Introduction to Differentiation Waves

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Embryo Physics Course, January 9, 2013

Welcome to our Embryo Physics Course!

- Please take a seat (right click on seat, left click on "Sit here")
- Turn your voice off* (it's off if no "O" or "((()))" over your avatar)
- Do NOT change slides unless you are the speaker (green arrows)

- *Two microphones open simultaneously can lead to screeching feedback loops

Dialogue

- Ask questions via chat (note Δ to expand chat window)
- Speaker may respond to questions as they come and/or at end (scroll back chat window)
- We try to finish within 1 hour
- You are welcome to stay here and chat with anyone after question period, and to explore Second Life
- We welcome your suggestions for speakers, including yourself

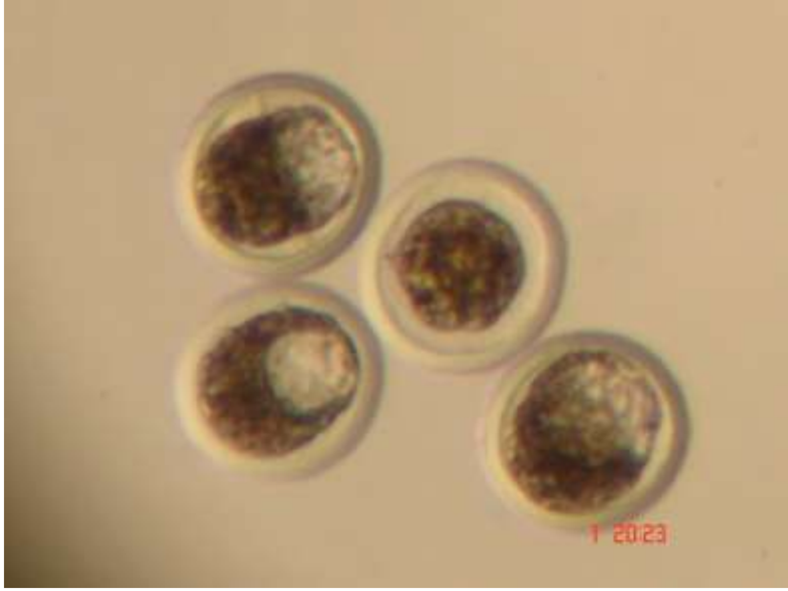
Graduate Credit

- Starting Fall, 2013, the Embryo Physics Course will probably be available for graduate course credit through Wayne State University
- Contact:
- Dr. Stephen A. Krawetz
- steve@compbio.med.wayne.edu

The Embryo Physics Approach:

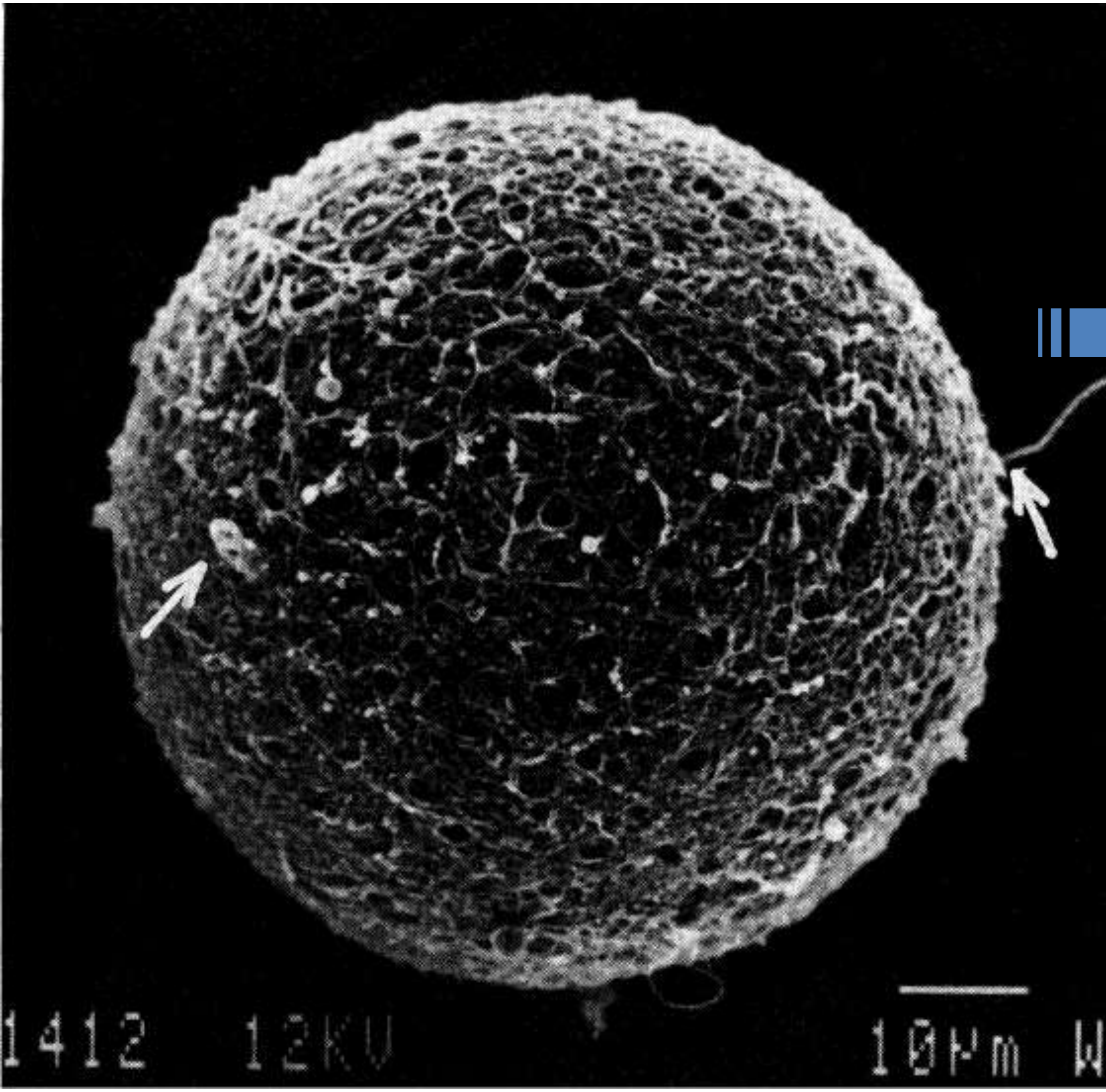
Consider a Spherical Cow…

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/

The Embryogenesis Problem



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.

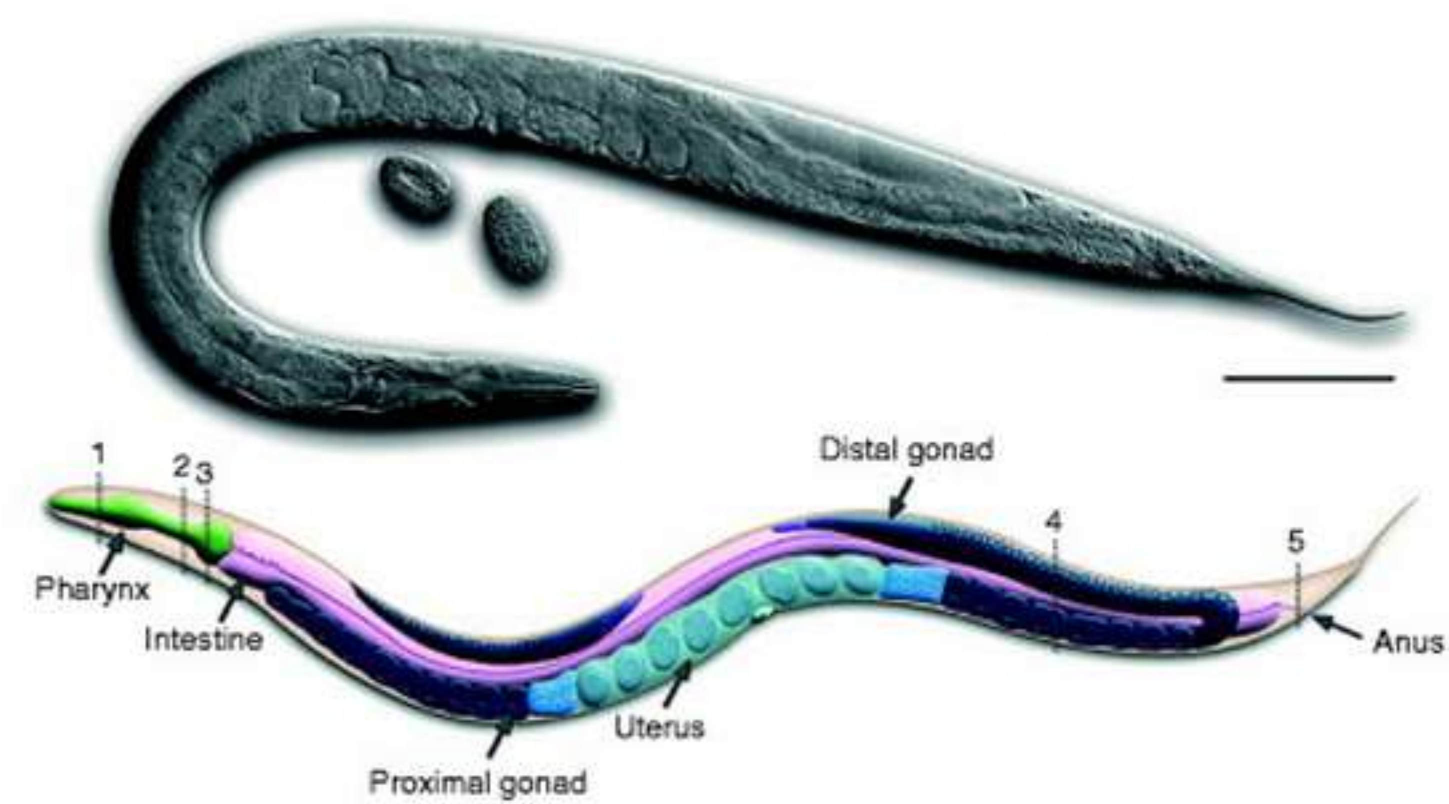
?

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 tiny spherically symmetrical 1-cell egg turn into your 20 trillion cell highly asymmetrical shape?

The Nematode *Caenorhabdititis elegans*, egg to adult



- Scale bar 100µm

- Zacchigna, S., C.R.d. Almodovar & P. Carmeliet (2007). Similarities between angiogenesis and neural development: what small animal models can tell us. *Current Topics in Developmental Biology* **80**, 1-55.

Basic Nematode Facts

- Adult mass: 4.7 µg = 4.7×10^{-6} g
- Ferris, H. (2011). Nematode Body Mass, Biomass and Metabolic Footprints. http://plpnemweb.ucdavis.edu/nemaplex/Ecology/nematode_weights.htm
- Mass of ~20µm *C. elegans* egg: 3.1×10^{-8} g
- Jorgensen, P. (2011). Volume of a *C. elegans* egg, from N2 worms grown at 24C. http://bionumbers.hms.harvard.edu/bionumber.aspx?&id=101423&ver=2
- Mongiovi, P. (2010). Area of a *C. elegans* egg. http://bionumbers.hms.harvard.edu/bionumber.aspx?s=n&id=101650&ver=3
- Ratio: 150

- Number of cells: 959 (hermaphrodite adult)
- Number of cell types: ~900
- Number of cells of each type: ~850 cells: 1 of each kind, ~50 cells: 2 of each kind

Basic Human Facts

- Average adult human mass: 62 kg = 62,000 g
- http://en.wikipedia.org/wiki/Body_weight#Average_weight_around_the_world
- Mass of 70µm human egg: 1.8×10^{-7} g
- Ratio: 345×10^{9}

- Number of cells: 380×10^{6}/g, yielding 23×10^{12} cells
- Osgood, E.E. (1955). Development and growth of hematopoietic tissues, with a clinically practical method of growth analysis. *Pediatrics* **15**(6), 733-751.
- Number of cell types: 200 to 7000 (what's wrong here?)
- Number of cells of each type: $3.3\text{-}115 \times 10^{9}$
- Bonner, J.T. (1988). *The Evolution of Complexity by Means of Natural Selection*. Princeton, Princeton University Press.
- Bard, J.B.L., R.A. Baldock & D.R. Davidson (1998). Elucidating the genetic networks of development: a bioinformatics approach. *Genome Res.* **8**(9), 859-863.
- Bard, J.B.L., M.H. Kaufman, C. Dubreuil, R.M. Brune, A. Burger, R.A. Baldock & D.R. Davidson (1998). An internet-accessible database of mouse developmental anatomy based on a systematic nomenclature. *Mech. Dev.* **74**(1-2), 111-120.

Can we have a theory of embryogenesis that applies over this huge range of size change and the differences in number of cells per cell type?

Differentiation waves as a Working Model for (All of) Embryogenesis

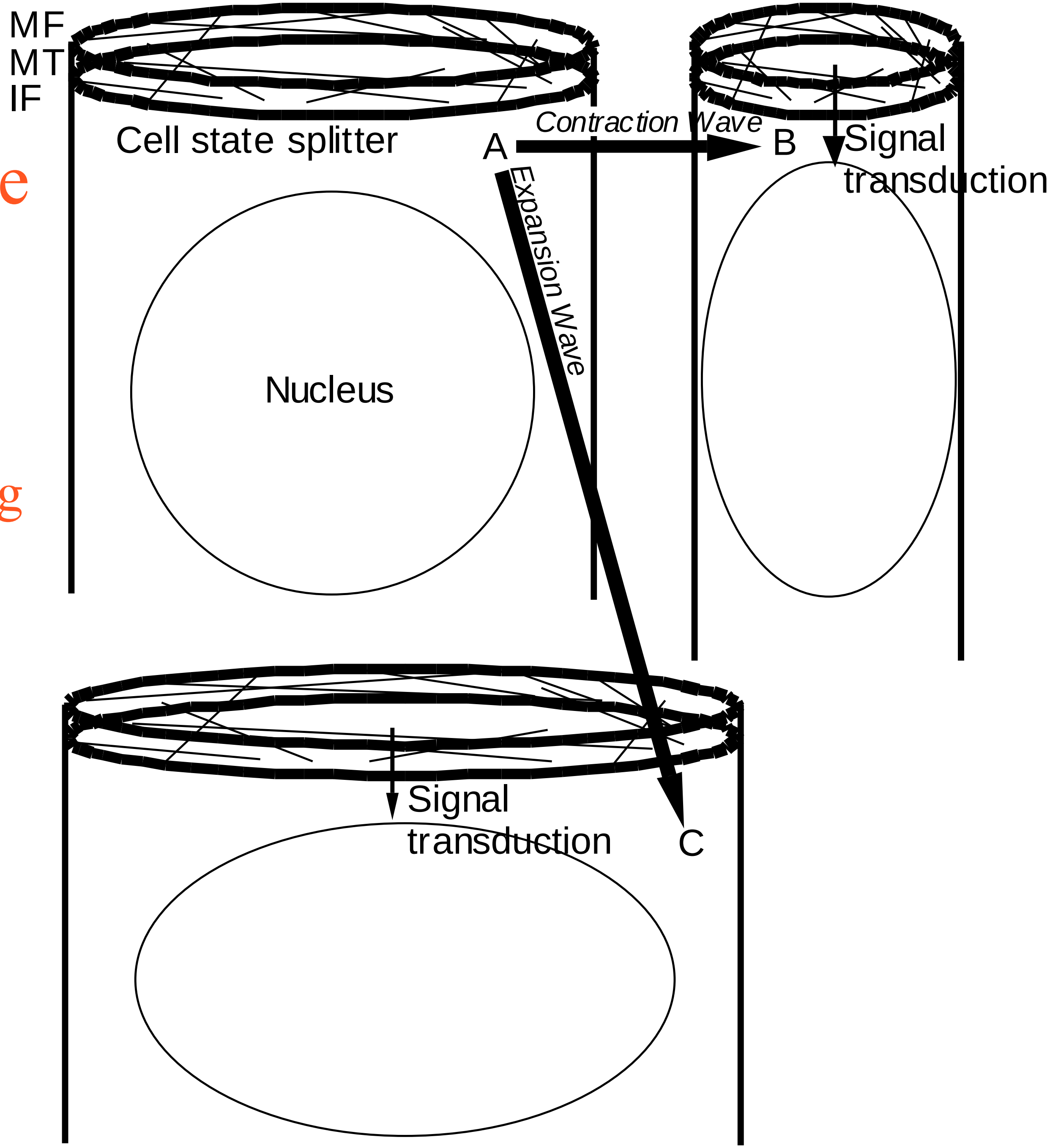
- Concepts to be introduced:
  - Cell state splitter
  - Differentiation wave
  - Contraction wave
  - Expansion wave
  - Differentiation code
  - Tissue
  - One-cell tissue
  - Differentiation tree
  - Single cell differentiation waves

Concept: *Cell state splitter*

- A cytoskeletal apparatus at the apical end of an epithelial cell consisting of:
  - A microfilament ring
  - Microtubules parallel to the surface
  - An intermediate filament ring

- It is an organelle that is not membrane bound
- Somewhat similar to the spindle apparatus

The Cell State Splitter



MF = microfilament ring

MT = annular apical microtubule mat

IF = intermediate filament ring

Resolution of the State of the Cell state splitter

- We assume that the cell state splitter is set up in a cell in a metastable state
- There is a radial tug of war between its microfilament ring and its microtubules
- One of them wins
- A one-bit signal is sent to the nucleus telling it which cytoskeletal component won
- The nucleus responds with a corresponding change in gene expression

Tug of War

OSU Special Collections & Archives. (1922). Tug-of-War, Morrow County, ca. 1922. http://www.flickr.com/photos/osucommons/5711360424/in/faves-nolancaudill/

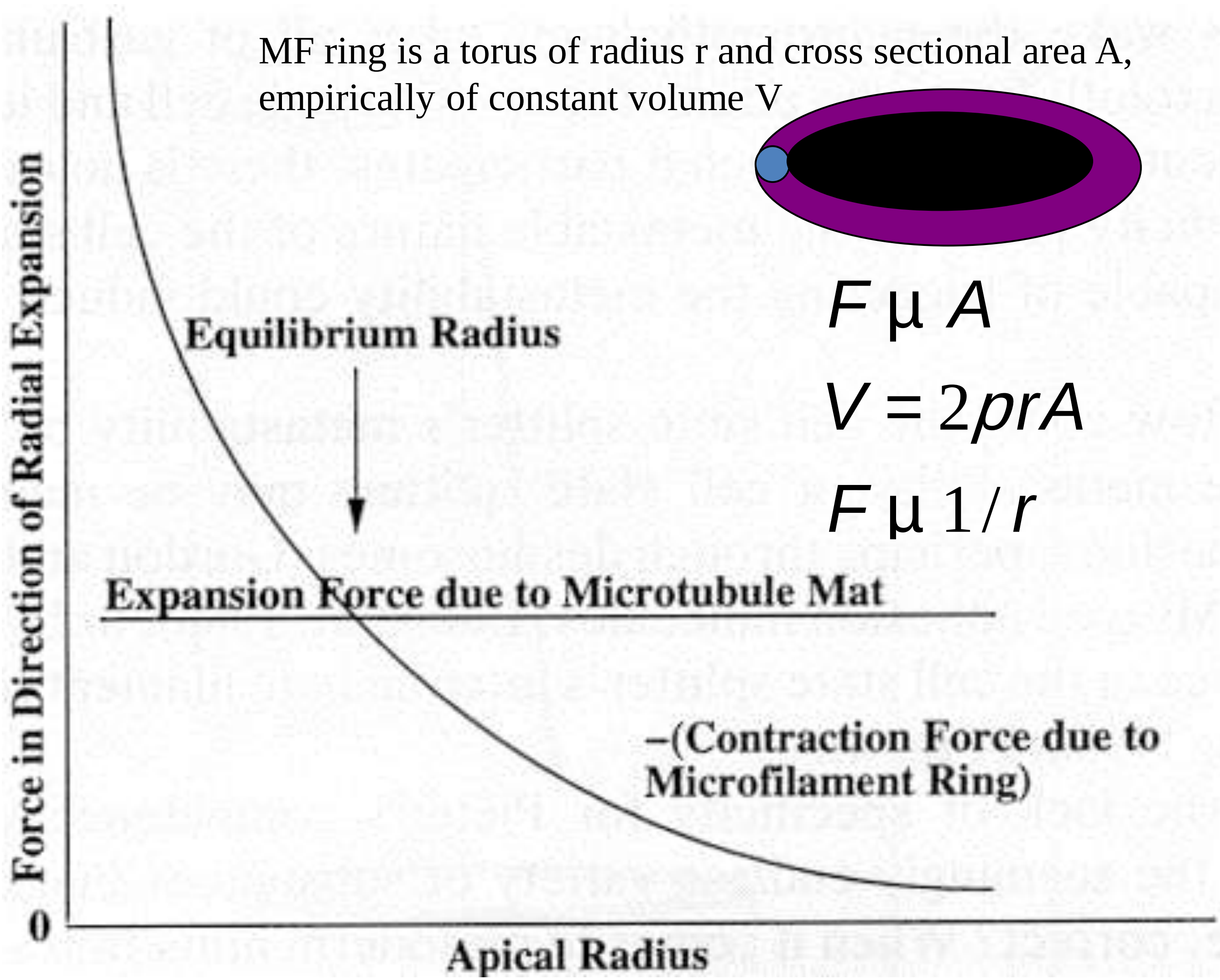
The Unstable (Bistable) Mechanical Equilibrium between the Microfilament Ring and the Microtubule Mat in the Cell State Splitter

MF ring is a torus of radius r and cross sectional area A, empirically of constant volume V

$$F \mu A$$

$$V = 2prA$$

$$F \mu 1/r$$

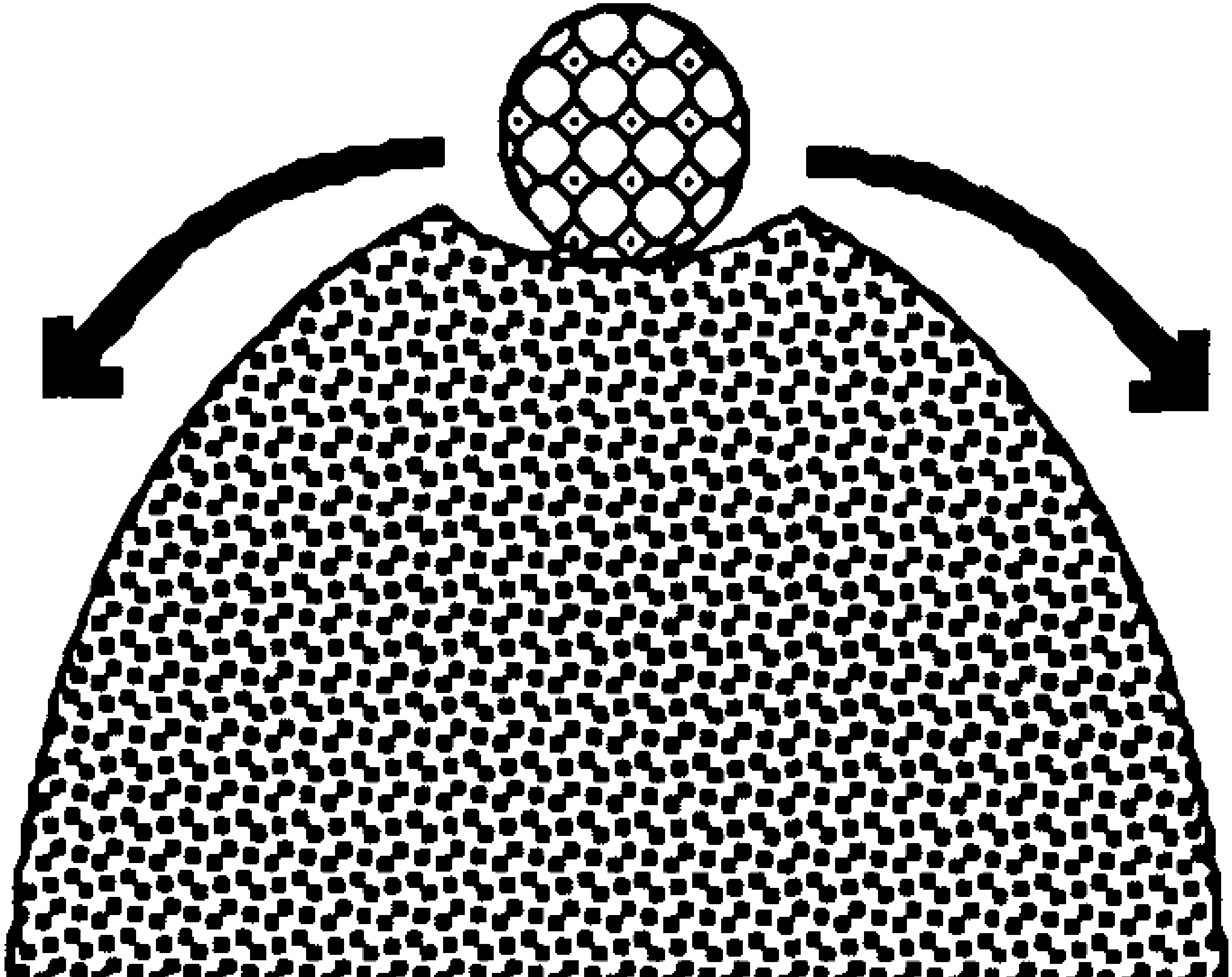


Gordon, R., N.K. Björklund & P.D. Nieuwkoop (1994). Dialogue on embryonic induction and differentiation waves. *Int. Rev. Cytol.* **150**, 373-420.

The Equilibrium Radius is a Point of Metastability

- The intermediate filament ring may be what maintains the metastable state
- It is elastic and small perturbations lead to restoration of the equilibrium radius
- But a large perturbation to a larger radius yields a microtubule force greater than the microfilament force, so the radius keeps increasing
- And vice versa: a perturbation to a smaller radius causes the radius to keep decreasing
- So the cell state splitter is bistable

A Metastability



Gordon, R. & G.W. Brodland (1987). The cytoskeletal mechanics of brain morphogenesis. Cell state splitters cause primary neural induction. *Cell Biophys* **11**, 177-238.

Concept: *Differentiation waves*

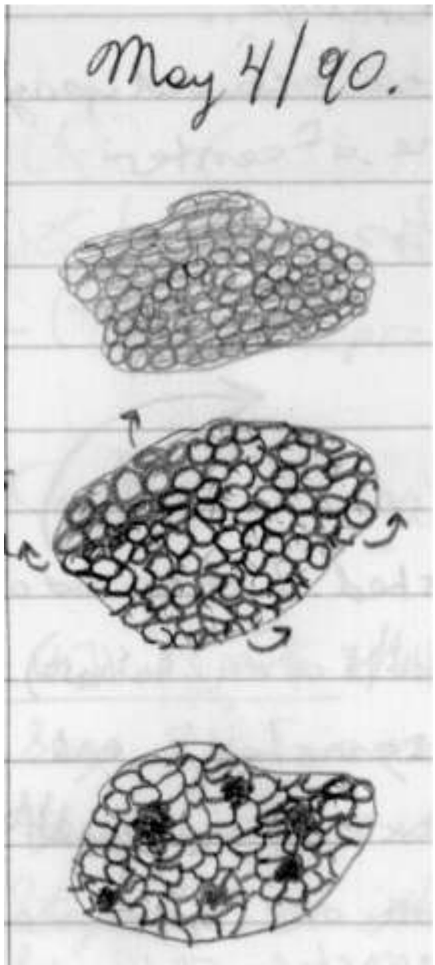
- Cells are organized into tissues
- In this model, this is accomplished via differentiation waves
- A differentiation wave is the propagation of the change in a cell state splitter from one cell to the next

Concept: *Contraction wave*

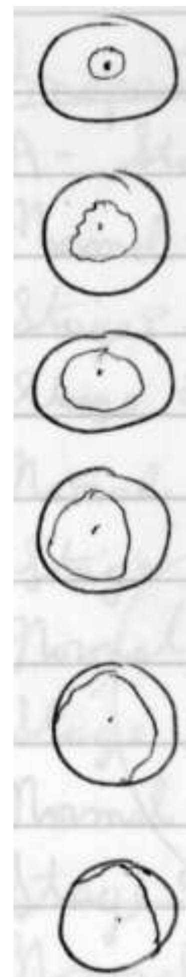
- A contraction wave is a differentiation wave in which each cell state splitter undergoes a contraction
- In a contraction, the microfilament ring wins the radial tug of war
- We can guess that the mechanism of wave propagation is stretch activated contraction

First Observation of a Contraction Wave

Found by Natalie K. Björklund
in ectoderm explants of axolotls… and in the intact embryo:



Perhaps a stretch-activated contraction?

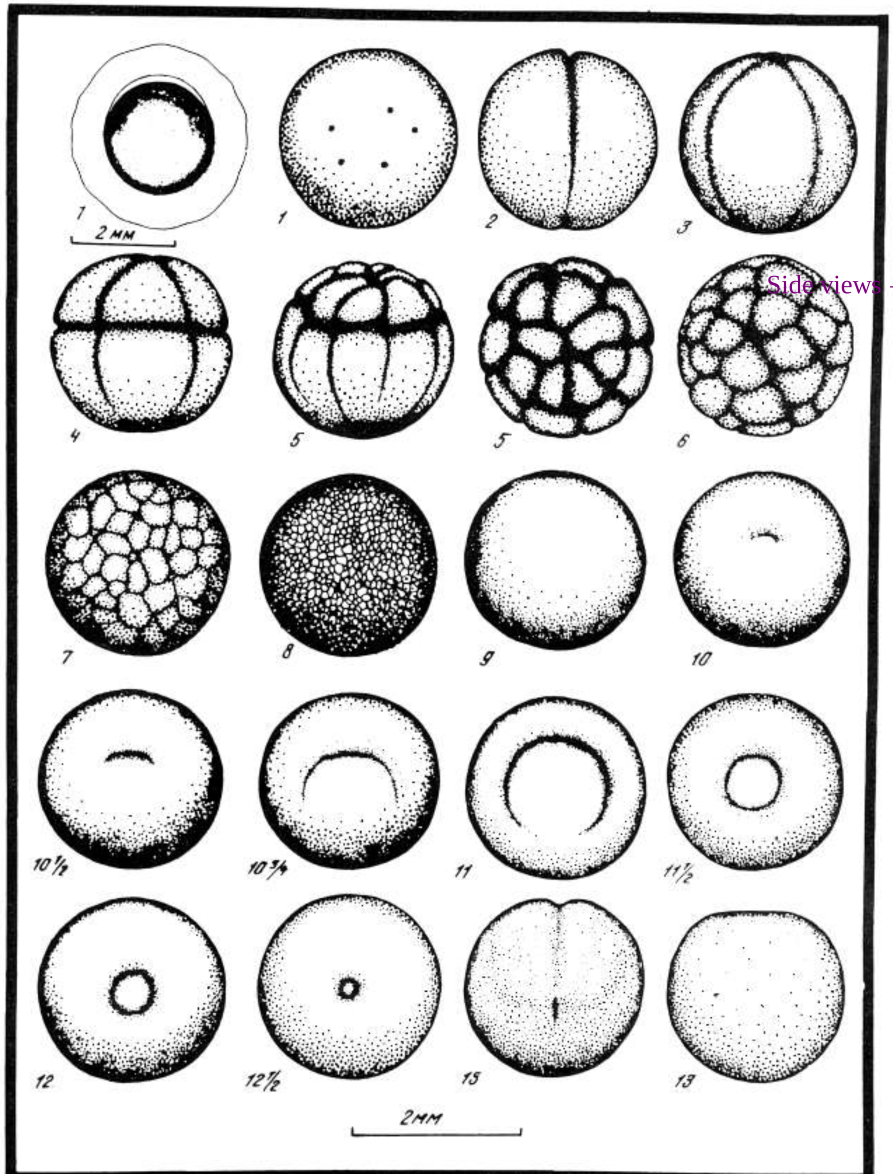


Gordon, R. (1999). *The Hierarchical Genome and Differentiation Waves Novel Unification of Development, Genetics and Evolution*, Singapore & London World Scientific & Imperial College Press.

The Axolotl
Ambystoma mexicanum

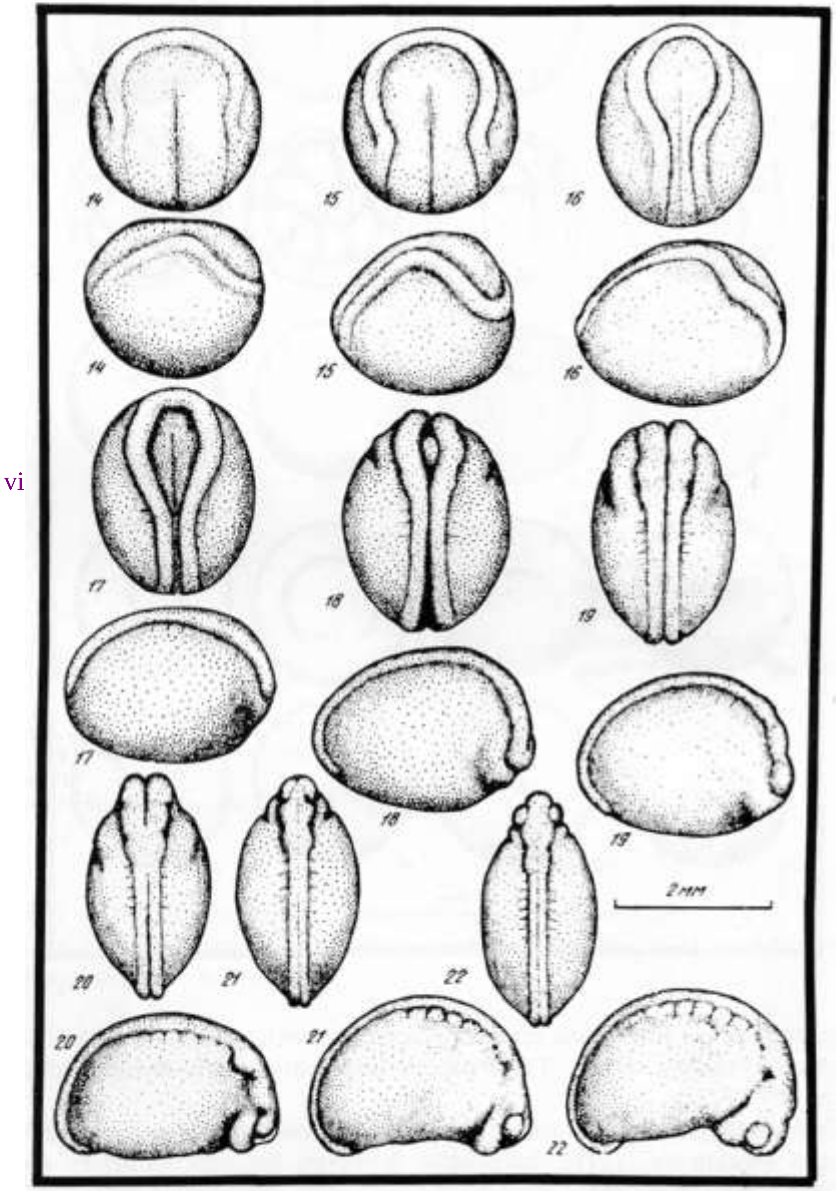
A rare piebald axolotl, 23 cm long, showing its external gills.

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.

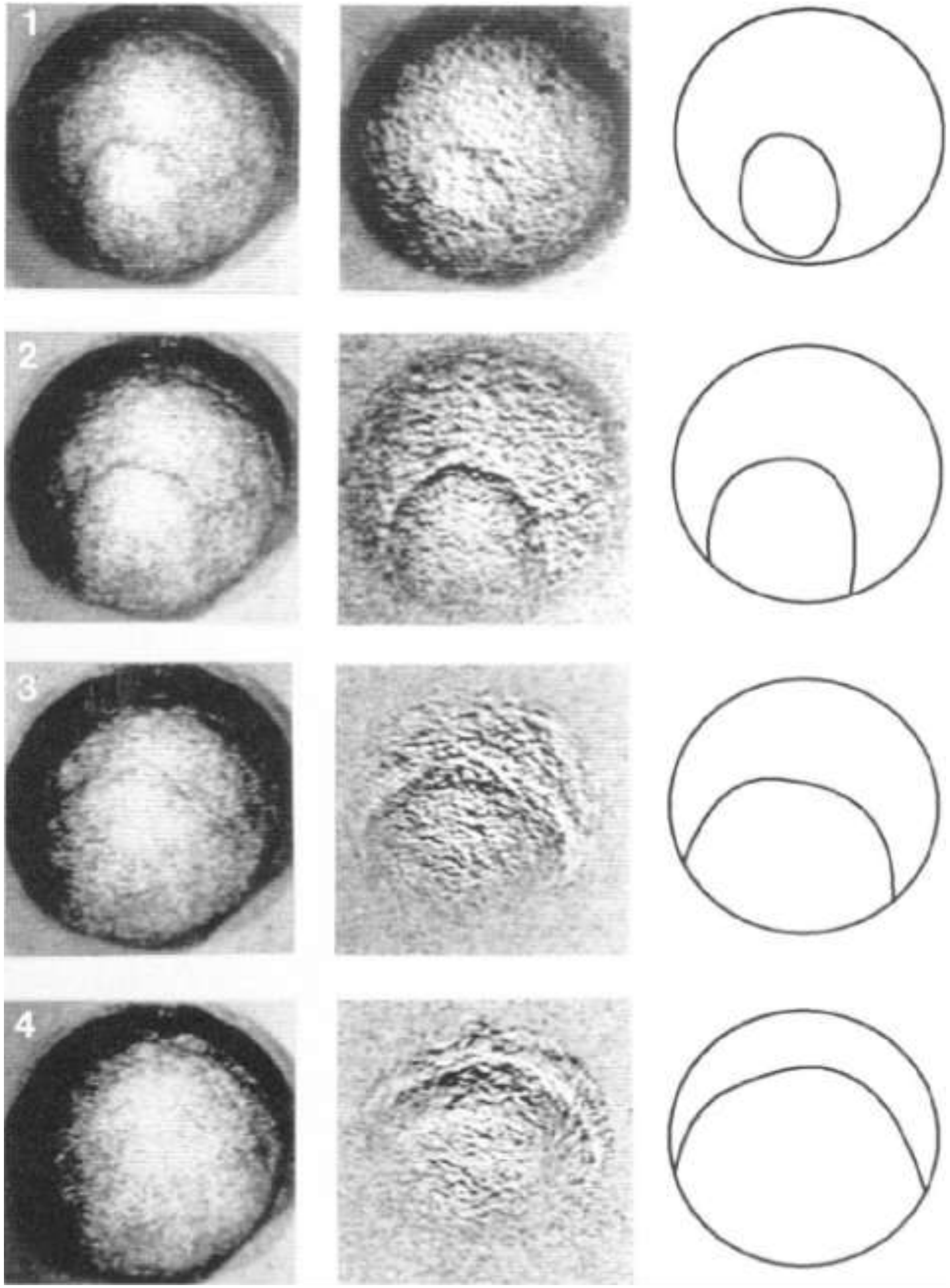
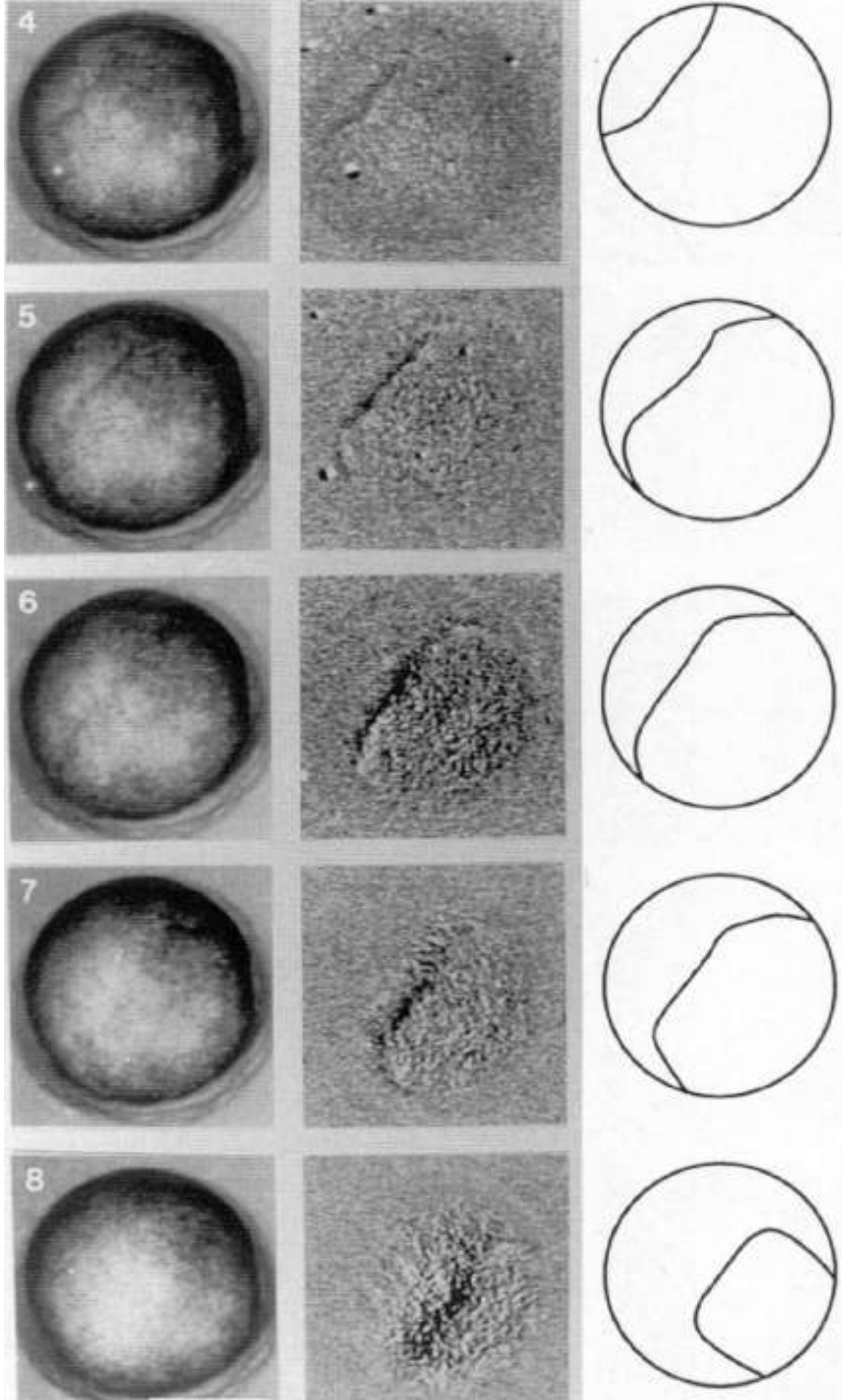
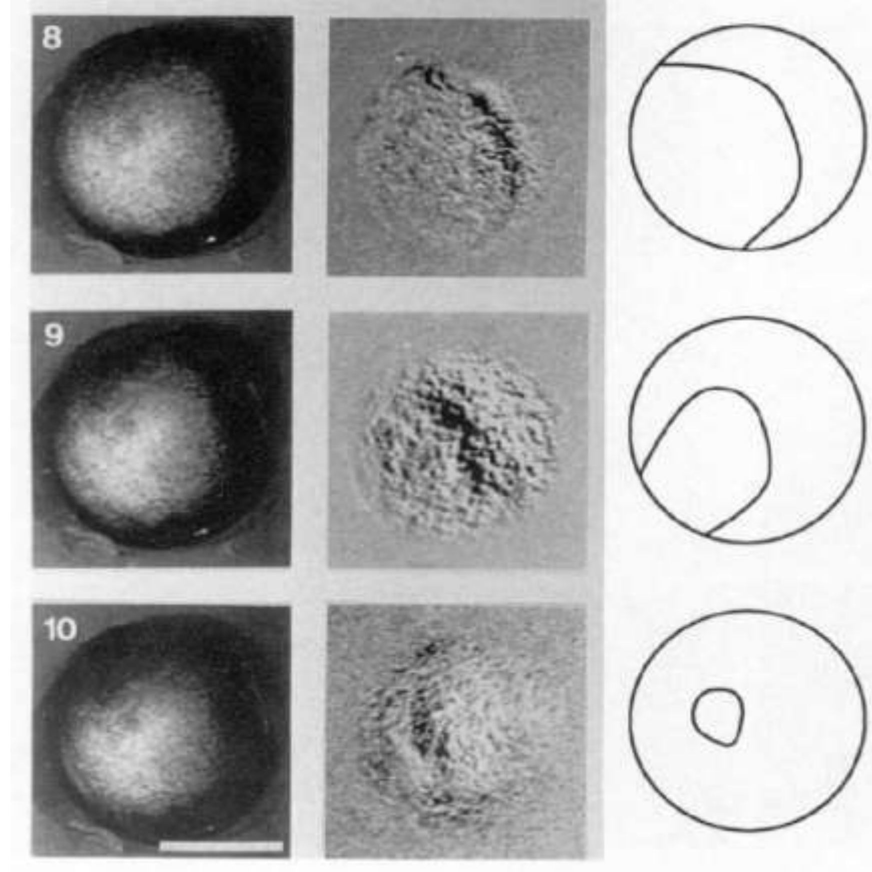


Image Processing

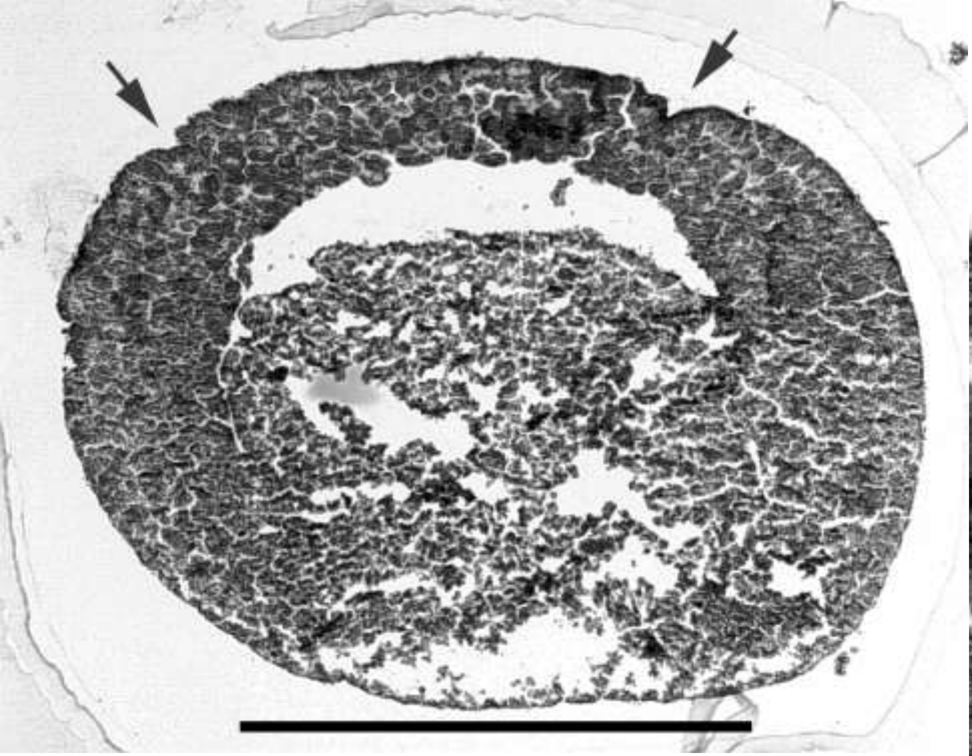
At hourly intervals, the image was digitally subtracted from the one 5 minutes earlier, showing the moving ectoderm contraction wave.

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.

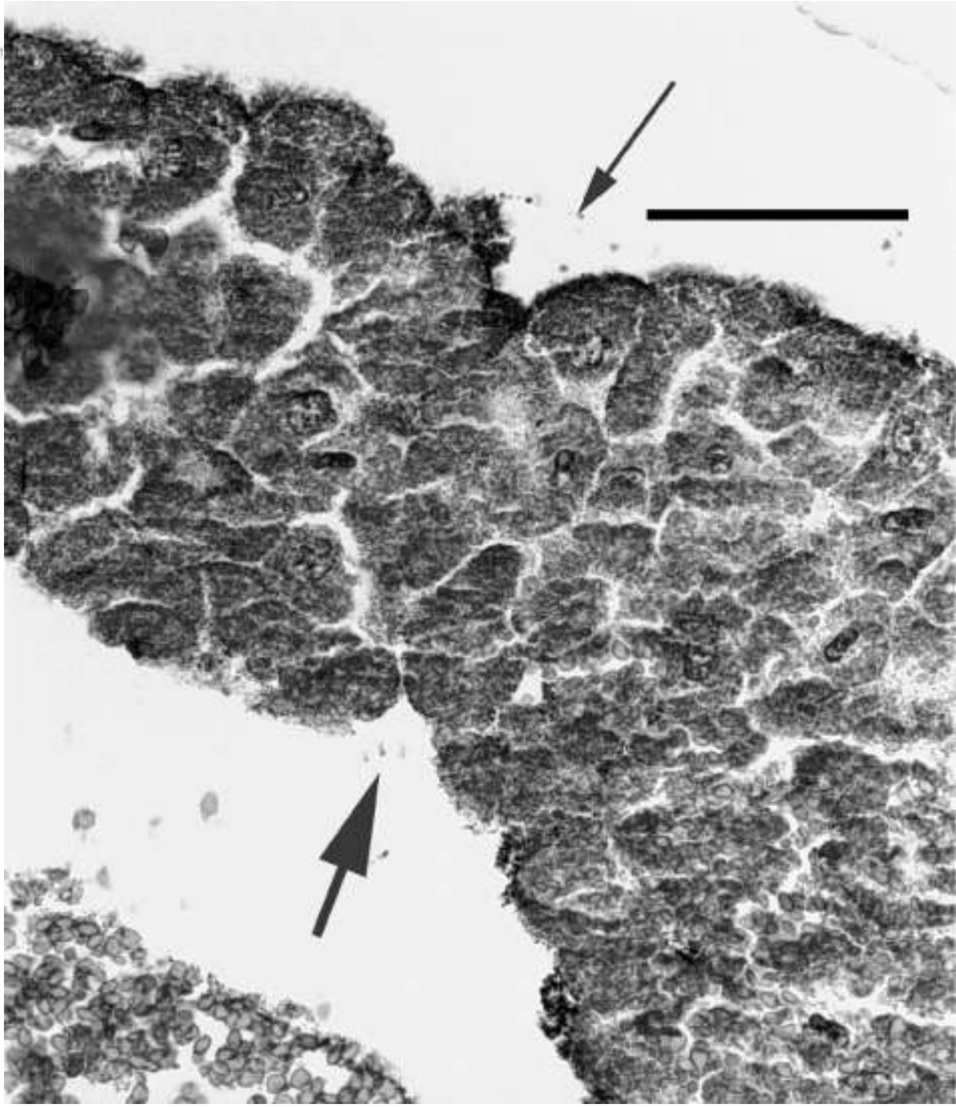




The arc shaped wave moves faster at its ends than in the middle, reforming a circle which then vanishes at what will be the anterior (head) end of the embryo. (These sets of images are from three different embryos.) Bar = 1 mm.

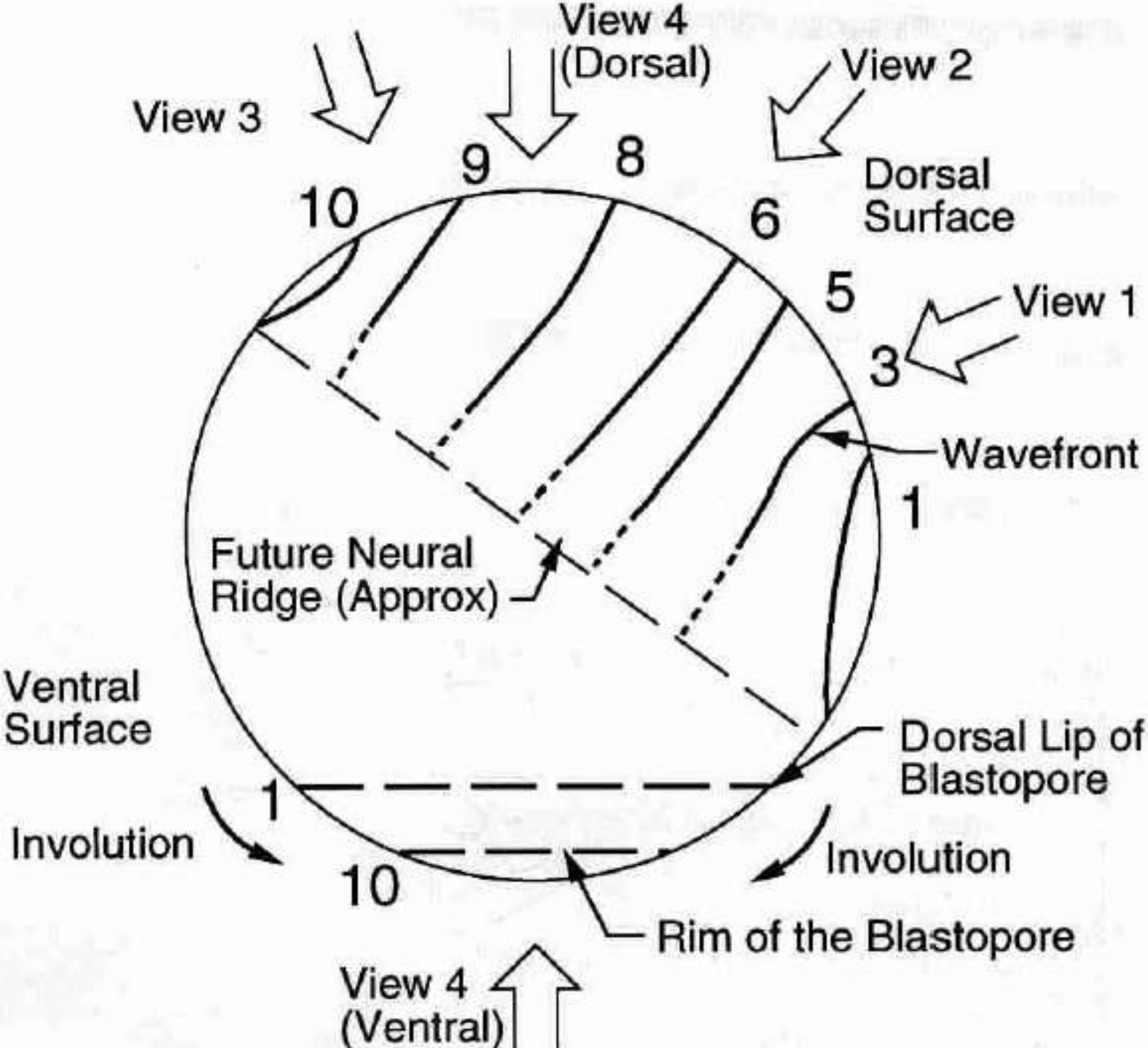


The Ectoderm Contraction Wave is a Morphogenetic Furrow



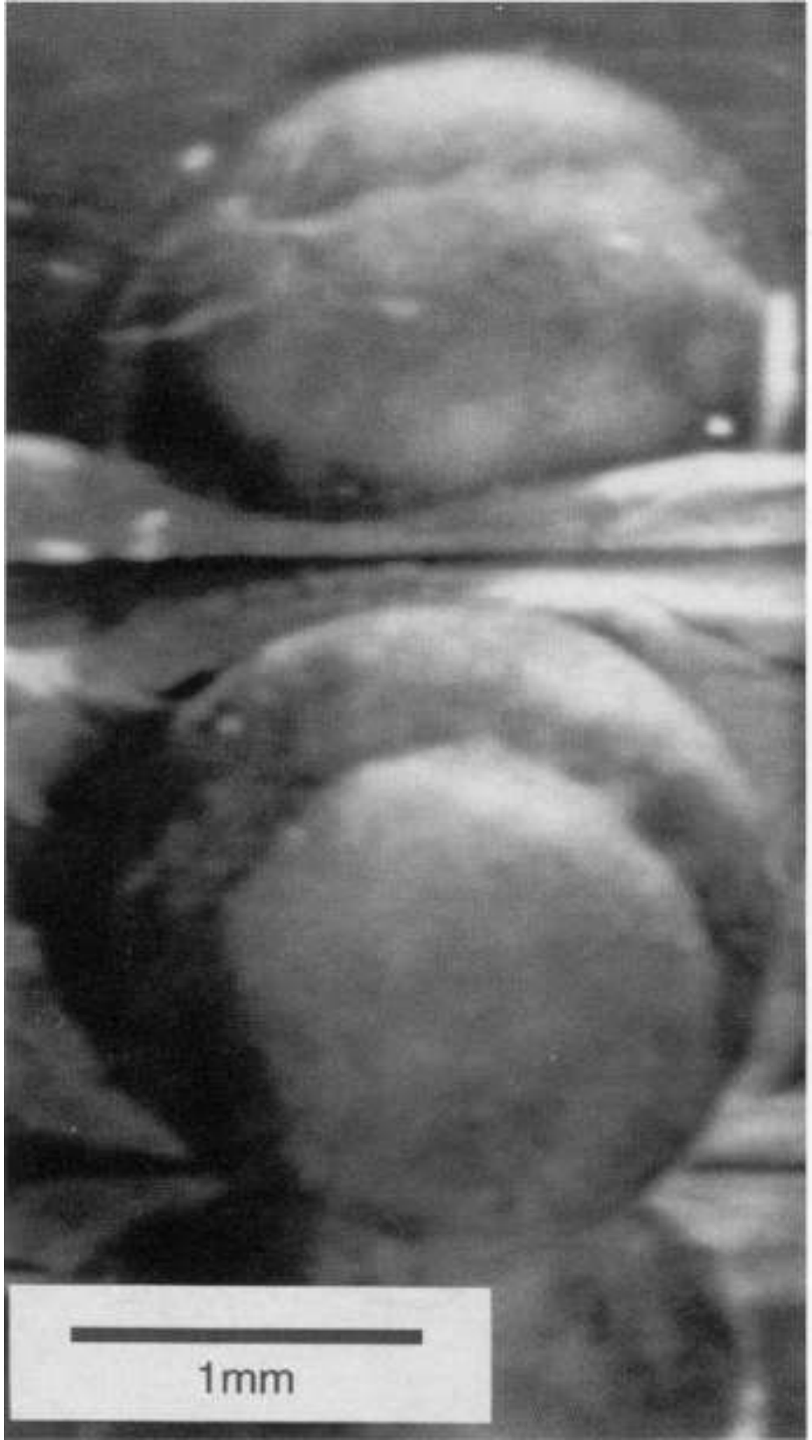
Microwave fixation developed in collaboration with Marc del Bigio, Natalie K. Björklund and Pierre Williot. Bars: 1 mm and 0.1 mm. There is a possibility (arrows on right) that furrowing occurs both on the apical and basal surfaces.

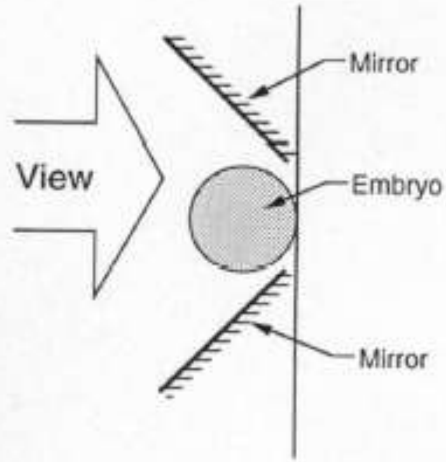
Gordon, R. (1999). *The Hierarchical Genome and Differentiation Waves: Novel Unification of Development, Genetics and Evolution*, Singapore: World Scientific and London: Imperial College Press, 2 vols.



Left side view during ectoderm contraction wave. 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.

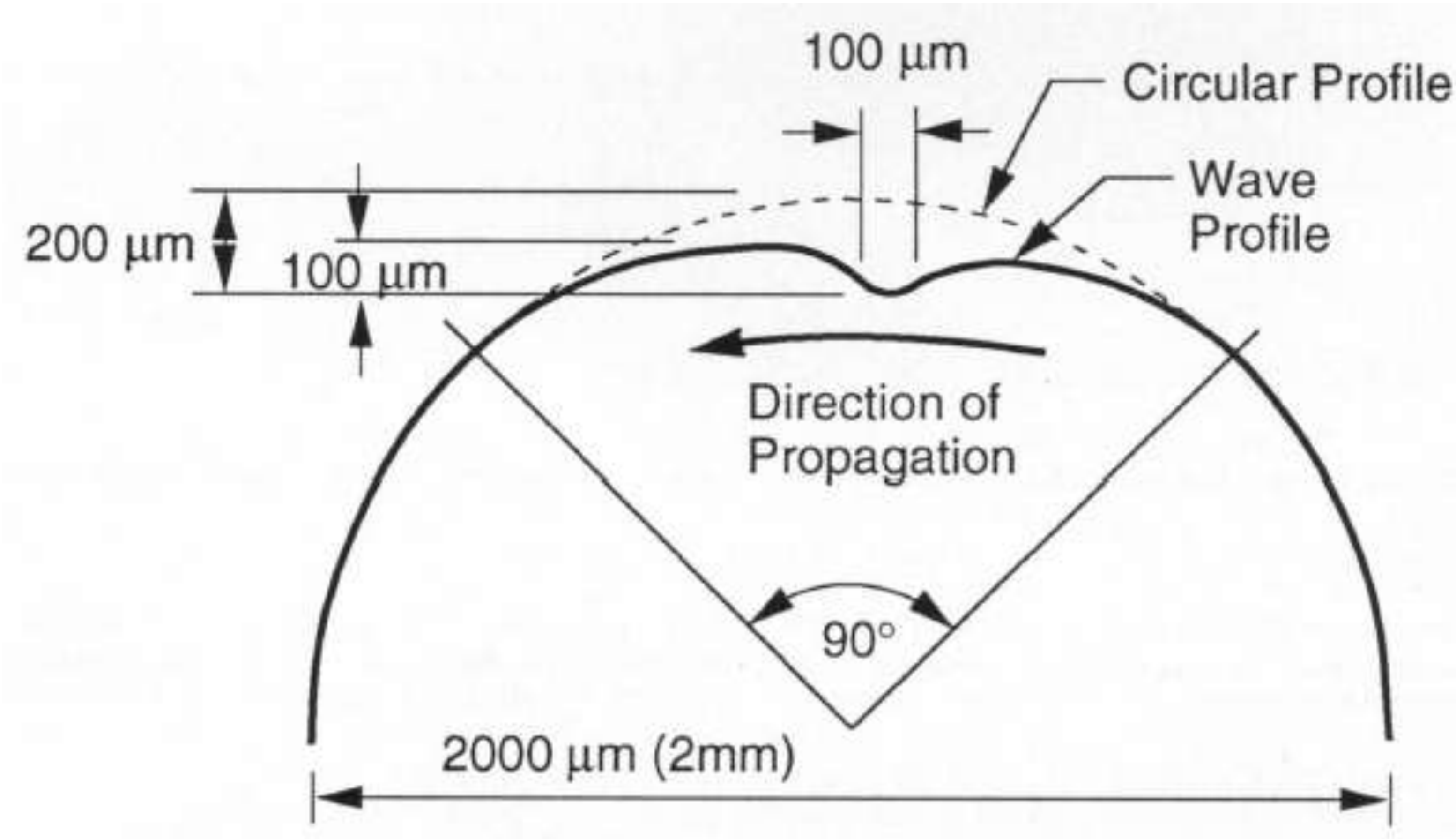




Front silvered mirrors show that the travelling furrow is an indent in the surface of the embryo.

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.

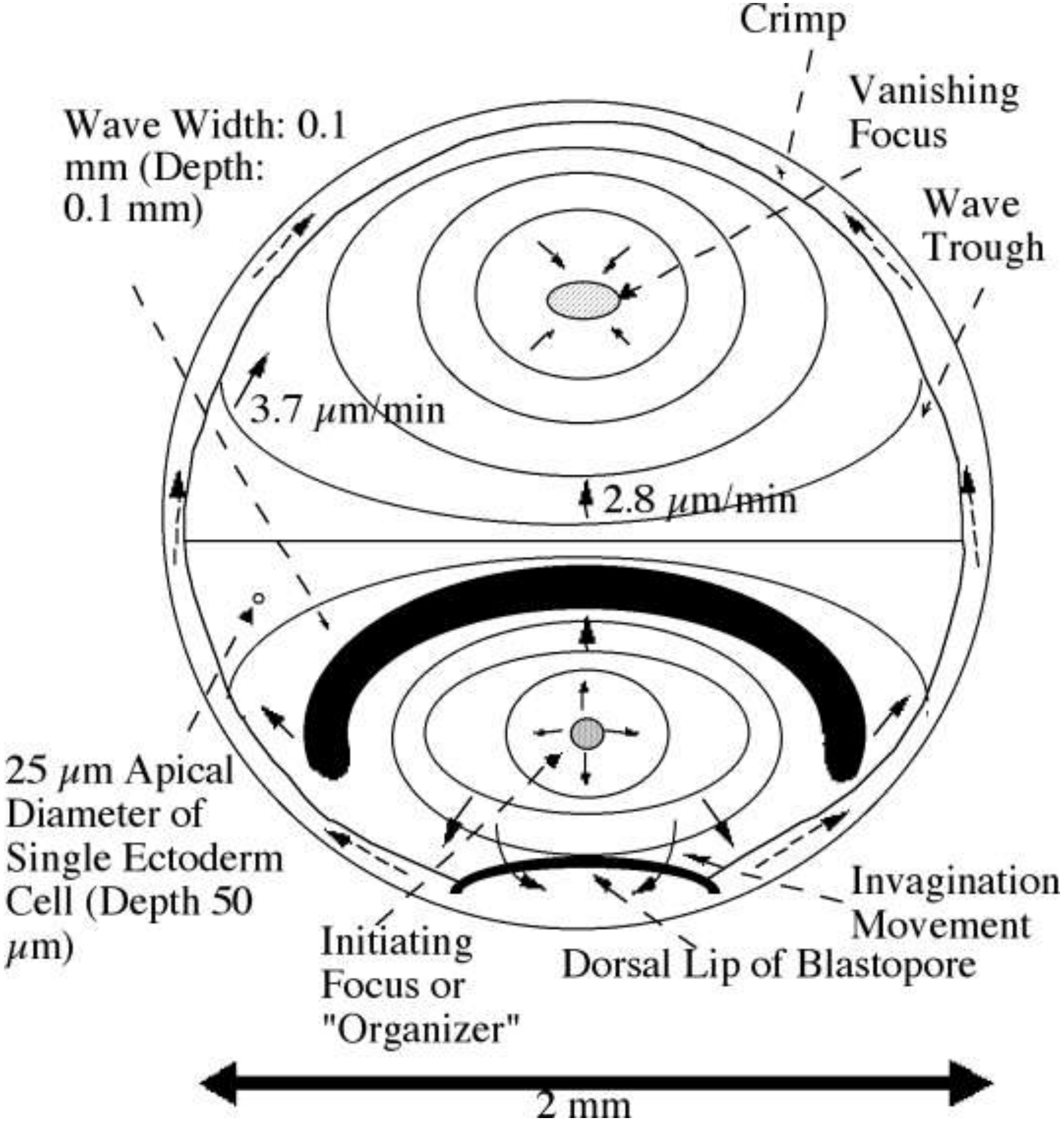
The Ectoderm Contraction Wave starts up the edge of the furrow. The furrow represents maximal apical contraction of the cells, which lasts about 10 minutes for each cell.



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

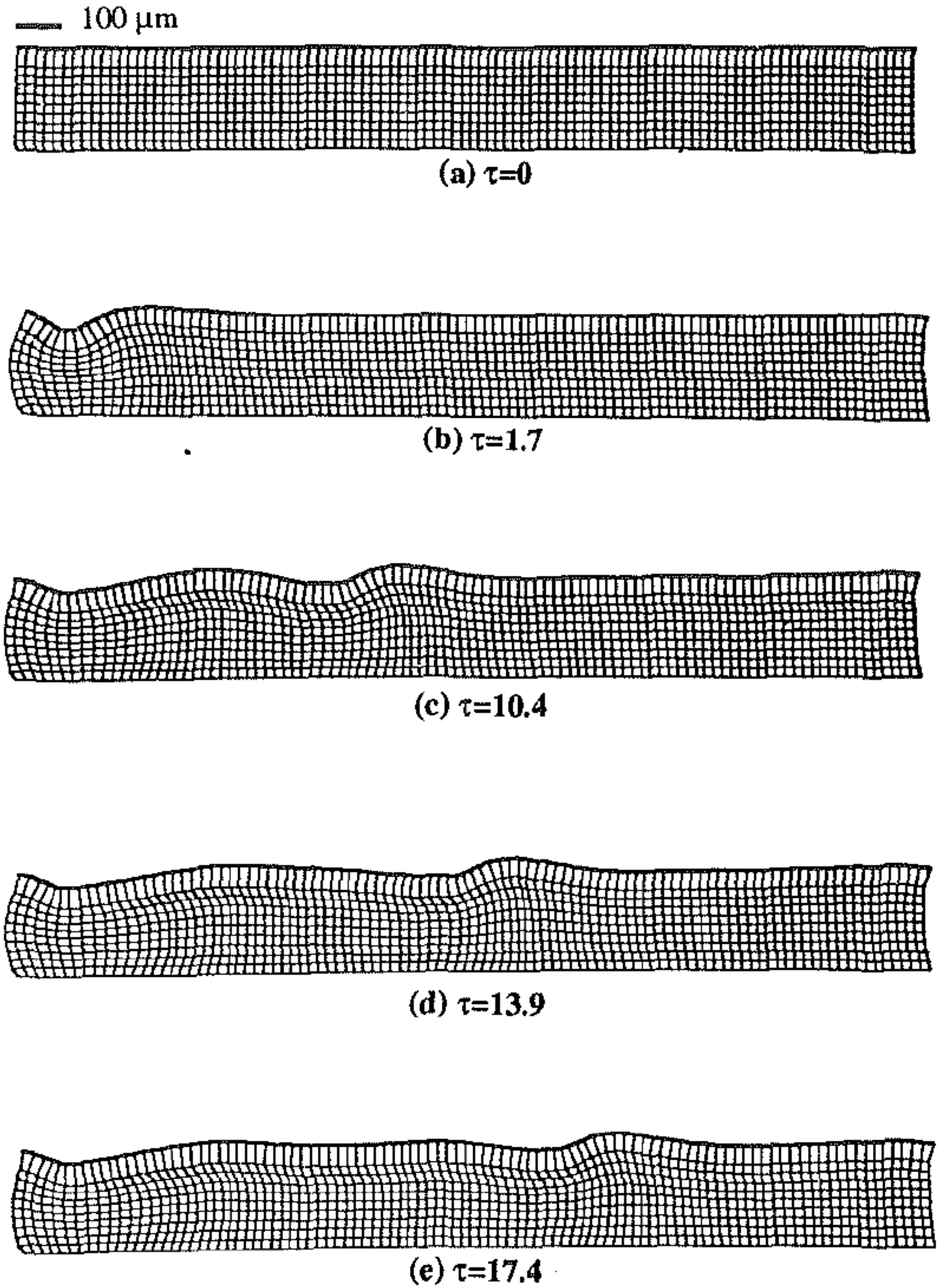
Does the invagination movement generate a strain field that restricts the wave to one hemisphere?



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.

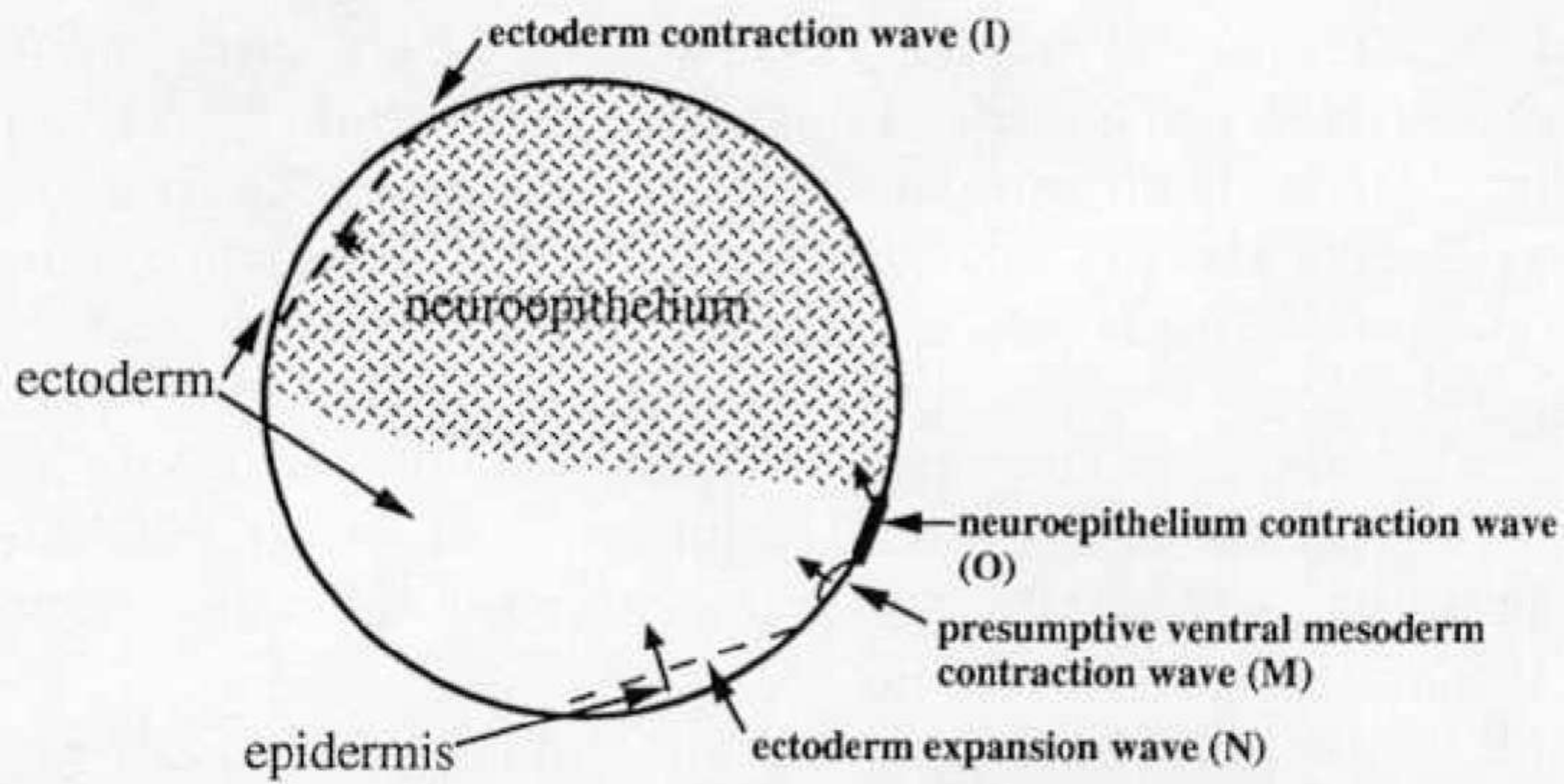


Computer simulation of a contraction wave based on stretch-activated contraction using the finite element method

Brodland, G.W. & D.A. Clausi (1994). Embryonic tissue morphogenesis modeled by FEM. *J. Biomech. Eng.* **116**(2), 146-155.

Concept: *Expansion wave*

- Expansion waves may be analogous to epibolic spreading of cell sheets
- They have yet to be investigated in any detail
- We hypothesize that they involve microtubule polymerization and/or motor molecules
- Propagation mechanism unknown (needs a model)
- No simulations have been done, but there are some analogous simulations

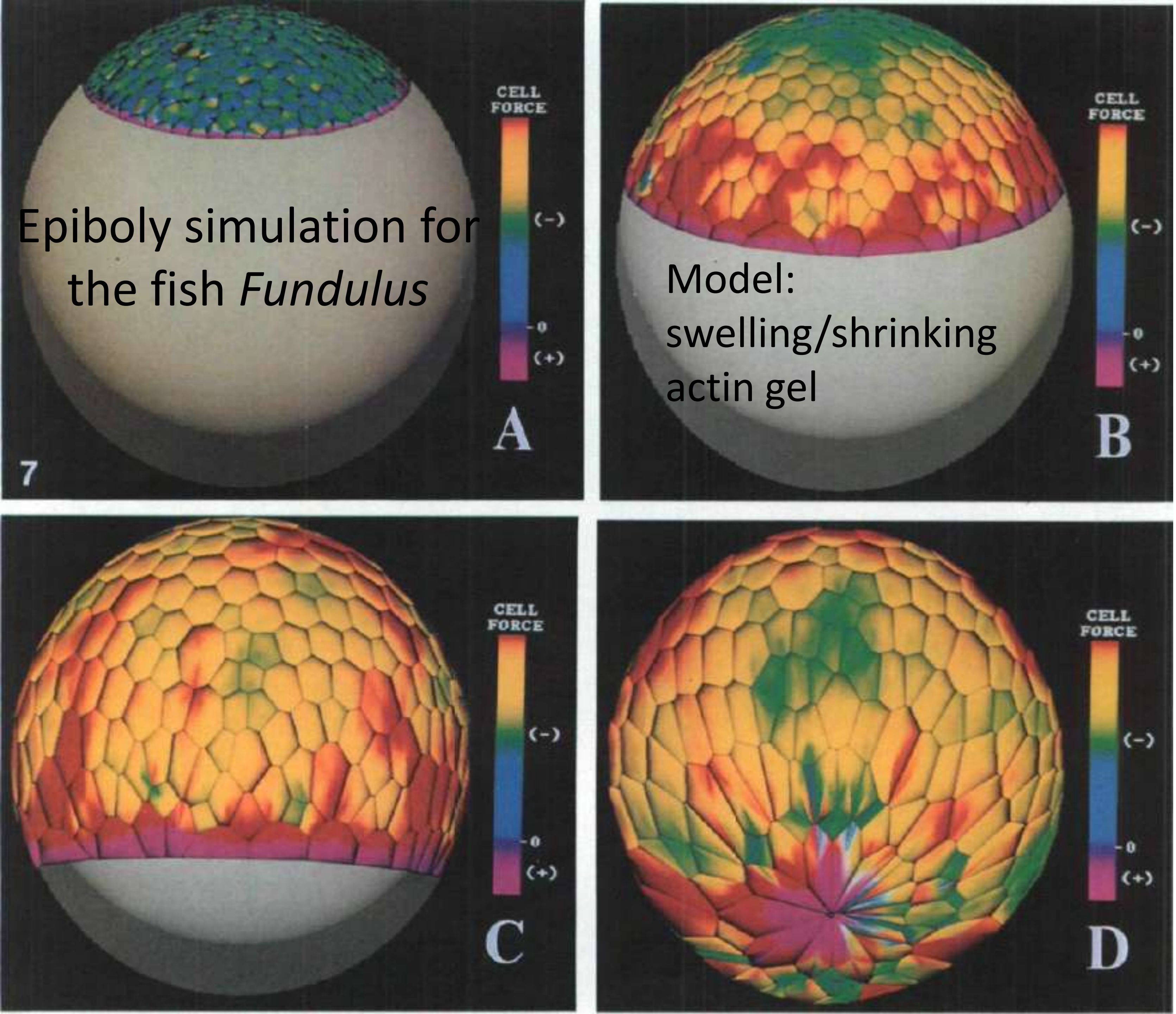


Left side view during ectoderm expansion wave, which starts as the ectoderm contraction wave ends.

Gordon, R., N.K. Björklund & P.D. Nieuwkoop (1994). Dialogue on embryonic induction and differentiation waves. *International Review of Cytology* **150**, 373-420.

Epiboly simulation for the fish *Fundulus*

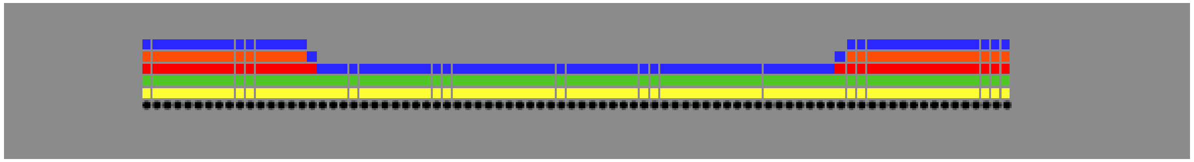
Model: swelling/shrinking actin gel



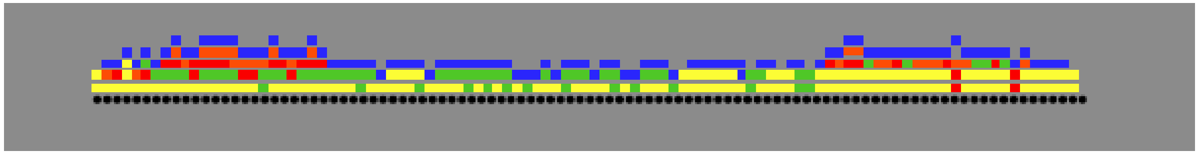
Weliky, M. & G. Oster (1990). The mechanical basis of cell rearrangement. I. Epithelial morphogenesis during *Fundulus* epiboly. *Development* **109**(2), 373-386.

Cellular automata simulation of thinning of the superficial epithelium in *Xenopus*

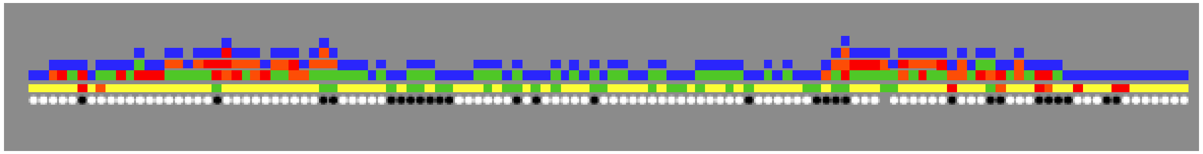
Longo, D., S.A. Peirce, T.C. Skalak, L. Davidson, M. Marsden, B. Dzamba & D.W. DeSimone (2004). Multicellular computer simulation of morphogenesis: blastocoel roof thinning and matrix assembly in Xenopus laevis. *Developmental Biology* **271**(1), 210-222.



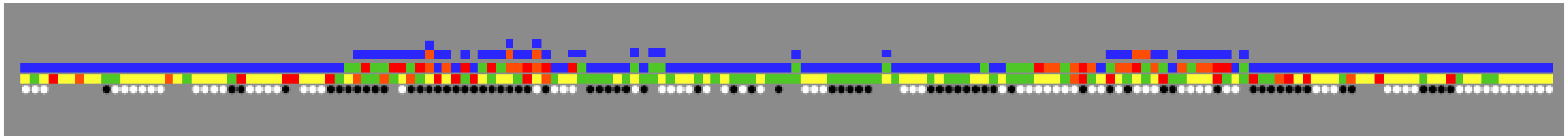
0 hours



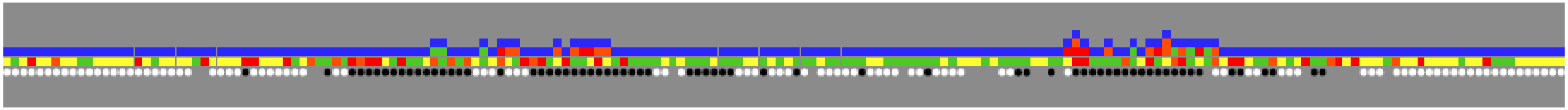
1 hour



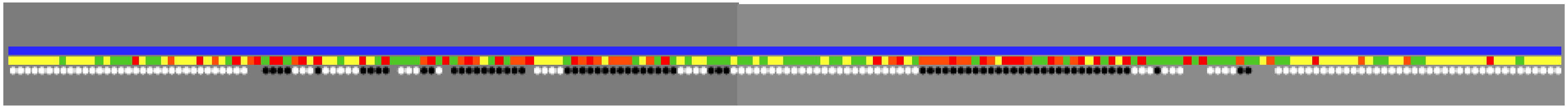
2 hours



3 hours



4 hours



4.8 hours

Concept: *Differentiation code*

- If we back track from a cell to its antecedents, we assume that the sequence of cells it came from participated in a sequence of:
- 0 contraction waves
- 1 expansion waves
- This can be summarized by a binary number:
  - Z01101
  - or
  - ZCEECE
- where “Z” is the fertilized egg (zygote), and consecutive differentiations are read right to left

Concept: *Tissue*

- All cells with the same differentiation code are equivalent from the point of view of differentiation and together constitute a “tissue”

Concept: *One-cell tissue*

- If there is just one cell with a given differentiation code, we call it a *one-cell tissue*

Concept: Differentiation tree

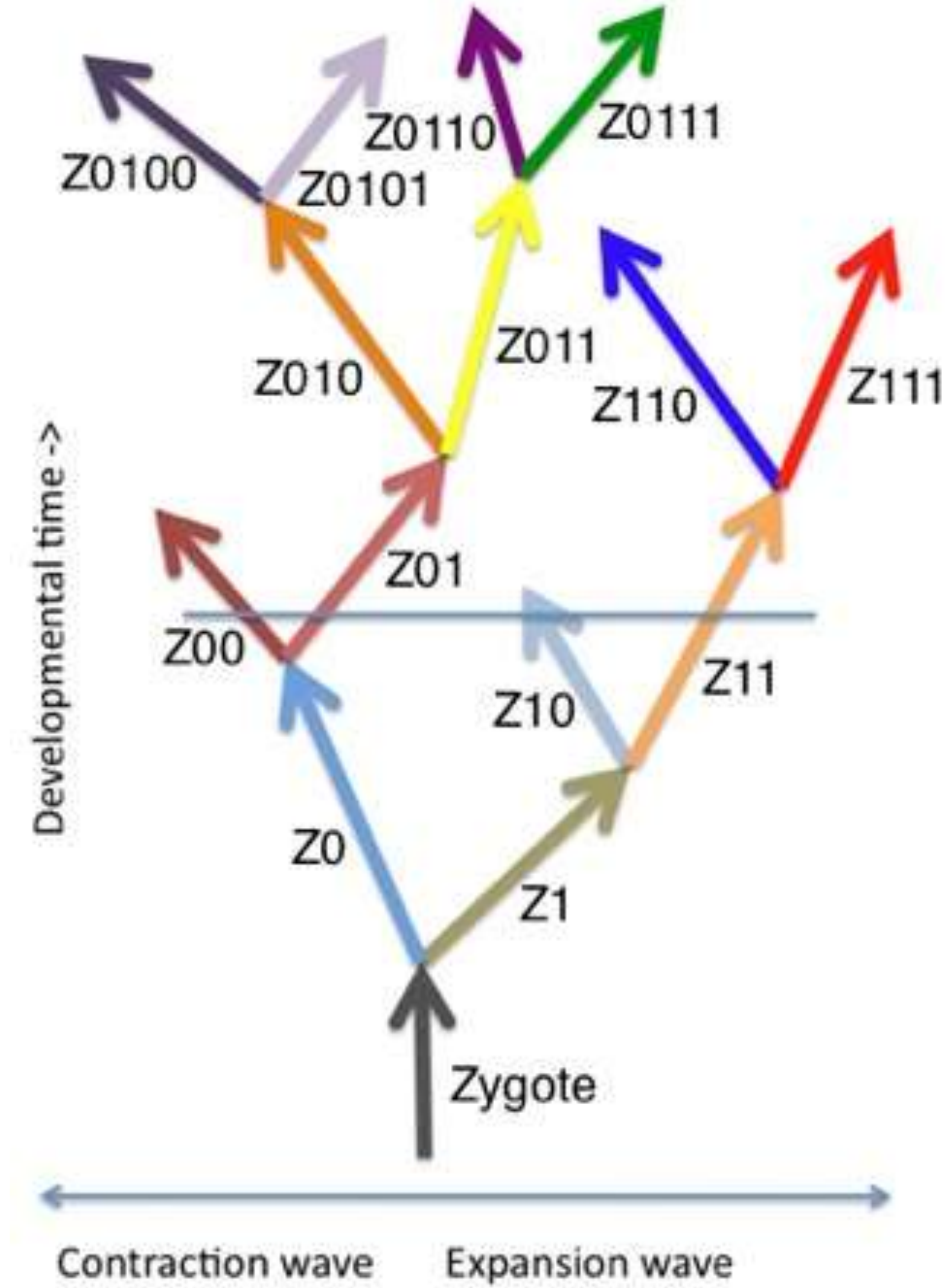
- Differentiation in an embryo can now be represented as a tree

The Differentiation Tree

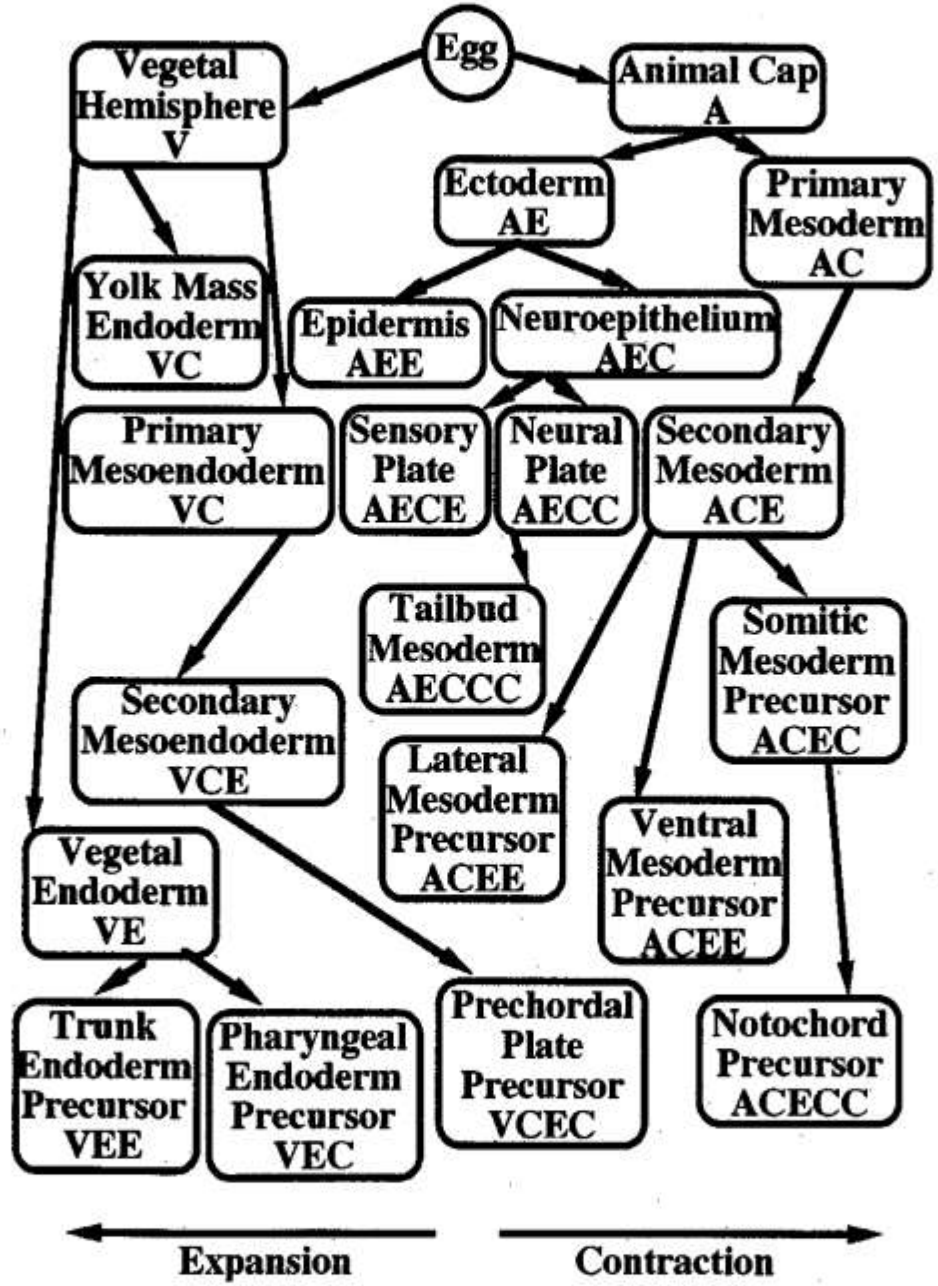
Each branch represents a distinct cell type

Note binary differentiation code

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



The Axolotl Differentiation Tree & Code, to open neural plate stage C=0=contraction E=1=expansion



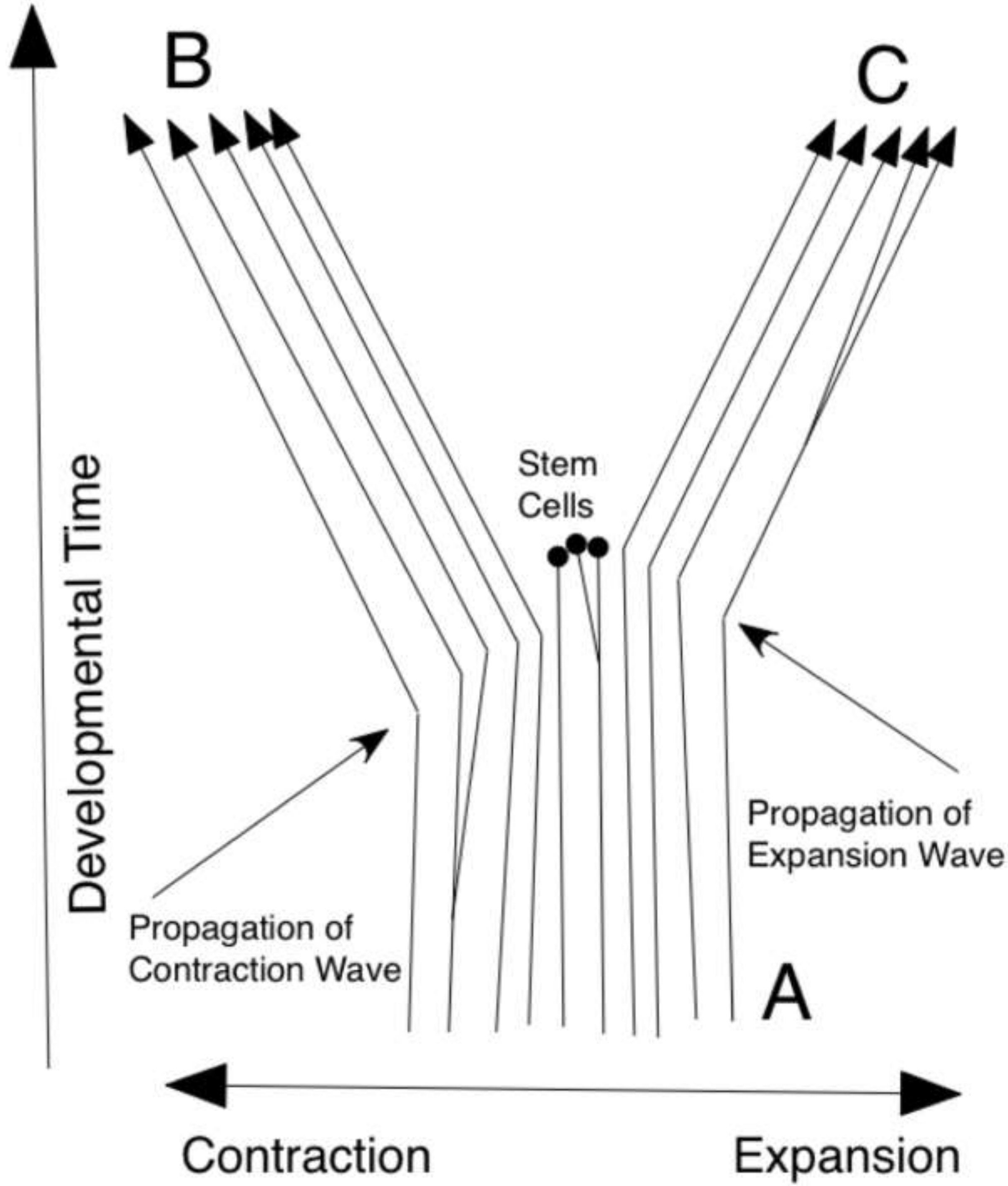
Björklund, N.K. & R. Gordon (1994). Surface contraction and expansion waves correlated with differentiation in axolotl embryos. I. Prolegomenon and differentiation during the plunge through the blastopore, as shown by the fate map. *Computers & Chemistry* **18**(3), 333-345.

Lineage tree vs Differentiation tree

- The lineage tree for an embryo is a tree that shows every cell division and tracks every cell, from zygote to adult
- The differentiation tree “bundles” groups of branches of the lineage tree of the embryo together
- The bundling is according to participation in contraction or expansion waves

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

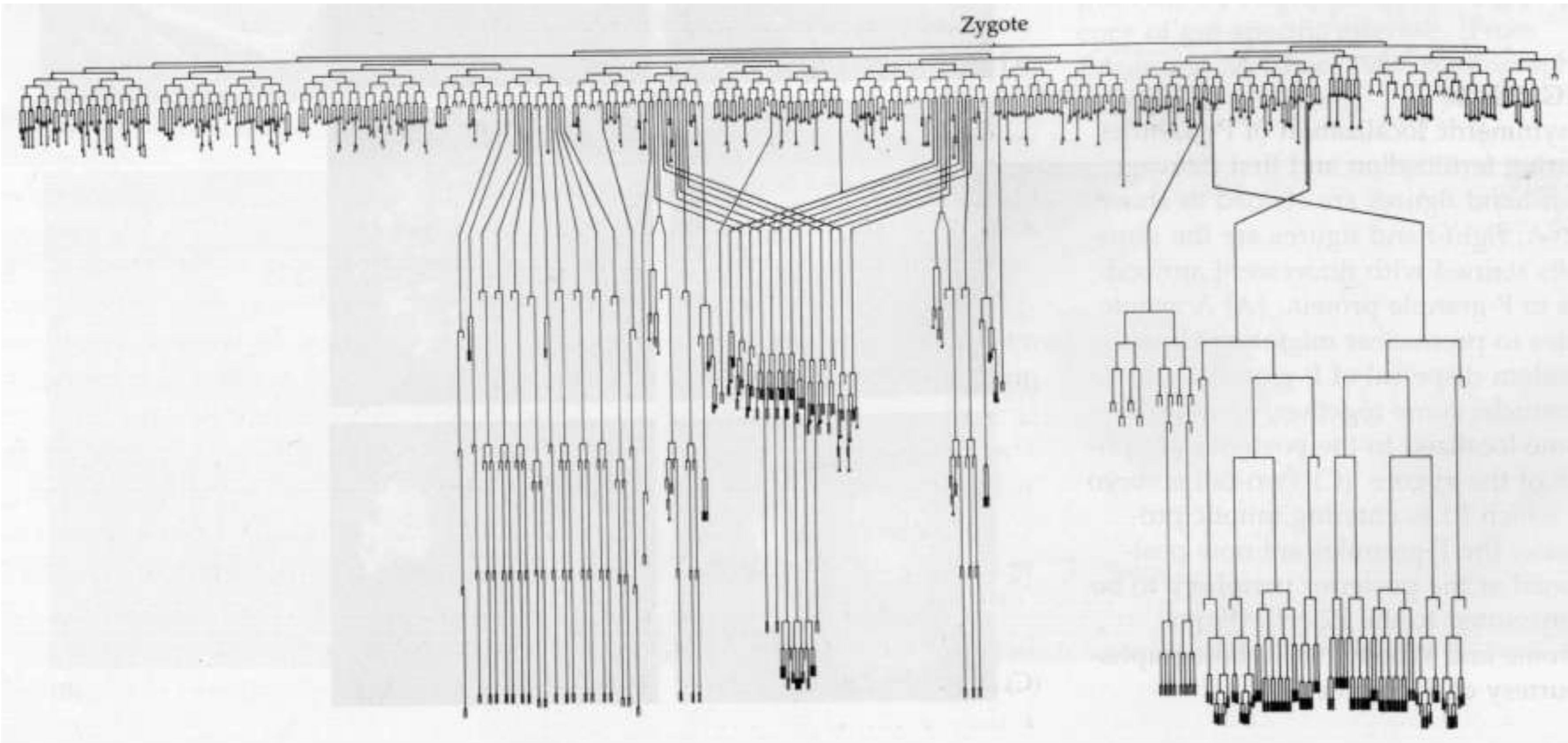
Gordon, R. (2011). Epilogue: the diseased breast lobe in the context of X-chromosome inactivation and differentiation waves. In: *Breast Cancer: A Lobar Disease*. Ed.: T. Tot. London, Springer: 205-210.



Concept: *Single cell differentiation waves*

- In mosaic development, as in the nematode *C. elegans*, most cells undergo asymmetric division, with simultaneous differentiation:
- cell A -> cell B + cell C
- It seems to be invariably the case that one daughter cell is smaller than the other in asymmetric division
- So we identify:
  - ◆BIG cell = expansion wave
  - ◆small cell = contraction wave
  - ◆Lineage tree = differentiation tree

Nematode Cell Lineage Tree
Caenorhabditis elegans



Need to reorder to make it a differentiation tree: small cell left, BIG cell right at each branch

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.

Summary

- Embryos with many or just one cell per cell type may develop by effectively the same mechanism, if we identify the action of differentiation waves as being essentially the same as single cell asymmetric cell divisions
- Both divide a tissue into two new tissues
- The differentiation waves model thus applies to both regulating and mosaic embryos