Cause and Effect in the Interaction between Embryogenesis and the Genome

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By

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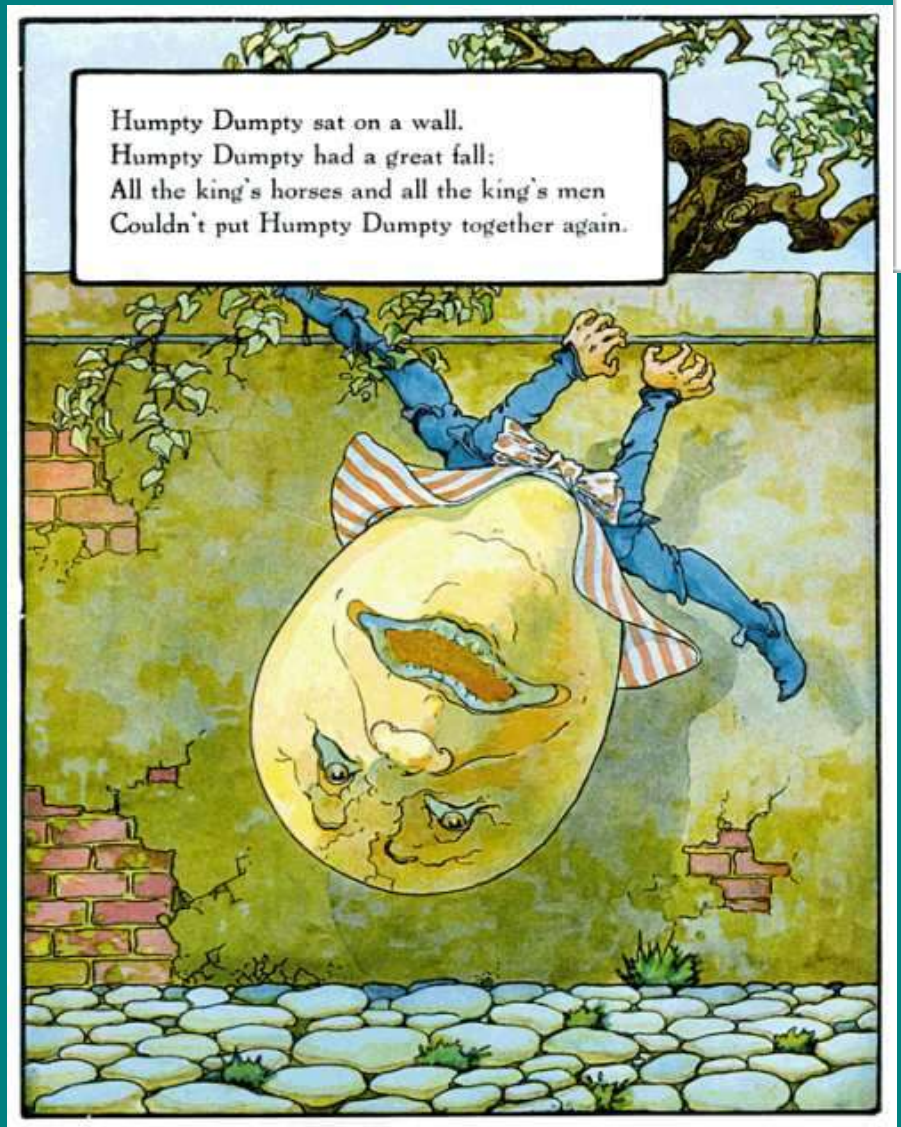
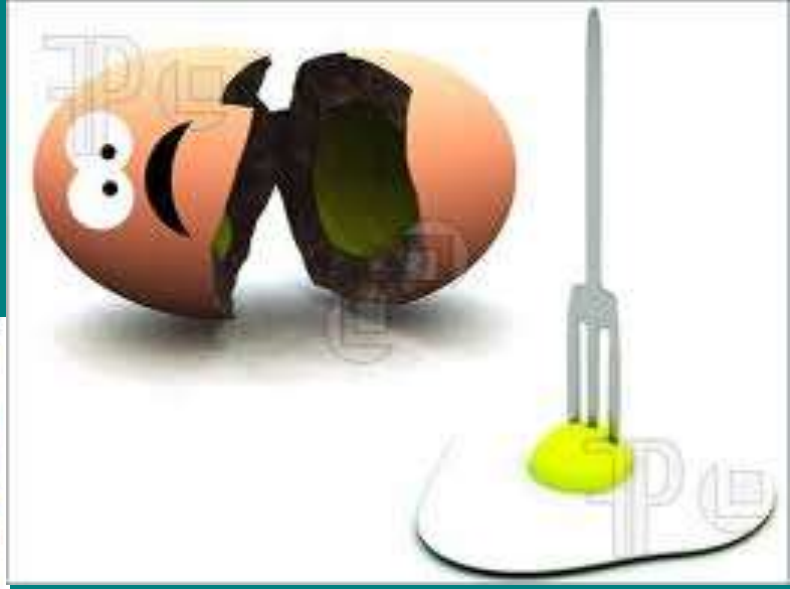
Consider a Spherical Cow…

But Cows are Spherical

http://reviews.presidentschoice.ca/6584/F19843/reviews.htm
http://www.sexingtechnologies.com/articles/in_vitro_fertilization
http://www.phgfoundation.org/news/2798/

Let's scramble eggs to make sure they are spherically symmetrical



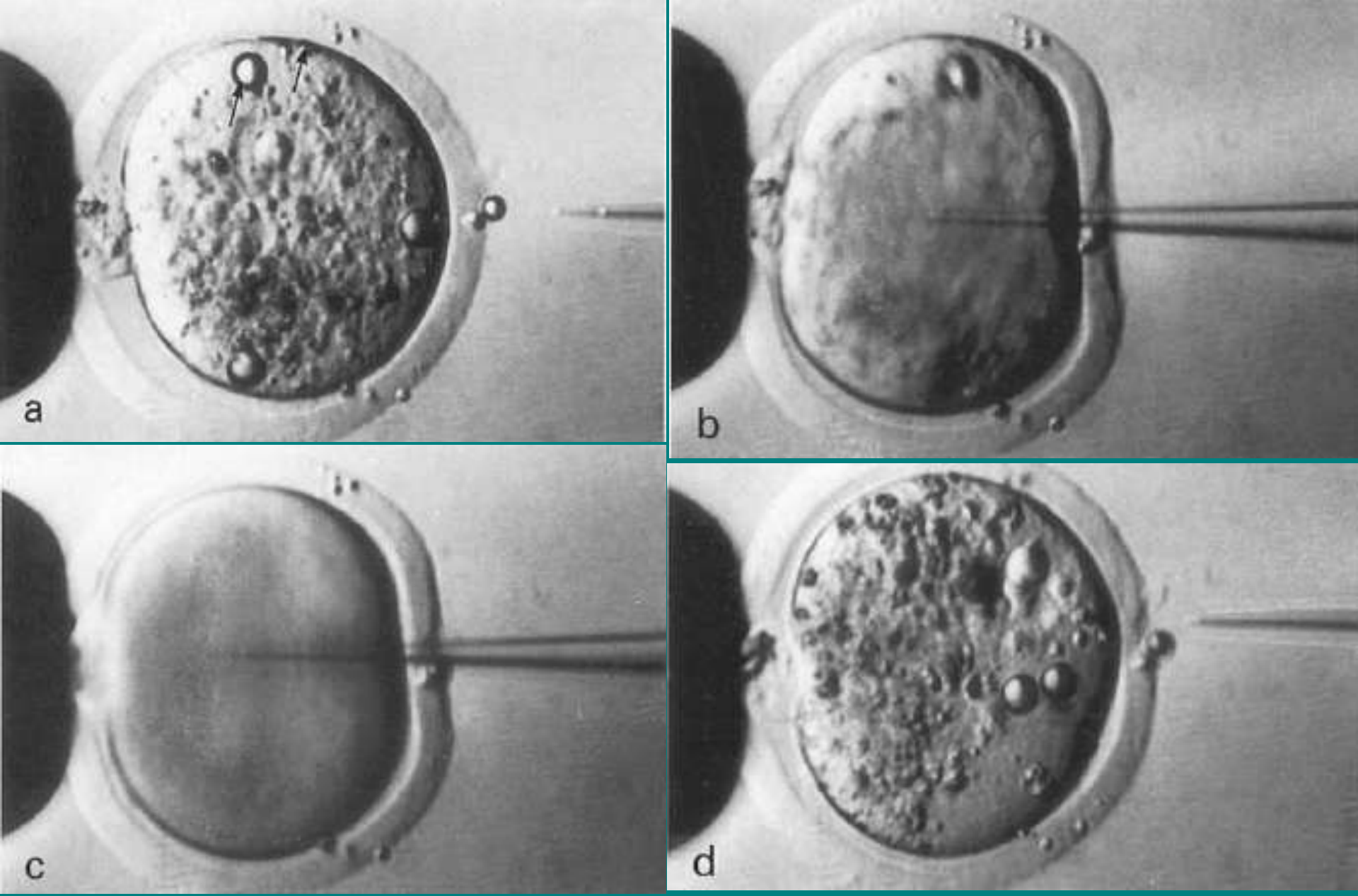


http://www.nurseryrhymesonline.com/humpty_dumpty_illustrated_by_frederick_richardson-3288.php
http://www.featurepics.com/online/Broken-Egg-Illustrations266617.aspx
http://www.tomatocasual.com/2011/01/24/tomato-basil-scrambled-eggs/

But Scrambled Eggs Develop Normally

- When the cytoplasm of a mouse egg was stirred around by Sergei Evsikov, using a miniature egg beater (actually a microscopic piezoelectric stirrer), while he was still in the Ukraine, he got a normal mouse.

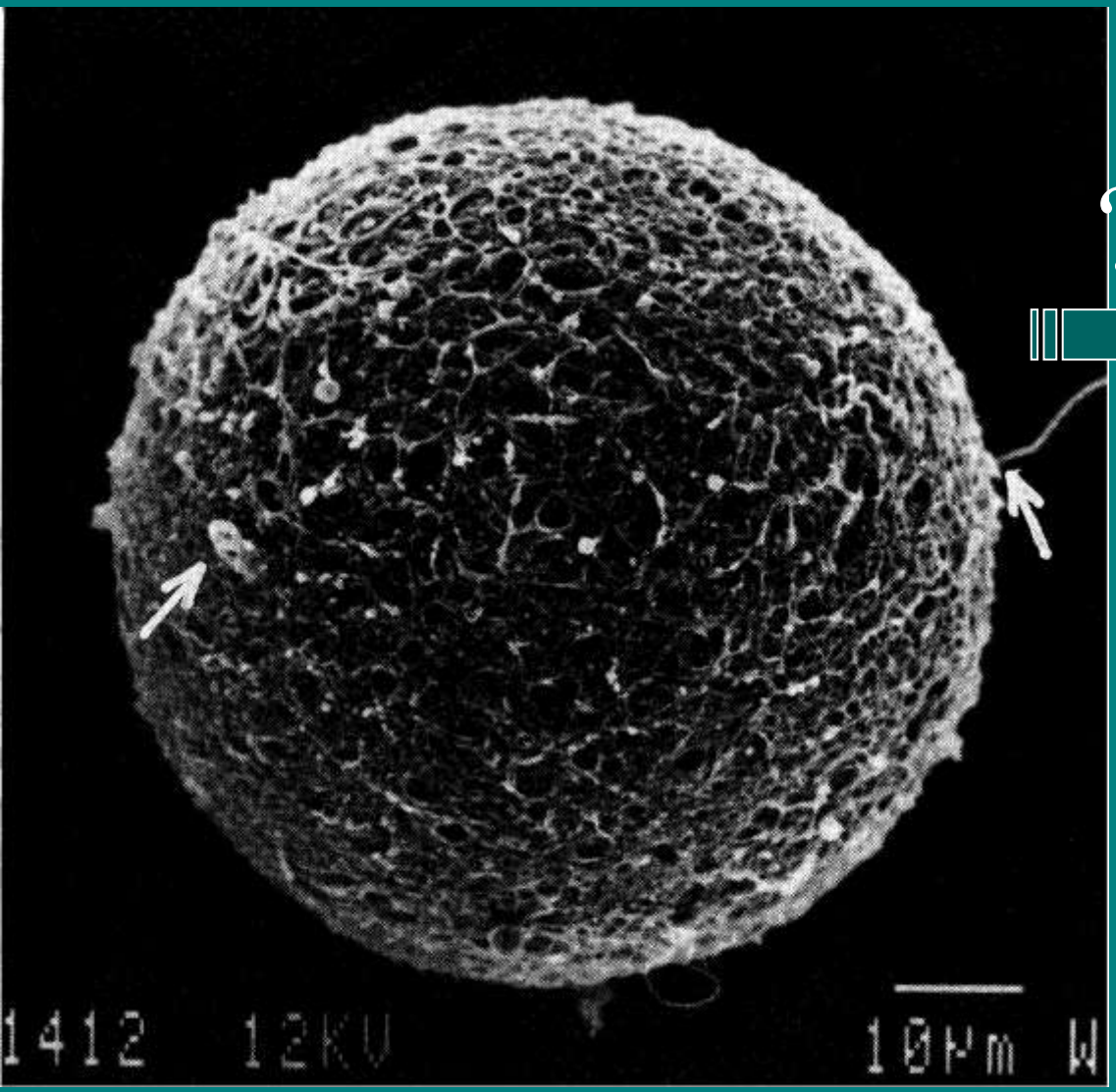
Evsikov, S.V., L.M. Morozova & A.P. Solomko (1994). Role of ooplasmic segregation in mammalian development. *Roux's Arch. Dev. Biol.* **203**, 199-204.



"Oil droplets *(arrows), injected beneath the plasma membrane* mark the peripheral regions of the cytoplasm, (b, e) Scrambling of the zygote cytoplasm, d Zygote immediately after cytoplasm scrambling." Evsikov et al. (1994)

No one else has pursued the question of the spherical symmetry of mammalian eggs

The Problem



?

1,000,000 µm = 1 meter

Egon Schiele, Kneeling Male Nude (Self-Portrait). 1910. http://www.moma.org/exhibitions/schiele/artistwork.html

How did your spherically symmetrical egg turn into your highly asymmetrical shape?

Nikas, G., T. Paraschos, A. Psychoyos & A.H. Handyside (1994). The zona reaction in human oocytes as seen with scanning electron microscopy. *Hum. Reprod.* **9**(11), 2135-2138.

Recent invitation to write:

- Gordon, R. 2011. The dilemmas of hierarchical instabilities in Turing's morphogenesis. In *The Once and Future Turing - Computing the World [in press]*. S. B. Cooper and A. Hodges (eds.): Cambridge University Press.

2012 THE ALAN TURING YEAR

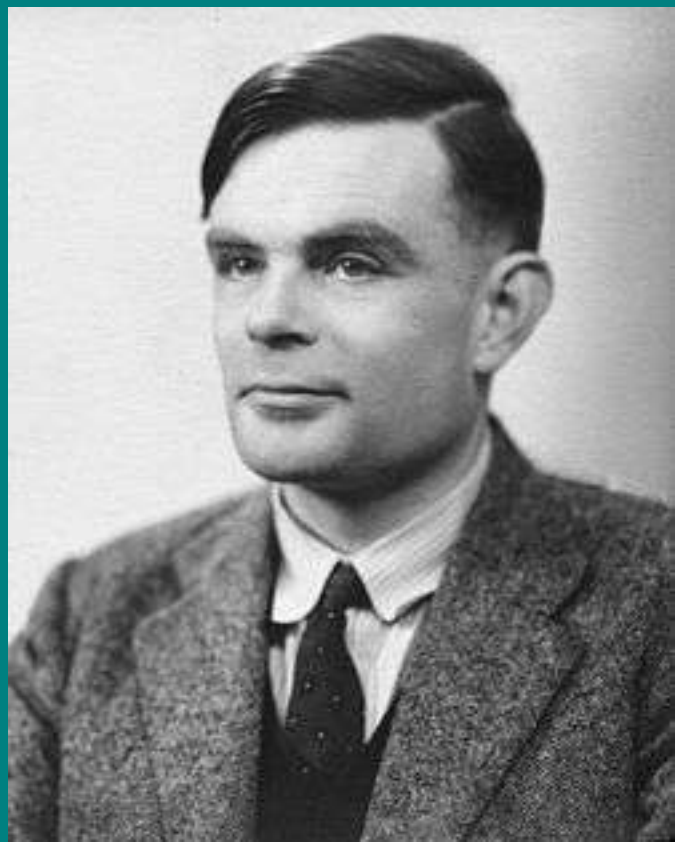
A Centenary Celebration of the Life and Work of Alan Turing

http://www.mathcomp.leeds.ac.uk/turing2012/give-page.php?302

Seminal papers:

Turing, A.M. (1937). On computable numbers, with an application to the Entscheidungsproblem. *Proc. London Math. Soc.* ***s2-42, 230-265.***

Turing, A.M. (1952). The chemical basis of morphogenesis. *Phil. Trans. Roy. Soc. London* ***B237, 37-72.***



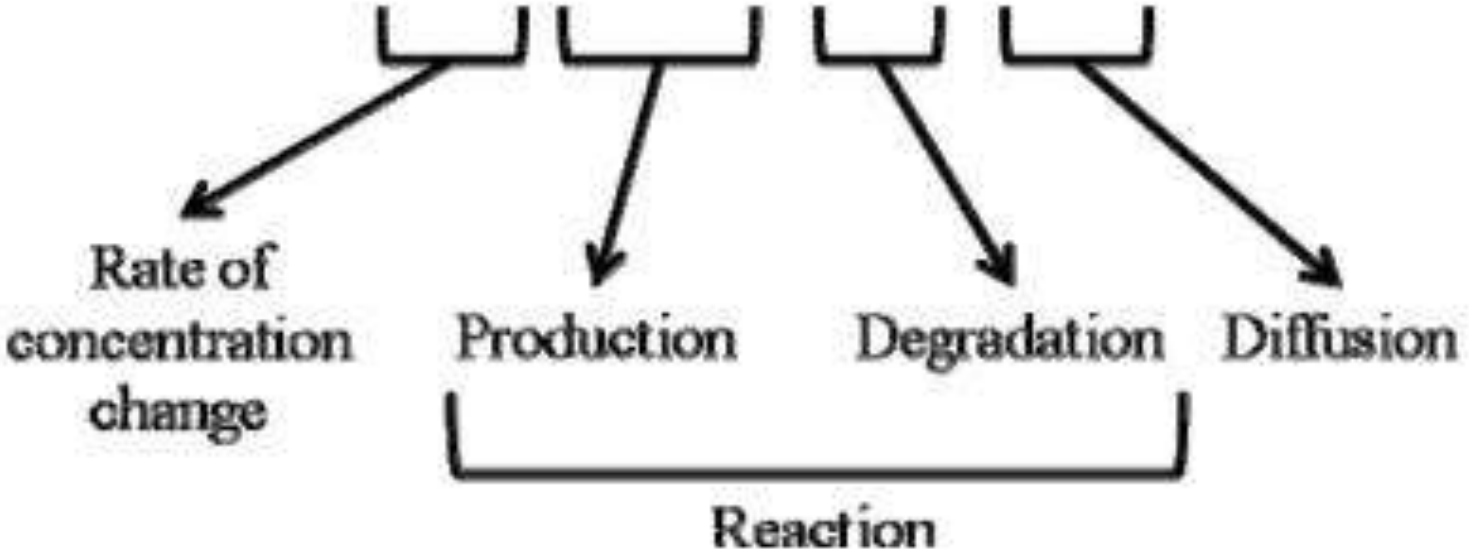
Bridging the Gap

- Alan Turing made a major contribution in putting forth a model for morphogenesis that attempts to bridge the gap between the molecules we are made of and how we look
- Volumes: 70 liters for an adult human and 0.15 nm^3 for an amino acid
- Ratio to $5x10^{26}$ to 1

Turing's Proposal: Coupled Reaction-Diffusion

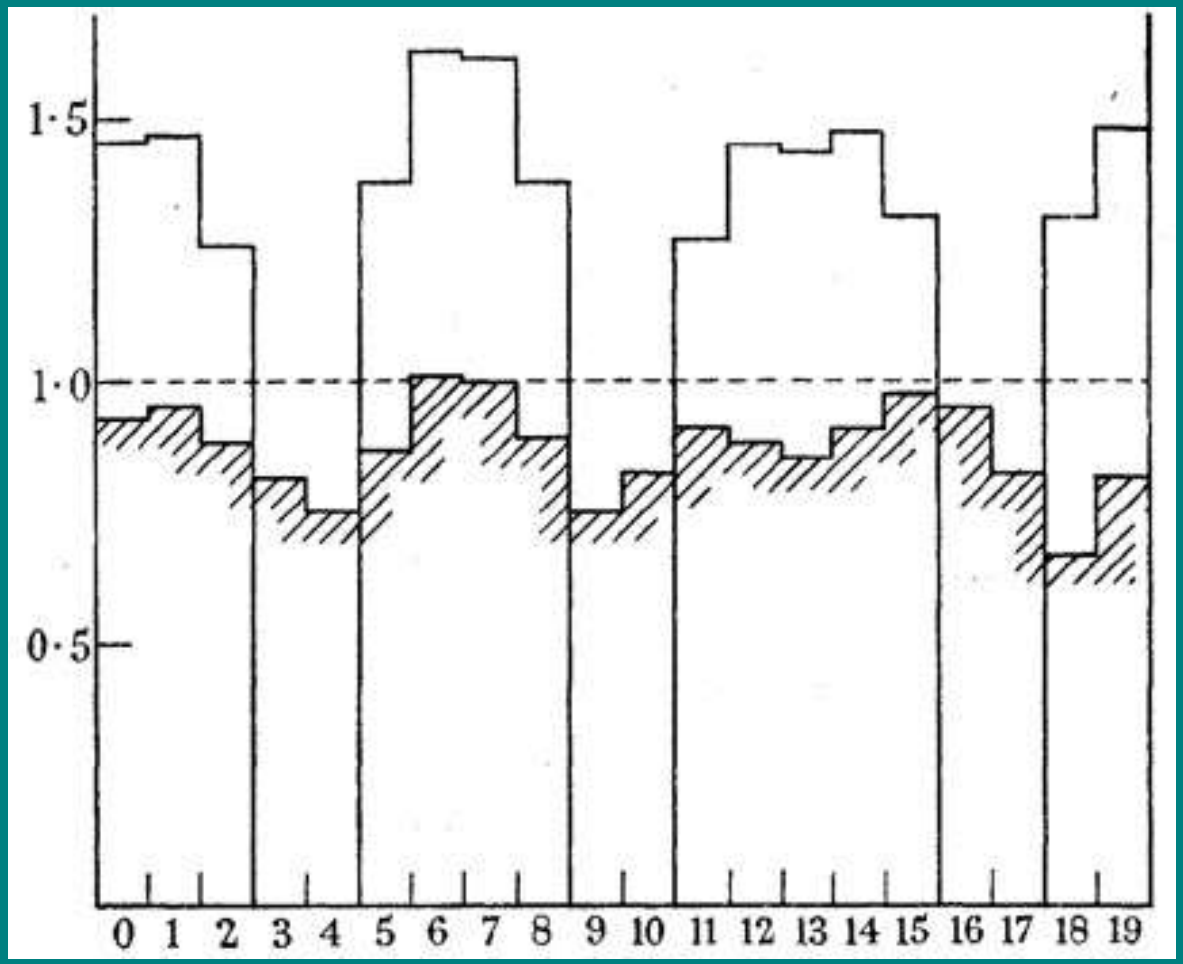
$$\frac{\partial u}{\partial t} = F(u,v) - d_u v + D_u \Delta u$$

$$\frac{\partial v}{\partial t} = G(u,v) - d_v v + D_v \Delta v$$



http://phylogenous.wordpress.com/2010/12/01/alan-turings-reaction-diffusion-model-simplification-of-the-complex/

Turing's Model for How Cells Change Type (Reaction-Diffusion Equation)



20 cells arranged in a ring

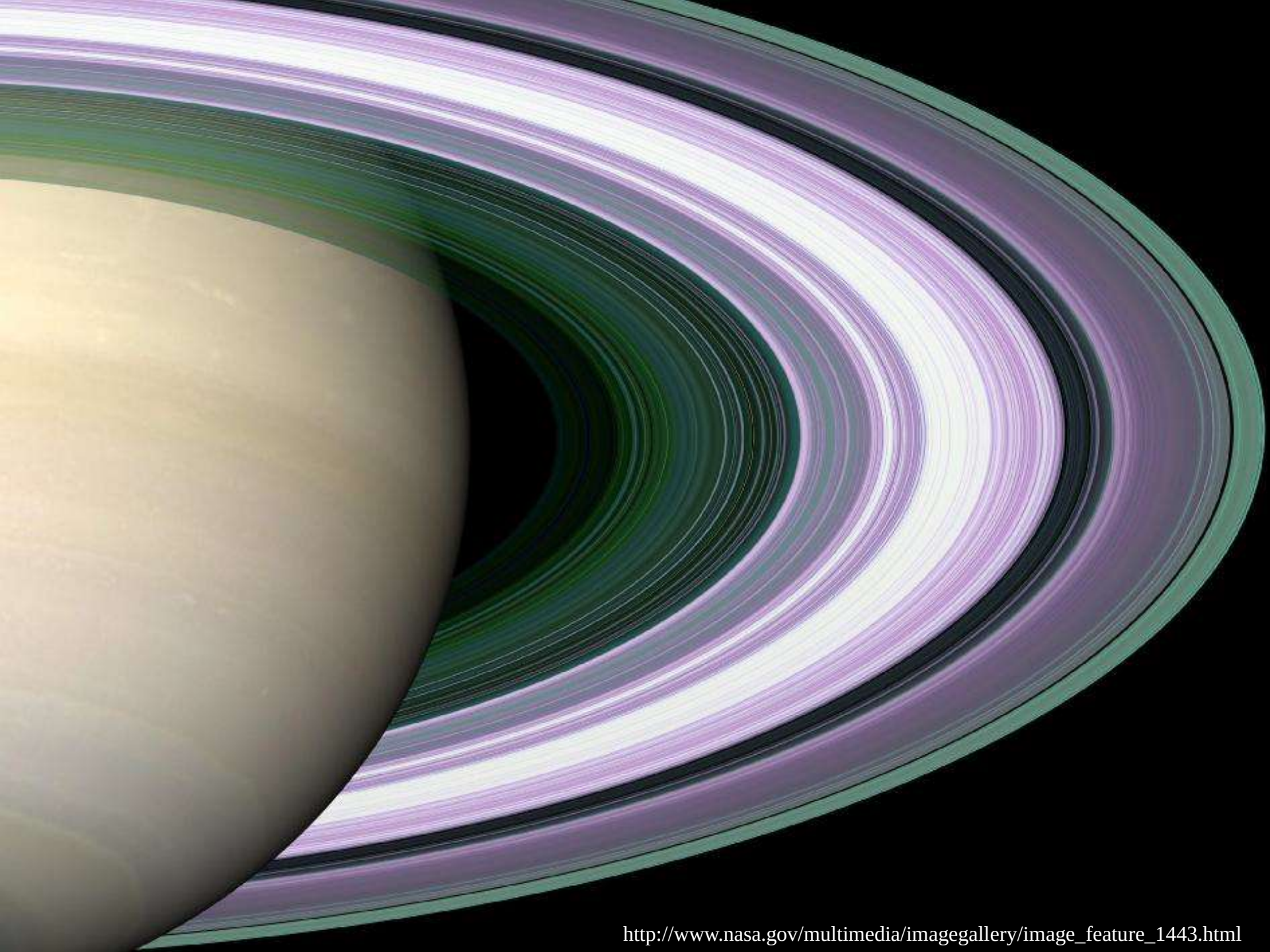
3. Final wave of morphogen concentration

1. Unstable "Equilibrium"

2. Initial random concentration of a morphogen

This is a Symmetry Breaking Model

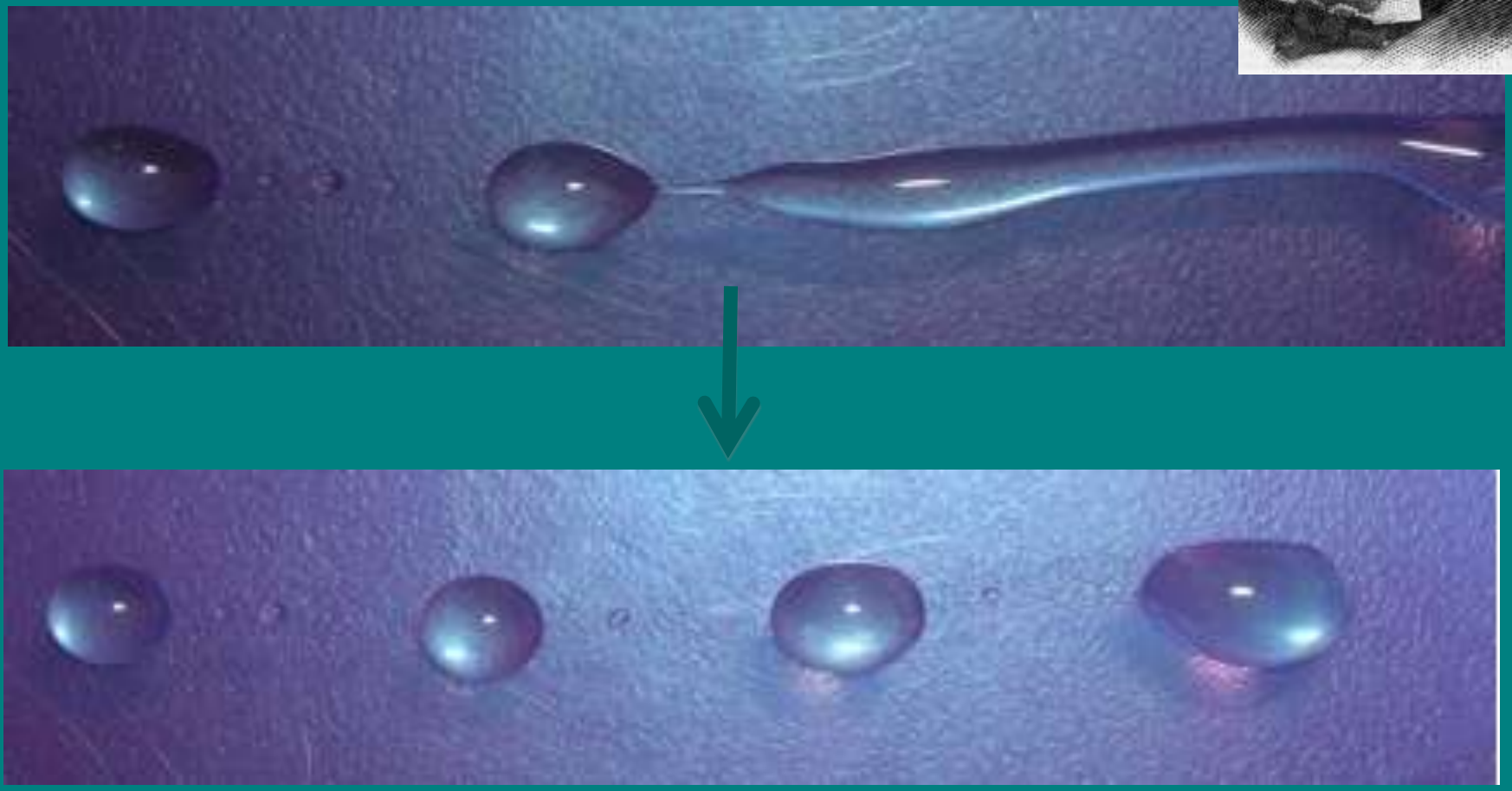
- Instability phenomena in “morphogenesis” have been known at least since 1859:
- James Clerk Maxwell (1859). *On the Stability of the Motion of Saturn's Rings. Cambridge, Macmillan and Co.*
- Lord Rayleigh (1892). On the instability of a cylinder of viscous liquid under capillary force. *Phil. Mag.*, **34,** 145-54.



http://www.nasa.gov/multimedia/imagegallery/image_feature_1443.html

http://th.physik.uni-frankfurt.de/~jr/gif/phys/rayleigh.jpg

Lord Rayleigh's Honey Experiment

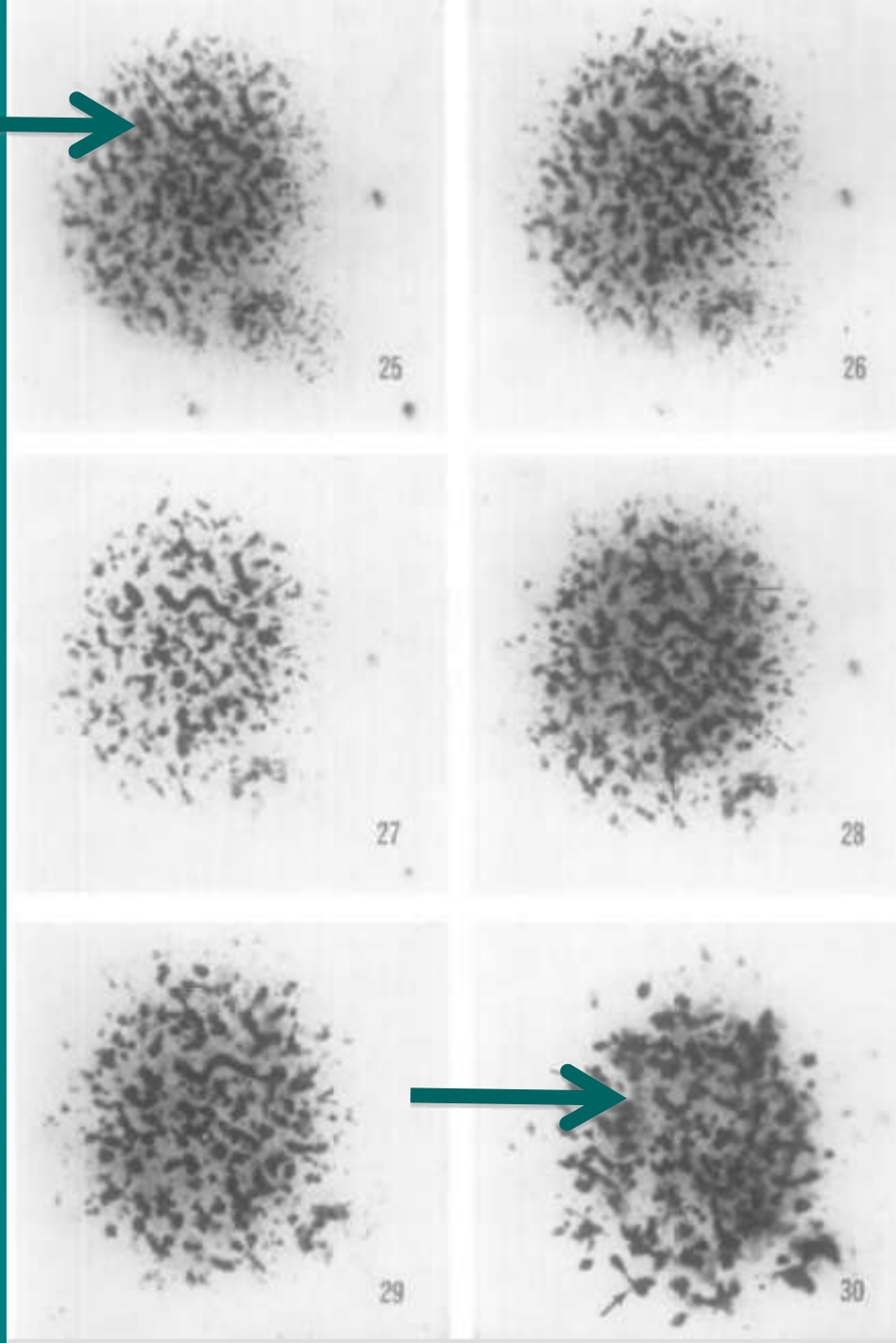


I applied this to self-sorting of embryonic cells in work with Narendra Goel, now of WSU

- Gordon, R., N.S. Goel, M.S. Steinberg & L.L. Wiseman (1975). A rheological mechanism sufficient to explain the kinetics of cell sorting. In: *Mathematical Models for Cell Rearrangement. Eds.: G.D. Mostow. New Haven, Yale University Press: 196-230.*
- Estimated tissue viscosities of 0.4×10^{6} to 0.7×10^{8} poise, compared to water at 10^{-2} poise

"…contraction of clusters will sometimes result in breaking the cellular bridge connecting fused clusters, with consequent separation of the clusters…"

Trinkaus, J.P. & J.P. Lentz (1964). Direct observation of type-specific segregation in mixed cell aggregates. *Developmental Biology* ***9(1), 115-136.***



Can Turing's model handle differentiation?

- Turing showed how just two cells, side by side, with the same morphogens inside, at nearly identical concentrations, can end up having different concentrations of each morphogen:
- "This breakdown of symmetry or homogeneity may be illustrated by the case of a pair of cells originally having the same, or very nearly the same, contents" (Turing, 1952).

Did Turing find the essence of the problem of cell differentiation?

- Start with one cell A, and let us suppose it divides to produce two daughter cells, B and C, that may each be different from A:
-
- A => BC
-
- In the next step, B divides into cells D and E, and C divides into cells F and G:
-
- BC => DEFG

Second Round of Differentiation Creates Problems

- But we have a problem here. Unless cells B and C can somehow influence one another, we could (by symmetry), just as well get:
-
- BC => EDFG
- BC => DEGF
- BC => EDGF

Could be Even Worse

- Furthermore (again by symmetry) cell A could just as well have produced:
-
- A => CB
-
- because BC can differ from CB if there is a left/right polarity to each cell. We thus see that there are even more possibilities:
-
- CB => FGDE
- CB => FGED
- CB => GFDE
- CB => GFED

So 2 steps of differentiation have 8 possible outcomes

- DEFG
- EDFG
- DEGF
- EDGF
- FGDE
- FGED
- GFDE
- GFED

Combinatoric Metasymmetry

- The problem gets exponentially worse with each step of cell division and its symmetry breaking.
- This shows that there is a symmetry to symmetry breaking, and if we are to have a specific organism result, we need to figure out how to break this “higher order” combinatoric “metasymmetry”

Turing's Contribution in Context

- Turing, in "The chemical basis of morphogenesis", laid the groundwork for one step of how differentiation might come about: symmetry breaking
- But there are metasymmetries to be broken at every level
- …with enough consistency to produce a viable organism
- We don't know yet how this is done.

Embryogenesis Combinatorics

- If half of the 7000 mouse cell types are present in the adult mouse, and we arranged them along a line, there would be 3500! (factorial) different arrangements *a priori*, or well over 10^{10000}.
- In three dimensions the possibilities are even greater.
- This is even without considering that there are many cells of each kind.

Number of Cell Types

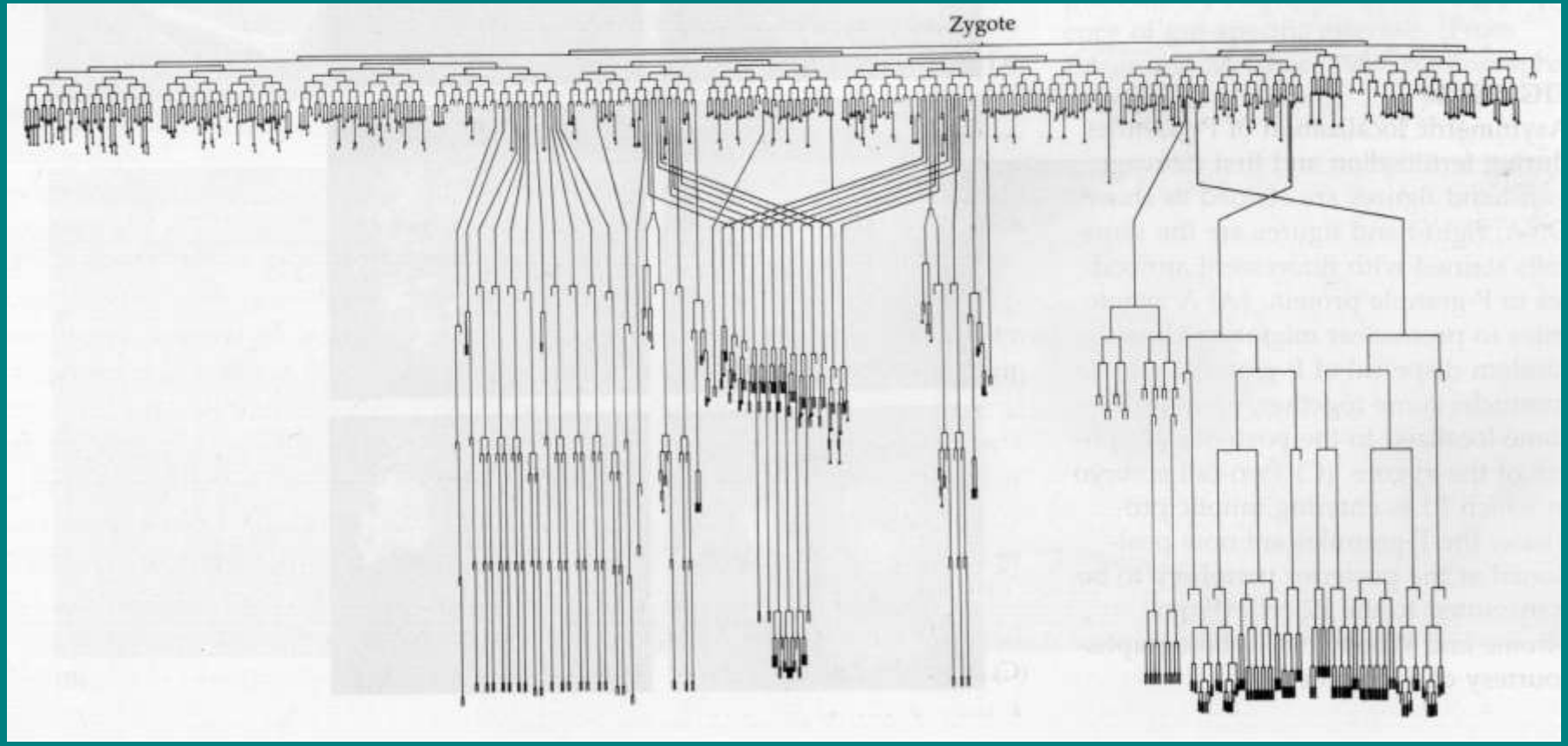
- In mouse development # cell types is estimated as high as 7000 with 8 hierarchical levels
- As $2^{12} < 7000 < 2^{13}$, the number of bifurcations in the cell lineage tree producing a mouse is around 12 to 13 on average, suggesting 4 or 5 more levels are to be found ("cryptic" cell types)
- # cells in humans is guesstimated at 10-100 trillion (10^{13}-10^{14}), yielding 10^{9}-10^{10} cells of each kind, if we have the same # of cell types as mice

- Bard, J. B. L., Baldock, R. A. and Davidson, D. R. 1998a. Elucidating the genetic networks of development: a bioinformatics approach. *Genome Res.,* **8**, (9)**,** 859-63.
- Bard, J. B. L., Kaufman, M. H., Dubreuil, C., Brune, R. M., Burger, A., Baldock, R. A. and Davidson, D. R. 1998b. An internet-accessible database of mouse developmental anatomy based on a systematic nomenclature. *Mech. Dev.,* **74**, (1-2)**,** 111-20.

Nematode *Caenorhabditis elegans*, for contrast

- 807 distinct cells
- +142 cells occur as 71 pairs
- 949 total # of cells
- So the number of cells of a given kind is mostly 1 and sometimes 2 in nematodes
- This is 9 to 10 orders of magnitude less than mammals
- Horvitz, H.R. & I. Herskowitz (1992). Mechanisms of asymmetric cell division: two B's or not two B's, that is the question. *Cell* ***68, 237-255.***

Nematode Cell Lineage Tree
Caenorhabditis elegans



Gilbert, S.F. (1991a). *Developmental Biology*, 3rd ed., Sunderland, Massachusetts: Sinauer Associates.

Sulston, J.E., E. Schierenberg, J.G. White & J.N. Thomson (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**(1), 64-119.

Modularity

- It is clear, then, that mammals have a mechanism for bundling many cells to be of the same kind that goes well beyond the mechanism used by nematodes to occasionally produce 2 cells of the same kind

Hierarchical Differentiation

- “Of course, to construct a metazoan body requires additional levels of organization of genes within the genome.”
- Valentine, J.W. (2004). *On the Origin of Phyla. Chicago, University of Chicago Press.*

“Of course”?!
The Great Module Hunt

- Can we find the proper modules for embryonic development, above the single cell level.

Modularity

- The search has been on to discover just what the modules of embryonic development are.
- Example:
- Newman, S. A. and Bhat, R. 2009. Dynamical patterning modules: a "pattern language" for development and evolution of multicellular form. *International Journal of Developmental Biology,* **53**, (5-6), 693-705.

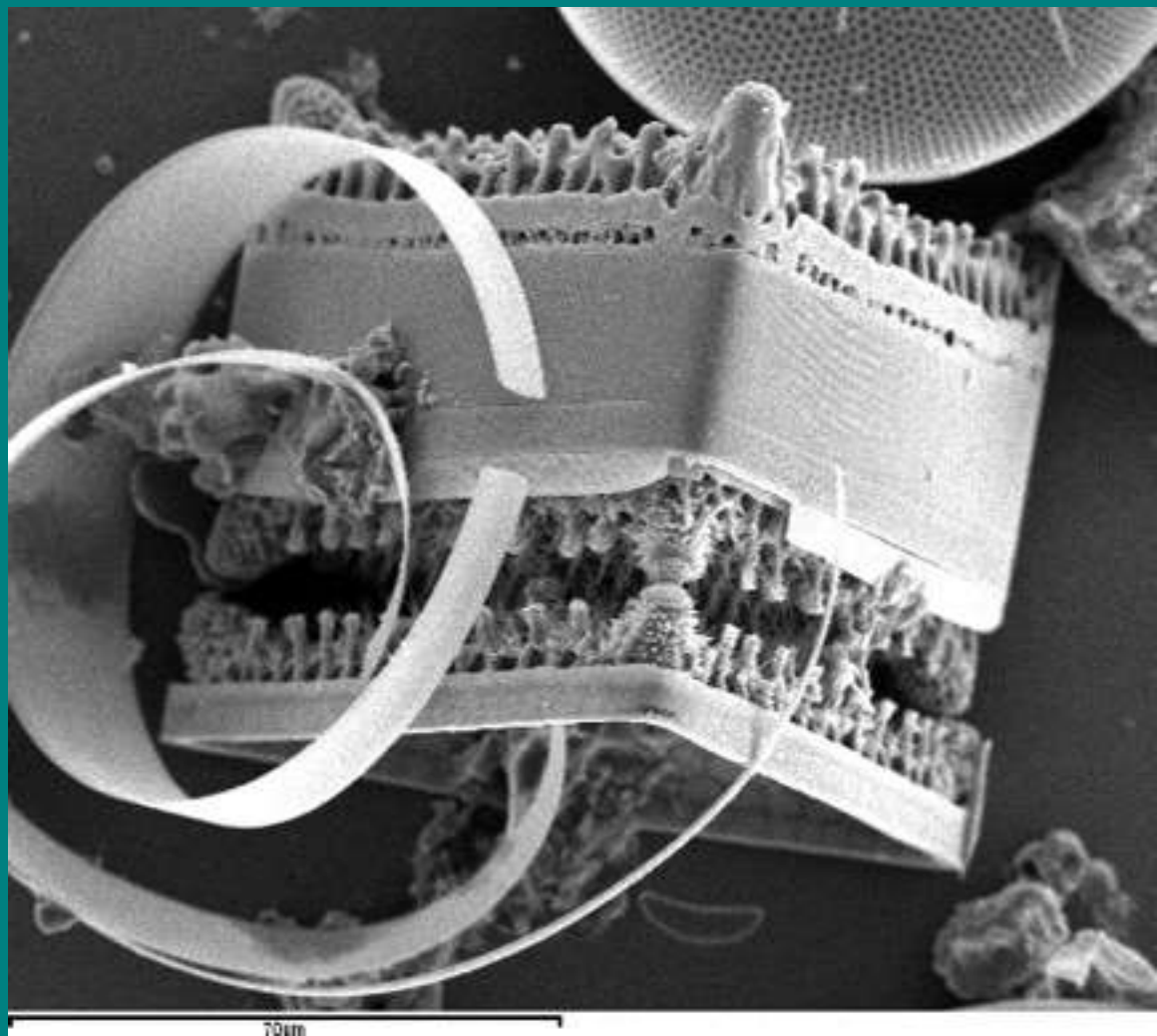
Modularity in Bridge Building: Spans & Braces

Cells are Not the Modules of Embryonic Development

- While this is clear by contrasting nematodes with mammals, many other lines of evidence lead to the same conclusion

Contradicting the "Cell Theory" that Cells are Modules in Development:

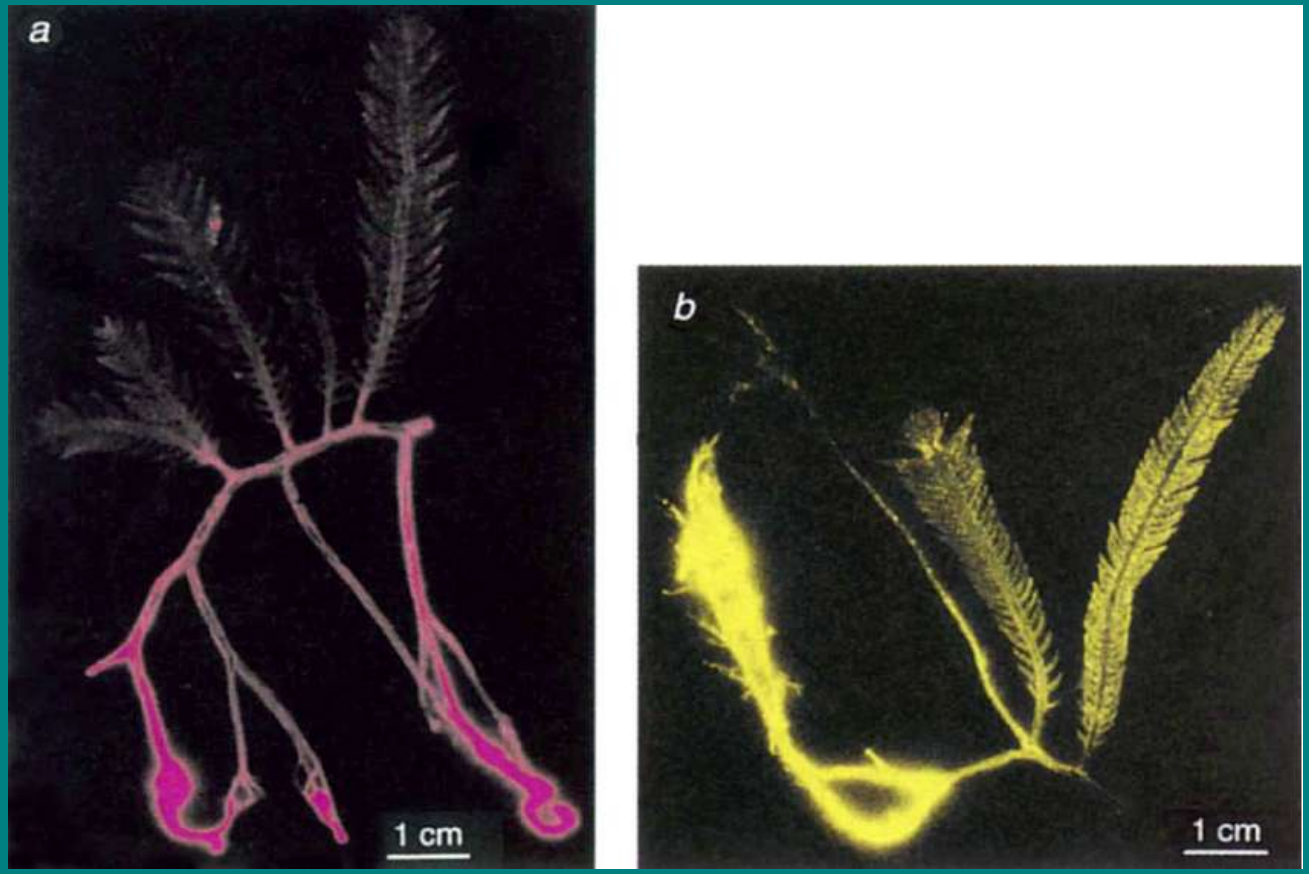
- 1. Single cell organisms can have quite complex morphologies, such as diatoms
- *Triceratium favus* with permission of Mary Ann Tiffany



Contradicting the "Cell Theory" that Cells are Modules in Development:

- 2. Some green algae have many nuclei that move in cytoplasmic streaming, unhindered by cell boundaries, yet "…exhibit morphological differentiation into structures that resemble the roots, stems, and leaves of land plants and even have similar functions".
- Cocquyt, E., Verbruggen, H., Leliaert, F. and De Clerck, O. 2010. Evolution and cytological diversification of the green seaweeds (Ulvophyceae). *Mol Biol Evol,* **27**, (9), 2052-61.

Example: Marine coenocyte (one cell) *Caulerpa taxifolia*



Chisholm, J. R. M., Dauga, C., Ageron, E., Grimont, P. A. D. and Jaubert, J. M. 1996. 'Roots' in mixotrophic algae. *Nature,* **381**, (6581), 382 + (6583) 565 erratum.

Autoradiographs showing transport from rhizoids to stolons and fronds, like roots to leaves

Contradicting the "Cell Theory" that Cells are Modules in Development:

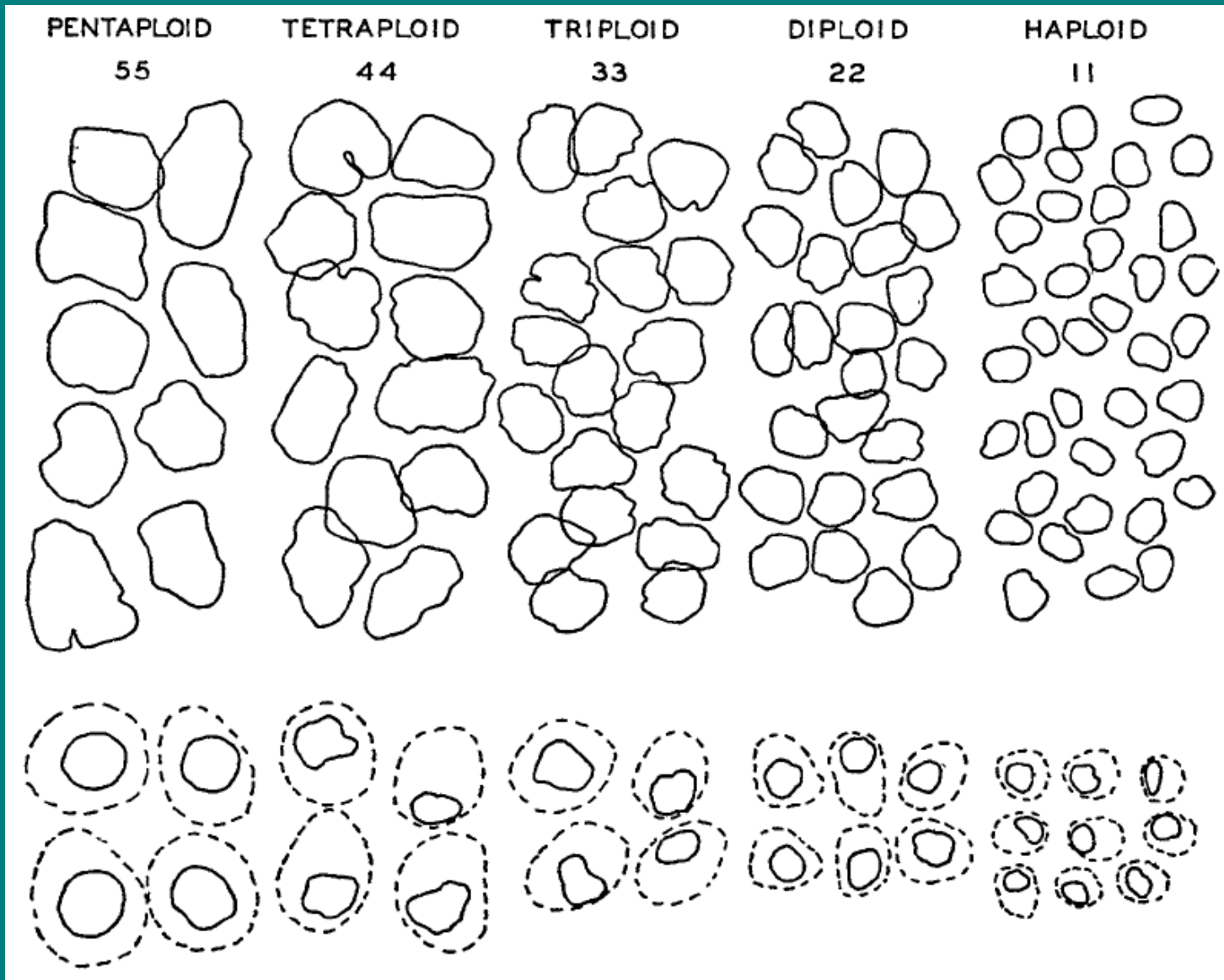
- 3. We can make "polyploid" salamanders with up to 7 copies of their genome per cell. The result is a normal looking adult with fewer larger cells that generally reaches the same size.
- So the adult's morphology is not dependent on how many cells it is made of (though its intelligence may be).

Example: newt
Notophthalmus (*Triturus*) *viridescens*

http://species.wikimedia.org/wiki/File:Notophthalmus_viridescensPCCA20040816-3983A.jpg

Example: Relative Cell & Nucleus Sizes in this Newt (Animal Size Unchanged)

Fankhauser, G. (1945). The effect of changes in chromosome number on amphibian development. *Quart. Rev. Biol.* ***20(1), 20-78.***



So if not cells,
What are the modules of development?

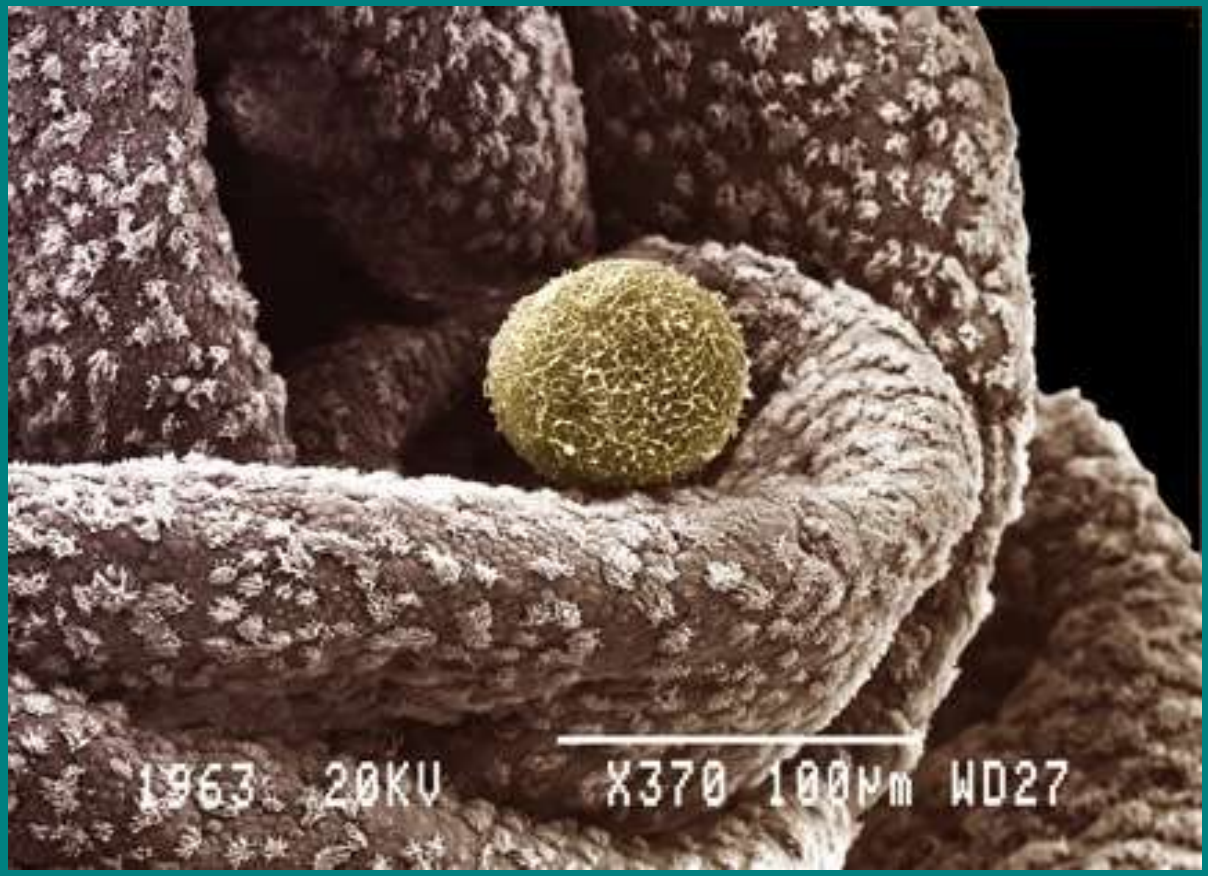
My answer: Tissues

- Organisms are partitioned into cells of different types
- Each group is a module called a"tissue"
- I redefine a "tissue" to be all the cells of a given type in an organism
- Note that mathematically a tissue is an equivalence class

Problem: there are no accepted criteria for placing cells into these equivalence classes

- **Solution**: we need a theory of how cell types form
- The theory will define the equivalence classes
- If the theory is consistent with all observations and its predictions, then the definition of the equivalence classes may be correct

Development starts from one cell: the fertilized egg (zygote)



Nikas, Y. 2011. *Human egg in the fallopian tube [#470]* http://www.eikonika.net/v2/photo_info.php?photo_id=470

Cells Change Types (or Don't) in Four Ways

- 1. *Symmetric cell division*.
- Result: two daughter cells of the same type
- 2. *Asymmetric cell division*.
- Result: two daughter cells of different types
- 3. *Stem cell asymmetric division*.
- Result: One daughter cell like the mother cell, the other different.
- 4. *No cell division*, but a hitherto mysterious process call "induction" changes the cell to a new type

Cell Differentiation

- The process (or processes) by which cells change type is called “differentiation”.
- Differentiation is one of the three major outstanding biological problems of our day
- (The other two are origin of life and consciousness)

The fundamental question in embryogenesis and differentiation: Hans Driesch (1867-1941)

- **How is it that cells in an embryo end up**:
- As the *right kinds*
- In the *right place*
- At the *right time*
- To which we now add:
- In the *right numbers*?

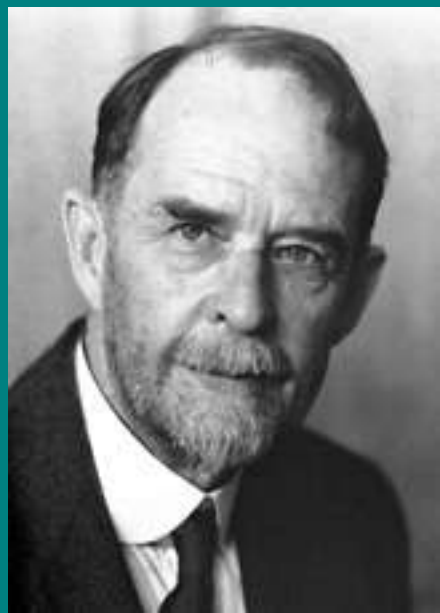
http://home.tiscalinet.ch/biografien/biografien/driesch.htm

*Sea Urchin's fertilized egg dividing and growing into a blastocyst, time lapse: http://amazingbeauty.org/Nature/ABbiology02-baby.html.
Also see: http://worms.zoology.wisc.edu/urchins/SUmainmenu.html.

Batteries of Genes: the Question, rather than "*The* Answer"

"An alternative view would be to assume that different batteries of genes come into action as development proceeds.... The idea that different sets of genes come into action at different times is exposed to serious criticism, unless some reason can be given for the time relation of their unfolding."

Thomas Hunt Morgan (1934). *Embryology and Genetics*, New York Columbia University Press.



http://www.nobelprize.org/nobel_prizes/medicine/laureates/1933/morgan-bio.html

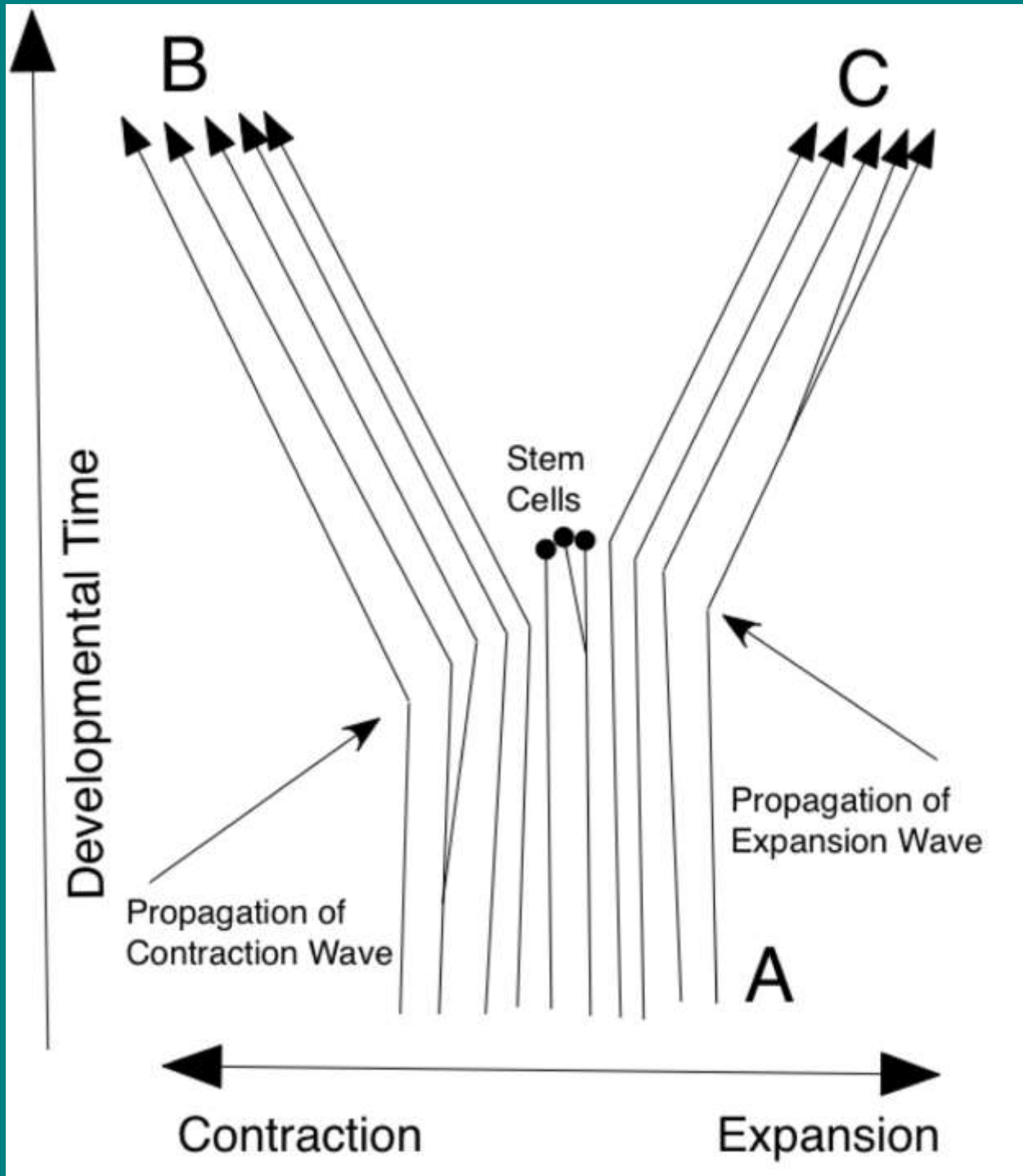
My own partial solution

- The cell lineage tree can be bundled into a more coarse *differentiation tree*
- Each branch of the differentiation tree represents a tissue consisting of cells of the same type
- These tissues are the fundamental modules
- Cells are organized into these bundles by differentiation waves

- Gordon, R. (1999). The Hierarchical Genome and Differentiation Waves: Novel Unification of Development, Genetics and Evolution. Singapore & London, World Scientific & Imperial College Press http://www.worldscibooks.com/lifesci/2755.html

A bundle of cells of type A is split into two bundles of cells B and C by a pair of differentiation waves

Natalie K. Gordon & Richard Gordon (2012). Embryogenesis Explained [in preparation]. Singapore: World Scientific Publishing Company.

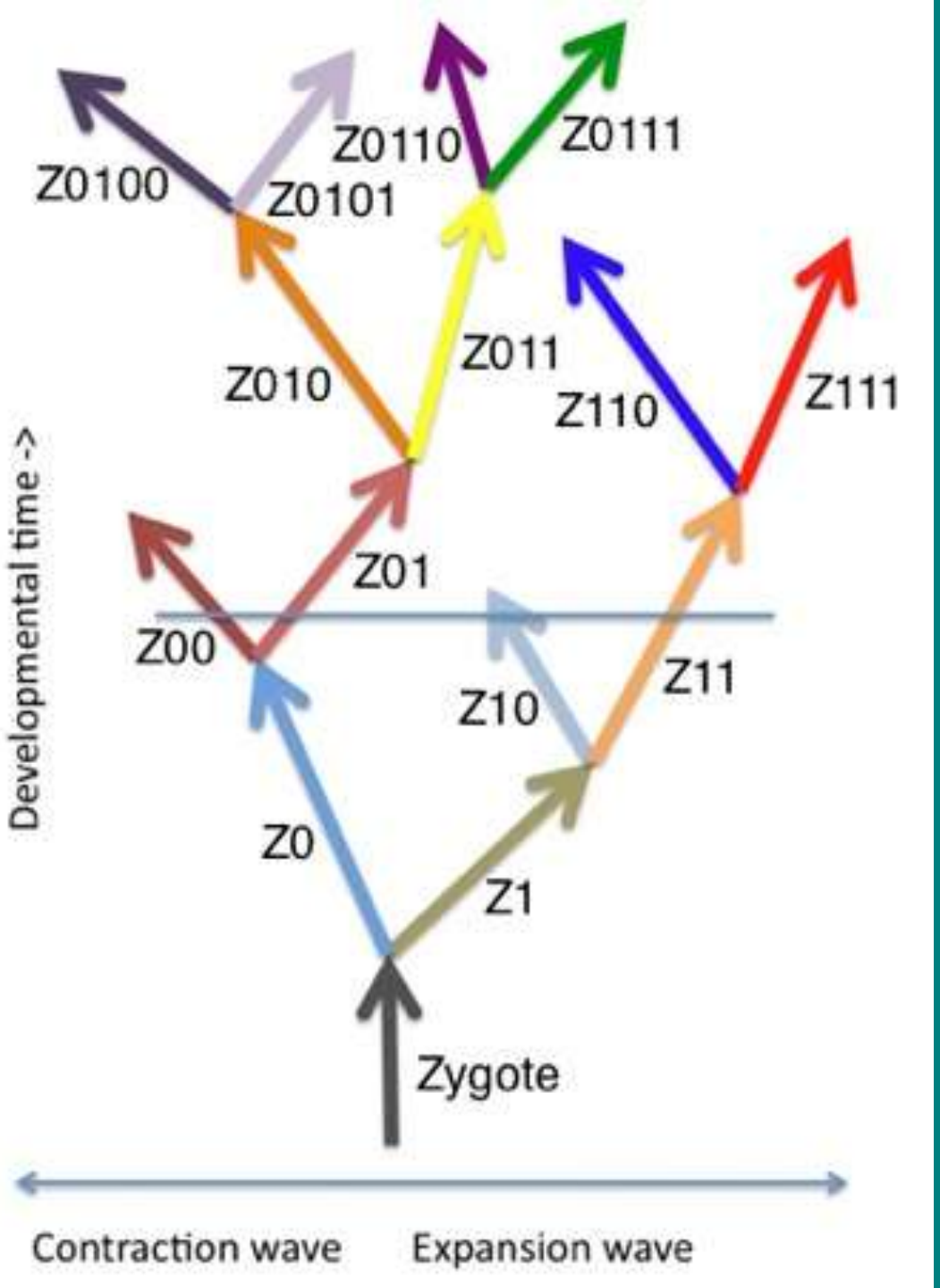


The Differentiation Tree

Each branch represents a distinct cell type

Note binary differentiation code

Natalie K. Gordon & Richard Gordon (2012). Embryogenesis Explained [in preparation]. Singapore: World Scientific Publishing Company.



Thomas Kuhn

1922-1996

http://www.bun.kyoto-u.ac.jp/phisci/Gallery/kuhn.html

- Kuhn, T.S. (1962). *The Structure of Scientific Revolutions*, Chicago University of Chicago Press. (3rd edition 1996)
- *Paradigm*: a way of thinking about a problem that most scientists agree on..
- *Anomaly*, something that doesn't fit the current paradigm, "subverts the existing tradition of scientific practice", leading to a scientific revolution, i.e., a change in paradigm
- "The successive transition from one paradigm to another via revolution is the usual developmental pattern of mature science". Kuhn had mostly physicists in mind when he wrote this.
- But: embryologists have merely accumulated paradigms without critically testing, comparing or eliminating any.

Paradigms for the Development of Organisms

- cell environment
- cell-cell interactions
- community effect
- complex systems dynamics
- cytoplasmic determinants
- deviation amplifying mutual causal processes
- differentiation waves
- dissipative structures
- divine intervention
- edge of chaos
- electrophoresis
- epigenesis
- gene regulation
- generic mechanisms
- genetic determinism
- gradients
- humunculus
- induction
- ionic currents
- maternal determinants
- morphogenetic fields
- morphogens
- negative feedback
- negentropy
- networks of interacting genes
- organicism
- plasmagenes
- positional information
- positive feedback
- predetermination
- prepatterns
- reaction-diffusion equations
- self-assembly or self-organization
- vitalism

I Suggest There Are:

- No Gradients
- No Morphogens
- No Cytoplasmic Determinants
- No Positional Information
- No Prepatterns
- No Inductions
- No Embryonic Regulation

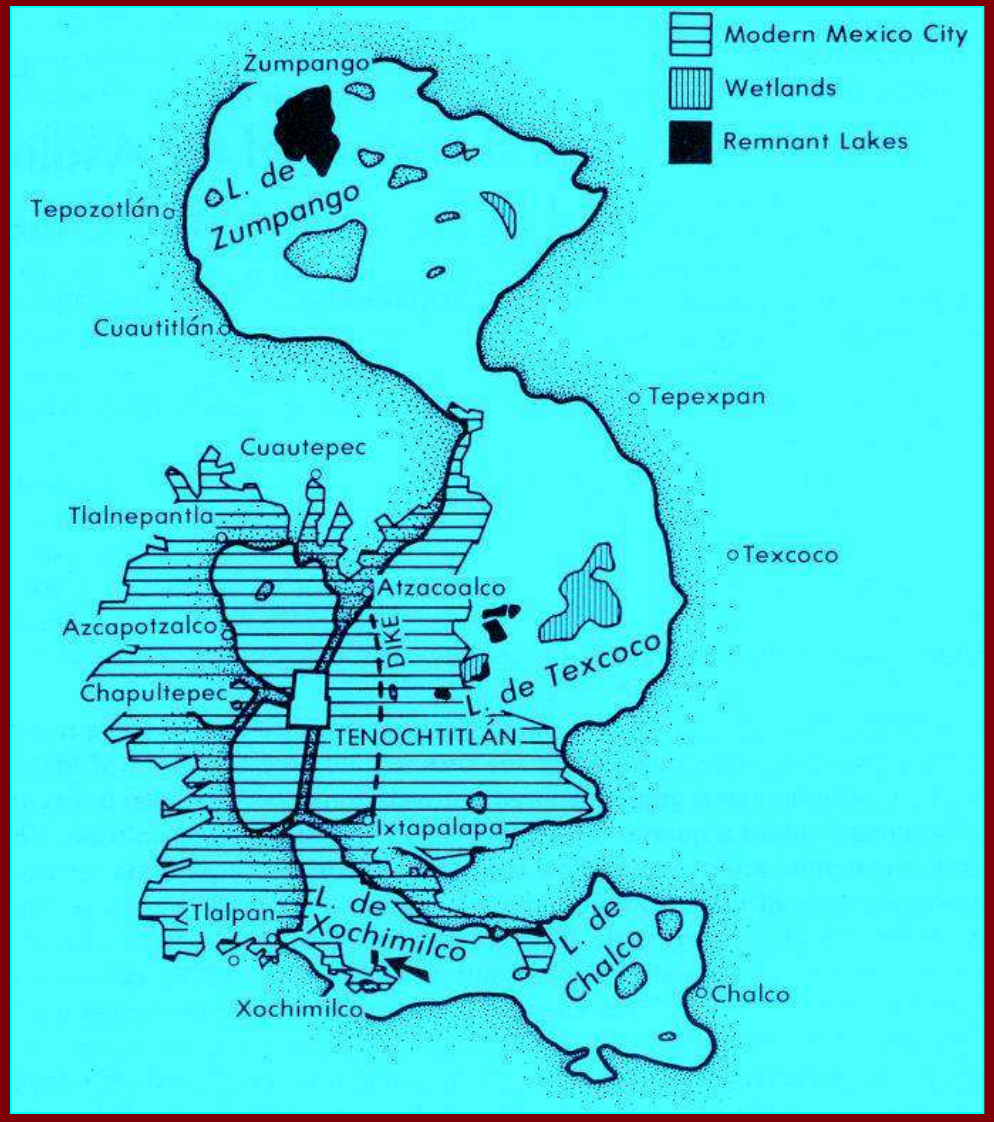
The Axolotl
Ambystoma mexicanum

A rare piebald axolotl, 23 cm long, showing its external gills.

Axolotl Wonders

- ✓Huge cells, 25µm x 50µm at neurulation.
- ✓Huge genome, 10x human genome.
- ✓Legs regenerate when bitten off.
- ✓Tail regenerates if lost.
- ✓Adults recover fully from spinal cord injuries.
- ✓One animal from two, fused half embryos.
- ✓Heart cut 75% regenerates, pumping in 24 hours.
- ✓Brain transplant can transfer memory.

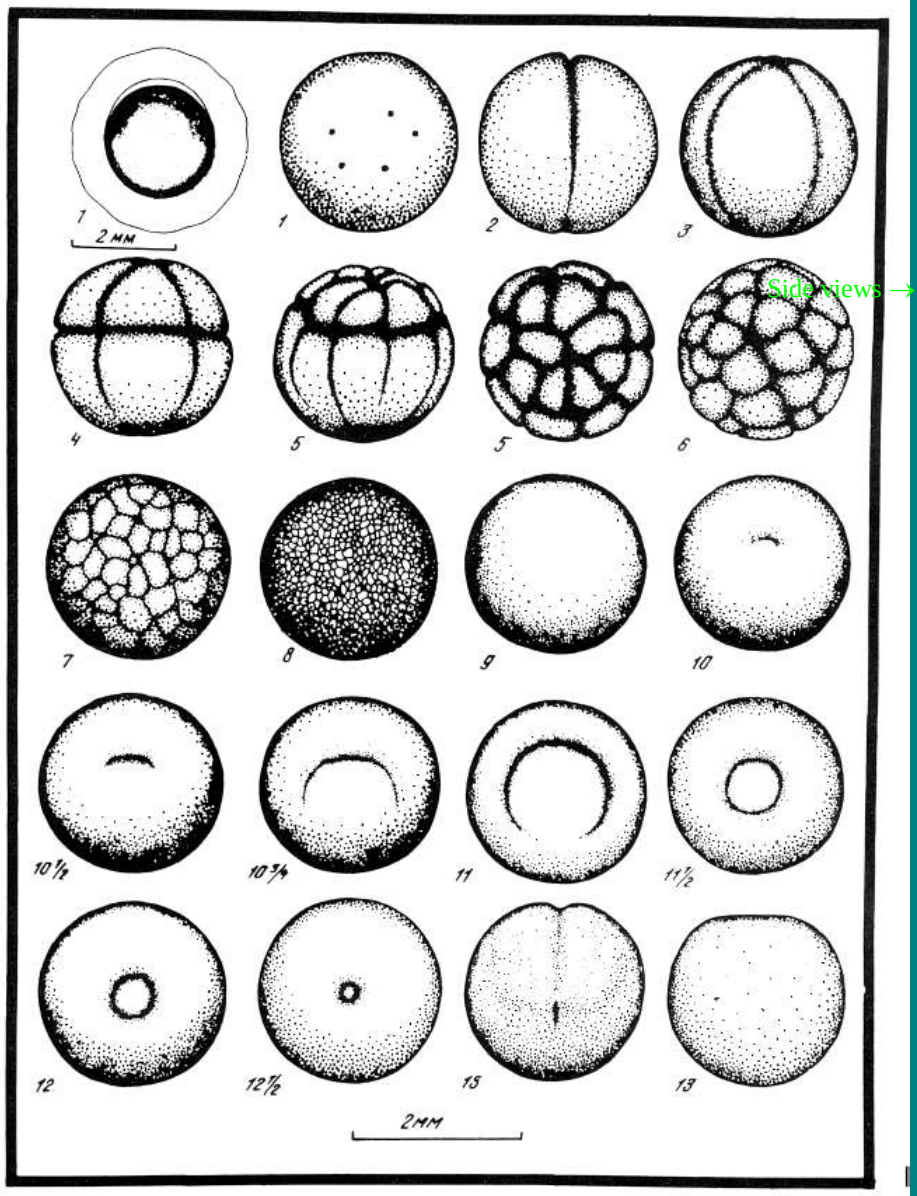
Stalking the Wild Axolotl



Graue, V., J. Sánches Robles, G. Castro, O. Cuamatzi, J. Márquez & M. Vázquez (1998). Breeding the axolotl in its native habitat. *Axolotl Newsletter* (27), 4-6. http://www.indiana.edu/~axolotl/newsletter/axnewsonline.html

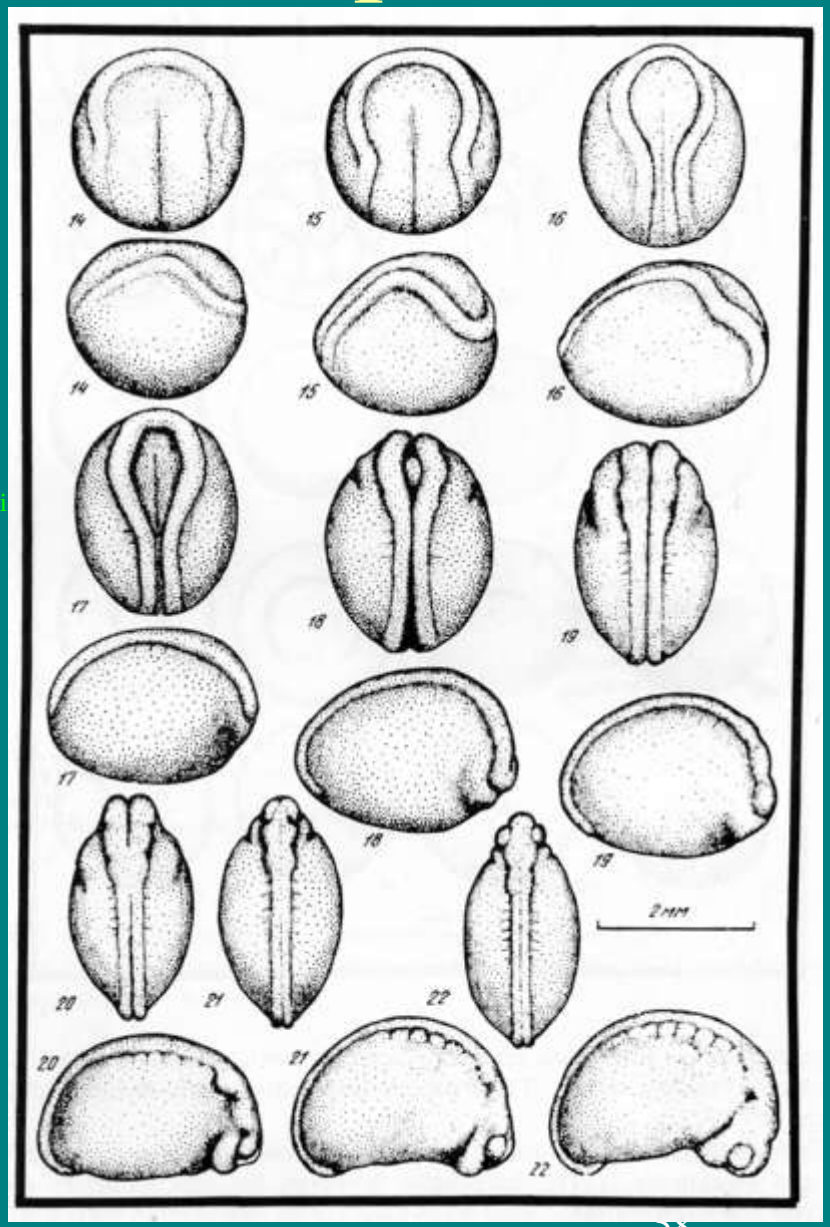
Brandon, R.A. (1989). Natural history of the axolotl and its relationship to other ambystomid salamanders. In Armstrong, J.B. & G.M. Malacinski, *Developmental Biology of the Axolotl*, New York Oxford University Press, p. 13-24.

Staging of Axolotl Development



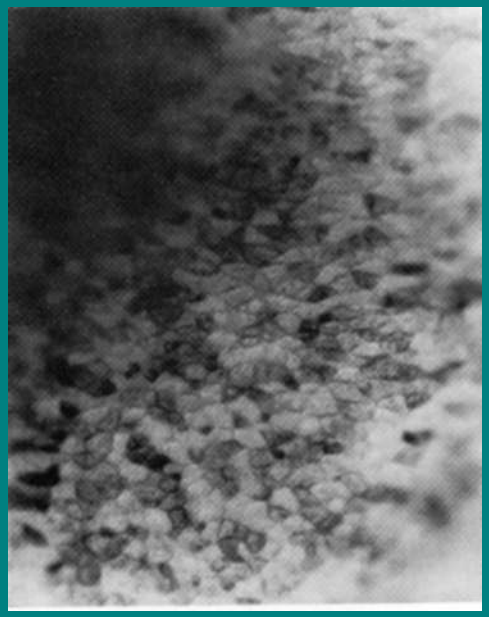
Side views →

Top vi



This and the subsequent six sketches are from Bordzilovskaya, N.P., T.A. Dettlaff, S.T. Duhon & G.M. Malacinski (1989). Developmental-stage series of axolotl embryos. In: Armstrong, J.B. & G.M. Malacinski, *Developmental Biology of the Axolotl*, New York: Oxford University Press, p. 201-219.

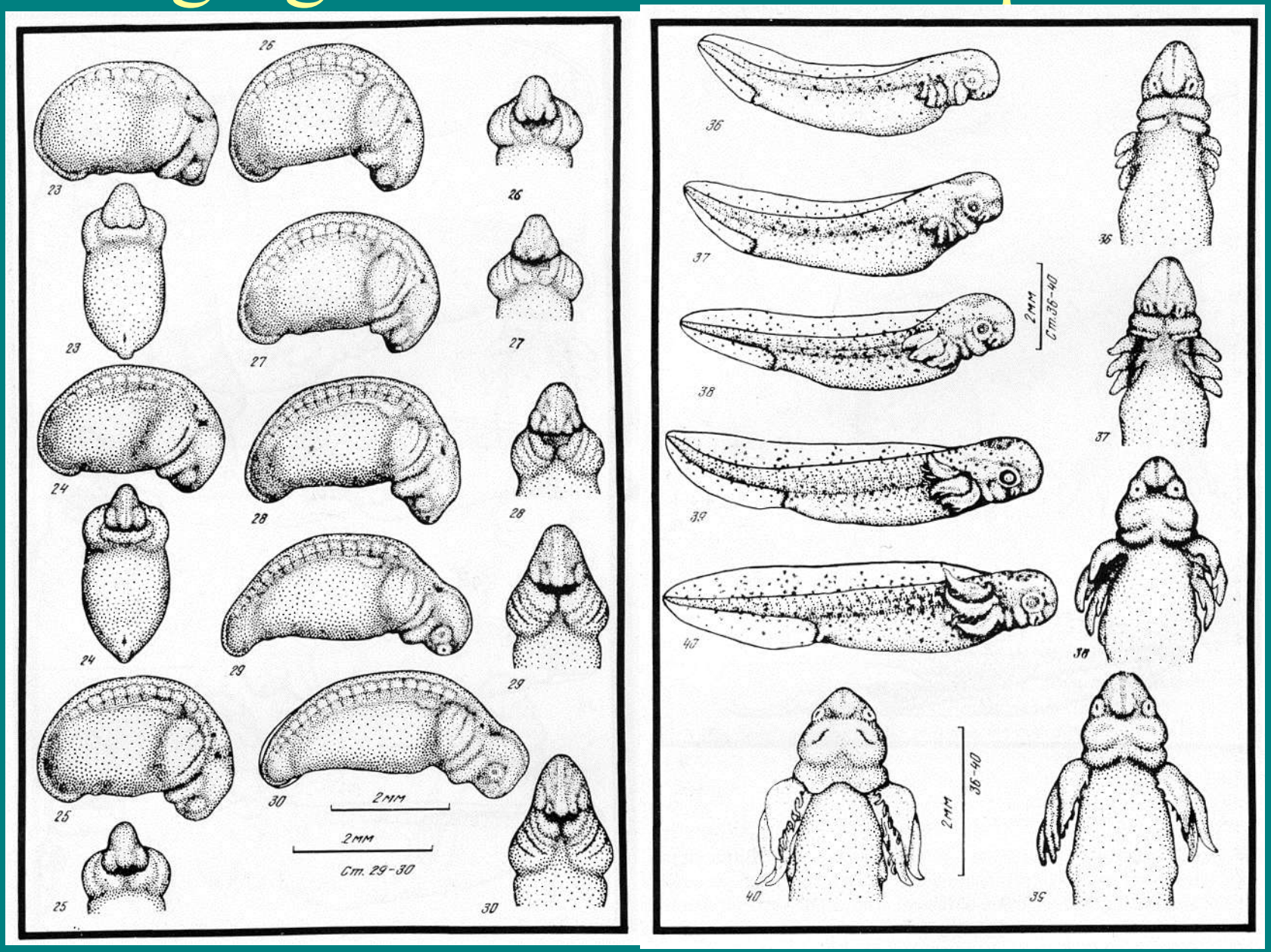
Newt/Axolotl Embryos Have Variegated Pigmentation: Permits Single Cell Tracking



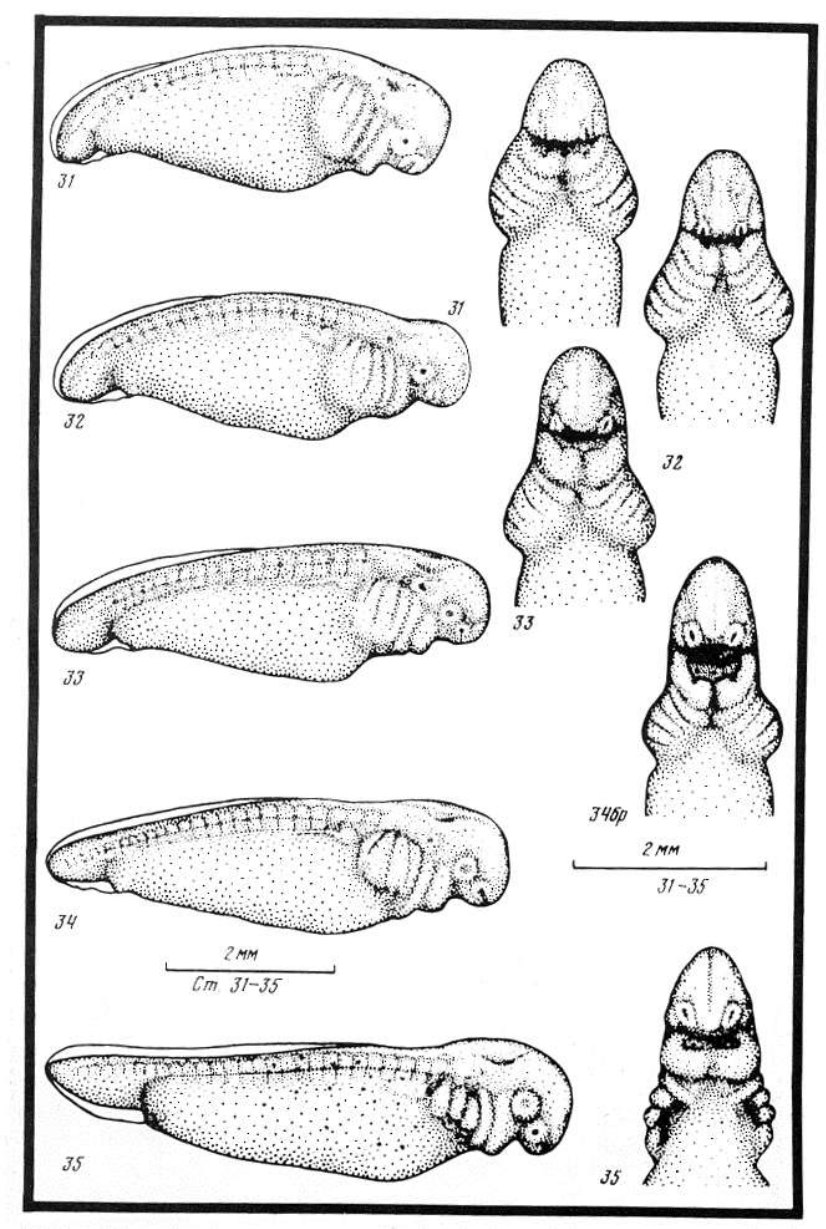
Jacobson, A.G. & R. Gordon (1976). Changes in the shape of the developing vertebrate nervous system analyzed experimentally, mathematically and by computer simulation. *J. Exp. Zool.* **197**, 191-246.

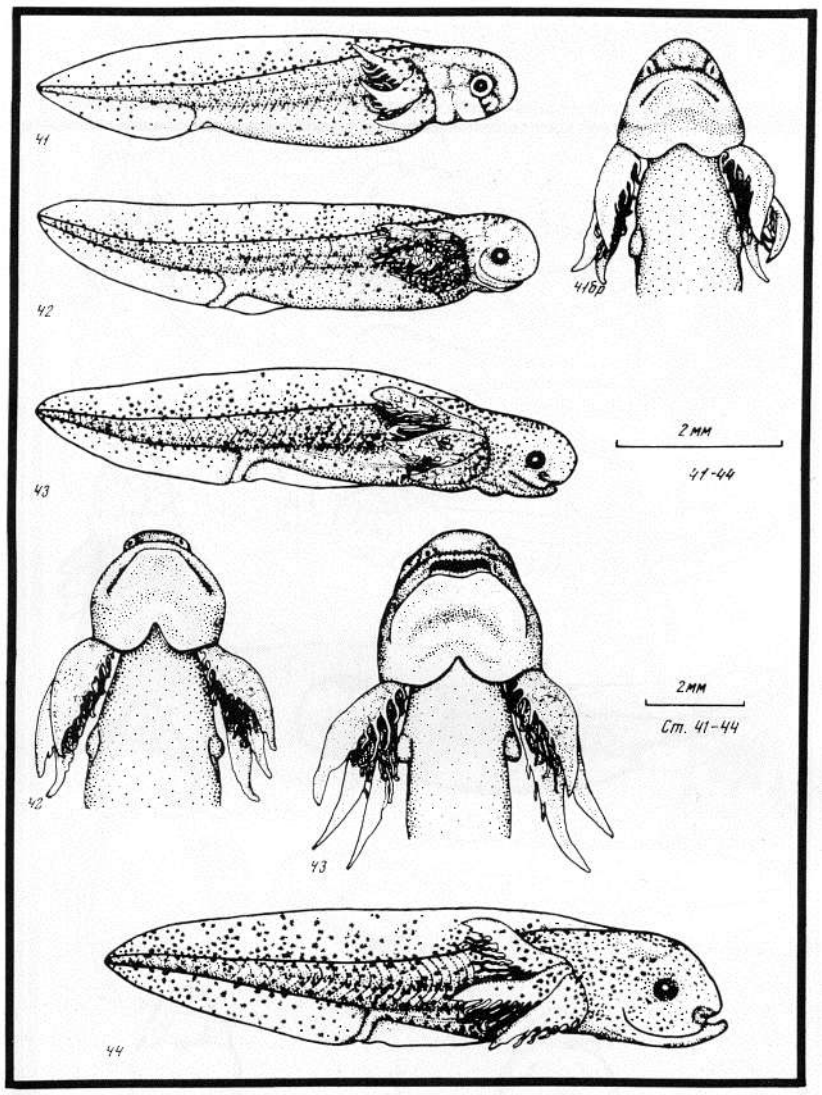
Gordon, R. & A.G. Jacobson (1978). The shaping of tissues in embryos. *Scientific American* **238**(6), 106-113.

Staging of Axolotl Development



Staging of Axolotl Development



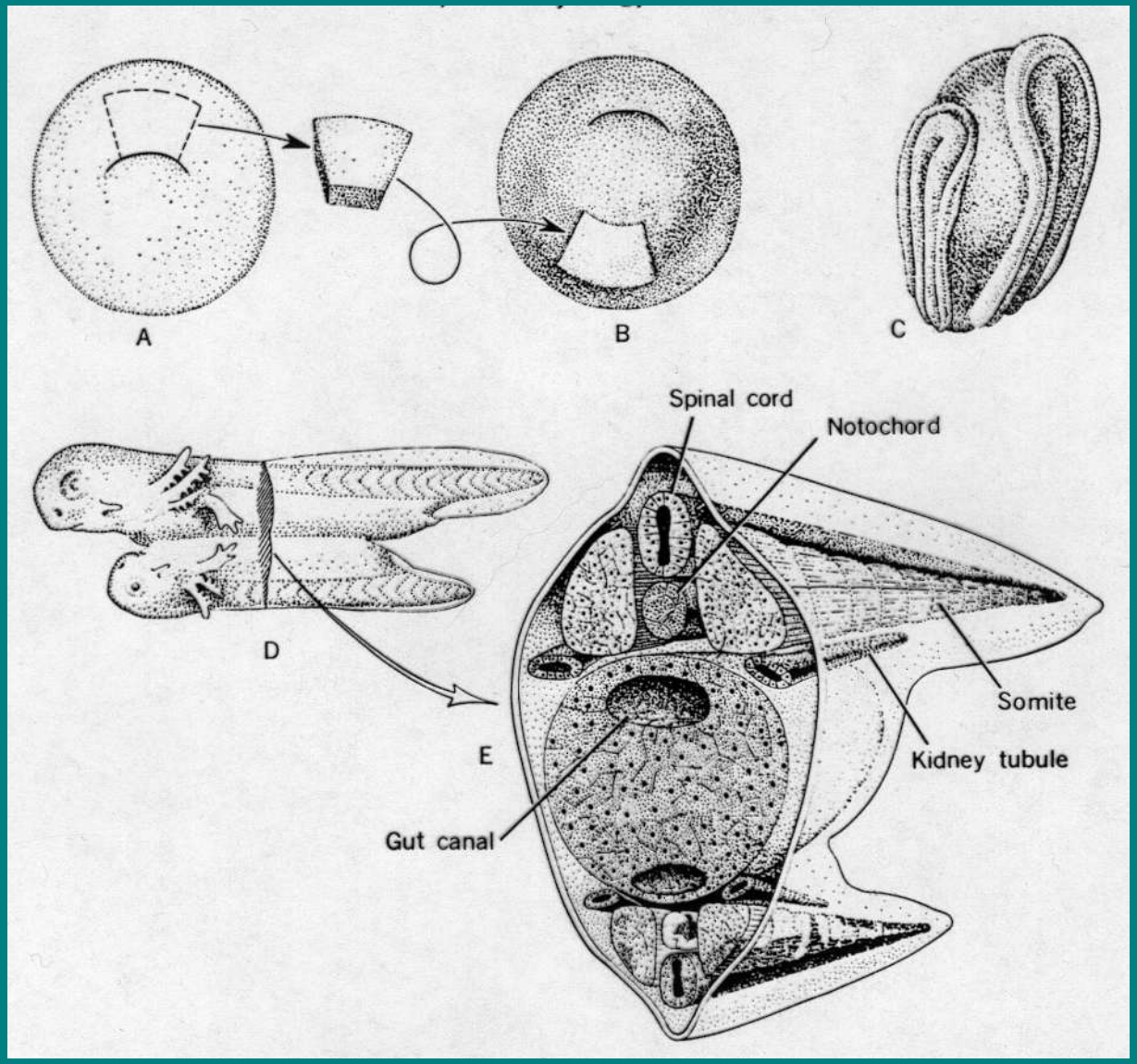


No increase in dry weight since it was an egg!

A newly hatched axolotl larva

Spemann, H. & H. Mangold (1924). Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren/On induction of embryo anlagen by implantation of organizers of other species. *Archiv mikroskop. Anat. Entwicklungsmech.* **100**, 599-638.

The Hans Spemann & Hilde Mangold experiment. Hilde died in a stove fire just after getting her PhD, and Hans got the Nobel Prize in 1935 for this work. On all other papers his male students were first authors.



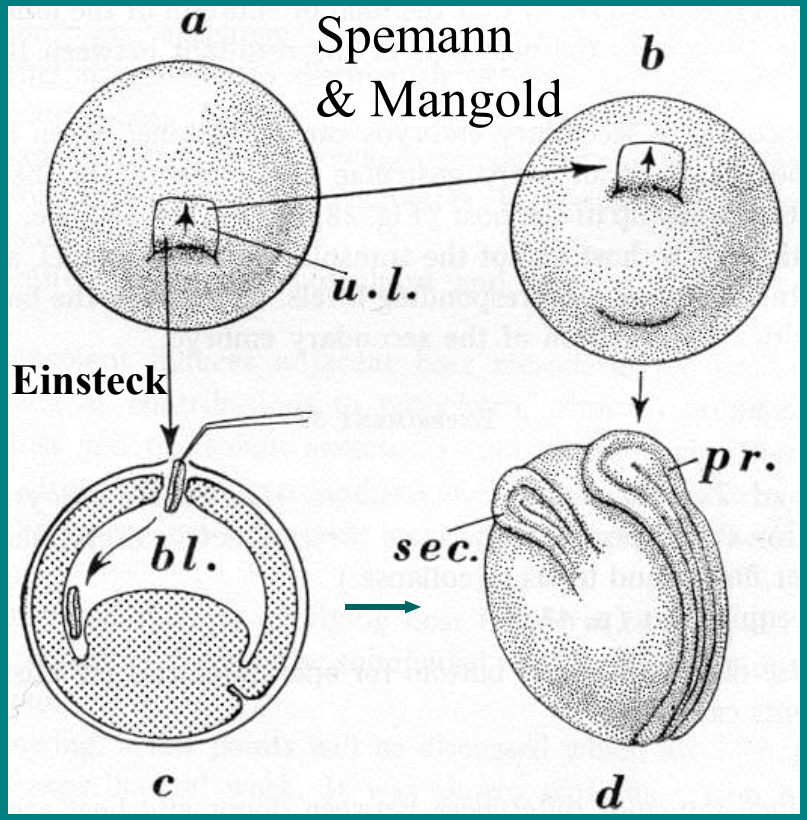
Twitty, V.C. (1966). *Of Scientists and Salamanders*, San Francisco W.H. Freeman and Co.

Hans Spemann & Hilde Mangold

Hamburger, V. (1988). *The Heritage of Experimental Embryology Hans Spemann and the Organizer*, New York Oxford University Press.

Browder, L.W., Erickson, C.A. & Jeffery, W.R. (1991). *Developmental Biology*, 3rd ed., Philadelphia Saunders College Publishing.

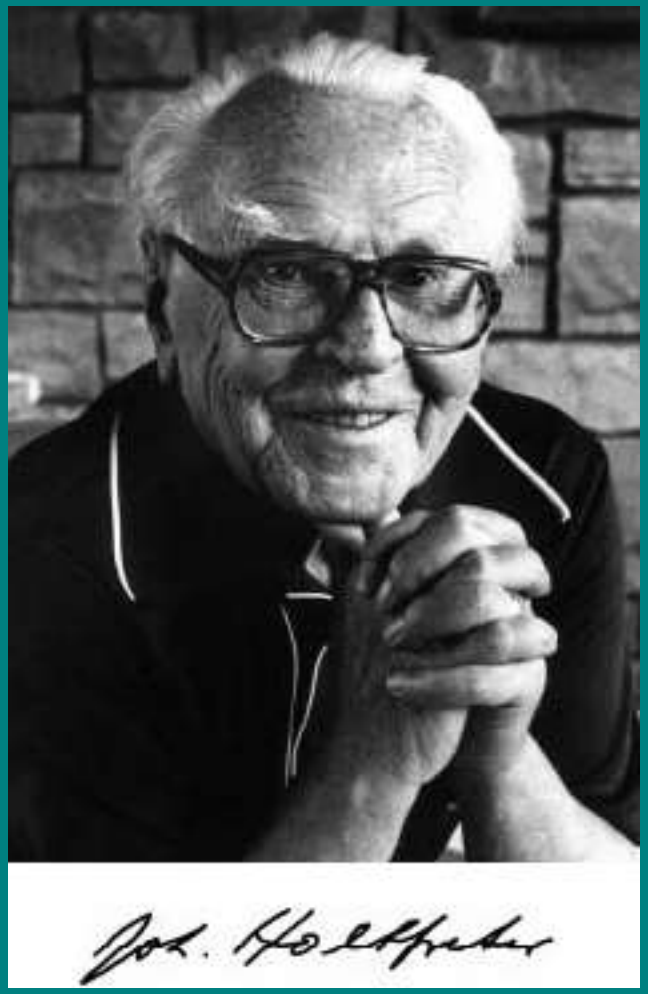
The Einsteck Method for the Surgically Less Adept



Hamburger, V. (1960). *A Manual of Experimental Embryology*, revised ed., Chicago University of Chicago Press.

Johannes Holtfreter

Spoiler: a dead organizer works just as well!



"Holtfreter was to remark despairingly that the analysis of organizer action was rapidly 'bringing chaos out of order.'"

Twitty, V.C. (1966). *Of Scientists and Salamanders*, San Francisco: W.H. Freeman and Co.

Gerhart, J.C. (1998). Johannes Holtfreter, January 9, 1901-November 13, 1992. *National Academy of Sciences of the United States of America Biographical Memoirs* **73**, http//stills.nap.edu/readingroom/books/bio73h/holtfreter.html.

Everything is an Inducer

- ectoderm killed by boiling
- high pH
- low pH
- calcium-free medium
- sodium chloride
- lithium chloride
- basic proteins from alcohol-fixed mouse kidney
- ribonucleoprotein particles from rat liver
- protein from guinea pig liver
- extract of 9 day chick embryos
- cyclic AMP

Everything is an Inducer

- Worms (*Enchytraeus*): body fragments.
- Snails (*Planorbis*, *Limnaea*): foot muscles, hepatopancreas.
- *Daphnia:* coagulated body extract.
- Lepidoptera (*Deilephila*): hemolymph and ganglia of pupa.
- Dragon-fly (*Libellula* larva): fat body, ganglia.
- Fishes (*Gasterosteus*): heart, liver, ovarian eggs, muscle, spleen.
- Amphibia (*Triton*, *Salamandra*, *Rana*): liver, heart, ovarian eggs, muscle, cartilage, brain, retina, regeneration blastema.
- Reptiles (*Lacerta*): liver, kidney, testis.
- Birds: liver, kidney, testis, thyroid, fat body, brain, retina; coagulated extract of chick embryos; fragments of primitive streak.
- Mammals (mouse): heart, liver, kidney, adrenals, brain, lens; calf's liver.
- Man: liver, brain, kidney, thyroid, tongue, sarcoma, carcinoma.

Today's Inducers in Fashion

- the homeobox gene *goosecoid:*
- TGFβ (transforming growth factor β) family
- FGF (fibroblast growth factor)
- activin
- noggin

But none of the previous inducers has been explained, nor explained away.

Upping the Ante (2001)

"A great number of genes are specifically expressed within the organizer, most of them encoding secreted proteins and transcription factors. The challenge is now to uncover genetic cascades and networks of interactions between these genes, in order to understand how the organizer functions."

Kodjabachian, L., A.A. Karavanov, H. Hikasa, N.A. Hukriede, T. Aoki, M. Taira & I.B. Dawid (2001). A study of Xlim1 function in the Spemann-Mangold organizer. *Int J Dev Biol* **45**(1 Spec No), 209-218.

Except…

- “No inductions were obtained with starch (prepared from wheat, potato, banana), agar, chick albumin, lard, wax, charcoal, gelatine, cholesterin, yeast, coagulated frog's blood” (Weiss, 1935).
- “Only such very inactive tissues as banana peel or insect wings failed to elicit a reaction!” (Brachet, 1974).

Weiss, P.A. (1935). The so-called organizer and the problem of organization in amphibian development. *Physiol. Rev.* **15**(4), 639-674.

Brachet, J. (1974). *Introduction to Molecular Embryology*, London: English Universities Press.

Quiz

- If each generation's favorite molecules have been successful inducers: steroids, RNA, specific genes, whole genetic networks, what do they have in common?
- Or, looking at what may be the key observations, why don't wax, lard, insect wings and banana peels work? What do these failed inducers have in common?

Eureka!

According to his memoirs, on 15 April 1726 William Stukely, an eccentric archaeologist, visited Isaac Newton at his home in Kensington. After dinner Newton and Stukely went into the garden to drink tea under the shade of some apple trees. As they sat chatting, Newton, who was then 83 years old, told Stukely that, *"he was just in the same situation as when, formerly, the notion of gravitation came into his mind. It was occasion'd by the fall of an apple, as he sat in contemplative mood".*

http://www.npl.co.uk/npl/about/newton.html

Under (a graft from) Newton's tree

Okay, so it was a willow tree at the University of Waterloo in 1985….

http://www.npl.co.uk/npl/about/newton.html

G. Wayne Brodland

http://www.civil.uwaterloo.ca/brodland/

Richard Gordon

http://www.umanitoba.ca/faculties/medicine/radiology/stafflist/rgordontitle.html

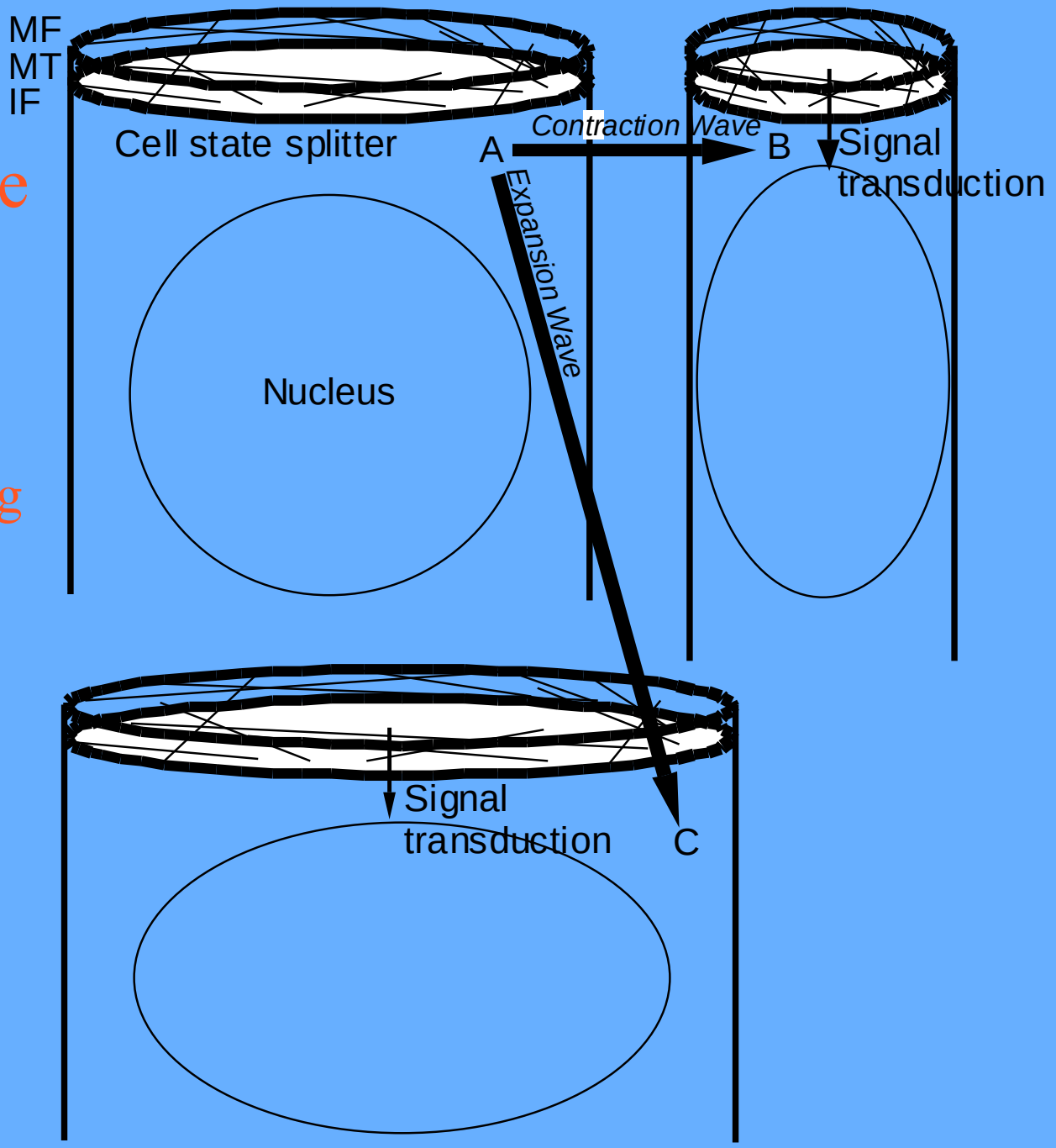
These photos 15 years later!

The Cell State Splitter

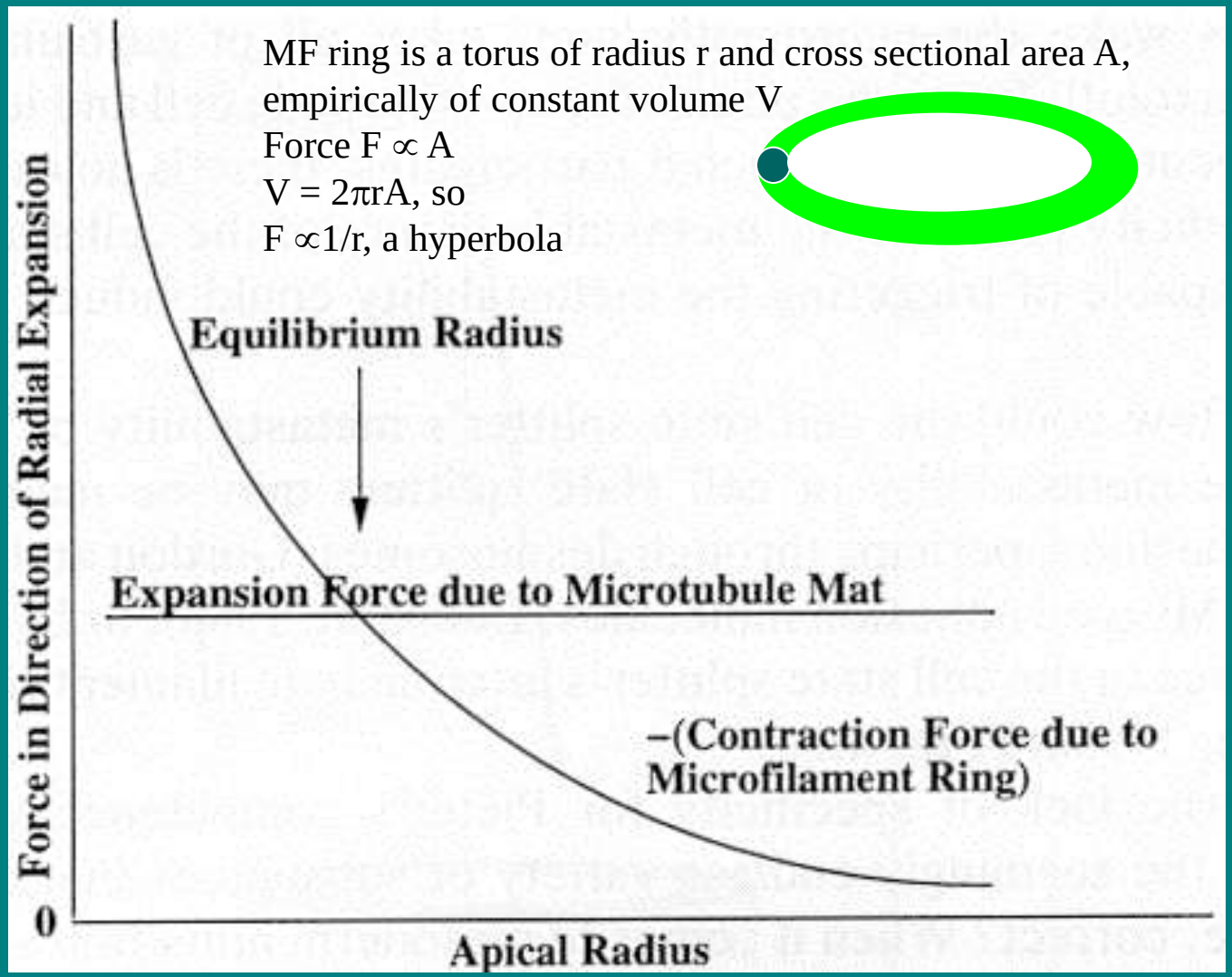
MF = microfilament ring

MT = annular apical microtubule mat

IF = intermediate filament ring



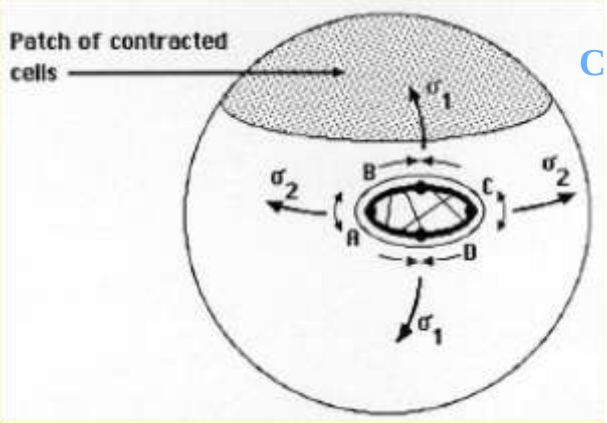
The Unstable (Bistable) Mechanical Equilibrium between the Microfilament Ring and the Microtubule Mat in the Cell State Splitter



Gordon, R., N.K. Björklund & P.D. Nieuwkoop (1994). Dialogue on embryonic induction and differentiation waves. *Int. Rev. Cytol.* **150**, 373-420.

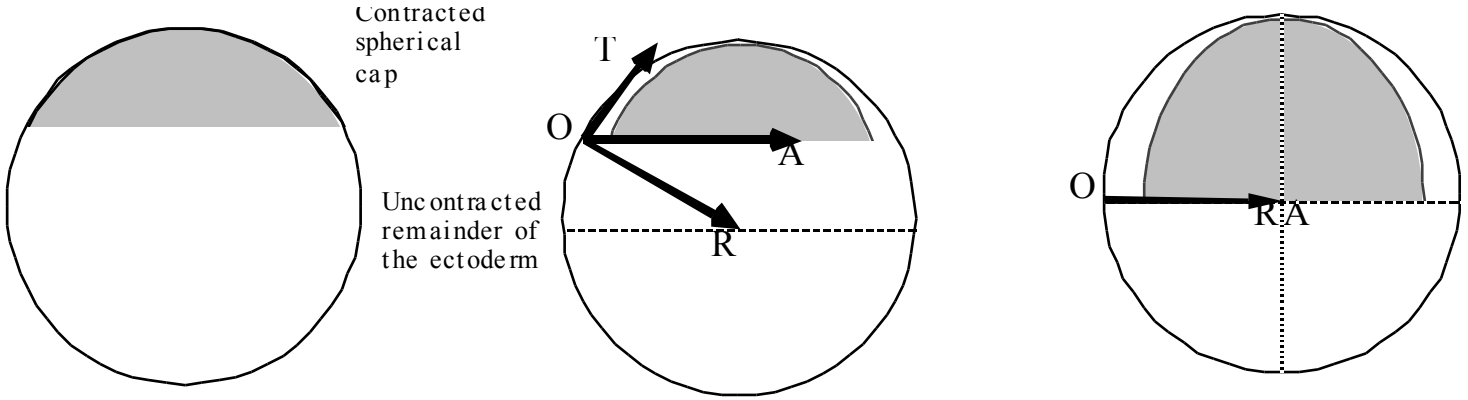
Needed a Wave to Carry the Contraction or Expansion from Cell to Cell: First Theory

The Hot/Cold Boiler Model



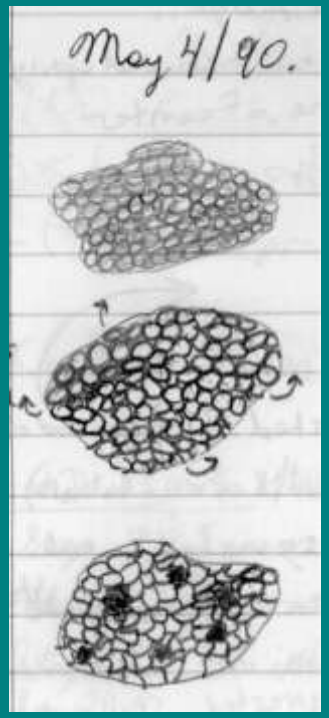
Cold: Contracting

Hot: Expanding



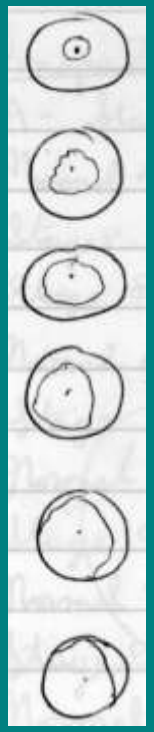
Needed a Wave to Carry the Contraction or Expansion from Cell to Cell: First Observation

Found by Natalie K. Björklund
in ectoderm explants…



Perhaps a stretch-activated contraction?

and in the intact embryo:



Gordon, R. (1999). *The Hierarchical Genome and Differentiation Waves Novel Unification of Development, Genetics and Evolution*, Singapore & London World Scientific & Imperial College Press.

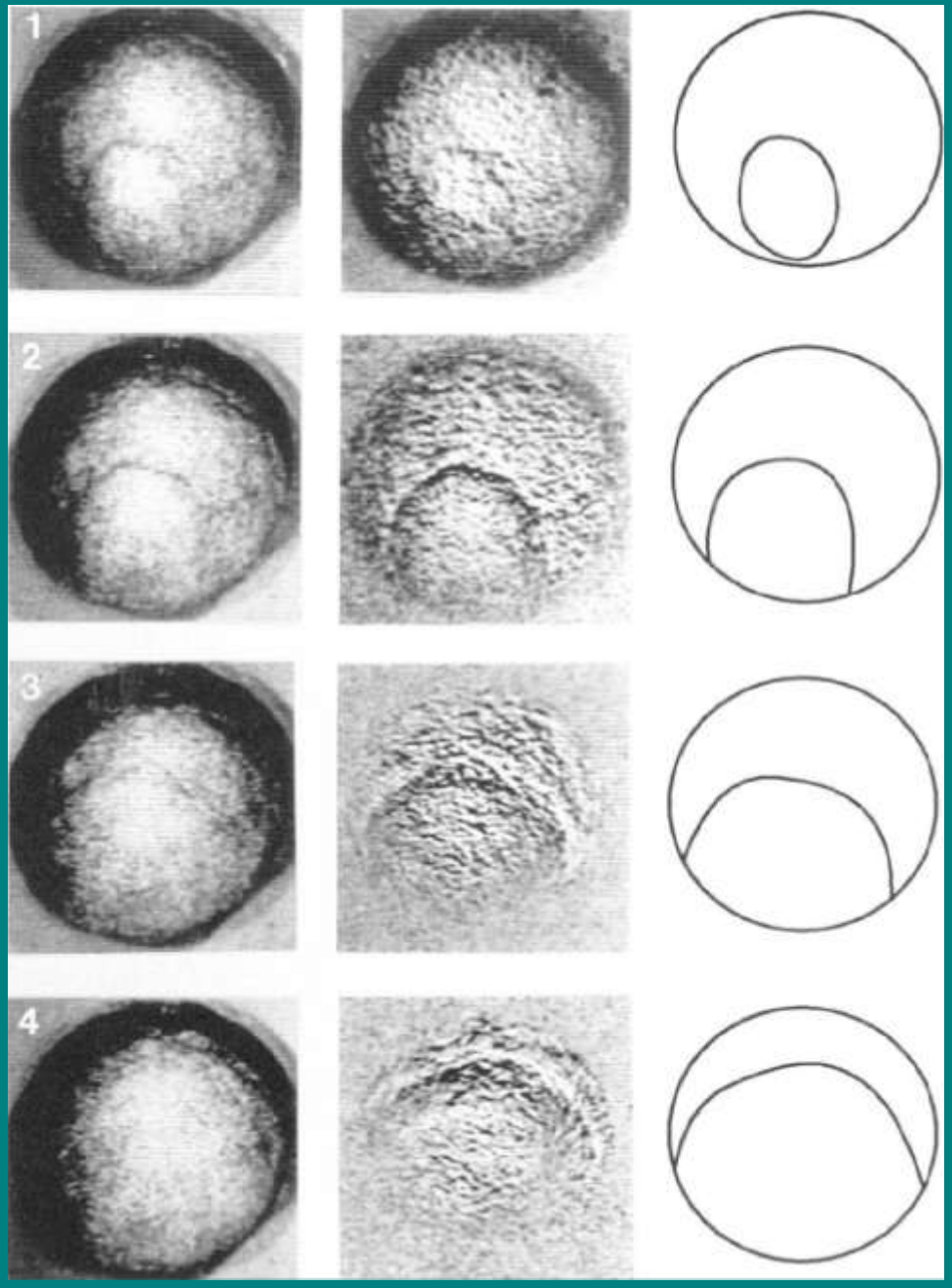
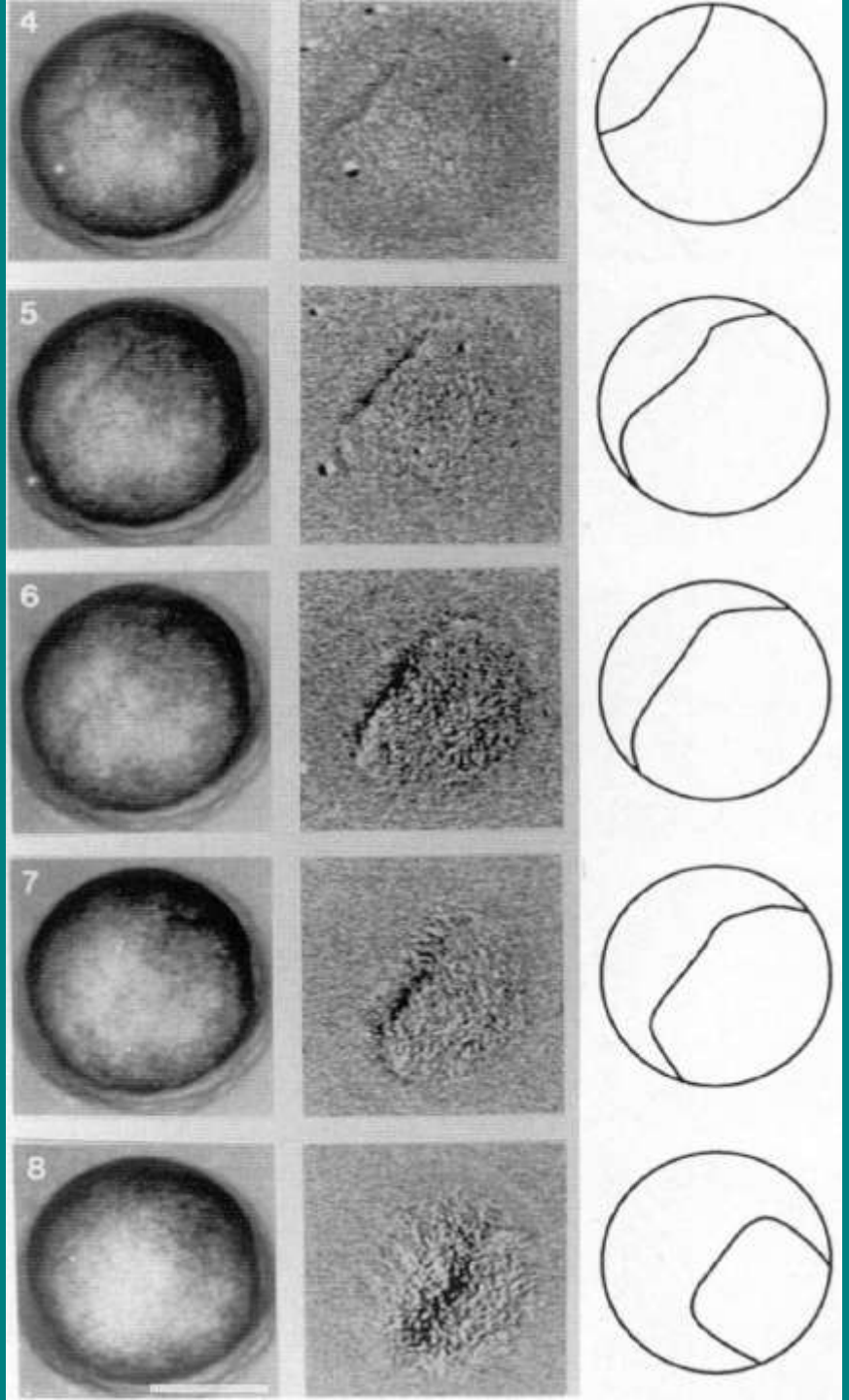
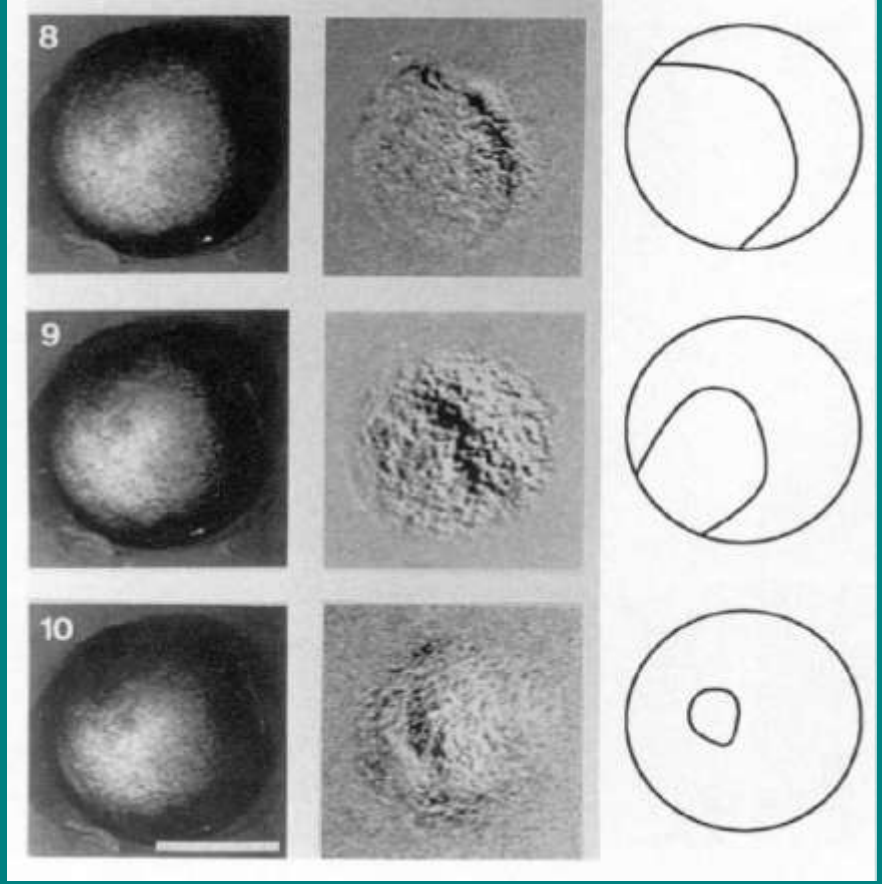


Image Processing

At hourly intervals, the image was digitally subtracted from the one 5 minutes earlier, showing the moving ectoderm contraction wave.

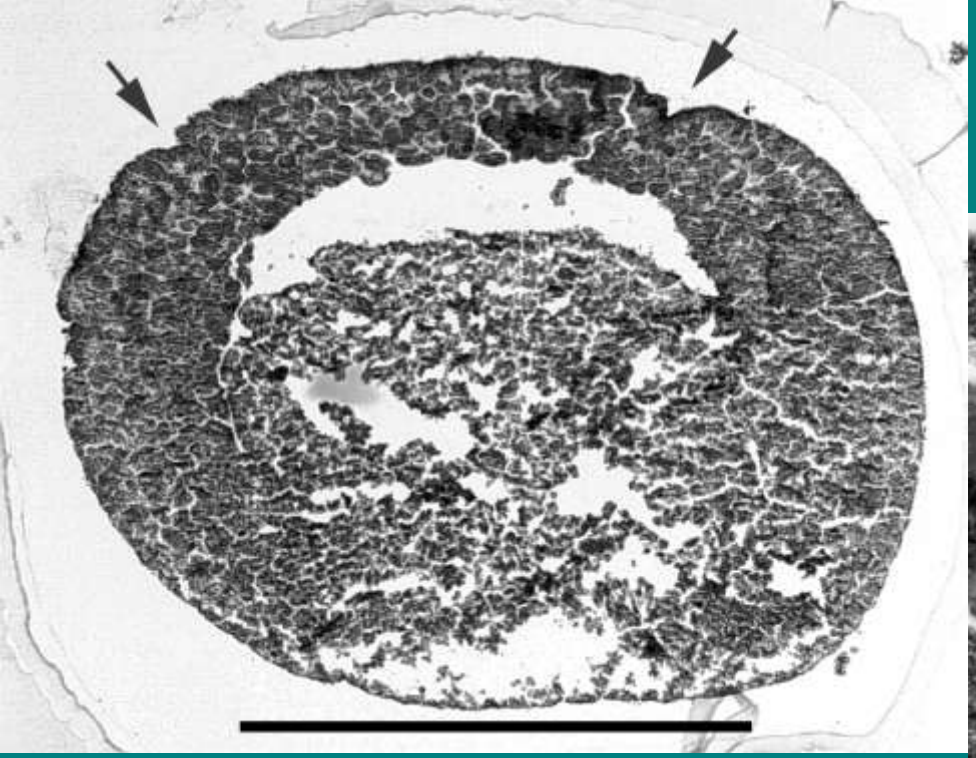
Brodland, G.W., R. Gordon, M.J. Scott, N.K. Björklund, K.B. Luchka, C.C. Martin, C. Matuga, M. Globus, S. Vethamany-Globus & D. Shu (1994). **Furrowing surface contraction wave coincident with primary neural induction in amphibian embryos**. *J. Morphol.* **219**(2), 131-142.

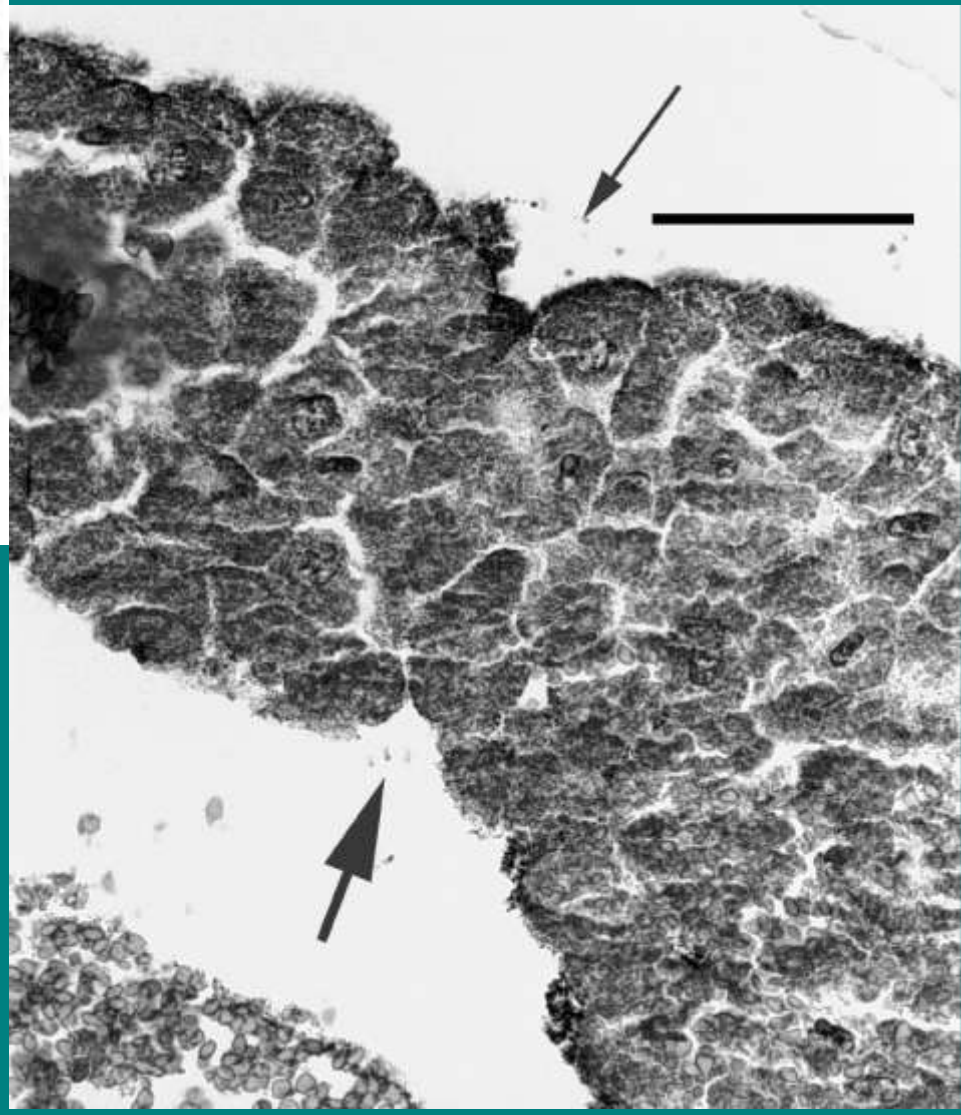




The arc shaped wave moves faster at its ends than in the middle, reforming a circle which then vanishes at what will be the anterior (head) end of the embryo. (These sets of images are from three different embryos.) Bar = 1 mm.

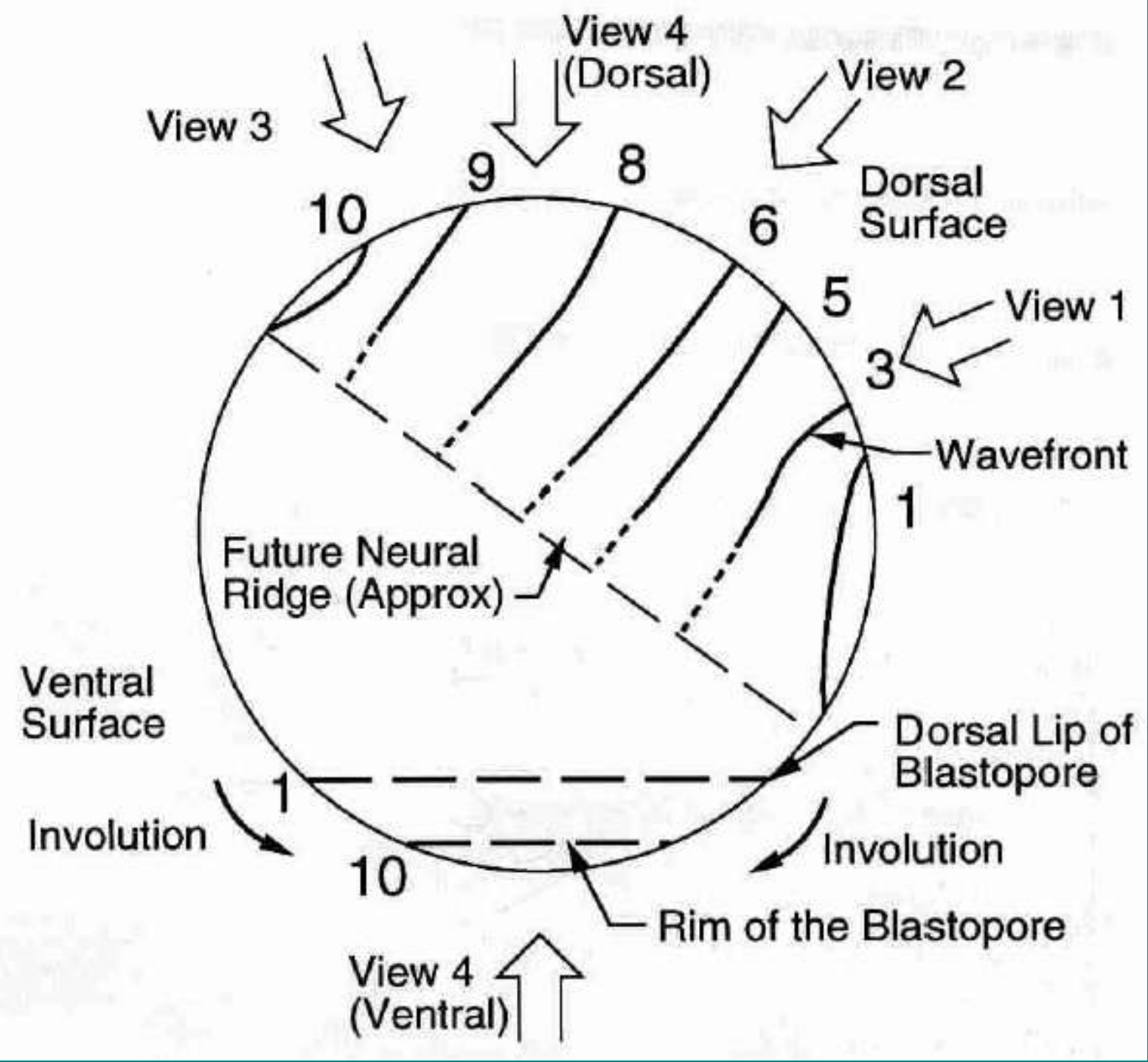
The Ectoderm Contraction Wave is a Morphogenetic Furrow





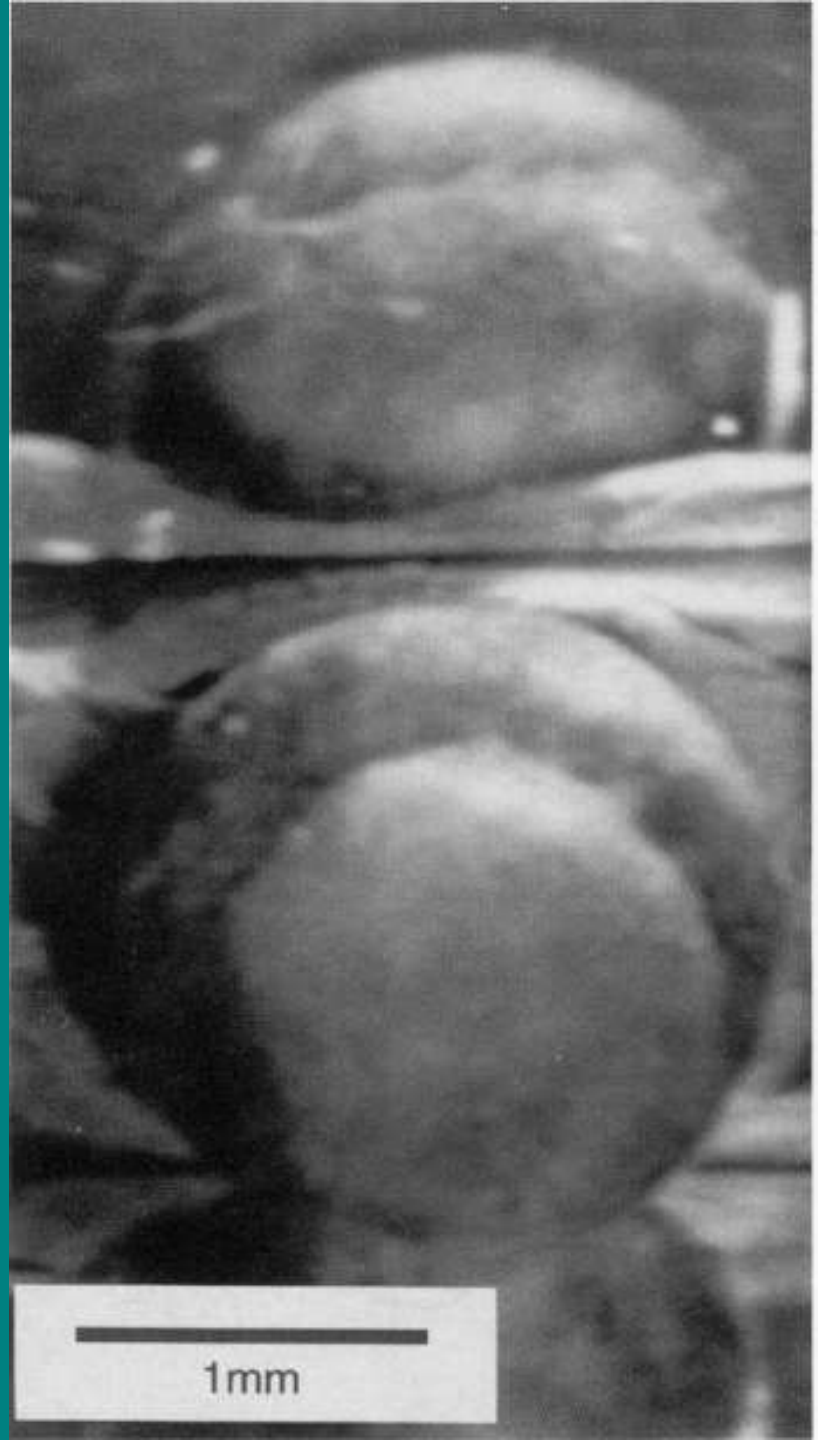
Microwave fixation developed in collaboration with Marc del Bigio, Natalie K. Björklund and Pierre Williot. Bars: 1 mm and 0.1 mm. There is a possibility (arrows on right) that furrowing occurs both on the apical and basal surfaces.

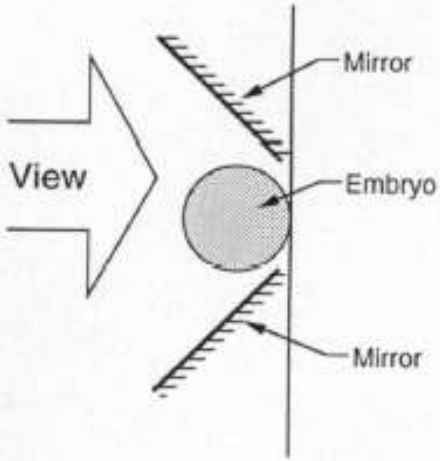
Gordon, R. (1999). *The Hierarchical Genome and Differentiation Waves: Novel Unification of Development, Genetics and Evolution*, Singapore: World Scientific and London: Imperial College Press, 2 vols.



Left side view during gastrulation. The wave takes 10 hours from start to finish.

Brodland, G.W., R. Gordon, M.J. Scott, N.K. Björklund, K.B. Luchka, C.C. Martin, C. Matuga, M. Globus, S. Vethamany-Globus & D. Shu (1994). **Furrowing surface contraction wave coincident with primary neural induction in amphibian embryos**. *J. Morphol.* **219**(2), 131-142.

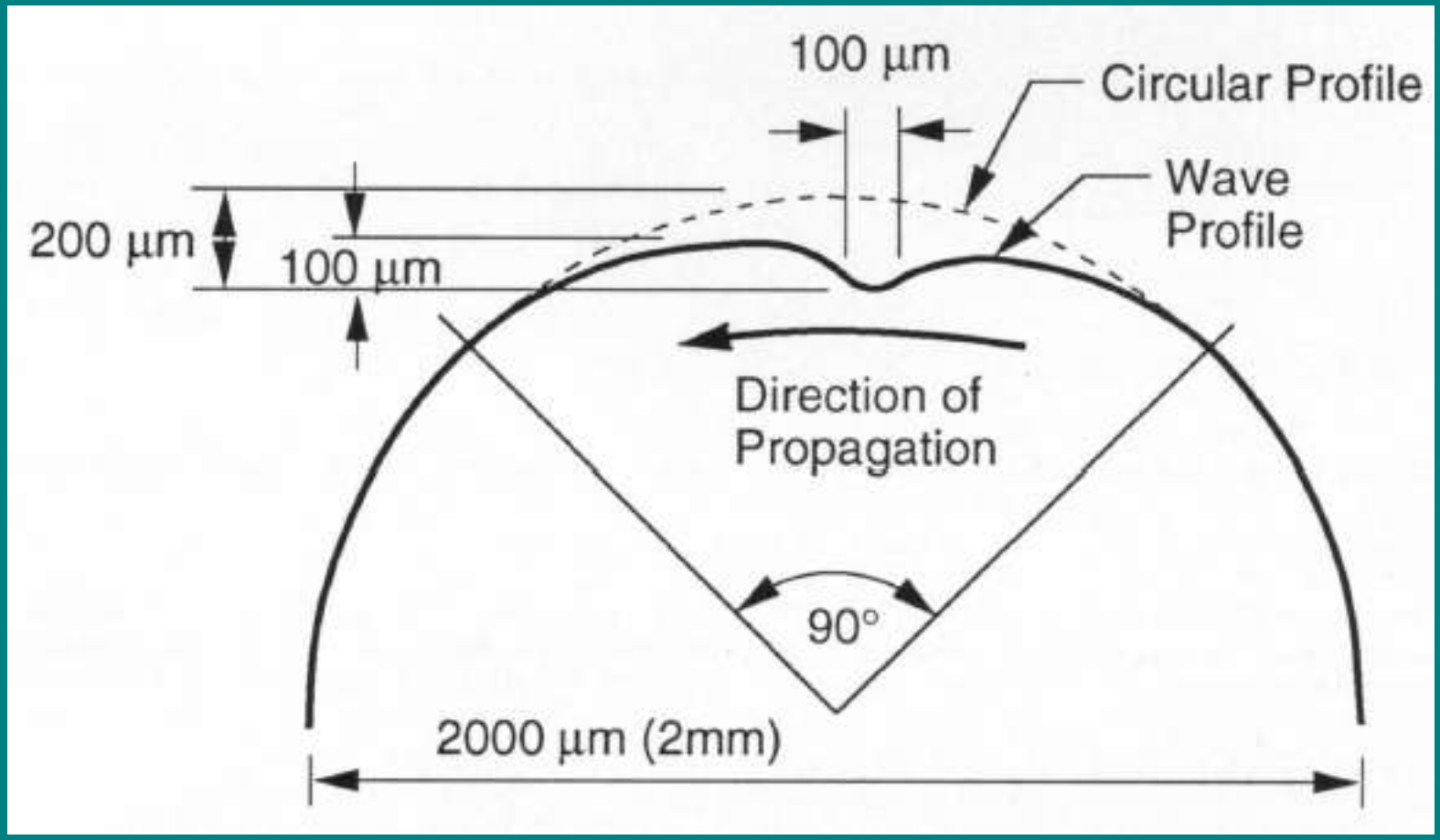




Front silvered mirrors show that the travelling furrow is an indent in the surface of the embryo.

Brodland, G.W., R. Gordon, M.J. Scott, N.K. Björklund, K.B. Luchka, C.C. Martin, C. Matuga, M. Globus, S. Vethamany-Globus & D. Shu (1994). **Furrowing surface contraction wave coincident with primary neural induction in amphibian embryos**. *J. Morphol.* **219**(2), 131-142.

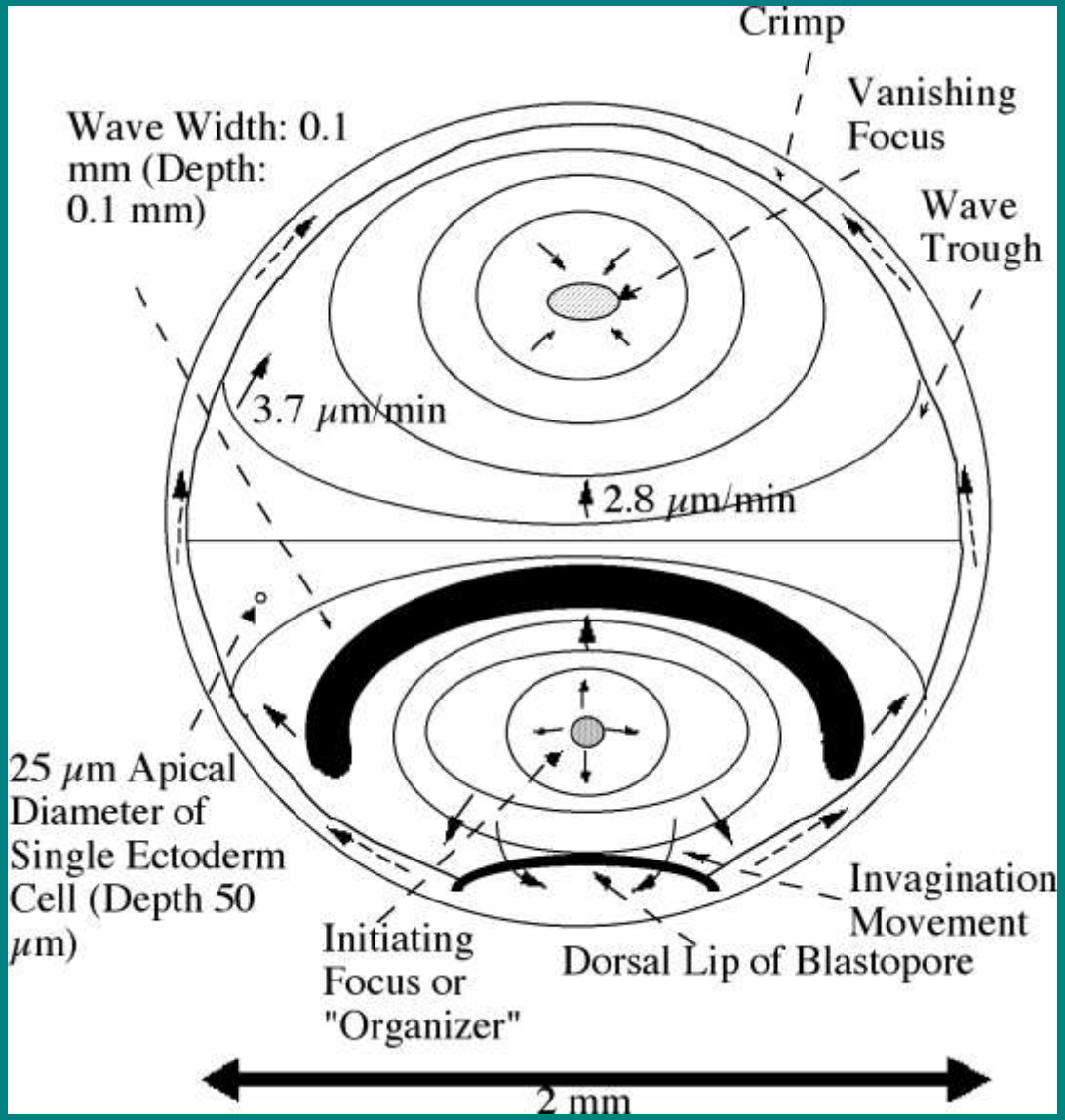
The Ectoderm Contraction Wave starts up the edge of the furrow. The furrow represents maximal apical contraction of the cells, which lasts about 10 minutes for each cell.



Brodland, G.W., R. Gordon, M.J. Scott, N.K. Björklund, K.B. Luchka, C.C. Martin, C. Matuga, M. Globus, S. Vethamany-Globus & D. Shu (1994). **Furrowing surface contraction wave coincident with primary neural induction in amphibian embryos**. *J. Morphol.* **219**(2), 131-142.

A Peculiar Trajectory: Why the Wave doesn't Turn the Whole Ectoderm into Brain

Does the invagination movement generate a strain field that restricts the wave to one hemisphere?



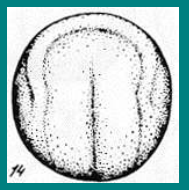
Head end

Tail end

Gordon, R., N.K. Björklund & P.D. Nieuwkoop (1994). Dialogue on embryonic induction and differentiation waves. *Int. Rev. Cytol.* **150**, 373-420.

Comments

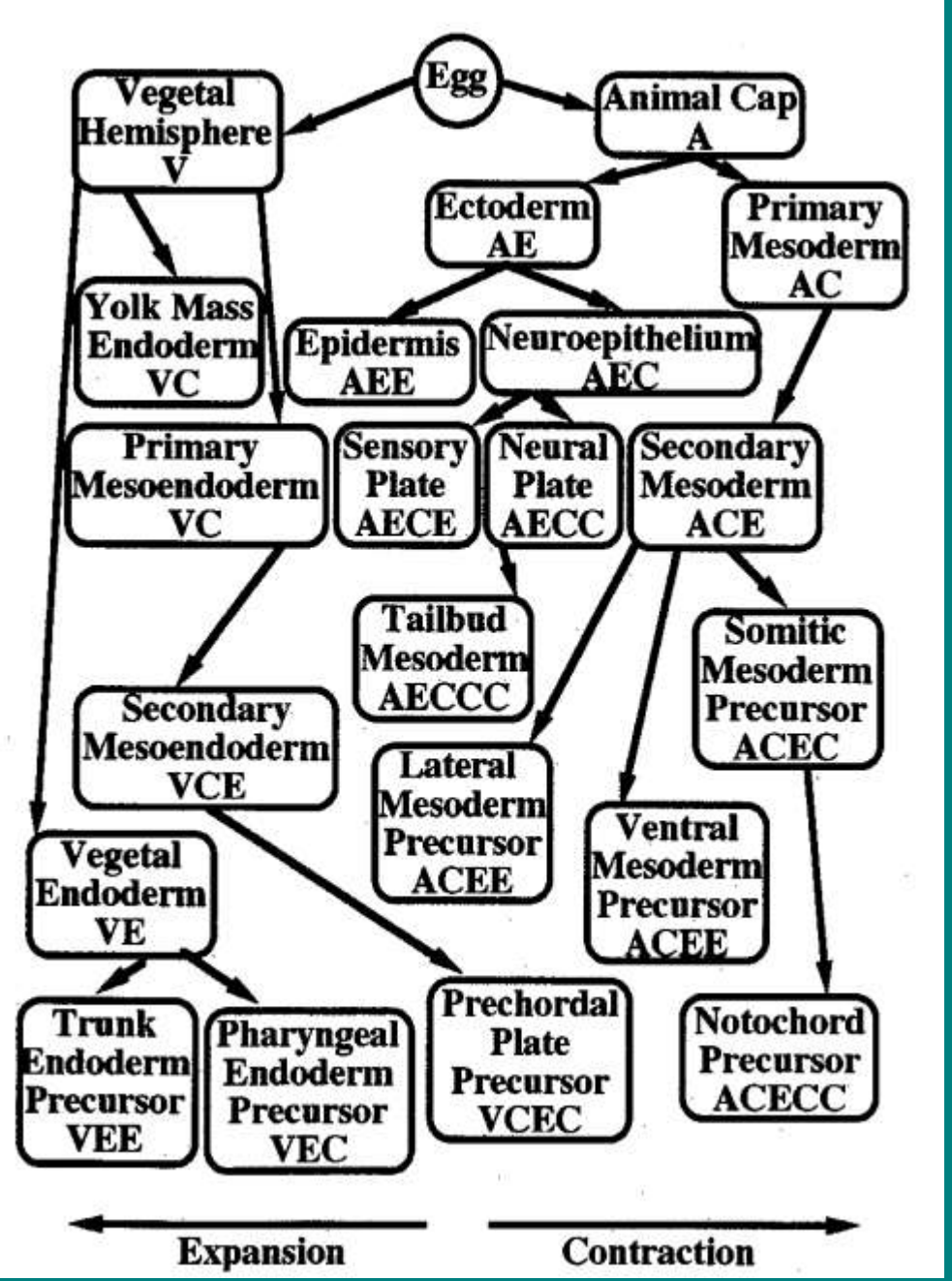
- The ectoderm contraction wave has a trajectory corresponding to what becomes the neural plate
- If it represents primary neural induction, then its launching site, not the dorsal lip of the blastopore, is the organizer of Spemann & Mangold
- The region covered by the wave may be taken as the physical representation of a "morphogenetic field"



Bordzilovskaya, N.P., T.A. Dettlaff, S.T. Duhon & G.M. Malacinski (1989). Developmental-stage series of axolotl embryos. In: Armstrong, J.B. & G.M. Malacinski, *Developmental Biology of the Axolotl*, New York: Oxford University Press, p. 201-219.

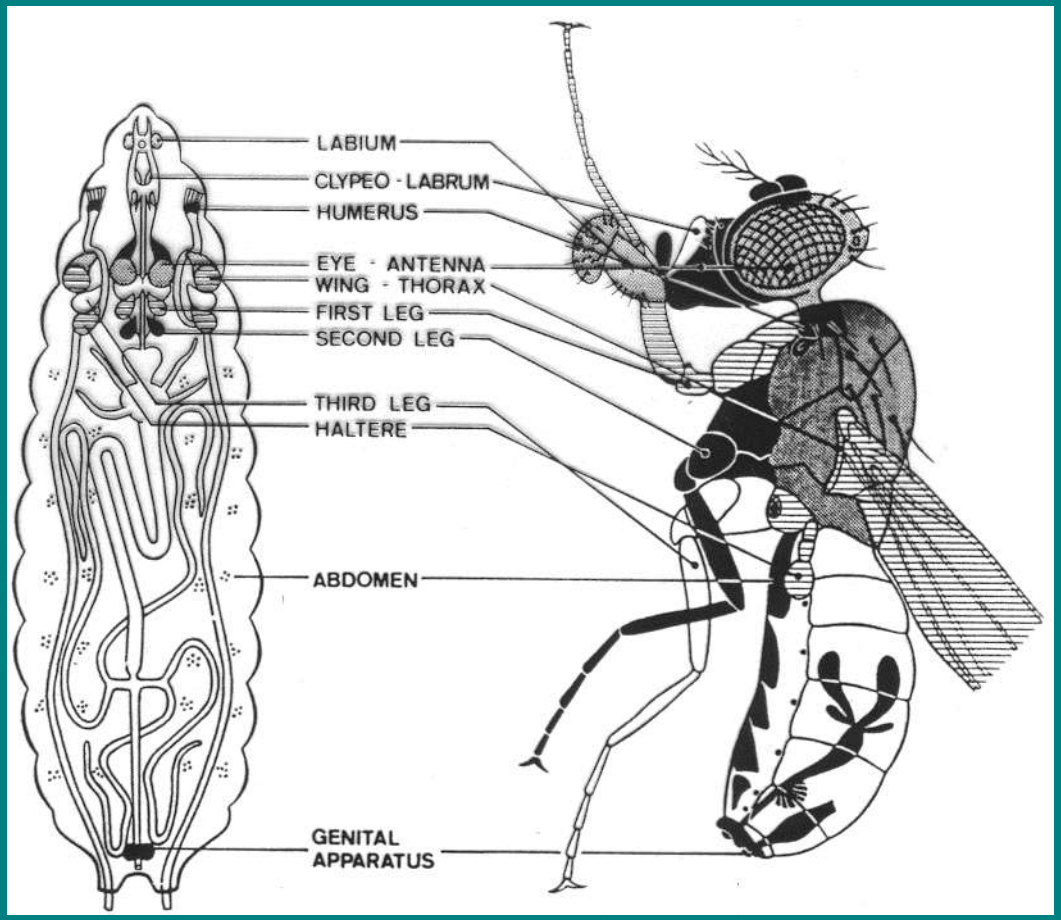
The Differentiation Tree and Code of the Axolotl through Neurulation

Björklund, N.K. & R. Gordon (1994). Surface contraction and expansion waves correlated with differentiation in axolotl embryos. I. Prolegomenon and differentiation during the plunge through the blastopore, as shown by the fate map. *Computers & Chemistry* **18**(3), 333-345.



Universality of Differentiation Waves: the *Drosophila* Eye Imaginal Disc

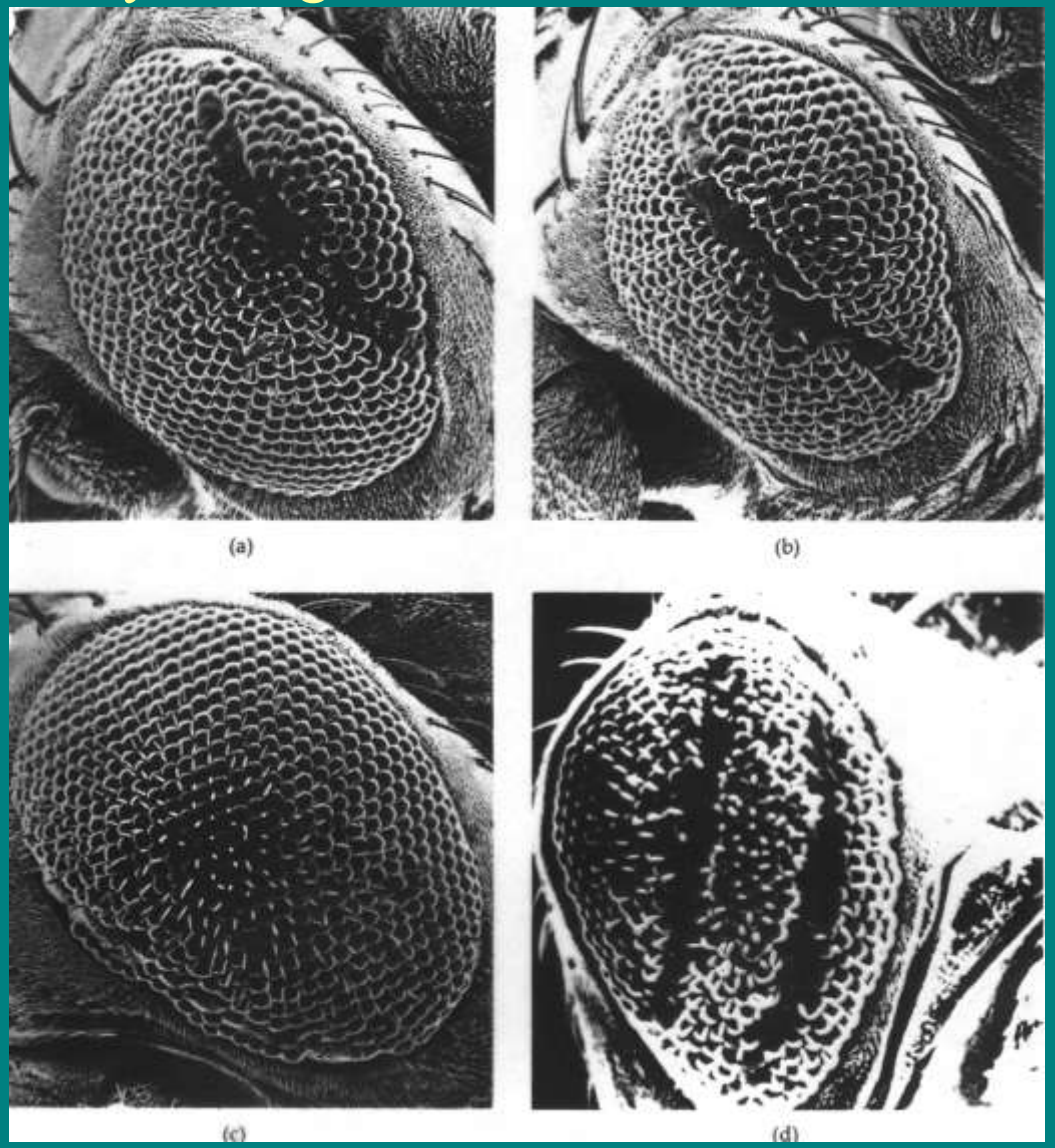
Imaginal discs in the insect larva (grub) form most of the adult parts, except for the brain



Nöthiger, R. (1972). The larval development of imaginal disks. In Ursprung, H. & R. Nöthiger, *The Biology of Imaginal Disks*, New York Springer-Verlag, p. 1-91.

Universality of Differentiation Waves: the *Drosophila* Eye Imaginal Disc

The temperature sensitive *shibire* mutation prevents ommatidia differentiation, but not wave propagation. Thus we are dealing with two signals: cell to cell propagation of the wave, and wave to nucleus to trigger differentiation.



Poodry, C.A., L. Hall & D.T. Suzuki (1973). Developmental properties of *shibire* ^{ts} a pleiotropic mutation affecting larval and adult locomotion and development. *Dev. Biol.* **32**(2), 373-386.

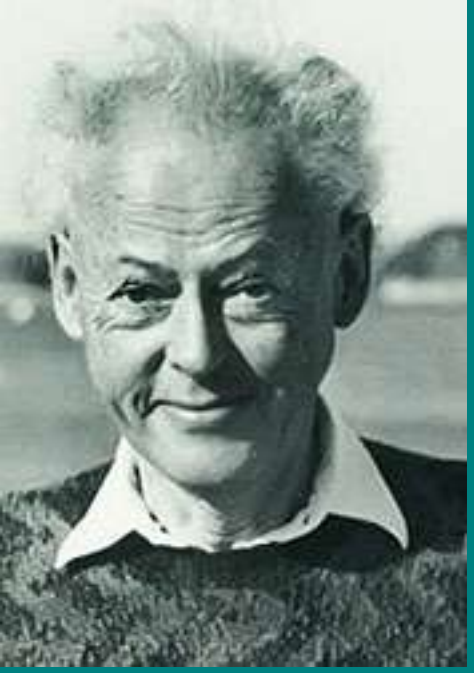
Suzuki, D.T. (1974). Behavior in *Drosophila melanogaster* a geneticist's view. *Can. J. Genet. Cytol.* **16**, 713-735.

Canadian content!

Universality of Differentiation Waves: Waves of comparable speed that might be involved in differentiation have been observed in:

- Zebrafish and goldfish retinas
- Sunflower heads
- Development of feathers and hairs
- Somites in frogs
- *Paramecium* (basal body differentiation)

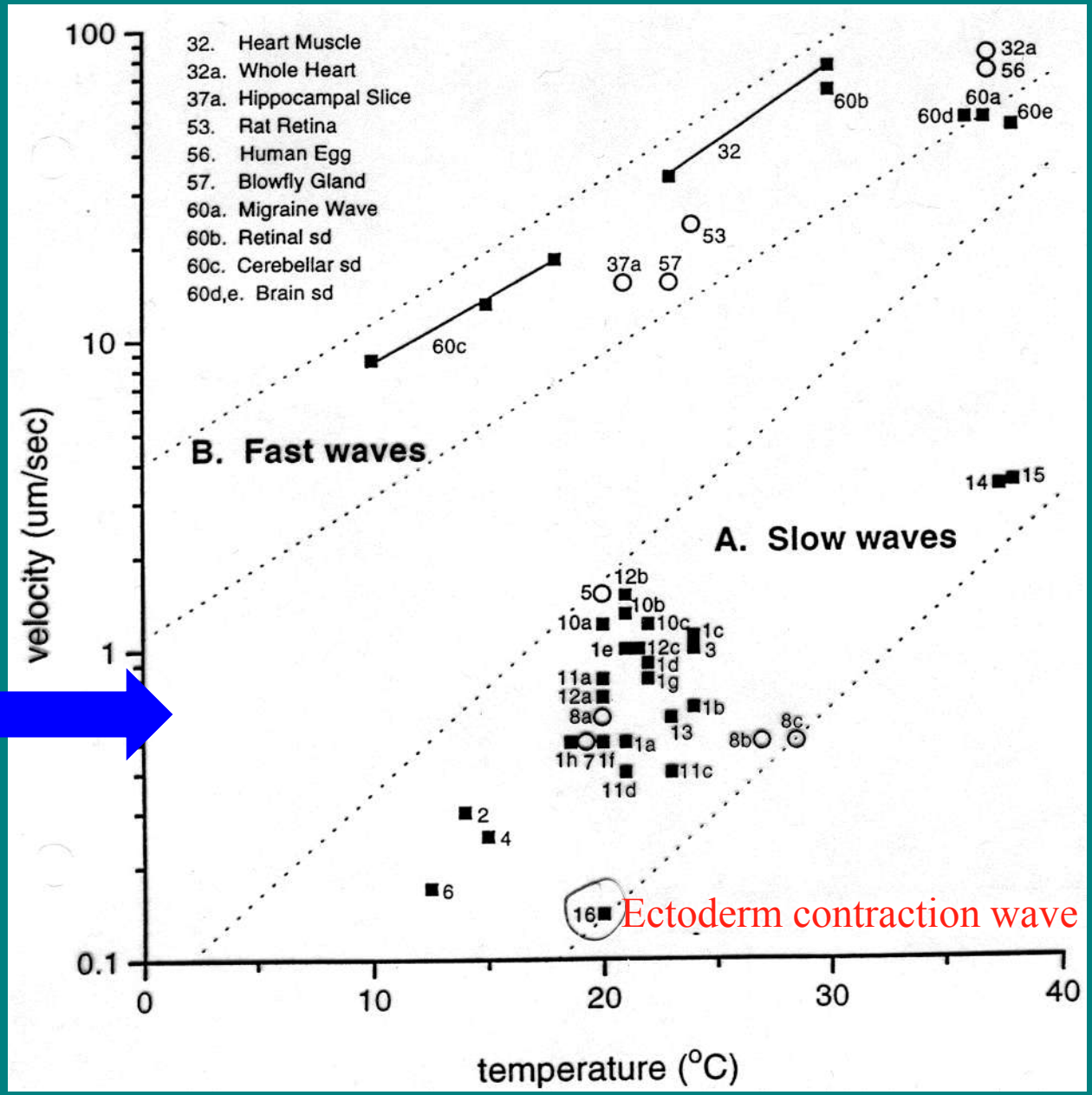
The last suggests that differentiation waves may predate the evolution of multicellular organisms



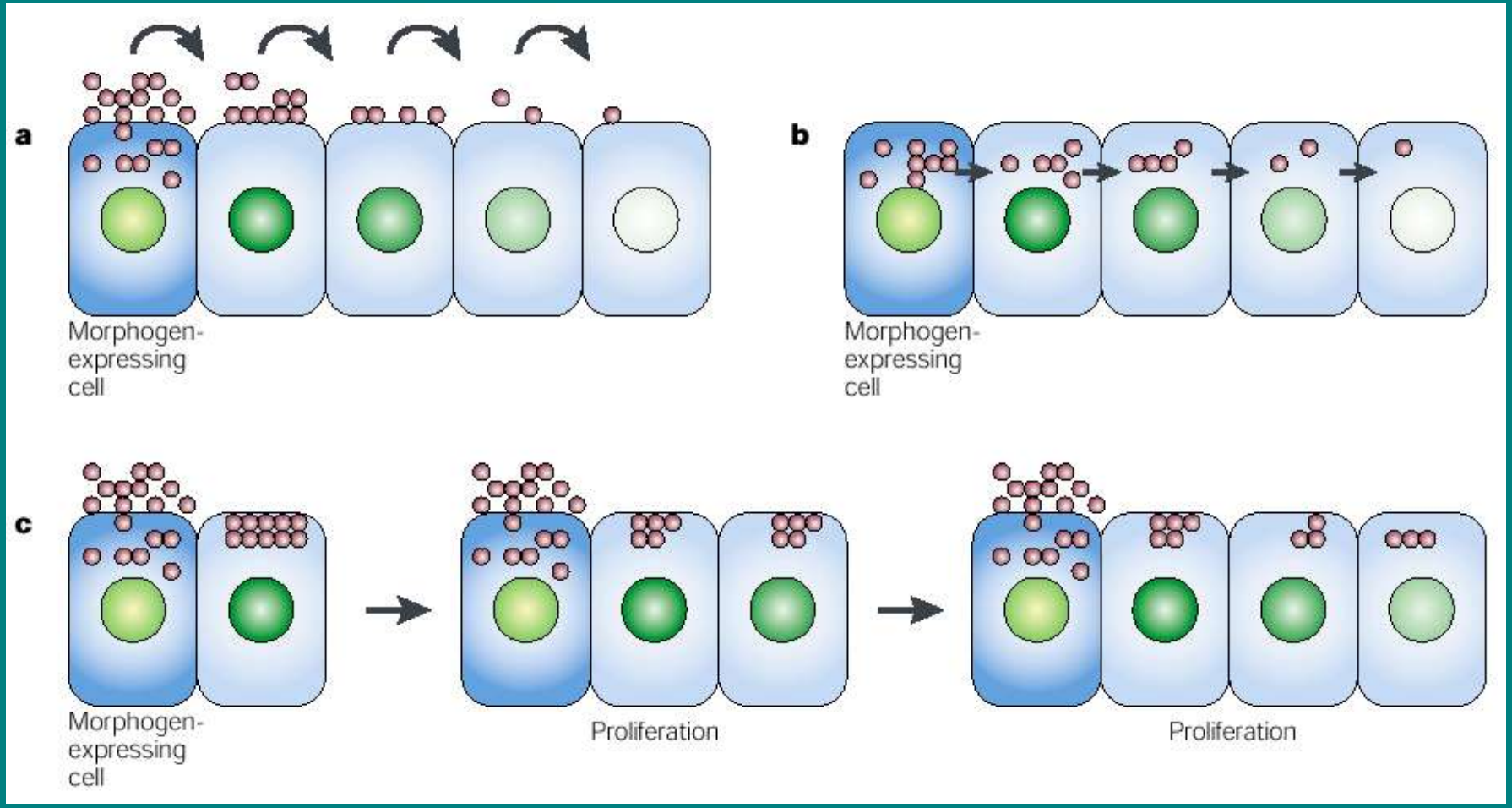
1927-2011

Calcium Waves in Development

Lionel Jaffe (1999). Organization of early development by calcium patterns. *BioEssays* **21**(8), 657-667.



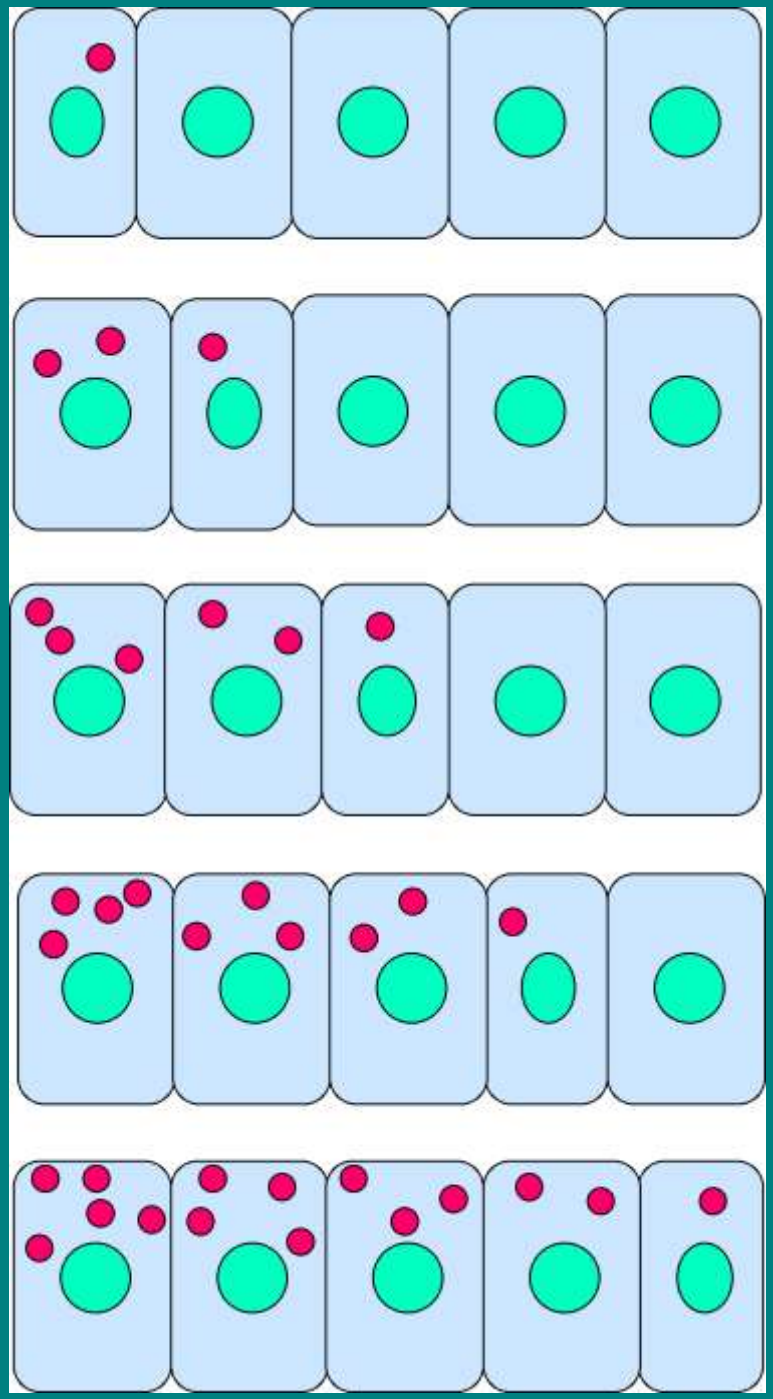
Today's Morphogen Hypotheses for Positional Information



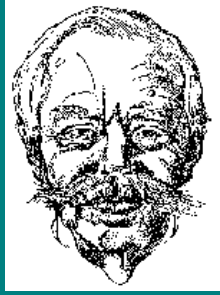
a) Diffusion through the extracellular space
b) Planar transcytosis
c) Displacement during growth

Tabata, T. (2001). Genetics of morphogen gradients. *Nat Rev Genet* **2**(8), 620-630.

The alternative: differentiation waves, with gradients as epiphenomena and *no positional information*: a cell does not "know" where it is. It merely responds to the differentiation waves in which it participates.



Jakob Johann von Uexküll's 1920 model anticipated differentiation waves



http://www.zbi.ee/~uexkull/cv.htm

"A gene or factor, then, is a ferment [enzyme, or, in modern language, an expressed gene] activated by an impulse [differentiation wave]....

An impulse-system can allow a whole series of cells to be simultaneously invaded by a fermentative action leading to a certain chemical change....

The number of cells within the mass is quite immaterial for the achievement of the final form....

The impulses... are fixed in space and time, but in themselves are still completely non-material."

[1]von Uexküll, J. (1926). *Theoretical Biology*, London Kegan Paul, Trench, Trubner & Co

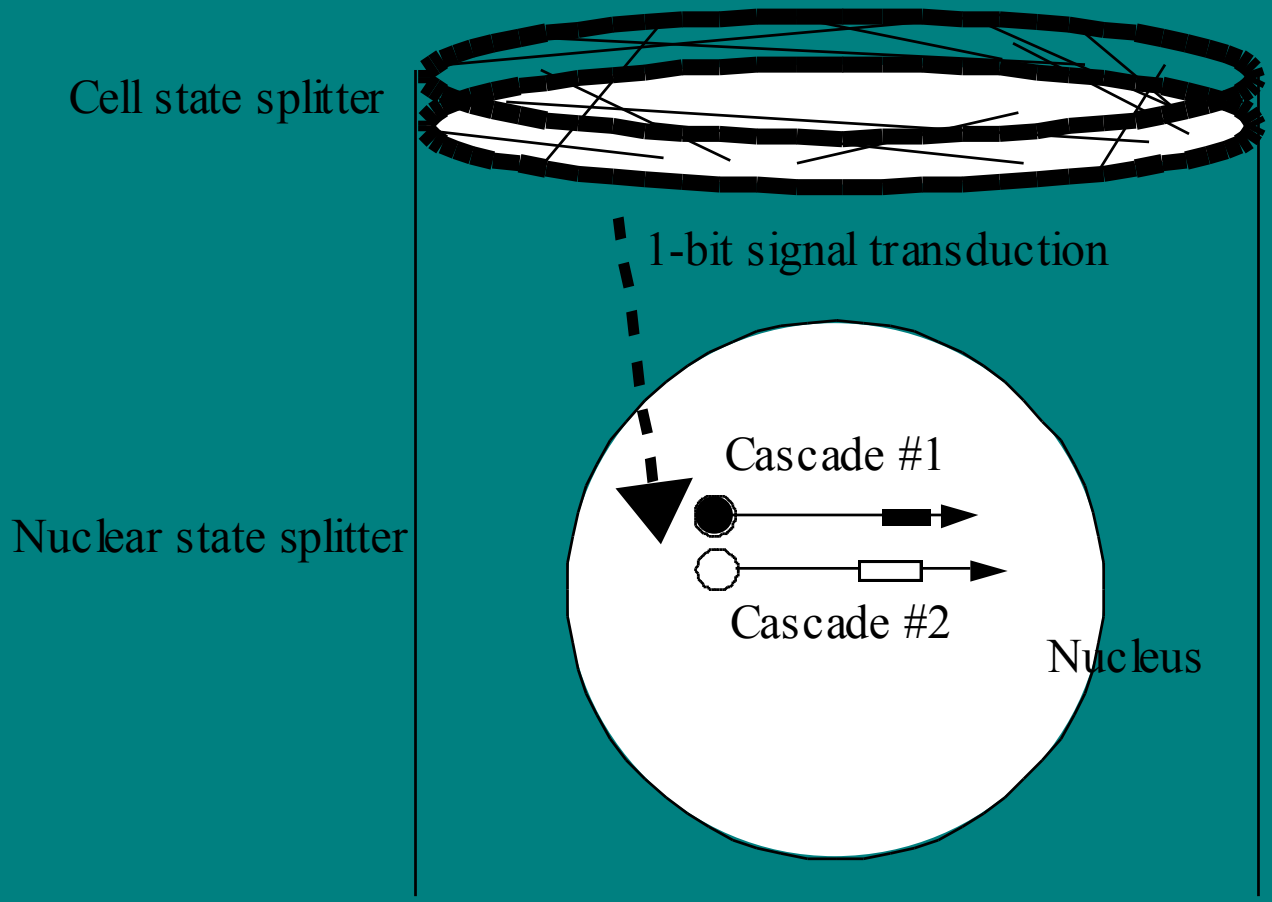
What's Going on in the Nucleus during Cell Differentiation?

- Hypothesis #1: Genetic networks have "basins of attraction" in a high dimensional space of reaction parameters, each basin corresponding to a state of cell differentiation
- Hypothesis #2: The nucleus has a different, discrete structure for each state of cell differentiation

Comments: Hypothesis #1 does not invoke any structure to the cell. It is the old biochemical model of the cell as a well stirred flask. A vast effort is under way to work out these genetic networks via DNA arrays and proteomics.

The Nuclear State Splitter

- *Hypothesis:*
- The nucleus has exactly two gene cascades ready to go.
- Which cascade is triggered depends on whether the cell just participated in a contraction wave or an expansion wave.



But what is it?

Toad Hall Toy Story (Winnipeg)

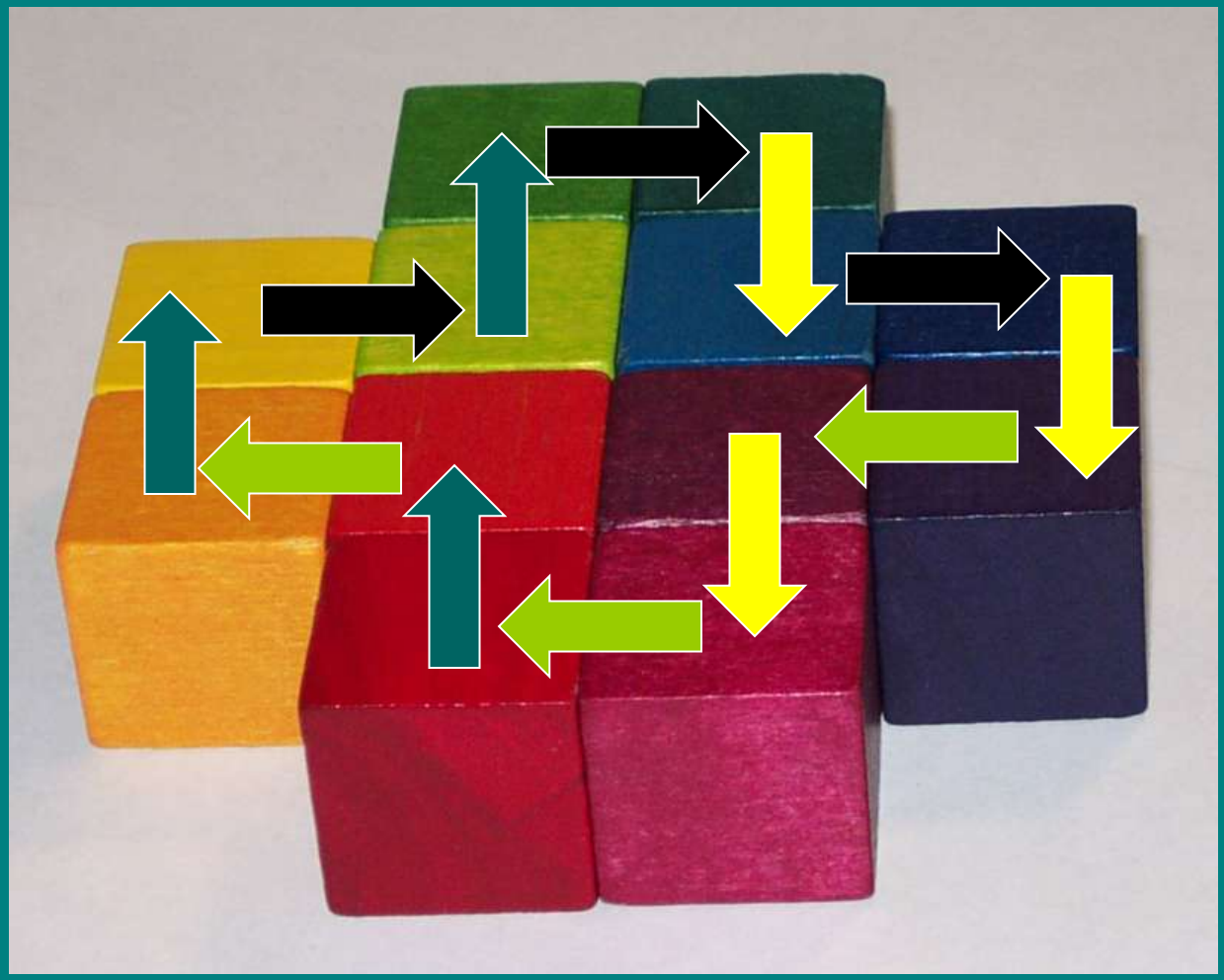
"May I help you?"

"I am looking for something that could be used to model functioning of the cell nucleus during embryogenesis."

"I think I'll leave you to it," the young clerk responded, backing away from Natalie as if she were insane.

The Wurfel

Invented by Pappa Geppetto's Toys Victoria Ltd., Victoria, Canada

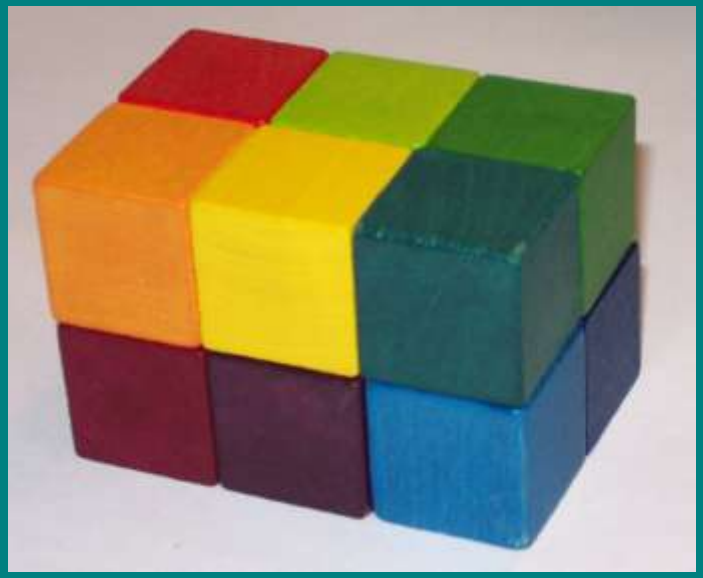


Arrows show the path of the taut elastic band through the blocks

A simple bracelet, but also a tensegrity structure

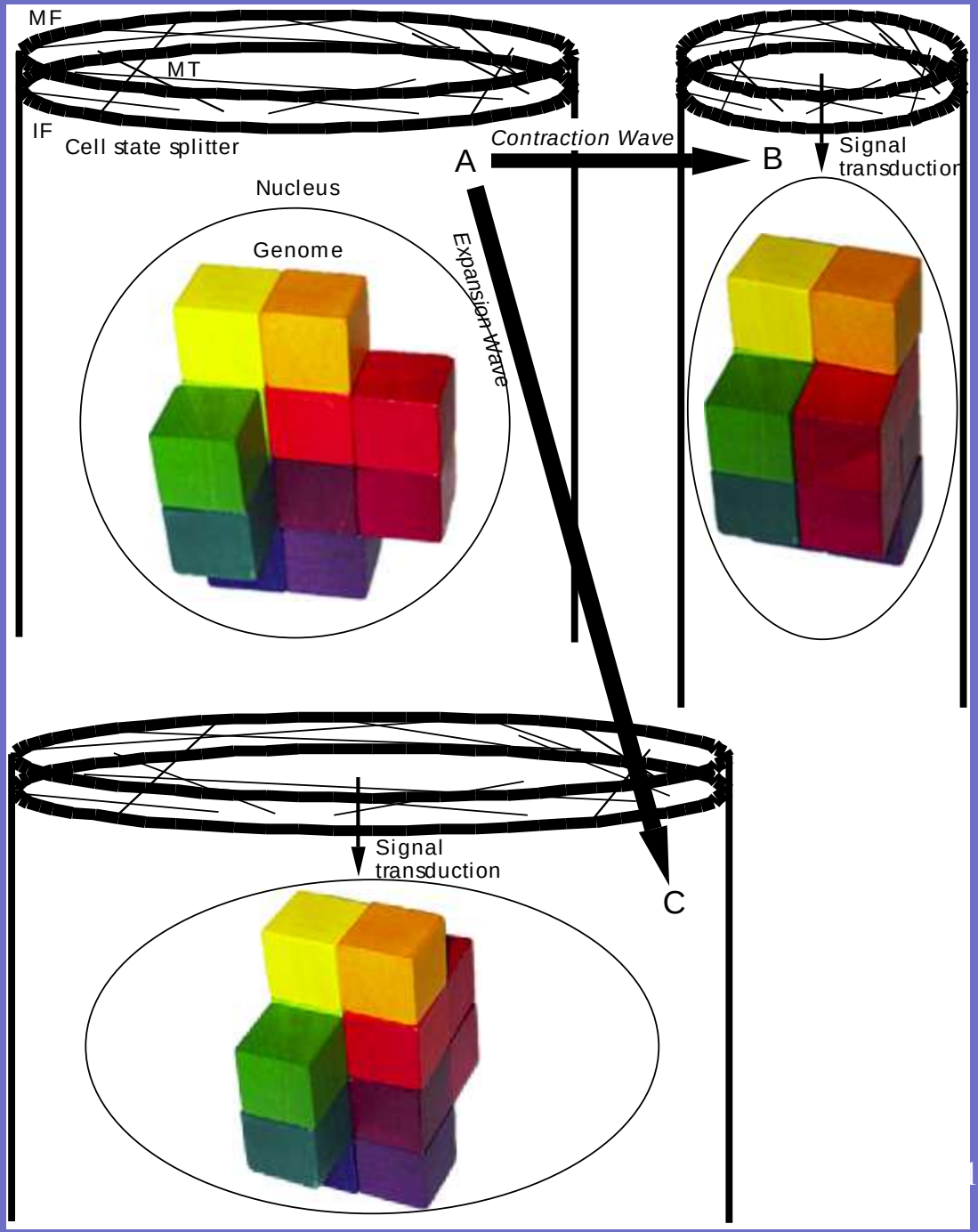
Canadian content!

More Wurfel Configurations



The Wurfel-like structure of the genome changes to one of two new configurations.

- This results in a change in gene expression, represented by the newly exposed and sequestered surfaces of the Wurfel.
- The change in genome structure may be reflected by a change in shape of the nucleus.



Evidence for a Role for the "Wholeness" of the Genome

- Tugging on one chromosome in a cell allows one to pull out all the chromosomes as clumps on a DNA string[1]
- Chromosomes have nonrandom positions in the nucleus[2]
- When two cells are fused to form a heterokaryon, certain sets of chromosomes are eliminated in the first few cell divisions[3]
- Chromosomes in nuclei in epithelia have a specific orientation with respect to the apical and basal surfaces of the cells[4]

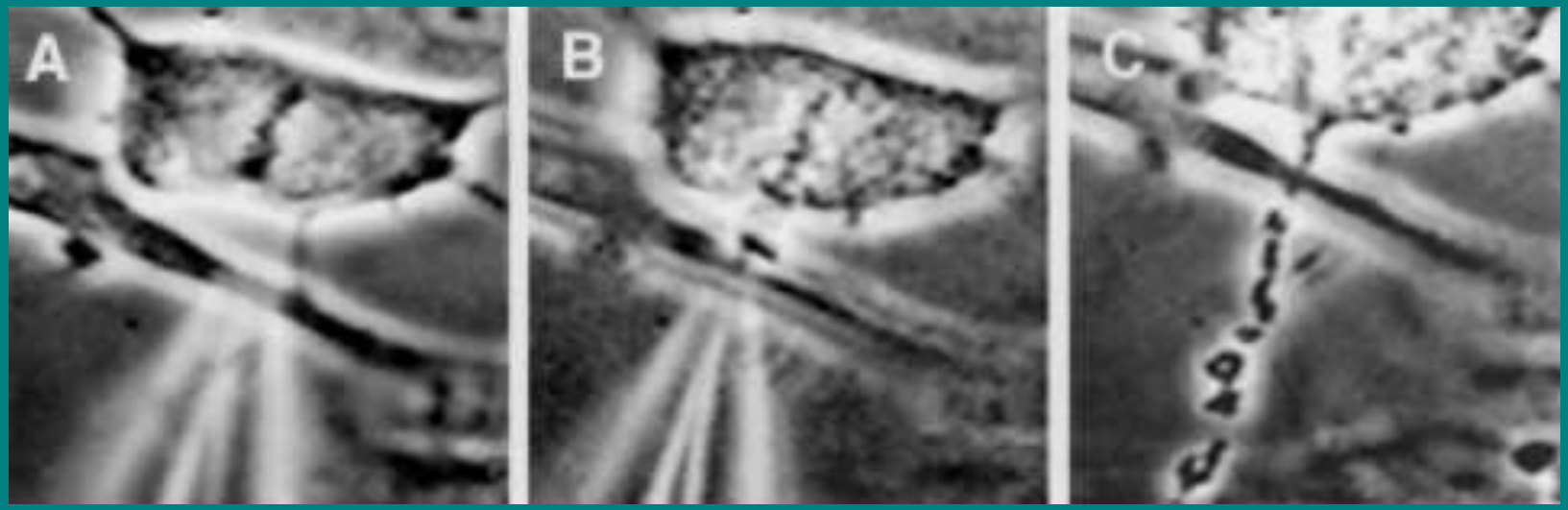
[1]Maniotis, A.J., K. Bojanowski & D.E. Ingber (1997). Mechanical continuity and reversible chromosome disassembly within intact genomes removed from living cells. *J. Cell Biochem.* **65**(1), 114-130.

[2]Nagele, R.G., T. Freeman, J. Fazekas, K.M. Lee, Z. Thomson & H.Y. Lee (1998). Chromosome spatial order in human cells evidence for early origin and faithful propagation. *Chromosoma* **107**(5), 330-338.

[3]Harris, H. (1995). *The Cells of the Body, A History of Somatic Cell Genetics*, Plainview, NY Cold Spring Harbor Laboratory Press.

[4]Francis-Lang, H., I. Davis & D. Ish-Horowicz (1996). Asymmetric localization of *Drosophila* pair-rule transcripts from displaced nuclei evidence for directional nuclear export. *EMBO J* **15**(3), 640-649.

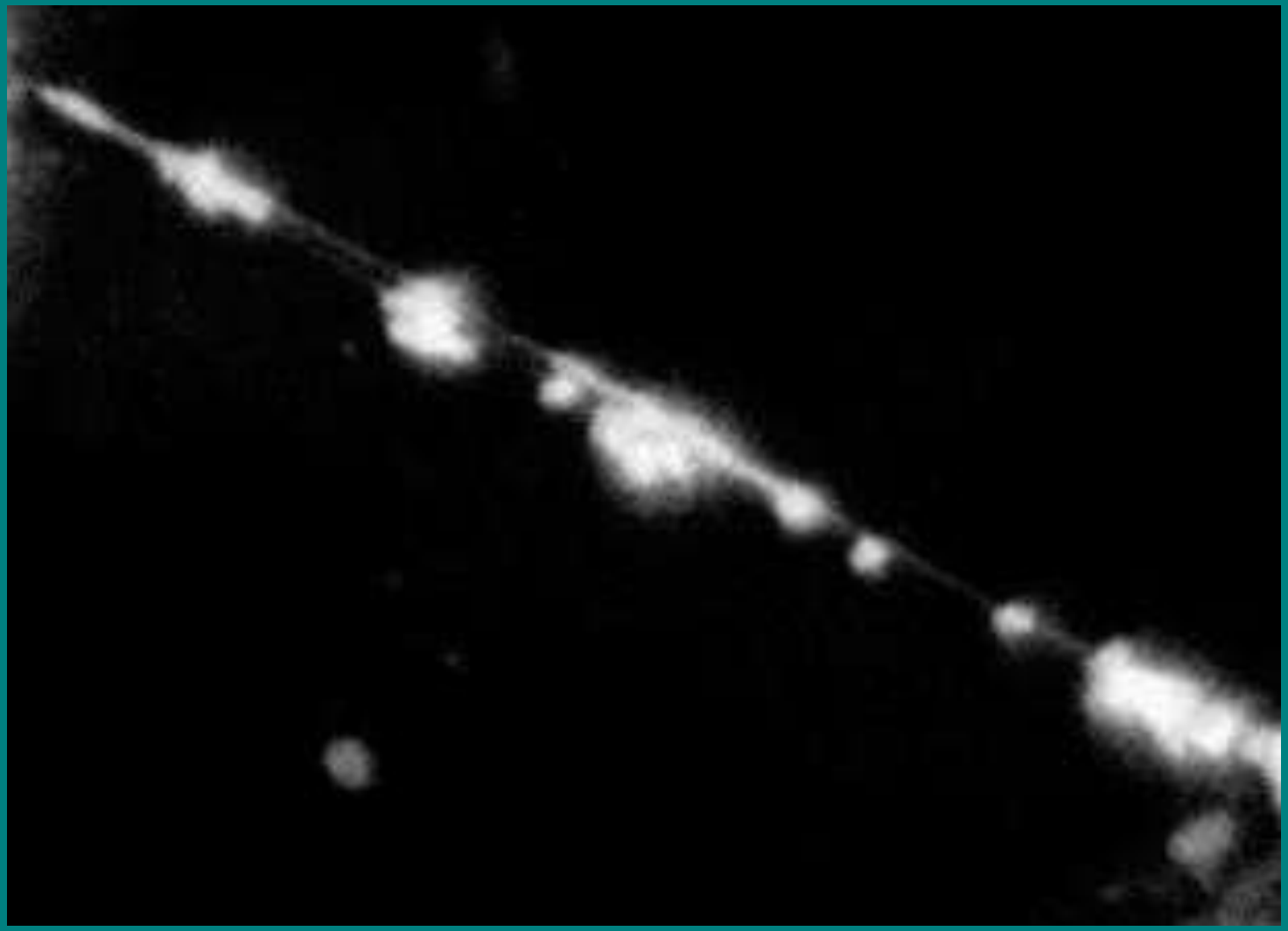
Pulling the Genome out of a Cell



By Andrew J. Maniotis, in:
Glanz, J. (1997). Force-carrying web pervades living cell. *Science* **276**(5313), 678-679.

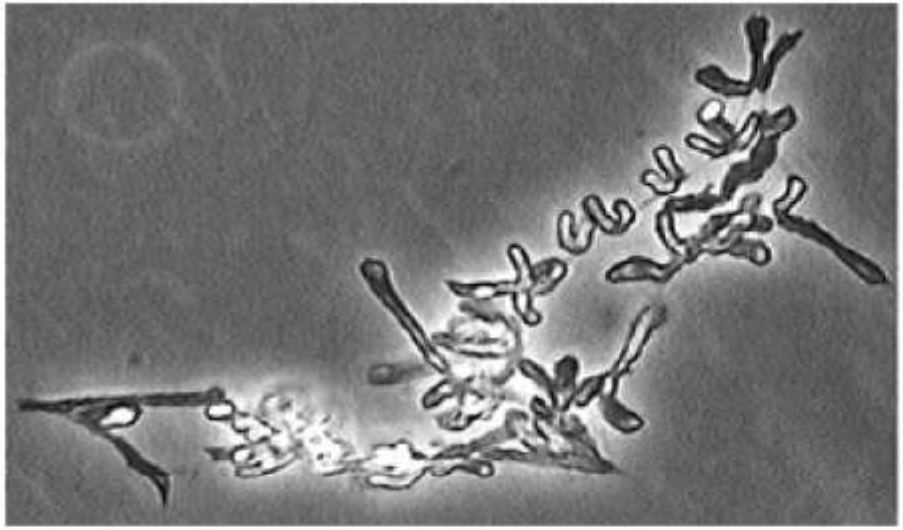
A Stretched Human Genome

Links are DNA

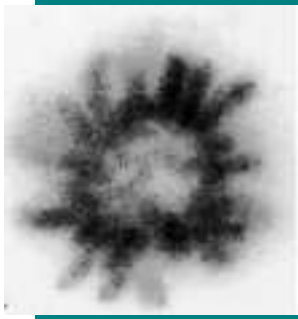


Maniotis, A.J., K. Bojanowski & D.E. Ingber (1997). Mechanical continuity and reversible chromosome disassembly within intact genomes removed from living cells. *J. Cell Biochem.* **65**(1), 114-130.

Better Technique Shows that (Mouse 3T3) Chromosomes
are Linked at their Centromeres
(from Andrew J. Maniotis, University of Illinois at Chicago).

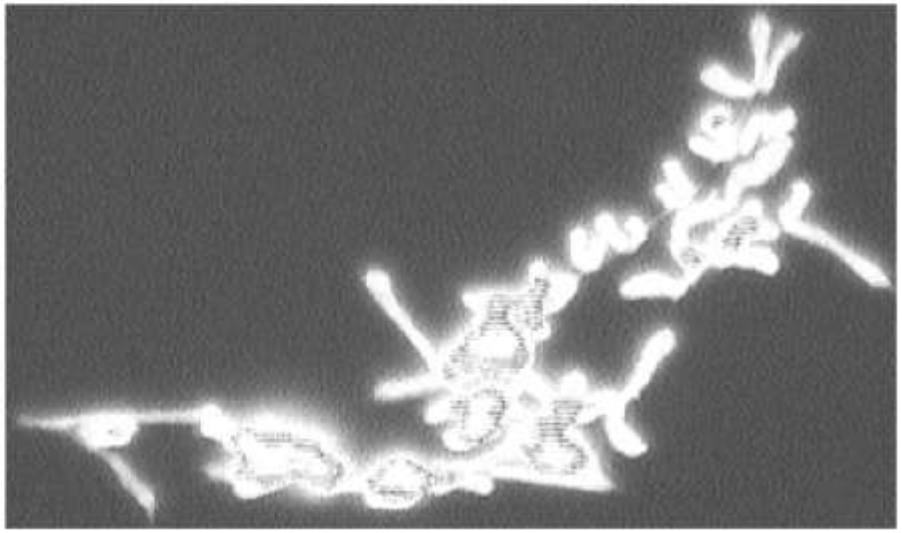


Prophase newt genome



A rosette arrangement of chromosomes persists throughout mitosis: Nagele, R.G., T. Freeman, J. Fazekas, K.M. Lee, Z. Thomson & H.Y. Lee (1998). Chromosome spatial order in human cells evidence for early origin and faithful propagation. *Chromosoma* **107**(5), 330-338.

Prophase newt genome
after 4ul bis benzamide
under flourescent light

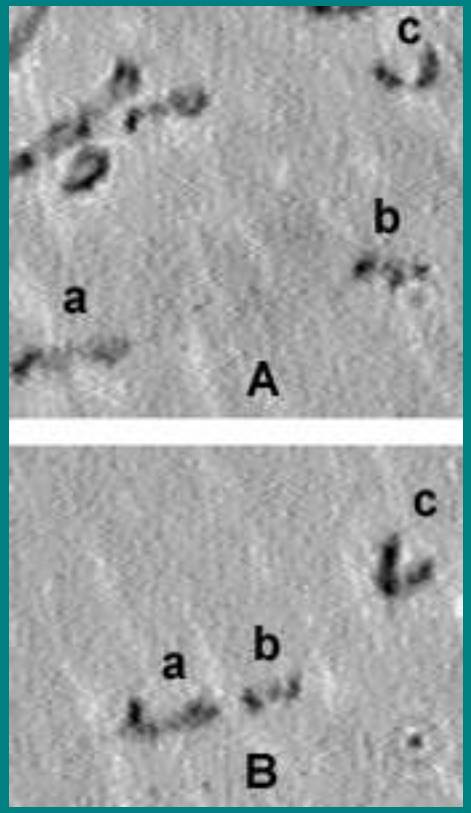


The individual chromosomes are

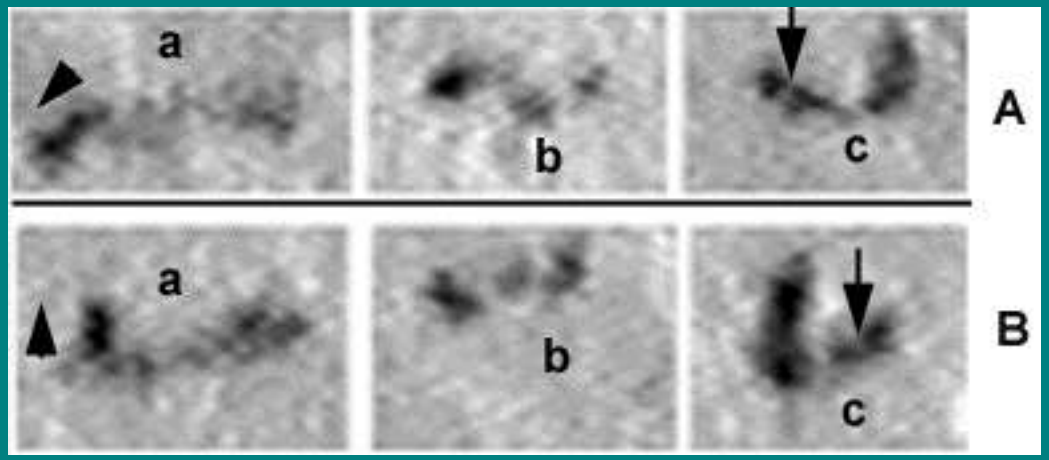
about 5 µm long.

Chromolinkers contain DNA. A whole newt genome (*Notophthalmus viridescens*) from a lung cell showing interchromosomal links ("chromolinkers") possibly forming a loop of all of the chromosomes. The chromolinkers, connecting at the centromeres, stain for DNA and are broken by restriction enzymes (from Andrew J. Maniotis, University of Illinois at Chicago).

Chromosomes are Linked in a Particular Order



Two cells of different kinds from the same organism show three chromosomes in the same order (from Andrew J. Maniotis, University of Illinois at Chicago).

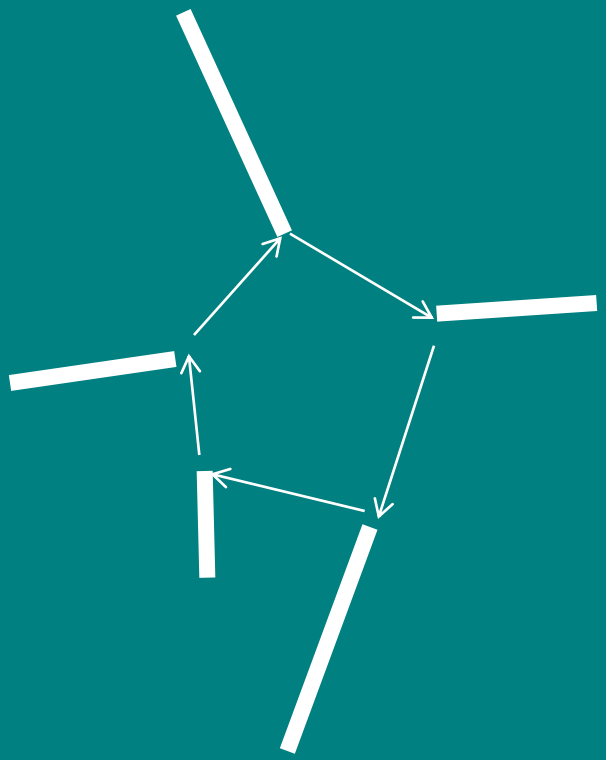


The two sets of chromosomes thresholded, with number of pixels:

Note: the extra chromosome 21 in human Trisomy 21 stays near the other chromosome 21:

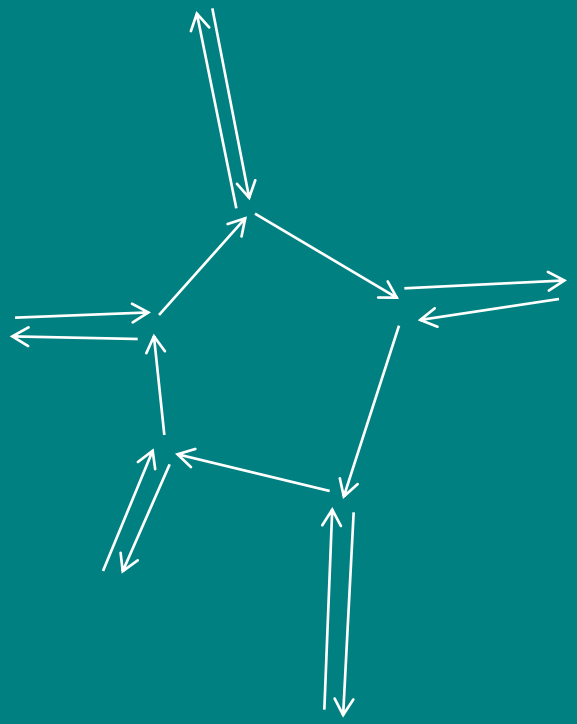
Nagele, R.G., T. Freeman, J. Fazekas, K.M. Lee, Z. Thomson & H.Y. Lee (1998). Chromosome spatial order in human cells evidence for early origin and faithful propagation. *Chromosoma* **107**(5), 330-338.

Models for Chromolinkers



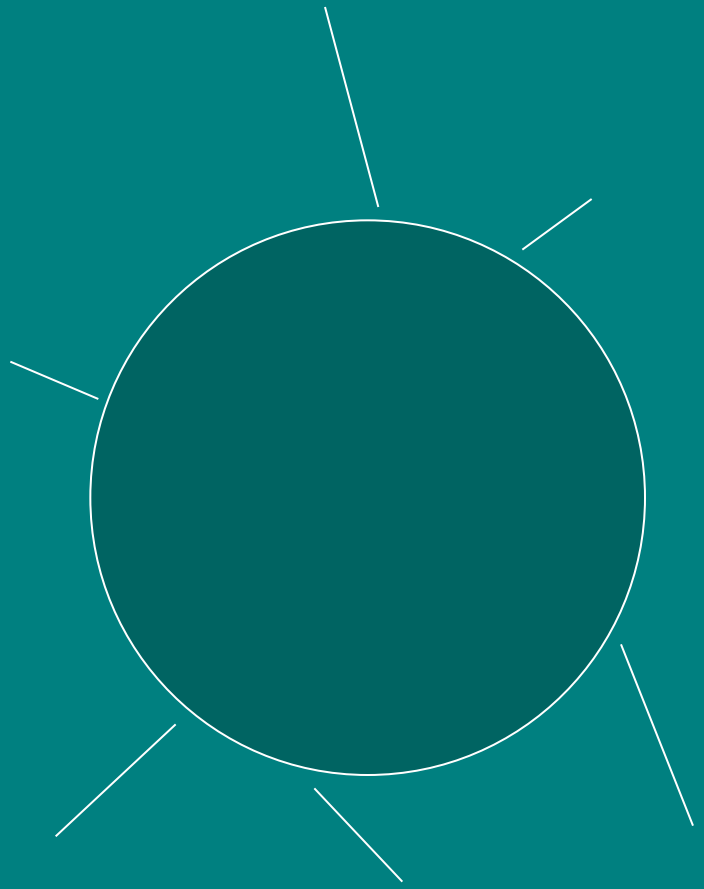
- Chromolinkers are extensions of chromosomes with specific binding sites to adjacent chromosomes
- Chromolinkers are separate DNA strands with specific binding sites to two specific chromosomes, at either end

Models for Chromolinkers



Chromolinkers are covalently integrated with the chromosomes so that the whole genome is a single loop of DNA

Models for Chromolinkers



Chromolinker is a separate loop of DNA to which the chromosomes are attached at specific sites

Why do Chromolinkers Exist?

- Represent intercentromeric connectors which mediate the rigid ordering of chromosomes in the prophase rosette
- Elaboration of telomeres that protect chromosomal breakage and end to end fusion of chromosomes
- A half genome-wide tension-sensing mechanism associated with the proper functioning of the metaphase-anaphase checkpoint
- Prevent entanglement of chromosomes

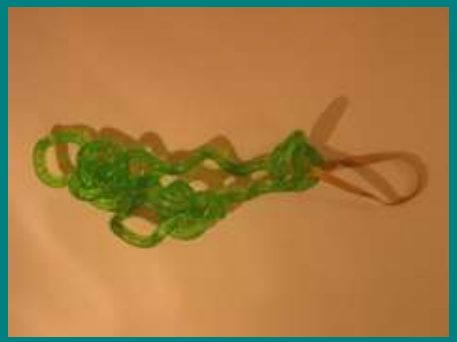
Model of Entanglement

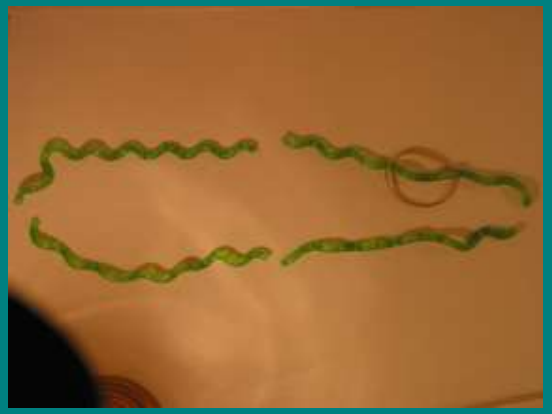
- Comes from reading:
- Qian, R. (2003). *Perspectives on the Macromolecular Condensed State*, Singapore World Scientific.
- Which is about the equilibrium and nonequilibrium properties of plastics

Full ring
(no ends)

Compacted and pulled out





4 Chromosomes

Compacted, more difficult to pull out, and left entangled

Evidence for a Role for the "Wholeness" of the Genome *in Cell Differentiation*

- Cells of the same kind have nuclei with the same shape
- Cells of the same kind in different species have nuclei with similar shapes
- Cells of different kinds that are closely related by lineage nevertheless can have nuclei of very different shapes
- The shape of the nucleus is a diagnostic characteristic in pathology
- Muntjak deer with different numbers of chromosomes look much the same

Whole Nucleus Structure Depends on Cell Type

Nuclear matrices

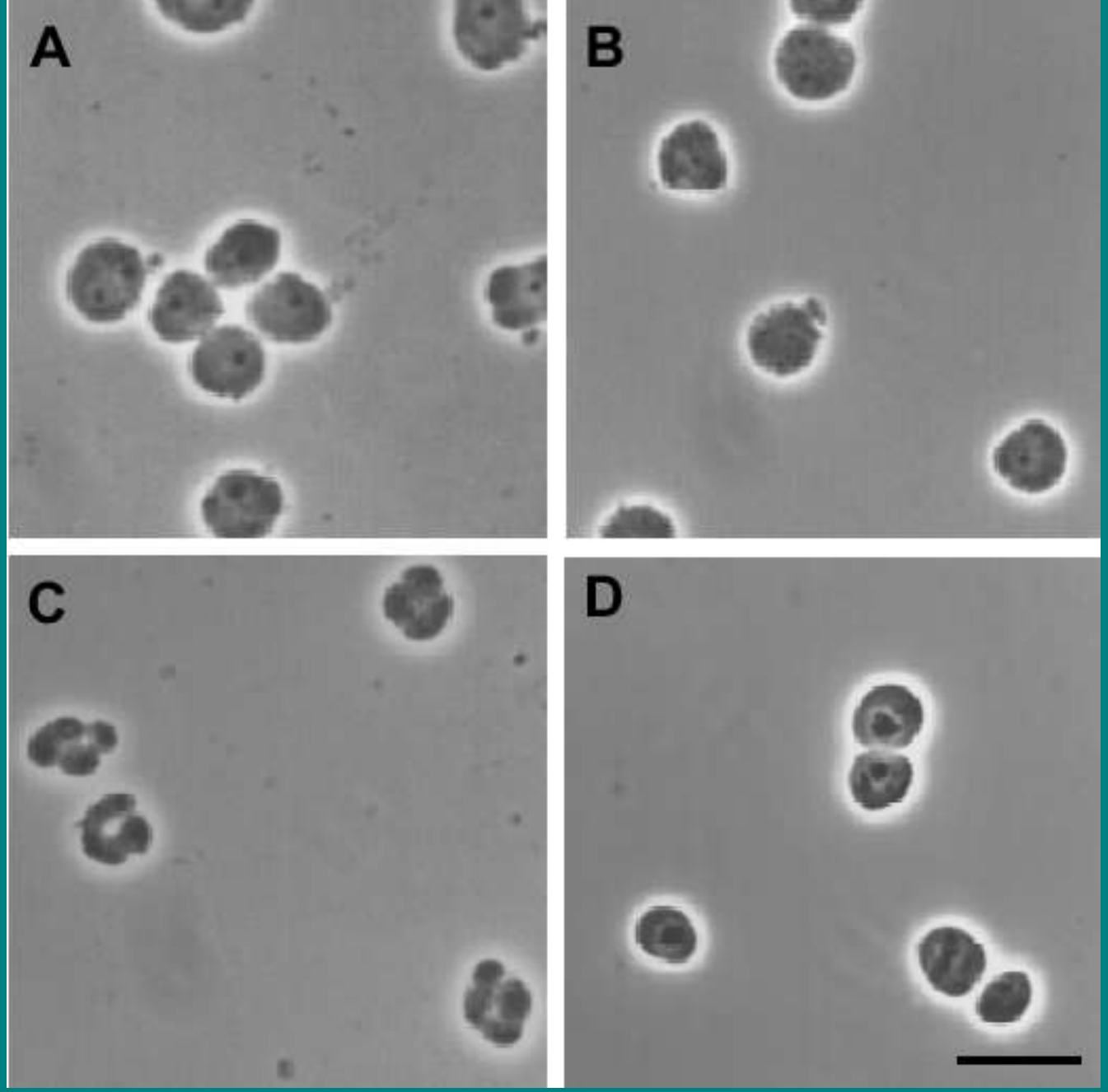
A: Lymphocytes

B: Monocytes

C: Neutrophils

D: HL-60 cells

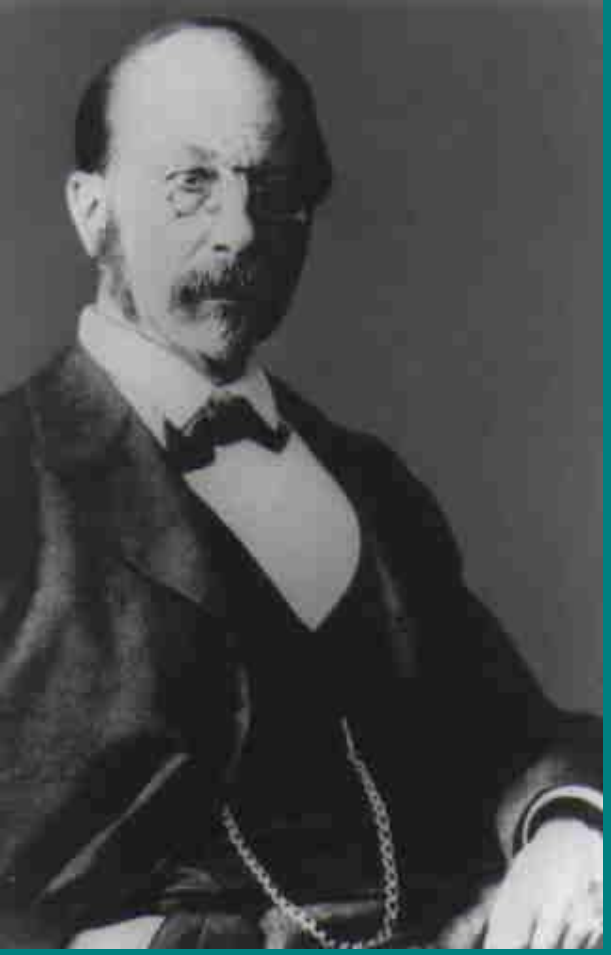
Bar = 10µm



Gerner, C. & G. Sauermann (1999). Nuclear matrix proteins specific for subtypes of human hematopoietic cells. *J Cell Biochem* **72**(4), 470-482

Six major events in the evolution of bacteria to people

1. Asymmetric cell division, resulting in differentiated cells, i.e., two cells of different kinds and sizes (single cell expansion and contraction waves)
2. Symbiotic union of bacteria to produce eukaryotic cells
3. Escape of differentiation waves from single cell trajectories to multiple cell trajectories
4. Invention of continuing differentiation, permitting unlimited cell types
5. Breaking of metasymmetries to produce fairly unique arrangements of the resulting many cell types, perhaps involving the physics of launching differentiation waves
6. Invention of an epigenetic differentiation code using alternative higher order configurations of the genome

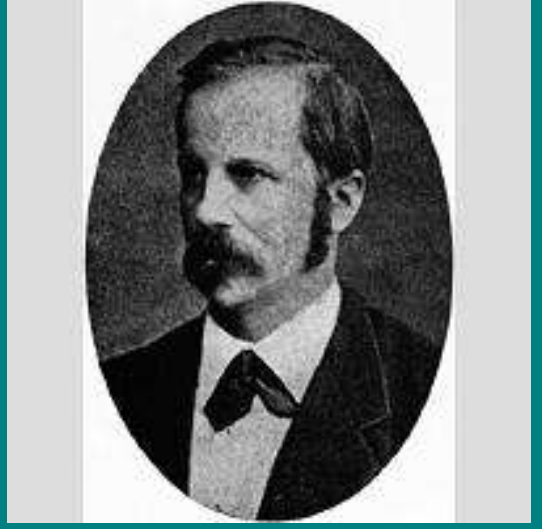


http://www.uni-leipzig.de/~psy/eng/his-e.html

Wilhelm His

(1831-1904), Prof. Dr. med., not his son, the cardiologist, **Wilhelm His** (1863-1934), also Prof. Dr. med.,

http://home.tiscalinet.ch/biografien/biografien/his.htm



http://vlp.mpiwg-berlin.mpg.de/people/data/per93.html

"To think that heredity will build organic beings without mechanical means is a piece of unscientific mysticism."

His, W. (1888). On the principles of animal morphology. *Roy. Soc. Edinburgh Proc.* **15**, 287-298.

Show buckling expts

Conclusion

- I conclude that embryogenesis alternates between physics, in the form of differentiation waves, and gene cascades that they trigger, which set up the conditions for the next bifurcating set of differentiation waves
- The process is summarized by the differentiation tree

Conclusion

- The nucleus is a finite state machine
- The number of states corresponds to the number of edges in the differentiation tree, i.e., the number of differentiated cell types, transient and terminal
- There is a ratcheting mechanism amongst the states of the nucleus that prevents going backwards during embryogenesis

Suggestions on Methodology

- The 3D structure of proteins took off when some of them were crystallized
- Nuclei sometimes have specific orientations, as in *Drosophila* epithelia
- Therefore crystallization of cell nuclei may be possible

Suggestions on Methodology

- Alternatively, soft x-ray micro-computed tomography (μCT) could be performed on individual nuclei, perhaps with synchrotron radiation, and perhaps with *in vivo* time-lapse
- As with metal labelling in x-ray computed tomography, labels could be added to the DNA-protein-DNA interchromosome bridges discussed in:
- Kalhor, R., *et al. (2011) Genome architectures revealed by tethered chromosome conformation capture and population-based modeling. Nat Biotechnol 30, 90-98*

Suggestions on Methodology

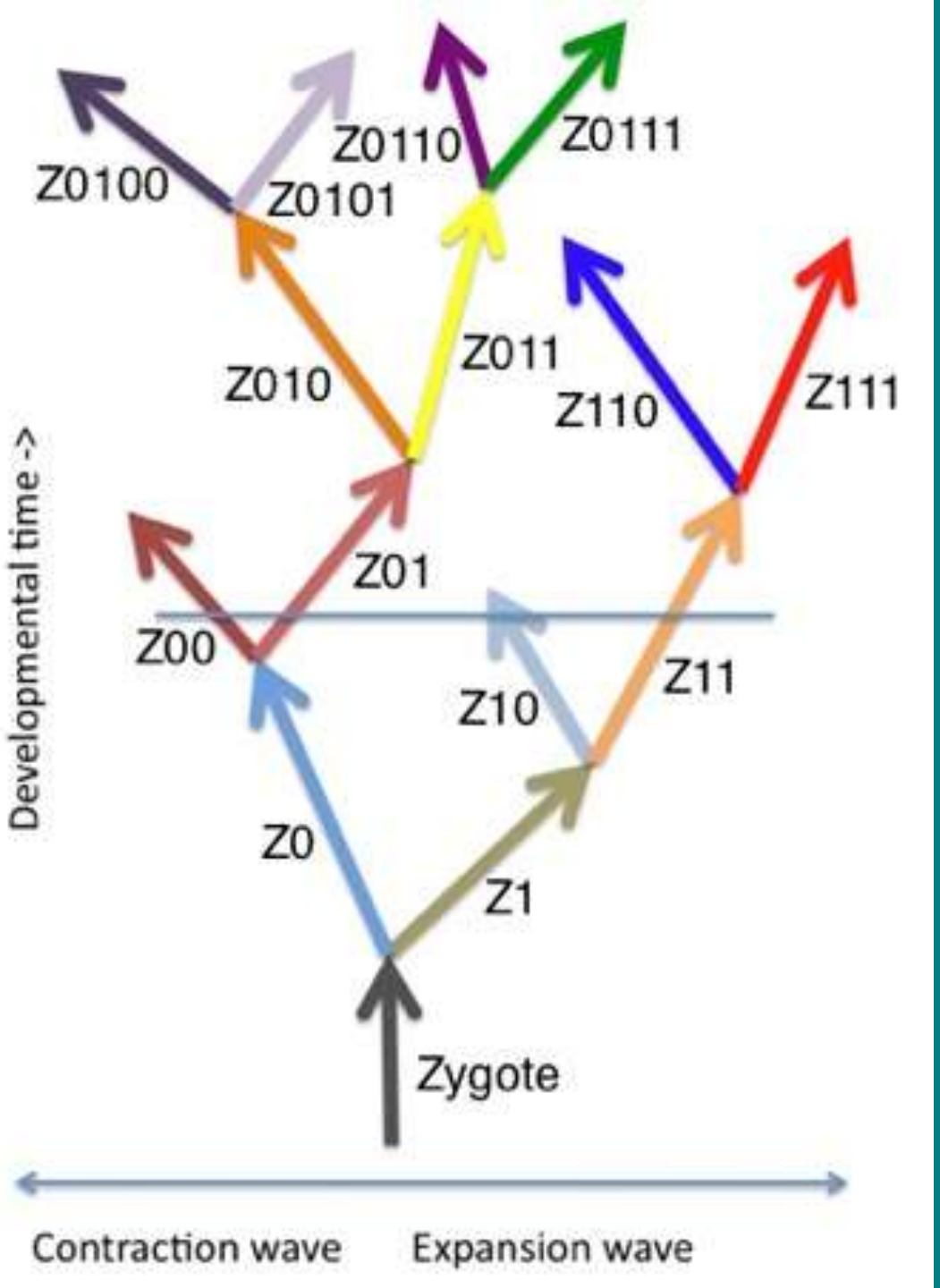
- Alternatively, superresolution light microscopy techniques might allow 3D time-lapse:
- Patterson, G., *et al. (2010) Superresolution imaging using single-molecule localization. In Annual Review of Physical Chemistry, Vol 61, pp. 345-367*

The Differentiation Tree

Each branch represents a distinct cell type

Note binary differentiation code

Natalie K. Gordon & Richard Gordon (2012). Embryogenesis Explained [in preparation]. Singapore: World Scientific Publishing Company.



- Dedicated to the memory of Jack Gordon, born in New York City March 21, 1921