University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 26

Done By: Sondos AL-khateeb

Corrected By: Russole Emad

Subject: Adhesion molecules and cell signaling .

Doctor: Diala Abu-Hassan

Price:

Date:

Cells interact with different components of ECM via their integrins and formation different types of junctions like hemidesmosome as well as adherence junctions , you can see these integrins interacting via different types of connecting molecules to the intracellular components of the cytoskeleton as well as interacting with fibrous ,protein and sugar component of the ECM .

integrins

these integrins are transmembrane heterodimers , composed of 2 subunits : alpha and beta with different types of alpha and different types of beta .
alpha subunit has 3 binding sites for metal ions (Ca , Mn and Mg) which are important in the dimerization process.

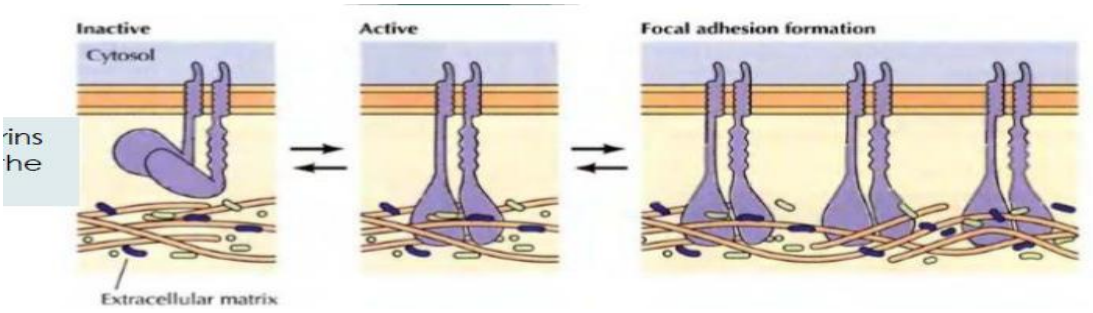
Functions:

1. The major cell surface receptors that attach cells to ECM, via binding to ECM proteins such as collagen, fibronectin,laminitis and proteoglycans.

2. Anchoring the cytoskeleton of focal adhesion and hemidesmosome.

focal adhesions assembly :

focal adhesions composed of integrins , these integrins are in the inactive form in this bent format , this form of integrins cannot interact with the ECM components , certain intracellular stimuli would open up the structure and allow the interaction of these integrins with ECM components , afterwords this will drag more integrin molecules to form focal complexes which will be further modified to focal adhesions by the recruitment of talin, vinculin and a-actinin.



Note: its not only integrins that participate in these interactions between cells and ECM components , other proteins as well can contribute such as selectins , IG superfamily (these are not immunoglobulins for immunity , but they are very similar to them in structure and that's why they called immunoglobulin superfamily) as well as cadherins .

homophilic VS heterophilic interactions

homophilic interaction :
if the interaction happens between molecule on cell surface and molecule on another cell surface , and these two molecules are from same type .like:cadherins with cadherins / N-CAM with N-CAM.

Heterophilic interaction :
if the interaction happens between molecule on cell surface and molecule on another cell surface or even ECM , and these two molecules are from different types.

selectins

role of selectins in linking cells to the ECM :
example :
selectins is expressed on the surface of leukocytes as well as surface of endothelial cells and platlets .
in the inflammatory reaction leukocytes have to be recruited to the site of injury and they have to be activated in order to eliminate the noxious stimuli. In order to recruit leukocytes these cells have to be rolled against the endothelium of blood vessels via week and transient interactions which are mediated by selecting family of adhesion molecules. Selectins are receptors or transmembrane protein expressed on leukocytes and endothelium that contain an extracellular domain that binds sugars(hence the lectin part of the name). The 3 members of this family are E-selectin on endothelial cells ,L - selectins on the surface of leukocytes and p-selectins which are present on platelets and endothelium.

Leukocytes rolling on endothelium mechanism of action:

these L selectins on the surface of leucocytes are going to identify carbohydrates on the surface of endothelium and interact with them as well as selectins on the endothelium and platlets are going to interact with carbohydrates on the surface of leucocytes . these would activate signaling pathways that brings I more integrin molecules that interact with intercellular adhesion molecules (ICAM :A member of IG superfamily) to bring this cell in very close proximity and attaching it to endothelium , this will activate opening a space inbetween the cells (intercellular spaces) and allow the passage of leukocytes to the site of injury across the blood vessel wall . (remember the process of recruiting leukocyte in an inflammatory reaction which we had taken in pathology course)

other proteins such as cadherins can interact by homophilic interaction between same molecules on different cell surfaces and connect the cells , there are many types of cadherins including :

1- classical cadherins like :

*E-cadherins in epithelial cells

*N-cadherins in neural cells

* P-cadherins in placental cells

2- desmosomal cadherins

3- fat-like cadherins

4- 7-tranemembrane cadherins

we have already discussed the contribution and presence of these cadherin molecules specifically the classical type depending on the cell surface they connecting , so if the cell is epithelial cell it is E-cadherin and so on .

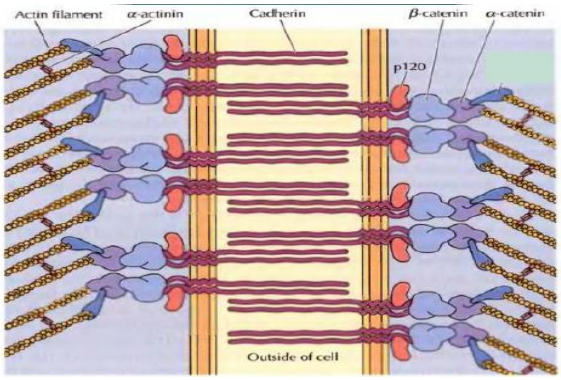
Cadherins can interact via heterophilic interactions such as interactions of cadherins in desmosomes.

junctions that cadherins contribute in their formation :

1- adherence junctions

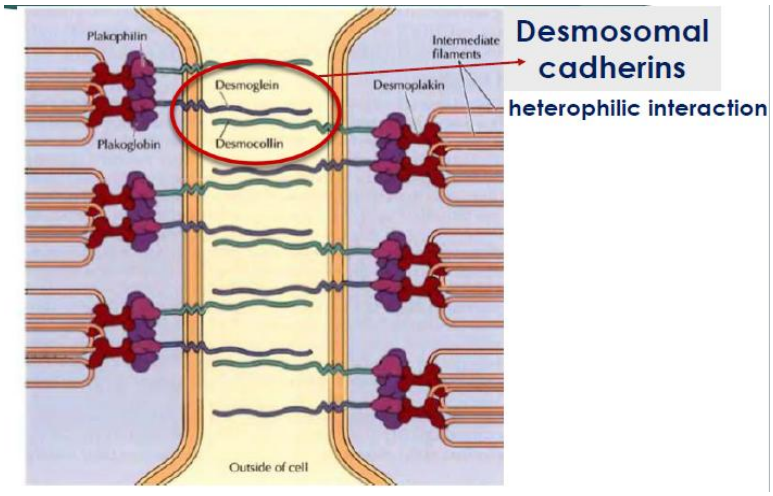
the interaction can be homophilic interaction (as we said previously) between cadherins to connect the actin fibers of cytoskeleton of one cell surface to the adjacent forming adherence junction .

we also have intermediate proteins connecting the actin filaments to cadherins such as beta catenin as well as alpha catenin .



2- desmosomes

in this junction the type of interaction is heterophilic since we have two types of desmosomal cadherins , which are desmoglein and desmocollin , these i nteract in the intercellular space and their



transmembrane proteins that can be associated to intermediate filaments by the presence of plako family proteins such as desmoplakin (which connect the cadherin molecules to the intermediate filaments) , plakoglobin and plakophilin .
all these 3 different types of plako family proteins can interact with intermediate filaments forming the desmosomal structure .

There are other types of junctions which cadherins don't play a role in, such as:

Tight junctions

tight junctions are basically strands of proteins that surround the whole periphery of the cell holding them together in very tight structures .
tight junction are made up of several protein types such as claudins , occludins and JAMS (junctional adhesion molecules) , then these are going to interact with the internal cytoskeleton of the cell by the presence of **zonula occludens proteins** (so these are medeiators between cytoskeleton and the protein component of tight junctions) .

function of tight junction

they are not the strongest junctions , but they are the impermeable ones that don't allow the passage of molecules inbetween the cells . in addition ,they separate the apical from basolateral surface in epithelial cells.

Gap junctions

gap junction are basically composed of channel like structures between the surfaces of adjacent cells . These channels help in synchronising the events that happens inside adjacent cells as well as transferring molecules of small sizes up to 1000 daltons between cells through this pore.
they are called gap junctions because they have an extracellular domains that will disperse the lipid component of cell membrane away from each other creating a gap in the membrane and these gaps will form a channel between adjacent cells.

structural component of gap junctions

they composed of proteins called connexins (there are many types of connexins) , these connexins can form homo or hetero hexamers called connexons , connexons that made up of 6 connexins in one cell surface will interact with a connexon on the surface of an adjacent cell forming a continuous gap junction that connect the cytoplasm of these two adjacent cells together as well as transmitting some compounds through this pore .
the presence of gap junction contribute in the development in some structures such as
1- development of the embryos where so many signaling molecules need to be

transmitted really quickly between cells rather than just release the molecule to the extracellular space then binding to a receptor and activating a signaling pathway . so , Its much faster when you transmit the compounds through the pore , and this is so important specifically in the development as we said .
2- development of synapses , as we all know that there are chemical synapses where vesicles are going to release neurotransmitters that's going to bind some receptors in the post synaptic neuron in a muscle or what ever . however during development these synapses were electrical synapses where we had these continuous channels of gap junctions that transmit signals from one neuron to another really quick , and they are going to transform into chemical synapses in mature cells , but they stay in some cells such as cardiac cells , we need them to contract all together so the presence of these gap junction will facilitate the transfer of metal ions (like Na which is important in action potential and contraction) specifically and the synchronization of contraction .

several diseases are associated with malfunction and problems in gap junctions :
1- Charcot-Marie-Tooth disease ----- peripheral nervous system
2- Deafness: if there is a problem in the channel that transmits potassium ions between the cells responsible for hearing
3- cataract : where the cells of the lens are basically avascular cells , they don't get any blood supply so they need gap junctions to transmit nutrients inbetween them , so if these gap junctions are obstructed that result in death of these cells and destruction of the transparent structure of the lens resulting in cataract
4- skin diseases

the next topic will be **cell signaling**
how cells can interact and transmit signals between each other ?
this is mediating by cell signaling , there are many modes of cell signaling which are :
1- direct cell-cell interaction as we said in the junctional proteins , one integrin for example is going to interact with another integrin or cadherin with another cadherin , and this happens only between neighboring cells .
2- paracrine signaling , one cell is releasing a ligand and this ligand is going to bind on receptor on the adjacent cell and induces some effect or activating certain signaling pathway . (this way happens between neighboring cells)
3- autocrine signaling where the cell itself releases a molecule that binds to its receptor and activates certain series of events .
4- signaling molecules that need to travel through the blood stream , released from one cell through circulation to be received and bound to a specific receptor in a target cell to induce some effect .

what are the **types** of signaling molecule that released from one cell to tell another cell a message ??

1- peptides (short sequences of amino acids) like growth factors ,peptide hormone like insulin and glucagon ,or neuropeptides like oxytocin and enkaphalins.

2- neurotransmitter like Ach , epinephrine and norepinephrine
3- steroids like vitamin D , cortisol and sex hormones
4- eicosanoids are derivatives of arachidonich acid like prostaglandins, leukotrienes and thromboxanes

5- gases such as CO and NO

some of these signaling molecules are hydrophobic (lipophilic) such as sex hormones , retinoic acid which is vitamin A , and vitamin D as well . so,
how do these induce an effect in a cell ??
by binding to an intracellular receptor ,,
these are going to be released from blood circulation , and because they are hydrophobic they can cross the cell membrane , once they are inside the cytosol they can bind their receptor .
but in the absence of the hormone their receptors are going to be held inactive by heat shock proteins which bind the receptor to inactivate it , then the entry of the hormone to cytosol is going to dissociate the HSP (heat shock proteins) , this way the hormone binds to its receptor , now ,, we have a hormone-receptor complex in the cytosol , this complex dimerizes with another hormone-receptor complex , then it is going to translocate into the nucleus through the nuclear pore where it will bind specific domain in DNA to activate the transcription of certain genes, production of mRNA and then translation on the ribosomes of the cytosol or RER to proteins that will have a certain effect.

how the hydrophilic signaling molecules induce effect in a cell ??

by binding to a cell surface receptors
1- these receptors are going to be bonded by a ligand , they already have a transmembrane segment , once the receptor is bound by its ligand it is going to induce some interaction or attraction of some molecules depending on the signaling pathway

2- we need this message to be transmitted from membrane to inside the cell , so we need to transduce the message to the cytosol by the activity of transducers as G protein or RAS protein
3- then other downstream molecules like effector molecules such as adenylate cyclase and MAPK will transmit the message by producing second messengers
4- second messengers like cAMP , cGMP and Ca are going to transfer this message in the cytosol until it activate specific transcription factor or inhibit it , and this transcription factor if it's activated it will activate or inhibit the expression of certain genes and induce a response in final target molecule like DNA ,channels, ...etc

Types of responses induced by a specific or a certain signaling pathway can be either

1- primary response :

once the lignd binds to its receptor it is going to activate a certain pathway that activate a certain set of genes or the expression of certain set of genes . (very direct)

2- secondary response :

it is depend on the primary response , we have to activate some genes by a primary response and proteins expressed by these genes are going to activate another set of target genes resulting in a secondary response

there are different type of receptors , we will talk about :

1- G protein coupled receptors

these are transmembrane proteins , composed of 7 helical structure inserted into the membrane , N terminus to the outside and T terminus to the inside
how do G proteins act ??
in normal situation where there is no ligand , its present by itself and there are some peripheral membrane proteins in the cyosolic side of the membrane (G protein) close to it. Once these receptor are activated the are going to bind to G protein.

G proteins are composed of 3 subunits (alpha ,beta and gamma) , the alpha subunit in the inactive form is bonded by GDP making it inactive , once we have the signaling molecule binding to the G protein coupled receptor this would bring and move this peripheral membrane attached complex (G protein by all of its subunits) in close proximity to the receptor , this would activate the exchange of GDP with GTP , now,, this complex can dissociate into two parts which are alpha subunit with GTP and the beta- gamma complex
what happens when I need to inactivate the G protein ?
simply by the hydrolysis of GTP to GDP by intrinsic GTPase activity, then this will allow the assembly of the whole complex (assembly of alpha , beta and gamma subunits all together) away from the receptor .

2- tyrosine kinases

there are so many types of these tyrosine kinases :

1.either receptor phosphorylate themselves (receptor tyrosine kinase)

2. or receptor get phosphorylated by other tyrosine kinase proteins (non receptor tyrosine kinases)

A- receptor protein tyrosine kinase (RTK)

. example on these receptors :
1- epidermal growth factor receptor
2- platlet derived growth factor receptor
3- insulin receptor

you can notice how different they are in the extracellular domains but they are very similar in the intracellular or cytoplasmic domains

How these receptors get activated ??

Insulin receptors for example: we have growth factor or ligand binding , it is going to bring another molecule and they will form dimer , these dimers are going to induce autophosphrylation of tyrosine kinase receptor and receptors dimerize. then I the receptor may bind other transducers that going to transmit the signal from the membrane to cytoplasmic structures , these called downstream signaling molecules .

B- non receptor protein tyrosine kinases like JAK and Src .

example to illustrate this type

cytokines : are chemicals that released in inflammatory reaction

once a cytokine binds to its receptor which called cytokine receptor superfamily , receptors are going to dimerize just like RTKs but this time it is not going to phosphorylate itself or phosphorylate the adjacent molecule (the other receptor in the dimer) , instead , the dimerization of cytokine receptors will bring tyrosine kinases that are not part of the cytokine receptor itself (out tyrosine kinases from the cytosol) which are the non receptor protein tyrosine kinases .

because the cytokine receptors are dimerized so we need two molecules of non receptor tyrosine kinases to bind the dimer , then each one of the non receptor tyrosine kinases is going to phosphorylate the other one (the first molecule of non receptor tyrosine kinase will phosphorylate the second molecule of non receptor tyrosine kinase and vise virsa) , this will make both of non receptor tyrosine kinases active . once they are active , each one of them will phosphorylate the cytokine receptor on which it is bind . so it is kind of indirect way to phosphorylation .

other types of tyrosine kinases :

1- tyrosine phosphatases

which inactivate these processes by removing the phosphate groups on tyrosine residues

2- serine/threonine kinases

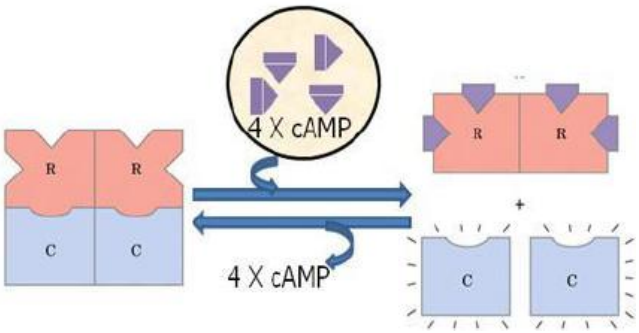
they phosphorylate on serine or threonine residues

3- guanylyl cyclases

4- Protease-associated receptor (tumer necrosis factor receptors TNF)

Second messengers

Now,, downstream of receptors we need other molecules (second messengers) to transmit the message . so , why do we need these second messengers ?
1- because receptors cant move from membranes , so we need more free molecules which are the second messengers to transmit the message to other compartments and organelles inside the cell
2- there might be several molecules of second messengers that can be activated by the receptor resulting in
amplification of the signal
3- second messengers are common in many signaling pathways, so this will result in cross talk inbetween different signaling pathways.



Examples on second messengers

1- cAMP

its made up of AMP with ring between phosphate and sugar converting it to a cAMP instead of the normal AMP without the presence of this ring , its derived from ATP by dephosphorylation process catalyzed by adenylyl cyclase enzyme

how does this act ??
cAMP downstream of a receptor is going to bind the regulatory subunit of specific kinase called protein kinase A , protein kinase A has 4 subunits , 2 regulatory subu nits and 2 catalytic subunits , once we have binding of these 4 cAMP molecules (2 on each regulatory subunit) this would separate and activate the catalytic subunits so now they can start phosphorylating or acting as a kinases , then catalytic subunits will translocation to the nucleus through nuclear pores , then they are going to phosphorylate the transcription factor (CREB), the phosphorylated CREB will bind to a specific region on the genome and activating transcription of certain target genes

how can we inactivate this signaling pathway ?
The phosphorylation of target proteins by protein kinase A is reversed by the action of phospho taste 1. For example ,CREP is inactivated by dephosphorylation via phosphatase 1.

2- phospholipids and Ca^{+2}

once we have ligand binding to a G protein , this would activate the G protein by exchanging GDP on alpha subunit with GTP , so it dissociate into alpha-GTP subunit and beta-gamma complex , this activated alpha subunit will activate enzyme called phospholipase C , from its name (phospholipase C) it will add phosphate groups on lipids , it acts on specific type of phospholipids called phosphatidyl inositol 4,5 bisphosphate (PIP2) , this PIP2 found in membranes , and by the action of phospholipase C it will be separated from the membrane (inositol and 3 phosphate groups (IP3) which are hydrophilic part of the PIP2 will disassociate from the membrane as result of phospholipase C action leaving the diacyl glycerol (DAG) which is the hydrophopic part of PIP2 in the membrane

after disassociation of IP3 it will go and bind a channel of endoplasmic reticulum , this will open the channel to exit of Ca ions to the cytosol , these Ca ions are going to bind protein kinase C activating it , once it is activated it is going to induce again other effect and activate another set of target genes

3- calmodulin and Ca^{+2}

straight calmodulin represent the inactive form , once Ca ions bind to calmodulin in will cause it to bend activating it , once calmodulin is activated it will bind calmodulin dependent kinase converting it from inactive form to the active form by taking the arm away from the active site and open up the active site of this enzyme

Signaling pathways

why do we have specific responses ?? why liver cells and muscle cells would response to insulin hormone by storing glycogen ? why specific signal only affect some tissues rather than others ??

1- because of expression of specific receptors which make this cell a target for this signaling pathway or hormone or whatever rather than other cells

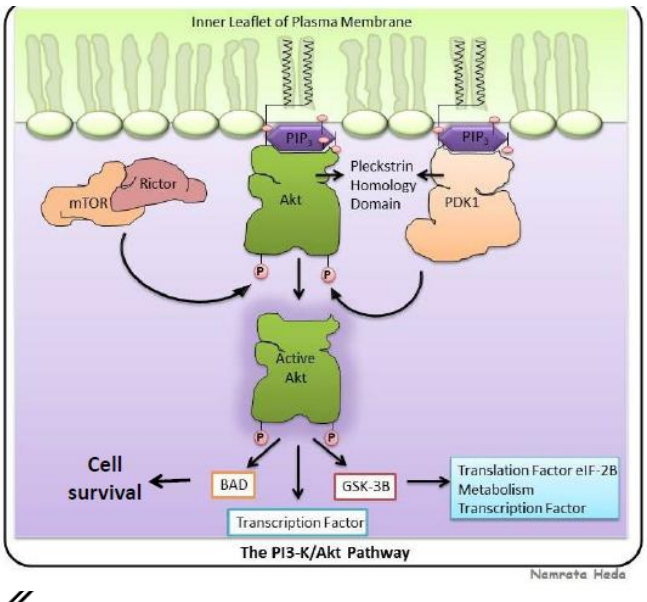
2- also inside the cell there are different combinations of regulatory proteins , downstream of a receptor there are other set of proteins that expressed in certain cell type , this will make the response of this cell different from the response of another cell . as we said before there is cross talk between these signaling pathways , but if a specific protein is not expressed it would not activate this pathway for example , but it would activate another pathway which its downstream effectors are present inside the cell .

3 - final effectors (transcription factor) must have access to its DNA biding site : if the chromatin is packaged tightly, the transcription factor wont be able to bind to DNA and hence activate transcription.also different final effectors and different transcription factors in each cell type would result in different responses even for the same pathway

Example on signaling pathways

1- PI-3 kinase and AKT pathway

PI-3 kinase pathway is mainly focus on AKT molecule (aka: protein kinase B) , AKT is a hup for interaction of so many signaling pathways . so many molecules and pathways and receptors can activate AKT , this



molecule once activated it is going to affect and induce changes in so many molecules downstream of it and this would result in activation or inactivation of gene expression , but these genes are involved in cell division , proliferation, survival and differentiation , so the activation of AKT signaling pathway results in all these processes to happen ,.
there are different types of AKT which are AKT1 , AKT2 and AKT3 . AKT1 is important mainly in the development of skeletal muscles , it helps in the differentiating of mesenchymal cells into skeletal muscle cells .
AKT2 in the other hand contribute to bone differentiation , so if it not present there is no bone differentiation .
AKT3 is mostly found in neuronal cells
what does AKT do ?
When a ligand binds to its receptor it will induce PI3K which will basically catalyze conversion of one phospholipid type wich is PIP2 which has 3 phpsphate groups , one original within phosphatidyl inositol and 2 addition al , PI3 kinase is going to phosphorylate it adding more phosphate so we would have PIP3 that has atotal of 4 phosphate groups , PIP3 will act like a binding site for AKT and then activation of AKT by the action of PDK1 which is dependant on the presence of PIP3.

Activation of AKT can be done by PDK1 as well as by mTOR , each of these (PDK1 and mTOR) will phosphorylate a specific residue within the AKT and would activate accordingly a different pathway branching from AKT

2- mTOR pathway

it is a downstream pathway under AKT pathway , once activated it is going to lead to cell survive and proliferation . so ,we will expect under certain conditions like if we need to induce apoptosis , the cell will start digest its internal organelles by autophagy . so autophagy will lead to cell death , so we need to inactivate mTOR under these conditions . the deffecincy of nutrients as a cause or stimulant for apoptosis is going to inhibit mTOR and once it is inhibitid this will induce autophagy and start the digestion of mitochondria as well as some other cellular components

3- RAS pathway

RAS is activated by a receptor tyrosine kinase , once the ligand (GF) binds to receptor tyrosine kinase it will dimerize then it will be phosphorylated on specific tyrosine residues , this would bring a transducers protein downstream of receptor tyrosine kinase like (Grb2 , SOS which is a guanosine exchange factor (GEF)) , Grb2 will bind to the receptor and SOS will bind the Grb2 molecule , then this SOS (GEF) will exchange the GDP on RAS with GTP , once we have RAS-GTP , this is the active form . if you remember when we said that RAS is a farnesylated protein attached by a farnesyl group to the inner leaflet of a membrane .
so, RAS cannot move because it is attached to the membrane , so the active form of it will bind Raf protein which will start phosphorylating a downstream molecules like MEC then MEC will phosphorylate ERK and this will phosphorylate other molecules

downstream resulting in target gene activation and activation of cell cycle.
also this activated ERK will translocate to the nucleus and phosphorylate a transcription factor called ELK-1 then the activated ELK-1 will phosphorylate serum response factor (SRF) then both of them will bind to specific region on DNA called serum response element (SRE) this will lead to activation of transcription of factors that lead to cell survival .

(this is the mechanism of RAS pathway as part of it is not mentioned in slides but it had been mentioned in record) .

THE END

P.S: please refer to the slides while studying this sheet

be always positive as much as you can

wish for you best of luck ☺