

The Physics of Cell Fate Branching:

Why a "Swedish Flag Model" is better than the French Flag:

by Albert Harris, UNC Chapel Hill

(With apologies to Lewis Wolpert, and to the Karolinska Institute)

"Things should be made as simple as possible, but not more so." Albert Einstein

In my opinion, the biggest obstacle to understanding embryonic development is the attractiveness of "How else could it work?"; and "What else could explain it?" as arguments in favor of various plausible theories or concepts. Two centuries ago, it was "Preformationism"; then, "Recapitulationism" ruled in the later 1800s, and early 1900s. Currently, "Positional Information" is the concept about which very many biologists say: "How else could it work". It must be true if we can't think of other possibilities.

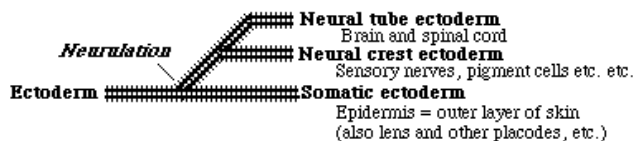
I will now try to clarify "How else?" And I will attempt this from the perspective of Wolpert's "French Flag Model".

Embryology works by mitotic division of the fertilized egg into hundreds and thousands of equivalent cells. The differentiation of these cells begins by a series of branching subdivisions combined with geometric rearrangements. For example, those in one subdivision become nerves; those in an adjacent branch of that subdivision become skin. The central goals of embryology are to describe this subdivision process, and to explain how it works - especially the interdependence of cell differentiation and cell rearrangements.

To represent this branching of embryonic fates, C. H. **Waddington** devised two analogies. One was his "Epigenetic Landscape", which was analyzed in a previous lecture. The other was branching railway tracks. Using the railway switch analogy, I have diagrammed the actual sequences of subdivision undergone by real embryos. By historical tradition, the first three branches are called the "**Primary Germ Layers**"; they are named the **ectoderm**, **mesoderm** and **endoderm**.

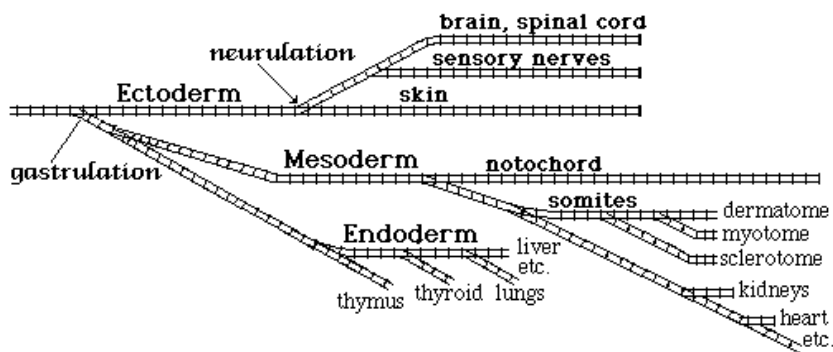


Having been subdivided from the other germ layers, the ectoderm is further split into the following three parts: #1) The **Somatic Ectoderm** (future outer layer of skin); #2) The **Neural Crest Ectoderm** (sensory nerves, pigment cells, etc.); and #3) The **Neural Tube Ectoderm** (the cells of which form brain and spinal cord).



Please note each of the following:

a) These subdivisions progressively narrow the range of cell types into which each part of the embryo can differentiate.



b) Each subdivision is accompanied by a geometric rearrangement of cells, usually folding of cell sheets. For example, neurulation subdivides the ectoderm into the three parts listed above (somatic ectoderm, neural tube, etc.).



c) All too few embryologists are curious why embryonic cells need geometric rearrangements in order to accomplish differentiation into future brain cells, future skin cells, etc. We just get used to it.

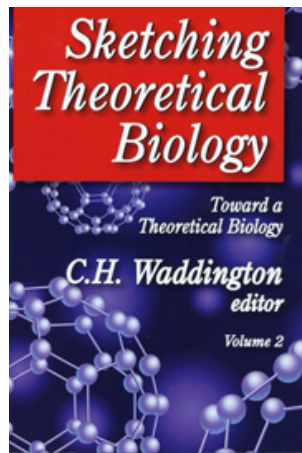
d) **Richard Gordon** invented the "**Cell State Splitter**" to explain causal links between subdivision of ectoderm and differences of physical contraction.

e) Because it was so much ahead of its time, mainline Developmental Biology textbooks and university courses neglect the "Cell State Splitter", and also neglect the parallel research of **Belousov** and **Belintzev** in Moscow.

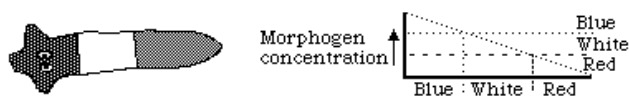
f) Instead, their rightful places have been usurped by Lewis **Wolpert**'s ubiquitous "**French Flag**"

Model". Wolpert's original invention of this analogy was for his presentation at a symposium organized by **C. H. Waddington**, in the late 1960s. It was much like the lecture series in which we are now participating. Waddington's symposium gave rise to a series of published books.

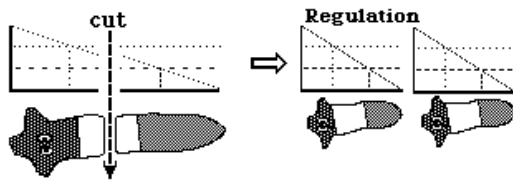
It was held at a palace on Lake Como in northern Italy, loaned by the Rockefellers. In fact, except for being somewhat up-market, the Rockefeller's Serbelloni palace is strikingly similar to the virtual world in which the present symposium is being held.



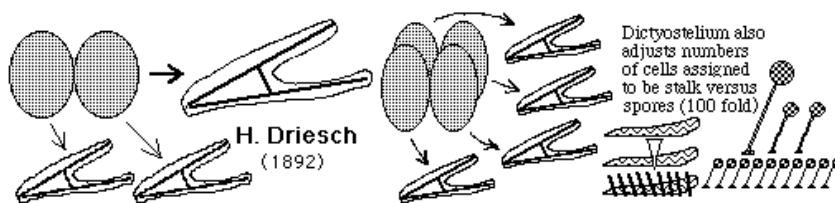
g) To explain how embryos generate consistent geometric patterns, independent of the amount of undifferentiated tissue to be subdivided, Wolpert visualized an imaginary flatworm (living along the coast of France), which has only three differentiated cell types. The anterior third of each worm consists of blue cells. The middle third consists of white cells. The rearmost third consists of red cells. Obviously, this is the geometry of the French flag tricolor. (I realize that the Russian flag has this same geometry, at a 90 degree angle.)

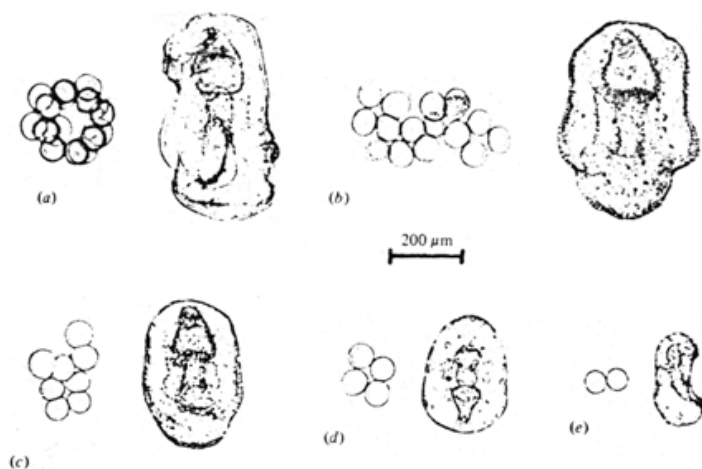


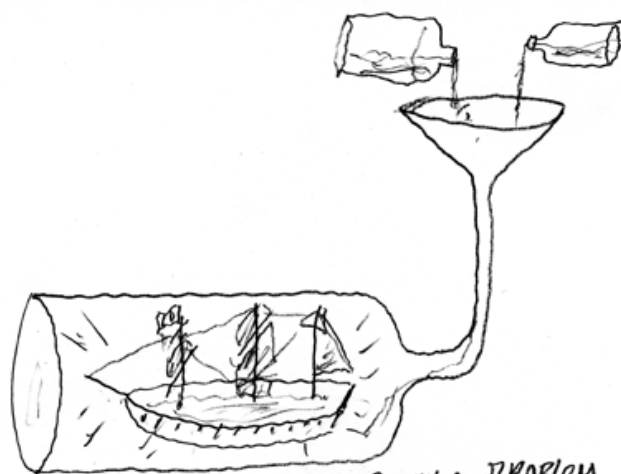
h) Here comes the key point. Wolpert figured out how to create (and to regenerate) his worm's colorful anatomy. His big idea was to make cell differentiation be controlled by local chemical concentrations in a diffusion gradient. Concentrations above a certain threshold would cause cells to differentiate into red cells; concentrations below another threshold would cause differentiation into blue cells, and those of the white cell type would be caused to differentiate from cells that detected intermediate concentrations of the gradient substance. Wolpert borrowed Alan Turing's word "**morphogen**", as his name for this chemical. (Turing had invented the word to mean a reactant chemical in a pattern generating instability.) Gastrulation and all other cell rearrangements are completely ignored by this French flag model.



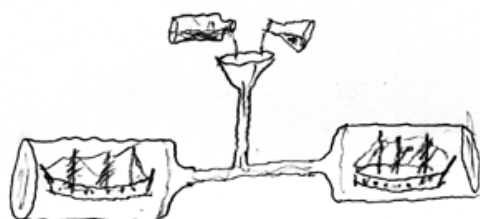
i) Here comes an even bigger point. Wolpert's wanted to explain a transcendently important phenomenon called "**Embryonic Regulation**". This phenomenon was discovered by **Hans Driesch**, in the 1890s. To his irreparable astonishment, Driesch discovered that normally-proportioned, half-size sea urchin larvae develop from embryonic cells separated at the 2-cell stage. Furthermore, he photographed quarter-sized urchin larvae that developed from cells separated at the 4-cell stage, and even eighth-sized larvae that developed when the first 8 cells were separated. Double-sized embryos resulted from fusion of two one-cell stage embryos. In other words, they "regulate" over a 16-fold size range.







SHIP IN THE BOTTLE PROBLEM



DRIESCH VERSION

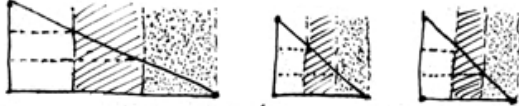
(SMALLER BOTTLES, LESS CHEMICALS,
SMALLER SHIPS FORM)



I note parenthetically, that these results are extremely repeatable. Jon Allen earned his PhD in my department by creating thousands of half-sized larvae, and using them in ecological experiments to test survival rates in the wild by different-sized larva of the same species. He also produced extremely tiny embryos from broken-off tips of arms of larvae. "Embryonic regulation" has remained the deepest conceptual riddle. It has been something of a "Gordian Knot" standing in the path of progress by developmental biologists for over a century.

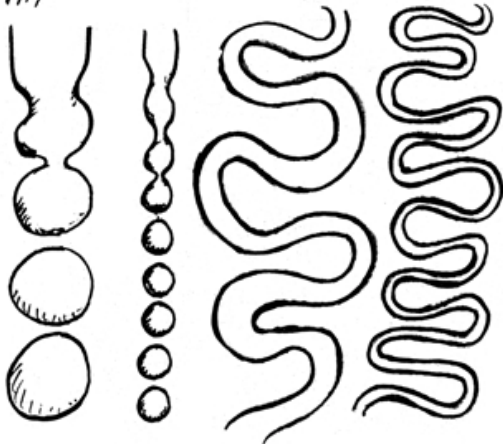
"Size Invariance" (L. Wolpert)

PROPORTIONAL ADJUSTMENT OF DIMENSIONS



AS EXPLAINED IN TERMS OF "POSITIONAL INFORMATION"

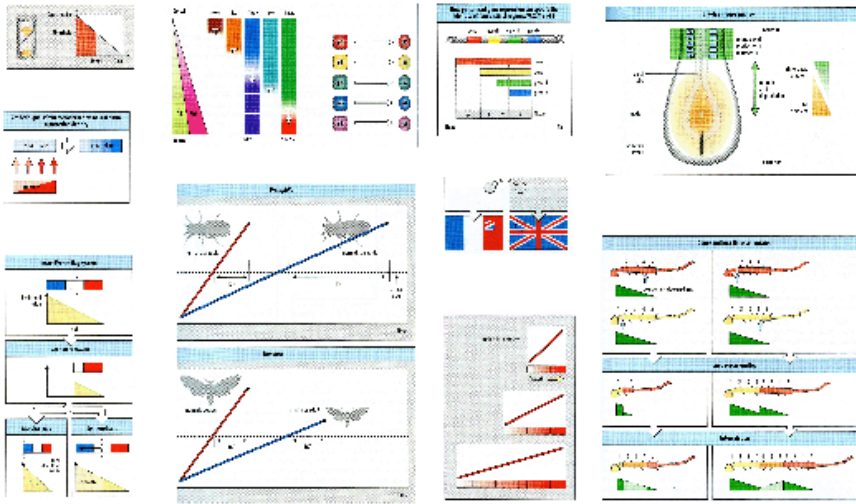
PHYSICAL or MECHANICAL ANALOGIES



WATER DROPS

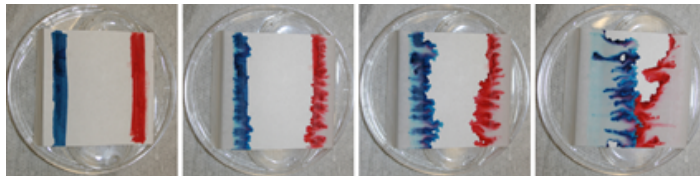
River Meanders

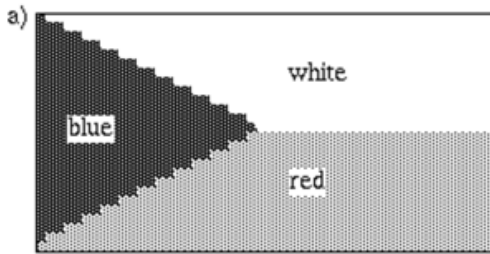
j) Wolpert uses his French flag concept to explain any number of embryological phenomena. Please consider the following replicas of diagrams in Wolpert's textbook, that I use in my current Embryology course (Biology 441, at UNC). This course has 110 students, and I hope the hundred book royalties will compensate him for my criticisms.



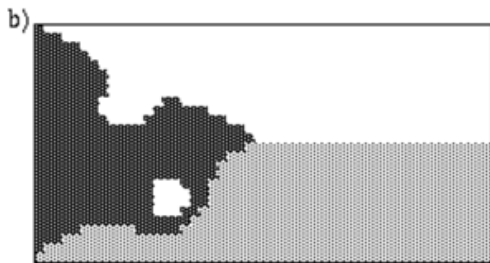
In that book, the word "principle" often refers to French flags. Wolpert's explanation has somehow become the dominant paradigm, accepted by 95% of developmental biologists. That's how nearly everybody explains pattern formation, and embryonic regulation (which means Driesch's half-sized larvae, and all that). I think this is largely for lack of showy enough opposition, rather than any originality or strength to his argument. Most people can't imagine any alternative. Their inability persuades them to accept whatever they are given.

To prove to ourselves the near-perfection of diffusion gradients for producing sharp boundaries, my wife and I have experimented with what happens when red and blue dyes diffuse inward from precisely linear sources at each end of a perfectly homogenous paper rectangle. We are forced to admit that tiny irregularities are detectable along the advancing boundaries (if you look close).





This is an approximation of the flag of Czechoslovakia. Basing your answer on Wolpert's concept of the "French Flag Model", imagine that there is a species of flatworm found along the coast of Bohemia that has this anatomical pattern. Explain what rules the cells would have to obey in order to generate this pattern, if they had a gradient of one morphogen with a source at the left and a sink at the right, and a second morphogen with a source at the top and a sink at the bottom.



Next, suppose that someone had introduced an artificial source of one morphogen and an artificial sink for the other morphogen (maybe an enzyme that digests it). The result was the generation of the abnormal pattern shown here on the left. Explain clearly which abnormality must have been caused by the abnormal source, and which must have been caused by the abnormal sink. Also state which of the two morphogens must have been affected in each case. Make your reasoning clear; when in doubt, make it clear that you understand the ideas.

WHY MOUNTAINS FORM WHERE THEY DO
(With apologies to Lewis Wolpert)



- 1) First, individual rocks determine their latitude and longitude.
- 2) Then those rocks having certain positions interpret this positional information by folding upward to form mountains.

(We know this because moving rocks from one location to another will determine whether or not they will become parts of mountains. However, it is also found that the precise contours of the mountains can be influenced by the chemical nature of the rocks.)

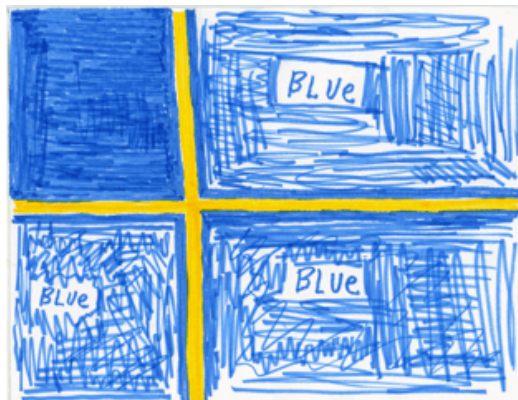
Although it is uncertain how rocks determine their position, GRADIENTS OF TEMPERATURE have been detected in the NORTH-SOUTH direction, and GRADIENTS OF SUNRISE-TIME in the EAST-WEST direction. It is hard to imagine what other purpose these gradients might have.

In honor of my grandmother, Martha Anderson, who spent her childhood in Sweden, I now propose a "Swedish Flag Model" as a superior analog to Wolpert's French flag. (Aesthetically superior, for one thing!). Imagine a flatworm, that would live in the Baltic Sea, if it lived anywhere. Instead of having three differentiated cell types, this flatworm has two: which are blue and gold. Most of its body consists of blue cells, but there is a cross-shaped zone made of gold-colored cells, displaced toward one end. This golden cross is caused to differentiate by means of a sequence of two morphogenetic movements. Large-scale active folding of cell sheets occurs during the worm's morphogenetic movements, somewhat as when a sea urchin embryo gastrulates and when a vertebrate embryo neurulates.

I leave aside the irrelevant complication that teleosts gastrulate somewhat differently, and so does the posterior sixth of the neural tube of bird embryos. For the same reason, I am not going to over-emphasize that my trusty Swedish Volvo automobile left me stranded with a discharged battery, for 3 days this past week, thereby trapping my older Volvo in the driveway. It will also go unmentioned that not all flatworms are rectangular, with exactly 90 degree corners.

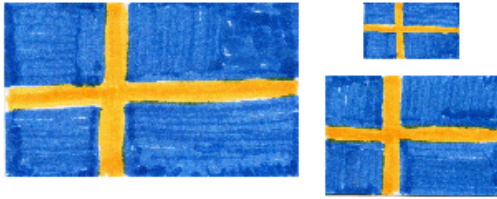


Another fact to mention in passing is that my thesis research began with *Botryllus schlosseri*, an actual species of colonial tunicate in which many have exactly the two beautiful colors of the Swedish flag, often arranged in a cross. Note that the word "colonial" refers to something other than the location of this species on the non-British side of the Atlantic Ocean.



The key idea of the "Swedish Flag model" is that gold cells are caused to differentiate wherever bending of the cell sheet is a maximum (instead of where chemical concentrations of diffusible chemicals exceed or are less than certain thresholds). Two morphogenetic folding events are postulated. One of these events folds a crease right down the horizontal (or anterior-posterior axis). This automatically divides the Swedish flatworm embryo into two halves. The principle is the same as

the familiar method by which you can divide any piece of paper into halves by folding two opposite edges together, and then creasing the bent part of the paper. The point is that there are plenty of methods (besides diffusion gradients) to adjust proportional dimensions. Furthermore, embryo cell sheets actually do undergo folding (which plays no role in the french Flag model).

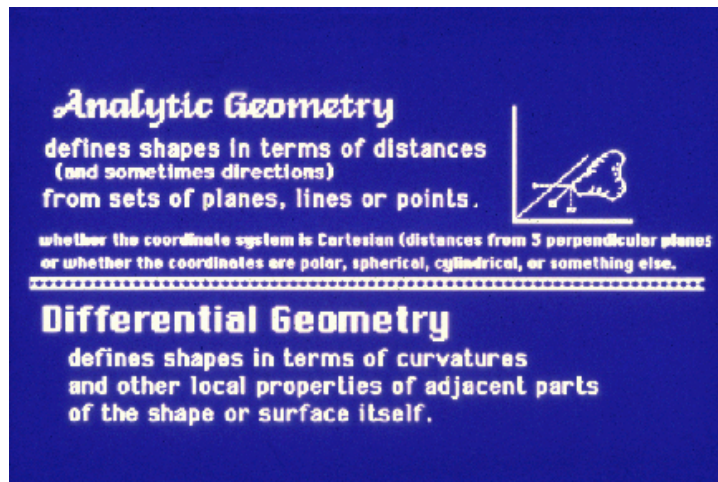


By pinching a crease along the most sharply bent part, you create a straight line along which the sheet of paper can be easily torn into exact halves. By differentiating into gold-colored cells wherever the worm's epithelial sheet is folded as a crease, the gold cross pattern of the Swedish flag can be created by two perpendicular folds. One fold (and therefore one yellow band), runs along the exact middle of the long axis. My imagination would have been subjected to less strain if the yellow cross was at the center of the Swedish flag. But really it's displaced a third of the width toward one end. Therefore, the morphogenetic folding that generates the vertical yellow line needs to be produced by a process like the method by which an ordinary letter is folded into thirds, to be put in an envelope. But the key idea is for amounts of folding of the sheet to control the locations where the gold colored cells will differentiate.

Notice the greater realism of my (Swedish flag) pattern creating method! Future boundaries between differentiated cell types are formed by folding of sheets, just as actually occurs in embryos. Folding is how embryos create the boundaries that separate future neural and pigmented retinal cells in the eye. Folding is also how the boundaries are created between neural and skin ectoderm, not only in neurulation, but also in separation of ectodermal placodes. Embryology contains dozens of comparable examples, where folds of cell sheets create boundaries between cells whose differentiated fate will be very different. Examples include folds in the brain surface that separate regions of different purpose. A large fold separates sensory areas of the cerebrum from motor areas. These can't be coincidences.

Even more important, this folding method can account for embryonic regulation. Whatever the size of the piece of paper, the folding method will generate scale model crosses. This is similar to the familiar method for tearing a piece of paper in two. You fold two opposite edges of the paper together, so that they become exactly aligned with each other; then you crease the fold enough to produce physical weakening; and then you tear along the crease. Everybody knows how to do this.

Despite everyone's familiarity with this method for separating paper into two equal-sized pieces (especially in contrast to the comparative rarity with which people cut paper in two by means of chemical diffusion gradients) nearly all developmental biologists cling to French flags. "Fifty million Frenchmen can't be wrong" is an old American saying.

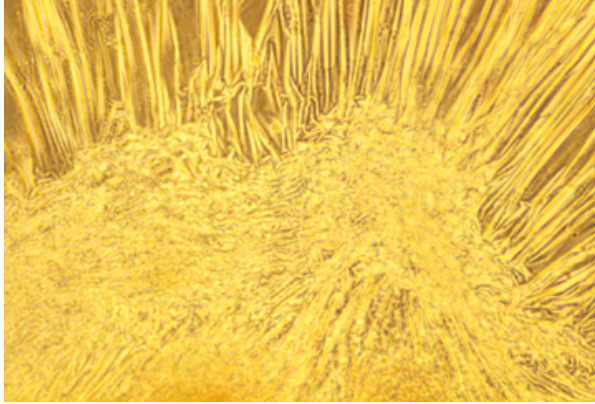


The preceding criticisms of flag models are a necessary foundation for the main subjects I was assigned. These subjects are the **contraction waves** discovered by **Natalie Björklund** and **Richard Gordon**. These waves may be the true means by which embryos control which cells will differentiate into skin and which into nerves. In my opinion, it is quite likely that they really are the cause. For one thing, contraction waves definitely occur in embryos. Also, they occur at the right times and the right places to be the creators of boundaries between cells that will branch along alternative pathways, some to become skin and the others to remain nerves.

These two correlations, of time and place, put contraction waves neck and neck with the Bicoid protein, in my estimation. The concepts of Belintzev, and the confirmatory experimental discoveries by Belousov, some of which were briefly described last week, pull contraction waves ahead of Bicoid.

Therefore we need to figure out what practical experiments on actual embryos could produce even more dramatic (and counter-intuitive) results. My lifetime experience in embryology is that logic doesn't win horse races. Faster horses may not even be enough. You need something dazzlingly dramatic enough that people can't resist conversion. A bright yellow photograph of thousands of tissue culture cells producing hundreds of rubber wrinkles that stretched all the way across a Petri dish: that

was what it took to eclipse Paul Weiss' (until-then) dominant paradigm about body cells creating pulling forces by capillarity. It's not fair, but something comparably startling will be needed to overcome decades of acceptance of the set of strong beliefs (and stronger wishes) that are symbolized by the French flag model.



We need something dramatic that can't plausibly be explained in any way other than by contraction waves and cell state splitters. This impossibility of any other kind of explanation needs to be self-evident. You don't just need to convince neural scientists, it's essential to convince the majority of your opponents. Scientists raised on a steady diet of French flags must be fed something indigestible to their previous imagination.

The great and influential philosopher of science, Karl Popper, successfully re-defined the border between science and non-science. His criterion was disprovability. By that he meant that some possible experimental criteria would (in principle) be able to disprove every hypothesis of any true science. His original targets were Freud, Adler and Marx.

Karl Popper's criterion can guide us toward whatever experimental results render alternative theories no longer defensible. What would those experimental results be for the concept of future cell differentiation being decided by whether a cell contracts or gets stretched?

One strategy would be to impose a geometrically incorrect pattern of stress on the surface of a gastrulating embryo (and test if this changes the pattern of cell differentiation). I know three ways to do this: micromanipulation; pulling on iron or nickel particles with a magnet; and (the best) draping a fine copper "magnet wire" across a tissue, in the presence of a strong, permanent magnet, and then putting brief pulses of direct-current electric field through the fine copper wire. A variation on the same theme is to dissect an old style ammeter out of its plastic case, and push on objects of interest with the tip of the needle.

Alternatively, you could connect wires to the ends of a commercial piezo-electric crystal, and zap occasional pulses of electricity through it. That would be a ten dollar, do-it-yourself Atomic Force Microscope. The goal is to produce artificial contractions in the right places, just before the natural contractions occur. Will your artificial waves be propagated by the surface cells of the gastrula? Or not? Can you initiate your own waves just before the normal time? Can you induce waves that

propagate on the opposite direction to the normally-occurring waves? Interference patterns of waves could produce arrays of moving dots. Lev Belousov may already have accomplished much of this. My bright ideas often show up in completed form in Lev's past publications - experiments that he had invented and completed before I even thought of them. I suggest to other inventive experimenters that they check Lev Belousov's papers to find out whether he has already finished what you are just now conceiving.

A very different strategy would be to take advantage of birefringence produced in collagen gels, in gelatin gels, or ideally in some non-toxic, non-reactive nematic liquid crystal substance. If this latter substance exists, it would be wonderful. Waves of either contraction or extension, or both, would light up like fireworks when viewed between crossed polarizers. With the addition of a quarter wave plate to the optical path, contractions would produce complementary colors (like yellow for tension and blue for compression). Think how strongly everyone is persuaded by those waves of fluorescent glow, spreading across the cortexes of just-fertilized fish oocytes. They prove the occurrence of waves of increased calcium ion concentrations. Don't forget, however, that it is human nature for people to be more readily persuaded of conclusions they already believe. They also have more admiration for the insight and originality of those who confirm whatever they already are sure of.

In the long run, however, minds can be changed. But often you need to convince you converts that the new idea was pretty much what they always knew.

(Like that sponges crawl: Nickel, M. (2006). Like a 'rolling stone': quantitative analysis of the body movement and skeletal dynamics of the spone *Tethya wilmelma*. J. Exp. Biol. 209, 2839-2846)