Possible mechanisms for initiating macroscopic left-right asymmetry in animals and plants

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[Support: U.S. Dept. of Energy]

KITP Morphodynamics Workshop, 23 Sept. 2009

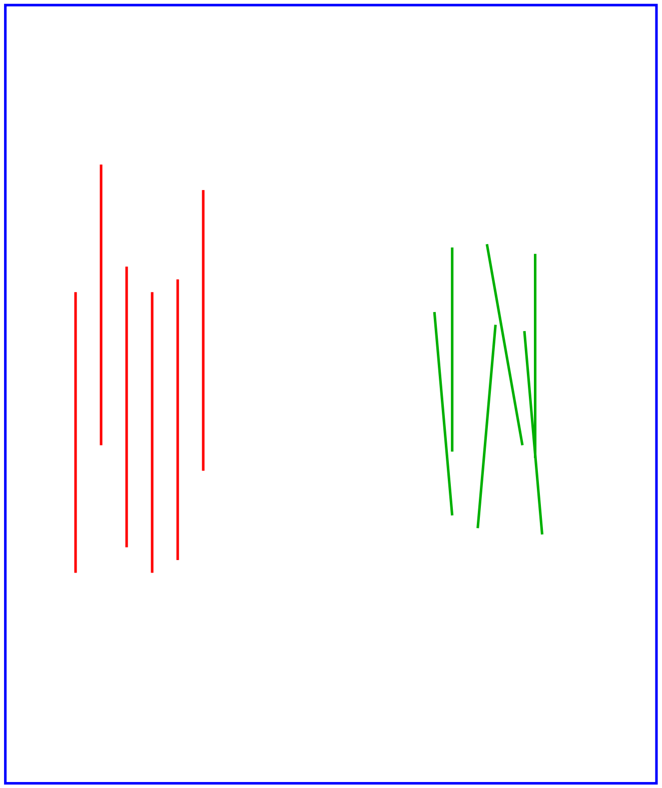
Outline

How get chirality from micro to macro scale? Deduce **physical** consequences from a priori, fundamental **symmetries**.

1. Introduction:
   Examples Assumptions/facts
   Classifying mechanism: cell/collective level, etc.
   SKIP review of vertebrate case (cilia-driven flow)
2. LR asymmetric transport (screw processive motors?)
3. Twist in cell division (shearing actin array?)
4. Plants
   Facts: circumnutation, root skewing, cell files
   Conjecture: rotating microtubule array?

6. Conclusions

Preview: dynamic cortical arrays?



Left side: shearing actin array (animals).

Right side: rotating microtubule array (plants).

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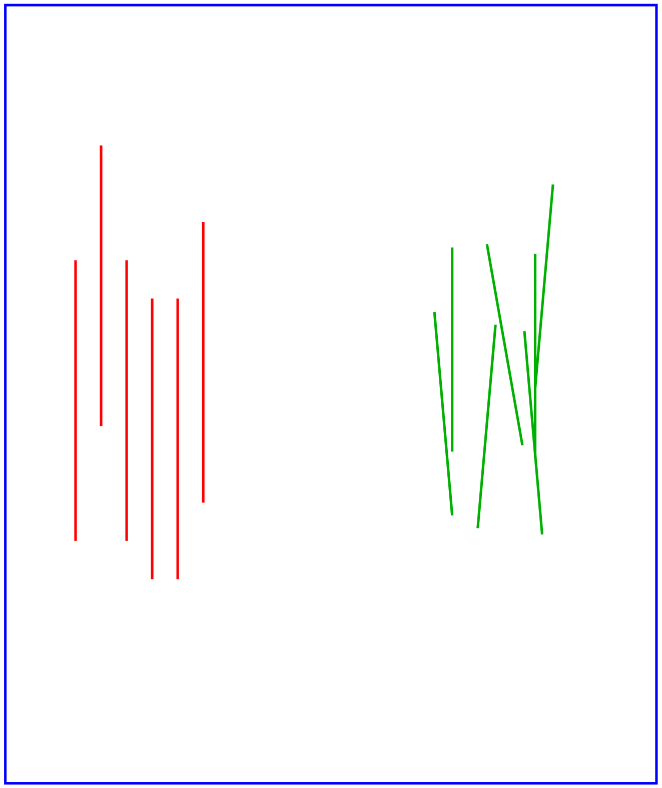

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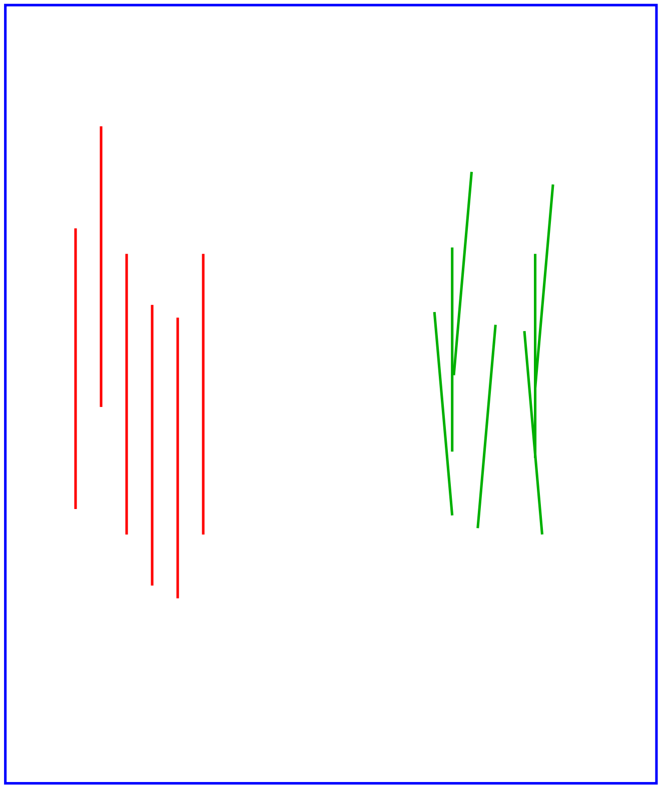

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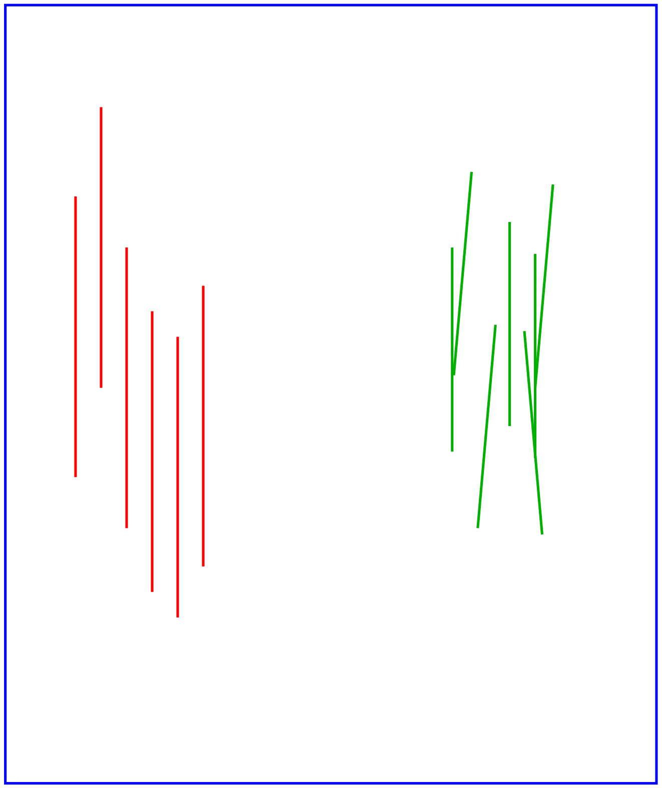

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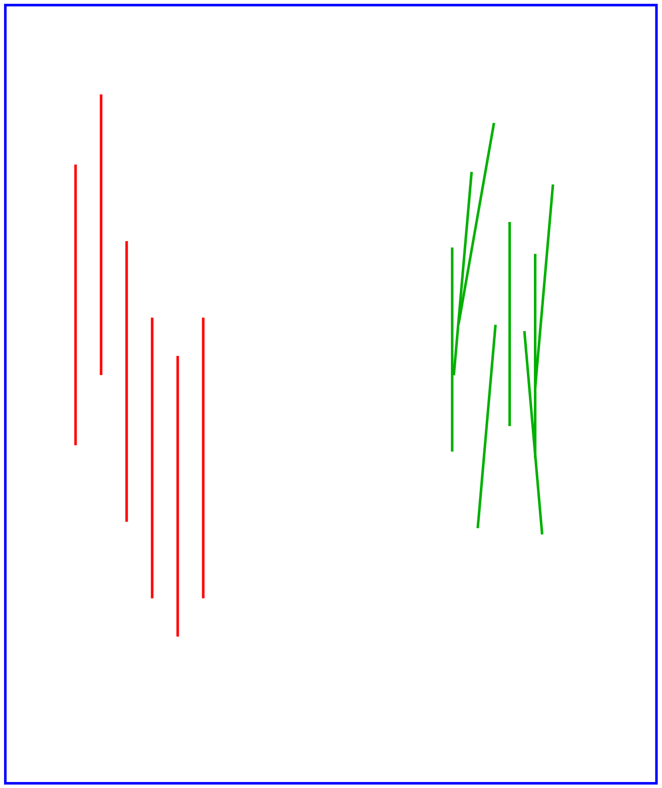

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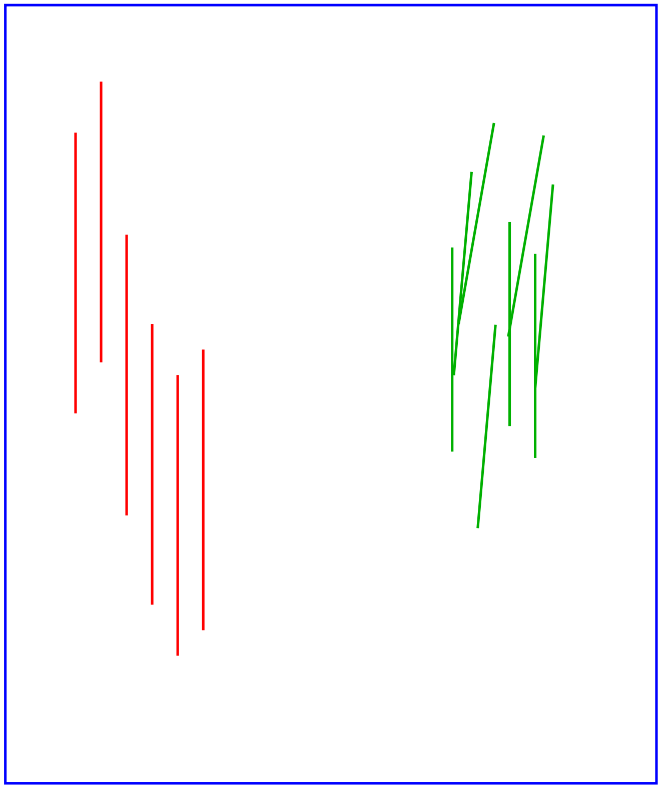

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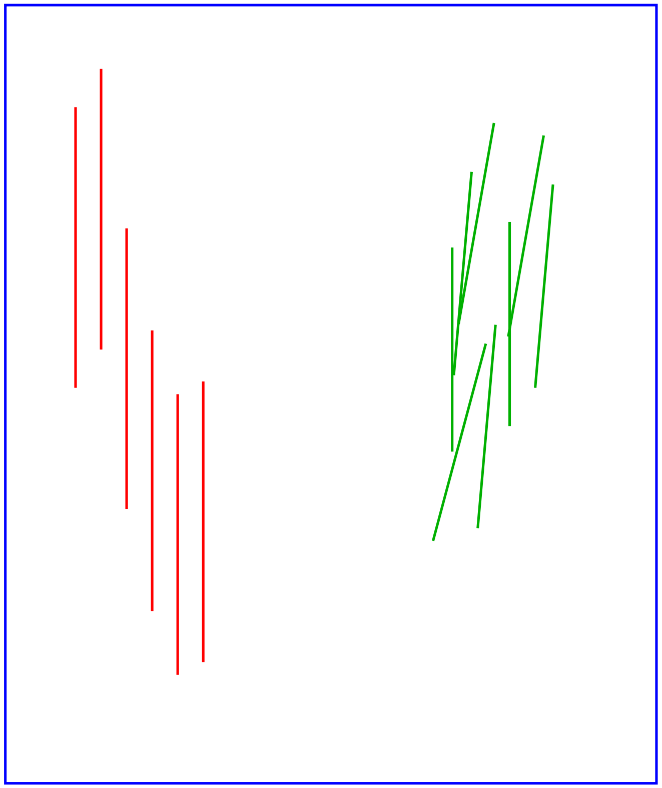

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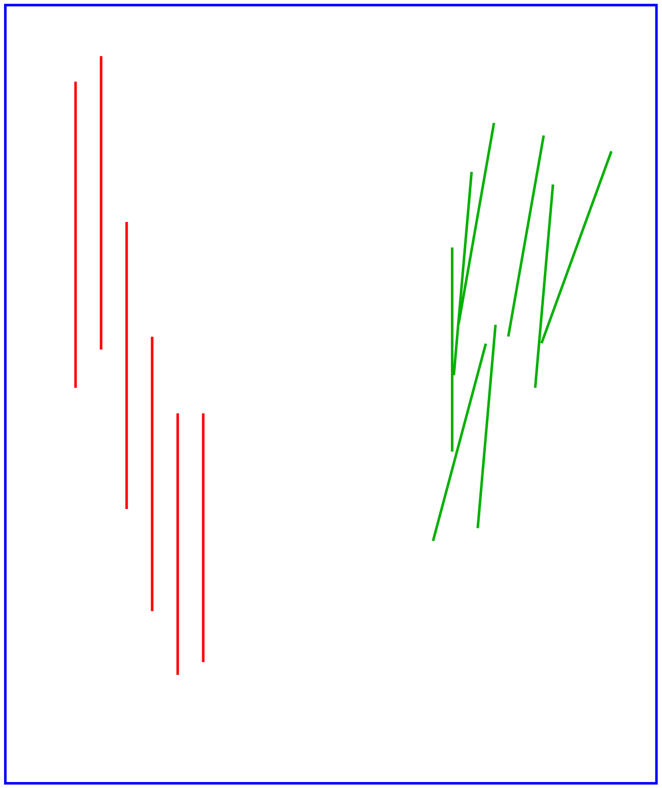

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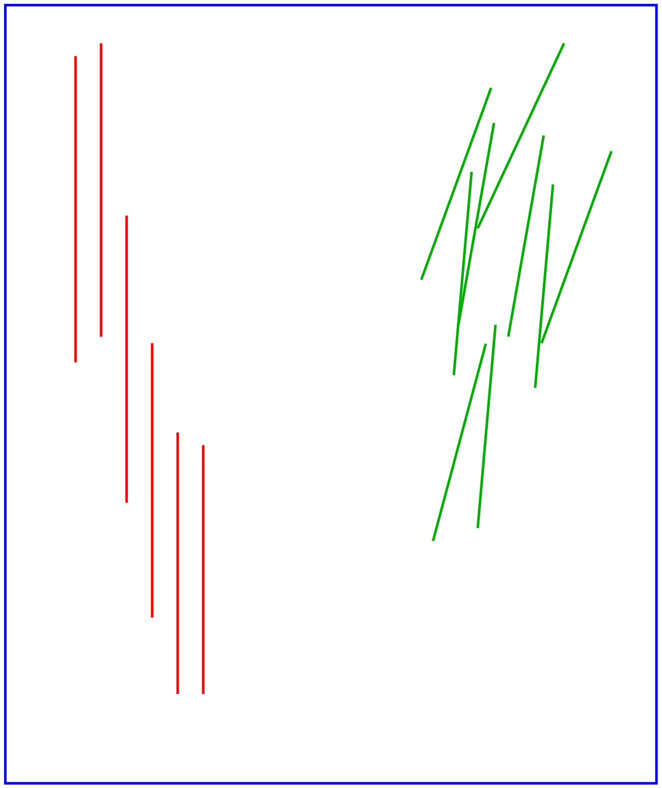

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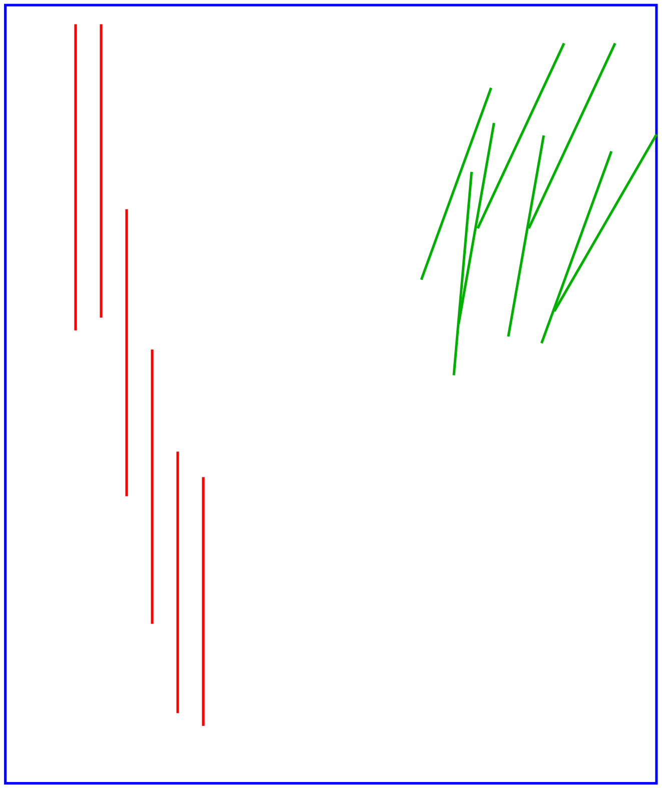

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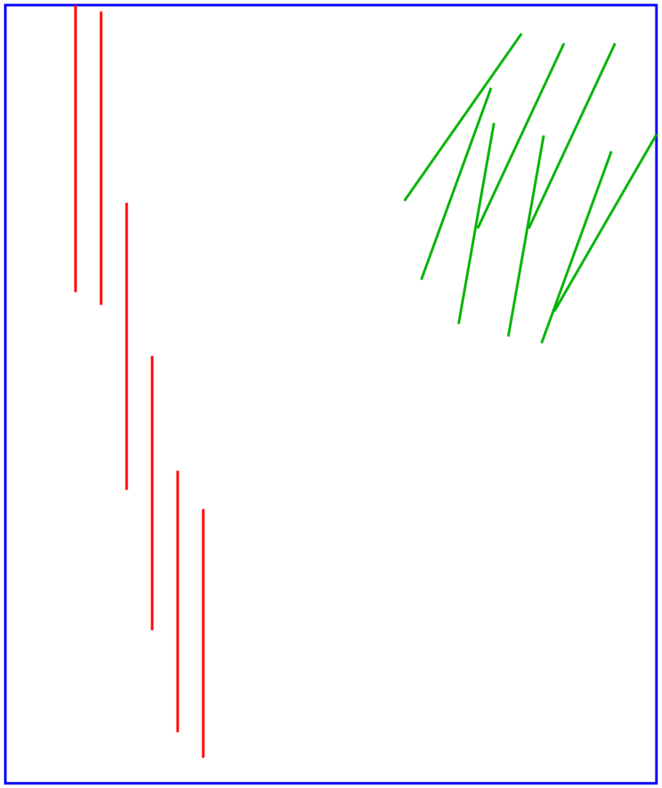

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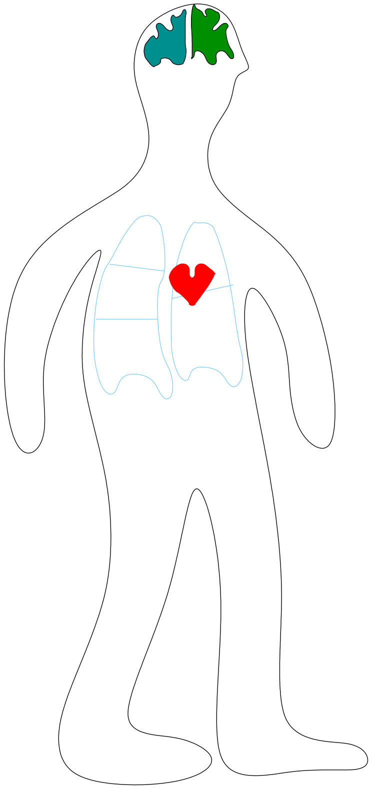
1. INTRODUCTION

1.1 Examples



a) Vertebrates: heart, lungs, etc.
(fraction reversed: $\sim 10^{-4}$)
Model species: chick, Xenopus frog, zebrafish, mouse

b) Human brain: left-hemisphere dominant / = “right-handed”
(fraction reversed: $\sim 10^{-1}$)

c) *C. elegans* (nematode “worm”):
twisting of gut (flies too: (– sorry, no image –)
[Spéder et al Nature 440, 803 (2006),]

d) molluscs: shell twists right-handed
Image: mating is awkward for R snail
and (rare) L snail

And crustaceans, etc.
No universal answer expected!
Different mechanisms (likely) apply to different cases.

Also Plants

Both roots and shoots spiral with a determined handedness (species dependent). Obvious in vines.

A corollary (seen): "Hall effect; roots grown vertically, on a surface, deviate horizontally

Mutants exist with reversed handedness.

[Note: "phyllotaxis" – Fibonacci spirals of leave placement, pine cones, sunflowers – are **not** handed.]

Poor little sucker...

1950s song “Misalliance” by Flanders & Swann

The fragrant honeysuckle spirals clockwise to the sun,
And many other creepers do the same,
But some climb anticlockwise; the bindweed does, for one,
Or Convulvulus, to give its proper name.

Said the right-hand-thread honeysuckle
to the left-hand-thread bindweed,
“Oh! Let us get married if my parents don’t mind.
Be loving and inseparable, inexplicably entwined. We’d
Live happily ever after,” said the
honeysuckle to the bindweed.

A bee who was passing remarked to them then:
“I’ve said it before and I’ll say it again:
Consider your offshoot, if offshoots there be.
They’ll never receive any blessings from me.

Poor little sucker! How will it learn
When it’s climbing which way to turn?
Right? Left? What a disgrace!
Or it may go straight upwards and fall flat on its face.”

Why we care about L/R?

My own motivation: just fascination w/ symmetry & symmetry breaking (in spirit of Landau)

- L/R as a biology topic has heated up in past 10 year
- **initial** L/R specification is (unusual?) case where physics is actually necessary
- Binary nature and approximate L/R symmetry make this "cleaner" than most development questions
  Yet L/R determination is likely to illuminate other development mechanisms.

1.2 Question and starting assumptions/fact:

An embryo develops 2 axes:

anterior/posterior= A/P = x,

dorsal/ventral=D/V=z

– by **spontaneous symmetry breaking**.
(Sometimes biased externally e.g. where sperm entered egg).

How to ensure 3rd axis (y) fulfills "right-hand rule" ($\mathbf{y}= \mathbf{z}\times \mathbf{x}$)?
Symmetry ⇒ **need all 3**: **x** and **z** axes, also (fibers') **chirality**)

Take as given:

1 From genetic control + chirality of molecules
(**not** shape of mother's womb/egg)

2 L/R, too, is **spontaneous** symmetry breaking (usually);
systematic bias amplified from **small** symm. breaking field.
⇒ a small L/R ($O(10^{-2}?)$) effect will suffice

3 Seek the **earliest** L/R asymmetry ⇒ whatever makes the
asymmetry, preceded it and was (**functionally**) symmetrical

1.3 Not your usual “biological” mechanism

Usual “biological” mechanism:

transport/diffusion of signaling molecules + reactions

⊗ **but** diffusion doesn’t distinguish handedness

Needs a “physical” mechanism?

semi-macroscopic elements: long fibers of each cell’s cytoskeleton)

forces and torques (molecular motors that run on the fibers)

Active motors: As mentioned, passive diffusion won't do it. If you have some kind of collision or reaction events, you need a change of k_BT at the key chiral event.

(Can static equilibrium elasticity do it? Bundles of actin fibers (in 3D) do twist ... can this lead to a maroscopic twist?)

1.4 Cartoon of cytoskeleton [for physicists]

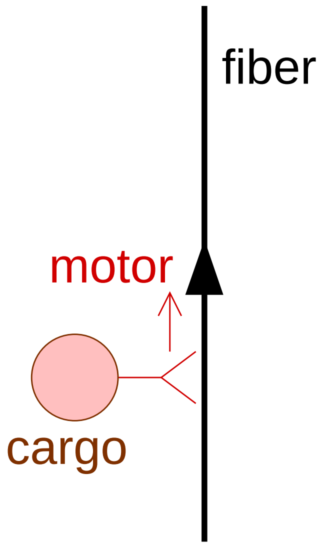
Cytoskeleton: Framework in each cell (of higher organisms), built from long, stiff, **directed, helical** macro-molecules ("fibers")

a) microtubules → motors dynein or kinesin

b) actin → motor myosin

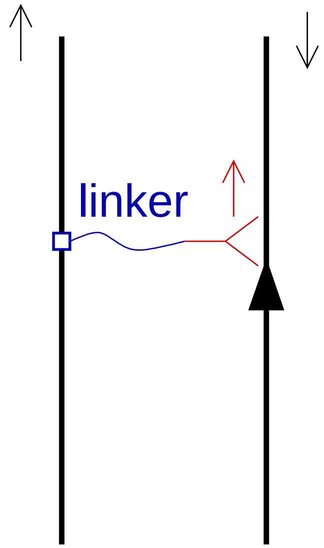
(Analogs of actin and mt's – similar modeling?)

Each motor (literally) walks in a fixed sense (dissipating energy) carrying cargoes (chemicals in vesicles = little membrane bags)



Rarely (by thermal fluctuation)
motor falls off its fiber;
→ diffuses till it reattaches to some fiber

In place of a cargo, motor can be attached to another fiber, so one moves relative to other. (e.g. muscles).



Fibers form networks with many crosslinks

Often, these networks are **dynamic**: fibers are always growing or shrinking (described by statistical mechanics)

There are many flavors within each kind of motor (used for specialized purposes)

1.5 Classifying mechanisms

(1) Two levels in any mechanism

a) intracellular

b) collective (intercellular): plan of the body's tissues

(2) Two styles in development (for different groups of animal):

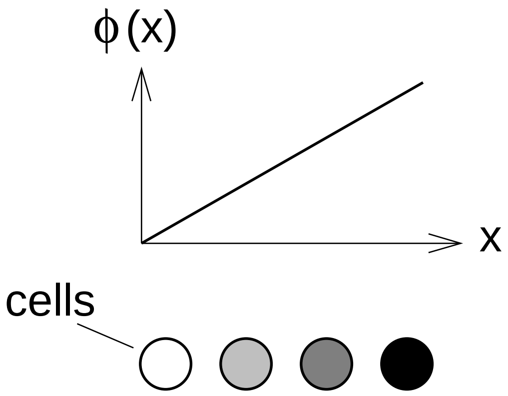
a) **early: (many) invertebrates**
as cells first divide, each gets a determining label
⇒ primarily cell-level mechanism suffices for L/R

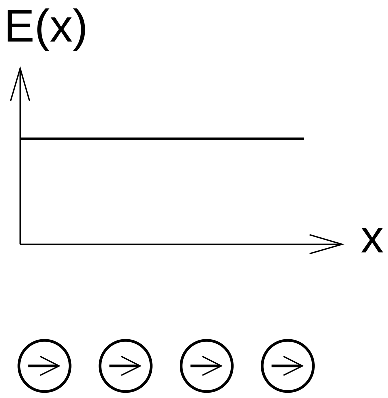
b) **late: vertebrates**
cells' fate isn't fixed early but pattern formation later
⇒ need an inter-cellular mechanism

(3) Two ways to represent L and R

“positional information”: chemical conc. $\phi(x,y,z) \propto x$.

polarization: local vector $\vec{E}(\mathbf{r})$.





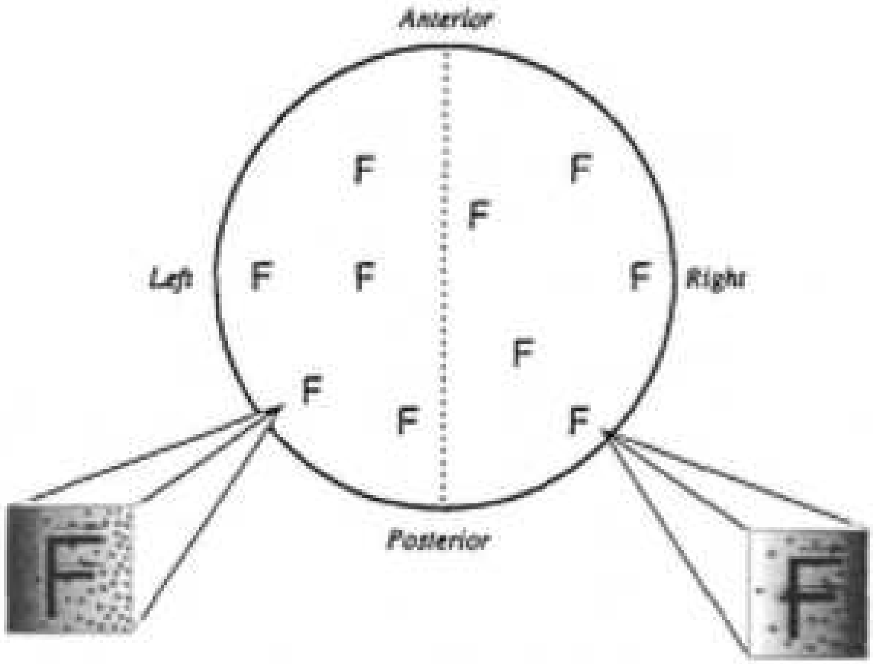
Of course $\vec{E}(\mathbf{r}) = \nabla\phi(\mathbf{r})$. Nontrivial to convert:

- $\phi(\mathbf{r}) \to \vec{E}(\mathbf{r})$: OK
  (Animal cells are big enough to sense
  conc. gradients between one side and the other)
- $\vec{E}(\mathbf{r}) \to \phi(\mathbf{r})$: HARD
  (To get imbalance in conc. $\phi(\mathbf{r})$ of a chemical,
  it must be actively transported with a bias along $\vec{E}(\mathbf{r})$)

Wolpert's F molecule

1st paper posing L/R question for developmental biologists.

[N. A. Brown and L. Wolpert, Development 109, 1-9 (1990).]



Imagined: an "F molecule:" devloping orientational order in cells throughout the embryo (polarization way)

2. ASYMMETRIC TRANSPORT?

An imaginary mechanism, for pedagogical purposes.

“Biological” mechanism: **transport** of signaling molecules.
Can we have a **late**-stage, transport-based mechanism?

Use: fiber molecules with motors

Consider for simplicity a planar geometry (dorsal/ventral sides)
(indeed embryos are flattish at an intermediate stage)

Aim for: analog of “Hall effect” (electrons moving in magnetic field): A/P transport direction gets rotated by small angle.

Cell level is the nontrivial level for this mechanism.

2.1 Single cell level

Assume “cortical” array of fibers **just under** cell’s membrane.
(There’s the **D/V asymmetry**!)

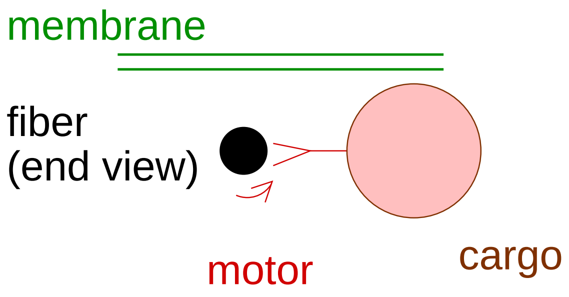
Assume fibers oriented exactly along $\pm z$.
(**not** (yet) the A/P asymmetry)

Active transport depending on motor motion
⇒ L/R **must** be due to the **generic helical component**
of any motor’s motion down any (microscopically) helical fiber.
(There’s the **chirality**.)

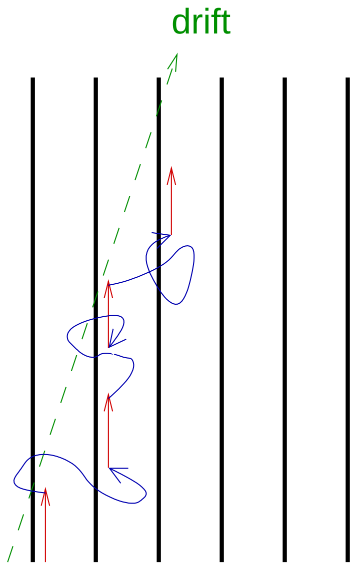
Myosin V is observed to spiral on actin:
[M. Y. Ali, Nature Struc. Biol. 9, 464 (2002).]

Note: although myosin II is non-processive, it too twirls actin filaments chirally: [J. F. Beausang, H. W. Schroeder III, P. C. Nelson, and Y. E. Goldman, Biophys. J. 95, 5820 (2008).]))

Say motor spirals **counter-clockwise**; ⇒ pulls cargo till jammed against membrane. i.e. travels on **right** side of road



If cargo reattaches to a neighboring fiber, it's more likely to attach to the fiber on left side.



Result: sideways drift: transport current at angle θ from z, where

$$\theta \sim r_{\text{cargo}}/\ell \tag{1}$$

Here $\ell \sim 1\ \mu m$ is typical distance that motor goes before falling off, and $r_{\text{cargo}} \sim 25\ nm$ is an effective distance of the cargo from the fiber axis (defined so r_{cargo}/d is probability of reattaching to the next fiber at a separation d)

So $\theta \sim 2.5 \times 10^{-3}$? **Might** suffice as a biasing field...

Note: "one side of the road" motion is conjectured for cellulose-synthesizing units moving along microtubules in plant cortical arrays. [A. Paradez, A. Wright, and D. W. Ehrhardt, Curr. Opin. Plant Biol. 9, 571 (2006)]

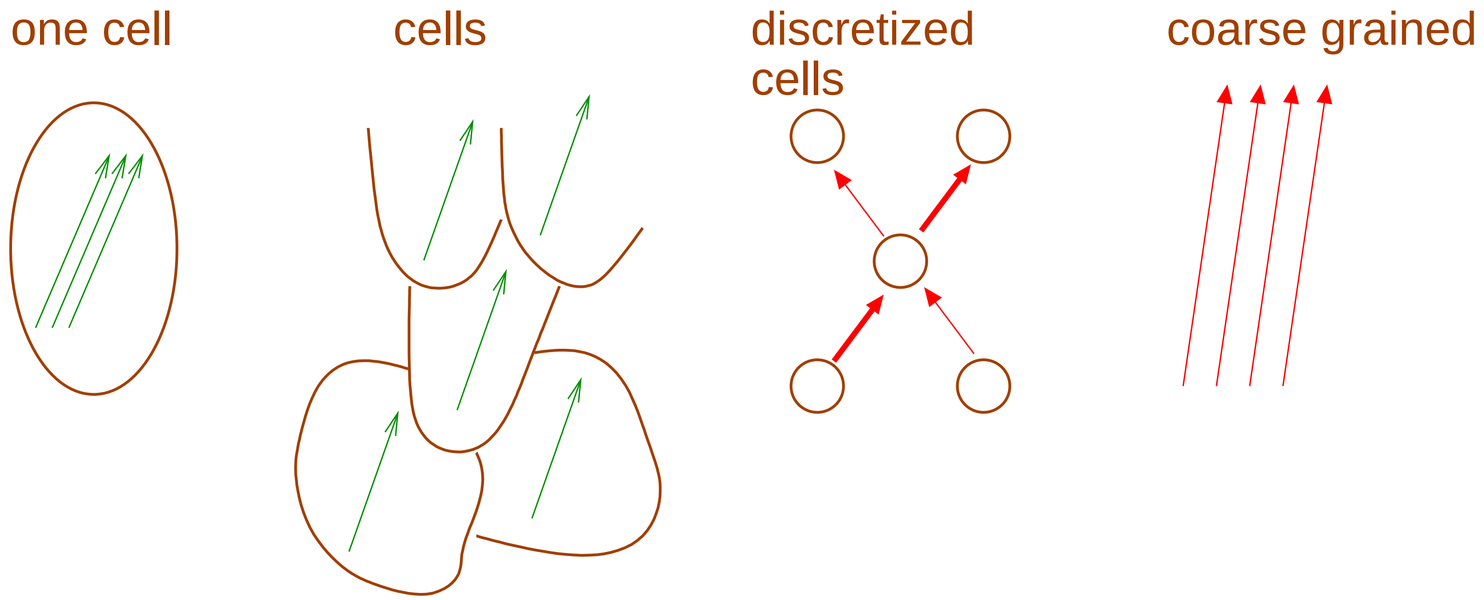
2.2 Collective level

Now this array is only on the **front** side of the **front** layer of cells
(Check: using D/V asymm. on organism level.)

Signal chemical is released from (say) posterior end.
(**Now** using **A/P** asymmetry.)

Then we argued signaling chemical has a **sideways bias**
relative to the array in each cell

$\Rightarrow$ **macroscopic** transport has a similar bias



Summary: it's not easy to generate late-stage asymmetry with a transport mechanism!

3. CELL DIVISION

Early-stage, **cell-level** mechanism (from actin array).

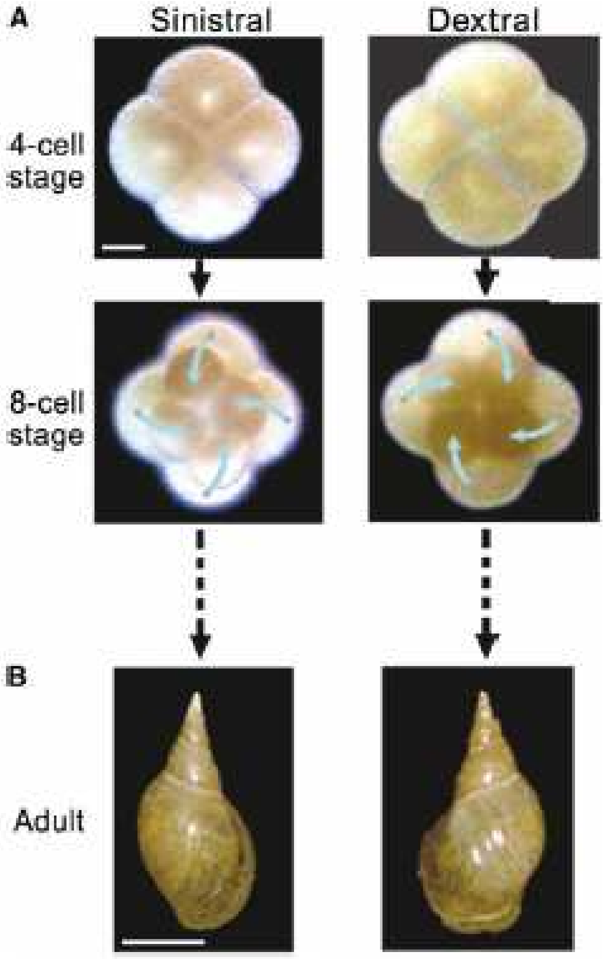
3.1 Experimental facts:

For **Molluscs** (snails).

1) 1st 4 cells in a square
2nd 4 cells form on top with twist
(normally in the sense shown).

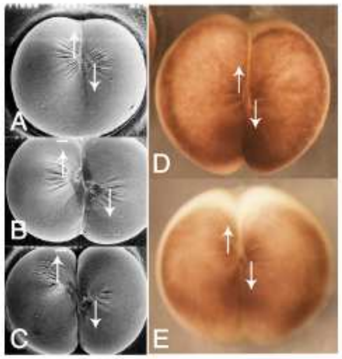
2) Twist depends on **actin**
but **not** on microtubules,
of the 2 kinds of fiber.
(?! cell division is driven by
an array of **microtubules**)

[Y. Shibazaki, M. Shimizu, and R. Kuroda, Curr Biol 14, 1462-1467 (2004)]

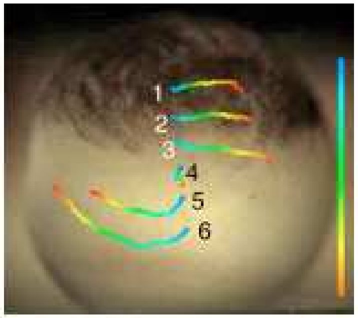


3) in frog eggs, same twist is observed and is due to actin;
the parallel actin molecules shear past each other
always in a clockwise sense; the motor mysin I-D is responsible

Actin forms a band pointing around the cell, in the plane where the split will occur;



Time lapse shows fluorescently labeled points along several fibers: uniform shear.



[M. V. Danilchik, E. E. Brown, and K. Riegert, Development 133, 4517-4526 (2006).]

3.1

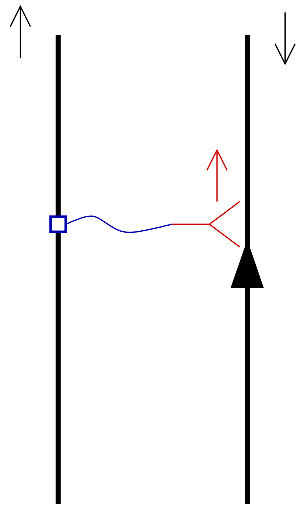
3.2 Model: asymmetric detachment

Start with array of parallel/antiparellel actin fibers.

Actin array's shear **must** be driven by the actin motor, myosin. Each myosin walks on one actin fiber; it **must** be connected and bonded (directly or indirectly) to another fiber, in order to make any shear. I'll assume these connections are permanent bonds.

Bottom line: I don't have a good picture how (non processive) myosin I or non-muscle myosin II actually drive shear. Toy picture how processive myosin V might.

To drive clockwise (CW) shear, dominant bridging bonds **must** be placed as shown: (connect to R or to L, according to polarity of walked-on fiber).



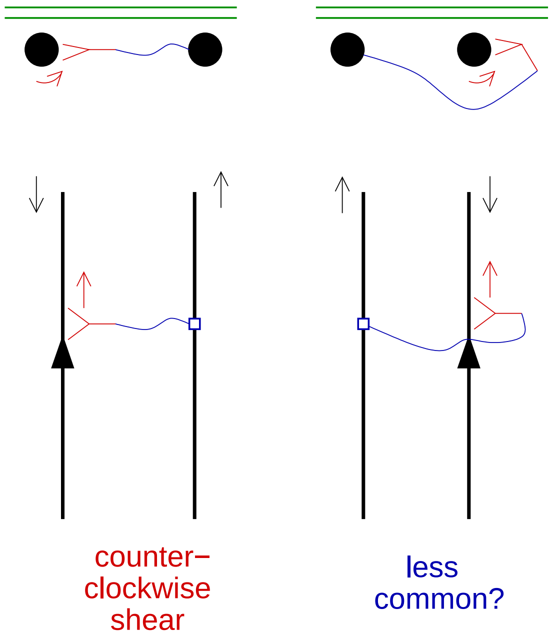
clockwise shear

[both ideas as in previous mechanism].

(1)How to get directed? Should depend on **helicity** of fiber, namely: myosin walks on actin with left-handed screw.

(2) Also, **must** depend on **front/back** difference; (probably) means interaction with **membrane**

Imagine a myosin V motor bonded
to fiber 1 and walking on fiber 2.
Motor spirals around fiber 2
till it comes against membrane
When motor is on **far** side of fiber 2,
linker strained ⇒ myosin falls off easier
⇒ near-side case dominates.
(Quantitative? depends on elasticity.)



4. CHIRALITY IN PLANTS

Phenomena in growing plants (inter-related)

1a **Circumnutation** circular motion of tip a it grows, period ~2 h [Darwin 1880, The Power of Movement in Plants]

1b **Spiral shape** of root/stem. (also **twining** by vines)

2 Skew of roots under gravity [$\mathbf{v} = \lambda(\mathbf{g} + \epsilon\mathbf{n} \times \mathbf{g})$.]

3 **cell file rotation** (defines L or R plant)/

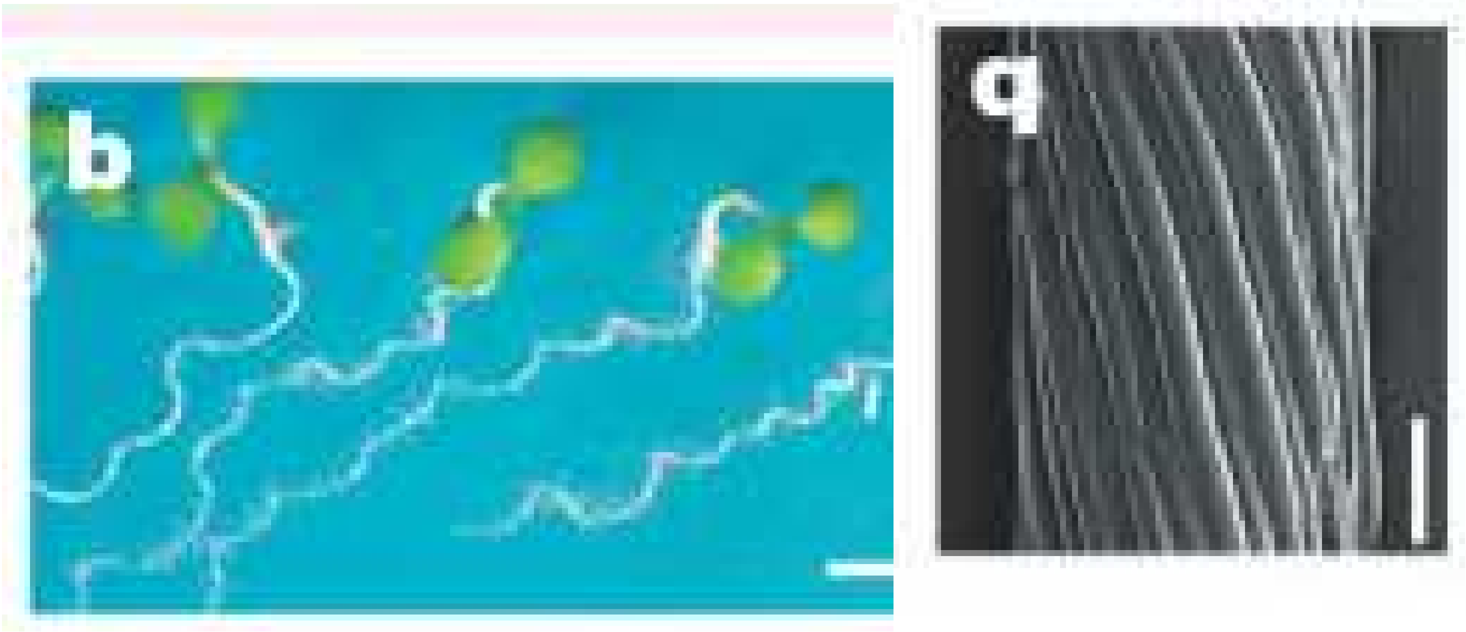
4a **microtubule arrays** under cell membrane, helical (~ transverse in elongating cells/ longitudinal eventually)

4a mt array orients helical array of cellulose fibers

Table 1: Chirality in Arabidopsis varieties

type	cell file rotation	root skew	mt array sense	remarks
WT	∅	L	R	
lefty1 lefty2 mt drugs	L	L+	R+	α-tubulin defects ... destabilizing mt's
spr1/sku6; spr2; wvd2-1	R	R	L	mt-mt interactions? or stabilizing mt's?

[S. Thitamadee, K. Tuchihara, and T. Hashimoto, Nature 417, 193 (2002)]

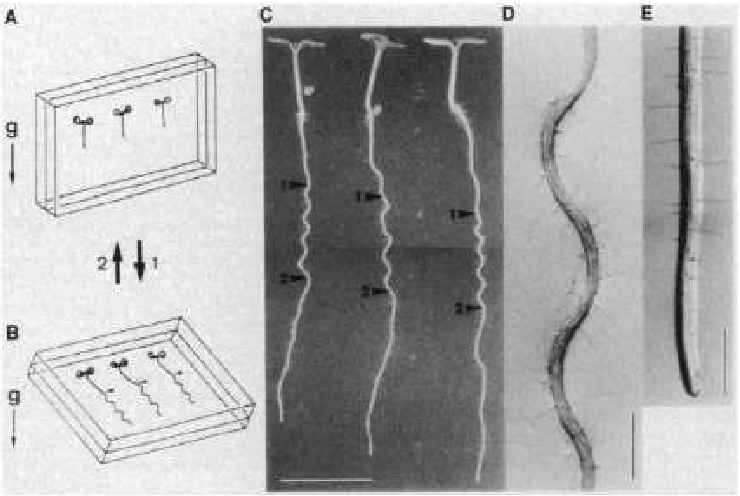


Roots skew on tilted agar.

Cell files on a root.

Both from left-handed “lefty1” mutant.

Root tip rotation is also associated with transverse waving when the root is grown on a hard agar plate.



[K. Okada and Y. Shimura, Science 250, 274 (1990)]

Adv’t: see videos of roots growing in agar, buckling spirally as they hit a resistive layer, by Sharon Gerbode (website of Itai Cohen group, Cornell).

5. ROTATING MICROTUBULE (mt) ARRAY

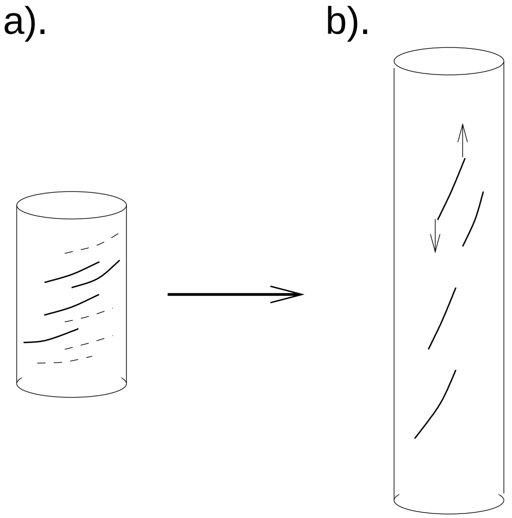
Dynamic fiber array # 2, for plants

5.1 Organism scale mechanism

Mechanical mechanism:

"twist-to-writhe" conversion instability: (partly) relieve torque stress when centerline goes helical. (example: DNA supercoiling).

Here: remember helical arrays of mt/cellulose. Cell elongation creates a torque stress
(fibers can't stretch as ends are pulled opposite ways)



Notes: orig. idea for Nitella algae.

[M. P. Wold and R. I. Gamow, J. Theor. Biol. 159, 39-51 (1992)]

Related (not same) mechanism for twist in elongating bacteria:

[C. W. Wolgemuth, et al Physical Biol. 2, 189-199 (2005)]

5.2 Cell level mechanism: dynamic mt array

+ end of mt grows (mostly)

− end of mt depolymerizes:

new mt branches at $\sim 30^\circ$ angle (maybe also at 0°)

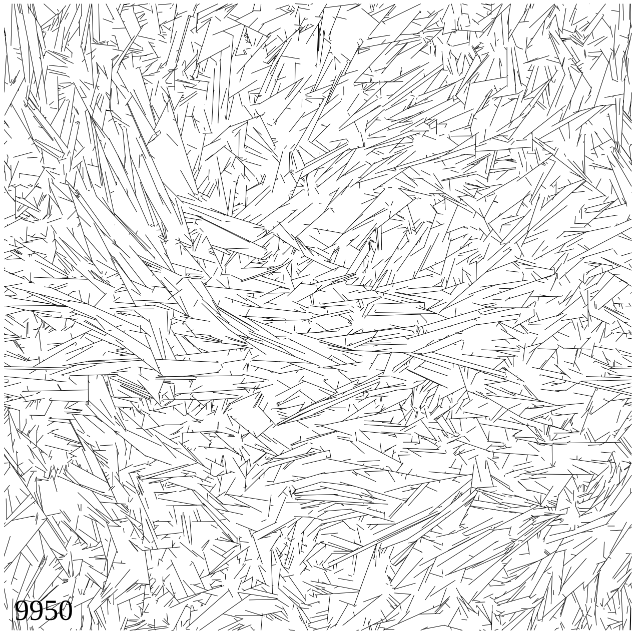
hit another mt at 30° (or less) $\Rightarrow$ align, bundle

hit another mt at $> 30^\circ$ $\Rightarrow$ depolymerize back from + end

Evolve to an aligned steady state

[Model from V. A. Baulin, C. M. Marques, and F. Thalmann, Biophys. Chemistry 128, 231-244 (2007)] Simulations by student Igor Šegota.

Model lacks branching – mt just halts if collision.



Symmetry breaking: orientation of array (within plane) is arbitrary

(mt not polarized, 50-50 either way in a domain)

Spontaneous rotation (=mechanism)

Spontaneous (slow) rotation of mt array orientation angle θ

$$\frac{d\theta}{dt} = \omega - (\alpha - \epsilon)\sin 2\theta$$

(Here $\theta = 0$ means transverse to long axis.)

ω = spontaneous rotation (radians/sec)

ϵ = elongation rate (1/time)

α = a transverse bias
... mechanism unimportant here; (a) curvature [anchoring proteins bend mt away from the membrane?] (b) transverse to tension?

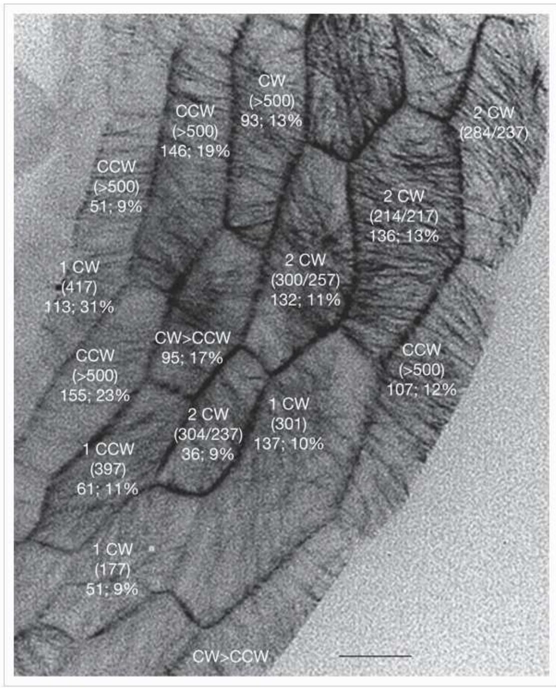
Comes to halt at: $\sin 2\theta_0 = \omega/(\alpha - \epsilon)$

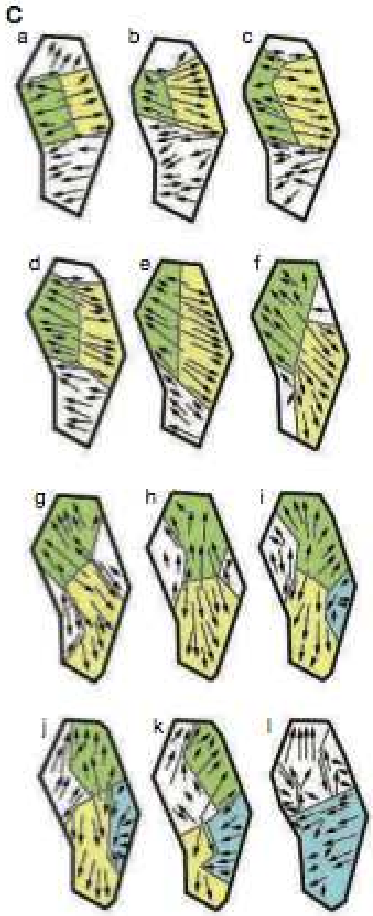
Change ω or α (mutations, or as tissue matures in WT)
$\Rightarrow$ remodel tilt θ_0.

Rotation of mt domains is observed in Arabidopsis

Left: raw images, taken 1/20 min (here labeled by rotation sense); right, mosaic of domains with mt motions drawn.

[J. Chan, G. Calder, S. Fox, and C. Lloyd, Nature Cell Biol. 9, 171 (2007).]





5.3 Possible mechanisms for rotation rate ω

Remember, membrane must be involved. (The upper and lower side of the mt itself are the same).

a **mt-mt collision** outcome depends on side you came from

b **membrane-mt-mt** protein nucleates a branch

c **membrane-mt** protein bends each mt to (say) its right

[mt events review: D. W. Ehrhardt, Curr. Opinion Cell Biol. 20, 107-116 (2008)]

Bulletin: I just saw rotating mt array proposed by a biologist. [G. O. Wasteneys and J. C. Ambrose, Trends in Cell Biology 19, 62-71 (2/1/09)

Reversal mutants?

Say competing contributions $\omega = \omega_1 - \omega_2$. If you knock out ω_1, then ω changes sign, but not exact reversal of mechanism

(a) mt array twist: Collision mech.?

How could an array get aligned with systematic twist θ?

A collective mechanism. Say a fiber nucleated at a random orientation at a certain rate per unit area; grows till it runs into some other fiber.
This favors $\sim$ parallel/antiparallel axes $\Rightarrow$ array.

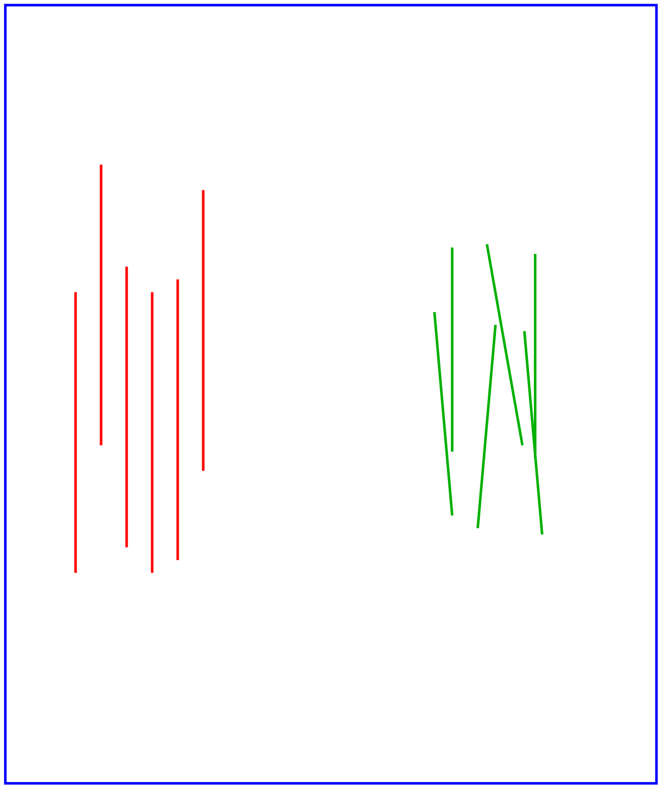
The screw thread of fiber 1 carries fiber hitting it up/down, depending which side that came from.
Say fiber 1 has a **right-hand** screw.
If fiber 2 hits from the right (resp. left)
it gets carried down (resp. up)
(here we used **helical** asymmetry!)

[skip]

Imagine next that if fiber 2 gets carried **up** (hitting the membrane) it is more likely to **stop** growing; but if carried **down**, it's more likely to get carried under and beyond fiber 1, and **continue** growing. (Write difference as a small dimensionless factor f)
(here we used up/down asymmetry!)

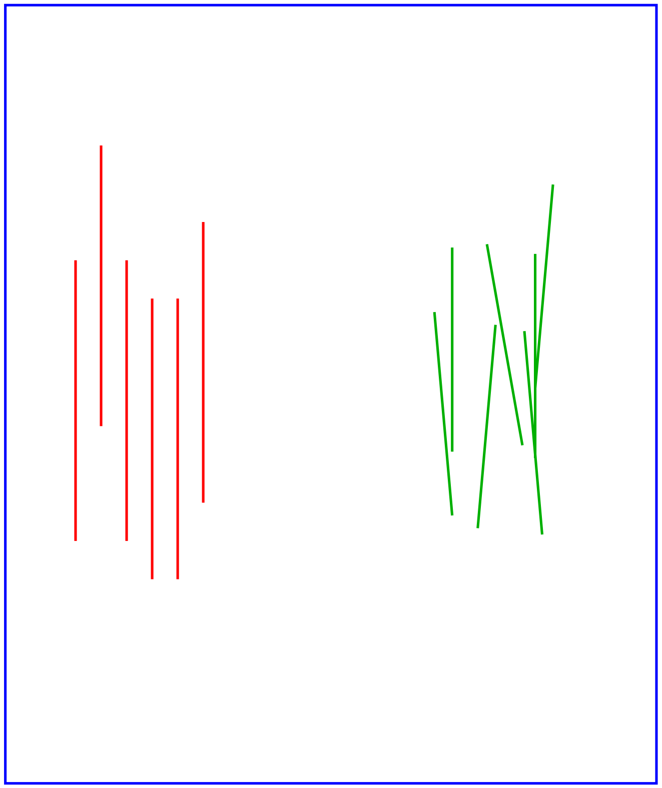
Hence: new fibers at an orientation where they hit old fibers from the right grow longer; the new fibers' orientation will be rotated **counter-clockwise** by an angle of order f. (I'm glossing over possible logarithmic factors.) So, if the array started out aligned in the z direction, its later orientation is rotated by an angle θ of order f times the number of generations of fiber growth since the start.

(b) mt array twist: branching mech.?



Right side: microtubule array treadmills forward and starts new filaments by branching on one side, so the orientation precesses. (ignore left side = shearing actin bundles.)

(b) mt array twist: branching mech.?



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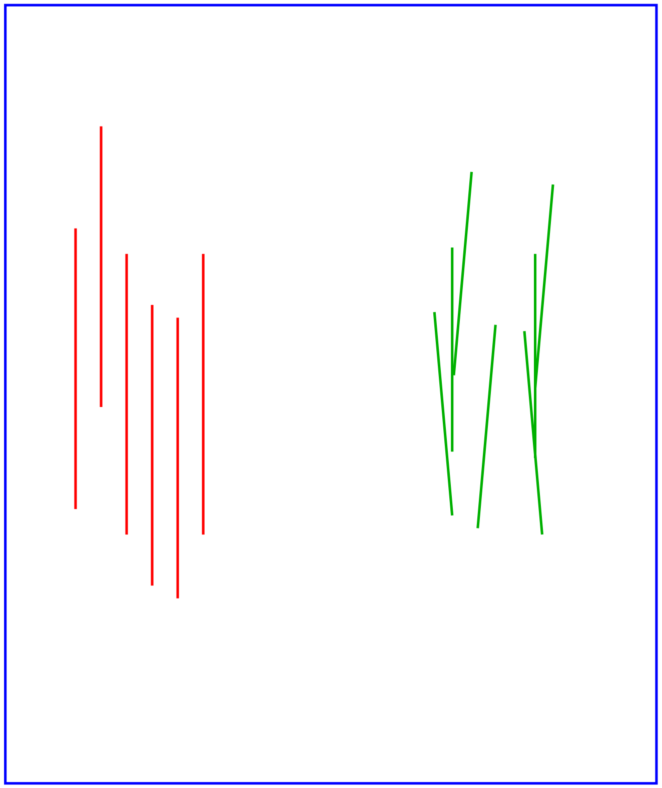

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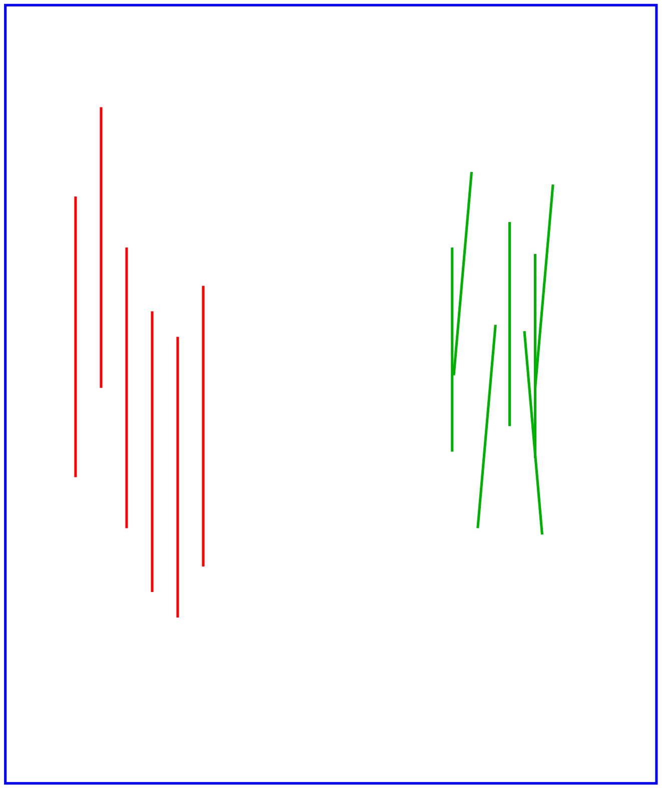

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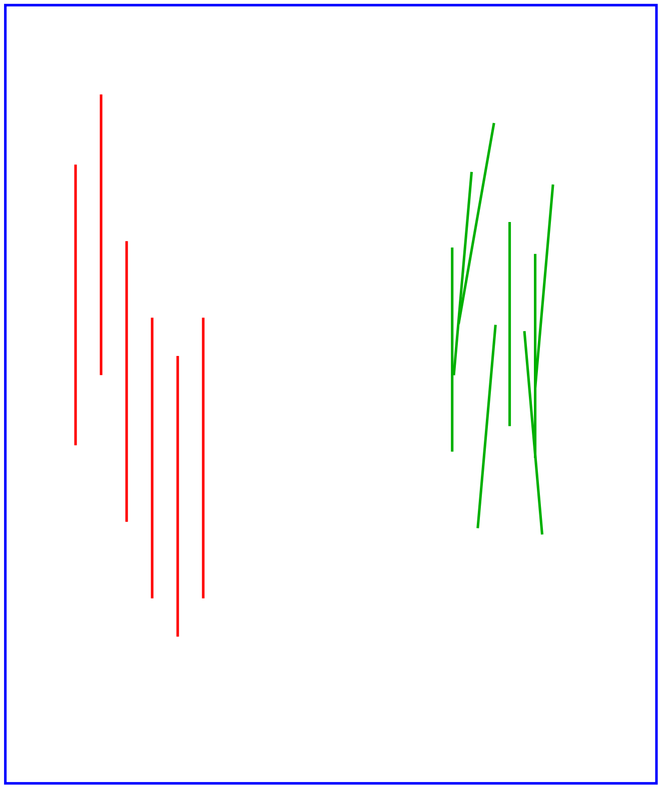

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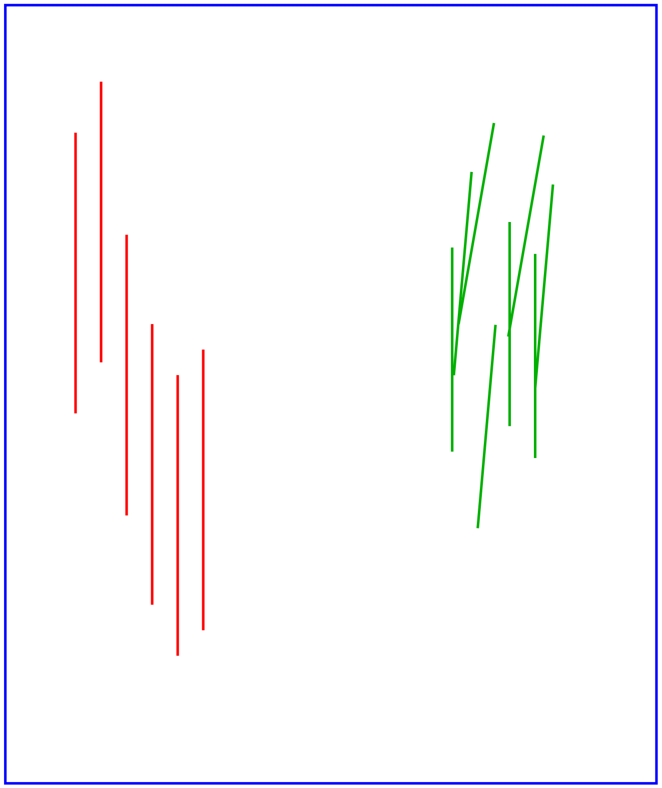

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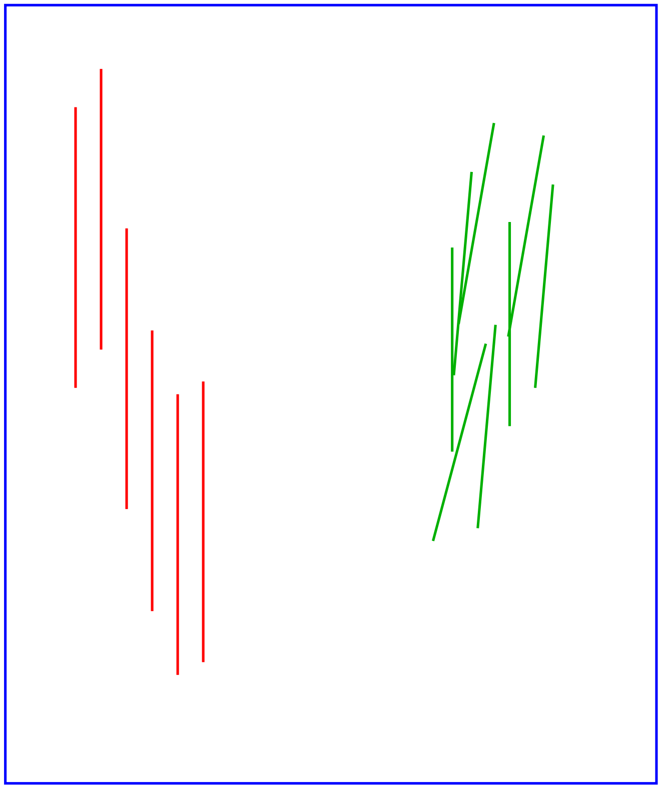

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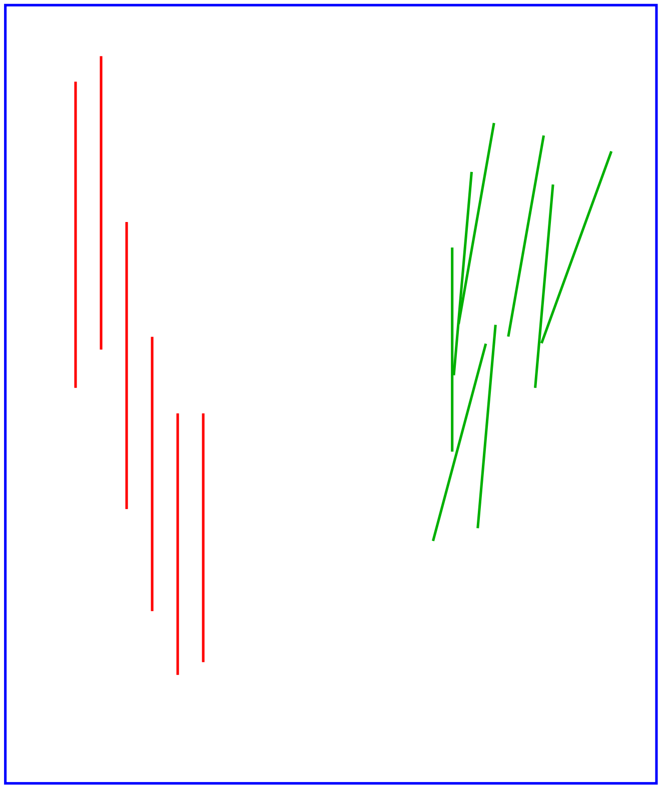

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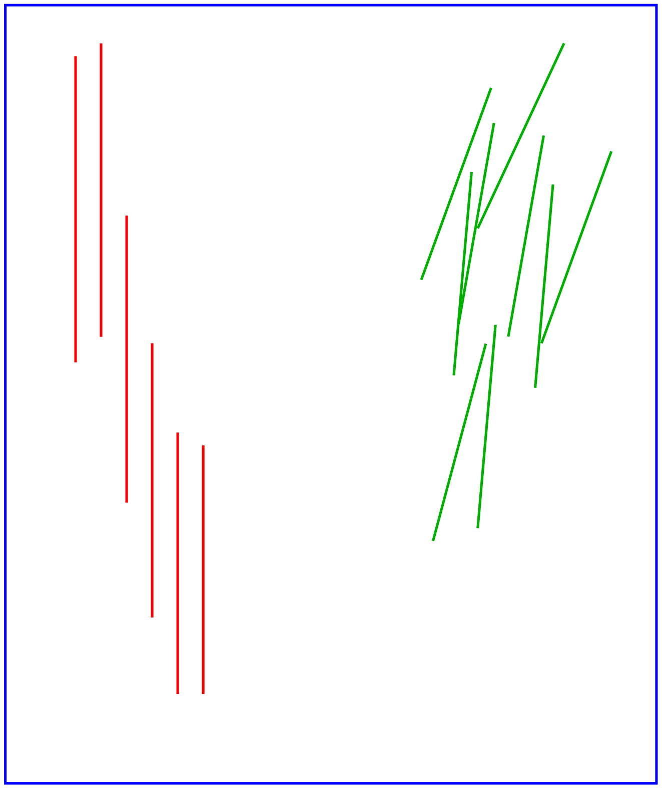

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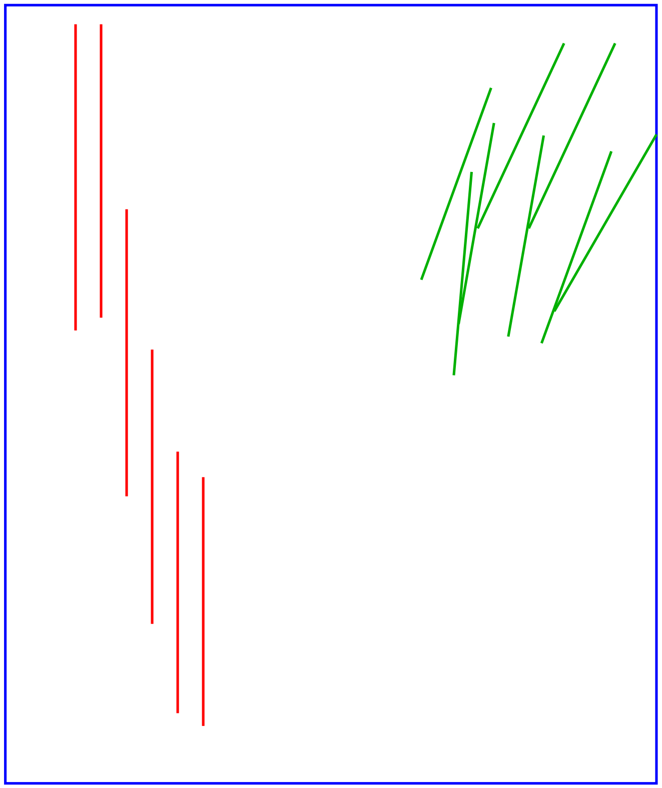

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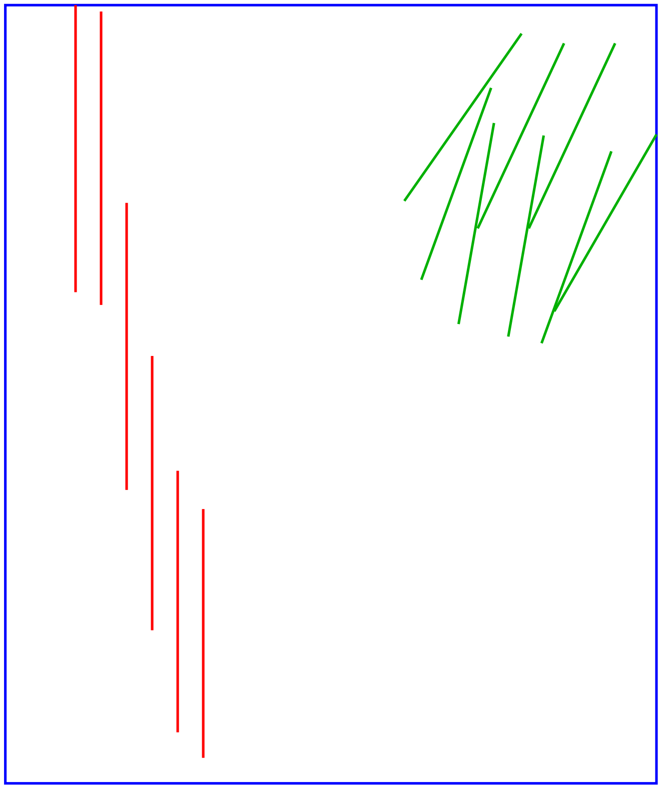

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6. CONCLUSIONS

Symmetry is key!

Any of the mechanisms to produce L/R asymmetry must explicitly use 3 ingredients:

dorsal/ventral asymmetry, or (in cell) **in/out** w.r.t membrane.

anterior/posterior asymmetry, or (in cell) **polarization**,

and **helicity** coming from the microscopic chirality of molecules.

Chirality can enter by

1 **screw mechanisms**: translation along **long helical fiber**
→ convert to rotation around axis
  (a) (processive?) motors
  (b) fiber-fiber collisions
  (c) changes of pitch of fiber

2 **membrane-fiber** binding proteins

Why arrays of long fibers? why membrane? why active motors?

Half-baked thoughts.

Long fiber array: make analogy to the Hall effect in E&M. Electrons must have a sufficiently long free path (in the absence of field) in order to see an appreciable deviation before their next collision. Similarly, we need a consistent direction (in the bulk of wherever the action is), which is easiest to arrange with a self-organizing array of fibers.

Membrane: Need to define a second direction, fixed in absolute coordinates. In the cytosol, that's hard! Along the membrane, you get one axis for free. (Note: either one membrane is different from the opposite, as in a animal embryo (nearly planar), or you produce a twisting more than an axis, as in plants and some embryos)