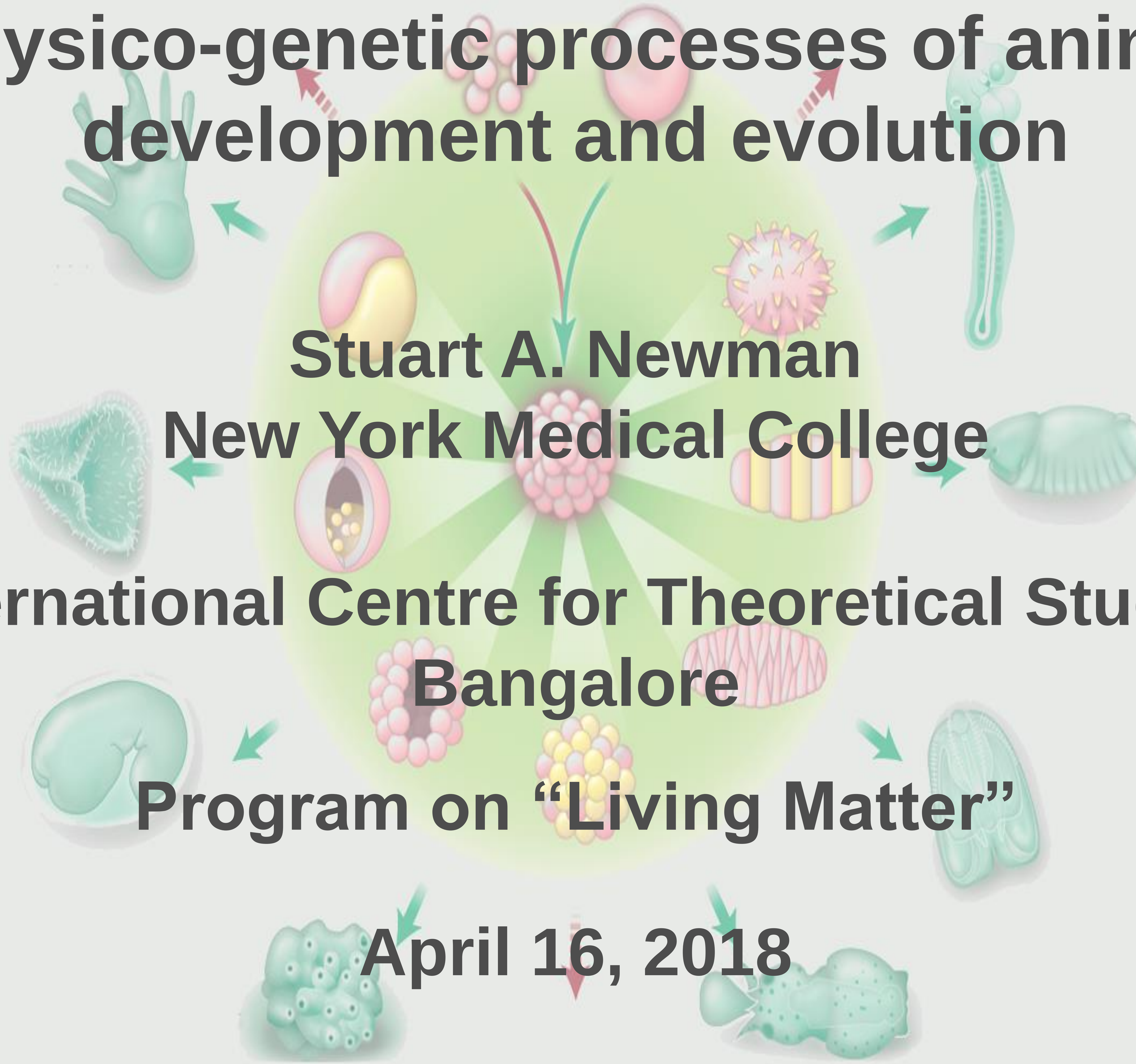


Physico-genetic processes of animal development and evolution

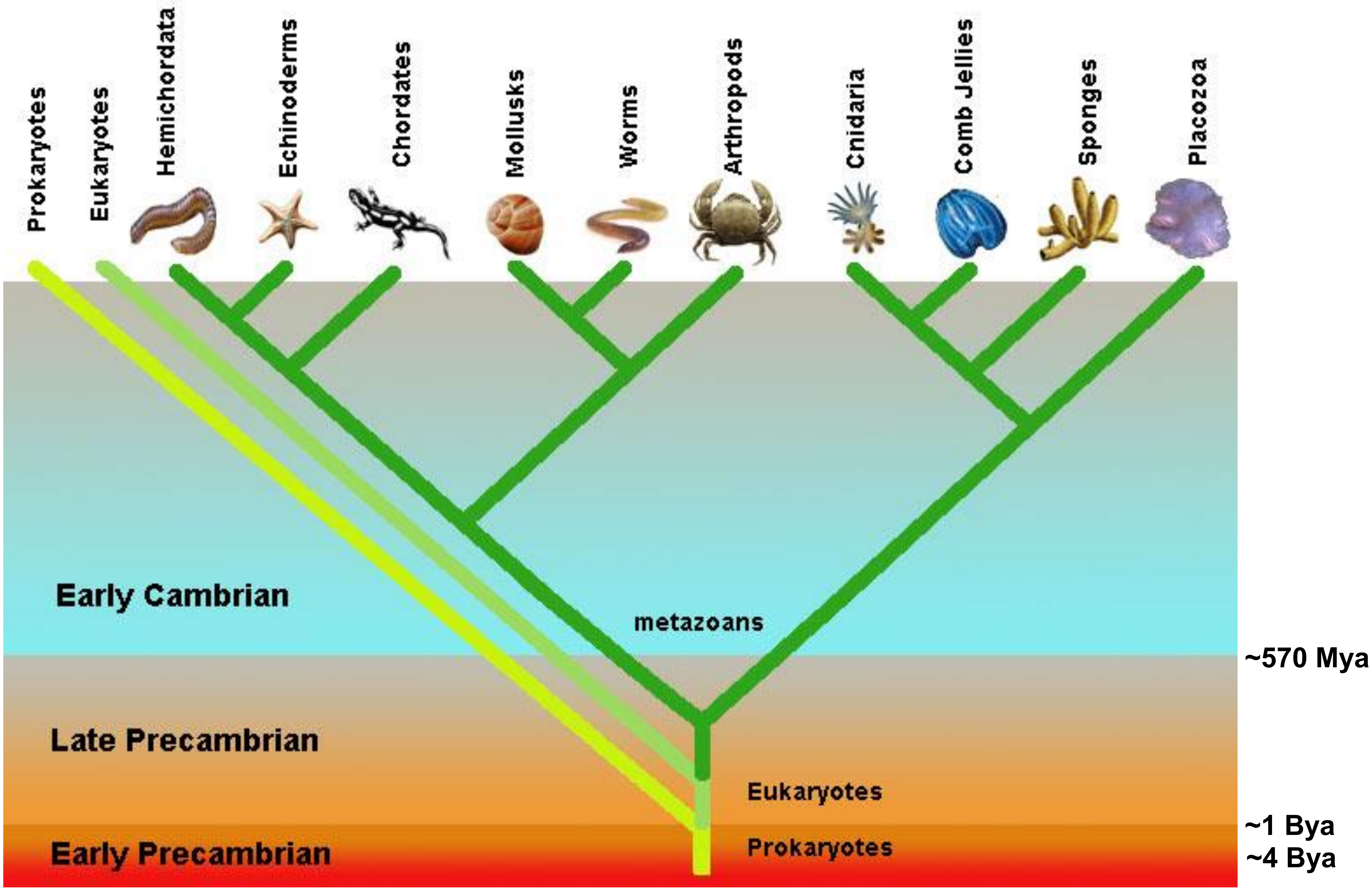
Stuart A. Newman
New York Medical College

International Centre for Theoretical Studies
Bangalore

Program on “Living Matter”

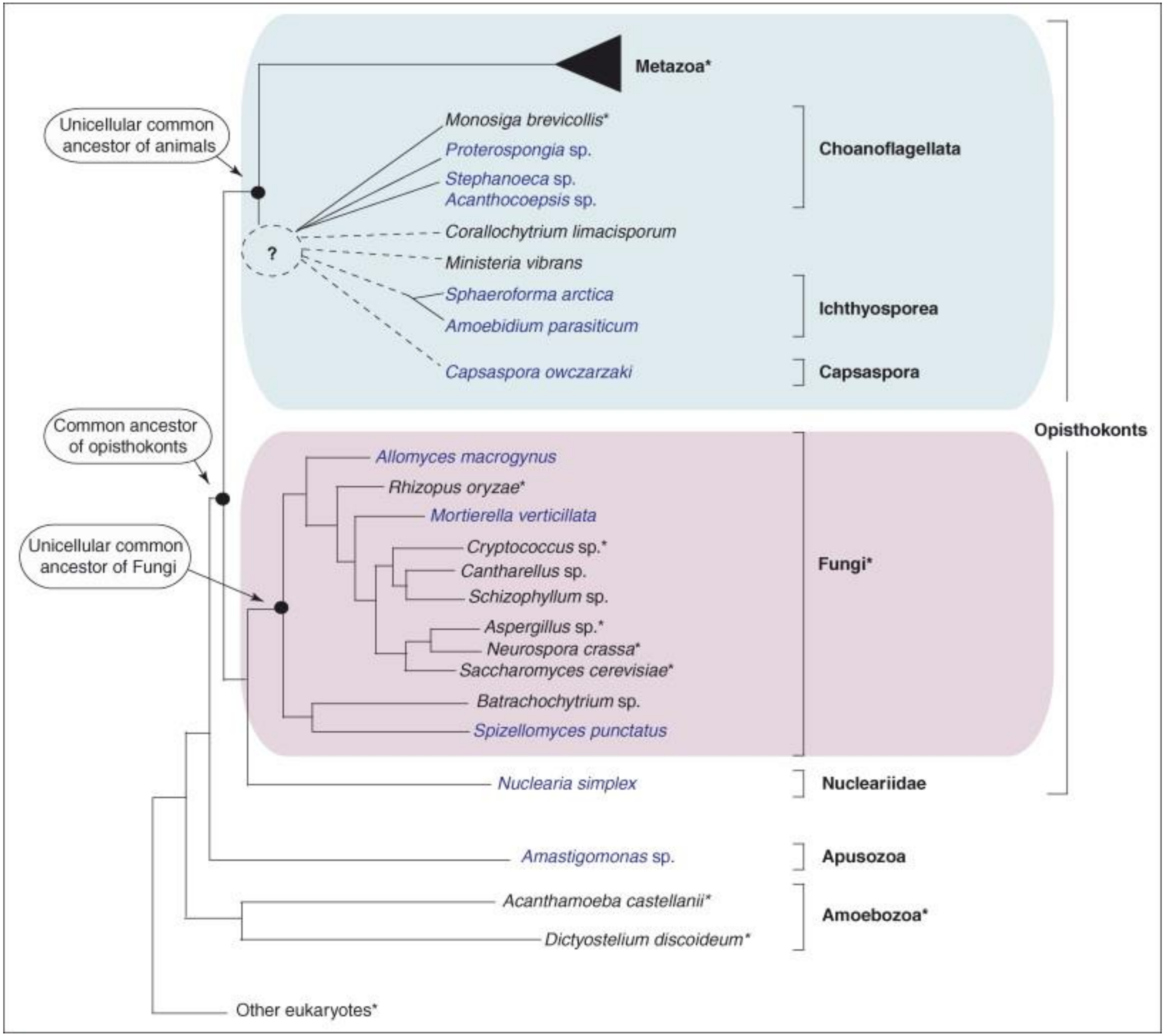
April 16, 2018

The Ediacaran and Cambrian Explosions



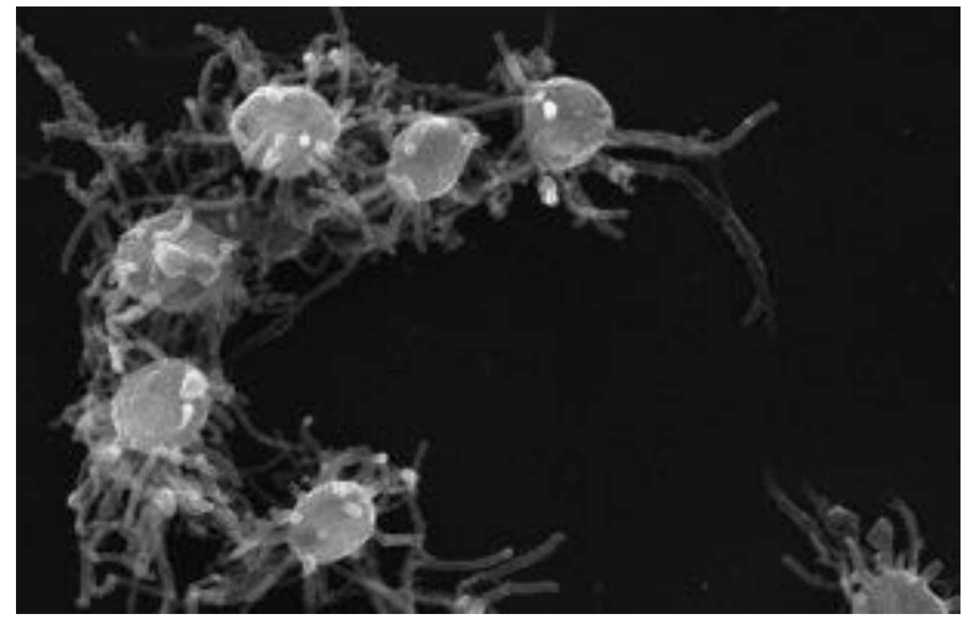
http://geologycafe.com/

The Holozoans

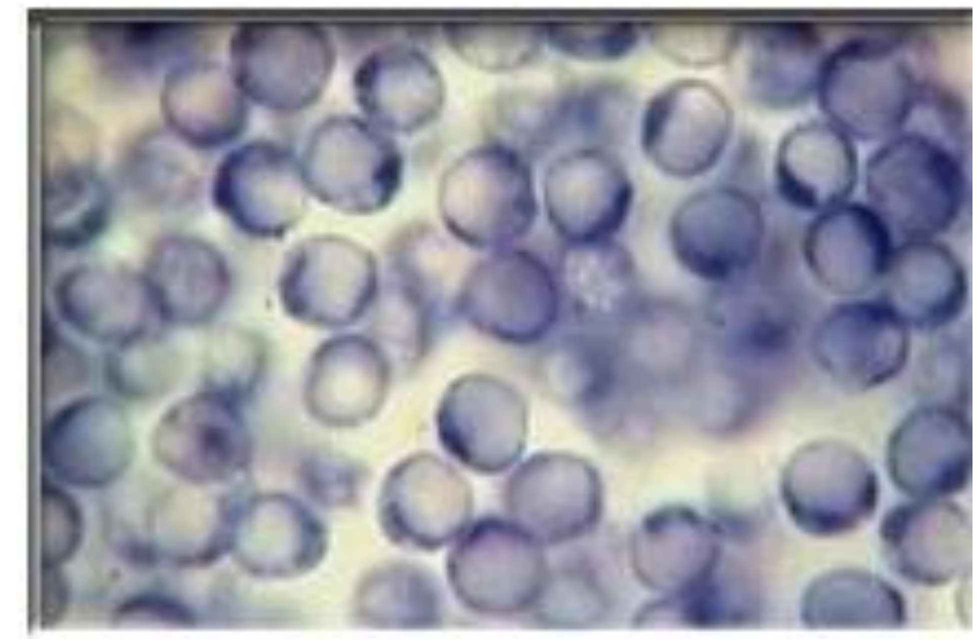


Ruiz-Trillo *et al. TIG* 2007

Monosiga



Capsaspora



Mesomycetozoea

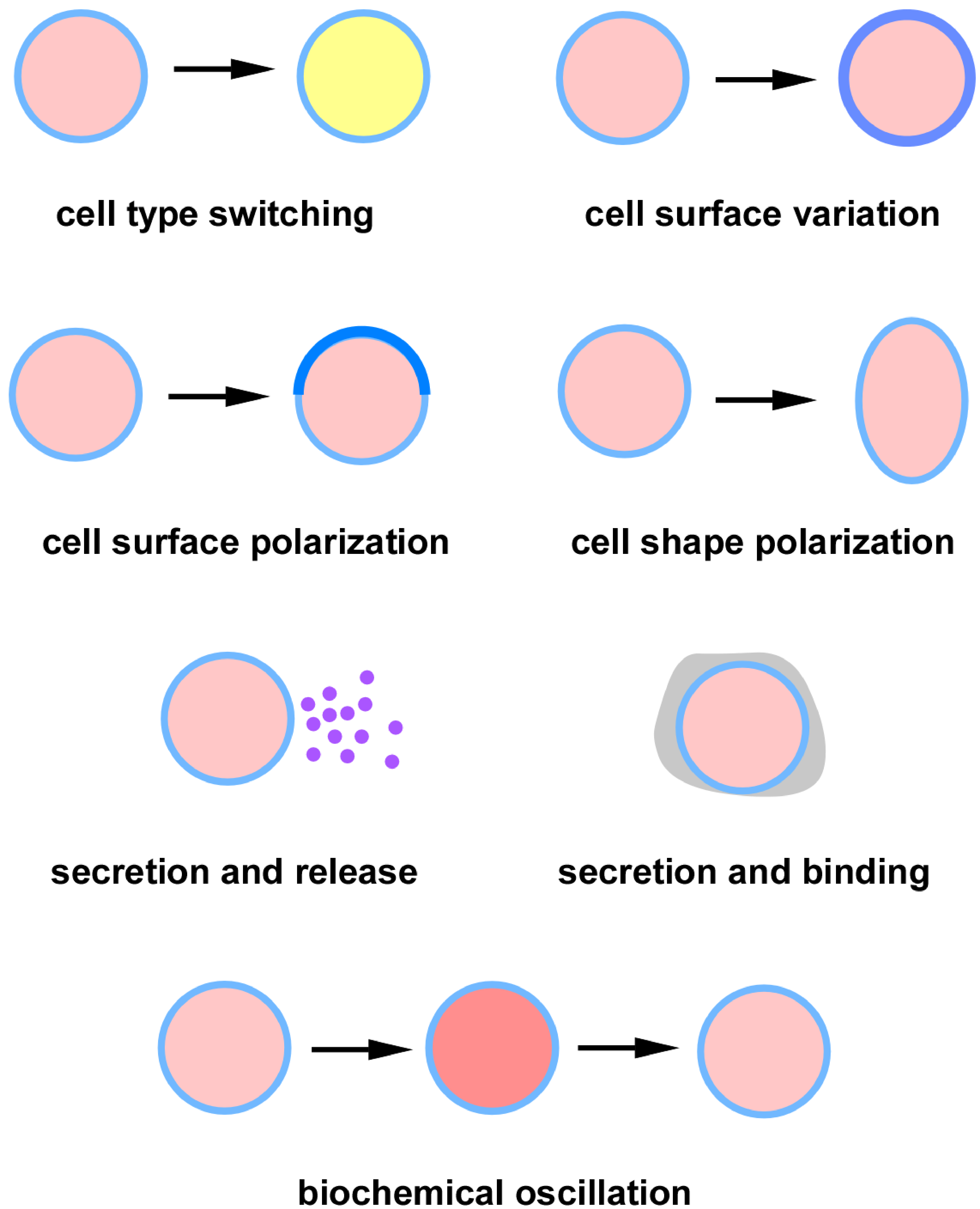
One or more of the extant holozoans and by inference, the unicellular ancestors of the metazoans, contain(ed) genes specifying cadherins, C-type lectins, Notch and Wnt pathway components, Hedgehog and other members of the metazoan developmental-genetic toolkit which eventually came to mediate cell-cell interactions (a.k.a. the **“interaction toolkit”**).

King *et al.*, *Nature* 451:783; 2008
Shalchian-Tabrizi *et al*., *PLoS ONE* 3:e2098; 2008
Sebé-Pedrós *et al. eLife 2:* e01287; 2013

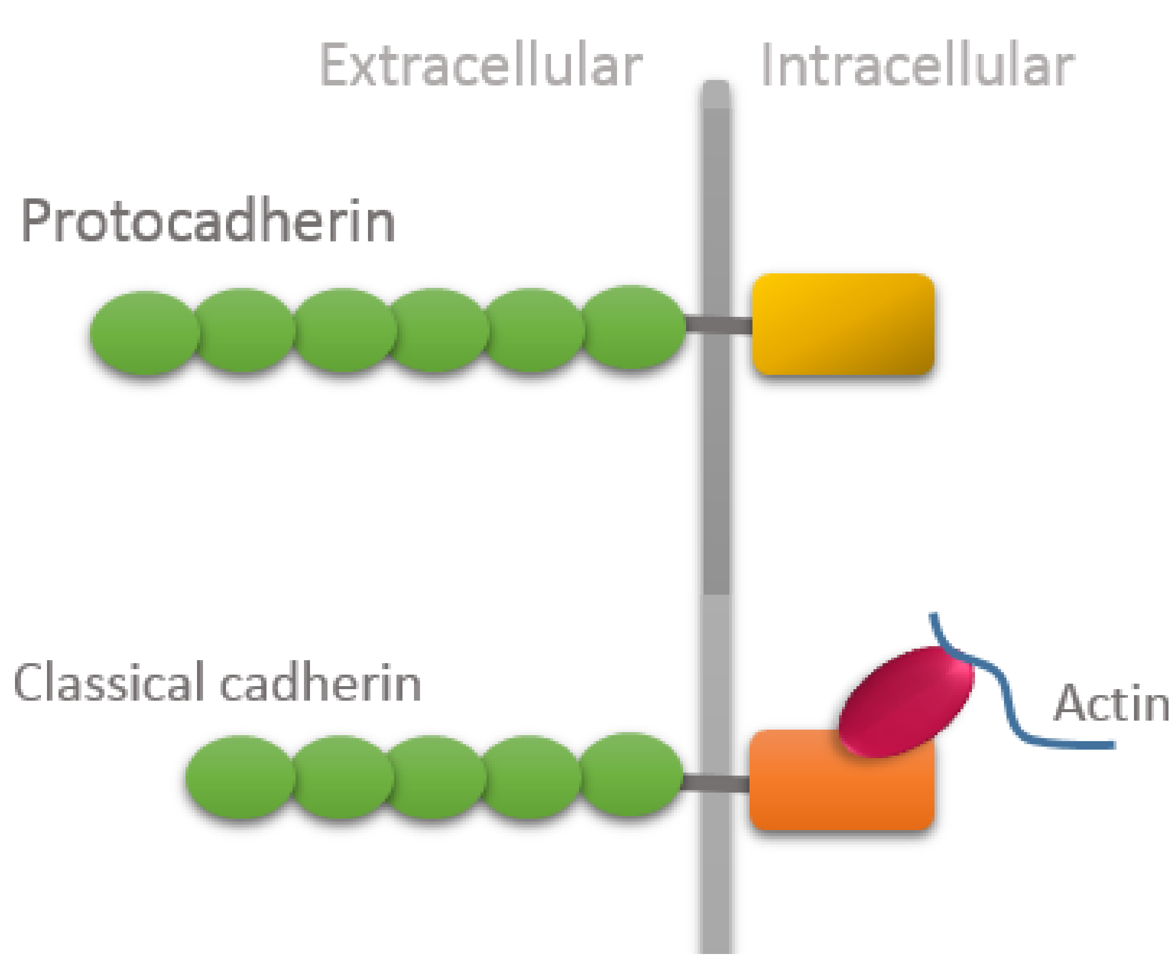
Origination of highly disparate animal body plans occurred with a pre-existing genetic toolkit and was compressed in time.

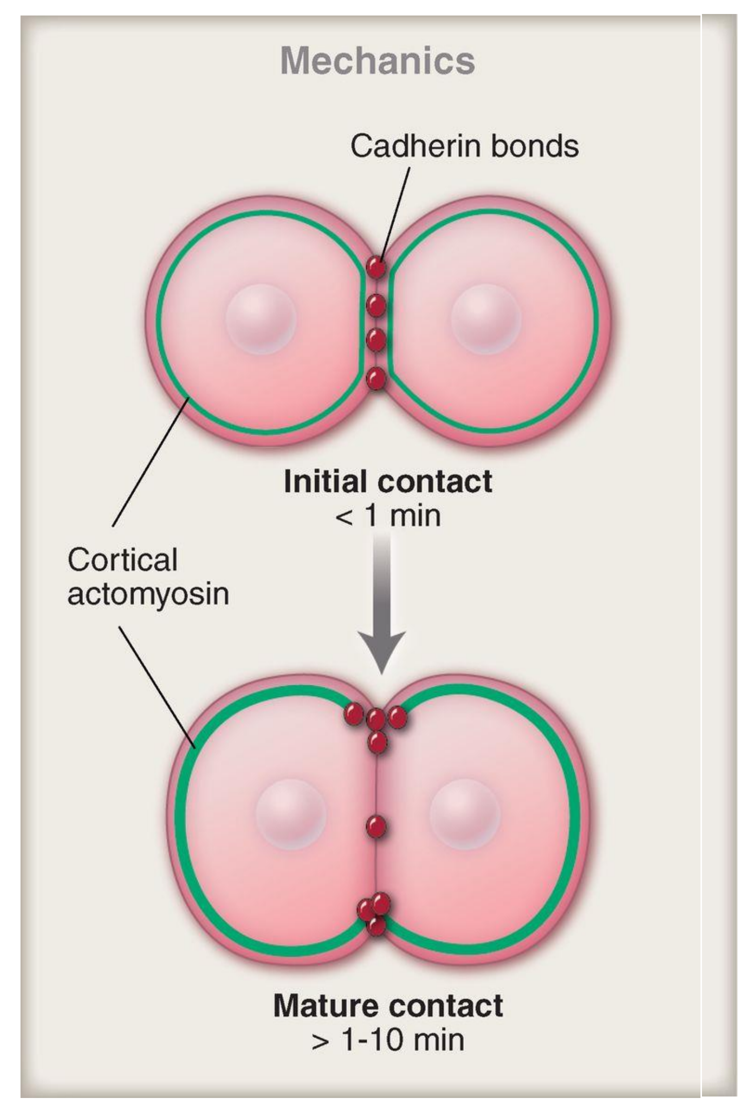
What additional causal agency was involved?

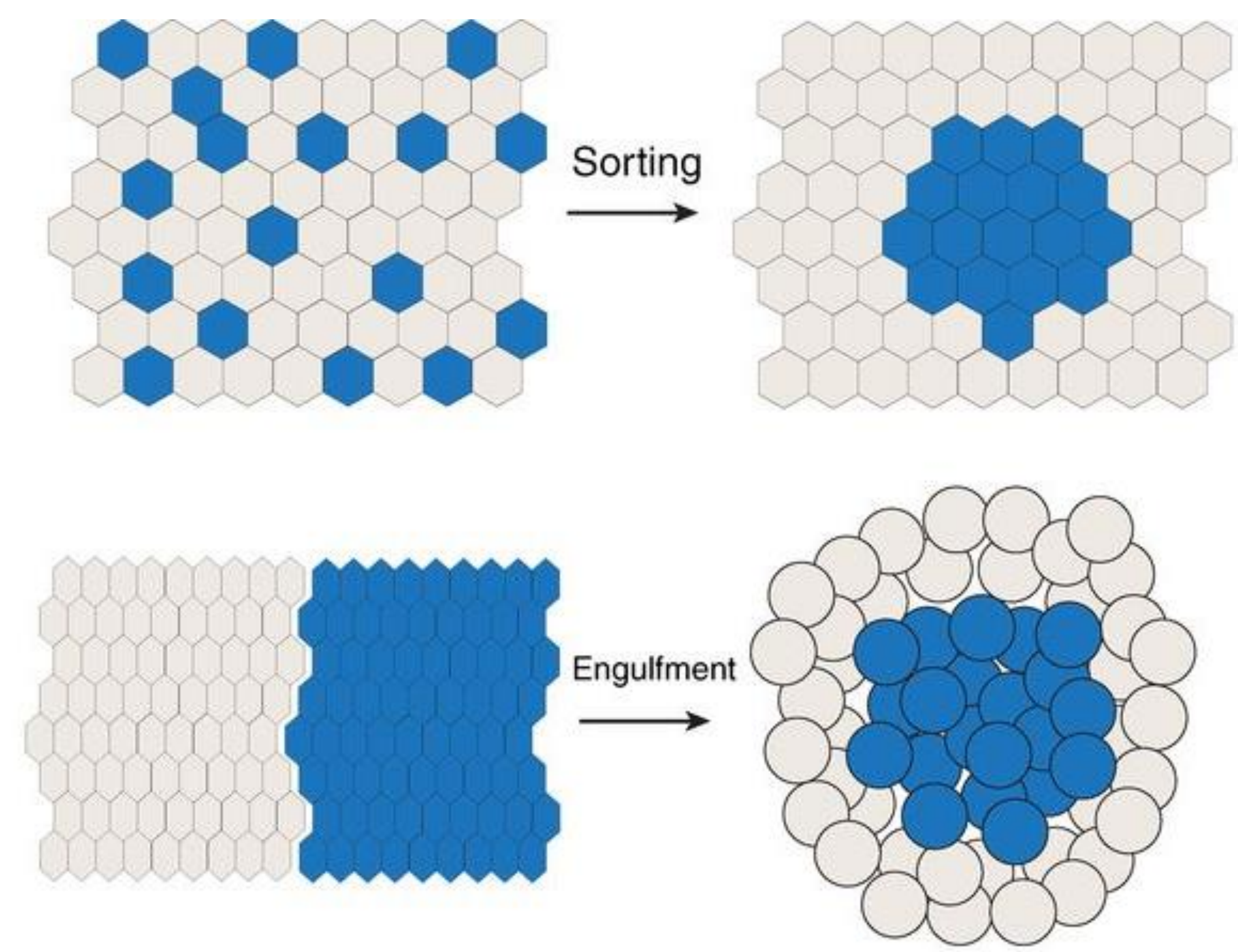
Toolkit-based functionalities in unicellular ancestors of the metazoans



Innovation of classical cadherins in the metazoans permitted cells to move autonomously without disrupting the cohesion of the cell mass







This created “liquid tissues”: a category of **biogeneric** matter

“Generic” mechanisms of development

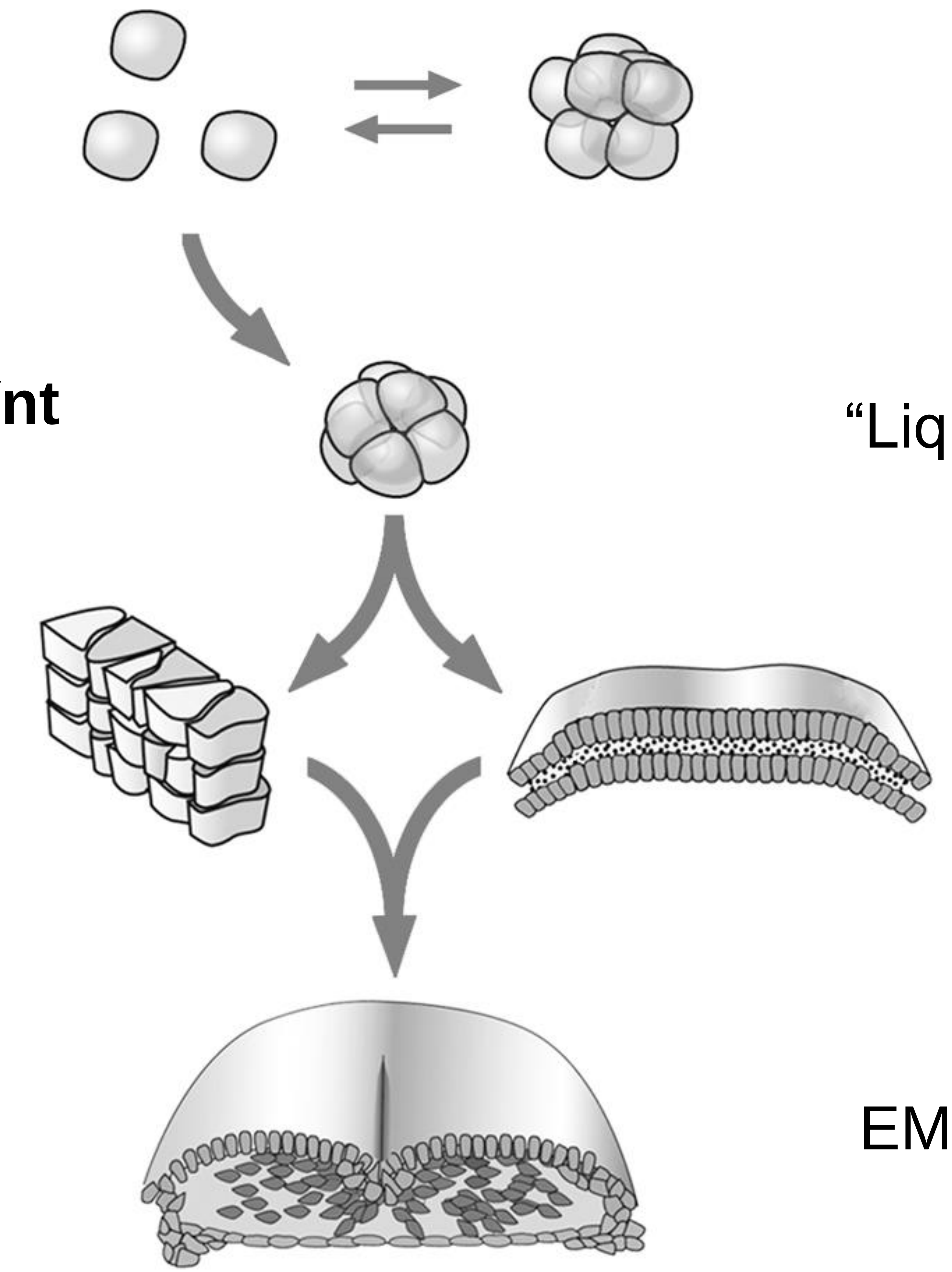
Form- and pattern-generating processes common to living and nonliving (mesoscale, viscoelastic, excitable) systems

“Biogeneric” materials and mechanisms of development

Multicellular materials in which cellular systems behave similarly to certain nonliving materials, but utilizing evolved, cell-based response functions

Biogeneric materials exhibit forms and patterns resembling those characteristic of generic processes.

Some novel genes were associated with emergence of novel biogeneric materials



Protocadherins — Cell clusters

Classical cadherins, Wnt — “Liquid tissues”

Vang/Stbm (PCP)
“Liquid crystalline” tissues

Peroxidasin
(basement membrane)
Planar tissues

Galectins, fibronectin, tenascin — EMT, mesenchyme

SAN *Phil. Trans. R. Soc. B*, 2016

“Physico-genetic” hypothesis
for body plan origination

Metazoan form originated and rapidly diversified by the action of **Dynamical Patterning Modules (DPMs)**.

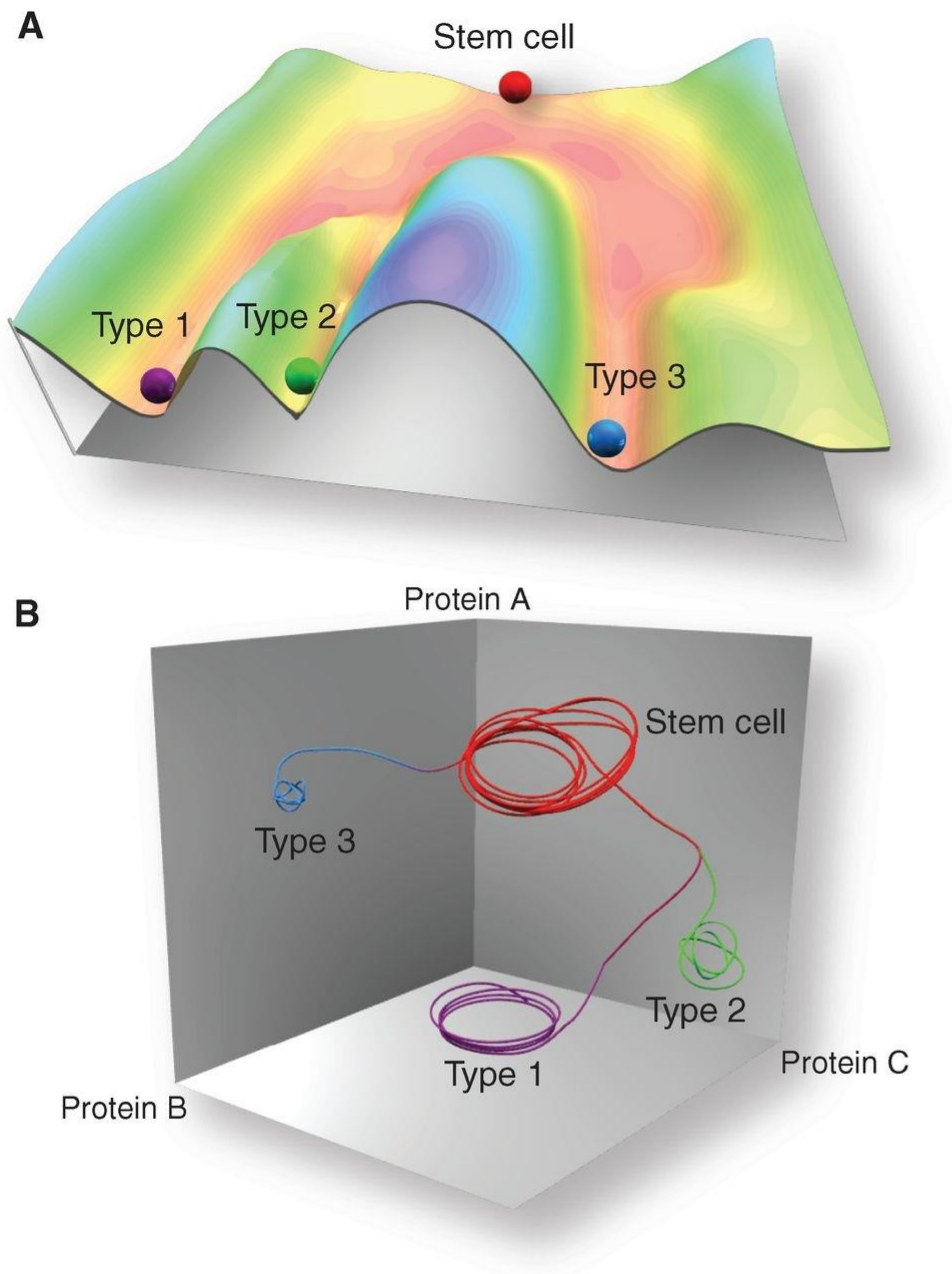
Definition: DPMs are specific molecules and pathways in association with specific physical forces or effects that pattern and shape multicellular aggregates.

Specifics: DPMs originated when ancient gene products or derivatives (many of which were present in unicellular ancestors of the metazoans) assumed novel physical functions due to the change of scale and context in the multicellular state.

Dynamical patterning modules generate the characteristic morphological motifs of biogeneric materials, analogously to the processes that generate waves and vortices in nonliving liquids, or the crystal structures of minerals.

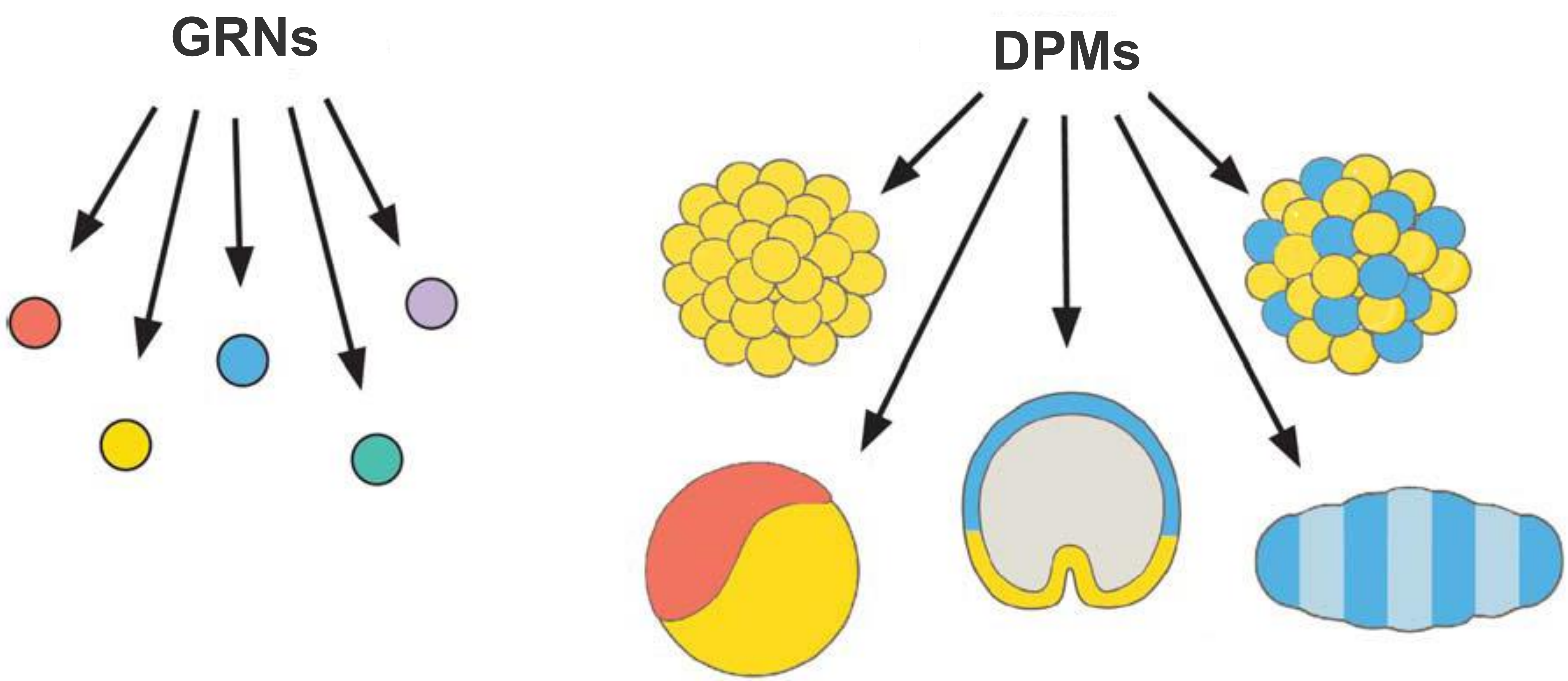


Dynamics of transcription factor-based gene regulatory networks (GRNs) specify alternative cell states



Furusawa & Kaneko *Science* 2012;338:215

GRNs mediate **cell differentiation**;
dynamical patterning modules (DPMs) mediate
pattern formation and morphogenesis



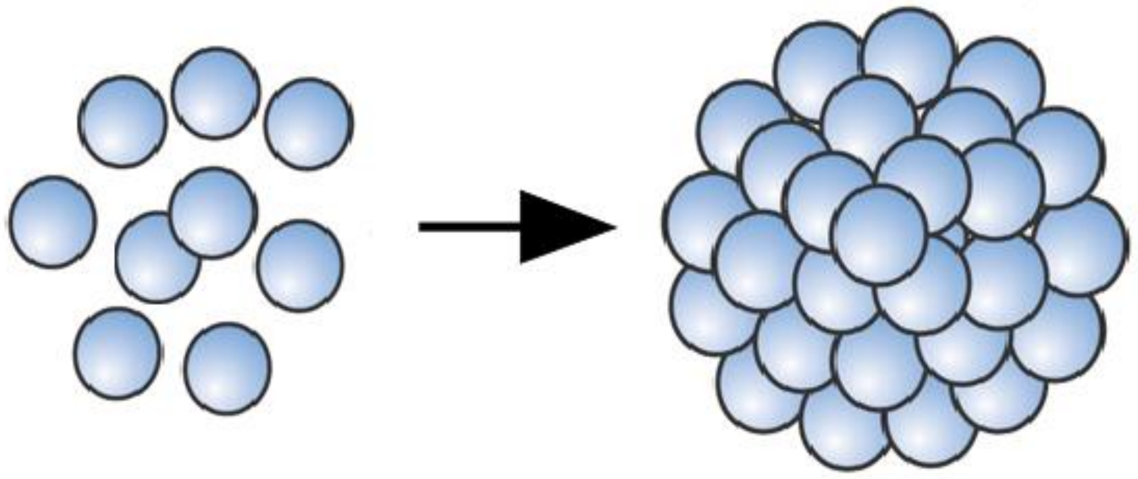
Newman et al. *J. Biosci.*; 2009

Mesoscale physical processes and mechanisms first studied during the 20th century

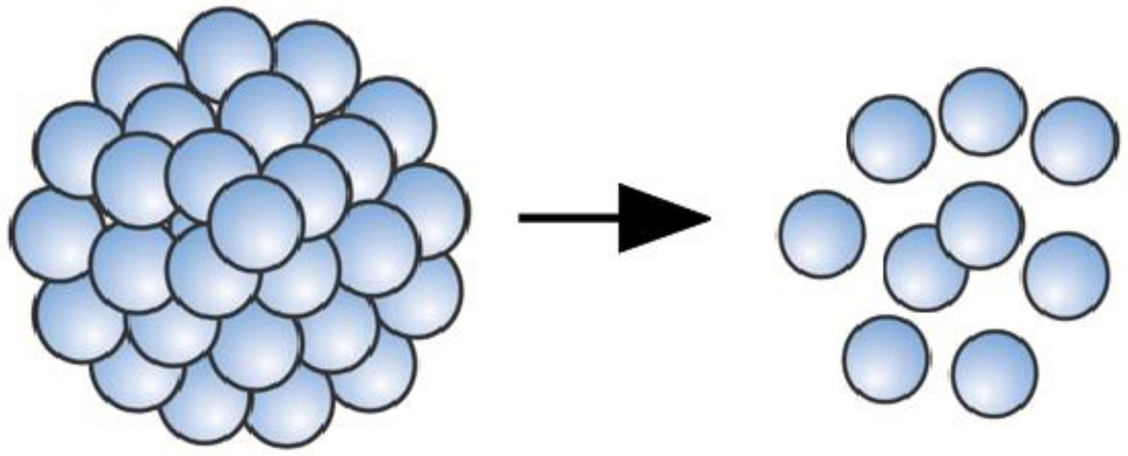
- Multistable dynamical systems (Poincaré; Lyapunov)
- Nonlinear oscillations (van der Pol)
- Reaction-diffusion instabilities (Turing)
- Phase separation and phase transitions (Ehrenfest)
- Thermodynamics of open systems (Prigogine)
- Nonlinear fluid mechanics (Batchelor)
- Deterministic chaos (Feigenbaum)
- Fractals (Mandelbrot)
- Percolation and scaling; soft matter (de Gennes)
- Mechanochemical coupling (Katchalsky)

By mobilizing **mesoscale physical effects** ancient molecules took on new functions in the multicellular context

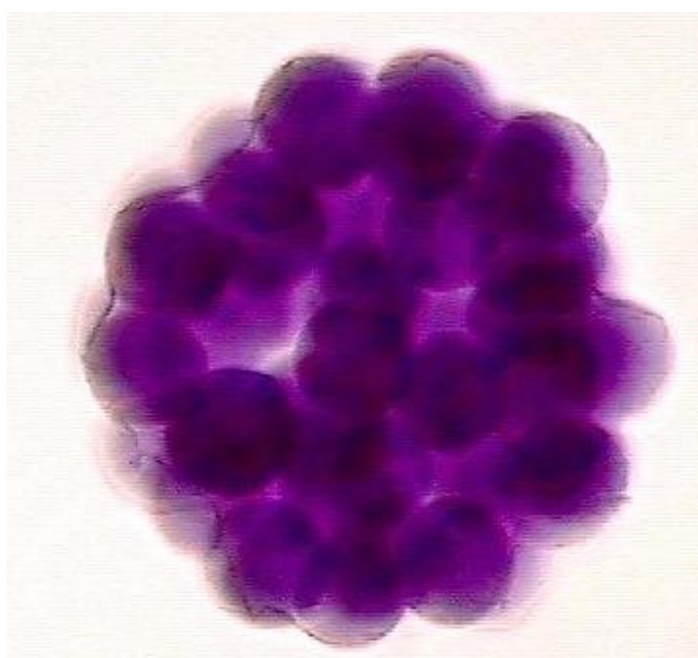
Aggregation is the necessary condition for multicellularity

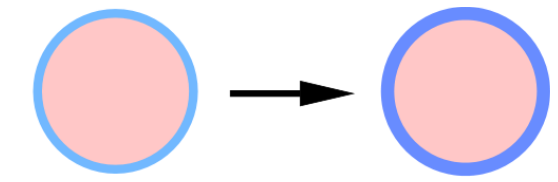


Its inverse is *epithelial-mesenchymal transformation* (EMT)

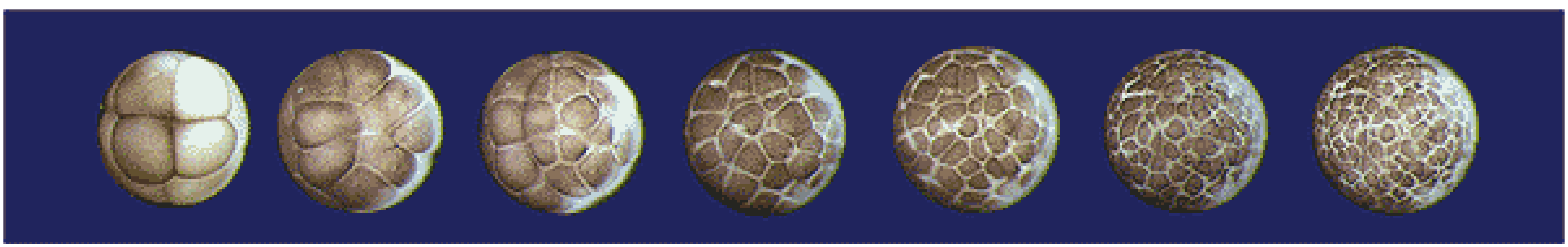


Metazoan cell clusters: ancient and modern



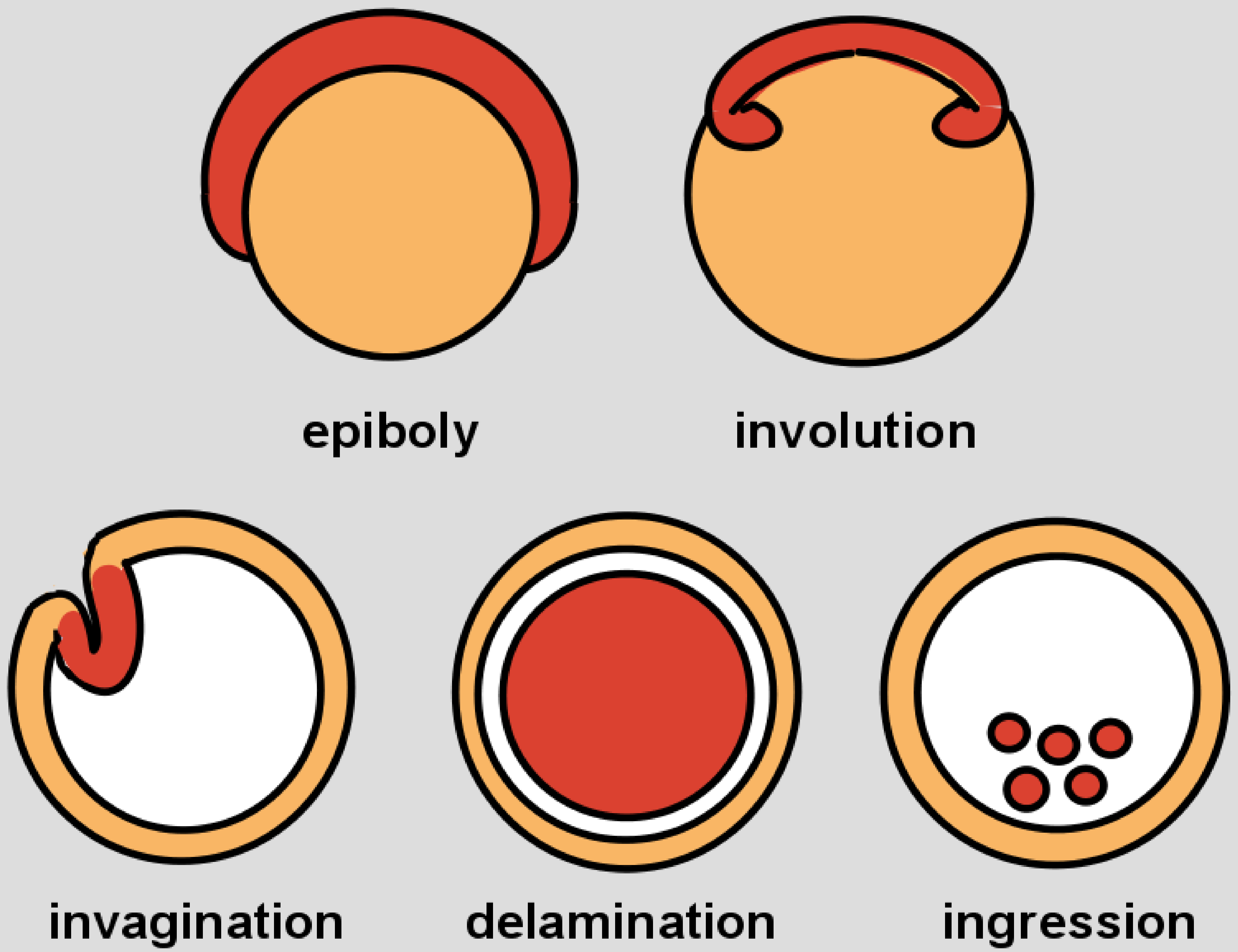


Toolkit molecules: cadherins; galectins

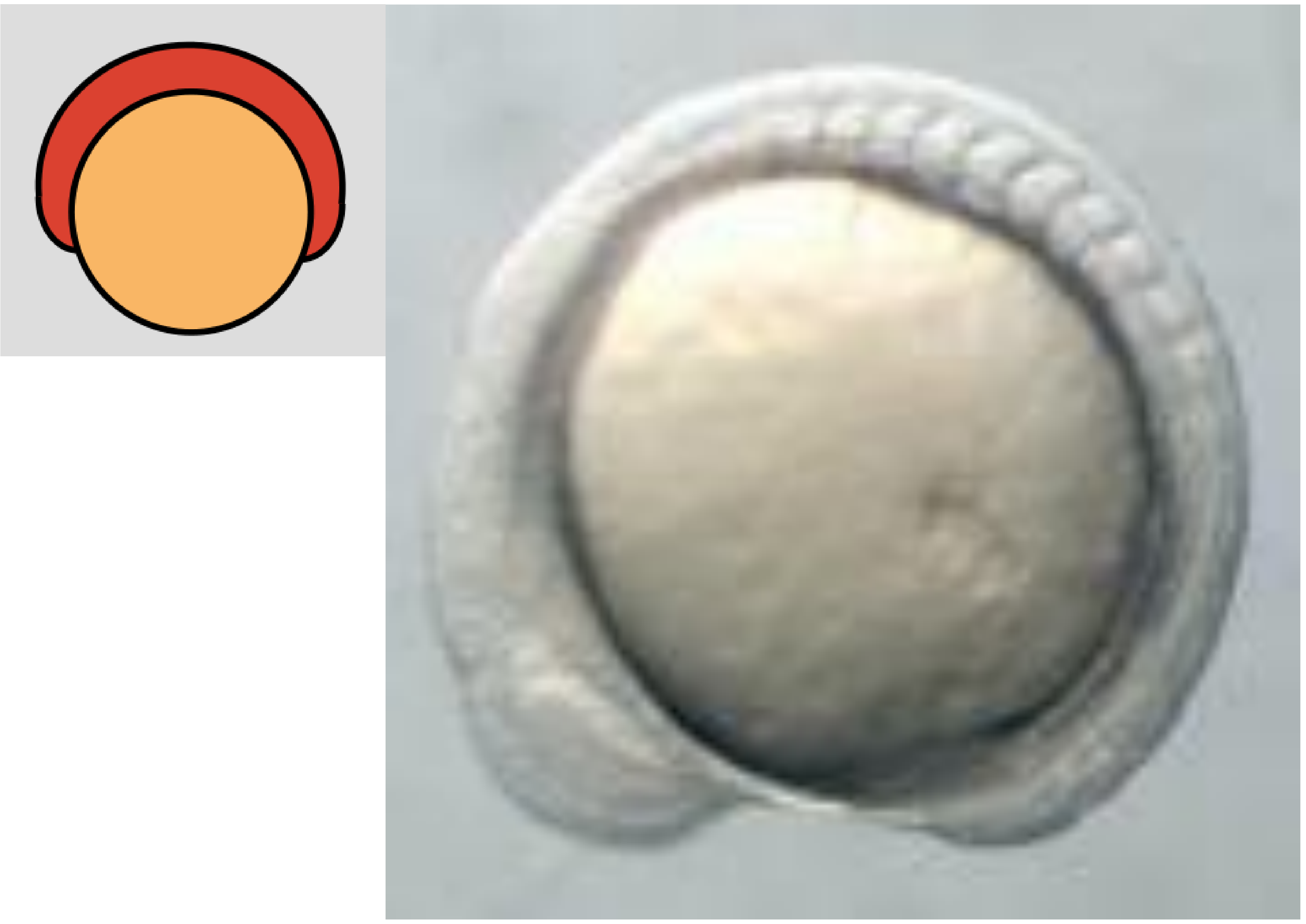


Upper left, Human morula; RWJMS IVF Program
Middle left, Starfish morula; K. Wynne, Tyler Junior College
Right, Fossil “embryos”; Ediacaran Doushantuo Formation; Shuhai Xiao, Virginia Tech
Bottom, Frog blastula; www.snv.jussieu.fr/bmedia/xenope1/xenope3.html

Gastrulation: establishment of multilayered embryos in all eumetazoan phyla

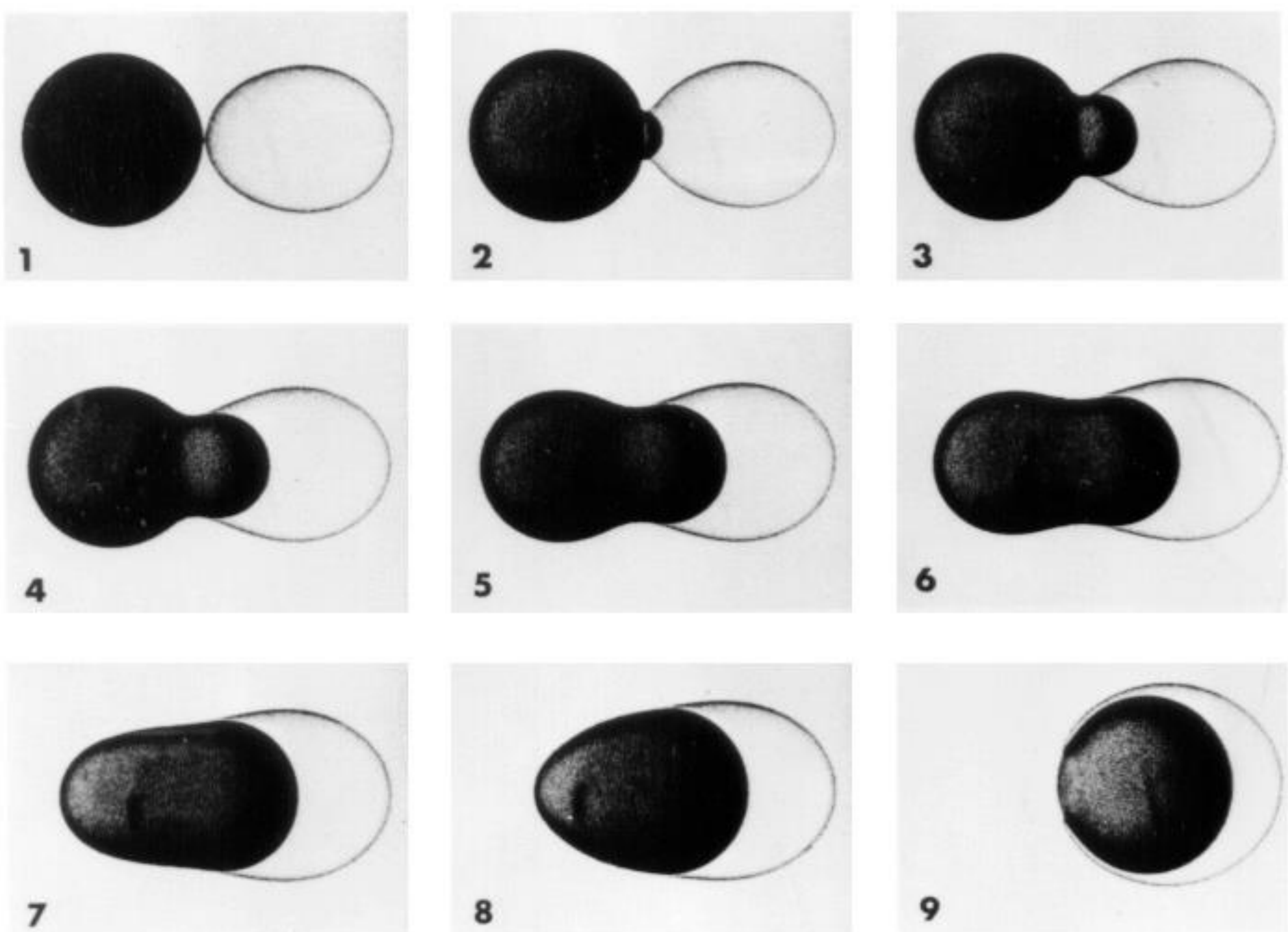


Gastrulation typically involves engulfment-like tissue rearrangement (e.g., zebrafish)

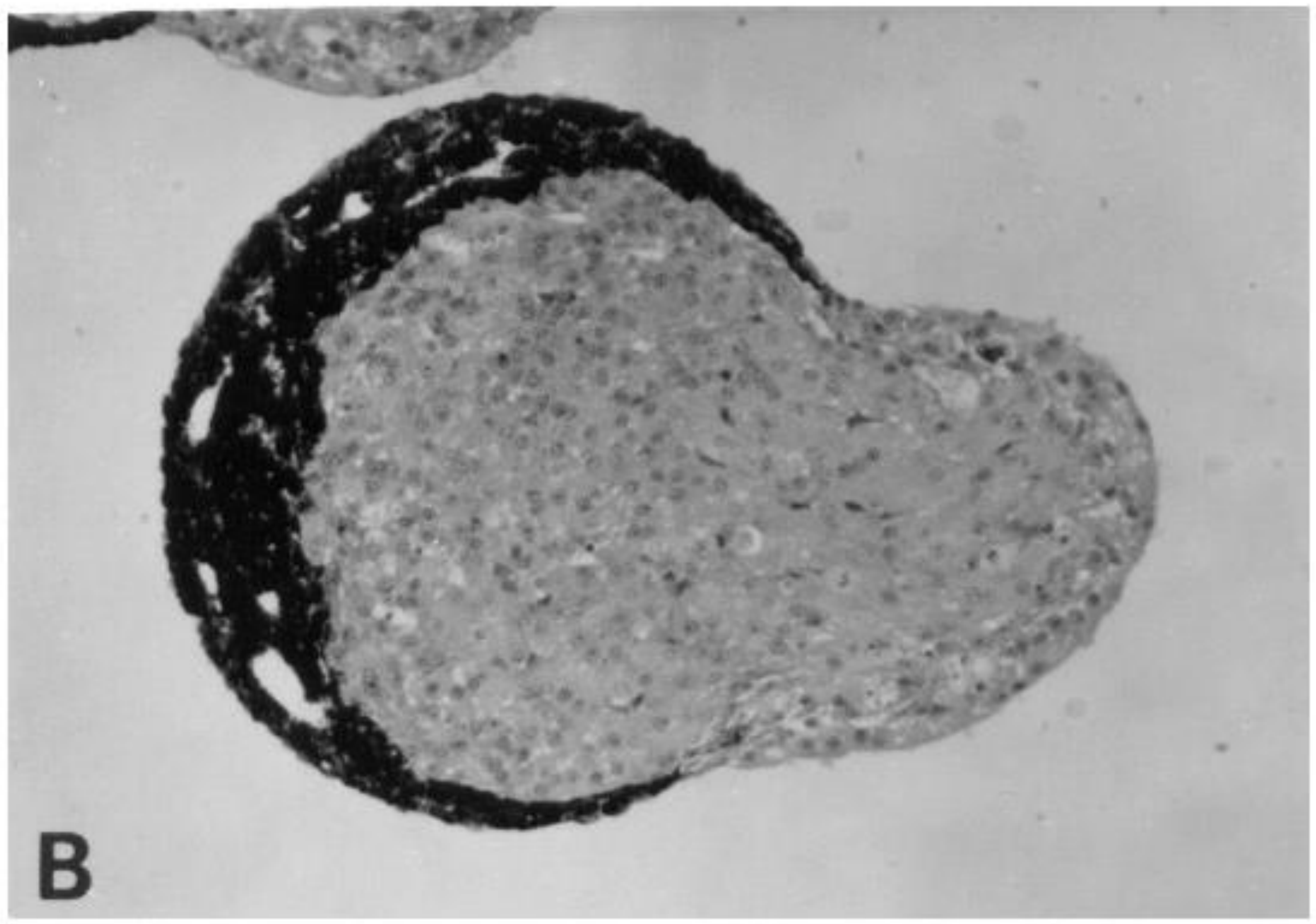


AG Gajewski; University of Cologne

Phase separation and engulfment behavior in liquids and tissues

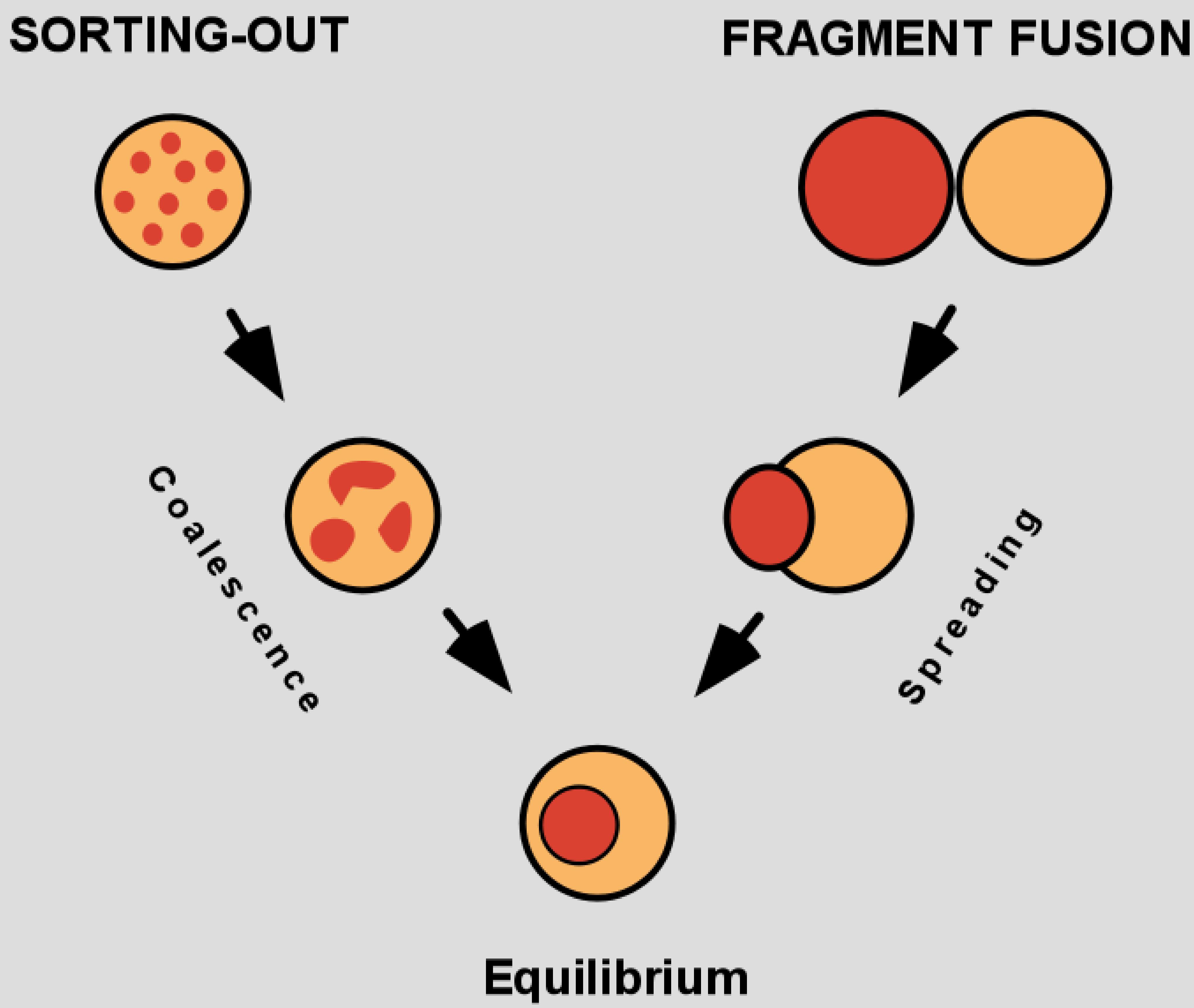


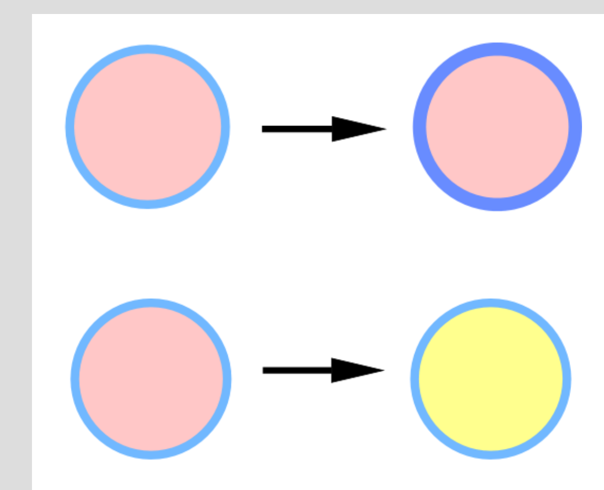
Torza and Mason *Science* 163: 813; 1969



Armstrong, *Crit Rev Biochem Mol Biol* 24:119; 1989

Differential interfacial tension can lead to cell sorting and tissue engulfment

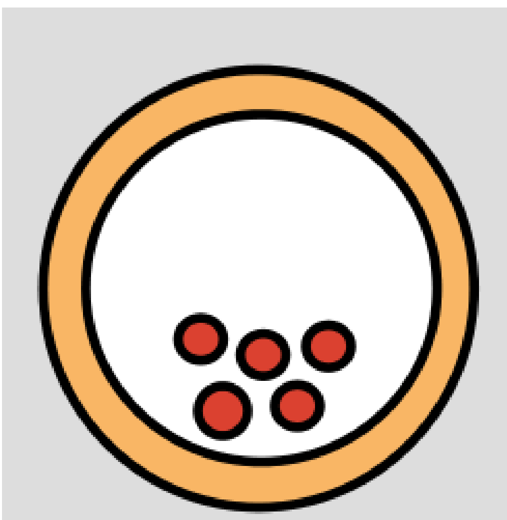


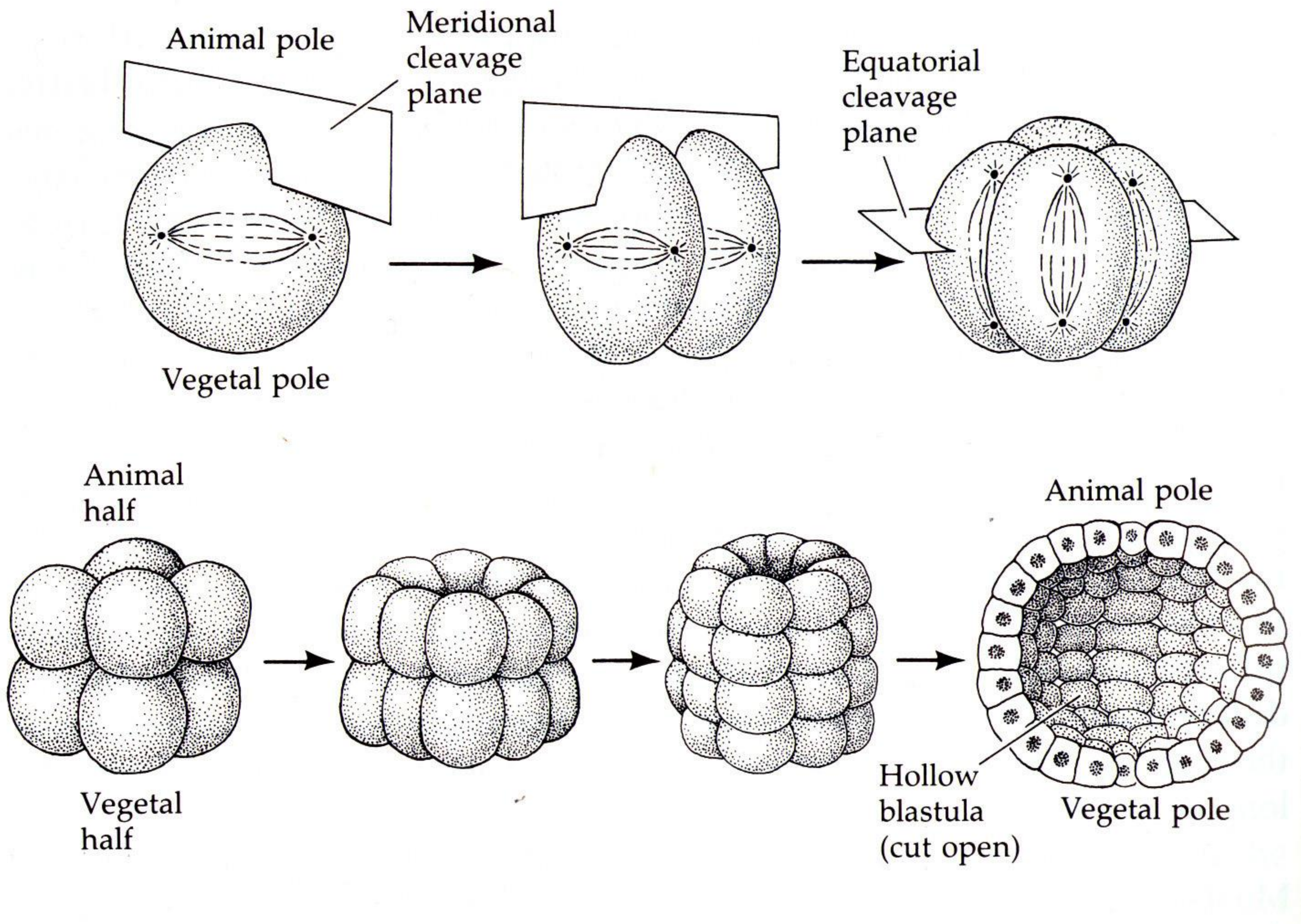


<u>Toolkit molecules</u>: cadherins; lectins; Notch

Based on Steinberg *Symp Soc Dev Biol*, 1978
See also Maître and Heisenberg *Curr Biol*, 2013

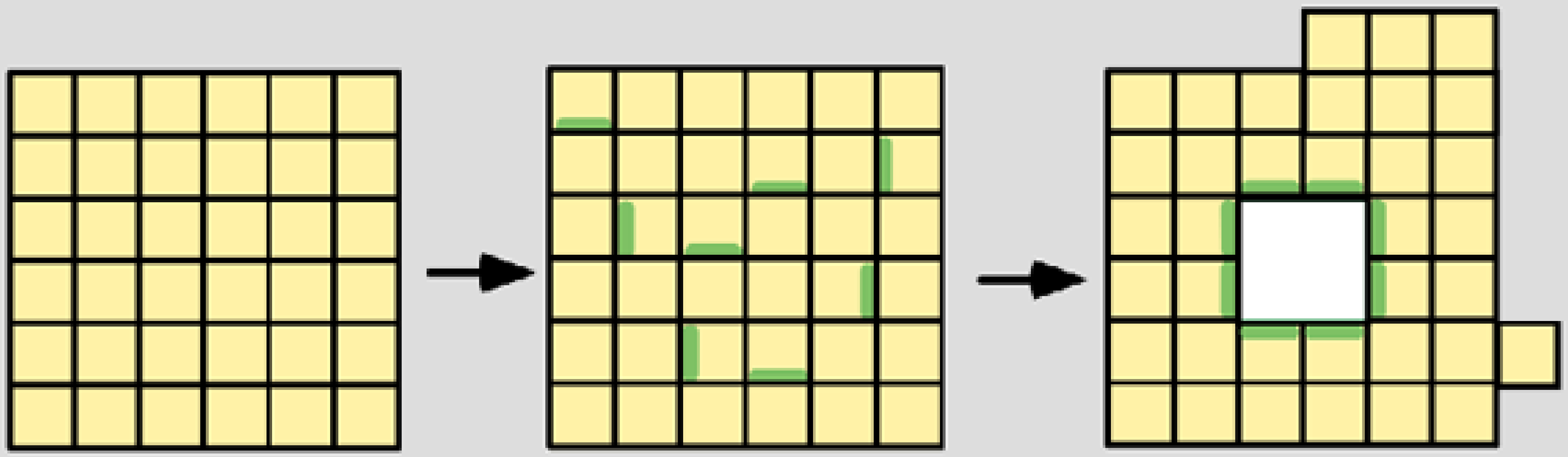
Gastrulation usually involves lumen formation (e.g., sea urchins)

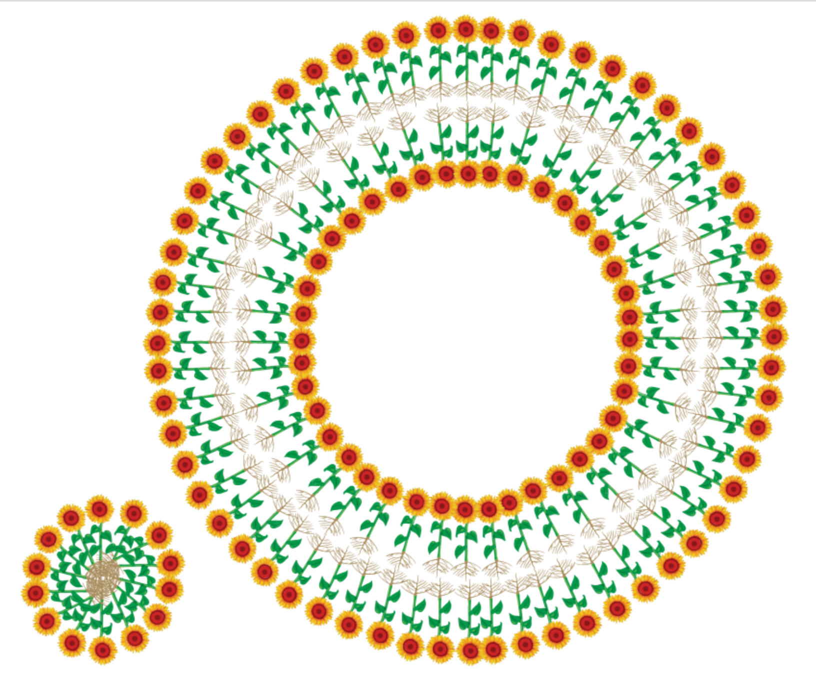




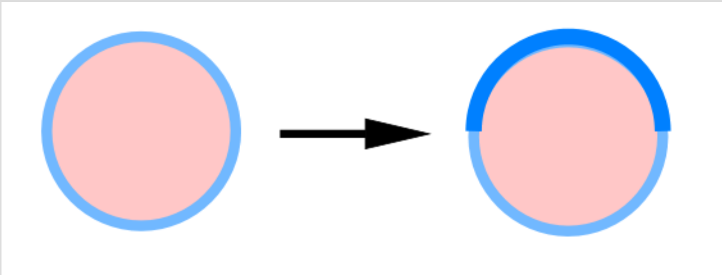
Gilbert, *Developmental Biology,* Sinauer, 2000

Lumens can automatically arise in clusters of cells that are individually apicobasally polarized





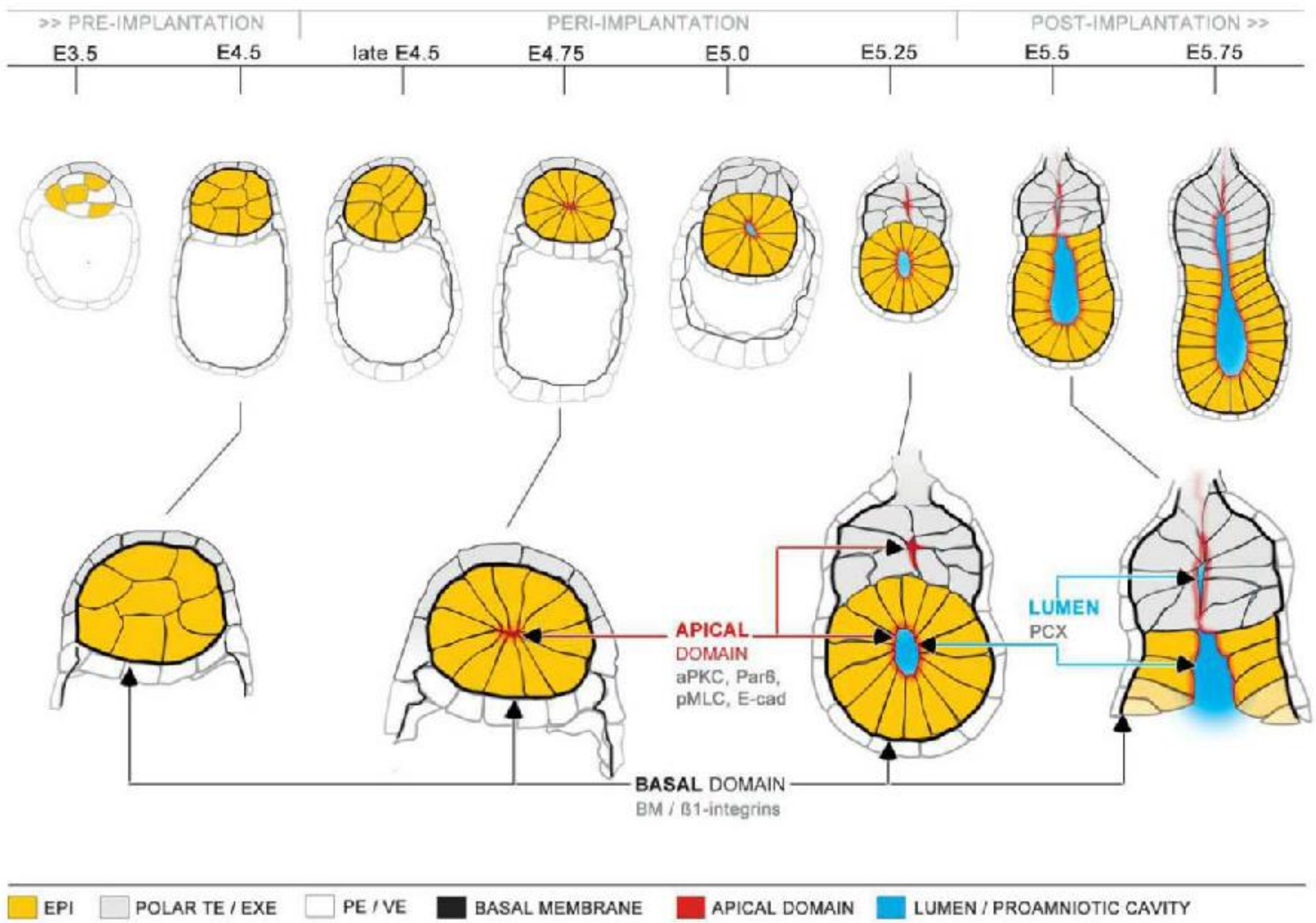
Physical analogue: micelles



Toolkit molecules: Wnt pathway; cadherins

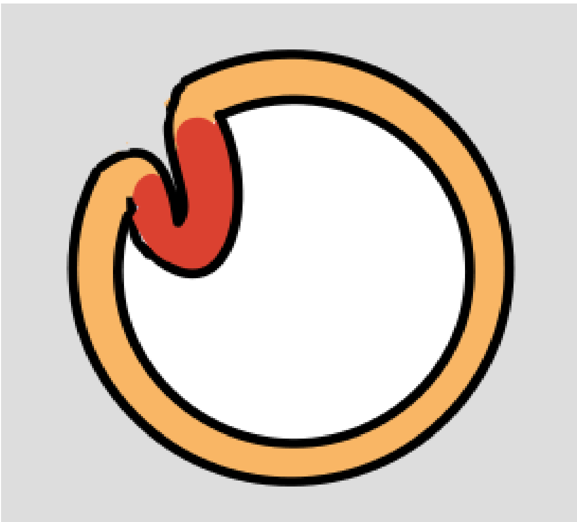
Self-Organizing Properties of Mouse Pluripotent Cells Initiate Morphogenesis upon Implantation

Ivan Bedzhov[1,2] and Magdalena Zernicka-Goetz[1,2,*]
[1]Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR, UK
[2]Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK
*Correspondence: mz205@cam.ac.uk
http://dx.doi.org/10.1016/j.cell.2014.01.023



Cell 156, 1032–1044, February 27, 2014 ©2014 Elsevier Inc.

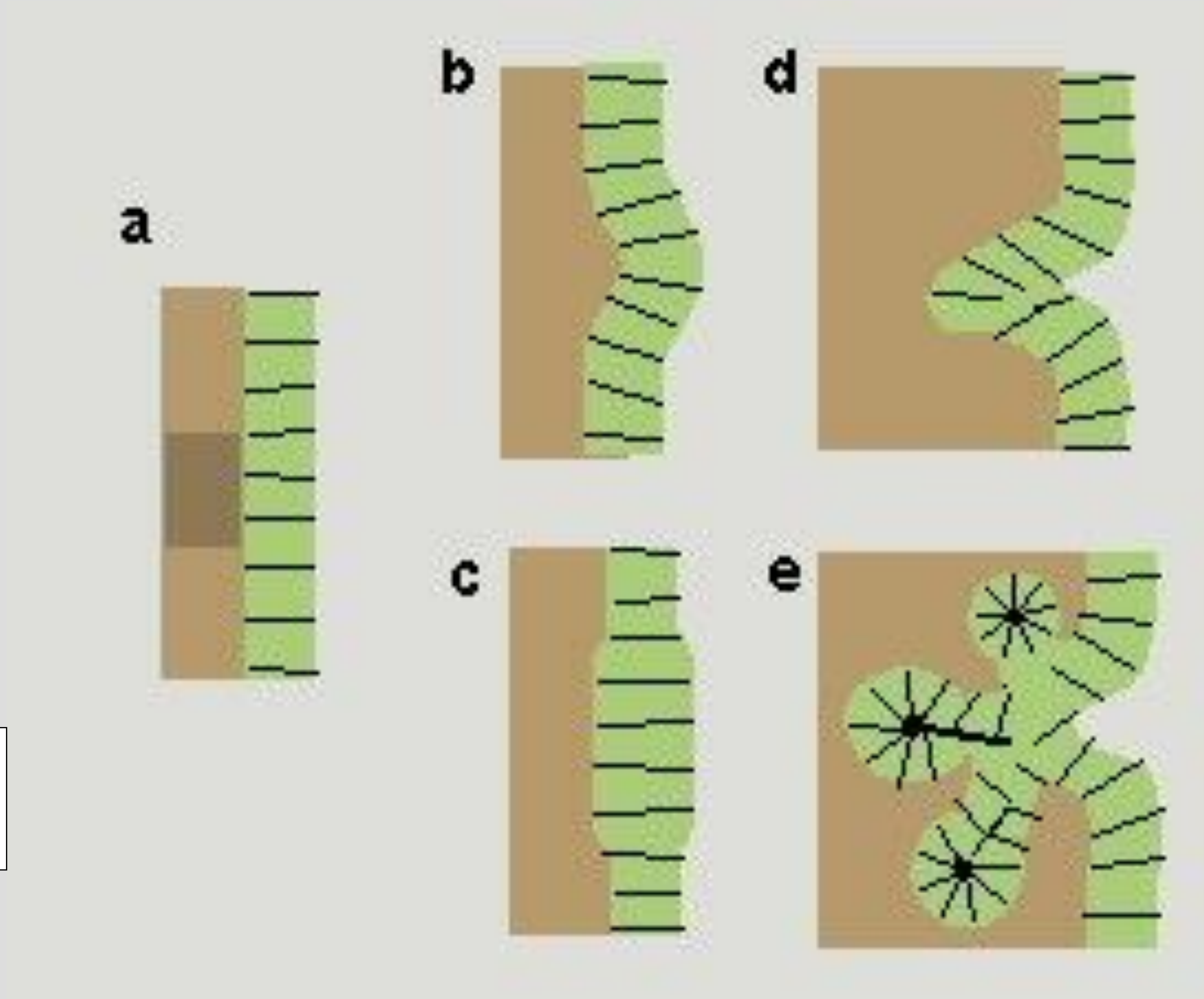
Gastrulation can involve buckling-like movements of tissue sheets (e.g., *Drosophila*)



Leptin, *In* Keller et al, eds., *Gastrulation*, Plenum; 1991

Epithelial morphogenesis by position-dependent modulation of stiffness and viscoelasticity (e.g., buckling, folding)

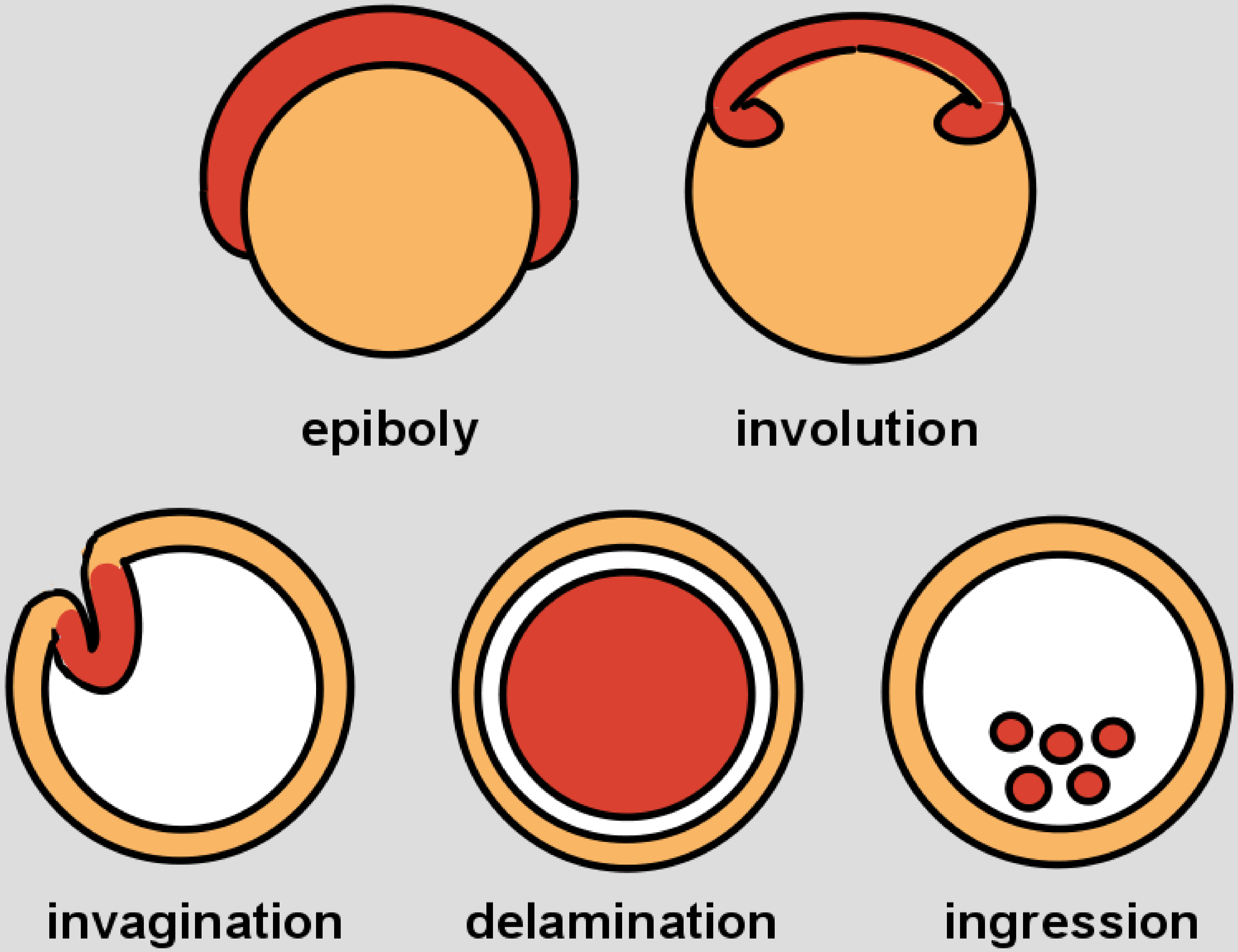
Physical analogue: dried paint



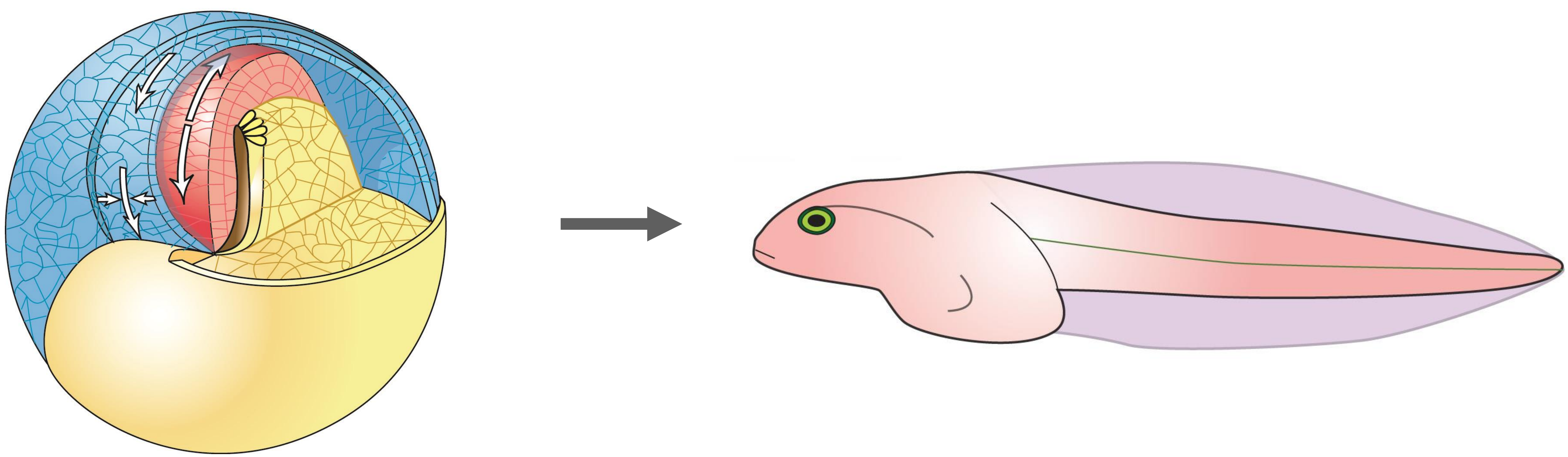
Toolkit molecules: collagen and other ECM molecules

Theory in: Gierer, *Q. Rev. Biophys*; 1977; Mittenthal and Mazo, *J. Theor. Biol*; 1983

Biogeneric bases of the origination of gastrulation: differential interfacial tension (phase separation and engulfment), ± cell polarity, ± epithelial folding, ± EMT

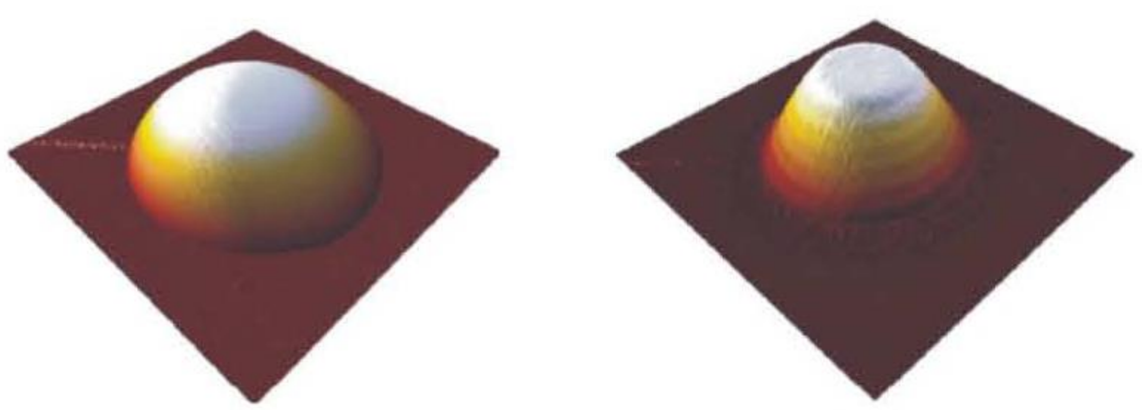


Embryos typically undergo elongation (e.g., during amphibian gastrulation, insect germ band extension)



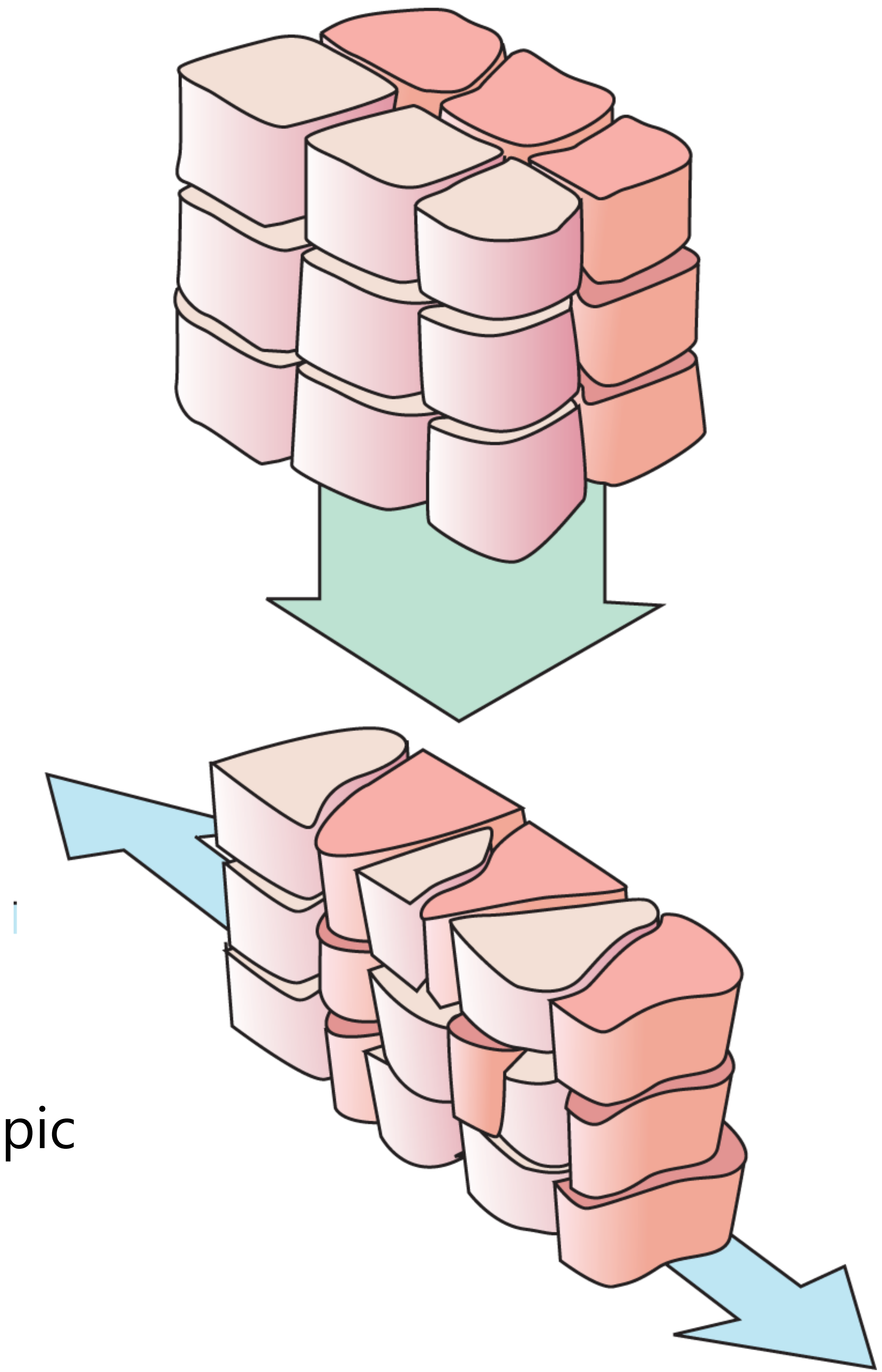
Forgacs & Newman, *Biological Physics of the Developing Embryo*; 2005 (After Keller et al., Philos Trans R Soc Lond B Biol Sci, 2000)

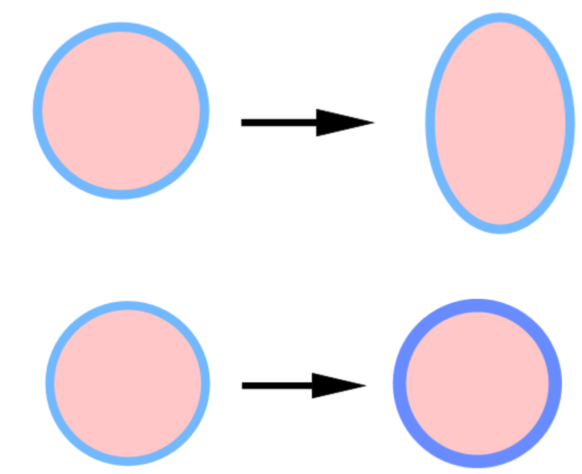
Tissue narrowing and elongation can arise from intercalation of (planar) polarized cells



Physical analogue: liquid crystal

Croll et al. Droplet shape of an anisotropic liquid. *PRL* 97, 204502; 2006





Toolkit molecules: noncanonical Wnt pathway; cadherins

Forgacs & Newman, *Biol Phys Develop Embryo*, 2005 (Based on Keller *et al. Philos. Trans. R. Soc. Lond.*, 2000)

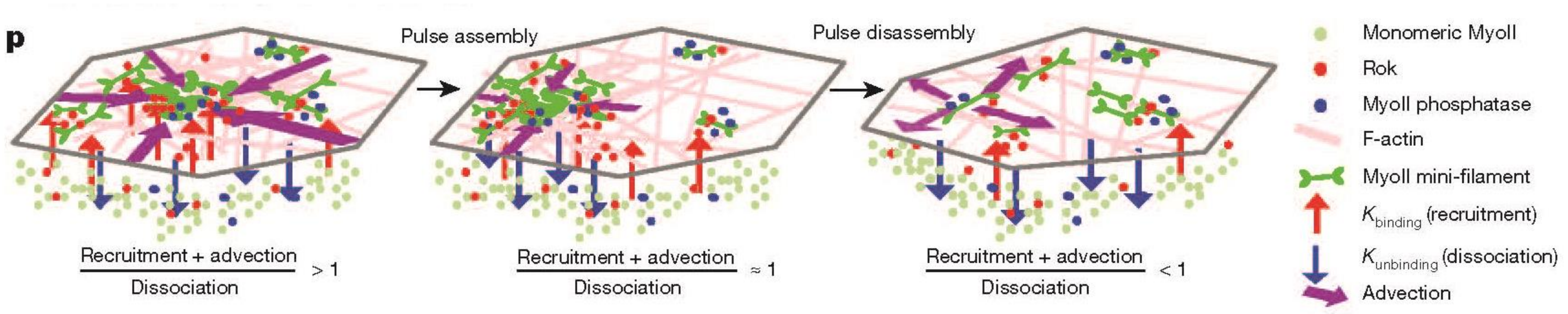
Planar cell polarity is *biogeneric*

20 AUGUST 2015 | VOL 524 | NATURE | 351

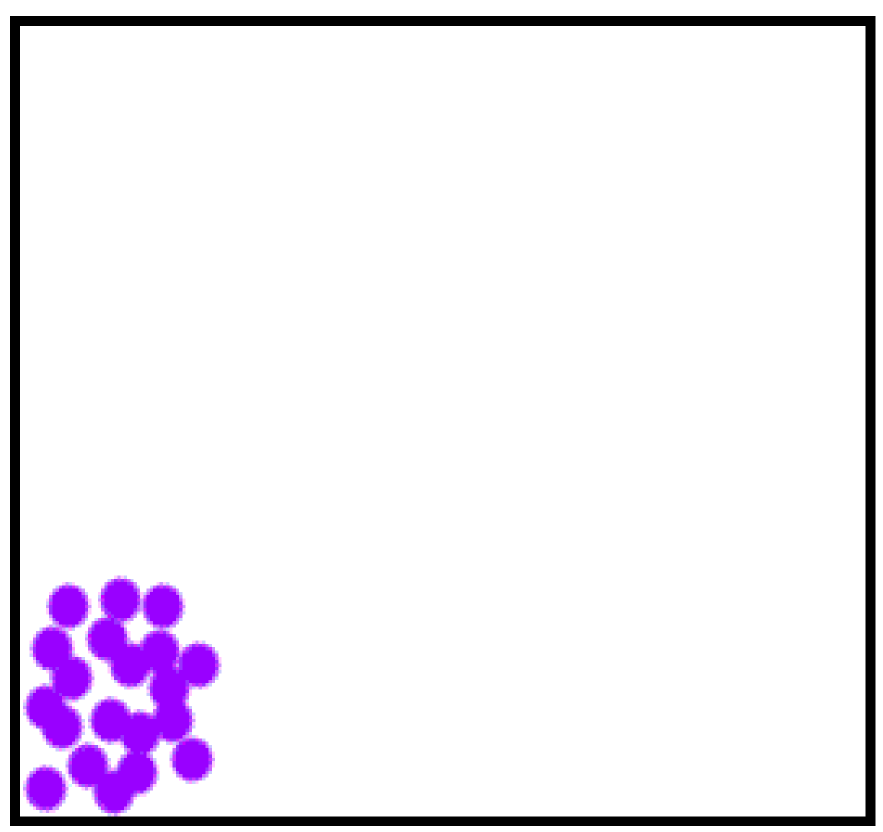
doi:10.1038/nature14603

A self-organized biomechanical network drives shape changes during tissue morphogenesis

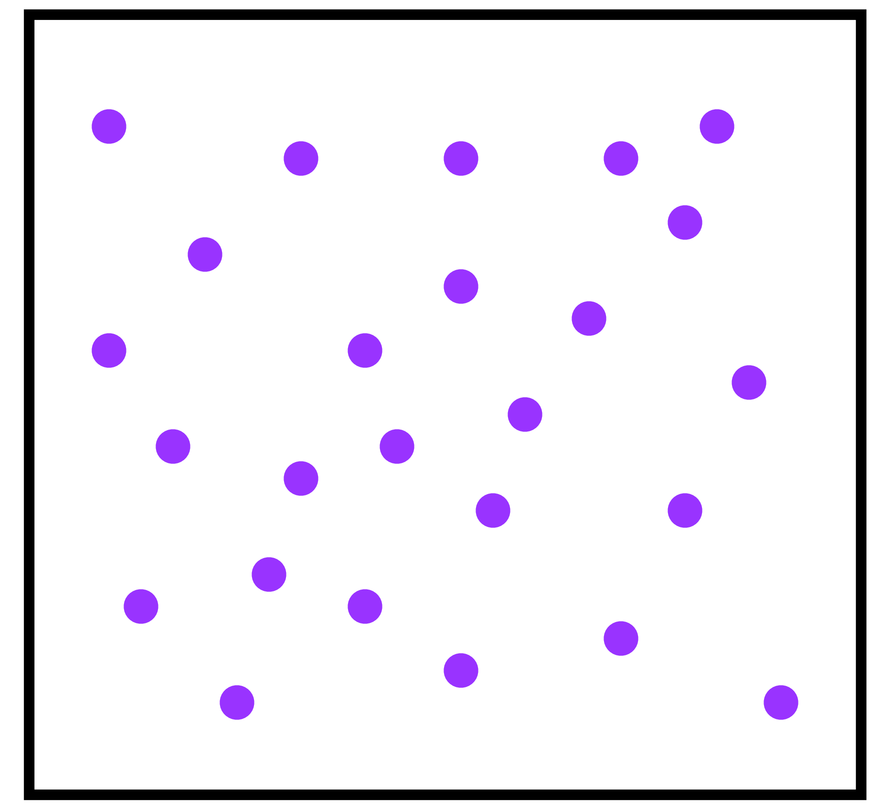
Akankshi Munjal[1], Jean-Marc Philippe[1], Edwin Munro[2] & Thomas Lecuit[1]

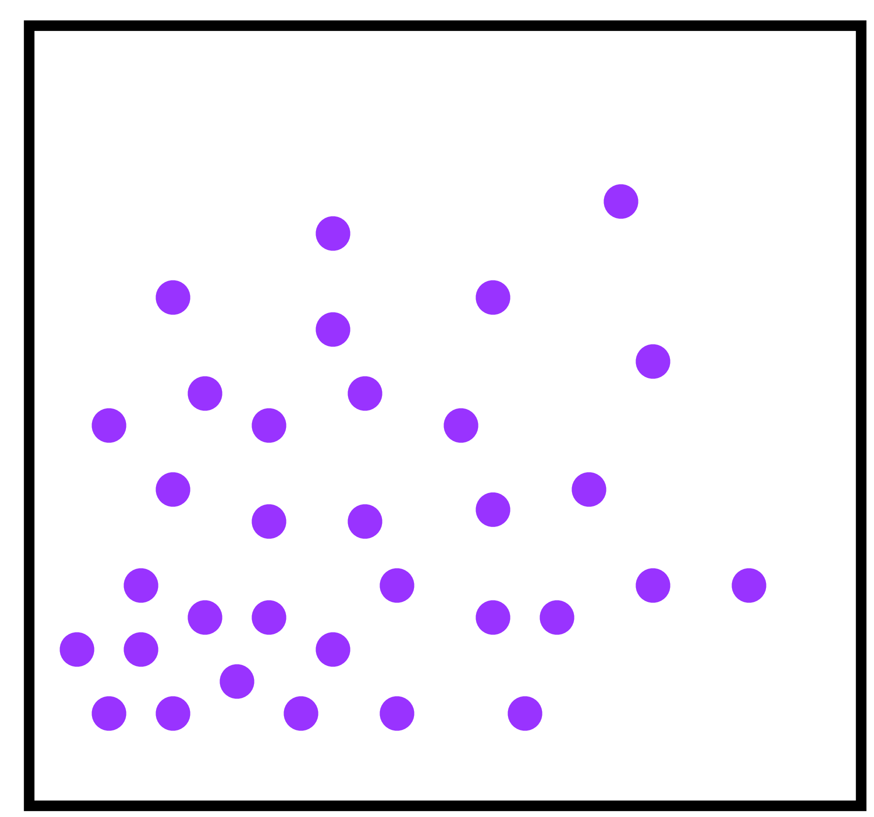


Diffusion, depending on rates, distances, etc., can…



even things out, or…

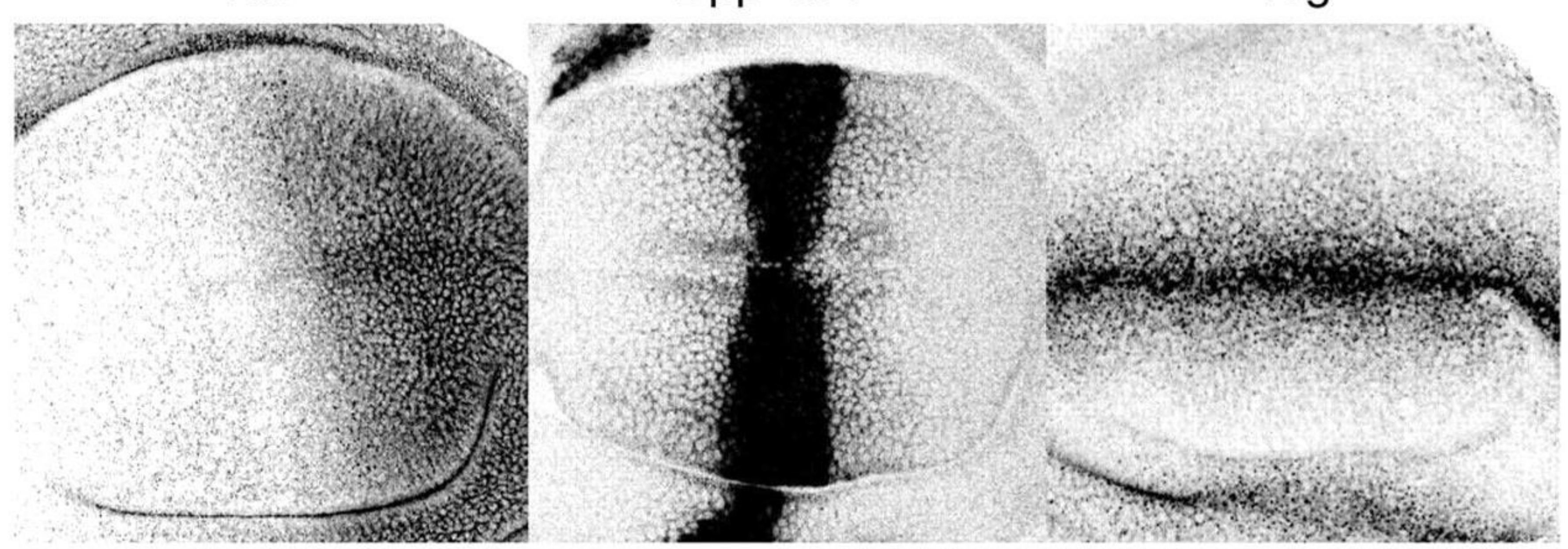




…produce gradients

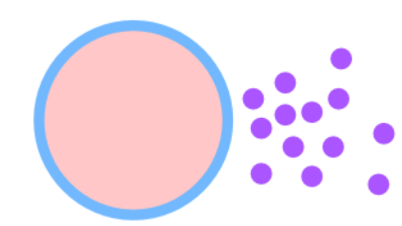
Pattern formation by morphogen gradients

Hedgehog (~Shh) Dpp (~BMP) Wg (~Wnt)



Drosophila imaginal disks

Tabata and Takei; *Development*, 2004



Toolkit molecules: Hh; FGF; Wnt; BMP/TGF-β

Generic diffusion

Vol 461|24 September 2009|doi:10.1038/nature08391

Fgf8 morphogen gradient forms by a source-sink mechanism with freely diffusing molecules

Shuizi Rachel Yu[1,3]*, Markus Burkhardt[2,3]*, Matthias Nowak[1,3], Jonas Ries[2,3], Zdeněk Petrášek[2,3], Steffen Scholpp[1]†, Petra Schwille[2,3] & Michael Brand[1,3]

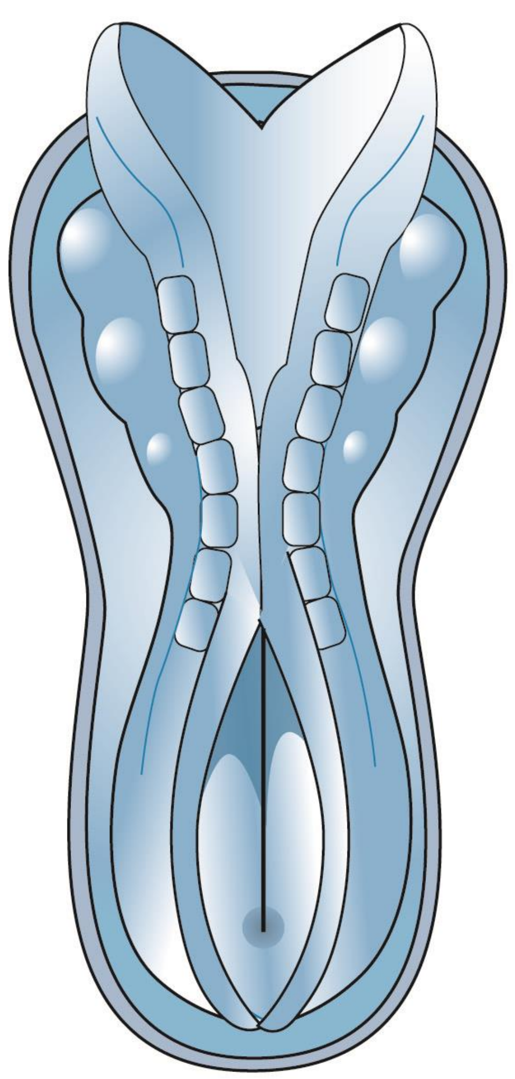
Biogeneric diffusion

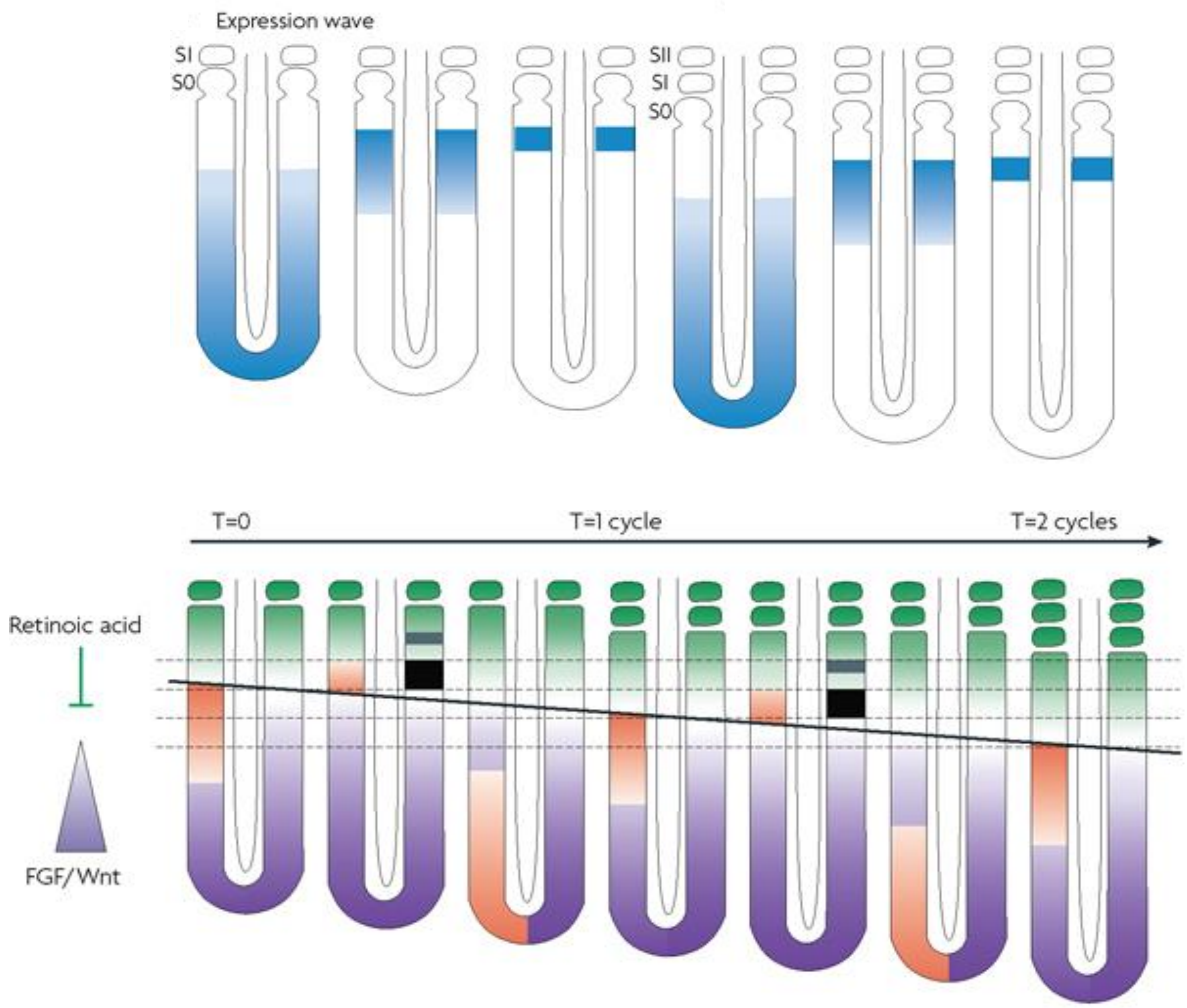
Current Biology *21*, 1391–1396, August 23, 2011

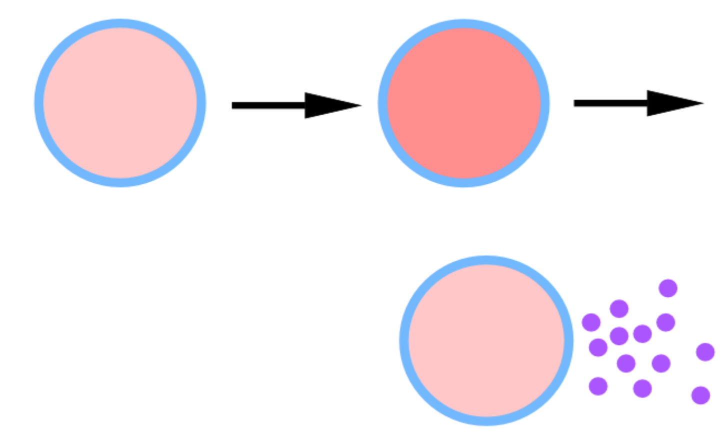
Expansion-Repression Mechanism for Scaling the Dpp Activation Gradient in *Drosophila* Wing Imaginal Discs

Danny Ben-Zvi,[1] George Pyrowolakis,[2,3] Naama Barkai,[1,4,*]
and Ben-Zion Shilo[1,*]
[1]Department of Molecular Genetics,
Weizmann Institute of Science, Rehovot 76100, Israel
[2]Institute for Biology I, Albert-Ludwigs-University of Freiburg
Hauptstrasse. 1, D-79104 Freiburg, Germany
[3]Centre for Biological Signaling Studies (BIOSS),
Albert-Ludwigs-University of Freiburg Albertstrasse. 19,
D-79104 Freiburg, Germany
[4]Department of Physics of Complex Systems,
Weizmann Institute of Science, Rehovot 76100, Israel

Somitogenesis results from biochemical oscillations operating in the presence of a morphogen gradient







<u>Toolkit</u>
<u>molecules</u>:
Notch; FGF/Wnt

Dequéant & Pourquié, 2008

Transition from a uniform state to hexagonal and striped Turing patterns

Q. Ouyang & Harry L. Swinney

Center for Nonlinear Dynamics and Department of Physics, The University of Texas, Austin, Texas 78712, USA

CHEMICAL travelling waves have been studied experimentally for more than two decades[1–5], but the stationary patterns predicted by Turing[6] in 1952 were observed only recently[7–9], as patterns localized along a band in a gel reactor containing a concentration gradient in reagents. The observations are consistent with a mathematical model for their geometry of reactor[10] (see also ref. 11). Here we report the observation of extended (quasi-two-dimensional) Turing patterns and of a Turing bifurcation—a transition, as a control parameter is varied, from a spatially uniform state to a patterned state. These patterns form spontaneously in a thin disc-shaped gel in contact with a reservoir of reagents of the chlorite–iodide–malonic acid reaction[12]. Figure 1 shows examples of the hexagonal, striped and mixed patterns that can occur. Turing patterns have similarities to hydrodynamic patterns (see, for example, ref. 13), but are of particular interest because they possess an intrinsic wavelength and have a possible relationship to biological patterns[14–17].

The reaction medium is a 2.0-mm-thick polyacrylamide gel disk (25.4-mm diameter), which is sandwiched between two 0.4-mm-thick porous glass disks (Vycor glass, Corning); similar reactors have been described previously[5,18]. The gel was prepared by the procedure in ref. 7. The gel and glass disks are transparent; the pattern can therefore be detected optically. The outer flat surface of each glass disk is in contact with a chemical reservoir, where reactant concentrations are kept constant and uniform by mixing and a continuous flow of fresh reagents. Components of the chlorite-iodide-malonic acid reaction[12] are distributed in the two reservoirs in such a way that neither is separately reactive. The chemicals diffuse through the porous glass disks into the gel where the reaction occurs. The gel, loaded with a soluble starch indicator (Thiodène, Prolabo), changes colour from yellow to blue with changes in concentration of I_3^- during the redox reaction. No starch is present in the porous glass; thus concentration changes in the glass disks are not visible. The pattern is monitored in transmitted light (580 nm) with a video camera.

Beyond critical values of the control parameters (chemical concentrations and temperature), patterns emerge spontaneously from an initially uniform background. Initially, after the parameters are switched into a regime where patterns arise,

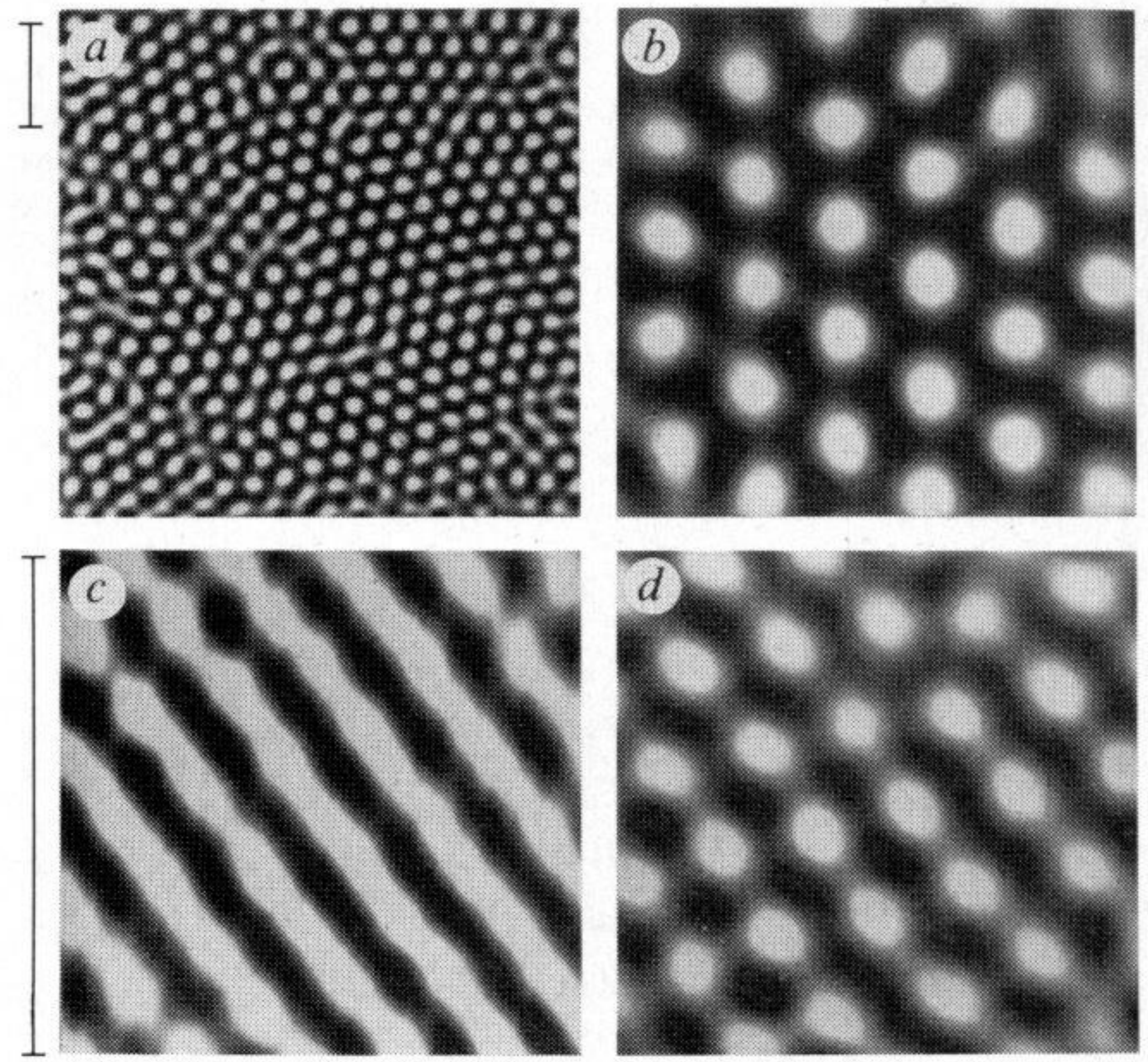
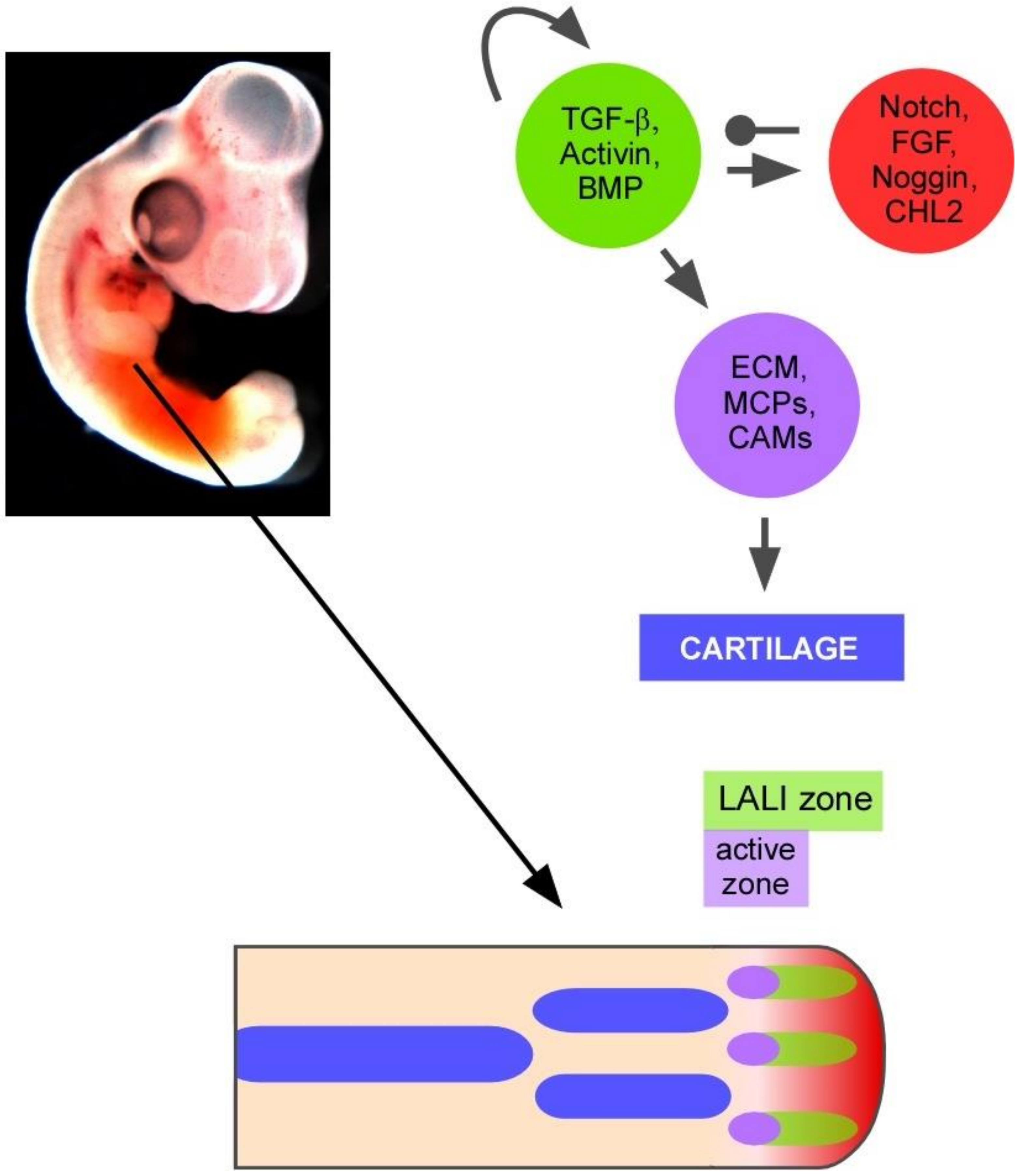


FIG. 1 Stationary chemical patterns formed in a continuously fed laboratory reactor. *a*, *b*, Hexagons; *c*, stripes; *d*, mixed state. The bar beside each picture represents 1 mm; the reactor is 25 mm in diameter. The concentrations in reservoirs A and B on the two sides of the reactor were: $[I^-]_0^A$, $[I^-]_0^B$, $[CH_2(COOH)_2]_0^B$ (in 10^{-3}M) in *a* and *d*, 3.0, 3.0, 13; in *b*, 3.5, 3.5, 8.3; in *c*, 5.0, 5.0, 8.3 (for the conditions given, the mixed state in *d* coexists with the hexagons in *a*). The parameters common to all observed patterns were: $[NaOH]_0^A = [NaOH]_0^B = 3.0 \times 10^{-3}$ M, $[Na_2SO_4]_0^A = [Na_2SO_4]_0^B = 3.0 \times 10^{-3}$ M, $[ClO_2^-]_0^A = 1.8 \times 10^{-2}$ M, $[ClO_2^-]_0^B = 0$, $[CH_2(COOH)_2]_0^A = 0$, $[H_2SO_4]_0^A = 2.0 \times 10^{-3}$ M, $[H_2SO_4]_0^B = 1.0 \times 10^{-2}$ M, temperature 5.6 °C.

Turing network for limb skeletal pattern formation



Zhu *et al.*, *PLoS One*; 2010;
Glimm *et al.*, *J Theor Biol.*, 2014

A *biogeneric* Turing-type mechanism

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Modeling the morphodynamic galectin patterning network of the developing avian limb skeleton

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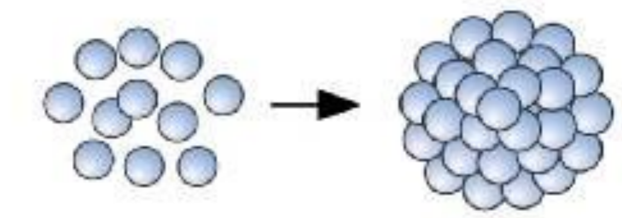
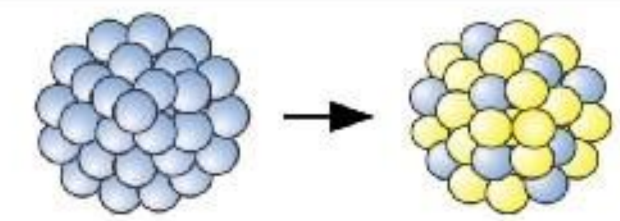
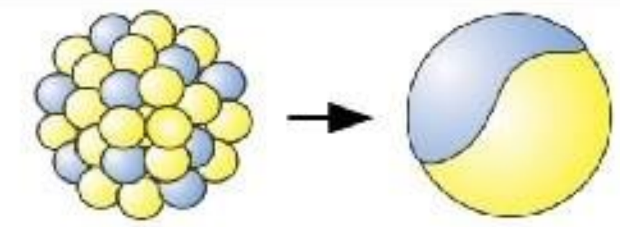
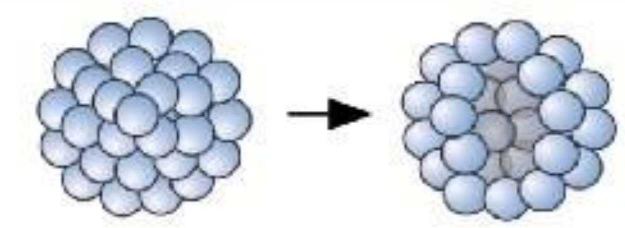
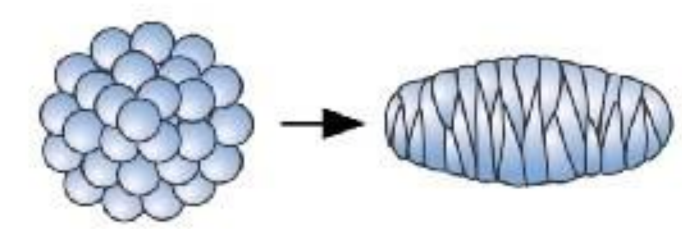
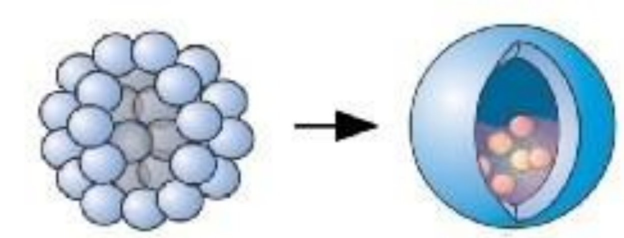
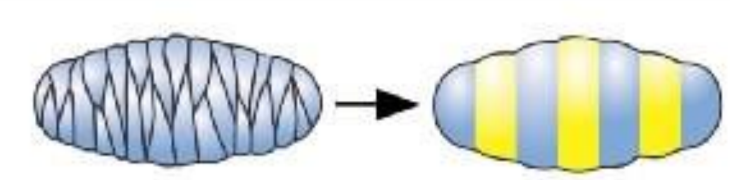
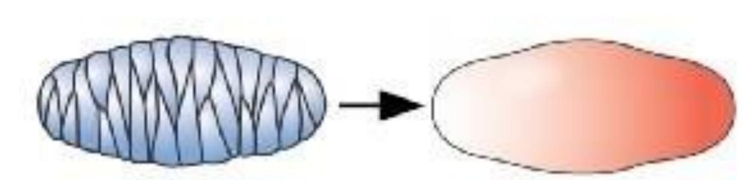
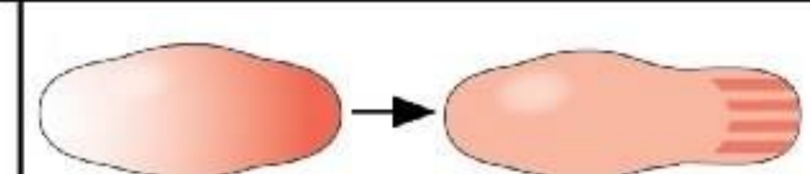
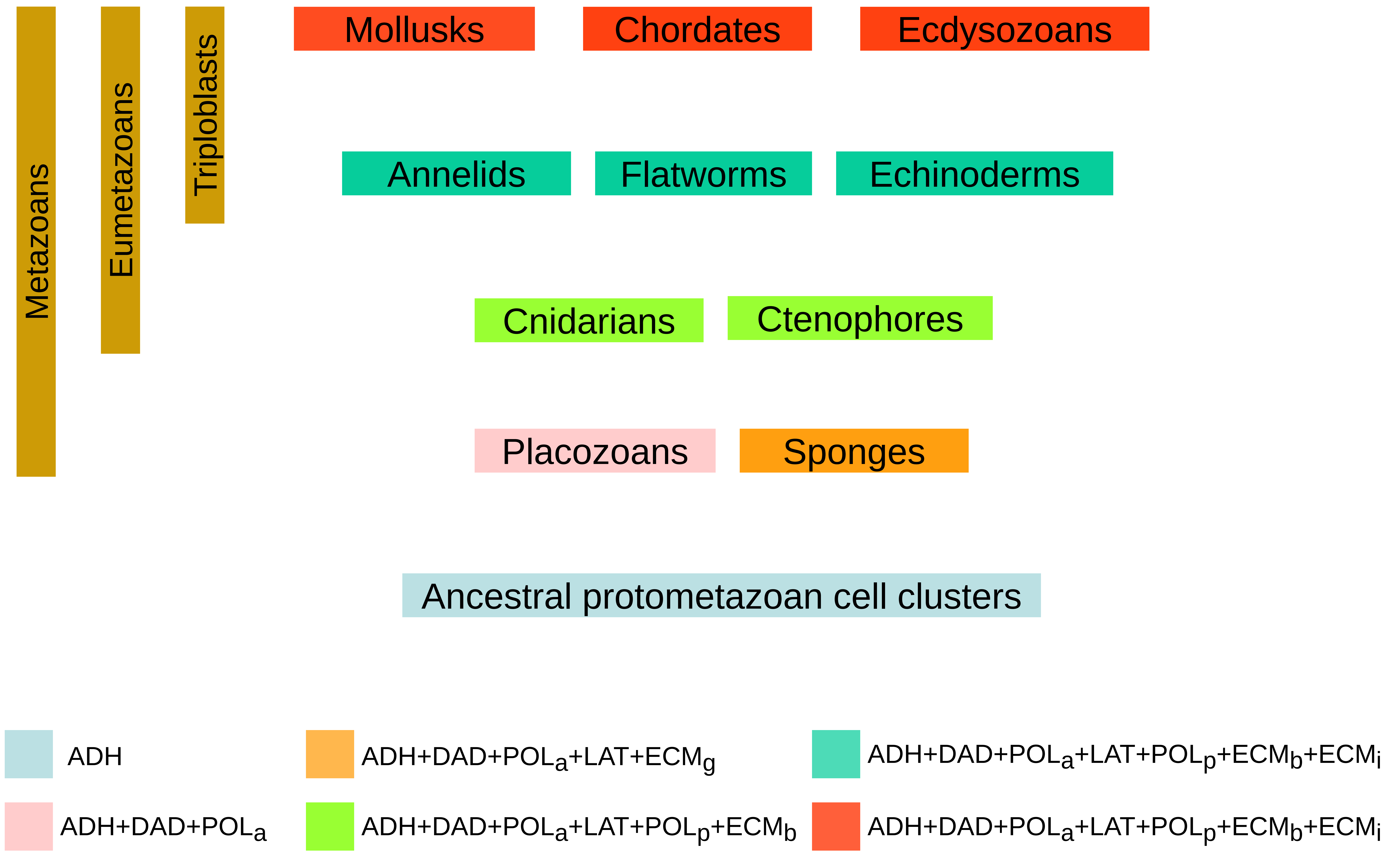
ABSTRACT

We present a mathematical model for the morphogenesis and patterning of the mesenchymal condensations that serve as primordia of the avian limb skeleton. The model is based on the experimentally established dynamics of a multiscale regulatory network consisting of two glycan-binding proteins expressed early in limb development: CG (chicken galectin)-1A, CG-8 and their counterreceptors that determine the formation, size, number and spacing of the "protocondensations" that give rise to the condensations and subsequently the cartilaginous elements that serve as the templates of the bones. The model, a system of partial differential and integro-differential equations containing a flux term to represent local adhesion gradients, is simulated in a "full" and a "reduced" form to confirm that the system has pattern-forming capabilities and to explore the nature of the patterning instability. The full model recapitulates qualitatively and quantitatively the experimental results of network perturbation and leads to new predictions, which are verified by further experimentation. The reduced model is used to demonstrate that the patterning process is inherently morphodynamic, with cell motility being intrinsic to it. Furthermore, subtle relationships between cell movement and the positive and negative interactions between the morphogens produce regular patterns without the requirement for activators and inhibitors with widely separated diffusion coefficients. The described mechanism thus represents an extension of the category of activator–inhibitor processes capable of generating biological patterns with repetitive elements beyond the morphostatic mechanisms of the Turing/Gierer–Meinhardt type.

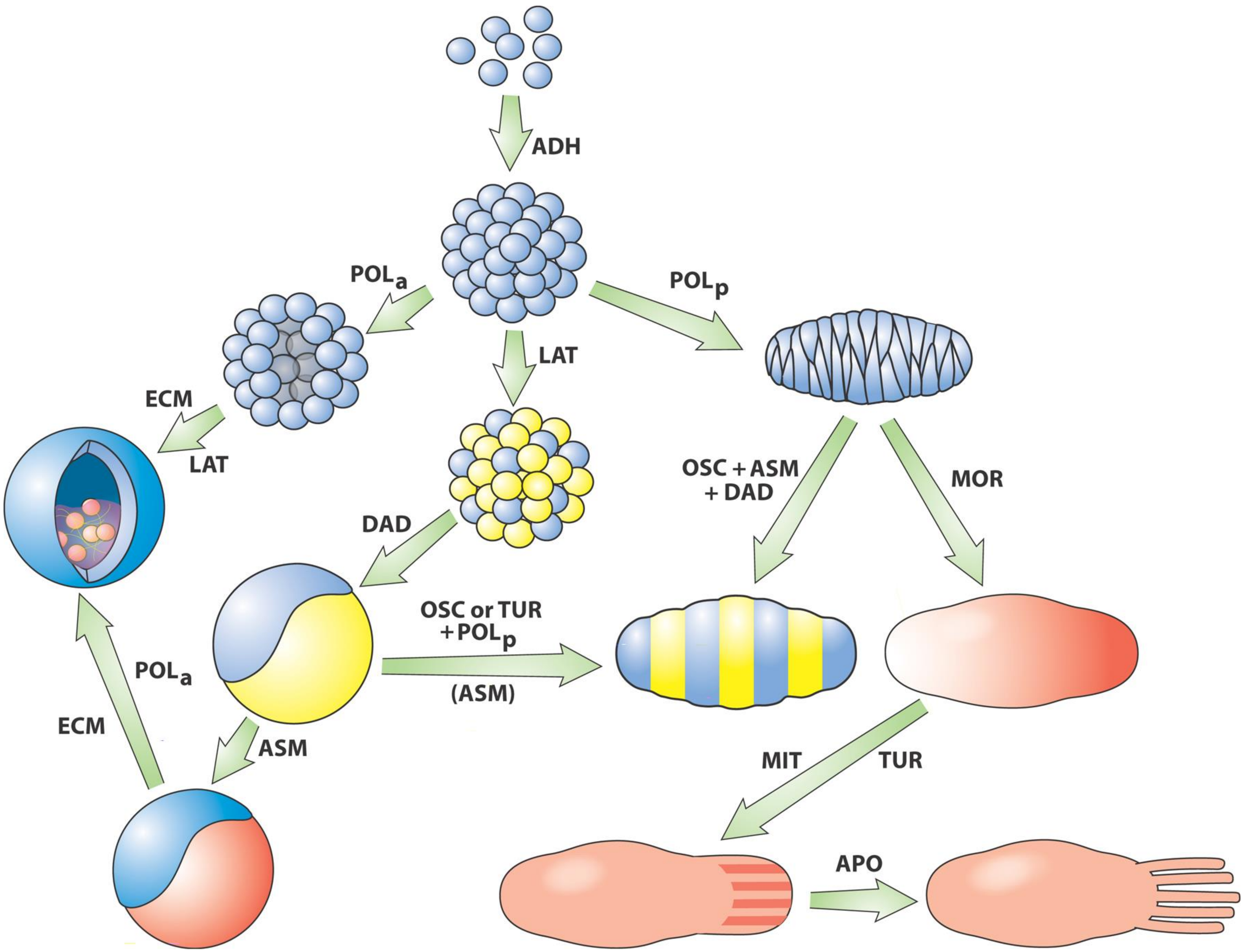
DPM	molecules	physics	evo-devo role	effect
ADH	cadherins	adhesion	multicellularity	
LAT	Notch	lateral inhibition	coexistence of alternative cell states	
DAD	cadherins	differential adhesion	phase separation; tissue multilayering	
POL_a	Wnt	cell surface anisotropy	topological change; interior cavities	
POL_p	Wnt	cell shape anisotropy	tissue elongation	
ECM	chitin; collagen	stiffness; dispersal	tissue solidification; elasticity; EMT	
OSC	Wnt + Notch	**synchrony of oscillation**	**morphogenetic fields; segmentation**	
MOR	TGF-β/BMP; FGF; Hh	diffusion	pattern formation	
TUR	MOR + Wnt + Notch	dissipative structure	segmentation; periodic patterning	

Newman and Bhat, *Phys. Biol.*; 2008

Metazoan morphological complexity tracked successive addition of DPMs over the course of evolution



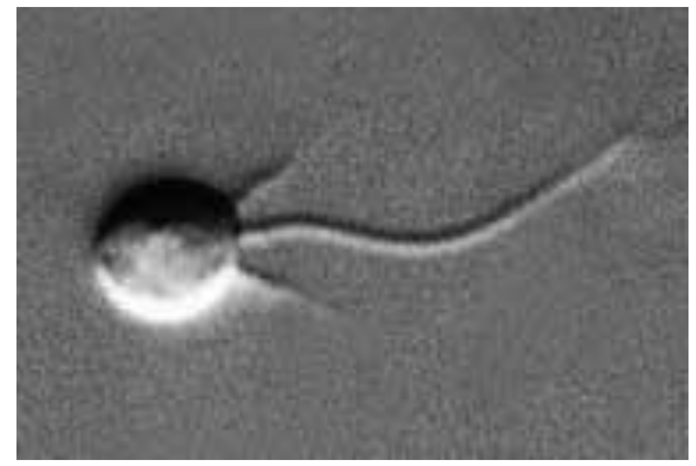
Combinatorial use of DPMs in the origination of animal body plans



Newman & Bhat, *Int J Dev Biol*; 2009

Presence of DPM-associated toolkit genes in choanozoans and metazoans

Choanozoa:
(unicellular sister clade of Metazoa)



ADH; DAD: cadherins; C-type lectins
LAT: Notch (only some species)
MOR: Hh
ECM: collagen

All of above, plus
POL: Wnt
no Notch

Placozoa Porifera
("basal" metazoans)

All of above, plus
MOR: TGF-β
POL: Wnt

Cnidaria:
(eumetazoans)

All of the above, plus
MOR: FGFs

Arthropods; chordates:
(Bilateria)

All of the above

Different combinations of non-collagenous ECM and matricellular proteins

The physico-genetic hypothesis implies that characteristic body plan motifs first appear (evolutionarily and developmentally) at the “morphogenetic stage”

Definition: The stage of development at which the embryo consists of a cluster of several dozen or more cells of equal size and shape.

Examples: The blastula of a sea urchin or amphibian, the blastoderm of an insect, the inner cell mass of a mammal.

DPMs are implemented at the morphogenetic stage

Evolutionary Biology
https://doi.org/10.1007/s11692-018-9448-9

SYNTHESIS PAPER

Are Planaria Individuals? What Regenerative Biology is Telling Us About the Nature of Multicellularity

Chris Fields[1] · Michael Levin[2]

Received: 26 November 2017 / Accepted: 6 March 2018

Abstract
Freshwater planaria (Platyhelminthes, Turbellaria, Tricladida) pose a challenge to current concepts of biological individuality. We review molecular and developmental evidence suggesting that mature intact planaria are not biological individuals but their totipotent stem cells (neoblasts) are individuals. Neoblasts within a single planarian body are, in particular, genetically heterogeneous, migratory, effectively immortal, and effectively autonomous. They cooperate to maintain the planarian body as an obligate environment but compete to make this environment maximally conducive to the survival of their own neoblast lineages. These results suggest that planaria have not fully completed the transition to multicellularity, but instead represent an intermediate form in which a small number of genetically-heterogeneous, reproductively-competent cells effectively "farm" their reproductively-incompetent offspring.

Keywords Bioelectricity · Cooperation · *Dugesia japonica* · *Dugesia ryukyuensis* · Germ cells · *Girardia tigrina* · Regeneration · *Schmidtea mediterranea* · Stem cells

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Review

Why are there eggs?

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A R T I C L E   I N F O

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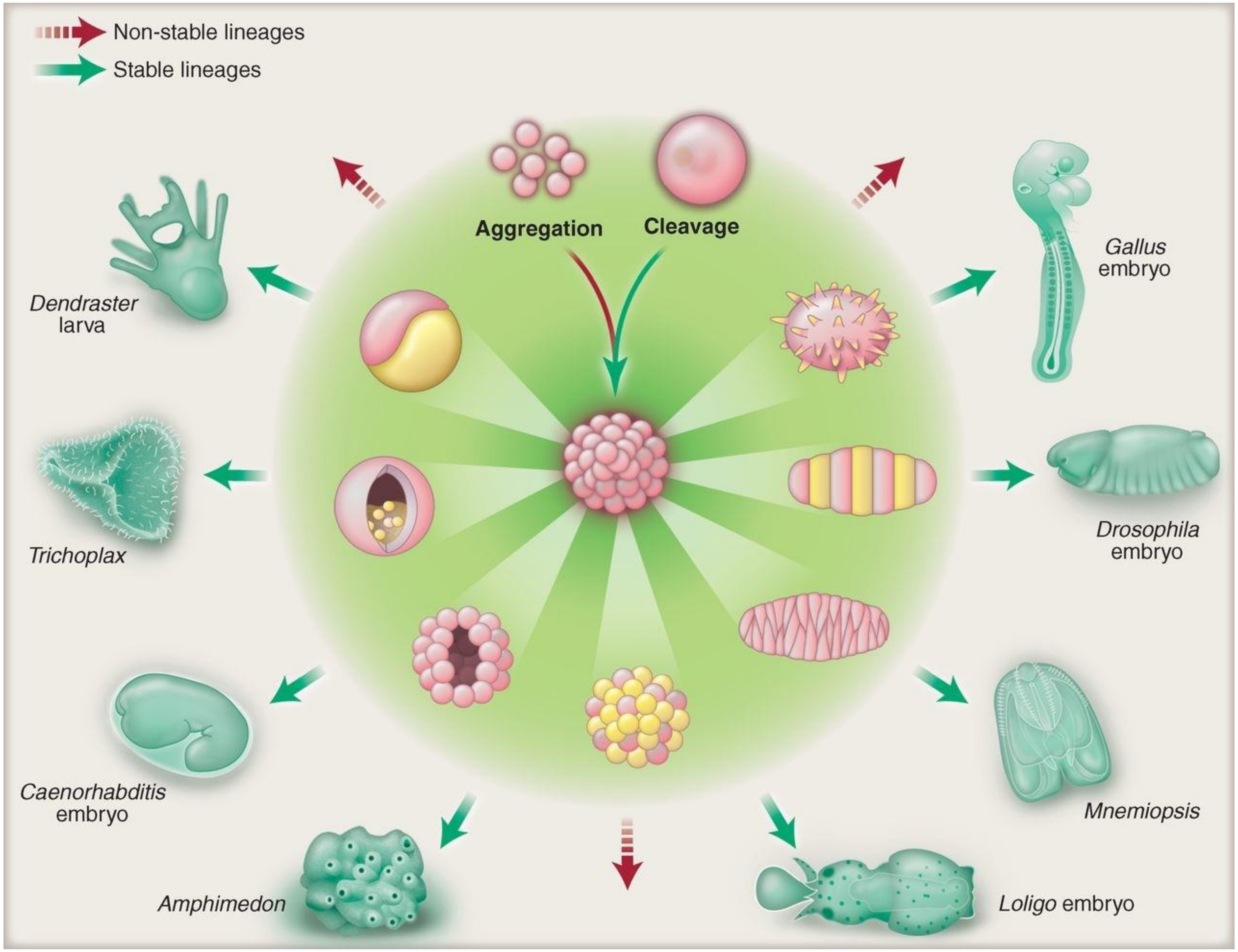
Keywords:
Dynamical patterning modules
Egg-patterning processes
Ooplasmic segregation
Pre-adaptation
Evo-devo

A B S T R A C T

A description and update of the "egg-as-novelty" hypothesis is presented. It is proposed that the major animal phylum-characteristic suites of morphological motifs first emerged more than a half-billion years ago in multicellular aggregates and clusters that did not exhibit an egg-soma divergence. These pre-metazoan bodies were organized by "dynamical patterning modules" (DPMs), physical processes and effects mobilized on the new multicellular scale by ancient conserved genes that came to mediate cell–cell interactions in these clusters. "Proto-eggs" were enlarged cells that through cleavage, or physical confinement by a secreted matrix, served to enforce genomic and genetic homogeneity in the cell clusters arising from them. Enlargement of the founder cell was the occasion for spontaneous intra-egg spatio-temporal organization based on single-cell physiological functions – calcium transients and oscillations, cytoplasmic flows – operating on the larger scale. Ooplasmic segregation by egg-patterning processes, while therefore not due to adaptive responses to external challenges, served as evolutionarily fertile "pre-adaptations" by making the implementation of the later-acting (at the multicellular "morphogenetic stage" of embryogenesis) DPMs more reliable, robust, and defining of sub-phylum morphotypes. This perspective is seen to account for a number of otherwise difficult to understand features of the evolution of development, such as the rapid diversification of biological forms with a conserved genetic tool-kit at the dawn of animal evolution, the capability of even obligatory sexual reproducers to propagate vegetatively, and the "embryonic hourglass" of comparative developmental biology.

Two routes to metazoan-type morphogenesis: cell aggregation or cleavage of an enlarged cell



S. Newman, *Science;* 2012

Origination of multicellular body plans and organs according to the physico-genetic hypothesis

- Multicellular clusters arose by cell aggregation or failure to separate post-mitosis, due to mutations or environmental change, independently of adaptive advantage.
- Phylotypic morphological motifs arose in cell clusters also independently of adaptation, by primitive developmental processes based on **DPMs**, morphogenetic-stage mobilization of *biogeneric* mesoscale physical effects by the products of the interaction toolkit genes.
- "Proto-metazoans" that propagated by cleavage of enlarged cells (proto-eggs) were more evolutionary stable, due to genetic uniformity of their multicellular progeny, than similar forms that propagated by aggregation.

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