Course 3: From single to collective cell durotaxis

Thomas Lecuit

chaire: Dynamiques du vivant
Most models for substrate-based cell guidance have relied on the biochemical nature of the cues delivered to the cell. We propose here that the physical characteristic, namely the resistance to displacement of the substrate, is an additional cue that cells can use to orient during migration.
**Hypothesis:** rigidity as a guidance cue

Orientation of traction forces in response to environmental cues. (a) When there are no external cues, traction forces (small arrows) in the front of the cell are oriented rearward and traction forces in the back of the cell are oriented forward. For net forward movement to occur (large arrow), the forces in the front of the cell must exceed the forces in the rear by an amount equal to the fluid drag, which is the force imposed on the cell by the surrounding media. (b) When a migrating cell encounters an appropriate molecular cue in its environment (indicated as fibronectin (FN)), the receptors that recognize the cue associate with force-generating components of the cytoskeleton. The increase in traction force generated at that side of the cell (small arrows) causes the cell to turn (large arrow) towards the location of the ligand. (c) The stiffness of the extracellular matrix (ECM) in the cellular environment might also orient the direction of cell migration. The binding of integrins to pre-stressed ECM fibres (straight lines; relaxed ECM shown as wavy lines) would selectively strengthen the linkage between those receptors and the force-generating cytoskeleton at that side of the cell. The localized increase in traction forces (small arrows) causes the cell to turn (large arrow) towards the rigid substrate.

Durotaxis: cell guidance by the rigidity of substrate

Traction index depends only on gradient strength, not on absolute stiffness

Gradient of ~ 0.5-4 kPa/cell length depending on cell type (L= 25µm-100µm)

Vascular smooth muscle cells

Structure and composition of Focal adhesions

PALM/STORM microscopy

Clutch mechanism at Focal adhesions

- Clustering: Increased adhesion (effective affinity), discretization and compartmentation of mechanics
- Actin coupling and force transmission
- Adhesive function and tension transmission function
- Mechano-sensation and transduction: clutch mechanism
Rigidity sensing by mechanical tugging

- Traction forces at focal adhesion are asymmetric (i.e. shifted towards distal tip)

- Two modes of traction at focal adhesions:
  - stable traction is nearly centered and has low values
  - fluctuating traction is asymmetric: it is shifted towards the distal tip and has higher values.
  > Tugging

- On more rigid substrates (16-55kPa)
  highest traction is more centered
  This requires actomyosin contractility
Rigidity sensing by mechanical tugging

- The FAK/Paxilin/Vinculin actin coupling complex is required for fluctuating, asymmetric, high traction at focal adhesions.
The FAK/Paxillin/Vinculin actin coupling complex is not required per se for asymmetric traction fluctuation at focal adhesions.

FAK/Paxillin/Vinculin extend the range of rigidity sensing via traction fluctuation (8.6k-32kPa).

In absence of FAK/Pax/Vinculin, actomyosin contractility allows stable traction.
Rigidity sensing during Durotaxis

- Rigidity increases the size of focal adhesions
- Random cell motility is enhanced on stiffer substrates
- The FAK/Paxilin/vinculin complex extends this to a higher range of ECM stiffness

- Cells are durotactic on a wide range of bulk ECM rigidity
- FAK/Paxilin/vinculin is not required for durotaxis per se, but extends the range of ECM rigidity to which cells respond for durotaxis

\[ MSD(\tau) = 4D_\tau^\alpha \]

\( D \) diffusion coefficient
\( \alpha \) directional coefficient
Rigidity sensing during Durotaxis

- FAK/Paxilin/vinculin are not required for chemotaxis along a diffusible PDGF gradient
- Or along an ECM based Fibronectin gradient (Haptotaxis)

\[ MSD(\tau) = 4D\tau^\alpha \]

- \( D \) diffusion coefficient
- \( \alpha \) directional coefficient
Durotaxis: mechanism

- Focal adhesions experience mechanical tugging: fluctuating, asymmetric traction. This is a local phenomenon suggesting that FA can probe the local stiffness autonomously.

- Conditions that favor FA tugging reduce random migration (i.e., induce more directional motility) and support durotaxis.

- FAK/Paxilin/Vinculin is required for tugging over a broad range of rigidities, most likely by strengthening the « molecular clutch ».

- Conditions that caused symmetric, stable traction at focal adhesions, led to random cell motility on stiffer substrates.

- Cells can probe mechanically their environment and steer up stiffness gradient by integrating the map of rigidity landscape at the cellular scale.


Durotaxis: mechanisms

- **Polarized attachment of cells to substrate:**
  - Feedback between Focal adhesions and ECM via actomyosin contractility
  - Gradient of stiffness induces polarized positive feedback, and greater adhesion at the front, and movement towards higher stiffness despite symmetric traction forces

- **Polarized substrate deformation:**
  - Substrate displacement (indentations under FAs) is larger on the soft edge than the stiff one despite symmetric traction forces

A model of durotaxis: rigidity gradient sensing

- Cells can probe mechanically their environment and steer up stiffness gradient by integrating the map of rigidity landscape at the cellular scale.
- Model: Langevin equation: \( d\mathbf{v}(t) = -\beta \mathbf{v}(t) dt + d\mathbf{B}(t) \)
- Stochastic forces arise from sampling of environment.
- The local stiffness \( k_x(\theta) \) is probed by cells as reciprocal of radial displacement component.

Probability density function used for the angular component of the stochastic force:

\[
P_{k}(\theta) = \frac{k_{x}(\theta)}{\int_{0}^{2\pi} k_{x}(\zeta)d\zeta}
\]

Isotropic

Biphasic

Biphasic

Bias speed
For example, contractile forces of stress fibers cause FAs to mature and grow, with its chemical potential reduced, making it thermodynamically favorable (She-mesh et al., 2005). Under these circumstances, durotaxis would be a phenomenon of stress fibers, in which FAs become more stable on stiffer substrates than on softer ones (Figure 3B) because mechanical stress is higher at the front of the cell than the rear (Rens and Merks, 2020; Lazopoulos and Stamenovic, 2008; Shemesh et al., 2005). When stress is above a critical threshold, the adhesion assembles; when it is lower, the adhesion disintegrates. Thus, this model proposes durotaxis is driven by differential adhesion between the front and the rear of the cell, similar to the classical models of cell migration (Ron et al., 2020; Tanimoto and Sano, 2014); indeed, adhesion is often stronger to stiff substrates, compared with soft substrates (Plotnikov et al., 2012).

Importantly, this asymmetric substrate adhesion does not involve an imbalance of traction forces, as it is sometimes misinterpreted in the literature. Since cells migrate at small scale in dissipative circumstances, the inertial forces are negligible, and therefore, the summation of tractions stresses on the substrate must equal the viscous stress applied by the medium on the cells, which is also negligible.

A third physical model of cellular force transmission is based on the motor-clutch hypothesis (Mitchison and Kirschner, 1988) in which intracellular molecular motors, like myosin II, transmit force to the ECM through rigid actin filament bundles and compliant transmembrane molecular clutches like integrins (Chan and Odde, 2008). A generalized clutch model simulating the dynamics of cell-matrix adhesions suggests that when stress fibers apply an equal force to the substrate at the front and rear, it would lead to durotaxis.

Persistence and substrate rigidity: evidence

- The persistence of cell motility depends on rigidity of the substrate (« universal » property)
- Velocity can increase or decrease with rigidity in a cell type specific manner

\[ \Delta(t) = \sqrt{\langle \| \vec{r}^2 \| \rangle(t)/(v_c t)} \propto (\tau_p / t)^{1/2} \]

**Directionality index:**

- \( \tau_p \): persistence time
- \( v_c \): linear velocity

Directionality increases by \( \sim 3 \) as stiffness increases by \( \sim 10 \)


D. House, —and M. Betke,
Model of durotaxis: rigidity-dependent persistence

- Persistent random walk (PRW) on a 2D substrate:
  - Persistence time is a function of rigidity: PRW

  Mean square displacement:
  \[
  \langle |\mathbf{r}|^2 \rangle(t) = 2v_c^2 \tau_p^2 \left( \frac{t}{\tau_p} + e^{-t/\tau_p} - 1 \right).
  \]

  \(\tau_p\)  Persistence time
  \(v_c\)  Linear velocity

  At short times, ballistic motion
  \[
  \langle |\mathbf{r}|^2 \rangle(t) \approx (v_c t)^2.
  \]

  At longer time, random walk
  \[
  \langle |\mathbf{r}|^2 \rangle(t) \approx 2v_c^2 \tau_p^2.
  \]

- A gradient of persistence induces a soft-to-stiff motility flux
  \[
  \frac{\Delta \tau_p}{\Delta x}
  \]

  Dimensionless number characterizes durotactic motion
  \[
  V = v_c \times \left( \partial \tau_p / \partial x \right)
  \]
Model of durotaxis: rigidity-dependent persistence

- Asymmetric distribution increases over time

\[ \bar{D}I(t) = \{D_{I_x}(t), D_{I_y}(t)\} \equiv \frac{\langle r_r(t) \rangle}{v_c t}. \]

\[ \bar{D}I(t) = 0. \] For RW and PRW

- Durotactic index increases over time

\[ \bar{P}(x, y) \]

Probability distribution

wave front drift velocity \( v_d \)

Random walk

constant increases with persistence
decreases with persistence

Analogies with bacterial chemotaxis?

- Biased (persistent) random walk in a spatial gradient
- Temporal gradient sensing
- Memory

Log of fraction of runs of length greater than length x

- up the gradient: runs are longer than is expected from the concentration dependence of the runs (i.e. tumbles are postponed)

Analogies with bacterial chemotaxis

STRATEGIES FOR CHEMOTAXIS

M. J. SCHNITZER*, S. M. BLOCK†, H. C. BERG†, E. M. PURCELL*

Departments of Physics*, and Cellular and Developmental Biology†,
Harvard University, Cambridge MA 02138, and
The Rowland Institute for Science†, Cambridge MA 02142, USA

CONCLUSIONS

We re-examined the problem of migration of motile organisms in spatial gradients of chemical attractants. We showed analytically and by Monte-Carlo simulation that organisms whose turning frequencies (tumble probabilities) depend solely on the local concentration of an attractant, but whose speeds remain constant, do not accumulate at the top of such a gradient: once uniformly distributed, they remain uniformly distributed. On the other hand, organisms whose swimming speeds depend on the local concentration of an attractant do accumulate in regions where the speeds are low.

We extended the Monte-Carlo simulation to non-local strategies and found that cells that respond (by suppressing tumbles) to concentrations of an attractant sensed over the recent past, but do not make temporal comparisons, drift down rather than up the gradient. Cells that compare concentrations sensed over the recent past with those sensed earlier are able to drift up the gradient. This is the strategy used by E. coli for chemotaxis.
Analogies with bacterial chemotaxis

1D theoretical model of stochastic motion:
Semipermeable barriers that reflect \( \frac{1}{2} \) of particles (eg. bacteria), and let \( \frac{1}{2} \) pass through

This models the idea that particles change randomly their trajectory every time \( \tau \) and distance \( \delta \)

Their motion is defined locally in space and time (no memory)

Flux: \( J = -(1/4)[C(x_2)(\delta_2/\tau_2) - C(x_1)(\delta_1/\tau_1)]. \)

\( \delta/\tau: \) velocity

- **Case 0**: \( \delta \) and \( \tau \) are constant. Fick’s law \( J = -D(\partial C/\partial x) \), with diffusion coefficient \( D = \delta^2/4\tau \)
  
  At equilibrium \( C \) is uniform

Let us consider cases where \( D \) changes in space ( \( \delta \) and/or \( \tau \) vary in space):  

- **Case 1**: velocity \( \delta/\tau \) is constant, but \( \delta \) and \( \tau \) vary in space
  
  we still have \( J = -D(\partial C/\partial x), \quad D = \delta^2(x)/4\tau(x) \) is not constant in space

Thus, whatever the distribution of barriers, provided that velocity is constant the distribution of particles at equilibrium will always be uniform

If bacteria have a uniform velocity, changing in space the probability of changing direction (tumbling) will not lead to spatial accumulation of cells. So if an attractant were to simply change the tumbling frequency (ie. the duration of run, or the persistence) there would be no chemotaxis.
Analogies with bacterial chemotaxis

Let us consider cases where $D$ changes in space:

- **Case 2**: distance $\delta$ is constant
  \[ J = -D(\partial C/\partial x) - C(\partial D/\partial x) = -\partial(\partial C/\partial x), \quad D = \delta^2/4\tau(x) \]
  At equilibrium $DC$ is uniform, and $C$ is inversely proportional to $D$
  Therefore, particles accumulate where their velocity is lowest

- **Case 3**: time $\tau$ is constant
  \[ J = -D(\partial C/\partial x) - C(\partial D/\partial x)/2, \quad D = \delta^2(x)/4t \]
  At equilibrium $D^{1/2}C$ is uniform, and $C$ is inversely proportional to $D^{1/2}$
  Therefore, particles accumulate where their velocity is lowest

- **Case 4**: all parameters vary in space
  \[ J = -(\delta/4)[v(\partial C/\partial x) + C(\partial v/\partial x)], \quad \delta/\tau \]
  At equilibrium, the density of particles is still inversely proportional to velocity

  - When speed is not constant, cells accumulate in regions of low speed
  - When speed is constant, cells remain uniformly distributed whatever the frequency of tumbling as a function of stimulus.
  - If the chemoattractant increases the persistence time (reduces the frequency of tumbling, there is no chemotaxis)
  - This is a generic result which should also apply to durotaxis.
Clarifications needed: data and model:
— without memory, no durotaxis is possible (Schnitzer et al)
— with memory, may be possible…

Collective durotaxis

Asymmetric expansion of a population of epithelial cells on a gradient of gel stiffness

Collective durotaxis

Single cells do not exhibit durotaxis on the same stiffness gradient

Cell speed increases with stiffness


Downloaded from https://www.science.org on September 21, 2022
Collective durotaxis

- Supracellular organisation via cell-cell mechanical coupling within cluster:
- Increase the length scale to sample the stiffness gradient

- Collective durotaxis

- Single cell durotaxis

Mechanosensitivity and strengthening of actin coupling at E-cadherin based adhesion junctions  
(See also course 14 Nov 2017)

Mechanical coupling at Junctions in a cell layer

Mechanosensitivity and strengthening of actin coupling at E-cadherin based adhesion junctions  
(See also course 14 Nov 2017)
Collective durotaxis requires integrity of cell cell contacts

This is not based on a local gradient sensing but on a long range collective sensing
Emergent property of the cell collective (ie. supracell) that requires cell-cell adhesion

Collective durotaxis

Probing the distribution and transmission of forces
Traction forces are exerted at the edge and propagate within the bulk of the cell layer

- Traction forces on ECM are concentrated at the edge of the cell cluster and are symmetrically distributed
- Substrate deformation is asymmetric in the gradient (lower in stiffer regions): given symmetric actin polymerization at the edge, expansion is asymmetric

- Tensile forces in the bulk

Collective durotaxis- Supracellular durotaxis

- Similar to cellular model
- The tissue bulk is an elastic material
- If viscous behavior in the bulk (i.e., cell adaptation to strain), the asymmetry in substrate deformations at the edge does not necessarily give rise to net cluster displacement.

Model:
- clutchlike cell-ECM dynamics at focal adhesions
- long-range force transmission through cell-cell junctions
- actin polymerization at monolayer edges

Collective durotaxis

- **Prediction and Tests:**
  - durotaxis increases with difference in substrate deformation of both sides of cluster (i.e., stiffness gradient steepness, mean stiffness)

Durotaxis is quantified by the cluster center of mass translation after 10 hours. This is assessed as a function of the initial stiffness of the center of mass of cell cluster.

- **Durotaxis requires cell contractility and mechanical transmission**
Collective durotaxis

- Supracellular guidance:
  - Increased length scale, increased sensitivity, global ordering from edges of cell cluster

- Collective durotaxis: shallow gradient sensing

- Single cell durotaxis: steep gradient sensing

Case Studies of collective cell migration

- Collective migration with leaders:
  
  **Case Study 1**: Neural crest cell migration (*Xenopus*)
  
  **Case Study 2**: Sensory organ primordium migration in fish lateral line (*Zebrafish*)

- Collective migration without leaders:
  
  **Case Study 3**: Egg chamber rotation (*Drosophila*)
Case Studies of collective cell migration

- **Collective migration with leaders:**
  
  *Case Study 1:* Neural crest cell migration (*Xenopus*)

  *Case Study 2:* Sensory organ primordium migration in fish lateral line (*Zebrafish*)

- **Collective migration without leaders:**
  
  *Case Study 3:* Egg chamber rotation (*Drosophila*)
Possible mechanisms of collective motility

- Motility guided by chemoattractant
- Motility guided mechanically
Case Study 1: Neural crest cell migration

Laura S. Gammill & Marianne Bronner-Fraser, 2003
Case Study 1: Neural crest cell migration (chick, *Xenopus*)

- Neural crest cells give rise to different important cell lineages in vertebrates:
  - melanocytes, craniofacial cartilage and bone, smooth muscle, peripheral and enteric neurons and glia
Case Study 1: Neural crest cell migration

- Dorsal view of *Xenopus* embryo
- Lateral view of *Xenopus* embryo

In vitro culture system

When in contact with NC, placode is motile as a whole (Supplementary Fig. S3 and Movie S6). NC cells were attracted to Fig. 2d), which is however insufficient to promote directional placode Fig. 2h).

Case Study 1: Neural crest cell migration

—Chase and run interactions between two co-migratory cell populations

- In vivo displacement of two adjacent tissues in *Xenopus*:
  - Neural crests (NC) and placode cells, epithelial cells that contribute to sensory organs

- In vitro culture system
  - In isolation NC cells are motile but not placode cells
  - When in contact with NC, placode is motile as a whole
  - This requires sensing of SDF1 chemokine by the GPCR CXCR4

Displacements

**Chase-and-run assay**  **Displacement**

NC

Placodes

PL + NC

PL + NC Cxcr4MO

ARTICLES

Case Study 1: Neural crest cell migration

Displacements

**Chase-and-run assay**  **Displacement**

NC

Placodes

PL + NC

PL + NC Cxcr4MO


**Thomas LECUIT 2022-2023**
Case Study 1: Neural crest cell migration

— Mechanical interactions between cell populations induce symmetry breaking

• Neural crest cells induce a symmetry breaking of traction forces exerted by placode cells

• Adhesion between NC and Placode cells
  • Mediated by N-cadherin

• Contacts between NC and Placode cells cause collapse of cell protrusions
  • This requires N-cadherin

Case Study 1: Neural crest cell migration

— Contact Inhibition of Locomotion underlies coordinated migration

- Induced upon collision of NC and PL cells
- Requires N-cadherin

Single cell analysis

Cell populations

<table>
<thead>
<tr>
<th>Controls</th>
<th>+NCD2</th>
<th>+E-cadh Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL 0 &gt; 340</td>
<td>PL 0 &gt; 340</td>
<td>PL 0 &gt; 340</td>
</tr>
<tr>
<td>150 µm</td>
<td>150 µm</td>
<td>150 µm</td>
</tr>
</tbody>
</table>

Angles after NC-PL collisions

- Control NC + Control PL
- Control PL + Control NC

N-cad morpholino (NMO)

Observations on the social behaviour of cells in tissue culture

1. Speed of movement of chick heart fibroblasts in relation to their mutual contacts

M. Abercrombie and Joan E. M. Hewatman
Department of Anatomy and Embryology, University College, London, England
Resumed August 20, 1953

Mean velocity in µm/hr

<table>
<thead>
<tr>
<th>Contact number</th>
<th>Mean velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

— Placode cells attract a cell population (Neural crest) that breaks the symmetry via adhesive contacts.

NC cells are attracted to placodal cells via Sdf1-dependent chemotaxis.

CIL: Contact inhibition of locomotion

- Contact between NC and placodal cells induces CIL.
- This breaks the symmetry of the placodal tissue, and induces movement.
- The system self-sustains owing to chemotaxis and CIL.
Case Study 1: Neural crest cell migration

— Role of the mechanical environment in migration

• Neural crest cell migration is induced by SDF1 dependent chemotaxis, but is also dependent on some environmental factors: the nature/stage of the host dictates NC migration

• Explants from different stages are equally attractif by an SDF1 source

Case Study 1: Neural crest cell migration

— Role of the mechanical environment in migration: stiffness

- The stiffness of the mesoderm increases over time from the non-migratory to the pre-migratory stage

- Chemotaxis towards SDF1 does not operate on a soft matrix in vitro.

- Matrix stiffness potentiates chemotaxis in vitro

Case Study 1: Neural crest cell migration

— Role of the mechanical environment in migration: stiffness

- Mechanical relaxation of tissue stiffness *in vivo*
  
  Strain dependent stiffening of matrix and tissues
  
  Stress relaxation following tissue ablation softens the mesoderm
  
  This affects NC migration *in vivo*

- Genetic relaxation of tissue stiffness *in vivo:*
  
  Tissue softening via genetic inhibition of Myosin2
  
  This reduces NC migration

- Increase of tissue stiffness *in vivo:*
  
  Atomic Force Microscopy (AFM) increases tissue stiffness and promotes cell motility
Case Study 1: Neural crest cell migration

— Role of the mechanical environment in migration: stiffness

- Convergence-Extension (C/E) movement in the mesoderm increase cell density and tissue stiffness

- Inhibition of C/E movements (DshDEP+) softens the mesoderm and reduces NC migration

- Tissue compression with AFM rescues NC migration in embryos where C/E is blocked

Case Study 1: Neural crest cell migration

— Tissue stiffening promotes migration of neural crest cells

Q: permissive or instructive (ie. stiffness gradient and durotaxis?)

Case Study 1: Neural crest cell migration

—Collective durotaxis along a self-generated stiffness gradient in vivo

- AFM is used to measure tissue stiffness in vivo
- A gradient of stiffness appears
- The gradient moves ventrally as cells migrate
- The placode and NC are required for stiffness gradient formation.

- An ectopic graft of NC induces a new gradient of stiffness
- This requires N-cadherin dependent softening.

Case Study 1: Neural crest cell migration

—Collective durotaxis along a self-generated stiffness gradient in vivo

- Relaxation of tissue stress by ablation of the ectoderm leads to disappearance of the stiffness gradient

- A NC graft is no longer able to migrate ventrally when the ectoderm is ablated, suggesting that the stiffness gradient is required for NC migration

Case Study 1: Neural crest cell migration

—Collective durotaxis along a self-generated stiffness gradient *in vivo*

- NC clusters exhibit global polarity (Myosin2 at the rear and Rac1GTP at the front) along a chemical of stiffness gradient

Contractility at the back:
Myosin 2 polarity

Actin nucleation at the front:
Rac1GTP polarity

Case Study 1: Neural crest cell migration

— Synergy between chemotaxis and durotaxis in vivo

- Disentangling chemotaxis and durotaxis:
  An ectopic source of SDF1 causes ectopic NC migration.
  Softening the tissue with ectoderm ablation blocks chemotaxis towards SDF1.

- Synergy:
  Ectopic SDF1 together with tissue stiffening causes ectopic NC migration.

Case Study 1: Neural crest cell migration

Summary

- **Durotaxis (stiffness gradient sensing)** operates in vitro and in vivo in cell populations.

- **Collective migration with leaders:**
  
  *Case Study 1: Neural crest cell migration (Xenopus)*

- **Guidance and Symmetry breaking** requires a combination of chemical and mechanical interactions/cues.