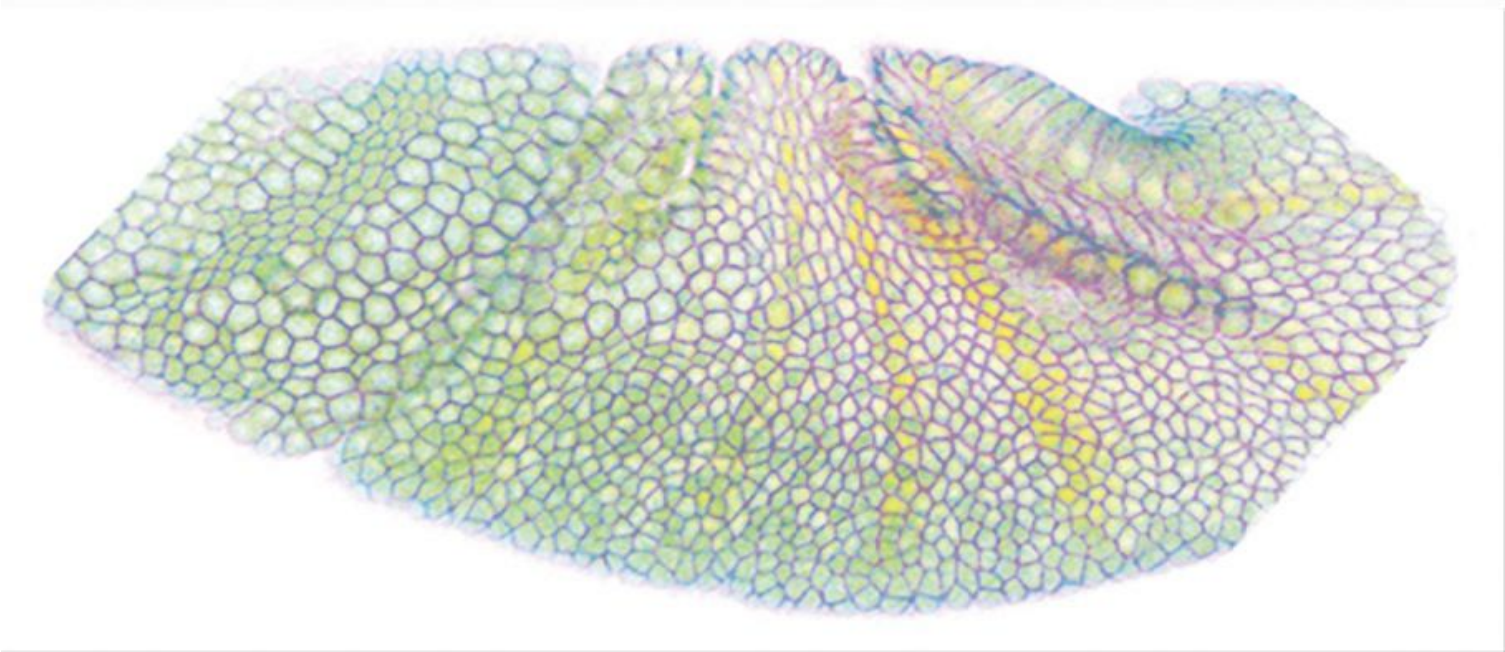
Mechanics of Morphogenesis



Lecture 4: Adhesion as an active, dissipative and regulated process

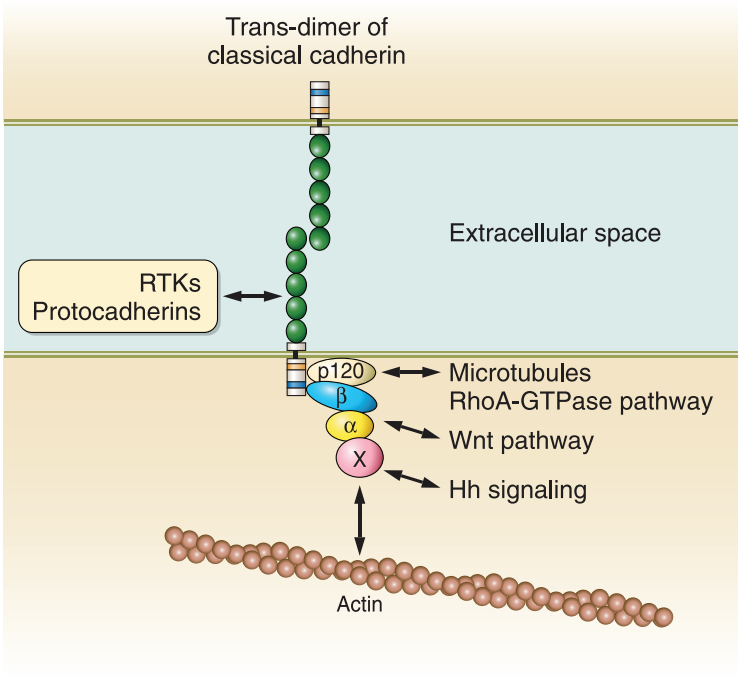
Thomas Lecuit
chaire: Dynamiques du vivant

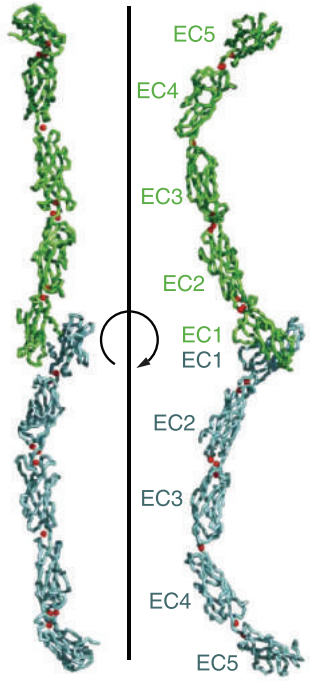
COLLÈGE DE FRANCE
1530

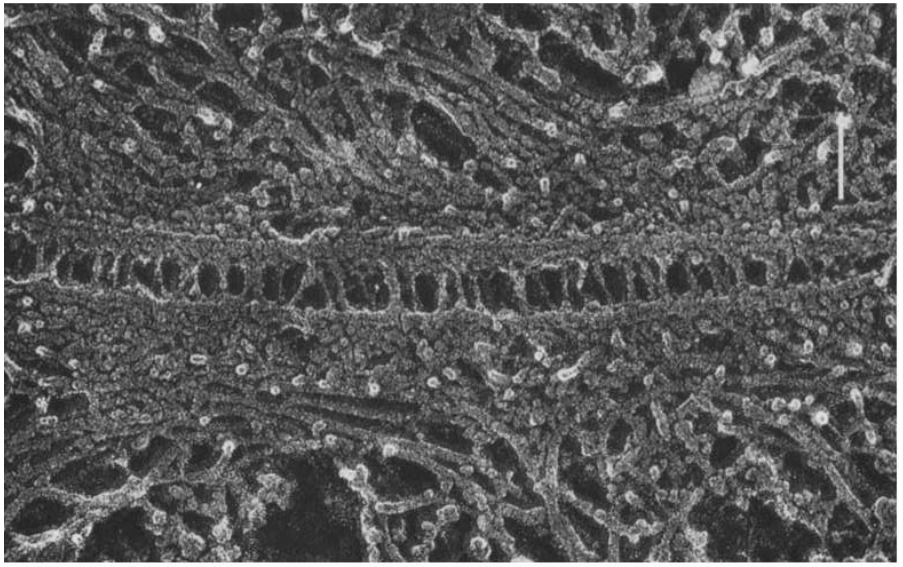
Molecular Model of Cadherin-based Adhesion

1. Extracellular homophobic ligation

Physiol Rev 92: 597–634, 2012
Hirano & Takeichi







Hirokawa N. and Heuser J.E. *J. Cell Biol* (1981) 91:399

Molecular Model of Cadherin-based Adhesion: Summary

Conclusions

- Cell-cell adhesion energy cannot be straightforwardly extrapolated from single molecule Cadherin interaction energy.
- Other features than extracellular Cadherin/Cadherin interaction kinetics and binding energy are required to account for cell sorting behaviour.
- Low affinity of single molecule Cadherin homodimerisation: role of molecule organisation in clusters?
- Interaction with F-actin affects diffusivity of Cadherins: impact on clustering?
- Interaction with F-actin accounts for cell-cell force separation and cell sorting: integration of intra-/extra-cellular coupling.

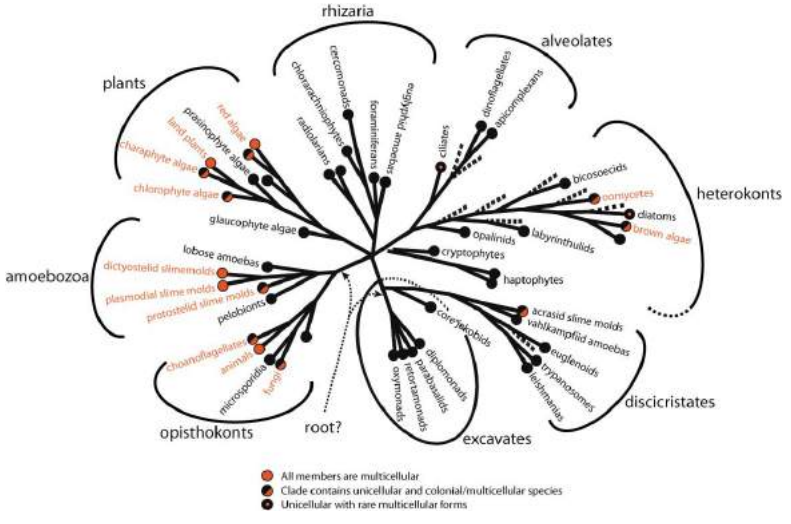
Evolution of Cadherin based Adhesion: Summary

- **Cadherin based Adhesion** most likely evolved in 3 parallel steps:

1. **Emergence of Cadherins** involved in sensing external environment and signalling in organisms with facultative multicellularity (LCA to Metazoa and Choanoflafelates ?).

2. **Emergence of Catenins** and actin coupling involved in cell-cell interactions and epithelial organisation (possibly in LCA to slime molds, Metazoa, choanos etc ?)

3. **Functional coupling of Catenins and Cadherins** (LCA to Sponges and Bilateria ?)



Adhesion in multicellular organisms

1. Affinity and Adhesion: a specificity problem

2. Adhesion: a thermodynamic model

3. The molecular framework of adhesion

4. Evolutionary origin of adhesion mechanisms

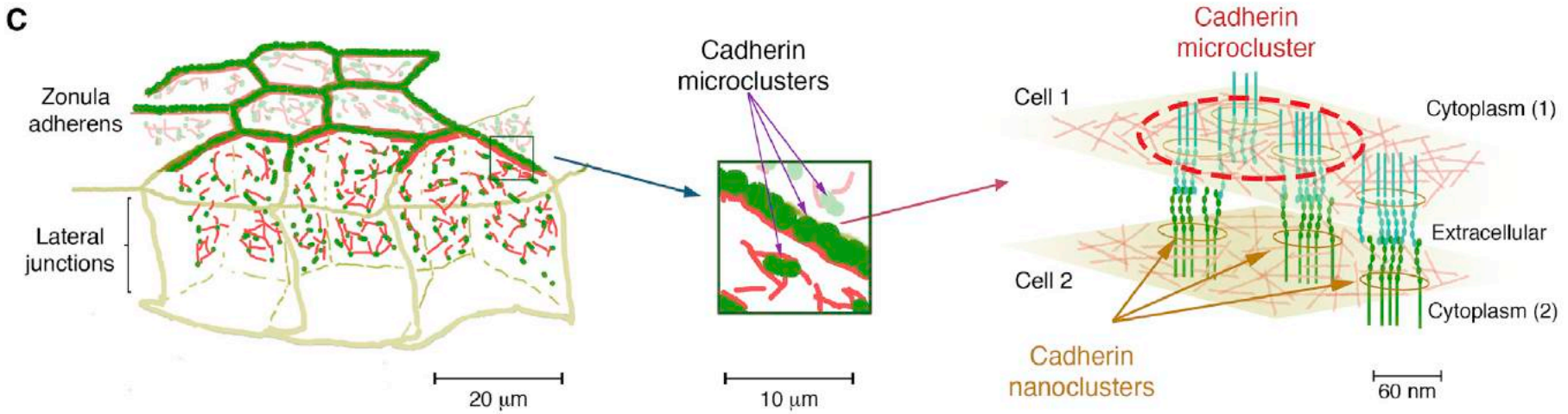
5. Adhesion as an active mechanism

- 4.1. Clustering
- 4.2. Mechanosensation - Mechano-transduction

6. Adhesion and *dissipation*

Adhesion as an *active, regulated* system

1. Active partitioning of Cadherin in finite sized clusters



Observed for all Cadherins

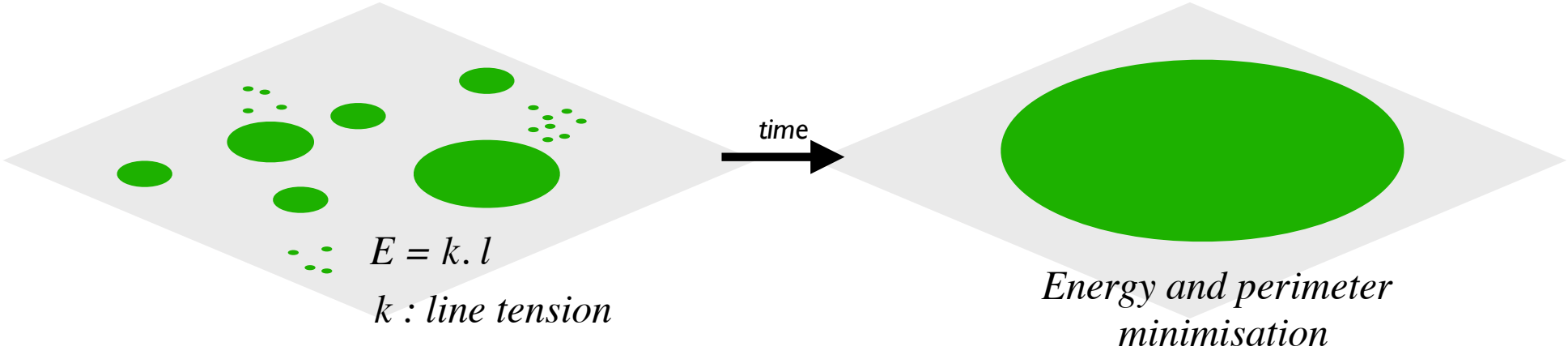
Yap A. et al *Dev. Cell*, 35: 12-.20 2015.

Adhesion as an *active, regulated* system

1. Cadherin clusters are out-of-equilibrium dynamic structures

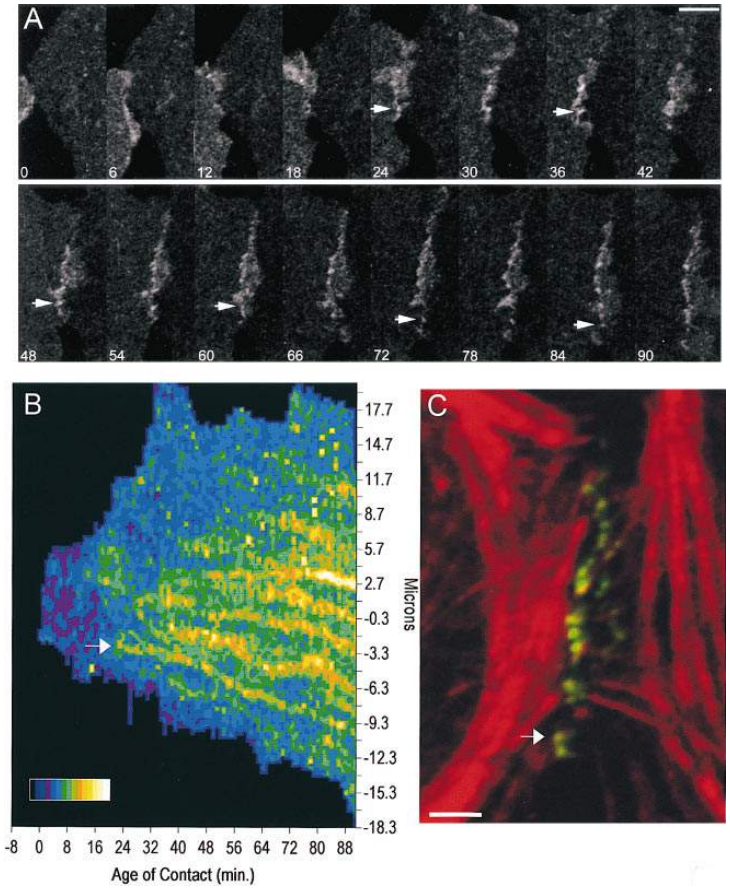
-Thermodynamics predict complete phase separation of Cadherin
2D « molecular sorting »

-Active processes must constantly seed, break and limit the growth of E-cadherin clusters



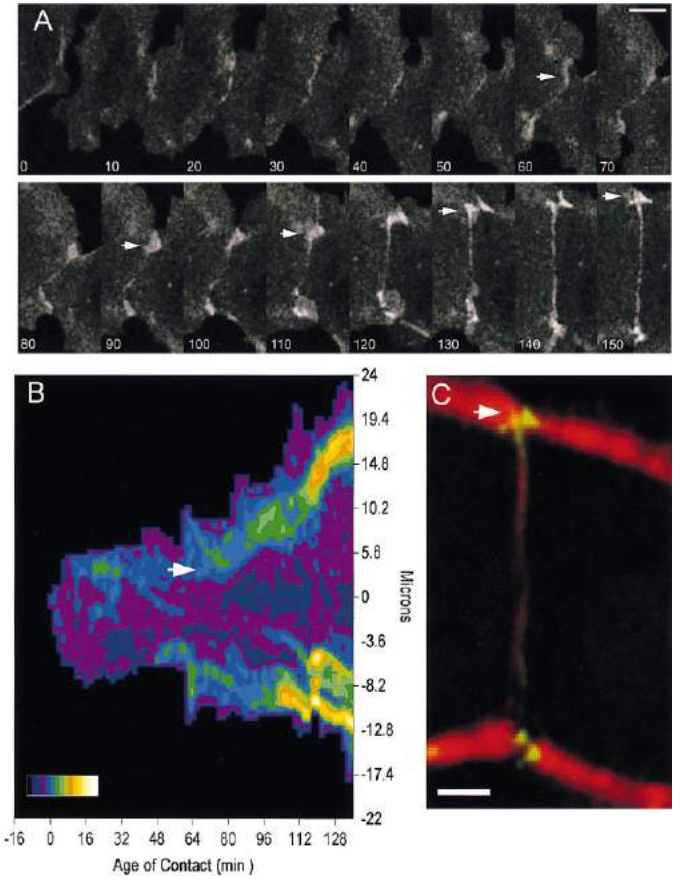
Adhesion as an *active, regulated* system

1. Cadherin forms « puncta »



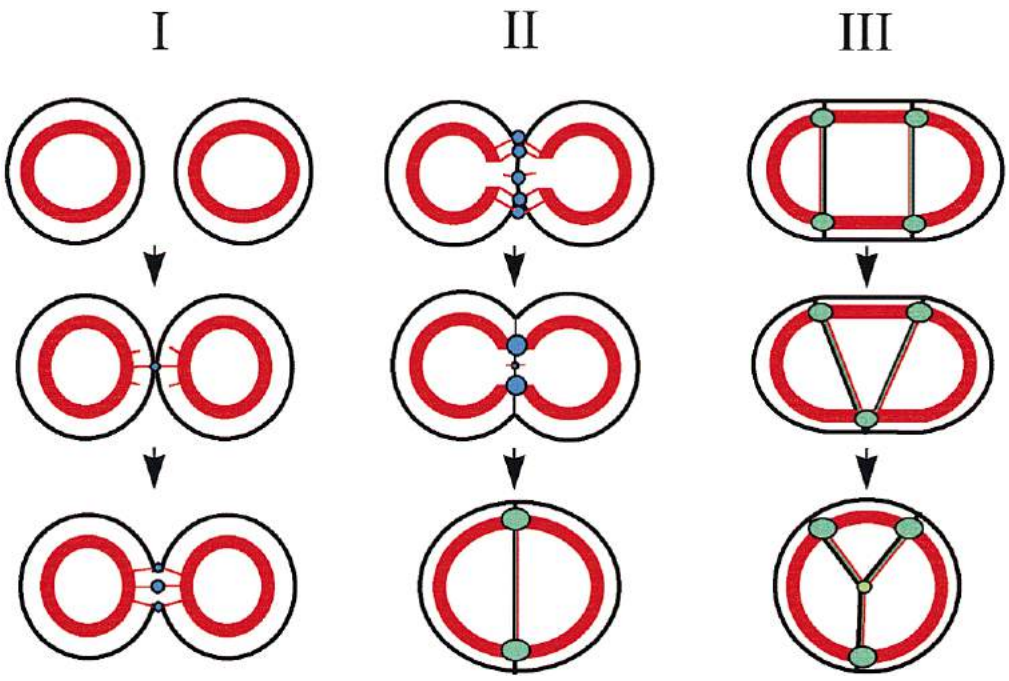
Adams et al JCB, 1998

As cell contacts form, E-cadherin::GFP forms small sized clusters
(few 10s of minutes in vertebrate MDCK cells)



Adhesion as an *active, regulated* system

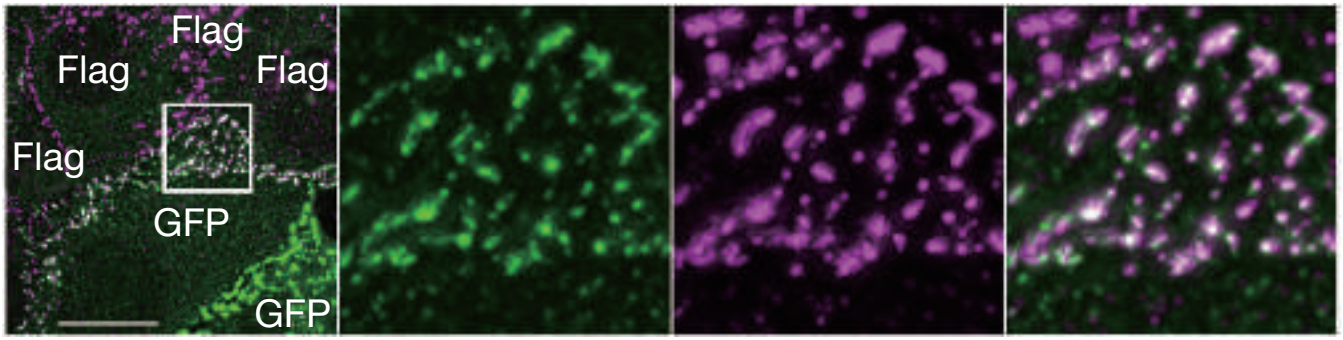
1. Cadherin forms « puncta »

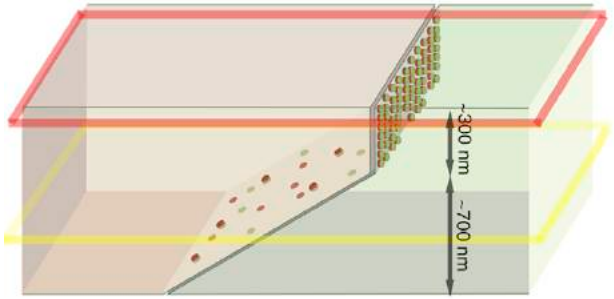


Adams et al *J. Cell Biol*., 1998. WJ Nelson

Adhesion as an *active, regulated* system

1. Cadherin puncta are homophilic clusters





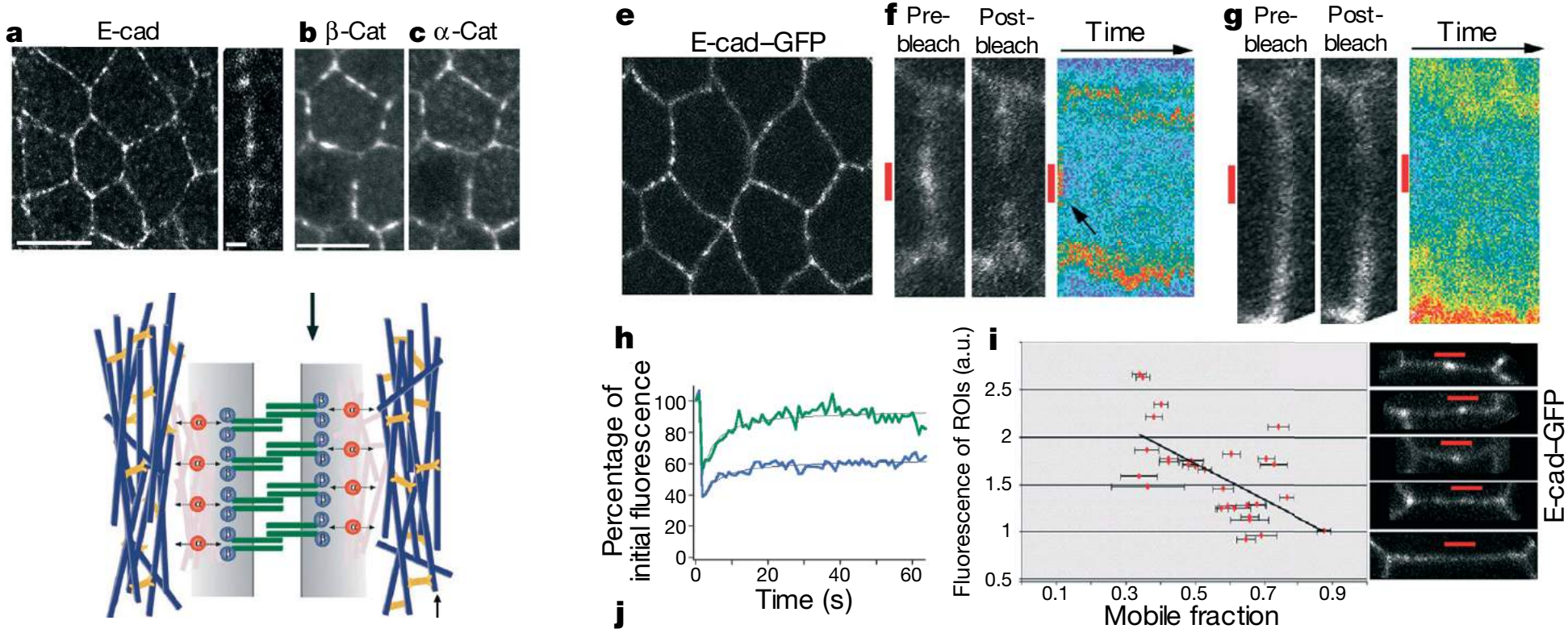
Wu Y. et al *Dev. Cell*, 32: 139-154. 2015.

Kametani & Takeichi, *Nature Cell Biology*, 9:92. 2007.

Adhesion as an *active, regulated* system

1. Cadherin puncta are homophilic clusters

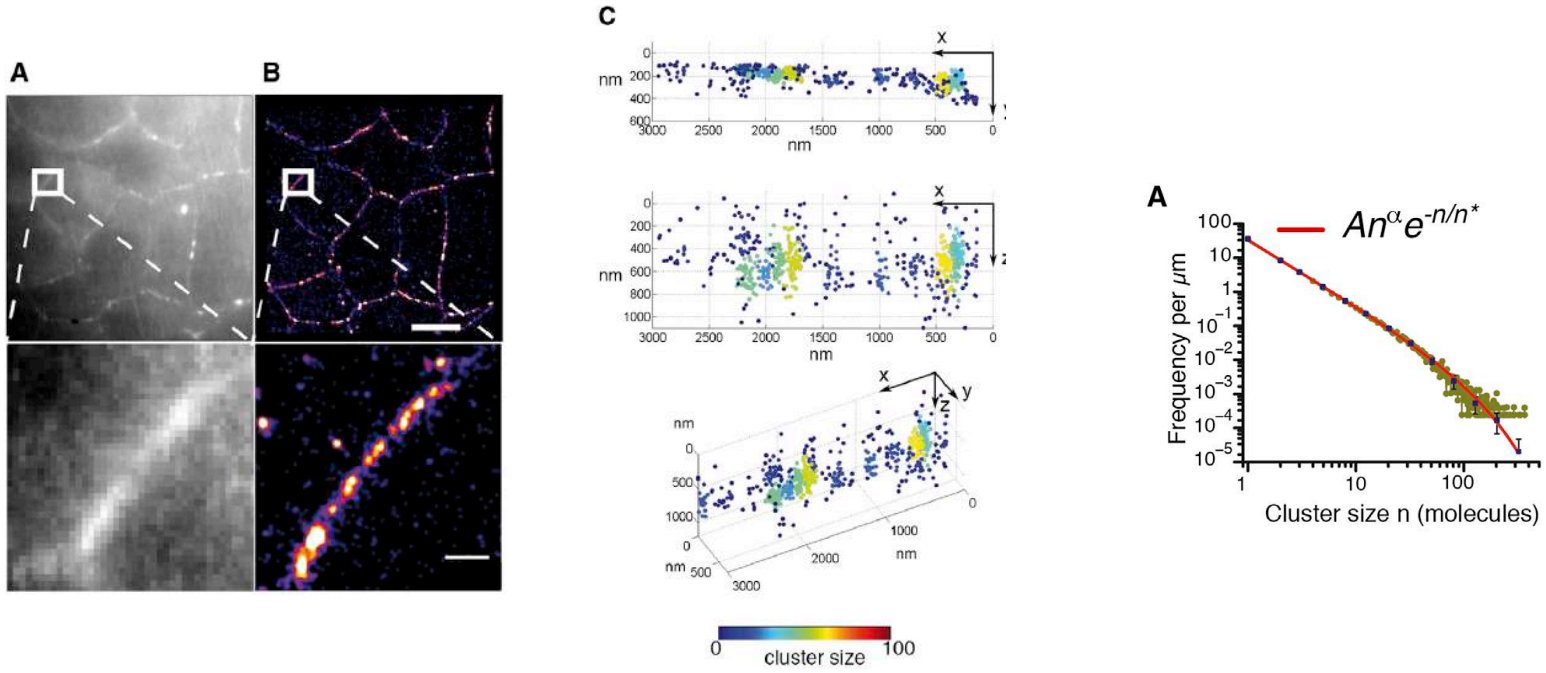
Compositional turnover using Fluorescent Recovery After Photobleaching (FRAP)



Cavey M. et al *Nature*, 453:751. 2008.

Adhesion as an *active, regulated* system

1. Cadherin clusters are out-of-equilibrium dynamic structures

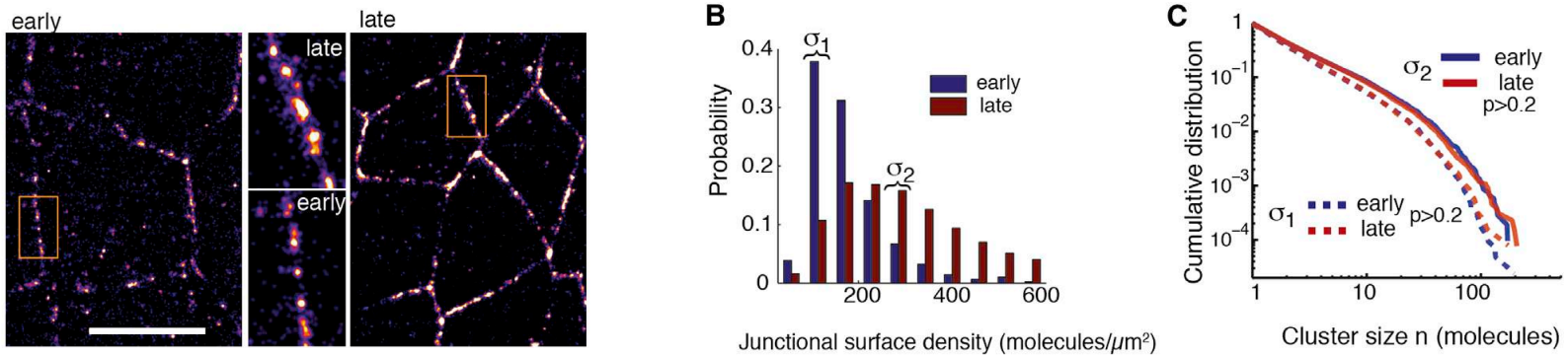


- Analysis of E-cadherin clustering using PALM: Photoactivation localisation microcopy
- E-cadherin forms nano-clusters: defined according to proximity (below resolution ~30nm)
- Power-law distribution of E-cadherin clusters

Truong-Quang BA. et al *Current Biol*, 23: 2197-2207. 2013.

Adhesion as an *active, regulated* system

1. Cadherin clusters are out-of-equilibrium dynamic structures

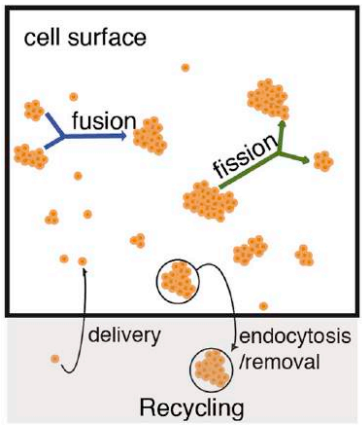


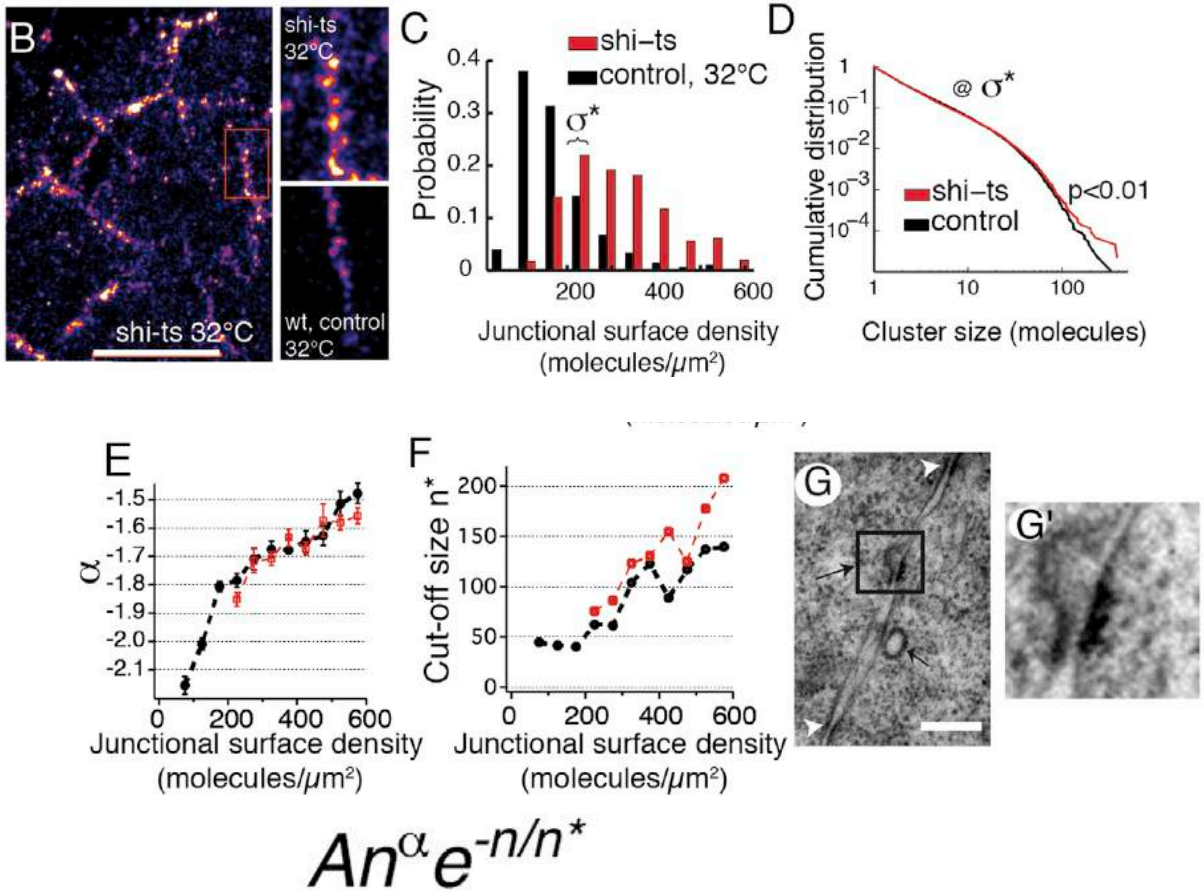
- The distribution of E-cadherin nano-clusters is set by density of molecules at cell junctions (but not by the age of the junction).

Truong-Quang BA. et al *Current Biol*, 23: 2197-2207. 2013.

Adhesion as an *active, regulated* system

1. Endocytosis influences cluster size



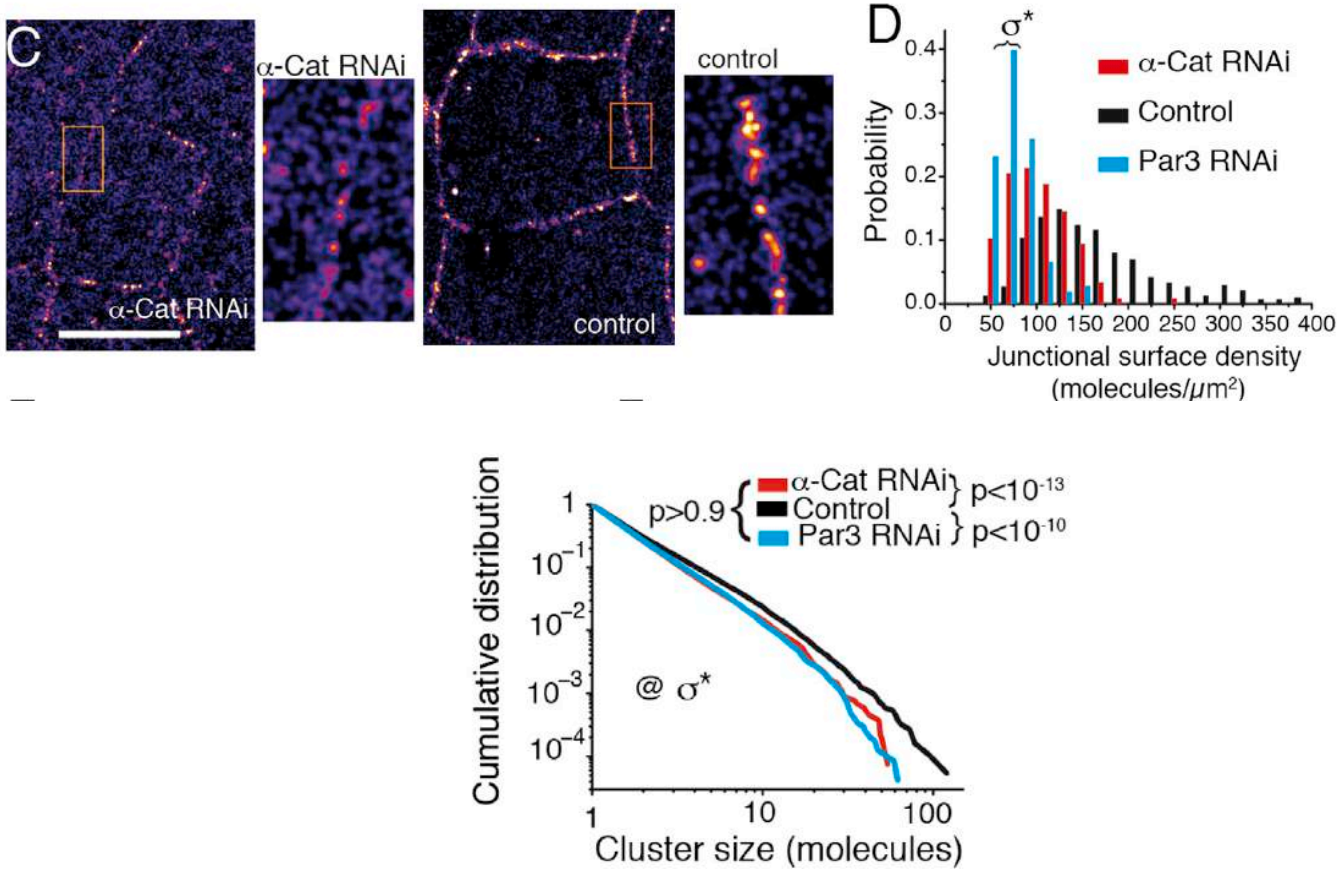


$$An^{\alpha}e^{-n/n^*}$$

Truong-Quang BA. et al *Current Biol*, 23: 2197-2207. 2013.

Adhesion as an *active, regulated* system

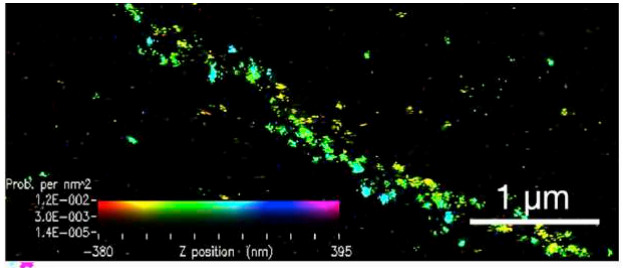
1. Actin coupling influences cluster size

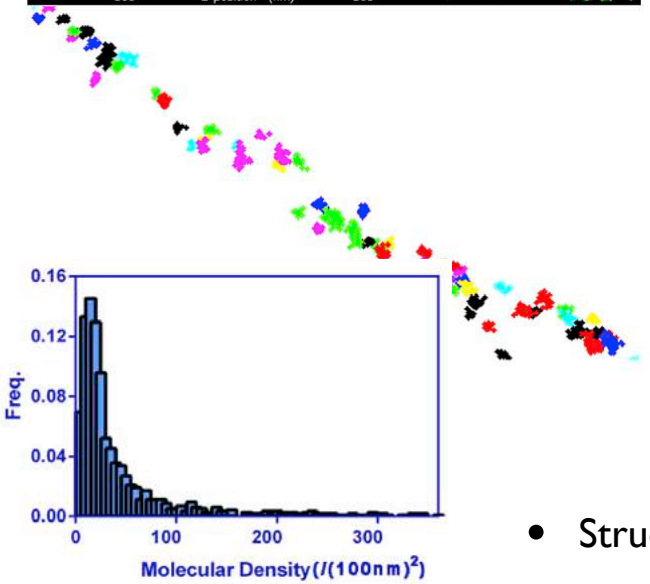


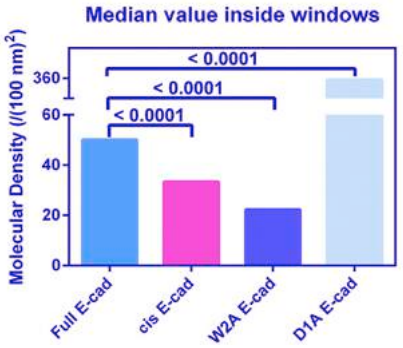
Truong-Quang BA. et al *Current Biol*, 23: 2197-2207. 2013.

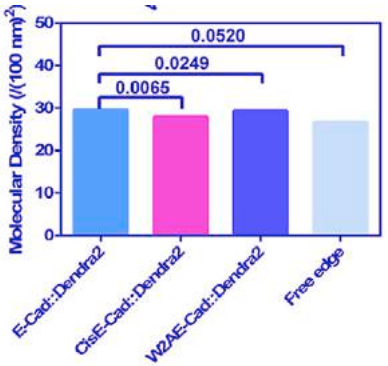
Adhesion as an *active, regulated* system

1. Impact of Cis- and Trans binding in cluster size





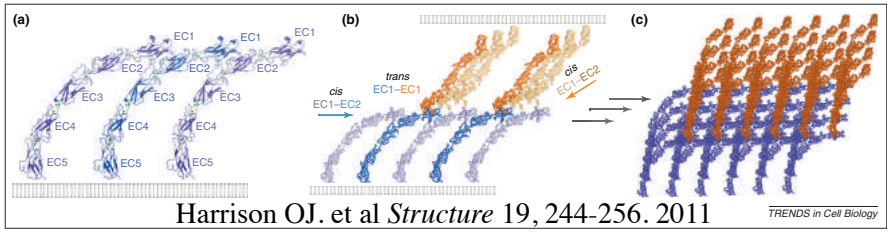




Cis-interaction *abolishing* mutant

Trans-interaction *abolishing* mutant

Trans-interaction *enhancing* mutant



Harrison OJ. et al *Structure* 19, 244-256. 2011

- Structural information predict cristal like clusters with density ~360molecule/100nm2

- Sub-regions (30x30 nm) of crystal like high density form and require cis- and trans-interactions

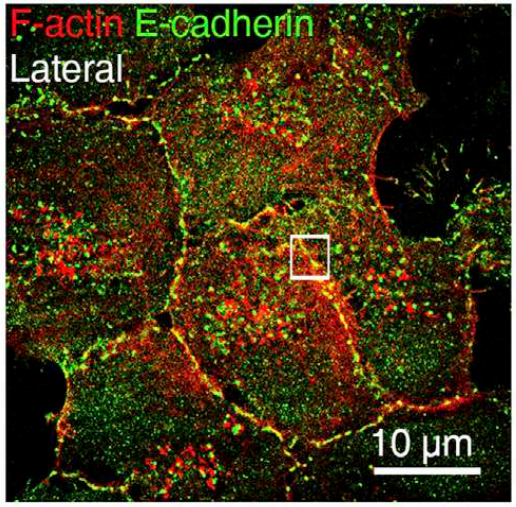
- However, clusters form independent of cis- and trans-interactions

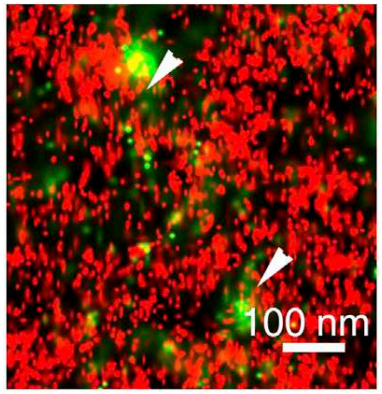
Wu Y. et al *Dev. Cell*, 32: 139-154. 2015.

Adhesion as an *active, regulated* system

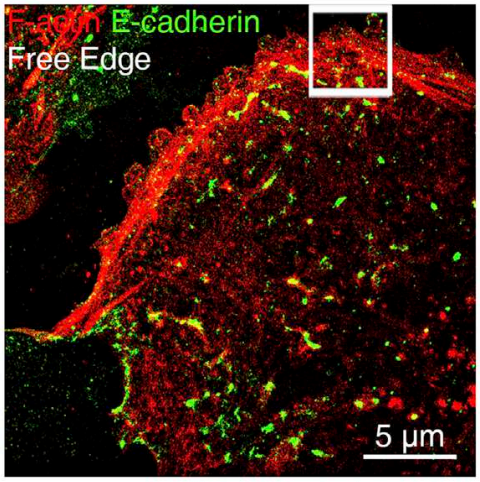
1. Actin corrals around E-cadherin cis- and trans- clusters

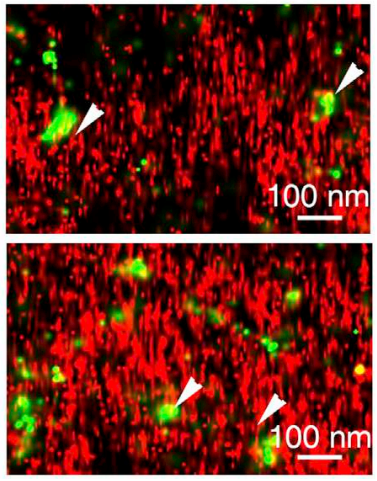
trans- clusters





cis- clusters

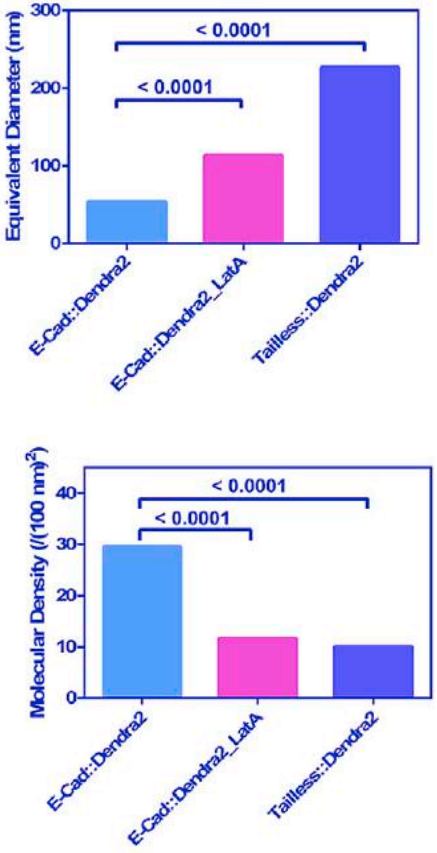


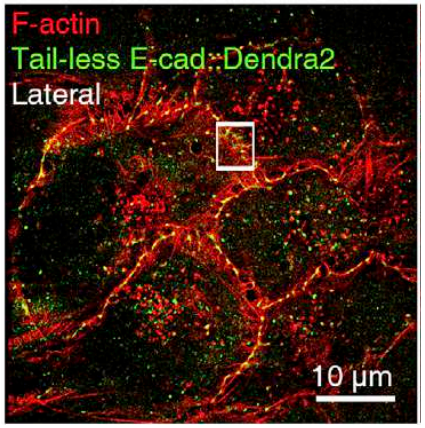


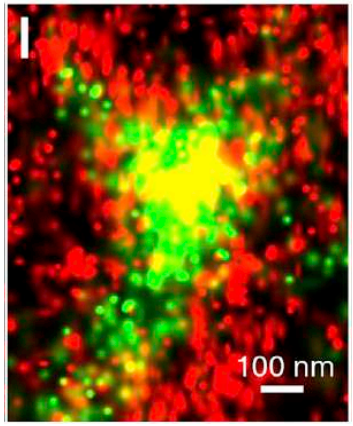
Wu Y. et al *Dev. Cell*, 32: 139-154. 2015.

Adhesion as an *active, regulated* system

1. Actin restricts the size and increases the density of clusters







- Clusters form independent of F-actin

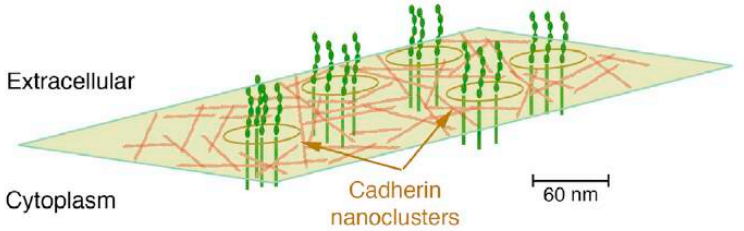
- The size of clusters is limited by F-actin corrals
- The density increases in small clusters

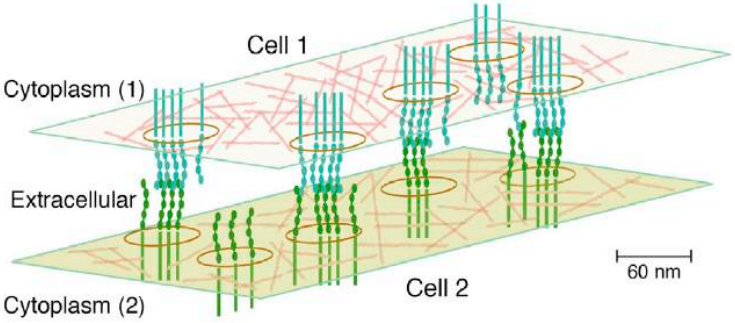
Wu Y. et al *Dev. Cell*, 32: 139-154. 2015.

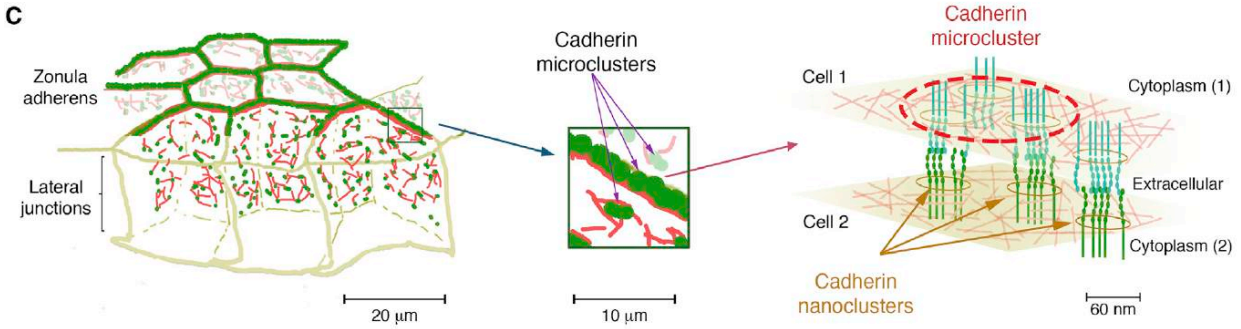
Adhesion as an *active, regulated* system

1. Current Working Model Cadherin clustering

- Formation of cis- non adhesive clusters are corralled by actin
- Actin corrals may be diffusion traps facilitating trans-cluster formation (Wu et al *Nature* 2011)(Sako et al *JCB 1998)*
- Cis-/trans-interaction and F-actin cooperate to form high density clusters (Truong Quang et al *CB 2013*, Wu et al *DevCell* 2015)
- F-actin stabilises E-cadherin clusters: compositional turnover
- F-actin immobilises E-cadherin clusters at cell junctions (Cavey et al, *Nature* 2008)







Yap A. et al *Dev. Cell*, 35: 12-.20 2015.

Adhesion as an *active, regulated* system

1. Conclusions

- **Cadherins form small aggregates**

This is a general property of all cadherins, found in all organisms

- **Mechanisms of cluster formation**

- ligation of ectodomains: cis and trans and diffusion trap (Honig, Shapiro, Troyanovksy, Zaidel-Bar)
- actin interaction (Kusumi, Nelson Dufour, Lecuit, Lenne, Yap, Troyanovsky, Zaidel-Bar etc)
- endocytosis:
  - clustering induces endocytosis (Levayer et al 2013)
  - endocytosis affects clustering (Truong Quang et al 2013)
- lipid interactions: in mammals.

Adhesion as an *active, regulated* system

2. Cadherin, mechano-sensing and -transduction

- **Function of clusters**

-strengthen adhesion: local on/off association.

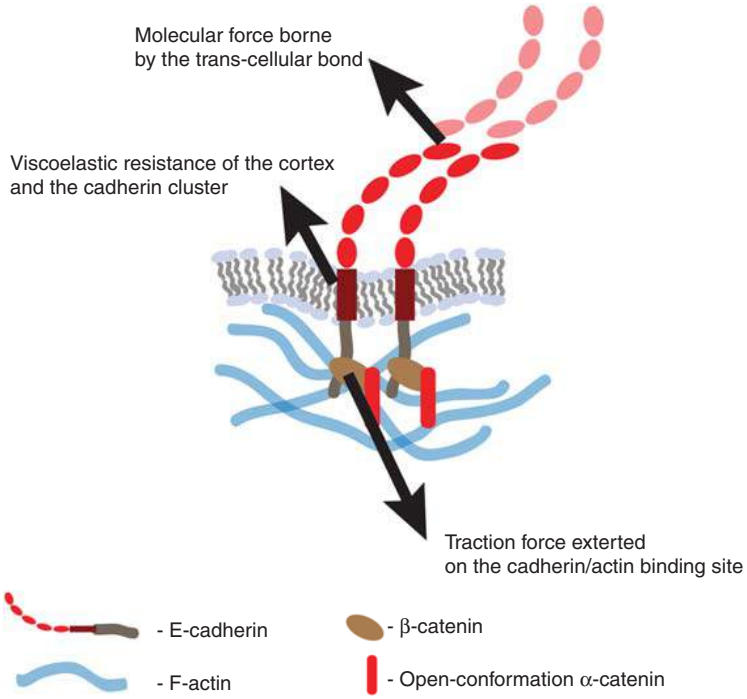
-regionalise tension transmission at cortex

-signalling: local actin organisation for instance.

- **E-cadherin as local mechanical « integrators »**

Adhesion as an *active, regulated* system

2. Cadherin, mechano-sensing and -transduction



- Mechano-sensation
- Mechano-transduction

Adhesion as an *active, regulated* system

2. Questioning E-cadherin mechanical coupling to actin?

Deconstructing the Cadherin-Catenin-Actin Complex

Soichiro Yamada,[1,3] Sabine Pokutta,[1,2,3] Frauke Drees,[1,3] William I. Weis,[1,2,*] and W. James Nelson[1,*]
[1]Department of Molecular and Cellular Physiology
[2]Department of Structural Biology
Stanford University School of Medicine, Stanford, CA 94305, USA

Cell *123*, 889–901, December 2, 2005

The E-cadherin/ß-catenin/a-catenin/F-actin complex cannot be identified biochemically in bulk or at membrane surfaces.

The interaction between all components are much more dynamic than anticipated.

BUT let's consider that:

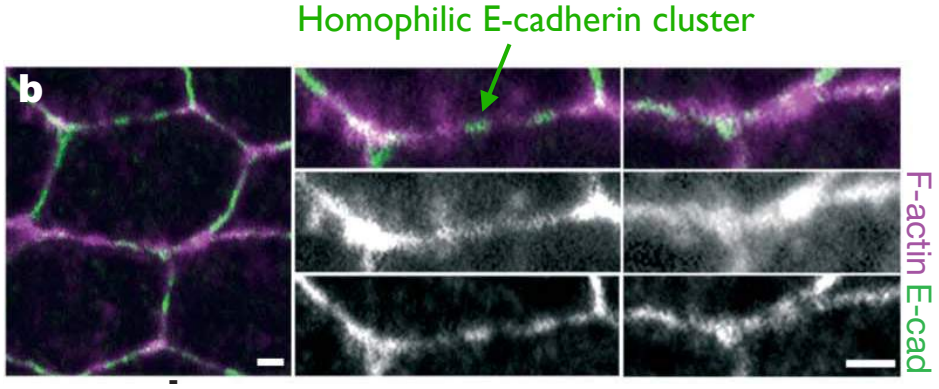
1. Clusters of E-cadherin may allow high affinity interaction while monomers diffuse without interacting with F-actin

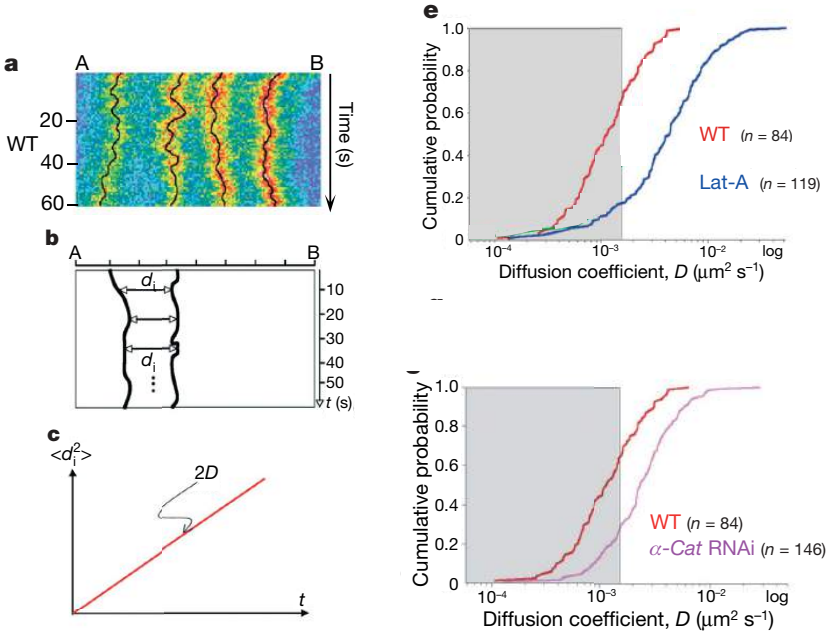
2. Interaction among adhesive complex components may be force dependent: e.g. catch-bond.

Adhesion as an *active, regulated* system

2. Coupling to F-actin: cluster immobilisation at junctions

Though single molecules diffuse at the membrane E-cadherin clusters are immobilised by actin filaments at the cortex.





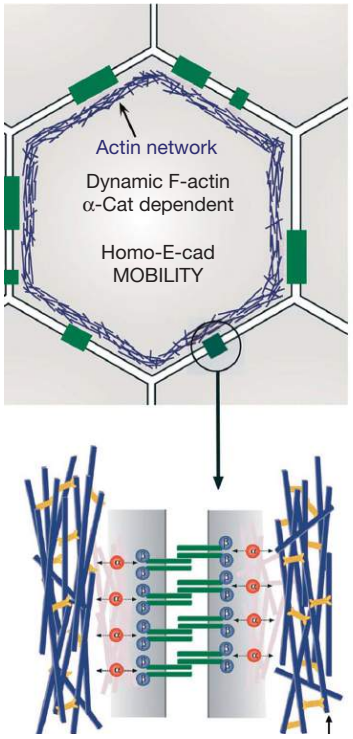
Matthieu Cavey[1], Matteo Rauzi[1,2], Pierre-François Lenne[2] & Thomas Lecuit[1]
Nature. 453:751. 2008

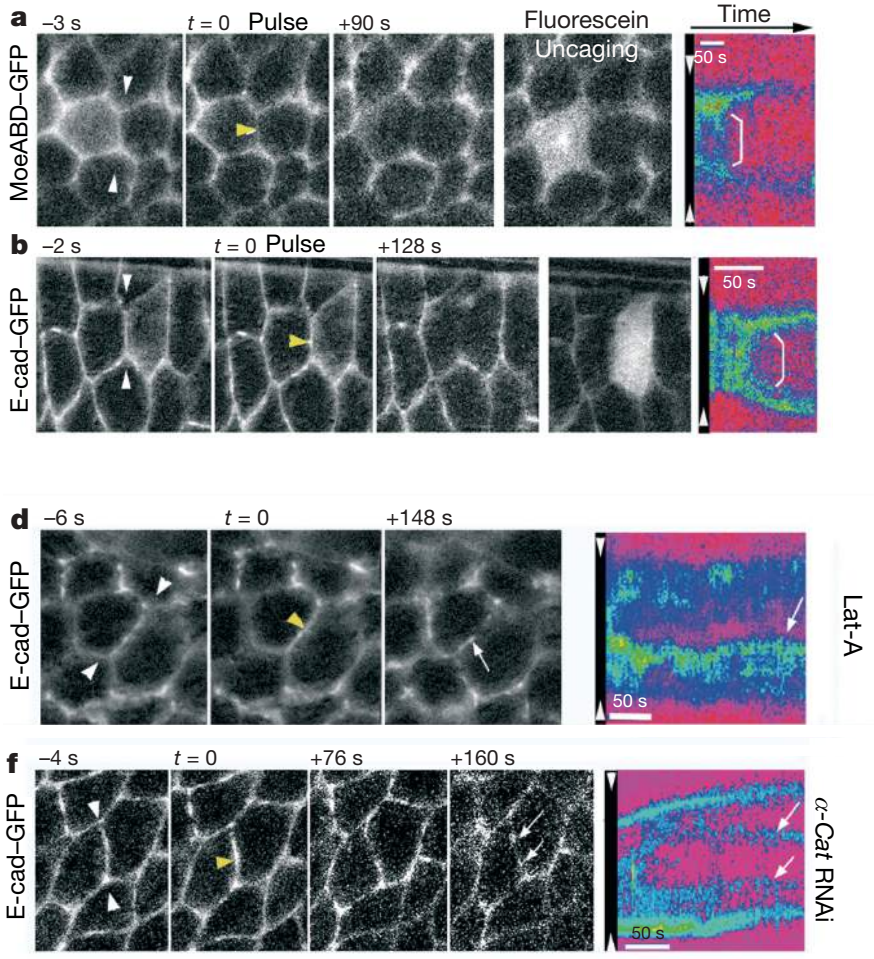
Adhesion as an *active, regulated* system

2. Coupling to F-actin: clusters are tethered to cortical actin

Actomyosin relaxation following laser nano-ablation

E-cadherin cluster redistribution requires F-actin and a-catenin



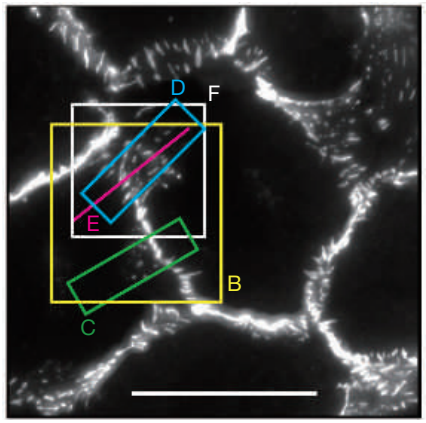


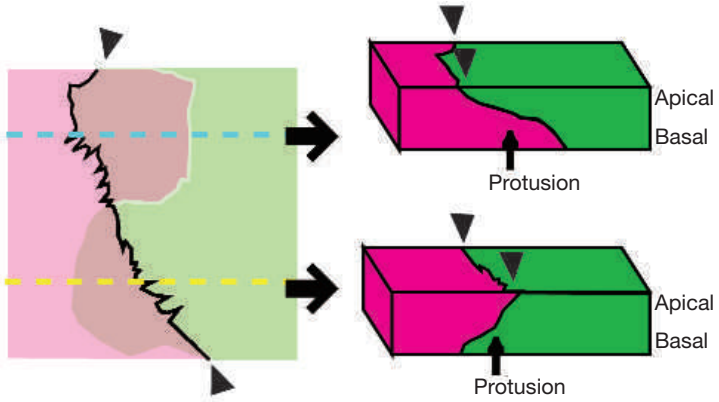
Matthieu Cavey[1], Matteo Rauzi[1,2], Pierre-François Lenne[2] & Thomas Lecuit[1]
Nature. 453:751. 2008

Adhesion as an *active, regulated* system

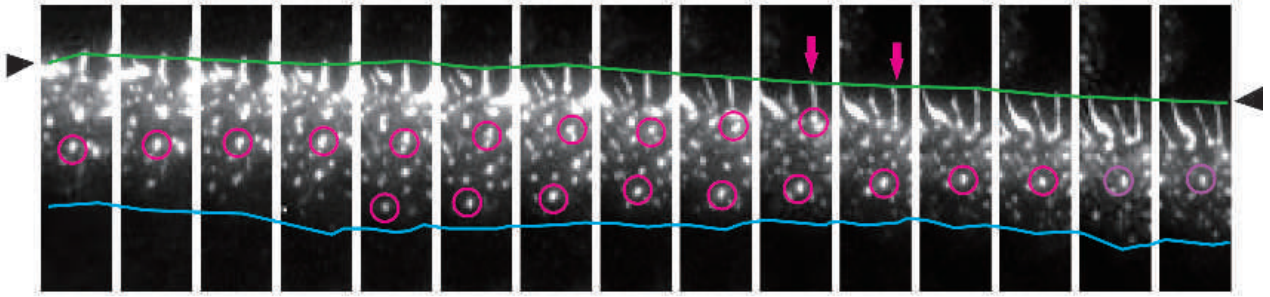
2. Coupling to F-actin: Actin driven flow

E-cadherin clusters are advected by actin flows





VE-Cadherin::GFP

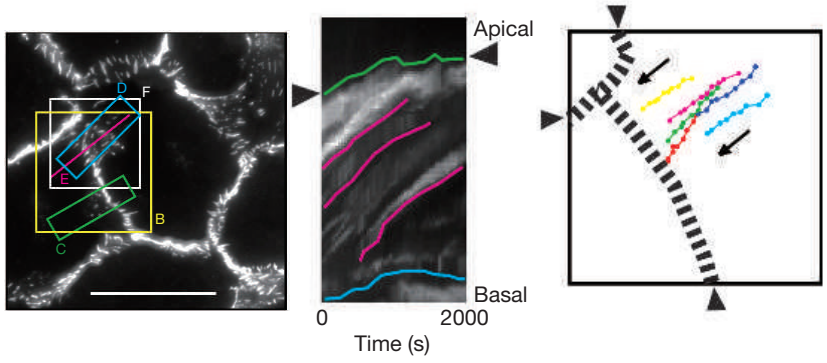


VE-Cadherin::GFP

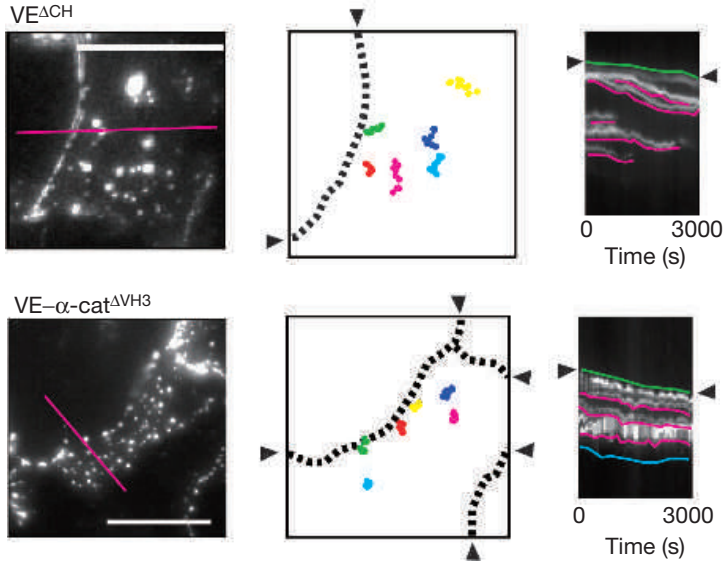
Kametani & Takeichi *Nature Cell Biology*, 9:92. 2007.

Adhesion as an *active, regulated* system

2. Coupling to F-actin: Actin driven flow



Flow requires interaction with F-actin



Kametani & Takeichi *Nature Cell Biology*, 9:92. 2007.

Adhesion as an *active, regulated* system

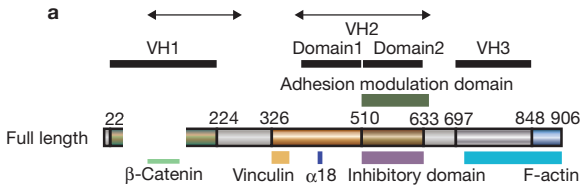
2. a-Catenin is mechanosensitive

Non-Muscle Myosin-II (NMII) is a present at cell junctions

Vinculin recruitment requires a-catenin binding to F-actin

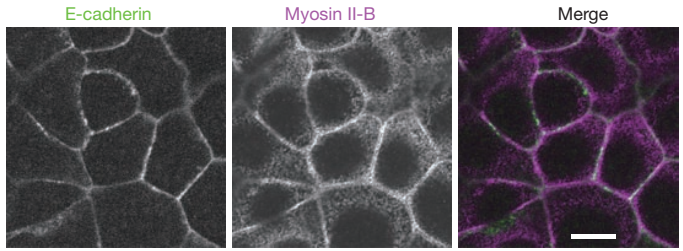
Vinculin recruitment requires NMII

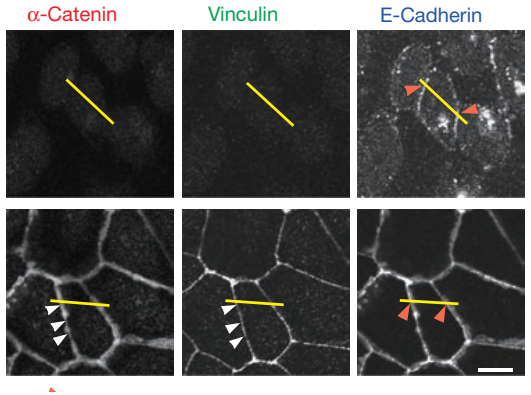
Auto-inhibitory domain

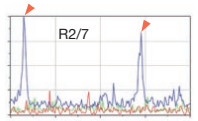


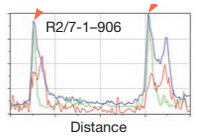
Inhibition of Vinculin recruitment

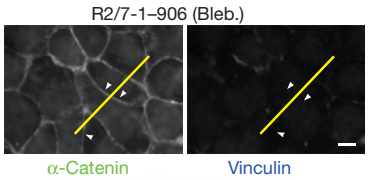
Constitutive Vinculin recruitment

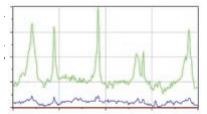




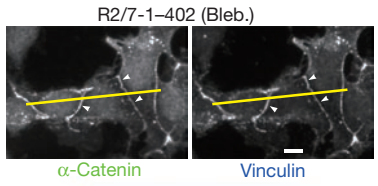


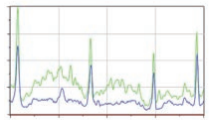






Myosin-II inhibitor



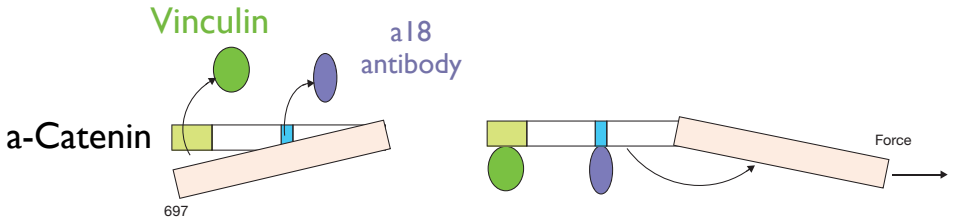


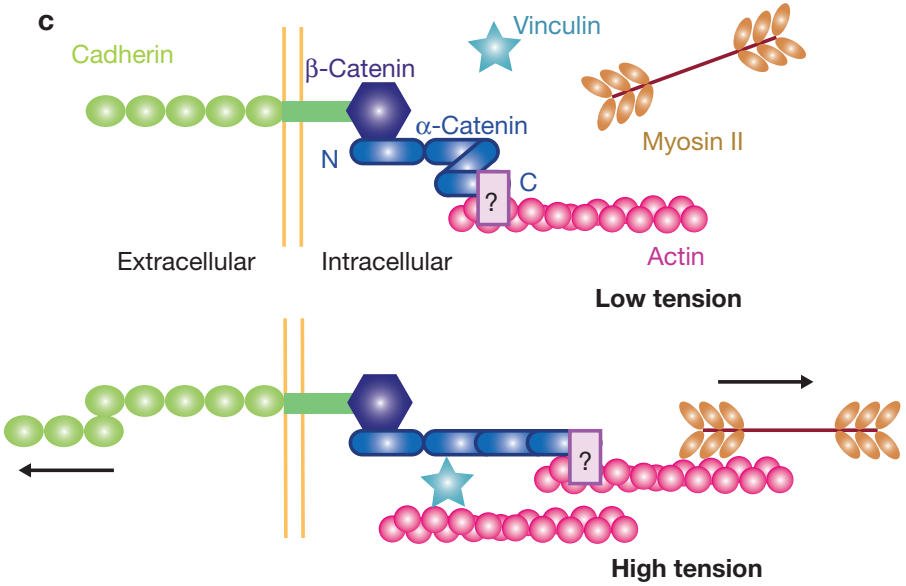
Myosin-II inhibitor

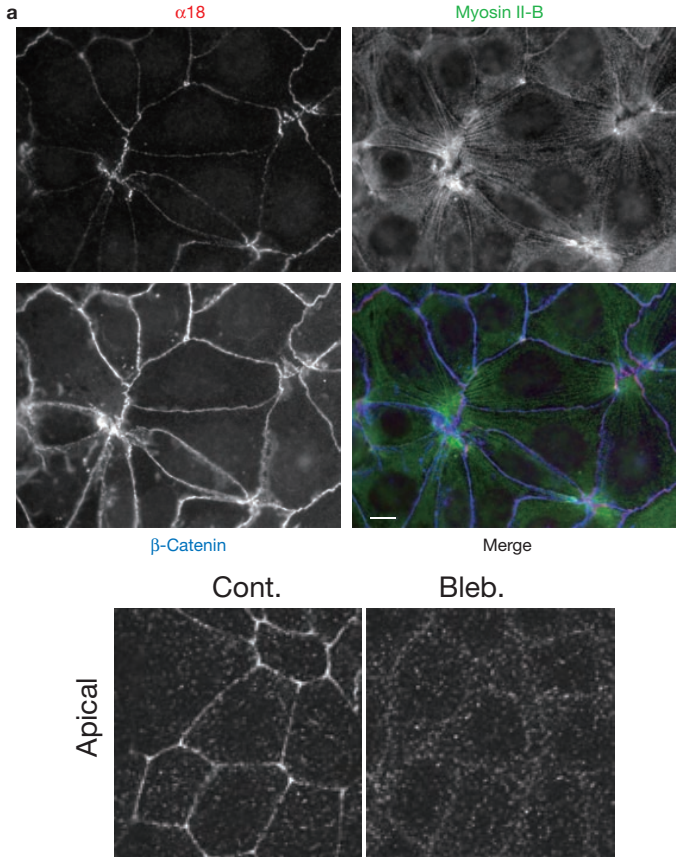
Yonemura et al , *Nature Cell Biology*, 12:533. 2010

Adhesion as an *active, regulated* system

2. a-Catenin is mechanosensitive







Yonemura et al , *Nature Cell Biology*, 12:533. 2010

Adhesion as an *active, regulated* system

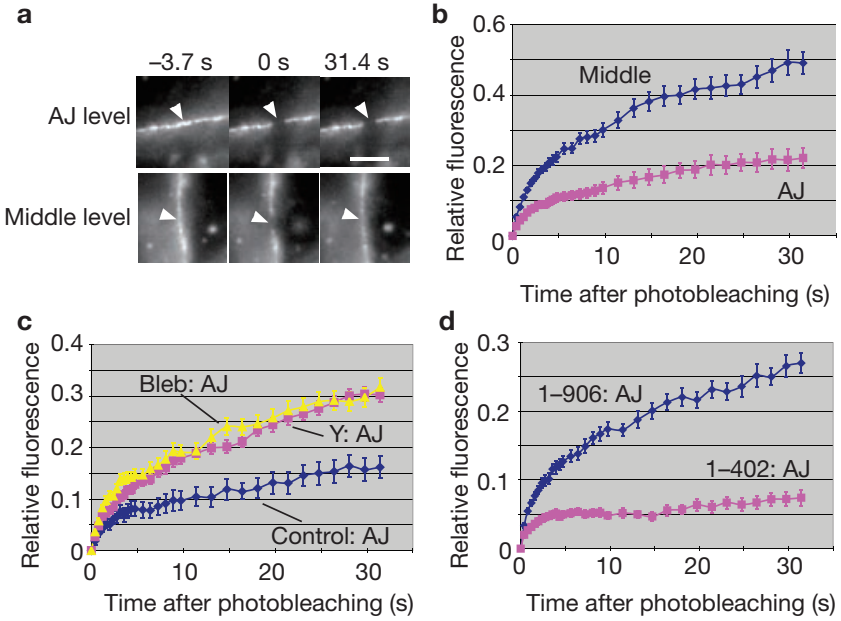
2. a-Catenin is mechanosensitive

Fluorescence Recovery after Photobleaching (FRAP):

- Probe recovery dynamics of a-catenin at lateral cell surfaces and cell junctions

- The return of fluorescence is lower at junctions: a-catenin is more stably bound
- This stabilisation requires Myosin-II based tension

- Deletion of auto-inhibitory domain enhances protein stabilisation



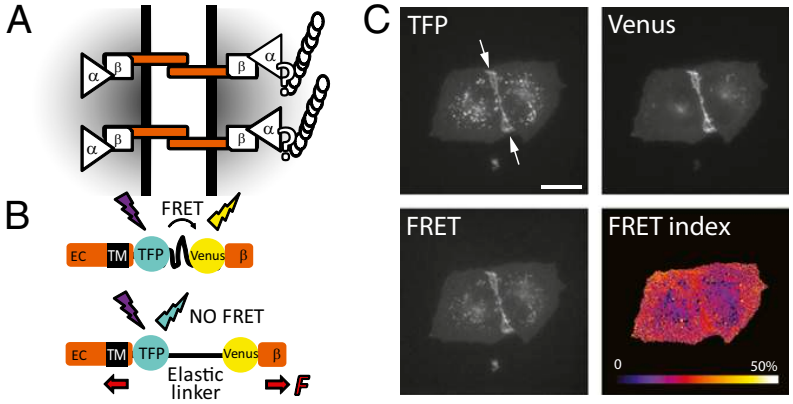
Yonemura et al , *Nature Cell Biology*, 12:533. 2010

Adhesion as an *active, regulated* system

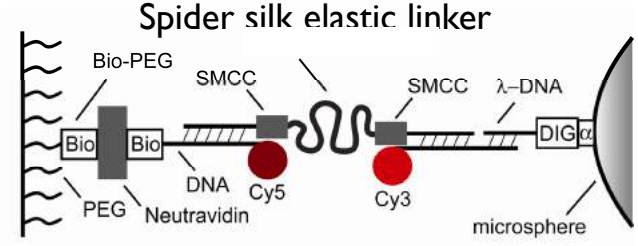
2. E-cadherin is under molecular tension

Borghi N. et al Nelson WJ. and Dunn A., *PNAS*, 109:12568. 2012

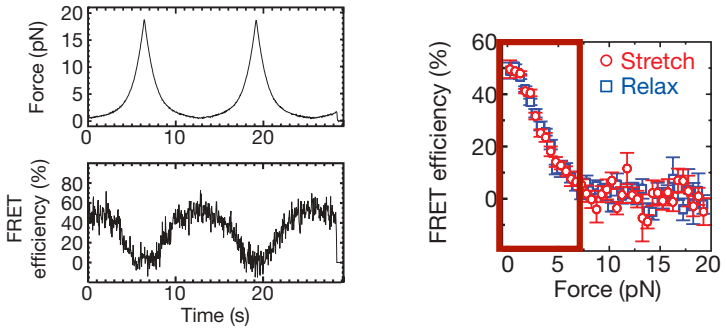
FRET-based sensor of molecular tension in the intracellular domain of E-cadherin



Force-FRET curve of the TS-Mod: based on the elastic properties of the spider silk domain.



1-6pN force sensitivity

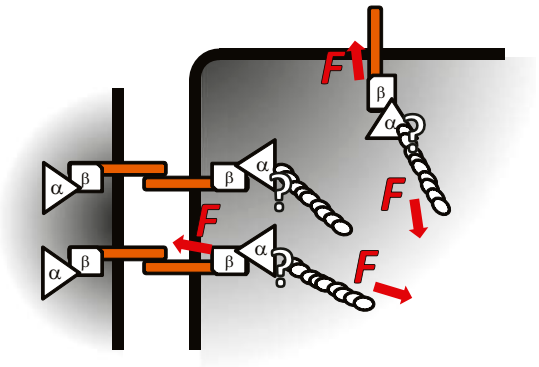


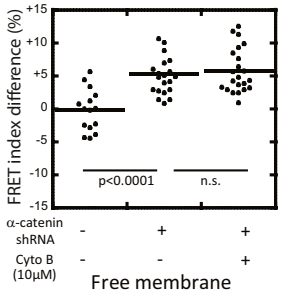
Grashoff C. et al., Schwartz M. , *Nature*, 466:263. 2010

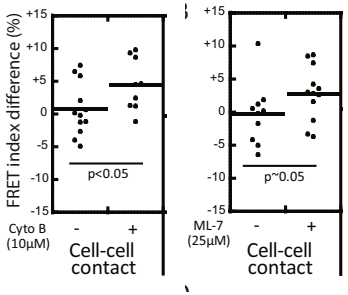
Adhesion as an *active, regulated* system

2. E-cadherin is under molecular tension

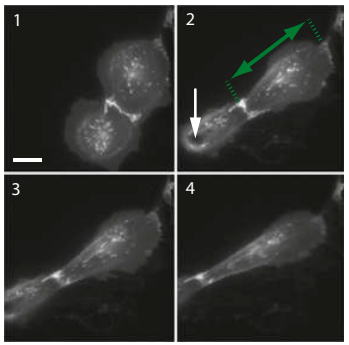
Probing **internal stress** on E-cadherin:
- free membrane E-cadherin under tension
- depends on Actin and Myosin-II

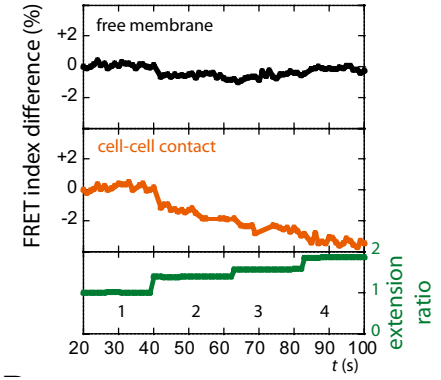






Probing **external stress** on E-cadherin:
- cell stretching increases tension on E-cadherin





Borghi N. et al Nelson WJ. and Dunn A., *PNAS*, 109:12568. 2012

Adhesion as an *active, regulated* system

2. Force dependency of binding to F-actin

The minimal cadherin-catenin complex binds to actin filaments under force

Craig D. Buckley,[1*] Jiongyi Tan,[2*] Karen L. Anderson,[3] Dorit Hanein,[3] Niels Volkmann,[3] William I. Weis,[2,4,5†] W. James Nelson,[5,6†] Alexander R. Dunn[1,2,7†]

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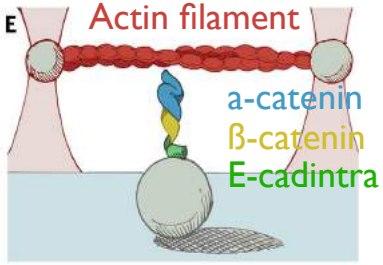
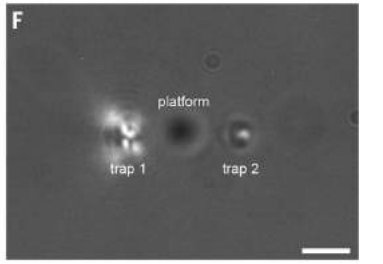
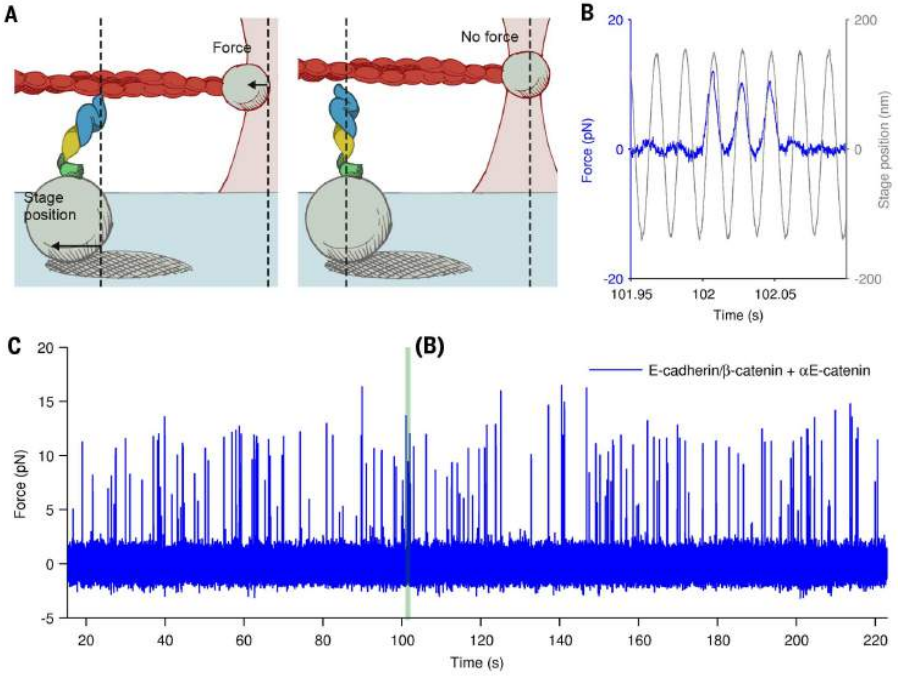


a-cat/F-actin *Kd* = 1µM

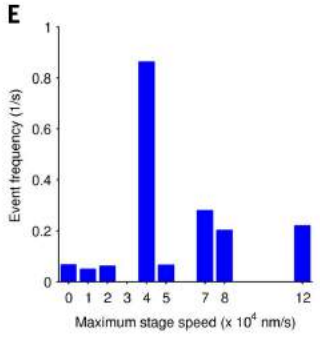
a-cat/ß-cat *Kd* = 1nM



Binding of a-cat to F-actin causes a restoring force on trap
Force is measured based on trap stiffness and displacement

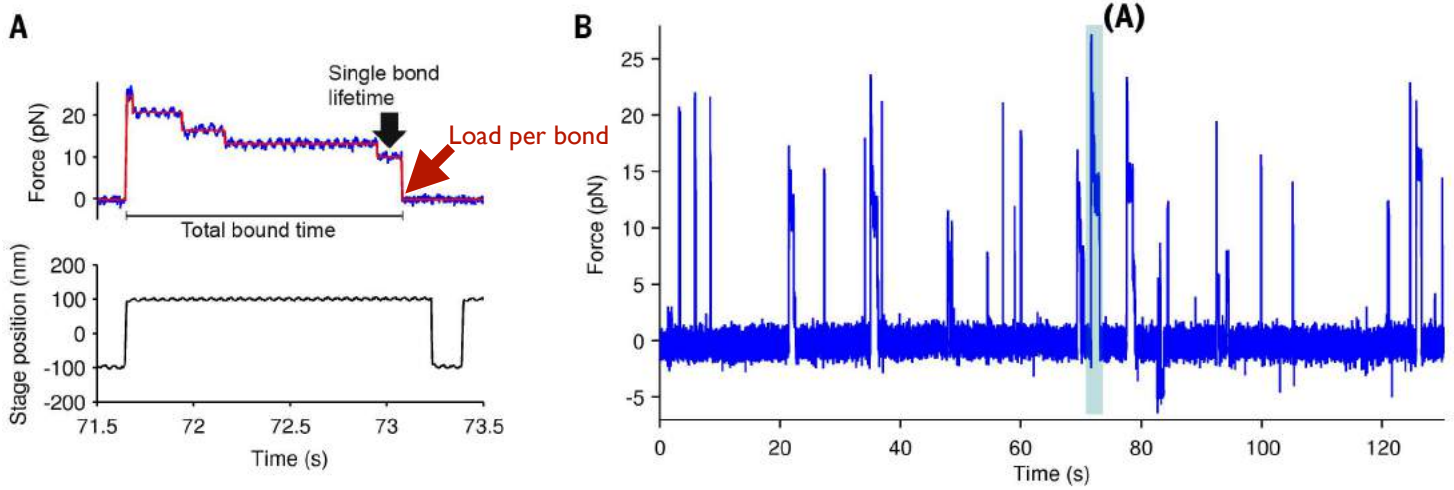


Binding is more frequent at intermediate than low displacement speed

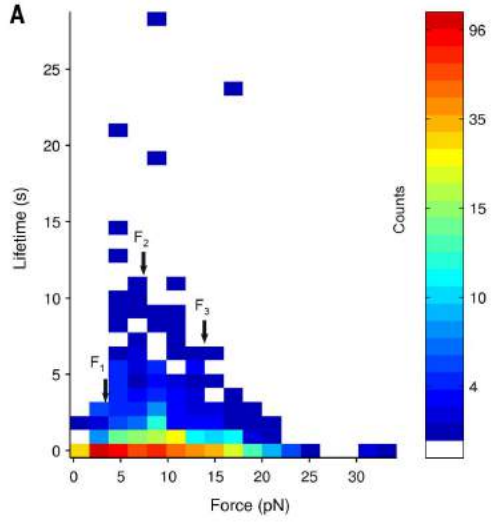


Adhesion as an *active, regulated* system

2. Force dependency of binding to F-actin



Many short-lived bonds at all force
A few long-lived bonds at 5-10 pN forces

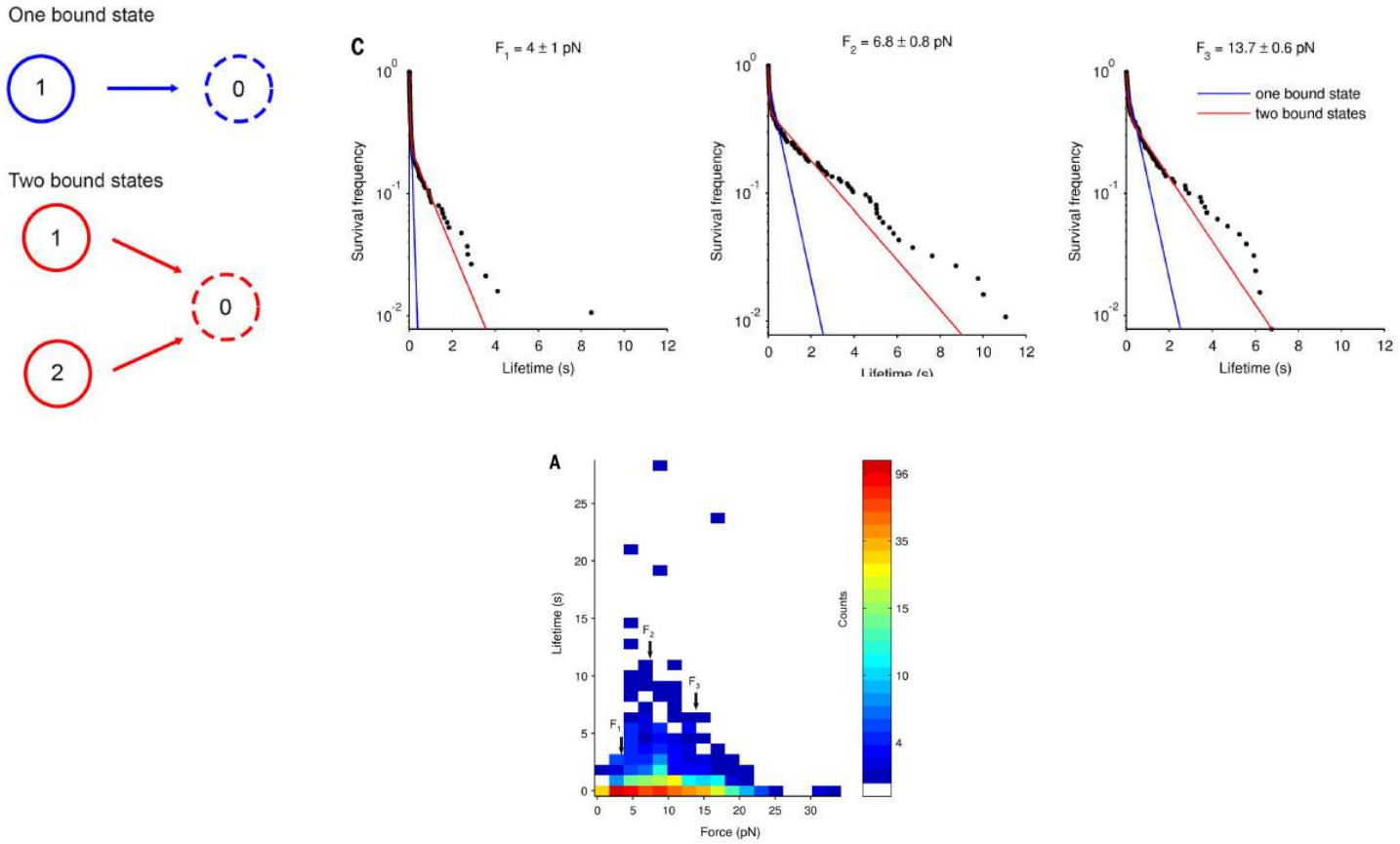


Buckley CD et al Nelson WJ. and Dunn A., *Science*, 346:6209. 2014

Adhesion as an *active, regulated* system

2. Force dependency of binding to F-actin

Falsification of a one bound state model
A two bound state model fits the lifetime distribution at 3 force bins



Buckley CD et al Nelson WJ. and Dunn A., *Science*, 346:6209. 2014

Adhesion as an *active, regulated* system

2. Force dependency of binding to F-actin

Bell's equation: $k_{ij}(F) = k_{ij}^0 \exp(F x_{ij}/k_B T)$ Bell GI. *Science* 200:618. 1978

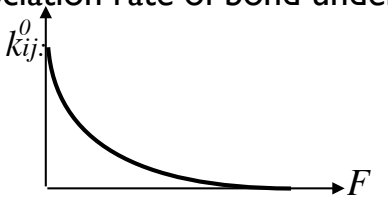
k_{ij}: rate of transition from i to j under force F

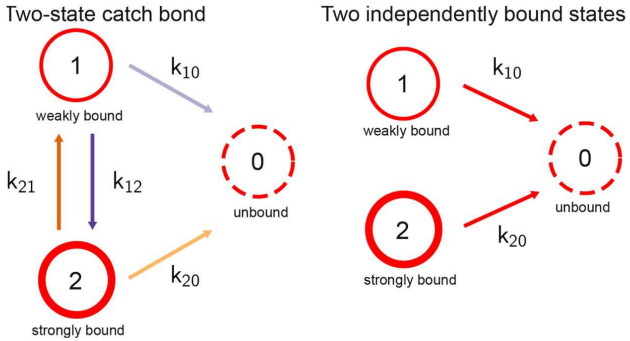
x_{ij}: distance to transition state

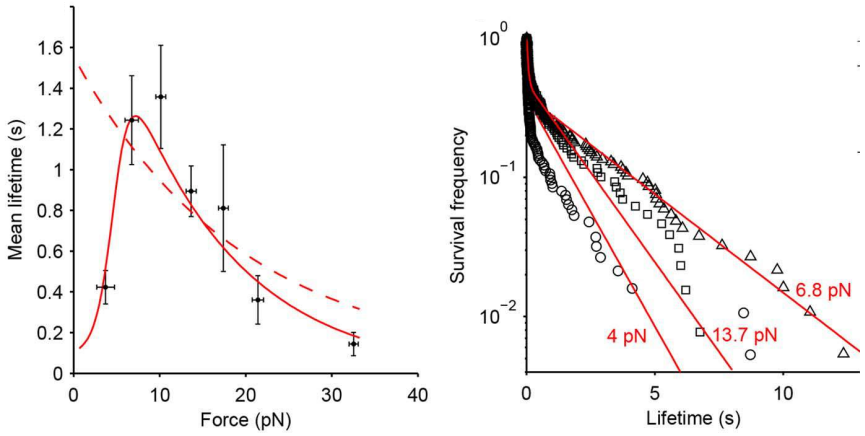
- slip-bond model: exponential increase of dissociation rate of bond under force F

- catch-bond model: exponential decrease of dissociation rate of bond under force F

$$k_{ij}(F) = k_{ij}^0 \exp(-F x_{ij}/k_B T)$$







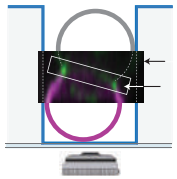
2 state catch bond

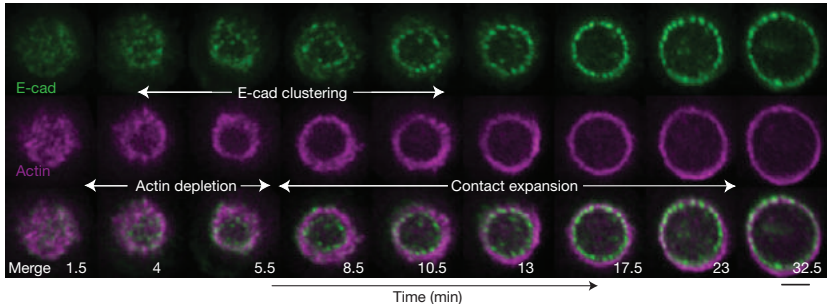
2 independently bound states

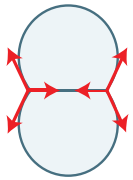
Buckley CD et al Nelson WJ. and Dunn A., *Science*, 346:6209. 2014

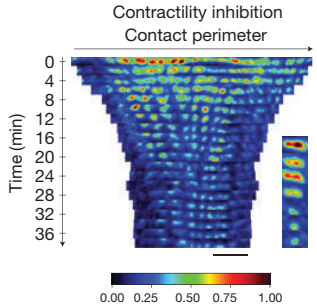
Adhesion as an *active, regulated* system

2. Mechanotransduction by Cadherins

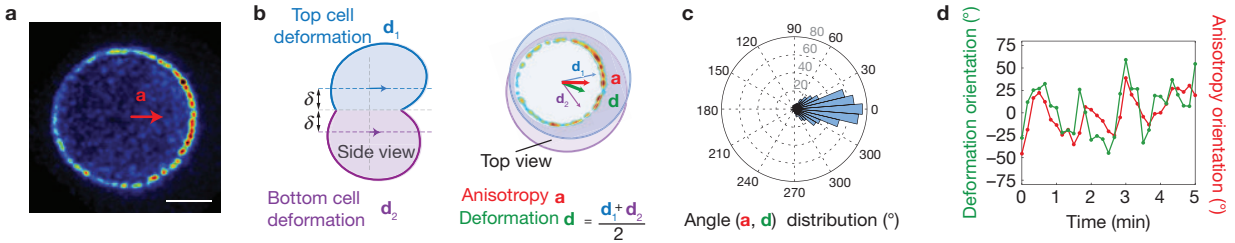




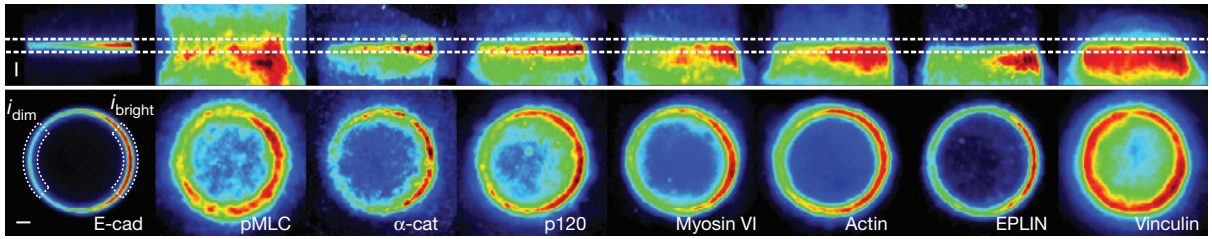




- E-cadherin clusters form a ring stabilised by cellular contractility



- Cell deformation correlate with anistropic distribution of E-cadherin clusters

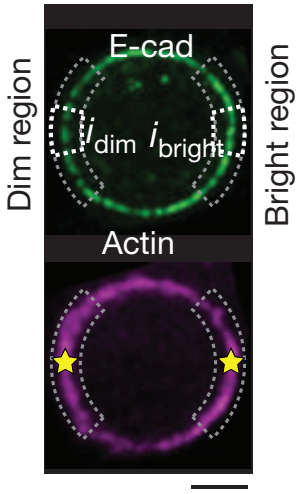


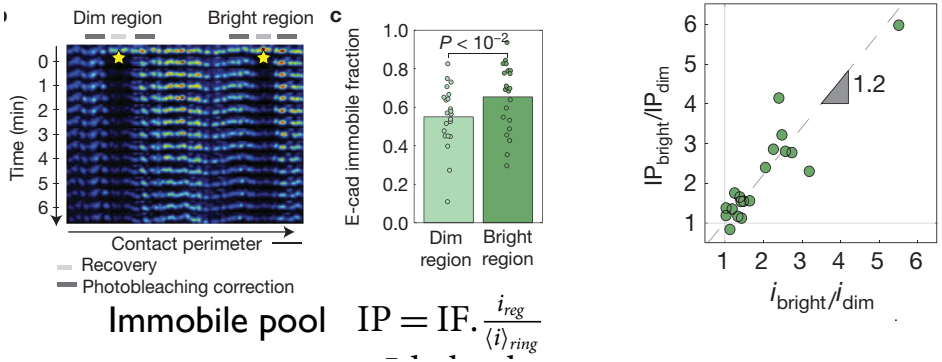
- A gradient of contractility associated with E-cadherin anisotropy

Engl W. Yap LL., Thiery JP. and Viasnoff V., *Nature Cell Biol*, 16:584. 2014

Adhesion as an *active, regulated* system

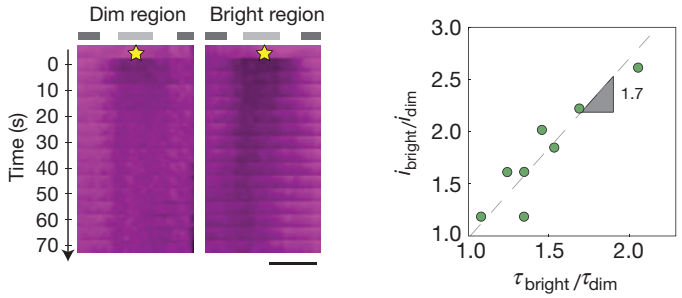
2. Mechanotransduction by Cadherins





Immobile pool $\mathrm{IP} = \mathrm{IF}.\frac{i_{reg}}{\langle i \rangle_{ring}}$

Increment of E-cad intensity proportional to increment of immobile pool



Increment of E-cad intensity proportional to increment of F-actin turnover time

E-cadherin immobilisation correlates with reduced F-actin turnover

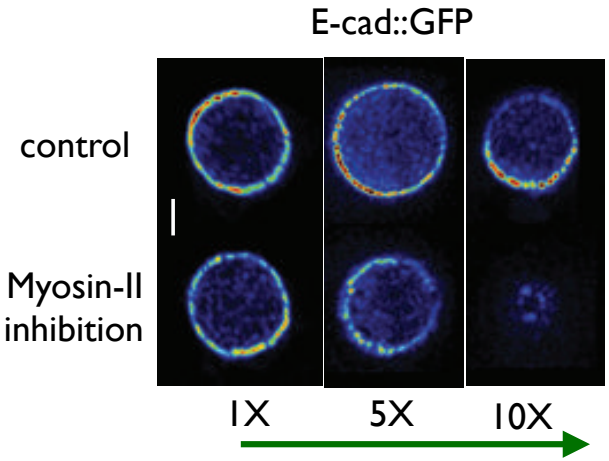
$$\Delta IP/IP_{dim} = (2.0 \pm 0.3)\ \Delta\tau/\tau_{dim}$$

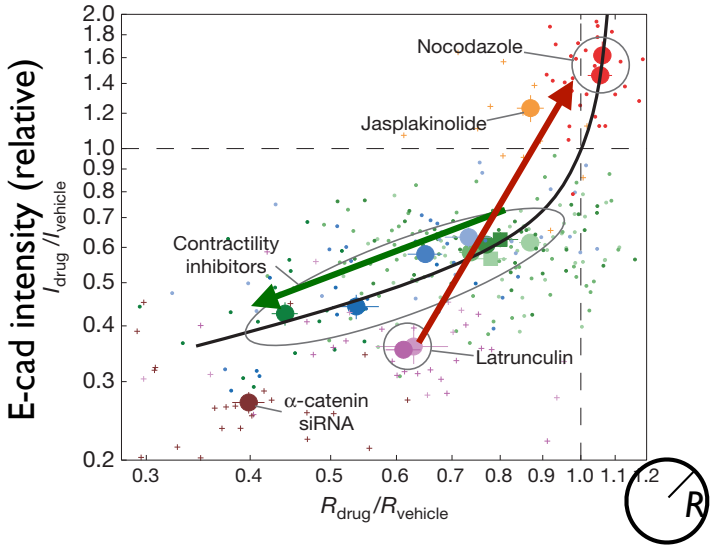
Engl W. Yap LL., Thiery JP. and Viasnoff V., *Nature Cell Biol*, 16:584. 2014

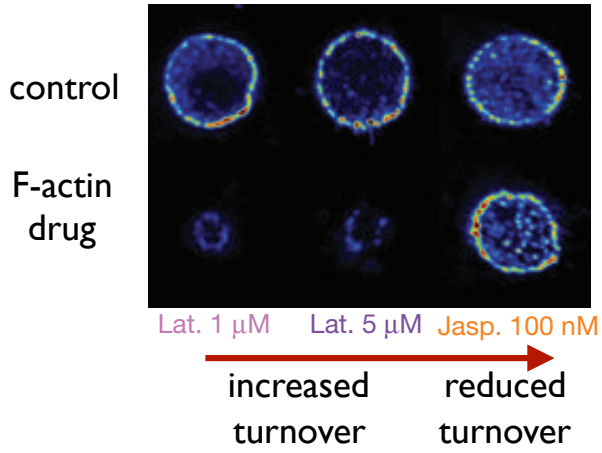
Adhesion as an *active, regulated* system

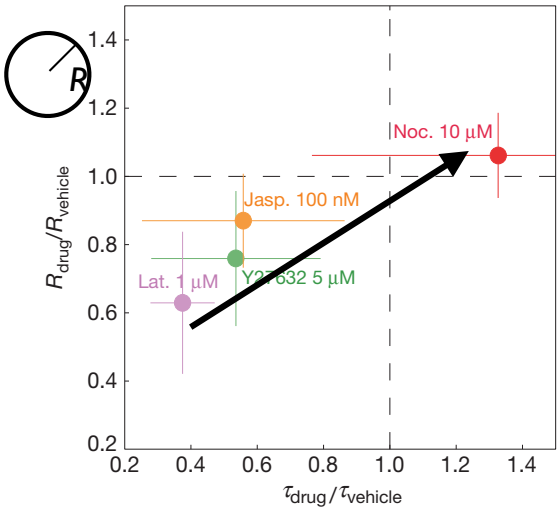
2. Mechanotransduction by Cadherins

- Increased cortical tension stabilises E-cadherin
- Reducing actin turnover stabilises E-cadherin







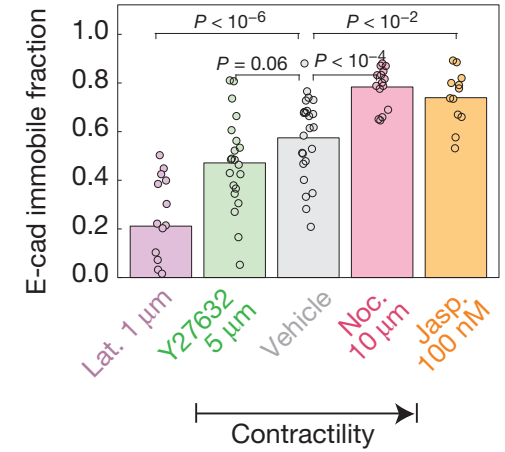


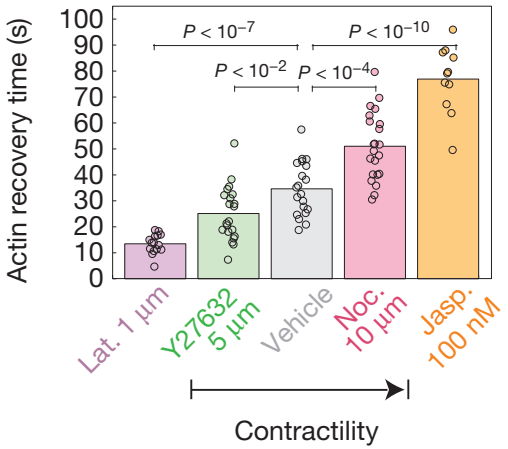
Engl W. Yap LL., Thiery JP. and Viasnoff V., *Nature Cell Biol*, 16:584. 2014

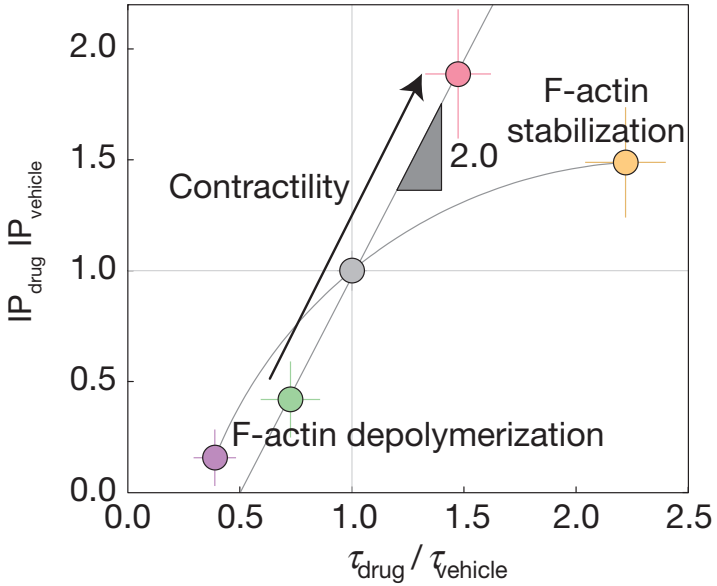
Adhesion as an *active, regulated* system

2. Mechanotransduction by Cadherins

- E-cadherin is stabilized by cortical tension via an effect on F-actin turnover







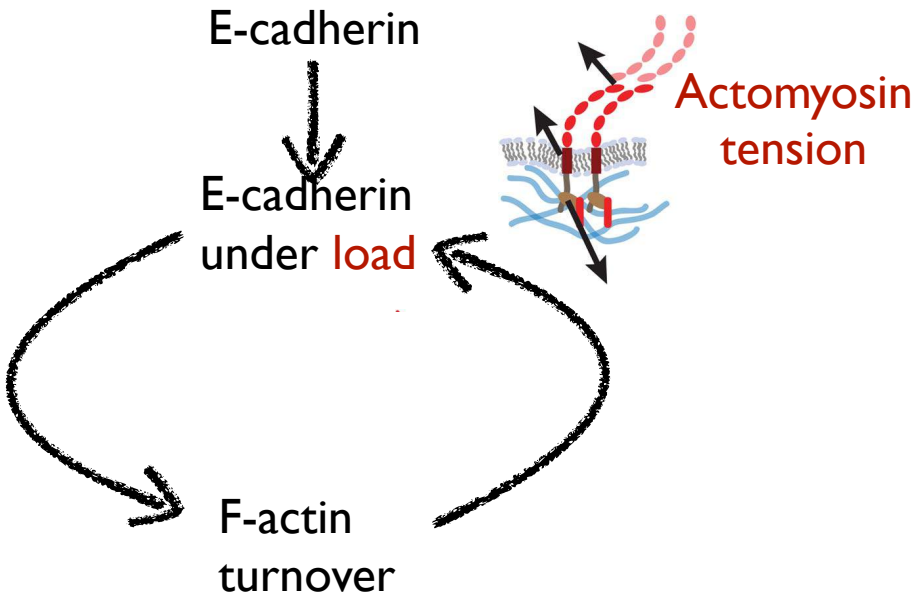
$\Delta IP/IP_{vehicle} = (2.0 \pm 0.2)\ \Delta\tau / \tau_{vehicle}$

Engl W. Yap LL., Thiery JP. and Viasnoff V., *Nature Cell Biol*, 16:584. 2014

Adhesion as an *active, regulated* system

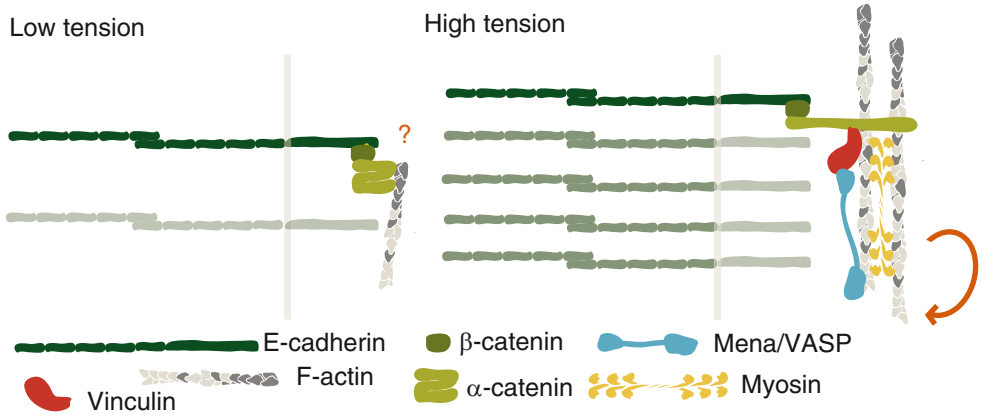
2. Mechanotransduction by Cadherins

- Suggests a positive Feedback Loop: E-cadherin under load modifies the actin cytoskeleton which in turn stabilises E-cadherin



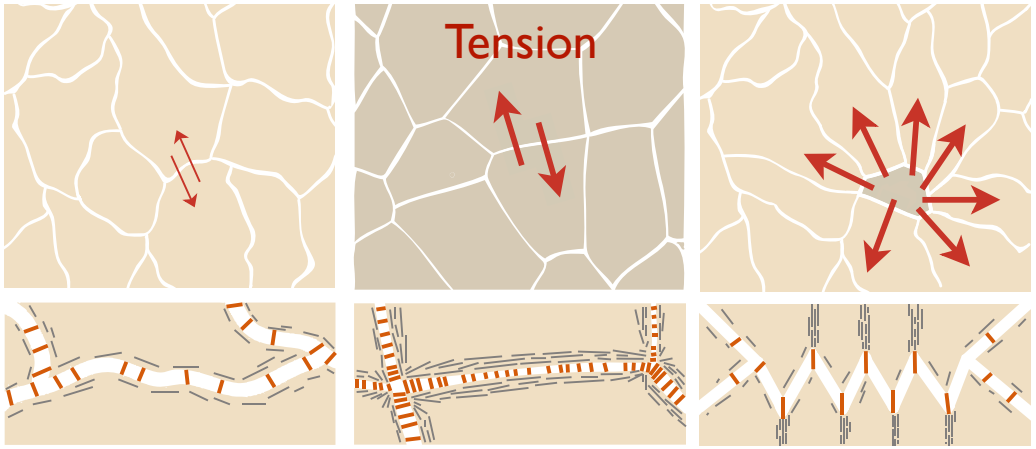
Adhesion as an *active, regulated* system

2. Mechanotransduction by Cadherins: tension feedback loop



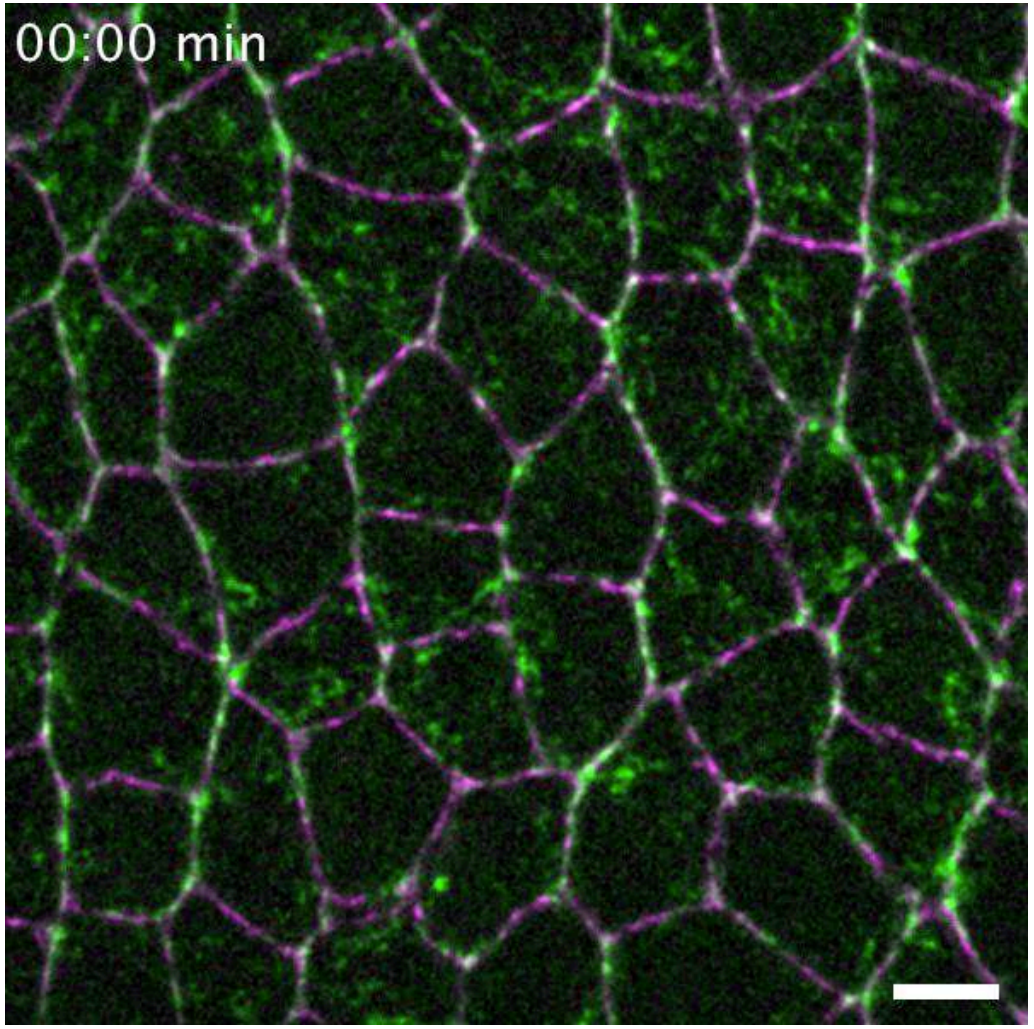
Leerberg JM et al Yap, AS., *Curr Biol*, 24:1689. 2014

- Tension feedback loop involves tension sensing by a-catenin and vinculin, and recruitment of actin elongation factor VASP.



Adhesion as an *active, regulated* system

Dynamic interplay between E-cadherin and Actomyosin networks at cell contacts



E-cadherin::GFP Myosin-II::Cherry Girish Kale

Adhesion in multicellular organisms

1. Affinity and Adhesion: a specificity problem

2. Adhesion: a thermodynamic model

3. The molecular framework of adhesion

4. Evolutionary origin of adhesion mechanisms

5. Adhesion as an active mechanism

   4.1. Clustering

   4.2. Mechanosensation - Mechano-transduction

6. Adhesion and *dissipation*

Adhesion and the regulation of dissipation

What are the determinants of adhesion strength?

- Strength of Molecular interactions: E-cad-E-cad, E-cad-Factin coupling
  - Number of bonds
  - Strength of bonds: but this depends on how fast one pulls on a chemical bond.
  - Nature of bonds: catch versus slip bonds.
  - Mechanical feedback: vinculin based reinforcement

- Molecular organisation:
  - Clusters enable phase transition between seemingly « free » monomers and clusters. Change effective affinity be few orders of magnitude.
  - Avidity effect: entropic effects vs binding energy.
  - Molecular clutch: strong coupling to F-actin and feedback control of tension on cluster density.

However:

- All interactions have relatively low affinity: mM to µM
- « Design »: many dynamic, weak bonds.

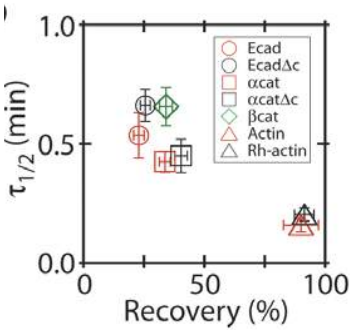
Adhesion and the regulation of dissipation

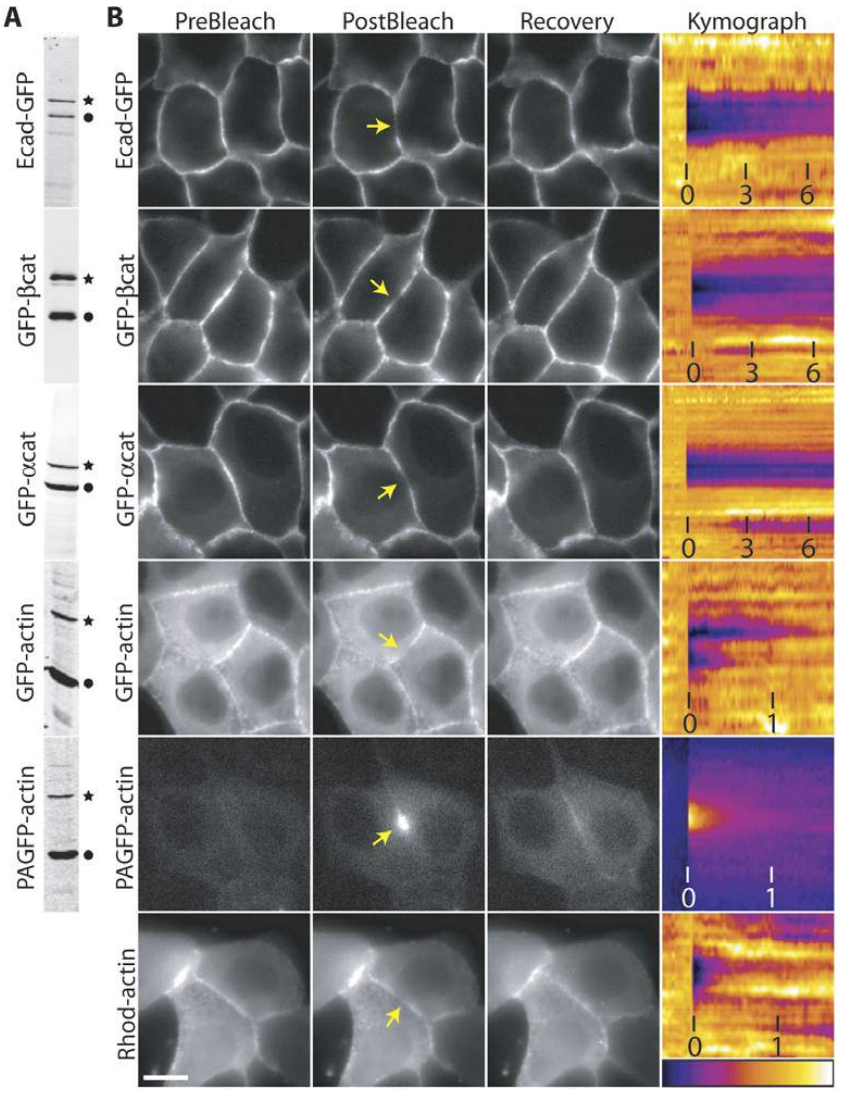
- Adhesion clusters with actomyosin cortex are viscoelastic aggregates.
  - elastic on short time scale (seconds), but behave like viscous fluids on longer times scales than turnover time.
  - on short time scale, depends chiefly on strength of molecular bonds. But:
    - The faster stress is applied, the greater the force to rupture interactions (Evans).
    - Stress induces molecular strain (a-catenin, Talin, Titin etc) that reinforces molecular interactions.
  - on long time scales (morphogenetic time scales (10 s - minutes), energy is dissipated and adhesive interface remains strong.

- Energy dissipation at adhesion clusters: many weak bonds
  - E-cadherin turnover: 20s. Rebinding and bond exchange
  - Actin turnover: <10s. Polymer dissipation
  - Active (motor) and passive cross linkers turnover: 5-20s

Adhesion and the regulation of dissipation

All component of the Adhesion complexes undergo extensive and rapid turnover





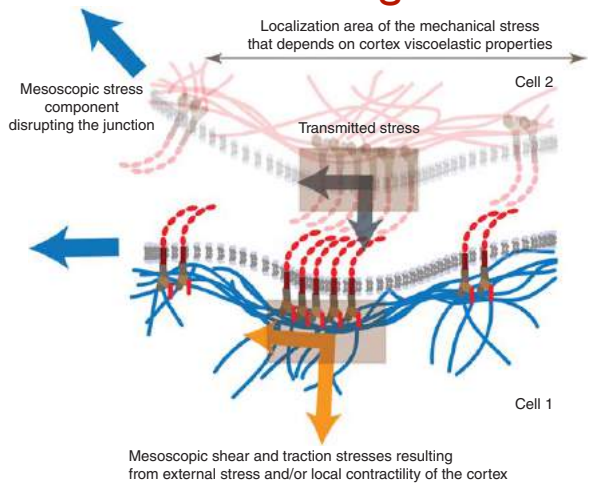
Yamada et al , Nelson WJ. *Cell*, 2005

Adhesion and the regulation of dissipation

- Cells have evolved means to control the modes of dissipation at adhesive interfaces to prevent formation/propagation of fractures:
  - orientation: tension or sheer stress
  - length scale. $l = (\eta/\gamma)^{1/2}$ η : viscosity γ : friction

- Cells can actively control the rheology of the adhesive cortex and thereby actively control dissipation:
  - Cortex stiffening: actin polymerisation (ENA/Vasp).
  - Cortex fluidisation: motors (Rho1 regulation)
  - E-cadherin stabilisation (via actin?)
  - E-cadherin turnover: endocytosis (actin and motor dependent)

- Clusters of E-cadherin: weak interactions (mM range), but many (10^3 - 10^4) within 10-100nm, with rapid rebinding kinetics ensure high local dissipation while maintaining sufficient coupling between cell surfaces

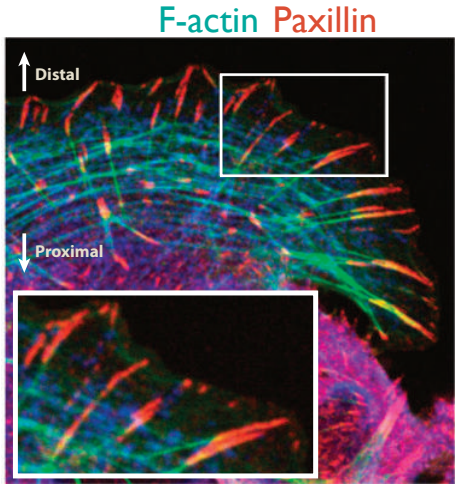


Yap AS. Duszyc K. and Viasnoff V. *Cold Spring Harbor Perspective Biology*. Aug. 2017

Similarities between cell-cell and cell-substrate adhesion

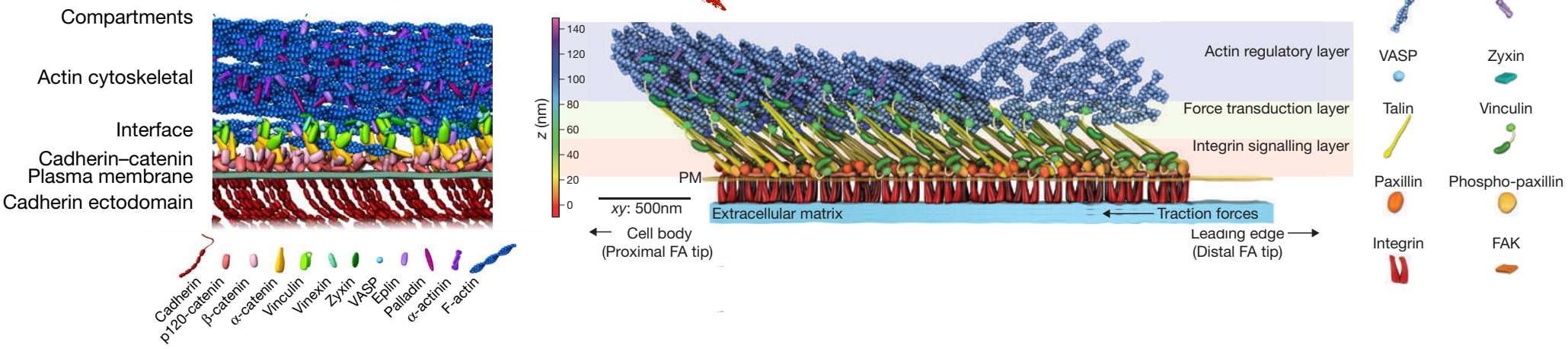
Cadherin Adhesion

Integrin Adhesion



Actomyosin Tension

Actin Flow

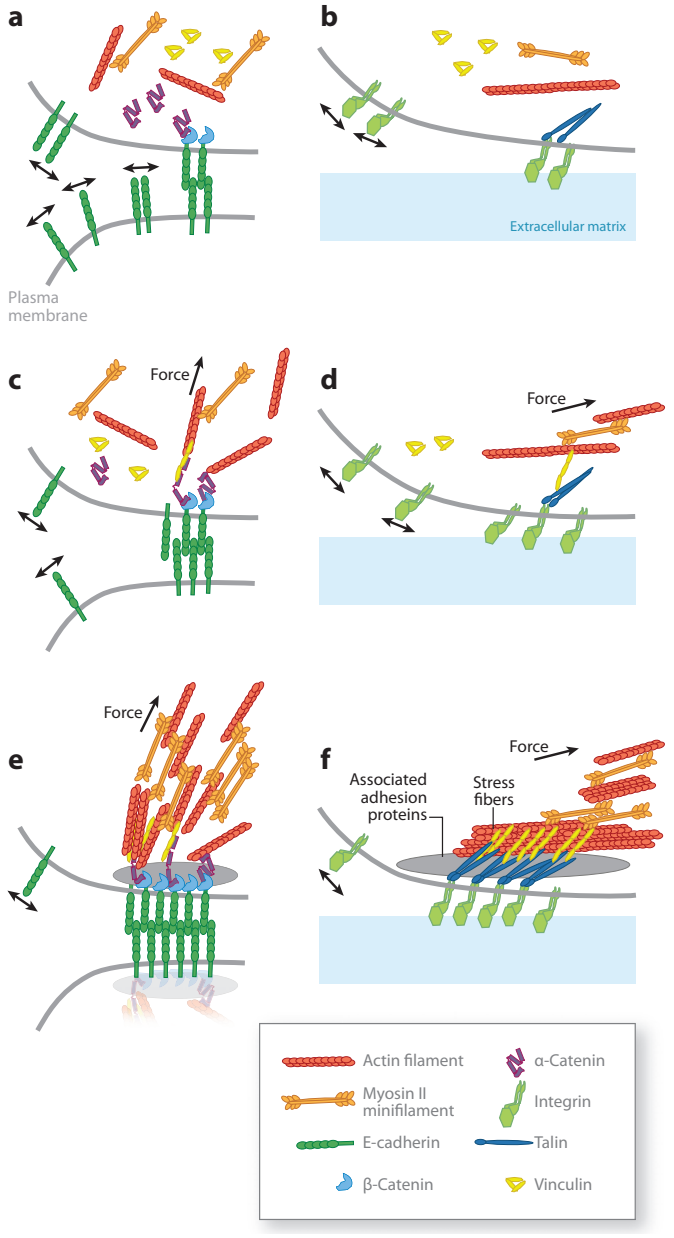


Bertocchi et al, *Nature Cell Biology* 2016

Gardel ML. et al, Waterman CM. *Ann Rev of Cell & Dev Biol*, 2010
Case L. and Waterman CM. *Nature Cell Biol*, 2015

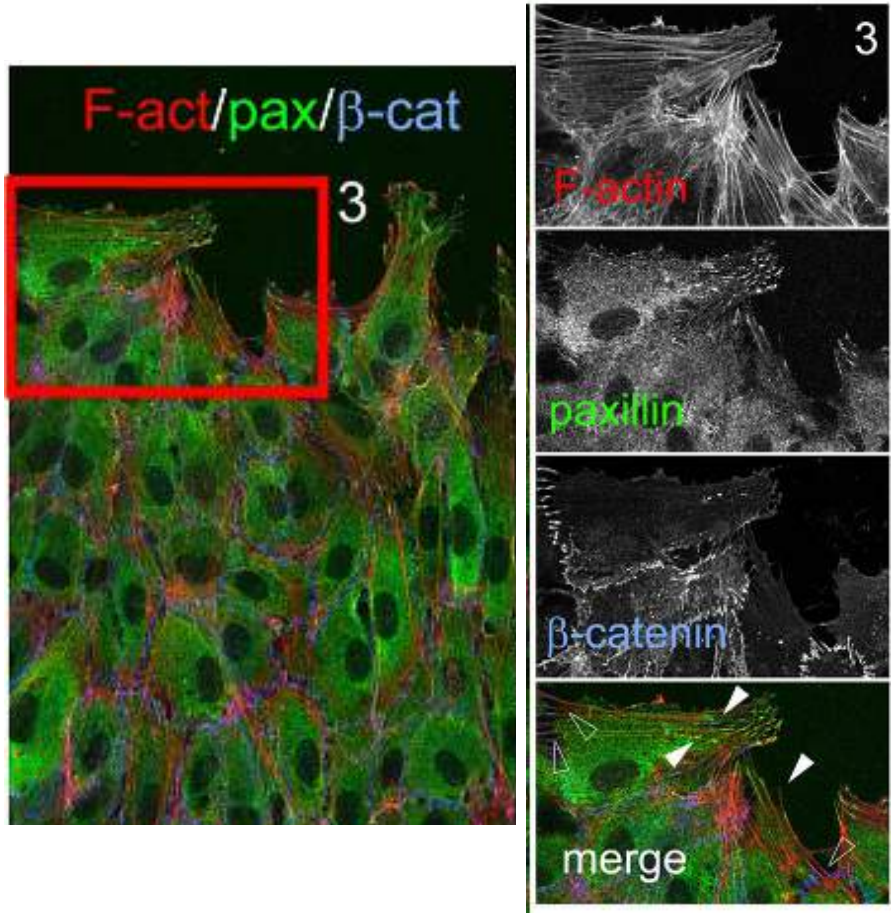
Similarities between cell-cell and cell-substrate adhesion

- Clustering: discretisation and compartmentation of mechanics
- Actin coupling and force transmission
- Adhesive function and tension transmission function
- Mechano-sensation and transduction: clutch mechanism
- Control of dissipation



Similarities between cell-cell and cell-substrate adhesion

Cells often exhibit both types of adhesion



Millan J. et al. *BMC Biol*. 8:11. 2010

Conclusions

- Cadherin based adhesion is an out-of-equilibrium system whereby active processes control the dynamic organisation in clusters.

- Cadherin clusters transmit cortical tension and response to force:
  -cluster organisation: turnover, density
  -molecular coupling: catch bond , strain dependent reinforcement etc

- Energy is constantly dissipated at adhesion sites:
  -turnover of all molecular components (~10 seconds)
  -many weak bonds (low affinity interactions) concentrated locally

- Viscoelastic properties of adhesion sites underlie organisation/plasticity paradox of tissue dynamics (see Lecture 1).

ADHESION: Questions/Problems

- Why do multicellular adhesive systems adopt near thermodynamic equilibrium configurations?
  - separation of time scales between molecular and cellular processes (1 to 2 orders of magnitude)?
- What underlies the modes of energy dissipation at adhesive interfaces?
  - spatial modes: xy, xz?
  - time scales of energy dissipation?
  - impact of molecular organisation?
  - mechano-chemical feedbacks?
- What underlies the mechanical adaptation of adhesion sites?
  - time scales of feedbacks.
- What sets tissue mechanical length scale and how is this tuned by the viscoelastic properties of adhesion and the modes of energy dissipation?
  Does this scale with tissue size? e.g. during tissue growth?

Conclusions

Next Lecture: 21 November 2017

« Cellular Tension »