

7.016 Recitation 14 – Fall 2018

(Note: The recitation summary should NOT be regarded as the substitute for lectures)

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Summary of Lectures 20 (10/29) and 21 (10/31):

Cell signaling: A cell responds to signals from its surrounding environment. In general, the signal molecule binds to a receptor, conveys the message to the inside of the cell and then the cell changes its activity in response to the signal. The signals can be...

- **Physical** (i.e. light, aroma, touch) or
- **Chemical**, which mediates its effect by binding to a receptor.

The chemical signals (often called **ligands**) are of four major types: autocrine (chemicals act on the same cells that produce them), paracrine (diffuse to and out of a nearby cell), endocrine (hormones that travel through blood to reach the target cells) or juxtacrine (chemical secreted by one cell acts on a cell that it is in contact with).

Signal receptors can be classified based on their location and whether or not their ligands can diffuse through the membranes. They are broadly of two major types:

- **Cytosolic** (such as estrogen and other steroid hormone receptors that have intracellular location) or
- **Membrane bound** (such as ion channels, protein kinase receptors and G protein-linked receptors).

The ion channels, on binding to their ligand undergo conformational change that allows the passage of ions. The protein kinases (such as receptor tyrosine kinases i.e. RTKs), when bound to their ligand, may undergo auto-phosphorylation to become activated. These kinases can then phosphorylate other proteins, thus changing their conformation and functions. In comparison, the G-protein coupled receptors exist in their GTP bound active state when bound to the ligand and GDP bound inactive state in the absence of any signal.

The same signaling molecule can elicit different responses in different cell types. These responses may either be...

- **Direct** i.e. it is the function of the receptor itself and occurs at the plasma membrane or
- **Indirect**, which are more common and involve second messenger like cAMP, nitric oxide (NO), Ca^{++} etc.

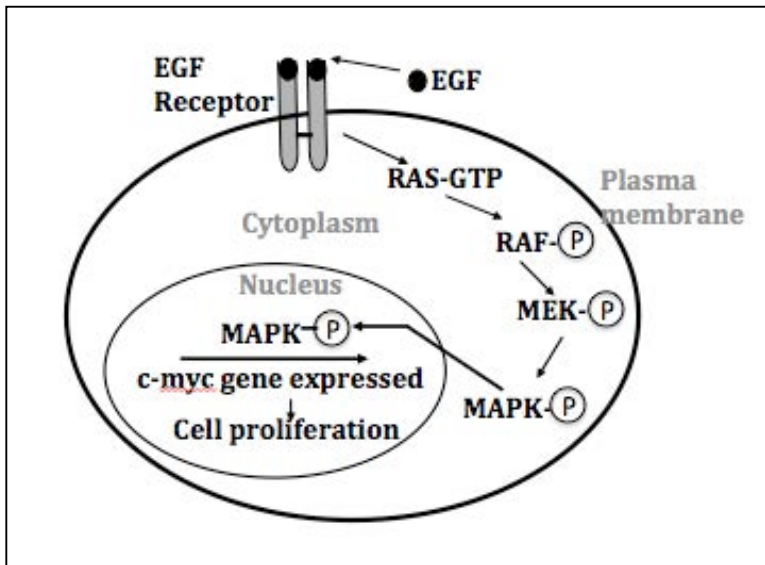
It is to be noted that a 2nd messenger is a small molecule that mediates later steps in the signal transduction pathways after the 1st messenger- the signal or the ligand- binds to the receptor. Thus a 2nd messenger serves to amplify the signal, which then activates many enzyme targets by binding to them non-covalently. In either case, cascade of signals gets initiated, each step adding towards amplification of the signal. The result is a change in the activities of the cell.

Signal transduction pathways within a cell cross-talk with each other and this plays a critical role in their tight regulation. Often the end product of signaling pathway may serve as an inhibitor for that pathway (feedback inhibition). Any alteration in these regulatory mechanisms disrupts cellular homeostasis resulting in diseases like cancer, cystic fibrosis, Alzheimer's etc.

Questions

1. Following is the schematic of a signal transduction pathway that is activated by the binding of a Epidermal Growth factor (EGF), produced by one cell type, to its specific membrane receptor of a target cell. The major steps involved in this pathway are outlined below:

- EGF ligand binds to the EGF receptor.
- Ligand bound EGF receptors become active through phosphorylation and homodimerization.
- Active EGF receptor causes Ras to exchange its bound GDP for GTP and become active.
- Active Ras activates the kinase cascade (RAF, MEK and MAPK) through phosphorylation.
- This increases the expression of c-myc gene, which **results** in cell proliferation.



You decide to engineer mammalian cell lines (**Cell line- 1 & Cell- line 2**), each expressing a specific mutant variant of either the EGF ligand or the EGF receptor (EGFR).

- **Cell line-1** has a mutation that results in the deletion of **only** the signal sequence of **EGF ligand**.
- **Cell line-2** has a mutation that results in the deletion of **only** the transmembrane domain (TMD or ALL transmembrane helices) of **EGFR**.

You incubate each of these mutant cell lines with fluorescent antibodies that specifically bind either to EGF or the EGFR. You then observe these cell lines under the fluorescent microscope to study the localization of EGF ligand or EGFR.

a) In cell line- 1 where do you expect to find the EGF ligand: **Cell membrane/ cytosol/ cell culture medium**? **Explain** your choice.

b) If cell line-2 is incubated with EGF ligand, do you expect these cells to proliferate? **Why or why not?**

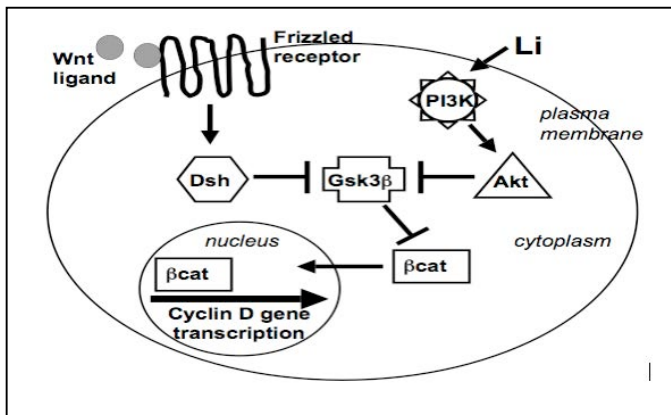
c) Consider the following cells that have mutations in different components of the EGF signal transduction pathway.

- **Mutant 1 (m1):** Ras protein that continues to stay in its GDP bound form.
- **Mutant 2 (m2):** RAF protein that lacks its kinase domain.
- **Mutant 3 (m3):** EGF receptor that lacks its extracellular domain.
- **Mutant 4 (m4):** MAPK that is constitutively phosphorylated at its active site.
- **Mutant 5 (m5):** c-myc gene that has a constitutively active promoter.

Complete the table for each of the following cells. Indicate whether c-myc is expressed and state the change in cell proliferation relative to wild type cells **in the presence of EGF**.

Homozygous mutations in the cell (i.e. both alleles of the relevant gene are mutated)	c-myc expressed (Yes/No)?	Cell proliferation increased//unchanged/ no proliferation?
Both m1 and m2		
Both m4 and m5		
Both m3 and m5		

2. The *wnt* signaling pathway is one of the most important in biology. It is required for cell proliferation and its inhibition leads to programmed cell death. As diagrammed below;



- Wnt ligands bind to frizzled receptors.
- These receptors bind to disheveled (Dsh) and activate its function.
- The Dsh inhibits GSK3β, a kinase.
- GSK3β phosphorylates the transcription factor β-catenin (βcat). The phosphorylated β- catenin is unstable and gets degraded.
- Thus wnt signaling inhibits GSK3β, promotes β-catenin stability and translocation to the nucleus.

- GSK3β can also be inhibited by addition of Lithium, acting through PI3 kinase/ Akt pathway.
- Cyclin D gene transcription is activated by β-catenin.

Compare the expression of cyclin D protein levels in the pairs of cells described below and explain your reasoning. In each case **both cells of the pair are treated with wnt ligand**. *For your answers you should consider only those components that are shown in the schematic above or listed as bullet points in the explanation and explain only in the space provided. You may ignore the effect of Lithium while answering the questions. **State the changes that will be elicited by the following mutations.***

a) Mutant cells having a Frizzled receptor that lacks its ligand binding domain. **Note:** Ignore the effect of lithium.

b) Mutant cells that express a constitutively active Dsh protein. **Note:** Ignore the effect of lithium.

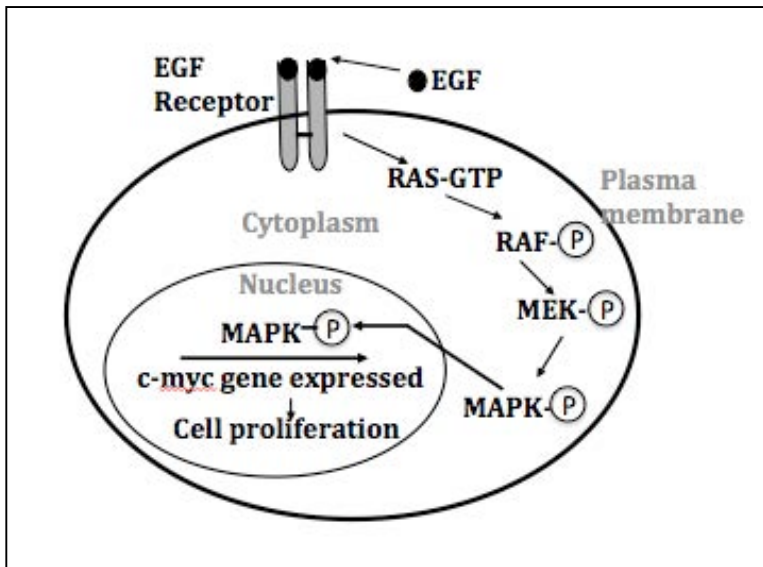
c) Mutant cells in which PI3K is inactive.

d) Mutant cells in which β -catenin lacks its GSK3 β phosphorylation site. **Note:** Ignore the effect of lithium.

The key

1. Following is the schematic of a signal transduction pathway that is activated by the binding of an Epidermal Growth factor (EGF), produced by one cell type, to its specific membrane receptor of a target cell. The major steps involved in this pathway are outlined below:

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You incubate each of these mutant cell lines with fluorescent antibodies that specifically bind either to EGF or the EGFR. You then observe these cell lines under the fluorescent microscope to study the localization of EGF ligand or EGFR.

a) In cell line- 1 where do you expect to find the EGF ligand: **Cell membrane/ cytosol/ cell culture medium**? **Explain** your choice.

In the absence of the signal sequence the protein will be translated and stay in the cytosol since the protein translation machinery will never be directed to the Rough endoplasmic reticulum (RER).

b) If cell line-2 is incubated with EGF ligand, do you expect these cells to proliferate? Why or why not?

The EGF receptor variant in this cell line has the signal sequence but lacks the hydrophobic / transmembrane domain sequence. Therefore, this variant of protein will be secreted into the medium. Thus the EGF receptor will not be available at the cell membrane to bind to EGF ligand and activate Ras-Raf–MEK-MAPK kinase pathway that is required for cell proliferation.

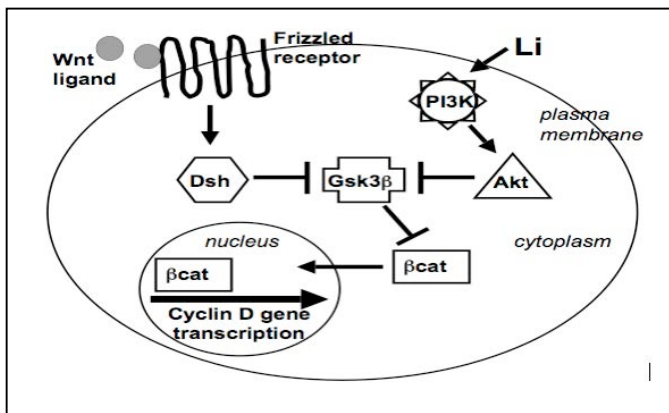
c) Consider the following cells that have mutations in different components of the EGF signal transduction pathway.

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- **Mutant 5 (m5):** c-myc gene that has a constitutively active promoter.

Complete the table for each of the following cells. Indicate whether c-myc is expressed and state the change in cell proliferation relative to wild type cells **in the presence of EGF**.

Homozygous mutations in the cell (i.e. both alleles of the relevant gene are mutated)	c-myc expressed (Yes/No)?	Cell proliferation increased//unchanged/ no proliferation?
Wild type	Yes	
Both m1 and m2	No	No proliferation. Both Ras and Raf kinases are constitutively inactive and so the cascade is never turned on to enhance c-myc gene expression that promotes cell proliferation.
Both m4 and m5	Yes	Increased proliferation. c-myc will be constitutively expressed owing to its constitutively active promoter irrespective of the presence or absence of active MAPK in nucleus.
Both m3 and m5	Yes	Increased proliferation. c-myc will be constitutively expressed owing to its constitutively active promoter. This will not require upstream signaling that is initiated by the binding of EGF with its receptor.

2. The *wnt* signaling pathway is one of the most important in biology. It is required for cell proliferation and its inhibition leads to programmed cell death. As diagrammed below



- Wnt ligands bind to frizzled receptors.
- These receptors bind to disheveled (Dsh) and activate its function.
- The Dsh inhibits GSK3β, a kinase.
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- Cyclin D gene transcription is activated by β-catenin.

Compare the expression of cyclin D protein levels in the pairs of cells described below and explain your reasoning. In each case **both cells of the pair are treated with wnt ligand**. For your answers you should consider only those components that are shown in the schematic above or listed as bullet points in the explanation and explain only in the space provided. You may ignore the effect of Lithium while answering the questions. **State the changes that will be elicited by the following mutations.**

a) Mutant cells having a Frizzled receptor that lacks its ligand binding domain. **Note:** Ignore the effect of lithium.

This mutation will prevent the Frizzled receptor from binding to the wnt ligand. The Frizzled will not be able to activate Dsh, which is required for the inactivation of GSK3 β . Therefore GSK3 β will remain constitutively active and will phosphorylate and promote degradation of β -catenin and prevent its nuclear translocation that is required for the transcription of cyclin D gene. So no cyclin D will be produced.

b) Mutant cells that express a constitutively active Dsh protein. **Note:** Ignore the effect of lithium.

Dsh will always be active irrespective of the presence or absence of wnt ligand. The active Dsh will constitutively inactivate GSK3 β . Hence the GSK3 β will not be able to phosphorylate and degrade beta catenin, which will now translocate to nucleus to enhance the transcription of Cyclin D gene. So cyclin D will be constitutively produced.

c) Mutant cells in which PI3K is inactive.

In the absence of a functional P13K, Lithium would not be able to mediate its effect. So the Cyclin D expression would be caused only through the Wnt ligand mediated signaling pathway.

d) Mutant cells in which β -catenin lacks its GSK3 β phosphorylation site. **Note:** Ignore the effect of lithium.

β -catenin will not be degraded by GSK3 β irrespective of the presence or absence of wnt ligand. Hence it will always translocate to the nucleus to enhance the transcription of cyclin D gene. So cyclin D will be constitutively produced.

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7.016 Introductory Biology
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