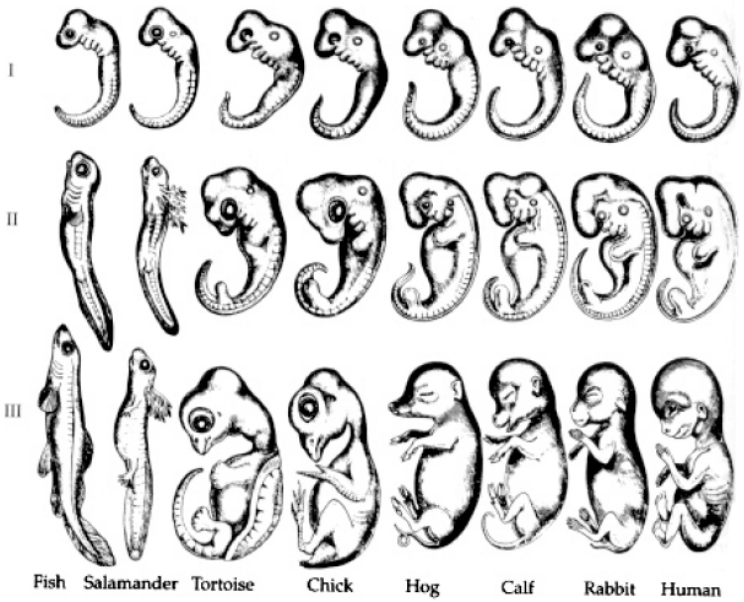
胚胎發育及幹細胞

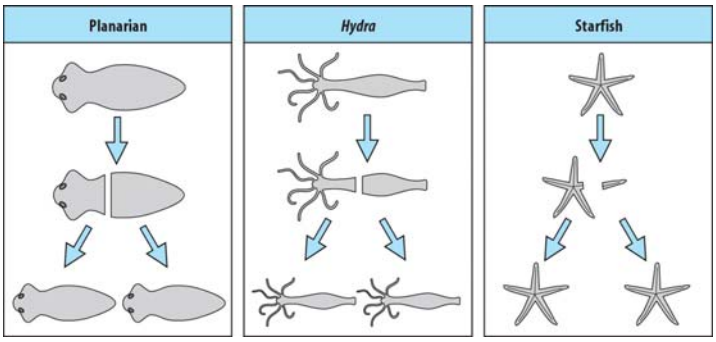
中山大學
生物科學系
吳長益

Embryos



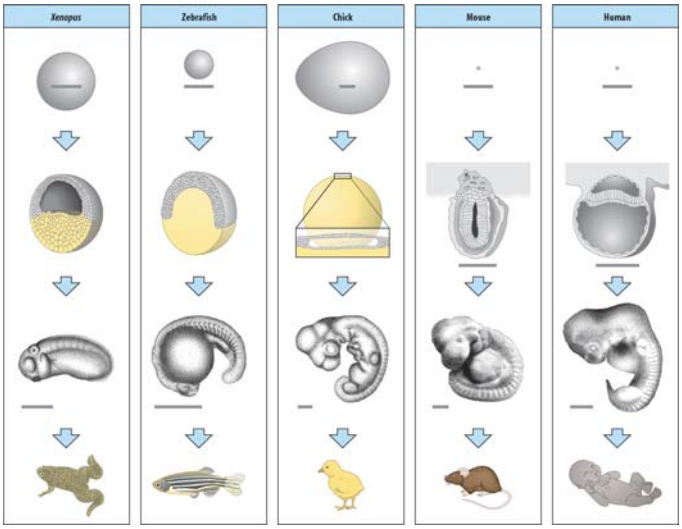
Outline

1. 胚胎學 **(embryology) vs.** 發育生物學 **(developmental biology)**
2. 模式生物 **(model organisms)**
3. 動物發育的主要階段
4. 發育過程之機制
5. 組織再生與幹細胞



胚胎學 **(Embryology) :** The development of an embryo from the fertilized egg

發育生物學 **(Developmental Biology)** is to understand how multicellular organism develop



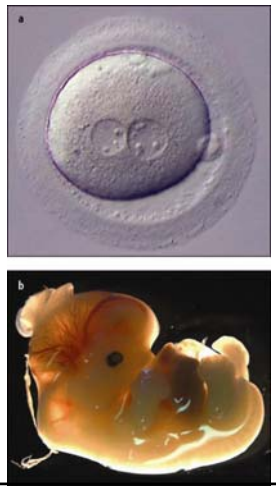
何謂發育生物學？ A Body-Building Plan

- 發育生物學(developmental biology) 源自於胚胎學(embryology)
- 胚胎學：探討受精卵到出生的過程
- 發育生物學包含：

  胚胎學

  +

個體生長、器官及組織受損的修復與再生、 老化、疾病的發生與治療等

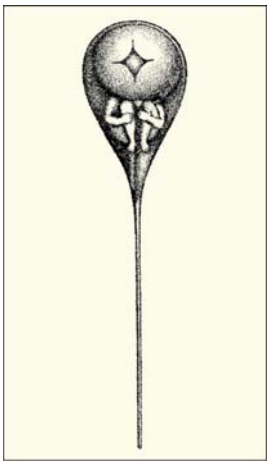


胚胎學上古老的爭論

Preformation theory (先成說)

Epigenesis theory (漸成說)

胚胎的各部份是一開始就存在?
還是在發育過程中逐漸形成的？



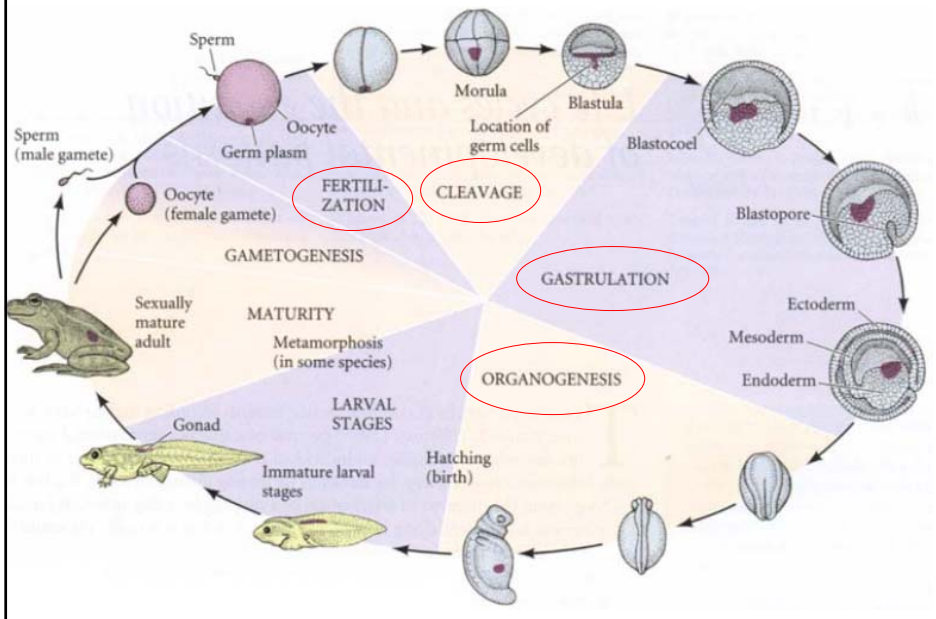
顯微鏡的發明

細胞學說的確立

基因概念的形成

胚胎發育符合漸成說的理論

發育的觀念: **A Life cycle of Frog**



發育的三要素:

1. 生長/分裂 growth/cleavage

   Increase in cell number
   Increase in cell size

2. 分化 Differentiation

   The fate of daughter cells

3. 形態發生 Morphogenesis

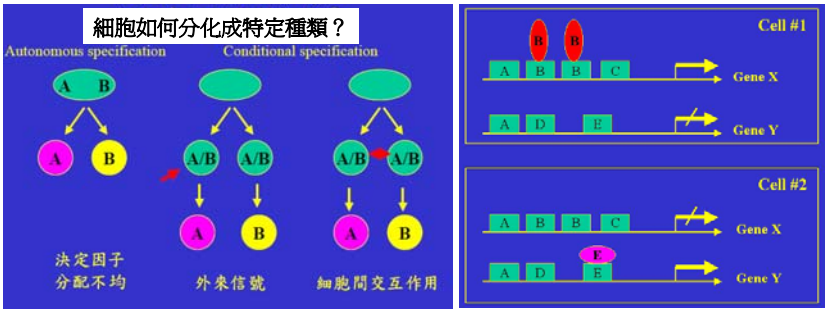
   The formation of shapes and patterns

發育的中心主題:

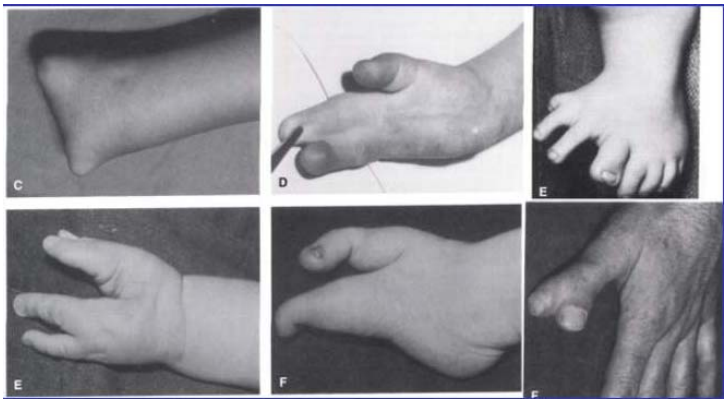
細胞如何分化成特定種類？

細胞如何移動到特定位置？

如何控制這些細胞發育成爲不同類型的細胞組織？



What happen if genetic control goes wrong in limb development?



發育出問題, 有些會遺傳, 因爲基因控制發育

模式生物

– nematode (*Caenorhabditis elegans*)
– fruit fly (*Drosophila melanogaster*)
– sea urchins
– South African Frog (*Xenopus laevis*)
– Zebrafish (*Danio Renio*)
– chick
– mouse
– plant (*Arabidopsis thaliana*)

用以研究發育生物學, 人類疾病

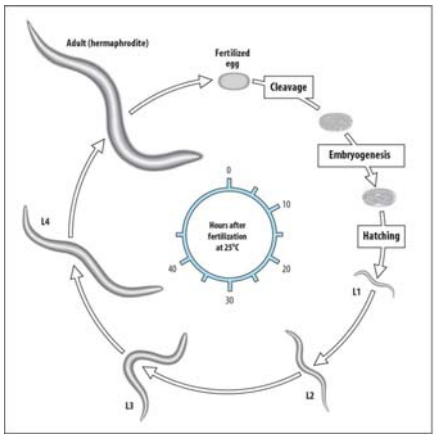
人類不利於作發育, 疾病模式之原因：

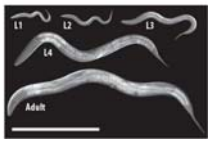
- 1.壽命長。
- 2.再生時間緩慢。
- 3.子代少。
- 4.極度異質性。
- 5.無法長期控制飼養方式及社經生活。
- 6.定量資料如飲食、飼養方面缺乏。
- 7.道德上的問題。

錯誤的觀念／有關實驗動物模式

- 1.動物是人類的迷你種。
- 2.動物模式是人類疾病的精確複製品。
- 3.在演化上居較高等級之動物，越能預測人類疾病。
- 4.囓齒類太小，不適合於大多數研究。
- 5.發現一個人類疾病動物模式相等於對人類疾病的了解有重大進展。

nematode (*Caenorhabditis elegans*)





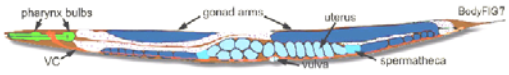
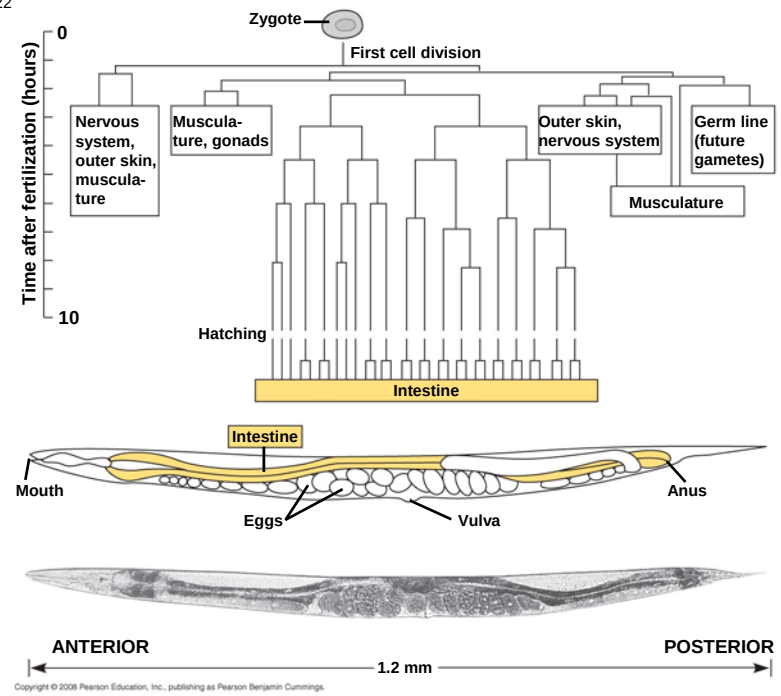
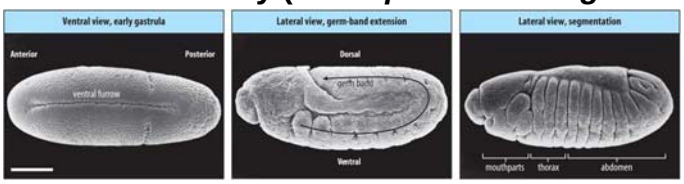
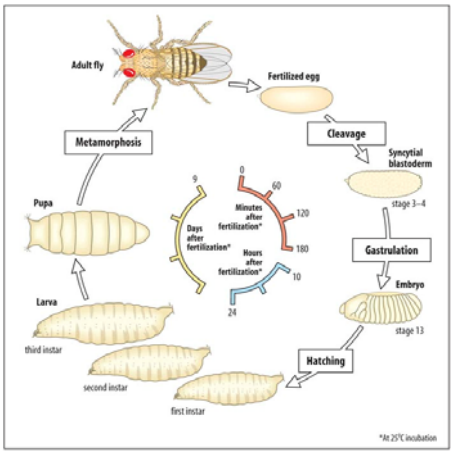


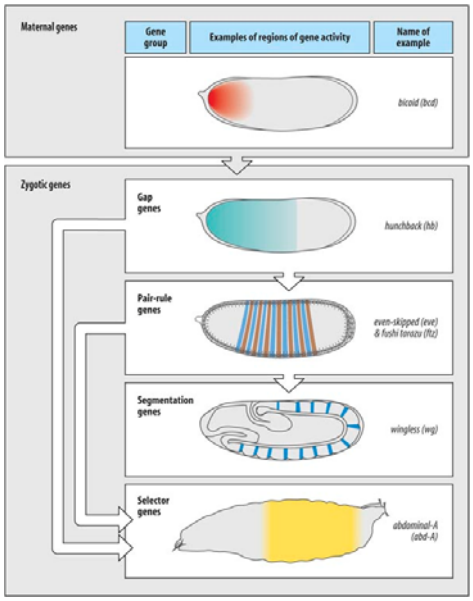
Fig. 47-22

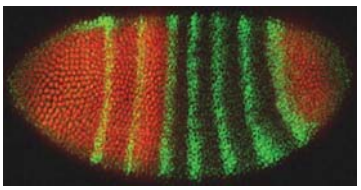


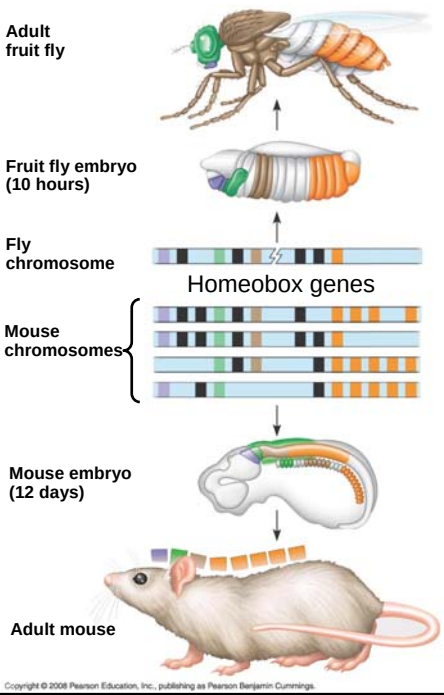
fruit fly (*Drosophila melanogaster*)

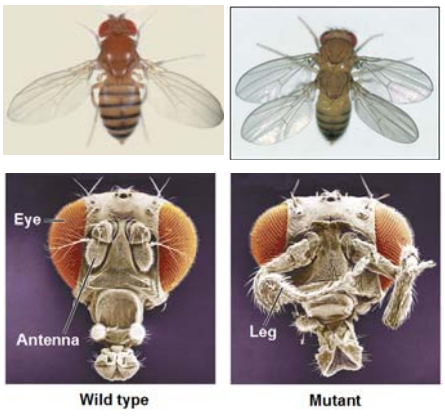




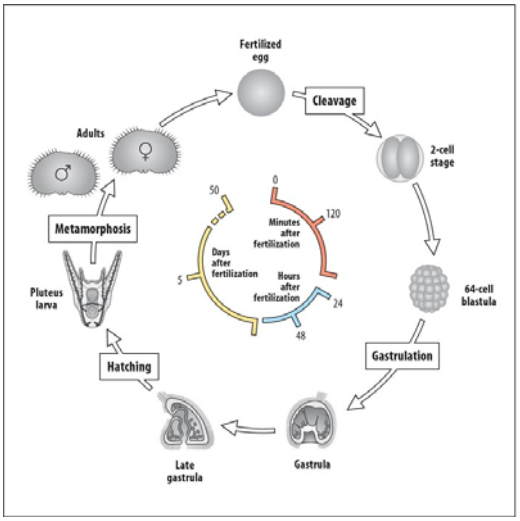


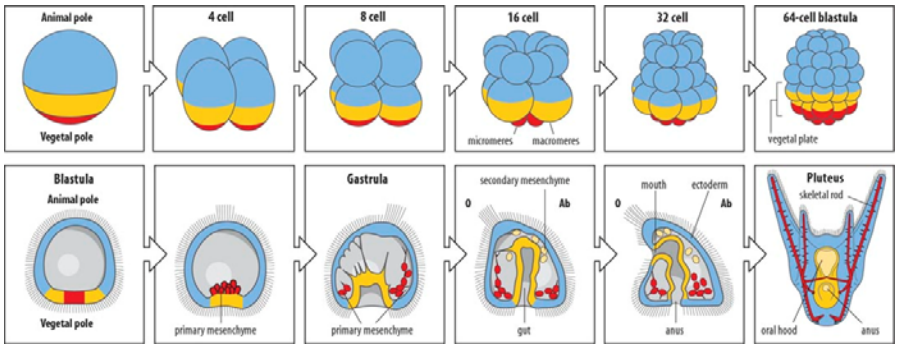




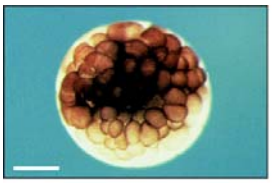


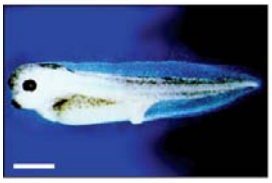
Sea urchin

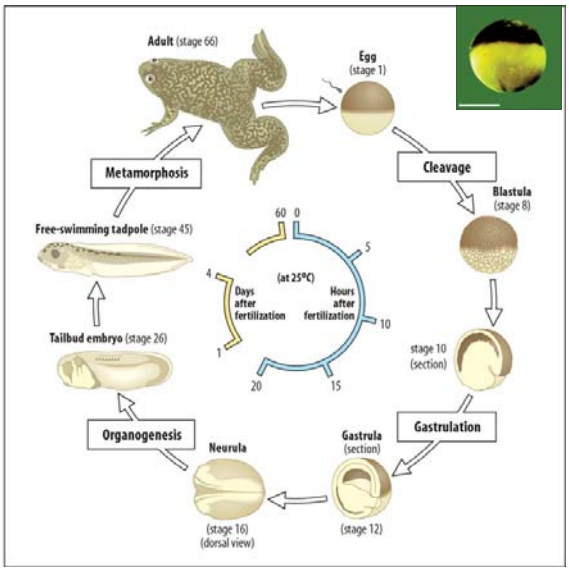

Fertilized egg
Cleavage
2-cell stage
64-cell blastula
Gastrulation
Gastrula
Late gastrula
Hatching
Pluteus larva
Metamorphosis
Adults
♂
♀
Minutes after fertilization
Hours after fertilization
Days after fertilization
0
120
24
48
5
50


Animal pole
Vegetal pole
4 cell
8 cell
16 cell
micromeres
macromeres
32 cell
64-cell blastula
vegetal plate
Blastula
Animal pole
Vegetal pole
primary mesenchyme
Gastrula
primary mesenchyme
secondary mesenchyme
0
Ab
gut
mouth
ectoderm
0
Ab
anus
Pluteus
skeletal rod
oral hood
anus

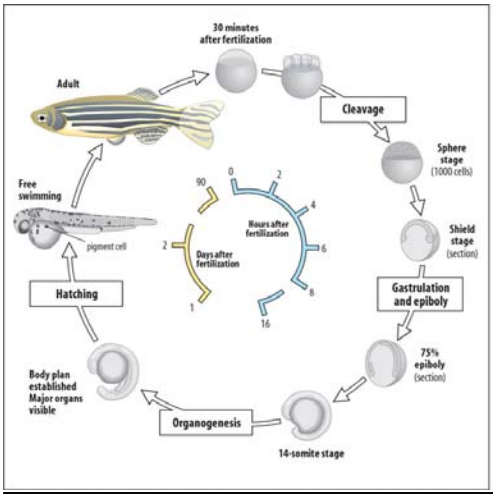
South African Frog (*Xenopus laevis*)

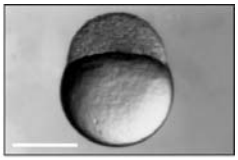


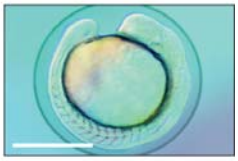


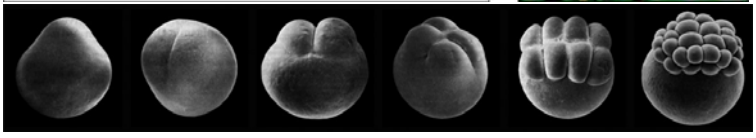


Zebrafish (*Danio Renio*)

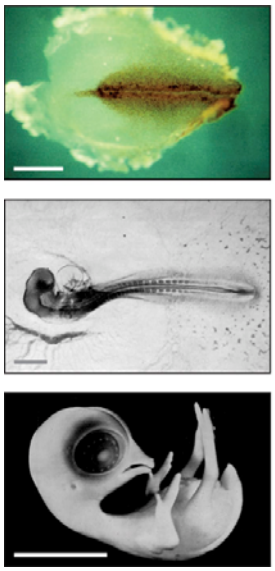


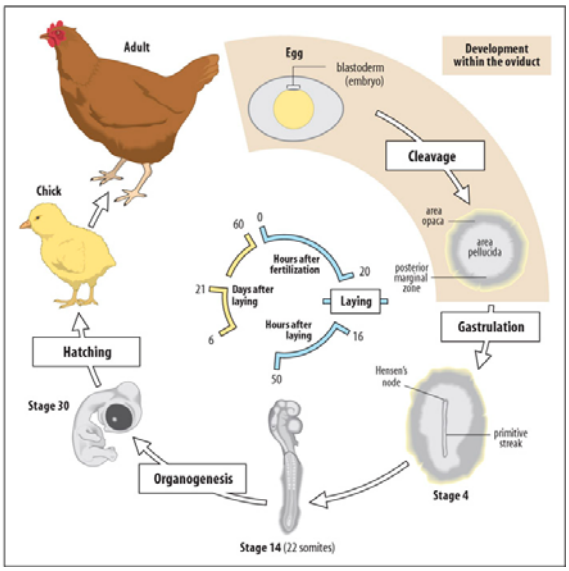


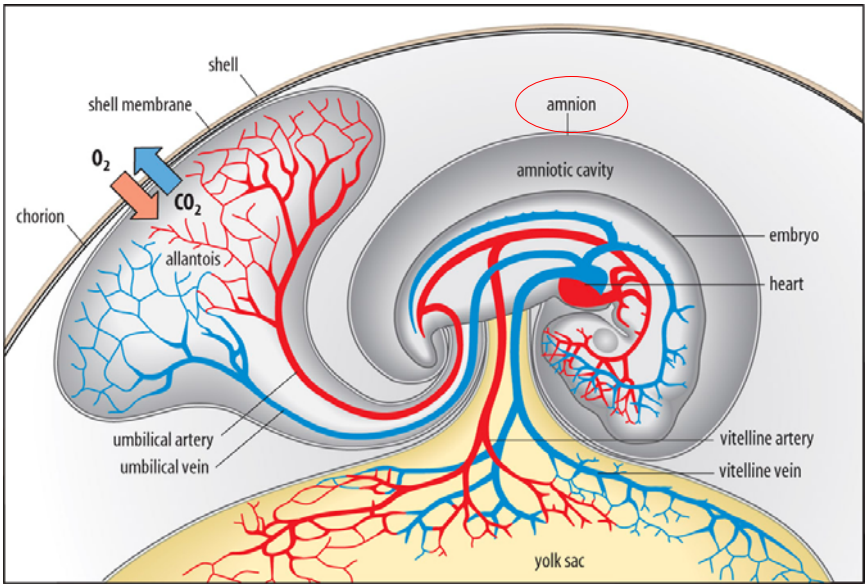




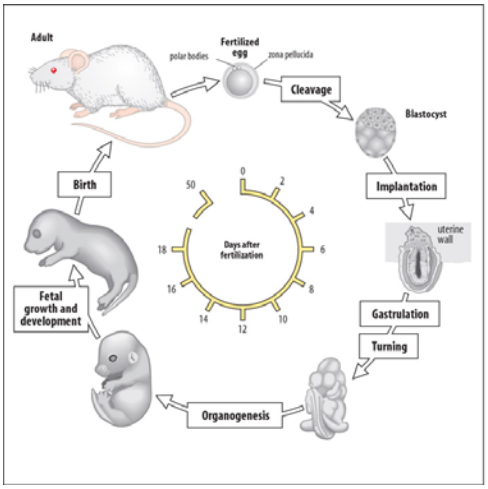
chick

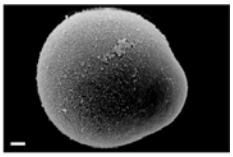


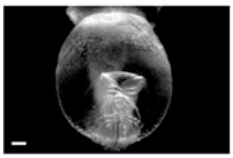

Adult
Egg
blastoderm (embryo)
Development within the oviduct
Cleavage
Chick
area opaca
area pellucida
posterior marginal zone
60
0
Hours after fertilization
20
21
Days after laying
Laying
6
Hours after laying
16
50
Gastrulation
Hatching
Hensen's node
primitive streak
Stage 30
Organogenesis
Stage 4
Stage 14 (22 somites)

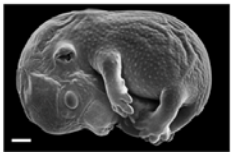

shell
shell membrane
amnion
O_2
amniotic cavity
CO_2
chorion
embryo
allantois
heart
umbilical artery
umbilical vein
vitelline artery
vitelline vein
yolk sac

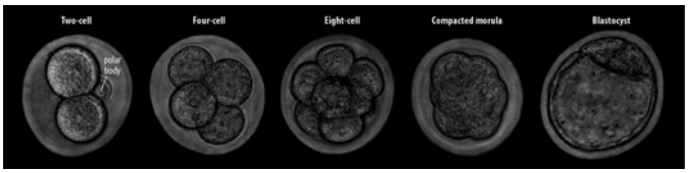
mouse



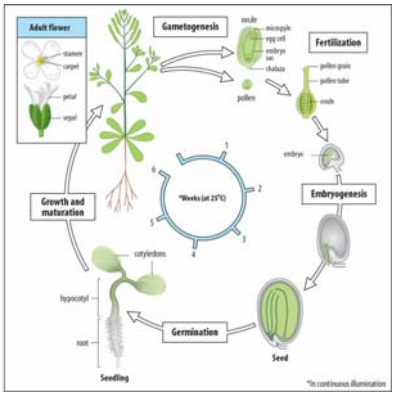


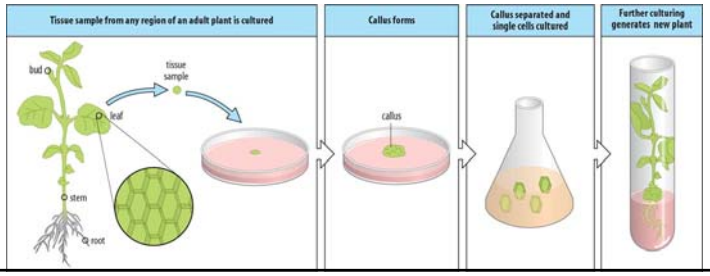






plant (*Arabidopsis thaliana*)





模式生物的特性

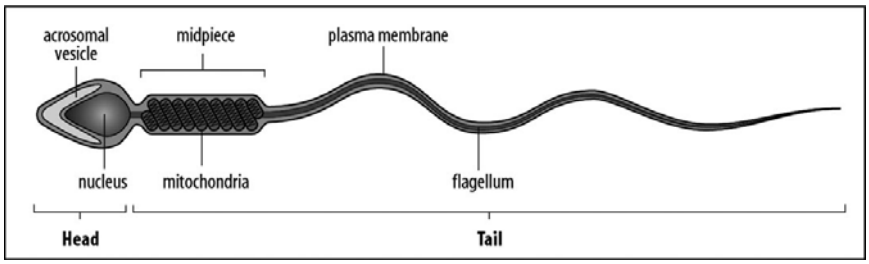
1. 與人類有相似處 (功能相似, 有基因保留性)
2. 易培養 (經費少, 空間小, 生長周期短, 繁殖快, 子代多)
3. 易於實驗操作 (體外受精, 胚體透明, 容易進行基因操控)
4. 資料多 (基因已解碼)

動物發育的主要過程

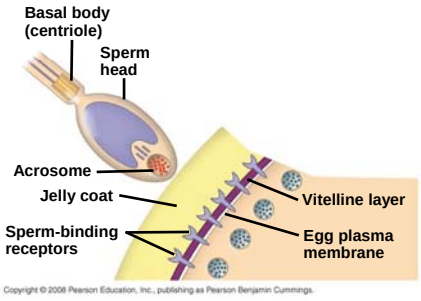
1. 受精 (Fertilization)
2. 卵裂 (Cleavage)
3. 原腸化 (Gastrulation)
4. 神經 (Neurulation)
5. 器官生成 (Organogenesis)
6. 個體生成 (Growth)

Fertilization

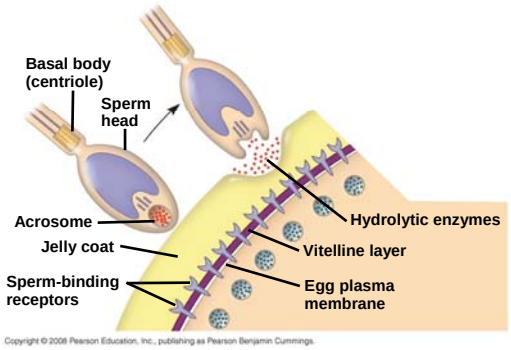
- Fertilization brings the haploid nuclei of sperm and egg together, forming a diploid zygote
- The sperm's contact with the egg's surface initiates metabolic reactions in the egg that trigger the onset of embryonic development



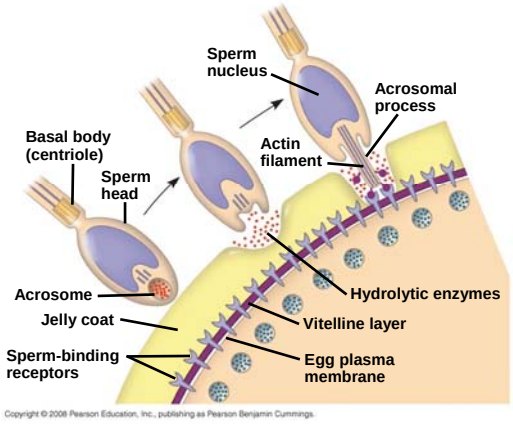
The acrosomal and cortical reactions during sea urchin fertilization



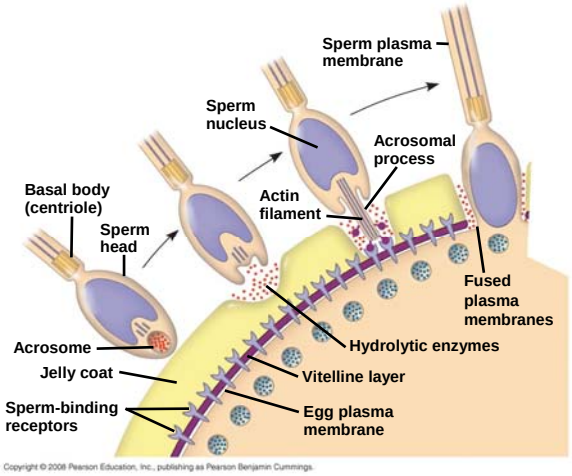
The acrosomal and cortical reactions during sea urchin fertilization



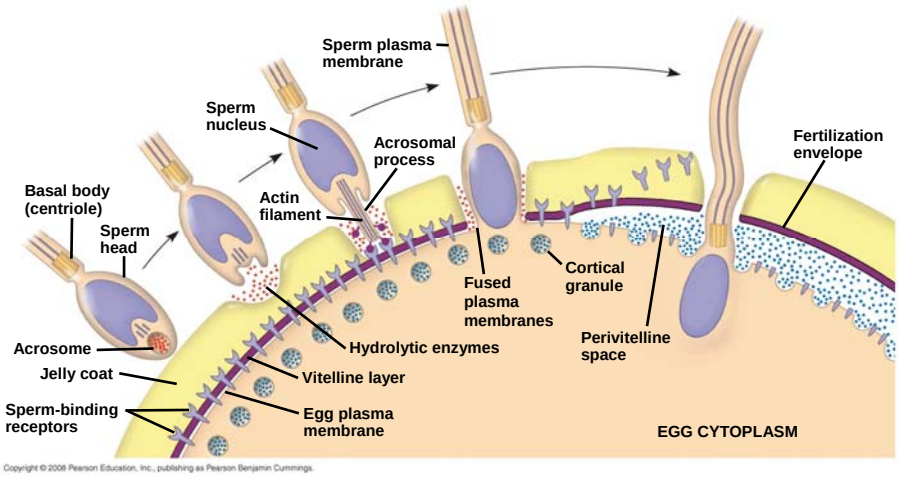
The acrosomal and cortical reactions during sea urchin fertilization



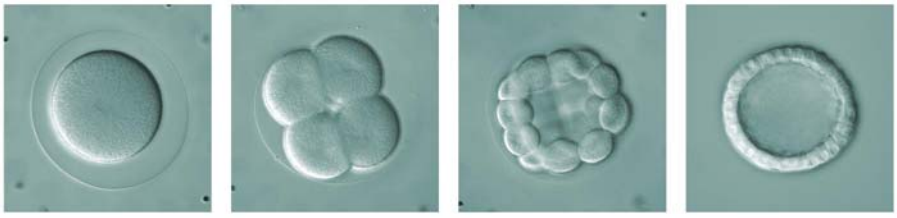
The acrosomal and cortical reactions during sea urchin fertilization



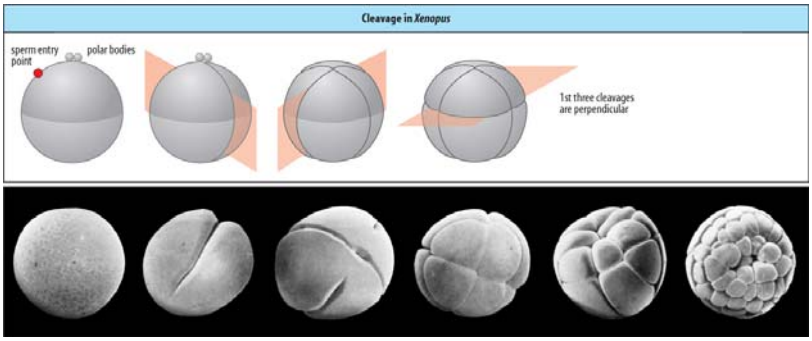
The acrosomal and cortical reactions during sea urchin fertilization

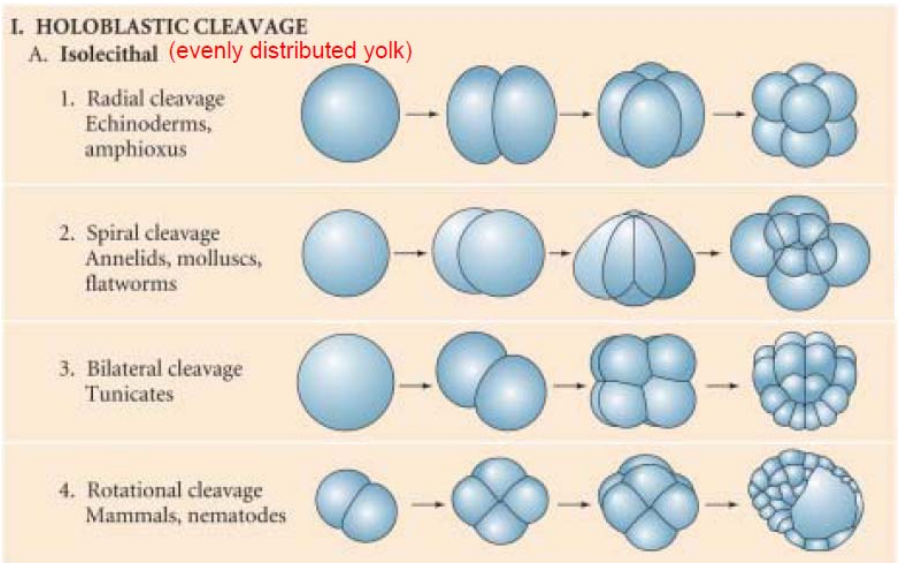


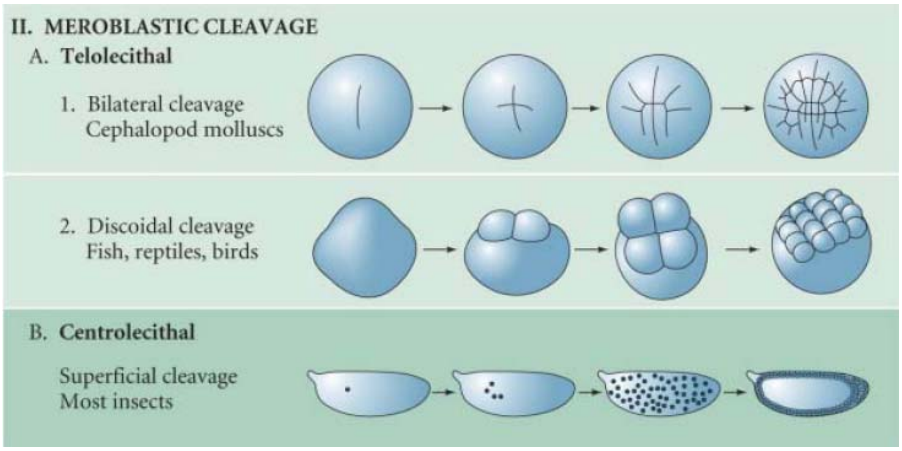
Cleavage

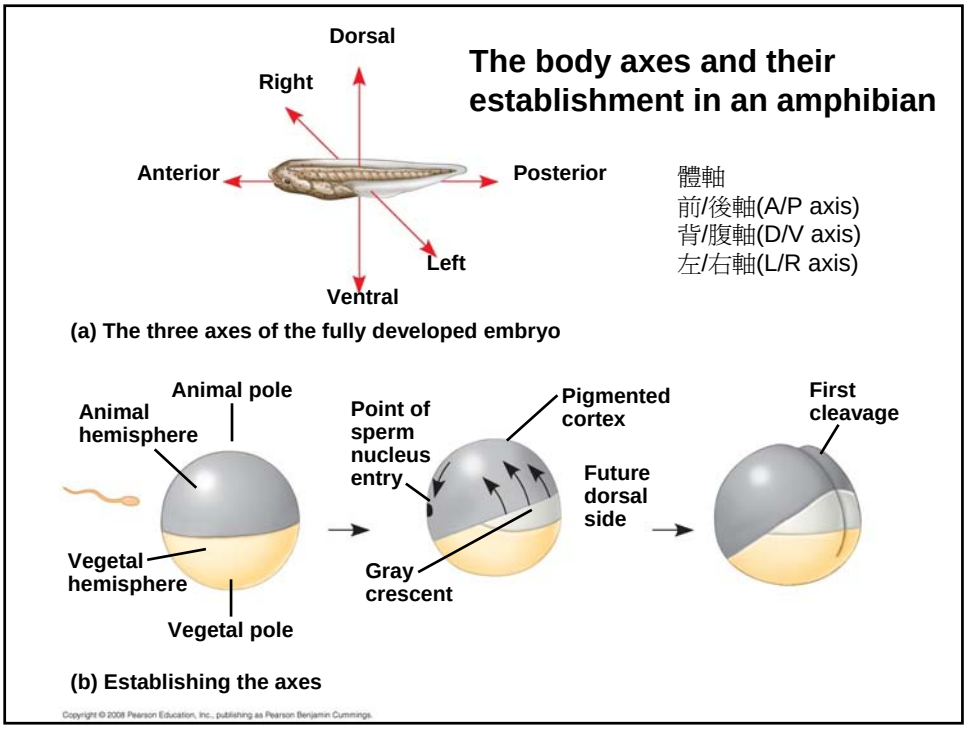


(a) Fertilized egg (b) Four-cell stage (c) Early blastula (d) Later blastula






II. MEROBLASTIC CLEAVAGE
A. Telolecithal
1. Bilateral cleavage
Cephalopod molluscs
2. Discoidal cleavage
Fish, reptiles, birds
B. Centrolecithal
Superficial cleavage
Most insects


The body axes and their establishment in an amphibian
Dorsal
Right
Anterior
Posterior
Left
Ventral
體軸
前/後軸(A/P axis)
背/腹軸(D/V axis)
左/右軸(L/R axis)
(a) The three axes of the fully developed embryo
Animal pole
Animal hemisphere
Vegetal hemisphere
Vegetal pole
Point of sperm nucleus entry
Pigmented cortex
Gray crescent
Future dorsal side
First cleavage
(b) Establishing the axes
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Cleavage in a frog embryo

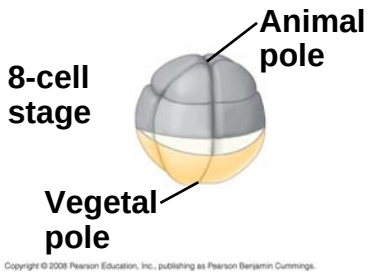
Zygote

Cleavage in a frog embryo

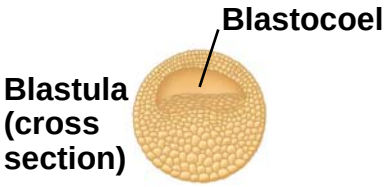
2-cell stage forming

Cleavage in a frog embryo

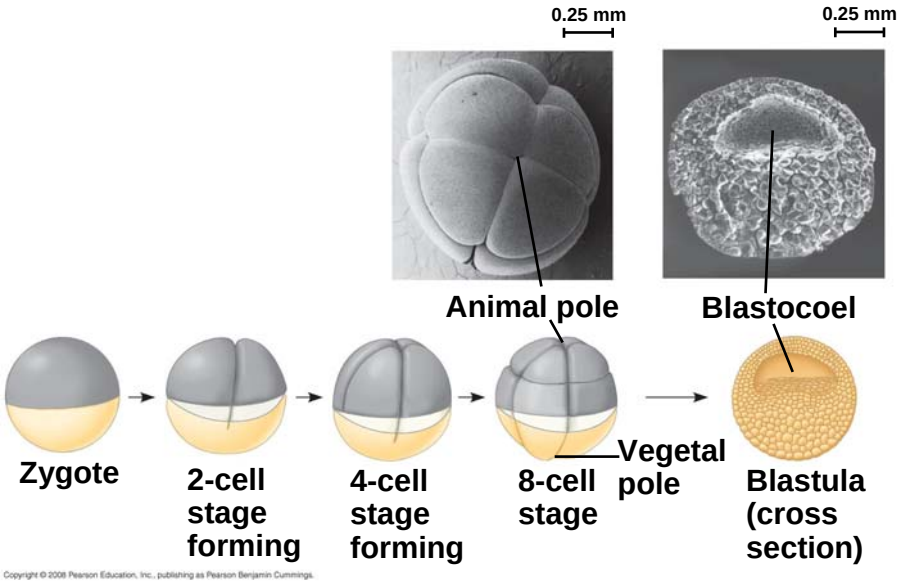
Cleavage in a frog embryo



Cleavage in a frog embryo



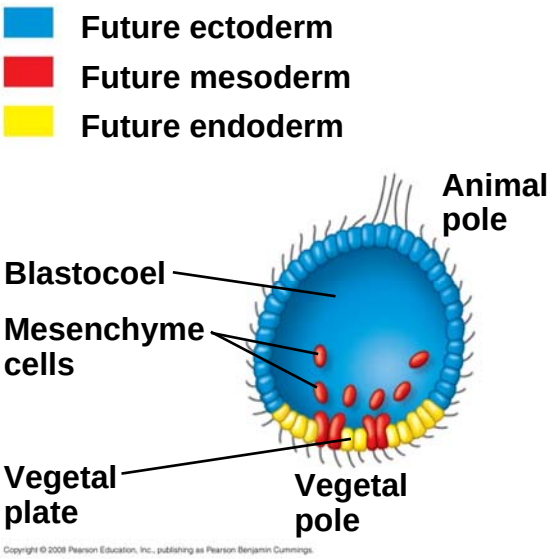
Cleavage in a frog embryo



動物發育的主要過程

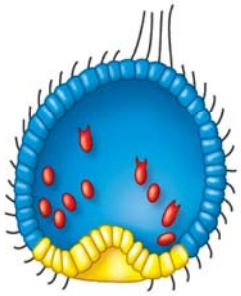
1. 受精 (Fertilization)
2. 卵裂 (Cleavage)
3. 原腸化 (Gastrulation)
4. 神經 (Neurulation)
5. 器官生成 (Organogenesis)
6. 個體生成 (Growth)

Gastrulation in a sea urchin embryo



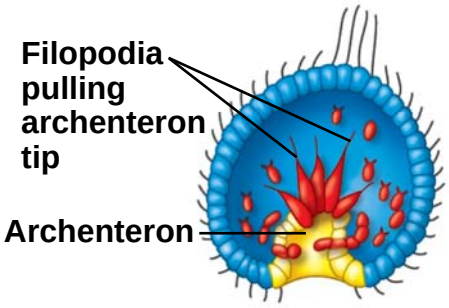
Gastrulation in a sea urchin embryo

Future ectoderm
Future mesoderm
Future endoderm



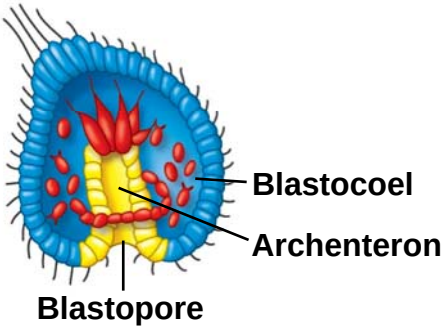
Gastrulation in a sea urchin embryo

Future ectoderm
Future mesoderm
Future endoderm



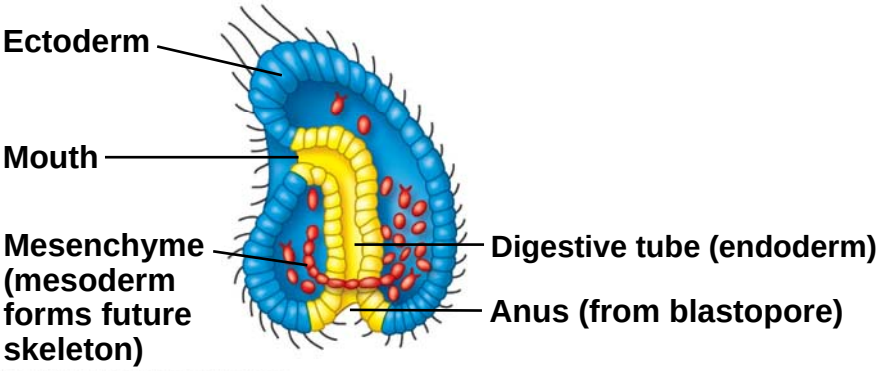
Gastrulation in a sea urchin embryo

Future ectoderm
Future mesoderm
Future endoderm

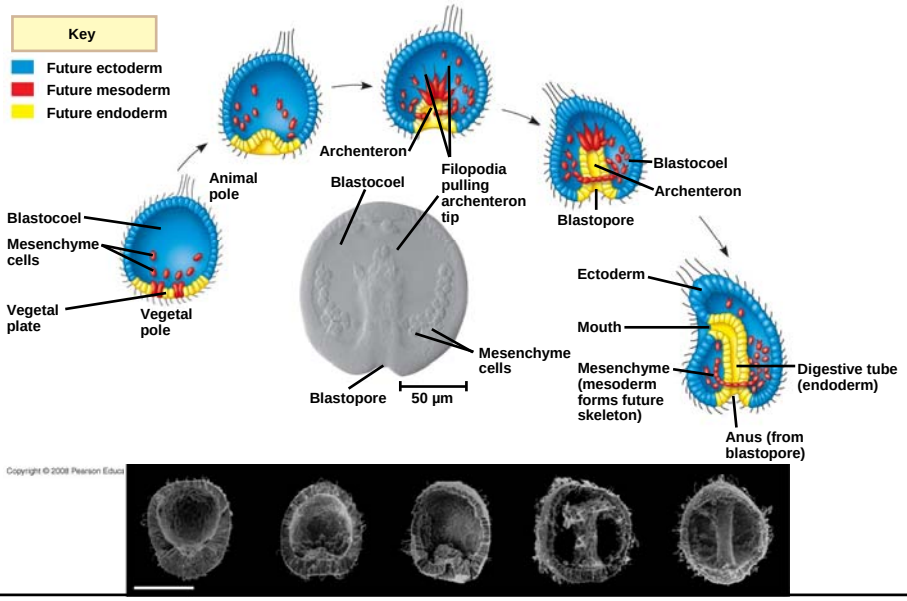


Gastrulation in a sea urchin embryo

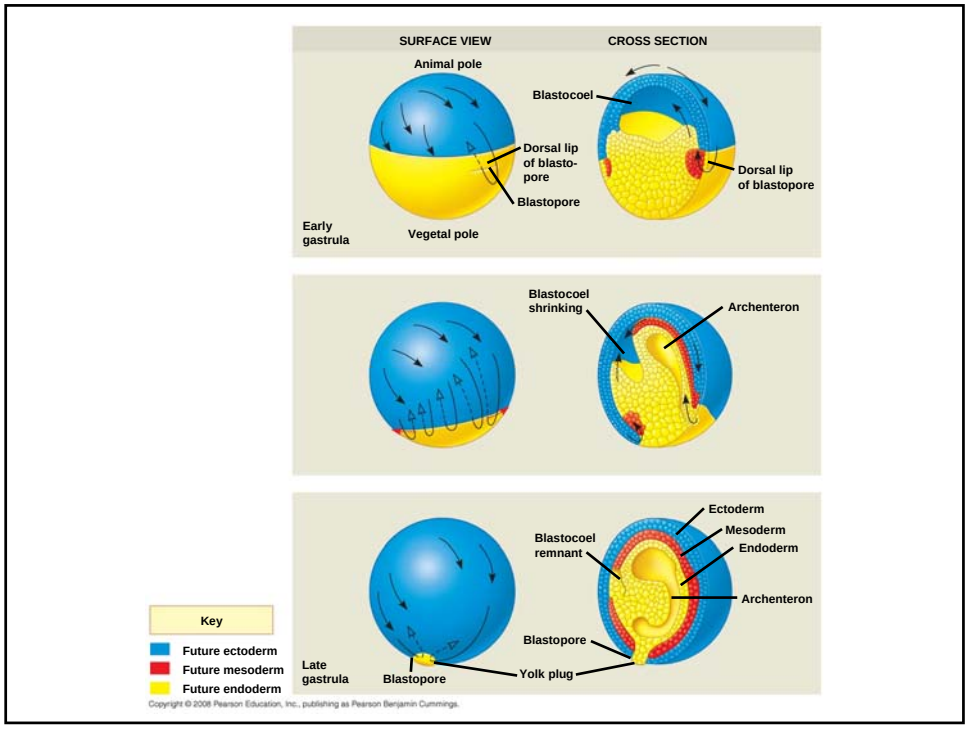
Future ectoderm
Future mesoderm
Future endoderm



Gastrulation in a sea urchin embryo



- Gastrulation in the frog
  - The frog blastula is many cell layers thick
  - Cells of the **dorsal lip** originate in the gray crescent and invaginate to create the archenteron
  - Cells continue to move from the embryo surface into the embryo by **involution**
  - These cells become the endoderm and mesoderm
  - The blastopore encircles a **yolk plug** when gastrulation is completed
  - The surface of the embryo is now ectoderm, the innermost layer is endoderm, and the middle layer is mesoderm


SURFACE VIEW
CROSS SECTION
Animal pole
Blastocoel
Dorsal lip of blasto-pore
Dorsal lip of blastopore
Blastopore
Early gastrula
Vegetal pole
Blastocoel shrinking
Archenteron
Ectoderm
Mesoderm
Endoderm
Blastocoel remnant
Archenteron
Blastopore
Late gastrula
Blastopore
Yolk plug
Key
Future ectoderm
Future mesoderm
Future endoderm
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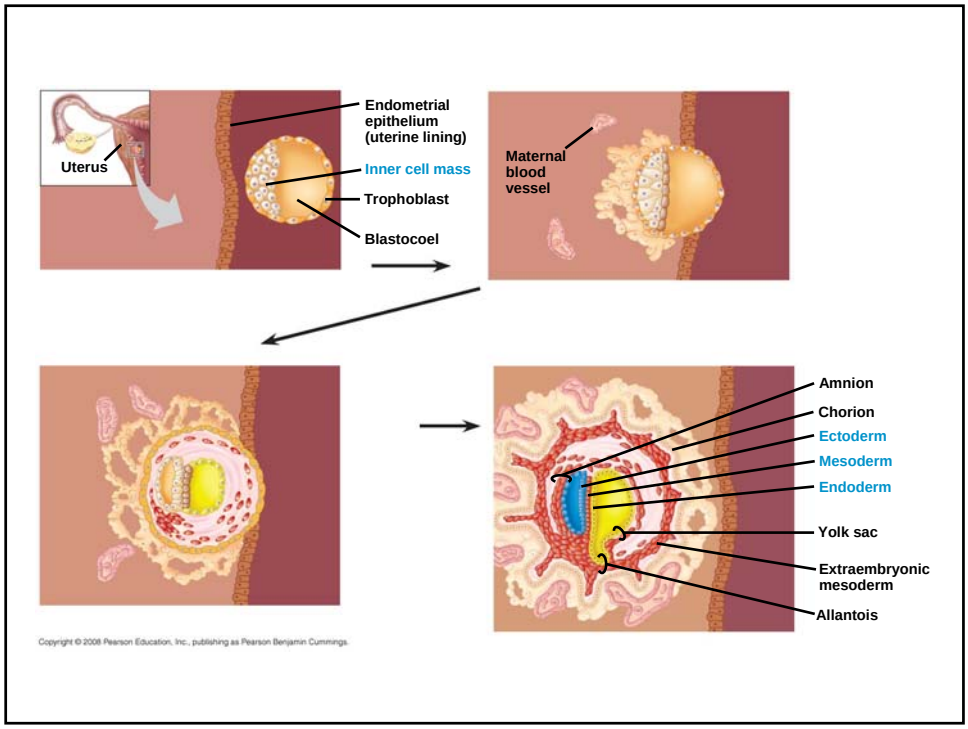
Uterus
Endometrial epithelium (uterine lining)
Inner cell mass
Trophoblast
Blastocoel
Maternal blood vessel
Amnion
Chorion
Ectoderm
Mesoderm
Endoderm
Yolk sac
Extraembryonic mesoderm
Allantois
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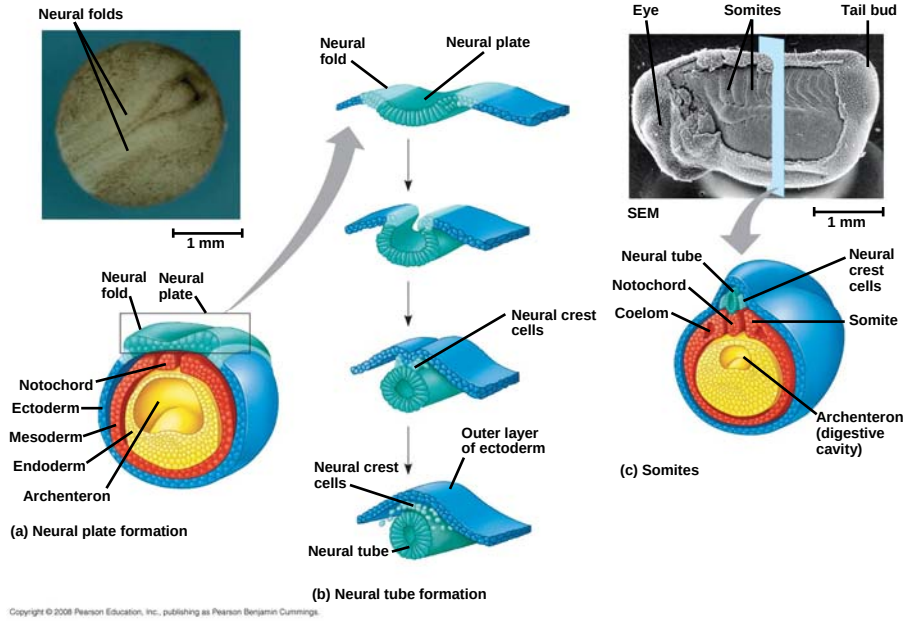
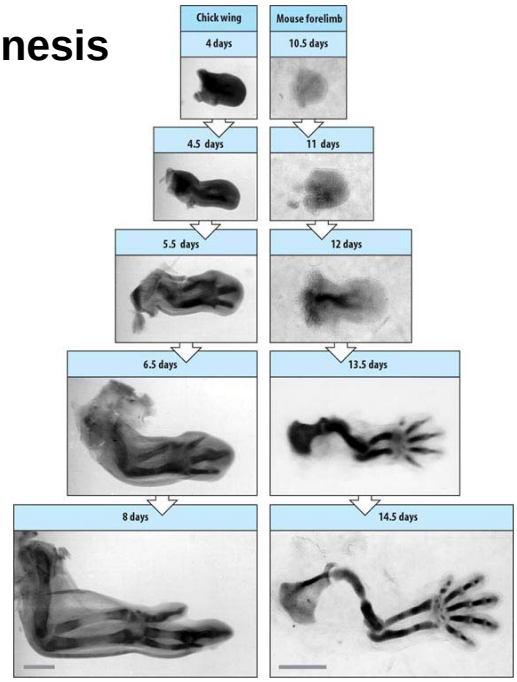
neurulation



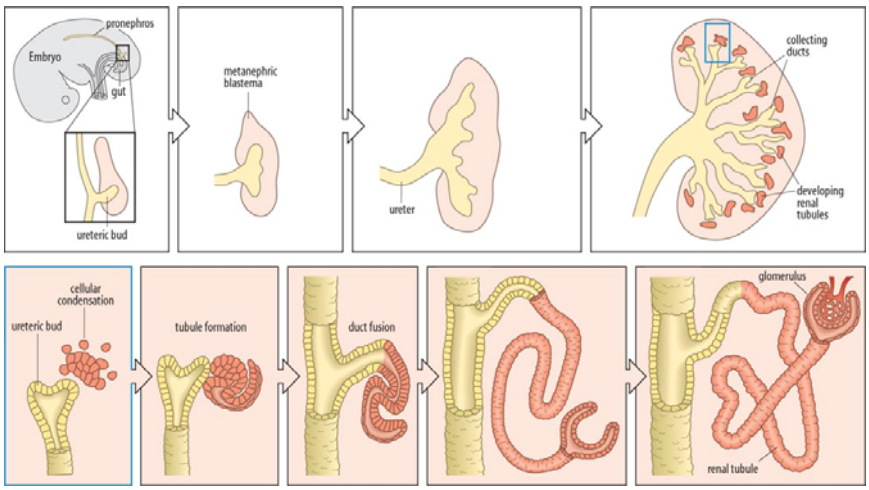
ECTODERM	MESODERM	ENDODERM
• Epidermis of skin and its derivatives (including sweat glands, hair follicles) • Epithelial lining of mouth and anus • Cornea and lens of eye • Nervous system • Sensory receptors in epidermis • Adrenal medulla • Tooth enamel • Epithelium of pineal and pituitary glands	• Notochord • Skeletal system • Muscular system • Muscular layer of stomach and intestine • Excretory system • Circulatory and lymphatic systems • Reproductive system (except germ cells) • Dermis of skin • Lining of body cavity • Adrenal cortex	• Epithelial lining of digestive tract • Epithelial lining of respiratory system • Lining of urethra, urinary bladder, and reproductive system • Liver • Pancreas • Thymus • Thyroid and parathyroid glands

Organogenesis

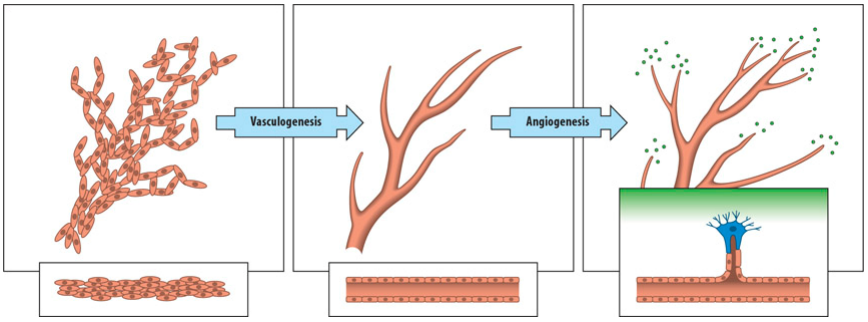
Limb



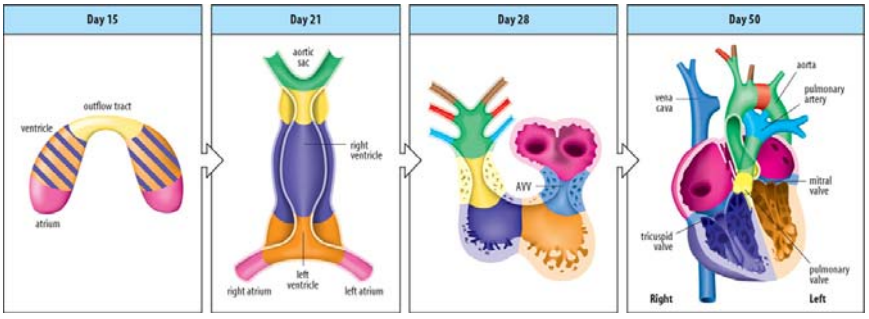
kidney



Blood vessels



Heart



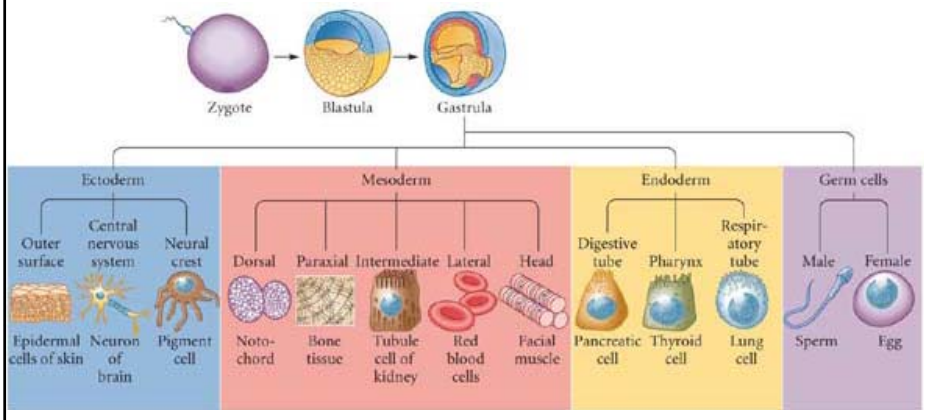
發育過程

Pattern formation
Morphogenesis
Cell differentiation
growth

發育過程之機制

1. Differential gene expression/signaling
2. Cell fate determination
3. Induction
4. Pattern formation
a. Positional information
b. Lateral inhibition
5. Asymmetric division

1. Differential gene expression

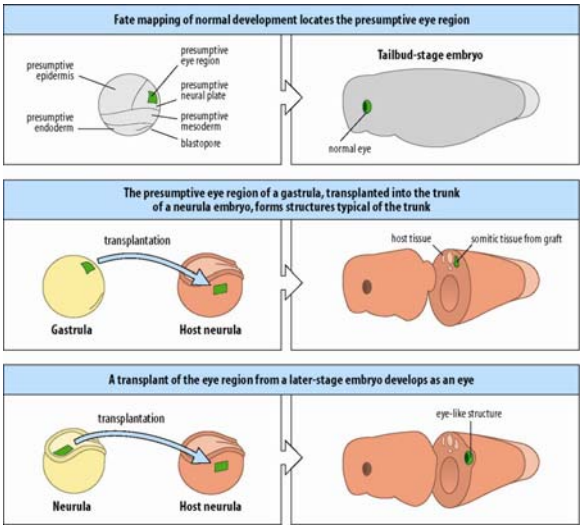


Representative cell types of a vertebrate

cell differentiation

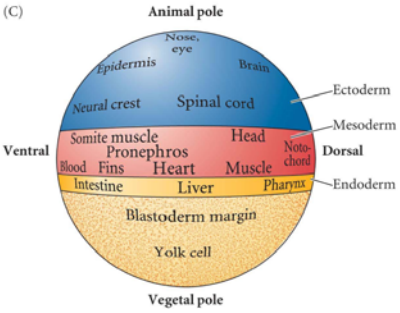
2. Cell fate determination

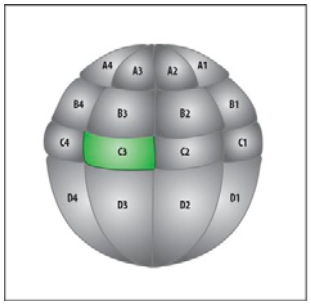
Cell transplantation

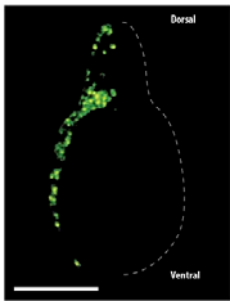


2. Cell fate determination

GFP labeled or Laser caged

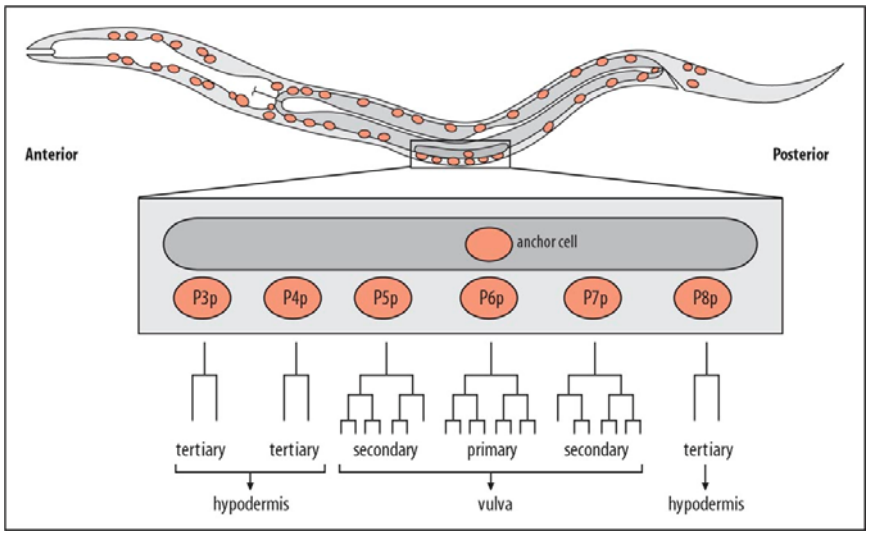




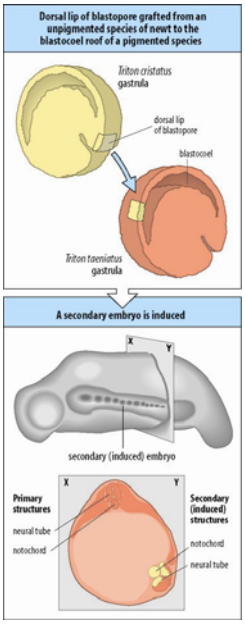


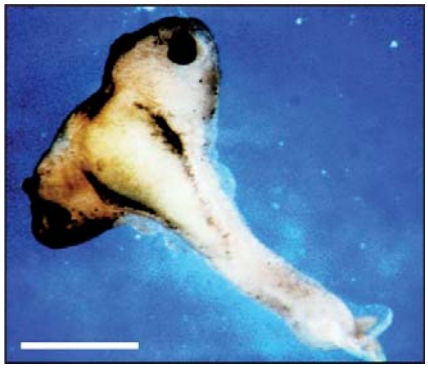
2. Cell fate determination

Cell-linage analysis



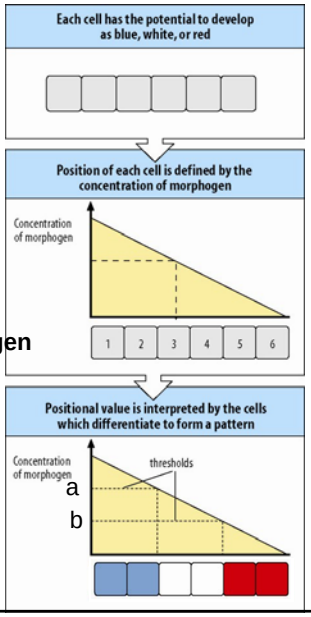
3. Induction





4. Pattern formation
a. Positional information

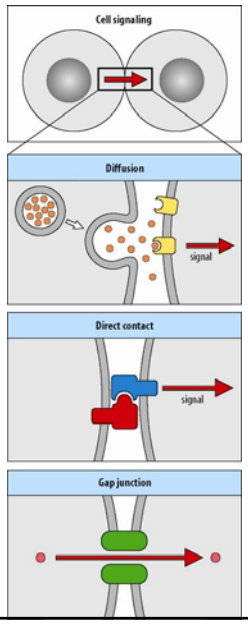
4. Pattern formation
a. Positional information

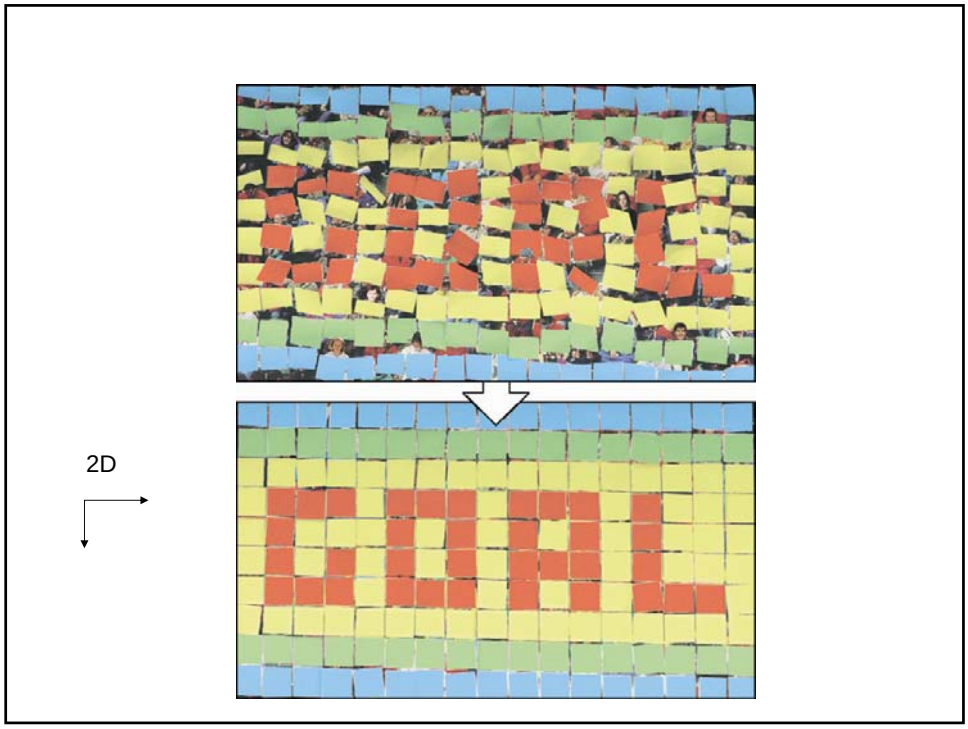


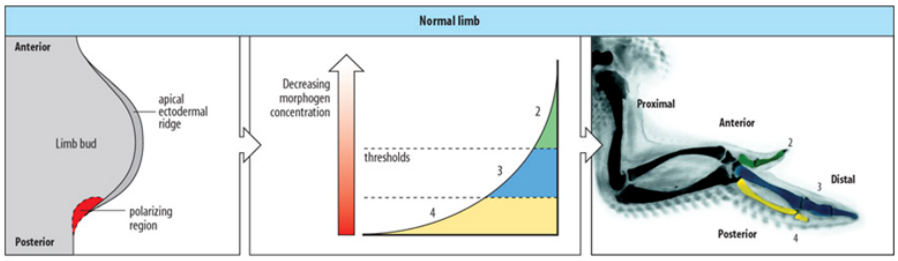
morphogen

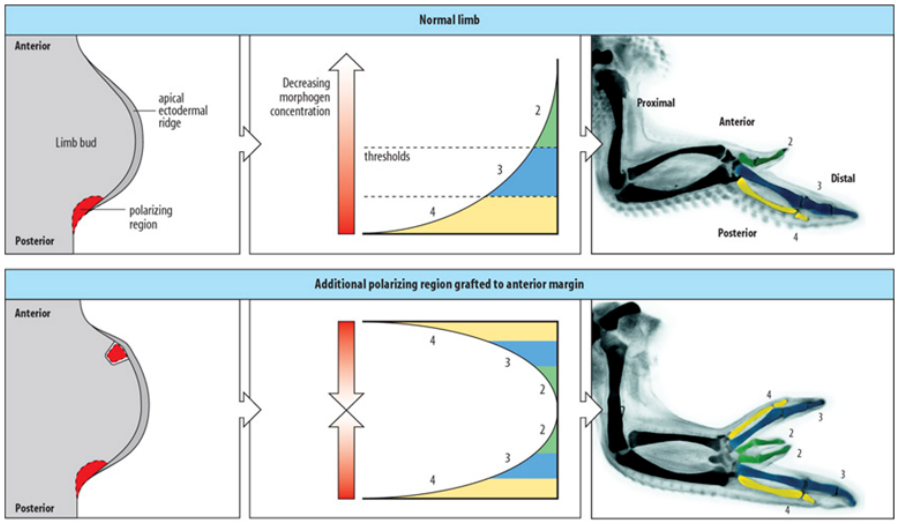
Set >a : Blue
<b : Red

cell-cell communication

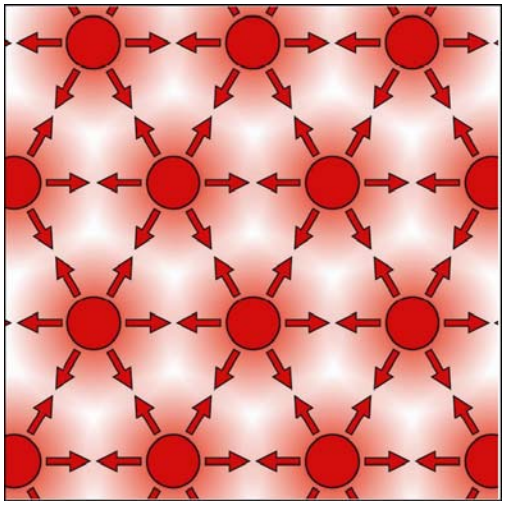



2D

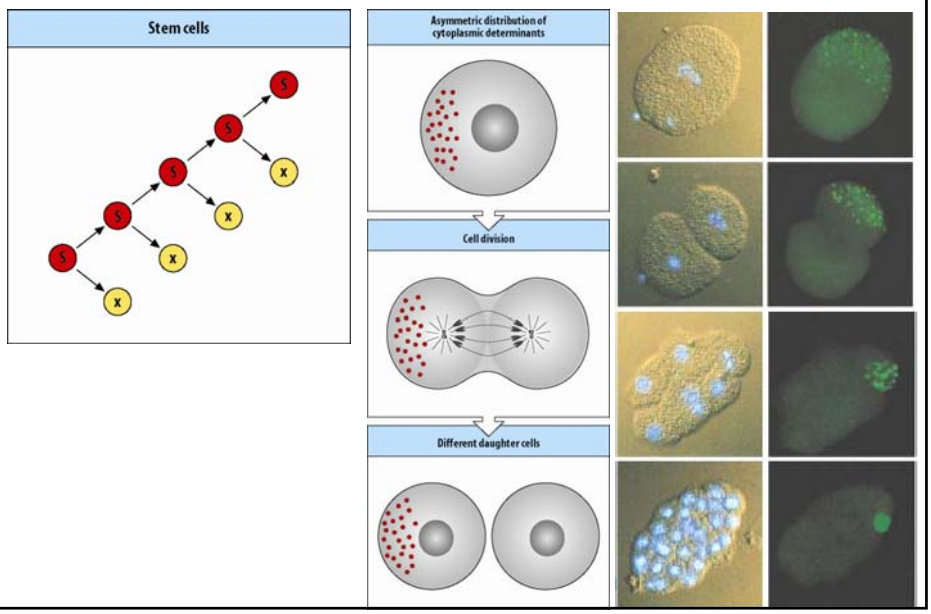

Normal limb
Anterior
apical ectodermal ridge
Limb bud
polarizing region
Posterior
Decreasing morphogen concentration
thresholds
2
3
4
Proximal
Anterior
Distal
Posterior

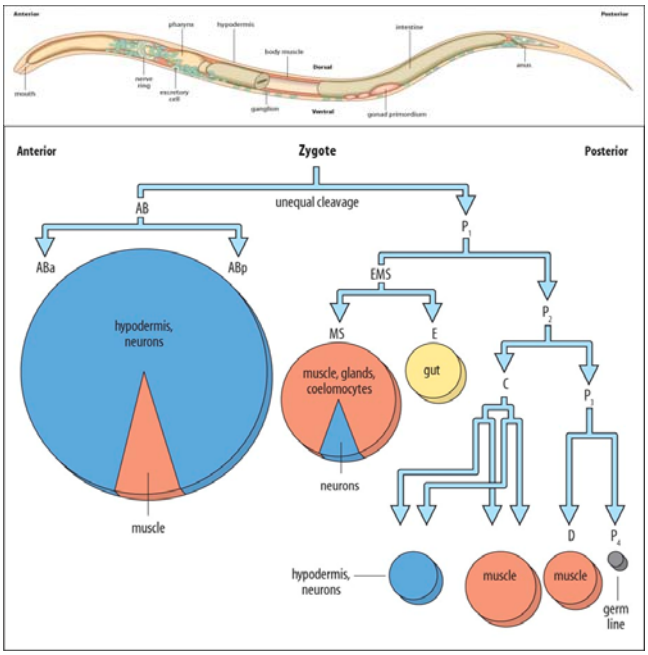


4. Pattern formation
b. Lateral inhibition



5. Asymmetric division





幹細胞

1.甚麼是幹細胞/ 與發育生物學之關係

2.幹細胞來源

a. 成體幹細胞 (adult stem cell):
骨髓，成體週邊血 (取得極爲困難）

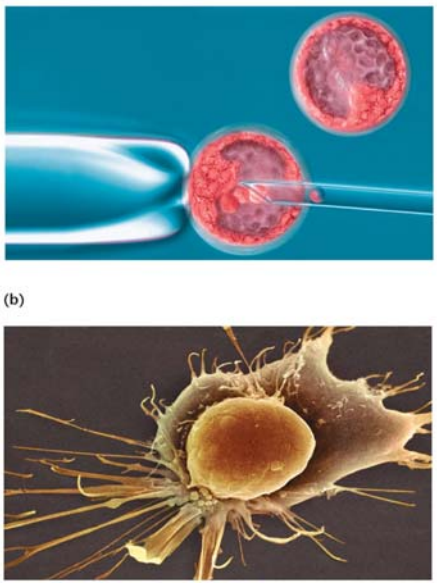
b. 胚胎幹細胞 (embryonic stem cell):
胚胎，臍帶血 (取得容易)

3. 幹細胞之運用 (複製, 組織再生)

4. 幹細胞研究之道德探討

幹細胞

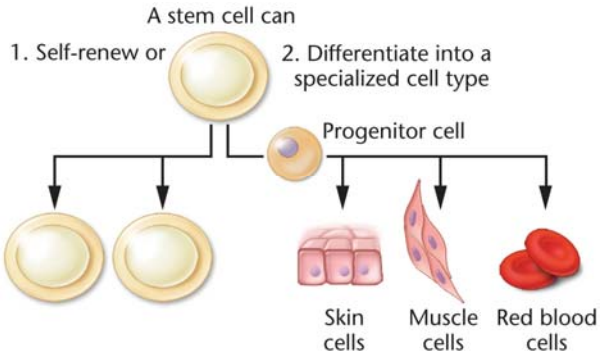
- The tiny cluster of cells is an early-stage human embryo that is being used to isolate **stem cells** under microinjection (a) and growing in culture (b).



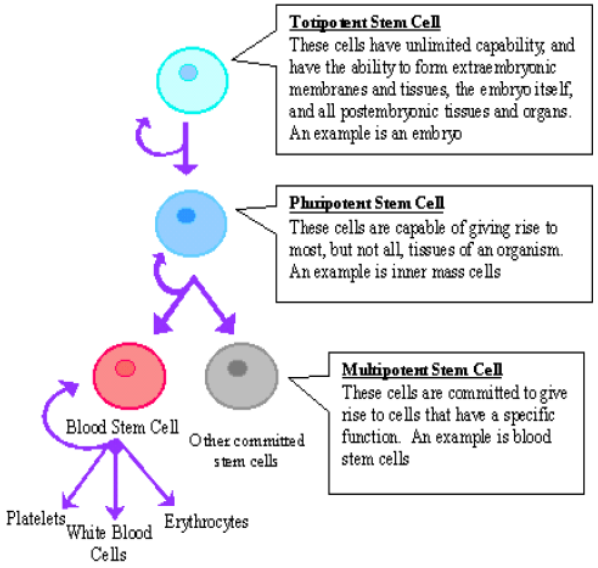
(b)

What Are Stem Cells?

- Stem cells share two basic characteristics that make them distinctive from other cell types : **self-renewal** and **differentiation** into specialized cell types



Ability of stem cell differentiation



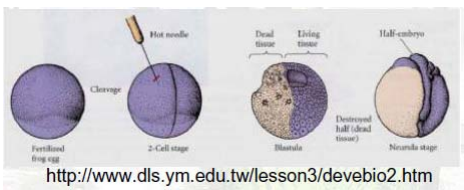
全能性幹細胞

複能性幹細胞

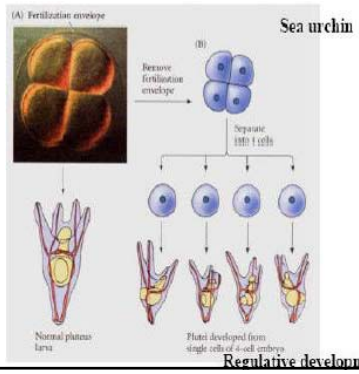
多能性幹細胞

幹細胞研究之先驅

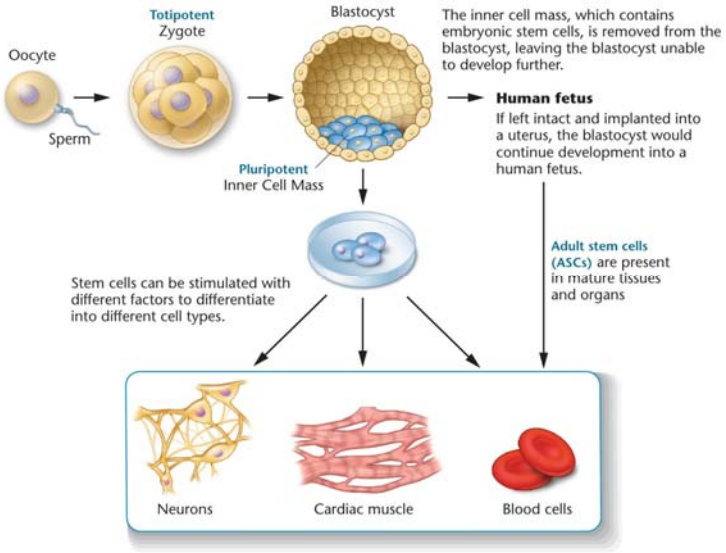
1998年美國威斯康辛大學的James Thompson以及Johns Hopkins醫院的John Gearhart兩位教授，成功地將人類多功能性 (pluripotent) 的胚胎幹細胞 (embryonic stem cell) 在**體外培養與繁殖**，開啓了全球對於幹細胞研究的熱



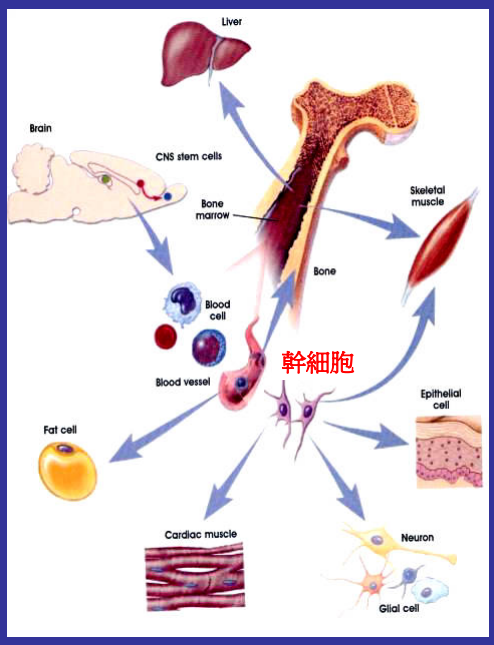
http://www.dls.ym.edu.tw/lesson3/devebio2.htm



human embryonic stem cells (hESCs)



ST Figure 4.3



The Major Types of Stem Cells

A. Embryonic Stem Cells (胚胎幹細胞 ESC)– 道德！
- From blastocysts left over from In-Vitro Fertilization in the laboratory

---Fetus Stem Cells (胎兒幹細胞)--道德！
- From aborted fetuses

---Umbilical Cord Stem Cells (臍帶幹細胞)

B. Adult Stem Cells (成體幹細胞)
- Stem cells have been found in the blood, bone marrow, liver, kidney, cornea, dental pulp, umbilical cord, brain, skin, muscle, salivary gland . . . .

成體幹細胞 (Adult Stem Cell)

- 又稱爲組織專一幹細胞 (Tissue-specific stem cell)
- 取自自體, 免疫相合, 不會排斥
- 無道德倫理爭議
- 增生能力有限, 隨年齡而降低
- 特定分化能力有限制
- 功能與應用上遜於胚胎幹細胞

幹細胞依功能分類

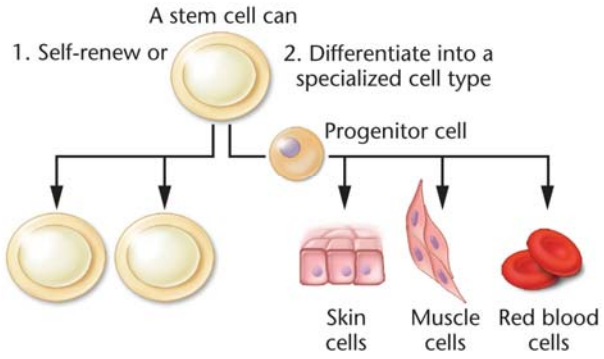
- 造血幹細胞 (hematopoietic stem cells，HSC)
- 間葉系幹細胞 (mesenchymal stem cells，MSC)

可分化成軟骨細胞, 骨細胞, 脂肪, 神經, 肝細胞等

- 這兩種幹細胞皆存在於骨髓、嬰兒臍帶血、以及成人週邊血液中。
- HSC在重建血液及免疫系統的研究中倍受重視
- MSC 在組織再生的臨床應用，則在近年來吸引了許多的注意力。
- 因無道德倫理爭議, 未來發展潛力大

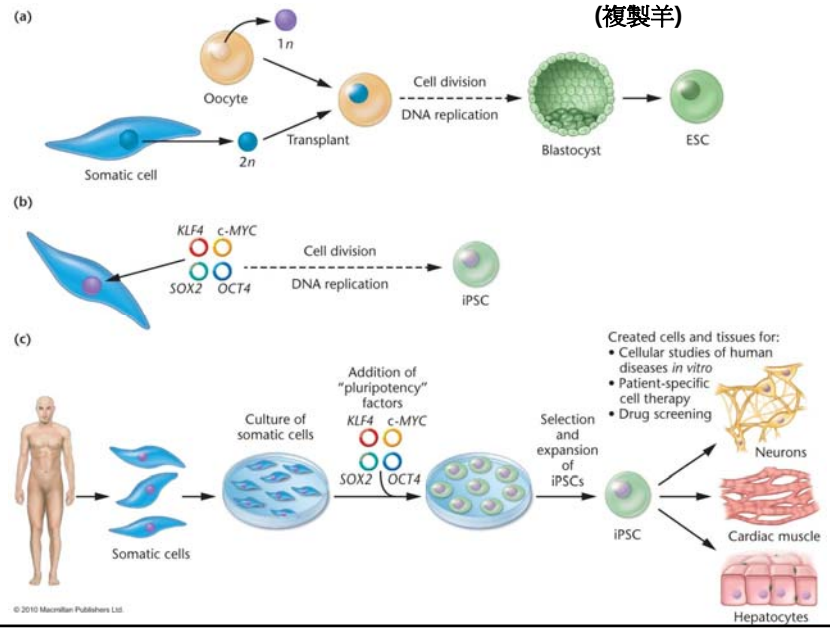
分化是不可回頭的發育路徑？

體細胞可否返老還童？



Nuclear Reprogramming Approaches for Producing Pluripotent Stem Cells

(複製羊)



- **Stem cells research-regenerative medicine**

ST TABLE 4.1

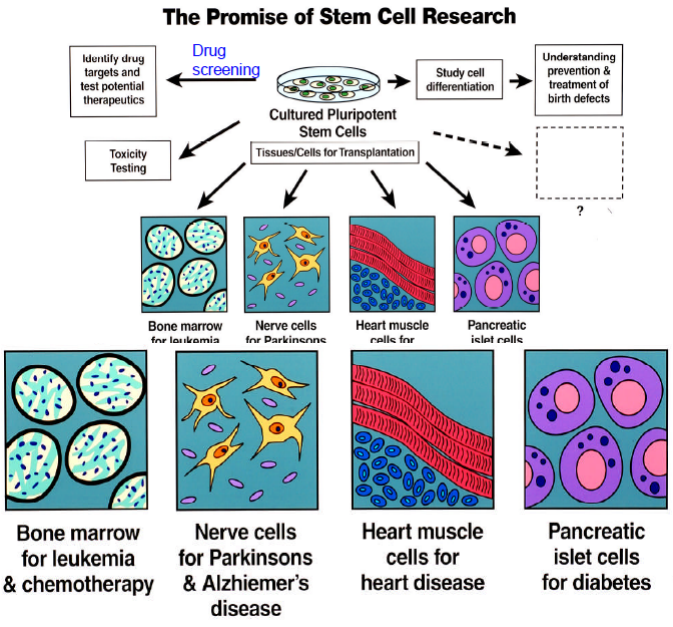
Potential U.S. Patient Populations for Stem Cell-Based Therapies

The conditions listed below occur in many forms and thus not every person with these diseases could potentially benefit from stem cell-based therapies. Nonetheless, the widespread incidence of these conditions suggests that stem cell research could help millions of Americans.

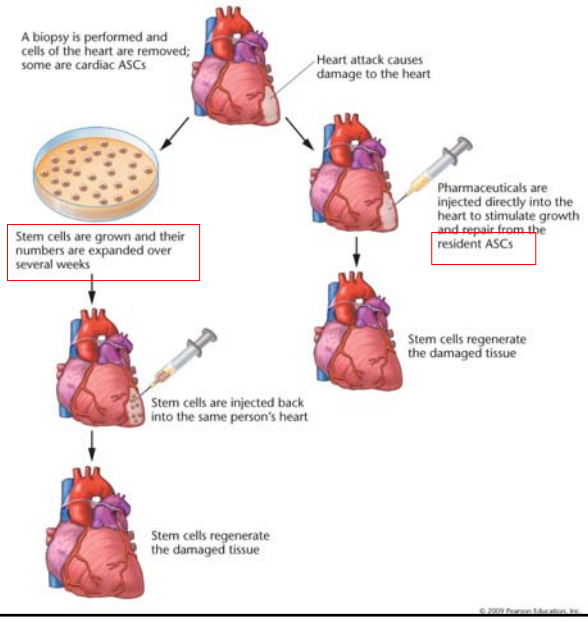
Disease condition	Number of patients in the United States
Cardiovascular disease	58 million
Autoimmune diseases	30 million
Diabetes	16 million
Osteoporosis	10 million
Cancers	8.2 million
Alzheimer's disease	5.5 million
Parkinson's disease	5.5 million
Burns (severe)	0.3 million
Spinal-cord injuries	0.25 million
Birth defects	0.15 million/year

Source: Derived from Perry (2000).

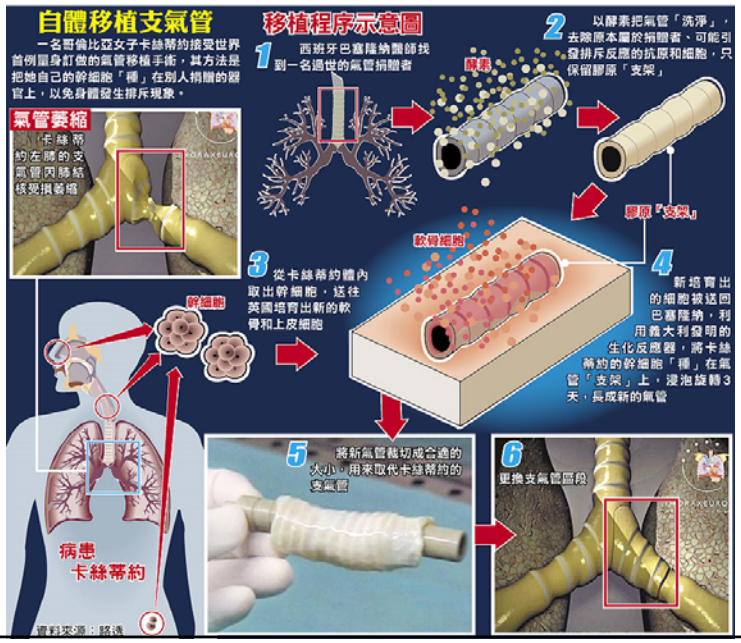
Parkinson's disease（巴金森氏症）
Alzheimer's disease（老人癡呆症）
Spinal cord injury（脊髓損傷）
Stroke（中風）
Burns（燒傷）
Diabetes（糖尿病）
Liver failure（肝衰竭）
Heart disease（心臟病）
Osteoarthritis（骨關節炎）
Rheumatoid arthritis
（風濕性關節炎）
End-stage kidney disease
（末期腎臟病變）

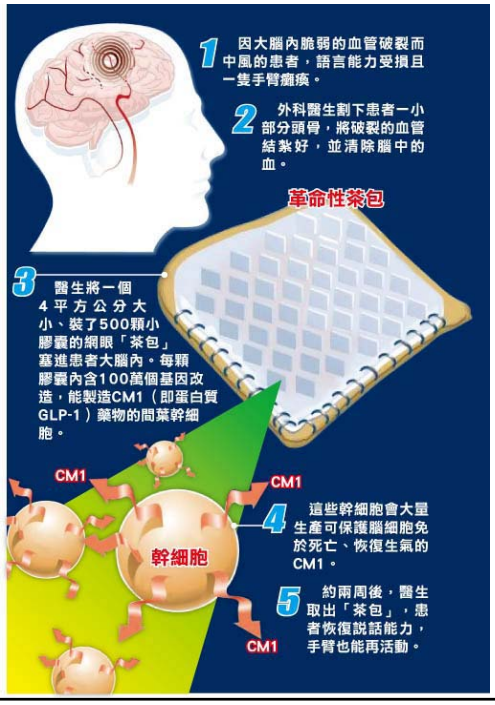


以幹細胞修補受損老鼠心肌



2008 自體幹細胞培育氣管移植





血性腦中風患者

2001年8月，美國總統布希針對「人類胚胎幹細胞」的議題，同意美國政府可以有條件地資助幹細胞的研究，但是他限制了胚胎幹細胞來源必須是現有的64株，不可以再取得新的胚胎幹細胞，他認爲這樣子就沒有毀掉胚胎的疑慮。

幹細胞,複製與組織再生

- 何謂複製 (cloning)
  - 是指藉由一個細胞、器官或是去氧核醣核酸（DNA）持續不斷的分裂成另一個在基因型態上完全相同的細胞、器官或是去氧核醣核酸的一種過程。

複製生命 (Dolly the sheep)

- 複製動物則是把成年動物的一個細胞，植入一個已經拿掉基因物質的卵子。複製作業整體的成功率不到3%。
- 撇開複製技術的困難度不談，「複製」本身所帶來的社會倫理衝擊則更引人爭議。

Dolly (sheep)	1 live birth out of 29 cloned embryos	3%
Cloned mice	31 live births out of 2468 cloned embryos	1%
Cloned pigs	5 live births out of 335 cloned embryos	1%
Cloned goats	3 live births out of 85 cloned embryos	3%
Cloned cattle	30 live births out of 496 cloned embryos	6%
Cloned cat	1 live birth out of 87 cloned embryos	1%
Cloned rabbits	6 live births out of 371 of cloned embryos	1%

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複製人與器官複製

複製動物
・1997年2月27日英國Roslin研究所科學家於Nature雜誌上首先發表複製羊桃莉(Dolly)複製成功

(Somatic Cell Nuclear Transfer)

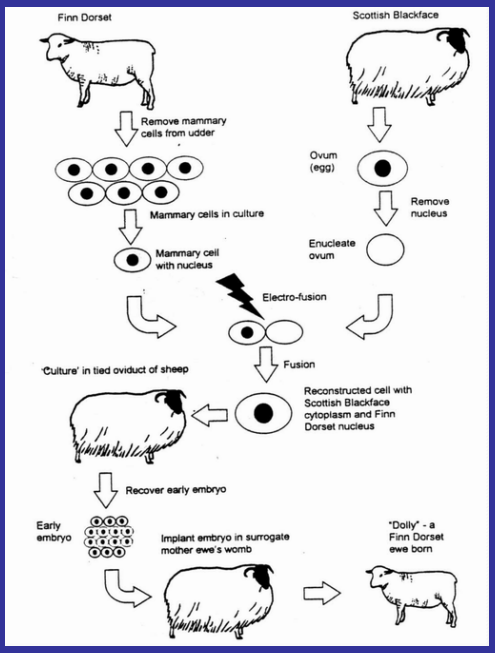
Nature 385: 810

複製羊技術

Somatic cell nuclear transfer (SCNT)

Removing the maternal nucleus before nuclear transfer

Nuclear transfer embryo about to be activated



治療性複製 (組織與器官複製)

包括三個部分

1. 核移植
2. 胚胎幹細胞製備（human embryonic stem cell）
3. 控制分化（control of differentiation）

- 目前這三部分都已有了初步成果，雖然離成功尚遠，但以人類過去科技發展的軌跡來看，既然可行性已獲得證實，則成功率與改進應指日可待

- 再生醫學(Regenerative Medicine) －
  培養細胞與組織，用來修補受損傷的組織與器官
- 細胞、組織、與器官移植

組織工程(Tissue Engineering)
- 皮膚移植（燒燙傷、美容）
- 器官再造
  - 1997 Dr. Charles Vacanti (University of Massachusetts)

利用小鼠製造人工義耳
- 以可吸收性聚合物製造耳朵形狀，以牛膠原細胞包覆
- 埋放到裸鼠皮下，生長成人形耳朵
  - 其他可能應用：膀胱、腎臟…等