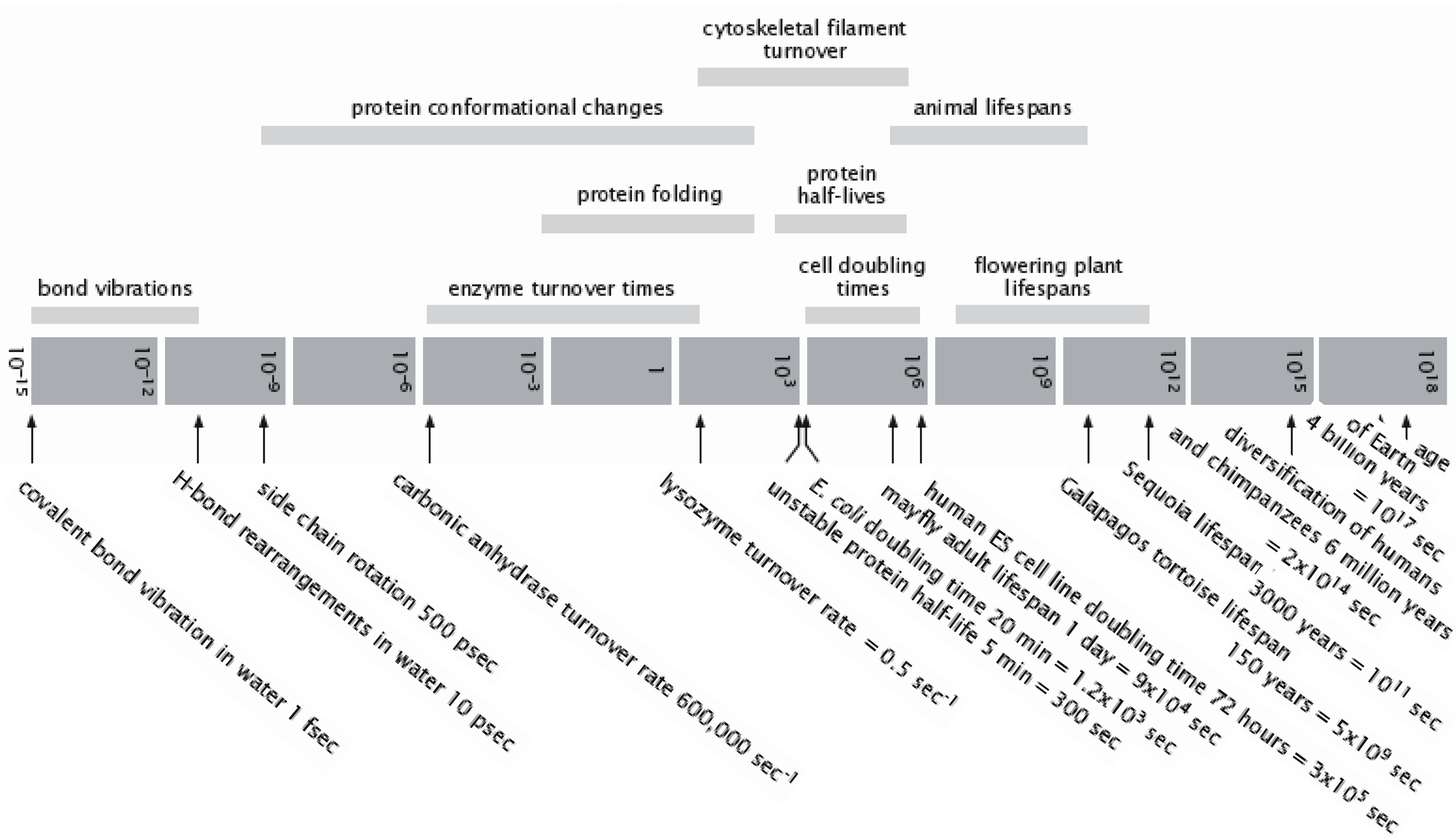
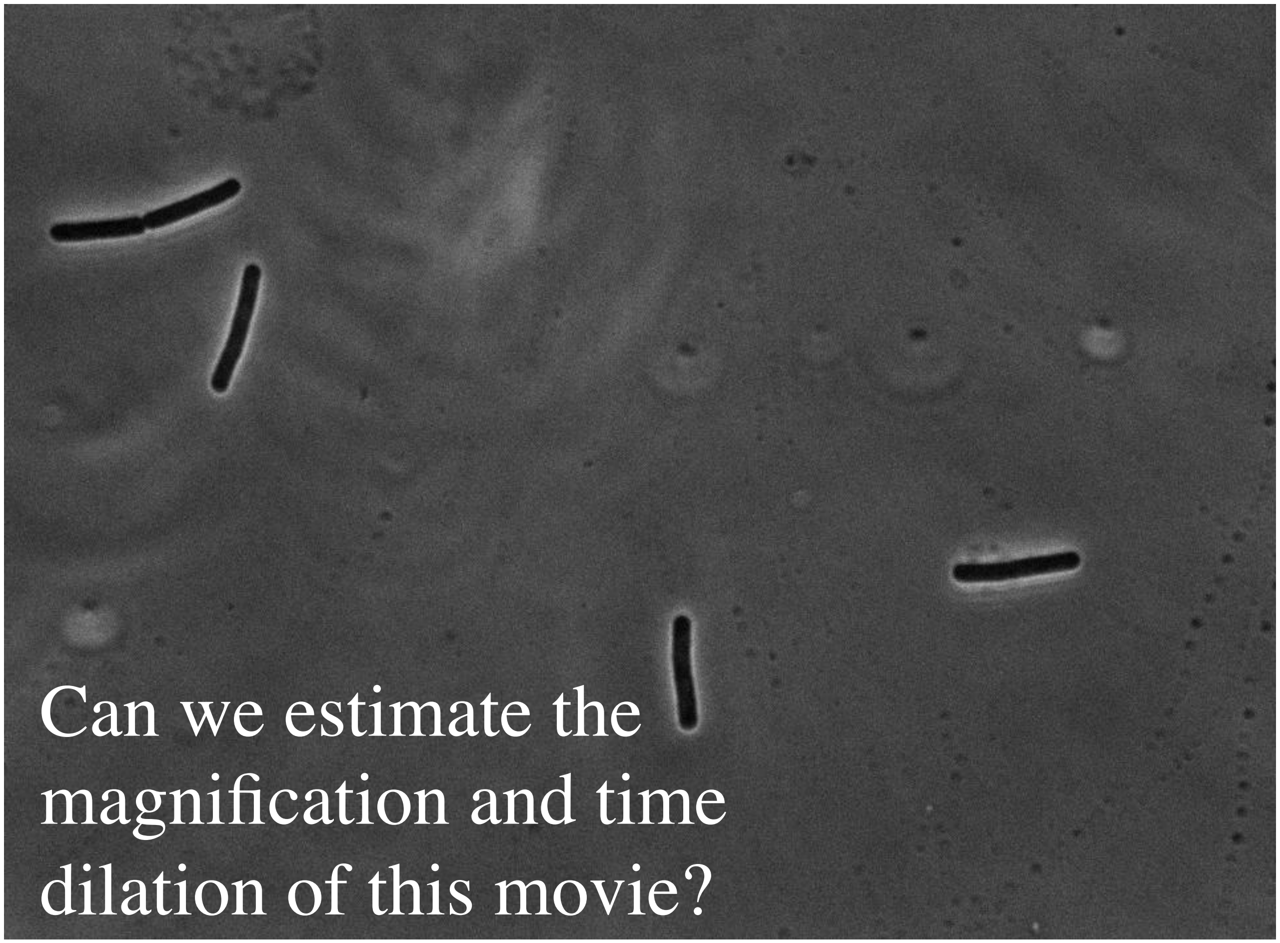
# THE HIERARCHY OF TEMPORAL SCALES




*Molecular motion of biochemical species as they interact and change identity* → *Unfolding of the lives of individual cells* → *Trajectories of entire species*

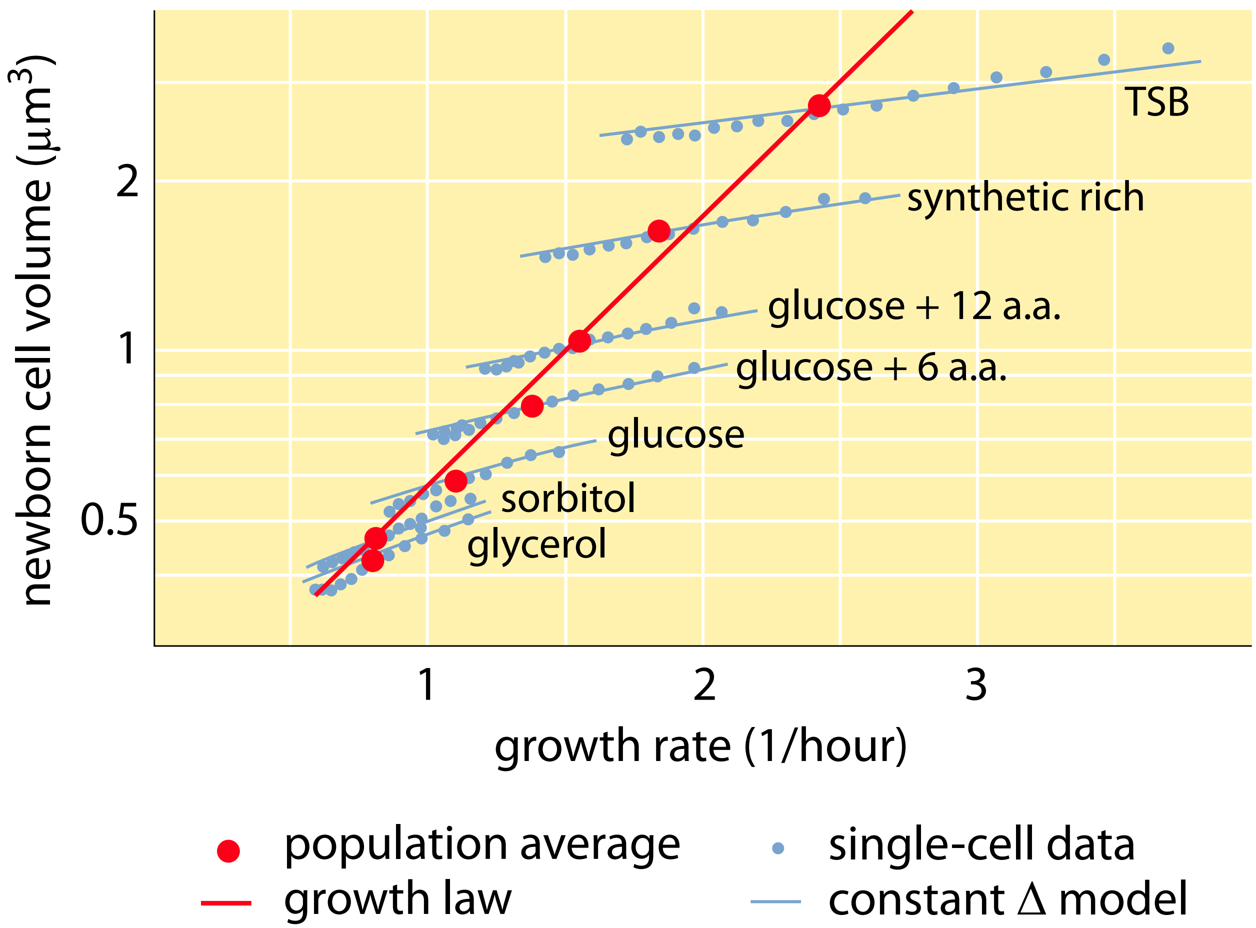
# What sets the limit on how fast one cell can become two cells?




*Bacillus subtilis* in rich medium

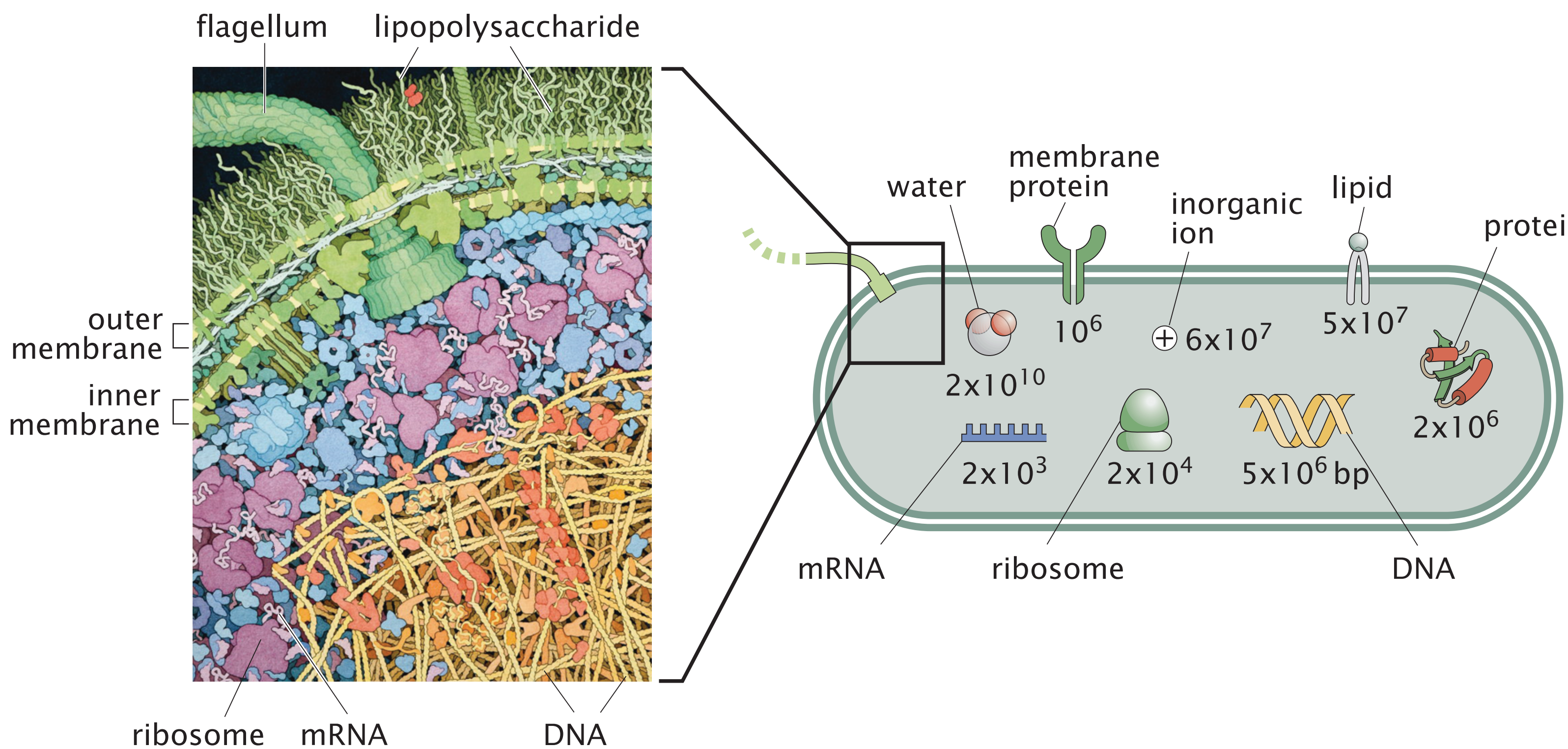
Rico Rojas

# What sets the growth rate under different conditions? *E. coli* data...




Data from Suckjoon Jun

# What is in a cell? We will need to make a second copy of everything




Which components are likely to take the longest time to make?
What numbers do we need to know?

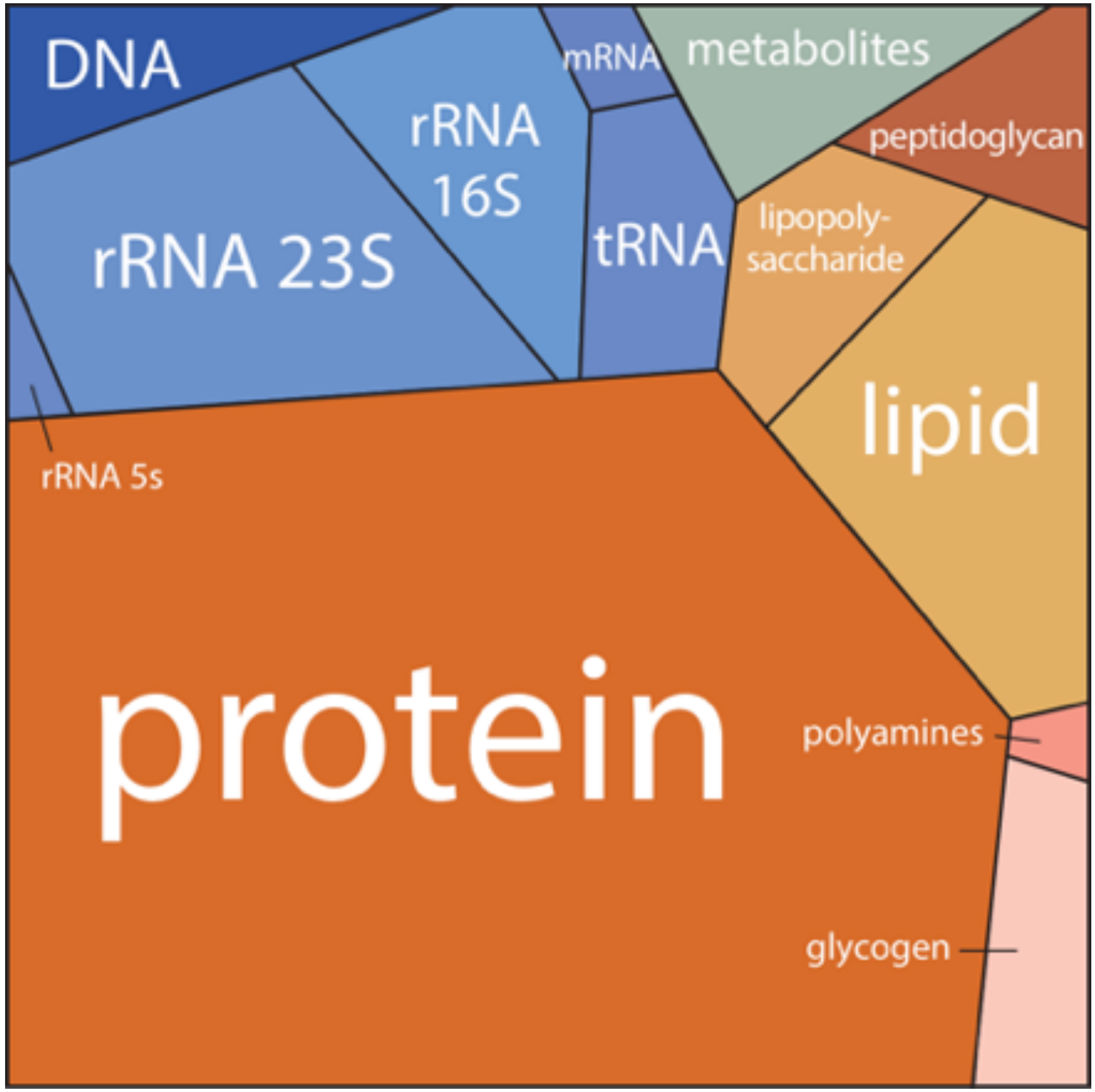
# Molecular census of *E. coli*

| macromolecule | percentage of total dry weight | weight per cell (fg) | characteristic molecular weight (Da) | number of molecules per cell |
|---|---|---|---|---|
| protein | 55 | 165 | $3 \times 10^4$ | 3,000,000 |
| RNA | 20 | 60 | | |
| 23 S rRNA | | 32 | $1 \times 10^6$ | 20,000 |
| 16 S rRNA | | 16 | $5 \times 10^5$ | 20,000 |
| 5 S rRNA | | 1 | $4 \times 10^4$ | 20,000 |
| transfer | | 9 | $2 \times 10^4$ | 200,000 |
| messenger | | 2 | $1 \times 10^6$ | 1,400 |
| DNA | 3 | 9 | $3 \times 10^9$ | 2 |
| lipid | 9 | 27 | 800 | 20,000,000 |
| lipopolysaccharide | 3 | 9 | 8000 | 1,000,000 |
| peptidoglycan | 3 | 9 | $(1000)_n$ | 1 |
| glycogen | 3 | 9 | $1 \times 10^6$ | 4,000 |
| metabolites and cofactors pool | 3 | 9 | | |
| inorganic ions | 1 | 3 | | |
| total dry weight | 100 | 300 | | |
| water (70% of cell) | | 700 | | |
| total cell weight | | 1000 | | |

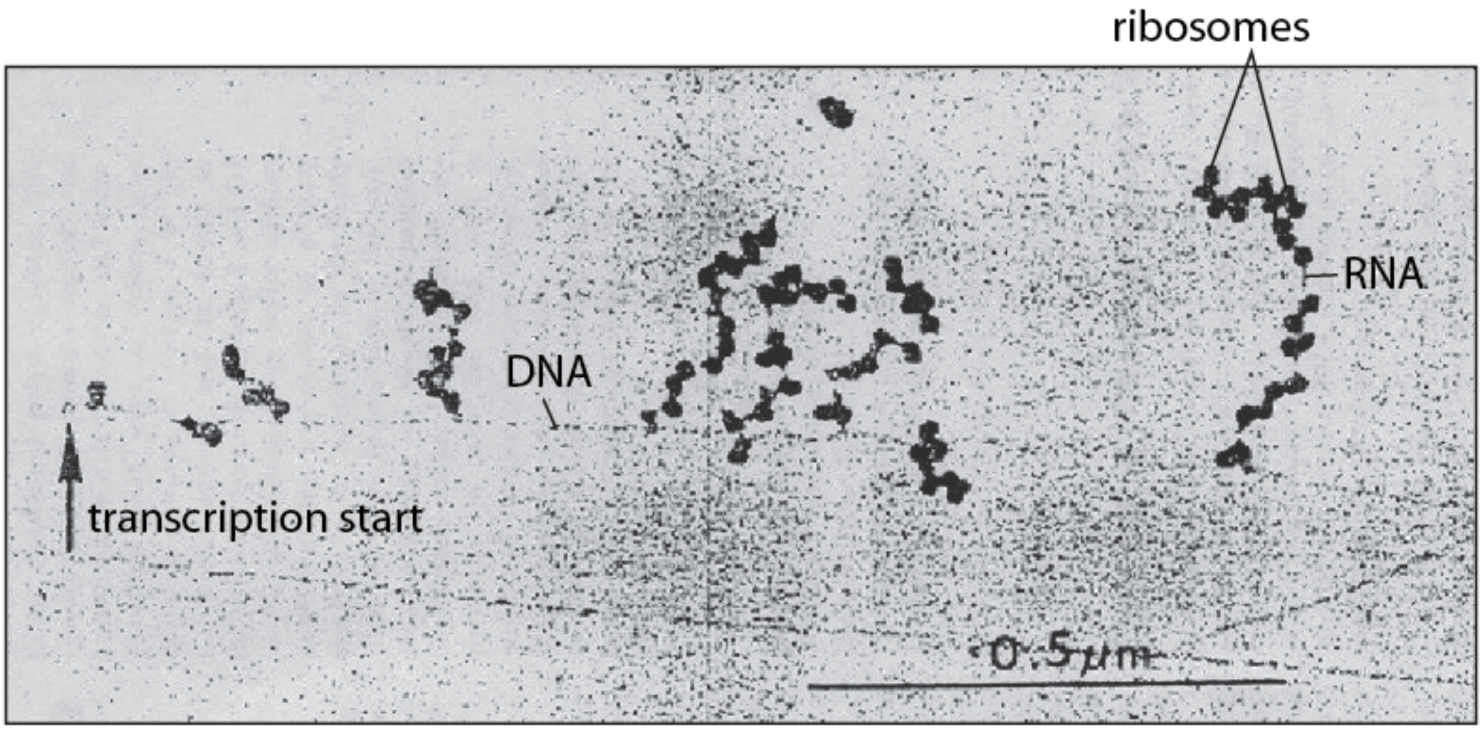
**composition rules of thumb**

- carbon atoms ~$10^{10}$
- 1 molecule per cell gives ~1 nM conc.
- ATP required to build and maintain cell over a cell cycle ~$10^{10}$
- glucose molecules needed per cell cycle ~$3 \times 10^9$ (2/3 of carbons used for biomass and 1/3 used for ATP)

# Another view of the *E. coli* data



# The Central Dogma in action…




What do you need to know to estimate the speed of RNA polymerase? Of the ribosome?

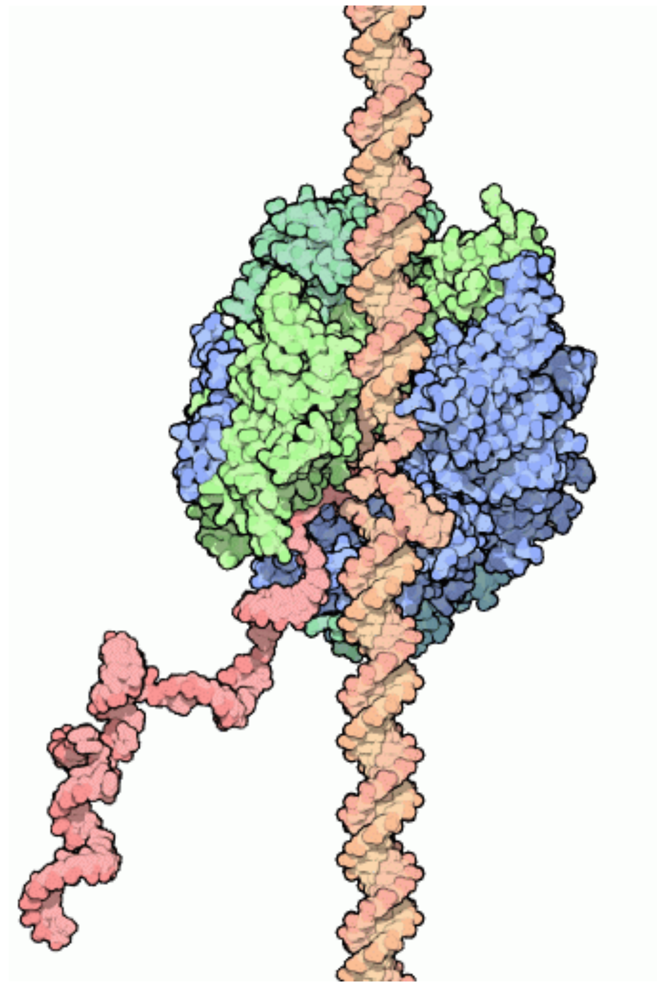
Miller et al., 1970, Science 169:392

# Enzymes of the Central Dogma

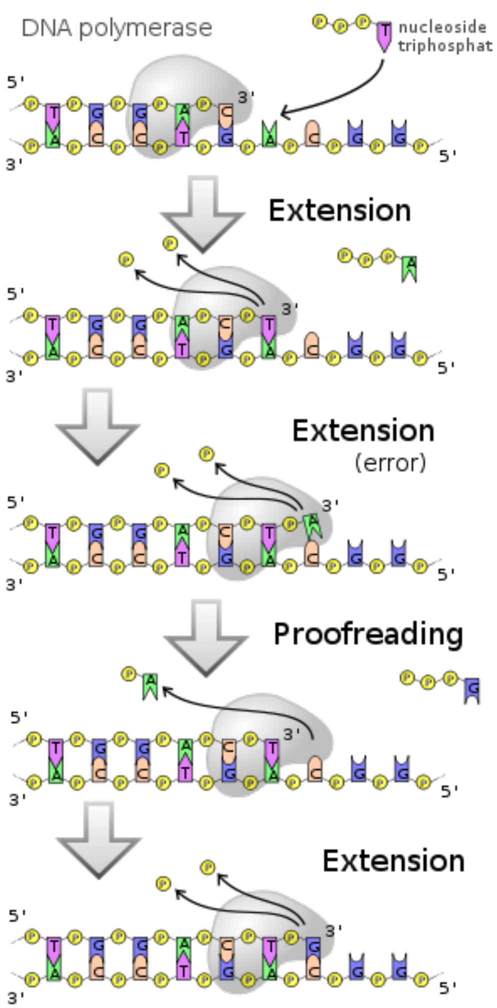
DNA polymerase
~500-1000 nucleotides per second
(for *E. coli*, probably ~600)

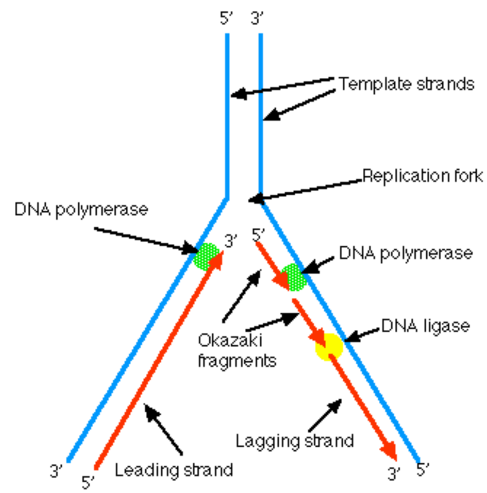
RNA polymerase
~30-90 nucleotides per second
(weak!)

Ribosome
~10-20 amino acids per second

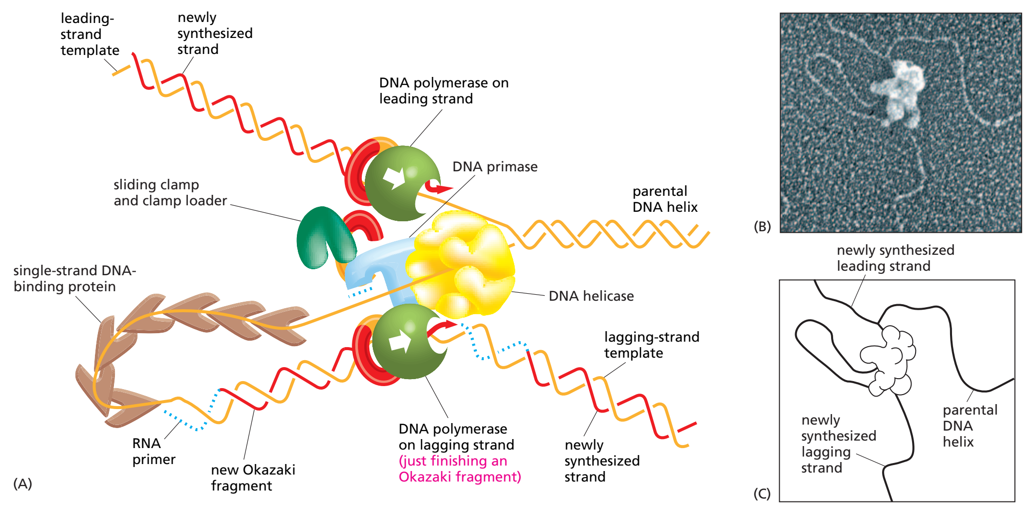


# DNA polymerase synthesizes DNA in the 5’ to 3’ direction



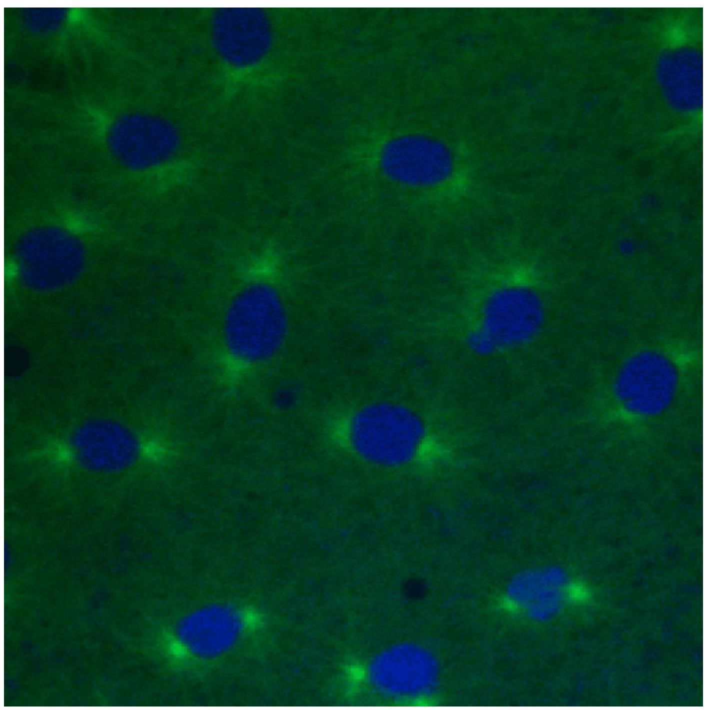

“Lagging strand” has to continually reinitiate replication and ligate fragments together



# How fast can a cell replicate all of its DNA?

*E. coli*? Fastest cell division time ~20 minutes

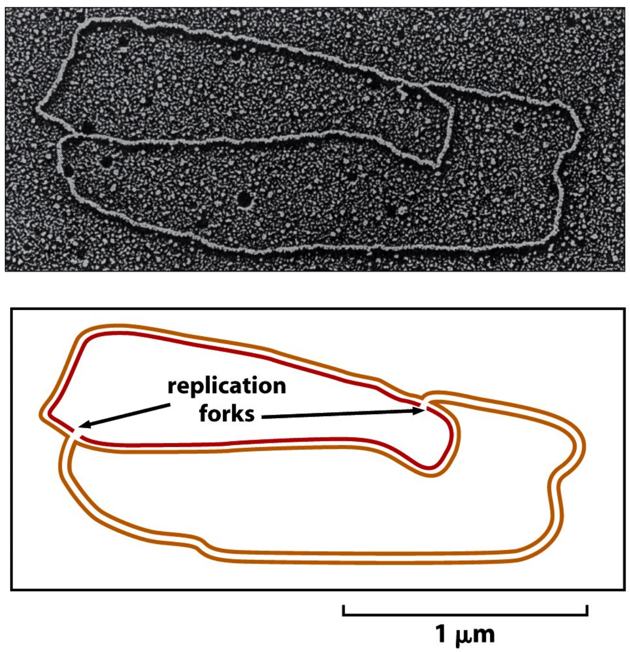
*Drosophila*? Fastest cell division time ~8 minutes (!)



Does this make sense?
Let’s calculate!

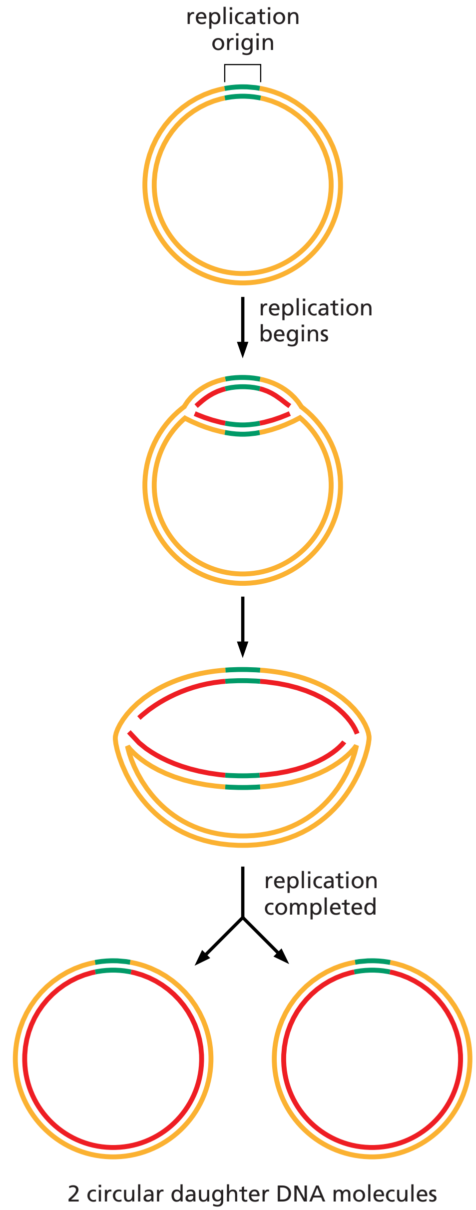
Fastest bacterial division times:
*Vibrio natriegens* - 9.8 minutes
*Clostridium perfringens* ~6.3 min

# *E. coli* has a single circular chromosome; replication starts at a single origin






Two replication forks on a bacterial plasmid




*E. coli* genome size ~4.6 x $10^6$ base pairs

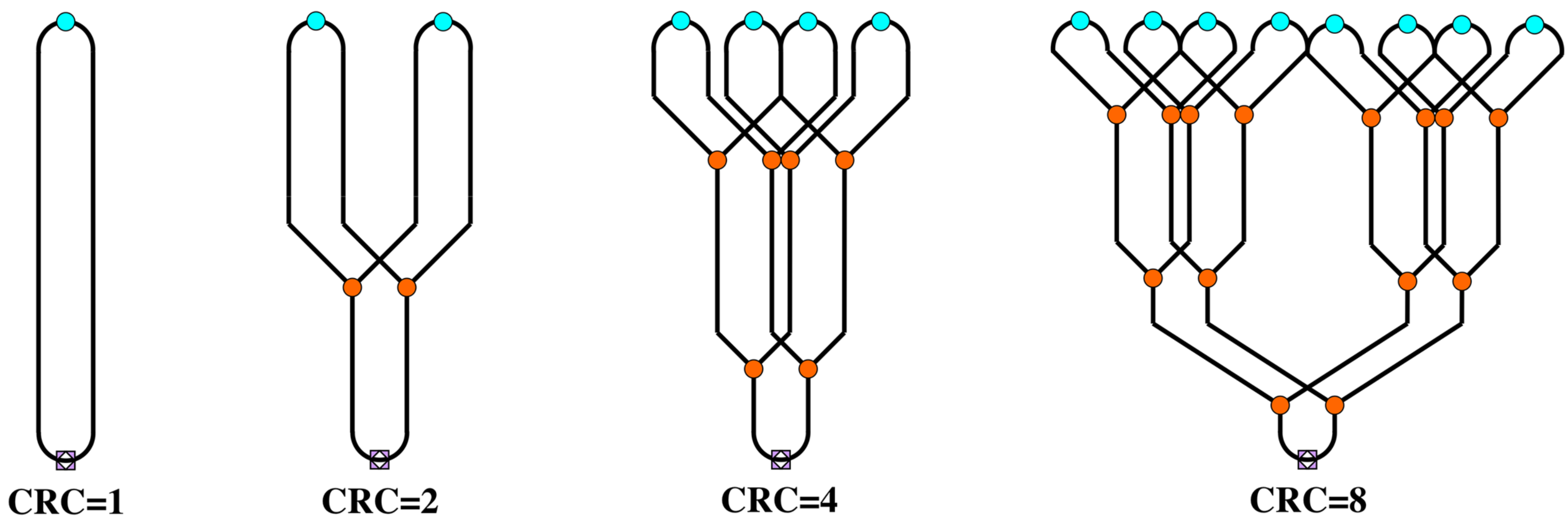
DNA polymerase speed ~600 nucleotides/sec

# How long will it take for *E. coli* to replicate its chromosome?

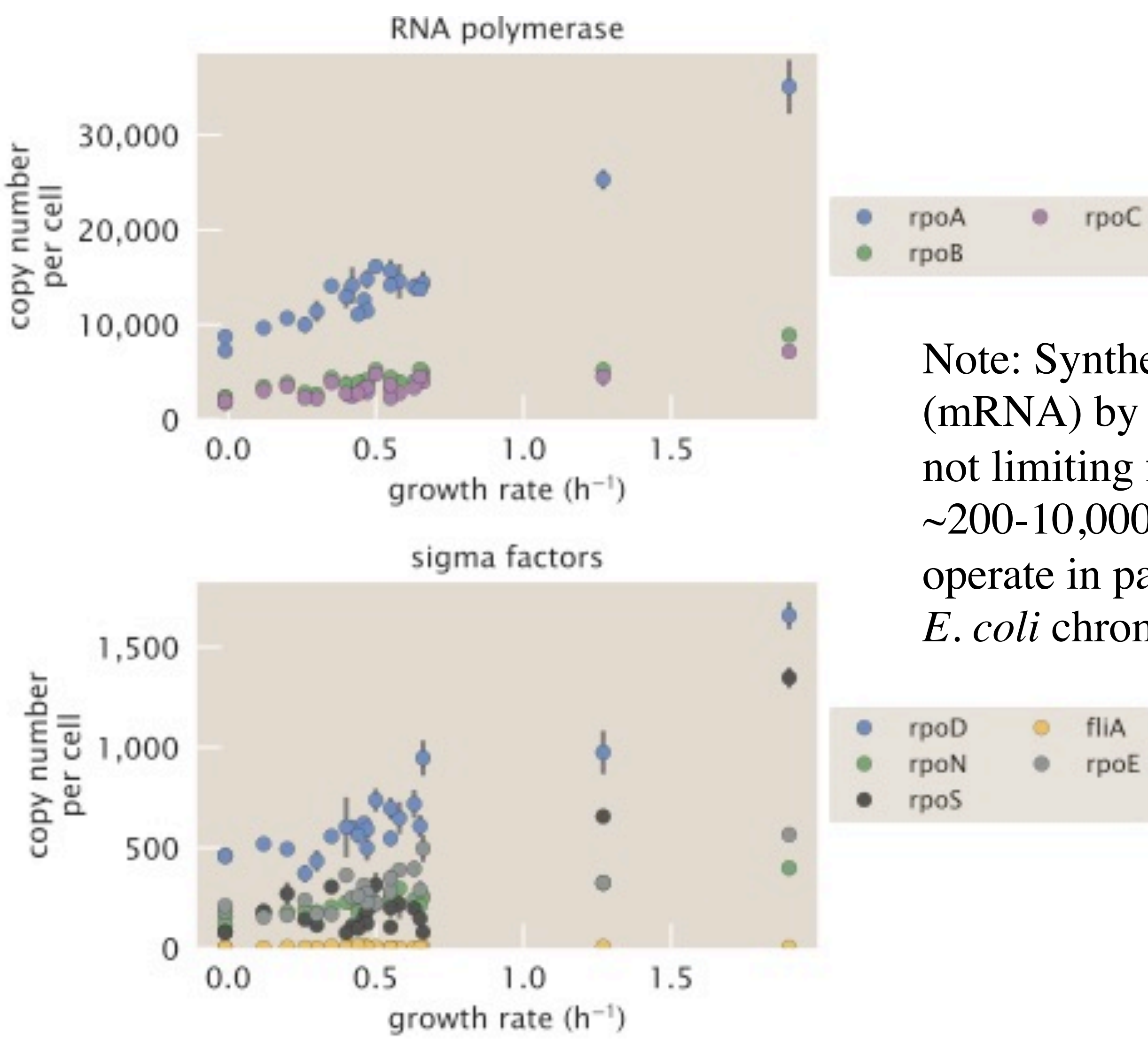
*E. coli* genome size ~4.6 x $10^6$ base pairs
DNA polymerase speed ~600 nucleotides/sec

Replication time ~60-70 minutes (!)

How does this work? Parallel processing / rereplication…



# RNA polymerase components as a function of cell growth rate




Note: Synthesis of messenger RNA (mRNA) by RNA polymerase is probably not limiting for bacterial growth; there are ~200-10,000 copies per cell, and they can operate in parallel on all the genes in the *E. coli* chromosome

# Importing carbon skeletons

What is the mass of an *E. coli* cell?

What fraction is dry mass? (~30%)

How much of dry mass is carbon? (~half)

Gives ~ $10^{10}$ carbon atoms per cell

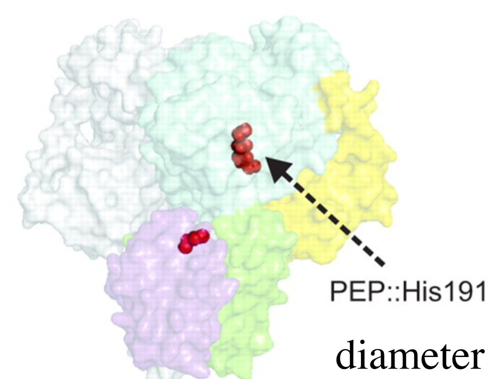
Import glucose - 6 carbons per sugar

Glucose transporter:

- Turnover rate ~200 molecules/sec
- How many copies do you need?
- Can they fit without crowding?

Note: expression data suggests 3000-15,000 copies
Higher expression in rich media (does this make sense?)

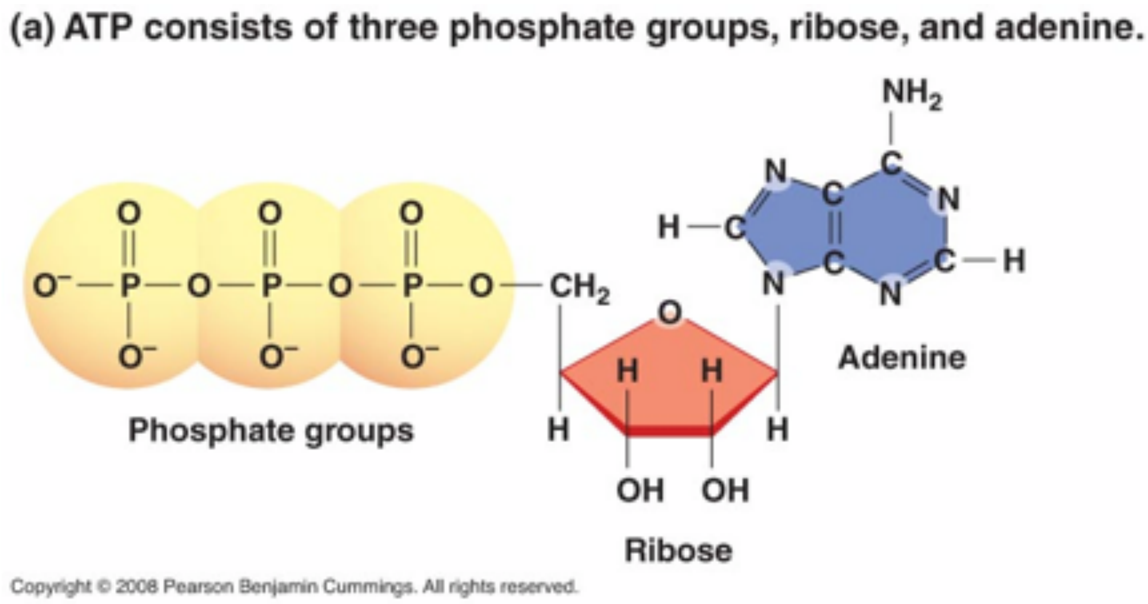
PtsI

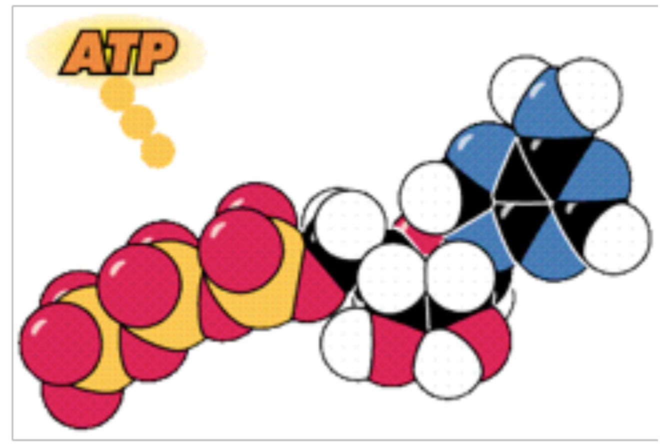



diameter ~5 nm

PEP ↔ P-His
*E. coli*

# How much ATP is needed per cell cycle?



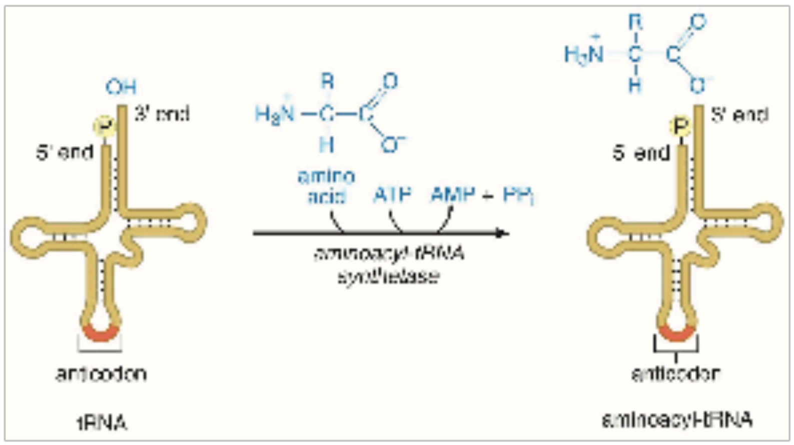

# What is most of the ATP used for?

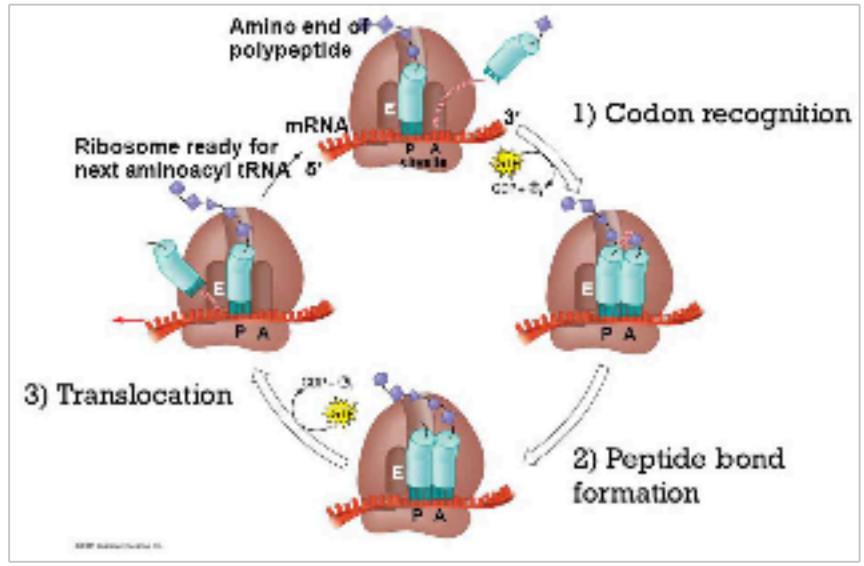
# Translation is very expensive!

"One ATP->AMP (2 ~P) for activation, one GTP->GDP for EF-Tu mediated transfer on the aminoacyl-tRNA to the A site, one GTP->GDP for the EF-G translocation of peptidyl-tRNA to the P site"

load amino acid onto tRNA

elongation cycle



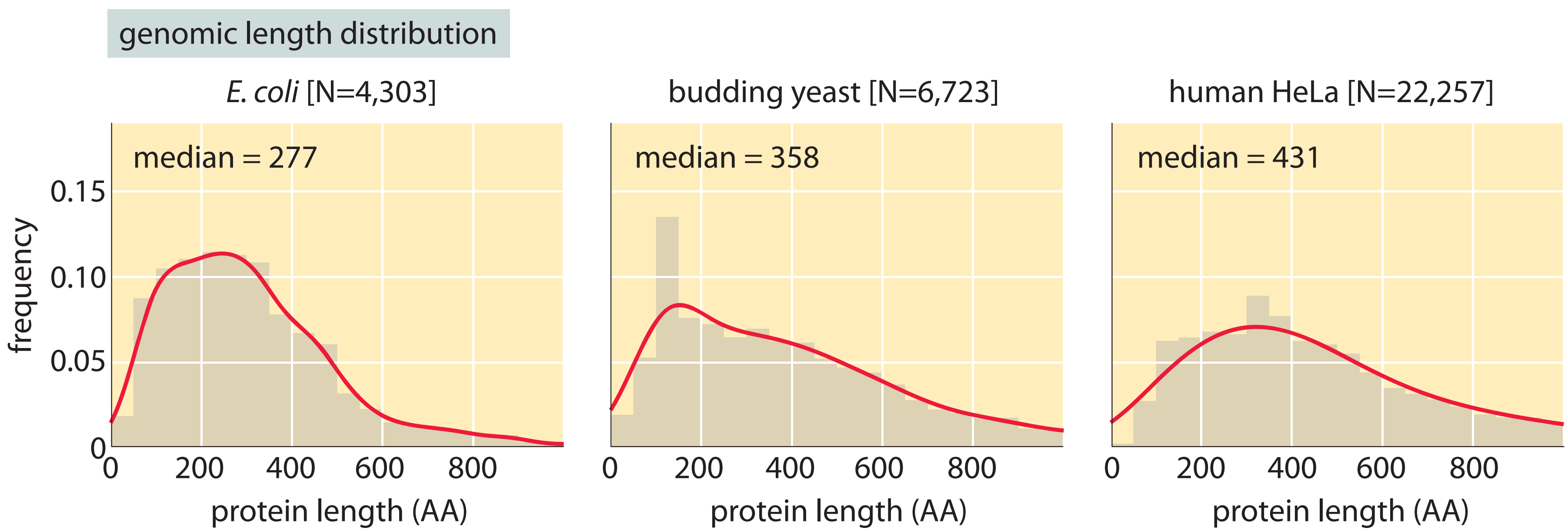




ATP ⟶ AMP

2GTP ⟶ 2GDP

4 ATPs worth of energy

# How big is a protein? How many proteins are there in one cell?



# Quantitative estimates for ATP usage

*Antonie van Leeuwenhoek* **39** (1973) 545–565

A theoretical study on the amount of ATP required for synthesis of microbial cell material

A. H. STOUTHAMER

*Biological Laboratory, Free University, de Boelelaan 1087, Amsterdam, the Netherlands*

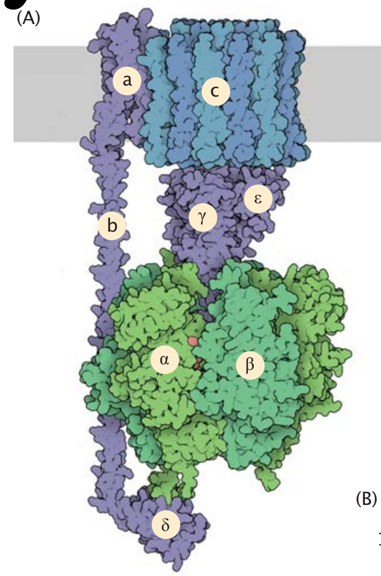
Table 3. ATP requirement for the formation of microbial cells from preformed monomers (glucose, amino acids and nucleic acid bases). Formation of lipids from acetate or glucose are separately considered.

| Macromolecule | Amount of monomer moles × $10^{-4}$/g cells | ATP required per monomer moles/mole | | ATP required moles × $10^{-4}$/g cells | |
|---|---|---|---|---|---|
| | | Lipids formed from | | | |
| | | Acetate | Glucose | Acetate | Glucose |
| Polysaccharide | 10.26 | 2 | 2 | 20.52 | 20.52 |
| Protein | 47.85 | 4 | 4 | 191.40 | 191.40 |
| Lipid | 1.40 | 33 | 1 | 46.20 | 1.40 |
| RNA[1] | 4.60 | 5 | 5 | 23.00 | 23.00 |
| DNA | 0.96 | 6 | 6 | 5.76 | 5.76 |
| Turnover RNA | | | | 13.90 | 13.90 |
| Total | | | | 300.78 | 255.98 |
| ATP required for transport of | | | | | |
| Amino acids | | | | 47.85 | 47.85 |
| Acetate | | | | 22.40 | – |
| Potassium ions | | | | 1.92 | 1.92 |
| Phosphate | | | | 7.74 | 7.74 |
| Total ATP requirement | | | | 380.69 | 313.49 |
| $Y_{ATP}^{MAX} = \frac{10\,000}{\text{Total ATP requirement}}$ | | | | 26.4 | 31.9 |

[1] For the calculation of the RNA content the UMP content (Table 1) has been taken as basis.

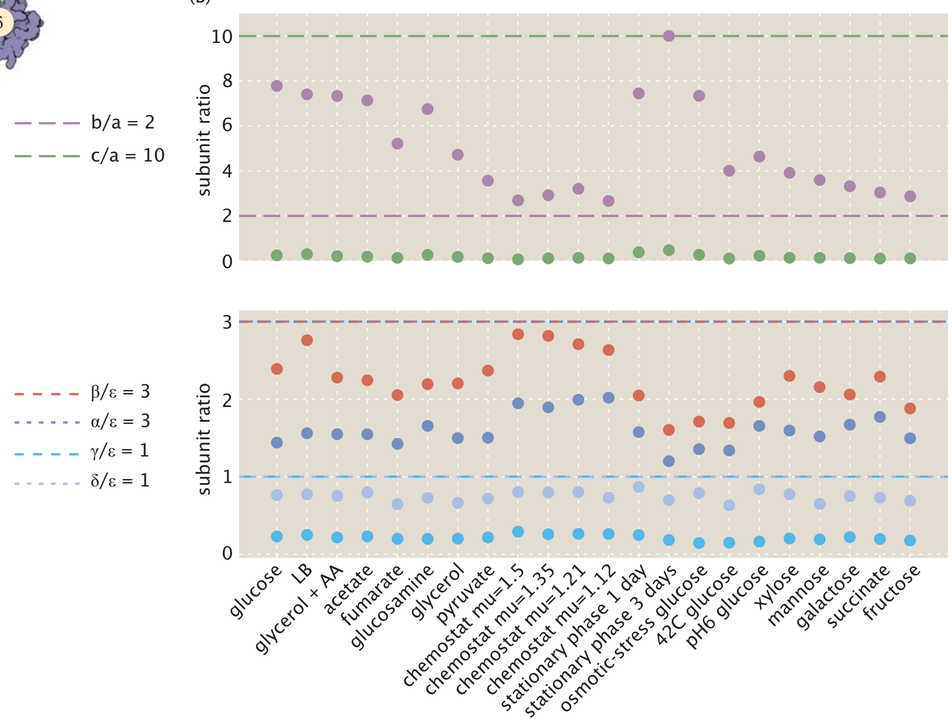
# ATP synthase copy numbers per cell

(A)

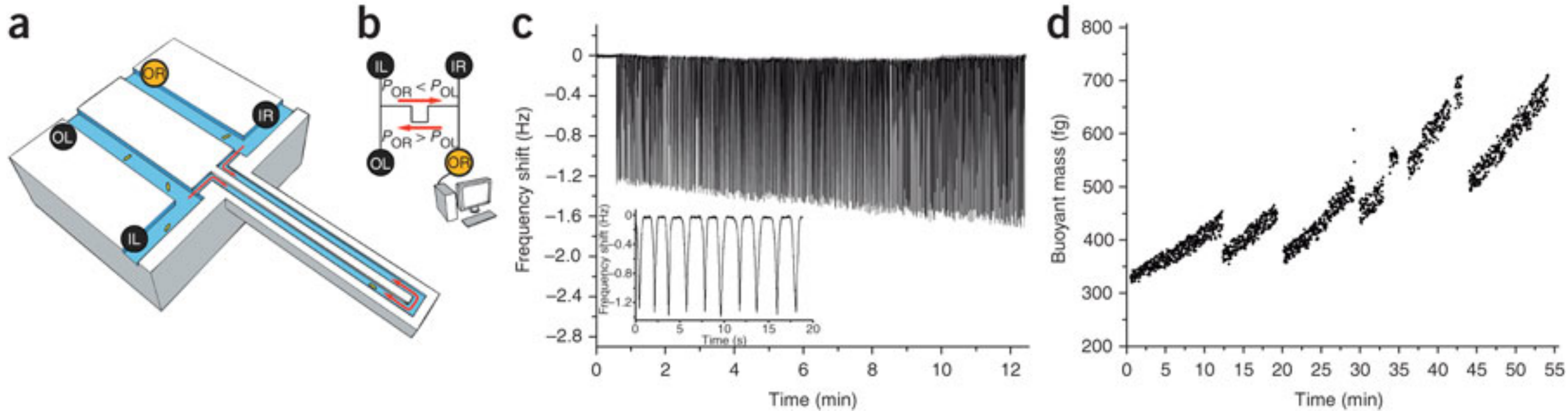


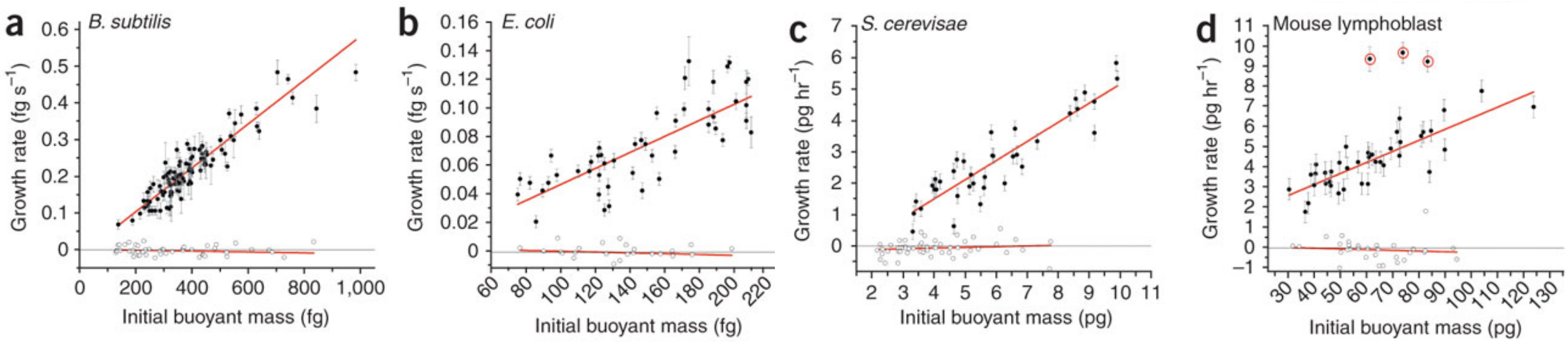

| | ribosomal profiling | | mass spectrometry | | |
|---|---|---|---|---|---|
| | MOPS minimal | measured ratio | M9 glucose | measured ratio | expected ratio |
| a | 3471 | 1.0 | 1155 | 1.0 | 1 |
| b | 4477 | 1.3 | 8988 | 7.8 | 2 |
| c | 23624 | 6.8 | 286 | 0.3 | 10 |
| α | 9565 | 2.6 | 14552 | 1.4 | 3 |
| β | 8213 | 2.3 | 24156 | 2.4 | 3 |
| γ | 3131 | 0.9 | 2307 | 0.2 | 1 |
| δ | 2677 | 0.7 | 7700 | 0.8 | 1 |
| ε | 3640 | 1.0 | 10112 | 1.0 | 1 |

(B)



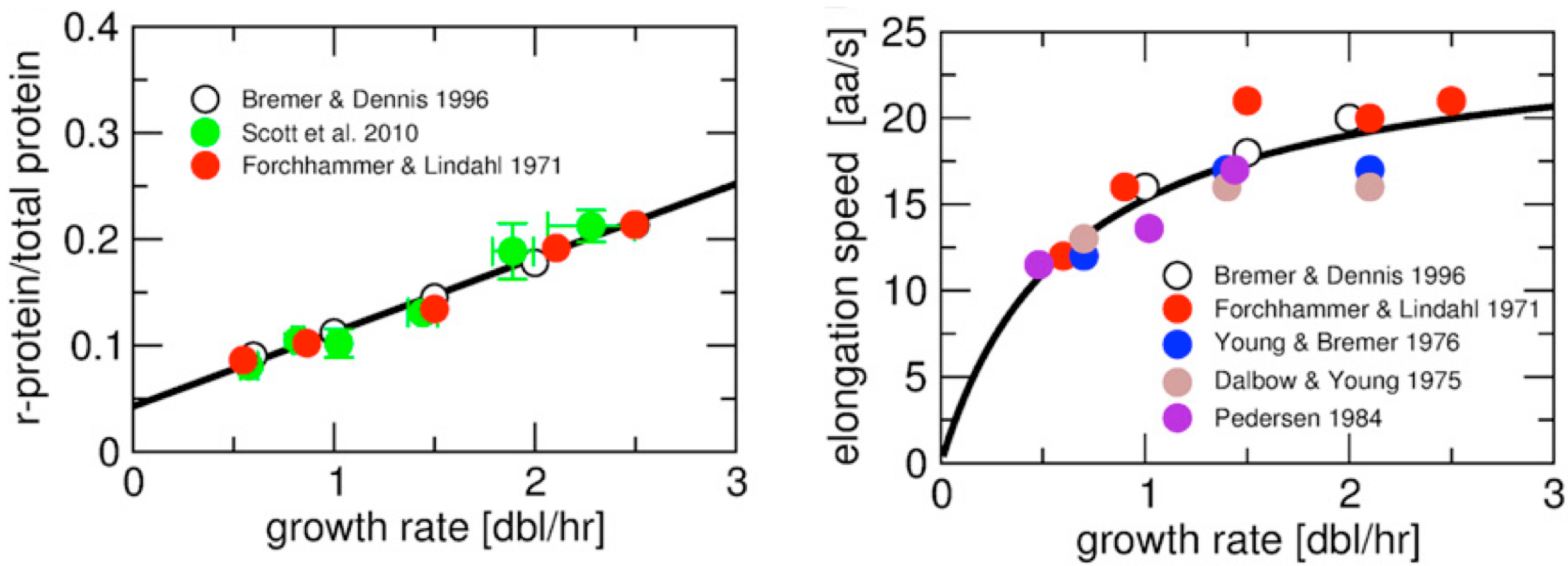
# Bigger cells grow faster; what does this imply?



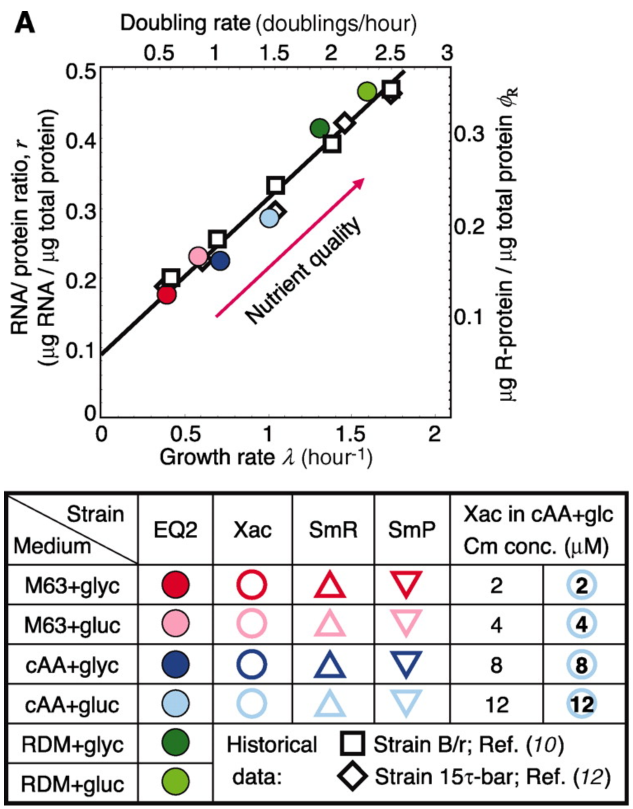

Godin et al., 2010, *Nature Methods* **7**: 387

# *E. coli* ribosome fraction, ribosome elongation speed, and cell growth rate

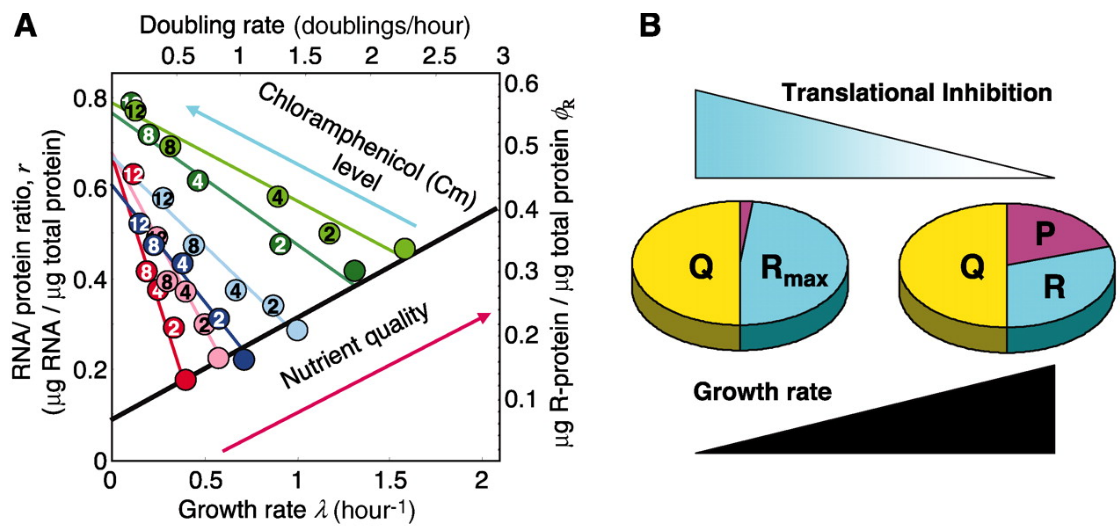



Klumpp et al., 2013, *PNAS*, **110**: 16754

# Faster-growing cells have more ribosomes

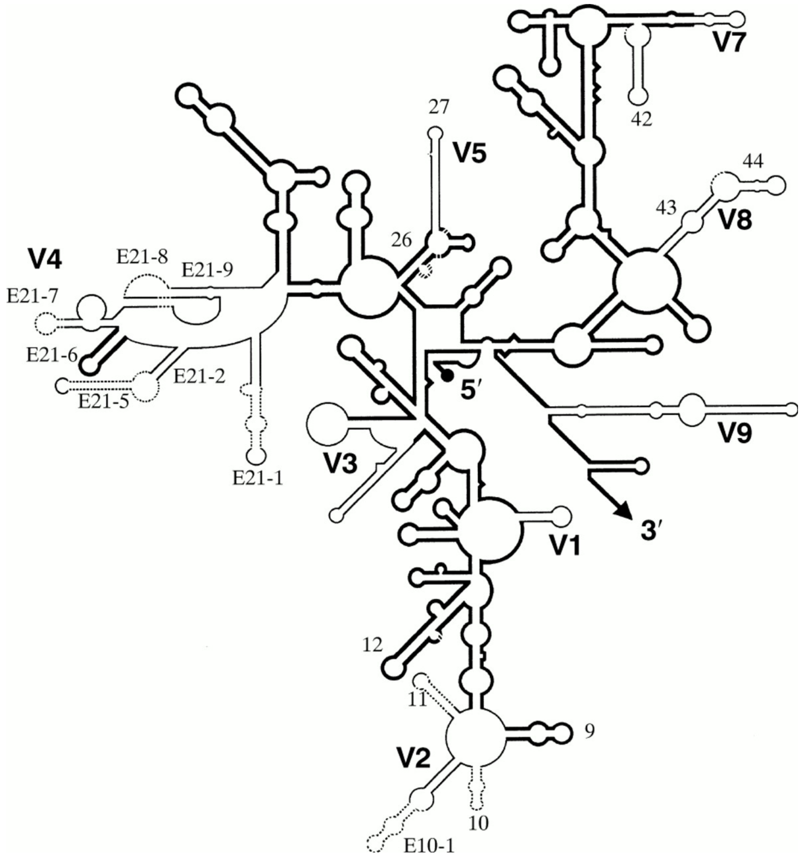



Also, inhibiting ribosomes with antibiotics drives the ribosome content higher




Scott et al., 2010, *Science* **330**: 1099

# Ribosomes!



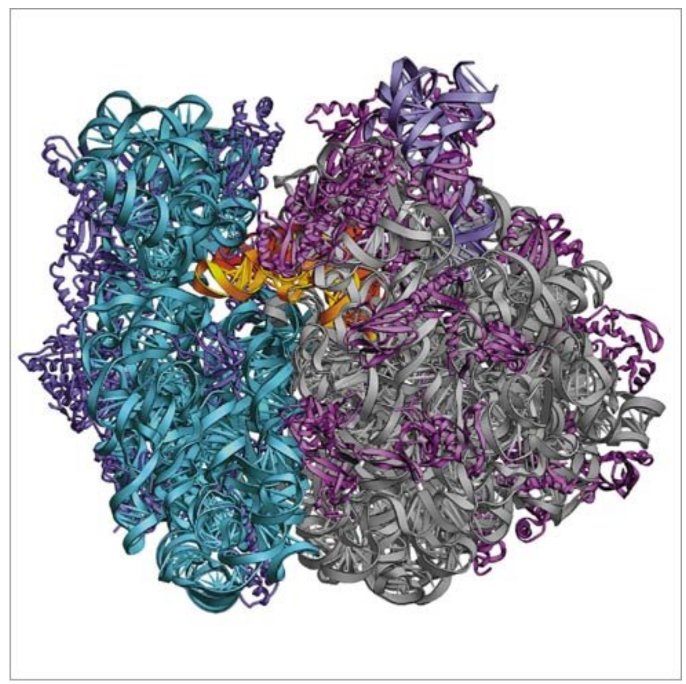

25 nm machine

Bacterial: 3 large RNAs, 55 proteins

Longest RNA ~2900 nucleotides

Eukaryotic: 4 large RNAs, >80 proteins

Function requires tRNAs, aminoacyl-tRNA synthetases, etc.



Small subunit RNA:
Vx = variable regions (alignment)

Total mass ~ 2.5 MDa
~2/3 RNA, ~1/3 protein

# How many ribosomes are there in a single cell?

What are some approaches for making this estimate?

Overall protein synthesis rate

Volume and volume fraction (what % is ribosomes?)

Others?

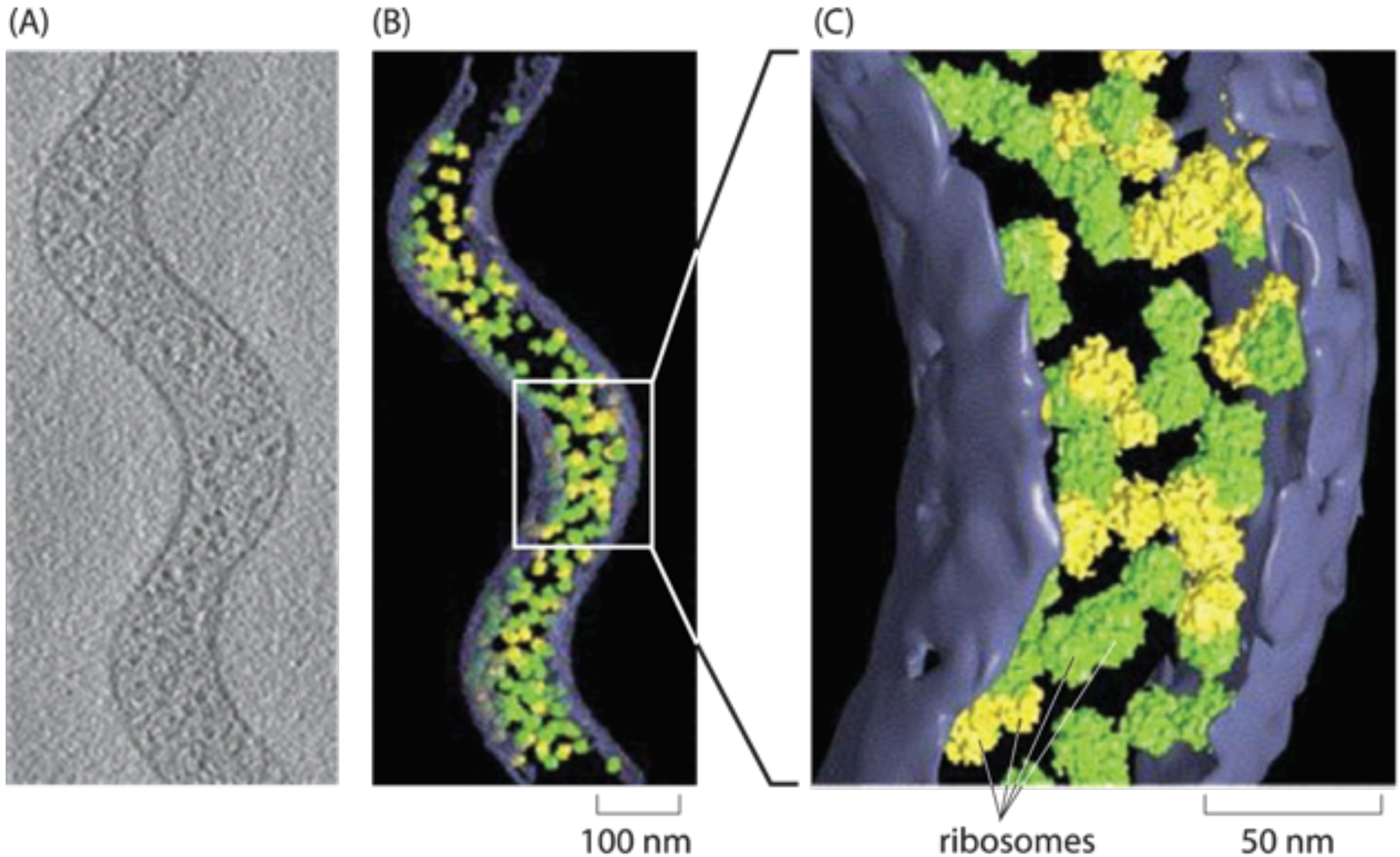
Mass and mass fraction (what % is ribosomes?)

Use the Miller spread; how many ribosomes are associated with one gene?

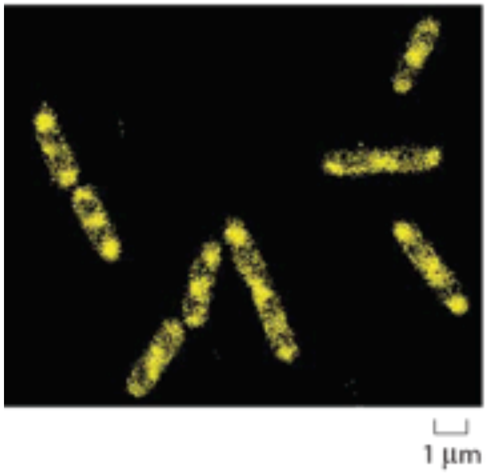
~80; if representative and all are fully saturated, and all genes are equally transcribed/translated, then get $2 \times 10^4$

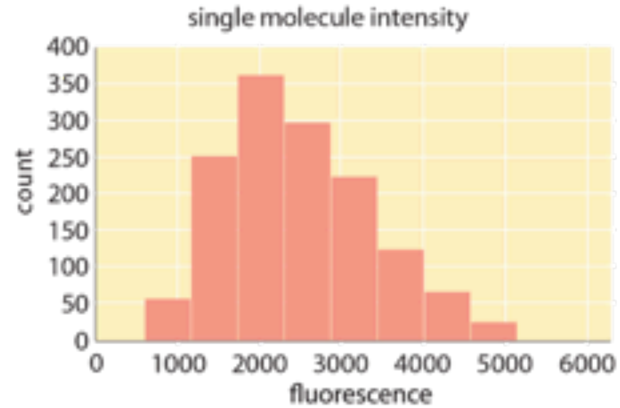
# Estimating ribosomal density

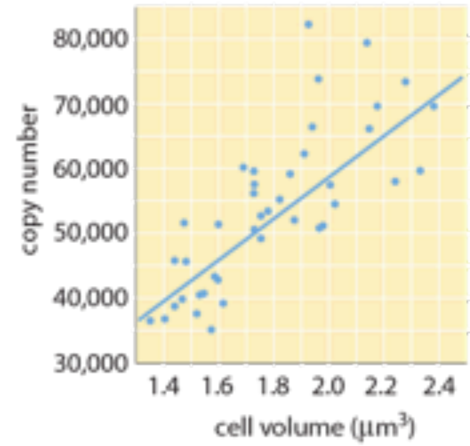
Cryo Electron Microscopy image of *Spiroplasma melliferum*



# Number of ribosomes in *E. coli*




Calculating from the dry mass

| doubling time (min) | ribosomes per cell | dry mass per cell (fg) | ribosome dry mass fraction (%) | ribosome fraction x doubling time (min) |
|---|---|---|---|---|
| 24 | 72000 | 870 | 37 | 9.0 |
| 30 | 45000 | 640 | 32 | 9.5 |
| 40 | 26000 | 430 | 27 | 11 |
| 60 | 14000 | 260 | 24 | 14 |
| 100 | 6800 | 150 | 21 | 20 |

*E. coli and Salmonella handbook*

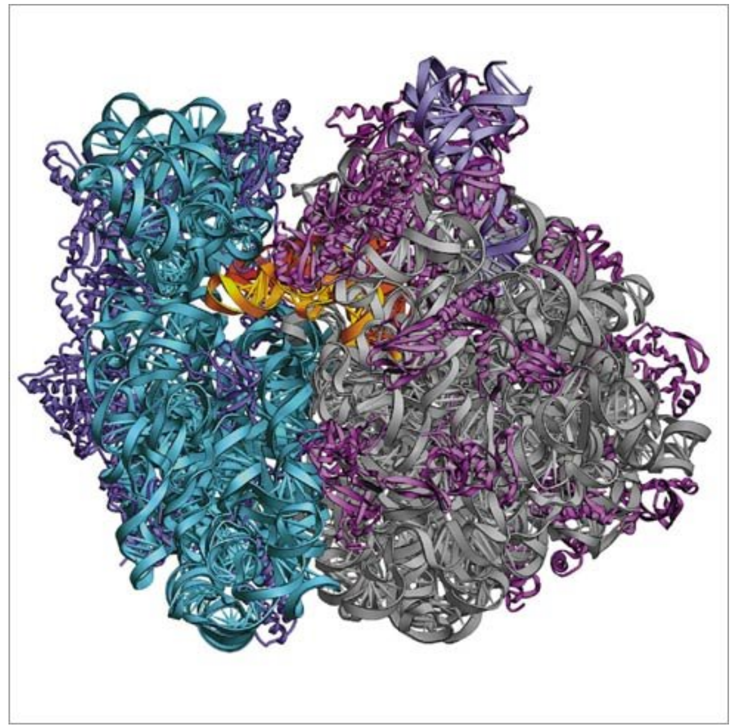
# How long does it take to make the ribosomal RNA?

In *E. coli*, RNA lengths are:
1542 (16S) - small subunit
120 (5S) - large subunit
2904 (23S) - large subunit

~2 x $10^4$ ribosomes per cell

RNA polymerase speed
~40 nucleotides/sec

How close together can the polymerases pack?

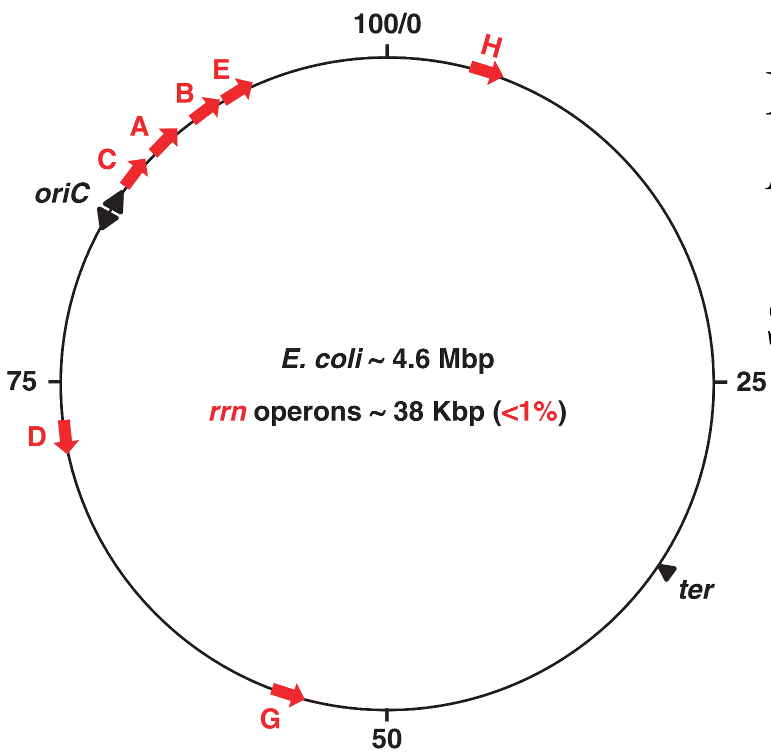


Initiation bubble ~50 bp
Assume RNAP can load 1 per sec

Let's calculate!

# How is this possible?

$2 \times 10^4$ ribosomes • $3 \times 10^3$ nucs (large RNA) / 40 nucs/sec = $1.5 \times 10^6$ sec total = 17 days
Assume multiple RNA polymerases per gene, load ~1 per sec (40-50 bp spacing)
= up to 70 polymerases working in parallel per gene at steady state
…brings it down to ~6 hours
Need ~20 copies of the rRNA genes all being transcribed in parallel




More clever parallel processing:
*E. coli* has SEVEN copies of the ribosomal RNA genes
Some of these copies are located quite near the origin of replication, so they are overrepresented in fast-growing cells
(remember, up to 8-16 copies of the origin for cells growing at maximum rate)

# What does a ribosome have to do to make (the proteins for) another ribosome?

Observation of *Escherichia coli* Ribosomal Proteins and Their Posttranslational Modifications by Mass Spectrometry

Randy J. Arnold and James P. Reilly
*Department of Chemistry, Indiana University, Bloomington, Indiana 47405*
Analytical Biochemistry 269, 105–112 (1999)

How long does this take?
Let's calculate!

Total mass ~ 2.5 MDa
(One amino acid ~110 Da)
~2/3 RNA, ~1/3 protein

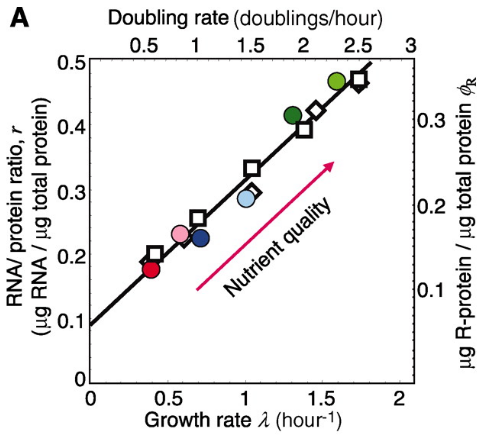
Speed:
~10-20 amino acids per second

| Subunit | Sequence mass (Da) | Experimental mass (Da) |
|---|---|---|
| S1 | 61158.3 | — |
| S2 | 26612.6 | 26610.5 |
| S3 | 25852.0 | 25851.9 |
| S4 | 23338.0 | 23339.5 |
| S5 | 17472.3 | 17514.8 |
| S6 | 15187.1 | 15187.2 |
| S7 | 19888.0 | 19888.7 |
| S8 | 13995.4 | 13993.2 |
| S9 | 14725.1 | 14723.3 |
| S10 | 11735.6 | 11734.5 |
| S11 | 13713.8 | 13727.7 |
| S12 | 13605.9 | 13651.3 |
| S13 | 12968.3 | 12968.1 |
| S14 | 11449.3 | 11449.3 |
| S15 | 10137.6 | 10137.6 |
| S16 | 9190.6 | 9190.5 |
| S17 | 9573.3 | 9573.0 |
| S18 | 8855.3 | 8897.0 |
| S19 | 10299.1 | 10299.6 |
| S20 | 9553.2 | 9553.6 |
| S21 | 8368.8 | 8368.8 |
| S22 | 5095.8 | 5095.9 |

| Subunit | Sequence mass (Da) | Experimental mass (Da) |
|---|---|---|
| L1 | 24598.6 | 24598.9 |
| L2 | 29729.4 | 29732.3 |
| L3 | 22243.6 | 22257.2 |
| L4 | 22086.6 | 22086.2 |
| L5 | 20170.5 | 20169.8 |
| L6 | 18772.7 | 18772.7 |
| L7 | 12164.1 | 12206.7 |
| L9 | 15769.1 | 15769.7 |
| L10 | 17580.5 | 17581.1 |
| L11 | 14744.3 | 14870.2 |
| L12 | 12164.1 | 12174.4 |
| L13 | 16018.6 | 16018.0 |
| L14 | 13541.1 | 13540.2 |
| L15 | 14980.5 | 14980.1 |
| L16 | 15281.3 | 15326.2 |
| L17 | 14364.7 | 14364.7 |
| L18 | 12769.7 | 12769.8 |
| L19 | 13002.1 | 13001.7 |
| L20 | 13365.8 | 13366.9 |
| L21 | 11564.4 | 11562.7 |
| L22 | 12226.4 | 12225.3 |
| L23 | 11199.2 | 11198.0 |
| L24 | 11185.1 | 11186.5 |
| L25 | 10693.5 | 10693.4 |
| L26 | 9553.2 | 9553.6 |
| L27 | 8993.3 | 8993.5 |
| L28 | 8875.3 | 8875.0 |
| L29 | 7273.5 | 7273.4 |
| L30 | 6410.6 | 6410.3 |
| L31 | 7871.1 | 7871.0 |
| L31 frag | 6971.1 | 6971.1 |
| L32 | 6315.2 | 6315.1 |
| L33 | 6240.4 | 6254.1 |
| L34 | 5380.4 | 5380.5 |
| L35 | 7157.8 | 7158.0 |
| L36 | 4364.4 | 4364.2 |

(800 kDa protein / 110 Da per amino acid) / 10-20 aa/sec = 6-12 minutes
Note each ribosome also has to make ~50-100 other proteins
Can we improve parallel processing by just making more ribosomes?

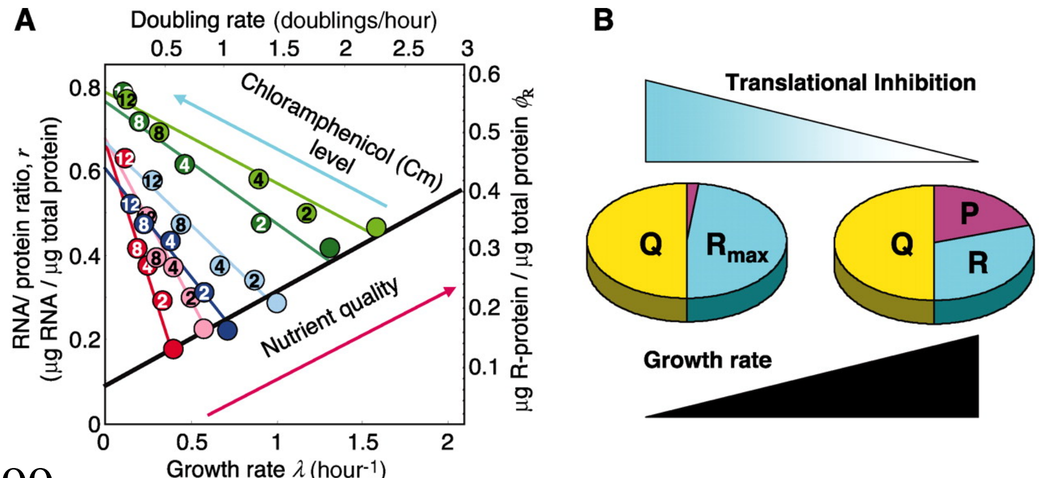
# Faster-growing cells have more ribosomes




| Medium \ Strain | EQ2 | Xac | SmR | SmP | Xac in cAA+glc Cm conc. (µM) | |
|---|---|---|---|---|---|---|
| M63+glyc | | | | | 2 | 2 |
| M63+gluc | | | | | 4 | 4 |
| cAA+glyc | | | | | 8 | 8 |
| cAA+gluc | | | | | 12 | 12 |
| RDM+glyc | | Historical data: | □ Strain B/r; Ref. (*10*) | | | |
| RDM+gluc | | | ◇ Strain 15τ-bar; Ref. (*12*) | | | |

Scott et al., 2010, *Science* **330**: 1099

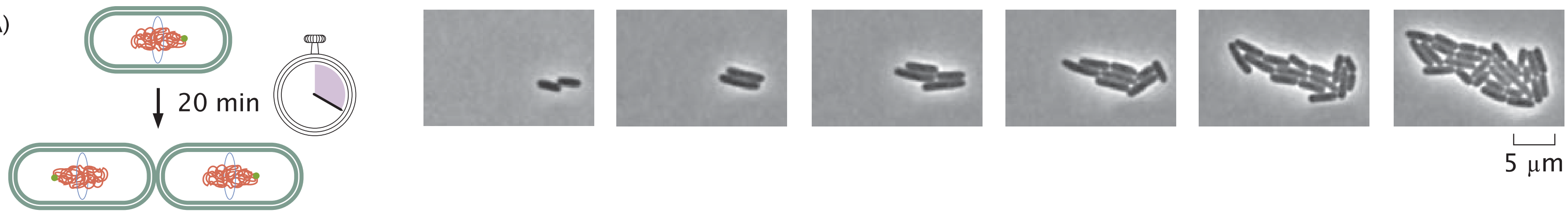
Also, inhibiting ribosomes with antibiotics drives the ribosome content higher



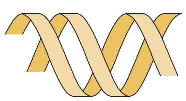
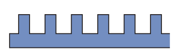
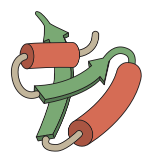
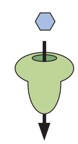
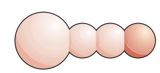
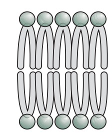
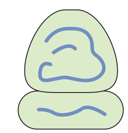

Our conclusion: Bacterial doubling time is limited by the amount of time it takes for a single ribosome to make all the ribosomal proteins for another ribosome.  Question: Why can't the cell just make more aminoacyl tRNAs?

# Putting it all together

(A)




(B)

| | chromosomes | mRNA | proteins | carbon import | ATP | lipids | ribosomes | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | RNA | proteins |
| |  |  |  |  |  |  |  | |
| HOW MUCH? | $5\times10^6$ bp | $2\times10^3$ mRNAs $\times\ 10^3$ nucs | $2\times10^6$ proteins $\times$ 300 aas | $10^{10}$ carbon atoms | ~$10^{10}$ ATP per cell cycle | $5\times10^7$ molecules | $2\times10^4$ ribosomes | |
| | | | | | | | $6\times10^4$ | $10^6$ |
| HOW MANY? | 40 DNA polymerases | 3000–5000 RNA polymerases | $2\times10^4$ ribosomes | 3000 glucose transporters | 3000–5000 ATP synthases | 10,000–20,000 fatty acid synthase complexes | 3000–5000 RNA polymerases | $2\times10^4$ ribosomes |
| HOW FAST? | 600 nucleotides per second | 40 nucleotides per second | 10–20 amino acids per second | 200 molecules per second | 1000 molecules per second | 1–2 molecules per second | 40 nucleotides per second | 10–20 amino acids per second |