Lessons from *Trichoplax adhaerens*:

The Emergence of Tissue in Evolution

Axel Krinner

IZBI, University of Leipzig

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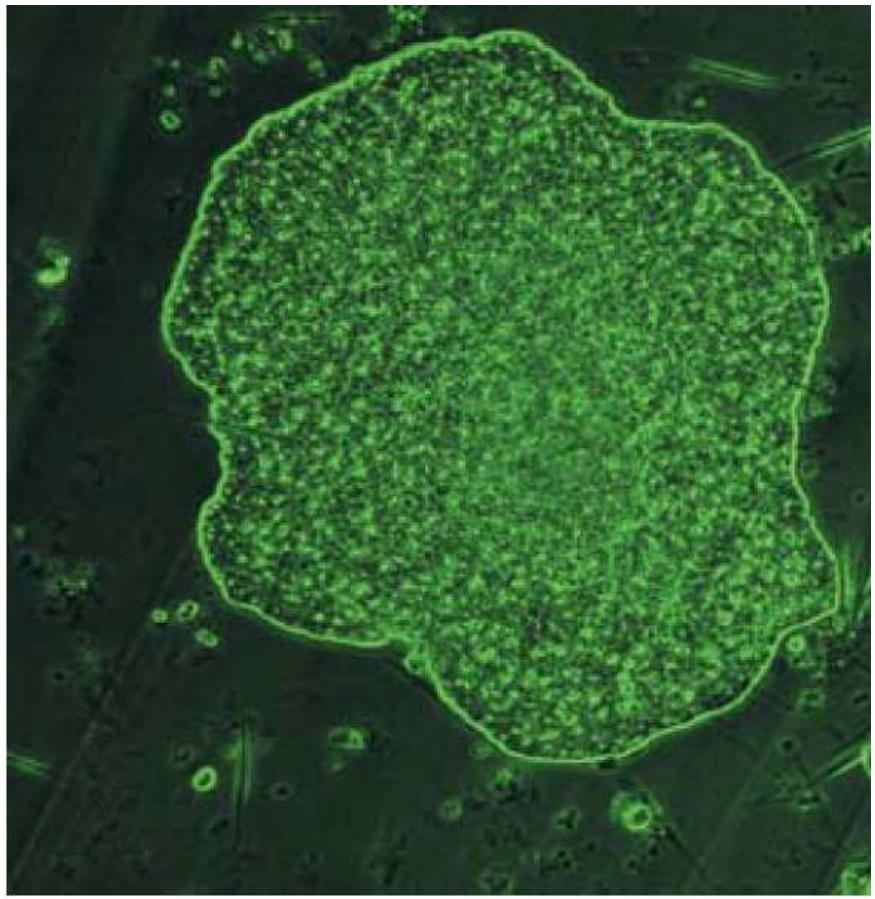
Outline

Hi, I'm *Trichoplax adhaerens*

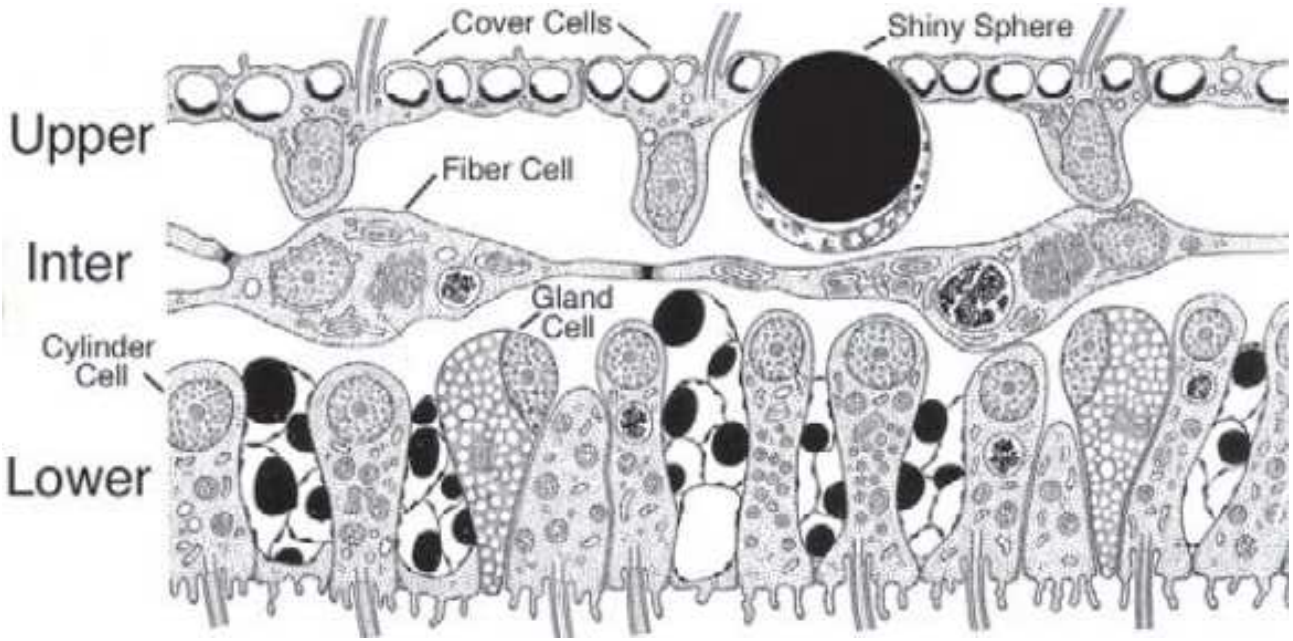


An FITC stained *Trichoplax*.

I'm a nice, hairy guy!

- Name: *Trichoplax adhaerens*, which can be translated as sticky, hairy plate.
- Age: Probably a couple of (tens of hundreds of) thousands of years.
- Size: About 2mm in diameter, but only 25μm in thickness. A more sexy body size occurs during reproduction: up to 20mm in length.
- C.V.:
  - First contact to scientists in 1883 with F.E. Schulze, but the relation ended in a cruel misinterpretation of myself.
  - I was hiding in the depth of the oceans for half a century to recover.
  - The 70's: time for my revenge. I was going to challenge scientists again. And it's still going on.

And this is my interior



The four classically recognized cell types of Trichoplax: cover cells of the upper epithelium, fiber cells of the intermediate layer, and cylinder cells and gland cells of the lower layer.

How I behave

- Motion:
  - Smooth gliding on surfaces using my ciliated ventral epithelium cells.
  - Pulsing shape changes using my fiber cells.
  - Brownian-particle like movement.
  - Floating weightless in the sea water.
- Feeding:
  - Small algae like cryptomonad to cyanobacteria.
  - I love crawling on my meal and the gland cells digest it extracorporeal.
  - Sometimes I also feed myself by transepithelial cytophagy. I open small apertures in my dorsal epithelium and make my food enter the interior by ciliated agitation and is taken up by the fiber cells.

How I reproduce

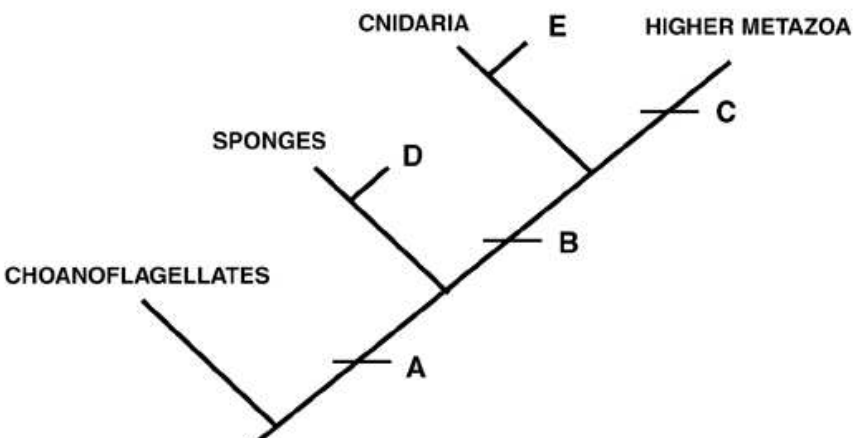
- Reproduction:
  - If a part of myself wants to go into another direction, I simply divide.
    No, it is supposed to depend on the ratio marginal cells/interior cells.
  - Sometimes I want to discover new habitats, so I start the formation of swarmers at my dorsal surface, which leave floating.
  - Probably I must have sex, because in the laboratory I produce female gametes, but they do not overcome the 64 cells stadium
    $\Rightarrow$ Life cycle remains enigmatic.

I am enigmatic

- I am the most primitive metazoan, incorporating the most simple bauplan: no organs, nerve or muscle cells, symmetry or axis; the only polarity is the dorsal-ventral one.
- I have the smallest metazoan genome ($\sim$ 40Mb = 3$\times$yeast gnome).
- I have epithelial cells without a basal lamina, and I do not possess any extra cellular matrix, which means no metazoan histology.
- I lack nerve cells, but I produces some neuro transmitter and express nerve related genes, both known from the cnidarian nervous system.
- I am the only representative of my phylum, because I do not not fit the patterns of cnidarian, porifera, and ctenophora.
- You can not observe me in my kingdom, I simply hide.
  $\Rightarrow$ ecology remains enigmatic, too.

Some more information

- There are many very different (on a molecular level), but by other means indistinguishable brothers in all (sub)tropical waters around the world.
- The analysis of molecular data on allelic varation does not give support of either sexual or vegetative reproduction.
- Phylogenetic insights are expected from the unveiling of the life cycle.
- Molecular approaches to phylogenetic positioning remain unsuccessful.
- rRNA studies created conflicting hypotheses



Confusion in the tree of lower metazoan: *Tricoplax* might be placed at A,B or C, while the other missing group ctenophora is possibly found in B or C. Morphological and most recent analyses indicate the basal site A for *Trichoplax*.

Why modelling *Trichoplax*?

To answer the question "What is a meatzoan?" it is intriguing to model some metazoan. This may be the framework of answering morphological questions *in silico*.

The most natural way to approach this topic is to start modelling of the simplest known metazoan: *Trichoplax*. Understanding the basal organisation patterns will clarify basic principles of the more complex structures.

Reported phenomena are the basis of modelling

Modelling always needs some information on observed phenomena in order to supply a framework. *Trichoplax* has shown:

- **Cell sorting**: After lysis cells sort into the original patterns. If the right ratio of functional cells is ensured, a living animal is produced again.
- **Motion**: Many physiological studies of movement from the 60's.
- **Vegetative reproduction**: Dividing and formation of swarmers.
- **Wound healing**: After cutting or punching a hole into *Trichoplax* wounds are closed by contraction of the interior.
- **Chopping into pieces** will produce several individua, if guaranteed, that an existential ratio of functional cells is given.

Approach

Off-lattice individual cell based modelling using a force based method:

- Every cell is modelled as an individuum, with certain characteristics like
  - position
  - orientation
  - age
  - elasticity
  - adhesion moecule density on the surface
  - reaction network(s) to control the above mentioned parameters

- Information has to be
  - collected form the immediate environment
  - processed via reaction networks

Equations of motion for the force based simulation

The center of mass of Cell i is described by a Langevin equation:

$$\Gamma_{cs}\frac{A_i^{cs}}{A_i}\frac{d\vec{x}_i}{dt} + \sum_{<j,i>}\Gamma_{cc}^{ij}\frac{A_i^{cc}}{A_i}(\vec{v}_i^{\,ij} - \vec{v}_j^{\,ij}) = \sum_{<i,j>}\vec{F}_{ij} + \vec{f}_i,$$

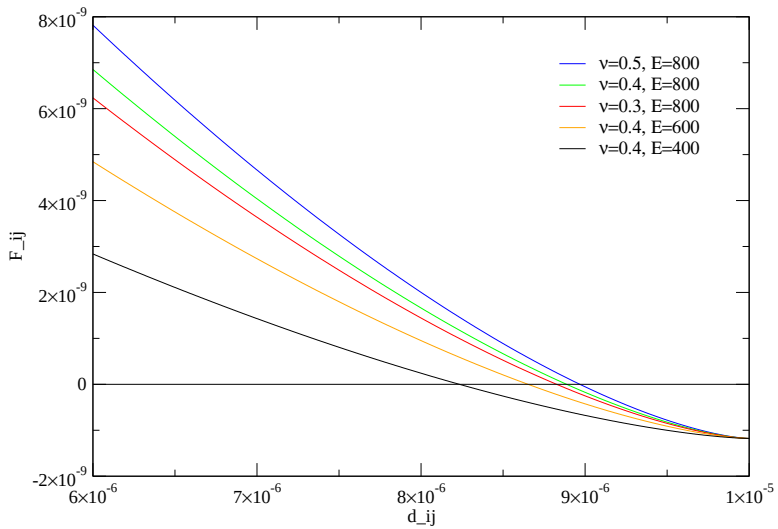
where $\vec{v}_i^{\,ij} = \frac{d\vec{x}_i}{dt} + \vec{\Omega}_i \times \vec{r}_i^{\,ij}$, $\vec{\Omega}_i = \vec{a}_i \times \frac{d\vec{a}_i}{dt}$ and $\vec{f}_i$ is a stochastic force, represented by white noise.

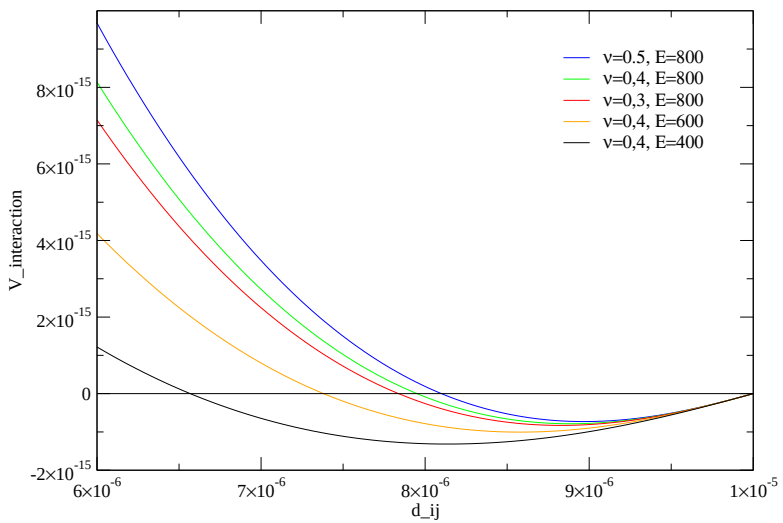
Remark: In general all forces also enter as torques in an analogue equations for the orientation, which is not needed for the first step of cell sorting.

Interaction

The cell-cell interaction is modelled by a slightly modified Hertz model, which includes an adhesion term:

$$V_{interaction} = V_{Hertz} + V_{adhesion}$$
$$= \frac{8}{15} \frac{1}{\frac{1-\nu_1^2}{E_1} + \frac{1-\nu_2^2}{E_2}} \sqrt{\frac{R_1 R_2}{R_1 + R_2}} (R_1 + R_2 - \delta)^{3/2} - \pi \varrho V_{sb} \frac{R_1 R_2}{R_1 + R_2} \delta.$$





a) Interaction force and b) potential for a variety of elastic constants.

One Simulationstep

1. Setting the system of equations (Contact Areas, Friction Coefficients, Deterministic and Random Forces).
2. Soving the system using the Euler method and "Einzelschrittverfahren".
3. Updating all positions and related parameters.

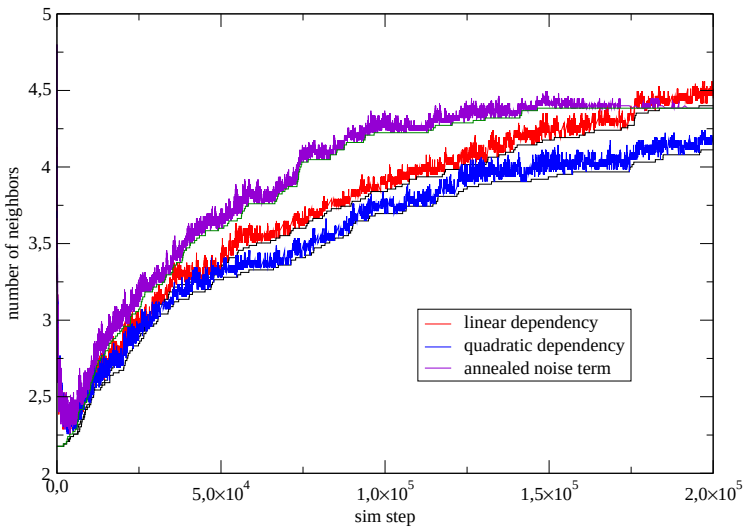
Cellsorting - First Simulations

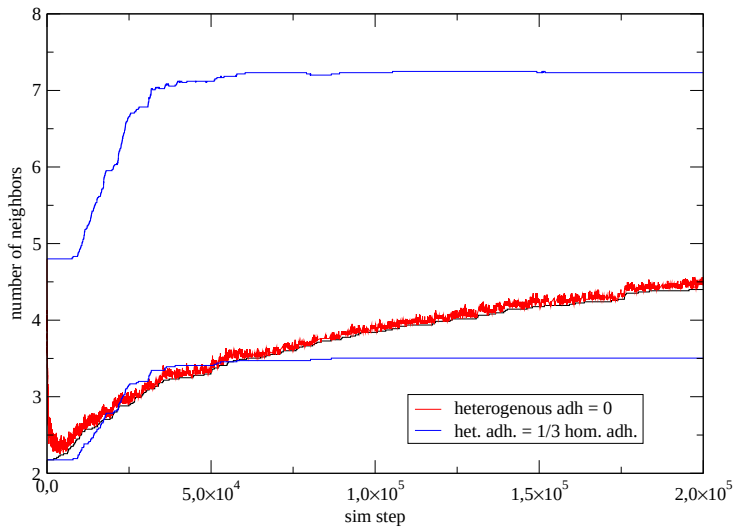
1. Differential adhesion only.
2. Regulation of the stochastic force via the number of contacts.

$\Rightarrow$ Still leads to separated clusters. Not sufficient

Cellsorting - First Simulations

Initial configuration is a cubic lattice filled with 125 cells of random type, where only two cell types occur.





Varying number of neighbors (total and equal neighbors) as a measure of sorting

Further steps

Improvment of the cell sorting model by inclusion of

- long range interaction
- (a) simple reaction network(s) in the cell.
- a reaction-diffusion equation in order to model chemotaxis.
- If necessary, deformable cells, e.g. via triangulation.

Somewhere later on the working plan:

- Modelling of *Trichoplax* as a sandwich of monolayers.
- Fiber cells will be modelled as a spring network.
- What happens if the constitution of particular cell types is suppressed?
- Many other ideas, realization depends on feasibility

Summary

- *Trichoplax* is an ideal model organism to unveil basic principles of tissue organization due to its simplicity.
- It may serve as a connection between the molecular regulatory and the phenotypic level.
- Thus the main question is: In which cases purely physical processes are sufficient to guarantee tissue organization, and where regulatory control mechanisms are necessary?
- Directed movement is essential for modelling living cells.
- Information of the immediate surrounding seems to be insuffictient to model cellsorting.