Eukaryotic Gene Expression: Basics & Benefits

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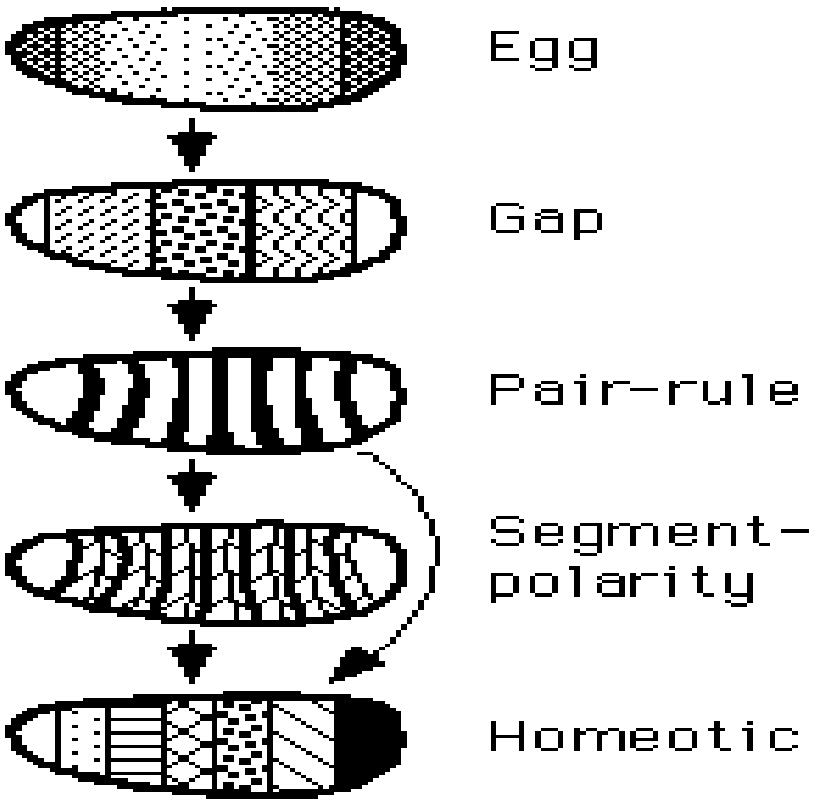
Lecture 25

Signal transduction pathways involved in embryonic development



Hedgehog is one of Drosophila's segment polarity gene products, involved in establishing the basis of the fly body plan.

The molecule is important during later stages of embryogenesis and metamorphosis as well.

Gap genes	Pair rule genes (8)	Segment polarity genes (16)
Hunchback	Runt	Engrailed
Kruppel	Hairy	Wingless
Knirps	Ftz	Goosgerry
Gaint	Evenskipped	Cubitus interruptus
tailless	Odd skipped	Patched
	Paired	Hedgehog
	Odd paired	Dishelved
	Sloppy paired	Costal2
		fused

Segment polarity genes are expressed in segments in which they affect anterior or posterior identification of the compartments

The expression of these genes are controlled by pair rule genes.

The products of segment polarity genes include secreted proteins, transmembrane proteins, kinases, cytoskeletal proteins as well as transcription factors.

Once the segment polarity gene expression is induced, cell-cell Interactions become very important

Embryogenesis is controlled by diverse signal transduction pathways

1) **TGFβ/BMP Serine/Threonine kinase receptors**
2) **Receptor Tyrosine kinases such as FGF, EGF, IGF, Insulin**
3) **Wnt**
4) **Sonic Hedgehog** 
5) **Notch**
6) **G protein-coupled receptors (7-transmembrane receptors)**
7) **Nuclear hormone receptors**

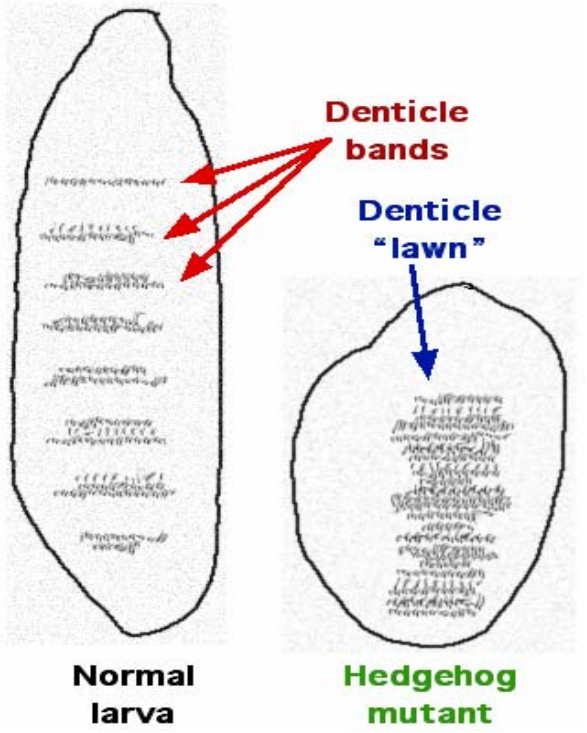
Through these signaling pathways, the same signals can trigger different types of cell differentiation responses in different embryonal cells thereby orchestrating diverse cell differentiation programs in the developing embryo.

The hedgehog family

Sonic hedgehog homolog (SHH)
Desert hedgehog (DHH)
Indian hedgehog (IHH).

SHH plays a key role in regulating vertebrate organogenesis, such as growth of digits on limbs and organization of the brain.

In fly mutants lacking hedgehog, the embryos are covered with denticles (small pointy projections), much like a hedgehog. The first two homologues of *hedgehog* were named after species of hedgehog and the third was named after a video game character (sega genesis)



Sonic hedgehog is a ‘morphogen’.

A morphogen is a molecule that diffuses to form a concentration gradient that differentially influences the cells of the developing embryo in a concentration-dependent manner

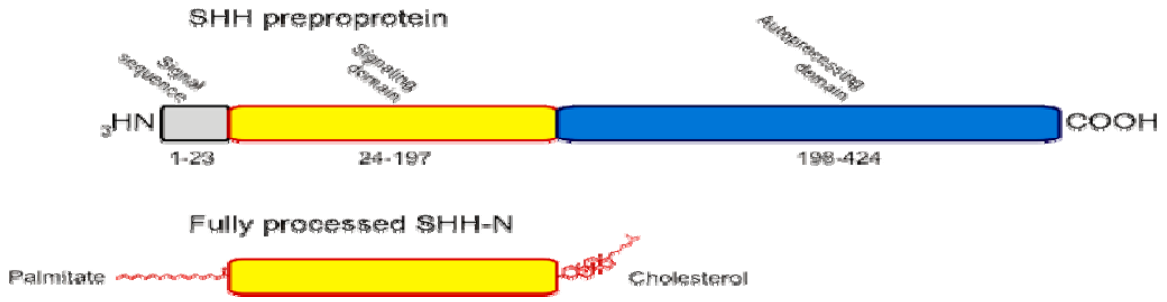
The hedgehog gene (*hh*) was first identified by Eric Wieschaus and Christiane Nusslein-Volhard in 1978.

Both were awarded the Nobel Prize in 1995 for the identification of genes that control the segmentation pattern of *Drosophila melanogaster* (fruit fly) embryos.

Mutations in the human sonic hedgehog gene, *SHH*, cause holoprosencephaly type 3 (HPE3) as a result of the loss of the ventral midline.

Sonic hedgehog is secreted by the zone of polarizing activity (ZPA), which is located on posterior side of a limb bud in an embryo.

The sonic hedgehog transcription pathway has also been linked to the formation of specific kinds of cancerous tumours.



Newly synthesised SHH is a 45 kDa protein. It is synthesized in the rough endoplasmic reticulum where it undergoes autoprocessing to generate a 20 kDa N-terminal signaling domain (SHH-N) and a 25 kDa C-terminal domain with no known signaling role.

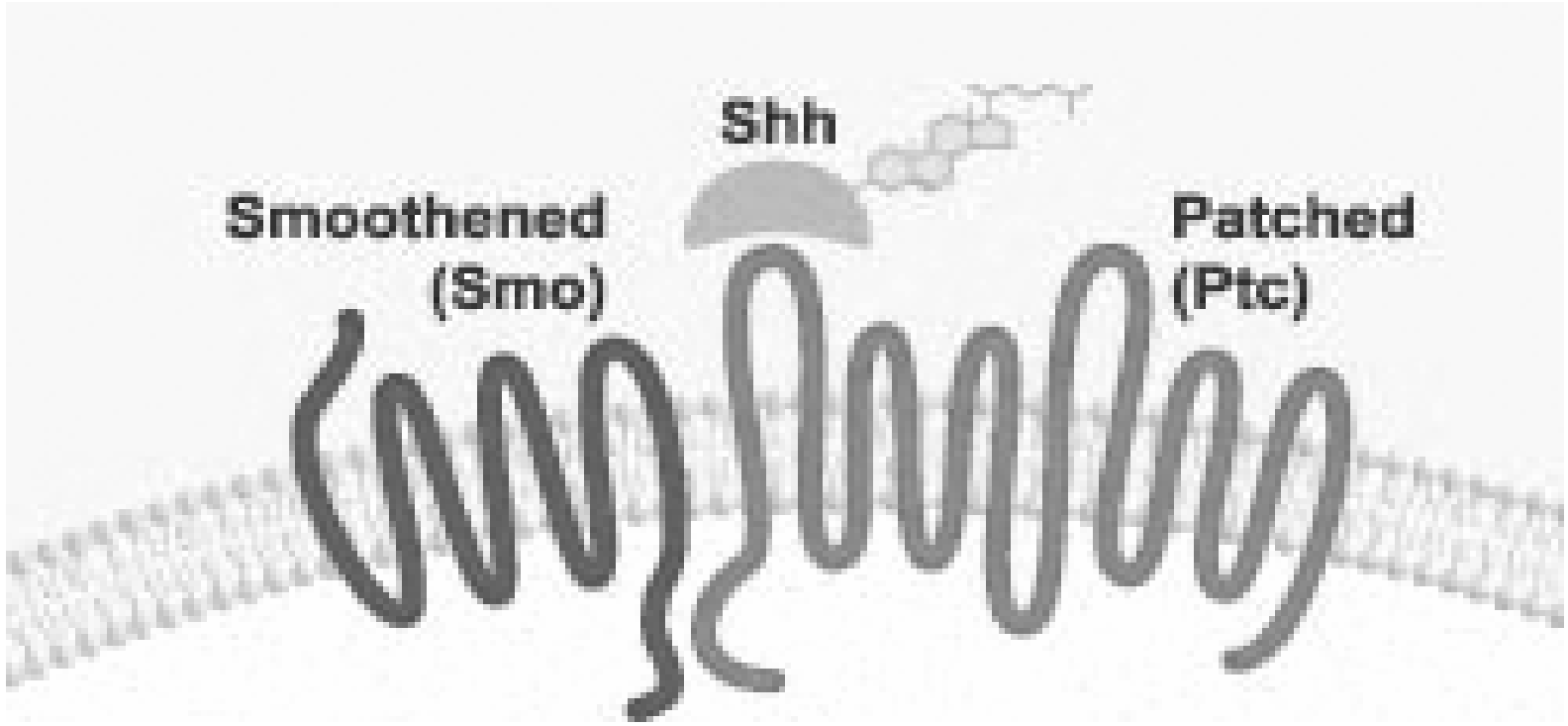
The cleavage is catalysed by a protease within the C-terminal domain.

During the reaction, a cholesterol molecule is added to the C-terminus of SHH-N.

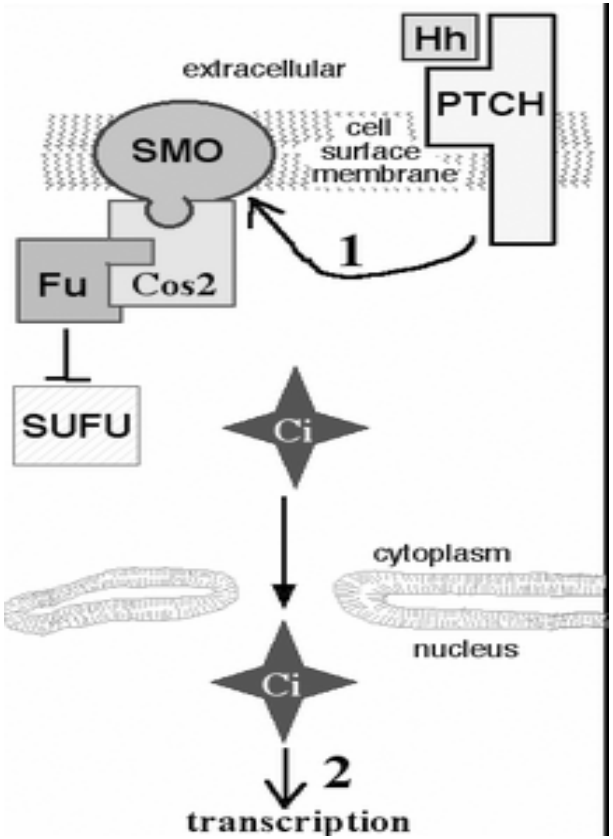
Thus the C-terminal domain acts as an intein and a cholesterol transferase.

Further, the N-terminal cysteine of SHH-N is palmitoylated and this modification is required for efficient signaling, resulting in 30-fold increase in potency over the non-palmitoylated form.

http://en.wikipedia.org/wiki/File:Shh_processing.png



The key players in Shh signalling are:
Two transmembrane proteins: Patched & Smoothened
A Transcriptoin factor: Ci/CiR



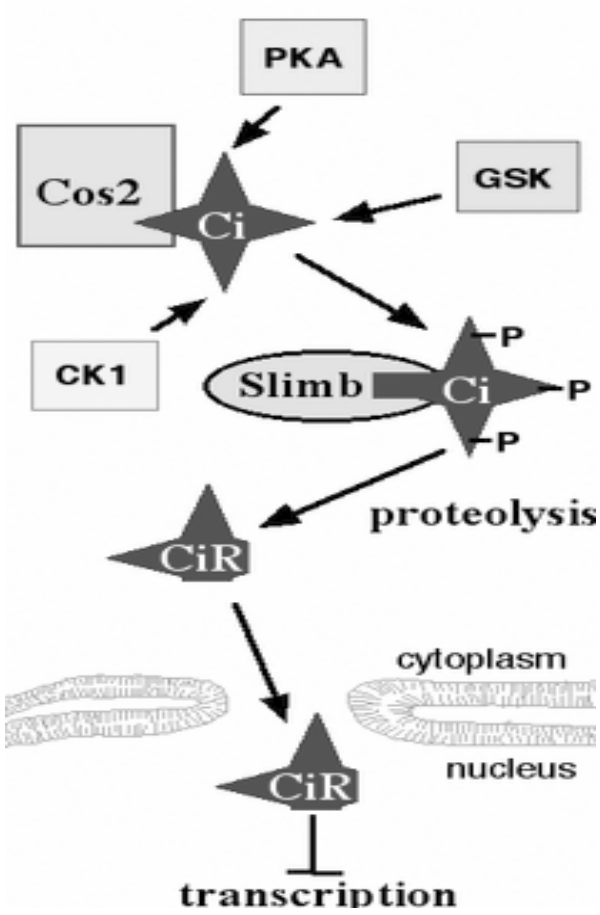
Shh interacts with a transmembrane protein known as Patched (PTCH)

In the absence of Hh, patched acts to prevent high expression and activity of the 7 membrane spanning receptor, Smoothened (SMO).

When extracellular Hh is present, it binds to and inhibits Patched, allowing Smoothened to accumulate and inhibit the proteolytic cleavage of a transcription factor known as the Cubitus interruptus (Ci).

Thus, when Hh is bound to PTCH, Ci protein is able to act as a transcription factor in the nucleus.

When Hh is not bound to Patched, Ci protein is proteolytically cleaved to produce a transcriptional repressor, CiR.



In the absence of Hh, Ci forms a complex with the kinesin- like protein Costal-2 (Cos2) which targets the Ci protein for proteosome-dependent cleavage leading to the generation of a fragment (CiR) that functions as a transcriptional repressor.

CiR enters the nucleus, where it acts as a co-repressor for Hh target genes.

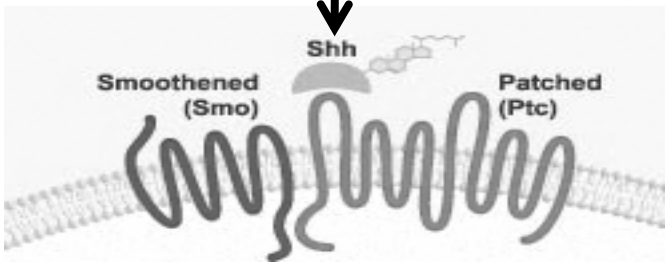
In cells with Hh-activated Patched, the intact Ci protein accumulates in the cell cytoplasm and levels of CiR decrease, allowing transcription of some genes such as ‘decapentaplegic’

For other Hh-regulated genes, expression requires not only loss of CiR but also the positive action of uncleaved Ci acting as a transcriptional activator.

One of the target genes involved in hedgehog signalling is ‘ wingless’.

The Wingless protein is called "wingless" because of the phenotype of some *wingless* fly mutants. Wingless and Hedgehog function together during metamorphosis to coordinate wing formation.

Hedgehog



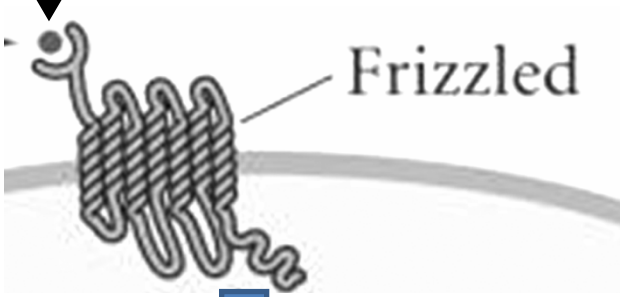
Cubitus interruptus (Ci)

Transcription of wingless

Wingless protein

Hedgehog

Wingless protein



Dsh

Zw3

Armadillo

Transcription of
engrailed, hedgehog

Wingless is secreted at the posterior boundary of a cell and it activates the Frizzled receptor in the adjacent cell which blocks the action of a cytioplasic serine/threonine kinase.

In the absence of kinase, the phosphorylation of Armadillo/β-catenin is blocked and this protein now translocates to the nucleus where it induces the expression of engrailed.

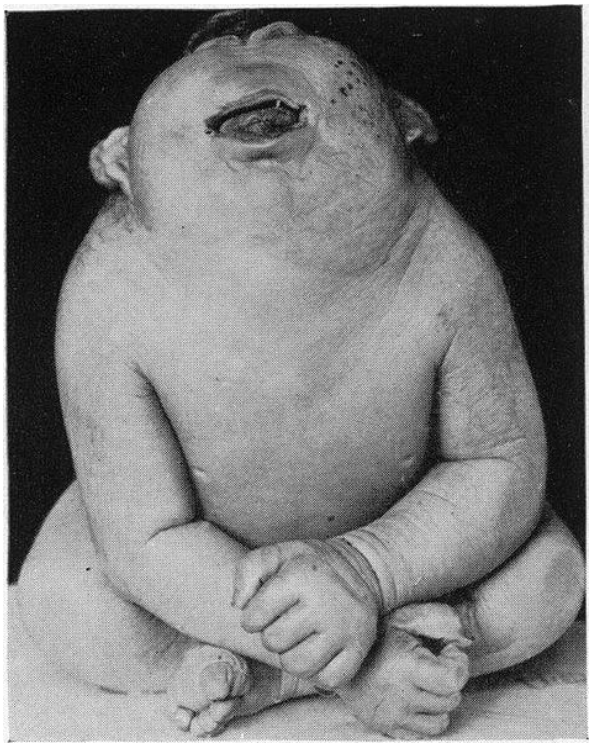
Engrailed causes the secretion of hedgehog at the anterior boundary where it interacts with the patched receptor and maintains wingless expression

Sonic Hedgehog (Shh) Function in early fly Development

Shh is involved in the separation of the single eye field into two bilateral fields.

Shh produced from the prechordal plate suppresses a protein known as *Pax6* and this results in the division of the eye field into two.

If the Shhgene is mutated, it results in cyclopia, a single eye in the center of the face.



http://commons.wikimedia.org/wiki/File:Cyclopia.jpg

Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function

Nature **383**, 407 - 413 (03 October 1996);

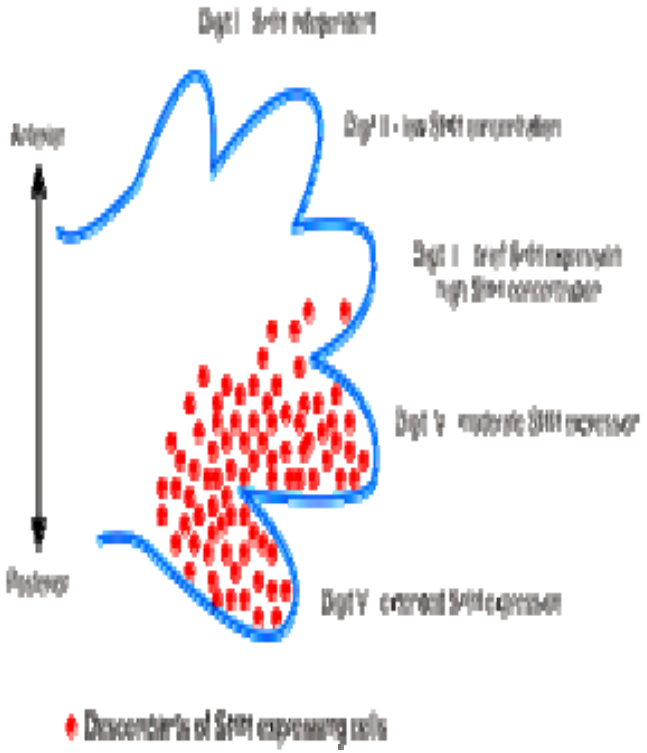
Shh also plays a role in a left-right axis patterning pathway.

Shh has been implicated in the growth of cutaneous appendages such as hair, feathers or scales.

Shh is thought to effect the specification of the mesoderm in gut formation.

Shh plays an important role in limb development, specifically effecting the zone of polarizing activity (ZPA).

Sonic hedgehog specifies digit identity in mammalian development.



Digits V, IV and part of III arise directly from cells that express SHH during embryogenesis.

These digits differ in the length of time that SHH continues to be expressed.

The most posterior digit V develops from cells that express the ligand for the longest period of time.

Digit IV cells express SHH for a shorter time, and digit III cells shorter still.

Digit II develops from cells that are exposed to moderate concentrations of extracellular SHH.

Finally, digit I development does not require SHH and is the default program of limb bud cells.

Hedgehog Pathway and Metastasis

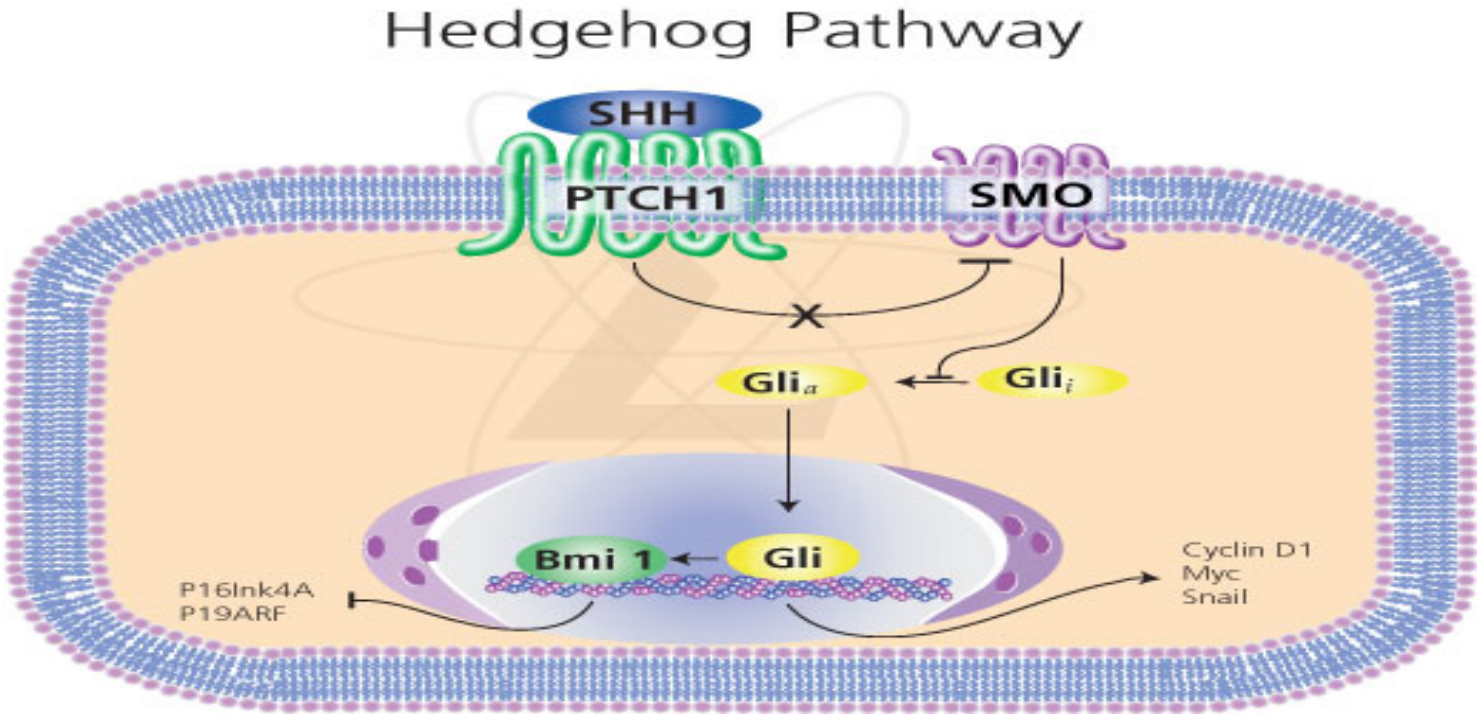
Activation of the Hedgehog pathway leads to an increase in a protein known as ‘Snail’ and a decrease in E-cadherin and Tight Junctions.

Hedgehog signaling also appears to be a crucial regulator of angiogenesis and thus metastasis.

Hedgehog Pathway and Tumor Regulation

Activation of the Hedgehog pathway leads to an increase in Angiogenic Factors (angiopoietin-1 and angiopoietin-2), Cyclins (cyclin D1 and B1), anti-apoptotic genes and a decrease in apoptotic genes (Fas).

Hedgehog signaling also has a key role in the regulation of adult stem cells involved in maintenance and regeneration of adult tissues.



Bmi1 is a polycomb gene that represses transcription through chromatin remodeling and down-regulates the expression of genes such as p16 Ink4A and p19 ARF, that are negative regulators of the cell cycle and are involved in stem cell quiescence and differentiation.

This enables stem cell proliferation and self-renewal via the Gli1- and Gli2-induced expression of the growth promoting genes cyclin D1, *Myc*, and *Snail* as well as upregulation of the Hh pathway elements PTCH1, Gli1, and Gli2.

*www.sigmaaldrich.com/etc/.../docs/.../**hedgehog**.../**hedgehog**_pathway.**ppt***

The hedgehog pathway has also been implicated in the development of some cancers.

Anti-cancer drugs that specifically target hedgehog signaling are being actively developed by a number of pharmaceutical companies.

Curr Opin Investig Drugs. 2007 Jun;8(6):457-61.
The Hedgehog pathway as a drug target in cancer therapy.
Lauth M, Toftgård R.

Clin Transl Oncol. 2009 Apr;11(4):199-207.
Hedgehog signalling as a target in cancer stem cells.
Medina V, Calvo MB, Díaz-Prado S, Espada J.

Activation of the hedgehog pathway is required for transition of the hair follicle from the resting to the growth phase.

Curis Inc. together with Procter & Gamble tried to develop a hedgehog agonist to be used as a drug for treatment of hair growth disorders. However, it failed die to toxicity.

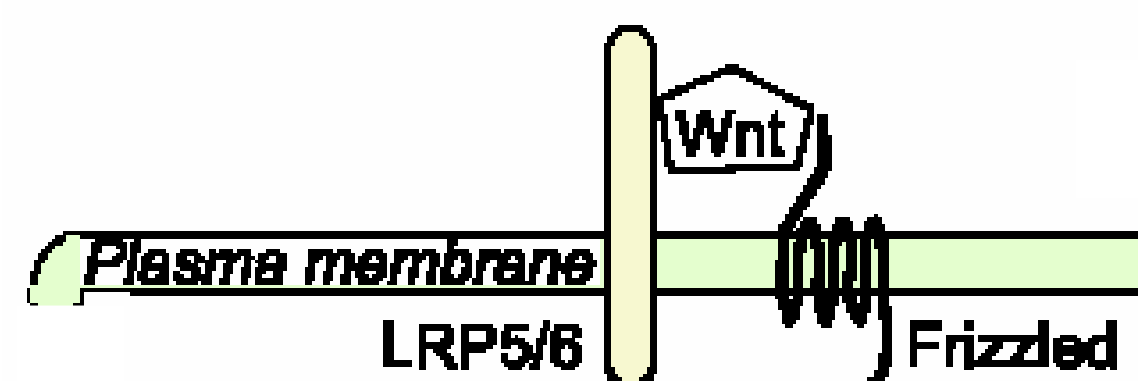
Biotech companies are also attempting to turn this pathway on after a patient has a stroke or heart attack.

In pre-clinical animal models it has shown that the pathway is up regulated upon a stroke or heart attack event.

The pathway provides a protective barrier against cell death and ischemia.

Agonizing the pathway this way allows the PTCH to be up regulated providing a negative feedback system thereby minimizing the side effects.

The wingless pathway in Drosophila is conserved in vertebrates and is known as Wnt signalling pathway.



In vertebrates, the binding of Wnt to Frizzled receptor can result in the activation of atleast three distinct signal transduction pathways.

The β catenin pathway

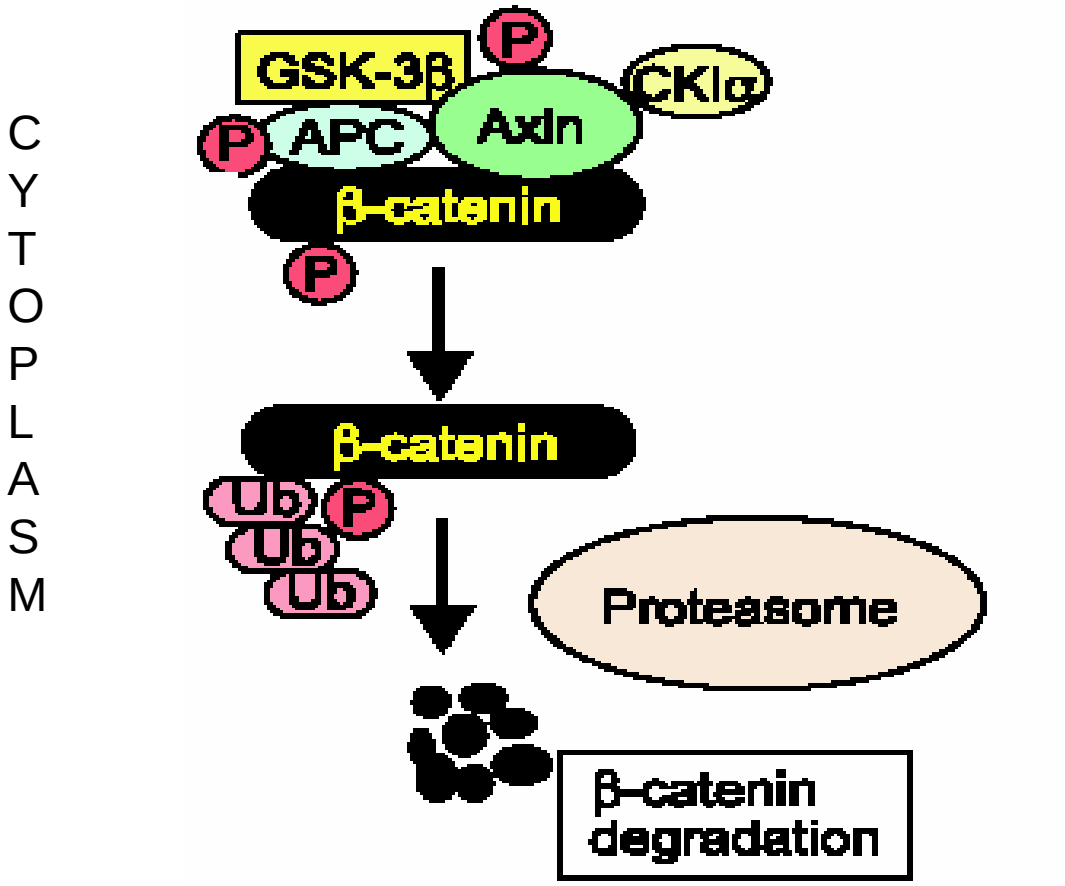
The PCP pathway

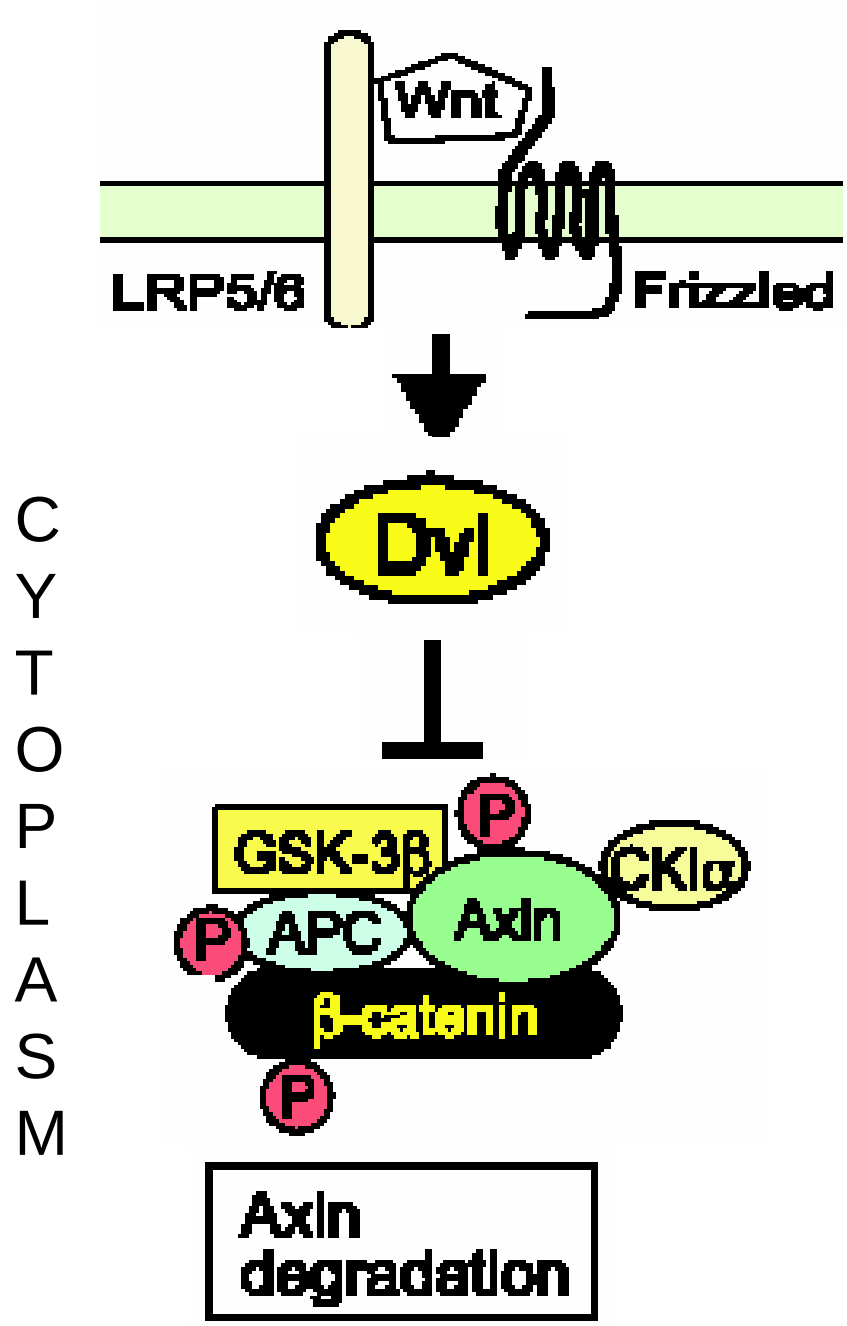
The calcium pathway

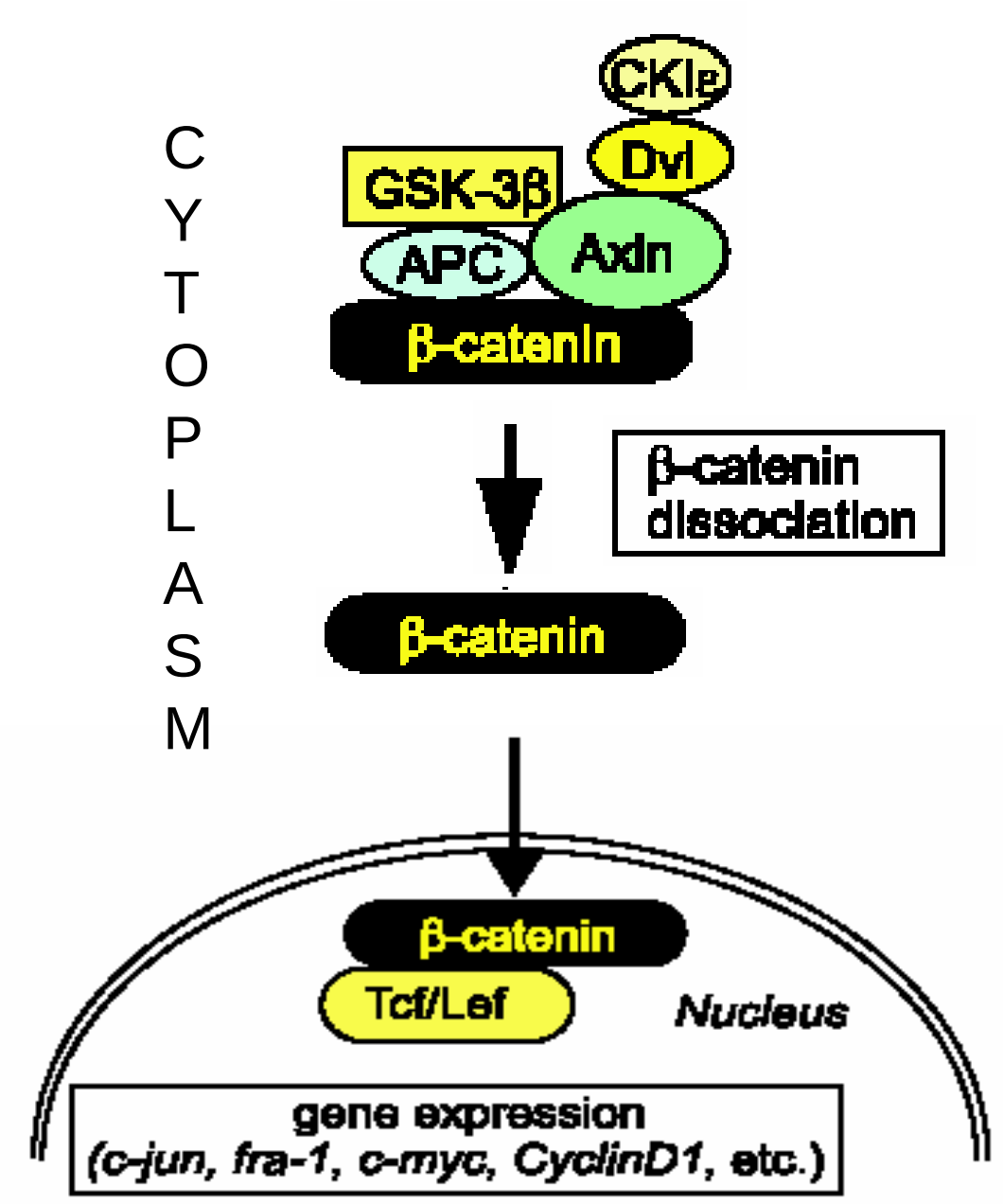
Wnt signalling

The β-catenin pathway

In the absence of Wnt, a transcription factor known as β-catenin (Armadillo in flies) is phosphorylated, ubiquitinylated and targeted for degradation in the cytoplasm




Wnt
LRP5/6
Frizzled
Dvl
GSK-3β
P
CKIα
P
APC
Axin
β-catenin
P
Axin
degradation
CYTOPLASM

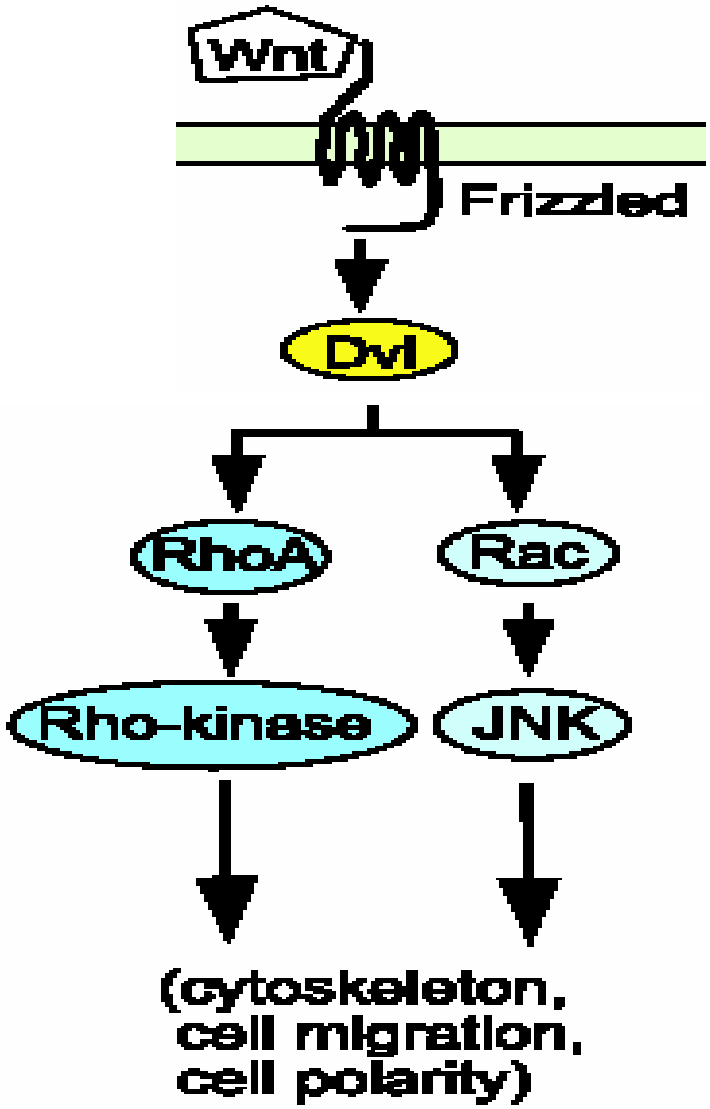


In the nucleus, in the absence of the Wnt signal,TCF acts as a repressor of Wnt/Wg target genes

β-catenin can convert TCF into a transcriptional activator of the same genes that are repressed by TCF alone

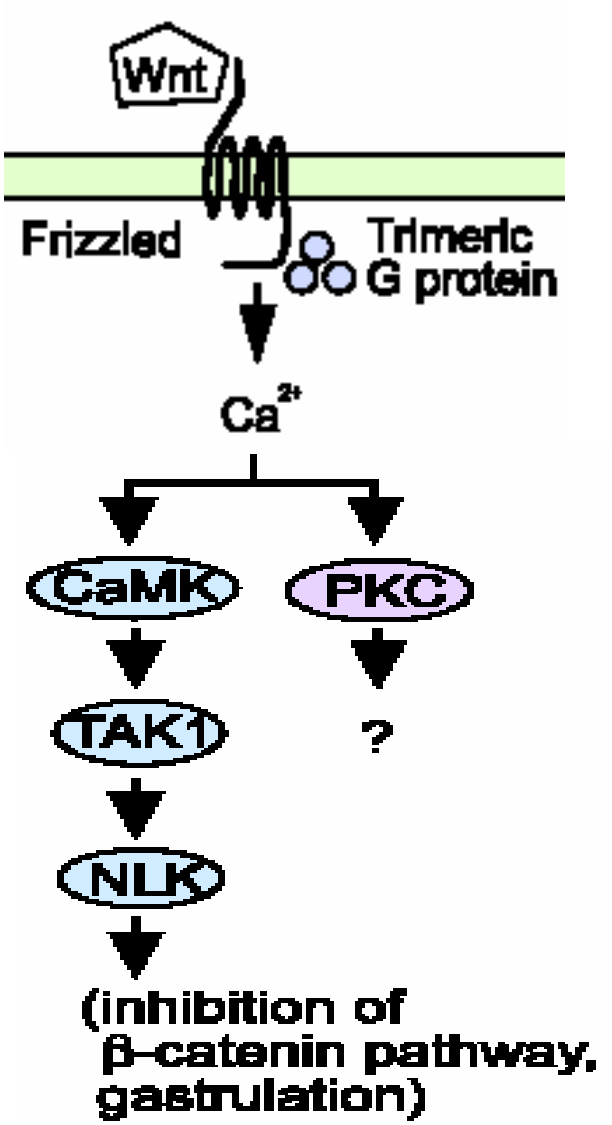
Wnt signalling

The PCP pathway



Wnt signalling

The calcium pathway



Wnt pathway and colon cancer

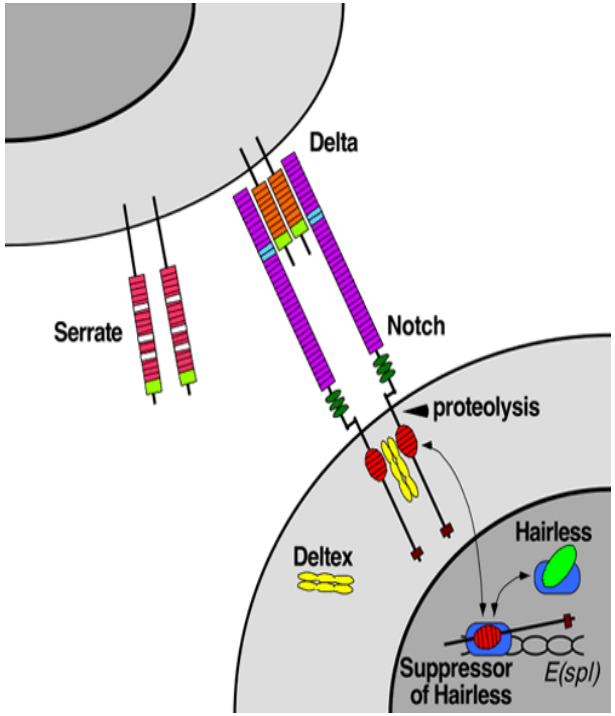
In colon cancer, mutations in a protein known as APC (adenomatous poluposis coli) are common.

APC binds to β-catenin and destabilizes it. The mutant APCs present in colon cancer cells allow the levels of β-catenin to increase

THE NOTCH SIGNALLING

The notch receptor is a single-pass transmembrane receptor protein.

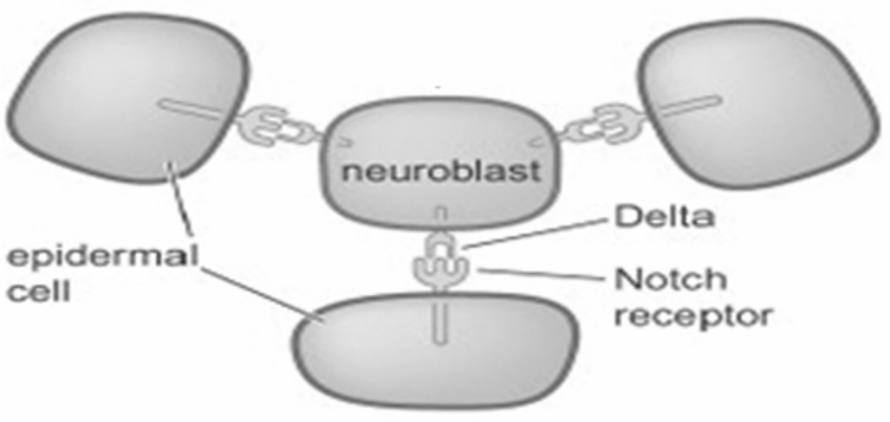
It is a hetero-oligomer composed of a large extracellular portion, which associates in a calcium-dependent, non-covalent interaction with a smaller piece of the notch protein composed of a short extracellular region, a single transmembrane- pass, and a small intracellular region.



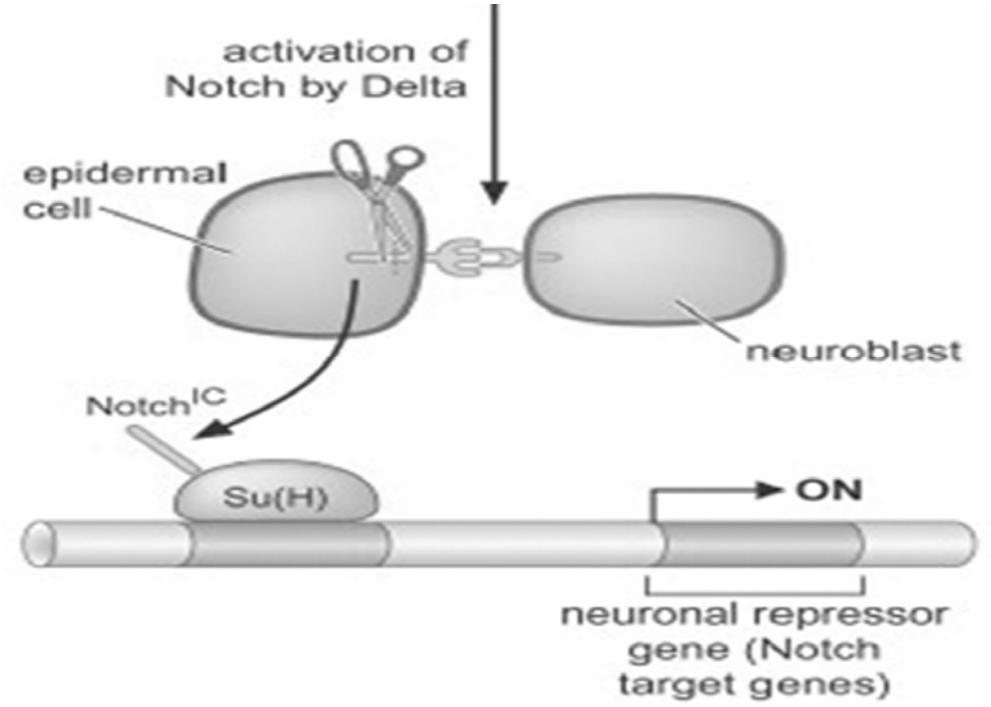
Ligand proteins binding to the extracellular domain induce proteolytic cleavage and release of the intracellular domain, which enters the cell nucleus to alter gene expression.

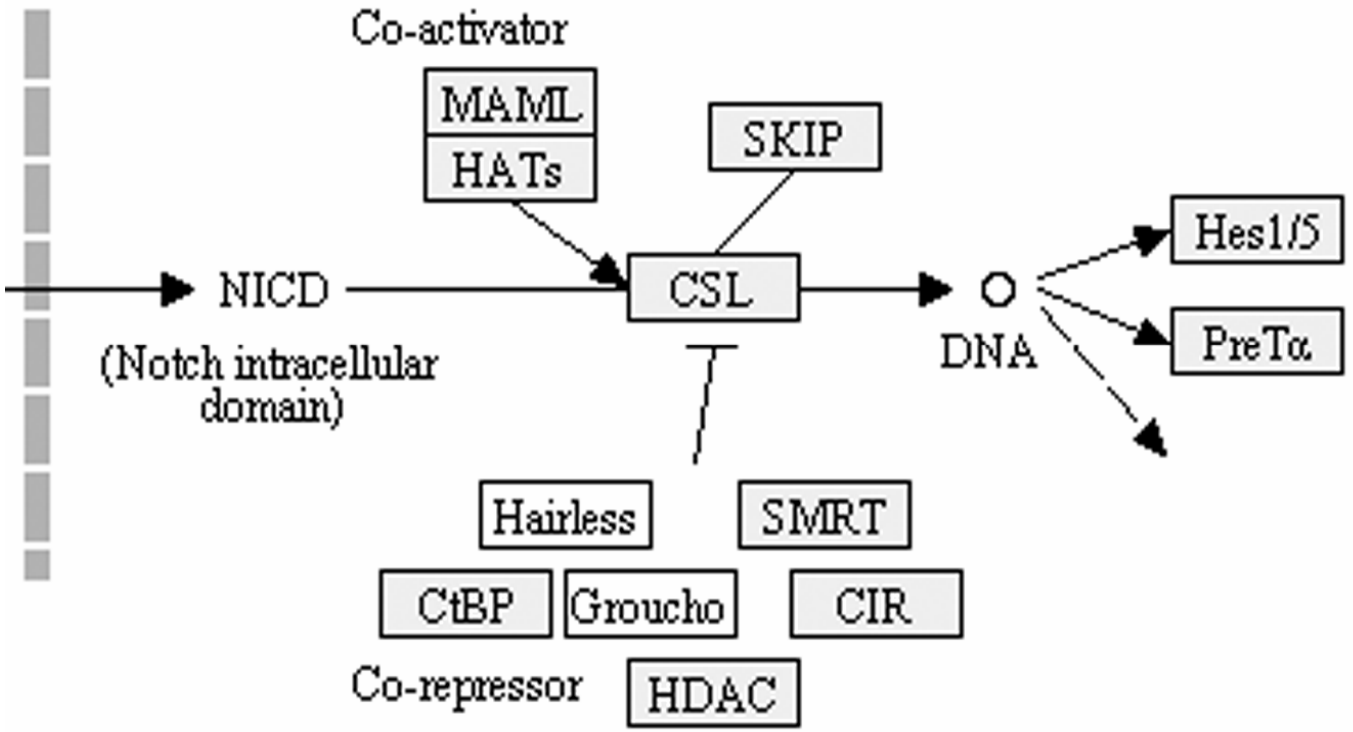
Because most ligands are also transmembrane proteins, the receptor is normally triggered only from direct cell-to-cell contact.

The developing neurons contain a signaling molecule **Delta** on their surface,which binds to and activates the **Notch** receptor on the skin cells.



Activation causes the intracytoplasmic domain of Notch (**NotchIC**) to be released from the cell membrane and enter the nuclei,then it associates with the DNA-binding protein Su(H).





The NICD translocates to the nucleus, where it forms a complex with the DNA binding protein CSL, displacing a histone deacetylase (HDAc)-co-repressor (CoR) complex from CSL. Components of an activation complex, such as MAML1 and histone acetyltransferases (HATs), are recruited to the NICD-CSL complex, leading to the transcriptional activation of Notch target genes

THE NOTCH SIGNALLING

The Notch signaling is an example of juxtacrine signalling or contact dependant signaling in which signal transduction requires physical contact between two adjacent cells

Juxtacrine signaling is a type of intercellular communication which is transmitted via oligosaccharide, lipid or protein components of a cell membrane and may affect either the emitting cell or immediately adjacent cells.

It occurs between adjacent cells that possess broad patches of closely opposed plasma membrane linked by transmembrane channels known as connexons.

The gap between the cells can only usually be between 2-4nm.

Unlike other types of cell signaling (such as paracrine and endocrine), juxtacrine signaling requires physical contact between the two cells involved.

Hedgehog Signaling in Development and Cancer

Jin Jiang and Chi-chung Hui

Developmental Cell 15, December 9, 2008