Cell to cell communication in early development

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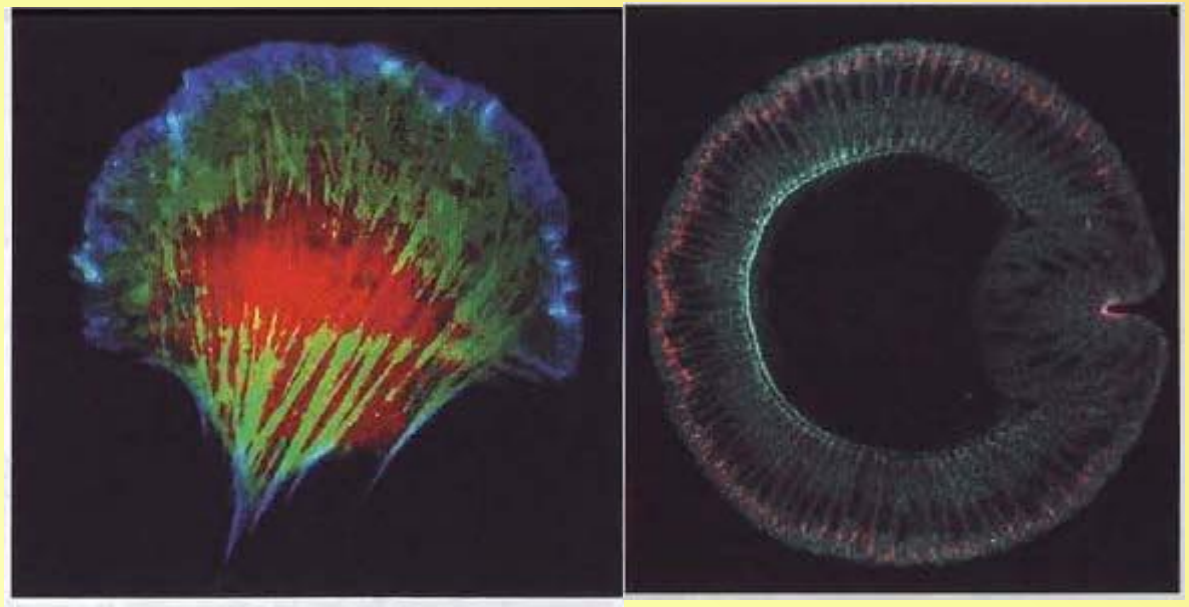


Dr. Subarna Ghosh

Development is more than just differentiation

- The different cell types don't exist as random arrays.
- The formation of organized form is called morphogenesis.
- Five questions that confront modern embryologists who study morphogenesis

Five questions that confront modern embryologists

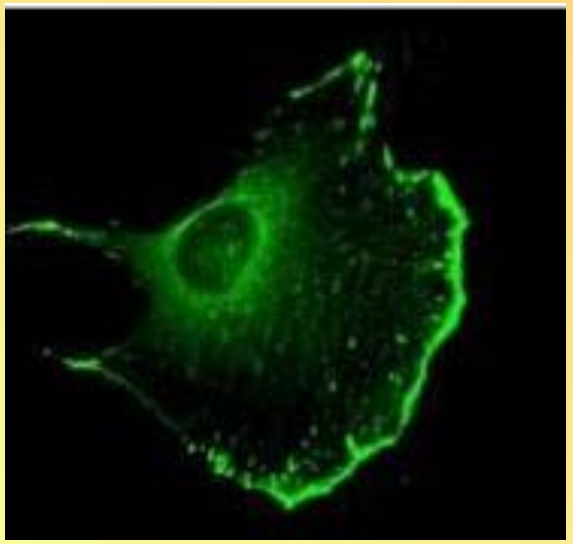
1. How are separate tissues formed from populations of cells?
2. How are organs constructed from tissues?
3. How do organs form in particular locations, and how do migrating cells reach their destinations?
4. How do organs and their cells grow, and how is their growth coordinated throughout development?
5. How do organs achieve polarity?

The topic contains the followings

- Cell adhesion
- Cell migration
- Cell signaling
- Paracrine Factors: The Inducer Molecules
- Juxtacrine Signaling
- The Extracellular Matrix as a Source of Developmental signals
- The Epithelial-Mesenchymal Transition

Differential cell affinity

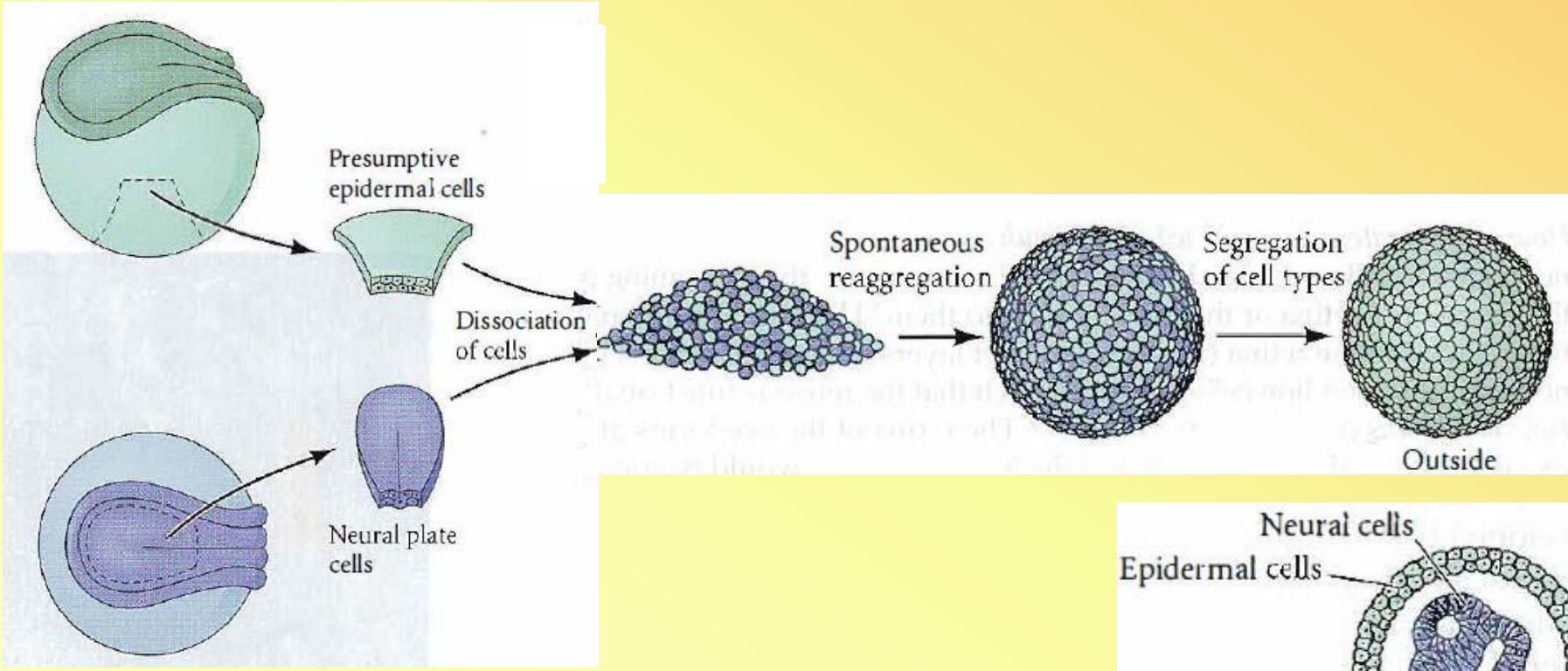
- Many of the answers to our five questions about morphogenesis involve the properties of the cell surface.
- Is the membrane identical in all cells?



Re-aggregation Experiment by Townes and Holtfreter, 1955

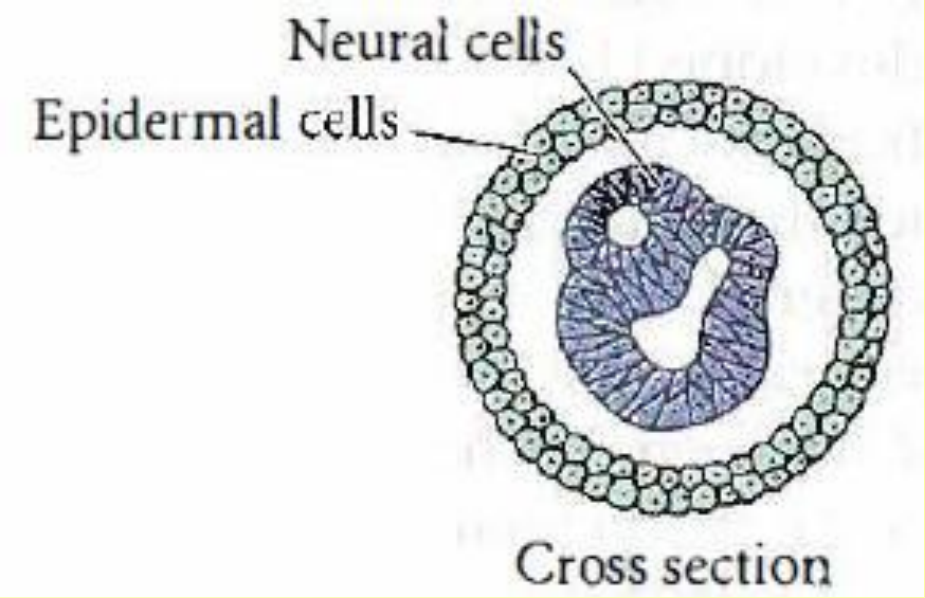
- Amphibian tissues become dissociated into single cells when placed in alkaline solutions.
- Prepared single-cell suspensions from each of the three germ layers after the neural tube had formed.
- When the pH of the solution was normalized, the cells adhered to one another.
- using embryos from species having cells of different sizes and colors to follow the behavior of recombined cells.

Reaggregation of cells from amphibian neurulae

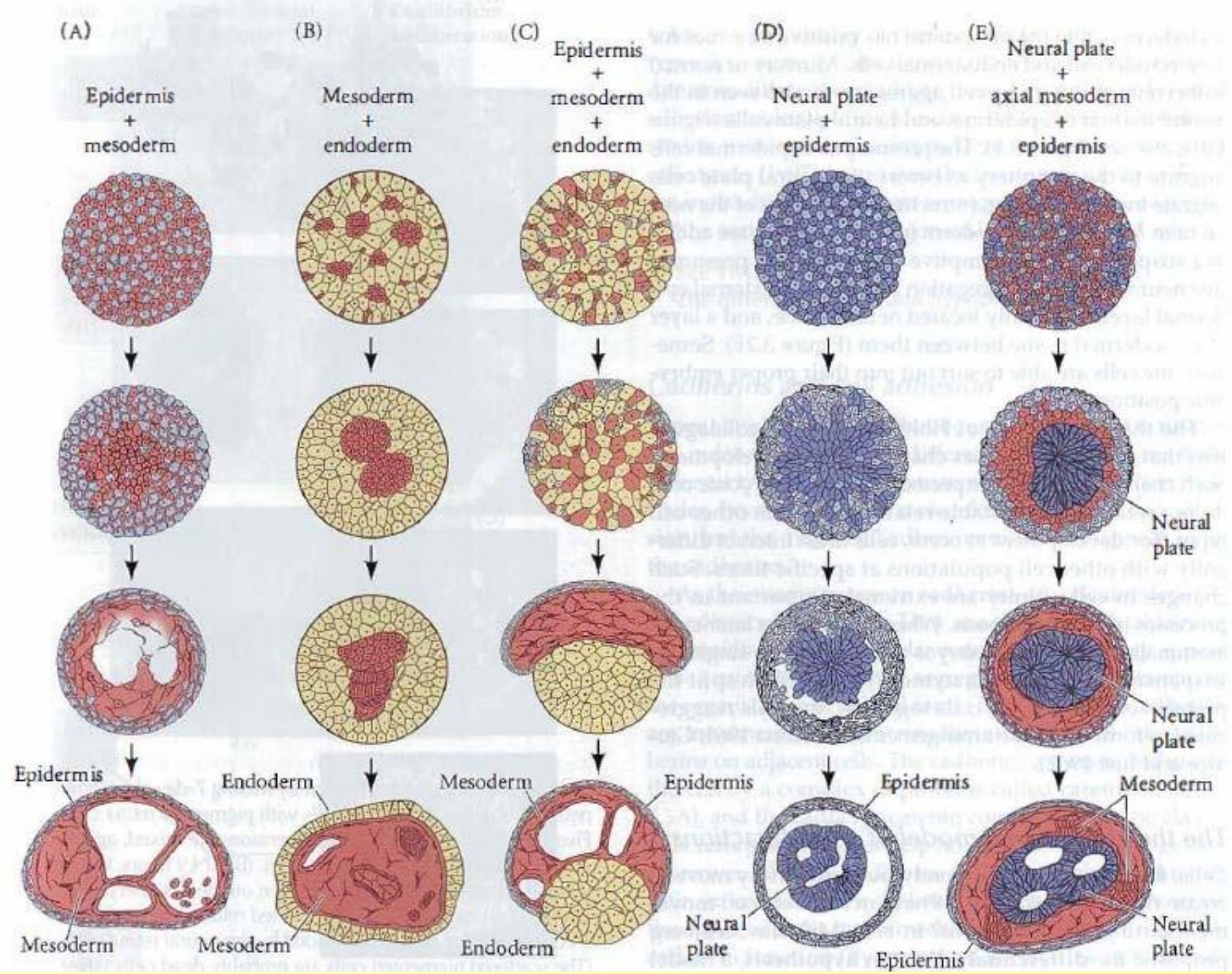


The conclusions

- Reaggregated cells become spatially segregated
- Instead of two cell types remaining mixed, each type sorts out into its own region.



Final positions of the reaggregated cells reflect their respective positions in the embryo.



Holtfreter's interpretation

- Holtfreter interpreted this finding in terms of selective affinity.
- The inner surface of the ectoderm has a positive affinity for mesodenmal cells and a negative affinity for the endoderm, while the mesoderm has positive affinities for both ectodermal and endodermal cells.

The conclusions

- Selective affinities change during development.
- Embryonic cells do not retain a single stable relationship with other cell types.

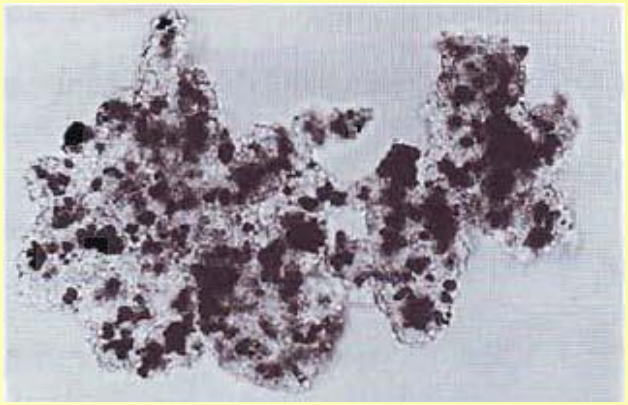
- What forces direct cell movement during morphogenesis?

Differential adhesion hypothesis

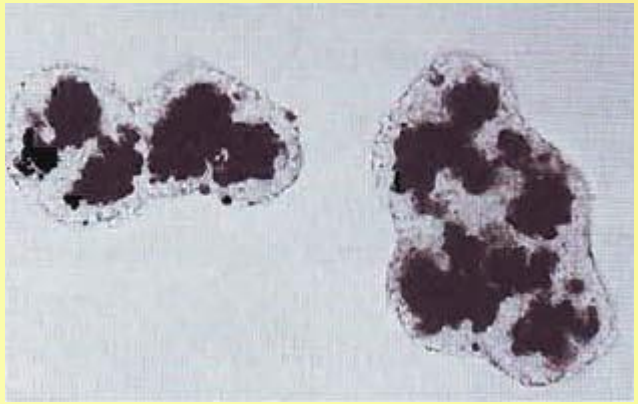
- By Malcolm Steinberg, 1964
- A model that sought to explain patterns of cell sorting based on thermodynamic principles.
- Certain cell types migrate centrally when combined with some cell types, but migrate peripherally when combined with others.

Interactions between pigmented retina cells and neural retina cells

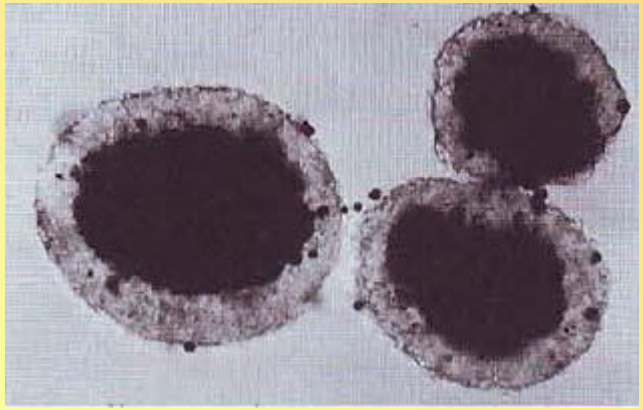
Aggregates formed by mixing 7-day chick embryo neural retina (unpigmented) cells with pigmented retina cells.



Five hours after mixture, aggregates of randomly distributed cells are seen.



At 19 hours, the pigmented retina cells are no longer seen on the periphery



At 2 days, a great majority of the pigmented retina cells are located in a central internal mass, surrounded by the neural retina cells.

Hierarchy of the cell-cell Interactions

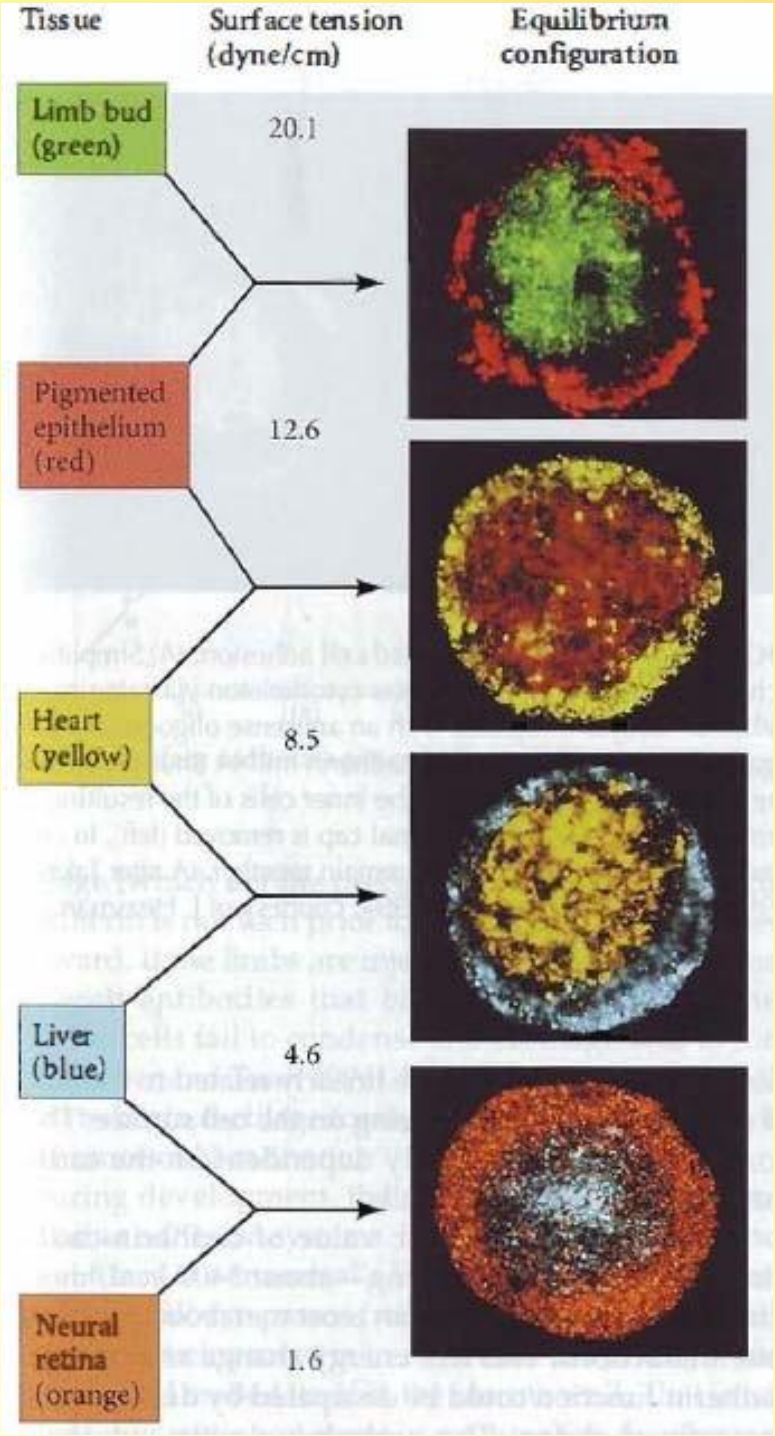
- If the final position of cell type A is internal to a second cell type B, and the final position of B is internal to a third cell type C, then the final position of A will always be internal to C.
- For example, pigmented retina cells migrate internally to neural retina cells, and heart cells migrate internally to pigmented retina cells. Therefore, heart cells migrate internally to neural retina cells.

The smallest interfacial free energy

- The cells rearrange themselves into the most thermodynamically stable pattern.
- if the strength of A-A connections is greater than the strength of A-B or B-B connections, sorting will occur, with the A cells becoming central.
- if the strength of A-A connections is less than or equal to the strength of A-B connections, then the aggregate will remain as a random mix of cells

According to this hypothesis

- the early embryo can be viewed as existing in an equilibrium state until some change in gene activity changes the cell surface molecules.
- The movements that result seek to restore the cells to a new equilibrium configuration. All that is required for sorting to occur is that cell types differ in the strengths of their adhesion.



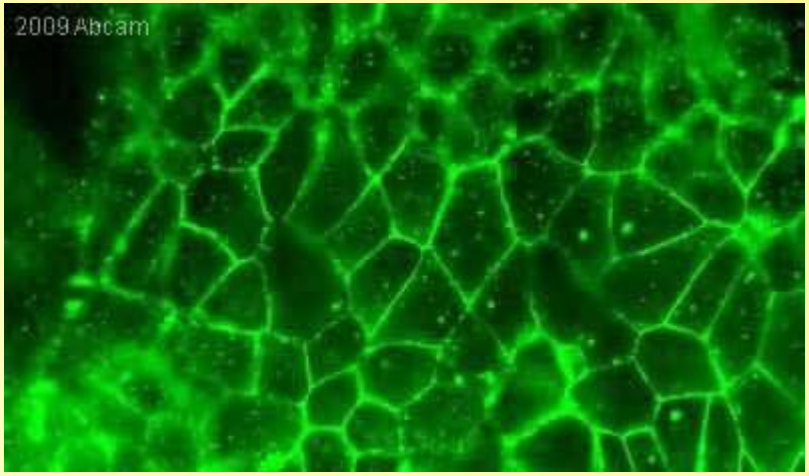
- In 1996, Poty and his colleagues in Steinberg's laboratory demonstrated that this was indeed the case.
- the cell types that had greater surface cohesion migrated centrally compared with those cells that had less surface tension.

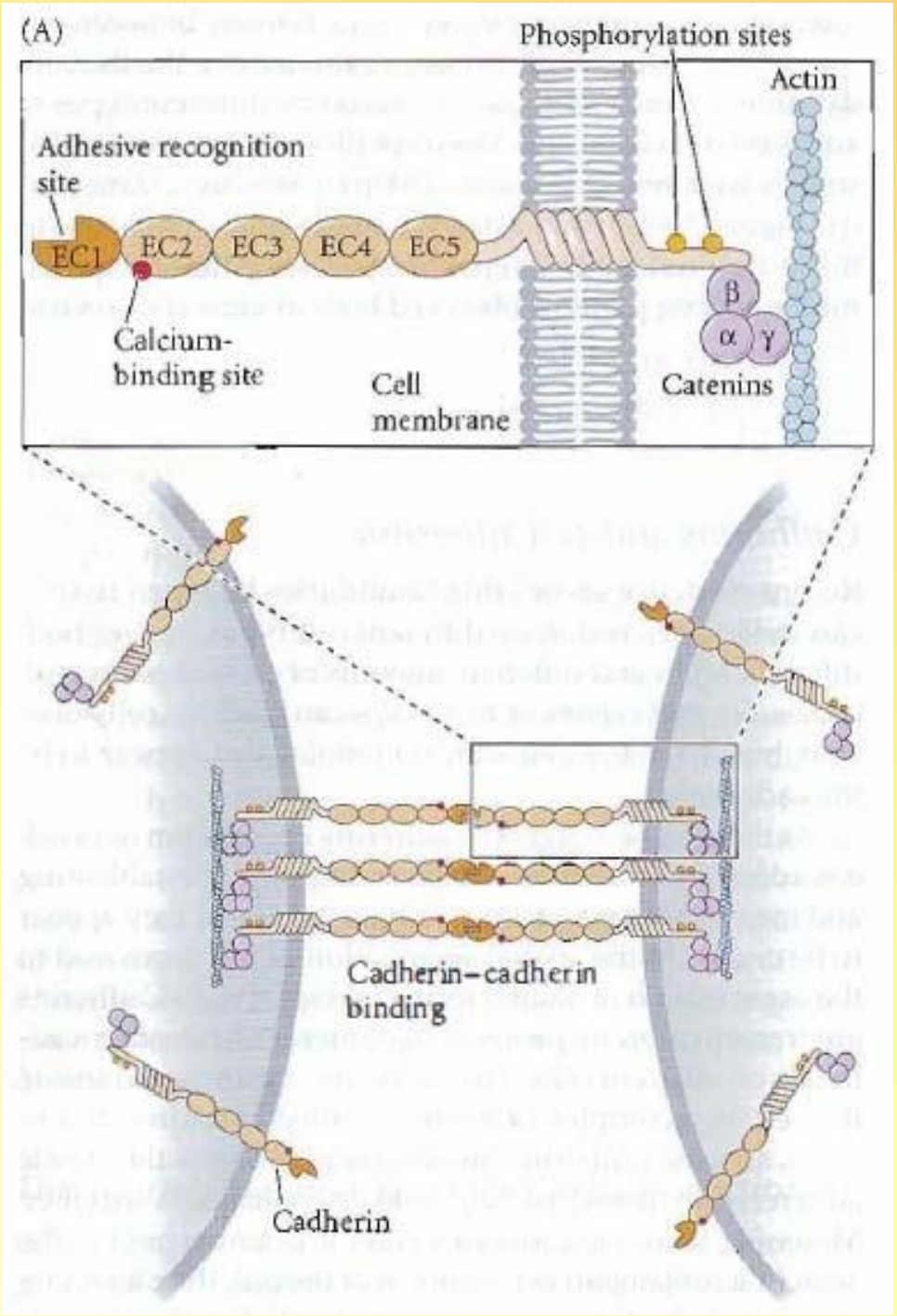
Cell “glue”

- Cells could have the same type of "glue" on the cell surface.
- The amount of this cell surface product (glue), could cause a difference in the number of stable contacts made between cell types.

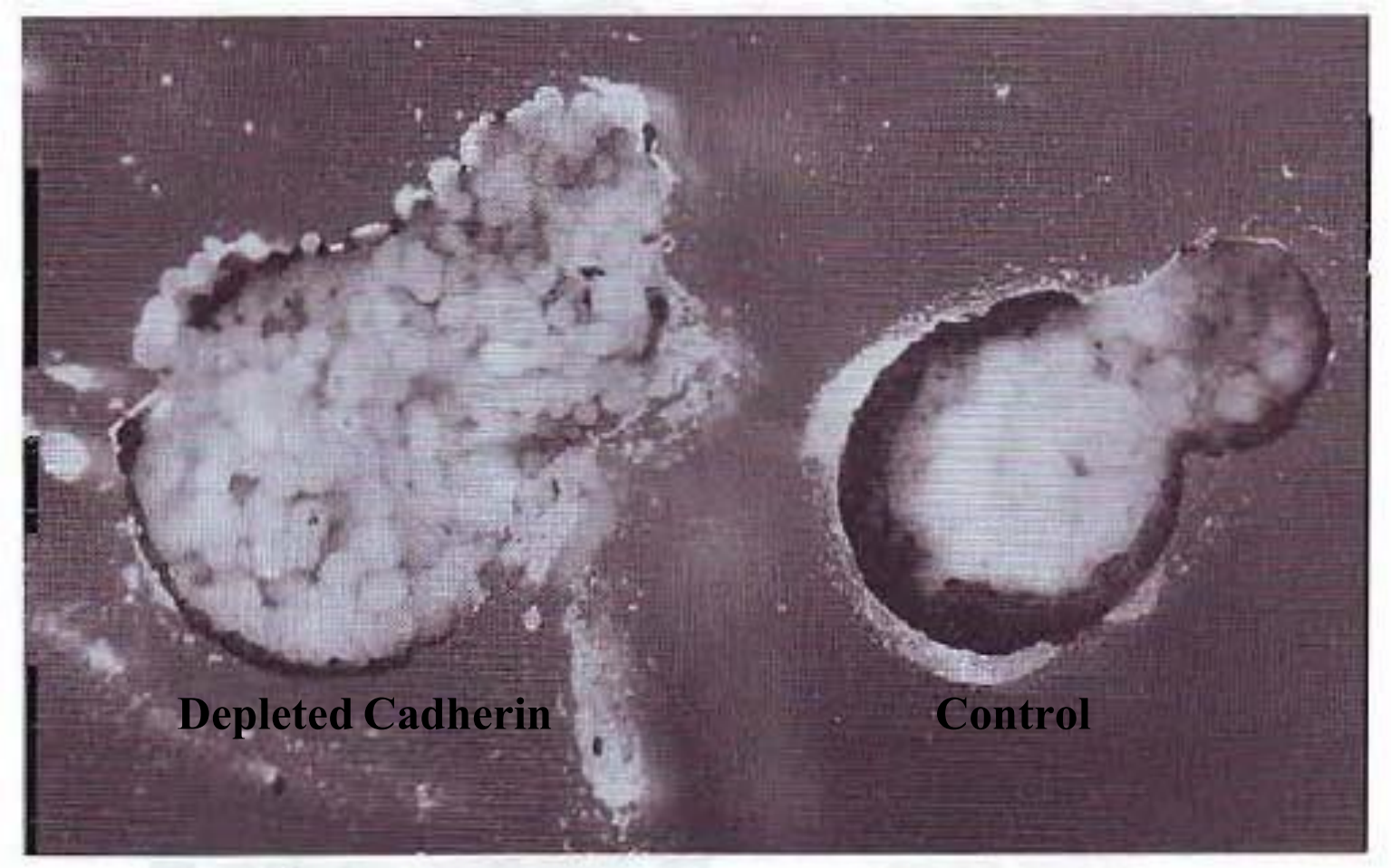
Cadherins and cell adhesion

- Major cell adhesion molecules : cadherins.
- 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




(A)
Phosphorylation sites
Actin
Adhesive recognition site
EC1
EC2
EC3
EC4
EC5
β
α
γ
Calcium-binding site
Cell membrane
Catenins
Cadherin–cadherin binding
Cadherin

Cadherin-mediated cell adhesion

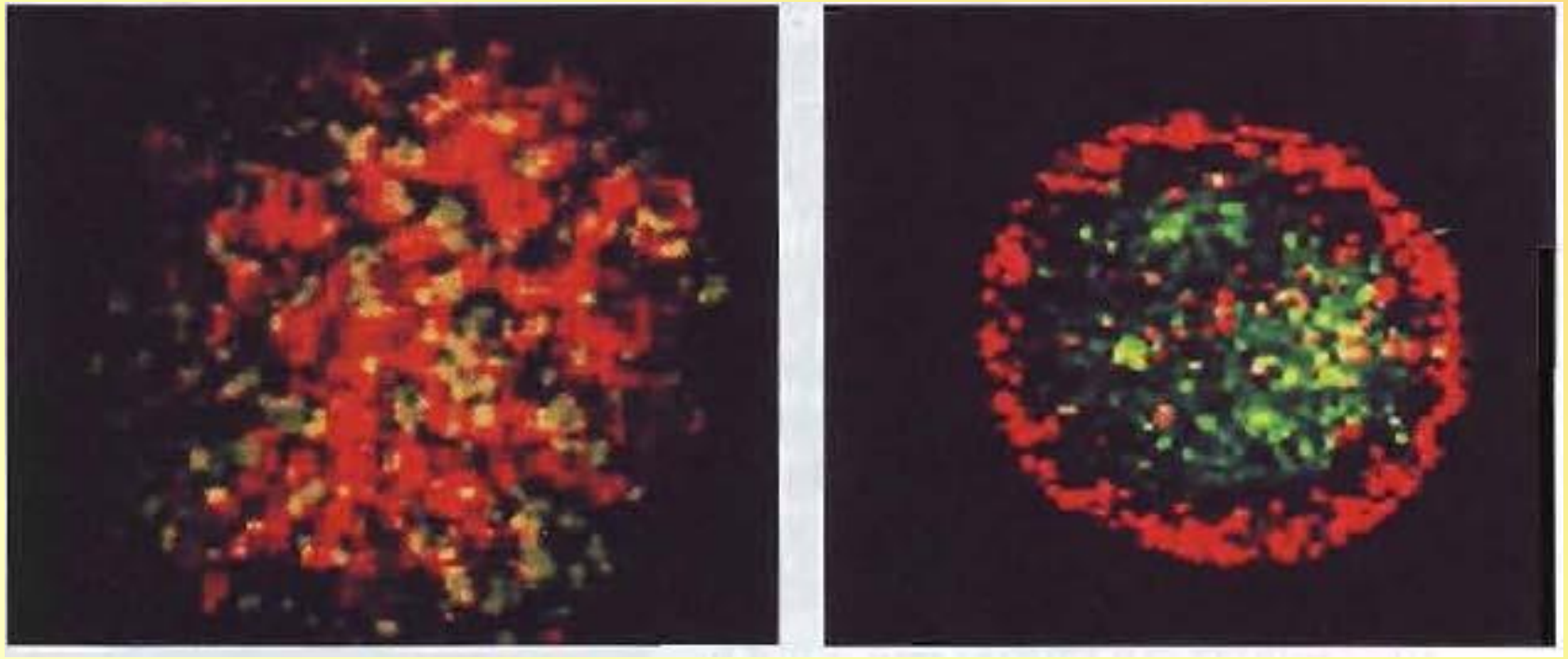


The tendency of cells to bind together depend on the strength of cadherin interactions

- Two cell lines that were identical except that they synthesized different amounts of cadherin.
- When these two groups of cells were mixed, the cells that expressed more cadherin had a higher surface cohesion and migrated internally to the lower-expressing group of cells.

Importance of the amount of cadherin for correct morphogenesis.

Green cells ： 2.4 times as many N-cadherin molecules



4 hours of incubation

Foty and Steinberg, 2005

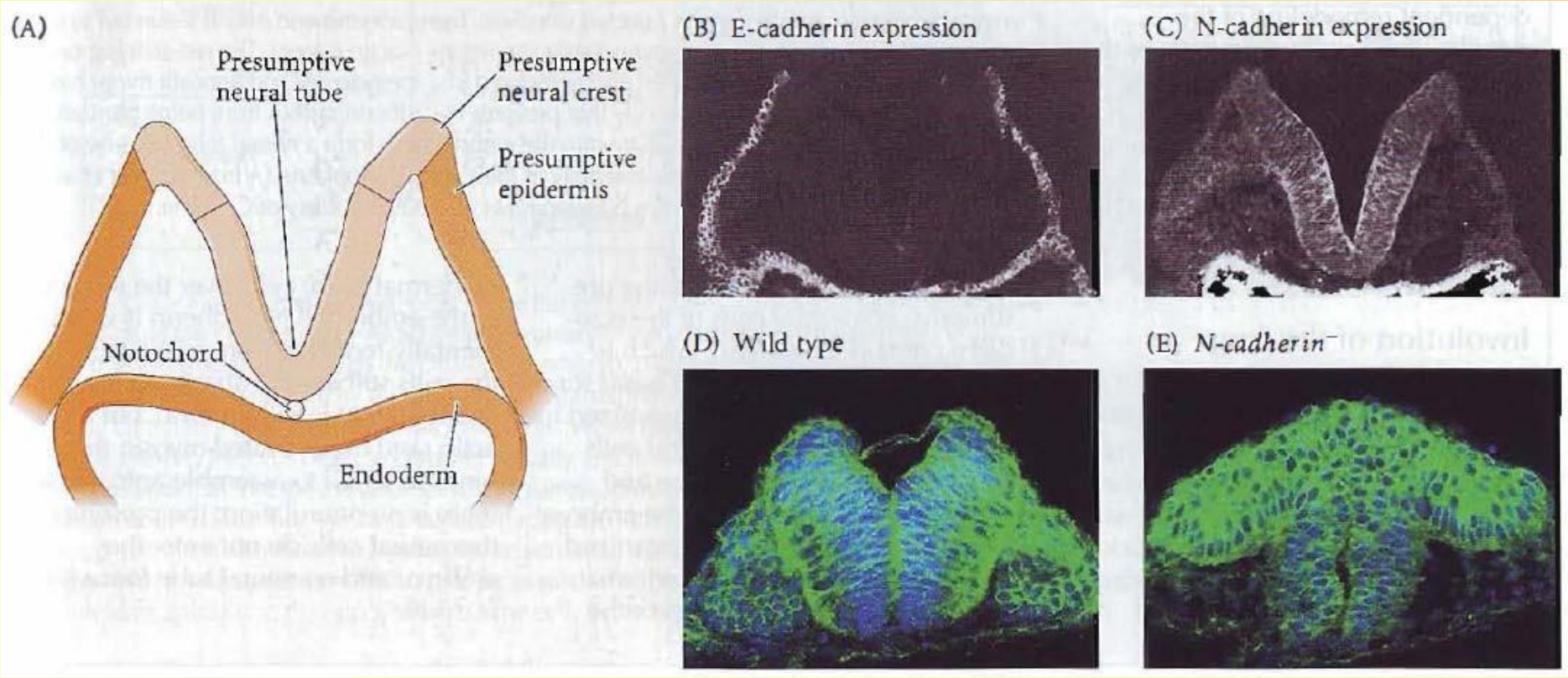
Several major cadherin types

- E-cadherin is expressed on all early mammalian embryonic cells, even at the zygote stage. Later in development, this molecule is restricted to epithelial tissues of embryos and adults.
- P-cadherin is found predominantly on the placenta, where it helps the placenta stick to the uterus (Nose and Takeichi 1986; Kadokawa et al. 1989).
- N-cadherin becomes highly expressed on the cells of the developing central nervous system (Hatta and Takeichi 1986), and it may play roles in mediating neural signals.
- R-cadherin is critical in retina formation (Babb et al. 2005).

Type and timing

- R-cadherin and B-cadherin do not bind well to each other.
- N-cadherin appears in the mesenchymal cells of developing chick leg just before condensation.

Importance of the types of cadherin for correct morphogenesis.



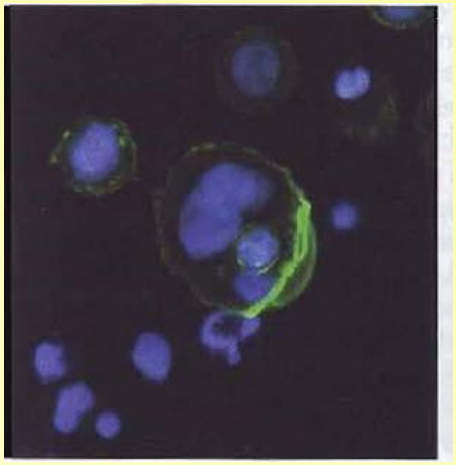
N-cadherin is critical for separation of presumptive epidermal and neural tissues during organogenesis.

Cell Migration

- Cell migration is a common feature of both epithelial and mesenchymal cells.
- In epithelia, the motive force for migration is usually provided by the cells at the edge of the sheet, and the rest of the cells follow passively.
- In mesenchymal cell migration, individual cells become polarized and migrate through the extracellular milieu.

Polarization

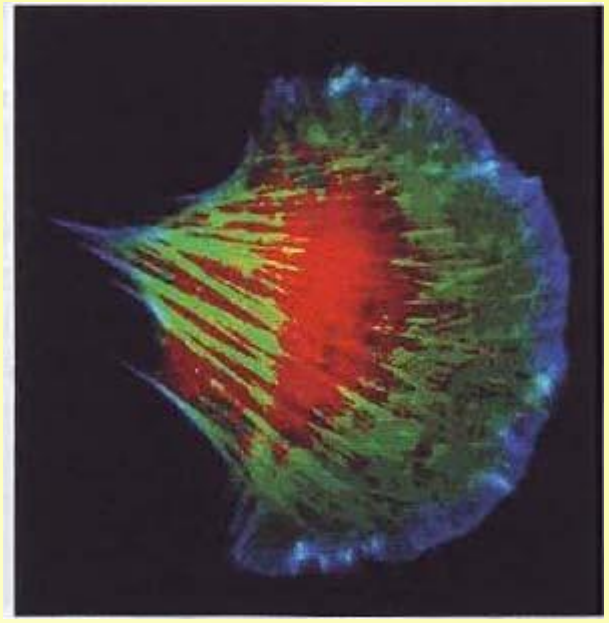
- The first stage of migration is polarization, wherein a cell defines its front and its back.
- The cell has a front and a back, and so that the front part of the cell becomes structurally different from the back of the cell.



Cell interiors are stained blue. The actin stained green) redistributed to the leading edge of the cell.

Protrusion of the cell's leading edge

- The second stage of migration.
- The mechanical force for this is the polymerization of the actin microfilaments at the cell membrane, creating long parallel bundles (filopodia) or broad sheets (Iamellipodia).

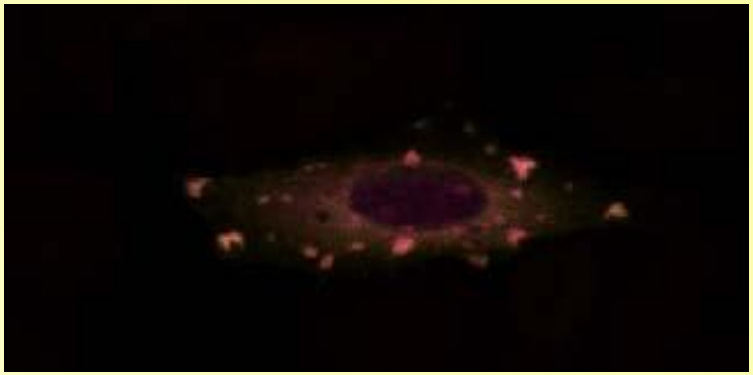


In the lamellipodium of a migrating mesenchymal cell,

Blue: the highest filamentous actin Red: the lowest filamentous actin

The adhesion of the cell to its extracellular substrate

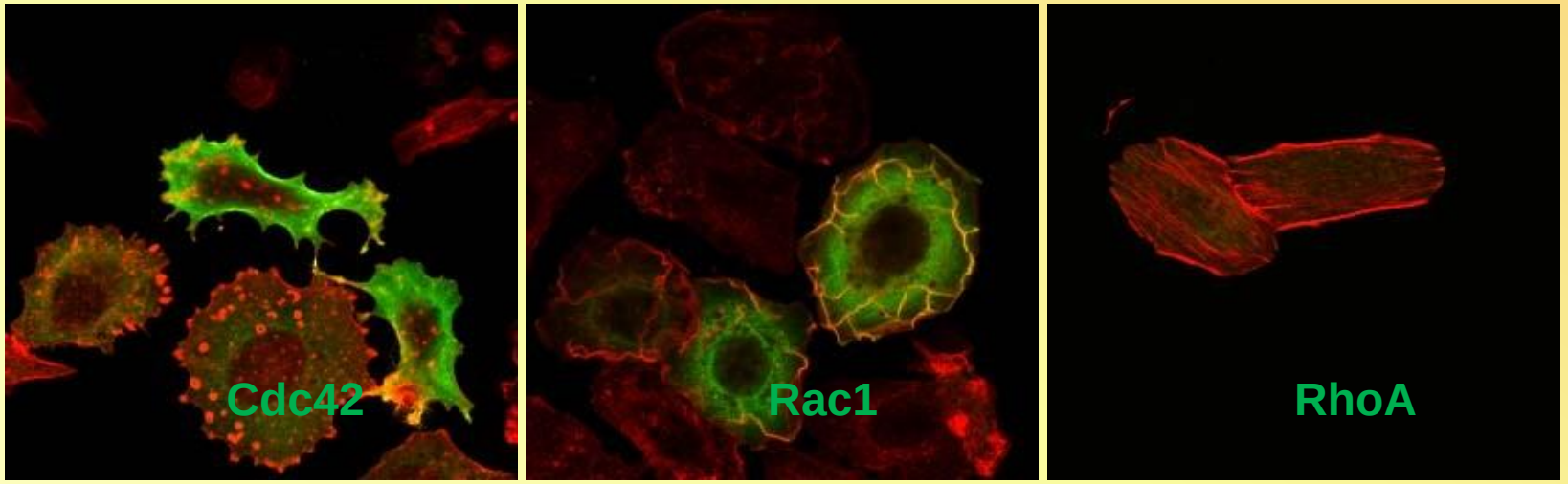
- The moving cell needs something to push on, and attaches to the surrounding matrix.
- Integrins span the cell membrane, connecting extracellular matrix outside to the actin cytoskeleton inside cells. The connections form focal adhesions on the cell membrane.



Focal adhesion sites of a HeLa cell

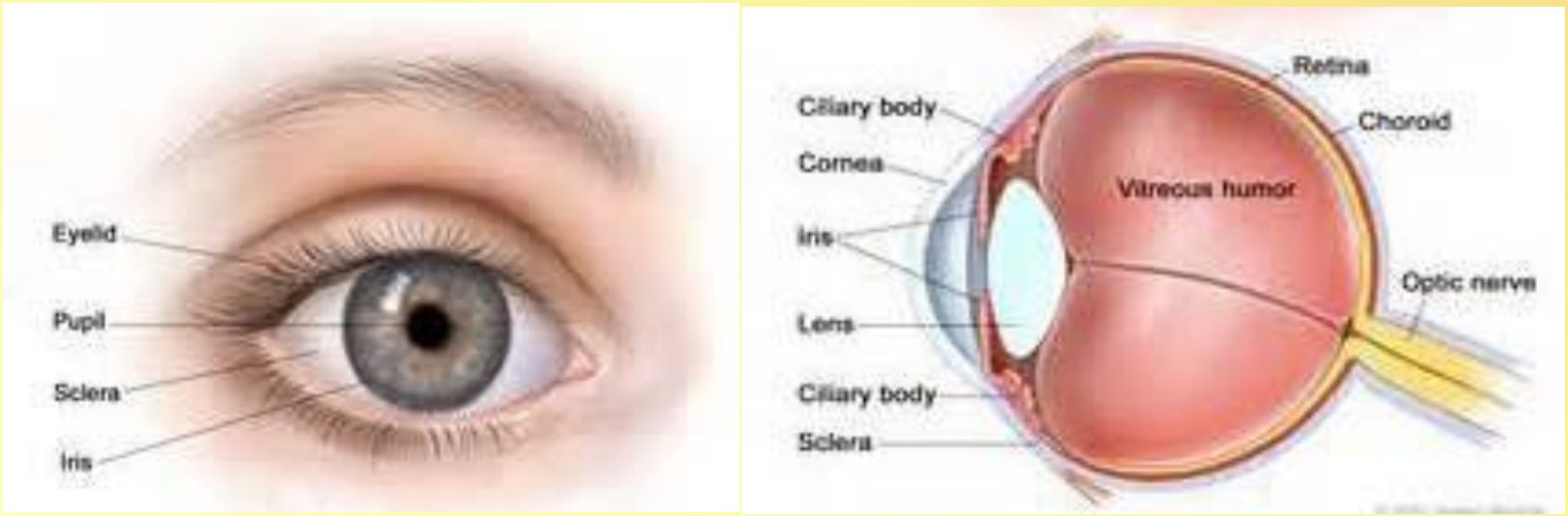
The release of adhesions in the rear

- Allowing the cell to migrate in the forward direction



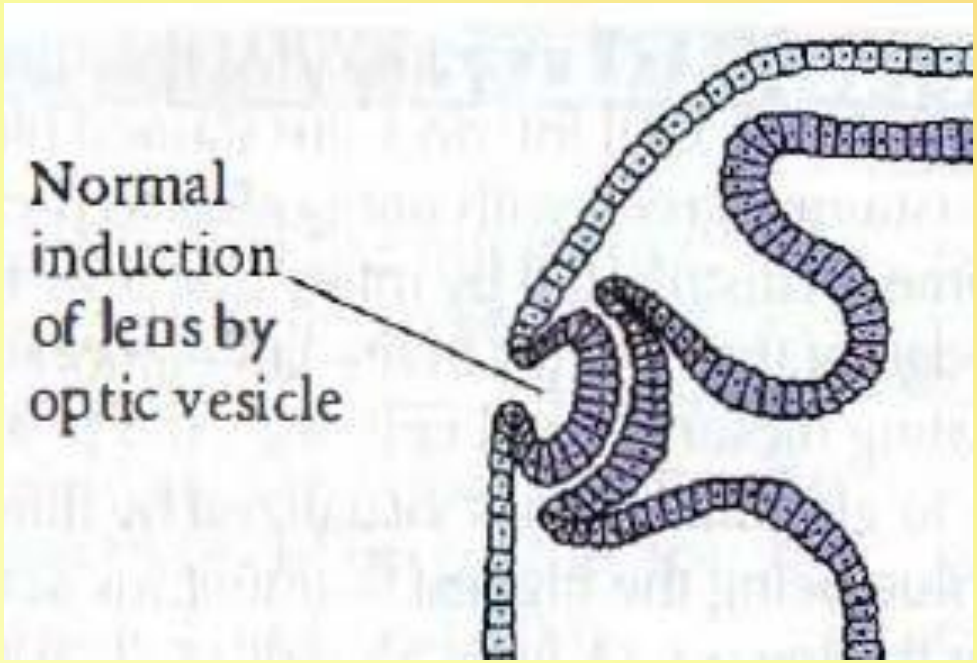
Cell Signaling

- Cell differentiation and behavior are regulated by signals from one cell being received by another cell.
- The development of the vertebrate eye is a classic example.



light is transmitted through the transparent corneal tissue and focused by the lens tissue, eventually impinging on the tissue of the neural retina.

Ectodermal competence and the abililty to respond to the optic vesicle inducer



Optic vesicle (the presumptive retina)

Induction

- Such coordination in the construction of organs is accomplished by one group of cells changing the behavior of an adjacent set of cells, thereby causing them to change their shape, mitotic rate, or cell fate.
- This kind of interaction at close range between two or more cells or tissues of different properties is called induction.

Two components to every inductive interaction

- The first component is the inducer: the tissue that produces a signal (or Signals) that changes the cellular behavior of the other tissue.
- Often, this signal is a secreted protein called a paracrine factor.
- The second component, the responder, is the tissue being induced.

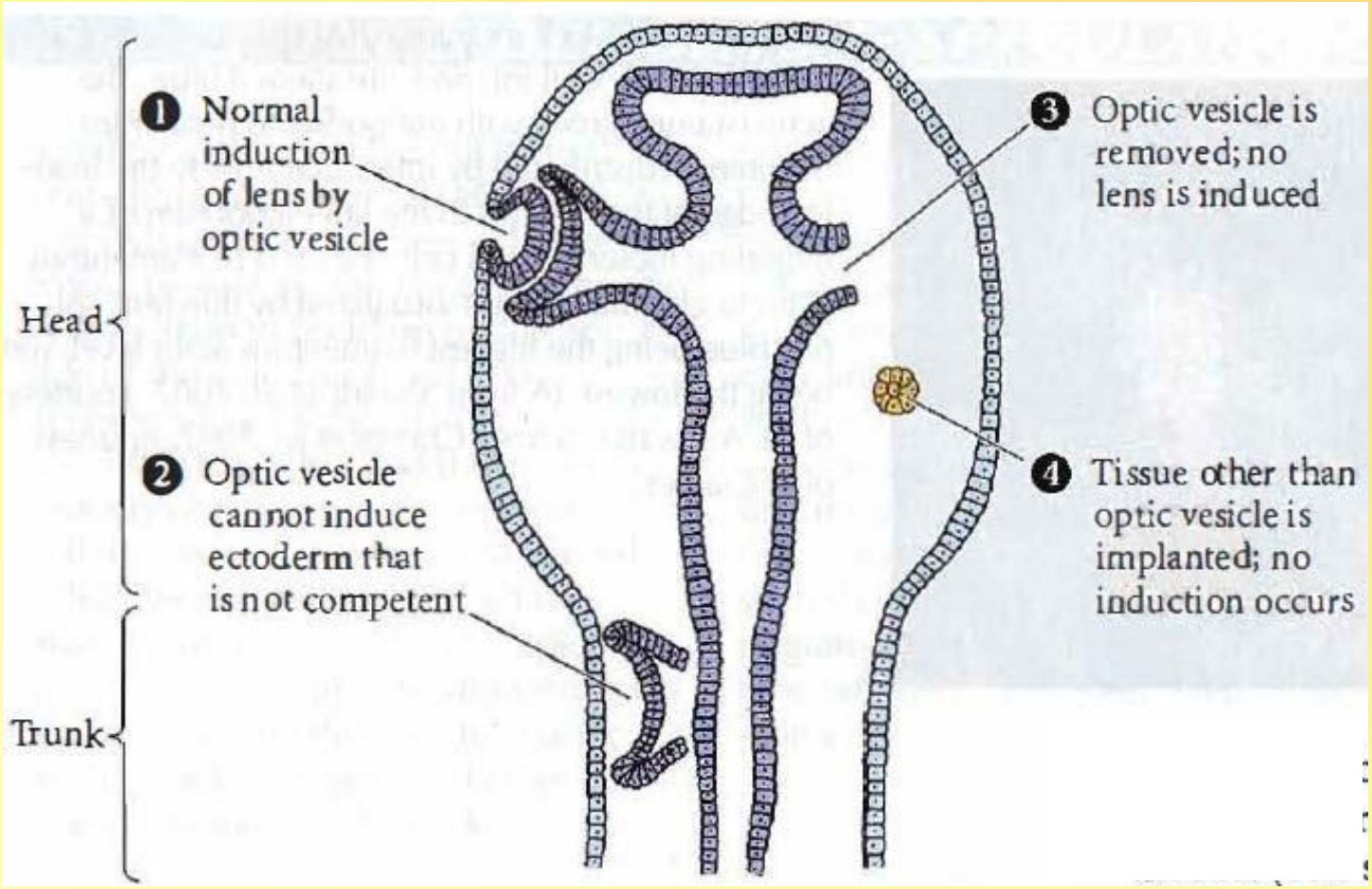
Paracrine factors

- Paracrine factors are proteins made by a cell or a group of cells that alter the behavior or differentiation of adjacent cells.
- Endocrine factors (hormones): travel through the blood and exert their effects on cells and tissues far away.
- Paracrine factors are secreted into the extracellular space and influence their close neighbors.

The responder

- Cells of the responding tissue must have both a receptor protein for the inducing factor and the ability to respond to the signal.
- The ability to respond to a specific inductive signal is called competence.

Ectodermal competence and the abililty to respond to the optic vesicle inducer

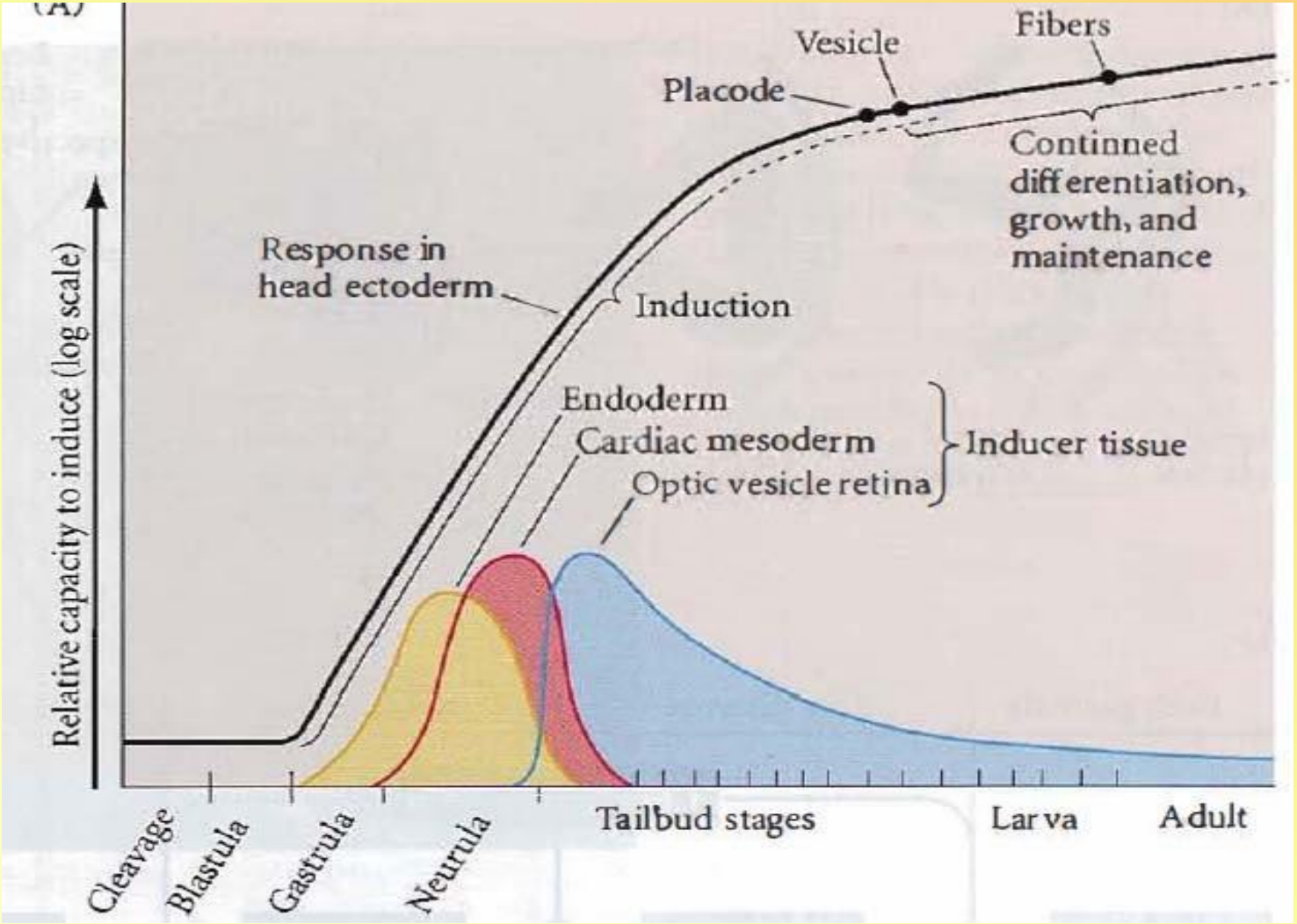


Optic vesicle (the presumptive retina)

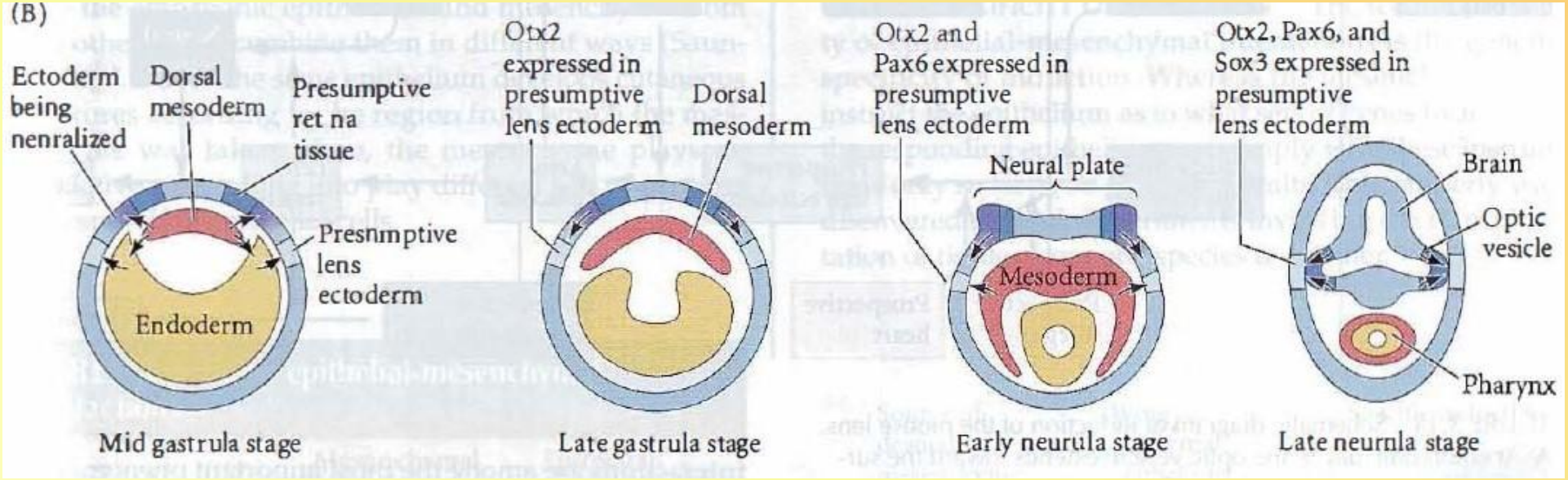
One induction will give a tissue the competence to respond to another inducer

- The ability to produce lens tissue is first induced by foregut endoderm, then by cardiac mesoderm, and finally by the optic vesicle.
- The optic vesicle eventually acquires the ability to induce the lens and retain its differentiation

Lens induction in amphibians

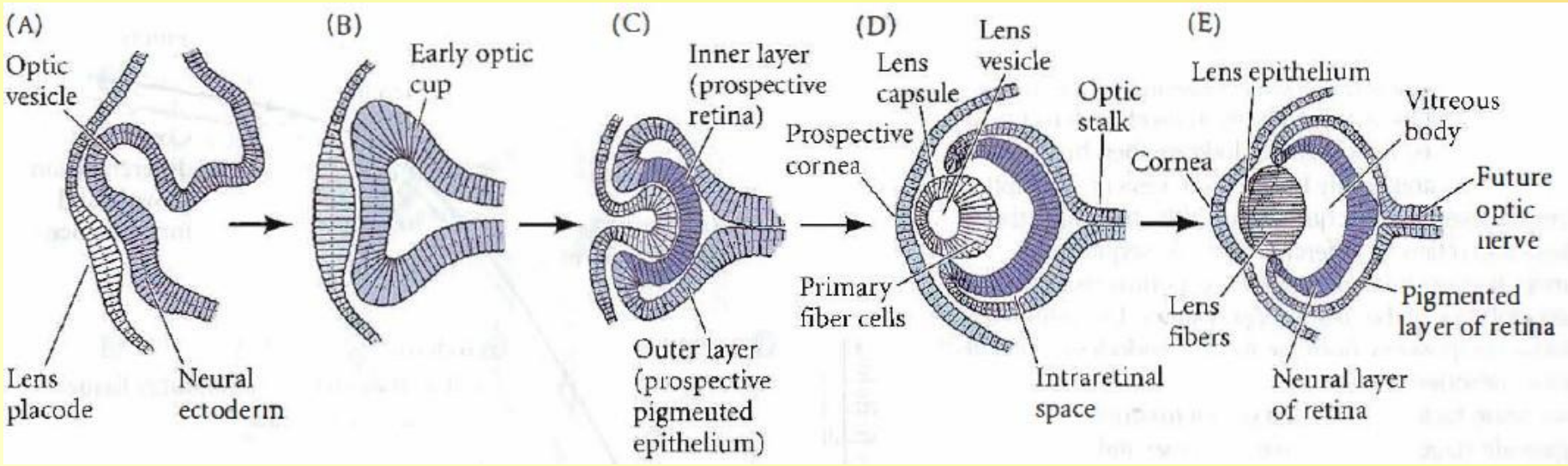


Sequence of induction



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 induces the optic vesicle itself; thus the inducer becomes the induced.



Sequential inductive events

- The lens is inducing the ectoderm above it to become the cornea.
- the corneal ectoderm cells become columnar and secrete multiple layers of collagen.
- Mesenchymal cells from the neural crest
- use this collagen matrix to enter the area and secrete a set of proteins that further differentiate the cornea.
- A third Signal, the hormone thyroxine, dehydrates the tissue and makes it transparent.
- Thus, there are sequential inductive events, and multiple causes for each induction.

A structure does not need to be fully differentiated in order to have a function

- The optic vesicle induces the lens placode before it becomes the retina;
- The lens placode (the prospective lens) reciprocates by inducing the optic vesicle before the lens forms its characteristic fibers.
- Thus, before a tissue has its "adult“ functions, it has critically important transient functions in building the organs of the embryo.

Instructive and permissive interactions

- In instructive interaction, a signal from the inducing cell is necessary for initiating new gene expression in the responding cell.
- Without the inducing cell, the responding cell is not capable of differentiating in that particular way.

Permissive interaction

- The second type of inductive interaction is permissive interaction.
- Here, the responding tissue has already been specified, and needs only an environment that allows the expression of these traits.
- For instance, many tissues need a solid substrate containing fibronectin or laminin in order to develop.

Epithelial-mesenchymal interactions

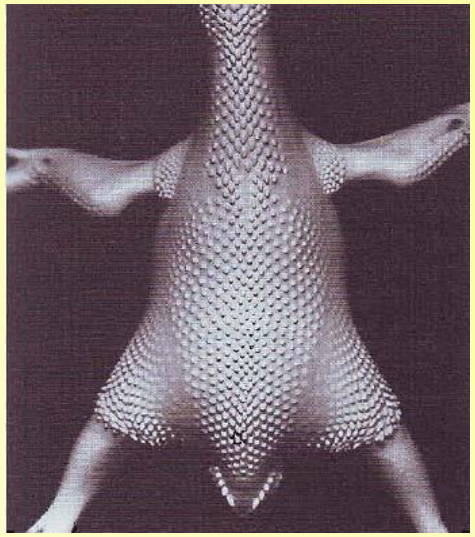
- All organs consist of an epithelium and an associated mesenchyme
- The properties of epithelial-mesenchymal interactions in the induction of cutaneous (skin) structures.

TABLE 3.1 Some epithelial-mesenchymal interactions

Organ	Mesenchymal component	Epithelial component
Cutaneous structures (hair, feathers, sweat glands, mammary glands)	Epidermis (ectoderm)	Dermis (mesoderm)
Limb	Epidermis (ectoderm)	Mesenchyme (mesoderm)
Gut organs (liver, pancreas, salivary glands)	Epithelium (endoderm)	Mesenchyme (mesoderm)
Foregut and respiratory associated organs (lungs, thymus, thyroid)	Epithelium (endoderm)	Mesenchyme (mesoderm)
Kidney	Ureteric bud epithelium (mesoderm)	Mesenchyme (mesoderm)
Tooth	Jaw epithelium (ectoderm)	Mesenchyme (neural crest)

REGIONAL SPECIFICITY OF INDUCTION

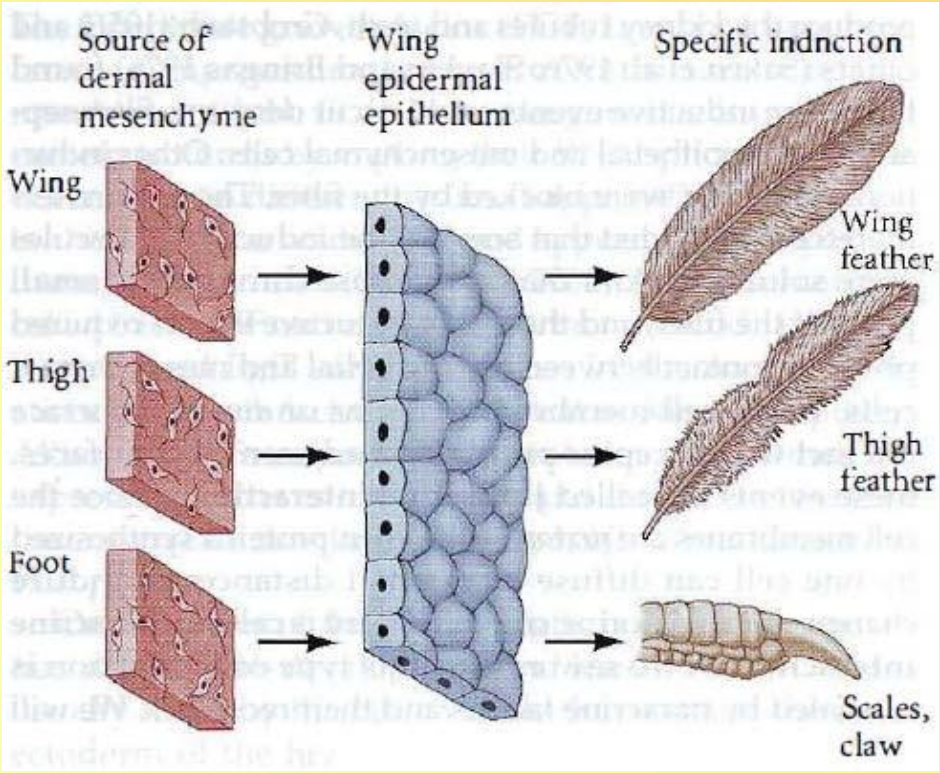
- Skin is composed of two main tissues: An outer epidermis (an epithelial tissue derived from ectoderm).
- A dermis (a mesenchymal tissue derived from mesoderm).



- Epidermis secretes proteins that signal the underlying dermal cells to form condensations,
- The condensed dermal mesenchyme responds by secreting factors that cause the epidermis to form regionally specific cutaneous structures

Regional specificity of induction in the chick

Instructive role

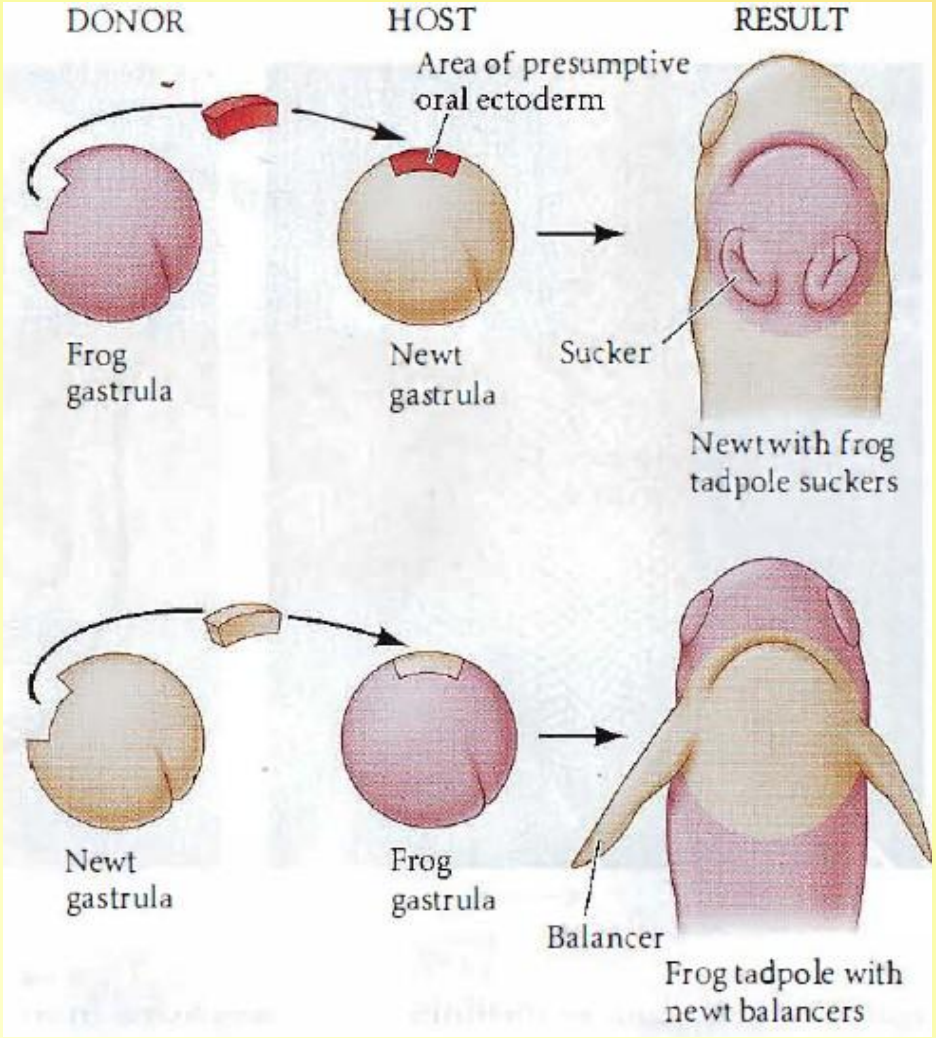


When cells from different regions of the dermis (mesenchyme) are recombined with the epidermis (epithelium), the type of cutaneous structure made by the epidermal epithelium is determined by the original source of the mesenchyme.

GENETIC SPECIFICITY OF INDUCTION

- Whereas the mesenchyme may instruct the epithelium as to what sets of genes to activate, the responding epithelium can comply with these instructions only so far as its genome permits.
- organ-type specificity (e.g., feather or claw) is usually controlled by the mesenchyme, species specificity is usually controlled by the responding epithelium.

Interspecific induction



Reciprocal transplantation between the presumptive oral ectoderm regions of salamander and frog gastrulae leads to newts with tadpole suckers and tad poles with newt balancers.

How are the Signals between inducer and responder transmitted?

- Grobstein (1956) and others found that some inductive events could occur despite a filter separating the epithelial and mesenchymal cells.
- Other inductions were blocked by the filter.
- Some of the inductive molecules were soluble factors that could pass through the small pores of the filter.

Juxtacrine and Paracrine interactions

- When cell membrane proteins on one cell surface interact with receptor proteins on adjacent cell surfaces, these events are called juxtacrine interactions.
- When proteins synthesized by one cell can diffuse over small distances to induce changes in neighboring cells, the event is called a paracrine interaction.

Paracrine factors

- Paracrine factors are secreted into the immediate spaces around the cell producing them.
- Endocrine factors* (hormones) travel through the blood to exert their effects,
- There is considerable debate as to the distances at which paracrine factors can operate.

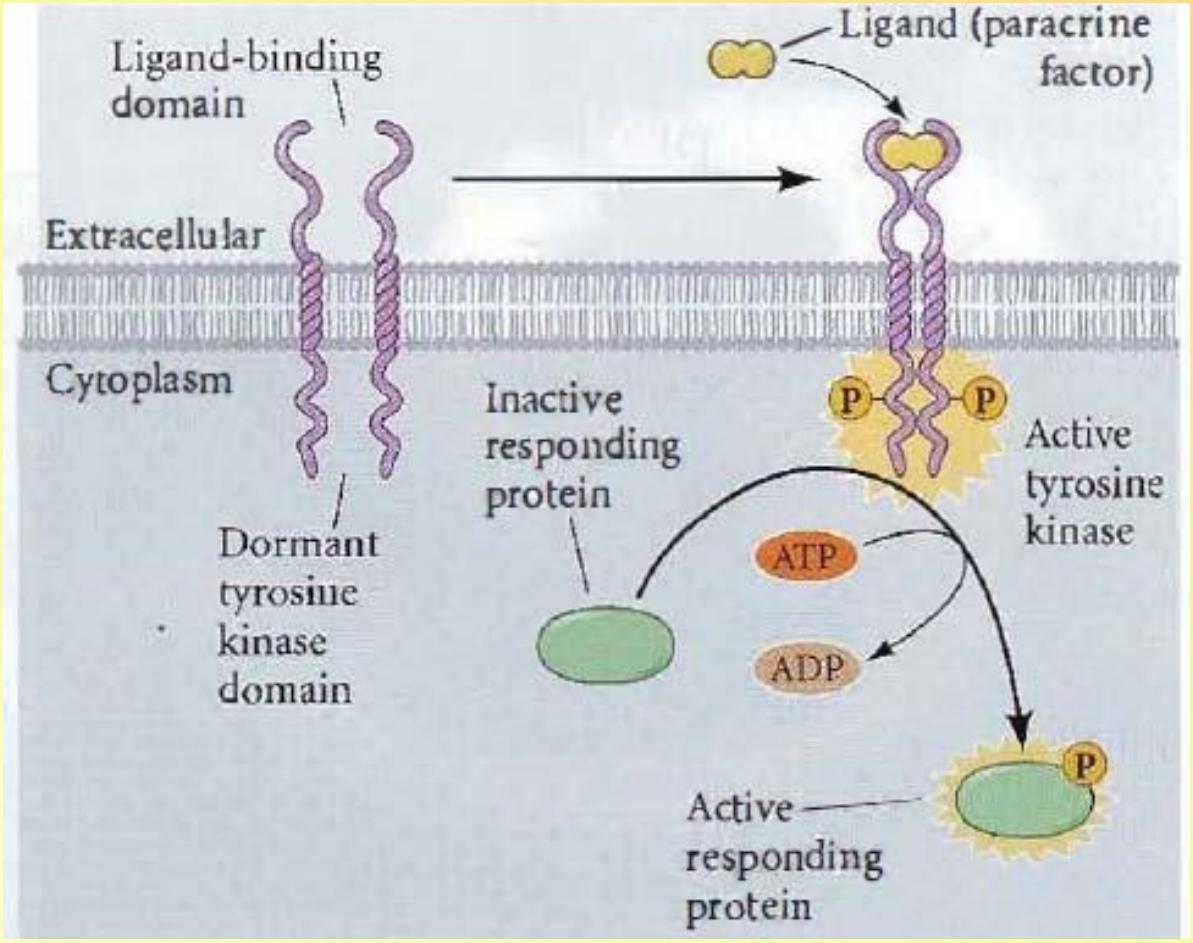
Autocrine interactions

- Autocrine interactions occur when the same cells that secrete paracrine factors also respond to them.
- The cell synthesizes a molecule for which it has its own receptor.
- Placental cytotrophoblast cells synthesize and secrete platelet-derived growth factor.

Signal transduction cascades: The response to inducers

- Four major families of the paracrine factors
- 1. The fibroblast growth factor (FGF) family
- 2. The Hedgehog family
- 3. The Wnt family
- 4. The TGF-□ superfamily, encompassing the TGF-□ family, the activin family, the bone morphogenetic proteins (BMPs), the Nodal proteins, the Vgl family, and several other related proteins.

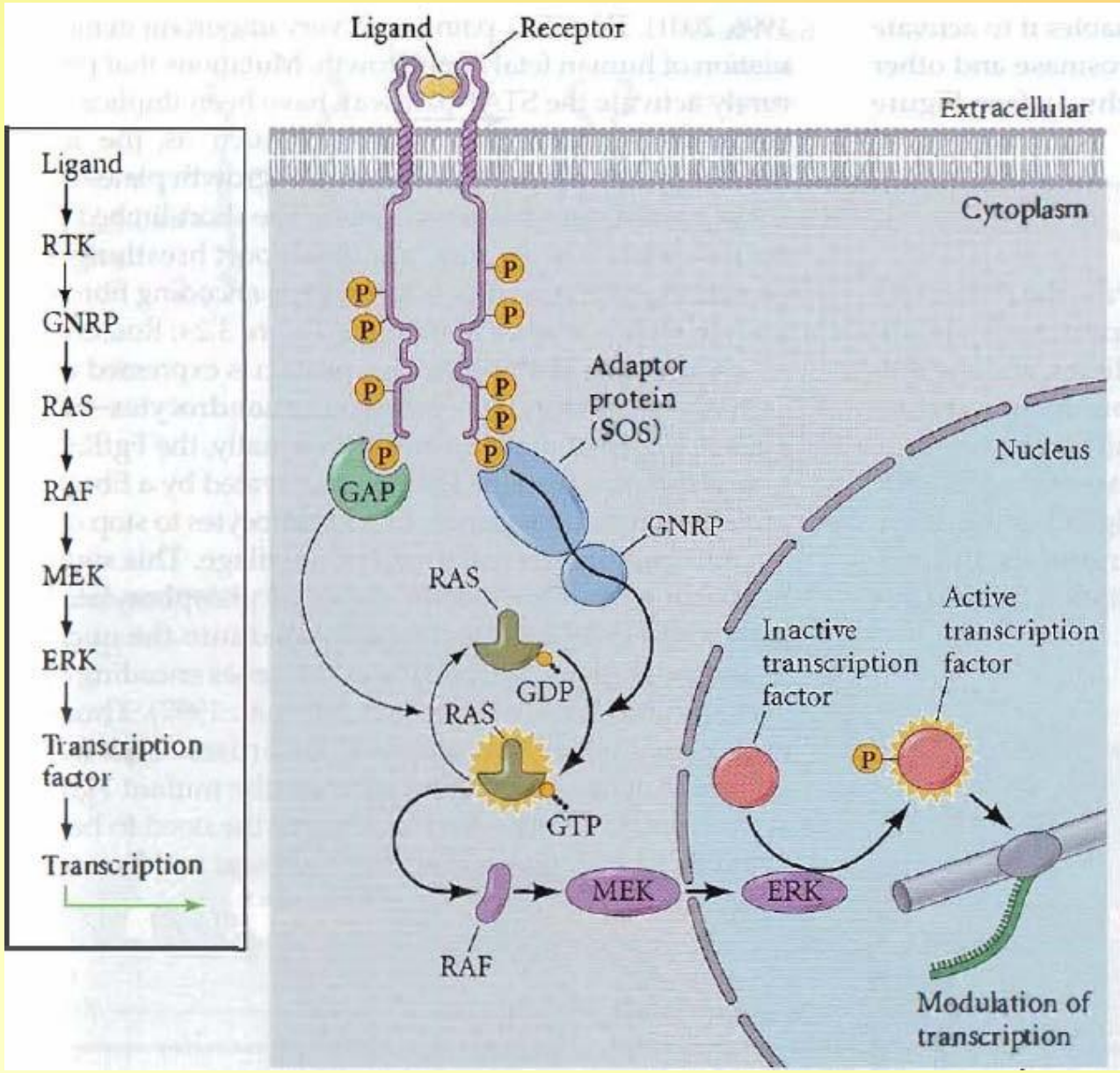
Structure and function of a receptor tyrosine kinase



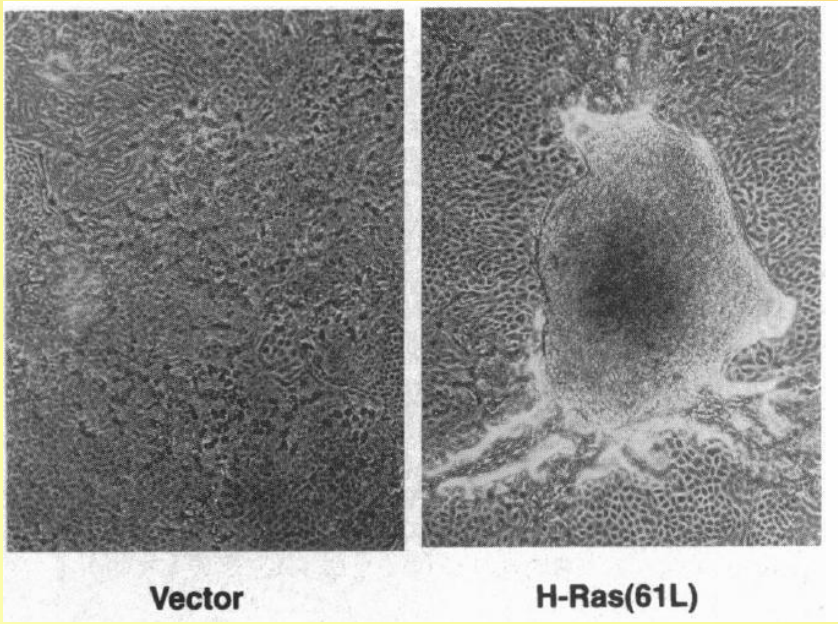
Fibroblast growth factors and the RTK pathway

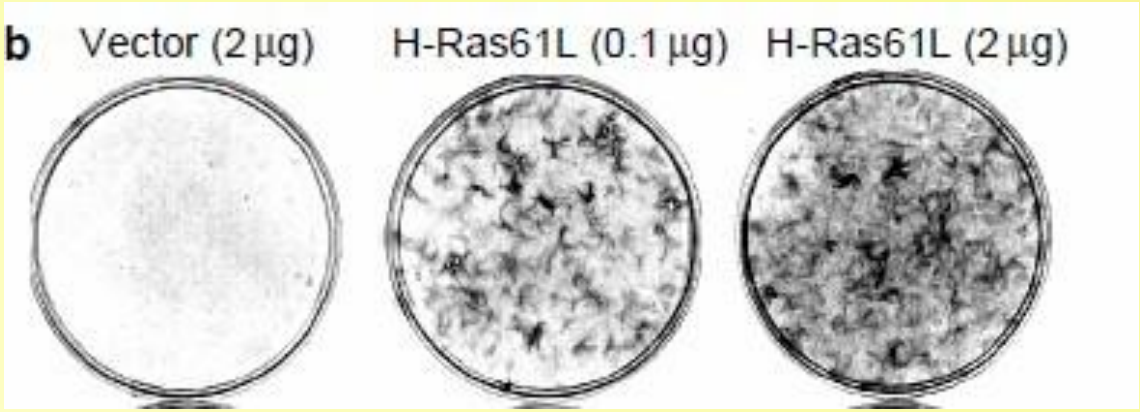
- The fibroblast growth factor (FGF) family of paracrine factors comprises nearly two dozen structurally related members
- FGFs often work by activating a set of receptor tyrosine kinases called the fibroblast growth factor receptors (FGFRs).

The RTK signal transduction pathway

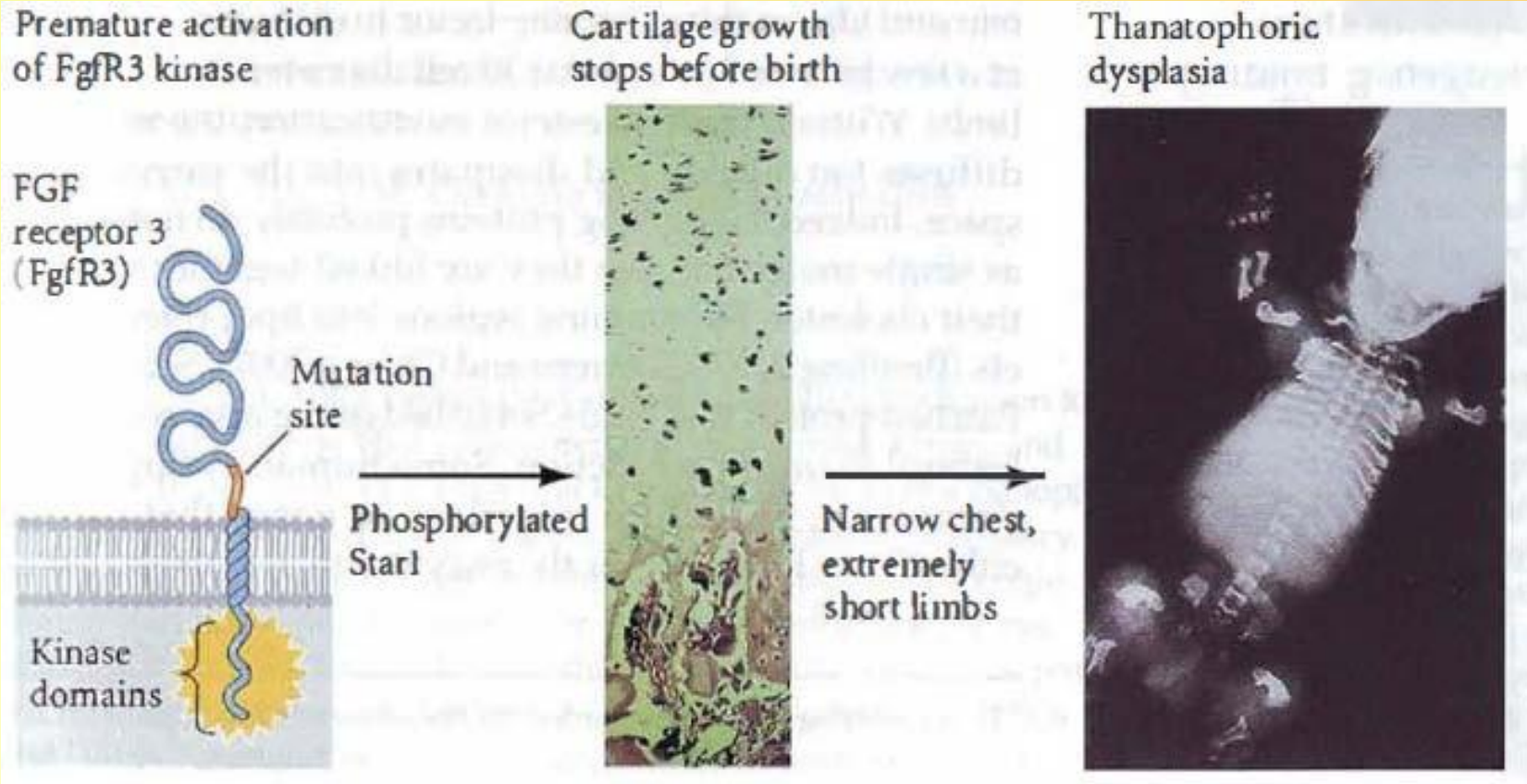


Ras transformed cells

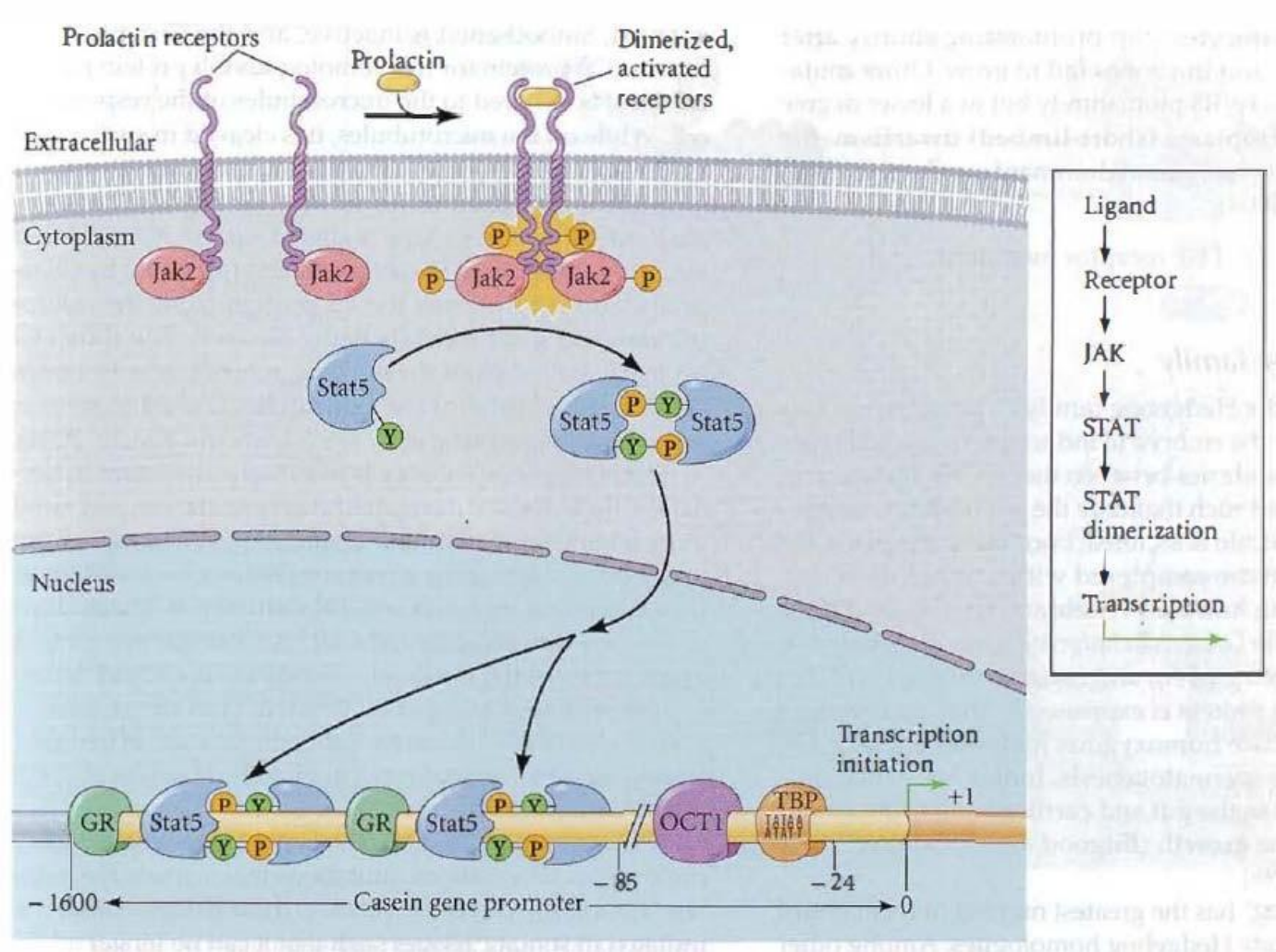




Thanatophoric dysplasia



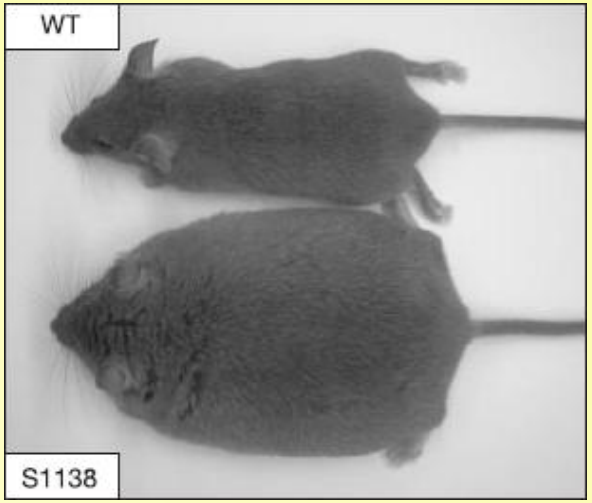
The JAK-STAT pathway

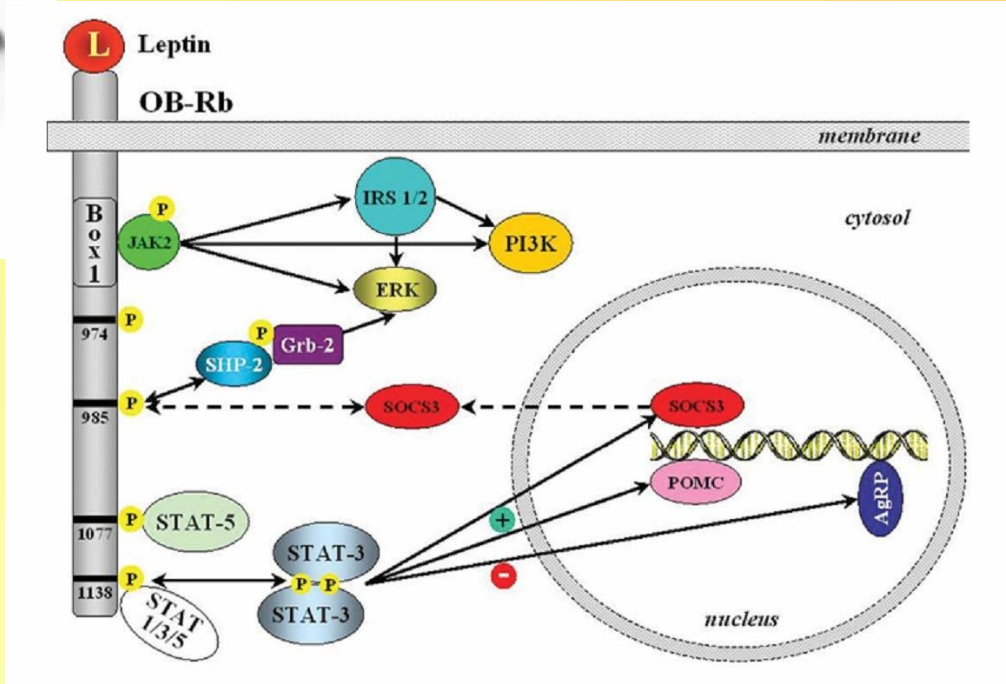


JAK: Janus kinase

STAT: Signal transducers and activators of transcription

Ob/ob mouse

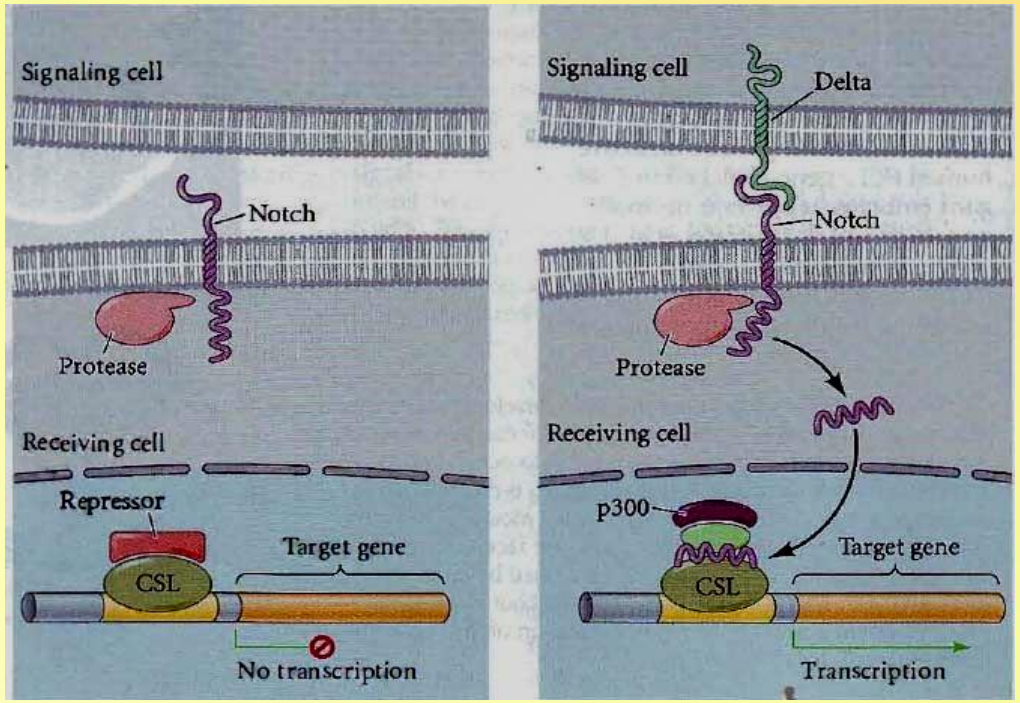

WT
S1138


L
Leptin
OB-Rb
membrane
cytosol
Box 1
JAK2
IRS 1/2
PI3K
ERK
Grb-2
SHP-2
974
985
1077
1138
SOCS3
SOCS3
POMC
AgRP
STAT-5
STAT-3
STAT-3
STAT 1/3/5
nucleus

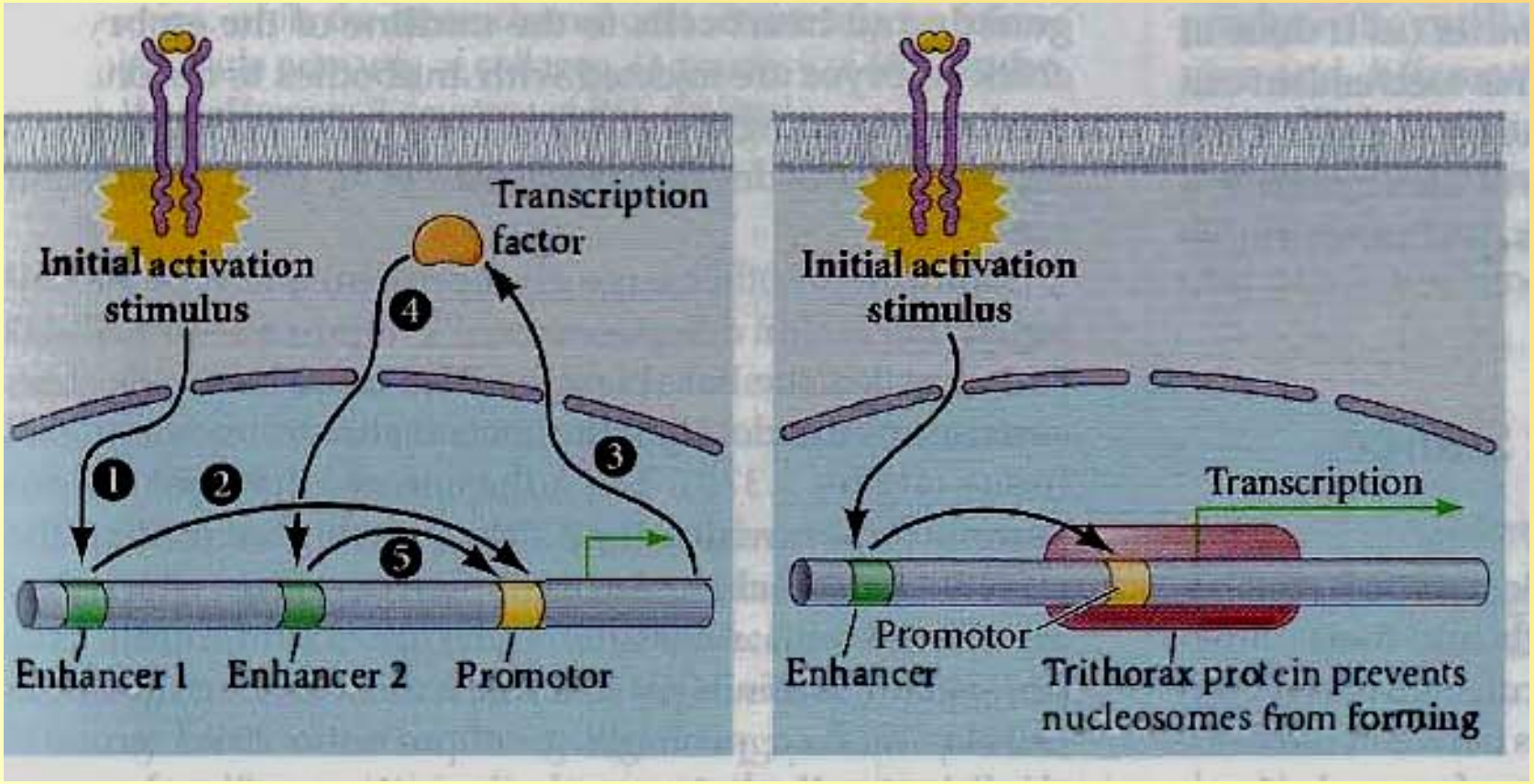
Juxtacrine Signaling

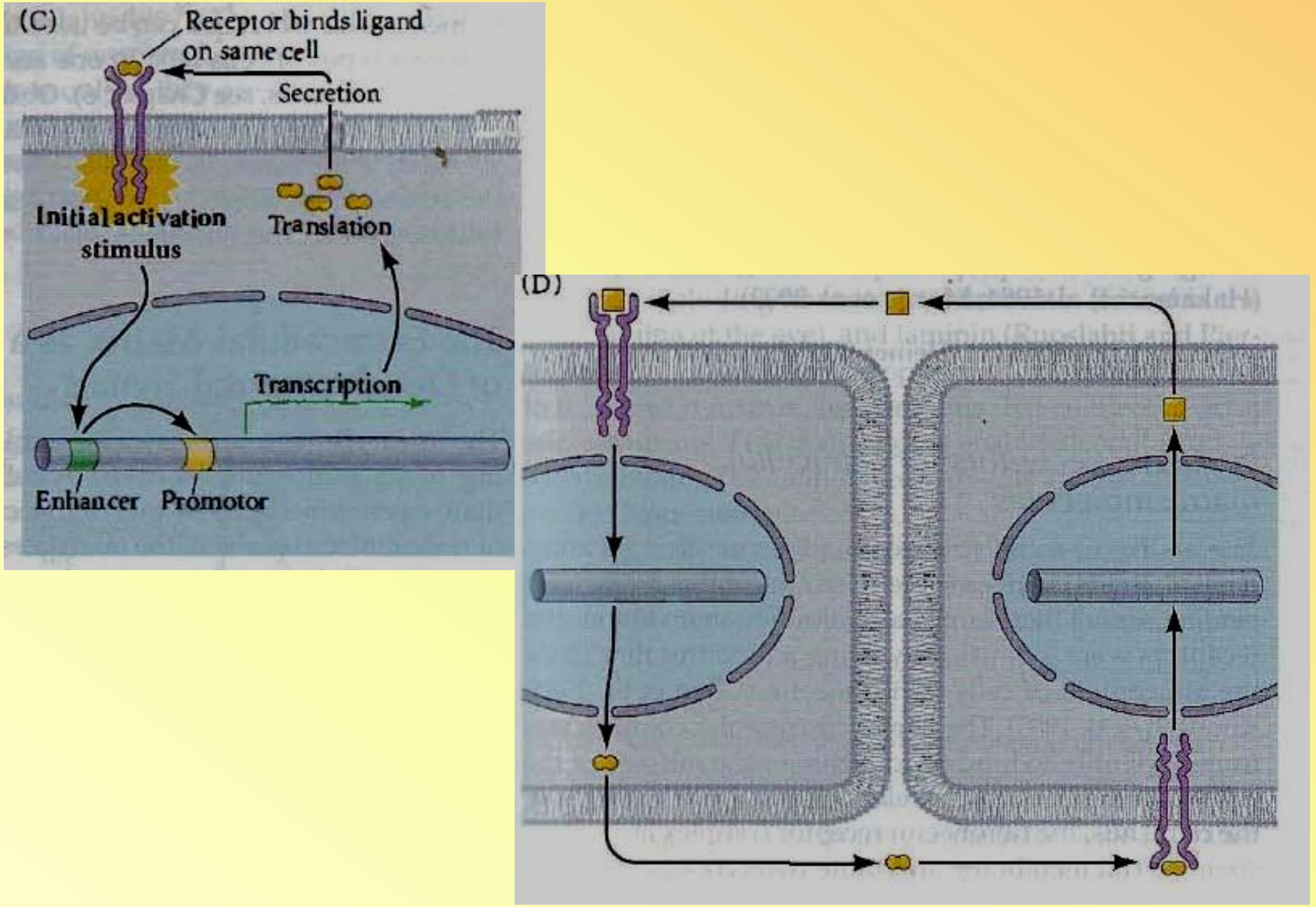
- Proteins from the inducing cell interact with receptor proteins of adjacent responding cells without diffusing from the cell producing it

The Notch pathway: juxtaposed ligands and receptors



Maintaining the Differentiated State




(C)
Receptor binds ligand on same cell
Secretion
Initial activation stimulus
Translation
Transcription
Enhancer
Promotor
(D)

The Extracellular Matrix as a Source of Developmental Signals

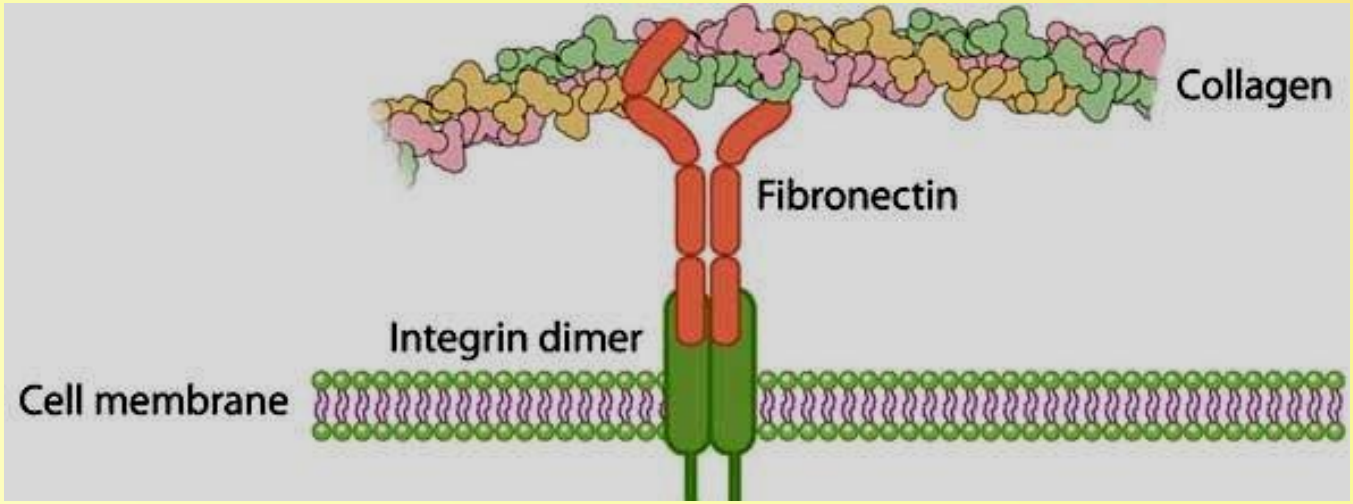
- Extracellular matrix is an insoluble network consisting of macromolecules secreted by cells into their immediate environment.
- Cell adhesion, cell migration, and the formation of epithelial sheets and tubes all depend on the ability of cells to form attachments to extracellular matrices.

Extracellular matrix

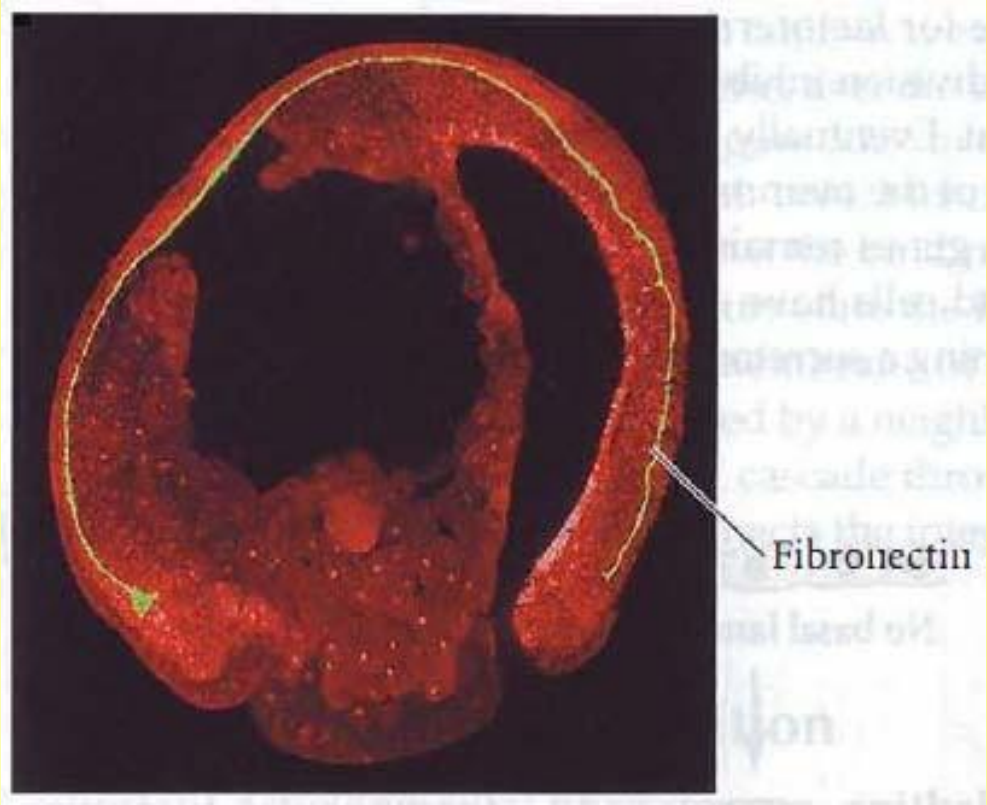
- Extracellular matrices are made up of collagen, proteoglycans, and a variety of specialized glycoprotein molecules such as fibronectin and laminin.
- Proteoglycans: Consist of core proteins (such as syndecan) with covalently attached glycosaminoglycan polysaccharide side chains.

Fibronectin

- A very large (460 kDa) glycoprotein dimer.
- Serve as a general adhesive molecule, linking cells to one another and to other substrates.



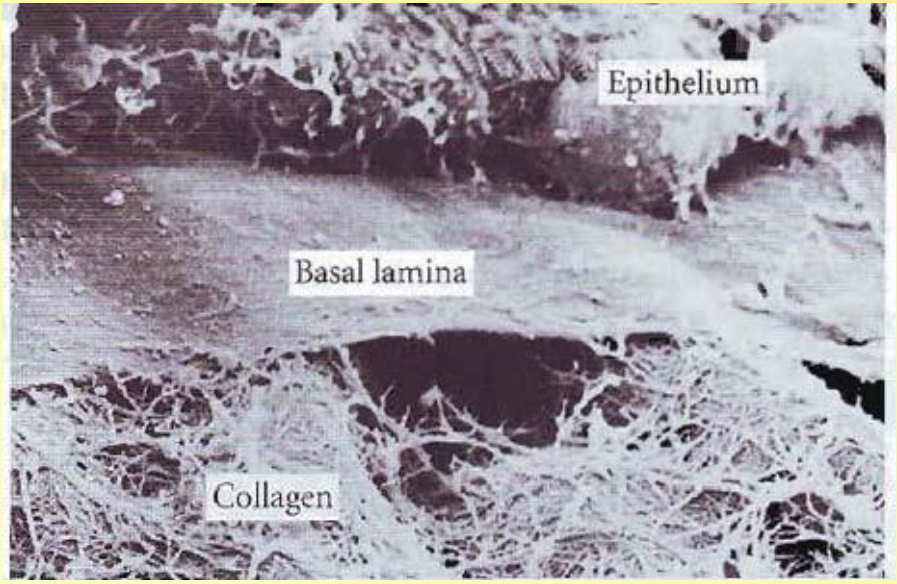
Orient the movements of the mesoderm cells



Fibronectin deposition as a green band in the Xenopus embryo during gastrulation.

Basal lamina

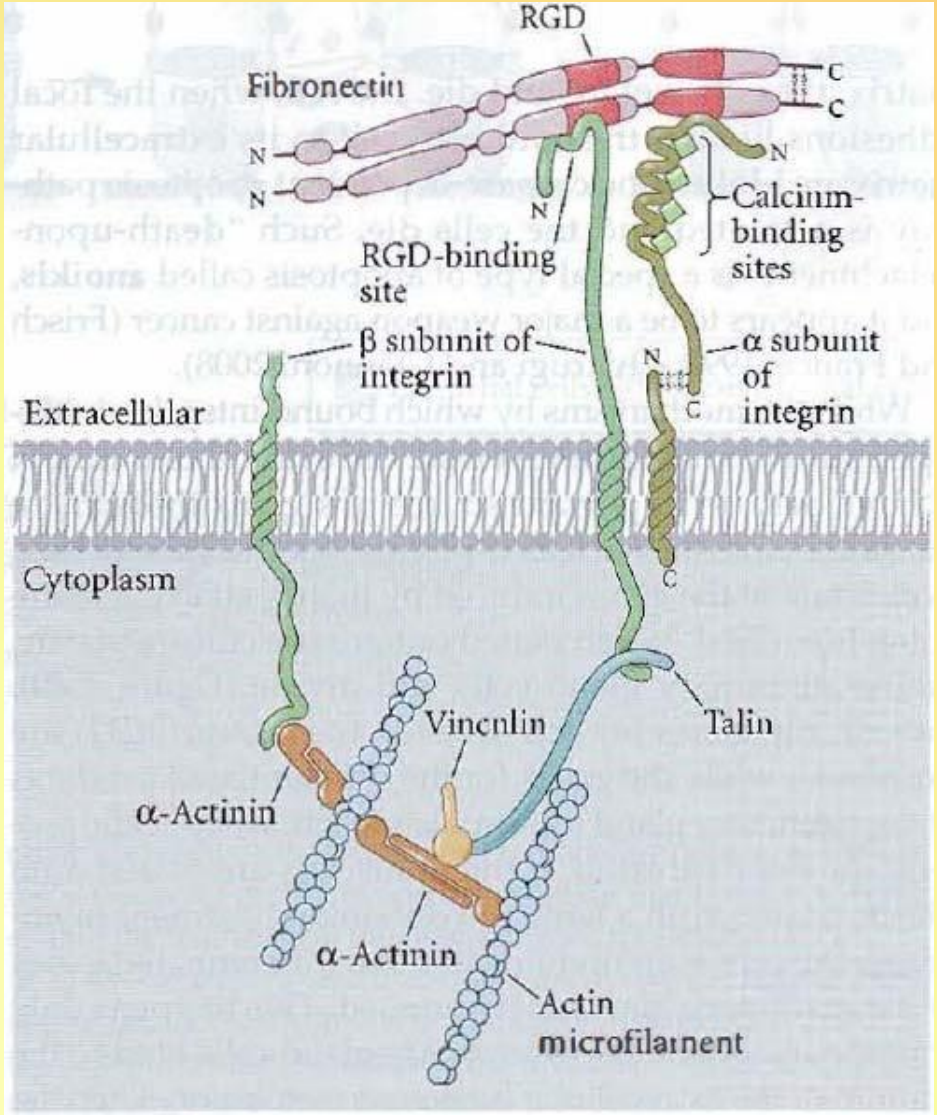
- Laminin and type IV collagen are major components of the basal lamina.
- The basal lamina is characteristic of the closely knit sheets that surround epithelial tissue.



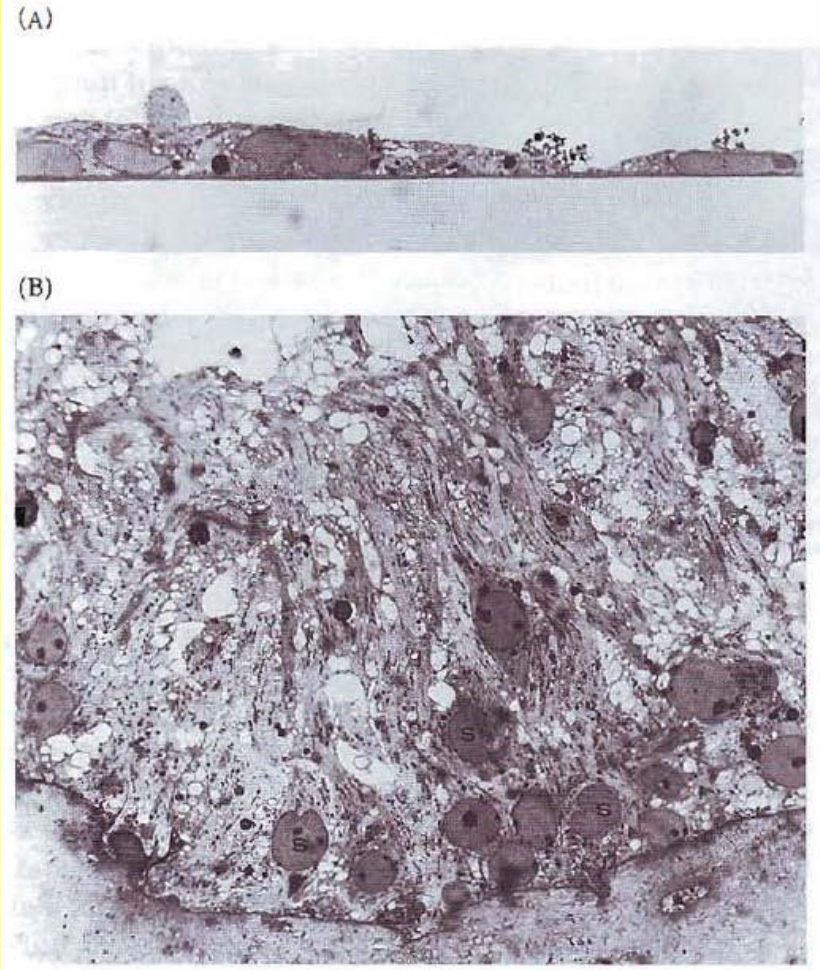
Integrins: Receptors for extracellular matrix molecules

- Integrins: they integrate the extracellular and intracellular scaffolds, allowing them to work together.

- The fibronectin receptor complex bind not only to fibronectin, but also to cytoskeletal proteins on the inside of the cell.



Role of the extracellular matrix in cell differentiation



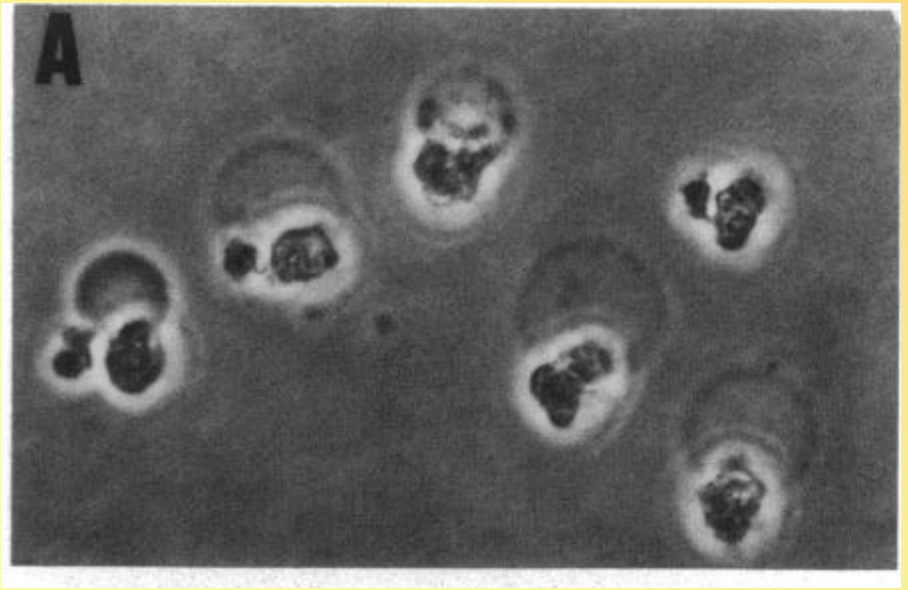
Rat testis Sertoli cells

on tissue culture plastic dishes

on dishes coated with basal lamina

Anoikis

- Chondrocytes can survive and differentiate only if they are surrounded by an extracellular matrix.
- "death-upon-detachment"



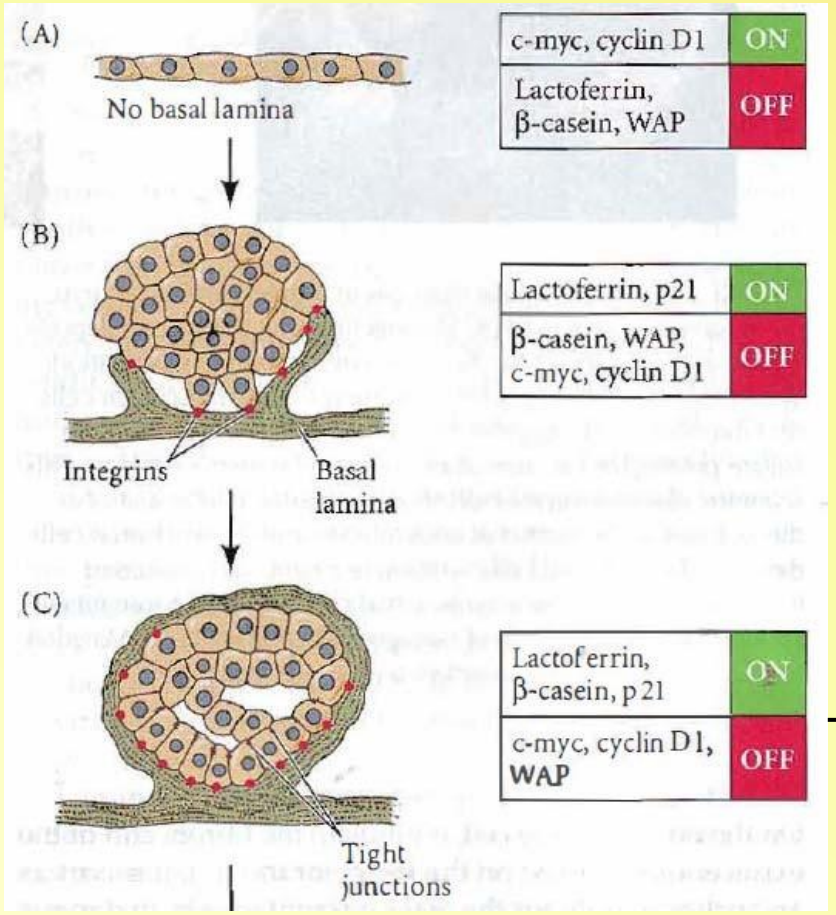
Molecular Biology of the Cell
Vol. 4, 953–961, September 1993

The Extracellular Matrix as a Cell Survival Factor

Jere E. Meredith, Jr., Babak Fazeli, and Martin A. Schwartz*

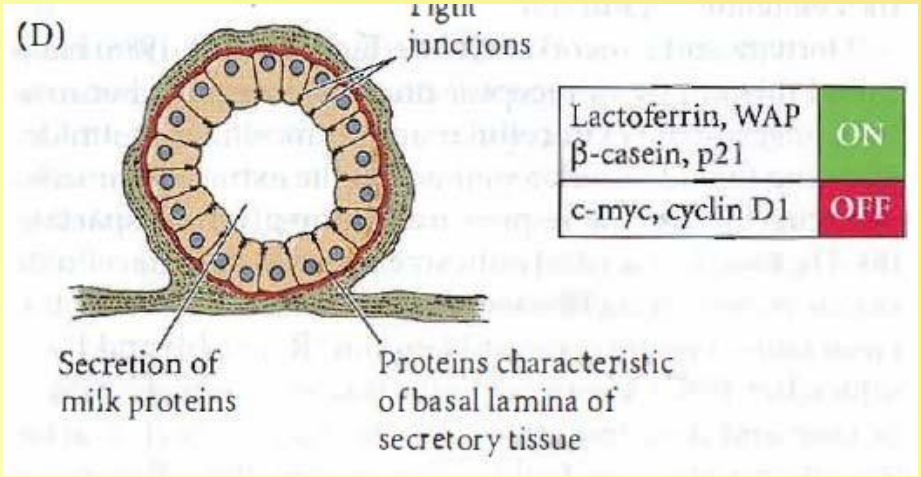
Basement membrane-directed gene expression in mammary gland tissue

Mouse mammary gland tissue



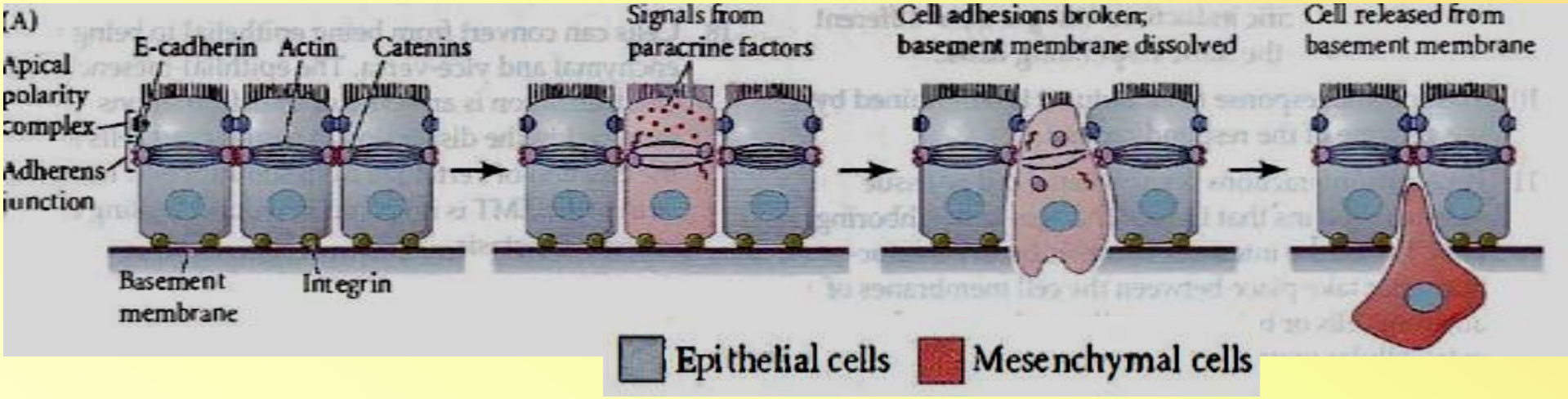
Divides on tissue culture plastic

Differentiates on laminin-containing basement membrane



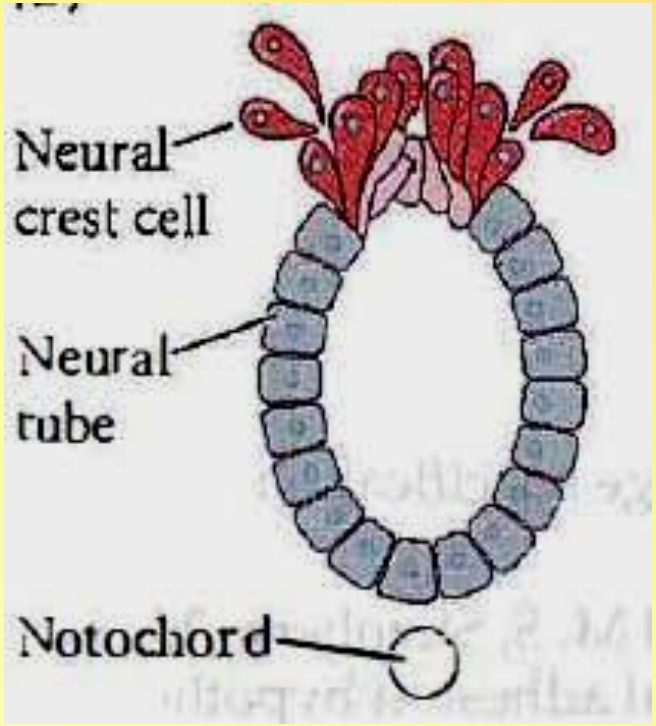
Epithelial-Mesenchymal Transition

- A polarized stationary epithelial cell, which normally interacts with basement membrane through its basal surface, becomes a migratory mesenchymal cell that can invade tissues and form organs in new places.



EMT is critical during development

The most critical adult form of EMT is seen in cancer metastasis



Summary: Cell-Cell Communication

- The sorting out of one cell type from another results from differences in the cell membrane.
- The membrane structures responsible for cell sorting out are often cadherin proteins that change the surface properties of the cells.
- Migration occurs through changes in the actin cytoskeleton.
- Inductive interactions involve inducing and responding tissues.
- The ability to respond to inductive signals depends on the competence of the responding cells.

Summary: Cell-Cell Communication

- Reciprocal induction occurs when the two interacting tissues are both inducers and are competent to respond to each other's signals
- Regionally specific inductions can generate different structures from the same responding tissue
- The specific response to an inducer is determined by the genome of the responding tissue.
- Paracrine interactions and Juxtacrine interactions

Summary: Cell-Cell Communication

- Paracrine factors are proteins secreted by inducing cells. These factors bind to cell membrane receptors in competent responding cells.
- Competent cells respond to paracrine factors through signal transduction pathways.
- The extracellular matrix is a source of signals for the differentiating cells and plays critical roles in cell migration.
- Cells can convert from being epithelial to being mesenchymal.

For further study follow the pdf note produced from GILBART along with this ppt.