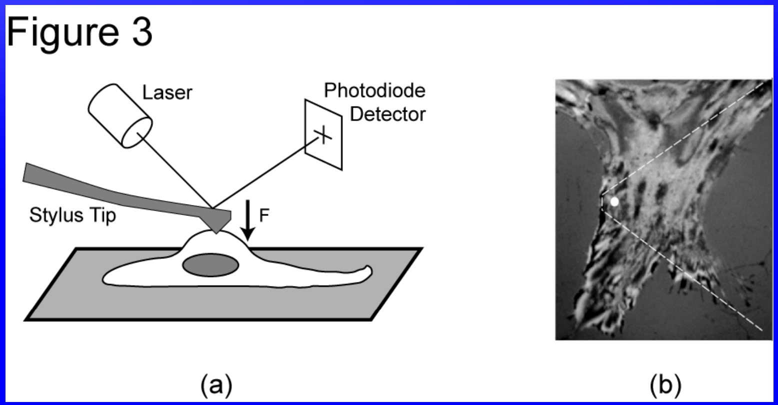
Session 24

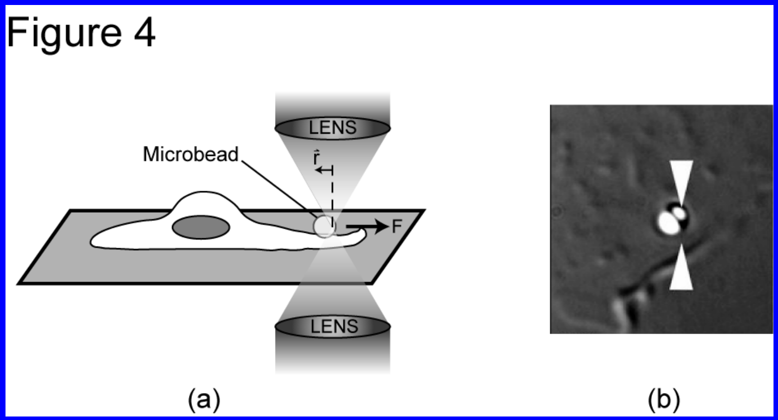
NANOSCALE APPROACHES

Nanoscale Mechanotransduction

- Atomic Force Microscopy

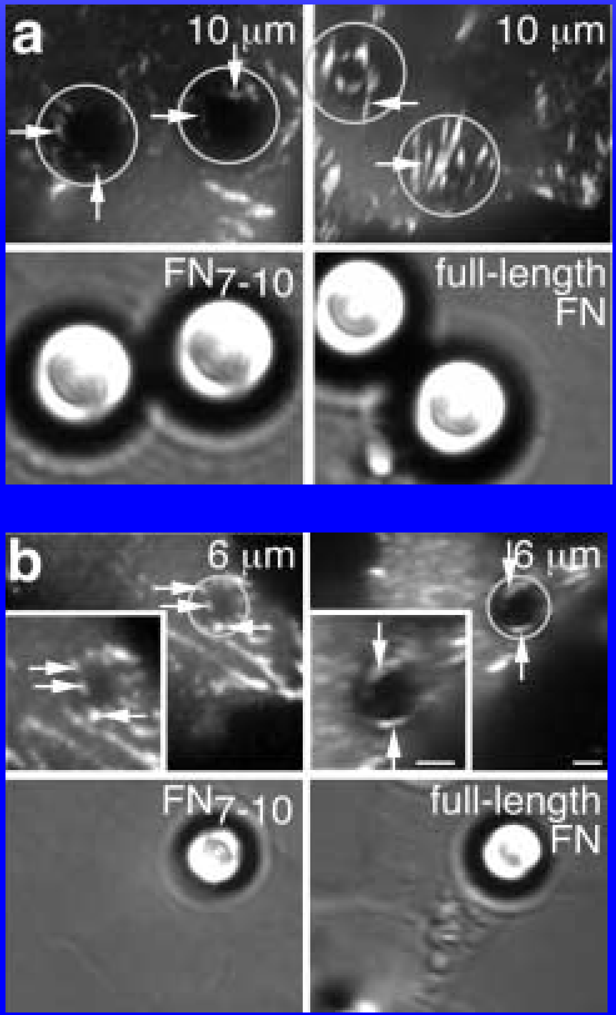


- Optical Tweezers



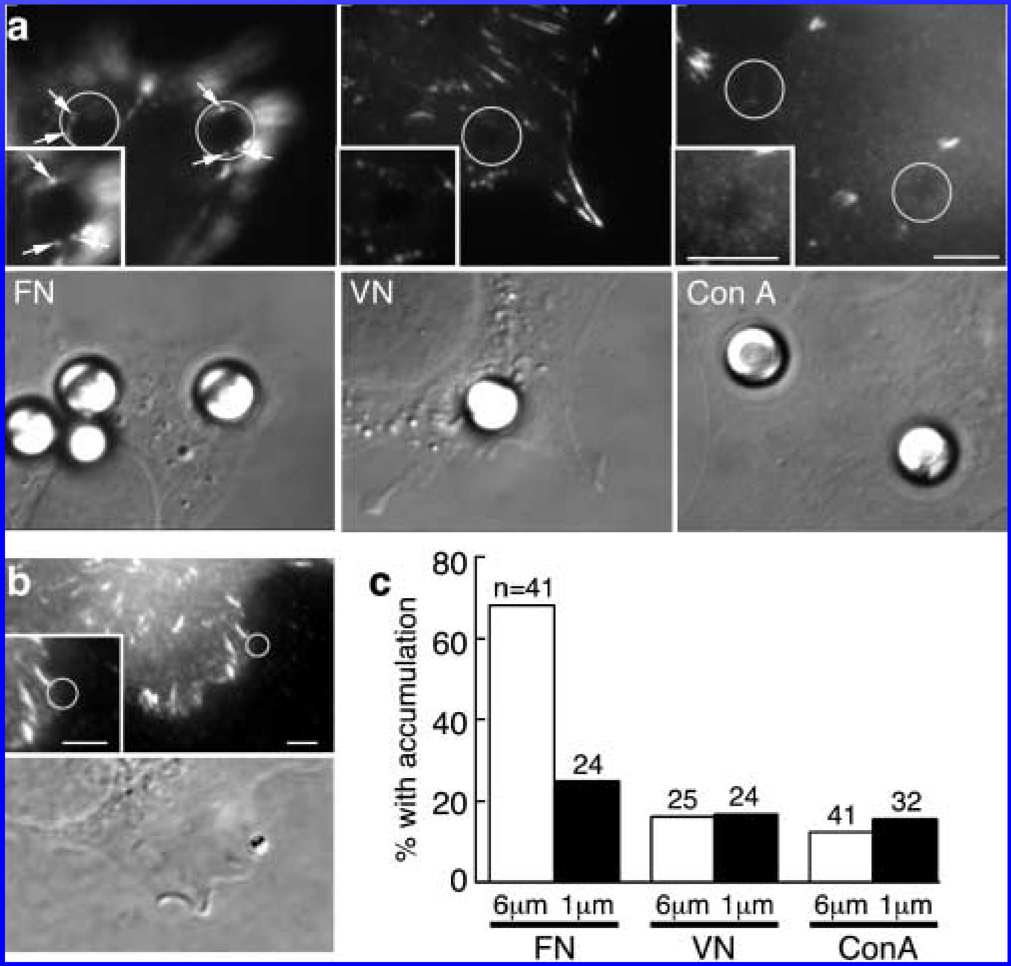
Adhesions form on Microbeads

- Beads coated with fibronectin fragment
  - Domains 7-10
  - RGD sequence located on 10
- Mature focal adhesions detected by vinculin immunofluorescence
- 10 and 6 μm beads permit FA growth



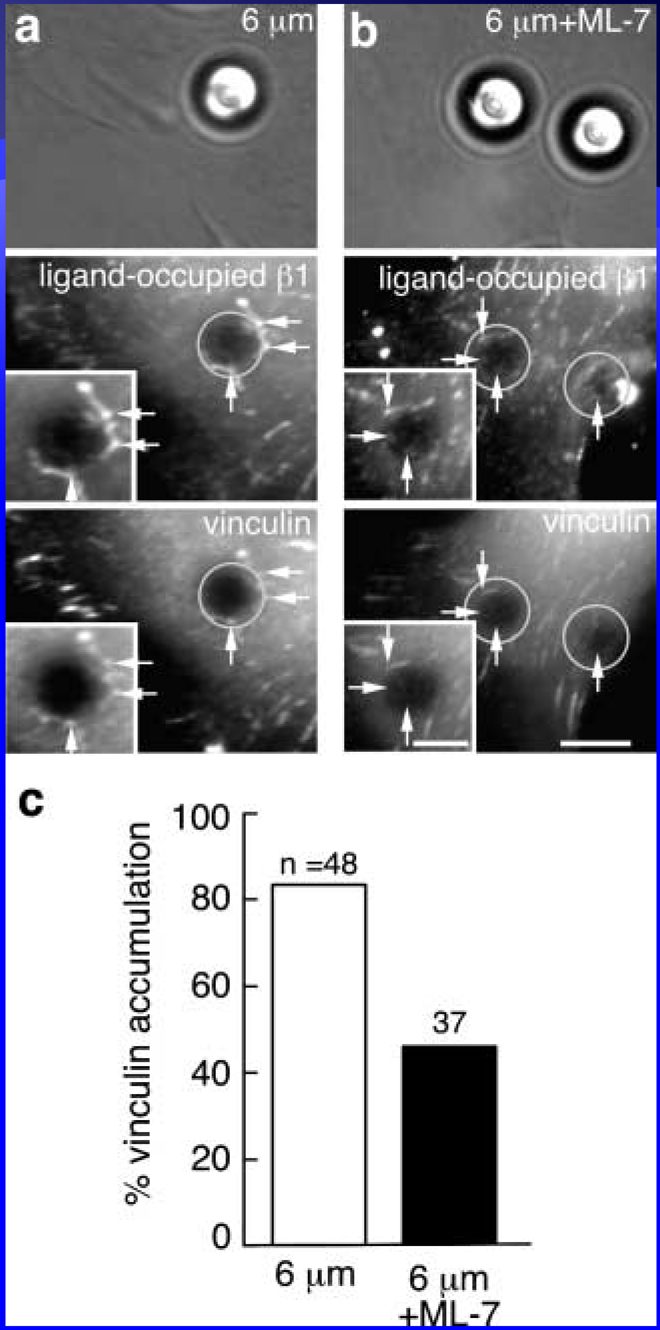
Ligand and area define FA formation

- Ligand
  - Fibronectin (integrin-binding)
  - Vitronectin (integrin-binding)
  - Concanavalin A (non-integrin-binding)
- Contact area
  - Reduced FA growth for 1 µm beads
  - Critical area needed for adhesion formation
  - > 0.1 µm² contact area



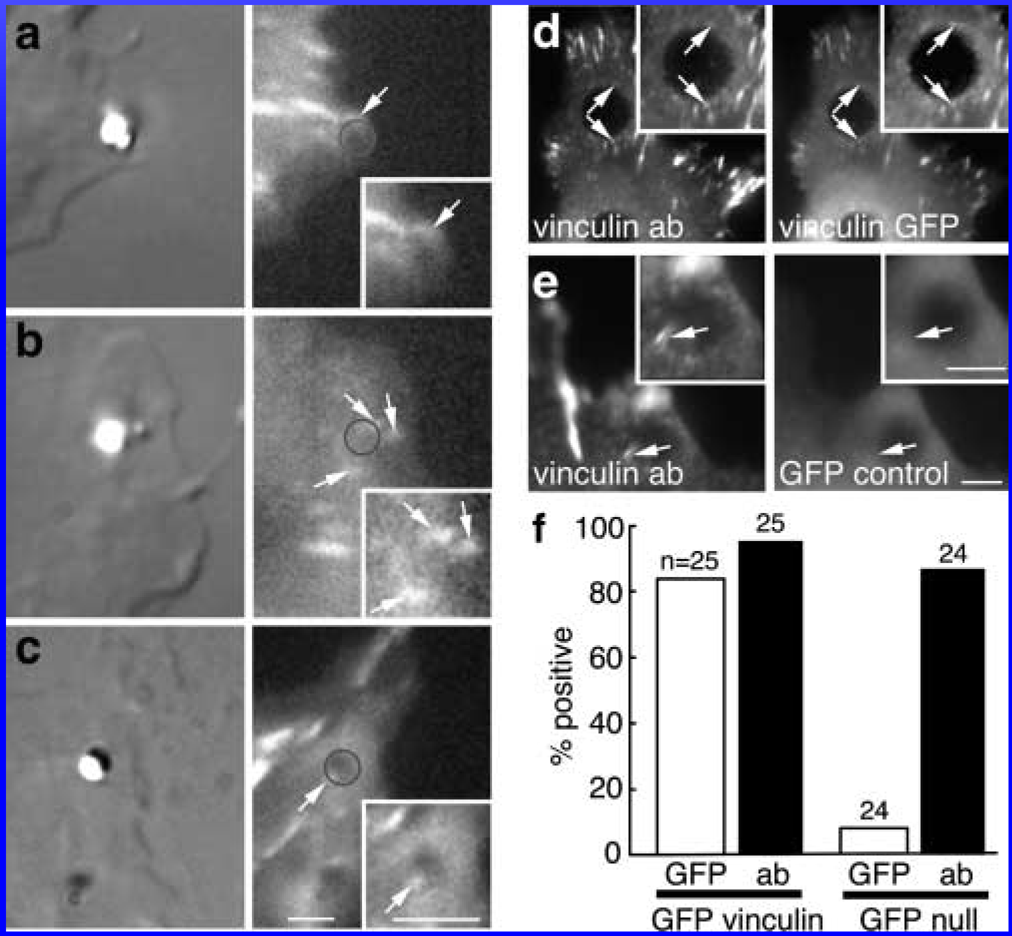
FAs need Internal Force

- ML-7 inhibits myosin light chain kinase (MLCK)
  - Myosin assembly inhibited
- Integrin β1 and vinculin
  - Co-localization in untreated cell
  - Myosin inhibition permits β1–FN binding but not accumulation of vinculin



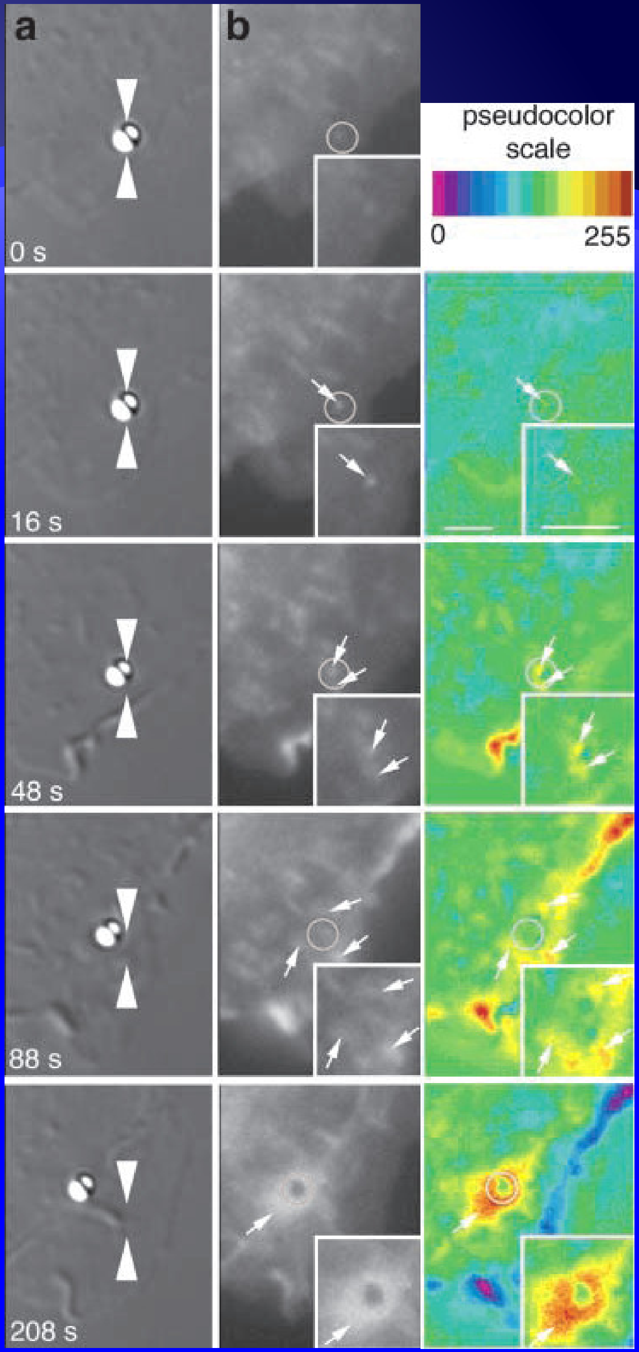
External Force induces FA growth

- Retrograde movement of beads on lamellipodia extensions
- Optical trap used to constrain 1 µm bead
- GFP-vinculin shows induced FA growth with trap force
- GFP-vinculin and antibody signal match



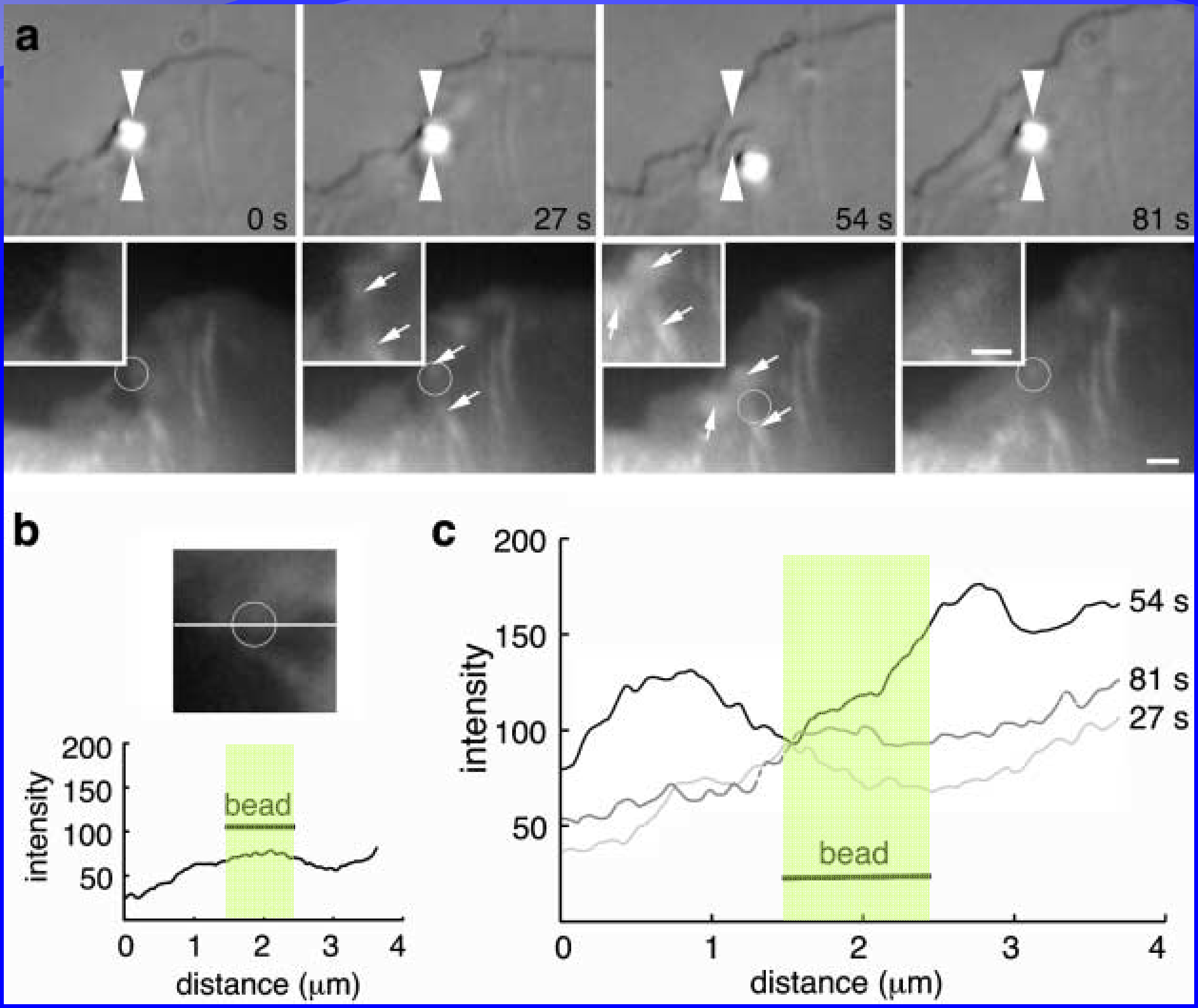
Spatiotemporal Response

- Punctate structure forms first
- Rearward motion causes vinculin accumulation on side of trap force
- Further pulling by cell shows vinculin diffusely surrounds bead



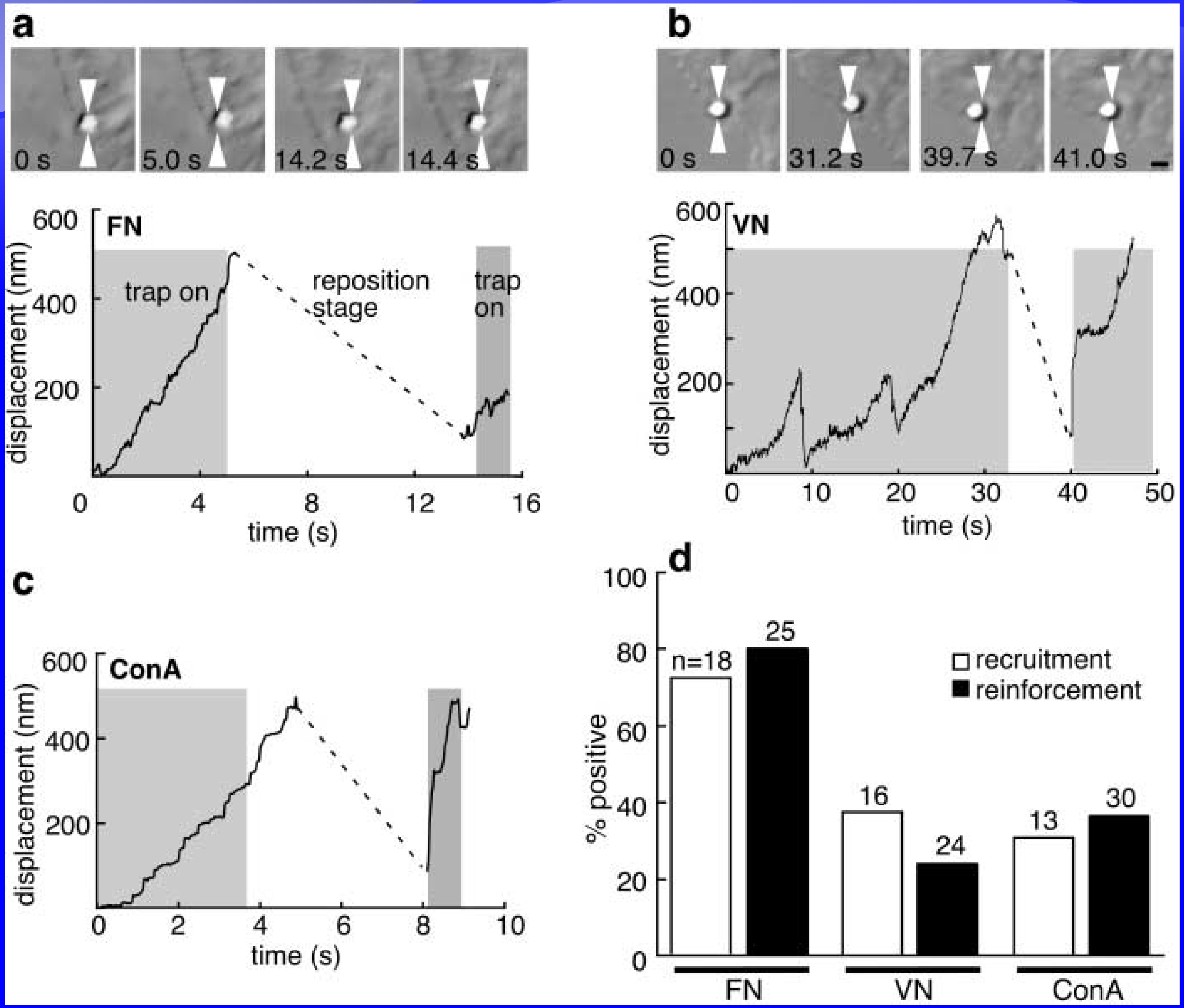
Intensity correlates with Force

- FA intensity rises when bead moves from trap center
- Vinculin dissipation causes loss of retro-grade force
  - Bead pulled back to center of trap (t=81 s)



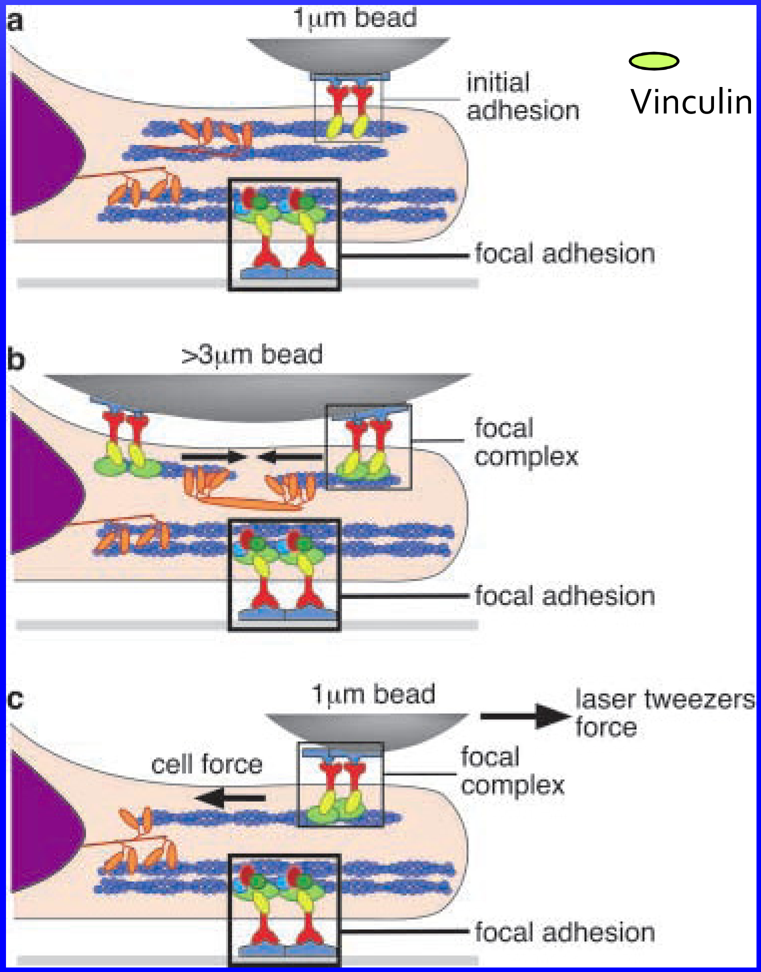
Reinforcement Assay of Cell Force

- Beads contained by trap have weak FAs and cell-forces
- Escaping the trap indicates strong FAs and force
- Strong forces
  - FN
  - VN
  - Con A



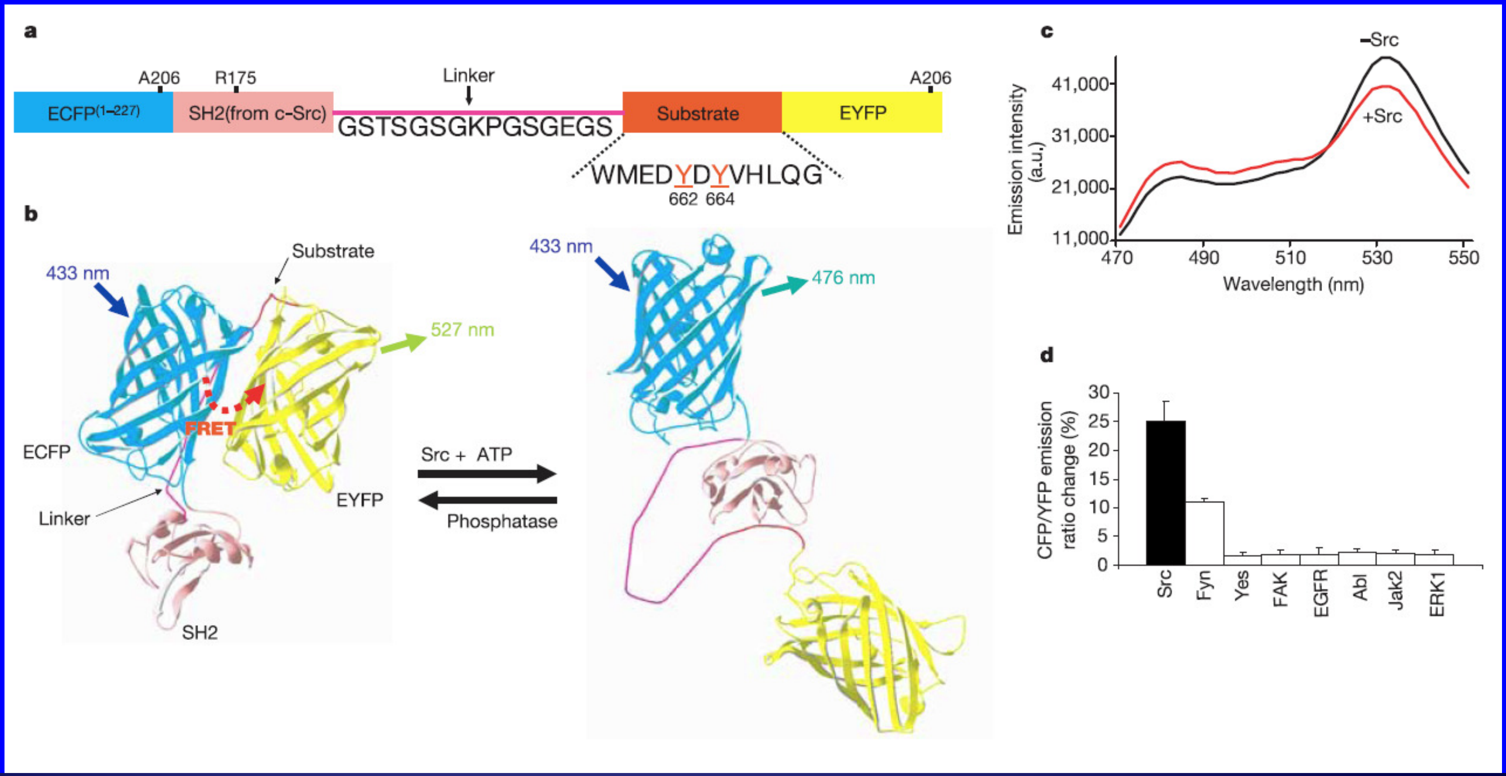
Physics of FA Mechanotransduction

- Small beads move under retrograde flow
- Large beads sufficient for FA growth by balanced internal forces
- External force can induce FA maturation



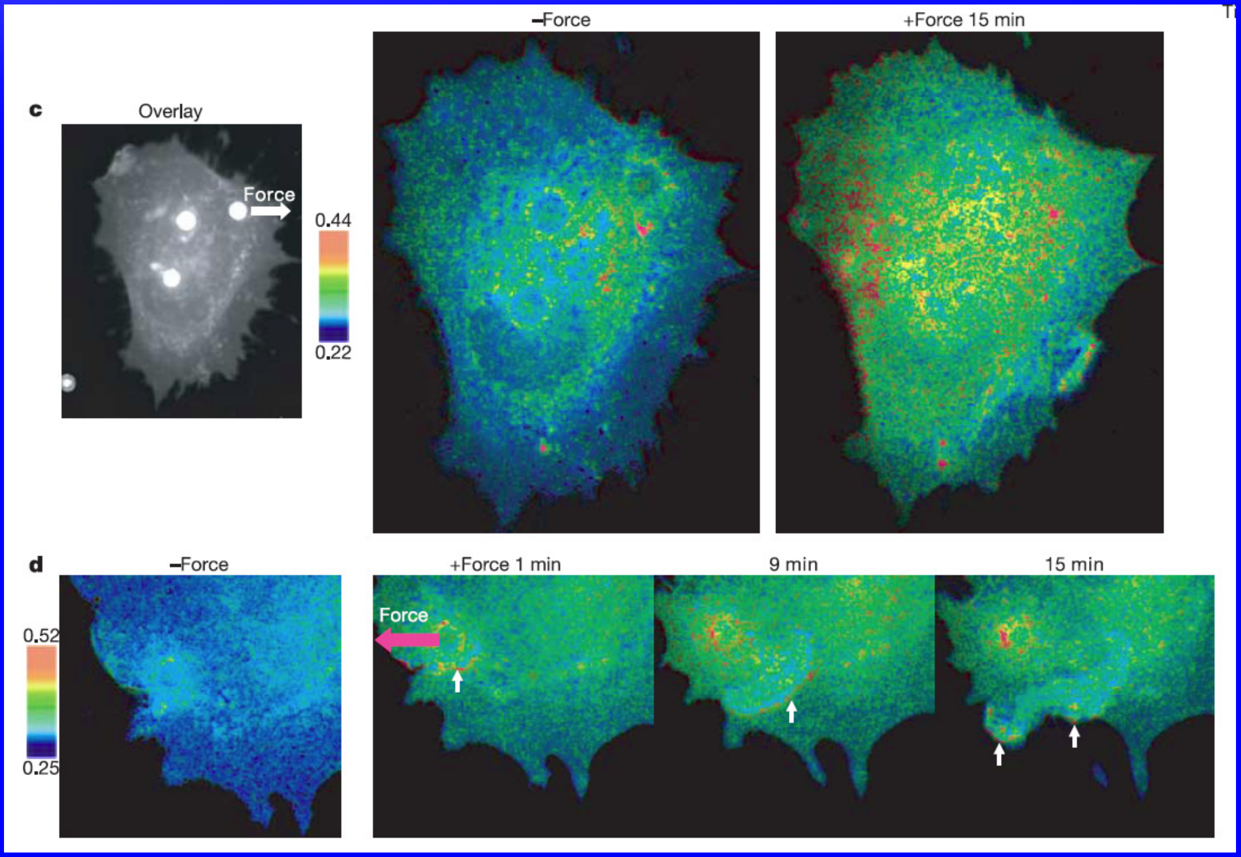
A possible biotrigger…

- Src known to regulate integrin-CSK interactions
- Ligand-activated β3 integrin phosphorylates Src
- FRET-reporter for active Src used with optical trap



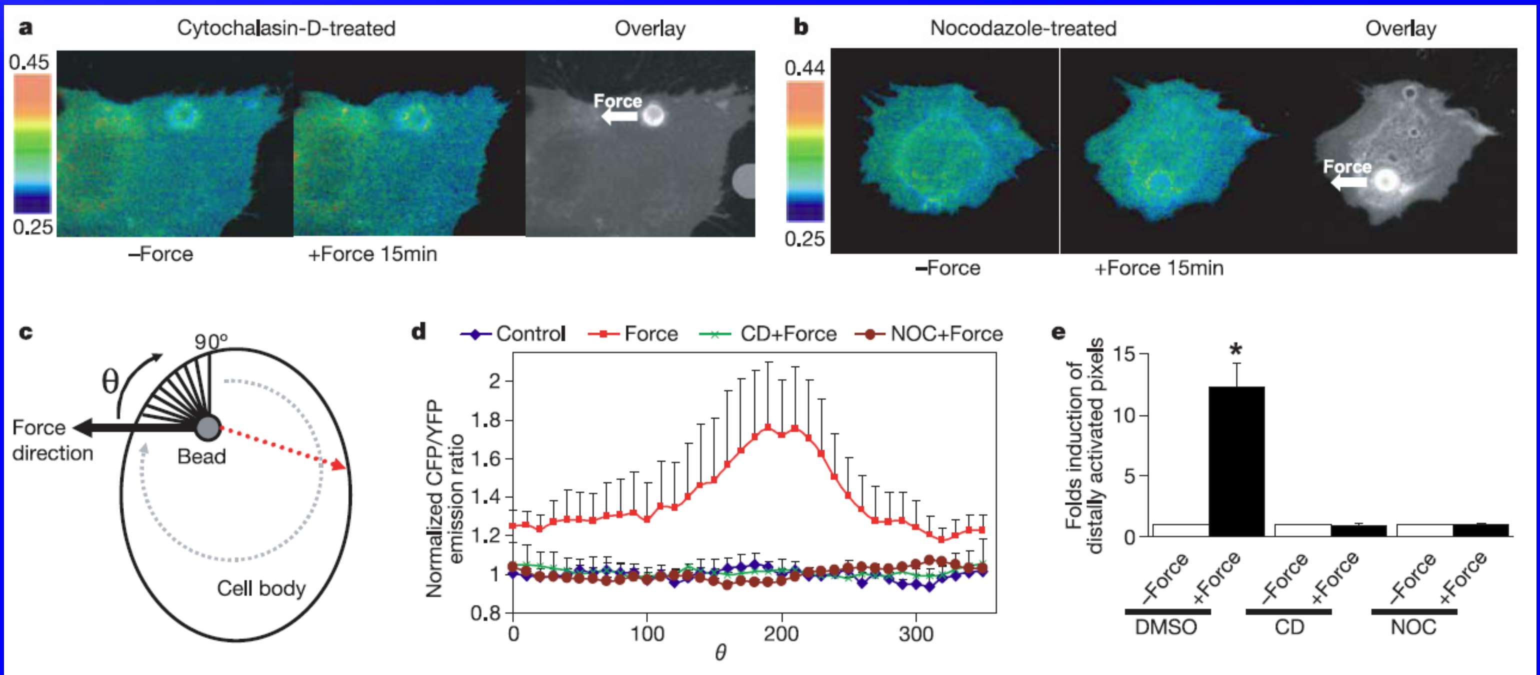
300 pN Force causes Src activation

- Directional and long-wave propagation of Src



CSK needed for long-range signaling

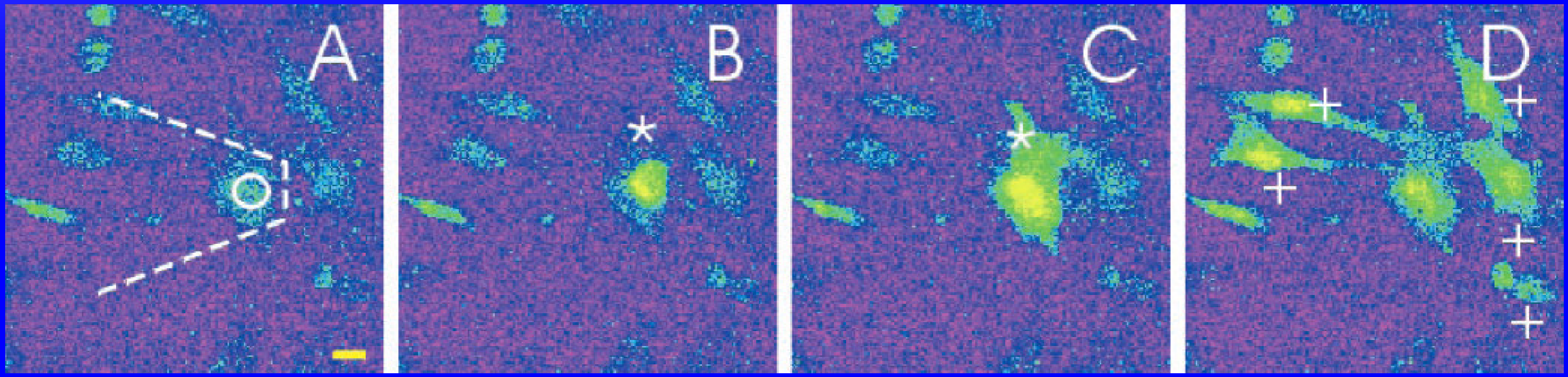
- Force transmission along filaments causes Src mechano-activation at distant sites



- Or, Src activation starts actin polymerization waves
  - Recruit and activate more Src at F-actin tips

Ca2+ spikes with AFM force

- Intercellular propagation in osteoblasts through gap junctions



- Response dependent on stretch-ion channels and MT network

QUESTIONS?