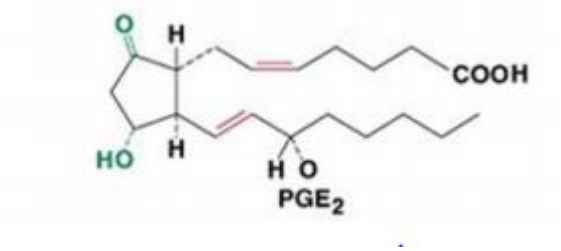


The three conjugating double bonds.

Its name is leuko**tri**ene because it has 3 *conjugating* (alternating) double bonds.

Conjugating Double Bonds = Double/Single/Double/Single/Double

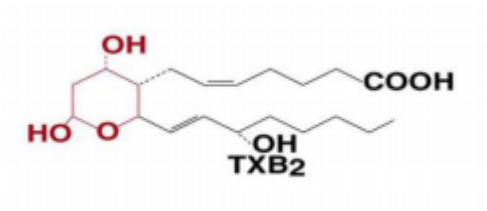
Prostaglandins



5 *membered ring.*

The doctor mentioned that we must be able to differentiate between the structures.

Thromboxanes



6 *membered ring.*

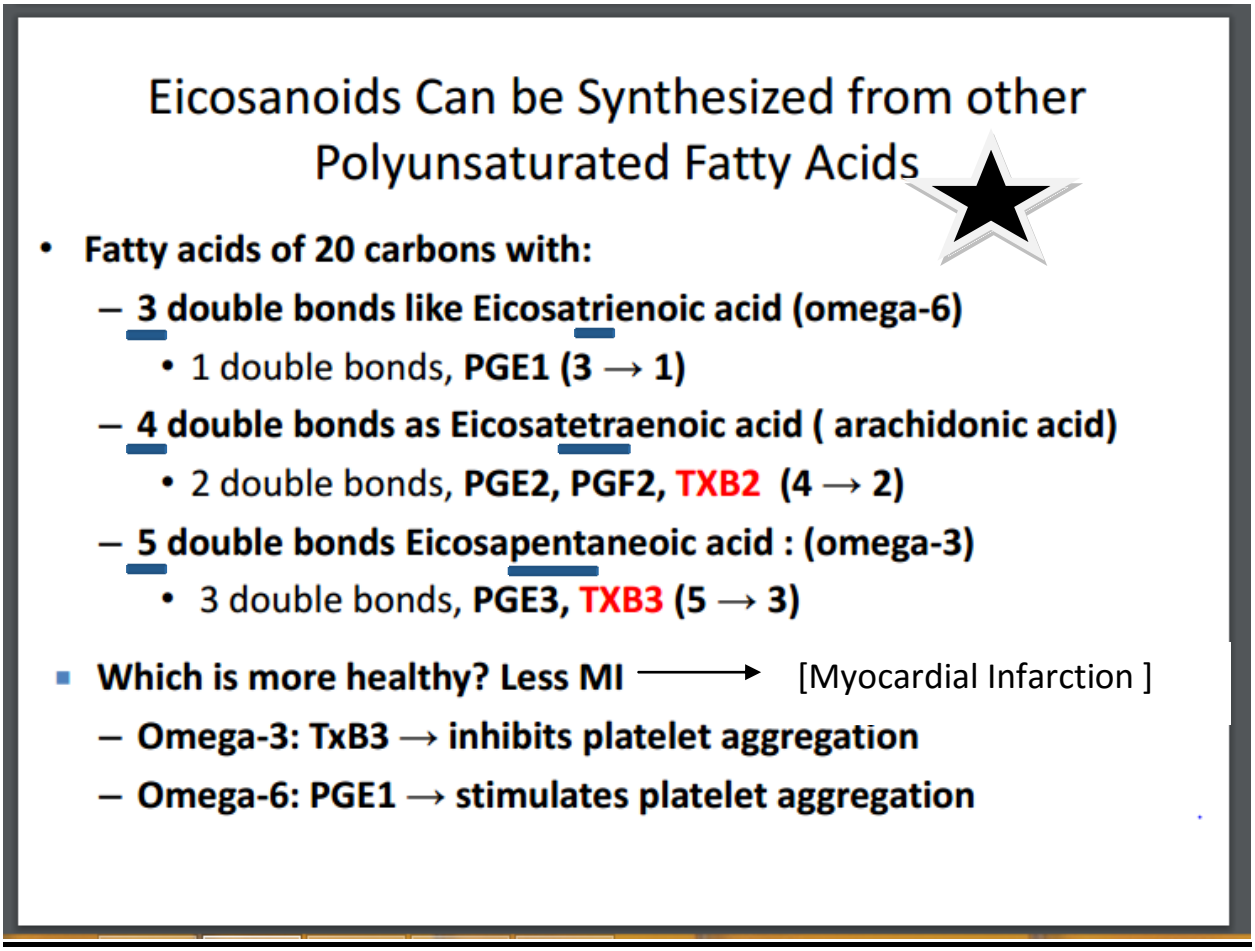
Some Functions of the Prostaglandins and Thromboxanes

- What 2 stands for?

- PGI2, PGE2, PGD2
  - Increase
    - Vasodilation, cAMP
  - Decrease
    - Platelet Agregation
    - Lymphocyte Migration
    - Leucocyte Aggregation

- PGF2α Increses
  - Vasoconstriction
  - Bronchoconstriction
  - Smooth Muscle Contraction

- Thromboxane Increases
  - Vasoconstriction
  - Platelet Agregation
  - Lymphocyte Proliferation
  - Bronchoconstriction



Arachidonic is the main source of all the eicosanoids; however it is not the only source.

Eicosanoids can be derived from other molecules , like the fatty acid in the membrane as long as it has 20 carbon atoms & being polyunstaturated [lots of double bonds].

Abbreviations : [The number at the end is the number of double bonds]

PGE1 – Prostaglandin E with 1 double bond

TXB3 – Thromboxane B with 3 double bonds.

Omega 6 is an eicosatrieonic acid with 3 double bonds, it is *bad* for the health because it produces PGE1 with induces platelete aggregation.

On the other hand. Omega 3 is an eicosapentaneoic acid , is *good* for the health since it extracts TXB3 which inhibits platelets aggregation preventing MI's.

END OF LECTURE.

Questions

[1] All of the following about Calcium Binding Proteins is true except:

(A) Contain a high content of both charged/uncharged polar amino acids

(B)Contain high content of negatively charged Aspartic Acid and Glutamic Acid

(C)Calcium fits in between the E alpha helix – Loop – F alpha helix

(D)The globular region of Calmodulin has one EF Hand

(E)The change of one amino acid will change the whole amino acid sequence.

[2] All of the following about Calcium/ATPase pump is true except:

(A) Returns the calcium back to the endoplasmic reticulum

(B) Its over activation may lead to Rigor Mortis

(C)Results immediately from the binding of calcium to calmodulin

(D) Uses 1 ATP Molecule for 2 Calcium Ions

(E)Used when the calcium's function in the cytoplasm is over.

[3] All of the following about Tyrosine Kinase is *false* except :

(A) May bind glucagon

(B)Growth Hormone receptor binds in a separate manner

(C)GH Receptor is only in the free monomeric form

(D)The GH itself may be both monomeric and dimeric

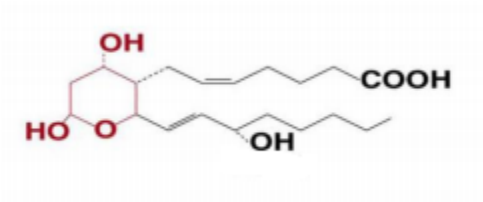
(E)A defect in the Janus Kinase enzyme may induce cancer.

Choose only one of these choices for the following two questions :

[A] PGE2
[B] TXB2
[C]TXB3
[D]LTB4
[E]ARACHIDONIC ACID

[4] A patient comes to your clinic and he has a cardiac arrest with previous myocardial infarction, what do you *ADD to* his diet?

[5]



The following structure is :

Answers

1	**D**
2	**B**
3	**E**
4	**A&C**
5	**B**

I really do apologize for any unintended mistake.

Hasan Saimeh.

اهدء خاص الى عمر خباز و يزن خريم .