Morphogenesis: pattern formation, growth, and cell movement
Morphogenesis: pattern formation, growth, and cell movement
“what about the horse part”

LAST TIME

Classical models of (pre)pattern formation in FIXED domain (except limb bud)

Themes: Hypothesis vs Search image; supervised modeling; evolutionary drift in mechanism/trajectory but conserved/converged outcome

TODAY

Morphogenesis ss
Include tissue growth, cell movement/ Tissue (de)formation

• Segmentation
  – Clock & wavefront model (imposed posterior elongations)
  – Elongation by segmentation

• Limbbud morphogenesis by differential cell growth rate possible/compatible with measurements?

• Multicellularity “by coming together” “from single cells to multicellular organism”
  through signaling, chemotaxis and differential adhesion
  (from data intensive to behavior intensive models)
clock and wavefront mechanisms for segmentation from temporal to spatial pattern
Cooke and Zeeman 1976 a common mechanism (?) in segmentation development in many organisms

clock:
internal cellular oscillations, phase synchronized between cells

wavefront:
competence wave moving from anterior to posterior at constant speed
Various mechanisms for wave arrest
FGF gradient slows wave (‘maturation’)

also no gradient
resistent to noise by coupling
proposed “implementation” as 3 tier mechanism in somitogenesis

single cell oscillator: delayed auto-feedback systems
delay determines number of segments
deed: intron deletion speeds up the clock
Harima et al Cell 2012
neighbour synchronization: with delay: longer period
reinvented or conserved, which genes oscillate?
GO terms: signalling and transcription

Krol et al Development 2011 orthologs
Only 2 overlapping orthologs involved in segmentation clock

first estimate:

after filtering:
Only 2 orthologs: but members of 3 pathways in all

(this analysis first to find member WNT pathway in zebrafish)
conclusion: very high plasticity!

Only small subset of the 3 pathways oscillate: enough for functional oscillations? “just in time assembly”

Similar (non) conservation pattern in cell cycle mechanisms yeast and pombe

Conserved HER/HES delayed oscillator also in medaka, Xenopus, and invertebrates (e.g. cockroach)!!

Segmentation lost? reinvented?
Is segmentation “the same” in the different organisms??

*RA knockout leads to asymmetric somatogenesis which is different for different vertebrate species*

**HOW/WHY??**

Model in more detail to find out which difference in regulatory network may explain difference in phenotype of RA knockouts
Vroomans & ten Tusscher 2017, Modelling asymmetric somitogenesis: Deciphering the mechanisms behind. species differences
Vroomans & ten Tusscher 2017:
Indeed, our results suggest that rather than focussing on a catch-all mechanism in all vertebrate species and assuming that species differences merely reflect neutral developmental systems drift, we should keep an open mind for the possibility of functionally significant species differences.

OR

Side-effects of neutral drift
But what about *Drosophila*?

2 (3) mechanisms in insects short vs long germband (+intermediate)

clock-wavefront (sequential) mechanism might be ancestral - reinvention of simultaneous mechanism long germband??
convergent extension, morphogenetic cell movement common to insects, fish, frogs,( mammals)

Elongation by intercalation but by different mechanisms, eg
- (Drosophila intercalation by contraction of those parts of the membrane that have a dorsal-ventral orientation)
- Xenopus: dorsal mesodermal cells polarize and change their adhesive properties; cells then crawl between each other in a zipper-like process (intercalation) axial adhesion
- Zebrafish: directed migration to the dorsal axis and intercalation follow a gradient in cadherin activity towards the central axis graded adhesion
Segment-Specific Adhesion as a Driver of Convergent Extension Renske M. A. Vroomans et al 2015
Convergent extension (CE)

(often) after segmentation;
How is segmentation conserved?

Segment specific adhesion
(here minimal)
Segmentation by itself sufficient for CE
(AND needed for CE (xenopus, drosophila)
more “realistic”: extension to posterior only
same results
For sorting strong persistence is needed; Weak persistence is sufficient in sorted tissue (WT)
Question: can limb bud MORPHOGENESIS be explained by gradient based differential cell proliferation?

The Role of Spatially Controlled Cell Proliferation in Limb Bud Morphogenesis Bernd Boehm1, Henrik Westerberg1, Gaja Lesnicar-Pucko1, Sahdia Raja1,2, Michael Rautschka1, James Cotterell1,2, Jim Swoger1, James Sharpe1,3* PLOS BIOL 2010
Measurement

Of 3D shape at 2 developmental stages

Of mitotic frequencies in different regions of the bud
colour cell cycle specific proteins
calculated cycle frequencies

DO these 2 measurement FIT?

(Is differential proliferation sufficient to explain growth/morphogenesis?

NO...
Finite element simulation of measured growth rates

<table>
<thead>
<tr>
<th>growth rates</th>
<th>tissue displacement</th>
<th>simulated shapes</th>
<th>comparison with real development</th>
</tr>
</thead>
<tbody>
<tr>
<td>dorsal view</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>posterior view</td>
<td>spatial distribution of ( S )</td>
<td>predicted shape change</td>
<td>real shape change</td>
</tr>
</tbody>
</table>

- \( 0.03 \) to \( 0.07 \)
Failure due to mistakes in growth rates measurements? Do growth rates exist such that shape emerges?
Yes differential growth CAN generate bud morphogenesis
BUT only for VERY different proliferation patterns (+ shrinkage)
conclusions

- Nice (because negative result!)
- Their hypothesis: directed cell movement plays a role

Use measured growth + fitted outward force (representing cell movement)
“How to compute an organism
Multilevel modeling of Morphogenesis
bridging levels of organization

Model premises

- Target morphogenesis ss (not only pattern formation)
- Cell basic unit (growth, division, movement, ...)
- Cell is NOT point, bead, homunculus
- Cells are deformable highly viscous objects
- Genes act through cells ’with a dynamics of their own’

use CPM as simple but basically correct representation of a cell
Finding Sufficient Conditions for complex behavior using only (subset of) known processes allowing many (open set) different observations

explicit 2-level model for implicit multilevel behavior

Dd morphodynamics:

From single cells (amoebae) to multicellular ’individuals’ with ’new’ ways of sensing and metamorphosis to groups of those

Dictyostelium phylogeny

Early offshoot:
shares protein domains otherwise exclusive for plants, fungi, and animals
Lifecycle Dictyostelium

- Mature fruiting body
- Spores
- Free-living amoebae
- Aggregation induced by starvation
- Slug formation
- Slug formation
- Cell division
- Germination
- Fruiting body formation
- Migration and direction
- Images of different stages of the lifecycle from 1 min 28 sec to 2 min 0 sec.
Goldbeter-Martel model of cAMP signaling

The Goldbeter-Martel model of cAMP signaling is described by the following equations:

\[
\frac{d\rho}{dr} = -f_1(\gamma)\rho + f_2(\gamma)(1 - \rho),
\]
\[
\frac{d\beta}{dr} = s_1\Phi(\rho, \gamma) - \beta,
\]
\[
\frac{d\gamma}{dr} = s_2\beta - \gamma.
\]

where

\( \rho \) = fraction of receptor in active state,
\( \beta = [\text{cAMP}]_{\text{intracellular}}/K_R, \)
\( \gamma = [\text{cAMP}]_{\text{extracellular}}/K_R, \)
\( t = k_1 \times \text{time}, \)

and

\[
f_1(\gamma) = \frac{1 + s_1\gamma}{1 + \gamma}, \quad f_2(\gamma) = \frac{L_1 + s_2\gamma}{1 + c_2\gamma},
\]
\[
\Phi(\rho, \gamma) = \frac{\lambda_1 + \rho^2}{\lambda_2 + \rho^2}, \quad Y = \frac{\rho\gamma}{1 + \gamma}.
\]

The parameters appearing in system (1)–(3) are explained and estimated in tables I and II; refer also to fig. 2.

Parameter set A in table II was used by Martiel and Goldbeter [16] to model autonomous oscillations of cAMP in stirred suspensions of Dictyostelium cells. The numerical solution of the
Martiel & Goldbeter, 1987 (cont'd)

\[ R \xrightleftharpoons[k_{-1}]{k_1} D \]
\[ R + P \xrightleftharpoons[a_1]{d_1} RP \]
\[ D + P \xrightleftharpoons[a_2]{d_2} DP \]
\[ RP \xrightleftharpoons[k_{-2}]{k_2} DP \]
\[ 2RP + C \xrightleftharpoons[a_3]{d_3} E \]
\[ E + S \xrightleftharpoons[a_4]{d_4} ES \xrightarrow[k_4]{d_4} E + P_i \]
\[ C + S \xrightleftharpoons[a_5]{d_5} CS \xrightarrow[k_5]{d_5} C + P_i \]
\[ P_i \xrightarrow[k_i]{d_5} \]
\[ P_i \xrightarrow[k_i]{d_5} P \xrightarrow[k_e]{d_5} \]
\[ S \xrightarrow[k_{1}]{d_5} \]
Parameter estimates of Goldbeter-model (Tyson 1989)

Table II
Model parameters.

<table>
<thead>
<tr>
<th>Name</th>
<th>Definition</th>
<th>Values used in calculations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_1$</td>
<td>$k_1/k_2$</td>
<td>10</td>
</tr>
<tr>
<td>$L_2$</td>
<td>$k_2/k_3$</td>
<td>0.005</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>$k_2/k_3$</td>
<td>18.5</td>
</tr>
<tr>
<td>$e$</td>
<td>$K_2/K_D$</td>
<td>3</td>
</tr>
<tr>
<td>$a$</td>
<td>[ATP]/K_m</td>
<td>10</td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>$v_{m}/K_m$</td>
<td>$10^{-4}$ $10^{-3}$ $10^{-3}$ $6.7 \times 10^{-4}$</td>
</tr>
<tr>
<td>$\lambda_2$</td>
<td>$v_{m}/K_m$</td>
<td>$0.26$ $2.0$ $2.0$ $1.0$</td>
</tr>
<tr>
<td>$s_1$</td>
<td>$v_{m}/K_m$</td>
<td>$690$ $950$ $950$ $80$</td>
</tr>
<tr>
<td>$s_2$</td>
<td>$v_{m}/K_m$</td>
<td>$0.033$ $0.05$ $0.05$ $0.13$ $0.13$</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>$v_{m}/K_m$</td>
<td>$20$ $47$ $47$ $47$ $28$</td>
</tr>
<tr>
<td>$\epsilon'$</td>
<td>$v_{m}/K_m$</td>
<td>$0.014$ $0.019$ $0.019$ $0.005$ $0.005$</td>
</tr>
<tr>
<td>$\epsilon''$</td>
<td>$v_{m}/K_m$</td>
<td>$0.0067$ $0.01$ $0.01$ $0.01$ $0.024$</td>
</tr>
<tr>
<td>Time-scale</td>
<td>$1/k_3$</td>
<td>$28$ $28$ $8.3$ $28$ $17$</td>
</tr>
<tr>
<td>Space-scale</td>
<td>$(k_4D)^{1/2}/k_3$</td>
<td>$10$ $8.2$ $4.5$ $8.2$ $4.1$</td>
</tr>
</tbody>
</table>

*All parameters (except the last two) are dimensionless. The time-scales are given in min, the space-scales in mm. When all four sets have the same value of a parameter, the symbol $*$ is used.

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> spirals
simplify dynamics - add cells:
Dd life cycle by excitable medium and differential adhesion

excitable medium
+ differential adhesion
Lifecycle of Dd by chemotaxis and adhesion

aggregation

streams

orientation

culmination
### Dd morphodynamics:
**multiple causes and multiple effects**

<table>
<thead>
<tr>
<th>Process</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregation</td>
<td>streams if wave propagation dep on density faster movement in streams</td>
</tr>
<tr>
<td>Mount/slug slug</td>
<td>cell sorting by differential adhesion AND chemotaxis slug shape attractor of energy minimization vs inward movement (wave shape) taxis (thermo- photo-taxis) via NH3 effect on excitability) slug shape and wave shape bi-directional mutant direction of movement vs momentum</td>
</tr>
<tr>
<td>culmination</td>
<td>needs dynamic cell differentiation downward movement of stalk cells caused by peristalsis caused by upward movement of spore cells pressure waves and wave shape self-correcting and self-terminating</td>
</tr>
</tbody>
</table>
stream formation requires
density dependent speed of cAMP wave propagation
(i.e. fast internal cAMP dynamics)

Why streams?

Fig. 4. Plot of velocity (each point averaged over 10 simulations) against the cell-medium bond energy for prespore amoebae. Given that the amoebae adhere to each other the group will always move faster than a single amoeba. Parameters are as described in the legend to Fig. 1. ■, Group of amoebae; ●, single amoeba.
Cell Sorting much faster in *moving slug* than in *fixed mount*

**WHY?**

Prestalk cells (Yellow) stronger adhesion
Prespore cells (Green)

\[ J_{yy} < J_{gg} < J_{yg} \]

Same chemotactic response

Savill and Hogeweg 1997
EQUAL chemotaxis speeds up sorting
(moving slug instead of fixed mount also faster sorting
(cf Kafer, “go against the flow” (binf4)
Movement Dd slugs: measured bead displacement and calculated force fields
cf Rieu, Baranth, Maeda and Sawada 2005

displacement field
outward directed forces!
stress field
similar forces in model Dd slugs?

Note: forces are (emergent) observables instead of model ingredients!

Can be measured (like in experiments)
cf From energy to cellular forces in the Cellular Potts Model: An algorithmic approach EG Rens, L Edelstein-Keshet - PLoS Computational Biology, 2019

Perpendicular forces expected because:
- wave shape (most concave in middle of slug)
- sideward push because of pressure gradient
conclusions

- Using simplifications which allows multilevel modeling we “can go for the horse part”
- Development as trajectory of dynamical system model minimizes regulation within cells
- Assumption of CPM seem very suitable to describe biological cells
- Relatively few parameters need to be specified; large set of 'new' observables
- Treating forces as observables rather than model assumption allow close comparison with experimental measurements

BUT WHAT ABOUT THE GENES?
Evolutionary “testing” of the model

who wants to be a stalk?, cf Queller
how to come become another dictyosteloid?

multiple levels needed to understand complexity
Who want to become a stalk?

Evolution of cooperation and why cheaters do not take over single gene greenbeard effect

Who depends on phase in cell cycle
Cell adhesion gene csA binds to csA
on agar csA knockouts become spores because wildtype cells have more adhesion -> go to front - become stalk
BUT
in soil csA knockouts are left behind during aggreg. phase

conclusion: who wants to become a stalk

Simple optimality reasoning often flawed

Important role of non-inheritable behaviour

stochasticity

environmental heterogeneity

selforganization
from Dictyostelium to other discyosteliids
Polysphondininium

Polysphondylium violaceum

A.R. Swanson, A Guide to the Common Dictyostelid Slime Molds of
Great Smoky Mountains National Park

continuous redifferentiation prestalk-stalk
sidebranches (polyshondininium)