Sviluppo, regolazione e morfogenesi

1) Lo sviluppo evoluzionario di una struttura o parte di un organismo
2) Lo sviluppo embrionico di una struttura o parte di un organismo
3) Il processo che avviene in un complesso sistema-ambiente che elabora la sua forma o struttura. Esempi: crescita , appprendimento e sviluppo di una societa'.

Un sistema morfogentico e' capace di mantenere la sua continuita' e integrita' cambiando aspetti essenziali della sua struttura o organizzazione.

Regolazione (regulation). Uno delle proprieta piu importanti e elusivi dei organismi viventi è la capacità di regolare forma e grandezza. (ad esempio la testa cresce di meno, le orecchie raggiungono la loro grandezza finale a 5-6 anni).

Alcuni aspetti cellulari della morfogenesi sono stati elucidati, e aspetti molecolari sono l'argomento di studio. Quello che manca e' una visione globale, che unisce il molecolare con il macro molecolare. Abbiamo le risposte a "come?", ma non a "perche?"

Development: understanding what happens will lead to new methods of regeneration, and give clues on how to engineer new tissues in the lab.

- Growth : increase of mass
- Morphogenesis: they move and rearrange
- Differentiation: emergent parts become different

FORM and FUNCTION

Differenze tra la morfogenesi e l'ingegneria tessutale

Morfogenesi	**Ingegneria Tessutale**
Utilizzo di cellule staminali	Cellule adulte o staminali
Le cellule producono il materiale extra-cellulare	Di solito viene introdotto uno scaffold
Il tessuto e l'ambiente vengono modulati in tempo reale dalla struttura stessa	L'architettura di una scaffold è pre-programmata
Crescita per aumento di massa da un punto sorgente	Crescita per demolizione progressiva dello scaffold e contemporanea produzione di EMC dalle cellule
La morfogenesi è dovuta a segnali interni alle cellule	La forma viene dettata dalla geometria dello scaffold

Fertilization begins when a sperm penetrates an oocyte (an egg) and it ends with the creation of the zygote.

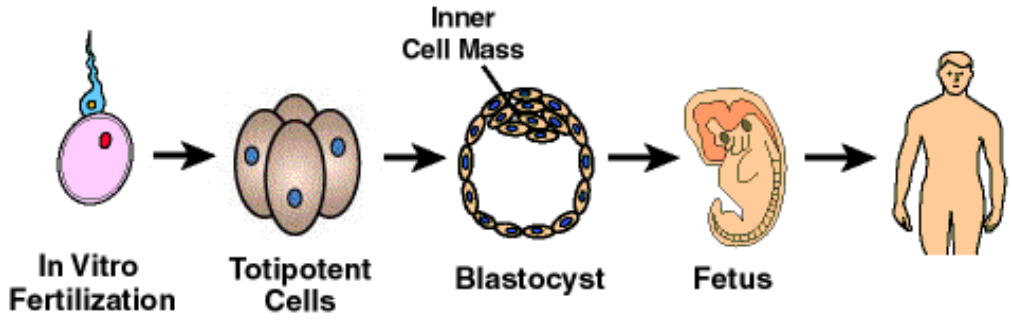
The early process of cell division is **Cleavage**

The First Cell Divisions produce Blastomeres, undifferentiated cells, each one is totipotent

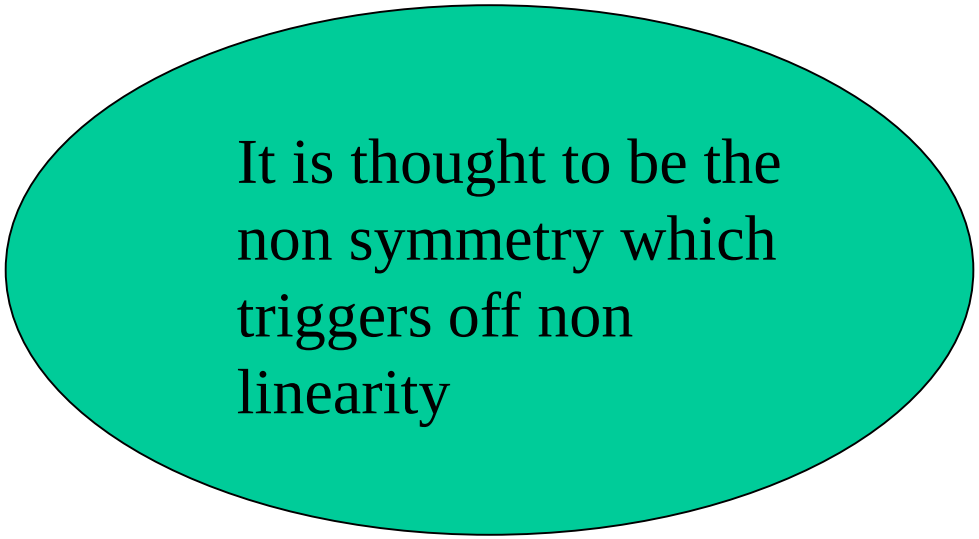
After about sixteen cells (and how many divisions?), the zygote becomes a morula (mulberry shaped) .

At this stage it is called a blastocyst.This is the first stage of morphogenesis.

The presence of the blastocyst indicates that two cell types are forming: the embryoblast (inner cell mass on the inside of the blastocele (inner cavity), and the trophoblast (the cells on the outside of the blastocele)



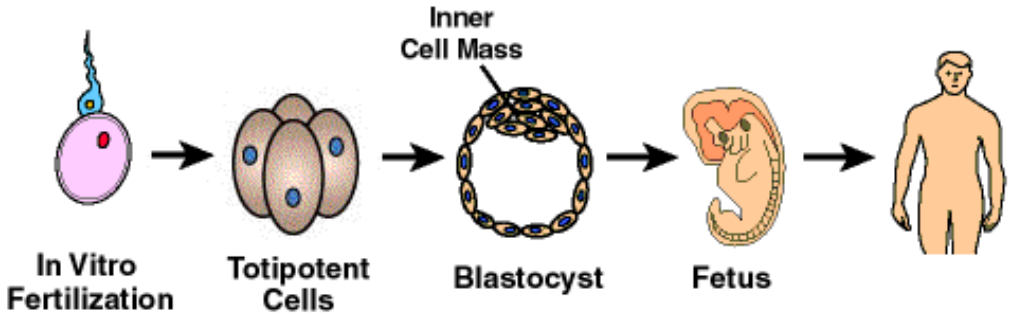
Zygote: not symmetrical (why?) - POLARITY.
Almost all have an ANIMAL pole (nr nucleus) and a VEGETAL pole (far from nucleus)



The trophoblast forms the placenta

The inner cell mass or embryoblast: the fetus

The blastocyst is pluripotent, or what we call **embryonic stem cells. (see stem cells)**



What's happening? The fertile egg is symmetrical. Each step leads to a greater level of asymmetry.

This is what we call morphogenesis.

The development of form and function.

The next step is called Gastrulation : a crucial time in the development of multicellular animals.

"It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life."

Lewis Wolpert (1986)

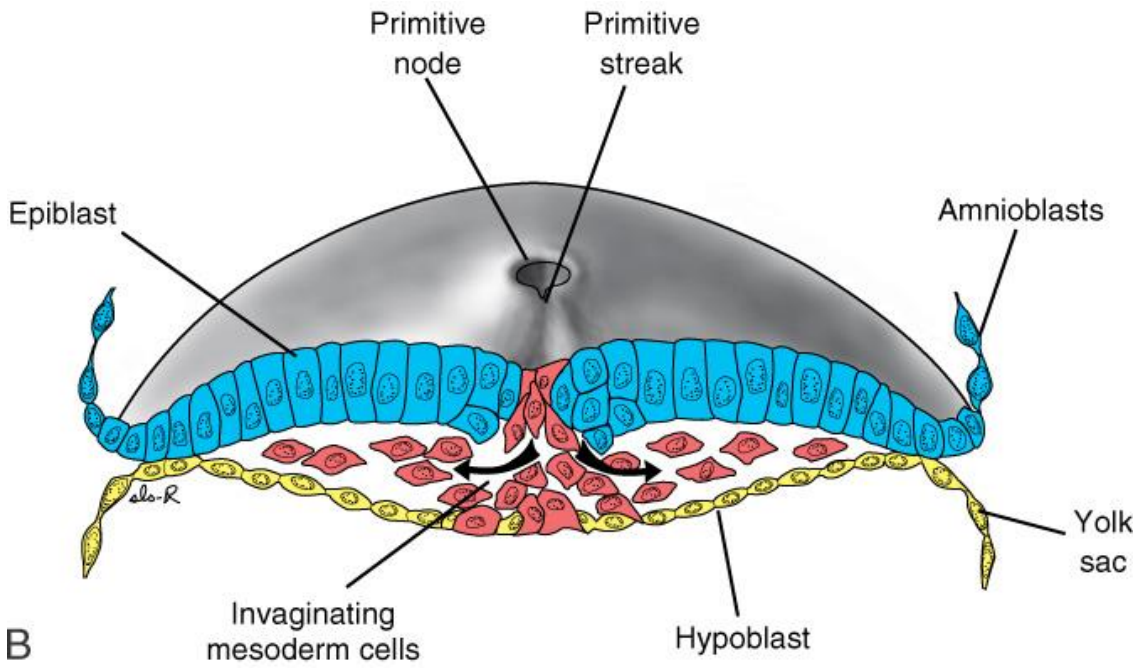
During gastrulation, the 3 germ layers of an embryo are formed and the body plan of the mature organism is established. Movements on a massive scale allow cells to establish great complexity from a very simple starting form .

1. The three primary germ layers (ectoderm, mesoderm & endoderm) are established.
2. The basic body plan is established, including the physical construction of the rudimentary primary body axes.
3. As a result of the movements of gastrulation, cells are brought into new positions, allowing them to interact with cells that were initially not near them.

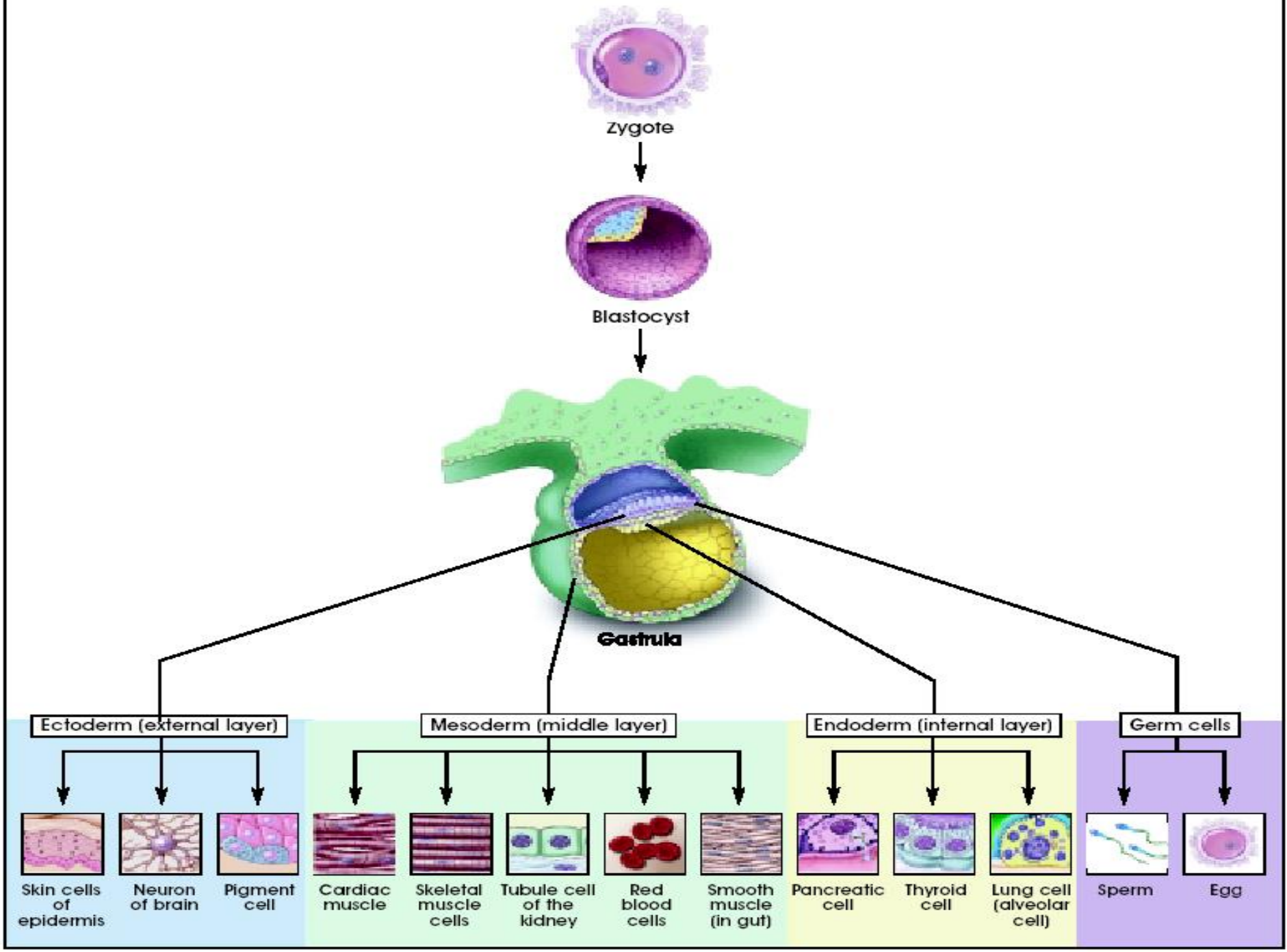
Endoderm: the most internal germ layer, forms the lining of the gut and other internal organs.
Ectoderm, the most exterior germ layer,forms skin, brain, the nervous system, and other external tissues.
Mesoderm, the the middle germ layer, forms muscle, the skeletal system, and the circulatory system.

There are two major types of cell arrangements in the embryo: **epithelial cells**, which are tightly connected to one another in sheets or tubes, and **mesenchymal cells**, which are unconnected to one another and which operate as independent units. **Morphogenesis is brought about through a limited repertoire of variations in cellular processes within these two types of arrangements: (1) the direction and number of cell divisions; (2) cell shape changes; (3) cell movement; (4) cell growth; (5) cell death; and (6) changes in the composition of the cell membrane or secreted products**

How it starts: at around 9 days, the inner cell mass (or embryoblast) divides into 2 layers, epiblast and hypoblast. The epiblasts migrate and invaginate to form an intermediate layer (later the mesoderm) . This process is correlated with changes in cell-cell adhesion through the *downregulation of cadherins and IgCAMs.*



So, even at 9 days, cell cell interaction is critical.



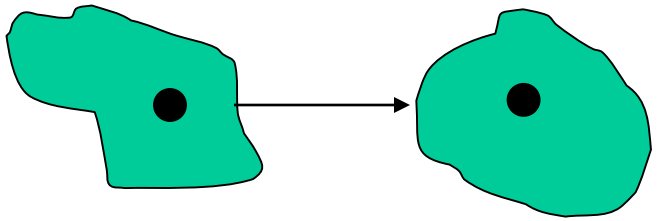
Gastrulation paves the way for inductive interactions, which are essential for neurulation and organogenesis.

organs

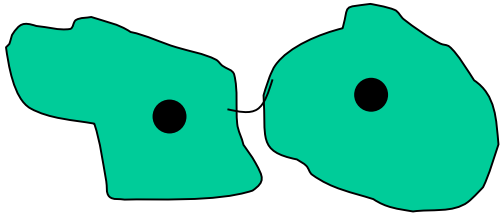
Neurulation : neural tube: Central Nervous System
Neural crest- migrates away forming different cell types
Epidermis covering neural tube

Control

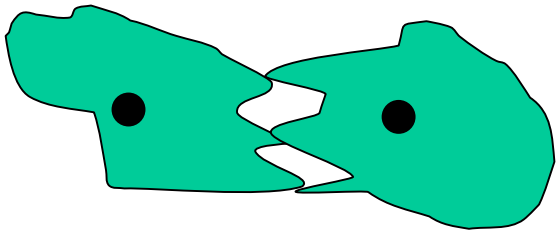
Induction: reorganisation of cells through interaction with other neighbouring cells



Diffusion of soluble agents



Contact through ECM



Contact through membrane receptors

Riassumendo, i processi principali sono

- Fertilizzazione
- Cleavage
- Gastrulation
- Organogenesi e neurogenesi
- Istogenesi

Come sa la cellula cosa diventare e dove andare?

I meccanismi di controllo

- Asimmetria
- Adesione e Coesione differenziale
- Informazione posizionale
- Campi embrionici/morfogenetici
- Induzione

I vincoli nella morfogenesi

- Partecipano da 10 a 1000 cellule
- Le dimensioni sono meno di qualche mm
- I tempi sono del ordine di diverse ore o giorni (tempi di diffusione, movimento e differenzazione cellulare)
- L’energia disponibile è ?

How do structures know where to form?

They require positional information.

In embryonic fields, cell fate often depends on the position of a cell within that field

How does a cell receive positional information?

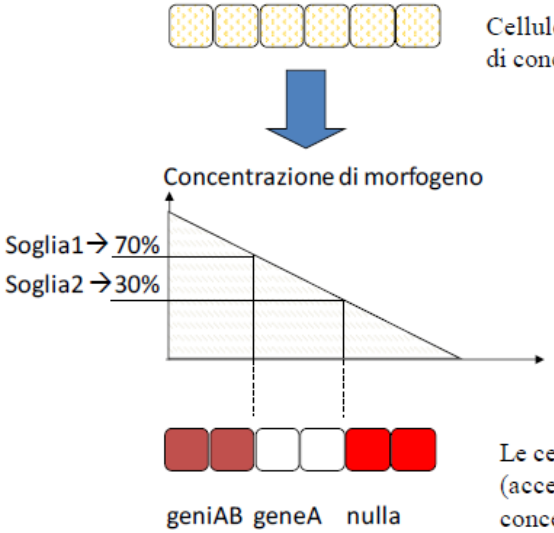
Wolpert proposed that pattern formation is a two-step process

- 1st, cells in a field receive positional values
- 2nd, cell interpret the positional information based on their genetic information and developmental history

- positional value = signals they receive from elsewhere

- interpretation = conversion of these signals into gene activity

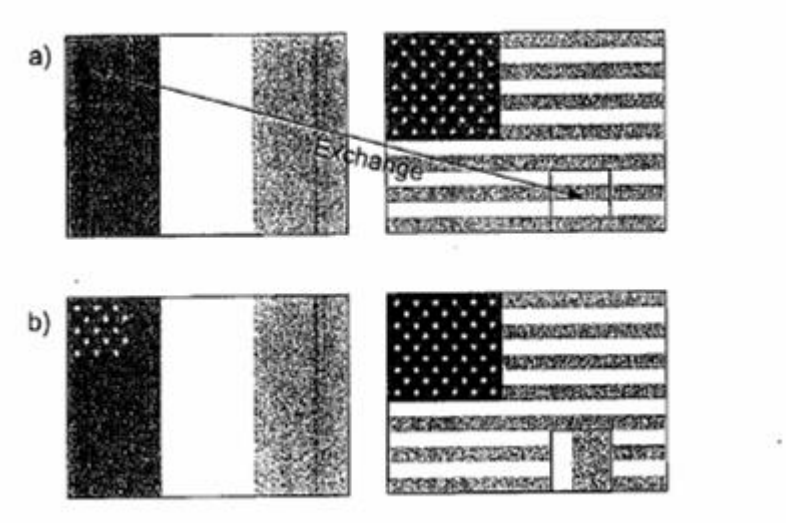
French Flag Hypothesis(Lewis Wolpert)



Cellule equipotenti sottoposte ad un gradiente di concentrazione di morfogeno

Le cellule aquisiscono un valore posizionale (accensione di particolari geni…) legato alla concentrazione. Esse differenzieranno in tipi differenti rappresentati dai colori della bandiera.
RISPOSTA A SOGLIA

Control : positional information



Questo tipo di esperimento fa vedere che l'informazione è contenuto nelle cellule ma viene anche da cellule intorno.
Es. Si trapianta la zona embrionale della coscia del drosofila sulla zona terminale dell' ala. Cosa forma il trapianto?

Morphogen and morphogen gradients

A morphogen: a chemical that propagates from one point of the embryo and acts on the cells in a concentration-dependent manner. It can trigger several different developmental pathways depending on its local concentration

Several morphogentic proteins have been identified in vertebrates (activin, BMP (bone morphogenic protein) , Sonic Hedgehog.

- long-ranges signals that specify positional value

Patterning depends on cellular interactions

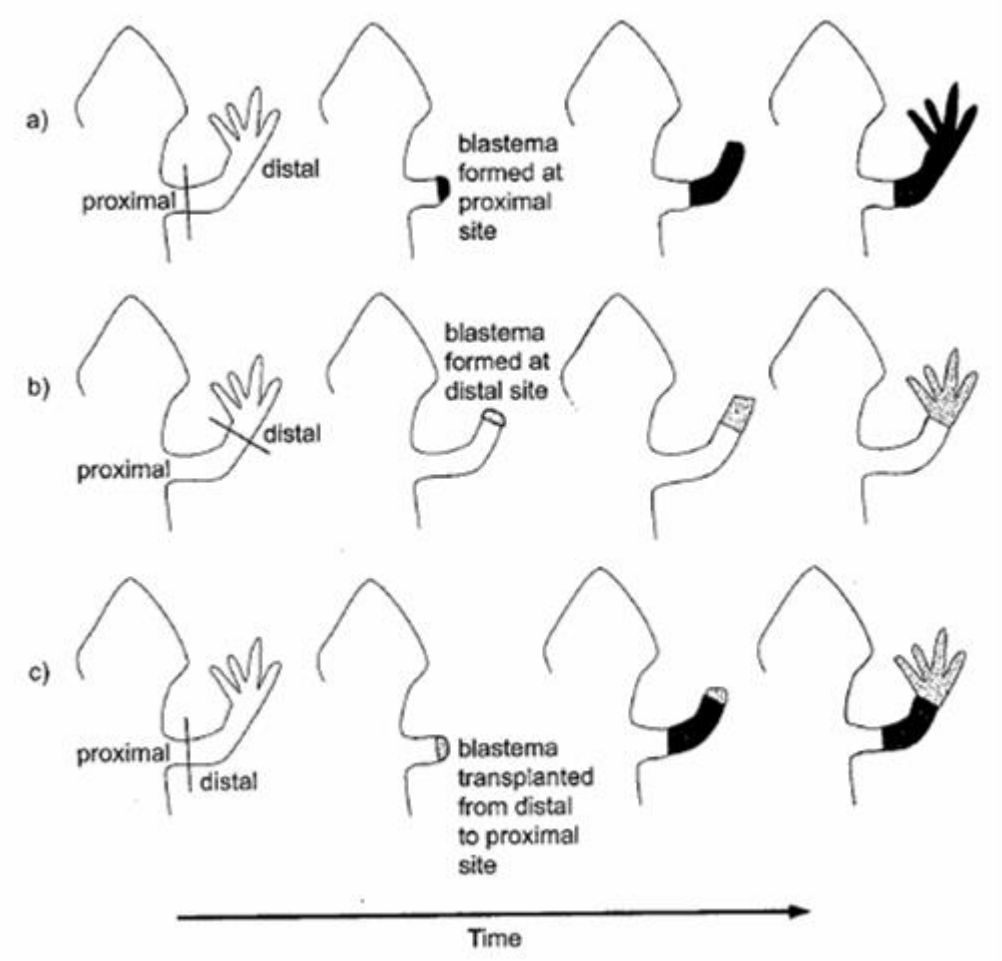
- classic studies demonstrated two forms of determination

a. Dependent determination

- surrounding tissue induces (regulates) the grafted tissue to form the structure of the specified location
- Example: exchange future belly epidermis with future mouth epidermis at early gastrula, each develops into the appropriate structure for the location

b. **Self determination**

- dorsal lip does not get reprogrammed by the local environment
- it reprograms the local environment to form a second embryonic axis.

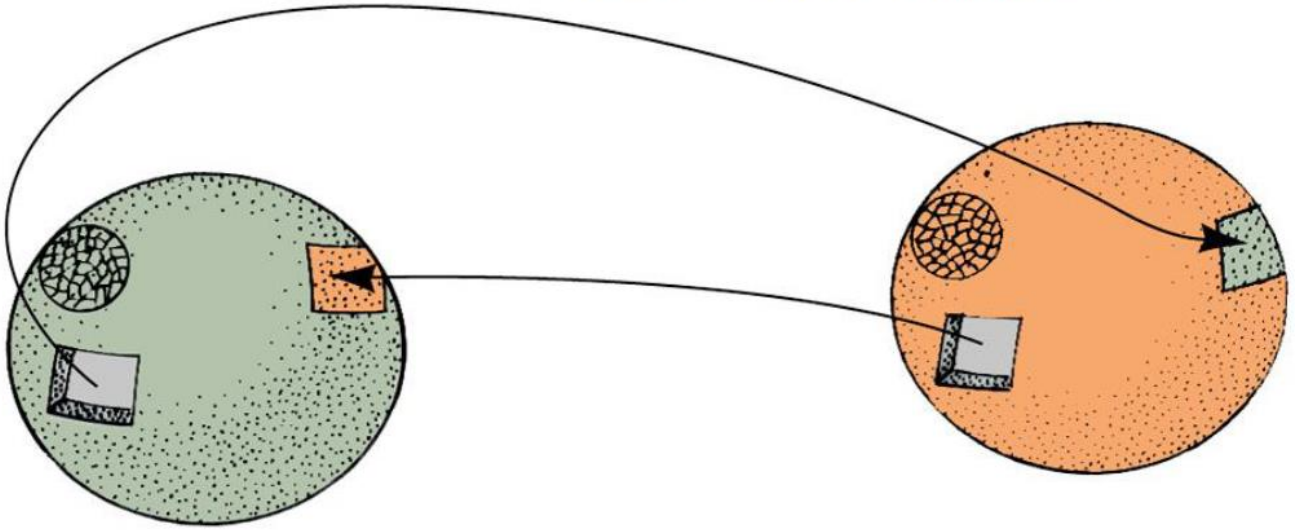


Blastema fate is self determined

Informazione posizionale

Classic experiment of Spemann and Schote (1932)

- exchanged belly epidermis to future mouth regions between frogs and salamanders
  - Salamander (*Triturus*) and frog (*Rana*)
    - belong to different subclasses of amphibians
      - urodeles –tailed amphibians
      - anurans – tailess amphibians



(a)

Donor Species of Prospective Belly Epidermis Graft	Structures Formed after Transplantation to Mouth Region of: Frog	Salamander
Frog	Horned jaw, sucker	Horned jaw, sucker
Salamander	Teeth, balancers	Teeth, balancers

RESULTS:
The grafts underwent dependent determination/differentiation

Conclusions from Spemann and Schote frog/salamander transplants.

- developing tissues were able to read the inductive signals from distant species, thus forming the correct structures.

- different species must use common signals to form mouth parts, but the type of mouth part formed *depended on the grafts genome*

- thus, the response of the grafted tissue was limited to ***its*** genetic ability

  - either the frog genome did not contain the gene(s) for salamader mouth parts (and/or visa versa)

-In other words, while common genes and common inductive signals are involved, *different species have unique patterns of gene expression*

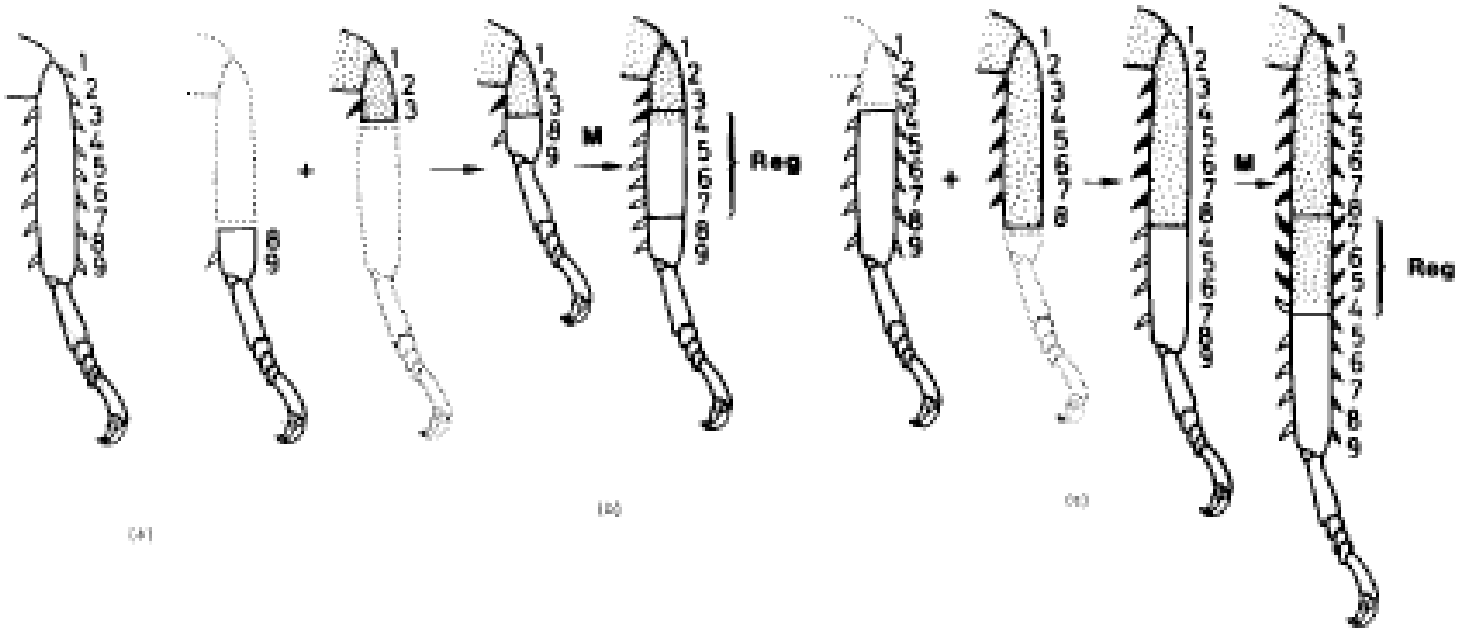


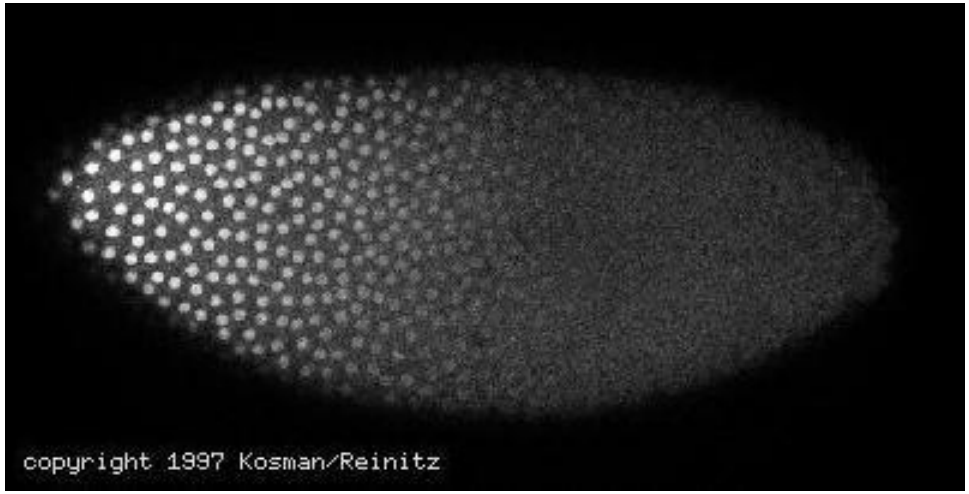
Figure 13.1: Intercalary regeneration in the proximo-distal dimension of cockroach legs (Bohn, 1970a,b, 1971; French, 1976a) (a) The levels of the tibia are denoted (arbitrarily) with 1,2...9. (b) The confrontation of a proximal 123- and a distal 89-piece leads, after one or two molds (M) to the regeneration of the missing elements. By using different species or mutants (stipled, clear) it has been shown that most of the regenerate is derived from the distal elements, indicating a distal to proximal respecification. (c) Surplus structures become duplicated. The regenerate is again derived mostly from those cells at the mismatching junction which carry the distalmost determination. The spines of the regenerate have a reversed orientation, indicating that the sequence of elements determines the polarity of the individual cells and not other way round. These experiments suggest a direct control of neighborhoods and argue against a long ranging positional information.

Morfogeno: molecola capace di indurre il differenziamento

Morfogeni e Controllo

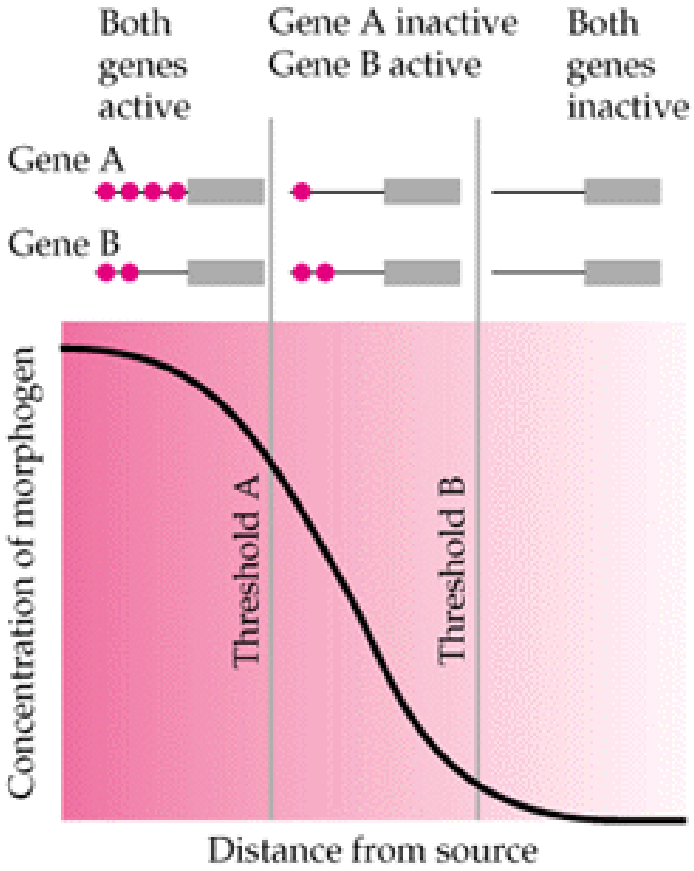
Il meccanismo della morfogenesi è diverso da quello dell'organizzazione iniziale dell'embrione. Formazione di pattern: piu complesso rispetto al sorteggio basato sui CAM

- Si sa che la differenza tra una cellula e l'altra è determinata da **CAM, posizione e gradienti molecolari.**
- Le differenze si evidenziano molto presto (es nel embrione dovute a polarità), e poi grazie ai **gradienti morfogeni** . Un esempio classico nella biologia dello sviluppo è il **gradiente biocoide**

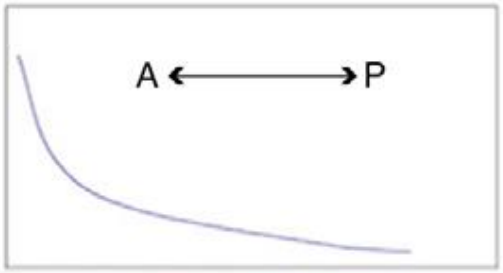


La proteina bicoide è un fattore di trascrizione → regola il livello di espressione di gnei nell'embrione (drosophila) . mRNA bicoide viene prodotto dalla madre, tradotto e poi difonde nel embrione dove regola diversi geni a secondo della sua concentratzione.

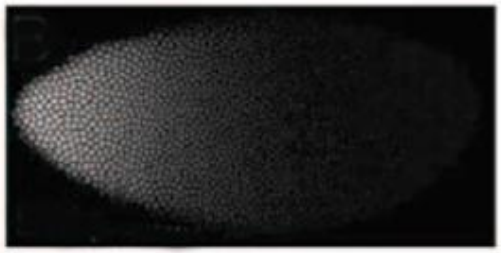
Transcription factor: protein which binds to DNA promotor regions and regulates the transcription of the gene. The affinity of the binding region determines the threshold concentration required for enhancing expression.



B Graded Bicoid and anteroposterior polarity



Bicoid distribution

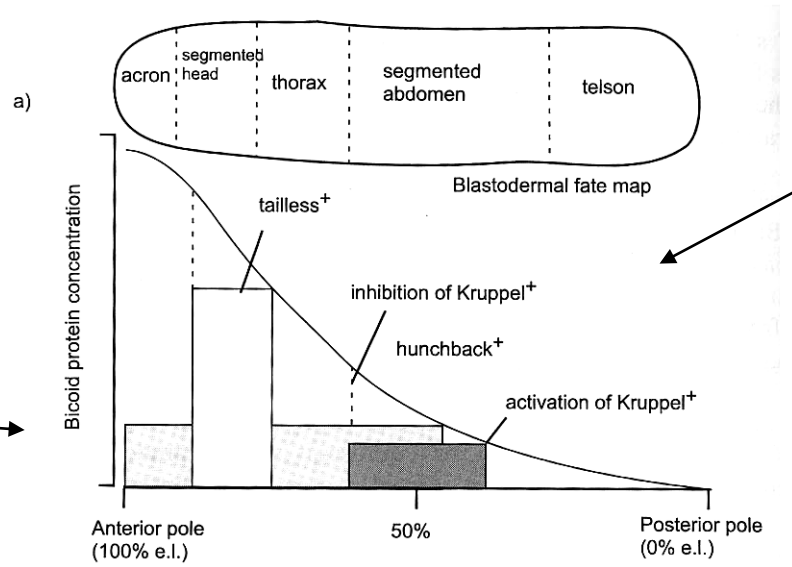


Bicoid protein

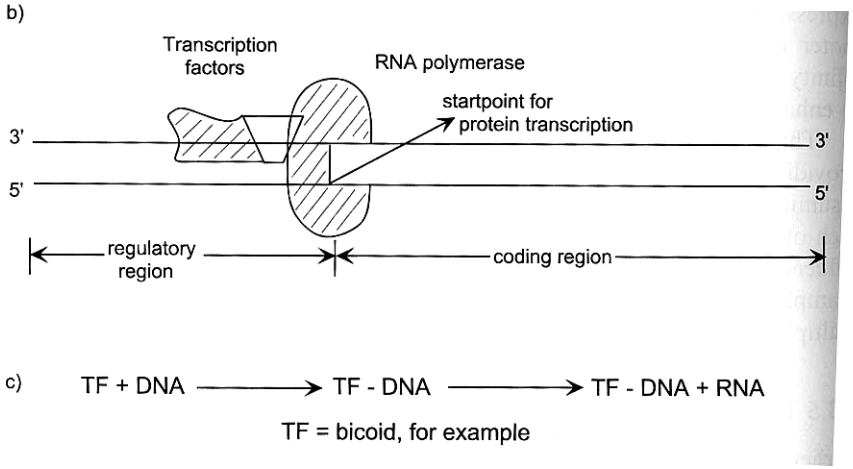
orthodenticle

hunchback

Expression level



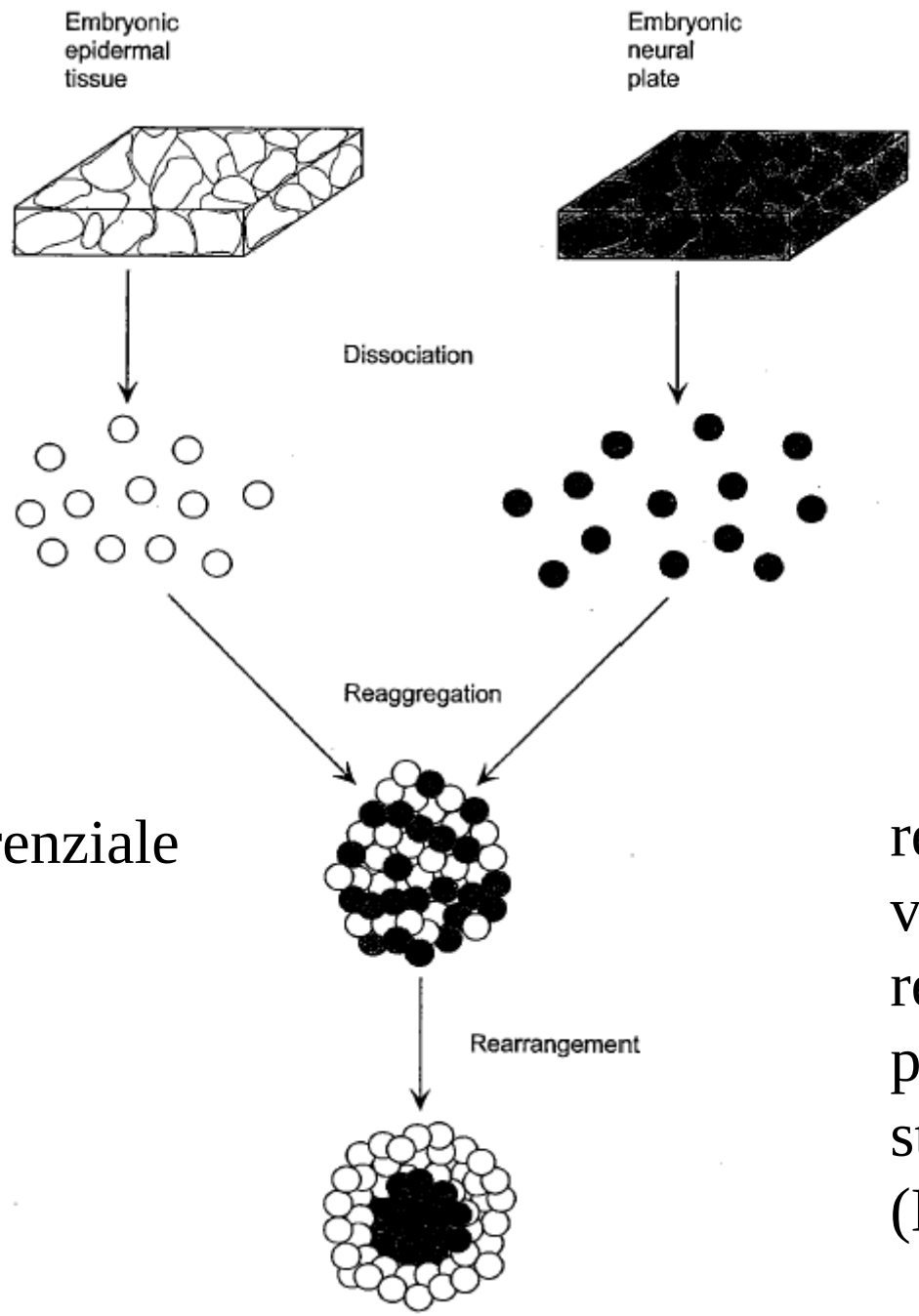
Different genes



Tf binds to DNA and inhibts or facilitates transcription

These are its charateristics: k about 1e-4 s^{-1}
Diffusion constant 1e-7 cm^2/s
Size of embryo:about 1000 micron. Biocoid protein diffuses freely into embryo.

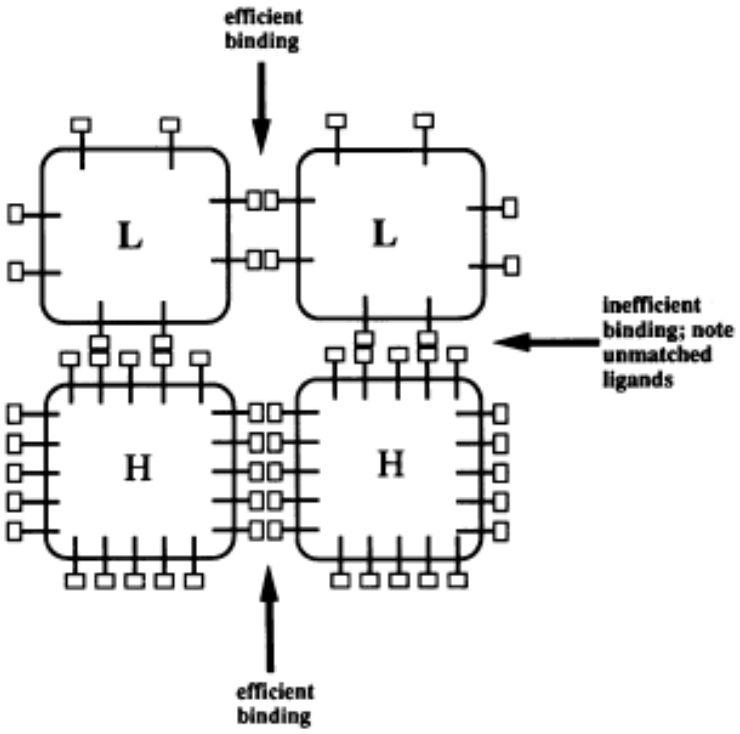
$$\frac{dC}{dt} = D\frac{d^2C}{dx^2} - kC$$

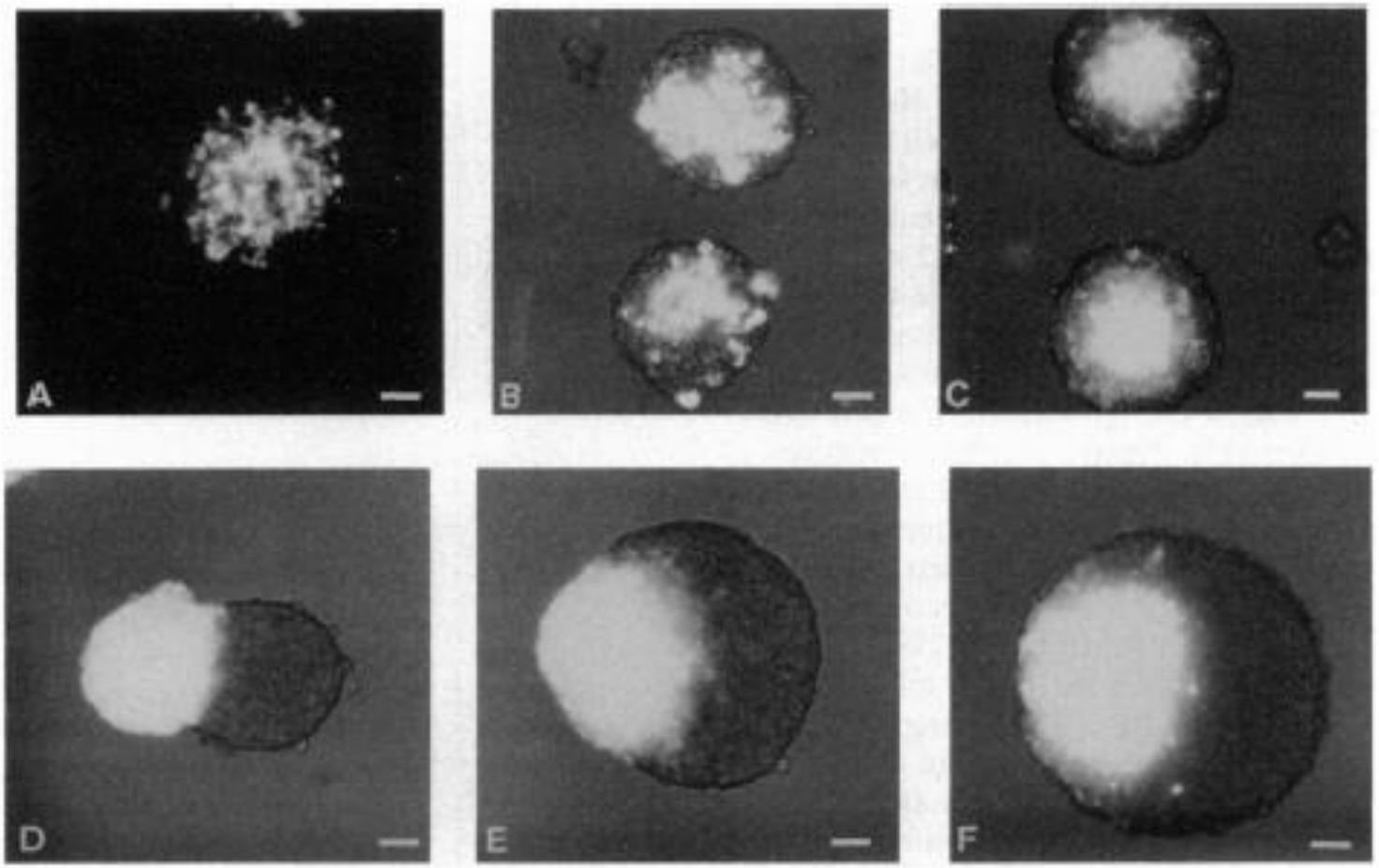


Adesione differenziale

reaggregation in vivo and recreation of primitive structures (Holtfreter)

Steinberg (1963) proposed that cell sorting and movement was simply due to differences in adhesion and cohesion between units. He demonstrated this using thermodynamics.


efficient
binding
L
L
inefficient
binding; note
unmatched
ligands
H
H
efficient
binding



2 cells with different levels of cadherin expression (1:20 expression ratio) . Aggregation and sorting in 4 days.

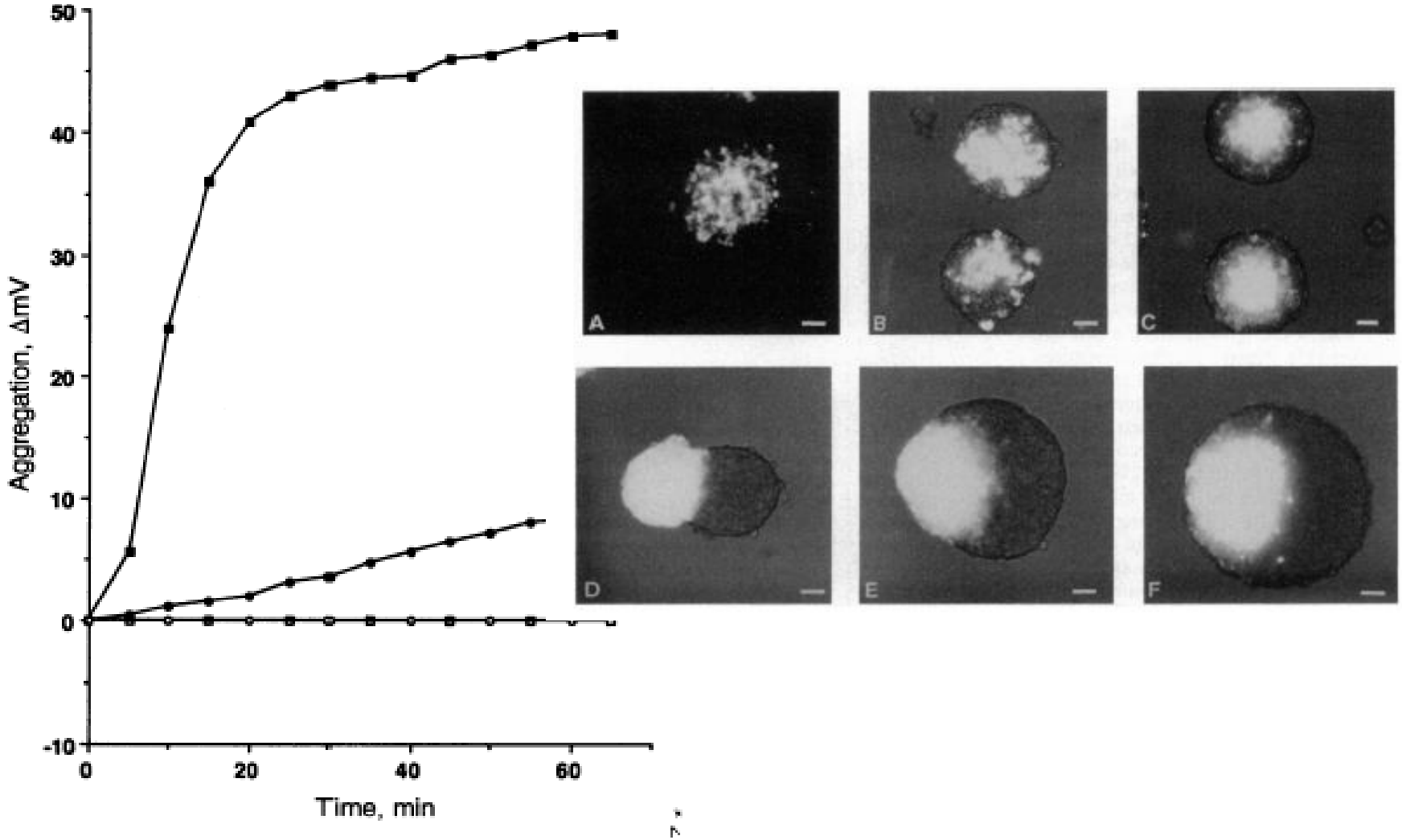
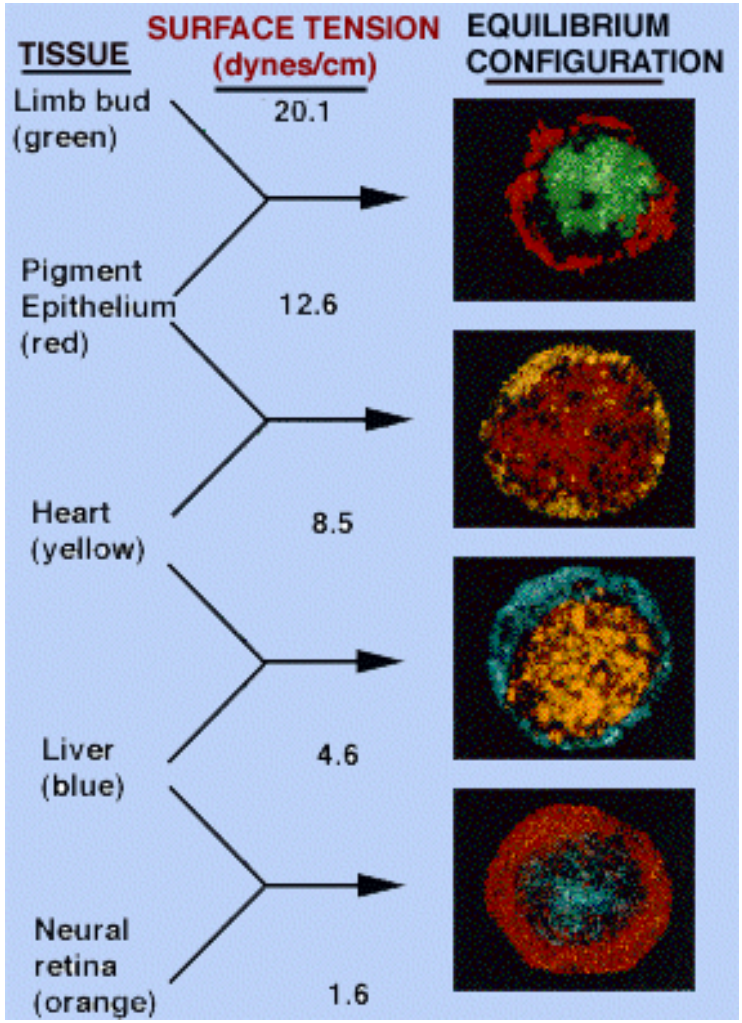
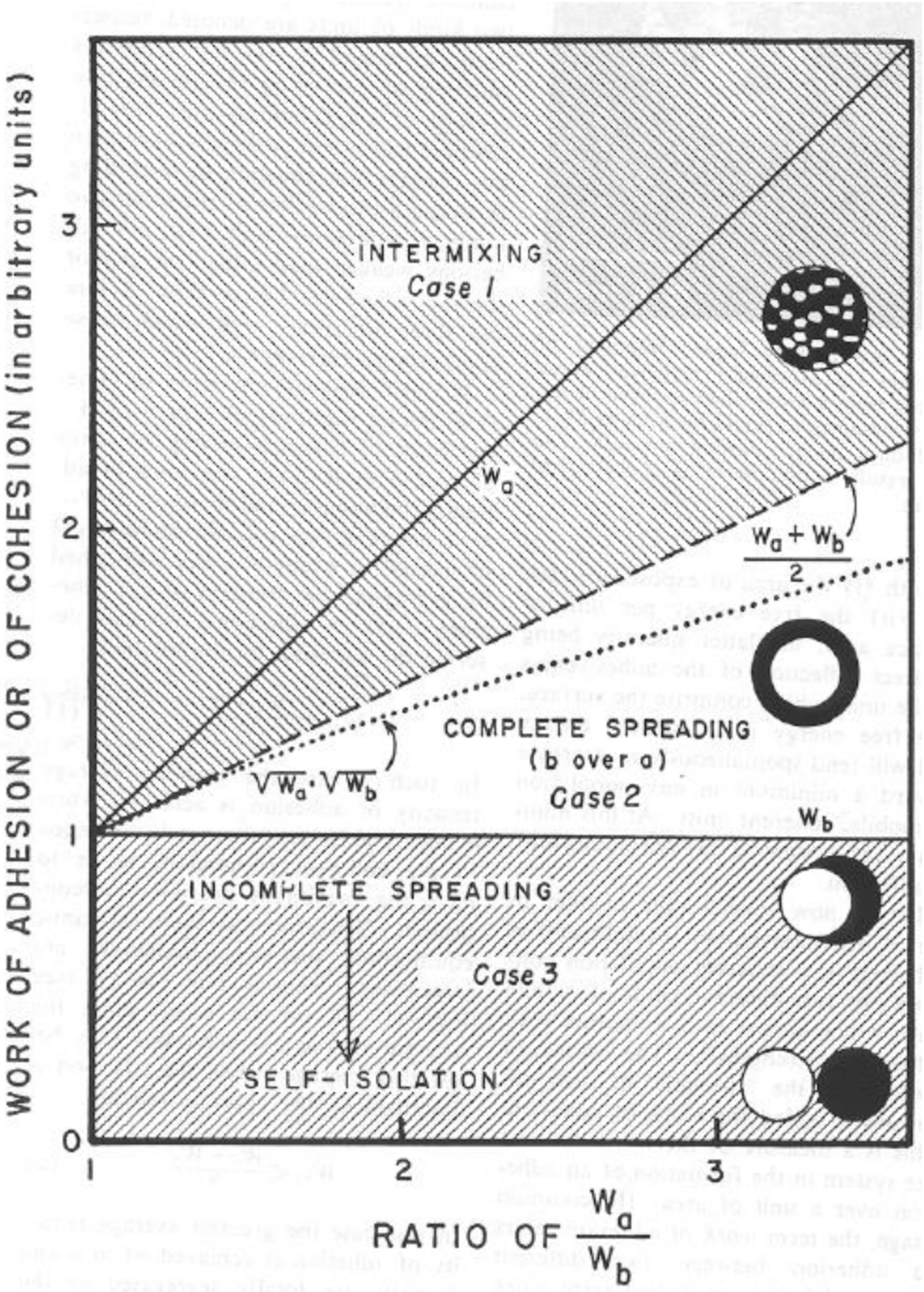


FIG. 1. Cell aggregation (expressed as ΔmV reading of the aggregometer). ■, PLβ2 cells + Ca^{2+}; □, PLβ2 cells in low Ca^{2+}; ●, PLs5 cells + Ca^{2+}; and ○, PLs5 cells in low Ca^{2+}.

PLs5- esprimono poco P-cadherin, PLβ2 ne esprimono 20 volte piu. Spiegare le curve

Control: positional information though differences in cohesion and adhesion




WORK OF ADHESION OR OF COHESION (in arbitrary units)
0
1
2
3
INTERMIXING
Case 1
W_a
$\frac{W_a + W_b}{2}$
COMPLETE SPREADING
(b over a)
Case 2
$\sqrt{W_a} \cdot \sqrt{W_b}$
W_b
INCOMPLETE SPREADING
Case 3
SELF-ISOLATION
1
2
3
RATIO OF $\frac{W_a}{W_b}$

I vincoli

No di cellule: 20 < 1000

Dimensione fisiche delle zone di riorganizzazione: 100 micron < 1mm

Tempo : ore, giorni

Densita' cellulare nell' uomo: 1-3. 10^9 cellule/ml. Calcolare il no totale di cellule in un uomo di 70 kg.

La subunita funzionale di un organo è di 100X 100 micron (nefrone, capillare, unità epatocitaria, ghiandola). Calcolare il no di cellule in una subunità.

* 100 micron è importante per O_2

La formazione di pattern è stato dibattuto per piu di 50 anni (Turing, 1952). Come fanno cellule vicine a sapere che devono essere diverse (es. i capelli)? La mano si forma cosi perche le cellule al confine diventao epiteliali.

Segnali

Morphogenesis :pattern, sorting

Cell-cell signalling

Cell-ECM signalling

Chemotaxis, hapotaxis, galvanotaxis

Contact guidance e inibizione del contatto

} Migrazione