Need a little extra help?

Extra office hours!

MWRF from 11:00 till 1:00 or by appointment: Marion.Brodhagen@wwu.edu.

Tutoring center in Old Main 387:

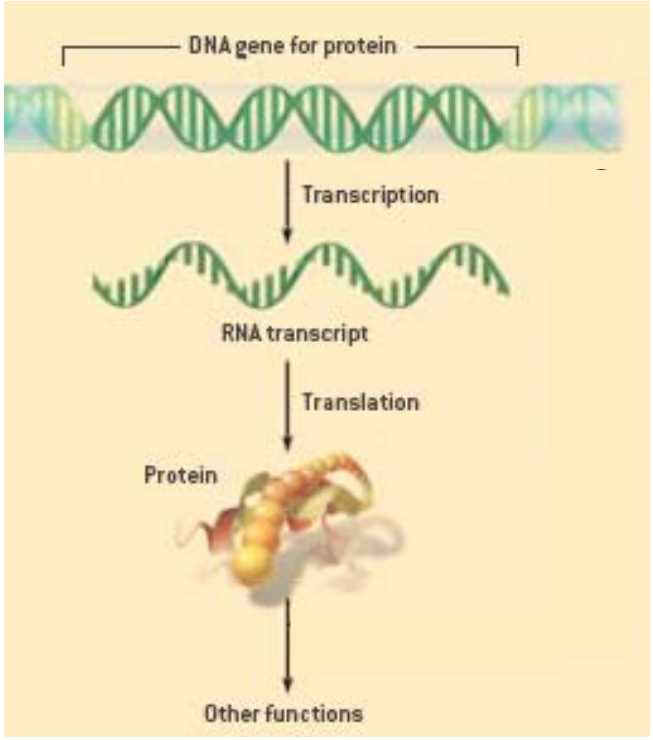
BIOL 205 drop-in tutoring at the following times:
Monday 10-12 & 6-8
Tuesday 10-12 & 6-8
Wednesday 1-4
Thursday 9-12 & 2-4
Friday 10-12

DNA, chromatin and chromosomes

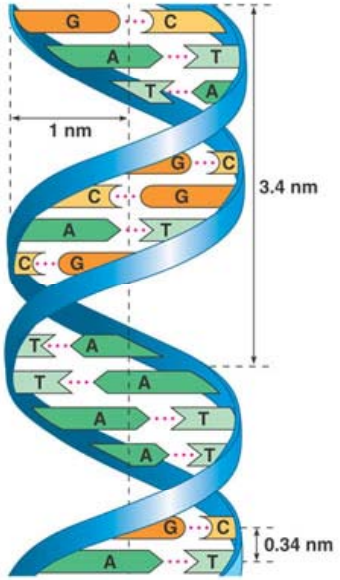
Readings: Review Chapter 2: pp 56-65
Chapter 5: read all.

What is a gene?

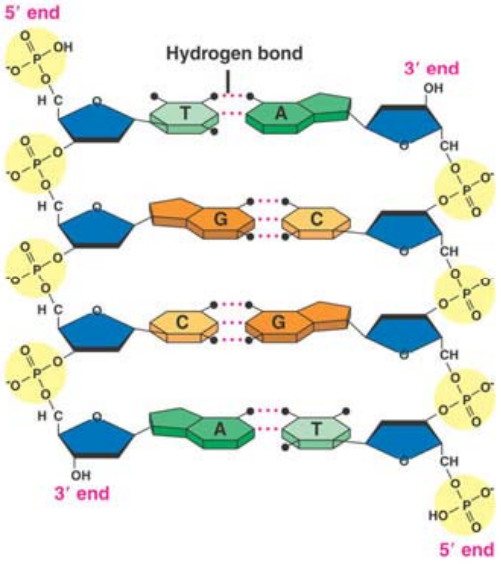
Central Dogma



Nucleic Acid Sequence

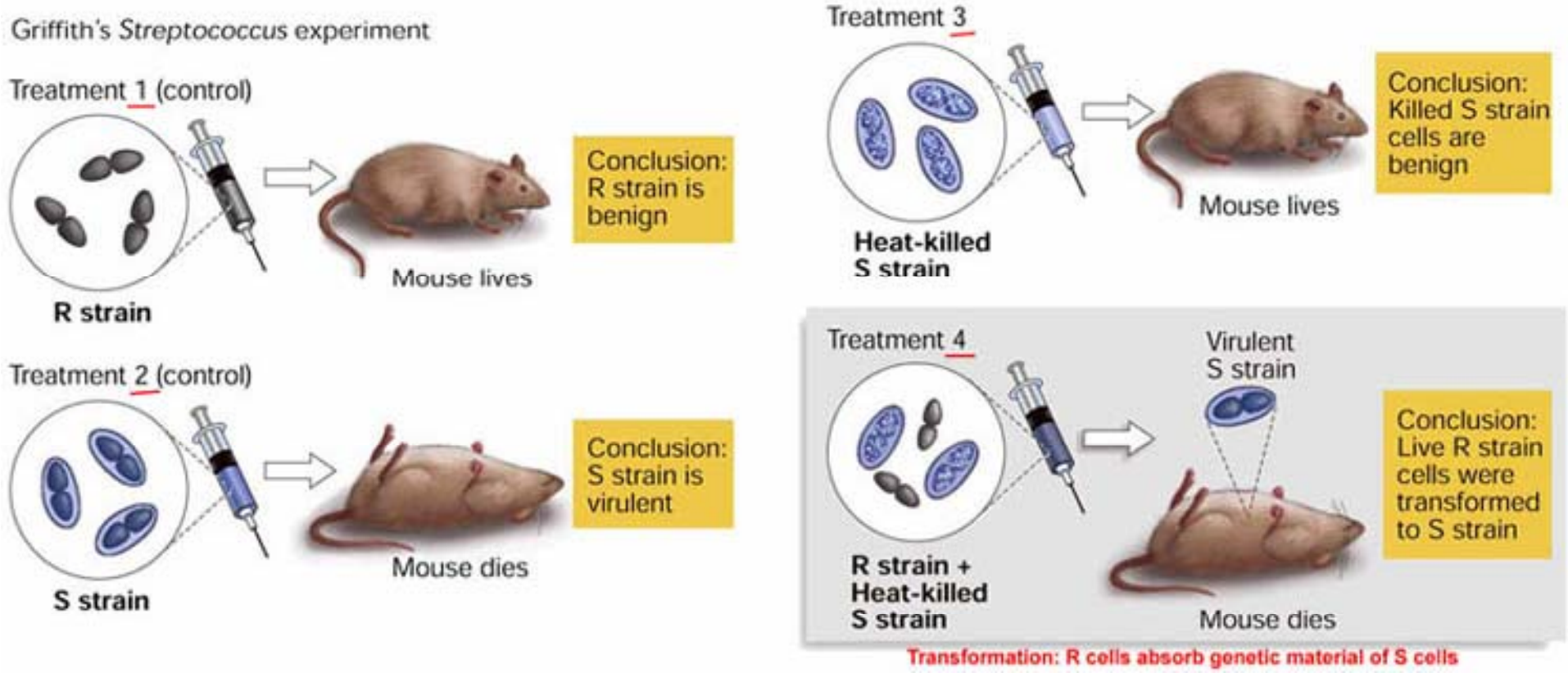


(a) Key features of DNA structure



(b) Part a c em ca structure

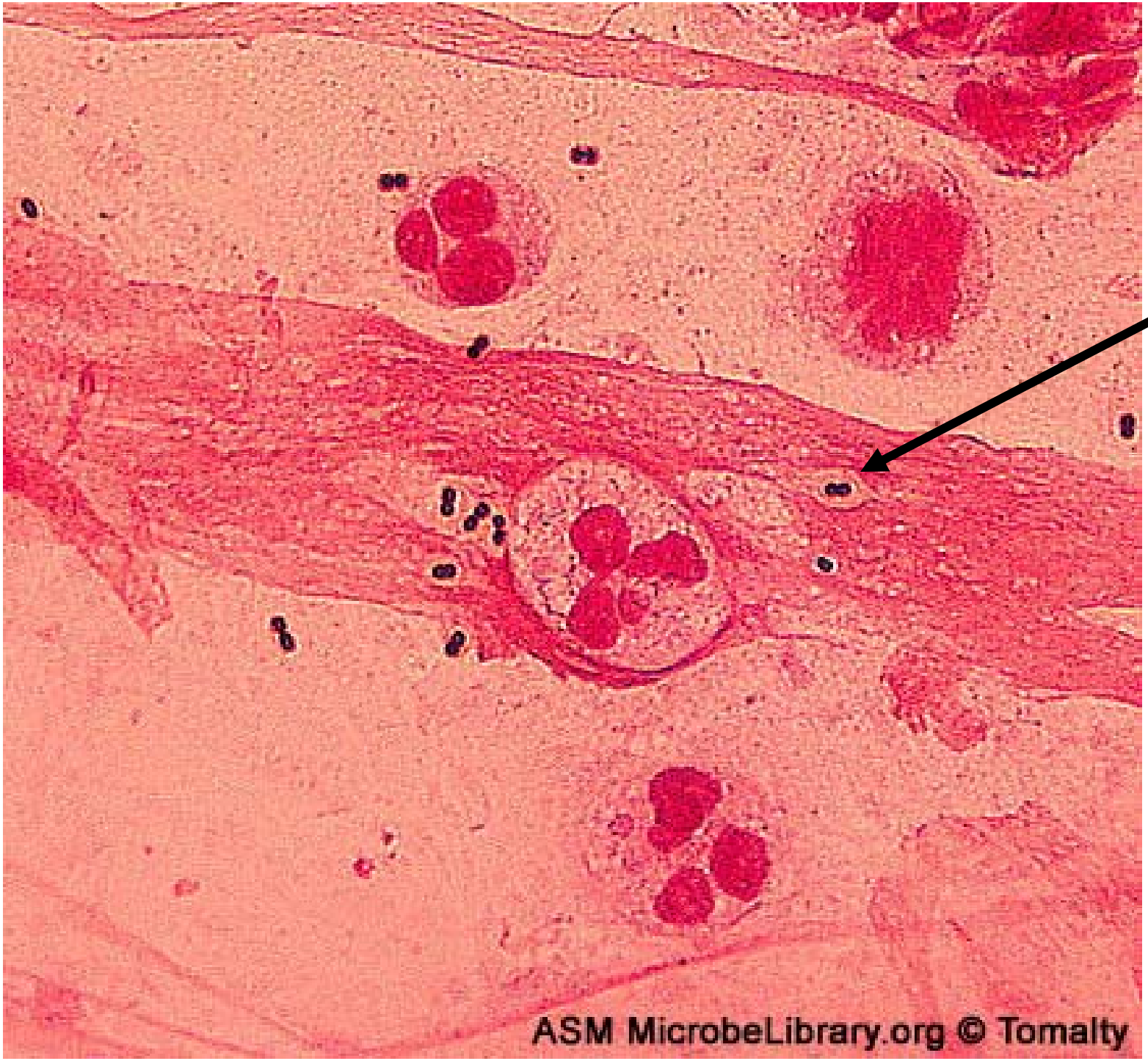
The "transforming principle" Fred Griffiths, 1928



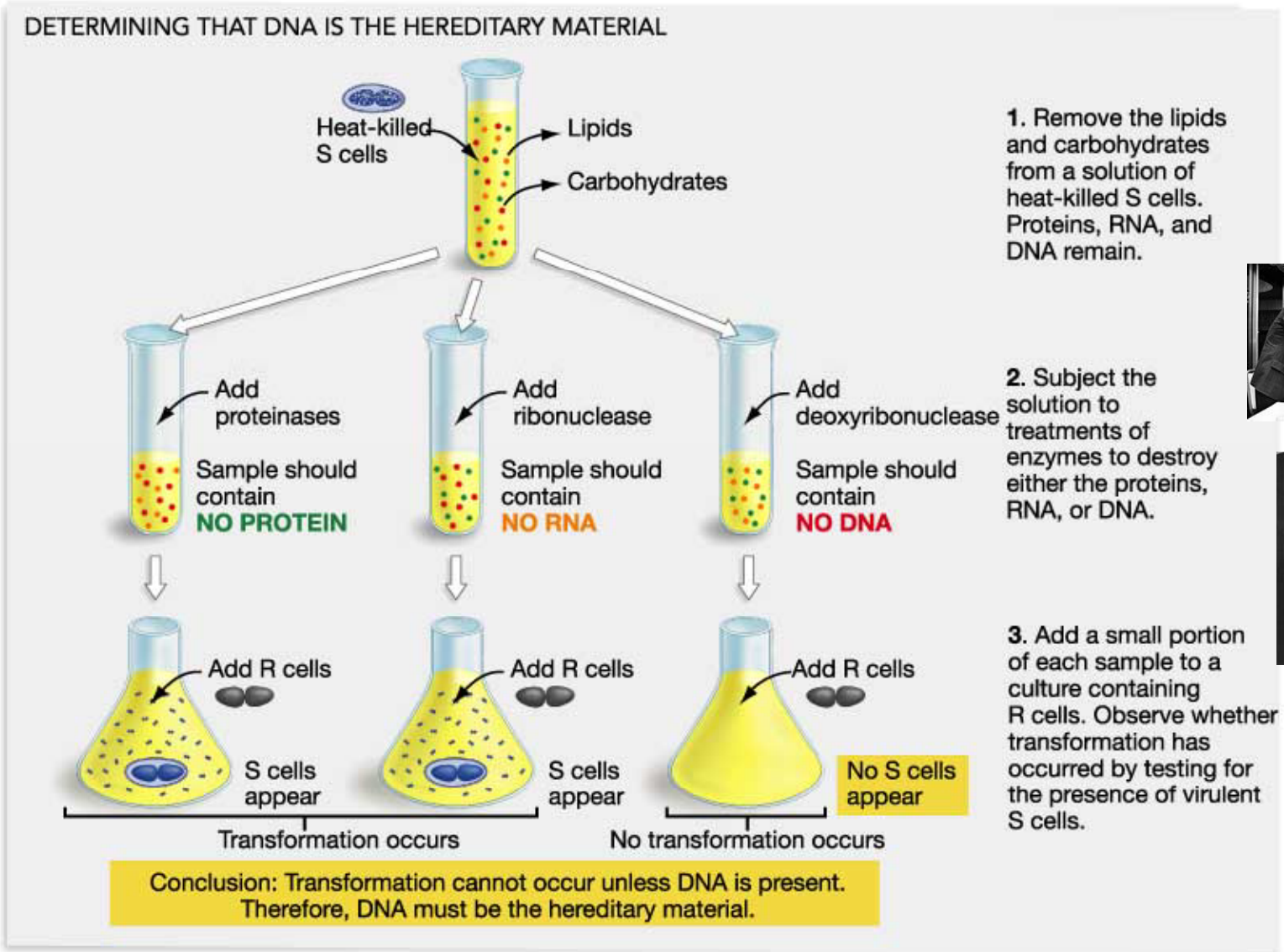
Streptococcus pneumoniae causes pneumonia.

R ➔ "rough"; less virulent

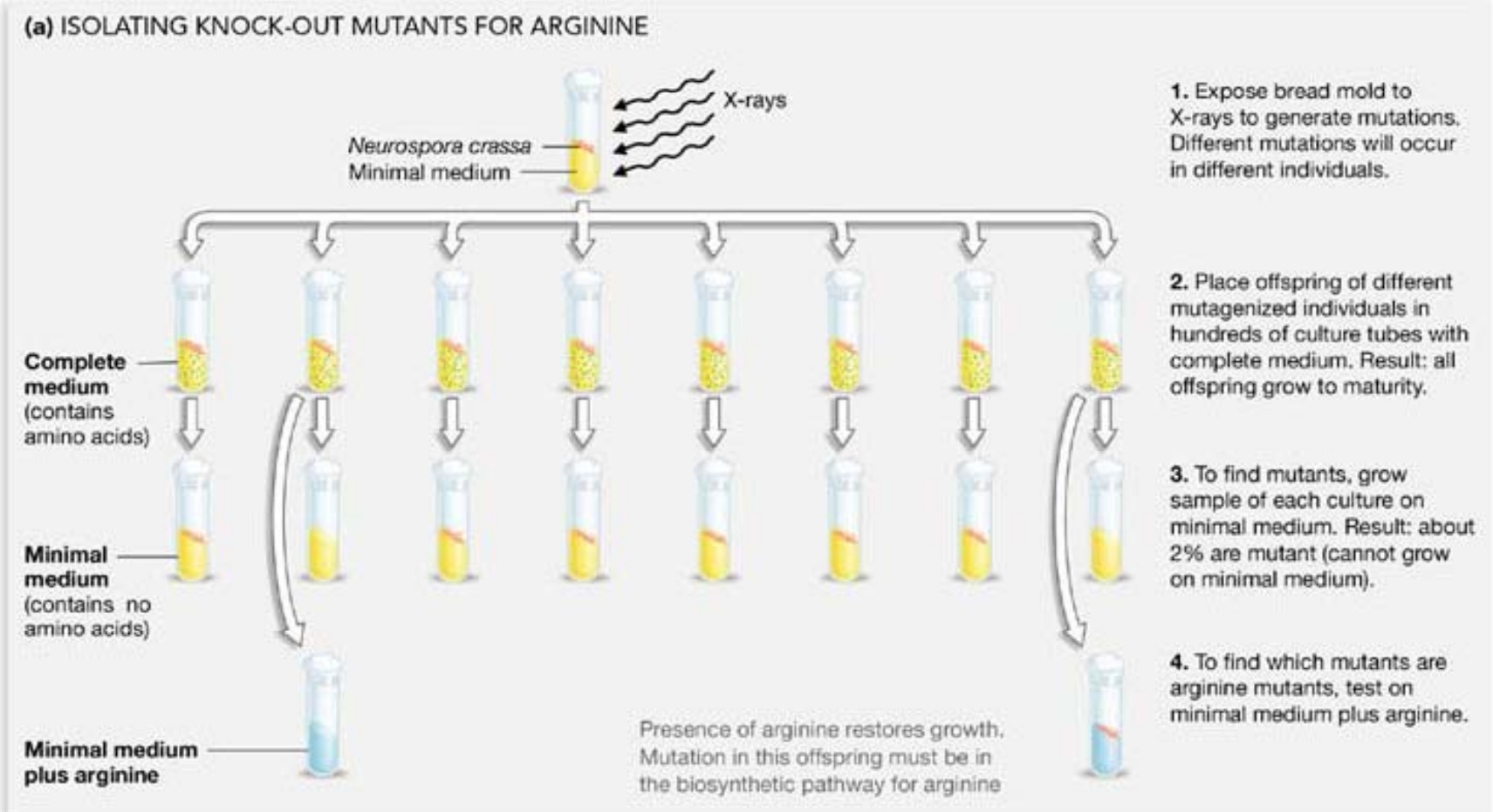
S ➔ "smooth"; due to polysaccharide coating that helps cells evade the mammalian immune system


Streptococcus pneumoniae surrounded by clear zone (capsule)
ASM MicrobeLibrary.org © Tomalty

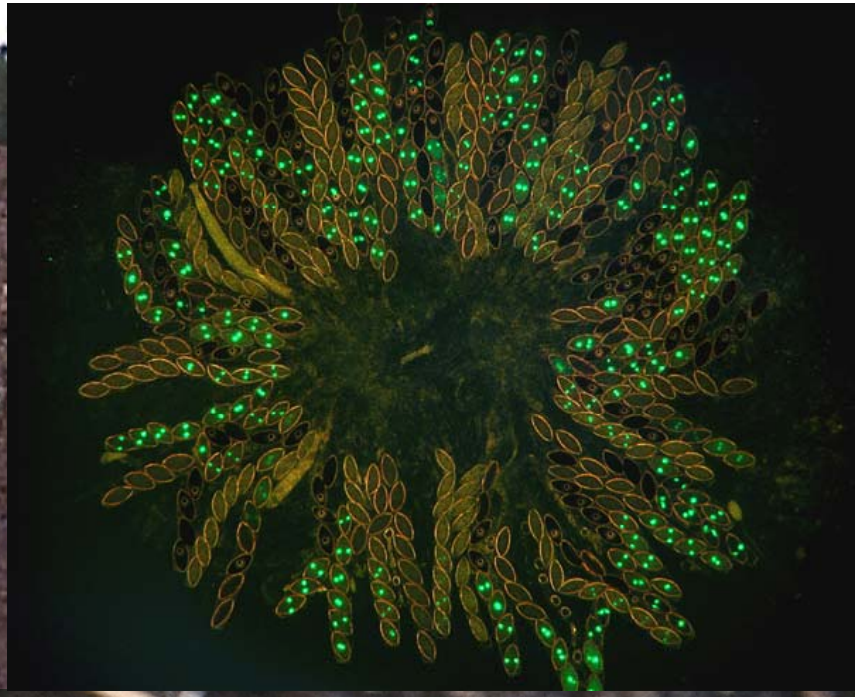
The transforming principle is DNA
Avery, MacLeod, and McCarty, 1944

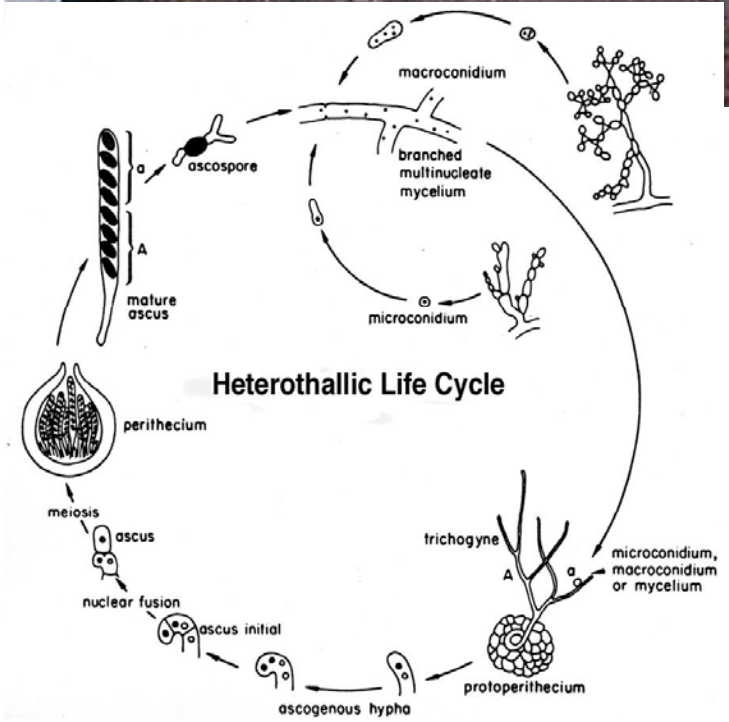


"One-gene-one-enzyme" hypothesis
Beadle and Tatum, 1941




NEUROSPORA

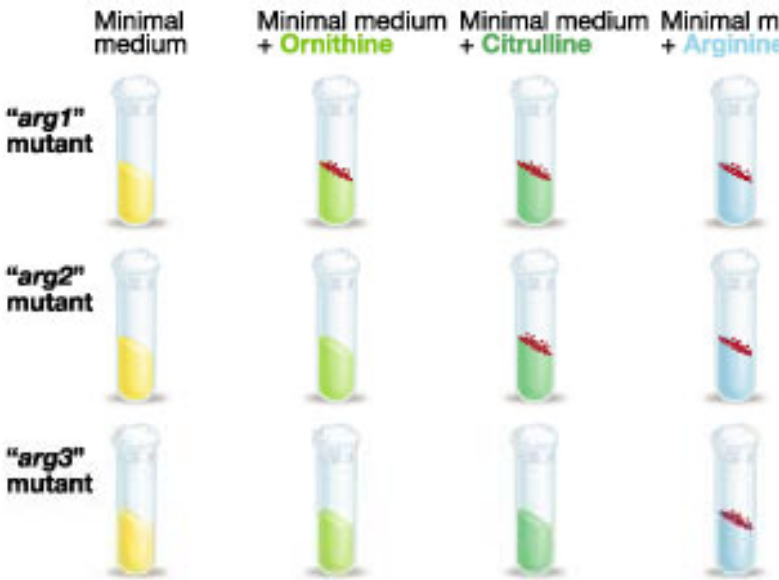



macroconidium
ascospore
branched
multinucleate
mycelium
a
A
mature
ascus
microconidium
Heterothallic Life Cycle
perithecium
meiosis
ascus
nuclear fusion
ascus initial
ascogenous hypha
protoperithecium
trichogyne
A
a
microconidium,
macroconidium
or mycelium


48
48

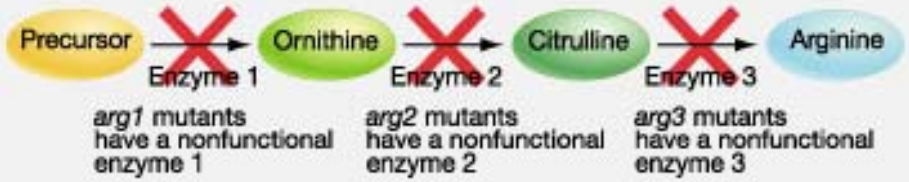
"One-gene-one-~~enzyme~~ polypeptide"

(c) Locating *arg* mutants within the arginine pathway



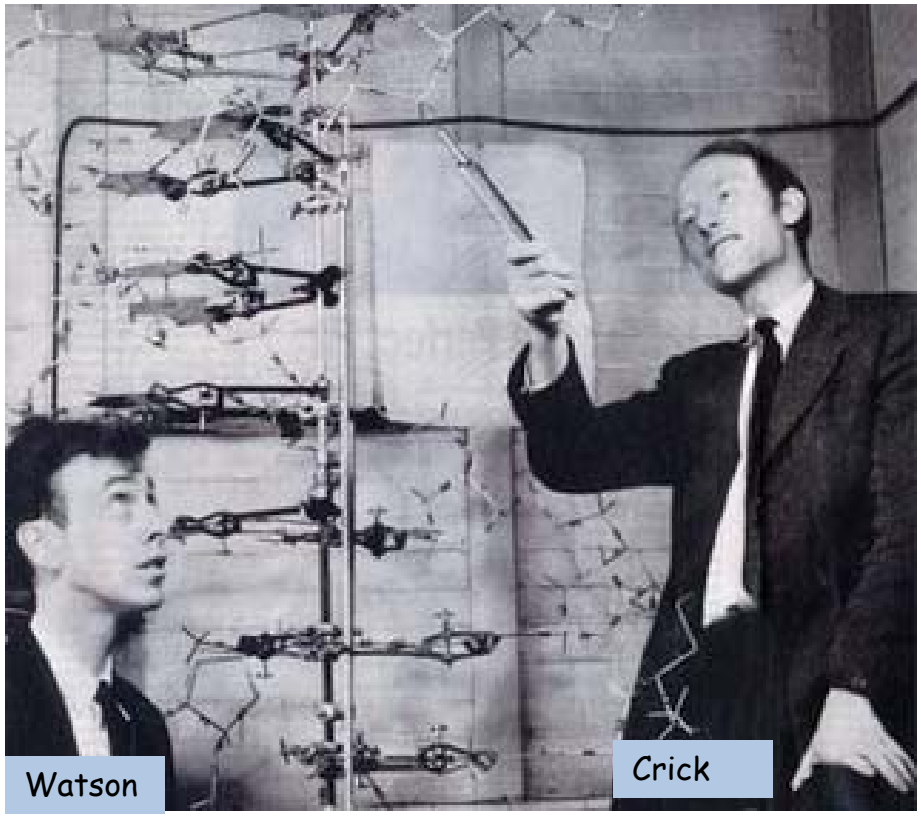
	Result	Conclusion
"*arg1*" mutant	Mutant *arg1* can grow if given *either* precursor.	Mutation must debilitate an enzyme early in the arginine pathway.
"*arg2*" mutant	Mutant *arg2* can grow if given citrulline but not if given ornithine.	Mutation must debilitate an enzyme between ornithine and citrulline in the pathway.
"*arg3*" mutant	Mutant *arg3* can grow if given arginine.	Mutation must debilitate an enzyme that creates arginine at the end of the pathway.

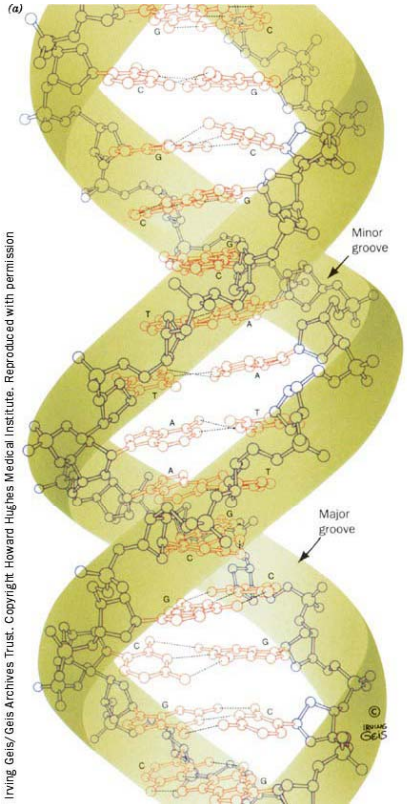
(d) THE ONE GENE–ONE ENZYME HYPOTHESIS

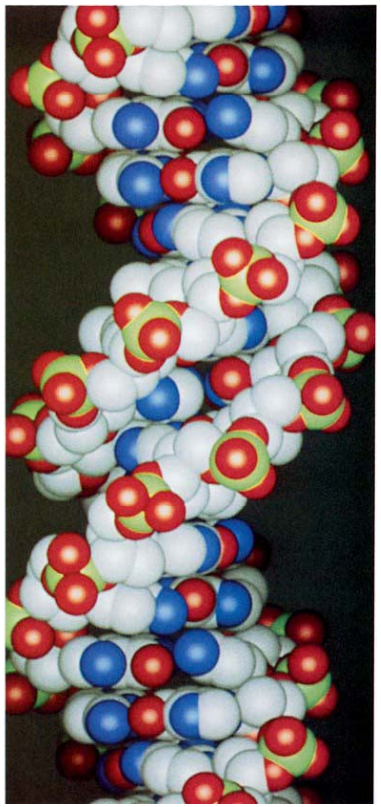


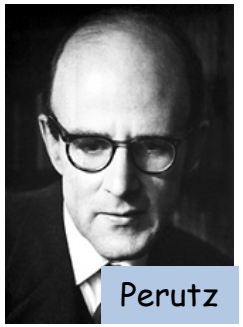
Hypothesis
Beadle and Tatum proposed that each gene in an organism is responsible for making a different protein, most of which function as enymes.

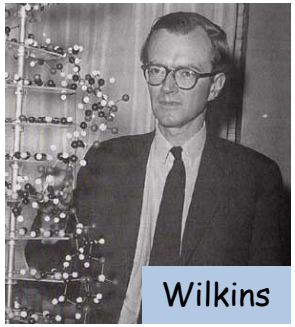
Standing on shoulders of giants

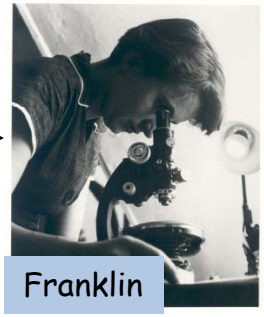












equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

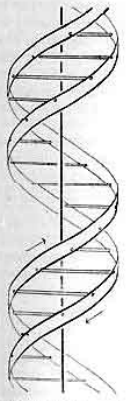
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the *z*-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

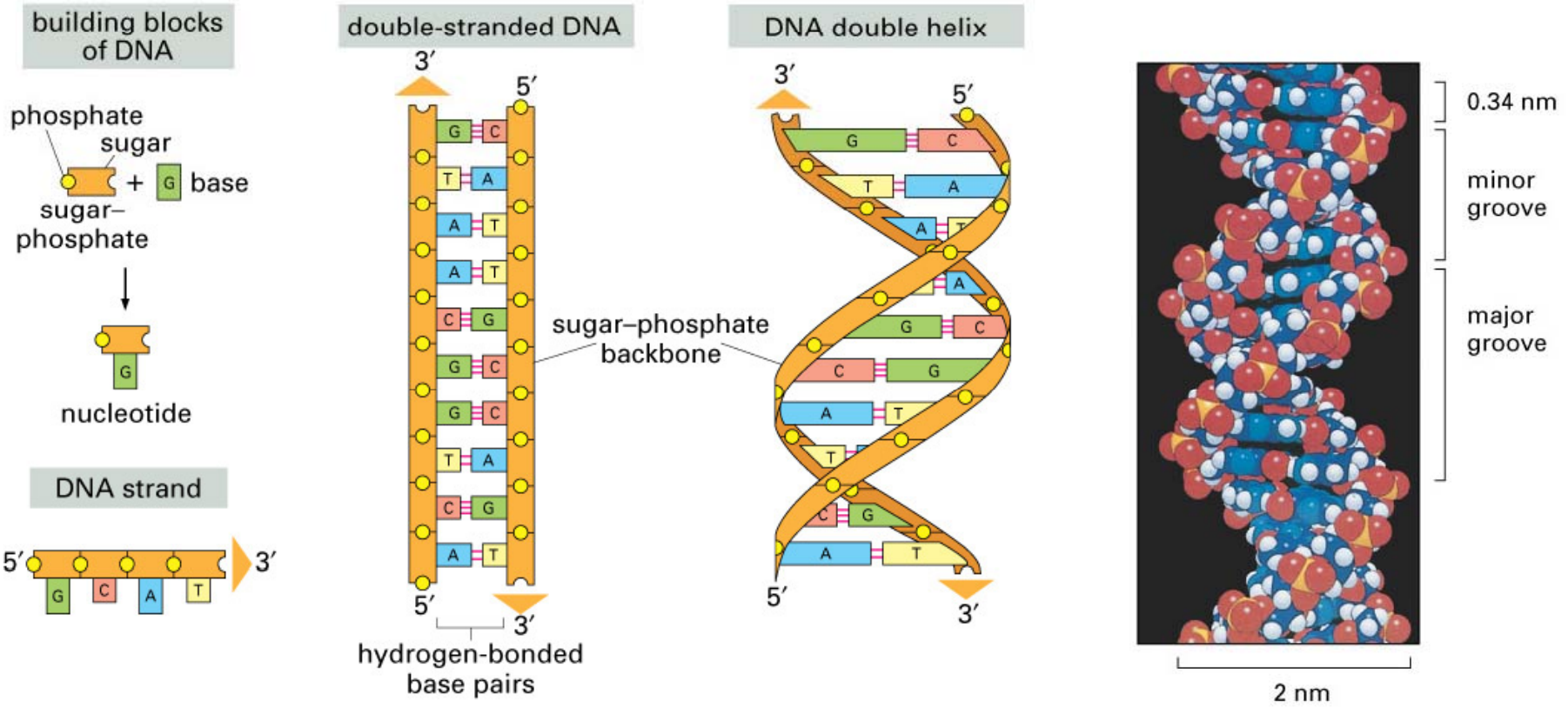
It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

"The Secret of Life" 1953

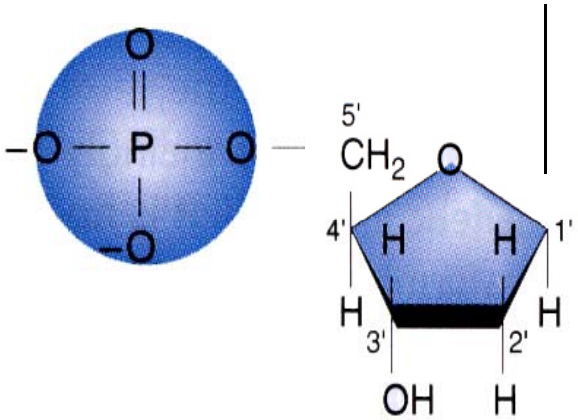
DNA structure

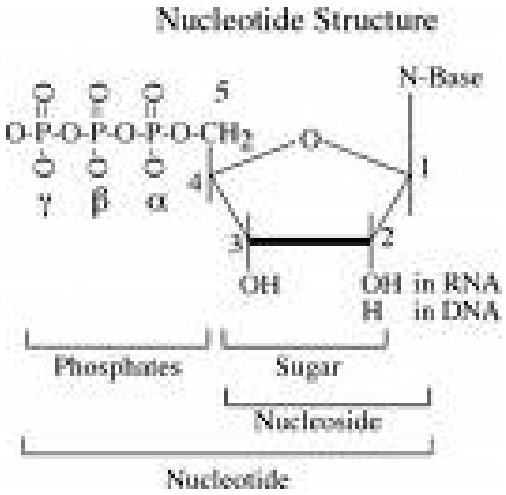


DNA (and RNA) are nucleic acids

- polymers consisting of monomers termed nucleotides.
- nucleotides: a molecule composed of:
  - a pentose sugar
  - a phosphate group
  - and an organic molecule called a nitrogenous base.

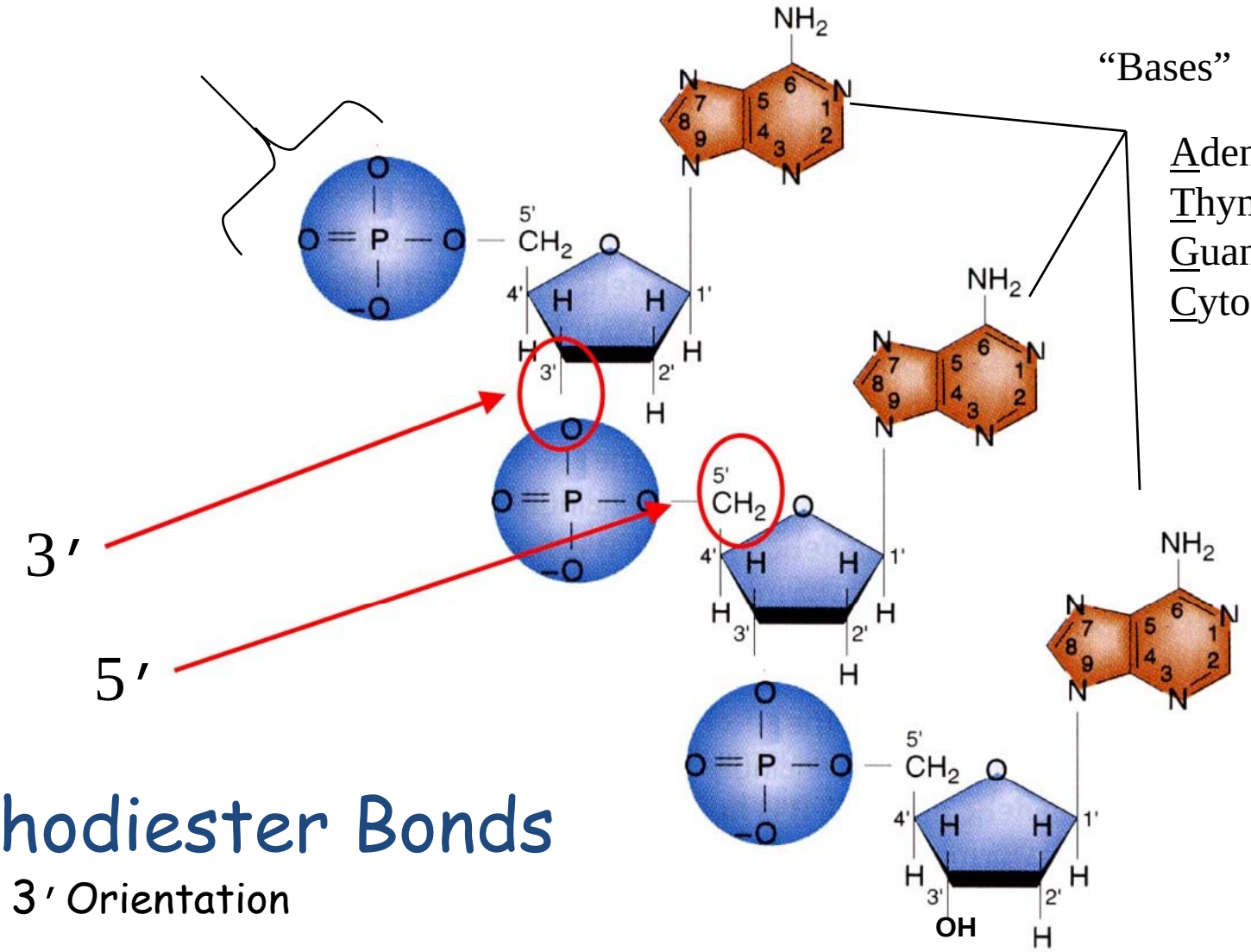
A, G, C, T/U





Polynucleotide

Phosphodiester Backbones
Mb long

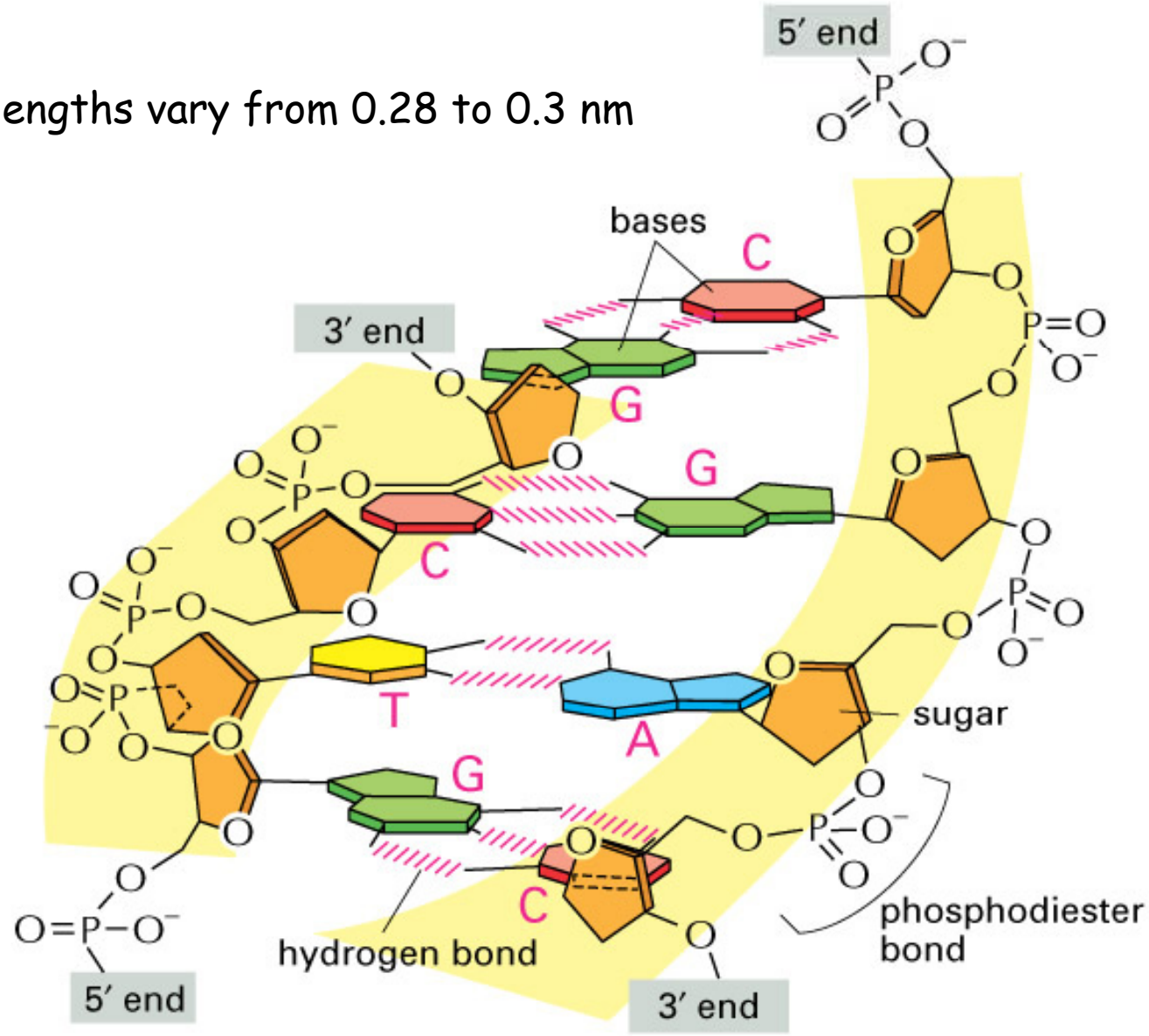


Phosphodiester Bonds

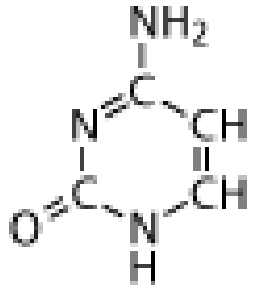
Note 5′ – 3′ Orientation

DNA structure

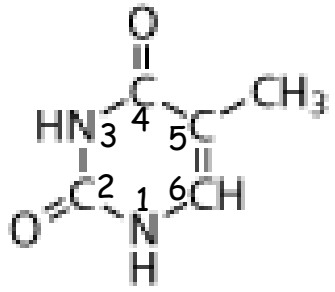
H-bond lengths vary from 0.28 to 0.3 nm



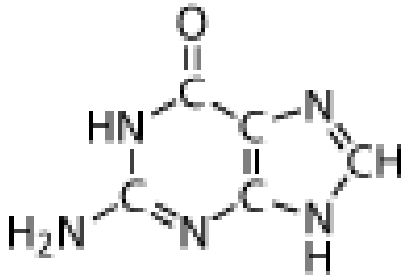
Bases: DNA



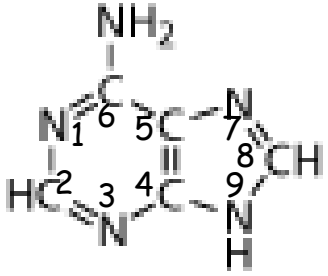
Cytosine



Thymine

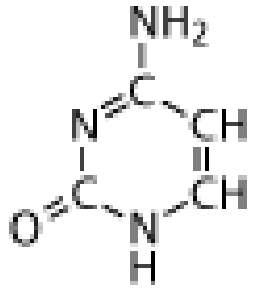


Guanine

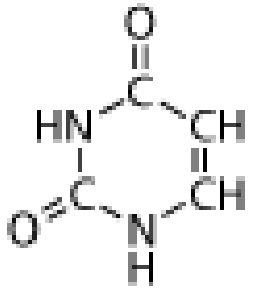


Adenine

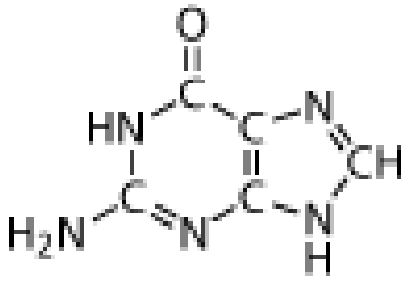
Bases: RNA



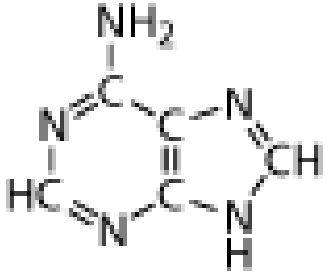
Cytosine



Uracil

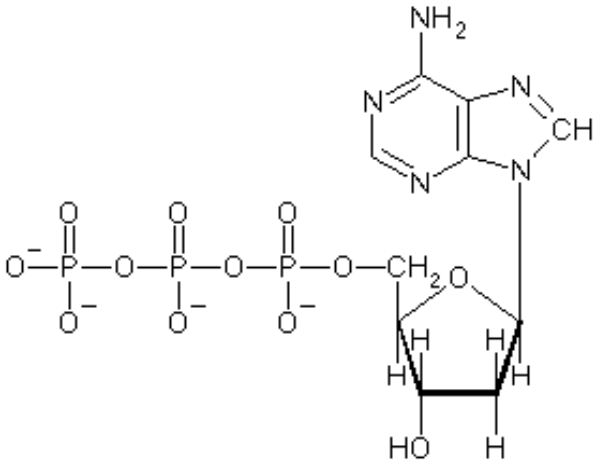


Guanine

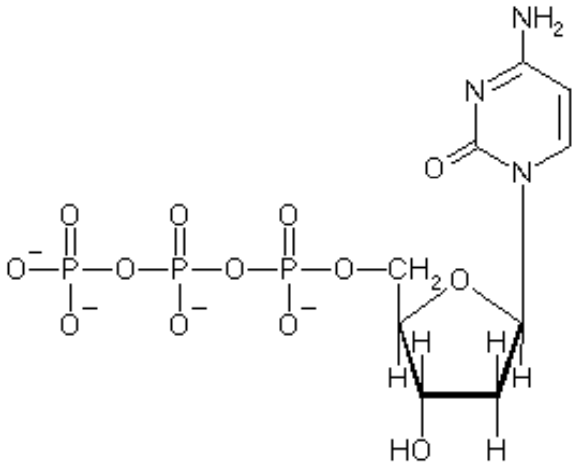


Adenine

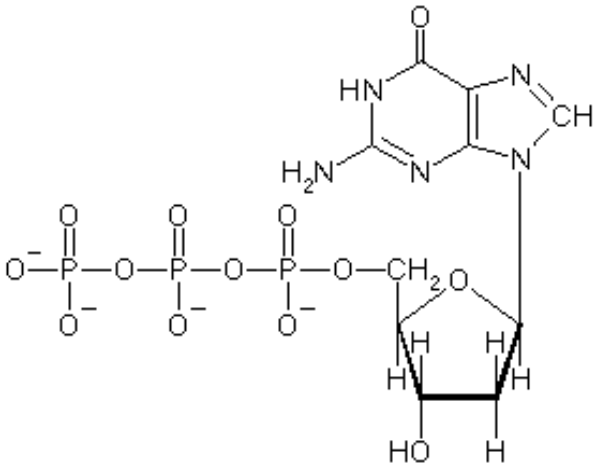
DNA nucleotides



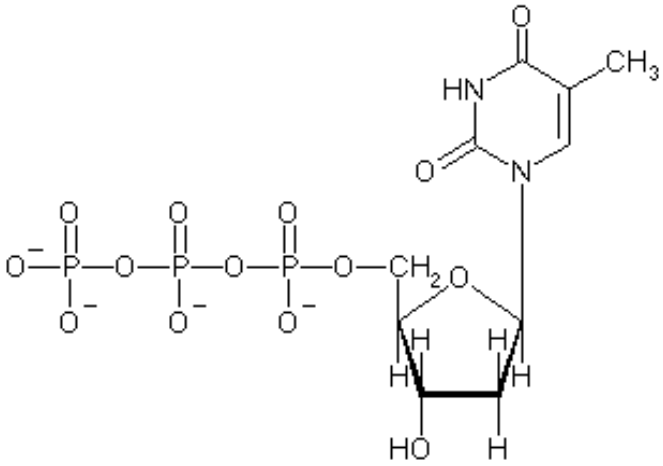
Deoxyadenosine 5'-triphosphate
(dATP)



Deoxycytidine 5'-triphosphate
(dCTP)

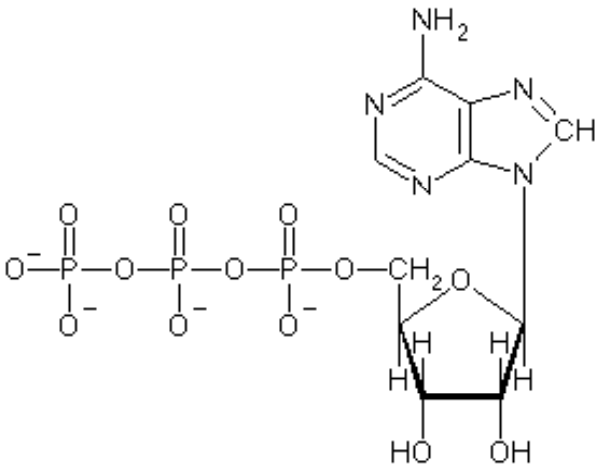


Deoxyguanosine 5'-triphosphate
(dGTP)

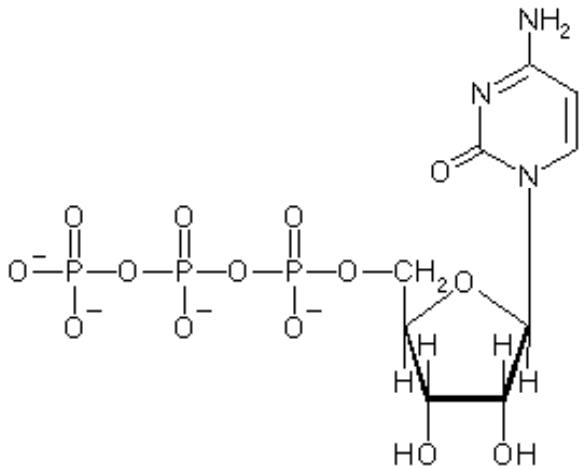


Deoxythymidine 5'-triphosphate
(dTTP)

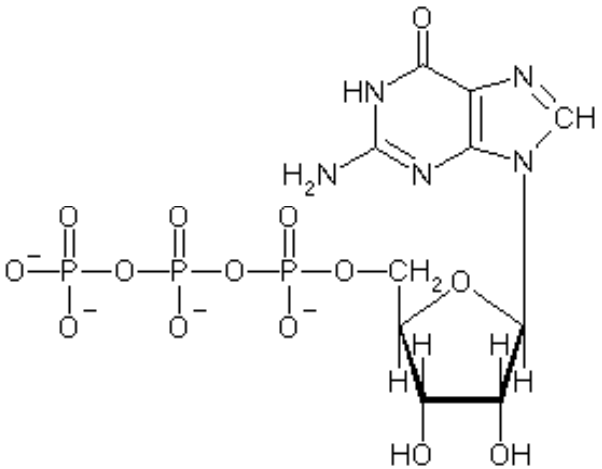
RNA nucleotides



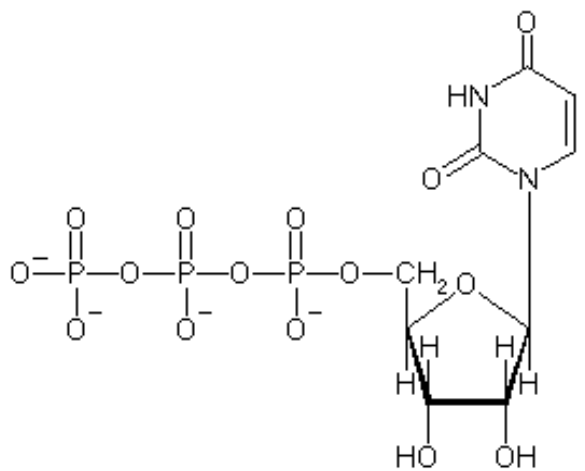
Adenosine 5'-triphosphate
(ATP)



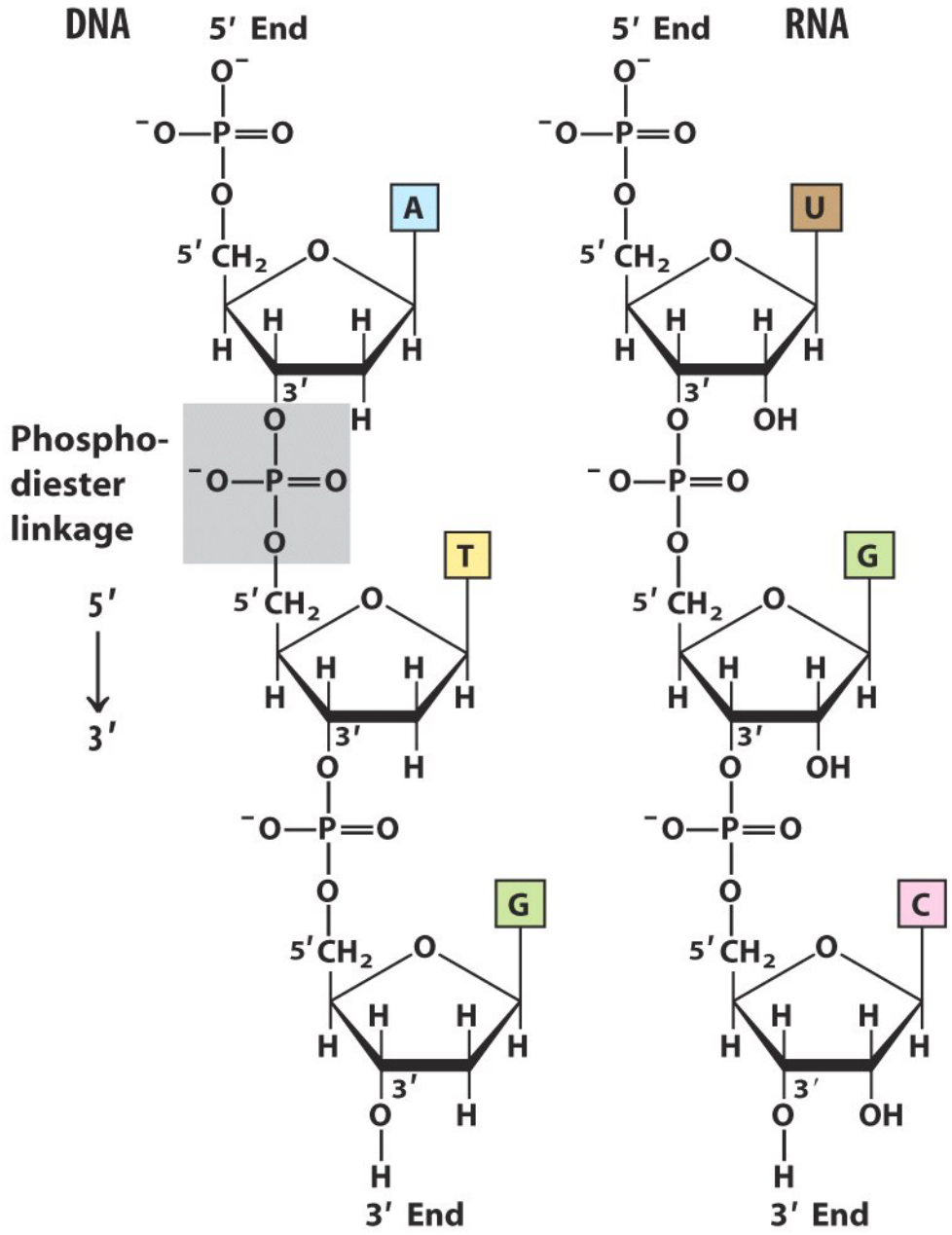
Cytidine 5'-triphosphate
(CTP)



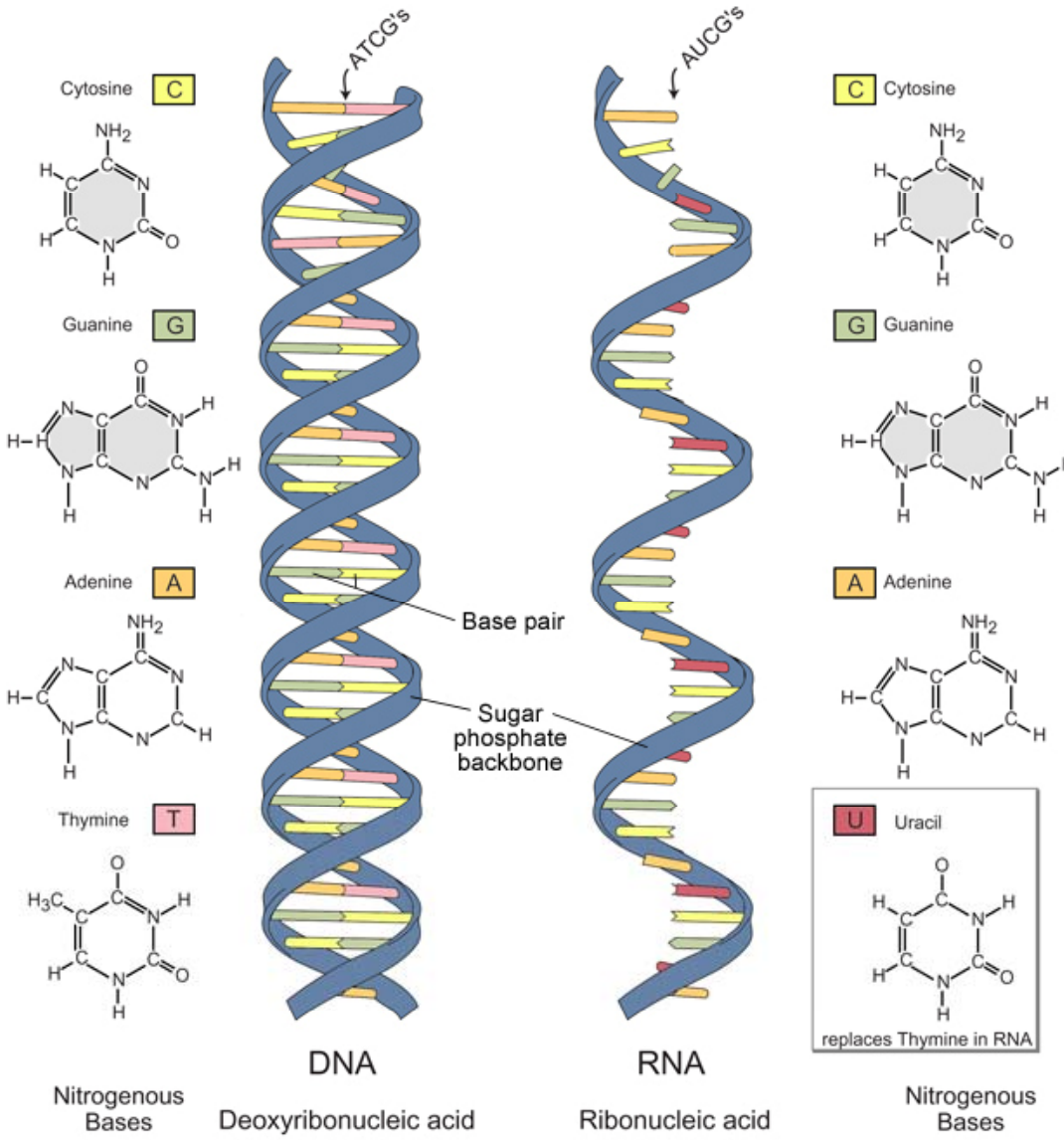
Guanosine 5'-triphosphate
(GTP)



Uridine 5'-triphosphate
(UTP)

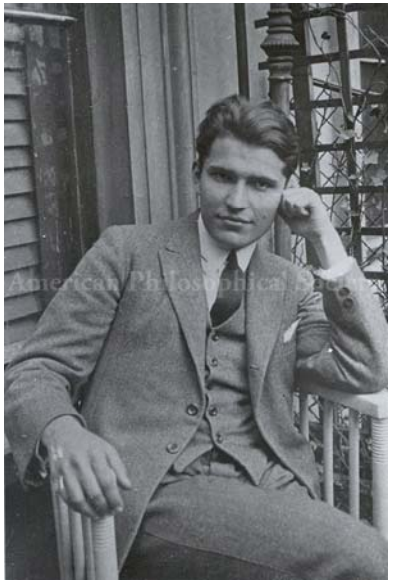

DNA
5′ End
RNA
5′ End
A
U
T
G
G
C
Phospho-
diester
linkage
5′
3′
3′ End
3′ End

Compare DNA and RNA

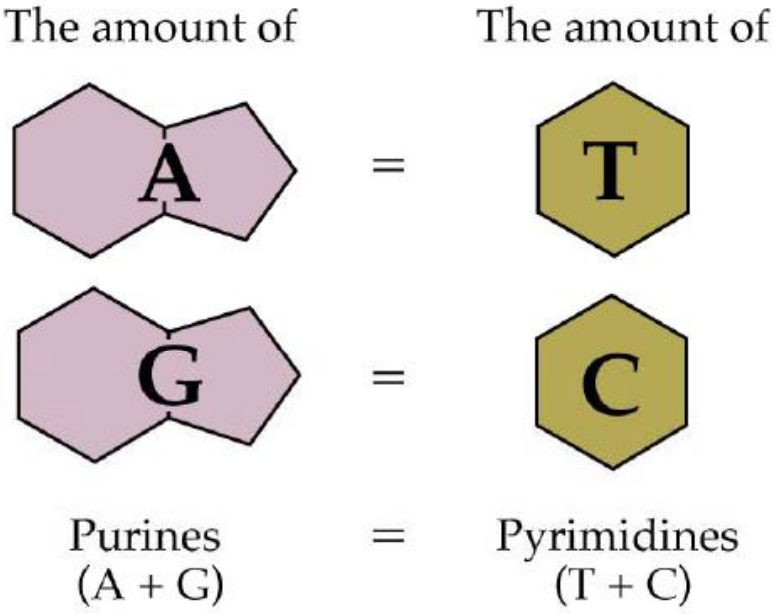


- Why 2' OH in RNA?
- Why is RNA single stranded?
- *Is* RNA always single stranded?
- Why Uracil instead of Thymine in RNA?

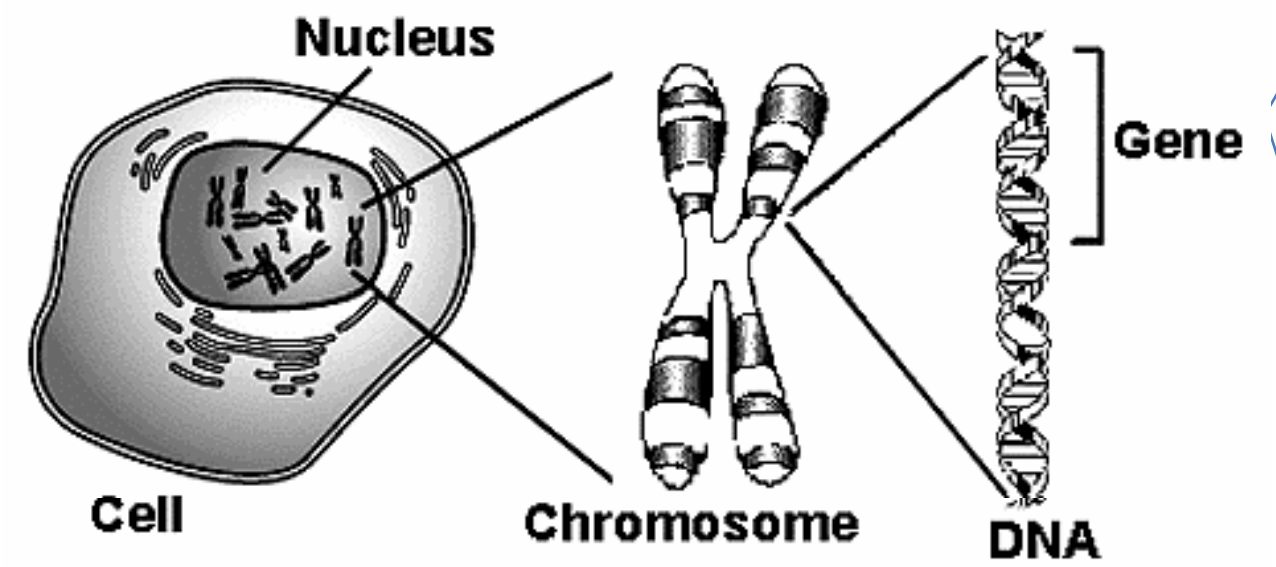
Chargaff's Rules

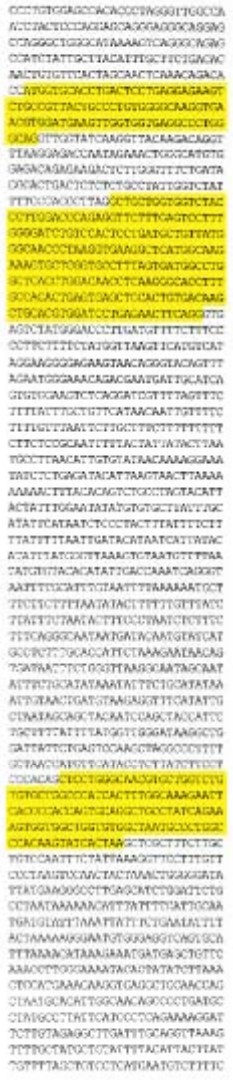


- Base composition varies among species
- BUT: there are *regularities.*
- The amount of guanine always equals the amount of cytosine.
- The amount of thymine always equals the amount of adenine.
- The amount of adenine + guanine = 50% of the total. (So 50% of the bases are purines).
- The amount of cytosine + thymine = 50% of the total. (So 50% of the bases are pyrimidines).

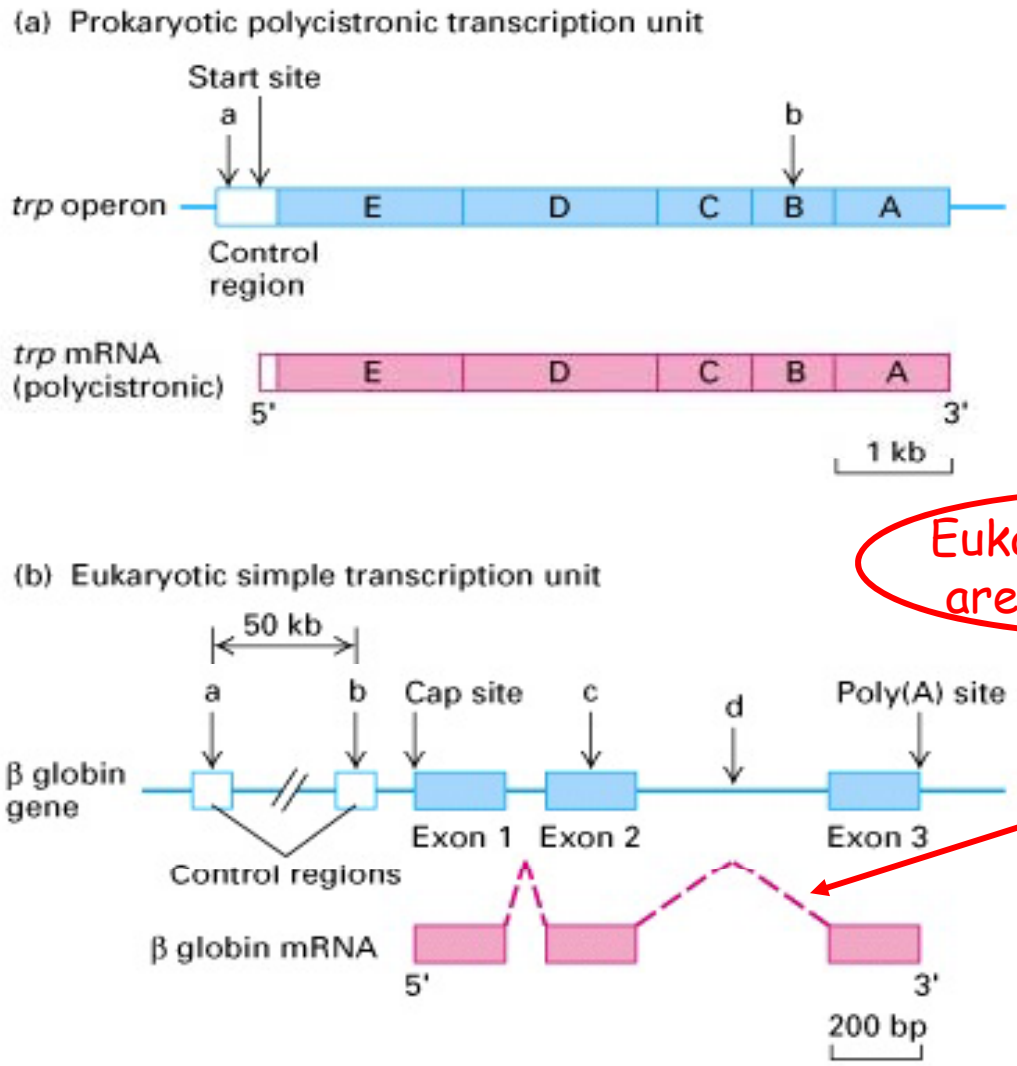


DNA function: the genetic blueprint





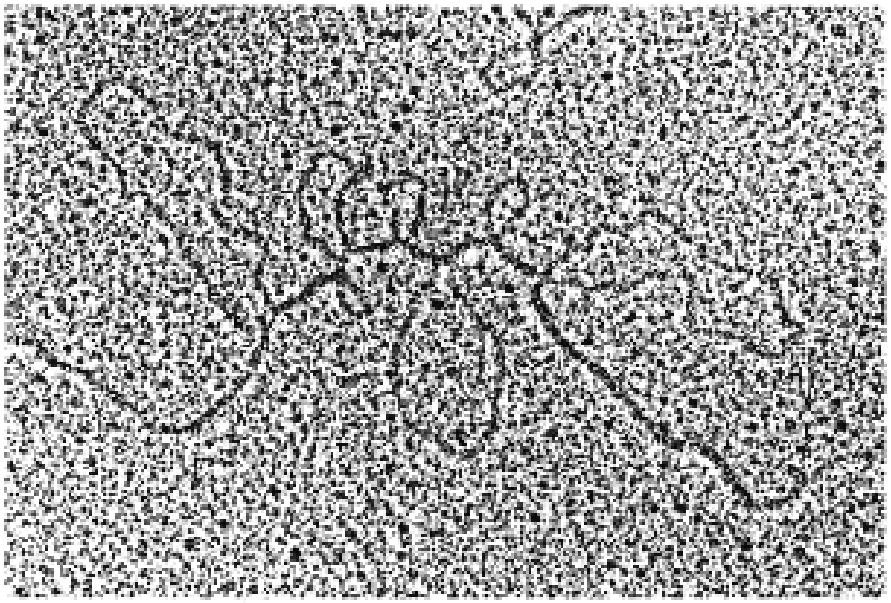
Prokaryotic vs. eukaryotic gene organization



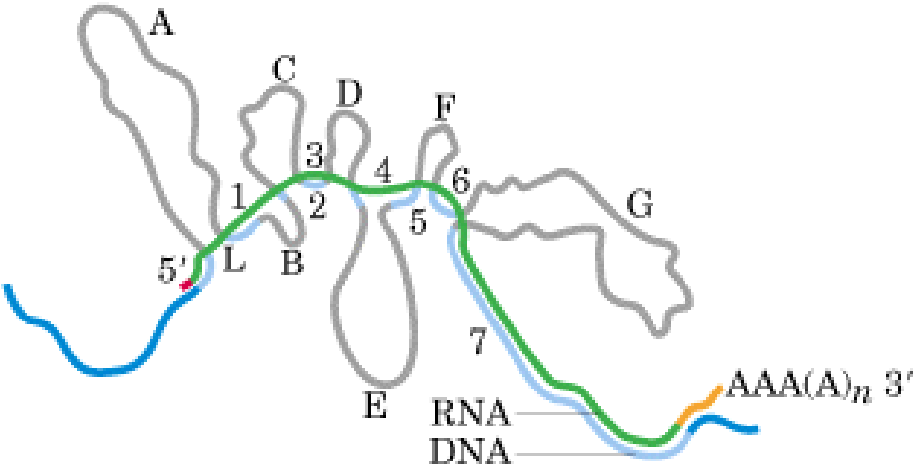
Polycistronic units are rare in eukaryotes

Eukaryotic genes are *interrupted*

Eukaryotic genes* have *introns* and *exons*



(a)

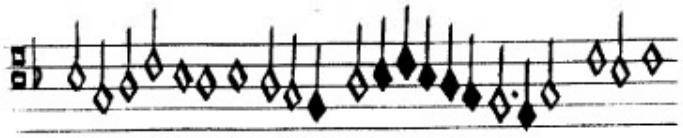


(b)

*A few Bacteria and Archaea have introns, too!

DNA function: the genetic blueprint

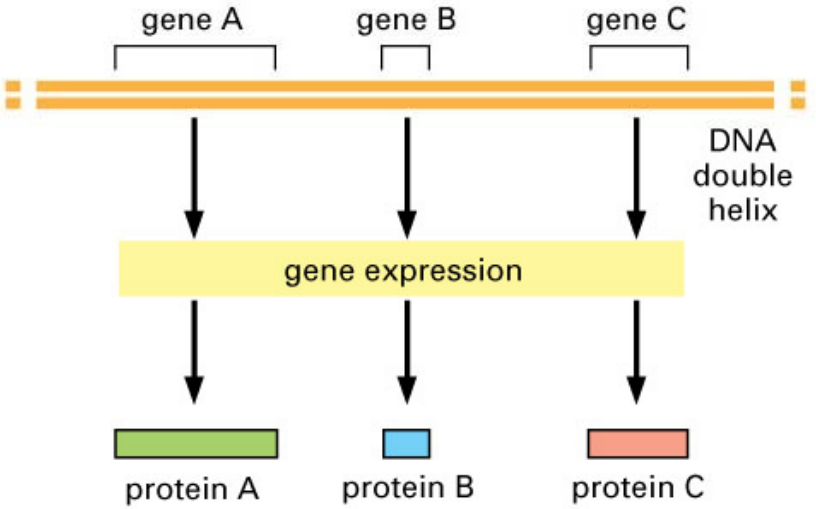
(A) molecular biology is....

(B) 

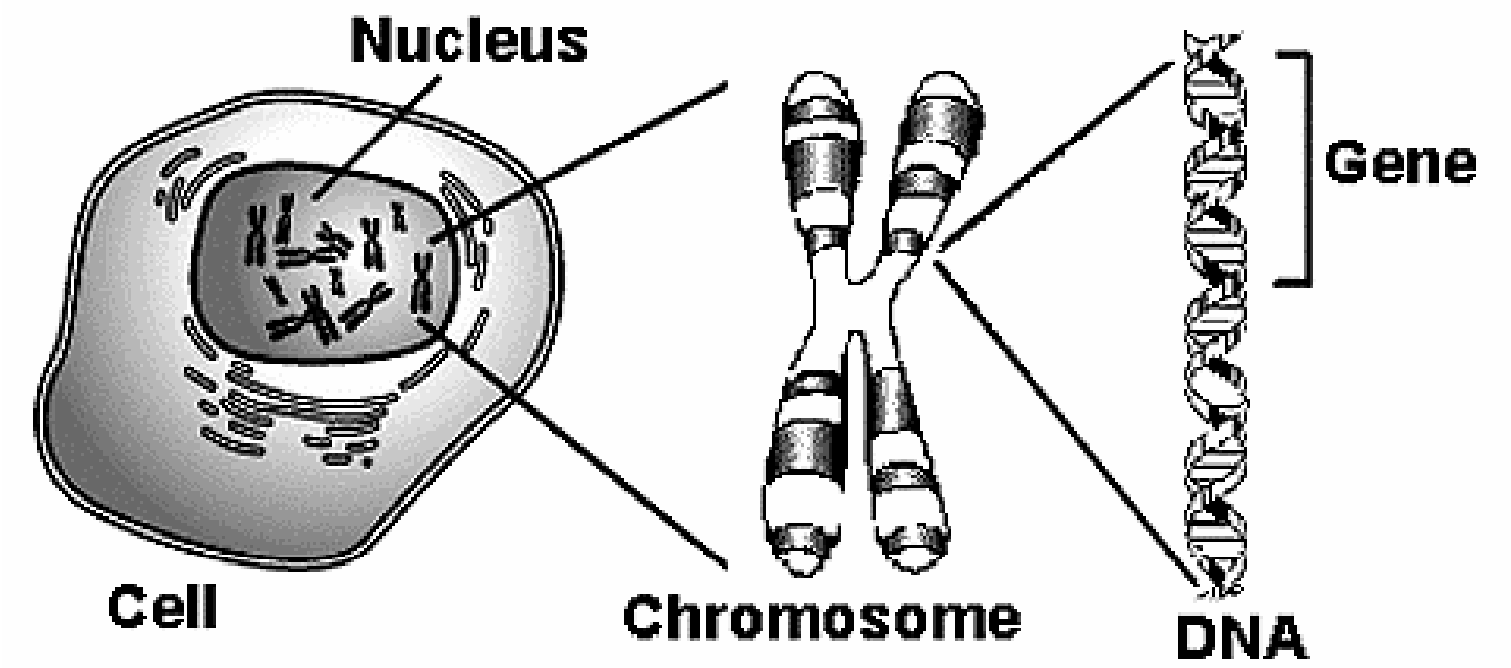
(C) •— •—•• •• —•—• •

(D) 乡细胞生物莫乐趣无穷

(E) TTCGAGCGACCTAACCTATAG



Genes, genomes, chromosomes, DNA How do they all connect????

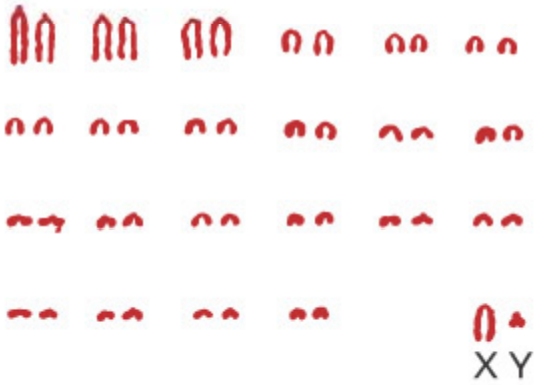


Genes, genomes, chromosomes, DNA

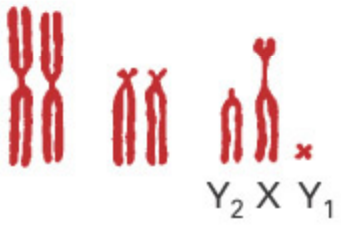
- The genome is the total information content represented by a single set (n) of chromosomes.
- Genome organization = the way in which this information is broken up and distributed over the chromosomes.
- Information includes genes which are sequences of DNA located at specific positions along the chromosomes.

Genome organization

Chinese muntjac



Indian muntjac



Genomes


Science
24 March 2000
The Drosophila Genome
nature
14 December 2000
International weekly journal of science
www.nature.com
Arabidopsis thaliana genome sequence
BSE in France
Infected beef in the food chain?
Fluid dynamics
The physics of flapping
Coral bleaching
Shielded by the glow
nature
STAR FORMATION
A massive protostar unveiled
CANCER IMMUNOLOGY
How tumours dupe T cells
AIR POLLUTION
China's NO2 build-up seen from space
NATUREJOBS
Membrane proteomics
THE CHIMPANZEE GENOME
Science
THE HUMAN GENOME
Science
11 December 1998
C. elegans
Sequence to Biology
nature
9 December 2004
International weekly journal of science
www.nature.com/nature
The chicken genome
Cracking the code
Stem-cell research
The religious dimension
Quantum physics
Exciton times
Hair-cell trigger
The channel for sound
nature
The mouse genome
Science
The RICE Genome

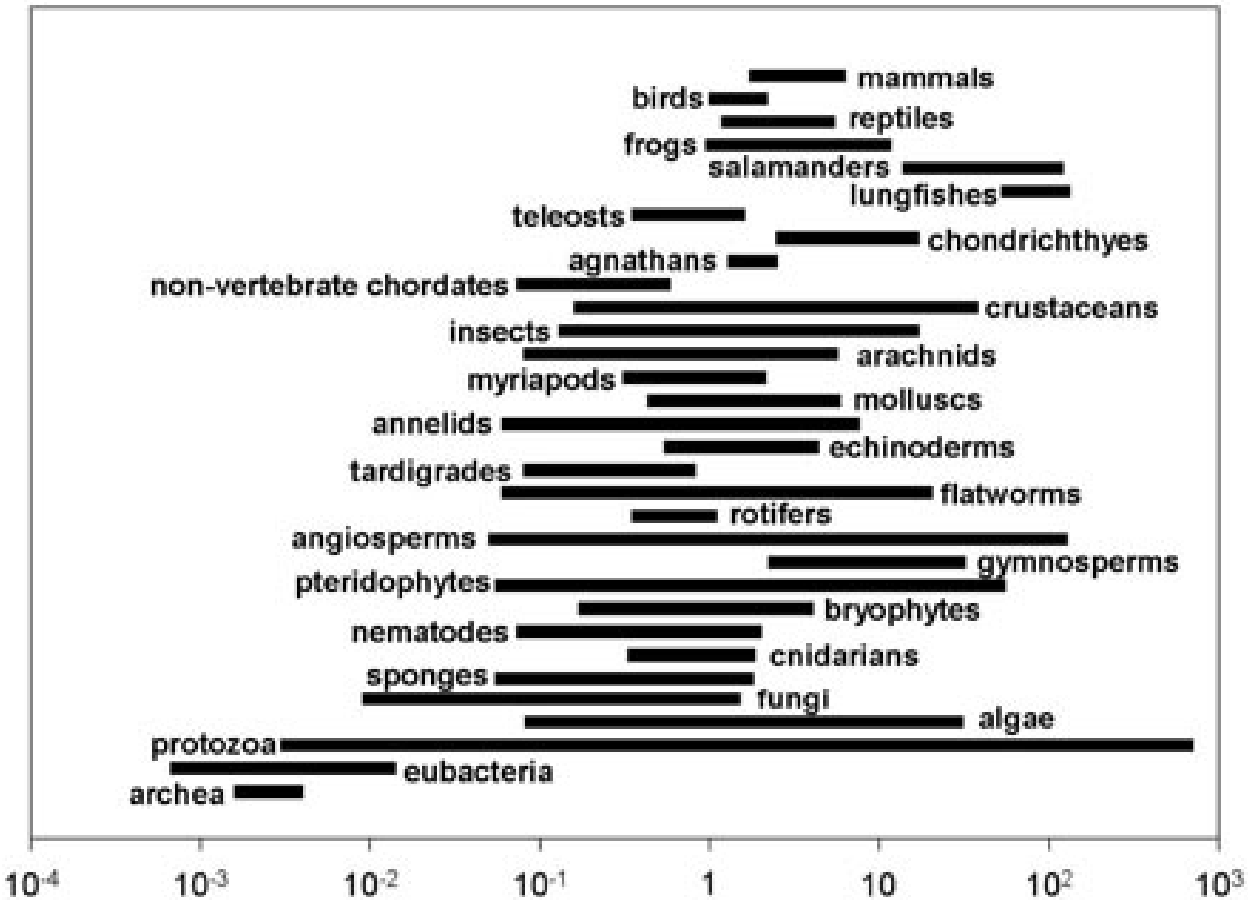
Comparison of gene number

	Species	Chromosomes	Genes	Base pairs
	**Human** *(Homo sapiens)*	46 (23 pairs)	28-35,000	3.1 billion
	**Mouse** *(Mus musculus)*	40	22.5-30,000	2.7 billion
	**Puffer fish** *(Fugu rubripes)*	44	31,000	365 million
	**Malaria mosquito** *(Anopheles gambiae)*	6	14,000	289 million
	**Fruit fly** *(Drosophila melanogaster)*	8	14,000	137 million
	**Roundworm** *(C. elegans)*	12	19,000	97 million
	**Bacterium** * *(E. coli)*	1	5,000	4.1 million

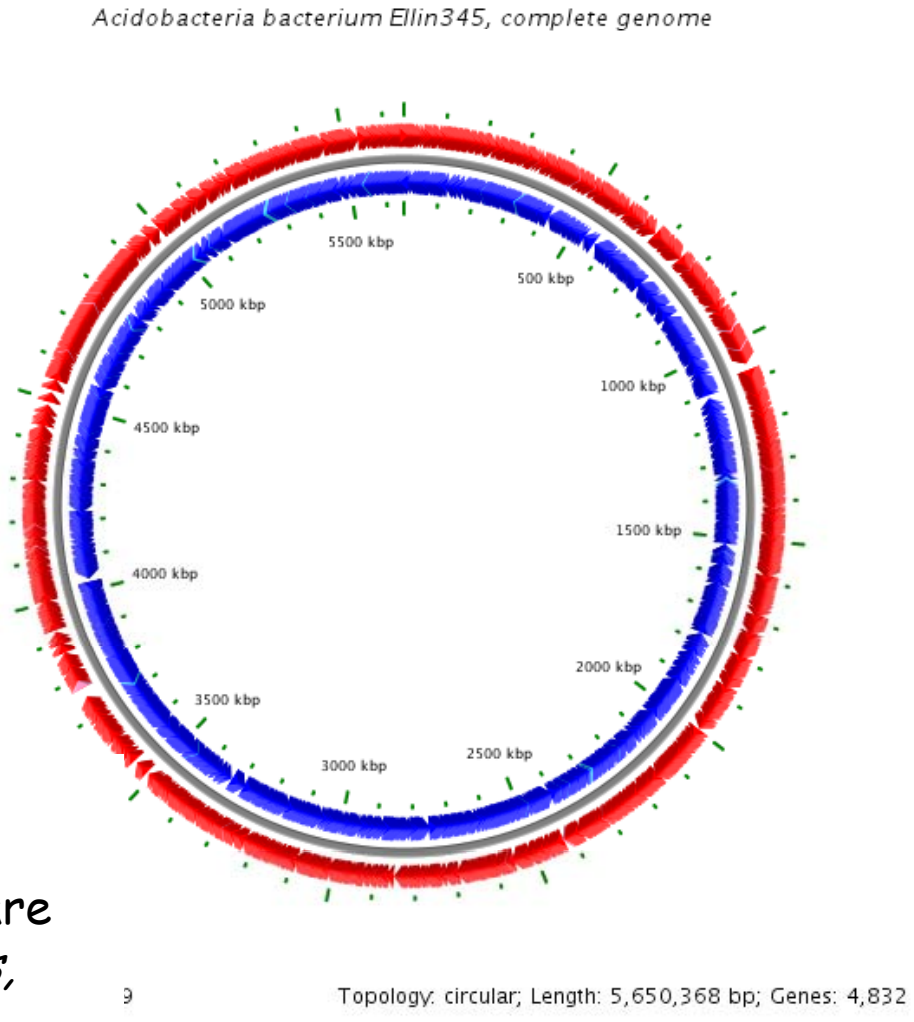
*Bacterial chromosomes are chromonemes, not true chromosomes

JOHN BLANCHARD / *The Chronicle*

Comparison of genome sizes

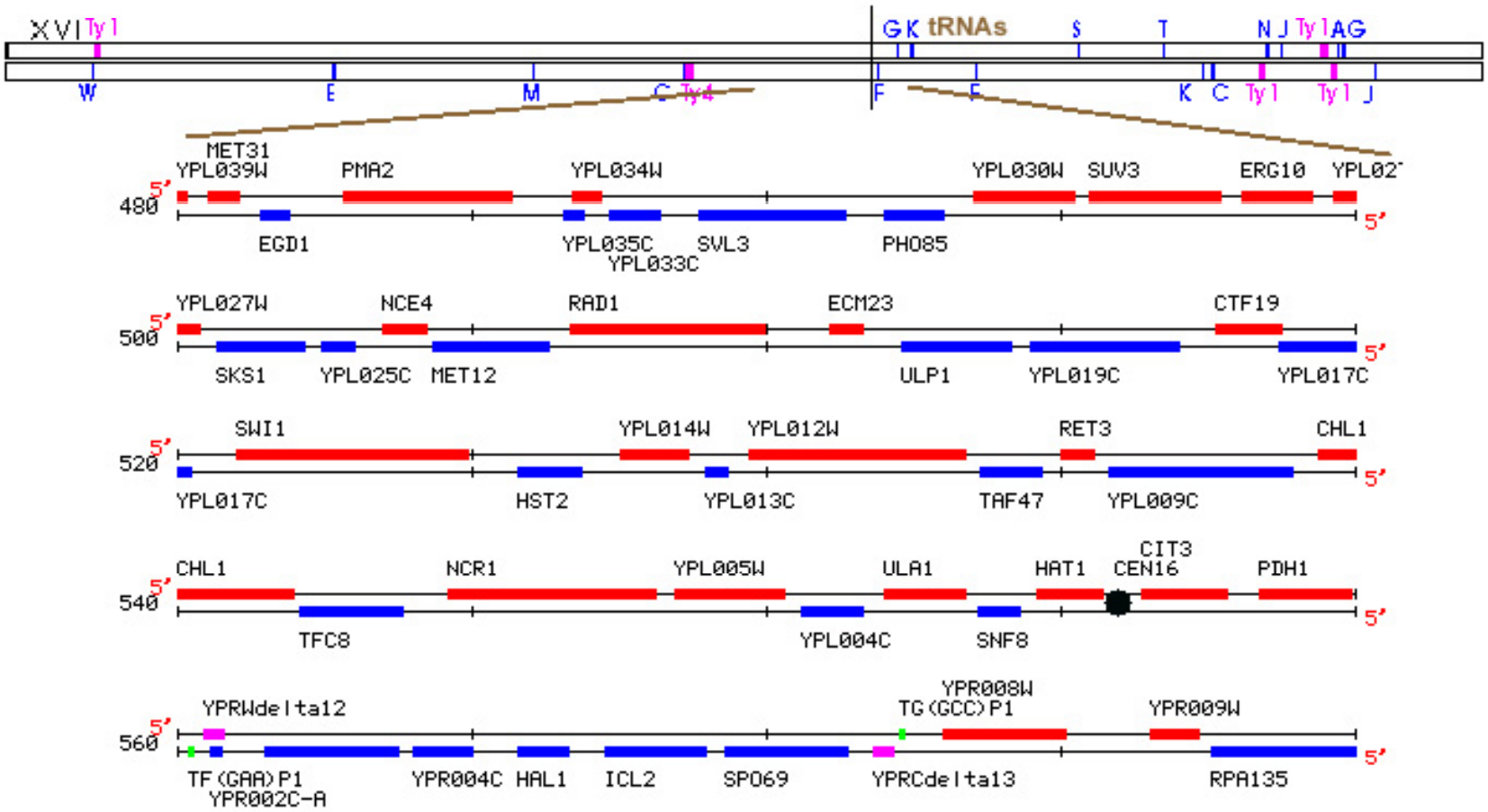


Prokaryotic genomes are very economically organized!



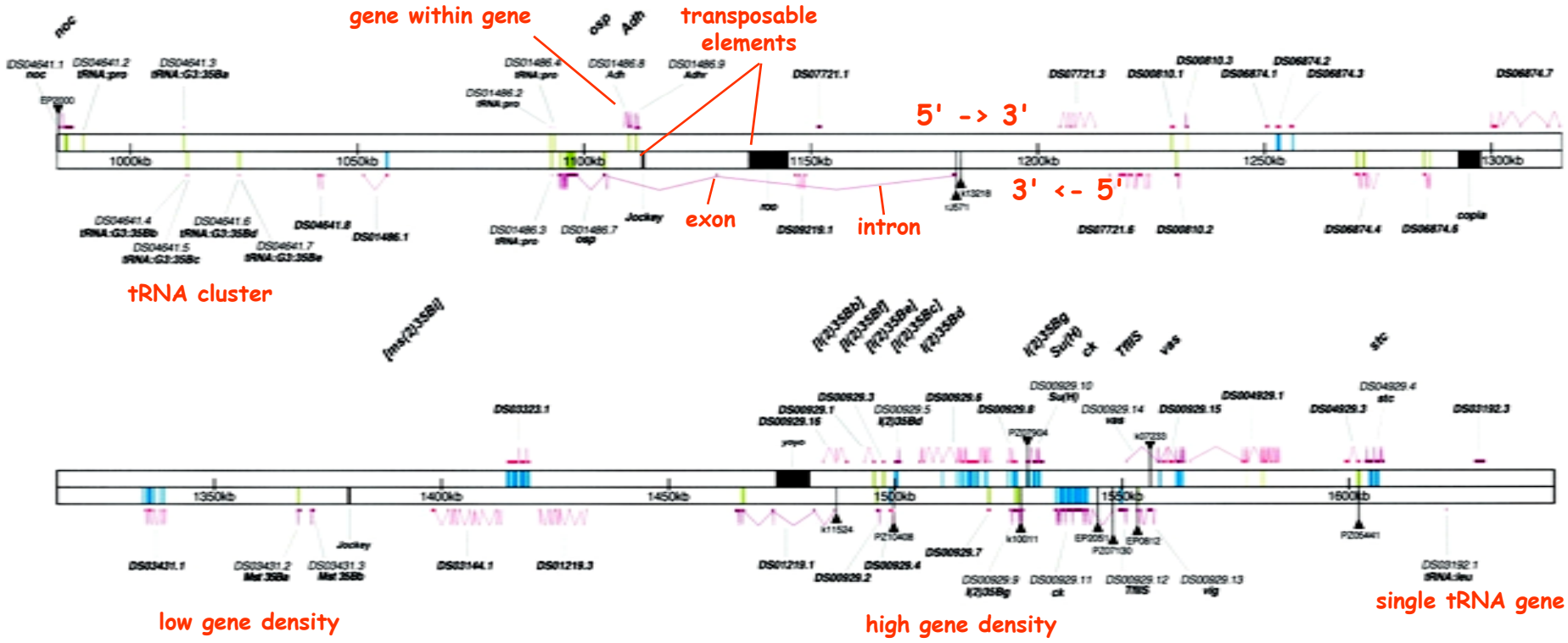
Bacterial and Archaeal chromosomes are usually circular, but sometimes are linear (e.g. *Streptomyces, Agrobacterium*).

Some unicellular eukaryotic genomes are economically organized too.



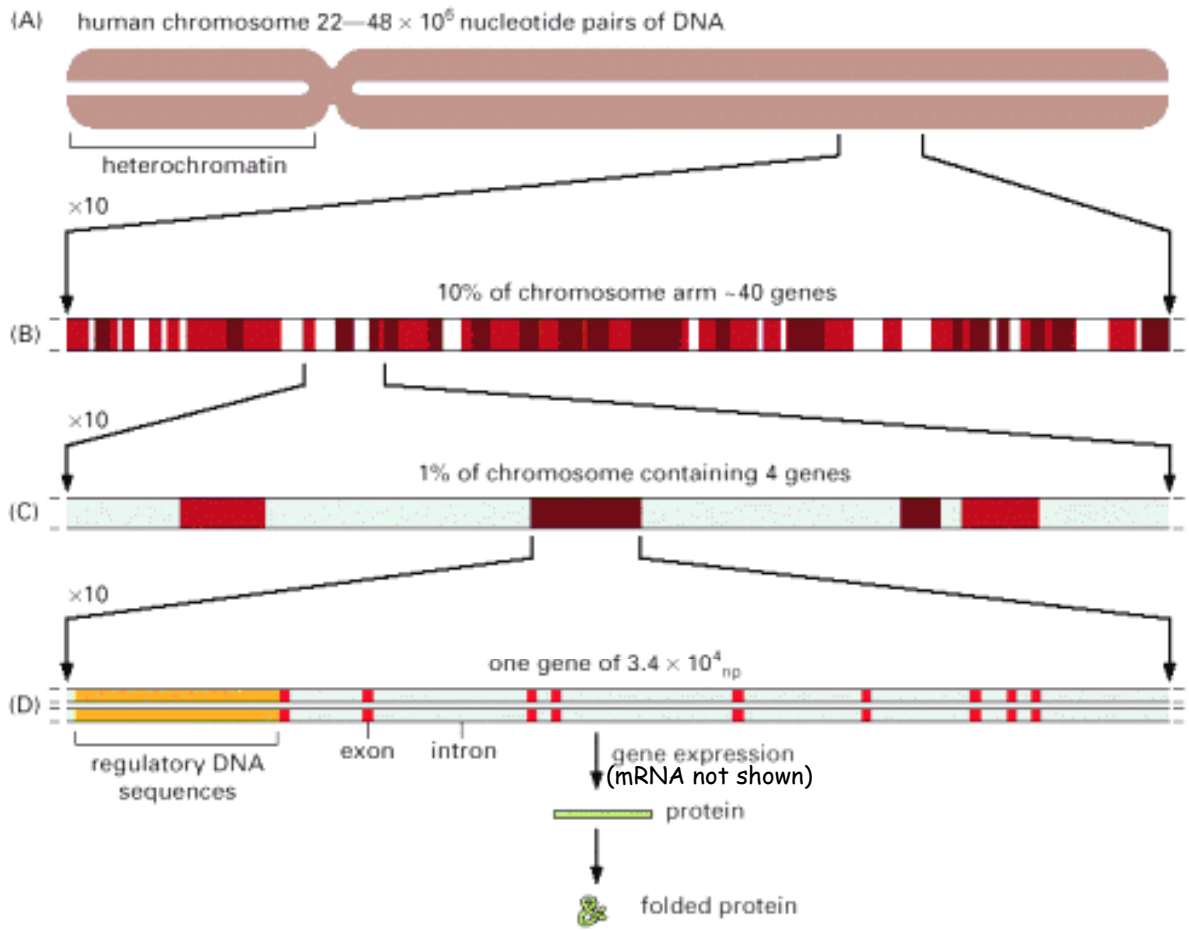
Section of chromosome 16 from *S. cerevisiae*

Eukaryotes tend to have very spacious genomes.

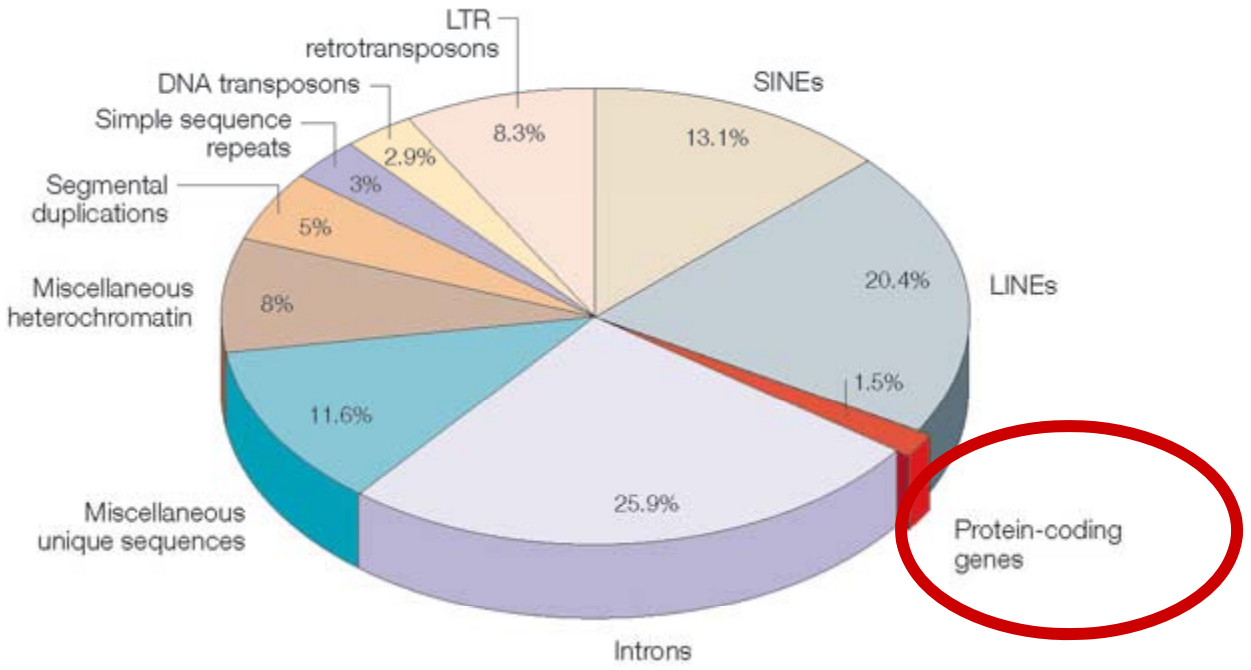


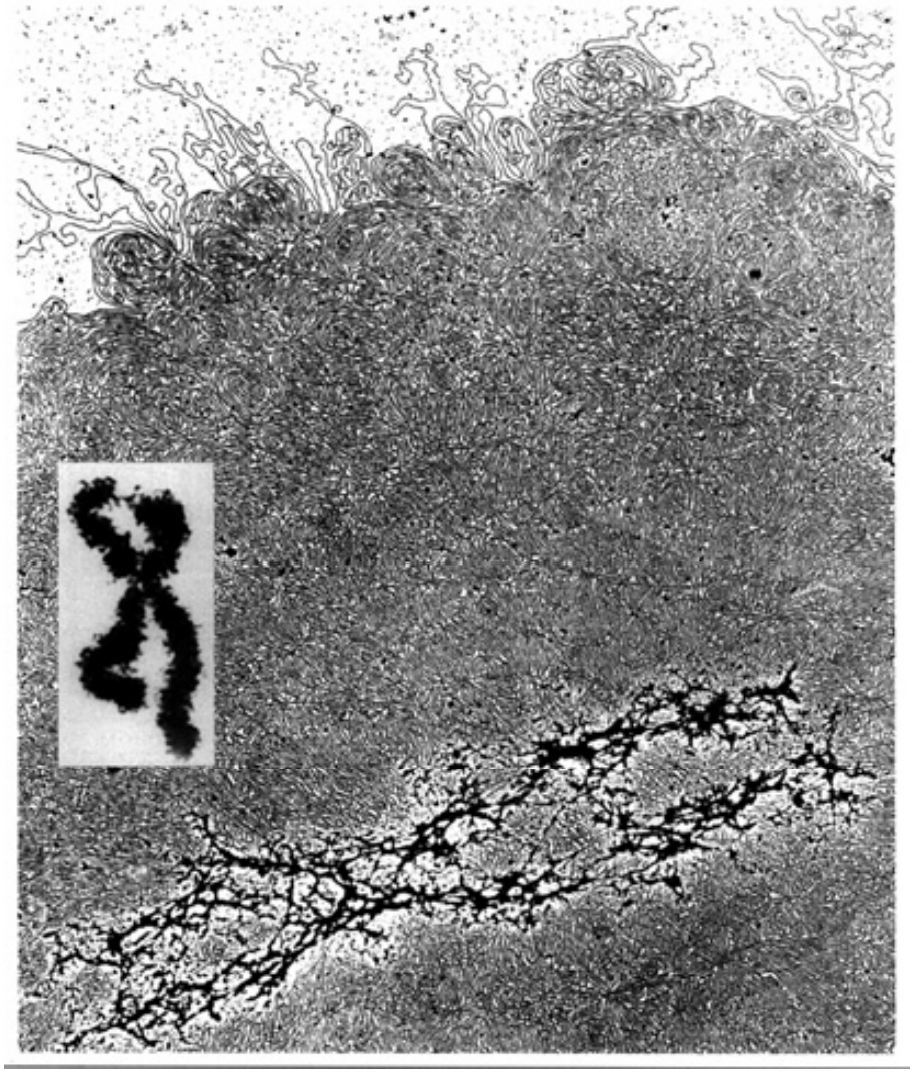
600,000 bp of *Drosophila* chromosome *2*

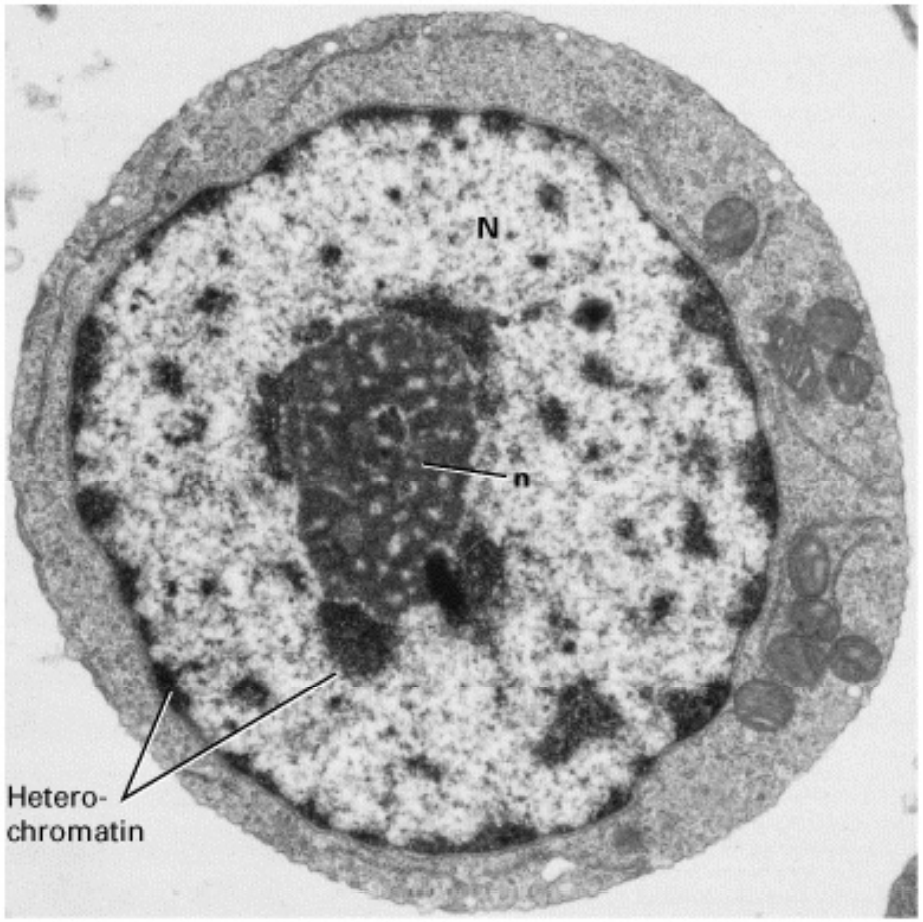
Eukaryotes tend to have very spacious genomes.



Eukaryotic genomes consist of a LOT of non-coding DNA!

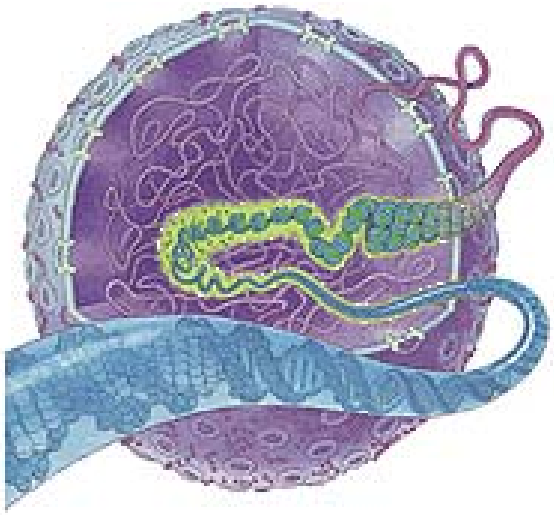


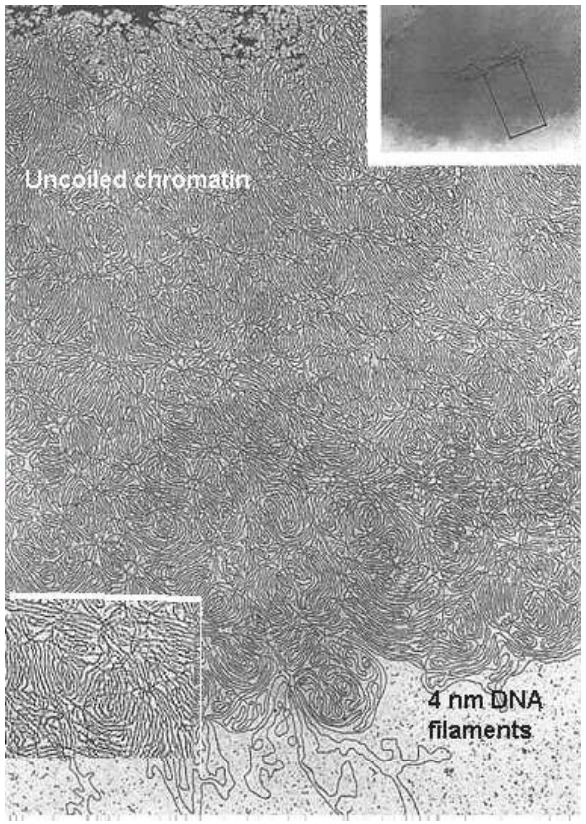


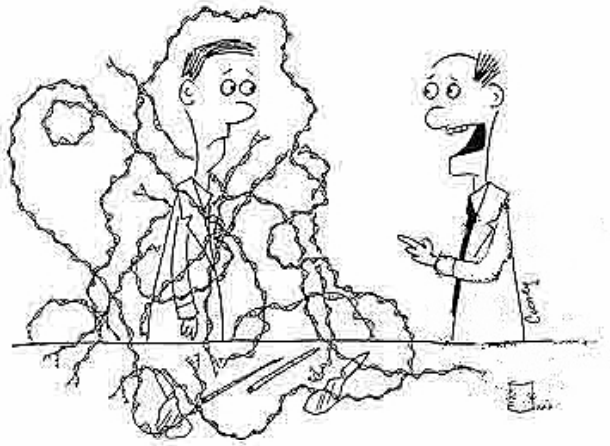


1 μm

Nuclei have a BIG packaging problem







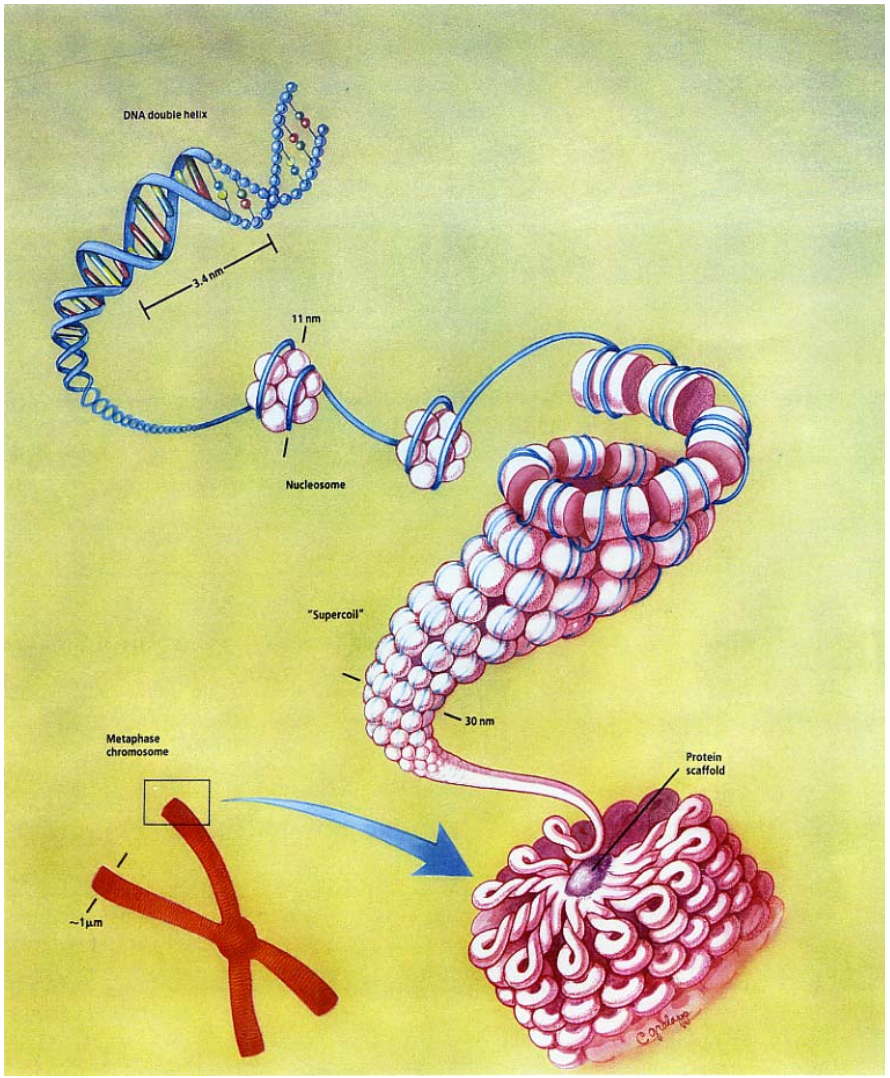
"From now on, take the Human Genome outside before you unravel it."

- How do you fit approximately 2 meters (human diploid nucleus) into a space that averages maybe 5 millionths (5 um) of a meter wide?
- How do you replicate, repair and transcribe tightly packaged DNA?

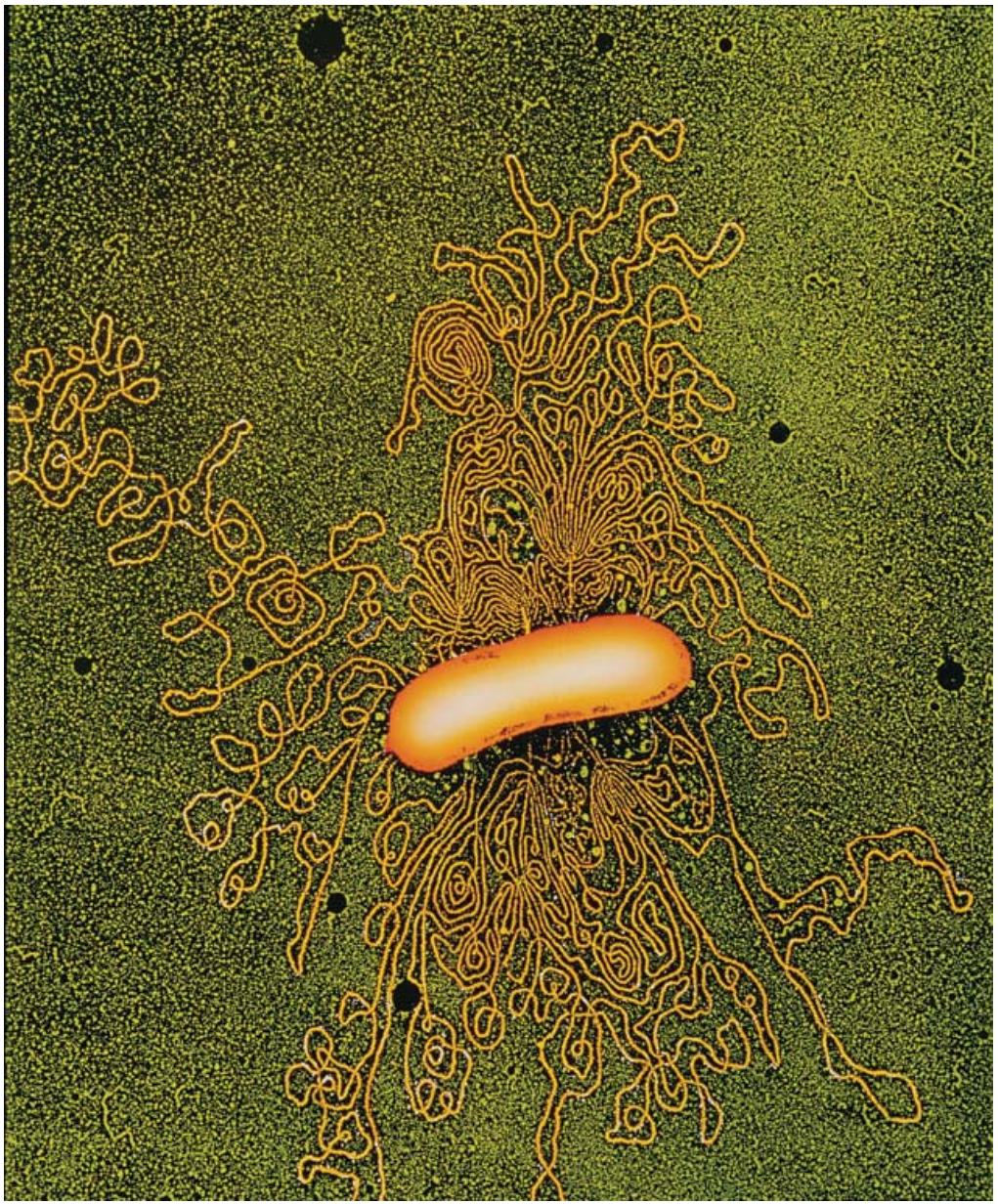
Solution: Chromatin!

- Chromatin is DNA packaged with specialized proteins (and even some RNA!) that serve to control the degree to which DNA sequences are accessible for synthesis and transcription

- These proteins include specialized structural proteins and enzymes



Bacteria have no nucleus, but also share problem: how to fit 1 mm long chromosome into a 1 uM wide cell?



Histone-like proteins!

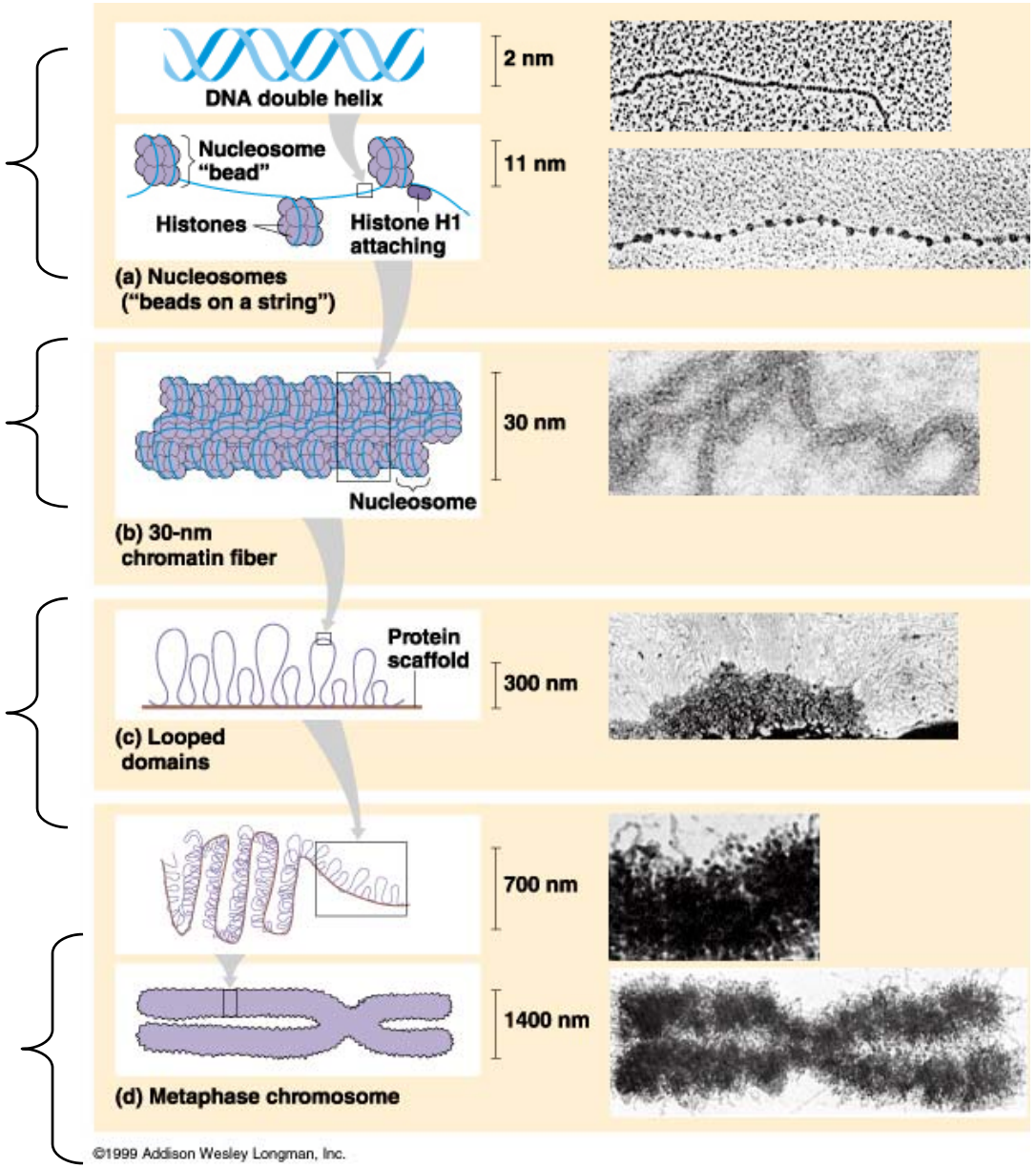
Chromatin packaging hierarchy

Level 1:
nucleosome formation

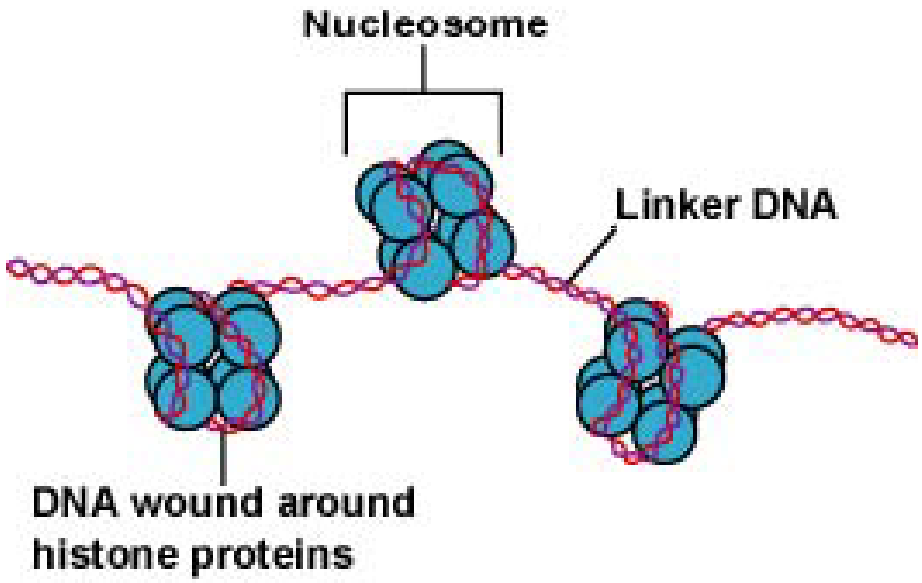
Level 2:
30 nm fiber

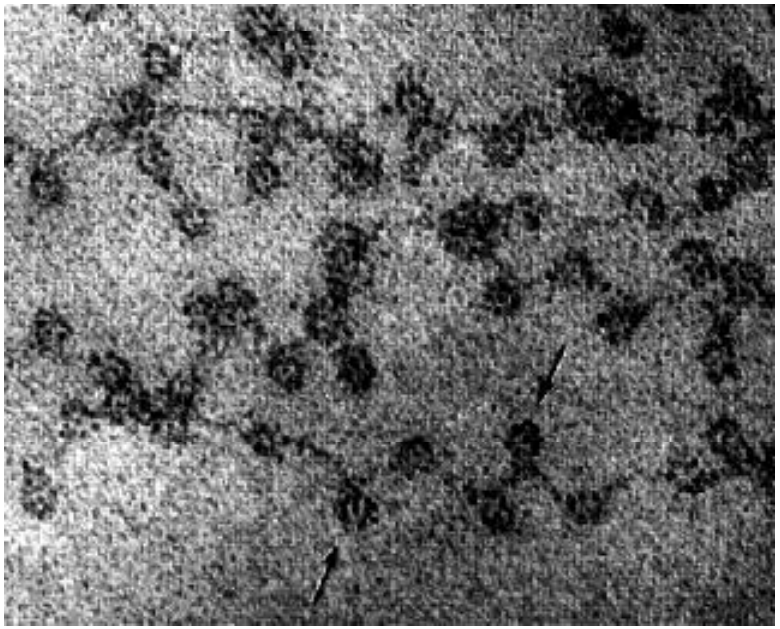
Level 3:
Nuclear scaffolding

Level 4:
Mitotic (metaphase)
chromosome



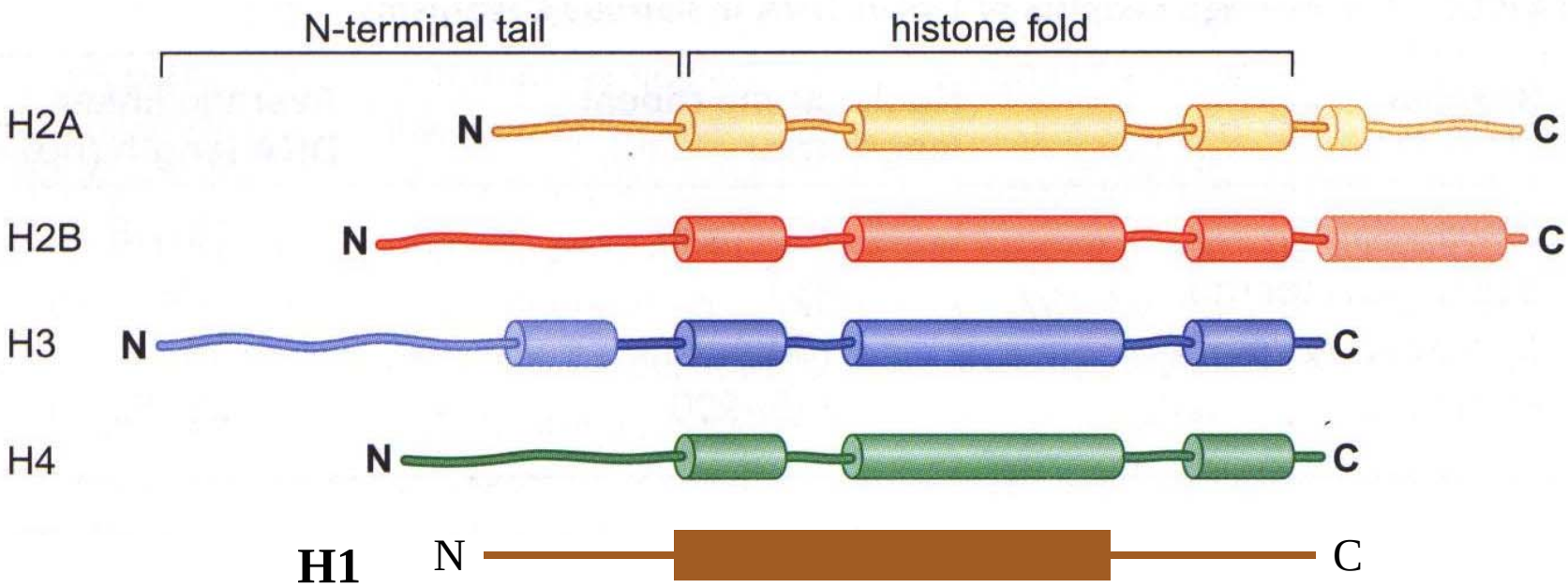
Level One: Building blocks of chromatin: nucleosomes





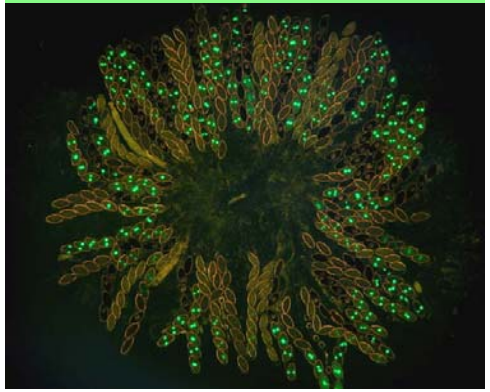
- "string on a bead" for obvious reasons
- Linker can vary (8-114 bp or more)
- Compaction ratio is approx. 7 fold

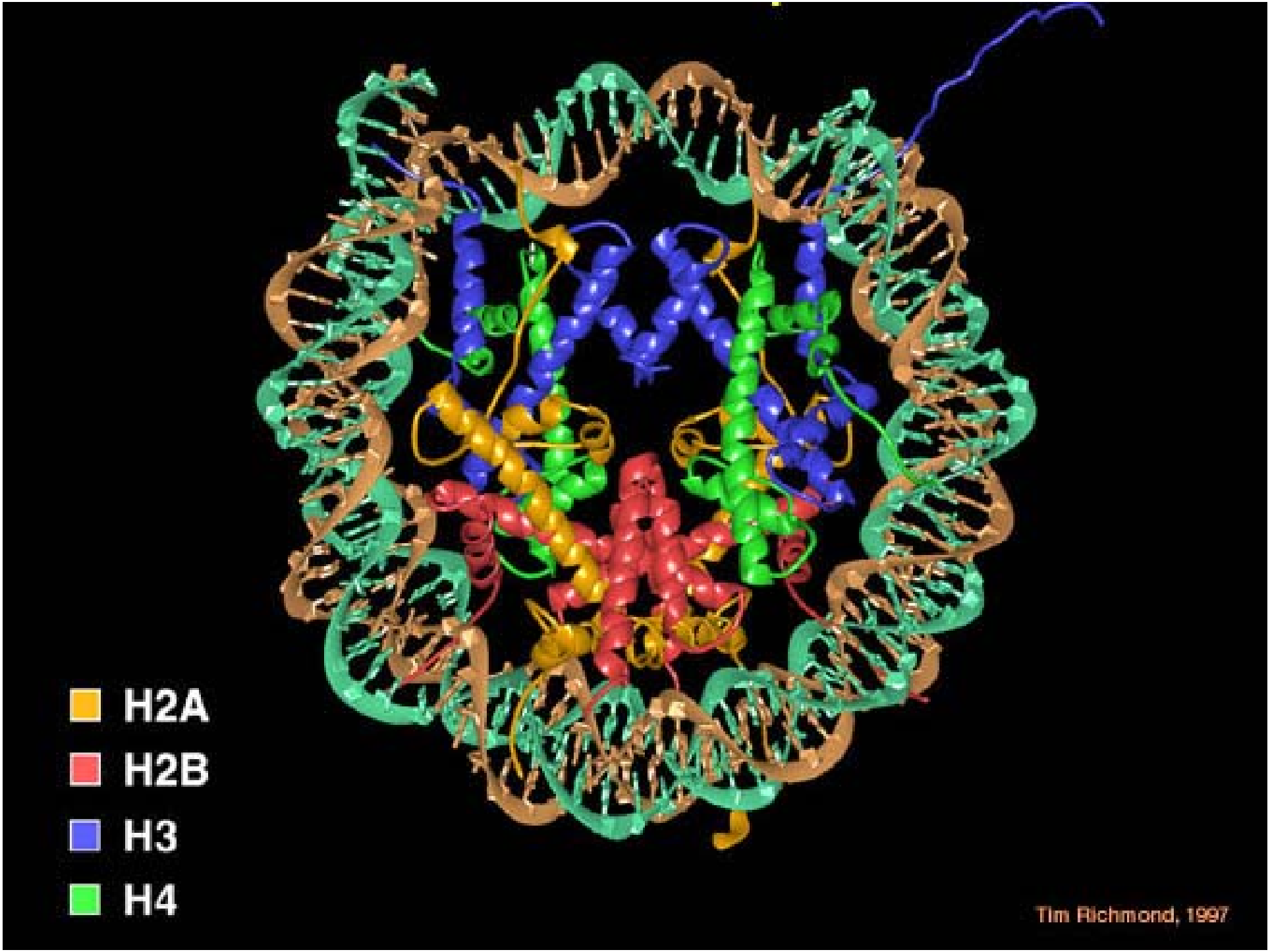
Nucleosomes are composed of histones

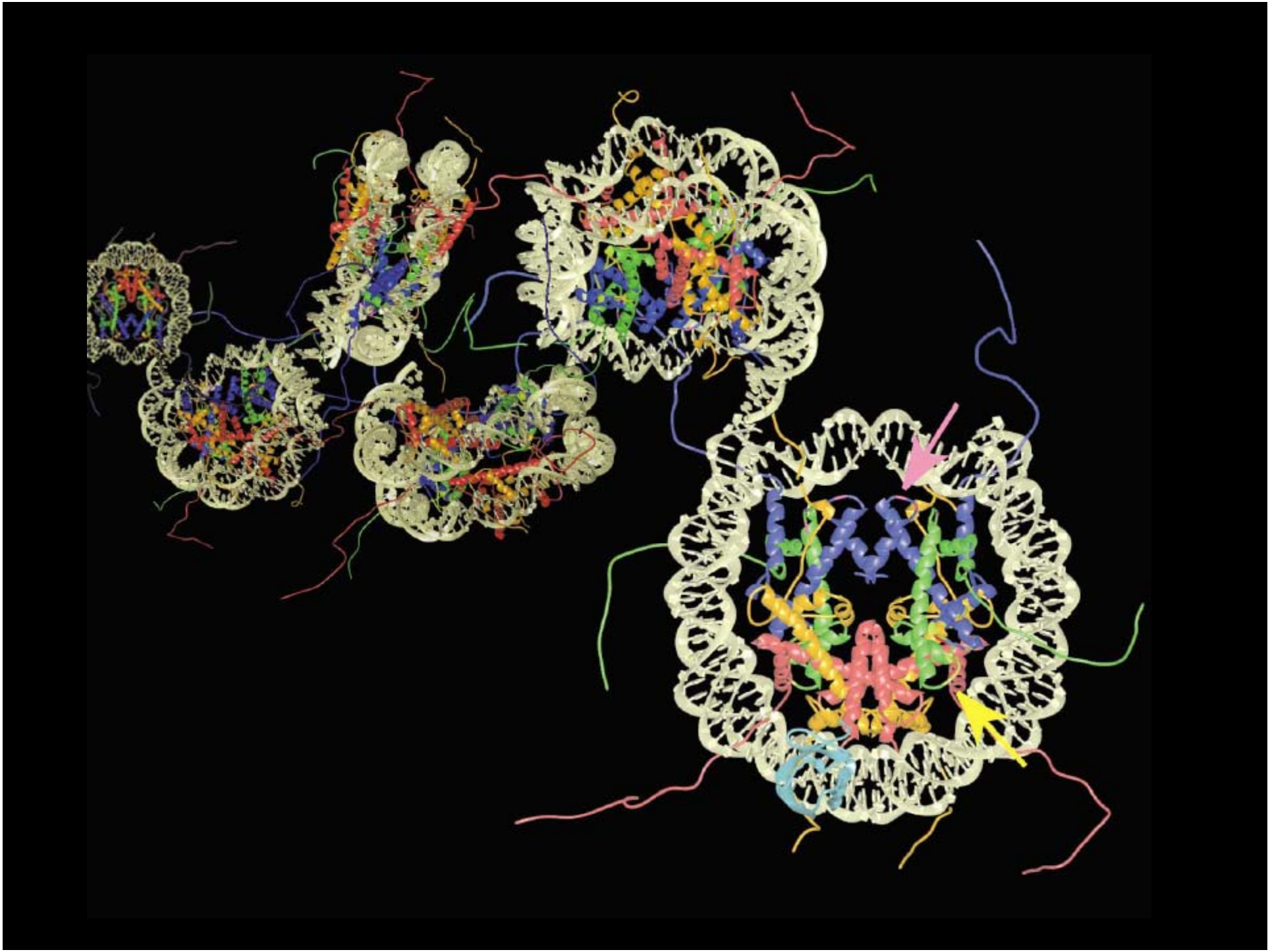


- 2/3 of chromatin mass is protein
- 95% of chromatin protein are histones
- H1, H2A, H2B, H3, H4

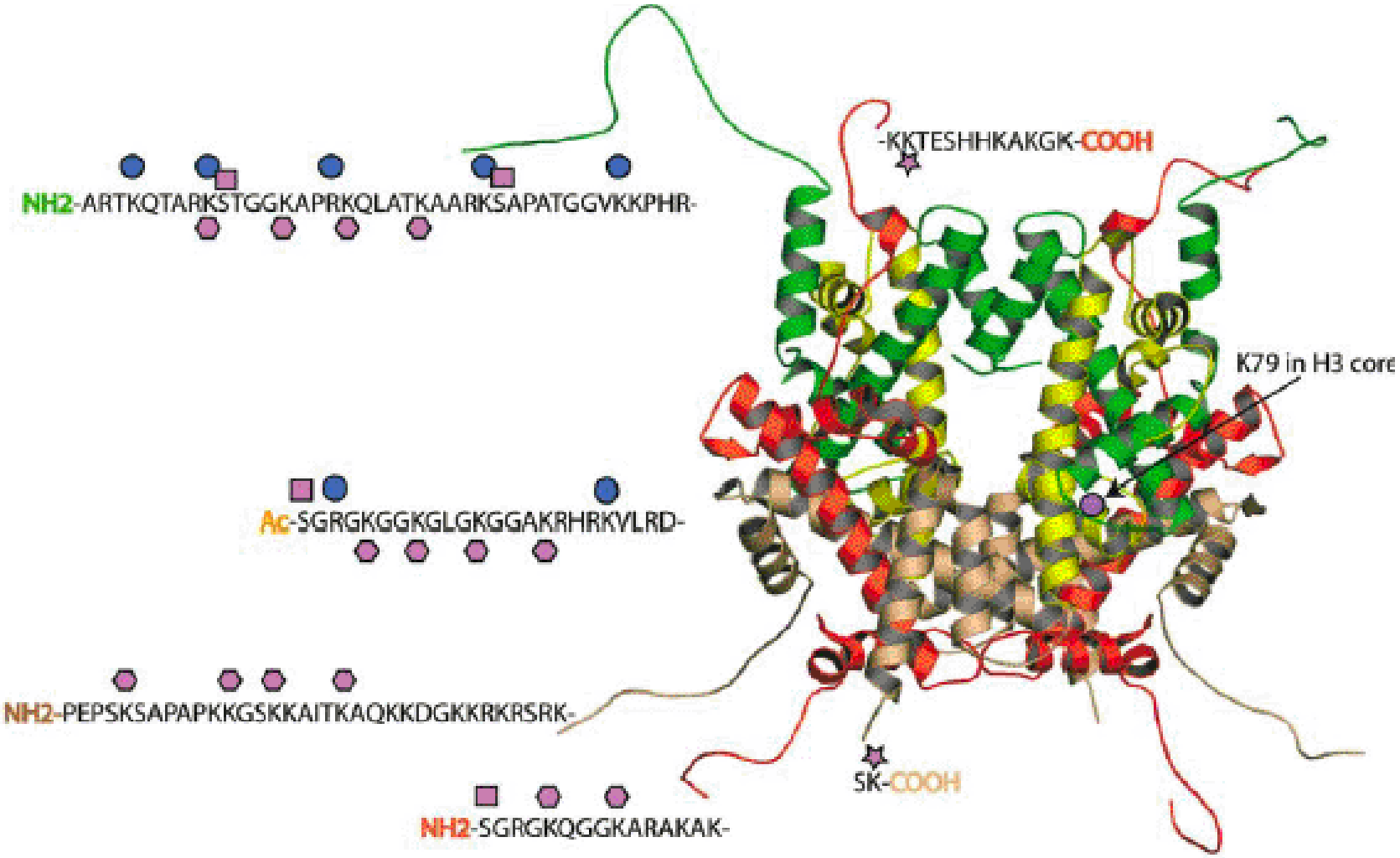
H1::GFP in *N. crassa*




H2A
H2B
H3
H4
Tim Richmond, 1997



Histone modifications



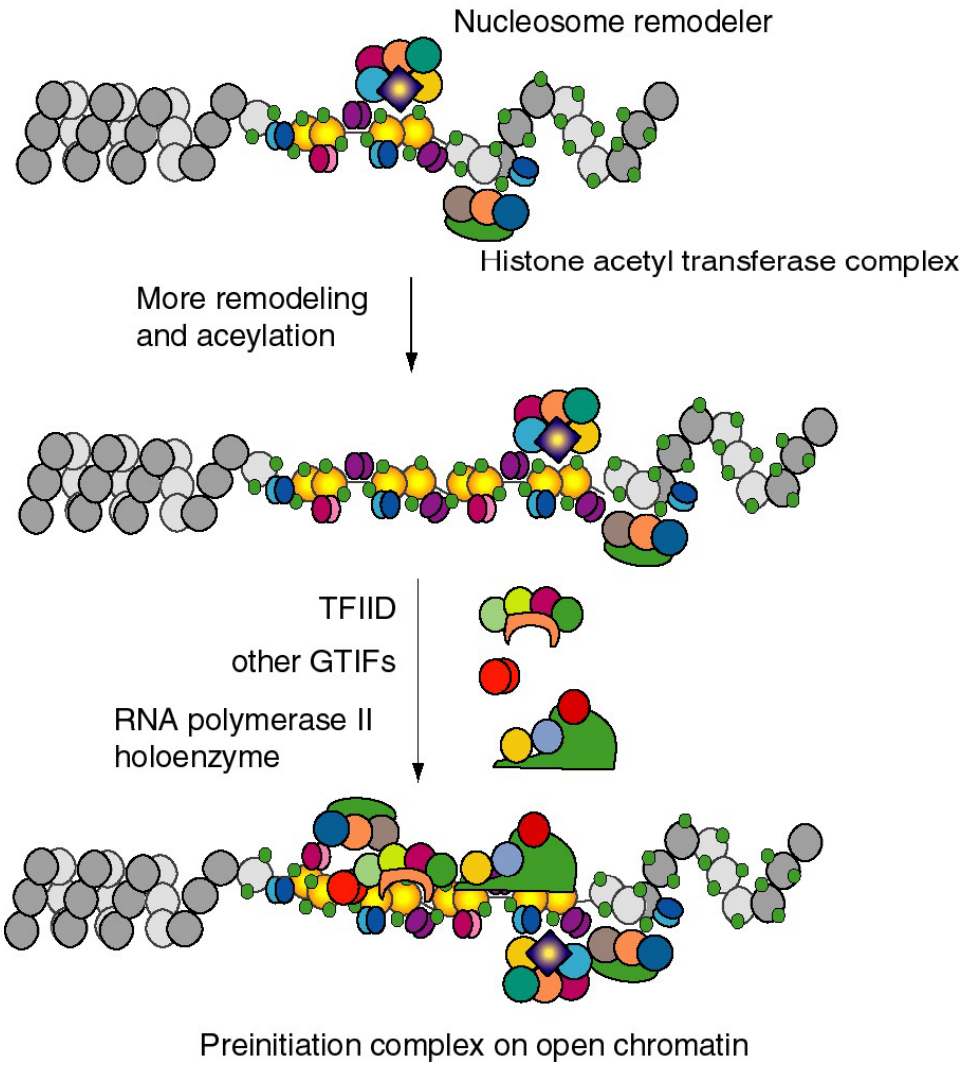
From: Khorasanizadeh, 2004.

Level Two: the 30nm fiber

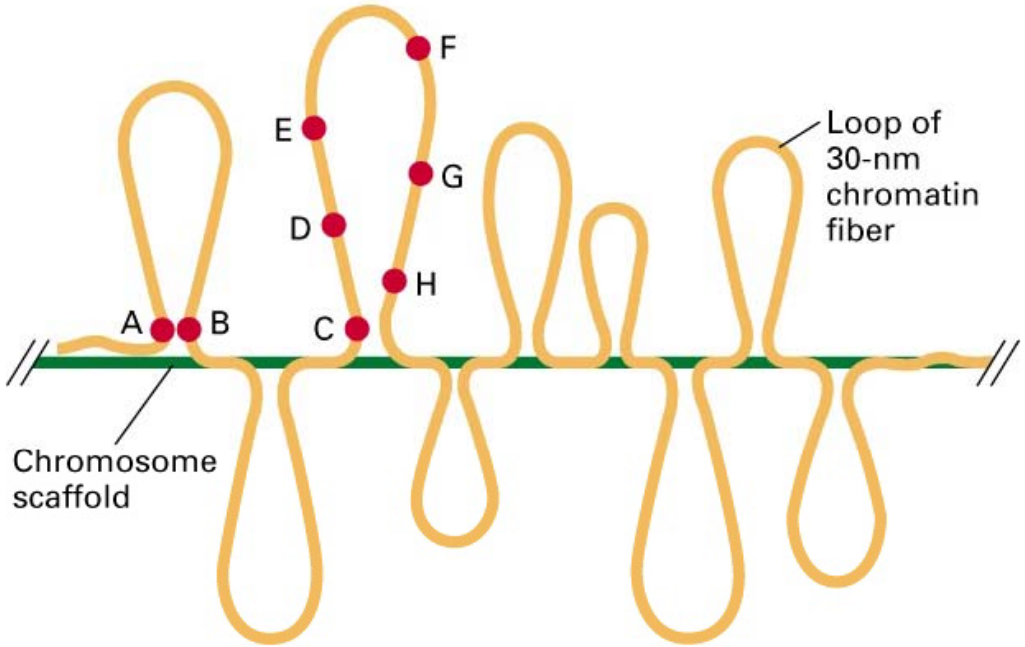
- Requires Histone H1
- Compaction ratio approx 100 fold

Lehninger

Chromatin REMODELERS (many are ATPases….)

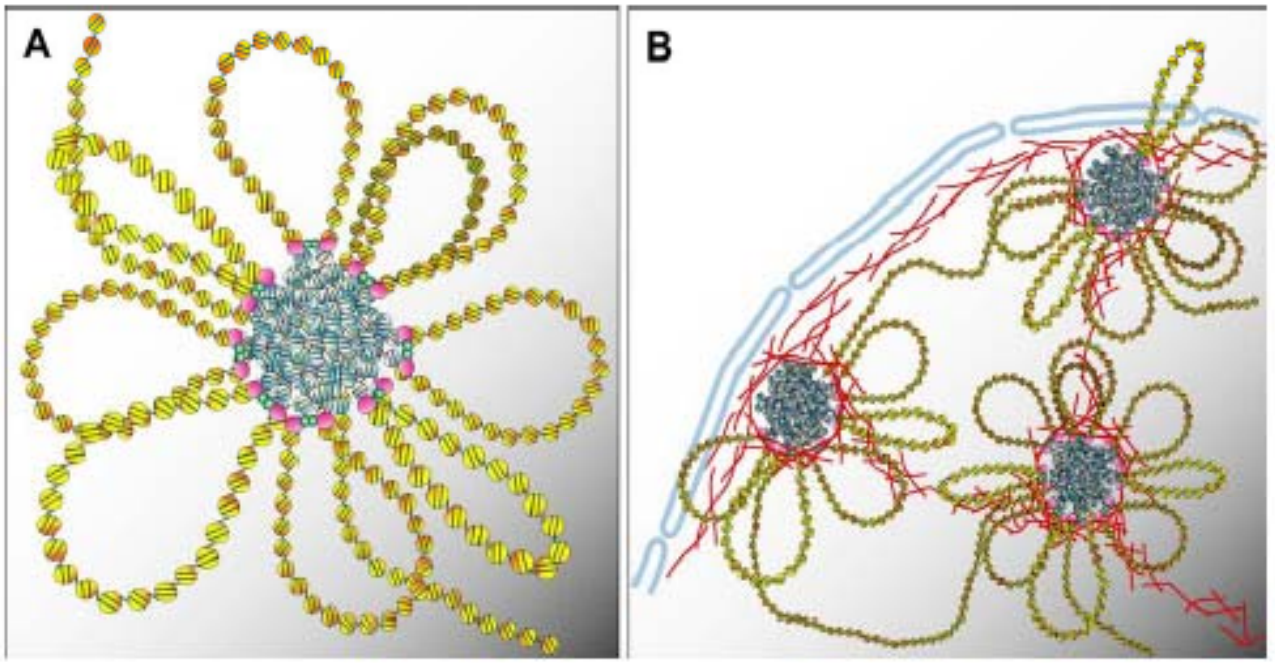


Level three: nuclear scaffolding



- Not well understood
- Organization is not random; involves sequence elements (red dots), more non-histone chromatin proteins and tethering to the nuclear envelope and matrix

Level three: nuclear scaffolding



- Not well understood
- Organization is not random; involved sequence elements (red dots), more non-histone chromatin proteins and tethering to the nuclear envelope and matrix

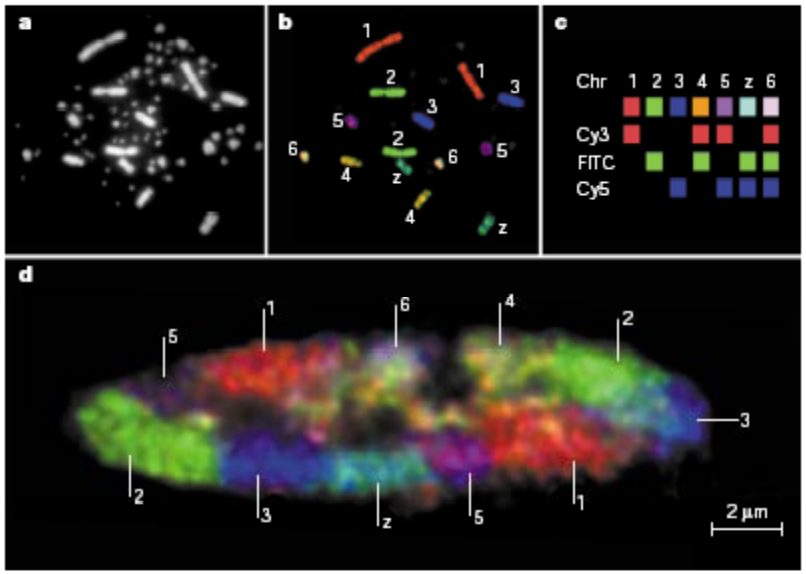
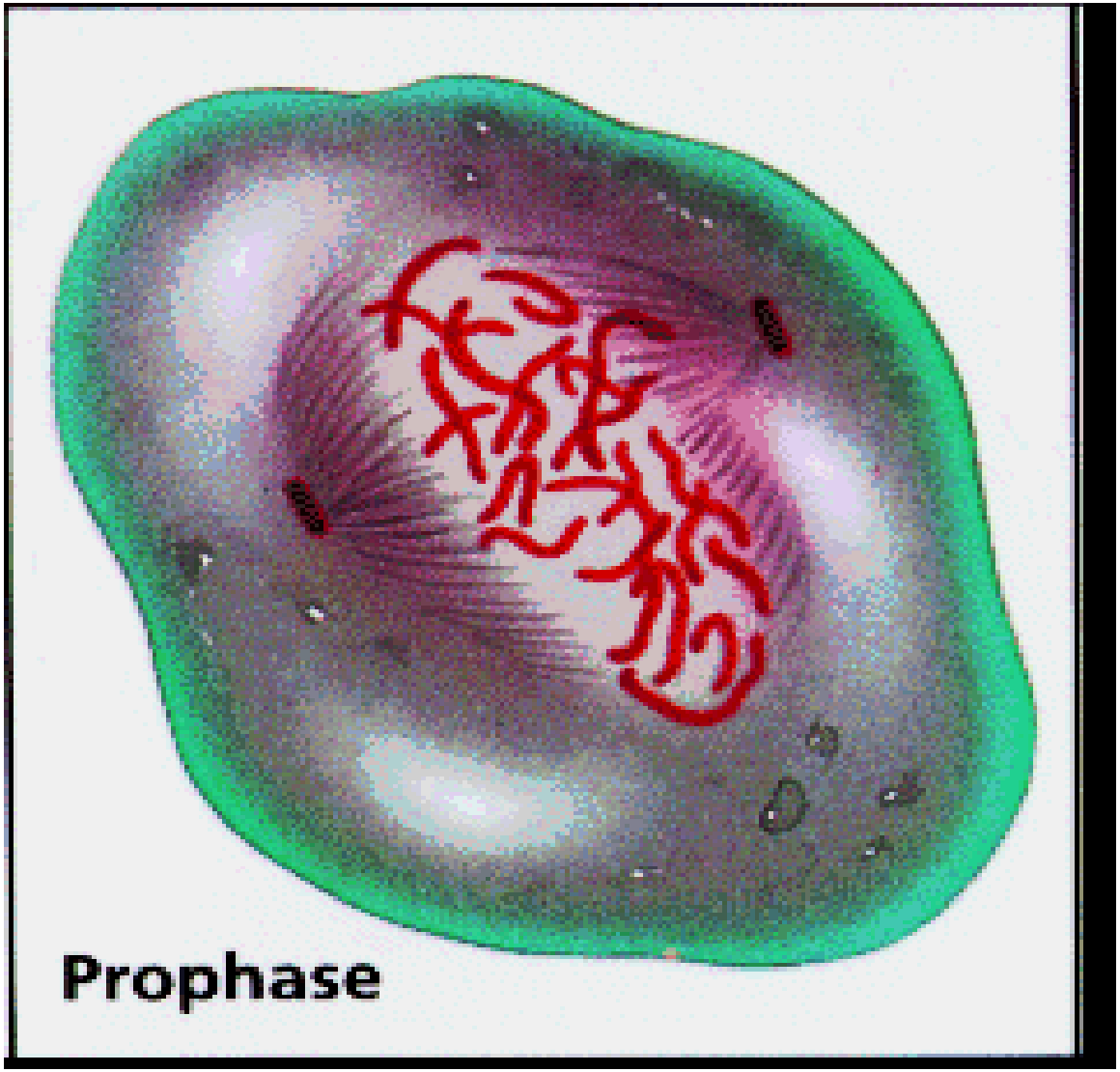


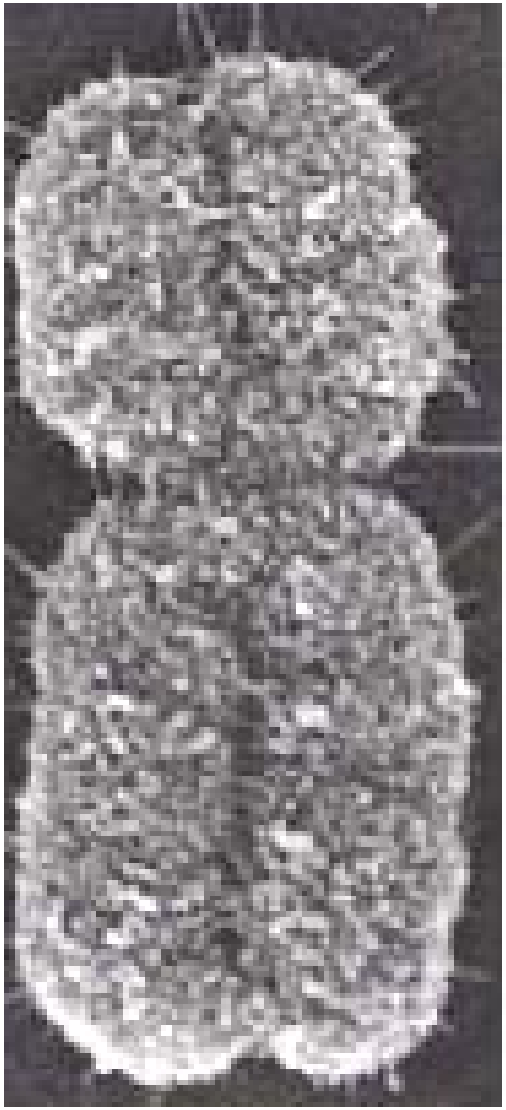
Figure 2 | **Chromosome territories in the chicken. a** | 4,6-diamidino-2-phenylindole (DAPI)-stained, diploid, chicken metaphase spread with macro- and microchromosomes. **b** | The same metaphase spread after multicolour fluorescence *in situ* hybridization with pseudocoloured chromosomes. Chicken chromosome paint probes (image courtesy of Johannes Wienberg) were labelled by a combinatorial scheme with oestradiol (1, 4, 5, 6), digoxigenin (2, 4, 6, Z) and biotin (3, 5, 6, Z). **c** | Oestradiol- and digoxigenin-labelled probes were detected using secondary antibodies labelled with Cy3 and fluorescein isothiocyanate (FITC); biotinylated probes were detected with Cy5-conjugated streptavidin. **d** | Mid-plane light optical section through a chicken fibroblast nucleus shows mutually exclusive chromosome territories (CTs) with homologous chromosomes seen in separate locations. (Note that only one of the two CTs for each of 4 and 6 is displayed in this section.) (Image courtesy of F. Habermann.)

Benefit:

1. Organization
2. Co-regulated genes together in 3D space, although not necessarily close in 1D DNA strand (or even on same chromosome)!

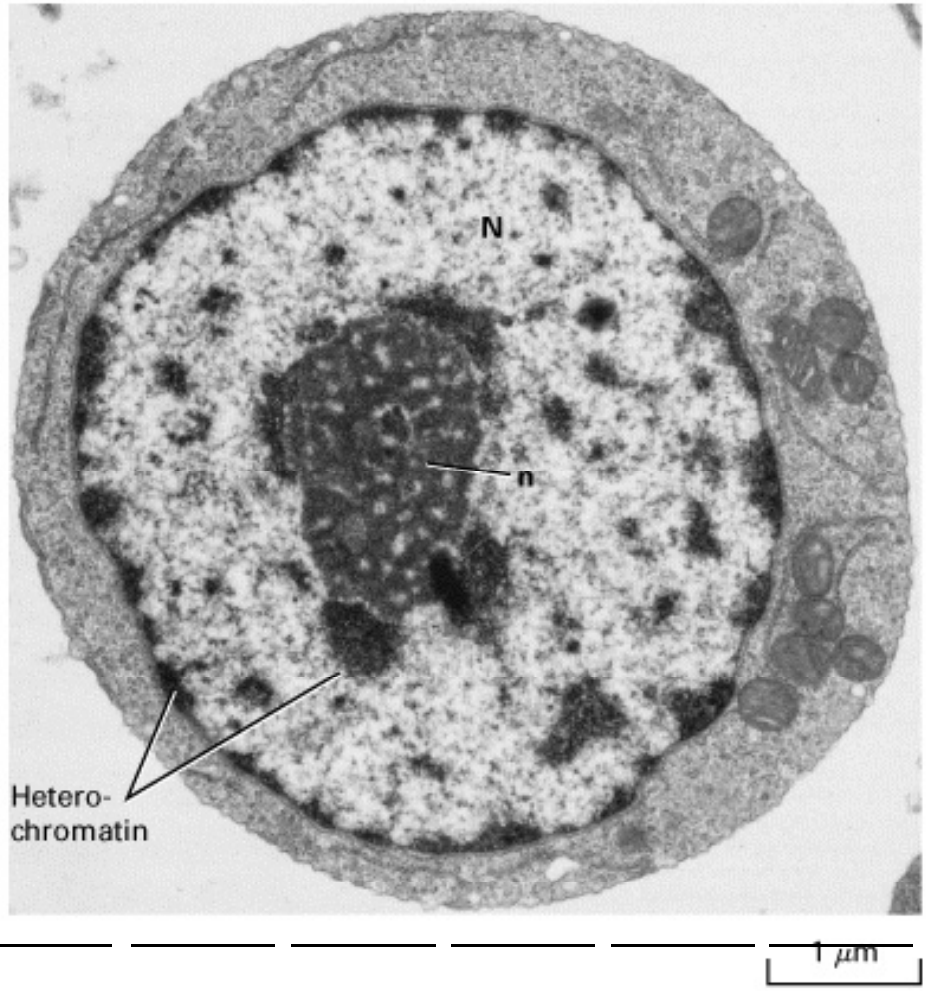
Metaphase chromatin: level four packaging: fully condensed





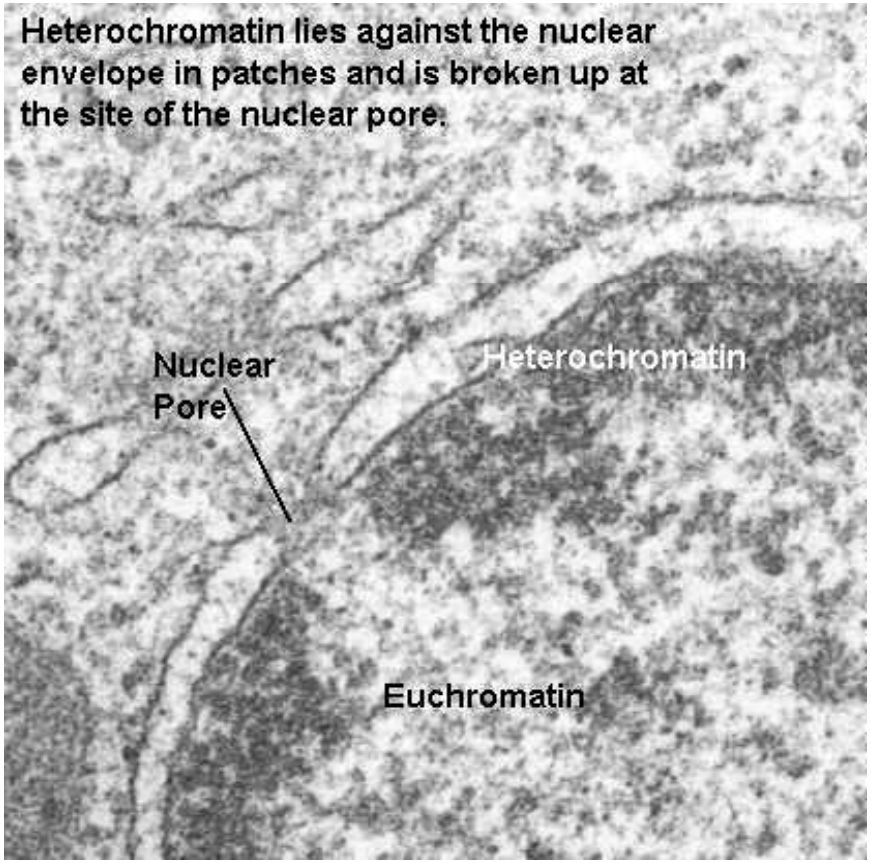
Interphase chromatin: levels 1-3 relatively decondensed chromosomes

- **Heterochromatin**: dark-staining, condensed (mostly simple-sequence, repetitive DNA)
- **Euchromatin**: light-staining, less condensed (complex sequence DNA: e.g. genes)

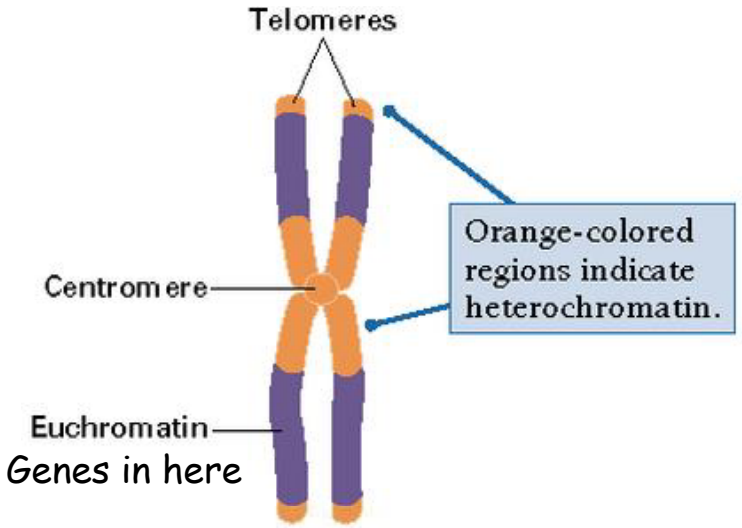


Expression reflects sequence, but also LOCATION, LOCATION, LOCATION

Constitutive heterochromatin

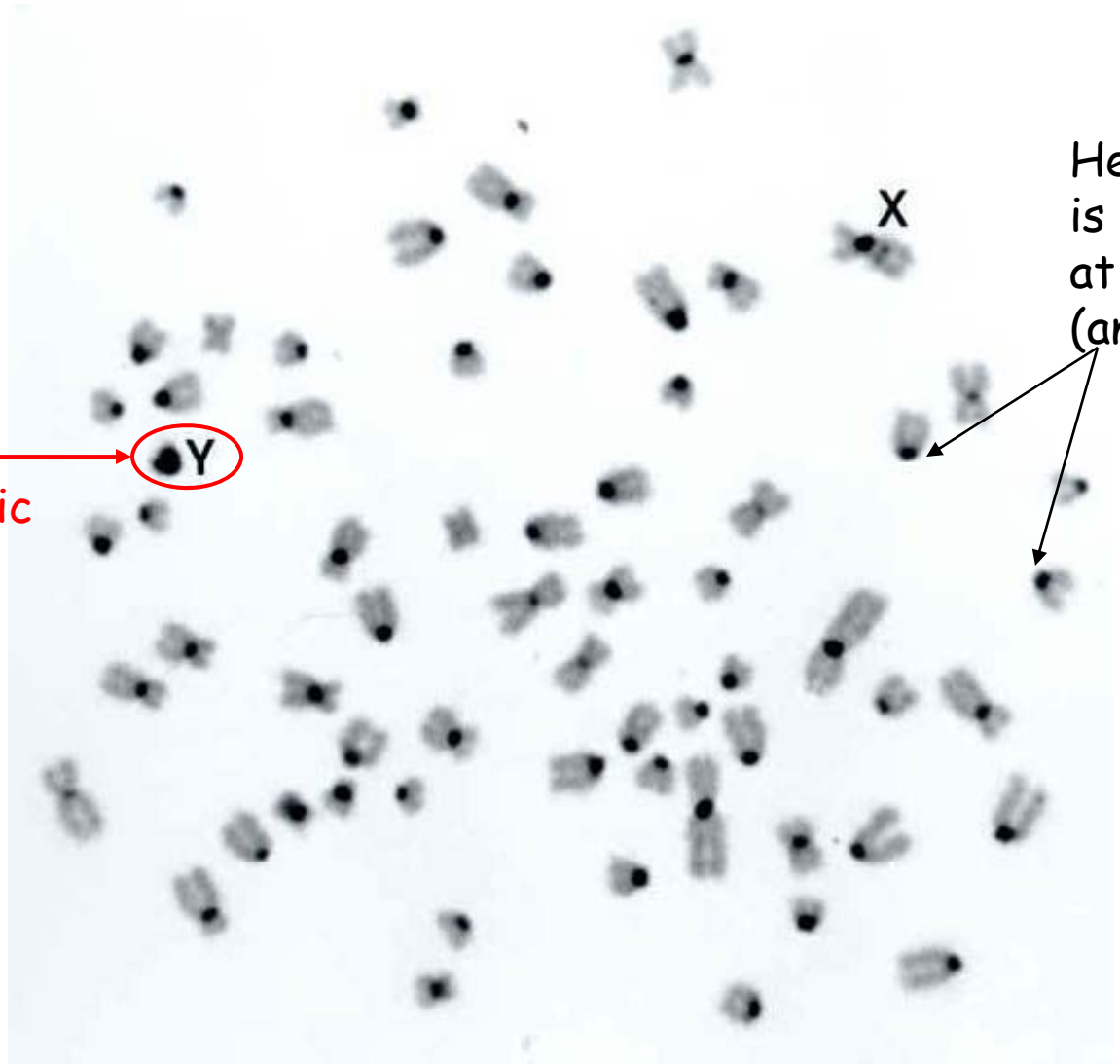


Interphase chromatin



Metaphase chromatin

Constitutive heterochromatin



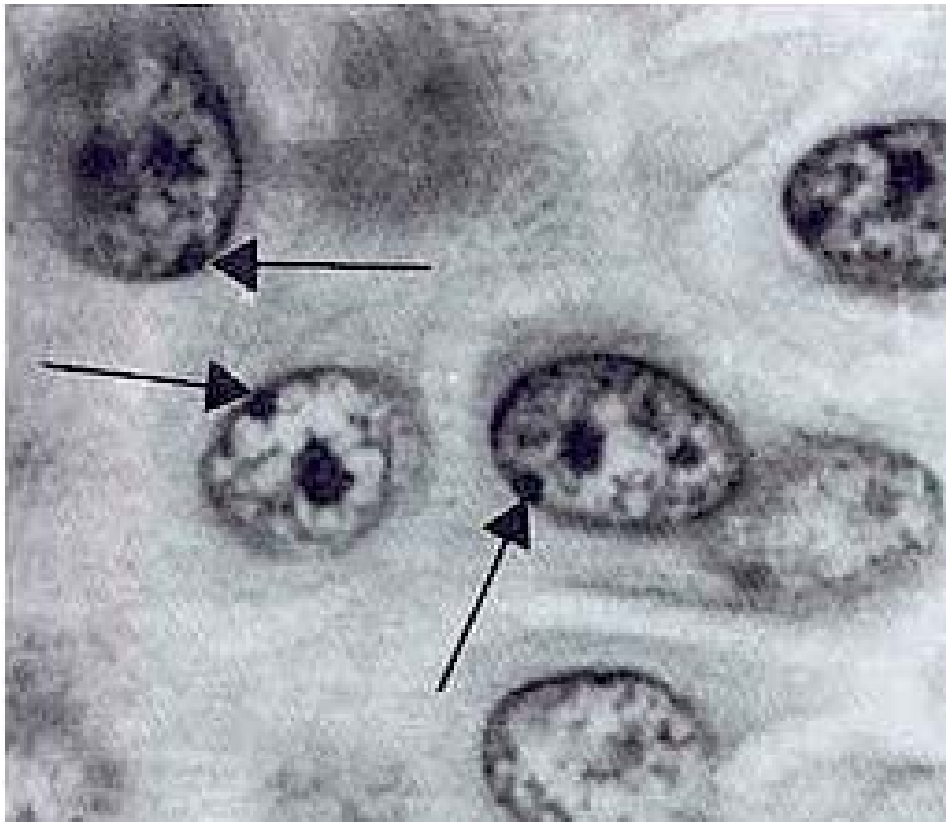
The Y chromosome is almost entirely heterochromatic

Heterochromatin is concentrated at centromeres (and telomeres)

horse

Facultative heterochromatin

Facultative heterochromatin XX females vs. XY males: Dosage compensation



- Barr body is the condensed, inactive X chromosome
- X chromosome chromatin looks *heterochromatic*
- Choice as to which X is inactivated is largely random (and stable)
- Choice is made early in development (humans: day 16 post fertilization)

Consequences of selective X inactivation