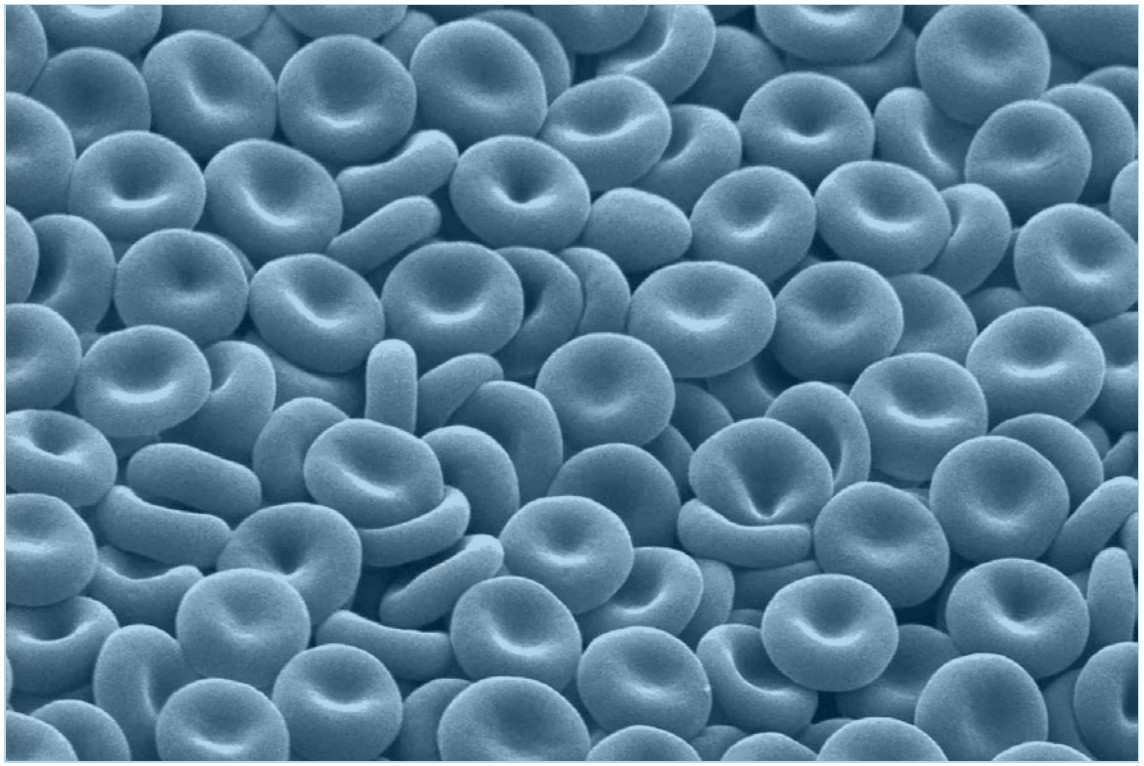
Cellular Growth and Form



DAVID MCCARTHY / Getty Images

Course 2: What sets cell volume?

Thomas Lecuit

chaire: Dynamiques du vivant

COLLÈGE DE FRANCE 1530

Growth of living and non living matter

« Pour tout corps inorganique, l'augmentation de volume et de masse est toujours accidentelle et sans bornes, et cette augmentation ne s'exécute que par *juxtaposition*, c'est-à-dire que par l'addition de nouvelles parties à la surface extérieure du corps dont il est question.
L'accroissement, au contraire, de tout corps vivant est toujours nécessaire et borné, et il ne s'exécute que par *intussusception*, c'est-à-dire que par pénétration intérieure, ou l'introduction dans l'individu de matières qui, après leur assimilation, doivent y être ajoutées et en faire partie. Or, cet accroissement est un véritable développement de parties du dedans au dehors, ce qui est exclusivement propre aux corps vivants ».

Lamarck, *Philosophie zoologique*, Part II, chap. I. (1809)

"For any inorganic body, the increase in volume and mass is always accidental and unbounded, and this increase is carried out only by juxtaposition, that is to say, by the addition of new parts to the outer surface of the body in question.
The increase, on the contrary, of any living body is always necessary and boundless, and it is only carried out by intussusception, that is to say, by the interior penetration, or the introduction into the individual of materials which, after their assimilation, must be added to it and become part of it. Now, this increase is a true development of parts from the inside to the outside, which is exclusively proper to living bodies".

Organism and Cell Growth

- Cell growth drives tissue and organism growth

- Discrete versus coarse-grained/continuous description of growth:
  Cell growth versus tissue growth
  Does multicellularity imply specific principles of growth of living matter in a tissue?
  Size measurement?

Cell size – volume

Statement of the problem:

- Cell size varies between cell types in Animals but within a given cell type there is very little size variation (ex. epithelial cell). So cells control their volume tightly.
  —Why is cell size regulated?

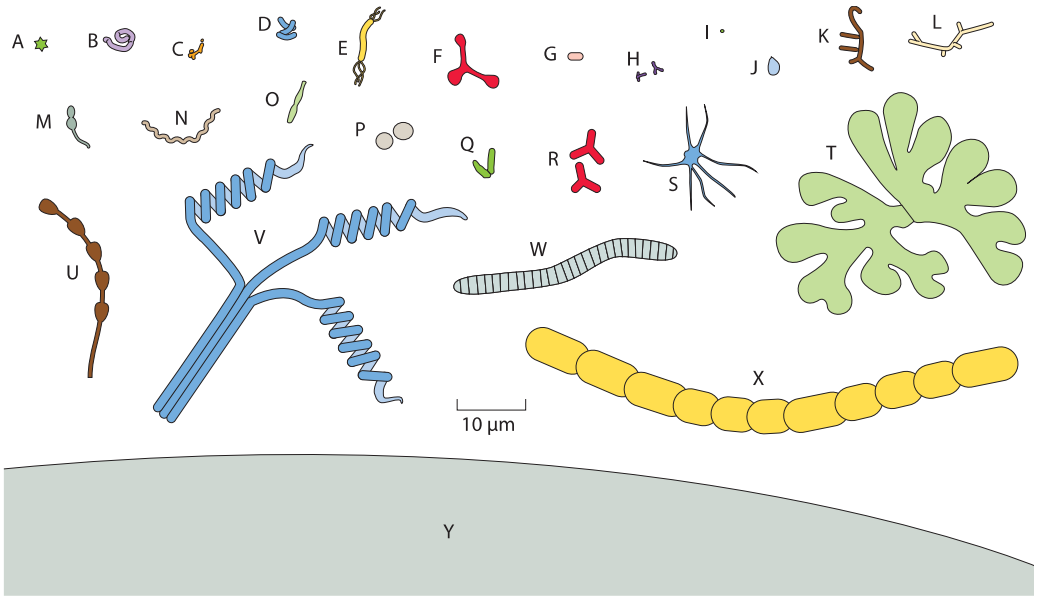
- Cell size is tightly coupled to cell function, for example, neuron, oocyte, red blood cells, Ciliates etc.

- Cell size is physically constrained, e.g.:
  —diffusion of metabolites limits cell size.
  —diffusion of signalling molecules limits communication within cells (unless other transport mechanisms operate such as motor driven or by trigger waves)
  —surface to volume ratio for exchange with environment
  —energetically: synthesis of ribosomes and translational capacity limits cell growth. Given maximal rate of rRNA transcription, there is a limit to cell growth, unless polyploidy or multinucleation (ex. muscle cells, ciliates etc).

- Cell size is governed by protein synthesis, osmotic flow and cell cycle which operate at different time scales: how is this integrated?

Cell size varies greatly between single cell organisms

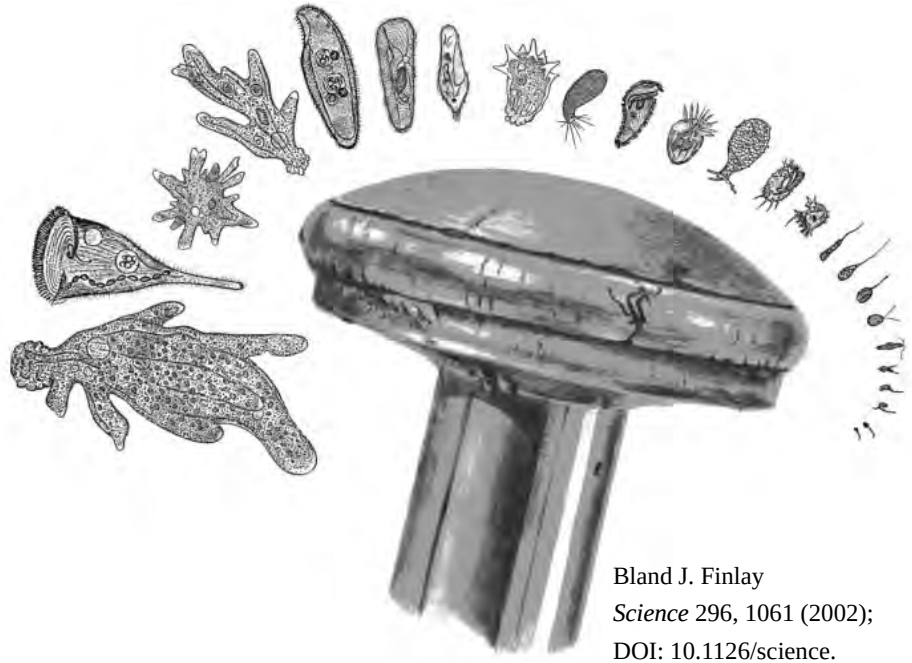
- Bacteria



Cell Biology by the numbers. Ron Milo, Rob Phillips, *illustrated* by Nigel Orme. *Garland Science* 2012

(A) *Stella strain* IFAM1312 (380); (B) *Microcyclus* (a genus since renamed *Ancylobacter*) *flavus* (367); (C) *Bifidobacterium bifidum*; (D) *Clostridium cocleatum*; (E) *Aquaspirillum autotrophicum*; (F) *Pyroditium abyssi* (380); (G) *Escherichia coli*; (H) *Bifidobacterium* sp.; (I) transverse section of ratoon stunt-associated bacterium; (J) *Planctomyces* sp. (133); (K) *Nocardia opaca*; (L) Chain of ratoon stunt-associated bacteria; (M) *Caulobacter* sp. (380); (N) *Spirochaeta halophila*; (O) *Prosthecobacter fusiformis*; (P) *Methanogenium cariaci*; (Q) *Arthrobacter globiformis* growth cycle; (R) gram-negative *Alphaproteobacteria* from marine sponges (240); (S) *Ancalomicrobium* sp. (380); (T) *Nevskia ramosa* (133); (U) *Rhodomicrobium vanniellii*; (V) *Streptomyces* sp.; (W) *Caryophanon latum*; (X) *Calothrix* sp. (Y) A schematic of part of the giant bacterium *Thiomargarita namibiensis* (290). All images are drawn to the same scale. (Adapted from K. D. Young, Microbiology & Molecular Bio. Rev., 70:660, 2006.)

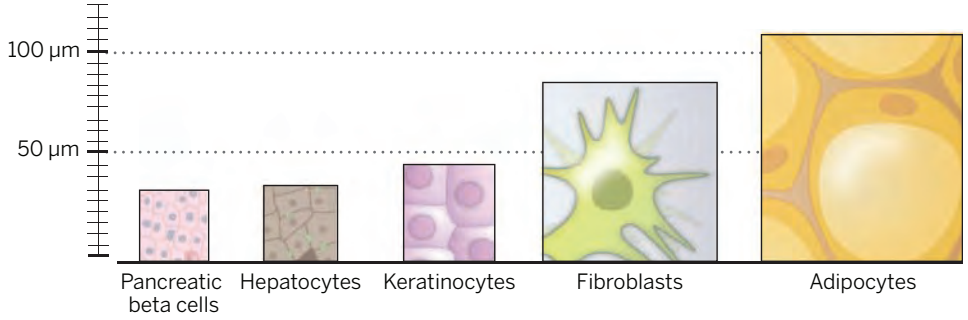
- Protozoans



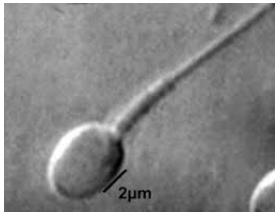
Bland J. Finlay
Science 296, 1061 (2002);
DOI: 10.1126/science.
1070710

0.2 - 2mm in length

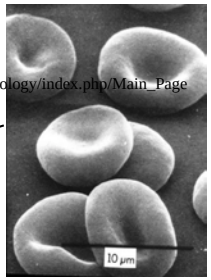
Cell size varies greatly between cell types in Metazoa



M Ginzberg R Kafri and M. Kirschner . *Science* 348, 1245075 (2015). DOI: 10.1126/science.1245075

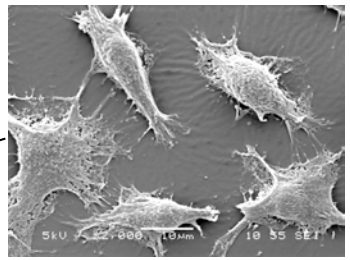


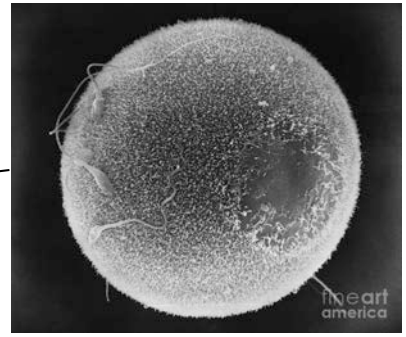
https://embryology.med.unsw.edu.au/embryology/index.php/Main_Page



https://www.quora.com/

cell type	average volume (µm^3)	BNID
sperm cell	30	109891, 109892
red blood cell	100	107600
lymphocyte	130	111439
neutrophil	300	108241
beta cell	1,000	109227
enterocyte	1,400	111216
fibroblast	2,000	108244
HeLa, cervix	3,000	103725, 105879
hair cell (ear)	4,000	108242
osteoblast	4,000	108088
alveolar macrophage	5,000	103566
cardiomyocyte	15,000	108243
megakaryocyte	30,000	110129
fat cell	600,000	107668
oocyte	4,000,000	101664

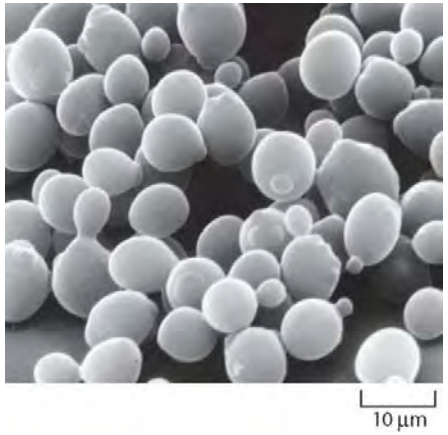


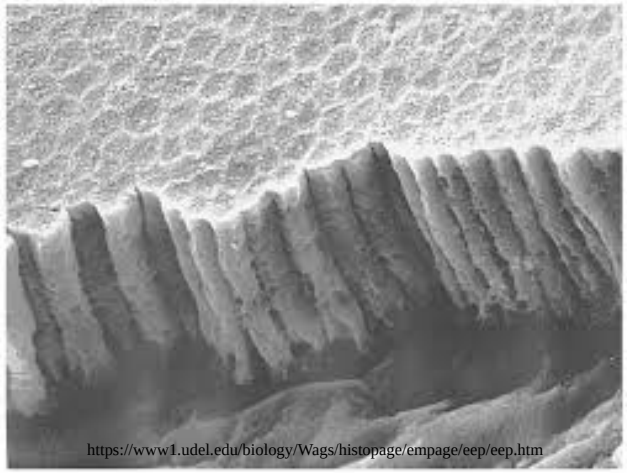


Cell Biology by the numbers. Ron Milo, Rob Phillips, *illustrated* by Nigel Orme. *Garland Science* 2012

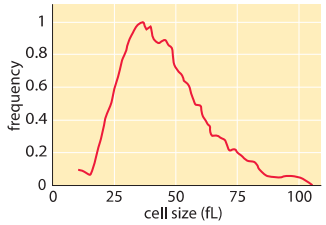
Cell size is constrained within a cell type

- Cell size shows little variation in cells placed in the same nutritional environment
- So there are mechanisms to regulate cell volume in normal physiological conditions

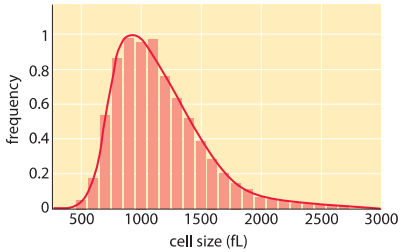




- The observed variation is mostly within the range of what is expected given cell growth during the cell cycle (2 fold change)



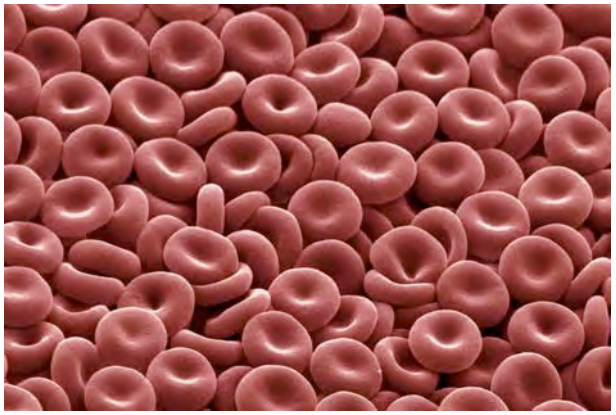
Yeast *S. cerevisiae*

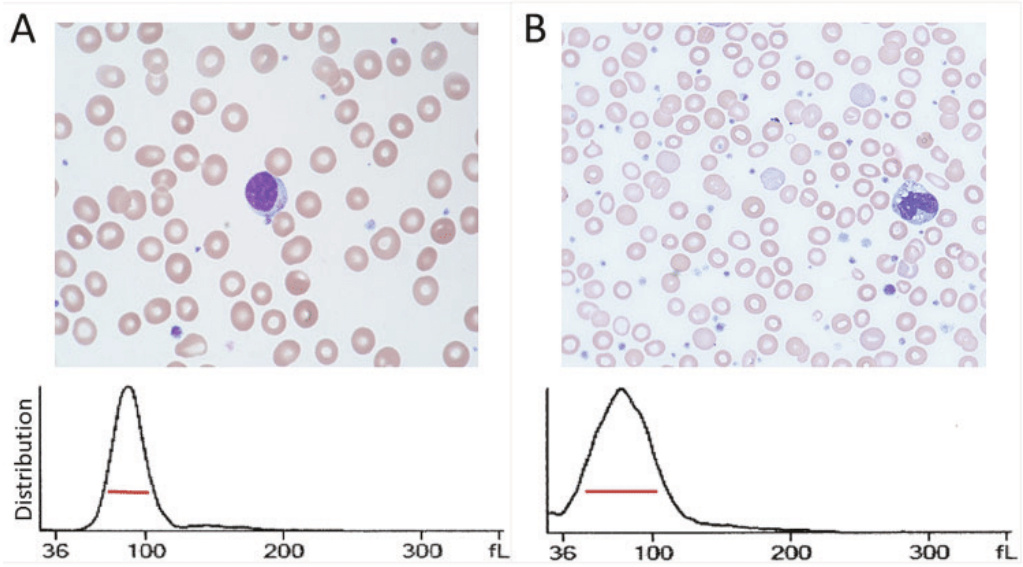


L1210, a mouse lymphoblast cell line

Variation in cell size is often a disease state

- Anisocytosis: iron deficiency anemia causes cell volume variation

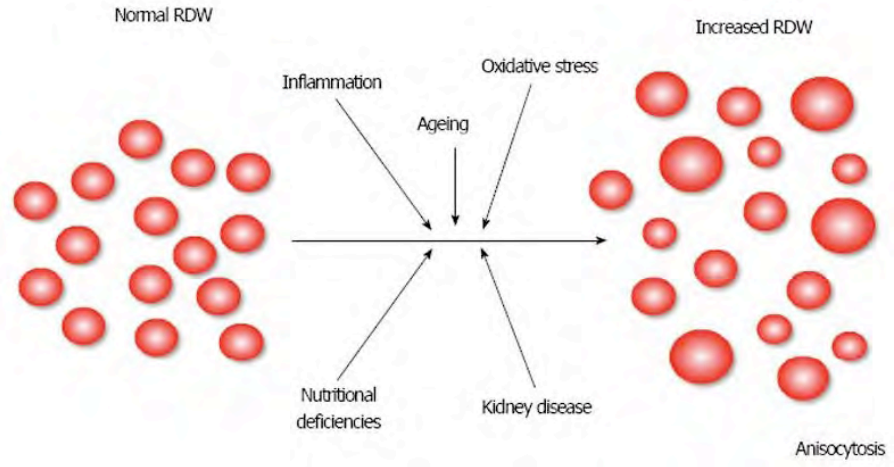




MCV: mean corpuscular volume (fL)

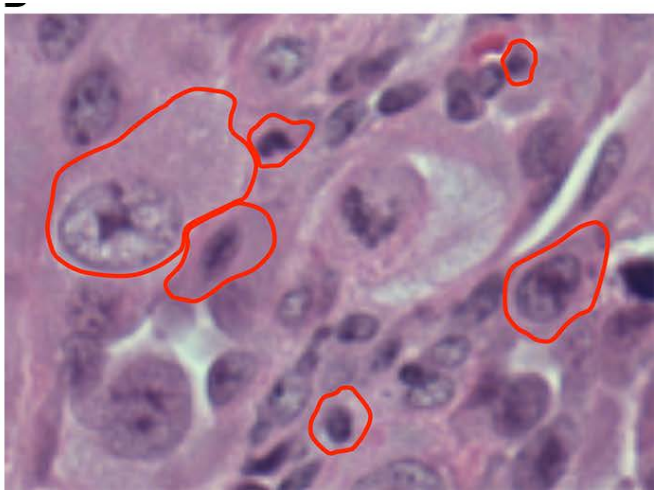
- Normal with of red blood cell: 6-8 µm
Volume: around 90fL

- RDW (red cell distribution width): measure of variability of volume of red blood cells (std / mean x100)
RDW normally: 11.5-14.5%



World J Cardiol 2018; 10(2): 6-14

Variation in cell size is often a disease state



Pleomorphic mammary tumor
Xenograft of transformed mammary epithelial cells

M Ginzberg R Kafri and M. Kirschner . *Science* 348, 1245075 (2015). DOI: 10.1126/science.1245075

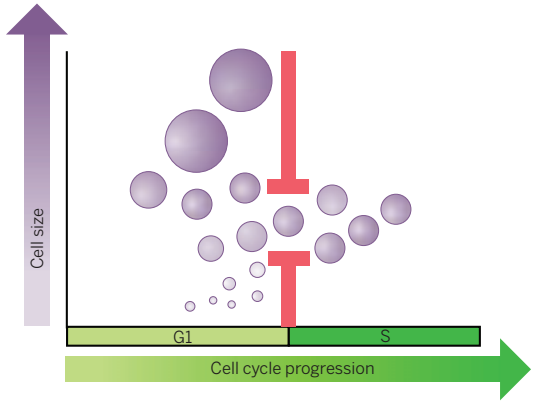
Cell Volume/Mass regulation

— Facts:

- Cell volume varies greatly *between* cell types
- Cell volume is constrained *within* a cell type
- So cell volume is regulated

— General mechanisms:

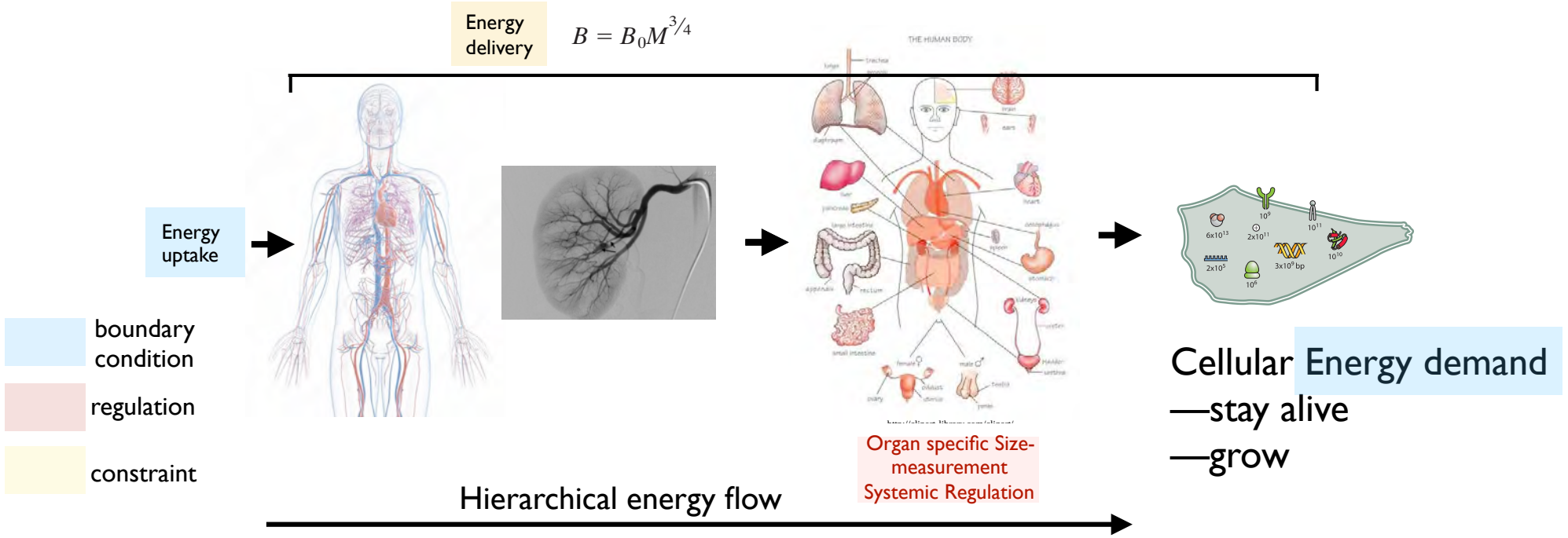
- Genetic encoding: cell type specificity
- Environment: nutrient, cytokines
- Homeostasis of cell volume on short time scales: eg. following internal or external perturbation /stress (Course 2)
- Coupling with cell division: cells grow in interphase and cells divide once cells reach a target size (Course 3)



Cell growth in the context of organism size regulation

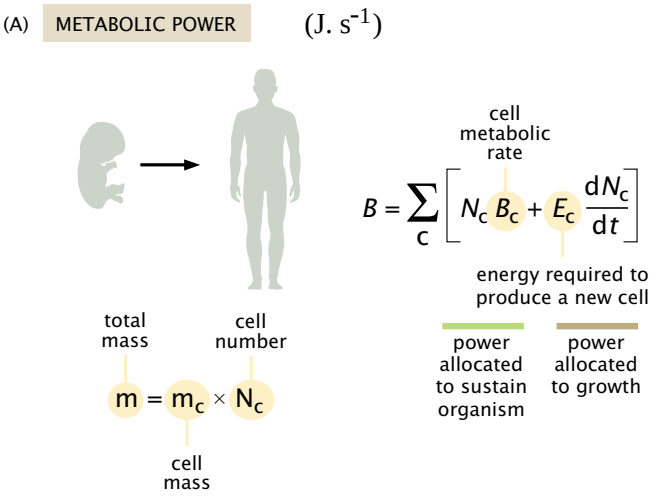
- Cell growth is dominant over cell division: when cell division is blocked, cells continue to grow and are larger (but organ size is unchanged)
- When the organ target size is reached, cells generally continue to grow and divide (i.e. gut, and stem cell derived organ). Cell growth is a sustained process

- Cellular energy demand to be alive (i.e. active state) and grow is continued in multicellular organisms



Cellular metabolic power and organism growth

- Cellular basal metabolic rate and energy required to grow a new cell underlie organism growth



(B)

GROWTH EQUATION

$$\frac{dm}{dt} = \left(\frac{m_c}{E_c}\right) B - \left(\frac{B_c}{E_c}\right) m$$

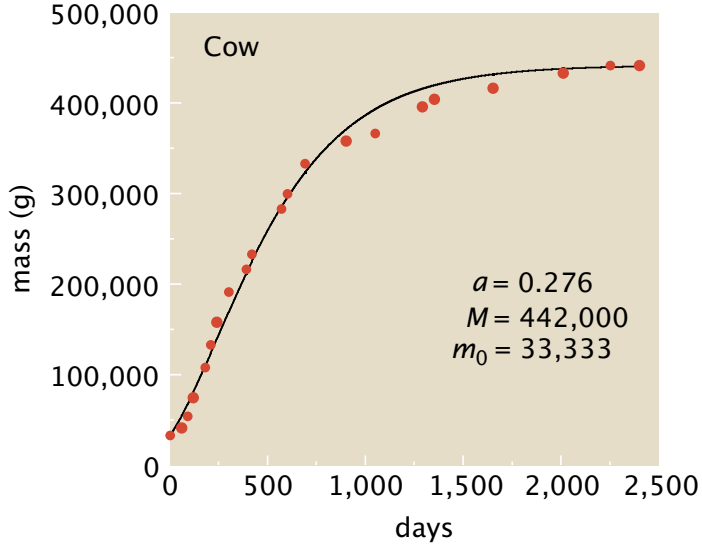
KLEIBER LAW

$$B = B_0 m^{3/4}$$

$$\frac{dm}{dt} = \underbrace{am^{3/4}}_{\propto \text{ energy supply}} - \underbrace{bm}_{\propto \text{ energy demand}}$$

$$a \equiv B_0 m_c / E_c$$
$$b \equiv B_c / E_c$$

asymptomatic mass:
$M = (a/b)^4 = (B_0 m_c / B_c)^4$

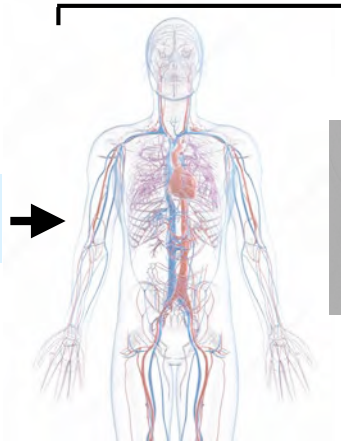


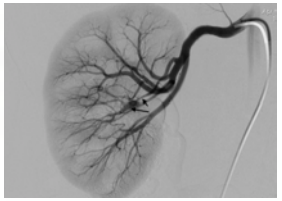
G. West, J. Brown and B. Endquist. NATURE | VOL 413 | 11 OCTOBER 2001

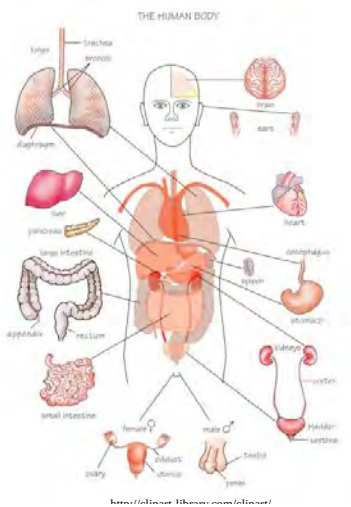
Cellular metabolic power/rate in number

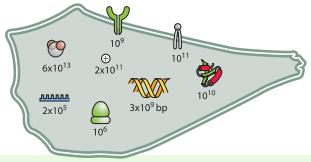
Energy delivery $B = B_0 M^{3/4}$

Energy uptake







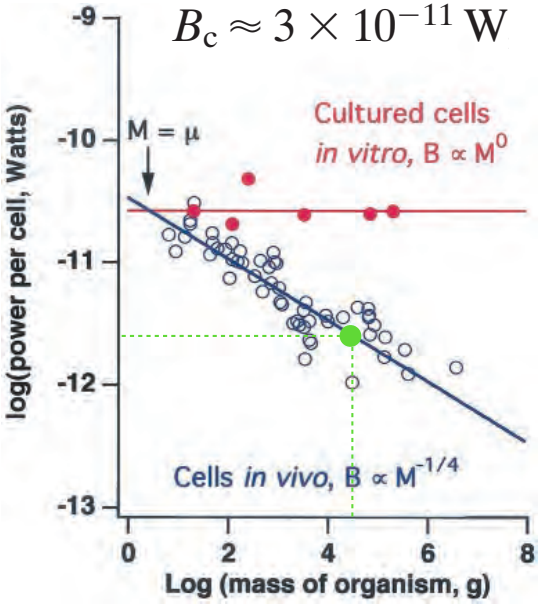


3. 10^7 ATP/cell/s $\sim 3.10^{-12}$ W
1 ATP hydrolysis ~20 $k_B T$

80 Moles ATP/human/day
(1000kcal/day)

Hierarchical energy flow

$B_c \approx 3 \times 10^{-11}$ W



West, G. B., Woodruff, W. H. and Brown, J. H. (2002). PNAS 99, 2473-2478.

Cellular metabolism and cell growth

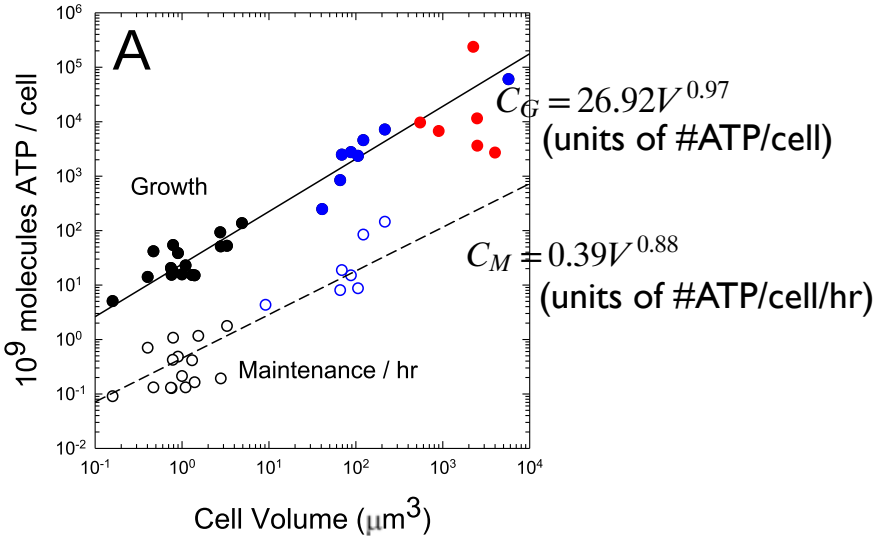
- Basal cellular metabolic rate and energy required for growth increase linearly with cell volume.
- Energetic cost of cell growth is largely dominant.

Total energy $C_T \simeq C_G + tC_M$

power of maintenance

energy to produce new cell

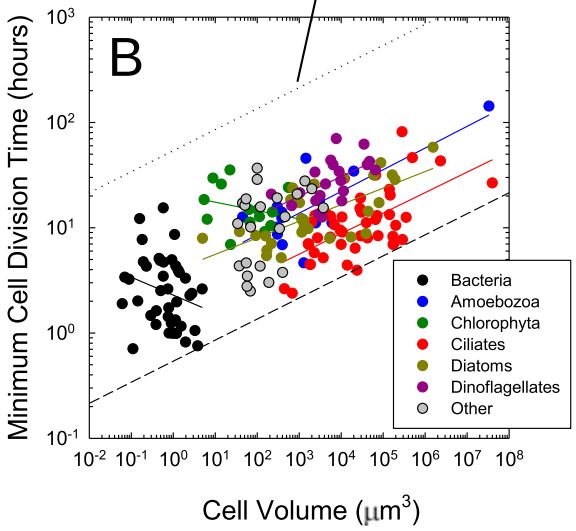
division time



$C_G = 26.92V^{0.97}$ (units of #ATP/cell)

$C_M = 0.39V^{0.88}$ (units of #ATP/cell/hr)

cell division time where $C_G = 0.5\ C_T$



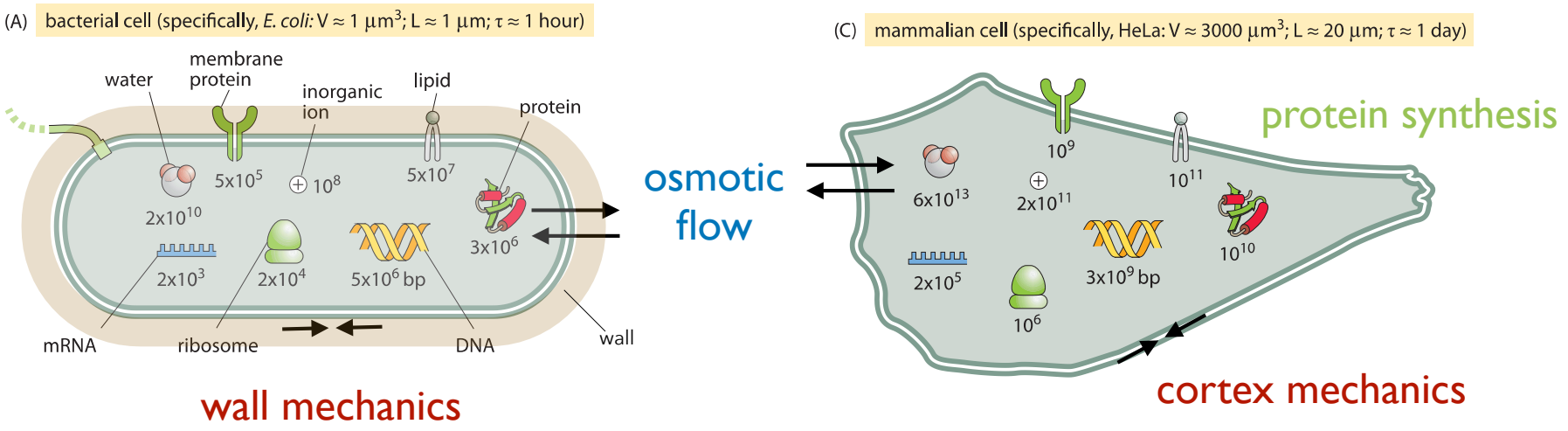
In *E. coli* (1 μm^3): 10^{10} ATP to grow and maintain cell per cell cycle
3. 10^9 glucose molecules are needed

M Lynch and G. Marinov (2015) *PNAS* 112: 15690–15695
www.pnas.org/cgi/doi/10.1073/pnas.1514974112

What sets cell size and cell growth?

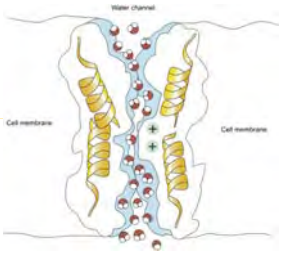
1. Short time scale: osmotic flow and cell mechanics
2. Long term regulation by protein synthesis
3. Coupling time scales

Plants, Fungi, Bacteria: Wall (surface) growth and Volume growth dictate total volume increase

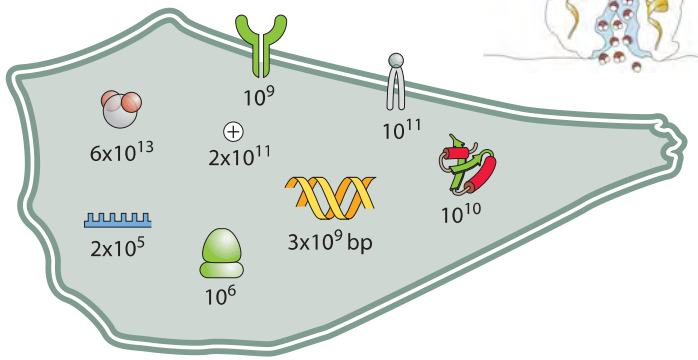


1. Short time scale: osmotic flow

- Cell contains 70% water and 30% dry mass (proteins, nucleic acids, lipids, metabolites, ions)
- So cell volume is chiefly contributed by water.



- Water flow through the membrane is mediated by water channels called Aquaporins
- Water flow is driven by 2 forces:
  - — hydrostatic pressure p
  - — osmotic pressure Π



- Water flow through a semi-permeable membrane (only water)

flow — force

$$J_w = L_p(\Delta p - \Delta \Pi)$$

L_p: filtration coefficient

van't Hoff relation:
Ideal gas law for osmotic pressure (dilute solution)

$$\Pi_i = k_B T.\, N_i/V_{cell}$$
$$\Pi_e = k_B T.\, C_e$$

ion concentrations (unit mM)

ion conc. (mM)	sea water	*E. coli*	*S. cerevisiae*	mammalian cell (heart or RBC)	blood plasma	BNID
K^+	≈10	30-300	300	100	4	104049
Na^+	≈500	10	30	10	100-200	104050
Mg^{2+}	≈50	30-100 (bound); 0.01-1 (free)	50	10 (bound) 0.5 (free)	1	104983, 100770, 101953
Ca^{2+}	≈10	3 (bound);100 nM (free)	2 (bound)	10-100 nM (free)	2	100130, 110746, 111366
Cl^-	≈500	10–200 media dependent		5-100	100	105409, 110744
BNID	106594	105926, 107033, 107114, 111425	107752	103966, 107187	105409	

protein concentrations : 200-300 g/l, which is about 10 mM (2-3. 10^4 Da/protein)
(median 200-300 aa/protein and 100Da/aa)

- Osmotic pressure in a cell is a function of the concentration of osmolites in the cytoplasm: namely mostly ions which are the most abundant molecules besides water (about 20 to 30 times the concentration of proteins)

C.Cadart L. Venkova, P. Recho, M. C. Lagomarsino and M. Piel *Nature Physics* 15: 993–1004 (2019)

Phil Nelson. *Biological Physics: Energy, information, life.* W.H.Freeman & Co Ltd (2013)

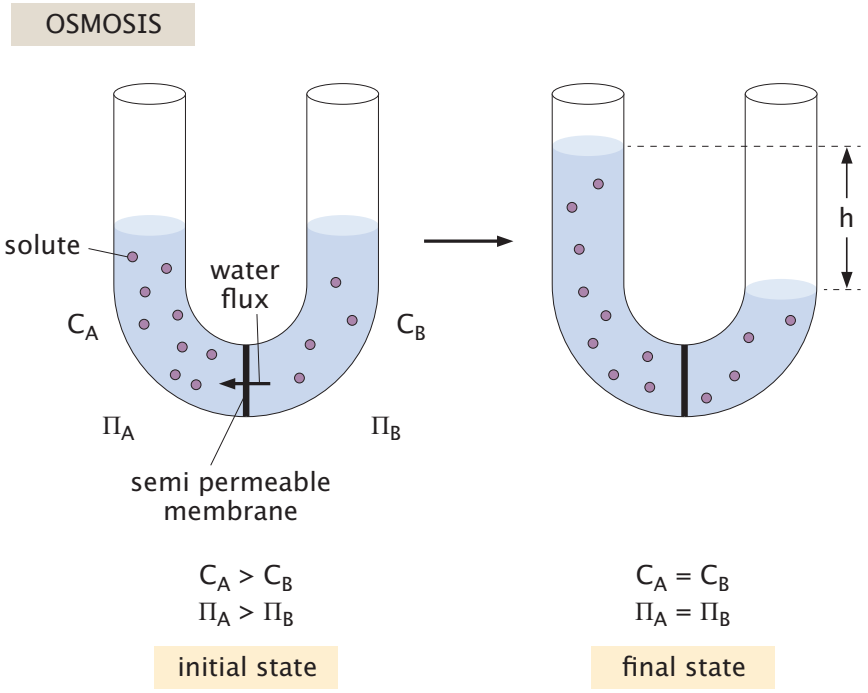
1. Short time scale: osmotic flow

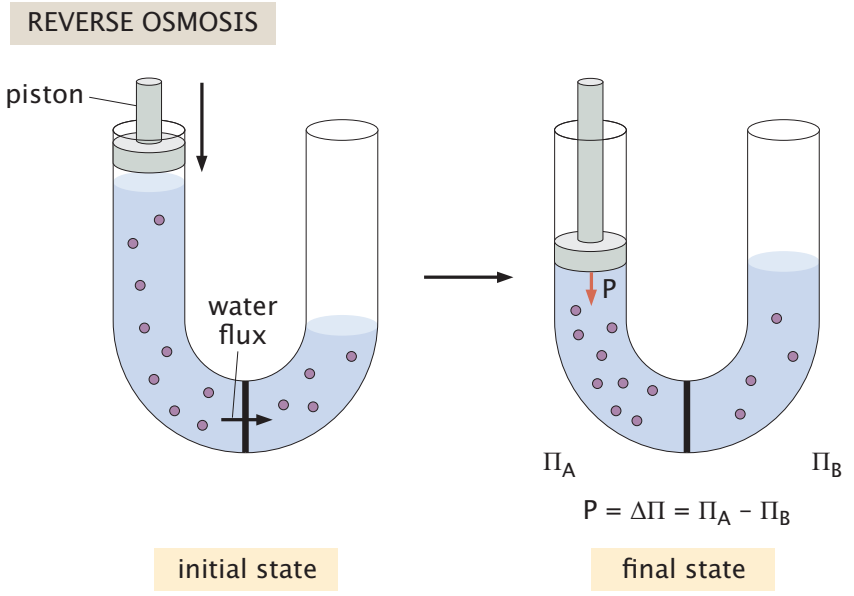
- **Osmotic flow through a semi-permeable membrane:** permeable to water but not to solutes such as ions or other small molecules (e.g. sugar, metabolites or proteins).
- Osmotic pressure can be measured as the pressure needed to oppose water flow through a semi-permeable membrane due to a difference in concentration of osmolites on both sides of the membrane
- At steady state, the applied pressure equals the osmotic pressure difference

- In equation: $\underbrace{J_w}_{\text{flow}} = L_p(\underbrace{\Delta p - \Delta\Pi}_{\text{force}})$

$$\Delta\Pi = k_B\, T.\, (C_A - C_B)$$

At steady state, $J_w = 0$ and $P = \Delta\Pi$





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Phil Nelson. *Biological Physics: Energy, information, life.* W.H.Freeman & Co Ltd (2013)

1. Short time scale: osmotic flow

- Osmotic forces have an entropic origin and rectify the brownian motion of water molecules across a semi-permeable membrane
- The entropic gain of rectifying brownian motion is compensated by the entropic cost of increasing volume due to flow (which costs order)

- A little calculation: $\Pi_i = k_B T.\ N_i/V_{cell}$

  A cell in pure water with 10mM concentration of proteins: $\Pi \approx 10^5$ Pa

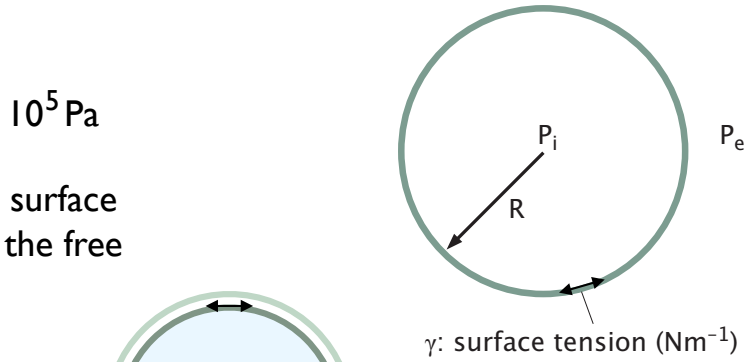
  Osmotic flow expands the cell volume and the cell will expand its surface until the cost of stretching the cell cortex (energy $\gamma.\ dS$) balances the free energy reduction of expanding the volume under pressure (p.dV)
  — this would give γ = 1.5 $N.m^{-1}$
  The membrane-rupture surface tension is: $10^{-2} N.m^{-1}$
  Even worst considering ion concentration which is 20-30 fold higher.

  A cell would lyse in pure water (e.g. red blood cells)

- The extracellular environment is NOT pure water.
  $[Na^+]$= 145mM
  $[K^+]$= 5mM
  $[Cl^-]$= 100mM

Laplace-Young Law

$\Delta P = P_i - P_e = 2\gamma(\frac{1}{R})$



Sens P and Plastino J. *J. Phys.: Condens. Matter.* 27:273103. 2015

Phil Nelson. *Biological Physics: Energy, information, life.* W.H.Freeman & Co Ltd (2013)

1. Short time scale: osmotic flow

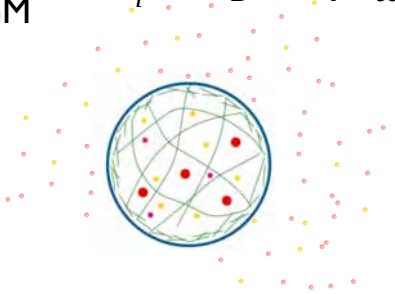
In Animal cells, ion channels balance *actively* ion concentrations across the membrane to minimise osmotic pressure difference between extra and intra-cellular environments

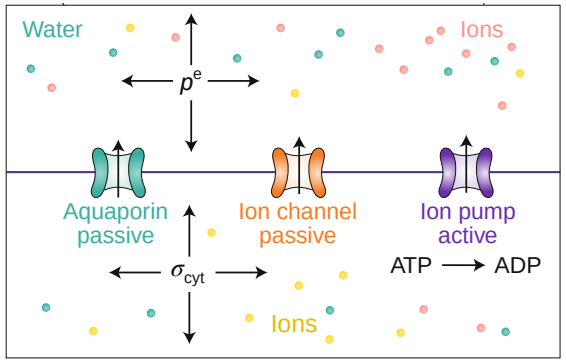
- Ion concentrations outside cells: $[Na^+]$= 145 mM, $[K^+]$= 5 mM, $[Cl^-]$= 100 mM

$$\Pi_e = k_B T.\, C_e$$

- Ion concentrations inside cells: $[Na^+]$= 5-15 mM, $[K^+]$= 140 mM, $[Cl^-]$= 5-15 mM

$$\Pi_i = k_B T.\, N_i/V_{cell}$$



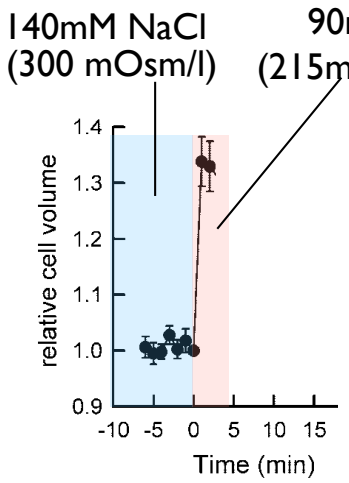


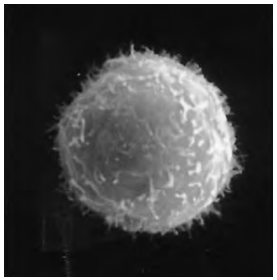
C.Cadart L. Venkova, P. Recho, M. C. Lagomarsino and M. Piel *Nature Physics* 15: 993–1004 (2019)

- As a result, the difference in osmotic pressure and the resulting hydrostatic pressure is minimal at steady state in animal cells and cells do not rupture.

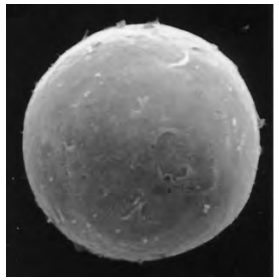
$$J_w = L_p(\Delta p - \Delta \Pi)$$

- An osmotic chock causes a rapid volume change





300 mOsm/l



150 mOsm/l

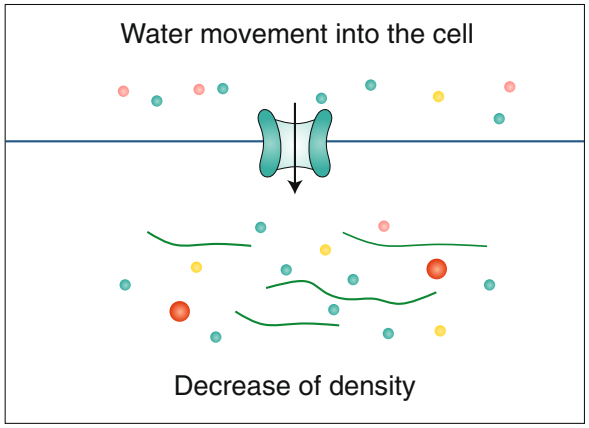
Fernandez-Fernandez et al . *Am J Physiol Cell Physiol* 283: C1705–C1714, 2002

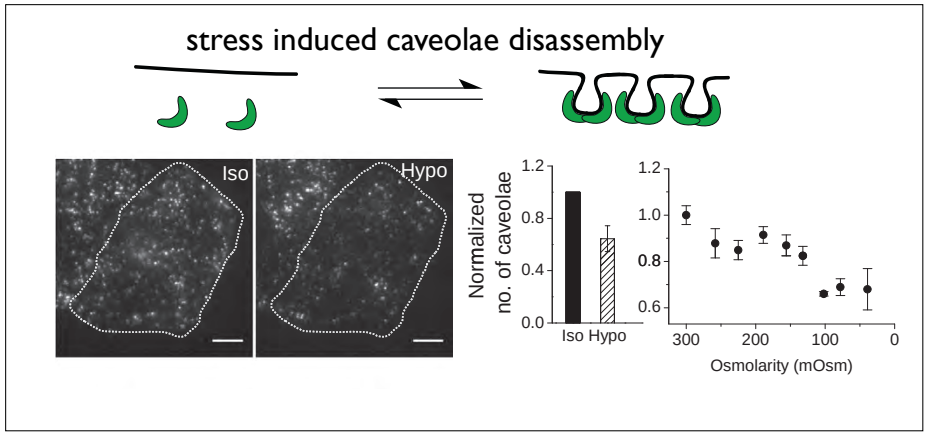
Guilak F. et al *Biophysical Journal* 82(2) 720–727

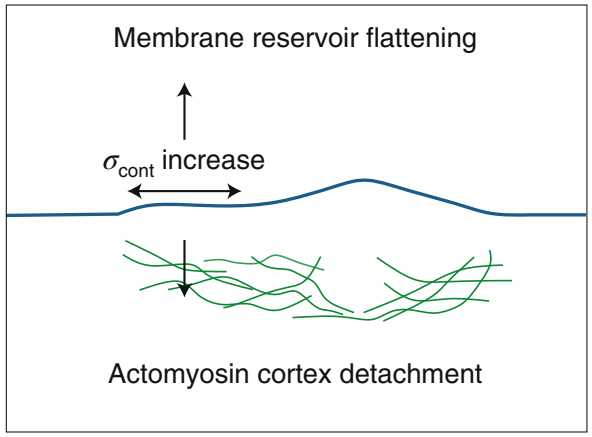
1. Short time scale: osmotic flow

- Osmotic shock: Fast passive volume change via ion transporters and membrane flattening

Guilak F. et al *Biophysical Journal* 82(2) 720–727







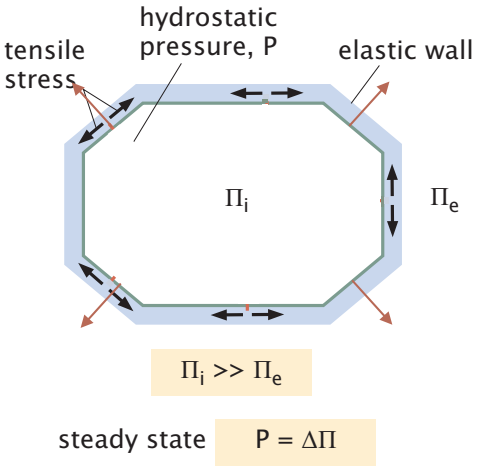
B. Sinha et al. and P. Sens, C. Lamaze and P. Nassoy *Cell* 144, 402–413

C.Cadart L. Venkova, P. Recho, M. C. Lagomarsino and M. Piel *Nature Physics* 15: 993–1004 (2019)

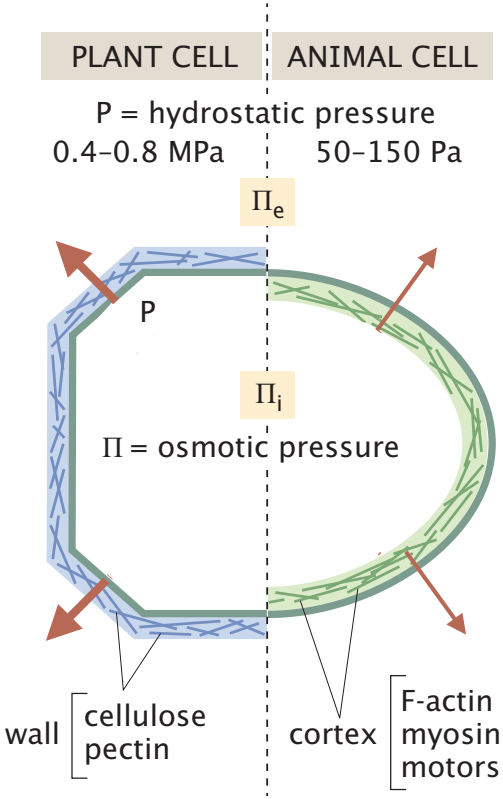
1. Short time scale: osmotic flow

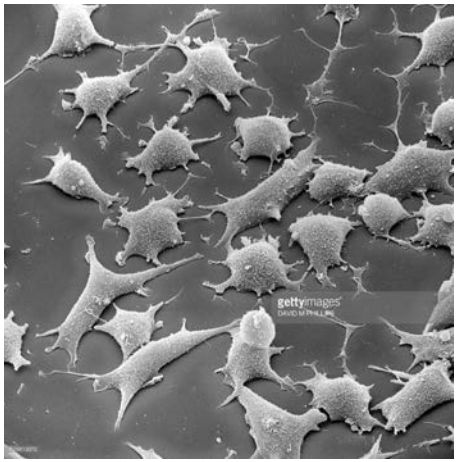
- Comparison of animal and plant cells in numbers:

Hydrostatic (also called Turgor) pressure can go up to several 10s of MPa in fungi and some plants



Π_e is kept to a minimal value in the apoplasm (cell wall) which contains water but a negligible concentration of solutes

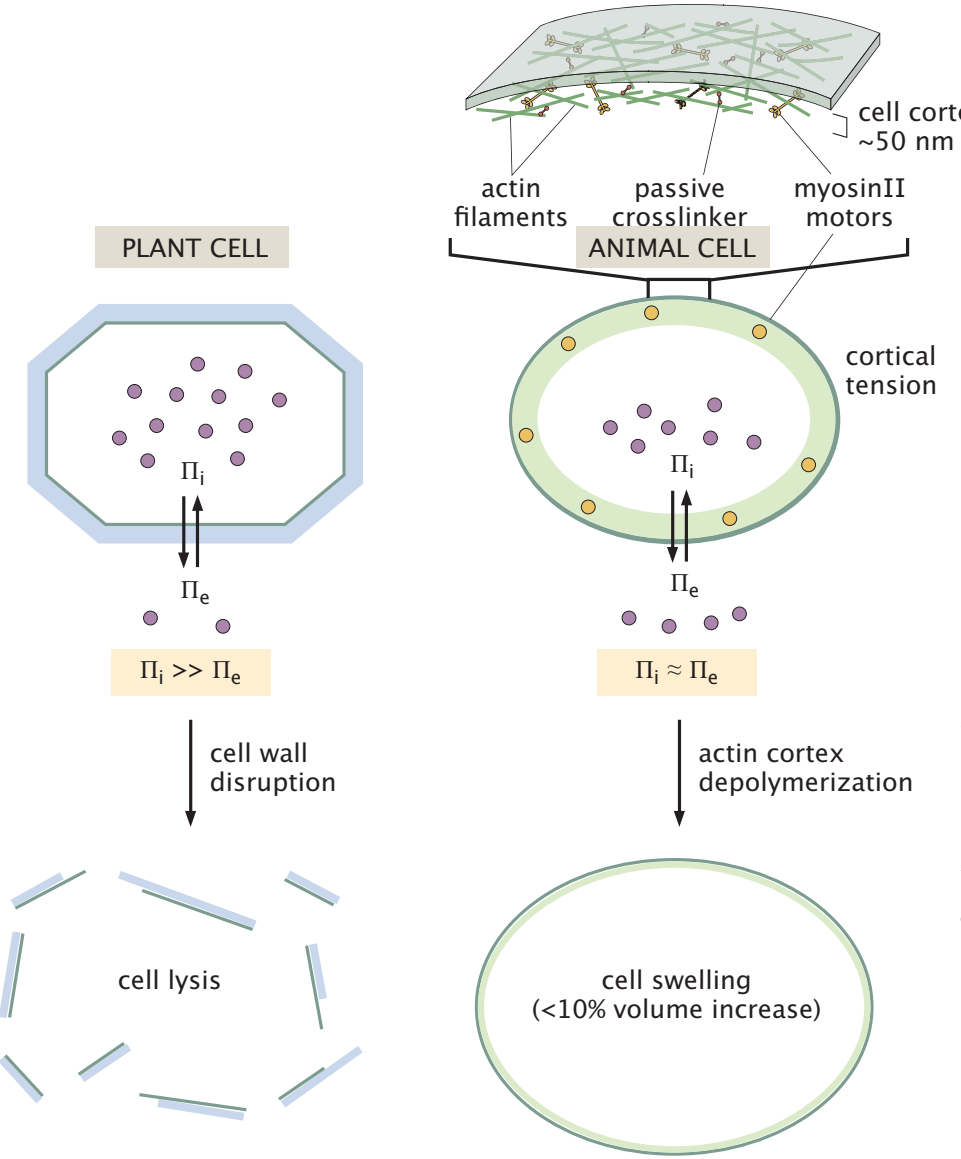




1. Short time scale: osmotic flow and cell mechanics

- Comparison of animal and plant cells: cell wall versus cell cortex mechanics

The integrity of the cell wall is essential to prevent cell lysis in a plant



Cell tension in animals:
$T_{memb} \approx 100pN/\mu m$

$T_{cortex} \approx 400pN/\mu m$

Membrane rupture threshold:
3000-10.000 pN/µm

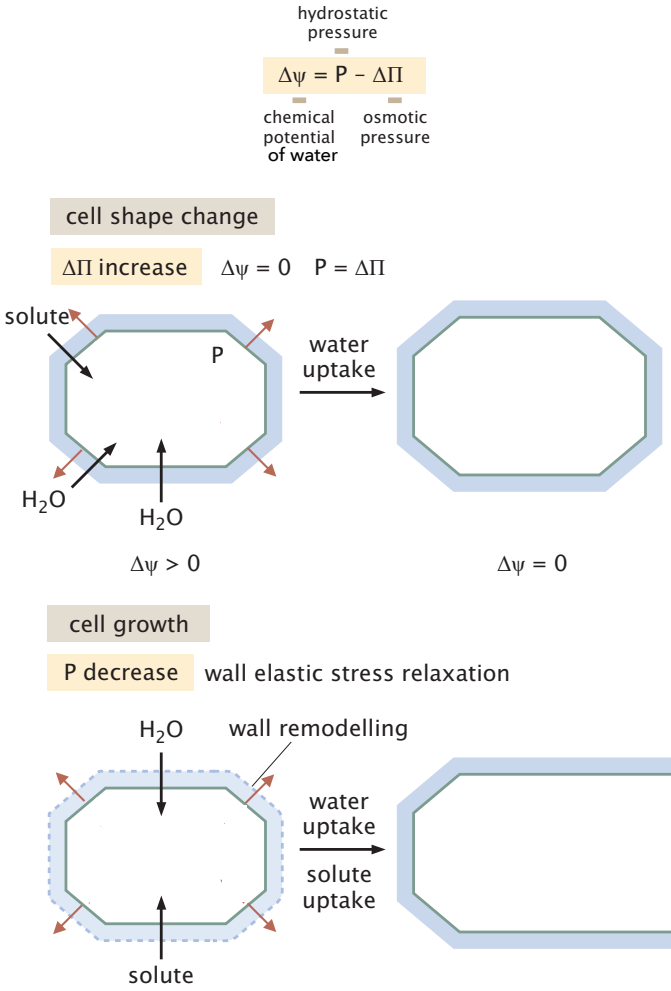
The cell actomyosin cortex cannot regulate the cell volume against a too high osmotic pressure difference.

A low osmotic pressure gradient is maintained in animals cells

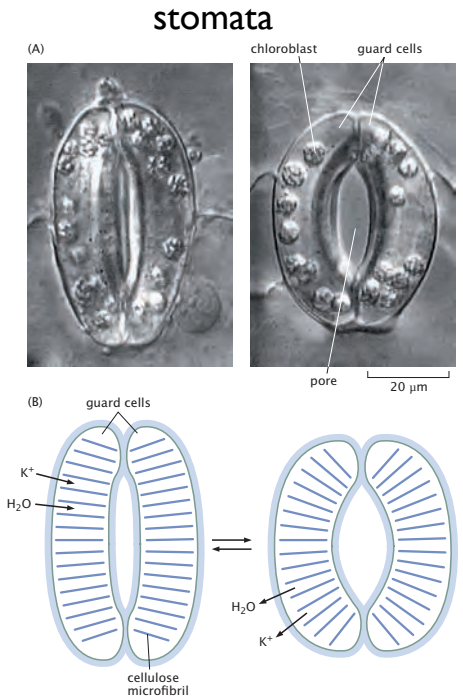
1. Short time scale: osmotic flow and cell mechanics

- The high osmotic pressure gradient in plant cells (and bacteria, fungi) requires a thick, elastic wall and remodelling of the wall content.
- The plant cell is a mechano-hydraulic machine

Reversible volume change induced by rise in ∏i (eg. ion transport, K+)
Water flows until water potential across the wall/membrane is equal



Irreversible volume change induced by lowering of hydrostatic pressure P through relaxation of cell wall under stress.
Water flows until water potential across the wall/membrane is equal (water flow dilutes solutes in cell)

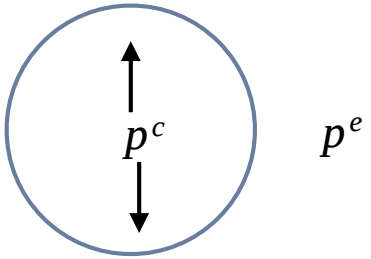


Peter Schopfer. *American Journal of Botany* 93(10): 1415–1425. 2006.

1. Short time scale: osmotic flow and cell mechanics

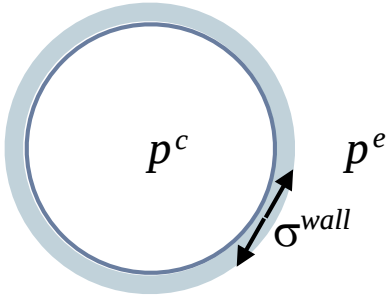
- Coupling osmotic flow and cortex mechanics: hydrostatic pressure links water flow and volume increase to the mechanical properties of cells

$$J_w = L_p(\Delta p - \Delta \Pi) \quad \text{and} \quad \Delta p = p^c - p^e$$
$$p^c - \sigma^{cortex} - \sigma^{mesh} = p^e - \sigma^{conf}$$



$p^c = p^e$

Bacteria
Yeast
single Plant cell

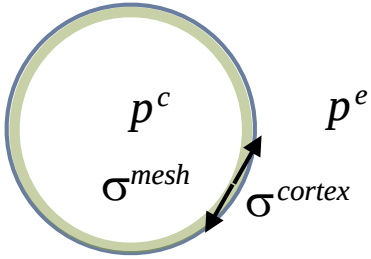


$p^c - \sigma^{wall} = p^e$

stress from cell wall

stress from cell cortex

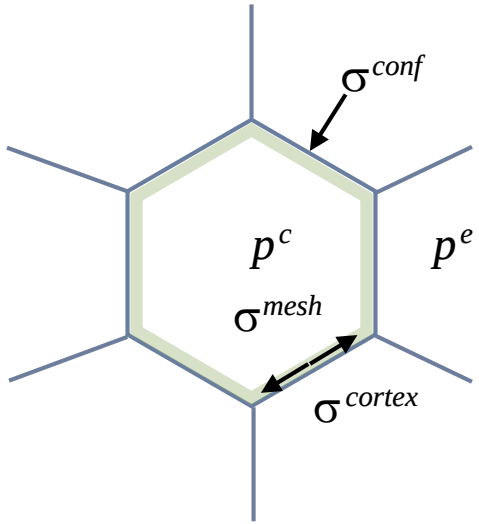
single Animal cell



$p^c - \sigma^{cortex} - \sigma^{mesh} = p^e$

stress from intracellular cytoskeleton

Animal cell in a tissue



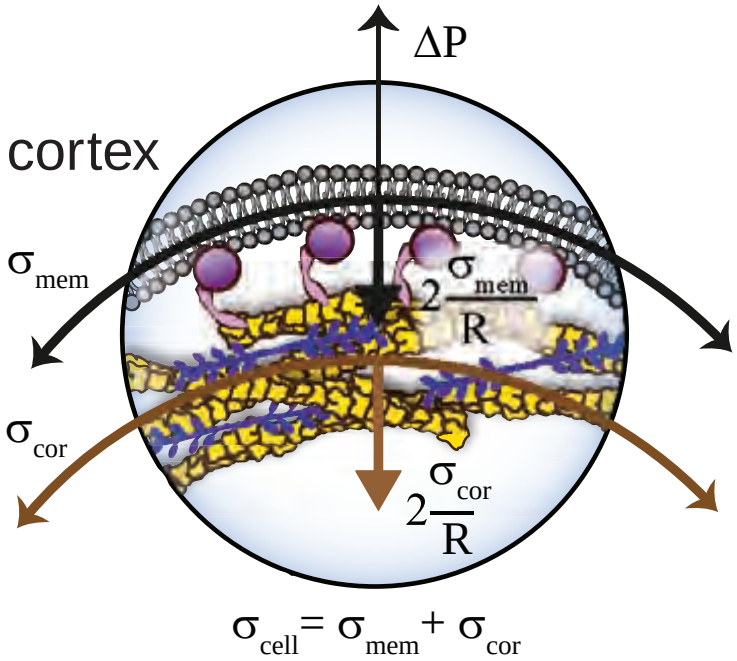
$p^c - \sigma^{cortex} - \sigma^{mesh} = p^e - \sigma^{conf}$

stress from cell surrounding
(neighbours, ECM)

C.Cadart L. Venkova, P. Recho, M. C. Lagomarsino and M. Piel *Nature Physics* 15: 993–1004 (2019)

1. Short time scale: osmotic flow and cell mechanics

- Animal cell: Membrane tension, cortical tension and dynamic coupling between the plasma membrane and the actin cortex



- Membrane is not permeable and bears most of pressure difference

$$\Delta P = \sigma_{\mathrm{mem}} C + f_{\mathrm{link}} \rho_{\mathrm{link}}$$

ρ_{link} : density of linkers

f_{link} : force per membrane/cortex linker

C : curvature

- Linkers are under tension due to cortical tension

$$f_{\mathrm{link}} \rho_{\mathrm{link}} = \sigma_{\mathrm{cor}} C$$

>> Cell tension contributed by both membrane and cortex tension

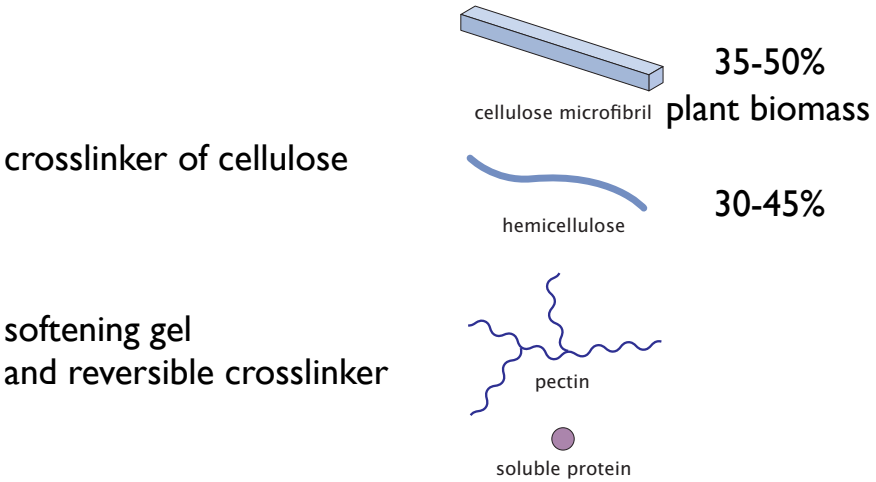
$$\Delta P = (\sigma_{\mathrm{mem}} + \sigma_{\mathrm{cor}}) C$$

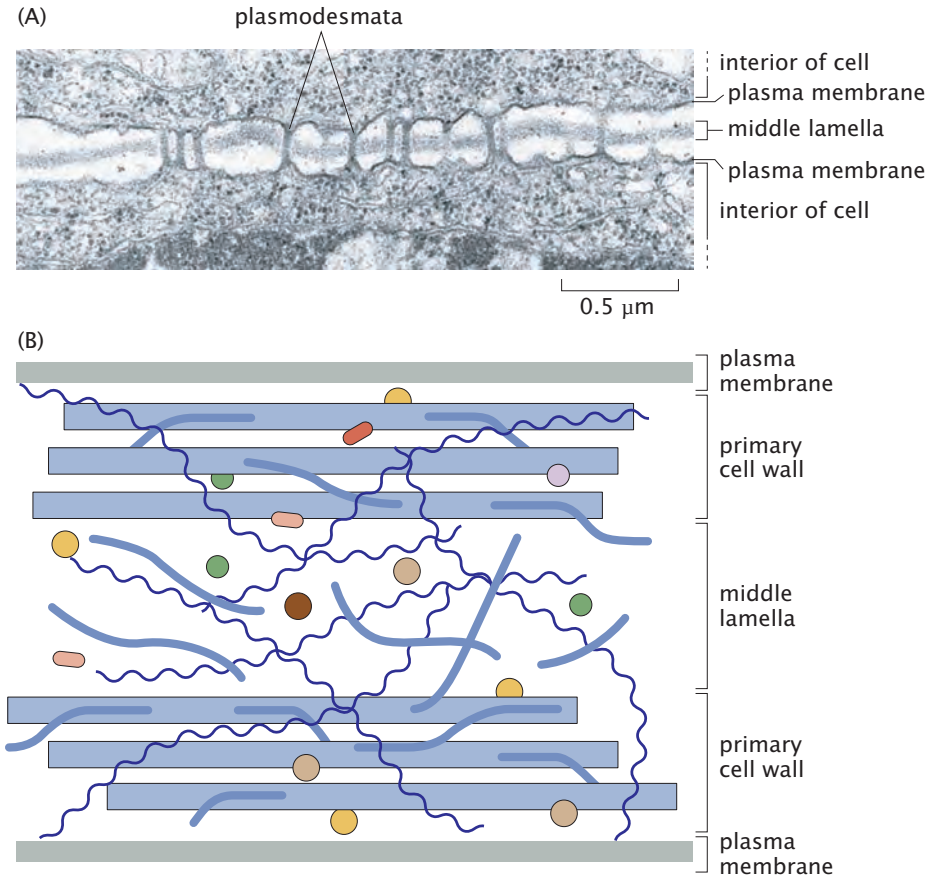
Sens P and Plastino J. *J. Phys.: Condens. Matter.* 27:273103. 2015

1. Short time scale: osmotic flow and cell mechanics

- Plant cell (and bacteria, fungi): the high osmotic pressure difference requires a thick, elastic wall and remodelling of the wall content.

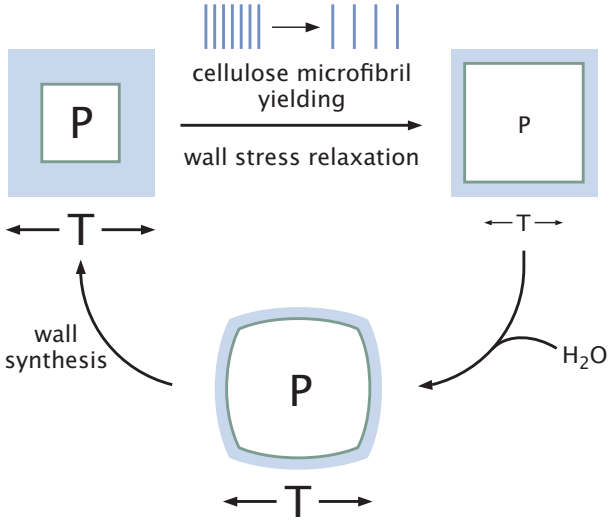
- Cell wall composition and mechanics



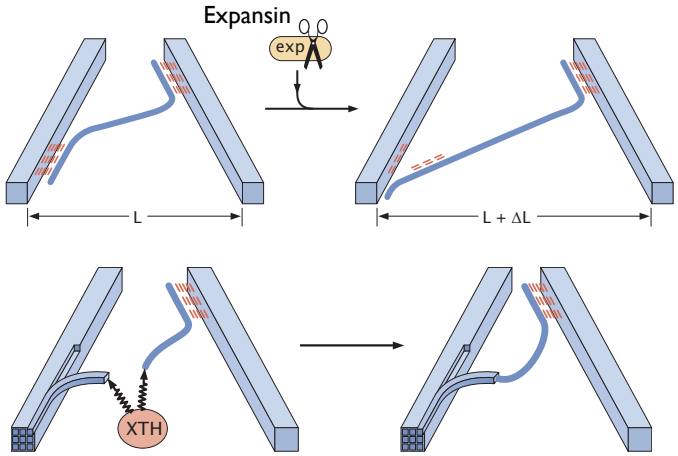


1. Short time scale: osmotic flow and cell mechanics

- Plant cell (and bacteria, fungi): Cell wall remodelling

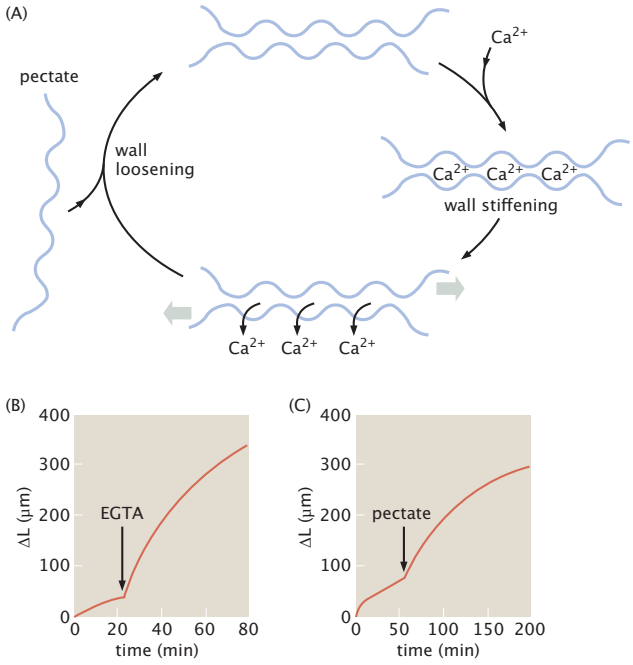


Cellulose-Hemicellulose fusion and crosslinking



XTH: Xyloglucan endotransglucosylase/endohydrolases

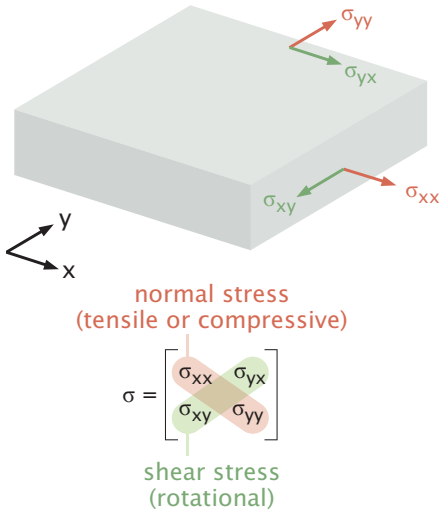
Pectate forms a hydrated gel that softens the wall
Ca2+ crosslinks pectate and stiffens the wall

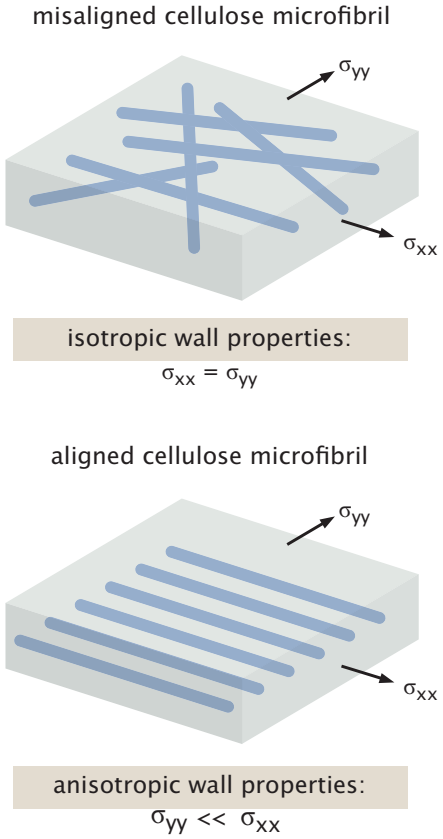


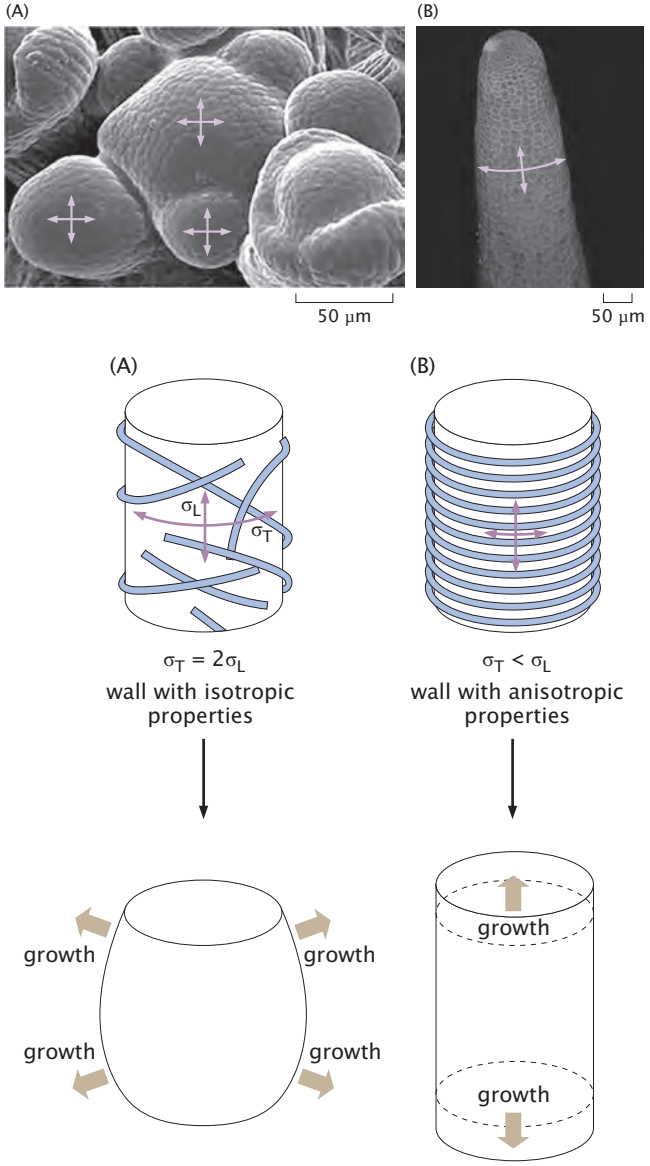
1. Short time scale: osmotic flow and cell mechanics

- Cell growth anisotropy is directed by anisotropy of wall components

Cell growth in plants is best characterised by a tensor
Stress tensor depends on wall organisation and geometry

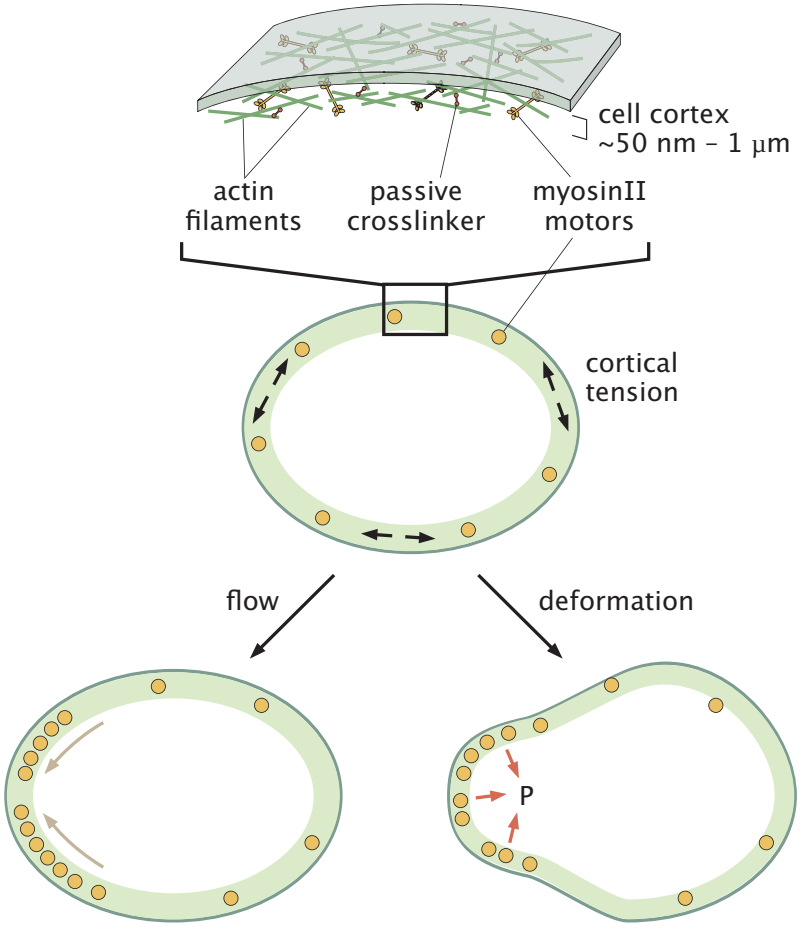


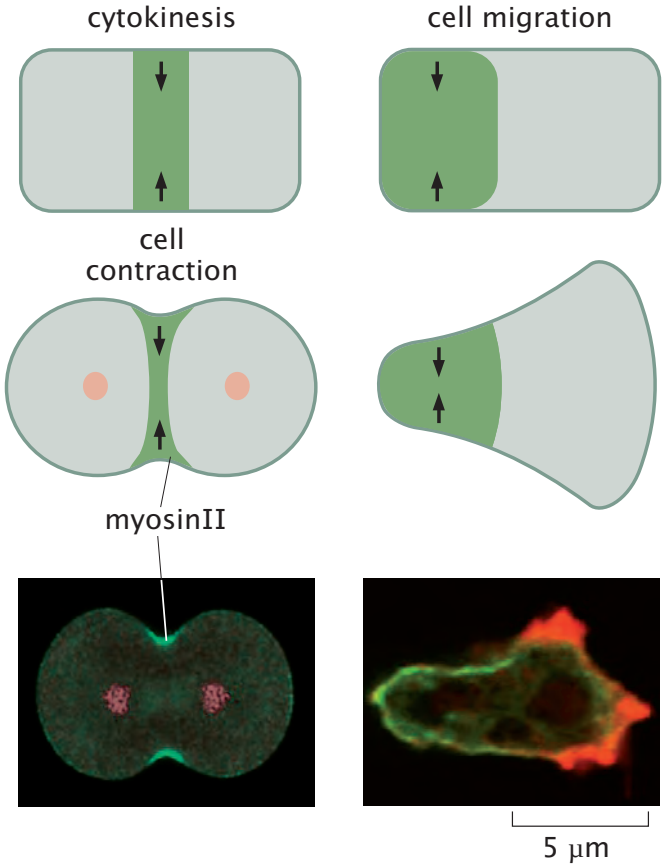




1. Short time scale: osmotic flow and cell mechanics

- In animal cells, the low osmotic pressure difference across the membrane results in low hydrostatic pressure
- Thus, the actomyosin cortex shapes cell (see courses 2017 and 2018)



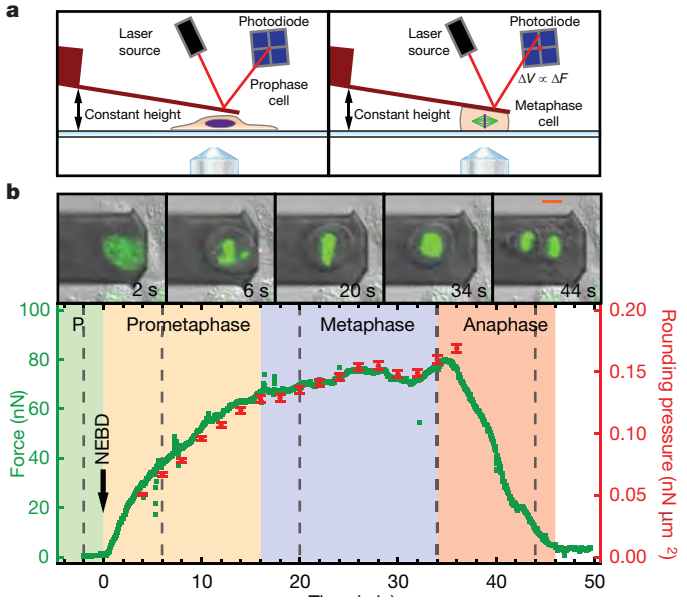


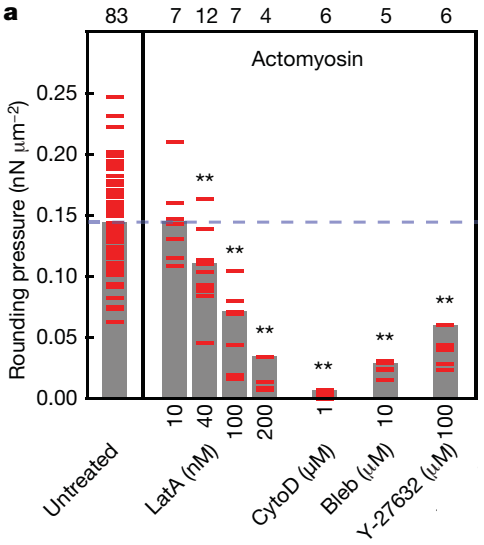
1. Short time scale: osmotic flow and cell mechanics

- Cell rounding during mitosis depends on osmotic pressure and actomyosin contractility

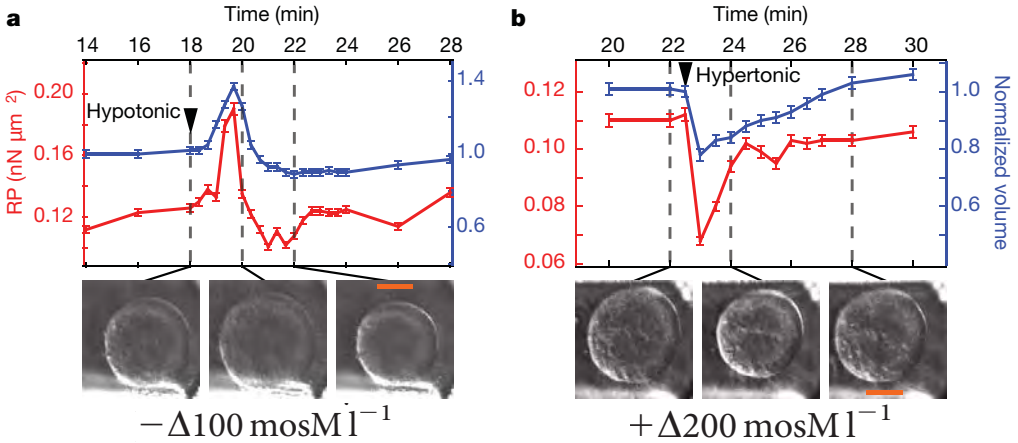
Upon entry into mitosis, cells round up and push against cantilever

The pressure requires an intact contractile actomyosin network at the cell cortex





A hypotonic (resp. hypertonic) shock in metaphase increases (resp. decreases) cell volume and the pressure on cantilever.



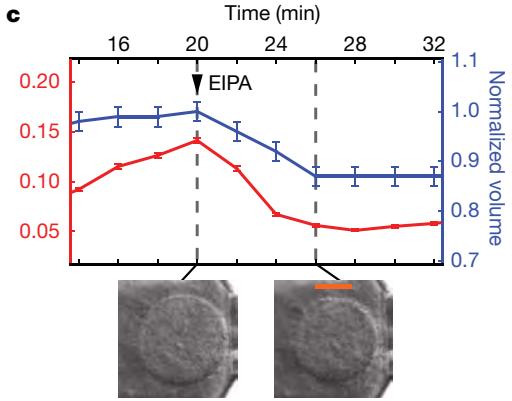
Martin P. Stewart et al. and A. Hyman. *Nature* 469: 226-230 (2011)

1. Short time scale: osmotic flow and cell mechanics

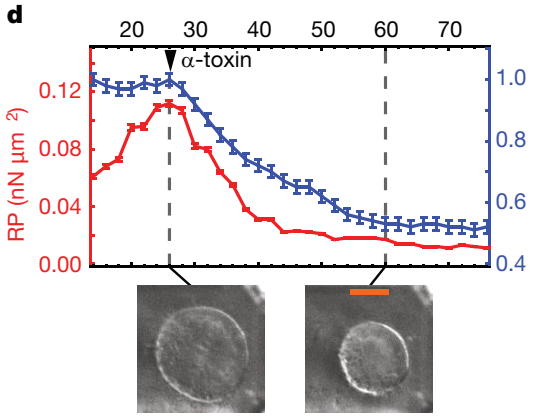
- Cell rounding during mitosis depends on osmotic pressure and actomyosin contractility

Blocking Na+/H+ antiporter reduces cell volume and rounding pressure in metaphase (ie. after cells have increased volume).

Ion channels are required for maintain osmotic pressure during mitotic rounding



EIPA: inhibitor of Na+/H+ antiporter.



EIPA: inhibitor of Na+/H+ antiporter.
Na+ contributes more to osmolarity than H+ because pH is buffered in cytoplasm

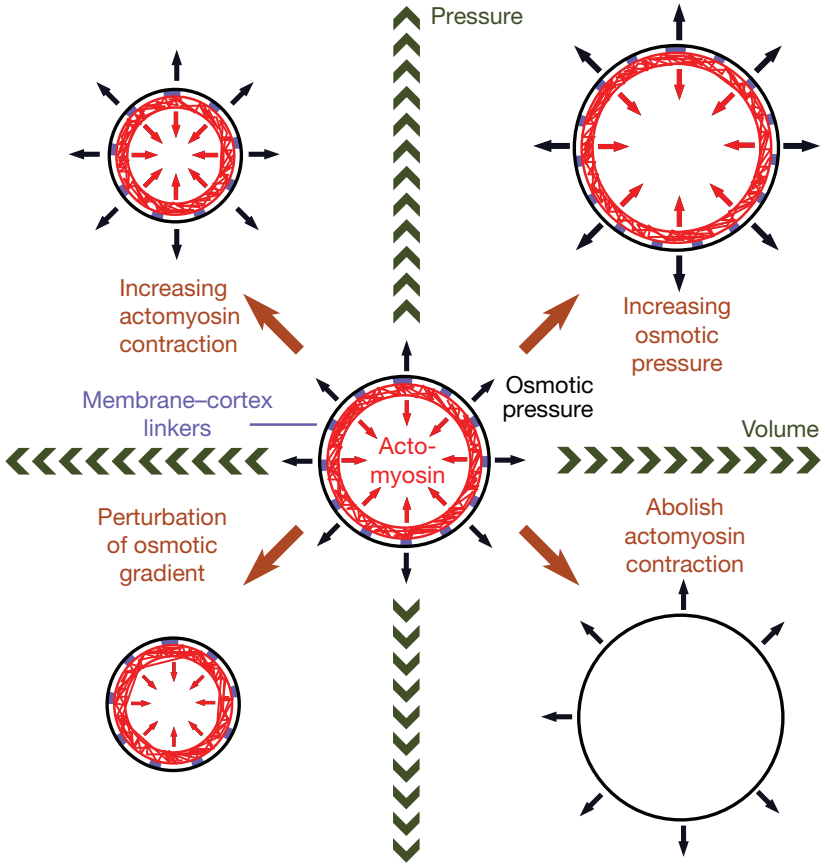
a-toxin from *Staphylococcus aureus* induces membrane permeability to monovalent cations and reduces volume and pressure.

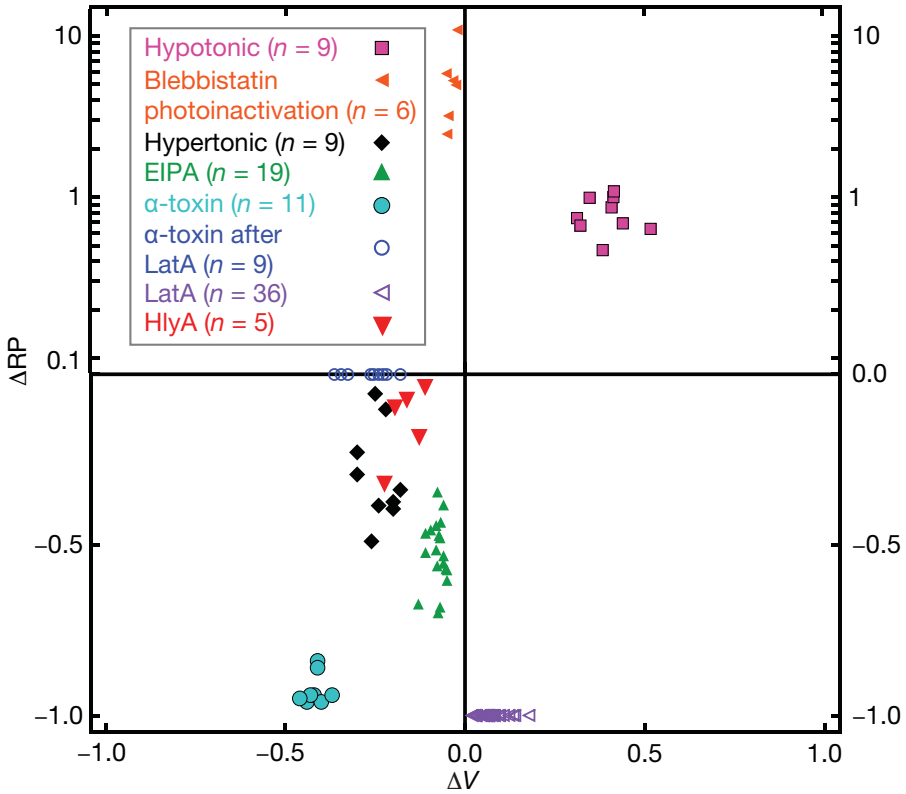
Martin P. Stewart et al. and A. Hyman. *Nature* 469: 226-230 (2011)

1. Short time scale: osmotic flow and cell mechanics

- Cell rounding during mitosis depends on osmotic pressure and actomyosin contractility

Cell rounding and volume is set by the opposite effects of osmotic pressure and cortical actomyosin contractility
However, the contribution of cortex contractility is quantitatively very modest compared to the gradient of osmotic pressure





Martin P. Stewart et al. and A. Hyman. *Nature* 469: 226-230 (2011)

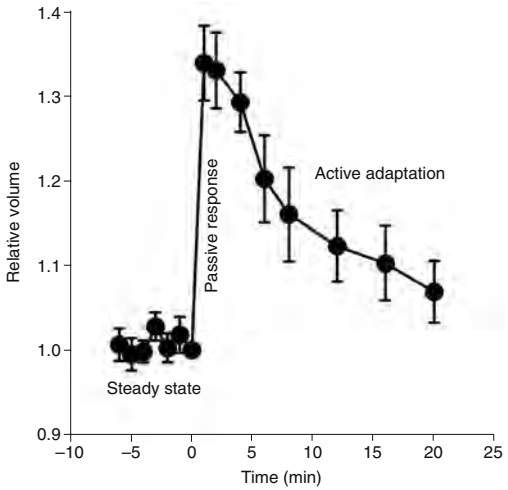
1. Short time scale: osmotic flow and cell mechanics

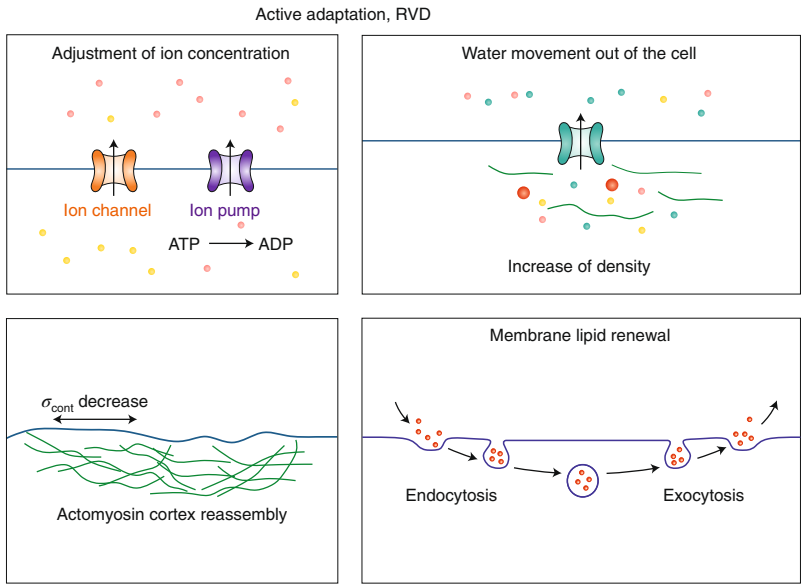
- Active adaptation to osmotic swelling via ion transporters, membrane flattening and vesicle trafficking

Mechanically gated ion channels respond to the increase membrane tension associated with rapid volume increase

Active export of ions decrease the osmotic pressure gradient, reverts water flow and decreases cell volume.

The membrane surface also adpats via endo/exocytosis.



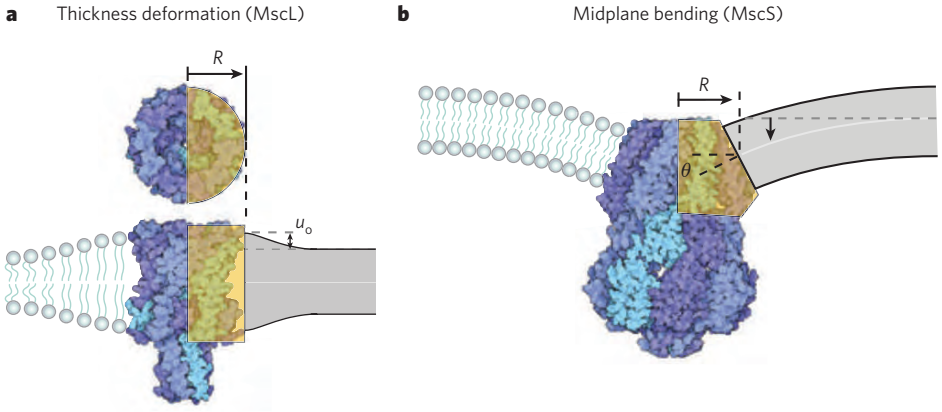


C.Cadart L. Venkova, P. Recho, M. C. Lagomarsino and M. Piel *Nature Physics* 15: 993–1004 (2019)

1. Short time scale: osmotic flow and cell mechanics

- mechanically gated ion channels

- Bacteria are exposed to rapid changes in osmolarity of environment and adapt rapidly
- This involves mechanically gated ion channels that respond to membrane tension induced by osmotic swelling
- Example: MscS and MscL
- Activated by membrane tension change in local membrane curvature, or hydrophobic mismatch at bilayer/protein interface



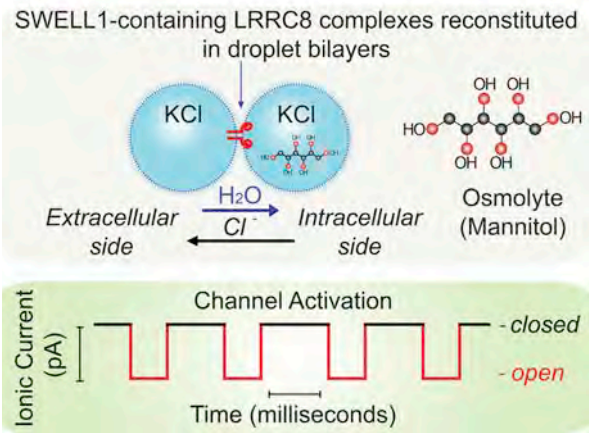
R. Phillips, T. Ursell, P. Wiggins and P. Sens *Nature* 459:379-385 (2009)

I. Booth and P. Blount *J. Bact.* 194: 4802–4809 (2012) Review

- low ionic strength gated ion channels

- Mammalian cells: Regulatory volume decrease involves swelling activated co-transport (efflux) of anions (Cl-) and cations (K+) outside the cell.
- Volume regulated anion channels (VRAC) is involved in this process. LRCC8 is a component of VRAC

- LRCC8 is not activated by swelling (ie. mechanically) per se.
- It is activated (gated) by an imposed osmotic gradient
- Low ionic strength in the absence of osmotic gradient induces anion current in vitro
- Hypotonic stress can activate LRCC8 by lowering of cytoplasmic ionic strength

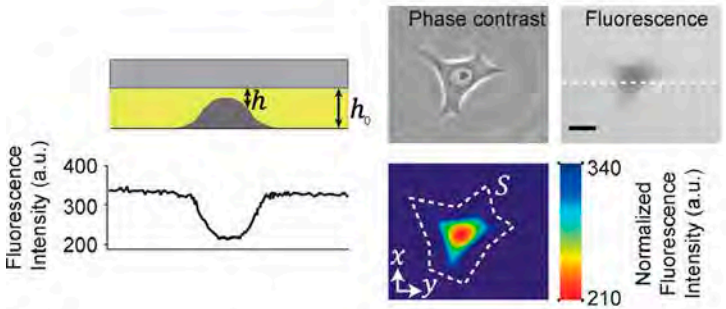


R. Syeda et al and A. Patapoutian. *Cell* 164, 499–511 (2016)

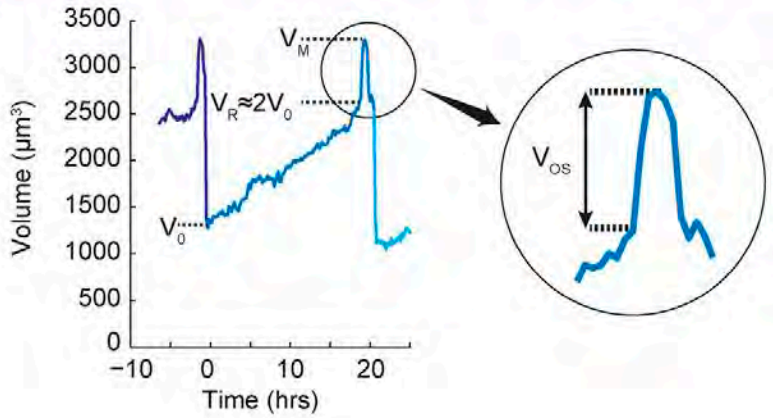
http://dx.doi.org/10.1016/j.cell.2015.12.031

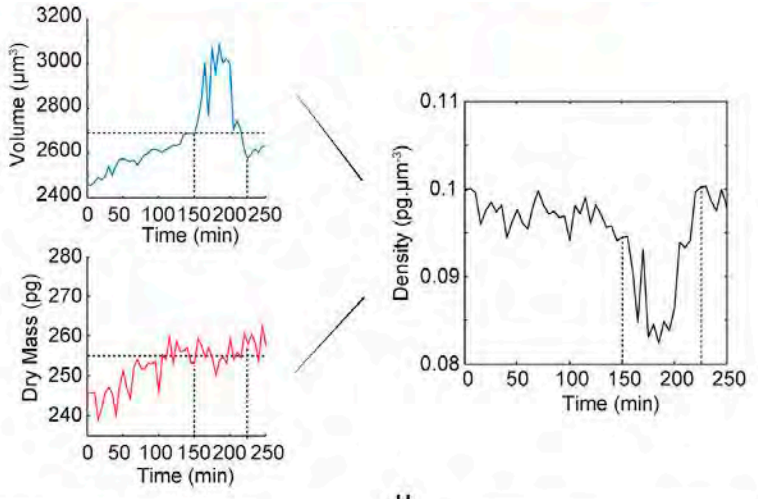
1. Short time scale: osmotic flow and cell mechanics

- Following cell rounding, cell volume increases (+20-30%) during mitosis while keeping a constant dry mass. Cell density decreases but is adjusted after mitosis.
- This process must involve regulation of osmolarity: a 10% volume increase would correspond to a 30 mOsm import



$$V_{cell} = \iint_S \frac{I_B(x,y) - I(x,y)}{\alpha} dS.$$



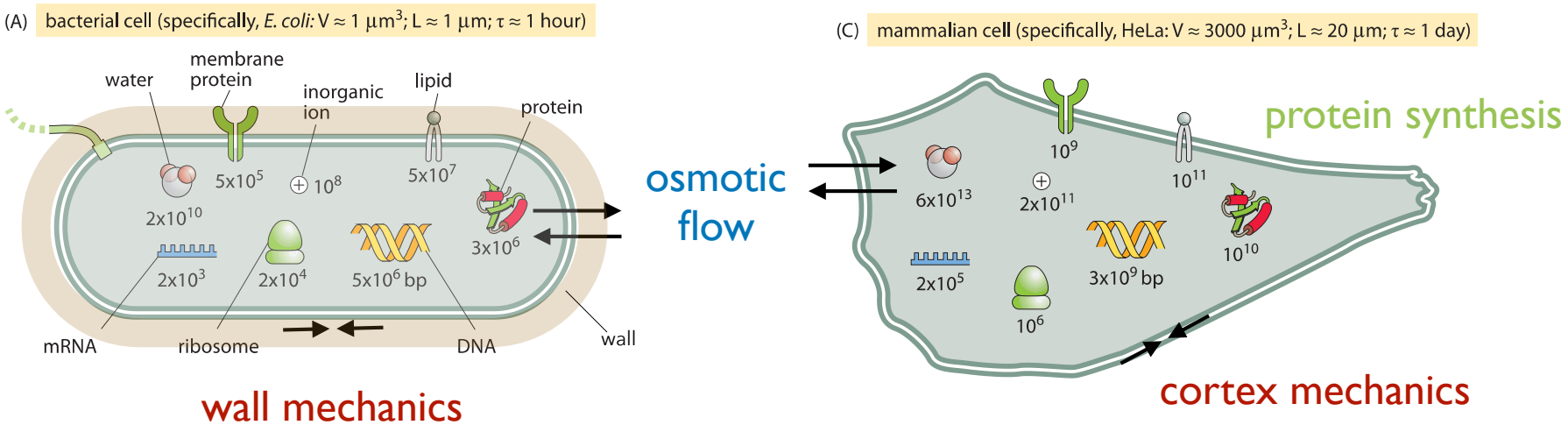


Zlotek−Zlotkiewicz E. et al. and M. Piel *J. Cell Biol.* 211:765–774 (2015)
www.jcb.org/cgi/doi/10.1083/jcb.201505056

What sets cell size and cell growth?

1. Short time scale: osmotic flow and cell mechanics
2. Long term regulation by protein synthesis during cell cycle.

Plants, Fungi, Bacteria: Wall (surface) growth and Volume growth dictate total volume increase

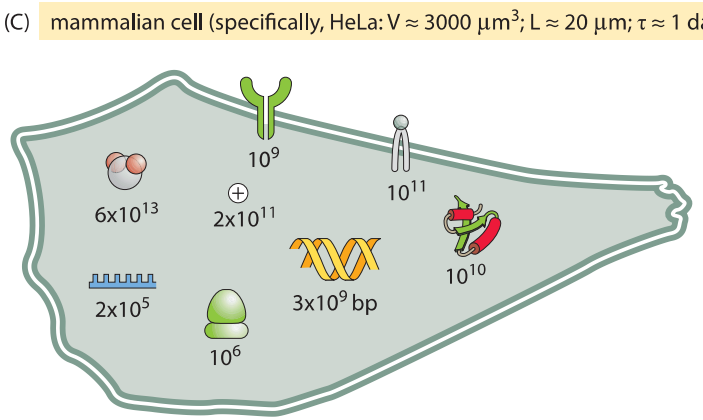


2. Long term regulation by protein synthesis

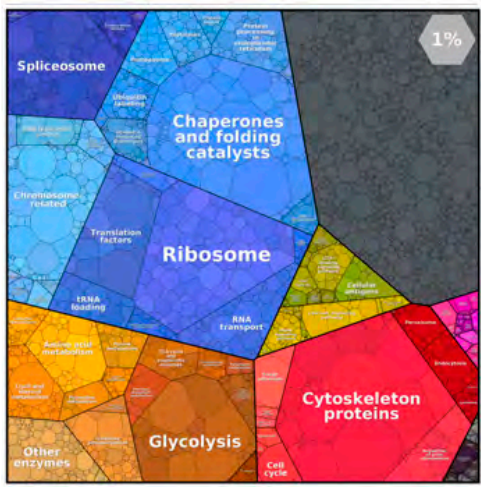
- Cell mass is made of approximately 30% dry mass and 70% water.
- A large part of the dry mass is made of proteins: (the rest is made of lipids and nucleotides)
  40-50% in Yeast and around 50% in other eukaryotes

  Yeast: proteins are at a concentration of few 100 g/L and occupy 20-30% of volume fraction in cytosol

- Protein density is highly regulated and changes very little.



H. sapiens (HeLa cell line)



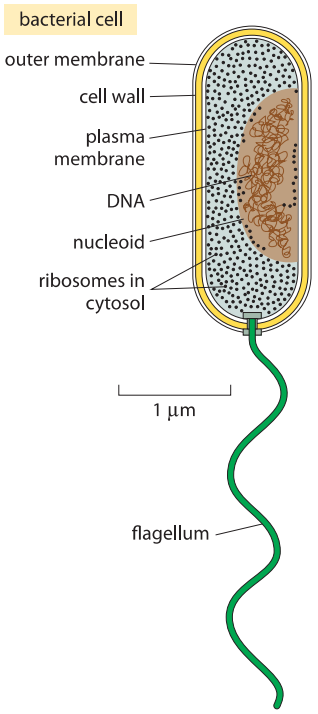
proteome

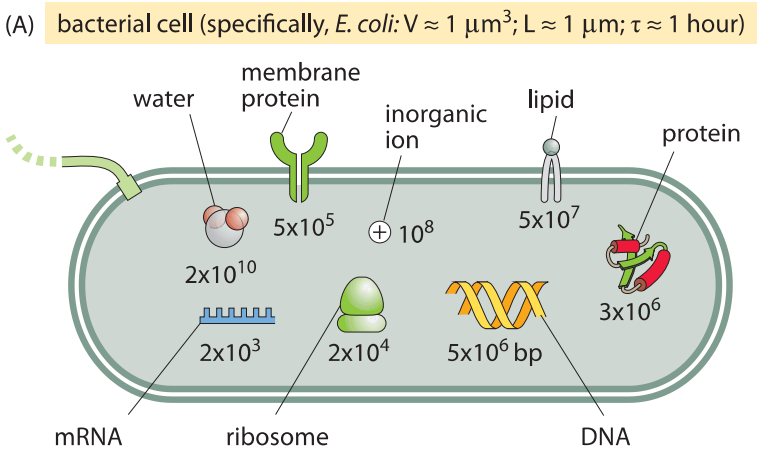
Liebmeister et al, R. Milo. PNAS (2013)
www.pnas.org/cgi/doi/10.1073/pnas.1314810111

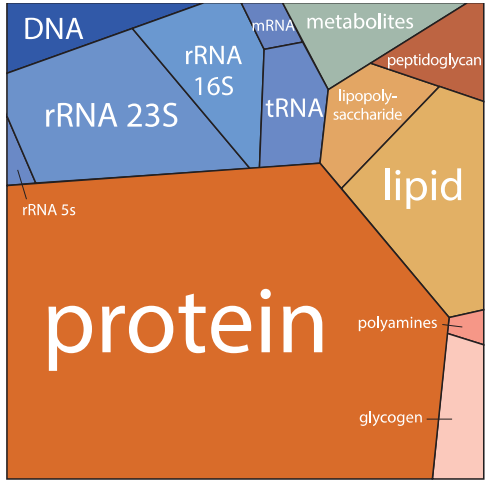
Cell Biology by the numbers. Ron Milo, Rob Phillips, *illustrated* by Nigel Orme. *Garland Science* 2012

2. Long term regulation by protein synthesis

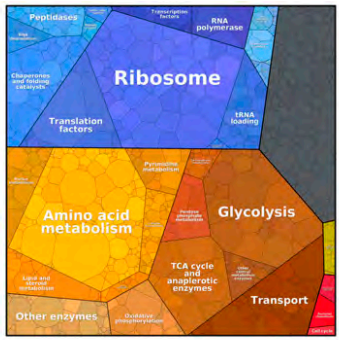
- Proteins are 55% of bacteria dry mass
- Ribosomes comprise a significant fraction of a cell dry mass: 10-15% of proteome, 15-25% of dry cell mass
- 20.000 ribosomes in *E. coli*







Proteome of *E. coli*

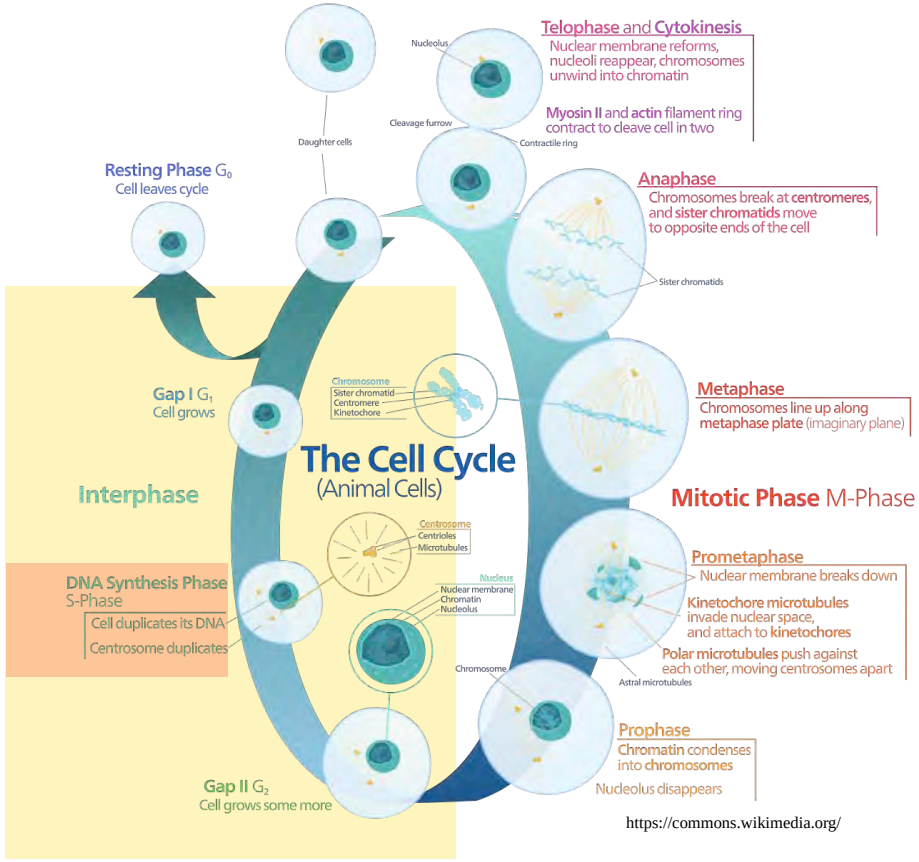


Cell Biology by the numbers. Ron Milo, Rob Phillips, *illustrated* by Nigel Orme. *Garland Science* 2012

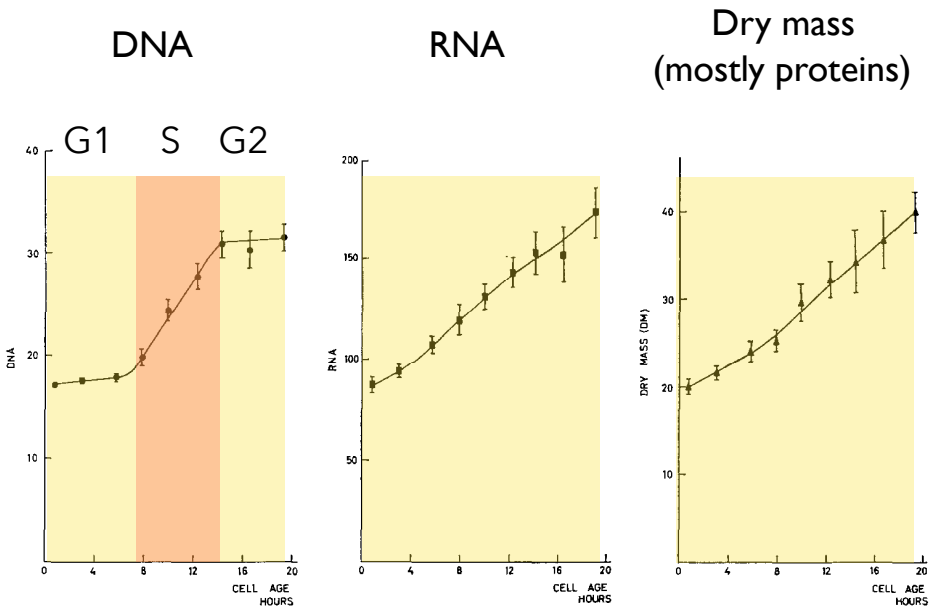
Liebmeister et al, R. Milo. PNAS (2013)
www.pnas.org/cgi/doi/10.1073/pnas.1314810111

2. Long term regulation by protein synthesis

- DNA, RNA and protein content doubles during interphase (growth phase)



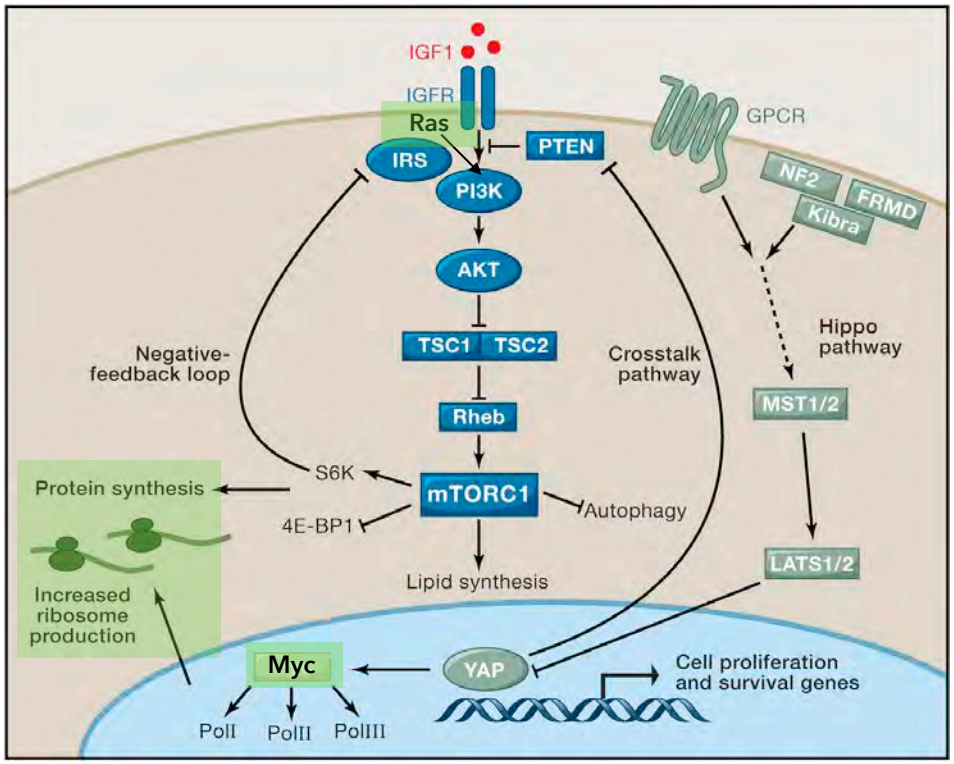
https://commons.wikimedia.org/



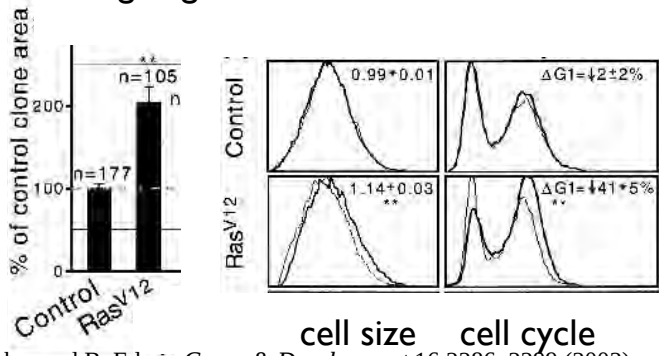
D. Killander and A. Zetterberg. *Experimental Cell Research* 40, 12-21) (1965)

2. Long term regulation by protein synthesis

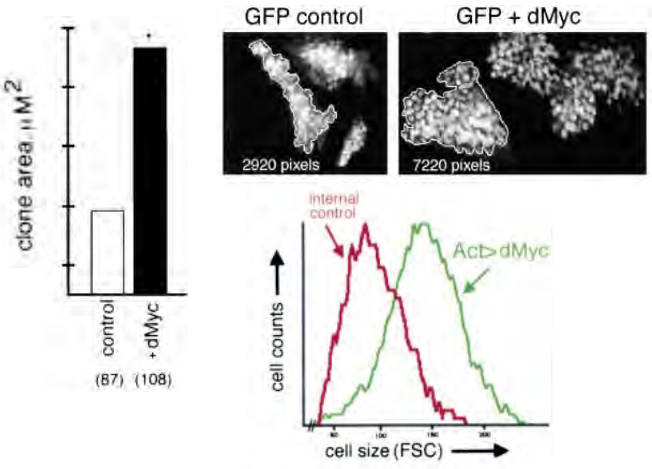
- Ras, Pi3K and TOR signalling control cellular anabolism and cell growth
  - In resting, non growing cell populations, the rate of synthesis and degradation of proteins is balanced
  - Growth pathways change this balance by promoting synthesis and/or inhibiting degradation



Laplante and Sabatini D. (2012) *Cell* 149:274



D. Prober and B. Edgar. *Genes & Development* 16:2286–2299 (2002)



Johnston LA, Prober DA, Edgar BA, Eisenman RN, Gallant P (1999) *Cell* 98: 779–790.

2. Long term regulation by protein synthesis

- Homeostasis of protein density:

— Protein density is highly regulated and changes very little.

- Importance of protein density:

— sufficient molecular crowding favours molecular interactions and reaction. Entropic effect of crowding agents.
(increase of molecular association and protein condensates in liquid-liquid phase separation)

— but too high crowding slows down signalling due to reduced diffusivity in cytosol (colloidal transition): biochemical *on rates* are often limited by diffusion.

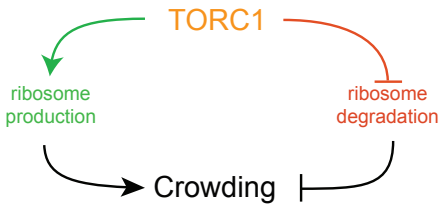
- Cell size regulation and cell growth require a constant balance between protein synthesis, total protein mass and water content via osmotic flow

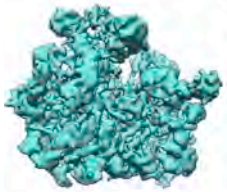
- What are the mechanisms of this coupling?

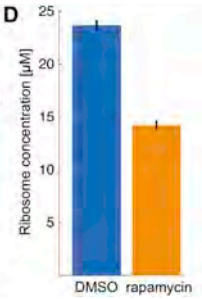
2. Long term regulation by protein synthesis

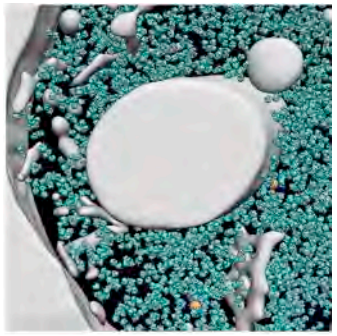
Tuning diffusion (and biochemical reactions) by the TOR protein synthesis pathway.

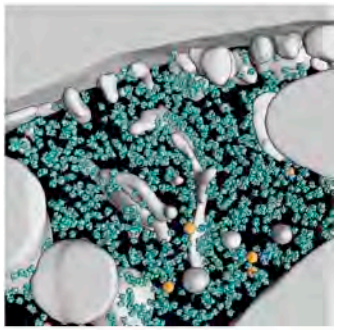
- Ribosomes constitute 20% of cytosolic volume
- The TOR pathway controls the density of ribosomes in the cytosol
- TOR pathway affects cytosol effective viscosity and molecular diffusion in Yeast and cell culture



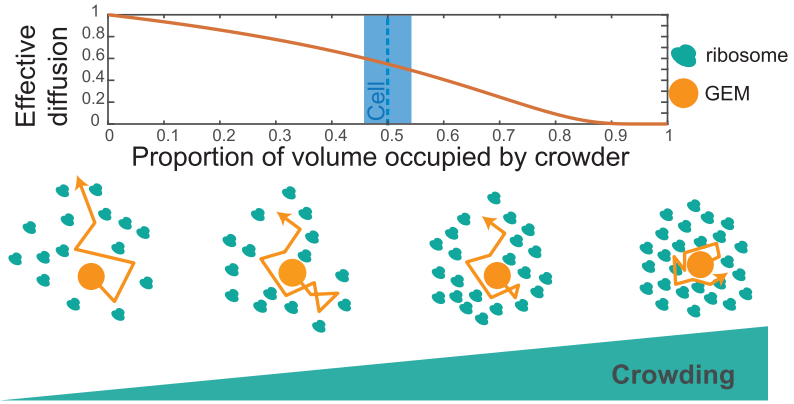


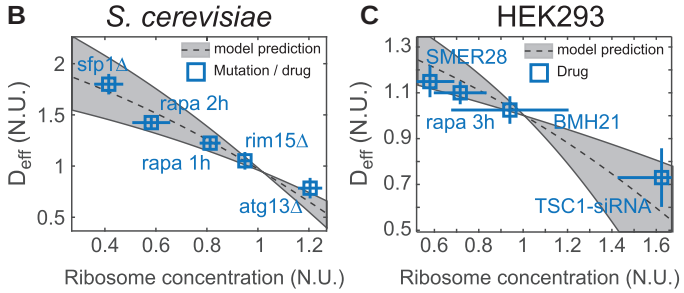






control (DMSO) TOR inhibition (rapamycin)

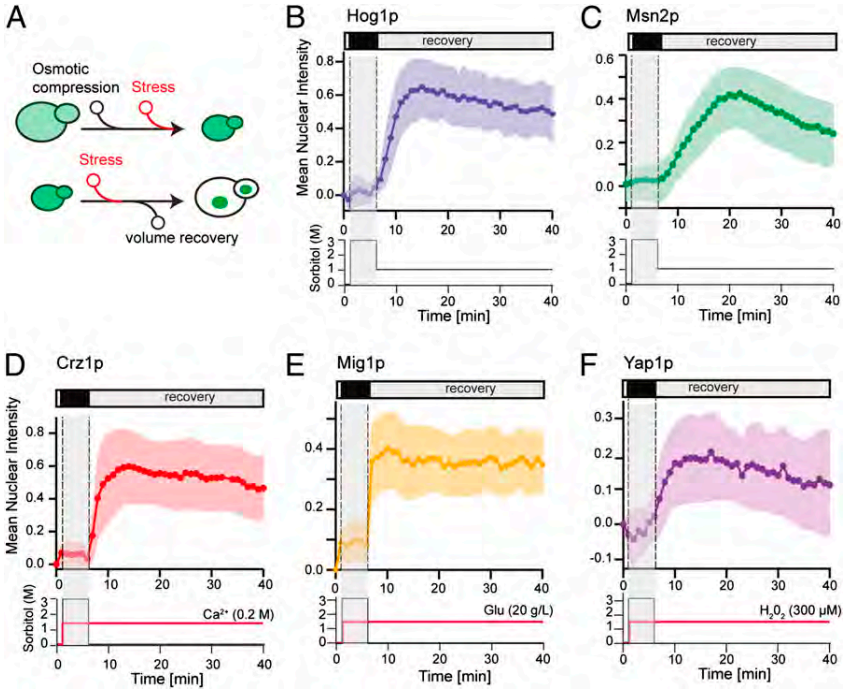




M. Delarue et al. and L.J. Holt *Cell* 174, 338–349, (2018)

2. Long term regulation by protein synthesis

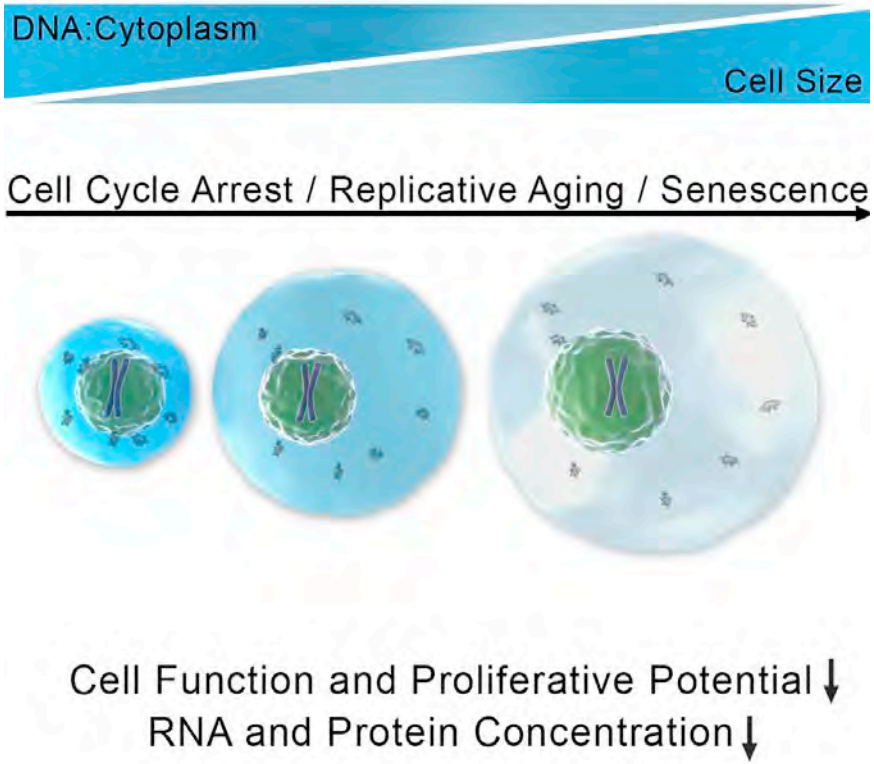
- Osmotic compression reduces signalling in Yeast
- Volume recovery is associated with restauration of signalling



Miermont, A., Waharte, F., Hu, S., McClean, M.N., Bottani, S., Léon, S., and Hersen, P. (2013). *Proc. Natl. Acad. Sci. 110*, 5725–5730. (2013)

2. Long term regulation by protein synthesis

- Excessive cell growth causes cytoplasm dilution and contributes to senescence
- This is due to uncoupling between protein synthesis and cell volume increase

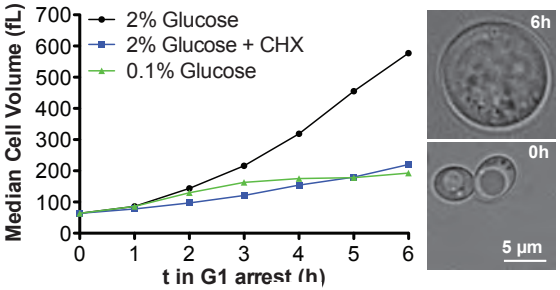


G. Neurohr et al. L. Holt and A. Amon, 2019, *Cell* 176, 1083–1097,

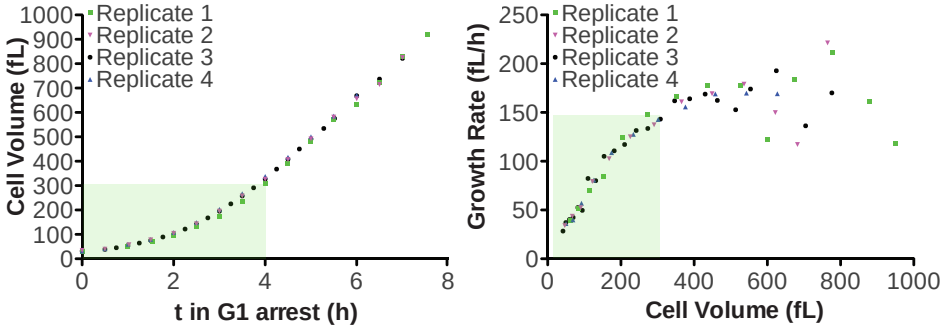
2. Long term regulation by protein synthesis

- Excessive cell volume growth reduces protein density

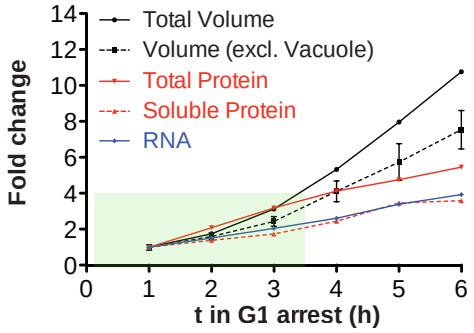
- Continued cell growth in cell cycle arrested mutant Yeast cells (*cdc28* mutants)

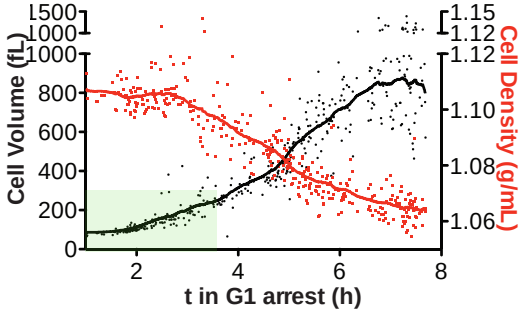


- Growth rate is initially proportional to cell volume (ie. exponential)
- But once cell size is too large, growth is linear.



- Total protein synthesis increases in exponential phase but decreases once volume is 300fl.
- Meanwhile volume growth continues (by water flux)
- Lack of scaling of protein to volume beyond a certain volume



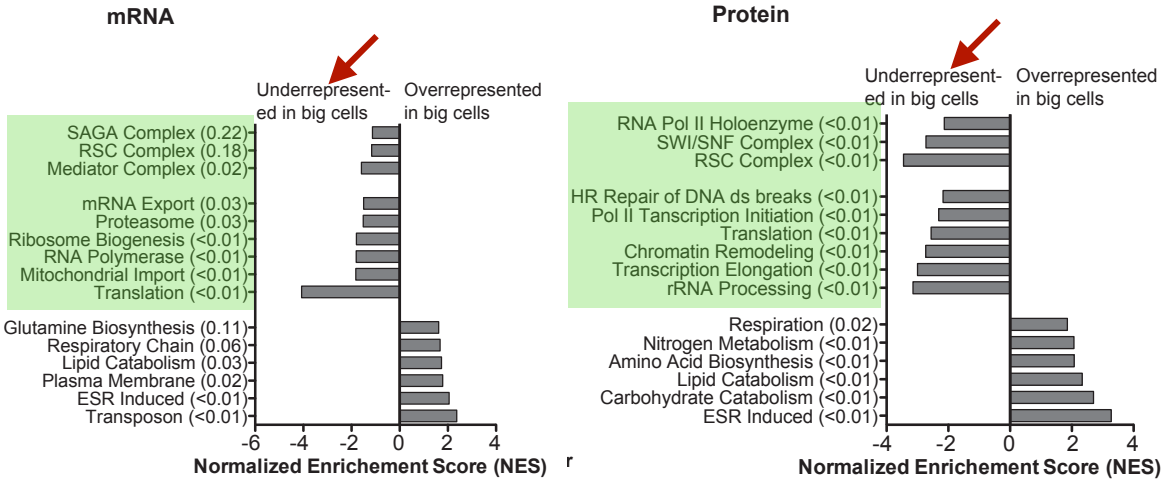


G. Neurohr et al. L. Holt and A. Amon, 2019, *Cell* 176, 1083–1097,

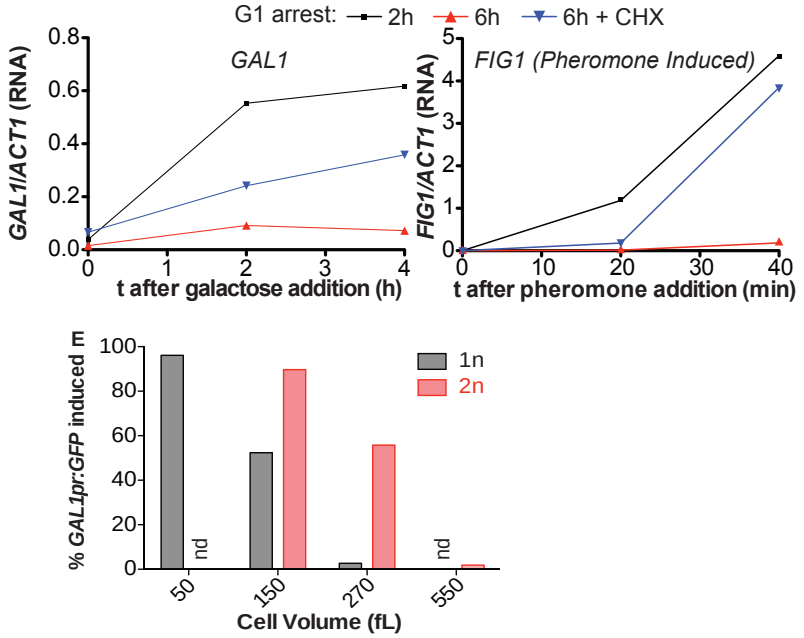
2. Long term regulation by protein synthesis

- In large cells, DNA becomes limiting for transcriptional and translational machinery

- Lack of scaling between volume increase and protein synthesis due to rate limiting transcriptional and translational machinery



- Impact of DNA/cytoplasmic ratio:
- haploid (n) and diploid (2n) *cdc48* mutants cells of similar size (using different duration of G1 arrest) were compared.
- *Background: cdc48* mutant (arrest in G1) fail to respond properly to Galactose addition (Gal1 expression) or exposure to hormone (alpha factor)
- Large cell phenotype was observed in diploid cells at twice the size as haploid cells.



G. Neurohr et al. L. Holt and A. Amon, 2019, *Cell* 176, 1083–1097,

Cell growth *rate* is limited by *kinetics* of transcription and ribosome synthesis

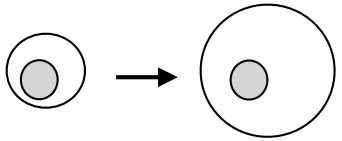
- As a cell grows, the nucleus becomes very small with respect to cell volume:
  — transcriptional capacity becomes limited (mRNA and rRNA)

- The translational capacity scales with cell cytoplasmic volume provided that ribosomal density remains constant

- But the assembly of ribosomes relies on rRNA transcription

- Time scale to double transcripts in a cell:

— at maximum polymerase loading a gene produces new transcript every 2 s
(1 polymerase every 60 nucleotides, rate of elongation of on average 30nt/s)



—100.000 to 1000.000 mRNAs and few 1.000.000 rRNAs
Some transcripts are present in a few thousand copies

—Doubling the amount of these transcripts can take several hours for a haploid genome

- Amplification of rRNAs (tandem arrays) to support large scale and fast assembly kinetics of ribosomes.

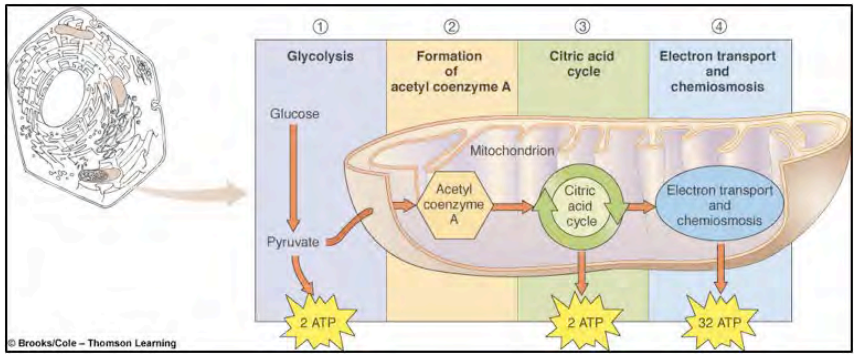
- Nurturing massive cell growth:

—some cells (oocyte) grow about 100.000 times in volume
—this would take >2000 days for haploid genome: polyploidy/multinucleation as a widespread solution

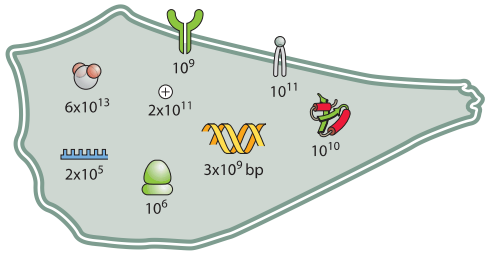
2. Long term regulation by protein synthesis

– Energetic cost

- Total cell energy budget is mostly used for protein synthesis



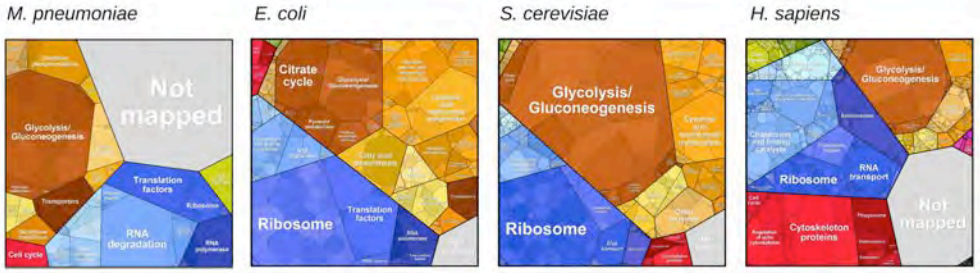
1 Glucose ⟶ 32 ATP

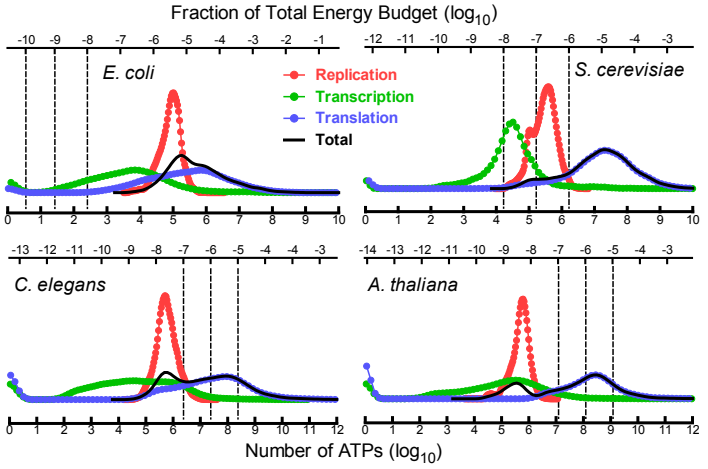


$3.\ 10^{7}$ ATP/cell/s $\sim 3.10^{-12}$ W

- Metabolism (especially glycolysis proteins) and Ribosomes are the most part of the proteome

Liebmeister et al, R. Milo. PNAS (2013)
www.pnas.org/cgi/doi/10.1073/pnas.1314810111

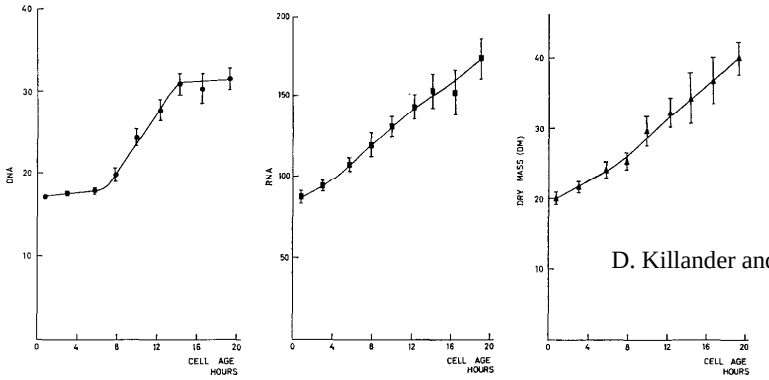




M Lynch and G. Marinov (2015) *PNAS* 112: 15690–15695
www.pnas.org/cgi/doi/10.1073/pnas.1514974112

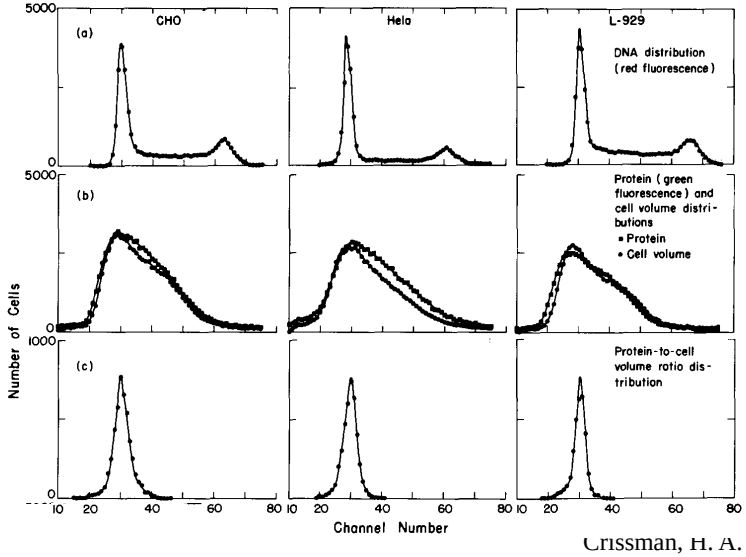
2. Long term regulation by protein synthesis

- DNA, RNA and protein content doubles during interphase (growth phase)



D. Killander and A. Zetterberg. *Experimental Cell Research* 40, 12-21) (1965)

- Protein concentration is constant through the cell cycle in different mammalian cell cultures

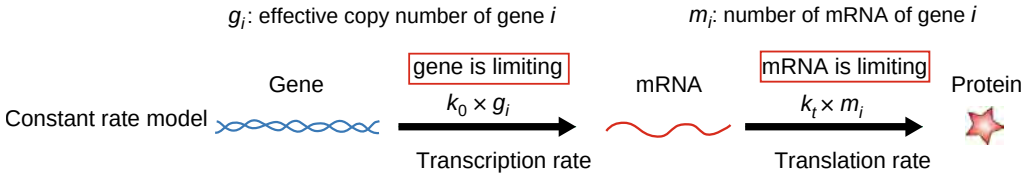


Crissman, H. A. & Steinkamp, J. A. *J. Cell. Biol.* 59, 766 (1973).

2. Long term regulation by protein synthesis

- A theoretical model explaining the empirical scaling of proteins and RNAs to exponentially growing cell volume during cell growth

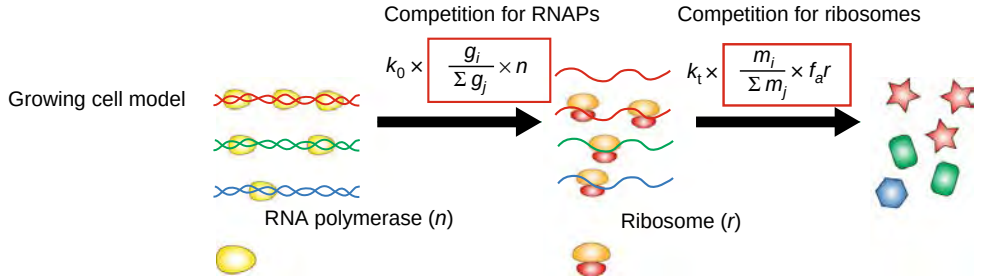
—The scaling of protein and mRNA numbers with an exponentially growing cell volume is incompatible with a constant synthesis rate in which DNA (gene copy number) and mRNAs are rate limiting



g_i effective gene copy number also accounts for promoter strength

—If exponential growth rate of mRNA, proteins and volume are the same on average, any noise would nonetheless accumulate. So homeostasis of mRNAs and protein concentration requires a regulatory mechanism

—Stochastic gene expression model where RNA Polymerases, RNAPs (number *n*) and ribosomes (number *r*) are rate limiting. f_a is the faction of active ribosomes (a constant)



gene allocation fraction $\phi_i = g_i / \sum_j g_j$: fraction of RNAPs working on genes
gene regulation is coarse-grained into ϕ_i

J. Lin and Ariel Amir. *Nature Communications* | (2018) 9:4496 | DOI: 10.1038/s41467-018-06714-z

2. Long term regulation by protein synthesis

$$m_i \xrightarrow{k_0\left(g_i/\sum_j g_j\right)n} m_i+1,$$

$$m_i \xrightarrow{m_i/\tau} m_i-1,$$

$$p_i \xrightarrow{k_t\left(m_i/\sum_j m_j\right)f_a r} p_i+1.$$

- A theoretical model explaining the empirical scaling of proteins and RNAs to cell volume during cell growth

- RNAP and ribosomes are limiting for transcription and translation.
- Ribosome synthesis is autocatalytic (exponential)

$$d\langle r\rangle/dt = k_t f_a \phi_r \langle r\rangle \quad \text{with growth rate } \mu = k_t f_a \phi_r$$

fraction of ribosomal gene in genome (ϕr)
(NB: amplification of rRNA increases ϕr)

- Therefore protein number grows exponentially, in particular RNAP

$$d\langle p_i\rangle/dt = k_t f_a \phi_i \langle r\rangle \qquad \langle p_i(t)\rangle = p_b(i)\exp(\mu t)$$

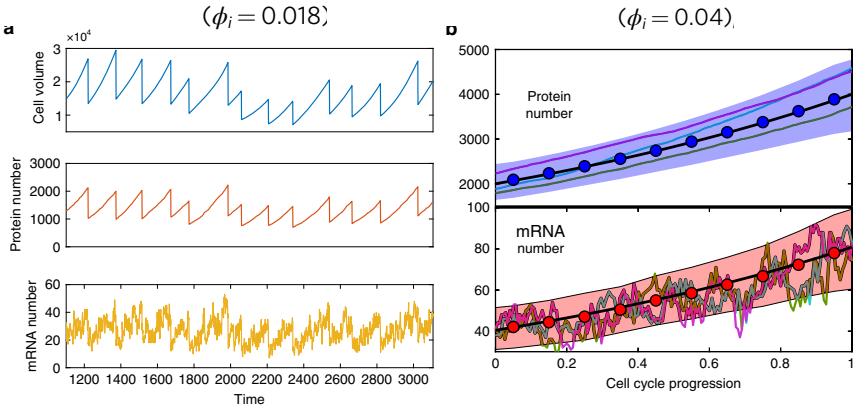
- So mRNA number too grows exponentially. $\langle m_i(t)\rangle = m_b(i)\exp(\mu t)$
- Volume is set proportional to total protein number,

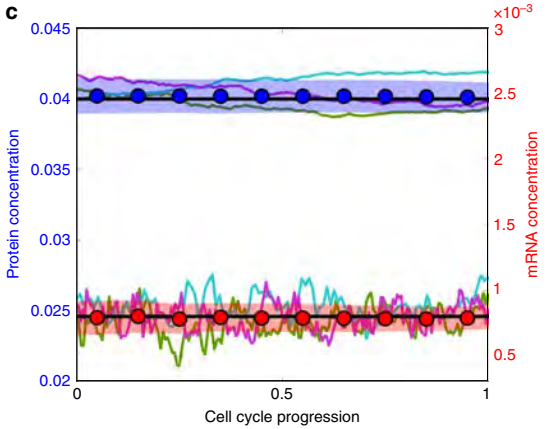
$$V \propto M = \textstyle\sum_j p_j$$

- Fluctuation in RNAP or ribosome number affects *all mRNAs or proteins* and so leaves invariant the *relative fraction* of one type of RNA (or protein) to the total pool of RNAs (proteins)

$$\frac{dc_i}{dt} \approx \mu(\phi_i - c_i). \qquad \text{stable fixed points } c_i = \phi_i$$

$$\frac{dc_i^m}{dt} \approx \frac{1}{\tau}\left(k_0\phi_i\phi_n\tau - c_i^m\right). \qquad c_i^m = k_0\phi_i\phi_n\tau$$





J. Lin and Ariel Amir. *Nature Communications* | (2018) 9:4496 | DOI: 10.1038/s41467-018-06714-z

2. Long term regulation by protein synthesis

- Exponential and linear modes of cell growth depend on saturation of DNA and RNA by RNA polymerases and ribosomes

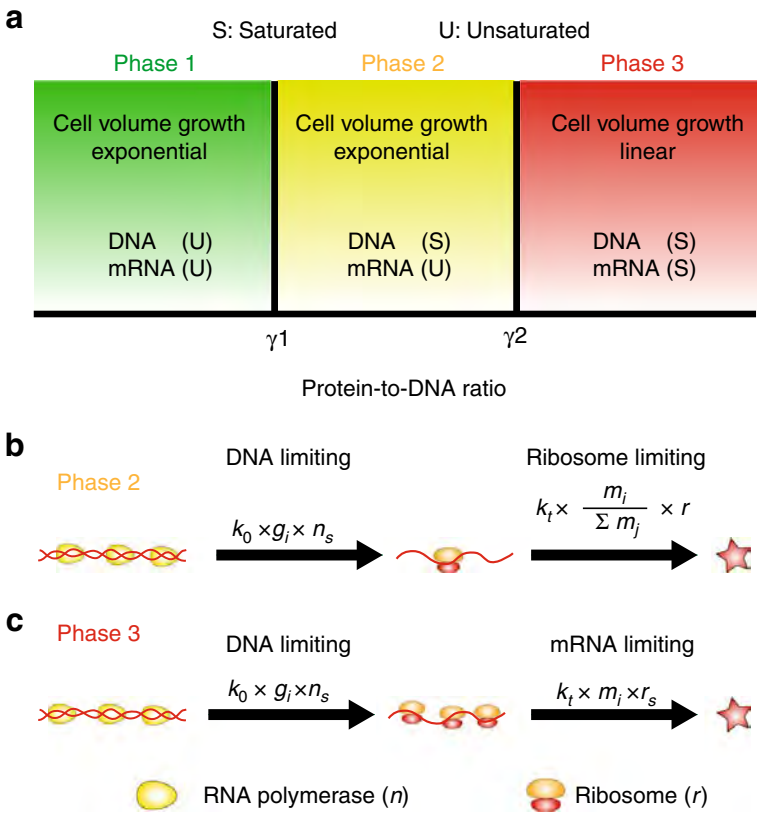
- When $\gamma < \gamma 1$, neither DNA nor mRNA is saturated. RNAP and ribosomes are limiting. The mRNA number, the protein number and the cell volume all grow exponentially with the growth rate set by the fraction of ribosomal gene in the total genome (ϕr)

- When $\gamma 1 < \gamma < \gamma 2$, DNA is saturated but mRNA is not. The protein number and the cell volume still grow exponentially while the mRNA number is a constant proportional to the gene number.

- When $\gamma > \gamma 2$, both DNA and mRNA are saturated. The protein number and cell volume grow linearly, and the cell volume growth rate is set by the genome copy number

(This is consistent with experimental data on decay of cell growth rate in G1 arrested cells)

G. Neurohr et al. L. Holt and A. Amon, 2019, *Cell* 176, 1083–1097,

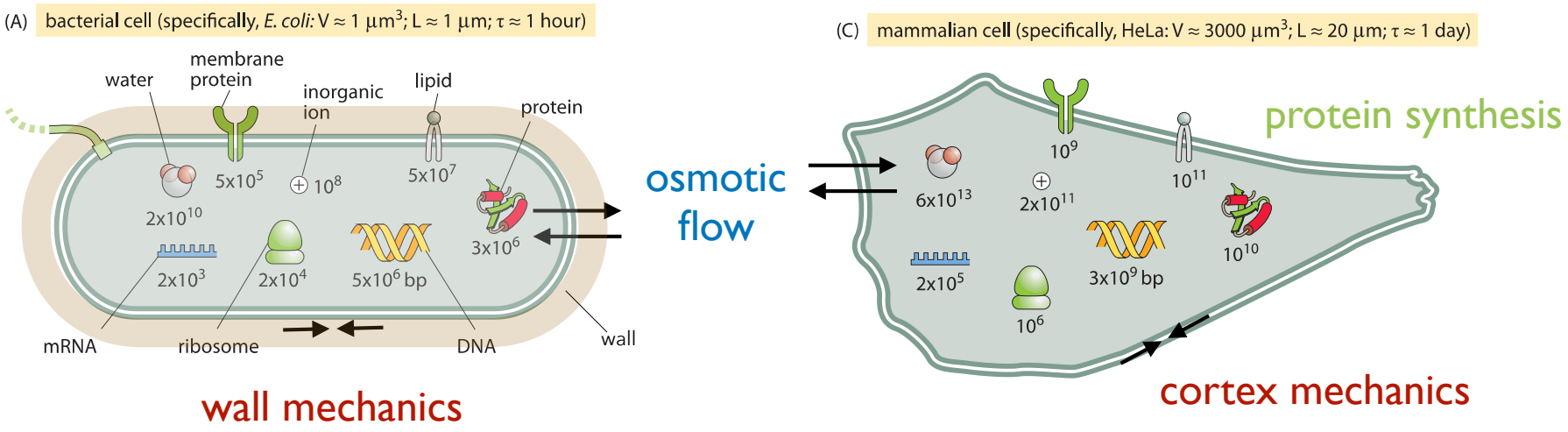


J. Lin and Ariel Amir. *Nature Communications* | (2018) 9:4496 | DOI: 10.1038/s41467-018-06714-z

What sets cell size?

1. Short time scale:osmotic flow and cell mechanics
2. Long term regulation by protein synthesis
3. Coupling time scales

Plants, Fungi, Bacteria: Wall (surface) growth and Volume growth dictate total volume increase

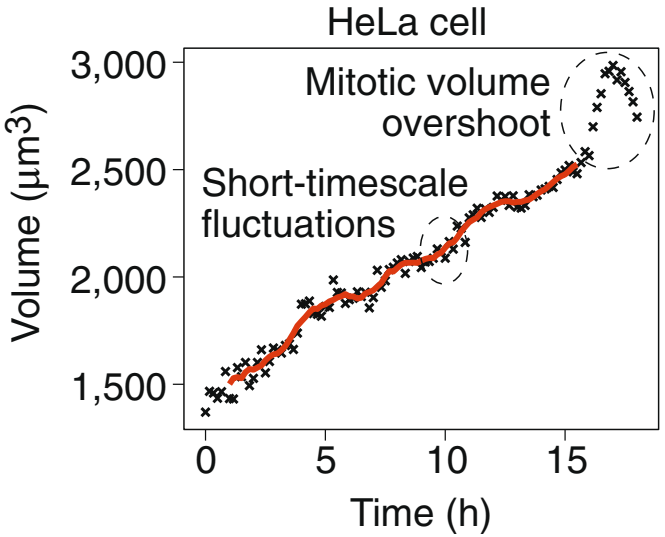


Coupling time scales

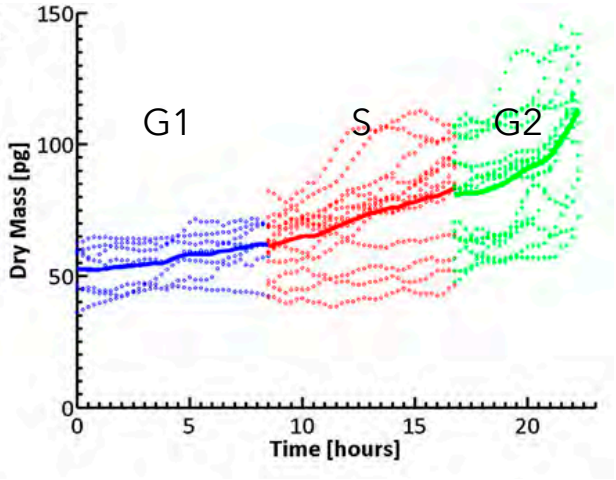
- On short time scales (a few minutes), cells adjust their volume based on osmotic gradient across the membrane/wall of cells
- A sudden change in osmolarity of environment causes rapid volume change
- Adaptive mechanisms through mechanical and electro-chemical feedbacks allow cells to return to their homeostatic volume

- Cells keep a nearly constant protein density and can respond to rapid changes in density to restore homeostatic density.
- Since the protein concentration is low compared to ions, their net contribution to osmolarity is negligible. Therefore, the mechanisms coupling protein density and cell volume are indirect.

- This is especially important due to protein synthesis during the growth phase of the cell cycle: the cell volume (water flow) must adapt to adjust the internal density as synthesis takes place:
  — negative feedback: increased density and molecular crowding could inhibit polymerases and ribosomes and protein synthesis.
  — membrane synthesis and wall synthesis should scale with volume (e.g. components are all synthesised in cytoplasm), this would increase ion channels/pumps and increase the rate of ion flux through the membrane…?



C.Cadart L. Venkova, P. Recho, M. C. Lagomarsino and M. Piel *Nature Physics* 15: 993–1004 (2019)



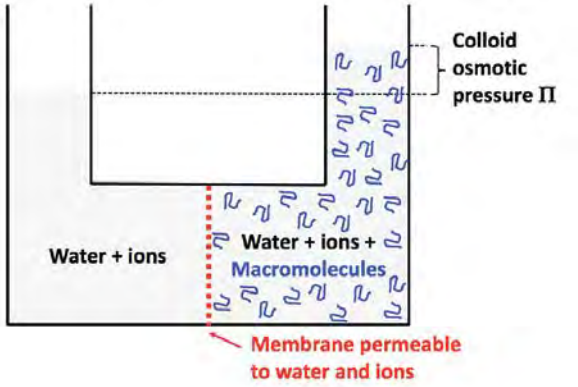
Mir, M. et al. *Proc. Natl Acad. Sci. USA* 108, 13124–13129 (2011).

Colloidal osmotic pressure (oncotic pressure)

- A possible mechanism to couple protein concentration and cell volume

- Pressure resulting from difference in protein concentration across a semipermeable membrane (permeable to water and ions but not to proteins)
- Water flow associated with difference in colloidal osmotic pressure
  Results from exclusion volume effect and depletion forces and depends on protein concentration
- At high protein concentration, proteins interact and van't Hoff law does not work. Non linear effect of protein concentration

colloidal osmotic pressure: $\Pi = cRT(1 + \alpha c + \beta c^2 \ldots)$



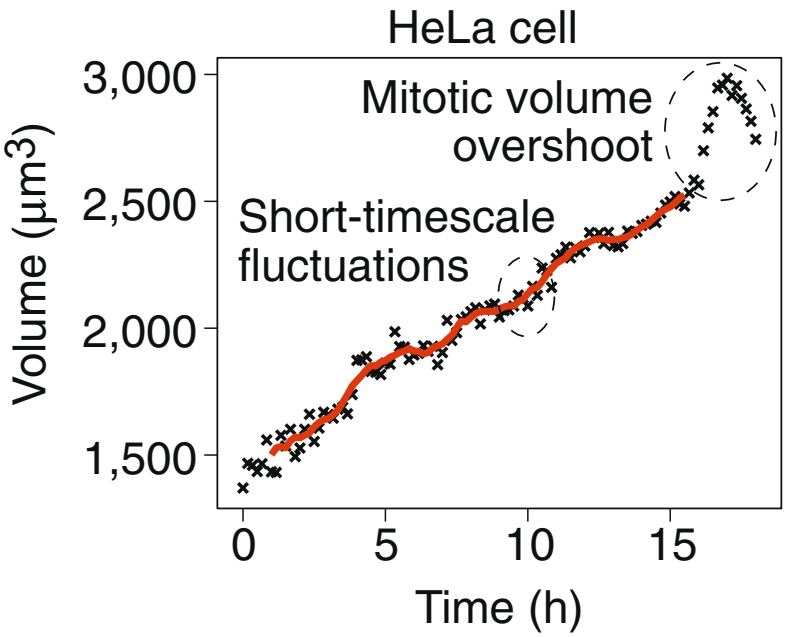
- Colloidal osmotic pressure would couple water flow and protein concentration:

Starling equation
Water flow driven by difference in colloidal osmotic pressure across a semipermeable membrane (permeable to water and ions but not to proteins)

$$J_v = L_p S([P_c - P_i] - \sigma[\pi_p - \pi_i])$$

T. Mitchison. *MBoC* 30: 173-180 (2018).

Cell size is set by co-regulation of cell division as a function of cell growth



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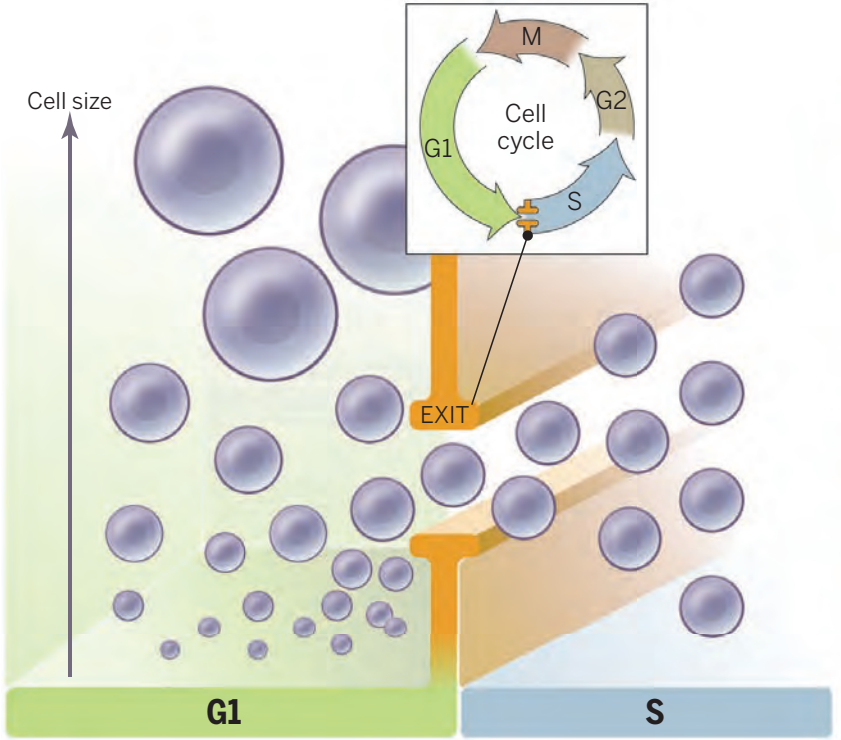

Miriam B. Ginzberg et al. and M Kirschner. *Science* 348, (2015); DOI: 10.1126/science.1245075

COLLÈGE DE FRANCE
1530

CHAIRE DYNAMIQUES DU VIVANT
Année académique 2020-2021

Thomas LECUIT

Taille, croissance et organisation cellulaires

Cours les mardis de 10h à 11h30
Amphithéâtre Guillaume Budé

Cours :

17 novembre 2020	Du tissu à la cellule : taille et complexité
24 novembre 2020	Volume cellulaire : déterminants physico-chimiques et régulation
01er décembre 2020	Croissance et division cellulaires : la cellule mesure-t-elle ses dimensions ?
08 décembre 2020	Lois de proportions cellulaires

Colloque :

Contraintes et plasticité au cours du développement et de l'évolution
(avec Denis Duboule, chaire Évolution des génomes et développement)

Les 03 & 04 juin 2021
Amphithéâtre Maurice Halbwachs