Quantitative Analysis of Forces in Cells

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Basic properties of forces in cells

Measurement methods and magnitudes of particular types of forces:

- Polymerization forces
- Forces from whole cells and cell aggregates
- Osmotic forces
Fundamental Cell Mechanobiology Fact: Net Forces Are Very Small in Comparison to Individual Force Contributions

Moving/accelerating cell: \( F_{\text{net}} = ma = (2000 \ \mu m^3)(1 \ gm/cm^3)(0.01 \ \mu m/s^2) = 2 \times 10^{-20} \ N \approx 0 \)

\[
F_{\text{net}} = 0 = \sum F_{\text{static}} + F_{\text{drag}}
\]

\[
F_{\text{drag}} = (6\pi \eta R)v = 6\pi \times (0.01 \ \text{Pa-s}) \times (20 \ \mu m) \times (0.1 \ \mu m/s)
\]

\[
= 2 \times 10^{-13} \ N \approx 0
\]

So \( \sum F_{\text{static}} = 0: \) **Force balance**

Also, \( F_{\text{grav}} = mg = (4\pi R^3/3)(\rho_{\text{cell}} - \rho_{\text{water}})g \)

\[
= [4\pi (20 \ \mu m)^3/3] (0.05 \ gm/cm^3) (10 m^2/s) = 2 \times 10^{-13} \ N \approx 0
\]
Force Balance

We always speak of forces **between** different entities, like tension or stress:

- **Tension (or compression)**

- **Shear stress and tensile stress**

- **Pressure is an isotropic compressive stress**
Force Balance in cells

(Baum lab Dev Bio 2015)
Basic Units Facts

\[ kT = 4.1 \text{ pN-nm} \]

\[ 1 \text{ Mpa} = 1 \text{ pN/nm}^2 = 10 \text{ atm} = 147 \text{ psi} \]
Biological Force Measurements Require a Reference Mechanical Scale, Like Temperature or Stiffness of a Filament/Rod

Persistence length $L_p$ quantifies the stiffness of a biopolymer filament. It is (roughly) the distance it takes a filament to bend 90 degrees by thermal fluctuations.

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Thermal fluctuations of two bundled actin filaments

(Dogic group Nat Matls 2015)
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Thermal fluctuations of two bundled actin filaments

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Polymerization Forces - the Brownian Ratchet

The thermal-ratchet prediction for this case is $v \propto \exp\left(\frac{F_a}{2kT}\right)$. It is seen that the corrections to the exponential dependence are much smaller for oblique incidence than for $i_0$. The largest discrepancies are about 30% in the range $F_a/kT \approx 1$ to 1.5. The decay rate at the highest force values actually seems to be slower than that in the exponential curve. We believe that this is caused by two factors. The first is that, as will be discussed below, there is a non-negligible probability of monomer addition even below the critical height for the obstacle. The second is bending of the filament as a result of the applied forces. For example, at $F_a/kT \approx 2.5$, we find that the tip of the fiber at the end of the growth process when it is 36 monomers long is bent about 15° relative to the 45° angle at the base. The bending is proportional to the square of the fiber length, and the observed velocities correspond to a weighted average of the bending between long and short fibers. The filament bending is expected to cause the growth rate to increase since the projection of the applied force on the growth axis becomes smaller, and also diffusion to the tip becomes less restricted.

We are plotting the monomer addition rate, not the rate of growth in the $z$ direction. We have in fact observed that the growth rates become larger for longer fibers. This may be partly due to such bending effects, and partly due to the fluctuations of the fiber tip. The latter may be estimated in terms of the effective elastic modulus of the fiber tip. As defined by Mogilner and Oster, the modulus is $\frac{kT}{l^3 \sin^2 i}$, where $l$ is the length of the fiber. The rms vertical fluctuation of the fiber tip is then $kT/\frac{l^3 \sin^2 i}$. With the persistence length $\frac{kT}{l^3}$, the effective elastic modulus is $\frac{kT}{l^3 \sin^2 i}$. The growth velocity is predicted to slow exponentially with opposing force.
Measuring Polymerization Force of a Single Actin Filament

So the growing filament could exert a force of at least 0.8 pN

Green end is attached to “formin”, red end is anchored by inactive myosin

Initial straight length is about 0.7 µm

Buckling force is

$$\pi^2 k T L_p / 4 L^2 = 0.8 \text{ pN}$$

So the growing filament could exert a force of at least 0.8 pN
**Microtubule Polymerization Forces**

V decays exponentially, but too rapidly

(Dogterom and Yurke 1997)
Polymerization Forces of Small Number of Actin Filaments Growing from a Bundle

Measured forces of 1-2 pN are smaller than expected from a bundle of filaments

(Footer et al 2007)
Actin “Comet Tail” Forces Measured by Micromanipulation

Bead with actin nucleator on surface

Growing actin “comet tail”

V₀ = growth velocity at zero force

Growth velocity drops gradually with opposing force

Growth is accelerated by pulling force

(Marcy et al 2004)
Measuring Forces Generated by Whole Cells

Cantilever approach

Since stiffness of cantilever is known, force can be obtained from measured displacement

Prass et al, JCB 2006
Cell Exerting a Force on a Barrier

1) Initial contact
2) Deflection of lamellum
3) Contact with nuclear mound
4) Maximum force
5) Release

(Prass et al 2006)
Quantifying Motion and Force

- Initial peak at 25s, 2.2 nN - lamellipodium (leading edge)
- Then lamellipodium sneaks around cantilever
- Later contact is with mound of the cell body
- Initial contact area is about 1 µm²
Measurements of Force Distribution on Substrate (Traction Force)

If the elastic properties of the gel are known, the bead displacement response to a given distribution of forces can be calculated.

This relationship is inverted to calculate the force distribution given from the bead displacements.

http://bam.lab.mcgill.ca/project_pages/TFM_Silicone.html
Cells pull inward on substrate, in time with spontaneous contractions

Strength of contraction increases with stiffness of substrate

(Hoffmann group, Biology Open 2013)
Stresses and Forces in Layers

Layer of cells or molecules with alternating displacement

Stress $\sigma$

Force $f$

Stress is positive when cells/molecules pull on each other (tension)
Force is largest where stress is changing most rapidly

\[ f \propto \frac{d\sigma}{dx} \]
Measured Stresses and Forces in Cell Layers

Migrating kidney cells on elastic substrate containing fluorescent beads

Displacements of beads are measured with (green) and without (red) forces from cells

(Trepot lab, Nat Cell Biol 2017)
x-direction traction force density obtained from bead displacements

Compressive (dark blue) and tensile (red/green/blue) stress corresponding to measured force density
Force Measurement Using Micropillars

http://ej.iop.org/images/0034-4885/75/11/116601/Full/rpp301965f09_online.jpg

Micropillar array

Forces from smooth muscle cell bend pillars inward

Scale bar = 10 microns

(C. S. Chen lab, PNAS 2003)
Molecular-Level Adhesion Forces
Molecular Force Sensors

“Donor” can emit green fluorescence

But by Förster resonance energy transfer (FRET), energy is instead transferred to the “acceptor” if it is close to the donor.

For low force, FRET occurs and the green fluorescence is not seen.

For high force, FRET is prevented and green fluorescence is seen.

The protein paxillin is concentrated in regions of high force.

(Dunn group, Nano Letters 2015)
Stress Measurement Using Ablation

Arrow indicates target of laser on a stress fiber

Scale bar = 10 microns

Ends of fiber retract immediately after laser pulse; initial rate is set by viscosity of medium

(Ingber lab, Biophys. J. 2006)
Ends of stress fiber continue to move apart; final displacement is determined by elastic properties of medium
Osmotic pressure difference (turgor pressure) is \( \Pi = kT(C_{in} - C_{out}) \)

Chemical measurement of \( \Pi \) : increase external ion concentration by an amount \( \Delta C_{out} = (C_{in} - C_{out}) \) so that \( \Pi \) vanishes. Then collapse of membrane away from cell wall can be observed.
Cusp in volume variation occurs when membrane leaves cell wall

\[ \Pi = kTC_{P=0} \]

(Budding Yeast)

\[ \Pi = 0.5 \text{ MPa} \]
Measuring Osmotic Pressure Difference in Walled Cells

Physics analysis shows that

$$\Pi = \frac{\text{(spring constant } k)}{\pi \text{(cell radius)}}$$

$$= 0.2 \text{ Mpa}$$
Conclusions

- We can only measure a limited range of forces in cells
- New technologies are pushing the field forward
- Measurement of stress inside cells remains a hard problem