Principles of embryonic patterning

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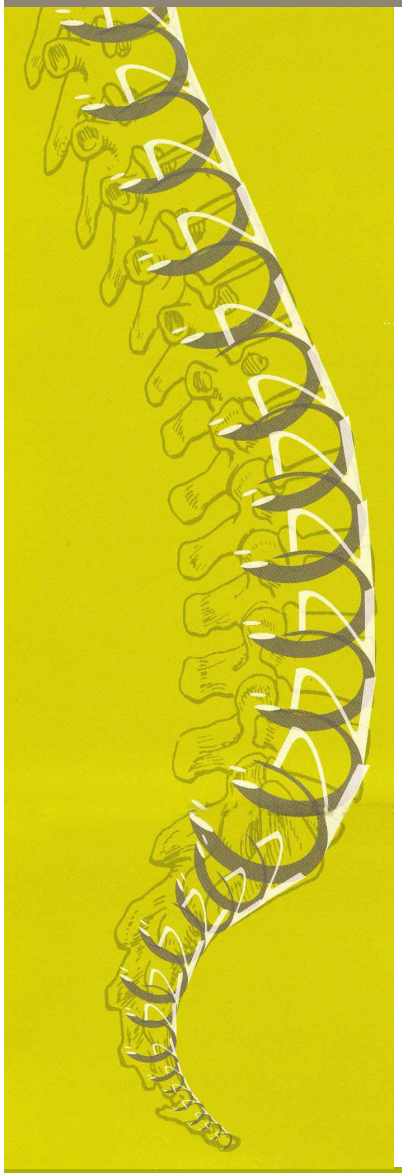
Outline



- Reproductive Biology
  - → Lessons from animal models
    - Totipotency
    - (Pre-)patterning and destiny
  - → Lessons from the human embryo
    - Totipotency and differentiation
- Conclusions

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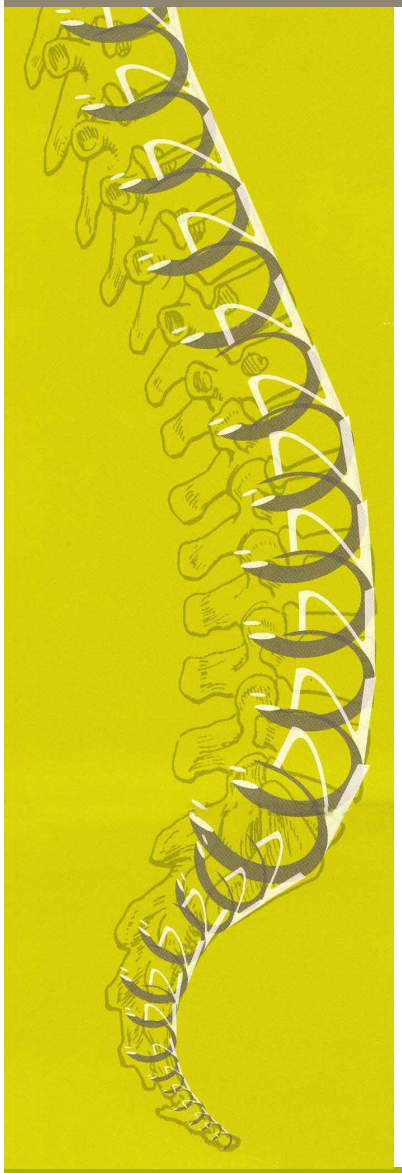

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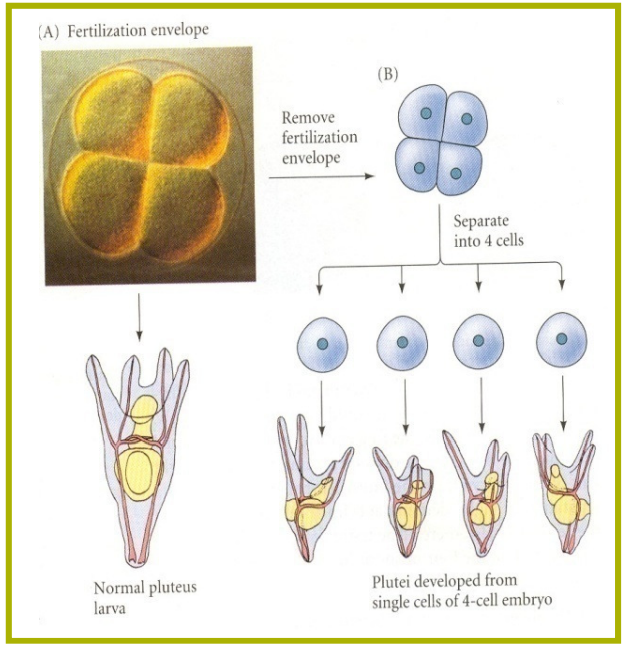
Totipotency

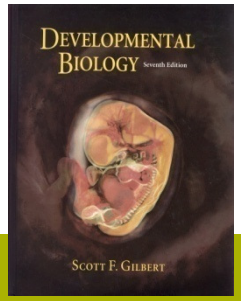
- Life starts with ...
  the totipotent cell
- The totipotent cell is ...
  able to develop into fertile offspring
- The ultimate totipotent cell is ...
  the zygote

Embryonic patterning?

Developmental biology: Echinodermata

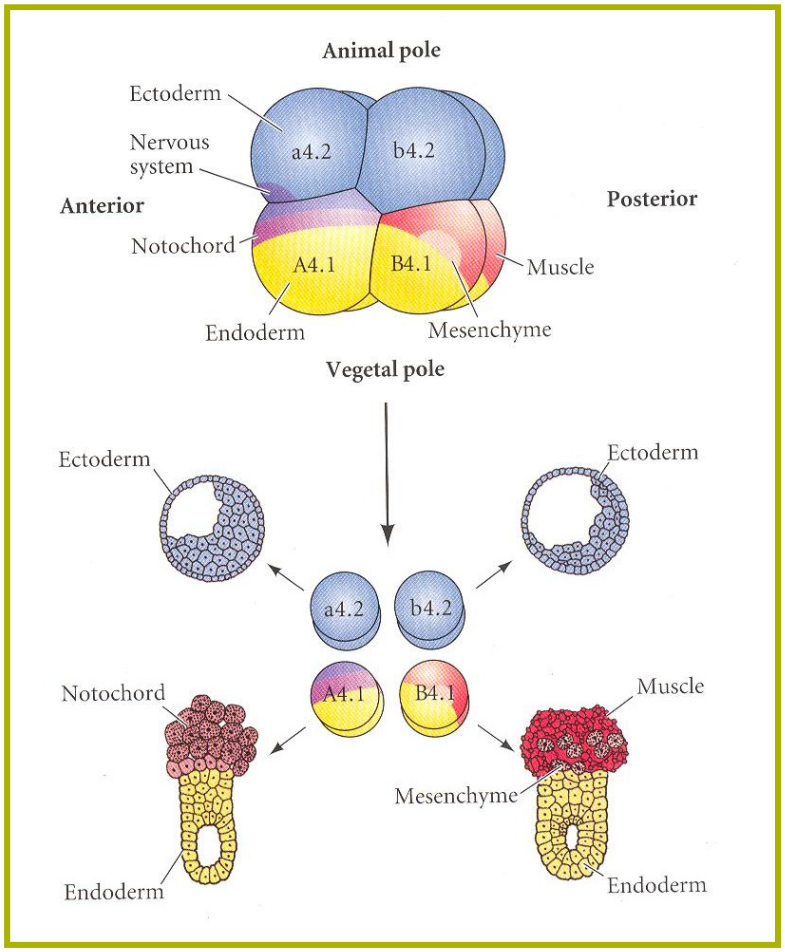


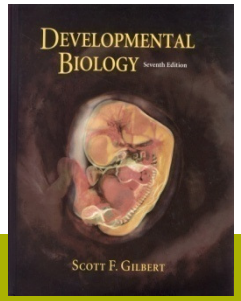
Sea urchin
Direction is reversible
Blastomeres are totipotent, plastic
Regulative development



Developmental biology: Invertebrates,Tunicata

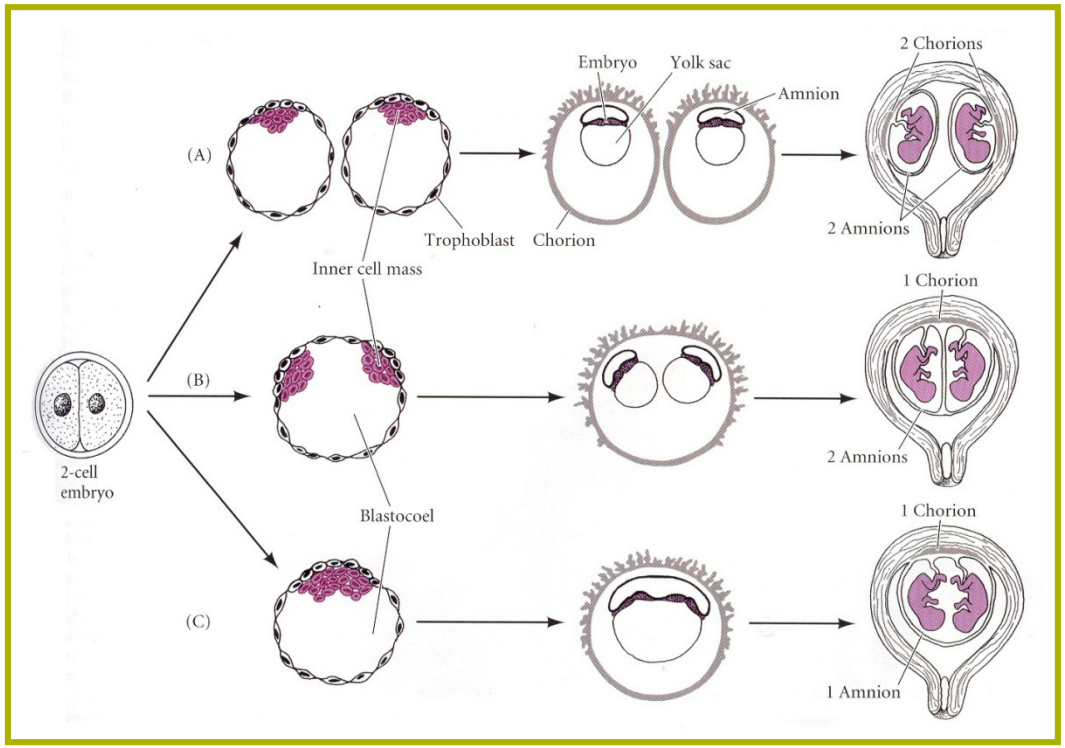
Sea squirt

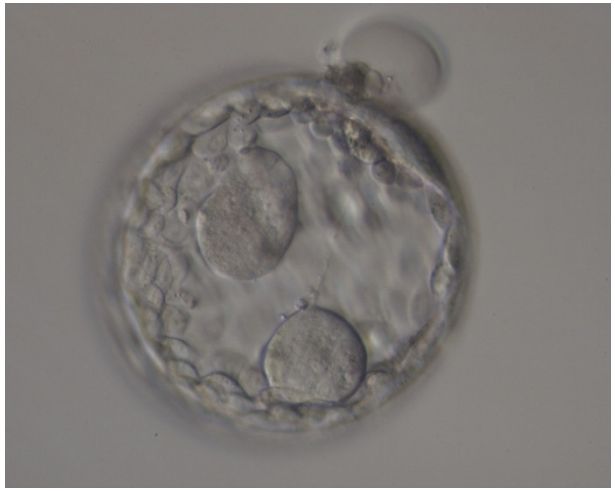


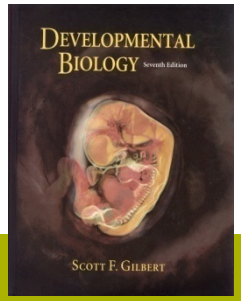


Direction is irreversible: commitment, destiny or fate
Restricted development

Developmental Biology: human monozygotic twinning

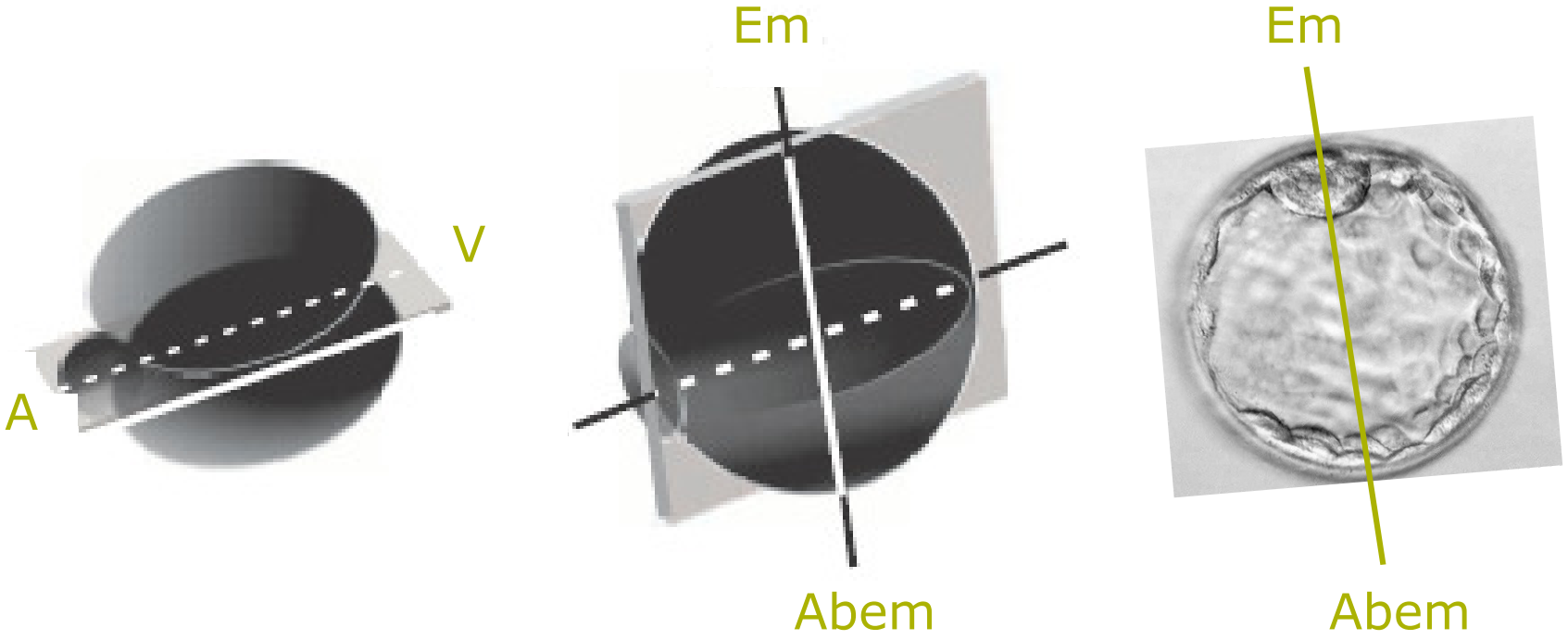






Lessons from the mouse embryo

- Pre-patterning in the zygote
  Gardner et al. 1997, 2001



2nd Pb is fixed

Lessons from the mouse embryo

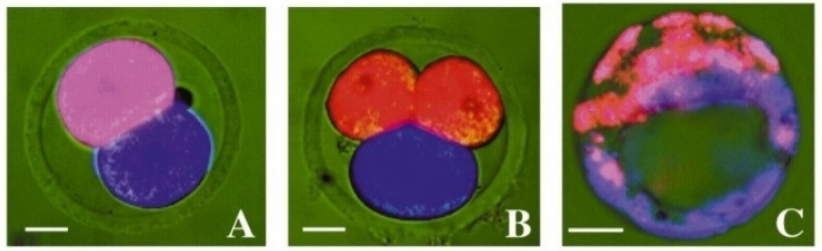
- Piotrowska et al. 2001

  → the 1st dividing blastomere contributes more to the Em part

  → pre-patterning at the 2-cell stage

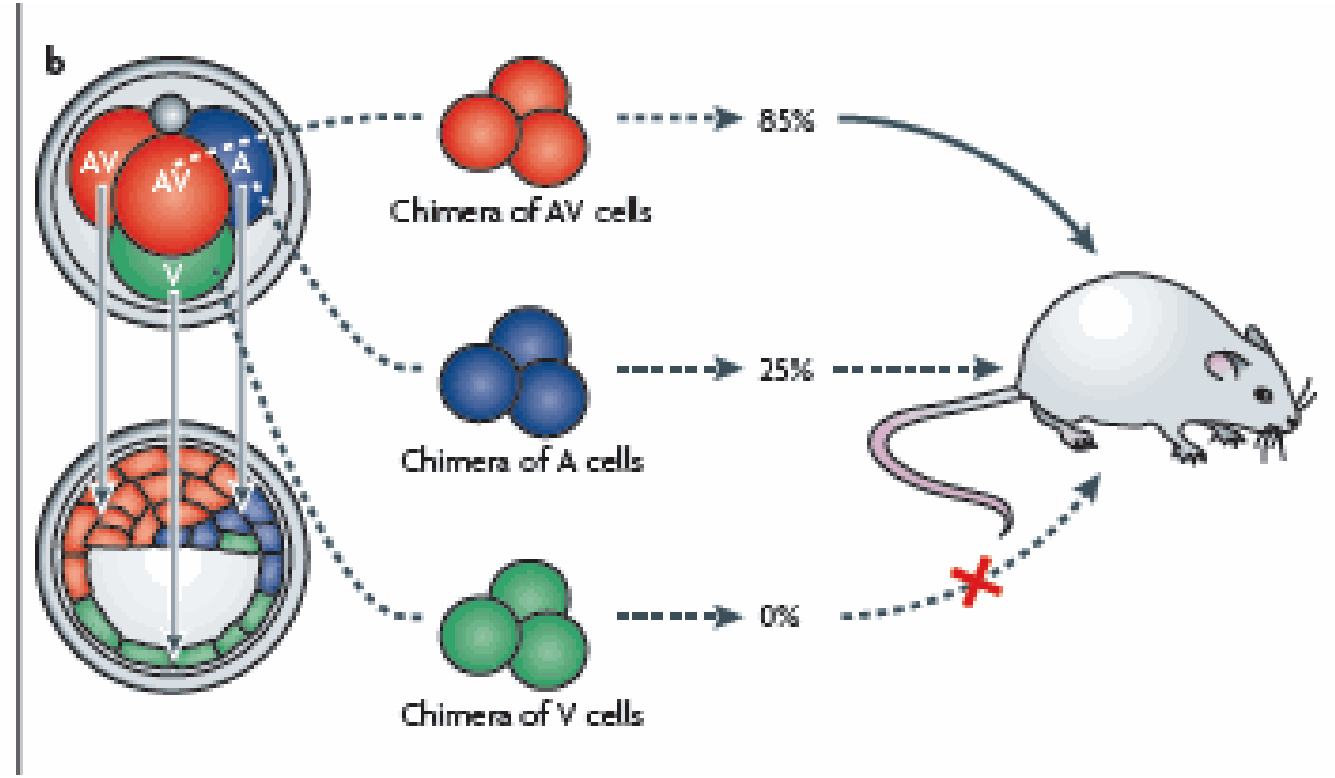
  → manipulations, in vitro experiments

ME



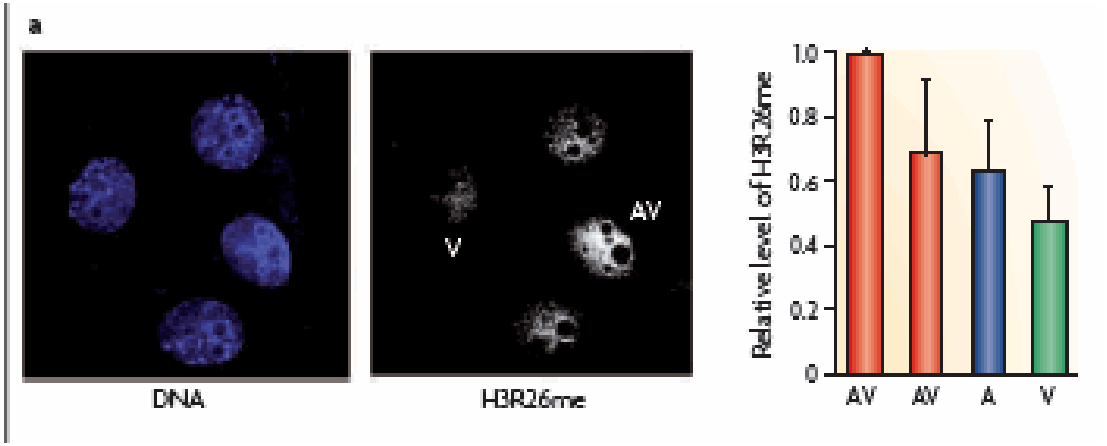
Lessons from the mouse embryo

- Piotrowska et al. et al. 2001

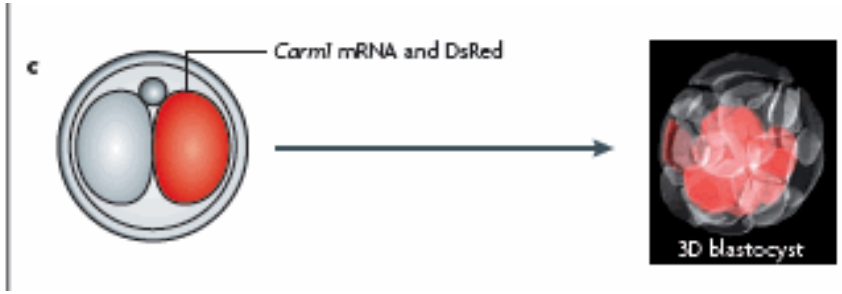


Lessons from the mouse embryo

- Torres-Padilla et al. 2007



V blastomere is less stained for H3R26me

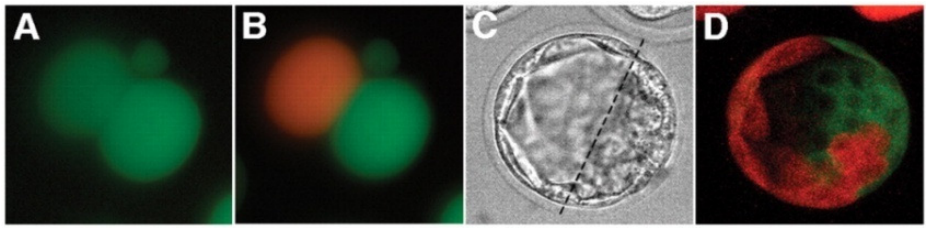


CARM1 (H3-specific arginine transferase) 88.5% ICM cells derived from injected blastomere (overexpression Nanog)

Lessons from the mouse embryo

- Regulative development
  - → The dance of the embryo (time lapse)

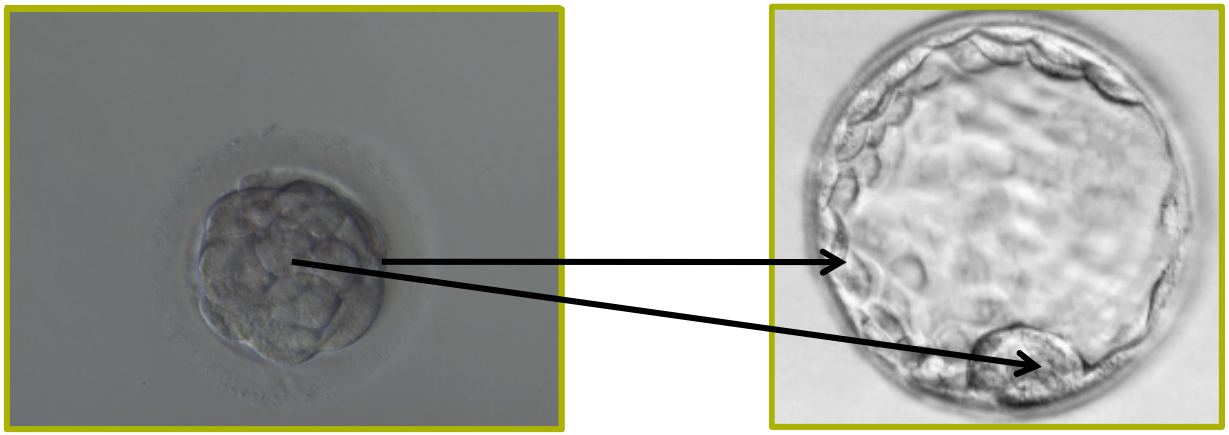
    Kurotaki et al. 2007
    - ZP (extrinsic factor) induced cavity
    - 2nd Pb is not fixed, embryo rotates in the ZP
    - In vivo experiments: photoconversion
    - No pre-patterning



Lessons from the mouse embryo

- Inside-outside hypothesis

  Tarkowsky and Wroblewska 1967



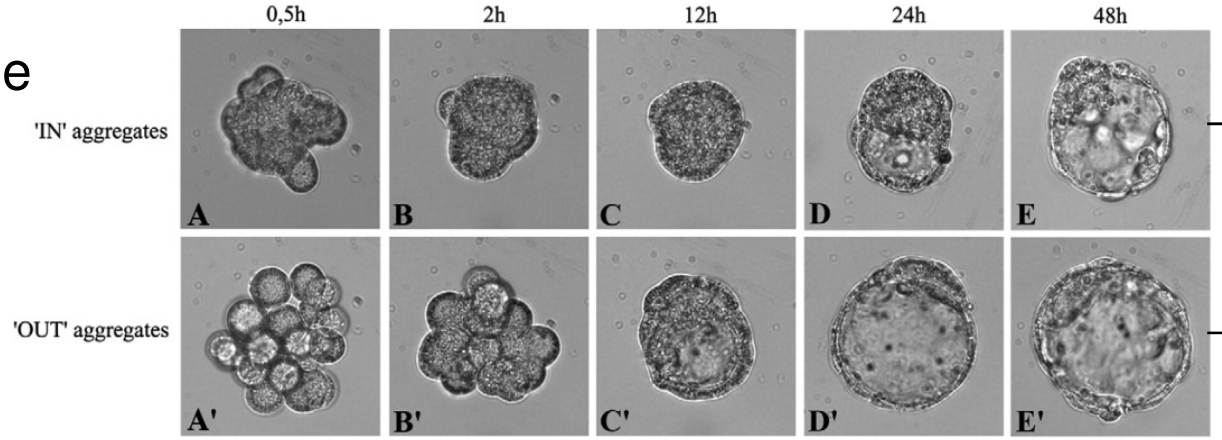
IN → ICM
OUT → TE

Lessons from the mouse embryo

- Potency of ‘IN’ and ‘OUT’ blastomeres

  Suwinska et al. 2008

16-cell stage
Compacted

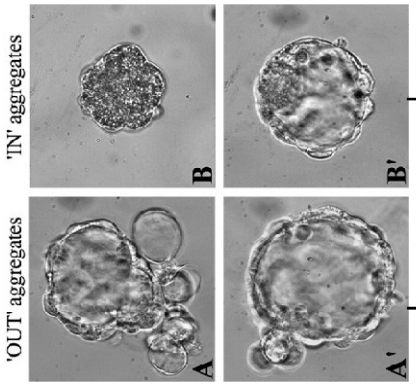


puppies

IΛ

puppies

32-cell stage
Early blastocyst



ICM+TE, no puppies

TE

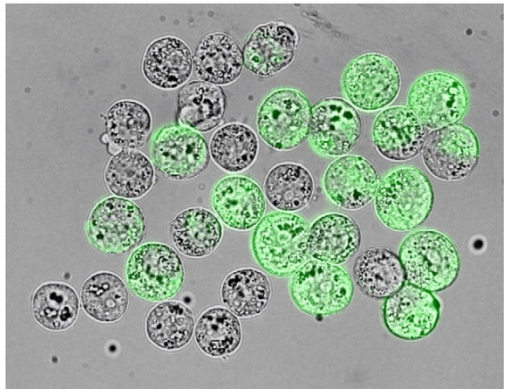
→ direction is reversible at compaction but irreversible at blastulation

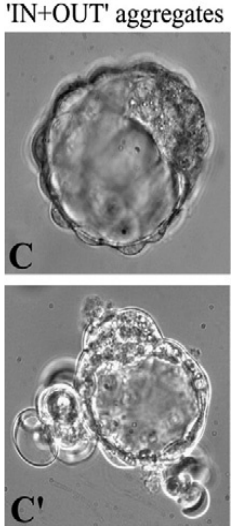
Lessons from mouse embryo

- Regulative development

  Suwinska et al. 2008

32-cell stage
Early blastocyst
→ sorting
→ puppies



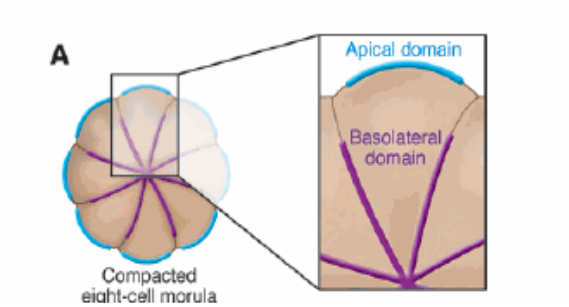


Lessons from the mouse embryo

- Polarization

  Cockburn and Rossant, 2010

  → Compaction: E-cadherin

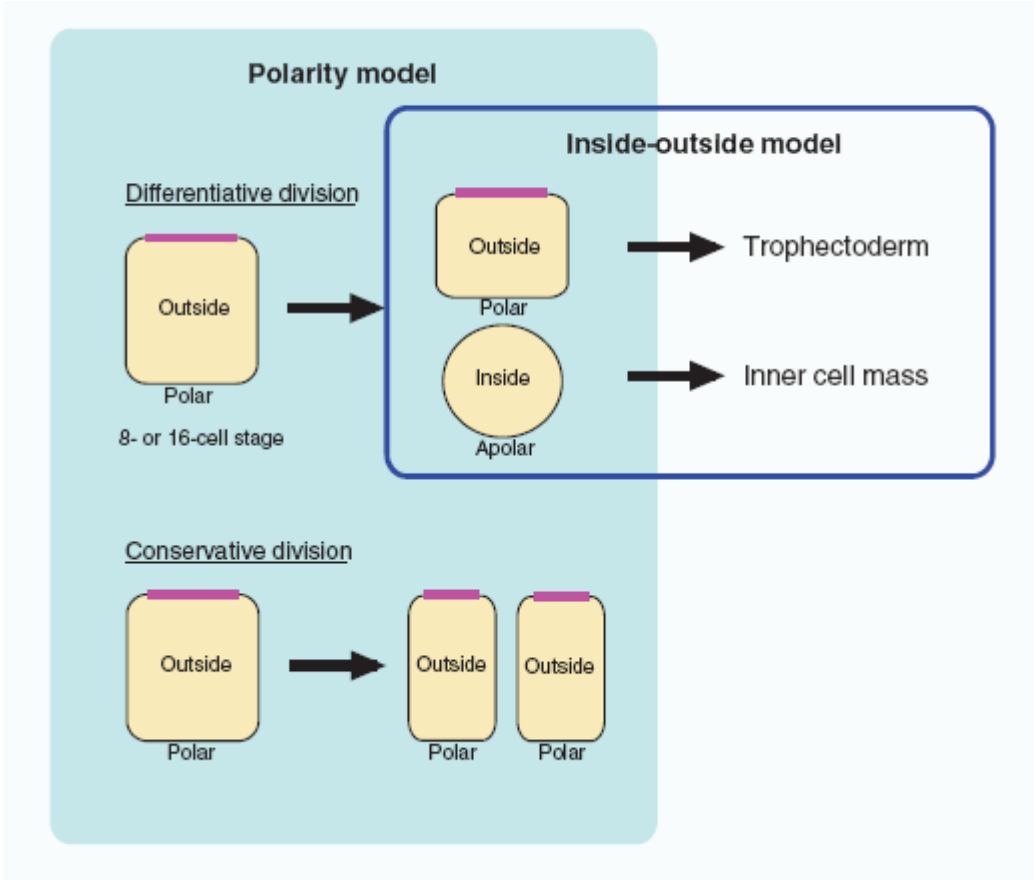


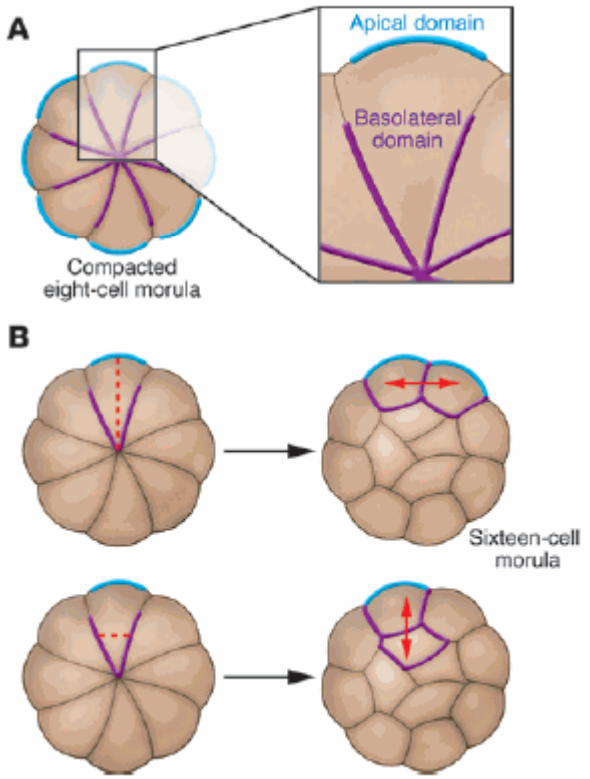
  - Basolaterally: nuclei; Par1, Lgl
  - Apically: endosomes; actin, aPKC, Par3

Lessons from the mouse embryo

- Polarization

  Johnson and McConnel, 2004; Cockburn and Rossant, 2010



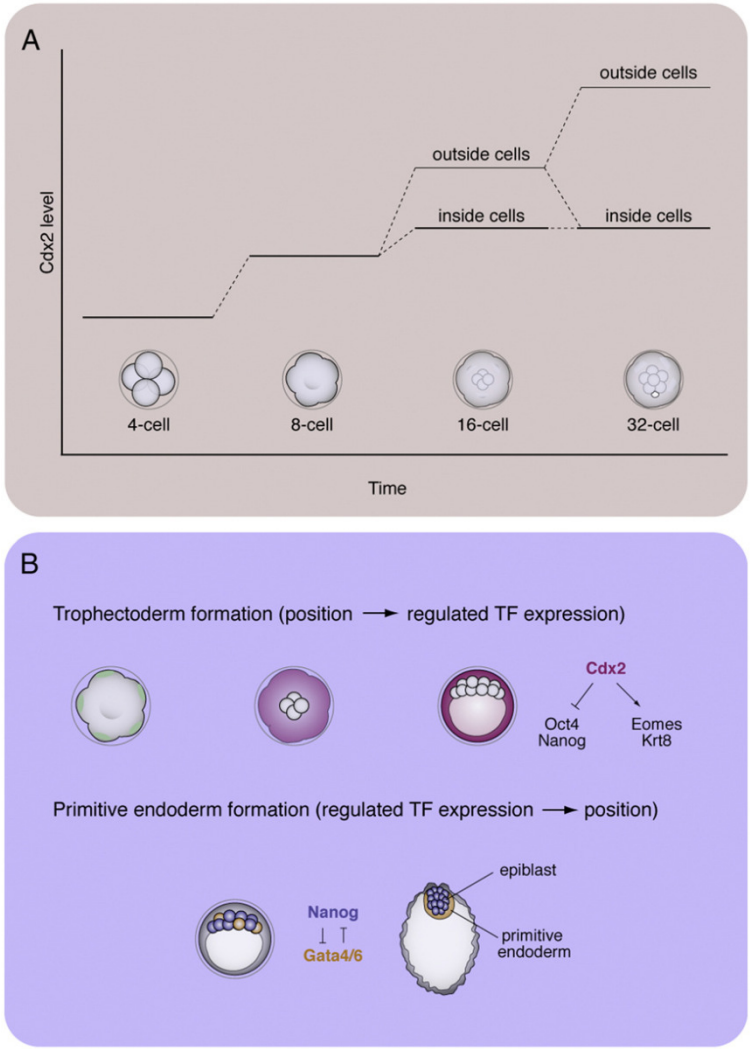


Lessons from the mouse embryo

- Lineage segregation
  Vallier and Rossant, 2005

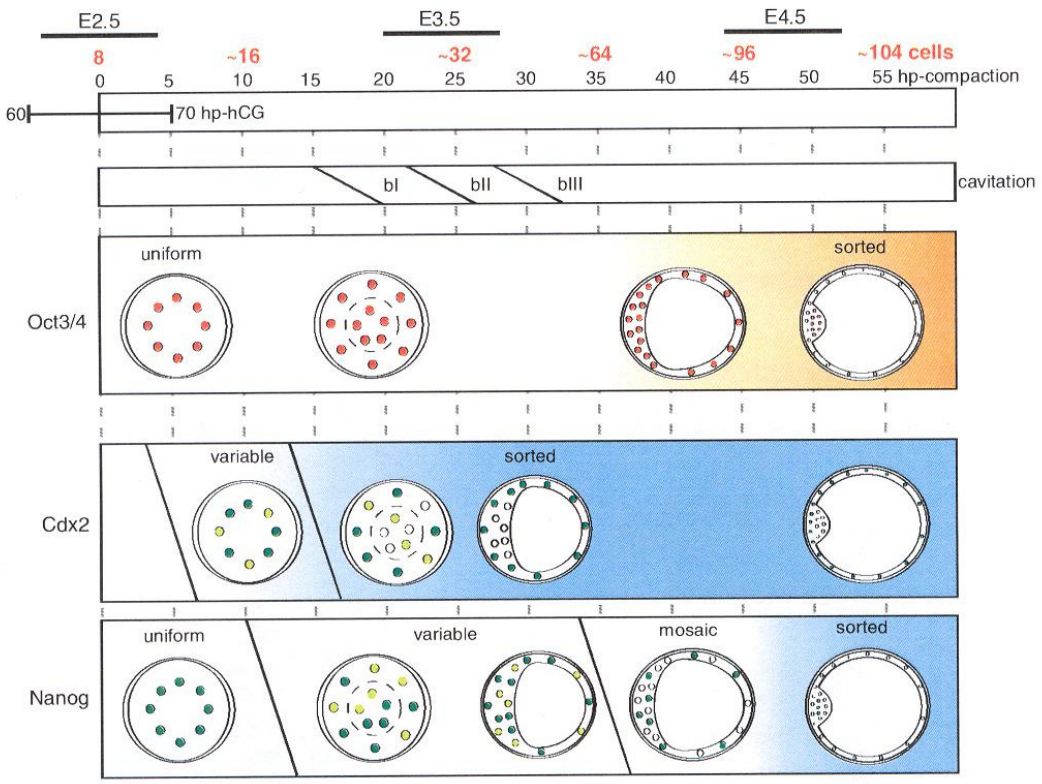
  1st lineage
  TE: Cdx2
  ICM: Pou5F1/Nanog

  2nd lineage
  Epi: Nanog
  PE: Gata4/6



Lessons from mouse embryo

- Stochastic model = regulative development
  Dietrich and Hiiragi, 2007

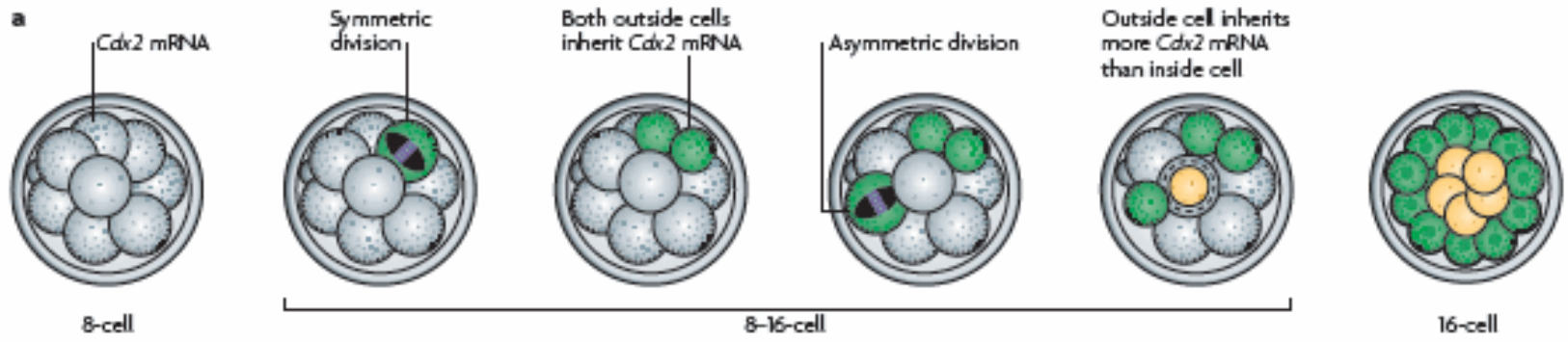


Oct-4: uniform and sorted

Cdx2 and Nanog: 2 phases
(1)Variable
(2)Sorted

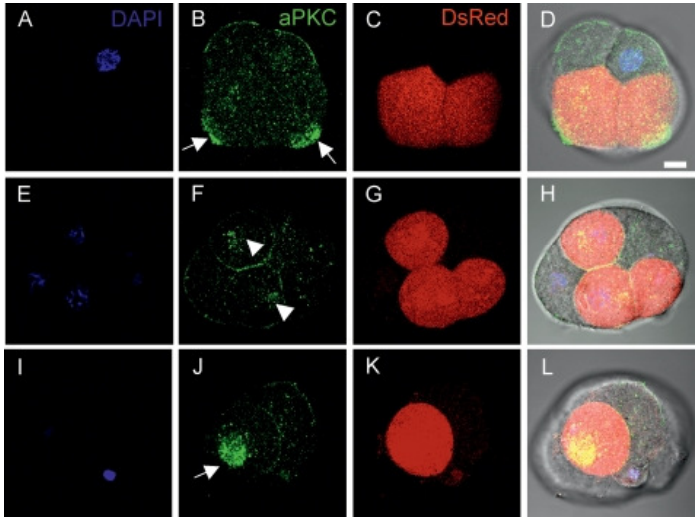
Lessons from the mouse embryo

- 1st lineage segregation
  - → Ralston and Rossant, 2005
    Cdx2 downstream of polarization
  - → Jedrusik et al. 2008; 2010
    Cdx2 mRNA is polarized in outside cells



Lessons from the mouse embryo

- 1st lineage segregation
  → Jedrusik et al. 2008; 2010
  - *Cdx2* mRNA: upregulation
    - Greater contribution to TE
    - More symmetric divisions
    - aPKC locatization
  - dsRNA Cdx2: downregulation
    - Greater contribution to ICM



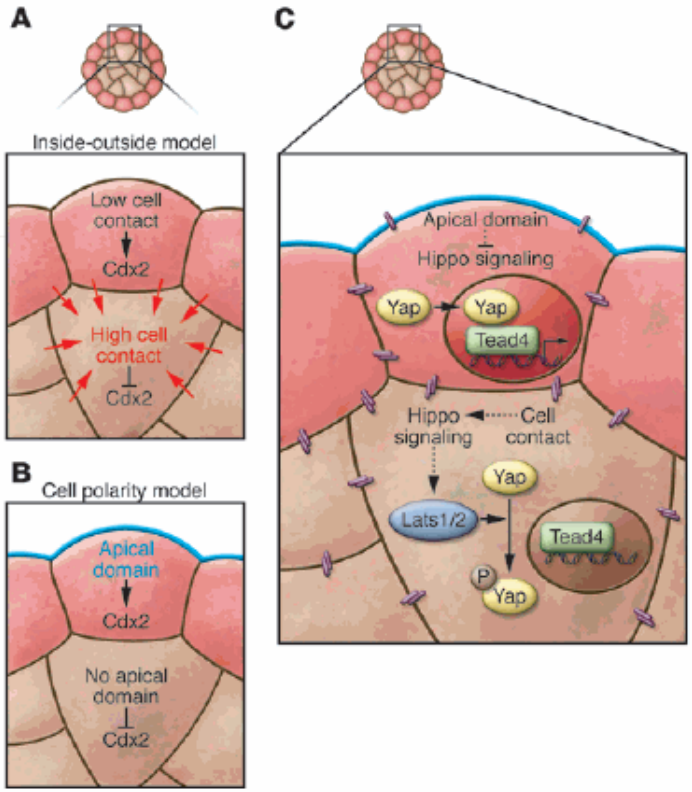
Lessons from the mouse embryo

- 1st lineage segregation

  Nishioka et al. 2009; Cockburn and Rossant, 2010

Different cell contact

Apical domain



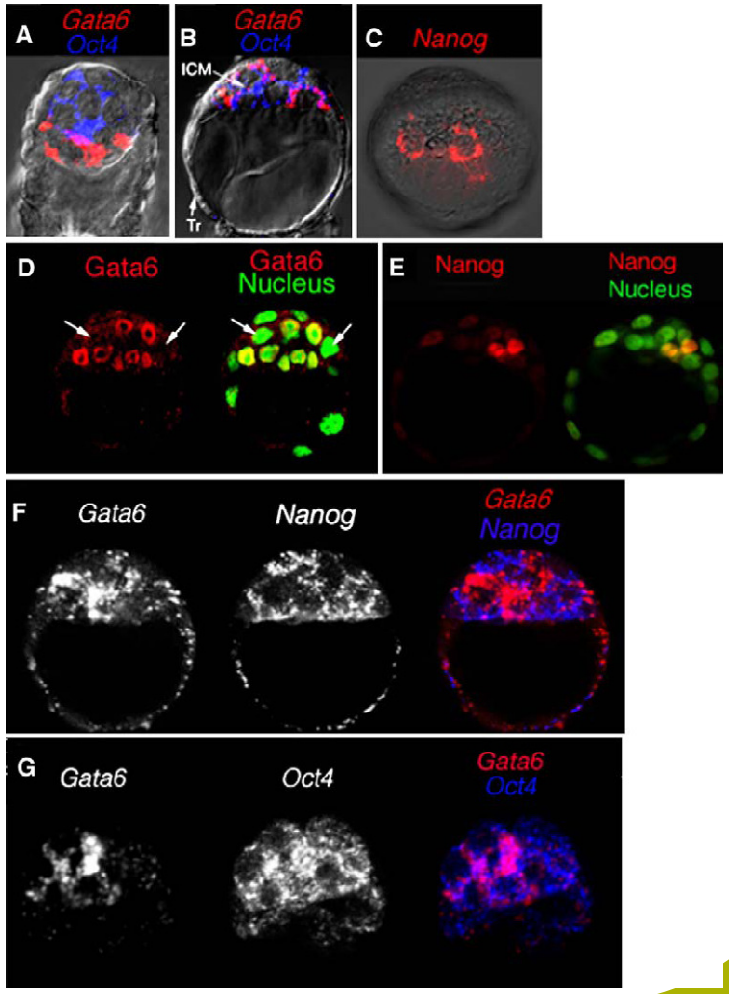
Lat1/2 kinases

Lessons from the mouse embryo

- 2nd lineage segregation
  Chazaud et al. 2006

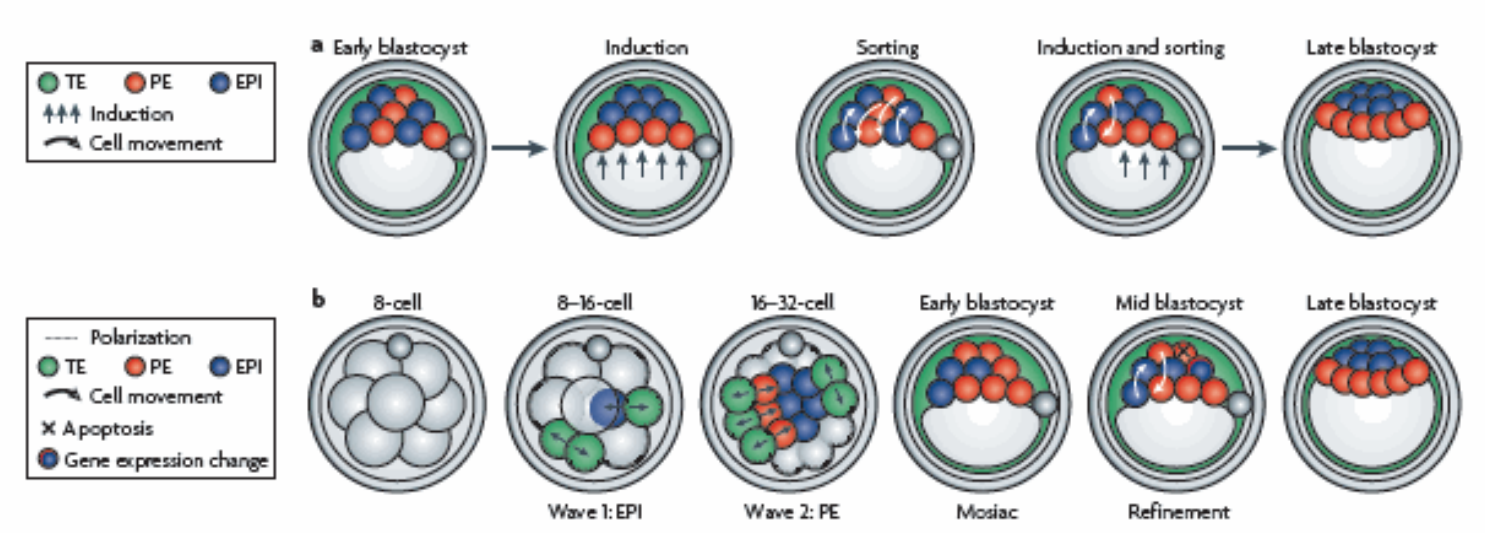
  Pepper-and salt-distribution
  Nanog and Gata6

  Sorting
  Epi: Nanog
  PE: Gata4/6



Lessons from mouse embryo

- Zernicka-Goetz et al. 2009
  - → Cell position
    - Cell movement according to gene expression pattern
    - Changes in gene expression
    - Apoptosis



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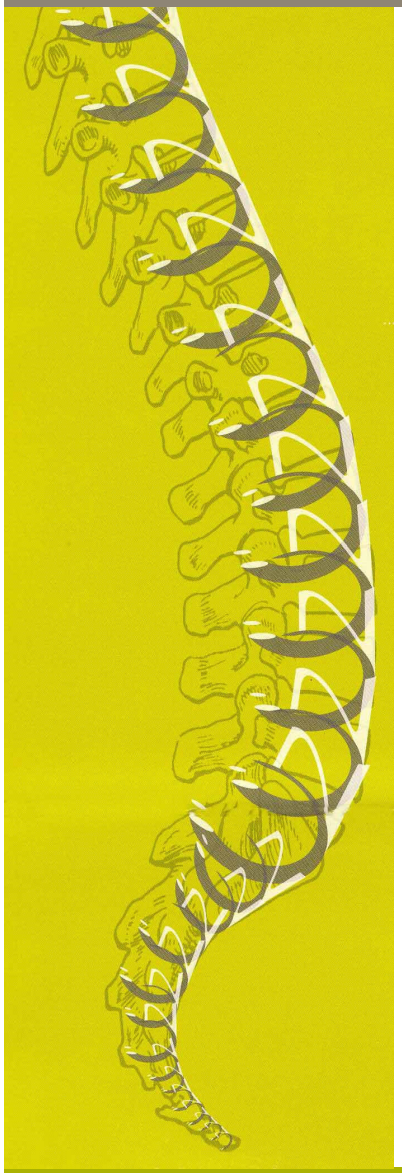

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Lessons from the human embryo

→ Embryonic genome activation (day 2/3)
(Braude et al. 1988; Dobson et al. 2004;
Cauffman et al. 2005; Cauffman et al. 2006)

→ 1st differentiation (day 5)

- → TE: differentiated
- → ICM: pluripotent
  - → Extra-embryonic endoderm, mesoderm and ectoderm
  - → 3 germ layers
  - → PGC
  - → embryonic stem cells (hESC)

Lessons from the human embryo

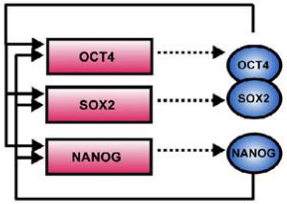
- Totipotency
  - → Marker for totipotency?
  - → When is totipotency lost?
    - When is the 1st differentiation irreversible?
    - Regulative development

Totipotency ↔ Differentiation

Lessons from the human embryo

- No “stemness” marker to identify totipotent cells

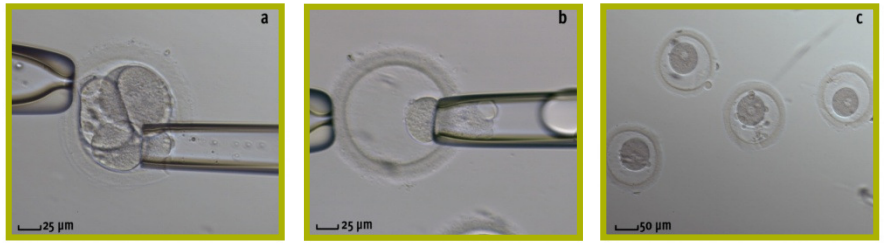
  Cauffman et al. 2005; 2006; 2009

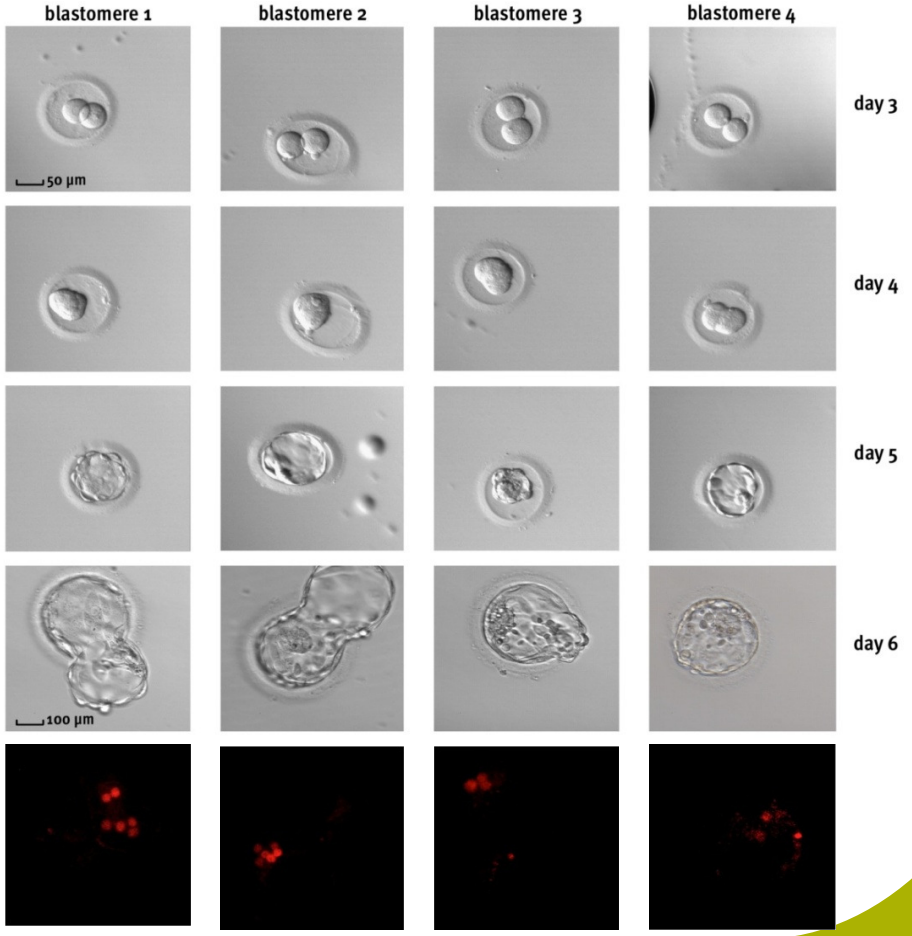


Boyer et al. 2005
hESC

Lessons from the human embryo

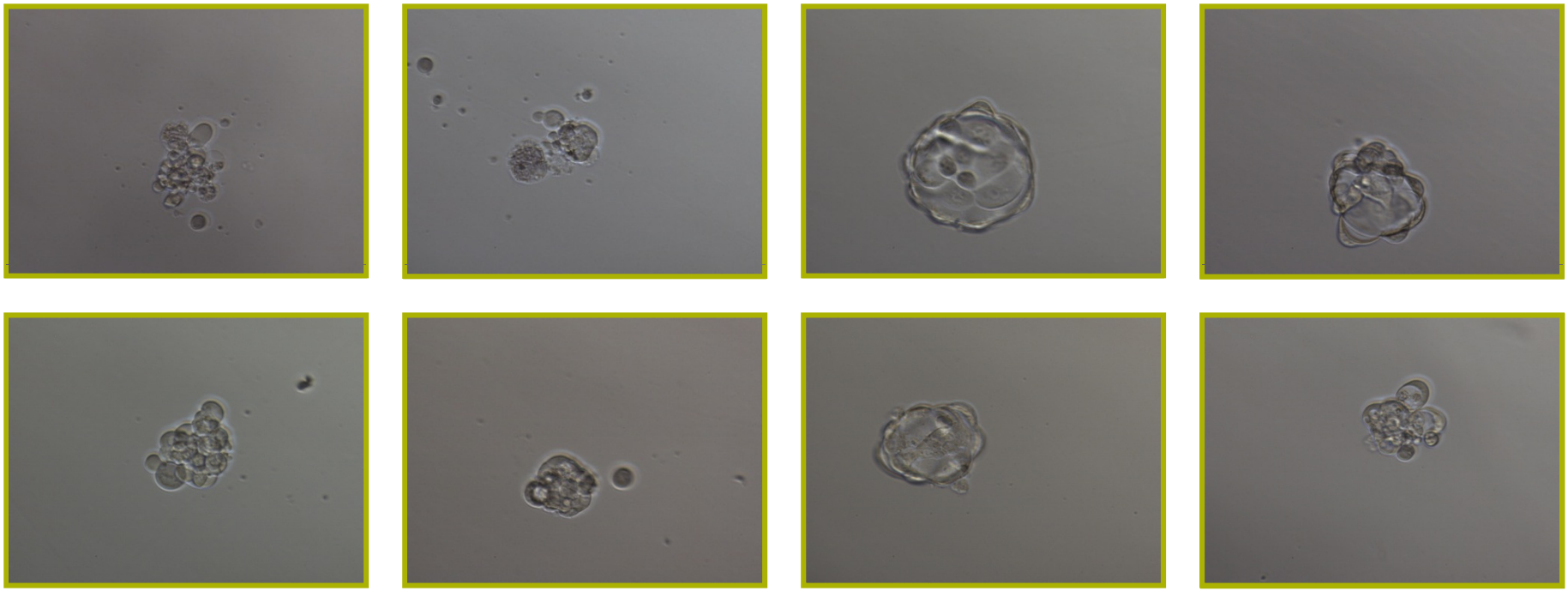
- Embryo splitting Van de Velde et al. 2008
  →Sister 4-cell stage blastomeres are potentially totipotent





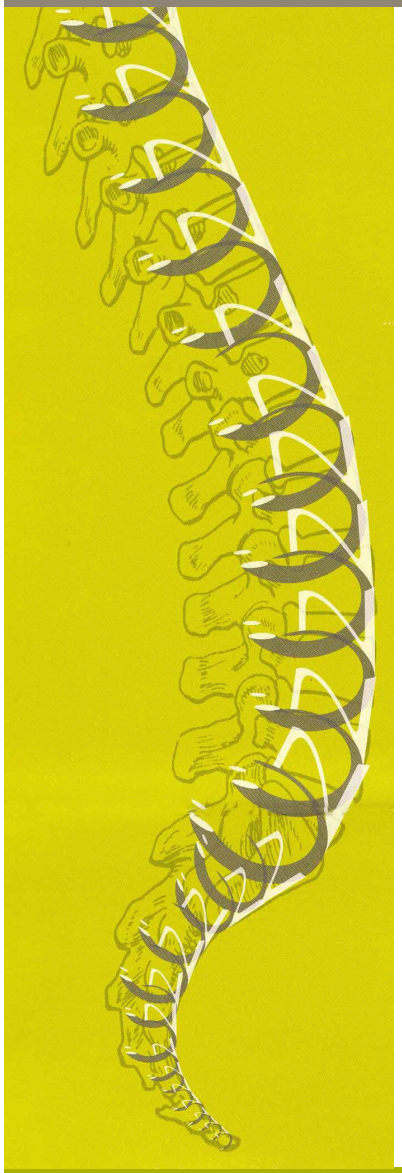
Lessons from the human embryo

- Splitting 8-cell stage embryo



No totipotent capacity
or not enough cells to form an inner cell population?

Outline



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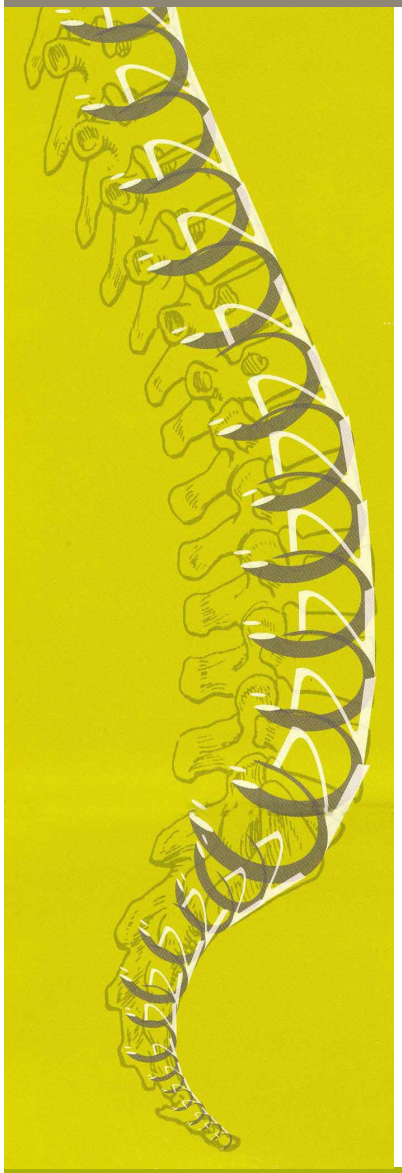
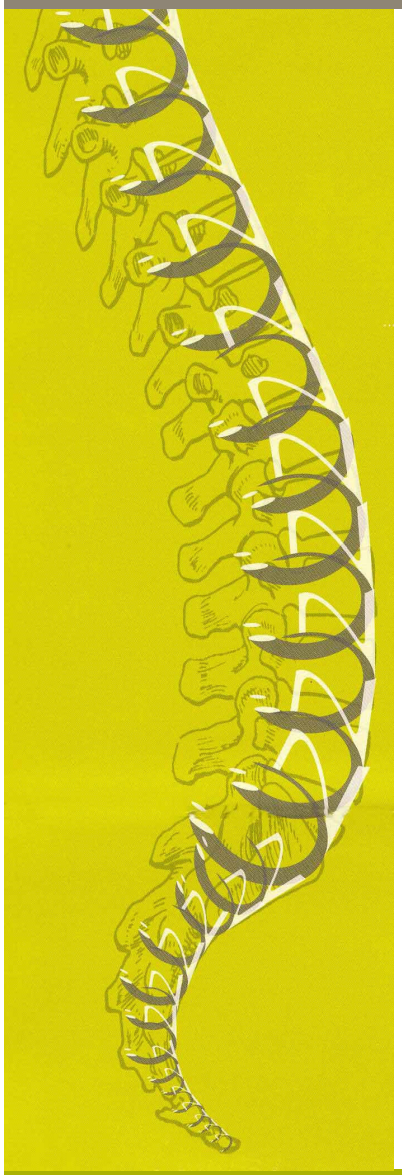


- Patterning in the mouse
  - → Regulative development
    - Polarity and position
  - → 1st and 2nd differentiation
    - Cell movement, changes in gene expression, apoptosis
- Patterning in the human
  - → Regulative development

Thanks



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Griet Meersdom

An Verloes

Martine Vercammen

Ewart Kuyck

Preimplantation genetic diagnosis

- De Vos et al. 2009

  Cohort of day 5 SET, 1-cell versus 2-cell biopsy PGD and PGS

  All embryos resulted from 8-cell stage embryos on day 3

	8 – 1 n=182	**8 – 2 n=259**	**8 – 0 (control) n=702**	**P value**
hCG per ET	46.7%	36.3%	48.6%	0.028
LBR per ET	37.4%	22.4%	35.0%	0.006

	8 – 1	**8 – 2**
Only 1 GTE	25.9%	18.2%
More than 1 GTE	46.5%	27.6%
Non-elective SET	34.1%	22.1%
Elective SET	55.0%	35.4%

Lessons from the human embryo

- Two hESC lines of distinct embryos

  Geens et al. 2009

  → VUB_26Quatro: 46 XX mosaic dup(7)(q33qter), del(18)(q23qter)

  → VUB_27Patru: 46 XY normal

  → At least one blastomere is pluripotent at the 4-cell stage