Eukaryotic Microbiology

MICROBE FOCUSED CELL BIOLOGY

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Cell-Cell Communication

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Overview of Cell Signaling

Dictyostelium discoideum, known as a slime molds, or social amoebae (singular: amoeba), is a model organism used to understand key cellular functions such as cell motility, signaling, and cell-cell interactions. *Dictyostelium*, known affectionately as 'dicty' to those who study them, has been extensively studied for over 75 years[1], providing important insights into cellular processes such as (but certainly not limited to) chemotaxis, the evolution of multicellular organisms, and cell adhesion. In other words, scientists better understand how cells move, how cells interact within a multicellular organism, and how cells stick together, all due to studies on a seemingly simple slime mold[2].

Dictyostelium is a eukaryotic organism. It has 6 gene dense chromosomes that contain between 8,000 and 10,000 gene with approximately 12,500 predicted proteins. In comparison, humans have 46 chromosomes (23 pairs), which are predicted to have between 20,000 and 25,000 protein-coding genes[3]. Not all genes within an organism will create protein products. For humans, however, it is estimated that approximately 20,000 genes encode a protein (so 20,000 proteins). Of these protein coding genes, we must take into account splicing variants, single amino acid polymorphisms, and post-translational modifications. This means that each protein coding gene could theoretically create as many as 100 different proteins (sometimes described as proteins species or proteoforms of a gene)[4]. Considering that the human genome can potentially code for 160-fold more proteins than *Dictyostelium*, it may be surprising that so much understanding of human cell biology has come from studies of *Dictyostelium*. Many *Dictyostelium* genes have orthologs (defined as genes descended from a common evolutionary ancestor) to human genes.

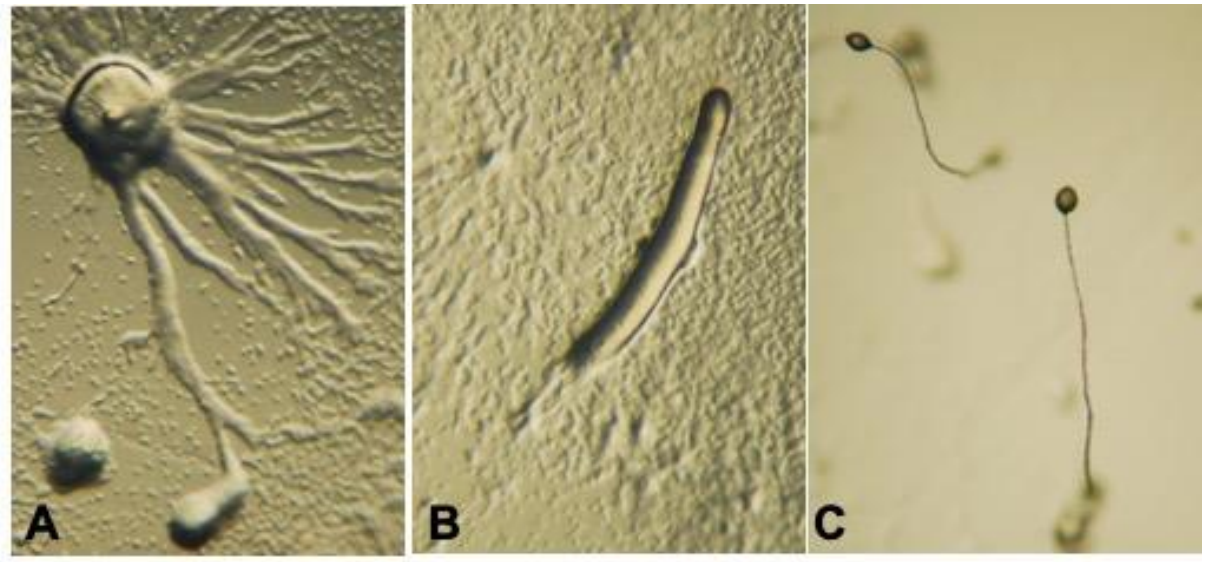

Figure 1. *Dictyostelium* viewed under a light microscope at distinct lifecycle stages. 1A. Aggregation stage. Under starvation conditions, single Dictyostelium cells begin the process of chemotaxis, streaming together to form an aggregation. 1B. Slug stage. Moving as one organism, Dictyostelium cells migrate before forming a fruiting body. 1C. Spore stage. Individual Dictyostelium cells develop into either a spore (black tips) or a stalk (long protrusion). Spores will geminate to move cells to better environments. Image credit: Medora Huseby, adapted from public domain images.

Several human diseases, such as Wiskott-Aldrich syndrome, are linked to defective proteins that were first identified in *Dictyostelium*, and then later in humans. Wiskott-Aldrich syndrome is characterized by a low platelet count (thrombocytopenia), bruising, bloody diarrhea and spontaneous nose bleeds. The low platelet count associated with Wiskott-Aldrich syndrome is thought to be associated with abnormally shaped T cells. T cells are a crucial immune system component, and similar to the amoeboid *Dictyostelium*, T cells must rearrange their actin cytoskeleton in order to move, in this case throughout the body. *Dictyostelium* cells that lack the ability to organize the actin cytoskeleton are unable to move and respond to stimuli. Immune system disorders, such as Wiskott-Aldrich syndrome, are the result of human T cells which lack the ability to organize the actin cytoskeleton. In this manner, the protein, and then the gene, that is dysregulated, was first discovered in *Dictyostelium* cells that could not properly move, and later a homolog in humans was found which exhibited the same lack of movement due to the inability to organize actin. The human and *Dictyostelium* protein is known as WASP (Wiskott-Aldrich Syndrome Protein), which is a binding partner for Cdc42, a Rho-GTPase, that will be discussed below[5].

Dictyostelium (**Figure 1**) typically inhabit soil, which is an environment teeming with many organisms living in close proximity to and feeding on one another. *Dictyostelium* feed on yeast and bacteria, both of which are plentiful in soil under conditions favorable for cell growth and division. *Dictyostelium* cells flourish and increase in number through binary fission when prey is plentiful. *Dictyostelium* must move through the soil to find microbes on which to feed. Though they lack eyes and

ears of a multicellular eukaryotic animal with which to hunt food, they still sense their food by signals secreted by their prey[6]. Bacteria secrete folic acid, which is a molecule that attract *Dictyostelium.* Or, if food is scarce, *Dictyostelium* cells can alert each other that starvation conditions are present. This alert will trigger cellular changes in nearby amoeba to move from individual amoeba to a multicellular organism. This survival mechanism ultimately allows the now multicellular organism to produce spores that can be dispersed in hope of finding a better feeding ground (**Figure 2**).

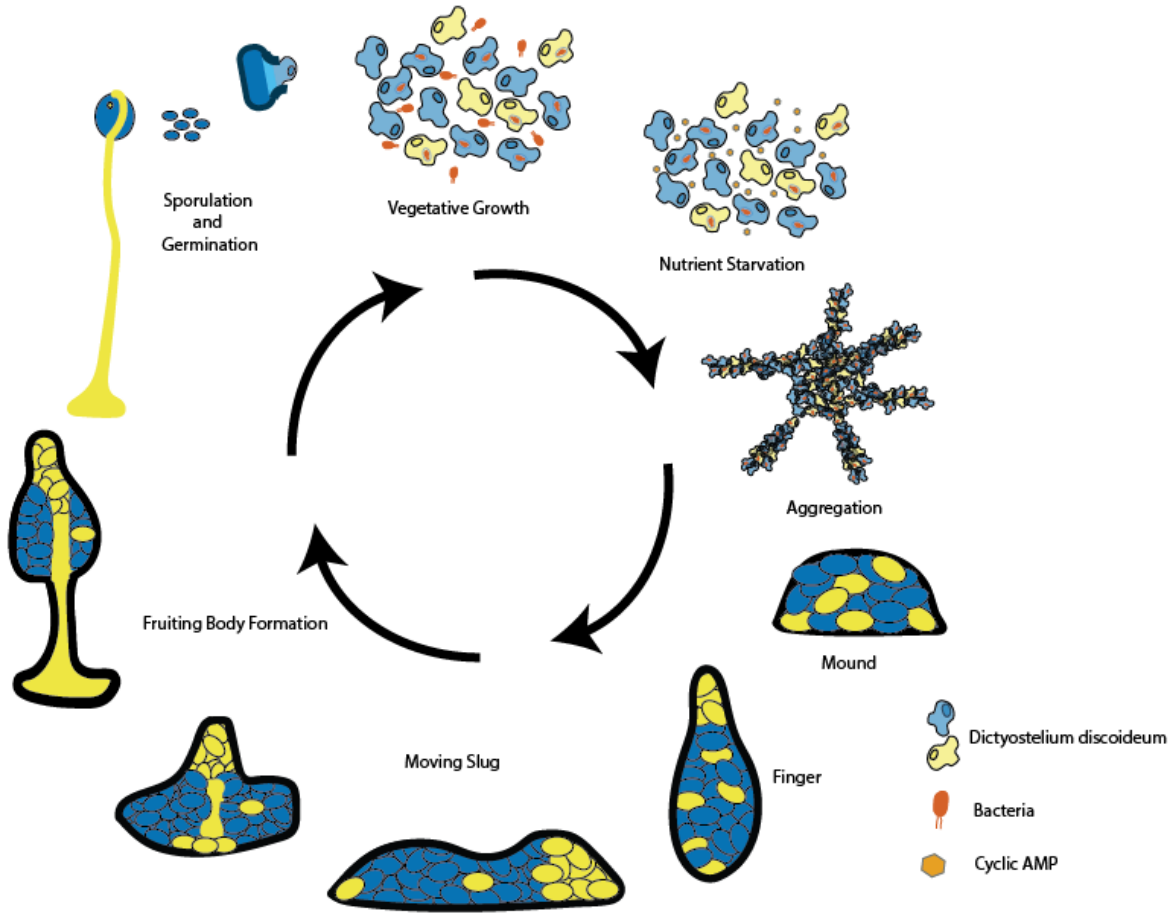

Figure 2. Depiction of *Dictyostelium* Life Cycle. Vegetative cells feed on bacteria until they are depleted. During nutrient starvation, cells send Cyclic AMP signals to induce aggregation. Some cells differentiate into a spore cell (blue) while others differentiate into stalk cells (yellow) which organize into structures allowing movement and eventual sporulation and germination when the environment is suitable for vegetative growth. Image credit: Joan M. Ryan, own work.

Hope, however, does not fill the nutrient requirements of any organism, *Dictyostelium* included. When spores are released, the cells must survive, even if they land in an environment which lacks a source of bacteria. *Dictyostelium* cells have addressed this issue by specializing certain cells. Some cells differentiate into a farmer type of cell, where they withhold, but do not kill for nutrients, approximately one-third of available

bacteria. These farmer cells stop consuming bacteria early in their lifecycle, and instead engage in bacterial husbandry, storing bacteria within cells of the fruiting body[7] (**Figure 2**). After *Dictyostelium* cells complete their lifecycle- from single cell, to slug, to fruiting body, and finally spore dispersal- the farmer cells will release the stored bacteria. These bacteria will seed the new environment, becoming a source of nutrients for newly germinated *Dictyostelium* cells.

This lifecycle is fascinating for many reasons, and also raises many questions. How do some cells become farmers, storing bacteria instead of eating bacteria? How do other *Dictyostelium* cells continue to eat bacteria and not store them? How does *Dictyostelium* convert from a single celled organism to a multicellular organism with as many as 100,000 cells? The answers depend on the signals that a given *Dictyostelium* cell receives, and then how that cell changes its behavior in response to those signals. These signals impact which genes are expressed, and hence, which proteins are made. Proteins (and RNA molecules) then determine how a cell functions– and if it becomes a farmer, or not. **The goal of cell signaling is to elicit a change in cell behavior that is designed to optimize cell survival and reproduction.**

This chapter will examine the following questions:

1. How do cells communicate using signals?

2. How do cells change their behavior in response to signals?

3. What is the molecular mechanism that governs a cellular response?

Signaling Molecules

In the previous examples, two signals were discussed, as well as their impact on *Dictyostelium* cell behavior. The first signal was folic acid, which is a chemoattractant, and allows *Dictyostelium* to track and move towards bacteria and yeast. Soil dwelling bacteria release folic acid[8]. *Dictyostelium* cells will respond to the folic acid signal by altering gene expression and protein activity within the cell, which ultimately allows the cell to move towards, and eventually, engulf the bacteria. This signal is **exogenous**, meaning that it came from a source other than the *Dictyostelium* cell.

The second signal discussed was a starvation signal. This signal is **c**yclic **a**denosine **m**ono**p**hosphate, abbreviated cAMP (**Figure 3**). cAMP is an **endogenous** signal molecule that is secreted by *Dictyostelium* cells in response to starvation conditions. When nutrients are lacking, *Dictyostelium* cells migrate towards each other and form a multicellular structure, with the end goal being to disperse spores to better feeding grounds.

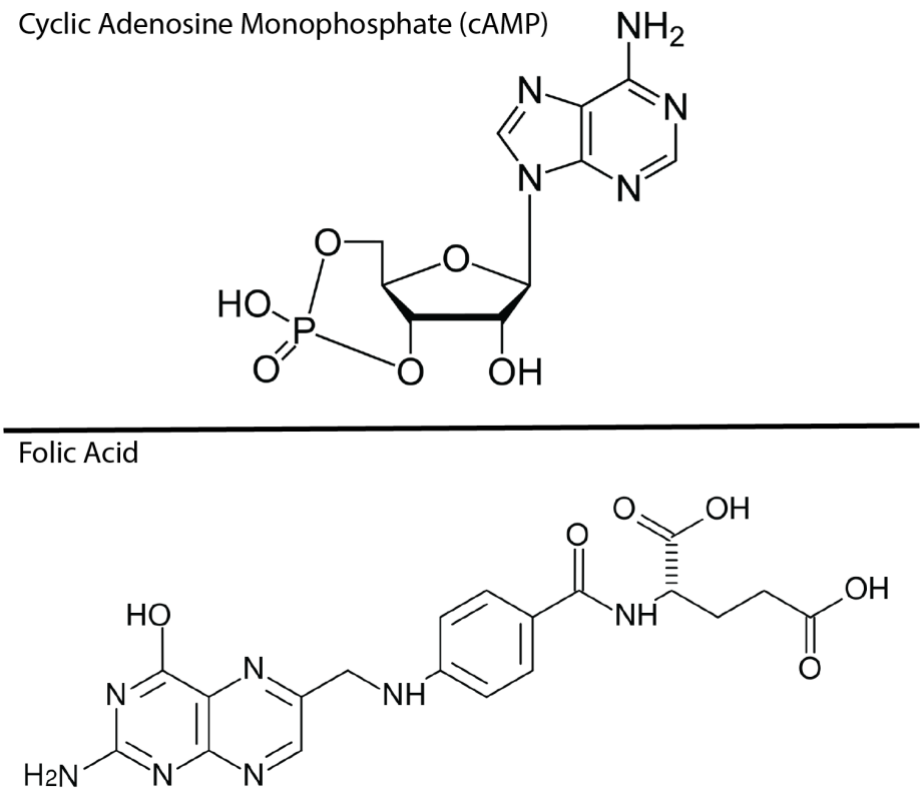

Figure 3: Molecular structures of cyclic adenosine monophosphate (cAMP) and folic acid. Upper panel: cAMP is secreted by *Dictyostelium* cells in response to starvation conditions. Lower panel: Folic acid is a chemoattractant secreted by soil dwelling bacteria. Image credit: Joan M. Ryan, own work.

Dictyostelium cells are not unique in their response to both exogenous and endogenous signals. All cells, from archaeal and bacterial prokaryotes to neurons of multicellular organisms, receive and respond to signals from and within their environment. The signals are molecules made either from the cell itself, or from another cell, not necessarily of the same species. Signal molecules are a broad class of substances, and have a wide range of structures, from a small, diffusible gas, to a complex protein. Cells that respond to a signal molecule will then move, differentiate, modulate gene expression, or undergo apoptosis (programmed cell death), to list a few potential outcomes.

A signal molecule, also known as a **ligand**, must bind to a **receptor** protein in order to initiate a change in cellular behavior. Receptors can be plasma membrane-anchored, organelle membrane-anchored, or as a non-membrane associated protein within a cell **(Figure 4)**. Only cells that express the specific receptor for a signal will be able to respond to that signal. If the cell lacks the receptor for a given signal molecule, the molecule may wash over the cell or enter the cell, but it will not impact cellular behavior. An absent signal molecule cannot elicit a cellular response, even if the cell has the correct receptor.

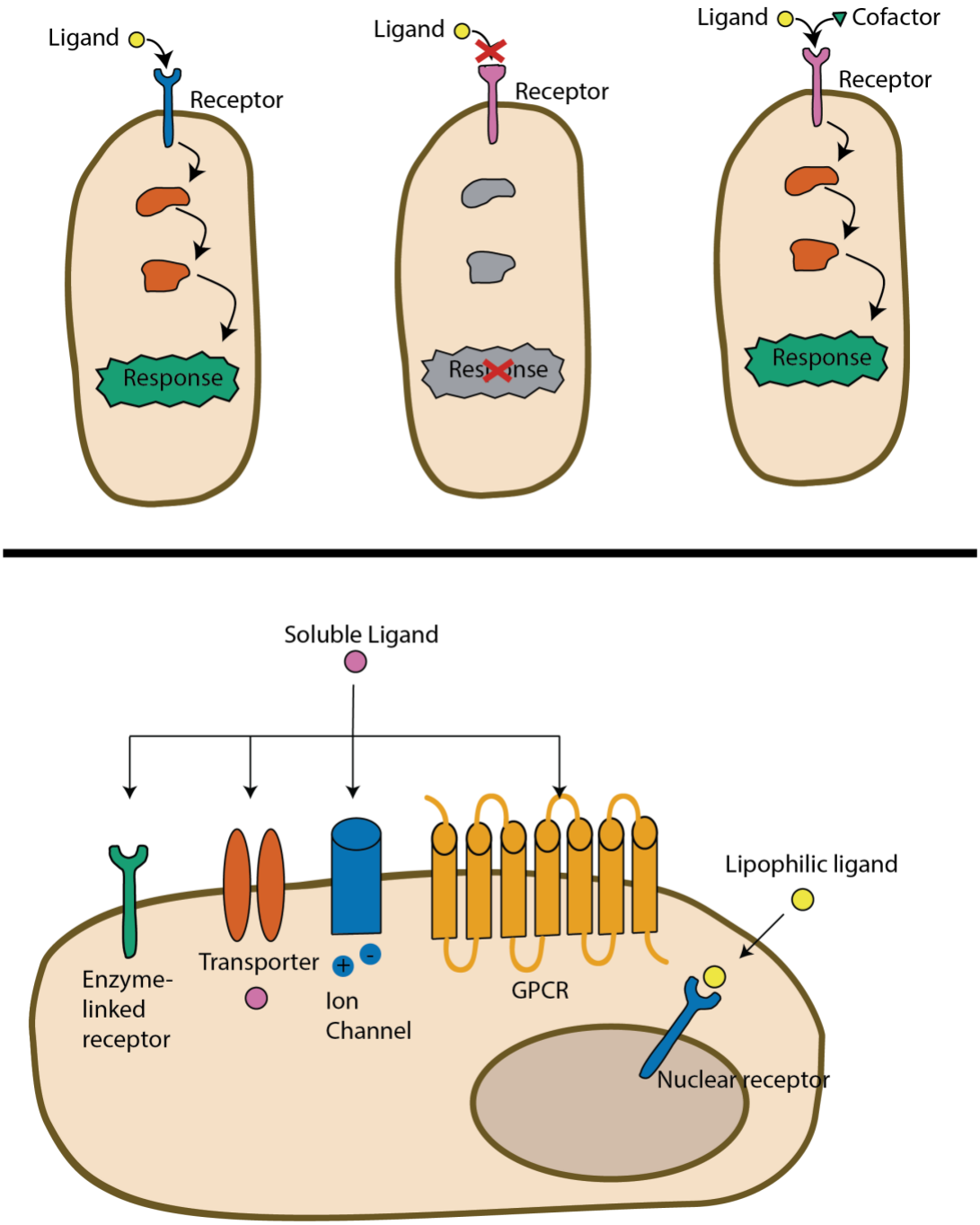

Figure 4. Cell response to receptor protein binding a ligand (signaling molecule). Upper panel: ligand-receptor specificity. If the ligand (yellow circle) matches the receptor (blue y shaped transmembrane protein), the signal is received by the cell and a response occurs (upper left image, arrows pointing to intracellular proteins (orange blobs) until a response is triggered). If the ligand does not match the receptor (purple transmembrane protein), that ligand signal is not received by the cell (represented by a red x) (upper middle image) and there is no cellular response. Sometimes ligand and a cofactor (green triangle) are required for the receptor to receive the signal and elicit a cellular response (upper right image). Bottom panel: Depiction of the four main receptor classes for soluble ligands including an enzyme-linked receptor (green y shaped transmembrane protein), a transporter (dual orange ovals), an ion channel (blue cylinder) and a G-protein coupled receptor (GCPR) (mustard colored seven cylinders linked together). Also shown is a lipophilic ligand (yellow circle) that can cross the cell membrane and interact with an internal nuclear receptor (blue y shaped transmembrane receptor) on the nucleus (dark tan circle). Not shown: non-membrane associated receptor protein. Image credit: Joan M. Ryan, own work.

Many types of signal molecules have been identified, and many remain to be identified. The known signaling molecules include, but are not exclusive to the following list:

- Gases
- Small, lipophilic molecules
- Chemicals
- Hormones
- Proteins
- Lipids
- Electrical impulses
- Pressure
- Humidity
- pH
- Light exposure
- Osmotic pressure

Sometimes a single signal molecule binds to a single receptor, which will elicit a change to cellular behavior. Other times multiple signals are required to trigger a change to cellular behavior (**Figure 4**). The second signal is referred to as a **co-factor**.

One type of signal used by Dictyostelium are chalones. **Chalones** are protein signals that inhibit the proliferation of the cell that secretes it. All of the previously mentioned signaling molecules have been documented as signals that alter cell behavior in *Dictyostelium* cells. However, it is likely the same signaling molecules will also elicit a change in cellular behavior in multicellular organisms such as humans.

Types of Cell-Cell Signaling

Consider being at a loud concert, and trying to have a conversation with your neighbor. In order to hear what your neighbor says, you must focus on their words, their body language, and even the way their mouth is moving, all while ignoring the loud music and voices of other concert goers. Cells constantly do the same. Though rather than attending concerts, cells are inundated with signals from their environment. However, cells do not respond to each and every signal molecule encountered. Instead, cells selectively respond to signals depending on which receptors are present on or within the cell. In addition, cells can change which receptors they display depending on the circumstances in the environment. For example, when *Dictyostelium* senses dangerous bacteria that it cannot consume, it will **up-regulate** gene expression for protein receptors that sense molecules released from the bacteria. In this way, *Dictyostelium* is hyper-sensitized to dangers in the environment, and poised to move away from it. Similar to up-regulation of gene products, cells can also **down-regulate** gene products that are not required for the cell in a given circumstance.

Cells use several types of signaling to respond to environmental changes, or to alert nearby cells of changes to the environment (**Figure 5**).

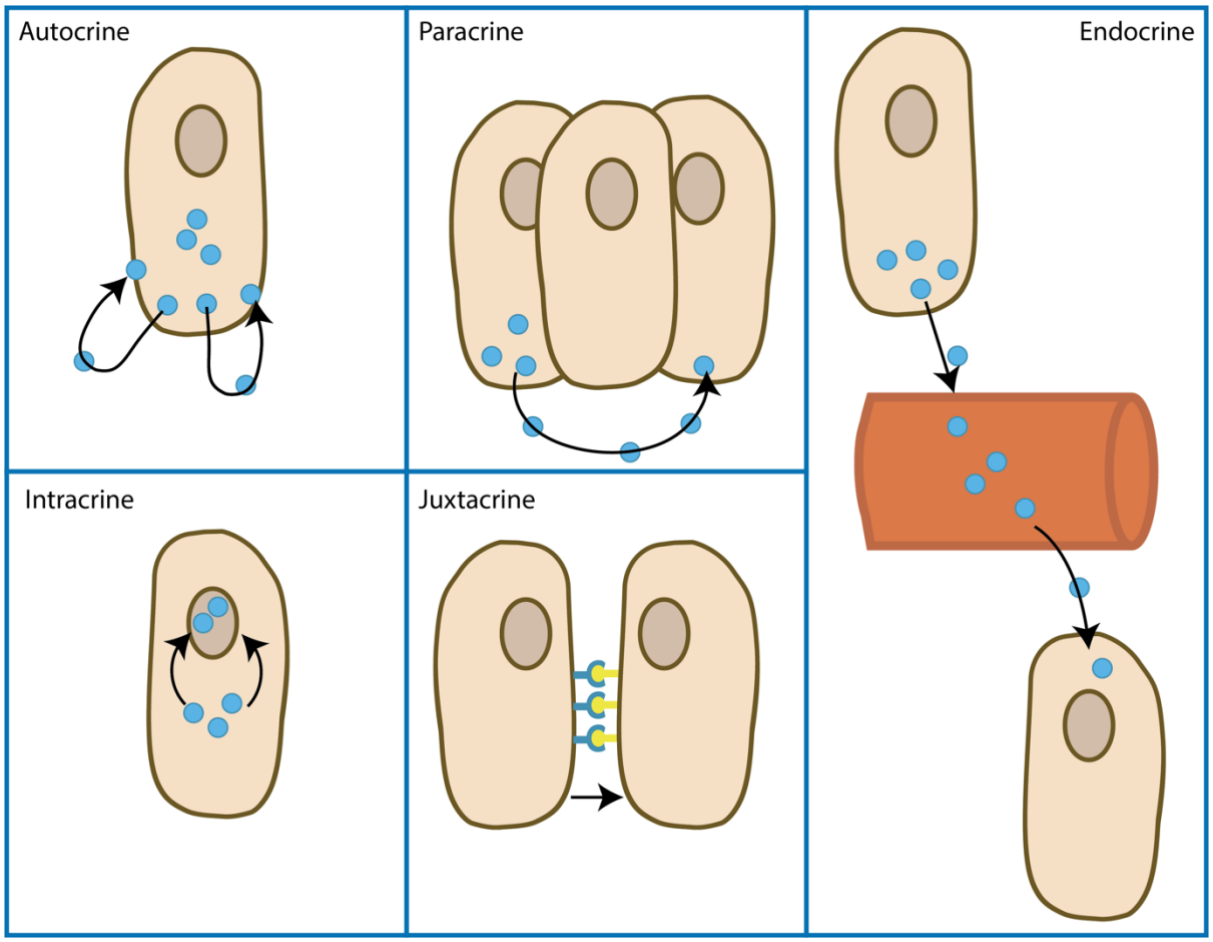

Figure 5. Types of cellular signaling. Upper left panel: Autocrine signaling occurs when cell secretes a signal (blue dot) that is then recognized by that same cell. Upper middle panel: Paracrine signaling occurs when a cell secretes a signal that is recognized by a nearby cell. Bottom left panel: Intracrine signaling occurs when a cell creates a signal that impacts its own behavior without leaving the cell. Bottom middle panel: Juxtacrine signaling is contact dependent signaling between neighboring cells. Right panel: Endocrine signaling is long distance signaling from one cell using a transport system like a vessel to reach a distant cell. Not shown: neuronal signaling, which occurs within neurons that use signals called neurotransmitters. Image credit: Joan M. Ryan, own work.

Autocrine signaling occurs when a cell secretes a signal, and then that signal binds to a receptor on the cell from which the signal was created. As with all signal-receptor binding, this will result in a change to cellular behavior. An example of autocrine signaling occurs within the lifecycle of *Dictyostelium.* One chalone signaling molecule is known as Autocrine Proliferation Repressor Protein A (ArpA). ArpA molecules bind to receptors on the same *Dictyostelium* cell that secreted them. This ArpA-receptor interaction causes the cell to slow proliferation (meaning that the rate by which cells divide is slowed)[11].

Paracrine signaling occurs when a cell secretes a signal, which then binds to a receptor on a different cell that is close proximity to the cell from in which the signal originated. This signal will impact the receiving cell's behavior. Paracrine factors (signals) diffuse a relatively small distance, so the source cell must be near the receiving cell. A concentration gradient is setup from the cell that secretes the paracrine factor, with the highest levels of signal reaching the cells nearest to the source of the signal. *Dictyostelium* uses paracrine signaling to trigger cells to differentiate from a single cell stage to the aggregation stage by secreting a protein called Prestarvation Factor (PSF). PSF is recognized by nearby cells (as well as the original cell that created and secreted the signal). Cells that have the PSF receptor will change their behavior by transcribing mRNA that will ultimately result in the transition to the aggregation stage of the *Dictyostelium* life cycle[12] (see **Figures 1 and 2**).

Juxtacrine signaling is also referred to as **contact dependent** signaling. Juxtacrine signaling occurs when the signal molecule is attached to the cell, and that signal molecule then binds to a receptor attached to another cell. In addition to binding to a cell, the signal molecule or the ligand could be part of the extracellular matrix of a multicellular organism. During development, *Dictyostelium* cells will become either a prestalk or prespore cell. Prestalk cells will become part of the stalk of the fruiting body, while prespore cells will ultimately become spores (see life cycle **Figure 2**). This process is partially mediated by the location of cells within the slug stage. Anterior cells differentiate into prestalk cells, and posterior cells differentiate into prespore cells. One way this process is mediated is through cell-cell contact. A transmembrane protein, known as TgrB1, will bind to another transmembrane protein, known as TgrC1. TgrB1 and TgrC1 are found on neighboring cells. The interaction of TgrB1 and TgrC1 will stimulate changes in behavior in both cells. One cellular change is rearrangement of the actin cytoskeleton network, which causes the cells to form protrusions. These protrusions are the beginnings of cell movement, which is necessary for the cells to proceed through the lifecycle[13].

Intracrine signaling occurs when a cell creates a signal that does is not secreted but still impacts the cell's behavior. This mode of signaling differs from autocrine signaling where the signal is secreted[14]. Intracrine signals, like all signaling molecules, can only impact cellular behavior if the correct receptor for the signal exists. With intracrine signaling molecules, this receptor protein must exist within the cell that creates the signal. In the absence of the correct receptor protein the cell will not alter behavior in response to an intracrine signal (or any signal).

Endocrine signaling occurs in multicellular organisms. Cells far apart must communicate with each other, and frequently do so through the release of endocrine signals (hormones). Hormones are released by endocrine cells and reach distant cells via the circulatory system. Some unicellular pathogens that colonize multicellular hosts can and do respond to endocrine signals. (See interkingdom signaling).

Neuronal signaling is used by neurons of multicellular organisms. Neurons communicate with other cells (including other neurons) by electrochemical signals. A neuron that is stimulated will create an electric potential that travels the length of the neuron. This electric potential will then trigger the release chemical signals. The primary chemical signal is called a neurotransmitter. Some eukaryotic parasites, such as *Trichinella spiralis*, a parasitic nematode and the causative agent of trichinosis, have been shown to impact the neurotransmitters norepinephrine and serotonin levels in infected mice. Animals infected with *T. spiralis* are known to exhibit behavioral, emotional, and motor changes, which may be explained by altered levels of neurotransmitters, though further studies are required[15].

Depending on the cell type, as well as the environment in which the cell is located, all forms of cell signaling may be used simultaneously. Sometimes using more than one type of cell signaling will augment a cellular response. Other times it can dampen the cellular response. And still, other sets of signals will cause competing responses. Consider contact-dependent signaling in *Dictyostelium*, which in part determines if *Dictyostelium* cells in the slug stage become prestalk or prespore cells, precursors to stalk or spore cells (see **Figure 2**). The same cells also undergo paracrine signaling using cAMP secreted by nearby cells, as well as autocrine signaling if the cAMP is secreted by source cell itself. cAMP can cause slug cells to undergo chemotaxis (movement towards, in the case of a chemoattractant molecule, or away from if the molecule is a repellent signal) towards the source of cAMP. When a cell is given both a paracrine source of cAMP, as well as a contact-dependent source of TgrB1/TgrC1, prestalk cells move towards the cAMP, while prespore cells ‘listen to’ the contact signal[16].

Signal Transduction

The goal of signal transduction is to use a signal molecule that will elicit a change in cell behavior. For example, *Dictyostelium* will respond to cAMP, and change its cellular behavior, causing it to aggregate with other starving *Dictyostelium* cells. The change in cellular behavior can be accomplished is different ways. The first step in a **signal transduction pathway** (a set of molecular reactions initiated by a ligand binding to a receptor protein that will impact cellular behavior) is a signal molecule binding to a receptor protein. This step is classified as signal reception. This interaction causes a shift in the receptor conformation that results in the signal molecule being transduced into a new type of message. **Transduction** is the process of converting one type of message into another; in this case, a signal molecule binding to a receptor protein causes the signal to be converted into a new message that will ultimately alter cell behavior (see **Figure 6)**.

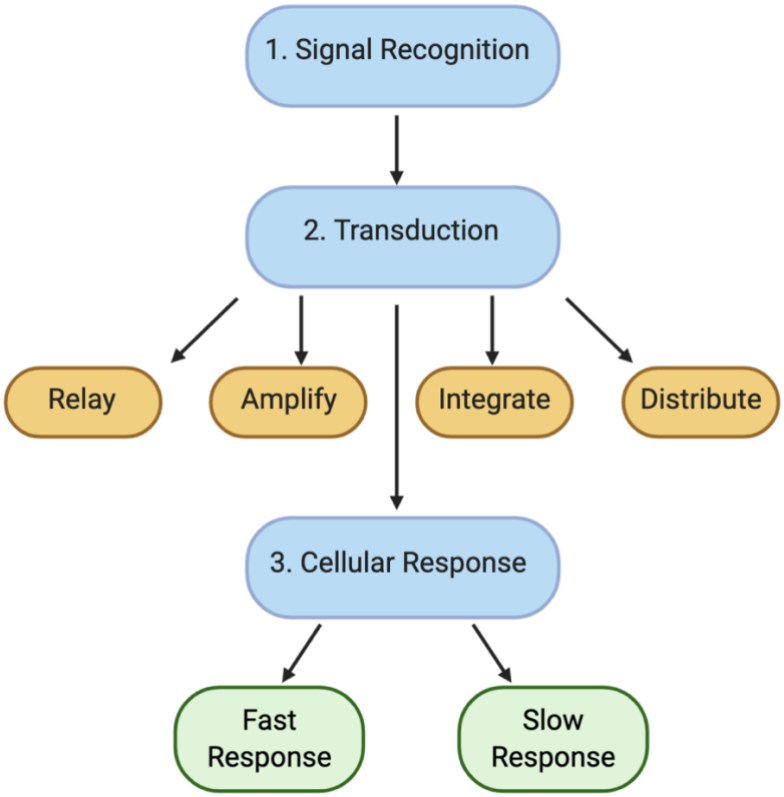

Figure 6. Signal transduction events. Signal transduction can be divided into three steps. Step one occurs when a signal binds to a receptor protein. Step 2 occurs when the original signal is transuded into a different message. During the transduction step, the signal can be relayed from intracellular signaling proteins, can be amplified (as well as transduced) by an enzyme to multiple copies of a new signal, can combine with other signals to be integrated into a response, or can be distributed to multiple intracellular receptors. Any combinations of relay, amplification, integration, and distribution can occur during a signal transduction event, depending on the original signal and the cellular response that is to be changed. Step 3 is a change to the cell behavior. This change is categorized as slow if a new molecule must be made (transcription followed by translation), or fast if the molecule already exists (and is modified by a functional group). Image credit: Medora Huseby, own work, created in Biorender.

Consider a signal molecule binding to a protein receptor that is located on the plasma membrane. If this signal molecule is large, hydrophobic, or a protein, it cannot directly pass through the plasma membrane to impact a change of cellular behavior. Instead, this type of signal will bind to a receptor, triggering a shift in the receptor conformation. This shift is then transduced across the plasma membrane, as a molecule within the cell will respond to that signal and then further relay the signal. In this manner, a signal binding outside the cell is converted to a different message within the cell. In *Dictyostelium*, cAMP binds to the Car1 receptor located on the plasma membrane (**Figure 7**). Upon binding, Car1 shifts conformation, and activates a G protein (see below for more detail on G protein activation) associated with the inside of the plasma membrane[17]. Once active, the G protein will relay the signal to another protein, which will then relay the signal to another protein, until the signal is relayed to an effector protein. An **effector protein** is a protein that will impact cell behavior. This could be

an enzyme, a cytoskeletal protein, or a transcription regulator, to name a few examples. The signal relays that take place within the cell are referred to as **intracellular signaling pathways**.

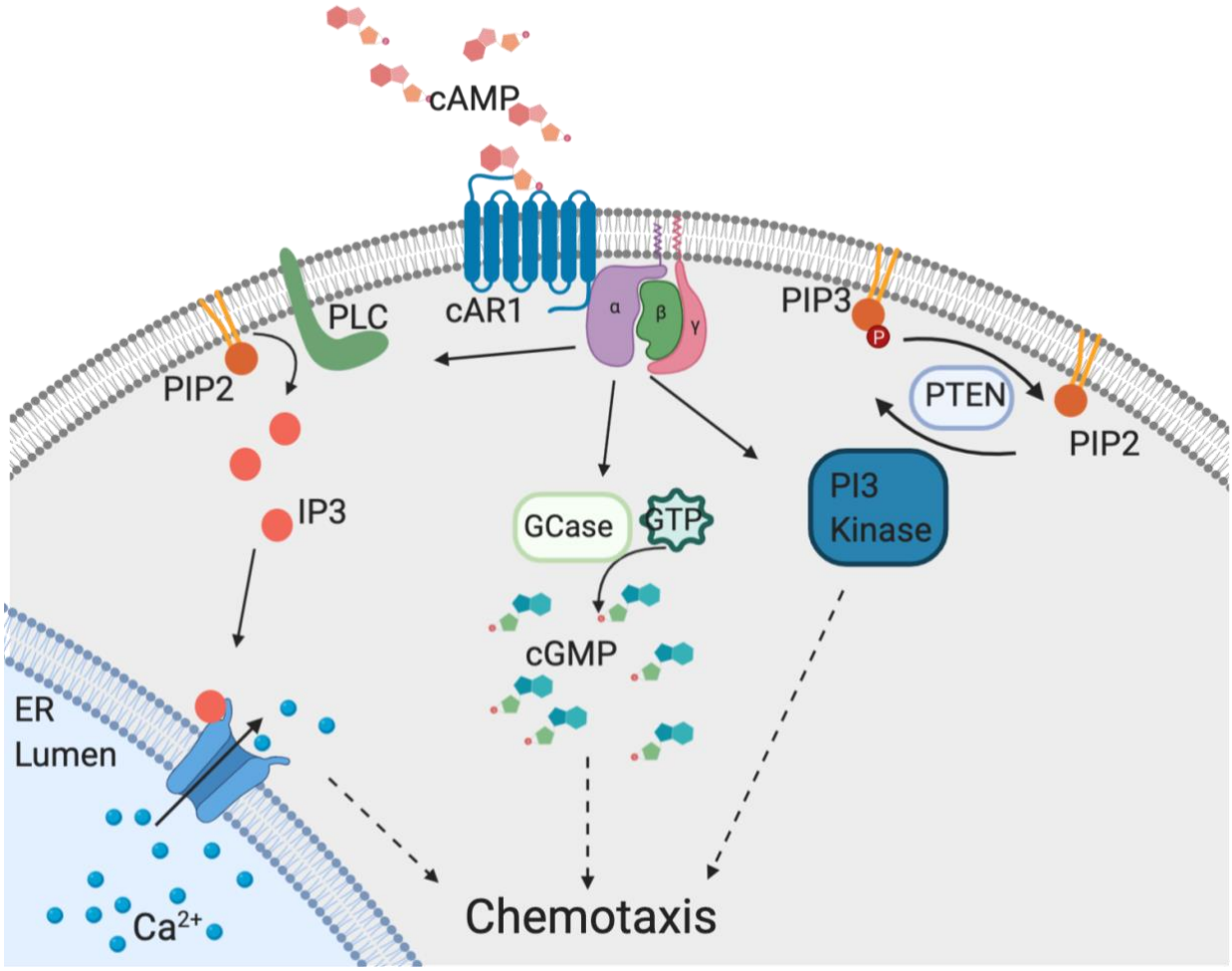

Figure 7. Model of signaling pathways leading to *Dictyostelium* chemotaxis. Signaling molecules such as lipid metabolites and cyclic nucleotides act as signal transducers to modify the cytoskeleton, which is required for chemotaxis. Extracellular cAMP (pink and orange hexagons in the white space) serves as a chemoattractant and binds to a G-protein coupled receptor called cAR1 (blue transmembrane protein with seven membrane domains). cAR1 activates a G protein (depicted as purple, green and pink subunits named α, β, and γ, respectively). The activated G protein will relay the signal via several different pathways. The G protein will activate phospholipase C (PLC, depicted in green as a transmembrane protein with one segment in the plasma membrane). PLC acts on phosphatidylinositol (PIP2, depicted as an orange ball attached to the plasma membrane with two orange lines), cleaving PIP2 into inositol (1,4,5)-triphosphate (depicted as orange circles) and DAG (not shown, but remain in the plasma membrane). IP3 binds to an ion channel located in the endoplasmic reticulum (ER) membrane (shown as a blue lipid bilayer). When bound to IP3, the ion channel (depicted as a blue transmembrane protein) will open, allowing calcium ions (depicted as small blue circles) to move out of the lumen of the ER into the cytoplasm (grey space). The increase in calcium ions will trigger further intracellular signaling events (depicted by the dashed arrow), leading to chemotaxis. The G protein will also activate a guanyly cyclase (light green circle with dark green boarder) known as GCase. GCase is an enzyme that creates cyclic guanosine monophosphate (cGMP, blue and green pentagons) from GTP (light blue star burst with teal boarder). cGMP will act on other signaling transduction molecules (depicted as a dashed arrow), ultimately leading to chemotaxis. Another

pathway activated by the G protein is through phosphoinositide 3-kinase (PI3 Kinase, shown as a blue rounded square), which is an enzyme that adds a phosphate to PIP2, creating phosphatidylinositol (3,4,5)-trisphosphate, or PIP3 (depicted as an orange circle with two orange lines projecting into the plasma membrane with a phosphate attached (red circle with a white P)). The phosphate can be removed by another enzyme called phosphatase and tensin homolog (PTEN, depicted as a light blue oval with blue boarder), converting PIP3 into PIP2. PI3 kinase will activate further intracellular signaling events (depicted as a dashed arrow) that will lead to chemotaxis. Image credit: Medora Huseby, own work, created in Biorender.

Intracellular signaling pathways are drawn by scientists with arrows and blocks (**Figure 7**). When an arrow is drawn between two proteins, it means that one protein has relayed the signal to the next protein in the intracellular signaling pathway. Arrows depict activation of the next protein in the signaling pathway. Some signaling pathways are inhibiting, rather than activating. If a protein inhibits the next protein in the pathway, it is drawn with a block. Proteins activated or inhibited after the signal is transduced are referred to as downstream in the pathway.

Intracellular signaling pathways can consist of many proteins that relay the signal in a sequential order. There are several ways the intracellular signaling pathway can impart the signal to trigger a change in cell behavior (**Figure 6**).

- **Relay:** The signal can be passed from protein to protein, onward in the pathway.
- **Amplification**: The signal can be amplified, which means it can be made stronger by creating more of the signal.
- **Transduction**: The signal can be converted into a new molecule. This can occur if an enzyme is activated to create a new signaling message (referred to as second messenger).
- **Integration**: More than one intracellular signaling pathway may be needed to impact a response to cell behavior.
- **Distribution**: A member of the intracellular signaling pathway may distribute the signal onward by activating (or inhibiting) more than one downstream protein.

If an enzyme is activated at any point during the pathway, then the signal can be transduced as well as amplified, as the enzyme will change the signal into a different component, and the enzyme can produce more of the product than the original relay did. For example, one of the downstream proteins activated via cAMP binding to cAR1 and thus activating the G protein is adenylyl cyclase[19]. Adenylyl cyclase is an enzyme that converts ATP into cAMP. When activated it transduces the original message into cAMP. If it produces an abundance of cAMP, then it also amplifies the original signal.

Within an intracellular signaling pathway the signal can be relayed and passed from one protein to another. This can occur, for example, in a phosphorylation cascade, where intracellular receptor proteins relay a phosphate group (by hydrolyzing ATP or GTP to ADP or GDP, respectively) in order to activate the next protein within the pathway (**Figure 8**).

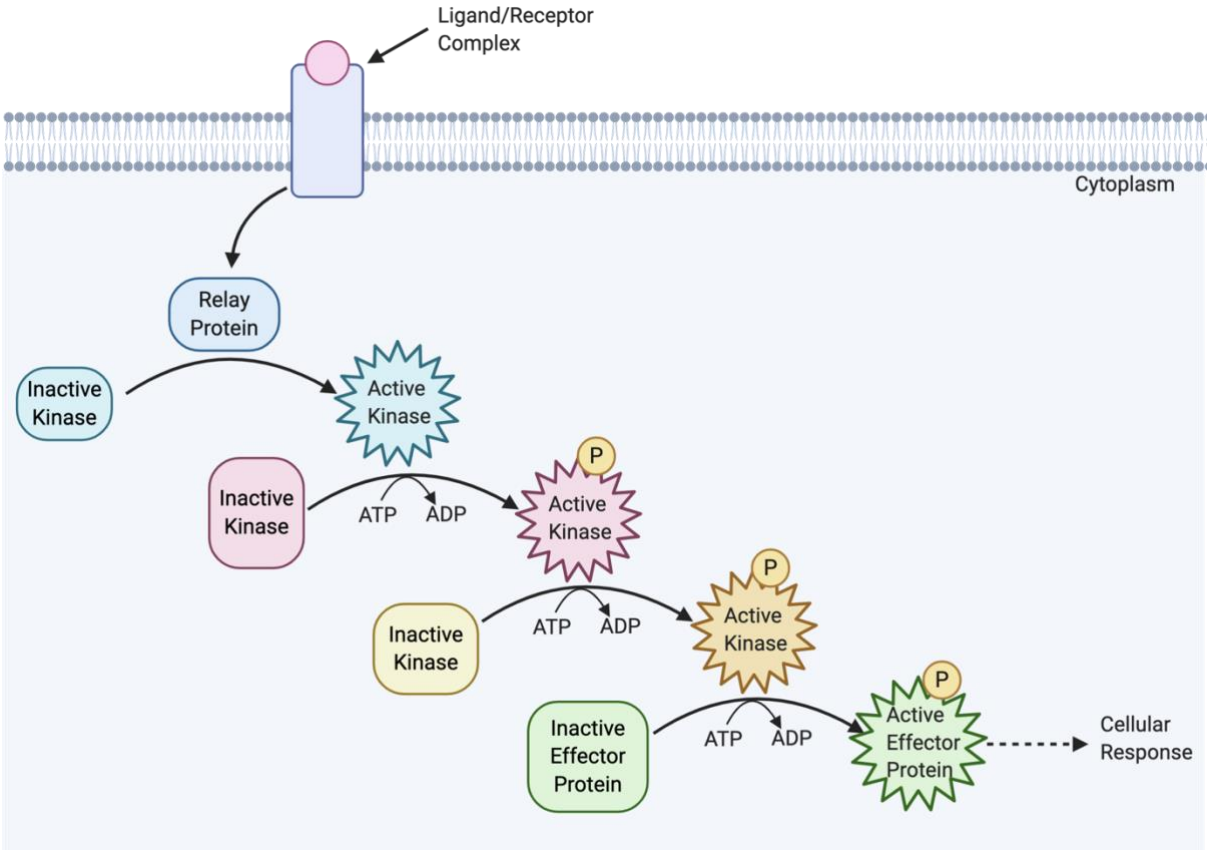

Figure 8. Phosphorylation cascade. A transmembrane receptor (light blue rectangle outlined in dark blue) is activated upon ligand (pink circle) binding. A conformational shift in the receptor will activate intracellular signaling pathways. In this example, a relay protein (blue oval outlined in dark blue) will interact with a kinase (teal oval outlined in blue) to convert it to an active form (teal starburst outlined in blue). The activated kinase will hydrolyze ATP to convert a different kinase (pink rectangle outlined in burgundy) to an active form (pink starburst outlined in burgundy). The active kinase is phosphorylated (yellow circle labeled 'P'). The active kinase will transfer this phosphate to a different inactive kinase (yellow oval outlined in tan), converting the kinase to an active form (tan star burst outlined in brown). This active kinase will then transfer the phosphate to another protein, in this example an effector protein (green square outlined in green) converting the effector protein to an active form (green starburst outlined in green). The effector protein will then impact cellular behavior (dashed arrow). Image credit: Medora Huseby, own work, created in Biorender.

Cell Surface Receptors

Three main families of cell surface receptors exist; these include the **G**–**P**rotein **C**oupled **R**eceptors (GPCRs), enzyme coupled receptors, and ion-coupled receptors. All of these receptor proteins bind to a specific ligand and become activated, transducing the ligand signal across the cell membrane. Recall some receptor proteins are found free in the cytosol and not associated with a membrane. Other receptors span the membrane of an organelle.

G-Protein Coupled Receptors

G**–**Protein Coupled Receptors (GPCRs) are membrane bound proteins that account for the vast majority of regulation of cellular functions in eukaryotic cells. GPCRs belong to a superfamily of proteins found in eukaryotic organisms ranging from the amoeba *Dictyostelium discoideum* to humans. GPRCs are linked to several human diseases such as diabetes, obesity, and Alzheizmer's[20]. As of 2019, there are 475 pharmaceutical drugs (~34% of all FDA approved drugs) which target a GPCR. Of the pharmaceutical agents in clinical trials, ~ 20% target potentially novel GPCRs[21].

GPCRs act as signal transductors, changing an outside signal (a ligand binding) into an inside relay that will ultimately impact cellular behavior. GPCRs respond to a wide variety of ligand types, depending on the specific GPCR. Some examples of signals that impact GPCRs in humans are photons, sugars, lipids, peptides, proteins, neurotransmitters, hormones, pheromones, ions, and odors. cAMP is one signal that will bind to and activate GPRCs in *Dictyostelium*[22]. Despite this wide variety of signal responsiveness and sequence differences between the six classes of GPCRs, GPCRs have a similar structure There exist six classes of GPCRs based on sequence homology and functional similarity[24] (**Figure 9**). All GPCRs share a common structural motif consisting of an extracellular N-terminus followed by seven transmembrane spanning alpha helices. Connecting the alpha helices are either extracellular or intracellular loops. The C terminus of GPCRs is found in the cytosol of the cell. Because of this structure, GPCRs are also referred to as 7TM receptors (seven TransMembrane receptors), seven-pass transmembrane domain receptors, serpentine receptors, and heptahelical receptors. This chapter will refer to them as GPCRs.

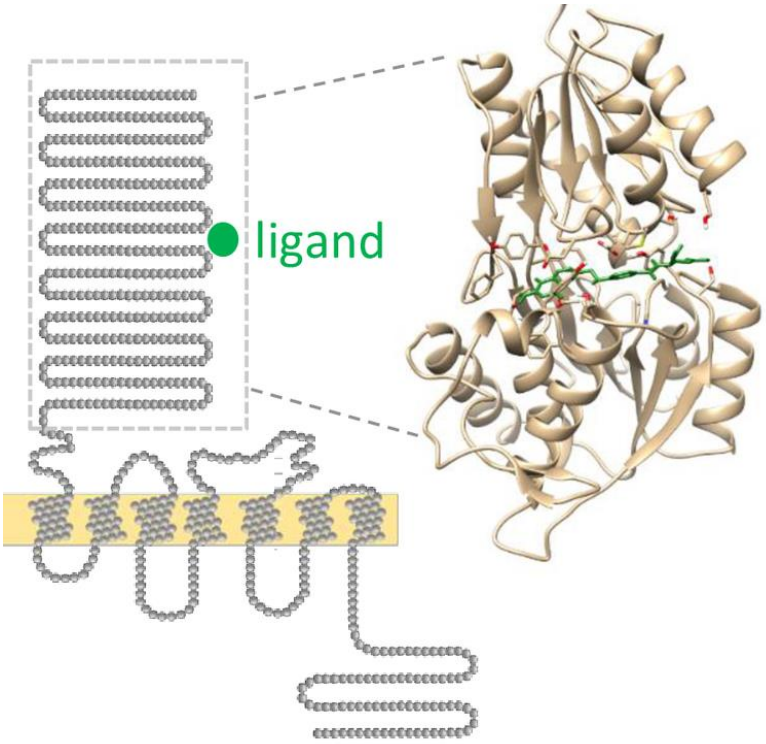

Figure 9. Structure of a G-protein coupled receptor. The extracellular domain of the GPCR is highlighted by a dashed box. On the right, structural modeling and computational docking predict that the extracellular domain is the binding site for ligand (green), which is folic acid[23]. The plasma membrane is represented by the yellow box through which the grey beads (which represent the GPCR) pass through (in the form of alpha helices) seven times. The N terminus of the GPCR is extracellular (and is shown to be involved in ligand binding) and the C terminus is intracellular. Image credit:

After binding the ligand, the GPCR shifts conformation, moving its transmembrane domains to relay a signal. This shift in conformation impacts a protein associated to the inner leaflet of the plasma membrane which will then further relay the signal transduction cascade. Most activated GPCRs (those which have altered their conformation) will then activate a heterotrimeric (three distinct peptides that interact and comprise the G protein) G protein that is associated with the inner (cytoplasmic) leaflet of the plasma membrane (**Figure 10**). G proteins are also known as Guanine nucleotide-binding proteins, and belong to a family of **molecular switches**.

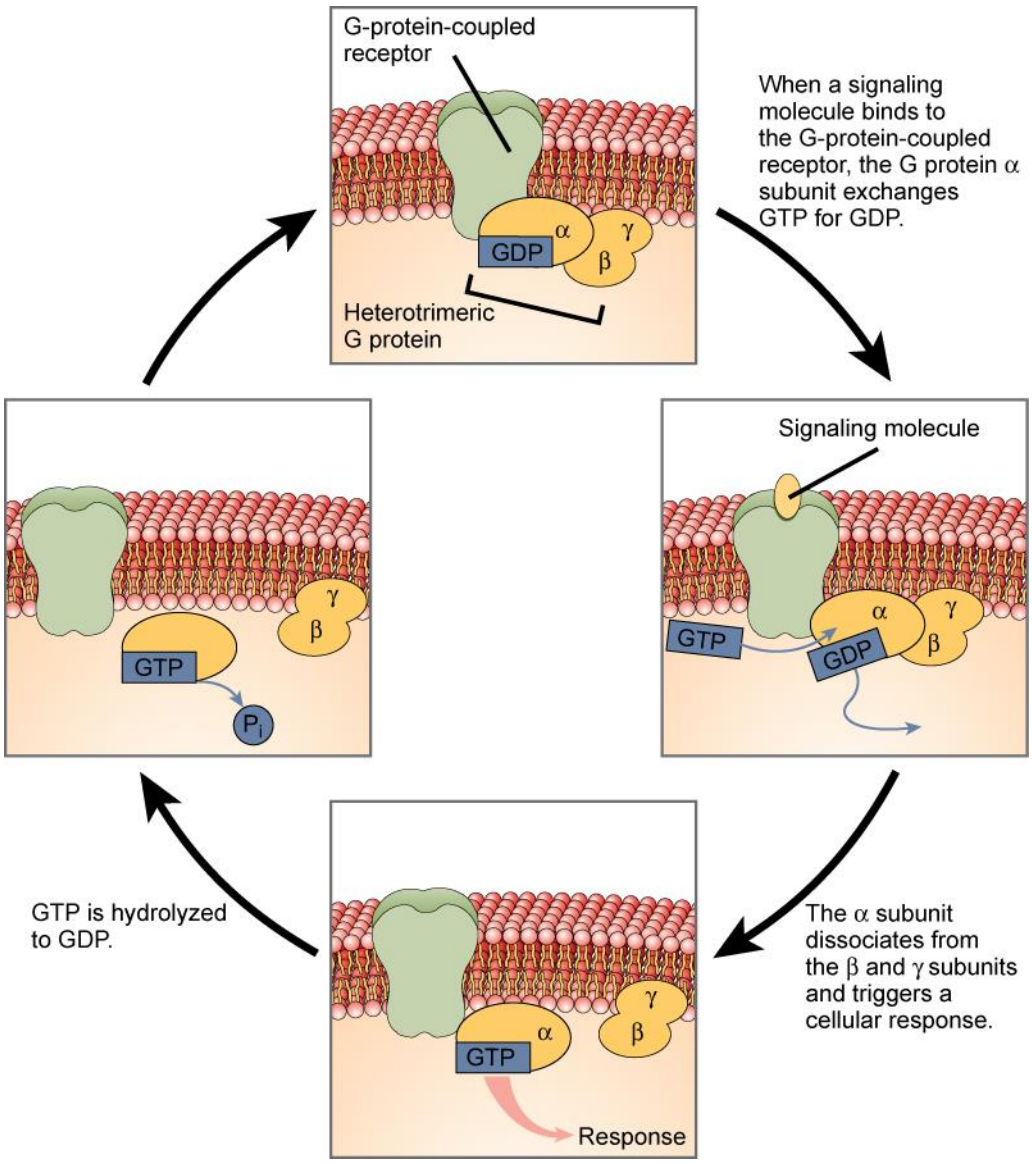

Figure 10. Activation of a G protein by a GPCR. Upon a conformational shift in the GPCR (light green), the heterotrimeric G protein (yellow) will be activated. The α subunit will exchange GDP (blue box) for GTP (right most image). When bound to GTP, the α subunit will dissociate from the β/γ subunits (lower most image). The α subunit can then enter into the intracellular signaling relay system to induce a cellular response. Similarly, the β/γ subunits, which remain associated with the inner leaflet of the plasma membrane, can further relay the signal to nearby protein (not shown in this image). The α subunit has intrinsic GTPase activity, and will hydrolyze GTP to GDP and inorganic phosphate (P_i) (leftmost figure). Once bound to GDP, the α subunit will re-associate with the β/γ subunits and the GPCR (topmost figure). When bound to GTD and the β/γ subunits, the G protein is inactive. Image credit: OpenStax. CC BY.

The subunits of a G protein are referred to as alpha (α), beta (β), and gamma (γ). The alpha subunit is associated with the inner leaflet of the plasma membrane through a lipid tail. The α subunit is also a GTPase, meaning that it can hydrolyze GTP to GDP through **intrinsic** enzymatic activity. It is a molecular switch that will shut itself off by

hydrolyzing GTP to GDP after a given amount of time. The α subunit is only active when bound to GTP and is inactive when bound to GDP. The G protein is nearby the GPCR and becomes activated when the GPCR changes conformation in response to the binding of a ligand. This shift in the GPCR causes the α subunit to decrease its affinity for GDP. After releasing GDP, the G protein can then become active when it binds to GTP. When bound to GTP, the active α subunit will dissociate from the β and γ subunits of the G protein. It should be noted that the G protein subunits do not always completely dissociate; in some cases, they shift apart far enough to allow for other proteins to be impacted, yet remain associated with the three subunits.

The active α subunit, bound to GTP, will associate with other proteins to activate or inhibit their activity, depending on the signal transduction goal. The β/γ subunit remains tethered to the inner leaflet of the plasma membrane though a covalent lipid tail on the γ subunit. The β/γ complex will either activate or inhibit proteins depending on the signal and proteins present in the cytoplasm.

The α subunit hydrolyzes GTP to GDP within seconds. Once the α subunit is again bound to GDP it will re-associate with the β/γ subunits, returning to a resting or inactive form until it again receives the signal to release GDP due to a conformational shift of the GPCR, which happens when the GPCR binds to its ligand (which is also called an agonist).

Activation of a G protein can activate membrane associated enzymes. If a new (non-protein) product is created by an enzyme it is considered a **second messenger**. If more of the enzymatic product is made, such as when adenylyl cyclase (an enzyme which synthesizes cAMP) is activated in *Dictyostelium,* this step would then be considered both a transduction as well as an amplification. Different second messengers will have different impacts on cellular activity. Not all active G proteins will activate or further a signal transduction cascade–some G proteins have inhibitor functions, which shut down a pathway rather than amplify it.

In addition, G proteins can activate transmembrane ion channels. Upon activation of a membrane channel, the G protein can either cause the channel to open or close. Channels are specific to ions and small molecules, but, once open, will allow an influx of ions until the activating signal is removed (in this case, the α subunit hydrolyzes GTP to GDP). This influx of ions can impact the membrane potential, or the ions can act as second messengers for other intracellular communication/signaling.

The α subunit of a G protein can be a target of microbial subversion of cell signaling. Pathogens will produce proteins that hijack host cell machinery to ensure that the pathogen survives despite host cell defenses[26]. See subversion section below.

The response of *Dictyostelium* to cAMP, the starvation signal, is a good example of a GPCR signal transduction pathway (**Figure 11**). cAMP also results chemotaxis in *Dictyostelium* due to GPCR signal transduction pathway. This pathway has been well

characterized, and many of the proteins involved have been identified and characterized. *Dictyostelium* has four GPCRs that bind to cAMP (called cAR1-4)[27]. On the side of the cell that will move towards a chemoattractant such as cAMP, cAMP binds the cAR1. This leads to the activation of two Ras molecules (RasG and RasC), which activates effector proteins required for cell movement[28].

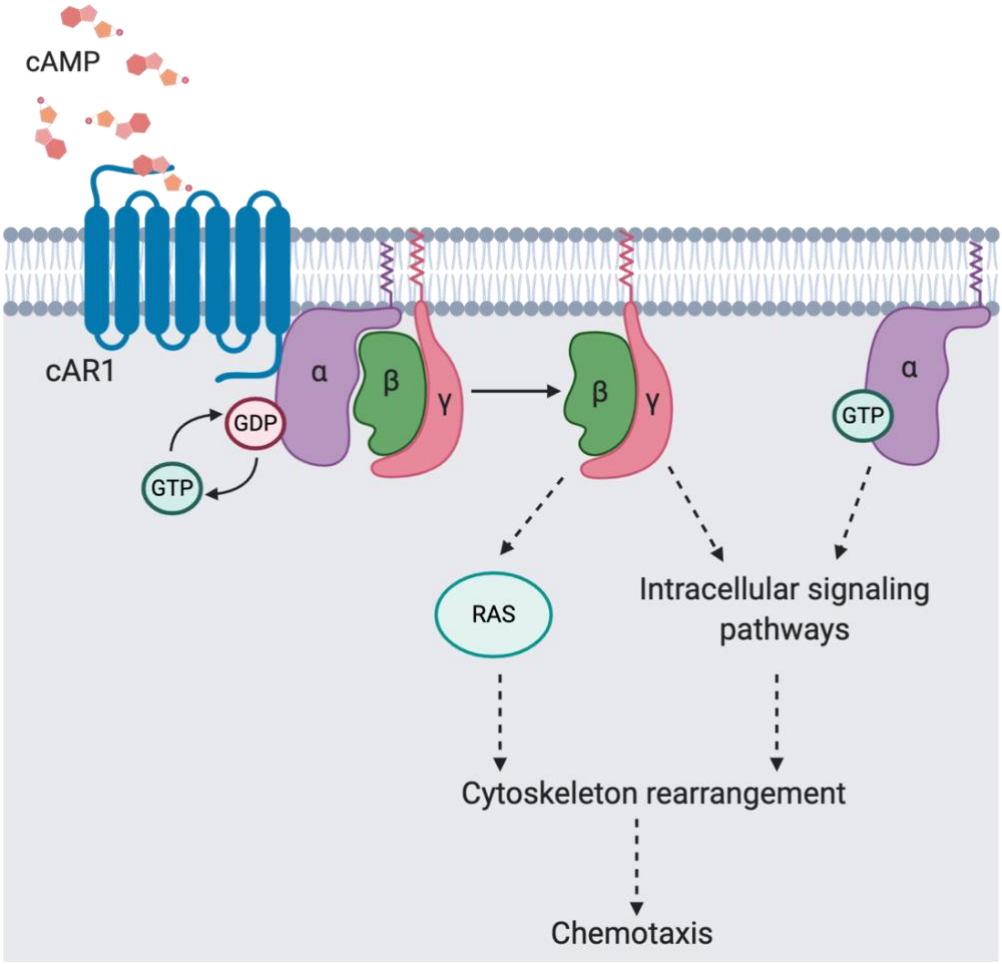

Figure 11. *Dictyostelium* chemotaxis signaling cascade. The GPCR cAR1 (blue) binds to cAMP (pink and orange hexagons), inducing a conformational shift which activates a G protein (purple, green and pink). The α subunit will swap GDP (small pink circle) for GTP (small teal circle), and dissociate from the β/γ subunits. The β/γ subunits will relay the signal to Ras proteins (light blue circle with dark blue edging), which induce further signaling until the cytoskeleton is rearranged, ultimately leading to chemotaxis towards the source of cAMP. The β/γ subunits will also relay the signal to other Ras independent signaling pathways. The α subunit will relay the signal to intracellular pathways until it hydrolyzes GTP into GDP. The plasma membrane is depicted by a bilayer of phospholipids (grey circles with lines protruding to indicate the heads and tails, respectively of the phospholipid.) White area represents extracellular space, and grey area represents intracellular space. Solid lines refer to direct events, dotted lines refer to signaling pathways with multiple signaling proteins not indicated by the image. Image credit: Medora Huseby, own work, created in Biorender.

This pathway begins with a GPCR named cAR1. cAR1 binds to cAMP, and undergoes a conformational shift into an active form. Activated cAR1 then activates a G-protein. The α subunit of the G protein exchanges GDP for GTP, and dissociate from the β/γ subunits. In this pathway, the GTP-bound α subunit, independent of the active β/γ subunits, activate **effector proteins** that cause actin polymerization at the front of the *Dictyostelium* cell and myosin II assembly at the rear of the cell. Myosin II helps the cell with retraction, while the actin polymerization causes the formation of structures (such as lamellipodia and filopodia) required for propulsion.[29] One of the protein components required for *Dictyostelium* chemotaxis is Ras.

The Ras superfamily of proteins are small GTPases. Members of the Ras subfamily (the other subfamilies are Rho, Ran, Rab, and Arf) can have a lipid tail that binds it to the inner leaflet of the plasma membrane[30]. Ras proteins are referred to as monomeric GTPases, as opposed to the trimeric G proteins which associate with GPCRs. Ras proteins function as molecular switches, similar to the alpha subunit of a G protein. When Ras is bound to GDP it is inactive. When GDP is switched out for GTP it becomes active, and can in turn activate other proteins in the signal relay (see **Figure 12**). GDP is swapped for GTP through proteins known as Guanine Nucleotide Exchange Factors (GEFs). GEFs activate monomeric GTPases, such as Ras. After a delay, an intrinsic GTPase will hydrolyze the bound GTP into GDP, and the Ras molecule will be inactivated and return to a dormant state[31]. Other proteins that regulate small GTPases are GTPase-activating enzymes (GAPs), and Guanine Dissociation Inhibitors (GDIs)[32]. GAPs negatively regulate the molecular switch by enhancing the intrinsic GTPase activity, causing enhanced hydrolysis of GTP to GDP, and hence a quick return of the protein to a GDP bound inactive state. GDIs block the GTPase cycle by binding to the GDP bound form of the protein, and preventing exchange of GDP for GTP as well as preventing Rho and Rab proteins from localizing to the plasma membrane[33]. Mutations in Ras genes which disrupts the GTPase activity have grave consequences for a cell. In *Dictyostelium*, Ras mutants interfere with chemotaxis and cell movement. Ras is considered an oncogene in humans, and, when mutated, can lead to abnormalities in cell proliferation and cancer formation[34].

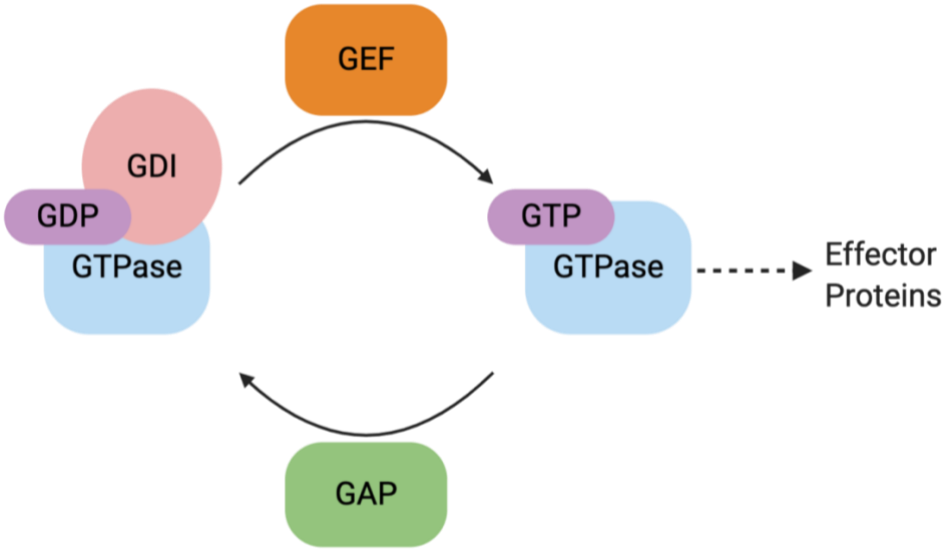

Figure 12. Small GTPase activity. Small GTPases, such as Ras and Rho, shown in blue, act as molecular switches that are active (on) when GTP bound, and inactive (off) when GDP bound (shown in pink). GAPs (GTPase Activating Enzymes), shown in green, enhance the intrinsic GTPase activity, quickly returning the GTPase to the GDP bound state. GDI proteins, shown in purple, are guanine dissociate inhibitors, bind to the GTPase in the GDP bound state, preventing the exchange of GDP to GTP. GEFs, or Guanine Nucleotide Exchange Factors, shown in rust, facilitate the exchange of GDP for GTP, and activate small GTPases. Image credit: Medora Huseby, own work, created in Biorender.

Rho-GTPases are a subfamily of the Ras superfamily. Cell Division Control Protein 42 (Cdc42) is a Rho-GTPase found in a range of organisms, from yeast to humans. When active, Cdc42 will further signal within the cell, ultimately leading to formation of lamellipodia, required for cell motility, as well as providing directionality for the cellular movement. When Cdc42 is nonfunctional, downstream intracellular signaling events remain dormant. One protein normally activated by Cdc42 is p21-activated kinase (PAK). PAK proteins are serine/threonine kinases that regulate the cytoskeletal component actin (see serine/threonine kinases)[35]. Without Cdc42 cells fail to form structures required for movement. Cdc42 is also crucial for establishment of cell polarity, as well as acting as a transcriptional regulator[36].Over expression of Cdc42 is associated with human cancers, such as melanoma, breast, and testicular cancers[37].

Signal ligands can either impact a slow response or a fast response in cellular behavior. (See signal transduction section and **Figure 6**). When a signal impacts existing proteins or mRNA molecules, this is considered a fast response, as no gene products are required *de novo*. An example of a fast response in *Dictyostelium* signaling occurs when the amoebae cells sense bacteria, which is their prey. Recall that folate (folic acid) is a signal released by bacteria. Folate will bind to a specific GPCR on the plasma membrane of the *Dictyostelium* cell. The folate signal is transduced inside of the cell by activating a heterotrimeric G protein, which then

activates downstream proteins, including Ras, to activate effector proteins which then alter the cytoskeleton, which will ultimately allow the cell to migrate and move towards the bacteria it intends to eat. *Dictyostelium* also has GPCRs that recognize the bacterial component **L**ipo**p**oly**s**accharide (LPS). LPS is part of the cell wall of Gram-negative bacteria. When Dictyostelium binds to LPS, it is generally close enough to the bacterium to attempt to phagocytose and engulf it. When LPS binds to the *Dictyostelium* specific GPCR, the goal of cytoskeletal modification is to form phagocytosis specific pseudopodia and engulf the bacteria (**Figure 13**).

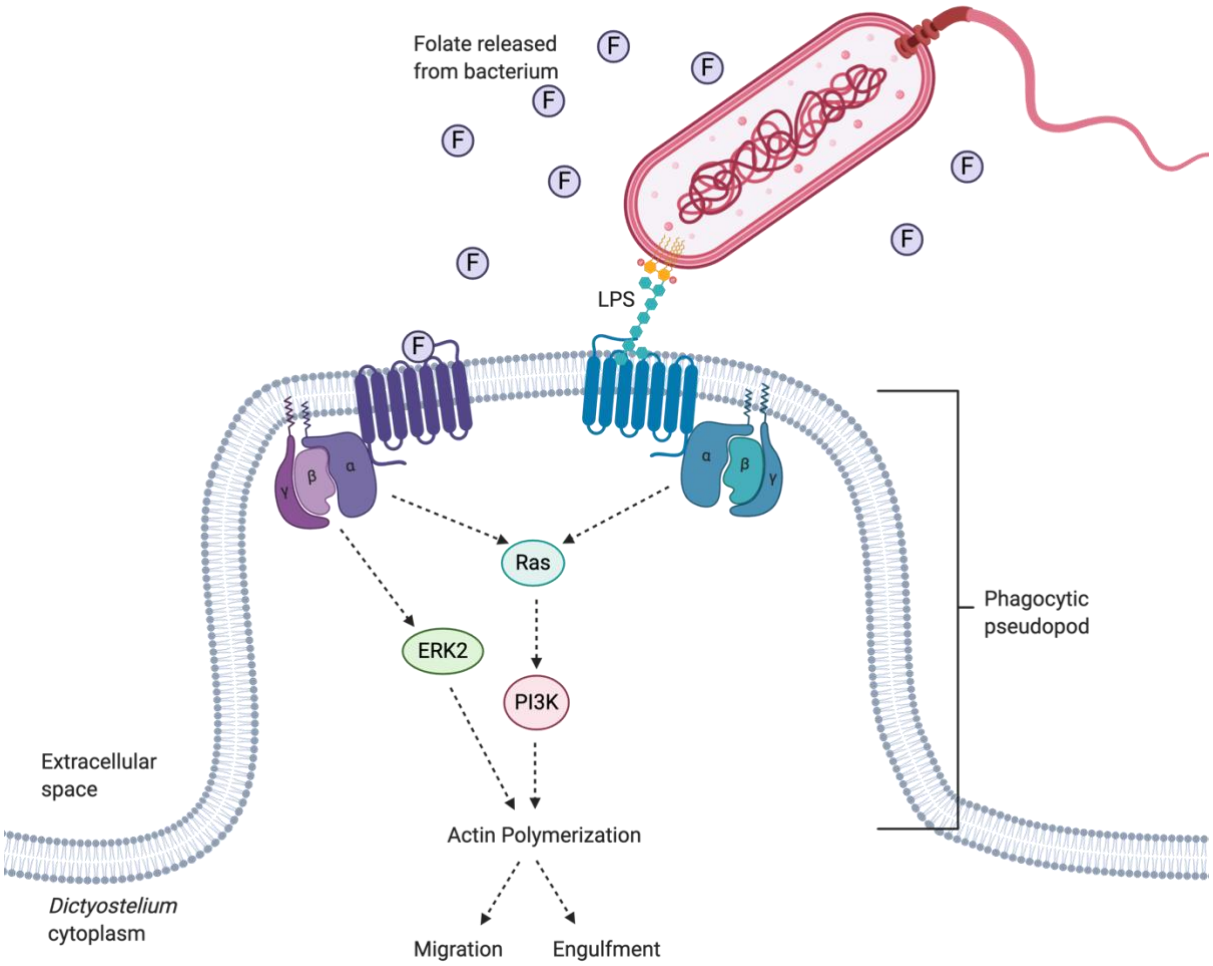

Figure 13. Fast responses in *Dictyostelium*: formation of a phagocytic pseudopod. *Dictyostelium* express receptor proteins to sense their environment. Bacteria, a food source for *Dictyostelium*, release folate (represented by purple circles with the letter F) into the environment. *Dictyostelium* contain folate receptors (purple transmembrane protein) which activate a G protein (purple α, β, γ subunits) which will activate two intracellular signaling pathways (indicated by dashed arrows). Ras (blue oval with teal outline) and ERK2 (green oval with green outline) are part of these intracellular signaling pathways. Activation of Ras and ERK2 lead to cytoskeletal rearrangement (through actin polymerization), and migration toward the bacteria which is releasing the folate. Once near the bacteria, receptors within the *Dictyostelium* membrane will bind to lipopolysaccharide (LPS- depicted as orange and blue hexagons)

which is a part of the bacterial cell wall. The LPS specific receptor (blue transmembrane protein) will active a G protein (blue purple α, β, γ subunits) which will activate an intracellular signaling pathway that activates Ras. Ras will activate PI3K (pink oval with burgundy outline), which will cause cytoskeleton rearrangement required for phagocytosis (engulfment). Phagocytosis is preceded by the formation of the phagocytic pseudopod, and extension of the *Dictyostelium* plasma membrane. The bacterium is represented as a pink oval with a dark pink flagellum. Image credit: Medora Huseby, own work, created in Biorender.

If gene expression is impacted such that a new molecule (either RNA or protein) must be synthesized *de novo*, it is considered a slow response to signal transduction. The response is slow because it takes a cell more time to create new gene products compared to altering existing gene products. The creation of new gene products often requires transcriptional regulator that must translocate to the nucleus, bind to a promoter region on the DNA, and then coordinate RNA polymerase machinery to transcribe mRNA. The mRNA must be processed and the mature transcript transported out of the nucleus. In the cytoplasm, the mRNA is then acted on by translation machinery. The cell behavior only changes once this protein product is made. This process typically takes hours and is considered a slow response. An example of this occurs during JAK/STAT signaling (see JAK/STAT signaling section).

If a GPCR is mutated or altered such that it becomes overactive, inactive, insensitive to ligand binding, or faulty in some other way, the resulting downstream effects are disastrous for a cell. Recall AprA (Autocrine proliferation repressor protein), which is an autocrine chalone signal used by *Dicytyoselium* to slow cell proliferation. This endogenous signal is released by growing *Dictyostelium* cells to regulate how many cells are present. Furthermore, AprA acts as a chemorepellent, causing *Dictyostelium* to move away from any source of AprA. The chalone AprA binds to the GPCR called GrlH. AprA binding to GrlH induces a cellular change (through activation of effector proteins) that slows down how fast *Dictystelium* cells divide and proliferate[39] (**Figure 14**). This cellular change is advantageous in harsh times, when too many cells in a nutrient depleted environment will not survive. Consider a scenario where there is a mutation in the gene that codes for GrlH such that it no longer recognizes AprA. These cells would always proliferate, even when it was detrimental to do so. Furthermore, these cells would also fail to undergo chemorepulsion. What if there are not enough nutrients present and the cells continue to proliferate? Ultimately the cells would have to enter the starvation stage, but might do so prematurely (**Figure 2**). What if the cells then are unable to move away from a noxious signal, or a predator? This would cause the cells to die, all due to a lack of signal transduction and communication from this GPCR.

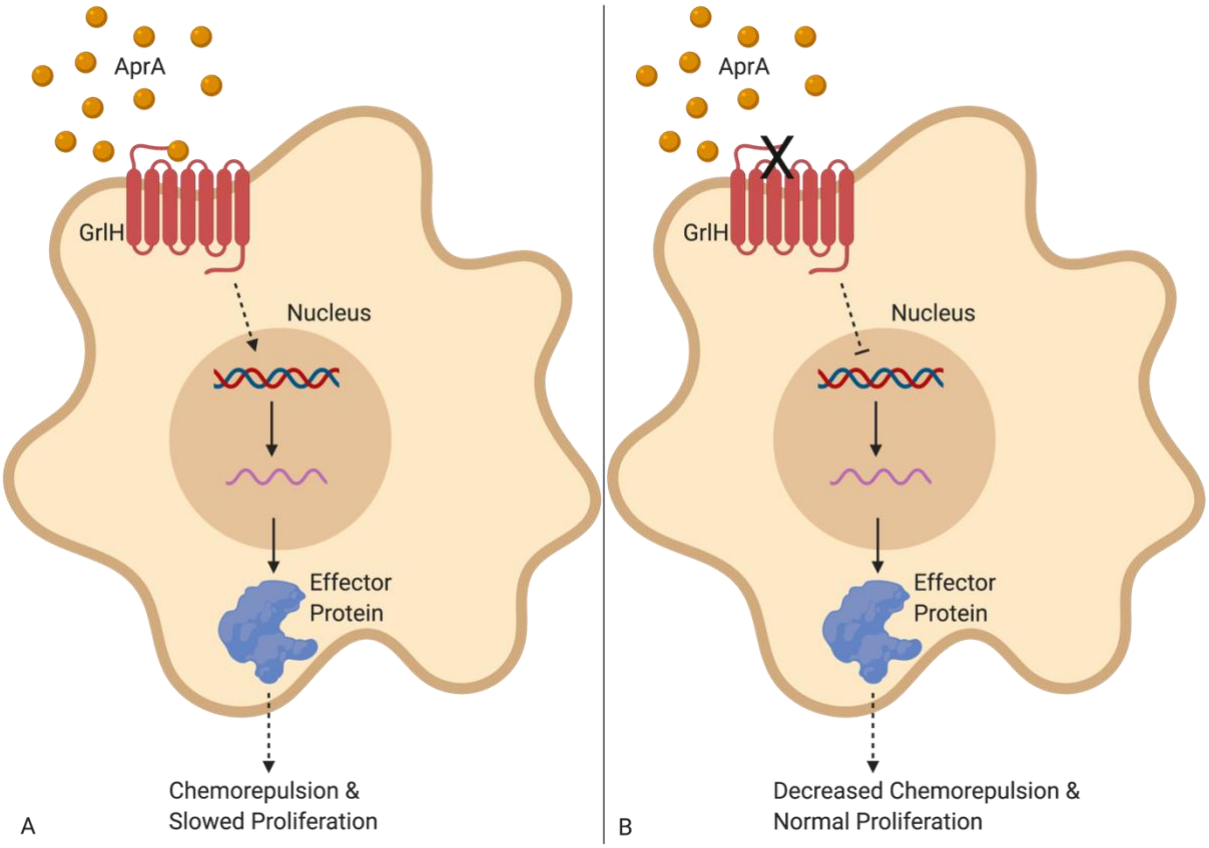

Figure 14. Slow response in *Dictyostelium*: chemorepulsion and slowed proliferation. A. Slowed proliferation in. AprA (orange circle) binds to the GPCR GrlH (red transmembrane protein) and initiates a signal transduction cascade that impacts gene expression in the nucleus (large tan circle). Specific genes on DNA (red and blue double helix) are transcribed into mRNA (purple line in the nucleus) which will then be translocated to the cytoplasm and translated into effector proteins (blue blob). B. Aberrant proliferation in. If the GPCR GrlH does not recognize/bind to AprA, then there is no signal transduction to cause the transcription and translation of effector proteins that slow the rate of cell division. The cell would proliferate normally, even though it would be harmful to the cell. Image credit: Medora Huseby, own work, created in Biorender.

Enzyme Coupled Receptors

Enzyme coupled receptors, sometimes referred to as enzyme linked receptors or as catalytic receptors, are transmembrane proteins. These receptors contain either intrinsic enzyme activity on their intracellular domain or associate directly with an intracellular enzyme [40].

Upon ligand binding to the extracellular domain, the receptor initiates enzymatic activity on the inside of the cell. Unlike GPCRs, enzyme coupled receptors do not associate with G proteins, and lack the seven pass structure of GPCRs.

There are five main types of enzyme coupled receptors:

1. Receptor tyrosine kinases (RTKs): intrinsic tyrosine kinase activity on their intracellular domain (see below for description) (**Figure 15**).
2. Receptor serine/threonine kinases: intrinsic serine/threonine kinase activity on their intracellular domain. These receptors phosphorylate serine or threonine residues, respectively, on their target proteins.
3. Receptor guanylyl cyclases: intrinsic cyclase activity on their intracellular domain. These receptors create cyclic guanosine monophosphate (cGMP), which acts as a second messenger similar to that of cAMP.
4. Tyrosine-kinase associated receptors (also referred to as nonreceptor protein-tyrosine kinases): receptor proteins that noncovalently associate with proteins which have tyrosine kinase activity, but the kinase activity is not intrinsic to the receptor itself.
5. Receptor tyrosine phosphatases: intrinsic phosphatase activity on their intracellular domain.

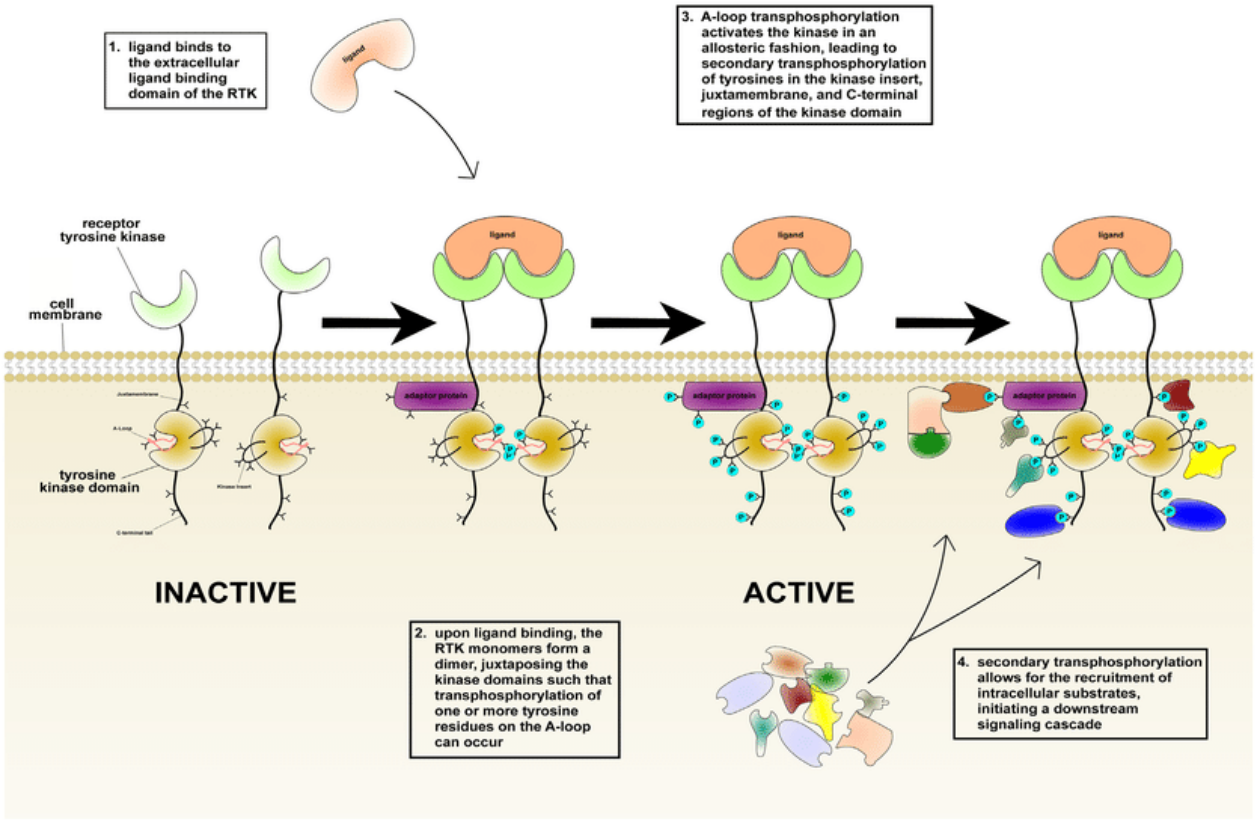

Figure 15. Receptor tyrosine kinase (RTK) activation. When inactive (left side of figure), RTKs exist as monomers. In this stage, they are not bound to a signal, nor dimerized. When a signal binds to the extracellular domain of an RTK (right side of the figure), the two RTK monomers form a dimer. This allows on monomer RTK partner to phosphorylate itself or its partner RTK on tyrosine residues. The phosphorylated tyrosine residues then act as docking areas for other proteins within the intracellular signaling pathway. Image credit: Openstax CC BY.

Enzyme coupled receptors span several families of protein receptors. Some of these proteins are associated with the plasma membrane, such as the **receptor tyrosine kinase** (RTK) superfamily. Kinases are enzymes that phosphorylate substrates, such as proteins. RTKs are protein receptors that phosphorylate a tyrosine residue on a target molecule in response to binding of a signal molecule (ligand). In many cases, the RTK will undergo autophosphoylation, meaning that it will phosphorylate specific tyrosine residues on its dimer partner (**Figure 15**).

RTKs are well conserved from organisms such as *Dictyostelium* to humans. One such RTK, known as Discoiden Domain Receptor (DDR), was first discovered in *Dictyostelium*, and found to be important in cell aggregation[41]. Mutations to human homologs of DDRs (DDR1 or DDR2) have been reported in several human cancers. DDR1 and DDR2 are implicated in several processes crucial to cancerous cells, such as proliferation and response to chemotherapies[41].

All members of the RTK superfamily have the same conformation. They consist of an extracellular ligand binding domain, followed by a single transmembrane alpha helix, and then an intracellular domain in the cytoplasm that contains the kinase domain[42]. **(Figure 15)** Most members of the RTK superfamily are made as protomers, but then associate as dimers upon extracellular ligand binding. The extracellular domains of many, but not all, RTK members allow the protomers to associate as dimers, which may be assisted by the transmembrane domain. As the transmembrane domain is a single alpha helix, RTKs lack the flexibility for confirmation change that GPCRs have to transduce extracellular signals into intracellular changes. Instead, upon ligand binding and dimerization of a RTK the intracellular kinase domain becomes activated[43] (**Figure 15**).

A ligand will bind to the extracellular domain of an RTK, effectively crosslinking the two RTK proteins into a dimer. Upon activation of the kinase domain, the intracellular regions of the RTK will phosphorylate one or more tyrosine residues on its dimer partner. Depending on which tyrosine residues are phosphorylated, different intracellular signaling proteins will be recruited to and assemble on the phosphorylated residues. Activated RTKs are intersections in a complex signaling network that will transmit information from the exterior to the interior of the cell[44]. These intracellular signaling proteins can act as adaptors to further allow other proteins to interact with the phosphorylated RTK, and can then further relay the signal (which started as an extracellular ligand binding to the RTK) into the cell. It should be noted that some RTKs will form dimers in the absence of an extracellular ligand, but remain inactive until their ligand binds[45]. Furthermore, some RTKs are believed to form larger oligomers than a dimer[46].

Many mammalian RTKs activate Ras (see GPCR section) proteins. Many RTKs respond to chemoattractants. Recall Wiskott-Aldrich Syndrome, which is a human disease that results from a mutation in the WAS gene, resulting in a defective protein, known as WASP (**W**iskott-**A**ldrich **S**yndrome **P**rotein). WASP is a protein that functions

to activate actin polymerization, which is a cytoskeletal rearrangement required for cellular movement. This protein is used both by human immune cells and *Dictyostelium* cells. WASP is a binding partner to CDC42, which is Rho-GTPase and a member of the Ras superfamily.

Not all receptor kinases are membrane bound, and instead may exist free of any membrane attachment within the cytoplasm. One family, known as JAK/STAT, are receptor kinases not bound to a membrane. JAK (JAnus Kinase) and STAT (Signal Transducer and Activator of Transcription) are proteins that are found in organisms ranging from *Dictyostelium* to humans. Dictyostelium have four STAT genes [47]. Dictyostelium STAT proteins have multiple functions during *Dictyostelium* development, including aggregation and chemotaxis. JAK proteins are kinases that are associated with transmembrane receptor proteins, or in other cases, recruited to the activated receptor. *Dictyostelium* lack orthodox JAK family members but do have many tyrosine kinase-like kinases (abbreviated TKL)[48].

Ligand binding to the receptor protein (which is associated with the JAK protein) will cause the receptor to dimerize with another receptor. This dimerization will recruit JAKs, which will result in their phosphorylation–either autophosphorylation by the JAK itself, or transphosphorylation by another kinase). Phosphorylated JAKs have increased kinase activity which is used to phosphorylate the receptor on target tyrosine residues. These phosphorylated sites on the receptor serve as docking sites where other proteins (generally through SH2 domains) can bind and interact with the JAKs. STATs are one such protein that are recruited to the phosphorylated tyrosine residues on the receptor proteins. (**Figure 16**).

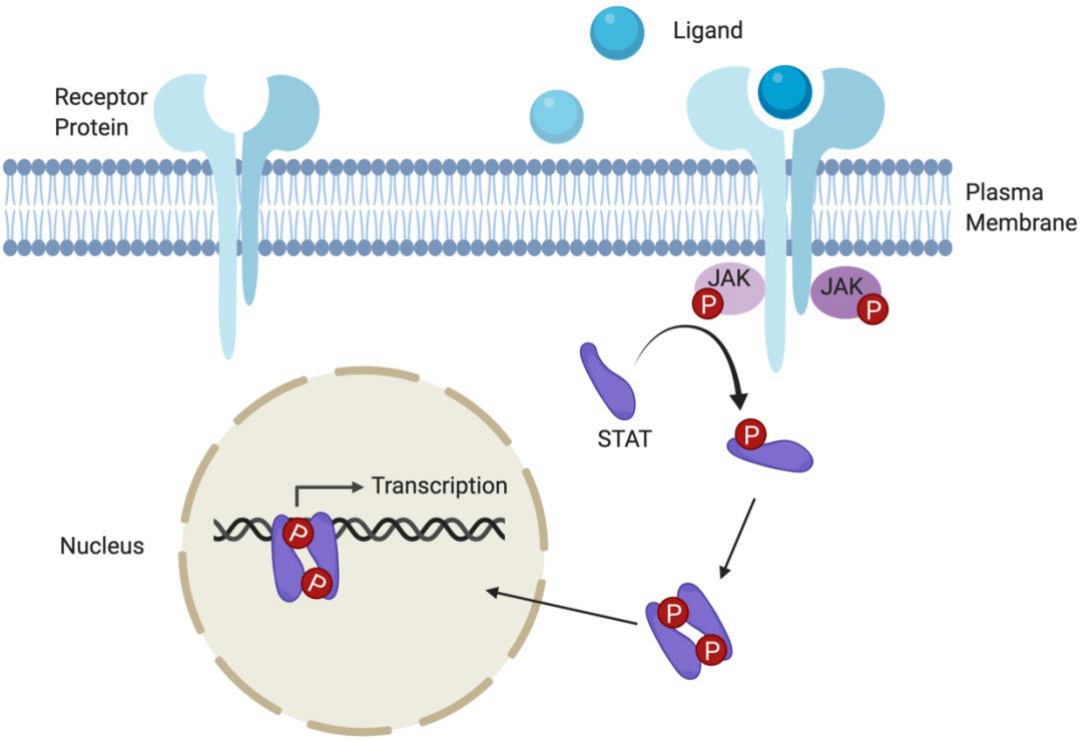

Figure 16. JAK/STAT signaling. JAK proteins (pink and purple spheres) are recruited to receptor proteins (teal and blue transmembrane proteins) bound to a ligand (blue sphere). Activated JAK proteins can autophosphorylate and transphosphorylate each other and the receptor protein (phosphate depicted as red circle with a white P). STAT proteins (purple) are recruited to phosphorylated JAK proteins, and are then phosphorylated. Phosphorylated STAT proteins dimerize and translocate to the nucleus, where they act as transcription regulators. This is a slow response. Not shown-importin proteins, which guide STAT proteins through the nuclear pore. Image credit: Medora Huseby, own work, created in Biorender.

JAKs can transphosphorylate STATs, causing the STAT to become activated. Inactive STATs exist as unphosphorylated cytoplasmic proteins. Other kinases can also activate STATs. Activated STATs will dissociate from the JAK/receptor complex, dimerize, and then translocate to the nucleus. STATS have a nuclear localization signal, which binds to proteins known as importins [49]. The importin/STAT dimer will then enter the nucleus through the nuclear pore. Once in the nucleus, activated STAT dimers will be released from the importin proteins and will then act as transcriptional activators, promoting transcription of a gene, either by directly binding to the promoter region of the gene– or by indirectly recruiting general transcription factors[50], which is also associated with another JAK protein. This dimerization event brings the JAK proteins in a close enough proximity to undergo transphosphorylation, an event characterized by the transfer of a phosphate group between a substrate and a receptor.

Though *Dictyostelium* and humans share STAT protein homology, no canonical JAKs have been identified in *Dictyostelium*[51]. Mutations to STAT genes that render the STAT protein non-functional in *Dictyostelium* cause a delay in cell aggregation due to inefficient chemotaxis to cAMP sources[52]. Furthermore, these cells display aberrant gene expression patterns that cause the cells to remain in the slug stage for several days. Wild type *Dictyostelium* form the slug stage around 17 hours after exposure to a cAMP signal.

Dysfunctional JAK/STAT signaling in human cells can result in different diseases, such as cancer, Severe Combined Immunodeficiency Disorder (SCID)[53] and psoriasis. JAK inhibitors are currently under investigation for drug therapy for some types of leukemia.

Cells carefully regulate JAK/STAT activation as well as the amount of downstream signaling that occurs from a phosphorylated (activated) STAT dimer. One family of protein inhibitors, PIAS (Protein Inhibitors of Activated Stats), add a functional group called SUMO (Small Ubiquitin-like Modifier) on to STATs. When a STAT monomer is modified with a SUMO group it cannot be phosphorylated, and hence activated, unless the SUMO group is removed[54]. Homologs to PIAS exist in *Dictyostelium*.

Protein tyrosine phosphatases (PTPs) remove phosphate groups from tyrosine residues. Members of the PTP protein family can remove phosphate groups from phosphorylated tyrosine residues on JAKs. If JAKs are not phosphorylated their kinase activity is not active, and they cannot undergo transphosphorylation to activate STATs[55]. *Dictyostelium* cells contain homologous PTP proteins.

Ion-Channel-Coupled Receptors

Ion channels are transmembrane pore forming proteins that allow ions to pass from one side of a membrane to another side (**Figure 17**). Ion channels represent the second largest target for existing drugs (after GPCRs)[56]. Ion channels undergo a conformational change, from a closed state to an open state, in order to allow ions to cross the plasma membrane, or the membrane of an organelle. Ion channels are selective, only allowing specific ions to pass through. Some ion channels remain open, permanently allowing ions to move across the plasma membrane with the electrochemical gradient. Such channels are referred to as leakage ion channels and are crucial to restore a resting membrane potential.

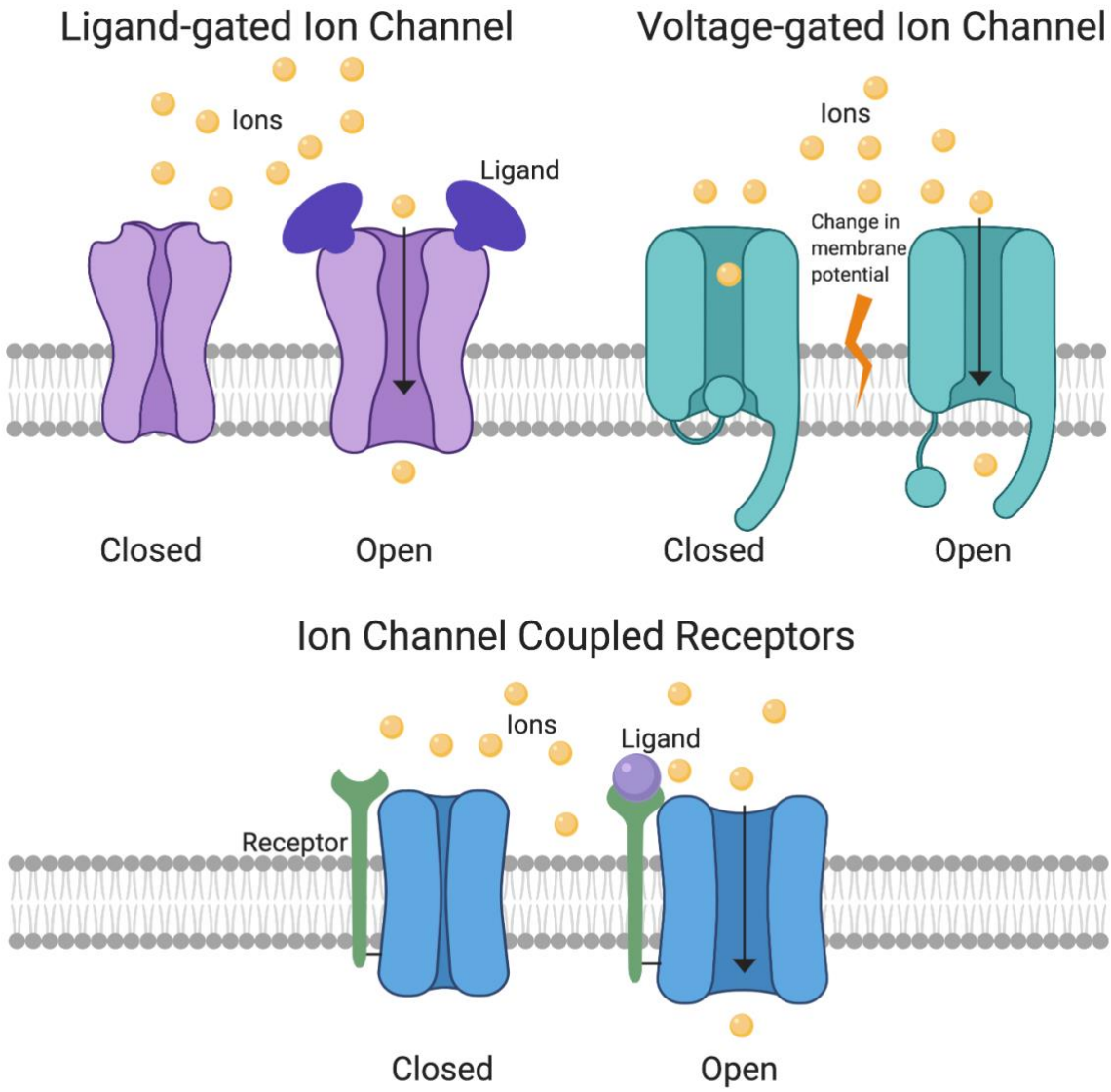

Figure 17. Open and closed state of ion channels. Ligand-gated ion channels (LGICs) remain open or closed (show in purple) until a ligand (dark purple) binds to the channel to change it from a closed to an open state (or vice versa). When open, specific ions (shown as yellow balls) move through the channel with their electrochemical gradient. Voltage-gated ion channels (shown in teal) change conformation to allow ions through in response to a change in the membrane potential. Ion channel coupled receptors (ICCRs) (shown in blue) open or close in response to a separate receptor protein (shown in green) binding to a ligand (purple sphere). The receptor may be covalently linked to the channel (shown as a black line). When the ligand is released from the LGIC, the receptor of the ICCR, or the membrane potential returns to a resting value, then the channel will switch conformation from open to closed, or vice versa. Not shown: leakage ion channels. Image credit: Medora Huseby, own work, created in Biorender.

Ion channels which are opened (gated) by binding of a ligand are referred to as **L**igand-**G**ated **I**on **C**hannels (LGICs). Ion channels which are gated by a ligand binding to a receptor that is physically coupled to an ion channel are Ion-Channel-Coupled

Receptors (ICCRs). Some ion channels are gated by a change in the membrane potential (voltage-gated ion channels)[57] (**Figure 17**).

LGICs are gated, meaning the conformation of the channel is shifted when a ligand binds to the channel. Once open, LGICs allow specific ions to passively move through the channel with the electrochemical gradient of the ion. In vertebrate cells, LGICs found on the plasma membrane mediate fast synaptic transmission[58], which is the process by which neurons communicate with other neurons. In this scenario, a neurotransmitter is frequently the ligand which will open the LGIC. *Dictyostelium* cells also contain LGICs. There are 5 ATP-gated channels on the contractile vacuole, an organelle *Dictyostelium* uses to control cell volume and regulate water flow due to changes in osmotic pressure. In response to ATP binding, the *Dictyostelium* P2X channels will release the ion Ca^{2+} from the contractile vacuole into the cytoplasm[59]. The release of Ca^{2+}, which is a second messenger (see second messenger section), will then trigger further steps in the signal transduction pathway, ultimately expelling excess water from the cell (**Figure 18**).

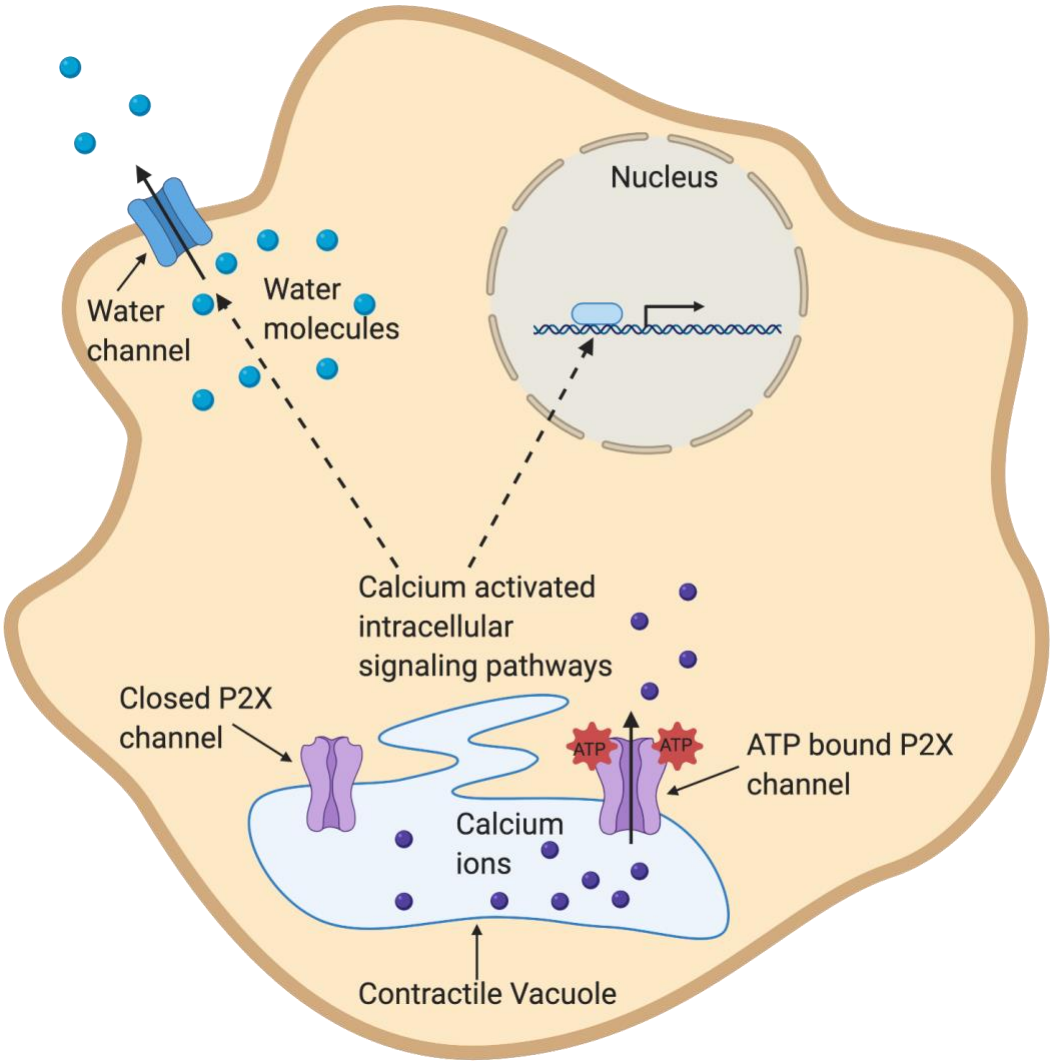

Figure 18. Calcium as a second messenger in *Dictyostelium*. The contractile vacuole (light blue organelle with a dark blue boarder) has P2X channels (purple, passing through the membrane of the contractile vacuole). When in the apo state (no ATP bound), the channel is closed. Upon ATP (8-pointed red star) binding, the P2X channel will open, allowing calcium ions (dark purple circles) to move from the lumen of the contractile vacuole into the cytoplasm (tan area inside the plasma membrane (brown boarder)). Calcium ions will activate intracellular signaling pathways (depicted by dashed arrows) that result in the efflux of water (blue circles) through a water channel (blue transmembrane protein that passes through the plasma membrane). The fast response to calcium would act on proteins already present in the cell. The slow response would translocate to the nucleus (grey circle with perforated line which depicts the nuclear membrane) to active transcription of a gene (blue and black double helix with arrow on top). The transcription factor is depicted as a light blue oval bound to the DNA. Image credit: Medora Huseby, own work, created in Biorender.

Similar to LGICs, Ion-Channel-Coupled Receptors (ICCRs) can both initiate and mediate cell signaling events. ICCRs are attached to a GPCR in such a way that a change in conformation of either protein will impact the other. When a ligand binds to a GPCR that is mechanically coupled to an ICCR the conformational shift within the

GPCR is directly transmitted to the ICCR, causing the ICCR to change its conformation. Not all ion channels are coupled to a GPCR. Some are independent molecules in the plasma membrane (or other cellular membranes). In this case, the ion channel can be activated by downstream effects of ligand activation of a receptor protein. For example, the G-protein, activated in response to a ligand binding to a GPCR, can then activate an ion channel (**Figure 19**).

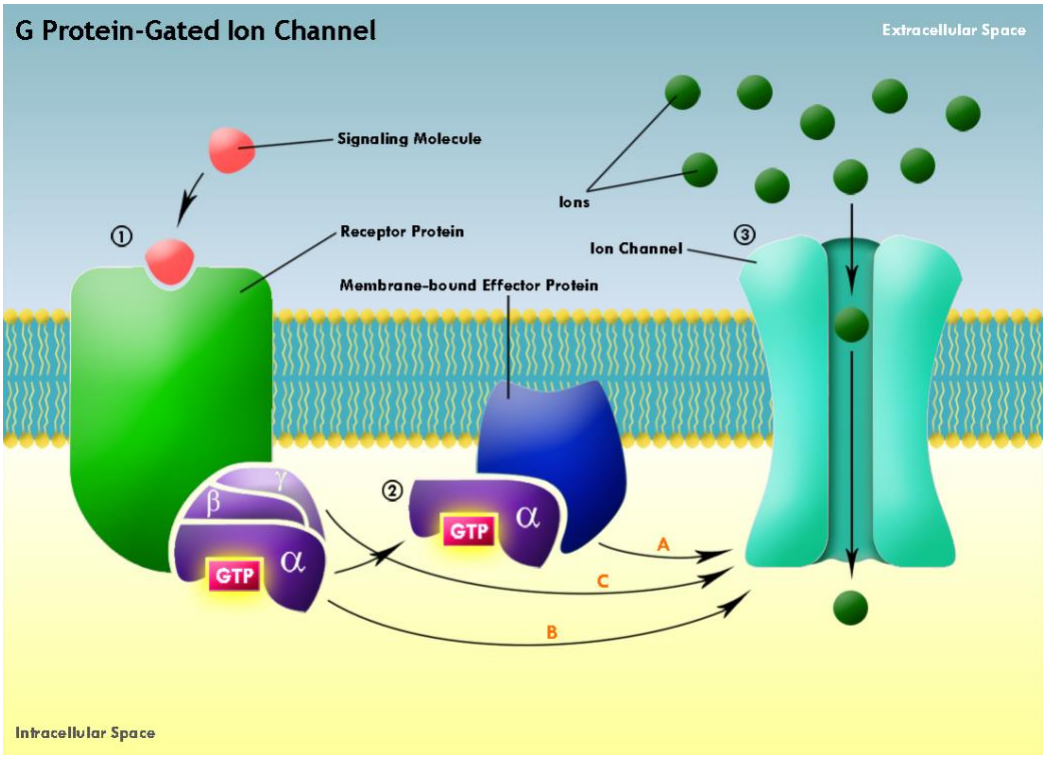

Figure 19. Activation of a G protein gated ion channel. The signal molecule (red circle) binds the receptor (green rounded cylinder) inducing a conformational change to the receptor. This allows the G protein's α-subunit (purple intracellular complex) to exchange GDP for GTP. This causes the α-subunit and βγ-complex to break free from the receptor and each other. 2) The GTP-bound α-subunit signals an effector protein which begins a signaling cascade which leads to the G protein-gated ion channel (teal transmembrane protein) eventually opening. When open the ion channel allows ions (green circles) to flow into the intracellular space (represented in yellow) 3) The G Protein-gated ion channel can be activated via pathways (A), (B), or (C). Image credit: OpenStax. CC BY.

Nearly all membranes have an electrical potential across them due to the inside and outside of a cellular membrane having different changes. Typically, the inside of the membrane has a negative charge associated with it, while the outside of the membrane has a positive charge associated with it. The difference in electrical potential across the membrane is referred to as the **membrane potential**. Changes to the membrane potential can induce signal transduction events and allow cells to

communicate. Changes in the membrane potential can activate voltage-gated ion channels. Voltage-gated ion channels will shift conformation when there is a change to the membrane potential. This will allow ions to move through the channel with their electrochemical gradient (**Figure 17**).

Changes in membrane potential, due to the activity of as of yet undefined voltage-gated ion channels, are speculated to have a possible role in signal transduction and differentiation in *Dictyostelium* cells[60]. A voltage-gated ion channel specific to K^+ ions is believed to be part of the signaling required for contractile vacuole regulation within *Dictyostelium*, however, the exact mechanism remains unknown[61].

Second Messengers

Signal transduction pathways are frequently relayed by **second messengers**[62]. Second messengers are small, non-protein molecules that relay the initial signal transduction within in the cell. Similar to signal molecules, there are many different types of second messenger molecules. Second messengers are divided into four classes:

1. Cyclic nucleotides (such as cAMP)
2. Lipid
3. Ions
4. Gases

Polar molecules, such as nucleotides and ions, are used to signal within the cytosol of the cell. Hydrophobic molecules, such as lipids and lipid derivatives, signal within the cellular membranes. Ions and gases can freely pass through membranes, signaling between compartments and organelles within a cell, or across the plasma membrane to a neighboring cell.

Second messengers are typically present in low concentrations, or sequestered within an organelle of a cell. Second messengers can also rapidly be created by a cell (microseconds for ions, and seconds for some lipids). Upon ligand binding a protein receptor shifts its conformation, sometimes activating a nearby enzyme. This enzyme, such as adenylyl cyclase, will amplify the initial signal by producing many copies of the second messenger, such as cAMP. The second messenger will rapidly diffuse to targets elsewhere in the cell. Second messengers can activate multiple effector proteins pathways, again amplifying the original signal **(Figures 7 and 18).** There exist many examples of second messengers modulating enzymatic activity. Cells carefully control duration of second messenger stimulation– as prolonged exposure to second messengers can cause intracellular signaling to occur inappropriately– leading to unwanted cellular behaviors. When the cell no longer requires modulation of specific effector proteins via second messengers, other protein systems are present to rapidly remove or inactivate the second messenger. Dysregulation of second messenger

signaling can result in a disease state for multicellular organisms. For example, chronic exposure to cAMP can cause uncontrolled/asynchronous growth of heart cells, referred to as pathological hypertrophy[64].

cAMP activates Protein Kinase A (PKA)[65] (**Figure 20).** When cAMP levels are low, PKA is inactive. However, upon an initial signal molecule binding to its receptor, such as a GPCR, the signal will be transduced and effector proteins, such as adenylyl cyclase, activated. Adenylyl cyclase will rapidly convert ATP into cAMP. Elevated levels of cAMP will activate PKA, which has been documented to phosphorylate 300-500 protein targets, depending on the cell type. Furthermore, a subunit of PKA can move into the nucleus and then phosphorylate transcription factors to regulate gene expression within the cell (a slow response). Because activated PKA has such as broad and varied response within the cell, PKA will be localized to specific locations by a group of proteins called **A**–**k**inase-**a**nchoring **p**roteins (AKAPs)[66]. This anchoring of active PKA limits the relay of the initial signal to a finite portion of the cell, similar to speaking to one person rather than to an auditorium of people.

Dictyostelium cells lack AKAPs with which to control PKA activity. Instead, *Dictyostelium cells* carefully regulate synthesis and degradation of cAMP, and hence PKA activity. (**Figure 20**). When cAMP is bound to PKA, PKA will phosphorylate and inhibit the activity of ERK2. ERK2 is a kinase that phosphorylates the phosphodiesterase RegA. RegA enzymatically cleaves cAMP into AMP, lowering cellular levels of cAMP. When ERK2 is inhibited it no longer inhibits RegA. When RegA is active, cAMP levels decrease and PKA is inactivated[67]. This is an example of an inhibitory feedback loop.

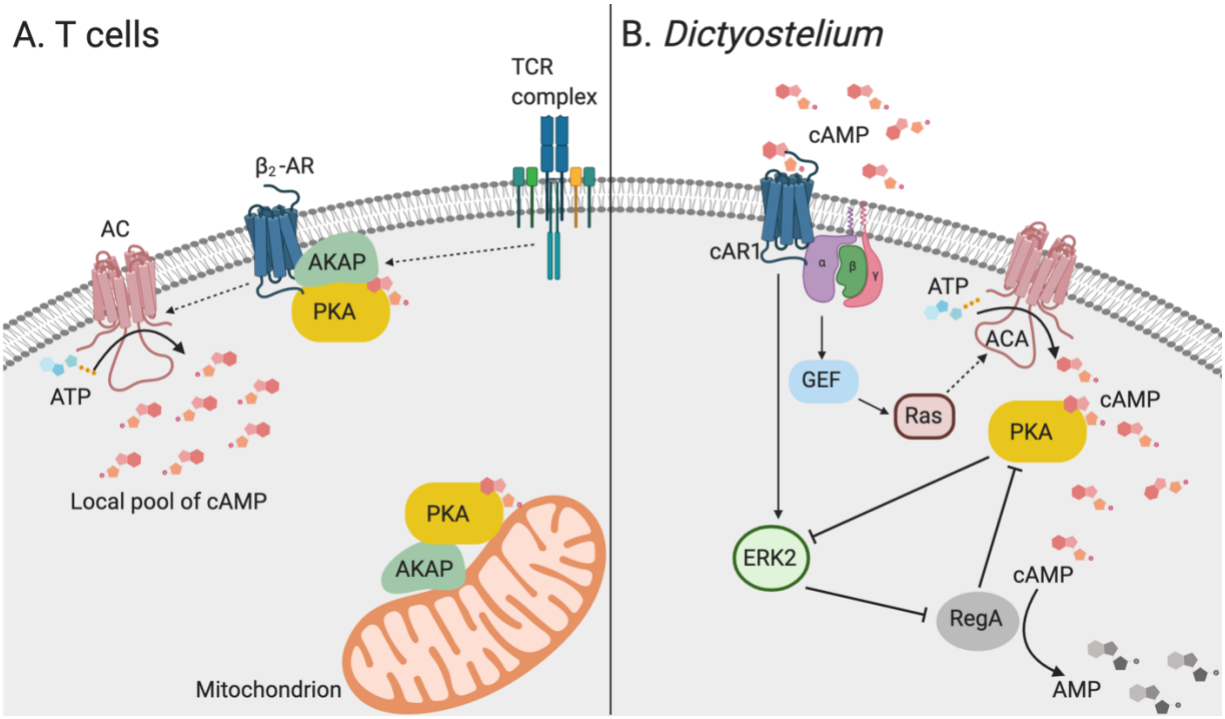

Figure 20. Control of PKA in T cells compared to *Dictyostelium*. A. In T cells, immune cells found in mammals, PKA activity is controlled by proteins which sequester PKA to specific cellular locations. Upon activation of the TCR complex (shown as a blue, green and yellow transmembrane complex of proteins), an intracellular signaling cascade (dashed arrow) will activate PKA (yellow oval with cAMP bound). AKAP (light green blob) will reversible bind to and sequester PKA. The beta-2-AR receptor (shown as blue cylinders passing through the cellular membrane) will interact with AKP and PKA, as well as commencing intracellular signaling events (dashed arrow) which will activate adenylyl cyclase (AC, shown as pink cylinders that pass through the plasma membrane). AC will covert ATP (blue hexagons with 3 orange phosphate groups attached) into a local pool of cAMP (pink and orange hexagons with one phosphate group attached). AKAPs can also sequester PKA to other cellular locations other than the plasma membrane, such as the mitochondria (orange organelle with light pink squiggles), as well as the nucleus, centrosomes, and the ER (not shown in this figure.) **B.** *Dictyostelium* cells control PKA by enzymatic control of intracellular cAMP concentrations. cAMP will bind to and activate the GPCR cAR1(blue cylinders which pass through the plasma membrane). The activated G protein (purple, green, and pink subunits associated with cAR1) will activate GEFs (guanine nucleotide exchange factors, depicted as a light blue circle), which will activate the small GTPase Ras (depicted as a pink oval with a burgundy boundary). When active, Ras will further intracellular signaling events (dashed arrow) that will activate ACA, an adenylyl cyclase found in *Dictyostelium* (depicted as pink transmembrane cylinders). ACA will covert ATP into cAMP. cAMP will bind to and activate PKA. Active PKA will inhibit ERK2 (shown as a green circle with a dark green boarder). ERK 2 normally inhibits RegA (shown as a grey oval), which is a phosphodiesterase that will cleave cAMP into AMP (grey pentagons with a phosphate group attached). Active RegA lowers intracellular levels of cAMP, which ultimately inhibits PKA activity. Image credit: Medora Huseby, own work, created in Biorender.

Subversion of Cell Signaling By Pathogens

Eukaryotic organisms, both multicellular and single celled, can be infected by smaller pathogens (such as bacteria and viruses). Pathogens target signaling pathways to co-opt cellular pathways for their own survival[68]. Some cellular targets include GTPases or regulators of GTPases which control vesicle trafficking, kinases cascades, or ubiquitin-dependent pathways which determine if certain cellular proteins and components will be targeted for degradation.

One well studied example of cell signaling subversion involves the cholera toxin, made by the bacterial pathogen *Vibrio cholerae*. Cholera toxin enters the host cell by endocytosis[69]. Once inside the host cell cytoplasm, the toxin interacts with the α subunit of a heterotrimeric G protein (**Figure 21**). It modifies the α subunit (via ADP-ribosylation), which locks the α subunit into an active state. When active, the α subunit signals adenylyl cyclase activity.[70] Activated adenylyl cyclase creates a surplus of cAMP. In an animal intestinal epithelial cell, high levels of cAMP cause an efflux of chloride ions and water, resulting in severe watery diarrhea and dehydration, better known as the disease cholera. Cholera is contracted by eating or drinking water contaminated with *V. cholerae*. The pathogen is transmitted to new hosts by causing watery diarrhea, which then contaminates more food and drinking water, quickly spreading through a population.

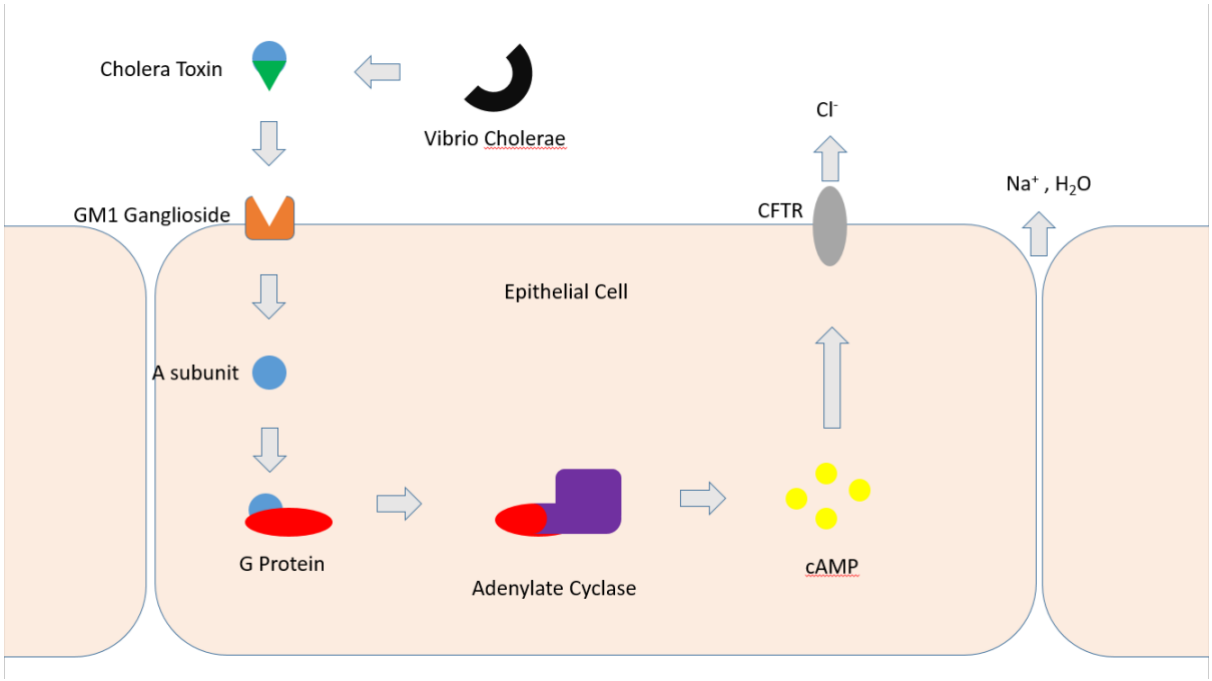

Figure 21. Cholera toxin mechanism. Cholera toxin (green/blue drop) binds to the receptor (GM1 ganglioside, orange with v cut out) on the target cell. After endocytosis, the toxin is processed (A subunit, blue circle) and then locks the α subunit of a G protein (red oval) into the active state. The active α subunit activates adenylyl cyclase (purple block), and enzyme that creates cAMP. High levels of cAMP cause further intracellular signaling, resulting in the phosphorylation of the CFTR (grey oval), a chloride channel protein. When phosphorylated (not shown), the chloride receptor moves the chloride ion out of the cell, leading to the secretion of water, sodium, and potassium into the intestinal lumen, leading to rapid dehydration and diarrhea.

Bacterial pathogens may contain Guanine-Nucleotide Exchange Factor (GEF) mimics. (see section on molecular switches). These proteins alter the molecular switch ability of a host GTPase. For example, *Salmonella*, which can cause typhoid fever, has two GEF proteins: SopE and SopE2. Both bacterial proteins stimulate actin polymerization because they activate the host cell Rho-GTPase Cdc42 by exchanging GDP for GTP, thus activating Cdc42, which then activates more intracellular signaling ultimately leading to actin polymerization[71] (**Figure 22**). This causes a membrane ruffling, which allows *Salmonella* to enter into the cell[72] (**Figure 23**).

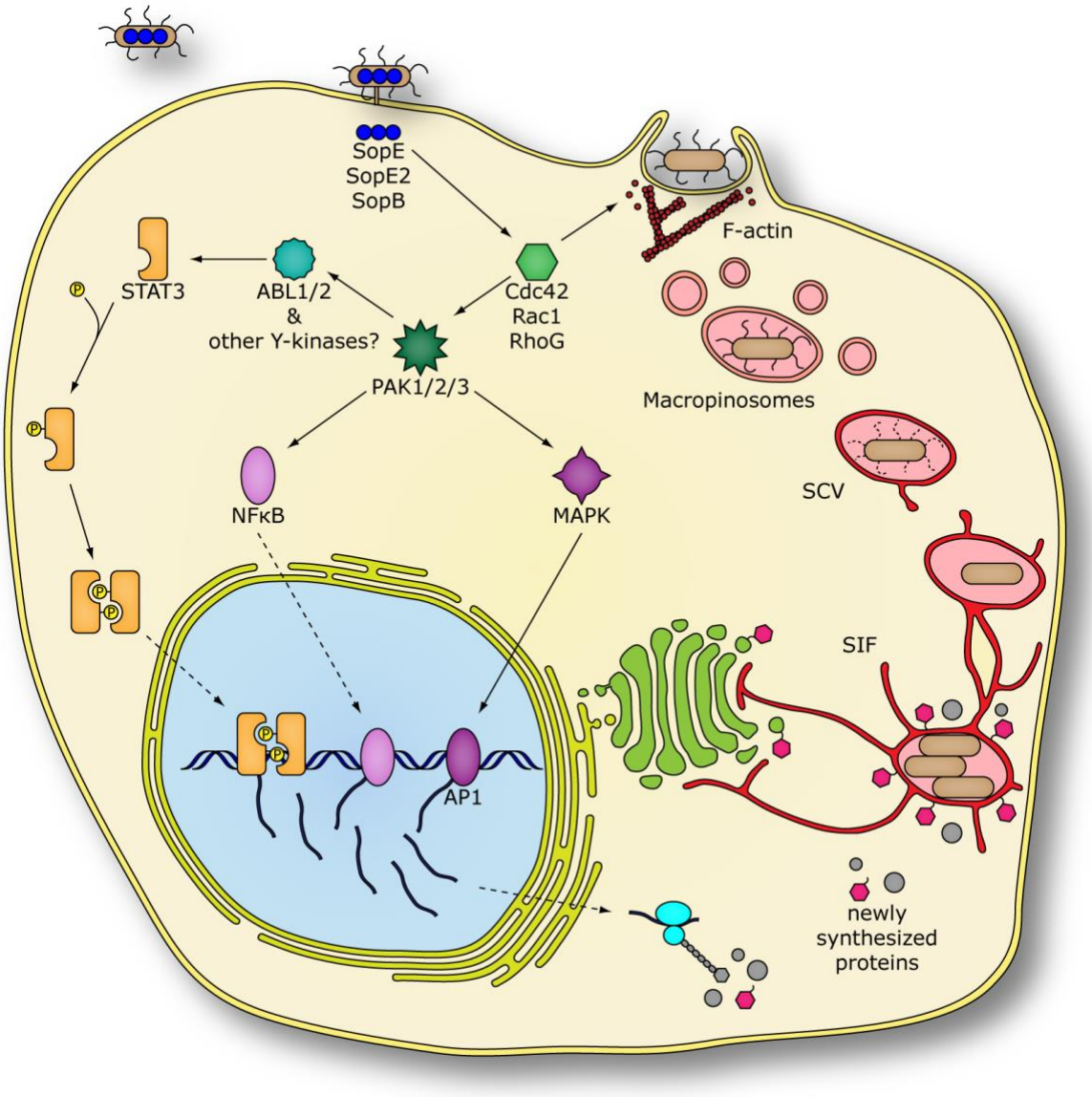

Figure 22. *Salmonella typhimurium* modulation of host cell signaling. *S. typhimurium* (small tan ovals with black lines representing flagella) infects intestinal epithelial cells (large cell shown in cream) by delivering effector proteins such as SopE and SopE2 (blue circles) into the host cell, using its SPI-1 T3SS. SopE and SopE2 activate the small GTPases Cdc42, Rac1 and RhoG to modify actin cytoskeleton (red branches near the top of the intestinal cell) to induce membrane ruffling and bacterial uptake. Internalized bacteria reside in macropinosomes (pink circles), which then maturate into Salmonella containing vacuoles (pink circles outlined by a red membrane). SopE and SopE2 stimulated small GTPases also activate members of the p21 activated kinase family (PAK) (green burst shape). These serine/threonine kinases trigger downstream signaling of mitogen-activated protein kinases (MAPKs) (purple circle with points) and nuclear factor kappa B (NFκB) (light purple oval) resulting in activation of additional transcription factors. In addition, activated PAK proteins also phosphorylate members of the Abl kinase family, thereby triggering auto-phosphorylation of Abl proteins for full activation, subsequently leading to the activation the cytoplasmic transcription factor STAT3. Products of STAT3-controlled genes ultimately influence vesicular trafficking between cellular compartments and the SCV, contributing to the formation of *Salmonella* induced filaments (SIFs), which characterize a fully mature, replication-competent *Salmonella*-containing vacuole[73].

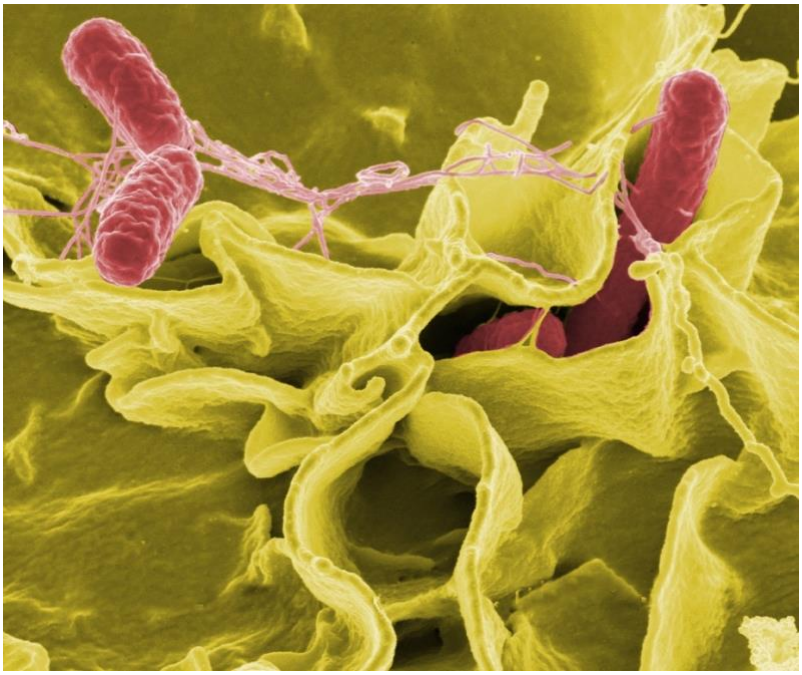

Figure 23. Membrane ruffling caused by *Salmonella*. Color-enhanced scanning electron micrograph showing *S. typhimurium* (red) invading cultured human cells. The waves on the cell membrane which enfold the bacteria are membrane ruffles caused by cytoskeletal rearrangement. Image credit: Rocky Mountain Laboratories, NIAID. Public domain image.

Microbes and hosts have interacted and co-evolved for as long as cells have existed. During this time, microbes and their hosts have been exposed to the signaling molecules released from each other. This type of signaling is referred to as **interkingdom signaling**, and mediates both beneficial and harmful relationships between microbes and their hosts[74]. The above examples of cholera toxin and GEF mimics are examples of pathogens which co-opt host signaling. Other microbes can sense, and respond to, host hormones, immunity factors, and metabolites to modulate the microbe's growth, metabolism, or virulence[75].

Adrenaline is a hormone that is involved in the flight-or-fight response animals experience in stressful scenarios. Adrenaline can be found in the central nervous system, as well as in the gut of humans. Enterohemorrhagic *Escherichia coli* (EHEC) can respond to adrenaline produced by host cells, which enhances growth conditions for EHEC cells within the host gut, as well as induces siderophore gene expression. Siderophores are bacterial proteins which scavenge iron[76]. Different compartments of the human gut contain different concentrations of adrenaline. EHEC cells prefer to colonize the colon, as opposed to the stomach or small intestine. EHEC could respond to local adrenaline concentrations to sense when they are in the colon. In this way,

EHEC cells respond to host hormone signaling through interkingdom signaling. Furthermore, host cells can sense and respond to signals made within and produced by microbial cells. *Pseudomonas aeruginosa* are bacteria that produce signals (known as AHLs, or N-acyl homoserine lactones) that cause either anti-inflammatory and pro-inflammatory host responses though host signaling of specific immune cells[77]. Bacterial AHLs can even induce apoptosis of host cell by stimulating apoptotic cell signaling cascades that result in cell death.

Eukaryotic pathogens, such as members of the genus *Plasmodium*, also signal and respond to host cells. Infection with specific species of *Plasmodium* can cause malaria, which is a mosquito-borne infectious disease with symptoms including fever, shaking chills, nausea, and sweating. Malaria infections occur at two stages; first in the liver, and later, in the blood. The liver stage of the infection is silent, meaning the host does not exhibit the aforementioned malarial symptoms. However, the host still detects the pathogen when it is in liver cells though PRR (pathogen recognition receptors), which will initiate an innate immune response through appropriate signaling pathways[78]. The blood stage of infections accounts for the symptoms associated with malaria. The *Plasmodium* cells respond to host signals such as glucose levels within the blood. This allows the pathogen to synchronize with other pathogen cells to rupture blood cells to release the pathogen. When the cell is lysed, parasitic signaling molecules are released en masse, and stimulate strong inflammatory host responses. These responses lead to the periodic recurrences of fever, chills, headache and malaise[79].

Summary

Cells sense their environment and respond to signals from their environment to perform all necessary cellular processes. Cells move, divide, or undergo apoptosis by sensing and responding to the signals around them. These signals can come from many places: the environment, other cells, or the cell itself. All cells-from single celled *Dictyostelium* to multicellular organism like humans-depend on signal transduction to change individual cell behaviors. Intracellular relay systems are exquisitely controlled, lest a cell overreact or induce an inappropriate response, and potentially cause other cells to do the same. There exist many ways for a cell to respond to a signal. The goal of cell signaling is to elicit cellular changes to optimize survival and reproduction.

Review Points:

- Cells communicate through signals from other cells, the same cell, or the environment.
- A signal (ligand) binds to a specific receptor protein.
- Signal transduction occurs when an extracellular signal is converted to an intracellular signaling cascade.
- Receptors can be membrane associated proteins or cytoplasmic proteins.
- GPCRs have seven transmembrane segments and shift their conformation to activate G proteins.
- Heterotrimeric G proteins are composed of an α, β and γ subunit. The α subunit is a molecular switch.
- Effector proteins cause changes to cellular behavior.
- A slow response occurs when a new protein (or RNA) product must be made *de novo* and takes several hours.
- A fast response occurs when an existing protein is modified and occurs within minutes.
- Secondary messengers are small, nonprotein molecules that further relay the initial signal within the cell.
- Enzyme-coupled receptors transduce a signal molecule by enzymatically modifying a protein target or activating an intracellular enzyme.
- Ion-channel-coupled receptors allow the movement of ions across the membrane when activated.
- Pathogens subvert host cell signaling pathways during pathogenesis.

Glossary Terms

ADP-ribosylation: The addition of one or more ADP-ribose molecules to a protein.

A-kinase-anchoring proteins (AKAPs): Proteins that anchor PKA to specific locations in cells.

Amplification: Creation of more signal to induce a stronger cellular response within an intracellular signaling pathway.

Autocrine: Signaling that occurs when a cell secretes a signal, which binds to a receptor protein on the cell from which the signal was created.

Cell division control protein 42 (Cdc42): A Rho-GTPase that leads to the formation of filopodia, a structure required for cell motility, as well as providing directionality for the cellular movement.

Chalones: Protein signals that inhibit cell proliferation of the cell that secretes it.

Chemoattractant: A substance that attracts motile cells, triggering a response that causes the cell to move towards the substance.

Chemotaxis: Movement of an organism in response to a chemical stimulus.

Co-factor: A molecule, other than the primary ligand, whose presence is required for activity.

Distribution: One of the ways signaling molecules impact intracellular signaling pathways through distributing the signal onward.

Down-regulation: Process by which a cell decreases the quantity of a cellular component.

Effector protein: A protein that will impact cell behavior.

Electrochemical gradient: The electrochemical potential of an ion consists of concentration of the ion across the membrane (concentration gradient) and the difference in charge across the membrane (the electrical gradient).

Endocrine: Signaling that occurs through the release of hormones.

Endogenous: Originating from within an organism.

Extracellular matrix (ECM): The ECM is a three-dimensional network of extracellular macromolecules, such as collagen, enzymes, and glycoproteins, that provide structural and biochemical support of surrounding cells.

Enzyme-coupled receptors: Transmembrane proteins receptors that contain either intrinsic enzyme activity on their intracellular domain, or associate directly with an intracellular enzyme

G–protein coupled receptors (GPCRs): Membrane bound receptors that share a similar structure with an extracellular N-terminus followed by seven transmembrane spanning alpha helices. Connecting the alpha helices are either extracellular or intracellular loops. The C terminus of GPCRs is found in the cytosol of the cell.

Guanine nucleotide exchange factors (GEFs): Proteins that exchange GDP for GTP.

GTPase: An enzyme that hydrolyzes GTP into GDP.

GTPase-activating enzymes (GAPs): Proteins that enhance intrinsic GTPase activity.

Guanine dissociation inhibitors (GDIs): Proteins that block the GTPase cycle by binding to the GDP bound form of the protein and preventing exchange of GDP for GTP.

Heterotrimeric G protein: A protein that consists of three subunits: alpha (α), beta (β), and gamma (γ), and that is associated with the inner (cytoplasmic) leaflet of the plasma membrane and interacts with GPCRs.

Hormone: A regulatory substance produced in an organism and transported in tissue fluids to stimulate specific cells or tissues.

Ions: Atoms or molecules with a net electric change (positive or negative).

Ion-channel-coupled receptors (ICCRs): Ion channels which are gated by a ligand binding to a receptor that is physically coupled to an ion channel.

Interkingdom signaling: Signaling communication between microbes and their hosts.

Intracellular signaling pathways: The signal relays that take place within a cell.

Intracrine: Signaling that occurs when a cell creates a signal which is never secreted that then impacts that same cell’s behavior.

Integration: More than one intracellular signaling pathway that is required to change cell behavior.

Intrinsic: Referring to enzymatic activity that originates within a protein.

Juxtacrine (contact dependent): Signaling that occurs when the signal molecule is attached to the cell, and that signal molecule then binds to a ligand attached to another cell.

Kinases: Enzymes that phosphorylate substrates.

Ligand: Molecule that binds to another (larger) molecule.

Ligand-gated ion channels (LGICs): Ion channels which are opened (gated) by binding of a ligand.

Lipophilic: Easily combined with or dissolvable in lipids and fats.

Lipopolysaccharide (LPS): A molecule found on the outer-membrane of Gram negative bacteria. Composed of O-antigen, lipid, and a polysaccharide.

Model organism: A model organism is a non-human species that is extensively studied to understand particular biological phenomena, with the expectation that discoveries made in the model organism will provide insight into the workings of other organisms.

Molecular switch: Molecules that can switch between two states, both of which are stable. One state is generally considered ‘on’, or active, and the other state is considered ‘off’, or in active.

Neuronal: Signaling that uses electrochemical signals between neurons and other cells.

Neurotransmitter: An endogenous chemical that enable neurotransmission. It is a type of chemical messenger which transmits signals across a chemical synapse from one neuron (nerve cell) to another “target” neuron, muscle cell, or gland cell.

p21-activated kinase (PAK): PAK proteins are serine/threonine kinases that regulate the cytoskeletal component actin.

Paracrine: Signaling that occurs when a cell secretes a signal, which then binds to a receptor on a different cell that is in close proximity to the cell from which the signal was created.

Post translational modification: The covalent modification of a protein after it has been synthesized. Typically, functional groups are added enzymatically to proteins to create post-translational modifications (PTMs).

Protein Kinase A (PKA): Protein family of enzymes dependent on cAMP for activity.

Protomer: The structural unit of an oligomeric protein.

Receptor: Protein which binds to ligands to initiate a cellular signaling pathway.

Receptor tyrosine kinases (RTKs): Enzyme coupled receptors with intrinsic tyrosine kinase activity on the intracellular domain.

Relay: Passing of a signal from protein to protein within an intracellular signaling pathway.

Second messengers: Small, non-protein molecules that relay the initial signal transduction within in the cell.

Signal transduction pathway: A set of molecular reactions initiated by a ligand binding to a receptor protein that will impact cellular behavior.

Single amino acid polymorphism: A substitution of a single nucleotide that occurs at a specific position in a genome.

Transduction: The process of converting one type of message into another.

Trichinosis: A parasitic disease caused by roundworms of the *Trichinella* type. Minor infection may be without symptoms. Complications may include inflammation of heart muscle, central nervous system involvement, and inflammation of the lungs.

Up-regulation: Process by which a cell increases the quantity of a cellular component.

Voltage-gated ion channels: Ion channels that are gated by a change in the membrane potential.

Wiskott-Aldrich syndrome: A syndrome is characterized by a low platelet count (thrombocytopenia), bruising, bloody diarrhea and spontaneous nose bleeds due to a mutation resulting in a dysfunctional protein (WASP).

Study Questions

1. Consider **Figure 7**. Identify the following:
    a. The ligand
    b. The receptor protein
    c. Signal distribution
    d. Signal integration
    e. Signal amplification
    f. Second messenger
    g. Cellular response

2. A cell secretes a signal, which binds to protein receptors located on both neighboring cells and the cell which originally secreted this signal. Choose the type of signaling from the following:
    a. Endocrine
    b. Juxtacrine and paracrine
    c. Paracrine and autocrine
    d. Autocrine
    e. Intracrine

3. G-Protein Coupled Receptors (GPCR) associate with which of the following proteins?
    a. Heterotrimeric G-protein
    b. cAMP
    c. GTP
    d. Effector proteins

4. Consider a heterotrimeric G-Protein. Which of the following is true? There may be more than one correct answer.
    a. It is composed of α, β and γ subunits.
    b. It is activated by a GPCR
    c. The γ subunit has intrinsic GTPase activity
    d. It is active when all subunits are bound together.

5. Which of the following are characteristic of Ras?There may be more than one correct answer.
    a. Monomeric GTPase
    b. Molecular switch
    c. Seven-pass transmembrane
    d. Has a β/γ subunit

6. Consider a cell that has a mutation such that all GEFs are nonfunctional. What is the fate of Ras proteins in this cell?
    a. Ras proteins are always bound to GTP

b. Ras protein are always bound to GDP
c. Ras proteins remain in an apo state, unable to bind to GTP nor GDP

7. In response to cAMP, *Dictyostelium discoideum* cells will undergo which of the following scenarios?
    a. Chemotaxis
    b. Spore dispersal
    c. Phagocytosis
    d. Apoptosis

8. When *Dictyostelium discoideum* senses LPS (Lipopolysaccharide) from bacteria, it will undergo intracellular signaling which results in phagocytosis of the bacterium. Is this a fast or a slow response? Explain your answer in one sentence.

9. Classify STAT receptor proteins as one of the following:
    a. GPCR
    b. Receptor tyrosine kinase
    c. Intracellular receptor and nuclear translocator
    d. Transmembrane protein
    e. Kinase

10. Which of the following removes a phosphate functional group?
    a. Kinase
    b. Ion channel
    c. Phosphatase
    d. GTPase
    e. SUMO

11. Which of the following ion-channel-coupled receptors are gated by changes in membrane potential?
    a. Ligand-gated ion channel (LGIC)
    b. Ion channel coupled receptors (ICCR)
    c. Voltage-gated ion channel
    d. GPCR

12. Which of the following steps occurs after ligand binding and dimerization of a receptor tyrosine kinase?
    a. The extracellular domain is phosphorylated by the dimer partner
    b. The intracellular domain is phosphorylated by the dimer partner
    c. Phosphate groups are removed from the extracellular domain by the dimer partner

d. Phosphate groups are removed from the intracellular domain by the dimer partner

13. Consider **Figure 18**. Identify the ligand and receptor protein that start the intracellular signaling pathway.

14. Consider **Figure 18**. Identify both the fast and the slow response.

15. T cells control PKA activity by AKAP proteins. How does *Dictyostelium* control PKA activity?

Study Questions Key

1. Consider **Figure 7**. Identify the following:
    a. The ligand: cAMP
    b. The receptor protein: cAR1
    c. Signal distribution: α subunit activating PLC, or GCase. β/γ subunit activating PI3 Kinase.
    d. α subunit activating PLC, or GCase. β/γ subunit activating PI3 Kinase.
    e. Signal integration: Ca^{2+}, cGMP, and PI3 Kinase impacting chemotaxis.
    f. Signal amplification: PLC creating IP3, GCase creating cGMP
    g. Second messenger: IP3 and Ca^{2+}
    h. Cellular response: Chemotaxis

2. A cell secretes a signal, which binds to protein receptors located on both neighboring cells and the cell which originally secreted this signal. Classify the type of cell signaling.
    Paracrine and autocrine

3. G-Protein Coupled Receptors (GPCR) associate with which of the following proteins?
    Heterotrimeric G-protein

4. Consider a heterotrimeric G-Protein. Which of the following is true?
    It is composed of α, β and γ subunits.
    It is activated by a GPCR

5. Which of the following are characteristic of Ras?
    Monomeric GTPase
    Molecular switch

6. Consider a cell that has a mutation such that all GEFs are nonfunctional. What is the fate of Ras proteins in this cell?
    Ras protein are always bound to GDP

7. In response to cAMP, *Dictyostelium discoideum* cells will undergo which of the following scenarios?
    Chemotaxis

8. When *Dictyostelium discoideum* senses LPS (Lipopolysaccharide) from bacteria, it will undergo intracellular signaling which results in phagocytosis of the bacterium. Is this a fast or a slow response? Explain your answer in one sentence.
    This is a fast response because the effector proteins exist in the cell and are not made *de novo*.

9. Classify STAT receptor proteins as one of the following:
   Intracellular receptor and nuclear translocator

10. Which of the following removes a phosphate functional group?
    Phosphatase

11. Which of the following ion-channel-coupled receptors are gated by changes in membrane potential?
    Voltage-gated ion channel

12. Which of the following steps occurs after ligand binding and dimerization of a receptor tyrosine kinase?
    The intracellular domain is phosphorylated by the dimer partner

13. Consider figure 18. Identify the ligand and receptor protein that start the intracellular signaling pathway.
    Ligand: ATP
    Receptor protein: P2X channel

14. Consider figure 18. Identify both the fast and the slow response.
    Fast response: Ca^{2+} activating pre-existing proteins which open the water channel and allow the efflux of water ions.
    Slow response: Ca^{2+} activating pre-existing proteins which translocate to the nucleus to act as transcription factors to transcribe then translate a new effector protein.

15. T cells control PKA activity by AKAP proteins. How does *Dictyostelium* control PKA activity?
    PKA bound with cAMP inhibits ERK2. When ERK2 is inhibited, RegA is active. RegA is a phosphodiesterase that cleaves cAMP into AMP. This lowers the level of cAMP and will ultimately inhibit PKA activity.

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