Protein self-assembly and cell organization

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Rudolf Lueckart (1822-1898)
How are eukaryotes different from prokaryotes?
Membrane-enclosed nucleus
...uncoupled transcription and translation
Extensive internal membrane systems and membrane-bound organelles
Expanded genome with multiple, large chromosomes
Much larger cell size (1-5 µm → 20-200 µm)
High degree of subcellular compartmentalization
Endosymbionts (mitochondria and chloroplasts)
Better, bigger, fancier multicellular organisms

Stromatolites:
Colonial cyanobacteria
Similar over 3.5 billion years

Coral

Membrane-enclosed nucleus...uncoupled transcription and translation
Extensive internal membrane systems and membrane-bound organelles
INTRACELLULAR MEMBRANE TRANSPORT WITH MOTOR PROTEINS ON MICROTUBULES CAN DRAW PLASMA MEMBRANE INSIDE, MODIFY SHAPE, LOCATION
NUCLEAR LAMINS STABILIZE NUCLEAR MEMBRANE
Expanded genome with multiple, large chromosomes
MITOTIC SPINDLE (MICROTUBULES, MOTORS) CAN SEGREGATE ACCURATELY, EFFICIENTLY
Much larger cell size
DIRECTED INTRACELLULAR TRANSPORT FREES THE CELL FROM THE DIFFUSION LIMIT
High degree of subcellular compartmentalization and specialization
MICROTUBULE ORGANIZING CENTER SETS UP A UNIVERSAL COORDINATE SYSTEM FOR CELL POLARITY
Endosymbionts (mitochondria and chloroplasts)
ACTIN CYTOSKELETON ENABLES PHAGOCYTOSIS, ALLOWING SELFISH PREDATION AND CAPTURE OF ENERGY-PRODUCING SERVANTS
Better, bigger, fancier multicellular organisms
ACTIN AND INTERMEDIATE FILAMENTS COOPERATE IN GENERATING STRONG, FLEXIBLE CELL-CELL JUNCTIONS IN METAZOANS CYTOSKELETON COORDINATES CELL WALL AND ECM DEPOSITION IN METAZOANS, FUNGI AND PLANTS

The plot thickens…

Bacteria have tubulin (FtsZ)

FtsZ (required for cell division):
- Is a GTPase with limited sequence similarity to tubulin (Mukherjee et al., 1993)
- Assembles into filaments in a GTP-dependent manner (Mukherjee and Lutkenhaus, 1994)
- Crystal structure is superimposable with either α or β-tubulin (Lowe et al., 1998; compare Nogales et al., 1998)

Sun and Margolin, 1998
And bacteria have actin (several kinds)

A, B wild-type *B. subtilis*
C, D mreB
E, F mbl

Jones et al. 2001

Mbl protein helices in cells

MreB filaments in vitro

van den Ent et al, 2001

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Strong structural conservation among actin superfamily

Vary in protofilament pitch, protofilament number (?), and coupling to ATP hydrolysis

van den Ent et al., 2002
Actin homolog used to organize magnetosomes

And some may have intermediate filaments….

Ausmees et al., 2003
Q: If bacteria have a cytoskeleton, why don’t they do something more interesting with it?

Hypothesis: The central feature of the cytoskeleton necessary to cellular life, large-scale cell organization, and cell division is the dynamic assembly and disassembly of helical protein filaments

- Eukaryotes enhance these features with specialized cytoskeleton-associated factors: NUCLEATORS and MOLECULAR MOTOR PROTEINS
- Corollary: Prokaryotes lack nucleators and molecular motor proteins (Q2: Why?)

Structural encoding: how to make a helix

On any (asymmetrical) protein surface, one of the possible pairs of interaction sites will yield the most favorable energy on binding. Therefore, any pure protein at high concentration will have some tendency to aggregate helically. Secondary favorable interactions will stabilize helices with multiple protofilaments.

Crane, 1950
Pauling, 1953

Actin filament reconstruction - Amy McGough
The accidental polymer: Hemoglobin S forms helical filaments (14 protofilaments)


A slight increase in the binding energy on one face tips the balance from deoxy-HbS being soluble at high concentrations to aggregating as a helical filament.

Stuart Edelstein
The beta trap: Prions and amyloid are other examples of accidental polymers that are energetically (too) stable.

Amyloid fibrils grow by addition of monomers at ends.

Collins et al., 2004
Cytoskeletal filament structures

Microtubule

Actin

Intermediate filament

Cytoskeletal polymers must be energetically UNSTABLE to allow cell structural changes, but STABLE for physical strength

One method to modulate stability/instability: couple nucleotide hydrolysis to a protein conformational change (compare HbS O$_2$ binding)

Actin is structurally related to hexokinase

Mitchison, 1995
Rapid in vivo dynamics are a general feature of cytoskeletal structures

Microtubules:
Mitotic spindles in marine eggs can be stable for hours but disappear within minutes after colchicine addition and reform within minutes after washout (Shinya Inoue, 1970s)
Both taxol (microtubule stabilizer) and colchicine/vinblastine/etc. (destabilizers) prevent chromosome segregation

Actin:
In vivo half-lives of actin filaments ~30 sec - 10 minutes, even within structures that persist for days (stress fibers)
Both jasplakinolide (actin filament stabilizer) and latrunculin (destabilizer) prevent cytokinesis and cell crawling

Intermediate filaments:
In vivo half-lives are a few minutes; turnover mechanism poorly understood
Mutations affecting filament assembly/disassembly are associated with structural damage (neuropathies, blistering diseases)

Rapid polymerization and depolymerization in vivo is characteristic of cytoskeletal polymers and important for function - also true for prokaryotic examples

GFP-FtsZ

Solid line - wild-type
Dashed line - mutant with slower GTPase activity slows slower turnover kinetics in vivo

Stricker et al., 2002
Coupling of polymerization to nucleotide hydrolysis can enable treadmilling and dynamic instability

Two distinct end dynamics in a constant biochemical environment

Treadmilling: two ends of the same filament behave differently (actin)

Dynamic instability: same structural end partitions between two states stochastically over time (microtubules)

Microtubule end structural change associated with nucleotide hydrolysis and catastrophe

Figure 10-11 part 1 of S. Molecular Biology of the Cell, 4th Edition.
Polymerization and depolymerization can generate force

Examples of kinetic models:

Hill and Kirschner, 1982
Forces predicted to be a few picoNewtons

Examples of kinetic models:

Peskin et al., 1993
Mogilner and Oster, 1996

Direct measurement of force generated by microtubule polymerization

Dogterom and Yurke, 1995
Nucleation is often the rate-limiting step in filament assembly

Actin nucleus ~3 subunits
Microtubule nucleus ~13 subunits
FtsZ: nucleus ~2 subunits

Oosawa and Asakura, 1974
Chen et al., 2005

Stabilized or crosslinked filaments are excellent nuclei

Compare kinetics of prions and “Ice-9”

Duplicate and specialize: The gamma tubulin ring complex (γ-TuRC) and the Arp2/3 complex are major nucleators for their cognate filaments

Note: bacteria could certainly do this if they so chose
Arp2/3 complex causes dendritic branching of actin filaments as well as nucleation

How do branches form? Are the ARPs behaving like actin?

Mullins et al., 1997
Crystal structure of inactive form shows Arp2 and Arp3 pointing apart

Robinson et al., 2001

3-D reconstruction of a branch

Volkmann et al., 2001

Conformational change affected by nucleotide, activating factors

Goley et al., 2004
Formins as actin nucleators

Large family (15 in mice), act as dimers
Involved in forming actin ring in cytokinesis, stress fibers, yeast actin cables
Forms bundles instead of dendritic branches
Remains associated with filament ends
Some family members can actually INCREASE the filament elongation rate
Some family members require profilin-bound actin

“End surfing” in live cells observed by Higashida et al., 2004

Kovar and Pollard, 2004

Centrosomes have many imbedded γ-TuRCs and nucleate dynamic microtubules from the minus end

The combination of localized nucleation and dynamic instability of polymers is a condition sufficient for a simple large-scale cellular organizing principle:
Self-centering to set up a global polar coordinate system
Centrosome plus dynamic microtubules can lead to a self-centering activity

Microfabricated square chamber
One centrosome plus tubulin added

Return to center after displacement by trap

Microtubule-wall-nudging self-centering activity in *Schizosaccharomyces pombe* keeps the nucleus in the middle

Yeast spindle pole bodies (centrosomes) are imbedded in the nuclear membrane
There’s more than one way to be self-centered

NO centrosome
Microtubules plus motors can still make asters and swirls

Nedelec et al., 1997

Self-centering activity of melanophore fragments requires dynamic microtubules and dynein

Cycles of dispersion and aggregation (by cAMP/adrenaline) do not require the centrosome but are inhibited by taxol and vanadate

Rodionov and Borisy, 1997
Melanophores from black tetras
Self-centering activity of melanophore fragments requires dynamic microtubules and dynein

Self-centering follows aggregation  Toroidal fragment

Rodionov and Borisy, 1997

Requires dynein-dependent nucleating activity (!)

Live cell imaging shows microtubule “fishtailing” but no actual transport

Nucleation by granules after cold shock

Vorobjev et al., 2001
Functions of self-centering:
Global coordinate system for positioning internal vs. external subcellular domains
(e.g. nucleus and Golgi near the middle, ER dispersed toward the periphery)
Basis for exploration and stabilization
Polarized cell growth can be a consequence of microtubule array polarity
Useful for division into two equal daughter cells
Note biological self-centering so far is dependent on nucleation, not on motor protein activity

Microtubule plus-end tracking proteins (+TIPS) associate only with growing ends
Directional delivery without motor proteins
EB1-GFP in a goldfish fibroblast
Vic Small
What about self-centering in bacteria? Dynamics of cell division

FtsZ is dynamic and can mark the equator: Finds the prospective division plane prior to completion of chromosome segregation

Sun and Margolin, 1998

How does the Z ring know where to assemble?

Oscillating Min - inhibitor of FtsZ assembly

MinC destabilizes FtsZ filaments, carried by MinD
MinD oscillates
MinE disassembles MinD, travels in waves

FtsZ assembly/nucleation is SUPPRESSED throughout the rest of the cell...nucleation is the default state
Contrast this to microtubules

Raskin and de Boer, 1999
Shih et al., 2003

Hale et al., 2001
**TUBULIN vs. FtsZ**

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<thead>
<tr>
<th></th>
<th>TUBULIN</th>
<th>FtsZ</th>
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<tbody>
<tr>
<td>Heterodimer</td>
<td>Monomer</td>
<td>Nucleation easy</td>
</tr>
<tr>
<td>Nucleation hard</td>
<td>13 protofilaments</td>
<td>None yet observed</td>
</tr>
<tr>
<td>Dynamic instability</td>
<td>Nucleotide trapped</td>
<td>Nucleotide exchangeable (??)</td>
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<tr>
<td></td>
<td></td>
<td>(If true, how does filament destabilization work?)</td>
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<tr>
<td></td>
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<td>Fusion of two distinct domains?</td>
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<td></td>
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<td>(Will reassociate in the presence of GTP)</td>
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Oliva et al., 2004

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**Modulating dynamics for function: ParM (actin homolog)**

Required for R1 plasmid segregation
Forms an inside-out “spindle” in bacteria
Similar growth kinetics at both ends
Performs dynamic instability!
Stabilized by binding ParR, which binds DNA

Moller-Jensen et al., 2002

Garner et al., 2004
Dynamics of protein self-assembly

Tweaking quantitative parameters gives very different large-scale behavior

Homologs may have different assembly properties and various kinds of coupling between nucleotide hydrolysis and assembly

First-line regulation of cellular organization:

Eukaryotes: Localization of nucleation sites
Prokaryotes: Selective stabilization or destabilization of spontaneously nucleated filaments

De novo micron-scale order from nanometer-scale components
References for January 12:

General: Alberts et al., 4rd edition, Chapter 16
        Current Opinion in Cell Biology, Cytoskeleton Issue
        (usually issue #1, February)

* = posted on course web site as recommended reading

Protein polymerization and polymer dynamics

Force generated by growing polymers

Nucleation by Arp2/3, formins and γ-TuRC


Goley ED, Rodenbusch SE, Martin AC, Welch MD. Critical conformational changes in the Arp2/3 complex are induced by nucleotide and nucleation promoting factor. Mol Cell. 2004 Oct 22;16(2):269-79.


Self-centering


The bacterial cytoskeleton


