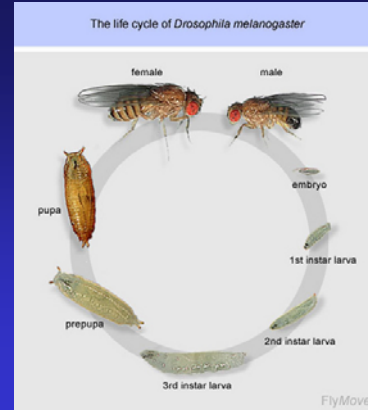
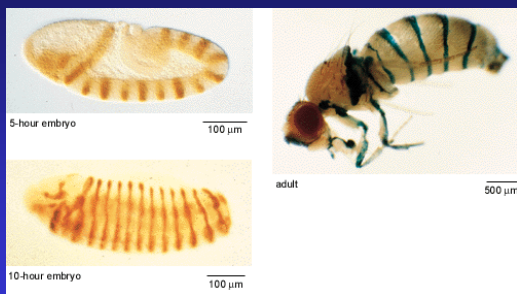


Hedgehog pathway

- First described in *Drosophila*
- Conserved signal transduction cascade
- Highly relevant to human development, disease, cancer, stem cell biology
- Important cell biology, gene regulation questions
- Therapeutic target, especially cancer



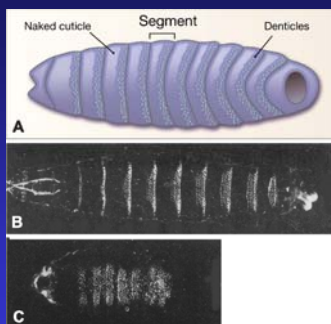
Embryonic patterning-early establishment of the body plan



The Nobel Prize in Physiology or Medicine 1995

Edward B. Lewis, Christiane Nüsslein-Volhard and Eric F. Wieschaus
for their discoveries concerning
"the genetic control of early embryonic development".

21

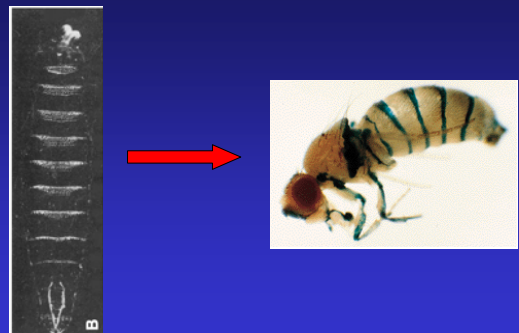


van den Brink G R Physiol Rev 2007;87:1343-1375

Physiological Reviews

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Simple Question: what genes control fly development?



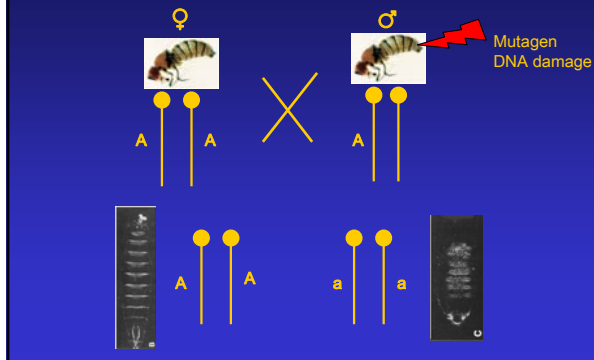
Forward Genetics

- How do you figure out how something works if you have little background knowledge about the players that could be involved?
- Disrupt something and see what happens

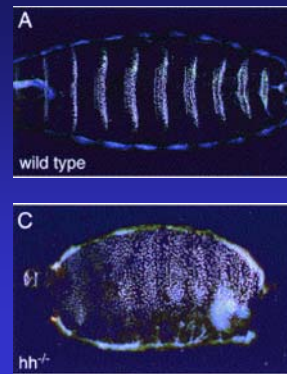
Forward Genetics

- Unbiased strategy of mutagenesis to disrupt the process that you are studying

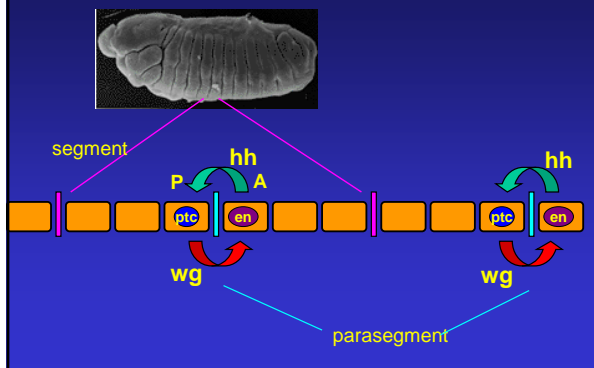
Screen for larval development genes in Drosophila



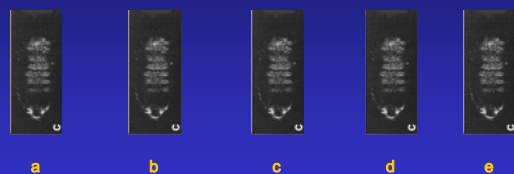
How did the hedgehog gene get its name?

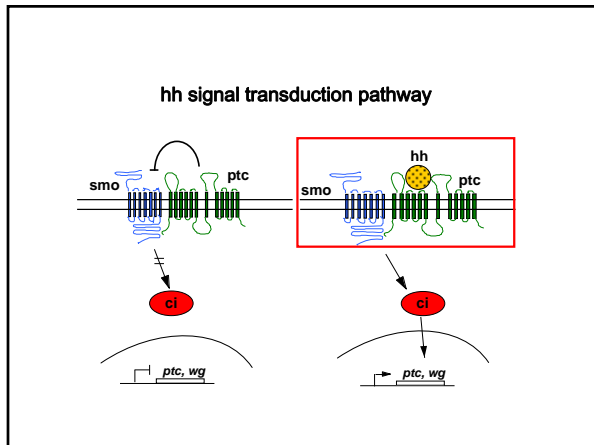
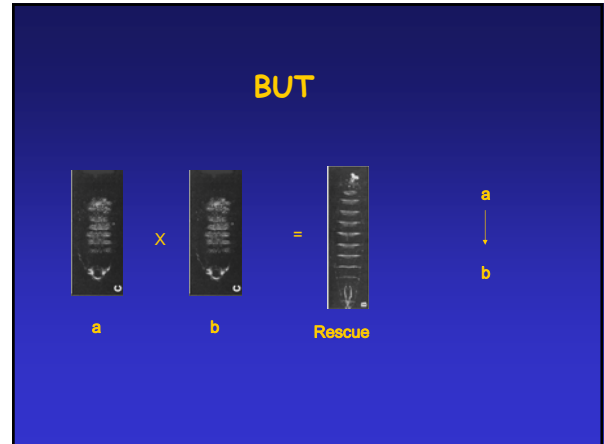


hedgehog signaling is required for the maintenance of engrailed expression at the parasegment boundary

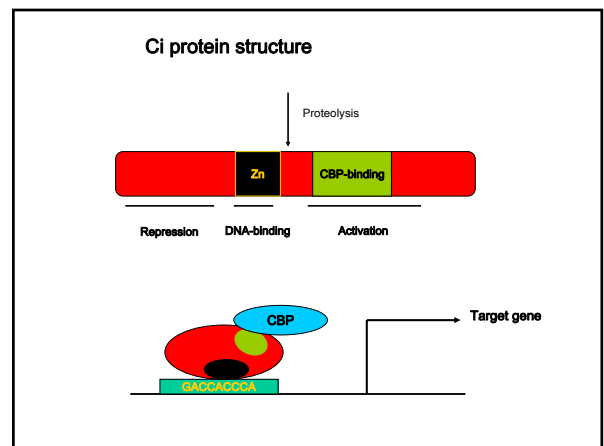
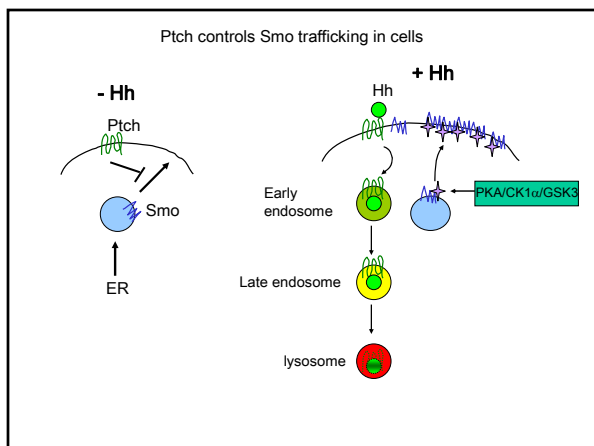


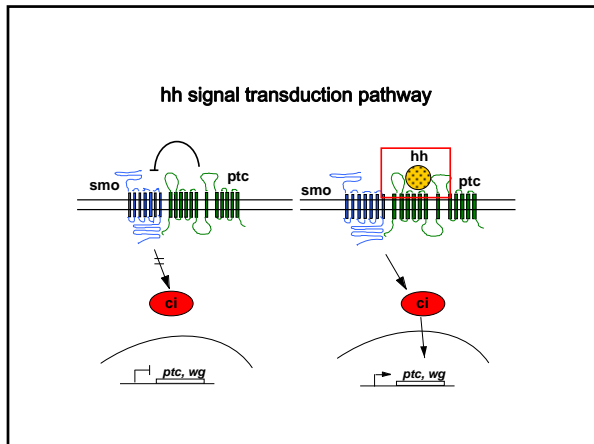
Epistasis (how to figure out the order of genes in a pathway)





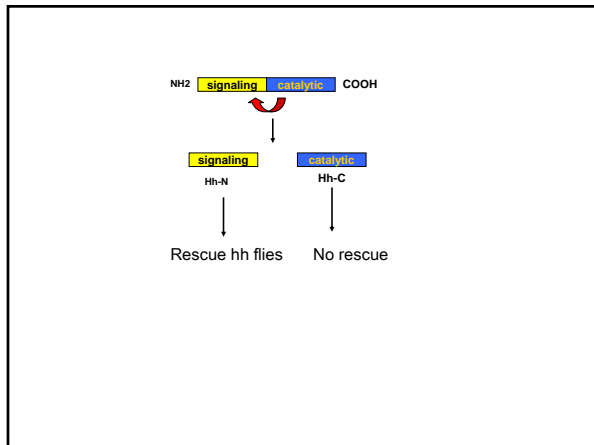
- ### Activation of the Hh signaling cascade
- *Ptch* and *Smo* never co-localize
 - *Ptch* negatively regulates the translocation of *Smo* to the cell surface
 - *Smo* activation requires conformational changes and phosphorylation





Hh signaling mediates long range patterning in the fly wing

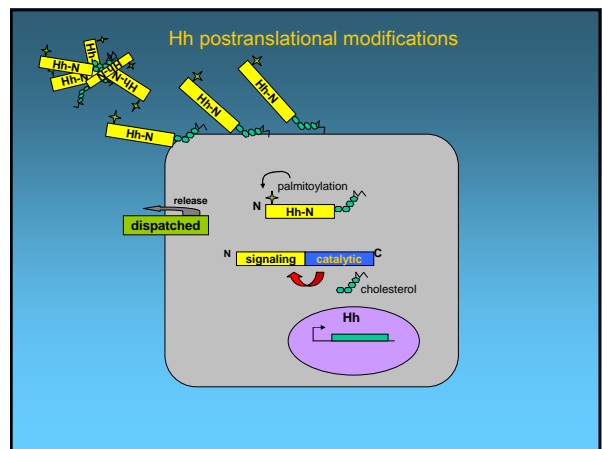
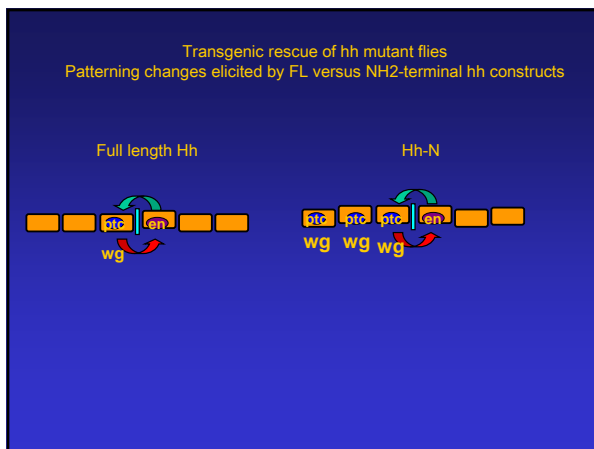
Cells adopt different fates in response to different concentrations of Hh



Localization and secretion of the Hh protein is depends on the carboxy terminus

Localization of the protein

Hh full length	Intracellular	cell surface	secreted
	+++	+++	+/-
	+	+	+++++



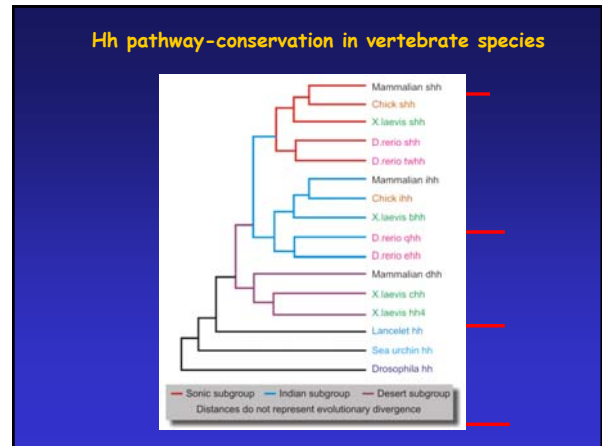
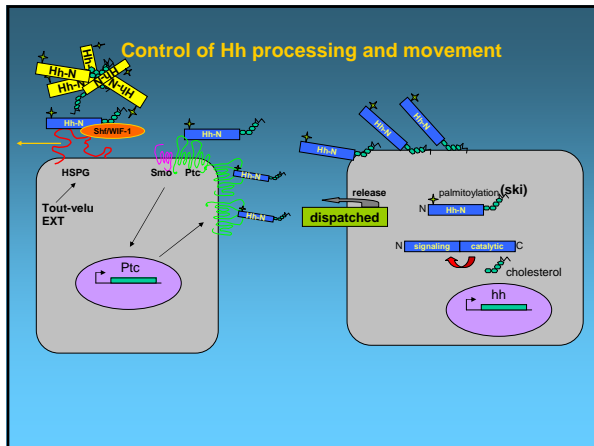


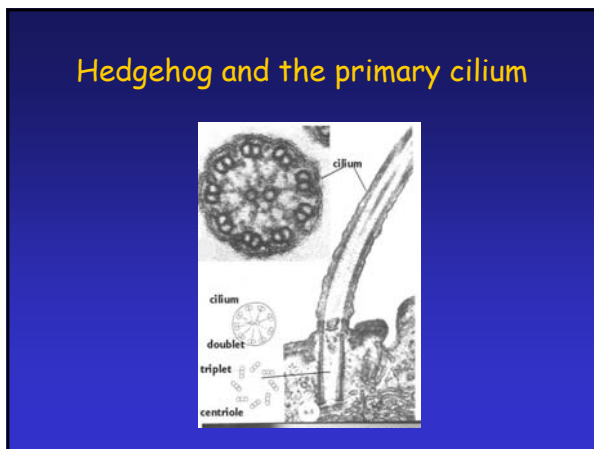
Table 1 | A comparison of the Hedgehog pathway in *Drosophila melanogaster* and mammals

	<i>D. melanogaster</i>	Mammals
Ligands	Hh	SHH, DHH, IHH
Acyl transferase	Rasp	SKN
Maturase	Disp	DISPA
Receptors	Ptc, PTC2	PTC1, PTC2
Glypican	Dip	?
Other hh binding factors	Suf, Pxb?	HIP, GAS1, megalin
Initiator	Smo	SMO
Smo regulators	PKA, CKI, GSK3 β	Lanosterol-2, GSK2
Cytoplasmic regulators	Cos2	KIF7, KIF3a, JFTBB, JFT172, Rab23
	Fu, Sufu	MM, RECK1, RECK2, SIL, FUR1, SIFU
Transcription factors	Ci	GLI1, GLI2 and GLI3

Hooper and Scott, 2005 *Nat. Rev.*

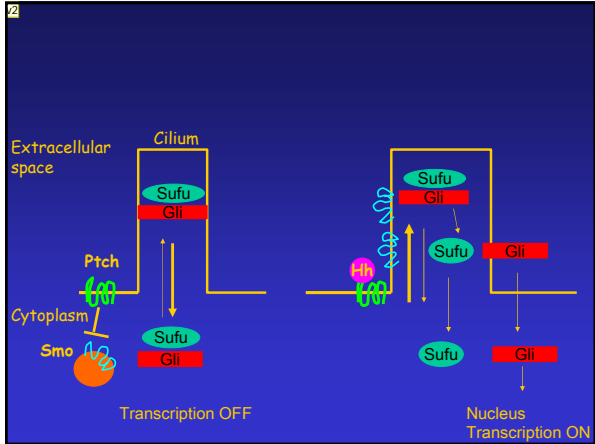
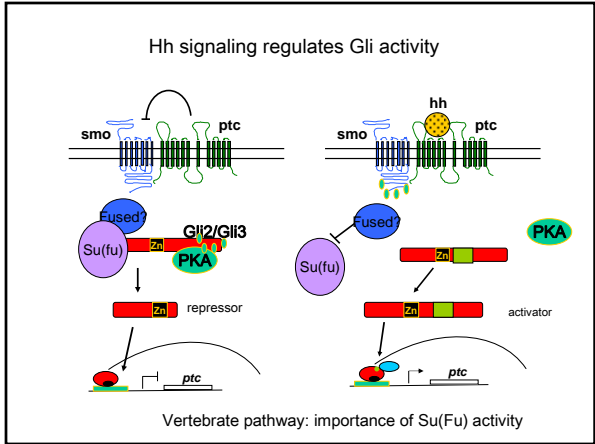
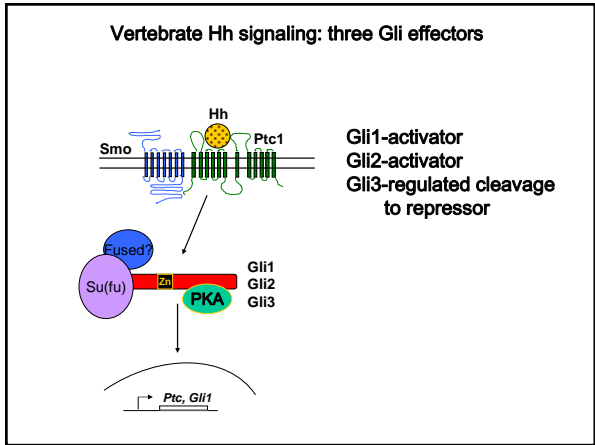
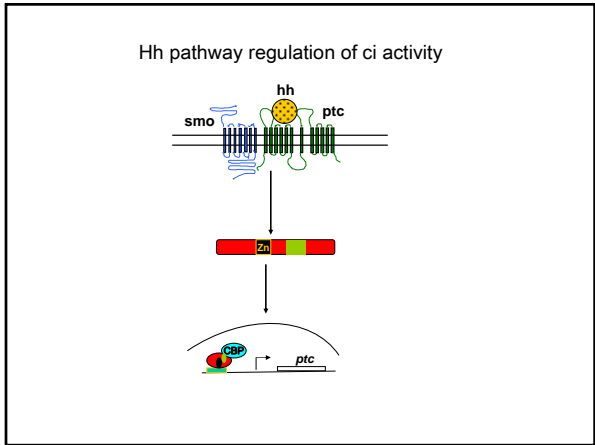
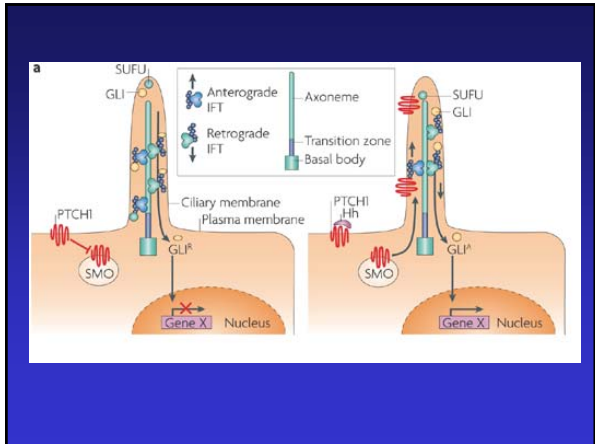
Divergence of fly and vertebrate signaling pathways

- Smoothened regulation
- Ci/Gli regulation



Cilia are required for Hh signaling

- Mutations in structural components of the cilia resemble loss of function for Hh signaling
- Intact cilia are required for Gli processing
- Ptc controls Smo entry into the cilium



Conservation of the Hh pathway components and function in vertebrates

Mammalian Hh homologues

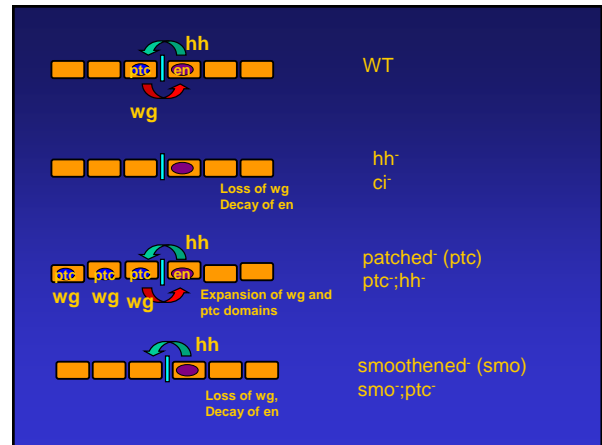
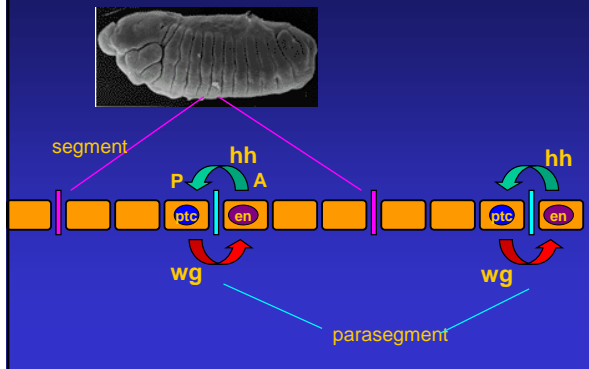
- **Dhh**: testes, peripheral nerve sheath
- **lhh**: chondrocyte development, gut, cardiac morphogenesis, kidney development, placentation
- **Shh**: neural tube patterning, muscle, proliferation, left-right asymmetry, limb polarity, epithelial-mesenchymal interactions (gut, lung) stem cell maintenance

Functional conservation in patterning

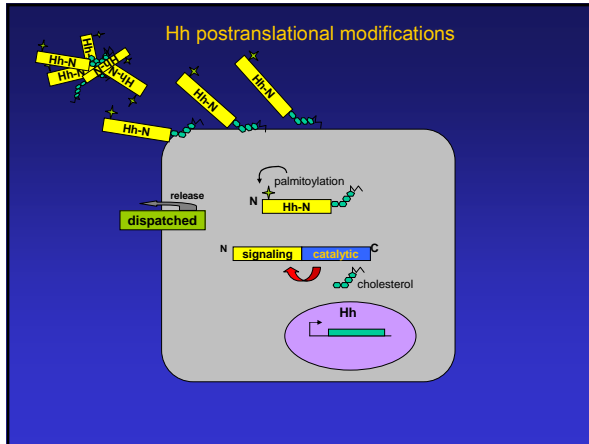
Vertebrate and *Drosophila* eye and limb

- Review Hh signaling pathway in flies and vertebrates
- Function of Hh and Gli3 in brain and limb development
- Basis for Hh induction of cancer

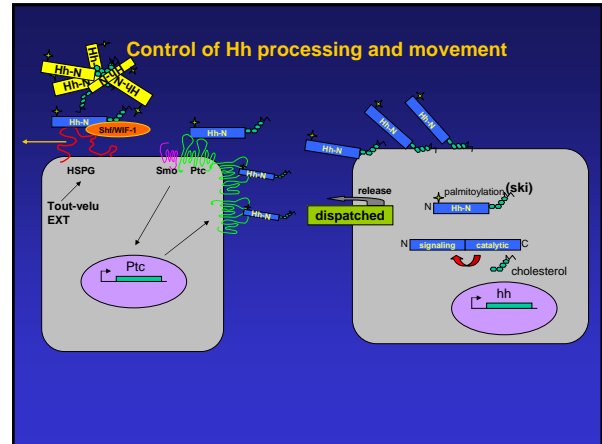
hedgehog signaling is required for the maintenance of engrailed expression at the parasegment boundary

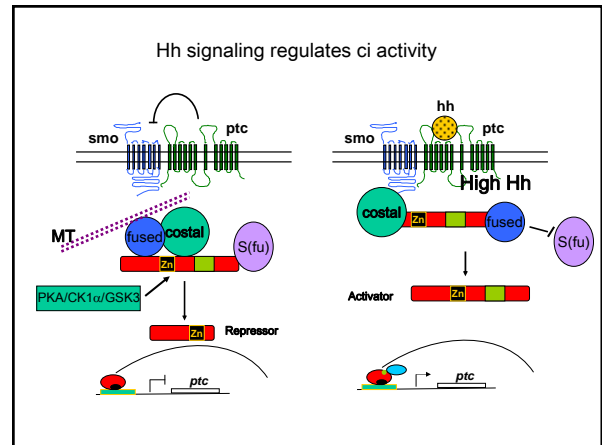
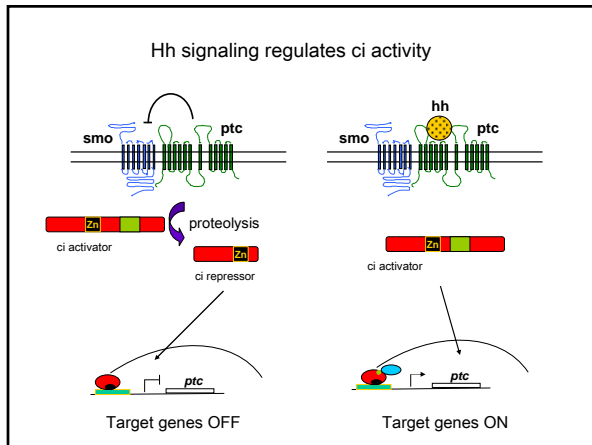
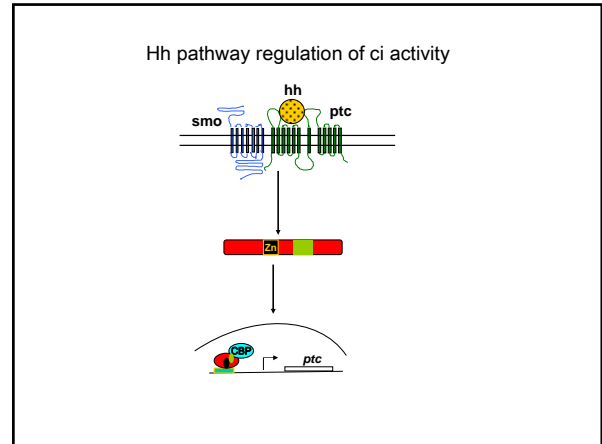
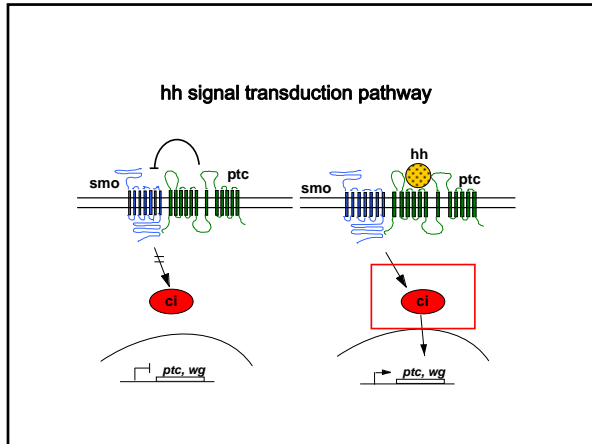


Hh posttranslational modifications



Control of Hh processing and movement





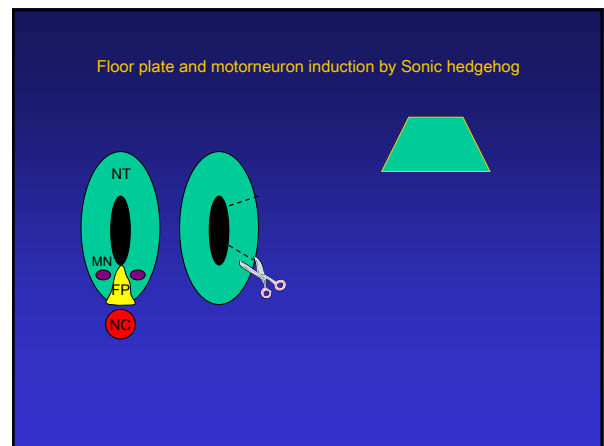
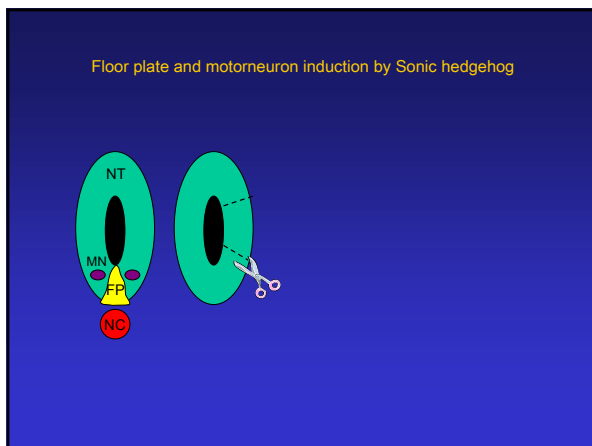
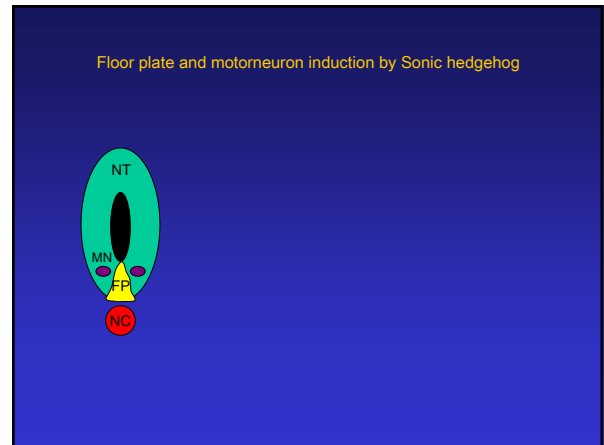
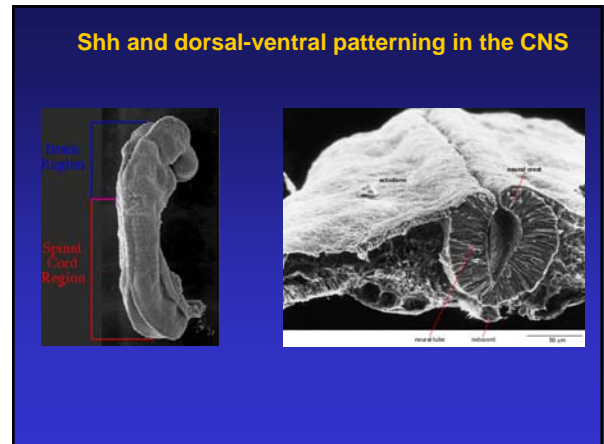
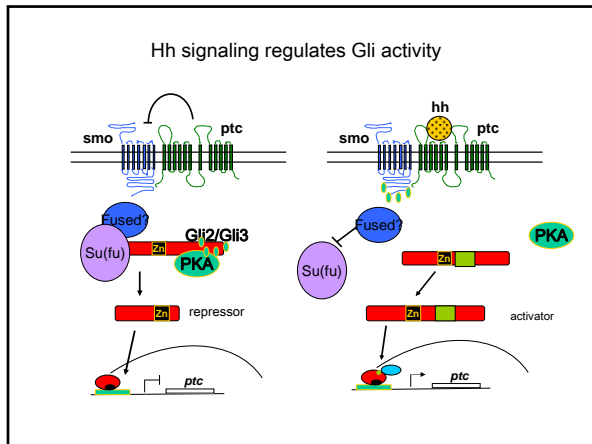
Loss of Hh and Ci not completely identical

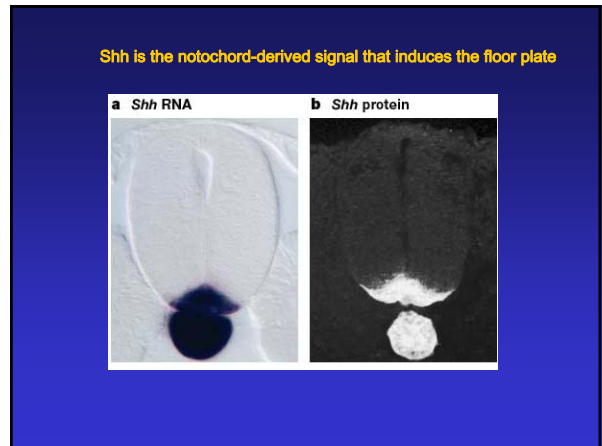
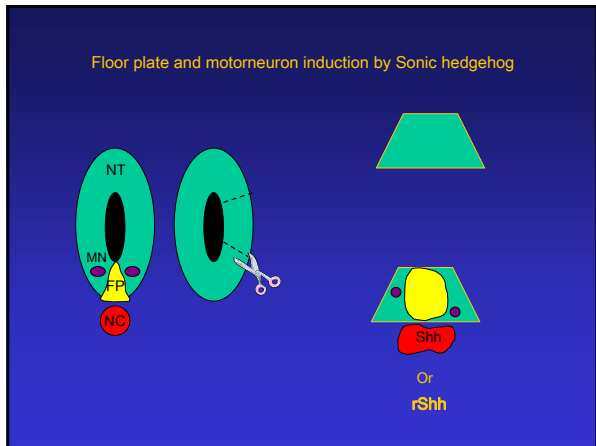
Ci mutant flies
No ci activator OR repressor

Hh mutant flies
Still have ci, cannot make ci activator but can make ci repressor

Hh in flies and vertebrates

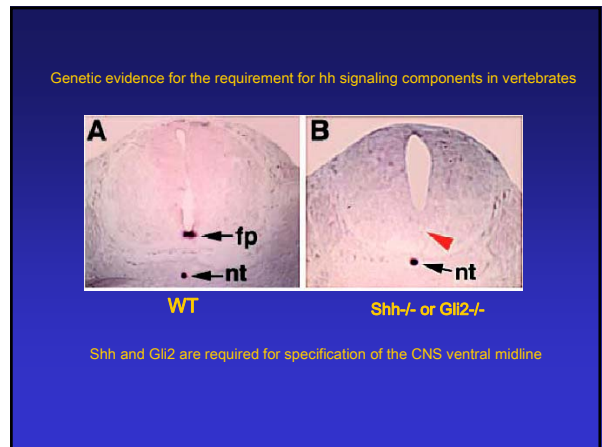
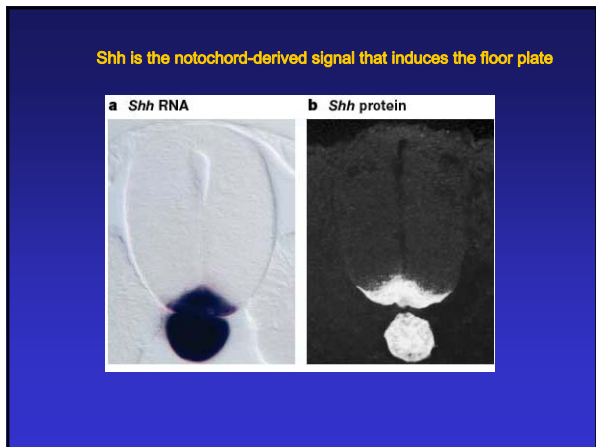
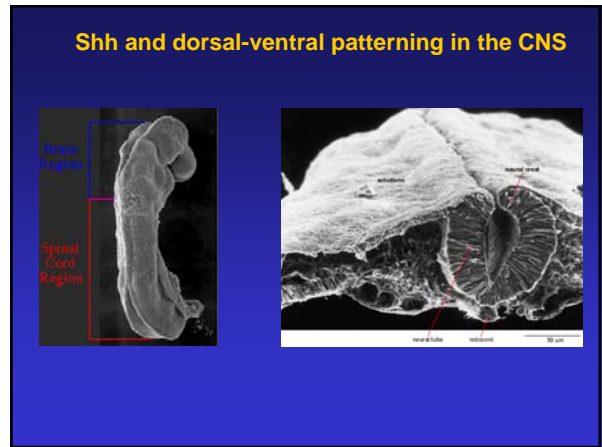
<ul style="list-style-type: none"> • Flies • One hh gene • One Smo, one Patched • One ci • Costal essential • Sufu not essential 	<ul style="list-style-type: none"> • Vertebrates • 3 Hh genes • One Smo, 2 patched • 3 Gli genes • Costal not essential • Sufu is essential • Primary cilium is essential
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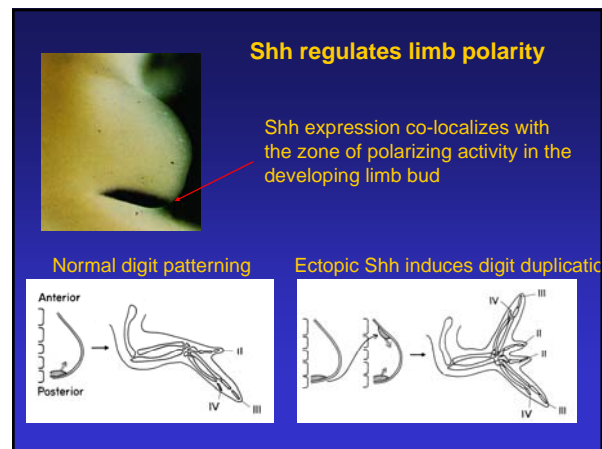
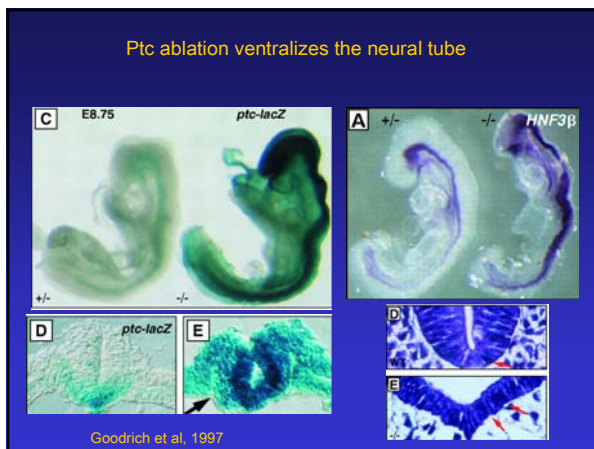
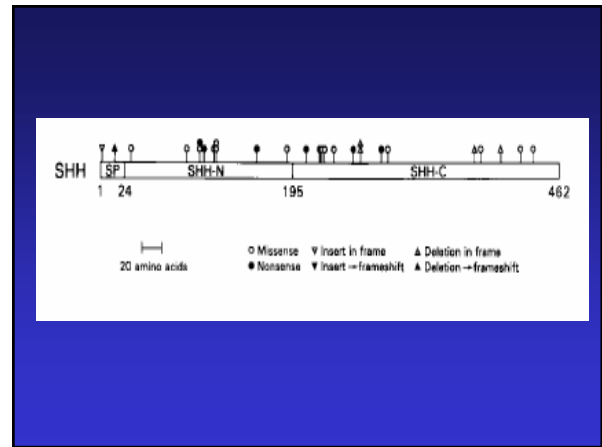
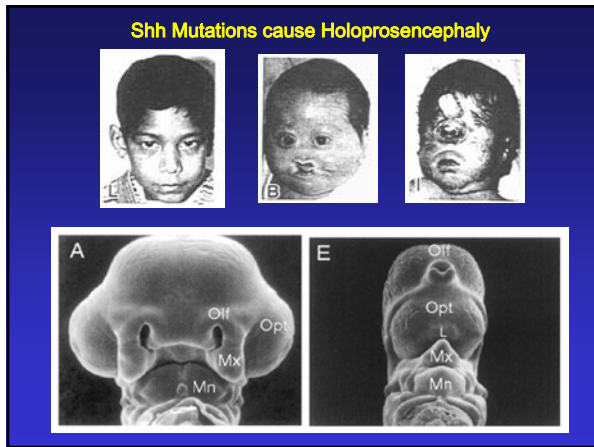
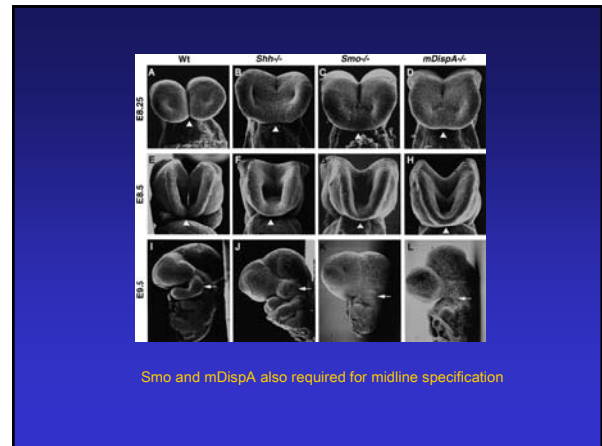
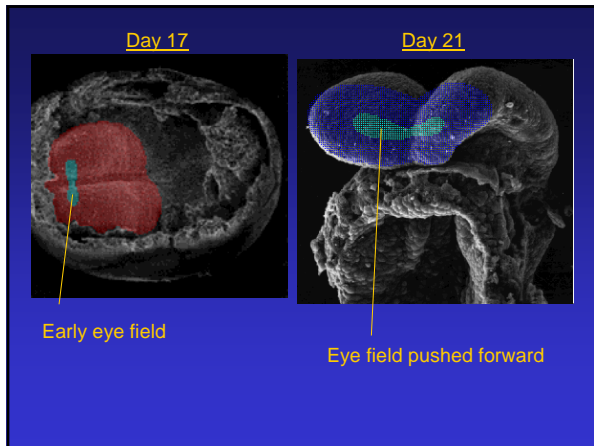




Genetic evidence for the requirement for Hh signaling components in vertebrates

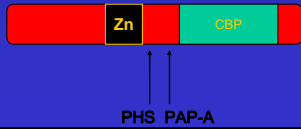
- Shh
- Smo
- Ptc
- Dispatched
- Gli



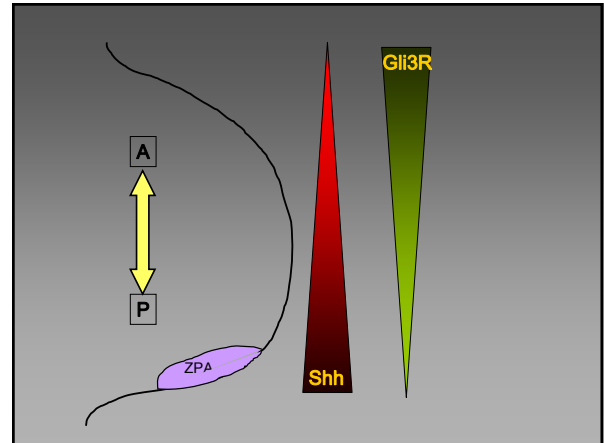
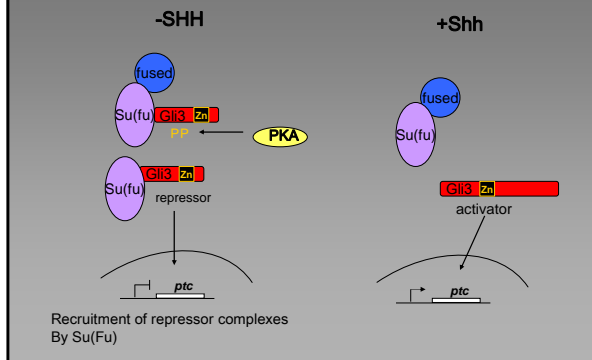


Malformations associated with Gli3 mutations

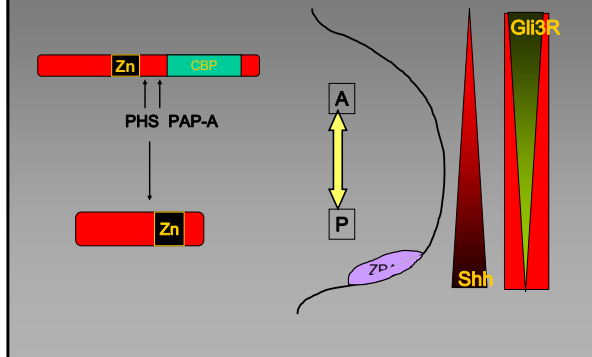
Condition	Gli status	Phenotype
Greig's cephalopolysyndactyly	dominant Loss of one Gli3 allele	Limb malformations and other defects
Pallister-Hall syndrome	Dominant Truncated Gli3 allele	Limb malformations and other defects
Postaxial polydactyly	Dominant Truncated Gli3 allele	Ectopic postaxial digit
Rubinstein-Tabi syndrome	Dominant Loss of CBP allele	Limb malformations and other defects



Shh-dependent processing of Gli3 in the limb bud



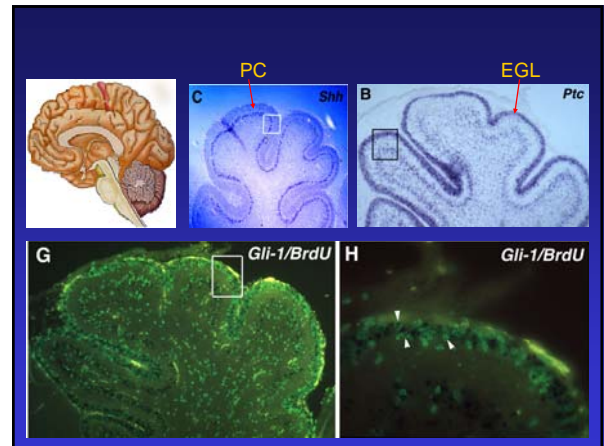
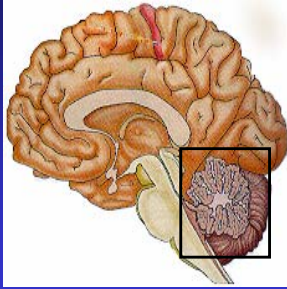
Limb malformations and disrupted Gli3R gradient



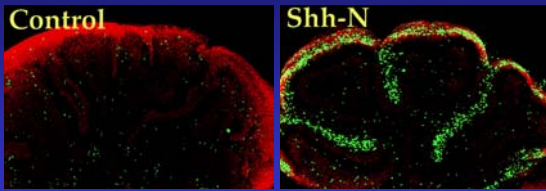
Shh/proliferation and cancer

- Cerebellum
- Retina
- Sclerotome
- Lung
- Muscle
- GI tract
- Hair follicle

Hedgehog signaling and proliferation and cancer in the brain



Shh protein can increase proliferation in the cerebellum

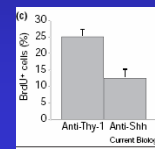


Green= dividing cells

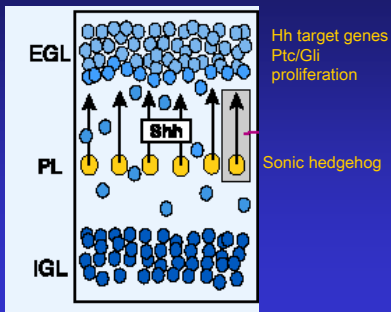
Blocking Hh signaling blocks proliferation in the Developing cerebellum



Localization of Gli mRNA

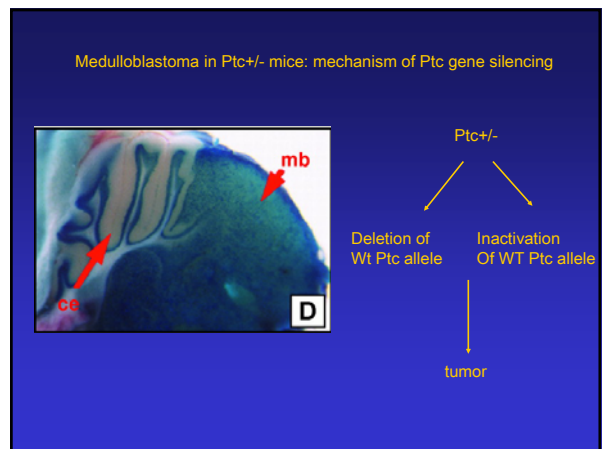
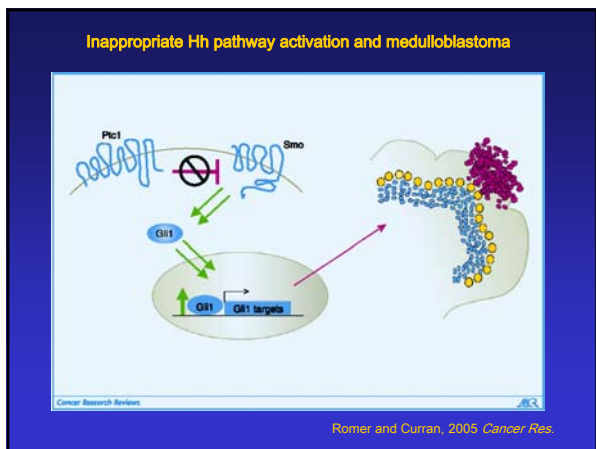
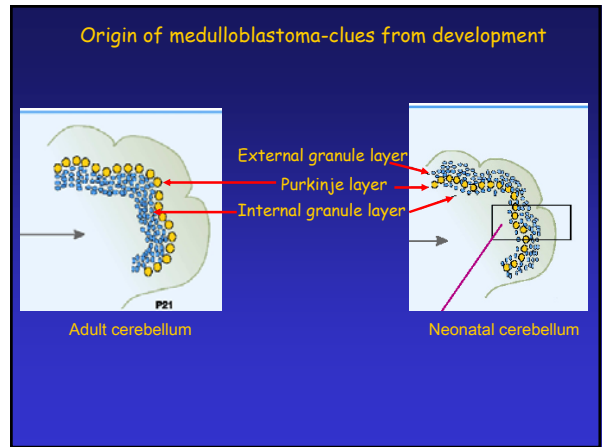
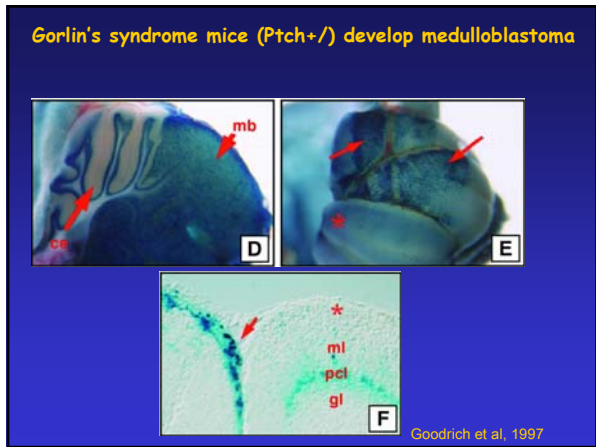
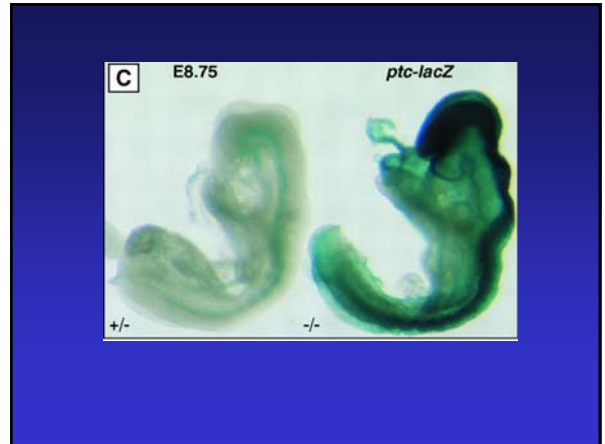


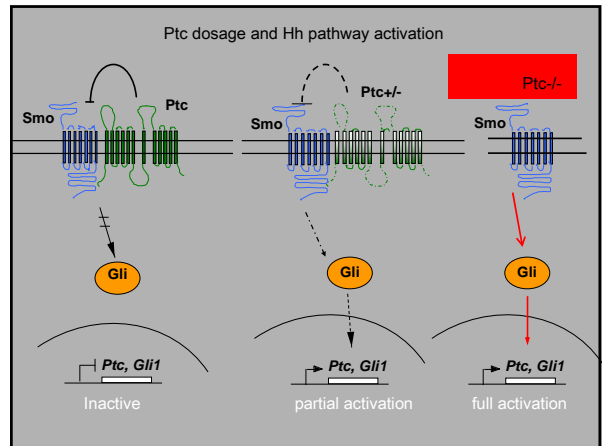
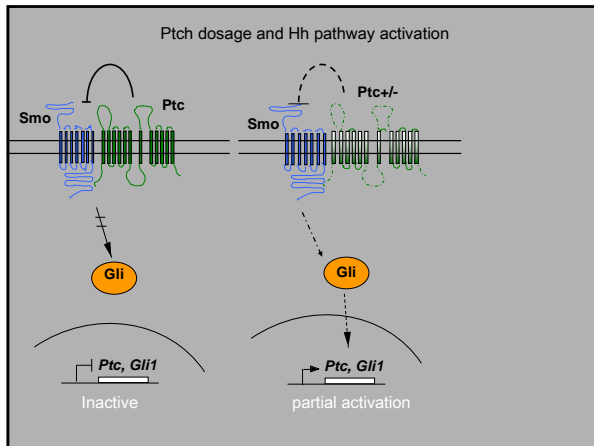
Sonic hedgehog is a mitogen for precursor Cells in the developing cerebellum



Gorlin's Syndrome

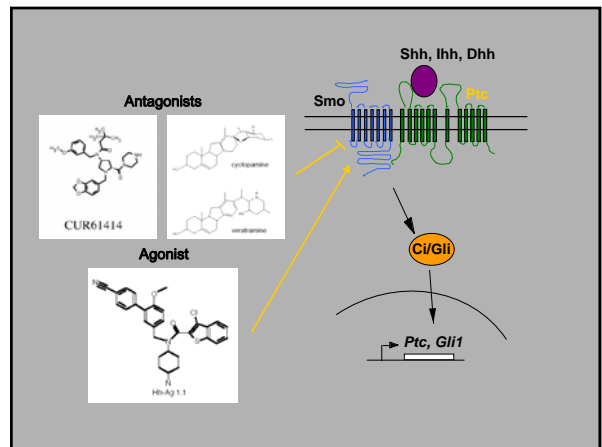
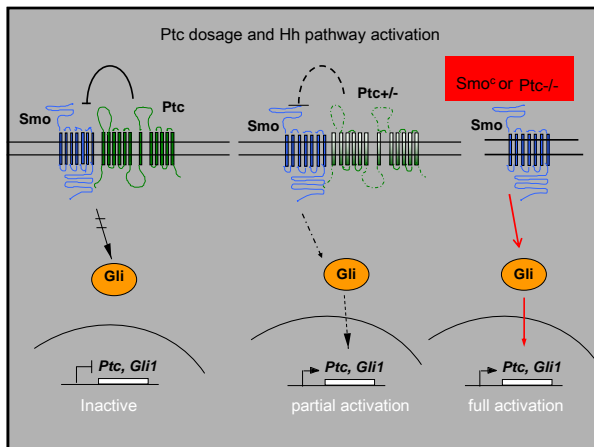
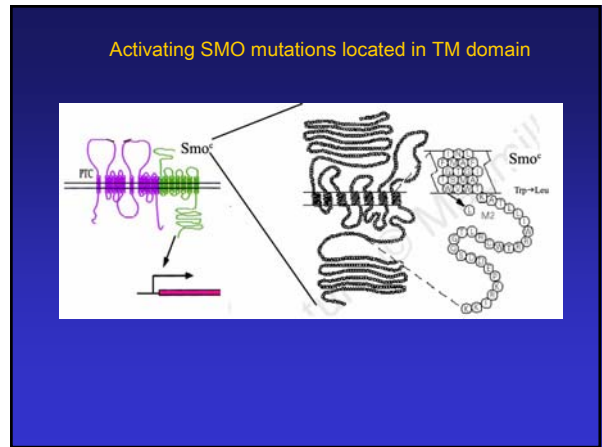
- Humans that have heterozygous deletion of Patched
- $Ptch^{+/-}$
- Congenital defects, overgrowth, Basal cell carcinoma, medulloblastoma and rhabdomyosarcoma





The Hh pathway and tumorigenesis

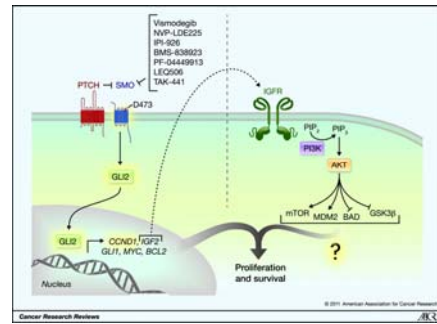
Tumour	Gene	Mutation
Basal cell carcinoma	Ptc1	Inactivating
Medulloblastoma	Su(Fu)	Inactivating
Rhabdomyosarcoma	Smo	Activating
	Gli1	Ectopic expression/amplification
Glioblastoma	Gli	Amplification
Osteosarcoma	Gli1	Amplification
B-cell lymphoma	Gli1	Amplification
Pancreas, stomach, lung, prostate	LIGAND DRIVEN	



Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/-)p53(-/-) mice.
 Romer JT, Kimura H, Magdaleno S, Sasai K, Fuller C, Baines H, Connelly M, Stewart CF, Gould S, Rubin LL, Curran T.
 Cancer Cell. 2004 Sep;6(3):229-40.

Smoothened mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma.
 Yauch RL, Dijkgraaf GJ, Alicke B, Januano T, Ahn CP, Holcomb T, Pujara K, Stinson J, Callahan CA, Tang T, Bazan JF, Kan Z, Seshagiri S, Hann CL, Gould SE, Low JA, Rudin CM, de Sauvage FJ.
 Science. 2009 Oct 23;326(5952):572-4. Epub 2009 Sep 2.

Multiple elements contribute to acquired resistance against SMO antagonists.



Metcalfe C, de Sauvage F J Cancer Res 2011;71:5057-5061

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