جامعة مولاي إسماعيل
UNIVERSITÉ MOULAY ISMAÏL

كلية العلوم والتقنيات
FACULTÉ DES SCIENCES ET TECHNIQUES

S6: biologie moléculaire

Introduction à la biologie moléculaire et au génie génétique: Chapitre 1

Généralités et définitions

Chapitre 1 généralités

http://www.medecine.unige.ch/enseignement/dnaftb/

Un site pédagogique qui doit vous permettre de faire le point sur vos connaissances et de les consolider……

Historique

1869: Johan Friedrich Miescher, **la nucléine**
1871: Phoebus Aaron Theodor Levene; nucléotides
1944: Erwin Schrödinger, concept du code génétique?
1952 : les travaux d'Avery, Hershey et Chase: l'ADN est de l'information génétique
1953: Watson et Crick & Co… double hélice de l'ADN. Nobel 1962

modèle du code génétique (Gamow)

1956 : découverte de l'ARN
Polymerase I (Kornberg A) Nobel 1959
1958: réplication de l'ADN, Meselson et Stahl

60: l'ARN messager ,Monod Nobel 1965
62-65: découverte enz de restriction, W Arber Nobel 1978
61-66: décryptage du code génétique (Nirenberg et Matthaei),
synthése de protéines *invitro* à partir d'ARNm poly-U

1965: opéron lactose (régulation de l'expression des génes, Monod et Jacob)

72-73: util des enzymes de restrictions, séquençage de l'ADN (Sanger)
86-88: La PCR Nobel 1980
90-98: interférence par ARN, Jorgensen/Andrew Z. Fire et Craig C. Mello Nobel 2006
2003: séquençage complet de l'ADN du génome humain

Historique

(1869)

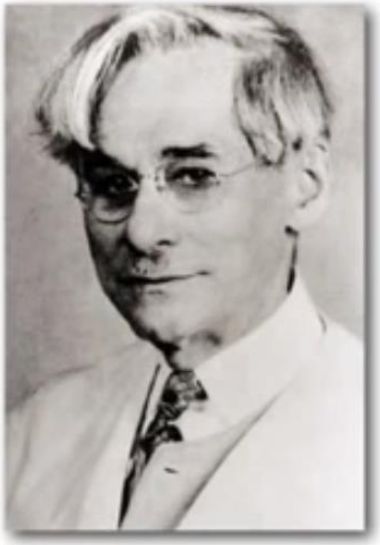
Isolated the genetic material from white blood cell nuclei. He noted it had an acidic nature and called it nuclein

Friedrich Miescher
Swiss physician / biologist
1844 - 1895

Historique

Discovery of DNA Components

- Determined the components of DNA:
  - adenine, guanine, thymine, cytosine, deoxyribose phosphate
- defined *phosphate-sugar-base* units called nucleotides

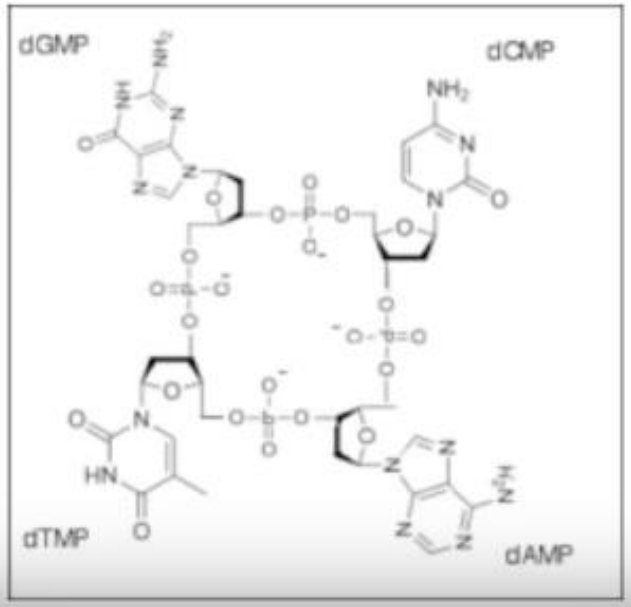


Phoebus Levene
Lithuanian-American Biochemist
(1869 - 1940)

Historique

Levene's Tetranucleotide
(1910)

- Levene proposed that there were four nucleotides per molecule
- DNA could not store the genetic code because it was chemically far too simple



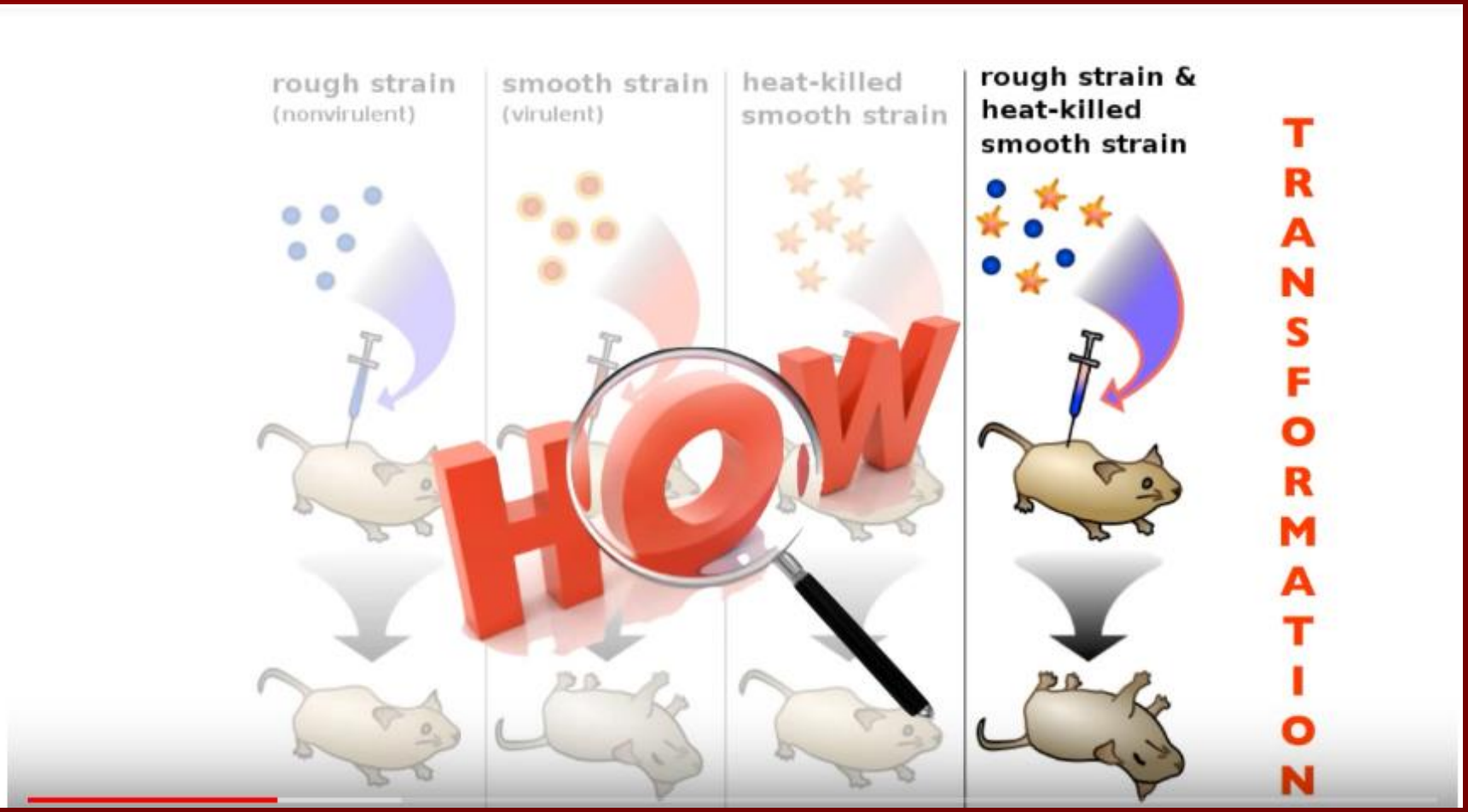
Historique

Frederick Griffith

- Studied the epidemiology and pathology of 2 strains of *Streptococcus pneumoniae*
- In January 1928 reported the first widely accepted demonstrations of bacterial transformation

Frederick Griffith
(1879 - 1941)
British bacteriologist

Historique


rough strain
(nonvirulent)
smooth strain
(virulent)
heat-killed
smooth strain
rough strain &
heat-killed
smooth strain
HOW
TRANSFORMATION

Historique

Oswald Avery
Canadian physician
(1877 - 1955)

Colin MacLeod
Canadian-American Geneticist
(1909 - 1972)

Colin MacLeod

Maclyn McCarty
American Geneticist
(1911 - 2005)

Historique

Journal of Experimental Medicine

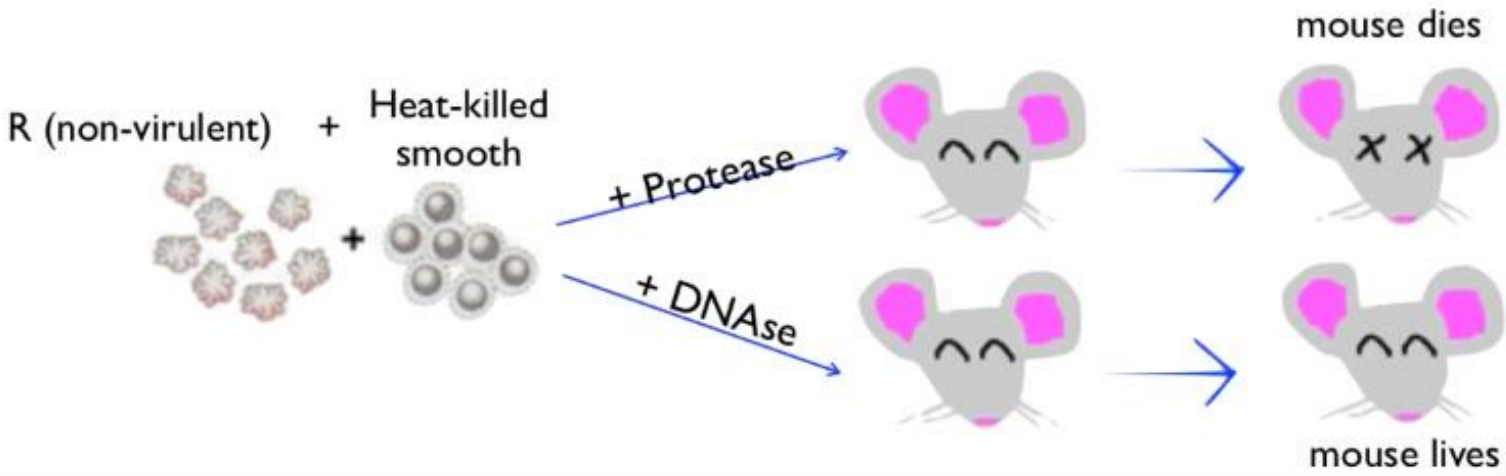
February, 1944

Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types: Induction of Transformation by a Deoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III

The paper suggested that DNA, rather than protein, may be the hereditary material of bacteria - and perhaps in higher organisms as well.

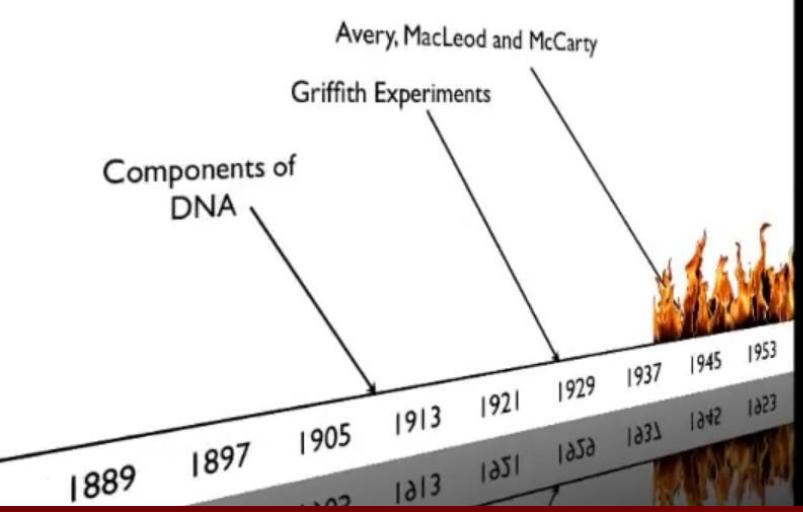
Historique

Avery, MacLeod and McCarty
1944



Historique

DNA Discovery Timeline



Counting Nucleobases
1952

Used paper chromatography and UV spectroscopy to examine the abundance of the nucleobases and he started to notice something VERY odd...

Erwin Chargaff
Austrian Biochemist
(1905 - 2002)

Historique

Organism	% Adenine	% Thymine	% Cytosine	% Guanine
 Octopus	33.2	31.6	17.6	17.6
 Sea Urchin	32.8	32.1	17.3	17.7
 Rat	28.6	28.4	20.5	21.4
 Grasshopper	29.3	29.3	20.7	20.5
 Human	29.3	30.0	20.0	20.7

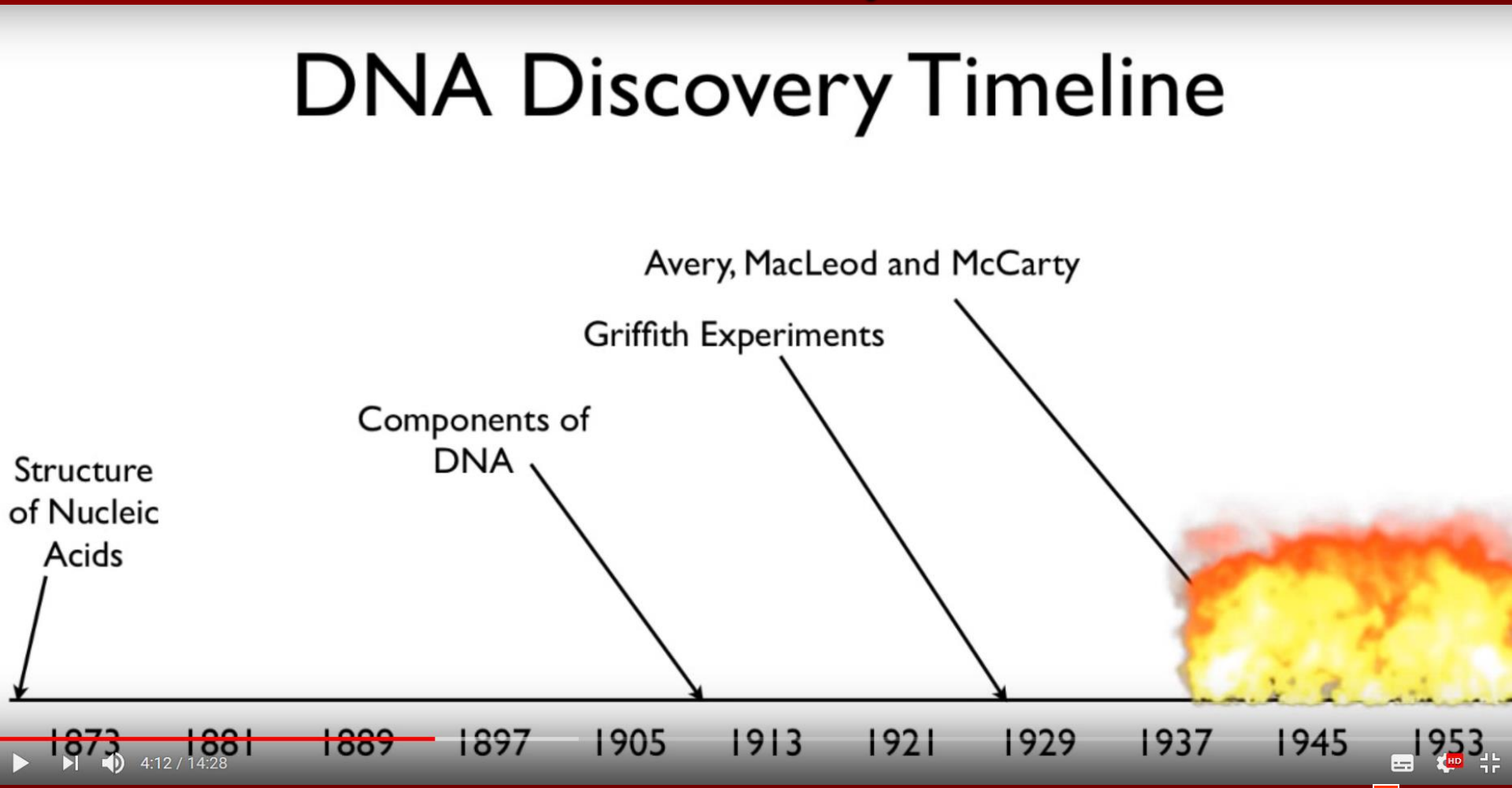
Unfortunately, Chargaff did not realize the importance of these findings; he did however share his discovery with Watson and Crick at Cambridge in 1952.

Chargaff's Rules

Adenine Thymine

Cytosine Guanine

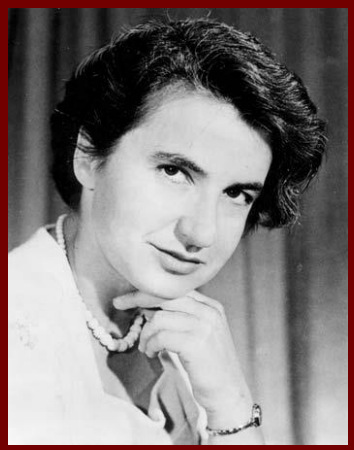
Historique



A suivre ...

Historique

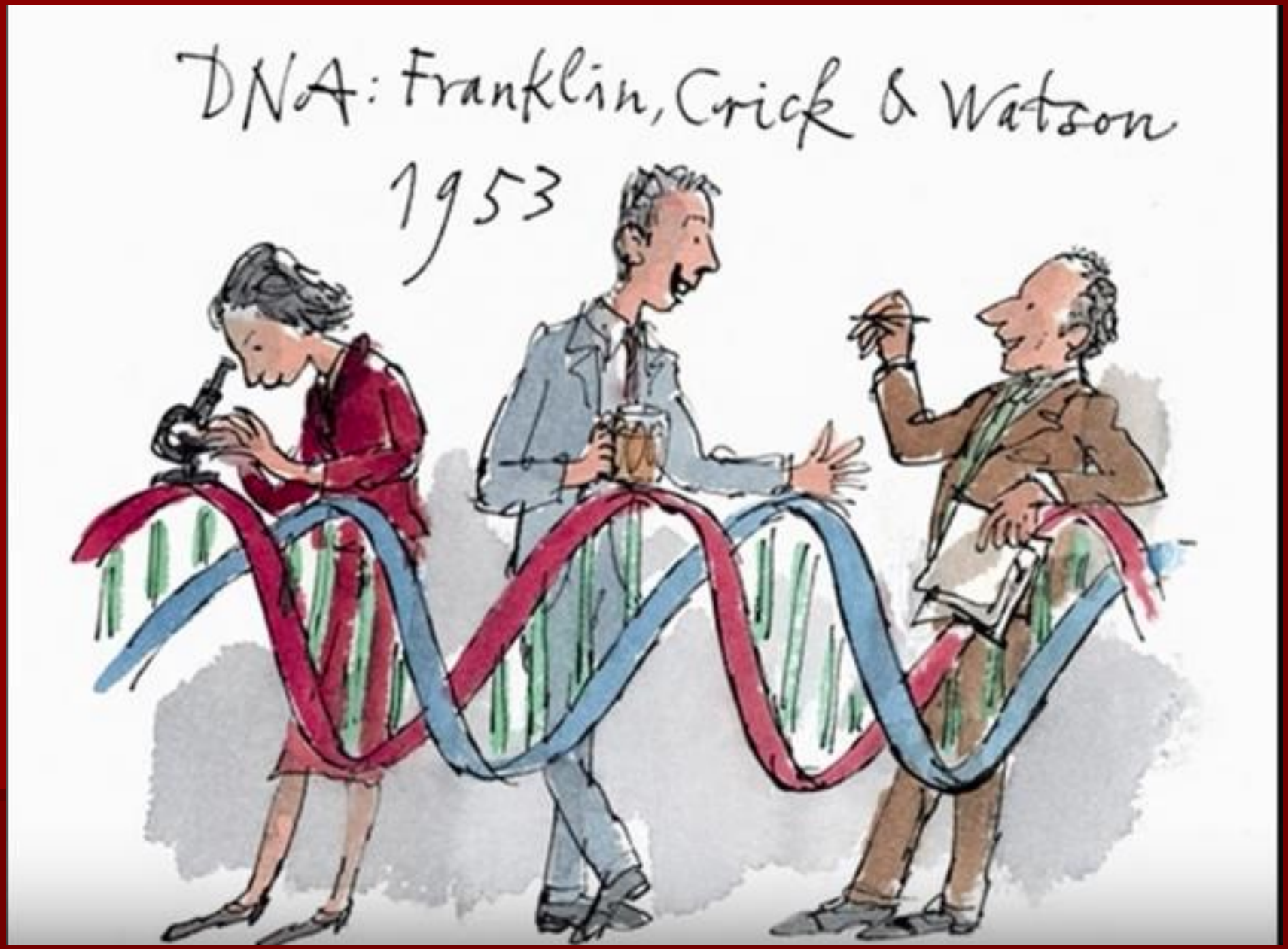
- Crick et Watson, Rosalind 1953
- Découverte de la structure de la molécule d'ADN
- ADN et ARN = acides nucléiques
- Les acides nucléiques = polymères de nucléotides



2011

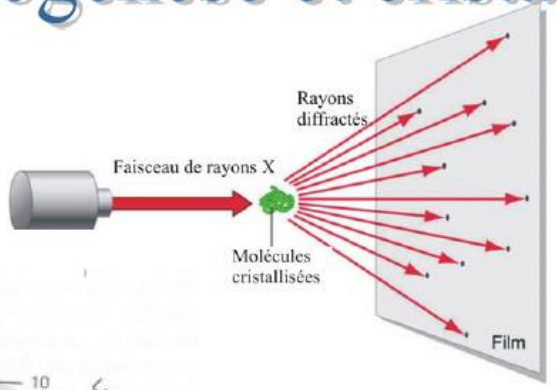
+ **Rosalind Elsie Franklin** (25 July 1920 – 16 April 1958)

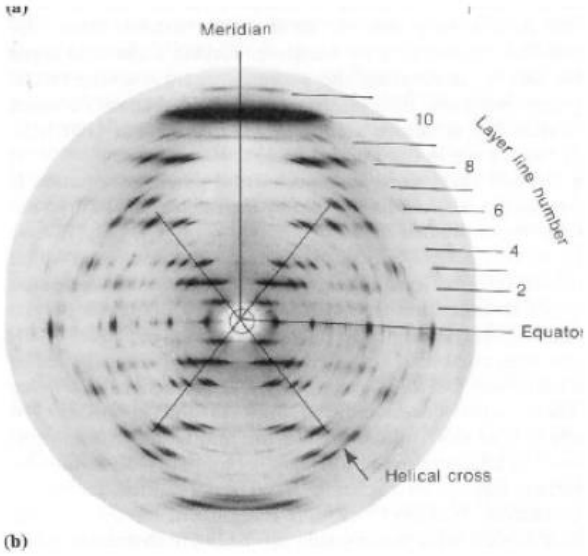
Histoire polémique????????

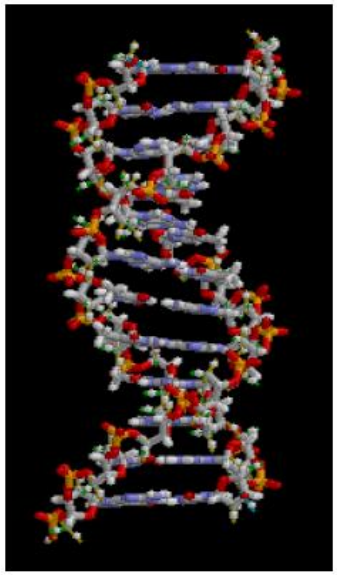


Historique

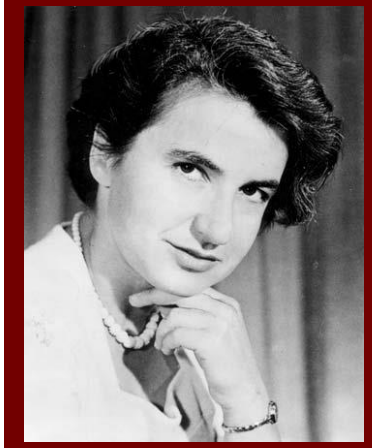
Cristallogenèse et cristallographie







Crick, Watson et Wilkins: Nobel 1962
avec l'aide des travaux de Rosalind Franklin



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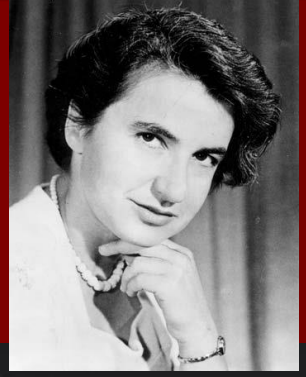
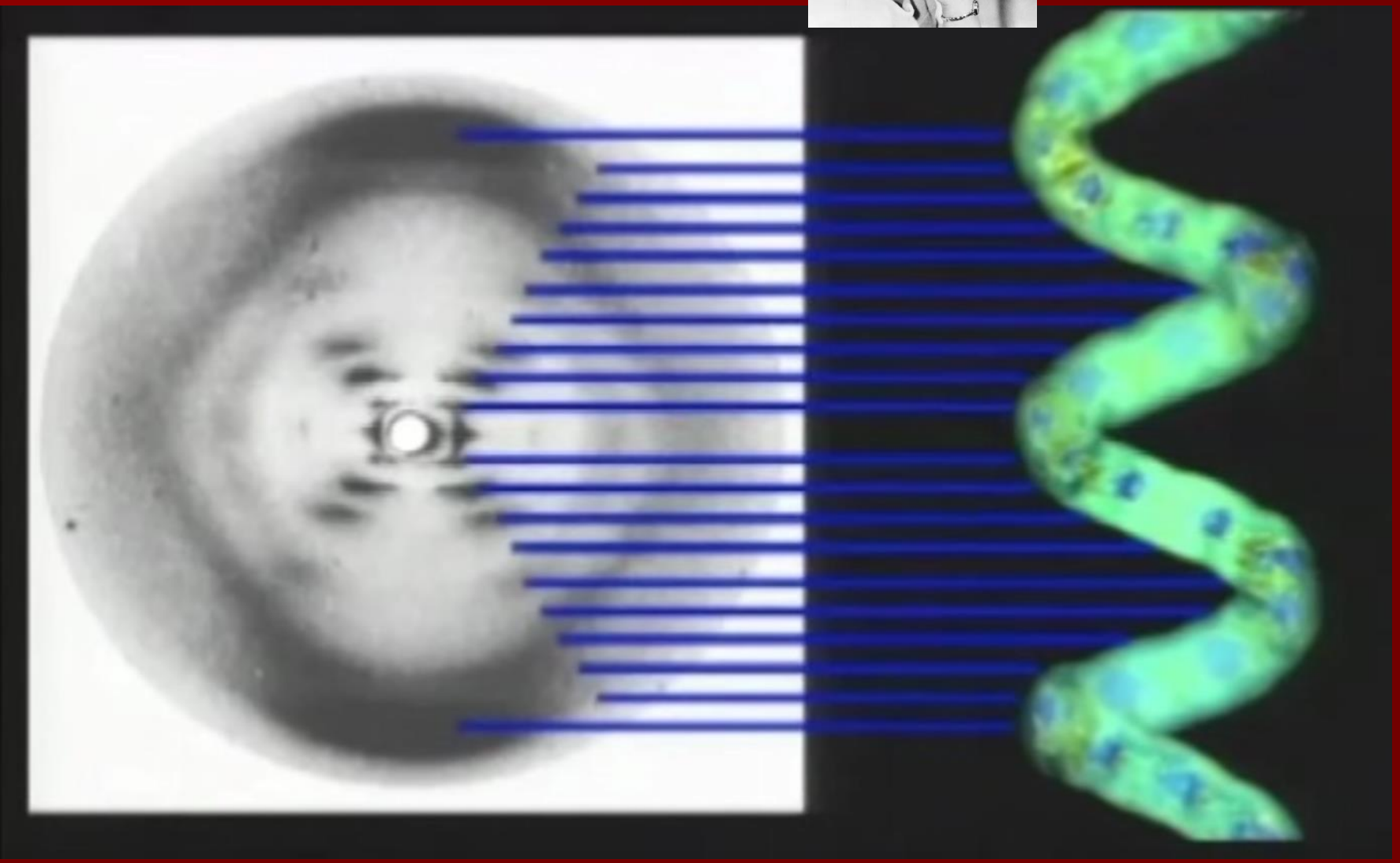




The double helix and the 'wronged heroine'

Brenda Maddox

9 Pitt Street, London W8 4NX, UK (e-mail: bmaddox@pitt.demon.co.uk)

In 1962, James Watson, Francis Crick and Maurice Wilkins received the Nobel prize for the discovery of the structure of DNA. Notably absent from the podium was Rosalind Franklin, whose X-ray photographs of DNA contributed directly to the discovery of the double helix. Franklin's premature death, combined with misogynist treatment by the male scientific establishment, cast her as a feminist icon. This myth overshadowed her intellectual strength and independence both as a scientist and as an individual.

"Science and everyday life cannot and should not be separated. Science, for me, gives a partial explanation of life. In so far as it goes, it is based on fact, experience and experiment." Rosalind Franklin, in a letter to her father, summer 1940.

In late February 1953, Rosalind Franklin, a 33-year-old physical chemist working in the biophysics unit of King's College in London, wrote in her notebooks that the structure of DNA had two chains. She had already worked out that the molecule had its phosphate groups on the outside and that DNA existed in two forms.

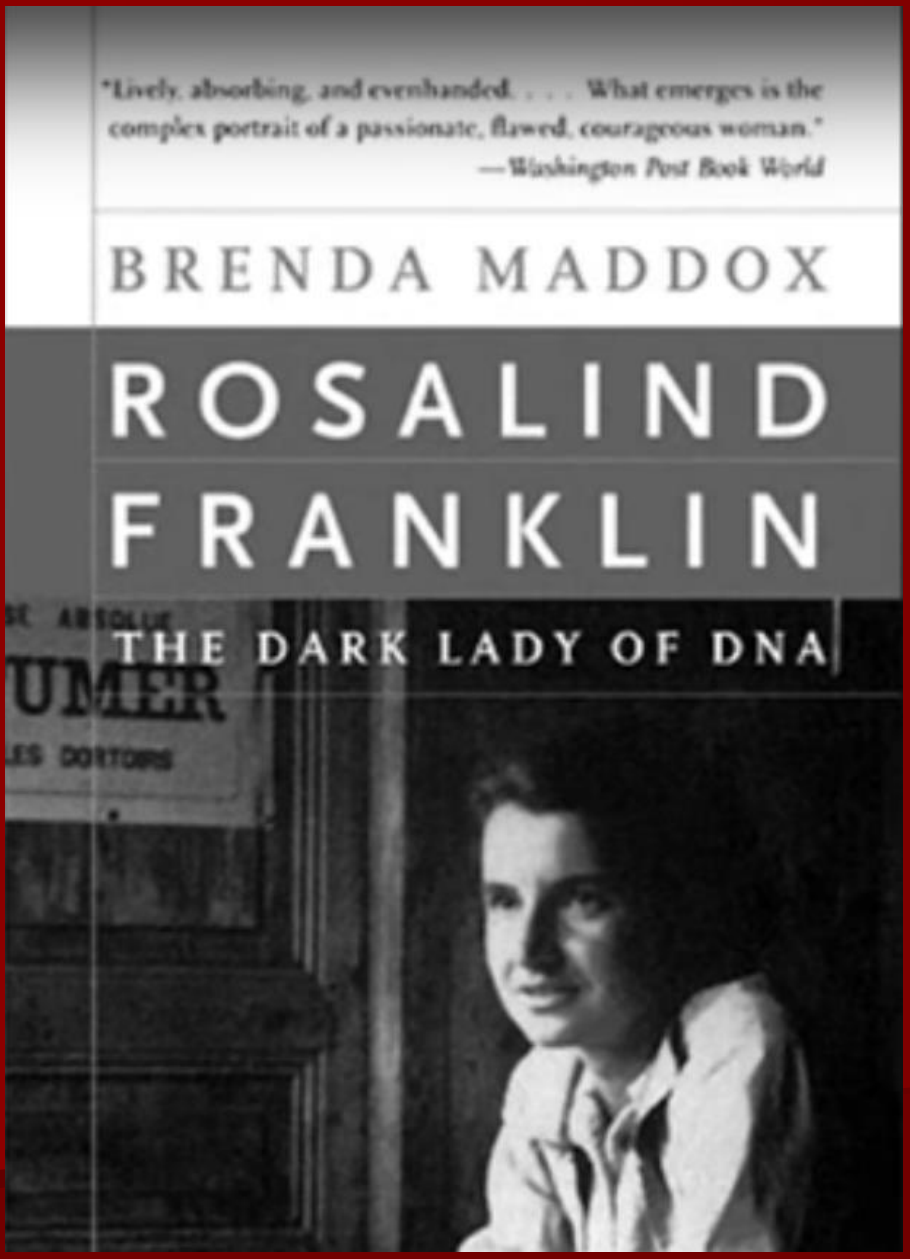
Two weeks later James Watson and Francis Crick, at the Cavendish Laboratory at Cambridge, built their now celebrated model of DNA as a double helix. They did it not only through brilliant intuition and a meeting of compatible minds, but also on the basis of Franklin's unpublished experimental evidence, which had reached them through irregular routes. She did not know that they had seen either her X-ray photograph (Fig. 1), showing unmistakable evidence of a helical structure, or her precise measurements of the unit cell (the smallest repeating unit) of the DNA crystal.

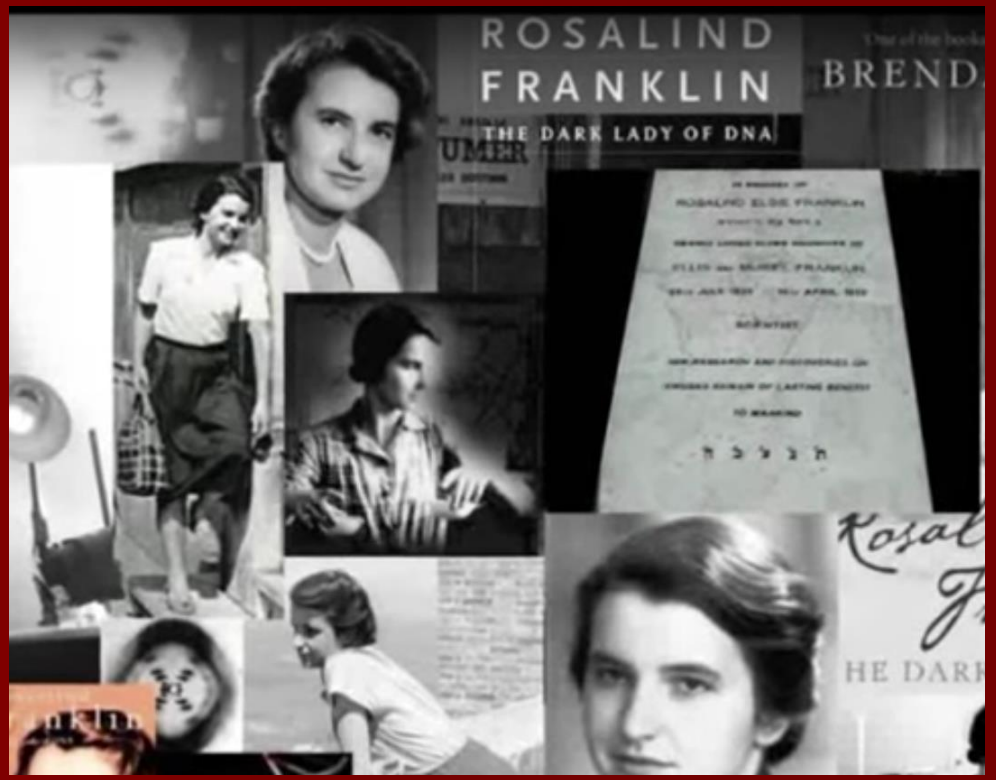
As Watson was to write candidly, "Rosy, of course, did not directly give us her data. For that matter, no one at King's realized they were in our hands." When this admission appeared in Watson's best-selling, much-acclaimed book of the discovery, *The Double Helix*, published in 1968 (ref. 1), he was a Harvard professor and Nobel laureate (he had shared the prize for medicine and physiology in 1962, with Crick and Maurice Wilkins of King's College.) By then Franklin had died — in 1958, at the age of 37, from ovarian cancer.

Other comments dismissive of "Rosy" in Watson's book caught the attention of the emerging women's movement in the late 1960s. "Clearly Rosy had to go or be put in her place […] Unfortunately Maurice could not see any decent way to give Rosy the boot". And, "Certainly a bad way to go out into the foulness of a […] November night was to be told by a woman to refrain from venturing an opinion about a subject for which you were not trained."

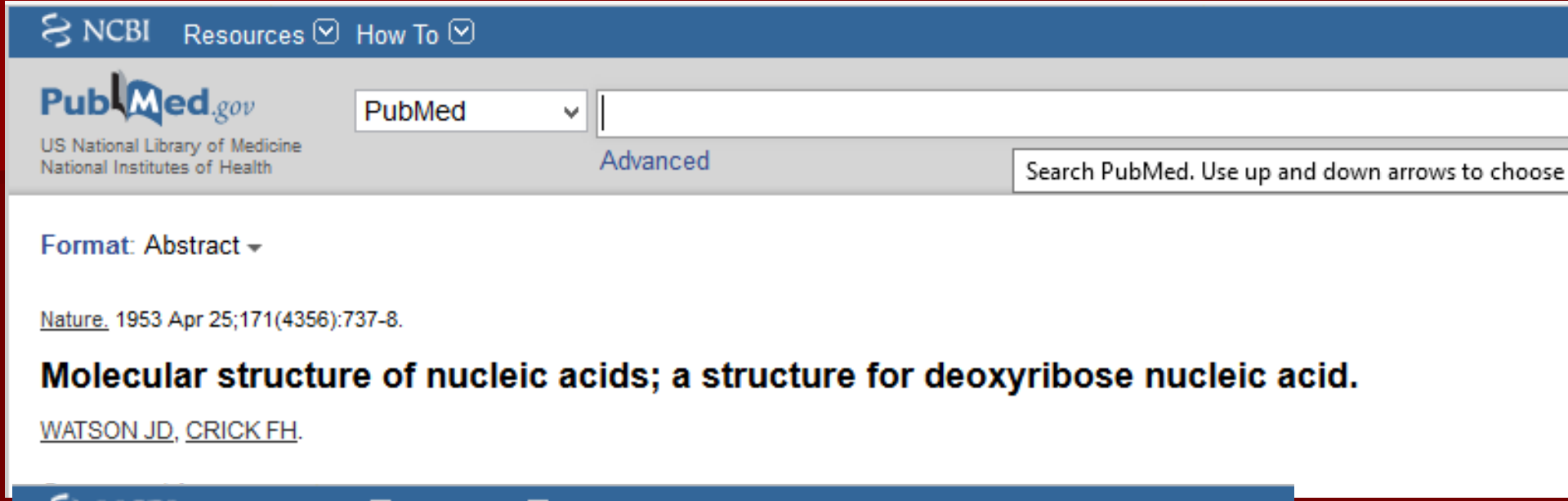
Son dernier prix c'était la vie….

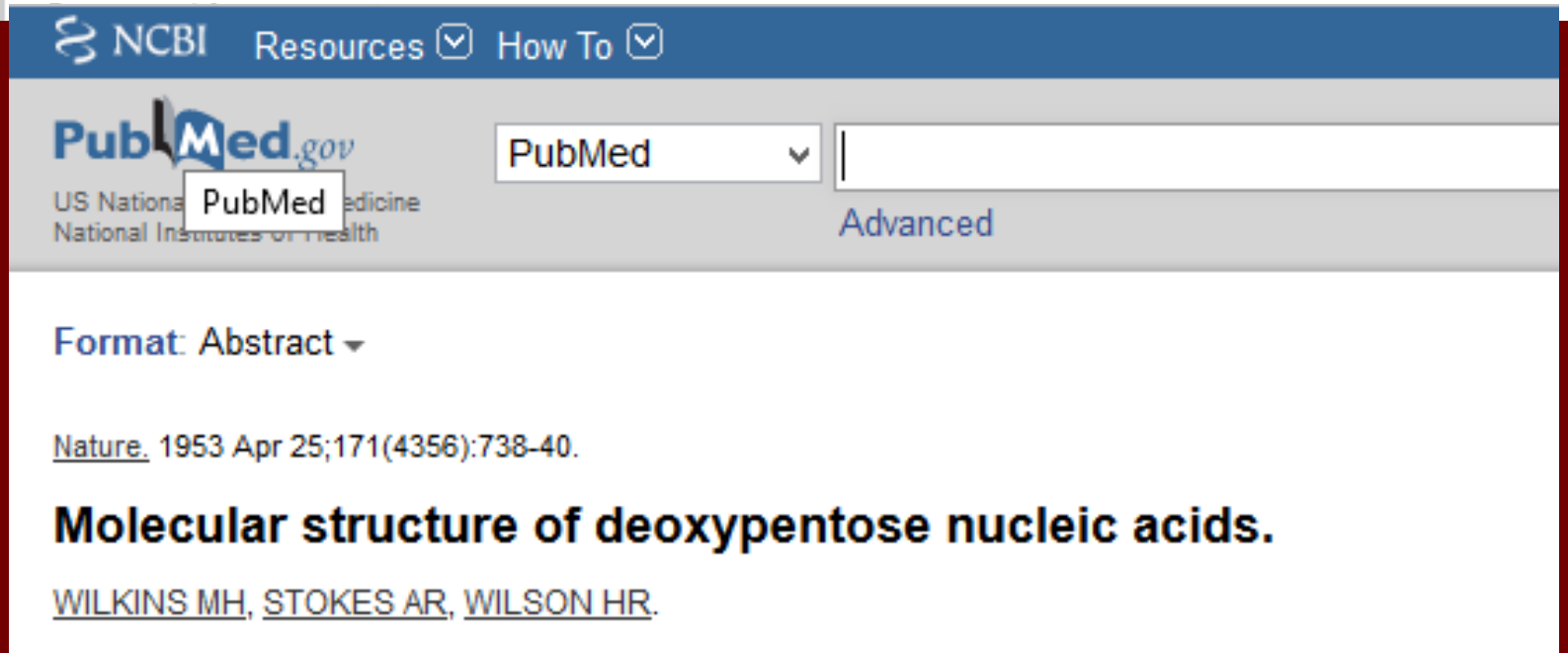
Brenda Madoxx, 2015


"Lively, absorbing, and evenhanded. . . . What emerges is the complex portrait of a passionate, flawed, courageous woman."
—Washington Post Book World
BRENDA MADDOX
ROSALIND FRANKLIN
THE DARK LADY OF DNA

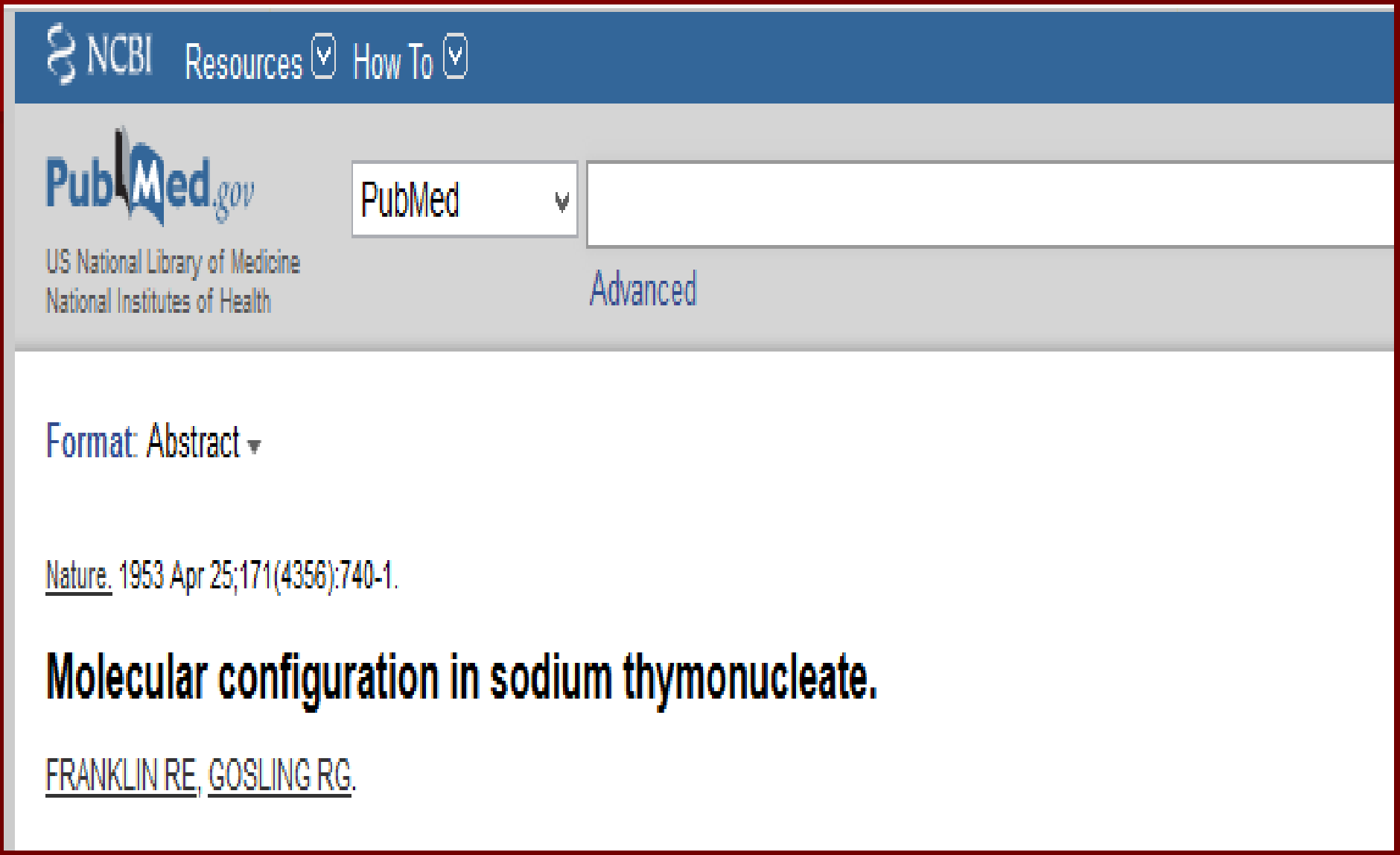

ROSALIND FRANKLIN
THE DARK LADY OF DNA

Publications dans nature

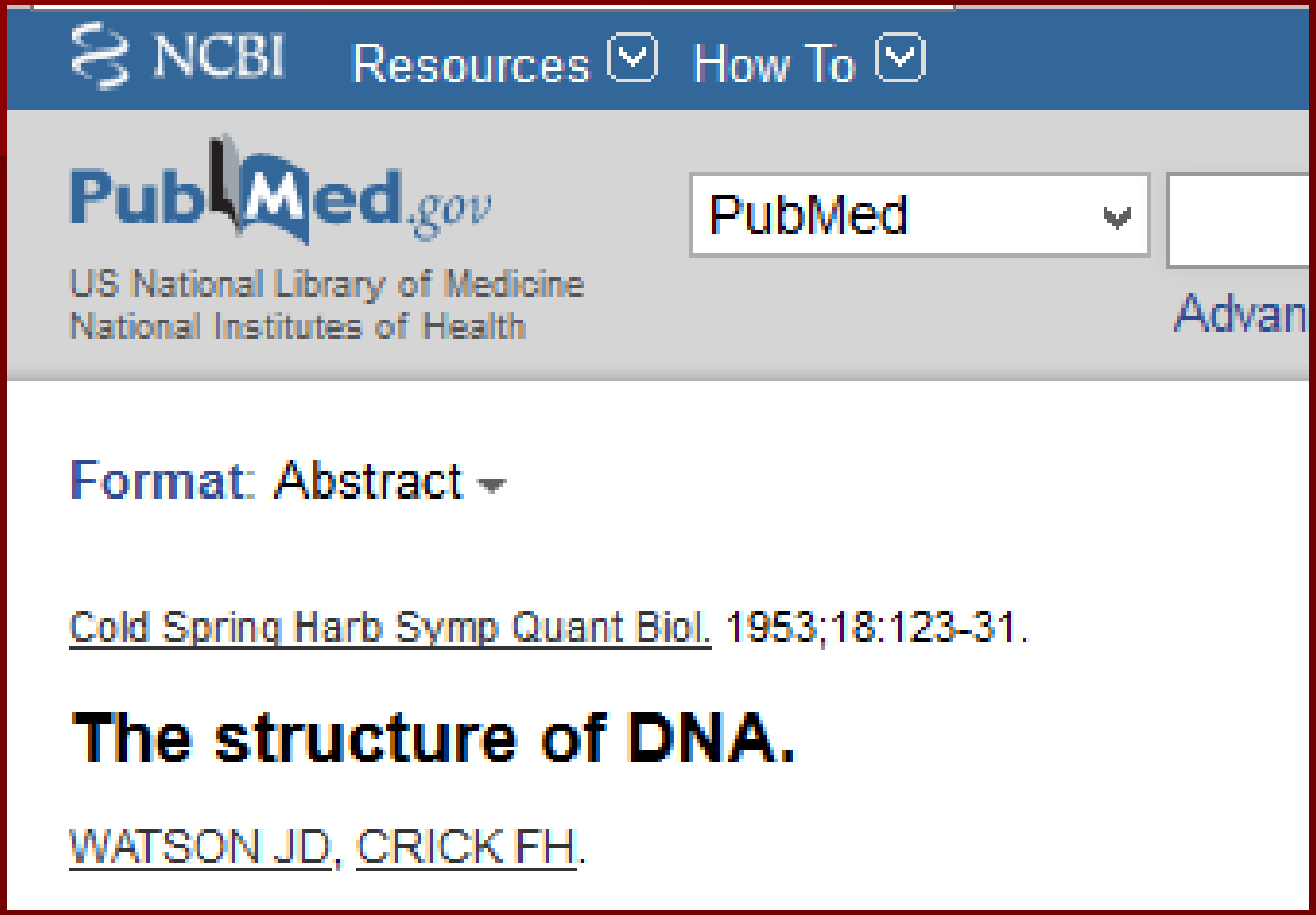




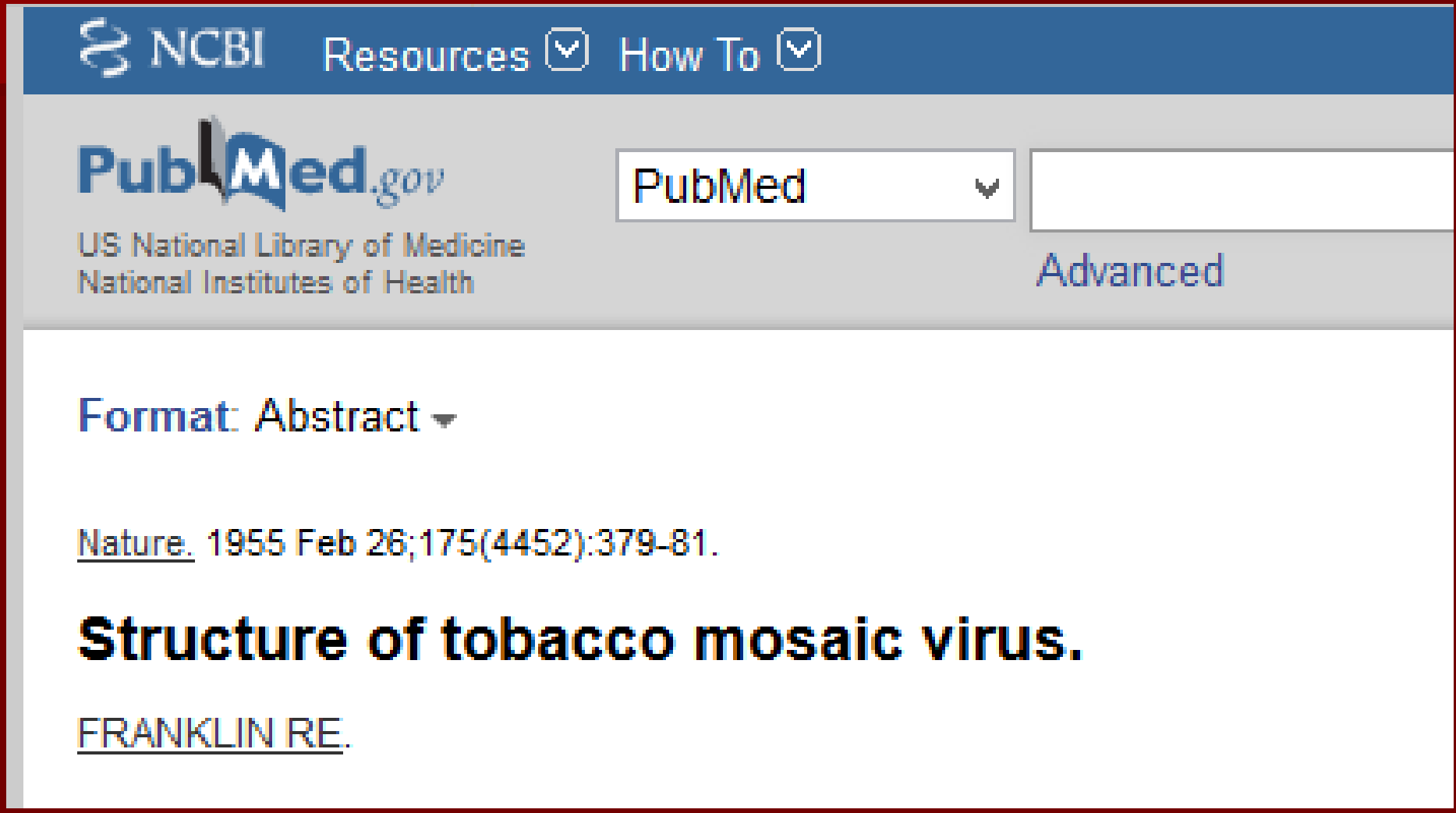
Publications dans nature



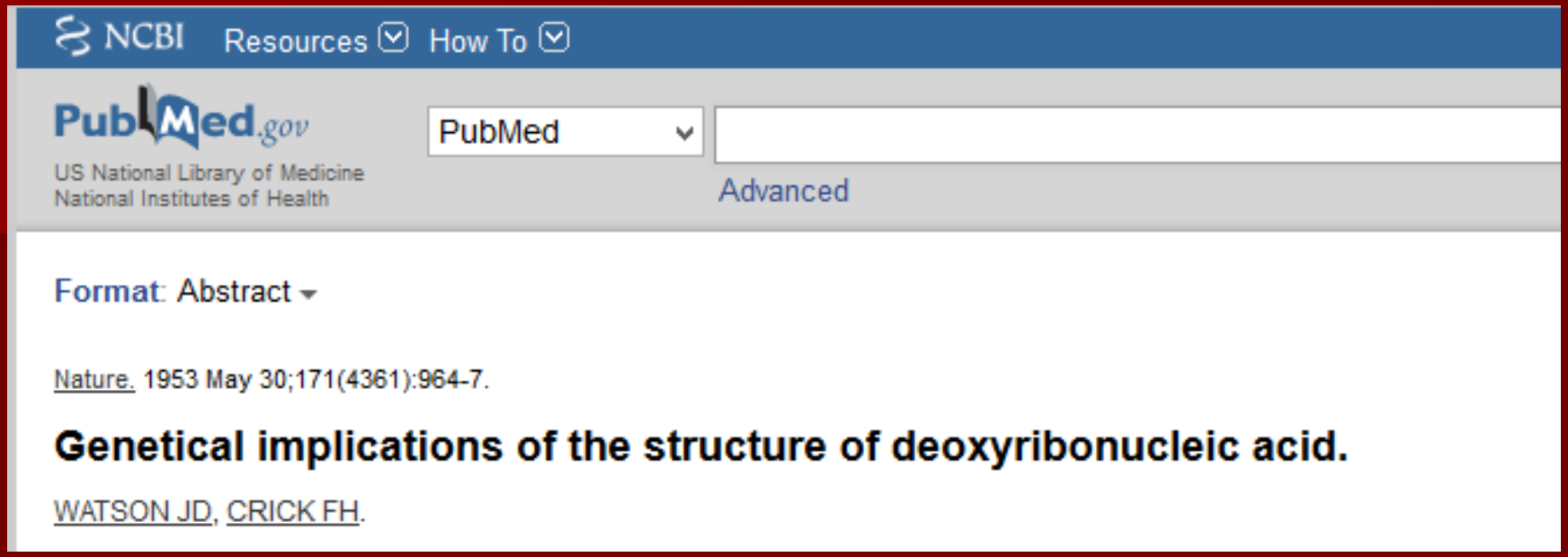
Publications

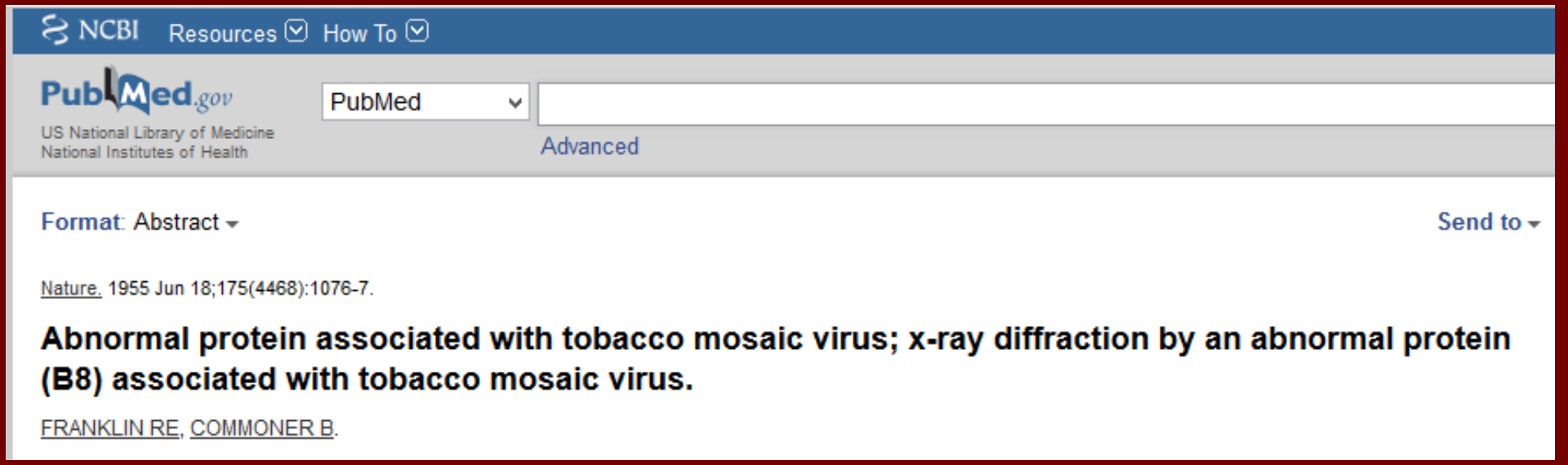


Publications dans nature



Publications dans nature





Nature 2003

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Nature. 2003 Jan 23;421(6921):407-8.

The double helix and the 'wronged heroine'.

Maddox B[1].

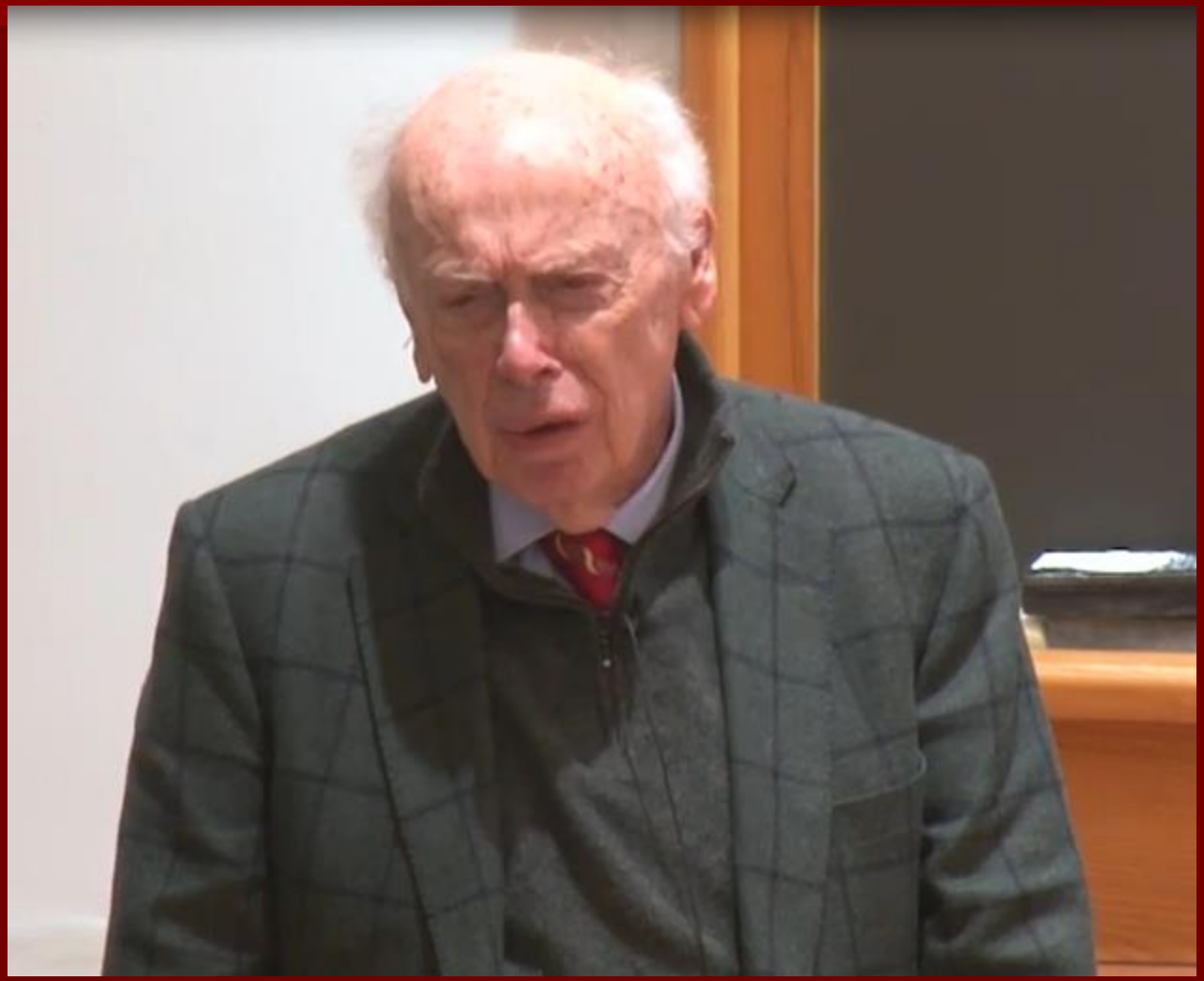
Author information

Abstract

In 1962, James Watson, Francis Crick and Maurice Wilkins received the Nobel prize for the discovery of the structure of DNA. Notably absent from the podium was Rosalind Franklin, whose X-ray photographs of DNA contributed directly to the discovery of the double helix. Franklin's premature death, combined with misogynist treatment by the male scientific establishment, cast her as a feminist icon. This myth overshadowed her intellectual strength and independence both as a scientist and as an individual.

Comment on

James Watson Double Helix Day 2016



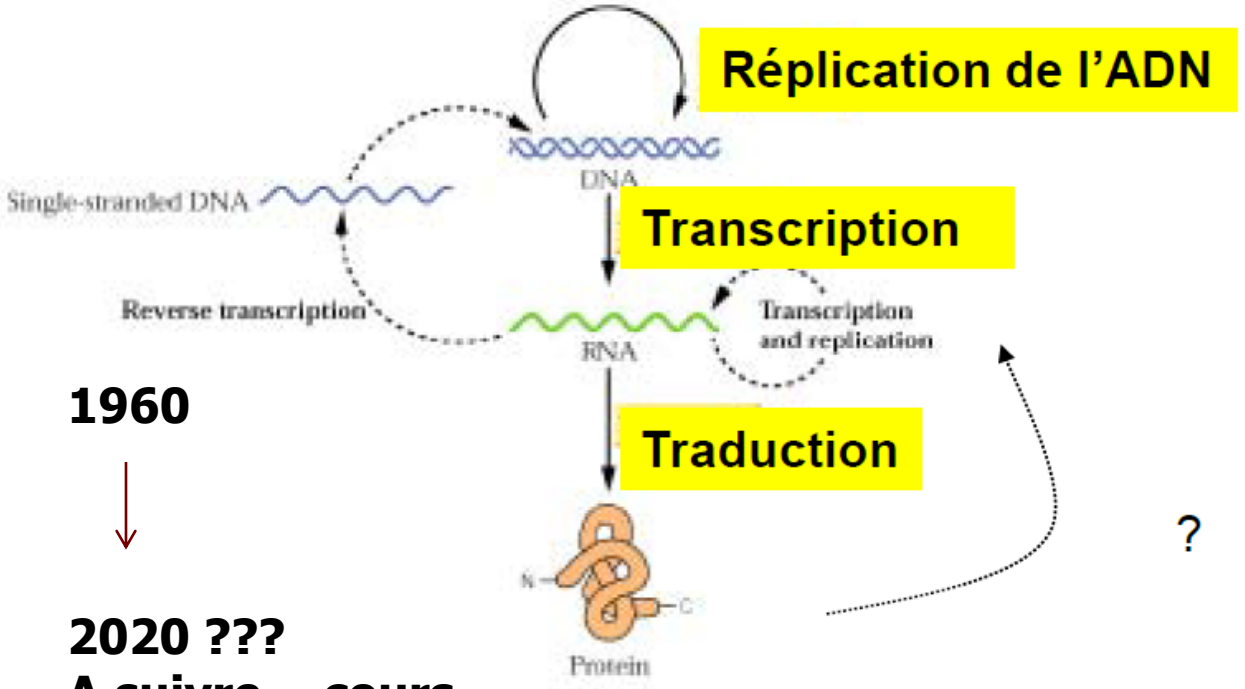
Du Dogme à la réalité

L'ADN dirige sa propre réplication en ADN identique,
ainsi que sa transcription en ARN, pouvant ou non être traduit en protéines.

☞ **Possibilité d'un « retour » à une forme ADN à partir de l'ARN**

ADN

Protéines



1960

2020 ???
A suivre… cours

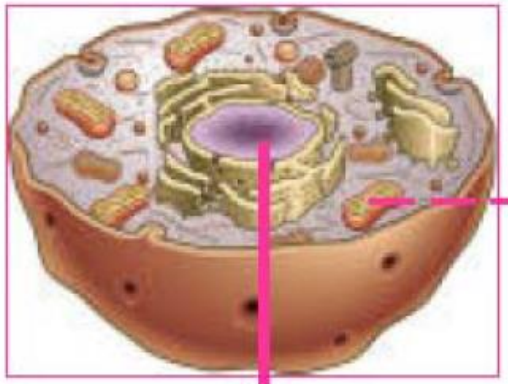
?

Le dogme central de la biologie moléculaire, énoncé par Crick en 1960.

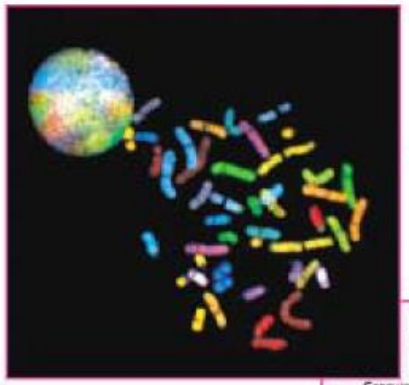
Comment se fait le contrôle de ces différentes étapes?

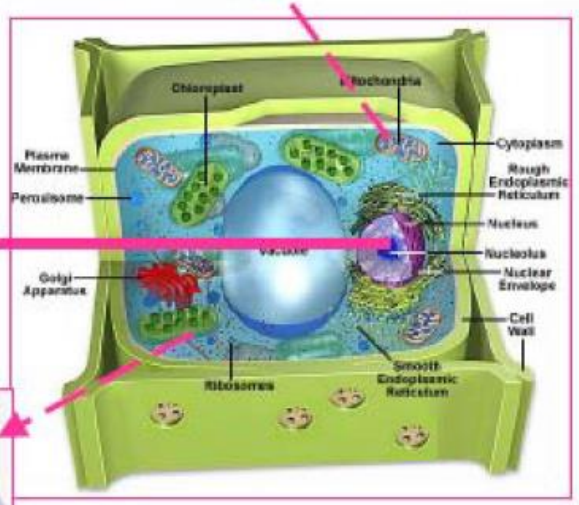
Génome-Définition

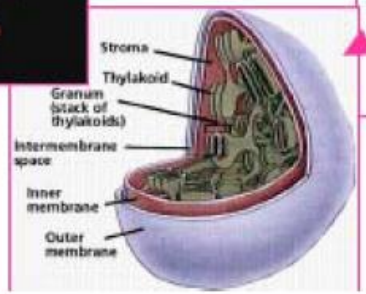
le gènome c'est....



... la ***totalité*** *des séquences du matériel génétique d'un organisme*







Génome-Définition

Evolution et organisation du génome

Procaryotes

Bactérie(*E.coli*) : 1 chromosome circulaire, 4.10^6 pb, 4 288 gènes

Eucaryotes

Levure (S. cerevisiae): 16 chromosomes, $12.\ 10^6$ pb, 6 000 gènes
nematode 6 chromosomes, $97\ 10^6$ pb 20000 gènes
Drosophile : 6 chromosomes,> 120.10^6 pb 13 700 gènes
Homme : 46 chromosomes, $3.\ 10^9$ pb, +/-30 000 gènes

Pour l'Homme????? A discuter

Génome-Définition

La taille des génomes

Petit Virus: 3000 nt, soit 1 page de 3000 caractères

Bactérie: $3x10^6$ nt (moyenne), soit 1 livre de 1000 pages

Homme: $3x10^9$ nt, soit 1000 livres

ou encore, 1 pile de livres de 50 m de haut

1,40-2 m d'ADN dans chaque cellule

10^{13} à 10^{14} cellules/ Homme

faites le calcul…

remarque: distance terre lune 384 400 km..

Génome-Définition

- matériel présent dans les virus, les bactéries et les cellules

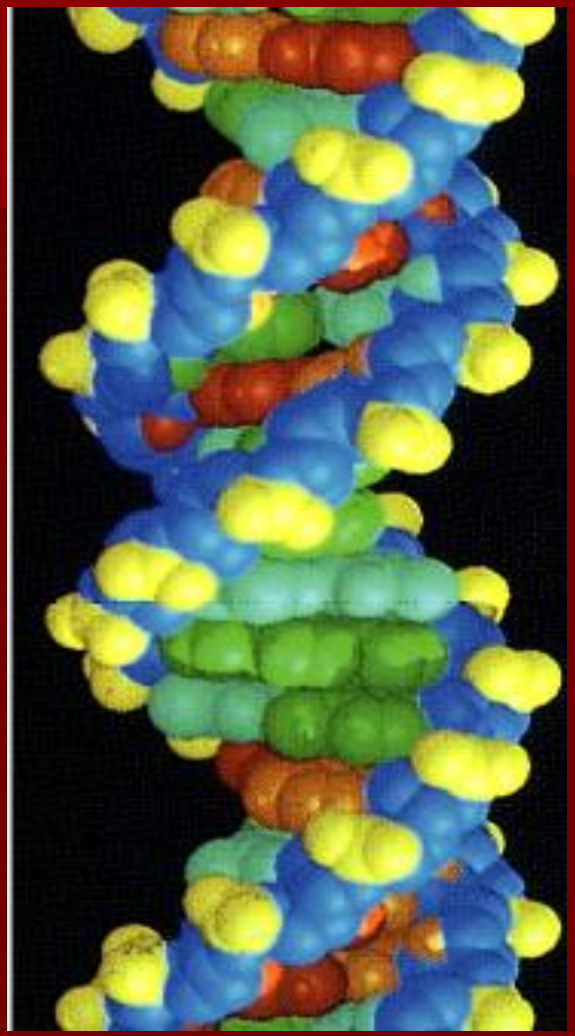
- ADN des cellules eucaryotes animales = ADN nucléaire + ADNmit

  - source : sang, tissus (biopsies), cultures cellulaires…
  - chez l'homme ≈ $(2 \times 3\ 10^{9}) \times 660 / 6{,}02\ 10^{23} \approx 6\text{-}7\ 10^{-12}$ gramme /cellule

    → 1 ml de sang ≈ $5\text{-}6\ 10^{6}$ leucocytes soit ≈ $30\ 10^{-6}$ g d'ADN

    et ≈ 10^{14} cellules dans le corps humain soit ≈ 600 g d'ADN !

ADN



- Acide désoxyribonuclèique
- Double helice
- Acide et polyanions
- Stable au cours de l'évolution
- Support de l'information génétique
- Localisation dans le noyau+++
- Le monomère est un nucléotide:
  - Phosphate
  - Sucre: Désoxyribose
  - base azotée

ADN

où trouvent-on de l'ADN?

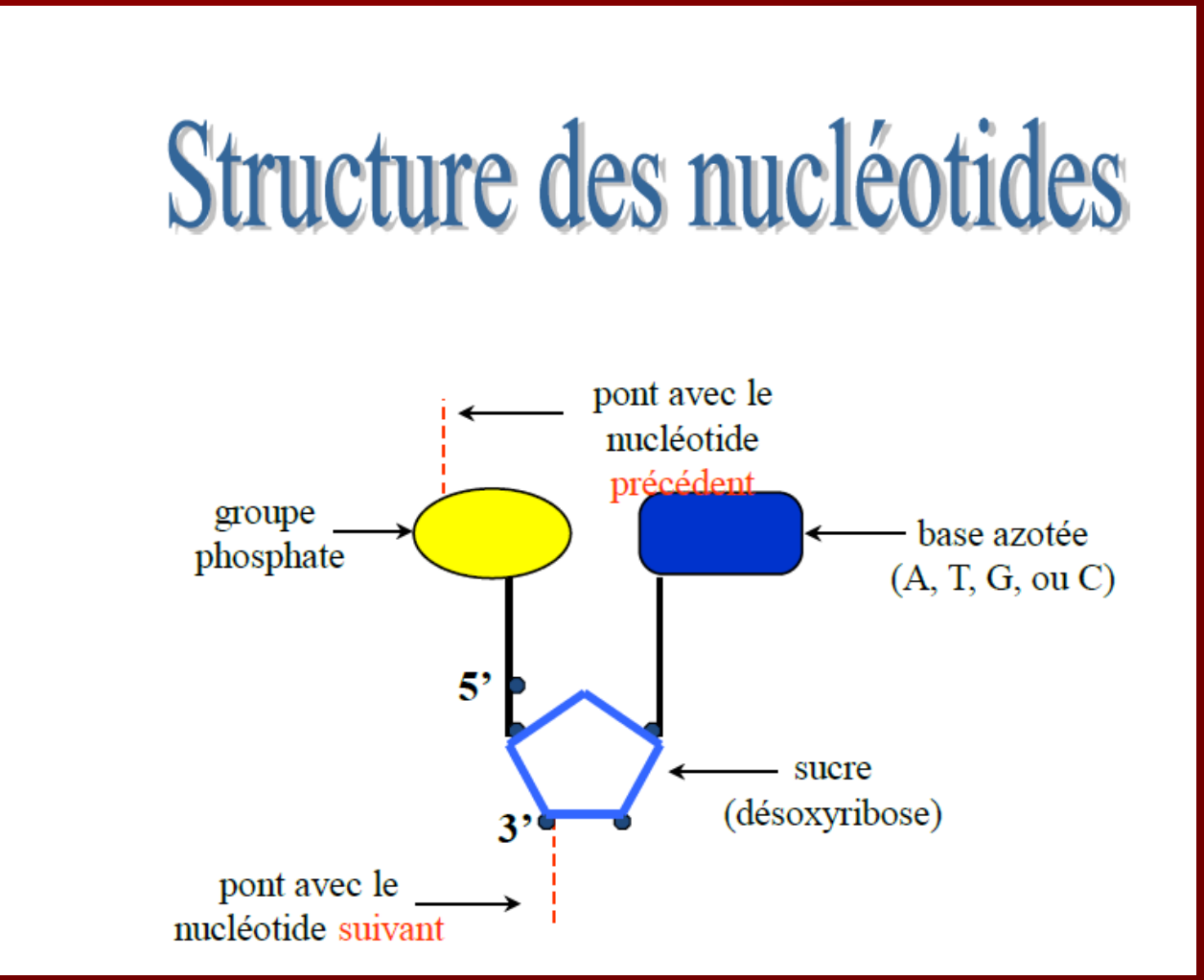
EUCARYOTES

Dans le noyau : molécules linéaires
Dans les organites : molécules en général circulaires

Le nombre de chromosomes n'a aucun rapport avec la complexité de l'organisme

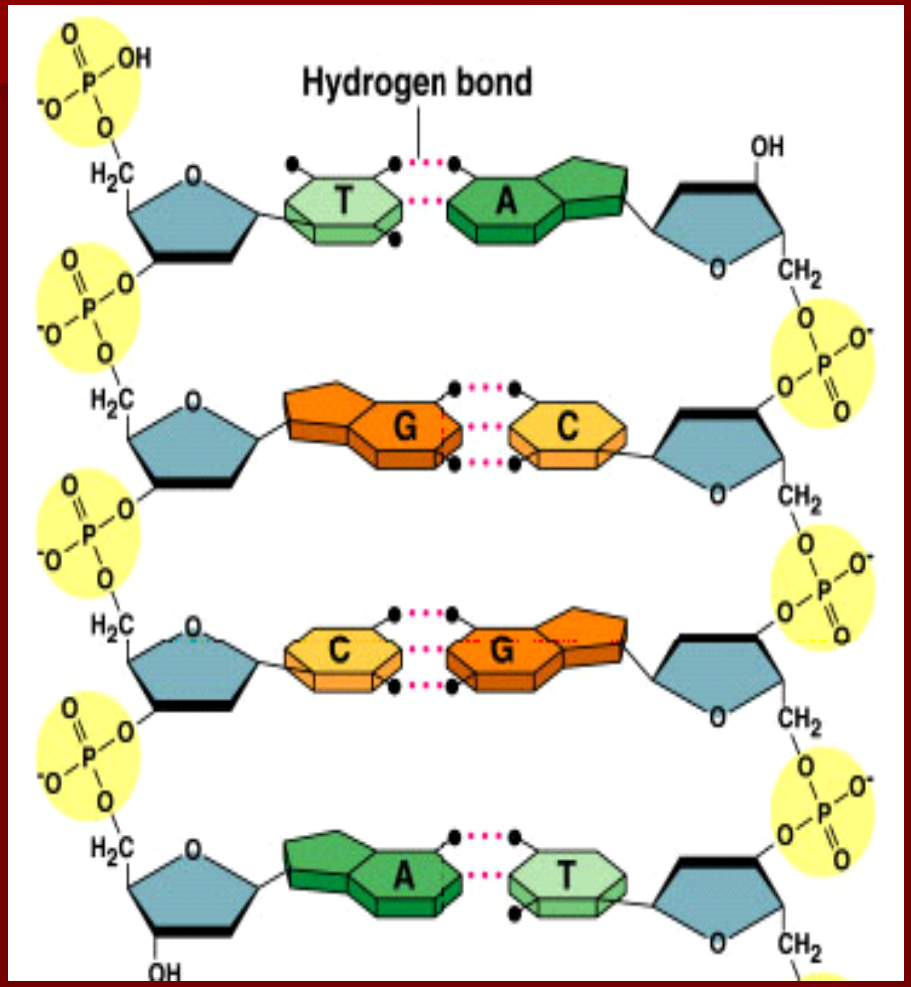
Le nombre de gènes (estimés) non plus semble –t-il:	
Arabidopsis thaliana (plante)	***27 379***
Homo sapiens (homme)	***26 517***
Caenorhabditis elegans (nématode)	***22 628***

Quelles sont les bases de l'ADN



- A – adenine
- T – thymine
- C – cytosine
- G – guanine
- Régles des paires de bases

Quelles sont les bases de l'ADN



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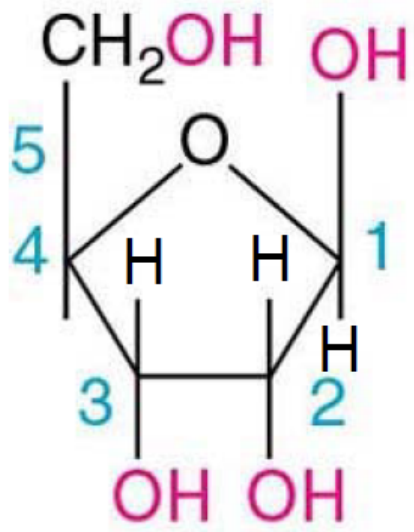
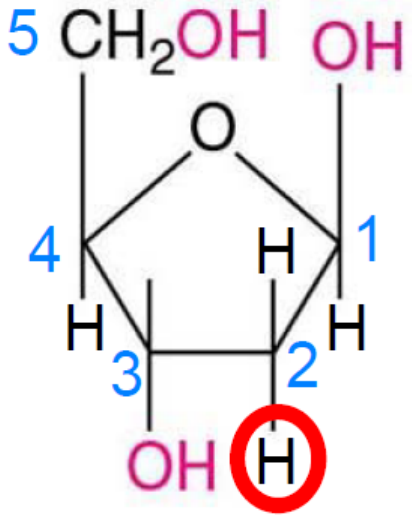
Quelles sont les bases de l'ADN



Ribose

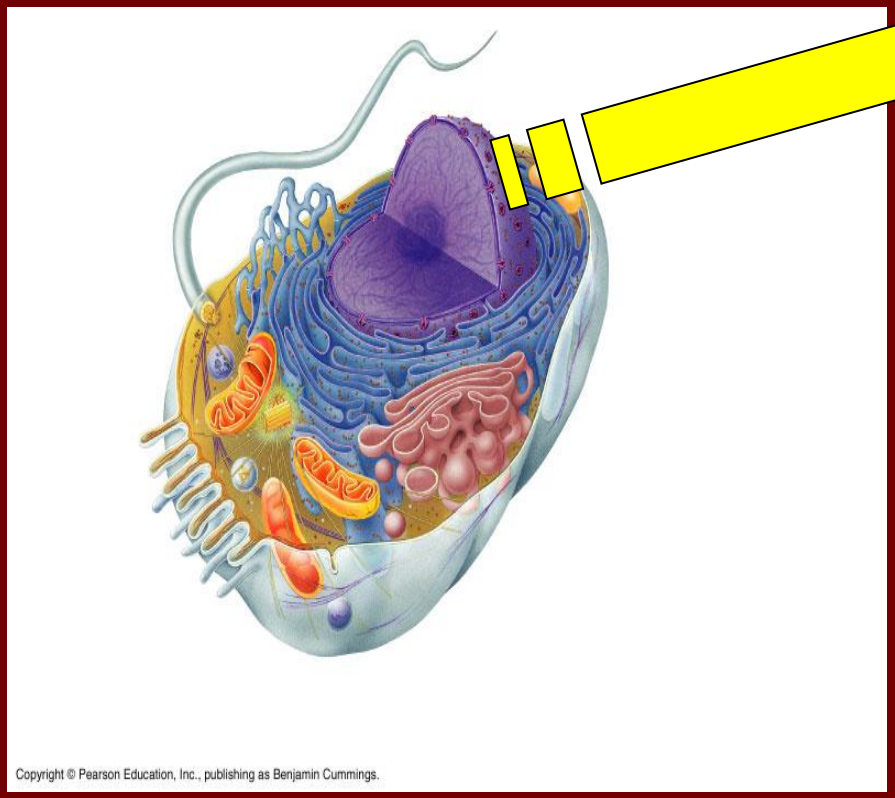
acide ribonucléique (ARN)

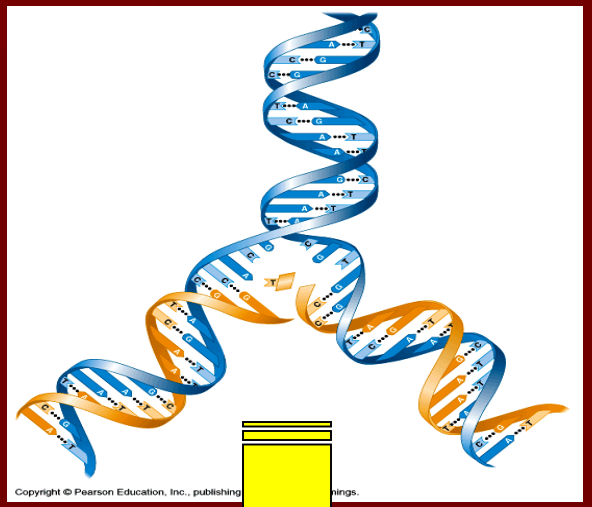


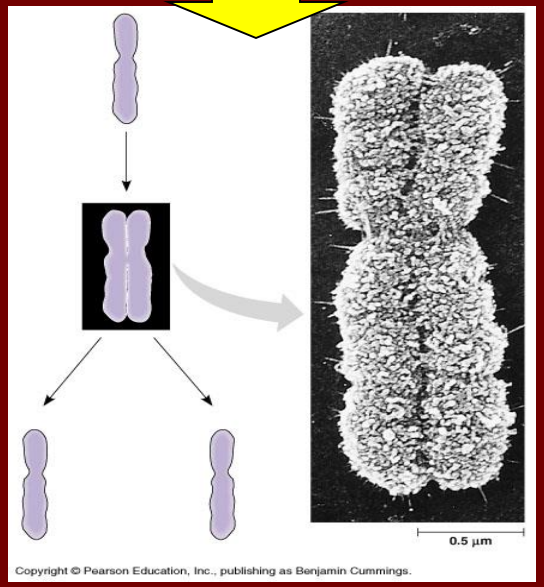
2-deoxyribose

acide desoxyribonucléique (ADN)

Ou l'ADN est localisé dans la cellule?



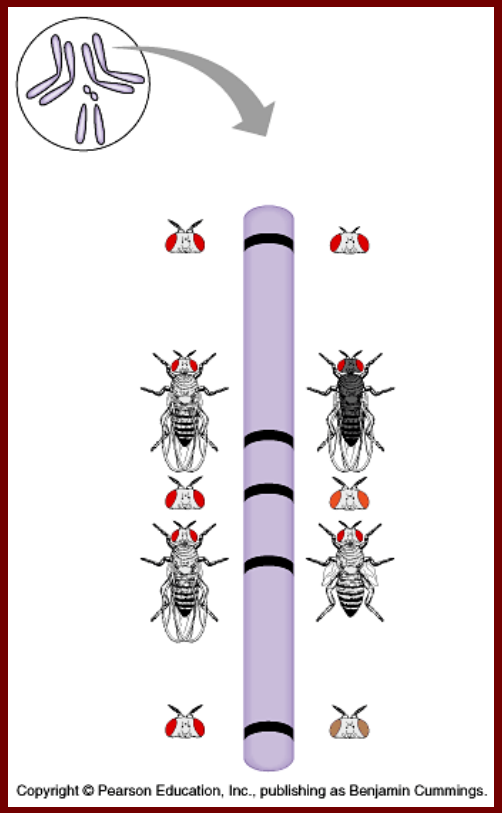




- Gènes sont localisés dans les chromosomes retrouvés dans le noyau de la cellule + mito+ chlorop.
- Les chromosomes sont visibles quant une cellule se prépare à la division.
- Les Chromosomes apparaissent quant la chromatine apparaît condensée et devient visible.
- La plus part du temps (90%), le matériel génétique est sous forme de chromatine.
- Un génome est l'information génétique complète contenue dans un individu.
  - (gene + chromosome)

C'est quoi l'expression des gènes?

L'expression des gènes est le résultat de son activation et il en résulte la synthèse de protéines.

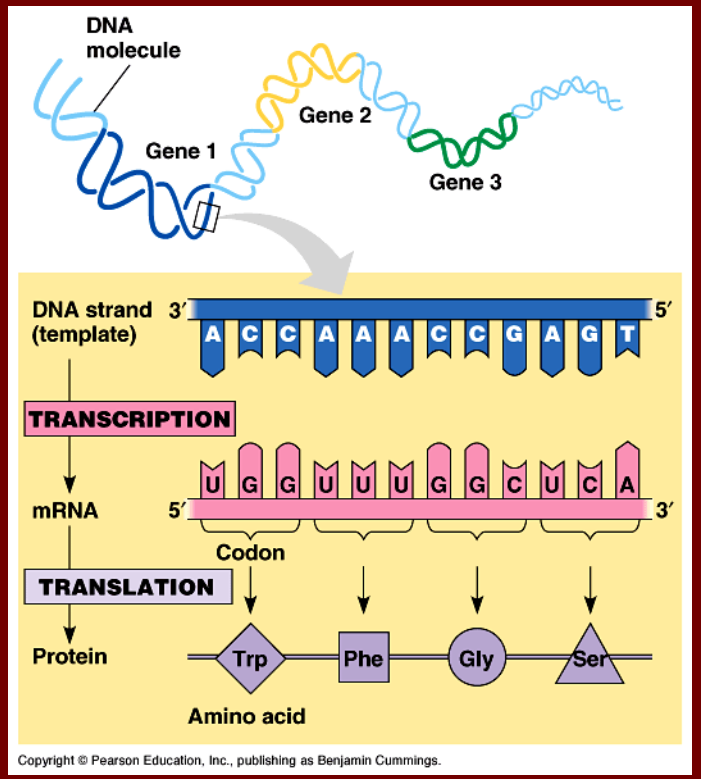


L'expression des gènes est différente chez les procaryotes et les eucaryotes.

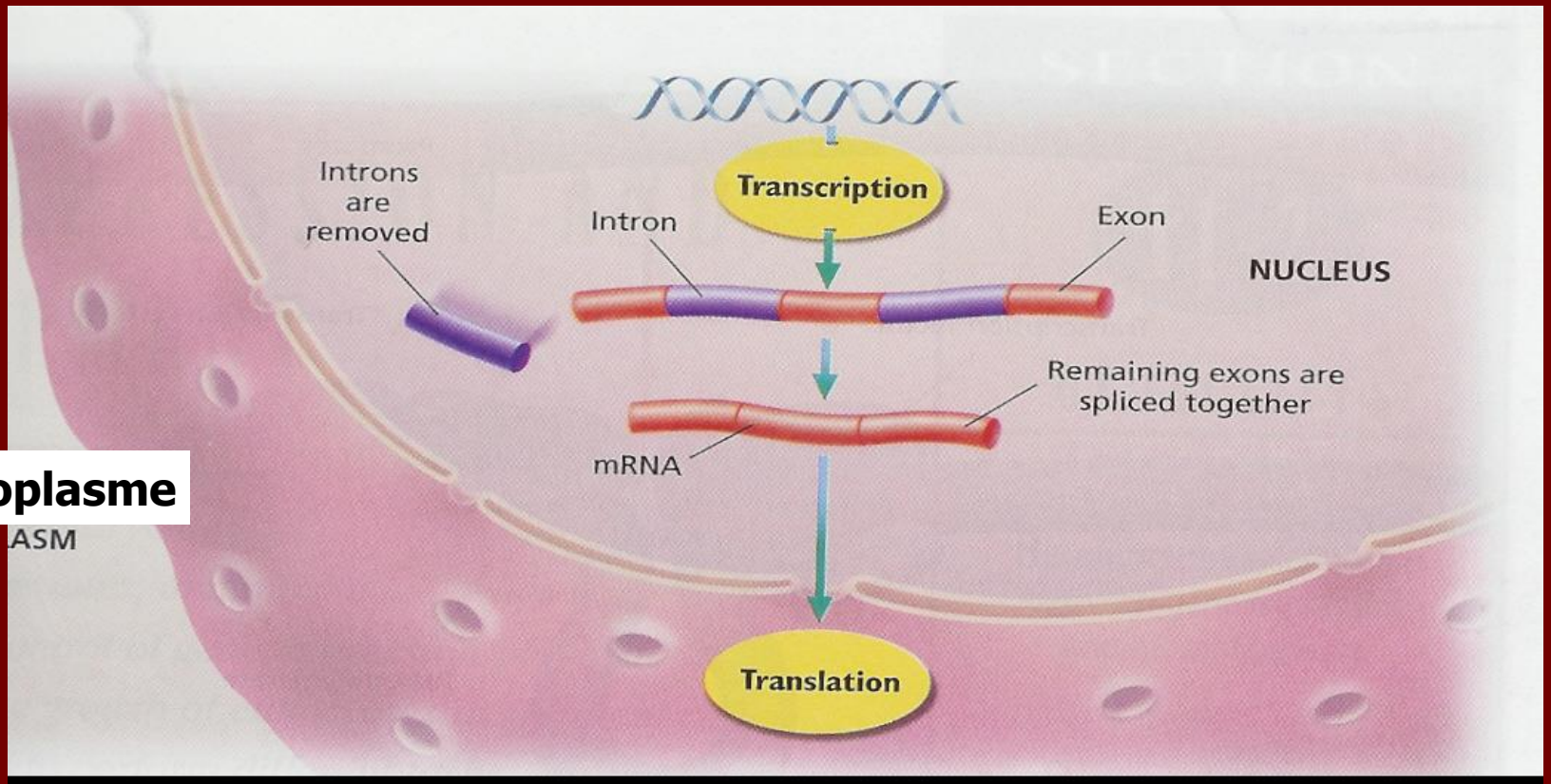
- Procaryotes
  - Auccune membrane ni d'organites (pas de noyau)
  - Organisme plus primitive
  - Seulement 1 "chormosome" circulare
  - Bactéries.

- Eucaryotes
  - Membranes bordant les organistes (fonction spécialisée –noyau, mitochondries, chloroplastes)
  - Les Chromosomes sont en paires et non circulaires
  - Tous les organismes non procaryotiques: protozoaires, levures, champignons, plantes, les animaux

- Chez les Eucaryotes, suivant la mitose ou la méiose, l'ADN est fermé mais certaines régions restent ouvertes pour la **transcription**. La zone relachée ou ouverte de l'ADN est appellée l' **euchromatine**.
- La transcription est la lecture de l'ADN et change le code en ARNm ou mRNA.
- La traduction change L'ARNm en protéines



- L'ADN chez les eucaryotes a des régions codantes et non codantes. Les régions de l'ADN qui codent pour des protéines sont appelés <u>exons</u>, tandis que les régions qui ne codent pas pour des protéines sont appelées <u>introns</u>. Attention ???



cytoplasme

- Chez les procayotes, la transcription et la traduction se produisent dans le cytoplasme.
- Chez les eucaryotes, la transcription se produit à l'intérieur du noyau en une séquence d'événements de deux étapes min.
  - Pré-ARNm comprend les introns et les exons du gène.
  - ARNm n'est que la partie codante (exons).
- Traduction se produit dans le cytoplasme via des ribosomes.

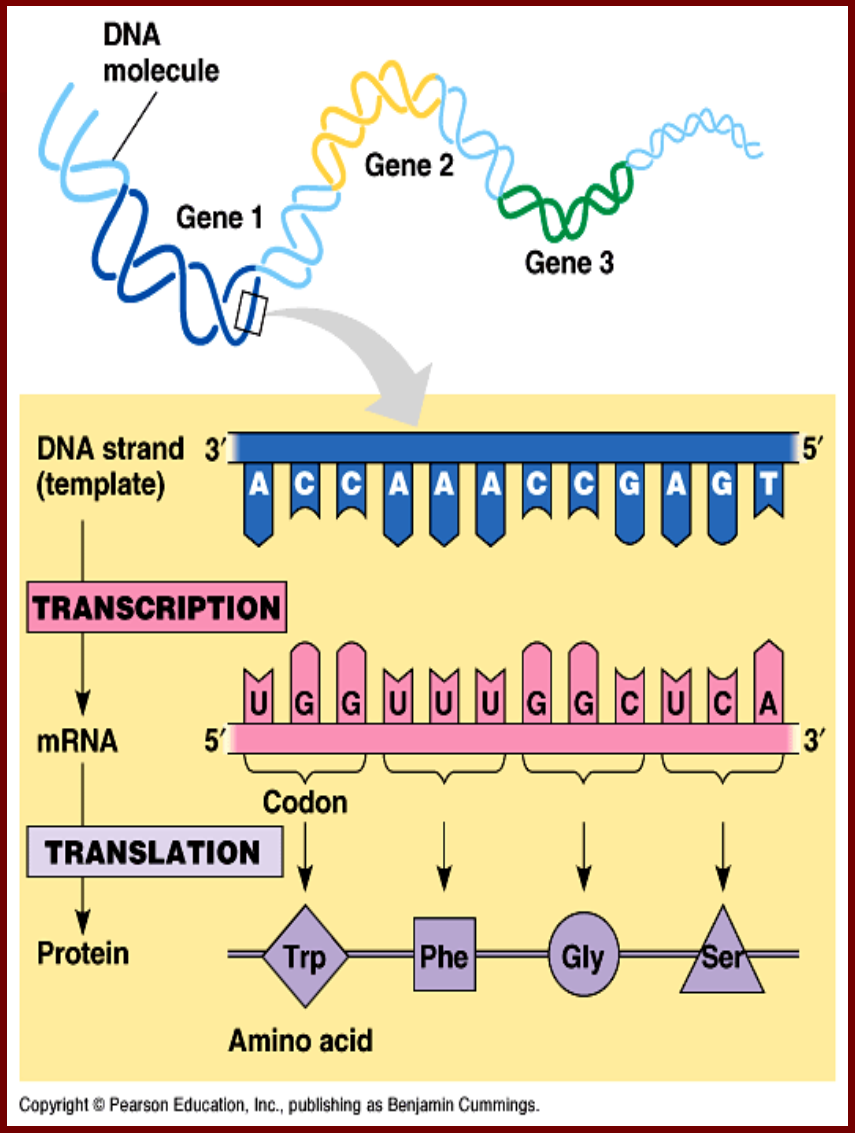
Rappel: Les trois (3) types d'ARN nécessaire à la traduction + autres (régulation de l'expression des gènes ?)

Messenager (ARNm), Transfert (ARNt) et Ribsomal (ARNr)

+ beaucoup d'autres (voir plus loin…)

Traduction

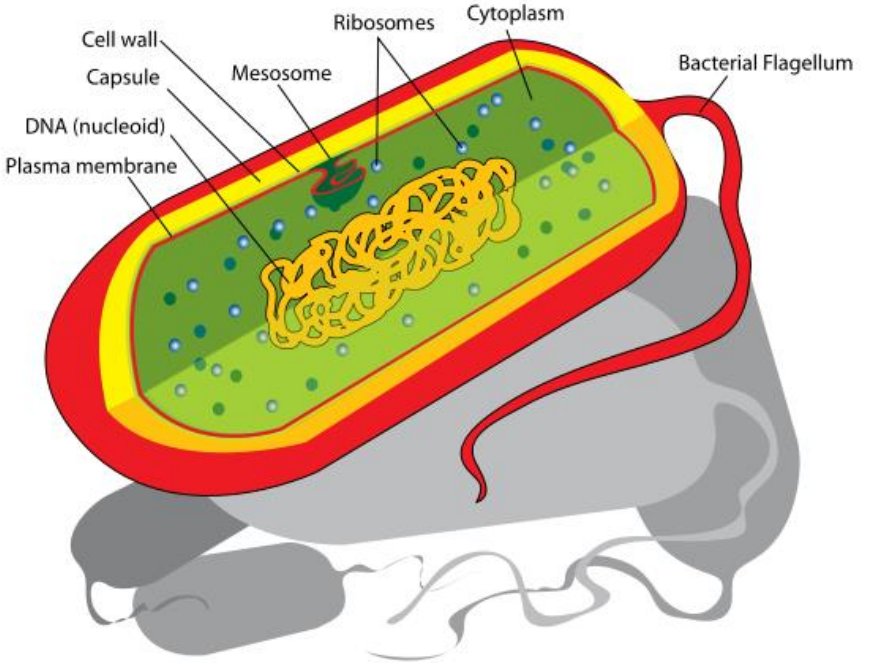
- mRNA
  - Simple brin, acide, polynanions
  - Fragile ??? À Retenir??
  - Pas de thymine mais uracile.
- tRNA ou ARNt dispose d'un code de 3 paire de base pour chauqe acide aminé.
- Acides aminés composent la chaine polypeptidique.
- Une ou plusieurs chaines polypeptidiques composent les protéines.
- Les protéines fournissent les "plans" pour les caractéristiques et les fonctions cellulaires.



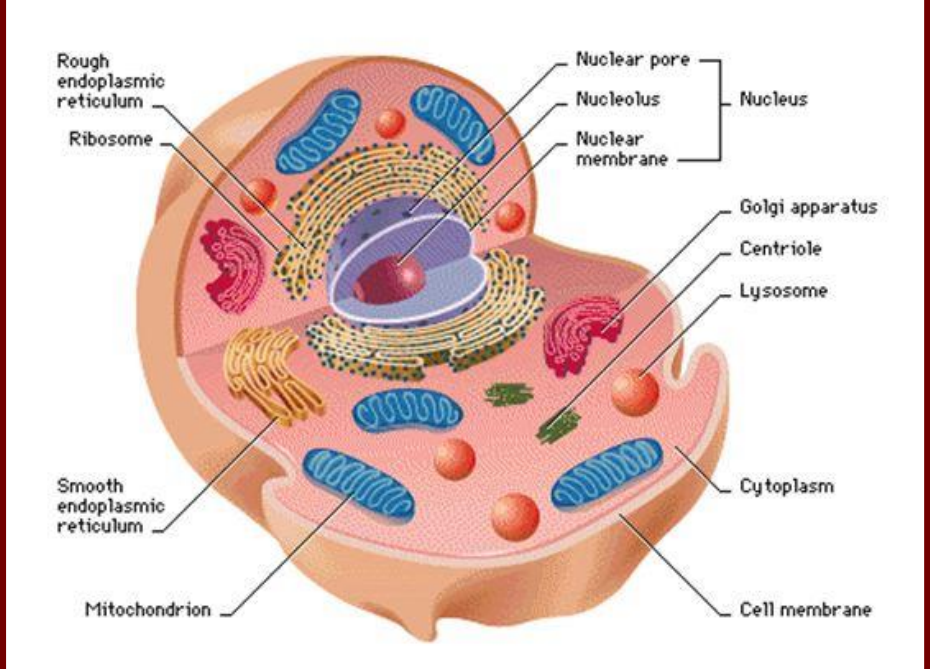
The cell: https://www.youtube.com/watch?v=URUJD5NEXC8

Similarities

Prokaryotes/Bacteria



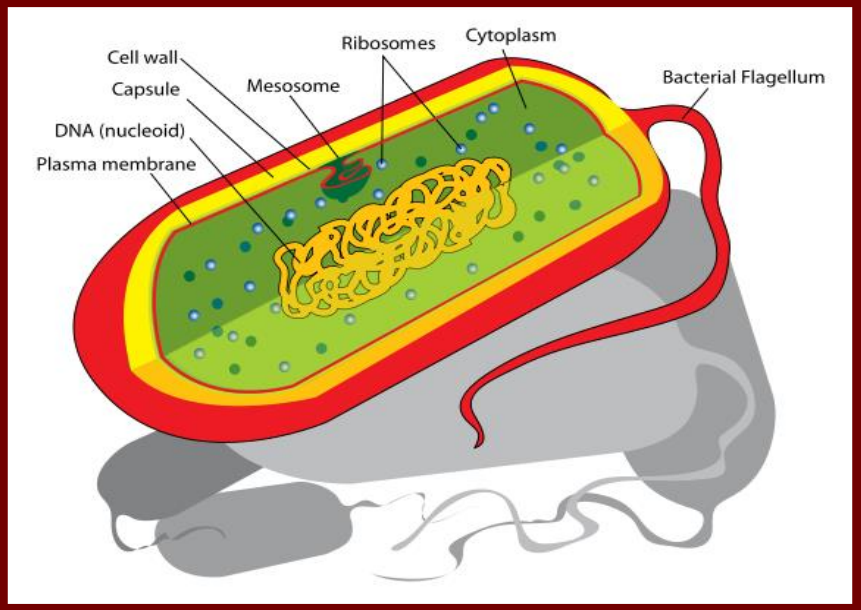
Eukaryotes

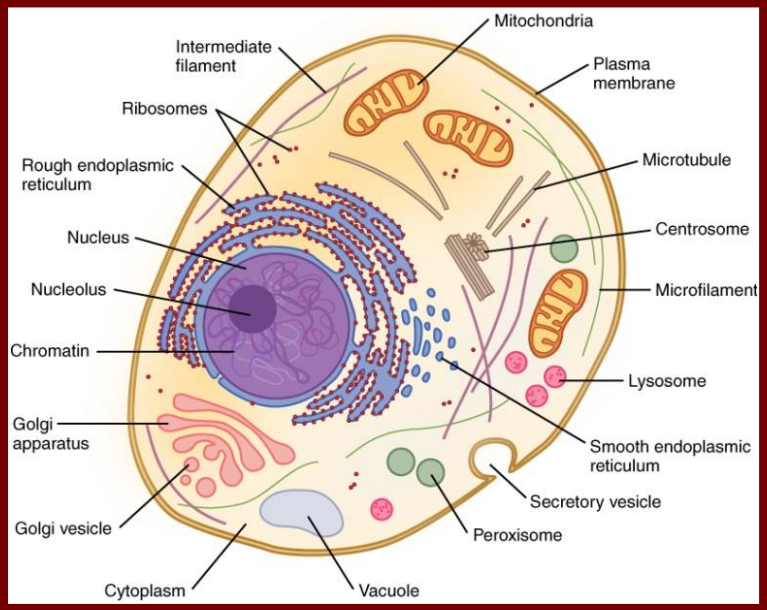


- **Plasma membrane** (phospholipid bilayer and regulates permeability)
- **Genetic material** – DNA
- **Cell Wall** – except animal cells
- **Ribosome** - catalyse protein synthesis
- **Cytoplasm/cytosol** – comprising of water, glucose, proteins and ions.

Differences

Prokaryotes/Bacteria





Eukaryotes

Differences

- **Bacteria**
- **Size:** 1-10μm
- Cell Wall (murein)
- **No distinct subcellular organelles**
- **Circular chromosome – nucleoid**
- Often plasmids, RNA and Ribosomes
- **Unicellular or multicellular**
- Escherichia Coli (*E. Coli*) is most studied bacterium

- **Plants, animals, fungi and protists (algae and protozoa)**
- **Size:** 10-100μm
- Cell Wall – only plants, fungi and protists (cellulose)
- **Well defined subcellular compartments bounded by lipid membranes**
- **Cytoplasm** consists of organelles, ribosomes, cytoskeleton (shape, movement and organises many metabolic functions)
- **Cytoskeleton:** microtubules made of tublin & microfilaments made of actin.
- **Most are multicellular**
- **Differentiate to specialized tissue/cells**

Looking at microbes: http://www.microbiologyonline.org.uk/students/microbe-passports-1#/home

Ce qu'il faut retenir

- Synthèse du chapitre
- Voir cours magistral…
- Différences pro/EU;
- Initiation mécanismes…