Croissance bactérienne: des molécules aux populations

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Plan du cours

- Cours I

Les bactéries: croissance, physiologie et antibiotiques

- Cours II

Réparation des chromosomes chez *E. coli* à l'échelle de molécules individuelles

- Cours III

La réparation de l'ADN dépend des condition de croissance chez *E. coli*

Les bactéries: croissance, physiologie et antibiotiques

Plan

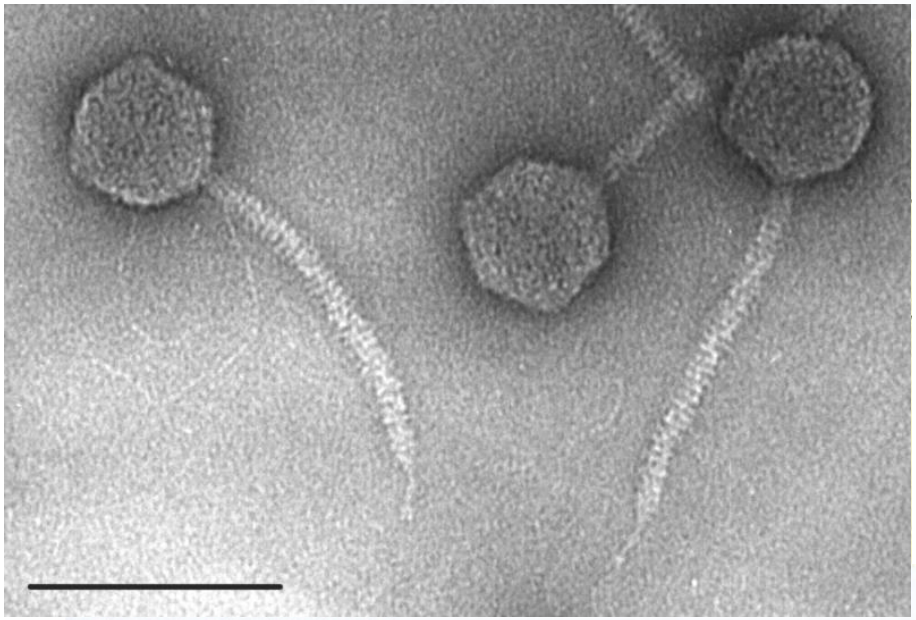
- Qu’est une bactérie?
- Qu’y-a-t-il dans une cellule?
- Notions de biologie moléculaire
- Régulation de l’expression des gènes
- Croissance et physiologie bactérienne

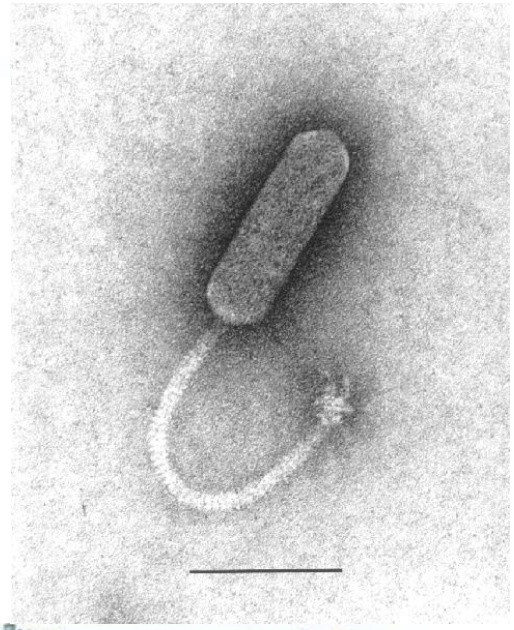
Le vivant

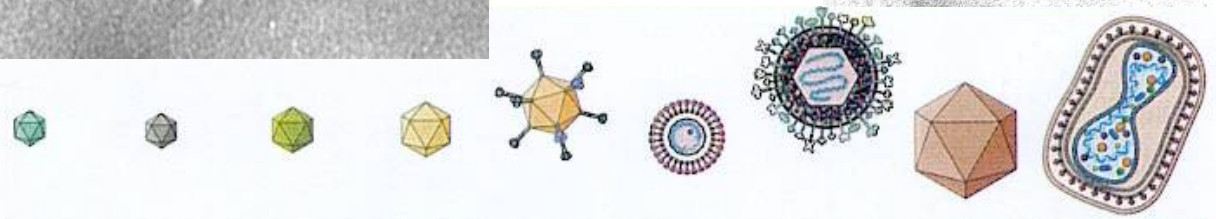
Trois définitions

- Organismes à reproduction autonome:
  - Capables de recopier l'information génétique (ADN)
  - Capables de traduire cette information en protéines
- Organismes soumis à « variation-sélection »
- Création « d'ordre » en utilisant de l'énergie

Les virus

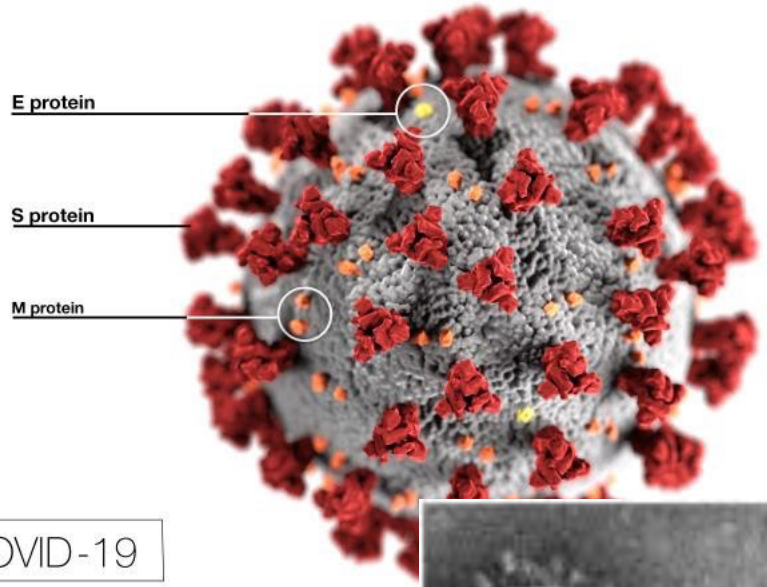






Properties									
Family name	Parvo	Circo	Polyoma	Papilloma	Adeno	Hepadna	Herpes	Irido	Pox
Virion polymerase	(–)	(–)	(–)	(–)	(–)	(+)	(–)	(–)	(+)
Virion diameter (nm)	18–26	12–26	40	55	70–90	42	150–200	125–300	170–200 × 300–450
Genome size (total in kb)	5	1.8–2.3	5	7–8	36–38	3.2	120–200	150–350	130–280

Un virus à éviter en ce moment: SRAS-Cov-2

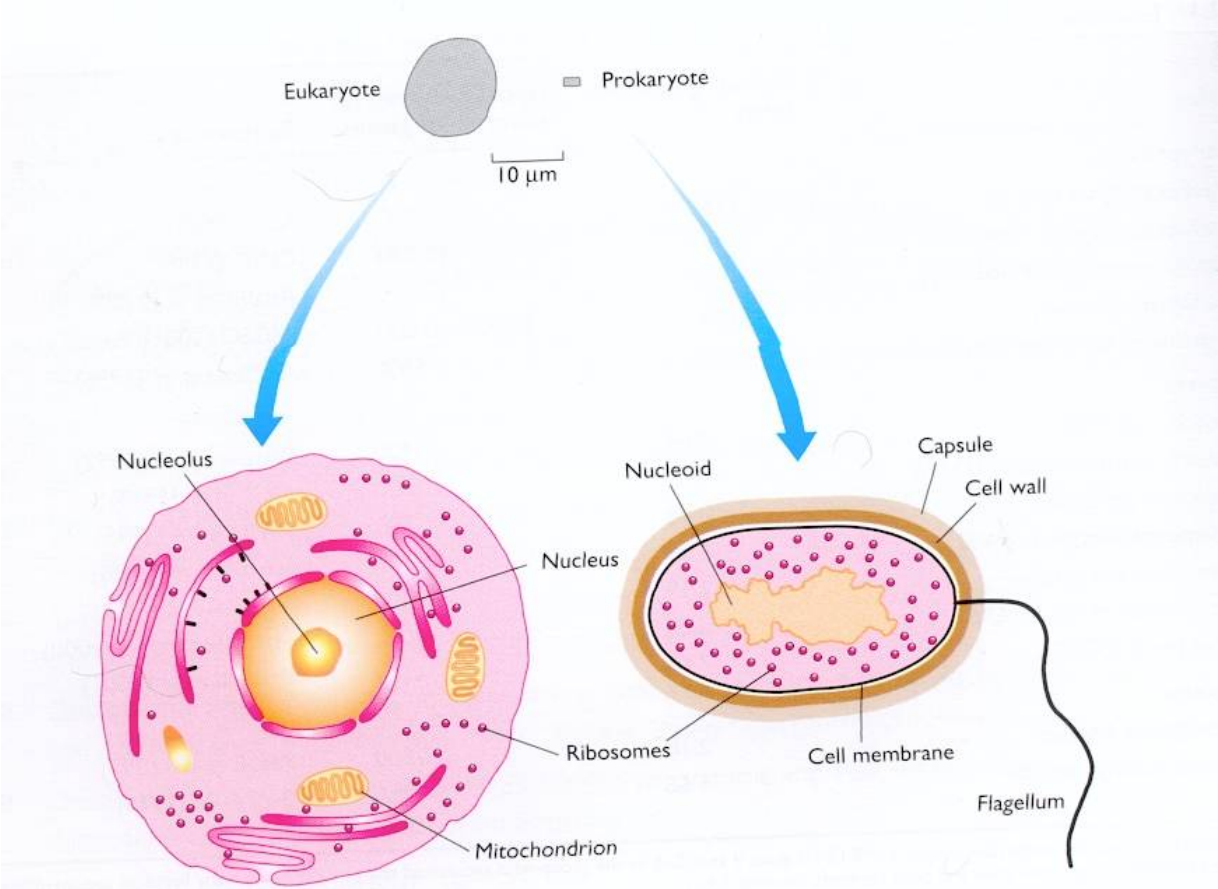


COVID-19

June Almeida

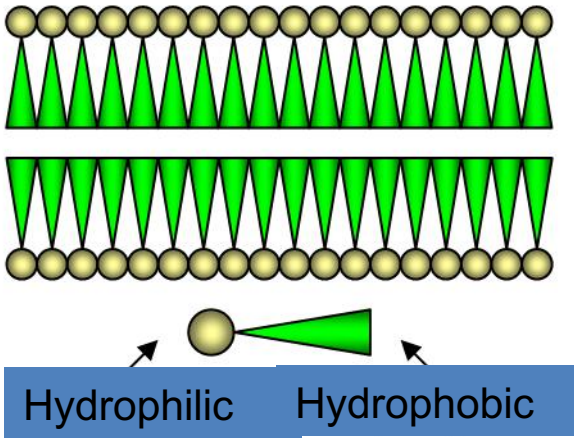
If it's not a virus, it's a cellular organism

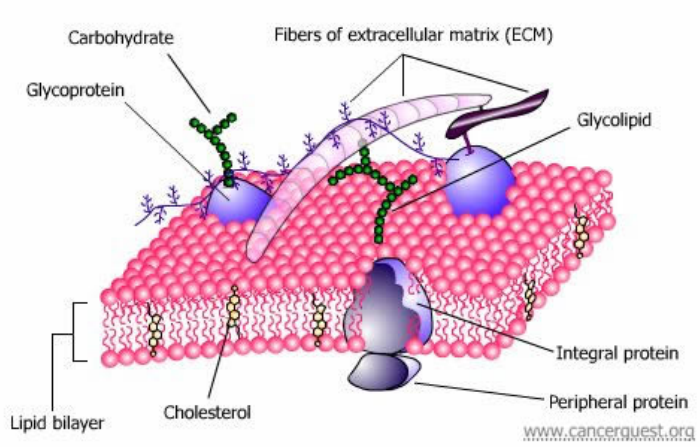
- Major categories of cellular organisms are:
  - Eukaryotes
  - Prokaryotes (bacteria & Archaea)



Structure cellulaire

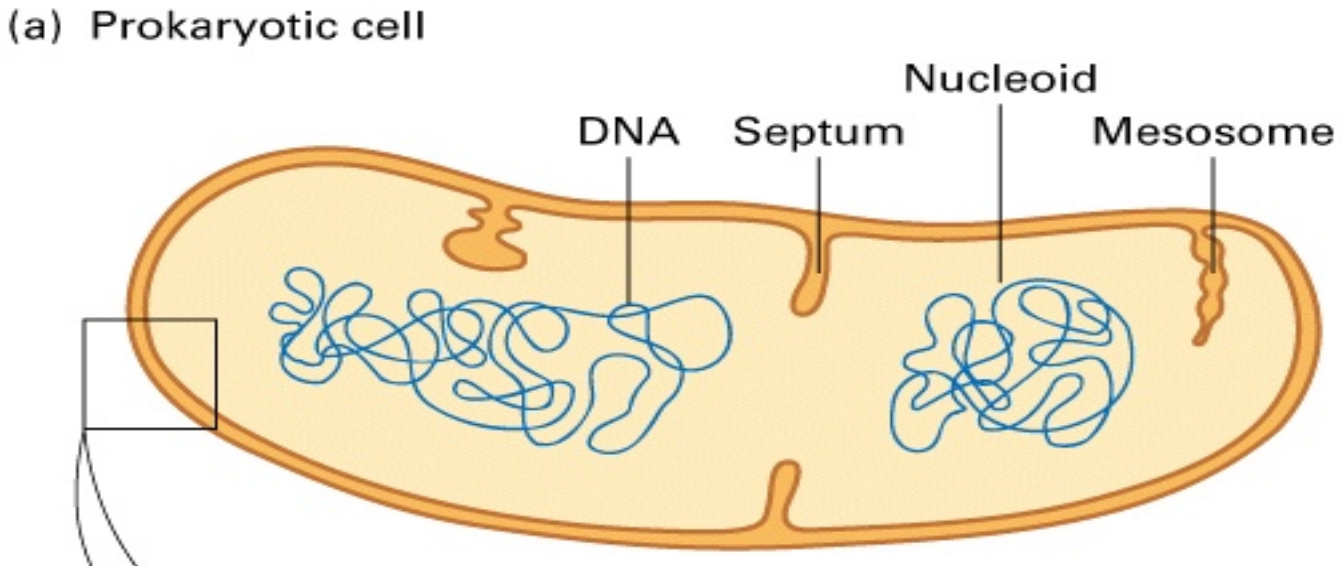
- Cellule est l'unité fondamentale
- Cellules délimitées par une membrane
  - Très approximativement un "sac d'eau"
  - En général l'extérieur est aqueux
- La Membrane est une bicouche lipidique



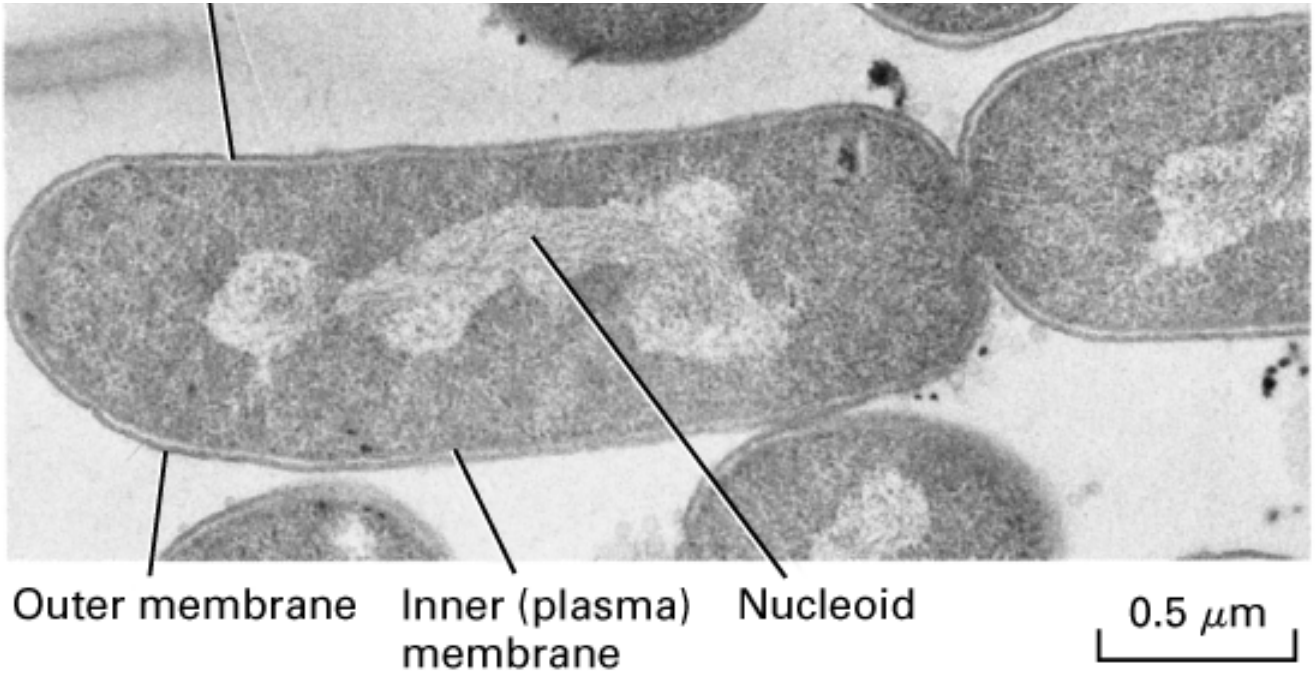


Prokaryotes

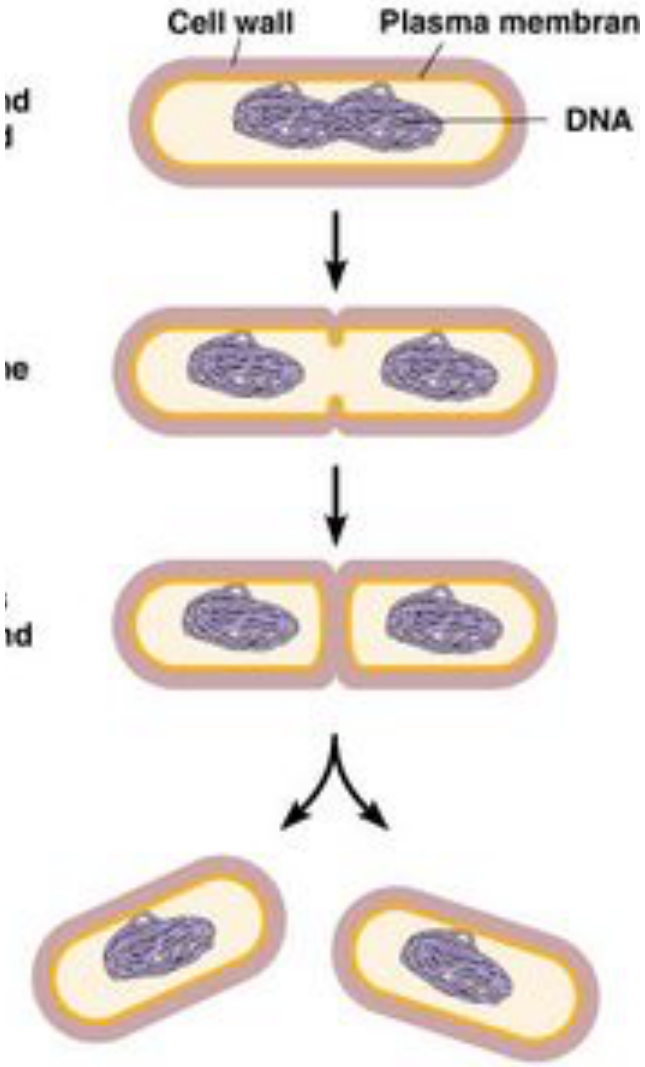
- Single-celled organisms
- Single membrane-bound compartment
- Typically about 1 micron diameter

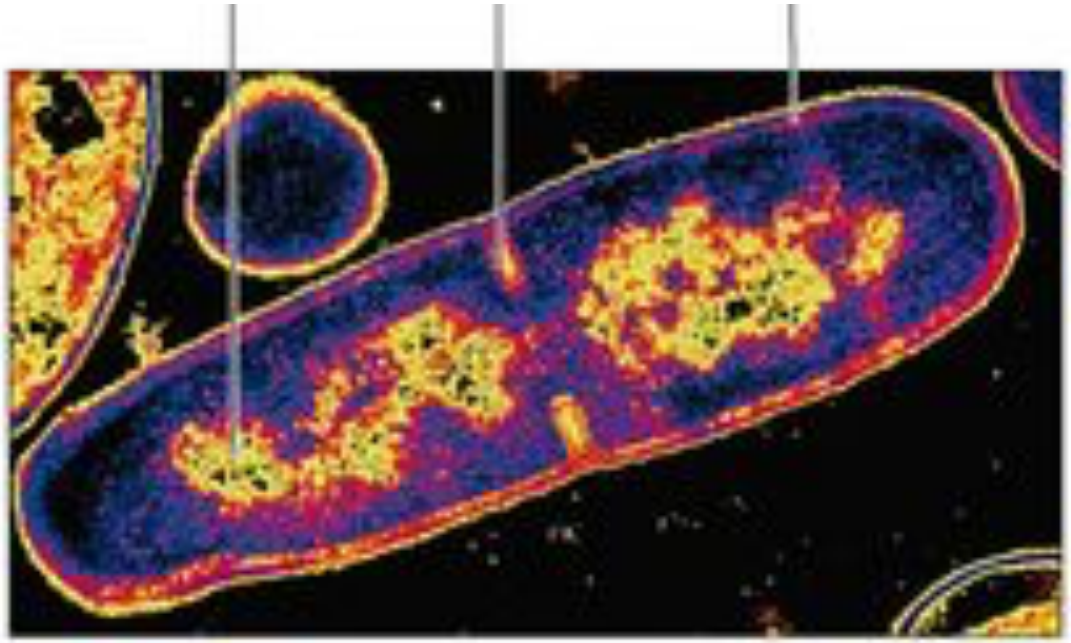


Prokaryotes



How do bacteria grow?

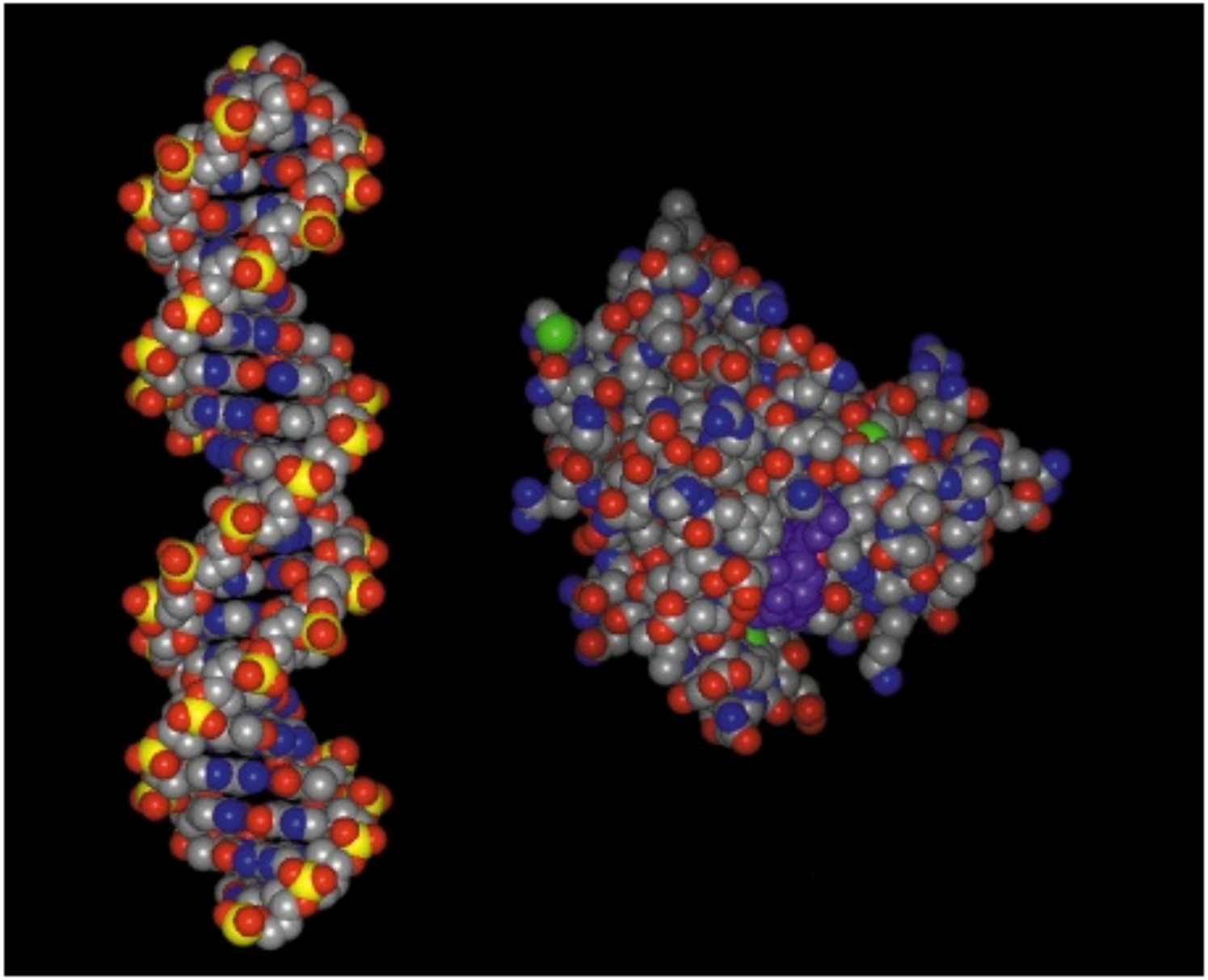




Qu'y-a-t-il dans une cellule?

Macromolecules (polymers)

- Synthesis
  - Molecular “strings” made in cell by linking *monomers* from a specified set (alphabet)
- Examples
  - Polysaccharides (sugar chains)
  - Proteins (amino acid chains)
  - DNA & RNA (Nucleic acids; nucleotide chains)



Nucleic acid
(DNA)

Protein
(Ras protein)

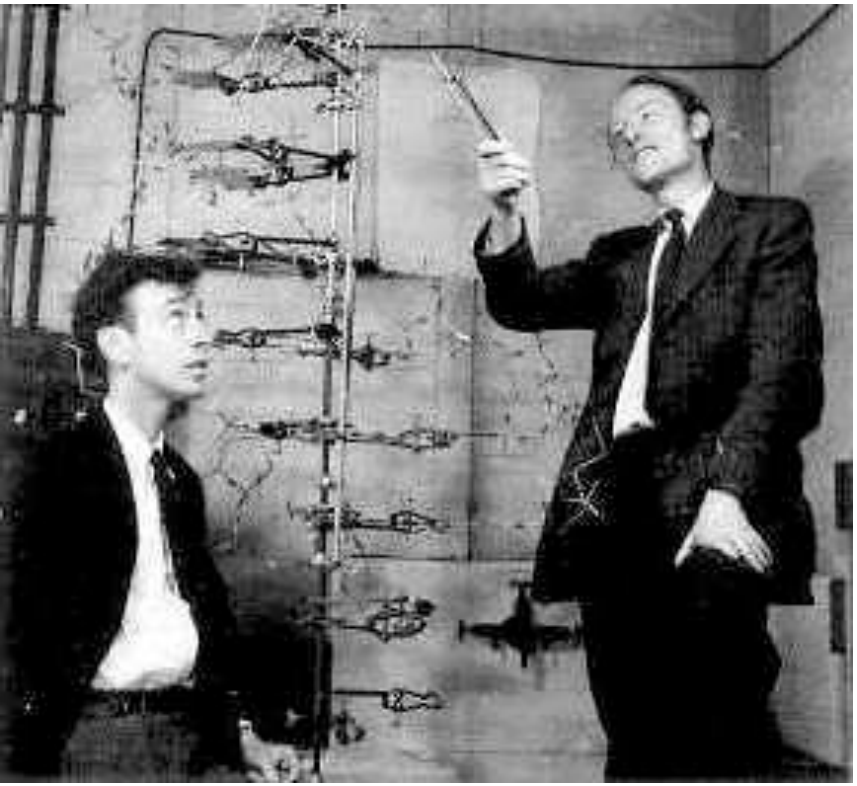
1 nm

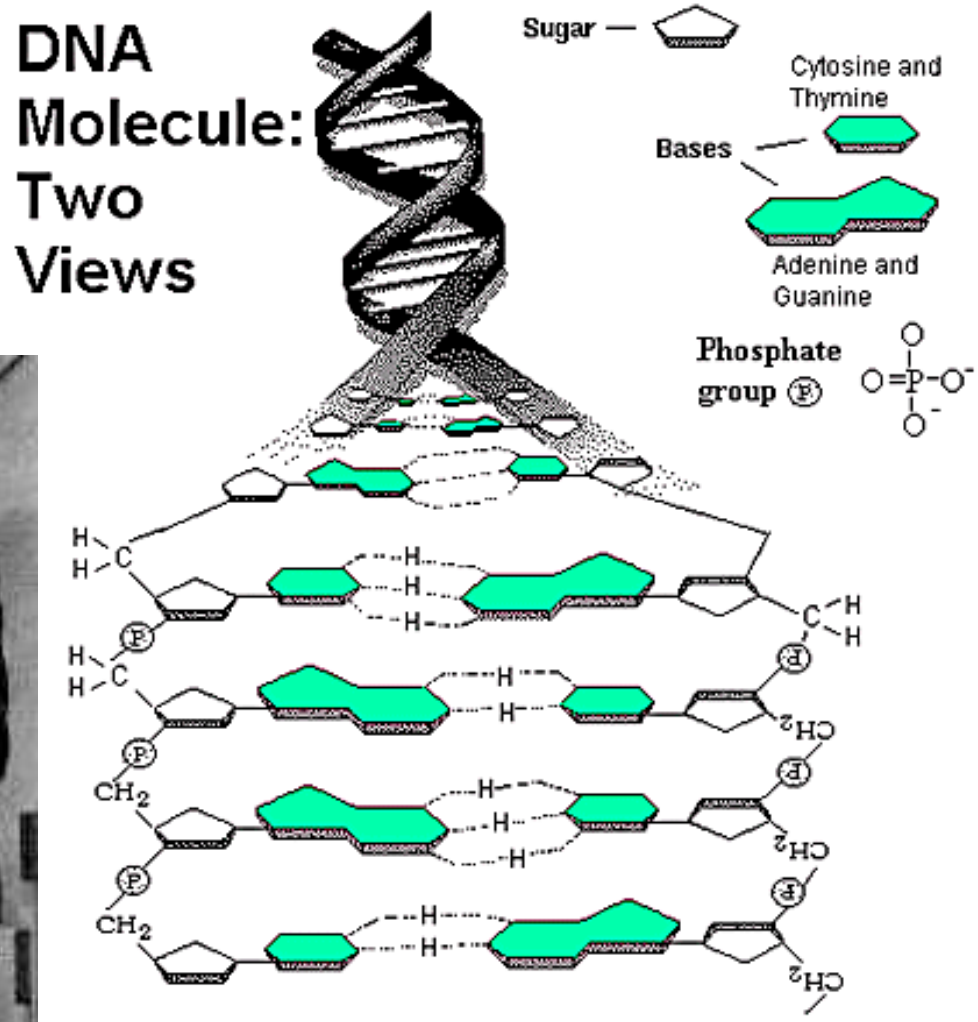
Protein functions

- Structural: E.g.,
  - Cytoskeleton gives membrane strength & rigidity
- Signaling (information transduction): E.g.,
  - receptor on cell surface senses a hormone
  - DNA binding protein turns genes on and off
- Enzymatic: speed up reactions to, e.g.,
  - Extract energy from nutrients
  - Interconvert small molecules

Nucleic acids

- Two major types of nucleic acid polymers
  - *Deoxyribonucleic acid* (DNA*)*
  - *Ribonucleic acid* (RNA).
- Composition
  - Four monomers called *nucleotides*
  - DNA: deoxy
    - Adenine (A), Guanine (G), Cytosine (C), Thymine (T)
    - Long term storage of information
  - RNA:
    - Adenine (A), Guanine (G), Cytosine (C), Uracil (U)
    - Short term information “transmission”



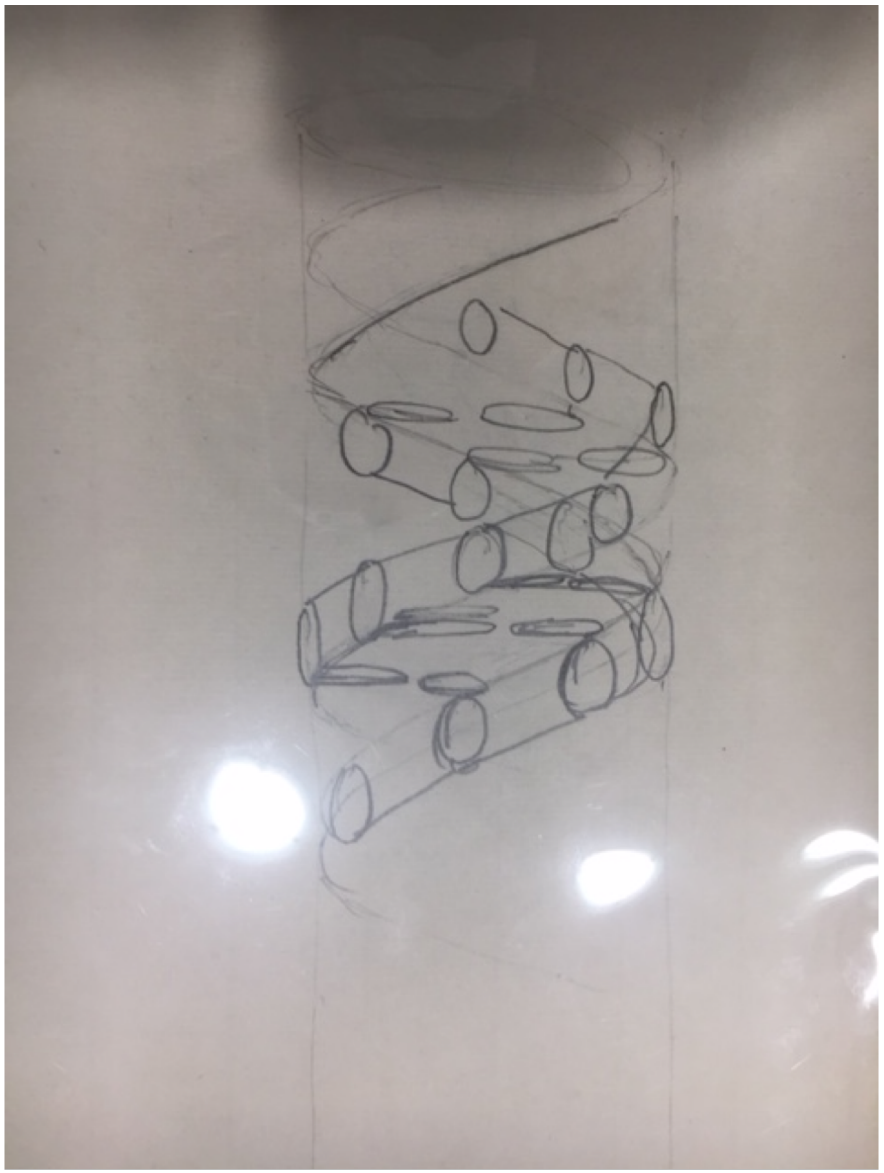


La structure en double hélice de la molécule d'ADN

James Watson and Francis Crick in 1953

Rosalind Franklin

The double helix by Rosalind Franklin



Wellcome Trust collection

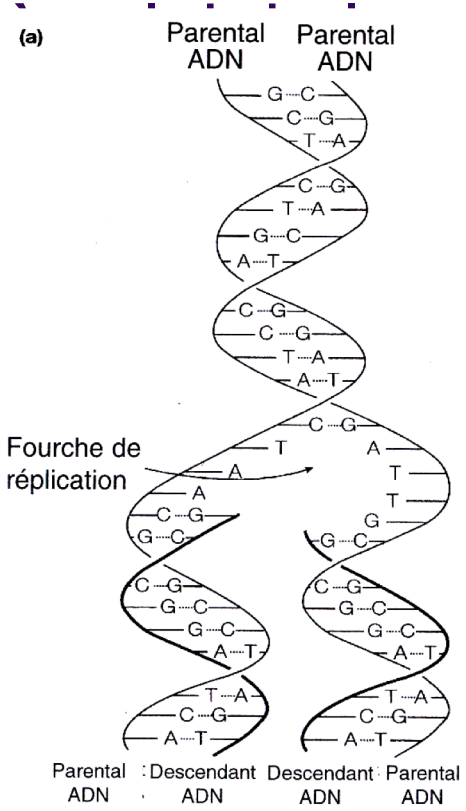
Réplication

C'est le mécanisme de transmission de l'information génétique

L'ADN des cellules filles est identique à celle de la cellule mère

La réplication est semi-conservative

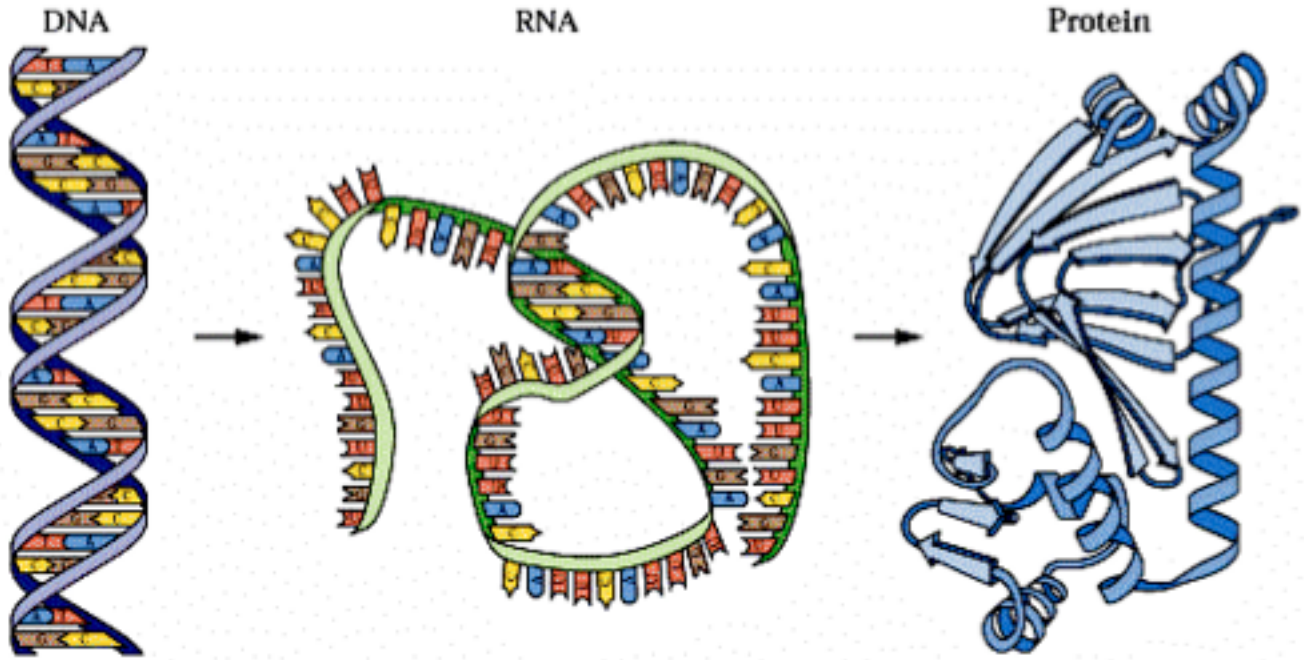
Chaque molécule d'ADN est constituée d'un brin parental et d'un brin néoformé



Notions de biologie moléculaire

Le dogme central de la biologie moléculaire

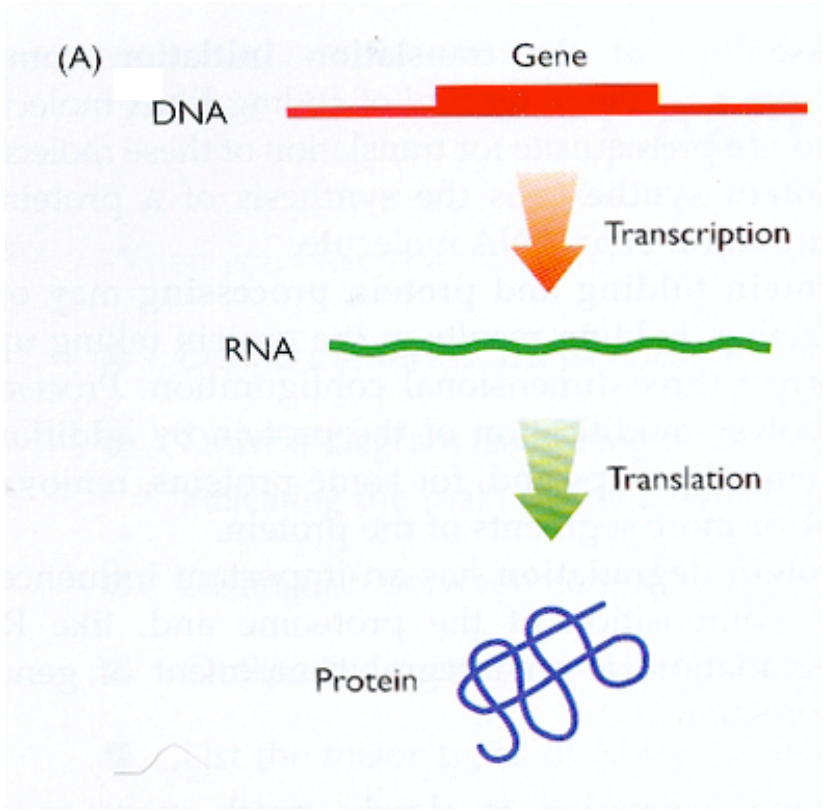
ADN ⟶ ARN ⟶ Protéine



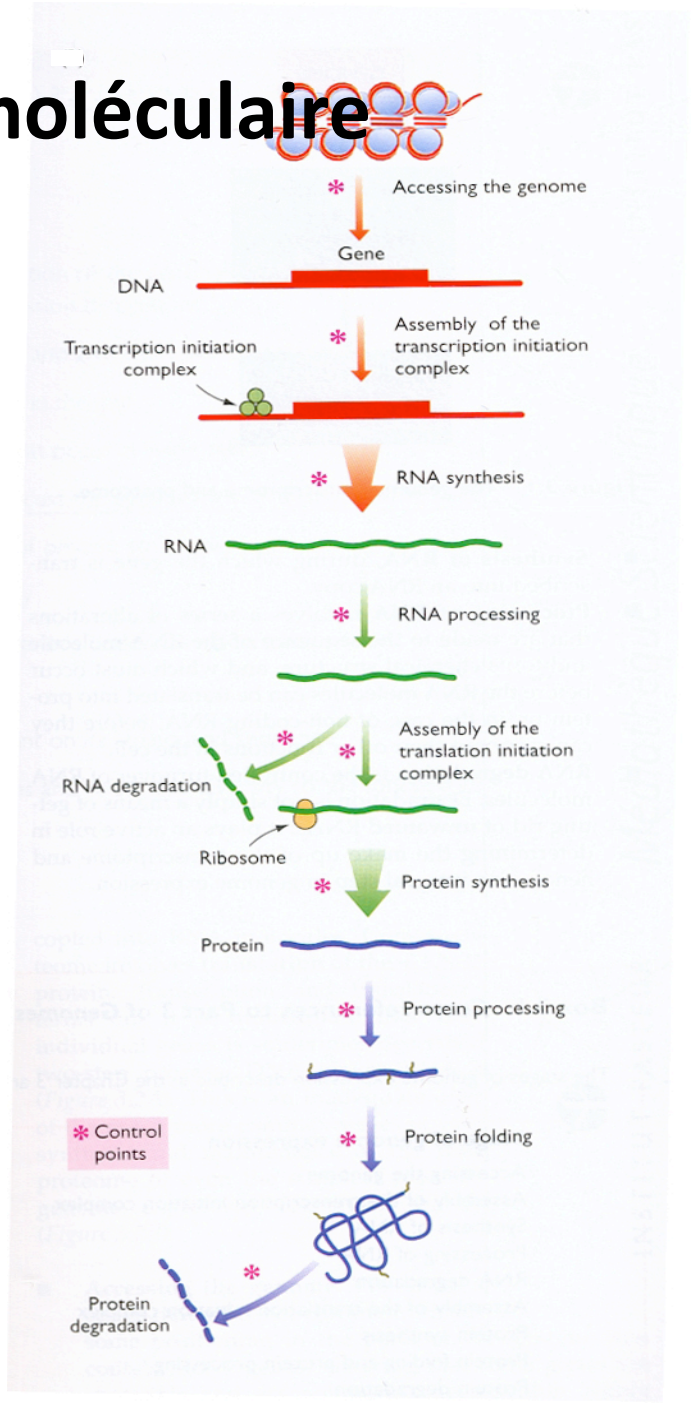
1960

Le dogme central de la biologie moléculaire

En 1960



En 2000



(d'après Brown, 2002, in genomes)

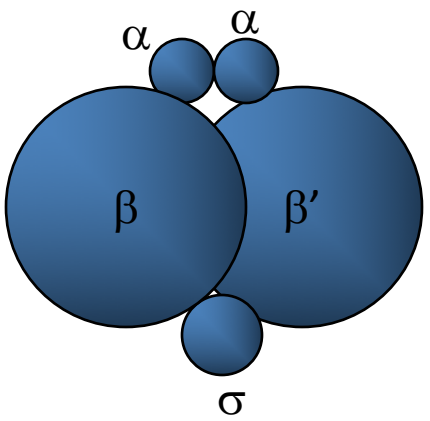
Transcription

La machinerie transcriptionnelle

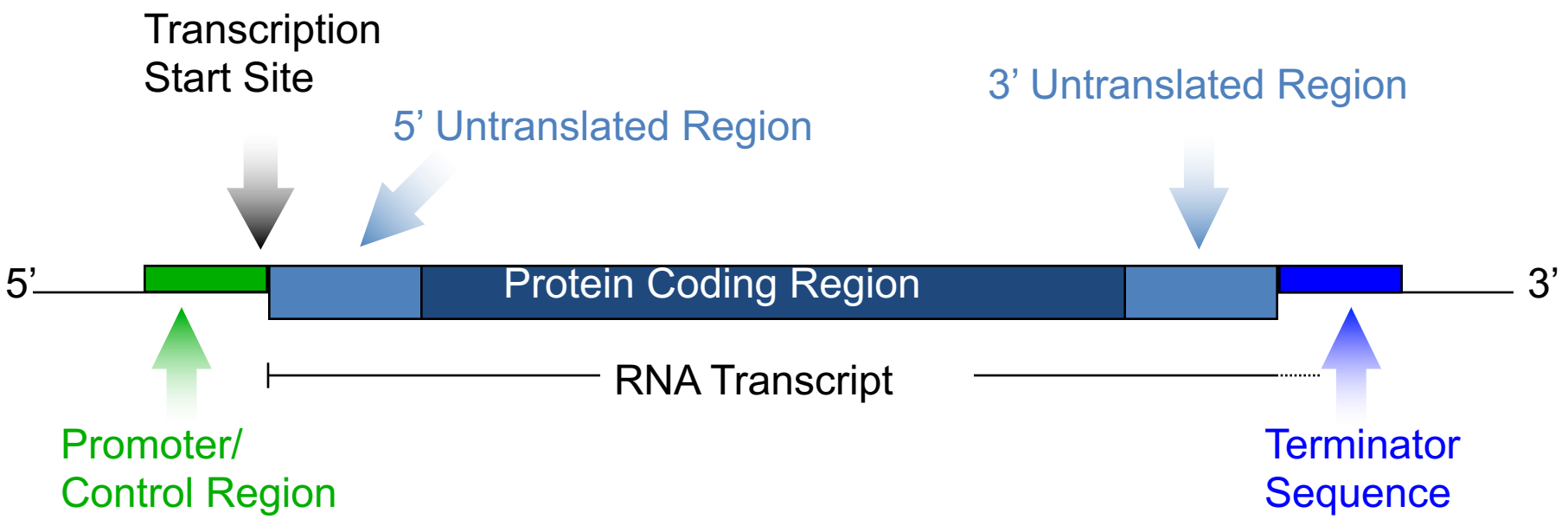
chez les procaryotes

Une seule ARN polymérase chez *E.coli* :

- **Découverte en 1958**
- **Contient 5 sous-unités**
- **Environ 7000 molécules par cellule**

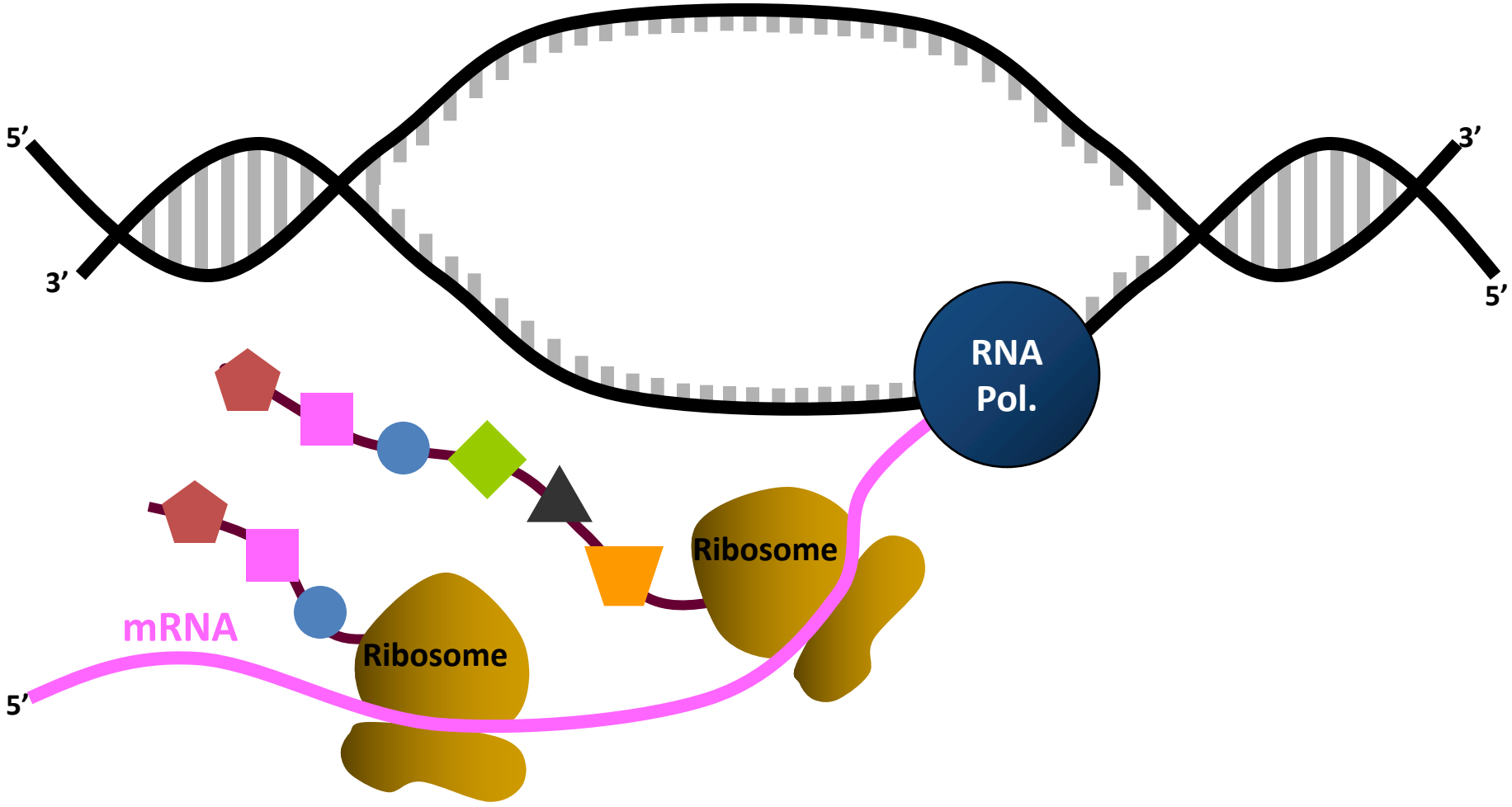


A “Simple” Gene

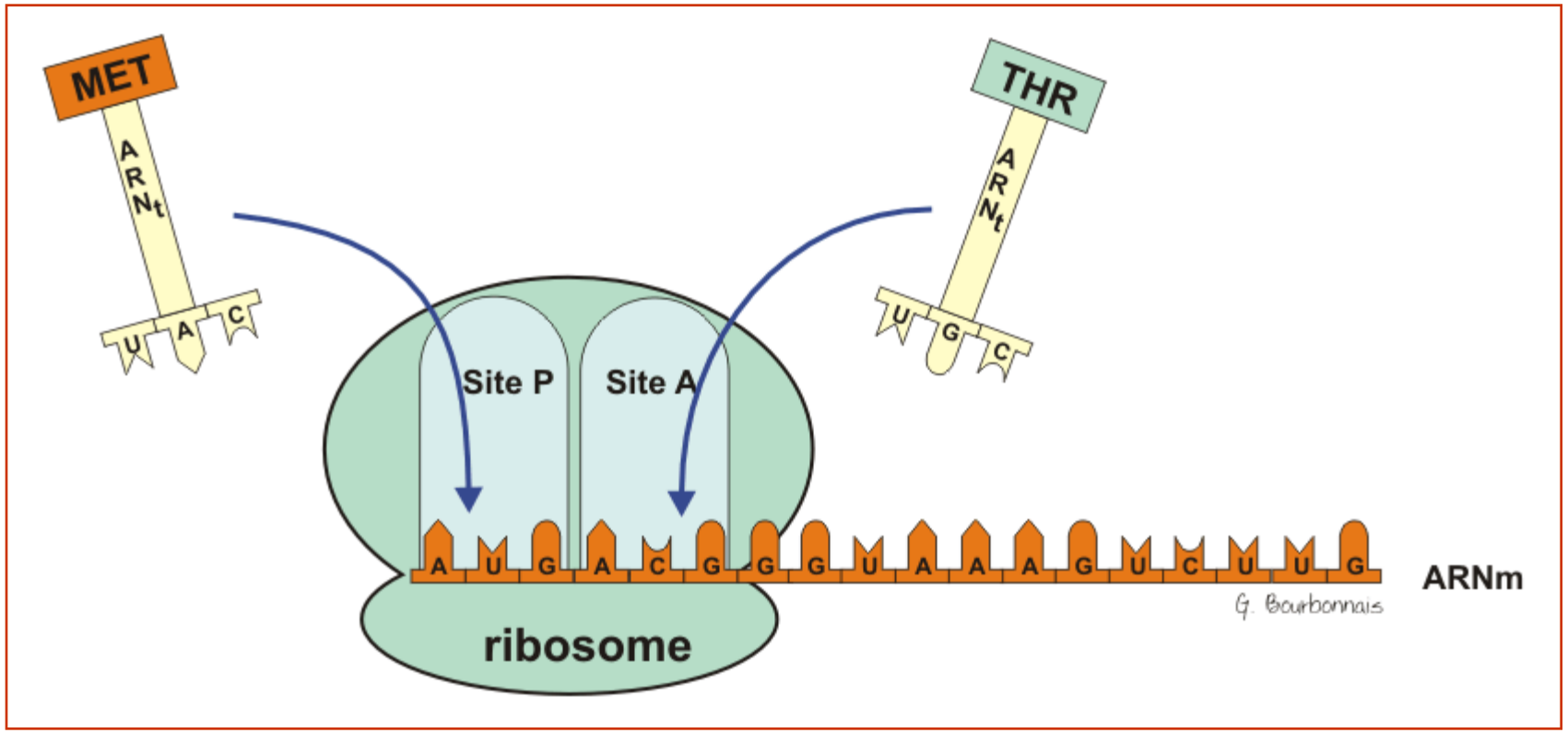


Traduction

Transcription And Translation In Prokaryotes



Mécanisme de la traduction



Le brin d'ARNm s'attache au ribosome.

Deux ARNt peuvent se fixer par leur anticodon sur l'ARNr au niveau du ribosome (un sur la zone appelée site P et l'autre sur la zone appelée site A).

Pause FILM

A retenir

- L’ADN est transcrit par l’ARN polymerase en ARN messager
- L’ARN messager est traduit par le ribosome en protéine
- Le code génétique (universel) permet de faire correspondre à un triplet de bases un acide aminé

Régulation

The lactose operon is negatively regulated

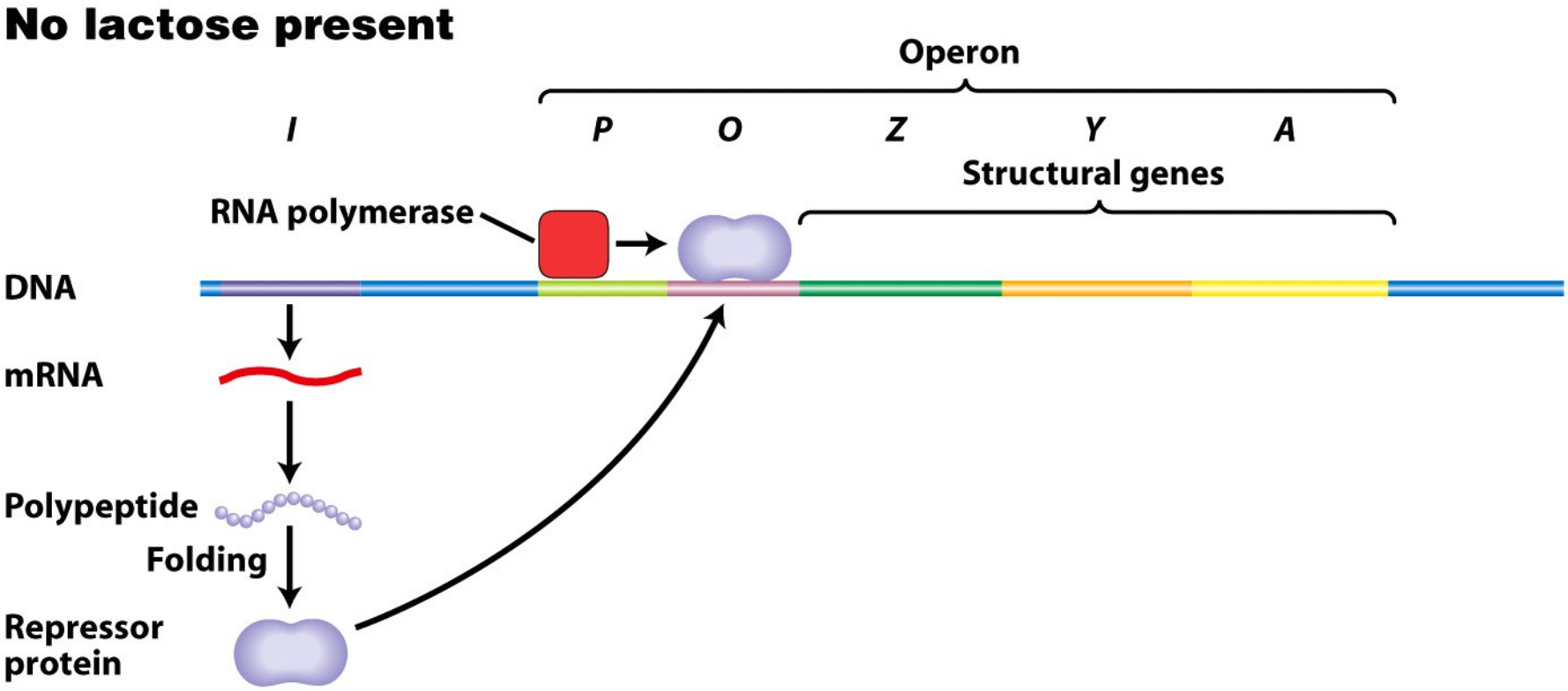
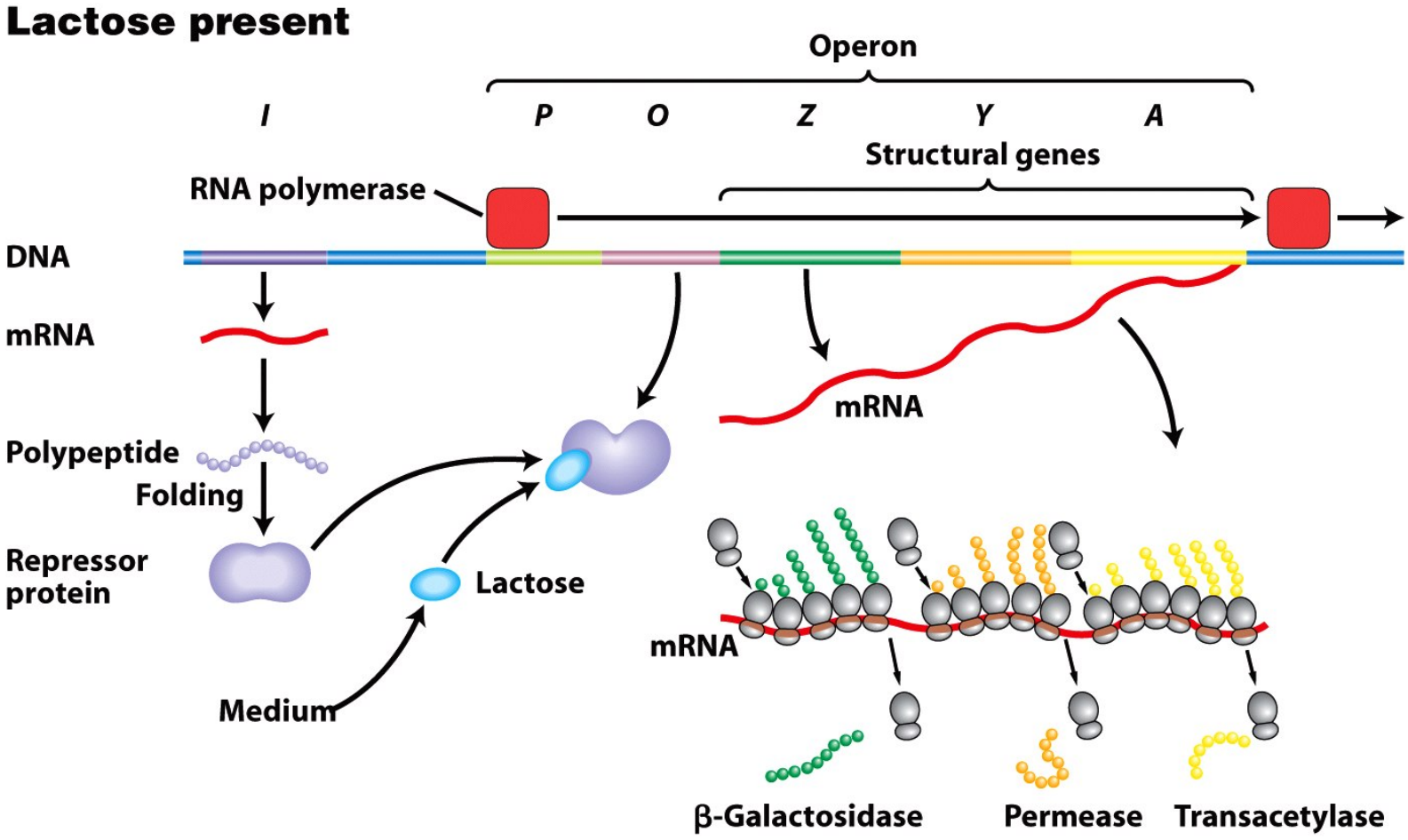


Figure 10-6a
Introduction to Genetic Analysis, Ninth Edition

Transcription of Z, Y, A genes are repressed by the *lacI* gene product which binds to the operator DNA sequence and transcription is blocked

The lactose operon is de-repressed by lactose.



Lactose (allolactose) binds to the LacI repressor and produces a conformational change (allostery) - the repressor cannot then bind to the operator, allowing transcription

A retenir

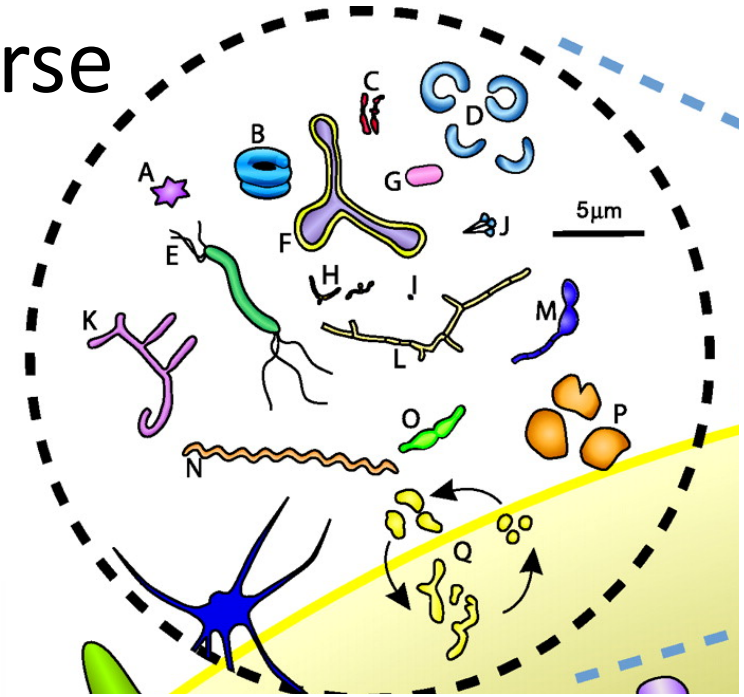
- Les ARNm ne sont pas formés en permanence (protéine régulatrices)
- Les régulateurs fonctionnent en réseaux

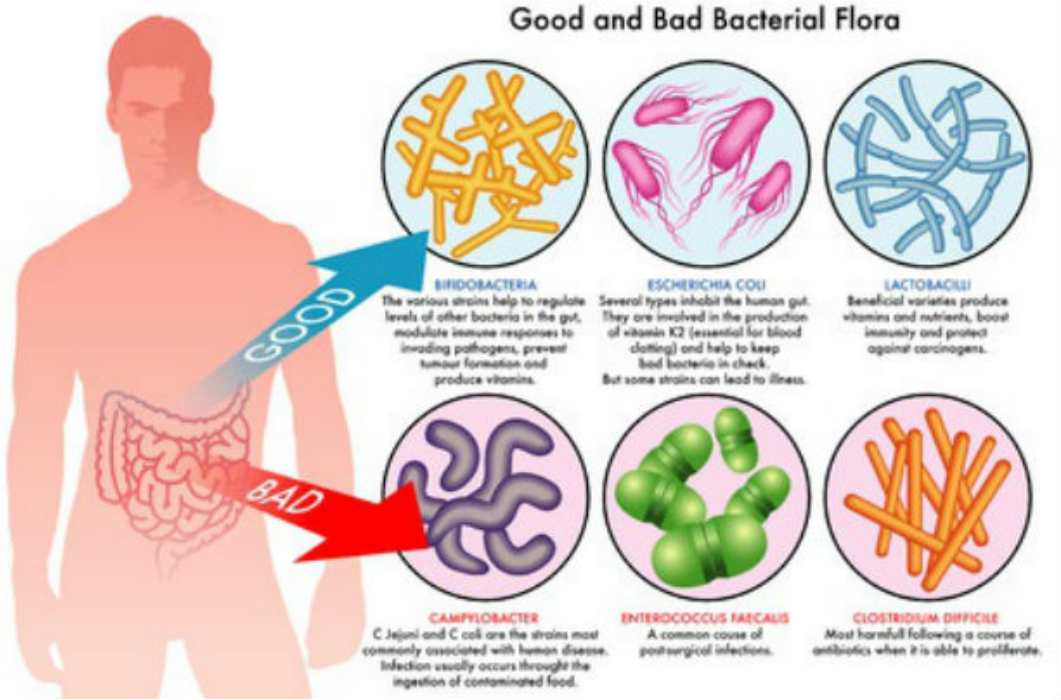
Des ARN peuvent être régulateurs

Le processus n'est pas déterministe

Croissance et physiologie bactérienne

Bacteria are extremely diverse



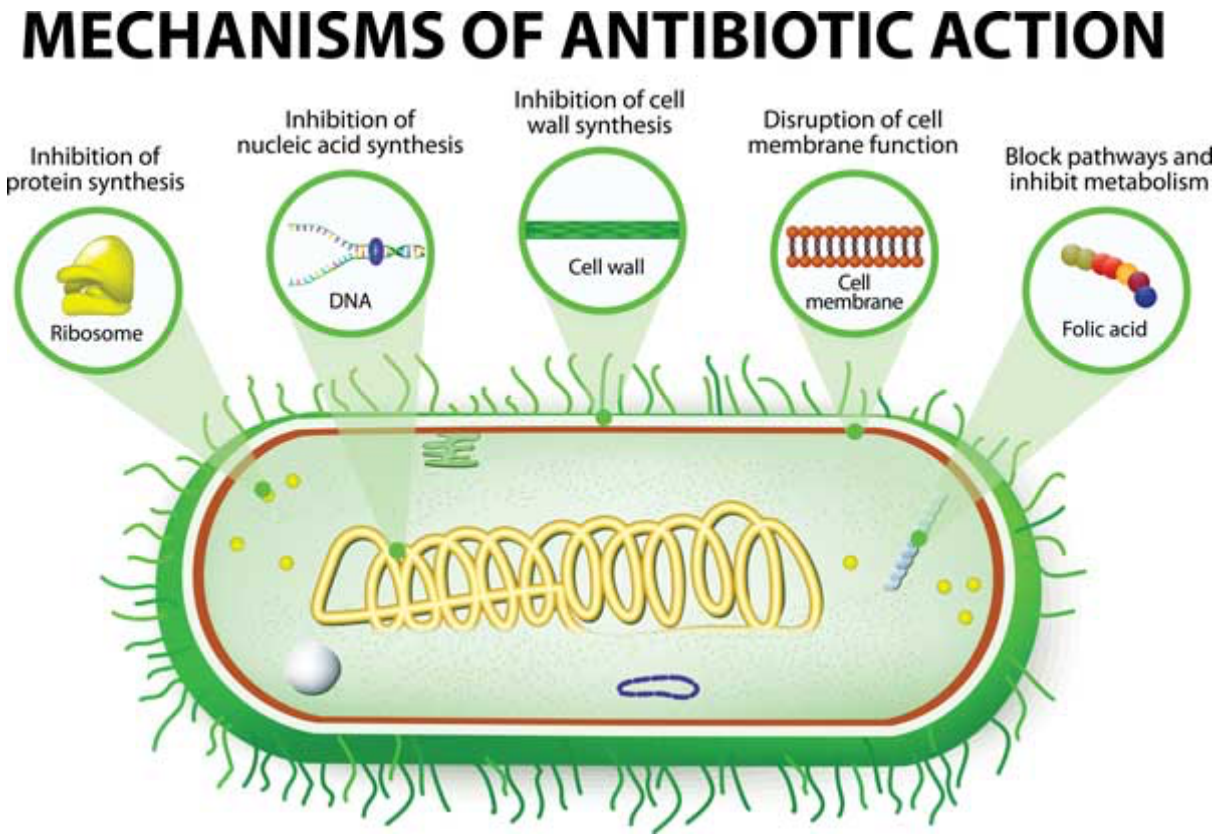


Kevin D. Young Microbiol. Mol. Biol. Rev. 2006; doi:10.1128/MMBR.00001-06

Population size:
Large!

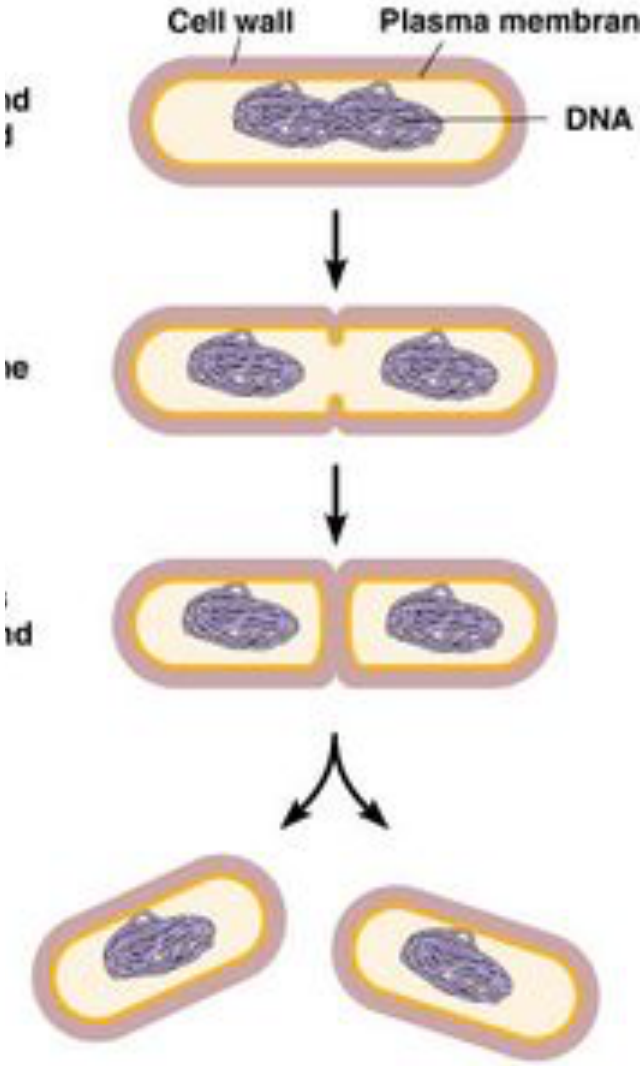
In the human body: 10^{13}

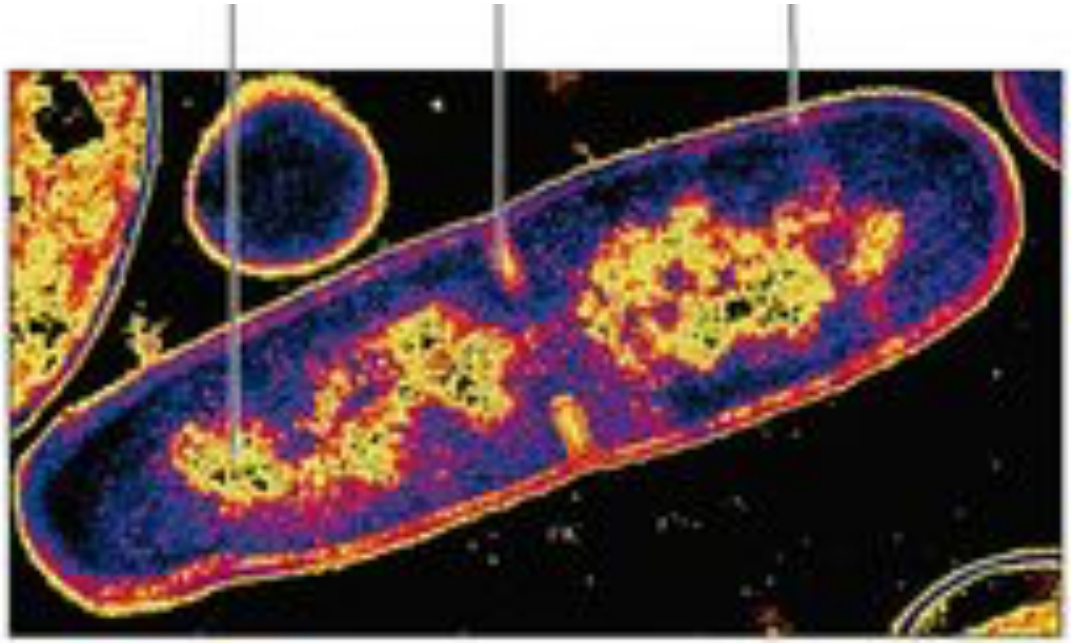
Antibiotics target all the molecular processes that we have described so far



Antibiotics only kill *growing* bacteria

To understand antibiotics we need to understand how bacteria grow

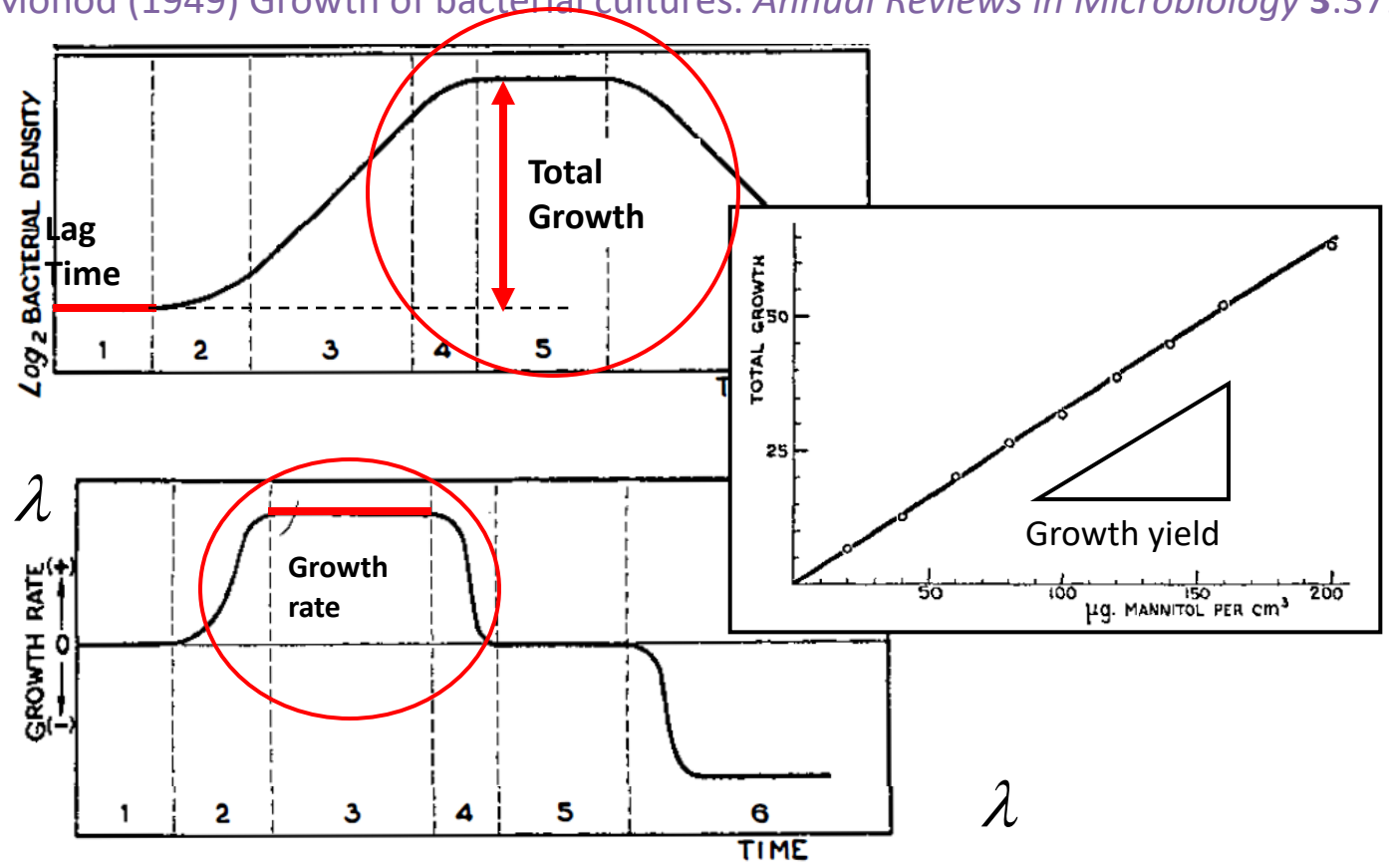




Growth of bacterial cells…an old story

1. Monod (1949): ‘Growth constants’ arising from nutrient assimilation
2. Schaechter, Maaløe and Kjeldgaard (1958): Empirical dependence of macromolecular composition (RNA, Protein, Mass/cell) as a function of growth rate.
3. Neidhardt and Magasanik (1960): Correlation between RNA content and proliferation rate.

J. Monod (1949) Growth of bacterial cultures. *Annual Reviews in Microbiology* **3**:371.



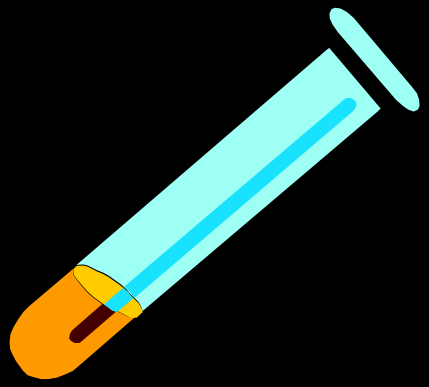
Despite immense underlying complexity, characteristics of bacterial growth obey simple laws.

BACTERIAL GROWTH

In balanced growth, the culture doubles at a constant rate.

Doubling rate

$$N(t) = N_0 \cdot 2^{\mu t}$$



Fast growth – rich medium

Number Density

$\log_2 N(t)$

Slope= doubling rate μ

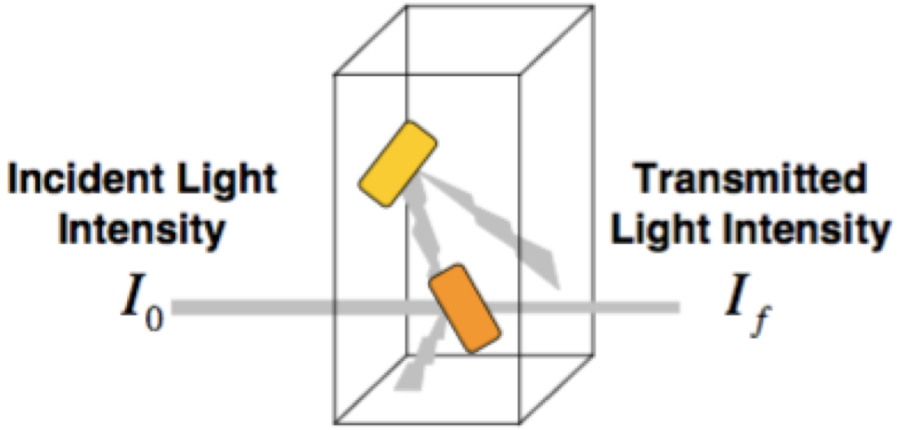
Slow growth poor medium

Time

E. coli can double every 20 min or several hrs

OD measurements

Figure 8: **Light scattering by bacteria.** In the dilute limit where multiple scattering events (yellow) are rare, the ratio of the incident (I_0) to transmitted (I_f) light intensity is proportional to the number of primary scatterers (orange).



In the dilute limit, where multiple scattering events are negligible, the ratio of the incident (I_0) to transmitted (I_f) light intensity is related to the number of bacteria N by,

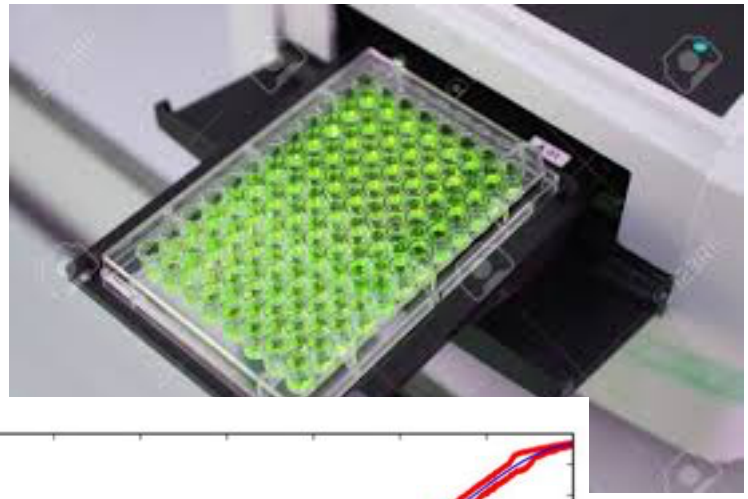
$$\log \frac{I_0}{I_f} = \ell N Q(\lambda),$$

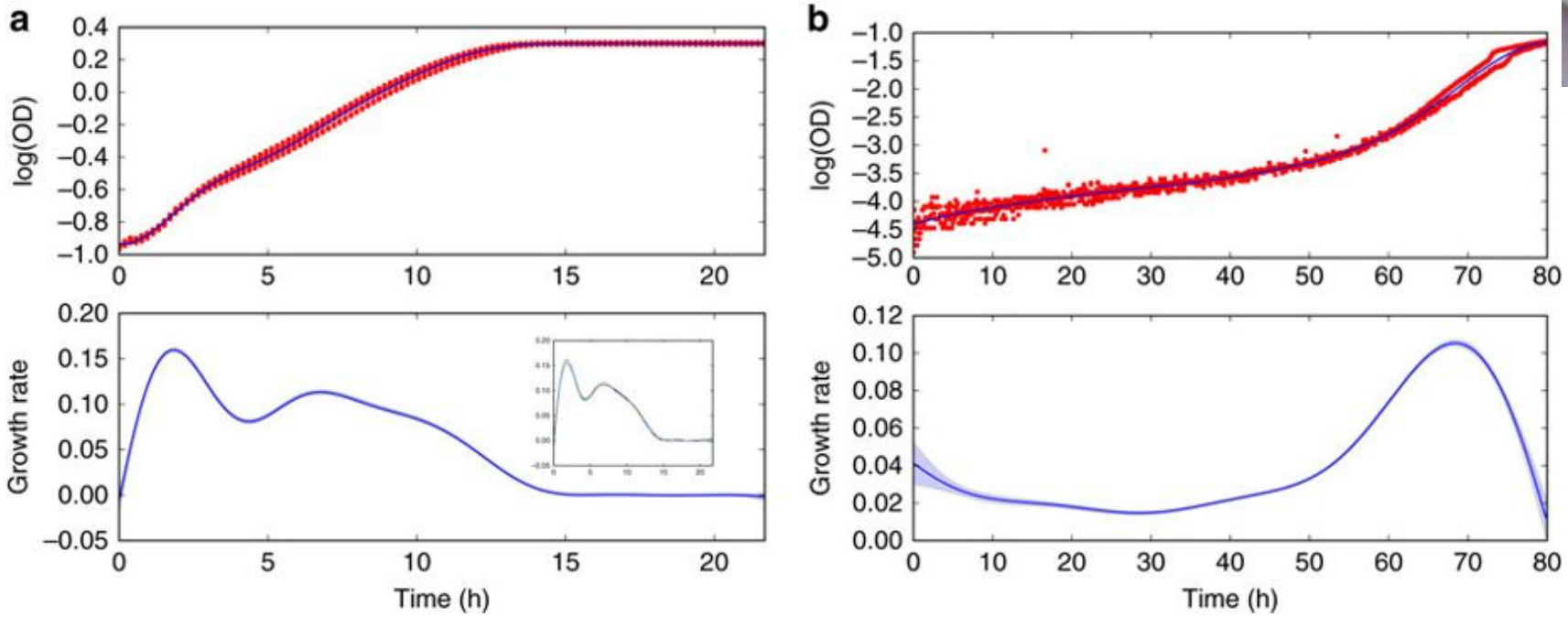
Caveats

- Calibration depends on apparatus
- Cell size changes!
- Plate readers need to be carefully monitored

Inferring growth rate from cultures

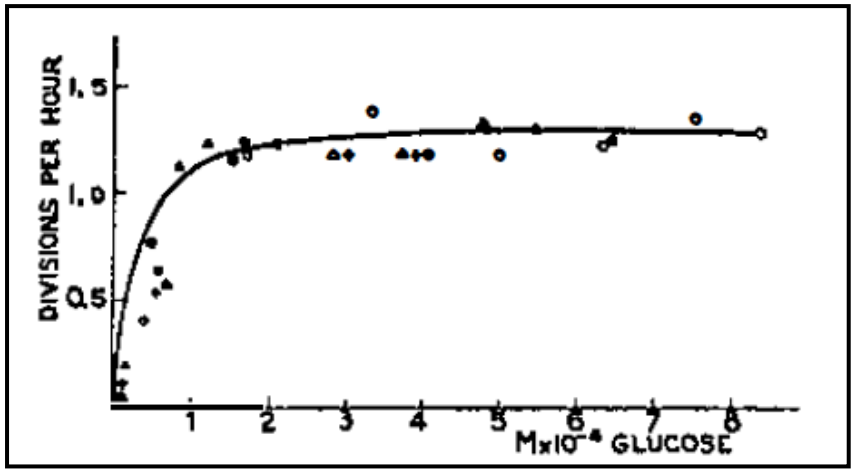
$$N(t) = N_0 e^{\lambda(t-t_0)}$$





1. growth rate (λ) depends on substrate concentration

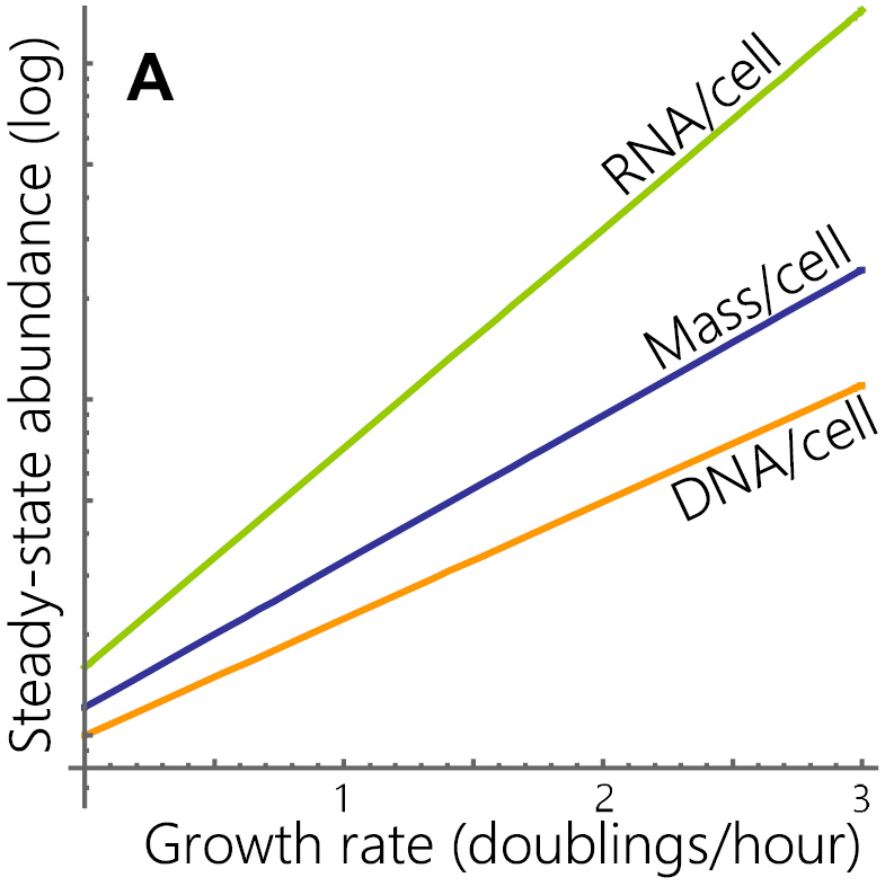
Empirically, hyperbolic relation between growth rate λ and the concentration of the growth-limiting substrate. (Monod 1949)



$$\lambda = \lambda_{\infty} \frac{[S]}{[S] + K_S}$$

Note: doubling rate $\mu = {}^{\lambda}/_{\ln(2)}$

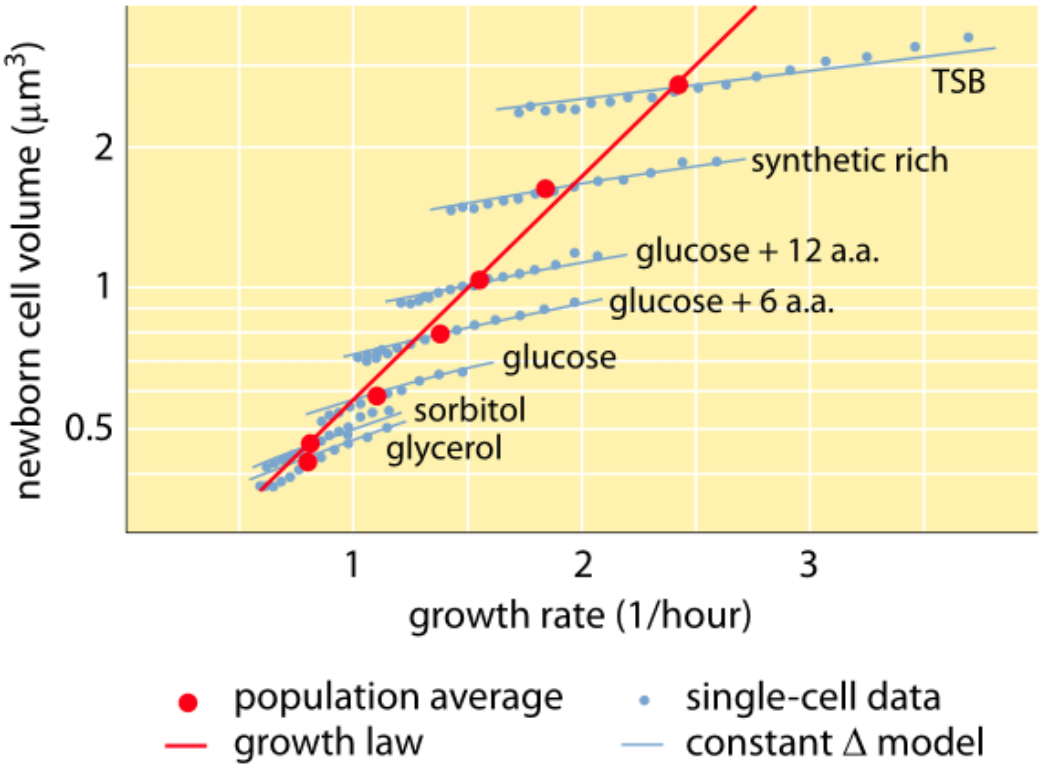
2. Macromolecular composition is a function of *growth rate alone*



RNA/cell increases more rapidly than mass per cell

Schaechter–Maaløe–Kjeldgaard experiments, 20 different nutrients (1958)

Obésité bactérienne!
Les bactéries qui mangent beaucoup sont plus grosses

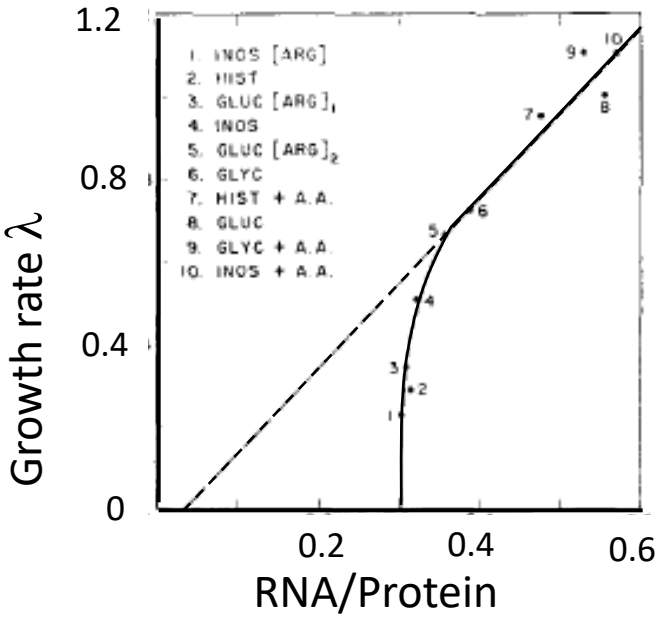


Et elles poussent plus vite

Cell biology by the numbers (Milo, Philipps)

3· Ribosomes and growth rate are coupled

- At moderate-to-fast growth rates, RNA/Protein correlates very strongly (linearly) with growth rate.
- After an upshift, protein synthesis increases but only AFTER RNA levels have increased.



Conclusion: RNA (i. e. Ribosomes) most likely plays a passive ("catalytic") role in protein synthesis rate.

Neidhardt and Magasanik (1960) Studies on the role of ribonucleic acid in growth of bacteria. *Biochimica et Biophysica Acta* **42**:99.

What does that mean mechanistically?

- If ribosomes translate at maximum rate (independently of growth rate), then protein synthesis (therefore growth rate) depends on number of ribosomes.
- This is consistent with ribosomes playing a catalytic role in protein synthesis
- Let's try and model the role of ribosomes in balanced growth

In exponential growth

- All cell constituents double at the same (constant) rate, λ. Noting M_p for protein mass we have
- $\frac{dM_p}{dt} = \lambda M_p$
- If ribosomes translate at rate k (independent of λ)

$\frac{dM_p}{dt} = \lambda M_p = \mathrm{k}N_{rb}$ (N_{rb} number of ribosomes)

Therefore: $\frac{N_{rb}}{M_p} = \frac{\lambda}{k}$

Which is consistent at moderate to high growth rates with Neidhardt and Magasanik

More precisely

- Not all ribosomes are active
- $\frac{dM_p}{dt} = \lambda M_p = \mathrm{k}(N_{rb} - N_{rb}^0)$
- Let's convert N_{rb} in mass, $M_{rb} = m_{rb} \times N_{rb}$

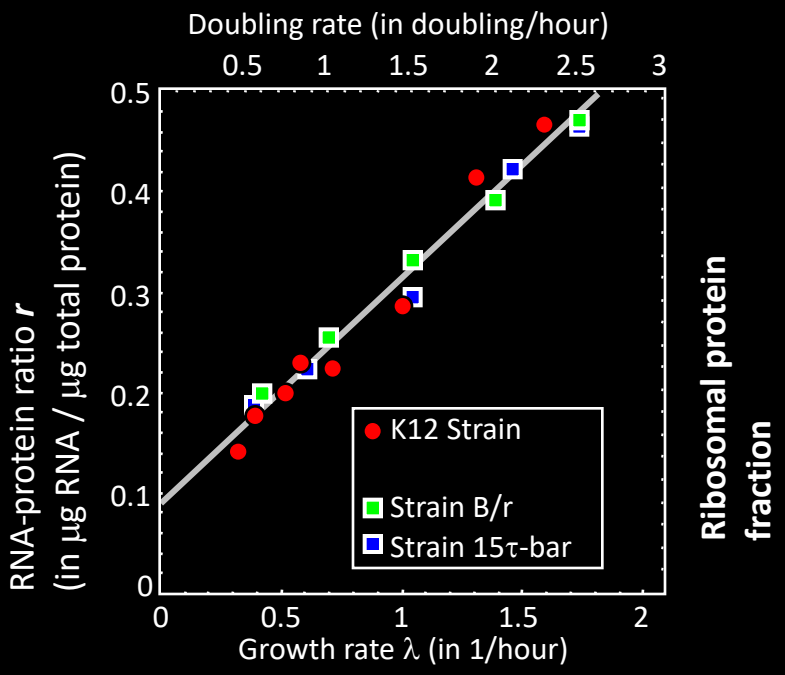
We get

$$\frac{\lambda}{{}^{k}/_{m_{rb}}} = \frac{M_{rb}}{M_p} - \frac{M_{rb}^0}{M_p}$$

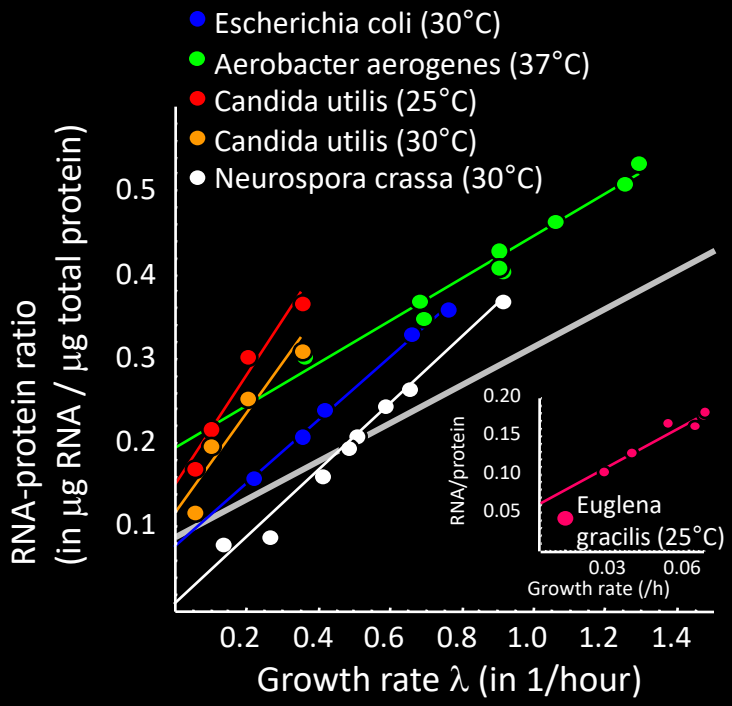
noting $\phi_r = \frac{M_{rb}}{M_p}$ and $\kappa_{trl} = {}^{k}/_{m_{rb}}$ we get

$$\phi_r = \frac{\lambda}{\kappa_{trl}} + \phi_{r_{min}}$$

Which is exactly what we observe experimentally



Ribosomal protein fraction



in other species

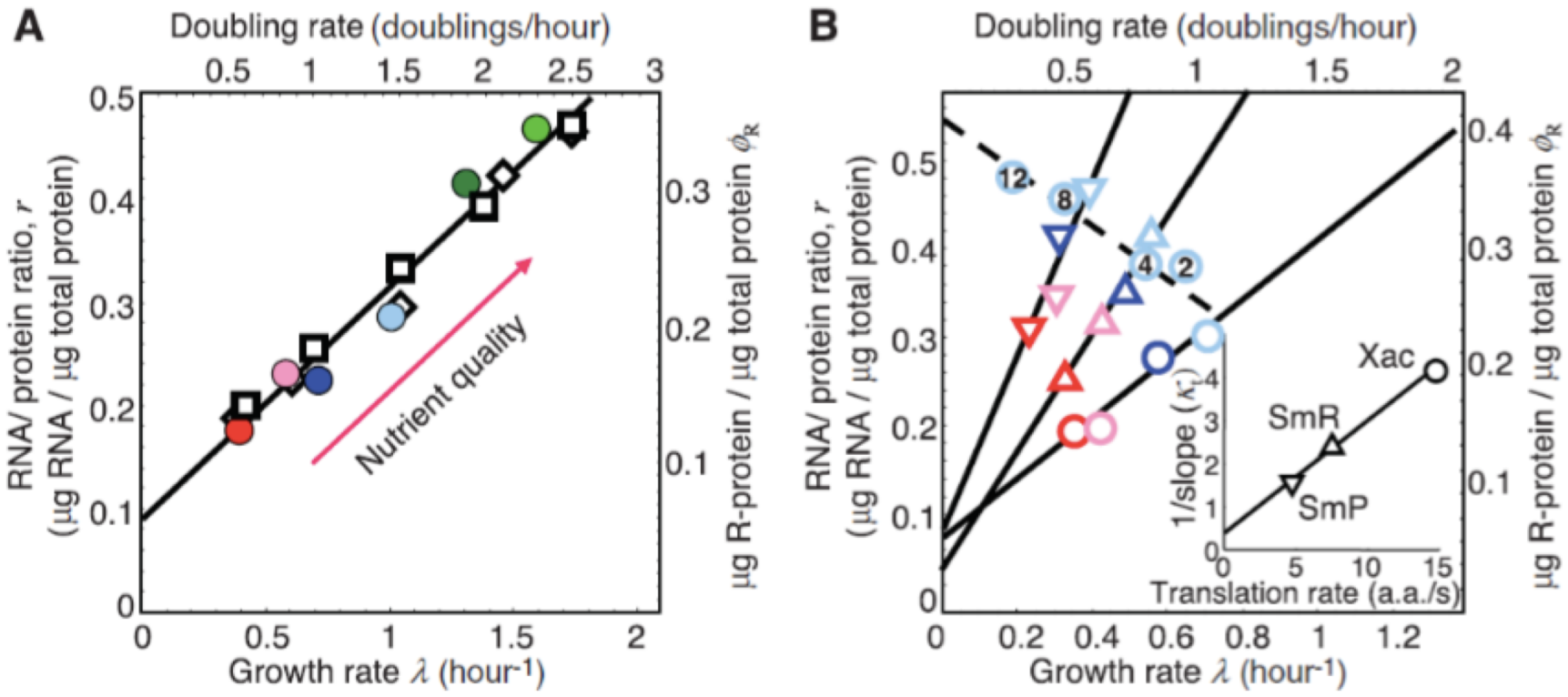
$$\phi_r = \frac{\lambda}{\kappa_{trl}} + \phi_{r_{min}}$$

Translational capacity

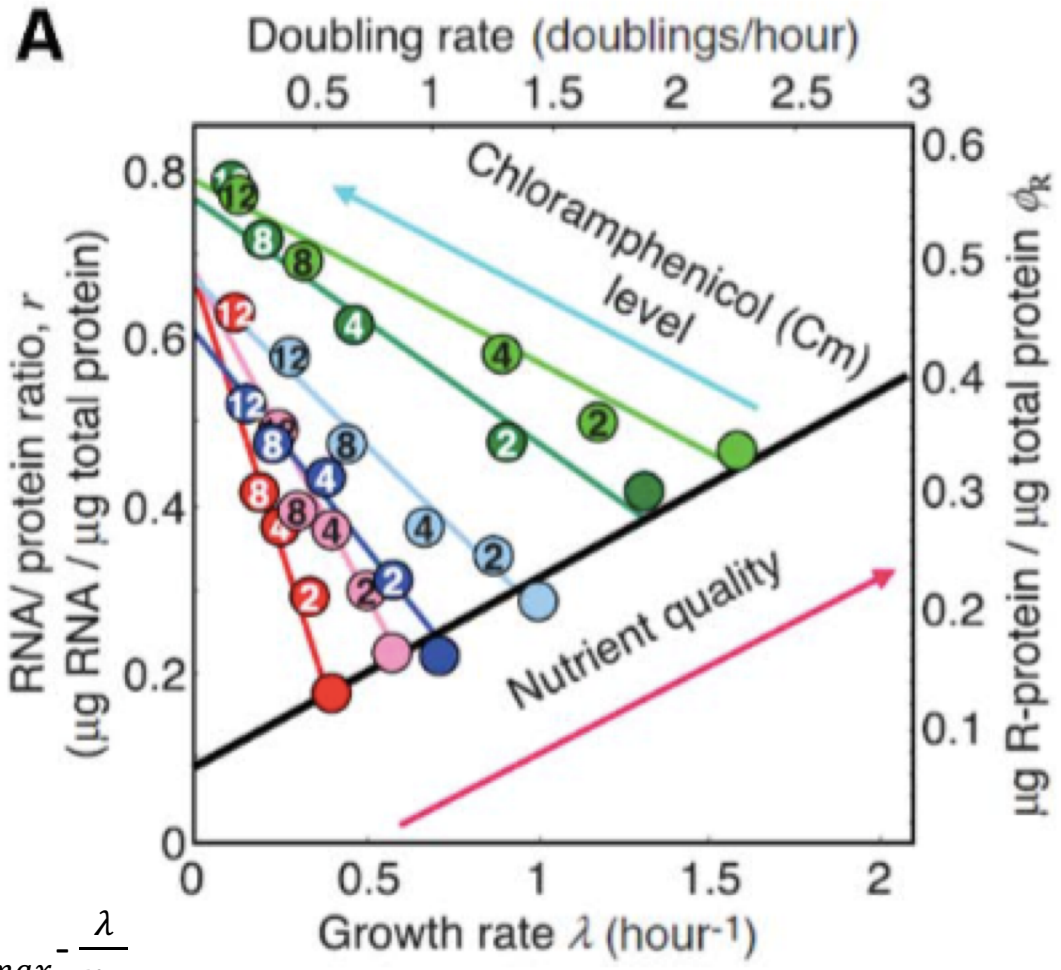
Scott, Gunderson, Mateescu, Zhang and Hwa (2010) *Science* 330:1099.

This can be tested experimentally

- Using ribosome mutants that translate at different rates

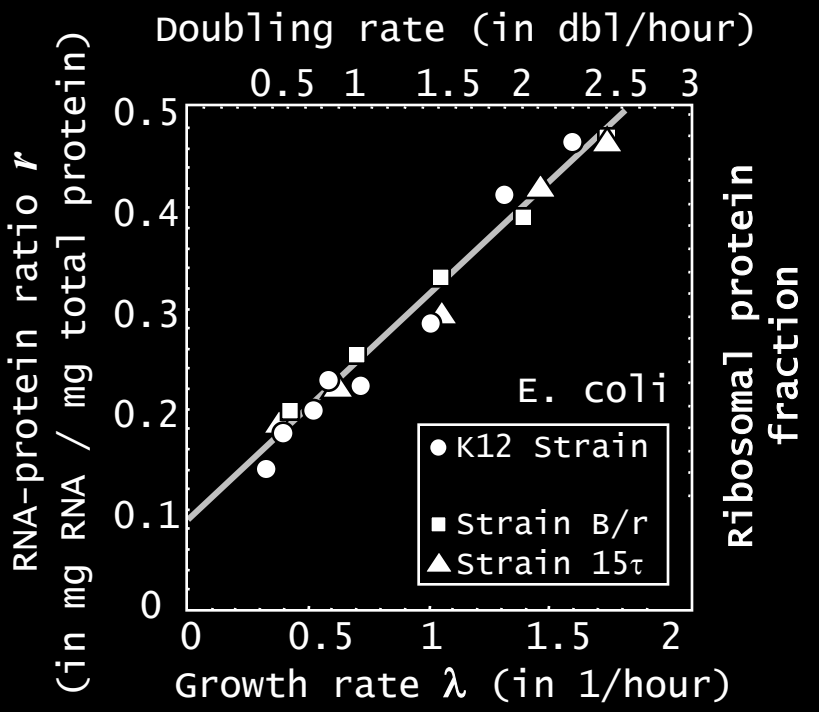


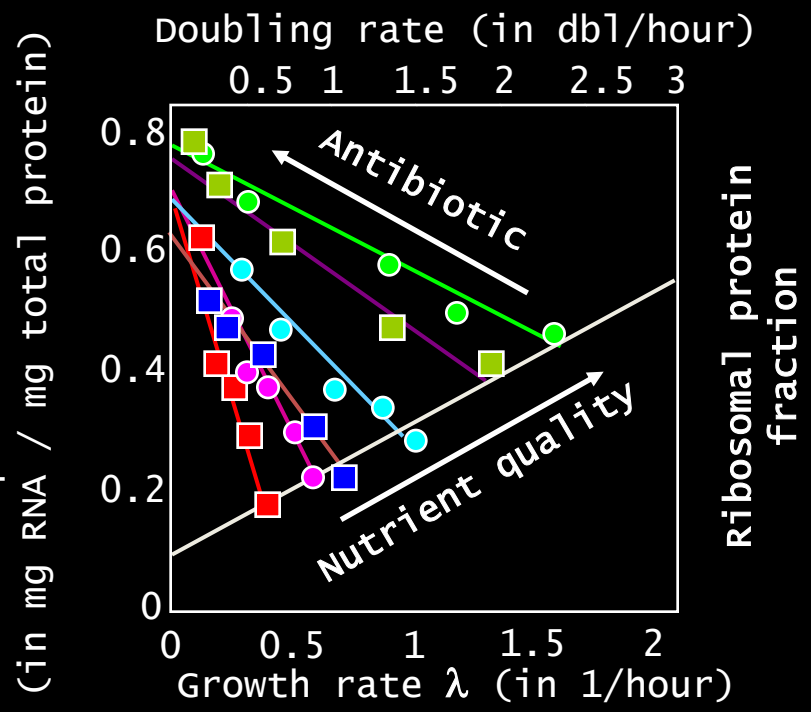
Or using antibiotics



$$\phi_r = \phi_{r_{max}} - \frac{\lambda}{\kappa_N}$$

TRANSLATIONAL GROWTH INHIBITION





$$r = r_{min} + \frac{\lambda}{K_{tsl}}$$

$$r = r_{max} - \frac{\lambda}{K_{nut}}$$

Scott, Gunderson, Mateescu, Zhang and Hwa (2010) *Science* **330**: 1099.

Two global constraints on bacterial growth

$$\phi_r = \frac{\lambda}{\kappa_{trl}} + \phi_{r_{min}}$$

"Phenomenological"
Not derived mechanistically

$$\phi_r = \phi_{r_{max}} - \frac{\lambda}{\kappa_N}$$

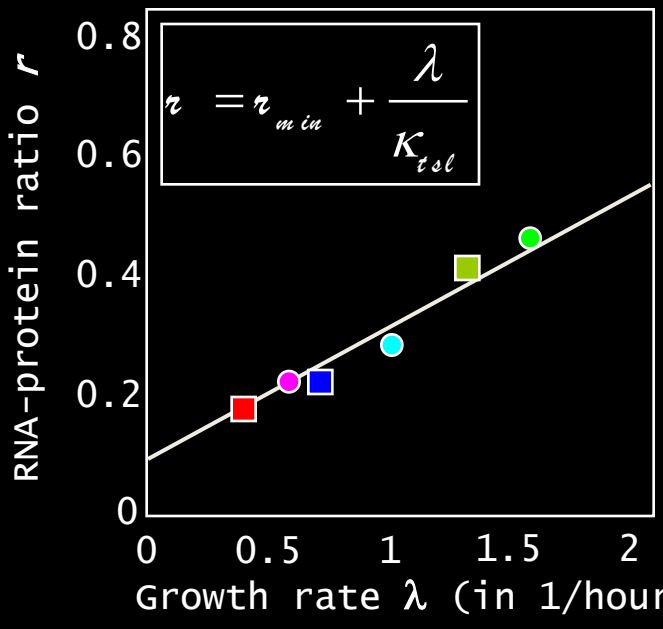
Eliminating ϕ_r we get something very close to Monod relationship

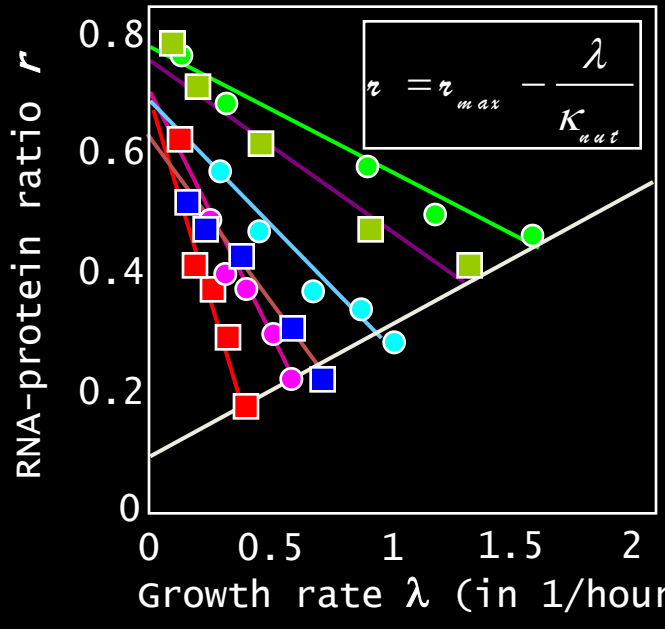
$$\lambda = \left(\phi_{\mathrm{R}}^{\max} - \phi_{\mathrm{R}}^{\min}\right) \frac{\kappa_{\mathrm{T}}\kappa_{\mathrm{N}}}{\kappa_{\mathrm{T}} + \kappa_{\mathrm{N}}}$$

Proteome partitioning

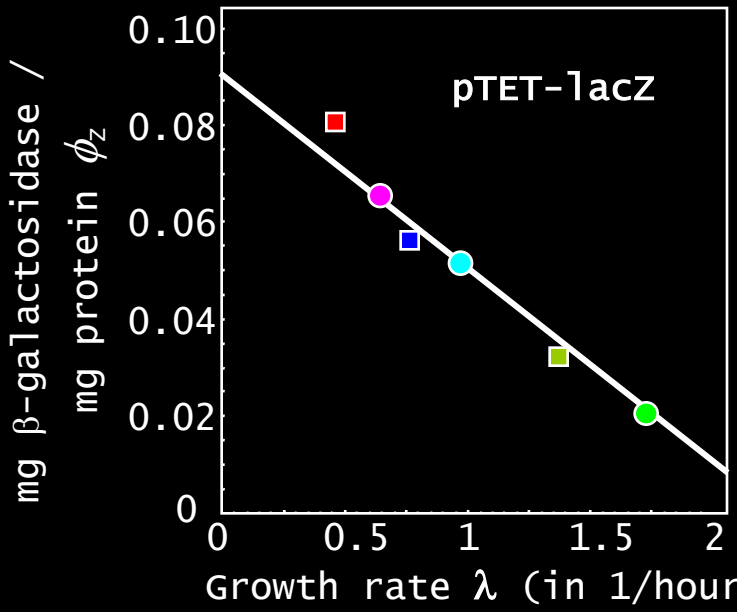
The two constraints observed above have very general consequences on protein expression

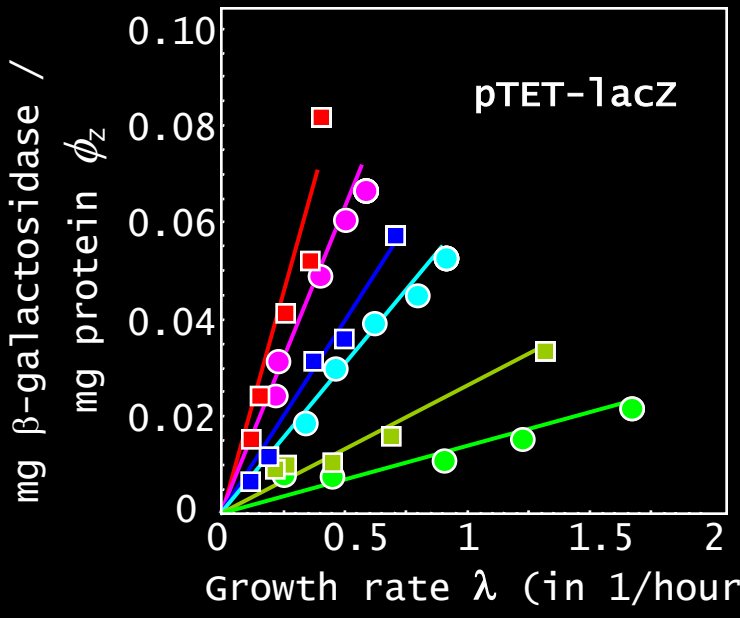
They have been established in **microbes** but probably hold also in higher eukaryotes





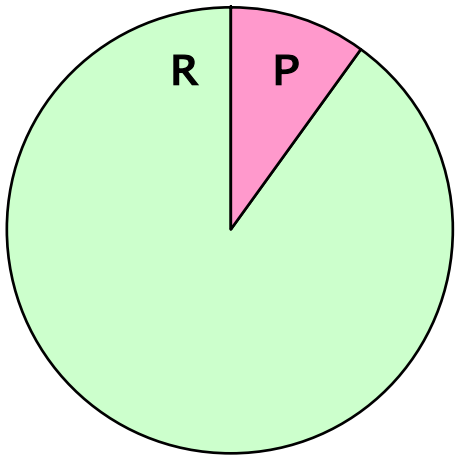
Mirror symmetry reflected in constitutive protein expression





Interpretation

- When I have a lot of ribosomes I have little of the rest and the reverse is true
- This suggest the idea of “proteome” partitioning



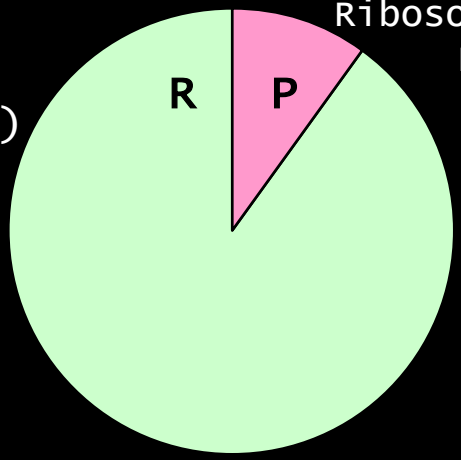
Proteome partition

Ribosome Affiliated Fraction (EF, IF, etc.)

ϕ_R

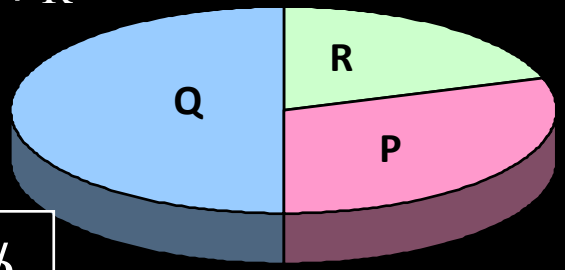
NOT Ribosome Affiliated Fraction

$1-\phi_R$



Have a pretty good estimate of the proportionality between RNA and R-fraction.

$$\phi_Q = 1-\phi_R^{max} \qquad \phi_R^{max} \approx 0.5$$

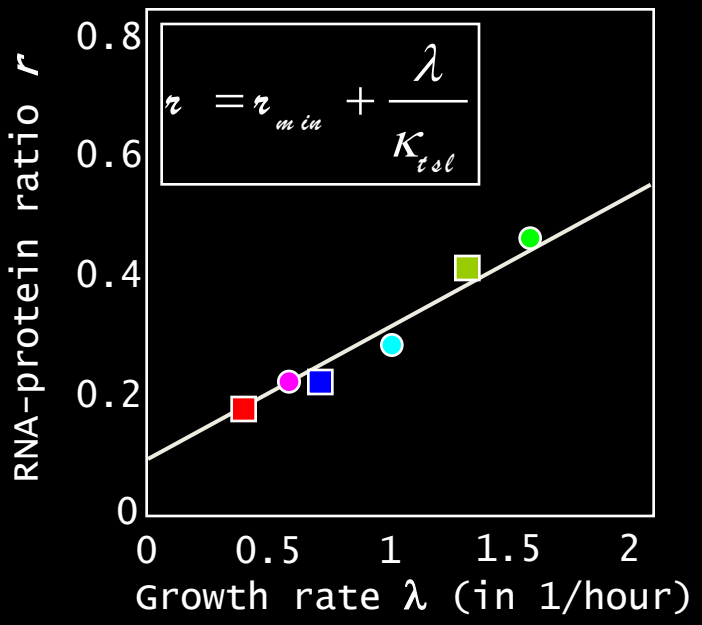


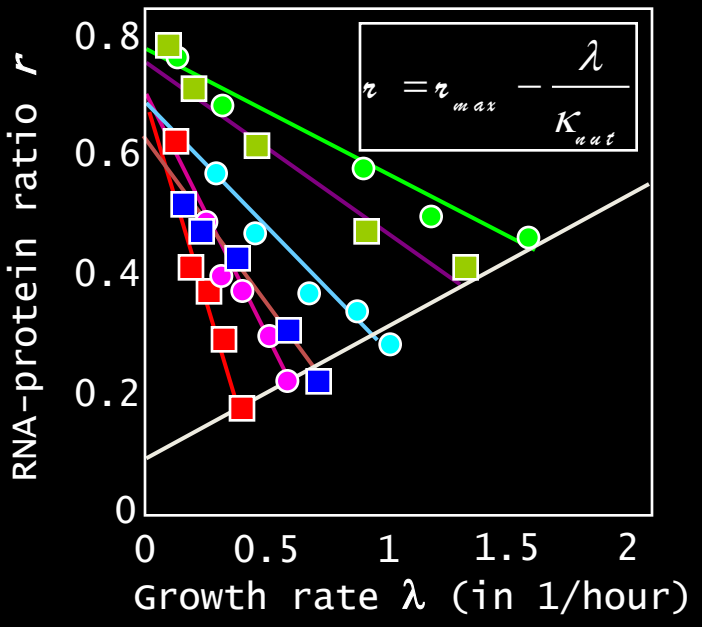
$$\phi_P = \phi_R^{max} - \phi_R$$

Fixed fraction

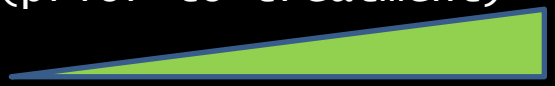
RIBOSOME CONTENT EXHIBITS GROWTH DEPENDENCE

Many antibiotics target the ribosome –
Is drug efficacy growth-state dependent?





Virulence of infection
(prior to treatment)

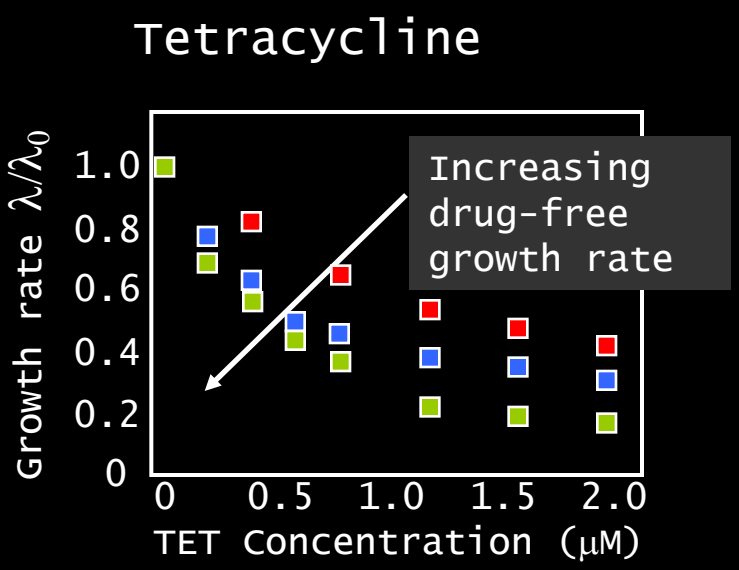


Antibiotic dose
(during treatment)

DRUG SUSCEPTIBILITY **IS** GROWTH DEPENDENT

Before treatment:

- Fast growth
- Medium growth
- Slow growth



IDEA: Combine a mechanistic model of drug action with empirical ‘growth laws’

Take home

- General constraint on growth have important consequences on gene expression
- Proteome allocation is constrained (in particular by ribosome content)
- This needs to be taken into account when deciphering genetic systems dynamics