Who am I?

Theoretical Biologist

B.Sc. In Math from U. Chicago (1963)

Ph.D. Chemical Physics, U. Oregon (1967)

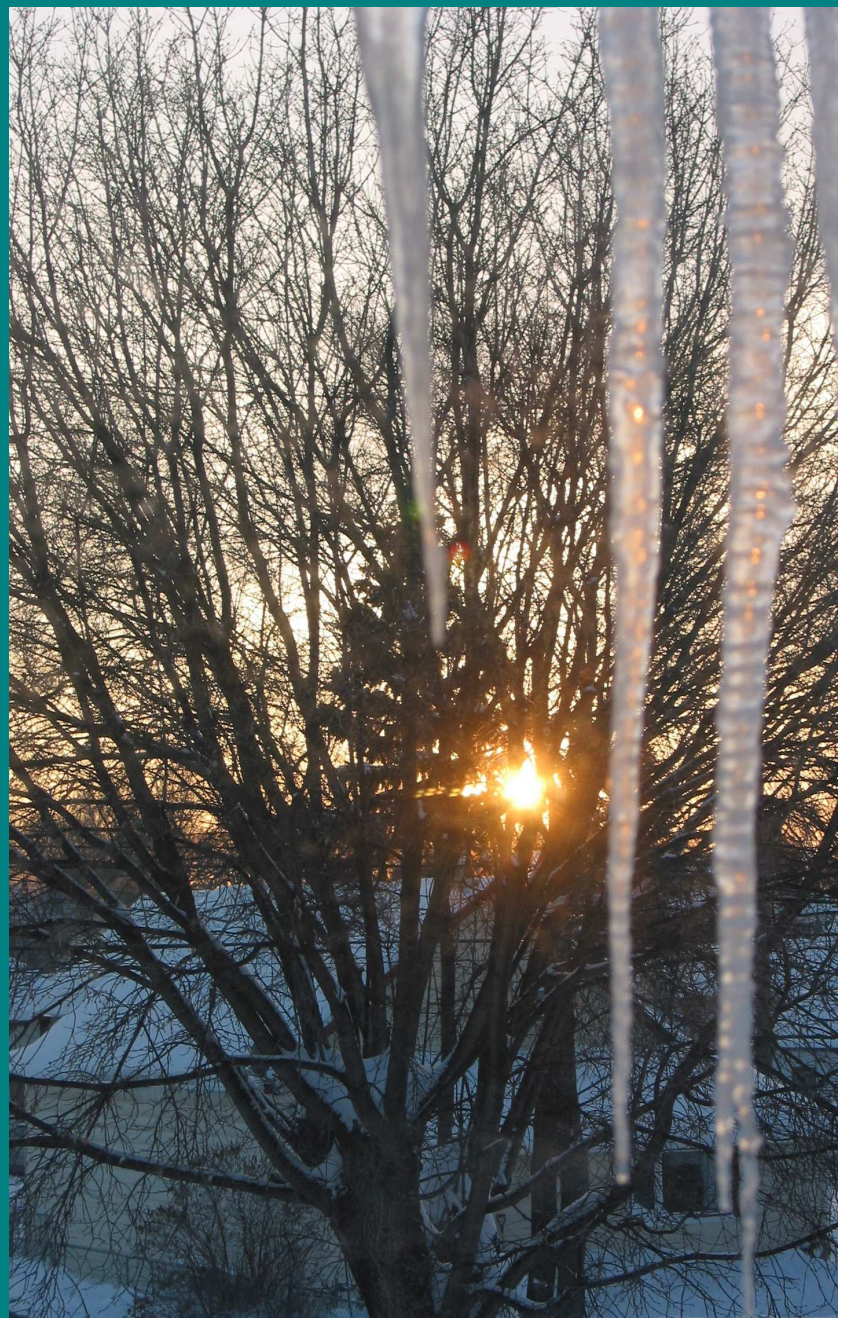
Academic appointments over the years:

Pathology, Radiology, Electrical & Computer Engineering, Botany, Zoology, Physics, Computer Science

Can we crack the differentiation code of embryos and cure breast cancer via 4D imaging?

Richard Gordon
Radiology & Biosystems Engineering
University of Manitoba, Winnipeg
(Winterpeg), Canada
GordonR@ms.Umanitoba.ca

February, 2004




OPEN: 1-5PM SAT.&S
NO SMOKING
NO ANIMALS

Present collaborators in embryology

Susan Crawford-Young, ECE

Tony Deluca, ECE

Rebecca Austman, Biosystems

David Levin, Biosystems, Visiting Prof.

Natalie Björklund, Biochemistry & Medical Genetics

Andy Maniotis, U. Illinois @Chicago

Wave & Genome

Collaborators past and present:
Andrew J. Maniotis, Department of Pathology, University of Illinois at Chicago
Antone G. Jacobson, Zoology, University of Texas at Austin
Cristofre Martin, Biology, University of Ottawa
David Eisenstat, Cell Biology, CancerCare Manitoba
Diana Gordon, Winnipeg
G. Wayne Brodland, Civil Engineering, University of Waterloo
Marc R. Del Bigio, Pathology, University of Manitoba
Mark G. Torchia, Department of Surgery, St. Boniface General Hospital
Natalie K. Björklund, Biochemistry & Medical Genetics, University of Manitoba
Pierre E. Williot, Pediatric Urology, Albert Einstein College of Medicine
Pieter D. Nieuwkoop, Hubrecht Laboratory for Embryology, Utrecht

Books by Richard Gordon

- 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 volumes.
- Gordon, R. editor (1994). *Mechanical Engineering of the Cytoskeleton in Developmental Biology*. International Review of Cytology **150**, 1-431.
- Rangayyan, R.M. & R. Gordon, editors (1990). *Proceedings IEEE WESCANEX '90, IEEE Western Canada Conference and Exhibition on Telecommunication for Health Care: Telemetry, Teleradiology, and Telemedicine*, Bellingham, Washington: International Society for Optical Engineering.
- Gordon, R. & Y. Chen, editors (1987-1988). *From Statistical Mechanics to Molecular Biology: a Festschrift for Terrell L. Hill*. Cell Biophysics **11**, **12**.
- Gordon, R. et al., editors (1984). *Topical Meeting on Industrial Applications of Computed Tomography and NMR Imaging*, Washington, D.C.: Optical Society of America.
- Gordon, R. & et al. (eds.) (1975). *Digest of Technical Papers, Topical Meeting on Image Processing for 2-D and 3-D Reconstruction from Projections Theory and Practice in Medicine and the Physical Sciences*, Washington, D.C. Optical Society of America.

More recent stuff

- February (2006) special issue: Morphodynamics: Bridging the Gap between the Genome and Embryo Physics, *International Journal of Developmental Biology* (IJDB), Guest editors: Lev Beloussov & Richard Gordon
- Ussing, A.P., R. Gordon, L. Ector, K. Buczko, A. Desnitski & S.L. VanLandingham (2005). The colonial diatom *"Bacillaria paradoxa"*: chaotic gliding motility, Lindenmeyer model of colonial morphogenesis, and bibliography, with translation of O.F. Müller (1783), "About a peculiar being in the beach-water". *Diatom Monographs* **5**, 1-140.
- Gordon, R., F.A.S. Sterrenburg & K. Sandhage (2005). A Special Issue on Diatom Nanotechnology. *Journal of Nanoscience and Nanotechnology* **5**(1).

Wave and Genome:

Reverse Engineering the Embryo

Richard Gordon

World Scientific
and
Harvard University Press*

*being negotiated

Consider a Spherical Cow…

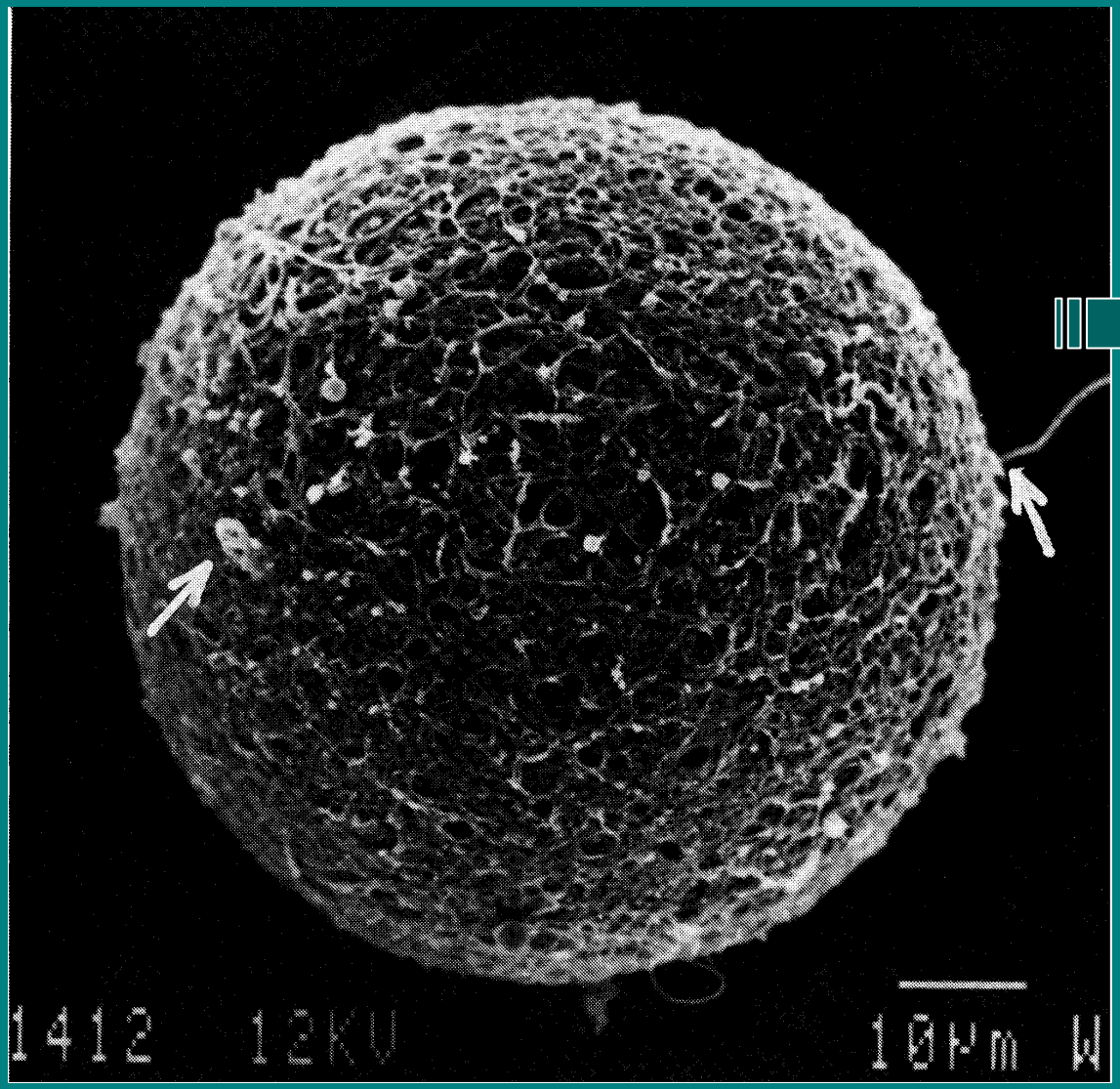
- At conception, when that lucky sperm, one in 200 million from your father, **fused** with the one egg released in your mother at ovulation (the as yet unfertilized oocyte, 1 of 40,000 available), you were a small sphere less than 1/10 of a millimeter in diameter, the size of a droplet of mist. The scanning electron micrograph (SEM, opposite) is a fertilized human egg showing the tails of two sperm who have just arrived. The scale bar is 10 micrometers, showing that your egg was about 70 micrometers or 70 thousandths of a millimeter (0.070 mm) in diameter, much smaller than the period at the end of this sentence. Shrunk back to their starting size, the 6 billion people on earth would just fill a 1 liter bottle.
- Physicists, who have a reputation for simplifying when theorizing about life, are often jokingly said to start off a lecture with: 'Consider a spherical cow...'. However, cows are indeed spherical, and not just when they are meatballs. In biology we give the same species name to every stage in the life cycle of an organism. Thus a fertilized, spherical cow egg *is* a cow (or a bull), *Bos taurus* or *Bos indicus*. The egg opposite is indeed a *Homo sapiens*.
- **The problem addressed in this book is how a spherically symmetric egg turns into a highly asymmetric organism like you and me.** My tentative solution, that it's done by differentiation waves, also permits us to speculate on how such eggs evolved from

- Comments:
- Yes, **fused**! As shown by a married couple of scientists, of course[1]. The cell membrane surrounding the haploid nucleus of the sperm fuses with the cell membrane of the haploid oocyte, somewhat like two bubbles in your bath water will fuse into one. The sperm's nucleus is drawn in, where it combines with the egg nucleus to form the first diploid cell, from which all other cells in our body arise.
- **Spherical symmetry** means that a spherical egg is the same in all directions from the center of the sphere. My guess is that the human egg is spherically symmetric. How do we know? We don't. The best we can say right now is that when the inside of a similar looking 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[2]. Sergei now works in reproductive technology in the USA. No one else has pursued the question of spherical symmetry of mammalian eggs. We will find that this lack of follow-up is a common problem in science. Which tends to rush along popular paths, rather than being thorough. My hope is that you, the reader, will take up one or more of these "side trails" that may lead to the main road: an explanation of how embryos build themselves.

[1] Colwin, L.H. & A.L. Colwin (1963). Role of the gamete membranes in fertilization in *Saccoglossus kowalevskii* (Enteropneusta). II. Zygote formation by gamete membrane fusion. *J. Cell Biol.* **19**, 501–518.

[2] 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.

The Problem



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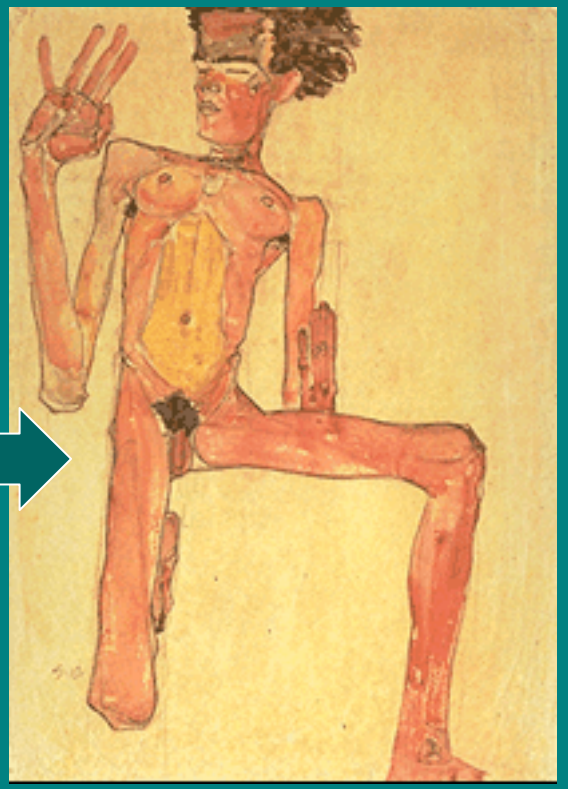


1,000,000 µm = 1 meter

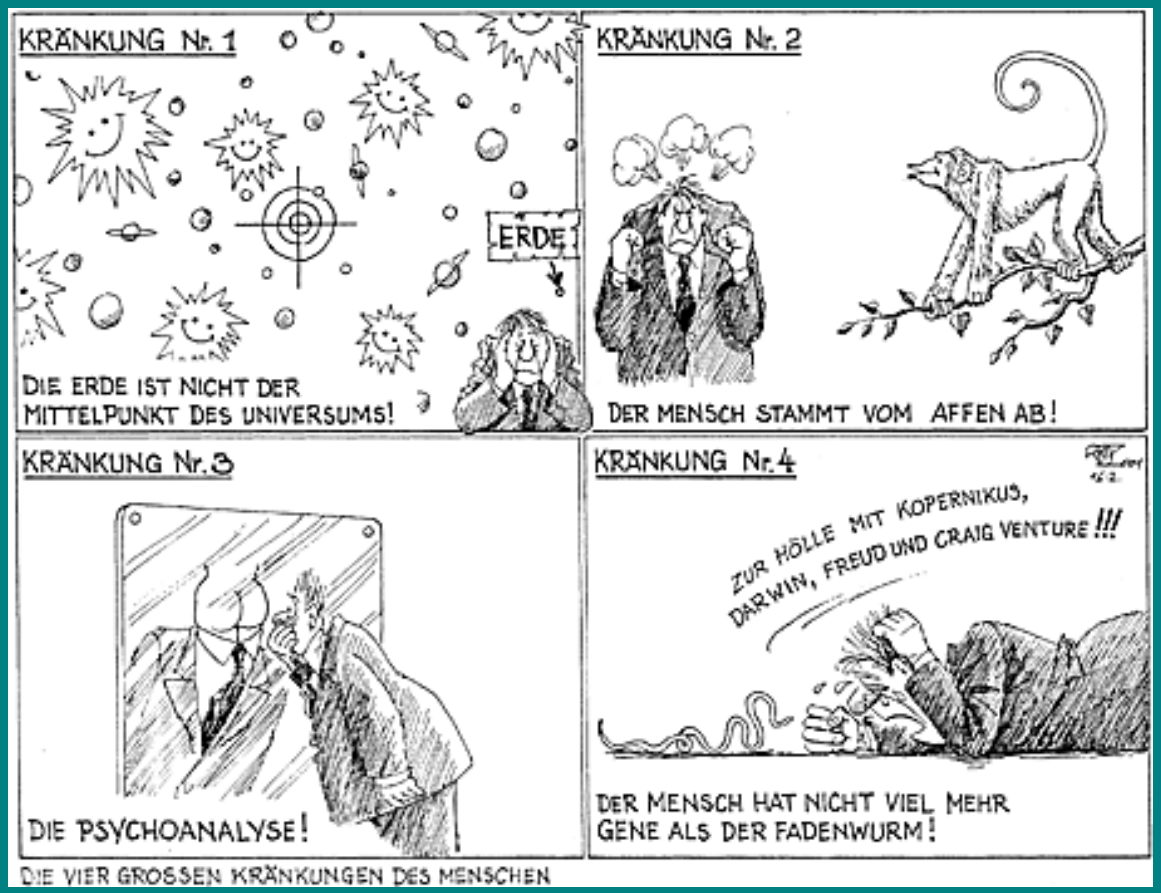
Egon Schiele
Kneeling Male Nude (Self-Portrait).
1910.
http://www.moma.org/exhibitions/schiele/artistwork.html

How did your spherically symmetrical egg turn into a highly asymmetrical shape? We're not even bilaterally symmetric, if you consider the brain your internal organs, and your left or right

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.

Genetic Determinism (Not)

We have just lived through a century in which the gene was king in biology[1]. Molecular biologists, starting with the then youthful antics of Watson[2] and Crick, rushed us through a heady plunge into what they conceived as the DNA makes RNA makes protein basis for life. A billion dollars spent and we had the sequence of the billion nucleotides in our own DNA. Then came the crushing blow to our dignity: we have no more genes than a worm!



Translation: Insult No. 1. The earth is not the center of the universe! Insult No. 2. Humans descend from the ape! Insult No. 3. Psychoanalysis! Insult No. 4. Humans have not many more genes than the thread worm! To hell with Copernicus, Darwin, Freud and Craig Venter[3]!!! Cartoon, by Dieter Zehentmayr, from *Der Standard* with permission.

Most of our concern about genetic determinism is the old nature/nurture problem: how much of what we do depends on the genes we inherit? It's actually a deeper problem, because what we really mean is: Do we have free will? Many genes have what is called limited "penetrance", which means that in organisms of the same species containing the same gene of the same allele (identical DNA sequence), one will show its effects and another individual will not. Usually the "environment" is blamed (such as smoking, eating habits, temperature, etc.), or crosstalk effects from other genes that may differ between the individuals. But the quotes opposite hint at another failure of genetic determinism: something besides genes must be involved in building embryos.

[1]Keller, E.F. (2000). *The Century of the Gene*, Cambridge Harvard University Press.

[2]Watson, J.D. (1968). *The Double Helix*, New York Atheneum.

[3]Venter, J.C. et al. (2001). The sequence of the human genome. *Science* **291**(5507), 1304-1351.

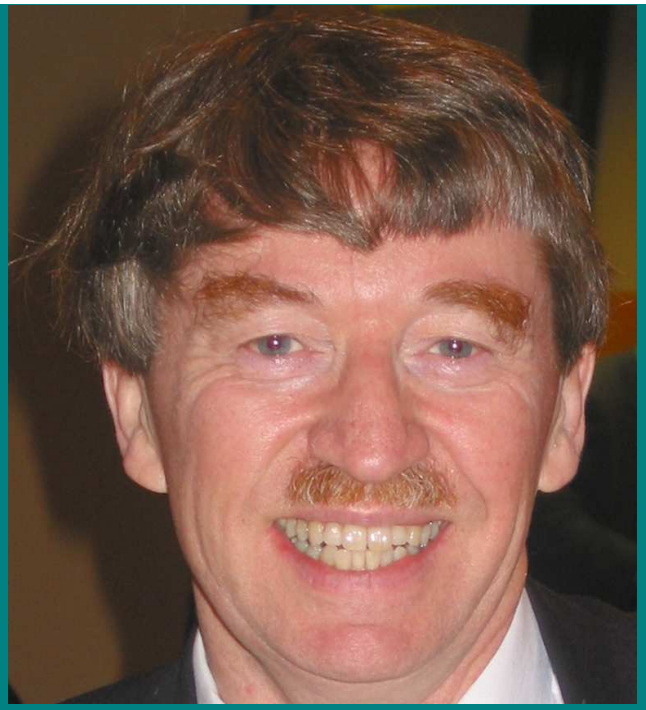
Left Side: Meet My Right Side

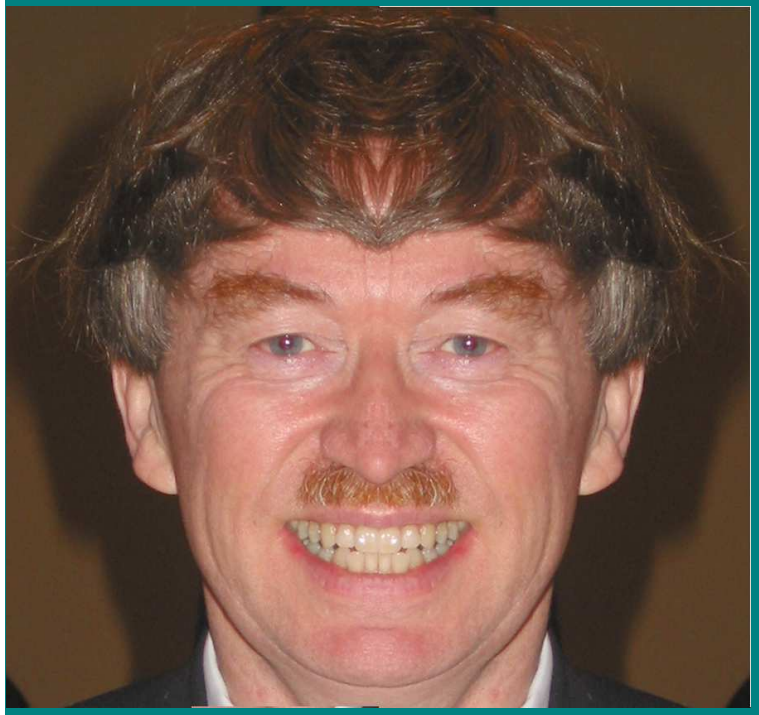
Jackson Beardsley self-portrait

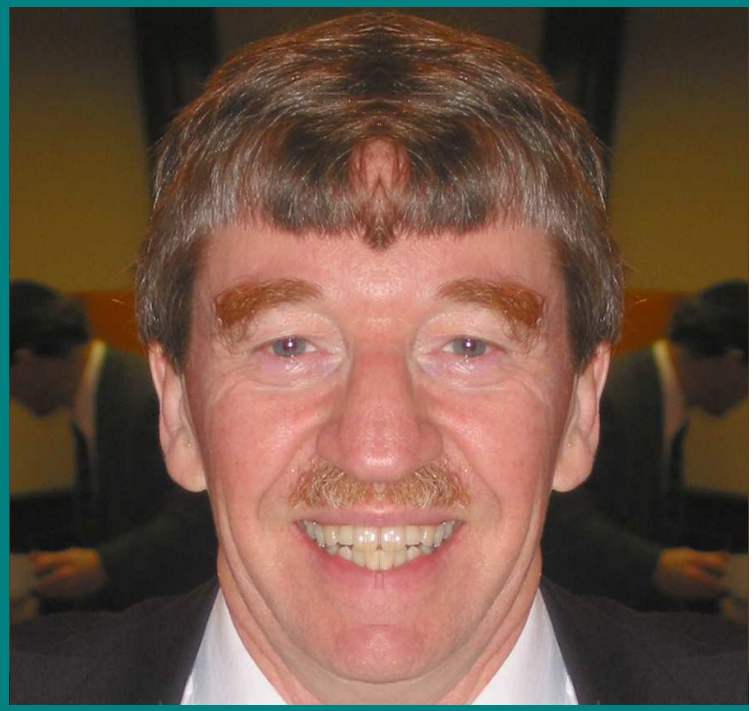
- One classical experiment done by photographers was to take a front on picture of a person, split it down the middle, and make mirror images of each half. Nowadays those of us inept with darkroom chemicals can do this easily with a digital camera and an image processing program. The result: each of us has two entirely different personalities: our left side and our right side.
- The point of this exercise is that even on the outside, our left and right sides are different. Lest you think that this has an "environmental explanation", just compare your fingerprints for corresponding fingers or your thumbs. No, we believe that the genomes in cells on our left and right sides are identical, yet anatomically we come out different left and right. This again proves that genetic determinism does not work.

The two sides of my friend and colleague, David Hoult, with whom I've worked on:

Tomanek, B., D.I. Hoult, X. Chen & R. Gordon (2000). A probe with chest shielding for improved breast MR imaging. *Mag. Res. Med.* **43**(6), 917-920.







Batteries of Genes (now observable with DNA arrays): 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."
Morgan, T.H. (1934). *Embryology and Genetics*, New York Columbia University Press.

"It seems evident at first sight that a mutation affecting the expression of a 'battery' of structural genes would lead to a much more profound difference in the development of an organism, and hence of its adult morphology and character, than one resulting in a change in the function of a single protein."
Delbrück, M. (1986). *Mind from Matter?*, Palo Alto Blackwell Scientific Publications.

"Some still unknown mechanism will turn on a set of genes in one cell and then turn it off, while orchestrating an entirely separate set of genes in another cell or group of cells (all of which were identical a few days ago)."

Schroeder, G.L. (2001). *The Hidden Face of God, Science Reveals the Ultimate Truth*, New York Simon & Schuster.

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)
- Chapter 10 of first edition: http://www.marxists.org/reference/subject/philosophy/works/us/kuhn.htm
- Thomas Kuhn is one of my heroes. He brought some clarity to understanding the process of scientific discovery, by defining a paradigm as: a way of thinking about a problem that most scientists agree on. I read his book a few years after the first edition came out, and he expressed surprise that anyone was still interested when I wrote to him. Later his fame took off. He suggested that an *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. He therefore also legitimized people who buck the current paradigm, which I find myself doing now and then, as in this book.
- "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. I'm going to try to eliminate some here, by claiming:

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

The biologist reading this list will cringe; for others I'll seem to be tilting at windmills. But for biologist and nonbiologist alike, the unbounded faith or fear that we can manipulate organisms to create anything we want is based on the mythology that, having read the sequence of letters of the genome, we understand its meaning. We are no further along than a three year old Talmudic "scholar" who has mastered the Hebrew alphabet, and can read a few words. For example, Carlson* extends the theory of positional information to the idea that we could grow pigmented leaves with pigmented cells that could substitute for the ink in any printed document. Not likely.

•Carlson, R. (2001). Open-source biology and its impact on industry. *IEEE Spectrum* **38**(5), 15-17.
http://www.spectrum.ieee.org/WEBONLY/resource/may01/spea.html

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
- mechanism
- morphogens
- negative feedback
- negentropy
- networks of interacting genes
- organicism
- plasmagenes
- positional information
- positive feedback
- predetermination
- prepatterns
- reaction-diffusion equations
- self-assembly or self-organization
- spirochete cortical colonies
- traction
- vitalism

Hans Driesch (1867-1941)

- Driesch was a clear thinker. One of his major contributions was to set our agenda, which is still in place, awaiting completion. 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

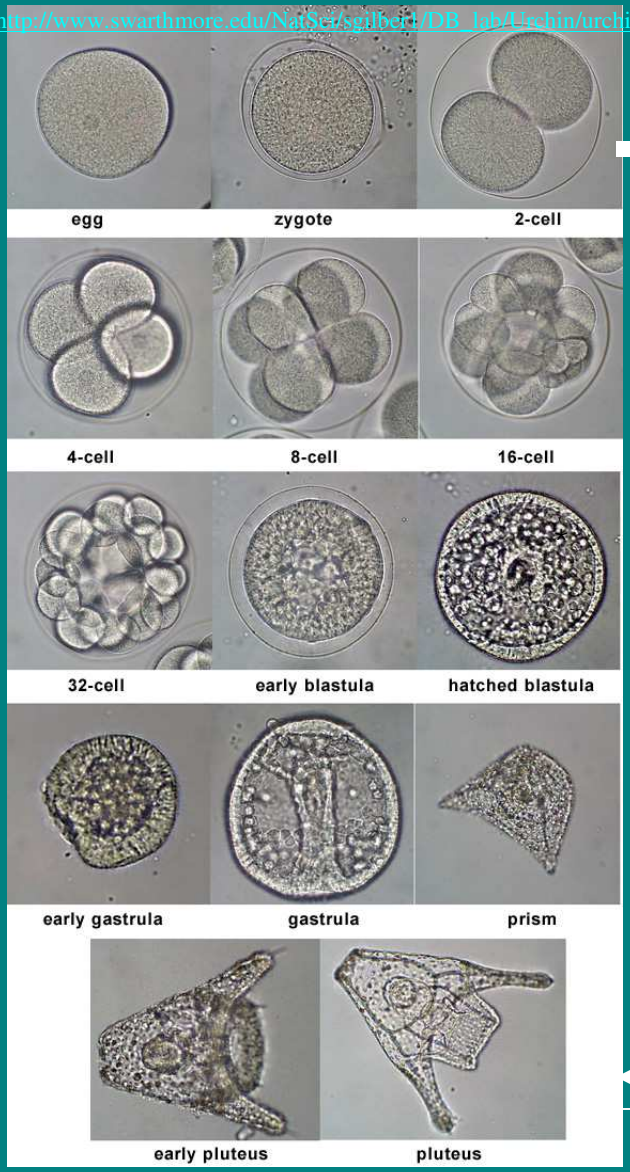
*Sea Urchin's fertilized egg dividing and growing into a blastocyst, time lapse: http://amazingbeauty.org/Nature/ABbiology02-baby.html.
Also see: http://worms.zoology.wisc.edu/urchins/SUmainmenu.html.

The Million $ Hans Driesch Prize is Awarded by MacDonald's Hamburger Restaurants!

The (Rail)Road to Vitalism

Sea Urchin staging series, taking one week

http://www.swarthmore.edu/NatSci/sgilbert/DB_lab/Urchin/urchin_stage.html



Shake apart

x2

B&O Steam Locomotive, from Sue Breakiron Wert

http://www.fay-west.com/connellsville/historic/picture2/steam_locomotive.shtml

- In the 1800's machines were made of gears, axles, cams, belts, wheels, etc., and were driven by steam, springs or gravity (as in clocks), wind or water mills. The workings of a machine were visible and much more obvious than today's computer driven appliances and robots. It is in that older context that we can appreciate the mental state of Hans Driesch when he tried to test if an embryo is a machine. His experiment was simple: he shook a sea urchin embryo apart when it reached the two cell stage, i.e., after the first cell division. No machine known to him would function as two identical machines if chopped in half. But the two cells turned into two whole pluteus embryos (albeit of half the volume each). Therefore embryos aren't machines*. Driesch turned to vitalism, the idea that there is something special about life that sets it apart from physics and chemistry, an "entelechy" that operates inside each cell, altering its physics and chemistry. He was the last professed vitalist. But his observation is still unexplained.
- * Driesch, H.A.E. (1892). The potency of the first two cleavage cells in echinoderm development. Experimental production of partial and double formation. In Willier, B.H. & J.M. Oppenheimer, *Foundations of Experimental Embryology*, New York Hafner, p. 39-50.

Baby, months later: http://ebiomedia.com/prod/BOechinoderms.html. Adult sea urchin

http://www.mbayaq.org/efc/living_species/default.asp?hOri=1&inhab=201

Would I Like My Clone?

- Cloning has been around for quite a while, but no one minded when it amounted to grafting trees, or making genetically identical frogs[1]. Cloning of cattle has been a lucrative business, though it was accomplished at first in the manner of Driesch's experiment on sea urchin embryos, by separating cells from the first few divisions. With the creation of the sheep Dolly via an actual transplantation of an adult's cell nucleus into a sheep egg, that had its original nucleus removed, the excitement built. But Dolly has had medical problems, such as arthritis and other signs of premature aging, which suggests that something more is involved in cloning than merely copying DNA. Subsequent work on cloning of cattle by nuclear transplantation may have given the opposite result: animals that live longer. Time will tell[2].
- The problem with a clone, of course, is that it is born as a baby that has to be raised. If the normal rebelliousness of toddlers and teenagers isn't daunting enough, consider whether you would actually get along with your clone? A clone doesn't have your memory, and so does not give you any greater degree of immortality than raising kids the usual way. The preliminaries certainly aren't as much fun. Nevertheless, I'm not against human cloning. While it's a bit perverse and narcissistic, as with other reproductive technologies, such as those that that give children to some infertile couples, it increases the value of human life. Only a few people are likely to indulge in it, and I doubt that it is of much harm in the long run. Besides, if the slightest problem develops, 18 years afterwards the clones will be suing their parents and the organizations that encouraged them for "wrongful birth", which will dampen enthusiasm considerably.
- Cloning is an excellent test for how tightly development is "controlled" by the genome, which, at least in its DNA sequence, everyone presumes is identical. The answer with cloned pets, such as cats, is now in: a clone need not look like nor behave like its "parent" at all. In a knee jerk reaction, this is attributed to the "environment". That's the least likely cause. I suspect that if we had a personality test for frogs that cloned animals would wide variations, just as they do in their pigmentation patterns[1].

[1]McKinnell, R.G. (1978). *Cloning, Nuclear Transplantation in Amphibia A Critique of Results Obtained with the Technique to Which Is Added a Discourse on the Methods of the Craft*, Minneapolis University of Minnesota Press.

[2]Vogel, G. (2000). In contrast to Dolly, cloning resets telomere clock in cattle. *Science* **238**(5466), 586-587.

Clones

- Cloned frogs showing variation in pigment patterns.
- Dolly.
- Cloned cats. http://msnbc.com/news/862384.asp?cp1=1

http://www.wowzone.com/clonecat.htm
Left: mother, right: surrogate mother and Copy Cat kitten, which is not only pigmented differently, but behaves differently, despite being genetically identical to its biological mother.2020

Shin, T., D. Kraemer, J. Pryor, L. Liu, J. Rugila, L. Howe, S. Buck, K. Murphy, L. Lyons & M. Westhusin (2002). A cat cloned by nuclear transplantation. *Nature* **415**(6874), 859.

A Face Only a Mother Could Love (to Eat)

Axolotls are much like us, being vertebrates with limbs, eyes, fingers and toes, and are probably similar to our amphibian ancestor who crawled out of the seas onto land. But they have some remarkable properties we wish we had. Cho(m)p off a leg (which these cannibals do to one another frequently), and it grows back in 6 weeks. Same for the tail. Sever their spinal cord, even in an adult, and they fully recover in 2 months[1]. (Why the medical community, hung up on the uniqueness of mammals, doesn't rush to understand how axolotls do this, is beyond me.) There's even one wonderful experiment, waiting for someone to repeat and extend, in which a chunk of brain of an axolotl trained to avoid dark places (which they ordinarily like) was transplanted to another axolotl, who subsequently avoided dark[2]. What a way to get an education!

I like to work with axolotls because their embryos have large, easily distinguishable cells on the surface. Their forming brain (actually CNS = Central Nervous System, consisting of brain and spinal cord) is one layer of cells thick (mathematically convenient), just like mammals, birds and fishes (but unlike frogs, the favorite of molecular biologists). This predilection for frogs (anurans) arose when molecular biologists were looking for a vertebrate in the 1950s, and found the South African clawed toad everywhere, because it was being used for pregnancy testing. They completely ignored the 50 year tradition in embryology of concentrating on axolotls, salamanders and newts (urodeles)[3]. Everything had to be rediscovered in *Xenopus laevis*, a much more distant relative of ours. So much for scientists reading the scientific literature. In fact, all of our "model organisms" in developmental biology, *Xenopus*, zebrafish (*Danio rerio*), fruit fly (*Drosophila melanogaster*), and nematode (*Caenorhabditis elegans*) are evolutionarily specialized in their embryogenesis, making one wonder about the universality

The Axolotl
Ambystoma mexicanum

A rare piebald axolotl, 23 cm long, showing its external gills. It occasionally comes up for a bubble of air, since it also has a single lung (an example of left/right asymmetry: our two lungs are also of different sizes and shapes).

Axolotls are very much like us: vertebrates with the same organs and tissues, distinct front and rear limbs, and an alertness similar to that of a stalking cat. Maybe they even share some form of consciousness. In our lab they come to the side of their tanks to stare at us, and seem to like having the glass cleaned of algae, so they can see out. Perhaps this is anthropomorphosing, but little is known about their intelligence, for lack of asking.

But axolotls can do some things we only wish we could, feats listed on the opposite page.

A newly hatched axolotl larva. Note the individually visible black pigment cells (melanocytes). A closer view of the translucent external gills would show blood cells circulating in single file.

Axolotl Wonders

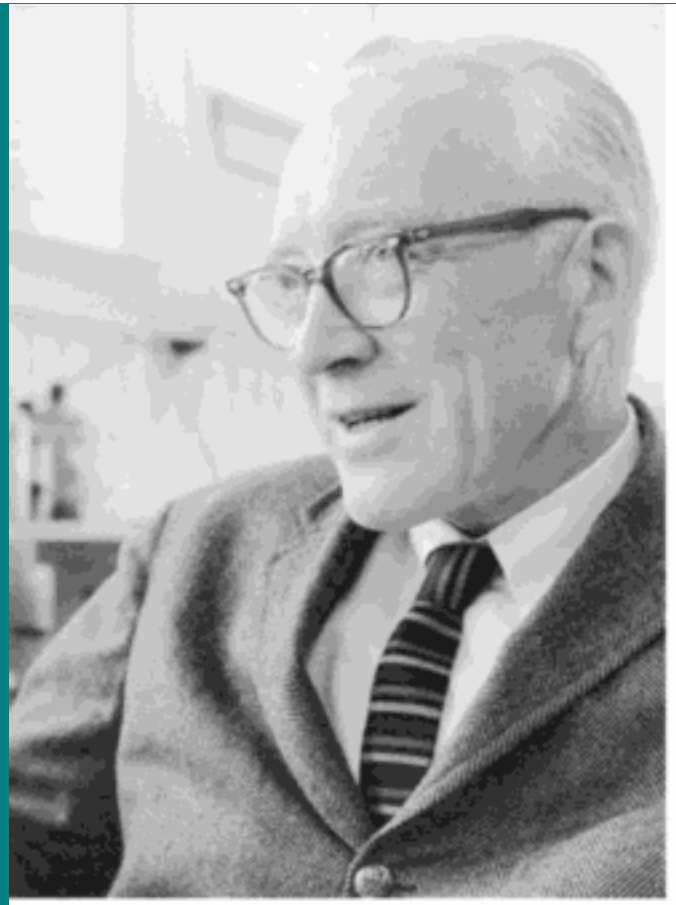
- ü Huge cells, 25µm x 50µm at neurulation.
- ü Huge genome, 10x human genome.
- ü Legs regenerate when bitten off.
- ü Tail regenerates if lost.
- ü Adults recover fully from spinal cord injuries.
- ü One animal from two, fused half embryos.
- ü Heart cut 75% regenerates, pumping in 24 hours.
- ü Brain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

Mexico City, Wilderness Habitat of the Axolotl

Victor Twitty, my intellectual grandfather on the embryology side (through Antone G. Jacobson, University of Texas), wrote a delightful book that I lend to every student interested in embryology[1]. In it he ranges over the puzzles of early embryological development of urodeles, to formation of cilia and pigment patterns, and their ecology, concentrating on the California newt.

The axolotl has been in labs for about 200 years. Most of the original animals came from the cold water Lake Xochimilco that is now Mexico City. They prefer 17°C, but tolerate and can develop over 8-24°C. (We ship them to other labs in ice water.) Axolotls persist in the "urban wild", with protection and aquatic breeding pens provided by Mexican embryologists.

Many people keep axolotls as pets. Their behavior is like that of a stalking cat, ever alert, ready to pounce if food swims by. We feed them turtle food, and occasionally raw beef heart for a treat. They have to be kept in groups all the same size, otherwise legs are chomped off the smaller ones. Bullies are transferred to tanks with larger axolotls. In Winnipeg we lend axolotls to anyone with an aquarium under our Animal Care approved Adopt-an-Axolotl program. This gives us a distributed colony that we can call back in case of fire in the lab over the weekend.



Courtesy of the Stanford News and Publication Service

V. C. Twitty

http://www.nap.edu/books/0309060869/html/332.html

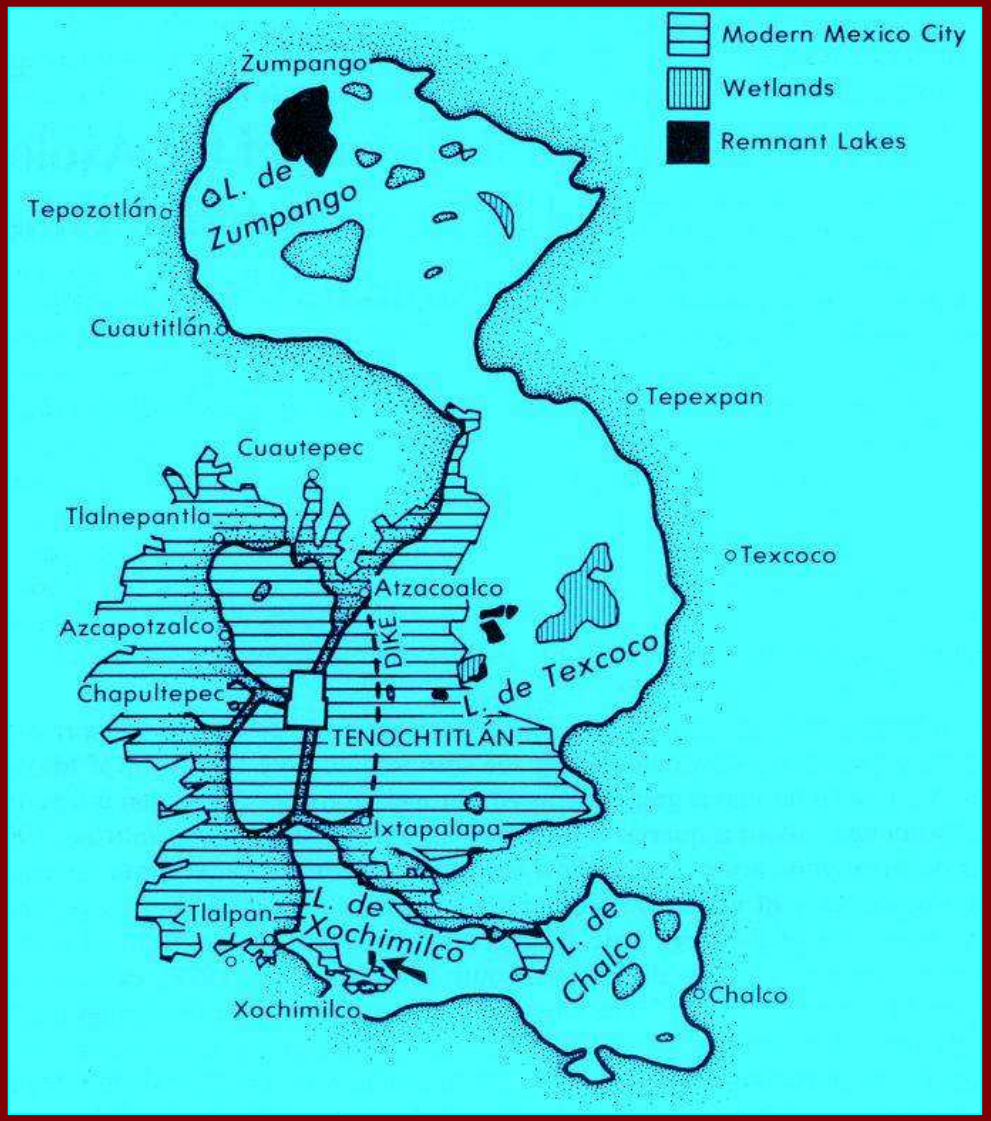
We used to inject human gonadotropin to force egg laying, but this was traumatic for the animals, and ran the risk of introducing abnormalities. Now we let nature take its course, and adjust our schedules to their behavior. In artificial 12 hour light/dark cycled tanks we get up to four spawnings per year per female. The females walk behind the reluctant males, picking up deposited sperm packets with their cloaca. Given the flushes suggesting whole body orgasms, visible in white and albino mutants, I doubt that we could convince axolotls that we do it better.

Juniors are raised from eggs in "pond tanks", aquaria kept continuously under bright light, to feed the algae that are eaten by rapidly reproducing *Daphnia* and cyclops, that are hunted by the axolotls who hatch at a length of

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

[2] Björklund, N.K. (1993). Small is beautiful: economical axolotl colony maintenance with natural spawnings as if axolotls mattered. In Malacinski, G.M. & S.T. Duhon, *Handbook of Experimental Methods*, Bloomington Department of Biology, Indiana University, p. 38-47.

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.

Descriptive Embryology

In embryology we must first discover what happens, before we can discuss how or why. These may seem to be an elementary philosophical prerequisite, but scientists often skip this step. For example if a gene is knocked out (suppressed or deleted in a genetically manipulated individual), and something fails to happen, say development of the eyes, most biologists conclude that this gene "controls" eye development. This is akin to saying that because a flat tire prevents a car from moving, "tires control cars". Now I can see that that gene is a component of some process that constructs the eye, and its discovery is part of descriptive embryology, but it doesn't control construction of the eye. This attitude, that genes control everything biological, is called genetic determinism[1]. I think it comes from a lack of training of many biologists in the fundamentals of logic, math, physics and chemistry, and from the enormous success of molecular biology in answering a narrow scope of important questions. A little humility while trying to understand the beauty of the self-construction of an embryo would be warranted. The presumption of molecular biology is that we know the nature of the answer, and need only work out the details.

So let's first just look. Embryologists have come upon a reasonably robust way of talking about the stages of development of an embryo. Stages are numbered not at uniform time intervals, but according to easily recognizable morphological features. This means that the time intervals between stages can vary greatly. For most so-called cold-blooded embryos, the speed of development increases rapidly as the temperature rises. Despite this, the same sequence of events seems to occur, so we can use the same staging at all temperatures. The following pages contain all the stages of development of the axolotl, up until hatching. Since the adult is essentially an overgrown (and mating capable) larva, the only major outward difference between the larva at hatching Stage 44 and an adult is the growth of the limb buds into legs with fingers and toes.

Like the human egg, the axolotl egg starts off as a sphere, only much bigger. In fact, at 2 millimeters (2 mm), in volume it is $2^3/(0.07)^3$ = 20,000 times bigger. Of course, the "reason" is that much of the volume is made of tiny, membrane surrounded sacks of yolk, which provide the food for the two week journey (at 20ºC) from stage 1 to stage 44. With a mouth, the larva can now eat and increase in "dry mass". Except for water, there is no increase in mass of an axolotl embryo until it can eat. In contrast, a human baby, 3 kg (7 lb) at birth has increased in dry mass about $20x10^9$ (20 billion) times compared to its egg. Prenatal nutrition does have benefits. On the other hand, we humans generally have just one baby at a time, whereas a female axolotl lays 300 to 1000 eggs per spawning. Both are routes to evolutionary success (being here, rather than extinct).

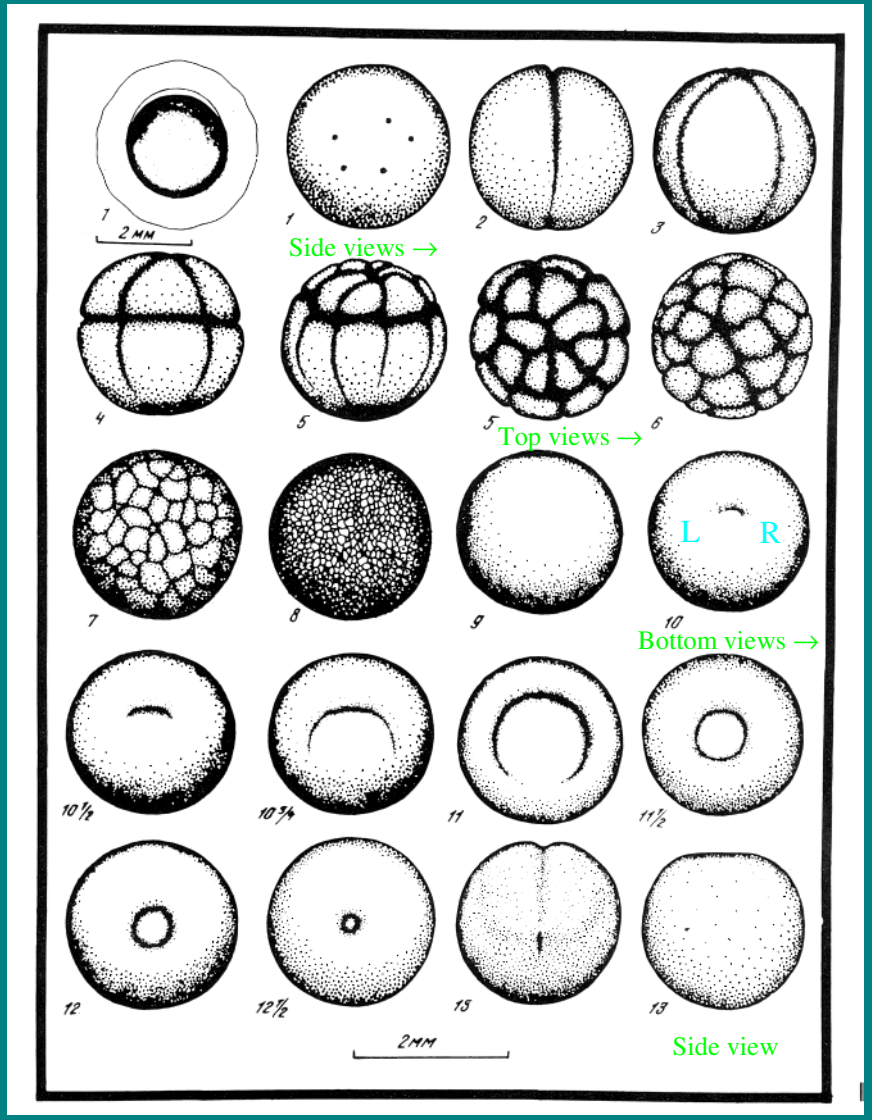
[1]Keller, E.F. (2000). *The Century of the Gene*, Cambridge Harvard University Press.

Staging of Axolotl Development

v The axolotl embryo starts off as the fertilized egg, Stage 1. The first drawing of Stage 1 shows the jelly coat that protects the embryo. It's easy to remove, and becomes cloudy after a while, so embryologists remove the jelly for a better view, and put the embryo in a dilute, sterilized salt solution to reduce the chance of infection by bacteria or fungi.

v At Stage 2 first cleavage has occurred, and the embryo now consists of two large cells called blastomeres, followed by four blastomeres at Stage 3.

v Cleavage occurs at right angles to the previous ones, resulting in 8 cells at Stage 4, but notice that the bottom blastomeres are larger. This is presumed to be due to the high viscosity of the yolk that is concentrated at the bottom of the embryo, but its physics has yet to be investigated.



In mammals, once they have an external nutrient supply, the embryo can increase both cell numbers and total dry mass together.

v Stage 5 has 16 cells, the result of more vertical cleavages. Note that the top cells are cleaved off first: cleavage actually occurs in a wave from top to bottom.

v The synchrony of cell division is lost over Stages 6 to 9. As the cells are getting smaller and smaller, the artist got tired of drawing them all.

v At Stage 10 a dimple appears on the surface, that acts as if you had pushed your thumb into a soft ball. The inside is actually hollow above this "dorsal lip of the blastopore". The left (L) and right (R) sides of the embryo are now obvious.

v Through Stage 13, half of the outer layer of cells moves inside via the blastopore, which visibly becomes a circle that then shrinks to a point.

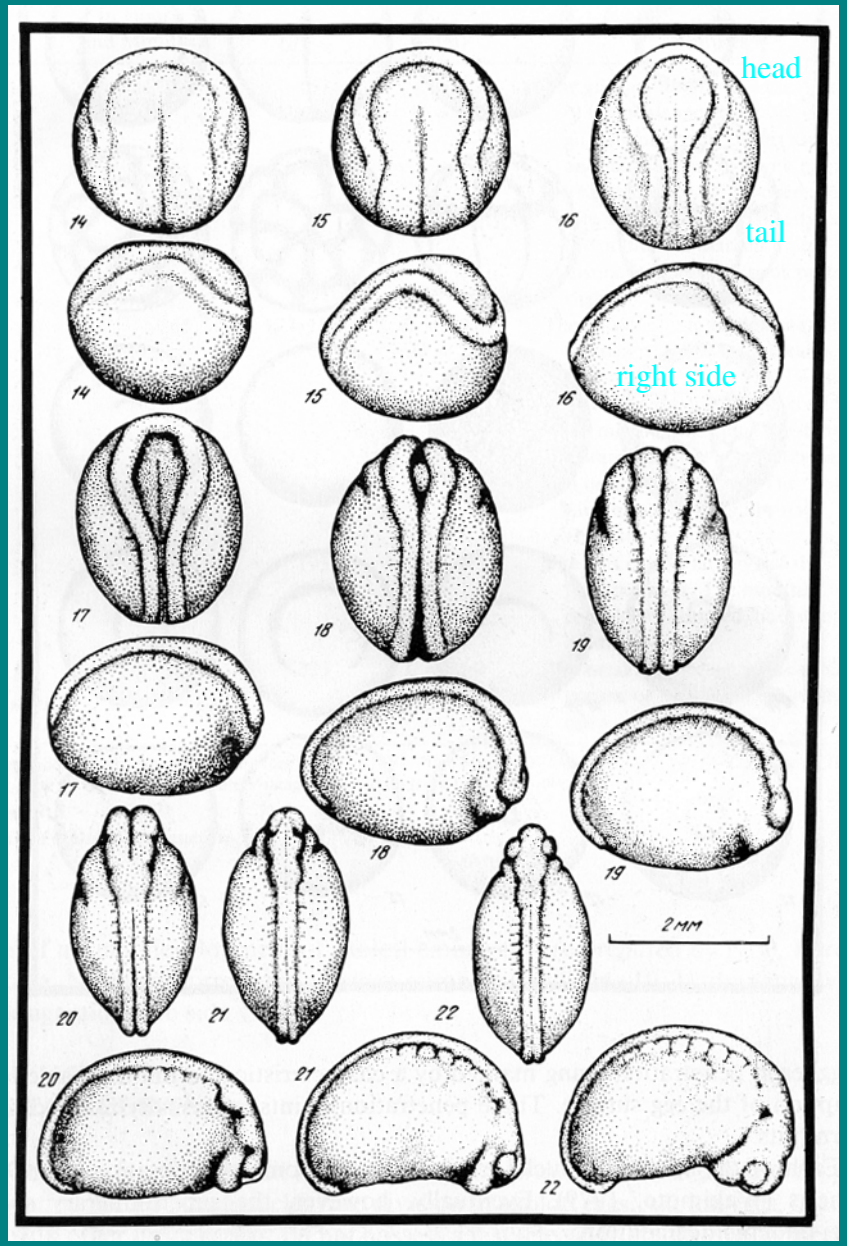
This and the subsequent six sketches are from Bordzilovskaya, N.P., T.A. Dettlaff, S.T. Duhon & G.M. Malacinski (1989). Developmental-stage series of axolotl embryos. In: Armstrong, J.B. & G.M. Malacinski, *Developmental Biology of the Axolotl*, New York: Oxford University Press, p. 201-219.

Staging of Axolotl Development

v The upper hemisphere at Stage 14 develops a neural ridge which demarks the neural plate from the epidermis. The neural plate narrows into a keyhole shape, Stage 16, and then seals as a tube at Stage 19. The epidermis has, in the meantime, expanded so that it covers the whole embryo, and, in fact, it even ends up over the neural tube. Later on the epidermis will become the skin of the animal.

v The round part of the neural plate at Stage 16 will later become the brain, and the narrow part will become the spinal cord. Thus the neural plate, a layer of cells one cell thick, is the source of the central nervous system (CNS).

v Three features of embryonic development are apparent in Stages 19-22: the eyes form, the brain segments, and many somites form to the sides of the CNS. The somites give rise to the segmented muscles and ribs of the animal, and the vertebrae.

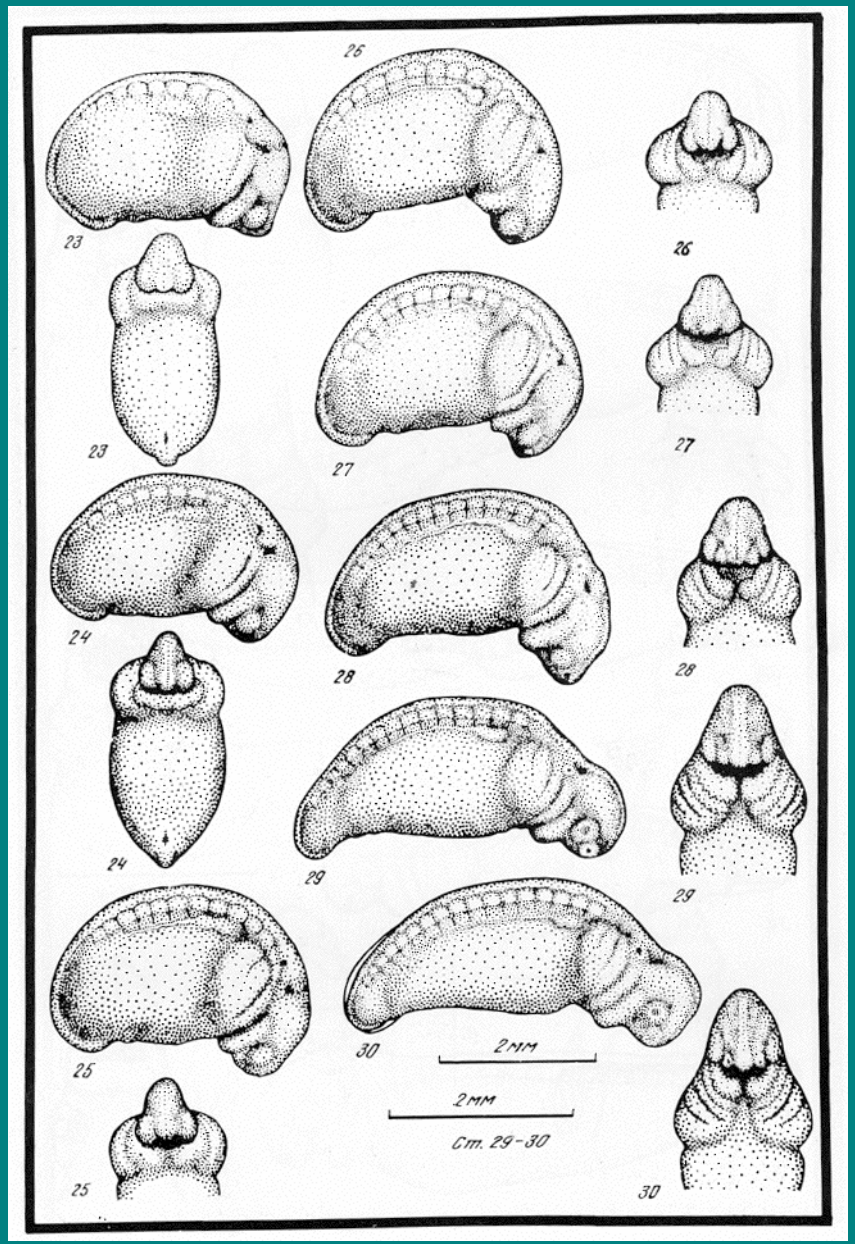


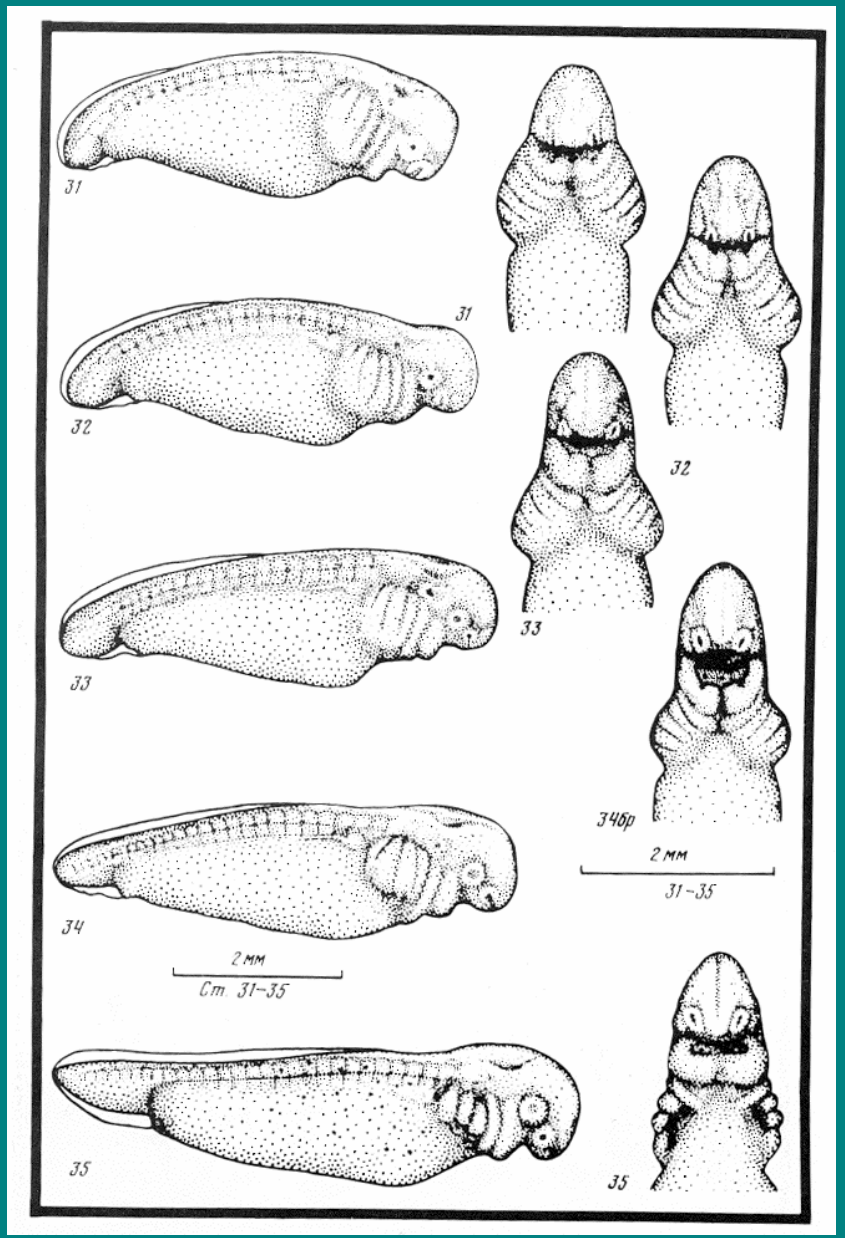
Timing, at 20°C:

Stage	Time in hours	What's starting
2-	0	Synchronous cleavage
2	0.6	2 cells
3	2	4 cells
8	16	Blastulation, asynchronous cleavage
10	26	Gastrulation
14	36	Neurulation
19	69	Neural tube, eyes, somites
44	340 = 14 days	Mouth opens, hatching

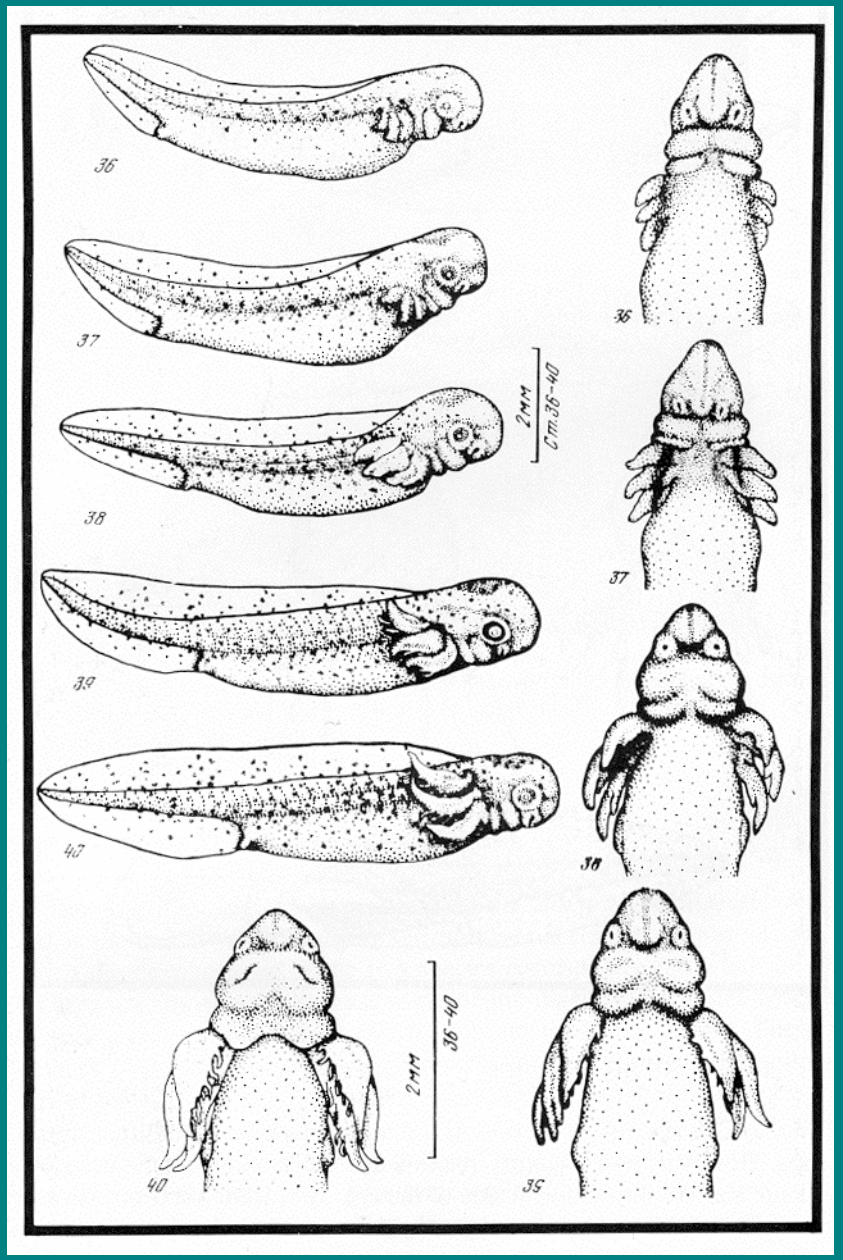
v The rest of the development, shown on the next two pages, takes the axolotl embryo to the hatching, larval stage. It looks, at Stage 44, pretty much like an adult, except that the four legs have yet to bud and grow out. Now it can eat and increase its dry mass.

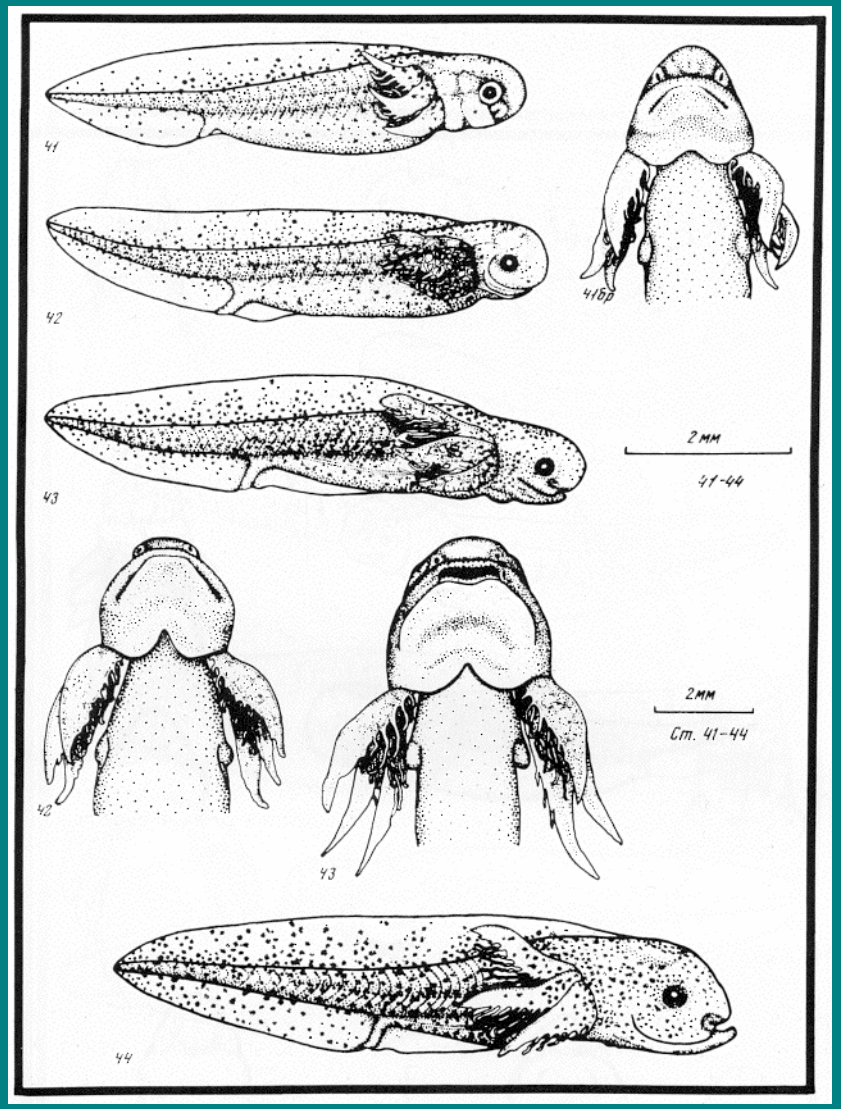
Stages 23-35 of Axolotl Development





Stages 36-44 of Axolotl Development





No increase in dry weight since it was an egg!

Take off shirt

Cortical Rotation Before First Cleavage

Stage 1 seems to merely result in a simple cell division, in terms of the outward morphology used to designate stages. A lot more is actually going on. First of all, the sperm and egg nuclei fuse. Actually, axolotl eggs (contrary to mammals) let many sperm in. Nevertheless, by a mechanism not yet understood, one sperm nucleus is "selected" and the others degenerate. Mechanically, the axolotl embryo is like the gross Jet Ball toy (meant to imitate an eyeball staring at you), which contains a bottom heavy ball floating in a fluid, inside a clear plastic spherical shell. I've colored the upper hemisphere of the clear, outer shell in the pictures below. The cortex is the membrane of the cell, stiffened by a few microns of actin, microtubules and other large molecules adhering inside. The cortex rotates as a unit. This is apparently driven by elongating microtubules and/or motor molecules that move along them. The microtubules become aligned by the fluid flow in the process. A few remarkable things are accomplished by cortical rotation: the left and right halves of the later animal are to the left and right of the equator of rotation, and the differences between left and right sides, what is called the bilateral asymmetry (such as which side the heart is on) are also probably determined at this time[1], even though they are not morphologically apparent until Stage 10. Thus cortical rotation "breaks" the symmetry from axial symmetry (symmetric around the axis between the North and South poles), to bilateral symmetry (mirror symmetry across the plane defined by the equator of rotation), to bilateral asymmetry (some fundamental difference between left and right sides).

The top, "animal" hemisphere has a dark pigment in the outer cortex.

The whole cortex, a layer a few microns thick, rotates about 30º before first cleavage (first cell division).

The "grey crescent" is so called because the cortex pigment is lifted here, allowing one to see into the yolk-poor animal hemisphere.

Equator of rotation.

The bottom, "vegetal" hemisphere has a lightly pigmented cortex. Thus one can see through to the dense yolk inside.

[1]Levin, M., T. Thorlin, K. Robinson, T. Nogi & M. Mercola (2002). Asymmetries in H^+/K^+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. *Cell* **111**(1), 77-89.

Problem: predict rotational jiggling

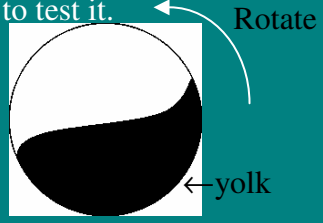
1. Assume microtubules are initially nearly randomly oriented
2. Microtubules generate torque on the cortex by polymerization and/or motor molecules moving along them
3. Cortical rotation flow aligns the microtubules: supercooperative phase transition of very long range coupling microscopic phenomena with macroscopic

Upside Down Doesn't Work

Suddenly turn an axolotl egg upside down, just after fertilization (beginning of Stage 1), and nothing happens. No cleavage. No further development. But if the egg is turned by just about any angle less than 180°, or develops in a space station with no gravity, development proceeds just fine. These are simple examples of experiments we can do with embryos, to prod them into giving up some of their secrets of self-construction. Russell Flint, a high school physics teacher, spent a sabbatical year with me, and we came up with a plausible explanation of this strange result. Basically, if we rotate the egg, the dense yolk sloshes, which means that the microtubules are all aligned in circular arcs in the cortex. However, if the egg is inverted, the yolk sloshes down the middle, and some microtubules are oriented one way, with equal numbers pointing in the opposite direction. No net movement of the cortex is possible, and bilateral symmetry doesn't emerge. The embryo is left axially symmetric, and stuck. This prediction is still a hypothesis, waiting for someone to test it.

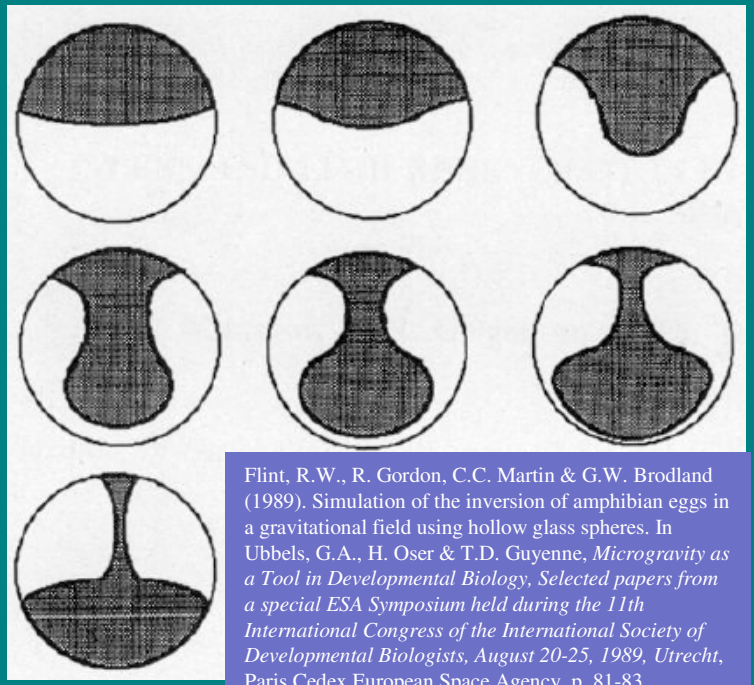
Is the axolotl egg spherically symmetrical, like the human egg? Well, it is certainly only axisymmetrical around the vertical axis, with respect to: a) the yolk density gradient, which makes it bottom heavy, b) the top ("animal") darkly pigmented and bottom ("vegetal") lightly pigmented hemispheres, and c) the different surface viscosities of the two hemispheres[1]. Yet, if we manually rotate it and hold it in a new position, the yolk sloshes back to the bottom, and development proceeds normally, even though the later individual has dark pigment in the "wrong" place. In fact, if we invert the egg slowly, we can get normal development of a larva: white on top and dark on its underside, the reverse of normal. What this suggests is that all parts of the cortex are essentially the same, i.e., that the axolotl egg is also fundamentally spherically symmetric.

Manual cortical rotation can substitute for natural rotation, and the equator of rotation *we choose* then determines where the left and right sides of the animal will be. This fits with the notion that flow alignment of the microtubules is what determines left and right. In normal development we have a bootstrap process: each microtubule pushes on the cortex, causing it to rotate. There is a slight net sum of all of the microtubule pushing. This causes the cortex to rotate in some direction. The rotation flow aligns the microtubules, which then enhances the rotation in that direction. If we rotate the embryo manually, we kick start the process.



Flow aligned orientation of the cortical microtubules

Yolk sloshes in this side view (pigmented cortex not shown)



Invert suddenly: yolk drips down center:

Flint, R.W., R. Gordon, C.C. Martin & G.W. Brodland (1989). Simulation of the inversion of amphibian eggs in a gravitational field using hollow glass spheres. In Ubbels, G.A., H. Oser & T.D. Guyenne, *Microgravity as a Tool in Developmental Biology, Selected papers from a special ESA Symposium held during the 11th International Congress of the International Society of Developmental Biologists, August 20-25, 1989, Utrecht*, Paris Cedex European Space Agency, p. 81-83.

[1]Dictus, W.J.A.G., E.J.J. van Zoelen, P.A.T. Tetteroo, L.G.J. Tertoolen, S.W. de Laat & J.G. Bluemink (1984). Lateral mobility of plasma membrane lipids in *Xenopus* eggs regional differences related to animal/vegetal polarity become extreme upon fertilization. *Dev. Biol.* **101**, 201-211.

Show sloshing simulation

Collaborators

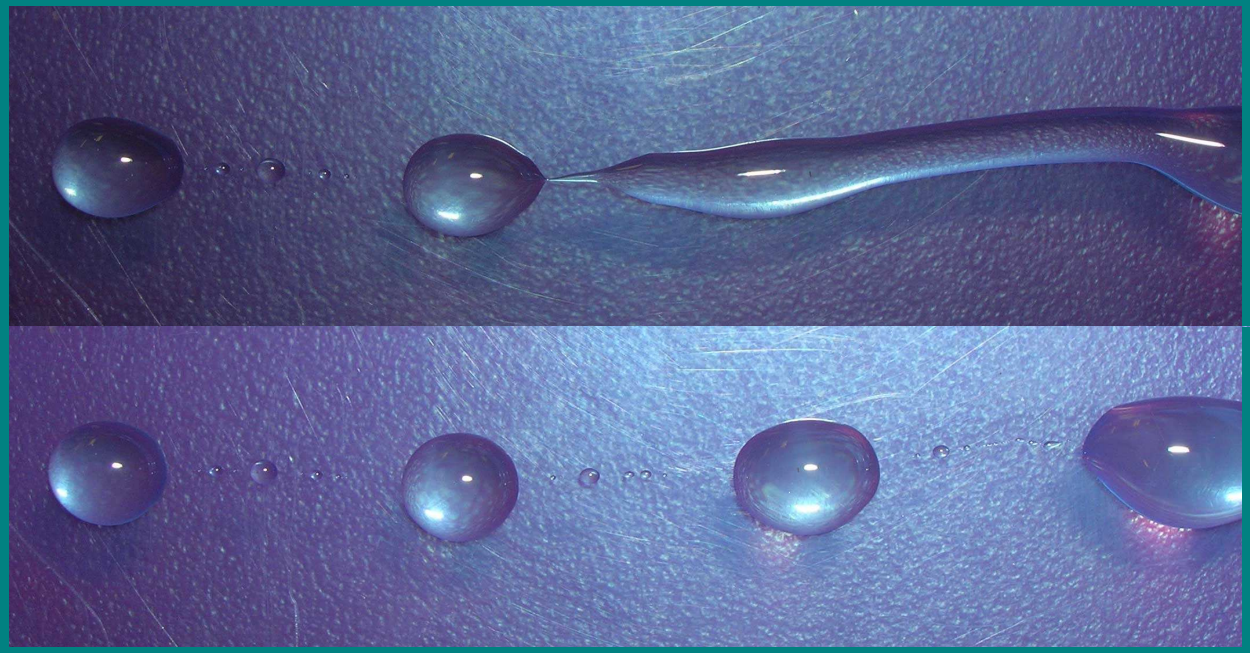
- Postdoc: Comron Nouri (Physics , UWO)
- Grad Student, Susan-Crawford-Young, 4D microscope (later)
- Grad Student, Tony De Luca, clinostat trajectories for tumbling embryos to roughly simulate zero gravity
- Roel Luppes & Arthur Veldman, U. Groningen, 2 phase FEM of viscous fluid flow
- Jack Tuszynski, U. Alberta, Microtubule simulations

Canadian Space Agency contract for “The Physics of Embryo Development in Altered Gravitational Fields”

Rayleigh Instability of the Inverted Egg: Sketch

Rayleigh instability occurs in a number of different situations. Examples are:

When a cylinder of viscous liquid is in zero gravity it breaks into individual spheres. These are pictures of honey stripes (trails) on a ziplock lid. (Made as a toy experiment in our lab for this presentation) (Comron Nouri)



The Mechanics of Embryos

Some scientists do research that is ahead of their time by not decades, but even a century or two. Why did their insights languish in the archives of libraries rather than inspire generations of research? We have such a case in Wilhelm His, who tried to explain neural tube closure, by actually buckling assorted laminated structures that he built. He was vilified by Ernst Haeckel, whose main view of embryos was their role in justifying what he called the biogenetic law, the claim that "ontogeny recapitulates phylogeny". Whether Haeckel's law was right or not was not the point. Haeckel saw evolution as "the cause" of why embryos do what they do, whereas His was after the physical causes of embryogenesis, and their relationship to heredity. The relationship between these two perspectives is still debated, as current scientists try to carve out a "new" field called "evodevo", short for "evolution and development". It is still hampered by lack of acceptance of His' perspective.

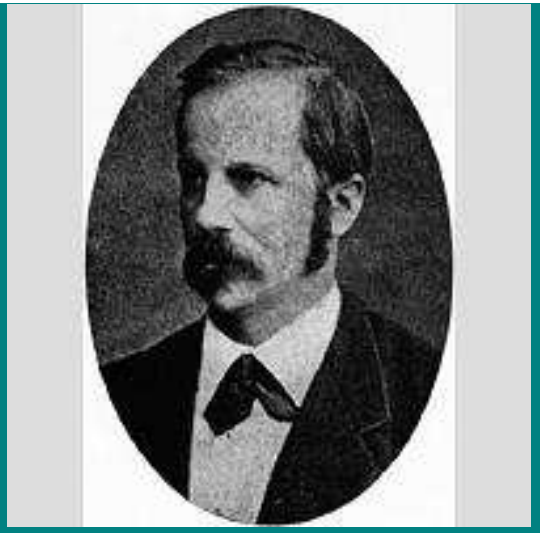
The 1911 Encyclopedia Britannica suggests that experimental embryology began with Wilhelm His in 1874 (http://44.1911encyclopedia.org/E/EM/EMBRYOLOGY.htm). But actually I think that it was *theoretical* embryology that began with His, and then languished in the rush of experimental results that followed, as scientists began to prod, cut and dissect the living embryo to see how it responded. Wilhelm Roux led this effort, and one of his results contradicted Wilhelm His' theory of neural tube formation

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

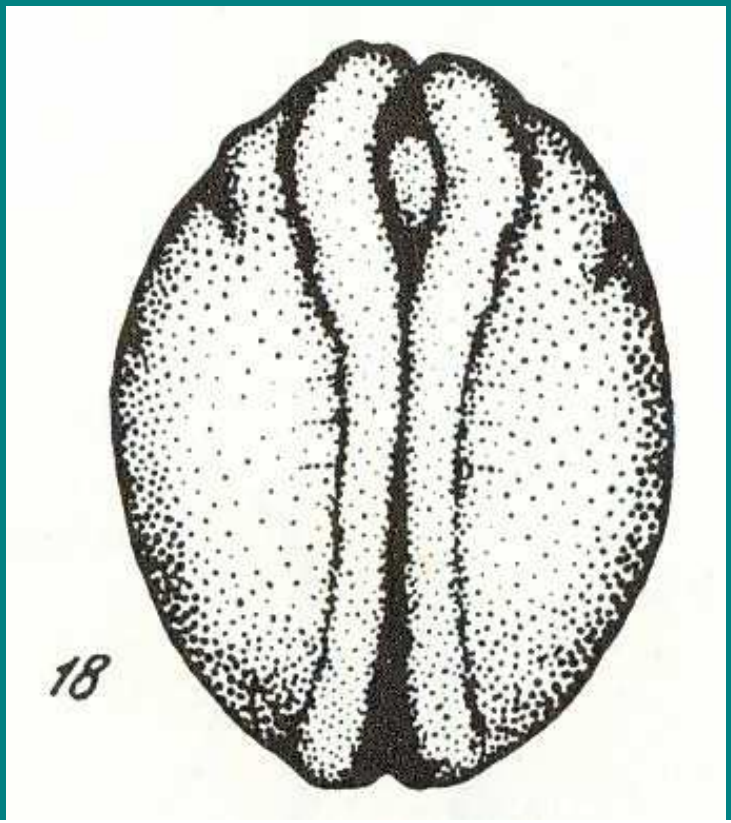
Wilhelm vs Wilhelm

Outcome: A Century without Embryo Physics

Wilhelm His (1874): lateral compression causes neural tube folding and closure, as demonstrated by sheets of material and laminates.

Wilhelm Roux(about 1890): cutting the embryo laterally still produces neural tube golding and closure.

Both were right: axial stretching causes lateral compression which results in folding and closure.



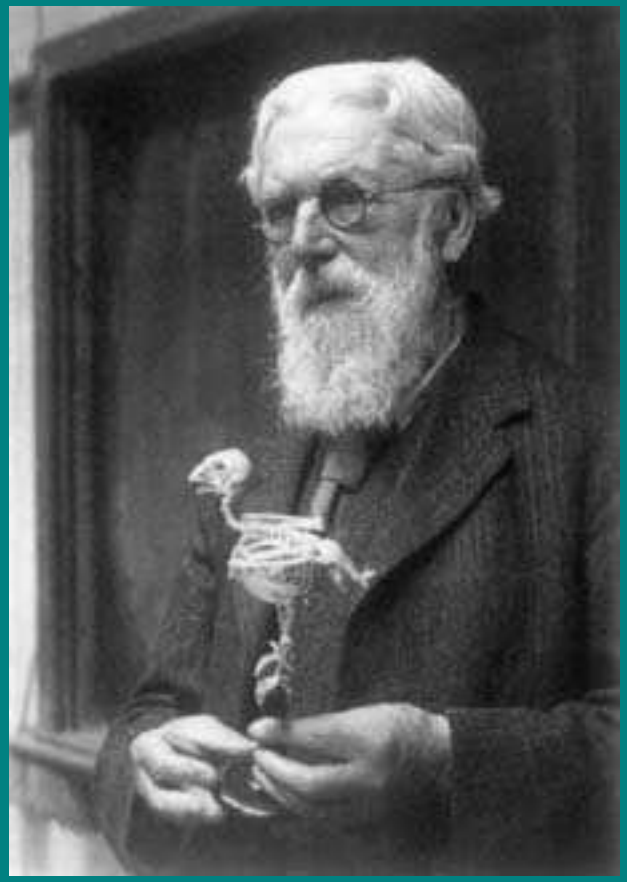
Mechanics or Molecules or Molecular Mechanics or Supramolecular Mechanics?

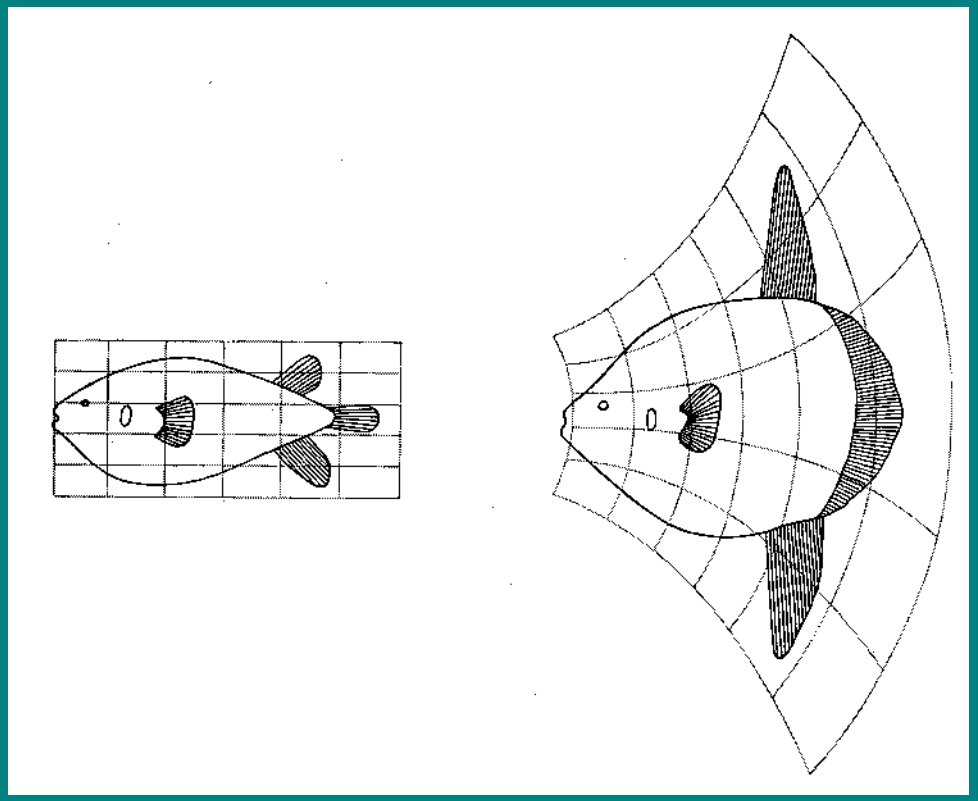
ØA small protein molecule is about 20 nanometers in diameter when folded into its working configuration. An axolotl embryo is 2 millimeters in diameter. Thus the embryo is 100,000 times wider than a protein molecule. The ratio of the volumes is $100{,}000^3$ = 100,000 x 100,000 x 100,000 = 10^{15} = 1 quadrillion. The biological literature is rife with claims that this or that molecule "controls" one or another aspect of embryogenesis. Let's make an analogy with buildings. From bricks about 20 centimeters wide, at 100,000 times we could construct a building 20,000,000 centimeters or 20 kilometers wide and tall. Without a lot of structure at all sorts of scales, it is hard to conceive how individual bricks could "control" the overall structure, though they might well contribute to it. To understand a building that large, we need to know the architecture of its large scale supports, its division into floors and rooms, its plumbing and wiring, and the details of those rooms. These are not all "brick level" concerns, although much of the structure might be made of bricks.

ØOf course, an embryos is as much a process as a structure, and we could well imagine that a small, defective part could stop a whole process. Lose the radiator cap for your car, and the fluid evaporates, the car overheats, and the engine block cracks. Does the radiator cap therefore control the car's engine? I'll let you answer that. In molecular embryology a molecular part is rendered defective or cut out by a variety of ingenious techniques including mutation, gene knockouts, gene dosage effects, etc. If a limb doesn't develop, or the neural tube fails to close, can we say that the corresponding gene, or its gene product (usually a protein) controls that aspect of embryogenesis?

ØBasically, unless we know how an embryo builds itself, each of the molecular parts that leads to faulty embryogenesis when removed does not fit into the overall picture. The overall picture includes something akin to the architecture of embryogenesis. But then, in a way, we could just as well say that it is the architecture that controls embryogenesis, and those individual proteins in the process. This is why reverse engineering may be the right approach to solving how an embryo builds itself. Reverse engineering does not merely determine the components of an unknown system, but also how they work together. A parts list is not adequate to understand how a machine works.

D'Arcy Thompson Grid

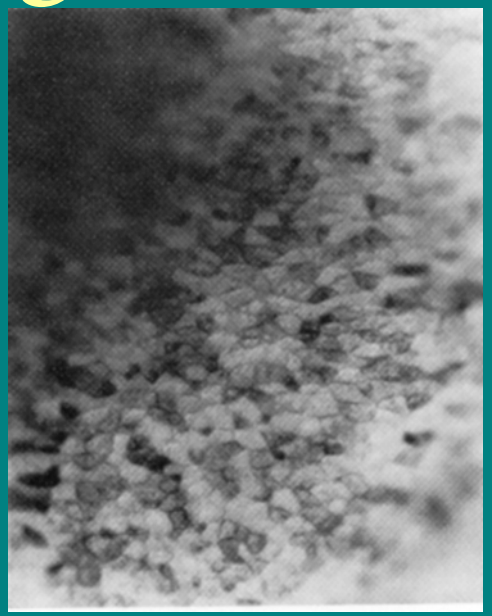




Thompson, D.W. (1917). *On Growth and Form*, Cambridge University Press.

O'Connor, J.J. & E.F. Robertson (2003). D'Arcy Wentworth Thompson, http//www-gap.dcs.st-and.ac.uk/~history/Mathematicians/Thompson_D'Arcy.html.

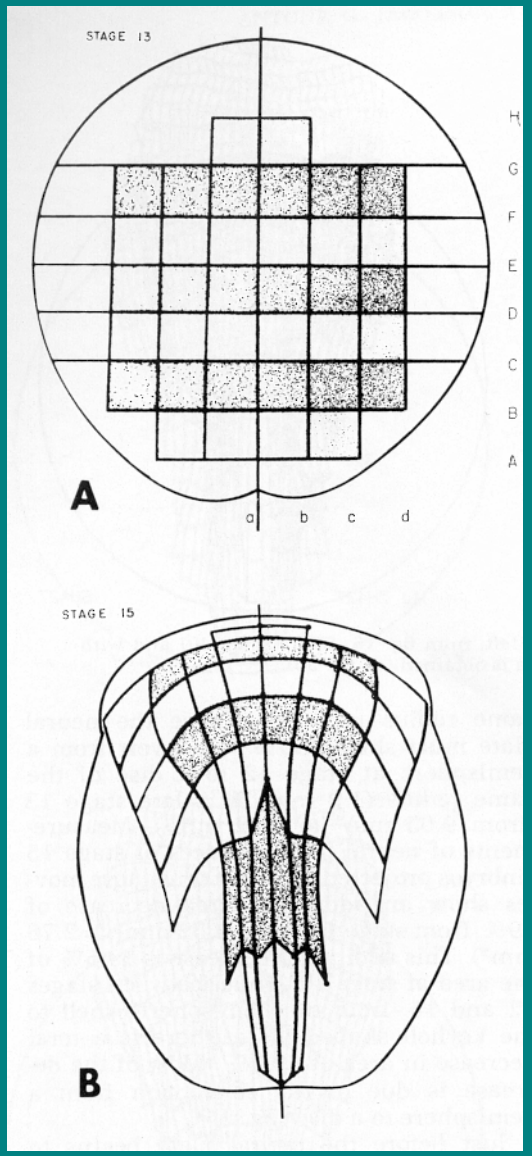
Urodele Embryos Have Variegated Pigmentation



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.

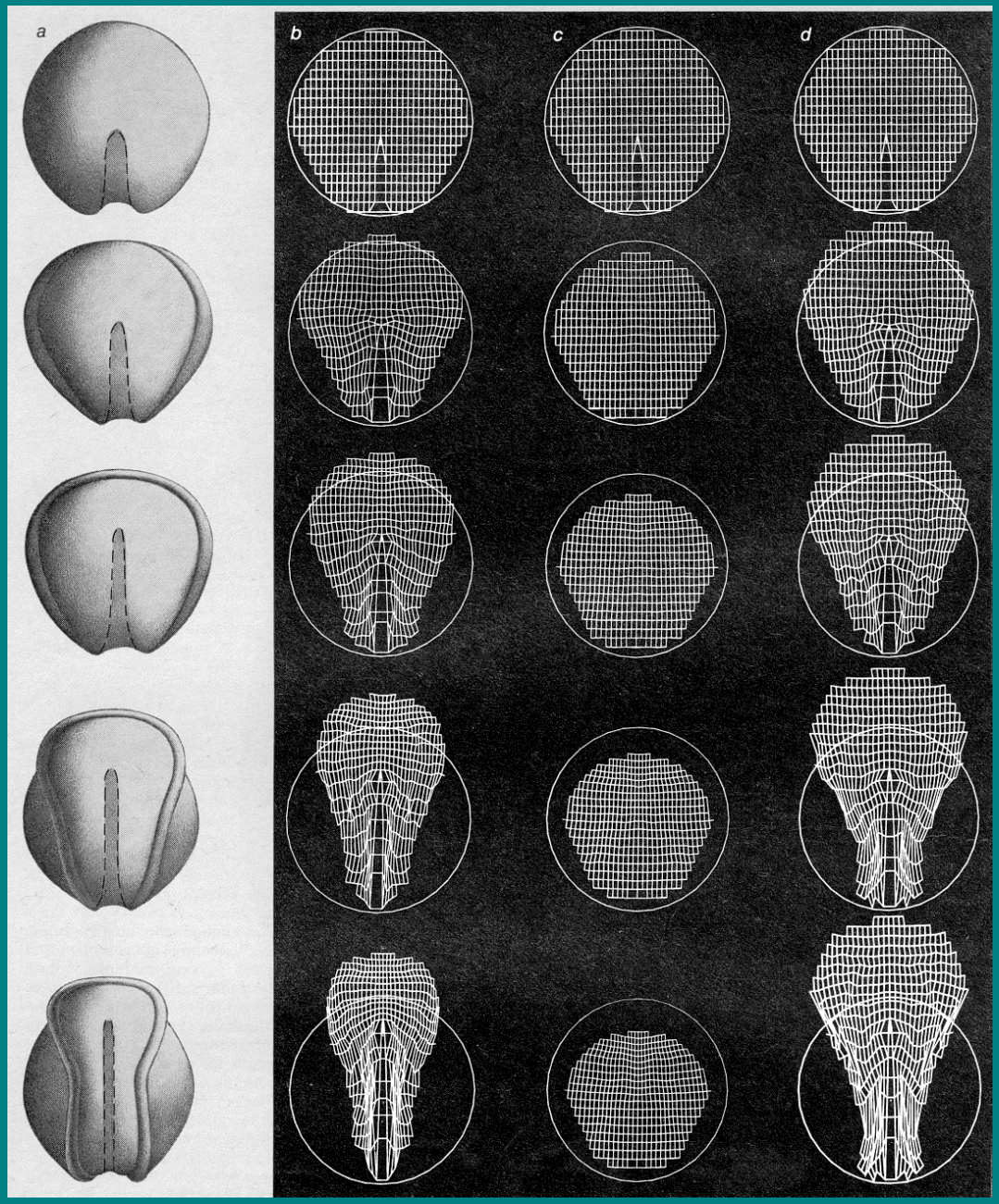
Gordon, R. & A.G. Jacobson (1978). The shaping of tissues in embryos. *Scientific American* **238**(6), 106-113.

Tracking Cells on the Neural Plate via a D'Arcy Thompson Grid



Burnside, M.B. & A.G. Jacobson (1968). Analysis of morphogenetic movements in the neural plate of the newt *Taricha torosa*. *Dev. Biol.* **18**, 537-552.

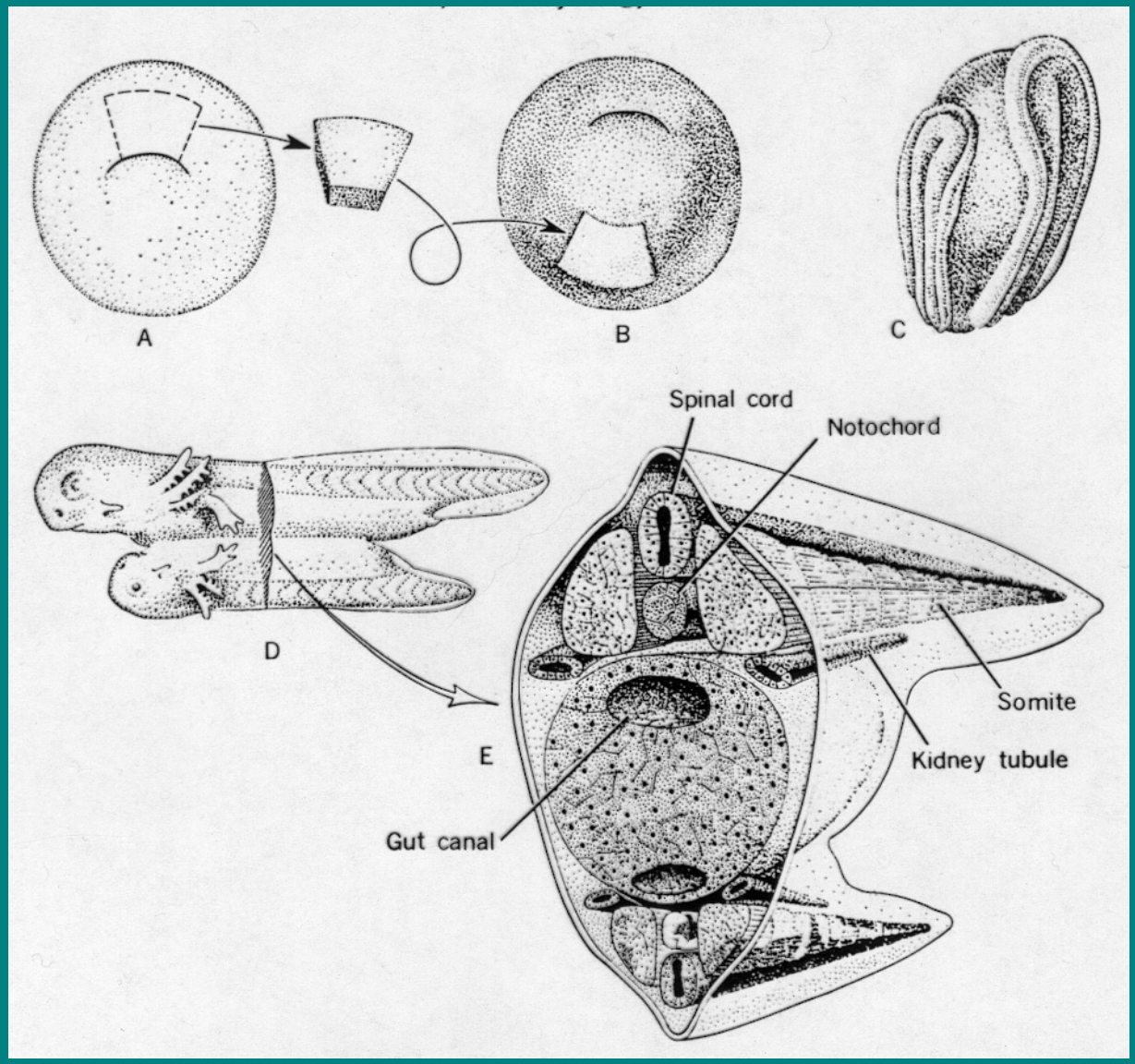
Computer Simulation of Shaping of the Neural Plate



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

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.



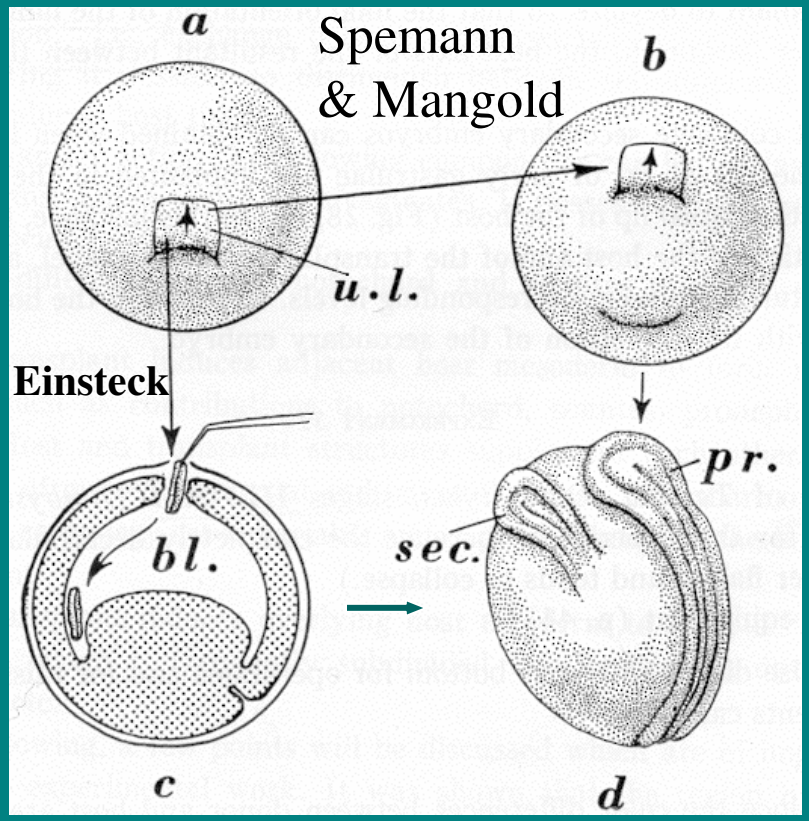
Twitty, V.C. (1966). *Of Scientists and Salamanders*, San Francisco W.H. Freeman and Co.

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



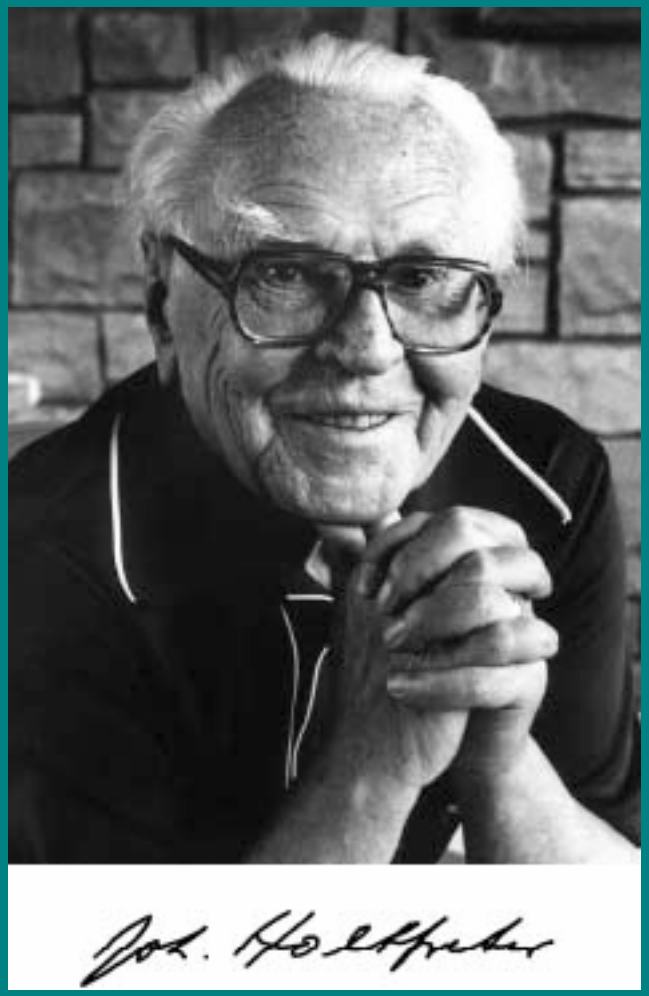
Hamburger, V. (1960). *A Manual of Experimental Embryology*, revised ed., Chicago University of Chicago Press.

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

Tutorial on the Cytoskeleton

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

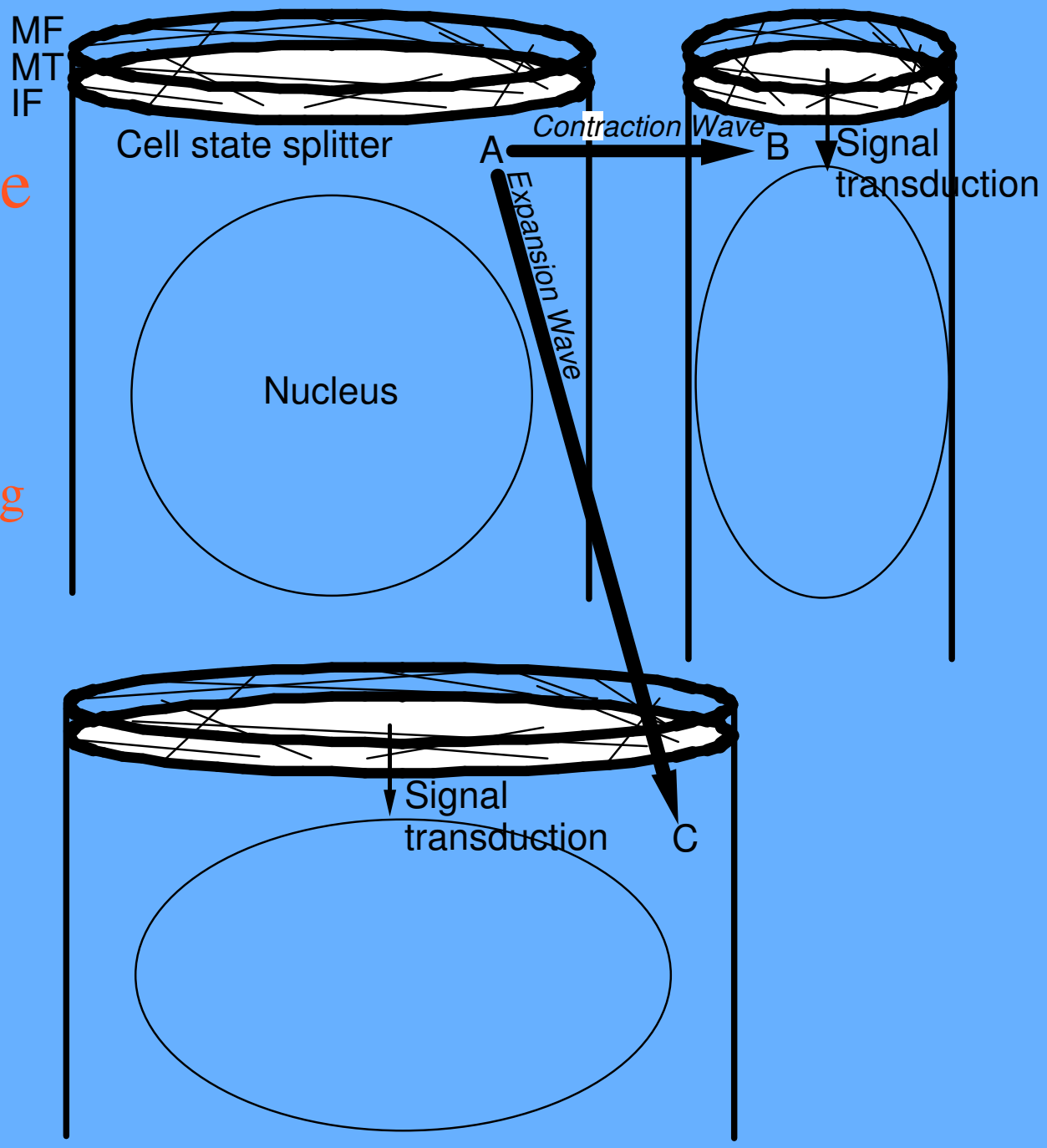
These photos 15 years later!

The Cell State Splitter

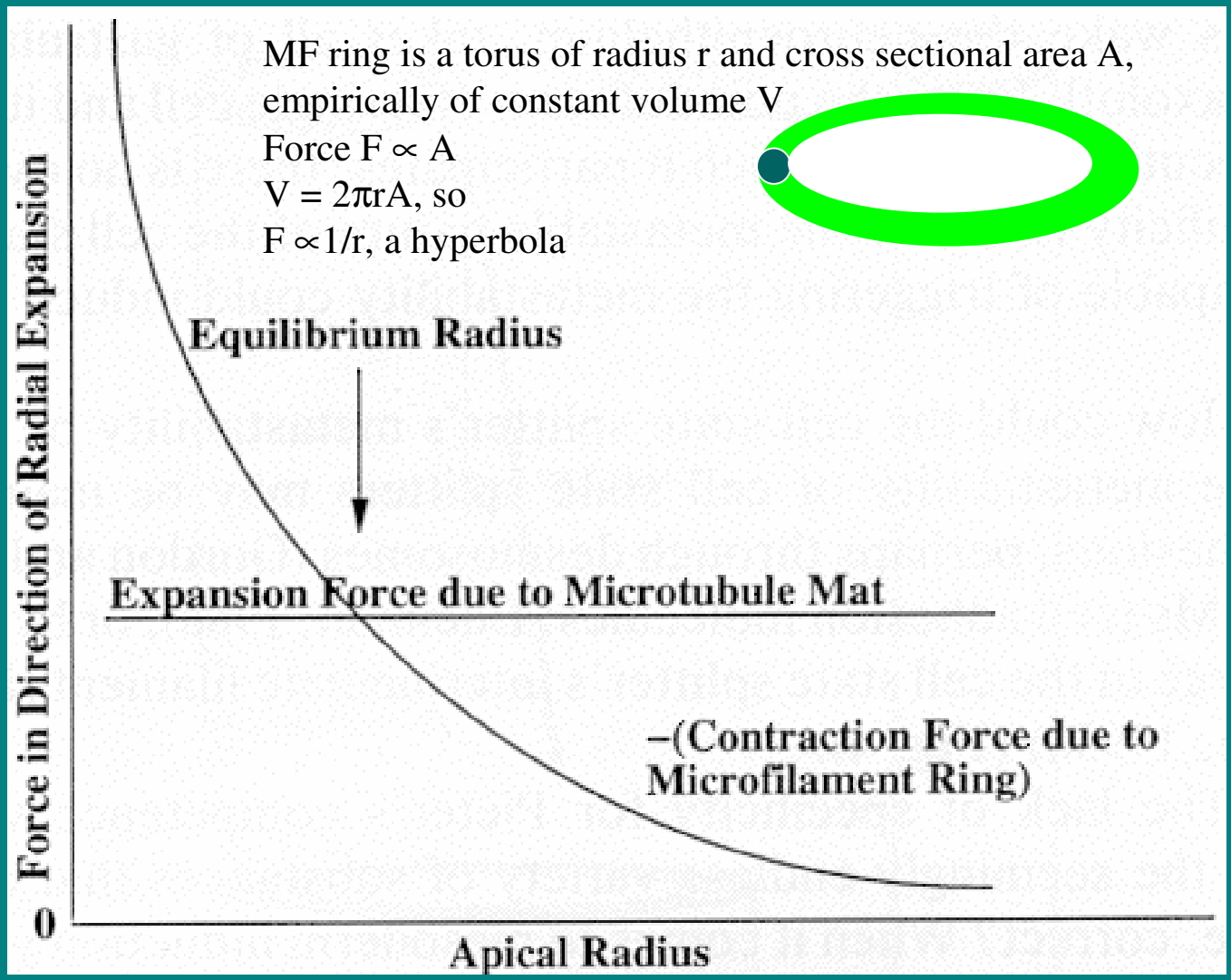
MF =
microfilament ring

MT =
annular apical
microtubule mat

IF =
intermediate
filament ring



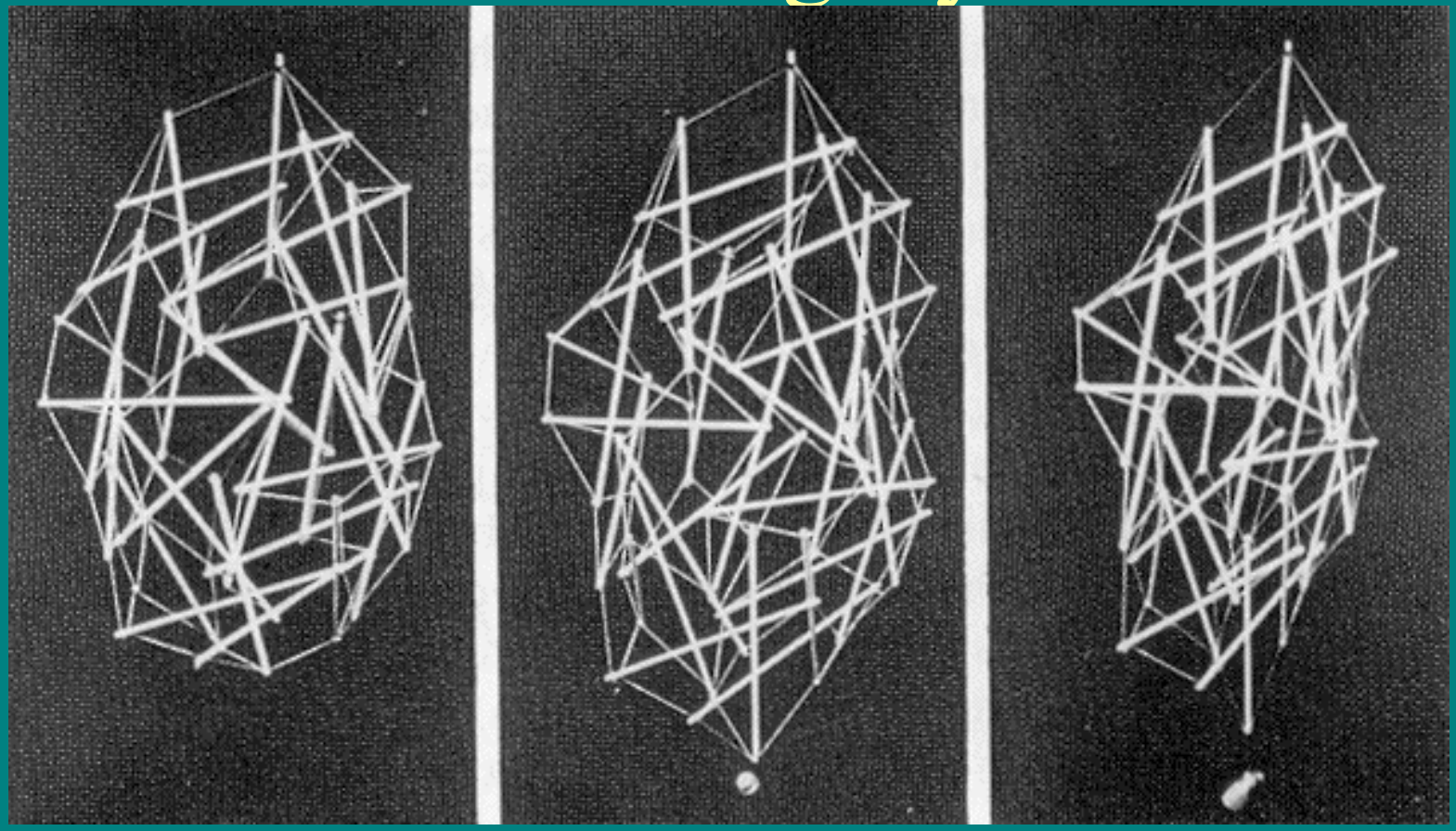
The Unstable (Bistable) Mechanical Equilibrium between the Microfilament Ring and the Microtubule Mat in the Cell State Splitter



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

Bottle cap as model of bistability

The Cell State Splitter is a Nonlinear Tensegrity Device

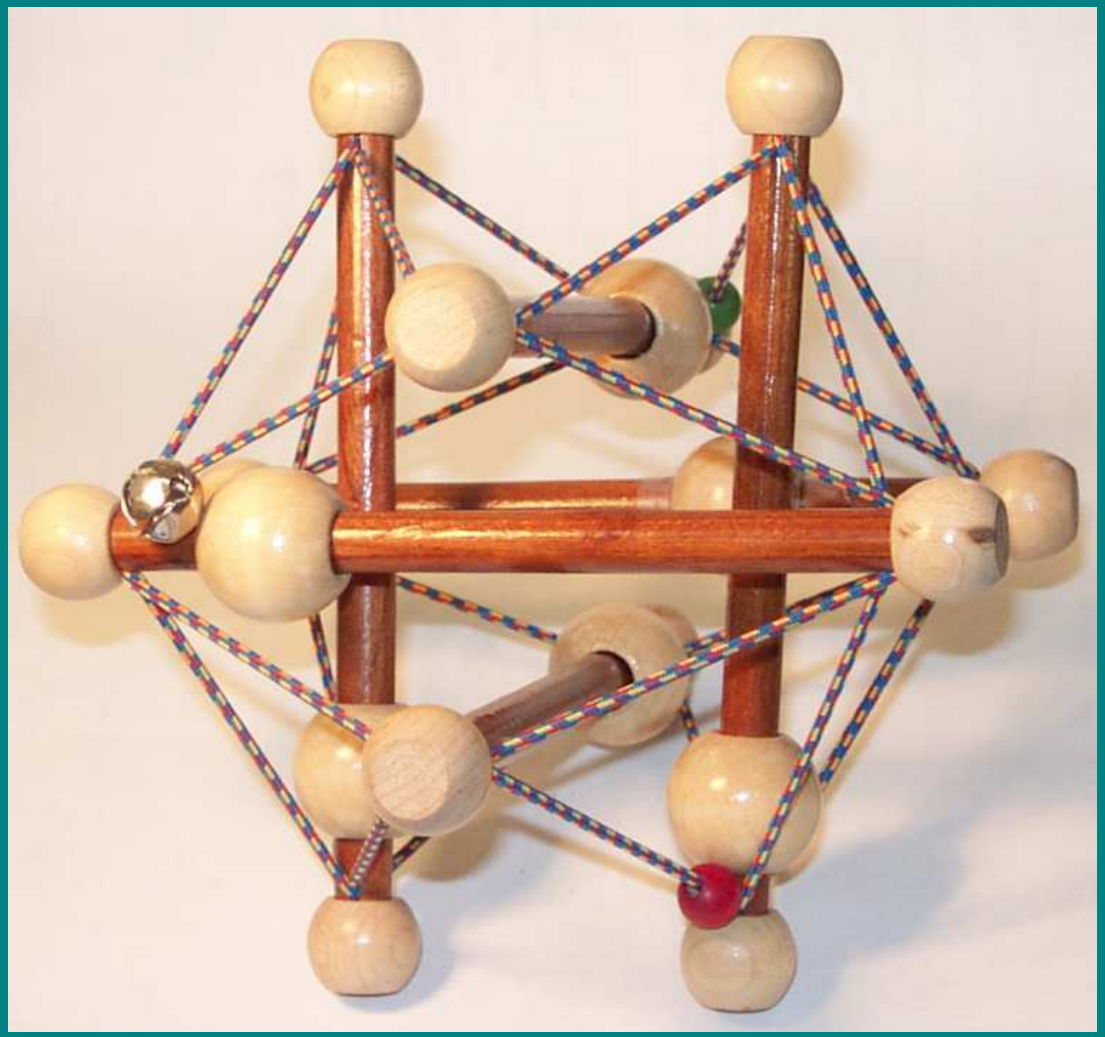


This one is linear: no snap through or bistability

Ingber, D.E., L. Dike, H. Liley, L. Hansen, S. Karp, H. Liley, A. Maniotis, H. McNamee, D. Mooney, G. Plopper, J. Sims & N. Wang (1994). Cellular tensegrity exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis. *Int. Rev. Cytol.* **150**, 173-224.

Tensegrity Toy

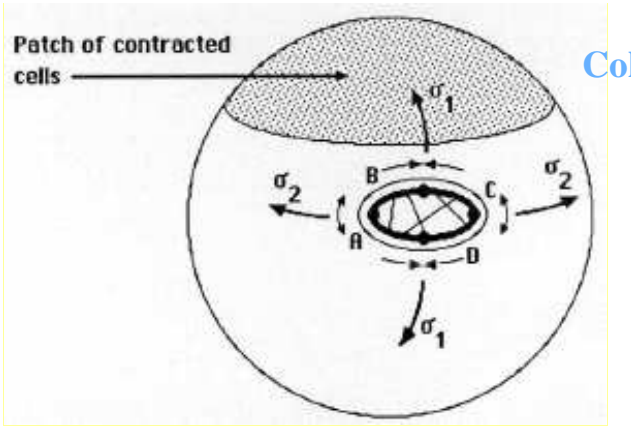
Most tensegrity structures, like this one, have a single stable state. The cell state splitter may be more like a snap through bottle cap, with two stable states. Here rods are microtubules supported laterally by intermediate filaments (not shown) and connected by microfilaments under tension (elastic strings.)



MT & IF demonstration
TAKE PICTURES

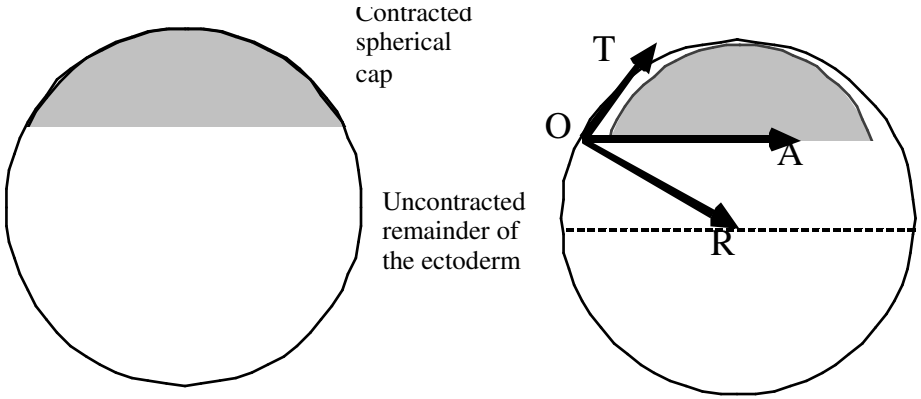
Needed a Wave to Carry the Contraction or Expansion from Cell to Cell: First Theory

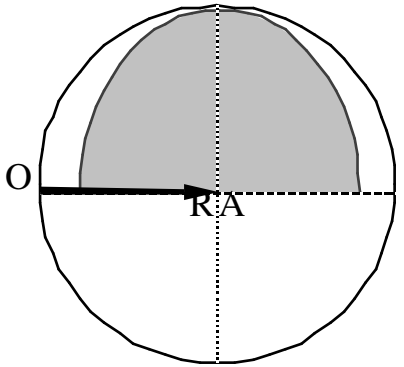
The Hot/Cold Boiler Model



Cold: Contracting

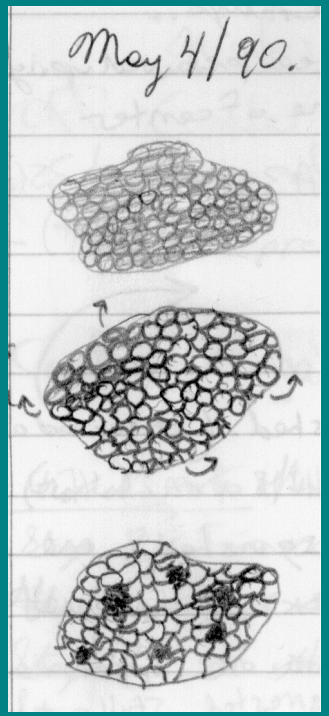
Hot: Expanding





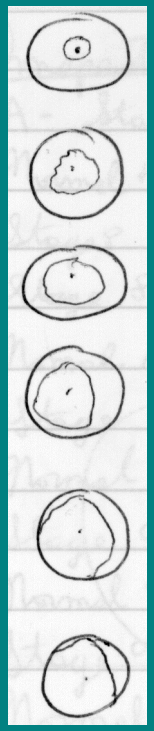
Needed a Wave to Carry the Contraction or Expansion from Cell to Cell: First Observation

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.

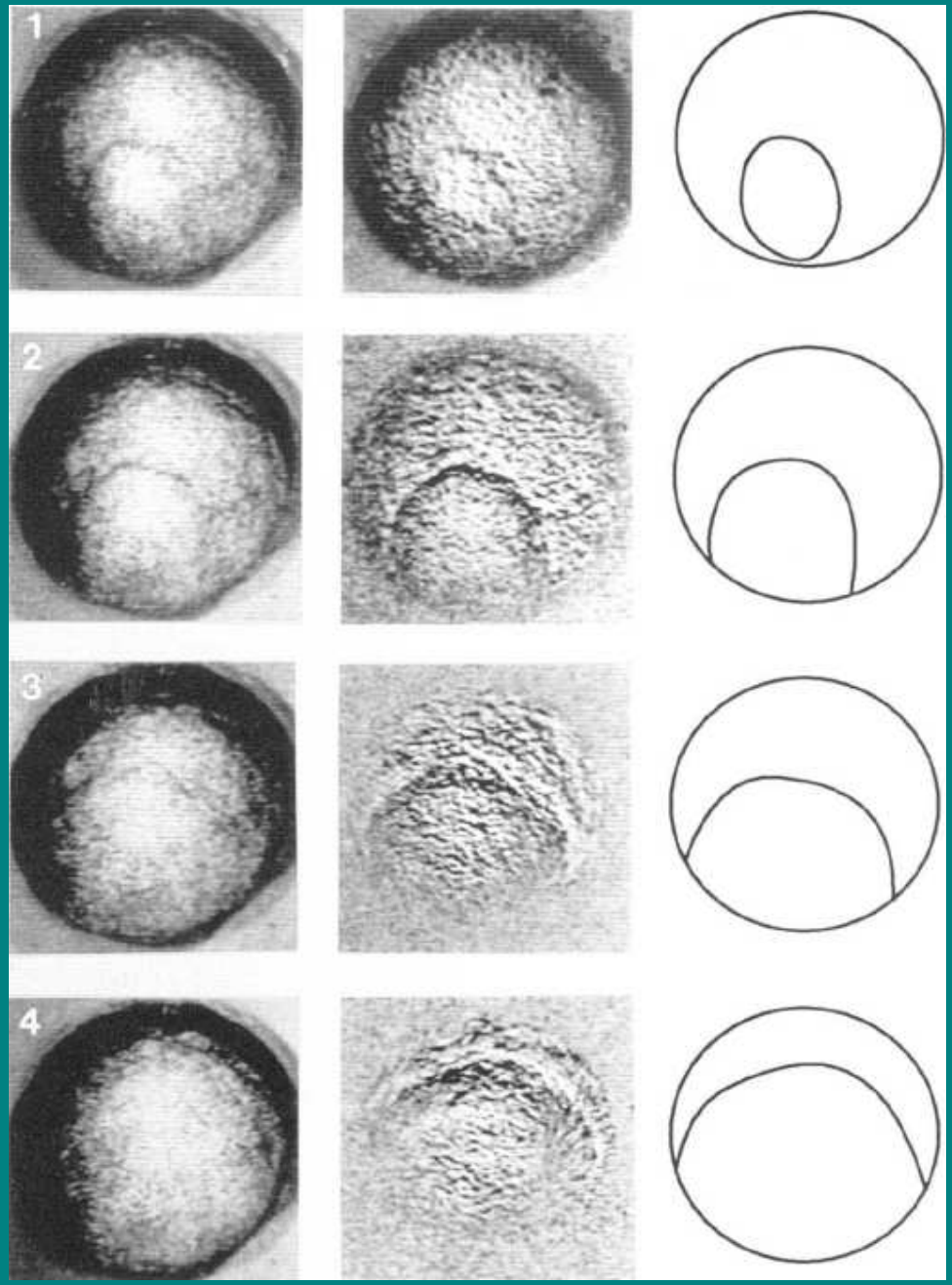
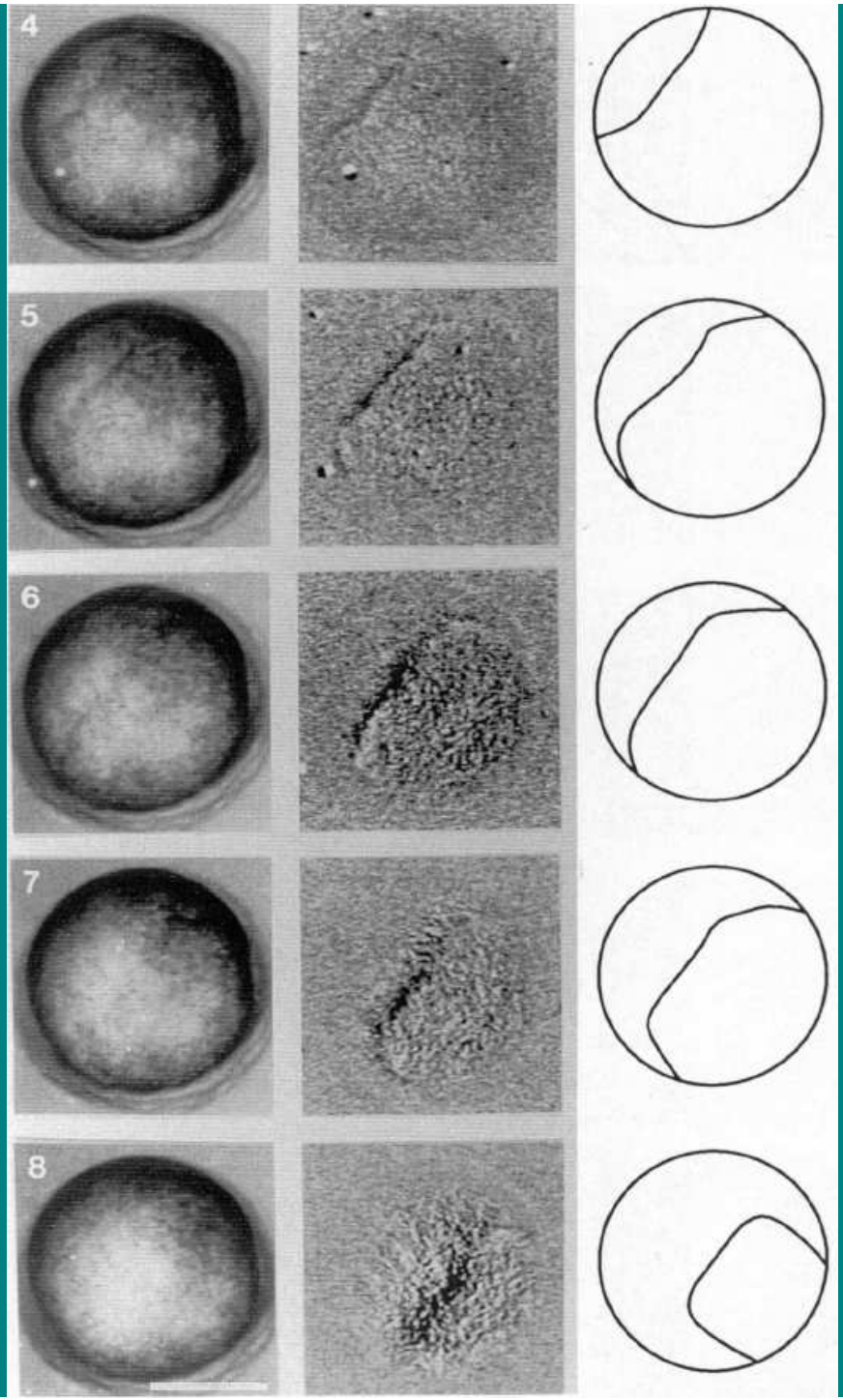
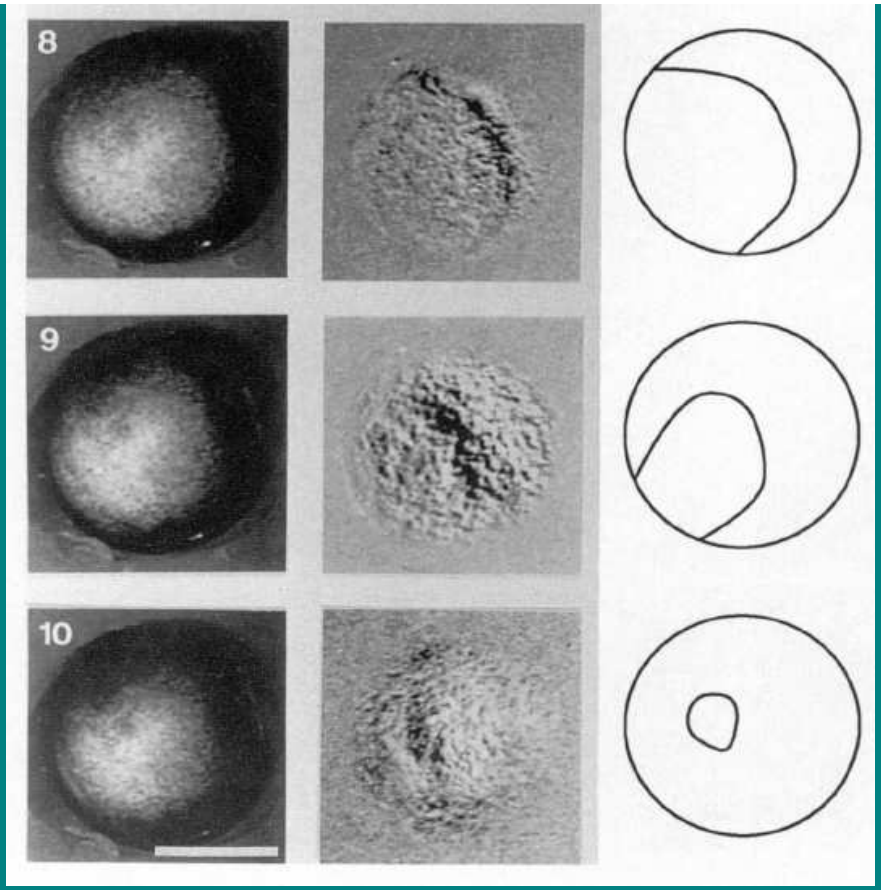


Image Processing

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.



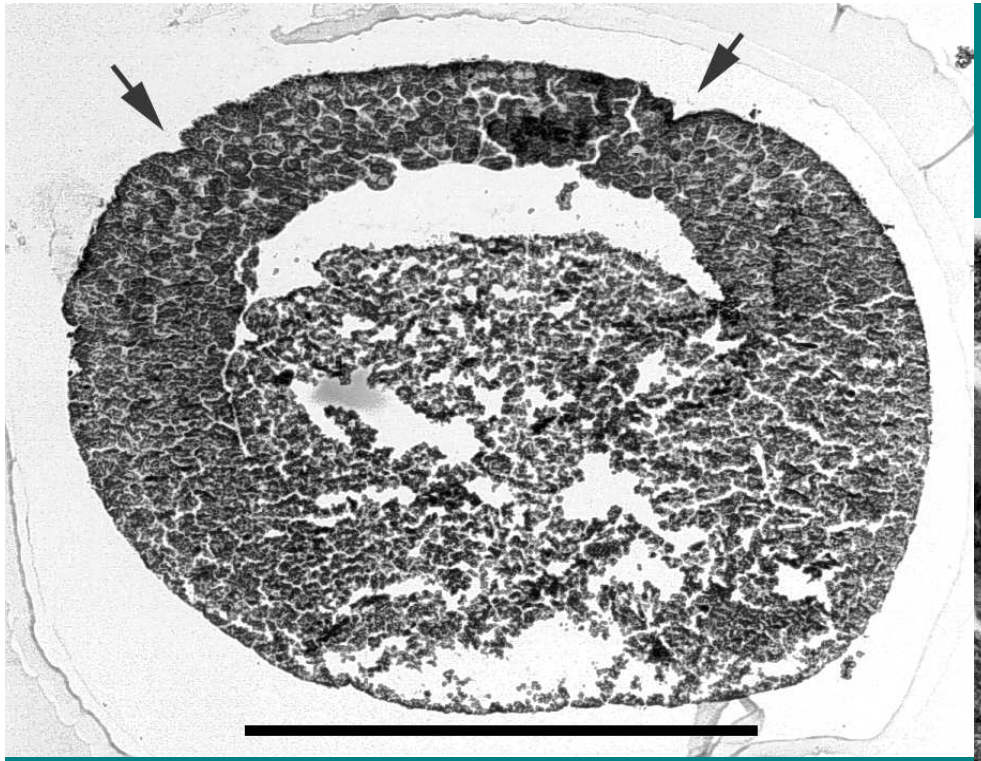


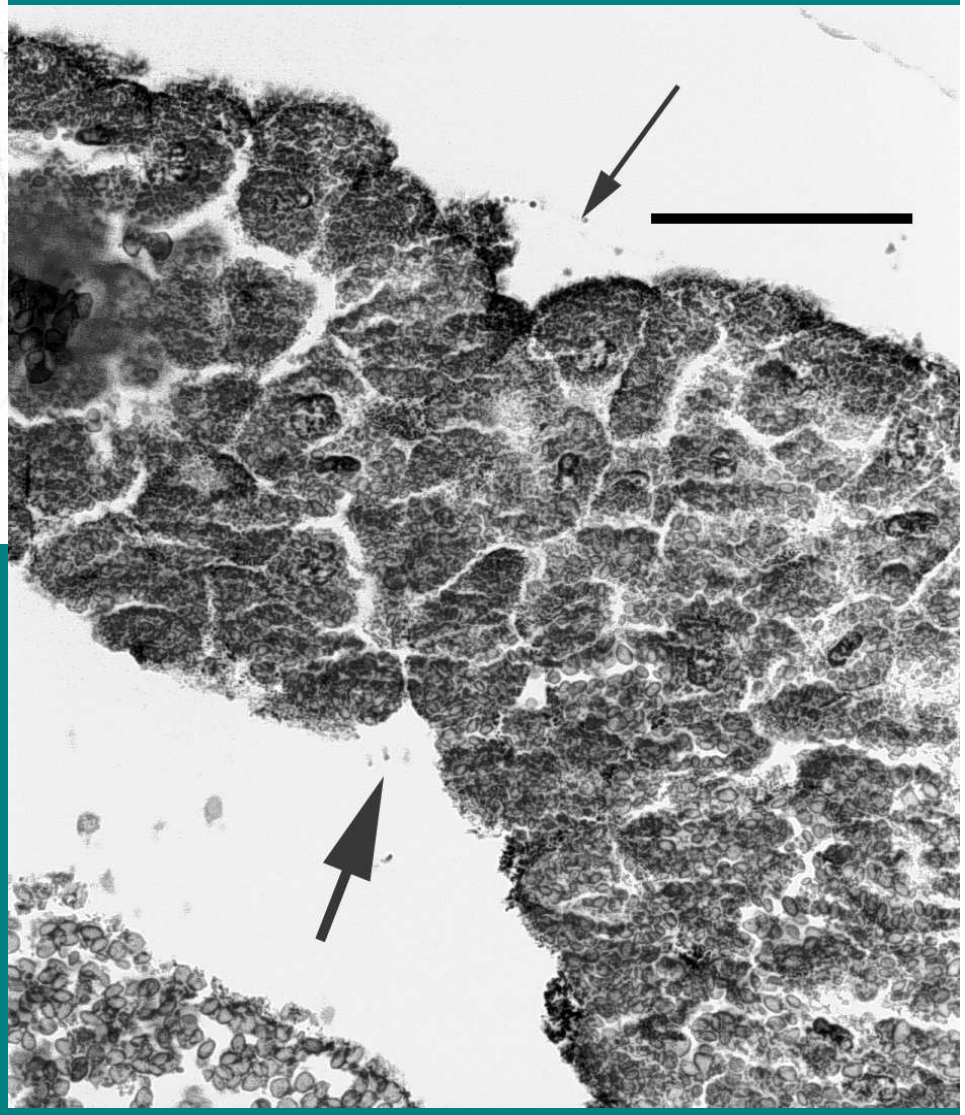
The arc shaped wave moves faster at its ends than in the middle, reforming a circle which then vanishes at what will be the anterior (head) end of the embryo. (These sets of images are from three different embryos.) Bar = 1 mm.

Show Movie

- Six embryos: 5 in gastrulation, the lower right in neurulation.
- 10,000 x speedup
- Repeated many times, but the ectoderm contraction wave traverses the ectoderm only once.
- Close up of the end of the wave, which looks like hyper-restoration
- Frog embryo showing no contrast due to high pigmentation

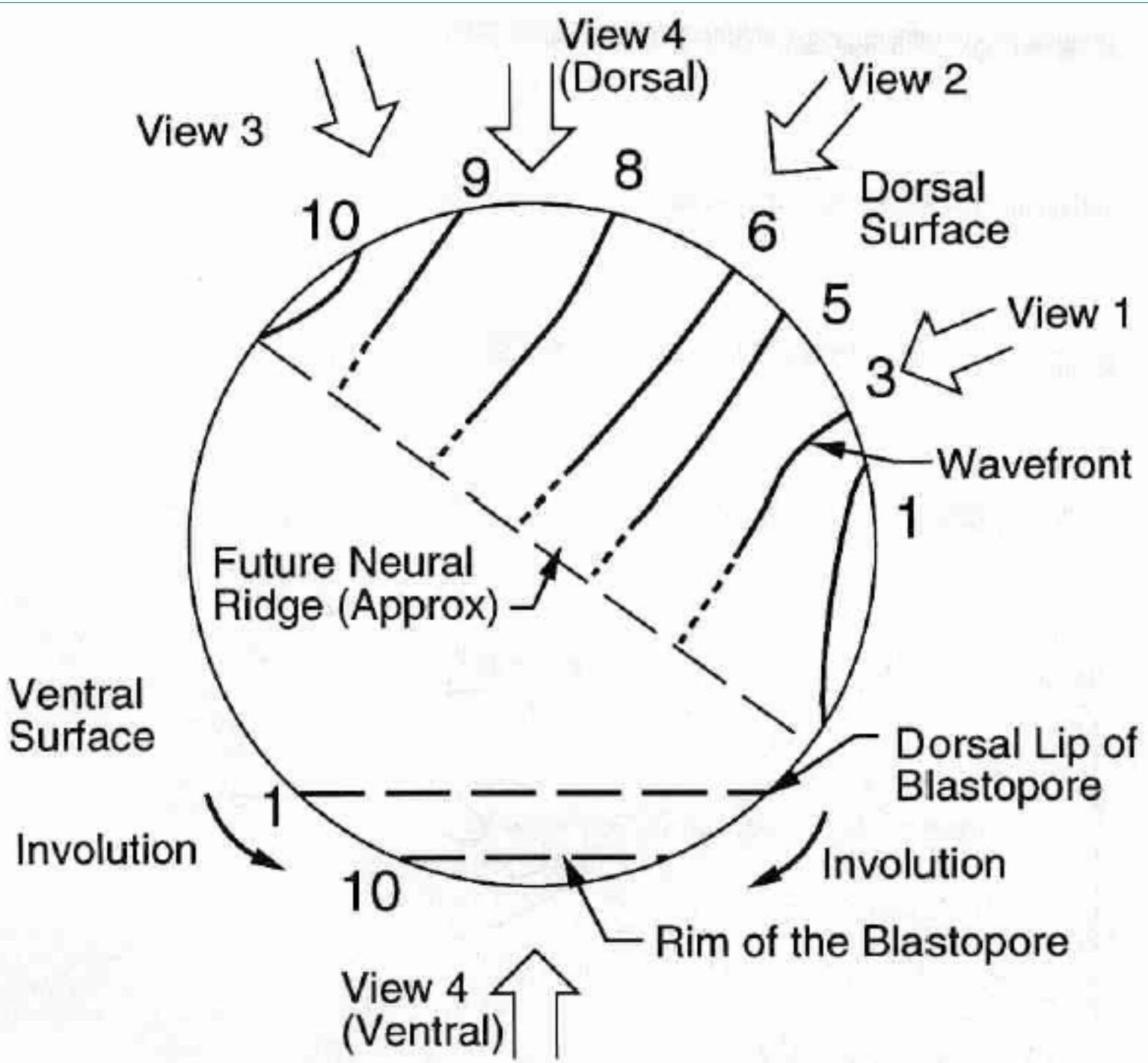
The Ectoderm Contraction Wave is a Morphogenetic Furrow





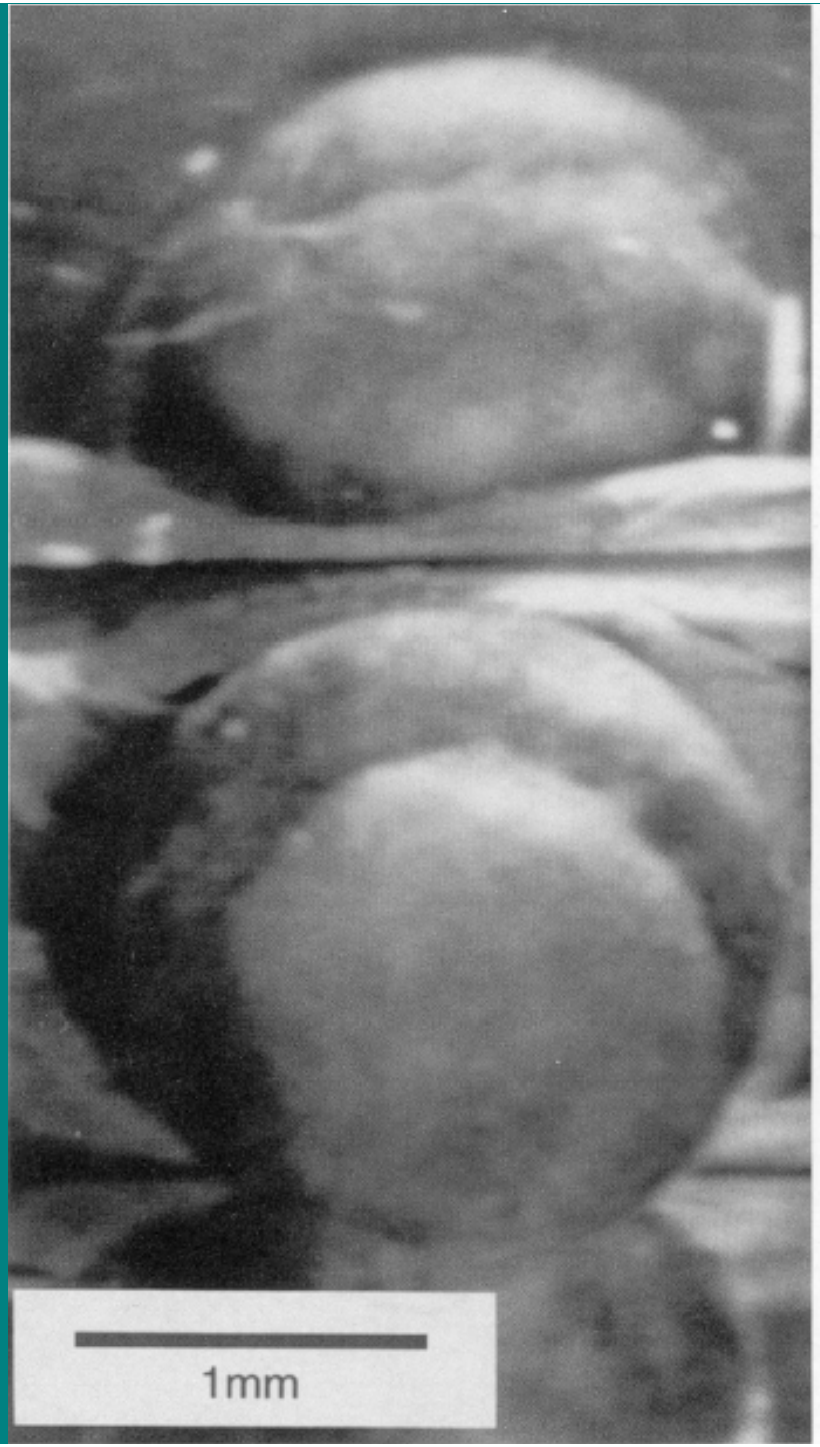
Microwave fixation developed in collaboration with Marc del Bigio, Natalie K. Björklund and Pierre Williot. Bars: 1 mm and 0.1 mm. There is a possibility (arrows on right) that furrowing occurs both on the apical and basal surfaces.

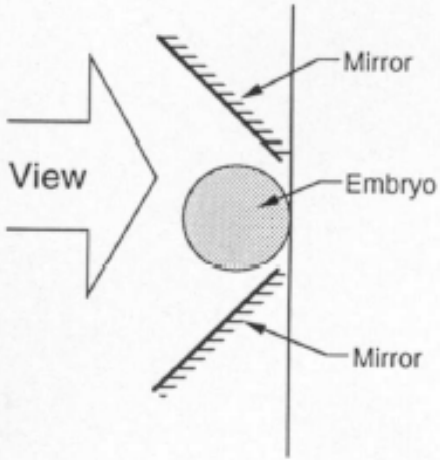
Gordon, R. (1999). *The Hierarchical Genome and Differentiation Waves: Novel Unification of Development, Genetics and Evolution*, Singapore: World Scientific and London: Imperial College Press, 2 vols.



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.

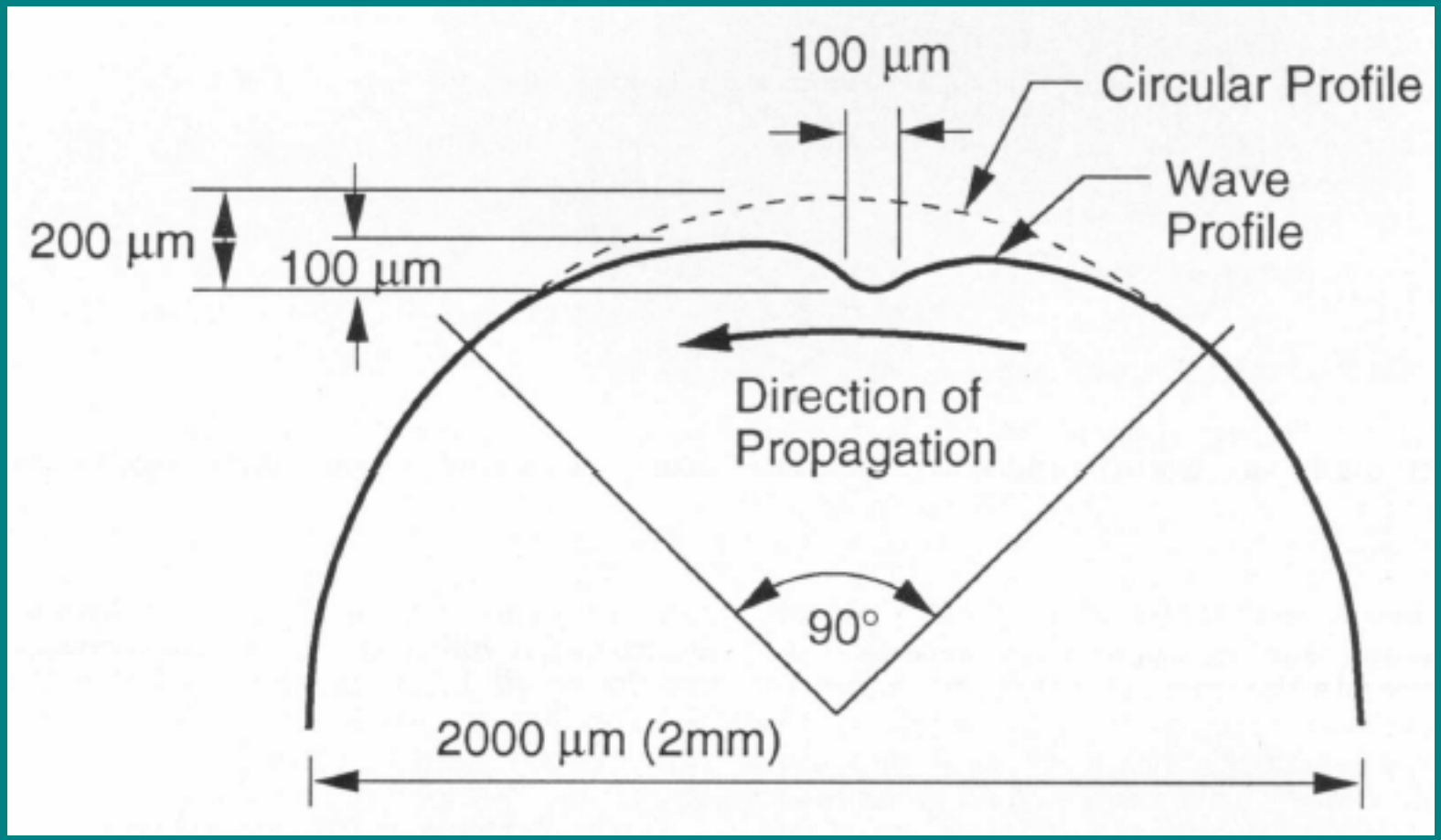




Front silvered mirrors show that the travelling furrow is an indent in the surface of the embryo.

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.

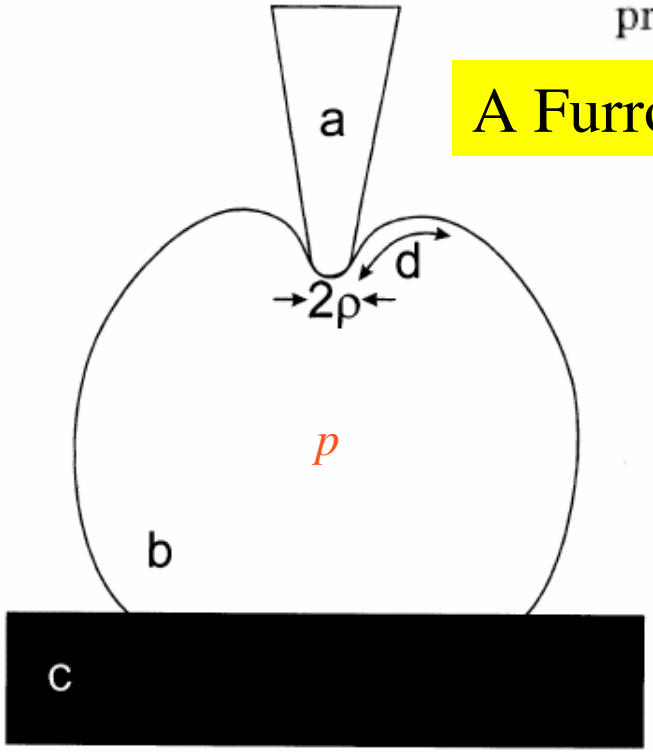


Brodland, G.W., R. Gordon, M.J. Scott, N.K. Björklund, K.B. Luchka, C.C. Martin, C. Matuga, M. Globus, S. Vethamany-Globus & D. Shu (1994). **Furrowing surface contraction wave coincident with primary neural induction in amphibian embryos**. *J. Morphol.* **219**(2), 131-142.

Boulbitch, A. (2000). Deformation of the envelope of a spherical Gram-negative bacterium during the atomic force measurements. *J. Electron Microscopy* **49**(3), 459-462.

AFM = Atomic Force Microscope

"To summarize, we introduced the energy describing deformation of the envelope of a Gram-negative bacteria and used it to study the indentation of the AFM tip into the bacterium during the measurements. We have shown that the response of the bacteria to the AFM tip indentation is linear and can be characterized by a spring constant. We finally find out that the spring constant of the bacteria is proportional to the bacterial turgor pressure."



"**Fig. 1** Schematic view of deformation of a spherical bacterial envelope by the AFM cantilever. (a) The cantilever; (b) the bacterial envelope, (c) the substrate. We also show the contact domain (diameter 2ρ), and the cut-off distance *d*."

A Furrow is a Dent Analogous to Poking a Cell

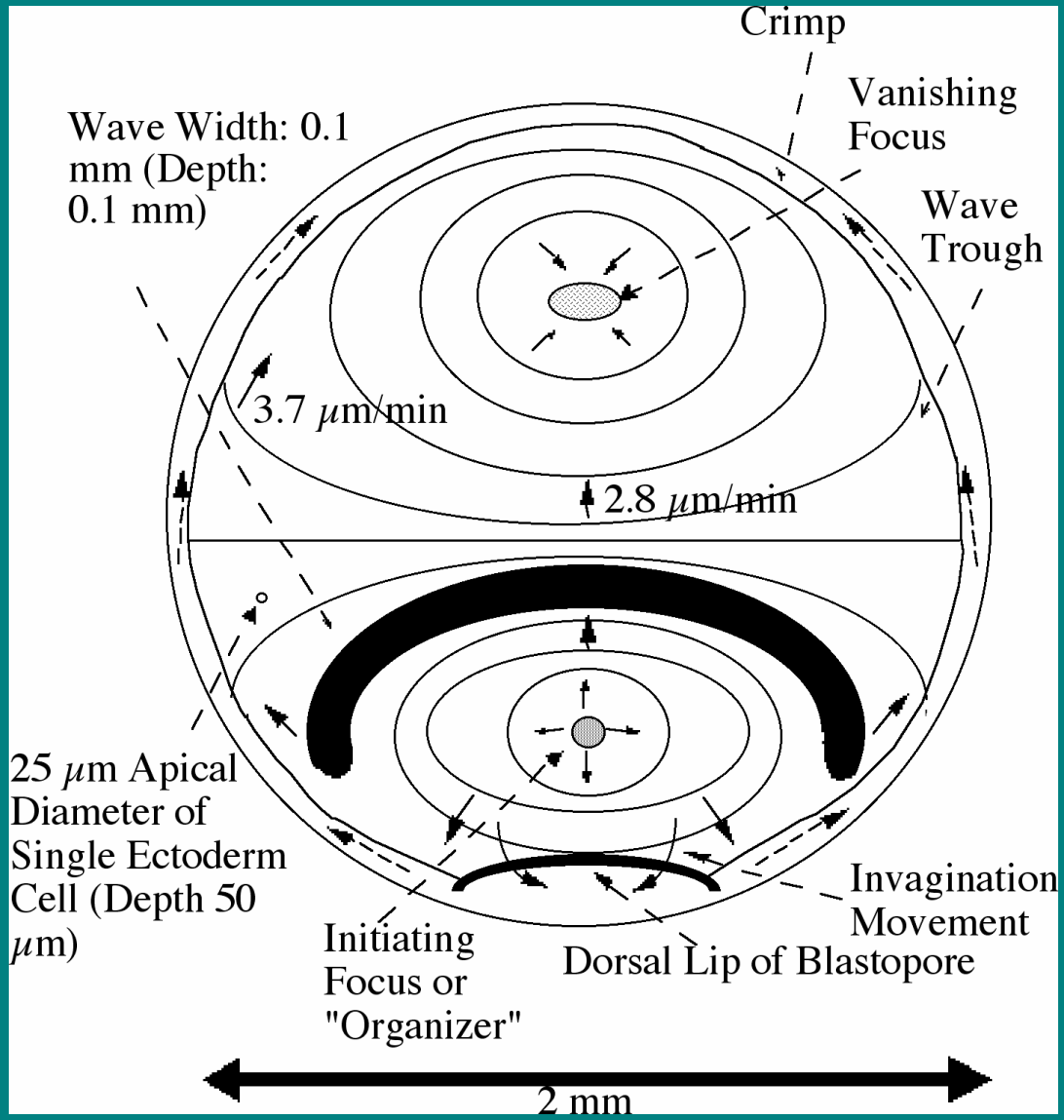
$p = 7$ atm in amphibians: p. 90 in Beloussov, L.V. (1998). *The Dynamic Architecture of a Developing Organism An Interdisciplinary Approach to the Development of Organisms*, Dordrecht Kluwer Academic Publishers.

Mathematical Wholeness: the free energy F is an integral over the whole surface of the surface tension σ, stretching in area A, and the applied force **f**, less an integral over the whole volume of the turgor pressure p:

$$F = \oiint \left[\sigma + \frac{\lambda}{2}\left(\frac{\delta A}{A}\right)^2 - (\mathbf{f}\cdot\mathbf{n})\psi \right] dA - p \iiint dV$$

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?

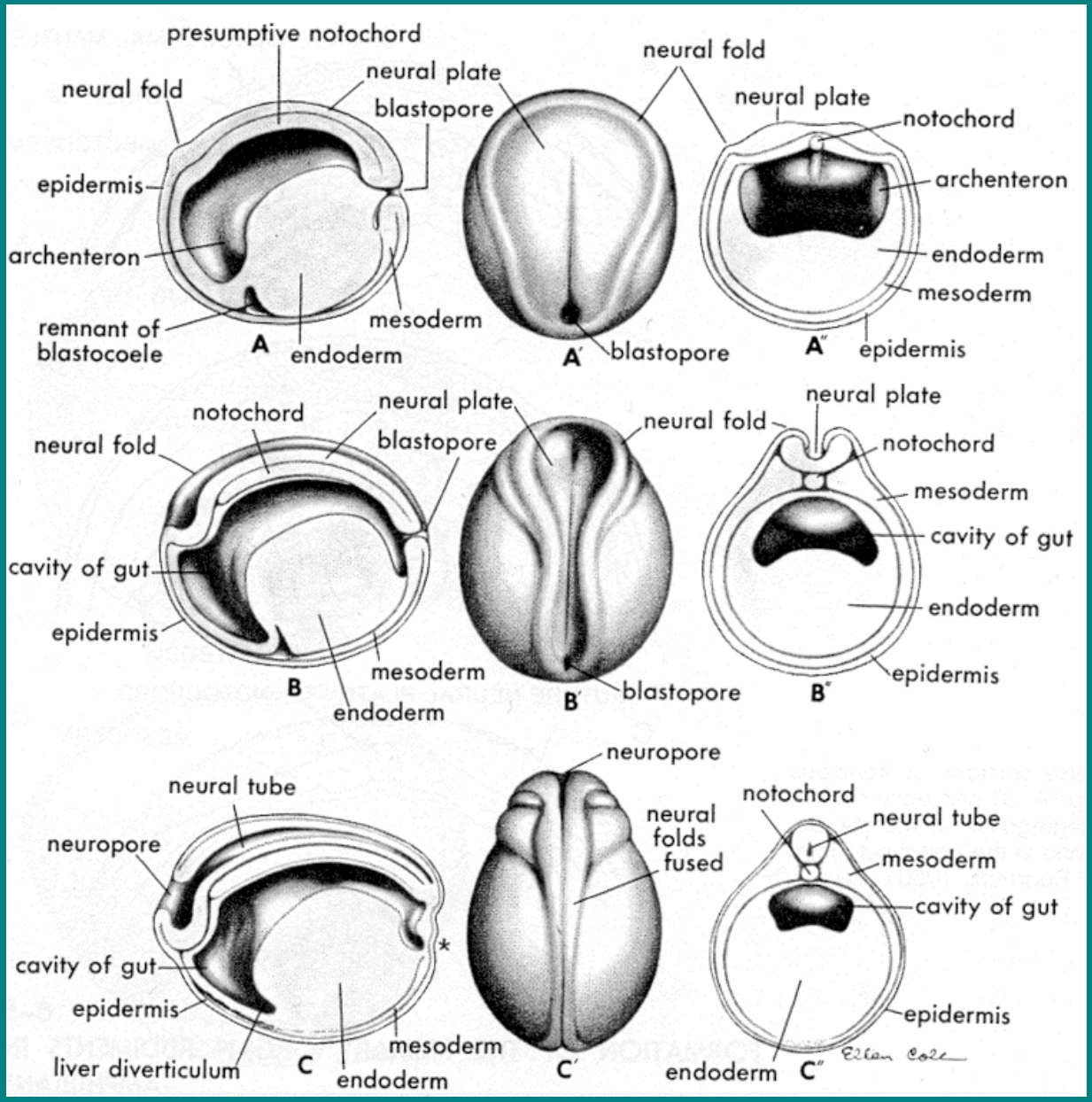


Head end

Tail end

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

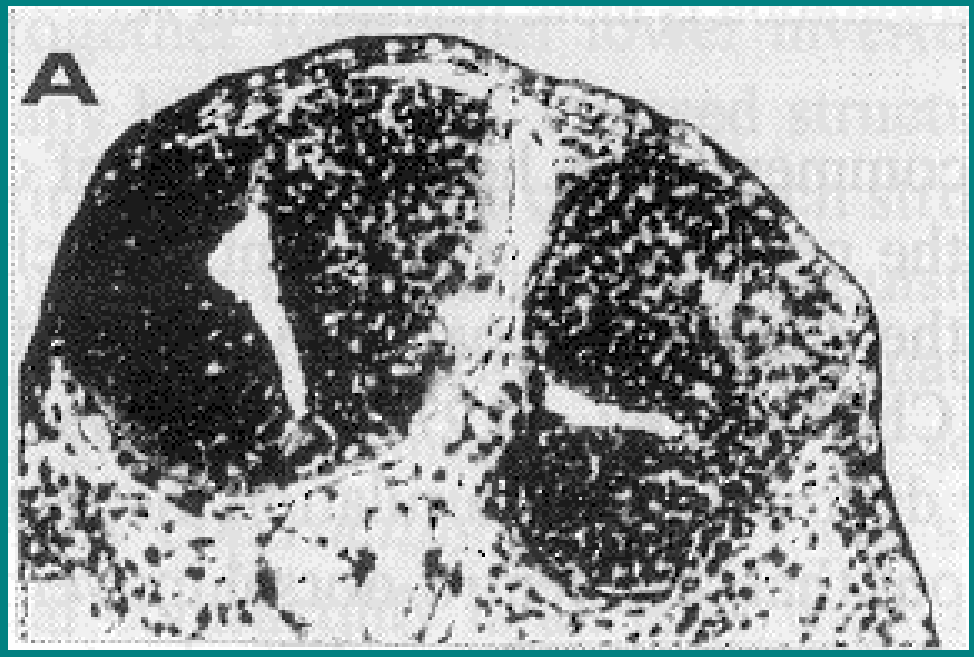
Normal Frog Neurulation



Balinsky, B.I. & Fabian, B.C. (1981). *An Introduction to Embryology*, 5th ed., Philadelphia Saunders College Publishing.

Indeed, if tension is relaxed, the whole surface, all of the ectoderm, turns into multiple neural plates

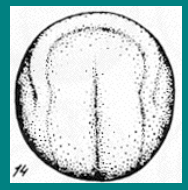
which roll into multiple neural tubes



Beloussov, L.V. (1998). *The Dynamic Architecture of a Developing Organism An Interdisciplinary Approach to the Development of Organisms*, Dordrecht Kluwer

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.

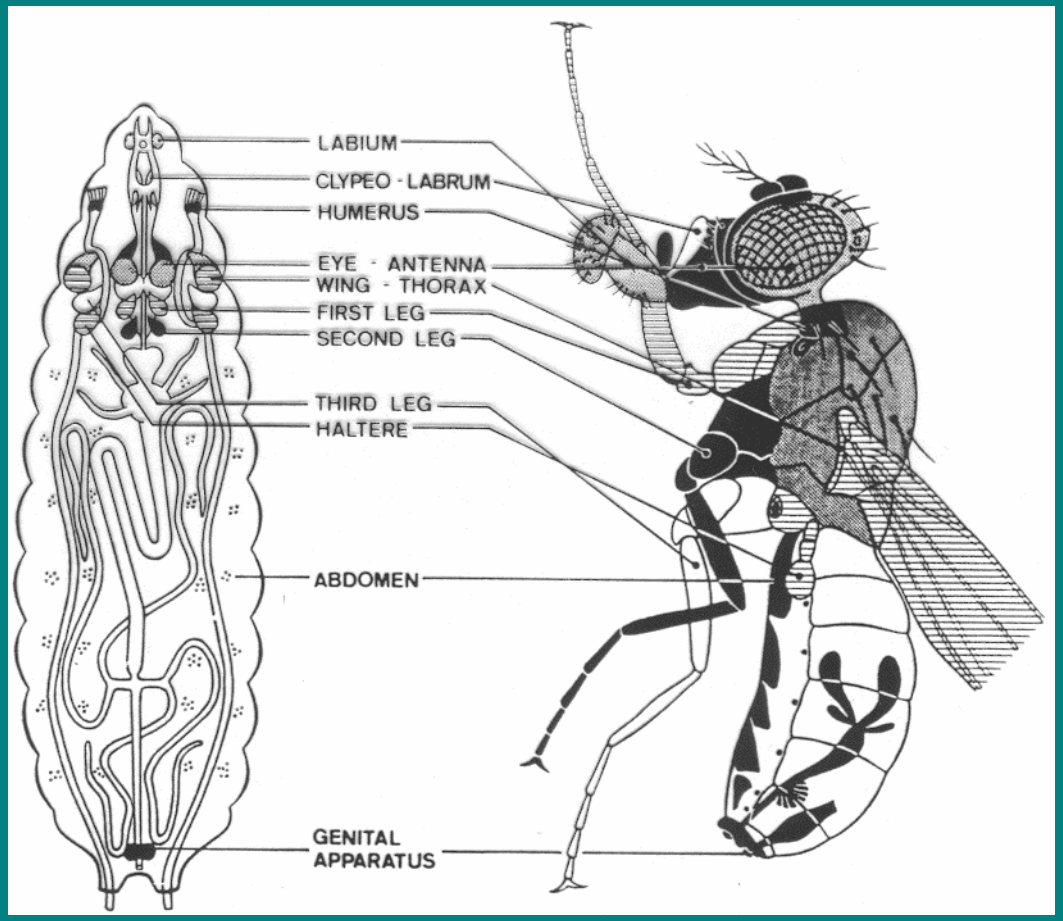
More Comments

- The wave self-annihilates, and thus is a wave in an active medium
- If the cells change kind as they participate in the wave, then this is a kink wave
- A forest fire is a wave in an active medium that is also a kink wave

http://www.smh.com.au:80/news/0201/04/gallery/snapshot8.html

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

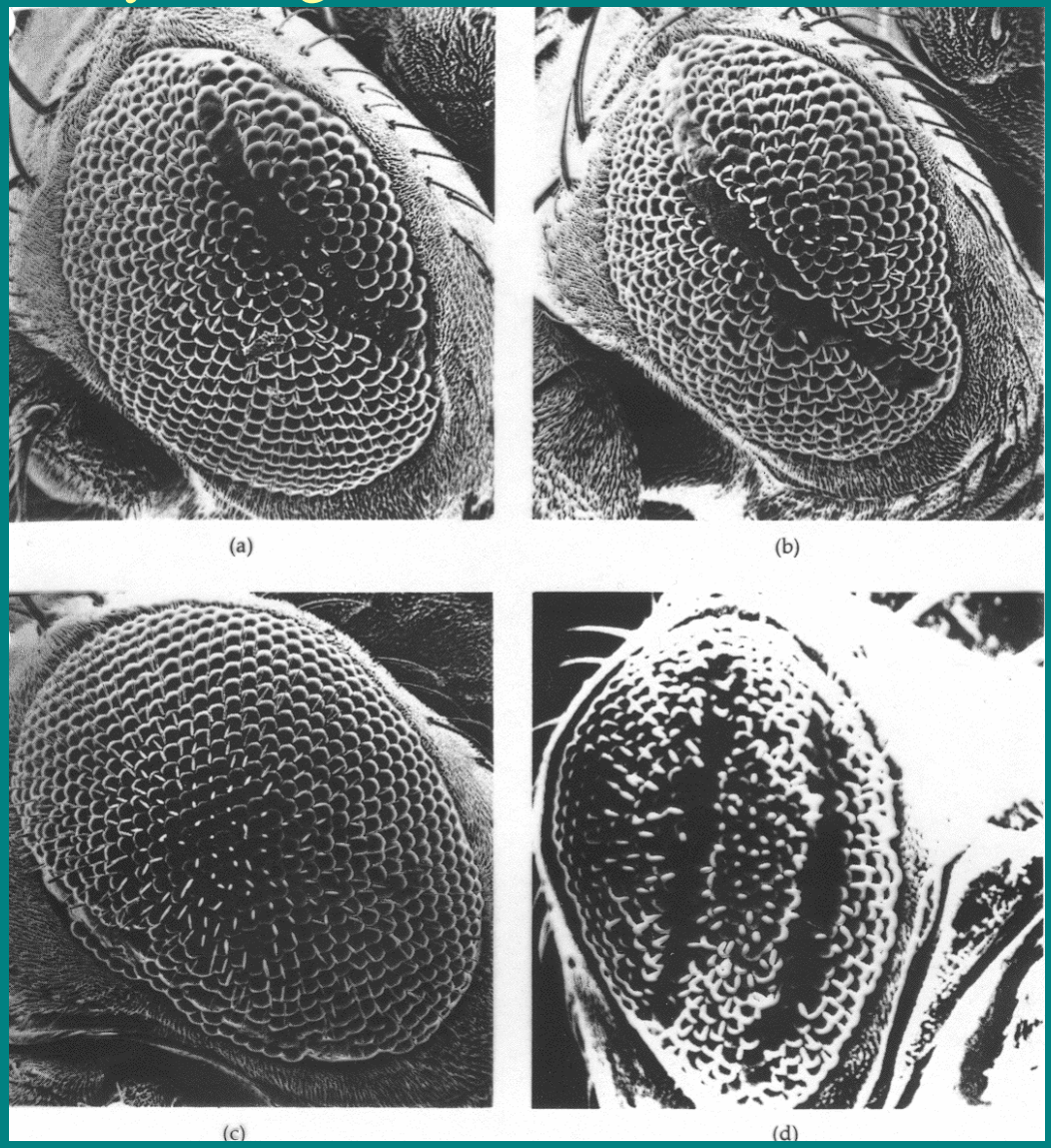
Imaginal discs in the insect larva (grub) form most of the adult parts, except for the brain



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.

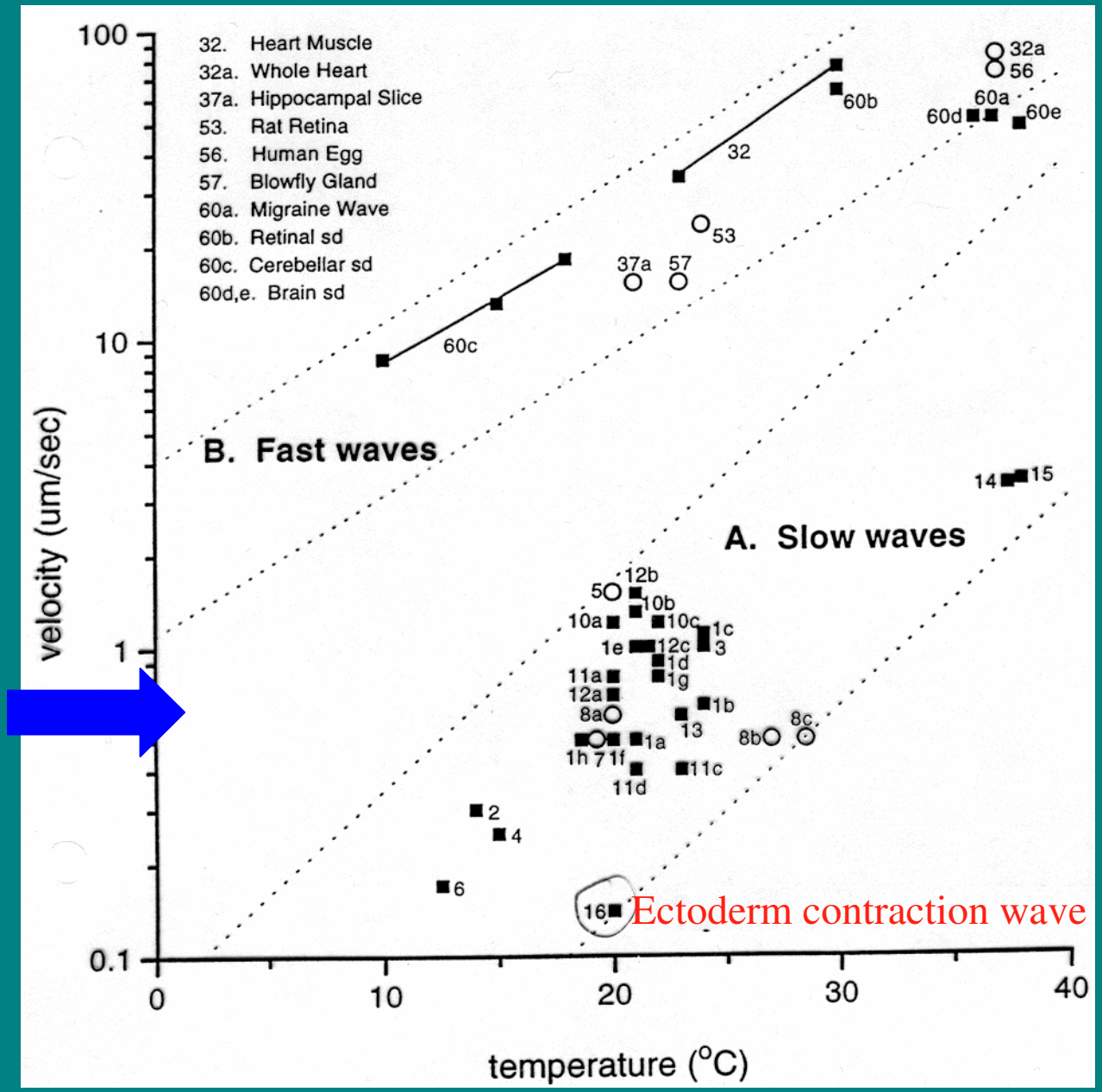
Canadian content!

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)

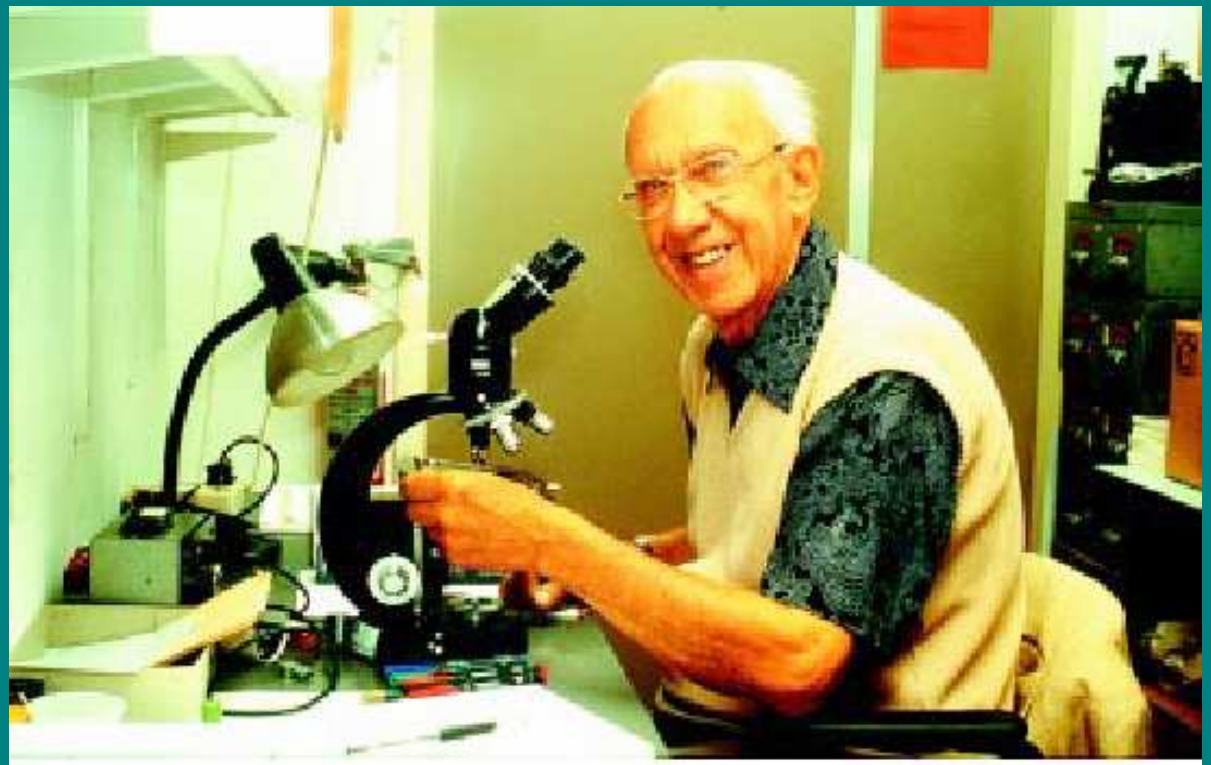
The last suggests that differentiation waves may predate the evolution of multicellular organisms

Calcium Waves in Development



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

Where is the Wave in *Xenopus*?



Pieter Nieuwkoop at work. *Photo by John Bluemink, August 1996.*

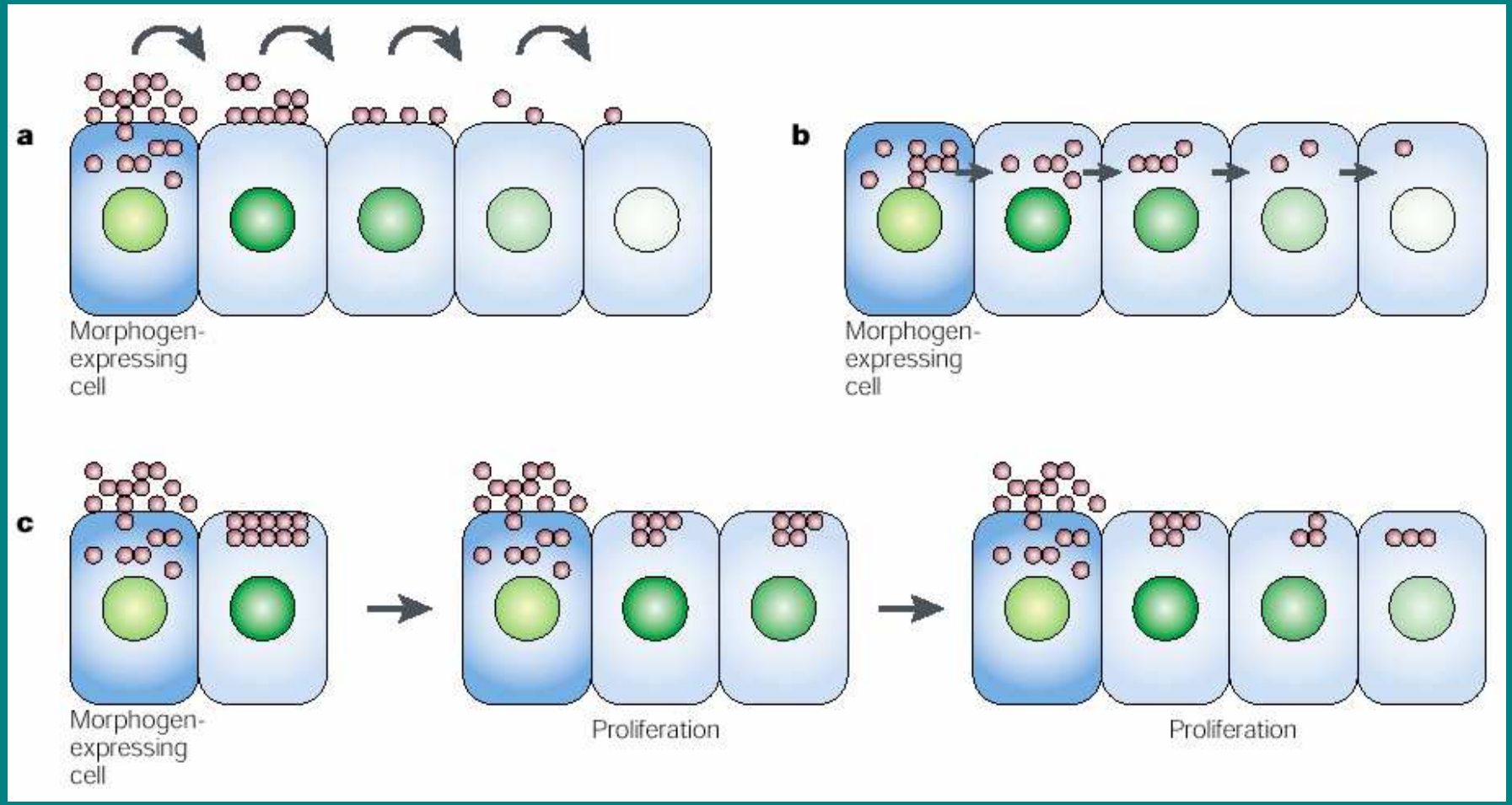
De Robertis, E.M. (1999). A nose for the embryo: the work of Pieter Nieuwkoop. *Int J Dev Biol* **43**(7 Spec No), 603-604.

A possible contradiction is lack of an observed ectoderm contraction wave in the South African clawed toad *Xenopus* laevis. This challenge to the theory came from our friend and collaborator, the late Pieter D. Nieuwkoop. We speculated with him[1] that, because the ectoderm is covered by an extra layer of cells in frogs and toads, the wave is obscured. So other methods besides simple time lapse microscopy will be needed to try to see if the predicted wave is there. Perhaps, as in the eye development of the flour beetle, *Tribolium*, in which the eye imaginal discs is similarly covered[2], the wave is at the bottom (basal) end of the cells, rather than at the apical end.

[1]Nieuwkoop, P.D., N.K. Björklund & R. Gordon (1996). Surface contraction and expansion waves correlated with differentiation in axolotl embryos. II. In contrast to urodeles, the anuran *Xenopus laevis* does not show furrowing surface contraction waves. *Int. J. Dev. Biol.* **40**(4), 661-664.

[2]Friedrich, M., I. Rambold & R.R. Melzer (1996). The early stages of ommatidial development in the flour beetle *Tribolium castaneum* (Coleoptera; Tenebrionidae). *Dev. Genes Evol.* **206**(2), 136-146.

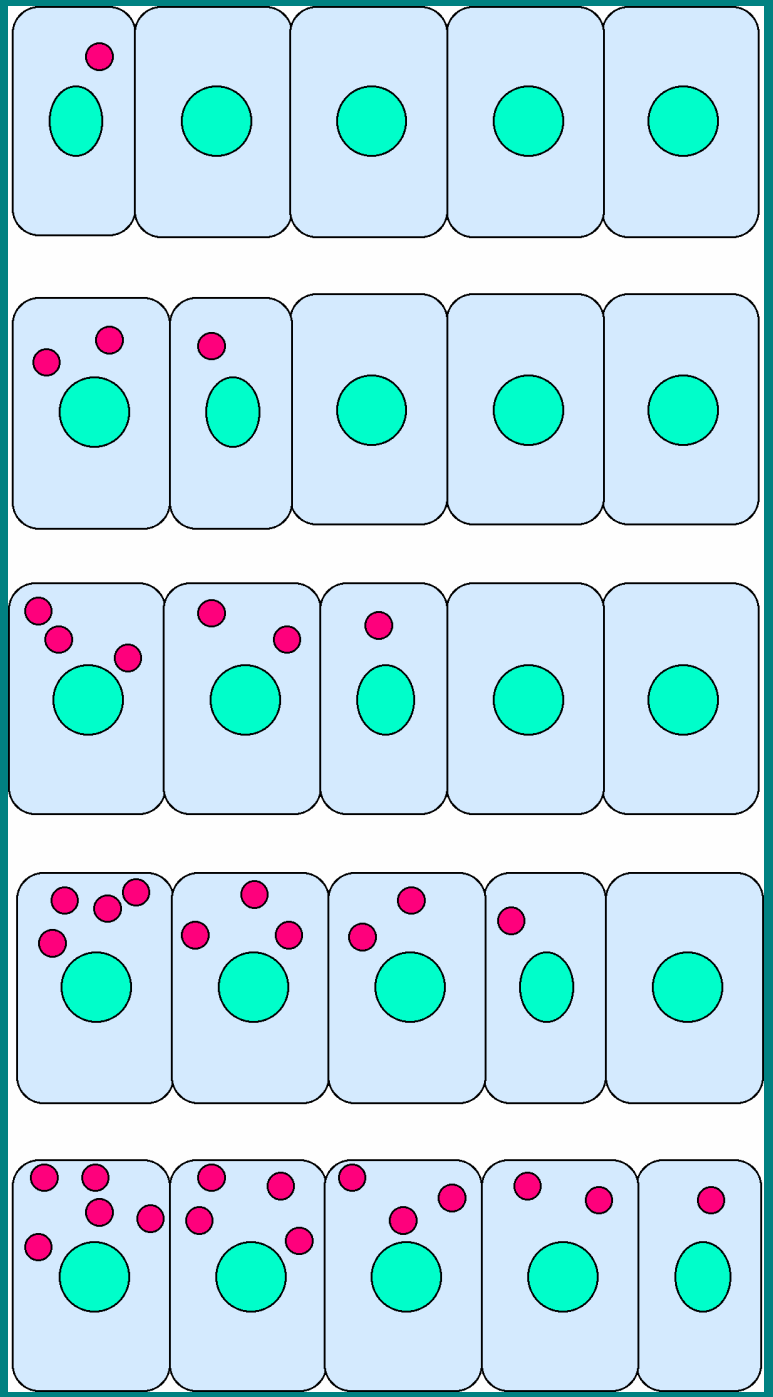
Today's Morphogen Hypotheses for Positional Information



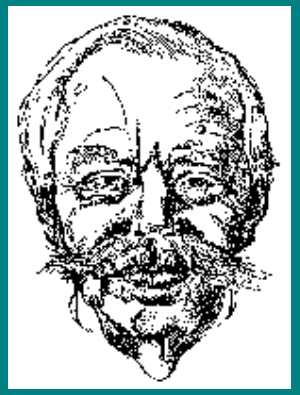
a) Diffusion through the extracellular space
b) Planar transcytosis
c) Displacement during growth

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 Clothesline



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

Sometimes one person thinks a problem through carefully, works it out, even writes it down, and is ignored. Decades or generations later their idea is rediscovered, and if dredged up from the archives, it is noted how quaint it is that that person had a glimpse of the idea first. So I will do that now for Jakob von Uexküll from Estonia (1864-1944), best known as the founder of semiotics. Here are his own words, published in German in 1920, and in English in 1926[1].

> "Employing a crude but very obvious comparison, we may picture the chromosomes in the nucleus of the germ-cell as washing-lines, on which factors for the absolute and relative properties hang, side by side, like articles of clothing which the subject will put on, one by one.... In contrast to what happens with machines, the builder resides within the organism itself.... A gene or factor, then, is a ferment [enzyme, or, in modern language, an expressed gene] activated by an impulse [differentiation wave].... Driesch succeeded in showing in sea-urchin larvae... that the half-size depended on each larva having the same-sized cells but only half the number. It follows that... the shaping impulses... are to a great extent independent of the quantity of material furnished them....An impulse-system can allow a whole series of cells to be simultaneously invaded by a fermentative action leading to a certain chemical change.... Simultaneous and equipotential impulses of this kind must produce in a mass of similar cells a differentiation with regard to position.... 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. But, since they are attached to the genes, they dominate the material, for that is set in motion by the fermentative action of the genes. The genes themselves represent a union of a latent ferment with an activating impulse...."

We can see that Uexküll solved poor Hans Driesch's dilemma: there is indeed a nonmaterial thing that coordinates cells, namely impulses. What Driesch missed is that physics is about more than matter. It is also about force. Force can be transmitted through matter, while the matter pretty much stands still. Pressure waves, sound waves, and waves in an active medium, can all move through matter, without being matter, and without being a mysterious "entelechy". Uexküll also solved Thomas H. Morgan's problem of how to turn batteries of genes on and off differentially even before Morgan put the problem in words. If differentiation waves do this, then we now know what Uexküll's impulses are. We are left with the unsolved problem of what we now call robustness: independence of the pattern (trajectories of the waves) from variations in embryo size.

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

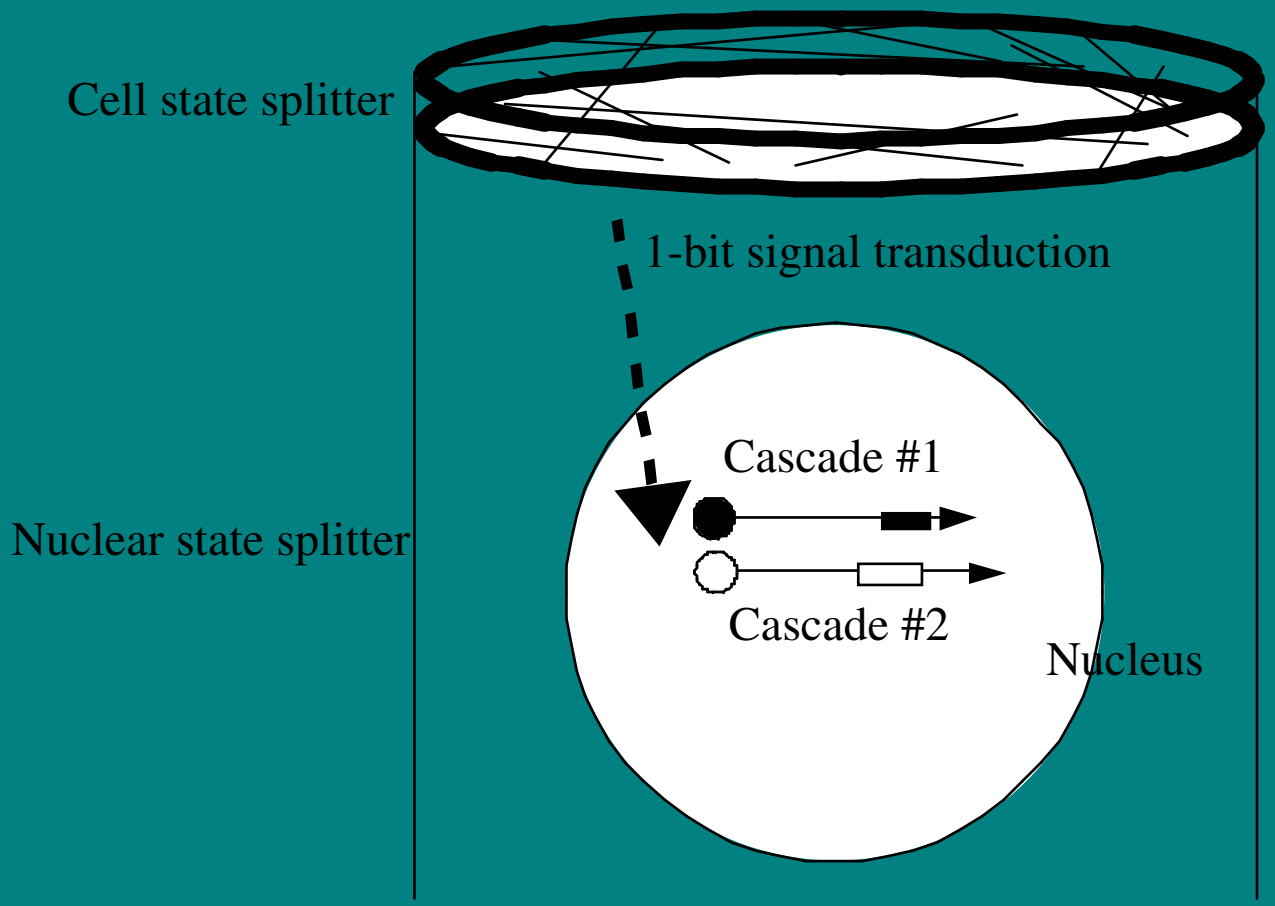
What's Going on in the Nucleus during Cell Differentiation?

- 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)

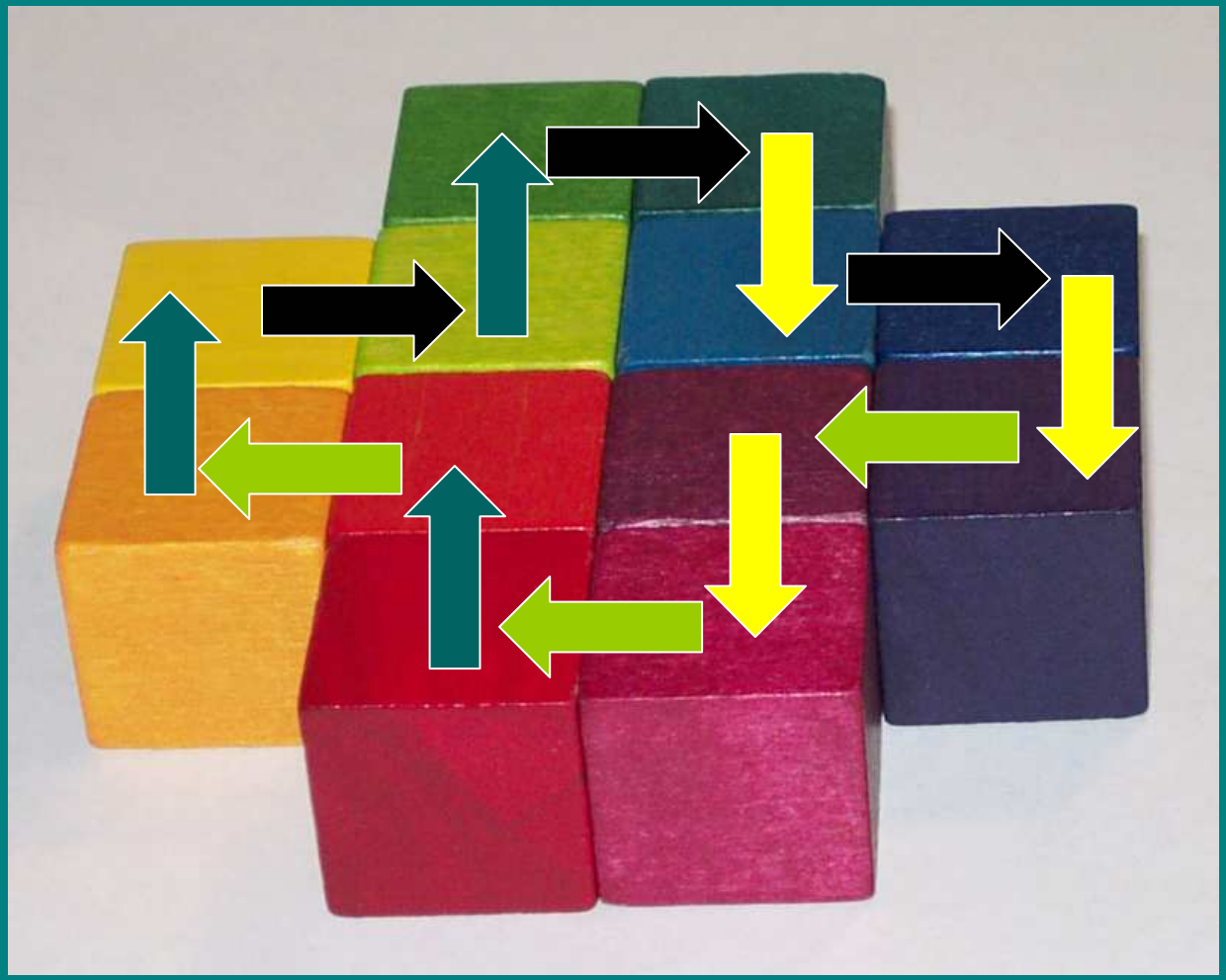
"May I help you?"

"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

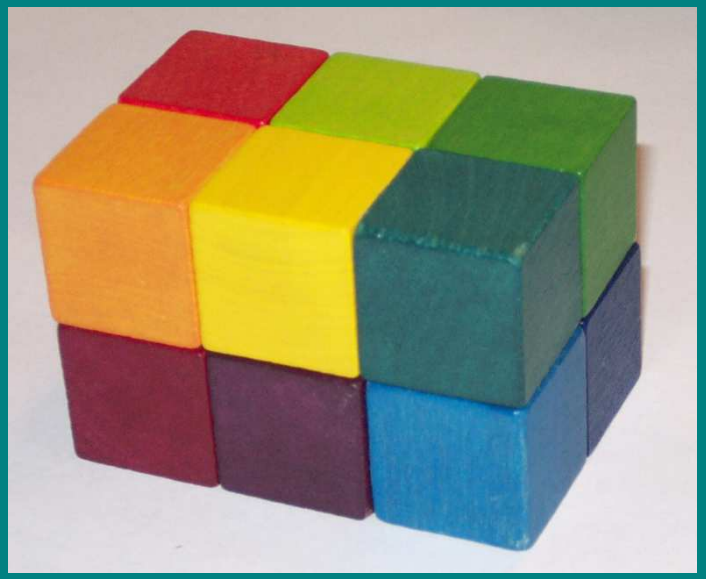


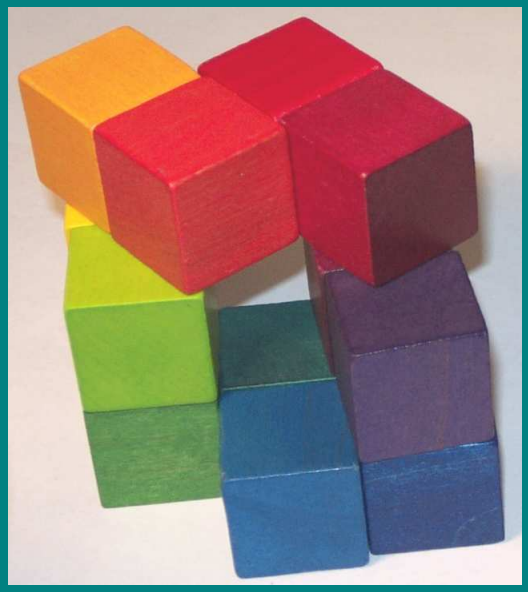
Arrows show the path of the taut elastic band through the blocks

A simple bracelet, but also a tensegrity structure

Canadian content!

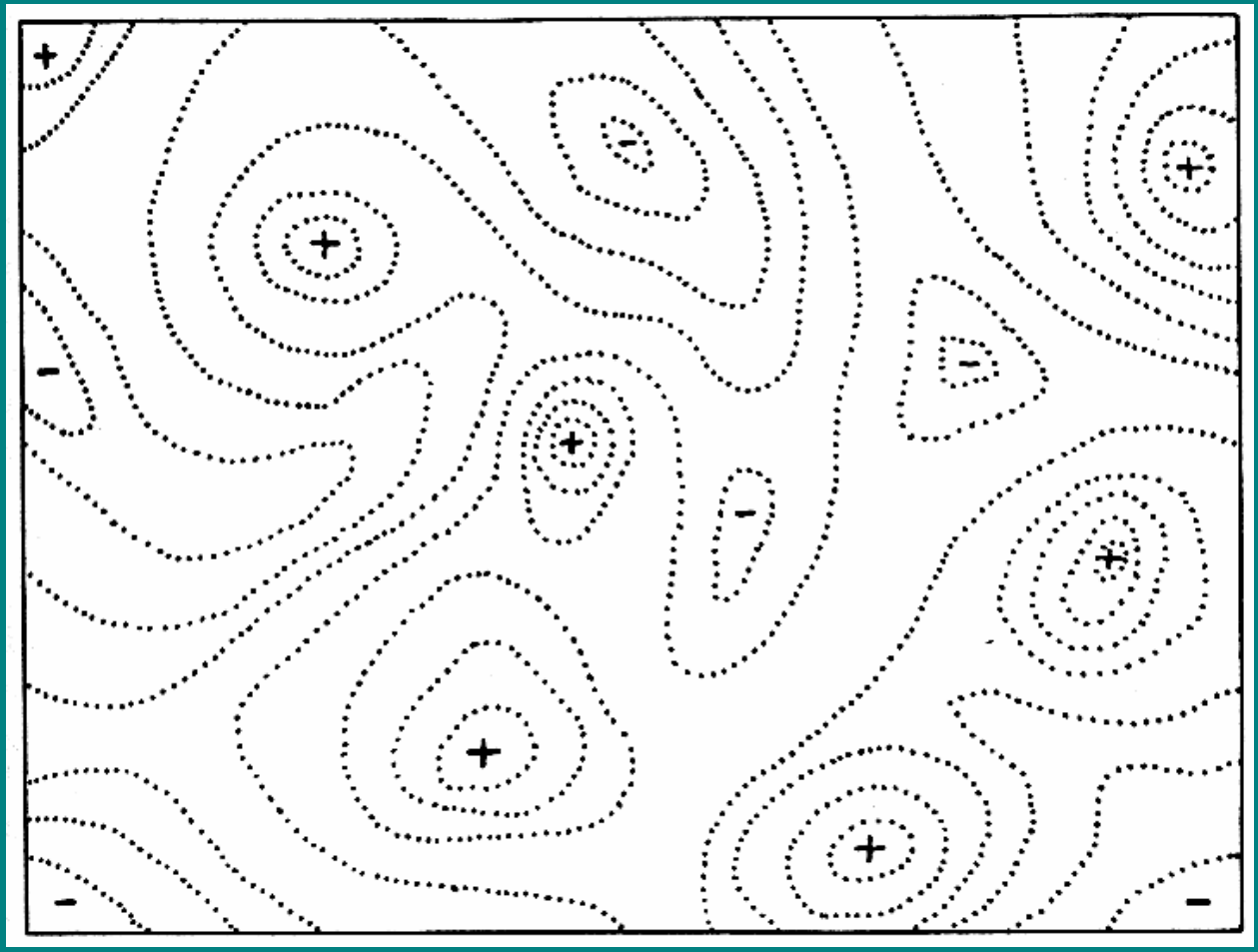
More Wurfel Configurations

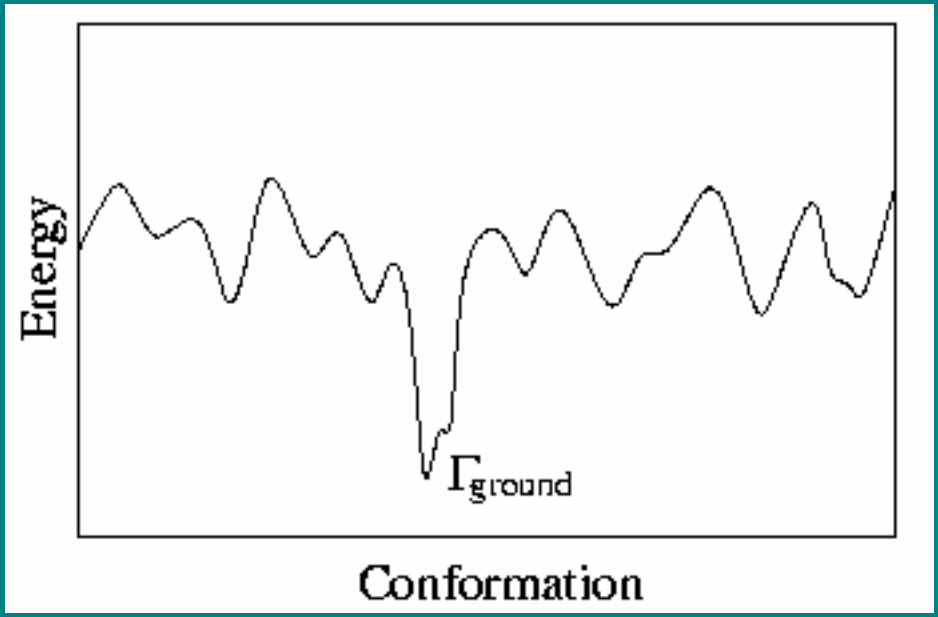




Original •••••Fitness Landscape Concept due to Sewall Wright

Wright, S. (1932). The roles of mutation, inbreeding, crossbreeding, and selection in evolution. *Proceedings of the Sixth International Congress of Genetics*, **1**, p. 356-366.



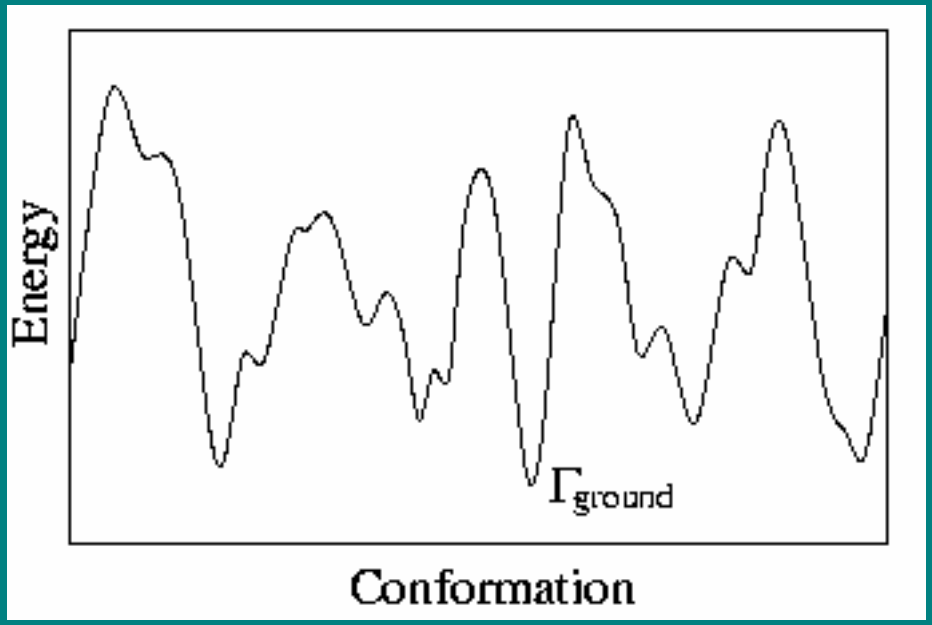


http://www.tcm.phy.cam.ac.uk/~tmf20/PHYSICS/thesis/node15.html

A protein has a landscape with a single major peak

http://www.geocities.com/Yosemite/4525/lbnm11.html

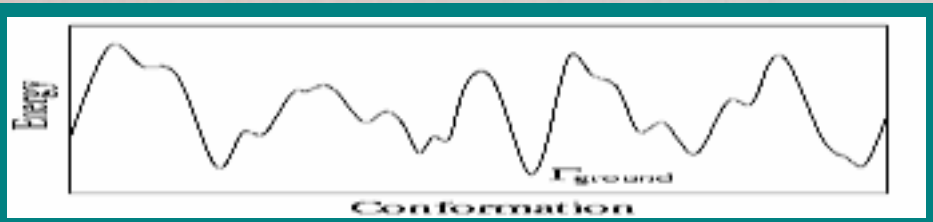
Wurfels have a rugged landscape, with many peaks of nearly equal height



http://www.tcm.phy.cam.ac.uk/~tmf20/PHYSICS/thesis/node15.html

http://www.llbean.com/parksearch/parks/pic_html/ls000628.htm

“Baby Beads” are threaded like Wurfels, but consist of spheres

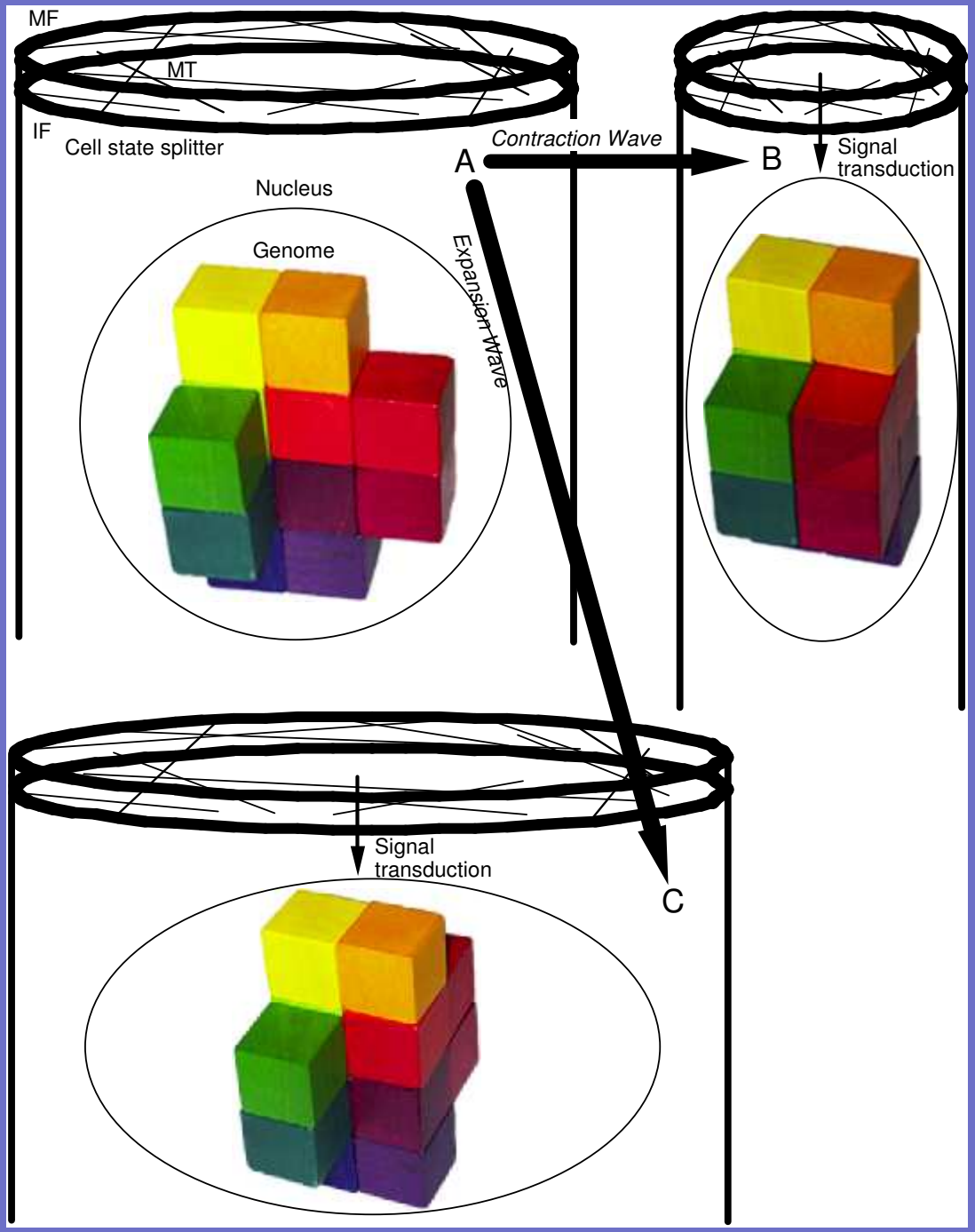


Their multiple peaks in the landscape are much reduced in height

A Wurfel with its corners clipped has a landscape with peaks of intermediate height

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]

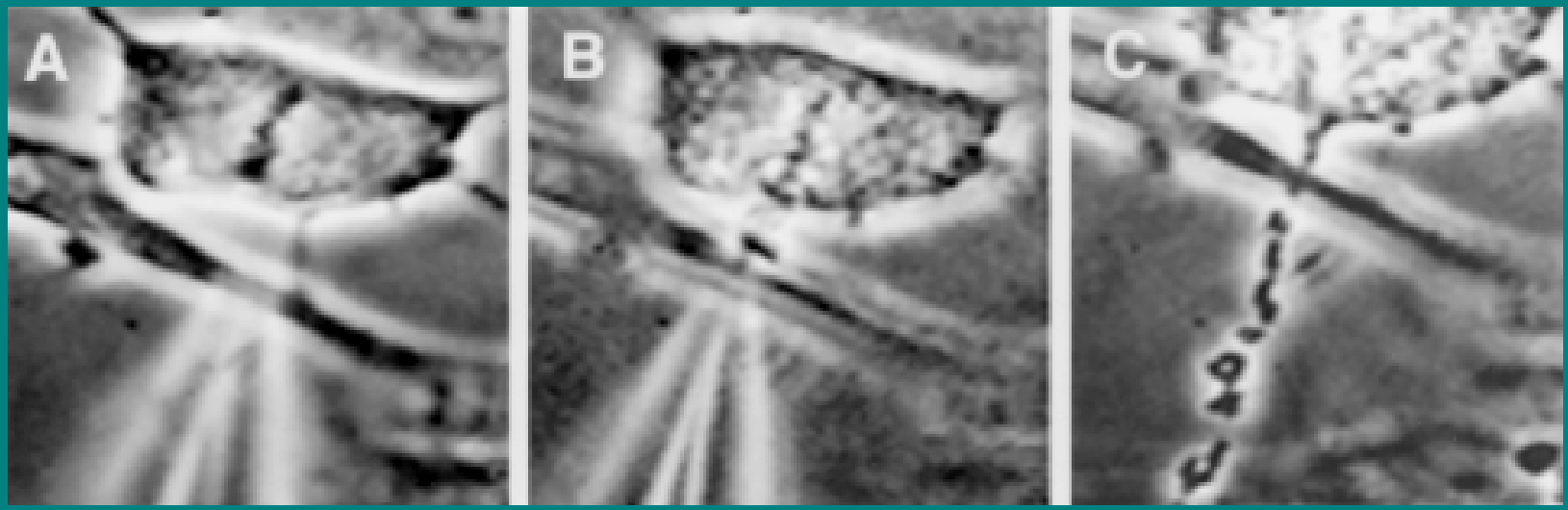
[1]Maniotis, A.J., K. Bojanowski & D.E. Ingber (1997). Mechanical continuity and reversible chromosome disassembly within intact genomes removed from living cells. *J. Cell Biochem.* **65**(1), 114-130.

[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.

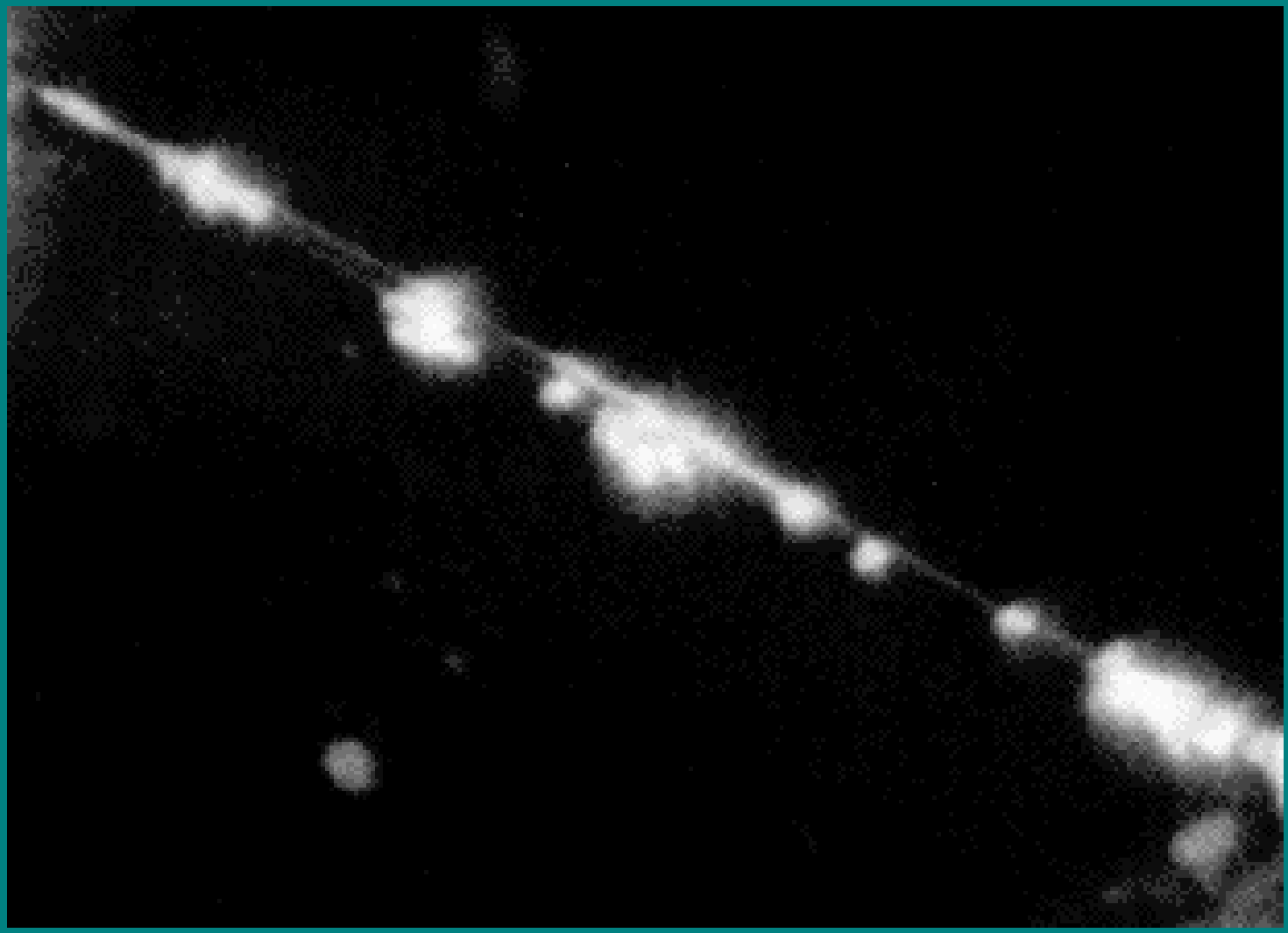
Pulling the Genome out of a Cell



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

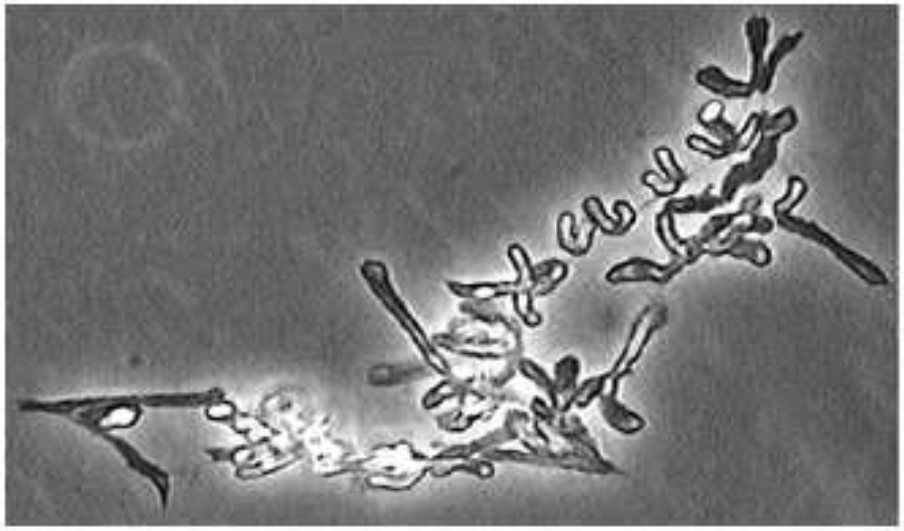
A Stretched Human Genome

Links are DNA

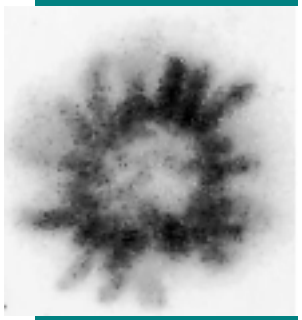


Maniotis, A.J., K. Bojanowski & D.E. Ingber (1997). Mechanical continuity and reversible chromosome disassembly within intact genomes removed from living

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

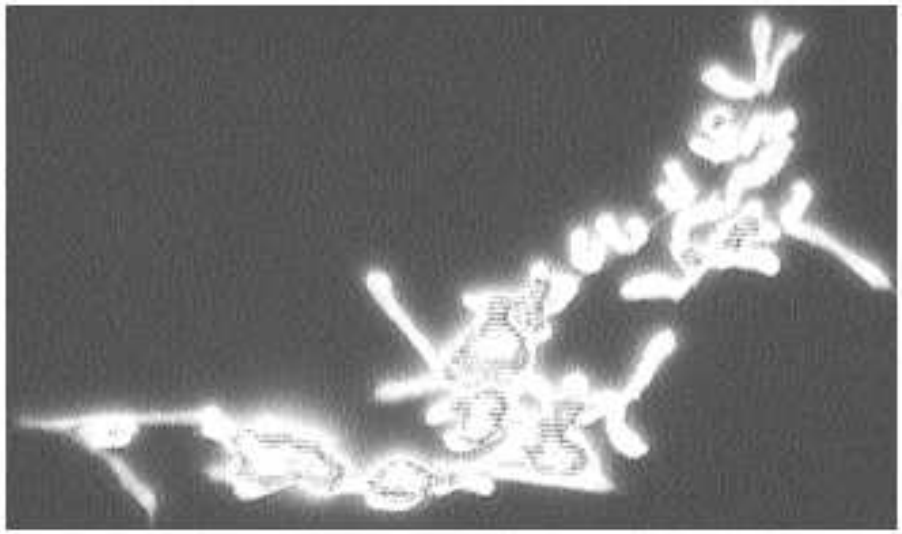


Prophase newt genome



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.

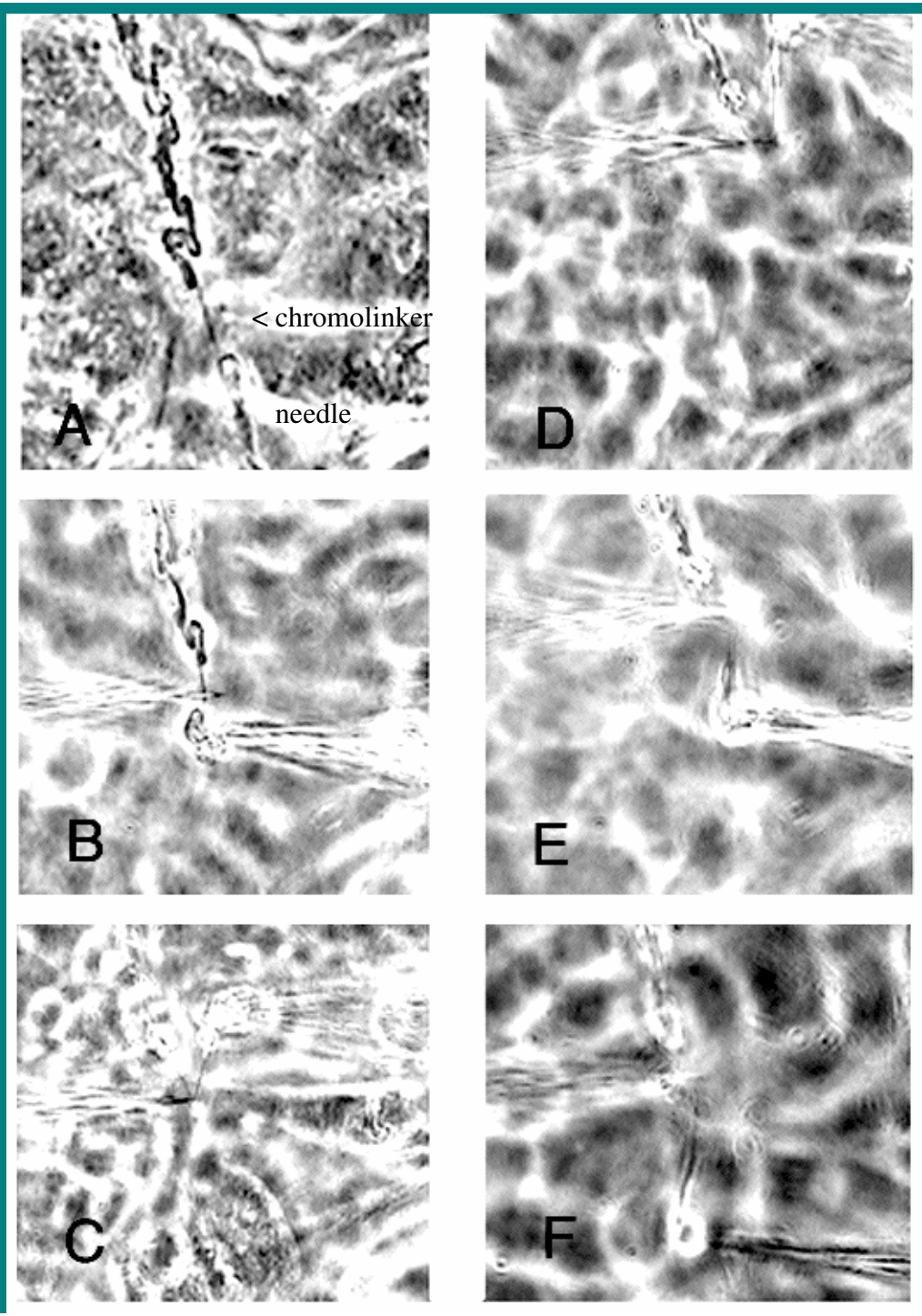
Prophase newt genome after 4ul bis benzamide under flourescent light



The individual chromosomes are

about 5 µm long.

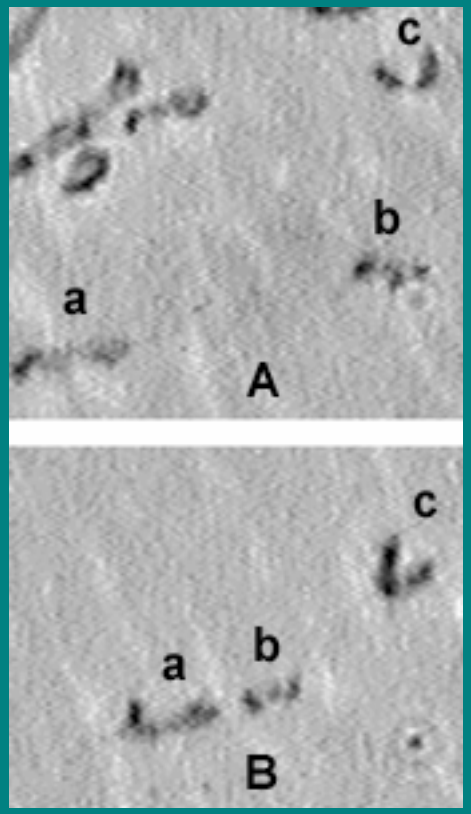
Chromolinkers contain DNA. A whole newt genome (*Notophthalmus viridescens*) from a lung cell showing interchromosomal links ("chromolinkers") possibly forming a loop of all of the chromosomes. The chromolinkers, connecting at the centromeres, stain for DNA and are broken by restriction enzymes (from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



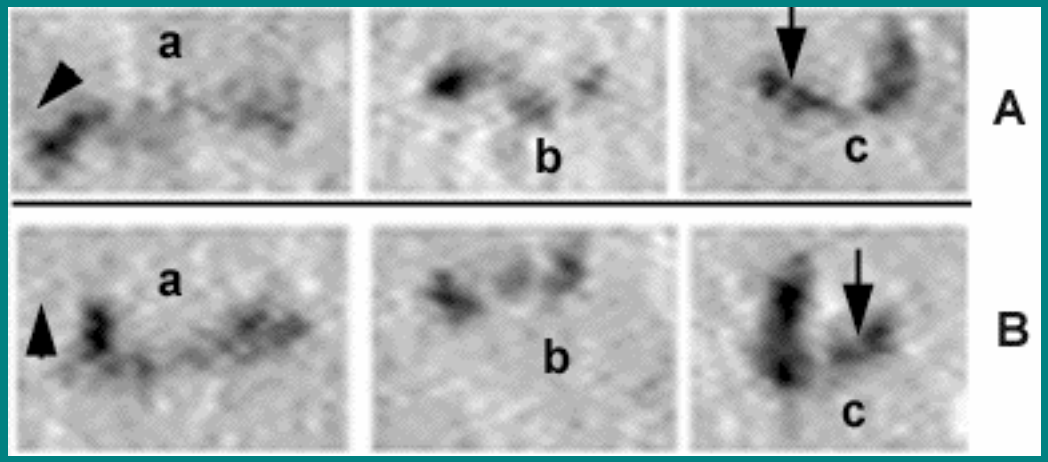
Wrapping up a Chromolinker

A genome is isolated in a flow chamber to avoid contamination by floating DNA. Two sterile, DNase treated needles are mounted over the chamber. A. Genome is partly removed from a mitotic cell with one needle (on right). B. Second needle contacts the chromolinker at 1/4th of its length, so as not to chance touching a chromosome, and is pushed down. C. The first needle is lifted and the chromolinker is wrapped around the top of the second needle. D-F. Wrapping continues. The end of the needle containing the chromolinker is physically broken off in a clean microfuge tube for cloning (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.

Show alpha-Wurfel bungee cord model, linear and circular

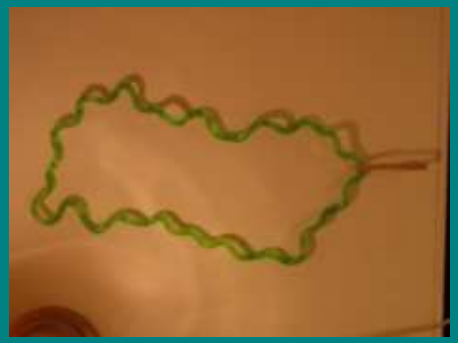
Our Daughter Lana (2002) with a Double-Ended Punching Bag (Chromosome Vibrating in its Genome)

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

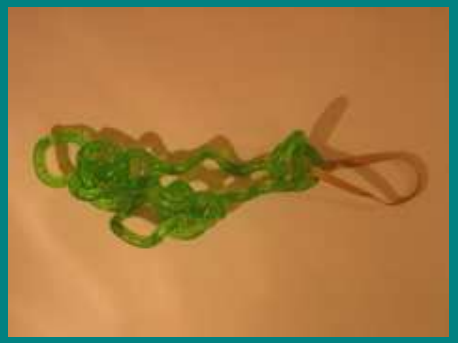
- Comes from reading:
- Qian, R. (2003). *Perspectives on the Macromolecular Condensed State*, Singapore World Scientific.
- Which is about the equilibrium and nonequilibrium properties of plastics

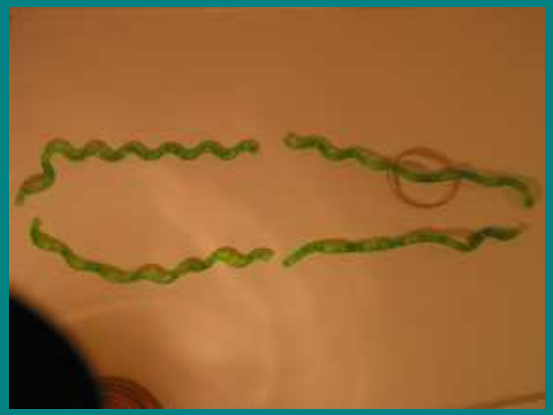


Full ring (no ends)

Compacted and pulled out





4 Chromosomes

Compacted, more difficult to pull out, and left entangled






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

- DNA available for transcription is typically in only one of two conformations: perinuclear, or in splotches
- The severity of an aneuploid phenotype is roughly proportional to the size of the extra chromosome
- The consequences of triploidy are generally worse than those of tetraploidy

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 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

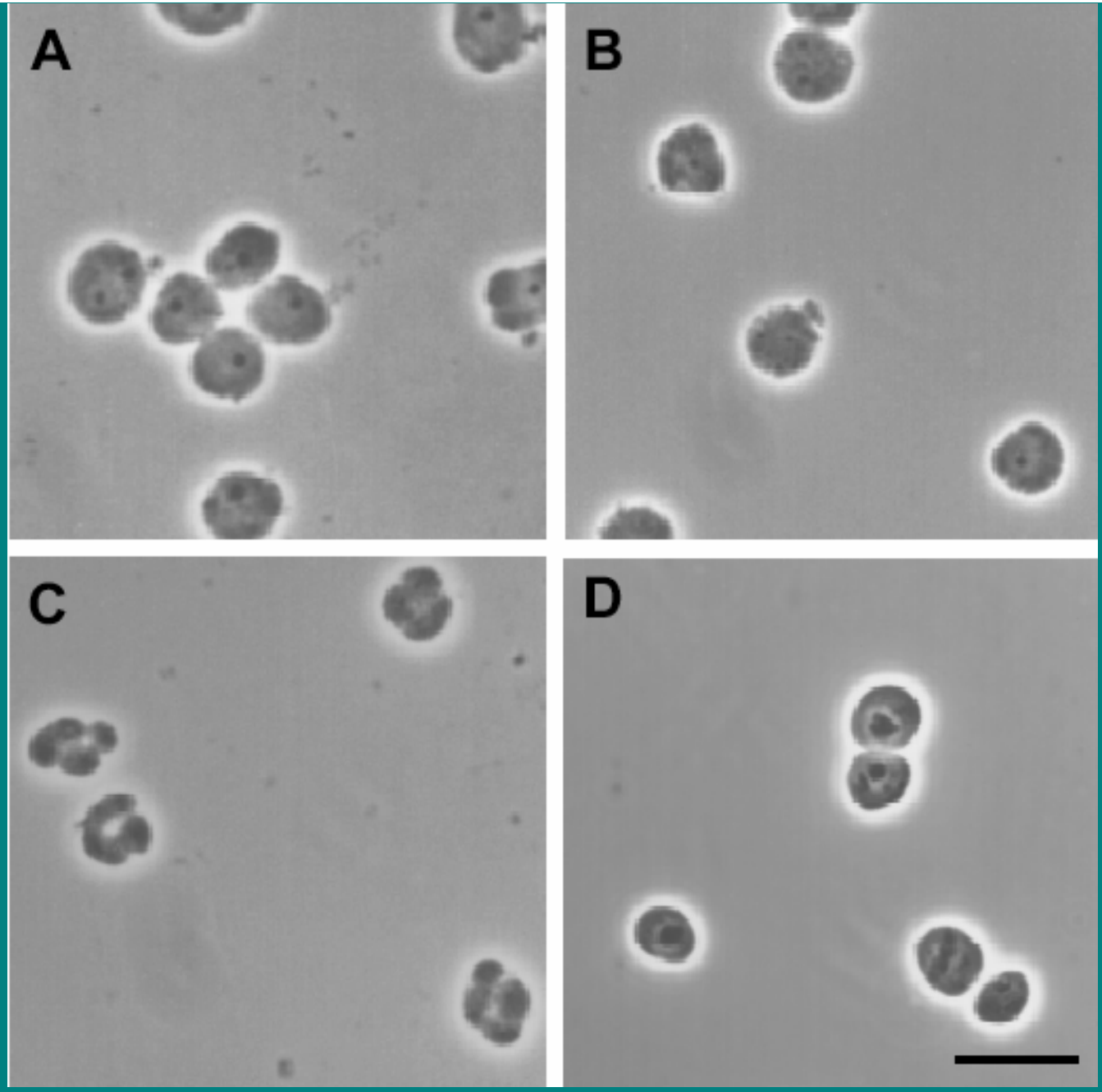
A: Lymphocytes

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

A Discrete Two State Toy Ball

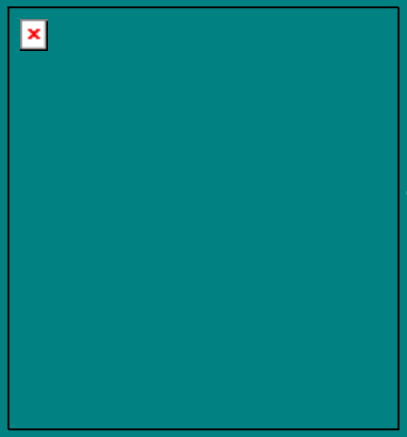
Waters, T. (1992). The unfolding world of Chuck Hoberman: the Buckminster Fuller of the 1990s. *Discover* **13**(3), 70-78.

The Hoberman Sphere

http://www.hoberman.com

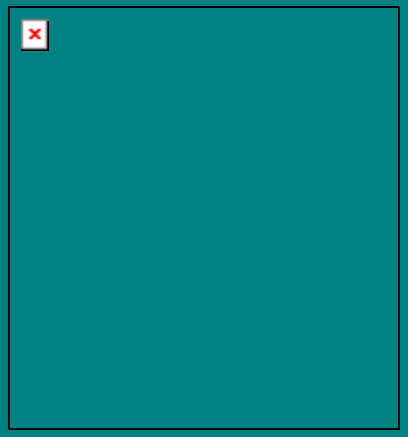
A New Model for Differentiation

Not



Some cells of a given type A change to type B when they are “induced” to do so by signals coming from a third tissue.

or



All cells of type A slowly change to type A’. Some are induced to change to type B.

but

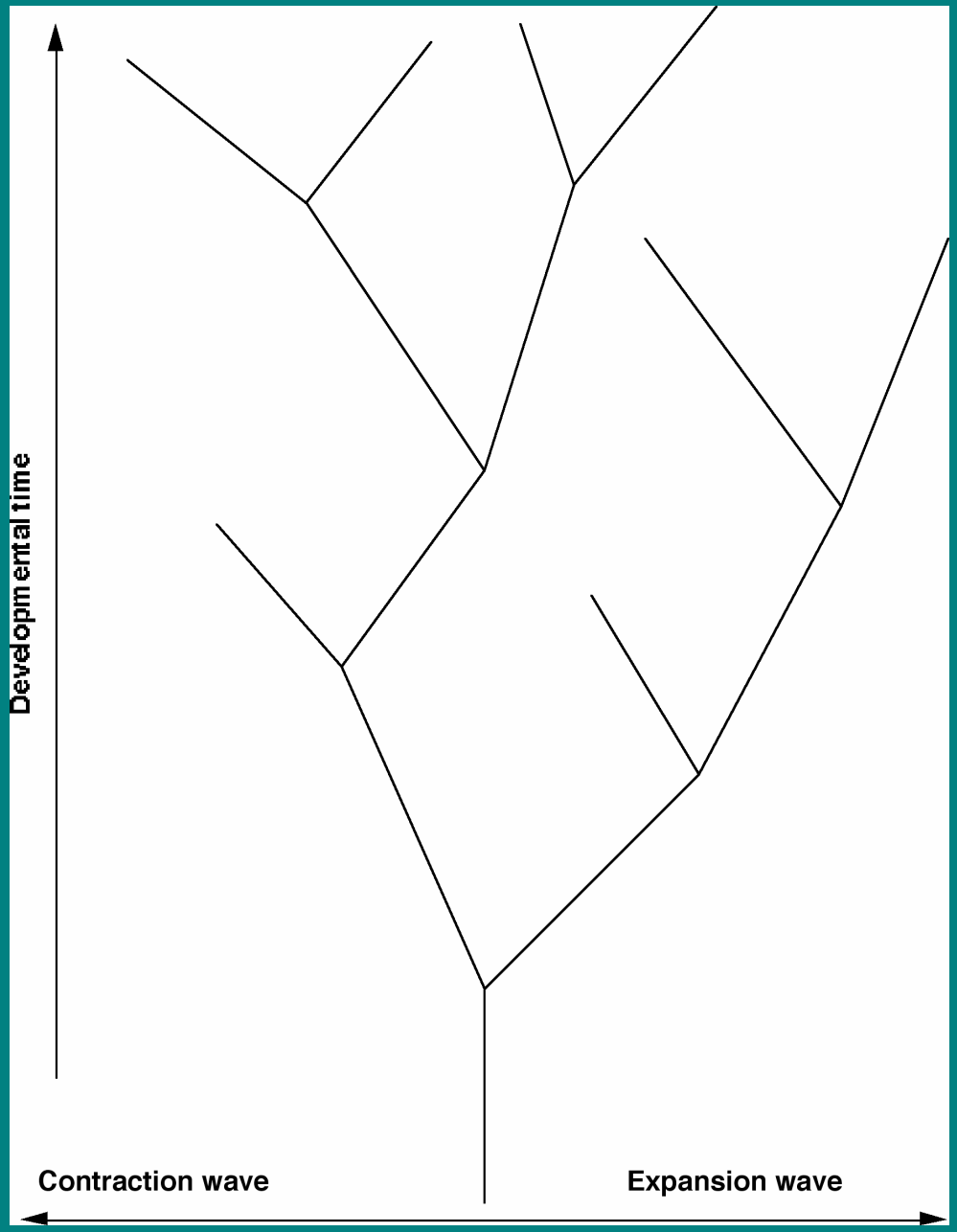
Cells of type A do not change until they participate in a differentiation wave. Those participating in a contraction wave become type B. Those participating in an expansion wave become type C. Any A cells missing both kinds of wave perhaps become stem cells.

The Differentiation Tree

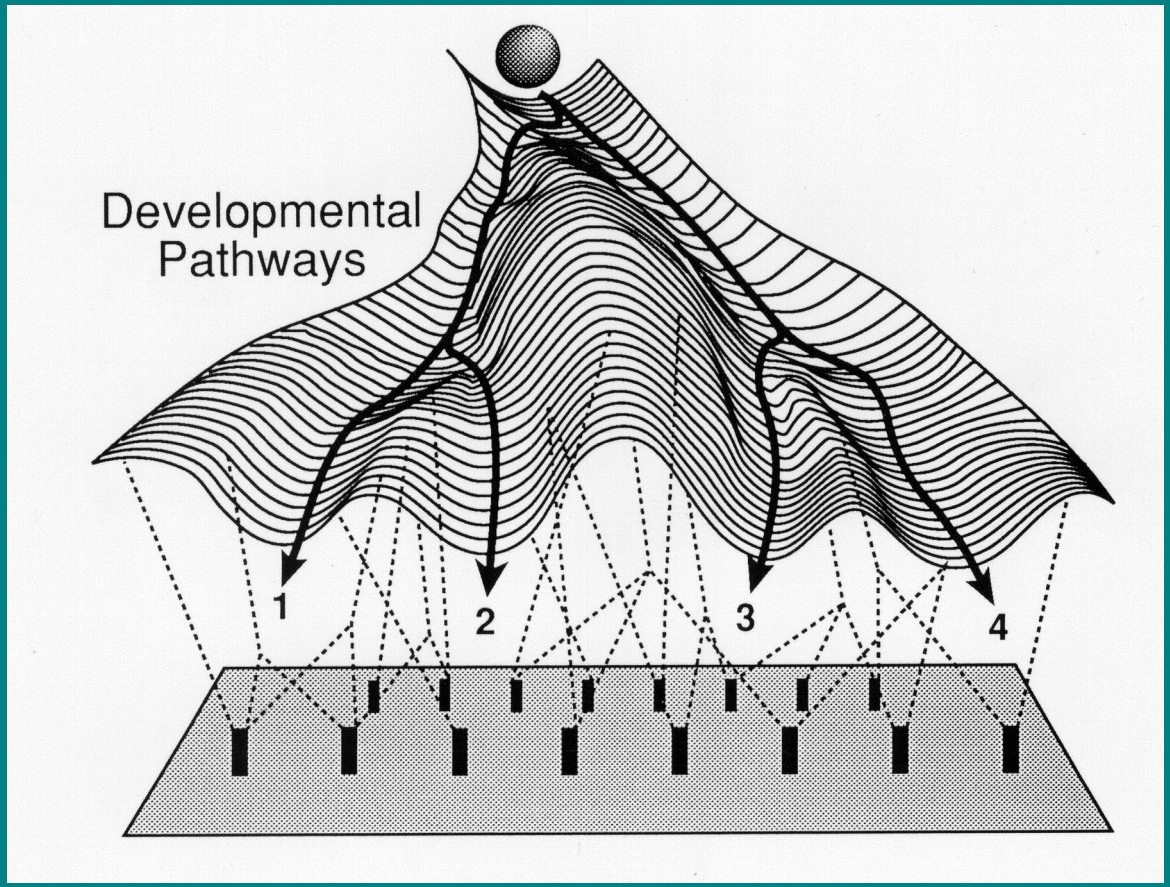
Embryogenesis may be modelled as a bifurcating sequence of tissues generated as each tissue is split into two new tissues by pairs of contraction and expansion waves.

Unsolved problems:

1. What launches these waves at specific times and locations?
2. What confines their trajectories?
3. What stops them?



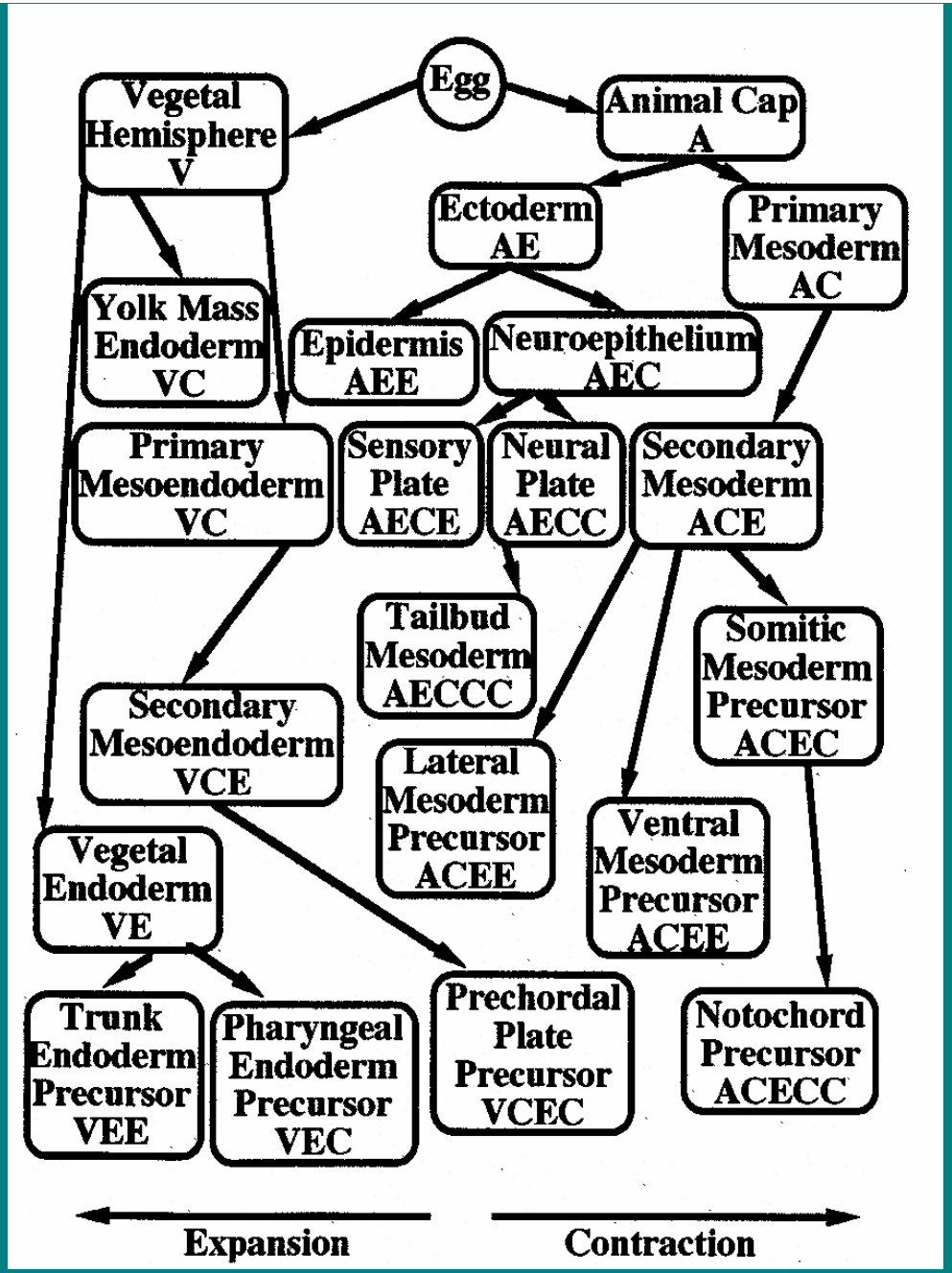
The Differentiation Tree is the Physical Embodiment of Conrad Waddington's Epigenetic Landscape



Held Jr., L.I. (1992). *Models for Embryonic Periodicity*, Basel: Karger.

The Differentiation Code

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*



Stem Cells: An Hypothesis

- Stem cells are cells in an embryonic tissue that missed participating in either a contraction or an expansion wave
- A tissue synthesizer could be built that would take stem cells through a series of expansions and contractions, dictated by the differentiation code of the desired tissue, and turn them all into that tissue

Scales of Explanation

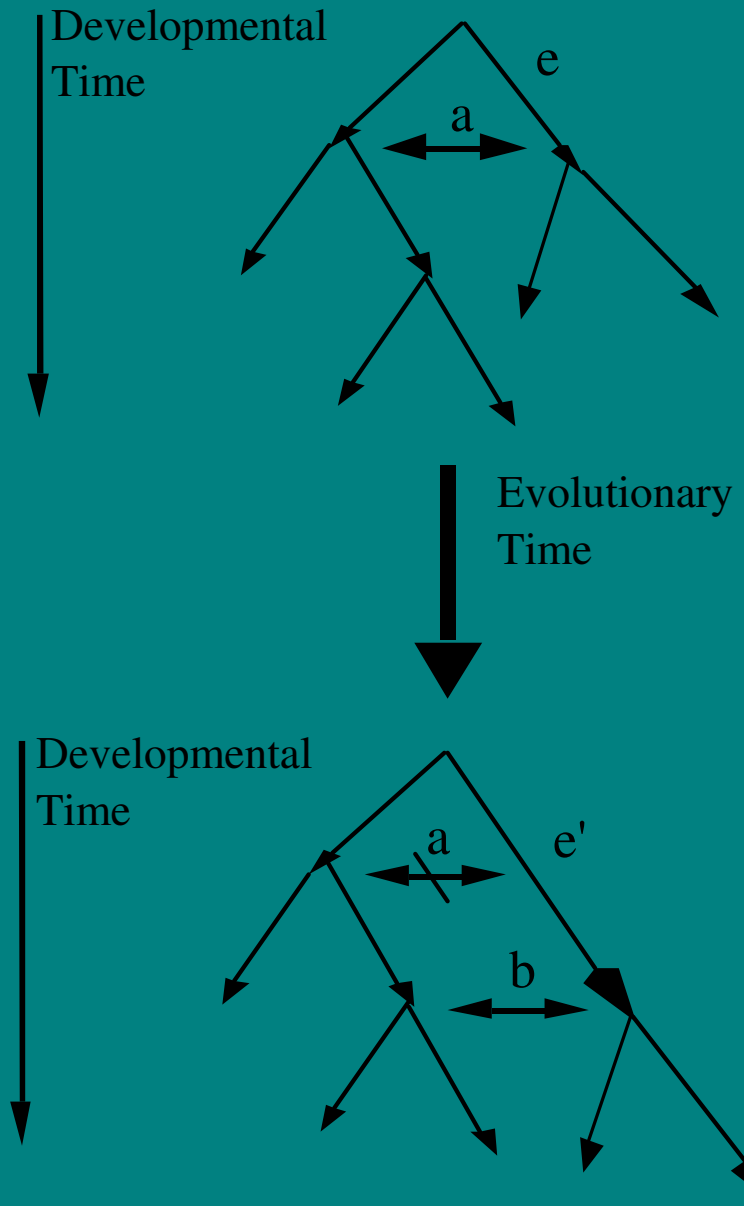
Structure	Order of magnitude in Angstroms
Hydrogen atom	1
Nucleotide	10
Nucleosome	100
Matrix Attachment Region (MAR)	1,000
Chromosome or domain	10,000
Whole genome	100,000

A New Model for Macroevolution

- Redefine *macroevolution* to be any favorable mutation that alters the topology of a species' differentiation tree
- Any mutation that leaves the topology unchanged is *microevolution*

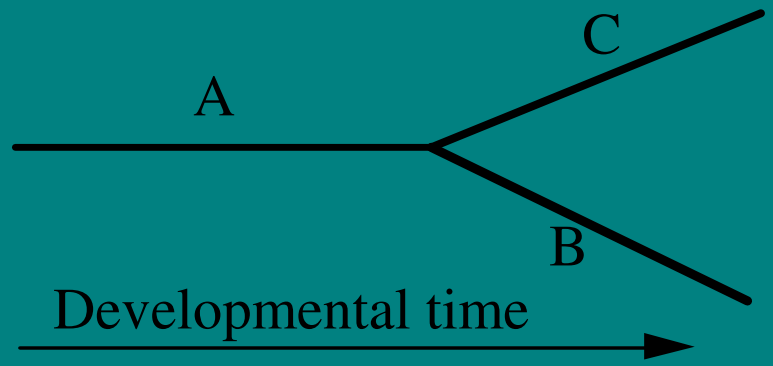
Heterochrony is Microevolution

- It involves simple stretching of the differentiation tree
- Inductive relationships between tissues are opportunistic and therefore not fundamental to evodevo

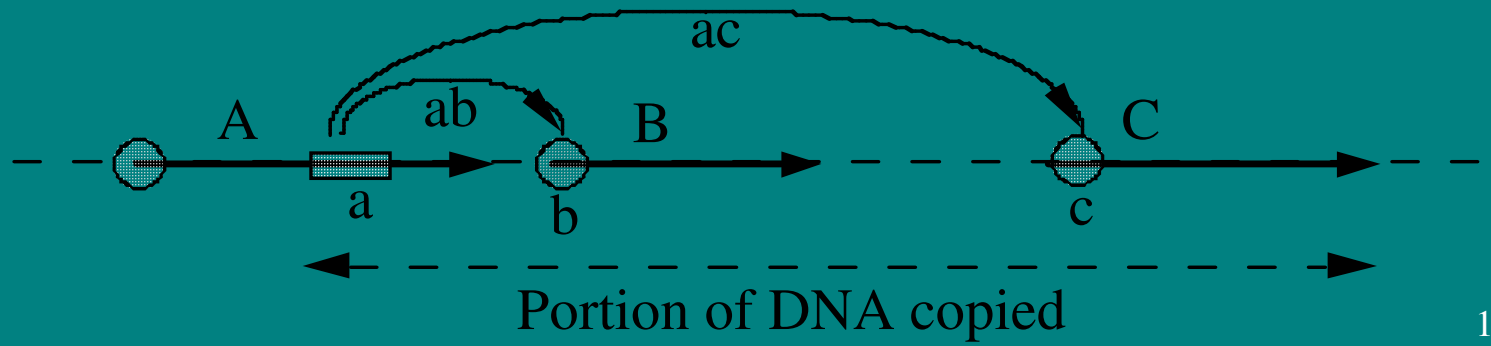


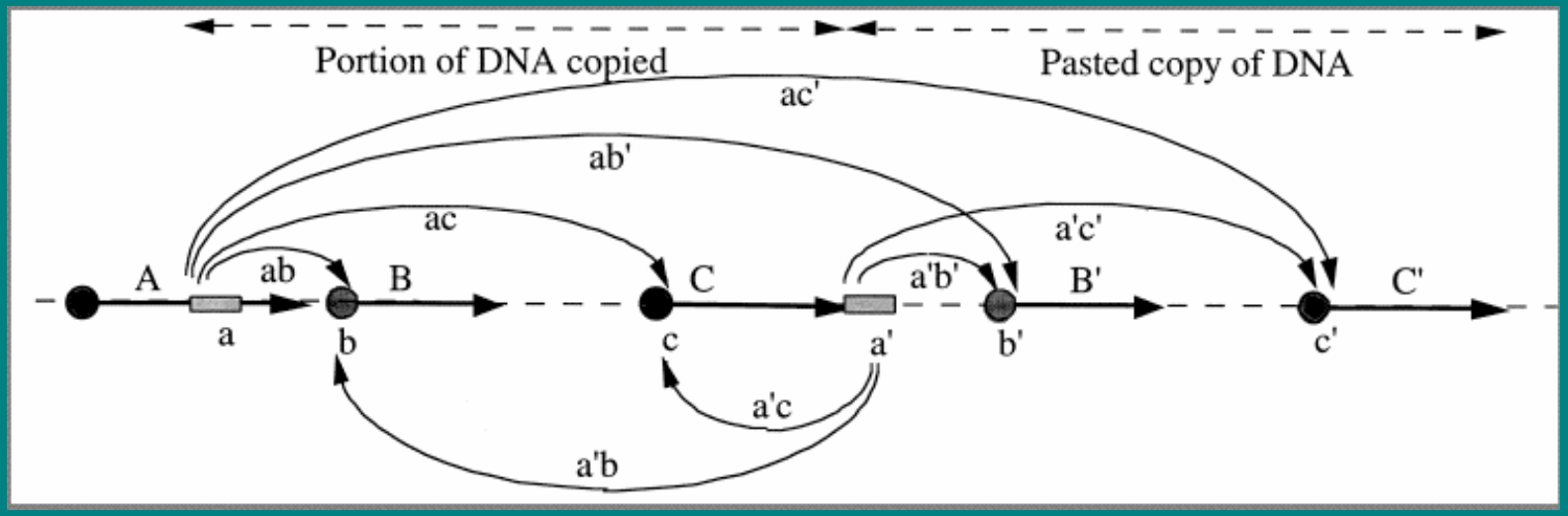
Macroevolution Duplicating a Branch of the Differentiation Tree could involve Cut, Copy & Paste at the DNA Level

Terminal branch of a differentiation tree

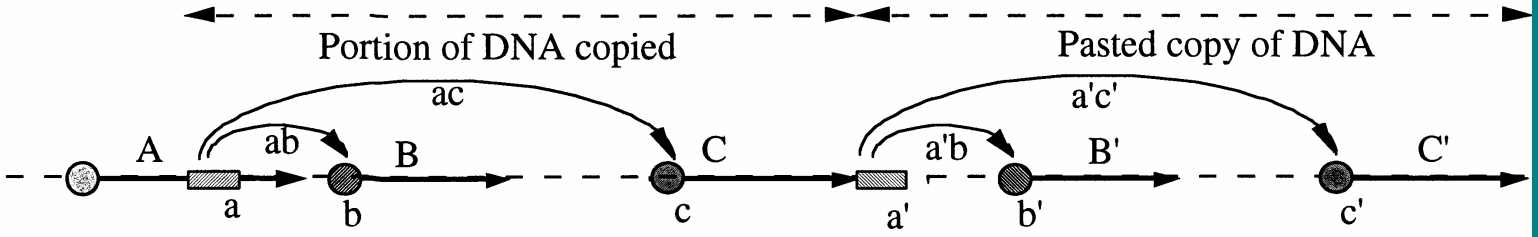


Its possible representation in DNA

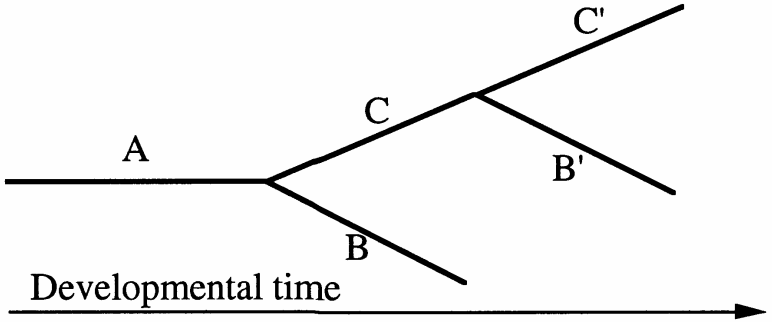




The evolved DNA



Its differentiation tree



Coevolution of the genes and DNA-binding proteins cleaning up the crosstalk

- An example of "post-adaptive adjustment":
- Seaborg, D.M. (1999). Evolutionary feedback a new mechanism for stasis and punctuated evolutionary change based on integration of the organism. *J Theor Biol* **198**(1), 1-26.

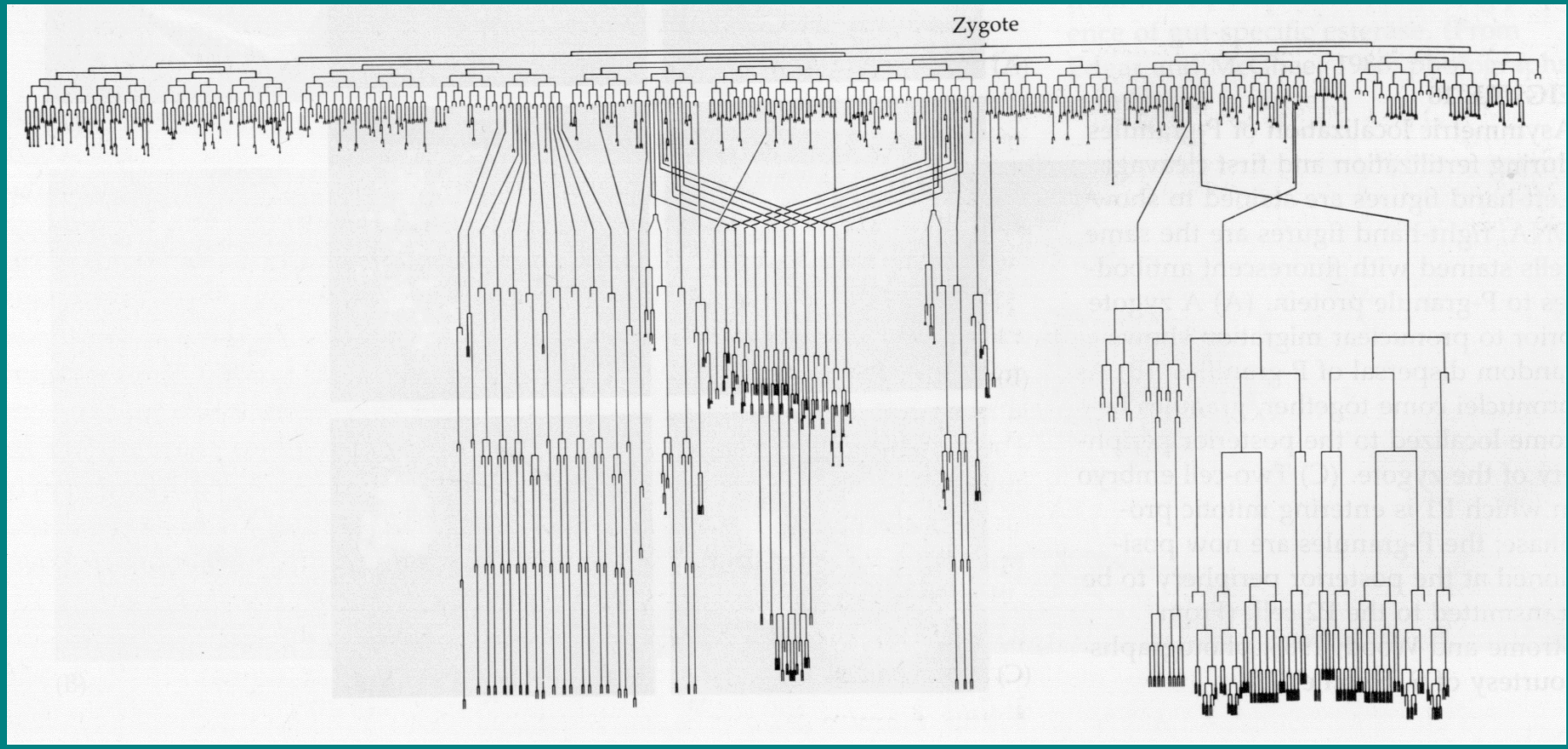
Properties of the Differentiation Tree

- The logic of the genome: at each branch point, an alternation of the physics and genetics of embryological development
- The physical basis for the epigenetic landscape
- The Bauplan for an organism
- The fundamental germ line replicator (Dawkins)
- The proper basis for phylogeny
- The basis for understanding the biogenetic law (ontogeny recapitulates phylogeny) (Haeckel)
- A basis for exploring gene expression along the differentiation tree
- The lineage tree of the tissues of an organism

Nematode Tree

Caenorhabditis elegans

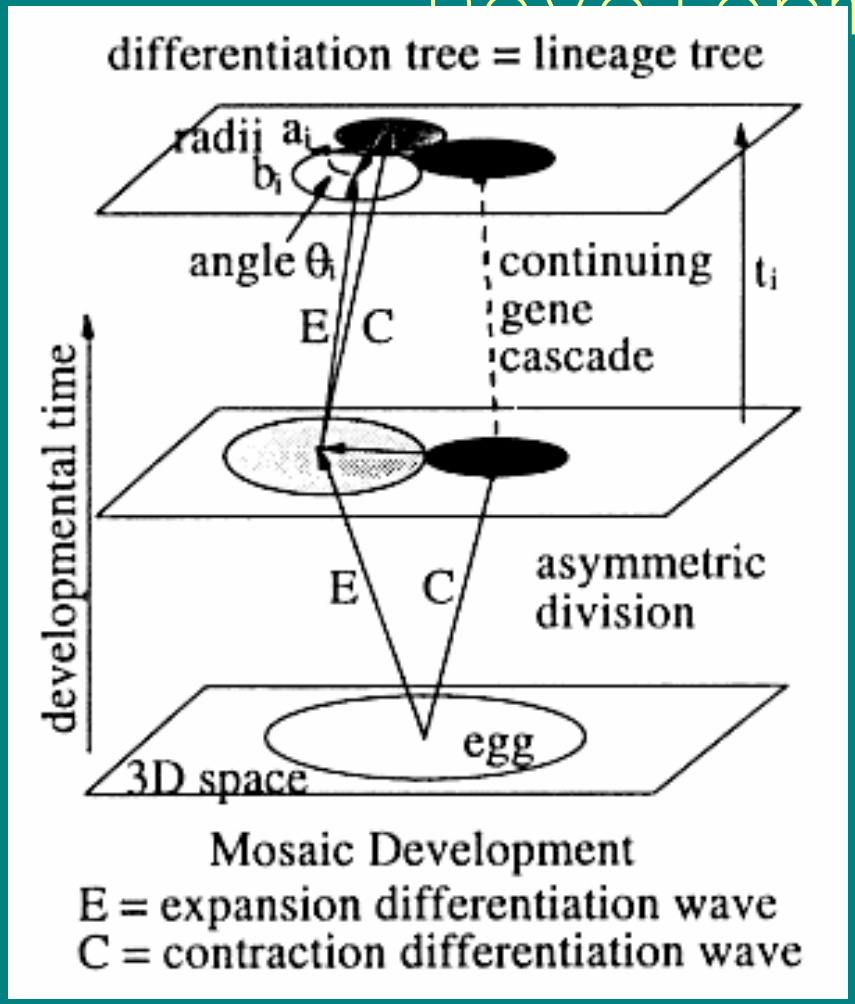
Each cell or pair is a tissue in a mosaic organism

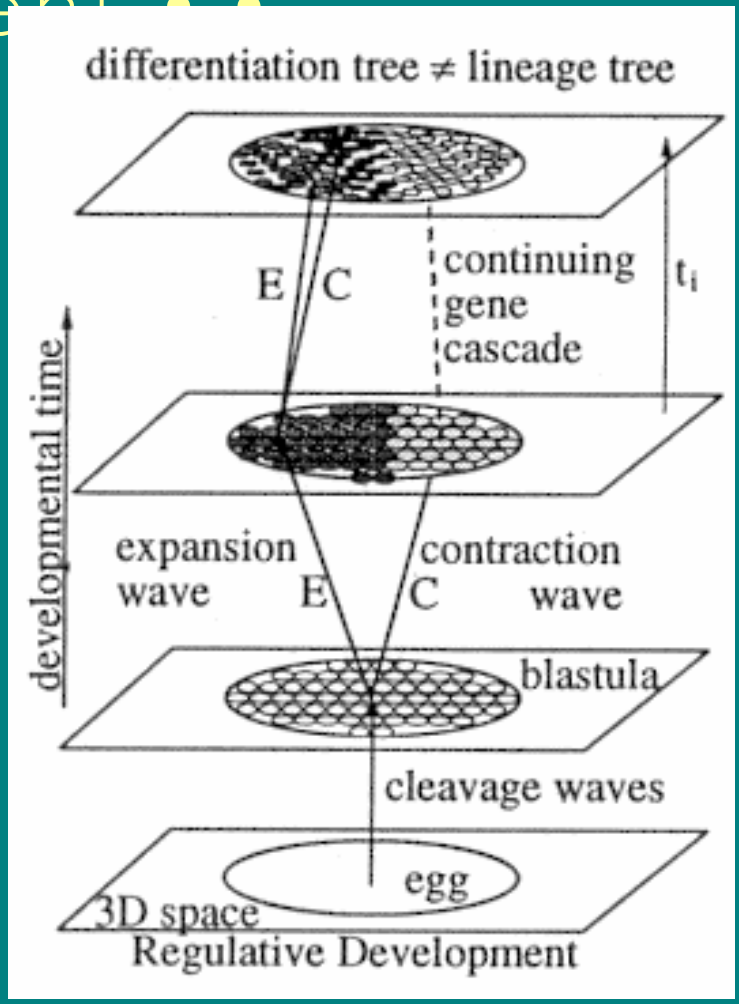


Gilbert, S.F. (1991a). *Developmental Biology*, 3rd ed., Sunderland, Massachusetts: Sinauer Associates.

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.

The Parallel between Mosaic and Regulative Development

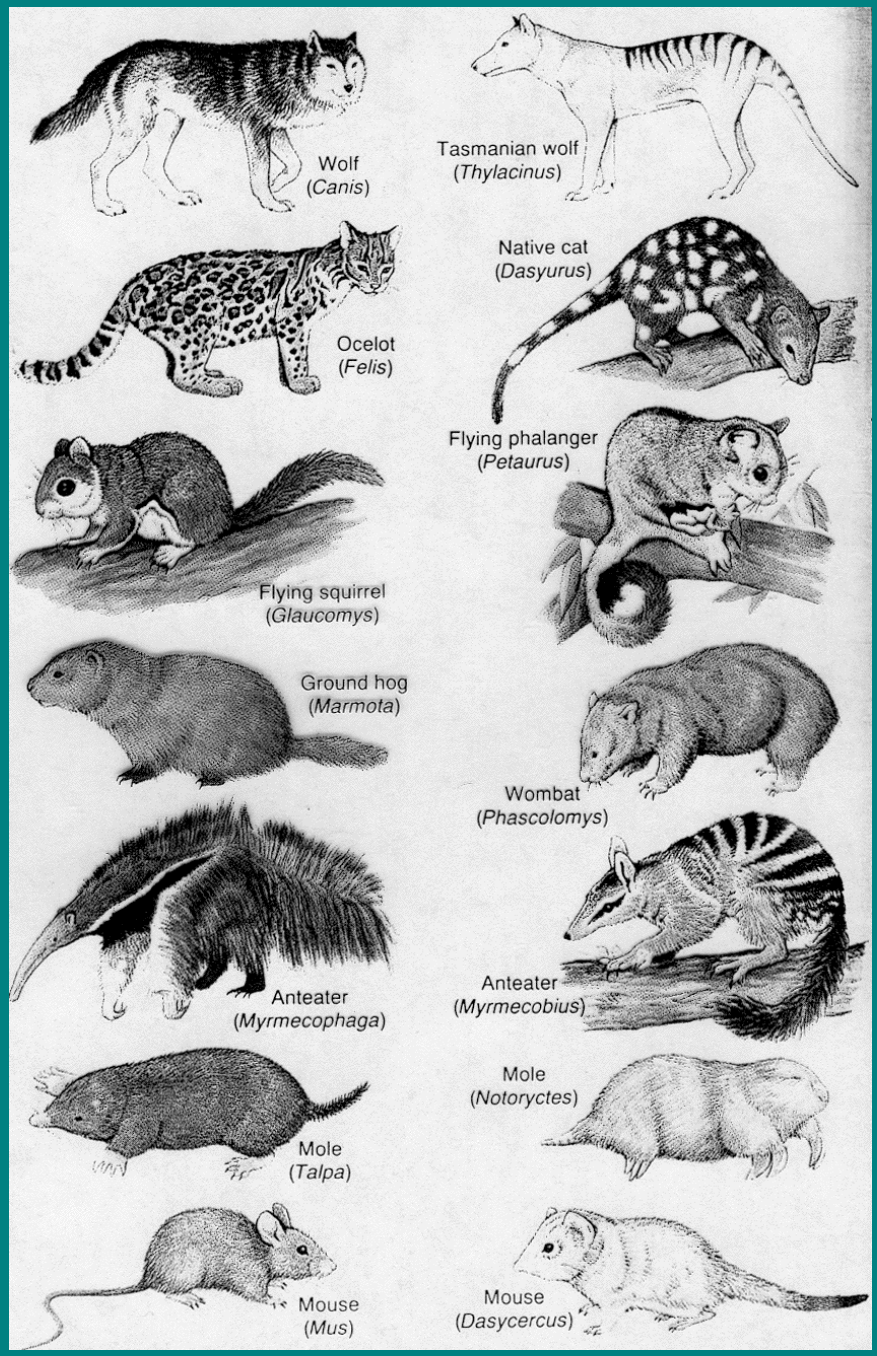




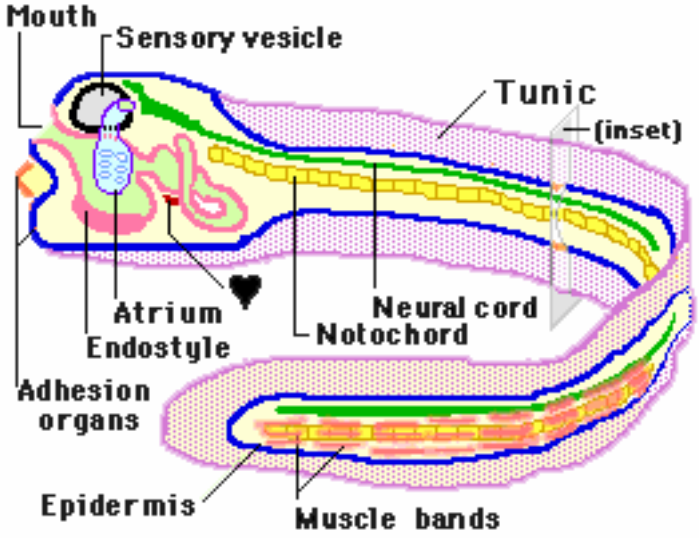
Some Higher Order Problems of Evolution

- Similarity of major radiations
- Metamorphosis as the concatenation of genomes
- Origin of multicellular organisms
- Explosive evolution of the brain
- Stasis
- Cope's Law and Bonner's Law
- The fractal nature of evolution
- Progressive evolution

Parallel Radiations: Placental and Marsupial Mammals

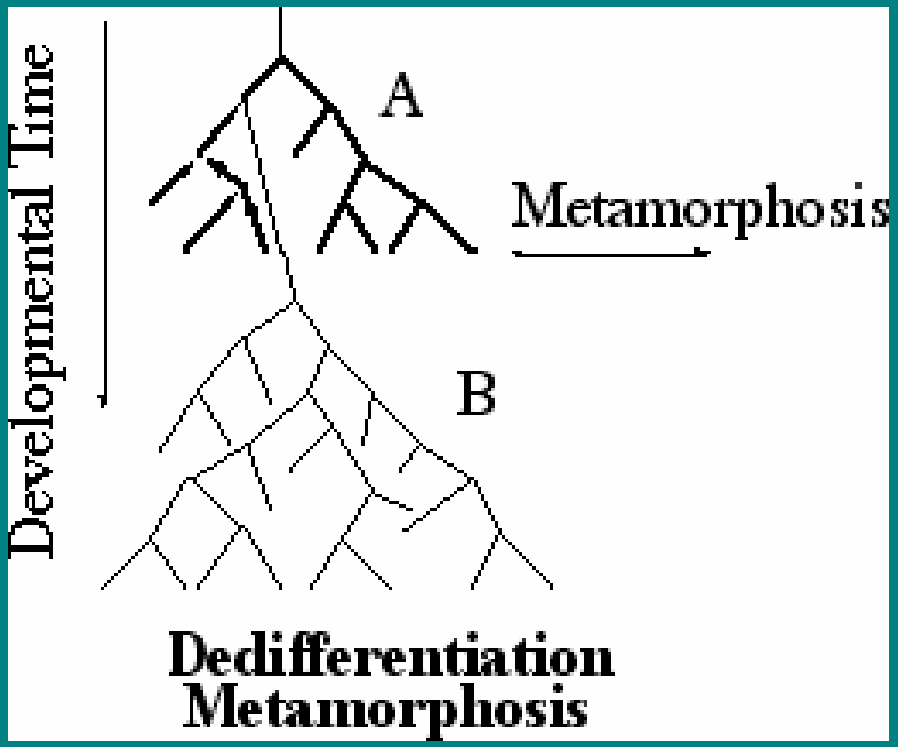


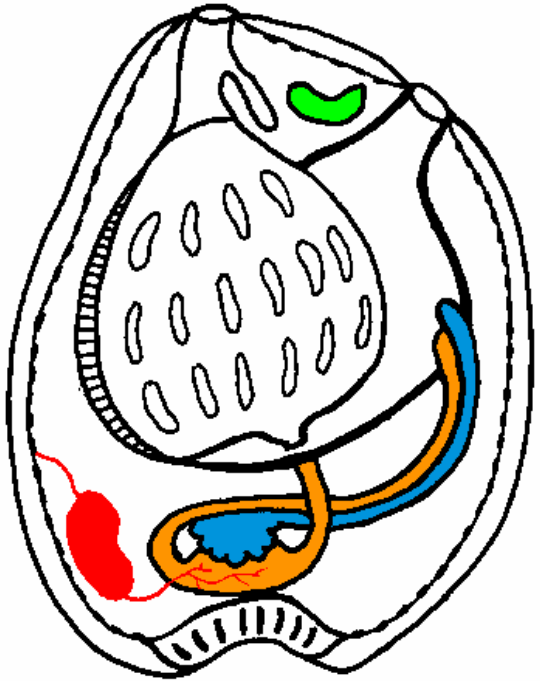
Dedifferentiation Metamorphosis



- Ascidians may be a concatenation of two genomes, i.e., of two differentiation trees

http://gwis2.circ.gwu.edu/~atkins/newwebpages/Embryo/Compembryo.html

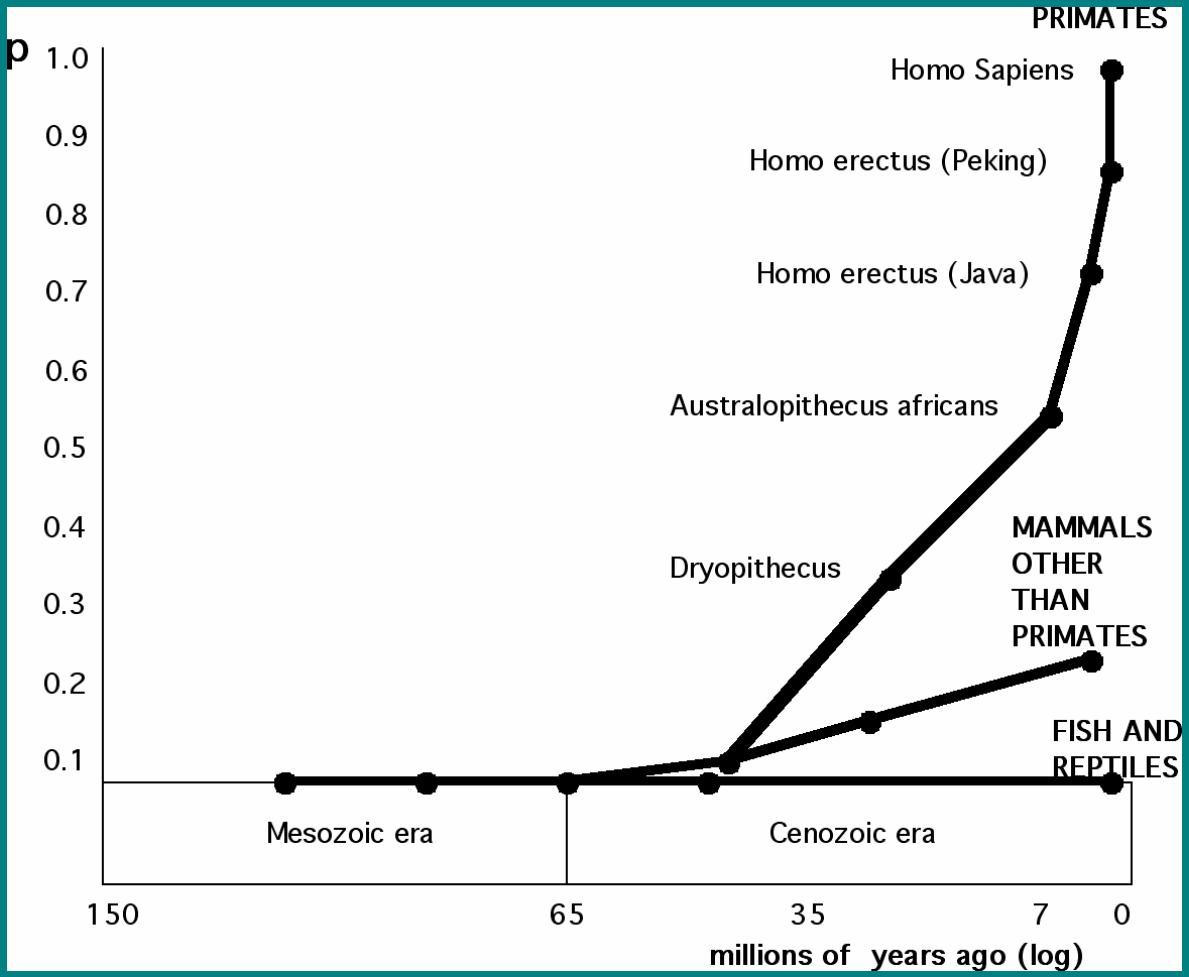




http://biodidac.bio.uottawa.ca/Thumbnails/UROC003C-GIF.htm

The Brain Differentiation Subtree?

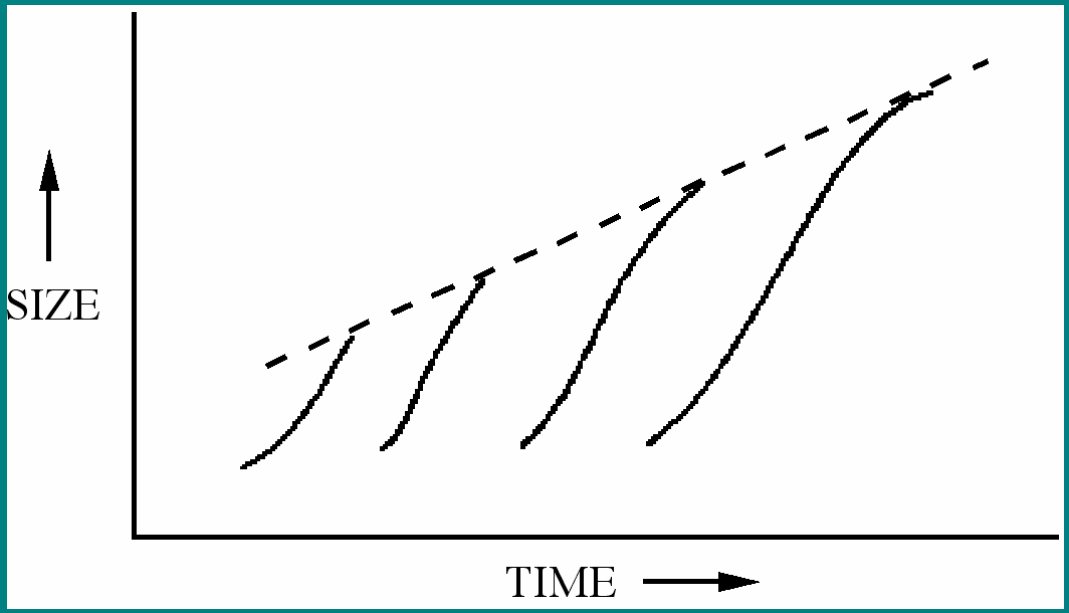
- Number of cell types in the human brain may exceed the # in the body



Fujita, S. (1990). Evolution and development of the central nervous system. *Tanpakushitsu Kakusan Koso* **35**(4 Suppl), 301-316.

Cope's Law and Bonner's Law

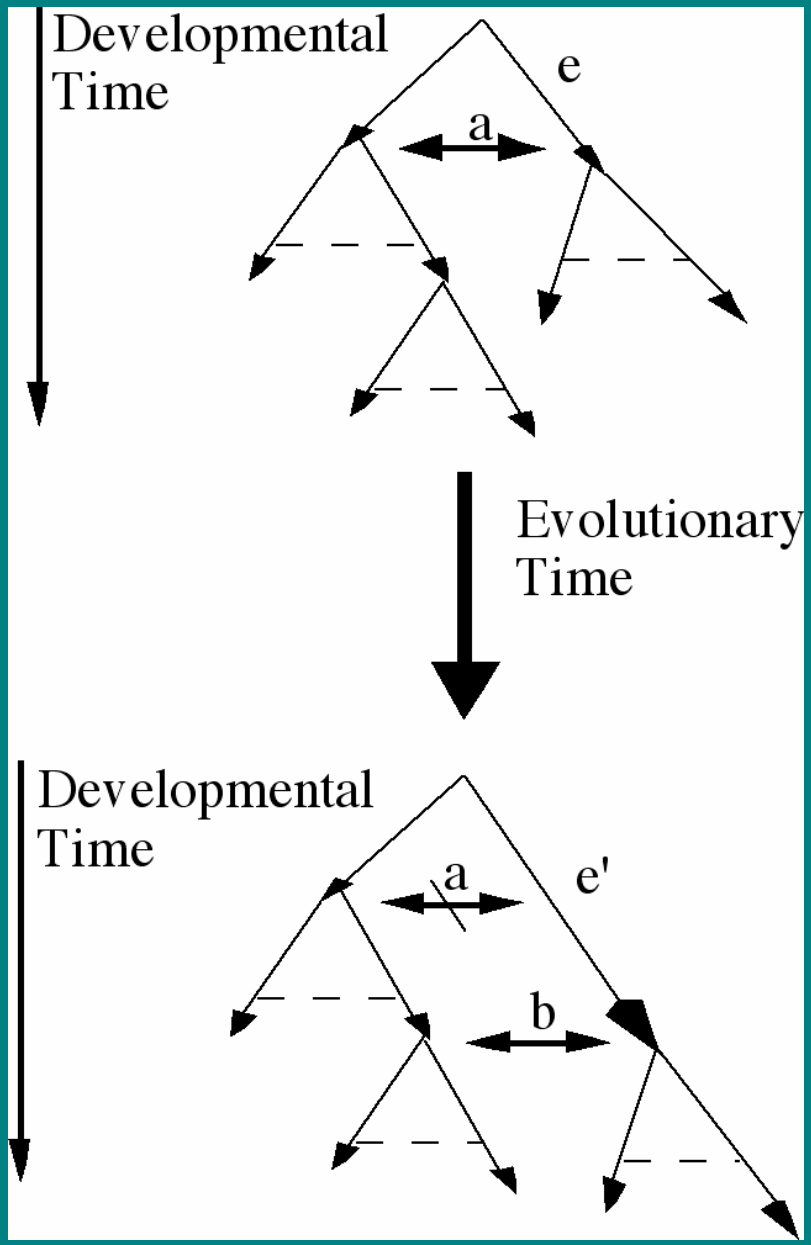
- In a Cope's Law sequence the differentiation tree grows
- When failure of the line occurs, both size of the organisms and the differentiation tree are reduced
- The pruned differentiation tree is more efficient
- Bonner's Law: the process repeats, with greater reach in size



Bonner, J.T. (1988). *The Evolution of Complexity by Means of Natural Selection*, Princeton: Princeton University Press.

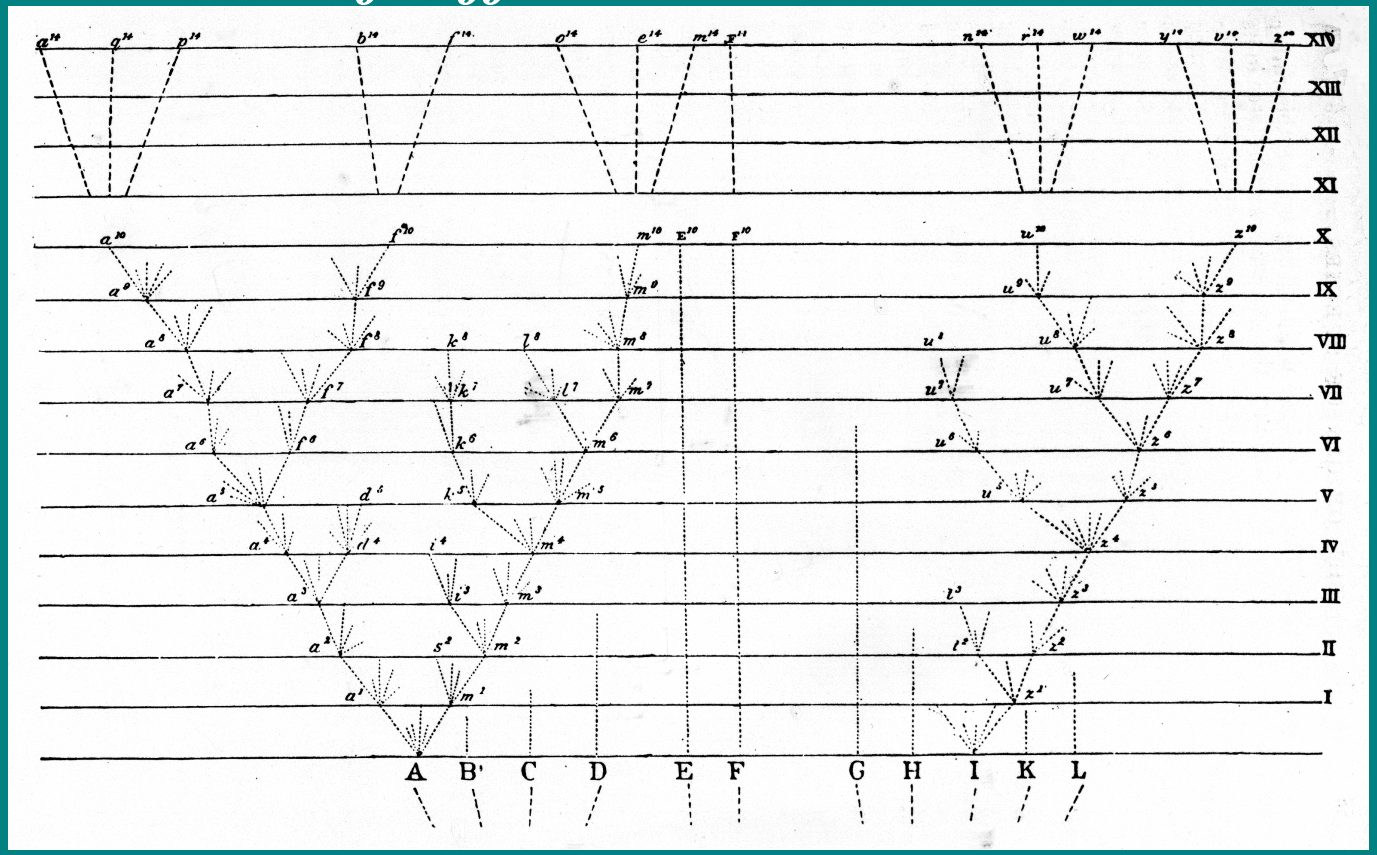
Stasis is due to Differentiation Webs?

- When opportunistic inductions become obligate, a portion of the differentiation tree becomes a web



Darwin's Fractal Phylogenetic Tree

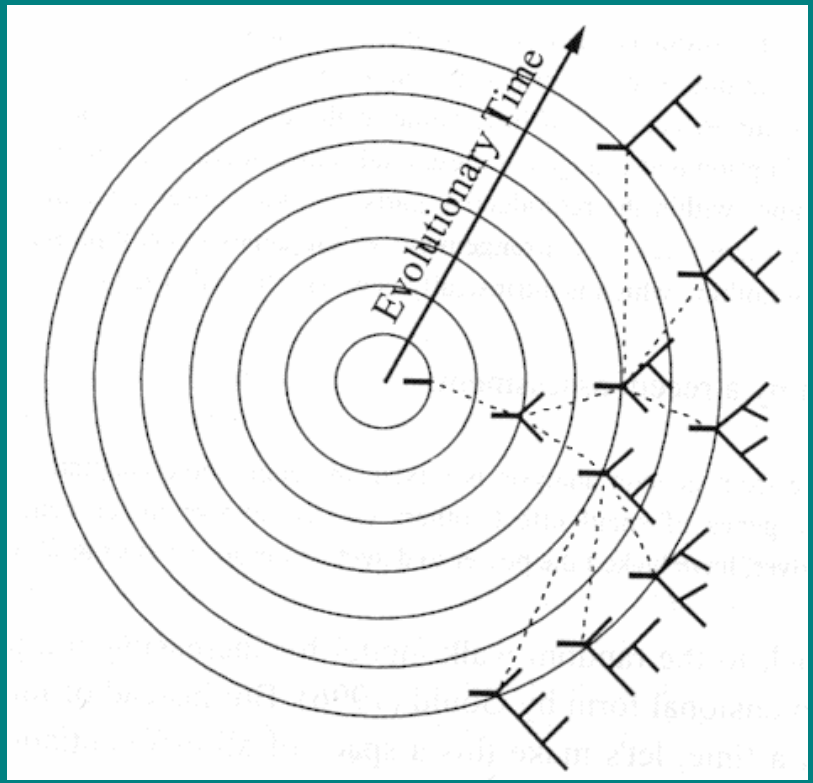
- He said this diagram is applicable to both genera and families, i.e., he said it was self-similar, and thus what we now call a fractal
- ***It is a tree of differentiation trees***



Progressive Evolution

- The mystery vanishes if the probability of growth of the differentiation tree exceeds that of pruning

Like frost on a window pane, and as inevitable



Deep Origins: We Are Bacteria

Living organisms are generally placed into two major categories; prokaryotes and eukaryotes. The prokaryotes include all bacteria and the blue-green algae, wrested by microbiologists from the botanists by changing their name to cyanobacteria. The distinctions between prokaryoteses and eukaryotes that are usually touted include:

Bacteria have one cell membrane, while most cells in the rest of us include organelles surrounded by membranes (mitochondria, chloroplasts, nuclei, etc.)

Most bacterial genomes consist of one circular double strand of DNA, while eukaryotic DNA is organized into chromosomes.

The DNA of eukaryotes is bound to histones, whereas bacteria lack histones.

Transcription (making an RNA copy of DNA) and translation (making a protein from the RNA) can occur simultaneously with the same strand of RNA being synthsized at one end while it is being translated at the other end, whereas eukaryotes separate these steps.

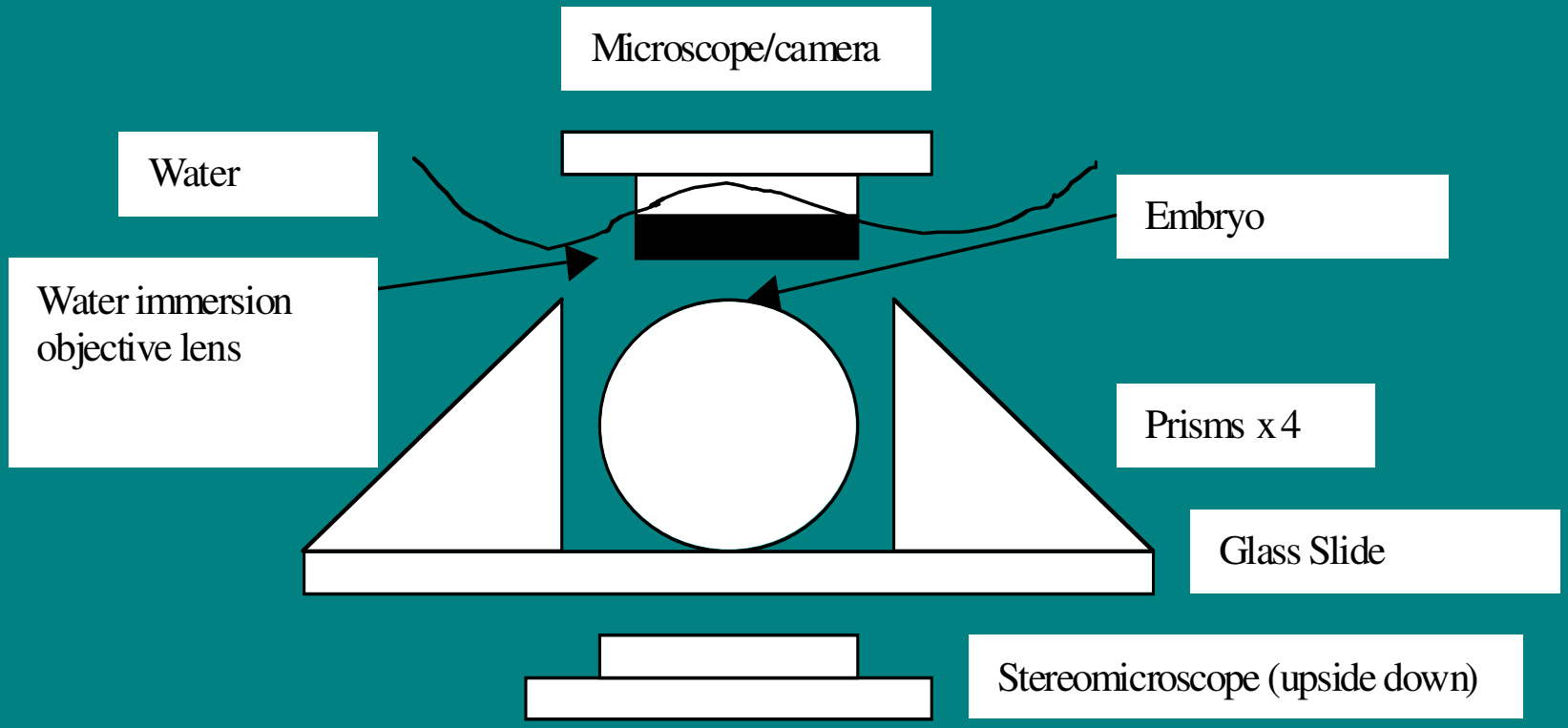
Prokaryotes lack a cytoskeleton, but eukaryotes have one (microfilaments, intermediate filaments, and microtubules).

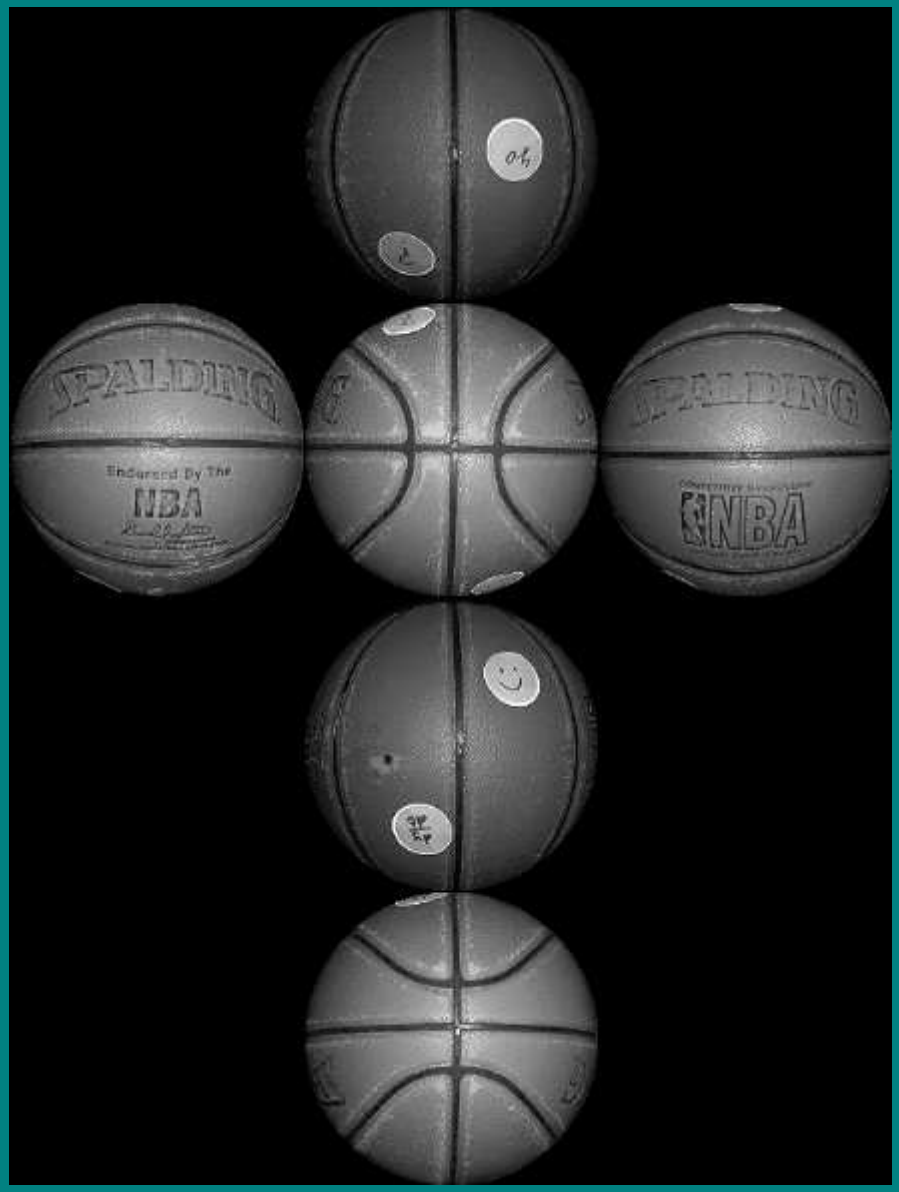
Eukaryotes include multicellular organisms whose cells take on different tasks (differentiation), whereas bacteria usually don't.

These distinctions would appear fine, except that they are wrong.Let's start with taxonomy, which we try to base on the lineage of organisms, i.e., on their evolutionary history. In general, if two species have a common ancestor, then we construct an umbrella group to include both. It is generally agreed that we only have evidence of bacteria in the fossil record from 3.465×10^9 years ago to 2.1×10^9 years ago. So by the logic of taxonomy, we are bacteria, since our common ancestor with today's bacteria were bacteria.

Deep Origins: We Are *Paramecia*

Challenges for 4D Microscopy





Unfolded box:

Back

Left Top Right

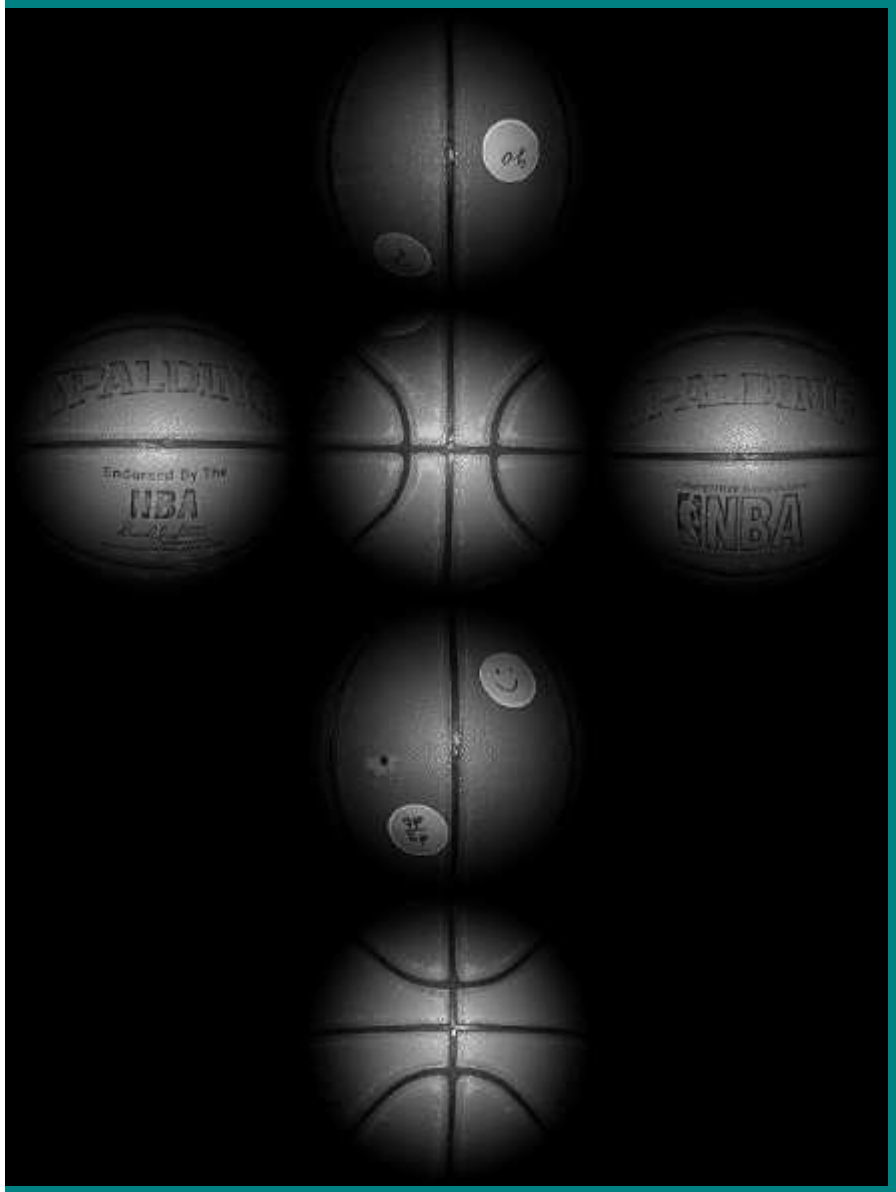
Front

Bottom

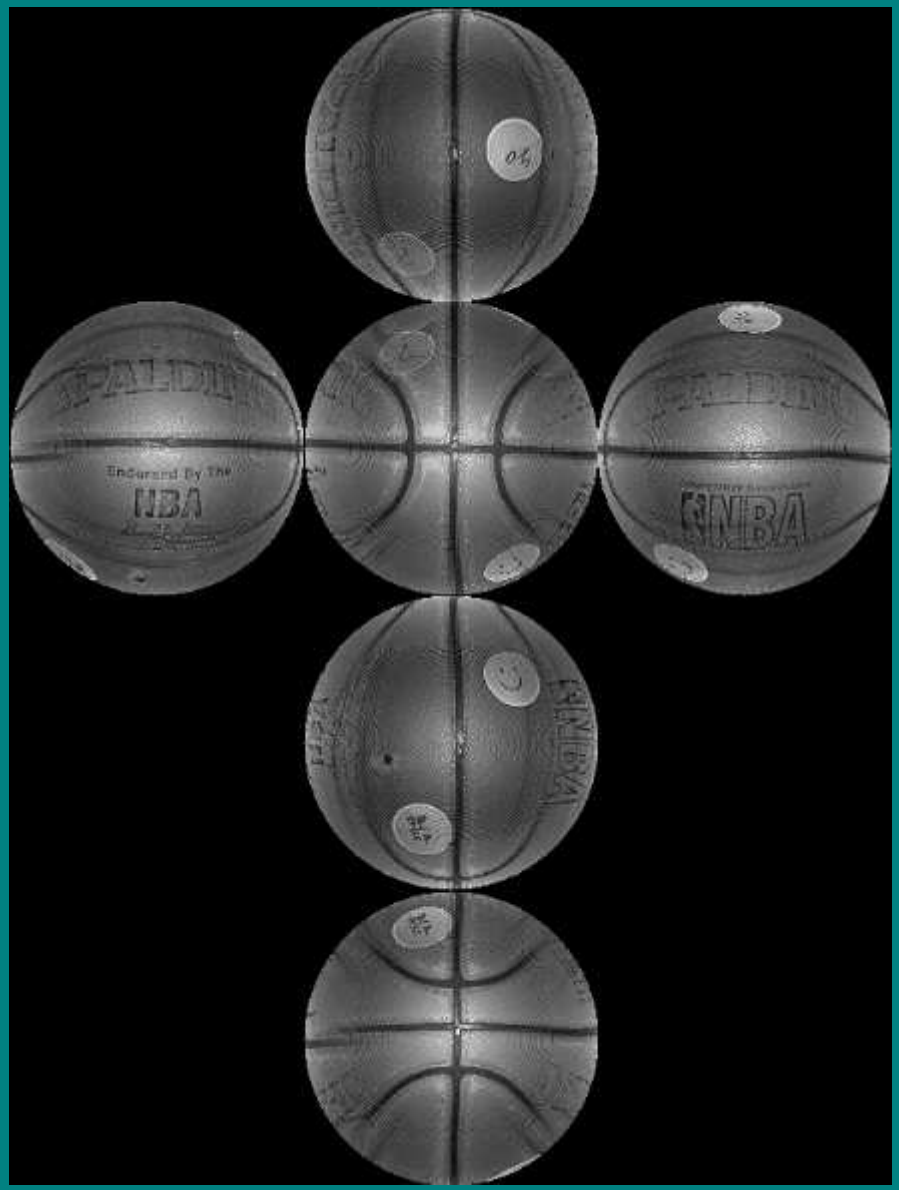
6 perpendicular views of the same basketball. From direction cosines of radii of the sphere, the weight of each plane is determined. The greyscale value of the voxel can be calculated as follows:

$$V(x, y, z) = \sum_{m} g_m \cos^2 \alpha_m$$

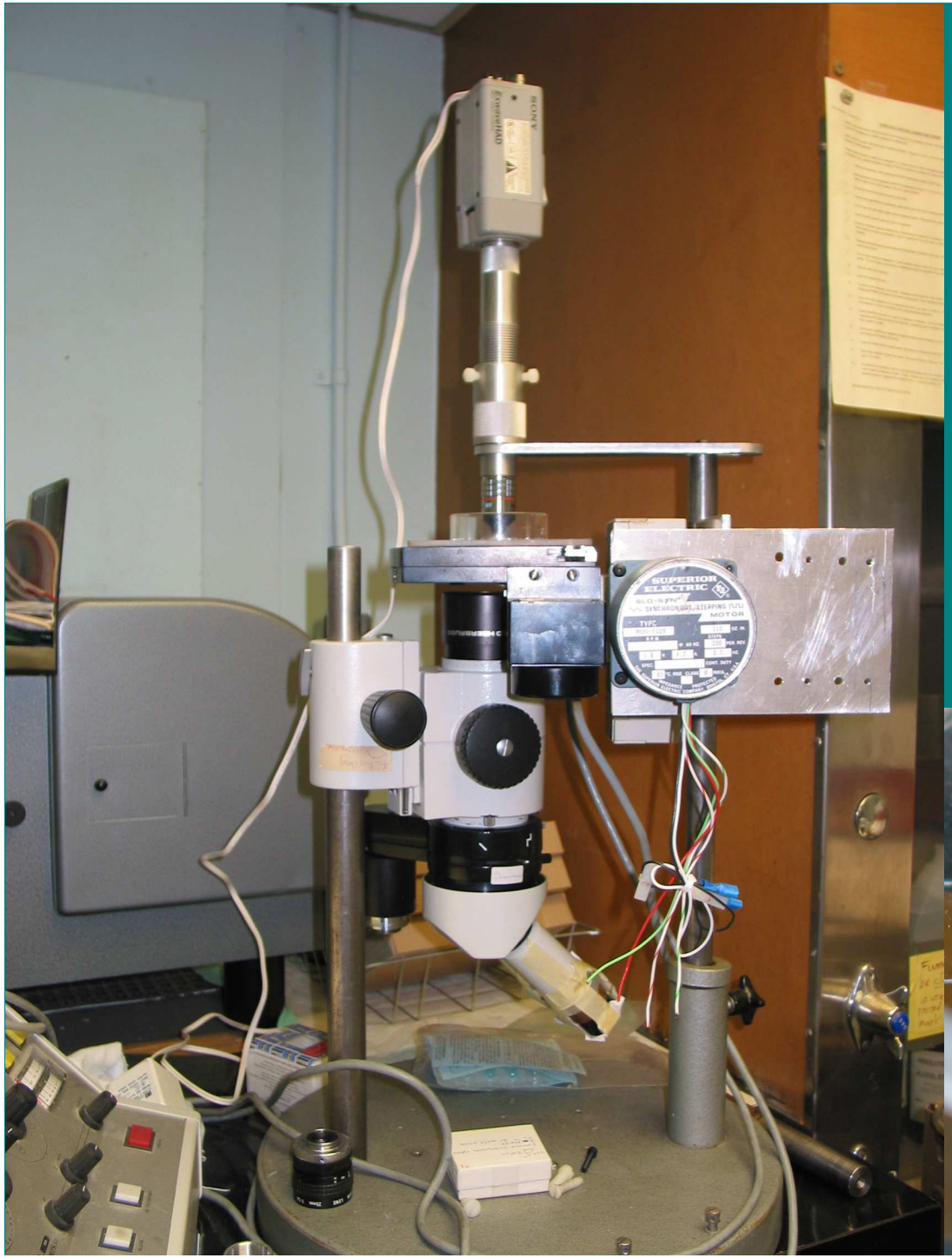
Tsang, C. & G. Kler (2002). *3D Imaging of Spherical Objects* [4th Year Undergraduate Thesis], Winnipeg Department of Electrical & Computer Engineering, University of Manitoba.



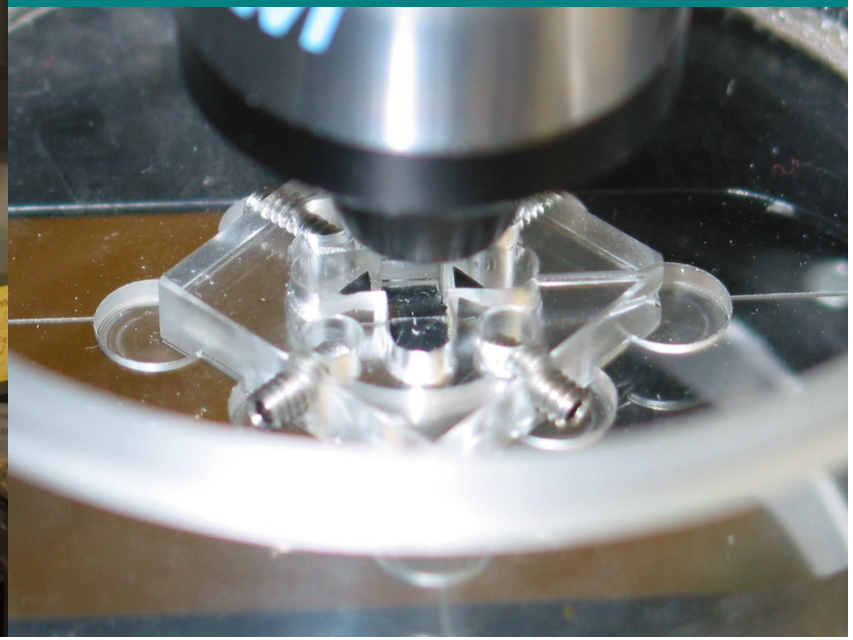
- Each projected view becomes less and less significant as it moves away from the center. The fading of pixels in one view corresponds to unfaded pixels in corresponding views. This weighted greyscale scheme allows for the view with the most direct view of the pixel (voxel) to have the most significance on its greyscale value.



- The 6 weighted images projected back from the 3D spherical image

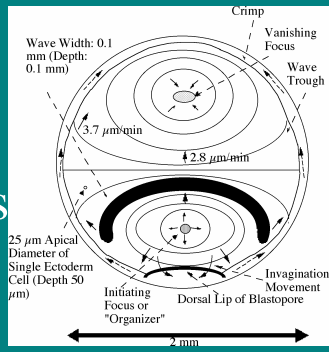


4 Prism Embryo Microscope (under construction by Susan Crawford-Young)



Why make a 4 prism embryo microscope?

- To track every cell on the surface, and check the dispersion of daughter cells (change in neighbors) during embryogenesis
- To follow up observations on waves of cytokinesis and their breakdown of synchrony by gastrulation
- To determine the degree of correspondence between amphibian differentiation waves and cytokinesis, which is apparently 100% in *Drosophila*
- To determine if there is a correlation between local wave speed and local strain, measured via change in great circle distance between pairs of cells. This might explain the reversal in curvature as the ectoderm contraction wave propagates.
- To see if there is a correlation between differentiation waves and failure of neural tube closure (NTDs = neural tube defects) at extremes of the temperature range for normal development (8-24 deg C)
- To formulate a global model for the physics of the embryo, in particular to understand the relay nature of wave-wave interaction that may permit normal development over a wide temperature range, and its breakdown at the extremes



Looking Inside the Embryo

- MRI microscopy
- Computed Tomography (CT)
- Acoustic microscopy
- Electrical Impedance Tomography (EIT) microscope
- Optical microscopy (CT of refractive index via wavefront reconstruction)
- Confocal microscopy (limited by yolk?)

Why look inside?

- To track endodermal cells during organogenesis, such as heart formation
- To measure volumes vs time of fluid chambers (blastocoele and archenteron), which would give hints about the role of pressure in embryogenesis
- To observe whether contacts of tissues correlate with launching of differentiation waves
- To see if internal organogenesis utilizes differentiation waves, i.e., to determine if differentiation waves represent a universal mechanism for cell differentiation

Back to Cortical Rotation

- Microtubules consist of α-tubulin and β-tubulin dimers stacked in a helical array that has a polarity and is chiral
- Therefore a microtubule is not its own mirror image
- Let us assume that the apical orientation of the microtubules oriented during cortical rotation is retained during cytokinesis
- Given two cells that are in mirror image positions during early gastrulation, they are therefore *not* mirror images of one

Mechanical Model for Left/Right Asymmetry

- Involution movement during early gastrulation generates a nonuniform strain state in the ectoderm
- This produces a torque on each cell
- The torque adds to the supercoiling on one side and subtracts on the other
- A polymerizing microtubule therefore has a different degree of supercoiling from a microtubule in its mirror image position
- Dynein binds to one supercoiled state, but not the other
- Free/bound dynein -> different subsets of gene expression left/right