CHAPTER 13

13 CELL SIGNALING

13.1 Introduction

a) Definition

Cell signaling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis.

b) General Importance of Signaling

i) Regulation of developmental processes during embryogenesis e.g. regulation of hair growth, cardiac development somitogenesis (somites are clusters of mesodermal cells that give rise to spinal nerves, striated muscles, blood tissues etc), haematopoiesis
ii) Required in self renewing systems of adult animals e.g. renewal of intestine topology villi (fingerlike protrusion in intestine, invagination into submucosa (known as crypts of Lieberkühn. Stem cells and proliferating transiently amplifying cells are found in crypt, and terminally differentiated cells are found in villi. Notch signaling in instestine results into conversion of undifferentiated proliferating cells crypt into post-mitotic musus-secreting goblet cells.
iii) Notch signaling result to gain in cell function e.g. forced notch signaling into intestine cells leads to undifferentiated cells.
iv) Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes. E.g. Failure to regulate Notch signaling precisely during T-cell development can lead to T-cell leukemia. Understanding cell signaling will enable effective treatment of these type of diseases.
v) Exchange of signals between early embryo cells and the cells of the uterus enable development.
vi) In the human gastrointestinal tract, bacteria exchange signals with each other and with human epithelial and immune system cells.
vii) For the yeast *Saccharomyces cerevisiae* during mating, some cells send a peptide signal (mating factor *pheromones*) into their environment. The mating factor peptide may bind to a cell surface receptor on other yeast cells and induce them to prepare for mating.

13.2 Unicellular and multicellular organism cell signaling

Cell signaling occurs between cells of a single organism and between the cells of two different organisms. Various types of types of signaling include;

13.2.1 Notch-mediated juxtacrine signal (also known as contact dependent signaling)

a) Properties Notch signaling

i) The **Notch signaling pathway** occurs between adjacent cells.
ii) It is a cell-to-cell communication and thus requires direct cell-cell contact.

iii) It is present in most **multicellular organisms**. Notch is present in all metazoans, and vertebrates

iv)Possess four different notch receptors, referred to as **Notch1 to Notch4**. The Notch receptor is a **single-pass transmembrane receptor protein**. It is a hetero-oligomer composed of a large extracellular portion, which associates in a calcium-dependent, non-covalent interaction with a smaller piece of the Notch protein composed of a **short extracellular region**, **a single transmembrane-pass**, and **a small intracellular region**.

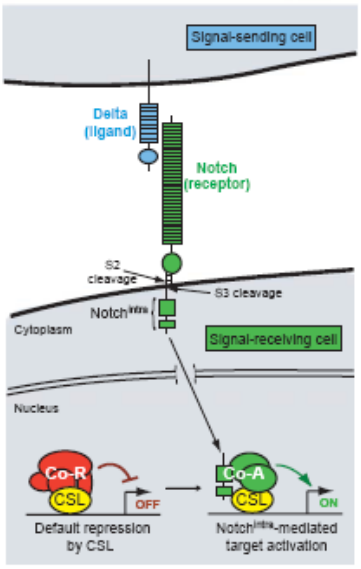


Figure 1. Basic operation of the Notch pathway. The key players are a Delta-type ligand, the receptor Notch and the CSL transcription factor (see Table 1). Delta and Notch are transmembrane proteins containing extracellular arrays of Epidermal Growth Factor (EGF) repeats (depicted by rectangles). Activation of Notch by its ligand triggers two proteolytic cleavages of Notch (S2 and S3). S3 cleavage releases the Notch intracellular domain ($Notch^{intra}$), which translocates to the nucleus. $Notch^{intra}$ activates CSL. The CSL co-repressor complex is displaced by a co-activator complex containing $Notch^{intra}$ (Co-A, green icons), which mediates Notch target gene activation. In the absence of nuclear $Notch^{intra}$, CSL associates with a co-repressor complex (Co-R, red icons), which actively represses the transcription of Notch target genes.

- DSL -Delta/Serrate/LAG-2
- tumor necrosis factor-α (alpha) converting enzyme (TACE)
- Notch intercellular domain (NICD)
- **S2 and S3 –secretases.** are enzymes that "snip" pieces off a longer protein that is embedded in the cell membrane.
- γ-secretase plays a critical role in developmental signaling by the transmembrane receptor Notch, freeing the cytoplasmic tail of Notch to travel to the cell nucleus to act as a transcription factor.

Names of core components of Notch signaling (ligand, receptor and transcription factor) in different species

Core component	*C. elegans*	*D. melanogaster*	Mammals
Ligand	LAG-2	Delta	Delta-like1 (DLL1)
	APX-1	Serrate	Delta-like2 (DLL2)
	ARG-2		Delta-like3 (DLL3)
	F16B12.2		Jagged 1 (JAG1)
			Jagged 2 (JAG2)
Receptor (Notch)	LIN-12	Notch	Notch1
	GLP-1		Notch2
			Notch3
			Notch4

Transcription factor CBF1/Su(H)/LAG1 (CSL)	LAG-1	Suppressor of Hairless [Su(H)]	CBF1/RBPJ□ RBPL

Mechanism of Notch Signaling

Following the suggestion that Notch is cleaved during Notch signaling in the early 1990s, the search for the 'Notch-ase' was on. Notch proteolysis turned out to be more complicated than anticipated, and involves successive cleavage events termed S1, S2 and S3 (Fig. 1, note that S1 is not shown) (reviewed by Fortini, 2002). Vertebrate Notch is constitutively cleaved in the Golgi complex (S1) by a furin convertase and is reassembled into a functional heterodimeric receptor at the cell surface, although the evidence for similar processing of invertebrate Notch is equivocal. Physical interactions between specific EGF repeats of the ligand and Notch then trigger the second cleavage of Notch (S2), which releases the majority of the extracellular domain (see Fig. 1). This is mediated by the metalloproteases TACE in vertebrates, and possibly Kuzbanian/SUP-17 in invertebrates. Truncated Notch is then a substrate for □-secretase, a multicomponent complex that cleaves Notch within its transmembrane domain (S3) and releases its intracellular domain ($Notch^{intra}$) (Fig. 1). Functional □-secretase has four principal transmembrane components: presenilin, nicastrin, Aph1 and Pen2 (reviewed by De Strooper, 2003). Presenilin is a putative aspartyl protease whose dysfunction also underlies the abnormal cleavage of the transmembrane □-amyloid precursor protein in Alzheimer's disease.

b) Functions / Importance of Notch signaling

i) Direct contact allows for very precise control of cell differentiation during embryonic development. e.g. in the worm *Caenorhabditis elegans*, two cells of the developing gonad each have an equal chance of terminally differentiating or becoming a uterine precursor cell that continues to divide.
ii) The choice of which cell continues to divide is controlled by competition of cell surface signals. One cell will happen to produce more of a cell surface protein that activates the Notch receptor on the adjacent cell. This activates a feedback loop or system that reduces Notch expression in the cell that will differentiate and increases Notch on the surface of the cell that continues as a stem cell.
iii) neuronal function and development
iv) stabilizing arterial endothelial fate and angiogenesis(growth of blood vessels)
v) regulating crucial cell communication events between endocardium and myocardium during both the formation of the valve primordial and ventricular development and differentiation
vi) cardiac valve homeostasis as well as implications in other human disorders involving the cardiovascular system.
vii) timely cell lineage specification of both endocrine and exocrine pancreas lineages in the gut
viii) expanding the hematopoietic stem cells (HSC) compartment during bone development and participation in commitment to the osteoblastic lineage, suggesting a potential therapeutic role for Notch in bone regeneration and osteoporosis.
ix) regulating cell-fate decision in mammary gland at several distinct development stages possibly some non-nuclear mechanisms, such as controlling the actin cytoskeleton through the tyrosine kinase Abl.

Gap junctions : Some cells can form gap junctions that connect their cytoplasm to the cytoplasm of adjacent cells. e.g. in cardiac muscle, gap junctions between adjacent cells allows for action potential

propagation from the cardiac pacemaker region of the heart to spread and coordinately cause contraction of the heart.

A gap junction or nexus is a specialized intercellular connection between certain animal cell-types. It directly connects the cytoplasm of two cells, which allows various molecules and ions to pass freely between cells.

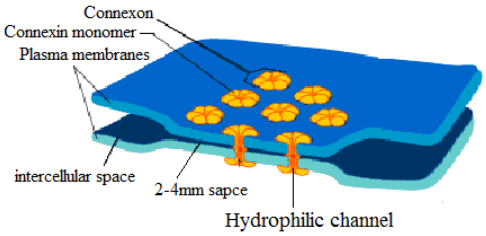


Figure 2
One gap junction is composed of two connexons (or hemichannels) which connect across the intercellular space. Gap junctions are analogous to the plasmodesmata that join plant cells. A notable use of gap junctions is in the electrical synapse found in some neurons.

13.2.2. Distant cell communication

i) Autocrine signaling Type of cell signaling in which a cell secretes signal molecules that act on itself or on other adjacent cells of the same type. Autocrine signaling occurs among the same cell,

ii) Paracrine signaling -short-range cell-cell communication via secreted signal molecules that act on adjacent cells. Uses local mediator which are secreted signal molecule that acts at short range on adjacent cells (same type or different types). *Paracrine* signals target only cells in the vicinity of the emitting cell e.g. Neurotransmitters. Paracrine signaling is a form of cell signaling in which the target cell is near ("para" = near) the signal-releasing cell.

Some signaling molecules degrade very quickly, limiting the scope of their effectiveness to the immediate surroundings. Others affect only nearby cells because they are taken up quickly, leaving few to travel further, or because their movement is hindered by the extracellular-matrix.

NB: Examples

- Growth factor and clotting factors are paracrine signaling agents. The local action of growth factor signaling plays an especially important role in the development of tissues. In insects, Allatostatin controls growth though paracrine action on the corpora allata.
- In mature organisms, paracrine signaling is involved in responses to allergens, tissue repair, the formation of scar tissue, and blood clotting.
- The overproduction of certain paracrine growth factors has been implicated in the pathology of cancer.
- Somatostatin and histamine are paracrine agents

iii) Hormone - general term for any extracellular substance that induces specific responses in target cells. Hormones coordinate the growth, differentiation, and metabolic activities of various cells, tissues, and organs in multicellular organisms. ***Endocrine* signals are called hormones**. Hormones are produced by endocrine cells and they travel through the blood to reach all parts of the body. Specificity of signaling can be controlled if only some cells can respond to a particular hormone.

NB: Some signaling molecules can function as both a **hormone and a neurotransmitter**. For example, epinephrine and norepinephrine can function as hormones when released from the adrenal gland and are transported to the heart by way of the blood stream. Norepinephrine can also be produced by neurons to function as a neurotransmitter within the brain. Estrogen can be released by the ovary and function as a hormone or act locally via paracrine or autocrine signaling.

13.2.3 Receptors for cell signals

Cells receive information from their environment through a class of proteins known as **receptors**. Notch is a cell surface protein that functions as a receptor. Animals have a small set of genes that code for signaling proteins that interact specifically with Notch receptors and stimulate a response in cells that express Notch on their surface. Molecules that activate (or, in some cases, inhibit) receptors can be classified as hormones, neurotransmitters, cytokines, growth factors but all of these are called receptor ligands. The details of ligand-receptor interactions are fundamental to cell signaling. As shown in Figure 1 (above, left), Notch acts as a receptor for ligands that are expressed on adjacent cells. While many receptors are cell surface proteins, some are found inside cells. For example, estrogen is a hydrophobic molecule that can pass through the lipid bilayer of cell surface membranes. Estrogen receptors inside cells of the uterus can be activated by estrogen that comes from the ovaries, enters the target cells, and binds to estrogen receptors.

Other signaling molecules are unable to permeate the hydrophobic cell membrane due to their hydrophilic nature, so their target receptor is expressed on the membrane. When such signaling molecule activates its receptor, the signal is carried into the cell usually by means of a second messenger such as cAMP.

Signaling pathways

a) Chromatin Modification

- Protein Acetylation
- Histone Methylation
- Histone Modification Table
- MAP Kinase Signaling

b) Mitogen-Activated Protein Kinase Cascades

- MAPK/Erk in Growth and Differentiation
- G-Protein-Coupled Receptors Signaling to MAPK/Erk
- SAPK/JNK Signaling Cascades
- Signaling Pathways Activating p38 MAPK
- Apoptosis

c) Apoptosis

- Inhibition of Apoptosis
- Death Receptor Signaling
- Mitochondrial Control of Apoptosis
- Autophagy Signaling
- Akt Signaling

d) Akt/PKB Signaling

- Akt Binding Partners Table
- Akt Substrates Table

e) Energy Metabolism

- Insulin Receptor Signaling
- AMPK Signaling

g) Translational Control

- Translational Control
- Translational Control: Regulation of eIF2
- Translational Control: Regulation of eIF4E and p70 S6 Kinase
- mTOR Signaling - new

h) Cell Cycle Control

- Cell Cycle Control: G1/S Checkpoint
- Cell Cycle Control: G2/M DNA Damage Checkpoint

i) Immunology

- Jak/Stat Signaling: IL-6 Receptor Family
- Jak/STAT Utilization Table
- NF-κB Signaling
- TLR Pathway
- B Cell Receptor Signaling
- T Cell Receptor Signaling

j) Development

- Wnt/β-Catenin Signaling
- Notch Signaling
- Hedgehog Signaling In Vertebrates
- TGF-β Signaling

k) Cytoskeletal Signaling / Adhesion

- Regulation of Actin Dynamics
- Regulation of Microtubule Dynamics
- Adherens Junction Dynamics

l) Other

- ErbB/HER Signaling
- Angiogenesis
- Ubiquitin/Proteasome

Signal transduction pathways.

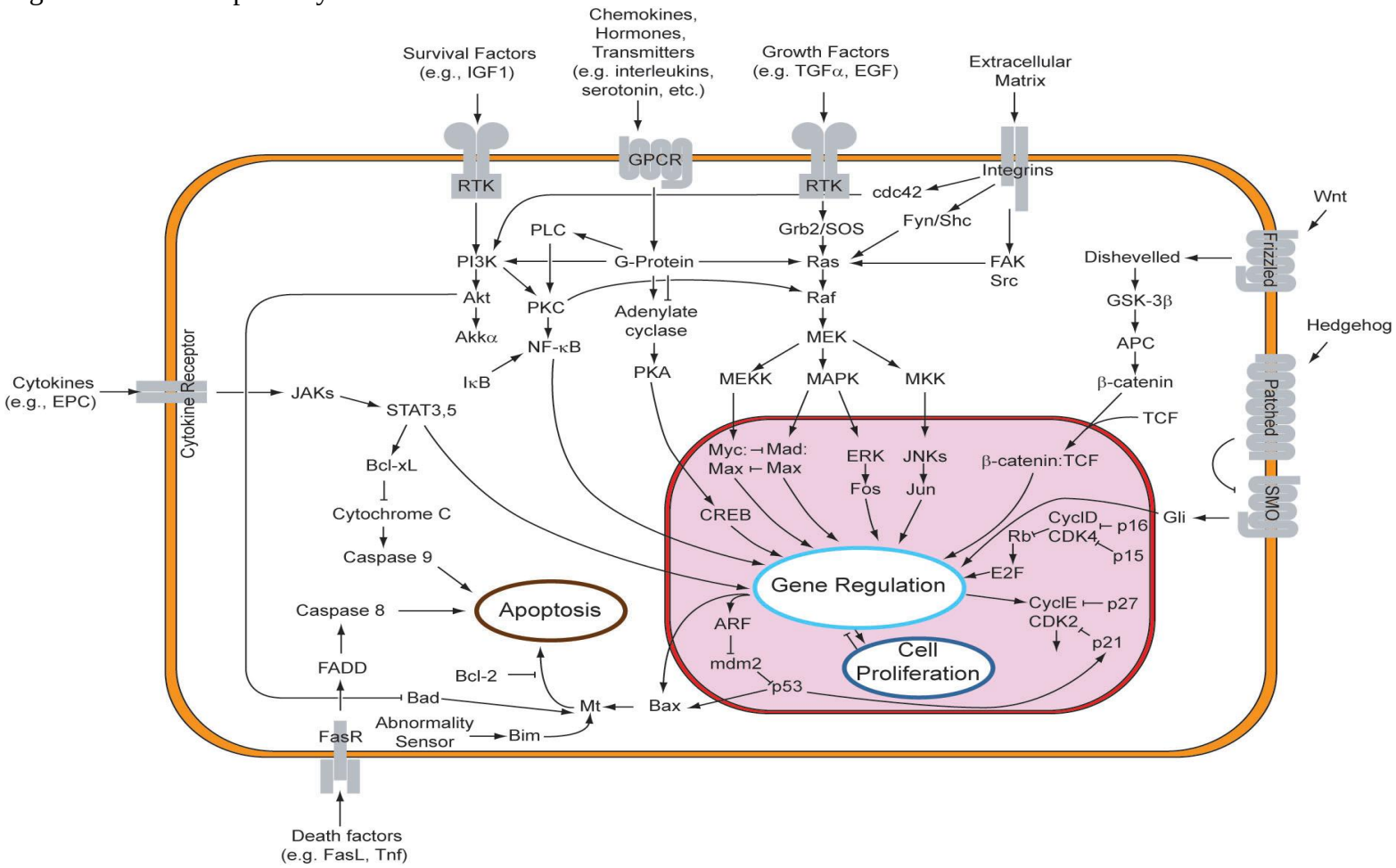


Figure 3. Mitogen-activated protein kinase / extracellular signal-regulated kinases (MAPK/ERK) pathway

*The **MAPK/ERK pathway** is a signal transduction pathway that couples intracellular responses to the binding of growth factors to cell surface receptors. This pathway is very complex and includes many protein components. The basic pathway shown in the figure (below) includes the major components of the pathway. In many cell types, activation of this pathway promotes cell division.*

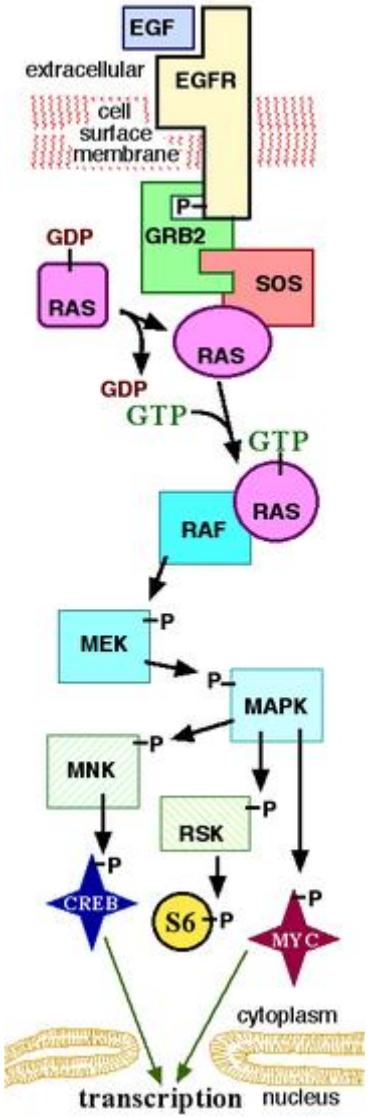


Figure 4.

Figure above is a diagram showing key components of a signal transduction pathway as extracted from MAPK/ERK pathway. In some cases, receptor activation caused by ligand binding to a receptor is directly coupled to the cell's response to the ligand. For example, the neurotransmitter GABA can activate a cell surface receptor that is part of an ion channel. GABA binding to a GABA A receptor on a neuron opens a chloride-selective ion channel that is part of the receptor. GABA A receptor activation allows negatively charged chloride ions to move into the neuron which inhibits the ability of

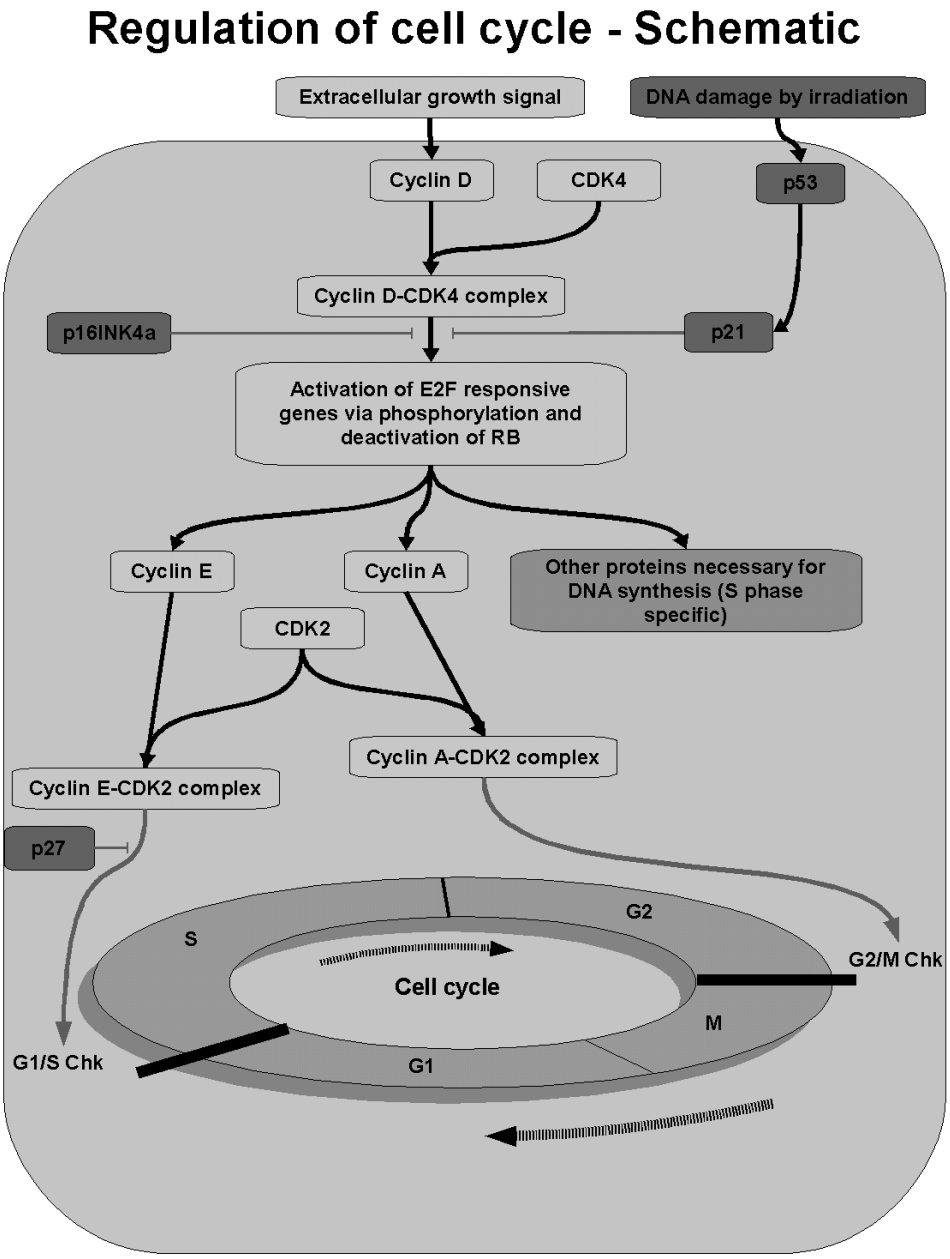
the neuron to produce action potentials. However, for many cell surface receptors, ligand-receptor interactions are not directly linked to the cell's response. The activated receptor must first interact with other proteins inside the cell before the ultimate physiological effect of the ligand on the cell's behavior is produced. Often, the behavior of a chain of several interacting cell proteins is altered following receptor activation. The entire set of cell changes induced by receptor activation is called a signal transduction mechanism or pathway.

In the case of Notch-mediated signaling, the signal transduction mechanism can be relatively simple. As shown in Figure above activation of Notch can cause the Notch protein to be altered by a protease. Part of the Notch protein is released from the cell surface membrane and can act to change the pattern of gene transcription in the cell nucleus. This causes the responding cell to make different proteins, resulting in an altered pattern of cell behavior. Cell signaling research involves studying the spatial and temporal dynamics of both receptors and the components of signaling pathways that are activated by receptors in various cell types.

A more complex signal transduction pathway is shown in Figure 4. This pathway involves changes of protein-protein interactions inside the cell induced by an external signal. Many growth factors bind to receptors at the cell surface and stimulate cells to progress through the cell cycle and divide. Several of these receptors are kinases that start to phosphorylate themselves and other proteins when binding to a ligand. This phosphorylation can generate a binding site for a different protein and thus induce protein - protein interaction. In Figure 4, the ligand (called epidermal growth factor (EGF)) binds to the receptor (called epidermal growth factor receptors EGFR). This activates the receptor to phosphorylate itself. The phosphorylated receptor binds to an adaptor protein (GRB2) which couples the signal to further downstream signaling processes. For example, one of the signal transduction pathways that is activated is called the mitogen-activated protein kinase (MAPK) pathway. The signal transduction component labeled as "MAPK" in the pathway was originally called "ERK" so the pathway is called the MAPK/ERK pathway. The MAPK protein is an enzyme, a protein kinase that can attach phosphate to target proteins such as the transcription factor MYC and thus alter gene transcription and, ultimately, cell cycle progression. Many cellular proteins are activated downstream of the growth factor receptors (such as EGFR) that initiate this signal transduction pathway.

Some signaling transduction pathways respond differently depending on the amount of signaling received by the cell. For instance the hedgehog protein activates different genes depending on the amount of hedgehog protein present.

Complex multi-component signal transduction pathways provide opportunities for feedback, signal amplification, and interactions inside one cell between multiple signals and signaling pathways.


Regulation of cell cycle - Schematic
Extracellular growth signal
DNA damage by irradiation
Cyclin D
CDK4
p53
Cyclin D-CDK4 complex
p16INK4a
p21
Activation of E2F responsive genes via phosphorylation and deactivation of RB
Cyclin E
Cyclin A
Other proteins necessary for DNA synthesis (S phase specific)
CDK2
Cyclin A-CDK2 complex
Cyclin E-CDK2 complex
p27
G2
S
G2/M Chk
Cell cycle
M
G1/S Chk
G1
CDK – Cyclin Dependent Kinase
G1/S Chk – G1/S checkpoint
G2/M Chk – G2/M checkpoint

Regulation of cell cycle

Regulation of the cell cycle involves steps crucial to the cell, including detecting and repairing genetic damage, and provision of various checks to prevent uncontrolled cell division. The molecular events that control the cell cycle are ordered and directional and impossible to "reverse" the cycle.

Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), determine a cell's progress through the cell cycle. Molecules are coded by genes e.g. in *Saccharomyces cerevisiae* these genes are *cdc* (cell division cycle) genes. Identifying number is used where there many such genes e.g., *cdc25*.

Cyclins form the regulatory subunits and CDKs the catalytic subunits of an activated heterodimer. Cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin. When activated by a bound cyclin, CDKs perform a common biochemical reaction called phosphorylation that activates or inactivates target proteins to orchestrate coordinated entry into the next phase of the cell cycle. Different cyclin-CDK combinations determine the downstream proteins targeted. CDKs are constitutively expressed in cells whereas cyclins are synthesised at specific stages of the cell cycle, in response to various molecular signals.

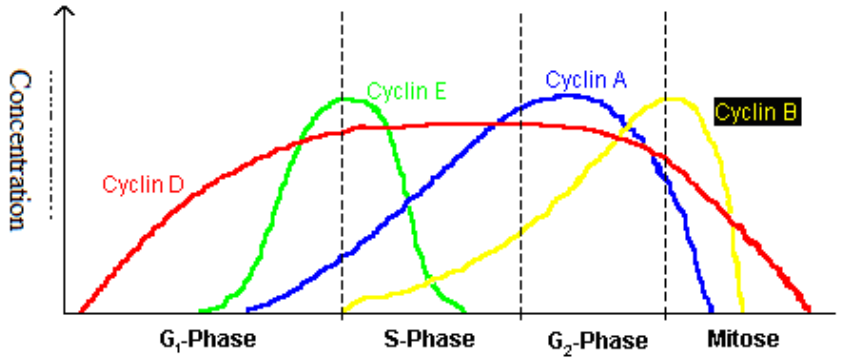
General mechanism of cyclin-CDK interaction

i) Upon receiving a pro-mitotic extracellular signal, G_1 cyclin-CDK complexes become active to prepare the cell for S phase, promoting the expression of transcription factors that in turn promote the expression of S cyclins and of enzymes required for DNA replication.
ii) The G_1 cyclin-CDK complexes also promote the degradation of molecules that function as S phase inhibitors by targeting them for ubiquitination.
iii) Once a protein has been ubiquitinated (every where), it is targeted for proteolytic degradation by the proteasome.
iv) Active S cyclin-CDK complexes phosphorylate proteins that make up the pre-replication complexes assembled during G_1 phase on DNA replication origins.
v) The phosphorylation serves two purposes: to activate each already-assembled pre-replication complex, and to prevent new complexes from forming.
vi) This ensures that every portion of the cell's genome will be replicated once and only once. The reason for prevention of gaps in replication is fairly clear, because daughter cells that are missing all or part of crucial genes will die. However, for reasons related to gene copy number effects, possession of extra copies of certain genes would also prove deleterious to the daughter cells.

Mitotic cyclin-CDK complexes, which are synthesized but inactivated during S and G_2 phases, promote the initiation of mitosis by stimulating downstream proteins involved in chromosome condensation and mitotic spindle assembly. A critical complex activated during this process is a ubiquitin ligase known as the anaphase-promoting complex (APC), which promotes degradation of structural proteins associated with the chromosomal kinetochore. APC also targets the mitotic cyclins for degradation, ensuring that telophase and cytokinesis can proceed.

Specific action of cyclin-CDK complexes

i) Cyclin D is the first cyclin produced in the cell cycle, in response to extracellular signals (eg. growth factors).
ii) Cyclin D binds to existing CDK4, forming the active cyclin D-CDK4 complex.
iii) Cyclin D-CDK4 complex in turn phosphorylates the retinoblastoma susceptibility protein (RB).
iv) The hyperphosphorylated RB dissociates from the E2F/DP1/RB complex (which was bound to the E2F responsive genes, effectively "blocking" them from transcription), activating E2F.
v) Activation of E2F results in transcription of various genes like cyclin E, cyclin A, DNA polymerase, thymidine kinase, etc.
vi) Cyclin E thus produced binds to CDK2, forming the cyclin E-CDK2 complex, which pushes the cell from G_1 to S phase (G_1/S transition).
vii) Cyclin B along with cdc2 forms the cyclin B-cdc2 complex, which initiates the G_2/M transition.
viii) Cyclin B-cdc2 complex activation causes breakdown of nuclear envelope and initiation of prophase, and subsequently, its deactivation causes the cell to exit mitosis.



Cell cycle inhibitors

Two families of genes, the *cip/kip* family and the INK4a/ARF (*In*hibitor of *K*inase 4/Alternative Reading Frame) prevent the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.

The ***cip/kip* family** includes the genes p21, p27 and p57. They halt cell cycle in G_1 phase, by binding to, and inactivating, cyclin-CDK complexes. p21 is activated by p53 (which, in turn, is triggered by DNA damage eg. due to radiation). p27 is activated by Transforming Growth Factor β (TGF β), a growth inhibitor.

The **INK4a/ARF family** includes p16INK4a, which binds to CDK4 and arrests the cell cycle in G_1 phase, and p14arf which prevents p53 degradation. And the amount of chromosomes are able to double at the same rate as in phase 2

Checkpoints

Cell cycle checkpoints are used by the cell to monitor and regulate the progress of the cell cycle. Checkpoints prevent cell cycle progression at specific points, allowing verification of necessary phase processes and repair of DNA damage. The cell cannot proceed to the next phase until checkpoint requirements have been met.

Several checkpoints are designed to ensure that damaged or incomplete DNA is not passed on to daughter cells. Two main checkpoints exist: the G1/S checkpoint and the G2/M checkpoint. G1/S transition is a rate-limiting step in the cell cycle and is also known as restriction point. An alternative model of the cell cycle response to DNA damage has also been proposed, known as the post replication checkpoint. p53 plays an important role in triggering the control mechanisms at both G1/S and G2/M checkpoints.

Role of cell cycle in tumor formation

A disregulation of the cell cycle components may lead to tumor formation. As mentioned above, some genes like the cell cycle inhibitors, RB, p53 etc., when they mutate, may cause the cell to multiply uncontrollably, forming a tumor. Although the duration of cell cycle in tumor cells is equal to or longer than that of normal cell cycle, the proportion of cells that are in active cell division (versus quiescent cells in G0 phase) in tumor cells are much more compared to that in normal cells. Thus there is a net increase in cell number as the number of cells that die by apoptosis or senescence remains the same.

The cells which are actively undergoing cell cycle are targeted in cancer therapy as the DNA is relatively exposed during cell division and hence susceptible to damage by drugs or radiation. This fact is made use of in cancer treatment; by a process known as debulking, a significant mass of the tumor is removed which pushes a significant number of the remaining tumor cells from G0 to G1 phase (due to increased availability of nutrients, oxygen, growth factors etc.). Radiation or chemotherapy following the debulking procedure kills these cells which have newly entered the cell cycle.

Synchronization of cell cultures

Several methods can be used to synchronise cell cultures by halting the cell cycle at a particular phase. For example, Serum starvation and treatment with Thymidine or Aphidicolin halt the cell in the G1 phase, Mitotic shake-off, treatment with colchicine and treatment with Nocodazole halt the cell in M phase and treatment with 5-fluorodeoxyuridine halts the cell in S phase.

Questions

1. Describe two main signals that control cell in G1 and G2.
2. Discuss the importance of cell signaling