modeling development (1)
classical models of pattern formation
segmentation patterns
Models of development

Pattern formation $\rightarrow$ shape

shape $\rightarrow$ pattern formation

pattern formation $\leftarrow$ shape

TODAY: pattern formation / segmentation

- Supervised modeling
- Top down modeling:
  - given observed pattern/behaviour $X$ and assumptions $A$
  - CAN $A \rightarrow X$
  - (AND does it generate $X++$)
- Data driven models, quantitative fitting

Theme: specific and/or general mechanisms and/or specific instantiations (?)
development: cell differentiation, pattern formation and morphogenesis

classicly most studied: pattern formation
prepattern $\rightarrow$ cell differentiation $\rightarrow$ morphogenesis

3 most discussed general mechanisms for stationary pattern formation for development

Turing patterns (Turing 1952) introduced term 'morphogen'

Positional information (Wolpert 1969) morphogen gradient - coordinate system

“Clock and wavefront” Cook and Zeeman 1976 temporal oscillation $\rightarrow$ spatial pattern

compare: “pattern is 'default’” however here specific positioning/orientation
Segmented bodyplans

A Overt body segmentation in the Bilaterian tree

Deuterostomia
- Echinodermata
- Hemichordata

Ecdysozoa
- Arthropoda
- Onychophora
- Tardigrada
- Nematoda
- Nematomorpha
- Kinorhyncha
- Priapulida

Bilateria
- Annelida
- Bryozoa
- Sipuncula
- Mollusca
- Nemertea
- Entoprocta
- Phoronida
- Brachiopoda

unsegmented

from Ten Tusscher EPJE

reinventions (?)
generic mechanism?
homologous at molecular, pathway level?
A generic regular pattern formation mechanisms
Turing Patterns

*Can DIFFUSION create patterns from homogeneous state?*

- 2 interacting substances
- stable homogeneous equilibrium in absence of diffusion
- unstable for spatial heterogeneous perturbations
- with diffusion: stable (+ regular) stable patterns
Turing patterns: formal requirements

\[
\begin{aligned}
\left\{ \begin{array}{c}
\frac{\partial A}{\partial t} = D_a \Delta A + f_1(A, I) \\
\frac{\partial I}{\partial t} = D_i \Delta I + f_2(A, I)
\end{array} \right.
\end{aligned}
\]

without diffusion stable:

\[
\text{tr} J = a_{11} + a_{22} < 0
\]
\[
\text{det} J = a_{11} \ast a_{22} - a_{21} \ast a_{12} > 0
\]

with diffusion unstable

\[
\begin{aligned}
\left\{ \begin{array}{c}
a_{11} + a_{22} < 0 \\
a_{11} \ast a_{22} - a_{21} \ast a_{12} > 0 \\
D_a a_{22} + D_i a_{11} > 2 \sqrt{D_a D_i \ast (a_{11} \ast a_{22} - a_{21} \ast a_{12})} > 0
\end{array} \right.
\end{aligned}
\]
simplified requirements

\[
\begin{align*}
\begin{cases}
a_{11} + a_{22} &< 0 \\
a_{11} * a_{22} - a_{21} * a_{12} &> 0 \\
D_a a_{22} + D_i a_{11} &> 0
\end{cases}
\end{align*}
\]

\[a_{11} > 0 \text{ and } a_{22} < 0 \quad \frac{D_i}{|a_{22}|} > \frac{D_a}{|a_{11}|}\]

Diffusion I >> Diffusion A:
short range activation, long range inhibition

Positive feedback system: \((+ +)\)

Variables vary over space in phase:

Activator – inhibitor system: \((+ -)\)
Turing patterns

In 2D:

NB wavelength
Not only regular patterns, but also domain dependence shifting with irregular domains.

Zebra: 'face recognition'

However sometimes “wrong” small domain: spots; large domain only 2 phases

“the stripes are easy, but what about the horse part?”, Turing
Strictly speaking:
Needs homogeneous initial state;
Needs diffusion
Needs large difference in diffusion;

HAS been sought but NOT BEEN FOUND

Less strictly speaking

Needs SOME mechanism of
local activation / longer range inhibition
fish stripes, Kondo-group

“looks like Turing patterns” (stripes)
“looks like turing patterns after ablation”
“short range activation, long range inhibition demonstrated by ablation experiments in pigment cells (no molecular interactions known)”
Early “hypothesis/model/theory”
Drosophila stripes == Turing pattern
WRONG + WRONG methodology

shame on 'us' theoreticians

Stripes: too degenerate pattern
to infer anything

not stable in 2/3D

scaling!
invariance vs uniqueness
de facto:
al all stripes 'own mechanism' of activation
Positional information/ French flag model
Wolpert 1969

“French flag”:
different morphogen concentrations → activate different genes

Alternative attractors:
maintain expression domains when morphogen gradient disappears
Positional information/ french flag problem
Wolpert 1969

Source/sink/diffusion for gradient formation
’read-out’ of concentration – > cell differentiation
(stabilization by mutual inhibition)

french flag problem: how to be scale invariant?

source/sink diffusion is scale invariant!
(but not a likely solution...)

problems: spatial/temporal scaling of diffusion
in tissue: cell boundaries may not allow gradients
how to have precise quantitative readout?
“simple mechanism may not be simple”
noise

“pathways which produce and use positional information”
receptors disturb gradient cf Kerzberg and Wolpert 1998

several potential solutions proposed
early patterning in Drosophila

Model 1: gap gene expression in Drosophila
(pre-gastrulation / pre cellularization)

paradigm system for positional information

Maternal gradient (Bicoid) (measured)
In syncytium stage (no cell walls to pass)

paradigm system for data driven quantitative modeling

Very precise description of pattern in space/time available
Much experimental knowledge about genes involved and their interactin

many papers main authors J. Reinitz anf J. Jaeger; here used:
Manu, .... Reinitz 2009 Canalization of Gene Expression in the Drosophila Blastoderm by Gap Gene Cross Regulation, Pos Biology
J. Jaeger .. Reinitz 2004. Dynamic control of positional information in the early Drosophila embryo Nature
modelled space-time frame

gap gene expression in late stage: black line: modeled area
modeling gene regulation: ODE for each nucleus

\[ \frac{dv_i^a}{dt} = R^a g \left( \sum_{b=1}^{N} T_{i}^{ab} v_i^b + m_i^a v_i^{Bcd} + \sum_{\beta=1}^{N_e} E_{i}^{a\beta} v_i^\beta(t) + h^a \right) \]

\[ + D^a(n) \left[ (v_{i-1}^a - v_i^a) + (v_{i+1}^a - v_i^a) \right] - \lambda^a v_i^a. \]

T interaction between gap genes; m interaction with biccid; E interaction of gap genes with time varying external factors; \( \lambda \) decay; \( D \) diffusion

interphase: production, diffusion and decay;
mitosis: only diffusion and decay
division: nuclei divide, inherit state,
distance between them halved

g(u^a) = \frac{1}{2} \left[ \left( \frac{u^a}{\sqrt{(u^a)^2 + 1}} \right) + 1 \right]
“data driven modeling”: massive fitting using simulated annealing

use: ’known genes’, initial conditions, spatial/temporal variation of non-regulated regulators.

Fit model output in all M nuclei, for all genes, at all N time-points for which data are available.

\[ E = \sum_{\text{all } a, i, t, \text{ and genotypes for which data exists}} (v_i^a(t)_{\text{model}} - v_i^a(t)_{\text{data}})^2 + \text{(penalty terms)} \]

Do this Z=65 times gives Z different outcomes; and select good fits, no major patterning defects, no known regulatory mistakes (23/65) similar networks
used example of 'good' network

<table>
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<tr>
<th>Target gene $a$</th>
<th>$bcd$</th>
<th>$cad$</th>
<th>$tll$</th>
<th>$hb$</th>
<th>$Kr$</th>
<th>$gt$</th>
<th>$kni$</th>
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<td>0.004</td>
<td>0.003</td>
<td>0.021</td>
<td>-0.001</td>
<td>0.022</td>
<td>-0.112</td>
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<tr>
<td>$Kr$</td>
<td>0.118</td>
<td>0.021</td>
<td>-0.203</td>
<td>-0.026</td>
<td>0.035</td>
<td>-0.042</td>
<td>-0.062</td>
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<tr>
<td>$gt$</td>
<td>0.256</td>
<td>0.023</td>
<td>-0.011</td>
<td>-0.028</td>
<td>-0.202</td>
<td>0.007</td>
<td>0.003</td>
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<tr>
<td>$kni$</td>
<td>0.012</td>
<td>0.020</td>
<td>-0.187</td>
<td>-0.082</td>
<td>0.000</td>
<td>-0.017</td>
<td>0.013</td>
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</table>

<table>
<thead>
<tr>
<th>Parameter</th>
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<td>15.000</td>
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<td>15.000</td>
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<td>$t^{1/2}$</td>
<td>9.529</td>
<td>15.908</td>
<td>9.438</td>
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</table>

above: model: early - late; below av. exp. early-late
classical question
developmental patterning very precise, despite

differences in

e.g. size of embryo or gradient noise

Manu et al 2009: is due to regulatory circuit.
robustness to variation in bicoid gradient
robustness to size variation (20%) without cross regulation gap-genes
model also reproduces shifts in expression patterns over time

Jaeger et al 2004 op.cit
neutral drift: change parameters, developmental trajectory but very similar final phenotype

Gap Gene Regulatory Dynamics Evolve along a Genotype Network Anton Crombach,*,,1,2 Karl R. Wotton,,1,2 Eva Jimenez-Guri,1,2 and Johannes Jaeger*, MBE 2016
discussion/conclusions

Fitting not very robust:
alternative “as good” fits with even opposite signs of interaction
(filtered to agree with experimental knowledge)

because of shifting “better” fitting because less degenerate supervised models: Fits

++ = scaling property and noise reduction

++ insight in evolutionary drift in conserved patterning
Positional information (?):

yes - gradient given and provides “coordinate system”

no - not simple concentration readout
readout itself ’makes the pattern’

scale invariant (tolerant) because of regulation / not invariant
bicoid gradient
However there appears to be a common mechanism in segmentation development in many organisms clock and wavefront mechanisms from temporal to spatial pattern

Cooke and Zeeman 1976

clock:
internal cellular oscillations, phase synchronized between cells

wavefront:
competence wave moving from anterior to posterior at constant speed
proposed “implementation” as 3 tier mechanism in somitogenesis

single cell oscillator: delayed auto-feedback systems
delay determines number of segments
indeed: intron deletion speeds up the clock
Harima et al Cell 2012
neighbour synchronization: with delay: longer period
gradients which appear to play a role

“arrest” can be autonomous (Hopf or other bifurcation or extern because of bistability Goldbeter 20.. 

\[ \text{similar result} \] resistant to noise 

distance governed by posterior rate of growth.
reinvented or conserved, which genes oscillate?

GO terms: signalling and transcription
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)
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
Table 3.1. Phenotypes of model organisms during somitogenesis

<table>
<thead>
<tr>
<th>organism</th>
<th>pErk dynamics</th>
<th>oscillating pathways</th>
<th>left-right phenotype</th>
<th>Slower osc</th>
<th>FGF8</th>
<th>delay (somite nr)</th>
<th>somite size diff</th>
<th>return to symmetry</th>
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<tbody>
<tr>
<td>chick</td>
<td>smoothly retracting</td>
<td>FGF, Wnt, Notch</td>
<td>right side</td>
<td>symmetric, more anterior</td>
<td>no; left somites smaller</td>
<td>yes</td>
<td>unclear</td>
<td></td>
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<tr>
<td>zebrafish</td>
<td>retract in jumps</td>
<td>Notch</td>
<td>right side</td>
<td>right side more anterior</td>
<td>right side 2-3 somites delayed</td>
<td>no</td>
<td>yes</td>
<td></td>
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<tr>
<td>mouse</td>
<td>oscillates</td>
<td>FGF, Wnt, Notch</td>
<td>right side</td>
<td>right side more anterior</td>
<td>right side 2-3 somites delayed</td>
<td>sometimes</td>
<td>yes</td>
<td></td>
</tr>
</tbody>
</table>

Vroomans & ten Tusscher 2017, Modelling asymmetric somitogenesis: Deciphering the mechanisms behind. species differences
Generic mechanism vs species specific differences
neutral drift or functional significant???

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??
ZOOM IN (1): bicoid gradient

Measured bicoid gradient (input of previous model) “nice” i.e. constant over time exponentiel shape characteristic length ($\lambda = \sqrt{D_{\text{eff}}/\alpha}$)) spanning the the embryo

HOWEVER: measured $D_{\text{eff}}$ differ orders of magnitude (too large or too small)

ARE $D_{\text{eff}}$ and $\alpha$ constant over time?

NO... # nuclei increase; bicoid shuttles between nuclei and cytoplasm; only degraded in cytoplasm ...

cf Multiscale modeling of diffusion in the early Drosophila embryo. Sample C, Shvartsman SY. PNAS 2010

HOW is the “right” bicoid gradient established / maintained?
Syncytium not homogeneous (time/space)! (furrows in membrane during mitosis)

Particle simulation of Brownian motion of particles given this geometry

Note: no other inhomogeneities implemented (nuclei etc.)
impact of furrows on diffusion, short timescales
Converges to normal diffusion with lower effective diffusion

\[
\frac{D_{\text{eff}}}{D_C} \approx \left(-0.0234 \frac{\omega}{\omega^*} - 0.0769\right)L^2 + \left(0.0369 \frac{\omega}{\omega^*} - 0.9681\right)L + 1,
\]
In vivo measurement of furrow dynamics and localized diffusion
This model predicts reduced diffusion during mitosis.

Previous modeling (Sample 2010 op. cit., DeLotto et al 2007 Development) predict reduced diffusion in interphase (nucleo-cytoplasmic shuttling).

Two effects may compensate each other (Daniels et al) (However not explicitly modeled).

Conclusion

Simple diffusion is not so simple
May need a lot of fine tuning to become “normal”
50 most abundant macromolecules => 85% content in weight

Steric models of all

Simulate 1008 molecules (including 8 GFP’s)

Concentration 275g/L

3 models:
Steric only: crowding phenomena
Long range electrostatic interactions
Short range potential to mimic hydrophobic interactions

Is D of tracer molecules 'correct'?
Who meets whom (and how often)
Can we explain stability of folded proteins?
macromolecules in the system

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Diffusion of GFP: model vs experiments + extrapolation to other proteins

in full model: parameter-fit of “well depth of Lennart Jones potential” $\epsilon$ allows fitting experimental data
Who meets whom? 15μs
change in folding stability because of cytoplasmic environment

Exp data available of Lrp construct: no change
Crabp (retinoic acid binding protein): destabilized

*insert folded/nonfolded form at random location in snapshot of simulation, measure thermodynamics stability*

exp. verified obtained for other prot.

energy calculation of full model in full model good agreement with experiment
conclusions

It is crowded!
Nevertheless fairly much movement
’diffusion’ ca 10% from diluted environment
changes neighbours frequently even in $15\mu s$
stabilization/destabilization dependent on protein

......... many observations possible

spectacular that it is possible

but 1 year real simulation time /run of $20\mu s$