DEVELOPMENT AS A PROCESS OF SELF-ORGANIZATION:

THE ARROW OF TIME, DETERMINISM AND VARIABILITY IN THE EMBRYO'S LIFE

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DEDICATED TO THE MEMORY OF

LEV V. BELOUSSOV

(18/07/1935 – 11/09/2017)

A PERSON WHO COMBINED BIOLOGICAL KNOWLEDGE WITH PHYSICAL UNDERSTANDING

THE MYSTERY OF THE EMBRYO DEVELOPMENT

Zygote

A small ball, 140 μm in diameter

Weight 1 μg

3 billion times smaller than a newborn

Adult human

Encephalon :

Surface size 1 m^2

86.000.000.000 neurons

500 TByte data capacity

= 1,5 km thick pile of books

Immune system:

Up to 100.000.000 types of Ig

Blood-vascular system:

100.000.000.000 capillaries

total thickness 50 m^2

total length 100.000 km

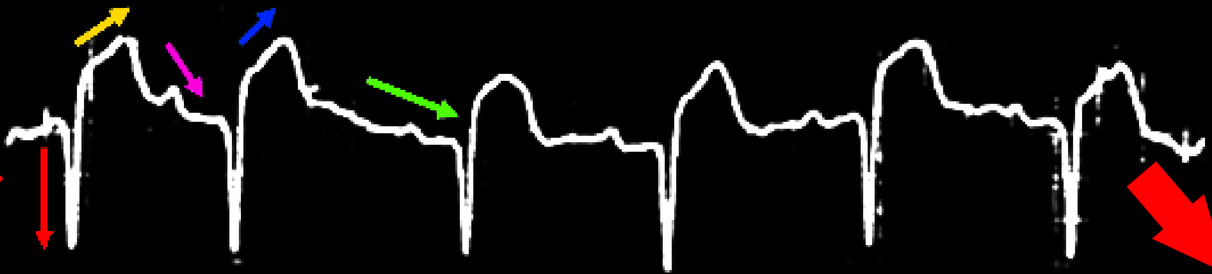
Embryo development is associated with the greatest complexity increase in the observable Universe

Why does it happen?

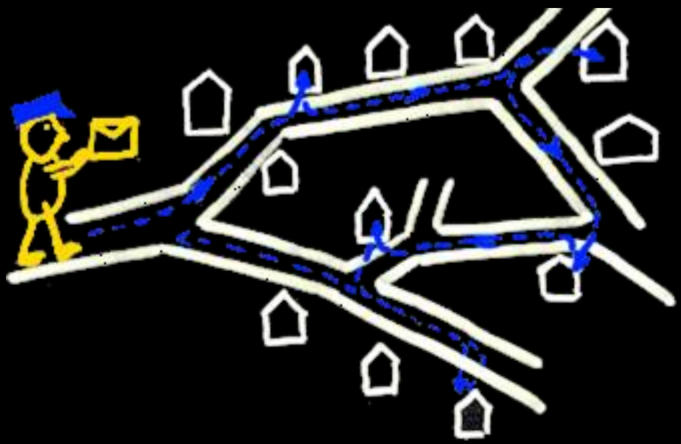
What drives it?

Nikica Zaninovic, PhD
WCMC

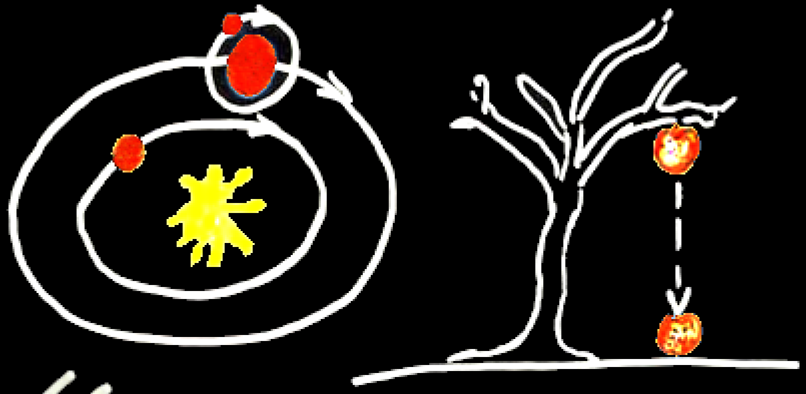
WHAT DRIVES BIOLOGICAL PROCESSES?



A sequence of causes?



Or a unified law?



Or a combination of these?

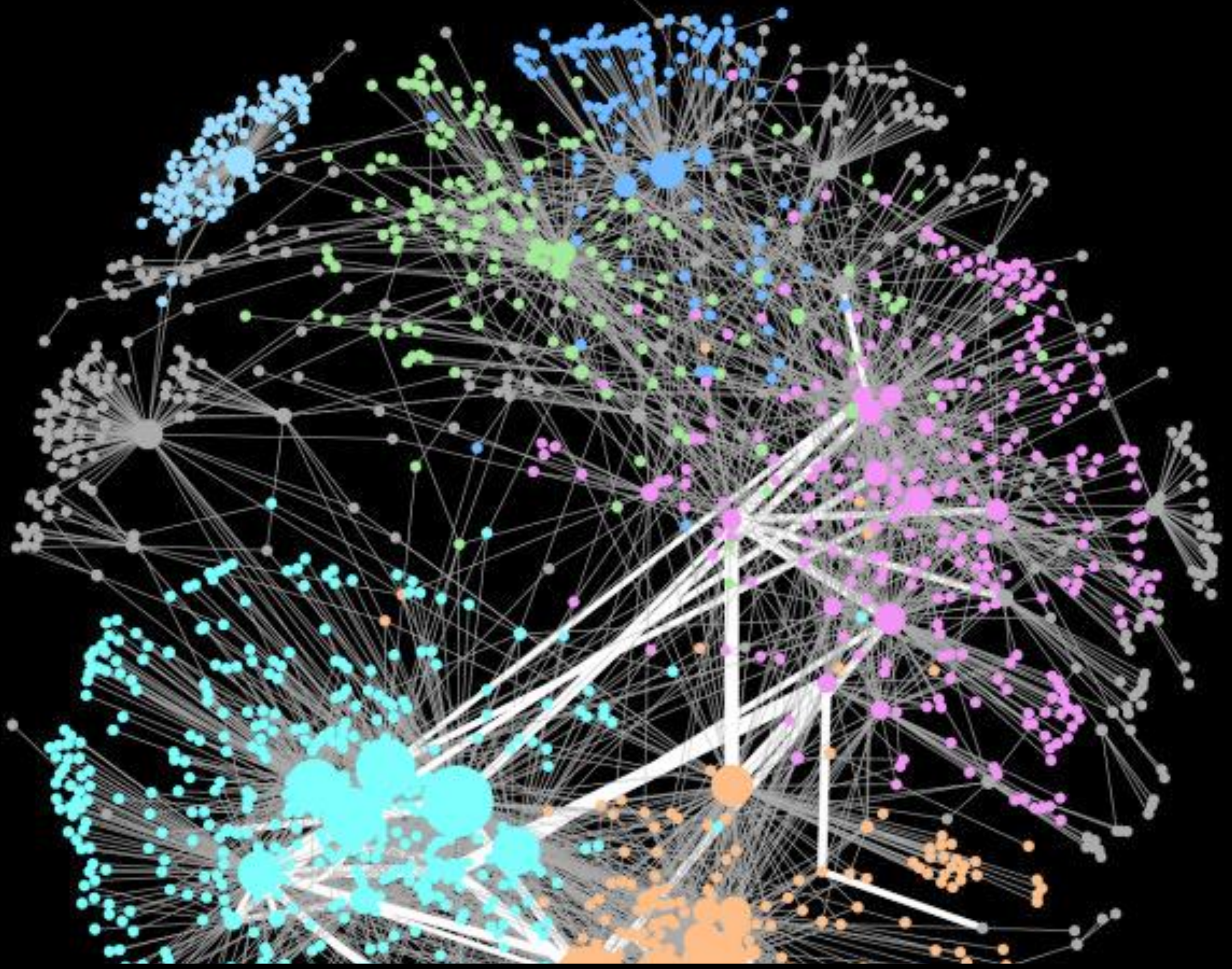
Lev Beloussov, presentation

SEQUENCE OF CAUSES

Gene networks, signaling cascades

Table 3
Genes critical to implantation and decidualization: res

Gene	Gene product
Bmp2	Bone morphogenetic protein 2
Cdh1	E-cadherin
Ctnnb1	β-catenin
Dicer	Dicer
Errfi1	ERBB receptor feedback inhibitor 1
Foxa2	Forkhead box A2
Gja1	Connexin 43
Hbegf	Heparin-binding EGF-like growth factor
Hand2	Heart and neural crest derivatives expressed transcript 2
Ihh	Indian hedgehog
Src2	Steroid receptor coactivator 2
Klf5	Kruppel-like factor 5
K-ras	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
Pten	Phosphatase and tensin homolog
Msx1/2	Muscle segment homeobox gene (Msh) family members 1/2;
Nodal	NODAL
Notch1	Notch1
Nr2f2	Chicken ovalbumin upstream promoter transcription factor II
p53	Transformation related protein 53

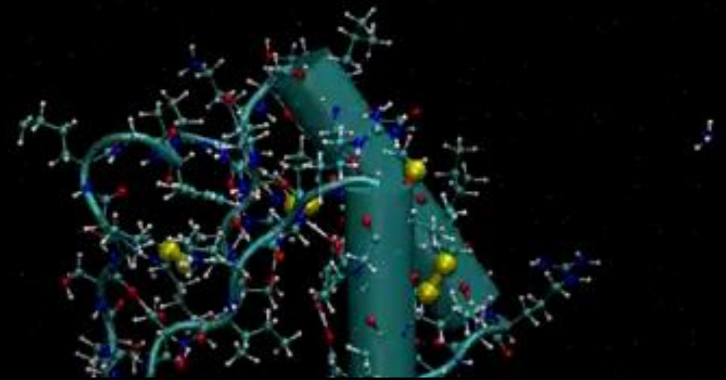


DOES IT ALWAYS HELP?

DOES IT EXPLAIN MECHANISMS?

UNIFIED LAWS

Deterministic processes



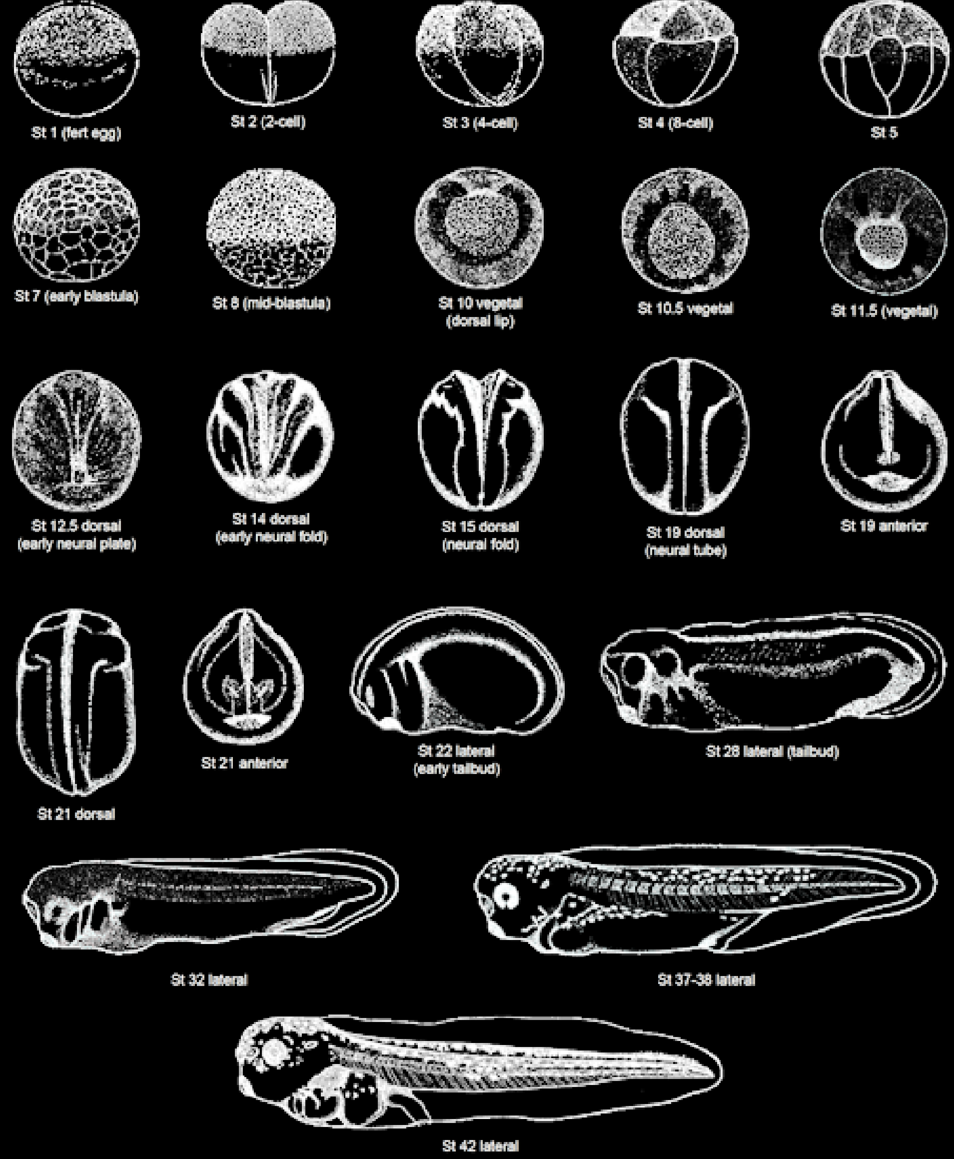
Predictable in the future

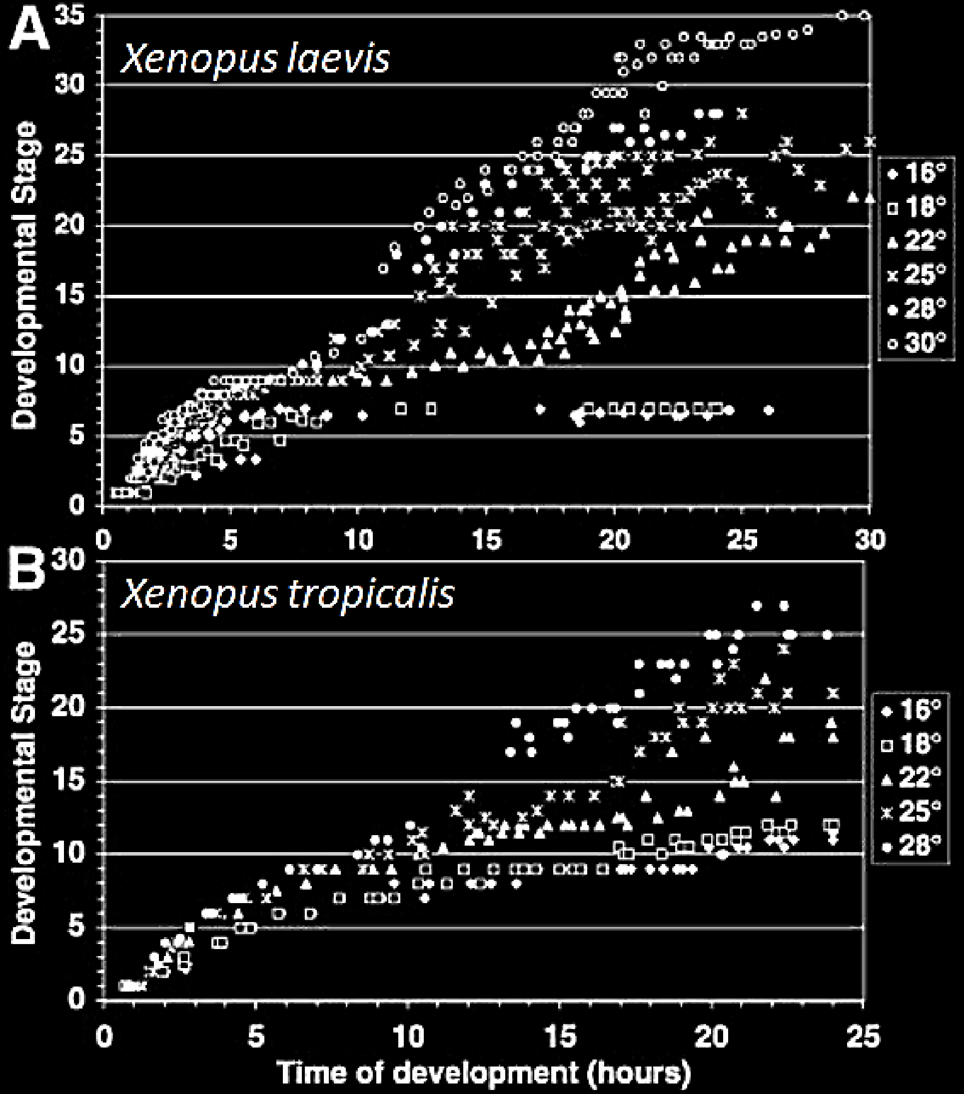
Reconstructable in the past

CAN IT DESCRIBE THE WHOLE EMBRYO DEVELOPMENT?

IS EMBRYO DEVELOPMENT DETERMINISTIC?

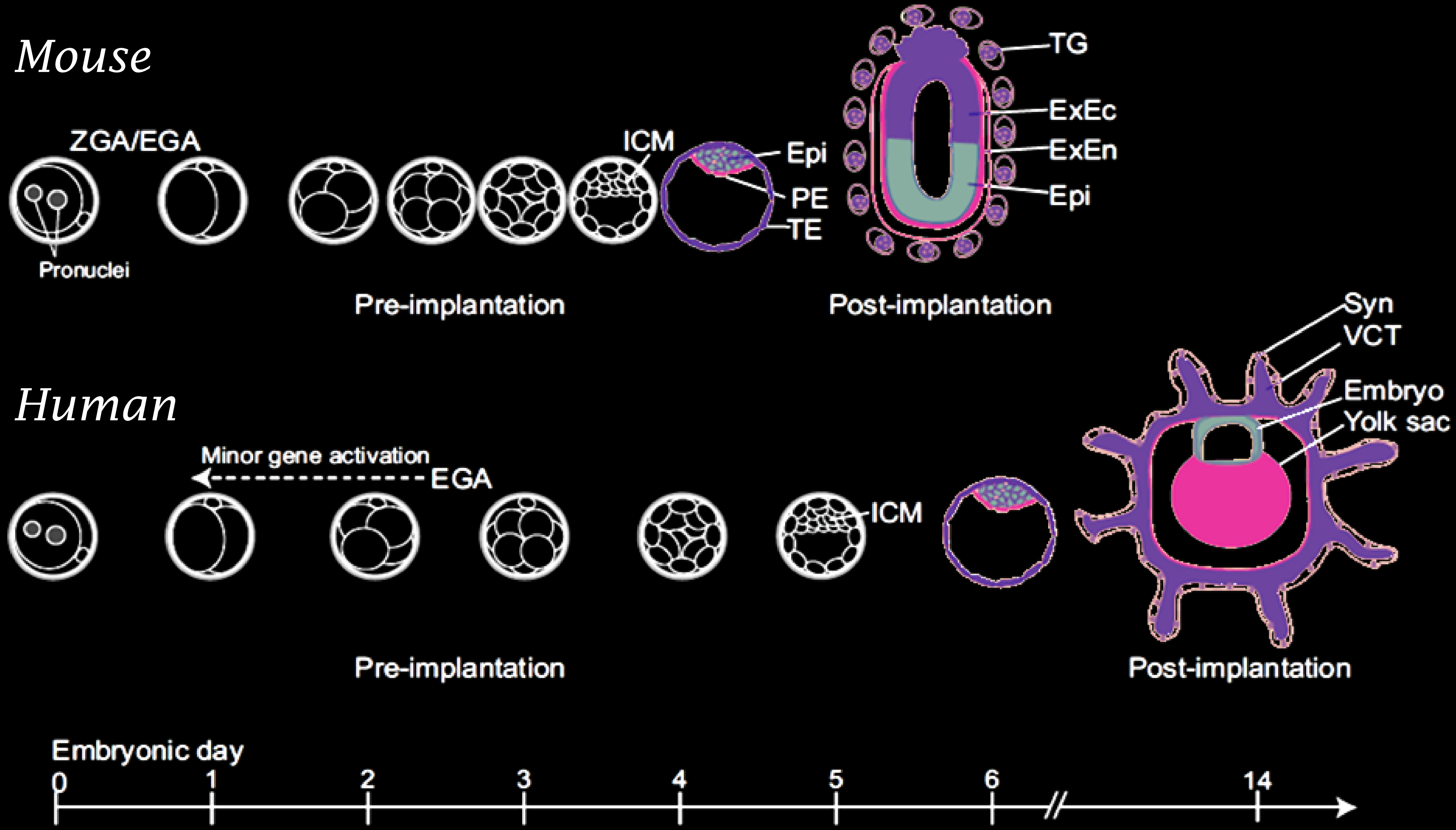
Xenopus laevis





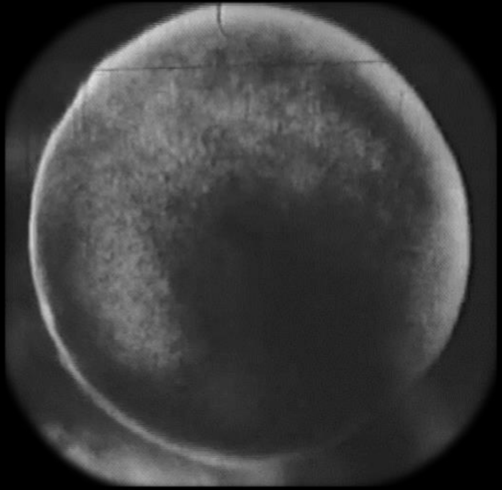
Nieuwkoop and Faber, 1956
Khokha et al, 2002

IS EMBRYO DEVELOPMENT DETERMINISTIC?

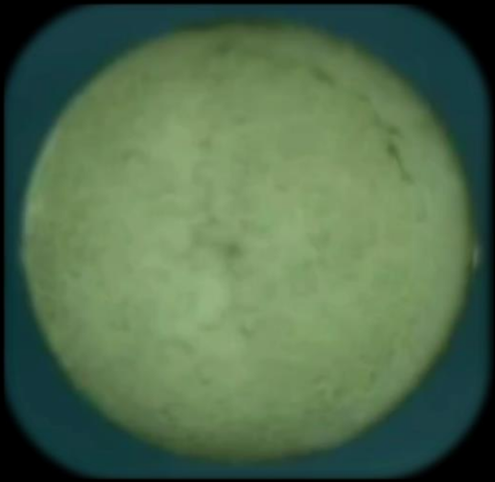


Niakan et al, 2012

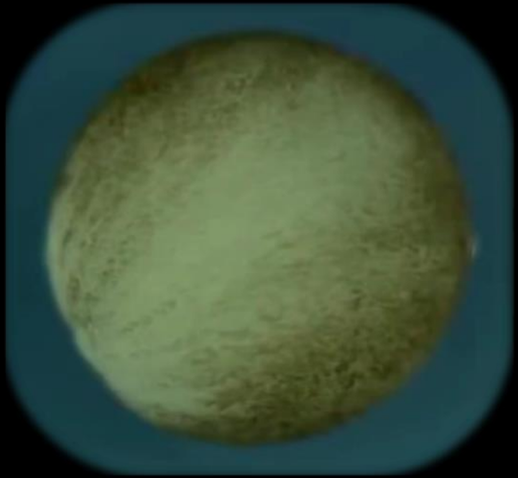
DETERMINED SEQUENCES OF STAGES



Cleavage
Xenopus laevis



Gastrulation
Xenopus laevis



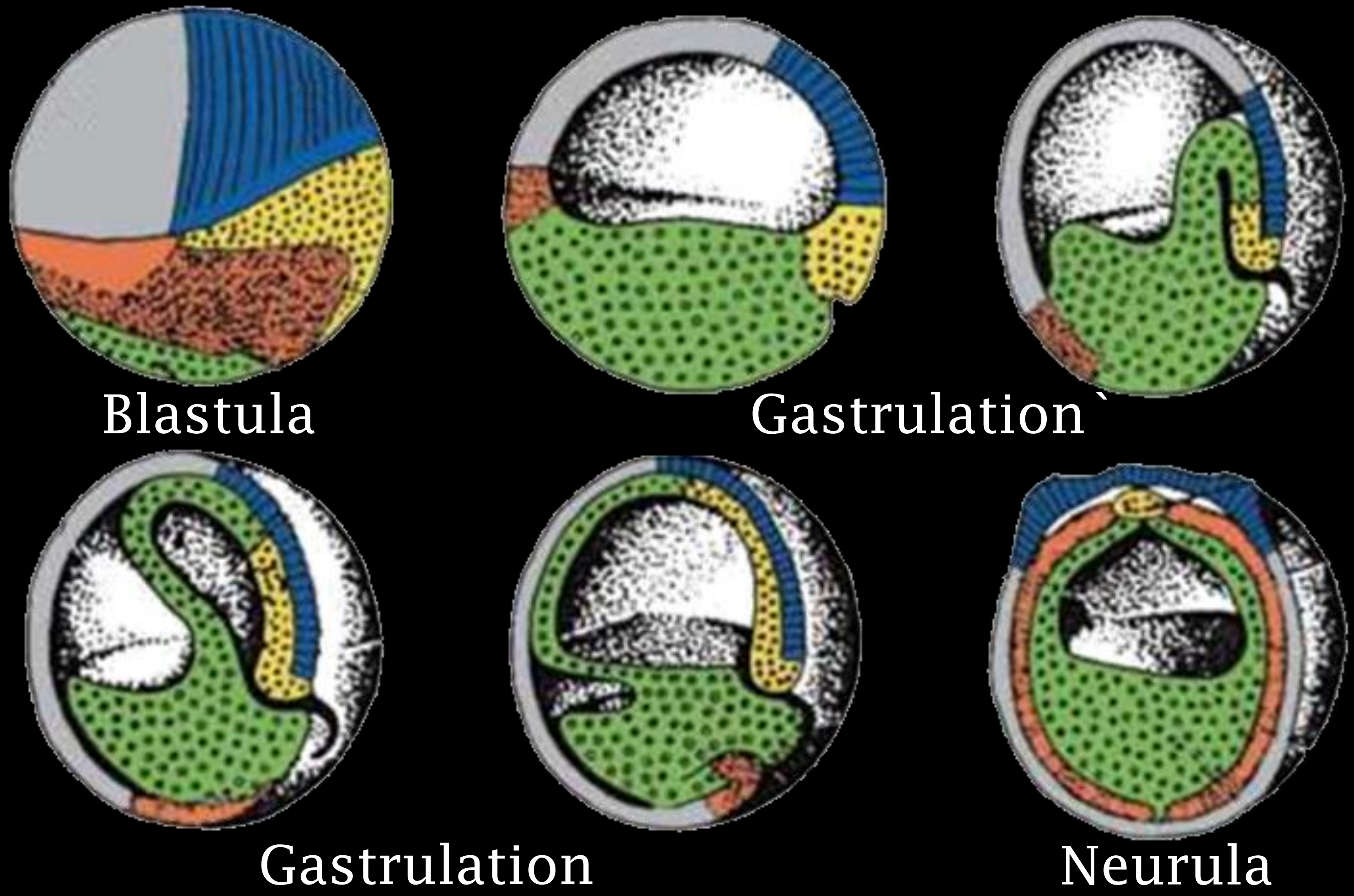
Neurulation
Xenopus laevis

Cleavage
Human

Blastocyst
development
Human

TOPOGRAPHY
OF PRESUMPTIVE RUDIMENTS

X. laevis



■ – **Endoderm**

Mesoderm: ■ – chordal ■ – somital ■ – ventral

Ectoderm: ■ – integumentary ■ – neural

IS EMBRYO DEVELOPMENT DETERMINISTIC?

DETERMINISM
LAPLACE'S DEMON

Pierre-Simon
de Laplace
(1749—1827)

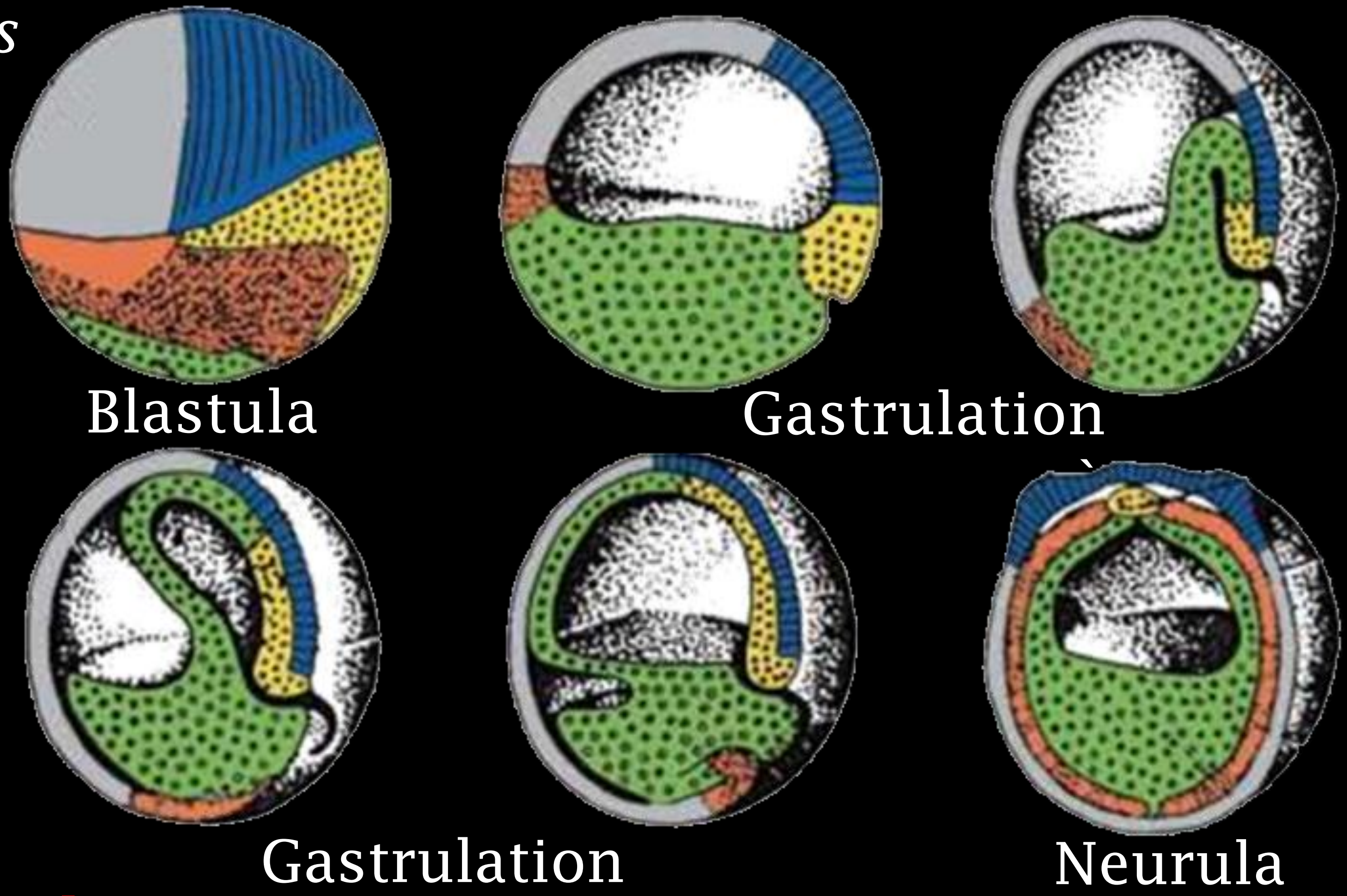
We may regard the present state of the universe as the effect of its past and the cause of its future.

An intellect... would embrace in a single formula the movements of the greatest bodies of the universe and those of the tiniest atom.

For such an intellect nothing would be uncertain and the future just like the past would be present before its eyes.

IS EMBRYO DEVELOPMENT DETERMINISTIC?

X. laevis



■ – **Endoderm**

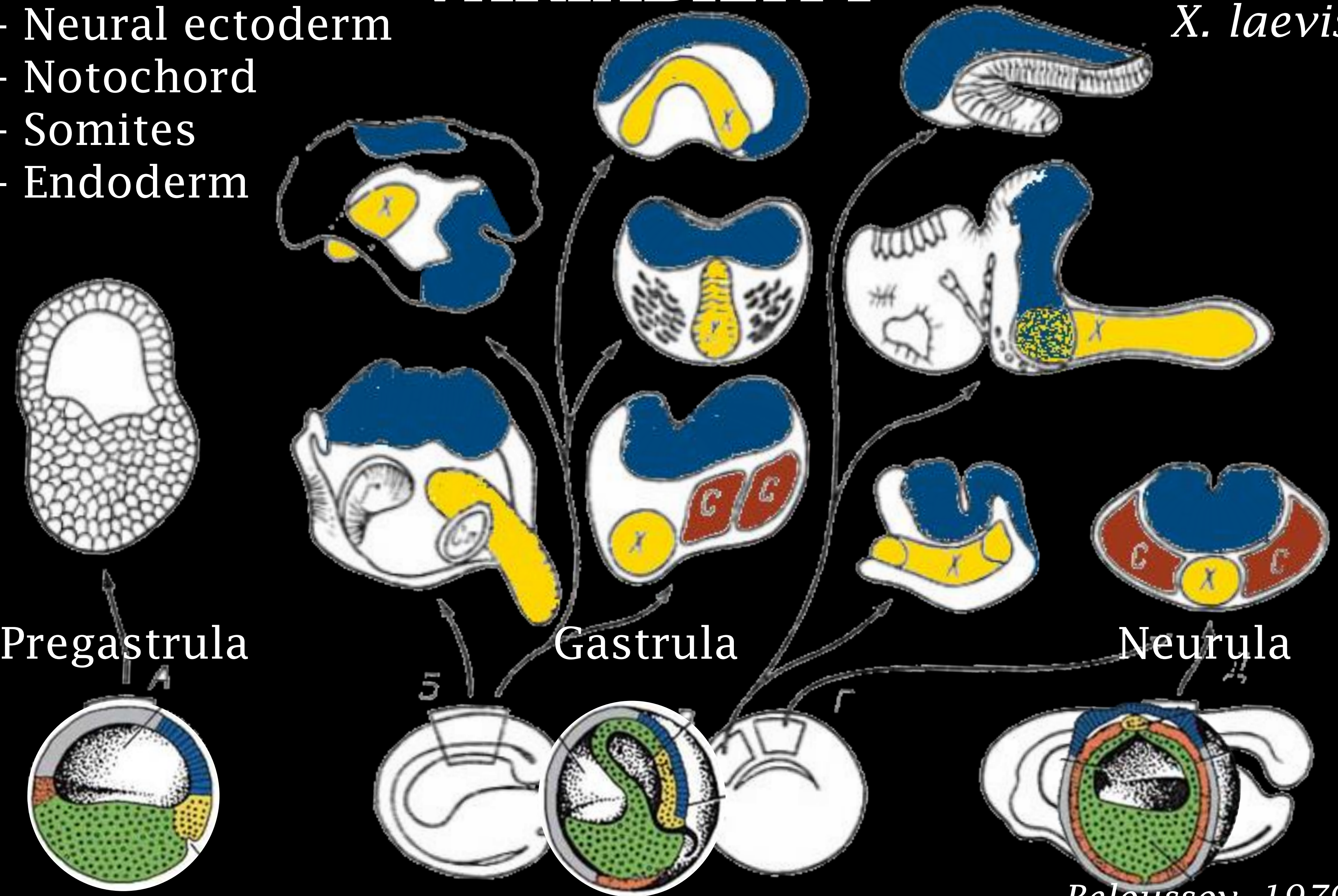
Mesoderm: ■ – chordal ■ – somital ■ – ventral

Ectoderm: ■ – integumentary ■ – neural

PRESUMPTIVE RUDIMENTS VARIABILITY

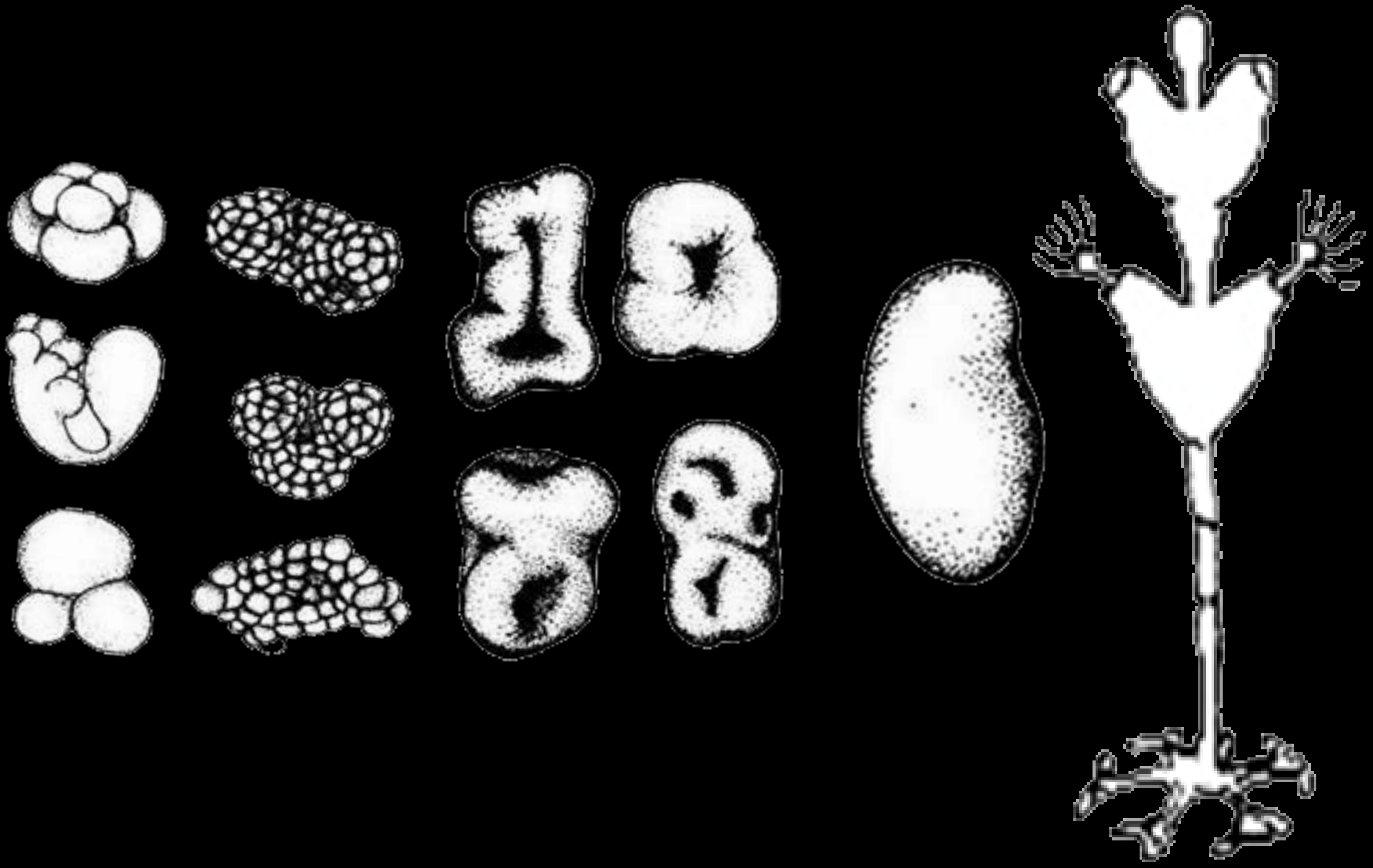
- ■ – Neural ectoderm
- ■ – Notochord
- ■ – Somites
- ■ – Endoderm

X. laevis



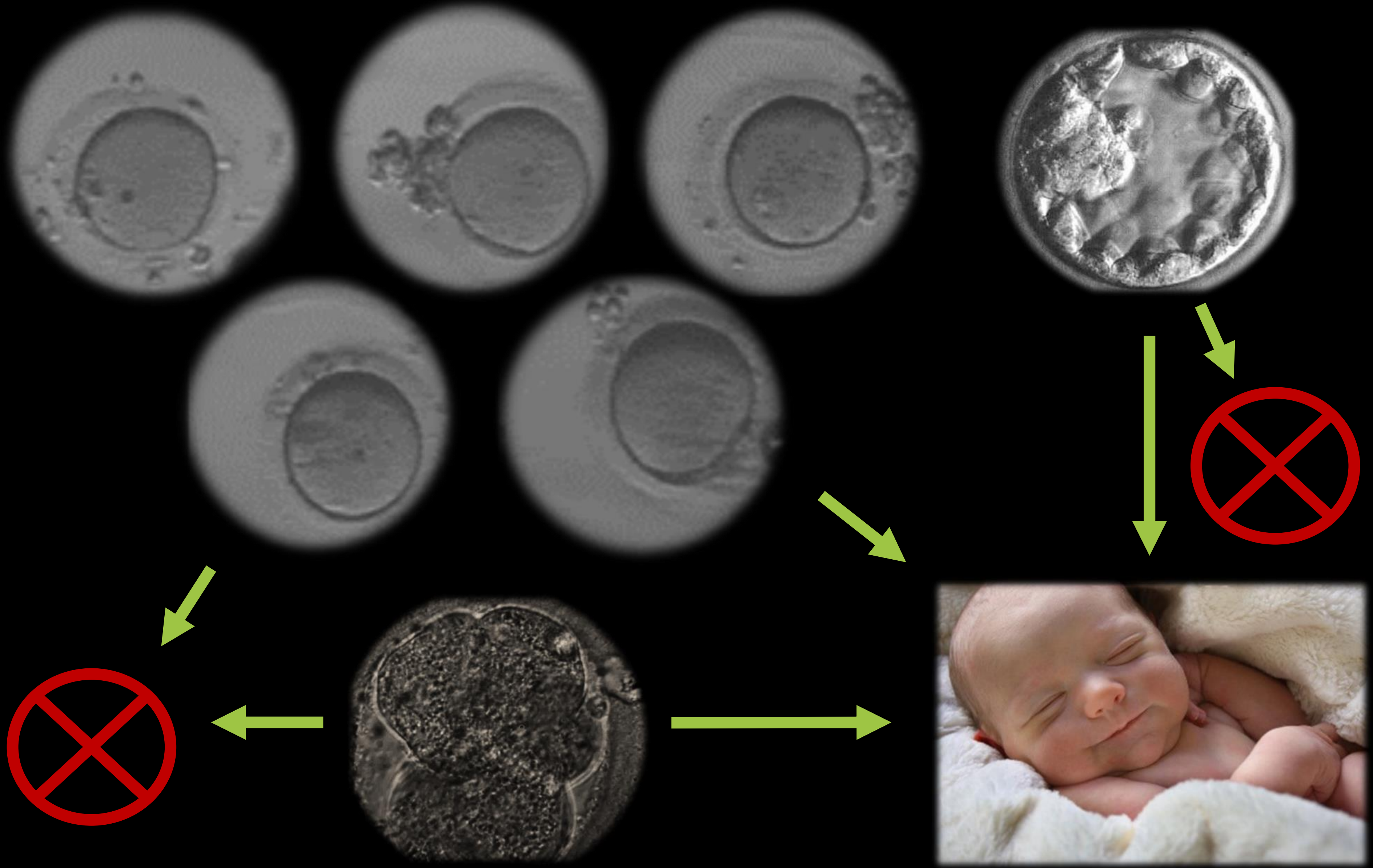
Beloussov, 1979

VARIABILITY AND EQUIFINALITY IN HYDROID DEVELOPMENT



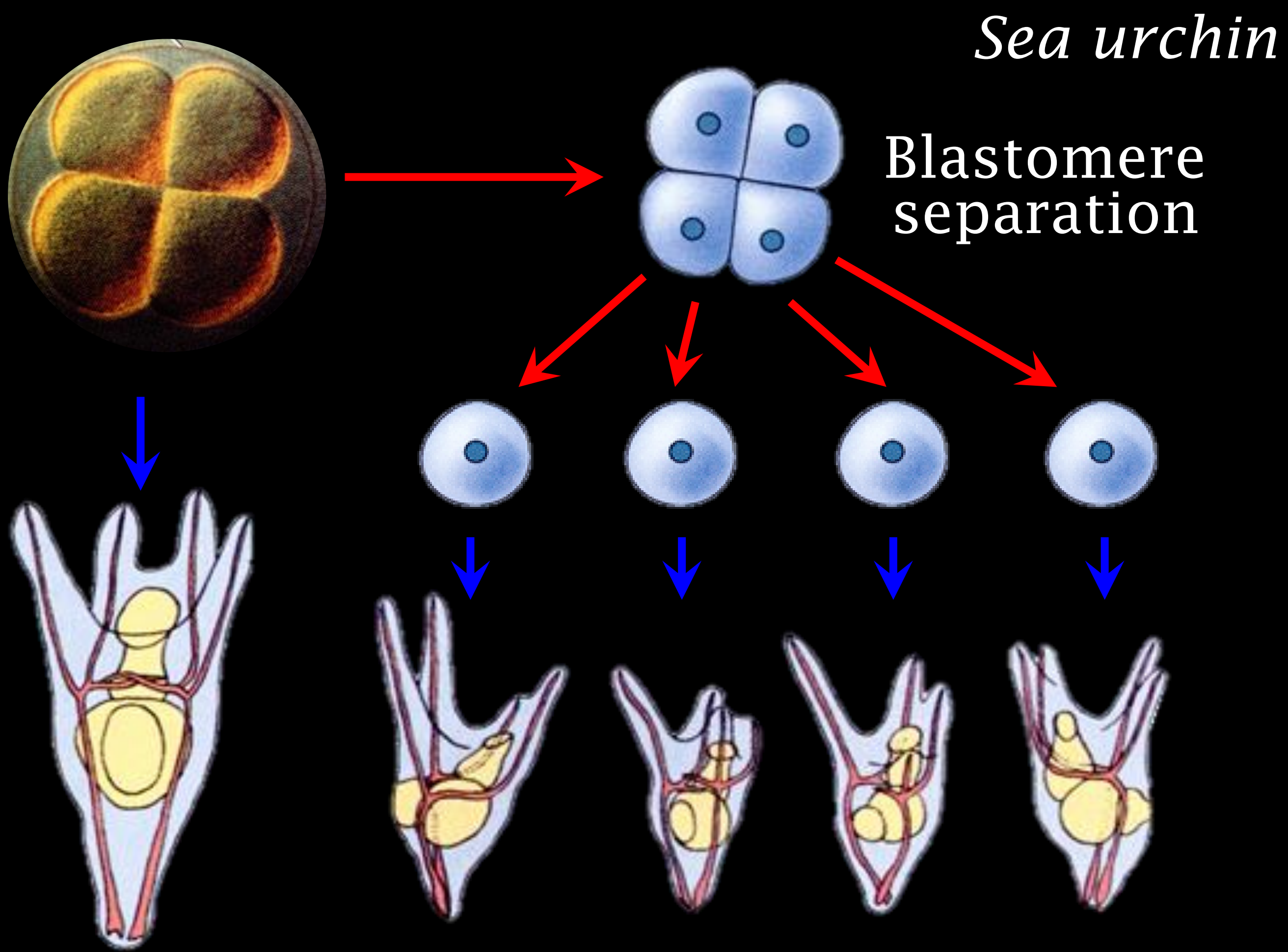
Beloussov, 1979

VARIABILITY IN HUMAN EMBRYO DEVELOPMENT



“SENSING OF THE WHOLE”
EMBRYONIC REGULATIONS

Hans Driesch
(1867—1941)

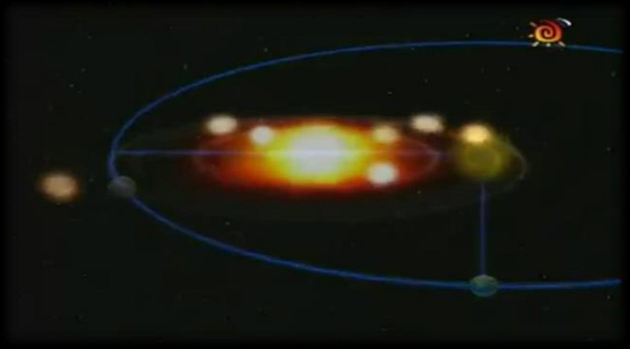


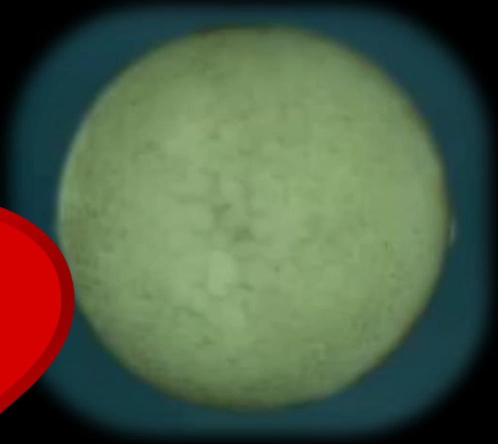
“THE FATE OF AN ELEMENT IS A FUNCTION OF ITS POSITION”

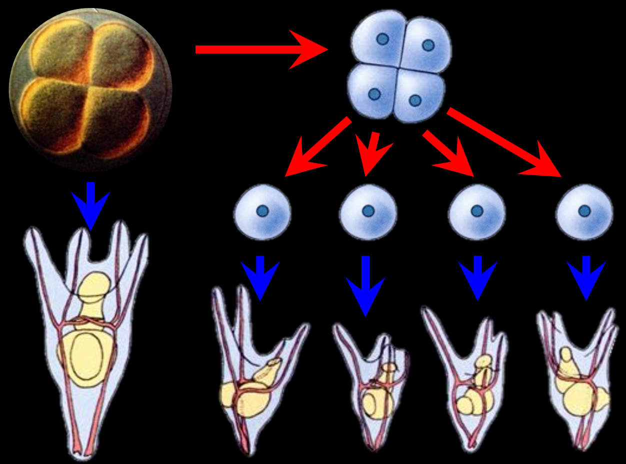
PHYSICS AND BIOLOGY

Determinism

Variability and regulations







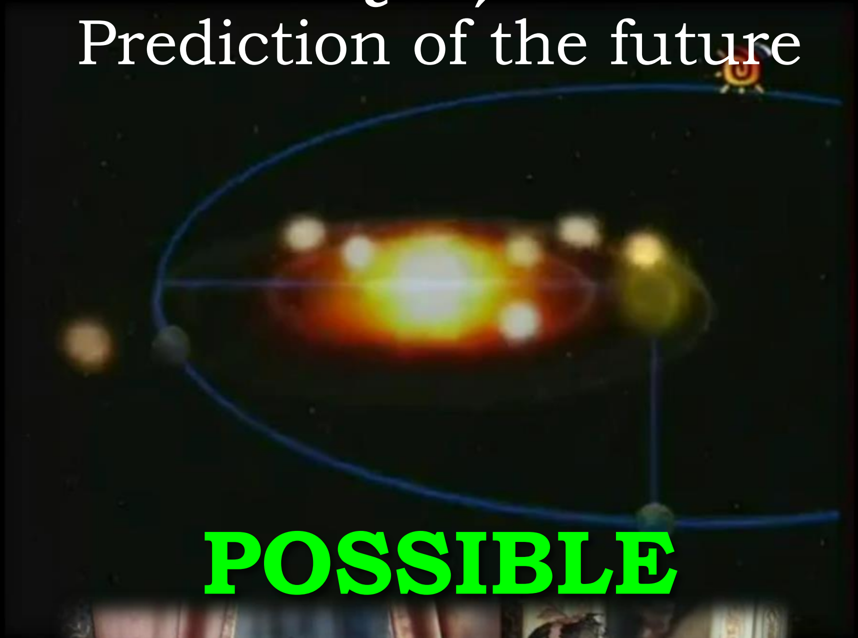
Are physical laws violated in biological systems???

TIME SYMMETRY

DETERMINISTIC PROCESSES

t →

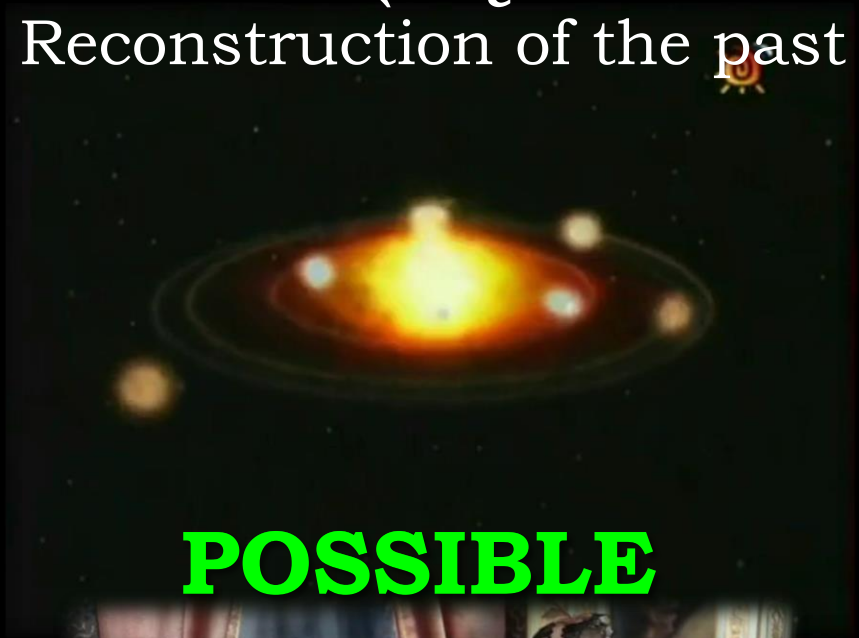
Prediction of the future



POSSIBLE

← t

Reconstruction of the past

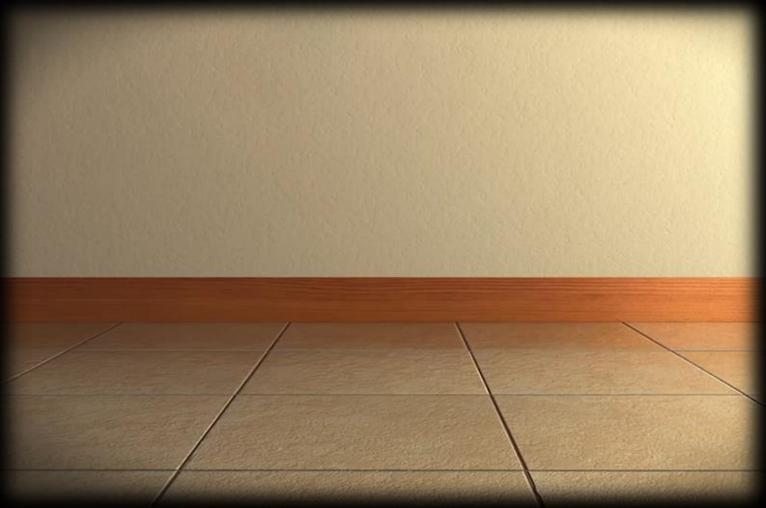


POSSIBLE

TIME SYMMETRY BREAKING

THERMODYNAMIC SYSTEMS

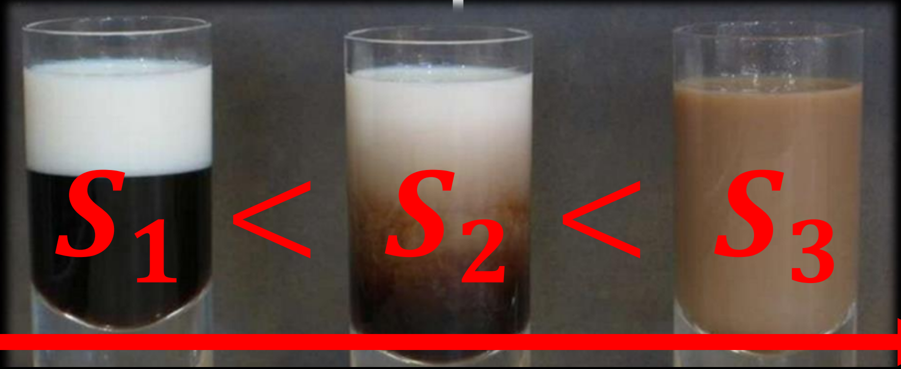
t →



POSSIBLE

← t

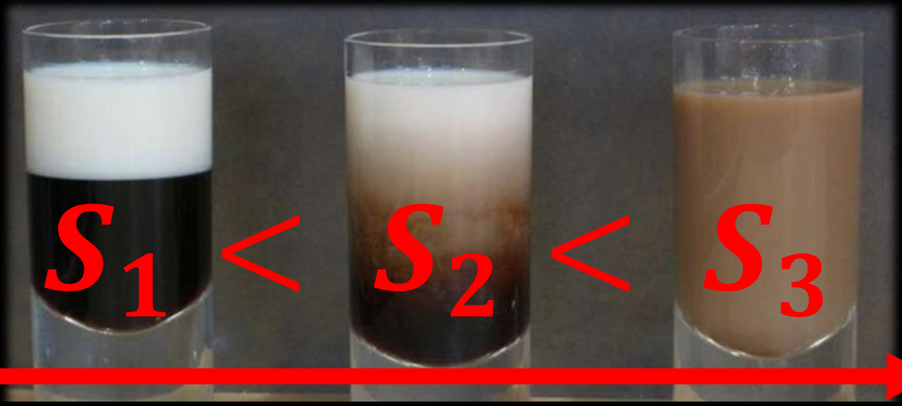
IMPOSSIBLE



past → future

Direction of time

TIME SYMMETRY BREAKING
THERMODYNAMIC SYSTEMS



past → future

Direction of time

For each molecule: $W(up) = W(down) = \frac{1}{2}$

For N molecules: $W(all\ are\ up) = \left(\frac{1}{2}\right)^N$

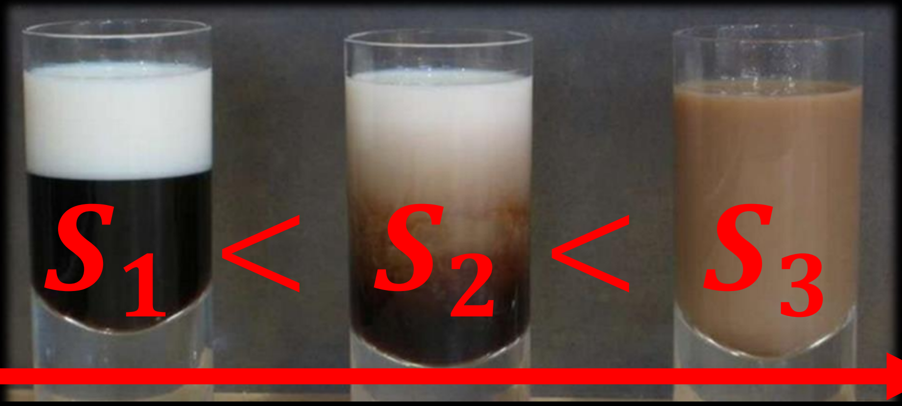
$$W_{3\rightarrow 1} \sim \frac{W_1}{W_3} \sim \underbrace{0{,}00\ldots001}_{10^{23}\ \text{zeros!!!}}$$

$$S = k \ln W$$

$$W_1 \ll W_2 \ll W_3$$

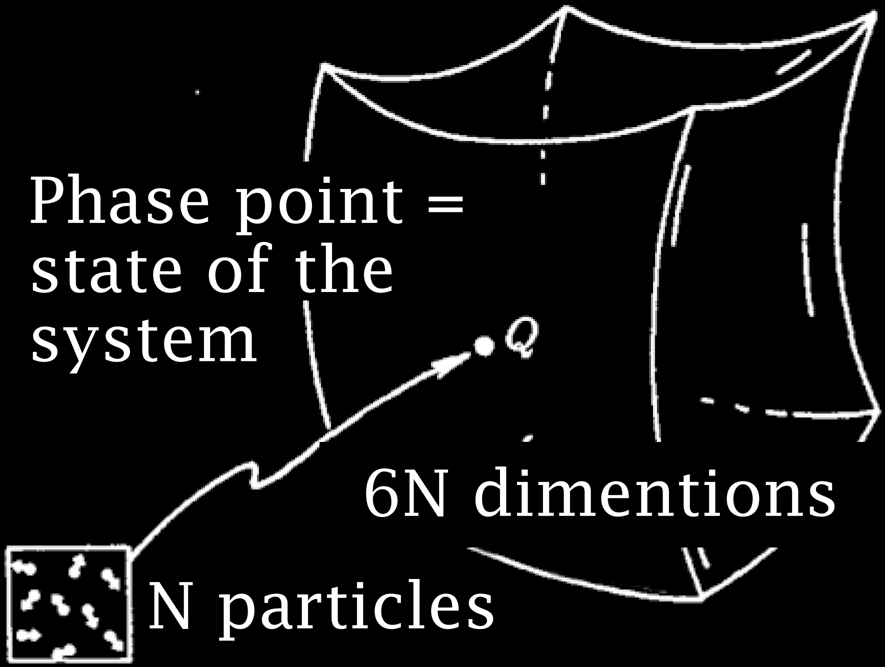
$$S_1 < S_2 < S_3$$

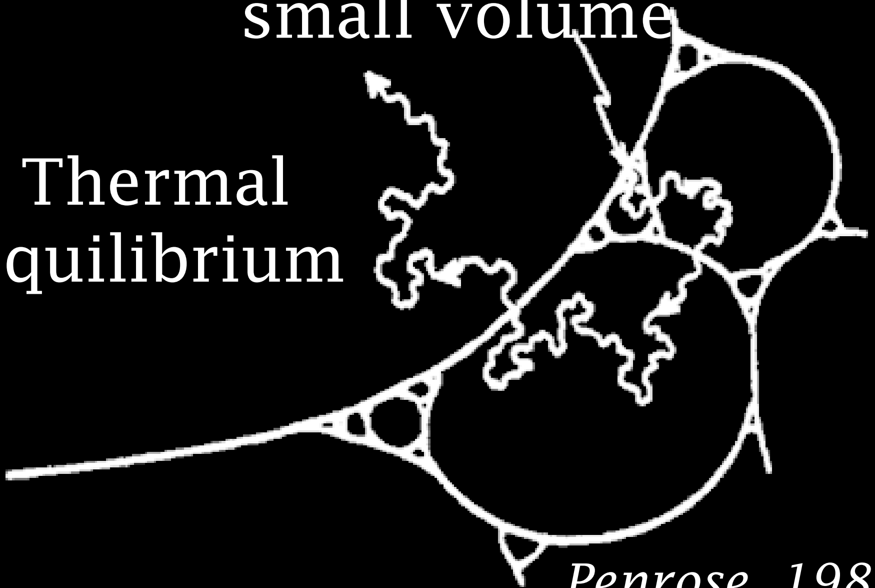
TIME SYMMETRY BREAKING
THERMODYNAMIC SYSTEMS



past → future

Direction of time





Penrose, 1989

THE ARROW OF TIME
THERMODYNAMIC SYSTEMS

Arthur Stanley Eddington (1882—1944)

Let us draw an arrow arbitrarily.

If as we follow the arrow we find more and more of the random element in the state of the world, then the arrow is pointing towards the future...

That is the only distinction known to physics.

I shall use the phrase ‘time's arrow’ to express this one-way property of time which has no analogue in space.

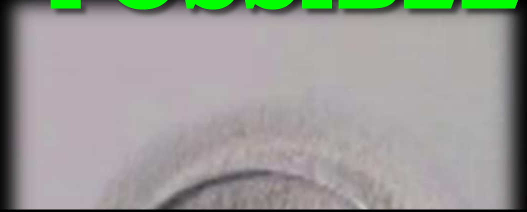
Eddington, 1928

TIME SYMMETRY BREAKING

BIOLOGICAL SYSTEMS

t → | ← t

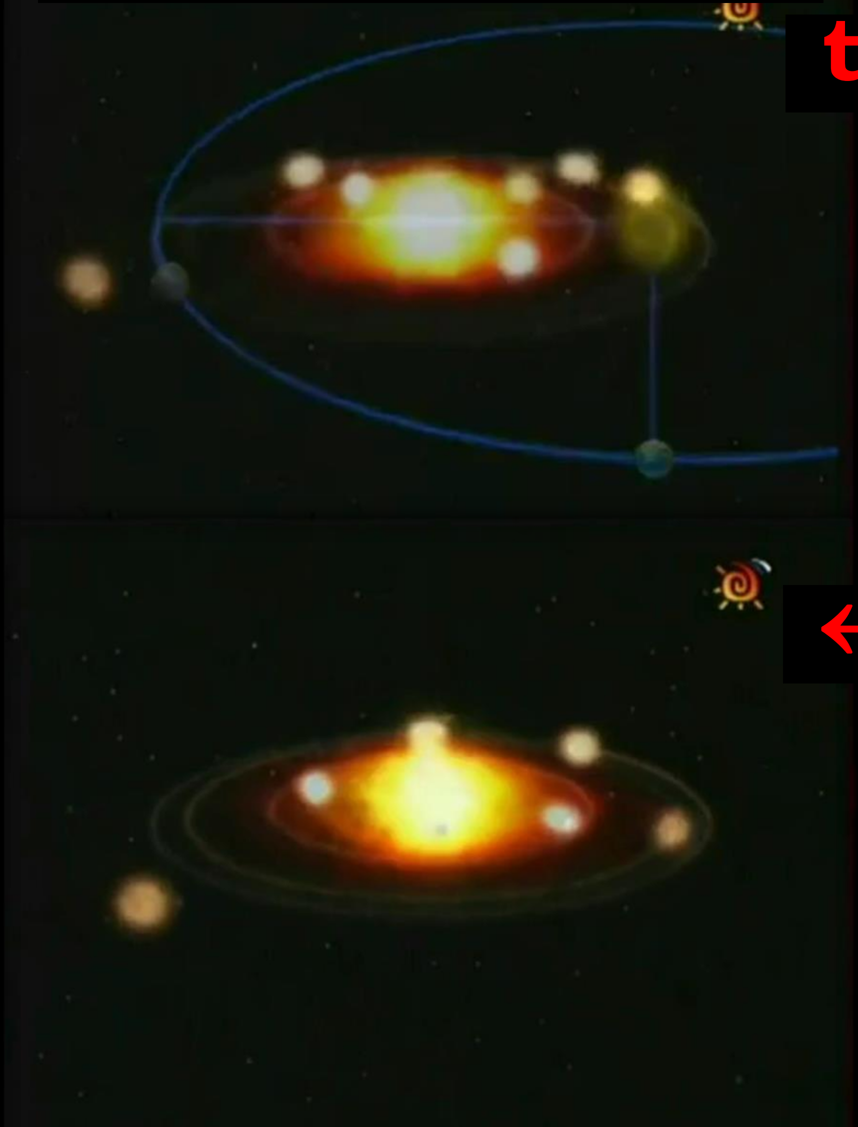
POSSIBLE | IMPOSSIBLE



IRREVERSIBILITY
IN EMBRYO DEVELOPMENT –
INCREASING COMPLEXITY

THE ARROW OF TIME APPEARS IN UNSTABLE SYSTEMS

Reversible



Irreversible

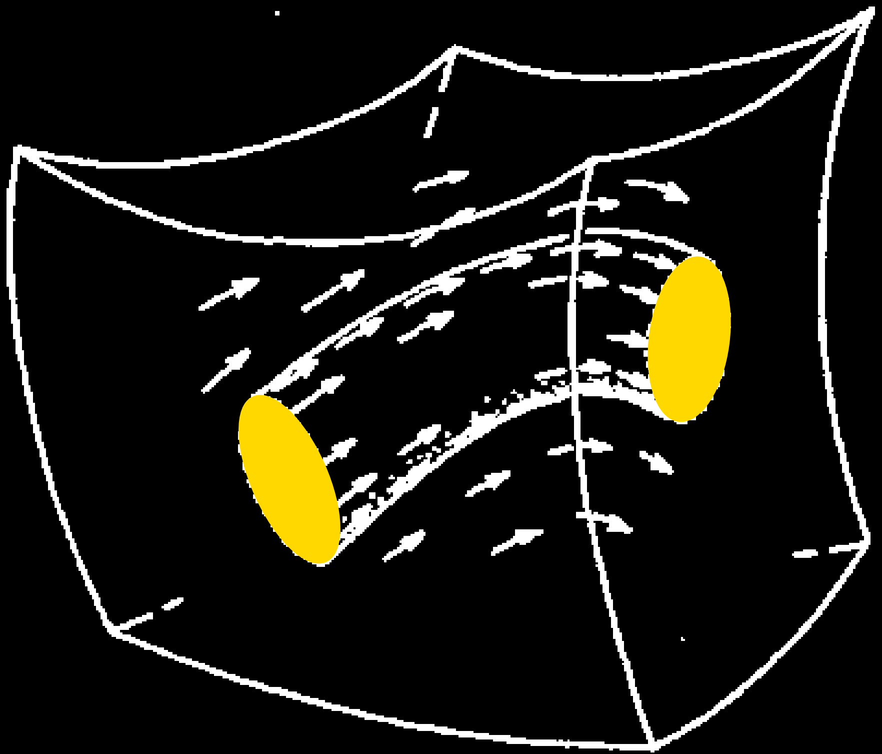
t →

← t

THE ARROW OF TIME APPEARS IN UNSTABLE SYSTEMS

Reversible | Irreversible

Phase portrait dynamics

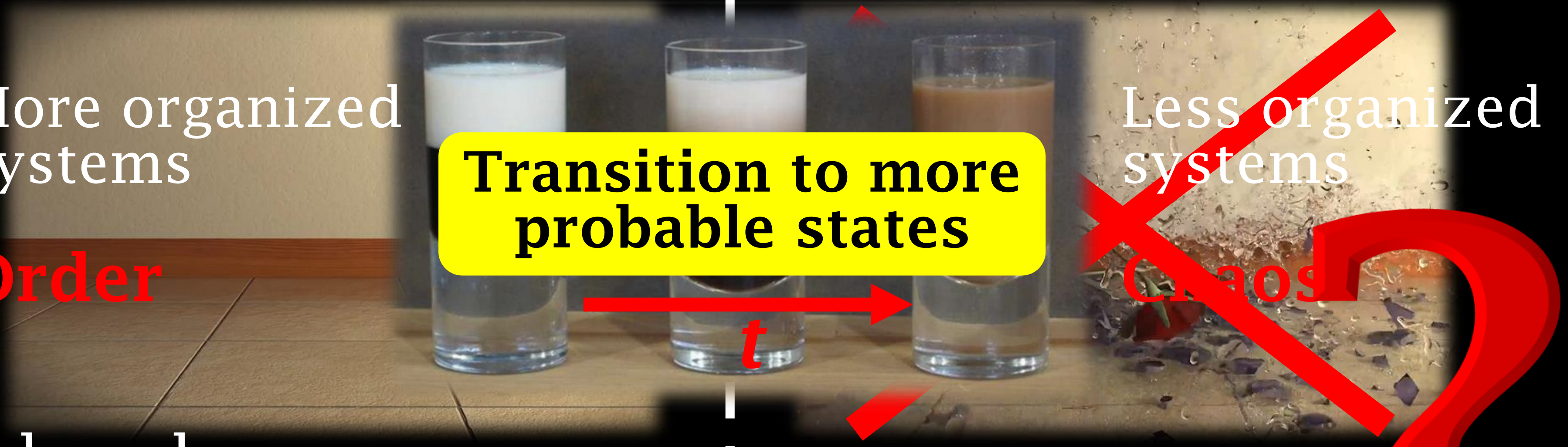


No ways to introduce asymmetric time

Universal measure of time: Fractal dimension of phase portrait

THE ARROW OF TIME IN IRREVERSIBLE SYSTEMS

Thermodynamics **t →** | **← t**



Embryology

Oppositely directed??! **Why???**

EMBRYOLOGICAL ARROW OF TIME
INCREASING COMPLEXITY

What is complexity?

What is more complex:
a drop of water or a snowflake?

EMBRYOLOGICAL ARROW OF TIME

INCREASING COMPLEXITY

Definitions

- **Neg-entropy**

  Increasing co

  Too high estimates for crystal-like systems
  Impossible to estimate for an embryo

- **Kolmogorov-Haitin complexity** – minimal length of an algorithm sufficient for the syst

  Increasing complexity = ↓ algorit

  Too high estimates for chaotic systems

Letter chains of 64 letters	Compacted description	Minimal length
4c1j5b2p0cv4w1x8rx2y39umgw5q85s7uraq bjfdppa0q7nieieqe9noc4cvafzf	—	64
George Gordon Byron, 6th Baron Byron FRS (22 January 1788 – 19 April 1824)	G.G.Byron's full name (dates of life)	37
Abababababababababababababababababa bababababababababababababab	ab 32 times	11

EMBRYOLOGICAL ARROW OF TIME
INCREASING COMPLEXITY
Definitions

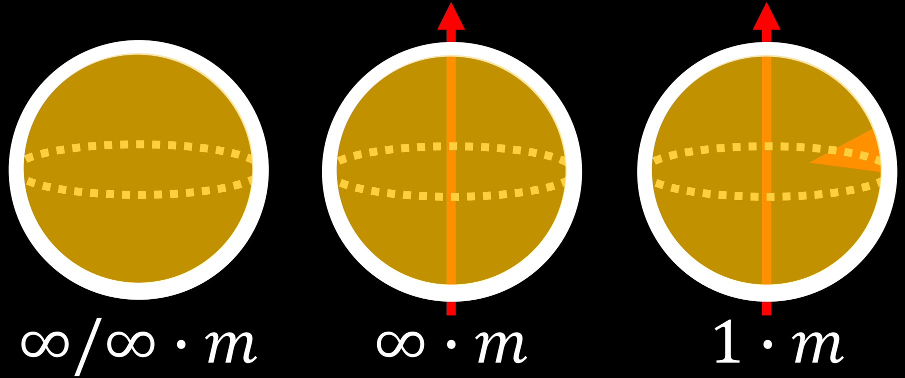
- **Asymmetry**
  Symmetric transformation $\hat{M}$ of an object O is any transformation leaving its properties P invariant:
  $$\forall \hat{M},\ P(\hat{M}O) = P(O)$$

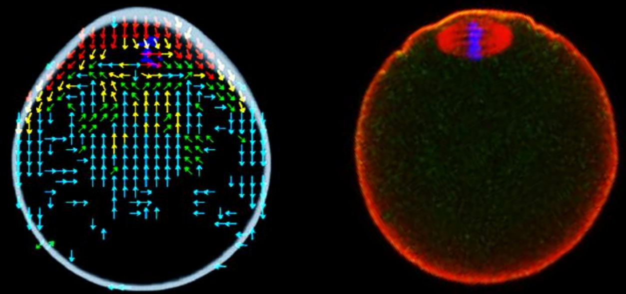
  Symmetry group of O is the set of all symmetric transformations $\hat{M}$: $M = \{\forall \hat{M}\},\ P(\hat{M}O) = P(O)$

  - ✓ Geometrical
  - ✓ Translational
  - ✓ Permutation
  - ✓ Functional (dynamic)

DEFINITIONS OF COMPLEXITY
SYMMETRY & SYMMETRY BREAKING

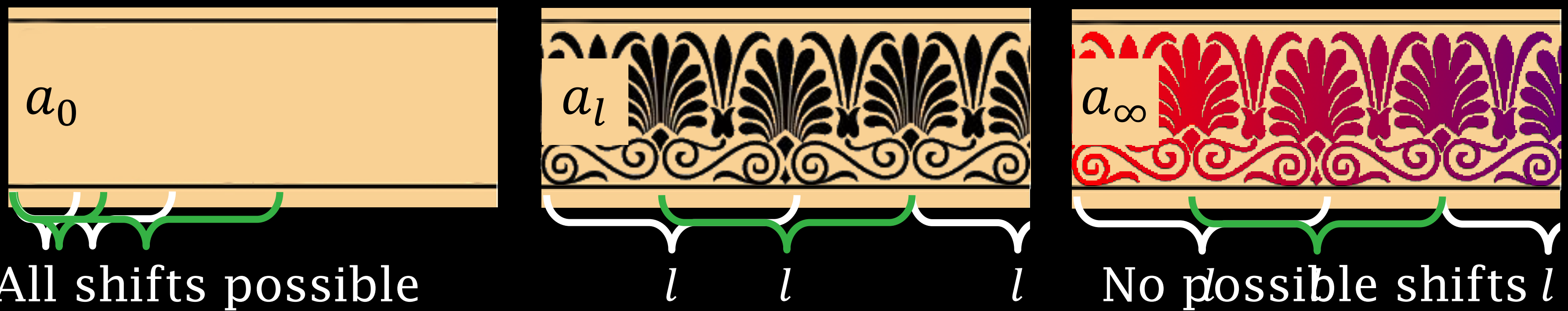
✓ Geometrical – meiosis, axes specification

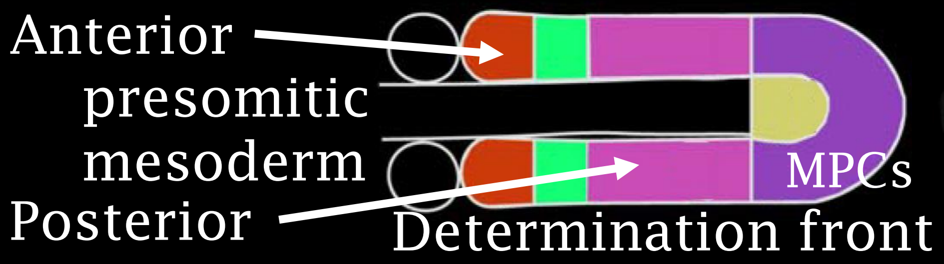


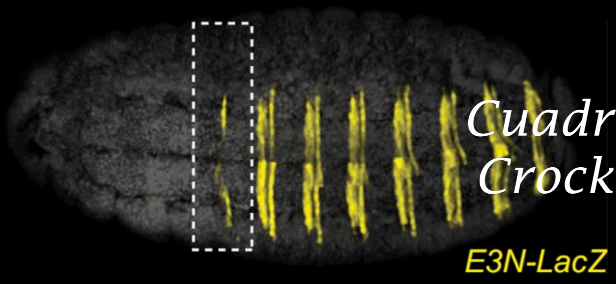


Yi et al., 2011

✓ Translational – segmentation





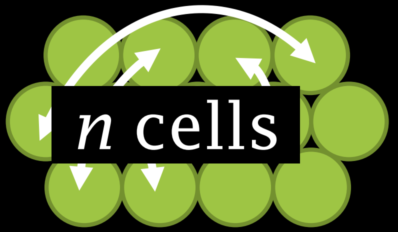


Cuadros et al., 2018
Crocker et al., 2014

DEFINITIONS OF COMPLEXITY

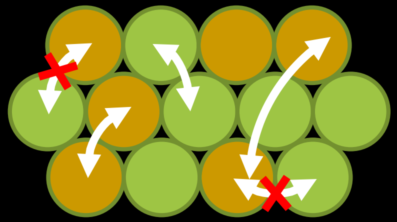
SYMMETRY & SYMMETRY BREAKING

✓ Permutational – cell fate decision



$n!$ variants

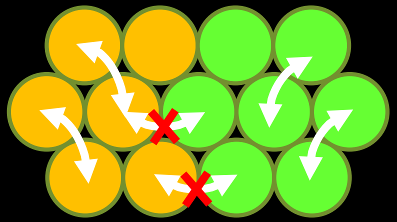
All interchanges possible



$m!\,(n-m)!$

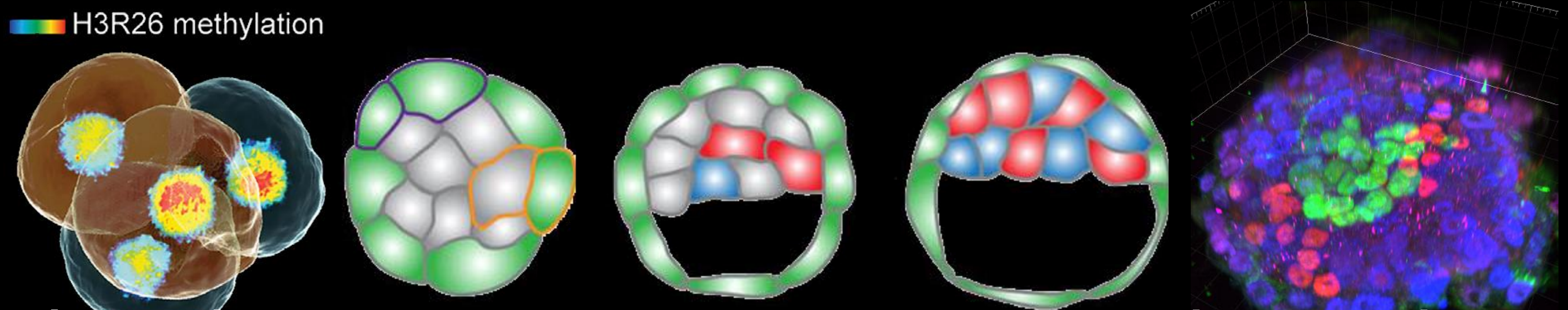
$m!\,(n-m)! < \mathbf{x} < n!$

Cell fate plasticity



$m!\,(n-m)!$

Only interchanges within one cell line



White et al., 2018 *Artus et al., 2014* *Deglincerti et al., 2016*

✓ Functional (dynamic) – symmetry of equations (in physics); cell fate potency and plasticity

SYMMETRY IN NATURE
CURIE'S PRINCIPLE

Pierre Curie
(1859—1906)

Dissymmetry is what creates the phenomenon.

When certain causes produce certain effects, the symmetry elements of the causes must be found in their effects.

When certain effects show a certain dissymmetry, this dissymmetry must be found in the causes which gave rise to them.

In practice... some causes can have so weak effects, that they are impossible to detect... <Thus>, the effects can be more symmetric than their causes...

Curie, 1894

SYMMETRY IN NATURE
CURIE'S PRINCIPLE

Pierre Curie
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Dissymmetry is what creates the phenomenon.

When certain causes produce certain effects, the symmetry elements of the causes must be found in their effects.

When certain effects show a certain dissymmetry, this dissymmetry must be found in the causes which gave rise to them.

Is actually a reframing of the principle of causality, but…

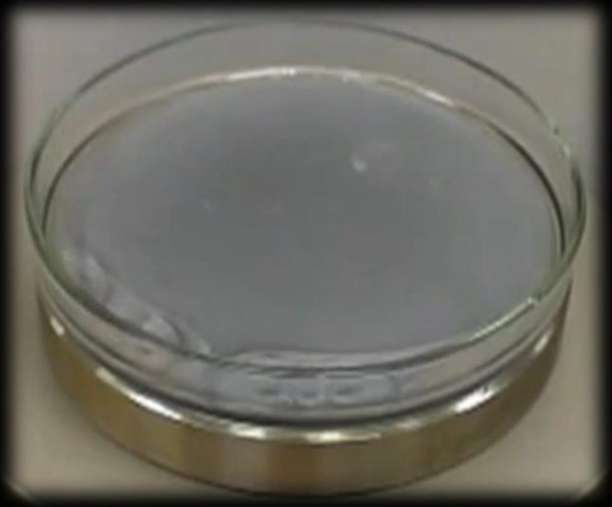
…applicable only to stable systems.

Curie, 1894

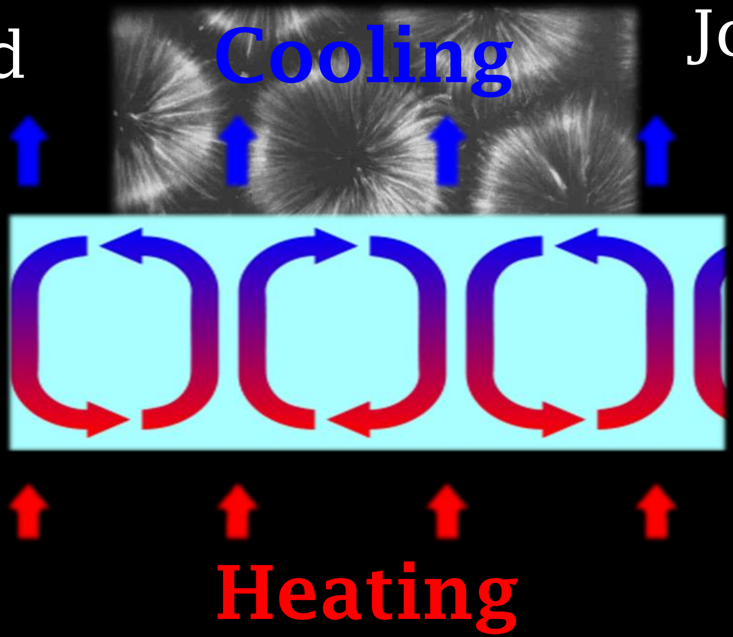
SELF-ORGANIZATION

Rayleigh-Bénard convection

Henri Claude Bénard
(1874—1939)



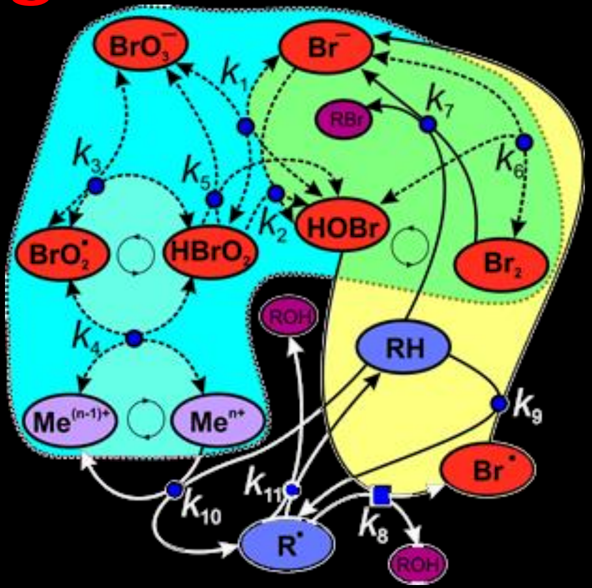
John William Strutt
Rayleigh
(1842—1919)



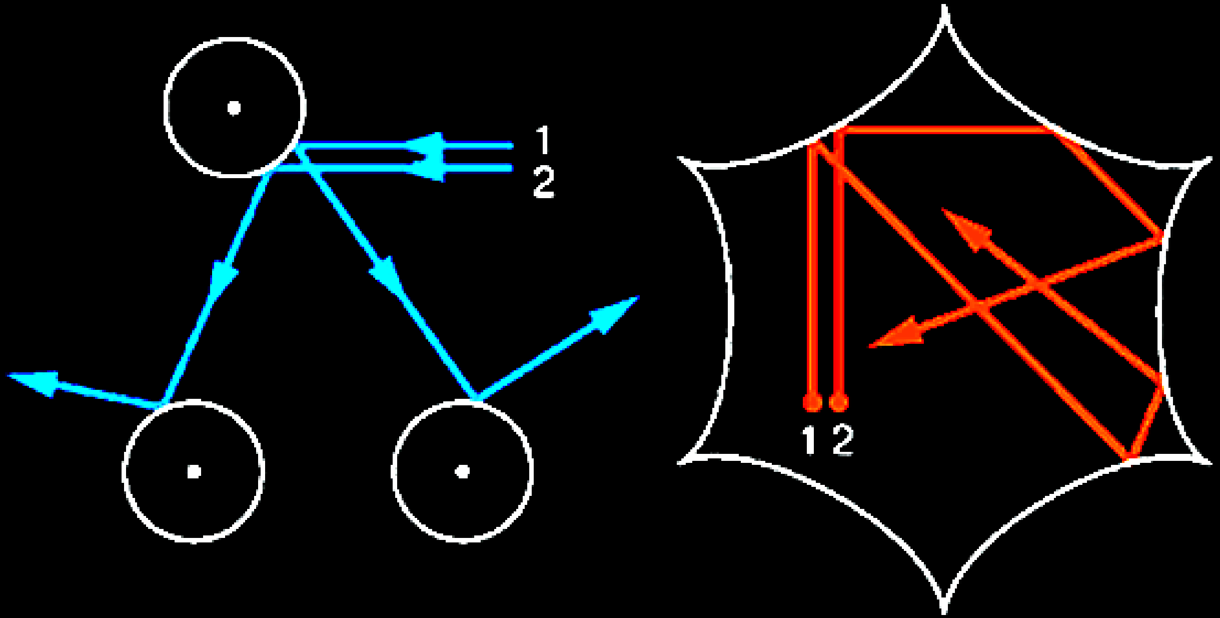
SELF-ORGANIZATION

Beloussov-Zhabotinsky (BZ) reaction

Appearance of new structures
Symmetry breaking



UNPREDICTABILITY IN UNSTABLE SYSTEMS



Scattering billiards (Sinai Billiards)

A negligible deviation from the initial conditions leads to measurable change in the consequence.

Trajectory becomes unpredictable after a number of collisions at any given (finite) precision of the initial conditions.

SELF-ORGANIZATION CONDITIONS:
ENERGY FLOW (SUPPLY)

Isolated systems

$$\frac{dS}{dt} \geq 0$$

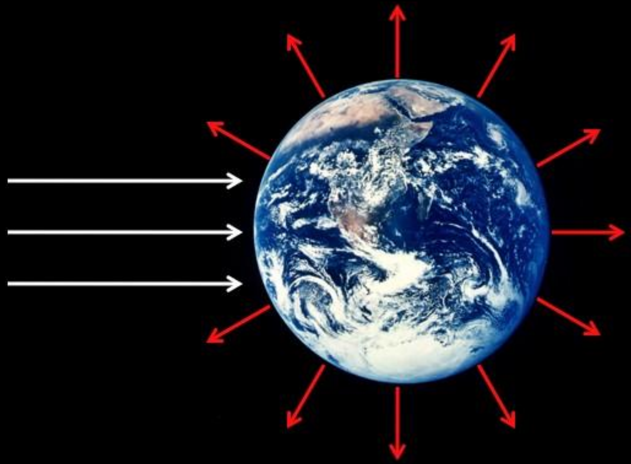
Processes
$S\uparrow$

Open systems

$$dS = d_iS + d_oS$$

Processes
$S_i\uparrow$

high S



high S

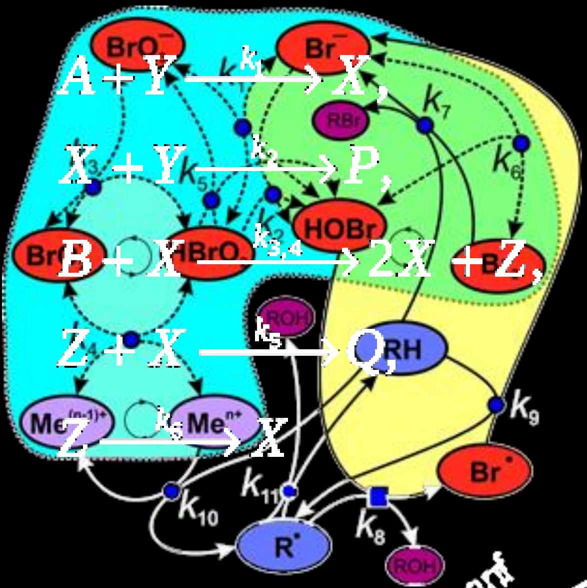
Organized
low S

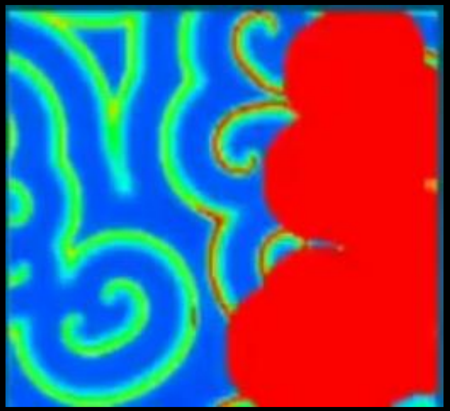
Energy and
material flow
$S_o\downarrow$

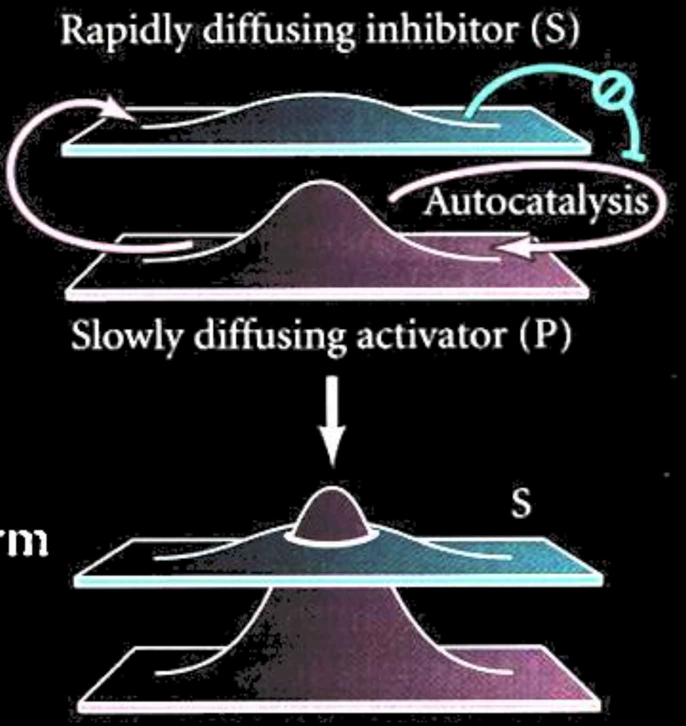
Disorganized
high S

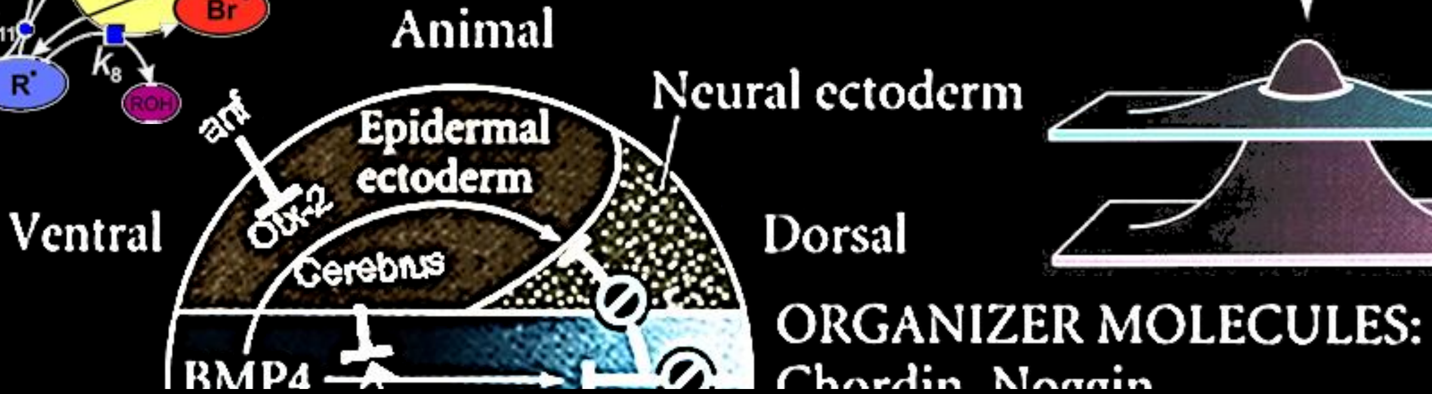
$$\frac{d_iS}{dt} \geq 0, \qquad \frac{dS}{dt} = \frac{d_iS}{dt} + \frac{d_oS}{dt} \leq 0$$

SELF-ORGANIZATION CONDITIONS:
FEEDBACK LOOPS









Self-organization – spontaneous increase of the system's complexity.
Characteristic for open non-equilibrium self-consistent systems.

SYMMETRY IN NATURE

ADDITION TO CURIE'S PRINCIPLE

Pierre Curie
(1859—1906)

In systems with unstable dynamics macroscopic effects can have so weak causes, that they are impossible to detect.

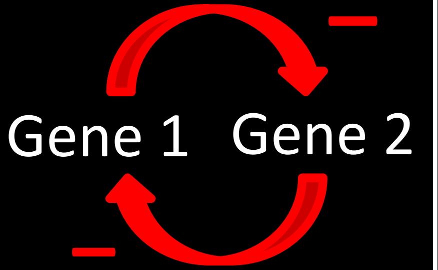
The system can go into *de facto* spontaneous symmetry breaking, without violating any physical laws.

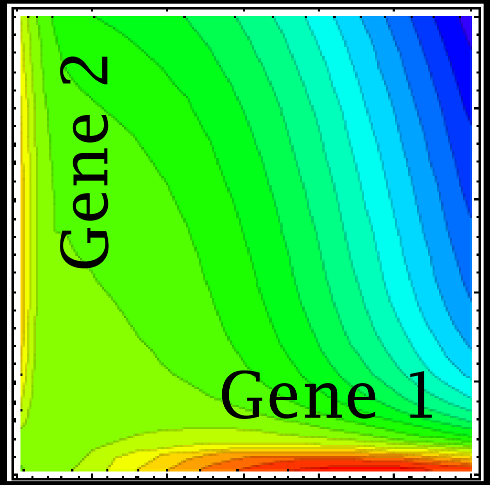
The "degree of applicability" of Curie's principle can be used as a measure of the system's stability.

GENETIC TRIGGER: CELL FATE

SYMMETRY BREAKING

Max Delbrück
(1906—1981)





Two **stable** states:

Gene 1 active

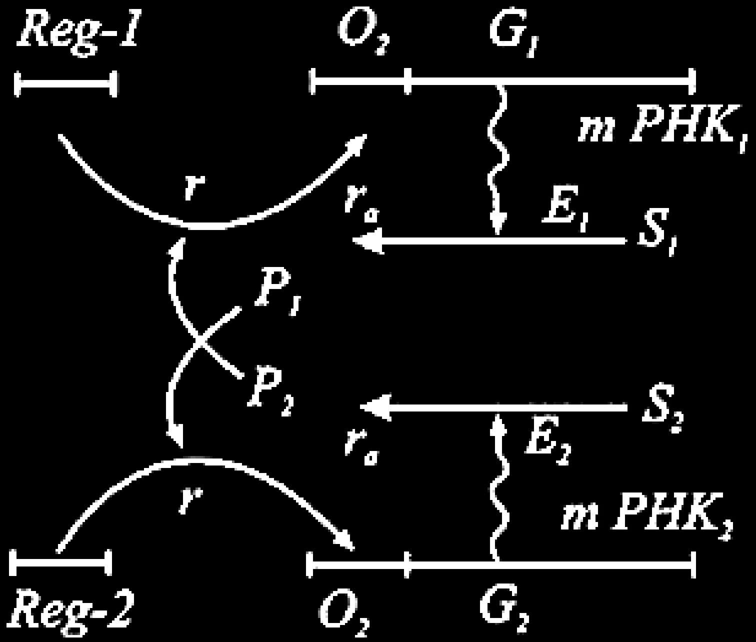
or

Gene 2 active

Intermediate states **unstable**

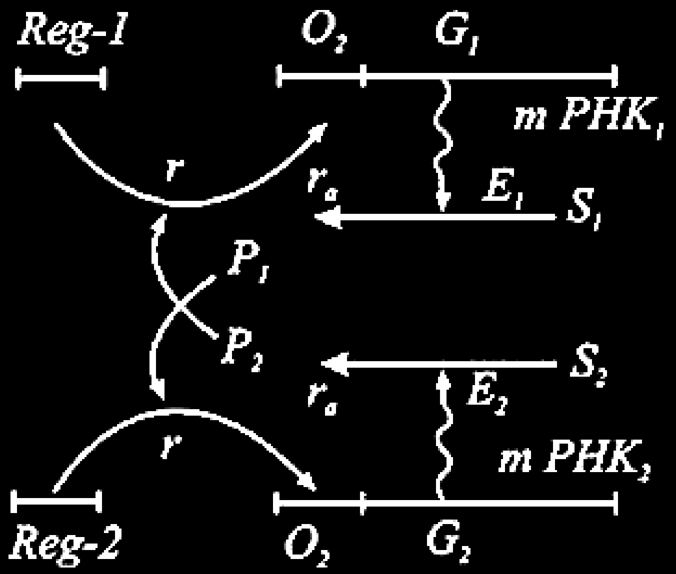
François Jacob
(1920—2013)

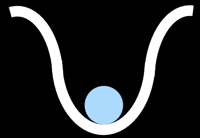
Jacques Monod
(1910—1976)



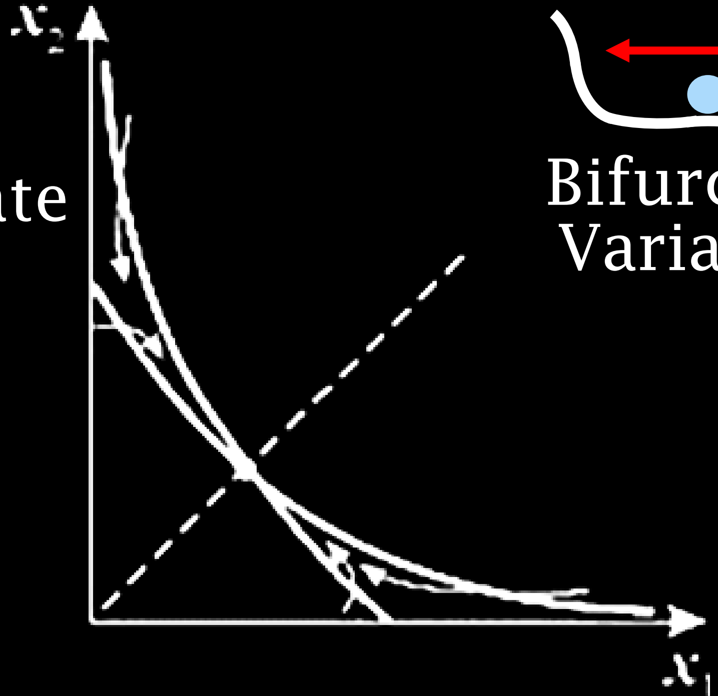
GENETIC TRIGGER: VARIABILITY

Dmitry S. Chernavsky
(1926—2016)

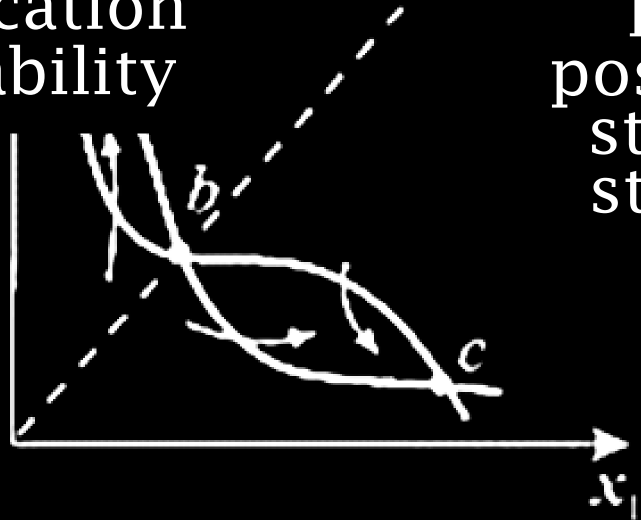


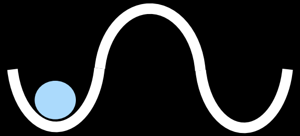


Stable state



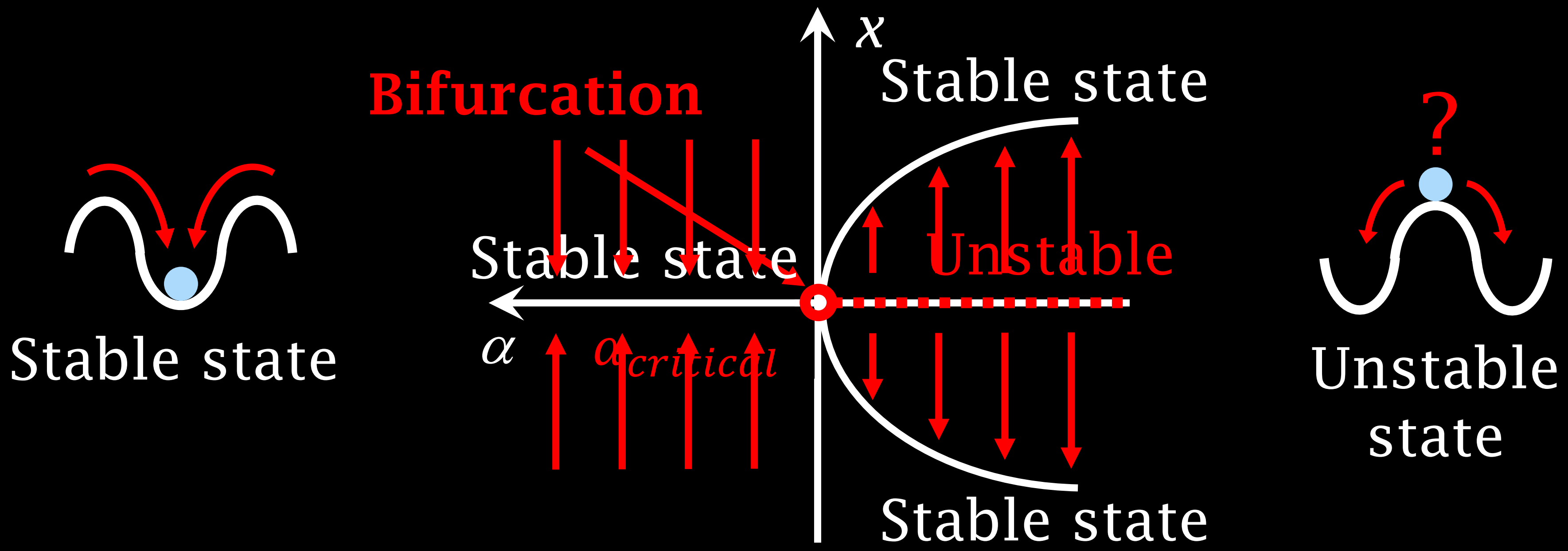
Bifurcation
Variability





Two
possible
stable
states

SYMMETRY BREAKING IN UNSTABLE SYSTEMS

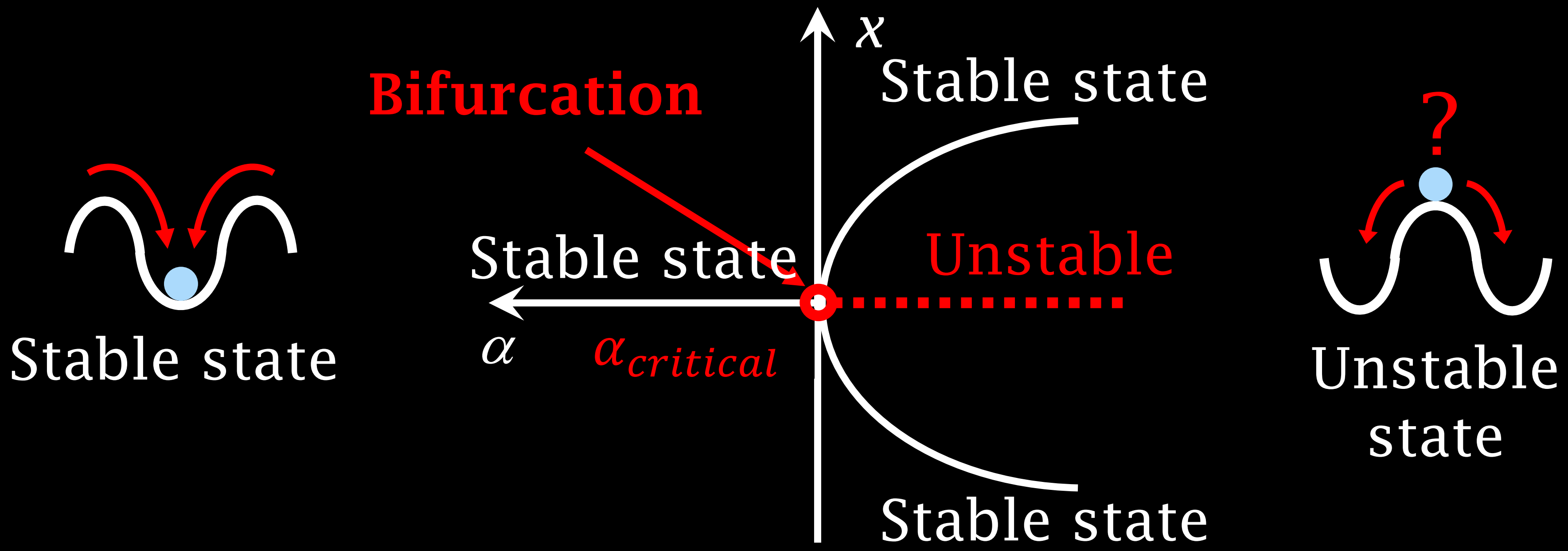


Bifurcation – birth of new possible states and transitions (change of phase portrait topology).

Selection of one state – symmetry breaking.

Structural instability – conditions for bifurcation.

SYMMETRY BREAKING IN UNSTABLE SYSTEMS



Competence – several attractors (stable states) in the system phase portrait.

Determination – selection of one attractor.

Differentiation – reaching the (pre-selected) attractor.

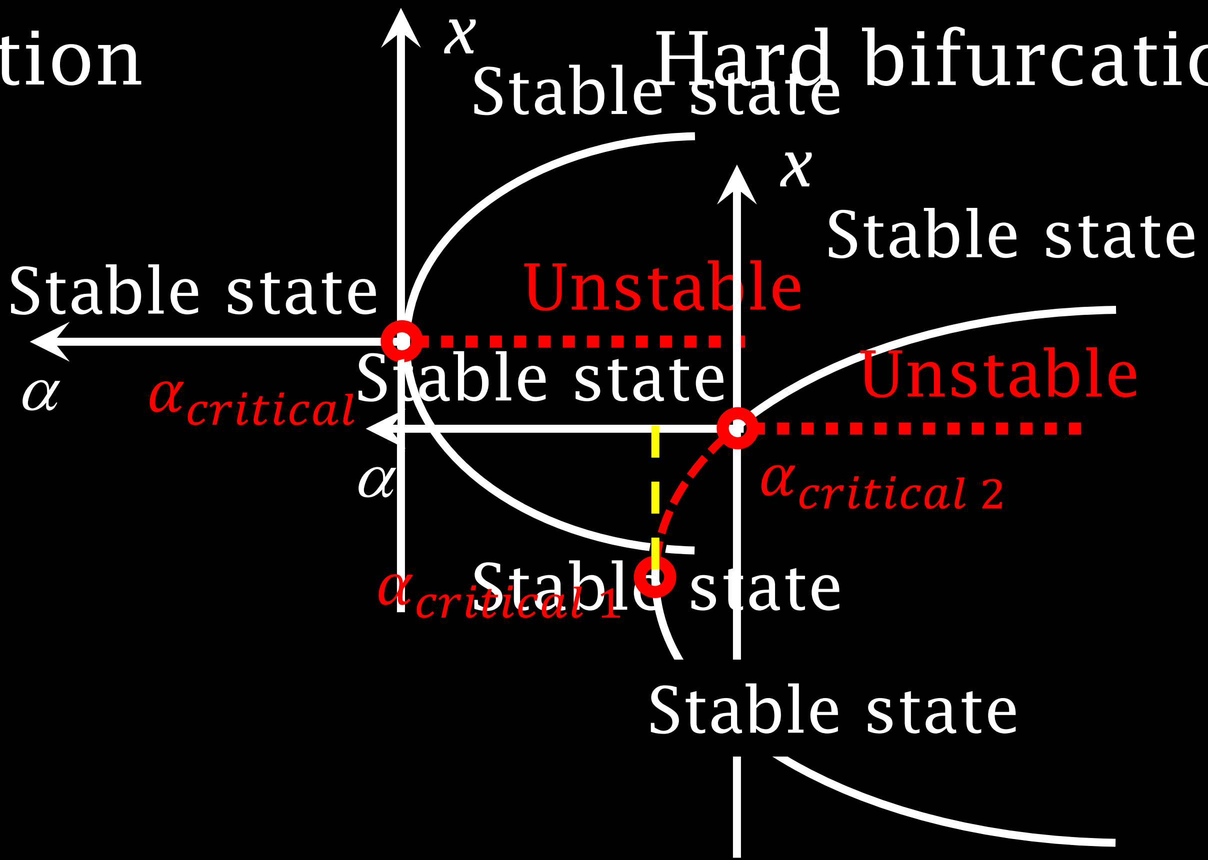
Induction – any influence, changing phase point position

Beloussov, lectures; 1975; 2015

SYMMETRY BREAKING IN UNSTABLE SYSTEMS

Soft bifurcation

Hard bifurcation

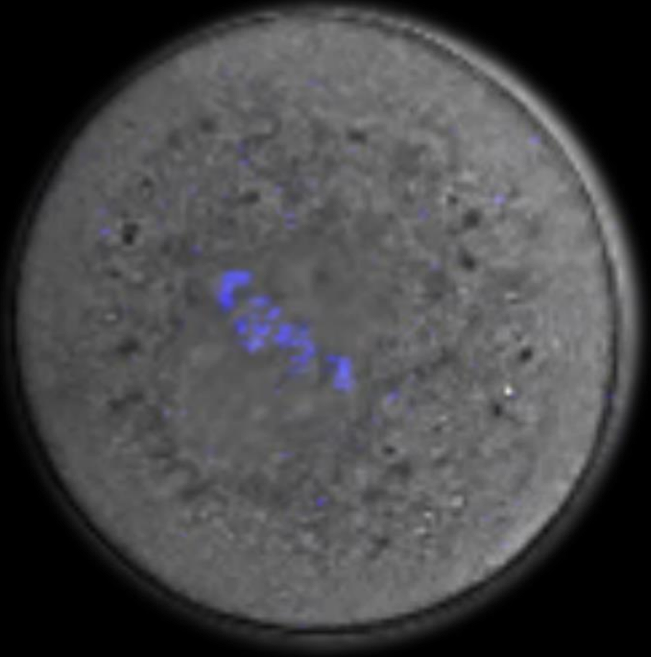


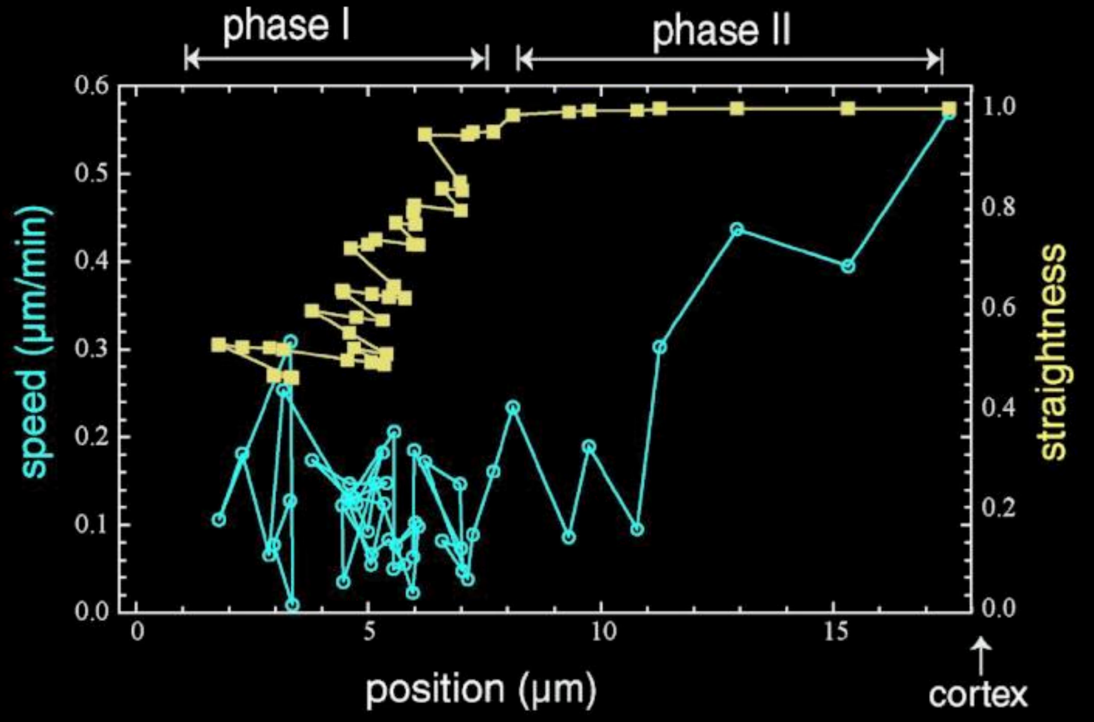
Final states equally achievable.
No predetermination.
Not biologically valuable.

Final states unequal.
“Upper” – main, “lower” – “minor”.
Frequently met in biological systems.

OOCYTE MATURATION (MEIOSIS)
GEOMETRICAL SYMMETRY

GV —NEBD→ **MI** —Spindle off-centering→ **MII**





- Phase 1: stochastic dynamics
- Phase 2: symmetry breaking

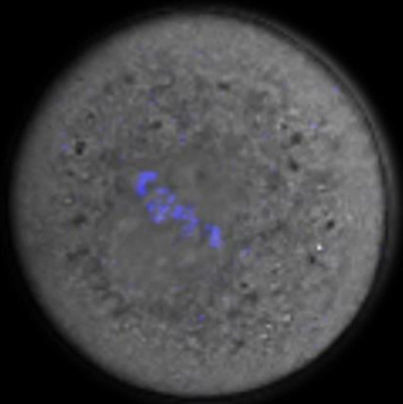
Yi et al., 2013

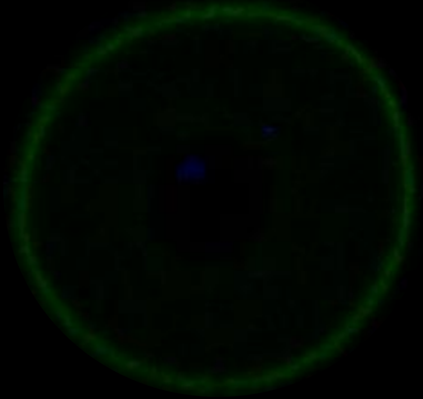
Yi et al., 2013

Blanchoin et al., 2014

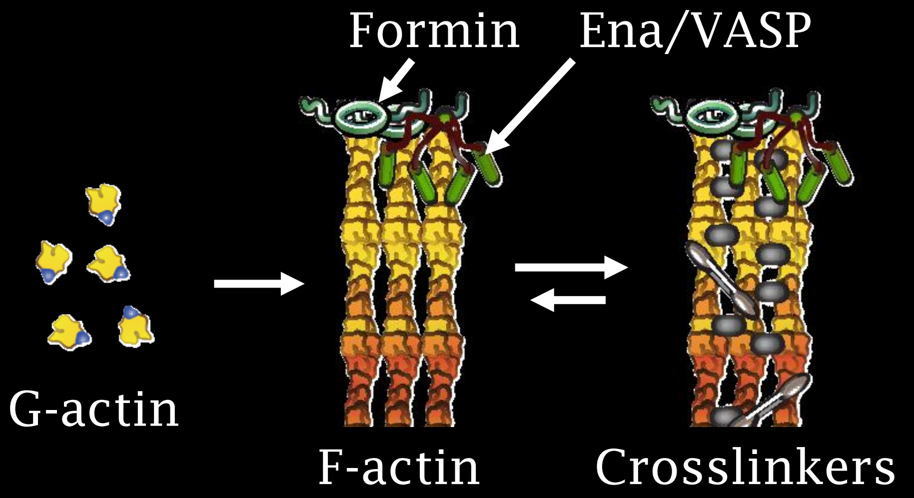
MEIOSIS

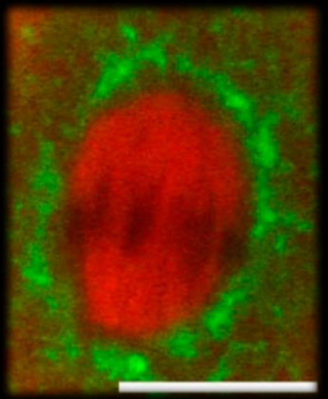
1: STOCHASTIC DYNAMICS



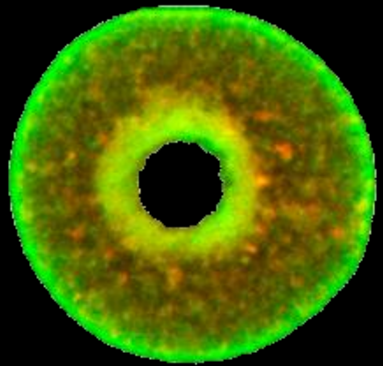


Chromosomes / Formin2

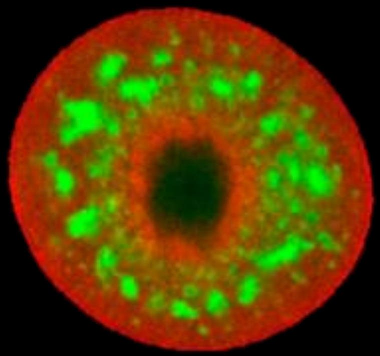




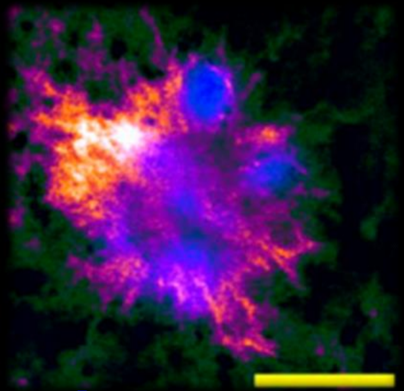
Spindle
Formin2



Formin2
Endoplasm.
reticulum



Formin2
Mitochondria



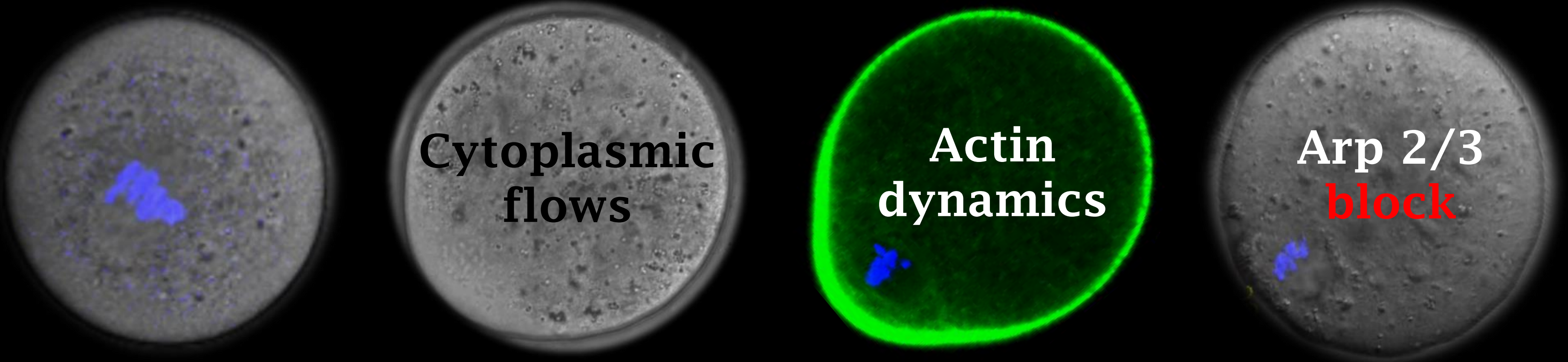
Chromosomes
Actin growth

Formin2: accumulates around chromosomes (connected to ER), nucleates actin growth

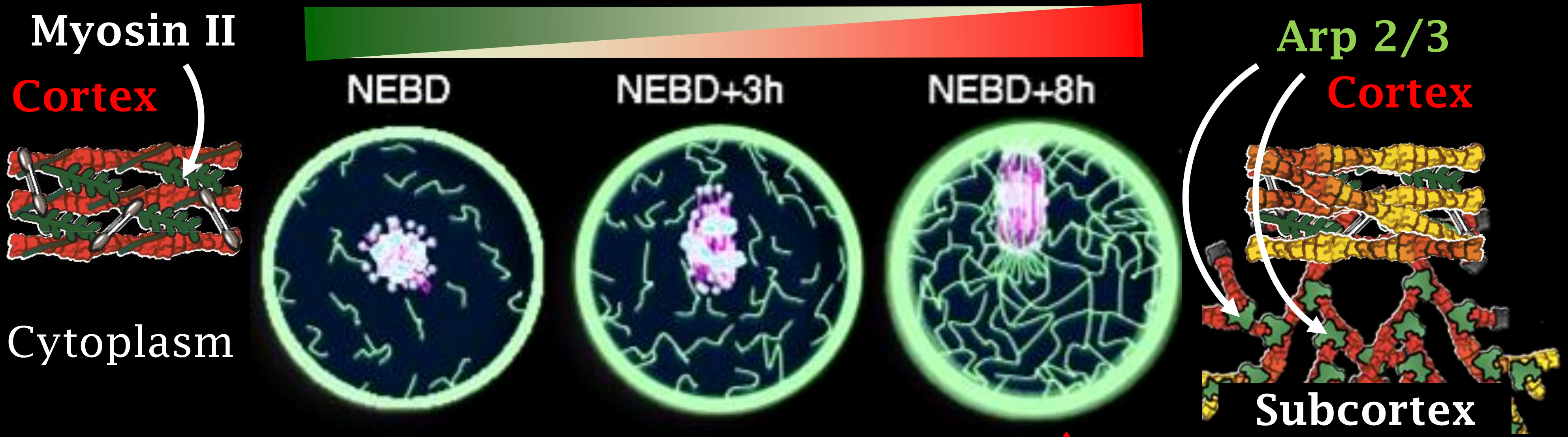
Yi et al., 2013
Chaigne et al., 2013

Blanchoin et al., 2014

MEIOSIS
2: SYMMETRY BREAKING



Cortex changes: ↑ Thickness, ↓ Stiffness, ↑ Plasticity, ↓ Elasticity

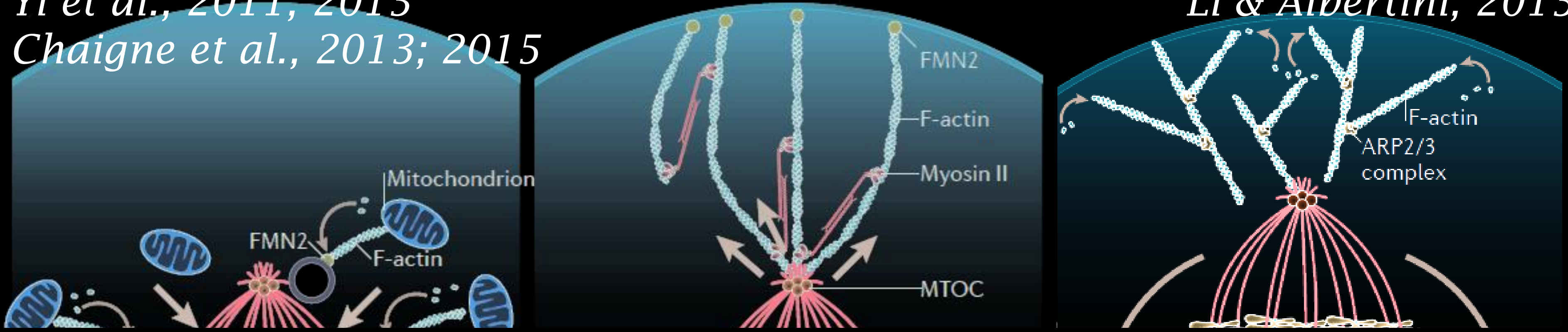


Arp2/3 accumulates in subcortex, ↑ plasticity
Myosin II is excluded from cortex, ↓ stiffness, ↓ elasticity

SYMMETRY BREAKING MECHANISMS

Yi et al., 2011; 2013
Chaigne et al., 2013; 2015

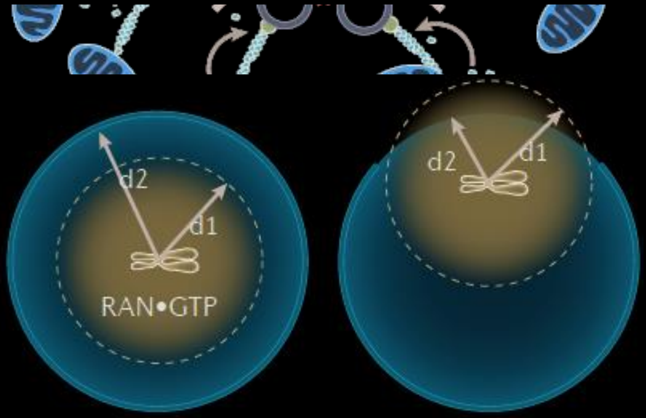
Li & Albertini, 2013

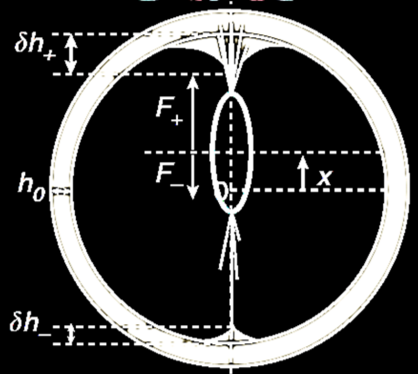


Sensing of the whole: boarders of the cell

Unstable dynamics ⇒

Balance of mechanical forces





Myosin II **Arp 2/3**

MECHANICAL PROPERTIES AND GEOMETRICAL SYMMETRY

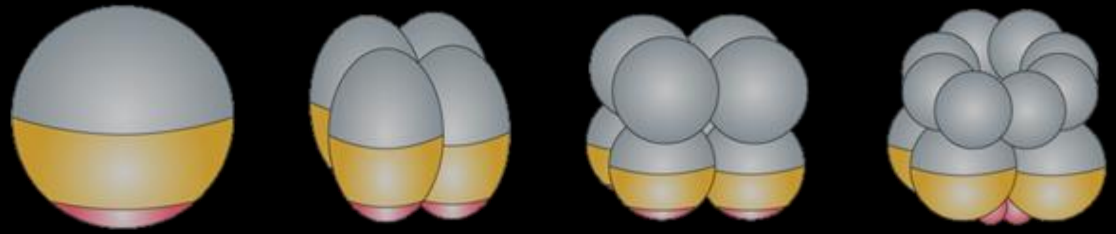
	Early meiosis I	Late meiosis I	Zygote (PN)	Cleavage (mitozis)
Cortical tension $\left(\frac{nN}{\mu m}\right)$	0.9 ± 0.2	**0.03 ± 0.1**	**0.35 ± 0.04**	0.6 ± 0.05
Cytoplasmic viscosity $(Pa{\cdot}s)$			440 ± 40	200 ± 300
Cortex	Myosin II	Arp2/3		Myosin II
Cytoplasm	Formin 2		Myosin Vb	Formin 2

Chaigne et al., 2013; 2015; 2016

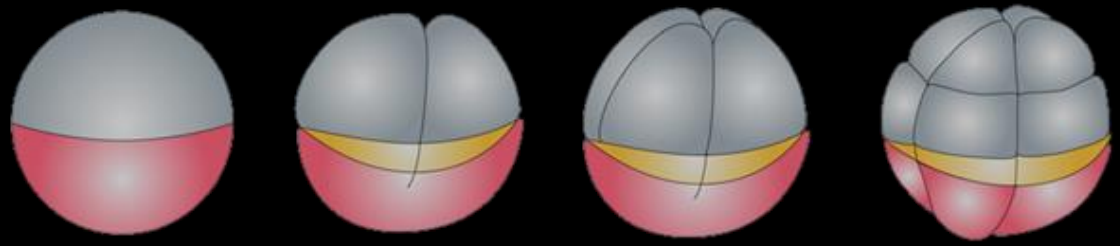
CELL FATE DECISION (CLEAVAGE)
PERMUTATION SYMMETRY

Worm (*C. elegans*)

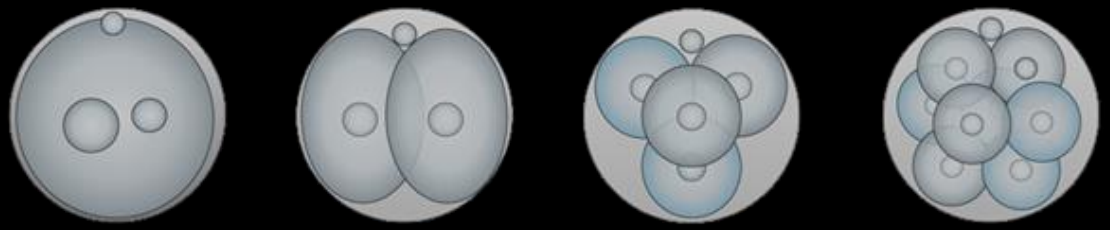
Sea urchin (*Echinoidea*)



Frog (*X. laevis*)



Mammal (*M. musculus*)



Chen et al., 2018; Bredov & Volodyaev, 2018

T_1

Determined cell fates

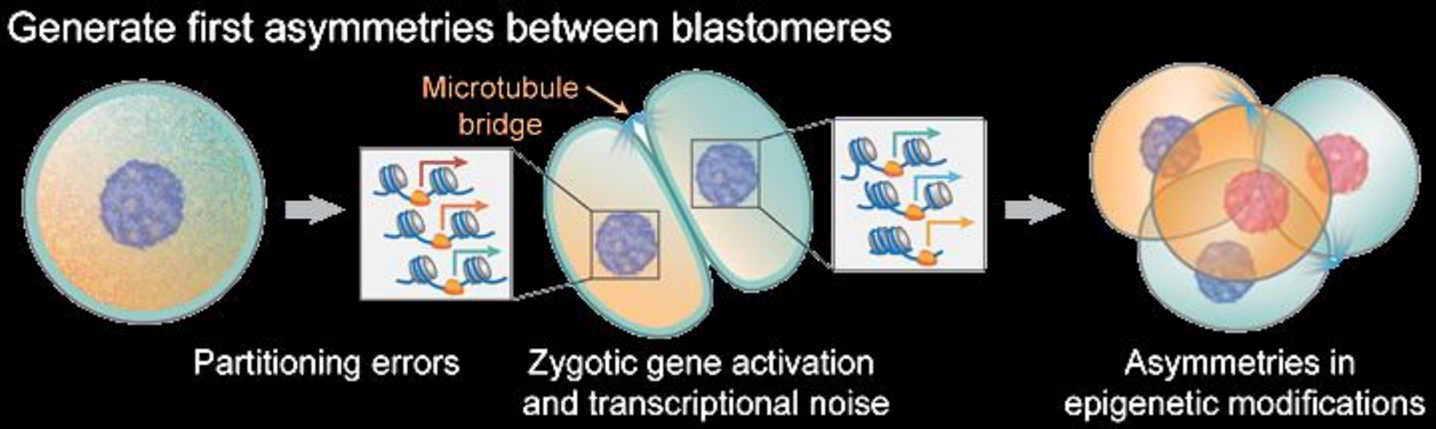
$T_{an} \cdot T_{veg}$

Blastomeres can be roughly divided in two groups: animal (grey) and vegetative (yellow + red)

$T_1 \ (T_n)$

Some differences appear at 2-cell. Yet, further fates are adjustable, and the cells are largely interchangeable till 4—8-cell.

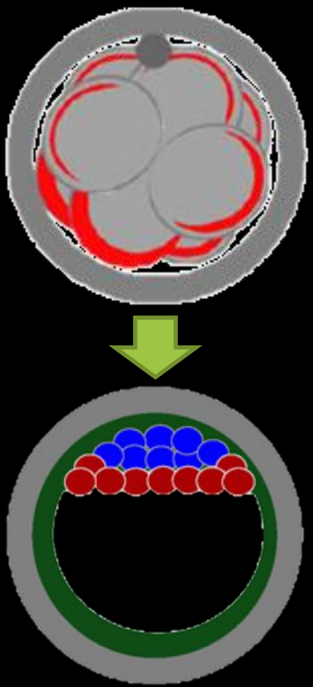
CLEAVAGE



White et al., 2018

4—8 blastomeres

- Cell polarization
  Apical pole – ↑ aPKC, Par3, Jam1
  Basal pole – ↑ Par1
- EGA – genome activation (human)
- “Cell fate decision”
  Epiblast / Hypoblast / Trophoblast

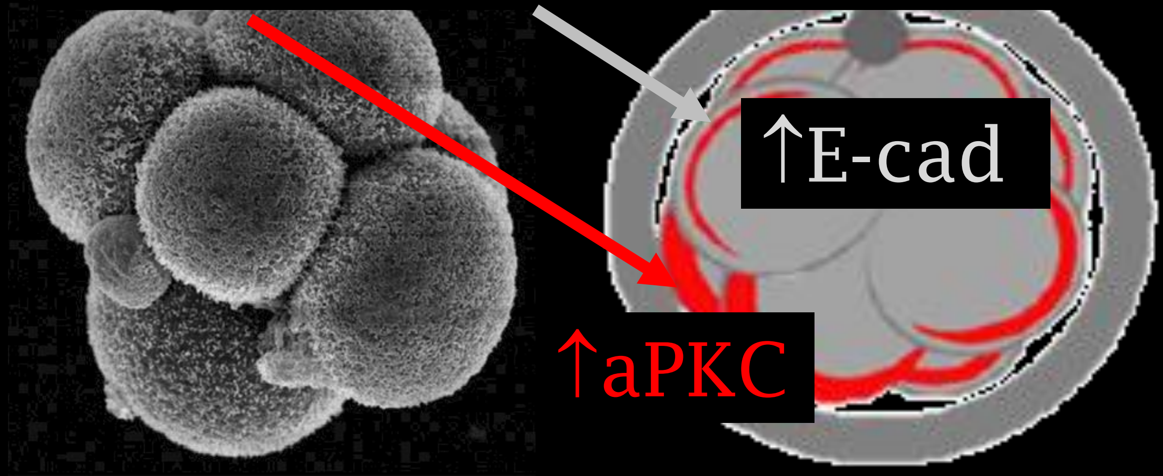


White et al., 2018

CELL POLARIZATION

4—8 blastoimeres

More / less polarized cells



Apical localization:

- Ezrin (actin-binding protein)
- Par3–Par6–aPKC

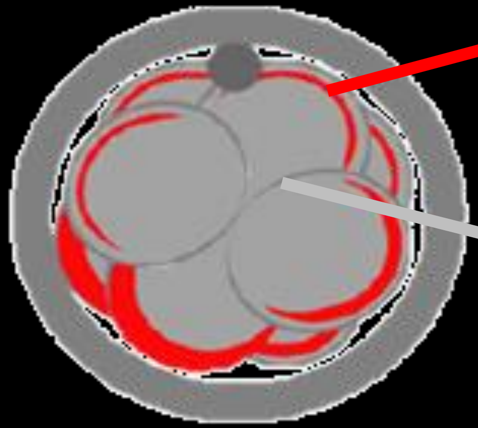
Baso-lateral localization:

- E-cad
- Par1, Jam1, Na^+/K^+ ATP-ase

Apical clustering of microvilli

"CELL-FATE DECISION"

Apolar cell



low aPKC ⇒ ↑ Cortical myosin

⇓

↑ Contractility

⇓

Cell internalization

high E-cad ⇓

Amot binds E-cad

Kinase Lats → Amot P

HIPPO

Yap → YapP

Nucleus active (Yap)

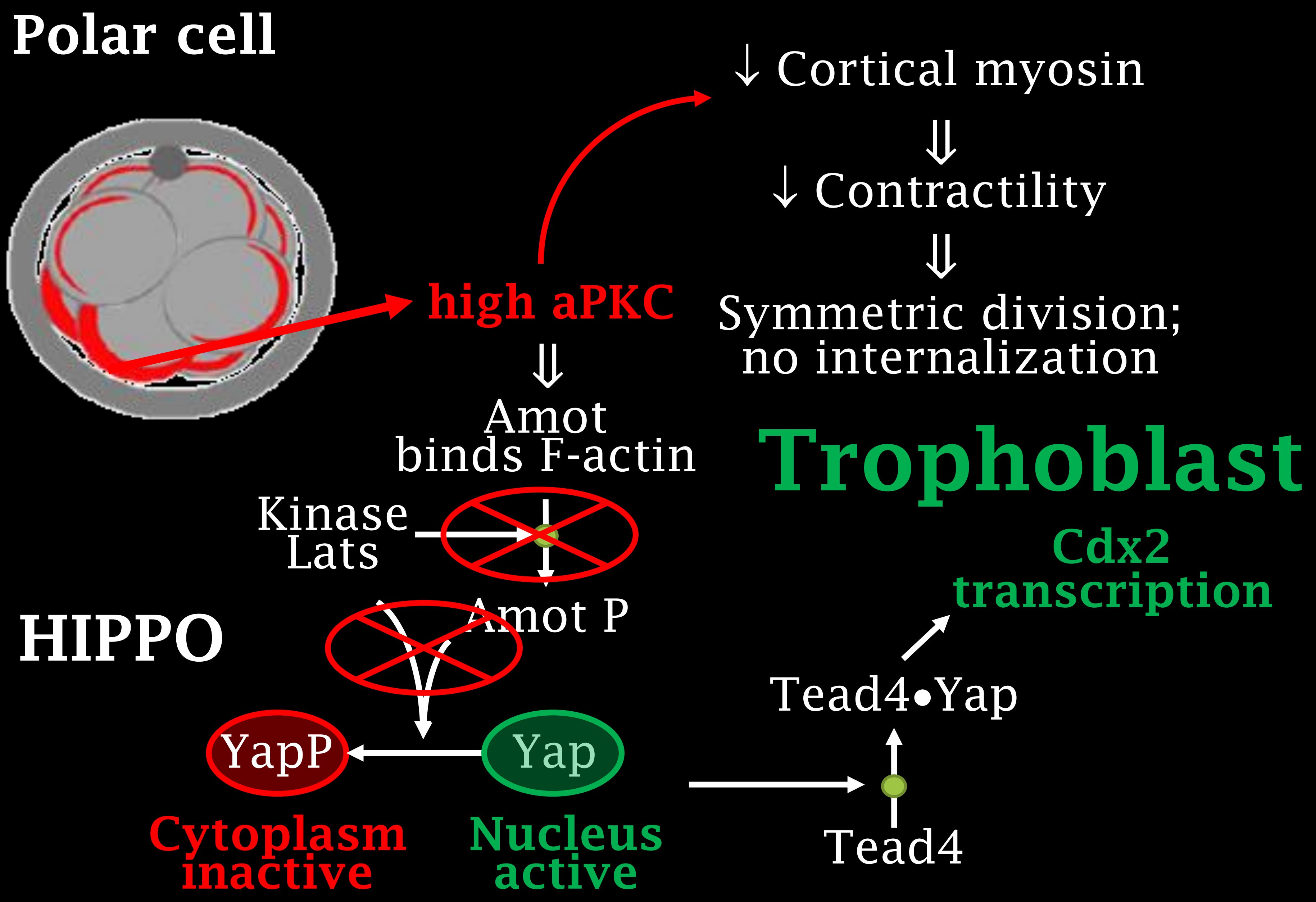
Cytoplasm inactive (YapP)

Epiblast

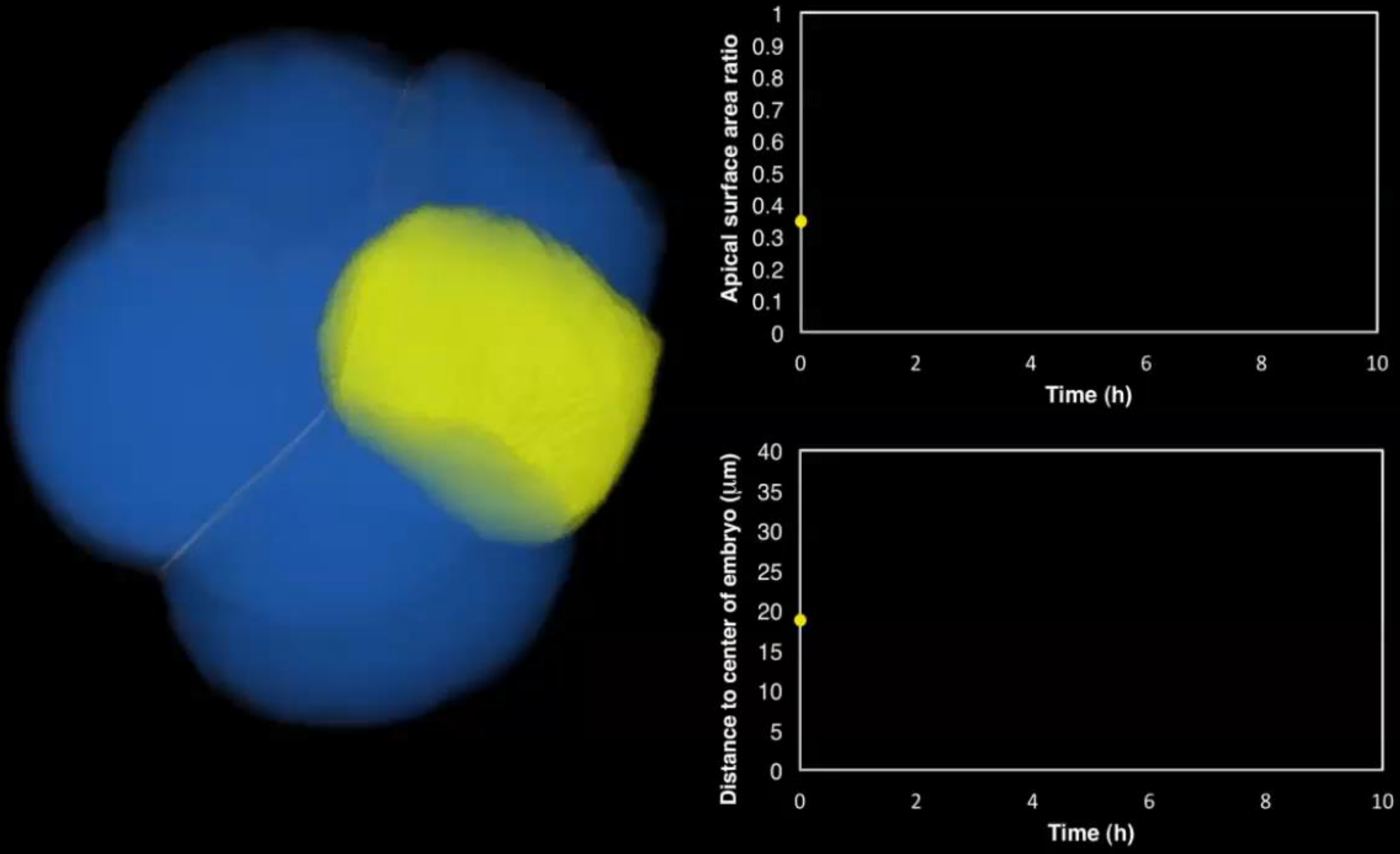
Two-photon imaging

Samarage et al, 2015

"CELL-FATE DECISION"

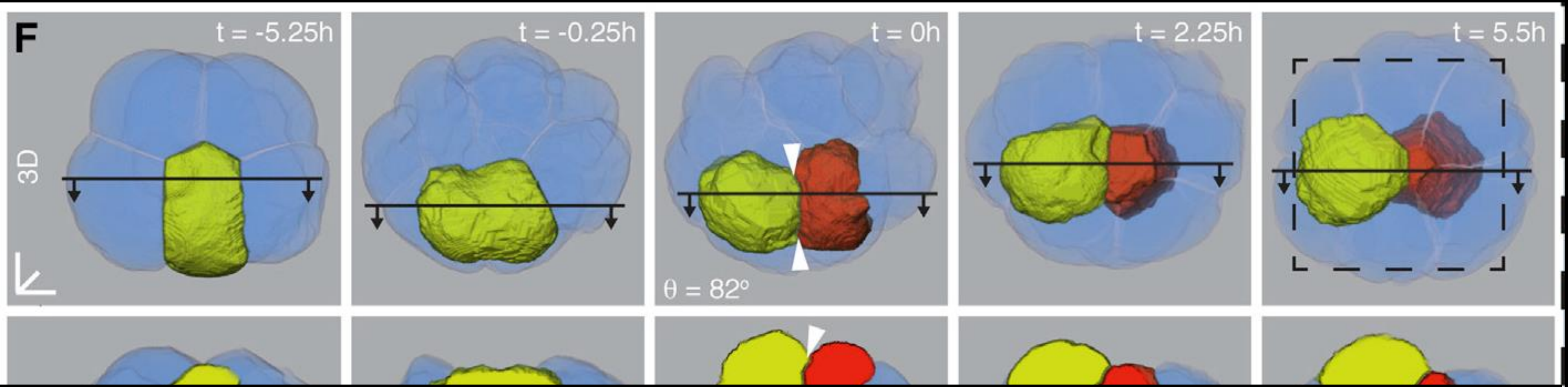


CELL INTERNALIZATION



Samarage et al, 2015

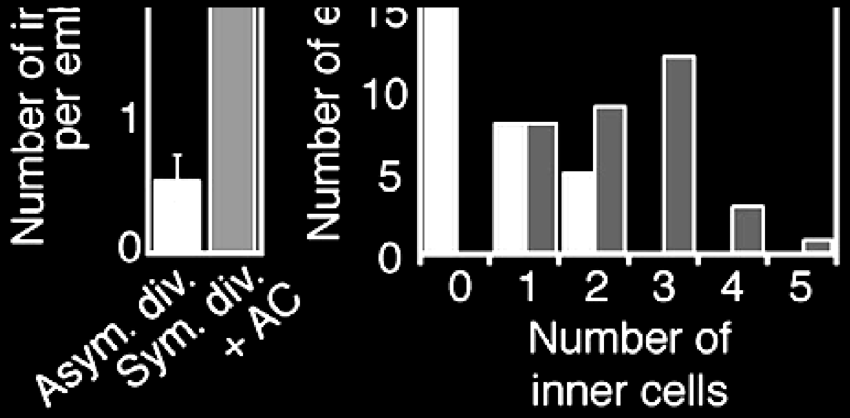
CELL INTERNALIZATION



Sensing of the whole: cell position and fate

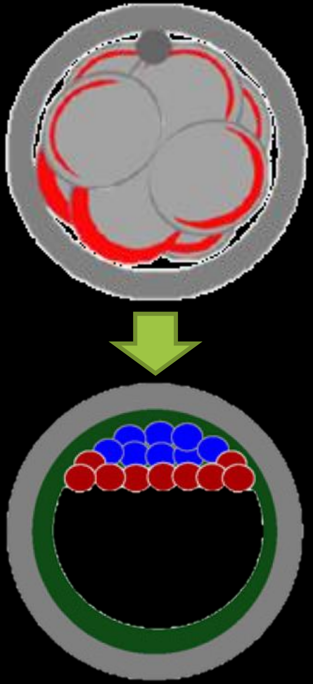
Unstable dynamics ⇒

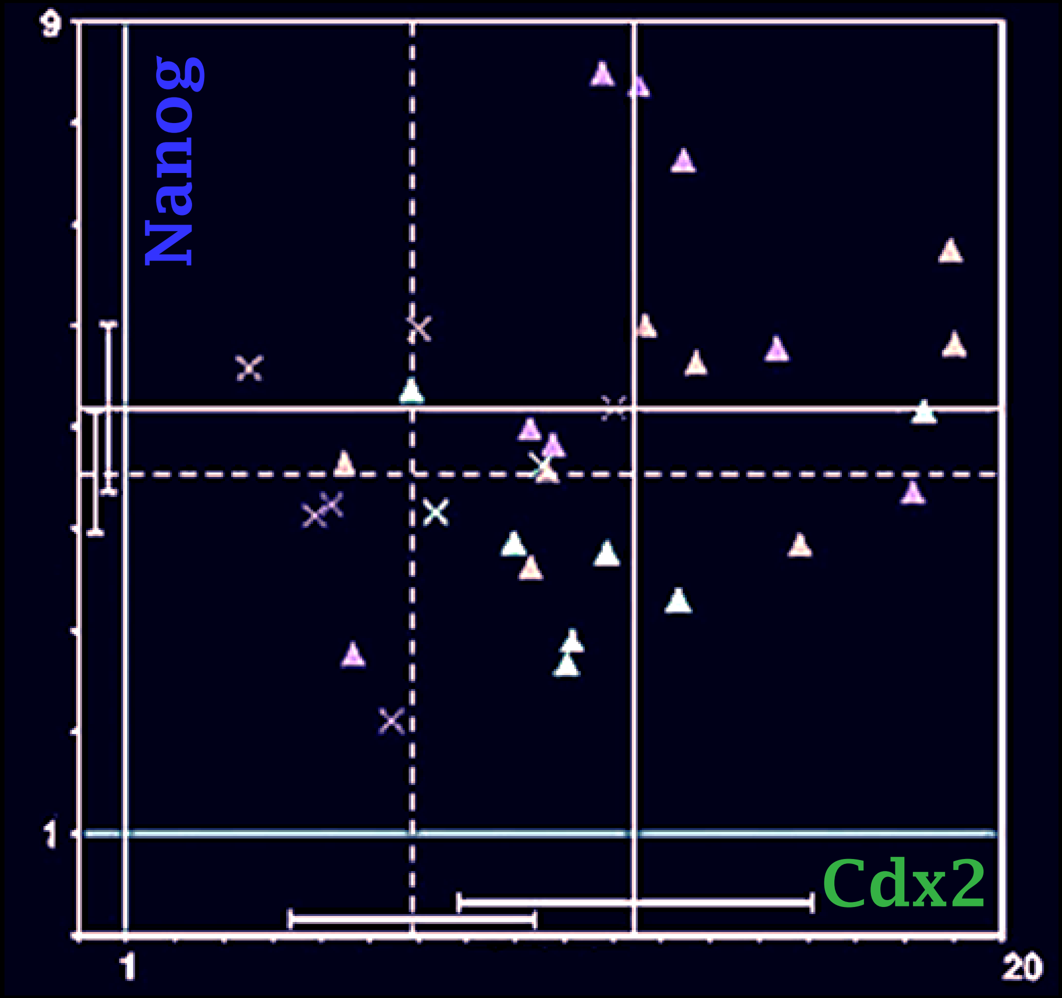
Cross-inhibition + balance of mechanical forces



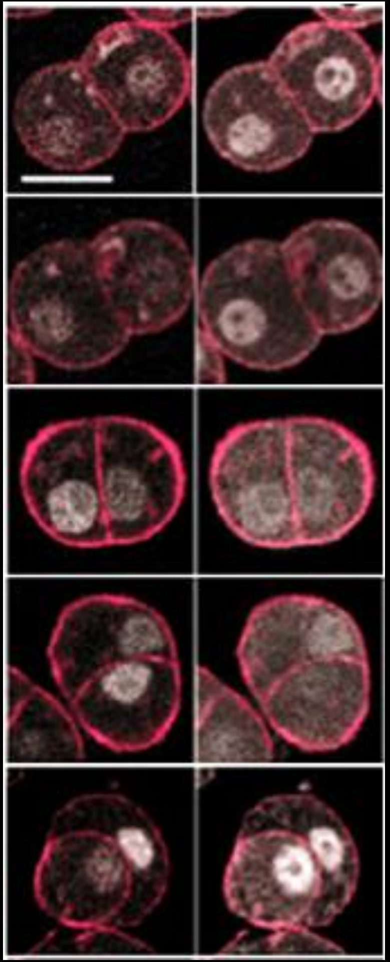
Samarage et al, 2015

CELL FATE DECISION
STOCHASTIC DYNAMICS





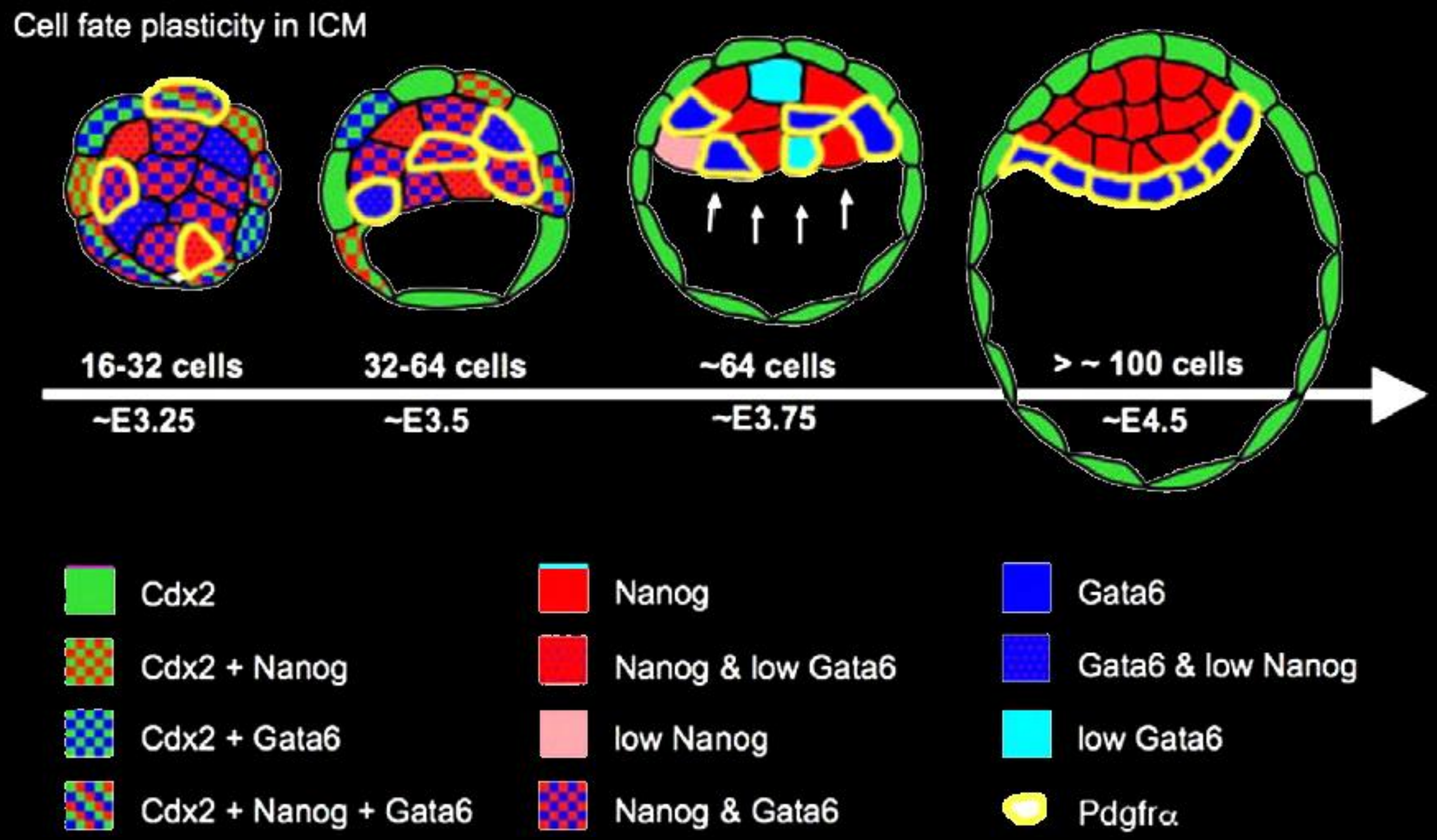
△ Polar blastomeres
× Apolar blastomeres



Cdx2 Nanog

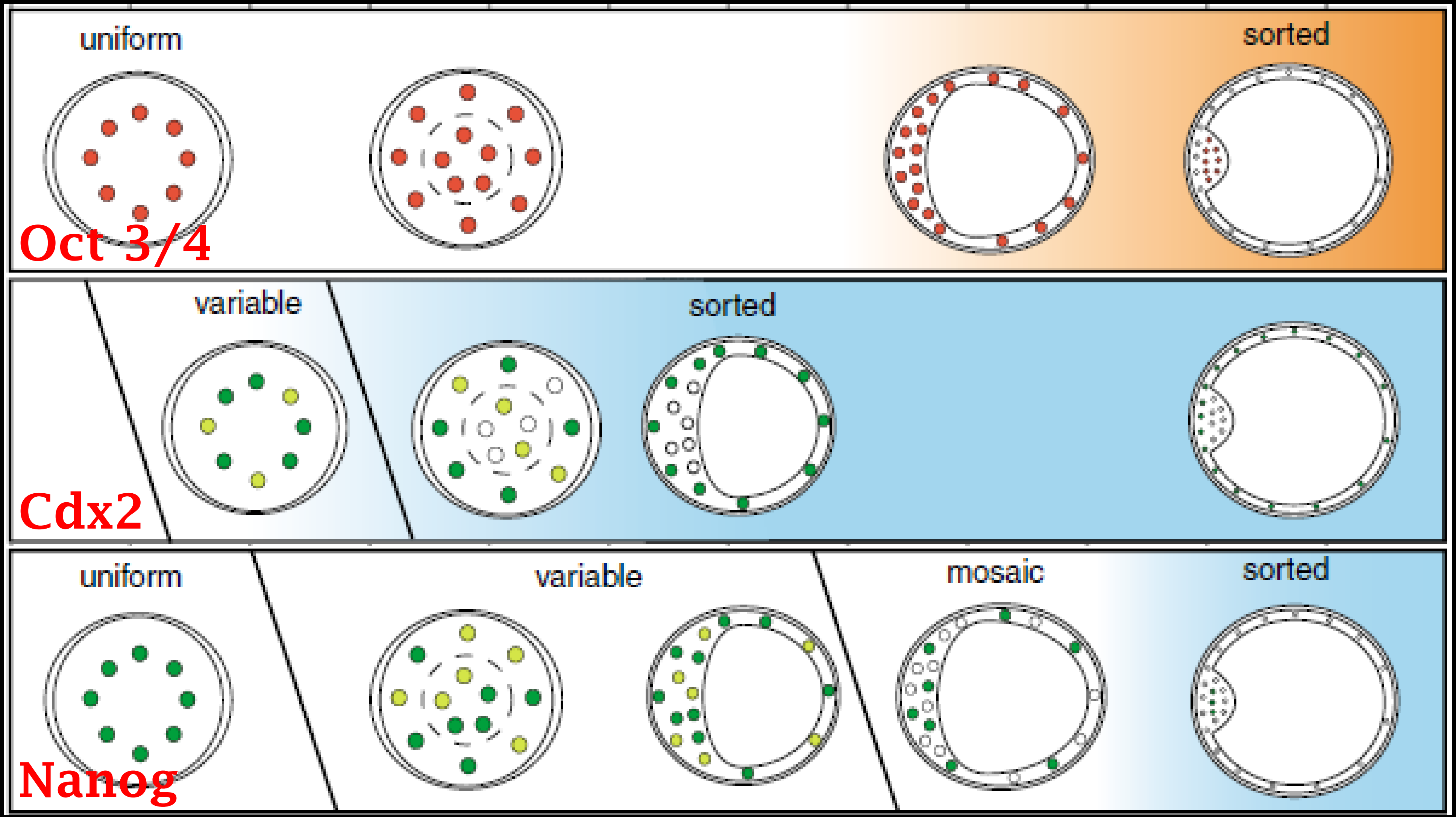
Dietrich et al., 2007

ORDER OUT OF CHAOS



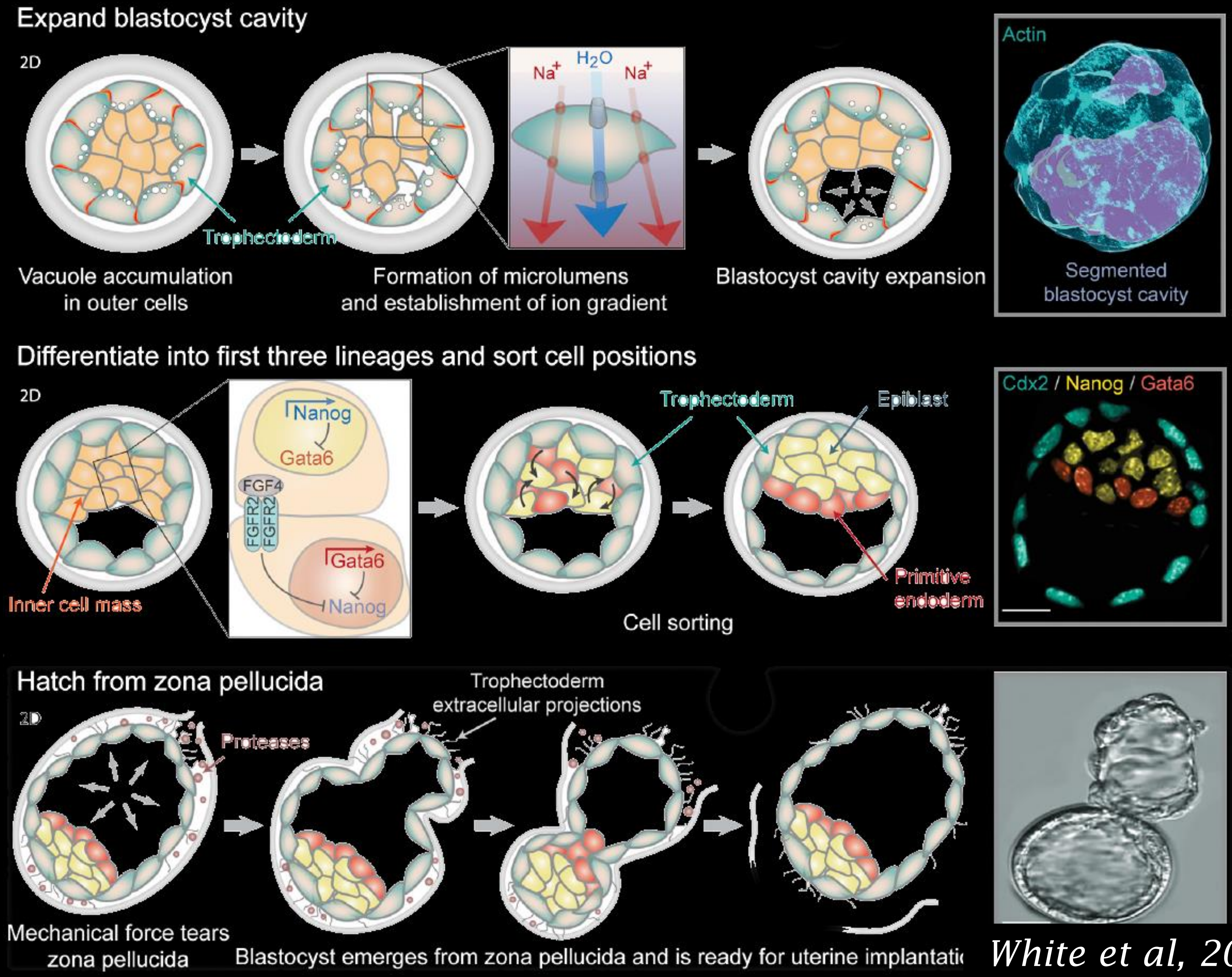
Plusa et al., 2008

DETERMINISM AND VARIABILITY IN EMBRYO DEVELOPMENT



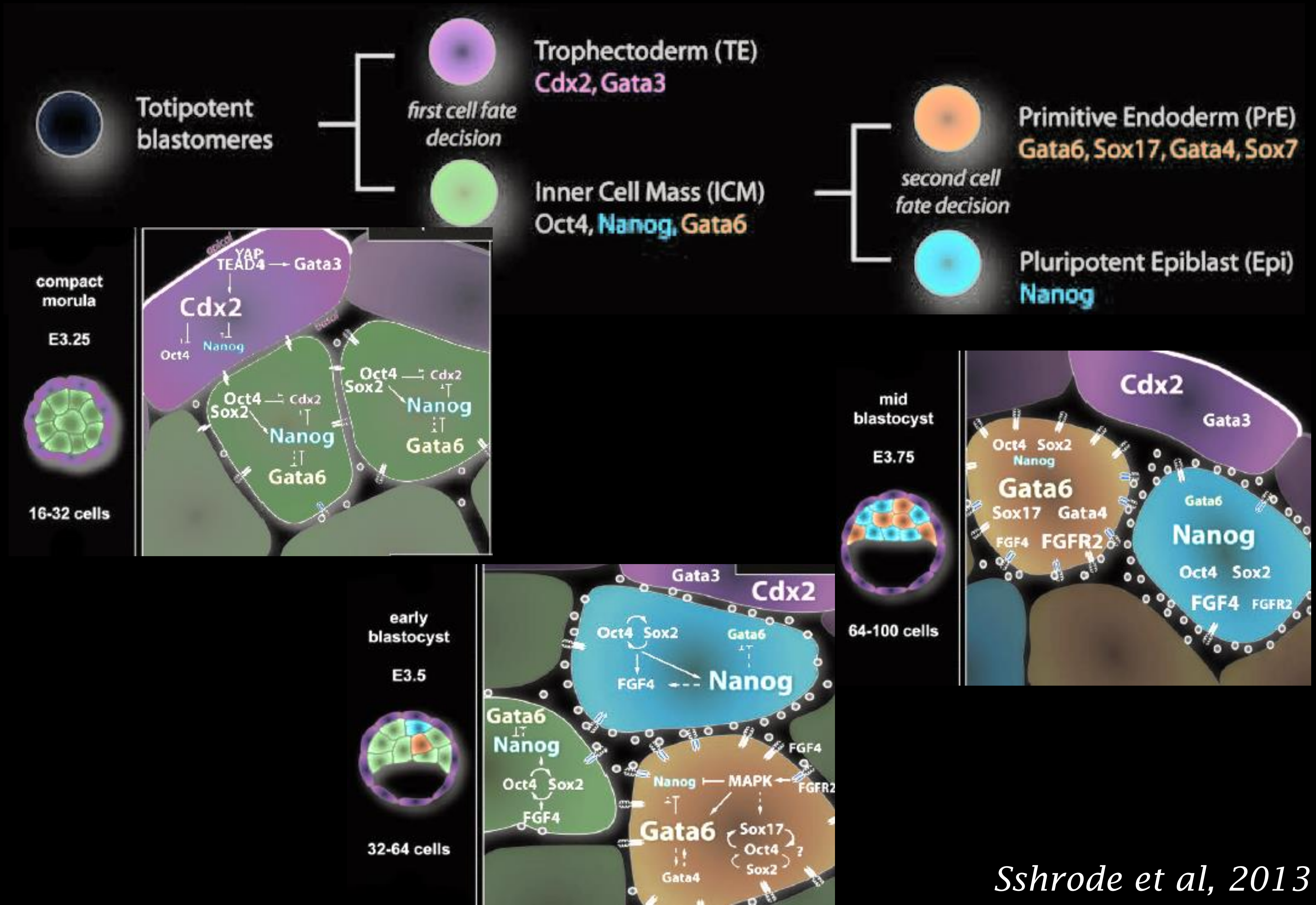
Dietrich et al., 2007

CAVITATION AND BLASTOCYST



White et al, 2018

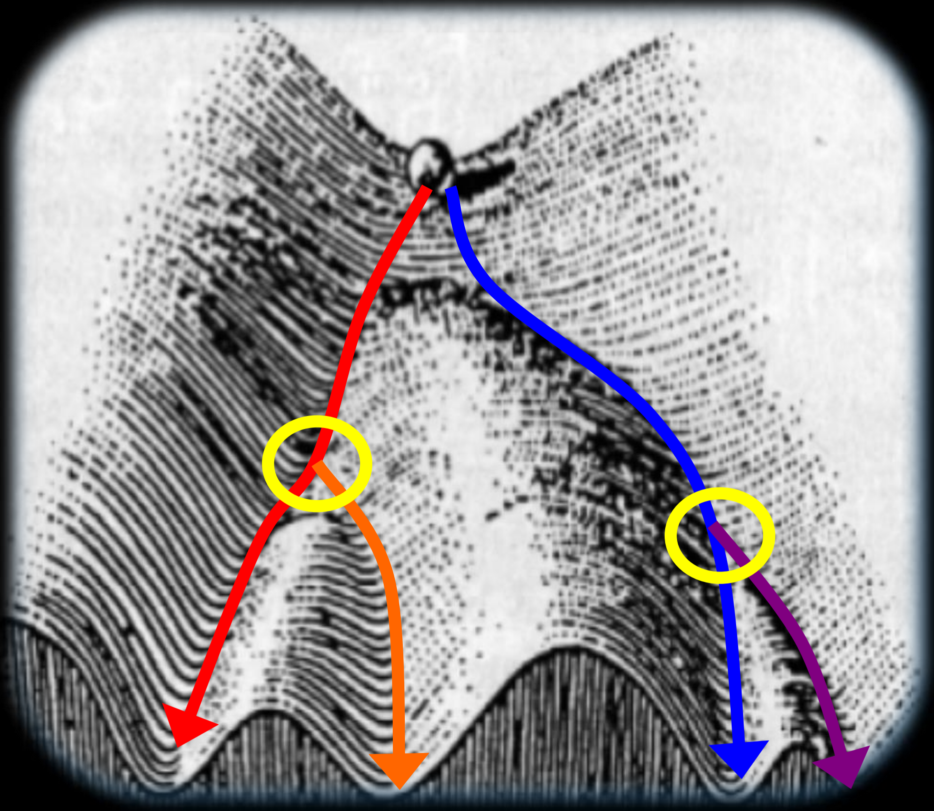
BLASTOCYST: CELL FATE DECISION

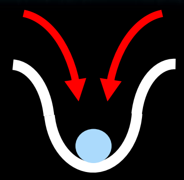


Sshrode et al, 2013

DETERMINISM AND VARIABILITY IN EMBRYO DEVELOPMENT

Konrad Waddington
(1905—1975)





Determinism – structurally stable paths (creods)

Variability – stochastic domains

THE PRESENT-DAY PHYSICS
LIGHTS UP LESS THEN A HALF
OF OUR OBJECT…

THANK YOU
FOR YOUR ATTENTION

…BUT THE CRESCENT IS GROWING