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3rd October 2019

Regulatory mechanisms of ontogenesis II.

Developmental biology lectures

Cell-cell communication in development

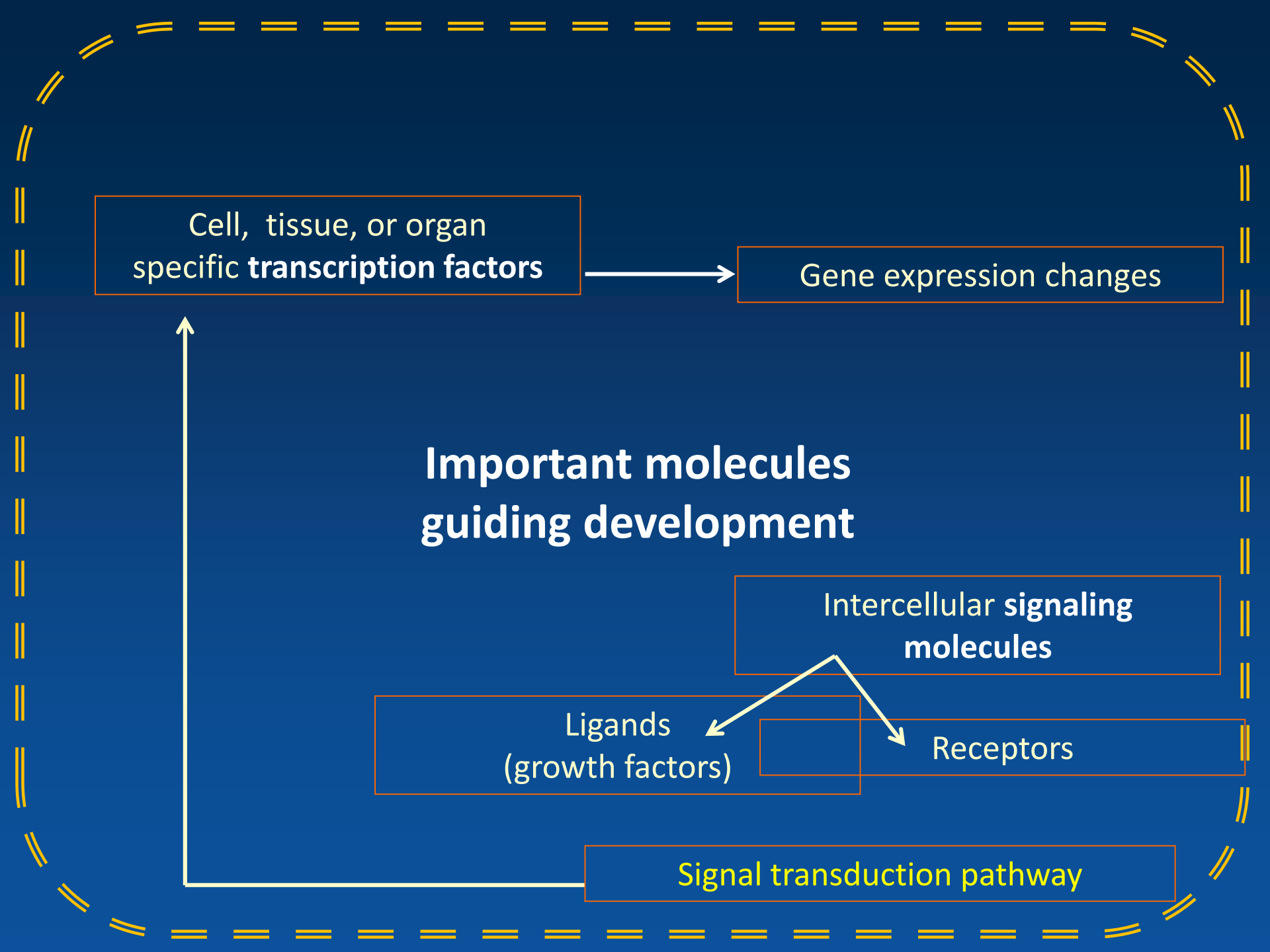
The twelfth-century rabbi and physician Maimonides (1190) framed the question of morphogenesis beautifully when he noted that the pious persons of his day believed that an angel of God had to enter the womb to form the organs of the embryo. How much more powerful a miracle would life be, he asked, if the Deity had made matter such that it could generate such remarkable order without a matter-molding angel having to intervene in every pregnancy? The idea of an angel was still part of the embryology of the Renaissance. The problem addressed today is the secular version of Maimonides' question:

How can matter alone construct the organized tissues of the embryo?

Cell, tissue, or organ specific **transcription factors** → Gene expression changes

Important molecules guiding development

Intercellular **signaling molecules**


Cell, tissue, or organ specific **transcription factors**
Gene expression changes
Important molecules guiding development
Intercellular **signaling molecules**
Ligands (growth factors)
Receptors
Signal transduction pathway

Induction and competence 1.

inducer → signal → responder

changes in cell shape
mitotic rates
cell fate

In neural induction:

notochord → ectoderm → neuroectoderm

Induction and competence 2.

The ability to respond to a specific inductive signal is called competence (Waddington, 1940)

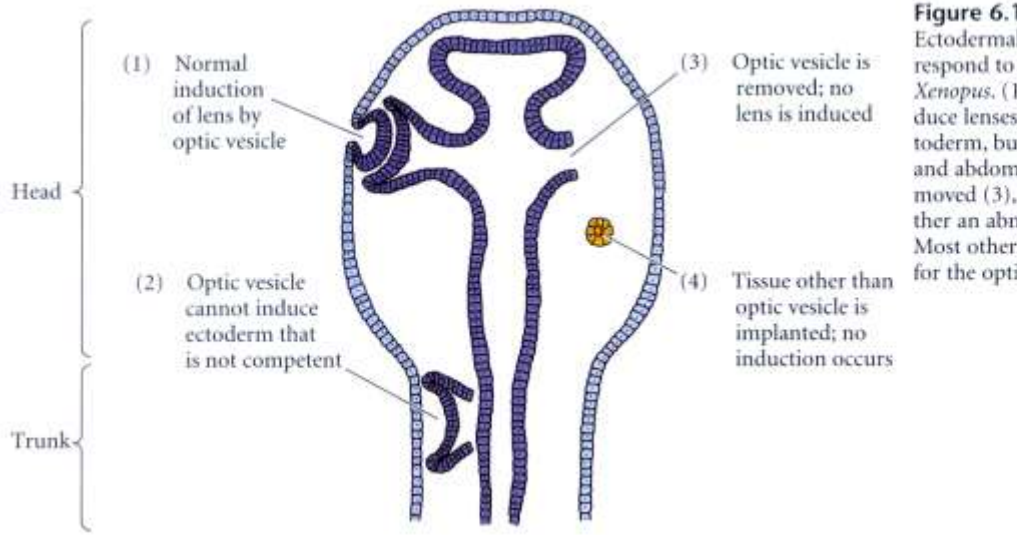


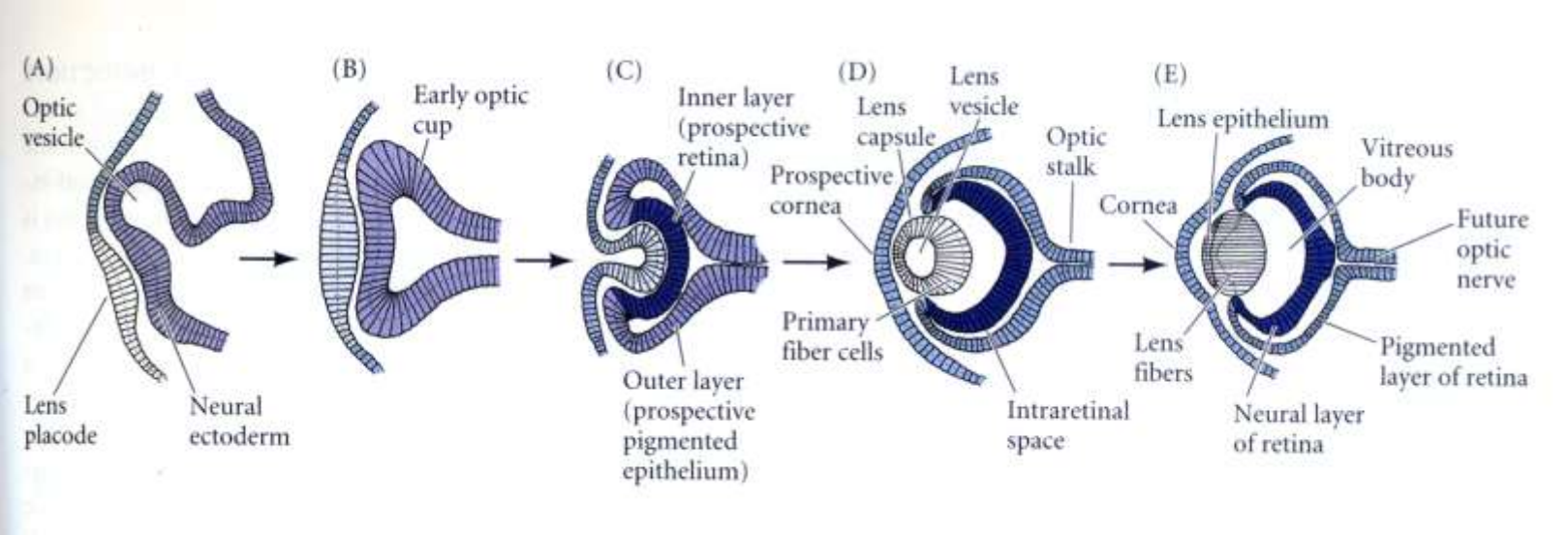
Figure 6.1
Ectodermal competence and the ability to respond to the optic vesicle inducer in *Xenopus*. (1) The optic vesicle is able to induce lenses in the anterior portion of the ectoderm, but not in the presumptive trunk and abdomen (2). If the optic vesicle is removed (3), the surface ectoderm forms either an abnormal lens or no lens at all. (4) Most other tissues are not able to substitute for the optic vesicle.

Competence is not a passive state, but an actively acquired condition. For example, in the developing chick and mammalian eye, the Pax6 protein appears to be important in making the ectoderm competent to respond to the inductive signal from the optic vesicle. Pax6 (paired domain containing TF) expression is seen in the head ectoderm, which can respond to the optic vesicle by forming lenses, and it is not seen in other regions of the surface ectoderm (Li et al. 1994). Moreover, the importance of Pax6 as a **competence factor was demonstrated by recombination experiments using embryonic rat eye** tissue (Fujiwara et al. 1994). Pax6 is needed for the surface ectoderm to respond to the inductive signal from the optic vesicle.

Induction and competence 3.

Cascades of induction: Reciprocal and sequential inductive events

Another feature of induction is the reciprocal nature of many inductive interactions. Once the lens has formed, it can then induce other tissues. One of these responding tissues is the optic vesicle itself. Now the inducer becomes the induced. Under the influence of factors secreted by the lens, the optic vesicle becomes the optic cup, and the wall of the optic cup differentiates into two layers, the pigmented retina and the neural retina (Figure 6.5; Cvekl and Piatigorsky 1996). Such interactions are called **reciprocal inductions.**



Cells receive and respond to extracellular cues through receptors. The first response is triggering complex signaling networks that relay extracellular cues into the cell, culminating in the reprogramming of various biochemical, genetic, and structural processes. The cellular signaling starts as soon as the first messenger (the ligand) binds to its receptor—a protein with the complementary structure on a transmembrane protein or within the cell. The binding of the ligand induces conformational changes to the receptor and activates well-controlled sets of reactions carried out by the second messengers or signaling intermediates that transduce the message from the receptor to the quantifiable effector functions.

Thus, cell signaling is a crucial cog in the cellular response system.

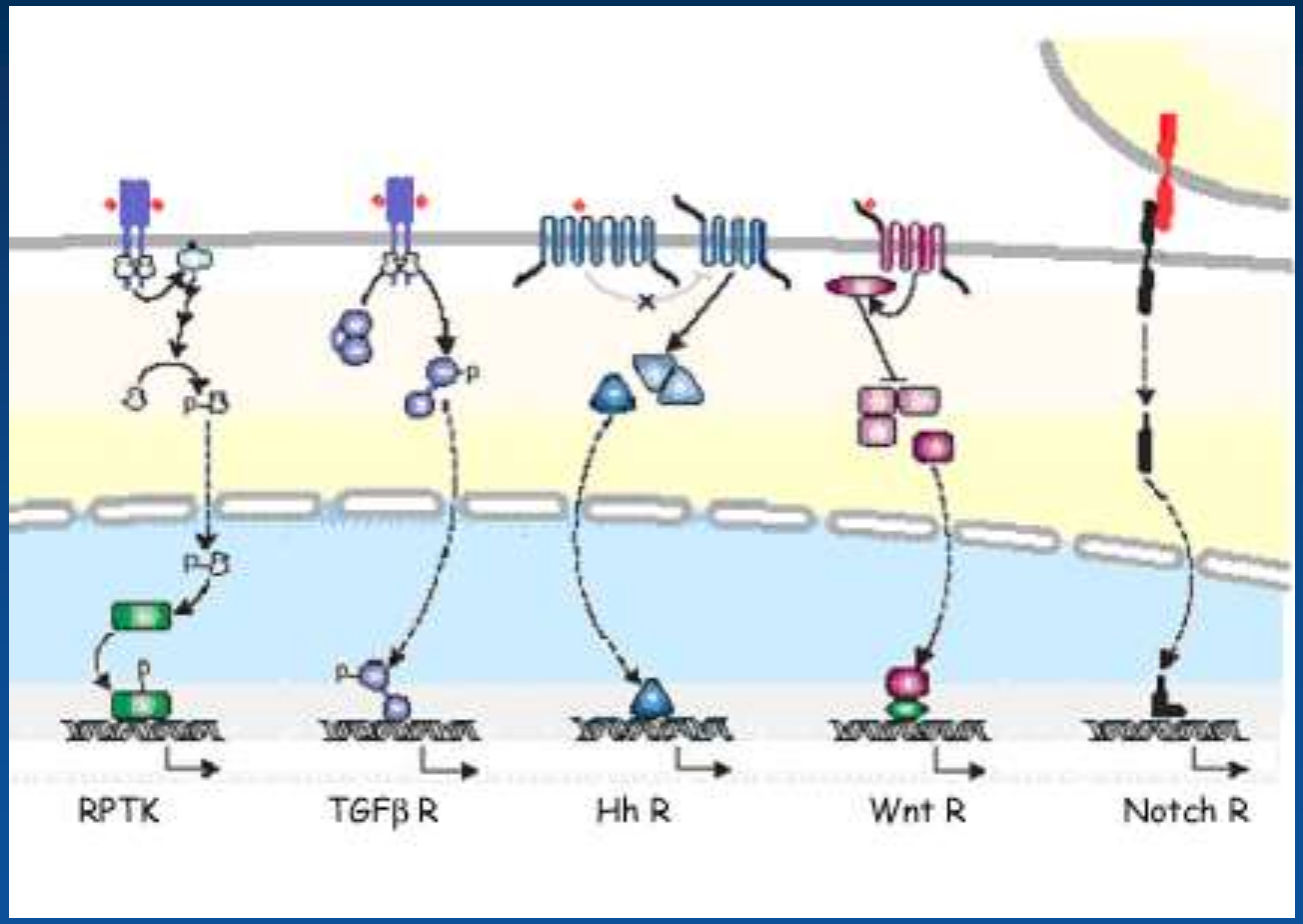
The discovery of cellular signaling dates back to 1855 when Claude Bernard described how certain ‘internal secretions’ of ductless glands, released into the bloodstream, can have effects on distant cells.

The messages are transferred from the first messenger (the ligand) to the receptor, and then decoded with the help of cascades of second messengers (kinases, phosphatases, GTPases, ions, and small molecules such as cAMP, cGMP, diacylglycerol, etc.).

The message is thus relayed from the membrane to the nucleus where gene expression ns, subsequent translations, and protein targeting to the cell membrane and other organelles are triggered. Although there are limited numbers of intracellular messengers, the specificity of the response profiles to the ligands is generated by the involvement of a combination of selected intracellular signaling intermediates.

Other crucial parameters in cell signaling are its directionality and distribution of signaling strengths in different pathways that may crosstalk to adjust the amplitude and quality of the final effector output.

Important signaling pathways in development



The same signaling molecule can be used at many different times and places as the embryo takes shape. Locally controlled factors, such as the concentration or duration of exprosure to a signaling molecule, are often important determinants of the fate of a group of responding cells. This reduces greatly the number of signaling molecules that need to be employed.

Signaling pathway:
Ligand, receptor, signal transduction pathway

Gene expression changes induced by the signal.

TGFβ ligands

The TGF-β family was named because its first-discovered member (transforming growth factor-β, TGF-β1) was isolated from virally transformed cells. Only later was it realised that many signaling molecules with greatly different functions during embryonic and postnatal life bear structural similarity to this molecule.

In the same family:

Activin	(mesodermal induction)
Inhibin	(inhibition of gonadotropin secretion by hypophysis)
Decapentaplegic	(Drosophila limb development)
Vg1	(mesodermal and primitive streak induction)
BMP molecules	(induction of neural plate)
Nodal	(formation of mesoderm and primitive streak, left-rigth axial fixation)
Lefty	(determination of body asymmetry)

TGFbetas generally are generally negative regulators of growth!

TGF-β activation

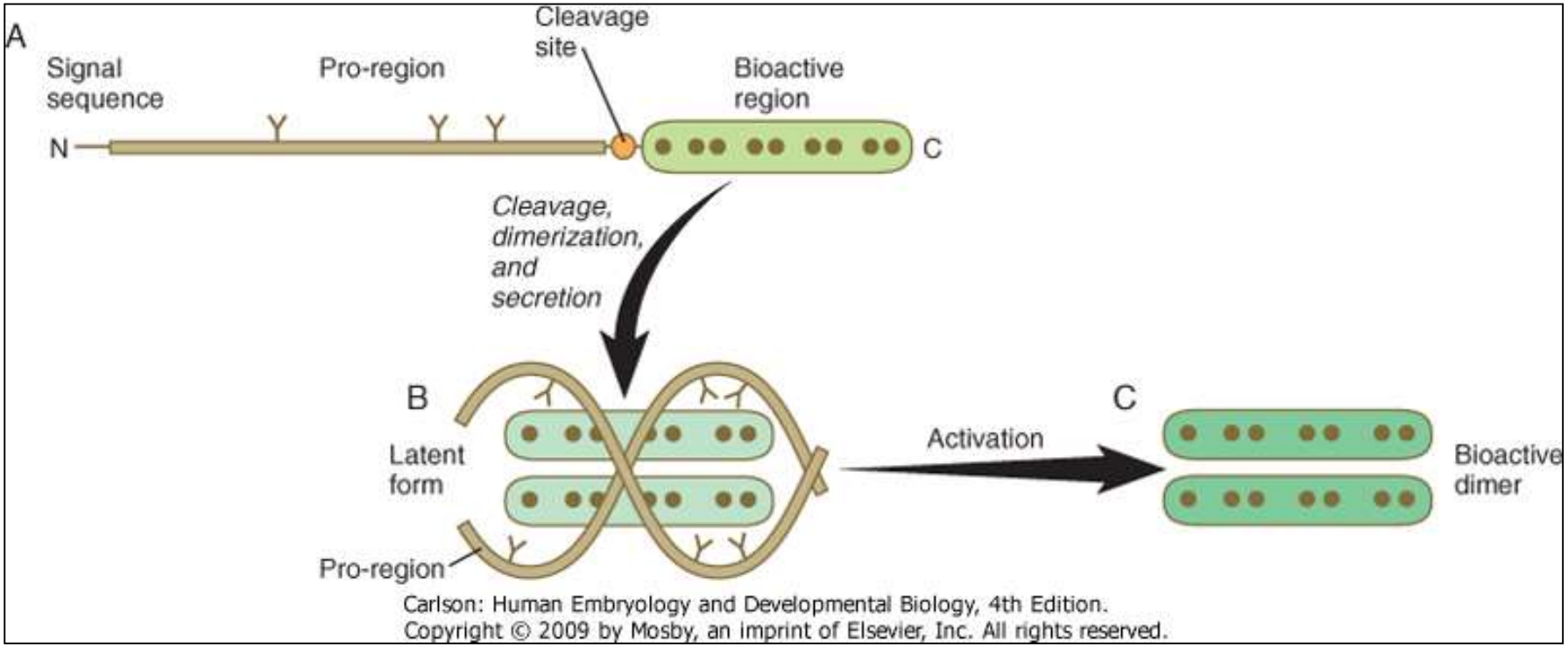
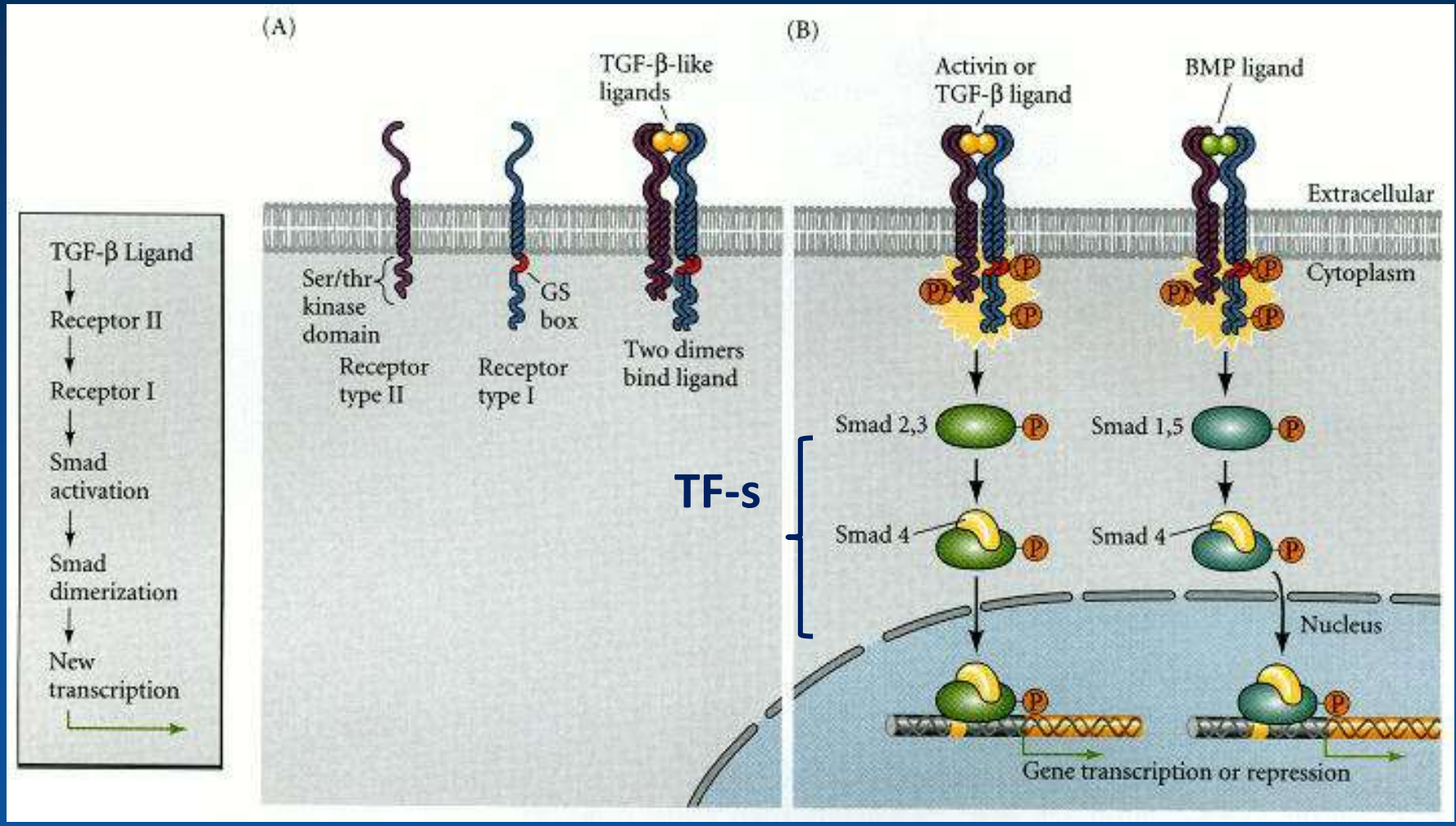


Figure 4-11 Steps in the activation of the growth factor, TGF-beta;1. A, The newly synthesized peptide consists of a C-terminal bioactive region, to which is attached a long glycosylated proregion and an N-terminal signal sequence. B, The proregion is cleaved off from the bioactive region, and two secreted bioactive regions form a dimer that is maintained in a latent form by being complexed with the separated proregions. C, Through an activation step, the bioactive dimer is released from the proregions and can function as a signaling molecule.

TGFβ/BMP signaling

-cytoplasmic serine/threonine kinase domain
-activates cytoplasmic Smad transcription factors by phosphorylation



One of the most important subfamilies is the bone morphogenetic proteins (BMPs).
Although BMP was originally discovered to be the active agent in the induction of bone during fracture healing, the 15 members of this group play important roles in development.

Inhibition of the inhibition (chordin, noggin, follistatin)

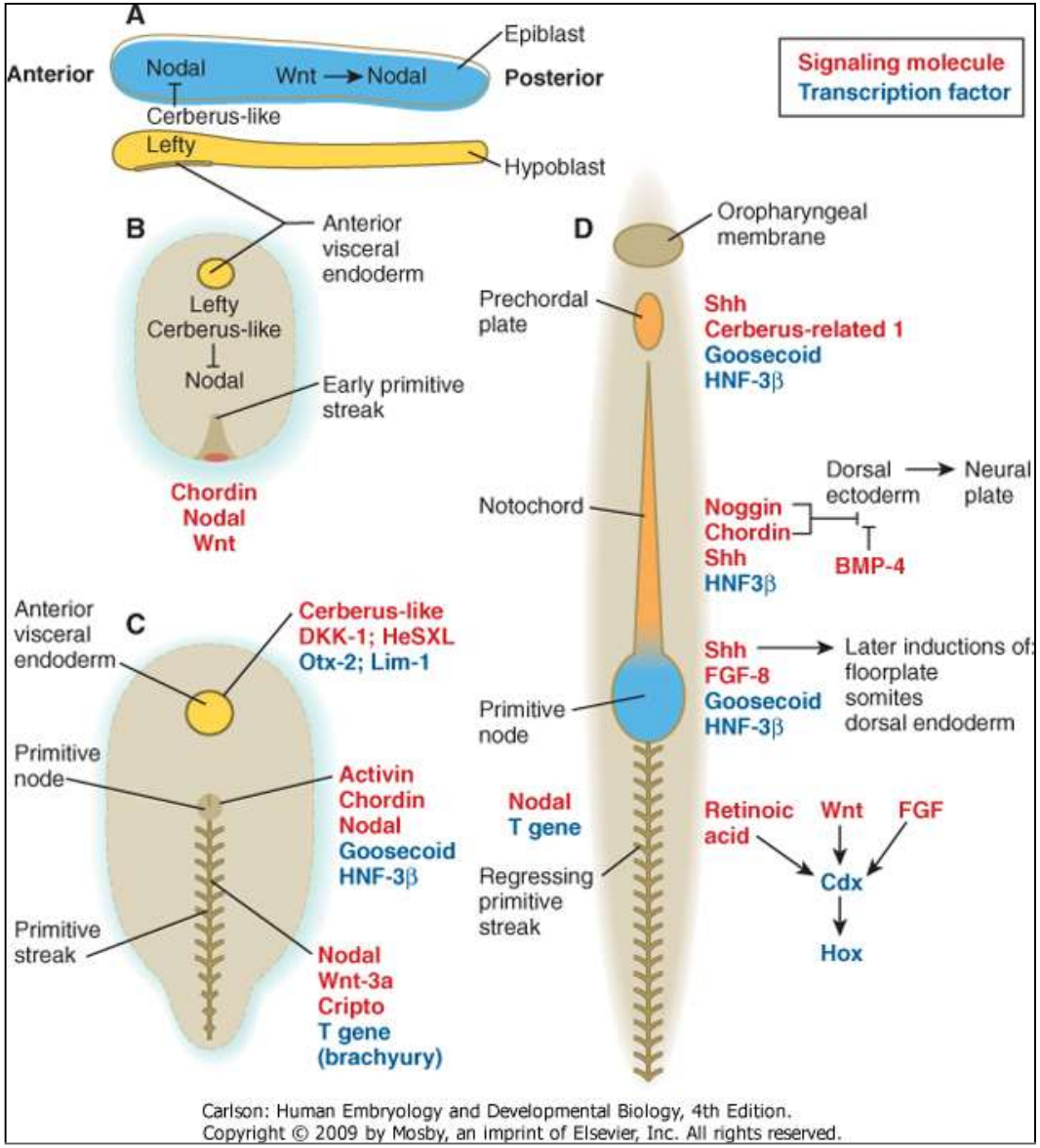
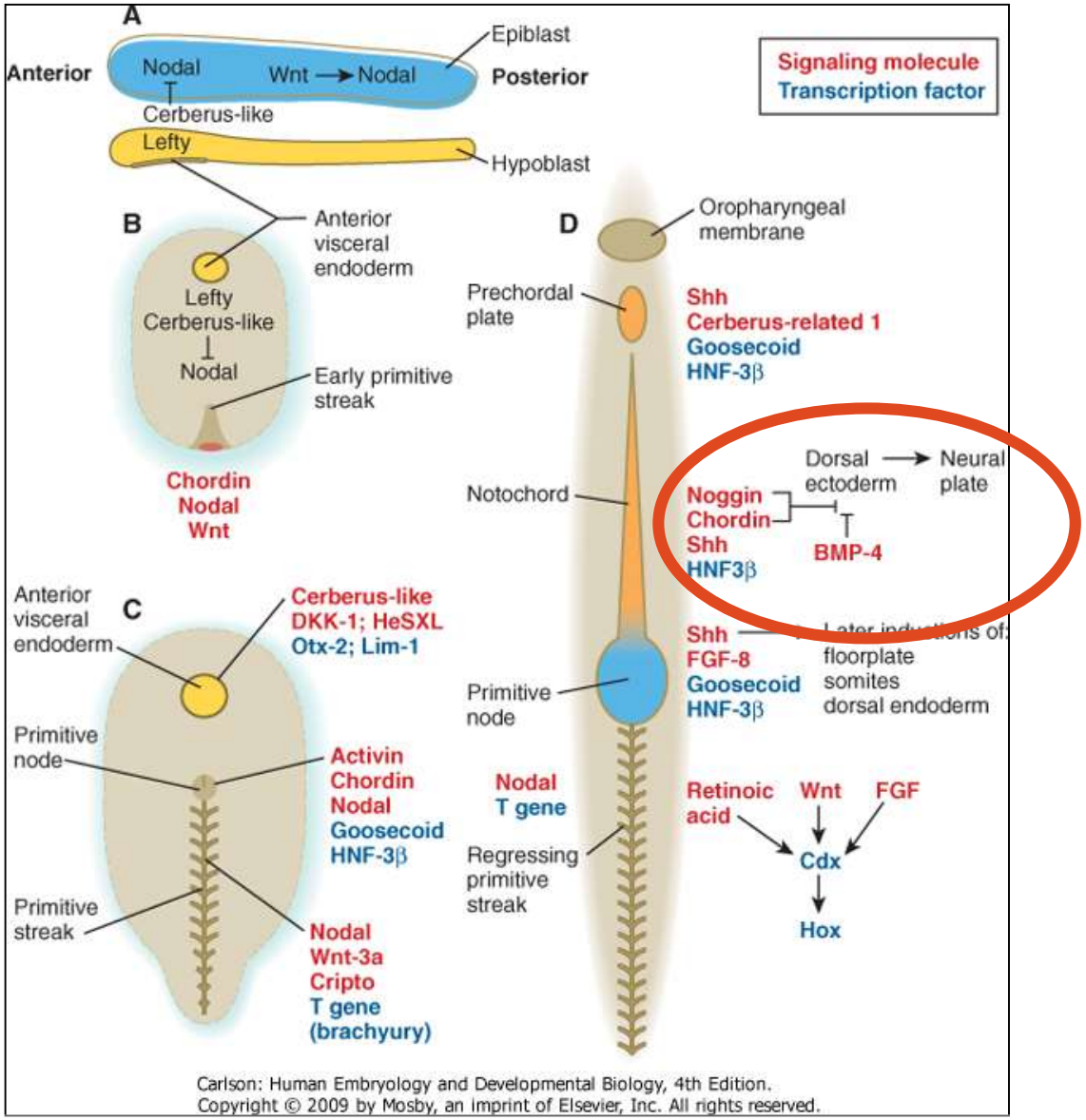


Figure 5-7 Summary of major genes involved in various stages of early embryonic development. A, Preprimitive streak (sagittal section). B, Early formation of the primitive streak. C, Gastrulation (period of germ layer formation). D, Late gastrulation and neural induction. The molecules in red are signaling molecules, and the molecules in blue are transcription factors. Names of specific molecules (bold) are placed by the structures in which they are expressed.



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FGF (fibroblast growth factor) ligands

Fibroblast growth factor (FGF) was initially described in 1974 as a substance that stimulates the growth of fibroblasts in culture. Since then, the originally described FGF has expanded into a family of 22 members, each of which has distinctive functions. Many members of the FGF family play important roles in a variety of phases of embryonic development and fulfilling functions, such as the stimulation of capillary growth, in the postnatal body.

Secreted FGFs are closely associated with the extracellular matrix and must bind to heparan sulfate to activate their receptors.

Regulation:

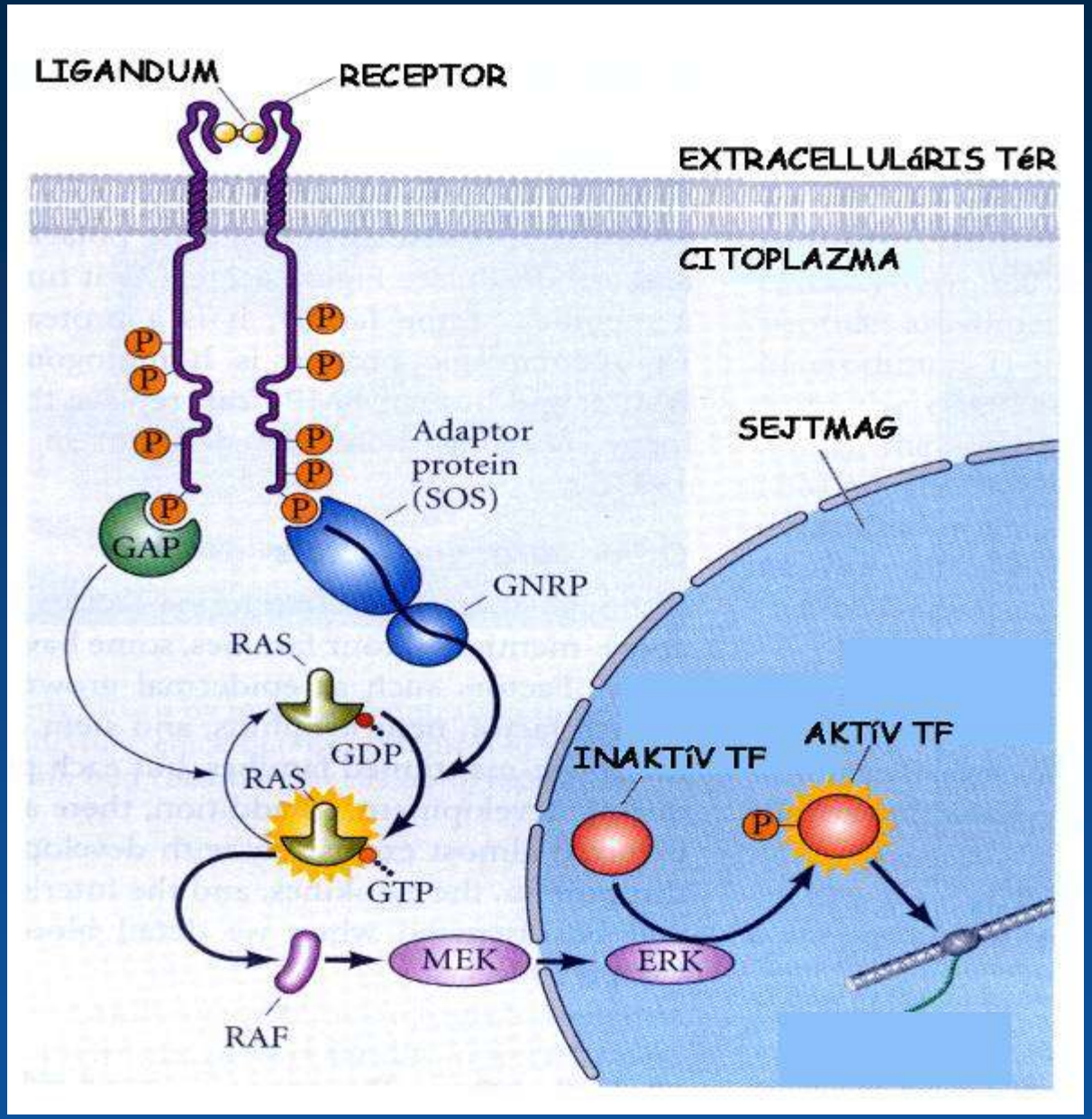
(1) modifications of their interaction with heparan proteoglycans in the receptor complex;
(2) regulation at the membrane of the responding cell through the actions of transmembrane proteins; and
(3) intracellular regulation by molecules, such as **sprouty**, which complex with parts of the signal transduction machinery of the responding cell.

FGFs generally are positive regulators of growth!

RTK (receptor tyrosine kinase) signaling pathways

-tyrosine kinase domain in cytosol

-activation of cytoplasmatic kinases (eg. MAP-kinases) which activates TFs in nucleus



VEGF ligands and their interaction with membrane-bound and soluble (secreted) VEGF receptors.

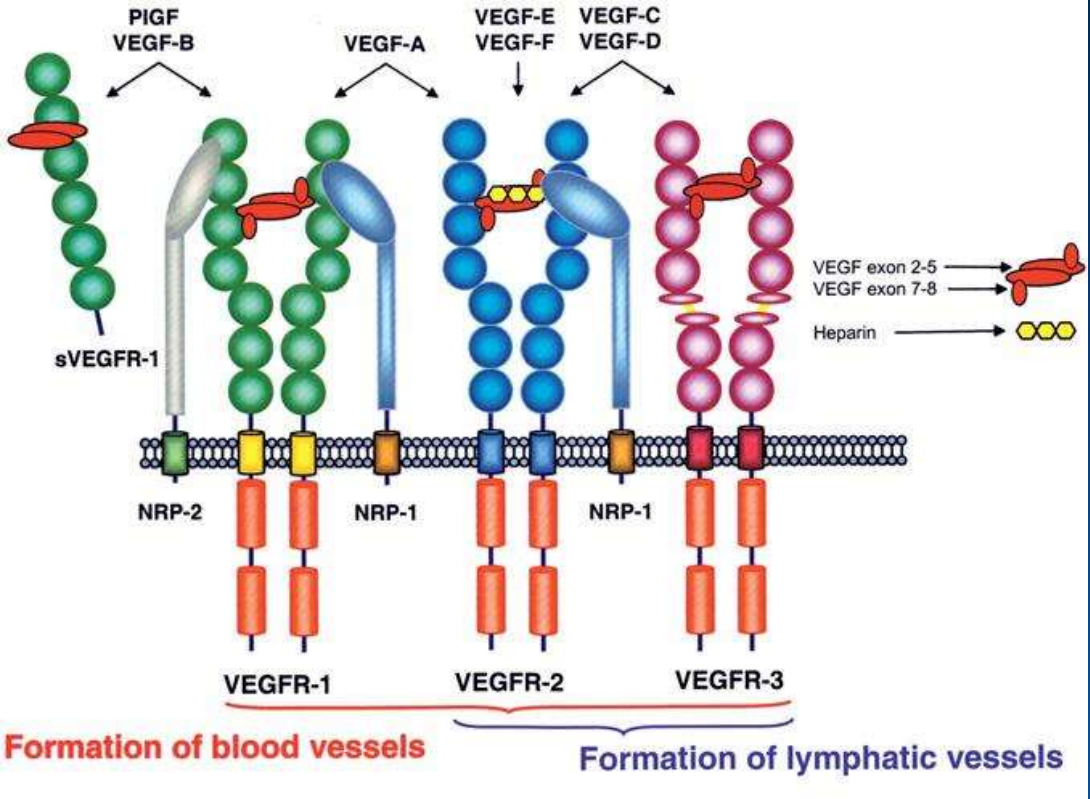


Figure 5.
Schematic presentation of the VEGF ligands and their interaction with membrane-bound and soluble (secreted) VEGF receptors. Notably, inhibitory effects are exerted by sVEGFR-1 (anti-hemangiogenesis) and sVEGFR-2 (anti-lymphangiogenesis). Neuropilin-2 (NRP-2) also interacts with VEGFR-3 on lymphatic endothelial cells, which is not shown in this scheme . NRP-1: Neuropilin-1.
Modified from:
http://www.rosenthallab.com/gallery/images/VEGF_VEGFR.jpeg
Pavlakovic et al. Page 14
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Ann N Y Acad Sci. 2010 October ; 1207(Suppl 1): E7–15. doi:10.1111/j.1749-6632.2010.05714.x.

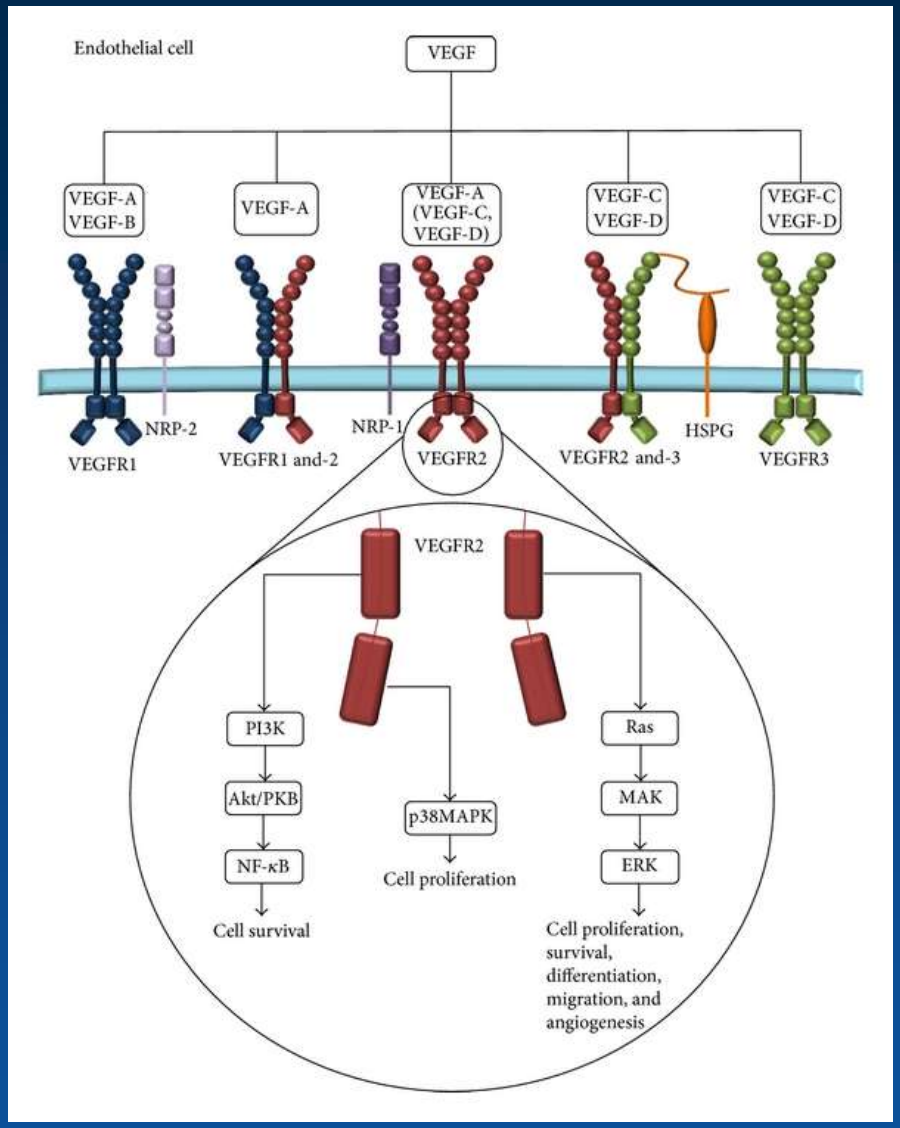
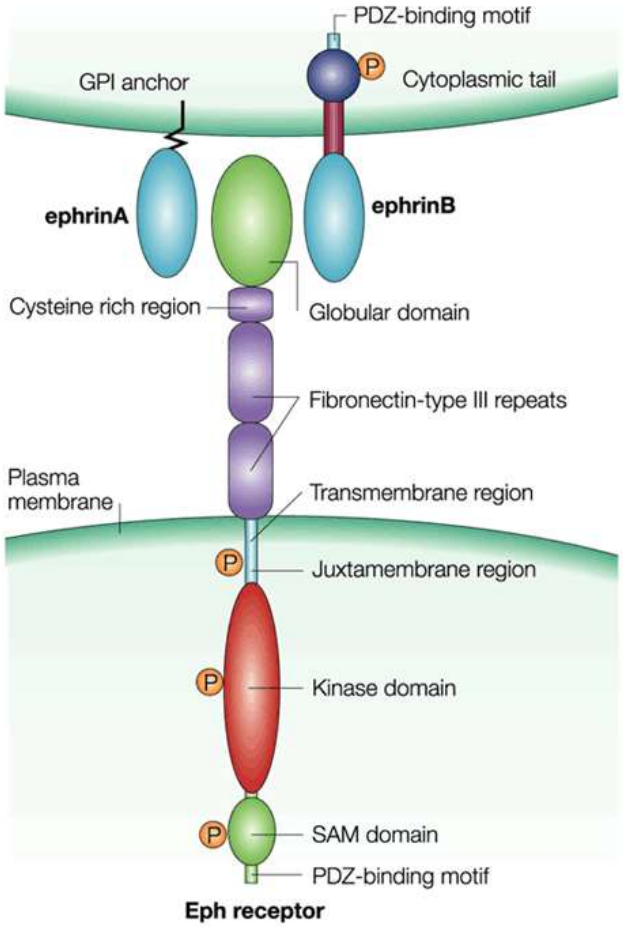


Figure 1: VEGF receptor binding. The five mammalian vascular endothelial growth factors (VEGF-A-D) bind to the receptor tyrosine kinases, VEGF receptor (VEGFR1-3 and co-receptors HSPG, NRP-1 and NRP-2). VEGFR-binding leads to the formation of homodimers and/or heterodimers. Proteolytic cleavage enables VEGF-C and -D to bind VEGFR-2 forming a homodimer. The binding and activation of VEGFR-2 lead to downstream signaling of the PI3 K, MAPK, and Ras pathways which promote cell survival, proliferation, differentiation, migration, and angiogenesis.

Nature Reviews Molecular Cell Biology **3**, 475-486 (July 2002) | doi:10.1038/nrm856



The tyrosine kinase receptors, Ephs (erythropoietin-producing hepatocyte kinases) and their ligands, ephrins (Eph receptor interaction proteins), are molecules known to be involved in the regulation of numerous biological systems in which cell-to-cell interactions are particularly relevant.

In contrast with most receptor tyrosine kinases, unidirectional signalling can originate from the ephrin ligands as well as from the Eph receptors. Furthermore, the concept of bidirectional signalling has emerged as an important mechanism by which Ephs and ephrins control the output signal in processes of cell–cell communication.

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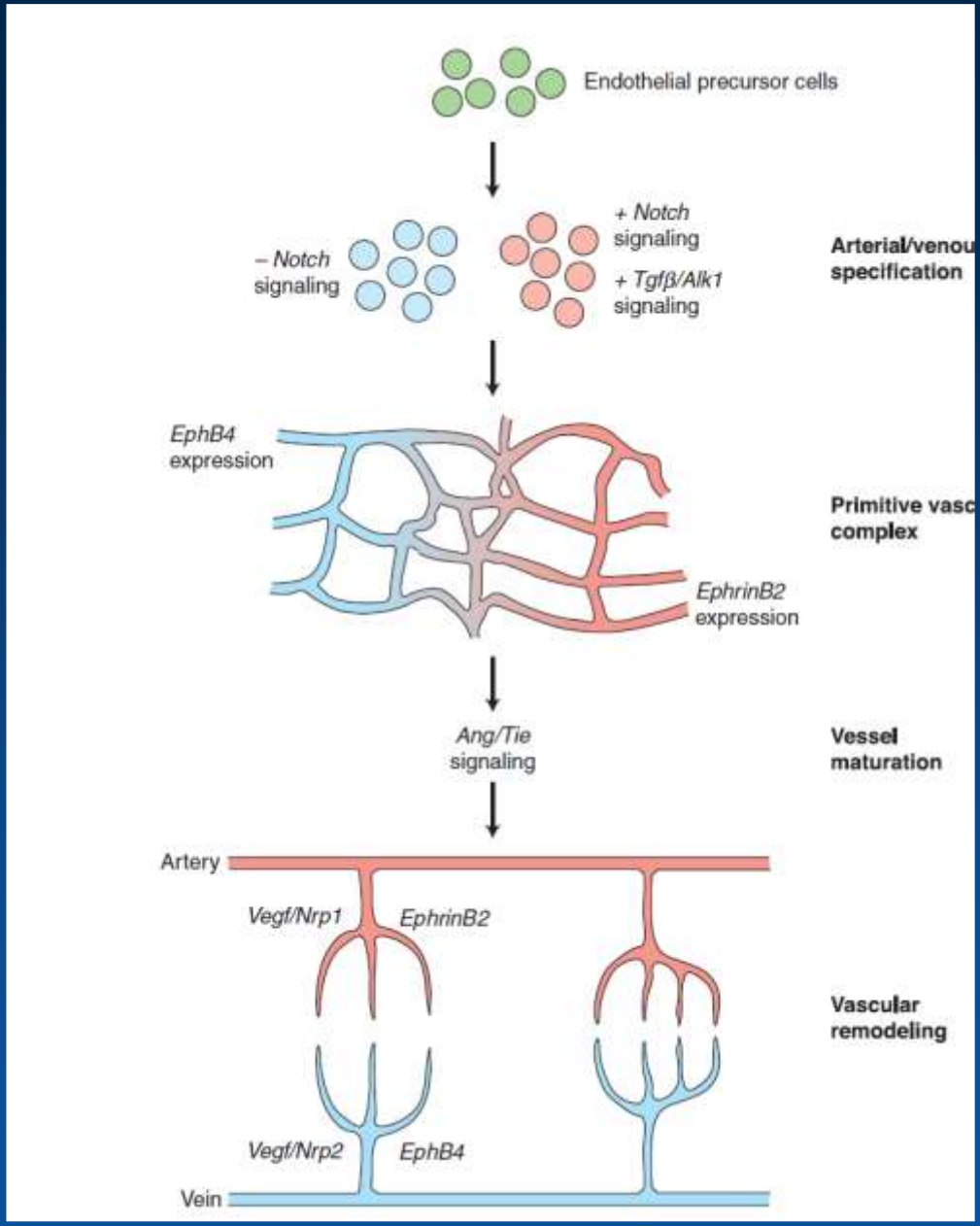

Endothelial precursor cells
– Notch signaling
+ Notch signaling
+ Tgfβ/Alk1 signaling
Arterial/venou specification
EphB4 expression
EphrinB2 expression
Primitive vasc complex
Ang/Tie signaling
Vessel maturation
Artery
Vegf/Nrp1
EphrinB2
Vegf/Nrp2
EphB4
Vein
Vascular remodeling

Comparison of RTK (receptor tyrosine kinase) and TGFβ-type signaling pathways

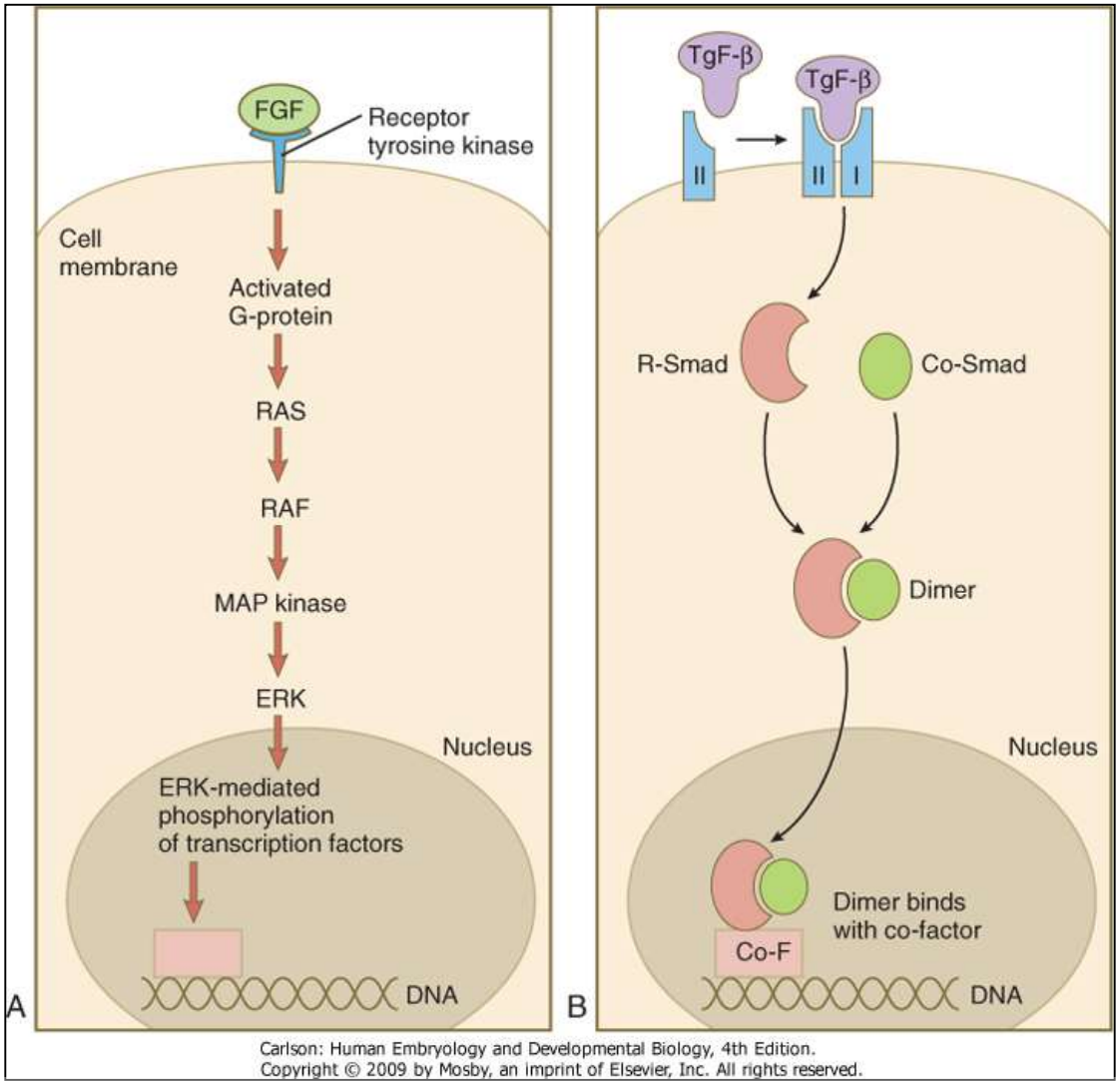


Figure 4-15 A, Fibroblast growth factor (FGF) and the receptor tyrosine kinase signal transduction pathway. B, Transforming growth factor-β (TGF-β) binding to a type II serine/threonine kinase receptor and activating a downstream pathway involving Smad proteins.

The Hedgehog ligands

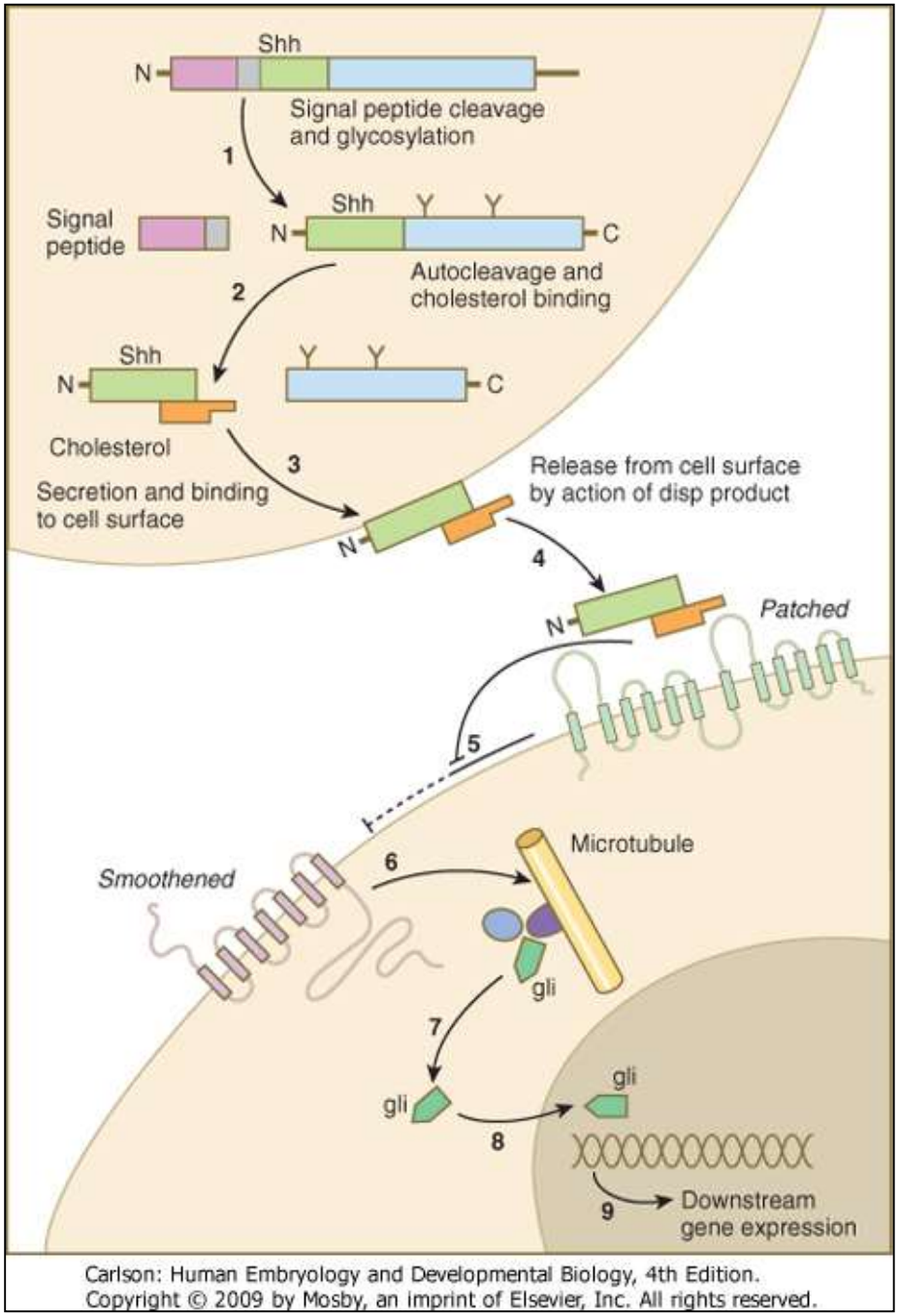
Segment-polarity molecule, hedgehog, in *Drosophila,*
the three mammalian hedgehogs: desert, Indian, and sonic hedgehog.
The name hedgehog arose because mutant larvae in *Drosophila* contain thick bands of spikey outgrowths on their bodies.

Sonic hedgehog (**shh**) is a protein with a highly conserved N-terminal region and a more divergent C-terminal region. After its synthesis and release of the propeptide from the rough endoplasmic reticulum, the signal peptide is cleaved off, and glycosylation occurs on the remaining peptide. Still within the cell, the shh peptide undergoes autocleavage through the catalytic activity of its C-terminal portion. During cleavage, the N-terminal segment becomes covalently bonded with cholesterol. The 19-kD N-terminal peptide is secreted from the cell, but remains bound to the surface of the cell that produced it. All of the signaling activity of shh resides in the N-terminal segment. Through the activity of another gene product (disp [dispatched] in *Drosophila*), the N-terminal segment of shh, still bound with cholesterol, is released from the cell. The C-terminal peptide plays no role in signaling.

Wild type

and poor mutant….

http://www.outdoorphoto.co.za/gallery/showphoto.php?photo=126954



Carlson: Human Embryology and Developmental Biology, 4th Edition.

Figure 4-12 **The sonic hedgehog (shh) signaling pathway.** (1) The signal peptide is cleaved off the newly synthesized polypeptide, and the remainder undergoes glycosylation. (2) The remaining peptide undergoes autocleavage under the influence of the C-terminal portion, and cholesterol binds to the N-terminal part, which is the active part of the molecule. (3) The N-terminal part is secreted and bound to the cell surface. (4) The bound shh molecule is released from the cell surface through the action of a product of dispersed (disp). (5) The released shh inhibits the inhibitory effect of Patched on Smoothened. (6) On release from the inhibitory influence of Patched, Smoothened emits a signal that (7) releases the transcription factor Gli from a complex of molecules bound to microtubules. (8) Gli enters the nucleus and binds to the DNA, (9) influencing the expression of many genes.

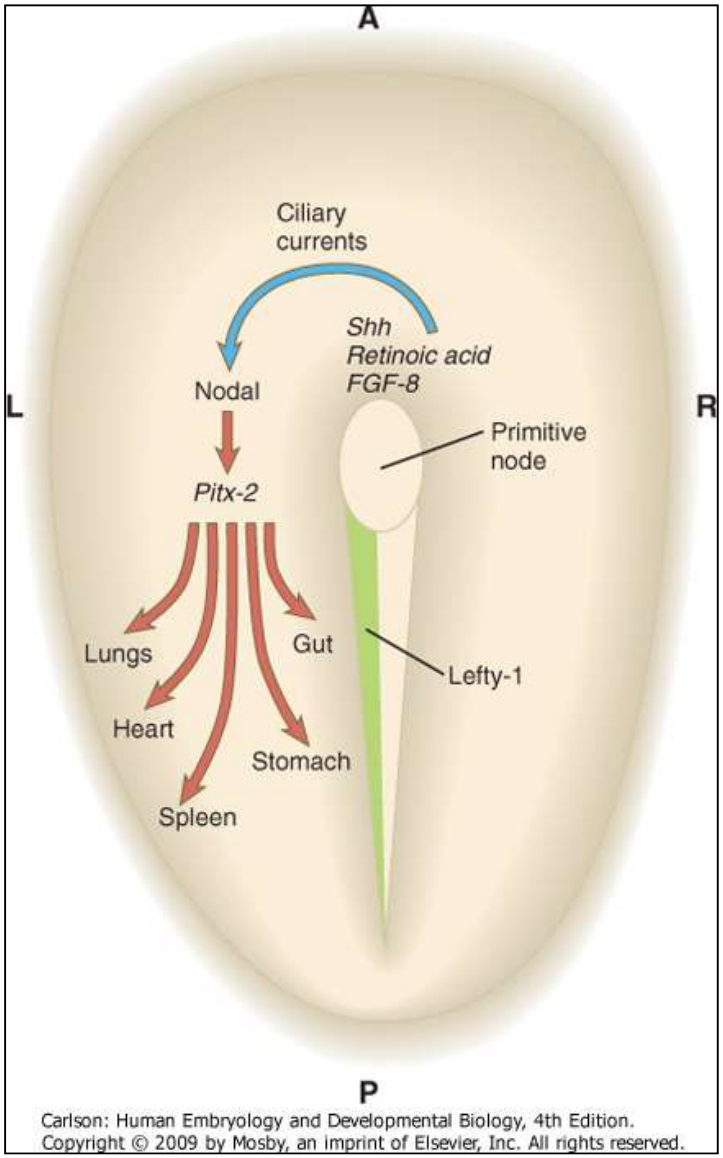


Figure 5-10 Summary of the molecular basis for body asymmetry. Ciliary currents at the primitive node sweep the symmetry-breaking molecules sonic hedgehog (shh) and fibroblast growth factor-8 (FGF-8) toward the left side of the embryo, where they stimulate an asymmetric cascade of gene expression starting with nodal. Lefty-1, expressed along the left side of the primitive streak, may prevent diffusion of molecules to the right side of the embryo. Only the most important molecules in a complex cascade are shown.

Wnt signaling pathway

The **Wnt** family of signaling molecules is a complex one, with **18 members** represented in the mouse. Related to the segment-polarity gene ***Wingless* in *Drosophila*,** Wnts seem to play dramatically different roles in different classes of vertebrates. In amphibians, Wnts are essential for dorsalization in the very early embryo, whereas their role in preimplantation mouse development seems to be minimal. In mammals, Wnts play many important roles during the period of **gastrulation.** As many organ primordia begin to take shape, active Wnt pathways **stimulate the cellular proliferation** that is required to bring these structures to their normal proportions. Later in development, they are involved in a variety of processes relating to cellular differentiation and polarity.

The **Wnt pathway** is a complex one that first involves the Wnt molecule binding to its transmembrane receptor, **Frizzled**. In a manner not yet completely understood, Frizzled interacts with the cytoplasmic protein **Disheveled**, which ties up a complex of numerous molecules (**destruction complex**), which in the absence of Wnt cause the degradation of an important cytoplasmic protein, **β-catenin** (Fig. 4-16). If β-catenin is not destroyed, it enters the nucleus, where it acts as a powerful adjunct to transcription factors that determine patterns of gene expression.

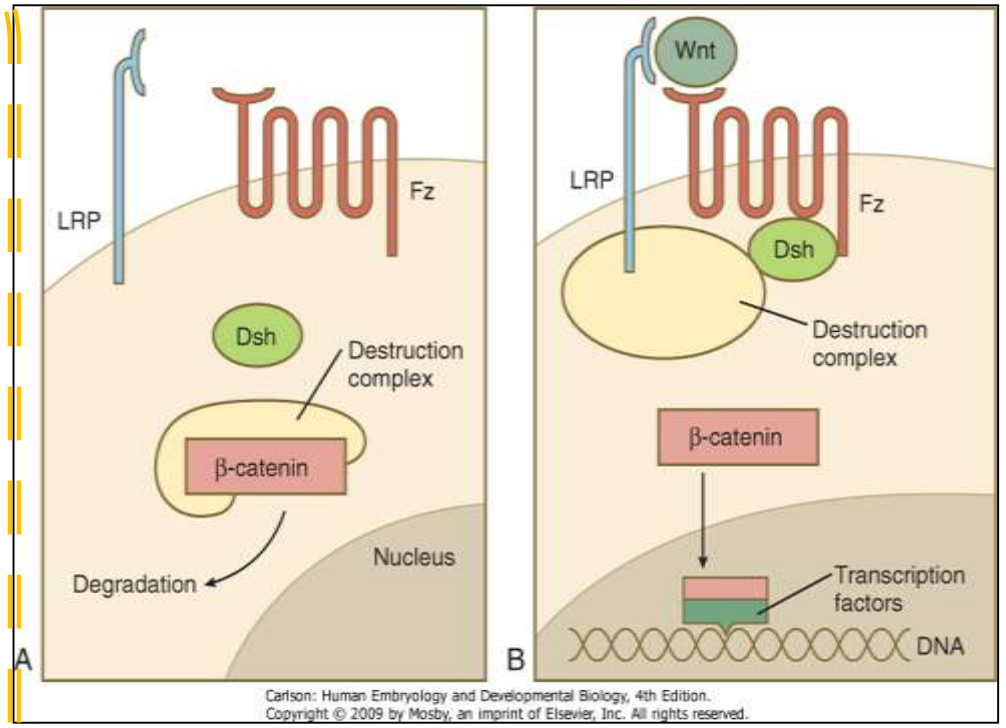
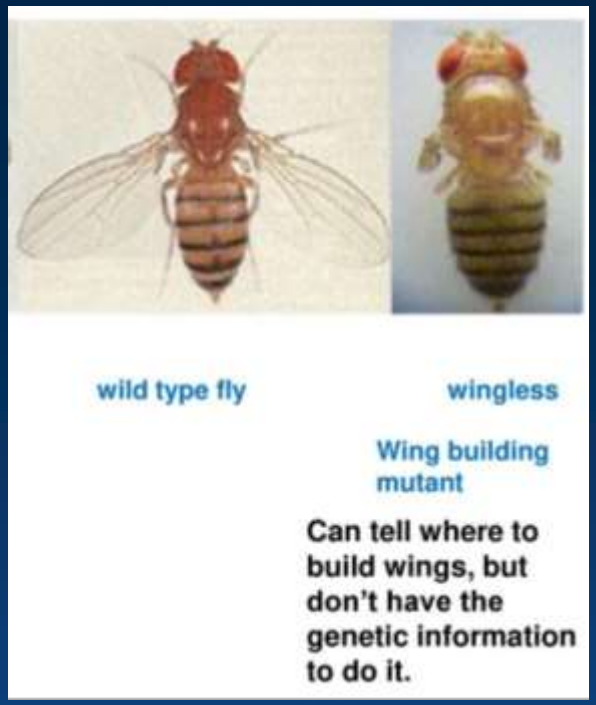
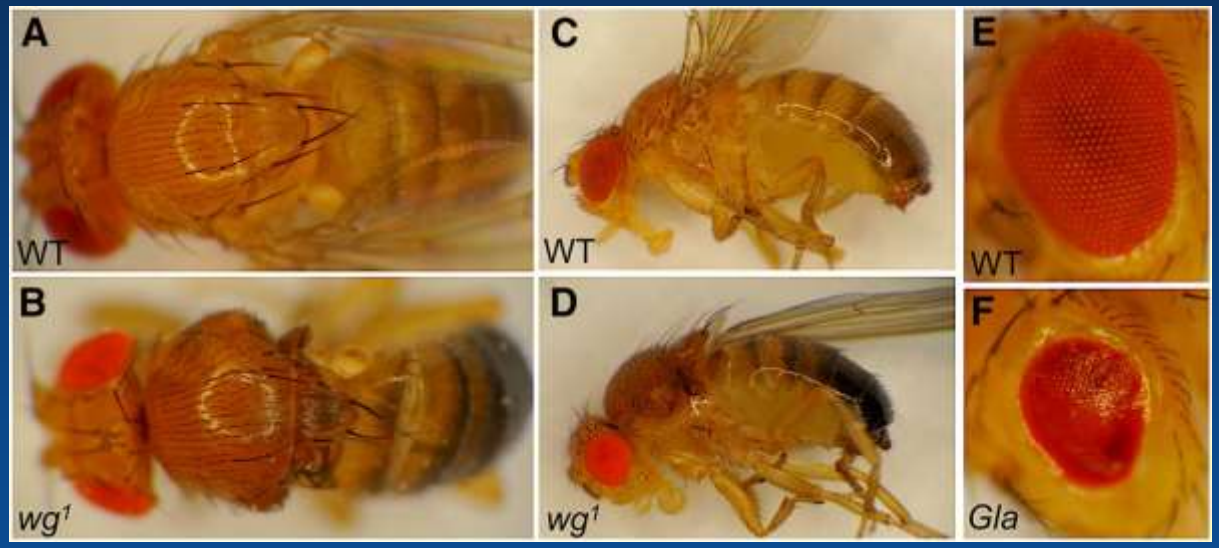


Figure 4-16 The Wnt signaling pathway, operating through β-catenin. A, In the absence of a Wnt signal, β-catenin is bound in a destruction complex and is degraded. B, In the presence of Wnt, the receptor Frizzled (Fz) activates Disheveled (Dsh), which prevents the destruction complex from degrading β-catenin. β-catenin then enters the nucleus, where it forms complexes with transcription factors.

Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility
https://www.nature.com/articles/nrg2042

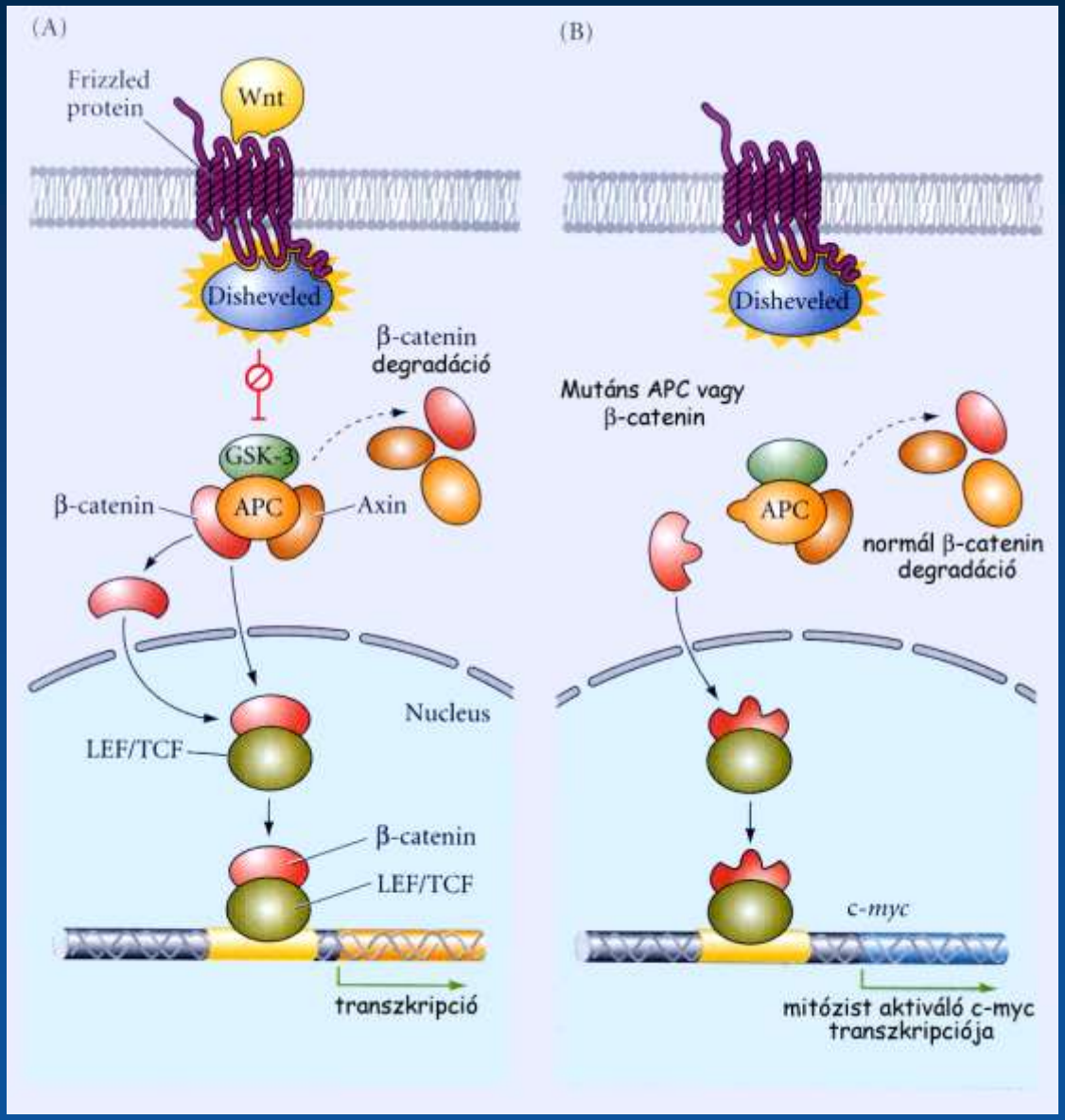
Wingless mutant





Viable *wg* mutant phenotypes. (A) Normal bristle pattern on the notum, the back of a fly's thorax, with both halteres visible out of focus at the posterior edge (in this and all images, posterior is to the right). (B) Notum of a wg^1 homozygous mutant showing disrupted pattern and absence of both wings and one haltere. (C) Side view of wild-type (WT) fly. (D) Side view of wg^1 homozygous mutant showing duplicated notum in place of one missing wing, and misshapen eye (*cinnabar* eye color is not part of the *wg* phenotype). (E) WT eye shows a regular pattern of ommatidia, the units of the compound eye. (F) The eye of a fly heterozygous for the *Gla* mutation shows a smooth "glazed" surface.

Wnt and APC (adenomatous polyposis coli)



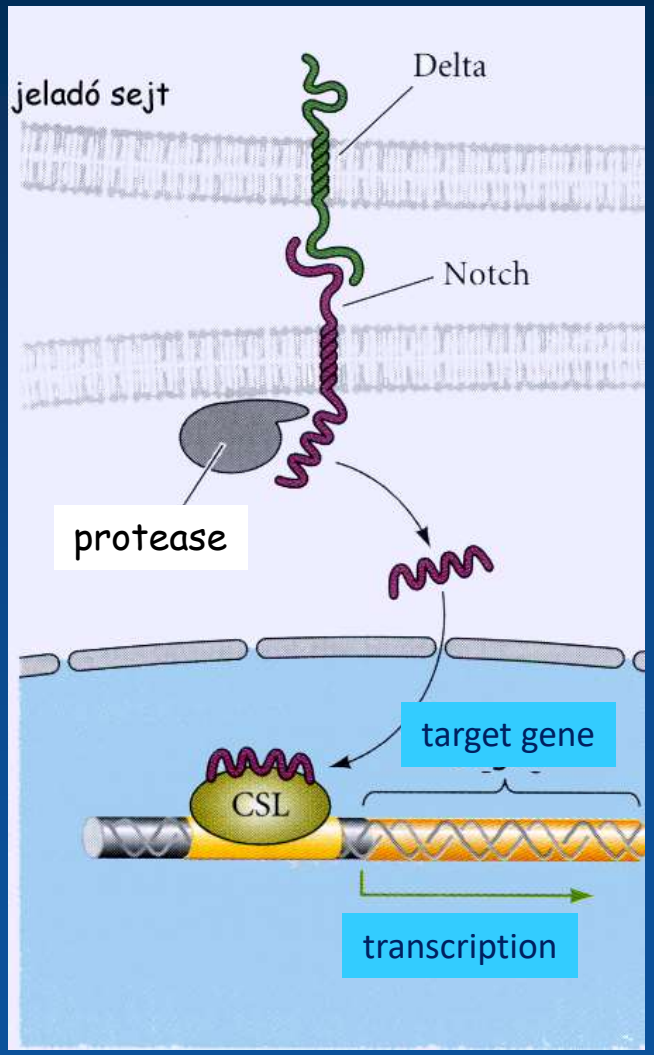
The Notch-Delta pathway: juxtaposed ligands and receptors

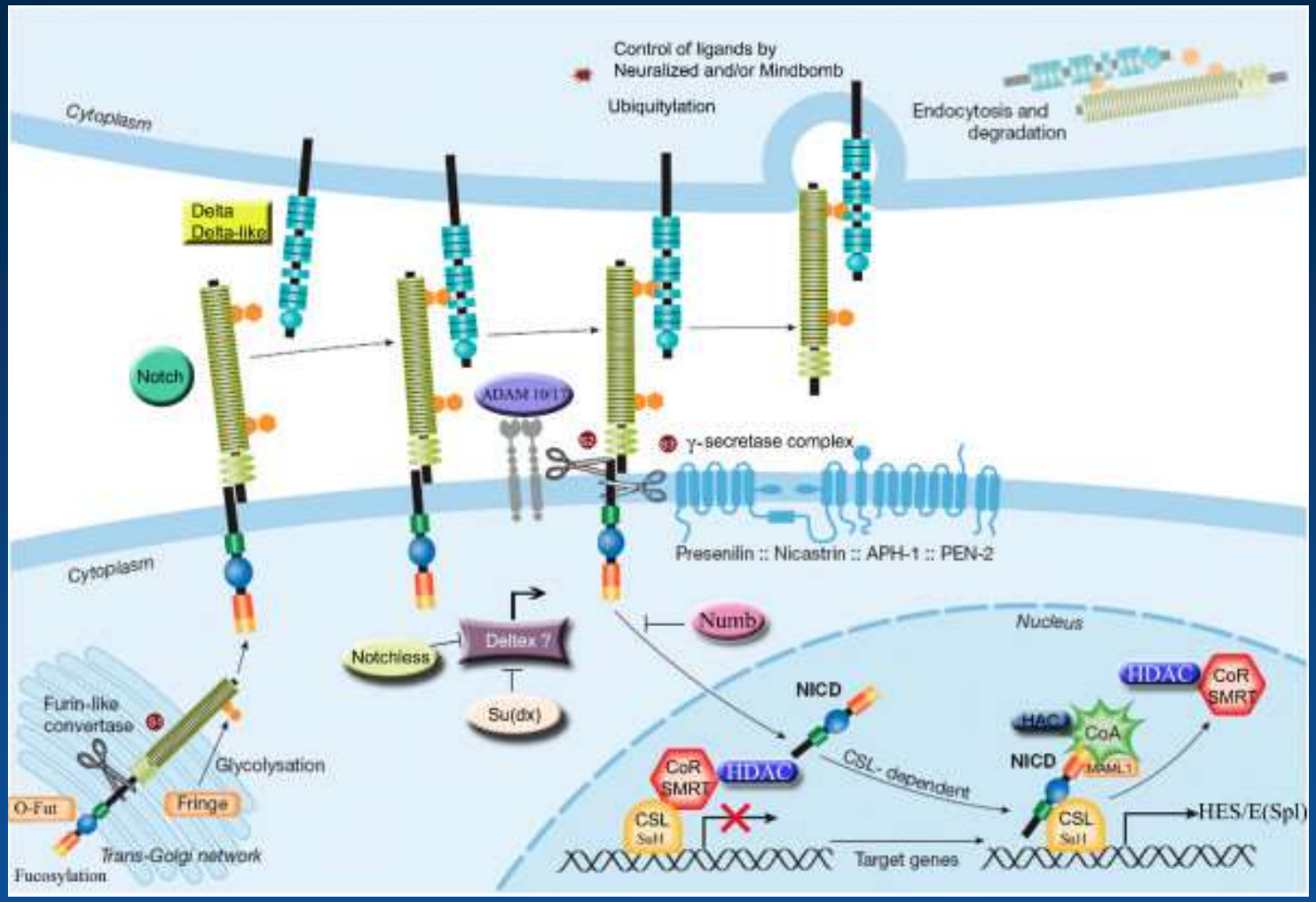
-juxtacrine signaling (between membrane-bound molecules)

-ligands in mammals: Delta, Serrate, Jagged
receptor: Notch)

Role:

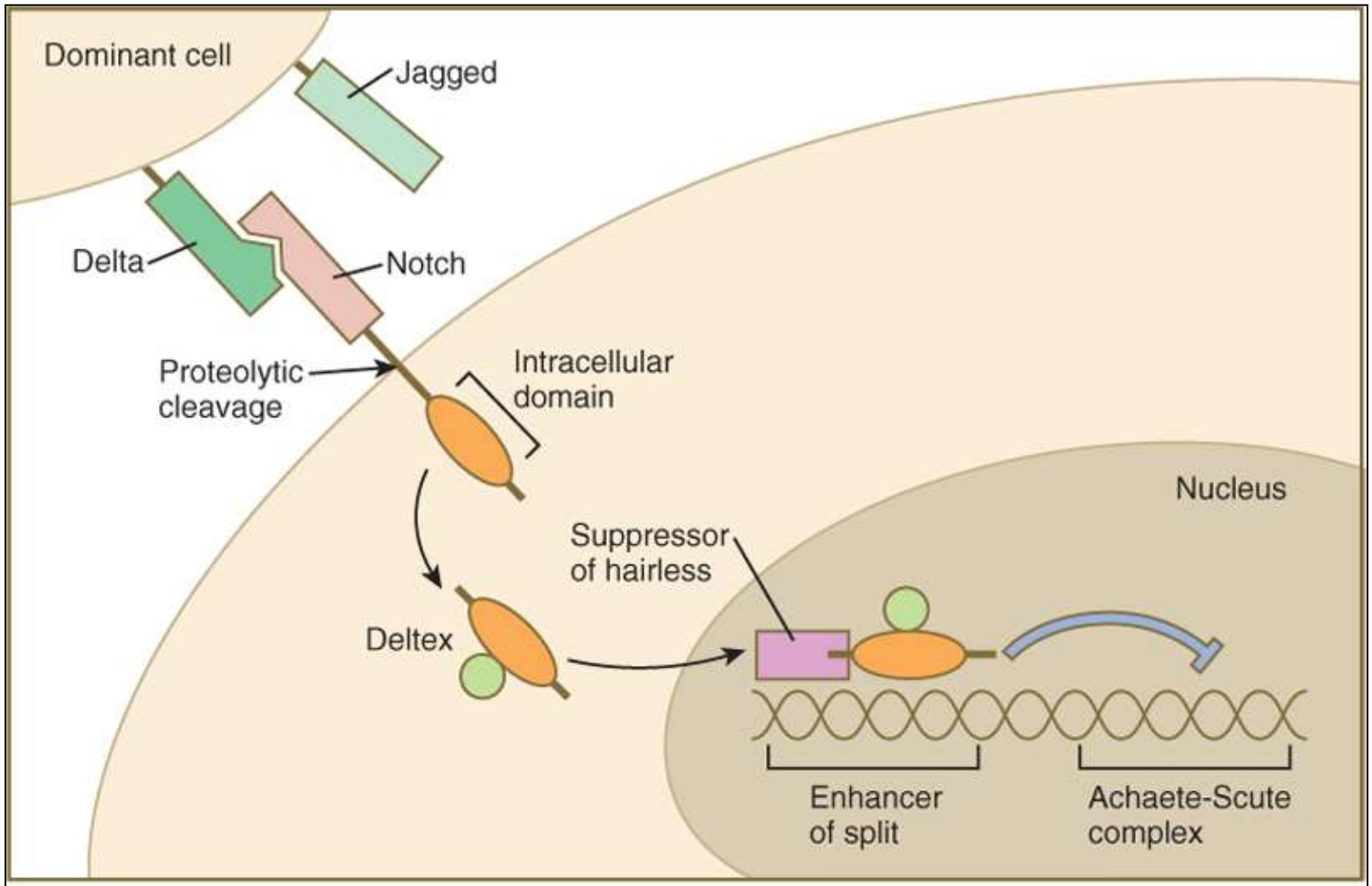
-Cell fate decisions by lateral inhibition
(eg. Drosophila neurogenesis)





http://deltanotch.webnode.com/introduction-to-delta-notch-signalling/

Figure 4-14 The Delta-Notch pathway. When Delta from a dominant cell binds to Notch on the surface of the neighboring cell, proteolytic cleavage releases the intracellular domain of Notch, which complexes with Deltex and enters the nucleus. There it becomes linked to Suppressor of hairless and serves as a transcription factor, which binds to Enhancer of split. This sends off an inhibitory influence that represses the expression of genes, such as the Achaete-Scute complex, which would otherwise promote differentiation (neurogenesis).



Lateral Inhibition and the Notch Receptor

The normal development of many tissues begins with a population of developmentally equivalent cells. At some point, one of these cells begins to differentiate into a dominant mature cell type, such as a neuron, and, in doing so, it transmits to its neighboring cells a signal that prevents them from differentiating into that same cell type. As a consequence, these neighboring cells are forced to differentiate into a secondary cell type, such as a glial cell in the central nervous system . This type of signaling of a dominant cell to its subservient neighbors is called **lateral inhibition**.

The common mechanism of lateral inhibition is the **Notch** signaling pathway, which is so basic that it has been preserved largely unchanged throughout the animal kingdom. Notch is a 300-kD cell surface receptor with a large extracellular domain and a smaller intracellular domain.
The Notch receptor becomes activated when it combines with ligands (**Delta** or **Jagged** in vertebrates) that extend from the surface of the dominant cell. This sets off a pathway that inhibits the neighboring cell from differentiating into the dominant phenotype.An abbreviated version of this pathway is as follows : The complexing of Notch with its ligand (e.g., Delta) stimulates an intracellular protease reaction that cleaves off the intracellular domain of the Notch molecule. The liberated intracellular domain of Notch becomes translocated to the nucleus, but on its way it may become associated with regulatory proteins, such as **Deltex**.
Within the nucleus, the intracellular domain of Notch combines with several helix-loop-helix transcription factors, and this complex binds to the DNA of a gene called ***enhancer of split***. The product of this gene is another transcription factor that regulates other genes. It represses certain genes of the ***Achaete-Scute*** complex, whose function is to promote neuronal development. By this complex pathway, the subservient cells are denied the opportunity to differentiate into neurons and they instead follow a secondary pathway, which leads to their becoming glial cells.

As complex as this seems, the above text is a greatly abbreviated version of this inhibitory pathway and its controlling elements. When more is learned about all of the elements involved in this pathway, it will likely look like a component of an immense network of regulatory pathways that interact in very complex ways to integrate internal and external environmental influences that determine the ultimate developmental fate of a cell.

Figure 4-13 **An example of lateral inhibition.** (1) A population of developmentally equivalent cells. (2) One cell, whether by its position or through stochastic (random) factors, begins to develop along a dominant pathway before its neighbors. (3) The selected cell gives off inhibitory signals (lateral inhibition) that prevent its neighbors from differentiating into the dominant cell type. (4) The selected cell differentiates into a mature cell type (e.g., a neuron), whereas its neighbors differentiate into secondary phenotypes (e.g., glial cells).

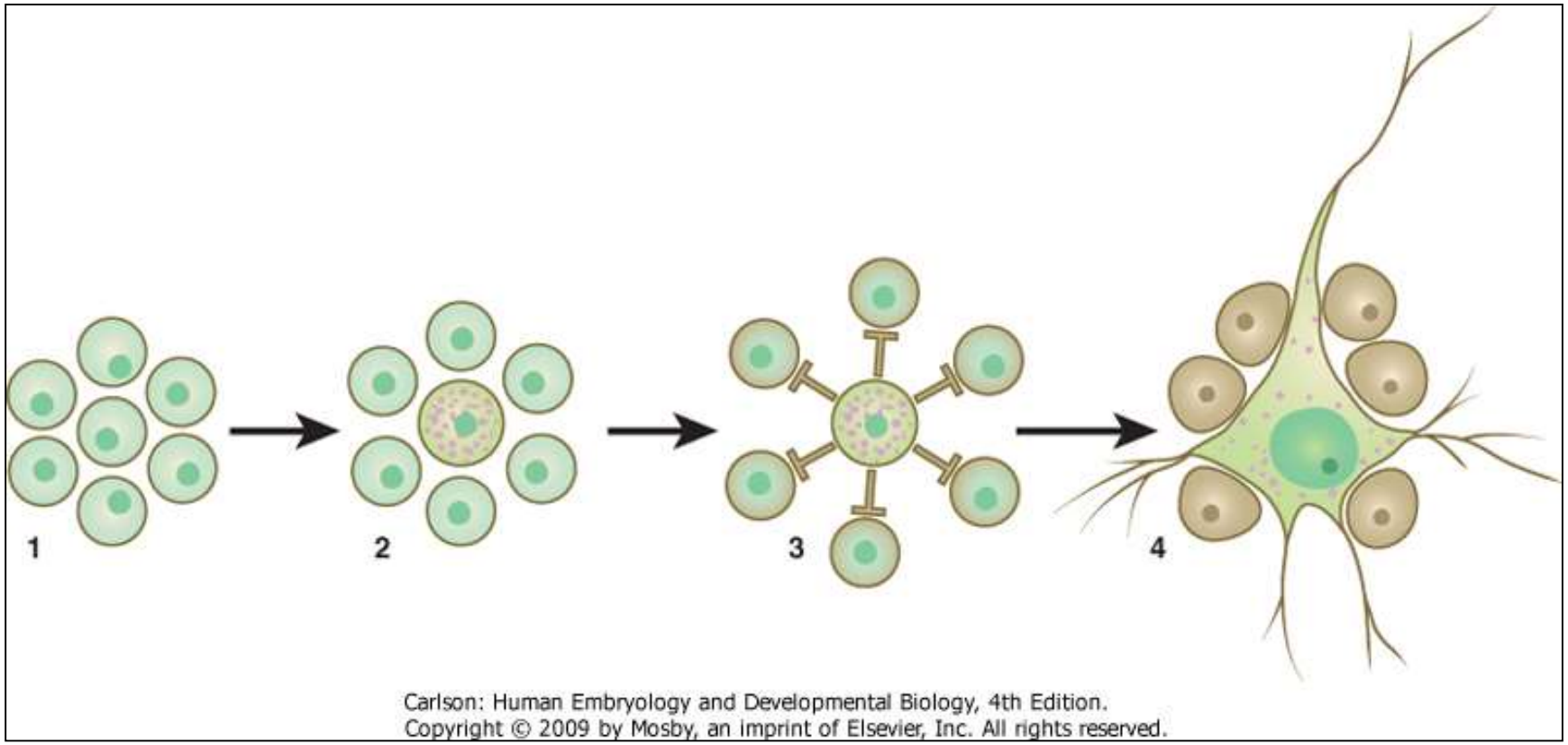
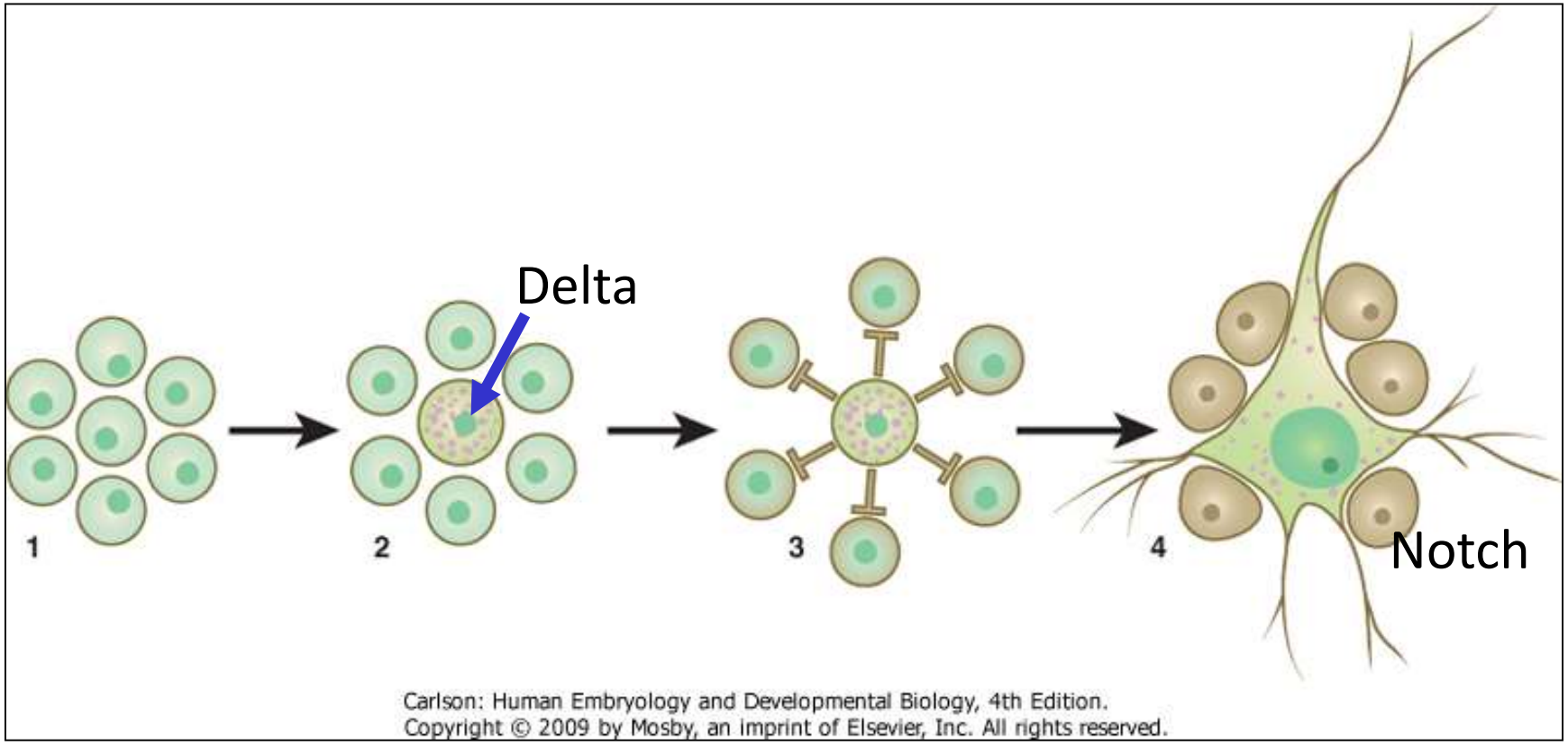
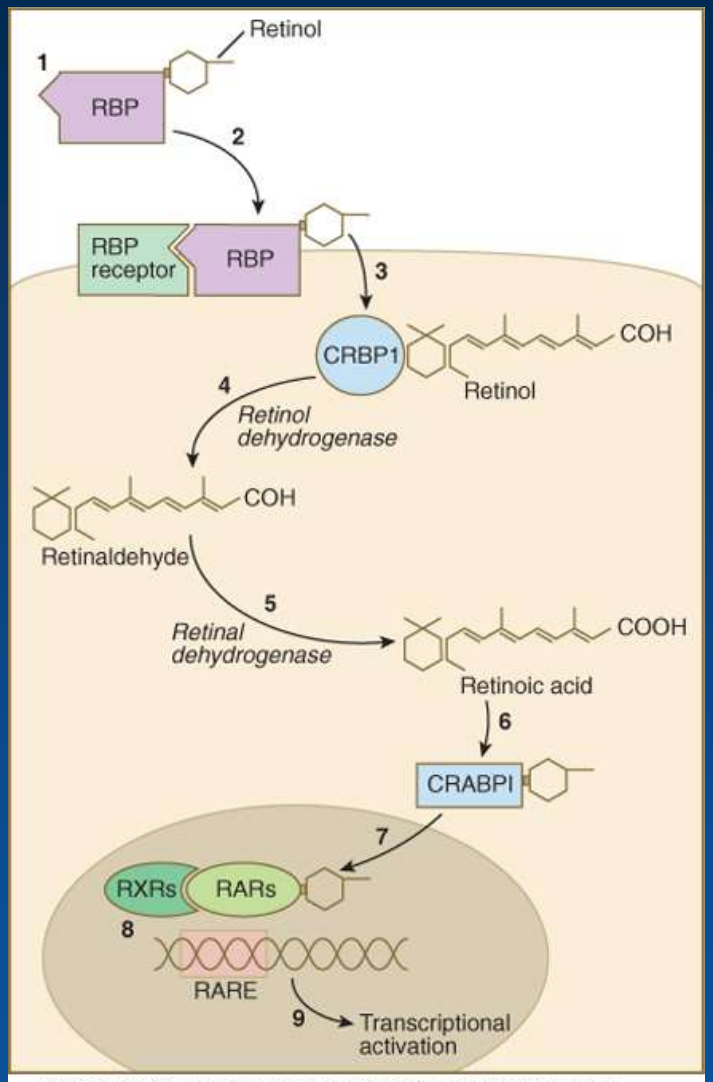


Figure 4-13 An example of lateral inhibition. (1) A population of developmentally equivalent cells. (2) **One cell, whether by its position or through stochastic (random) factors,** begins to develop along a dominant pathway before its neighbors. (3) The selected cell gives off inhibitory signals (lateral inhibition) that prevent its neighbors from differentiating into the dominant cell type. (4) The selected cell differentiates into a mature cell type (e.g., a neuron), whereas its neighbors differentiate into secondary phenotypes (e.g., glial cells).



Vitamin A (retinol) and its metabolite (retinoic acid,RA)



Either a severe deficiency or an excess of vitamin A results in a broad spectrum of severe congenital anomalies that can involve the face, eye, hindbrain, limbs, or urogenital system.

Vitamin A enters the body of the embryo as retinol and binds to a retinol binding protein, which attaches to specific cell surface receptors . Retinol is released from this complex and enters the cytoplasm, where it is bound to **cellular retinol binding protein (CRBP I)**. In the cytoplasm, the all-trans retinol is enzymatically converted first to all-trans retinalde-hyde and then to all-trans retinoic acid, the retinoid with the most potent biological activity.
CRBP and **CRABP I (cellular retinoic acid binding protein)** may function to control the amount of retinoids that enters the nucleus. When released from CRABP, retinoic acid enters the nucleus, where it typically binds to a heterodimer consisting of a member of the **retinoic acid receptor** (**RAR**) α, β, or γ family and a member of the **retinoid X receptor** (**RXR**) α, β, or γ family. This complex of retinoic acid plus receptor heterodimer binds to a **retinoic acid response element** (**RARE**) on DNA, usually on the enhancer region of a gene, and it acts as a transcription factor, controlling the production of a gene product.

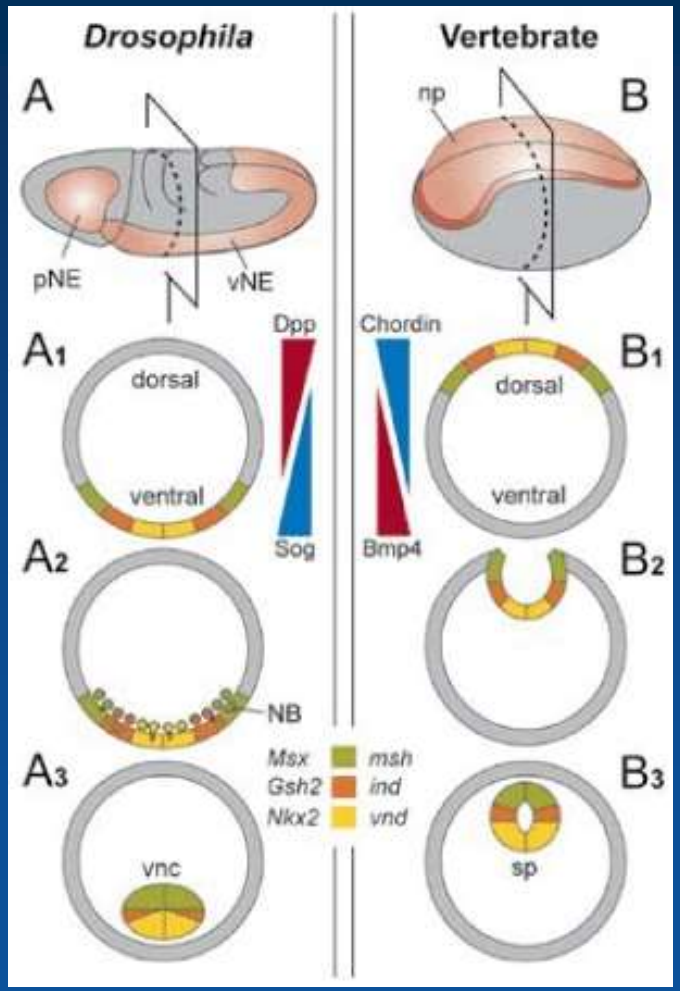
Retinoic acid is produced and used in specific local regions at various times during prenatal and postnatal life. Among its well-defined targets early in development are certain *Hox* genes (e.g., *Hoxb-1*), where misexpression caused by either too little or too much retinoic acid can result in serious disturbances in the organization of the hindbrain and pharyngeal neural crest.

RA acts on Hox genes and cause homeotic transformation

One of the most spectacular examples of the power of retinoic acid is its ability to cause extra pairs of limbs to form alongside the regenerating tails of amphibians (Fig. 4-18). This is a true example of a homeotic shift in a vertebrate, similar to the formation of double-winged flies or legs instead of antennae in *Drosophila*.

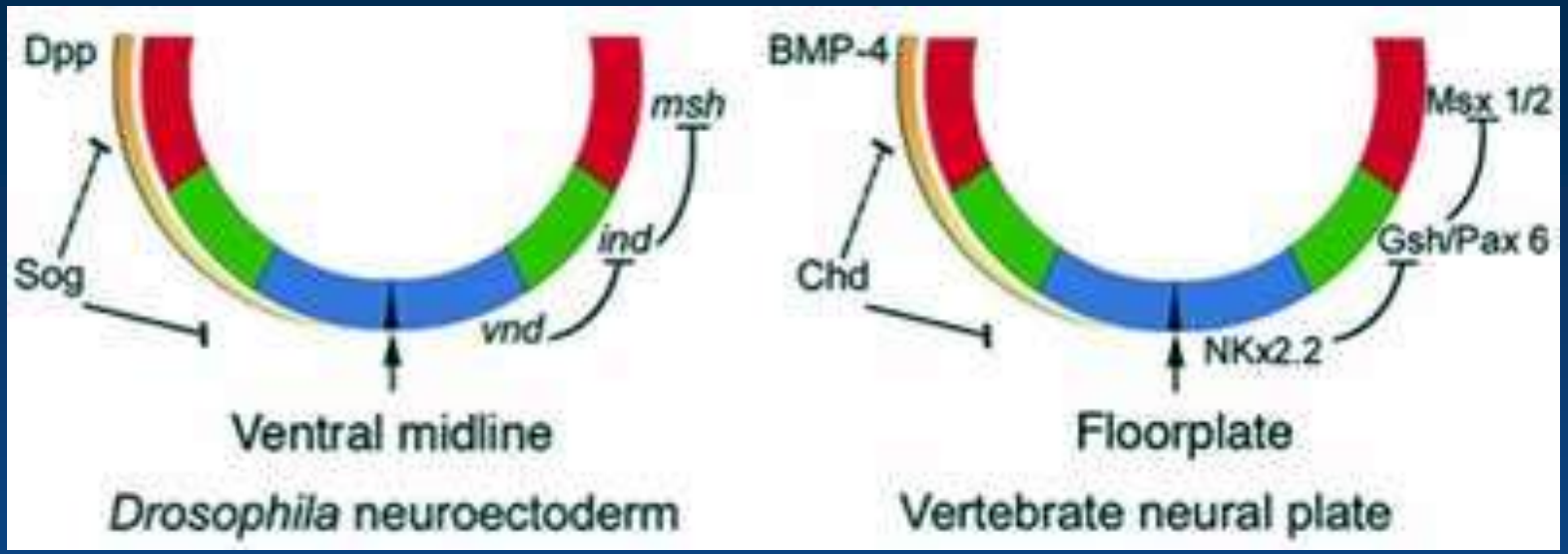
Signaling conservation between lower and higher animal groups

BMP signaling is conserved between insects and vertebrates in neurogenesis



Expression of Dpp/ BMP4 and Sog/Chordin as well as of the columnar genes support the inversion of the DV body axis. Simplified schemes of cross sections through the trunk of developing *Drosophila* and vertebrate embryos (indicated by frames in A,B; neurogenic ectoderm highlighted in brown) during successive stages of development (A1-A3 and B1-B3, respectively). (A1, B1) The border between nonneurogenic (grey) and neurogenic ectoderm (coloured) becomes defined by gradients of the antagonistically acting factors Short gastrulation (Sog)/Chordin (both in blue) and Decapentaplegic (Dpp)/Bone morphogenetic protein 4 (Bmp4) (both in red). The ectodermal region expressing *sog/chordin* forms the neuroectoderm, which is dorsal in vertebrates but ventral in *Drosophila*. DV patterning within the *Drosophila* neuroectoderm is achieved by the activity of the columnar genes: *msh, ind* and *vnd* (as indicated by the colour code), expressed in longitudinal columns at lateral, intermediate and ventral sites, respectively. A set of homologous genes *Msx, Gsh2* and *Nkx2*, is expressed in the vertebrate neuroectoderm in a corresponding medio-lateral sequence. (A2, B2) Two different modes of morphogenesis are apparent during ongoing development: The *Drosophila* neuroectoderm gives rise to neuroblasts (NB), which delaminate towards the interior of the embryo to form the ventral nerve cord (vnc). The vertebrate neuroectoderm invaginates to form the dorsal neural tube. (A3, B3) In the vertebrate spinal cord (sp) the columnar genes are nevertheless expressed in the same dorsoventral order as in the *Drosophila* ventral nerve cord. Further abbreviations: np, neural plate; vNE, ventral neuroectoderm; pNE, procephalic neuroectoderm.

Conserved signaling between insects and vertebrates in neurogenesis

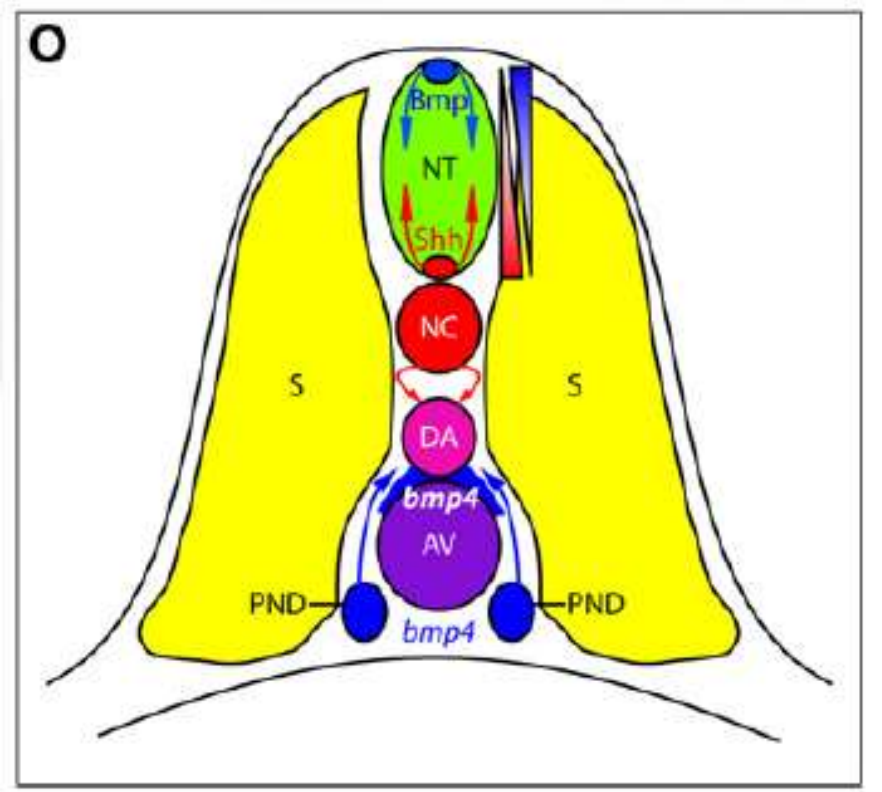


Simplified summary model indicating the proposed similarities in BMP-mediated patterning of the vertebrate and invertebrate neuroectoderm.

Two processes collaborate to establish the pattern of neural identity gene expression in *Drosophila* and vertebrates: graded BMP signaling preferentially represses expression of ventral neural identity genes (left), which then engage in a chain of ventral-dominant repression wherein more ventral genes prevail in repressing the expression of more dorsal genes (right). The indicated inhibition of *Msx1/2* by Pax6 remains hypothetical.

Not indicated on this scheme are additional levels of cross-inhibition (e.g., Vnd inhibition of *msh,* late Ind repression of *vnd,* and Pax6 repression of *Nkx2.2*), which are likely to help sharpen and refine the pattern created by the core mechanism of threshold-dependent BMP repression coupled to ventral dominance.

BMP-4 and Shh in dorsal aorta patterning



(O) Diagram of zebrafish trunk depicting polarization of the DA by dorsal Hedgehog and ventral Bmp signaling, mirroring the neural tube.

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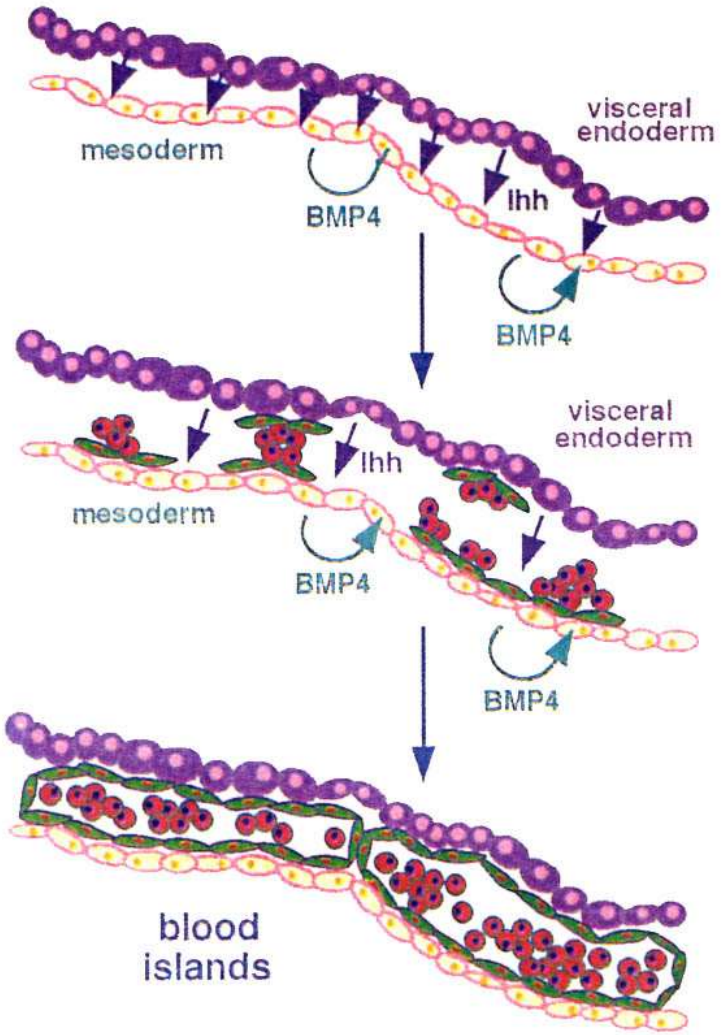
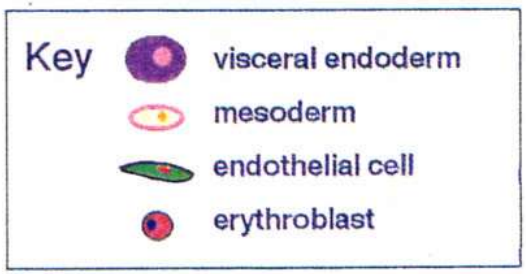
Induction of hematopoiesis in the yolk sac: BMP-4 and Ihh



Model for activation of primitive hematopoiesis and vasculogenesis in the yolk sac.

Ihh is secreted from visceral endoderm to target extraembryonic mesoderm, where it activates expression of BMP-4. BMP-4 protein in turn feeds back to extraembryonic mesoderm, activating VEGFR-2, CD34, and SCL and formation of hematopoietic/vascular stem/progenitor cells.



BMP-4 and Shh patterns the third pharyngeal pouch

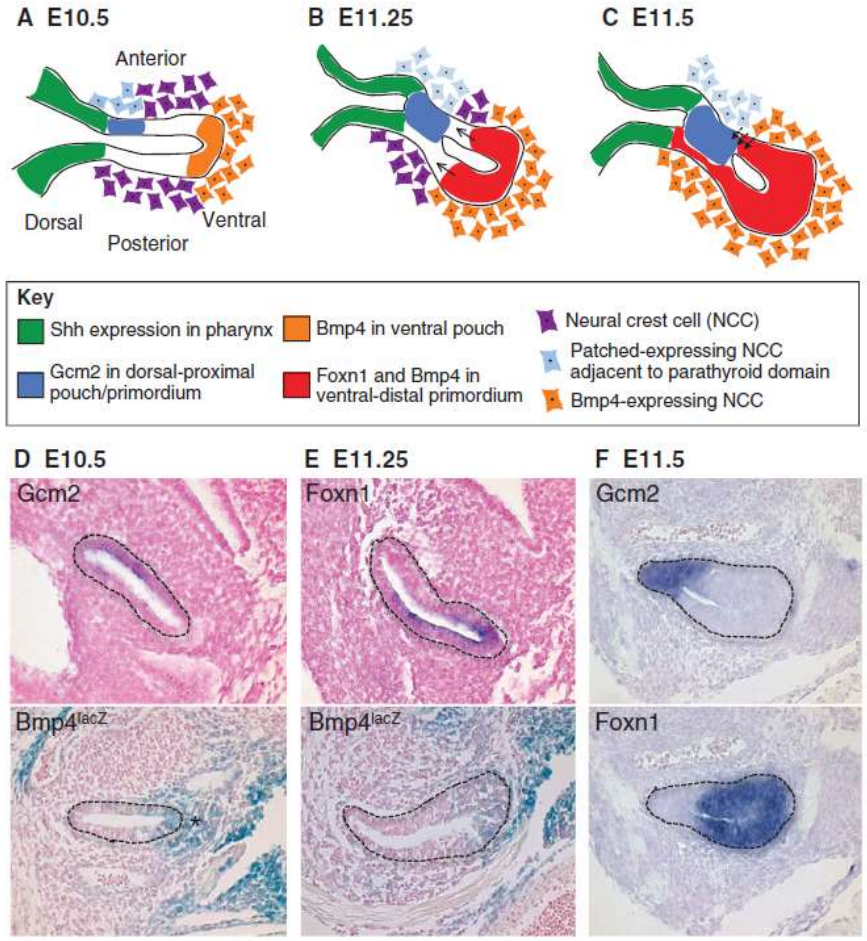


Fig. 3. Patterning the third pharyngeal pouch. (**A**) At E10.5, Gcm2 expression (blue) marks the parathyroid domain within the third pouch, Bmp4 (orange) is expressed at ventral tip of the pouch and adjacent mesenchyme, and Shh (green) expression is detected in the pharynx but is excluded from the pouch. Neural crest cells (NCCs; purple/light blue/orange) surround the pouch and some express regionalized markers (Bmp4, orange; patched, light blue). (**B**) By E11.25, Foxn1 and Bmp4 expression (red) spreads dorsally (arrows) towards the Gcm2-expressing parathyroid domain (blue). (**C**) By E11.5, the third pouch epithelial cells express either Foxn1 and Bmp4 (red) or Gcm2 (blue). Signals from adjacent NCCs refine the position of the border between domains (arrows). (**D**) In situ hybridization for Gcm2 (top) demonstrates its proximal-dorsal-anterior-restricted expression domain at E10.5. Analysis of $Bmp4^{lacZ}$ mice (bottom) demonstrates Bmp4 expression at the ventral tip of the pouch endoderm and in the adjacent mesenchyme (asterisk) at this stage. (**E**) Foxn1 in situ hybridization (top) and $Bmp4^{lacZ}$ expression (bottom) at the distal-ventral tip of E11.25 primordium. (**F**) In situ hybridization for Gcm2 (top) and Foxn1 (bottom) on adjacent sections show non-overlapping expression at E11.5. In D-F, third pouch endoderm is outlined by dashed line. All data panels are sagittal sections oriented with dorsal on the left and ventral on the right. Panels in F included with permission (Foster et al., 2010).

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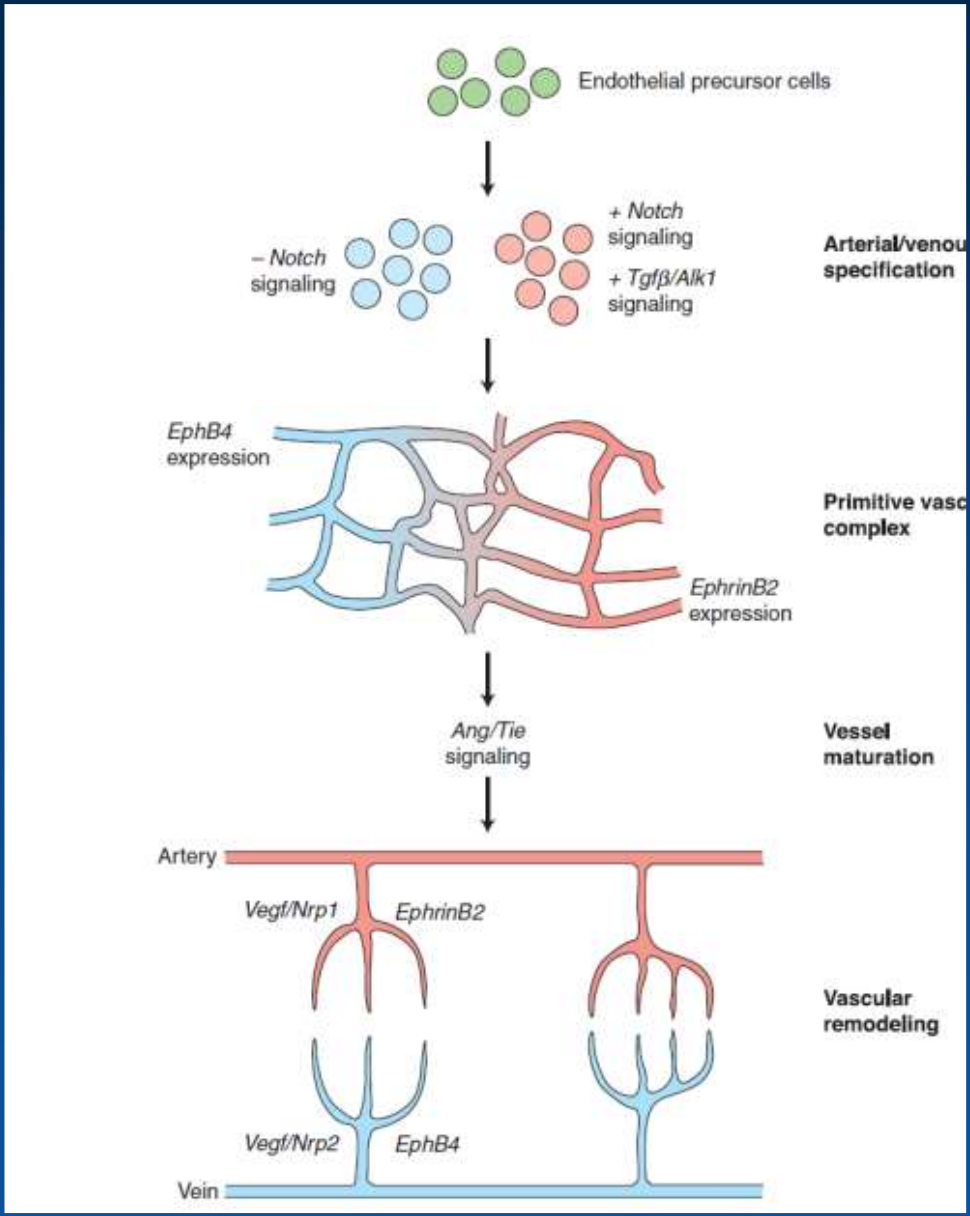
Signaling during vasculogenesis: arterio-venous specification by Notch/TGFβ/Ephrin/Tie/VEGF signaling



Thank you for your attention!

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