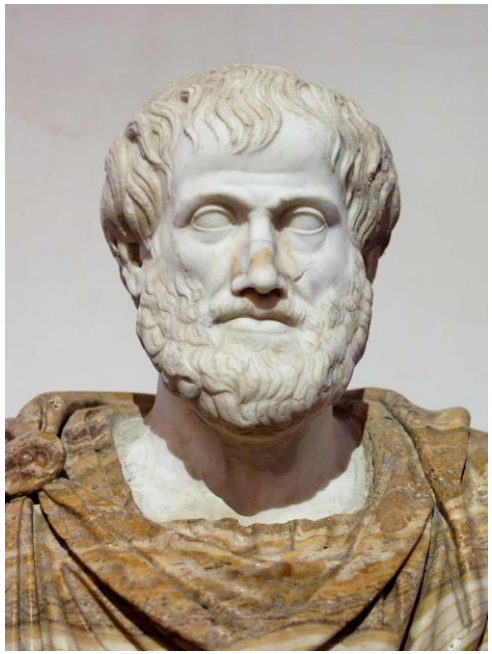
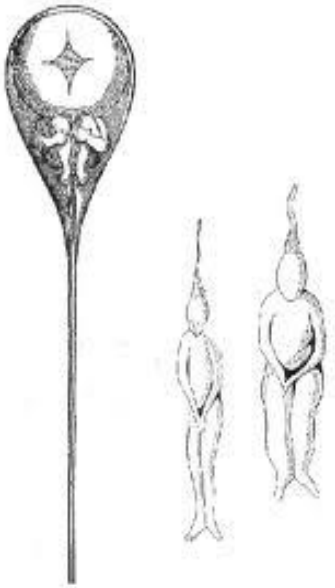
Corso di Embriologia

“Archeo-Embriologia”

Epigenesis - Aristotele 384 – 322 a.c.

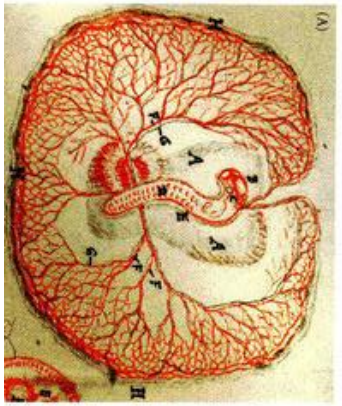


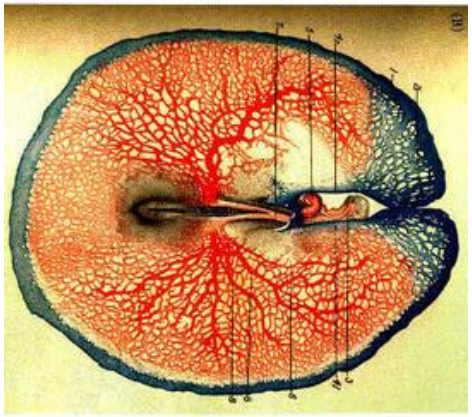
Pre-formazionismo



Marcello Malpighi

Marcello Malpighi
(1628-1694)





Teoria cellulare cambia la concezione del processo di differenziazione cellulare 1820-1880

Organismi multicellulari sono composti da cellule

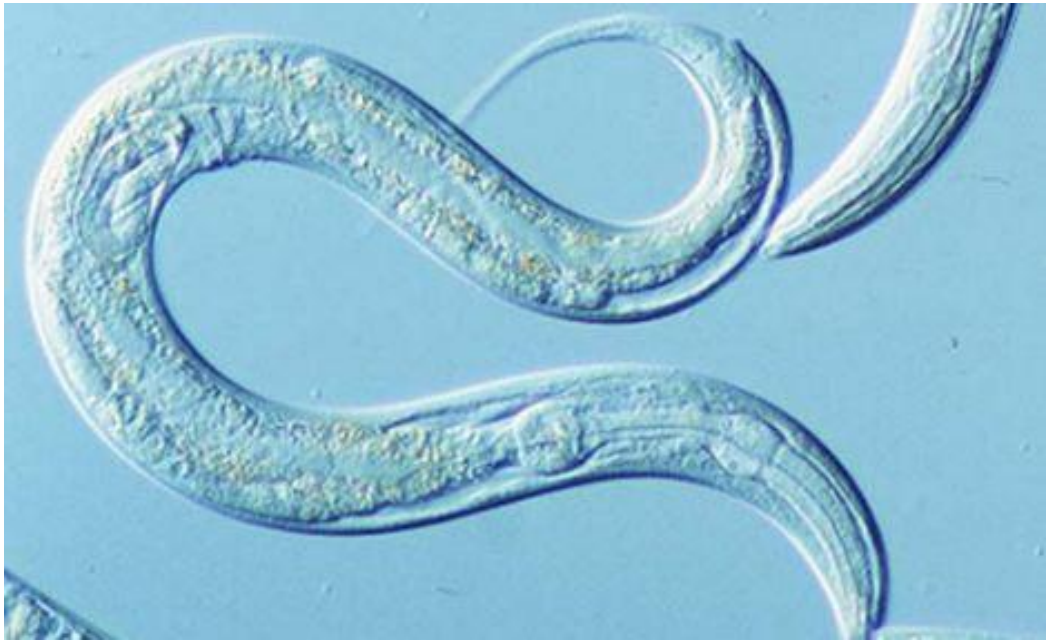
Tutte le cellule derivano da una sola, benché complessa: l'oocita fertilizzato (zigote)

Pionieri della teoria cellulare

Theodor Schwann

Theodor Schwann

Photo courtesy of
National Library of Medicine



Ex from an egg.

y)

izquotes.com

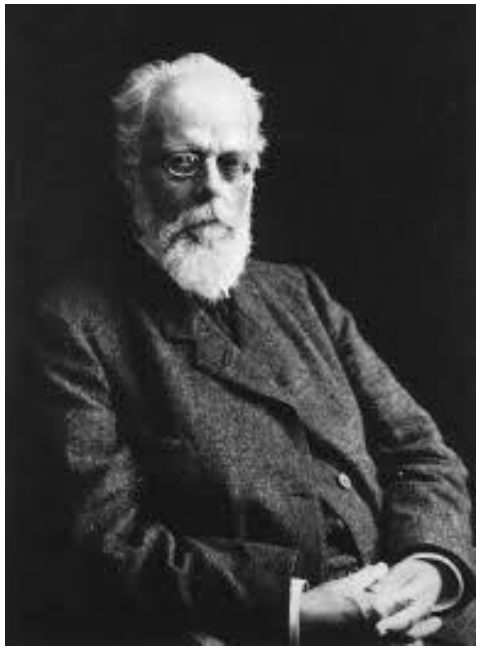

Morphol. Jahrbuch Bd III
Taf III
Fig 1.
Fig 2.

Primo postulato in biologia dello sviluppo: Soma e cellule germinali

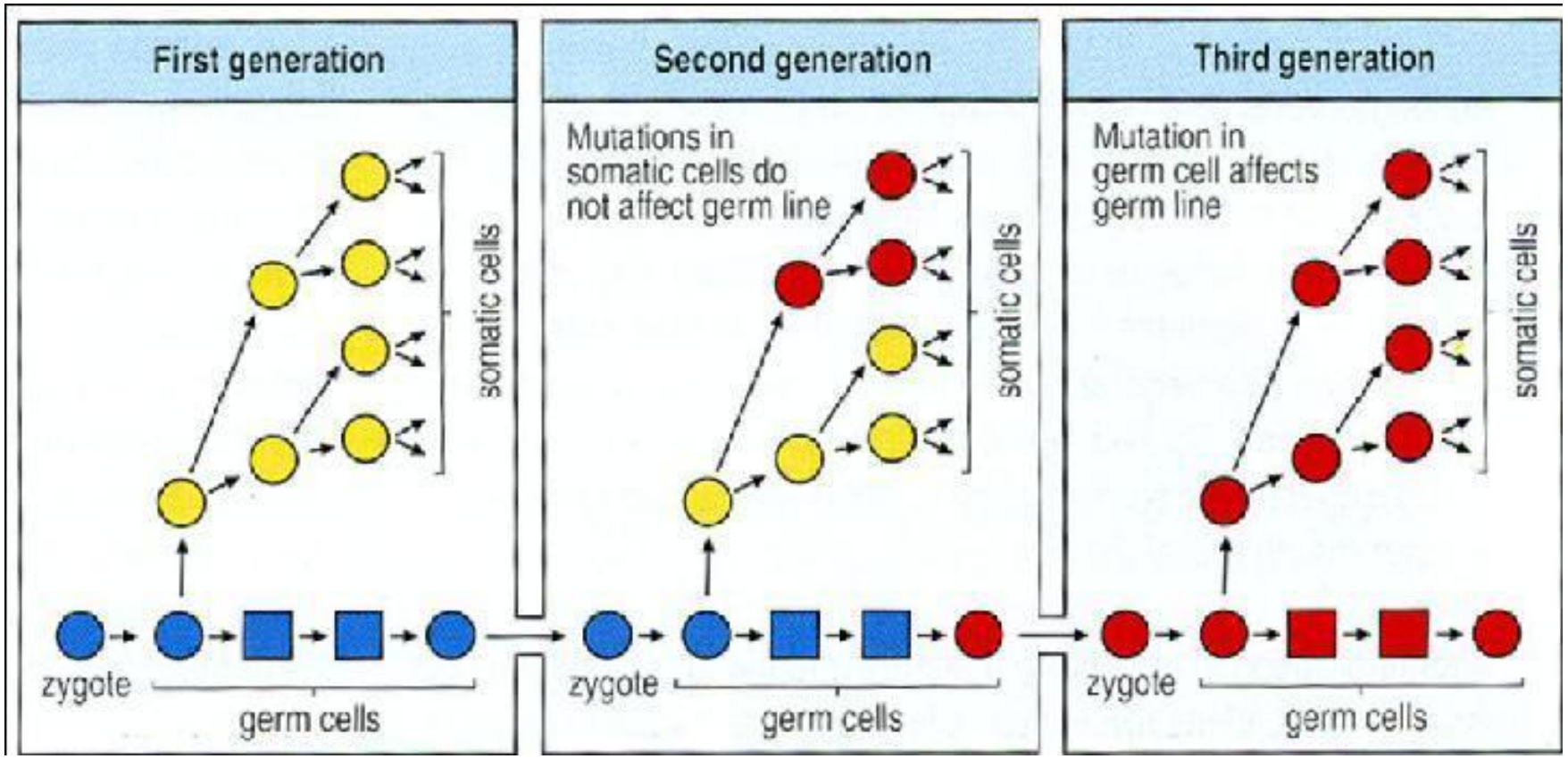
Il soma cellule germinali sono nettamente distinte
Il soma (corpo) contiene le cellule germinali e le passa da una generazione all'altra

"Mutazioni delle cellule somatiche non sono trasmessa alle cellule germinali
Mutazioni delle cellule germinali sono trasmessa a tutte le cellule somatiche"

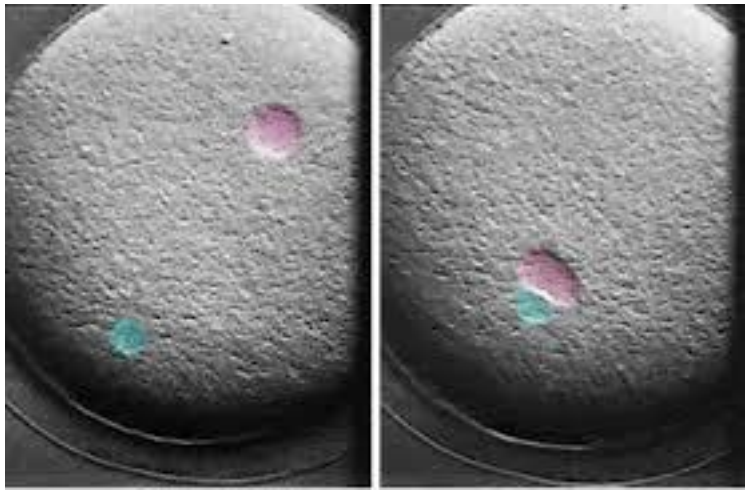
August Weissman



Mutazioni cellule germinali base del processo evolutivo

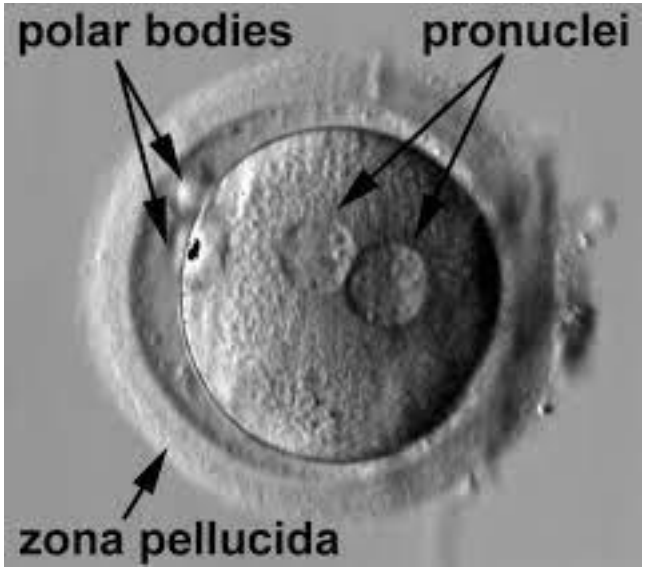


Due distinti pronuclei visibili in zigote di riccio di mare dopo la fecondazione:
Uno deriva dallo spermatozoo, l'altro dell'oocita



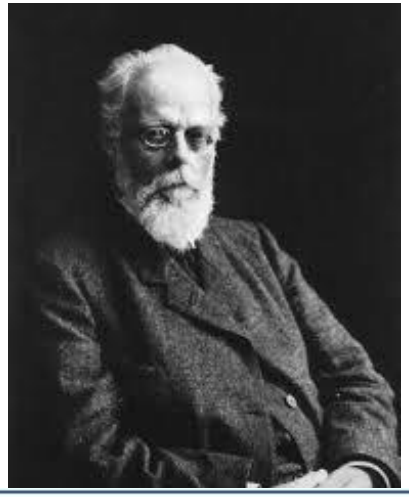
…inoltre, i cromosomi contenuti nello zigote derivano in numero uguale dallo spermatoz
Prima evidenza empirica che sostiene la trasmissione dei caratteri ereditari come postul
Siamo alla fine del 19 secolo.

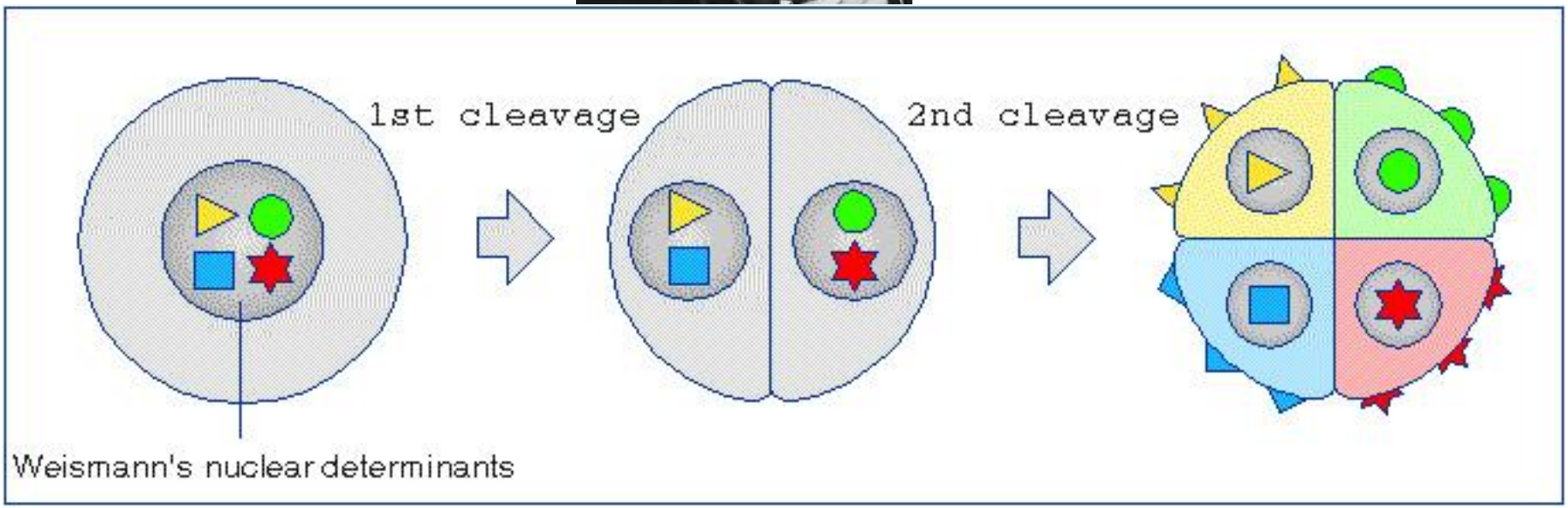
Domanda da un milione di $: come si arriva da uno zigote all'individuo "finito", Compsto da almeno ? Tipi cellulari diversi???



Sviluppo di tipo "mosaico" e "regolativo"

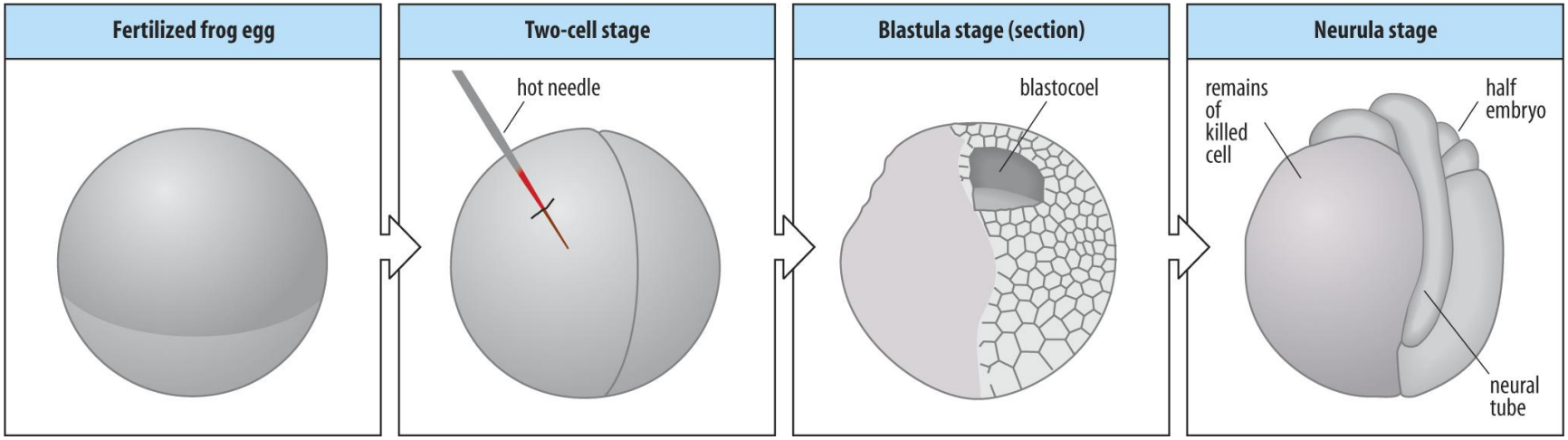
Weissman è il padre del modello di sviluppo “mosaico”



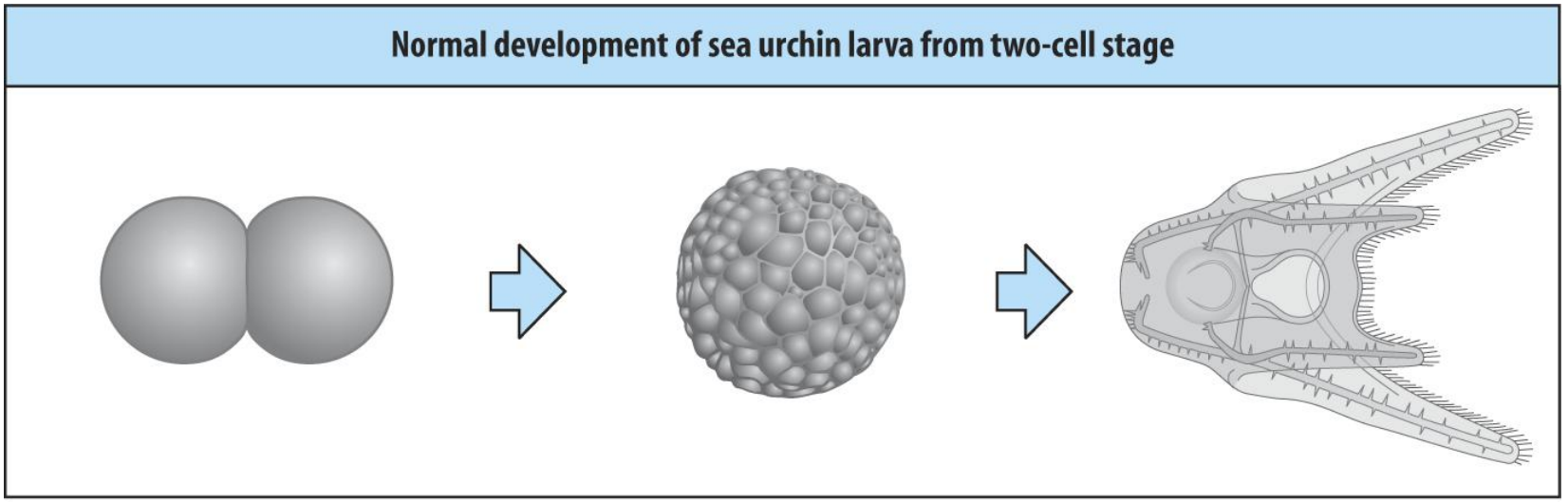


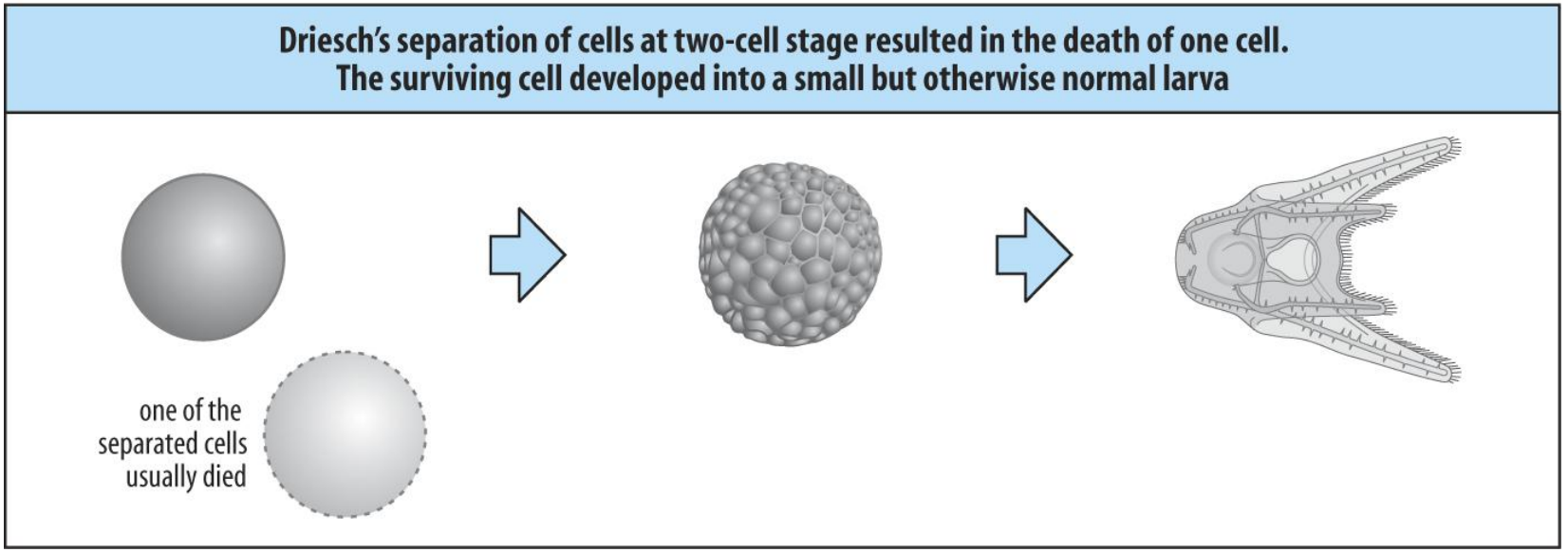
“Determinanti” asimmetricamente divisi durante lo sviluppo embrionale responsabili della differenziazione cellulare- siamo nel 1880

L’esperimento di Wilhelm Roux sostiene il modello mosaico di sviluppo

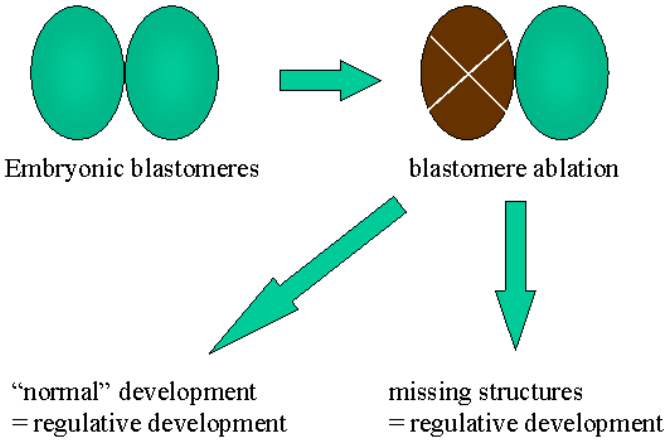


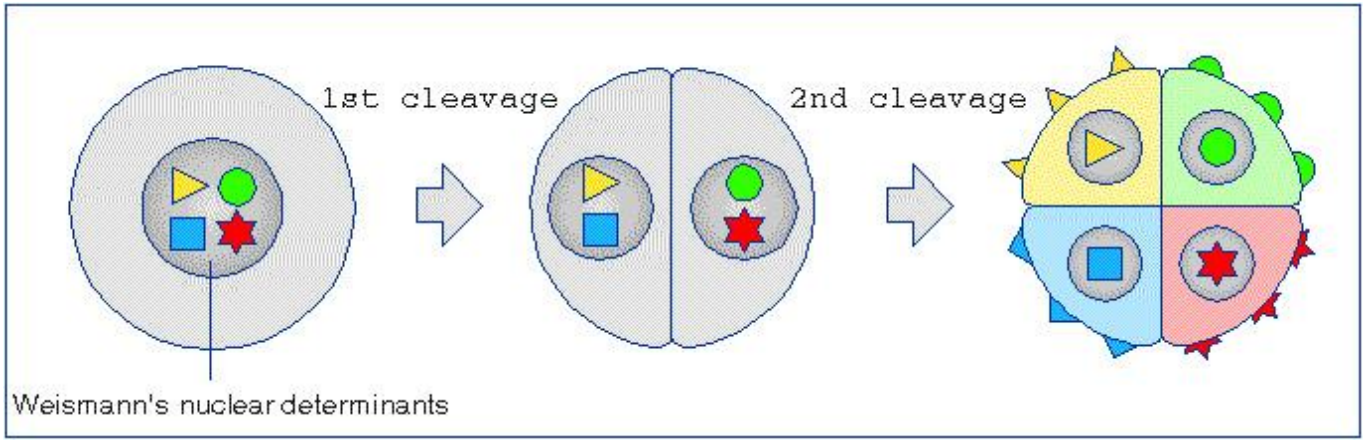
…ma il suo collega Driesh lo smentisce usando embrioni di riccio di mare….
Siamo nel 1892..





Mosaico o Sviluppo regolato: Quale dei due è corretto?





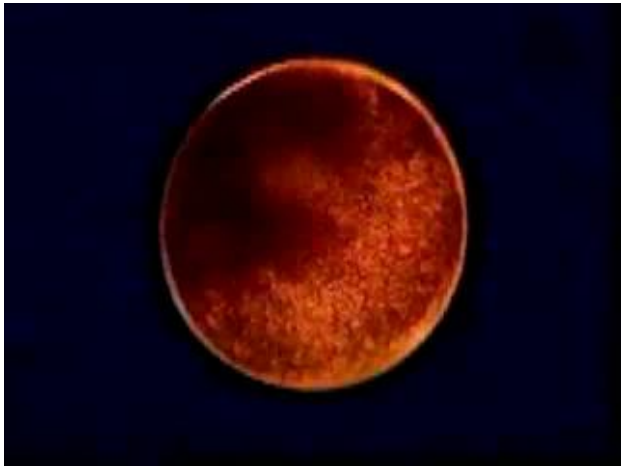
Introduzione all'embriologia , o biologia dello sviluppo

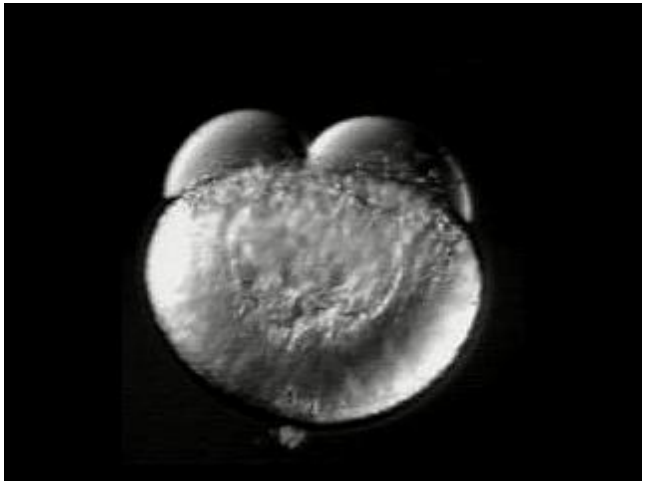
Lo sviluppo di un organismo avviene attraverso:

1) divisioni cellulari,
2) organizzazione di foglietti (tessuti) primordiali,
3) emergenza di assi di sviluppo
4) Differenziazione cellulare
5) Accrescimento
6) Morte cellulare programmata (apoptosis)

1) Divisioni cellulari

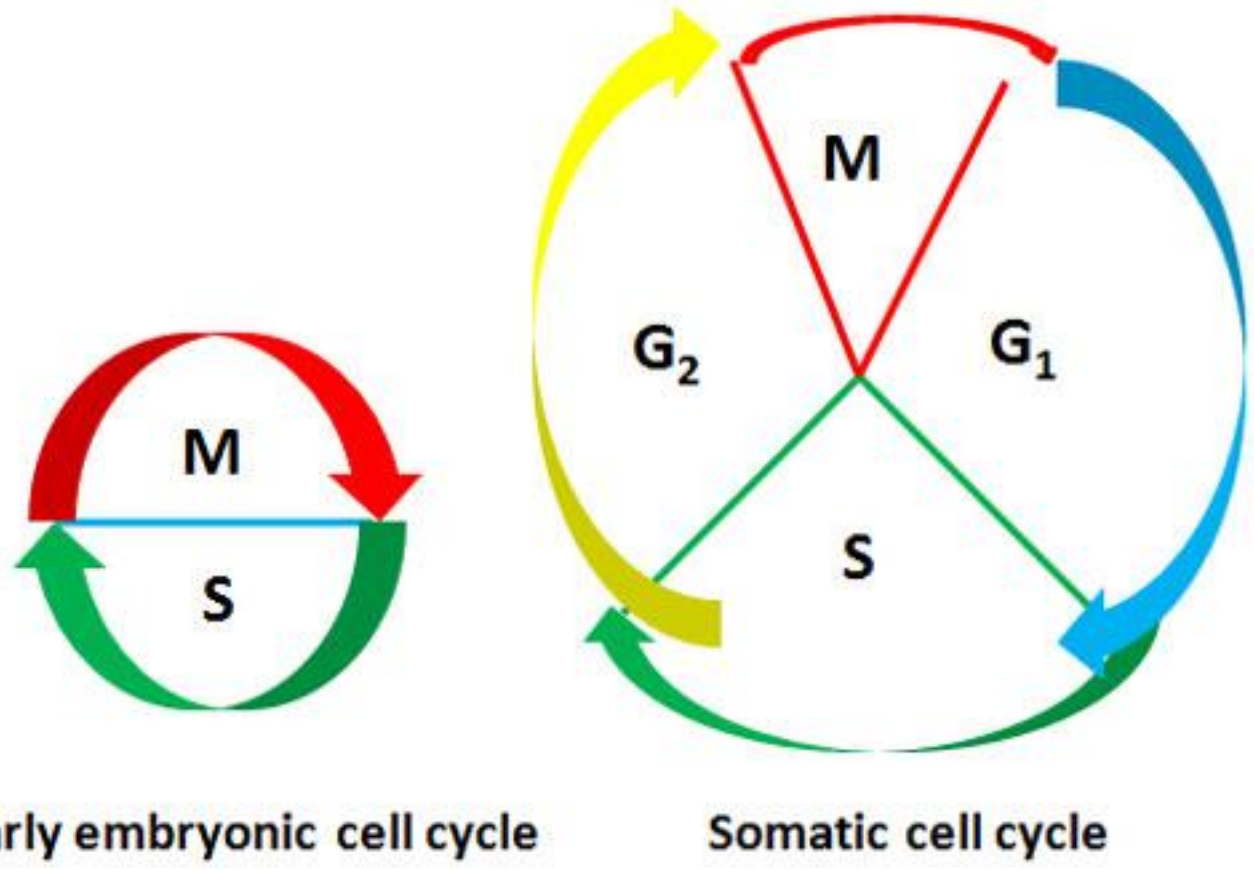
Dopo la fecondazione lo zigote si divide (+ o –, dipende dalla specie) rapidamente (divisioni riduzionali) -





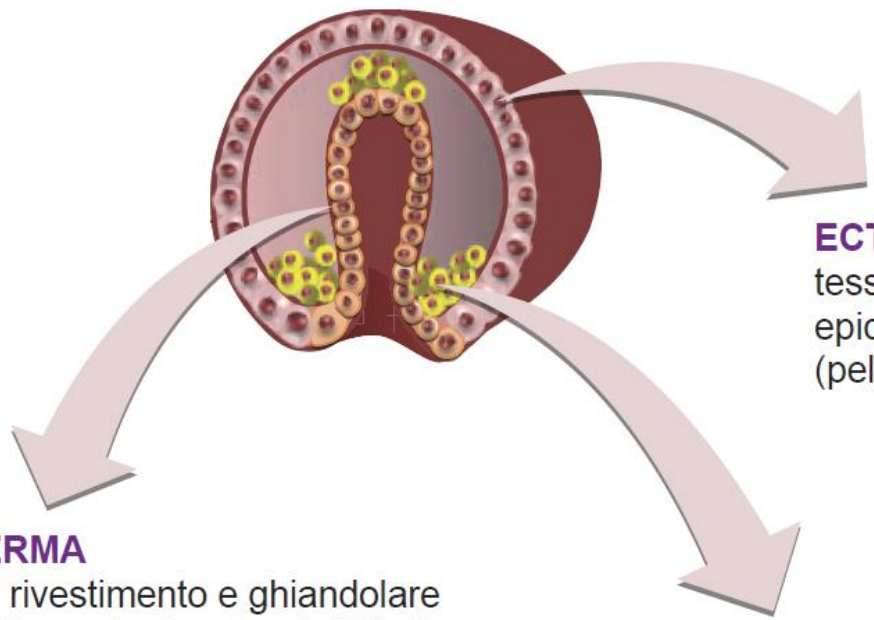
http://wn.com/Human_embryo_which_gave_a_vital_pregnancy_Embryo_Cleavage_Rating_ECR

Mancanza di accrescimento cellulare, ciclo cellulare embrionale alternanza di fasi S e M



2) organizzazione di foglietti (tessuti) primordiali

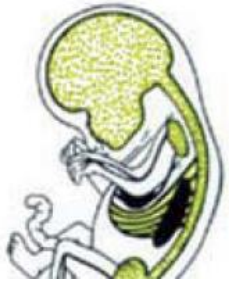
<u>"Gastrulazione" primo evento morfogenetico</u>



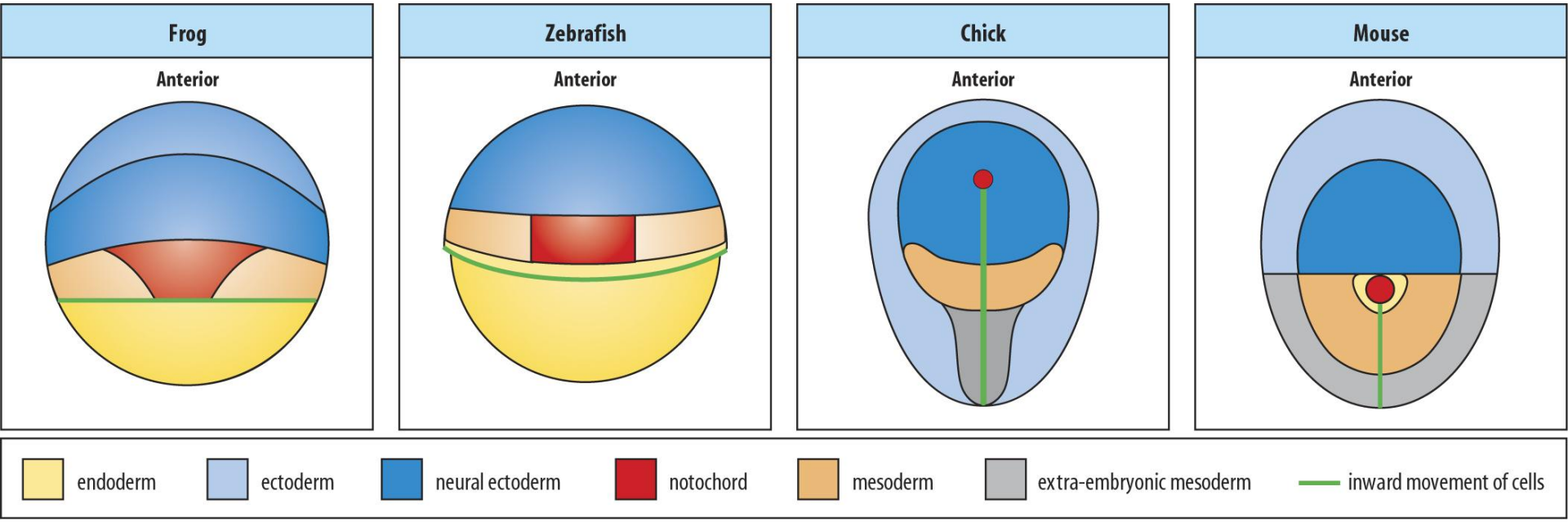
ECTODERMA
tessuto nervoso;
epidermide e suoi derivati
(peli, capelli, unghie, smalto dentario).

ENDODERMA
epitelio di rivestimento e ghiandolare
del tubo digerente, fegato, vie biliari
e pancreas; vie respiratorie; vescica,
uretra e prostata; tiroide,
paratiroide e timo;
cellule delle linee germinali
di ovociti e spermatozoi.

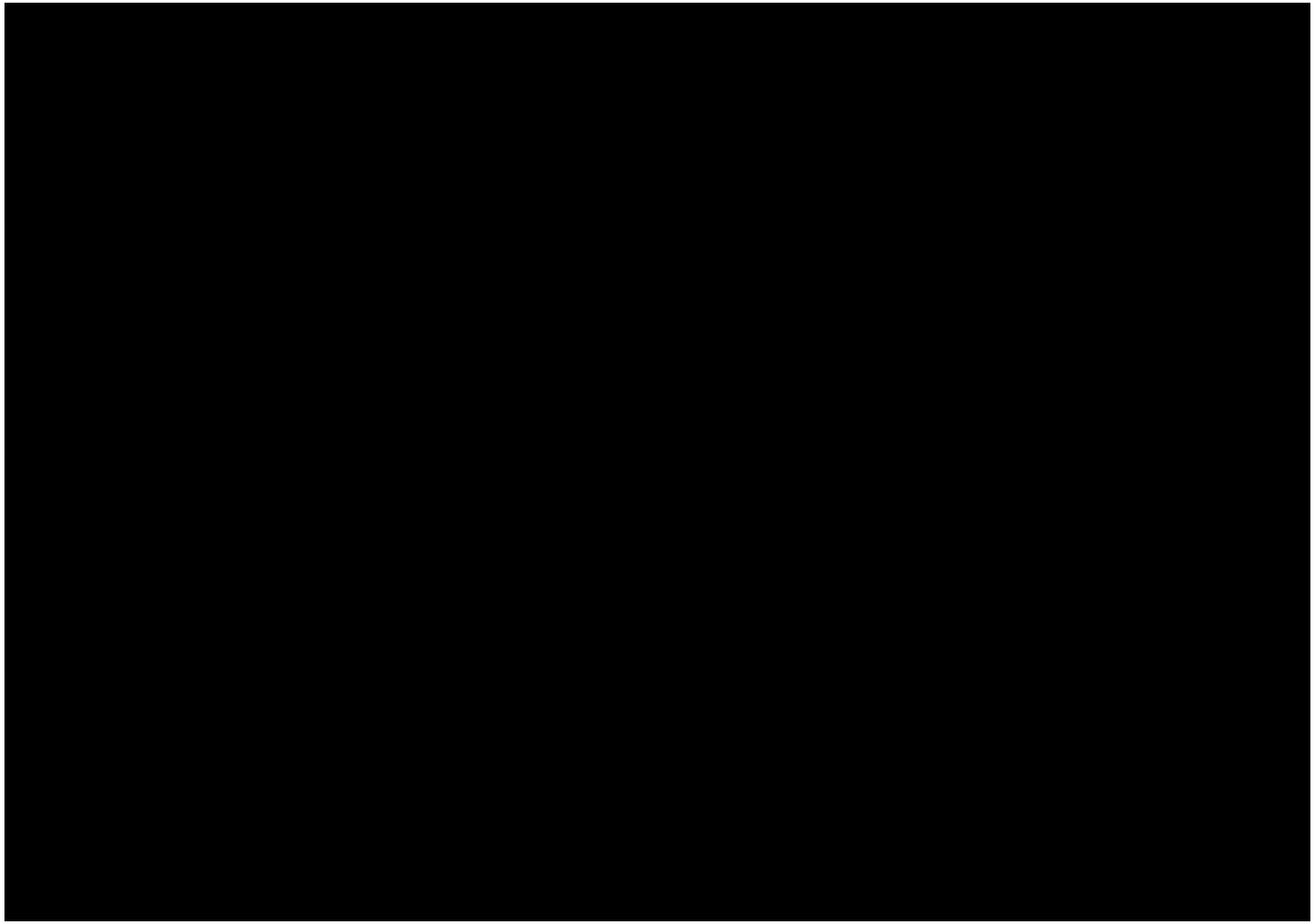
MESODERMA
scheletro; muscolatura;
tessuto connettivo;
apparato cardiocircolatorio;
apparato renale.



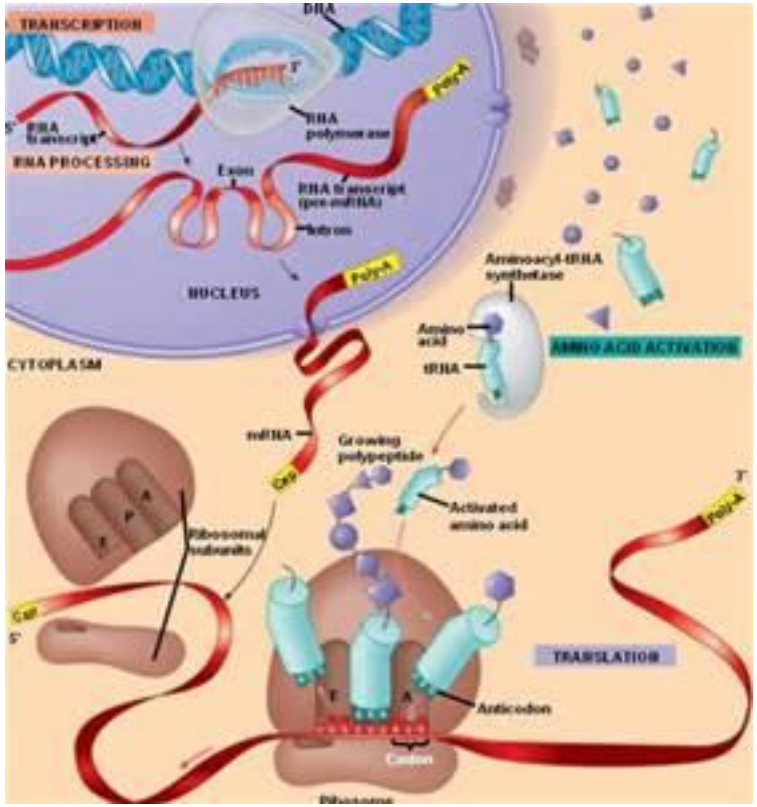
Differenze tra specie nell'organizzazione dei tre foglietti germinativi primari



ONTOGENESI: L'insieme degli stadi di sviluppo attraverso i quali
un organismo passa dallo stato iniziale di ovocellula
o di germe a quello di individuo completo

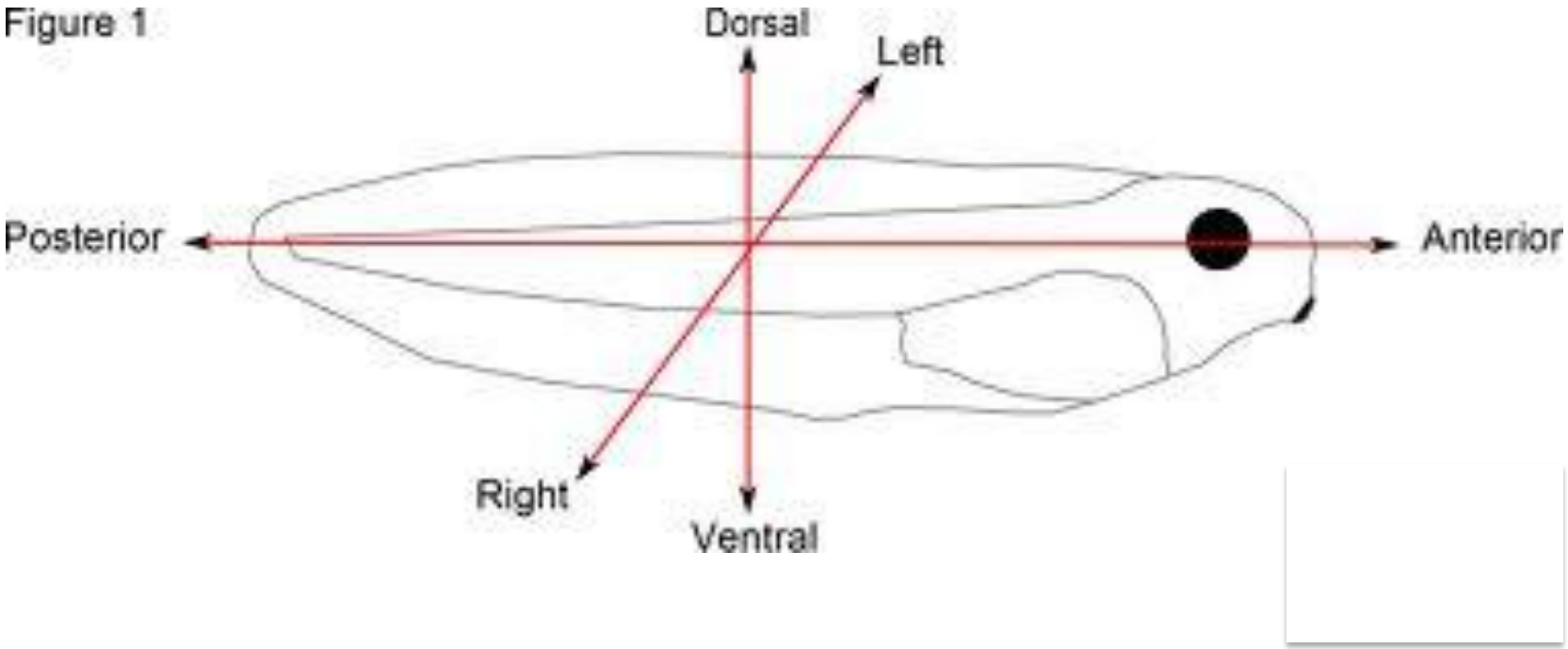


Programma di espressione genica che controlla proprietà e attività delle cellule

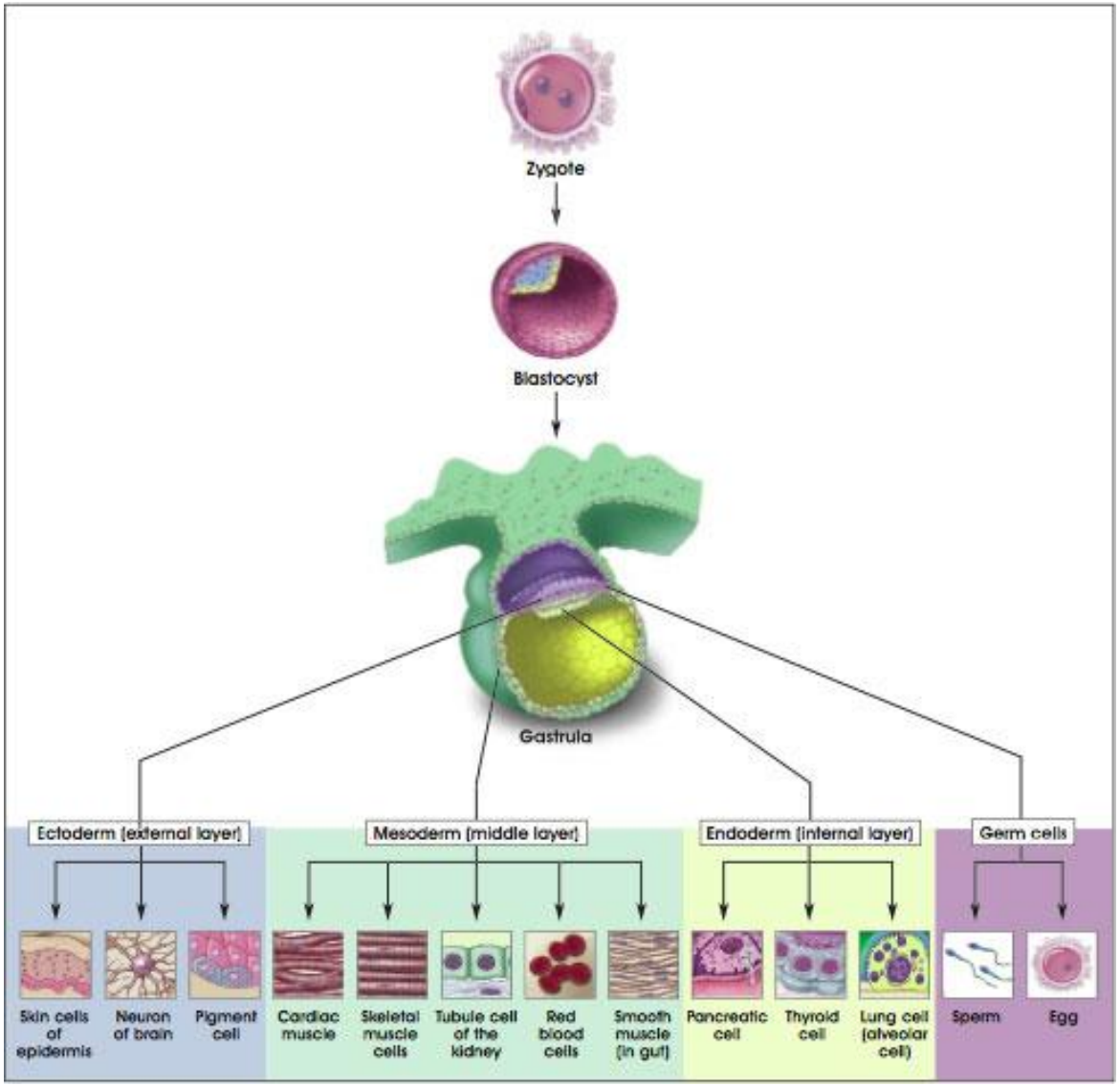


..prodotti genici controllano modificazioni della forma cellulare, contrazione localizzata di porzioni cellulari, produzione di molecole di adesione… forze principali nell'ontogenesi)

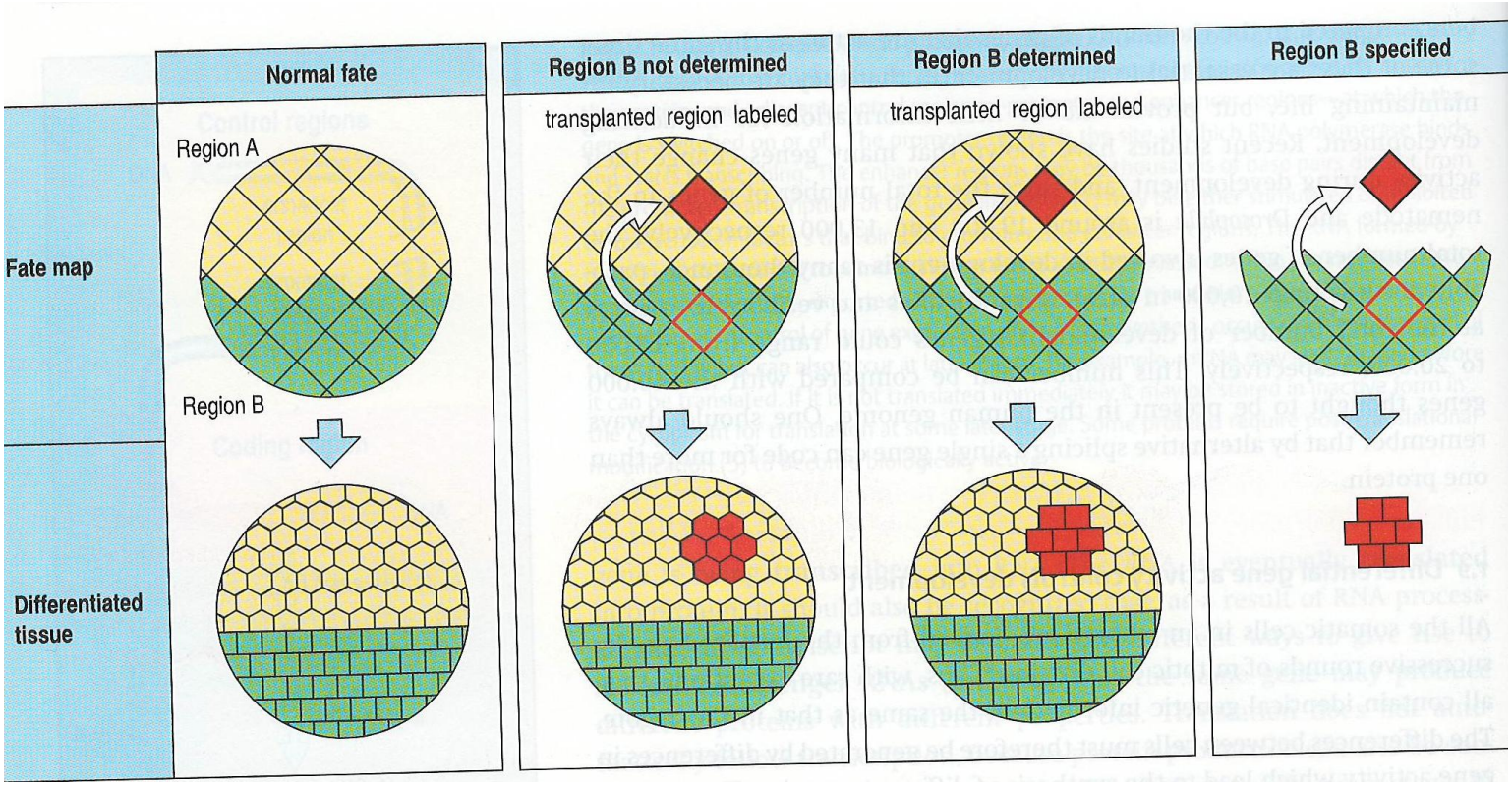
3) emergenza di assi di sviluppo



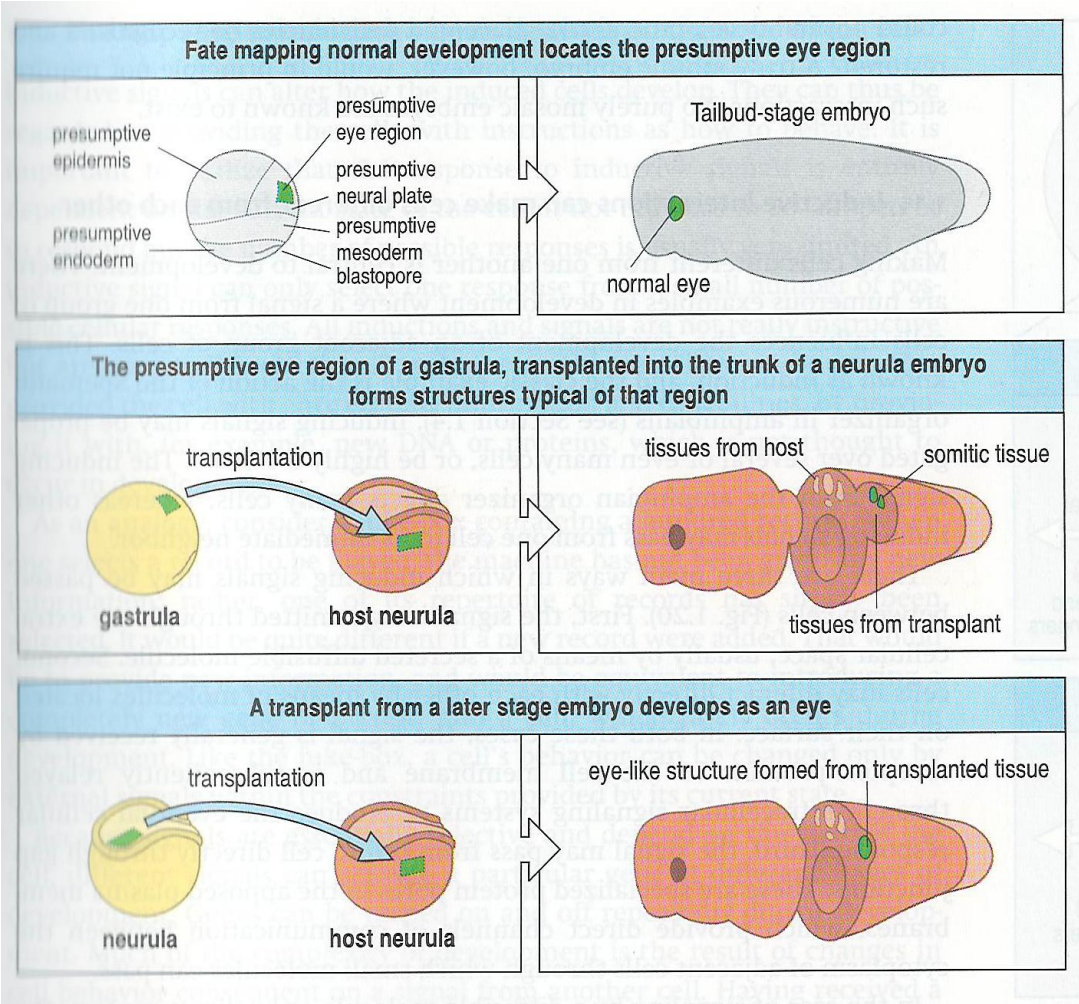
4) Differenziazione cellulare:
Quali sono i meccanismi che la inducono?



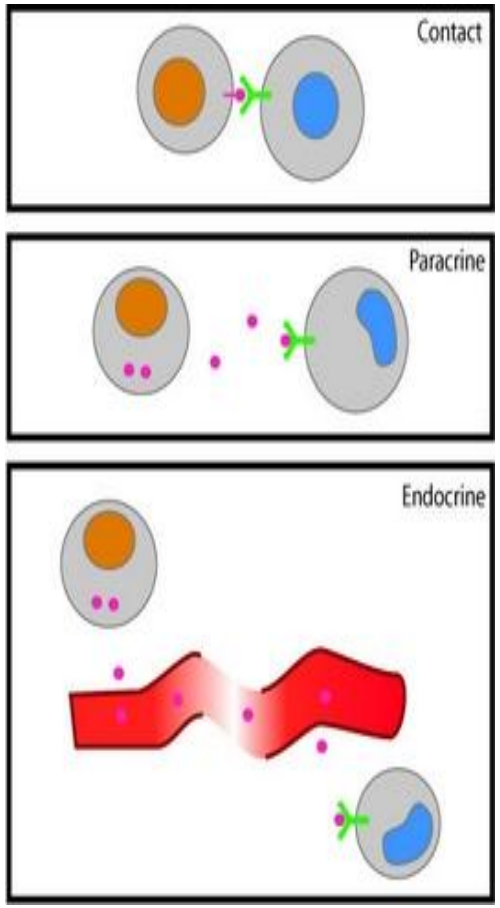
Determinazione e specificazione cellulare

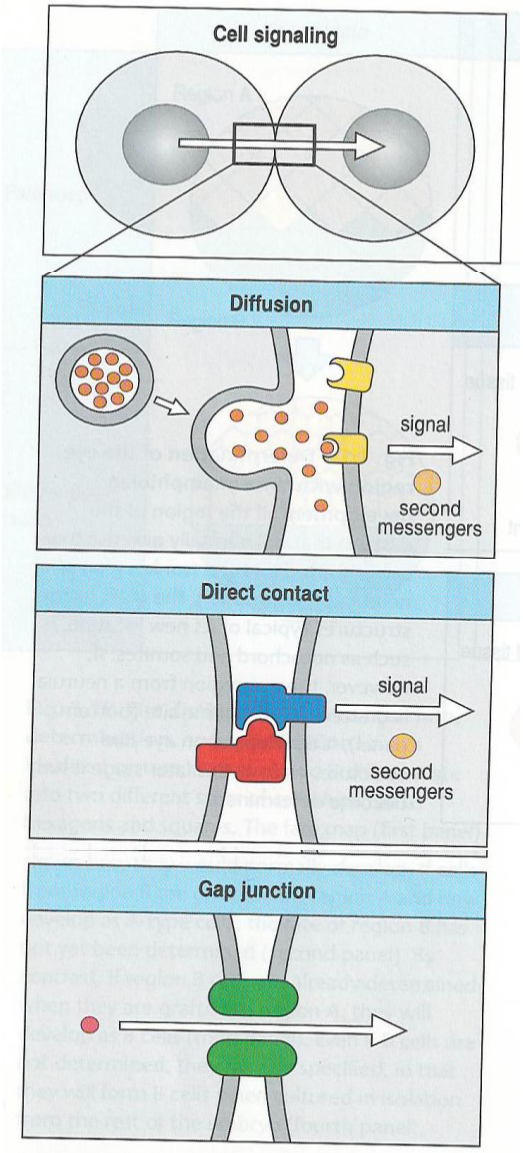


Determinazione e specificazione cellulare

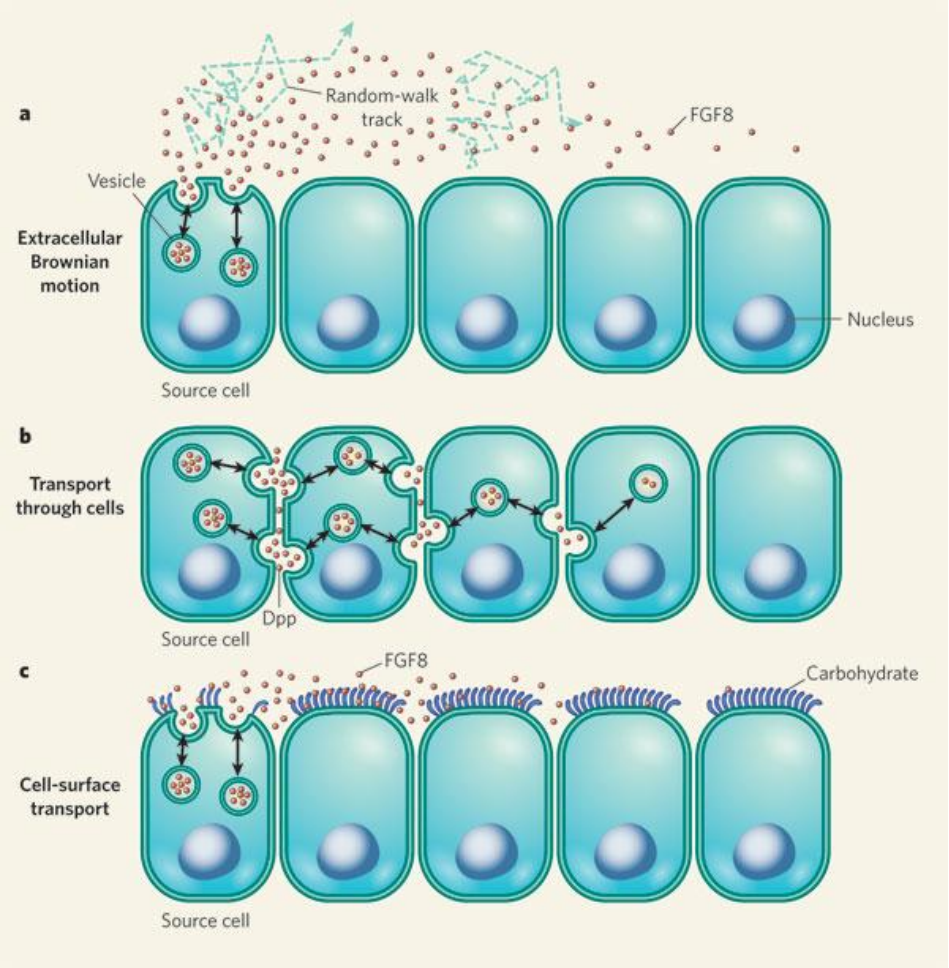


Modalità di trasmissione del segnale

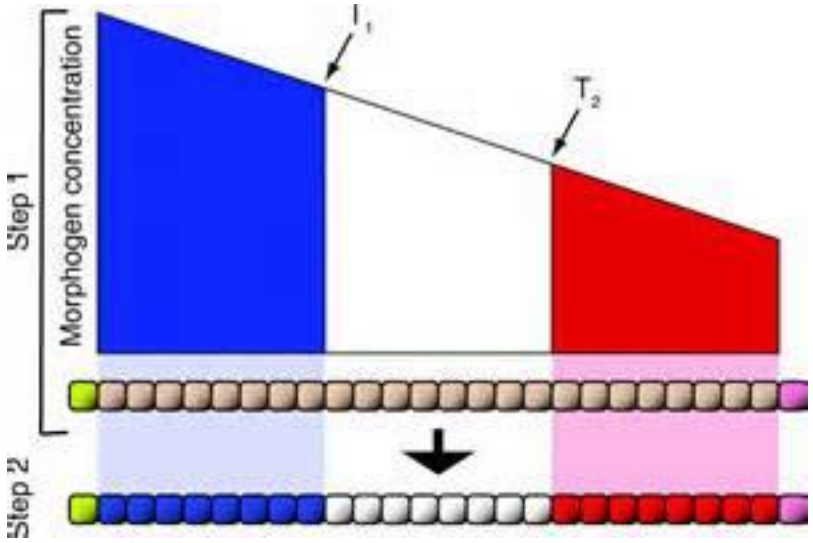


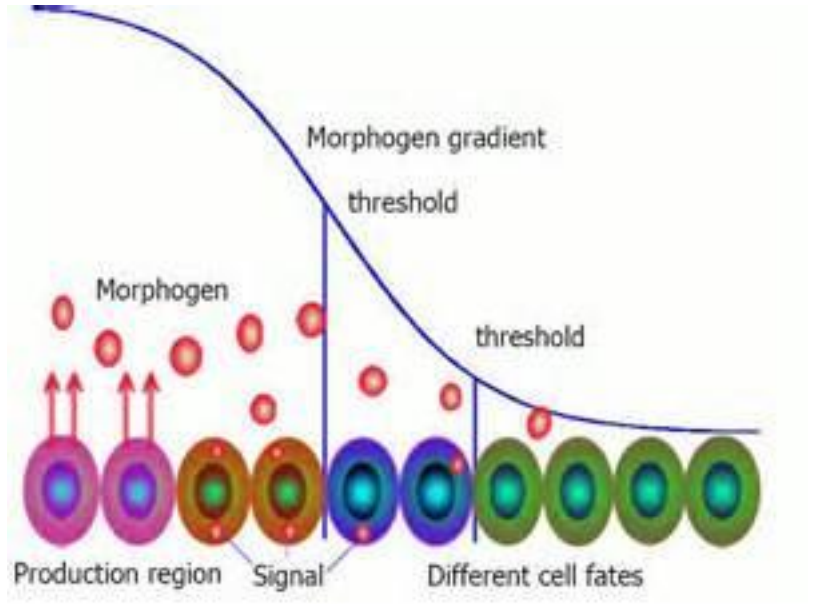


Modalità di Produzione di un “gradiente” di morfogeni…

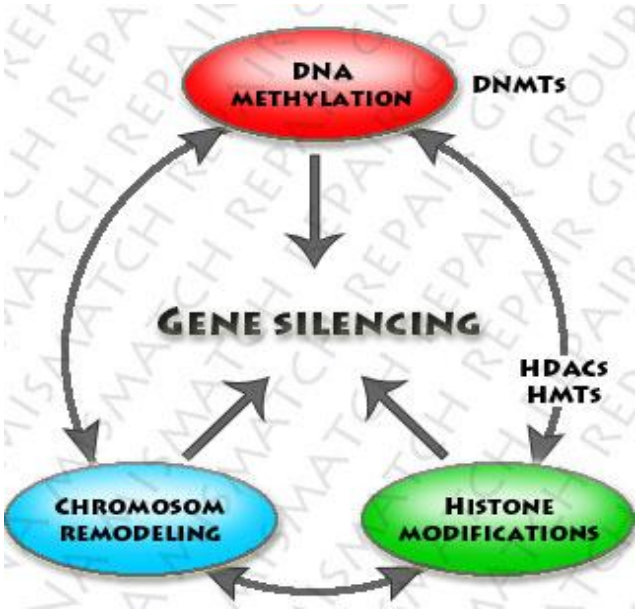


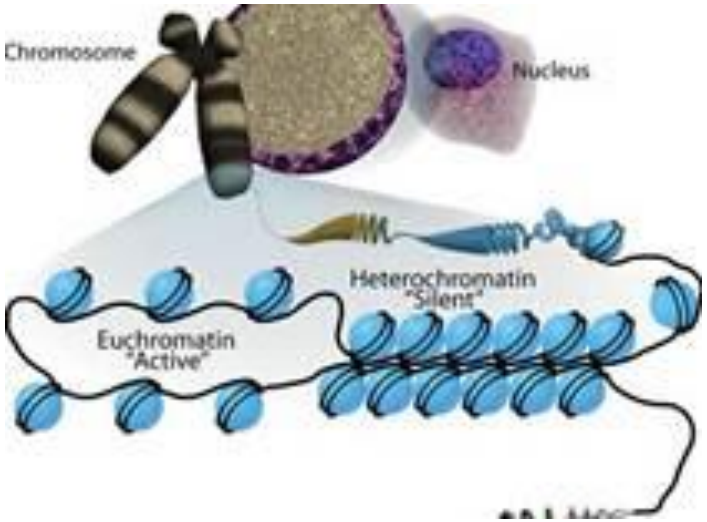
Gradienti come induttori di differenziazione



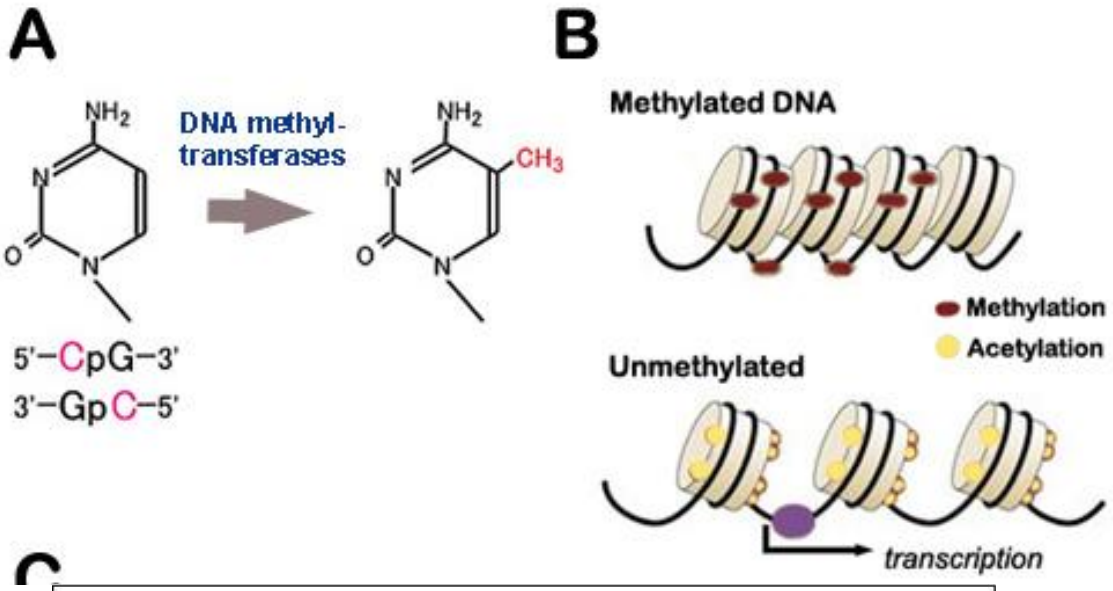


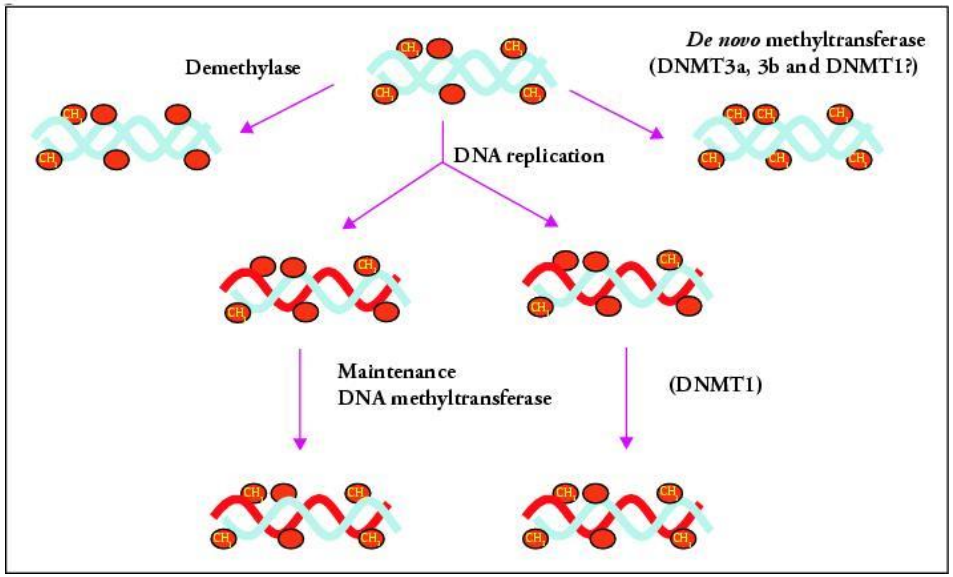
Meccanismi molecolari della differenziazione cellulare
Progressiva accumulazione di modificazioni epigenetiche sul DNA
Durante lo sviluppo

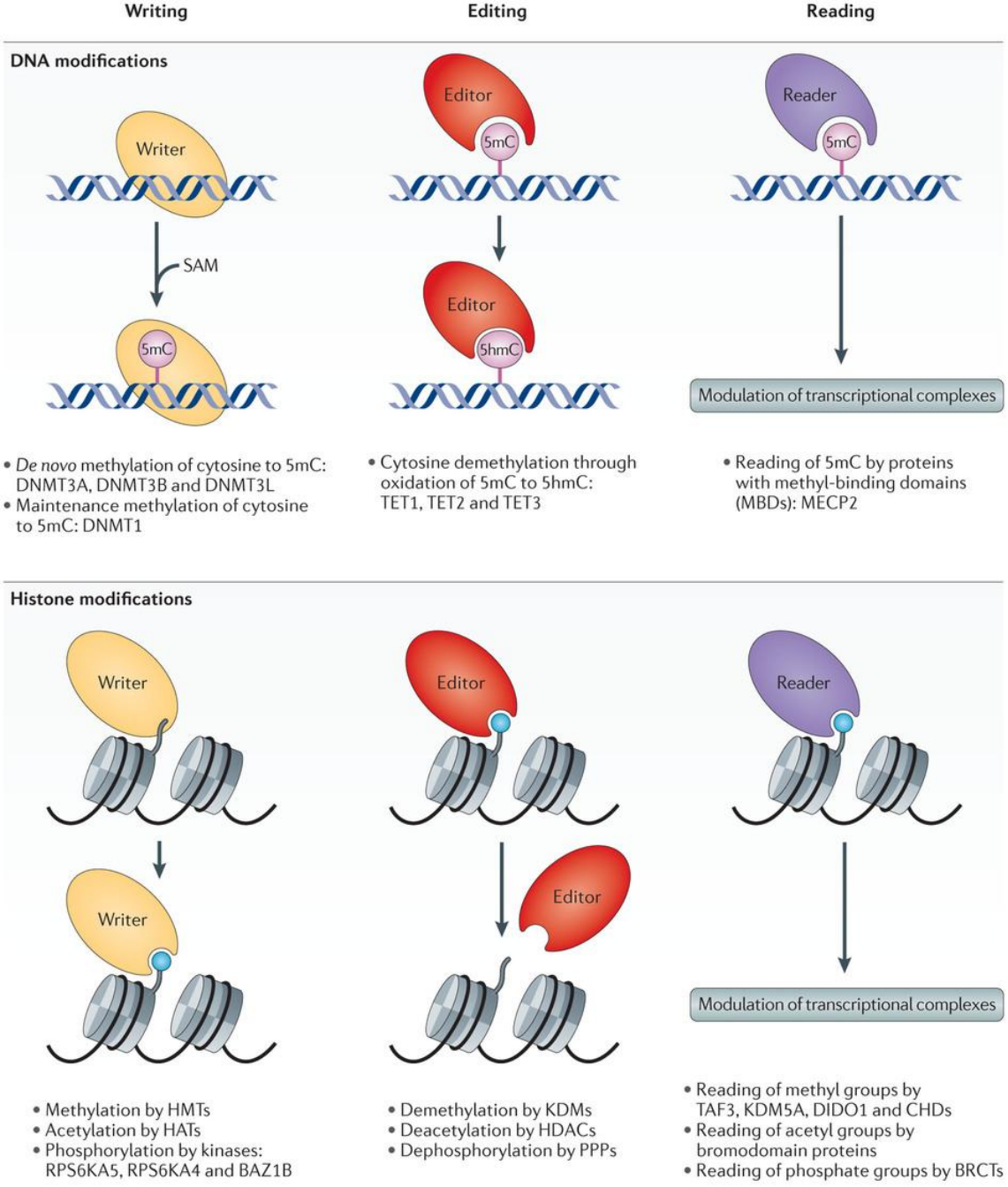




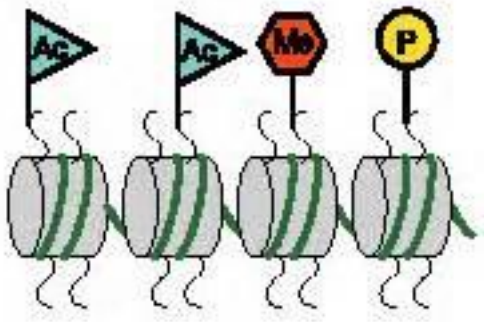
Metilazione del DNA

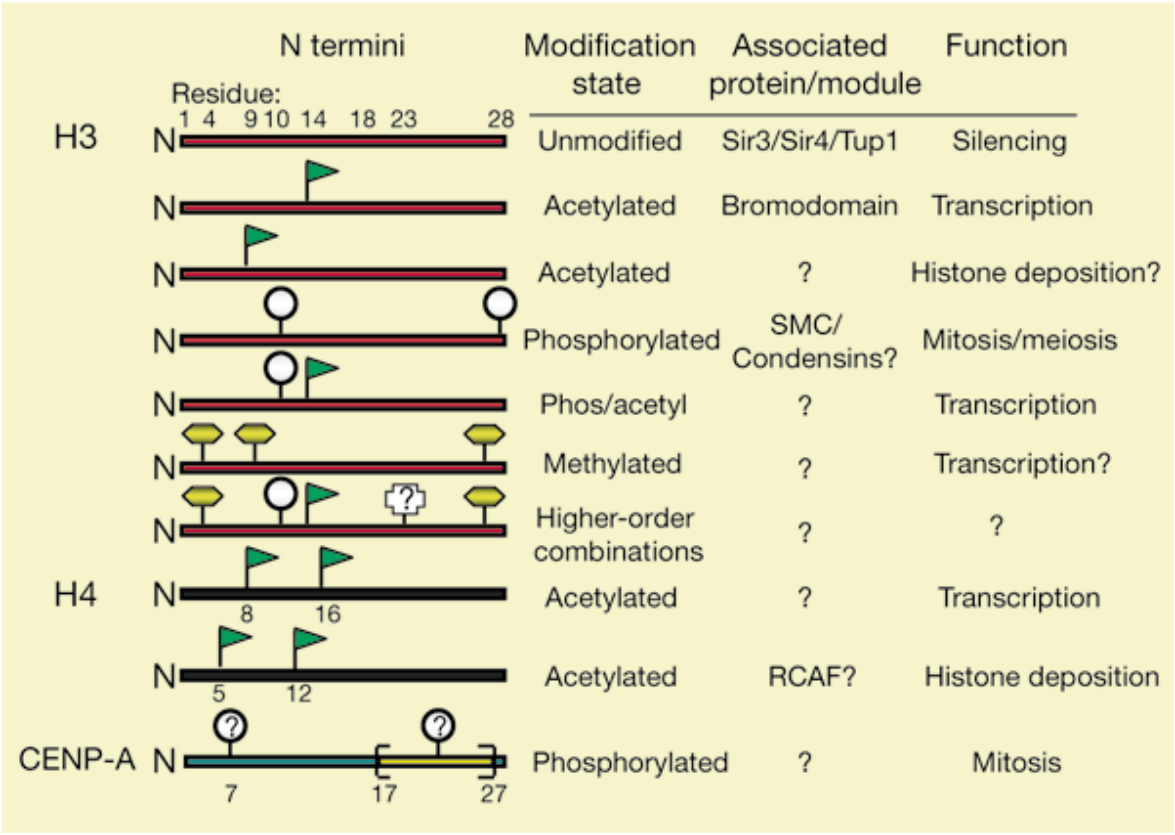





Writing
Editing
Reading
DNA modifications
Writer
SAM
5mC
Editor
5mC
Editor
5hmC
Reader
5mC
Modulation of transcriptional complexes
• De novo methylation of cytosine to 5mC: DNMT3A, DNMT3B and DNMT3L
• Maintenance methylation of cytosine to 5mC: DNMT1
• Cytosine demethylation through oxidation of 5mC to 5hmC: TET1, TET2 and TET3
• Reading of 5mC by proteins with methyl-binding domains (MBDs): MECP2
Histone modifications
Writer
Writer
Editor
Editor
Reader
Modulation of transcriptional complexes
• Methylation by HMTs
• Acetylation by HATs
• Phosphorylation by kinases: RPS6KA5, RPS6KA4 and BAZ1B
• Demethylation by KDMs
• Deacetylation by HDACs
• Dephosphorylation by PPPs
• Reading of methyl groups by TAF3, KDM5A, DIDO1 and CHDs
• Reading of acetyl groups by bromodomain proteins
• Reading of phosphate groups by BRCTs

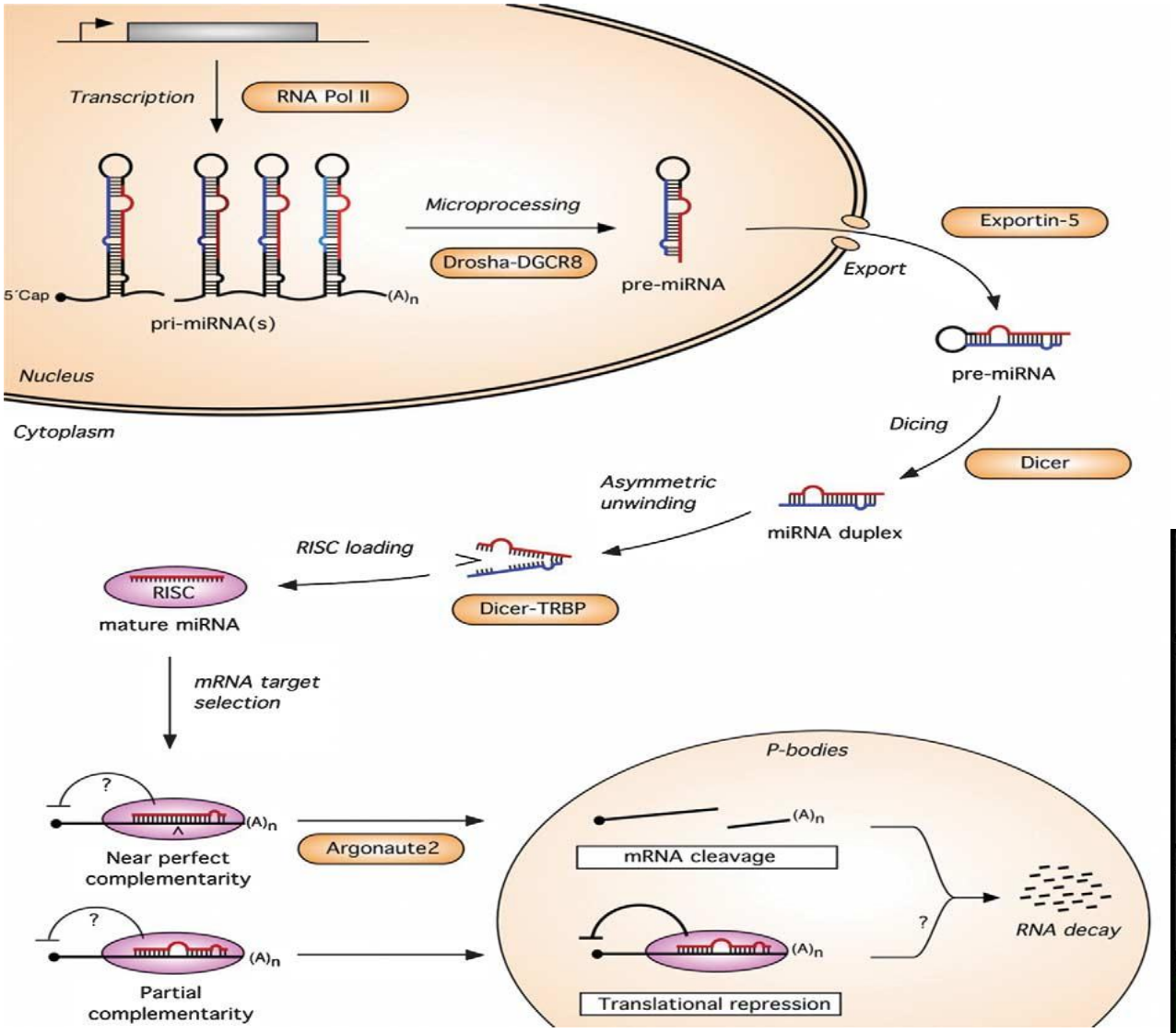
The Histone Code

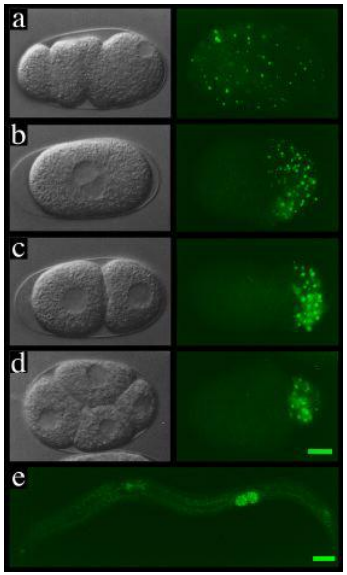




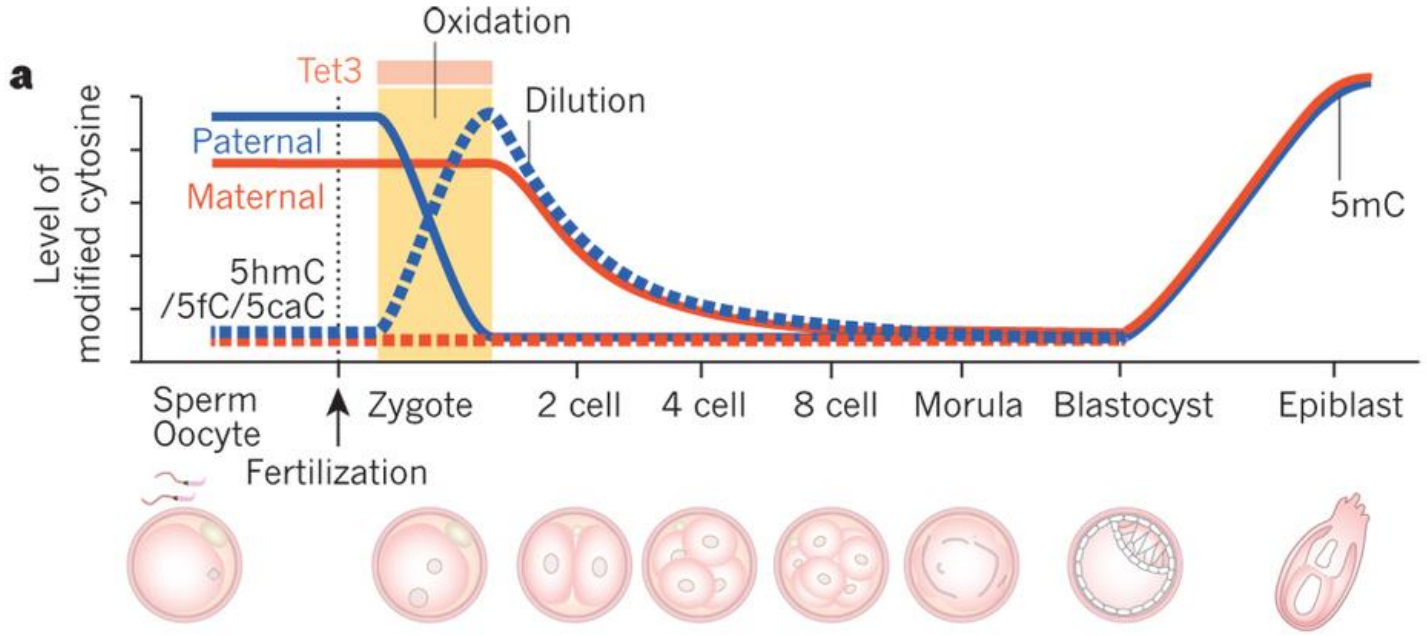
CENPA centromere protein A

New entry nei meccanismi di controllo dello sviluppo: MicroRNA (miRNAs) http://www.nature.com/nrg/multimedia/rnai/animation/index.html



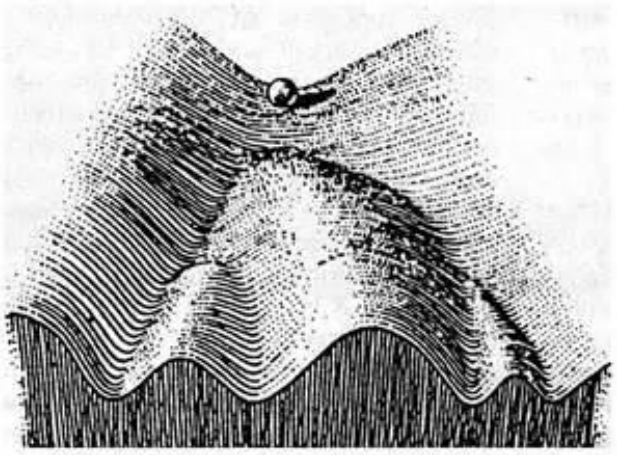


Dinamica della metilazione del DNA durante lo sviluppo



Legenda:
5hmC: HydroxymethylCytosine
5fC : FormylCytosine
5caC : CarboxylCitosine

Progressiva repressione genomica durante lo sviluppo dovuta a:

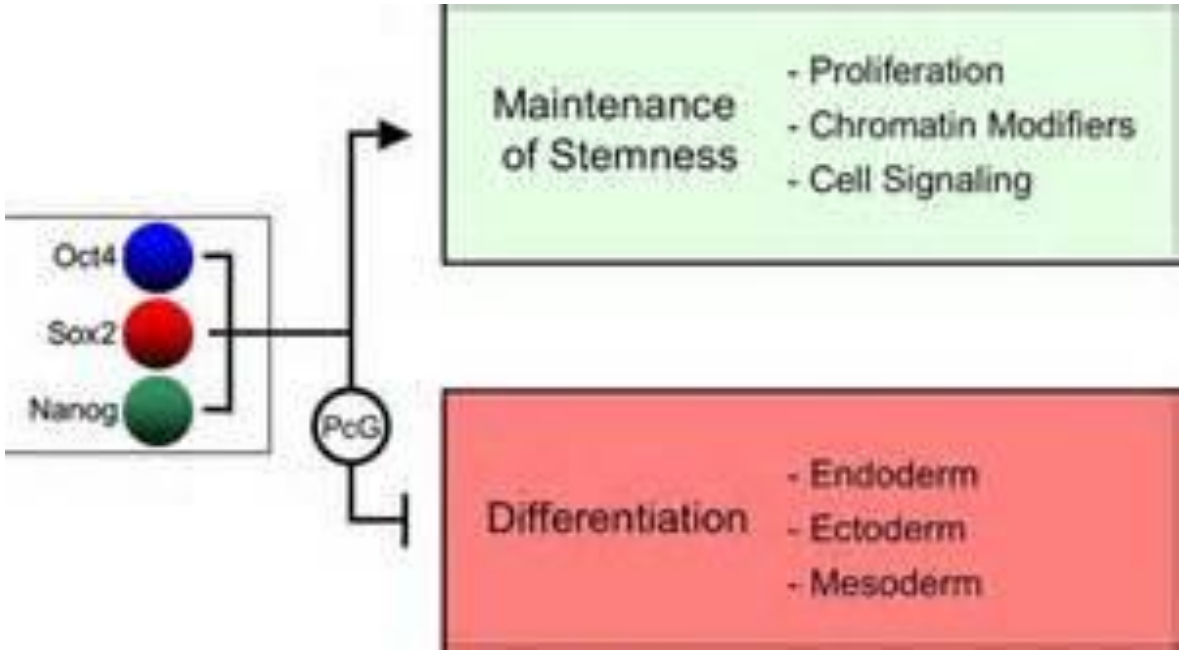


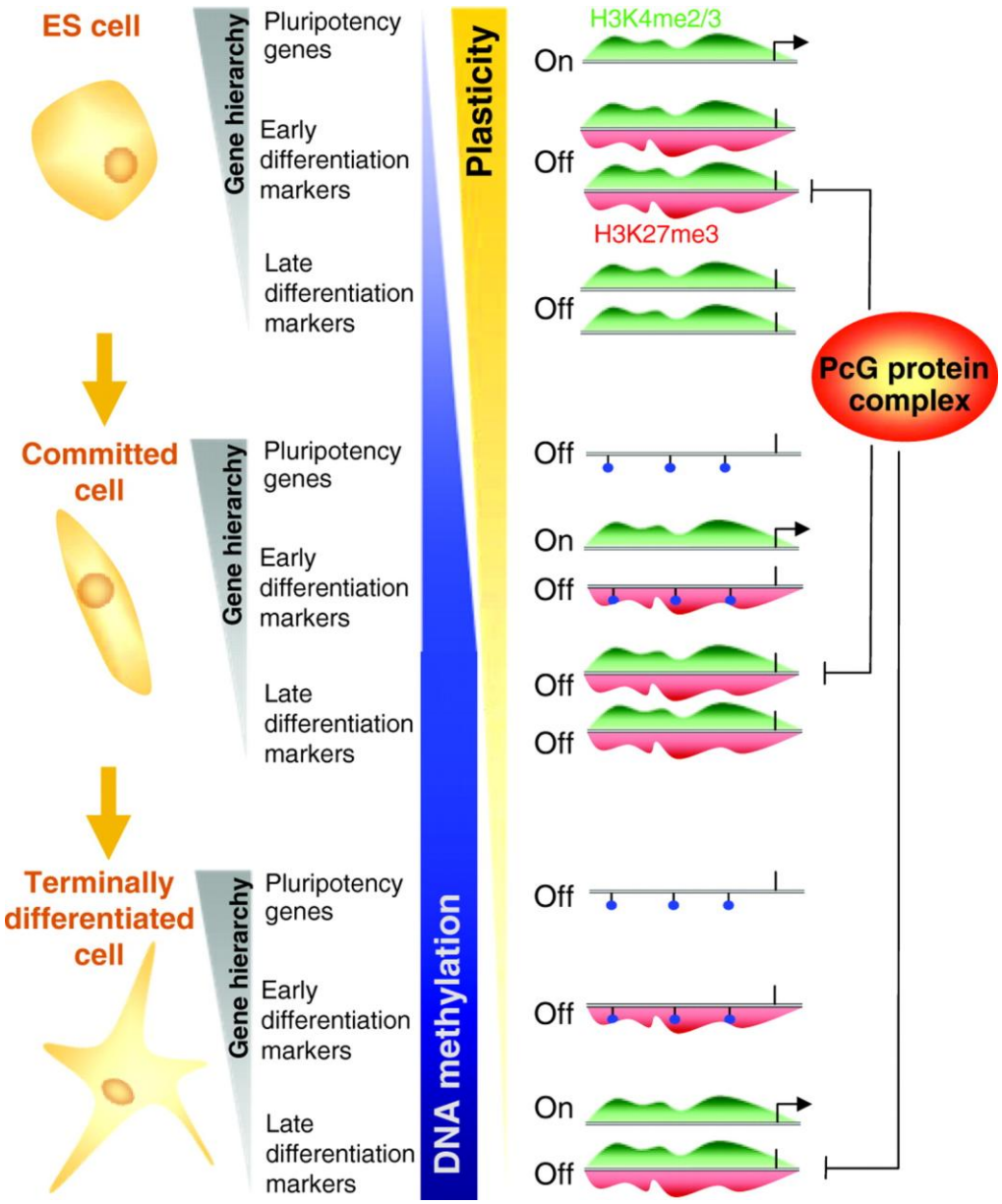
OCT4, SOX2, and Nanog: gene espressi nell'embrione responsabili della multi-potenza
Agiscono modificando l'asseto epigenetico dell'embrione
(modificazioni istoniche e metilazione cromatina)

Polycomb repressive complex (PRC2): gruppo delle proteine PolicombGroup (PcG), catalizza la di/tri metilazione dell'istone 3, residuo 27 della Lisina (H3K27me2/me3)

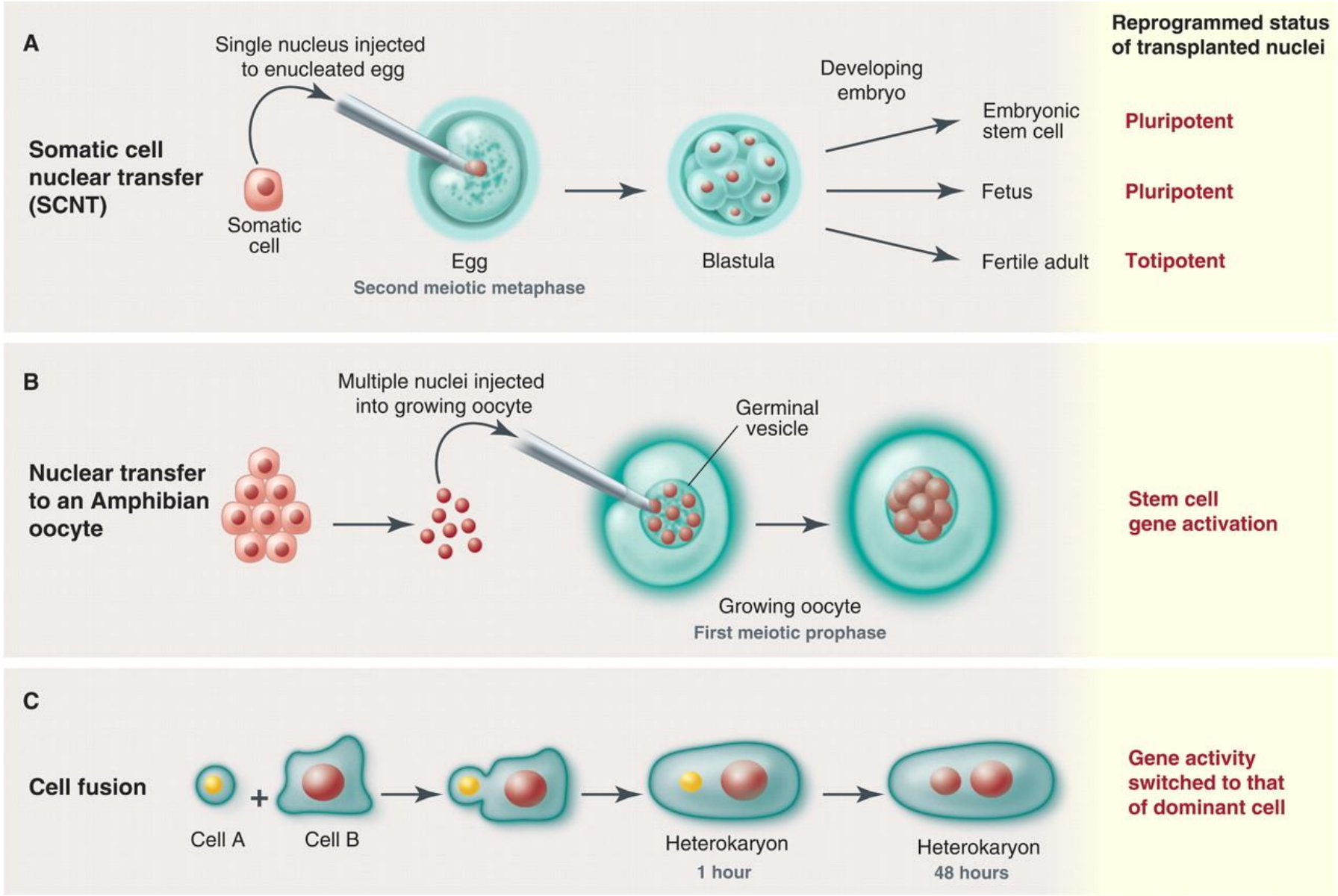
Trithorax group proteins (TrxG) TrxG sono reclutate a loci altamente trascritti, dove catalizzano la trimetilazione dell'istone 3 sulla lisina 4 (H3K4me3) promuovendo L'acetilazione del DNA e quindi la trascrizione

OCT4, SOX2, and Nanog

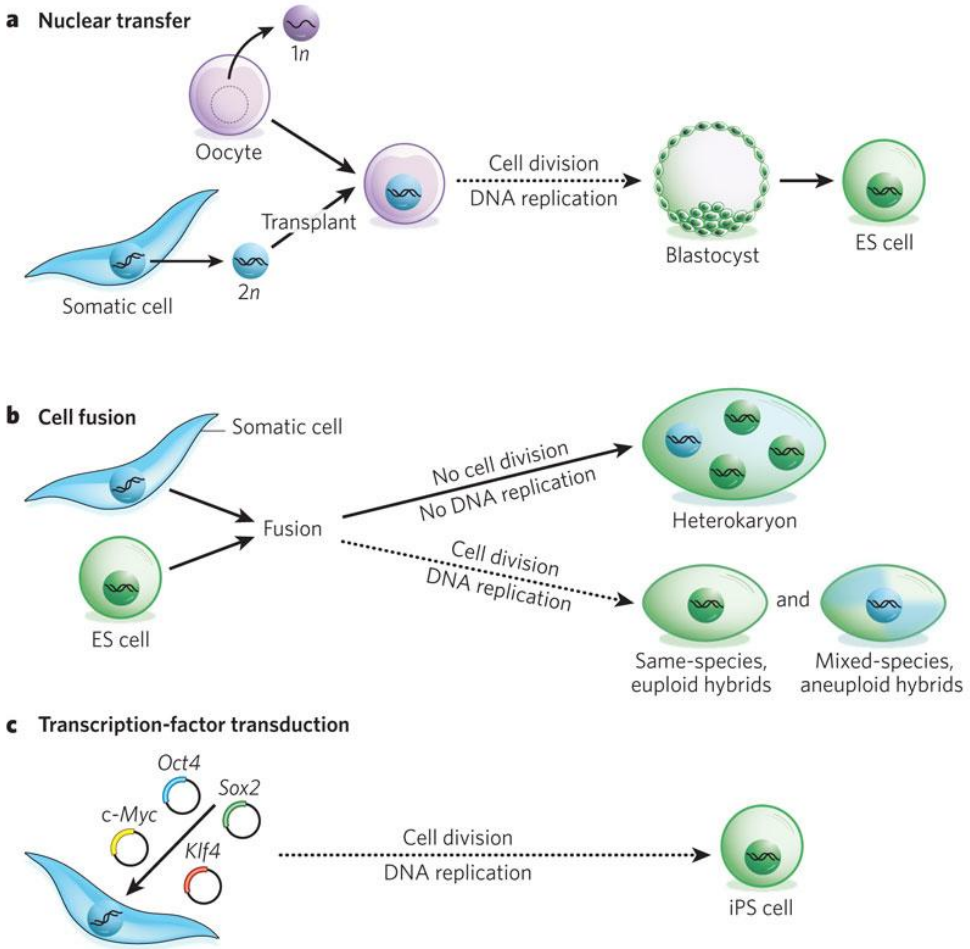



ES cell
Gene hierarchy
Pluripotency genes
Early differentiation markers
Late differentiation markers
Committed cell
Gene hierarchy
Pluripotency genes
Early differentiation markers
Late differentiation markers
Terminally differentiated cell
Gene hierarchy
Pluripotency genes
Early differentiation markers
Late differentiation markers
Plasticity
DNA methylation
H3K4me2/3
On
Off
Off
H3K27me3
Off
PcG protein complex
Off
On
Off
Off
Off
Off
Off
On
Off

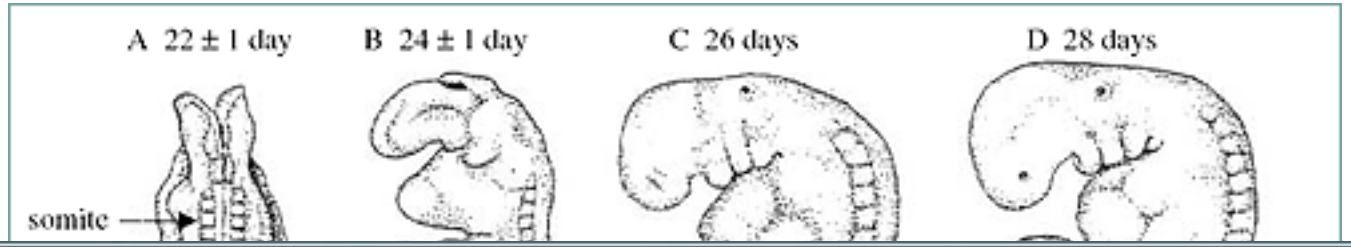
La differenziazione cellulare è un processo reversibile I

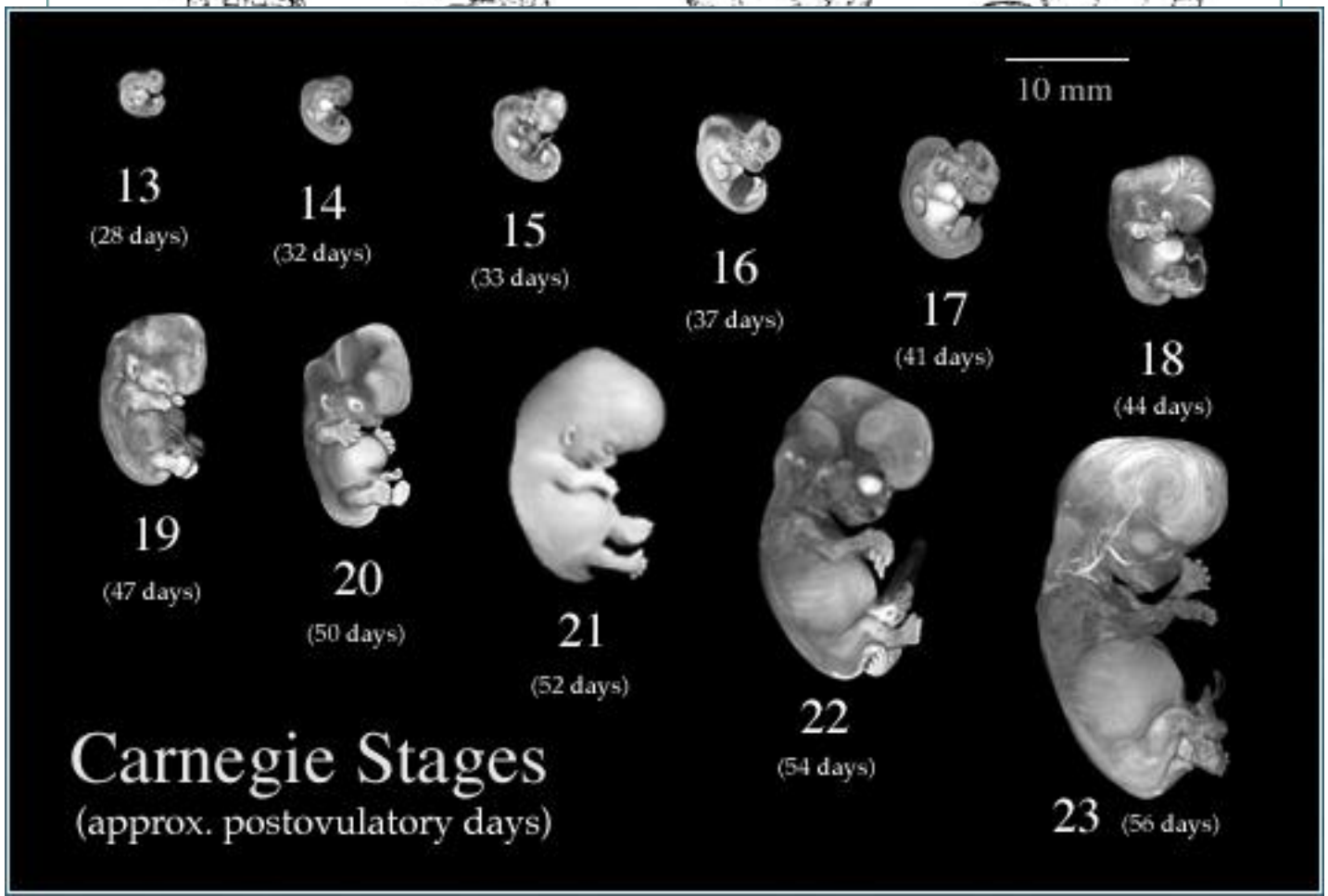


La differenziazione cellulare è un processo reversibile II

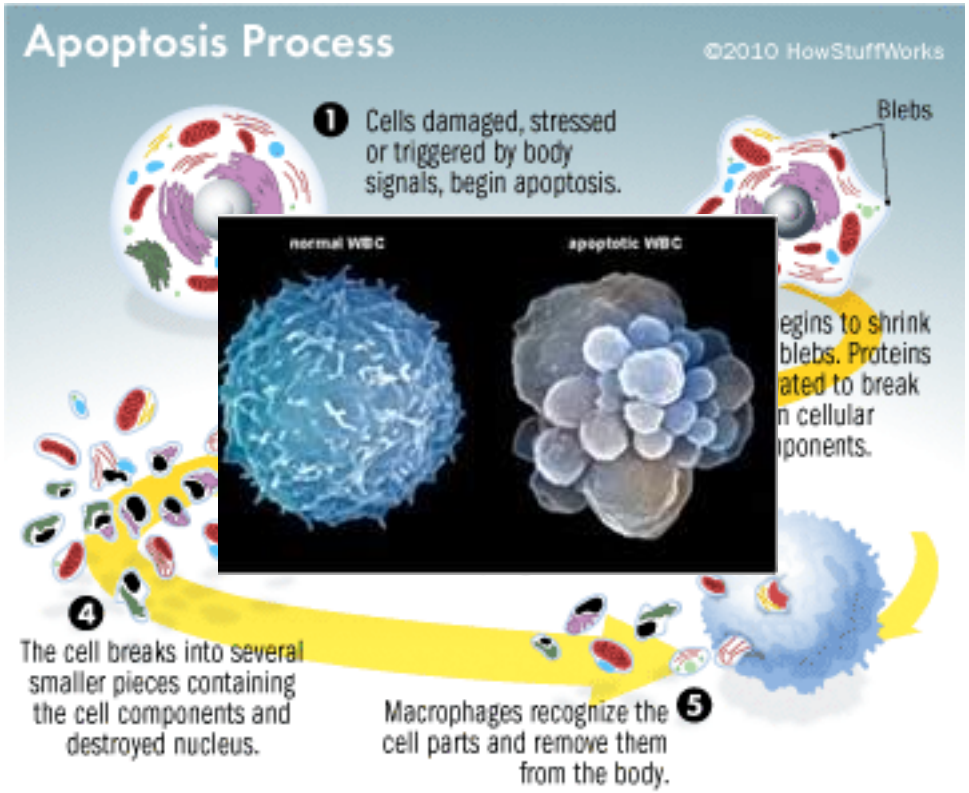


5) accrescimento





6) Morte cellulare programmata (apoptosi)



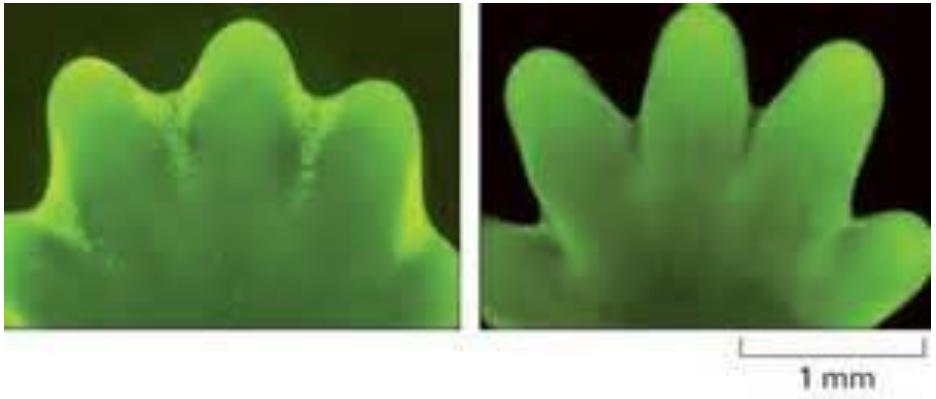
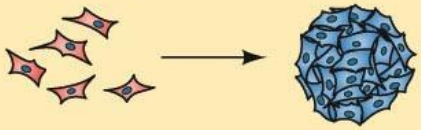
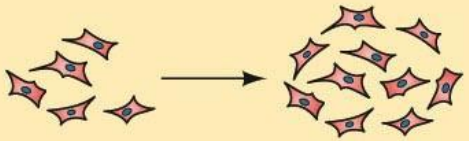
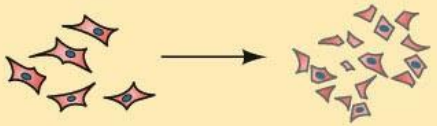
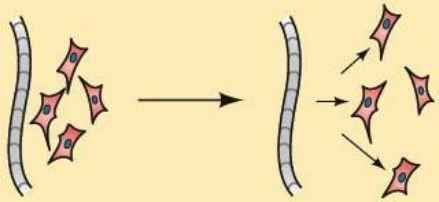
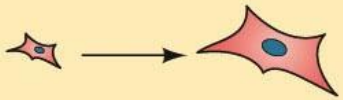


TABLE 1.1 **Summary of major morphogenic processes regulated by mesenchymal and epithelial cells** (Part 1)

Process	Action	Morphology	Example
MESENCHYMAL CELLS			
Condensation	Mesenchyme becomes epithelium		Cartilage mesenchyme
Cell division	Mitosis produces more cells (hyperplasia)		Limb mesenchyme
Cell death	Cells die		Interdigital mesenchyme
Migration	Cells move at particular times and places		Heart mesenchyme
Matrix secretion and degradation	Synthesis or removal of extracellular layer		Cartilage mesenchyme
Growth	Cells get larger (hypertrophy)		Fat cells

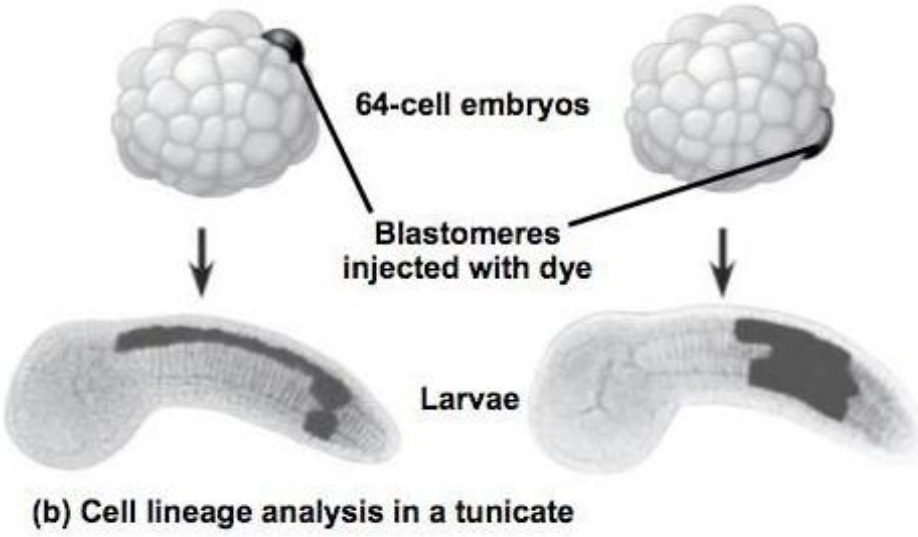
Modalità di studio della biologia dello sviluppo

Fondamentale la “mappatura” delle linee di discendenza cellulare

Strumenti:

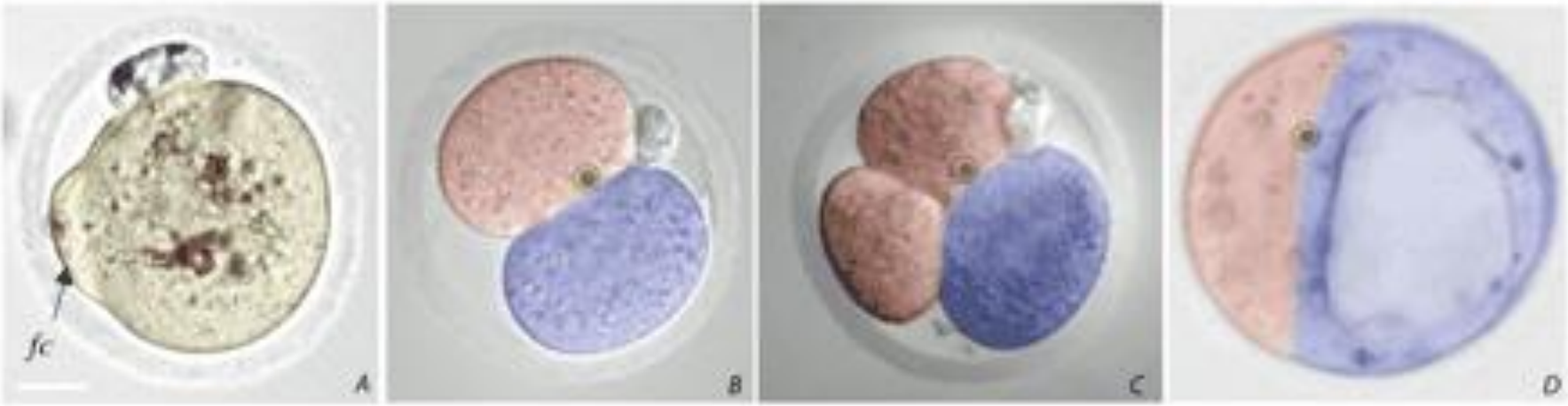
Osservazione diretta di embrioni viventi

Uso di coloranti “vitali”

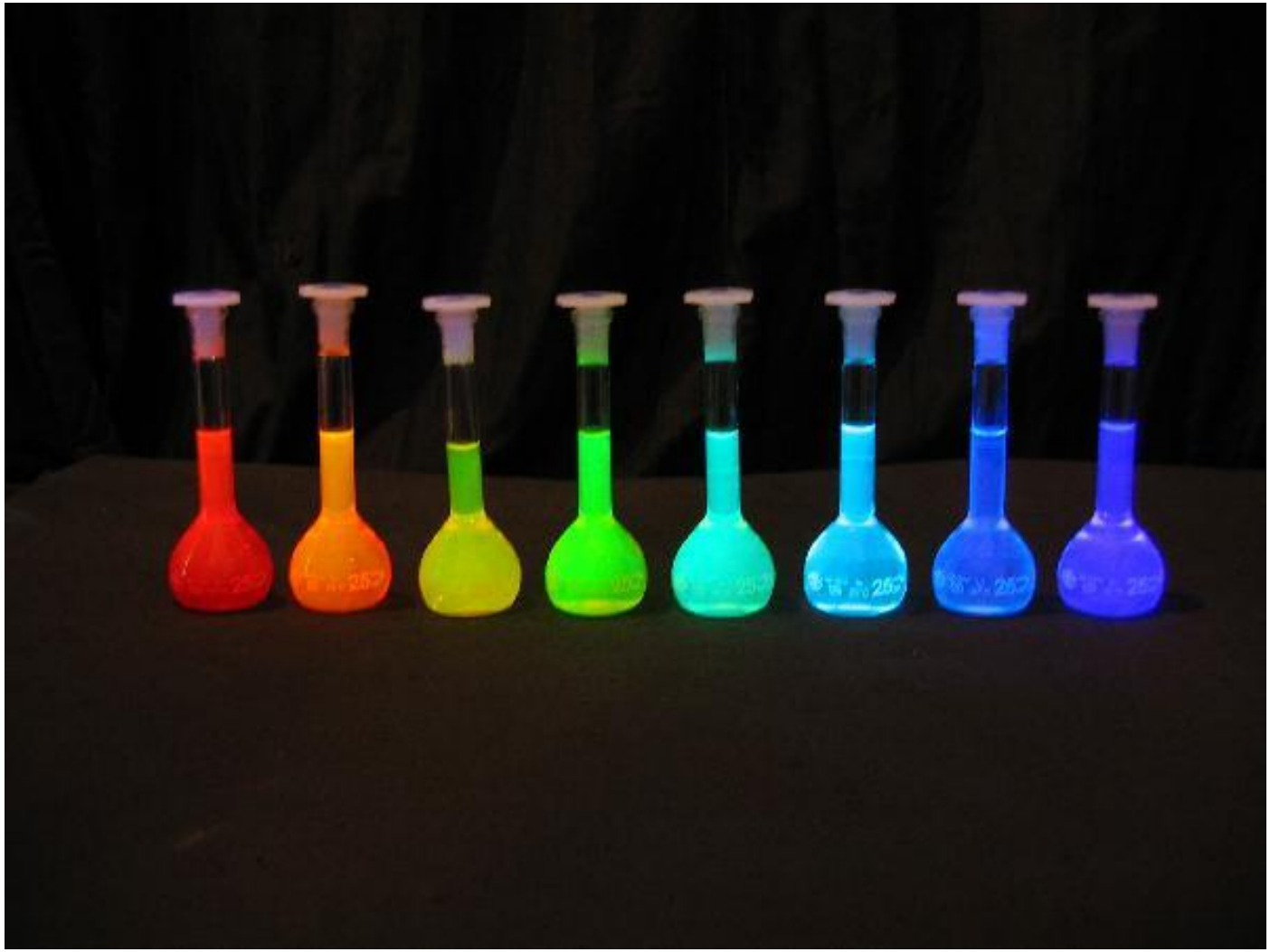


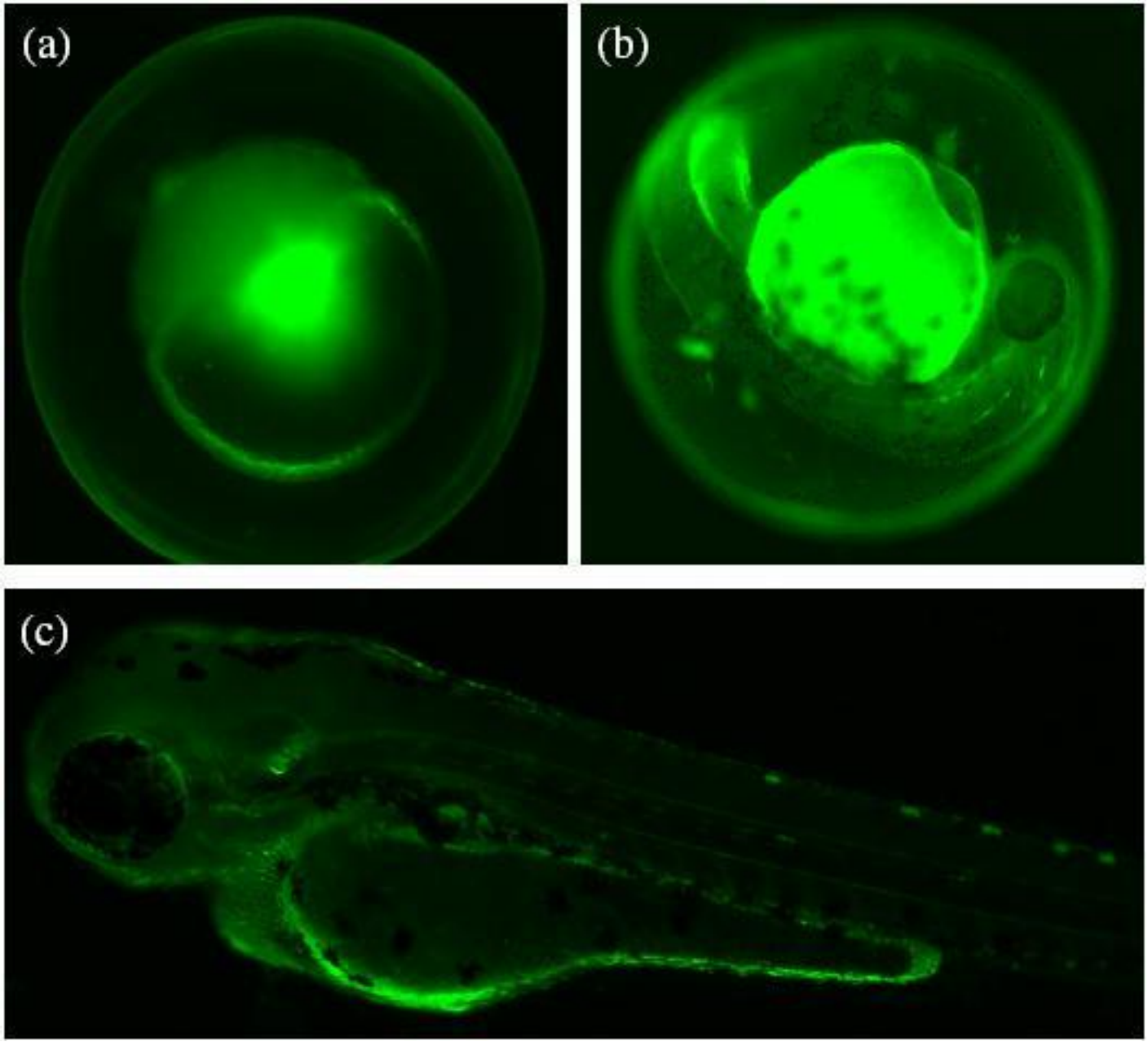
(b) Cell lineage analysis in a tunicate

Mappaggio cellulare mediante biglie e coloranti vitali lipofilici



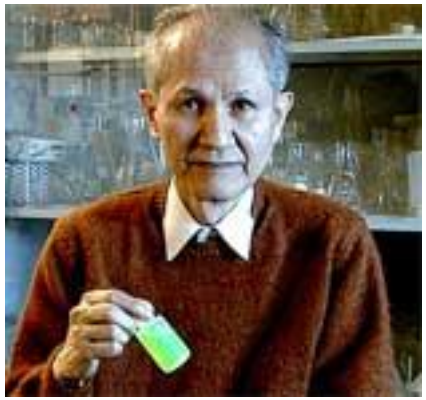
Uso di coloranti fluorescenti

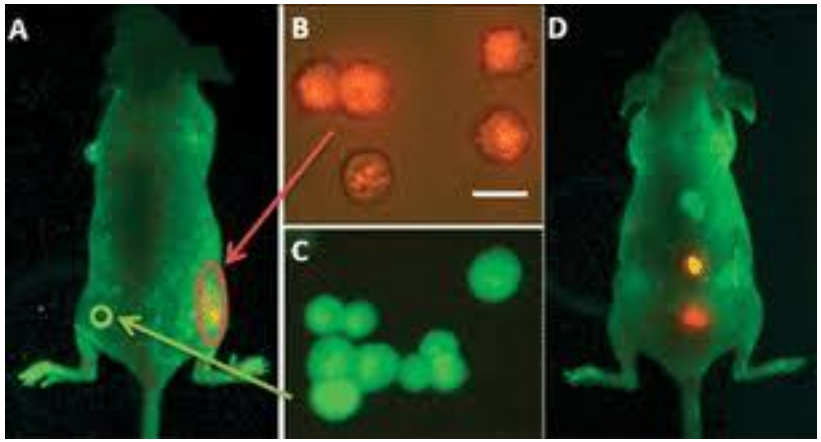


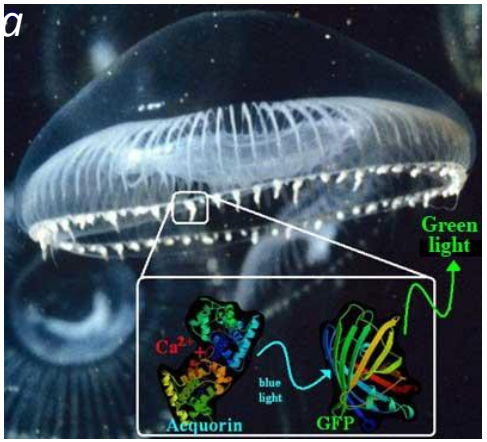

(a)
(b)
(c)

Una rivoluzione nello studio della biologia: Green Fluorescent Protein (GFP)

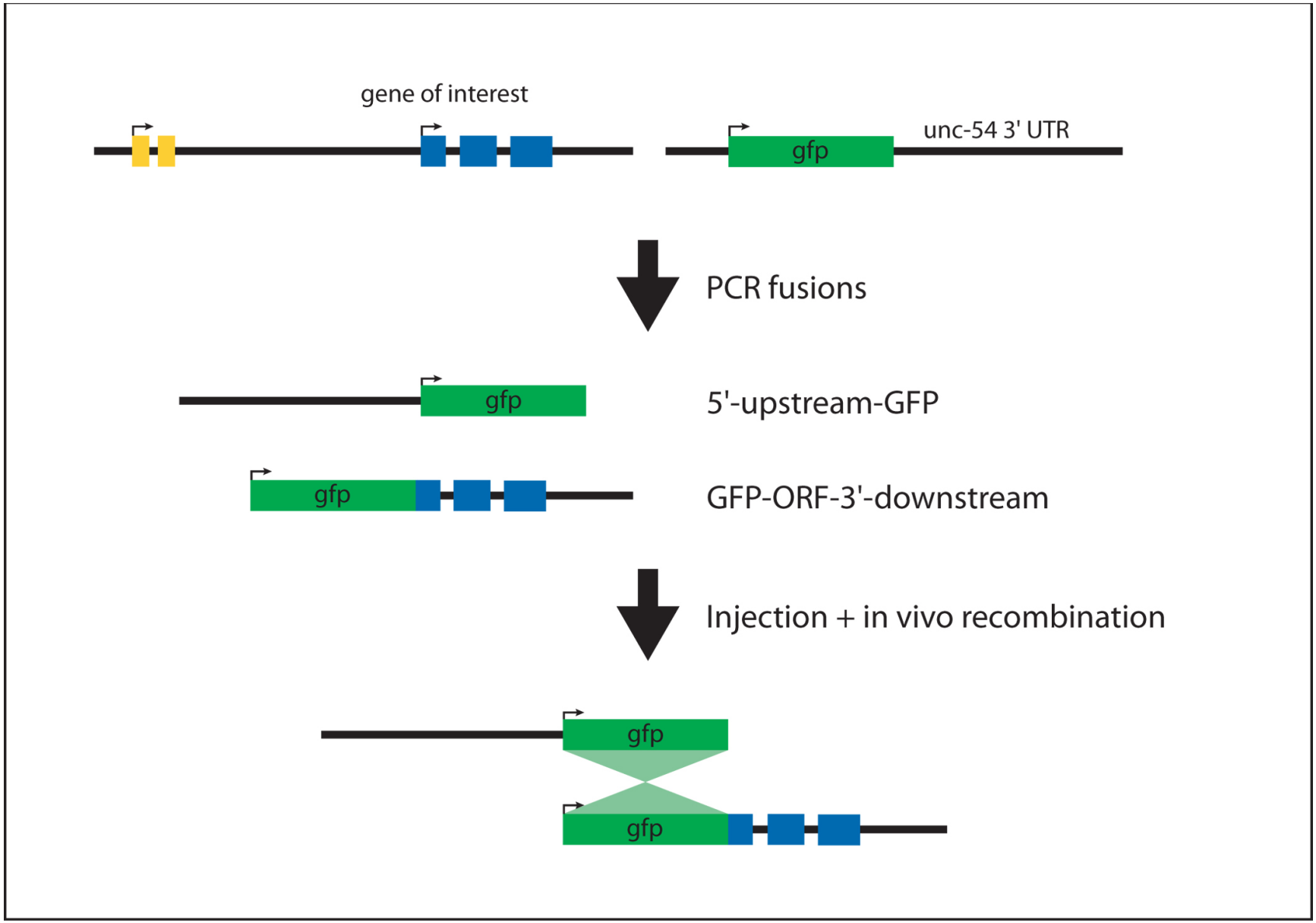
Osamu Shimomura
Premio Nobel 2008



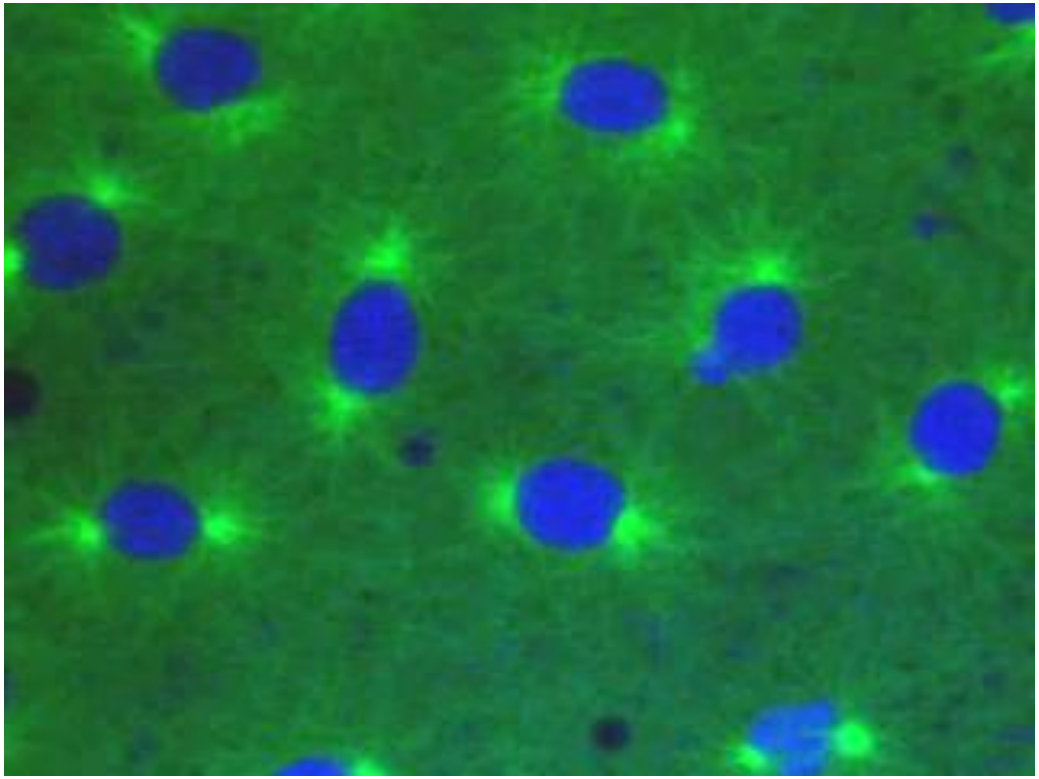




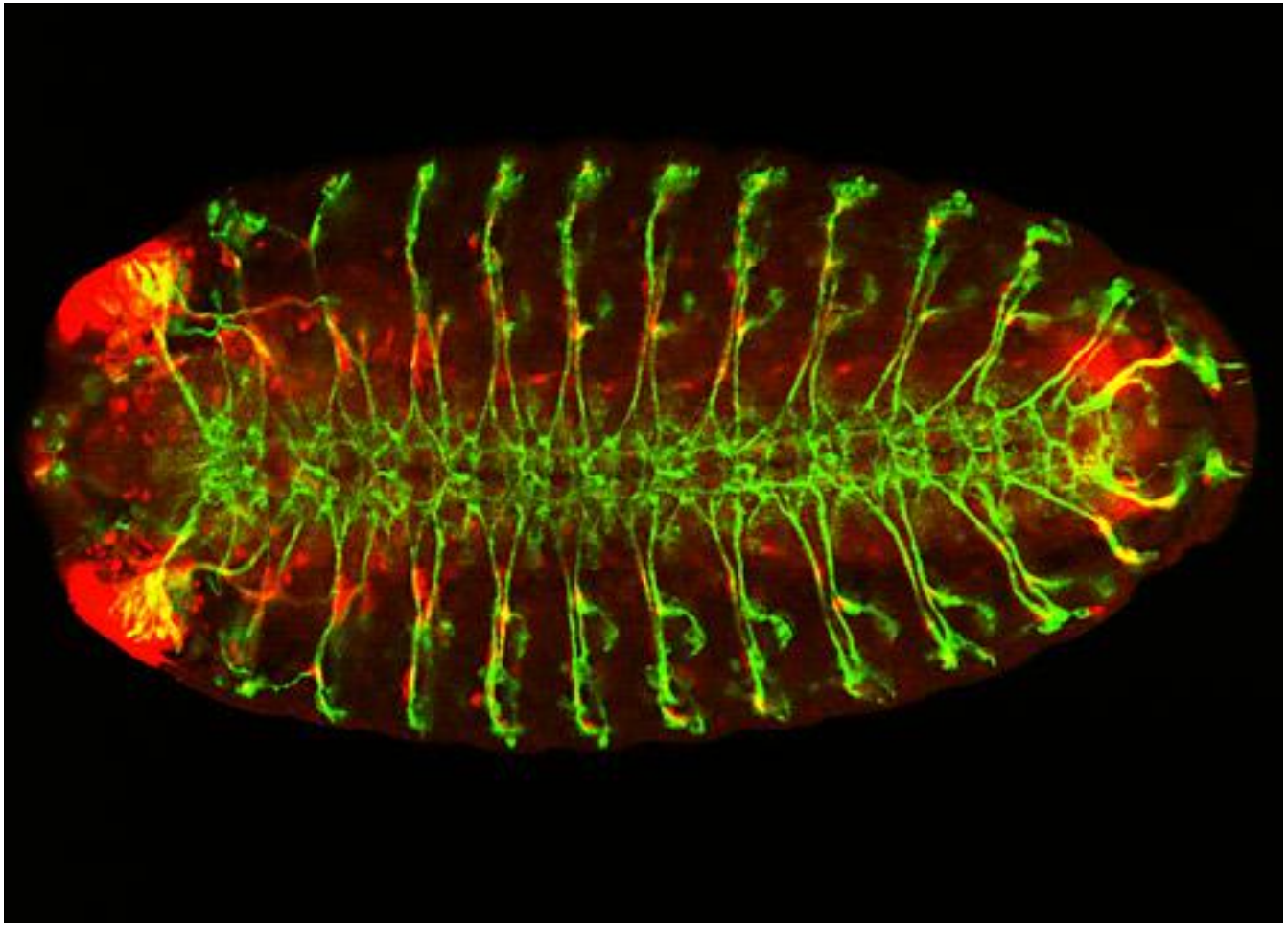
Produzione di un costrutto con la GFP



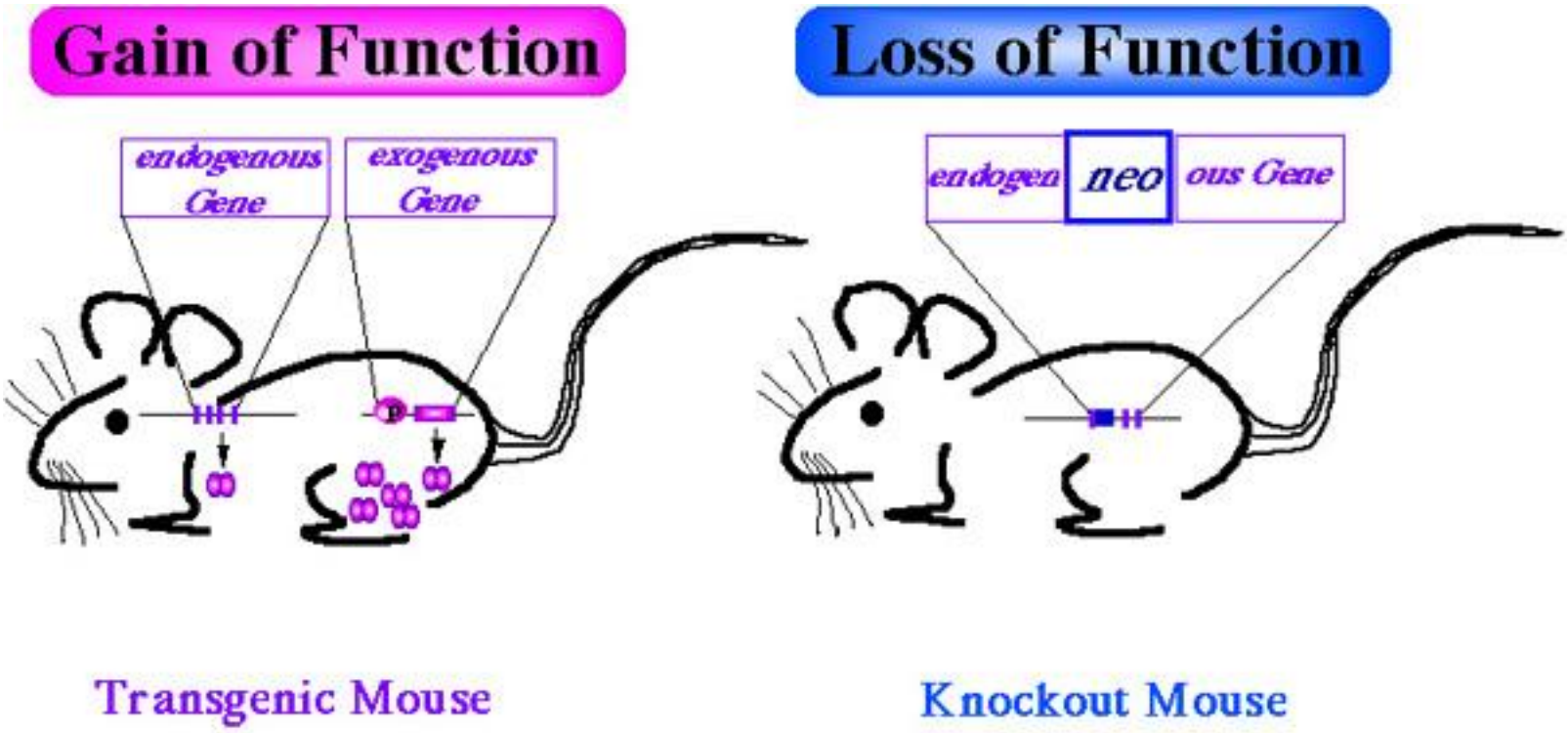
Embrione di Drosofila Melanogaster con tubulina "taggata" con GFP



Drosofila: visualizzazione del sistema nervoso (nestin GFP)



Ablazione/aumento funzione di un dato gene (Knockout - KO)

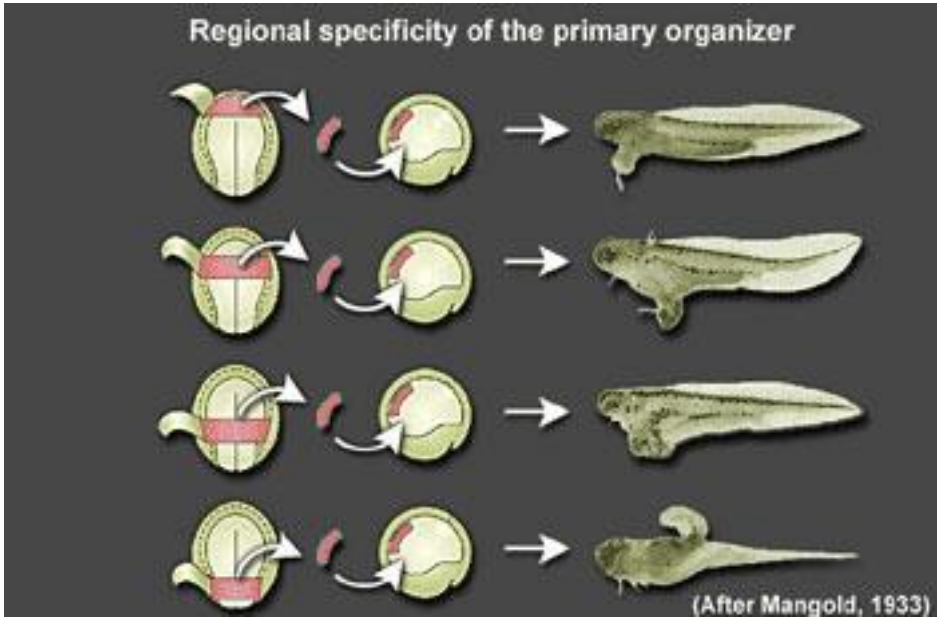


O

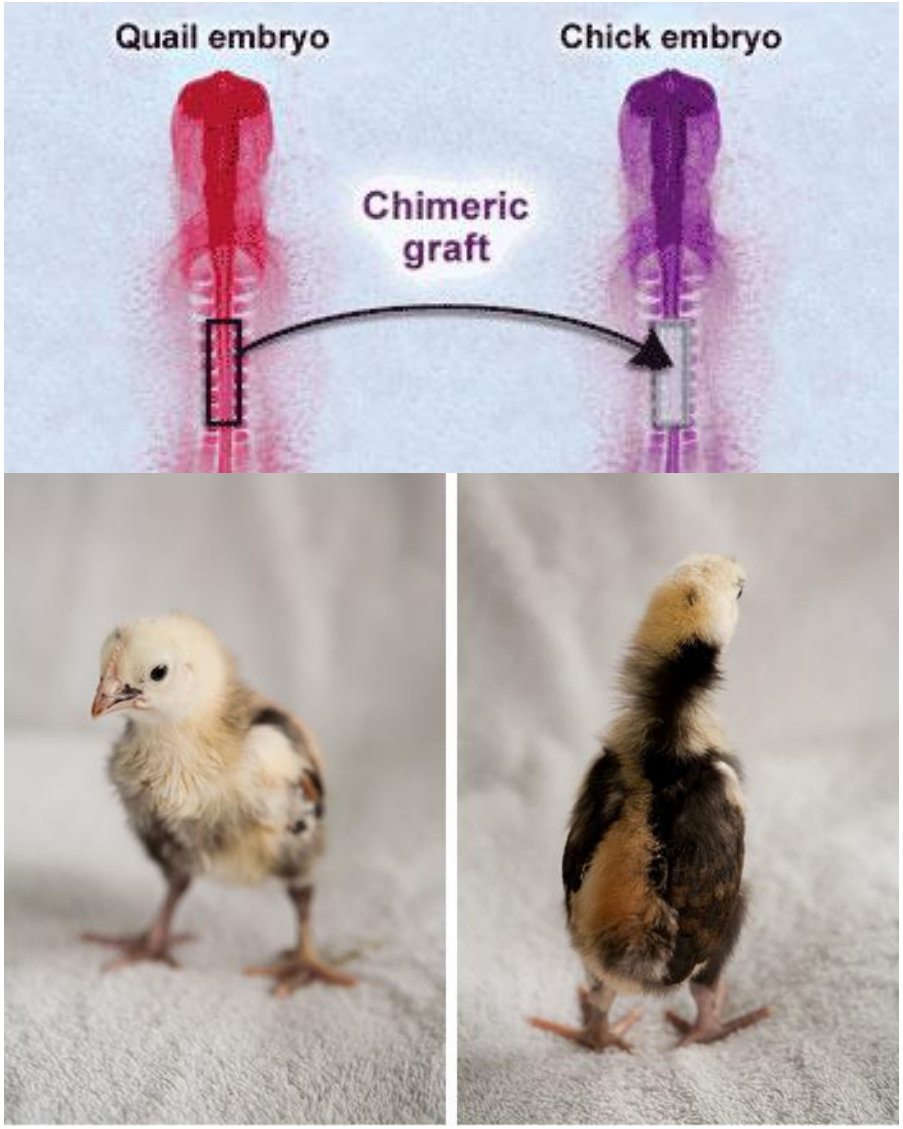
no.

Generazione di chimere

Una chimera è un individuo formato da due o più tipi
Di cellule di animali diversi



Chimere quaglia/pollo



Dissezione e micromanipolazione:
Trapianto nucleare