From Genes to Proteins

Structure-function in DNA and proteins

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Aims and Schedule

1) To understand basic principles in structural biology

2) To demonstrate a link between structure and function

Lectures:

Monday – 13/Feb/2017: Genes to Proteins

Friday – 17/Feb/2017: Principles of Structural Biochemistry

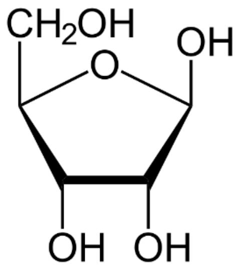
DNA in a 3D world

	Experiment	Conclusion
Late 19^{th} century	Removal of the nucleus resulted in cell death, but removal of an equal volume of cytoplasm did not	The nucleus is key to cell survival
Late 19^{th} century	Removal and transplantation of a nucleus can change cells shape and function	Nucleus is linked to phenotype
Griffiths – 1929	Virulent strains of *Pneumococcus* were able to transform non-pathogenic *Pneumococcus* into a disease causing organism	"Some" material has been exchanged between the two cells that is able to change the phenotype of the cells
Avery and MacLeod – 1944	DNA extracted from virulent *Pneumococcus*. On transformation to non-virulent strains, it conferred pathogenicity. Transfomation not affected by proteases, but by Dnase	DNA is suggested as the transforming agent
Hershey and Chase – 1953	Infected *E. coli* with T2 phage. DNA: 32P labelled, coat protein: 35S labelled. Only 32P detected inside cells	DNA alone is responsible for cells phenotype, no proteins involved

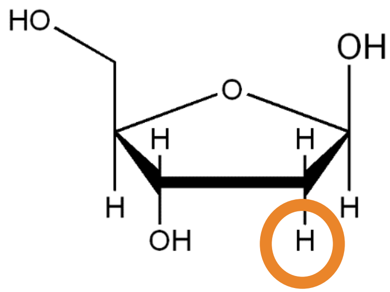
Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid, - Francis Crick, James D. Watson, Nature, **171**, 1953

Building DNA

- Both DNA and RNA contain:
  1. Pentose sugars (5C)
  2. Organic bases
  3. Inorganic phosphate

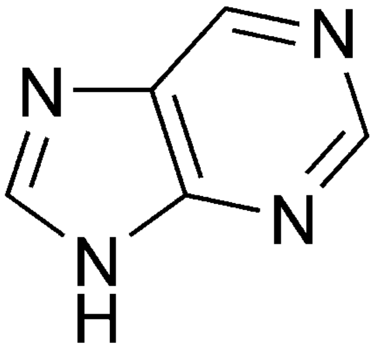


Ribose
(Forms ribonucleic acid – RNA)

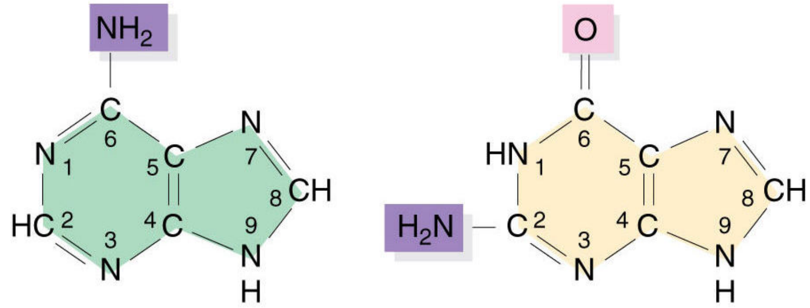


Deoxyribose
(Forms deoxyribonucleic acid – DNA)

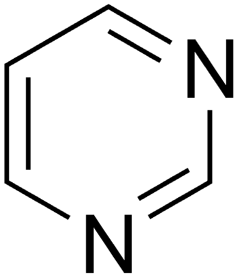
Bases



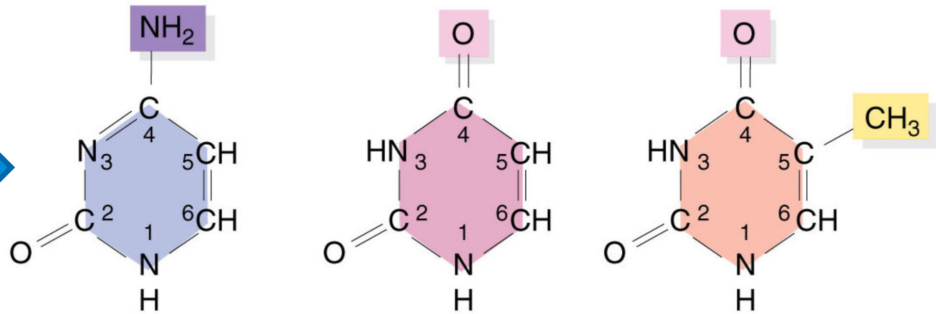
Purine



Adenine (A) Guanine (G)

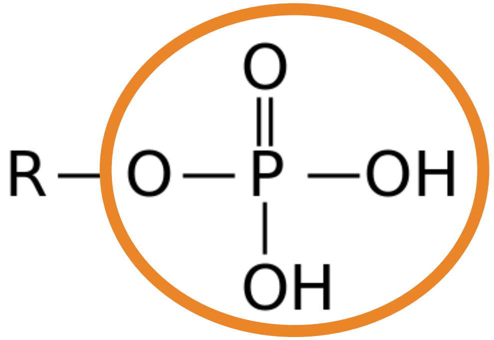


Pyrimidine

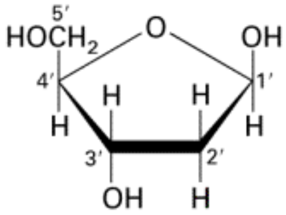


Cytosine (C) Uracil (U) (found in RNA) Thymine (T) (found in DNA)

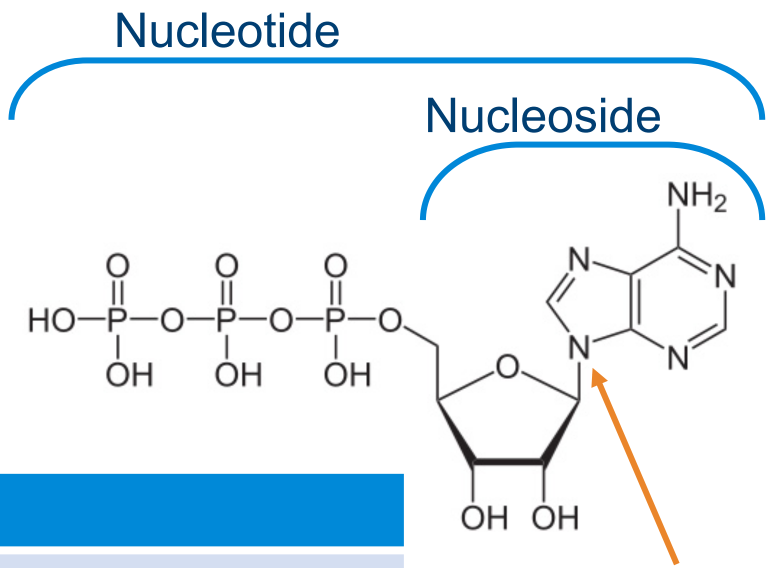
Nucleotides



Phosphate group



2-Deoxyribose



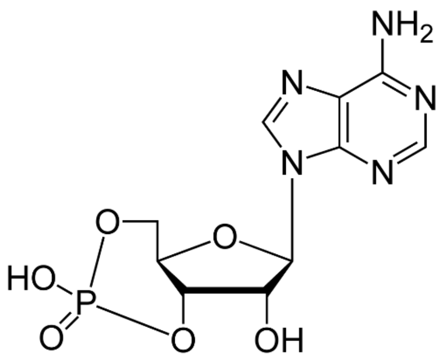
Glycoside link

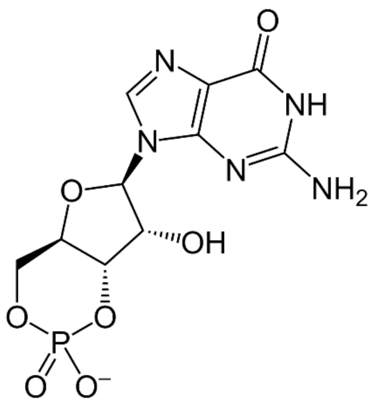
Base	Nucleoside	(Deoxy)ribonucleotide
Adenine	Adenosine	(Deoxy)adenosine 5’-mono/di/triphosphate
Guanine	Guanosine	(Deoxy)guanosine 5’-mono/di/triphosphate
Cytosine	Cytidine	(Deoxy)cytidine 5’-mono/di/triphosphate
Thymine	Thymidine	(Deoxy)thymidine 5’-mono/di/triphosphate
Uracil	Uridine	Uridine 5’-mono/di/triphosphate

Ribonucleoside
Deoxyribonucleoside

Regulatory nucleotides

- Nucleotides play other roles in a range of processes, including metabolism and cell signalling





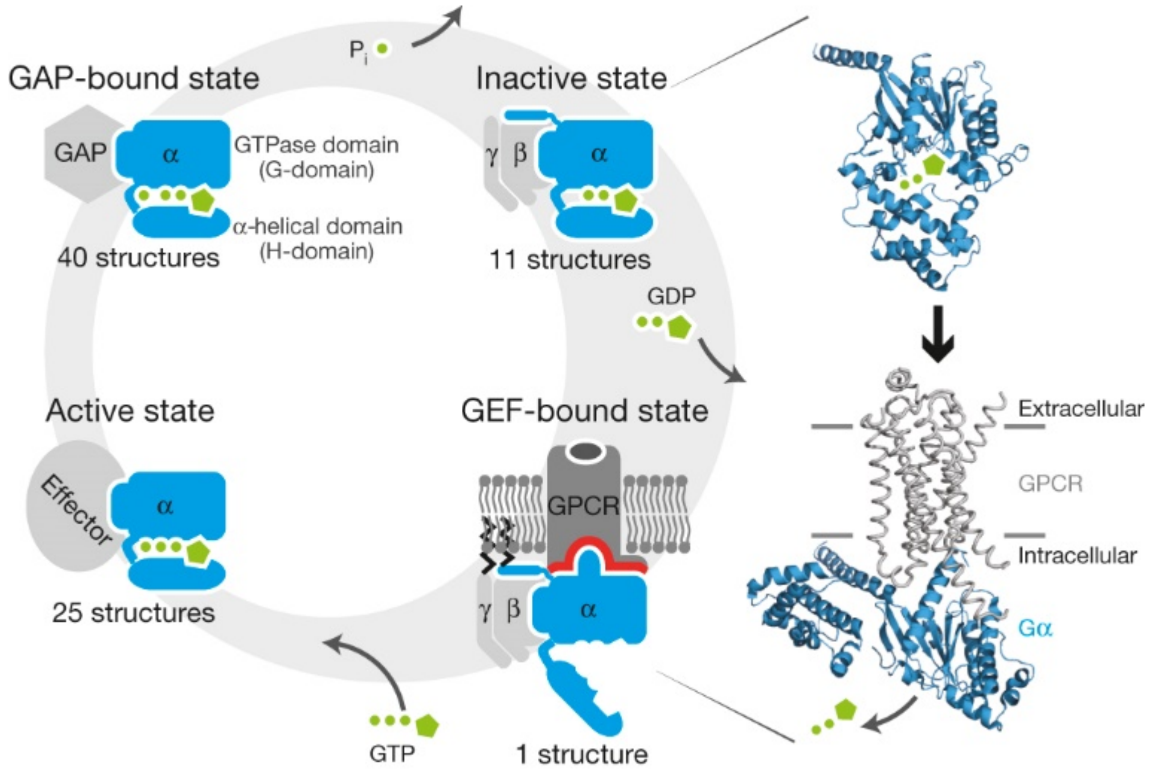
Cyclic AMP
(cAMP)

Second
messenger
GPCR→PKA

Cyclic GMP
(cGMP)

Second messenger
Photoreceptor→phosphodiesterase
→cGMP gated Na channel →hyperpolarisation

GPCRs and G-proteins

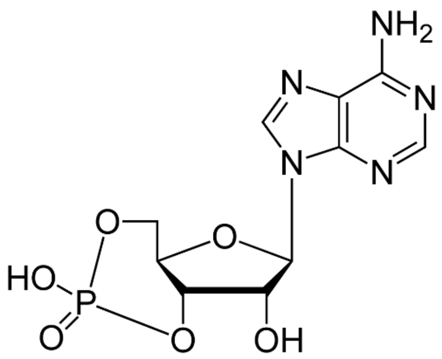


- Whole system of signalling based on GTP/GDP exchange

Doi:10.1038/nature14663

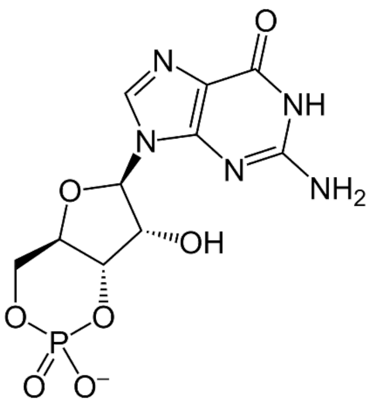
Regulatory nucleotides

- Nucleotides play other roles in a range of processes, including metabolism and cell signalling



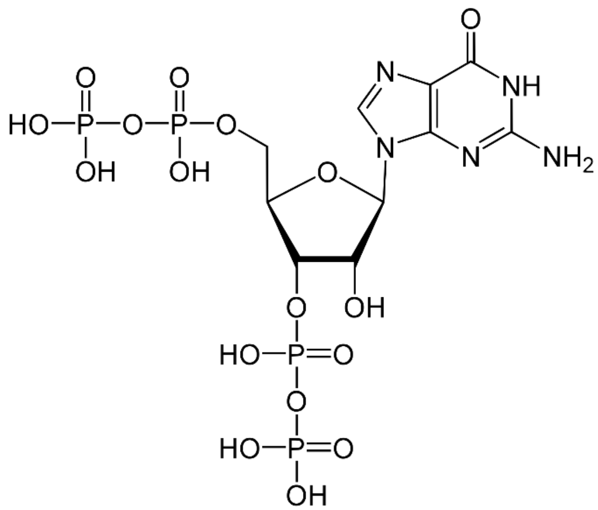
Cyclic AMP (cAMP)

Second messenger
GPCR→PKA



Cyclic GMP (cGMP)

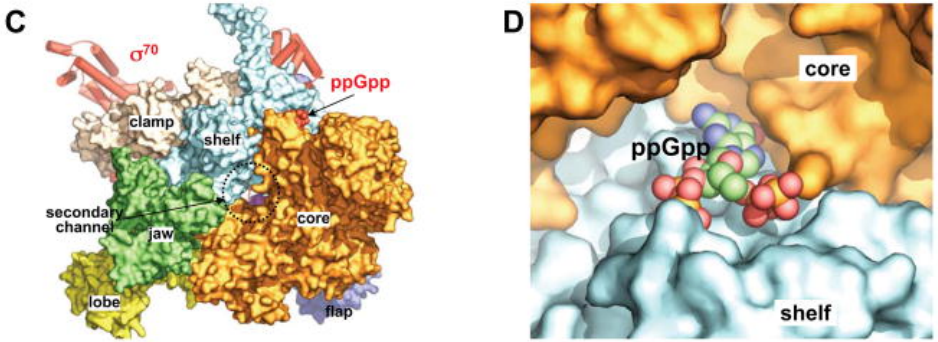
Second messenger
Photoreceptor→phosphodiesterase
→cGMP gated Na channel →hyperpolarisation

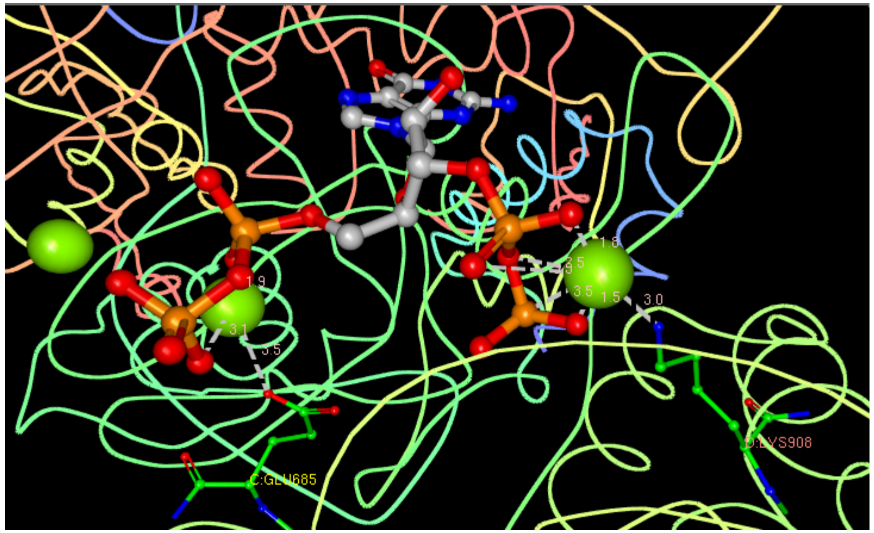


ppGpp

Bacterial alarmone,
Stringent response

ppGpp



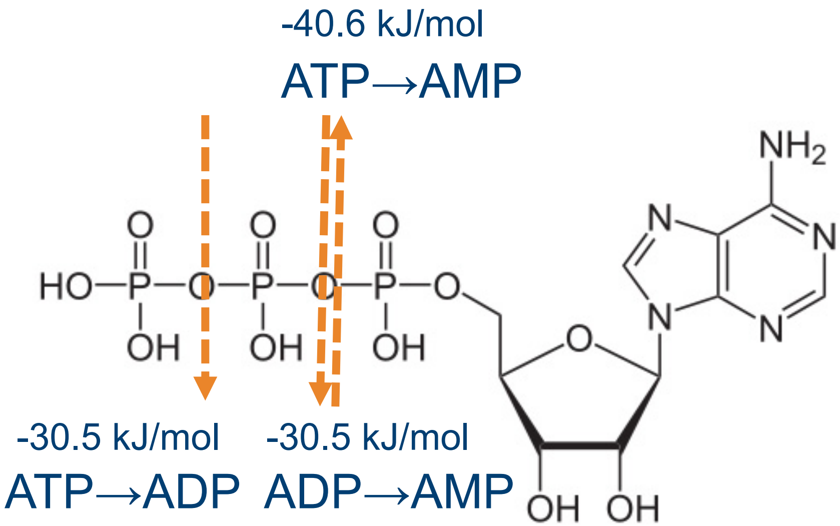


ppGpp in *E. coli (PDB: 4JKR)…*

…and in *Thermus thermophilus (PDB: 1SMY). Which is right?*

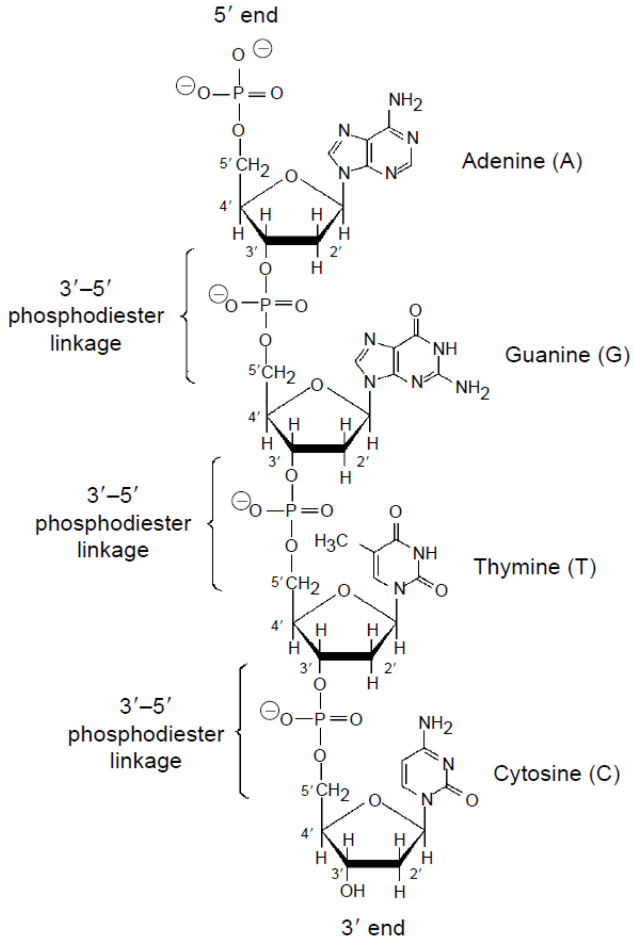
1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3677725/
   The Mechanism of *E. coli* RNA Polymerase Regulation by ppGpp Is Suggested by the Structure of Their Complex
   Mol Cell. 2013 May 9; 50(3): 430–436.
2. https://www.ncbi.nlm.nih.gov/pubmed/15109491
   Structural Basis for Transcription Regulation by Alarmone ppGpp
   Cell. 2004 Apr 30;117(3):299-310.

Nucleotide chains make oligonucleotides

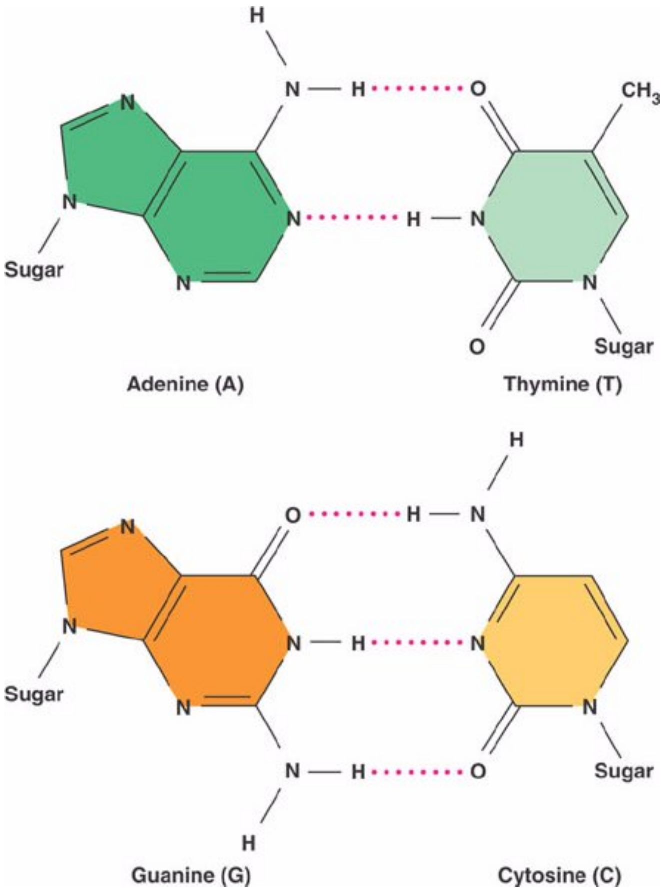


DNA has 5’-3’ directionality, always written:

5’-NNNNNN-3’



Base pairing

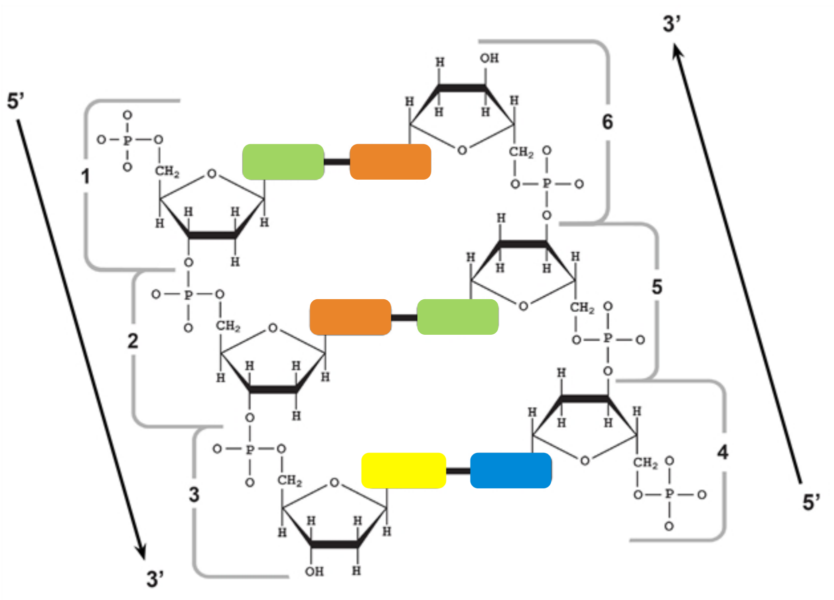


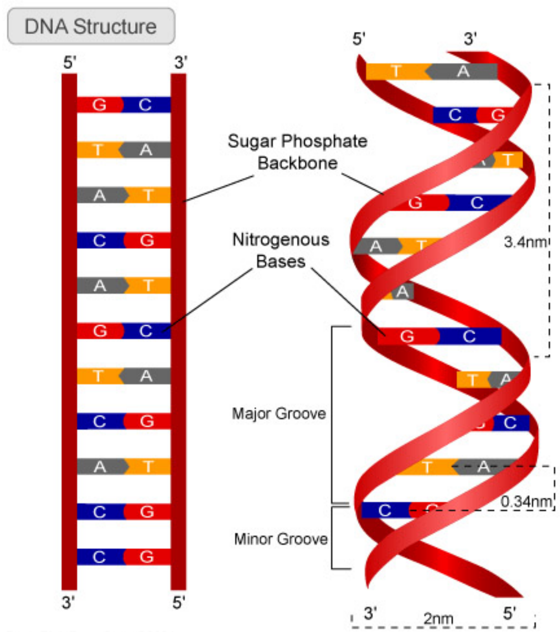
- Purine pairs a Pyrimidine
  - A=T, G≡C
- Chargaff's rules
  - A+G = C+T

Bacteria A: 32% Adenine
Bacteria B: 17% Adenine
What's the proportion of C, G and T?
One of these was isolated from a hot spring. Which one?

Antiparallel DNA helix

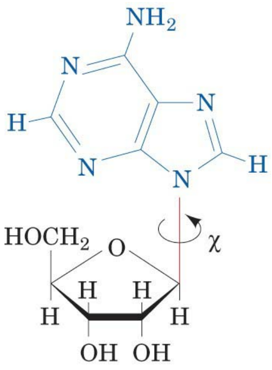
- Two strands: 5’-3’ : 3’-5’



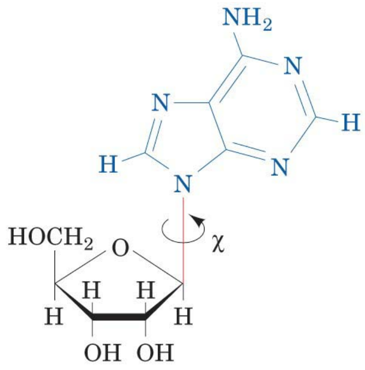


From sequence to 3D structure

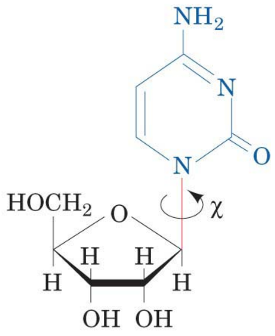
1) Rotation of C1'-N-Glycoside bond



syn-Adenosine



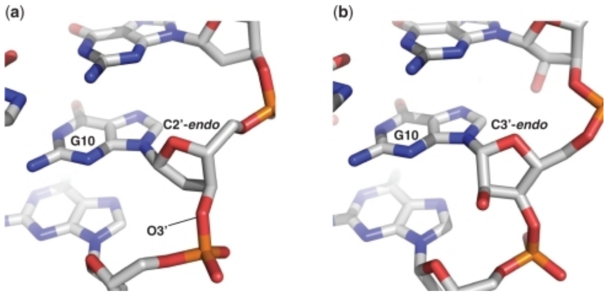
anti-Adenosine

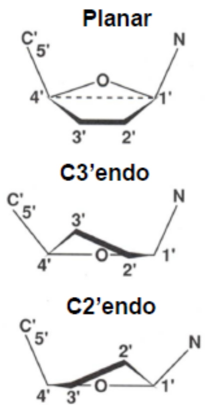


anti-Cytidine

- Purines (A, G) can adopt both syn and anti orientation of the base
- Pyrimidines: =O in steric hindrance with ribose O

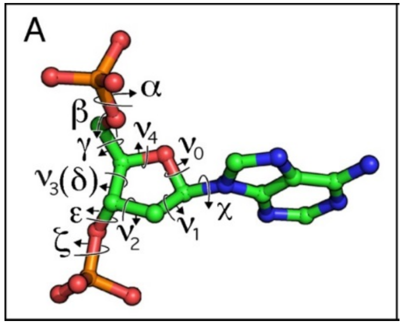
2) Sugar puckering





- C-2' endo or C-3' endo

3) Bond rotations in deoxyribose backbone



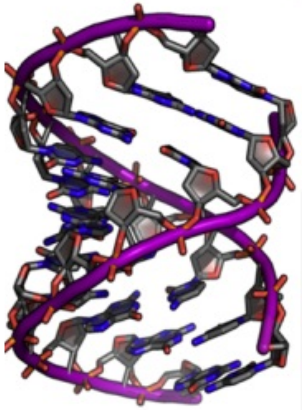
- Rotations mainly about 7 bonds
  Only ribose ring constrained

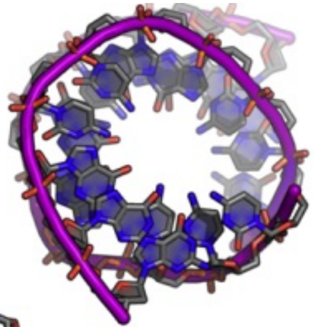
Forms of the double helix

Alternative forms of DNA exist: some artificially, some naturally

Dehydrated form

A-DNA



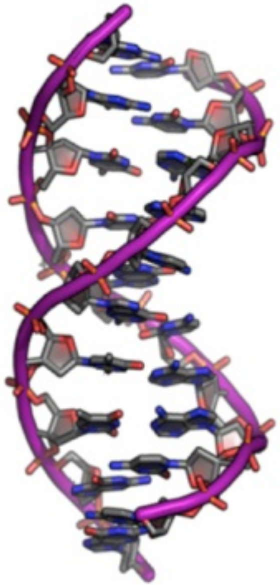


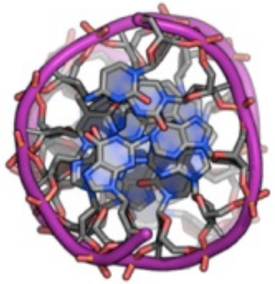
Sense	Right
Base/ turn	11
Rise/ bp	2.6 Å
Base tilt to helix	20°
Glycosyl bond	Anti
Sugar pucker	C-3' endo

Lack of water
Shallow minor groove
Deeper major groove

Watson-Crick form

B-DNA

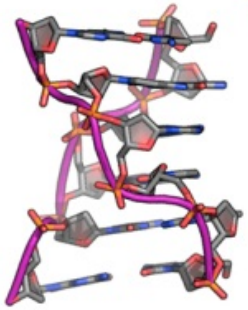


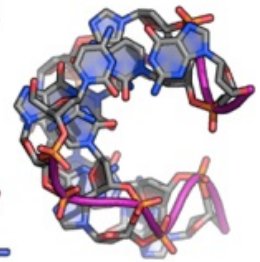


Sense	Right
Base/ turn	10.5
Rise/ bp	3.4 Å
Base tilt to helix	6°
Glycosyl bond	Anti
Sugar pucker	C-2' endo

Rare but observed

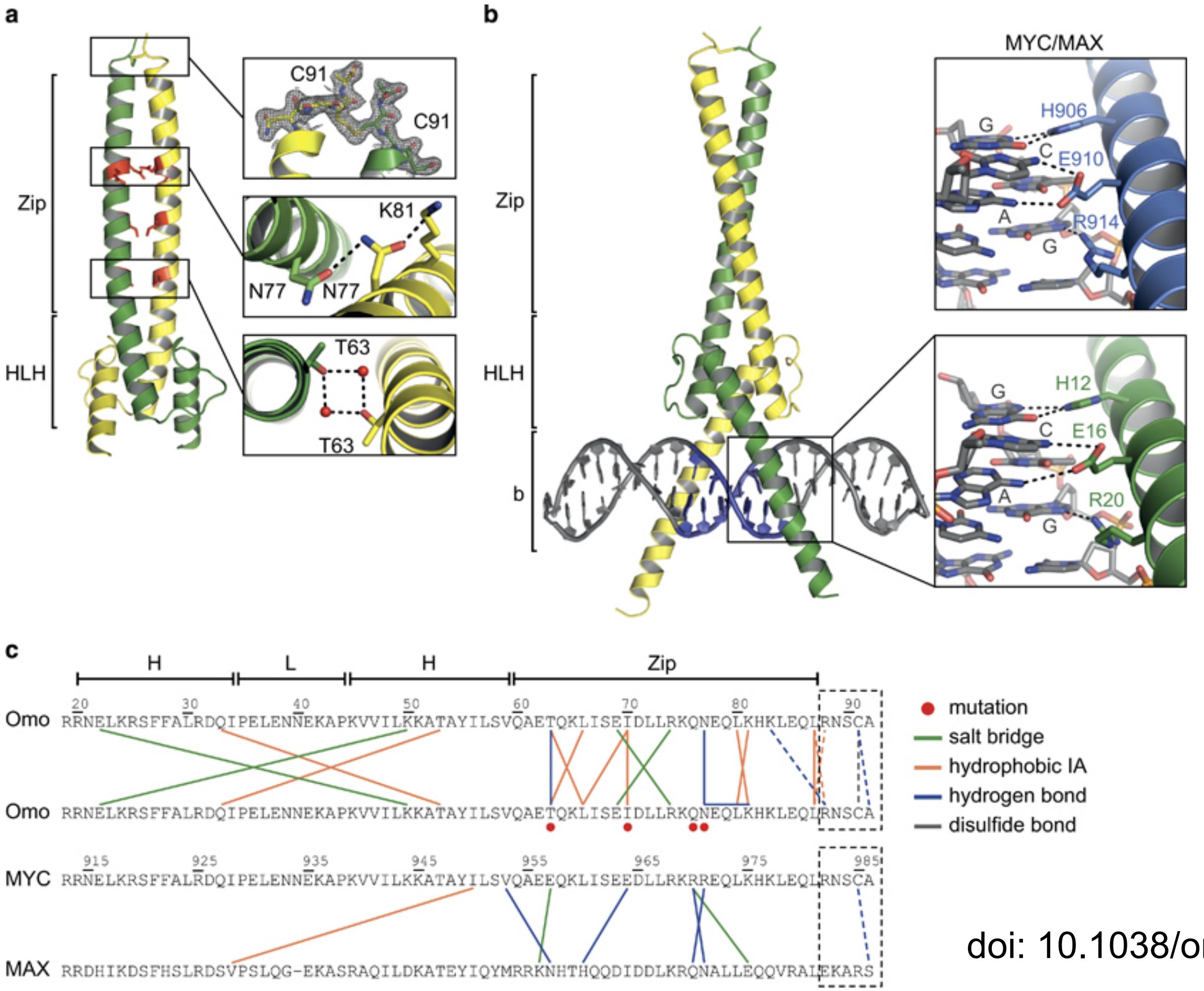
Z-DNA





Sense	Left
Base/ turn	12
Rise/ bp	3.7 Å
Base tilt to helix	7°
Glycosyl bond	Pyr:Anti Pur:Syn
Sugar pucker	Pyr: C-3' endo Pur: C-2' endo

Implications of DNA structure



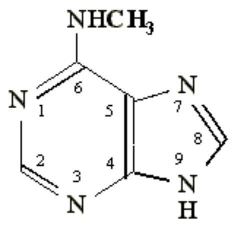
Myc
Essential role in development and tumorogenesis. Transcription factor

OmoMYC
Dominant negative variant. Tumour suppressor

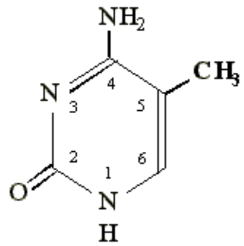
doi: 10.1038/onc.2016.354

DNA can be modified

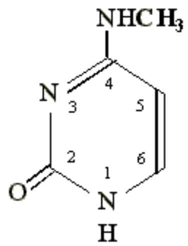
- DNA methylation
  - Enzymatic process
  - Adenine, Cytosine > Guanine, Thymine
  - CH3 source: always S-adenosylmethionine
  - Bacteria: Defence
    and Repair (Dam methylase in G**<u>A</u>**TC)
  - Eukaryotes: 5% methylcytidine
    CpG islands



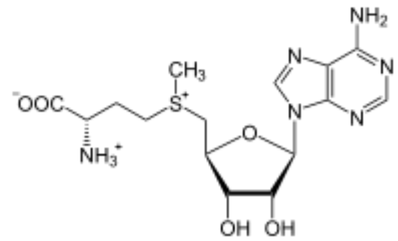
N_6 methyl adenine

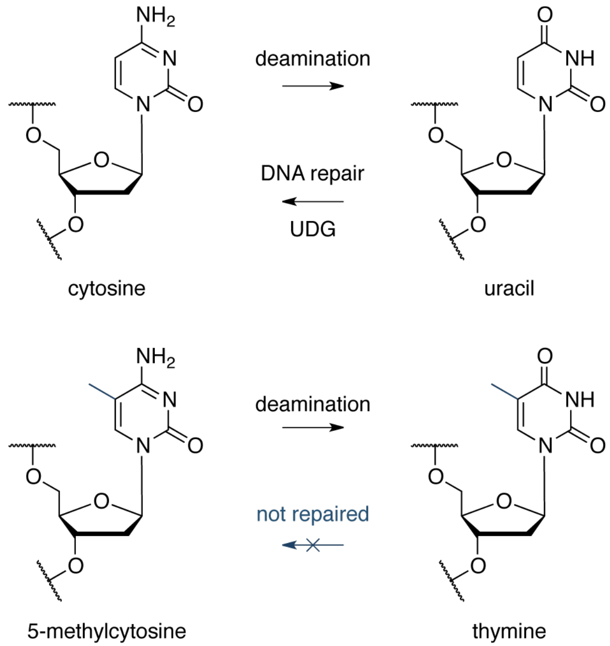


C_5 methyl cytosine



N_4 methyl cytosine





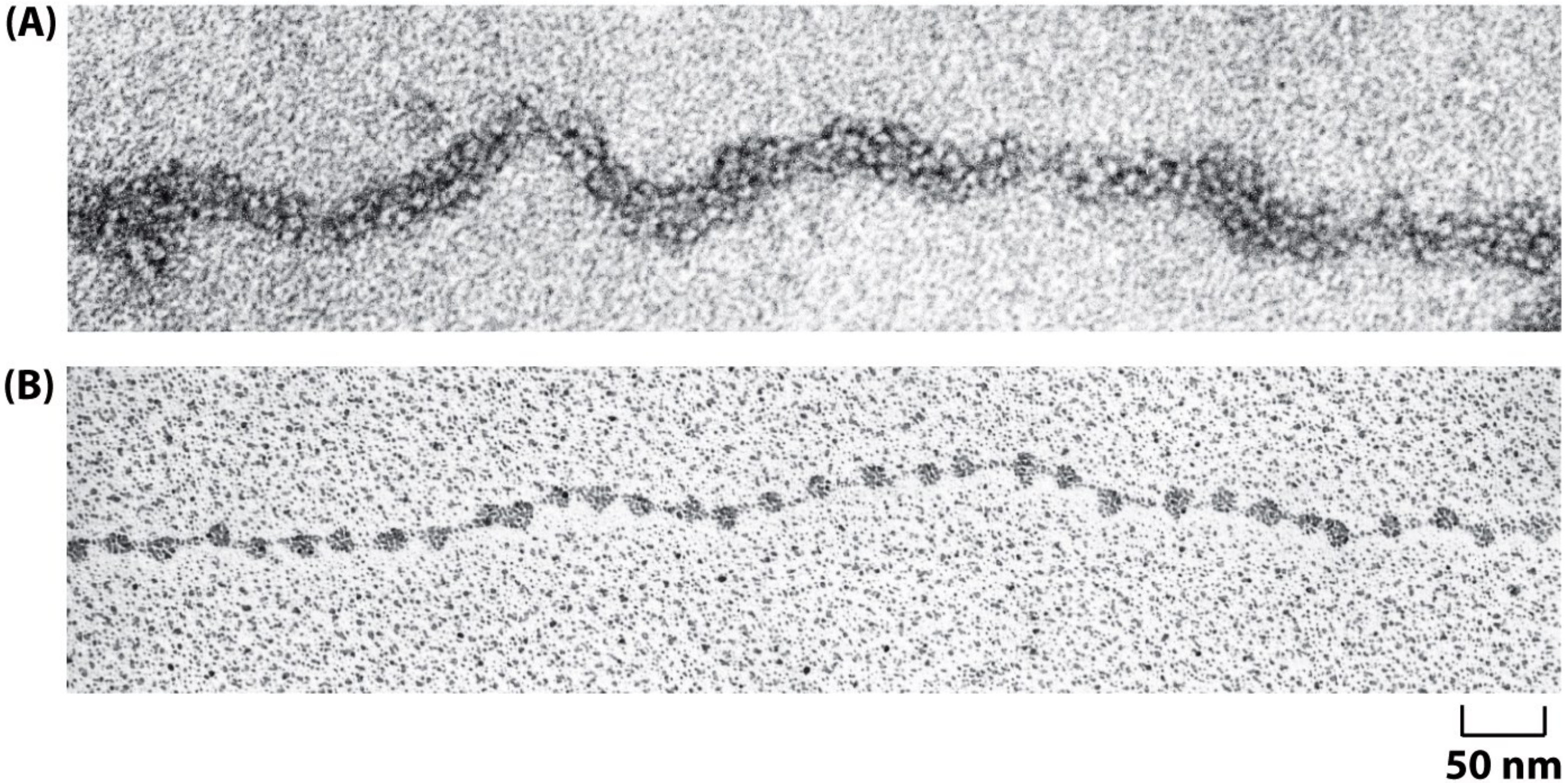
Epigenetics

“Stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence“

Cold Spring Harbor, 2008

DNA organisation

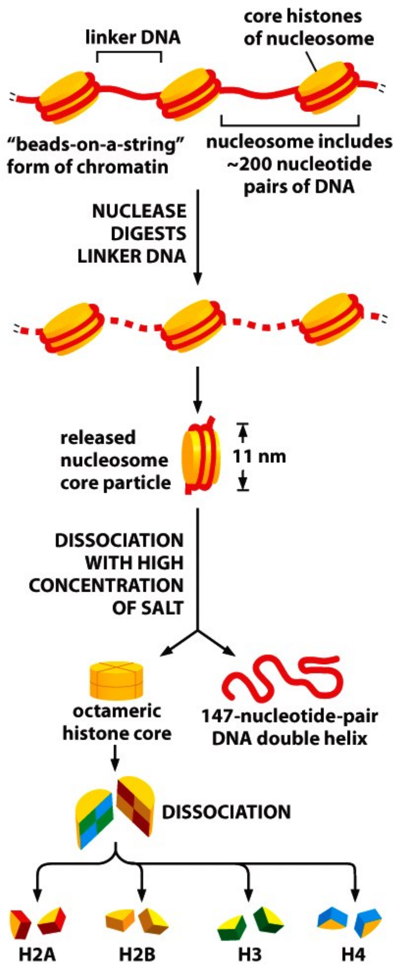
Chromosome 22: stretched, linear DNA: 1.5 cm
At metaphase: a mere 2 μm (10,000 compaction)



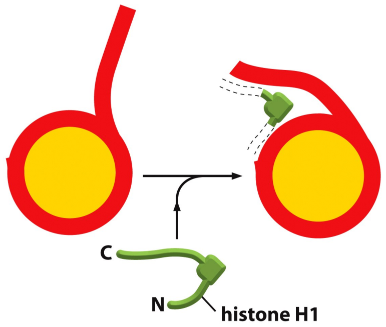
30 nm fibre

30 nm fibre (decondensed)

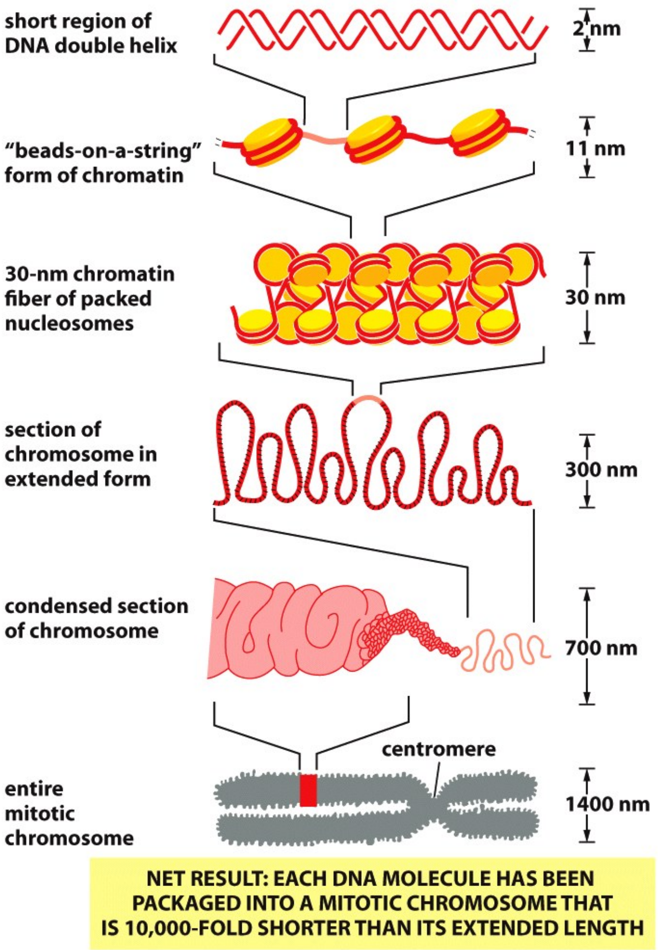
The chromatin (DNA+Histones)



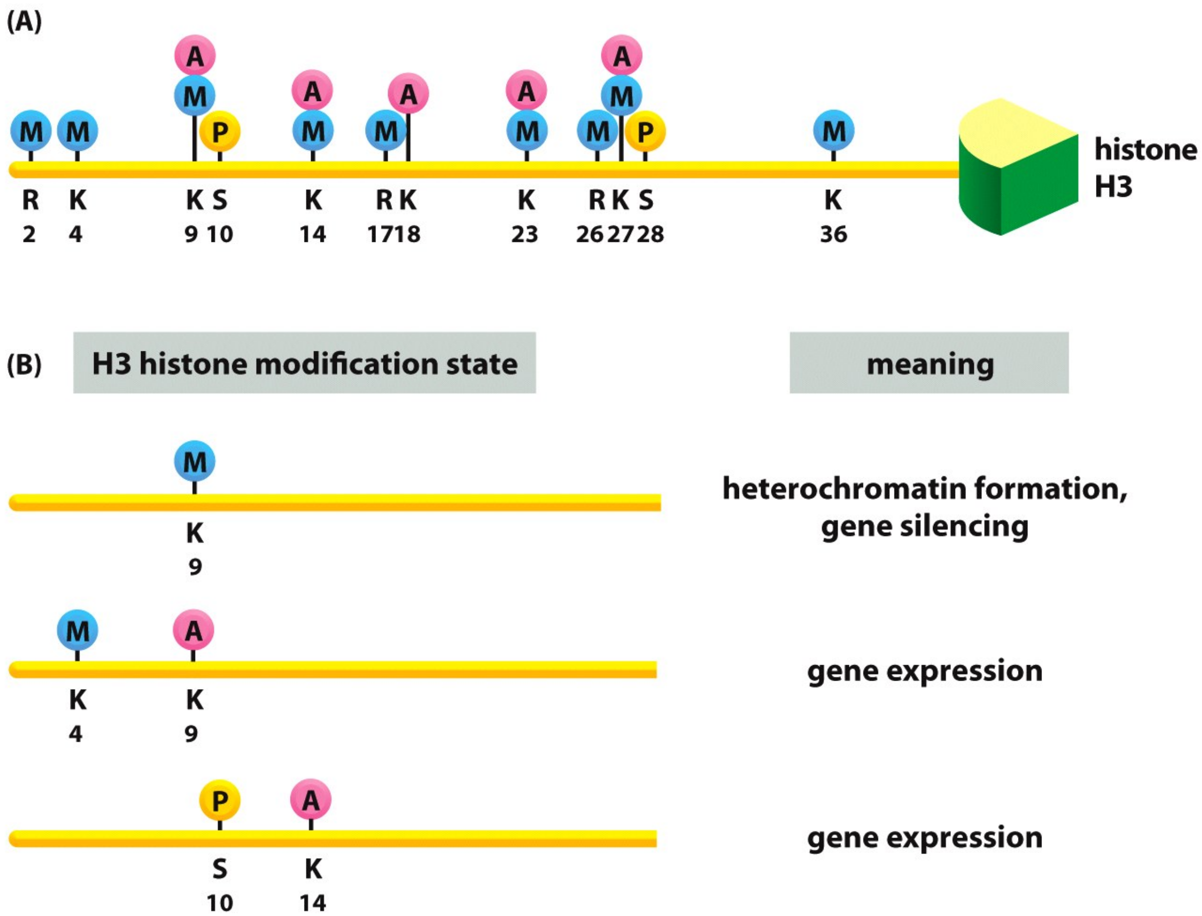
- Chromatin unwound
- Linker DNA digested
- DNA dissociated with high salt
- 147 bp: enough to be wrapped 2x round
- Histone: hetero-octamer + H1



DNA organisation on multiple levels



DNA modification at the chromatin level



Methylation (K1-3, R)

Acetylation (K)

Phosphorylation (S)

Summary

- DNA (genetic information) has transforming effect on cells/organisms
- DNA is constructed of nucleotides
- 2x 5'-3' antiparallel strands form double helix
- DNA is recognised by binding elements
- DNA can be modified, which has functional implication
- DNA is tightly packaged into chromosomes, with a further opportunity for epigenetic variation

Papers for next session

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2938214/
   A crystallographic and modelling study of a human telomeric RNA (TERRA) quadruplex
   Nucleic Acids Res. 2010 Sep; 38(16): 5569–5580

2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3677725/
   The Mechanism of *E. coli* RNA Polymerase Regulation by ppGpp Is Suggested by the Structure of Their Complex
   Mol Cell. 2013 May 9; 50(3): 430–436.

3. http://www.sciencedirect.com/science/article/pii/S0092867416317391
   Structures of the Human HCN1 Hyperpolarization-Activated Channel
   Cell. 2017 Jan 12;168(1-2):111-120.e11.