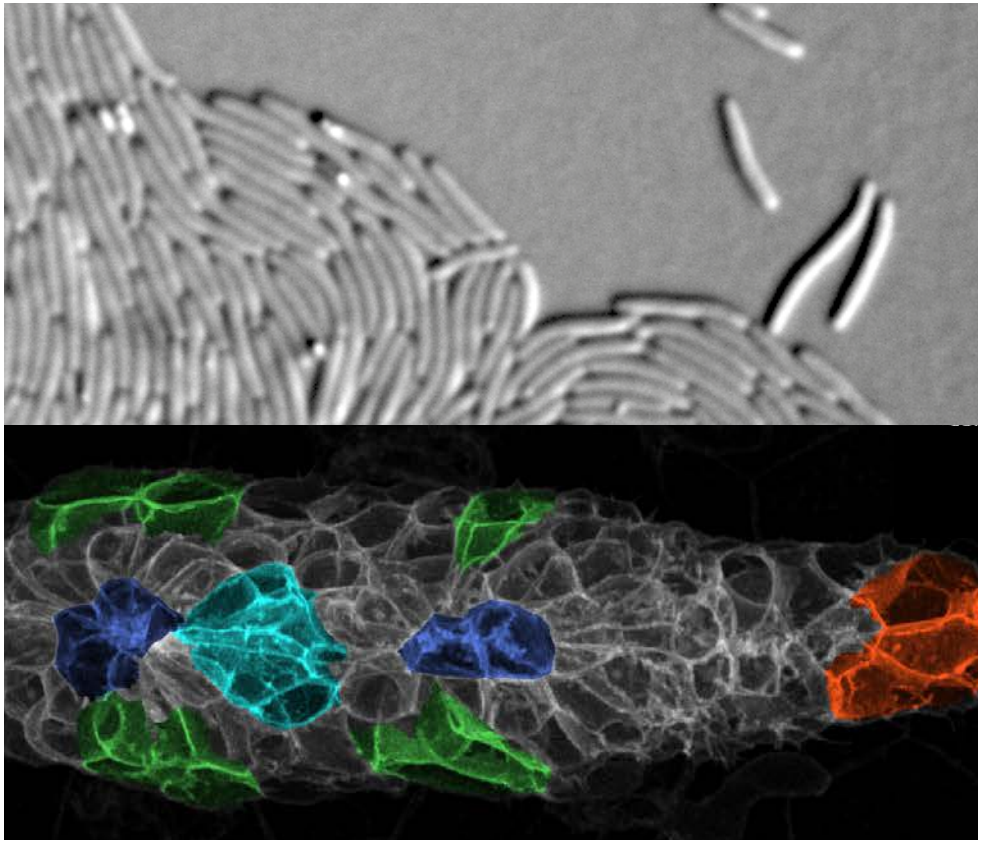
Cellular Motility



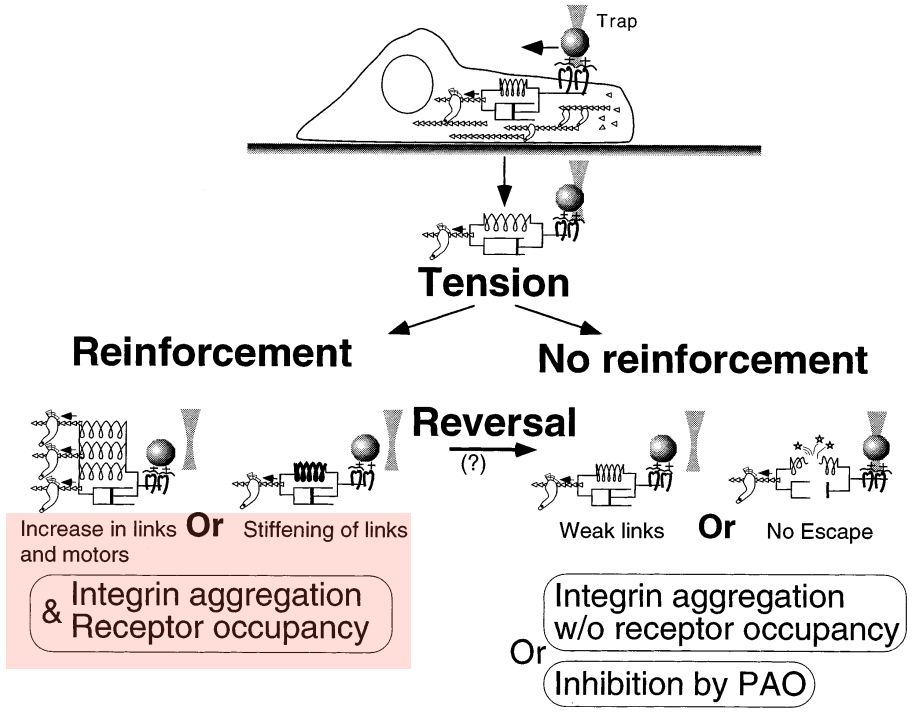
Course 3: From single to collective cell durotaxis

Thomas Lecuit

chaire: Dynamiques du vivant

COLLÈGE DE FRANCE 1530

Possible mechanisms of Rigidity sensing
Implications: rigidity as a guidance cue



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.

D. Choquet, D. Felsenfeld and M. Sheetz. *Cell*, 88: 39–48, (1997)

Hypothesis: rigidity as a guidance cue

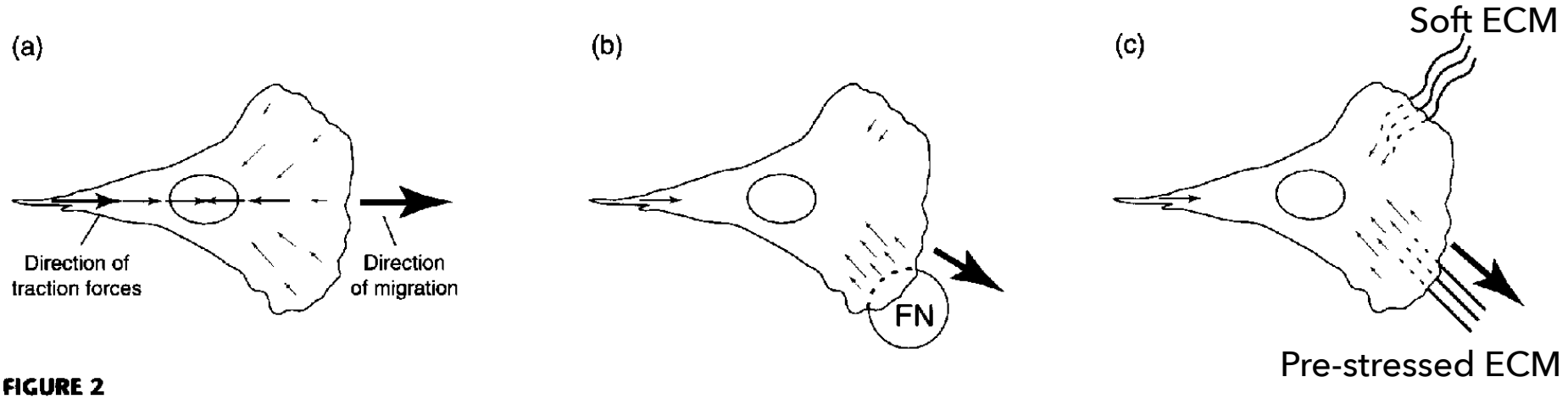


FIGURE 2

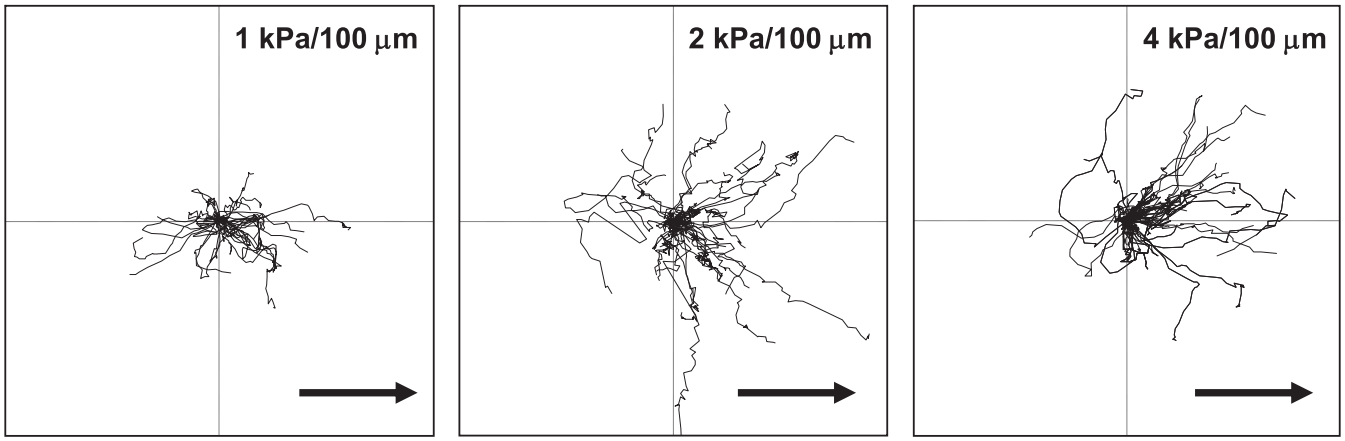
Orientation of traction forces in response to environmental cues. (a) When there are no external cues, traction forces (small arrows) in the front of the cell are oriented rearward and traction forces in the back of the cell are oriented forward. For net forward movement to occur (large arrow), the forces 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.

Sheetz, M. P., D. P. Felsenfeld, and C. G. Galbraith. *Trends Cell Biol.* 8:51–54. (1998)

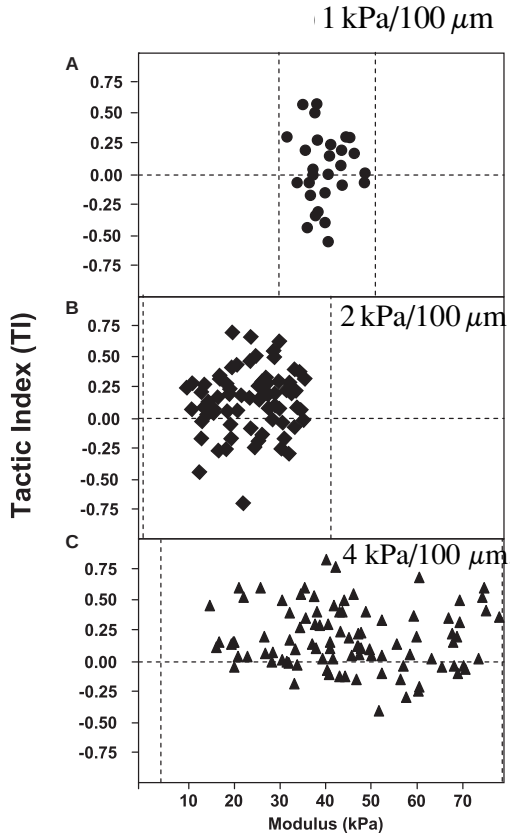
Durotaxis: cell guidance by the rigidity of substrate

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)

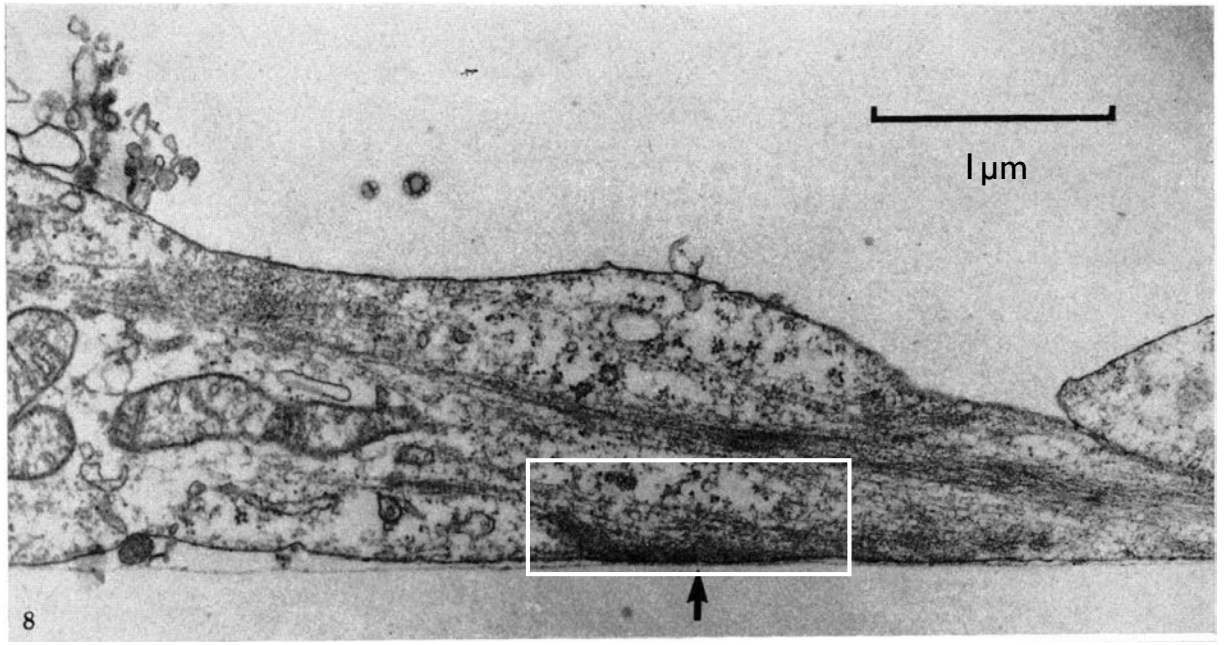


Vascular smooth muscle cells



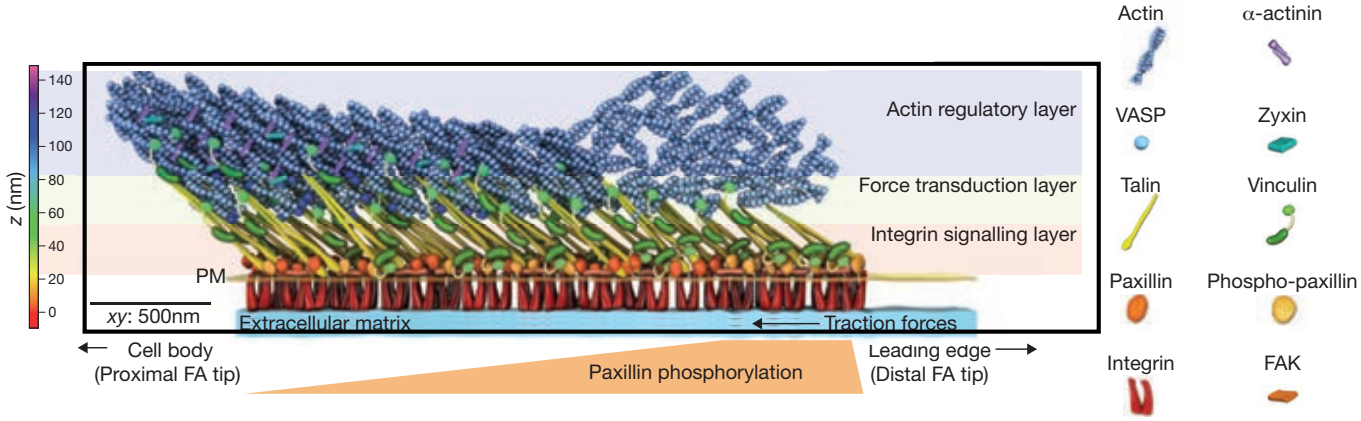
B. Isenberg et al, and JY Wong *Biophysical Journal* 97:1313–1322 (2009)

Structure and composition of Focal adhesions



M. Abercrombie *Proc. Royal Society.* 207:129-147 (1978)

PALM/STORM microscopy

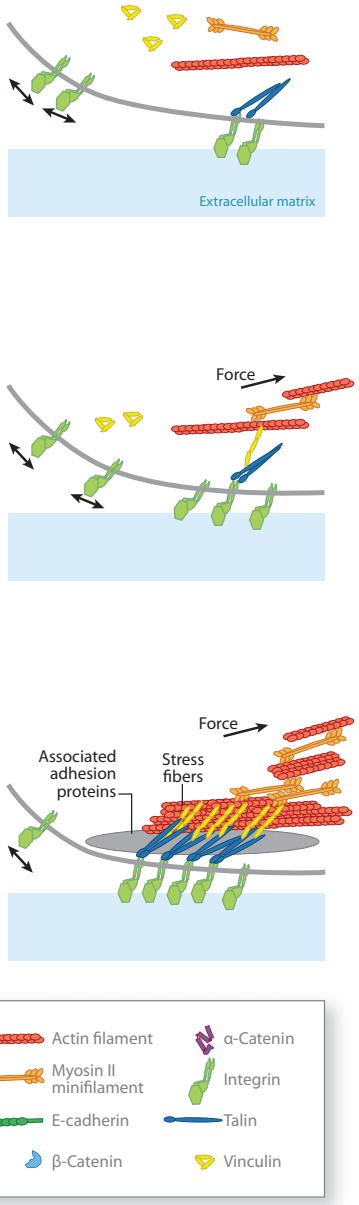


Lindsay B Case and Clare Waterman *Nat Cell Biol.* 17(4):955-963 (2015)

Kanchanawong, P. *et al.*. *Nature* **468,** 580–584 (2010).

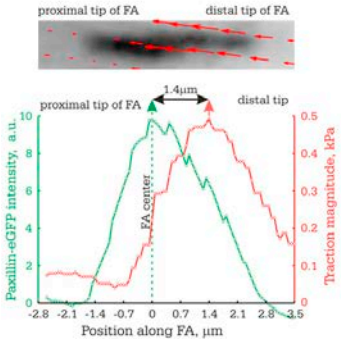
Clutch mechanism at Focal adhesions

- 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



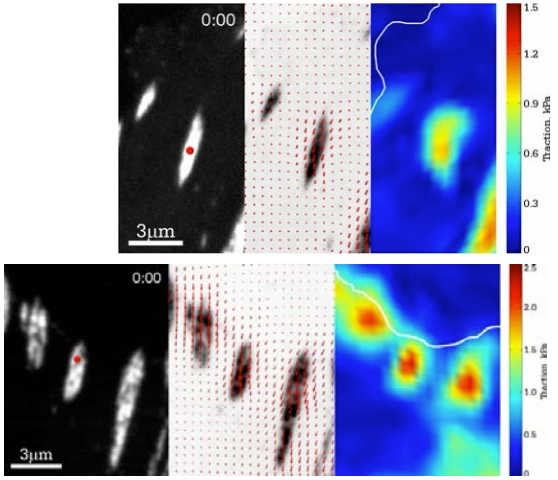
Rigidity sensing by mechanical tugging

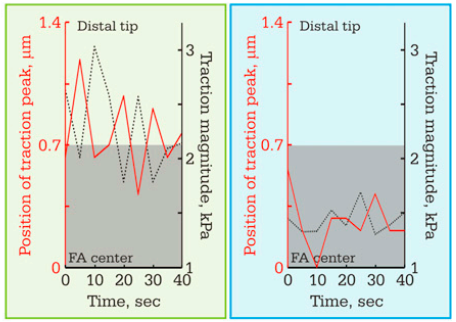
- Traction forces at focal adhesion are asymmetric (ie. shifted towards distal tip)



- Two modes of traction at focal adhesions:
  — **stable traction** is nearly centered and has low values

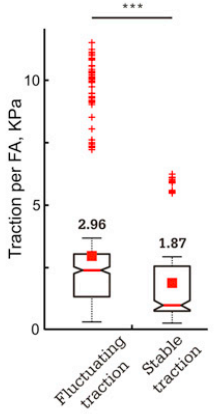
  — **fluctuating traction** is asymmetric: it is shifted towards the distal tip and has higher values.
  > Tugging

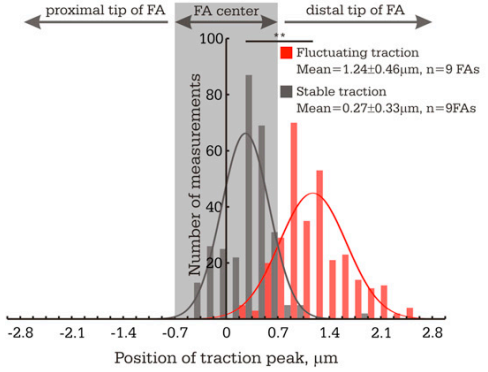


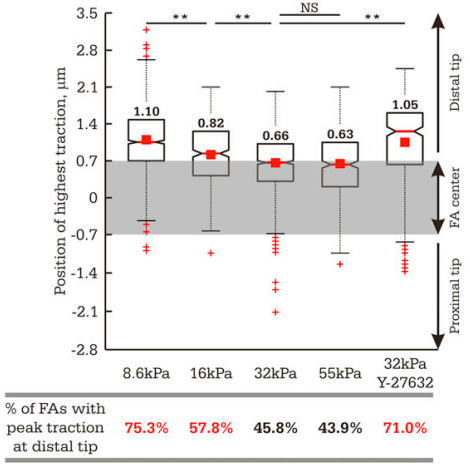


Fluctuating traction Stable traction

- On more rigid substrates (16-55kPa)
  highest traction is more centered
  This requires actomyosin contractility



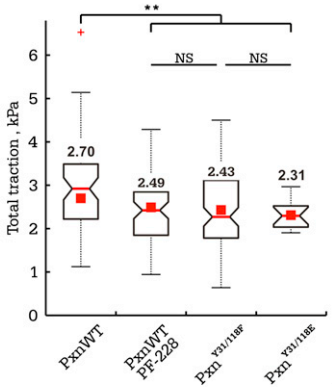


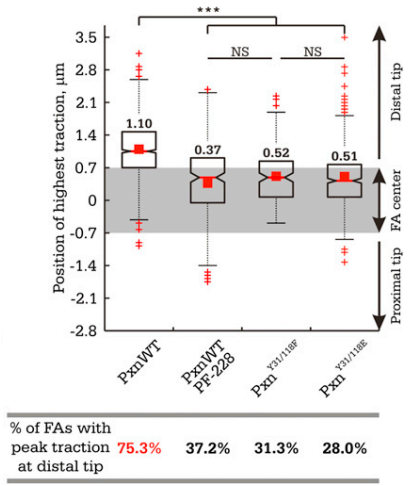


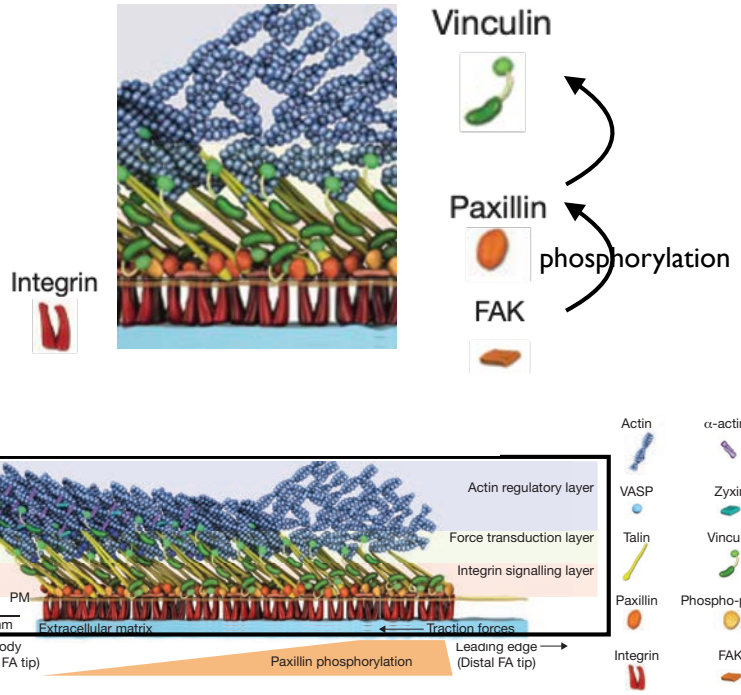
S. Plotnikov et al and C. Waterman. *Cell* 151: 1513–1527 (2012)

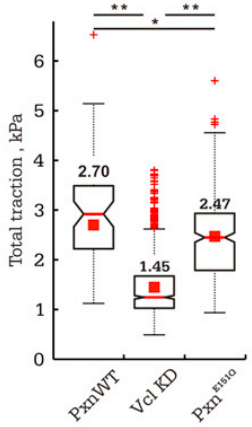
Rigidity sensing by mechanical tugging

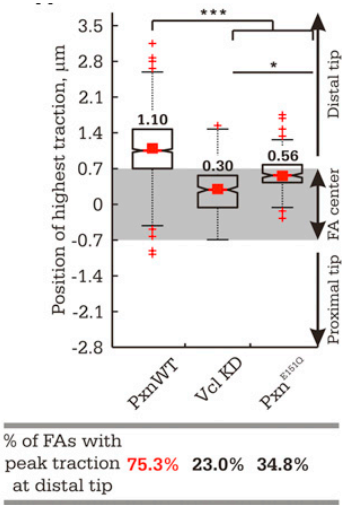
- The FAK/Paxilin/Vinculin actin coupling complex is required for fluctuating, asymmetric, high traction at focal adhesions











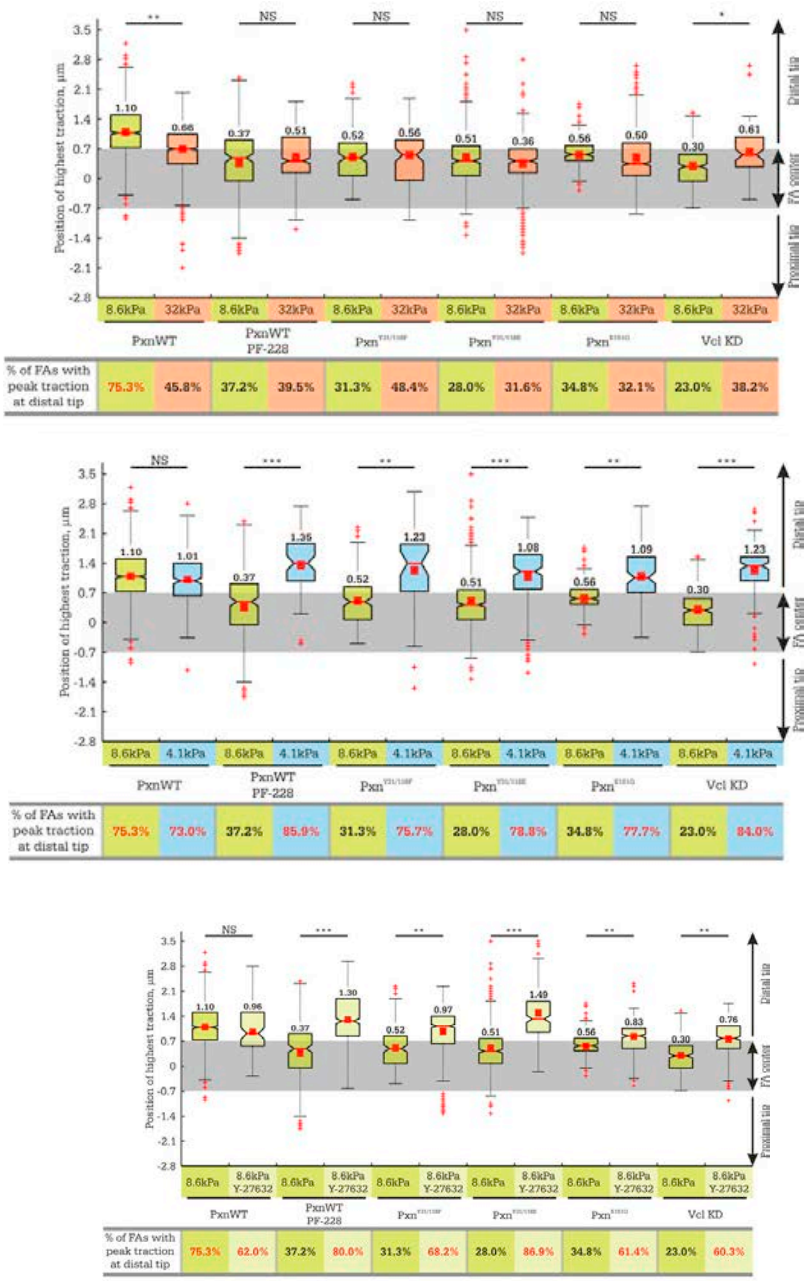
S. Plotnikov et al and C. Waterman. *Cell* 151: 1513–1527 (2012)

Dynamic range of rigidity sensing

- The FAK/Paxilin/Vinculin actin coupling complex is not required *per* se for asymmetric traction fluctuation at focal adhesions

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

In absence of FAK/Pax/Vinculin, actomyosin contractility allows stable traction

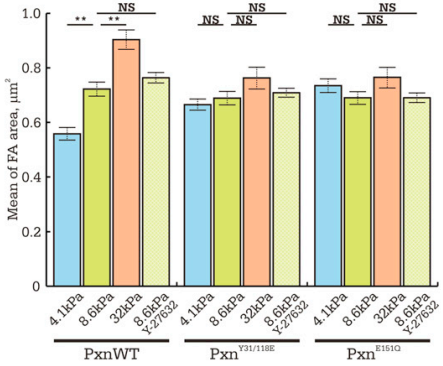


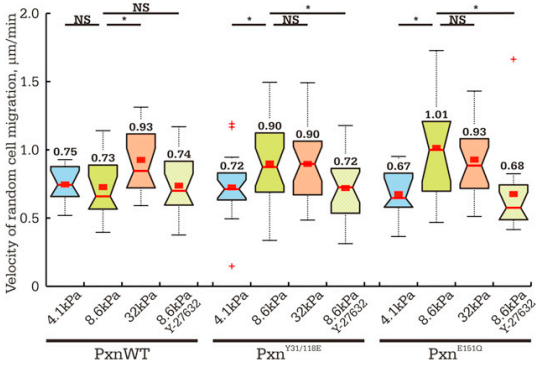
S. Plotnikov et al and C. Waterman. *Cell* 151: 1513–1527 (2012)

Rigidity sensing during Durotaxis

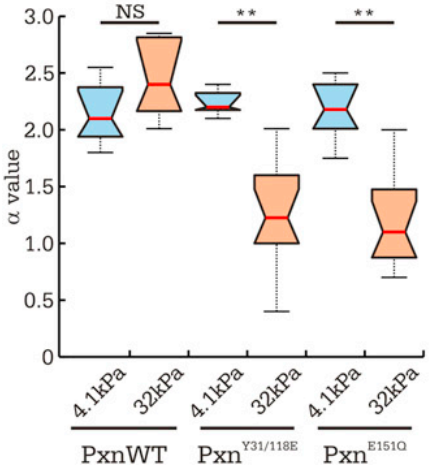
- Rigidity increases the size of focal adhesions
- Random cell motility is enhanced on stiffer substrates

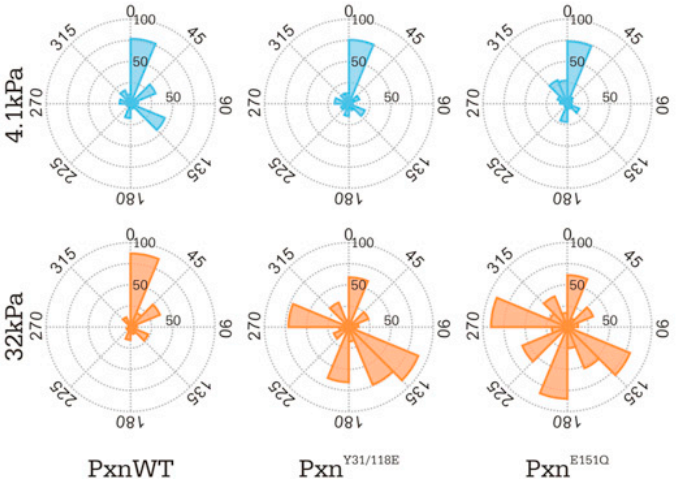
- The FAK/Paxilin/vinculin complex extends this to a higher range of ECM stiffness





- 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





$$MSD(\tau) = 4D\tau^{\alpha}$$

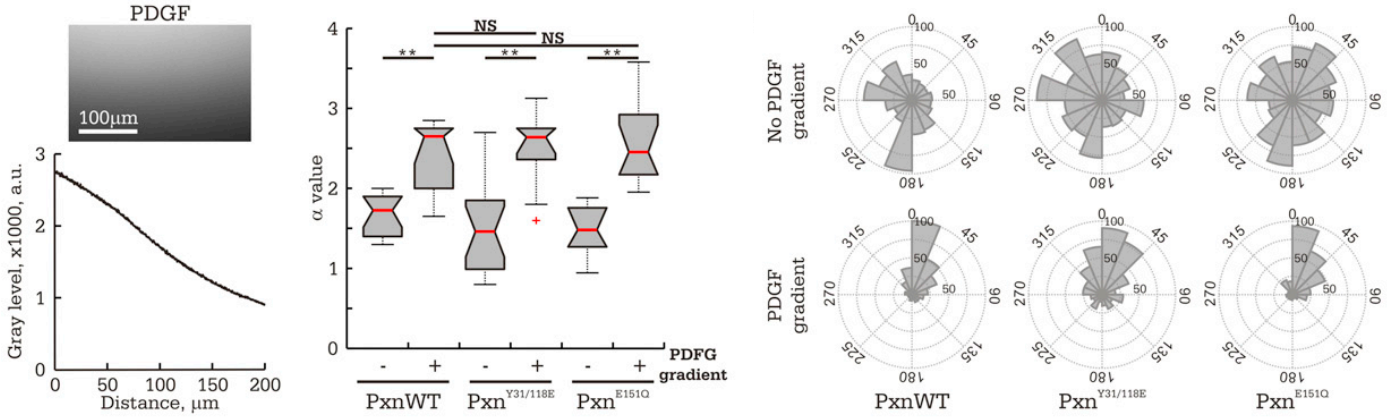
D diffusion coefficient

α directional coefficient

S. Plotnikov et al and C. Waterman. *Cell* 151: 1513–1527 (2012)

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

α directional coefficient

S. Plotnikov et al and C. Waterman. *Cell* 151: 1513–1527 (2012)

Durotaxis: mechanism

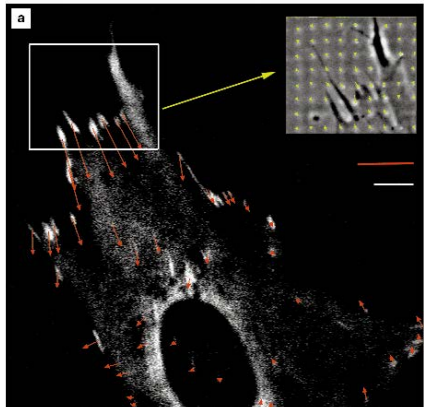
- Focal adhesions experience mechanical tugging: fluctuating, asymmetric traction. This is a **local phenomenon** suggesting that FA can probe the local stiffness autonomously

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

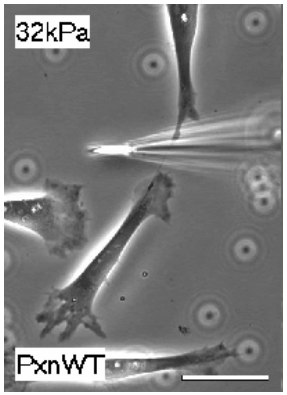
- FAK/Paxilin/Vinculin is required for tugging over a broad range of rigidities, most likely by strengthening the « molecular clutch ».

- 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**



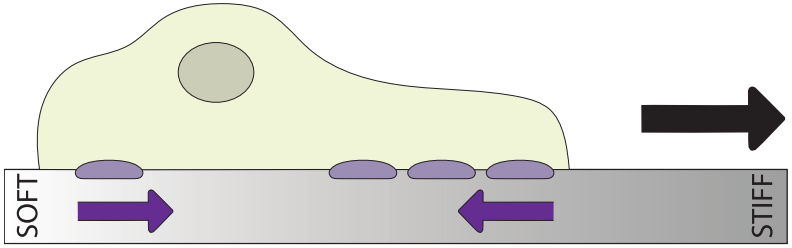
N. Balaban et al. and B. Geiger. *Nature Cell Biology*. 3: 466-472 (2001)



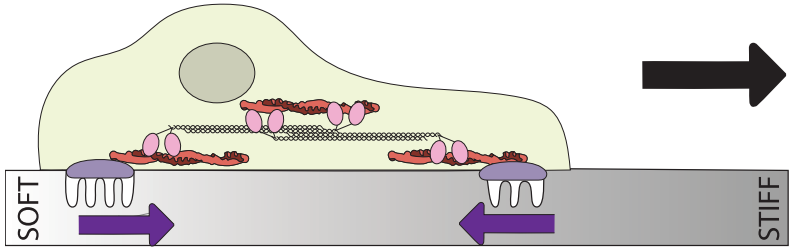
S. Plotnikov et al and C. Waterman. *Cell* 151: 1513–1527 (2012)

Durotaxis: mechanisms

- Polarized attachement 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



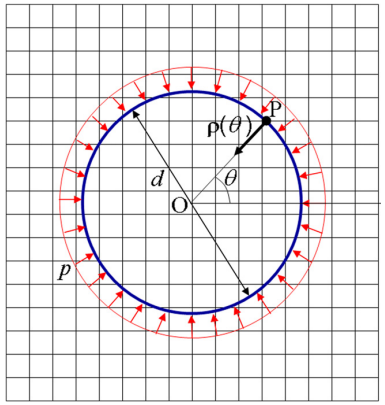
- Polarized substrate deformation:
- Substrate displacement (indentations under FAs) is larger on the soft edge than the stiff one despite symmetric traction forces



A. Shellard and R. Mayor. *Developmental Cell* 56: 227-239 (2021)

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

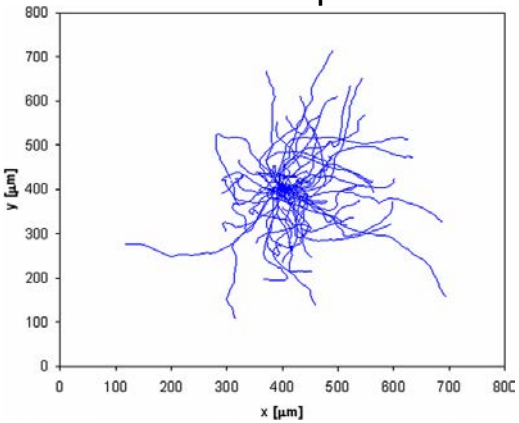


- Model: Langevin equation $\mathrm{d}\mathbf{v}(t) = -\beta\mathbf{v}(t)\mathrm{d}t + \mathrm{d}\mathbf{B}(t)$
- Stochastic forces arise from sampling of environment
- The local stiffness $k_{\mathbf{x}}(\theta)$ is probed by cells as reciprocal of radial displacement component

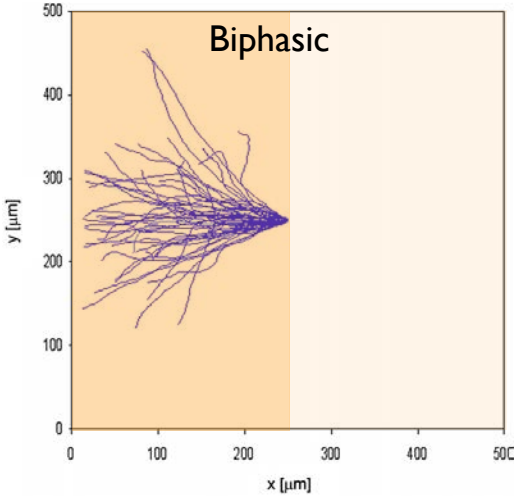
Probability density function used for the angular component of the stochastic force

$$P_k(\theta) = \frac{k_{\mathbf{x}}(\theta)}{\int_0^{2\pi} k_{\mathbf{x}}(\xi)\mathrm{d}\xi}$$

Isotropic



Biphasic



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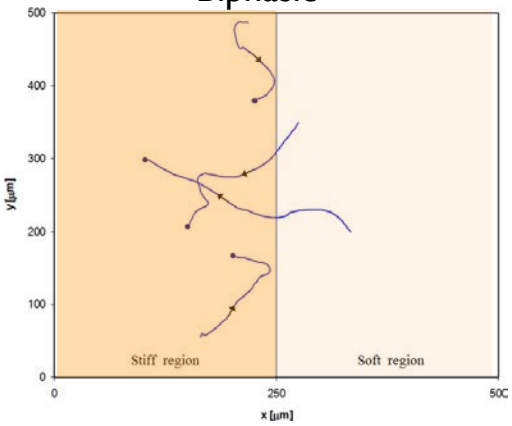
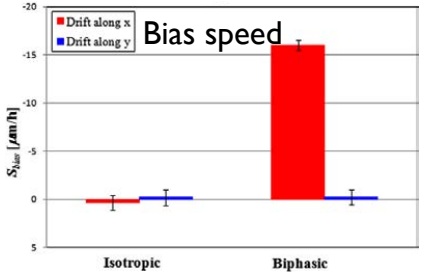
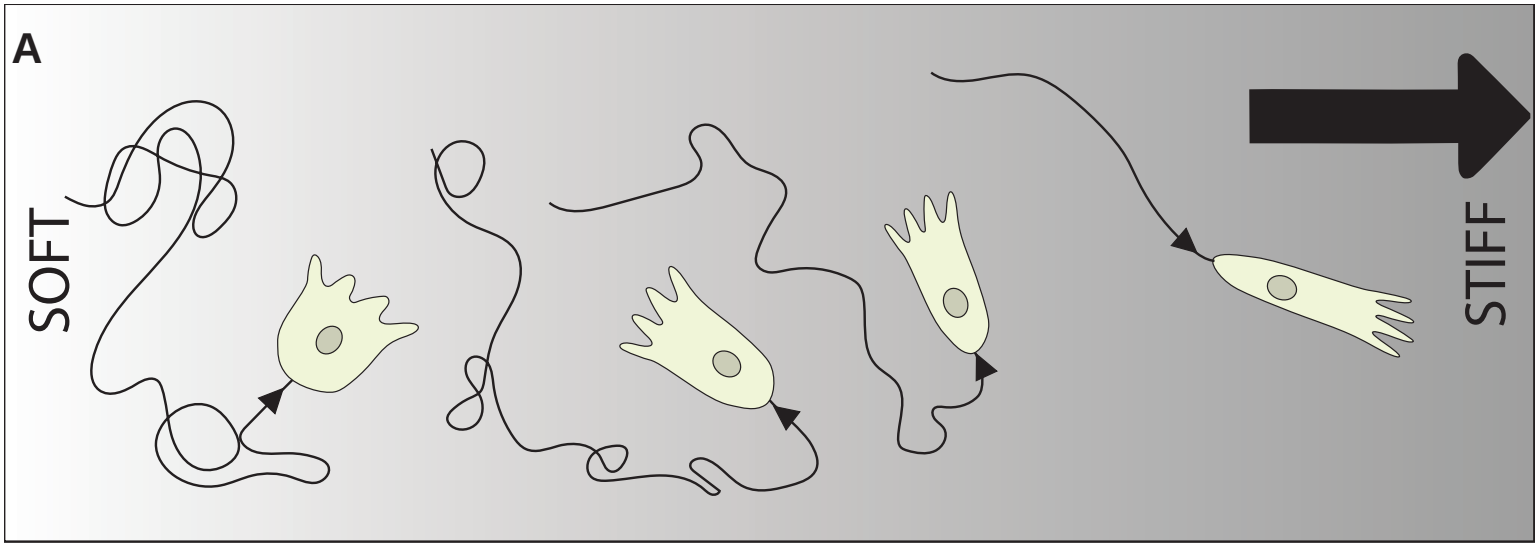




F. Stefanoni et al *Journal of Theoretical Biology* 280: 150–158 (2011)

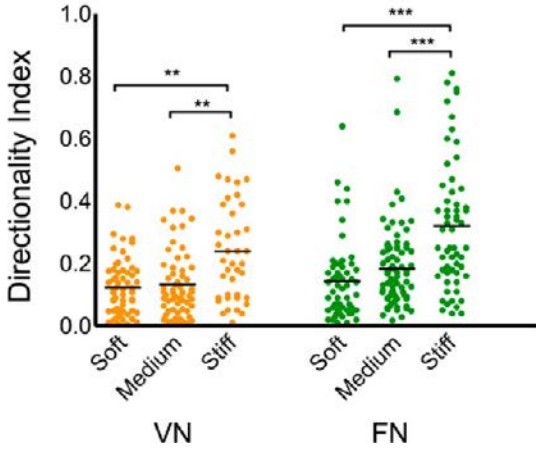
Durotaxis: Rigidity-dependent *persistence*

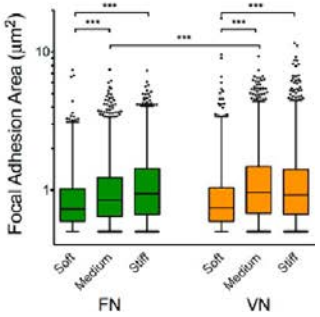


A. Shellard and R. Mayor. *Developmental Cell* 56: 227-239 (2021)

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





Fibronectin

Gel	Young's Modulus (kPa)
Soft PEGVS(20k)-PEGSH(5k)-5%	5.5 ± 0.7
Medium PEGVS(10k)-PEGSH(5k)-10%	33.2 ± 5.8
Stiff PEGVS(10k)-PEGSH(5k)-20%	65.4 ± 8.8

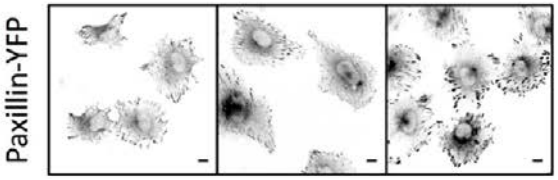
Synthetic hydrogel substrate based on radical free, cross linked PEG, with tunable stiffness

Directionality index:

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

τ_p : persistence time

v_c : linear velocity



D. Missirlis and J.P. Spatz, *Biomacromolecules* 15, 195 (2014).

Directionality increases by ~3 as stiffness increases by ~10

D. House, —and M. Betke,
Computer Vision and Pattern Recognition Workshops, 2009 (IEEE, Berlin, Germany, 2009), pp. 186–193.

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:

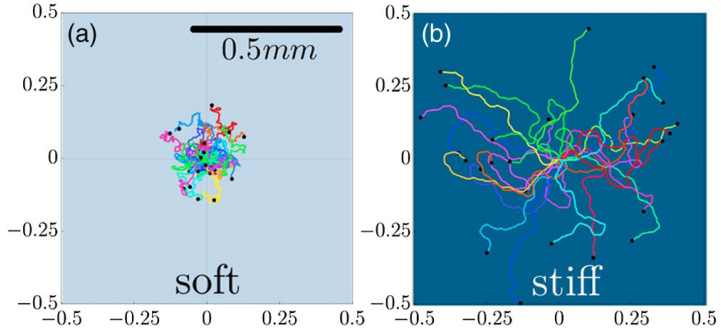
$$\langle|\vec{r}^2|\rangle(t) = 2v_c^2\tau_p^2\left(\frac{t}{\tau_p} + e^{-t/\tau_p} - 1\right).$$

τ_p Persistence time

v_c Linear velocity

At short times, ballistic motion $\langle|\vec{r}^2|\rangle(t) \approx (v_c t)^2$

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

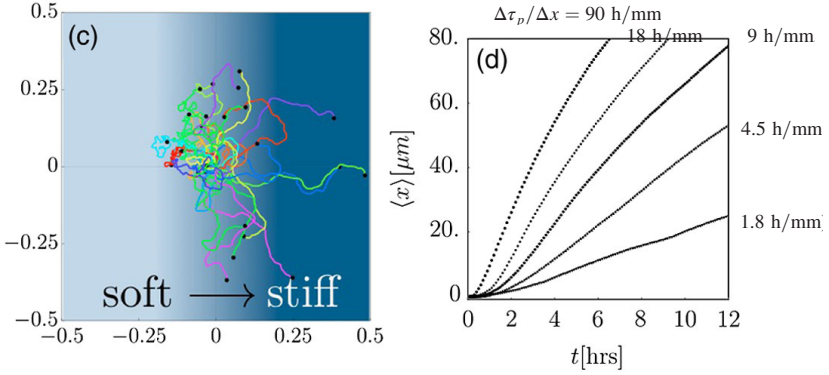


- A gradient of persistence induces a soft-to-stiff motility flux

$$\Delta\tau_p/\Delta x$$

Dimensionless number characterizes durotactic motion

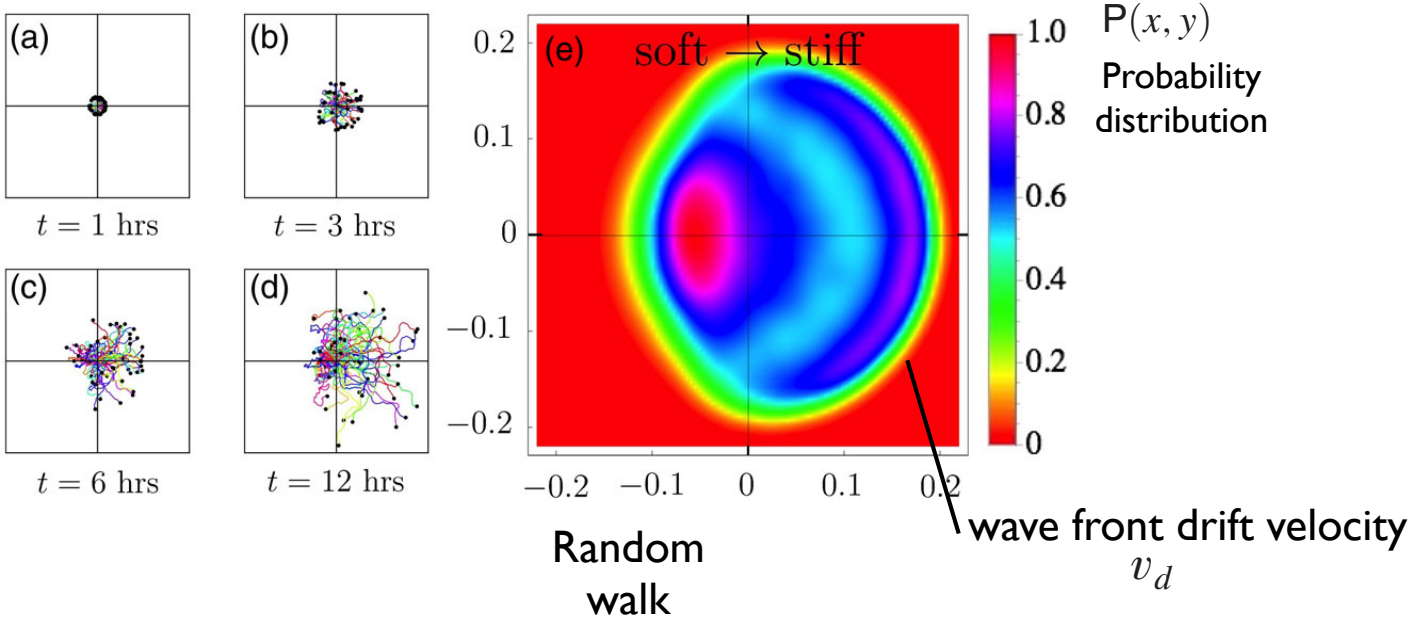
$$\mathrm{V} = v_c \times (\partial\tau_p/\partial x)$$



E. Novikova et al and C. Storm *PRL* 118, 078103 (2017)

Model of durotaxis: rigidity-dependent persistence

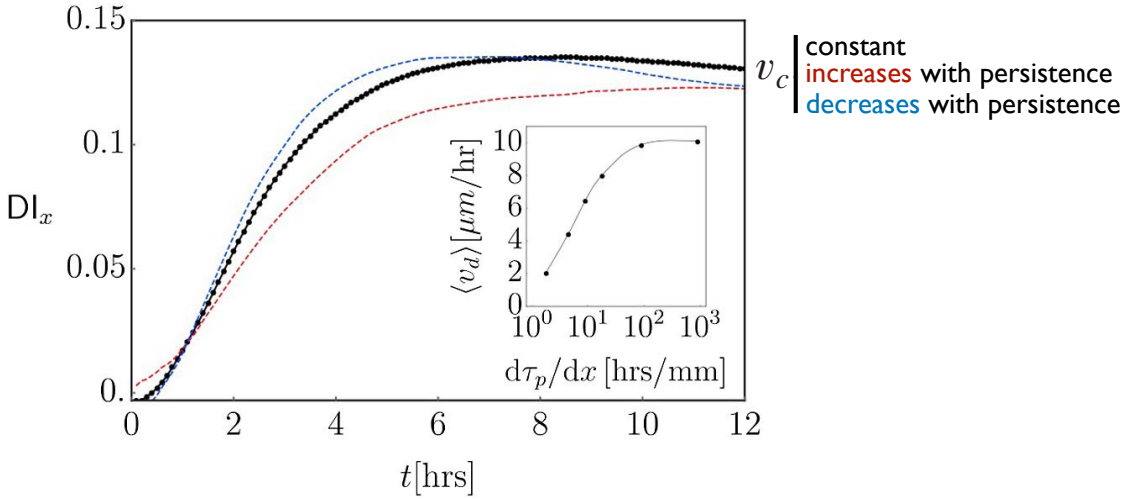
- Asymmetric distribution increases over time



- Durotactic index increases over time

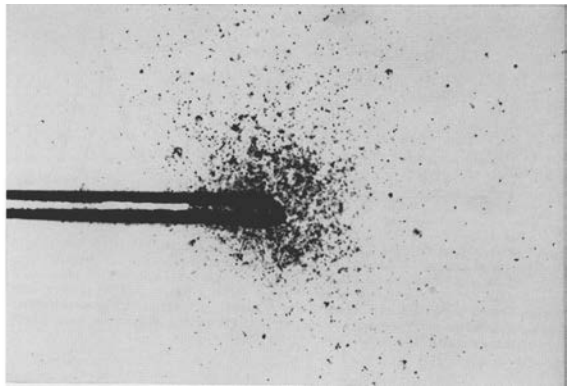
$$\vec{\mathrm{DI}}(t) = \{\mathrm{DI}_x(t), \mathrm{DI}_y(t)\} \equiv \frac{\langle \vec{r} \rangle (t)}{v_c t}.$$

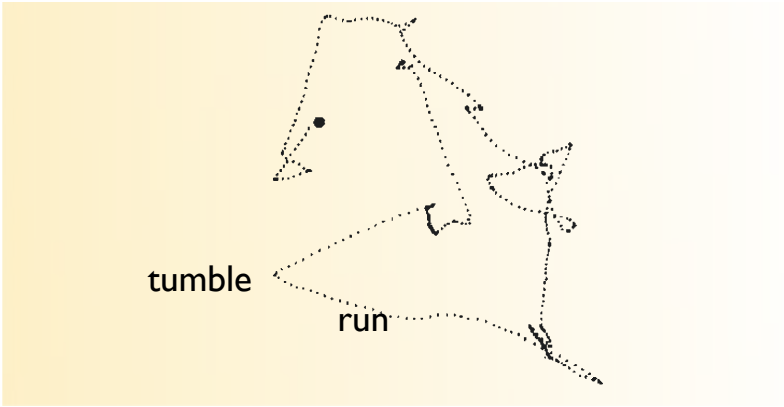
$\vec{\mathrm{DI}}(t) = \vec{0}$. For RW and PRW

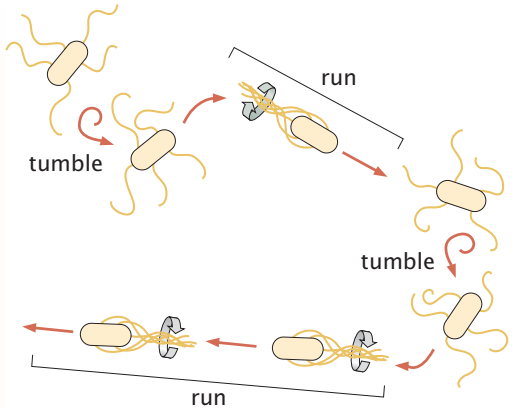


E. Novikova et al and C. Storm *PRL* 118, 078103 (2017)

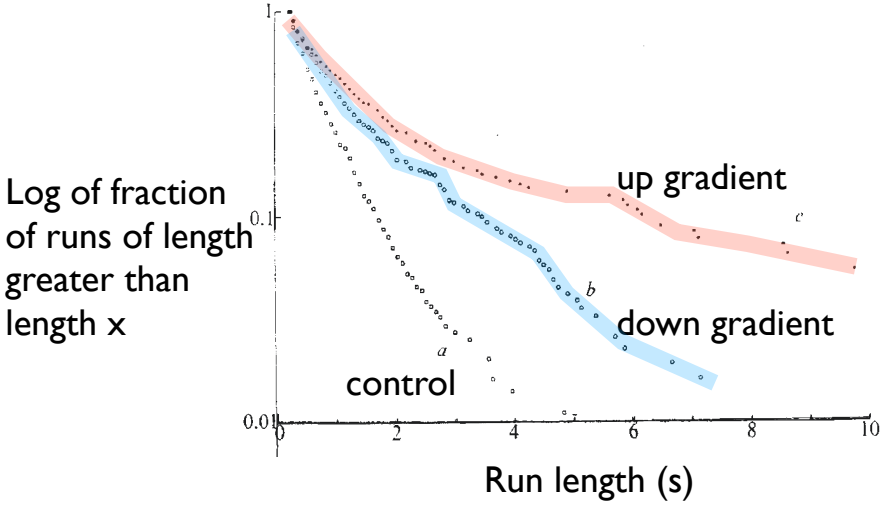
Analogies with bacterial chemotaxis?







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



— up the gradient: runs are longer than is expected from the concentration dependence of the runs (ie. tumbles are postponed)

Howard Berg and Douglas Brown. *Nature* 239, 500-504 (1972)
R. Macnab. D.E. Koshland. *PNAS.* 69:2509-2512 (1972)

Analogies with bacterial chemotaxis

STRATEGIES FOR CHEMOTAXIS

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CONCLUSIONS

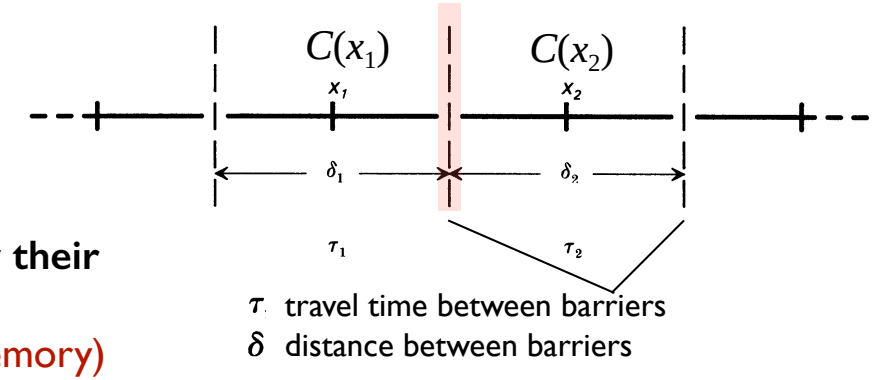
We re-examined the problem of migration of motile organisms in spatial gradients of chemical attractants. We showed analytically and by Montecarlo simulation that organisms whose turning frequencies (tumble probabilities) depend solely on the local concentration of an attractant, but whose speeds remain 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 Montecarlo 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.

Schnitzer M, Block S, Berg HC, Purcell E. *Symp. Soc. Gen. Microbiol.* 46:15–34 (1990)

Analogies with bacterial chemotaxis

1D theoretical model of stochastic motion:
Semipermeable barriers that reflect ½ of particles (eg. bacteria), and let ½ pass through



τ: travel time between barriers
δ: distance between barriers

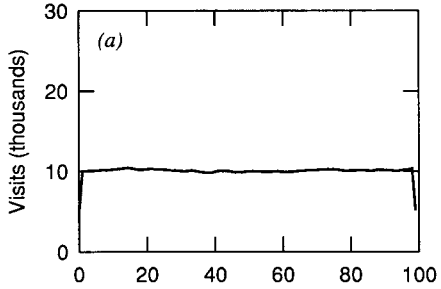
This models the idea that particles change randomly their trajectory every time τ and distance δ
Their motion is defined locally in space and time (no memory)

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

- **Case 0**: δ and τ 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 (** δ and/or τ vary in space**):**

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



Monte Carlo simulations

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.

Schnitzer M, Block S, Berg HC, Purcell E. *Symp. Soc. Gen. Microbiol.* 46:15–34 (1990)

Analogies with bacterial chemotaxis

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

- **Case 2**: distance δ is constant $J = -D(\partial C/\partial x) - C(\partial D/\partial x) = -\partial(DC)/\partial x,$ $D = \delta^2/4\tau(x)$

  At equilibrium DC is uniform, and C is inversely proportional to D
  Therefore, particles accumulate where their velocity is lowest

- **Case 3**: time τ is constant $J = -D(\partial C/\partial x) - C(\partial D/\partial x)/2.$ $D = \delta^2(x)/4t$

  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 $J = -(\delta/4)[v(\partial C/\partial x) + C(\partial v/\partial x)],$ (δ/τ)

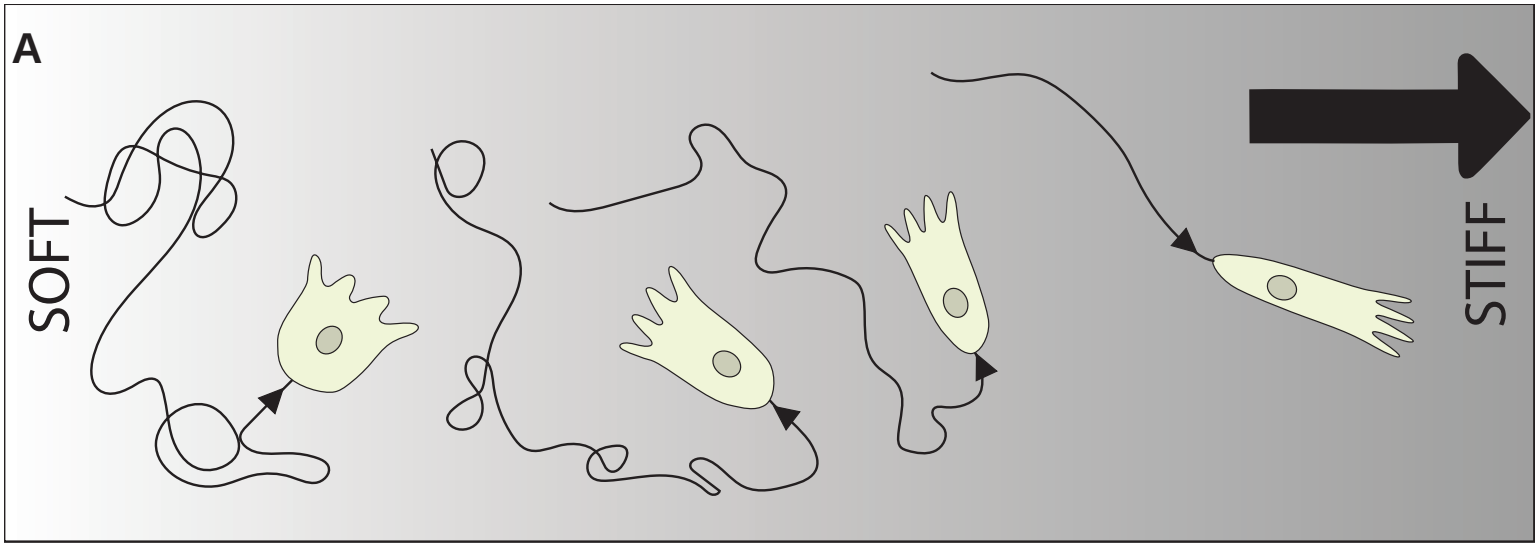
  At equilibrium, the density of particles is still inversely proportional to velocity

- 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.

Schnitzer M, Block S, Berg HC, Purcell E. *Symp. Soc. Gen. Microbiol.* 46:15–34 (1990)

Durotaxis: Rigidity-dependent *persistence* ??

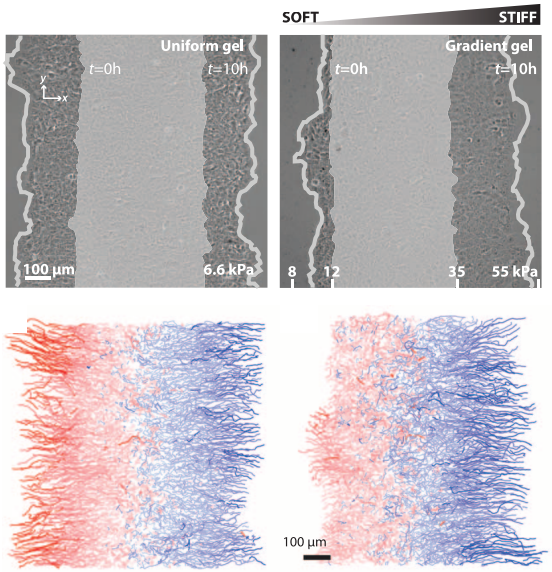
Clarifications needed: data and model:
— without memory, no durotaxis is possible (Schnitzer et al)
— with memory, may be possible…

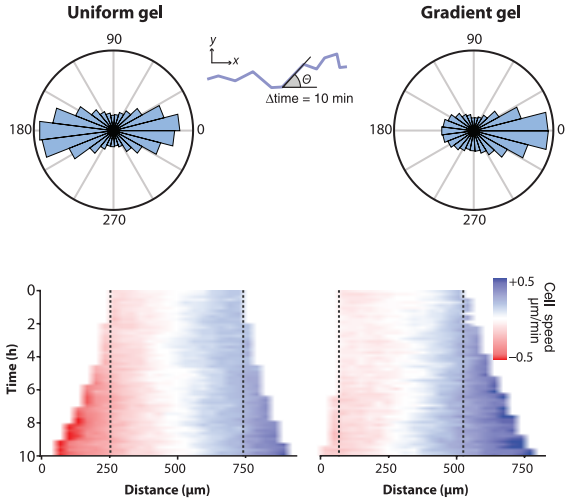


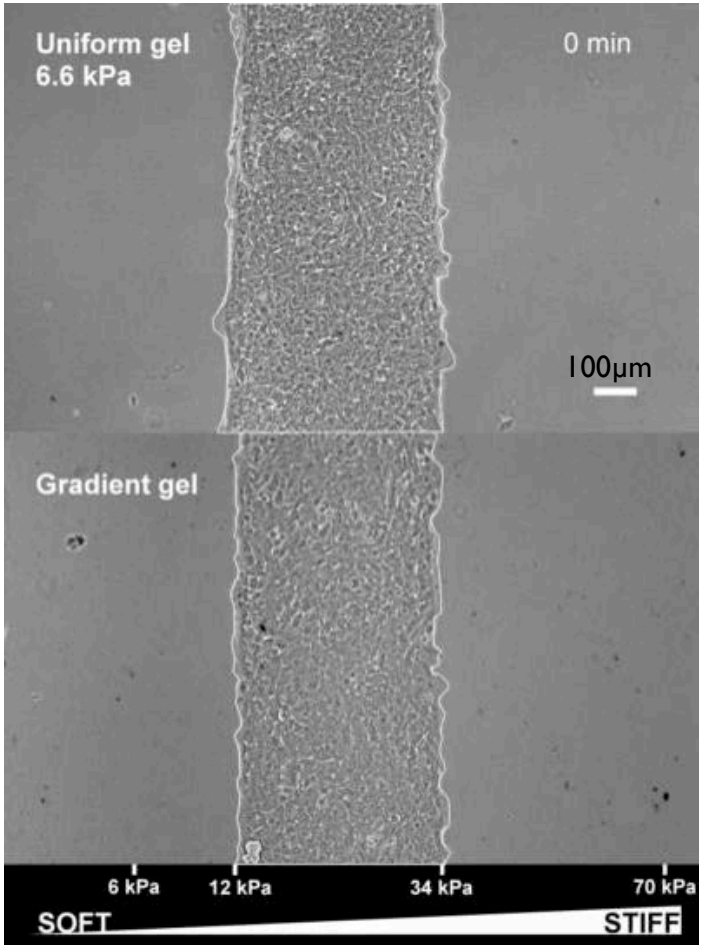
A. Shellard and R. Mayor. *Developmental Cell* 56: 227-239 (2021)

Collective durotaxis

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





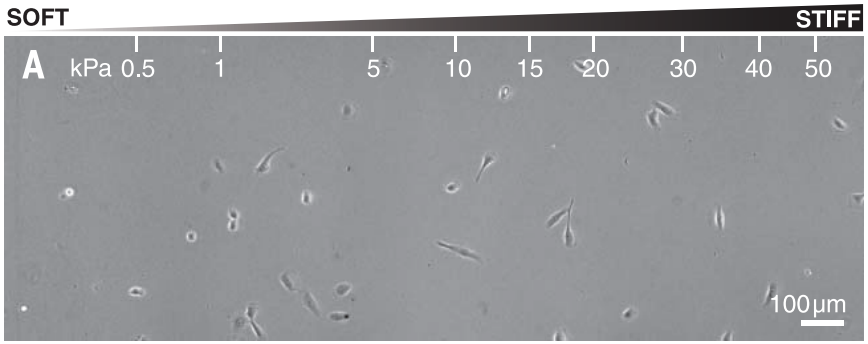


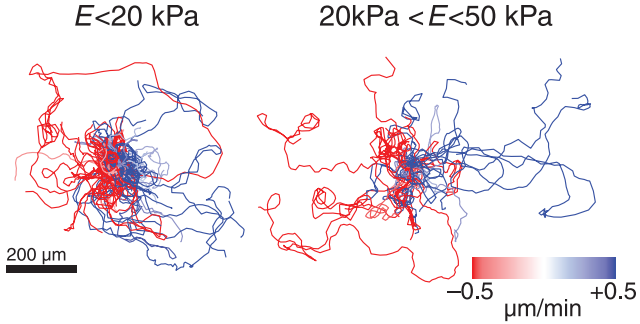
~22kPa / 450µm

Sunyer, R., et al and Roca-Cusachs, P., and X. Trepat. *Science 353*, 1157–1161 (2016)

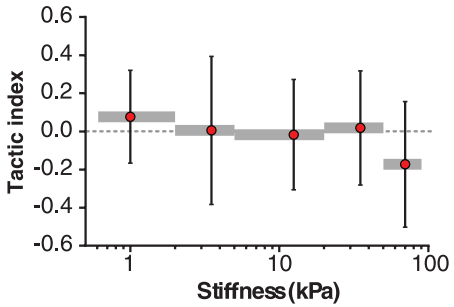
Collective durotaxis

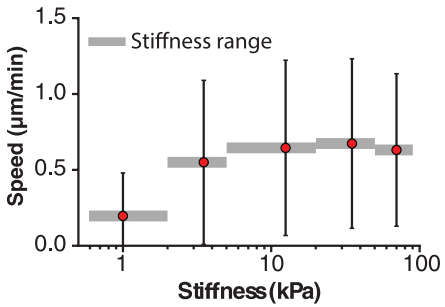
Single cells do not exhibit durotaxis on the same stiffness gradient

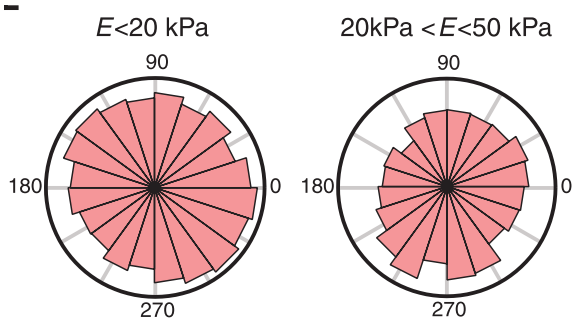




Cell speed increases with stiffness





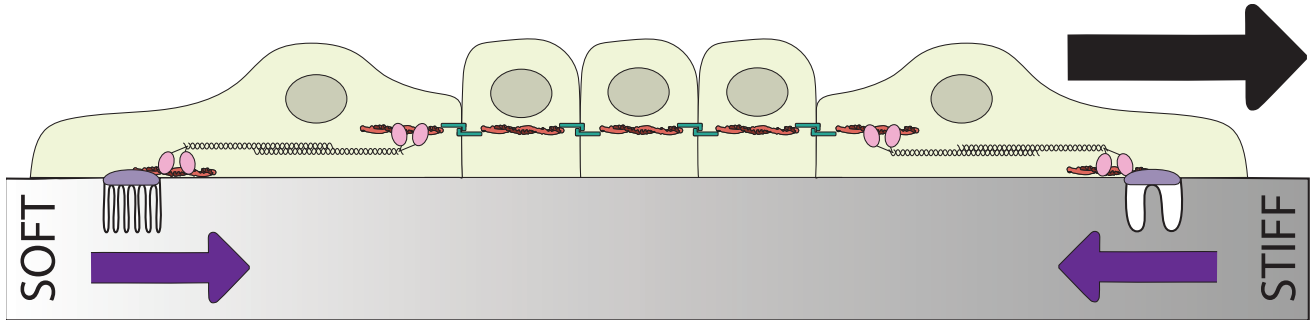


Sunyer, R., et al and Roca-Cusachs, P., and X. Trepat. *Science 353*, 1157–1161 (2016)

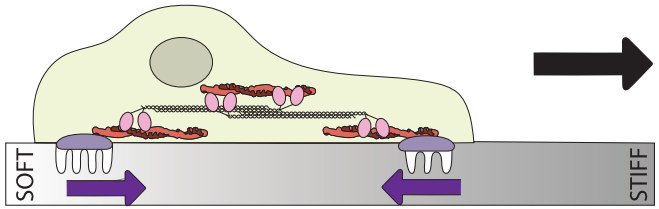
Collective durotaxis

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

- Collective durotaxis



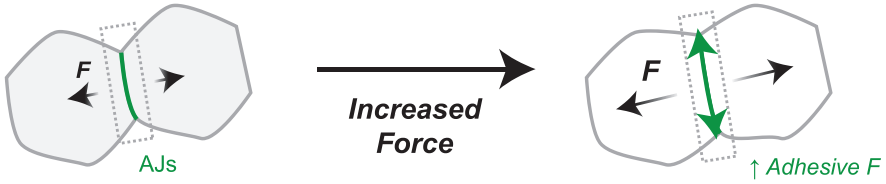
- Single cell durotaxis

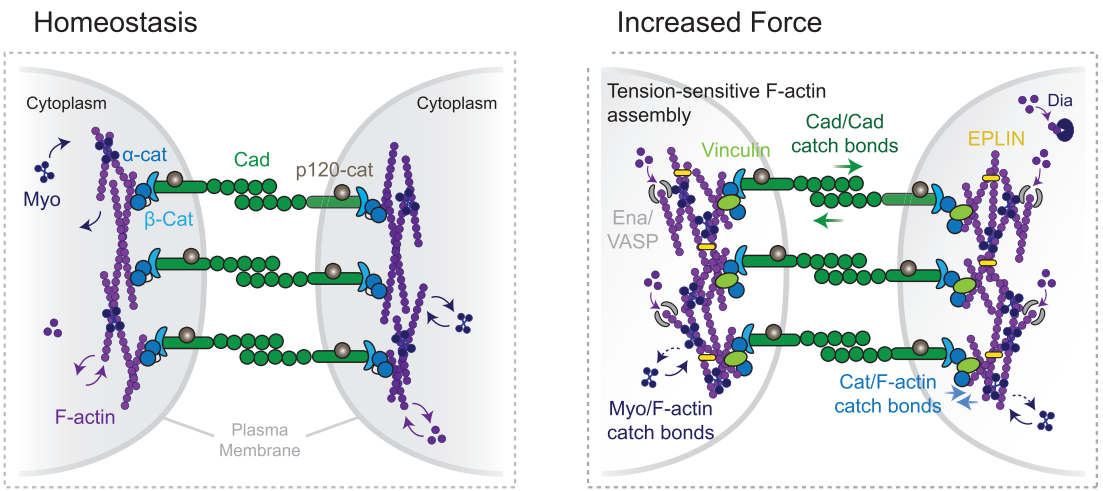


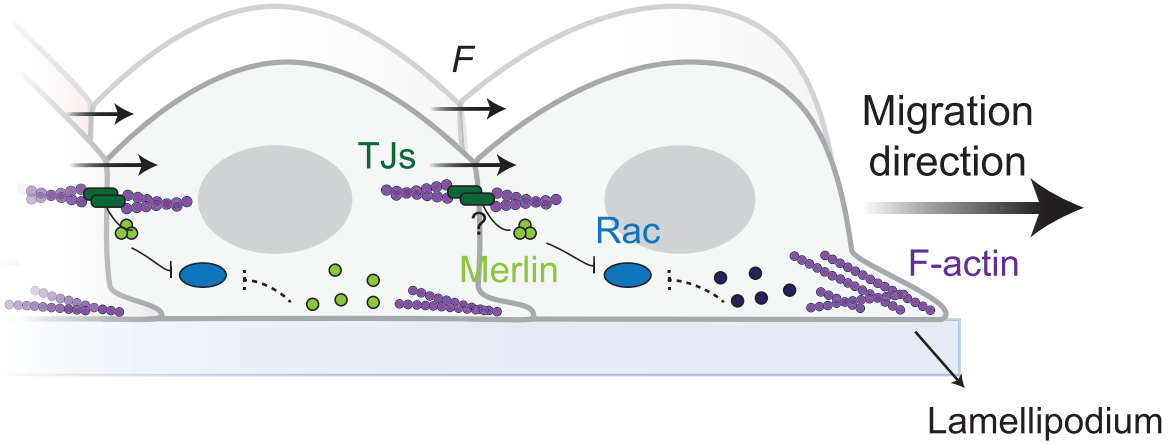
A. Shellard and R. Mayor. *Developmental Cell* 56: 227-239 (2021)

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)





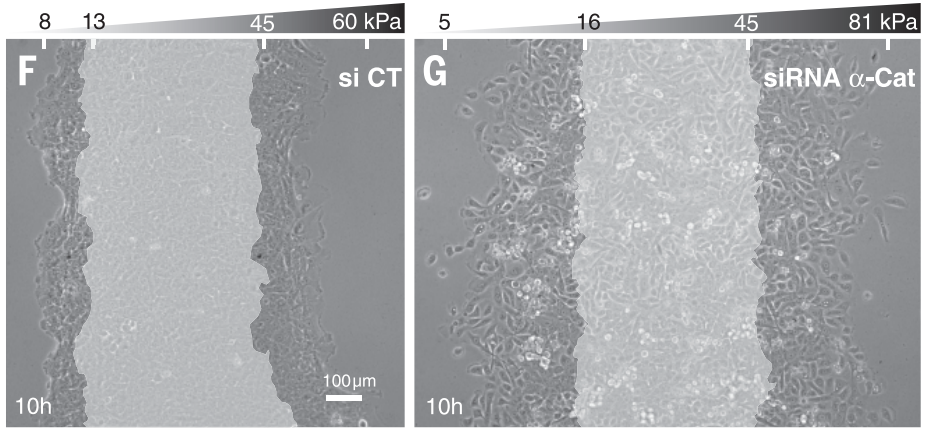


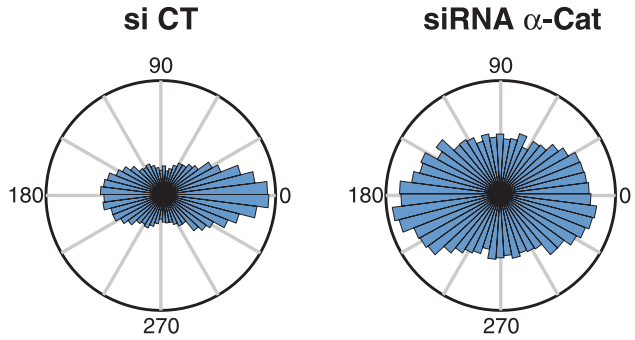
D. Pinheiro and Y. Bellaiche. *Developmental Cell* 47: 3-19 (2018)

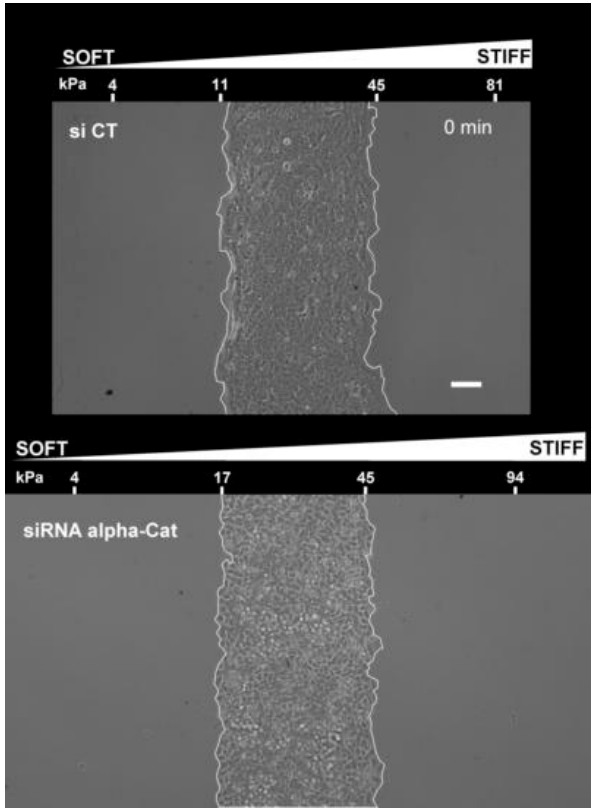
Collective durotaxis

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







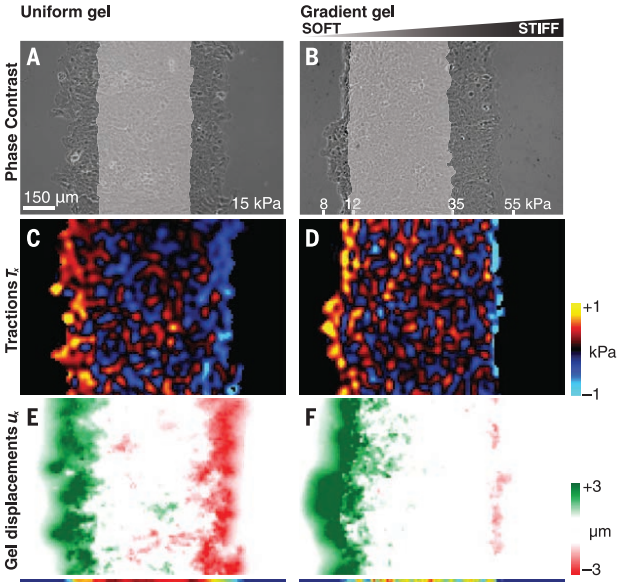
Sunyer, R., et al and Roca-Cusachs, P., and X. Trepat. *Science 353*, 1157–1161 (2016)

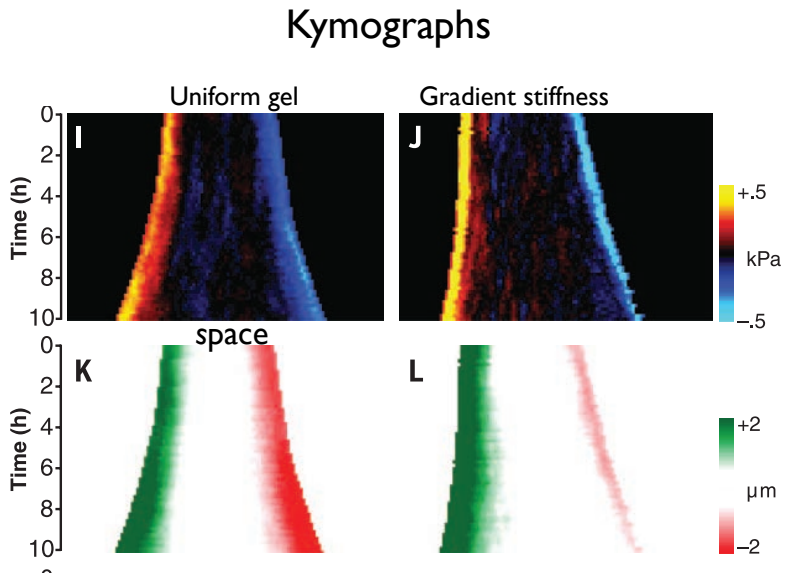
Collective durotaxis

Probing the distribution and transmission of forces

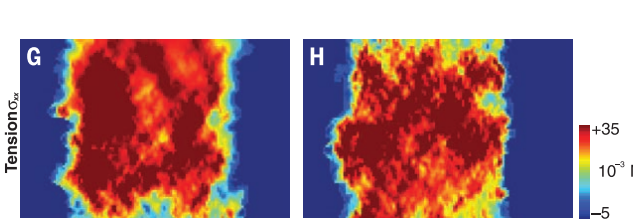
Traction forces are exerted at the edge and propagate within the bulk of the cell layer

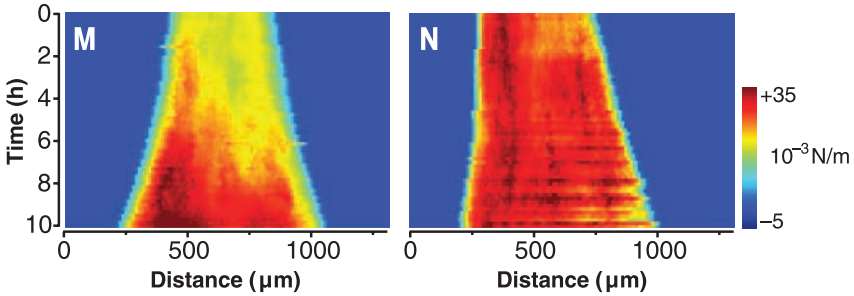
- Traction forces on ECM are concentrated at the edge of the cell cluster and are symmetrically distributed
- Substrate deformation is asymmetric in the gradient (lower in stiffer regions): given symmetric actin polymerization at the edge, expansion is asymmetric





- Tensile forces in the bulk





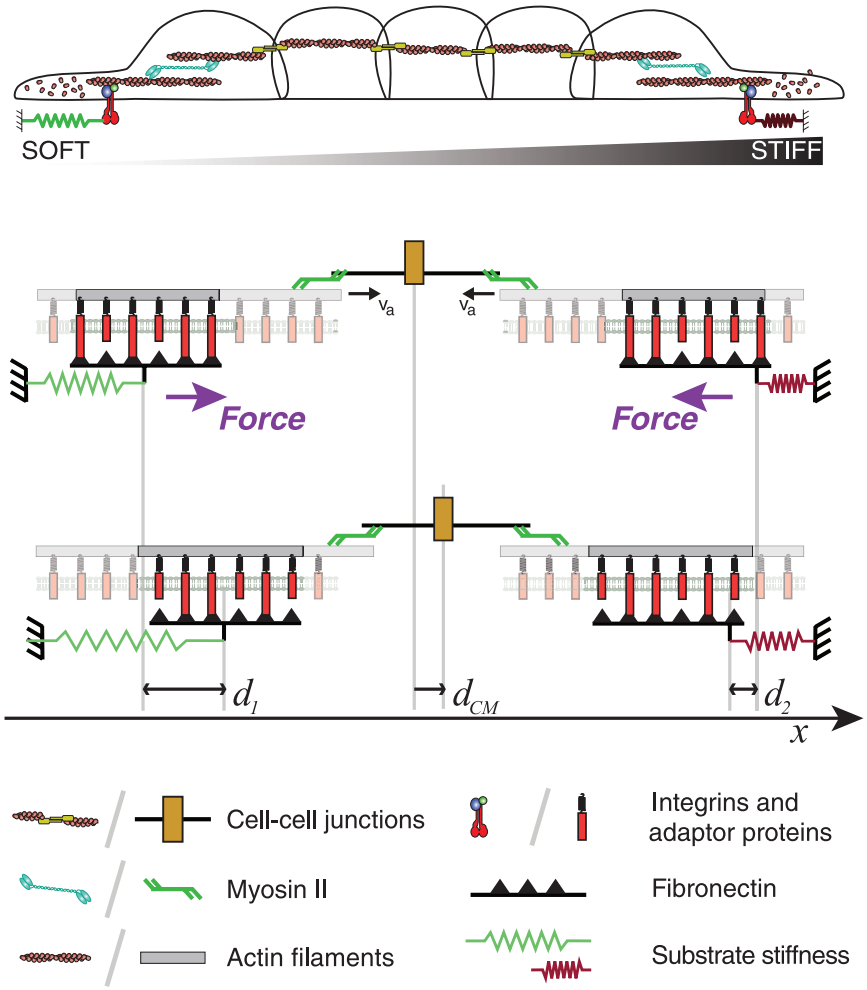
Sunyer, R., et al and Roca-Cusachs, P., and X. Trepat. *Science 353*, 1157–1161 (2016)

Collective durotaxis- Supracellular durotaxis

- Similar to cellular model
- The tissue bulk is an elastic material
- If viscous behavior in the bulk (ie. 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



Sunyer, R., et al and Roca-Cusachs, P., and X. Trepat. *Science 353*, 1157–1161 (2016)

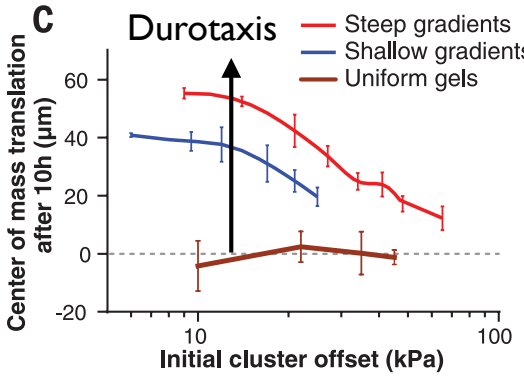
Collective durotaxis

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

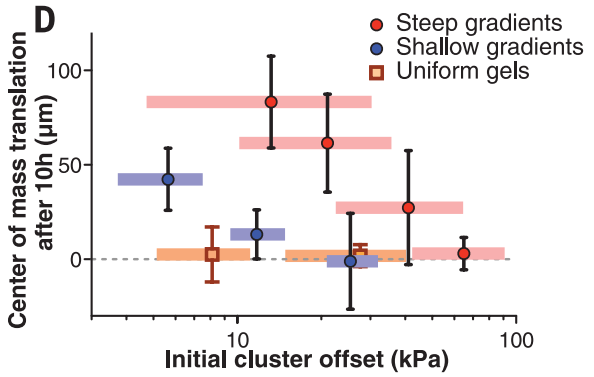
  Durotaxis is quantified by the cluster center of mass translation after 10 hours
  This is assessed as a function of the initial stiffness of the center of mass of cell cluster

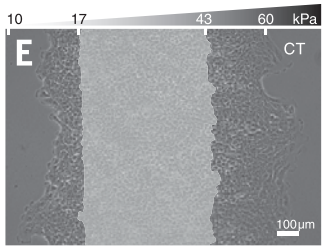
- Durotaxis requires cell contractility and mechanical transmission

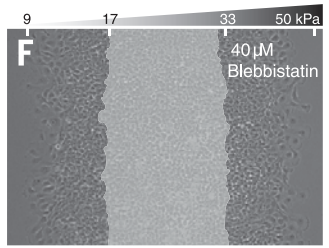
- Model



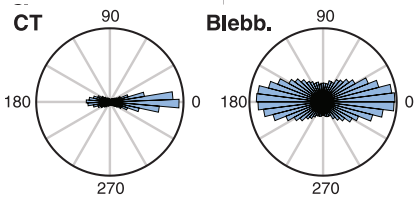
- Experiments

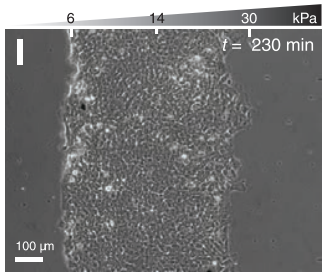


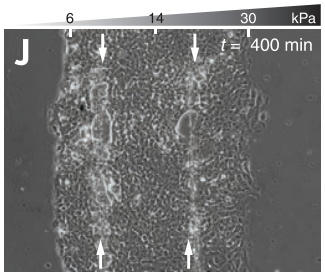




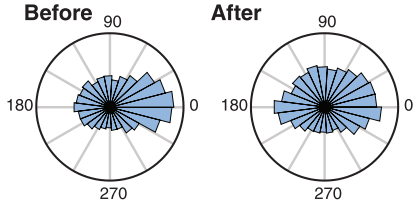
myosinII inhibitor







Laser ablation in gel

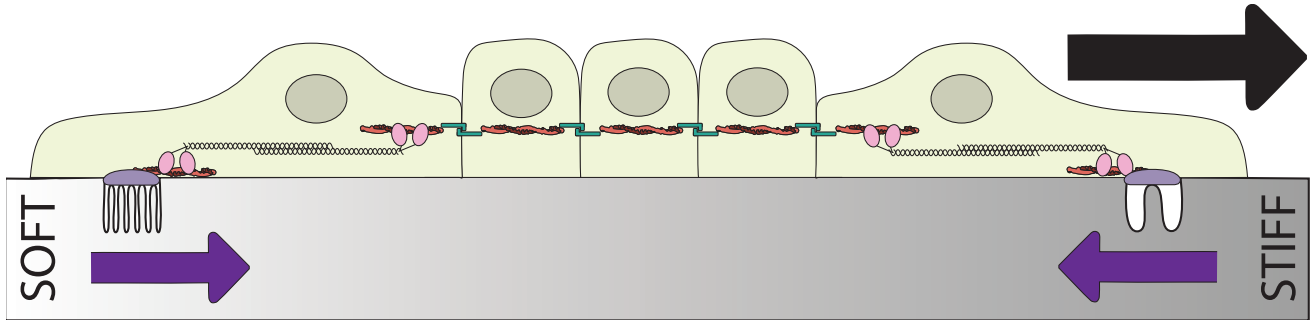


Sunyer, R., et al and Roca-Cusachs, P., and X. Trepat. *Science 353*, 1157–1161 (2016)

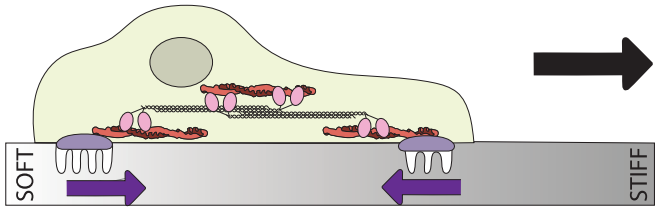
Collective durotaxis

- 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



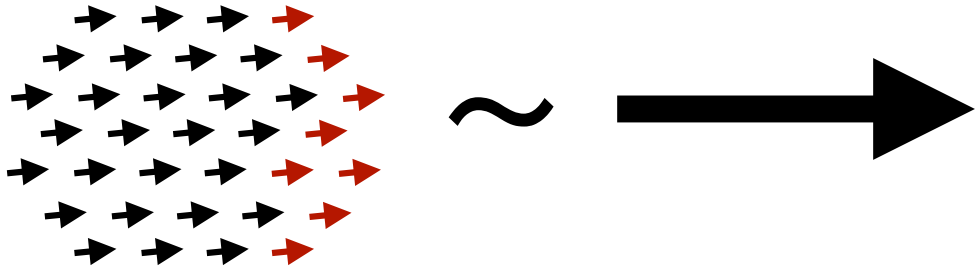
A. Shellard and R. Mayor. *Developmental Cell* 56: 227-239 (2021)

Case Studies of collective cell migration

- **Collective migration with leaders:**

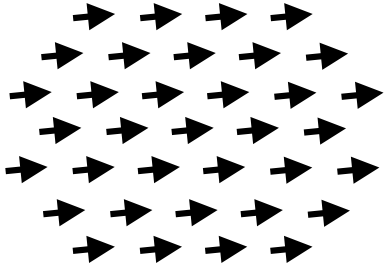
Case Study 1: Neural crest cell migration (*Xenopus*)

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



- **Collective migration without leaders:**

Case Study 3: Egg chamber rotation (*Drosophila*)



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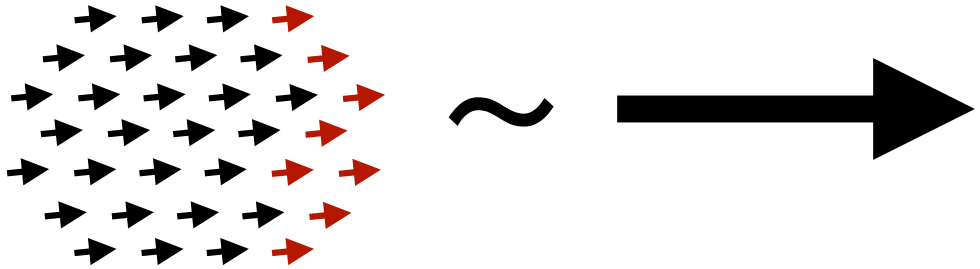

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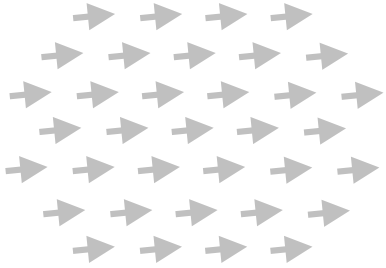
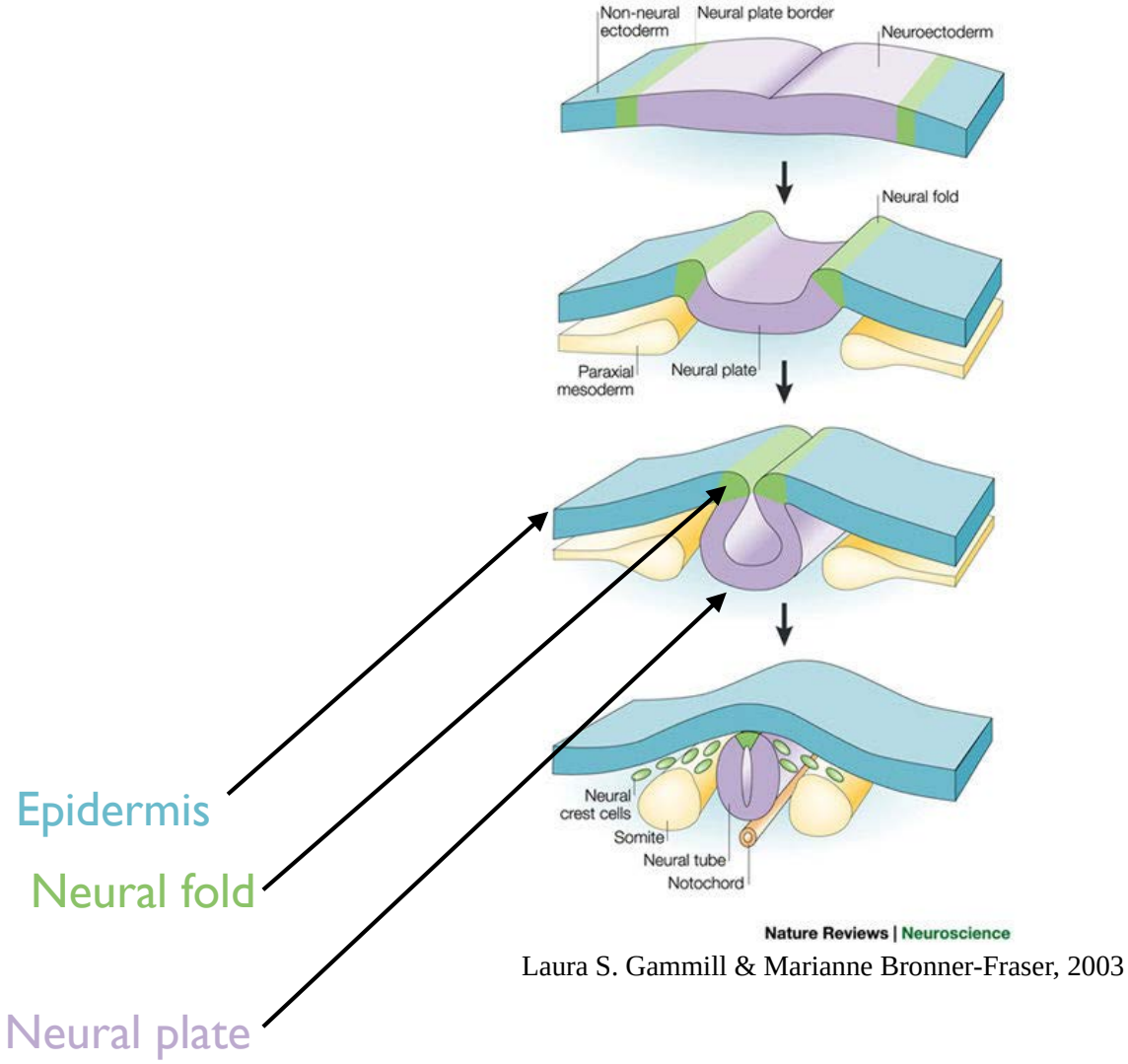


Possible mechanisms of collective motility

- Motility guided by chemoattractant
- Motility guided mechanically

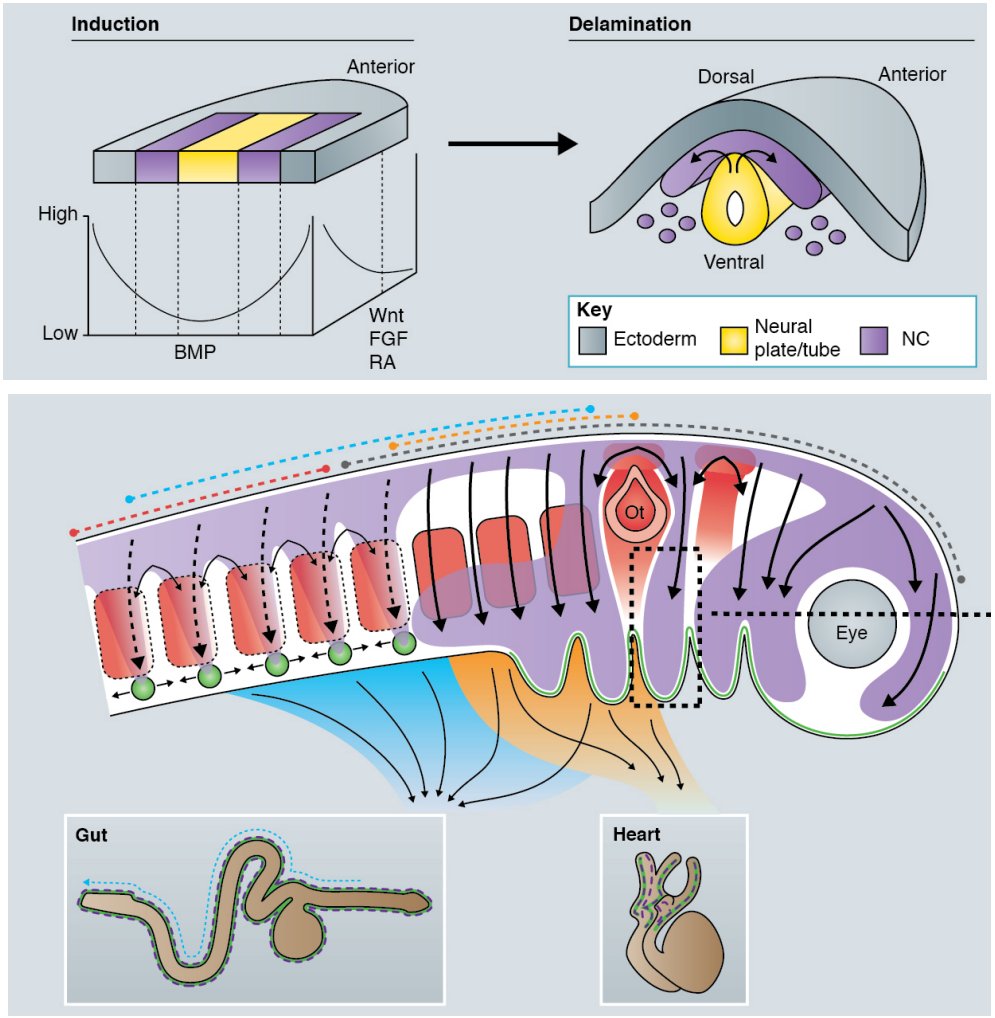
Case Study 1: Neural crest cell migration



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



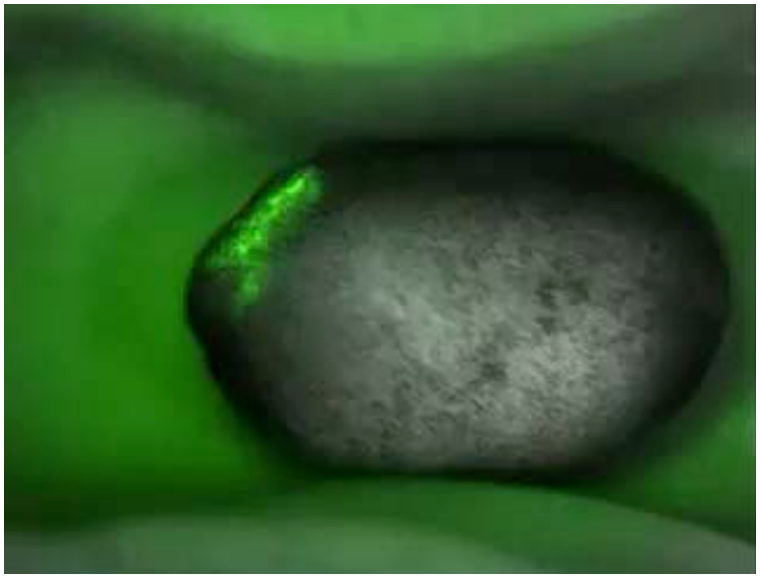
R. Mayor and E. Theveneau. *Development* (2013) 140 (11): 2247–2251.

Case Study 1: Neural crest cell migration

- Dorsal view of *Xenopus* embryo

Anterior

- Lateral view of *Xenopus* embryo



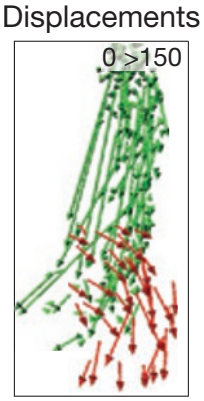
Anterior

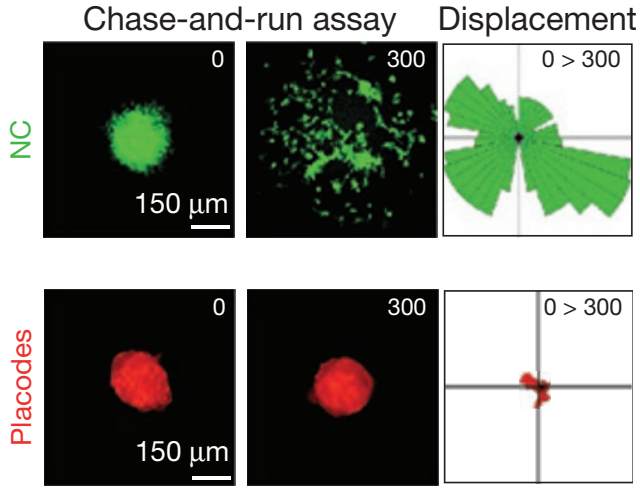
G. Abbruzzese. *J Cell Sci* (2015) 128 (6): 1139–1149.
https://doi.org/10.1242/jcs.163063

Case Study 1: Neural crest cell migration

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

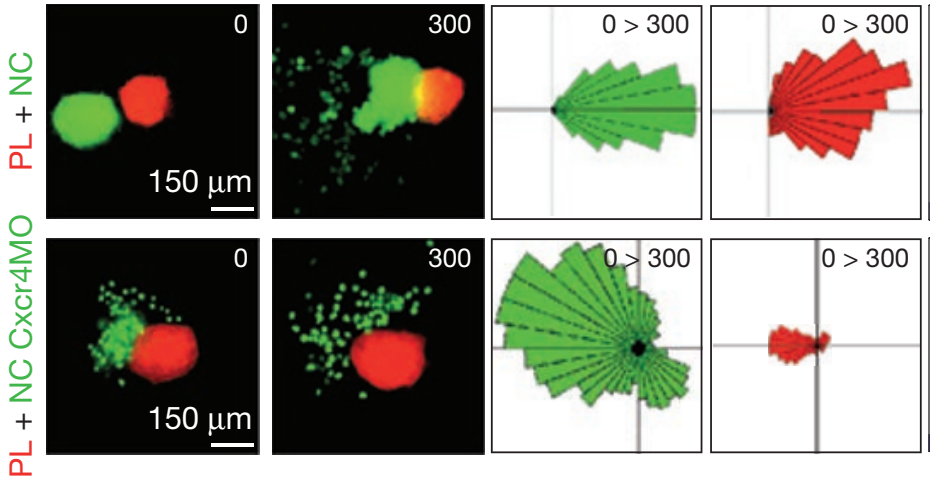
- In vivo displacement of two adjacent tissues in *Xenopus*:
- Neural crests (NC) and placode cells, epithelial cells that contribute to sensory organs





- 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

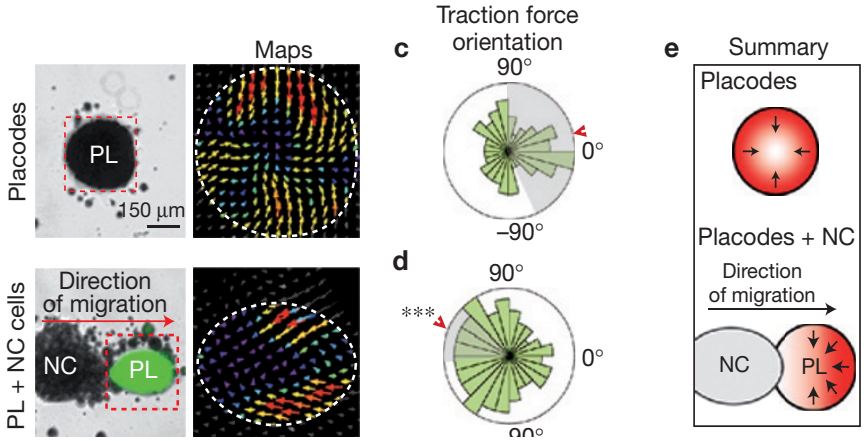


E. Theveneau et al, and R. Mayor. *Nature Cell Biol.* 15: 763-772 (2013)

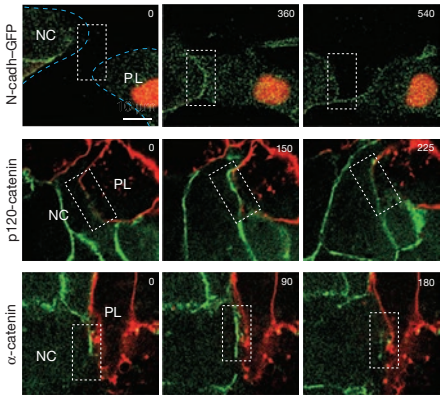
Case Study 1: Neural crest cell migration

—Mechanical interactions between cell populations induce symmetry breaking

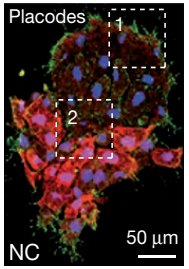
- Neural crest cells induce a symmetry breaking of traction forces exerted by placode cells

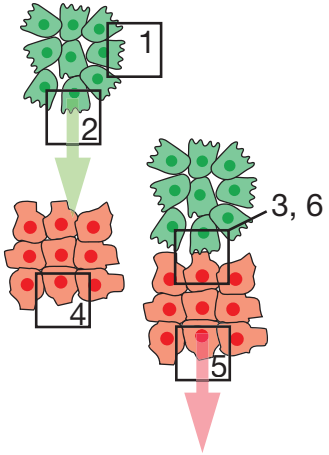


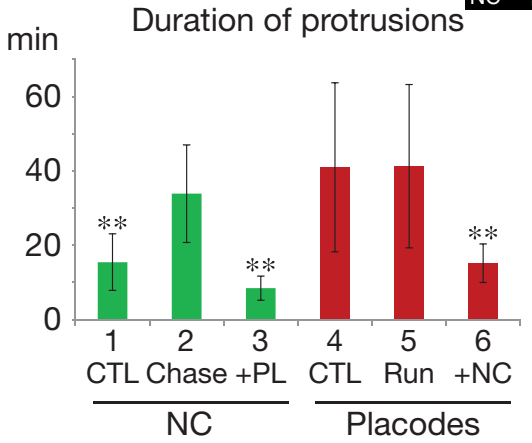
- Adhesion between NC and Placode cells
- Mediated by N-cadherin



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



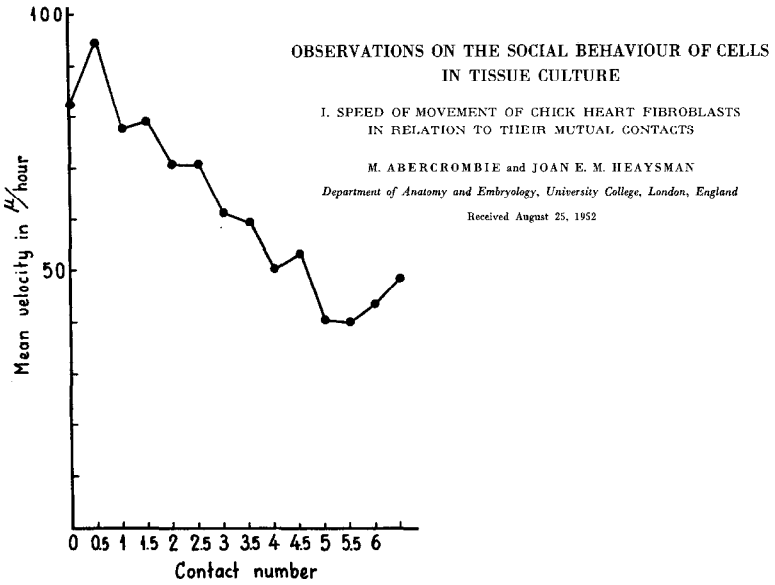




E. Theveneau et al, and R. Mayor. *Nature Cell Biol.* 15: 763-772 (2013)

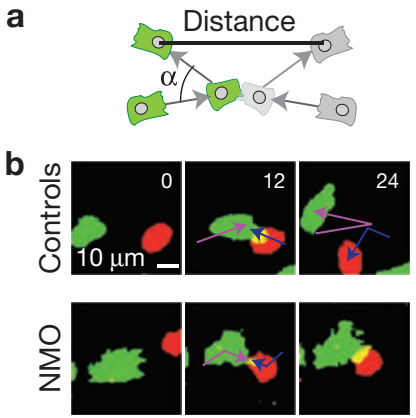
Case Study 1: Neural crest cell migration

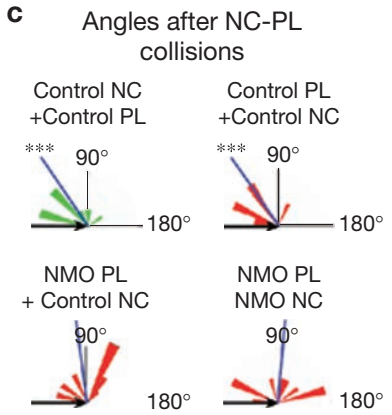
— Contact Inhibition of Locomotion underlies coordinated migration



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

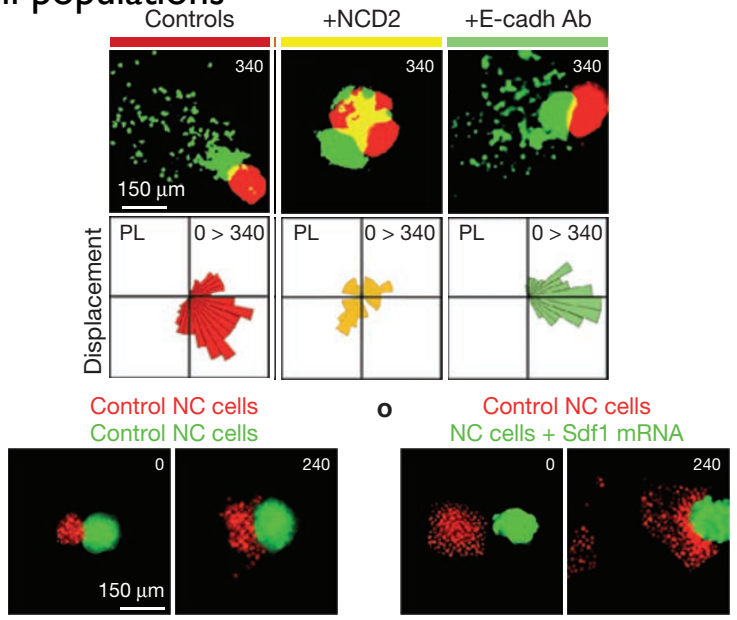
Single cell analysis





N-cad morpholino (NMO)

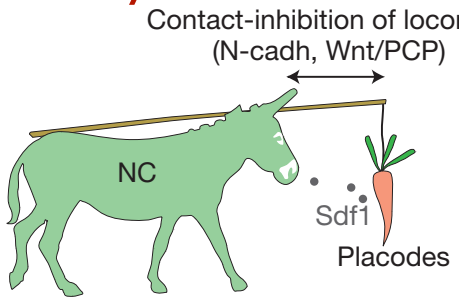
Cell populations

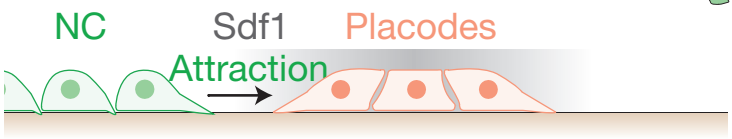


E. Theveneau et al, and R. Mayor. *Nature Cell Biol.* 15: 763-772 (2013)

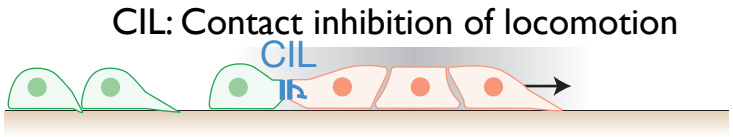
Case Study 1: Neural crest cell migration

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

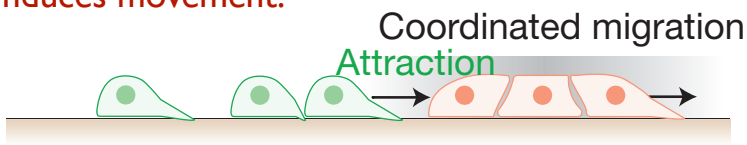




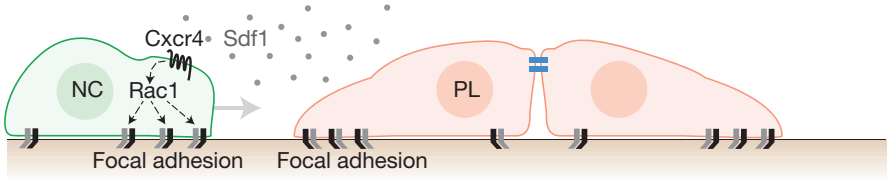
- NC cells are attracted to placodal cells via Sdf1-dependent chemotaxis

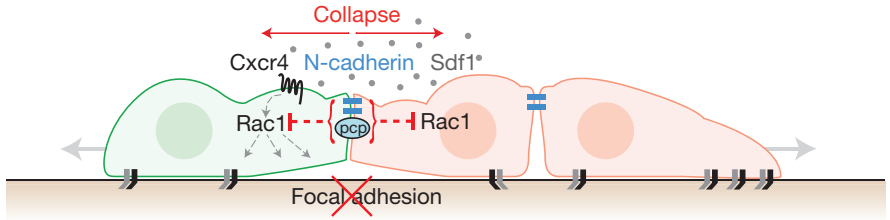


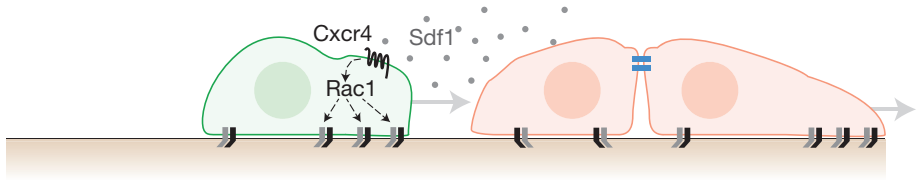
- Contact between NC and placodal cells induces CIL
- This breaks the symmetry of the placodal tissue, and induces movement.



- The system self-sustains owing to chemotaxis and CIL





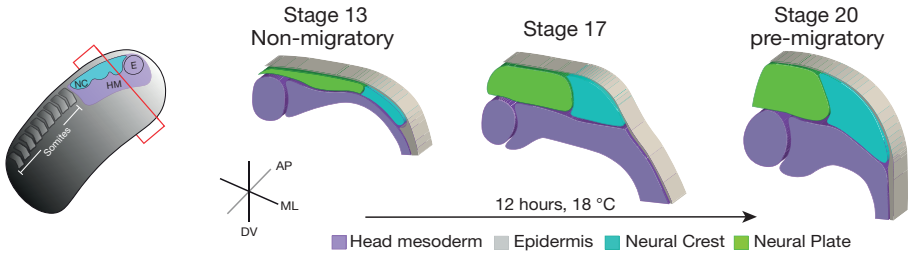


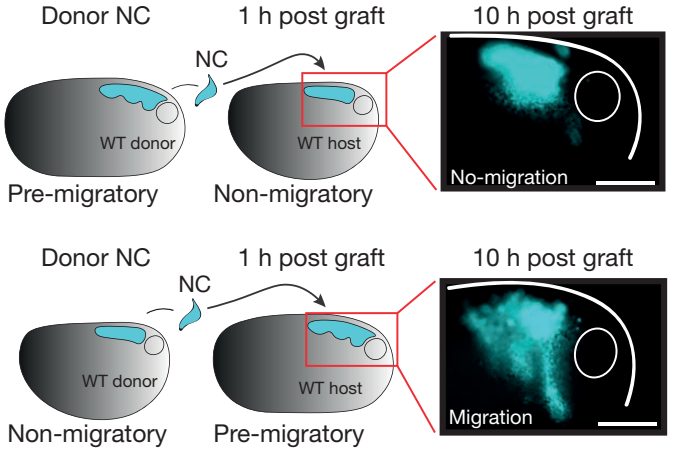
E. Theveneau et al, and R. Mayor. *Nature Cell Biol.* 15: 763-772 (2013)

Case Study 1: Neural crest cell migration

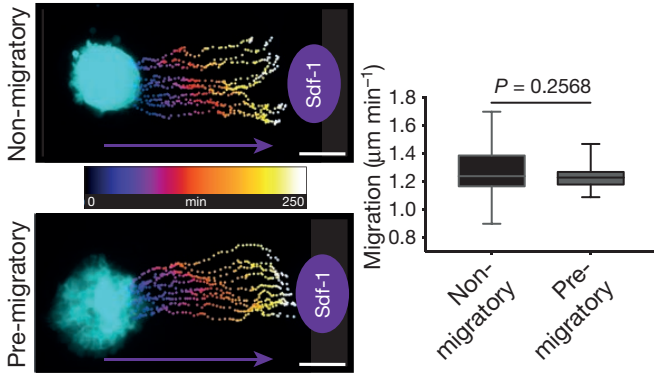
— Role of the mechanical environment in migration

- 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

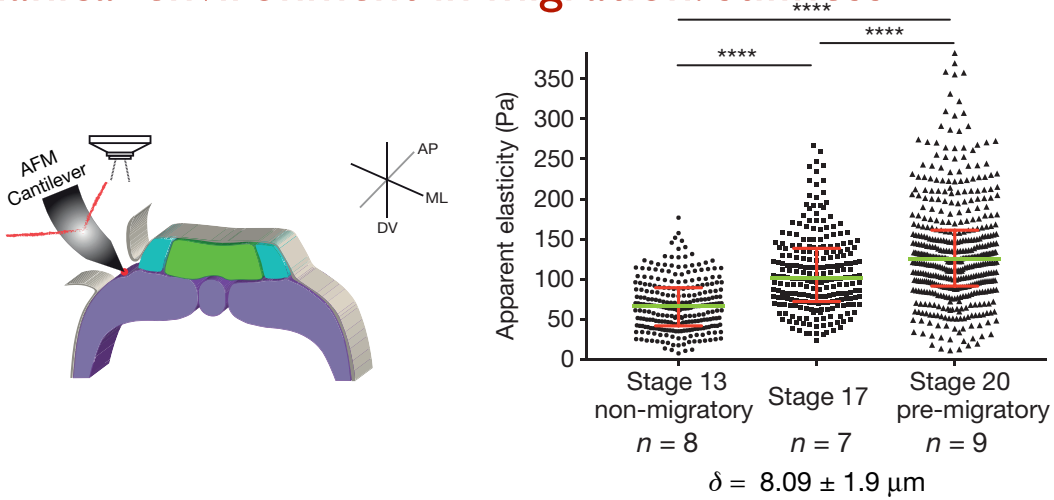


E. Barriga, K Franze, G. Charras and R. Mayor *Nature*, 554:523-527 (2018)

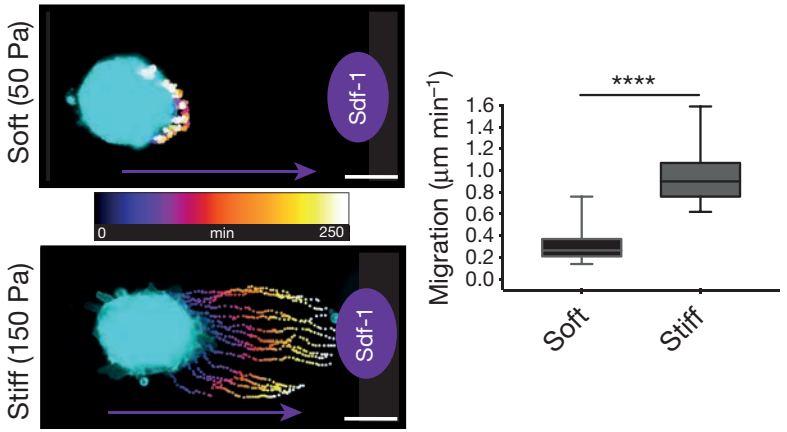
Case Study 1: Neural crest cell migration

— 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



E. Barriga, K Franze, G. Charras and R. Mayor *Nature*, 554:523-527 (2018)

Case Study 1: Neural crest cell migration

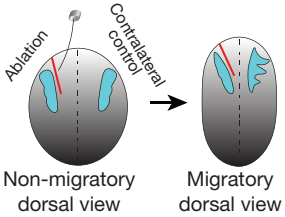
— Role of the mechanical environment in migration: stiffness

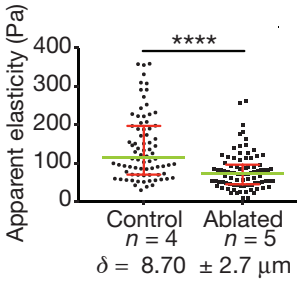
- Mechanical relaxation of tissue stiffness *in vivo*

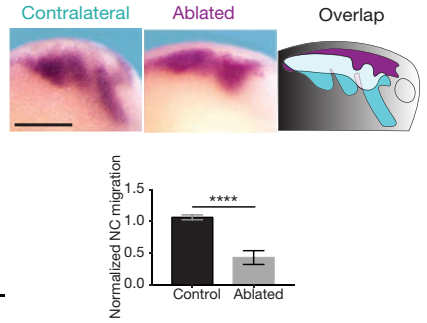
Strain dependent stiffening of matrix and tissues

Stress relaxation following tissue ablation softens the mesoderm

This affects NC migration in vivo



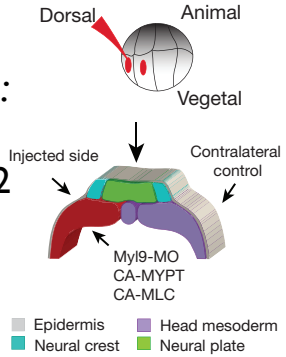


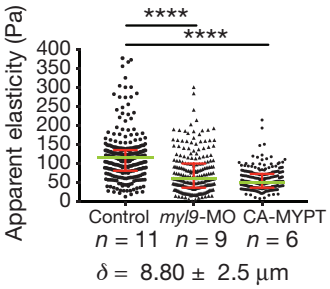


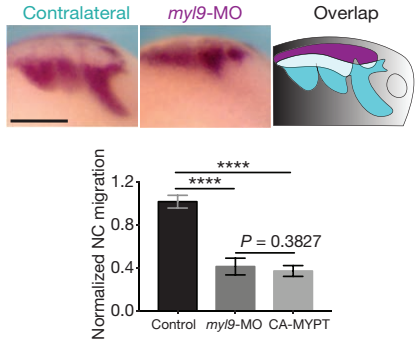
- Genetic relaxation of tissue stiffness *in vivo:*

Tissue softening via genetic inhibition of Myosin2

This reduces NC migration

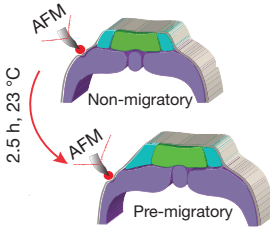


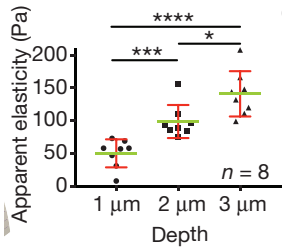


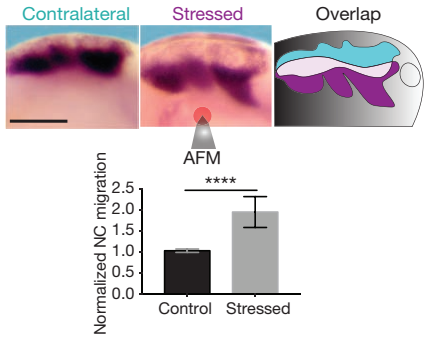


- Increase of tissue stiffness *in vivo:*

Atomic Force Microscopy (AFM) increases tissue stiffness and promotes cell motility





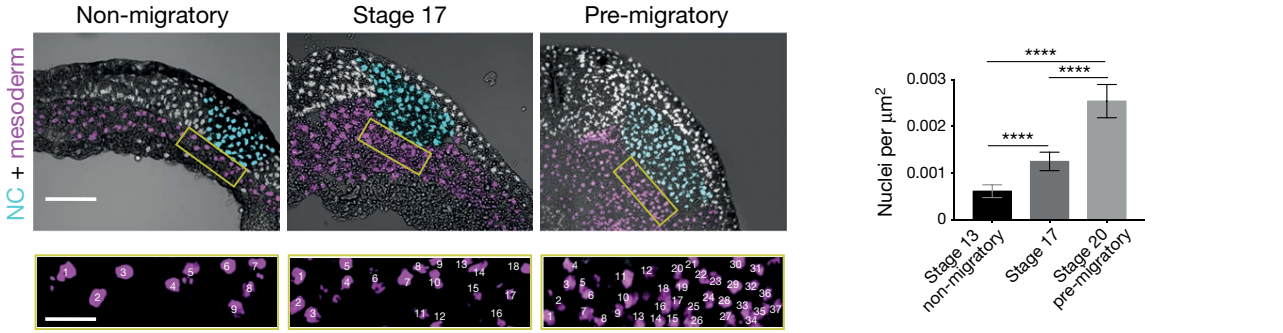


E. Barriga, K Franze, G. Charras and R. Mayor *Nature*, 554:523-527 (2018)

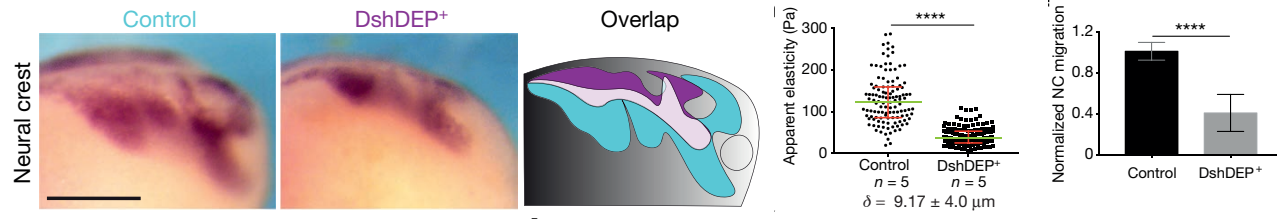
Case Study 1: Neural crest cell migration

— Role of the mechanical environment in migration: stiffness

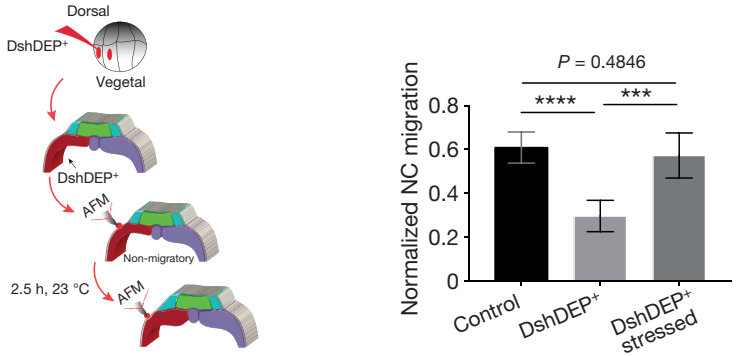
- Convergence-Extension (C/E) movement in the mesoderm increase cell density and tissue stiffness



- 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

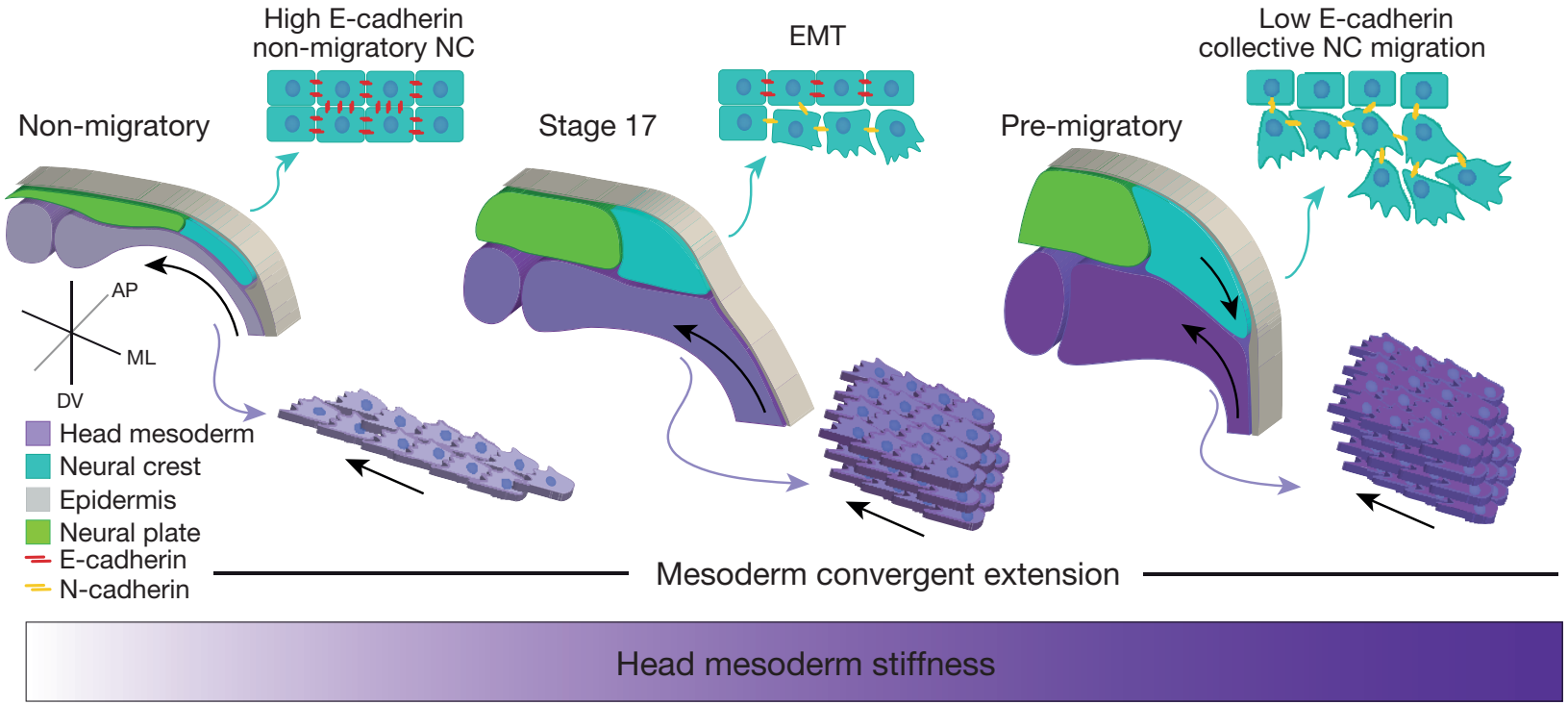


E. Barriga, K Franze, G. Charras and R. Mayor *Nature*, 554:523-527 (2018)

Case Study 1: Neural crest cell migration

— Tissue stiffening promotes migration of neural crest cells

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

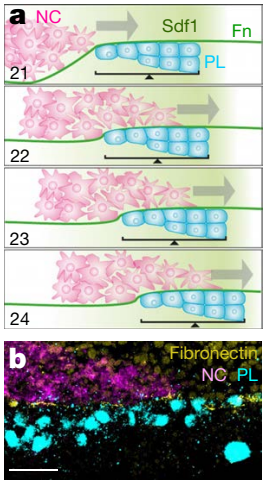


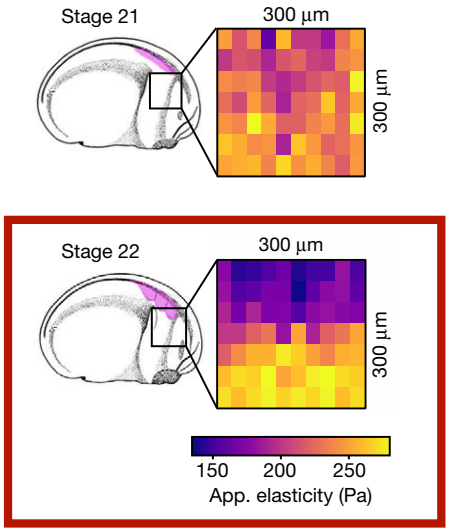
E. Barriga, K Franze, G. Charras and R. Mayor *Nature*, 554:523-527 (2018)

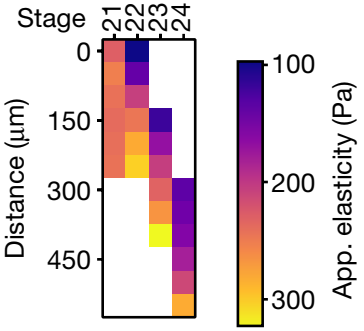
Case Study 1: Neural crest cell migration

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

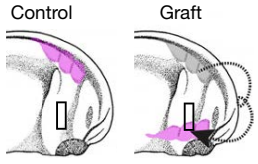
- AFM is used to measure tissue stiffness in vivo
- A gradient of stiffness appears
- The gradient moves ventrally as cells migrate
- The placode and NC are required for stiffness gradient formation.

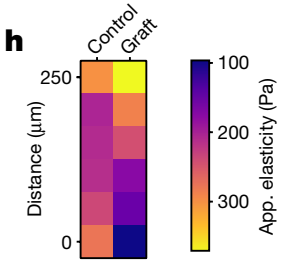


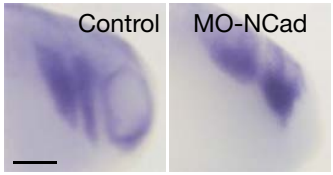


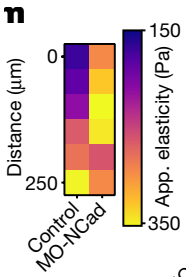


- An ectopic graft of NC induces a new gradient of stiffness
- This requires N-cadherin dependent softening.







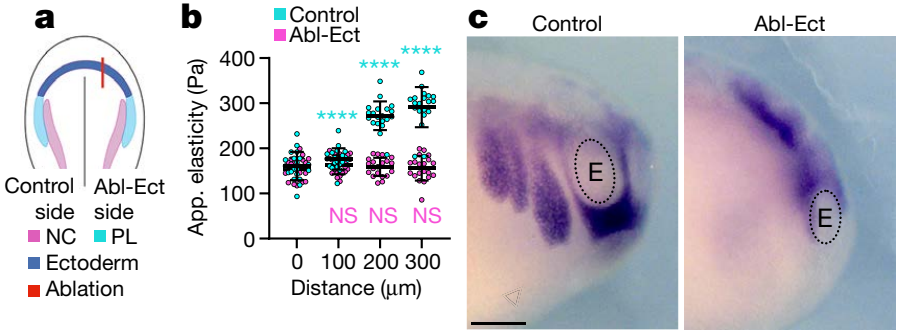


A. Shellard and R. Mayor. *Nature*, 600:690-694 (2021)

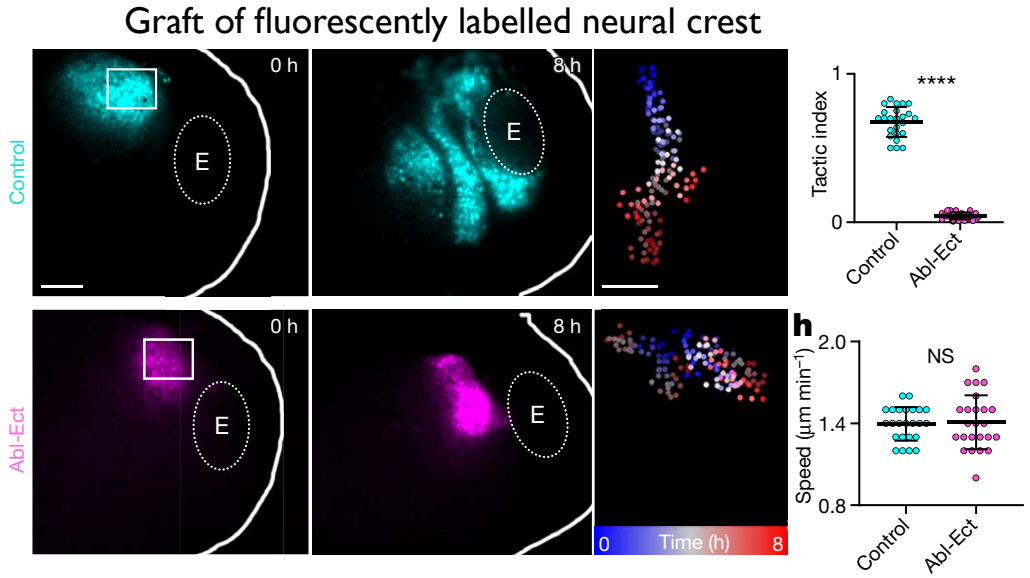
Case Study 1: Neural crest cell migration

—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

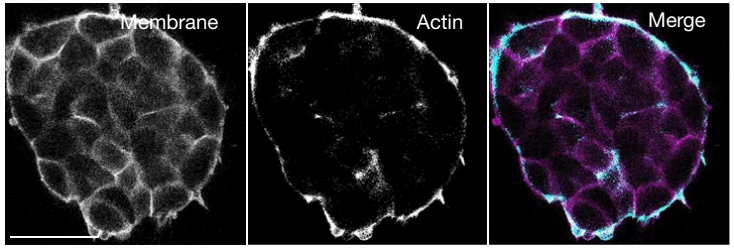


A. Shellard and R. Mayor. *Nature*, 600:690-694 (2021)

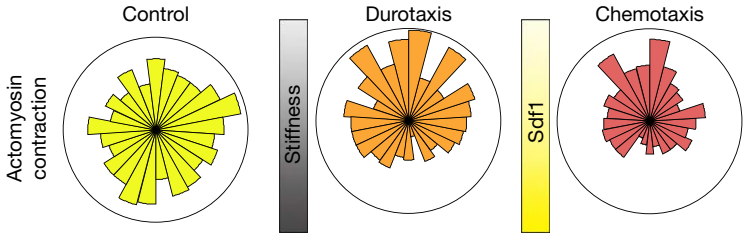
Case Study 1: Neural crest cell migration

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

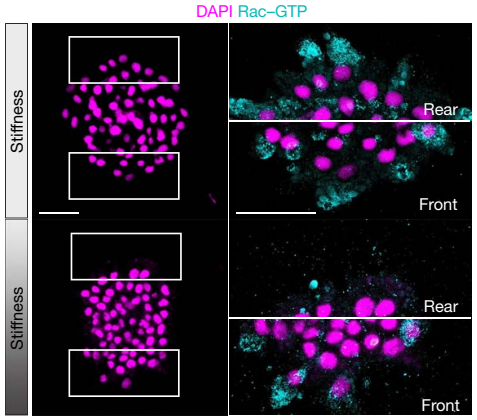
- NC clusters exhibit global polarity (Myosin2 at the rear and Rac1GTP at the front) along a chemical of stiffness gradient

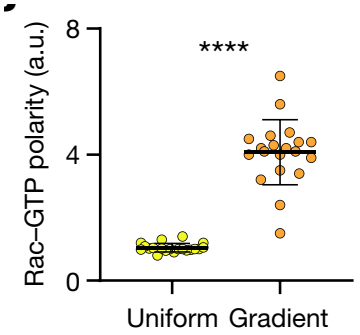


Contractility at the back:
Myosin 2 polarity



Actin nucleation at the front:
Rac1GTP polarity





A. Shellard and R. Mayor. *Nature*, 600:690-694 (2021)

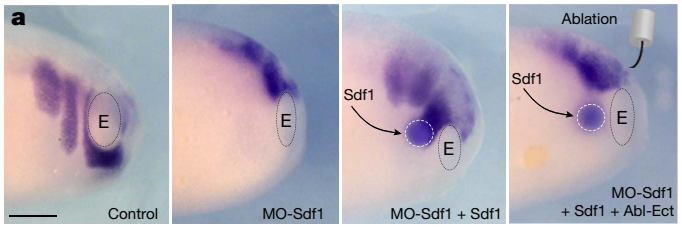
Case Study 1: Neural crest cell migration

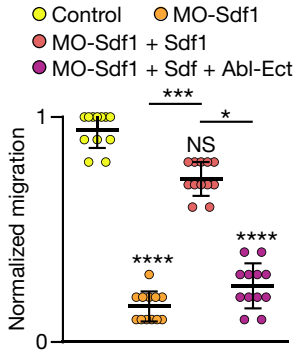
— Synergy between chemotaxis and durotaxis in vivo

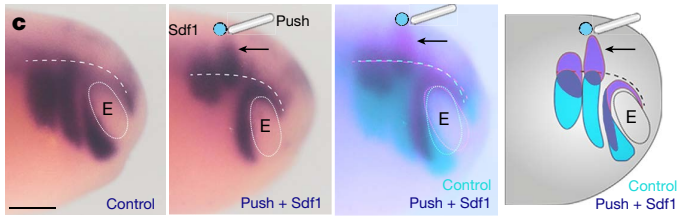
- Disentangling chemotaxis and durotaxis:

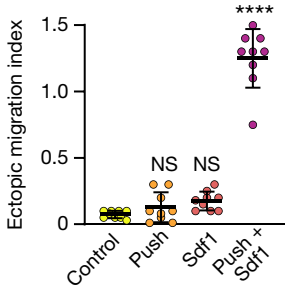
An ectopic source of SDF1 causes ectopic NC migration.

Softening the tissue with ectoderm ablation blocks chemotaxis towards SDF1.



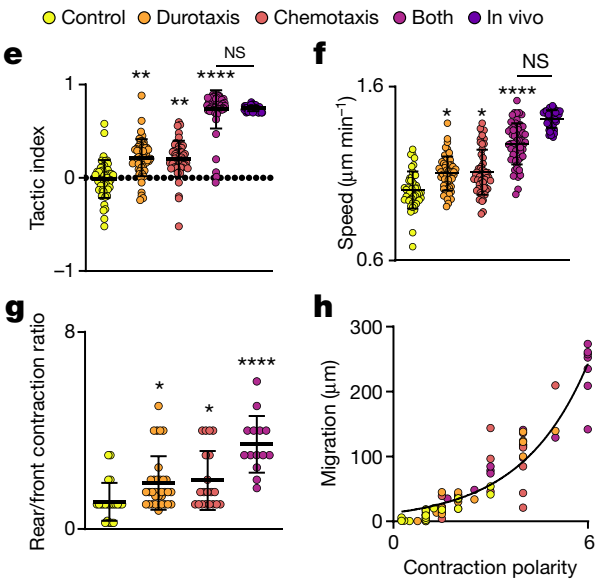






- Synergy:

Ectopic SDF1 together with tissue stiffening causes ectopic NC migration.



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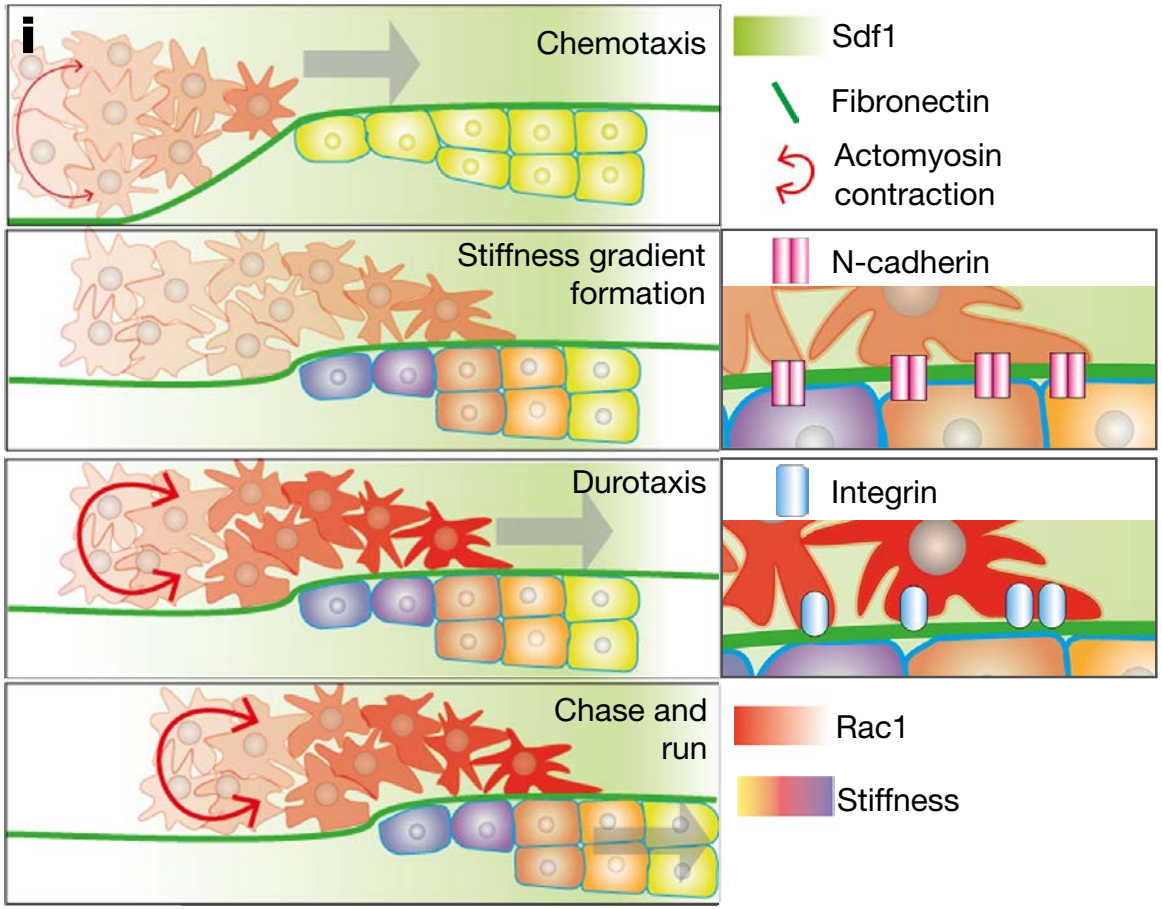
Case Study 1: Neural crest cell migration



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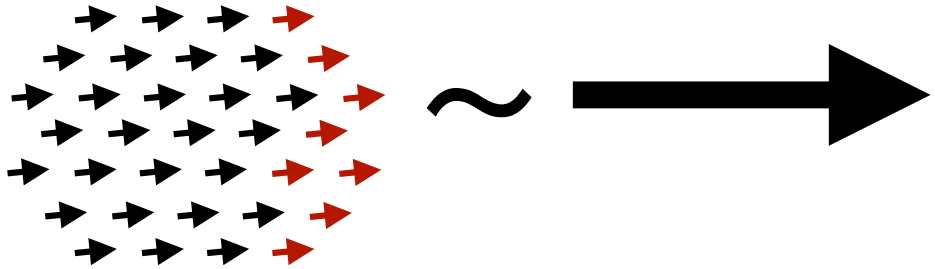
Summary

- 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