Lecture 2

Cell-to-Cell Communications

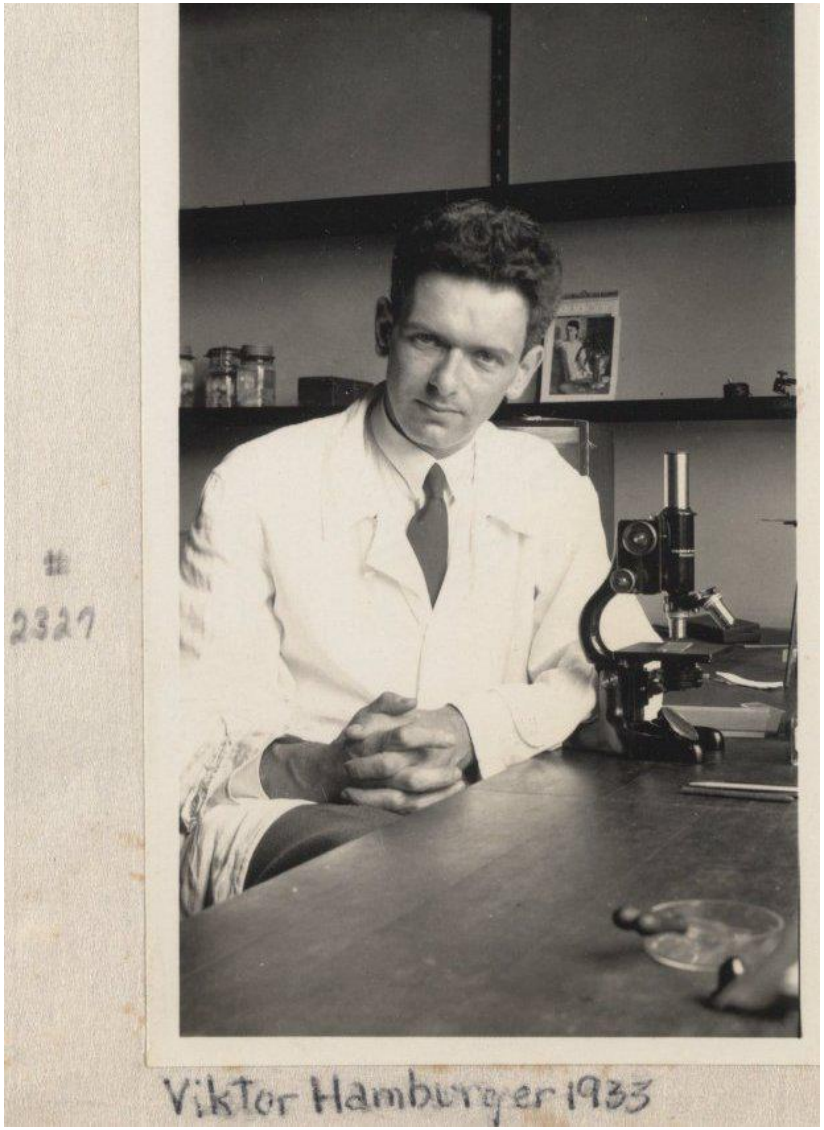
PART I

Discovery of Growth Factors in the Nervous System

I. Neurotrophin family (Neurotrophic Factors – NTFs**).**

1. The NGF story (R. Levi-Montalcini, Science 237,1154-1162, (1987).

Fig. Viktor Hamburger's experiment



Born	July 9, 1900 Landeshut, Silesia
Died	June 12, 2001 St. Louis, Missouri
Nationality	German - Amertican
Fields	Embryology
Institutions	Washington University in St. Louis
Alma mater	University of Freiburg
Doctoral advisor	Hans Spemann
Known for	Nerve growth factor

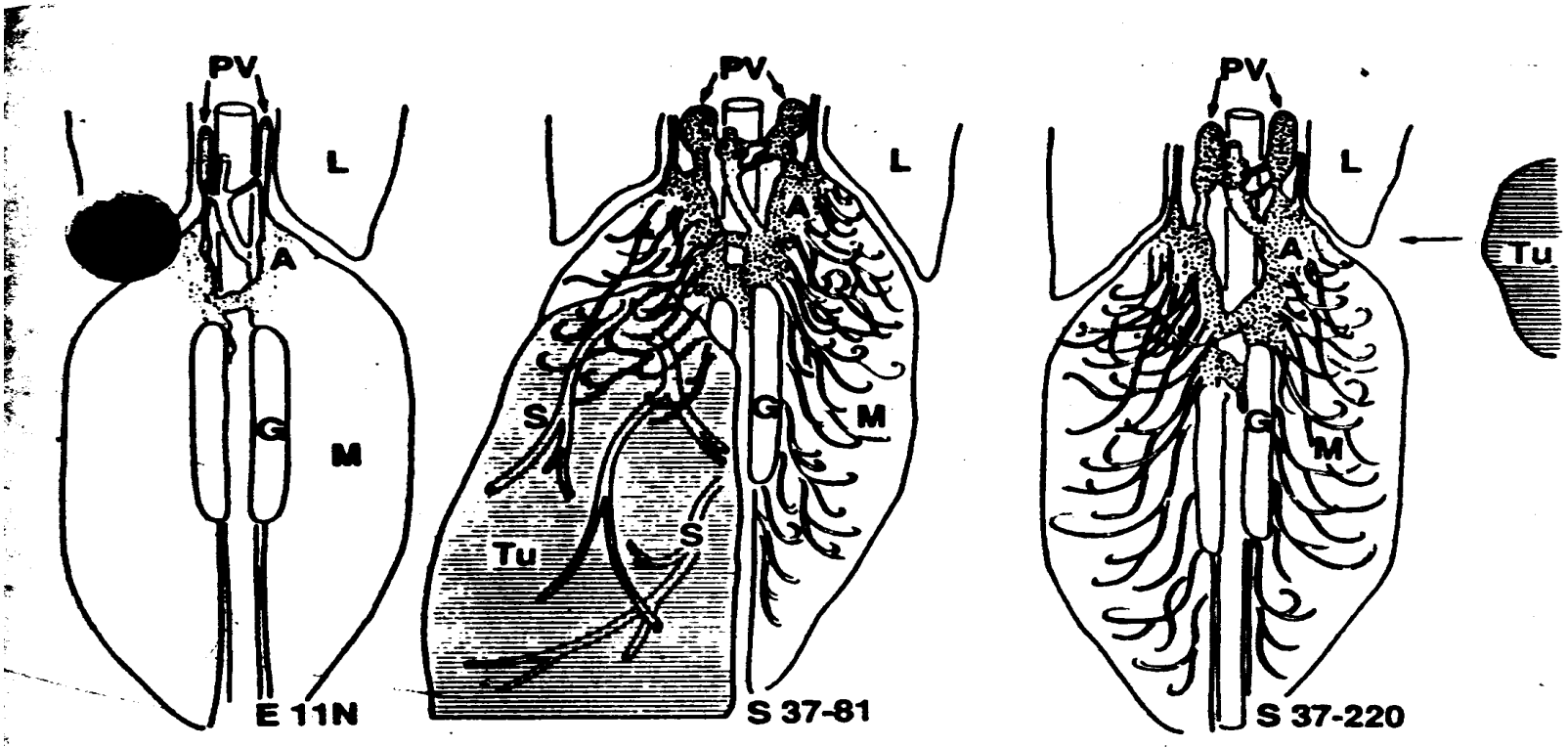
Rita Levi-Montalcini

Rita Levi-Montalcini died in her home in Rome on 30 December 2012 at the age of 103.[29]
Upon her death, the Mayor of Rome, Gianni Alemanno, stated it was a great loss "for all of humanity.

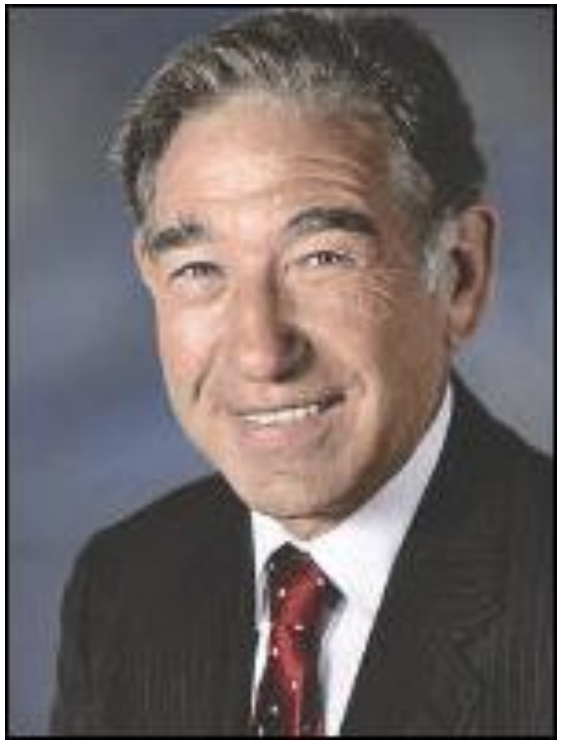
Born	22 April 1909 Turin, Italy
Died	30 December 2012 (aged 103) Rome, Italy
Citizenship	Italy
Nationality	Italian
Fields	Neurology
Institutions	Washington University in St. Louis
Alma mater	Turin Medical School, University of Turin
Known for	Nerve growth factor
Notable awards	Louisa Gross Horwitz Prize(1983) Nobel Prize in Physiology or Medicine (1986) National Medal of Science (1987

Fig. Viktor Hamburger's experiment

Fig. 1 Mouse sarcoma tumors produce substance(s) that stimulates growth of sympathetic nerves, ganglia and adrenals in the chick embryo but has no effect on motor fibers. This substance can diffuse long distances (compare intra-embryonic and intra-chorioallantoic membrane transplantation of the tumor)[*from Ann. N.Y. Acad. Sci., 55, 330 (1952*)]. Immuno-sympathectomy by Anti NGF antibody.



Stanley Cohen (born November 17, 1922) is an American biochemist who, along with Rita Levi-Montalcini, was awarded the Nobel Prize in Physiology and Medicine in 1986.

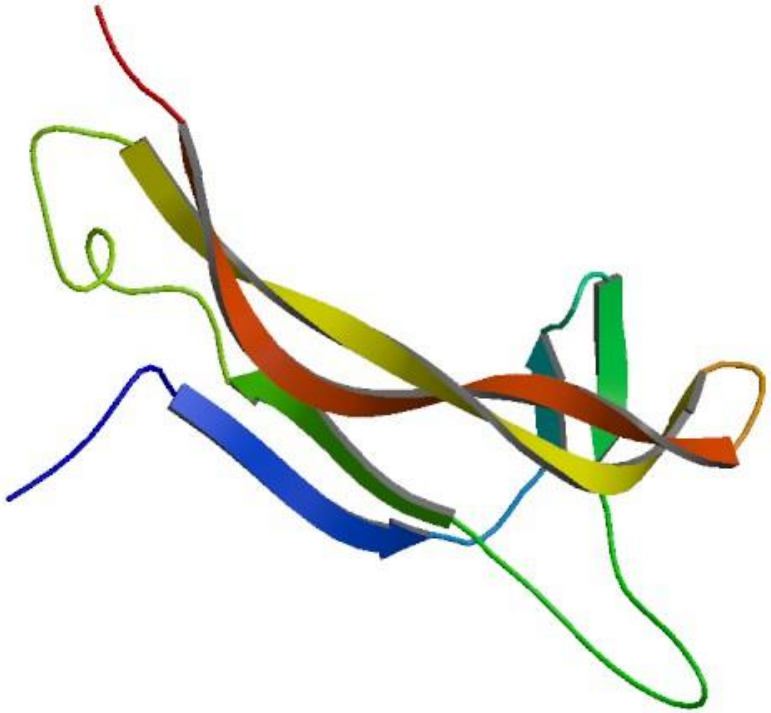


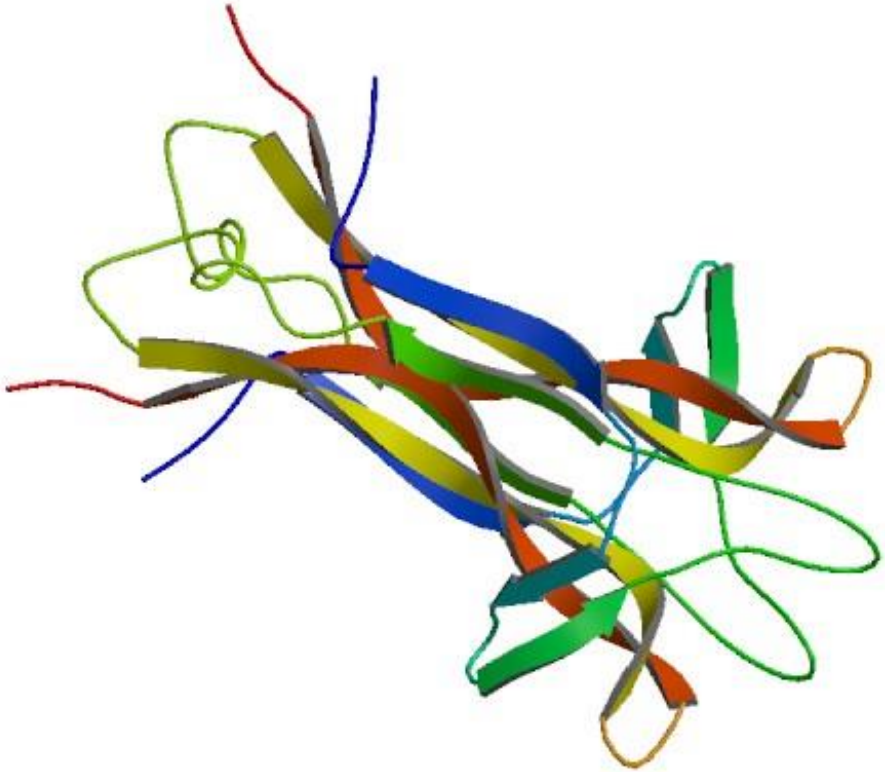
Born	November 17, 1922 (age 92) Brooklyn, New York
Nationality	American
Fields	Biochemistry
Institutions	Vanderbilt University Washington University in St. Louis
Alma mater	University of Michigan Oberlin College Brooklyn College
Thesis	*The Nitrogenous Metabolism of the Earthworm* (1949)
Doctoral advisor	Howard B. Lewis[1][2]
Known for	Nerve growth factor
Notable awards	Louisa Gross Horwitz Prize(1983) Nobel Prize in Physiology or Medicine (1986) Franklin Medal (1987

α β γ 130kDa NGF precursor complex

β pro NGF

β – 34 kDa + **26 kDa (monomer)**



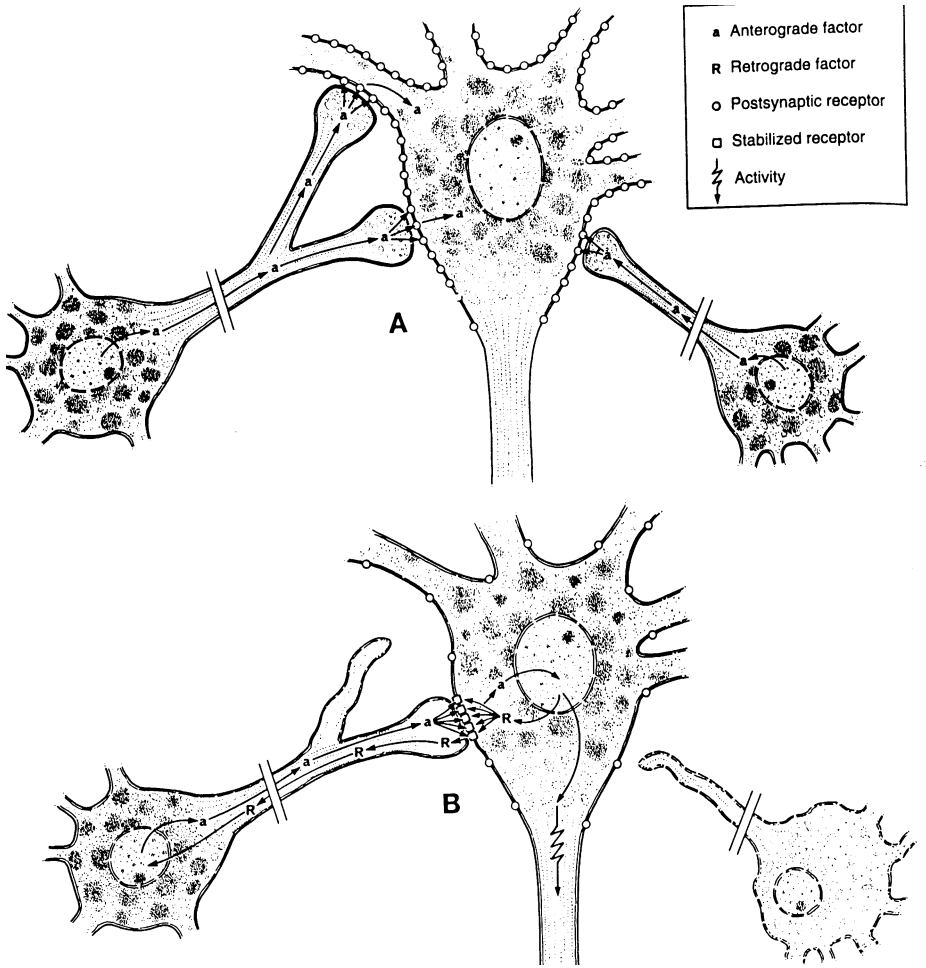


NGF monomer

NGF dimer

(6 cysteins)

Fig. 2 NGF as a retrograde messenger and trophic factor that helps to establish correct connections - model of GF action:



L. Marciano, M.K. Stachowiak, BNI, 1992

Other effects of NGF	mitogenic	differentiating (genomic)	survival	growth cone Effects (local)
SIF cells		+	+	
Adrenal medulla Chromaffin cells	+	+	+	
PC12 cells	-	+	+	
Sympathetic and			+	+
Sensory neurons			+	
Central Ach neurons			+	

1. Discovery of BDNF [Thoenen, Nature 341,149 (1989)].
Fig. 1

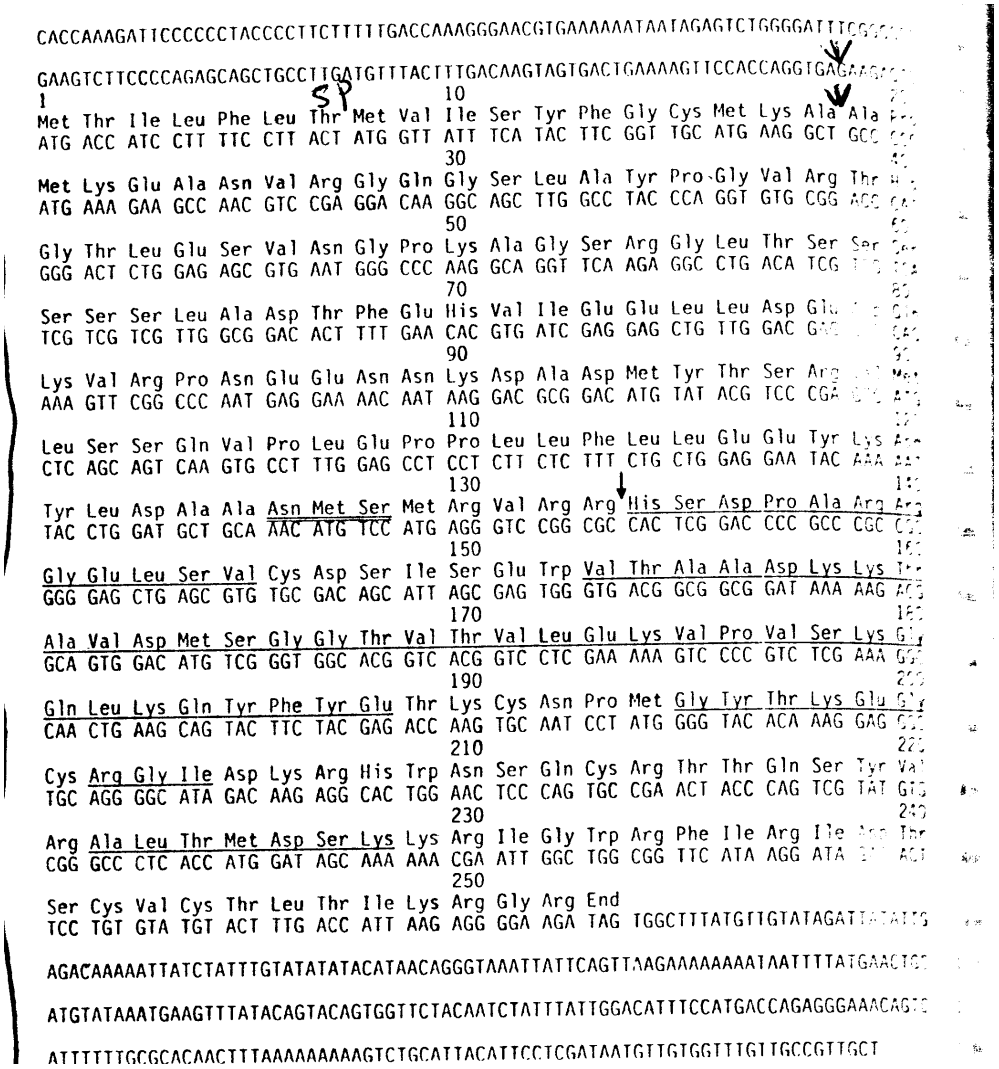


FIG. 1 Nucleotide sequence and deduced amino-acid sequence of BDNF. The peptide sequences obtained from microsequencing (Table 1) are underlined. The only consensus sequence for N-glycosylation is doubly underlined and an arrow indicates the start of mature BDNF.

1. Other NTFs: NT3, NT4/5, NT6, [Yp et al, J. Physiol. 85,123-130 (1991)]

Fig. 1

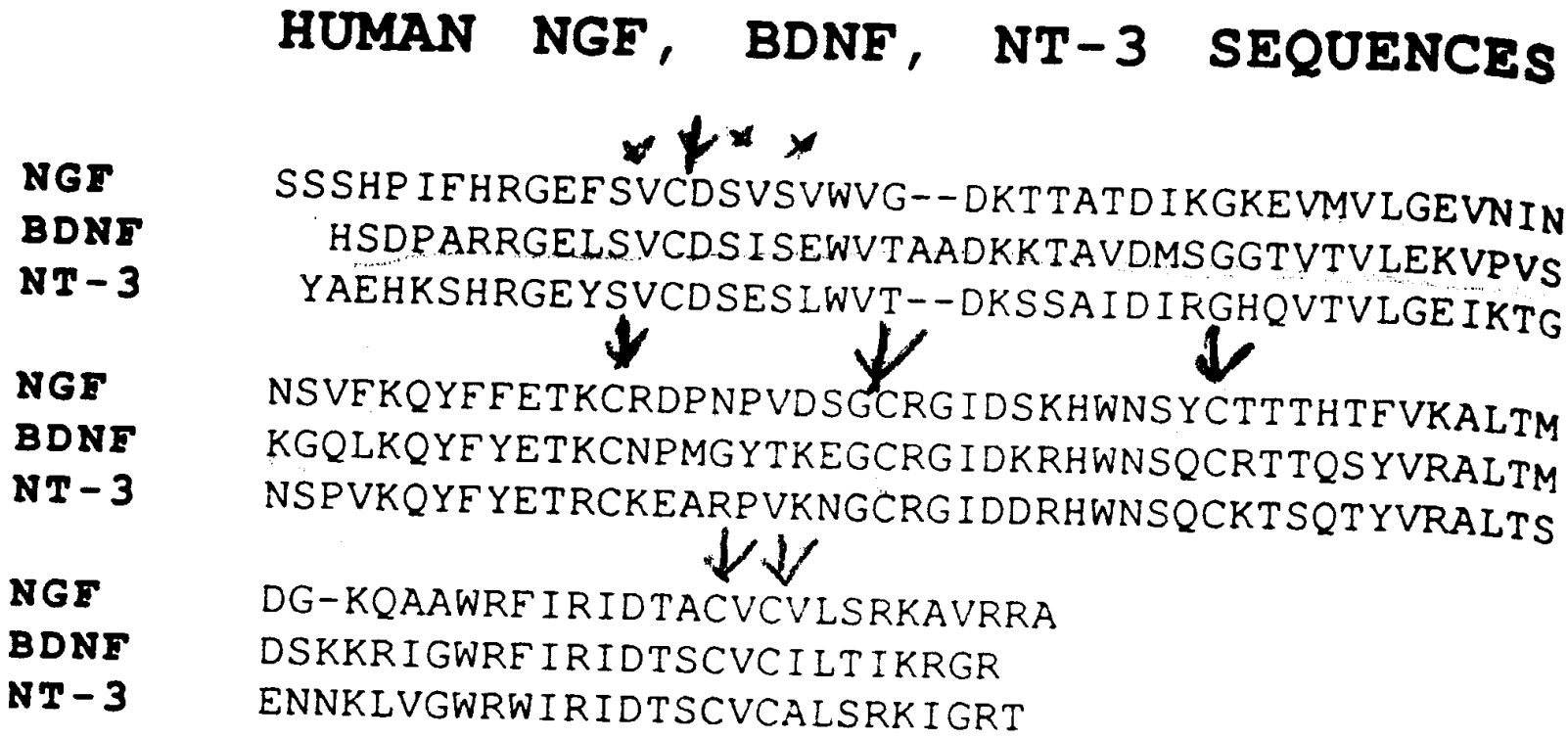
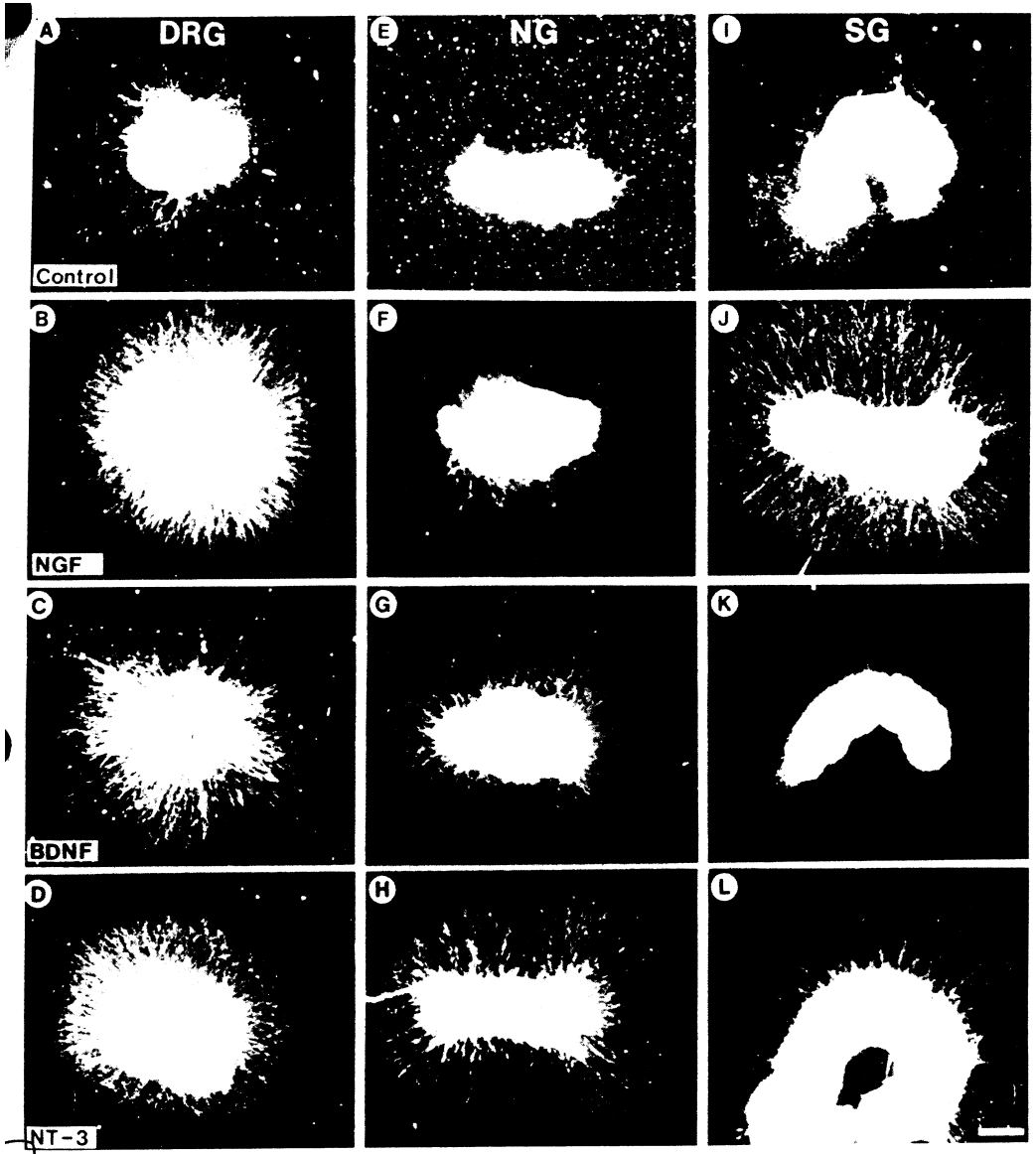


FIG. 1 — Sequence comparisons of the mature forms of human NGF, BDNF and NT-3. Sequences are aligned to maximize homology

1. NTFs can be distinguished from each other by their ability to affect distinct (partially overlapping) population of neurons in vitro.

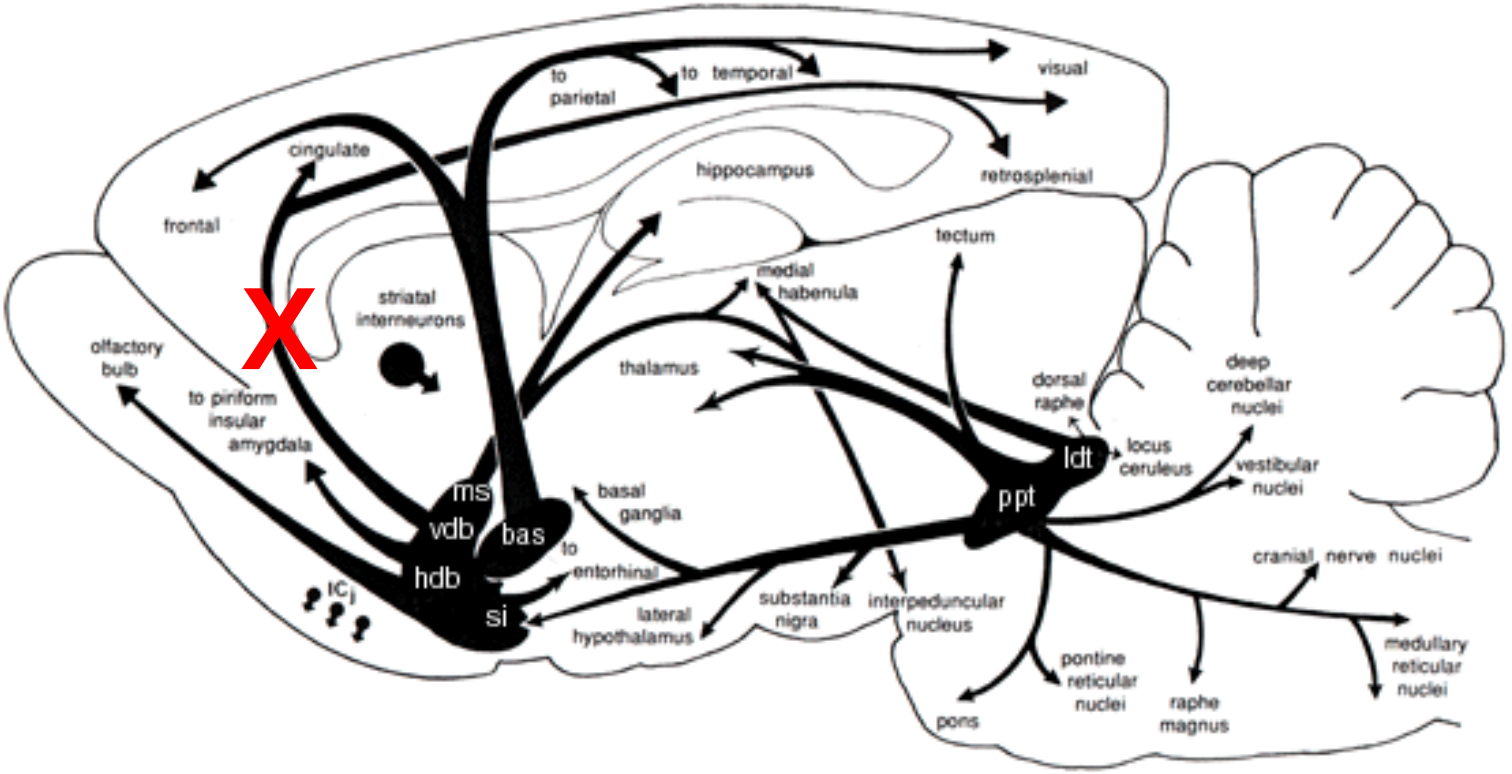
Fig.2 (Yp)



2. — Comparisons of the activities of NGF, BDNF and NT-3 on ganglionic explants from embryonic (E8) chick.

Action of NTFs in mature nervous system

Lesion of the forebrain cholinergic system rescue by NGF



Rescue of septal neurons by NGF

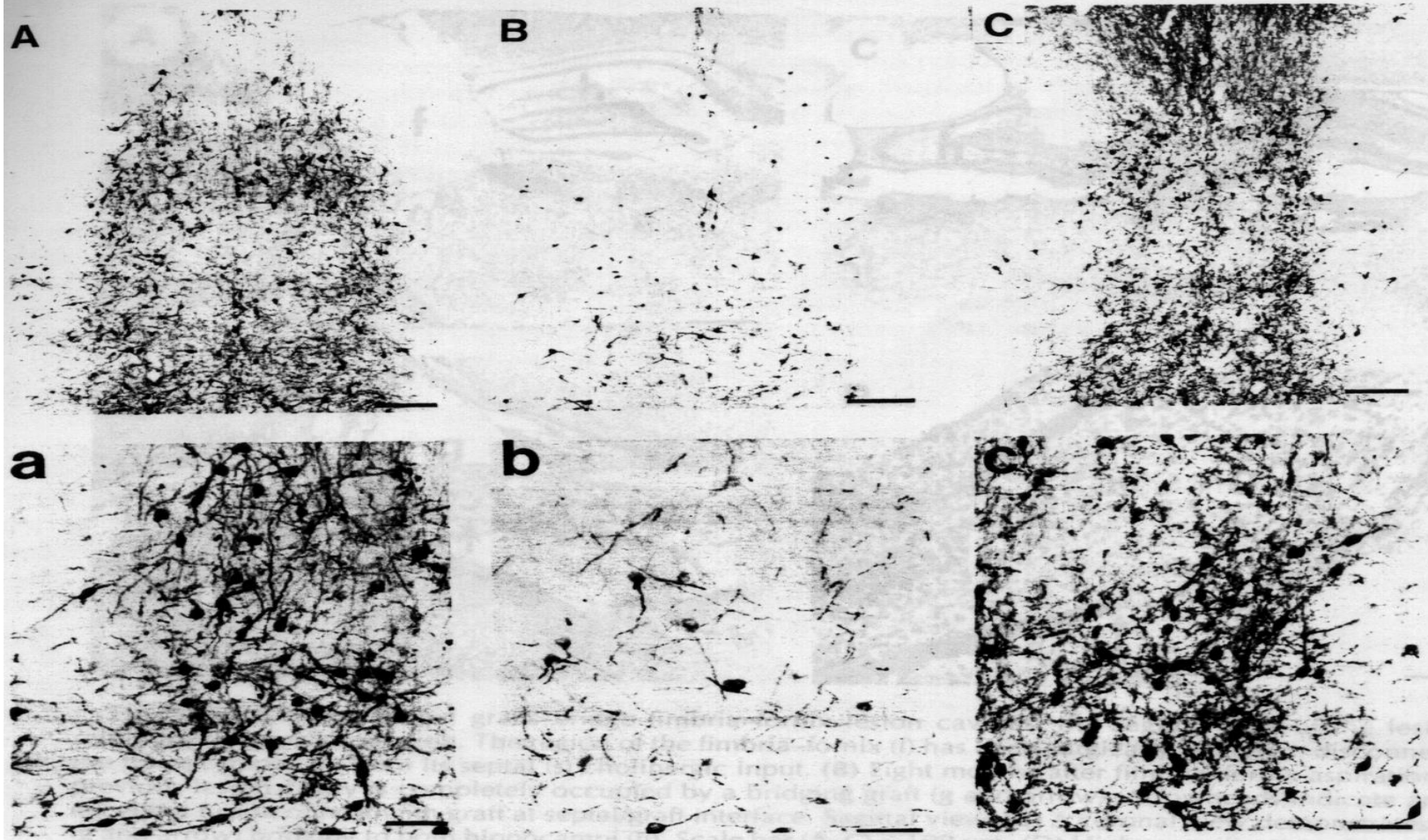
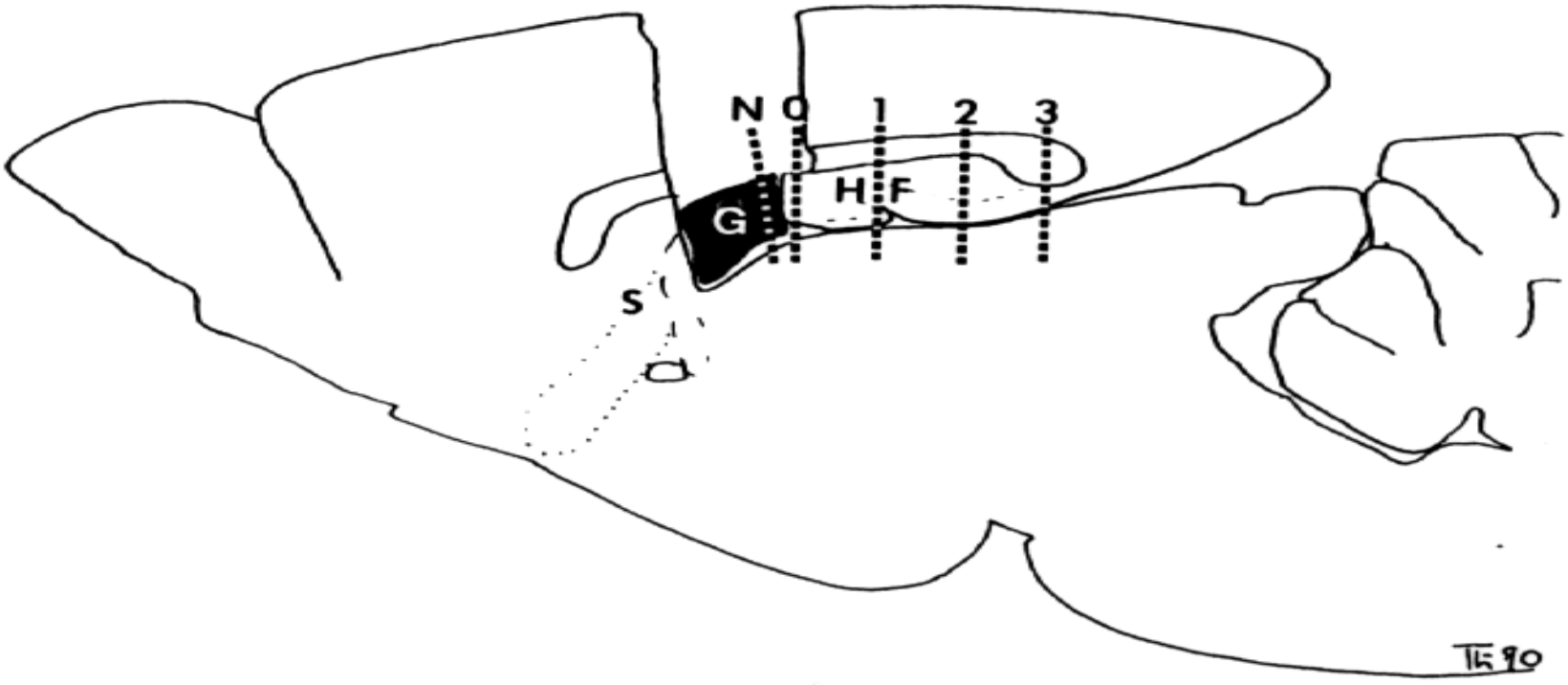


Fig. 2. Transient NGF infusions prevent long-term cholinergic neuronal degeneration. **(A,a)** Normal distribution of medial septal cholinergic neurons in the intact rat brain at low and high magnification. Immunocytochemical label for the p75 low-affinity NGF receptor. **(B,b)** After bilateral fimbria–fornix transections, medial septal cholinergic neurons undergo retrograde degeneration that persists up to 8 mo after the lesion. **(C,c)** Subjects that receive bilateral fimbria–fornix transections and transient 9-wk NGF infusions show long-term rescue of medial septal cholinergic neurons. Six months after NGF infusions are discontinued, a high proportion of neurons remain labeled for the p75 receptor. Scale bars (A,B,C) = 62 μm; (a,b,c) = 25 μm.

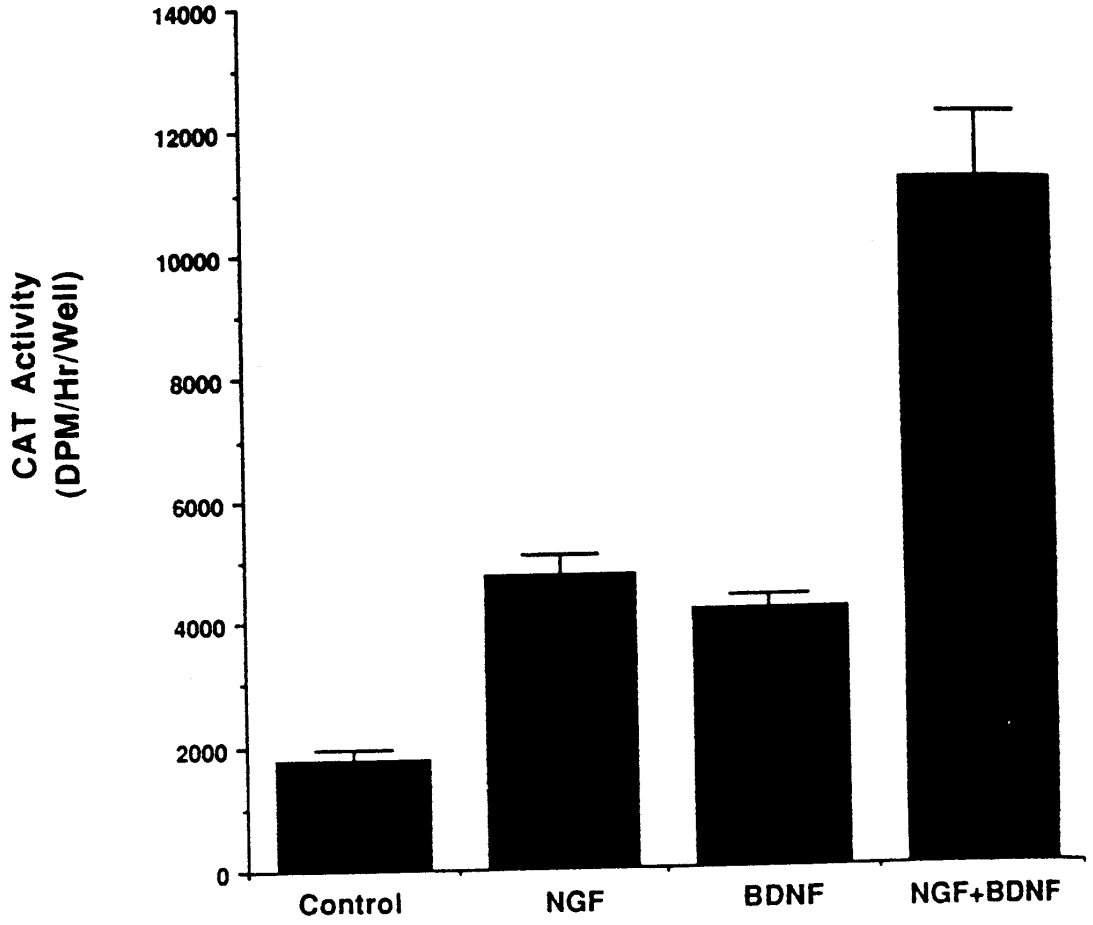
Action of NTFs in mature nervous system

Growth factor-rescued neurons require substrate molecules to extend neurites (1)



5. Neurotrophin receptors – [**Barbacid, Current Opinion in Cell Biol.7,148-155 (1995)]**

Fig. 4 (Yp)



Effects of NTFs on Choline Acetyltransferase (CAT) activity in cultured septal neurons.

Fig. 1 (Barbacid) – Types of receptors

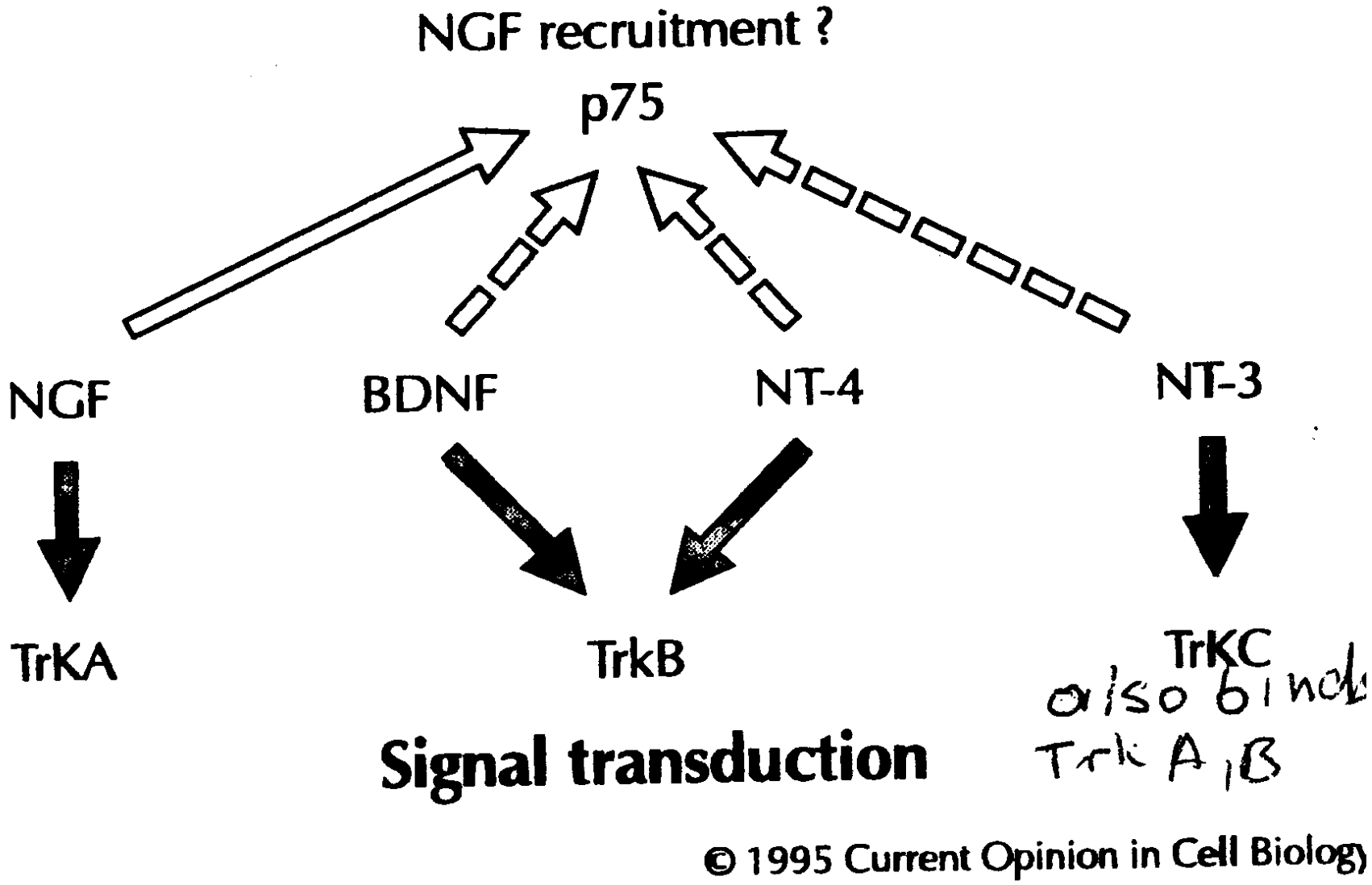


Table 1 (Barbacid – summary of knockout effects)

Table 1. Summary of the defects observed in mice targeted in genes encoding various neurotrophins and their receptors.*

Phenotype	Knockout strain						
	p75	NGF	TrkA	BDNF	TrkB	NT-3	TrkC
Sensory activity	Partial	Very low	Very low	Normal	Normal	Normal	Normal
Nociception	Normal	Normal	Normal	**Impaired****	**ND**†‡	Normal	Normal
Balance	Normal	Normal	Normal	Normal	Normal	Impaired	Impaired
Proprioception							
PNS defects‡‡							
Superior cervical ganglion	Normal	5%	5%	Normal	Normal	50%	75%
Trigeminal ganglion	Normal	30%	30%	60%	40%	40%	ND
Nodose–petrosal ganglion	ND	Normal	Normal	**40%**	**10%**(a)	60%	ND
Vestibular ganglion	ND	ND	ND	**15%**	**ND**‡	80%	ND
Dorsal root ganglia	Smaller	30%	30%	70%	70%	**35%**	**80%**
Ia Afferents	ND	Normal	Normal	Normal	Normal	Lost	Lost
CNS defects							
Facial motor neurons	ND	ND	ND	**Normal**	**30%**	Normal	ND
Spinal cord motor neurons	ND	ND	ND	**Normal**	**70%**	Normal	ND
Cholinergic projections	ND	Reduced(b)	Reduced	ND	ND	ND	ND

Fig. 2 (Barbacid) High and Low affinity receptors
P-75: transfection to PC12 not expressing tracks: NGF does not stimulate differentiation but it stimulates tyrosine phosphorylation and *c-fos*, inhibits apoptosis, facilitates binding to TRKs, shared by NTFs.

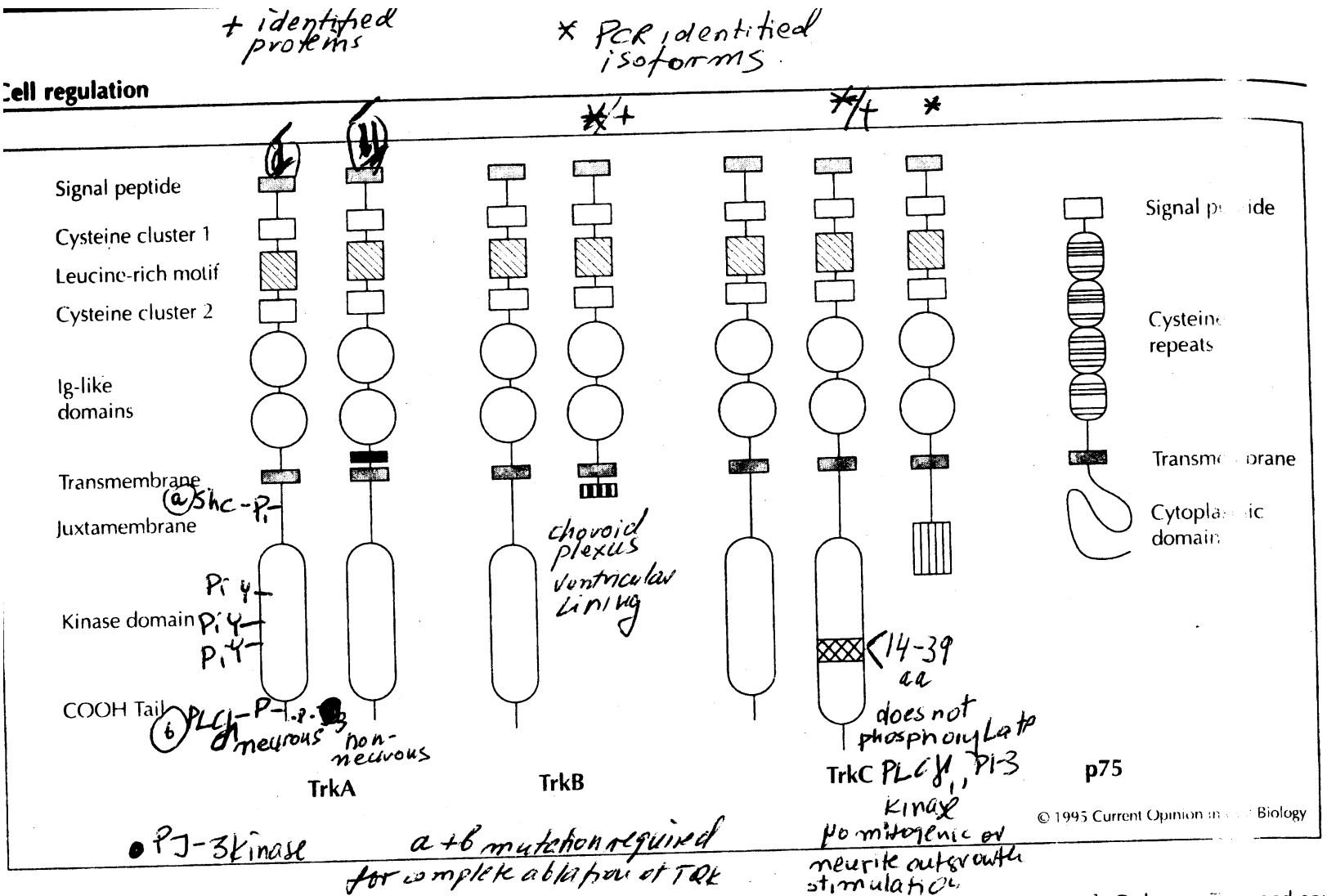
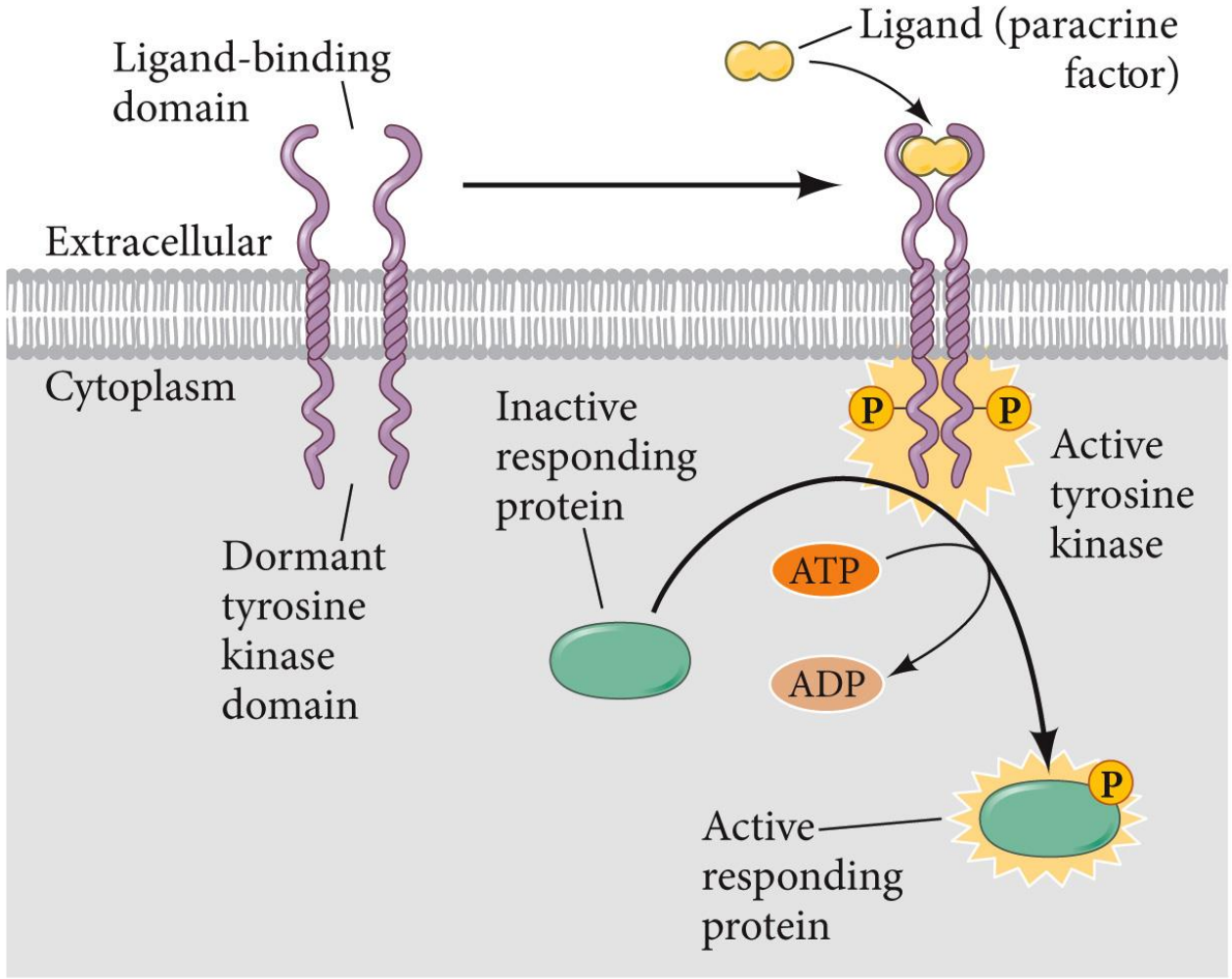
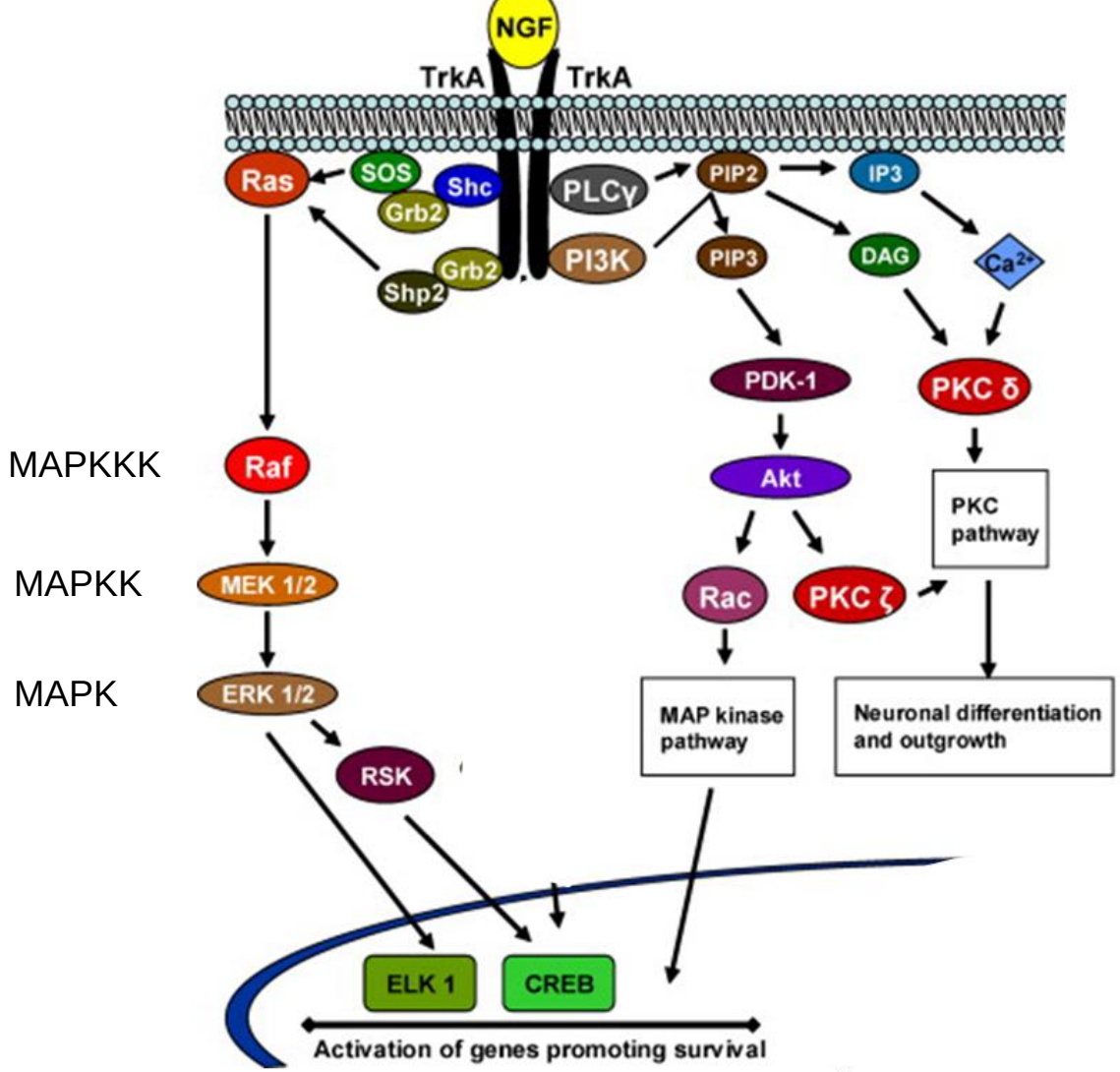


Figure 4.24 Structure and function of a receptor tyrosine kinase



DEVELOPMENTAL BIOLOGY 11e, Figure 4.24

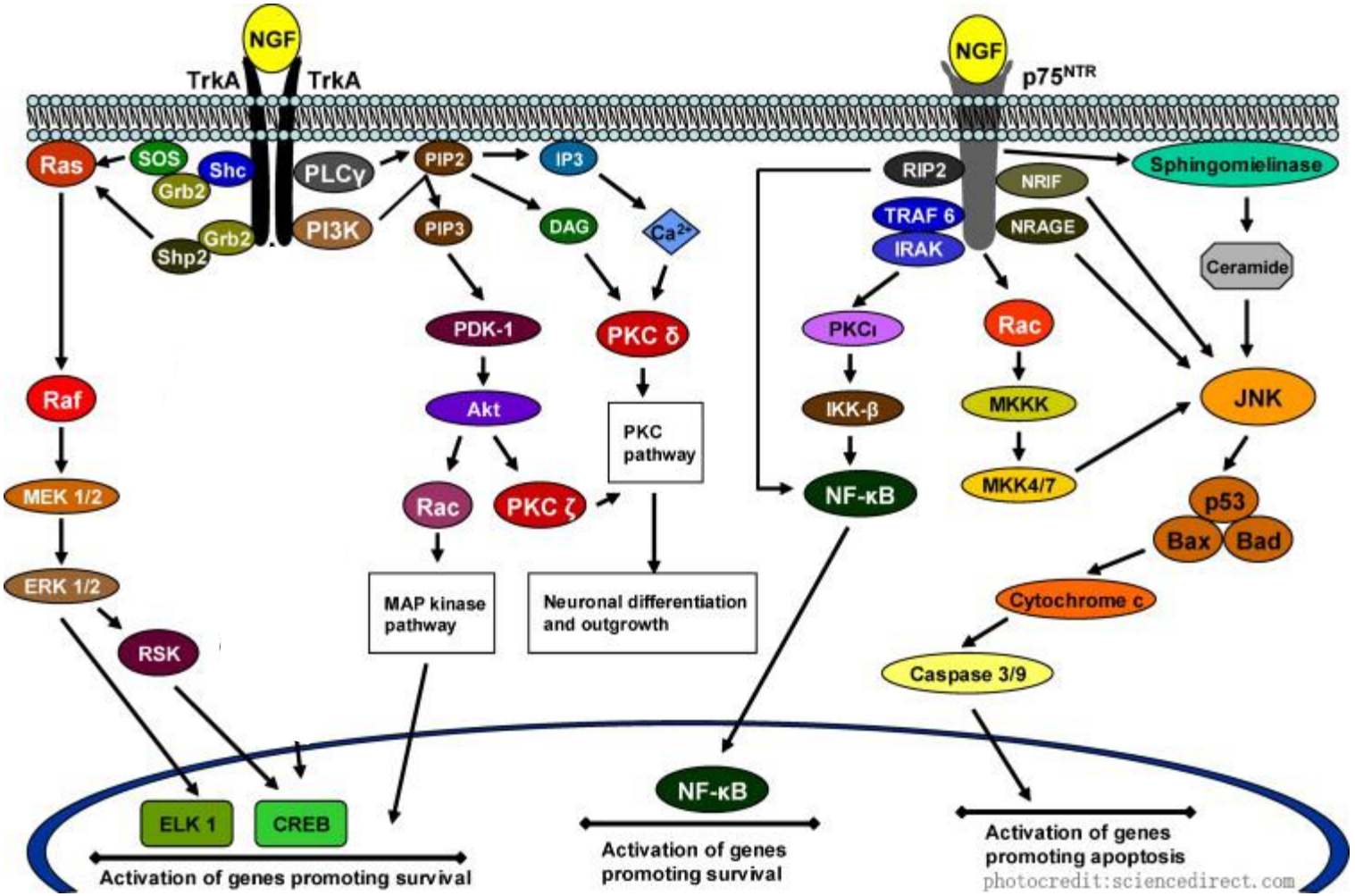
Figure 4.24 Structure and function of a receptor tyrosine kinase (type I TM protein). The binding of a paracrine factor (such as EGF) by the extracellular portion of the transmembrane (type I) protein - receptor activates the dormant tyrosine kinase, whose enzyme activity cross-phosphorylates its reciprocal receptor partner followed by specific tyrosine residues of certain intracellular proteins.



1. TRK signal transduction pathways [Segal and Greenberg, 19, 463-489 (1996)]:

(i). juxtamembrane Y490-Shc-Grb2-SOS>Ras>Raf>MAPK

(ii) C-terminal Y - PLC(, I3PK



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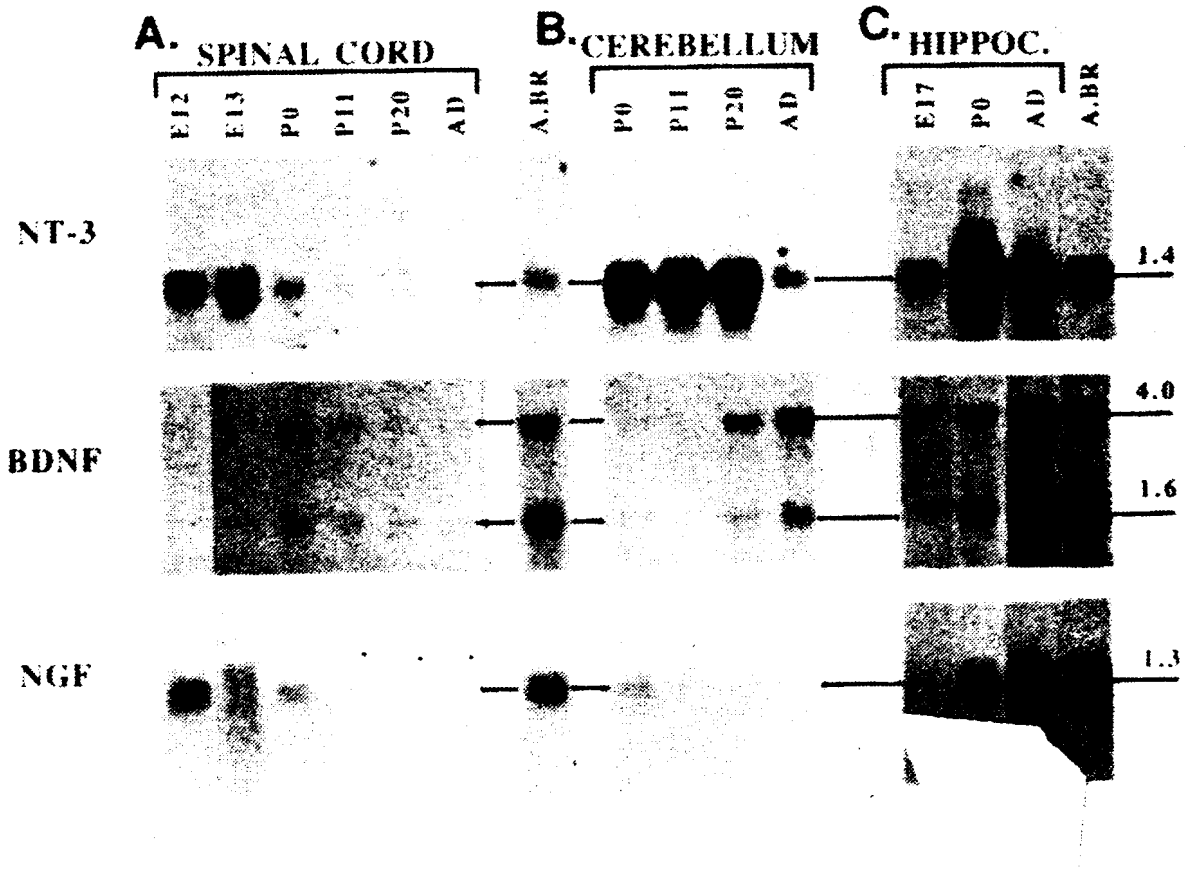
4. Expression of NTFs during **development and adulthood.**
Fig. 3 (Yp et al.)



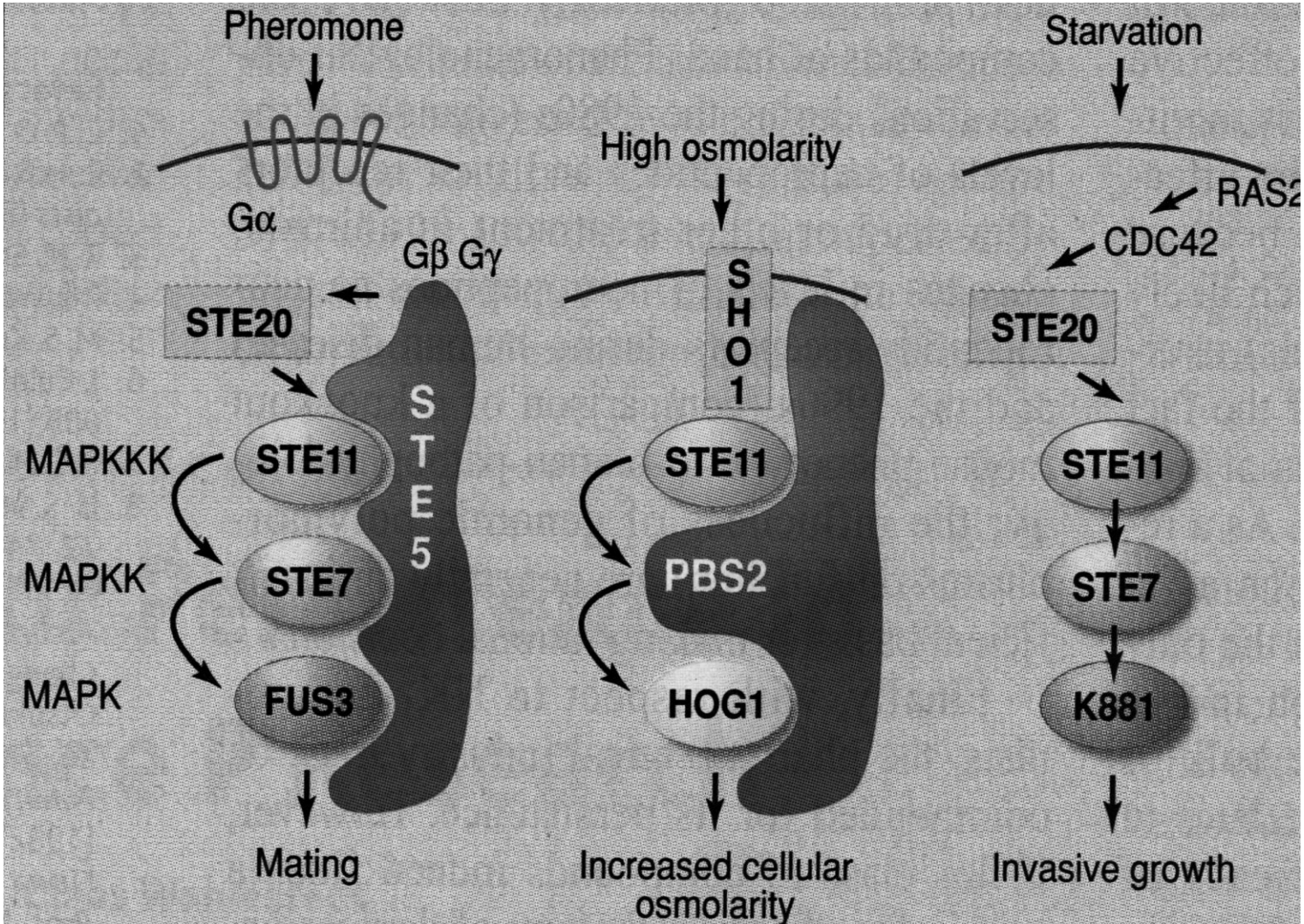
How to achieve specificity in signaling by different growth receptors

A. Scaffolding Hypothesis: [Routing MAP Kinase Cascades - Science 281, 1625 (1998)]

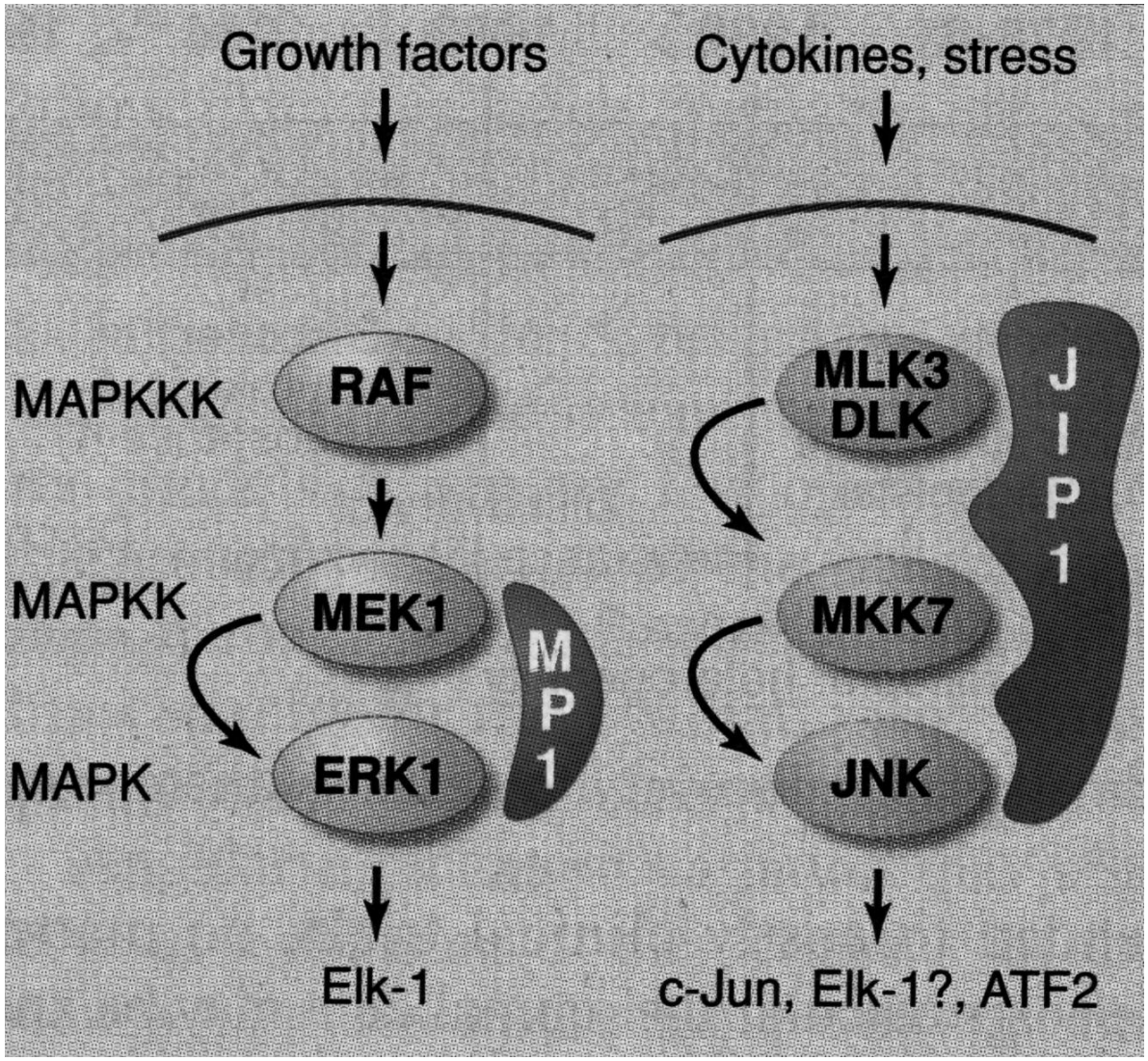
Cells use the same subsets of kinases (k) yet biological effects (e1-4) may be different depending on the stimulus (R1-2)

R1	R2
k1	k1
k2	k2
e1,	,e2

Scaffolding proteins in yeast to prevent cross talk between pathways.



Scaffolding in mammals



8. **Retrograde signaling by Neurotrophic factors in peripheral NS,** (Hendry, 1992, Pharmac. Ther. 56, 256-285) (Fig. 3)

Signaling back from the target to the presynpatic neuron genes requires a retrograde intra-axonal transfer of message . Models:

A. short-acting second messenger (Ca++, cAMP, PIP2 – a transport of message generating NTF-receptor complex to the cell body is necessary.

Long lasting second messenger (i.e., protein kinases able to survive the transport to cell body so that there is no need for the transport of the NTF itself.

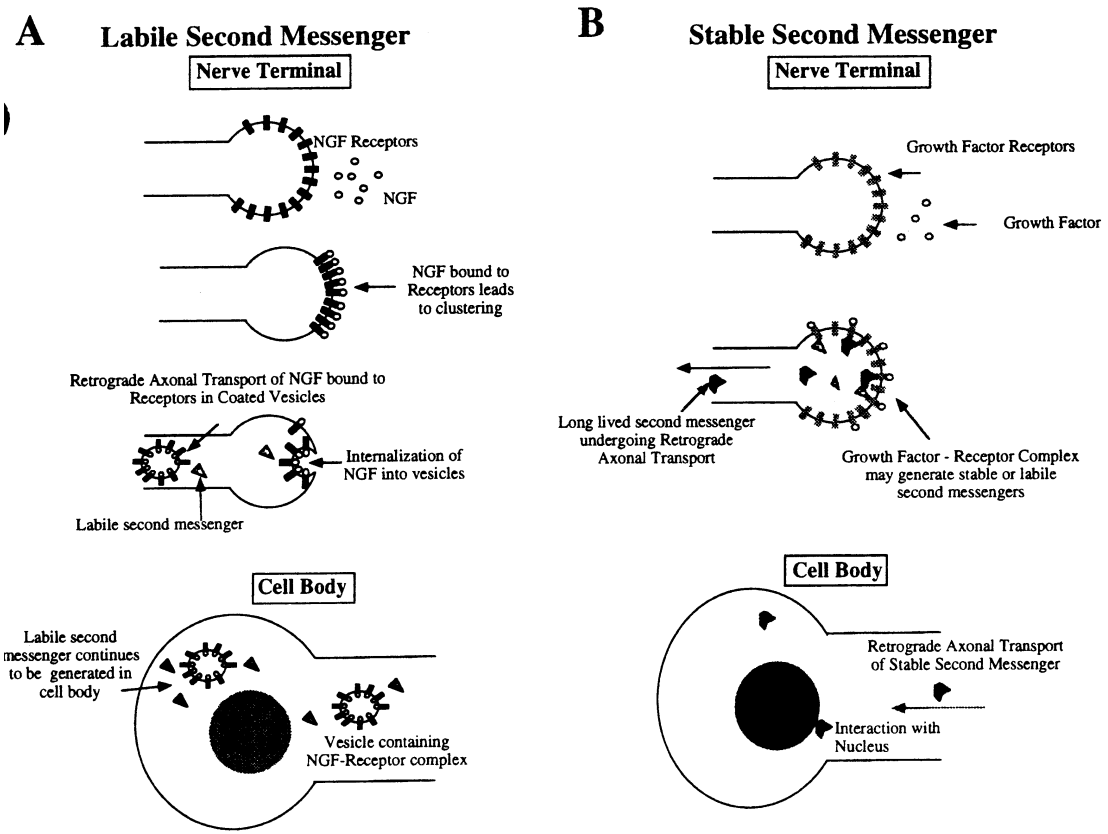
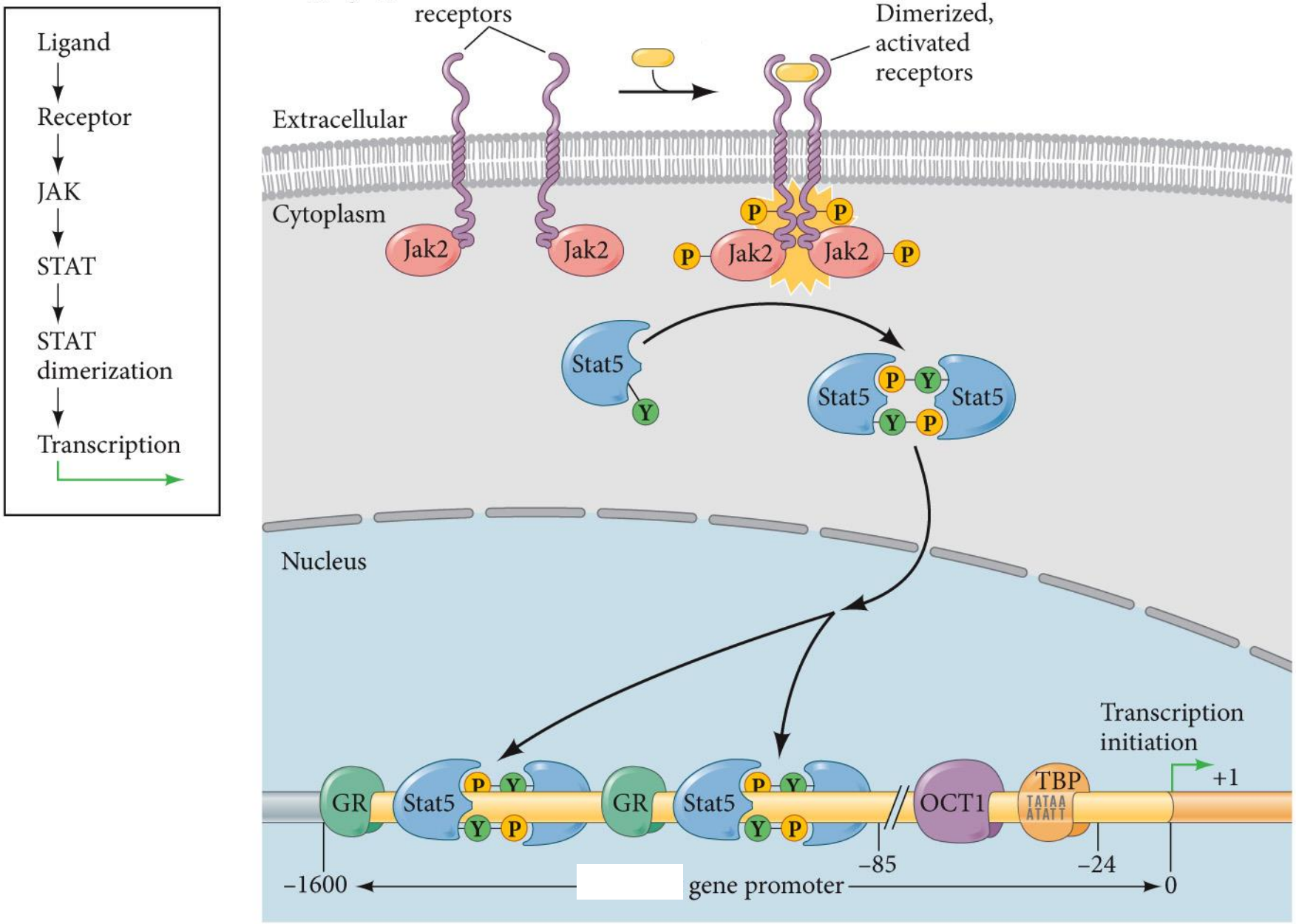
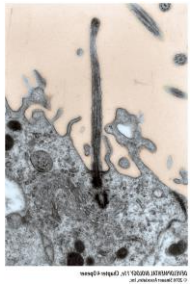


Figure 4.27 A JAK-STAT pathway:



DEVELOPMENTAL BIOLOGY 11e, Figure 4.27



PART II

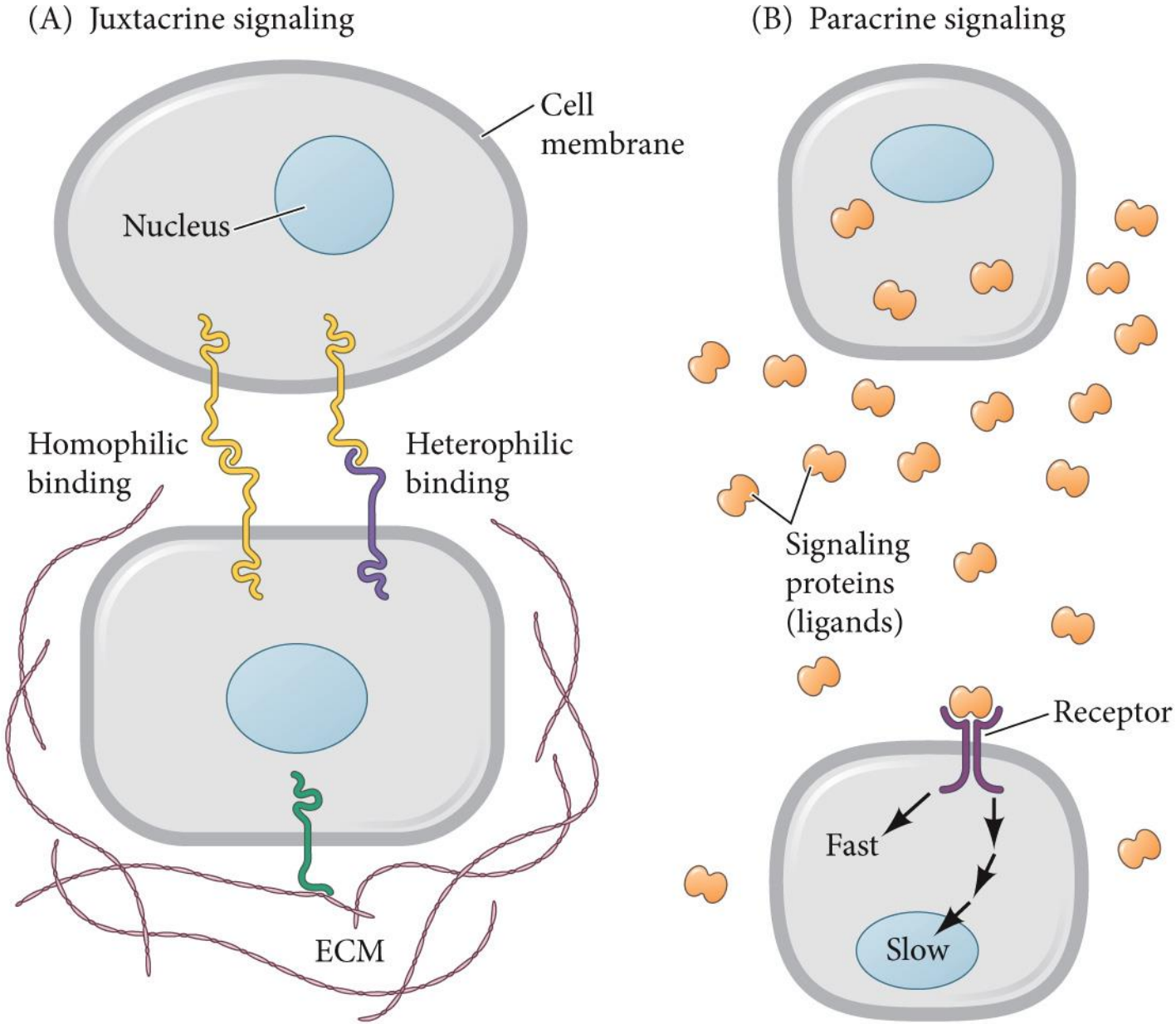
Mechanisms of Morphogenesis communication between cells

.- Embryo is held together, organized and formed by interactions between cells

- embryonic cells could must have differences in their membrane components that would enable the formation of organs.

E. Just (1939) and Johannes Holtfreter (Townes and Holtfreter 1955)

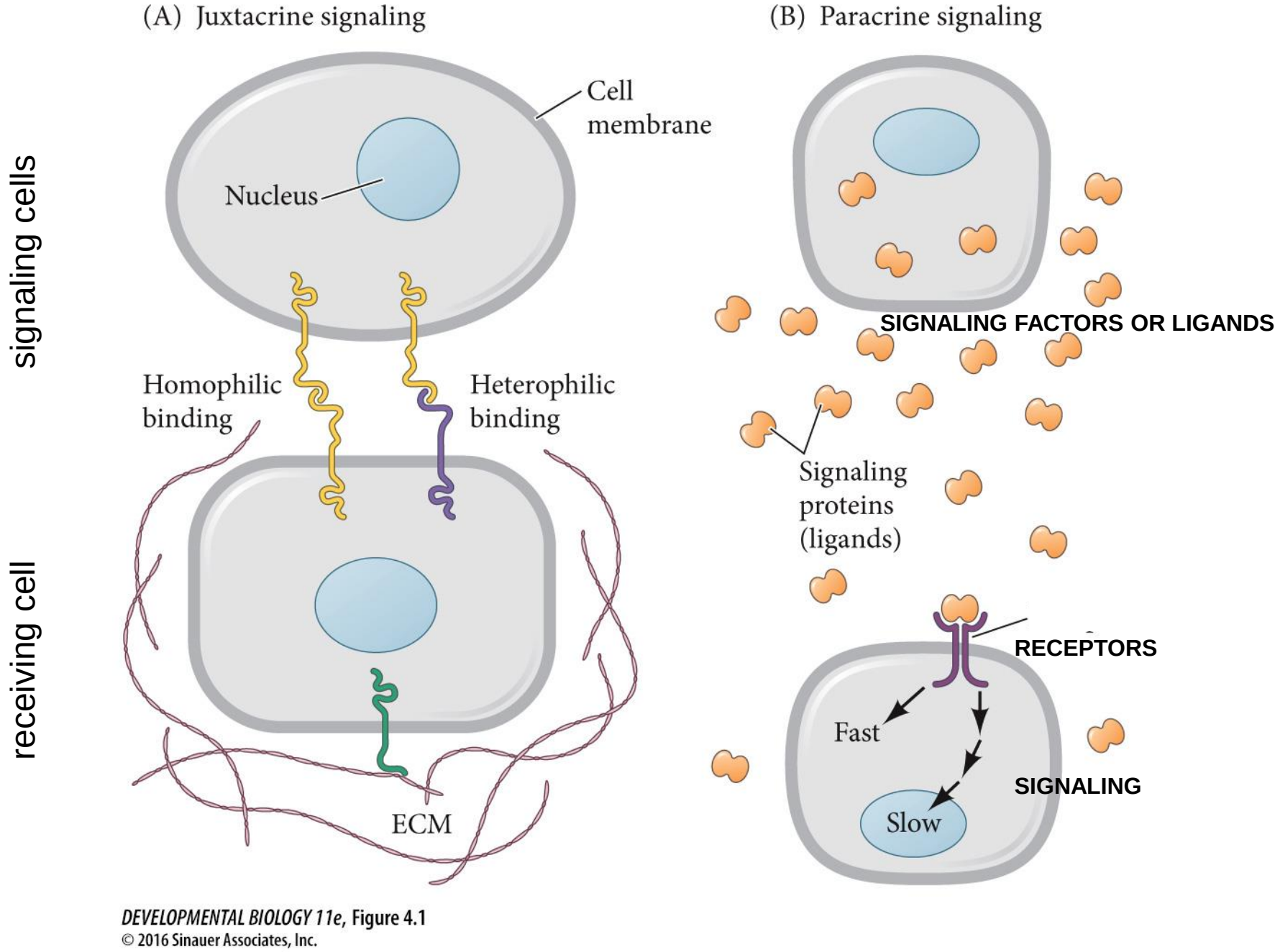
Figure 4.1 Local and long-range modes of cell-to-cell communication



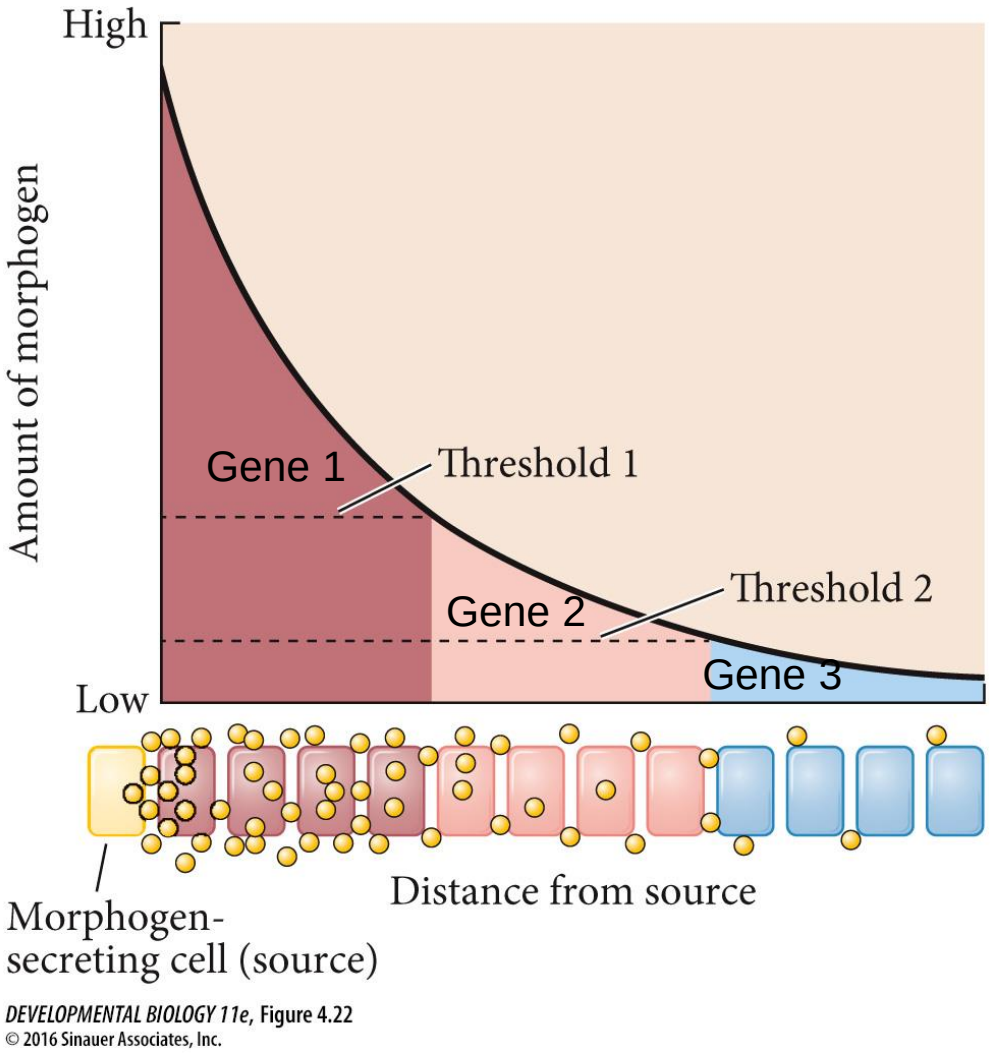
DEVELOPMENTAL BIOLOGY 11e, Figure 4.1

Grobstein (1956) (Saxén et al. 1976; Slavkin and Bringas 1976)

An embryo is held together, organized, and formed by the interactions between cells.



DEVELOPMENTAL BIOLOGY 11e, Figure 4.1



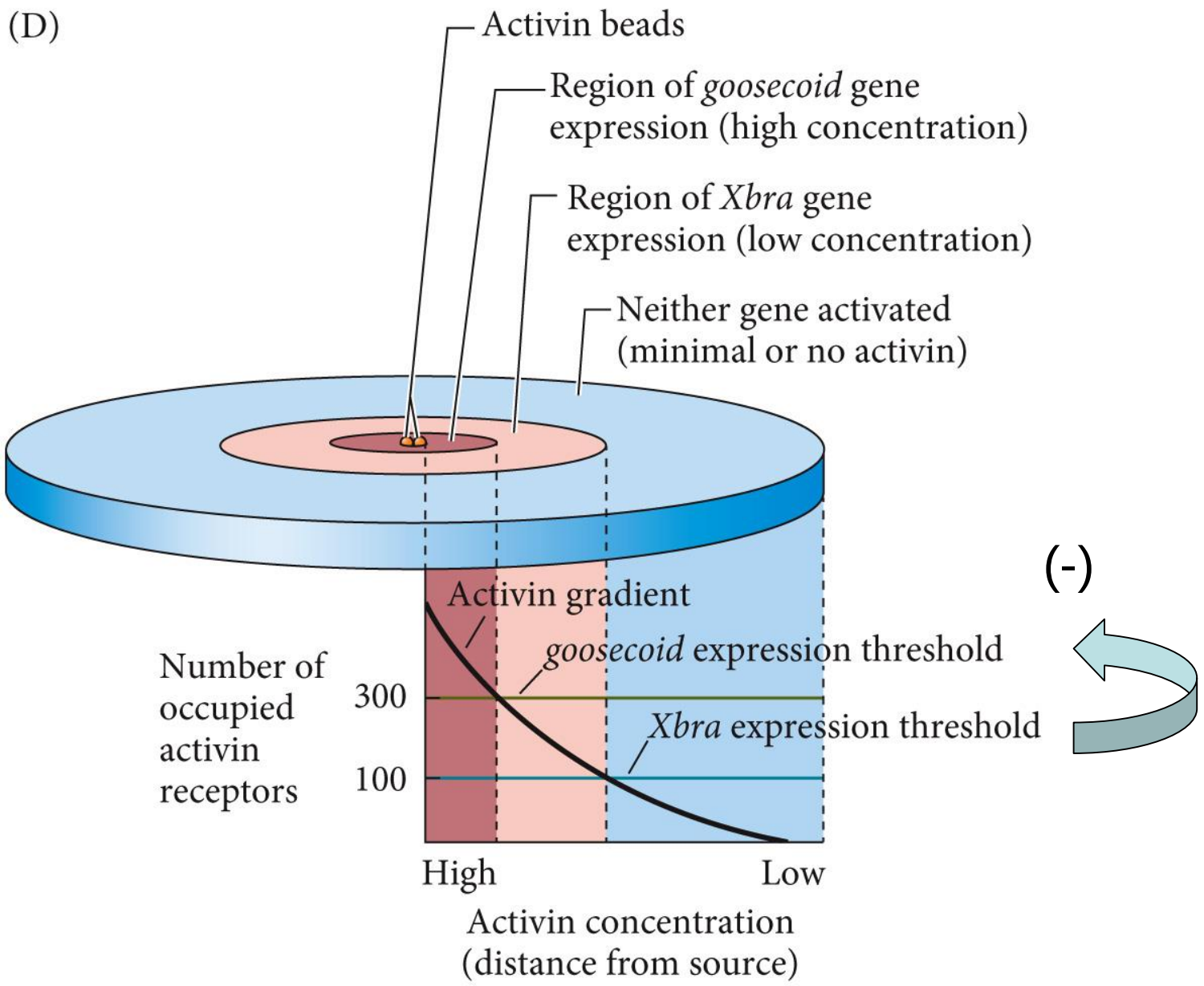
DEVELOPMENTAL BIOLOGY 11e, Figure 4.22

Morphogen gradients:

paracrine factors expressed in gradients that regulate gene expression and determine cell fate by concentration. Produced in one group of cells and then travel to another population of cells, specifying the target cells to have similar or different fates according to the concentration of the morphogen. –

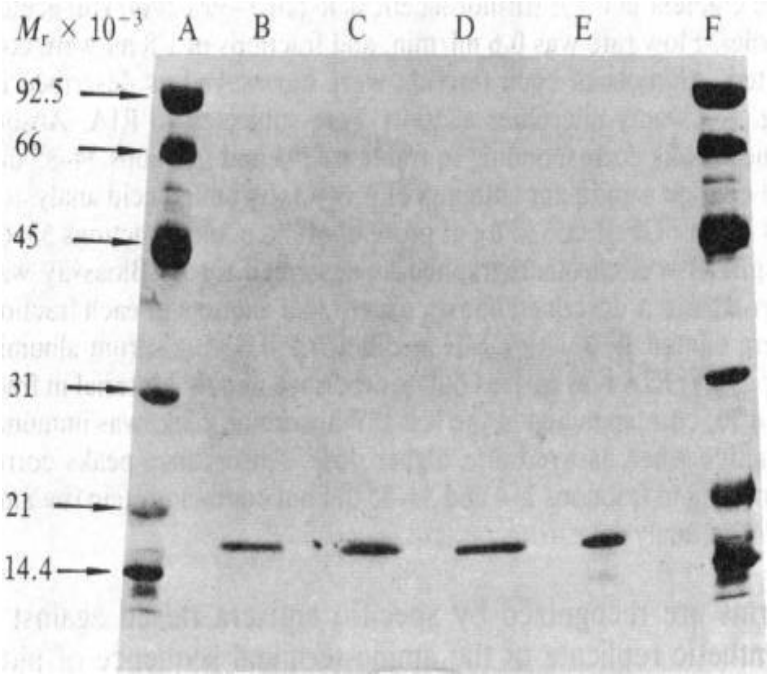
Figure 4.22 Specification of uniform cells into three cell types by a morphogen gradient.

Figure 4.23 A gradient of the paracrine factor activin, a morphogen, causes concentration-dependent expression differences of two genes in unspecified amphibian cells (Part 4)



(After Gurdon et al. 1994; Dyson and Gurdon 1998)

Fibroblast Growth Factors (FGFs) and FGF Receptors (FGFR)



Basic FGF (FGF-2) mitogenic factor

1. **Denis Gospodarowicz, G.Lui, and J. Cheng** ***1982***
Purification in High Yield of Brain Fibroblast Growth Factor by Preparative Isoelectric Focusing at p H 9.6
J. BIOL CHEM 257.*, *pp. 12266-12276,.

FGFs (n=21) - multifunctional signaling factors expressed in all tissues in multicellular organisms – Metazoa (Stachowiak eta.al., ‚Integrative Nuclear Signaling in Cell Development-A Role for FGF Receptor-1. *DNA Cell Biol* **2007,** 26, (12), 811-26.)

Nomenclature of FGF and Some Features of F

Expressed in all tissues.

Name	Alternative names	Genes cloned from[b]
FGF-1	Acidic FGF (aFGF)	Human, hamster, bovine, rat, pig, chick, mouse
		Human, opossum, bovine, rat, chick, mouse, she
FGF-2	Basic FGF (bFGF)	*Xenopus*, newt
FGF-3	INT-2	Human, chick, fish, mouse, *Xenopus*
FGF-4	HST-1, k-FGF (Kaposi)	Human, chick, bovine, mouse, *Xenopus*
FGF-5		Human, mouse, rat
FGF-6	HST-2	Human, mouse
FGF-7	KGF (keratinocyte GF)	Human, mouse, rat, sheep, dog
FGF-8	AIGF (androgen induce	Human, mouse, chick, *Xenopus*
FGF-9	GGF (glial)	Human, rat, mouse, *Xenopus*
FGF-10		Human, rat, chick, mouse
FGF-11	FHF-3	Human, mouse
FGF-12	FHF-1	Human, mouse, chick
FGF-13	FHF-2	Human, mouse, chick
FGF-14	FHF-4	Mouse
FGF-15		Mouse
EGL-17		*C. elegans*
BNL	Branchless	*Drosophila*

[a] Based on Emoto *et al.* (1997).
[b] Based on searches of Genbank through September 1997.

Functions of FGFs are controlled at the level of:

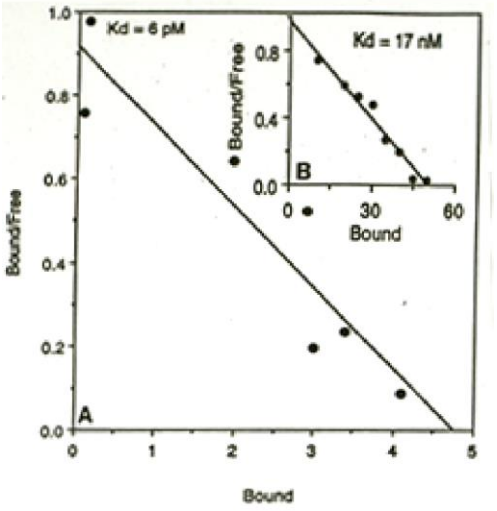
- gene transcription (promoter, antisense RNA),
- mRNA stability, translation,
- protein storage,
- Interaction with accessory proteins: Heparan Sulfate Proteoglycans (HSPG),
- serine proteases: **Thrombin** and **plasmin**

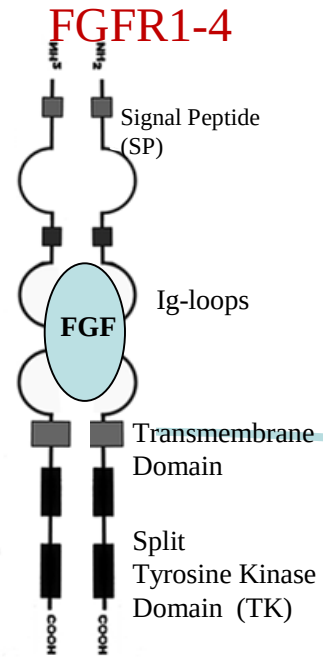
FGF receptors – 3 types: high affinity, HSPG, Cysteine FGF receptor (CFR).
(Table III)

TABLE III
Nomenclature of Genes Encoding FGF-Binding Proteins

FGF-binding proteins	Types of FGF-binding proteins	Alternative names	Genes cloned from
fgfr			
	FGFR1	*flg, fms, cek1*	Human, newt, rat, mouse, *Xenopus*, chick, bovine, fish
	FGFR2	*bek, K-sam, kgfr, cek-3*	Human, newt, rat, fish, *Xenopus*, chick, mouse
	FGFR3	*cek-2*	Human, newt, chick, mouse, fish
	FGFR4	*frek*	Human, newt, *Xenopus*, quail, fish, monkey
	C. elegans FGFR	*egl-15*	*C. elegans*
	Drosophila FGFRa	*dfr1*, breathless (*btl*)	*Drosophila*
	Drosophila FGFRb	*dfr1/dfgf-R2*, heartless	*Drosophila*
	Sea urchin FGFR	*spfgr*	Sea urchin
hspg			
	Syndecans-1, -2, -3, -4	2 = Fibroglycan 3 = N-syndecan 4 = Ryodocan, amphiglycan	*Drosophila*, mouse, chick, *Xenopus*, human, rat
	Glypicans-1, -2, -3, -4, -5	2 = Cerbroglycan 3 = OCI-5 4 = K-glypican dDLY (*Drosophila* gly)	Rat, human, mouse, chick
	Perlecans		Mouse, human, rat, *C. elegans*
	Betaglycans		Rat, human, chick, pig
cfr		MG-160, E-selectin ligand (ESL-1)	Chick, mouse, human, rat, bovine

High Affinity FGF Receptors (FGFR1-4) have a general structure of type I membrane proteins.



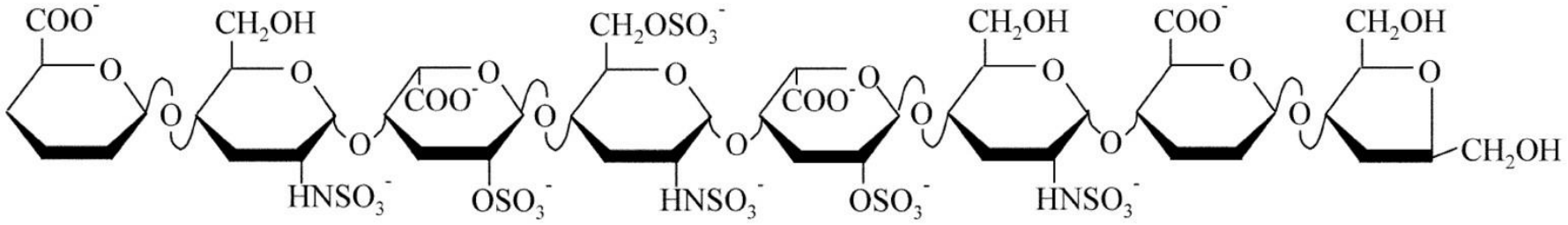


Kd=[nM]

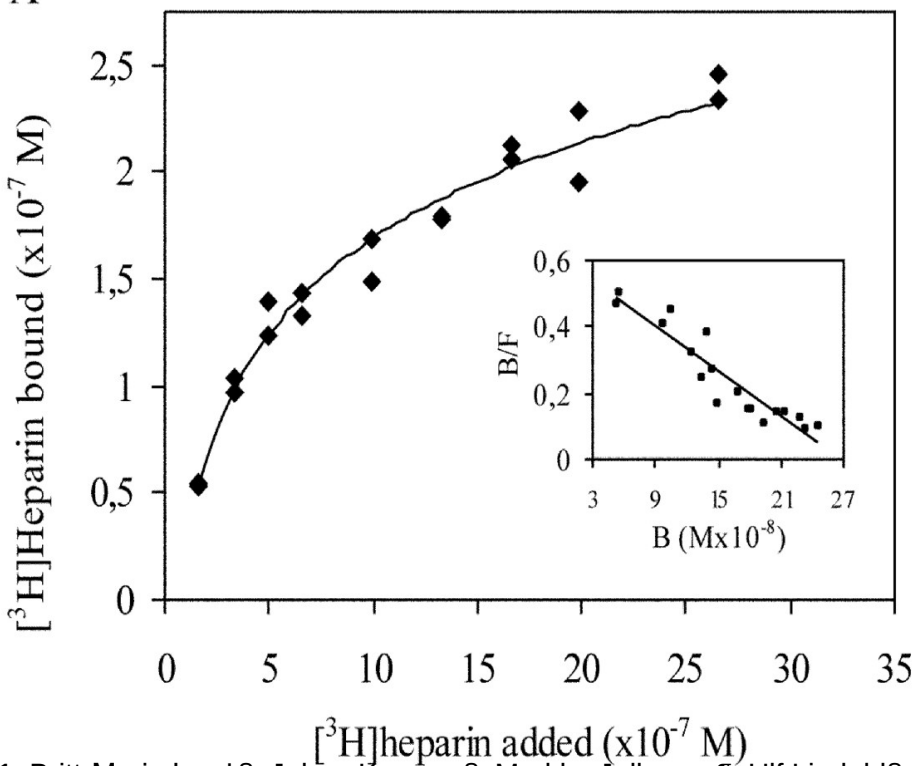
Kd=[pM]

Binding of Heparin/Heparan Sulfate to Fibroblast Growth Factor Receptor 4*

Octasaccharide8a



A



Vol. 276, No. 20, Issue of May 18, pp. 16868–16876, 2001, Britt-Marie Loo‡§, Johan Kreuger§, Markku Jalkanen¶, Ulf Lindahl§, and Markku Salmivirta‡§¶i

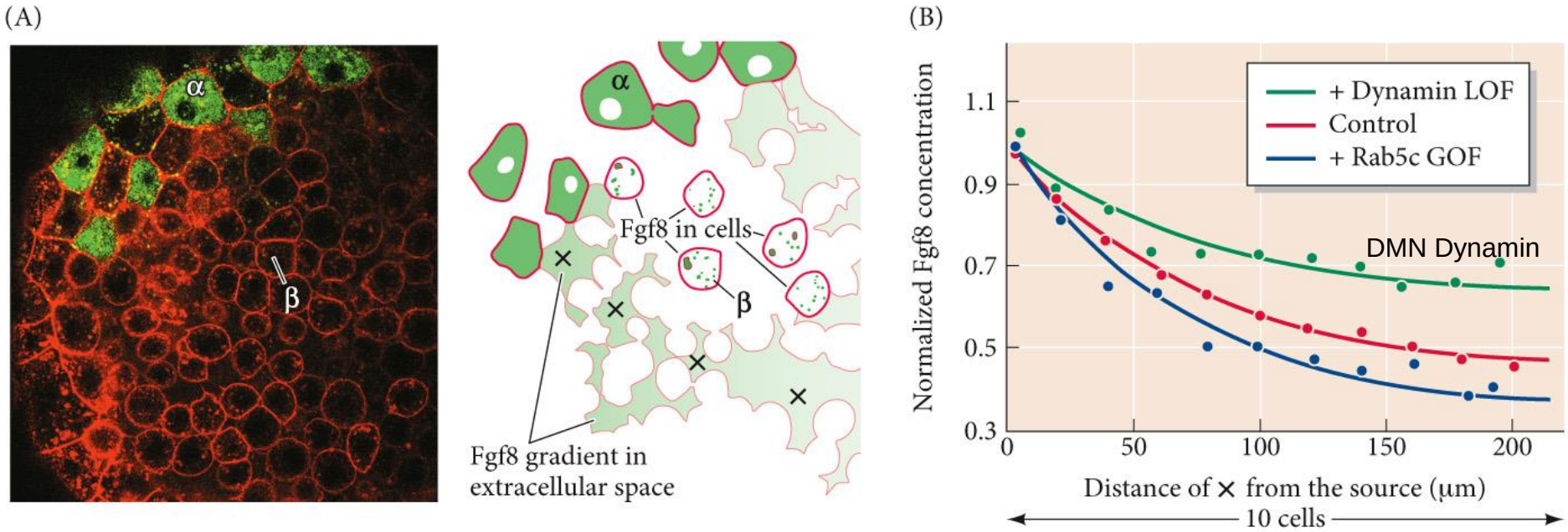
B

FGF8: FGF 8 (AIGF, androgen-induced growth factor), is secreted as a 28-32 kDa glycoprotein with multiple splice variants (at least eight isoforms in mouse and four in human).

Receptors: 8b activates FGFR 2 (IIIc0, FGFR 3 (IIIc), and FGFR 4, while 8e activates FGFR 3 (IIIc) and FGFR 4; **no FGF 8 isoform activates a IIIb form or FGFR 1.**
FGF 8b may be of prognostic value in prostate cancer.

In the fetus, regions known to express FGF 8 include the embryonic infundibulum, the apical ectodermal ridge of the limb bud and oral epithelium of the first bronchial arch, and the pre-primitive streak embryonic ectoderm, nephrogenic cords, Bowman's capsule and developing labyrinth. In the adult, it is found in prespermatogonia and antral follicles of the ovary

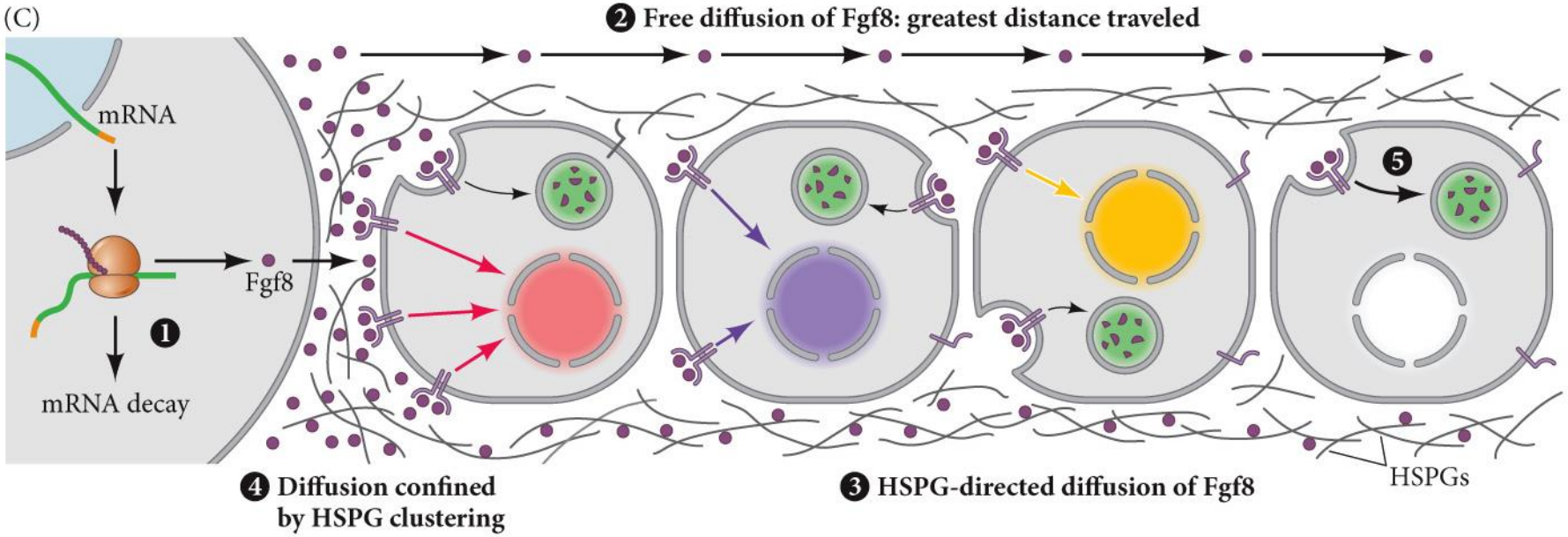
Figure 4.39 The Fgf8 gradient in Sebra fish gastrule



Yu et al. 2009; C after Bökel and Brand 2013; Balasubramanian and Zhang 2015.)

A) Zebrafish blastulae injected with Fgf8-GFP DNA (green) and mRFP-glycosyl phosphatidylinositol (GPI; red stain) - confocal image of a resulting gastrula, Fgf8 produced by and secreted away.
(B) schematic of Fgf8 in a gradient in the ECM as well as being internalized in receiving cells.

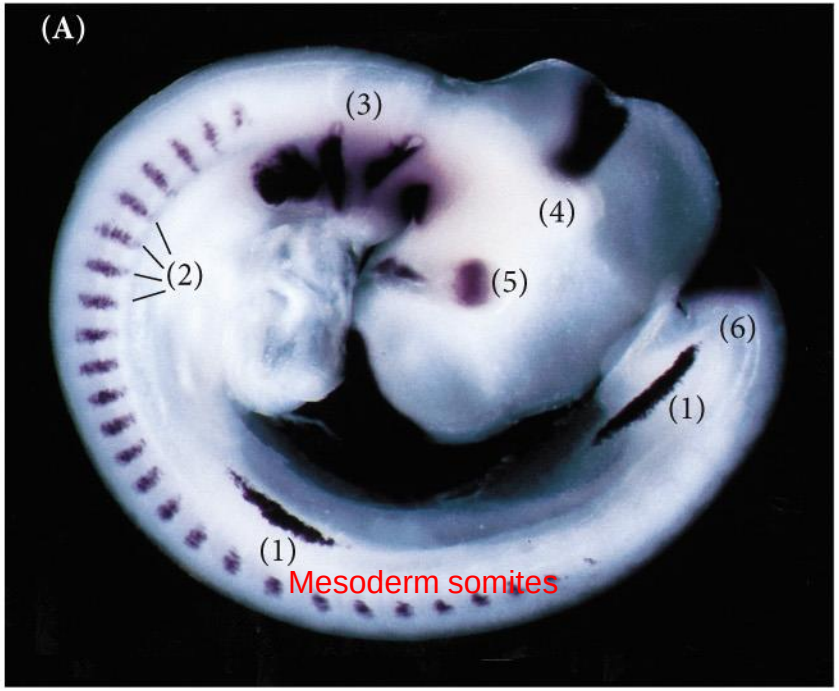
Five primary mechanisms for shaping the Fgf8 gradient.

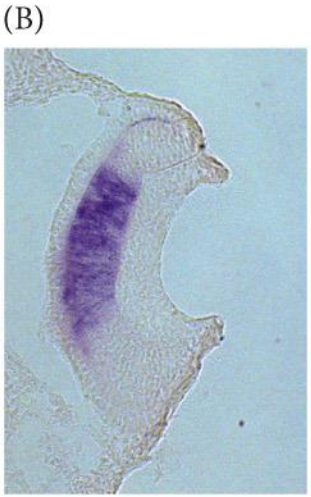


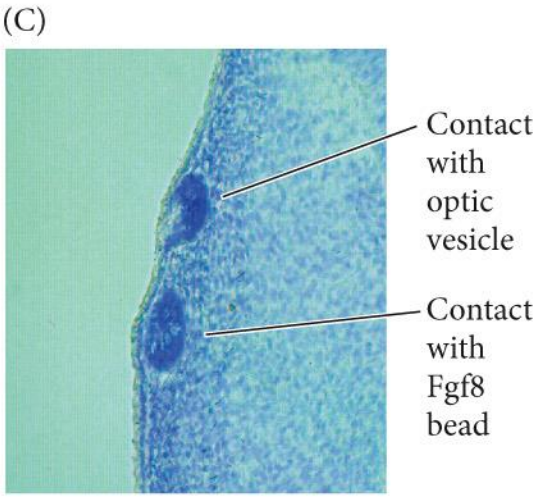
re 4.39

Yu et al. 2009; C after Bökel and Brand 2013; Balasubramanian and Zhang 2015.)

Figure 4.25 Fgf8 mRNA in the developing chick embryo (A) and eye (B, C)







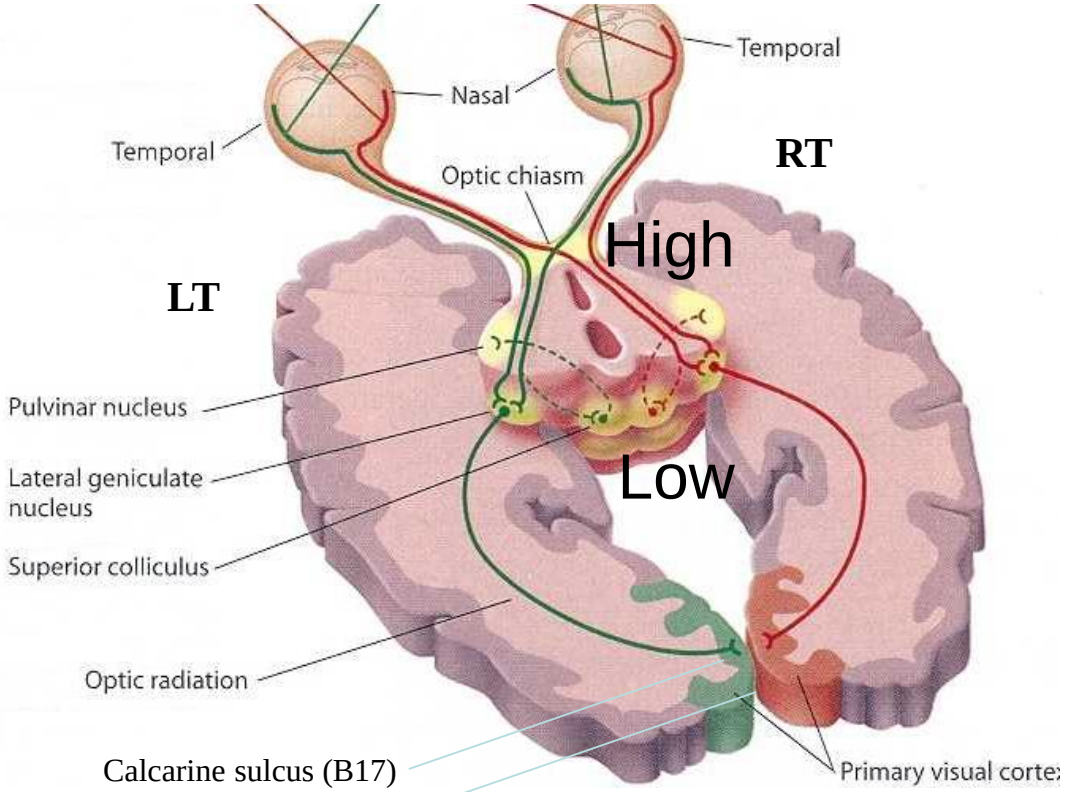
Fgf8 mRNA – retina

L-Maf - ectoderm presumptive lens

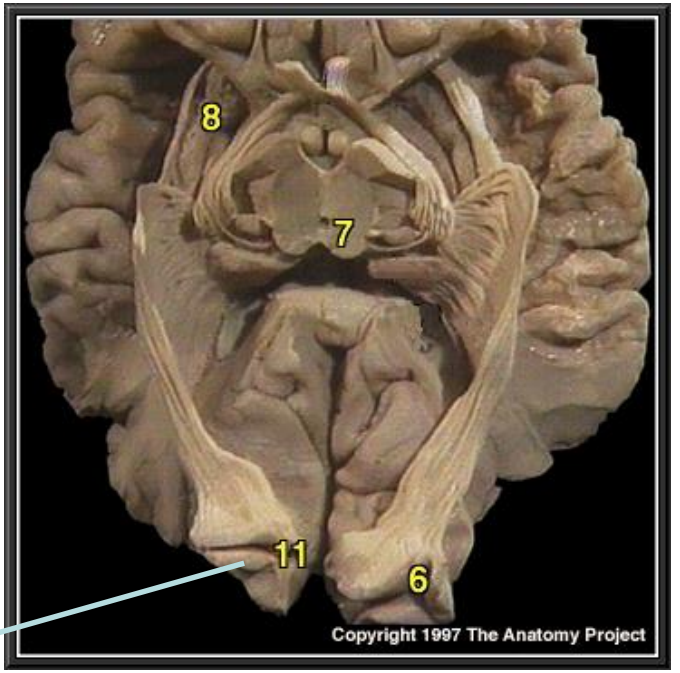
1) the distal most limb bud ectoderm
2) the somitic mesoderm segments
3) the branchial arches of the neck
4) midbrain/hindbrain boundary
5) The optic vesicle (also B and C)
6) the tail

E. Laufer, C.-Y. Yeo, and C. Tabin; B,C courtesy of A. Vogel-Höpker

FGF gradient in the Visual Pathway



B. 17; secondary B18,19



Calcarine sulcus (B17)

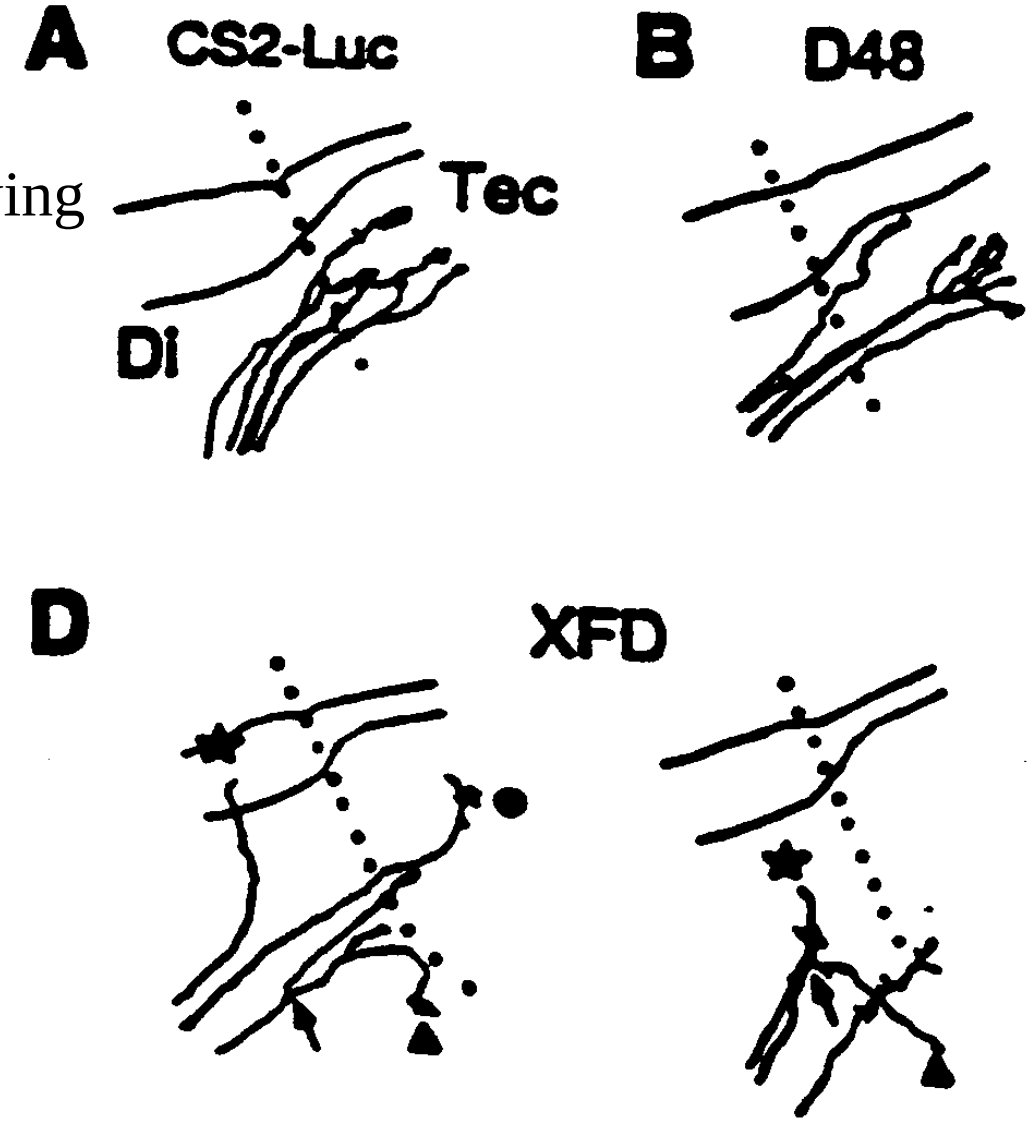
Development of Retino-tectal projection

axonal growth and targeting by FGF-2 gradient

Receptor expressed by growing axons.
FGF-2 gradient
Diencephalon > tectum

A,B - normal FGFR1

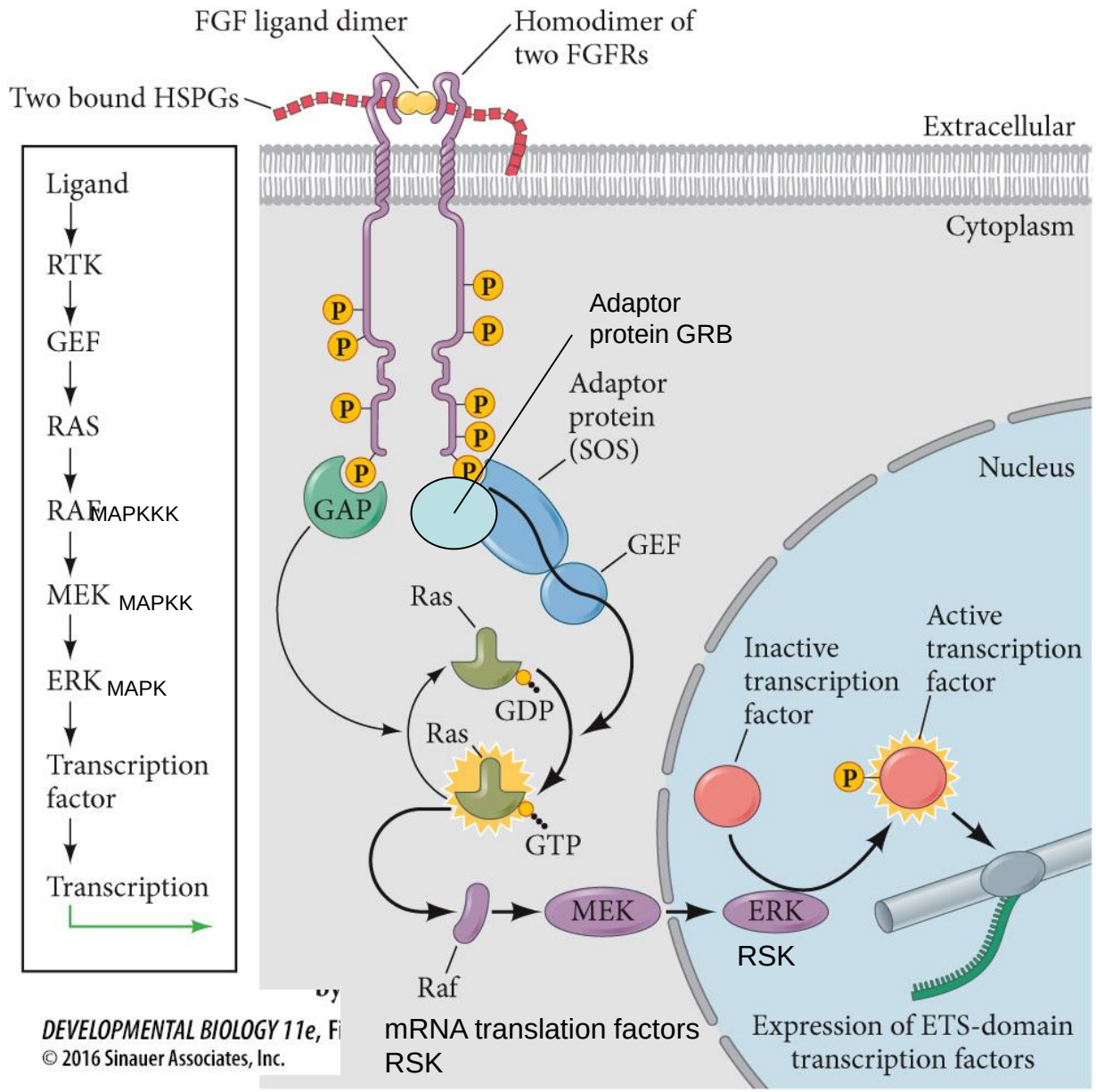
D - inactive FGFR1



FGF-2 and other FGFs are produced at some point of development by all tissues and play roles in:

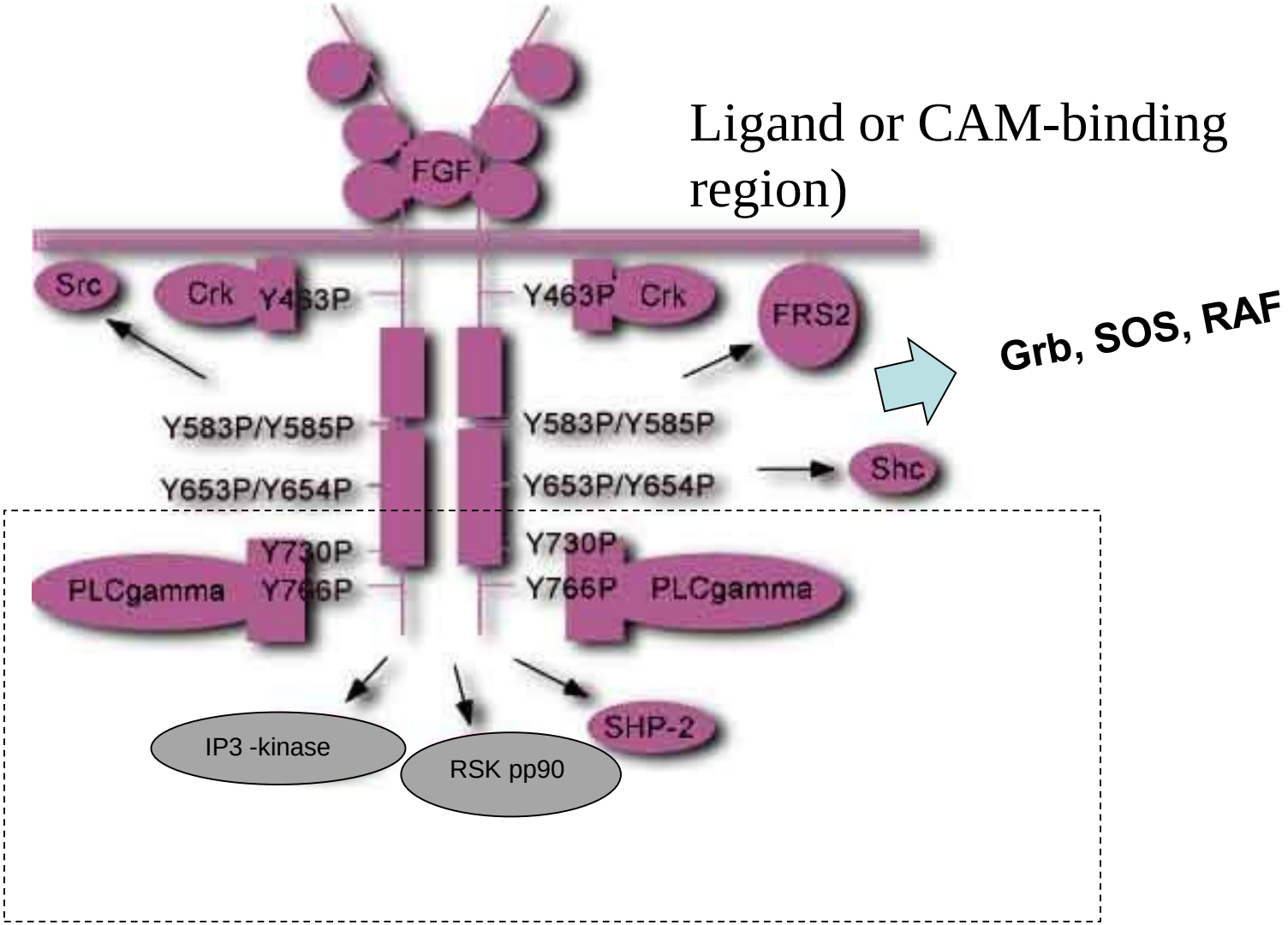
- Gastrulation
- Neurulation
- Anteroposterior specification
- Organ morphogenesis
- Axonal growth and guidance
- Neuronal survival

Figure 4.26 The widely used FGFR RTK signal transduction pathway can be activated by fibroblast growth factor



DEVELOPMENTAL BIOLOGY 11e, Figure 4.26
© 2016 Sinauer Associates, Inc.

FGFR1-4 additional signaling by via PLC, IP3, RSK1



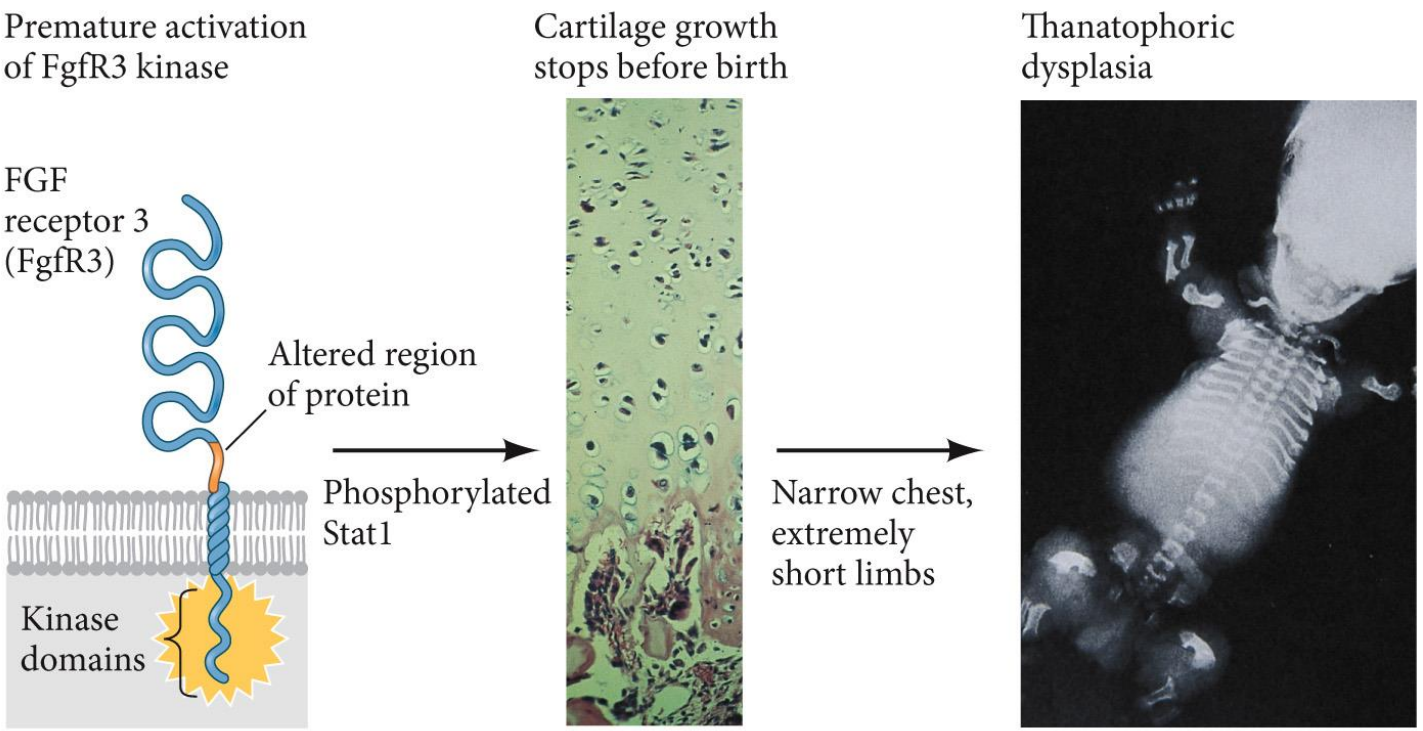
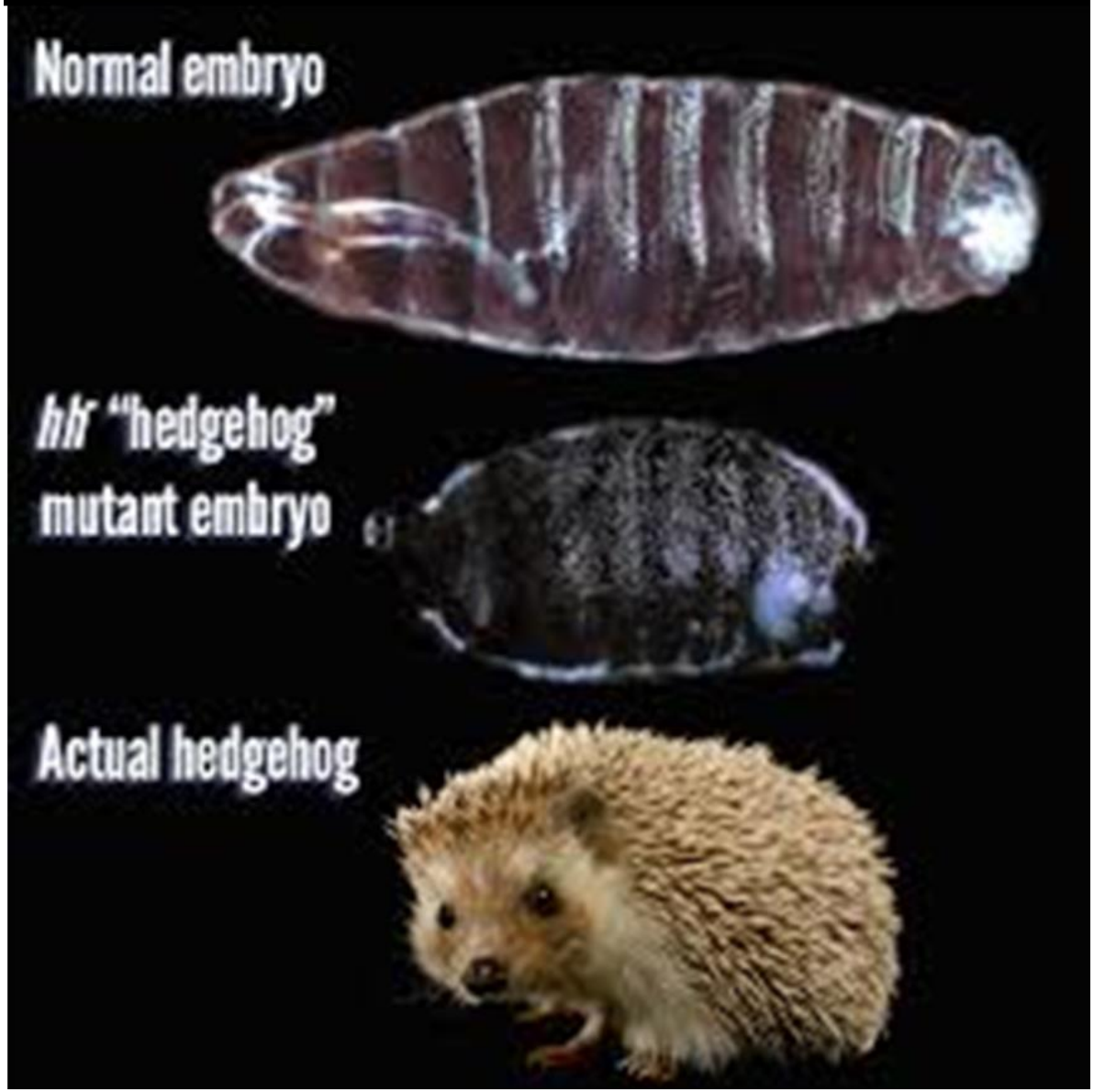


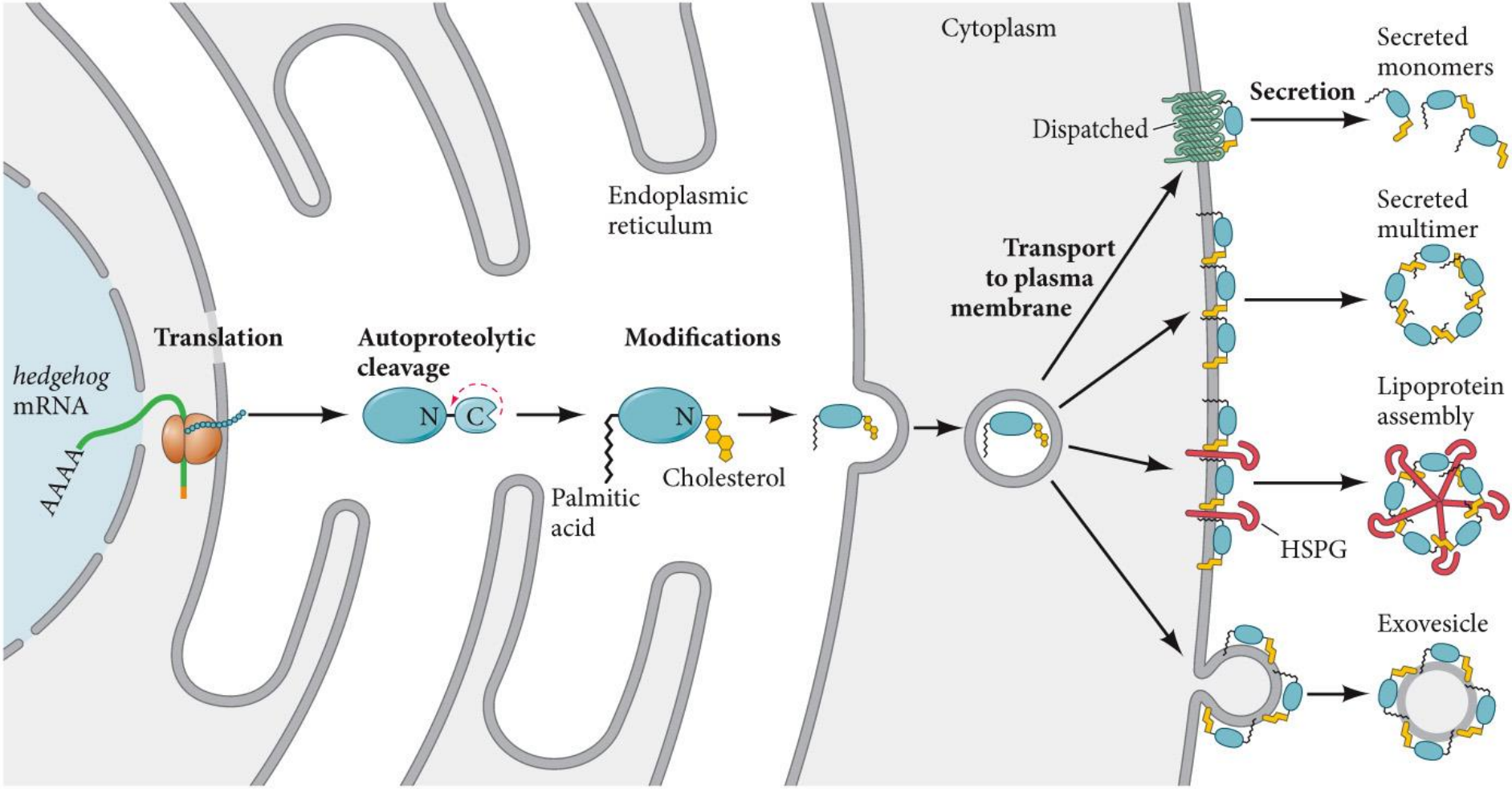
Figure 4.28 A mutation in the gene for FgfR3 causes the premature constitutive activation of the STAT pathway and the production of phosphorylated Stat1 protein. This transcription factor activates genes that cause the premature termination of chondrocyte cell division. The result is thanatophoric dysplasia, a condition of failed bone growth that results in the death of the newborn infant because the thoracic cage cannot expand to allow breathing. (After Gilbert-Barness and Opitz 1996.)

Hedgehogs



In Drosophila the *hh* acts as a segment polarity gene required for the development of dorsal embryonic structures. Low stringency screening of mouse, chick, and zebra-fish identified: *Desert hh (Dhh)*, *Indian hh(Ihh)*, and *Sonic hh (Shh)*. *Dhh* and *Shh* have a hydrophobic signal peptide like region suggesting secretion (therefore differ from hh, which is secreted by type II mechanism). *Dhh* most homologous with the Drosophila *hh* (approximately 50%).

Figure 4.29 Hedgehog processing and secretion

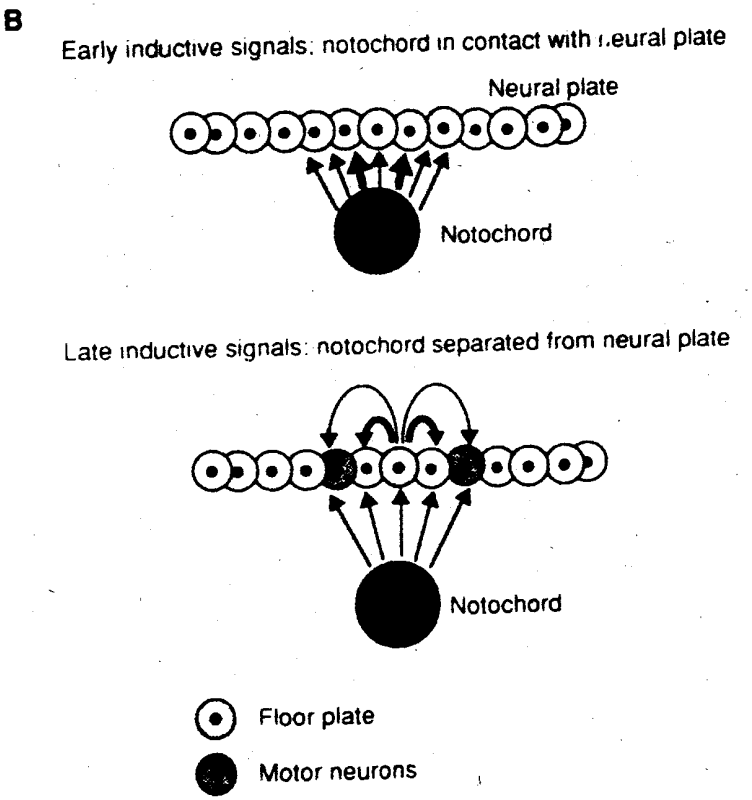


DEVELOPMENTAL BIOLOGY 11e, Figure 4.29

Unbound - Dissipates quickly
Bound - Form gradient
~ distance of 30 cells

Briscoe and Thérond 2013; Breitling 2007; Guerrero and Chiang 2007

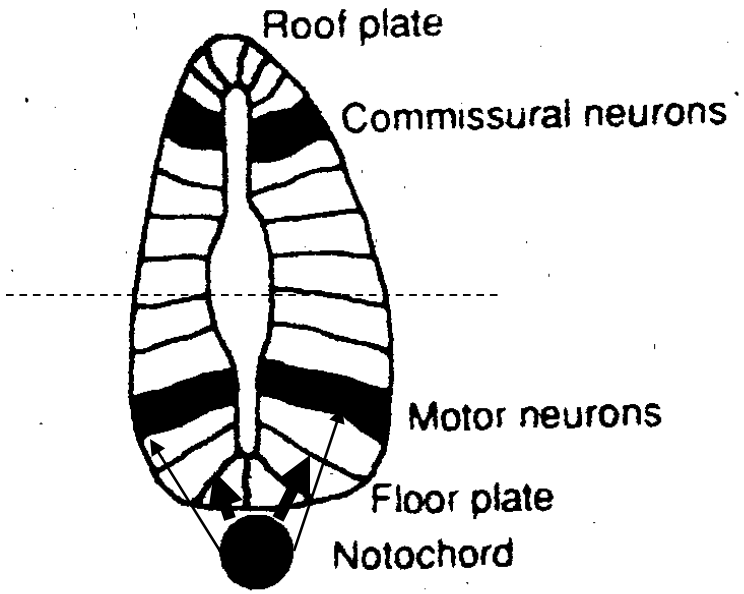
Contact-dependent and
Diffusible action of *shh*



NT is a source of contact-dependent and independent signals:
Cells immediately above NT receive both signals and form FP; more lateral cells receive diffusable factors and form motoneurons.
After FP moves apart from the NT, neuronal patterning is mostly induced by the FP. FP induces additional FP cells by contact-dependent signal and motoneurons by diffusable factors.

Patterning of Neural Tube:

Vertebrate spinal cord (SC) and hindbrain are bilaterally symmetrical with two ventral groups of motoneurons separated by specialized groups of cells and the ventral midline "floor plate" (FP). These cell types are induced by notochord (NT) (removal of NT prevents an induction of additional NT induces an additional FP). (FP extends from the spinal cord to caudal Diencephalon; in spinal cord and hindbrain induces several types of neurons including motoneurons)..



Contact-dependent and Diffusible action of *shh*

Midbrain – Shh action in Floor Plate:
Local effect – floor plate
Diffusable effect – DA neurons (motor function) in Substantia nigra

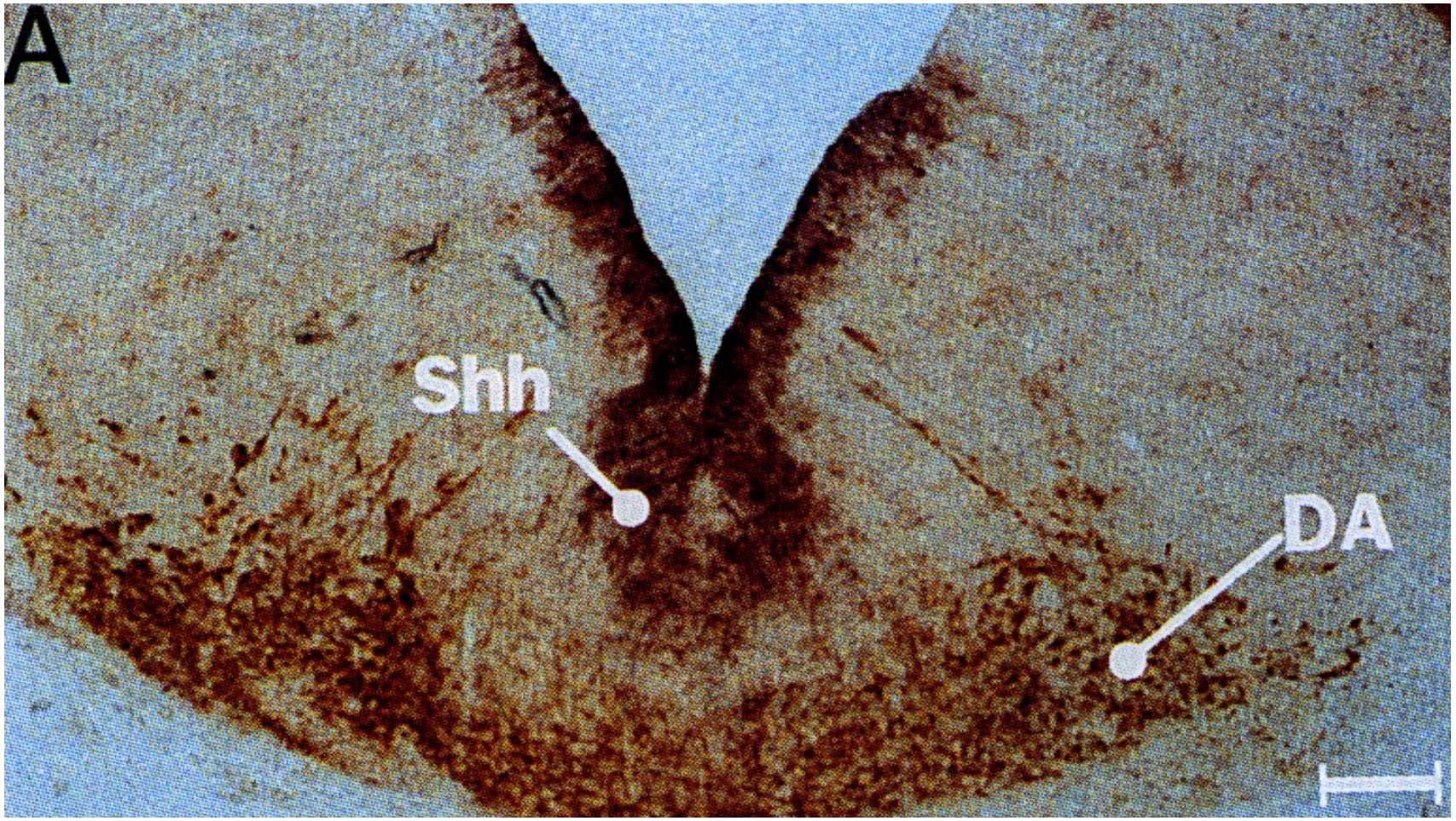
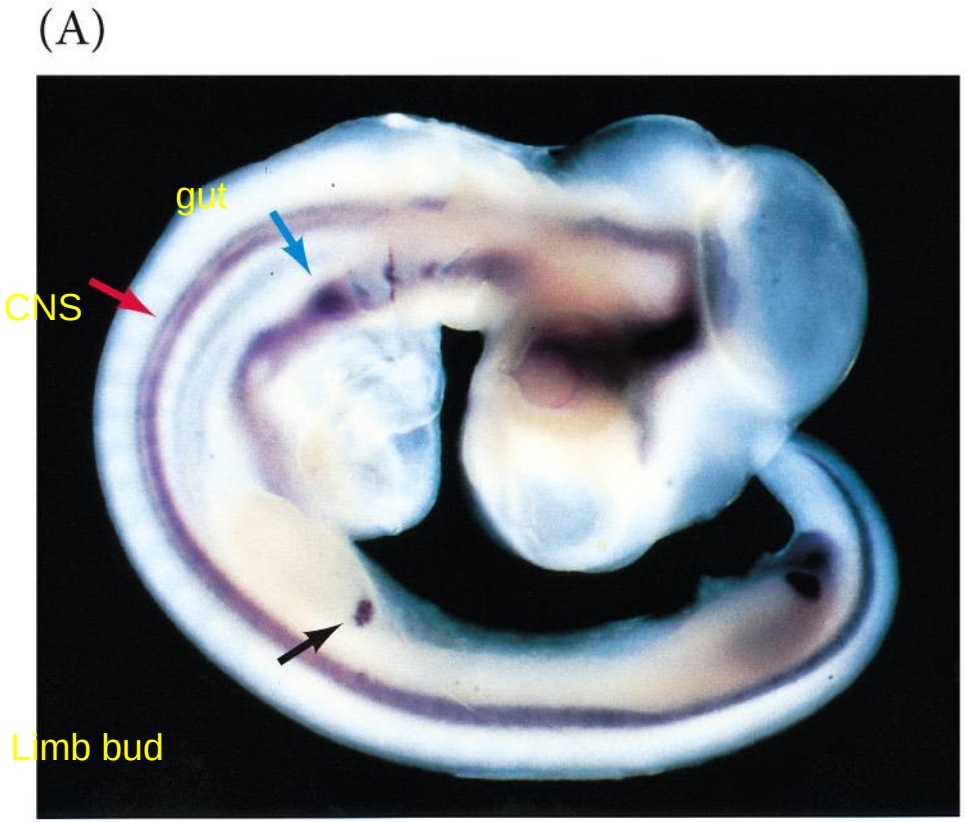


Figure 4.31 Sonic hedgehog



DEVELOPMENTAL BIOLOGY 11e, Figure 4.31

Figure 4.31 (A) Sonic hedgehog mRNA. Shh is important in pancreas development; limb and CNS patterning, neural differentiation and pathfinding, retinal craniofacial morphogenesis

(B) cyclopic lamb –**jervine** alkaloid from ***Veratrum californicum*** **"**corn lily"

C. Tabin; B courtesy of L. James and USDA Poisonous Plant Laboratory

Human Cyclopias - mutation of Shh or impaired cholesterol synthesis.

<u>Inactivating mutations</u> - malformations,

<u>Activating mutations</u> – (hyperactive smoothened) have mitogenic effects and cause cancers.

Patched mutations (no longer inhibit Smoothened) can cause tumors of the basal cell layer of the epidermis (basal cell carcinomas).

basal cell nevus syndrome, **Aberrant Sonic hedgehog (Shh) activation during adulthood leads to neoplastic growth,** rare autosomal dominant condition - developmental anomalies (fused fingers; rib and facial abnormalities) and multiple malignant skin tumors (Hahn et al. 1996; Johnson et al. 1996).

Vismodegib, a compound that inhibits Smoothened functio, is currently in clinical trials as a therapy to combat basal cell carcinomas

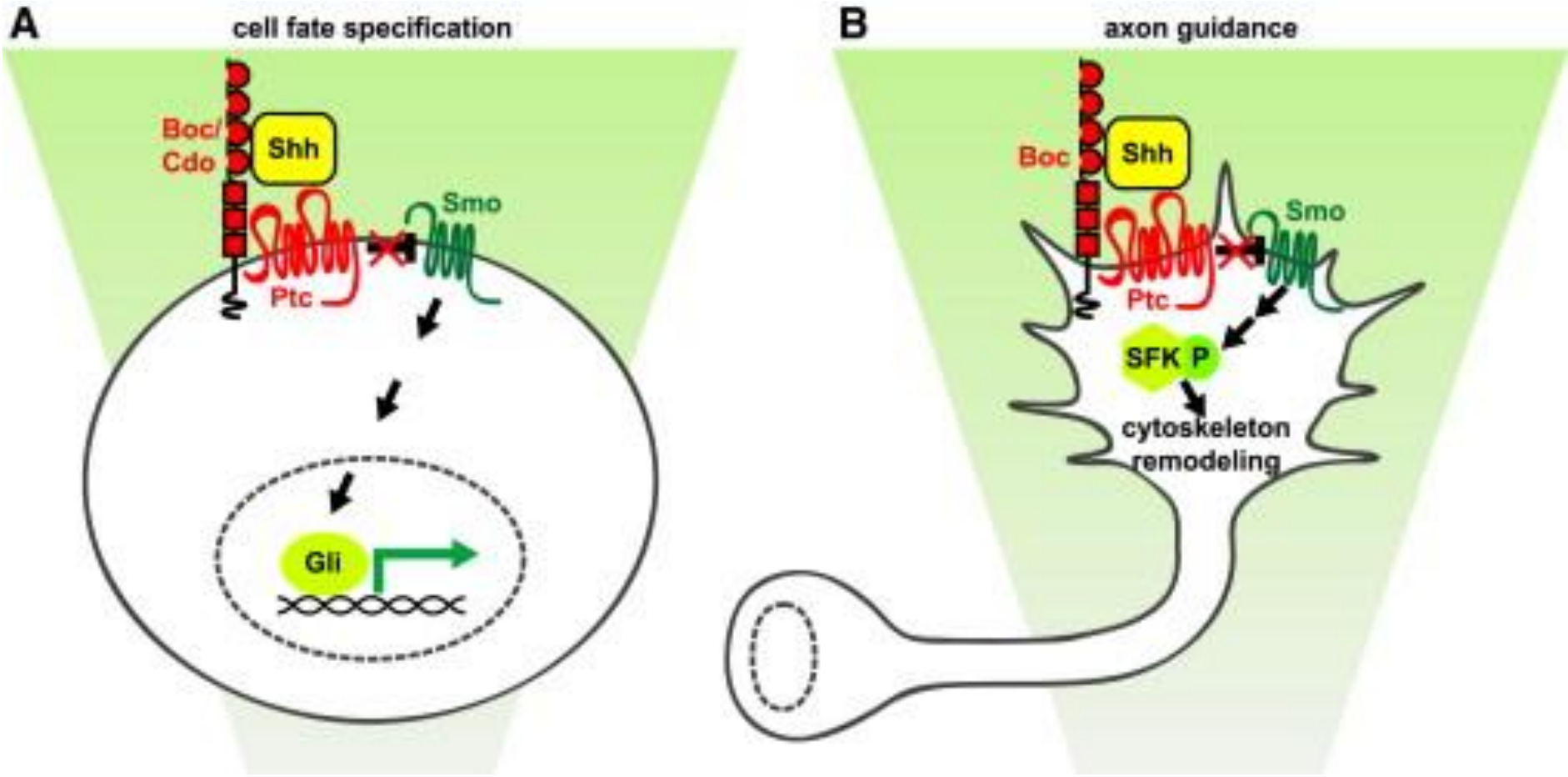
Cancer Res. 2014 Sep 15;74(18):4967-75. doi: 10.1158/0008-5472.CAN-14-1666. Epub 2014 Aug 29.

Sonic hedgehog signaling in Basal cell nevus syndrome.

Athar M[1], Li C[2], Kim AL[3], Spiegelman VS[4], Bickers DR[3].

The hedgehog (Hh) signaling pathway is considered to be a major signal transduction pathway during embryonic development, but it usually shuts down after birth. **Aberrant Sonic hedgehog (Shh) activation during adulthood leads to neoplastic growth.** Basal cell carcinoma (BCC) of the skin is driven by this pathway. Here, we summarize information related to the pathogenesis of this neoplasm, discuss pathways that crosstalk with Shh signaling, and the importance of the primary cilium in this neoplastic process. The identification of the basic/translational components of Shh signaling has led to the discovery of potential mechanism-driven druggable targets and subsequent clinical trials have confirmed their remarkable efficacy in treating BCCs, particularly in patients with nevoid BCC syndrome (NBCCS), an autosomal dominant disorder in which patients inherit a germline mutation in the tumor-suppressor gene Patched (Ptch). Patients with NBCCS develop dozens to hundreds of BCCs due to derepression of the downstream G-protein-coupled receptor Smoothened (SMO). Ptch mutations permit transposition of SMO to the primary cilium followed by enhanced expression of transcription factors Glis that drive cell proliferation and tumor growth. Clinical trials with the SMO inhibitor, vismodegib, showed remarkable efficacy in patients with NBCCS, which finally led to its FDA approval in 2012.

Hedgehog signaling independent of Gli, involves fast **remodeling of the actin cytoskeleton**



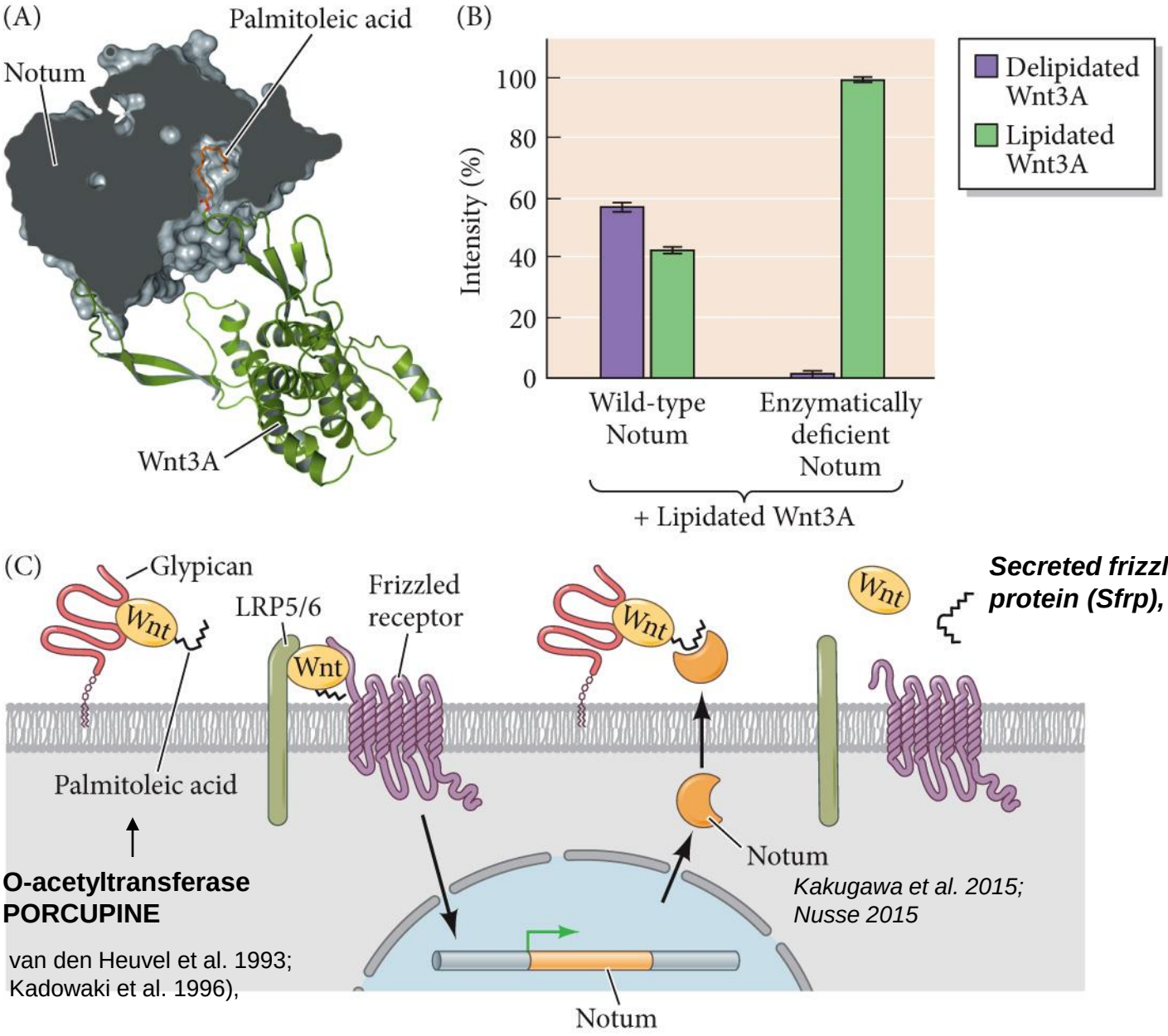
SRC FAMILY KINASES (SFKS)

WNT family - 19 Wnts in humans

WNT name = Drosophila *Wingless* (segment polarity) and vertebrates homologue *Integrated)*

Wnt proteins are critical in establishing the polarity of insect and vertebrate limbs, in promoting the proliferation of stem cells, in regulating cell fates along axes of various tissues, in guiding the migration of mesenchymal cells and pathfinding axons and in development of the mammalian urogenital system, and . How is it that Wnt signaling is capable of mediating such diverse processes as cell division, cell fate, and cell guidance?

Figure 4.33 Notum antagonism of Wnt



O-acetyltransferase PORCUPINE

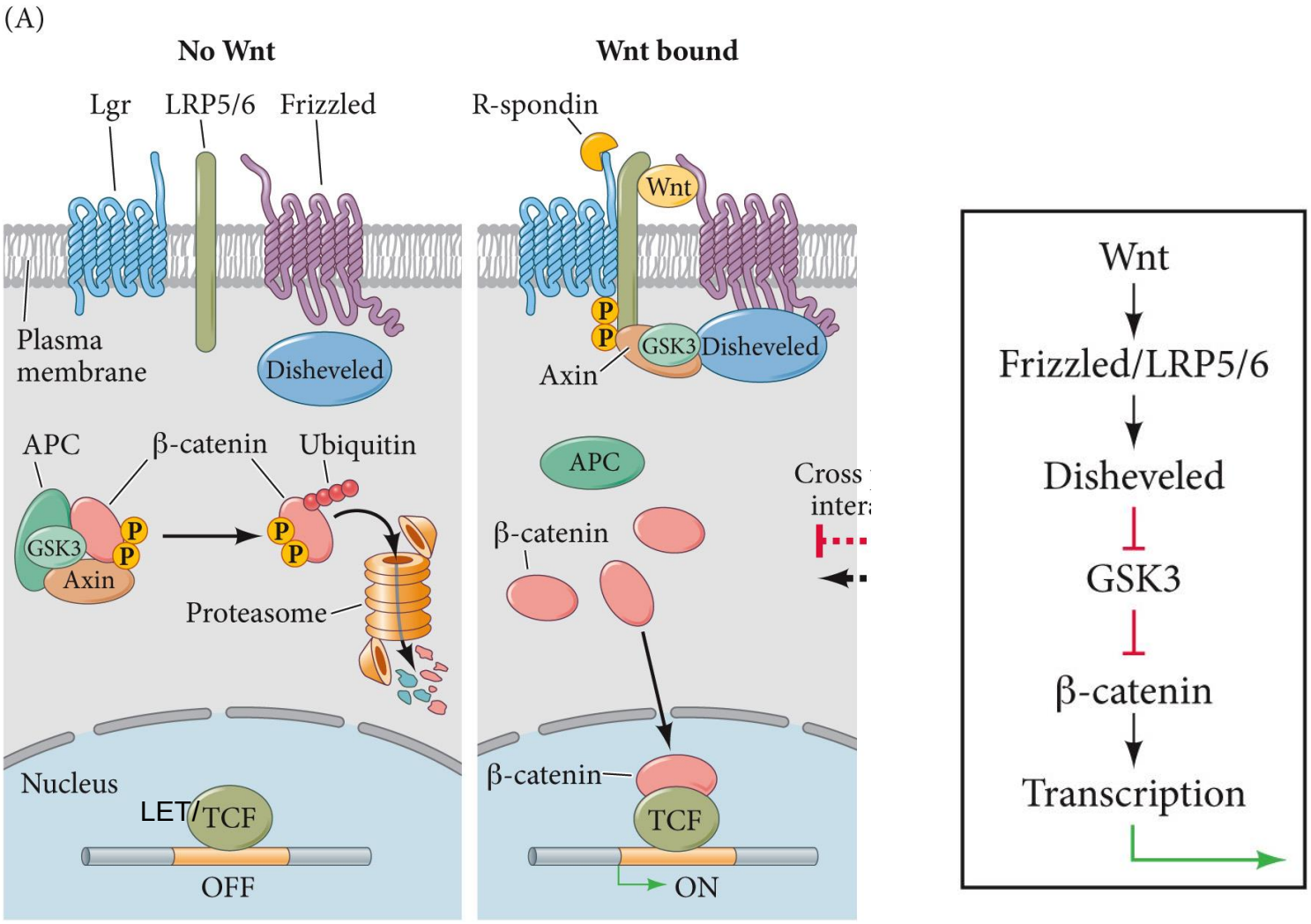
van den Heuvel et al. 1993; Kadowaki et al. 1996),

Secreted frizzled-related protein (Sfrp),

Kakugawa et al. 2015; Nusse 2015

DEVELOPMENTAL BIOLOGY 11e, Figure 4.33

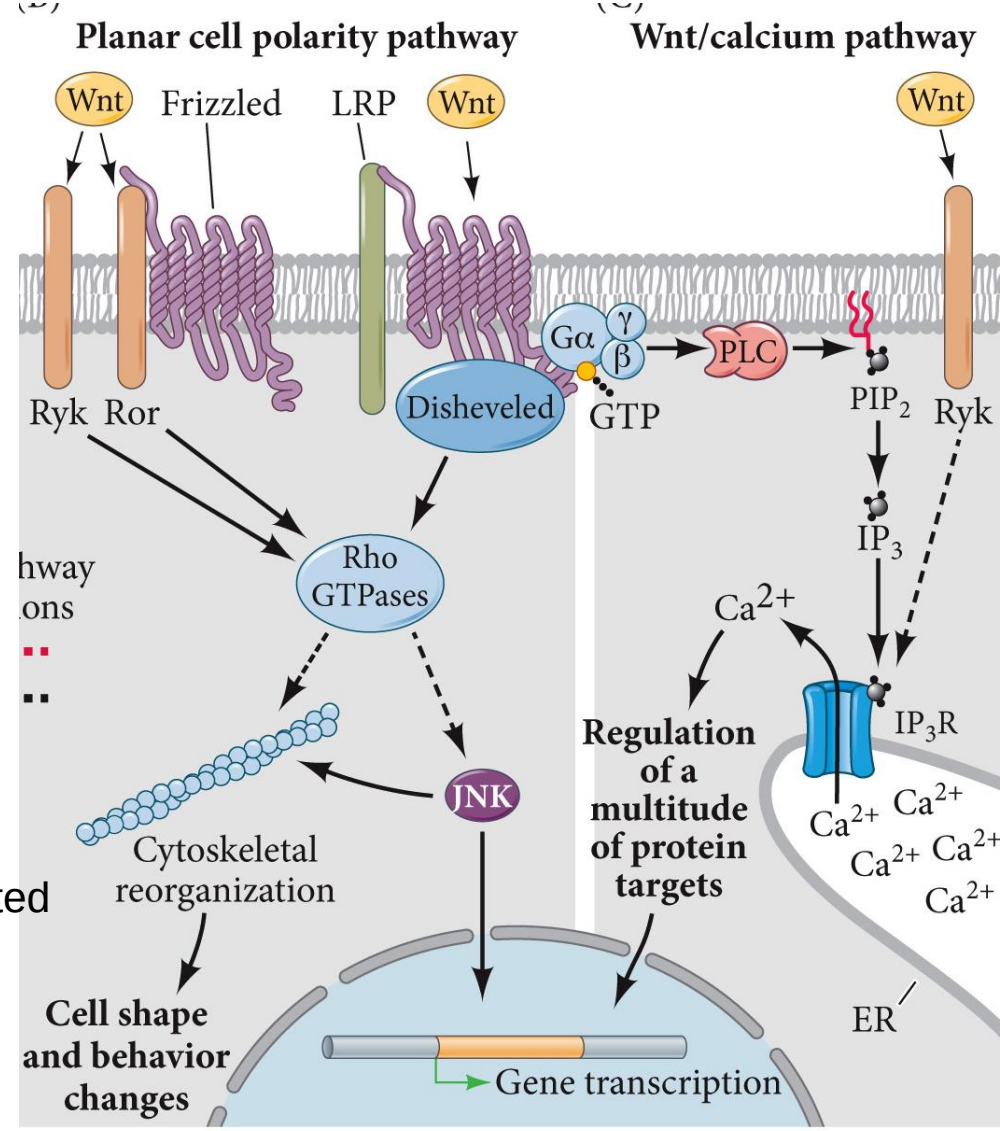
Figure 4.34 THE CANONICAL WNT PATHWAY (β-CATENIN DEPENDENT)



Cadigan and Nusse 1997; Niehrs 2012).

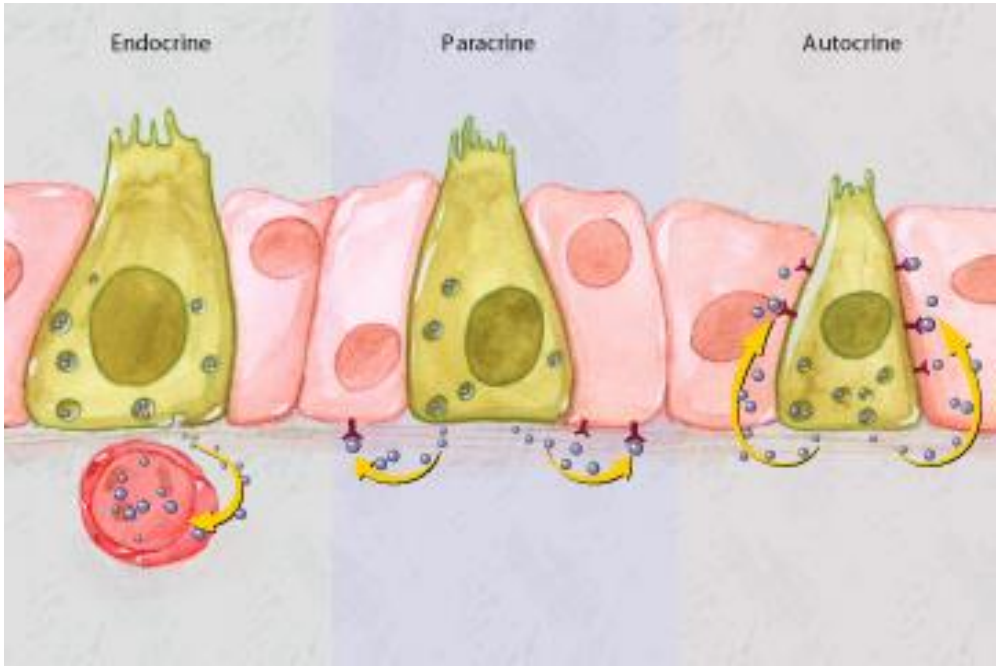
DEVELOPMENTAL BIOLOGY 11e, Figure 4.34

Figure 4.34 THE NONCANONICAL WNT PATHWAYS (B-CATENIN INDEPENDENT)



Actin & Microtubule cytoskeleton are phosphorylated by kinases

TBF-β superfamily



Assoian, R. *et al.* (1983) J. Biol. Chem. **258**:7155**.**

J. Massagué 2003.

TGF-β family: TGF-β1, 2, 3, and 5 formation of the extracellular matrix.

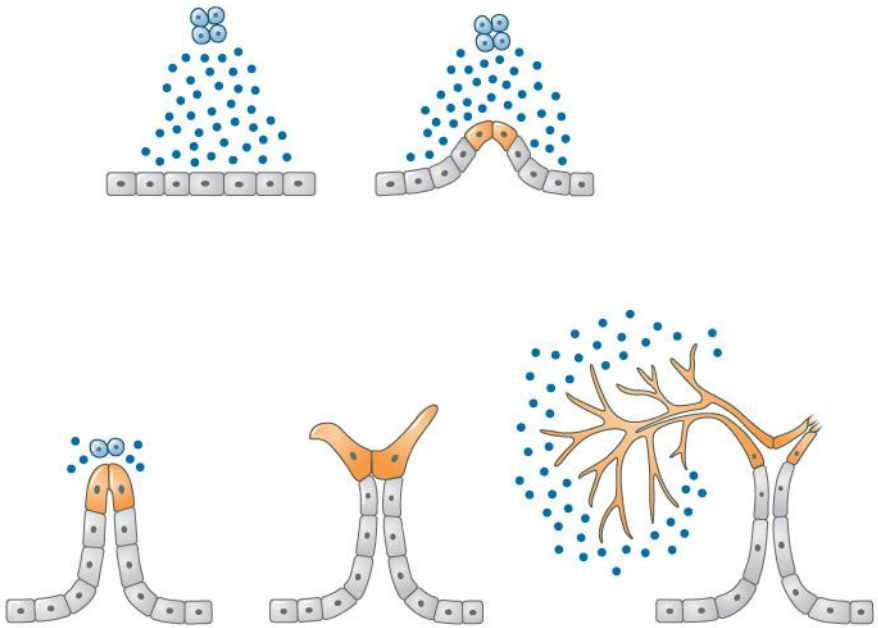
TGF-β1(and others) increases the amount of extracellular matrix that epithelial cells make (both by stimulating collagen and fibronectin synthesis and by inhibiting matrix degradation).

TGF-β1(and others) regulate cell division (both positively and negatively).

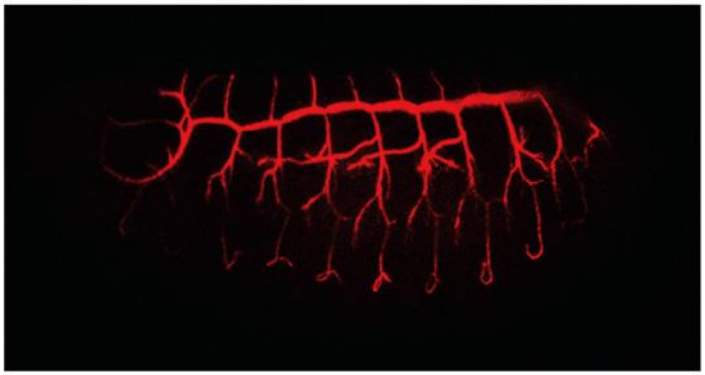
TGF-β proteins control where and when epithelia branch to form the ducts of kidneys, lungs, and salivary glands

Concept of branch formation

(A)



(B)



DEVELOPMENTAL BIOLOGY 11e, Figure 4.21

Table 1 The transforming growth factor β (TGF-β) family and representative activities[a]

Names [Homologues]	%	Representative activities (References)
Activin subfamily		
Activin βA	42	Pituitary follicle-stimulating hormone (FSH) production, erythroid cell differentiation; in frog, mesoderm induction. (3, 9, 10)
Activin βB	42	
Activin βC	37	
Activin βE	40	
TGF-β subfamily		
TGF-β1	35	Cell cycle arrest in epithelial and hematopoietic cells, control of mesenchymal cell proliferation and differentiation, wound healing, extracellular matrix production, immunosuppression. (11–14)
TGF-β2	34	
TGF-β3	36	
Distant members		
MIS/AMH	27	Müllerian duct regression. (15, 16)
Inhibin α	22	Inhibition of FSH production and other actions of activin. (9, 10)
GDNF	23	Dopaminergic neuron survival, kidney development. (17)

Table 1 The transforming growth factor β (TGF-β) family and representative activities[a]

Names [Homologues]	%	Representative activities (References)
BMP2 subfamily		
BMP2 [Dpp^D]	100	Gastrulation, neurogenesis, chondrogenesis, interdigital apoptosis; in frog: mesoderm patterning; in fly: dorsalization, eyes, wings. (1–3)
BMP4	92	
BMP5 subfamily		
BMP5 [60 A^D]	61	Along with BMPs 2 and 4, this subfamily participates in the development of nearly all organs; many roles in neurogenesis. (1, 2)
BMP6/Vgr1	61	
BMP7/OP1	60	
BMP8/OP2	55	
GDF5 subfamily		
GDF5/CDMP1	57	Chondrogenesis in developing limbs. (1, 4)
GDF6/CDMP2	54	
GDF7	57	
Vg1 subfamily		
GDF1 [$Vg1^X$]	42	Vg1: axial mesoderm induction in frog and fish. (4)
GDF3/Vgr2	53	
BMP3 subfamily		
BMP3/osteogenin	48	Osteogenic differentiation, endochondral bone formation, monocyte chemotaxis. (5)
GDF10	46	
Intermediate members		
Nodal [Xnr 1 to 3^X]	42	Axial mesoderm induction, left-right asymmetry. (1, 6)
Dorsalin	40	Regulation of cell differentiation within the neural tube. (7)
GDF8	41	Inhibition of skeletal muscle growth. (8)
GDF9	34	

2. General structure of disulfide-linked homo- or heterodimers of TGF-∃ and related GF. [Annu. Rev. Cell. Biol. 6, 697-641 (1990)]

Fig. 1 (TGF-Beta1 is a 390 aa precursor processed to 120 aa active peptide)

Latent complex = TGFBeta-1 dimmer + LAP (dimmer pro-region) + LTBP (cross-linked to extracellular matrix)

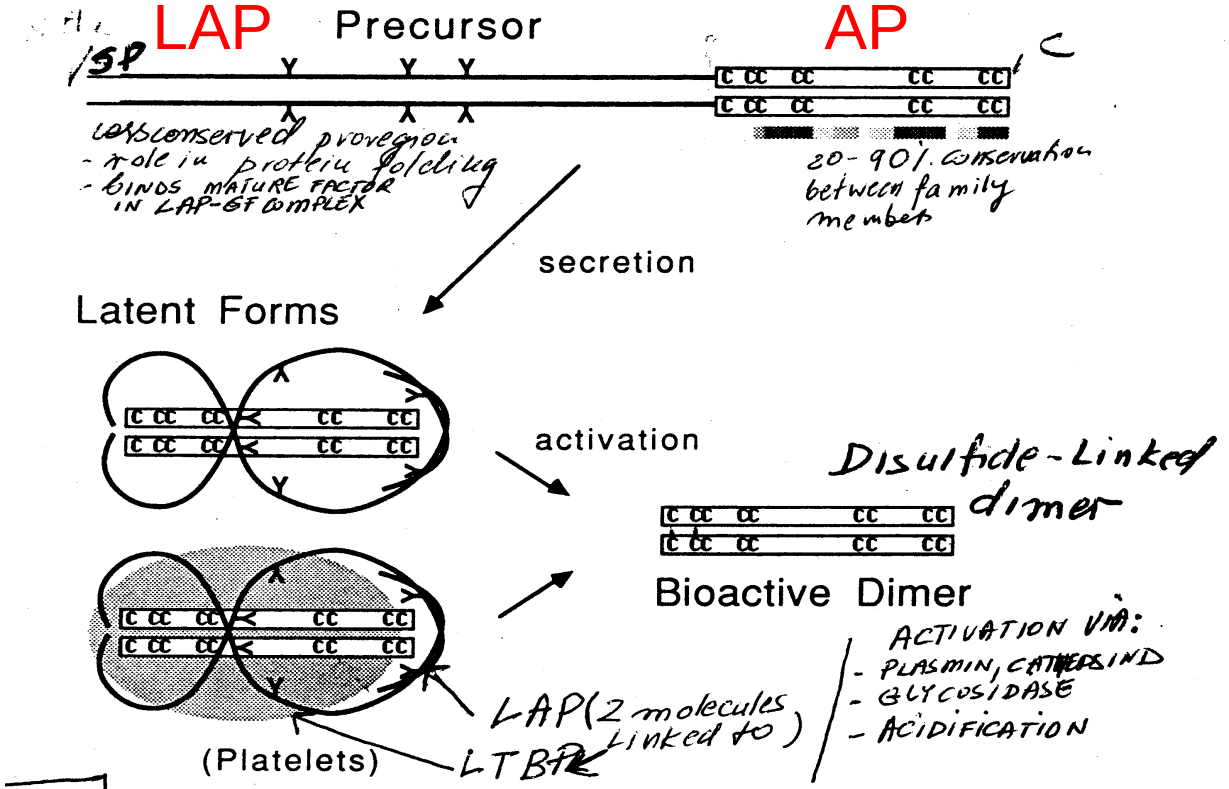


Figure 1 Precursor, latent, and bioactive forms of TGF-β1. The TGF-β1 precursor consists

BMP2,4,7 - (Bone Morphogenic Proteins):

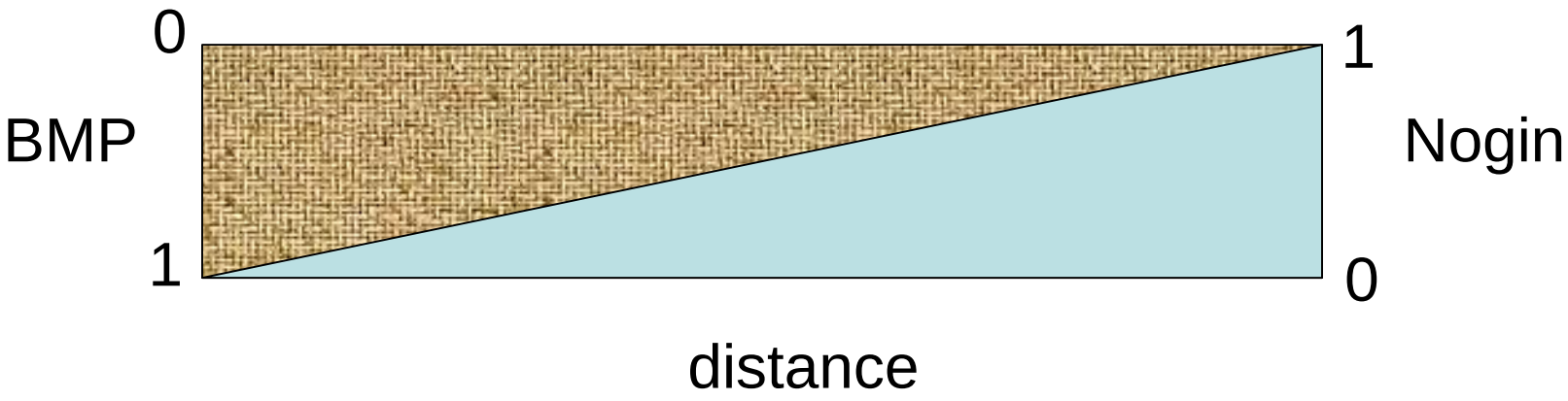
- homo- and hetero-dimers,

- precursors cleaved after dimerization and before secretion, but do not associate with LTBP into an inactive latent complex

- extracellular BMPs are sequestered by **follistatin**, **chordin** and **nogin** (secreted accessory proteins),

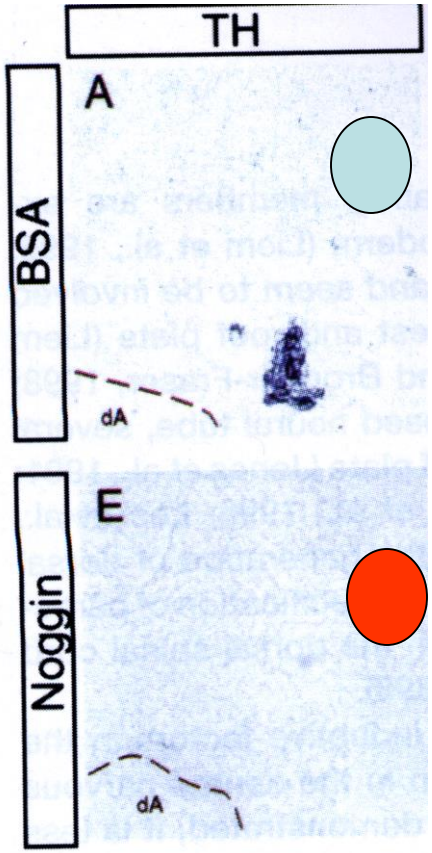
-DPP – is sequestered by SOG (Short Gastrulation)

Sequestration of BMP



development of Neural Crest and autonomic NS
[Schneider et al. Neuron 24, 861 (1999)]

Fig. 1: Transplantation of Nogin-loaded beads at E2 (adjacent to Notochord and Neural Tube). Analysis at E4-5 (when sympathetic ganglia should be formed).



BSA

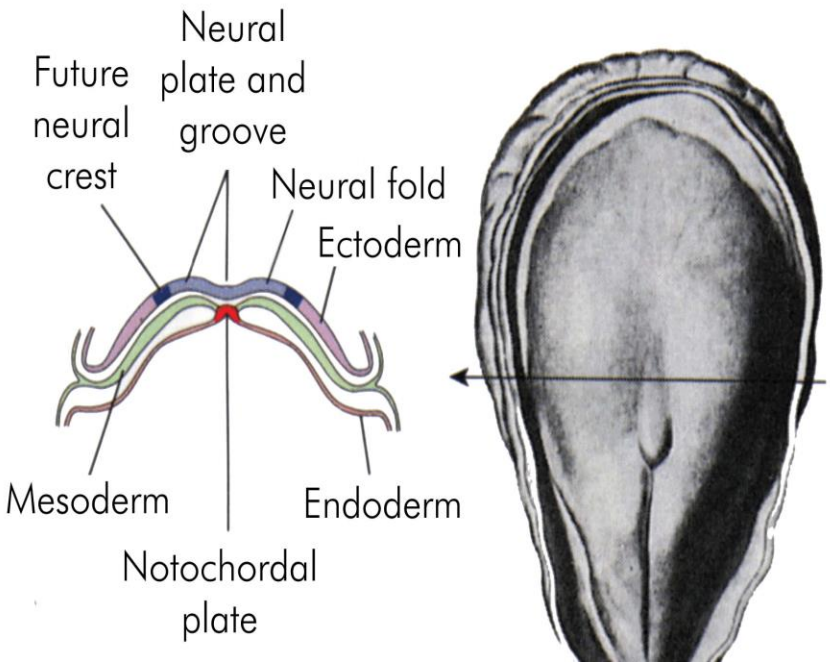
Nogin

Fig. 3 Nogin has no effect on cell migration

Fig. 4 Nogin has no effect on initial ganglion formation (Sox-10) but it prevents Cash-1/Mash-1 essential for axonal growth)

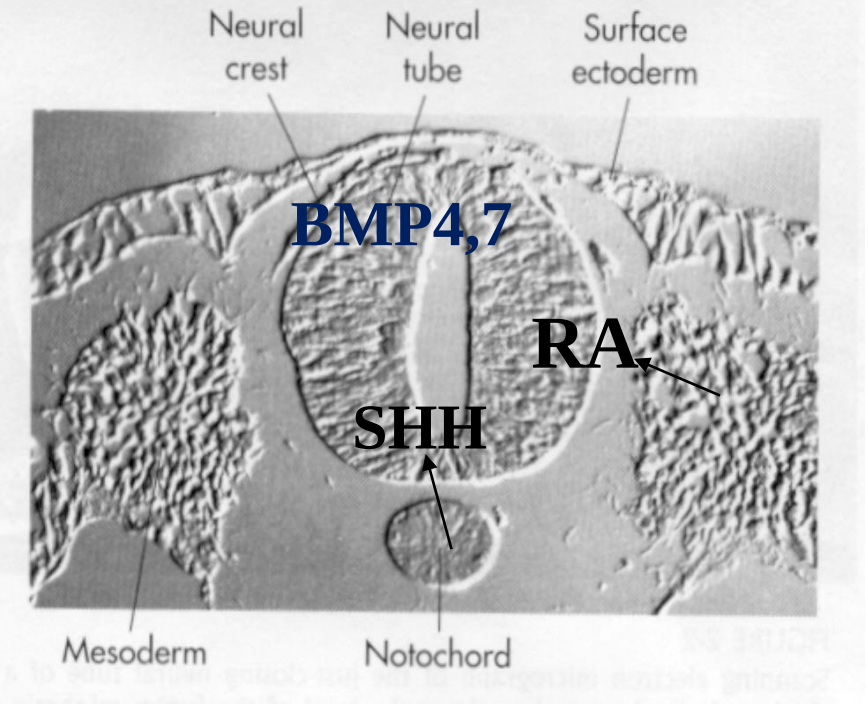
And induces apoptosis

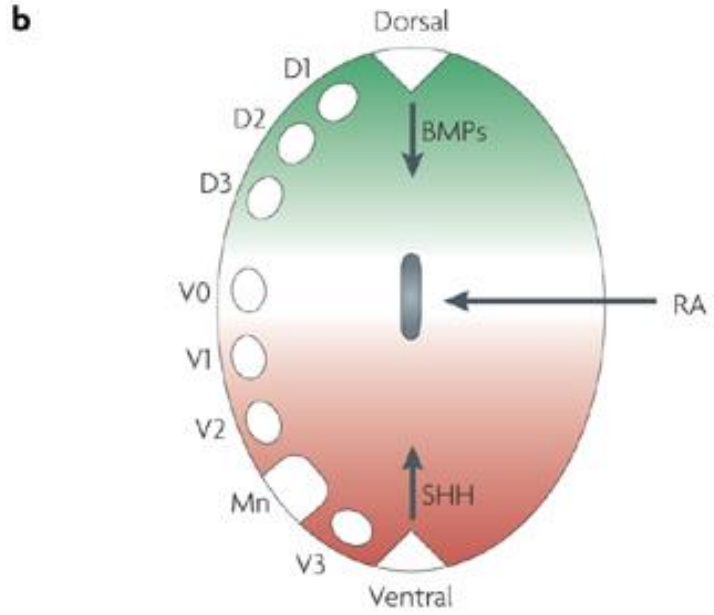
Induction of the Neural Plate requires neutralization of the BMP:



Neural Patterning:

a. Neural induction: Epidermalizing signal (BMP4)
– neuralizing signal (Nogin, Chordin – produced by Notochord)





Dorsal-Ventral patterning of Mouse spinal cord by morphogenes:

Bone morphogenetic proteins (BMPs), which are released from the dorsal region and neutralized by ventral Nogin.

Sonic hedgehog (SHH), which is released from the ventral region, have a role in patterning the dorsoventral specification of neural cell types (D1, D2, D3, V0, V1, V2, Mn, V3) in the spinal cord.

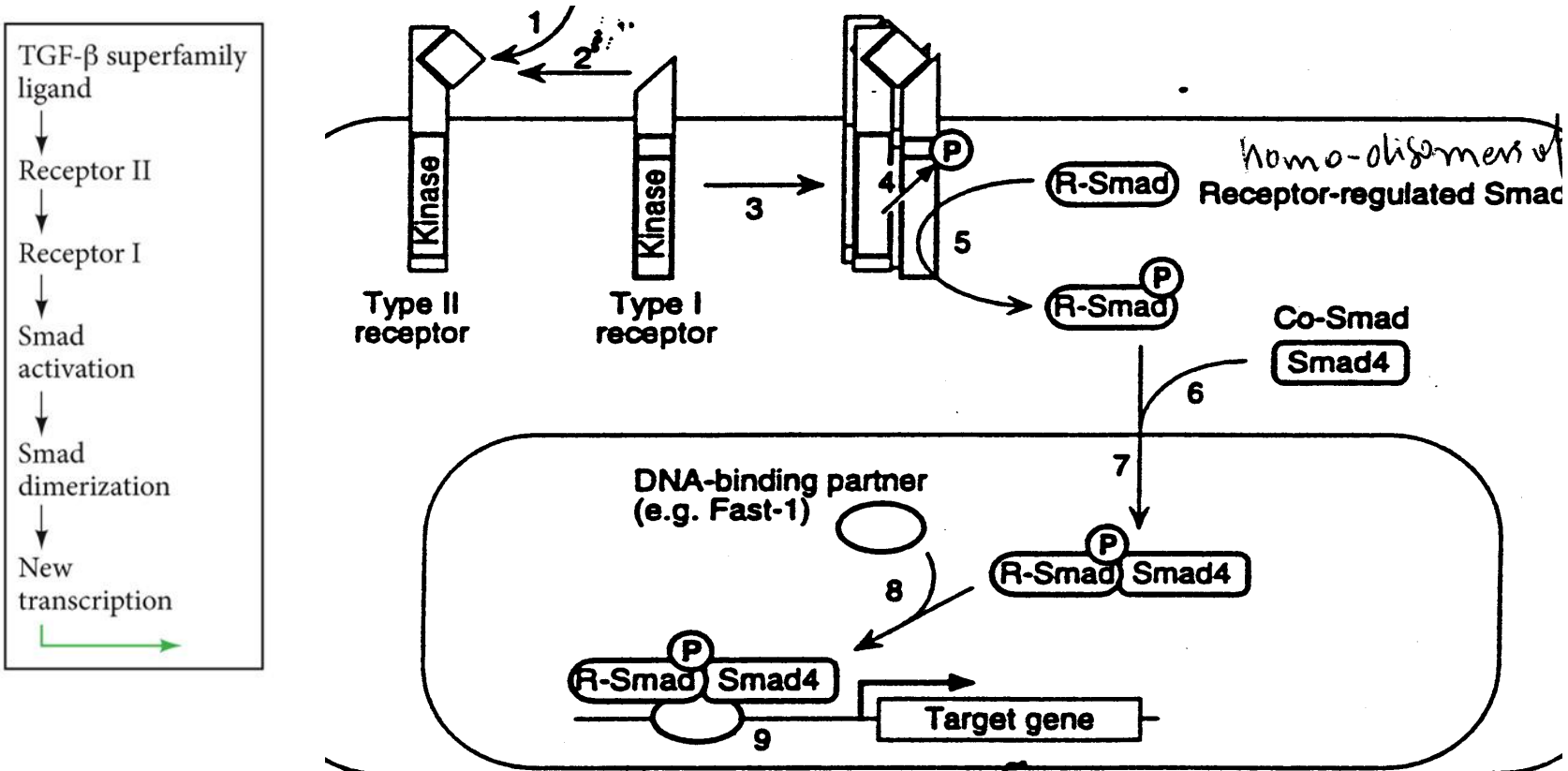
Retinoic Acid (RA), which is released from the adjacent somites (antero-posterior specification),

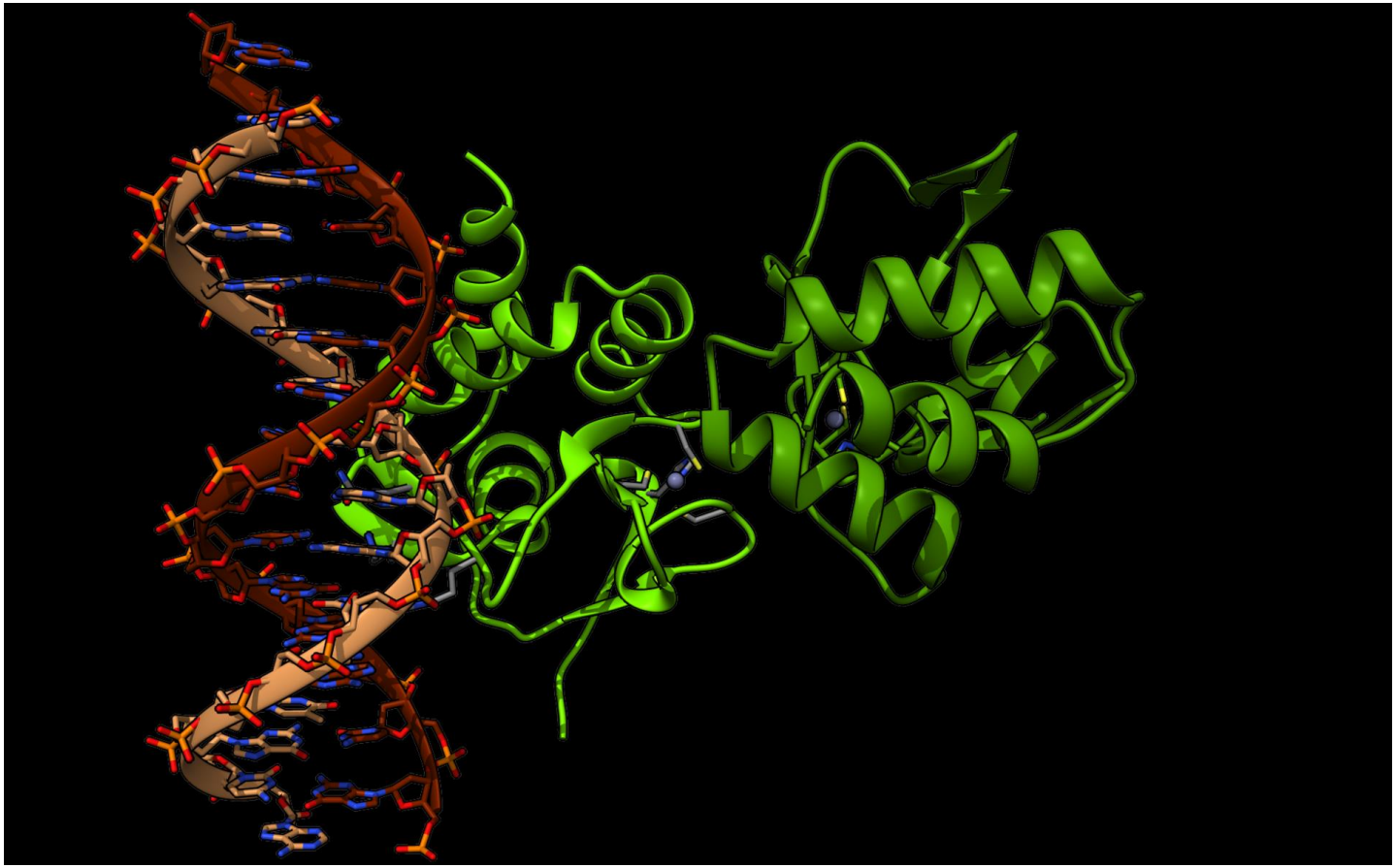
TGF-Beta1 superfamily receptors and signal transduction:

[J. Massaque, Ann. Rev. Biochem. 67, 753-791 (1998)

Fig. 1 (general Scheme)

High affinity serine-threonine kinase receptors (except GDNF – Re tyrosine kinase), cysteine rich glycosylated 1-transmembrane proteins with the signal peptide. Type I generally less specific than type II (no species specificity).

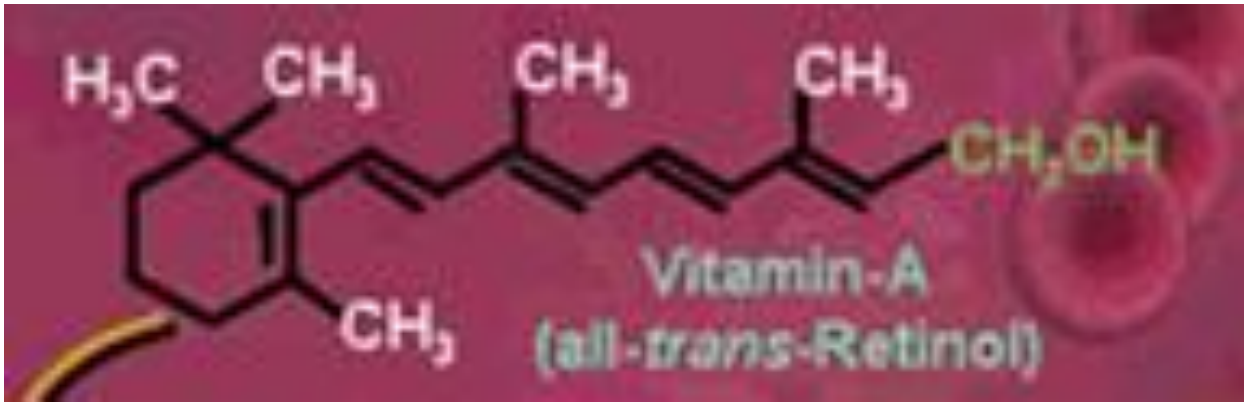




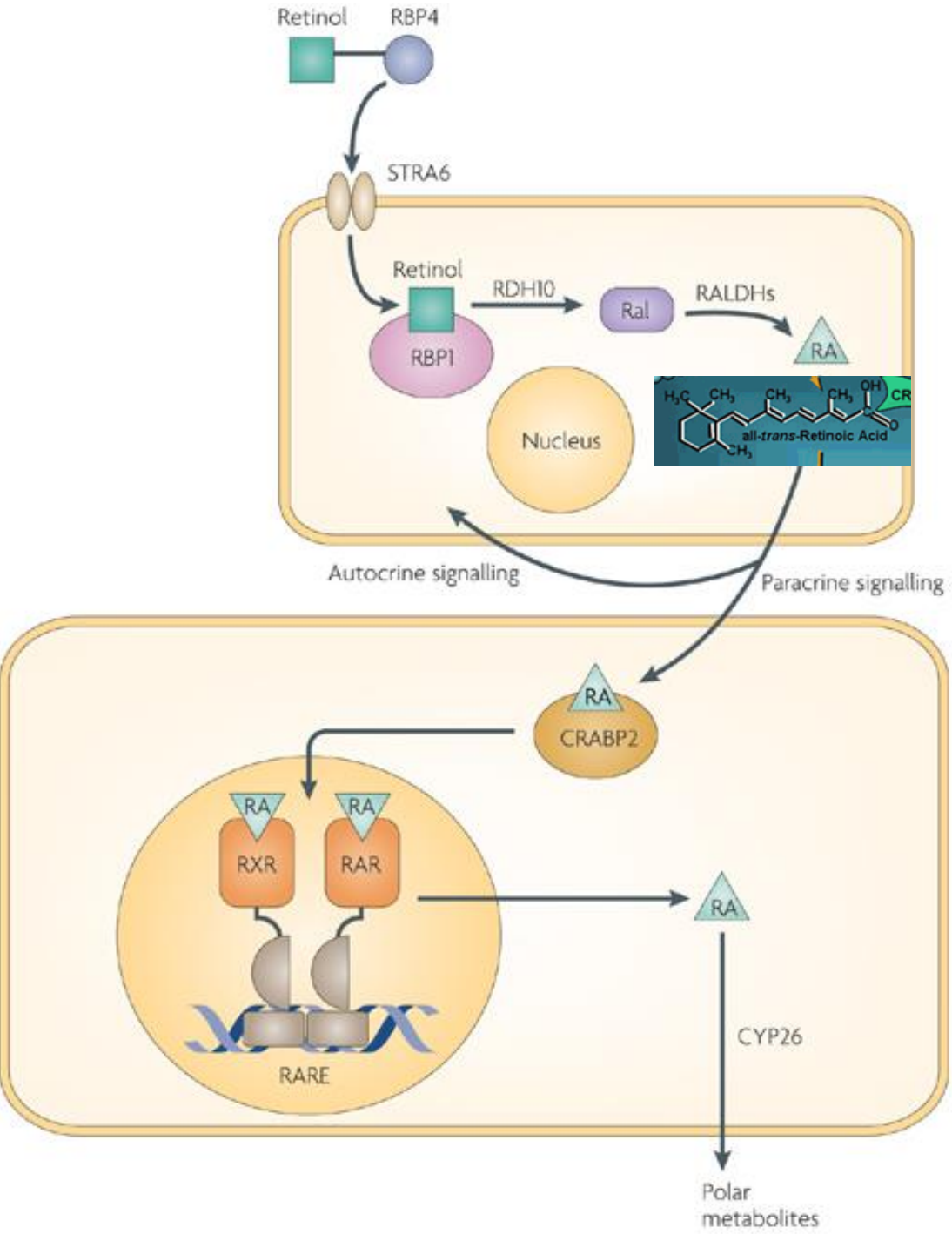
Myhre syndrome

Mutations in *SMAD4*

- Retinoic Acid (RA) as a morphogen



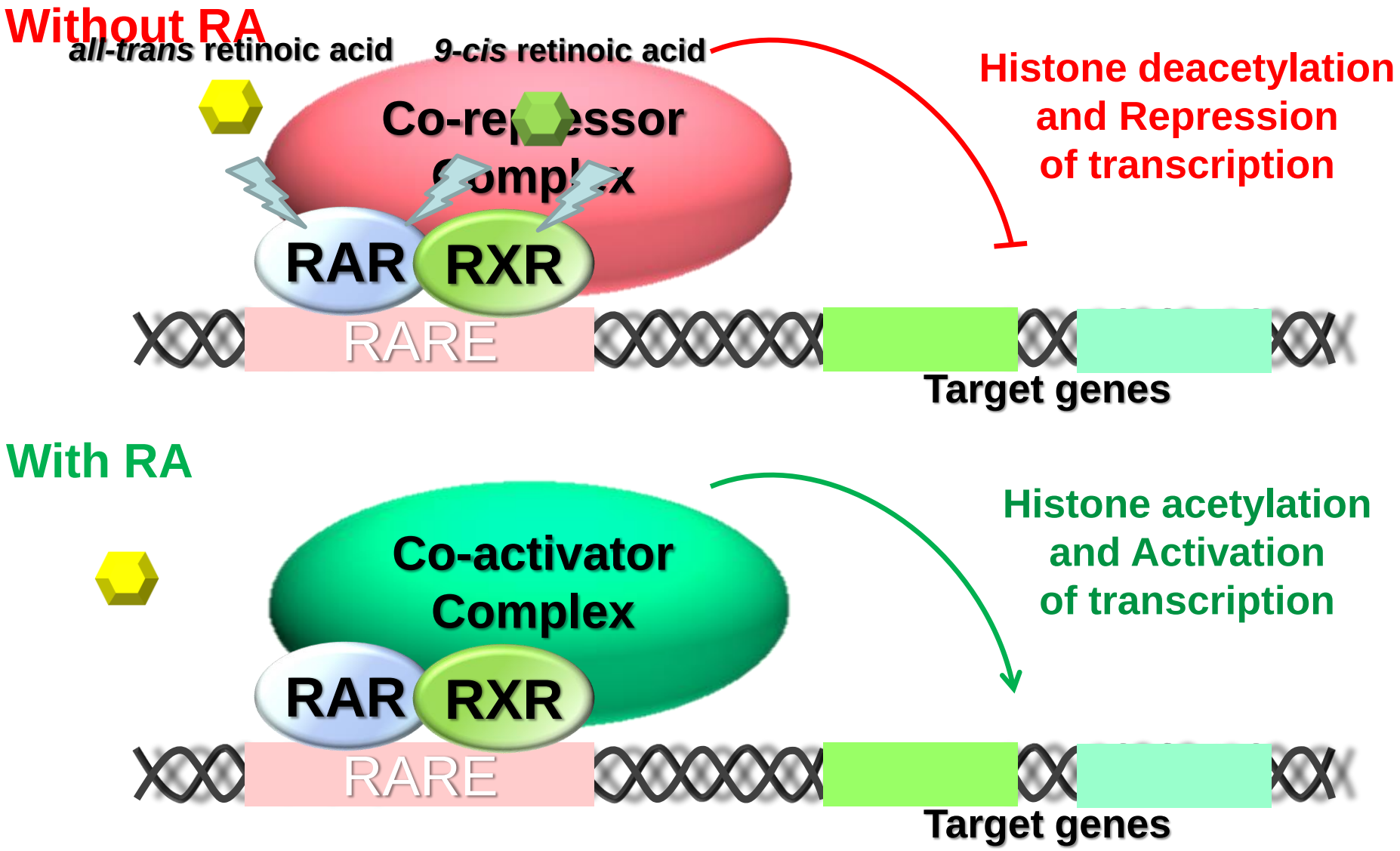
Biosynthesis of RA:



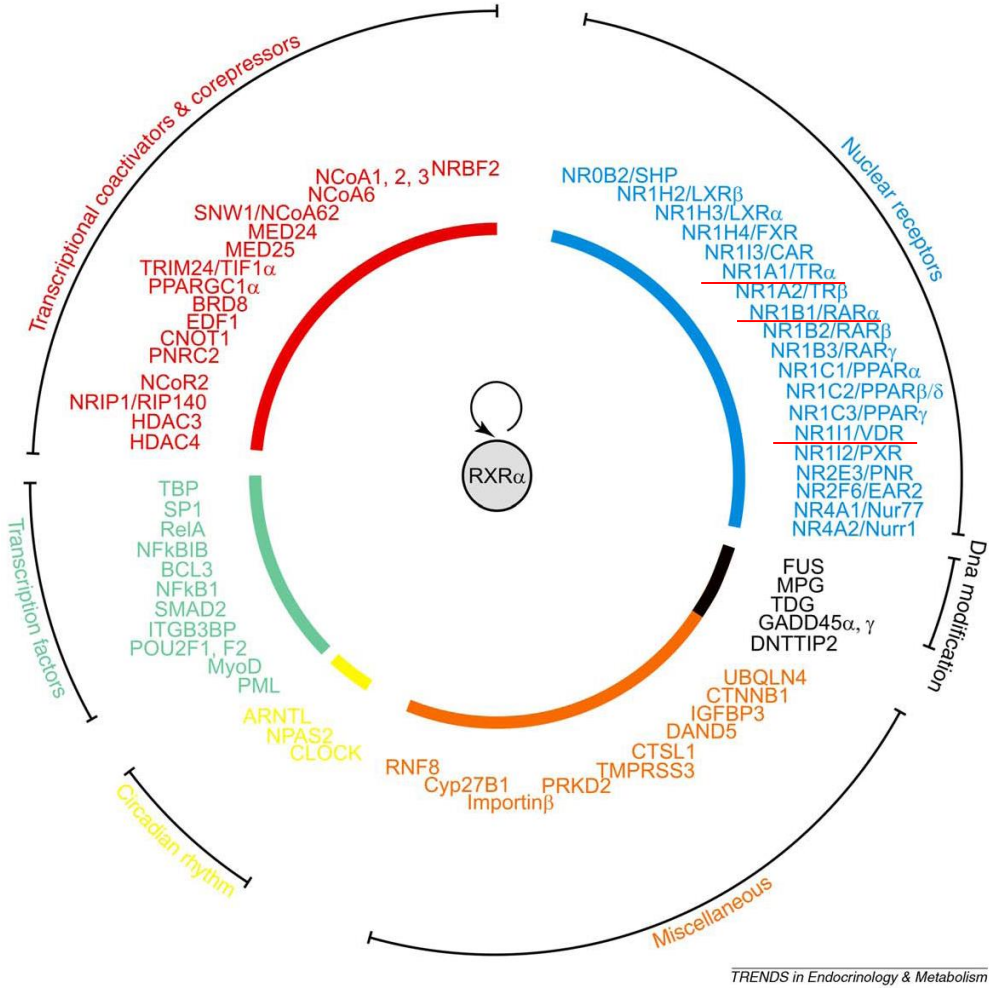
Transport and signaling:.

RAR and RXR function as retinoic acid receptors and transcriptional factors that promote cell differentiation

Förthmann B, Aletta JM, Lee YW, Terranova C, Birkaya B, Stachowiak M.K., Claus, P. Coalition of Nuclear Receptors in the Nervous System. Journal of cellular physiology. 2015; 230(12):2875-80.

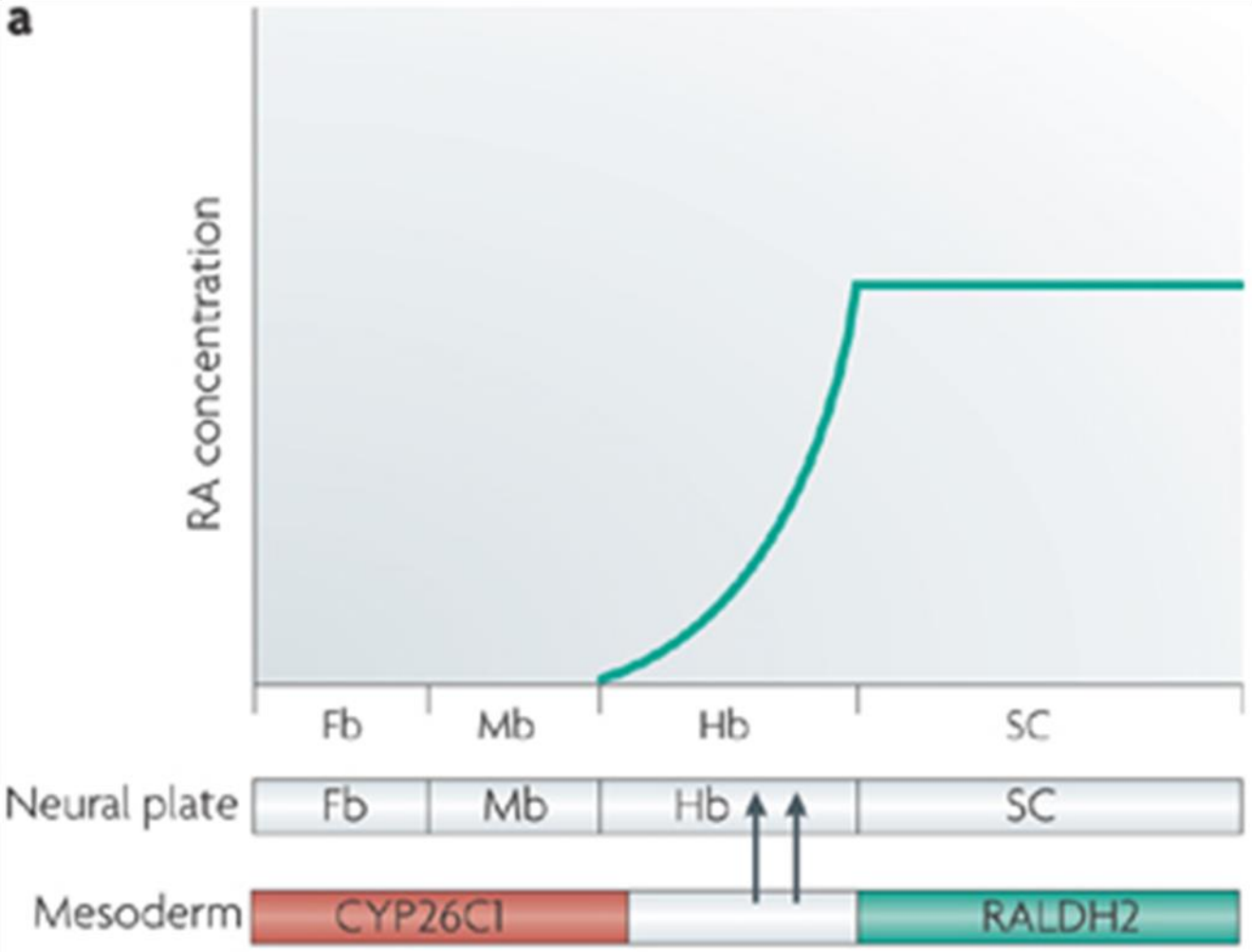


RXR dimerizes with diverse nuclear receptors



Different binding partner of RXR enables to activate multiple gene subsets in both ligand dependent and independent manners.

Anterior-Posterior of the neural plate and tube patterning by RA:
Experiments suggest that a gradient of RA in the mesoderm that is generated by retinaldehyde dehydrogenase 2 (RALDH2) (which is expressed posteriorly) and an RA-catabolizing enzyme CYP26C1 (that is expressed anteriorly) patterns the amniote hindbrain (Hb) and anterior spinal Cord (SC). Other brain areas: Fb, forebrain; Mb, midbrain



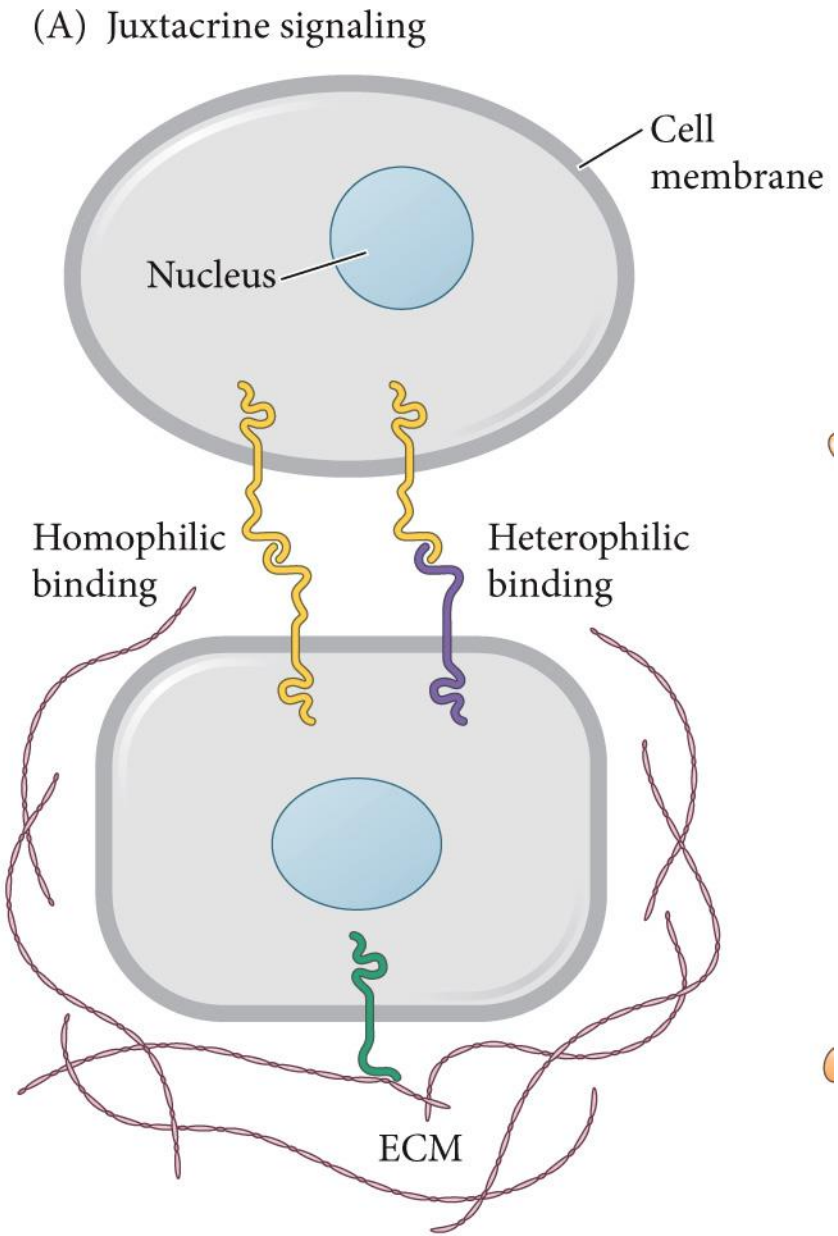
Part III

Juxtacrine Signaling for Cell Identity

Notch,

Eph ligands & Ephrin receptors

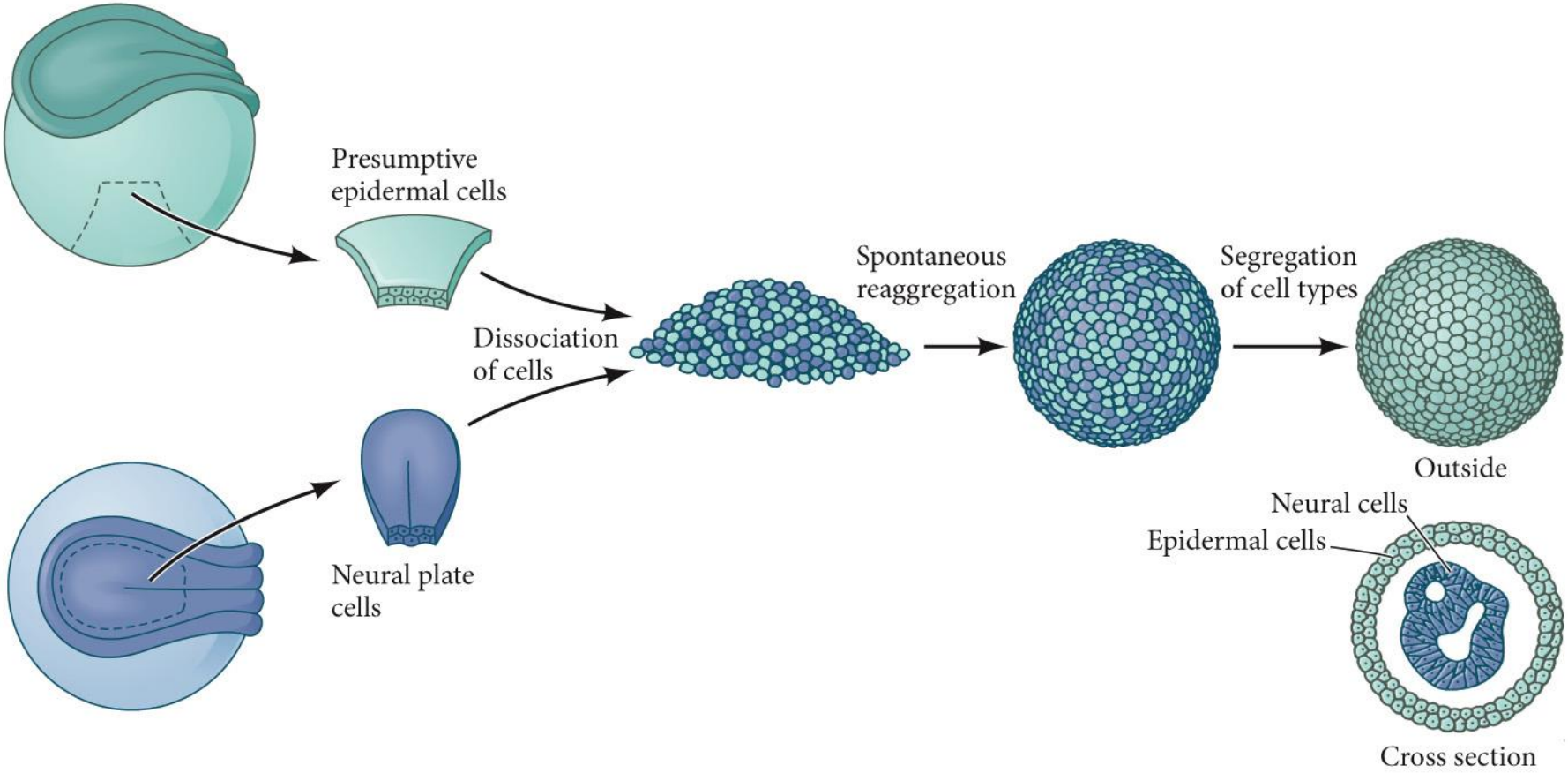
Figure 4.1 Local and direct signaling



DEVELOPMENTAL BIOLOGY 11e, Figure 4.1

Figure 4.3 Reaggregation of cells from amphibian neurulae – Illustrates differential cell affinity

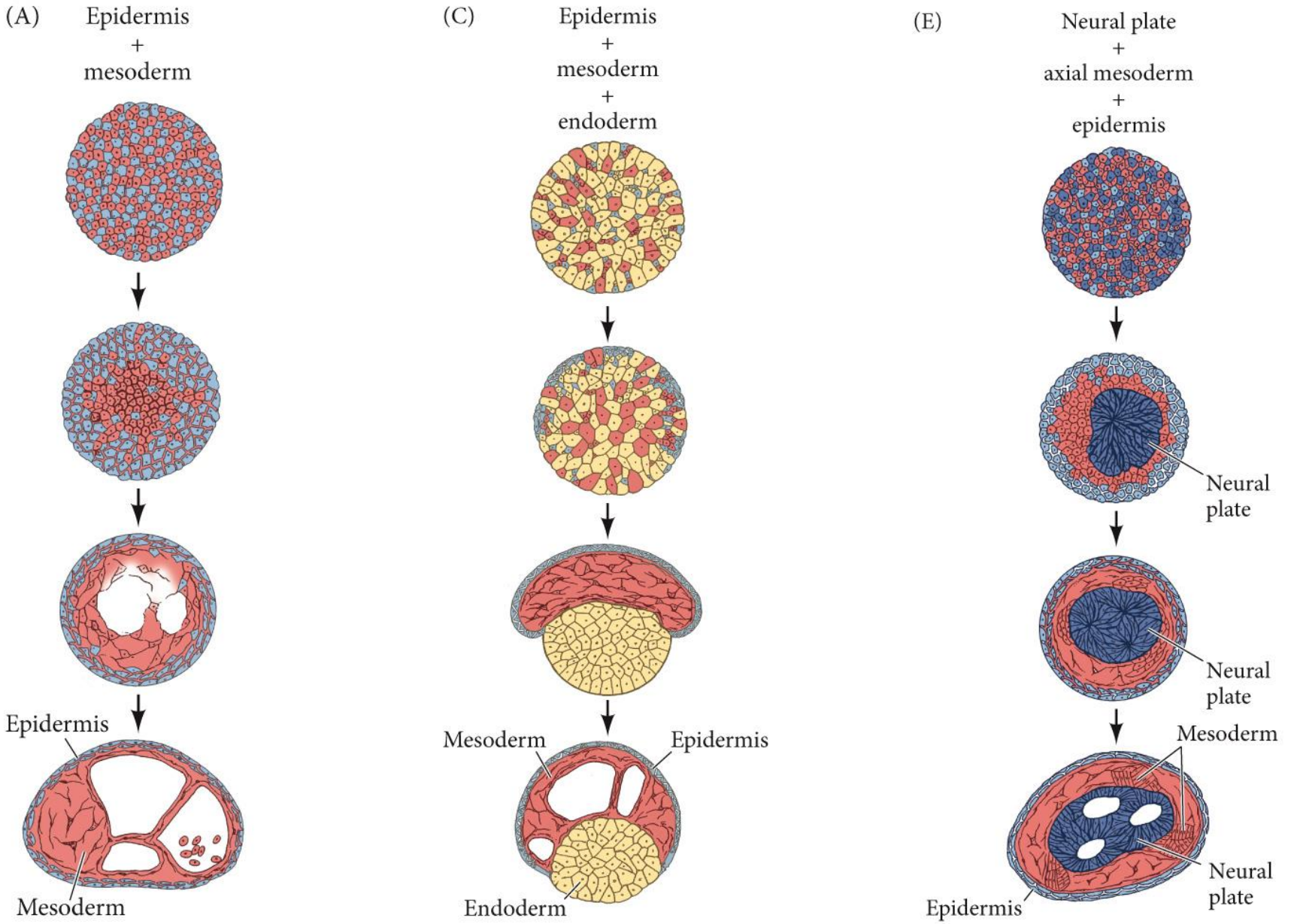
Differential cell affinity



DEVELOPMENTAL BIOLOGY 11e, Figure 4.3

Townes and Holtfreter 1955

Figure 4.4 Sorting out and reconstruction of spatial relationships in aggregates of embryonic amphibian cells (Part 3)

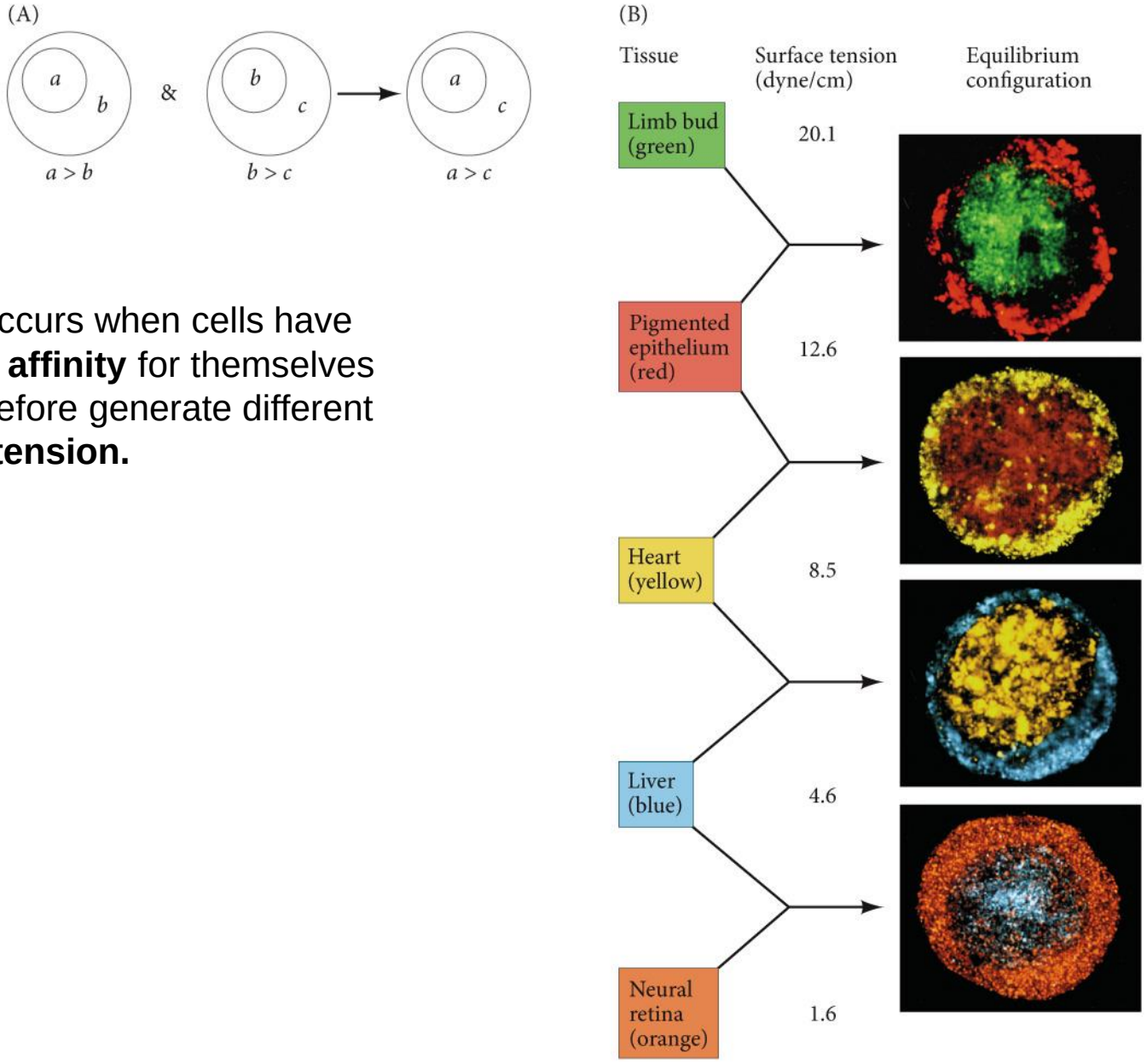


DEVELOPMENTAL BIOLOGY 11e, Figure 4.4 (Part 1)

DEVELOPMENTAL BIOLOGY 11e, Figure 4.4 (Part 3)

DEVELOPMENTAL BIOLOGY 11e, Figure 4.4 (Part 5)

Figure 4.5 Hierarchy of cell sorting of decreasing surface tensions



Sorting occurs when cells have **different affinity** for themselves and therefore generate different **surface tension.**

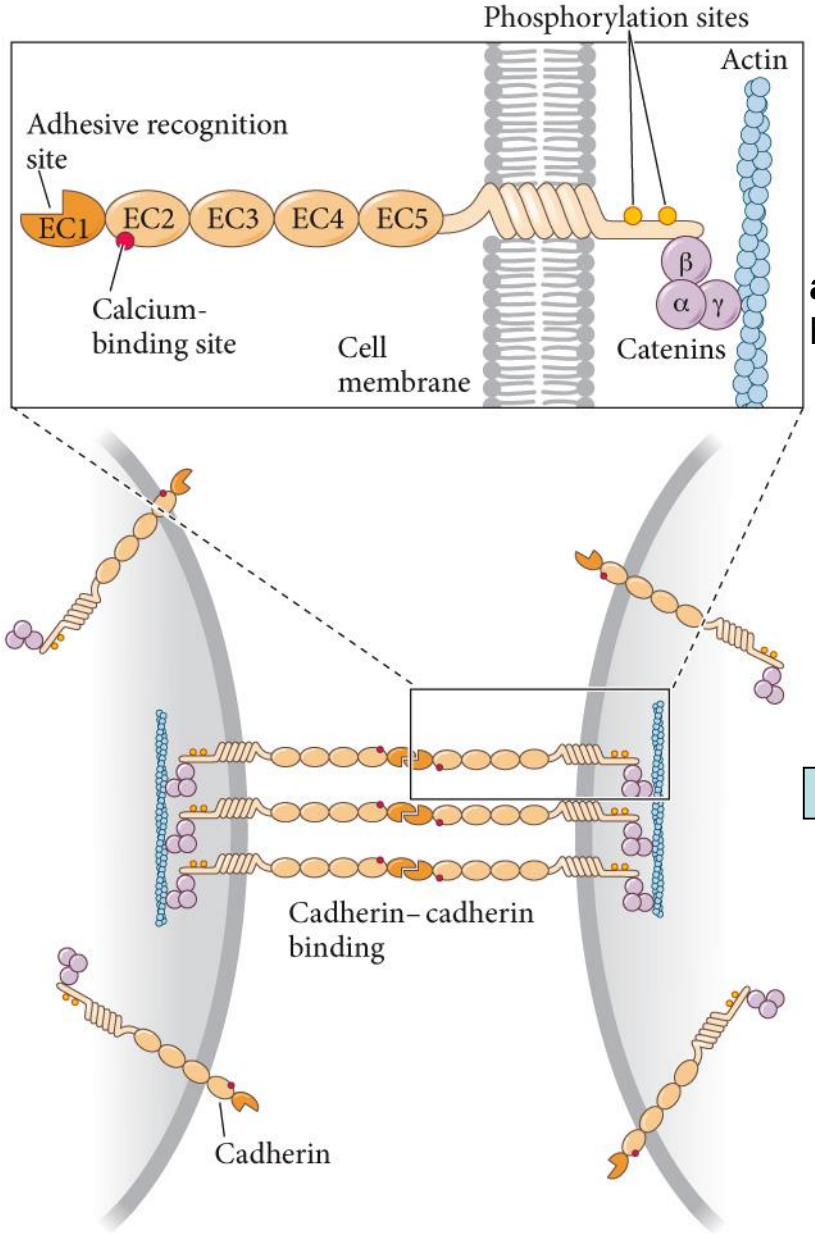
DEVELOPMENTAL BIOLOGY 11e, Figure 4.5

CADHERINS -
CALCIUM-DEPENDENT ADHESION MOLECULES major class of proteins that mediate cell-cell adhesion and sorting.
Cadherins are:

- transmembrane proteins that interact with other cadherins on adjacent cells
- The cadherins are anchored inside the cell by a complex of proteins called **catenins** (Wnt sig.)
- cadherin- catenin complex forms **adherens** junctions that hold **actin** (microfilament**) CELL TO CELL TO CYTOSKELETON A MECHANICAL UNIT.**
- Blocking cadherin prevents the formation of epithelial tissues and cause the cells to disaggregate
- **STRENGTH OF BINDING AND CELL SURFACE TENSION** depend on quantitatively on number of cadherin molecules (the more cadherins on the apposing cell surfaces, the tighter the adhesion) or qualitatively type of cadherins – some bind some don't

- **GENE REGULATION:** Cadherins initiate and transduce signals that can lead to changes in a cell's **GENE EXPRESSION.**

Figure 4.6 CADHERINS - CALCIUM-DEPENDENT ADHESION MOLECULES



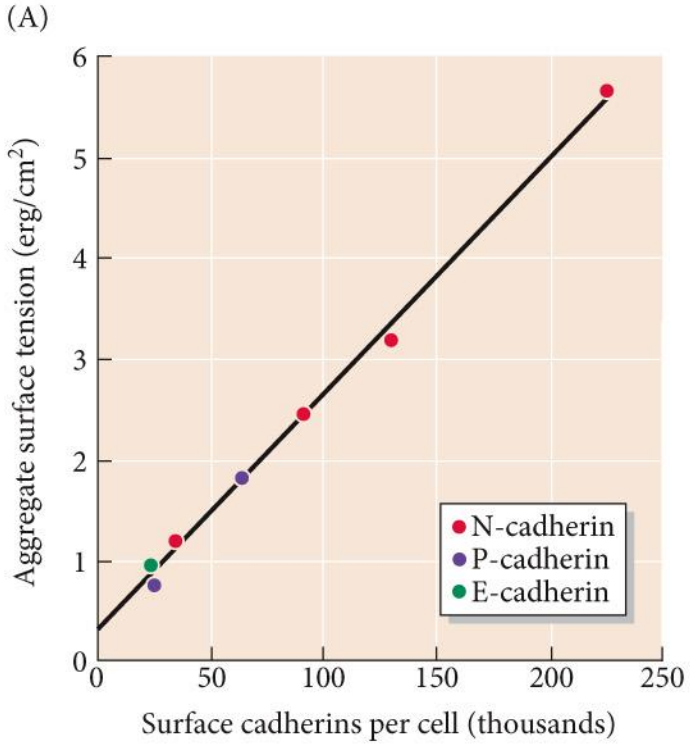
adherens junctions hold **actin**

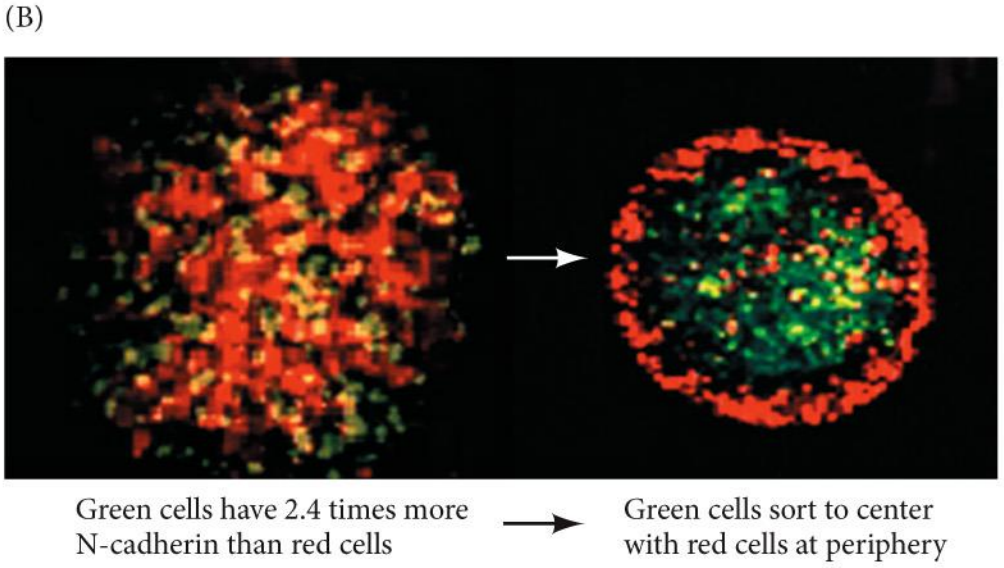
Gene regulation

DEVELOPMENTAL BIOLOGY 11e, Figure 4.6

Figure 4.8 Importance of the amount of cadherin for correct morphogenesis

Quantitative principle for cell sorting





Quantitative principle of cells interactions - Classes of cadherins - *notes*

- **E-cadherin** is expressed on all early mammalian embryonic cells, even at the zygote stage; needed for the formation and migration of the epiblast as a sheet of cells during gastrulation. Later in development, this E-cadherin is restricted to epithelial tissues of embryos and adults.

- **P-cadherin** is found predominantly on the placenta, so that sticks to the uterus.

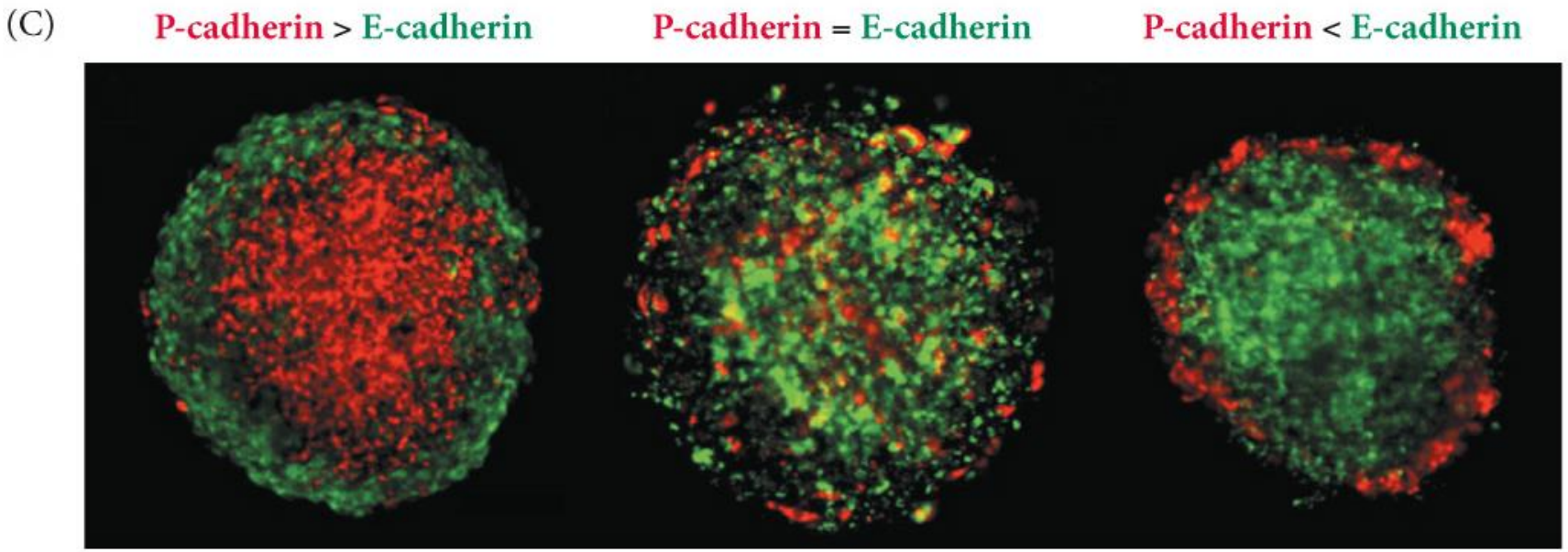
- **N-cadherin** on the cells of the developing central nervous system (CNS)

- **R-cadherin** is critical in retina formation.
-
- **Protocadherins** lack the attachment to the actin cytoskeleton through catenins. Expressing similar protocadherins keeps migrating epithelial cells together, and expressing dissimilar protocadherins is separats the mesoderm forming the notochord from the surrounding mesoderm that will form somites).

- Differences in types of cadherins expressed determine cell interactions/segregations

Figure 4.8 Importance of the amount of cadherin for correct morphogenesis

Quantitative principles of cells interactions



DEVELOPMENTAL BIOLOGY 11e, Figure 4.8

- **E-cadherin** is expressed on all early mammalian embryonic cells, even at the zygote stage; needed for t formation and migration of the epiblast as a sheet of cells during gastrulation. Later in development, this cadherin is restricted to epithelial tissues of embryos and adults.

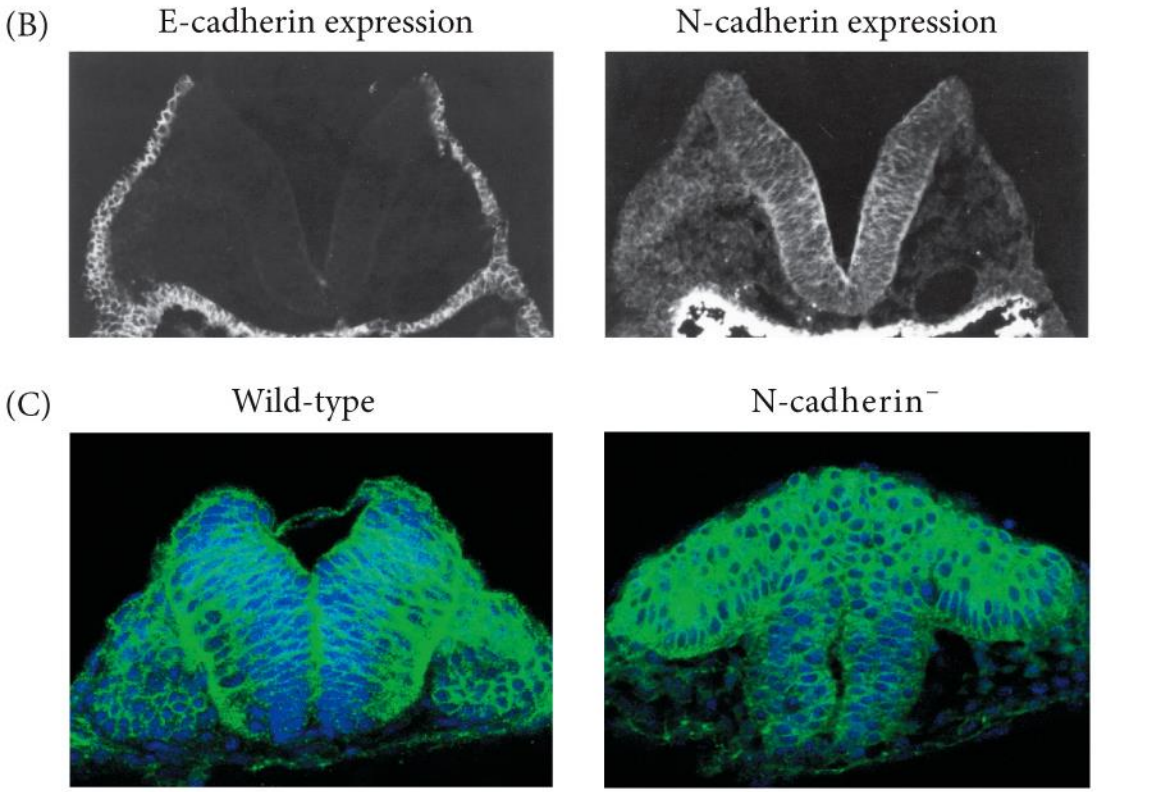
- **P-cadherin** is found predominantly on the placenta, so that sticks to the uterus.

- **N-cadherin** on the cells of the developing central nervous system (CNS)

- **R-cadherin** is critical in retina formation.

-

Qualitative principles of cells interactions



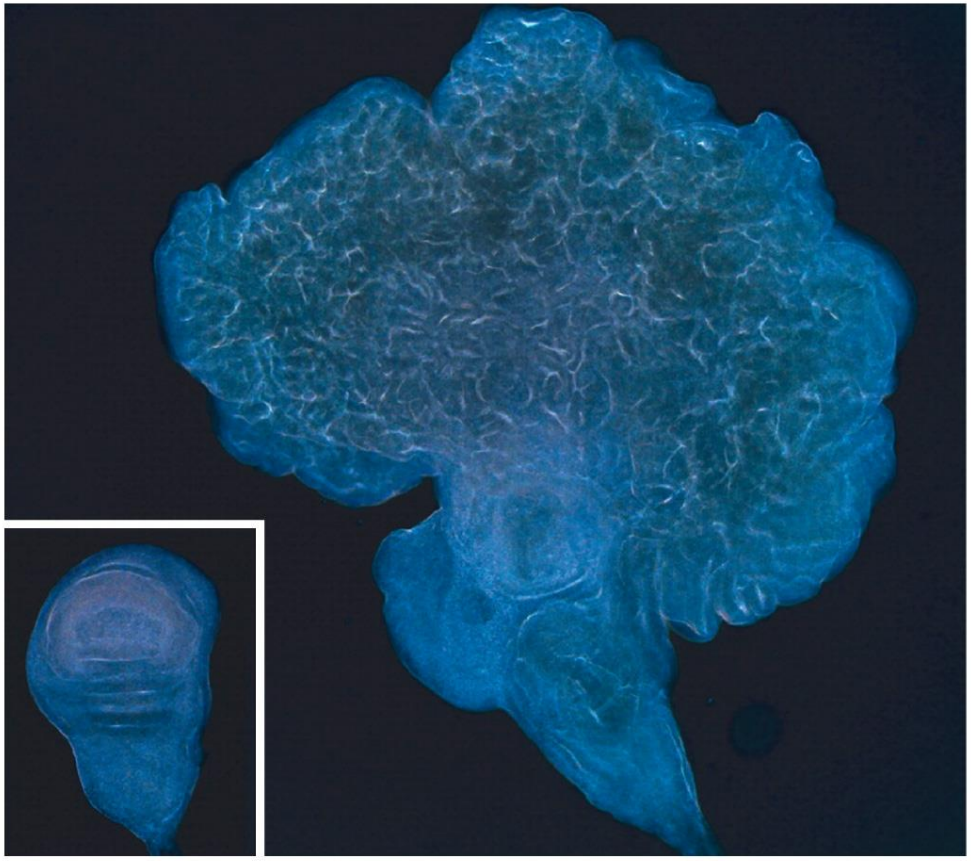
Hong and Brewster 2006

- **E-cadherin** - all early mammalian embryonic cells,
- **P-cadherin** i- placenta
- **N-cadherin** - developing central nervous system (CNS)
- **R-cadherin** - retina formation.
- **Protocadherins** lack the attachment to the actin cytoskeleton through catenins.

Figure 4.46 Hippo signaling is critical for controlling organ size

E-cadherins and Hipo integrator of pathways

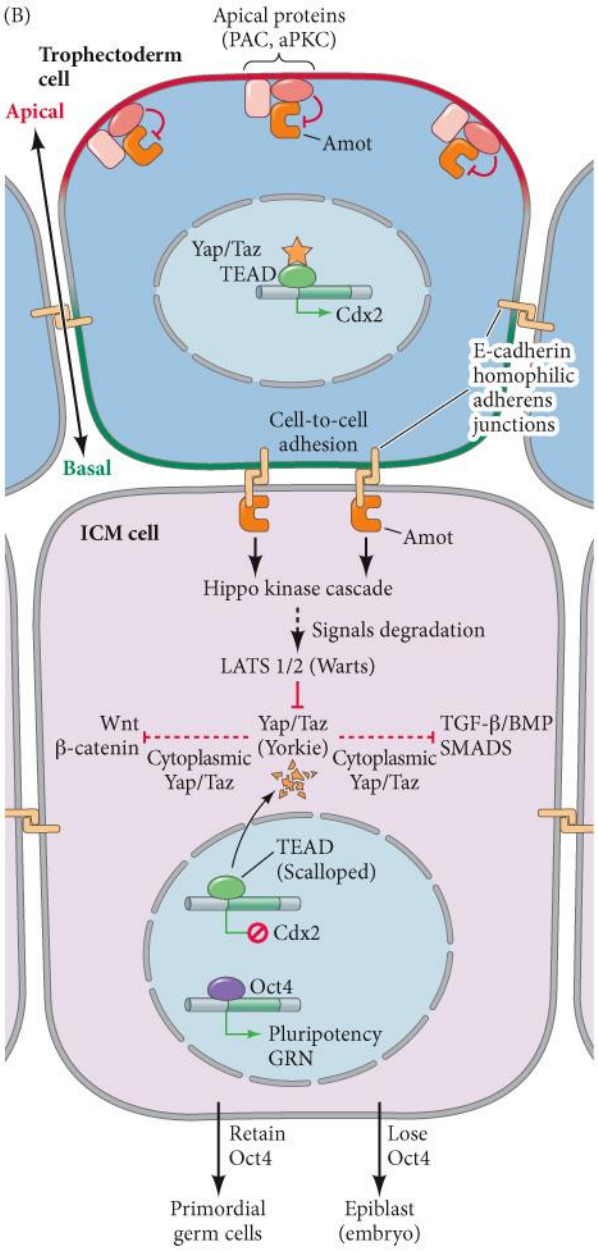
Although E-cadherins is one type of activator, the Hippo (kinase) signal transduction pathway does not have a dedicated ligand or receptor, is critical for organ size control (accelerates cell division and apoptosis)



Wild-type Overexpression of *yorkie*

DEVELOPMENTAL BIOLOGY 11e, Figure 4.46

Figure 5.7 Hippo signaling and ICM development (Part 2)



DEVELOPMENTAL BIOLOGY 11e, Figure 5.7 (Part 2)

Trophectoderm: Lack of Hippo signaling

Yap/Taz > nucleus > co-activator of Tead TF > Cdx2 trophectoderm phenotype.

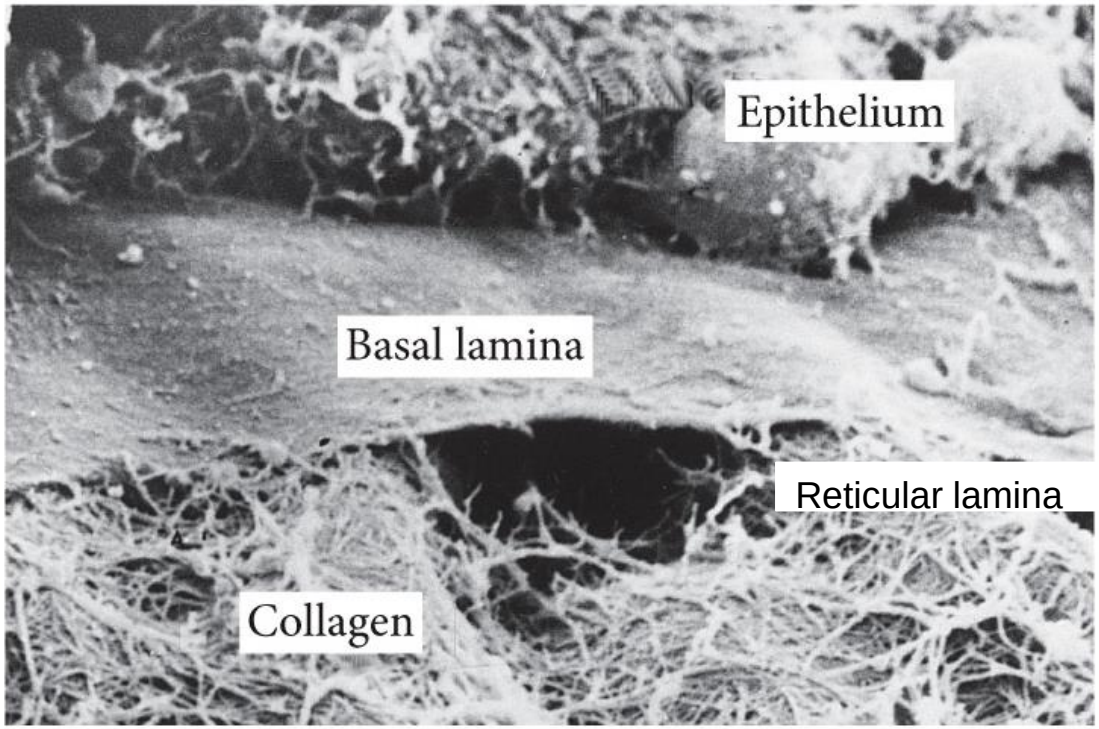
ICM: E-cadherins and Hipo pathways

Cell adhesion molecules such as E-cadherin interact with the F-actin binding **angiomotin**, > **Hippo kinase** cascade (LATS) > phosphorylates **Yap/Taz**> .degraded, Cdx2 not expressed>ICM (Oct4) pluripotent phenotype

Figure 4.11 **The extracellular matrix (EM) as a source of Developmental signals**

EM PROTEINS

- **proteoglycans** (i.e., heparan sulfate and chondroitin sulfate)
- **specialized glycoprotein (**fibronectin and laminin)
- **Basal lamina** (collagen, plus laminin)
- **Reticular lamina** (collagen)



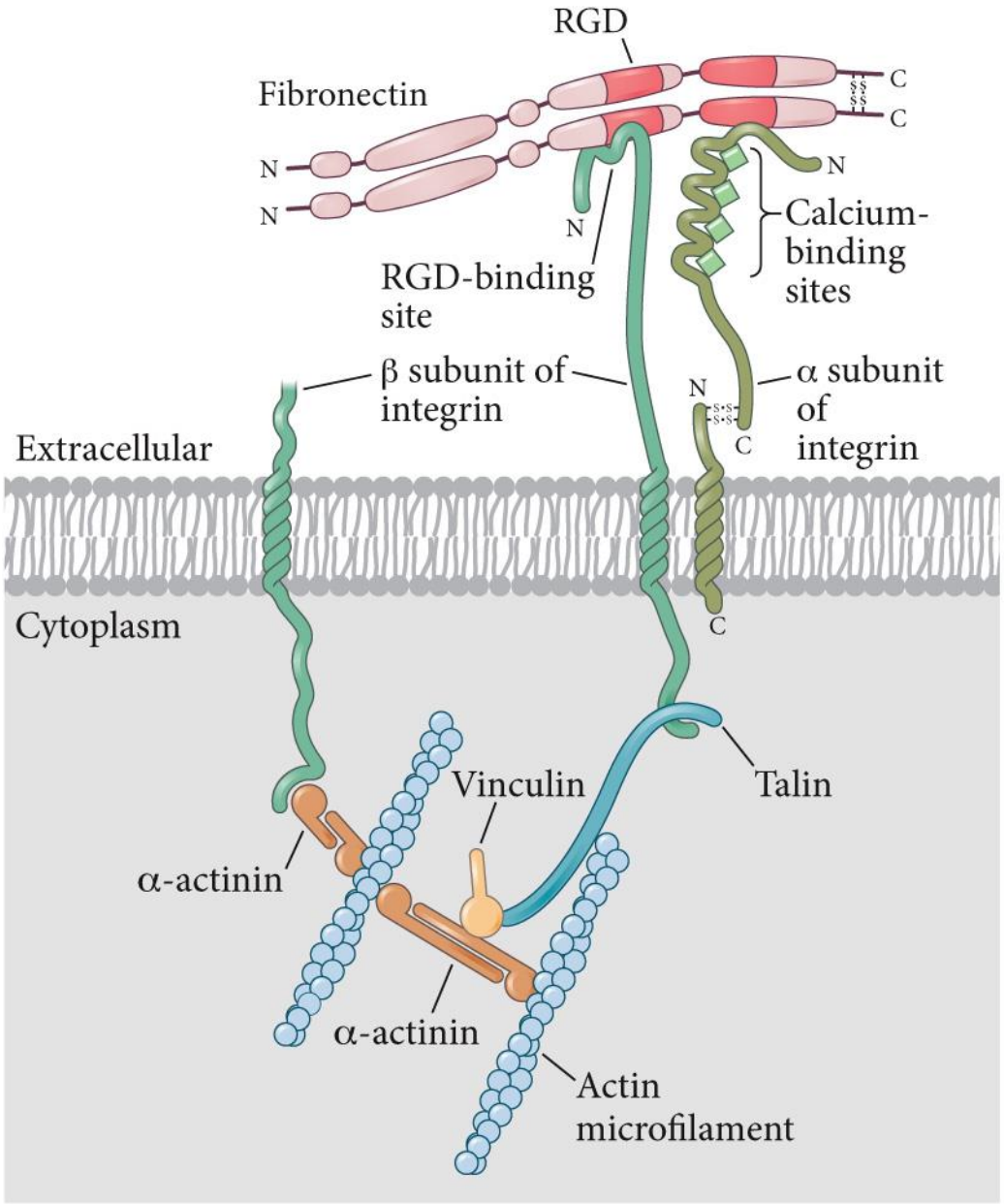
Mesenchymal cells

LAMININ OR FIBRONECTIN OR RELATED VITRONECTIN (basal lamina of the eye), have arginine-glycine-aspartate (**RGD) SITES** which bind to membrane receptors **INTEGRINS** (integrate the extra cellular and intracellular scaffolds),

Through the cytoplasmic side, integrins bind to **TALIN** and **A-ACTININ**, that connect to **ACTIN MICROFILAMENTS**. This dual binding to fixed EM and contracrting actin – cells move.

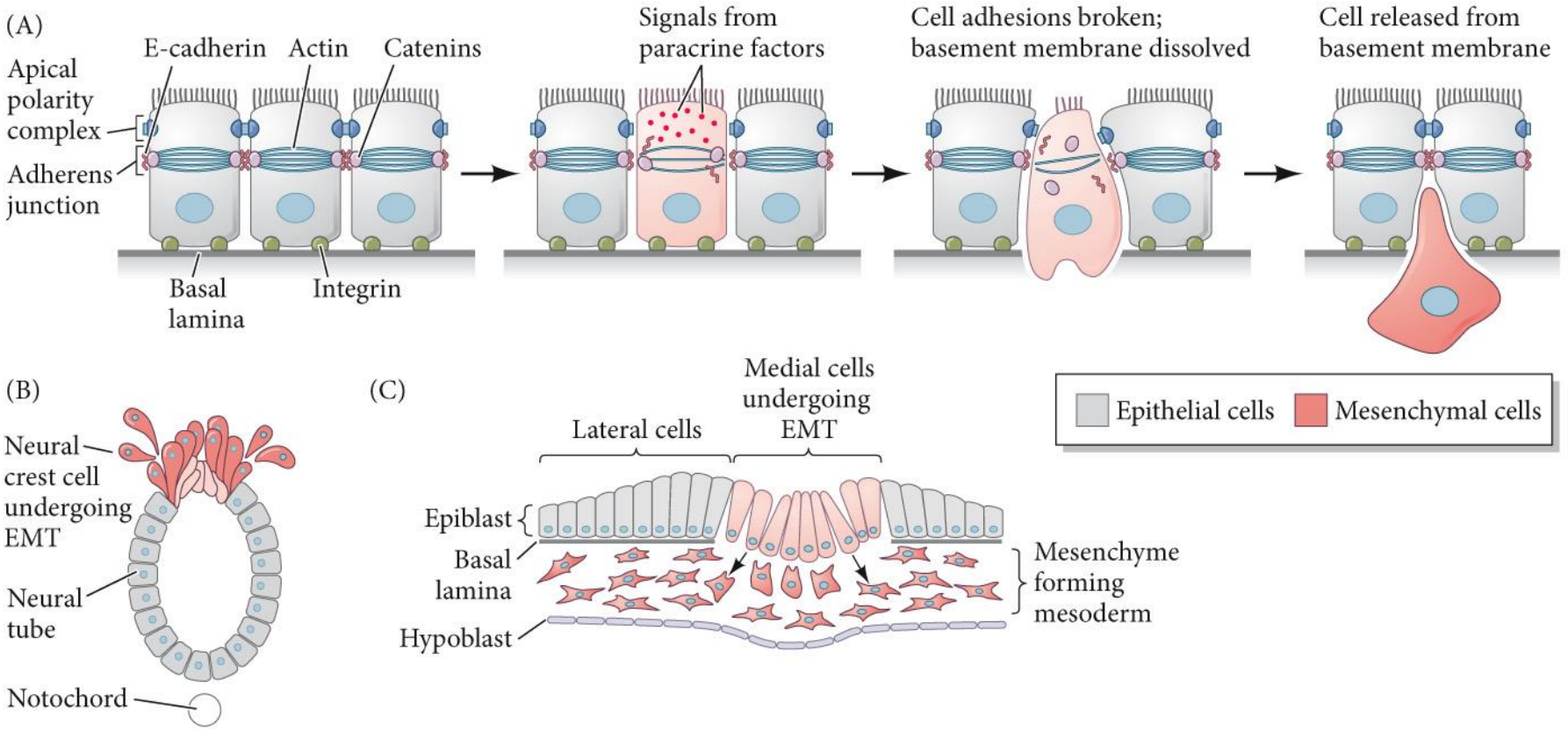
Integrins can also signal altering **GENE EXPRESSION** in developing tissues. Integrings> mammary gland integrins binding to laminin activate genes for differentiated products of the mammary gland (**CASEIN, LACTOFERRIN**,), genes for proliferation (c-myc, cyclinD1) are inhibited.

Integrins: Receptors for extracellular matrix molecules



DEVELOPMENTAL BIOLOGY 11e, Figure 4.12

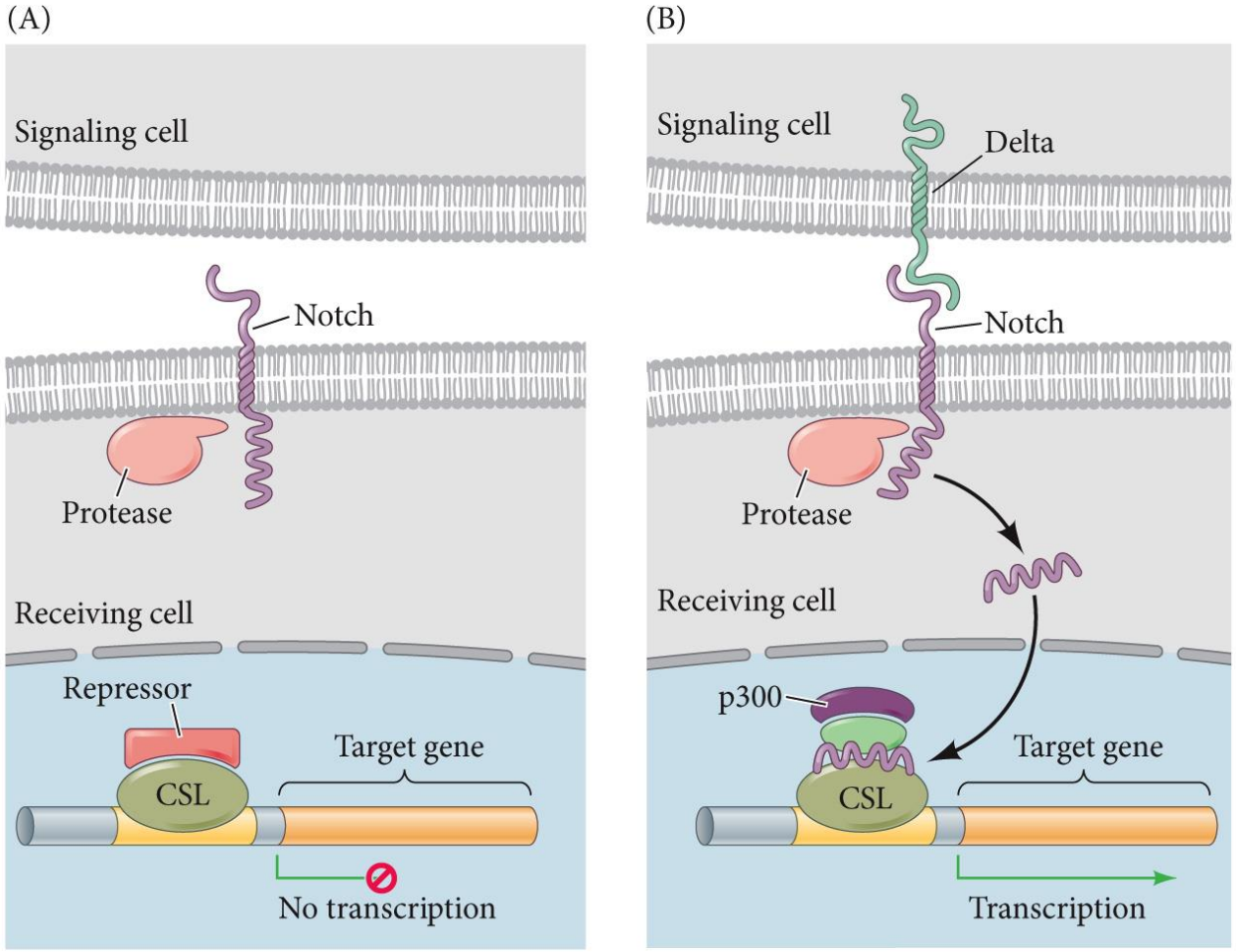
Epithelial-mesenchymal transition (EMT) - integrated regulation by cadherins and integrins.



DEVELOPMENTAL BIOLOGY 11e, Figure 4.14

Notch synthesis, signaling and function

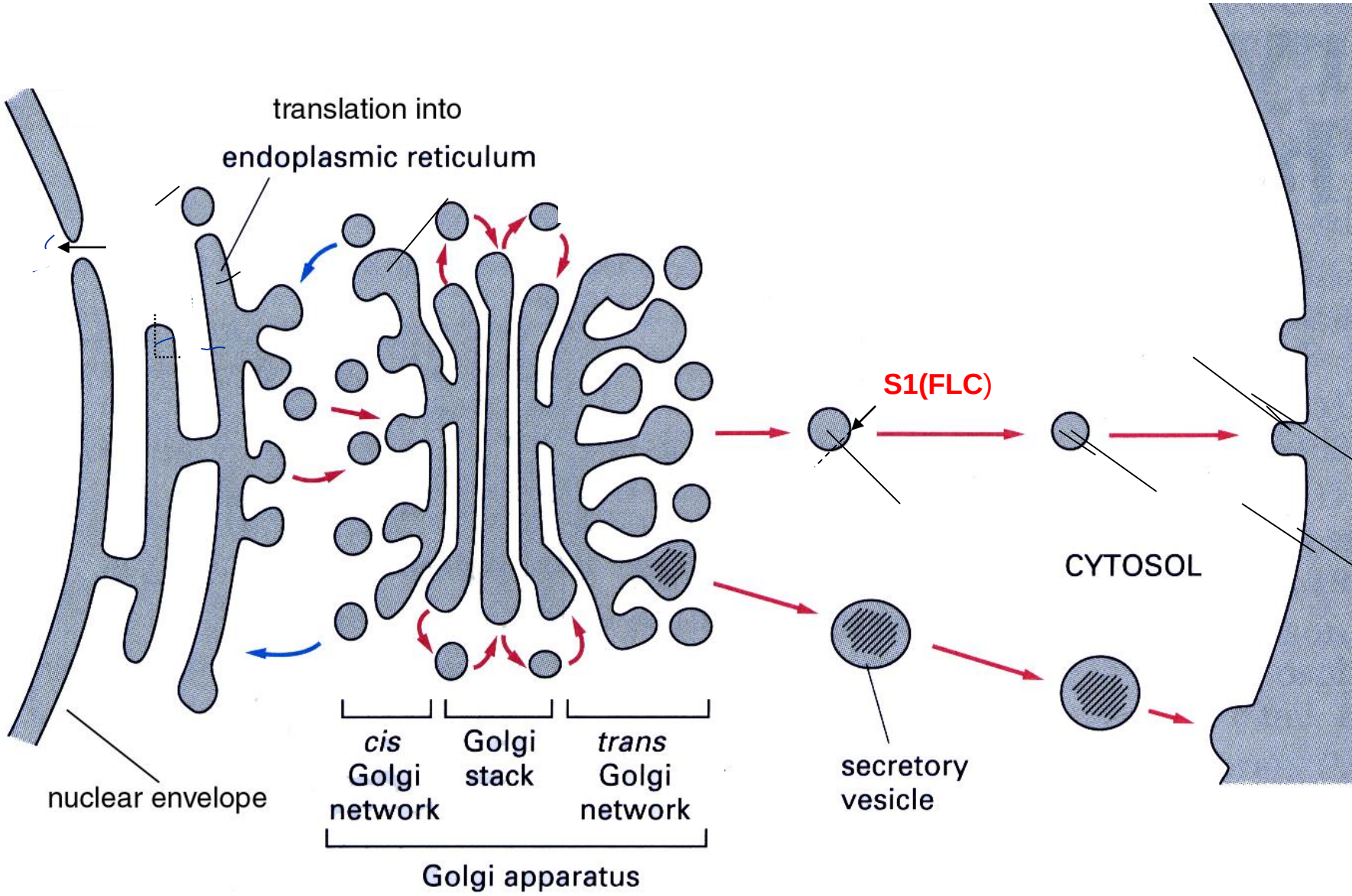
Figure 4.43 Mechanism of Notch activity



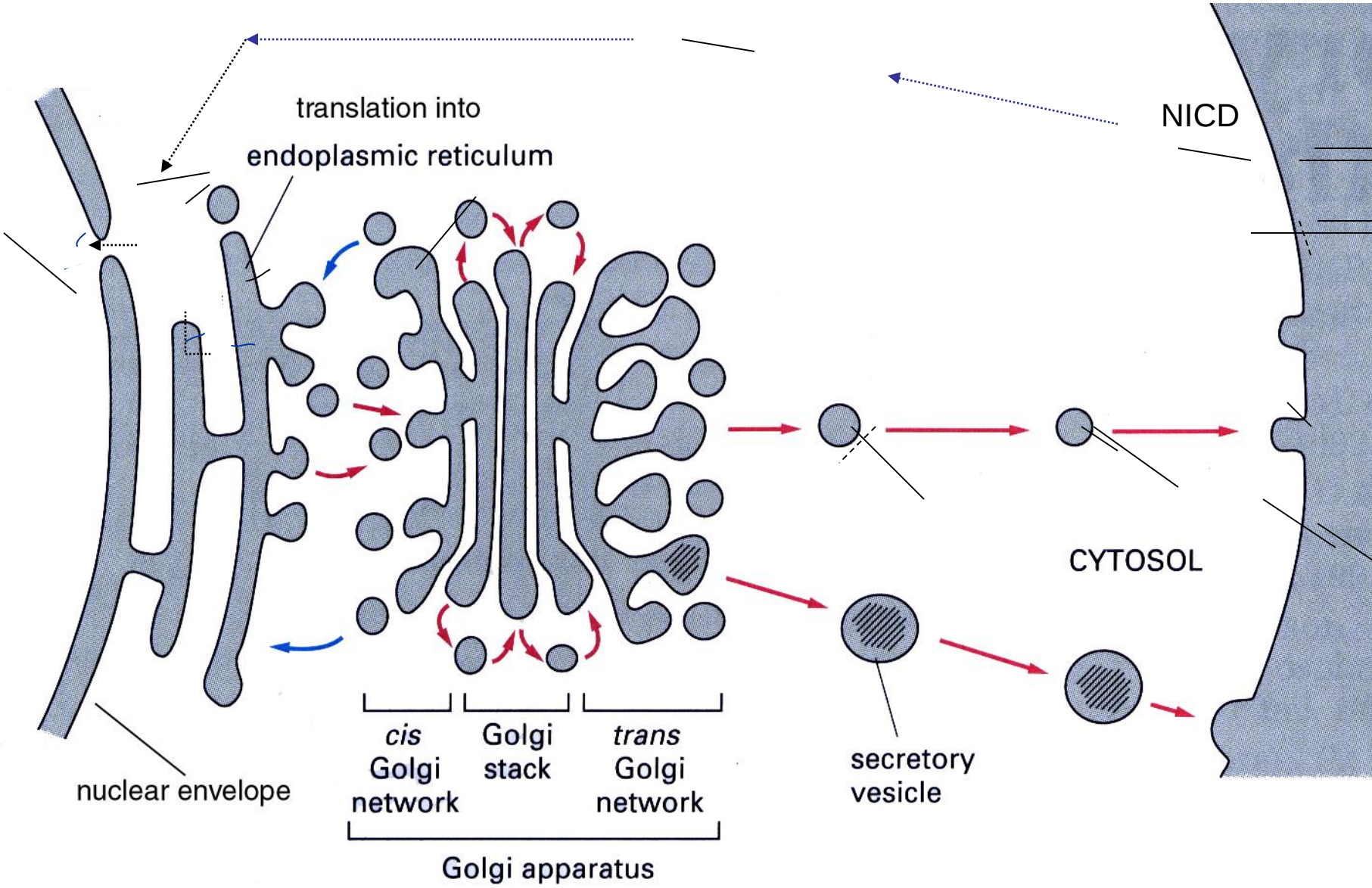
DEVELOPMENTAL BIOLOGY 11e, Figure 4.43

The Notch pathway: Juxtaposed ligands and receptors for pattern formation. remain bound to the inducing cell surface. Cells expressing ligands: Delta, Jagged, or Serrate proteins in their cell membranes activate Notch protein (receptor) on neighboring cells. Notch undergoes a conformational change, its cytoplasmic domain cut off by the presenilin-1 protease. The cleaved ICD enters the nucleus and binds to a dormant transcription factor CSL. When bound to the Notch, the CSL transcription factors activate their target genes by recruitment of histone acetyl- transferases (

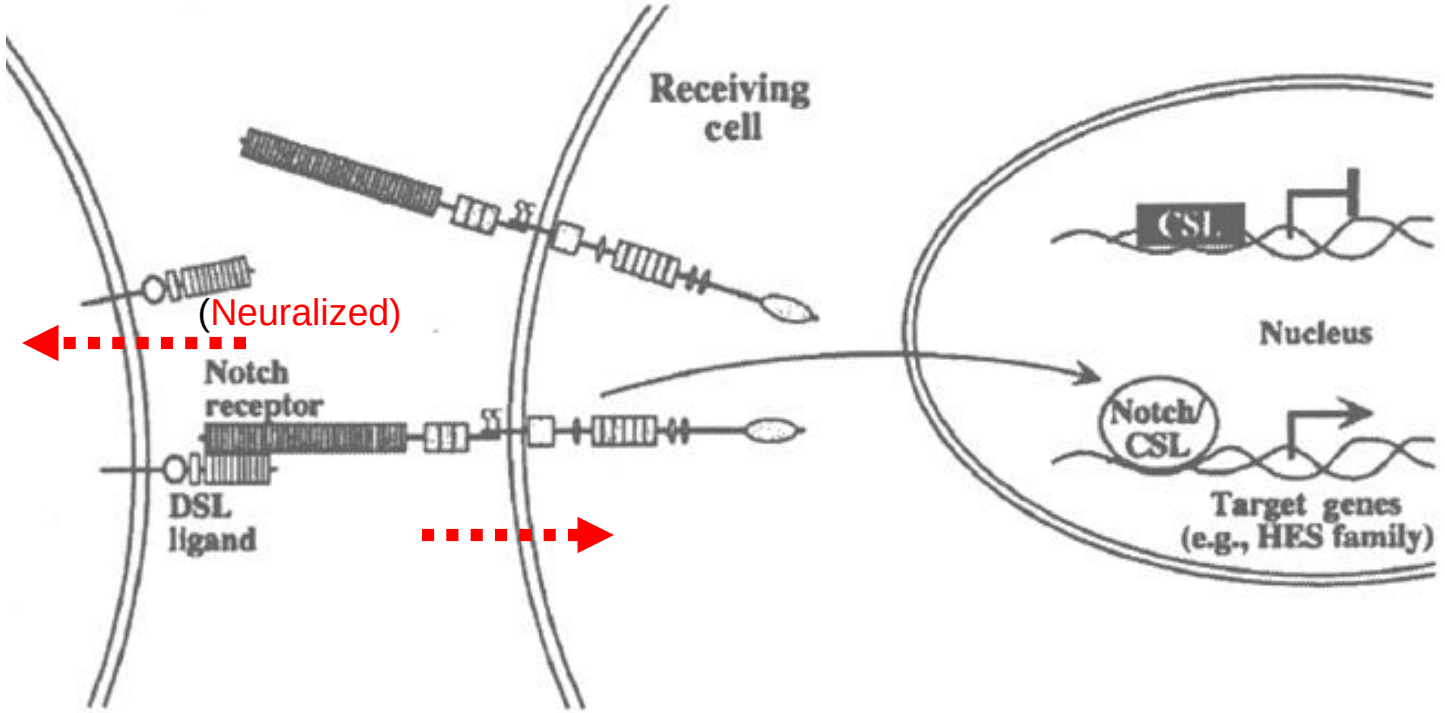
Before Notch arrives at membrane via secretory pathway it is cleaved by **furin-like convertase (FLC)** at S1 cleavage site upstream from the transmebrane domain. Peptides are reassociated in Ca++-dependent manner (EDTA chelation dissociates 2 polypeptides and causes Notch activation.



or Kuzbanian) metalloproteaeses and 2. by Gamma-secretase (GS) activity in early endosomal cis-endocytotic compartment [Presinilin (PS) + Nicastrin The freed Notch Intracellular Domain (NICD) enters the nucleus and switches DNA-bound co-repressor complex into activating comple leading to an activation of selected target genes.



Ligand binding results in trans- and cis-internalization

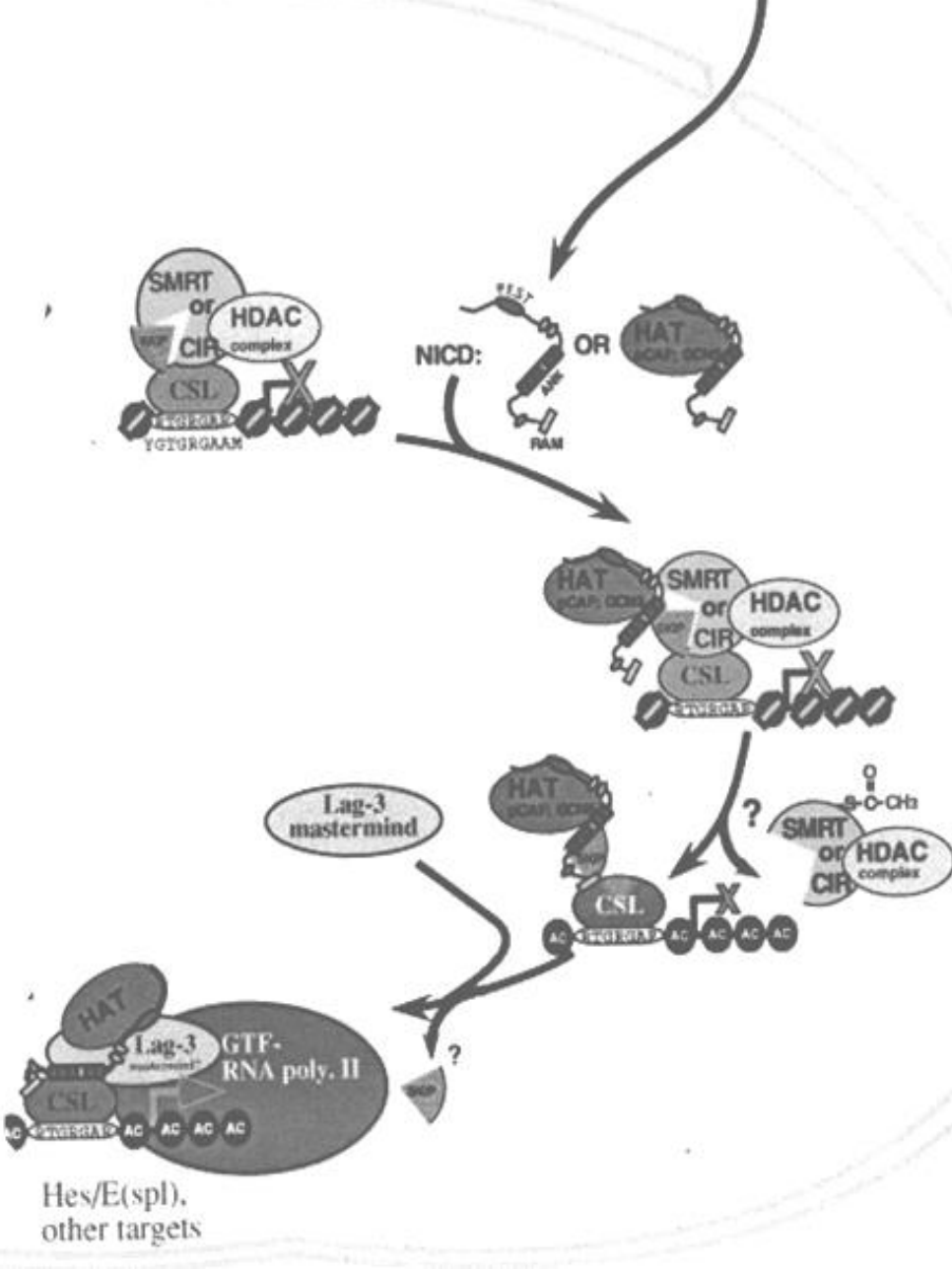


In CORE SIGNALING Delta Ligand binding is followed by its essential internalization mediated by Ubiquitin ligase “Neuralized” which triggers transient Ligand transendocytosis into the ligand cell. Later Notch is cis-internalized

Core signaling pathway: Nuclear Events controlled by NOTCH,

Nuclear target of core Notch signaling is **signal sequence-binding protein-J kappa [RBP-J kappa, also called CSL or Su(H)].**. CSL binds constitutively to **SMRT (Silencing of Retinoid and Thyroid hormone Receptor)** and to HDAC (histone deacetylase) repressing gene transcription.

After entering the nucleus, NICD causes dissociation of SMRT/HDAC and to recruiting HAT (histone acetyltransferase). This converts the CSL repressor into an **activator** complex.

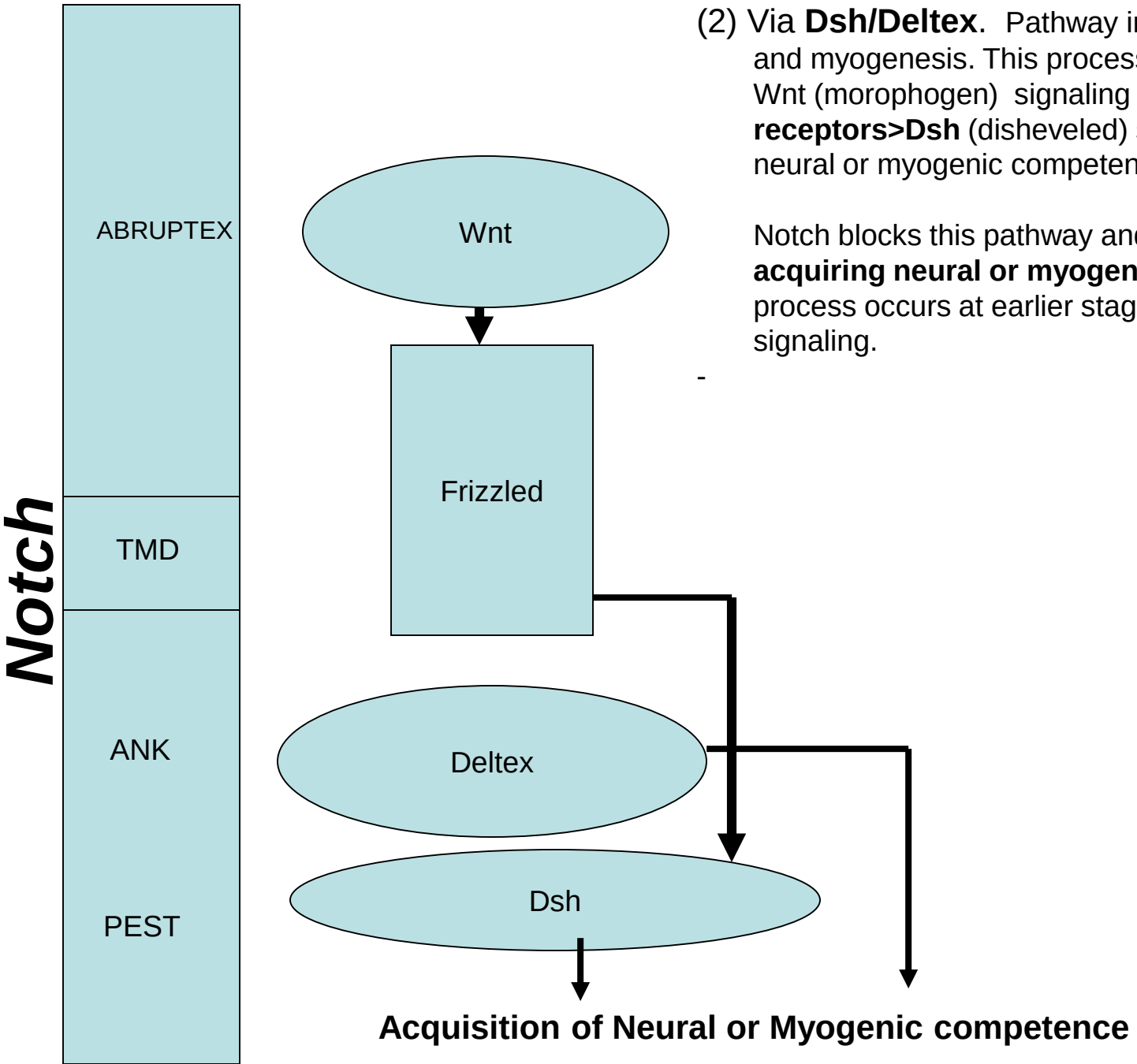


ALTERNATIVE NOTCH SIGNALLING PATHWAYS.

(2) Via **Dsh/Deltex**. Pathway involved in neurogenesis and myogenesis. This process in normally stimulated by Wnt (morophogen) signaling pathway: **Wnt>Frizzled receptors>Dsh** (disheveled) stimulates the acquisition of neural or myogenic competence. :

Notch blocks this pathway and **prevents cells from acquiring neural or myogenic competence**). This process occurs at earlier stage than the core Notch signaling.

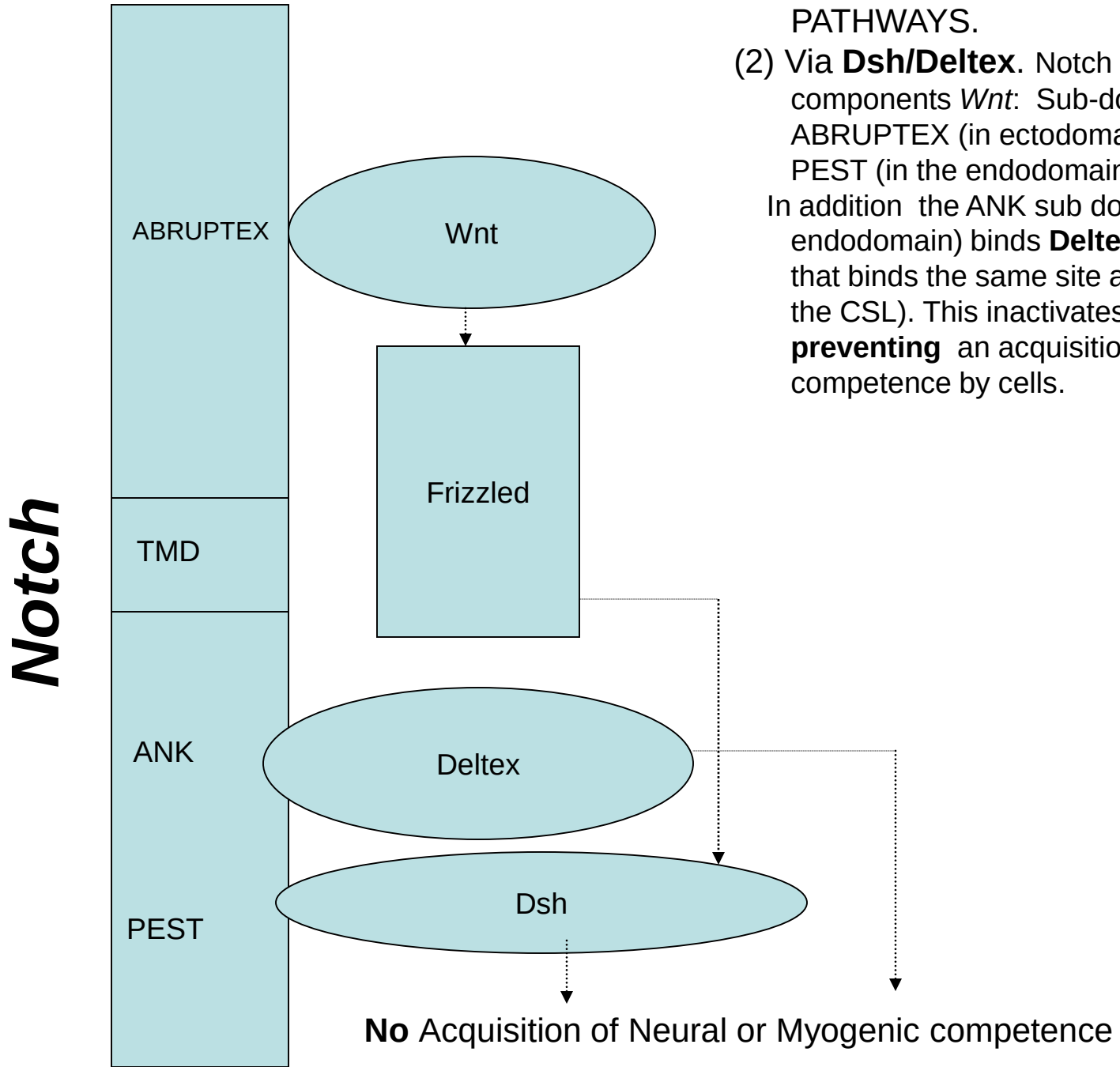
-



ALTERNATIVE NOTCH SIGNALLING PATHWAYS.

(2) Via **Dsh/Deltex**. Notch binds and inactivates the components *Wnt*: Sub-domains involved are ABRUPTEX (in ectodomain) which binds to **Wnt** and PEST (in the endodomain) which binds **Dsh**.

In addition the ANK sub domain (in Notch endodomain) binds **Deltex** (cytoplasmic ring protein that binds the same site as CSL and competes away the CSL). This inactivates Wnt/Dsh and Deltex **preventing** an acquisition of neural or myogenic competence by cells.

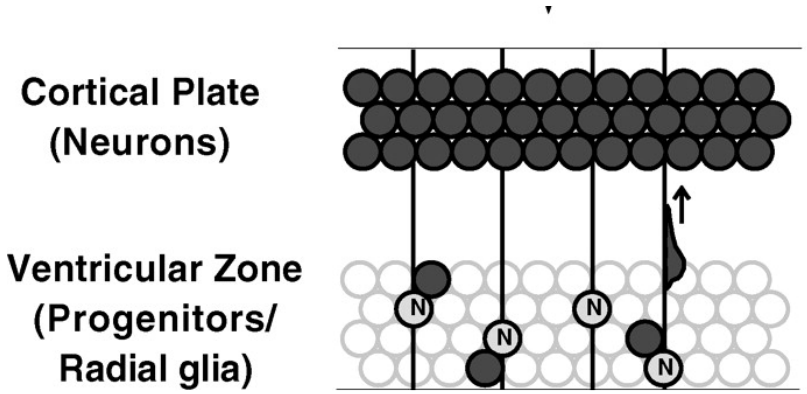


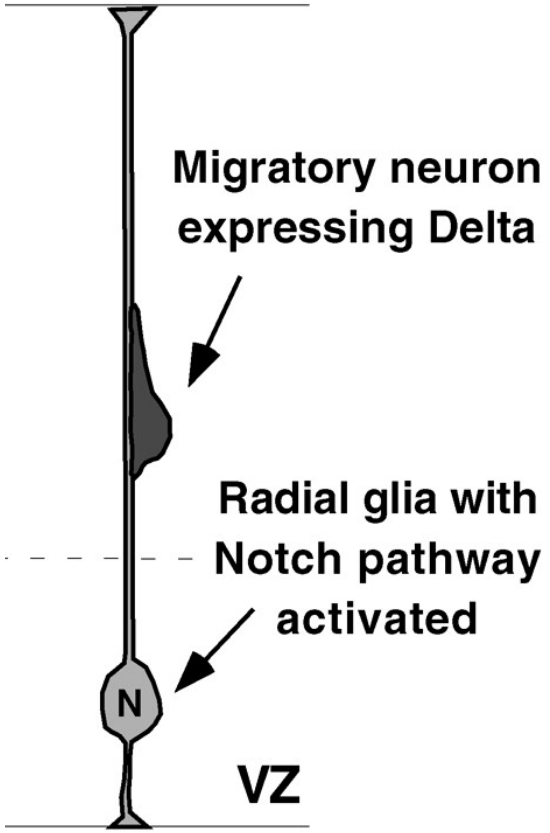
No Acquisition of Neural or Myogenic competence

Gaiano & Fishell, Ann. Rev. Neurosci. 25 (2002), 471-490

(a) - Notch promotes radial glia phenotype (Stem Cells) in developing brain

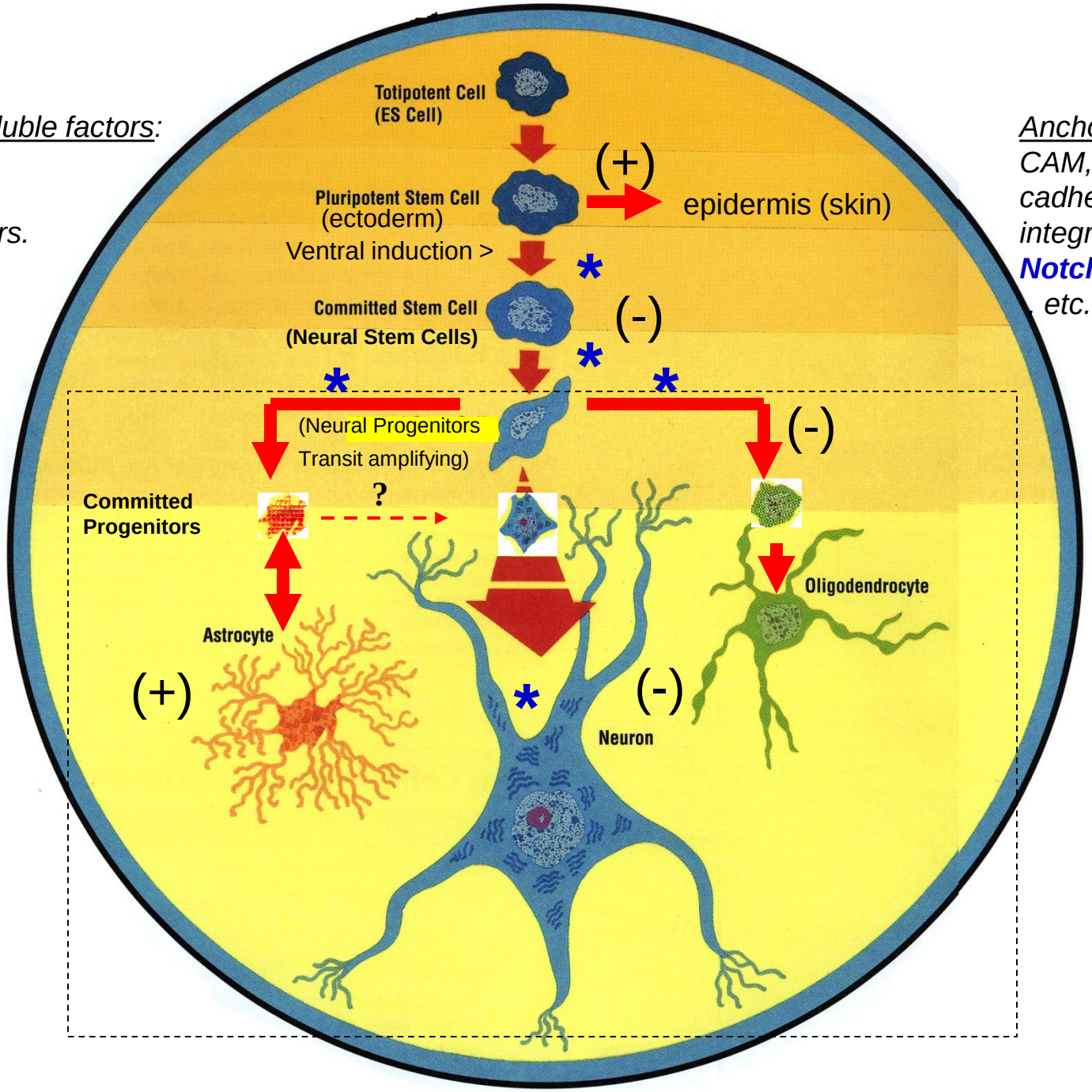
Developing brain:
(Notch receptive are radial glia)





Signals from soluble factors:
growth factors,
hormones,
neurotransmitters.

Anchorage signals:
CAM,
cadherines,
integrines,
***Notch* involved** *
, etc.,



Appendix – additional factors