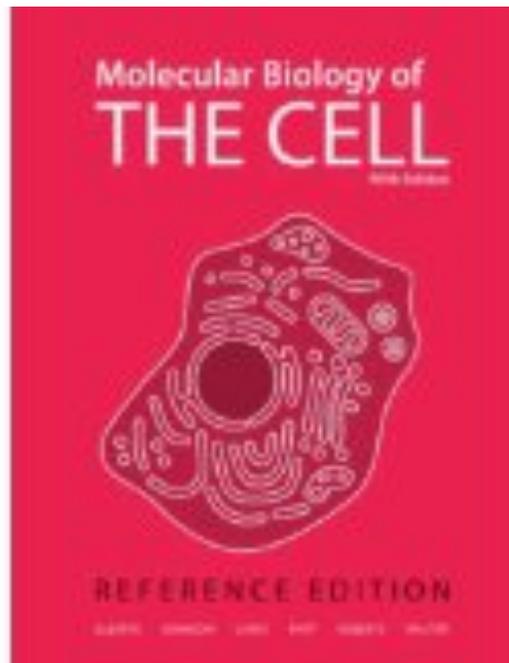


Today:

A few fundamental facts and notions of molecular biology + basic modeling of gene regulation



Some important molecules

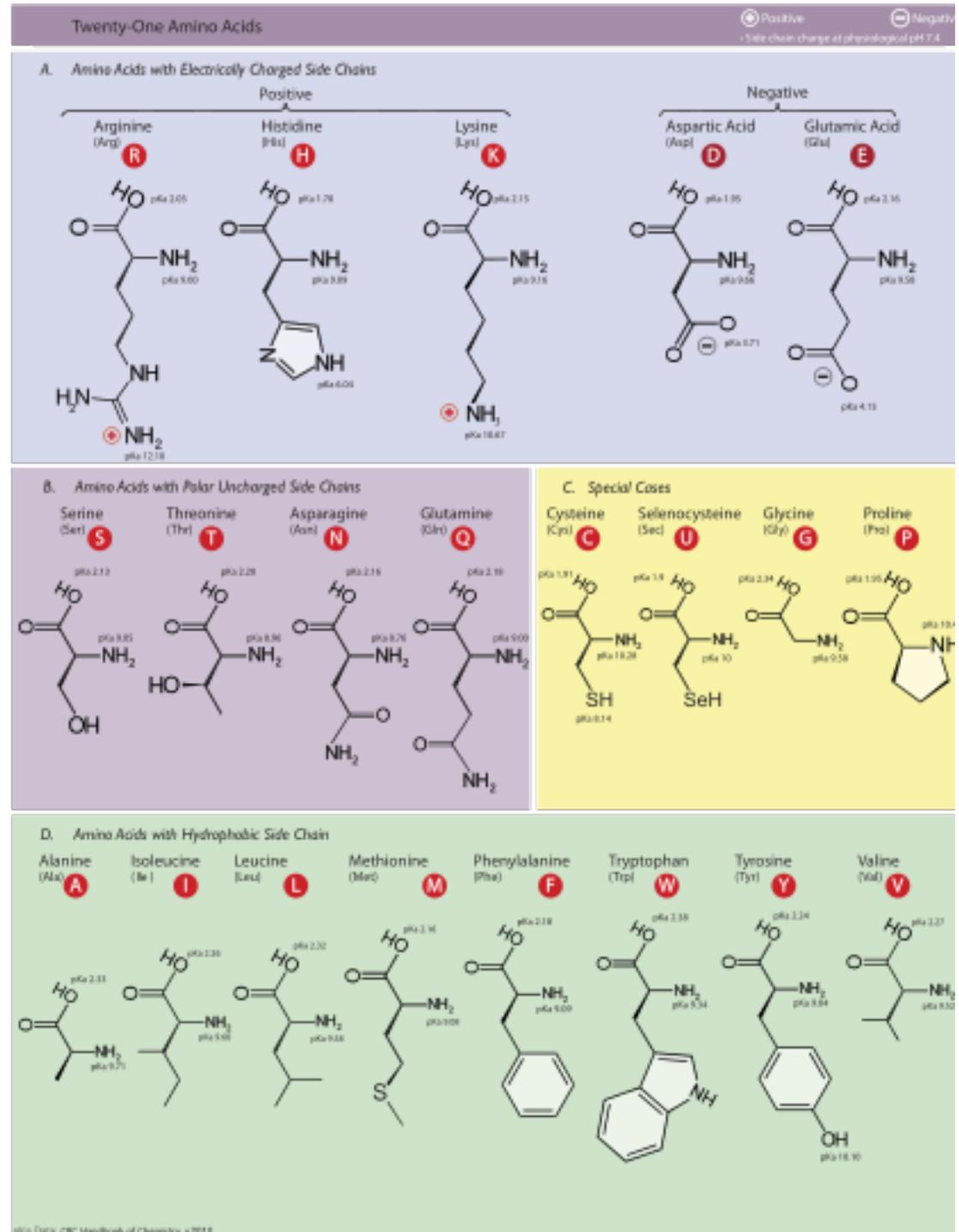
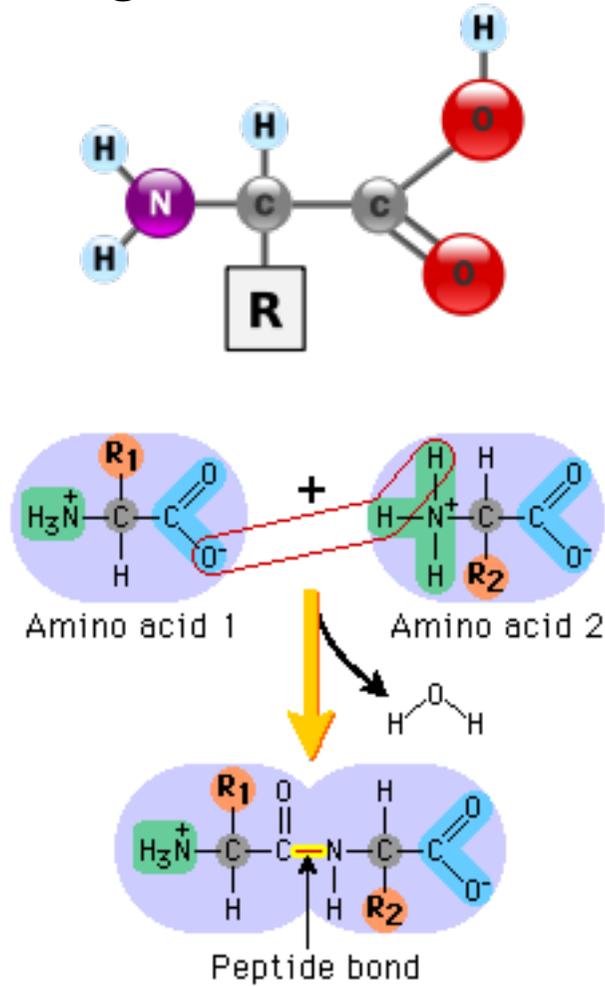
- . DNA

- . RNA

- . Proteins

(lipids, metabolites, ATP,...)

The main biological
 « machines »: **Proteins**
 Building blocks: **Amino Acids**

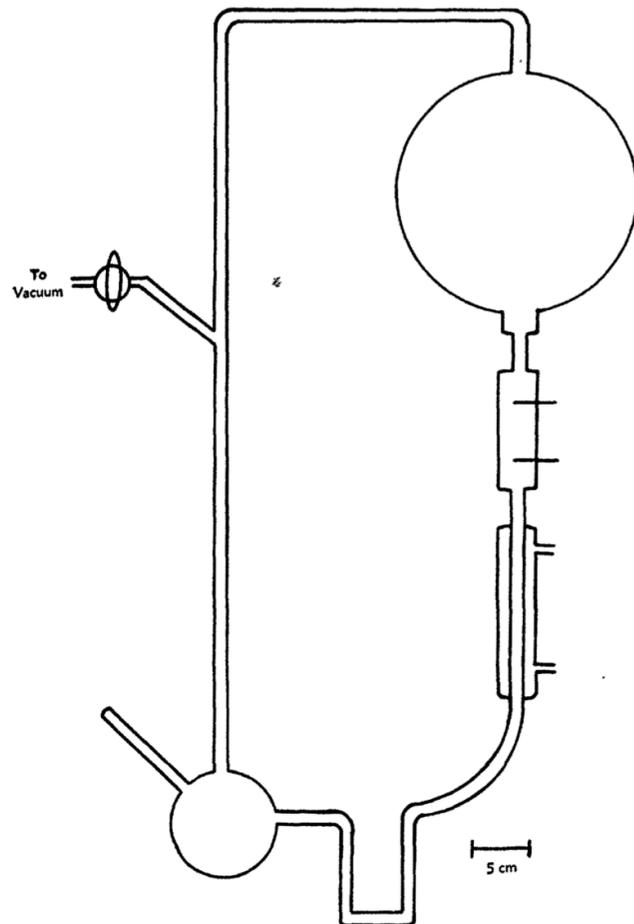


A Production of Amino Acids Under Possible Primitive Earth Conditions

Stanley L. Miller^{1, 2}

*G. H. Jones Chemical Laboratory,
University of Chicago, Chicago, Illinois*

Science, 117, 528-529 (1953)



H₂O, CH₄, NH₃, H₂ in gaseous form and electric discharges.

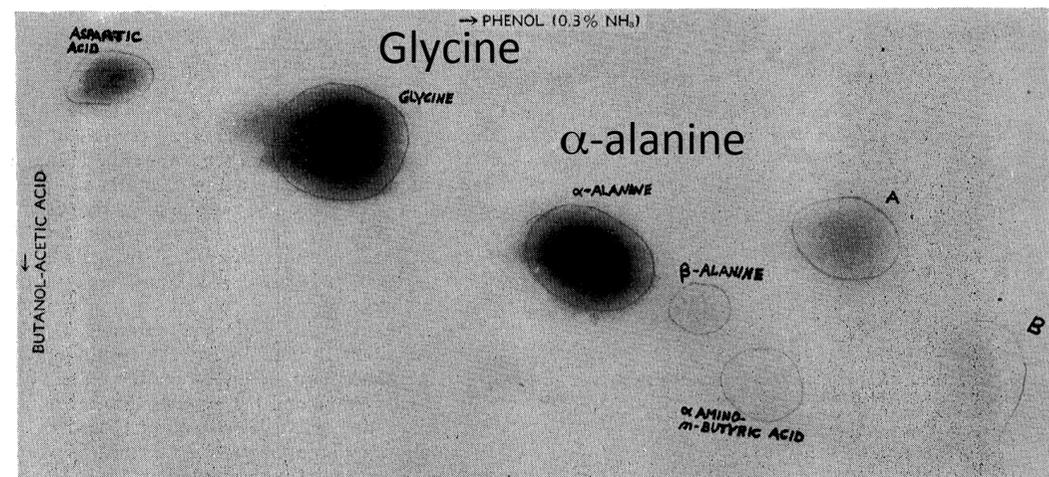
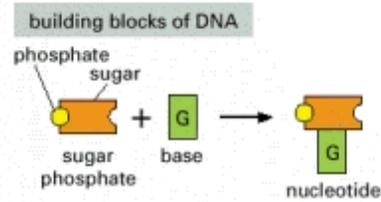
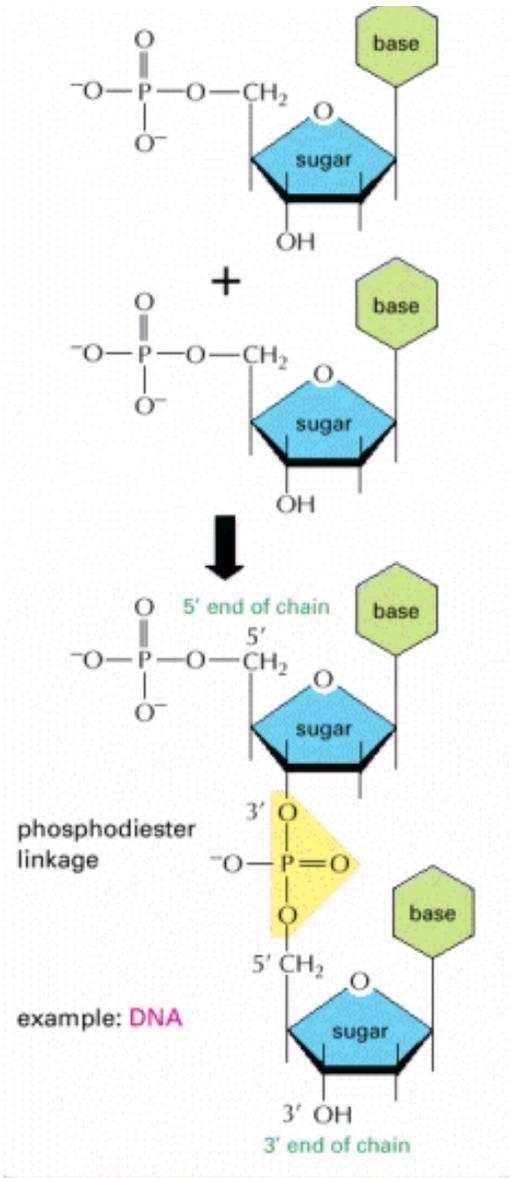
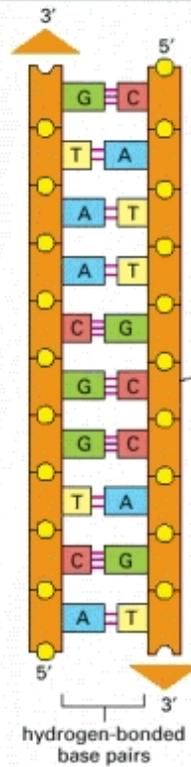


FIG. 2.

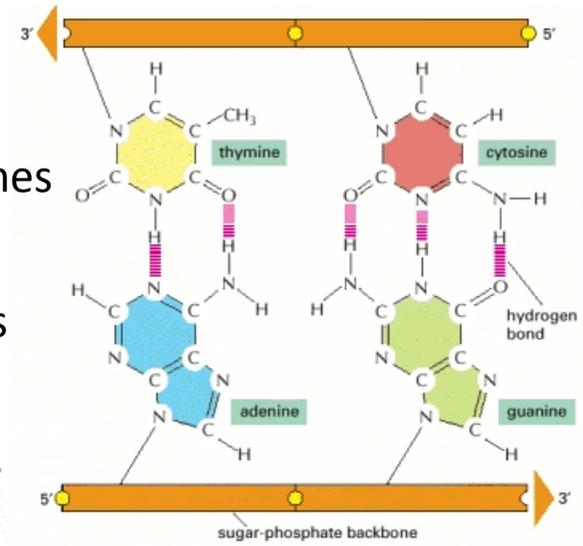
DNA, nucleotides and bases



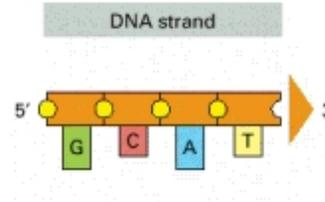
double-stranded DNA



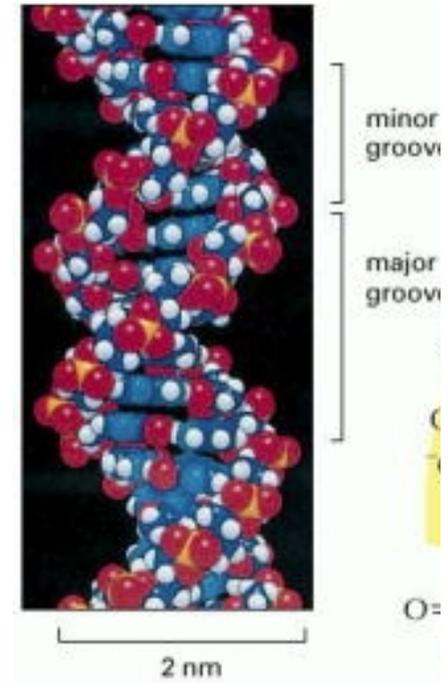
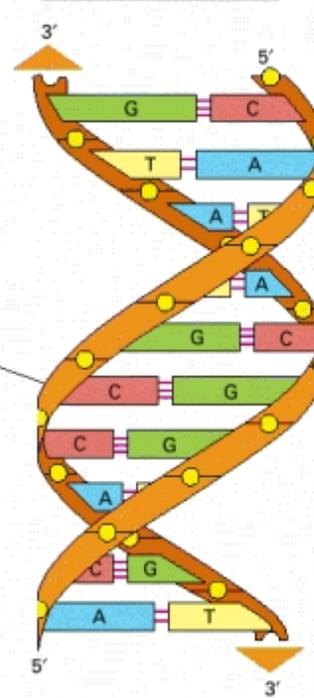
Pyrimidines



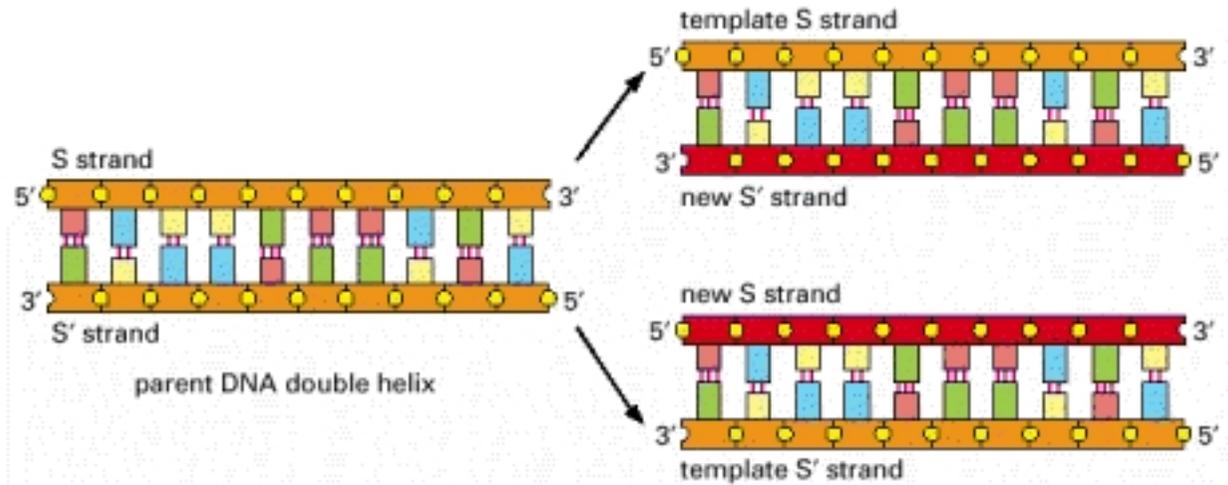
Purines



DNA double helix



Conservation of genotype when cells divide



"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material »
The famous last sentence in Watson and Crick, Nature, April 25, 1953.

Very high precision: error rate $\sim 10^{-9}$, how achieved?

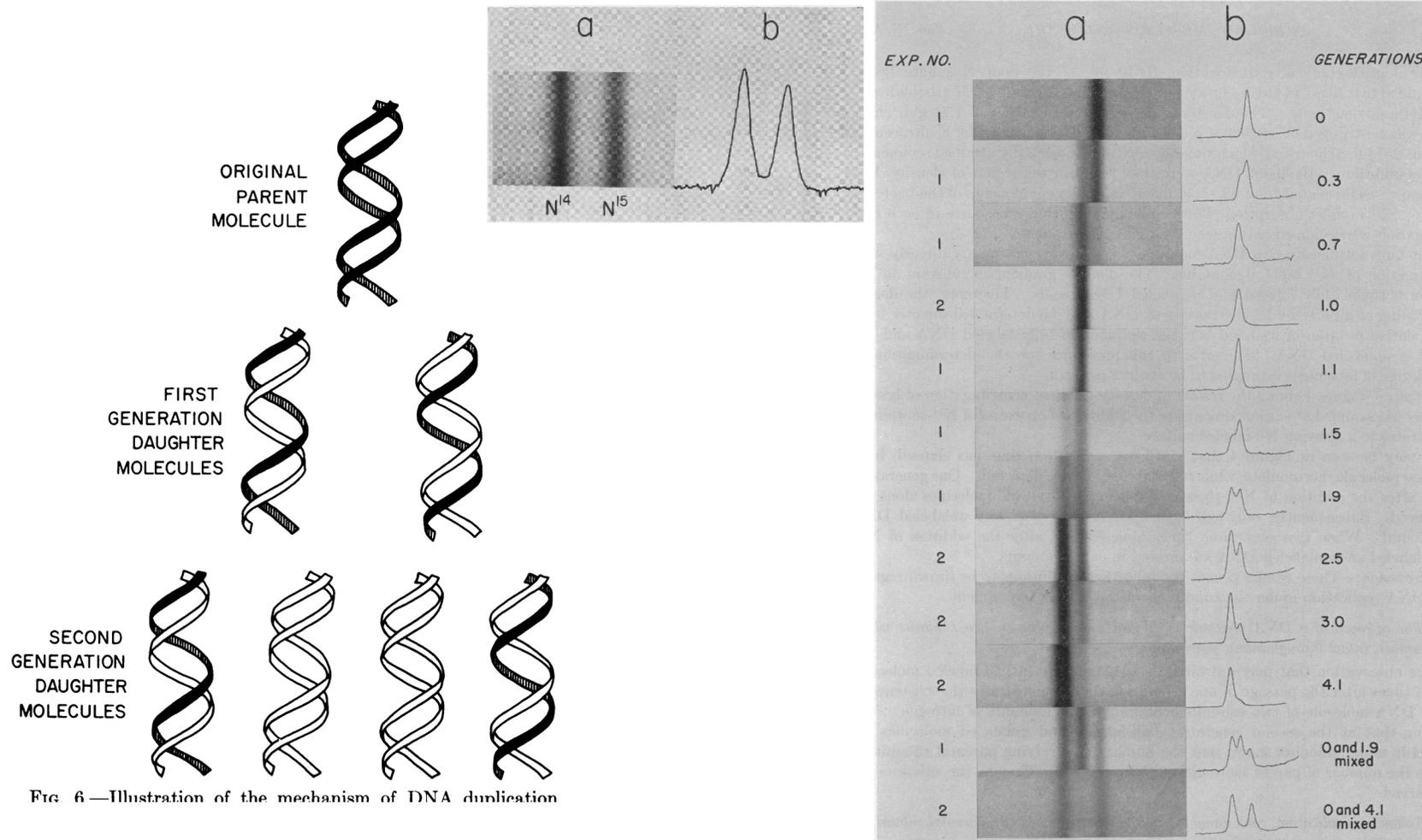
-> « Kinetic proof-reading » to be discussed later

THE REPLICATION OF DNA IN *ESCHERICHIA COLI**

BY MATTHEW MESELSON AND FRANKLIN W. STAHL

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,† AND NORMAN W. CHURCH LABORATORY OF CHEMICAL BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

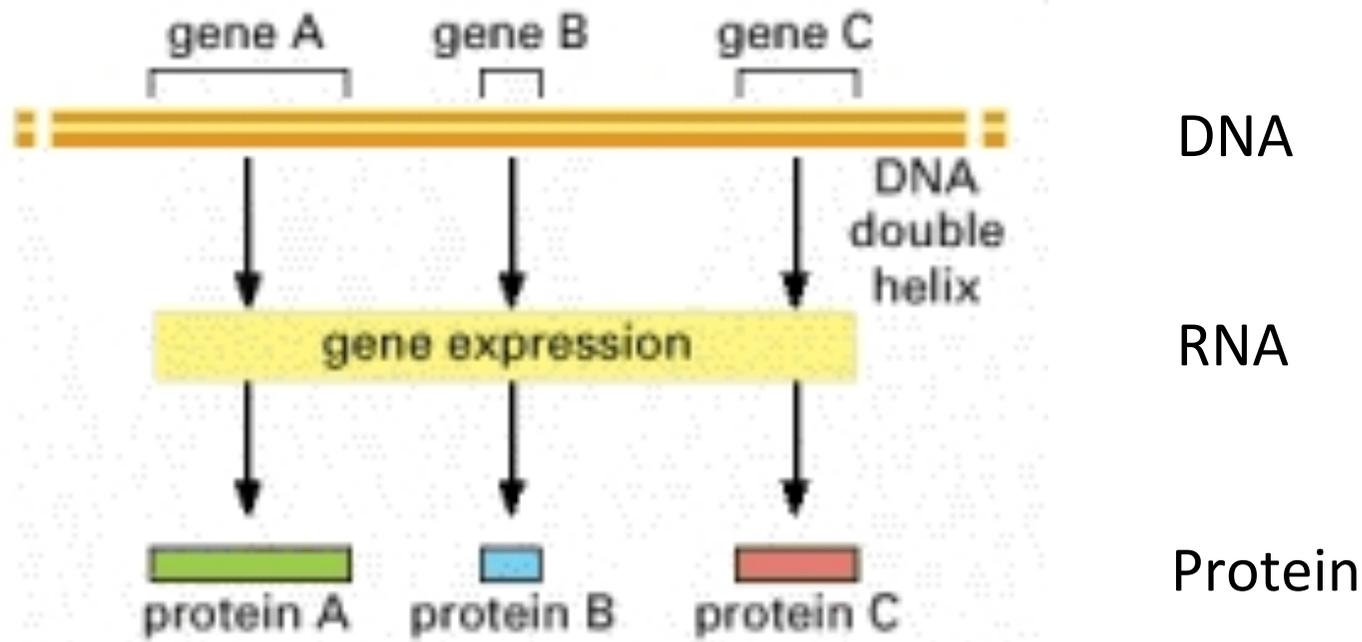
. Communicated by Max Delbrück, May 14, 1958



The classical view

« DNA makes RNA, RNA makes proteins, proteins make us »

F. Crick



RNA viruses: reverse transcription RNA → DNA

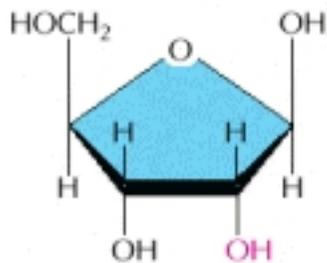
More recently: non-coding RNA with regulatory roles (miRNA, lncRNA,...)

Complementarity between mRNA and DNA

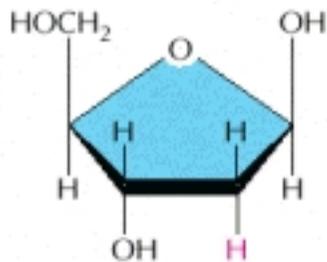


Transcription: synthesis of mRNA
(from 5' end to 3' end) by
RNA polymerases

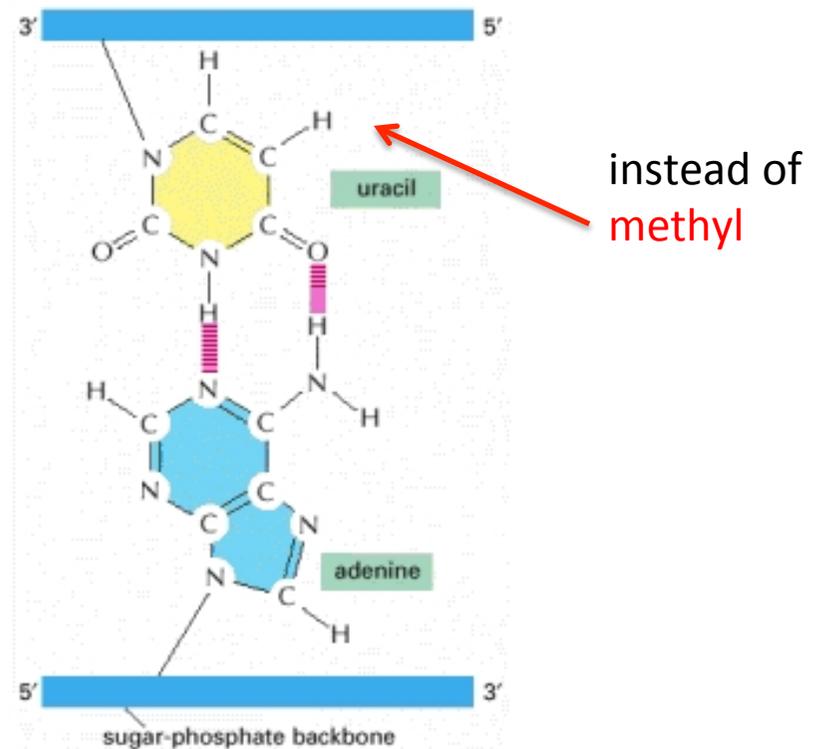
Biochemistry, 5th edition Berg JM, Tymoczko JL, Stryer L.



β -D-ribose
used in ribonucleic acid

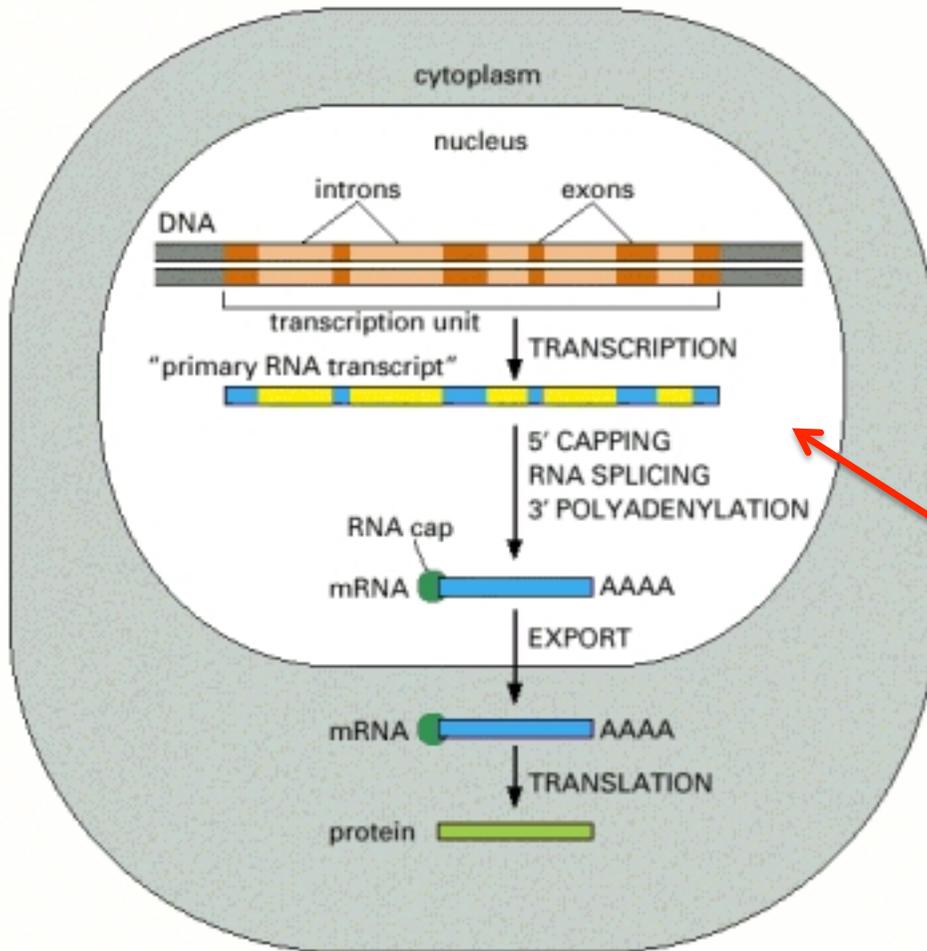


β -D-2-deoxyribose
used in deoxyribonucleic acid

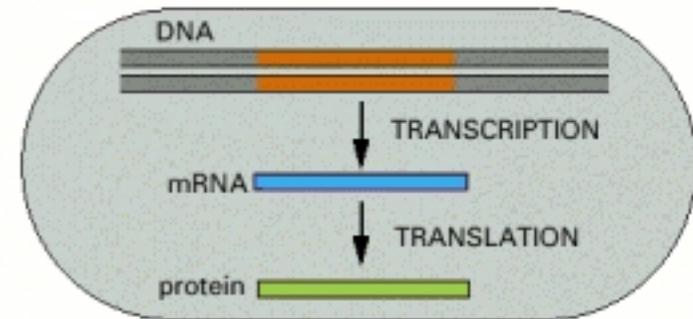


From gene to protein: summary.

(A) EUCARYOTES



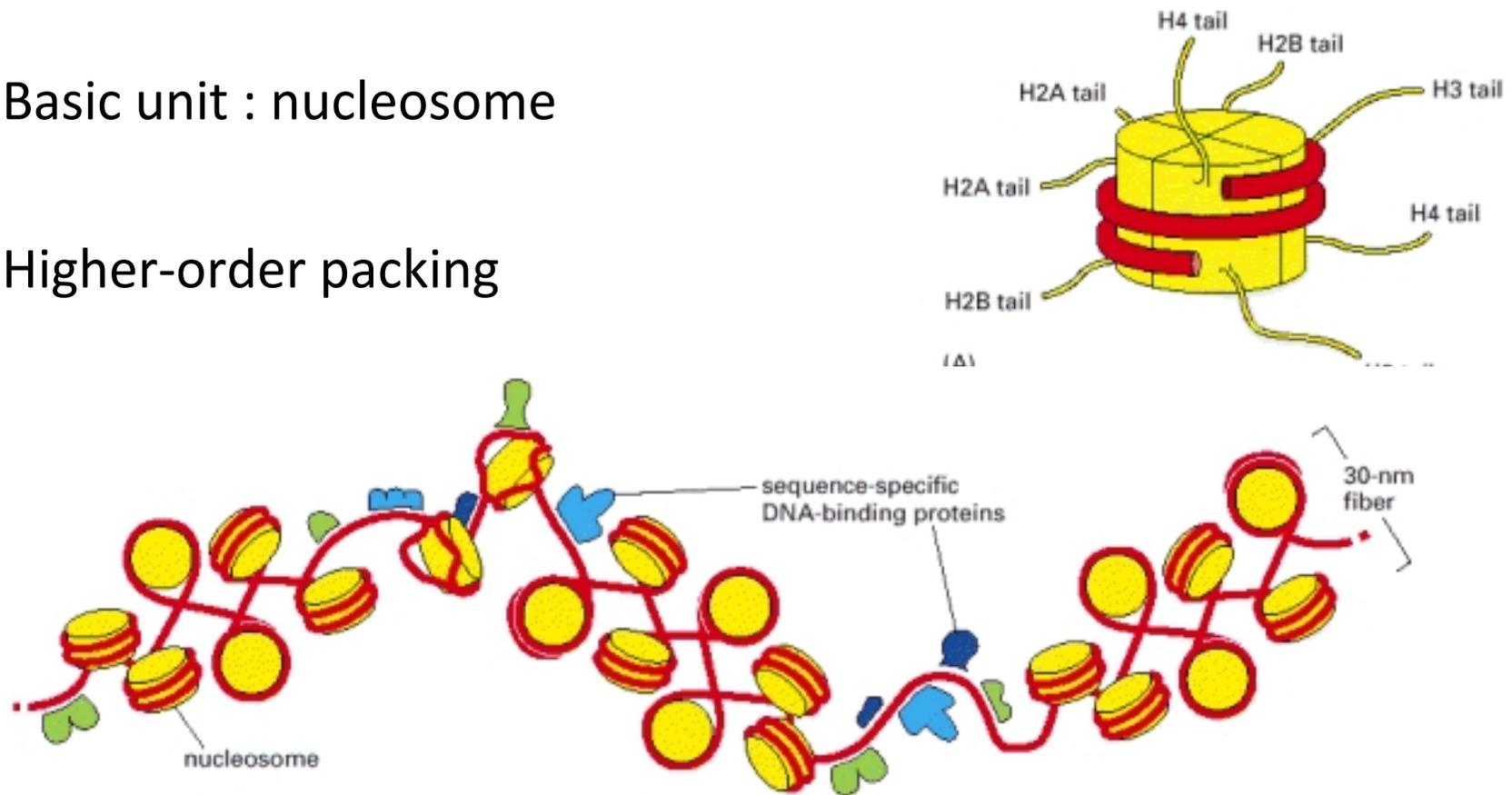
(B) PROCARYOTES



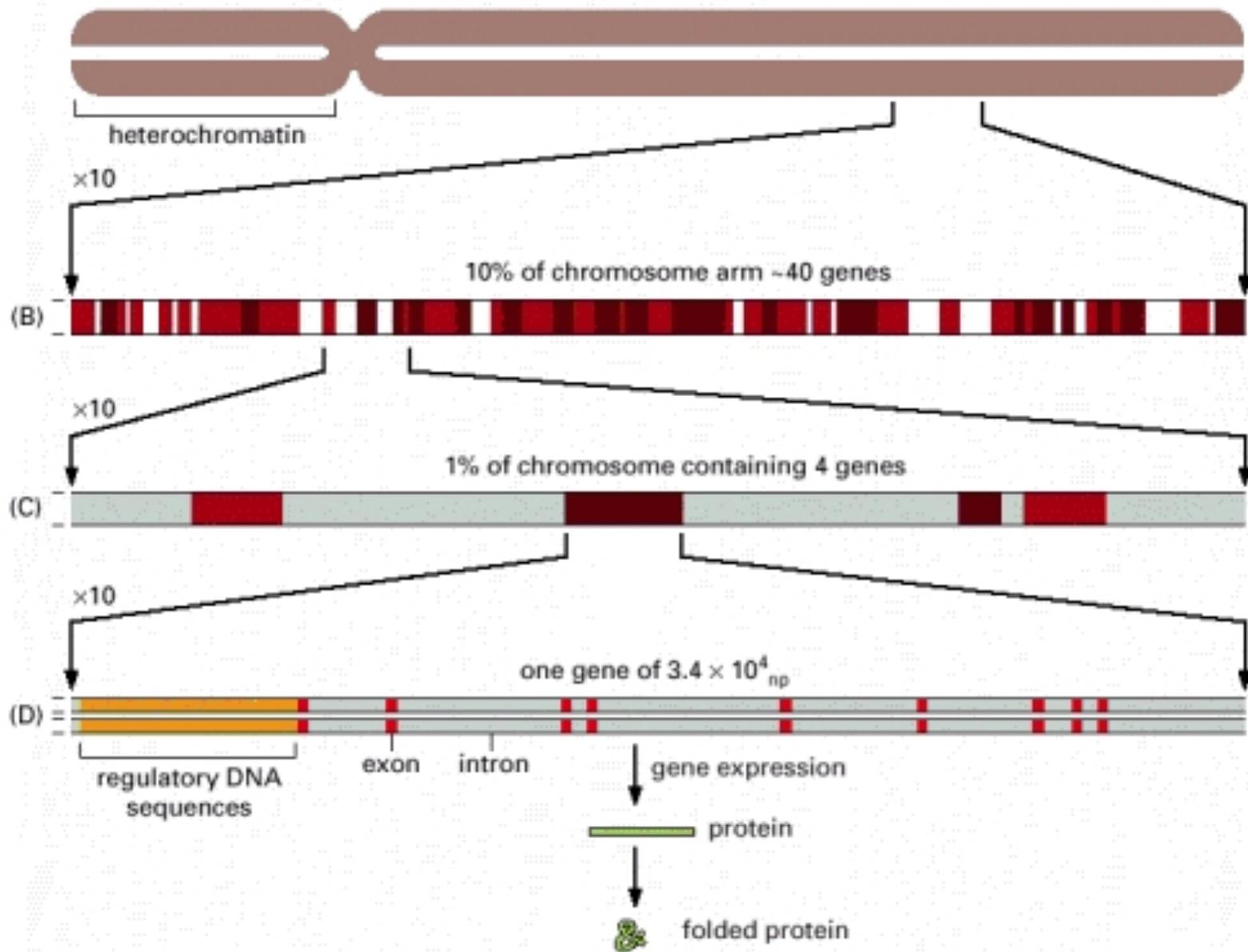
Exons and introns

Chromatin

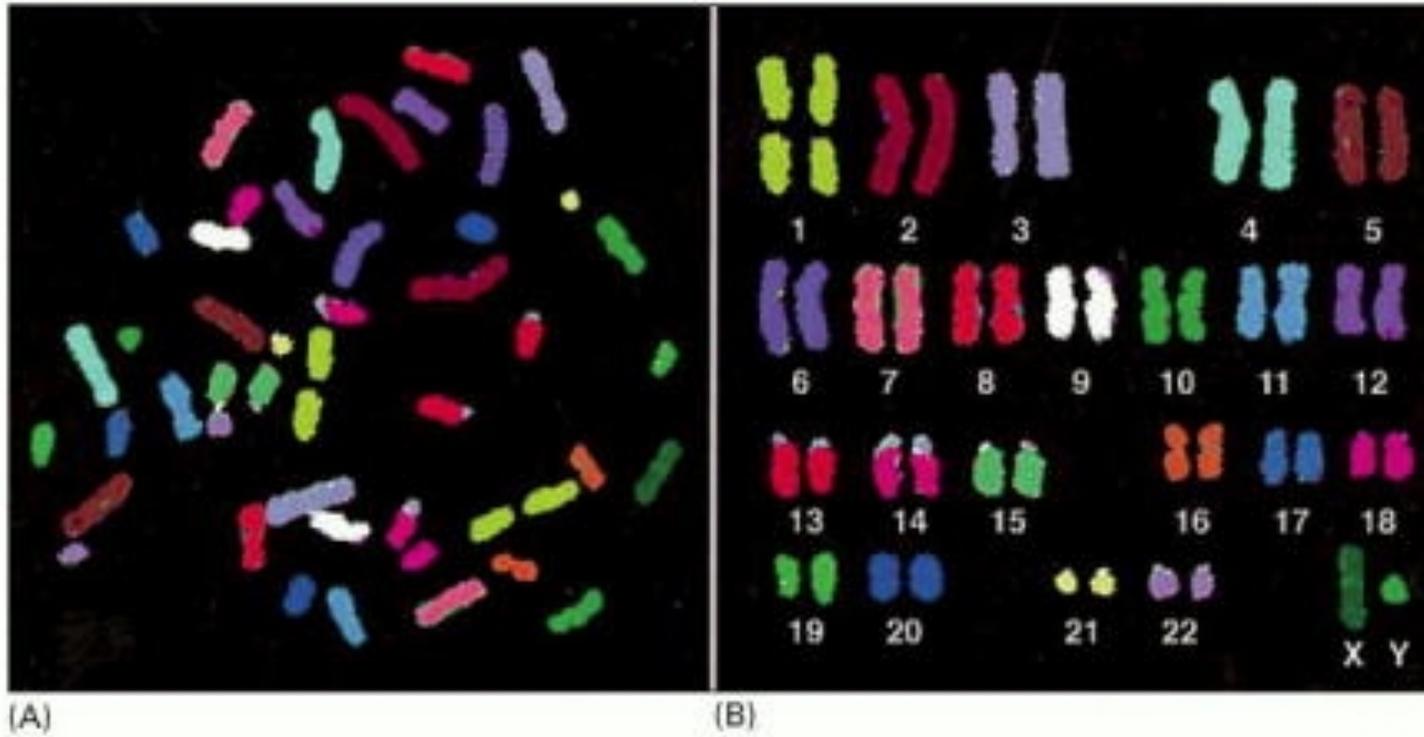
- Chromosomes: complexes of protein with DNA
- Basic unit : nucleosome
- Higher-order packing



(A) human chromosome 22— 48×10^8 nucleotide pairs of DNA

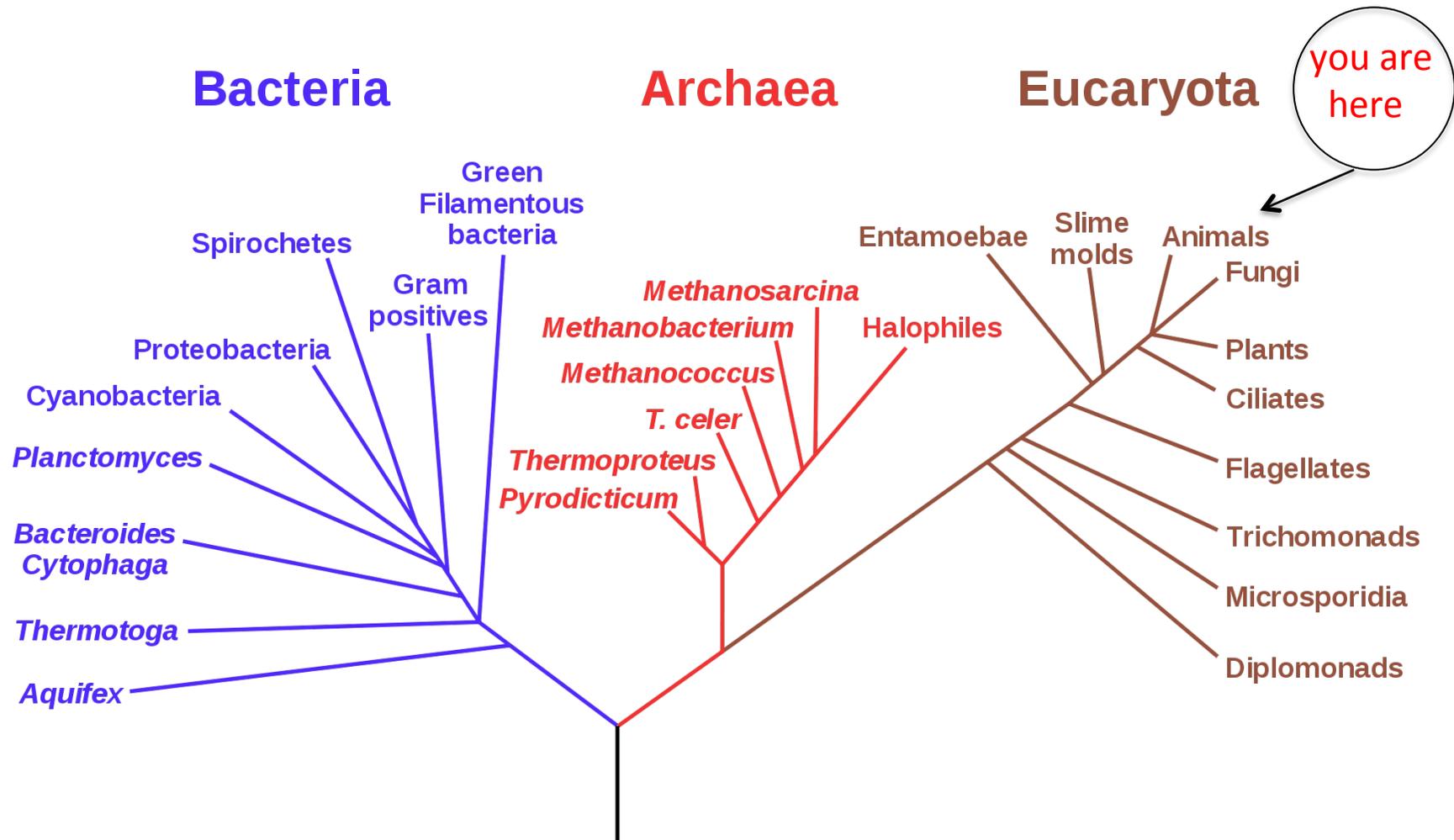


One DNA molecule is packaged into one chromosome



Human : 22 pairs of « autosomes » +X/Y

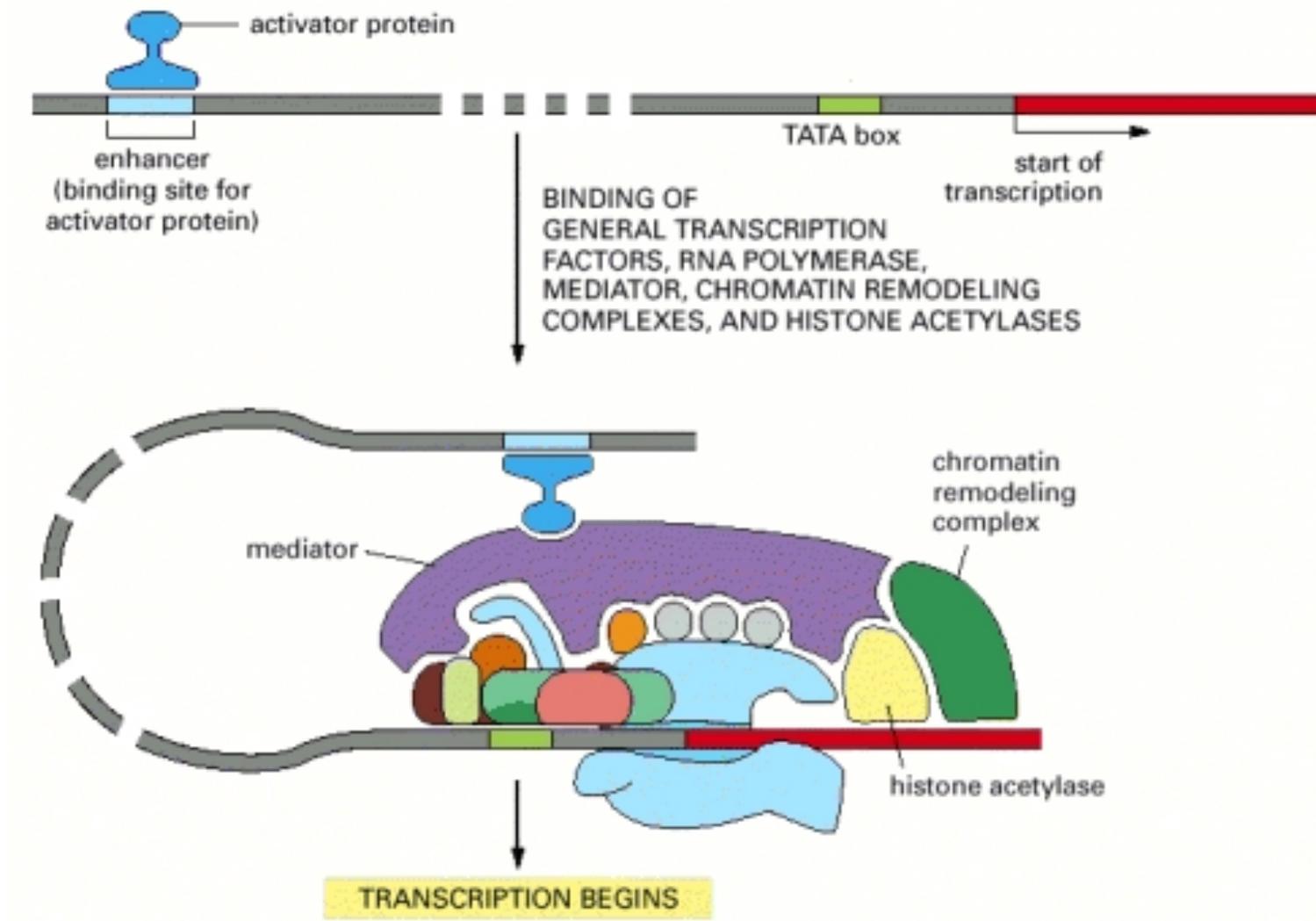
Phylogenetic Tree of Life



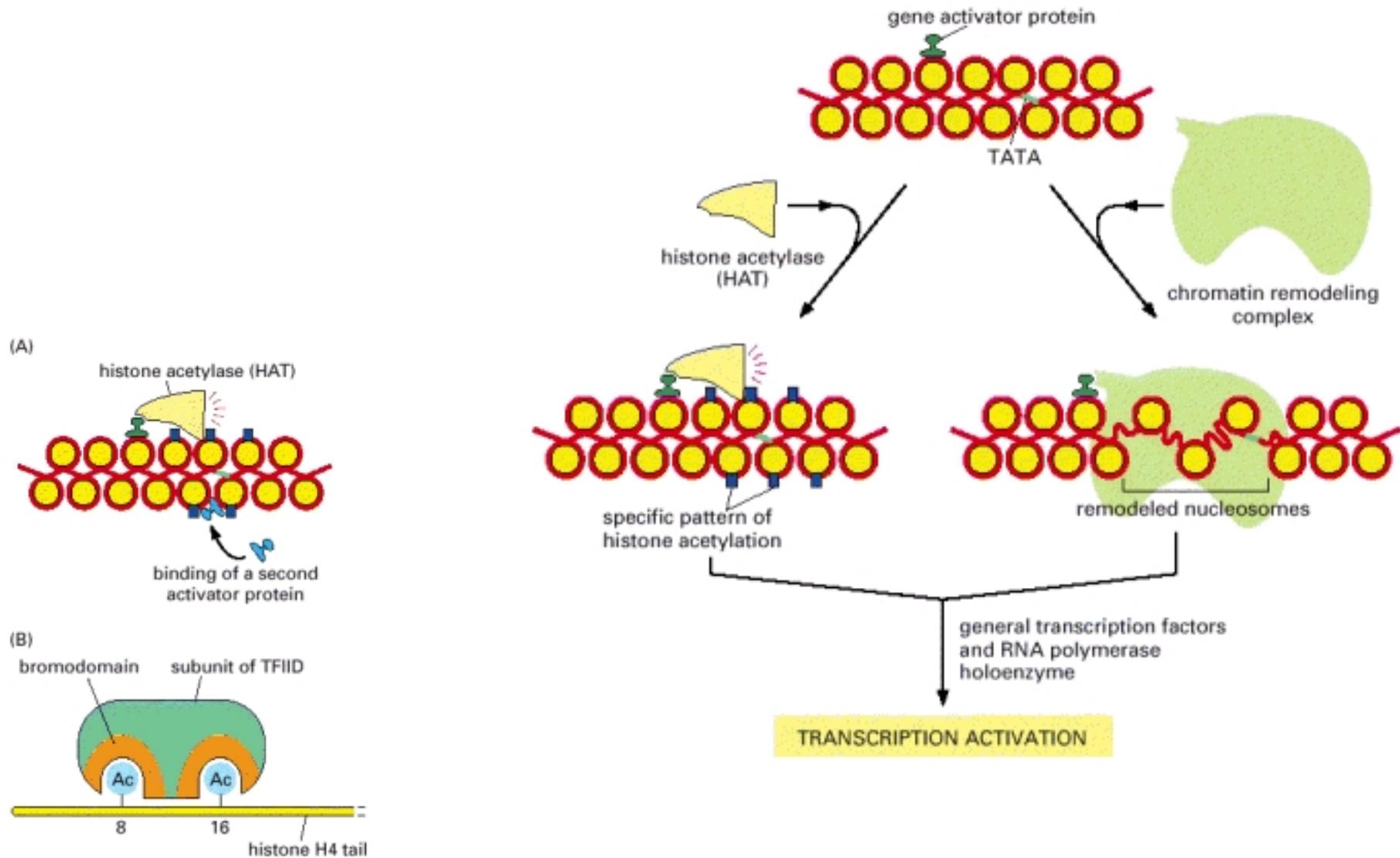
Woese C, Kandler O, Wheelis M (1990) *Proc Natl Acad Sci USA* **87 (12): 4576–9**

« Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. »

Transcription and its regulation



Modification of chromatin structure and accessibility

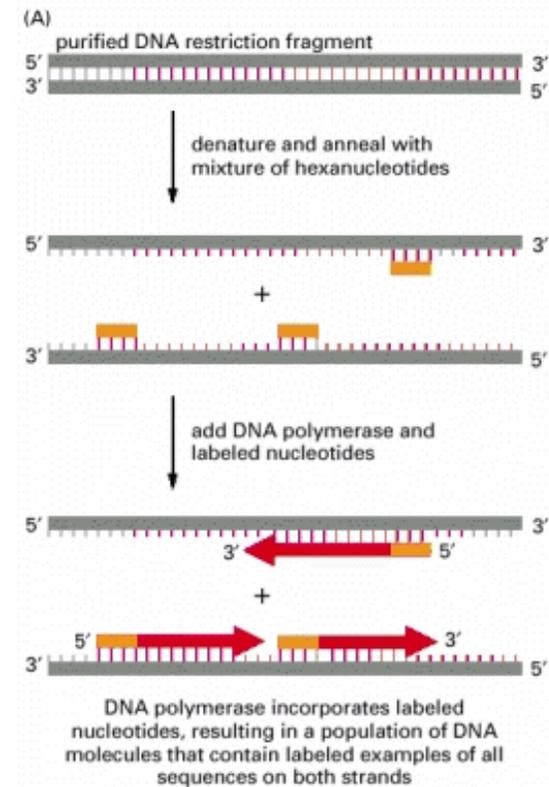
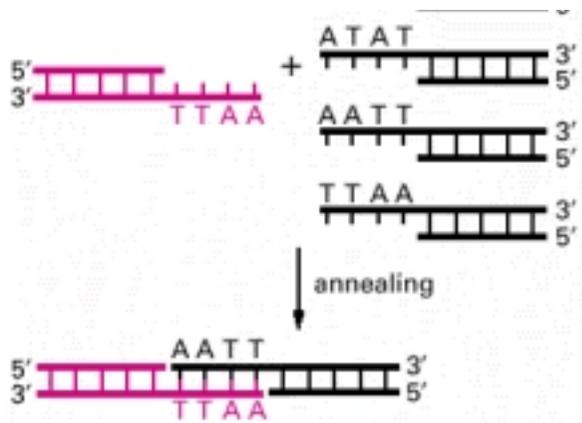
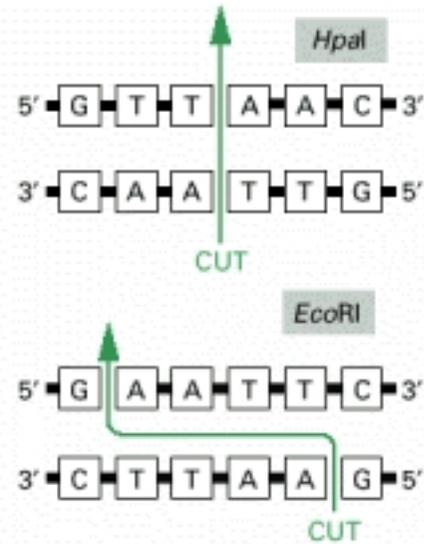


Regulation is possible and exists at all levels

- Regulation of **transcription**
- Regulation of **translation**
- **Post-translational** regulation:
protein modification, complexation,.....

Some tools (from nature) to manipulate DNA

- **Cut** : restriction enzymes
- **Copy** : primer + polymerase
- **Paste** : ligase

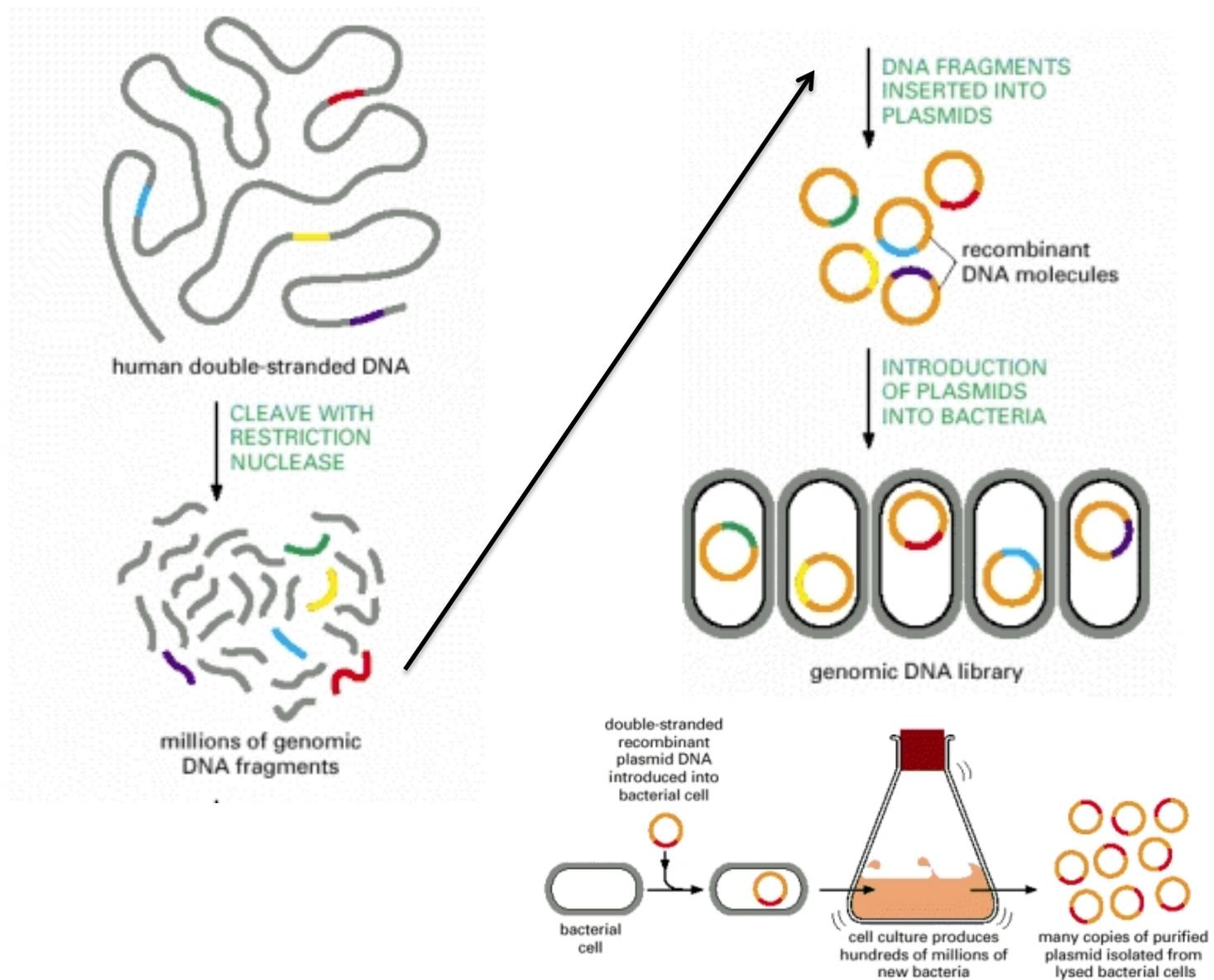


(C)

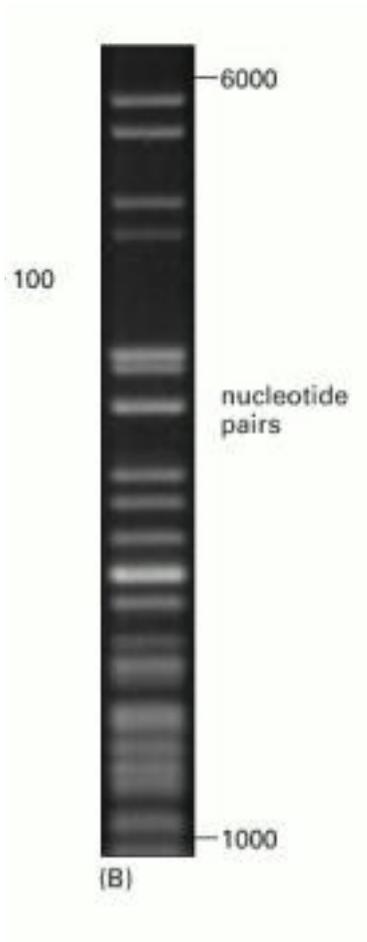
O

I

One application : cloning

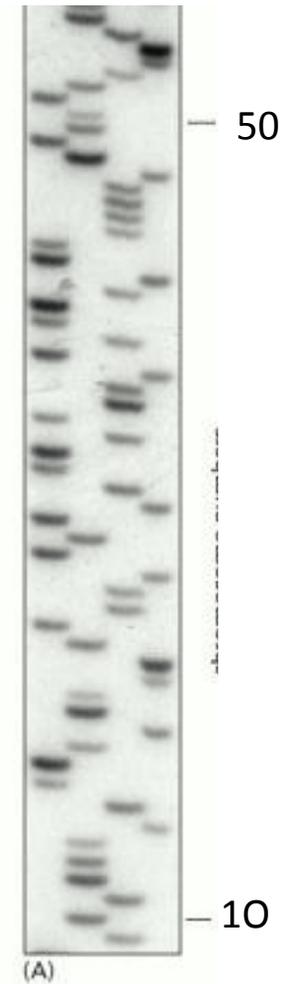


Measure and separate different sizes: gel electrophoresis



Migration in an electric field in a gel.

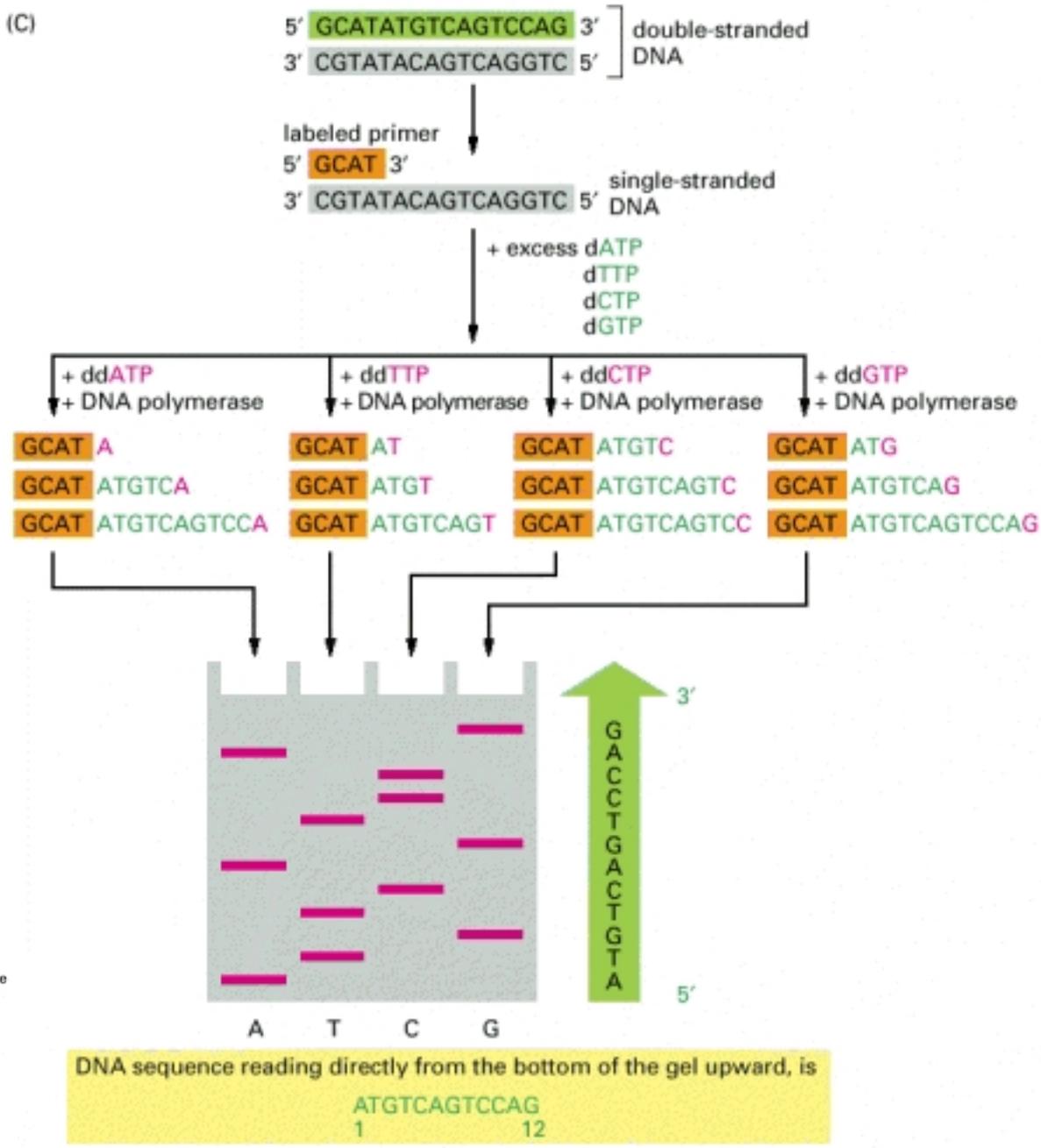
Different ranges of sizes can be resolved with different types of gel.



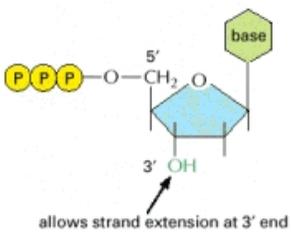
Some clever and useful applications

- Read DNA bases:
Sanger's **sequencing** technique (1975).
- Amplification:
Polymerase **C**hain **R**eaction (Mullis, 1985)
- Seeing in real time (and in color) inside cells:
Fluorescent proteins and protein fusion (1994)

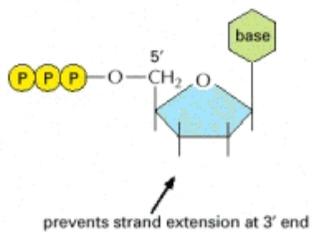
Sanger's sequencing technique



(A) deoxyribonucleoside triphosphate

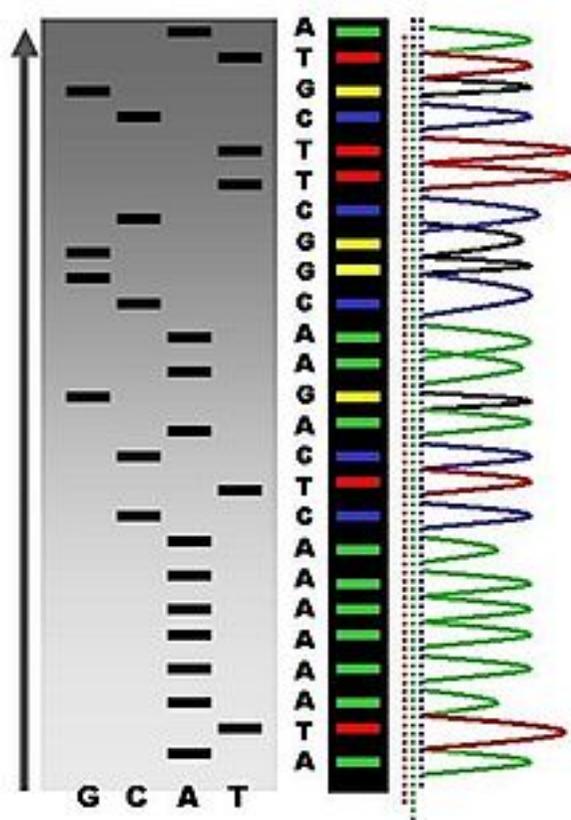


dideoxyribonucleoside triphosphate

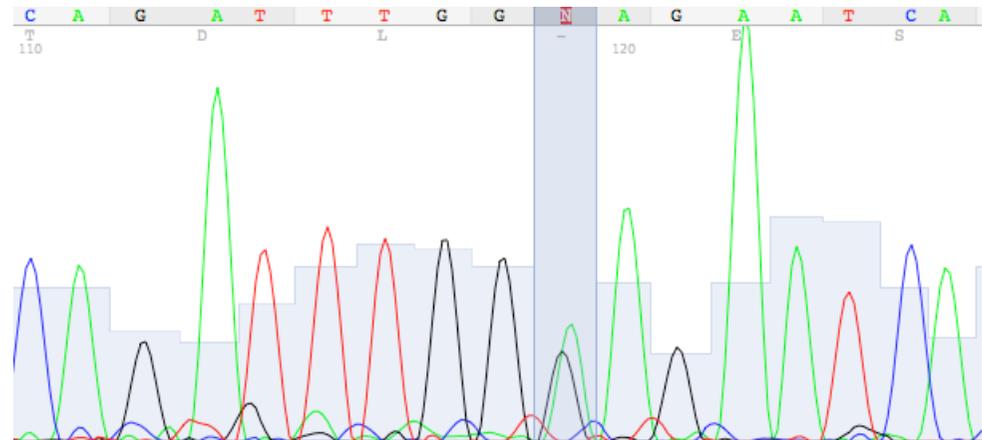


Automation: use of fluorescently-labelled chain terminators

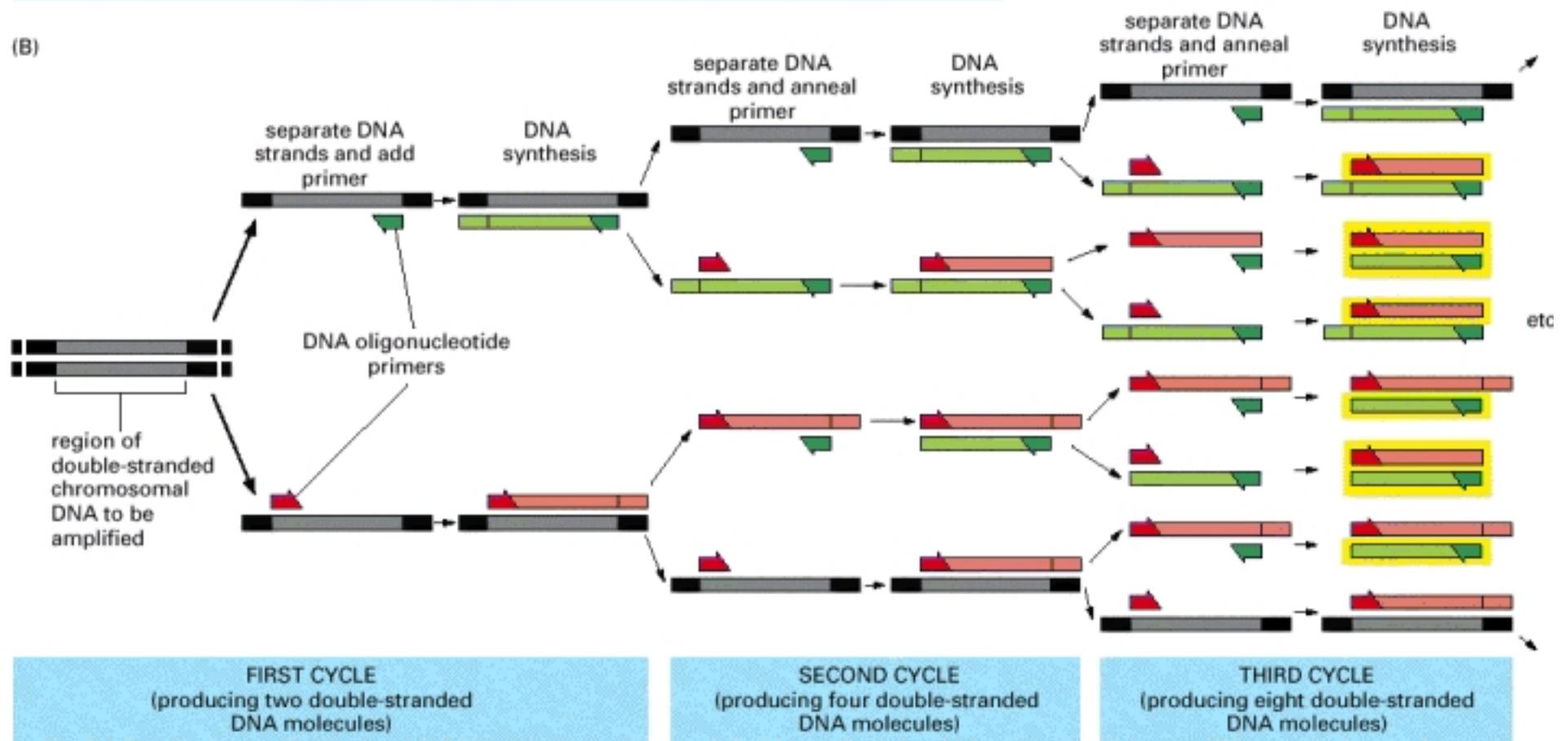
- ddNTPs marked with fluorescent dyes emitting at different wavelengths (color)



```
AATGTGTCAGAGCTGGCTCCGCTCTAACTGACCTCCCTTGCCCTCTAAAAAGTGACACATTGAATTTTACTGA  
CTCTGTGTTTCAAACATTTTGGGGACTGAAAACCACCAGGCCAAATCTCAGGCCTTAGAAAGCAGTTAAACTG  
AATTGCAAATAAAAGGCTTAGAAAGTCCTTAAATCATAAACTTGGCATCTCCAATAGGACAAATGAACTTTGTC  
TTCTCATCTGAGTTAACAGATTTGGTAGAATCAAAATCTTTTCTGTGTATAGACTACATGCTACCATTTCACA  
GGCAAGCTGCCCTAAATTTGAAAACTTTTCCTTCTGAAACCACCCAACTCTTTAGCTTGATTGGGCAACCG  
AATCTCTTAGGCTTTTGCTCTTTGTGTCCAAAACTAGCAGTGTTCCTGAGAGCAGGCTATCAATCATTTACATA  
TTAGTGTGACATTCAGTCAGCATCCATTTAAATAAATTTACTCAGATAAATATTTATTAAGCACCTACTTTGTTC
```



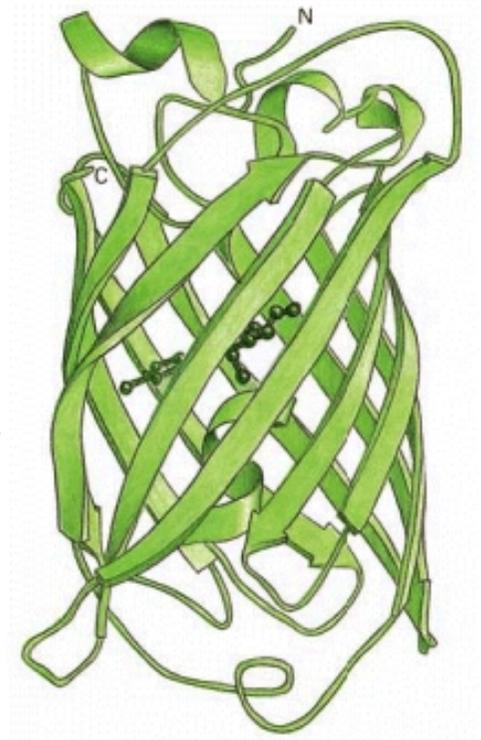
Amplification: Polymerase Chain Reaction



Exponential amplification of a particular sequence

Green Fluorescent Protein

- Isolated from a **jellyfish**
- Subsequently modified (brightness improved, other **colors**)
- Reporter molecule: DNA coding sequence under the control of a promoter
- Fusion protein: GFP-DNA coding sequence added before/after the coding sequence of another protein



A quantitative cell-centered approach to biology

- **Intra-cellular dynamics :**

how a cell state should be characterized?

how does it evolve in time, respond to external signals,...

- **Inter-cellular dynamics :**

how do cell cooperate to create functioning organs, organisms

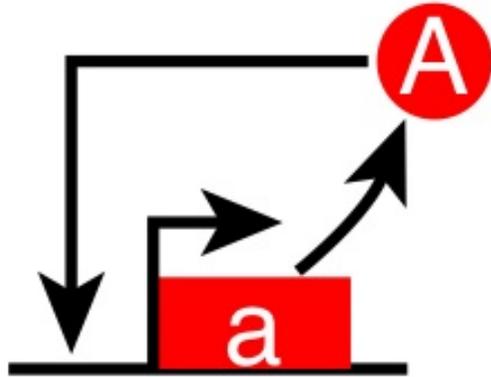
Dynamical processes can be hard to understand:

- see the details, perturb

- qualitative reasoning can be difficult **mathematical analysis can help**

Why is modelling useful?

The simplest network

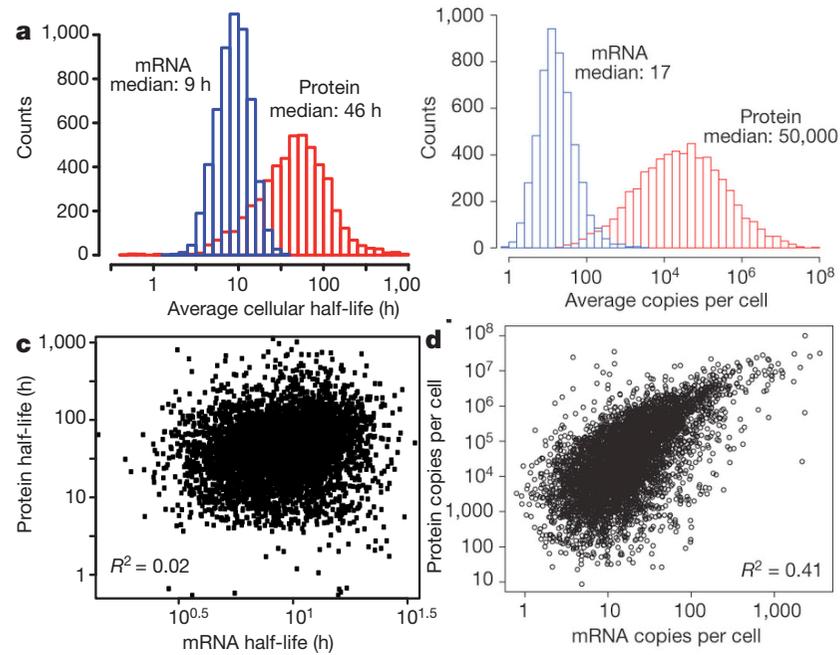


Can this network be in different states?

Helpful to model it mathematically

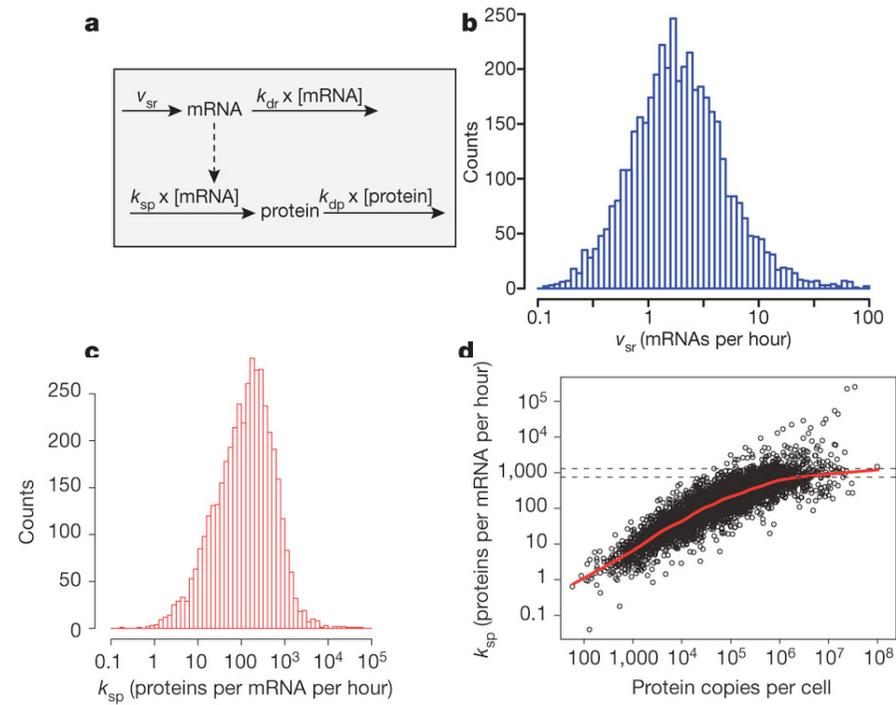
A feeling for the number of mRNA and proteins

B Schwanhäusser et al, Nature 473, 337 (2011); corrected 495, 126-127 (2013)



Mouse fibroblasts (NIH3T3)

Quantitative model of gene expression in growing cells.

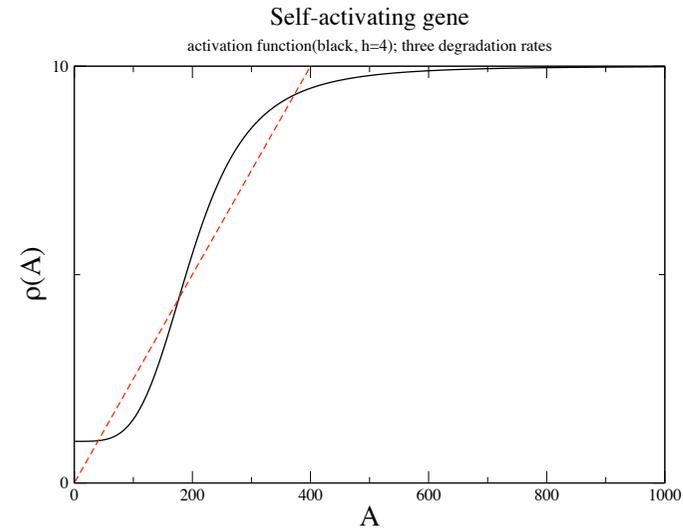
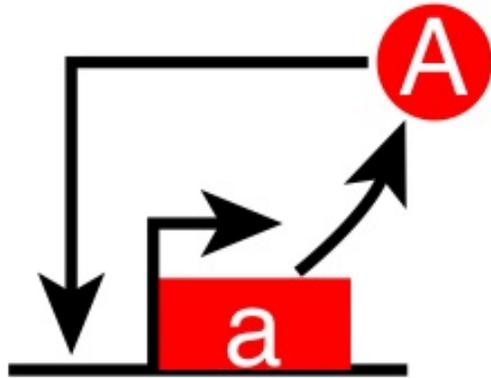


B Schwanhäusser *et al. Nature* **473**, 337-342 (2011) + correction (2013)

nature

Bistability

The simplest network



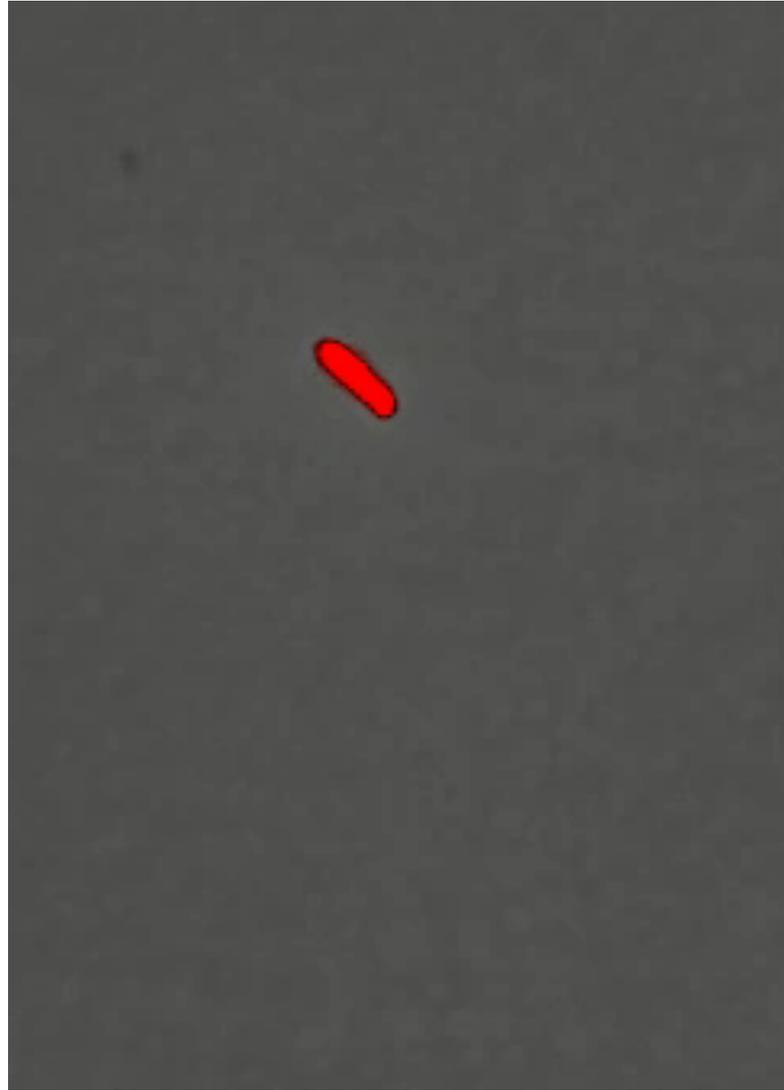
Can this network
be bistable?

It is interesting to quantitatively
measure gene regulation function
(two stable states, some unstable)

$$\frac{dA}{dt} = \frac{\rho_0 + \rho_1 (A/A_0)^h}{1 + (A/A_0)^h} - \delta_A A$$

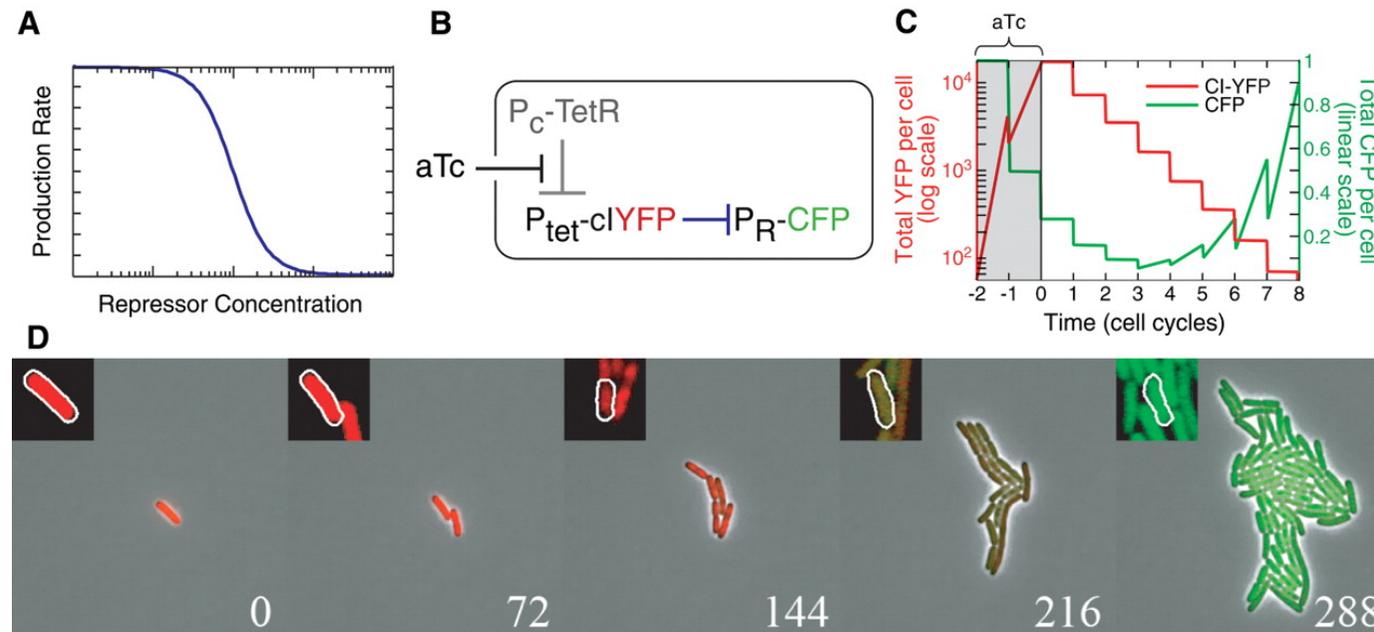
Measuring a promoter activity

Monitoring proteins in real time



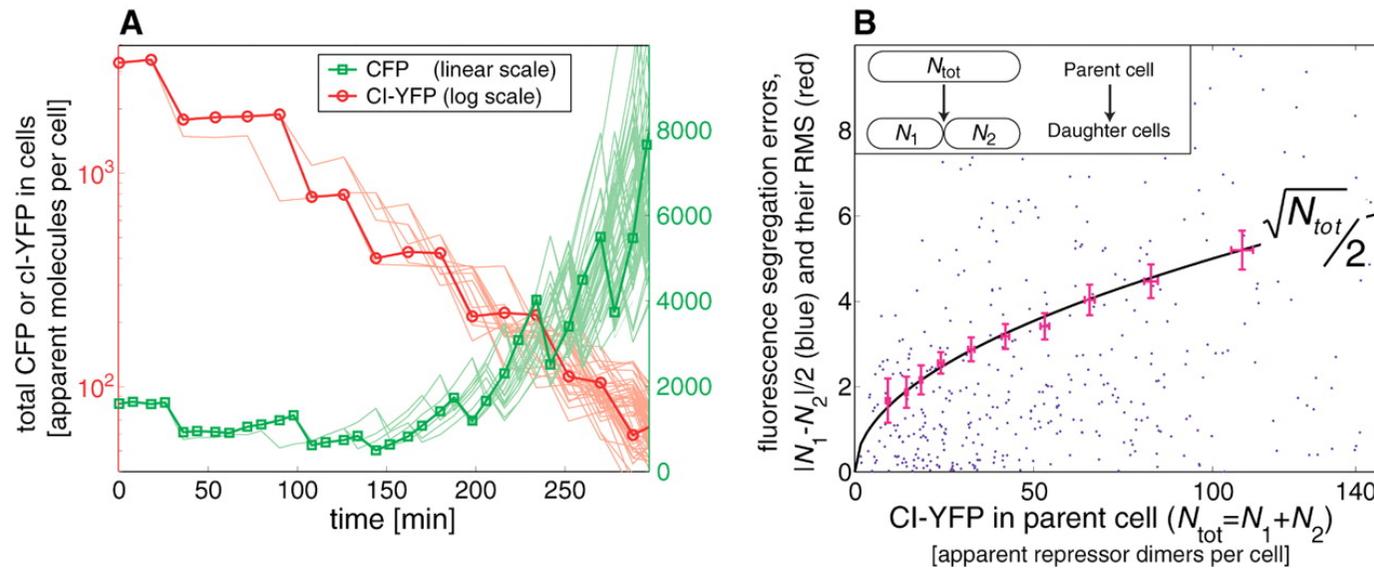
Rosenfeld et al, Science (2005)

Fig. 1. Measuring a gene regulation function (GRF) in individual E. coli cell lineages



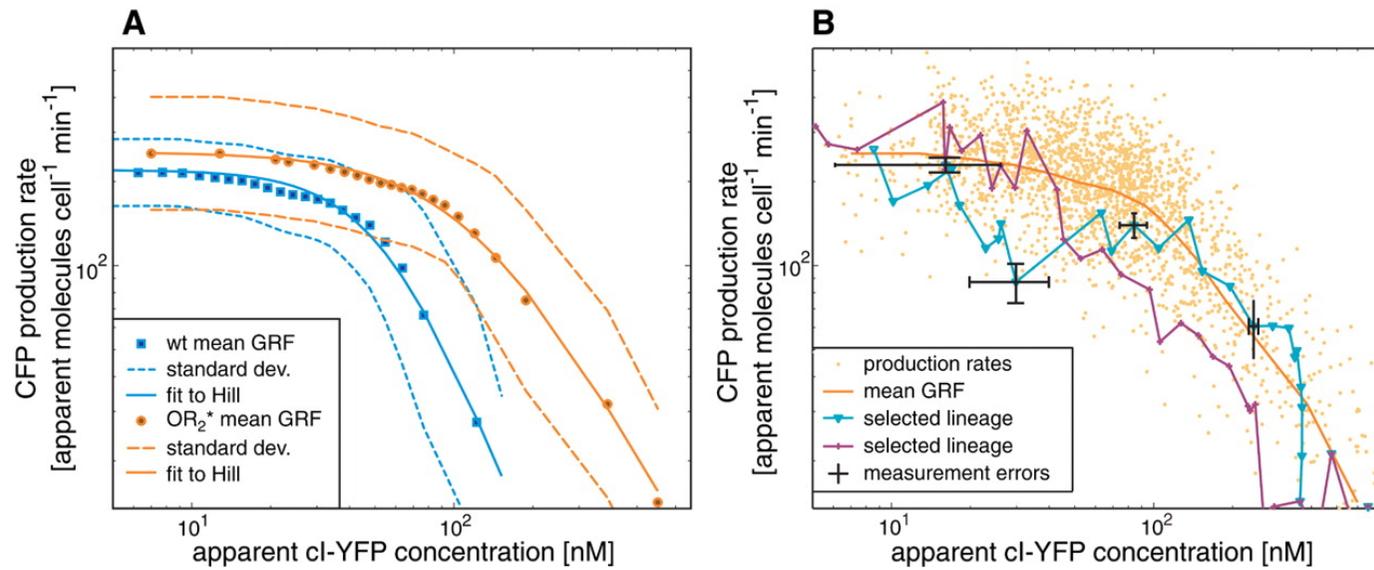
N. Rosenfeld et al., Science 307, 1962-1965 (2005)

Fig. 2. Data and calibration



N. Rosenfeld et al., Science 307, 1962 -1965 (2005)

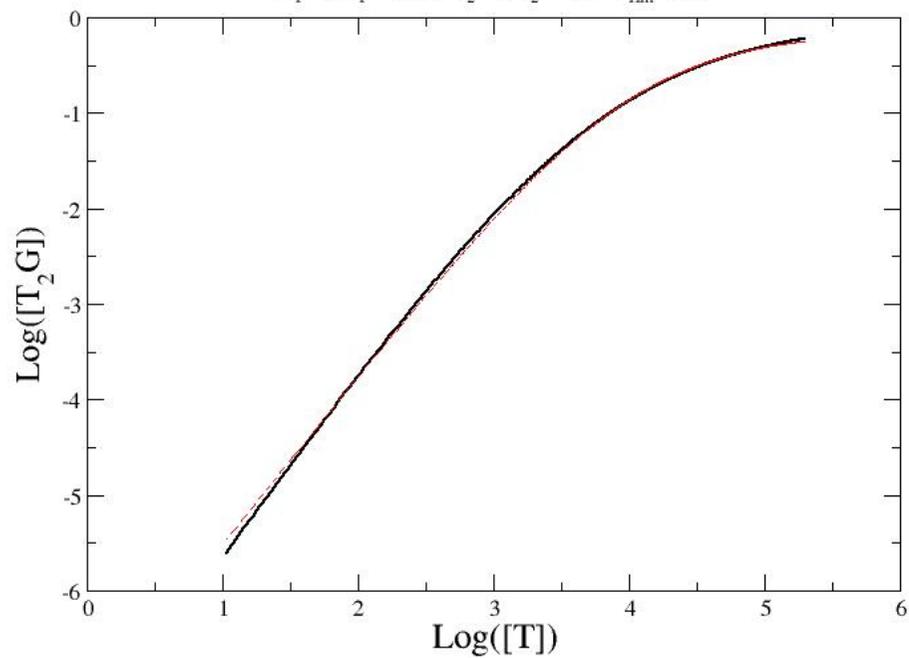
Fig. 3. The GRF and its fluctuations



N. Rosenfeld et al., *Science* 307, 1962 -1965 (2005)

Sequential attachment (black) vs Hill fit (red)

$k_1^p=1, k_1^m=100, k_2^p=2, k_2^m=40; n_{Hill}=1.77$



Sequential attachment (black) vs Hill fit (red)

$k_1^p=1, k_1^m=100, k_2^p=2, k_2^m=40; n_{Hill}=1.77$

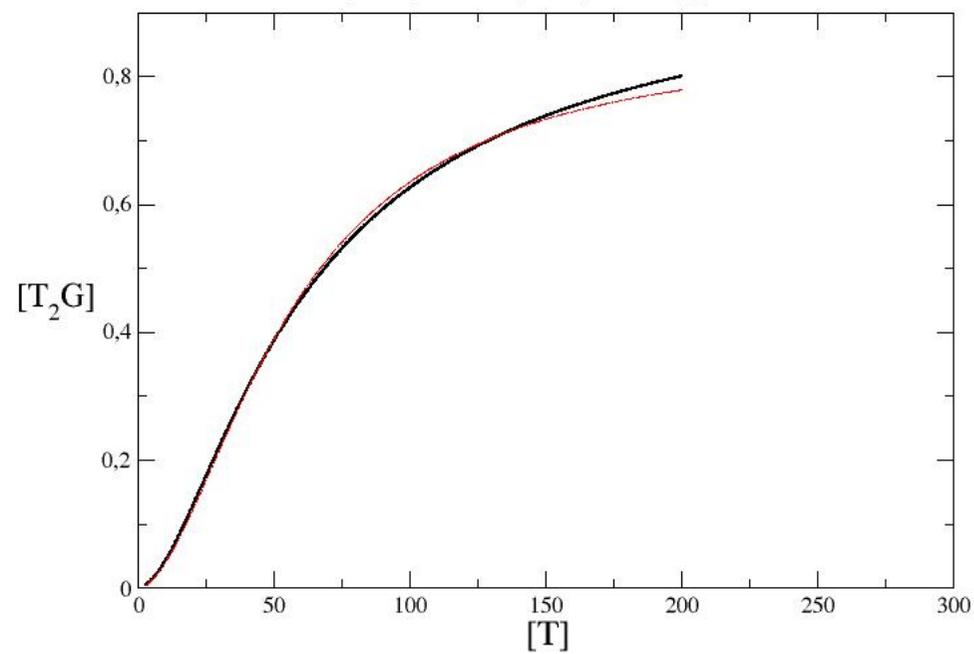
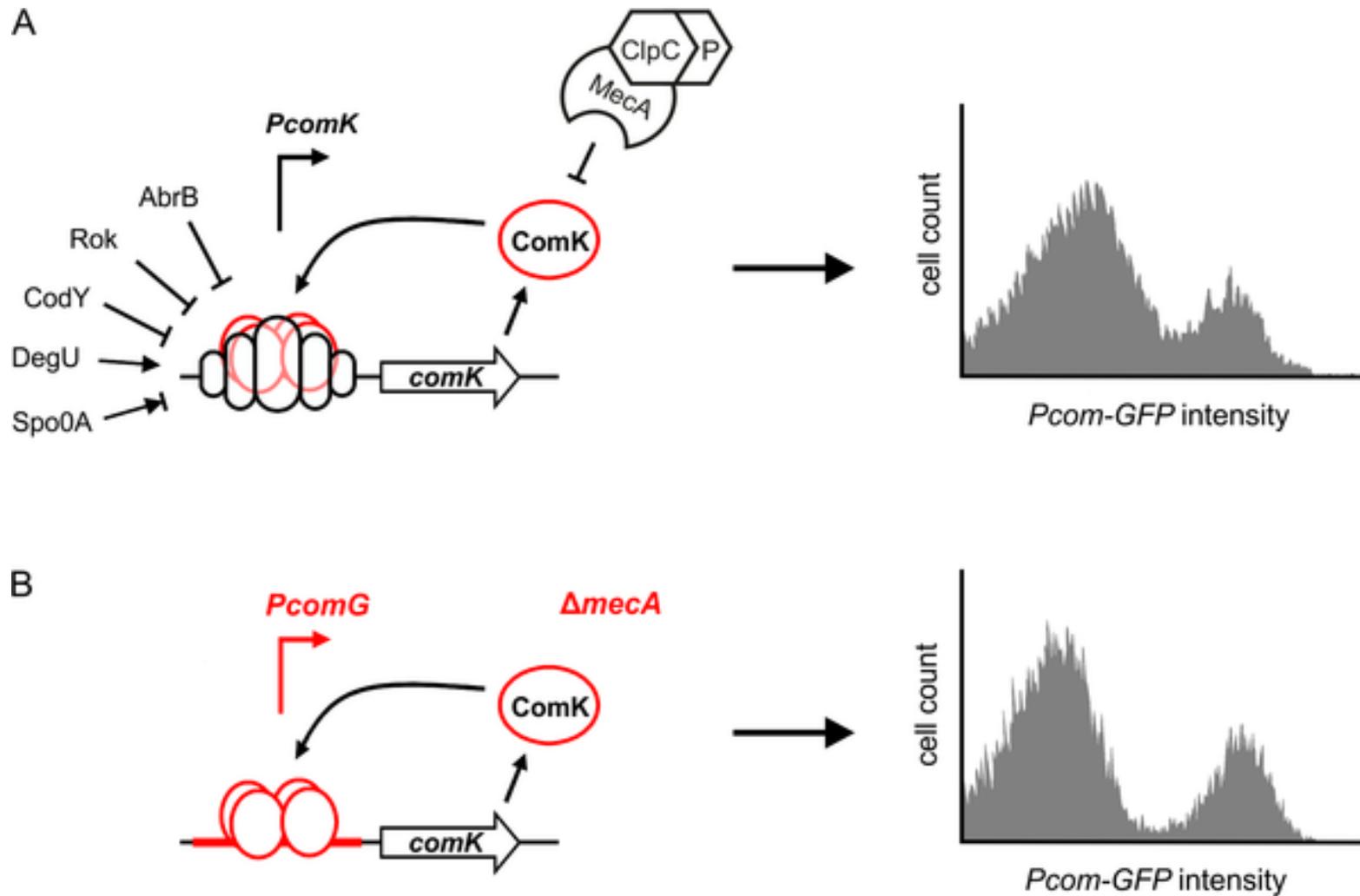


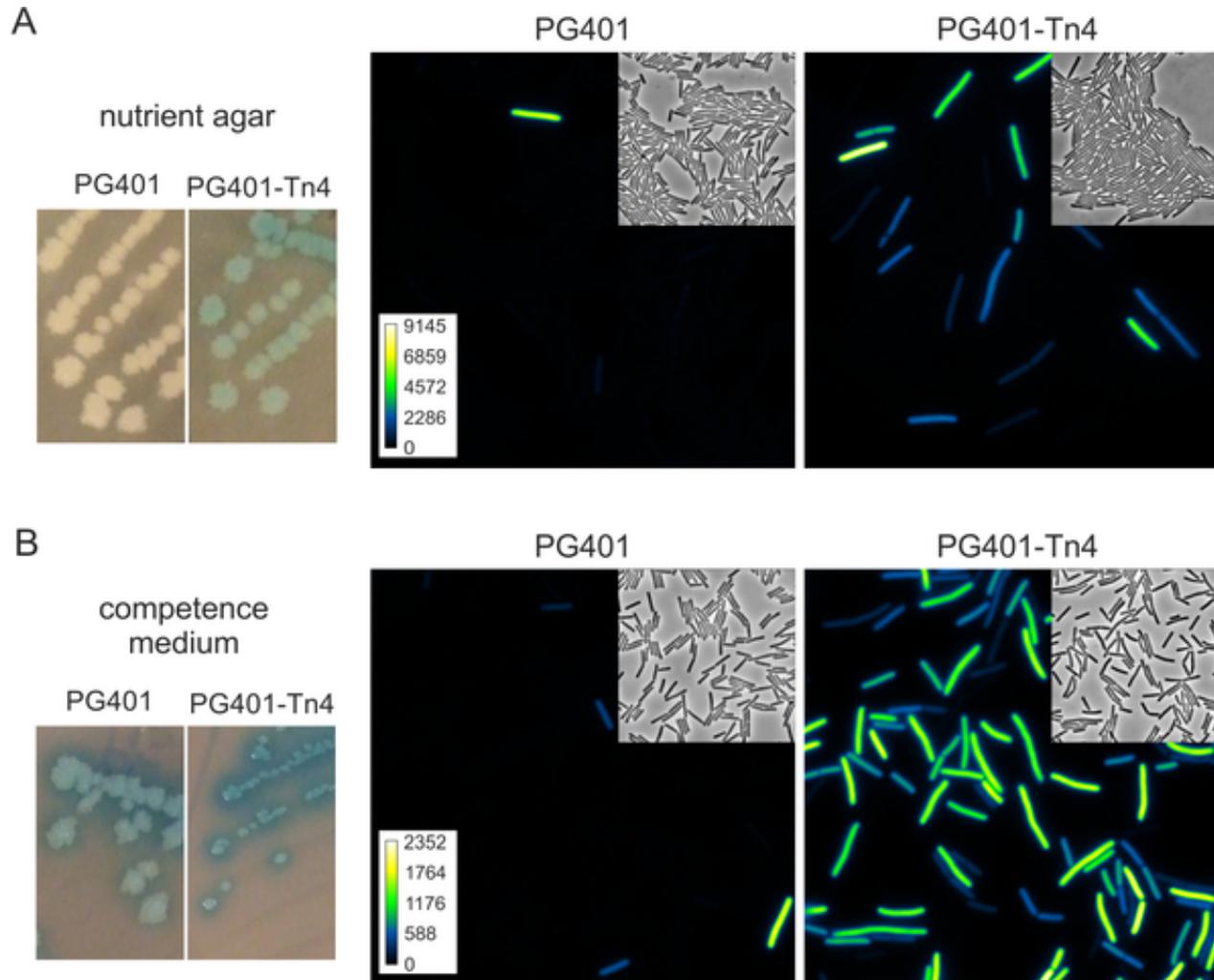
Fig 1. Autostimulation of ComK expression is sufficient for bimodal distribution.



Gamba P, Jonker MJ, Hamoen LW (2015) A Novel Feedback Loop That Controls Bimodal Expression of Genetic Competence. PLoS Genet 11(6): e1005047. doi:10.1371/journal.pgen.1005047

<http://journals.plos.org/plosgenetics/article?id=info:doi/10.1371/journal.pgen.1005047>

Fig 2. Transposon insertion in ykyB increases the activation of an artificial ComK feedback loop.

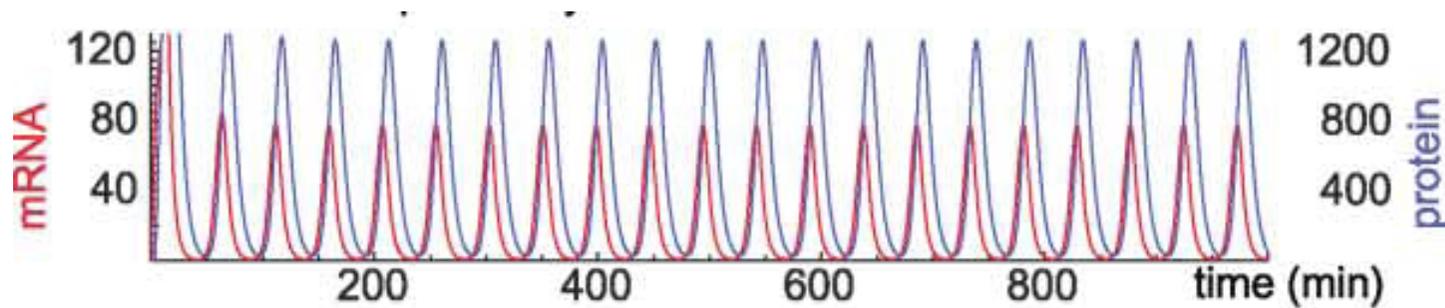
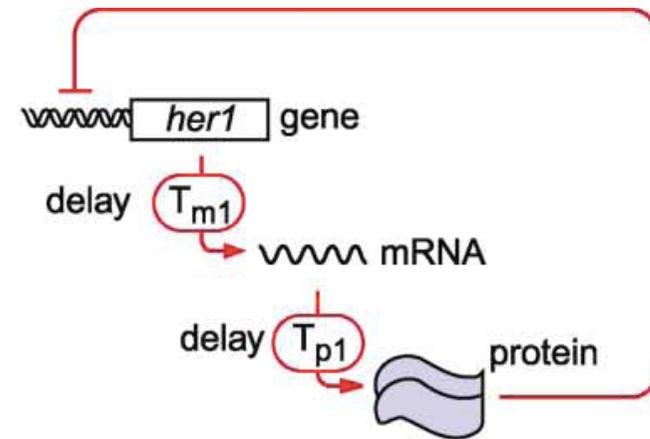
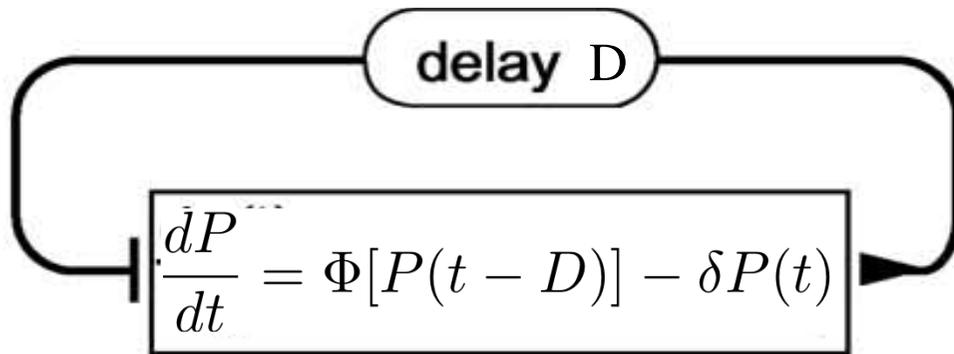


Gamba P, Jonker MJ, Hamoen LW (2015) A Novel Feedback Loop That Controls Bimodal Expression of Genetic Competence. *PLoS Genet* 11(6): e1005047. doi:10.1371/journal.pgen.1005047

<http://journals.plos.org/plosgenetics/article?id=info:doi/10.1371/journal.pgen.1005047>

Auto-inhibition with delay

...,Mackey,...,Goldbeter,...,Monk,..., Sneppen & Jensen,...



J Lewis, Current Biology (2003)

Auto-inhibition with delay

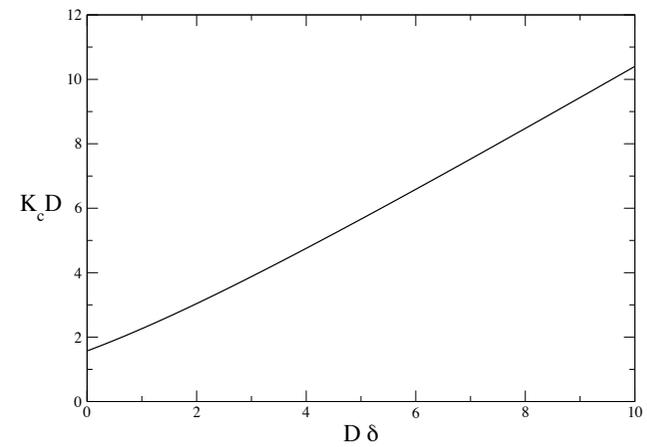
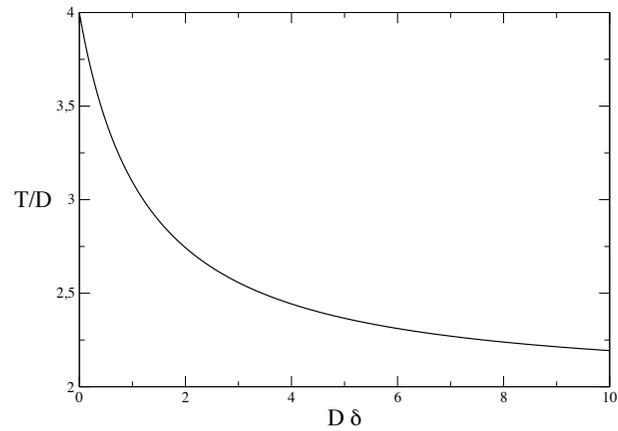
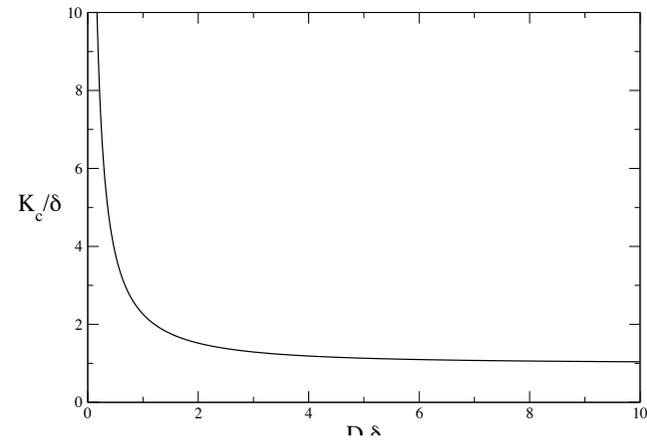
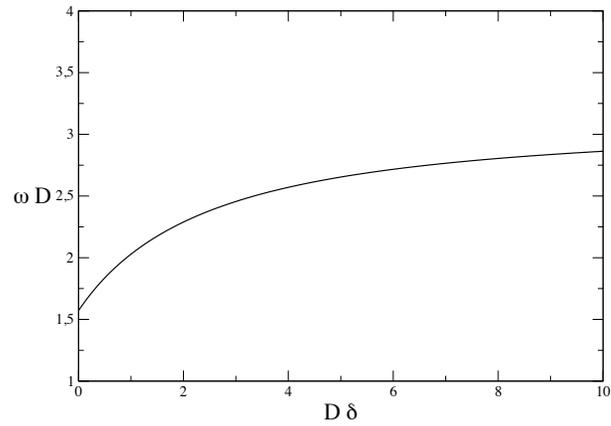
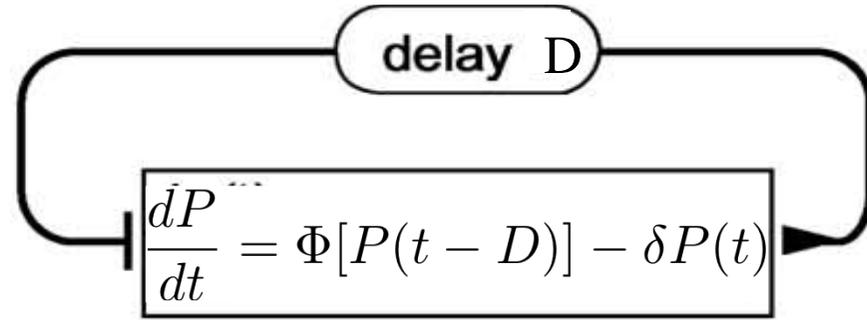
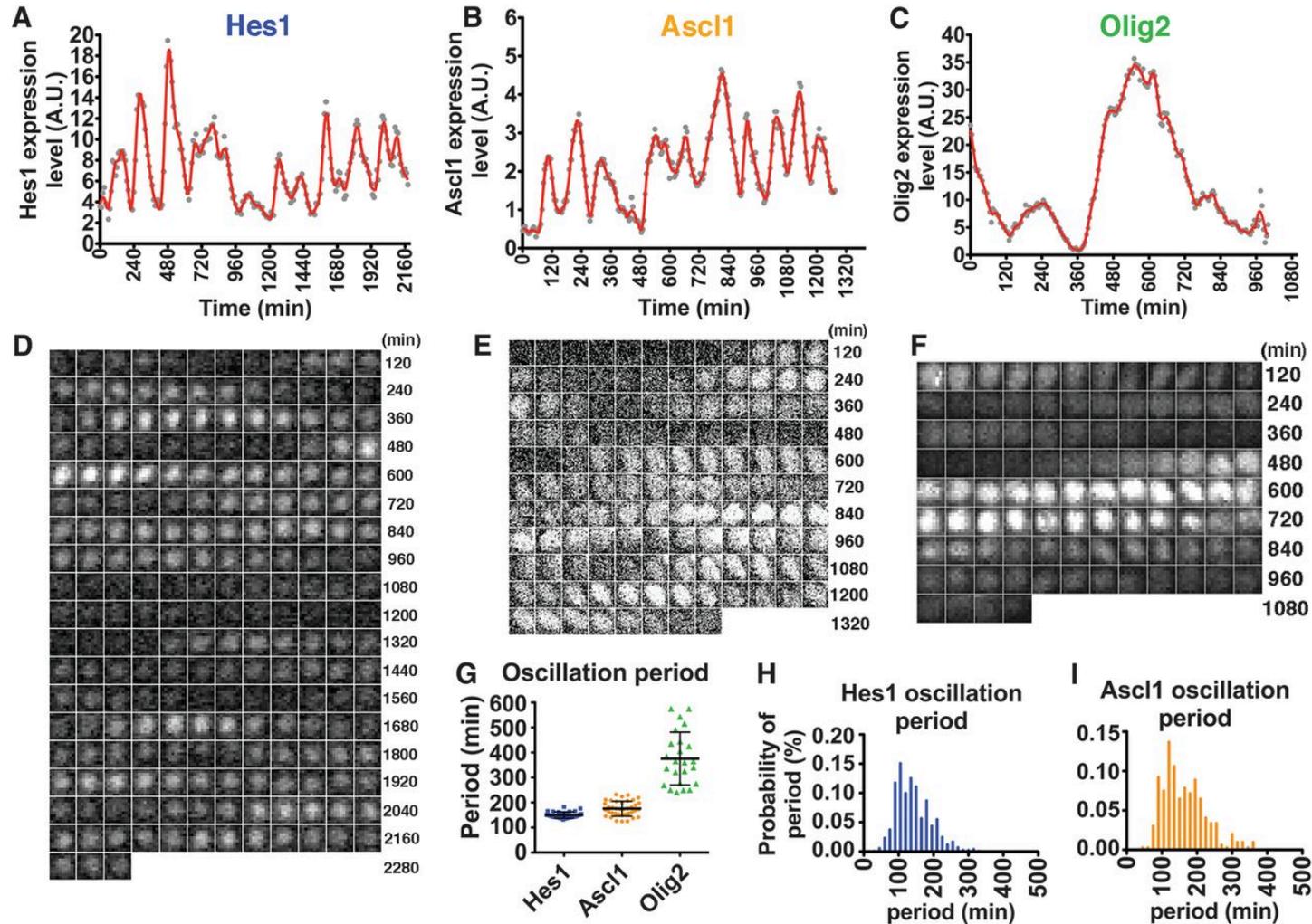


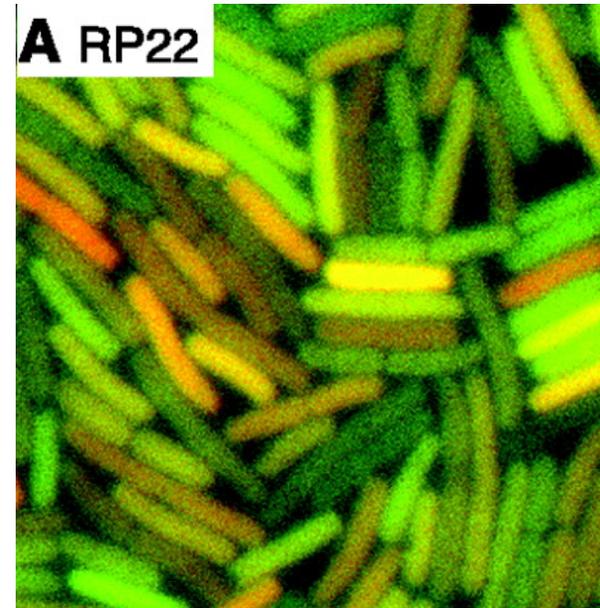
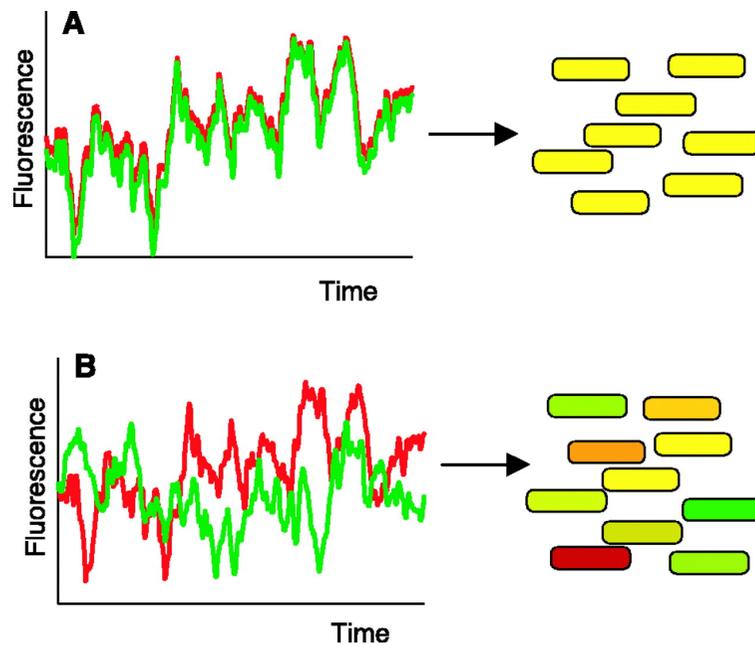
Fig. 2 Oscillatory expression of bHLH factors in self-renewing NPCs derived from the ventral telencephalon. (A to F) Bioluminescence images and quantification of Luc2-Hes1 [(A) and (D)], Luc2-Ascl1 [(B) and (E)], and Luc2-Olig2 [(C) and (F)] reporter expression.



Itaru Imayoshi et al. Science 2013;342:1203-1208

Noise

Intrinsic and extrinsic noise can be measured and distinguished with two genes (cfp, shown in green; yfp, shown in red) controlled by identical regulatory sequences.



M B Elowitz et al. Science 2002;297:1183-1186



The end (for today).

See you next week!