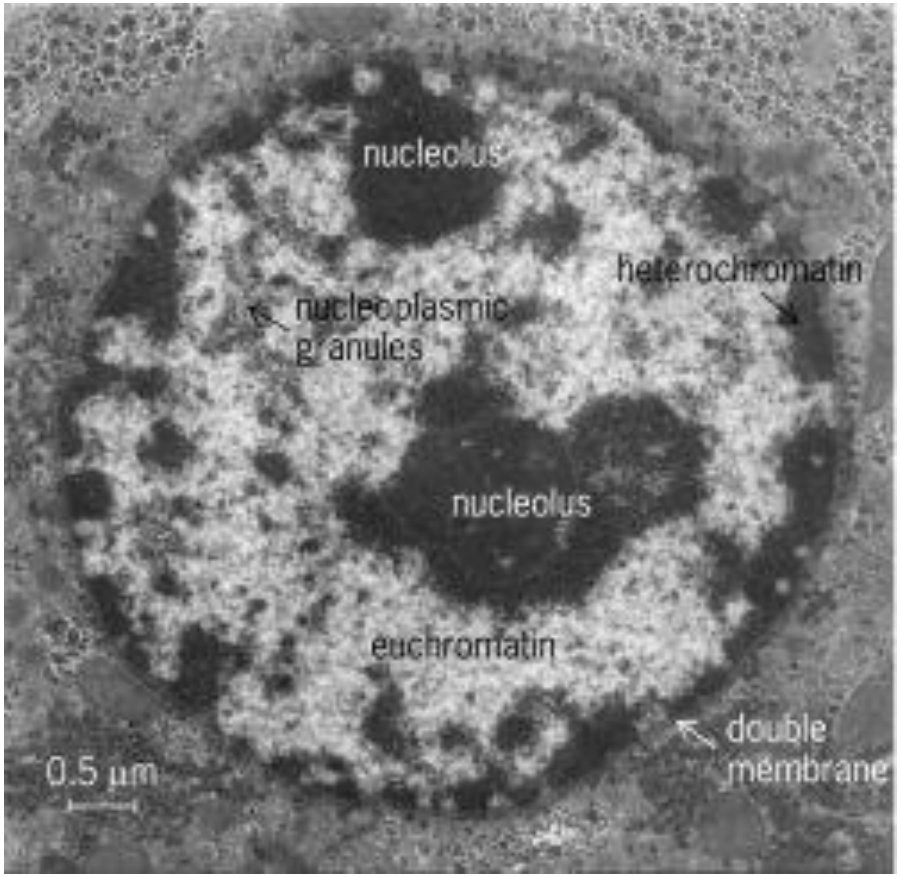
Ch 1 - L3.1

Chromatin Structure

Nucleosome positioning

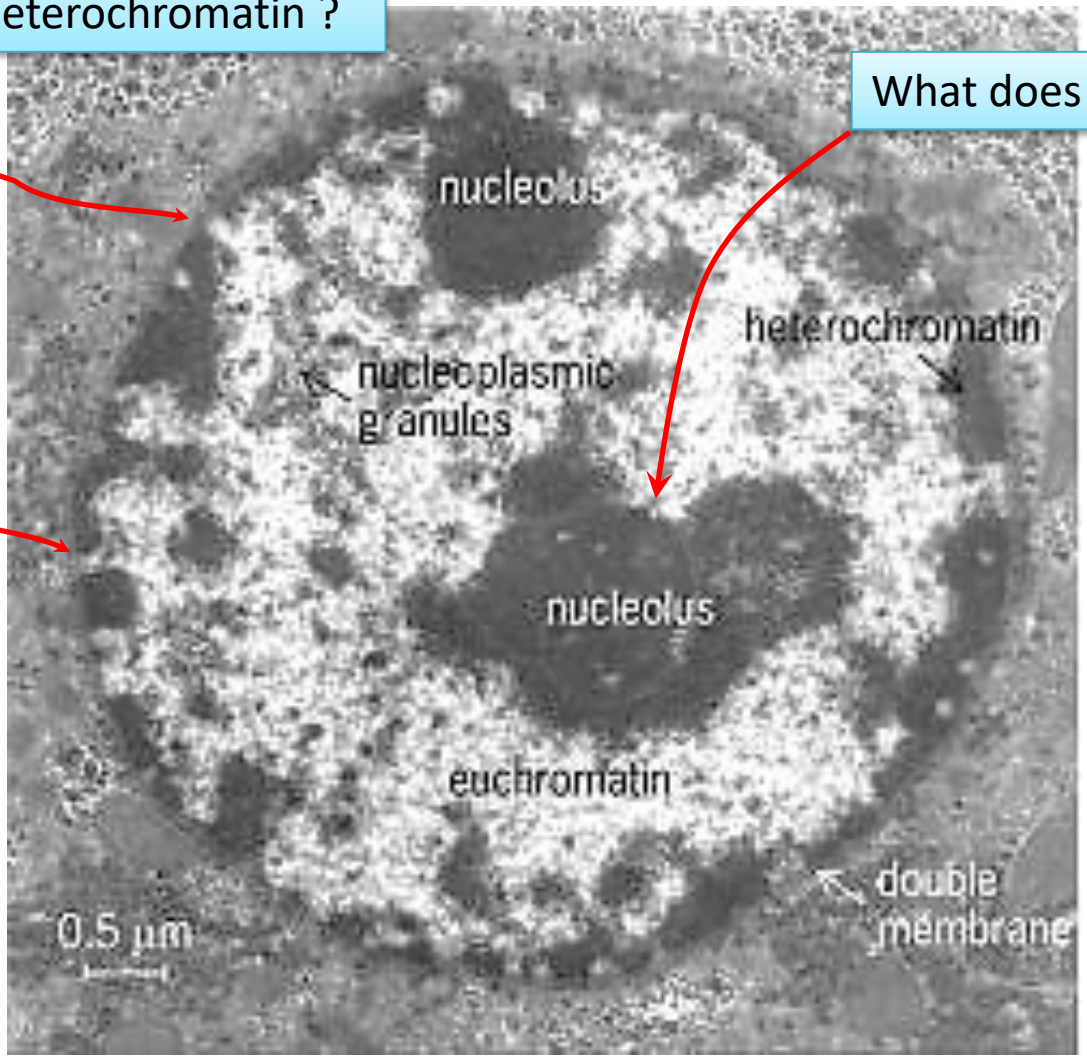
Accessibility

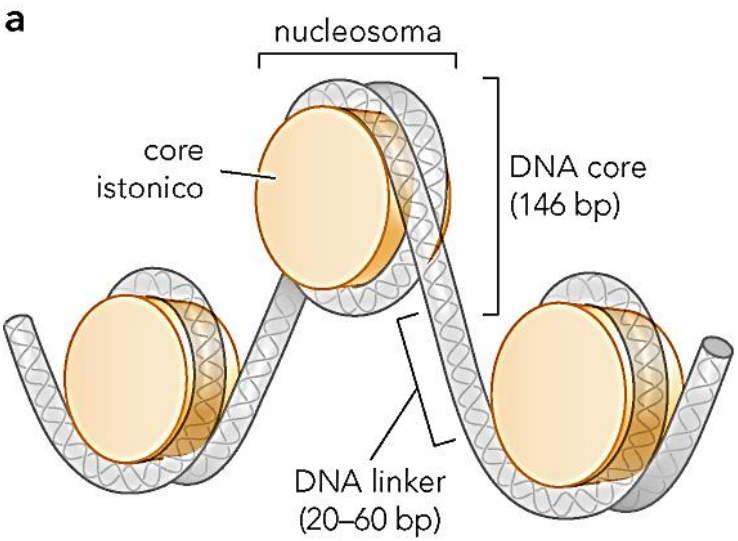


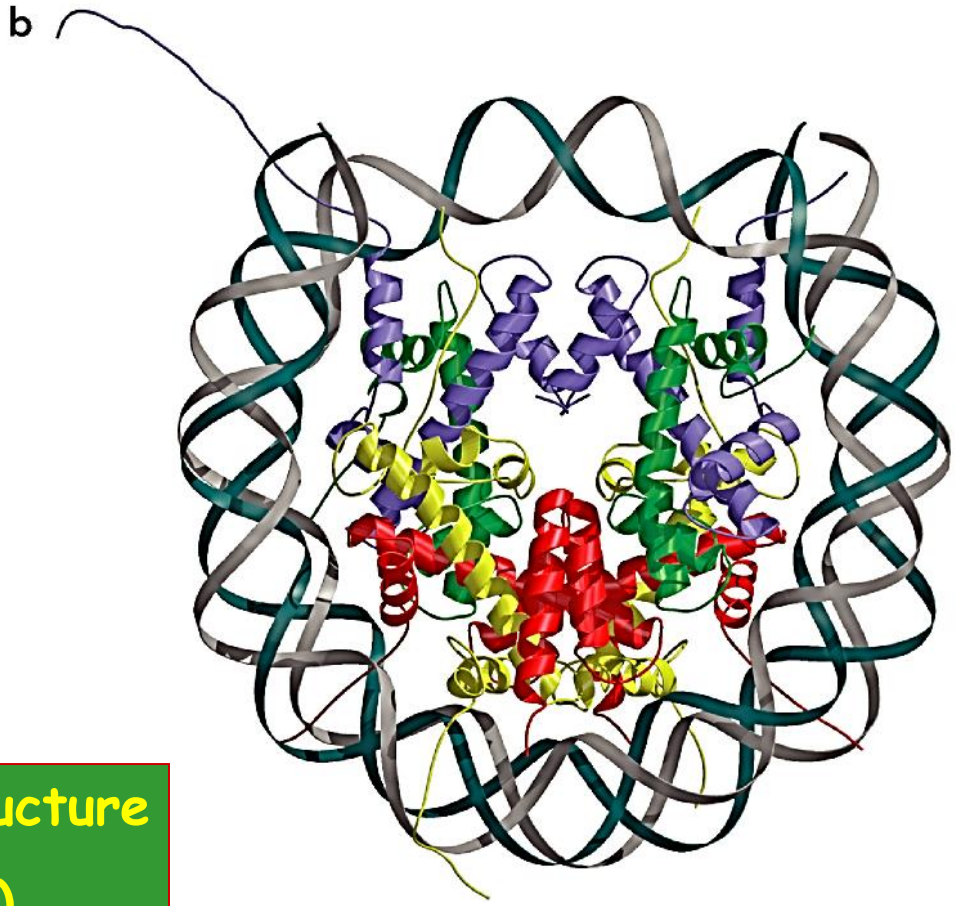
A rat liver cell nucleus at Transmission E.M.

What we find in peripheral spaces not occupied by Heterochromatin ?

What does the nucleolus do ?





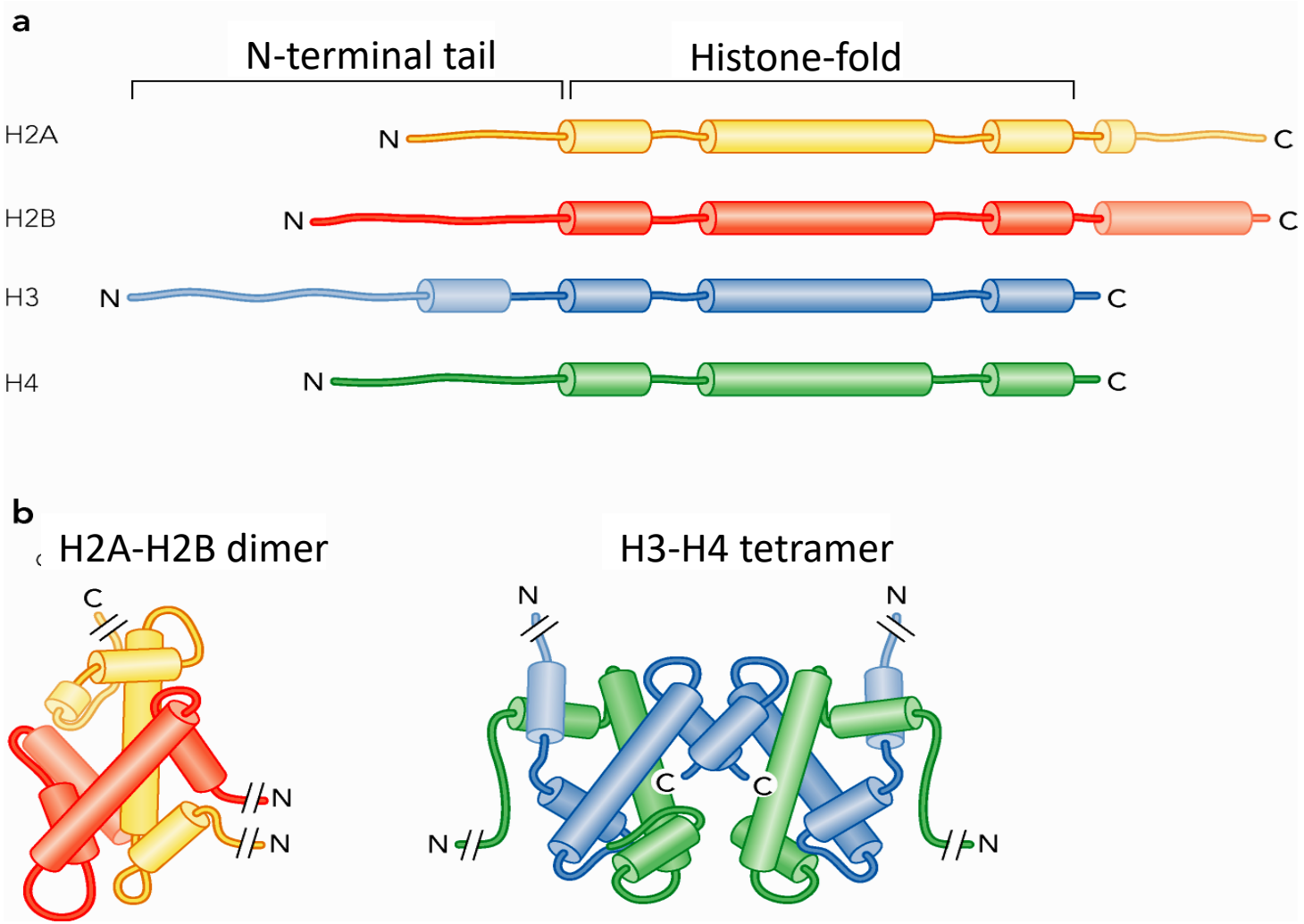


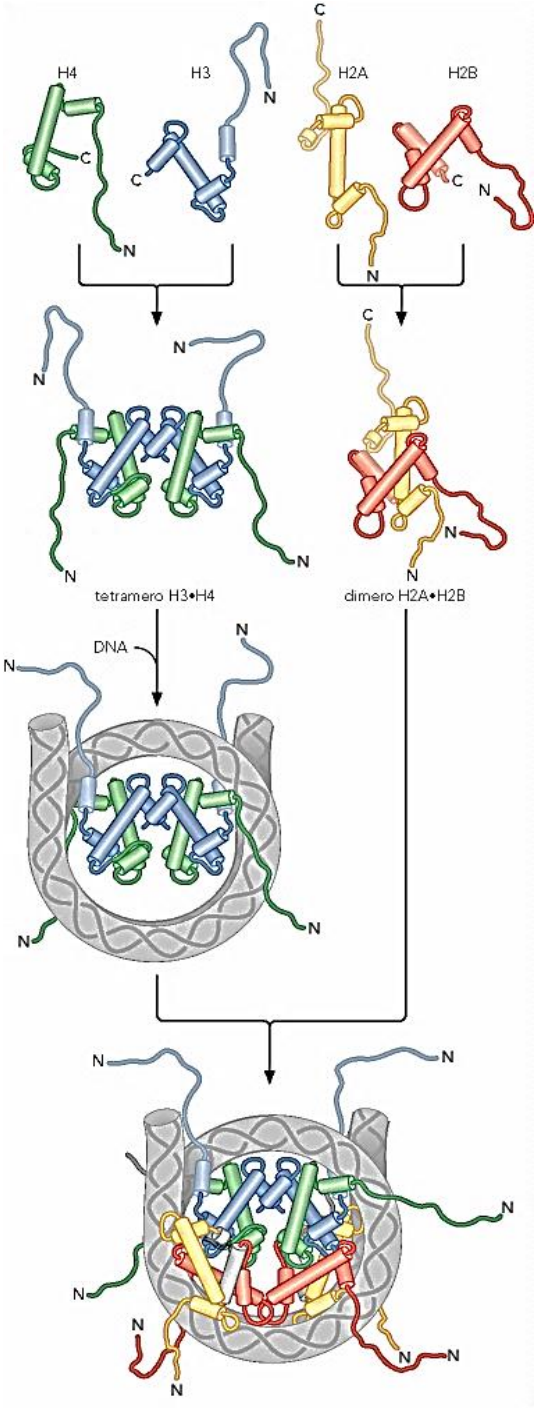
Nucleosome DNA structure

topology (supercoiling)

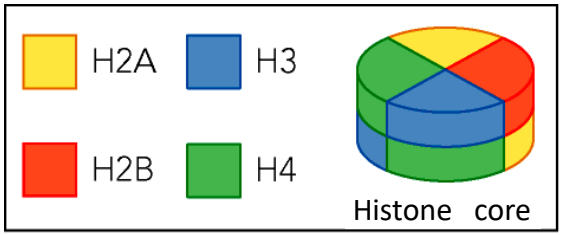
topoisomerases

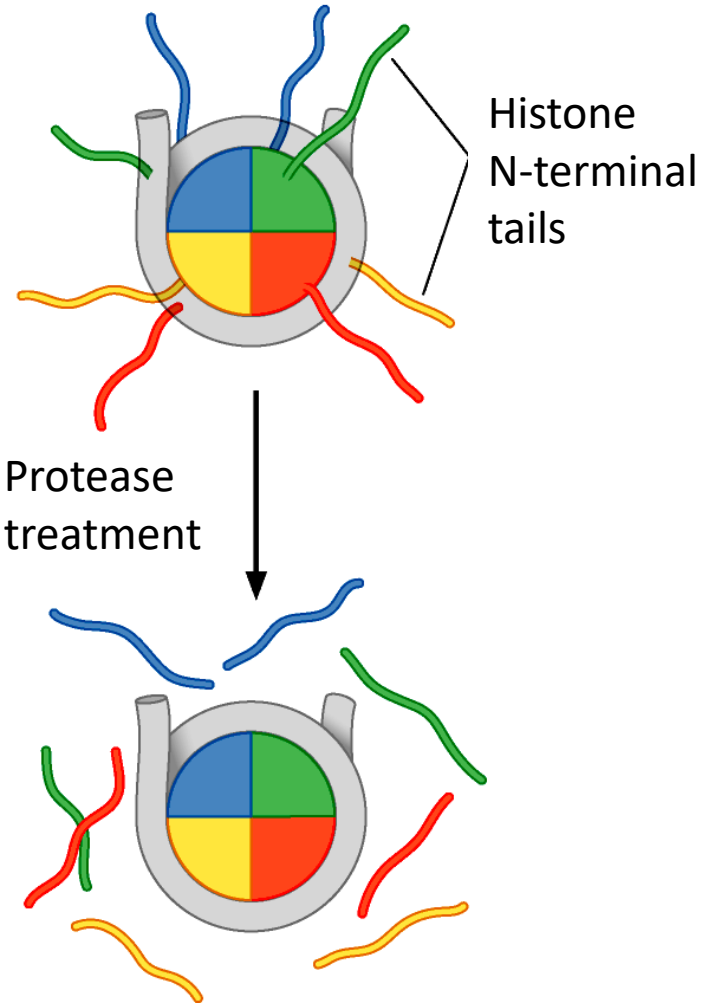
Histones


a
N-terminal tail
Histone-fold
H2A
H2B
H3
H4
N
C
b
H2A-H2B dimer
H3-H4 tetramer
C
N
N
N
N
C
C
N
N

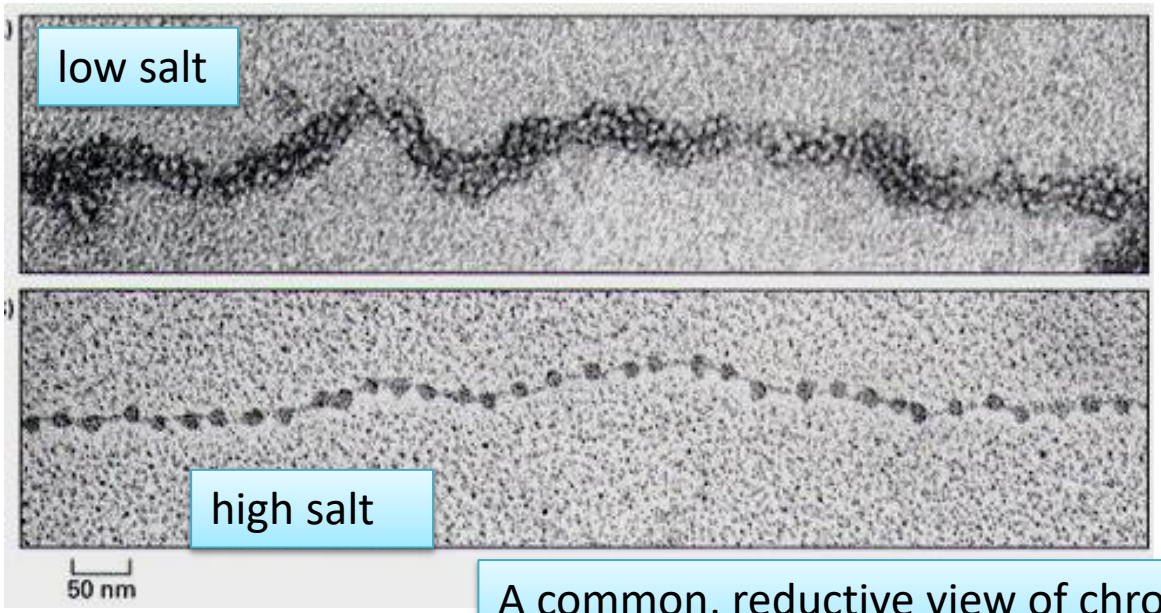


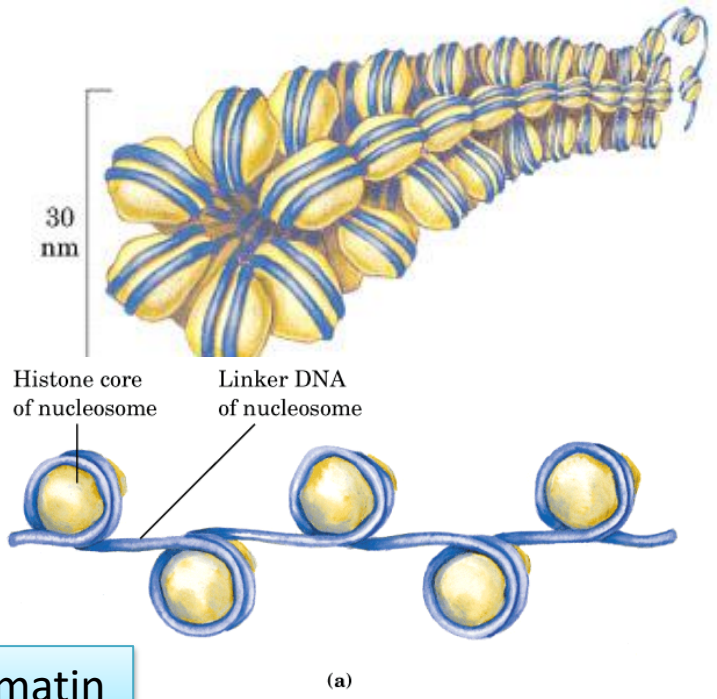
Nucleosome assembly & N-terminal tails



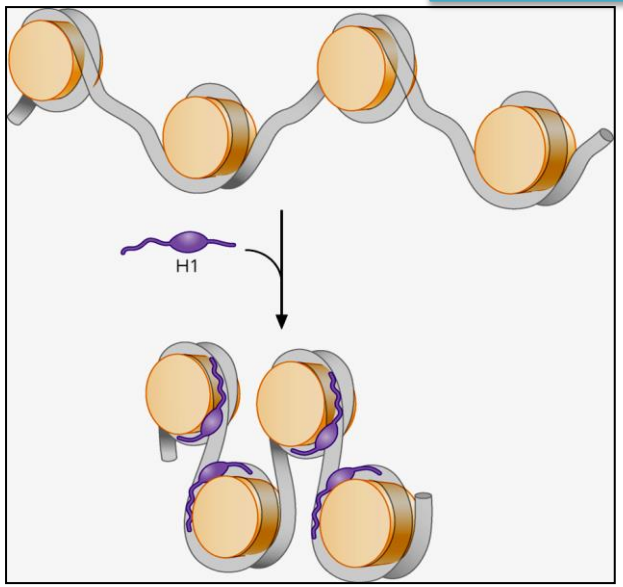


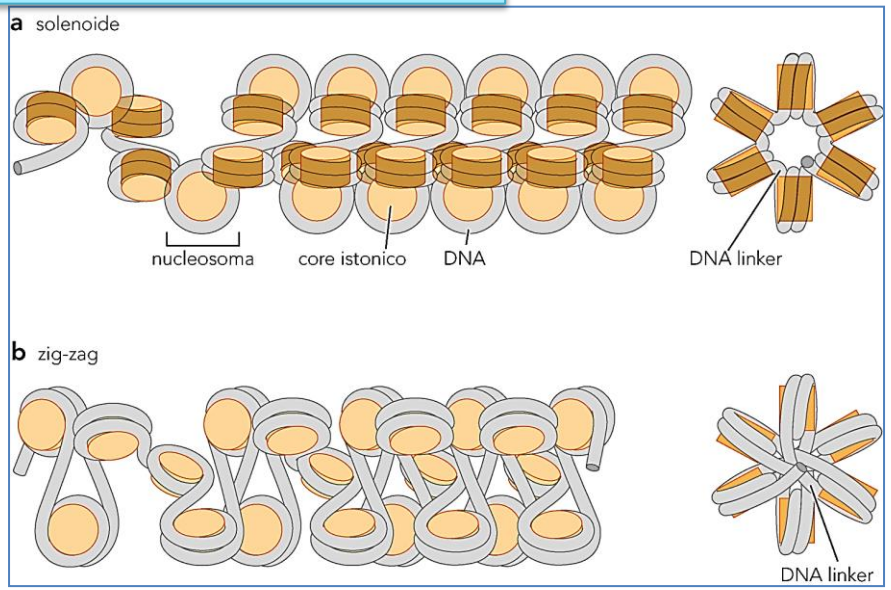
Basic chromatin structure





A common, reductive view of chromatin



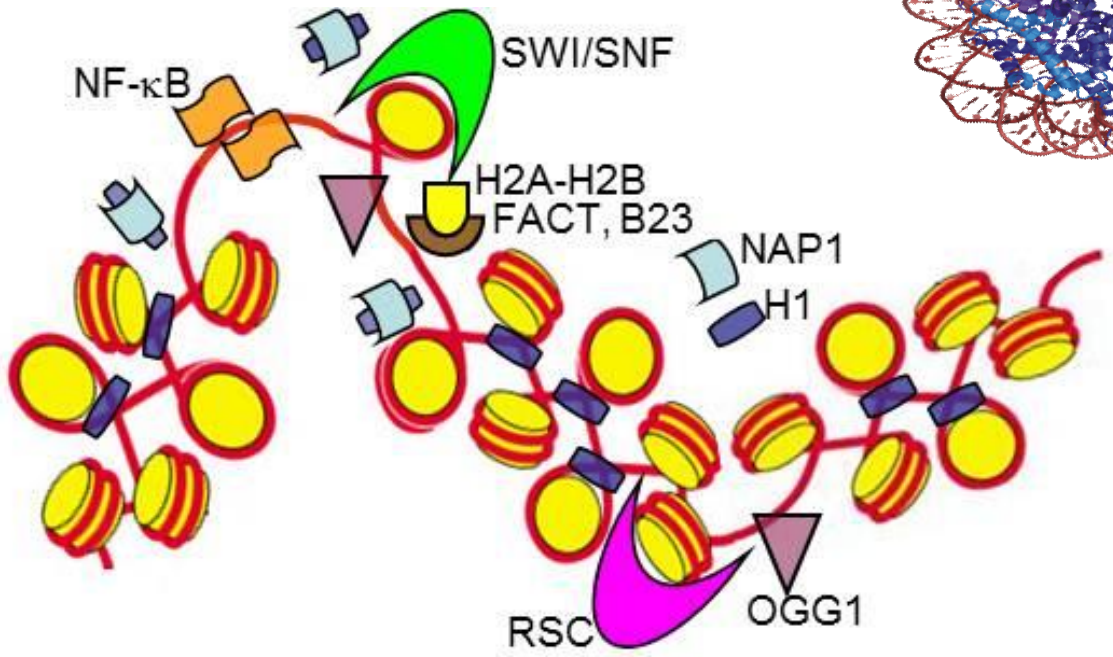


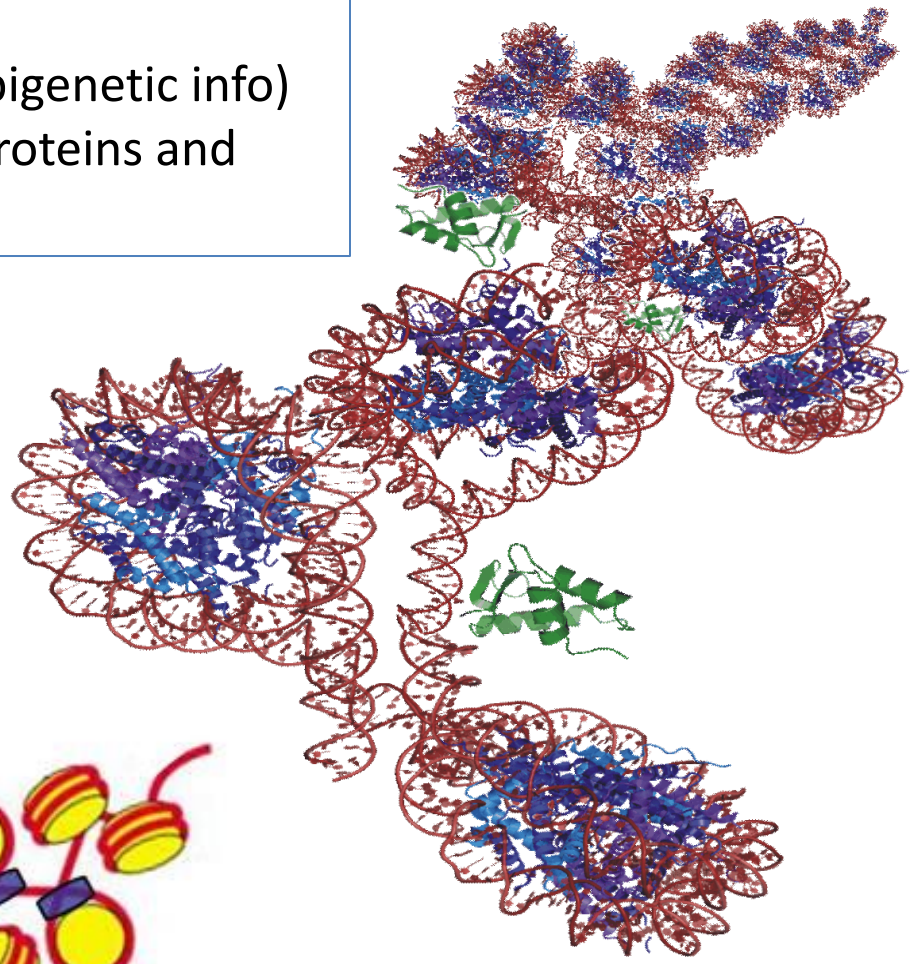
Heterochromatin

- **Facultative**
- **Constitutive at centromeres, telomeres (repetitive sequences)**

Chromatin is composed of

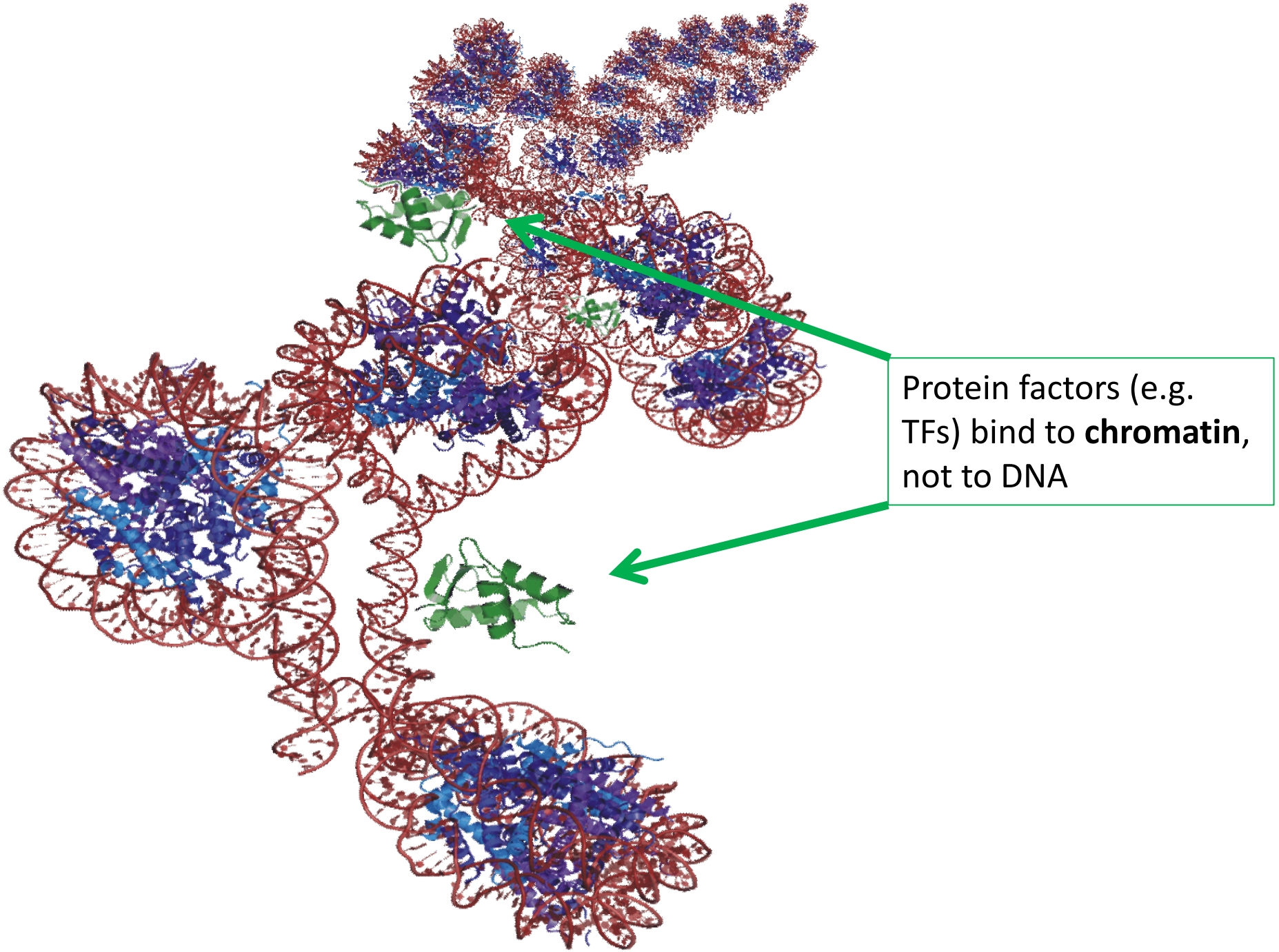
- DNA (genetic information)
- variously modified histone proteins (epigenetic info)
- DNA binding factors, enzymes, other proteins and noncoding RNAs (regulatory).





ENS, LYON

(Proteins in this cartoon are just examples)


Protein factors (e.g. TFs) bind to **chromatin**, not to DNA

Molecular organization of **heterochromatin** *versus* **euchromatin**

Features distinguishing HC from EC

1. Accessibility
2. Nucleosome positioning
3. Post-translational Modifications to Histones (PTM)
4. Histone-binding proteins and modifying enzymes
5. Histone variants

nucleosome

6. **DNA methylation**
7. **Noncoding RNA**
8. **Transcription factors**

1. Accessibility

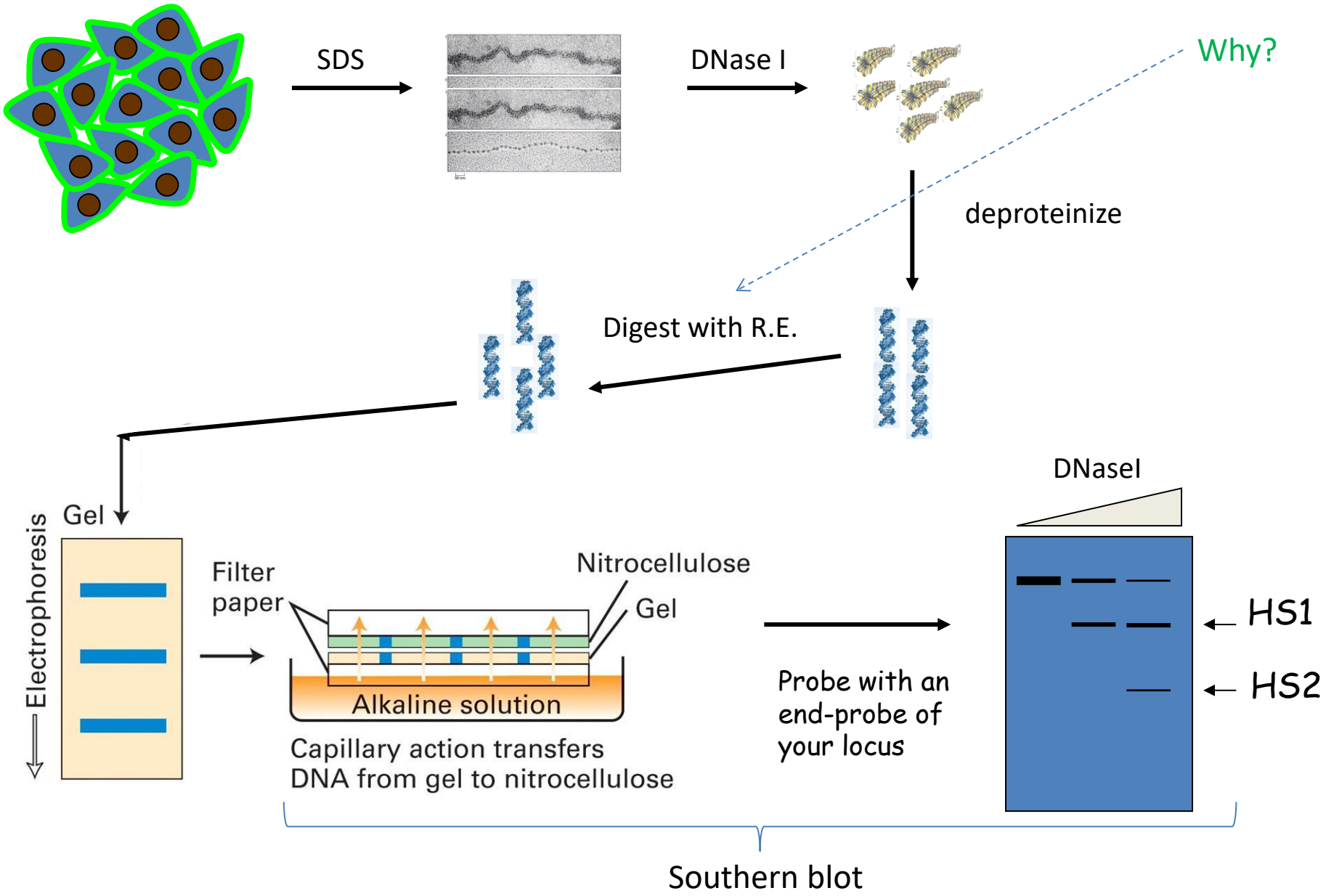
General principle: **enzyme accessibility**.

Dnase I assay → Dnase HS sites (hypersensitive)

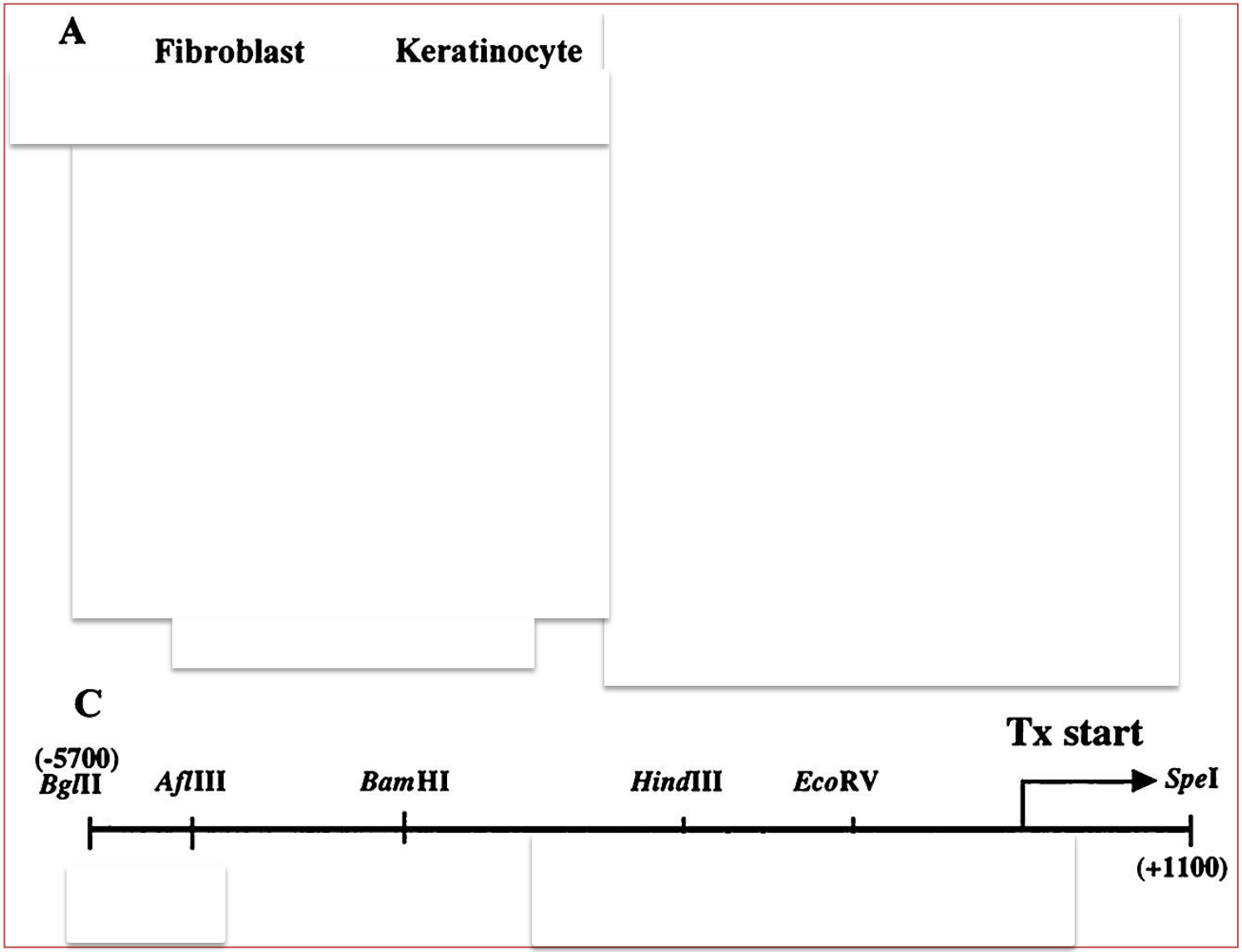
Let's see :

1. a scheme
2. an example taken from a quite old paper.

The classical assay to detect the gross organization of chromatin at a specified locus: the **DNase I Hypersensitivity** Assay



Keratin K14 gene, analysis of the 5’-flanking region



Tratto da: Sinha et al., (2000), Mol Cell Biol, 20: 2543-2555.

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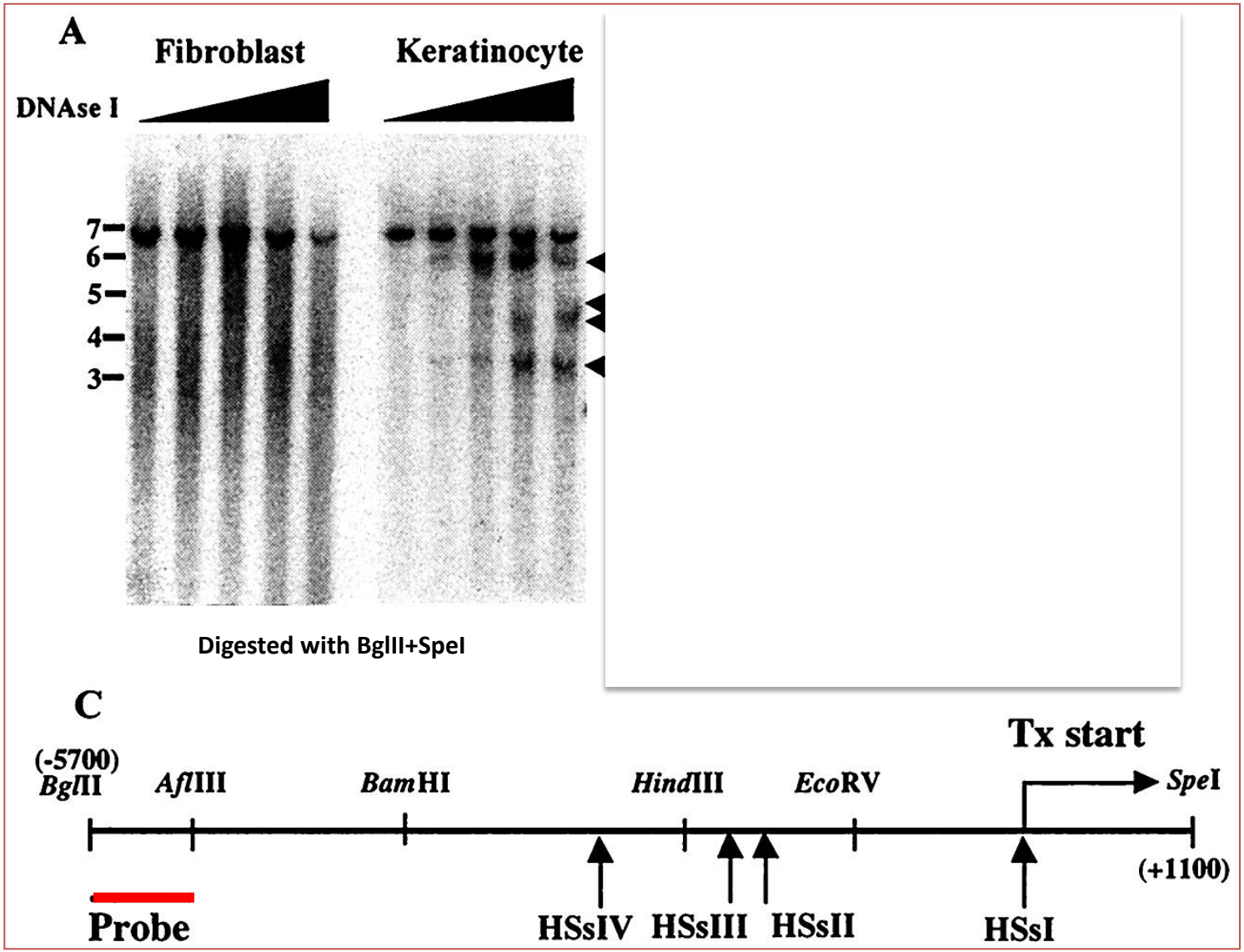

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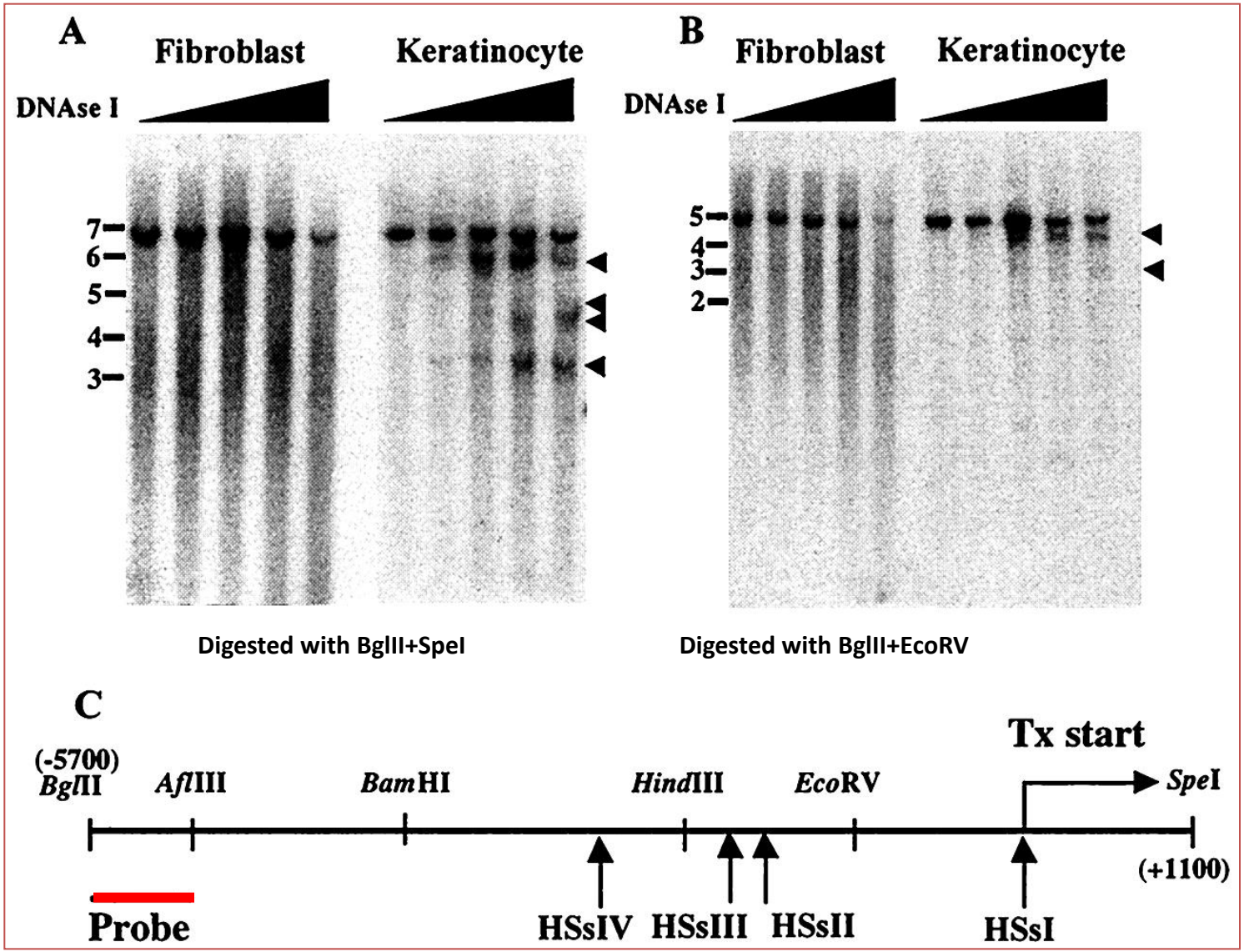


Tratto da: Sinha et al., (2000), Mol Cell Biol, 20: 2543-2555.

If accessibility is not the same in all parts of chromatin, may it depend (also) from different distribution of nucleosomes ?

2. Nucleosome positioning

Questions that are relevant to genome organization:

Do nucleosome display preferred positions in the genome ?
(are they just arrayed regularly spaced as they appear in pictures?)

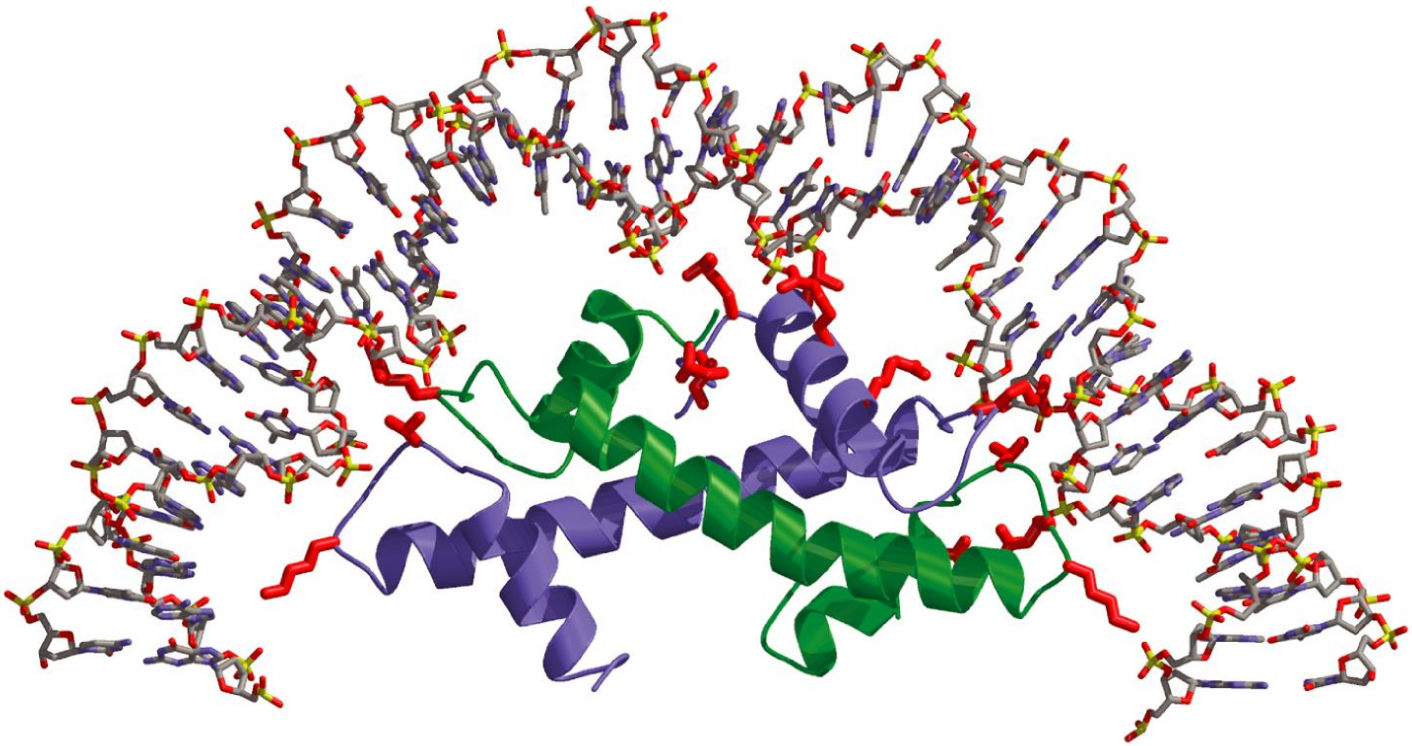
Do nucleosomes display any kind of **specificity** for nucleotide sequence?

Are nucleosome mobile ? Can they translate position over DNA ?
(are they stable on the same sequence they were assembled to ?)

Is nucleosome position **functional** to gene/genome regulation ?

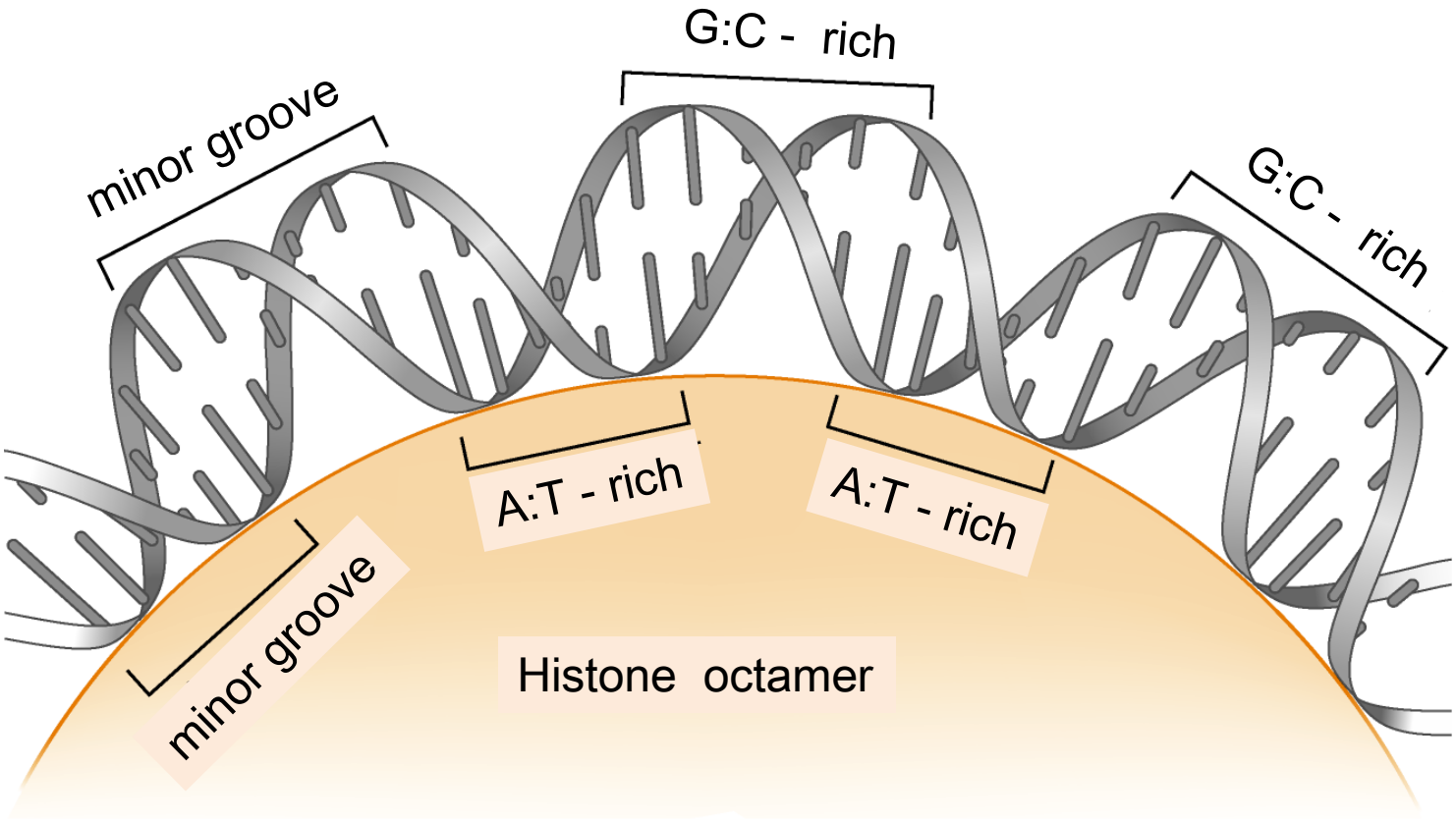
«Passive» positioning

Protein-DNA contacts are mainly due to basic aminoacids – backbone phosphates



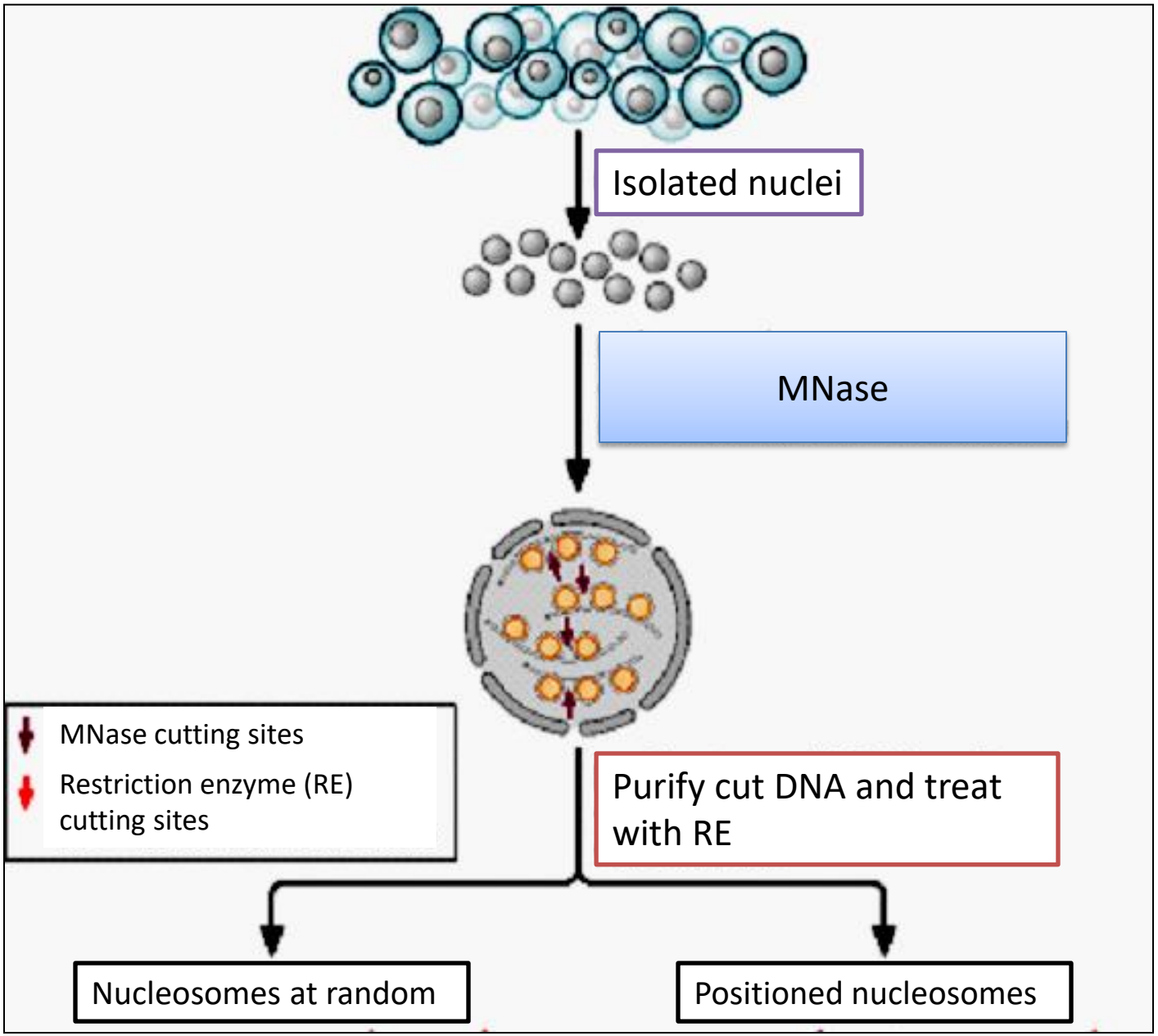
«Passive» positioning

Weak sequence specificity: in general, A/T rich sequences are in touch, whereas C/G rich stay outside.
This is due to intrinsic bending of DNA axis in different sequence contexts.



Nucleosome positioning analysis

1st step: digestion

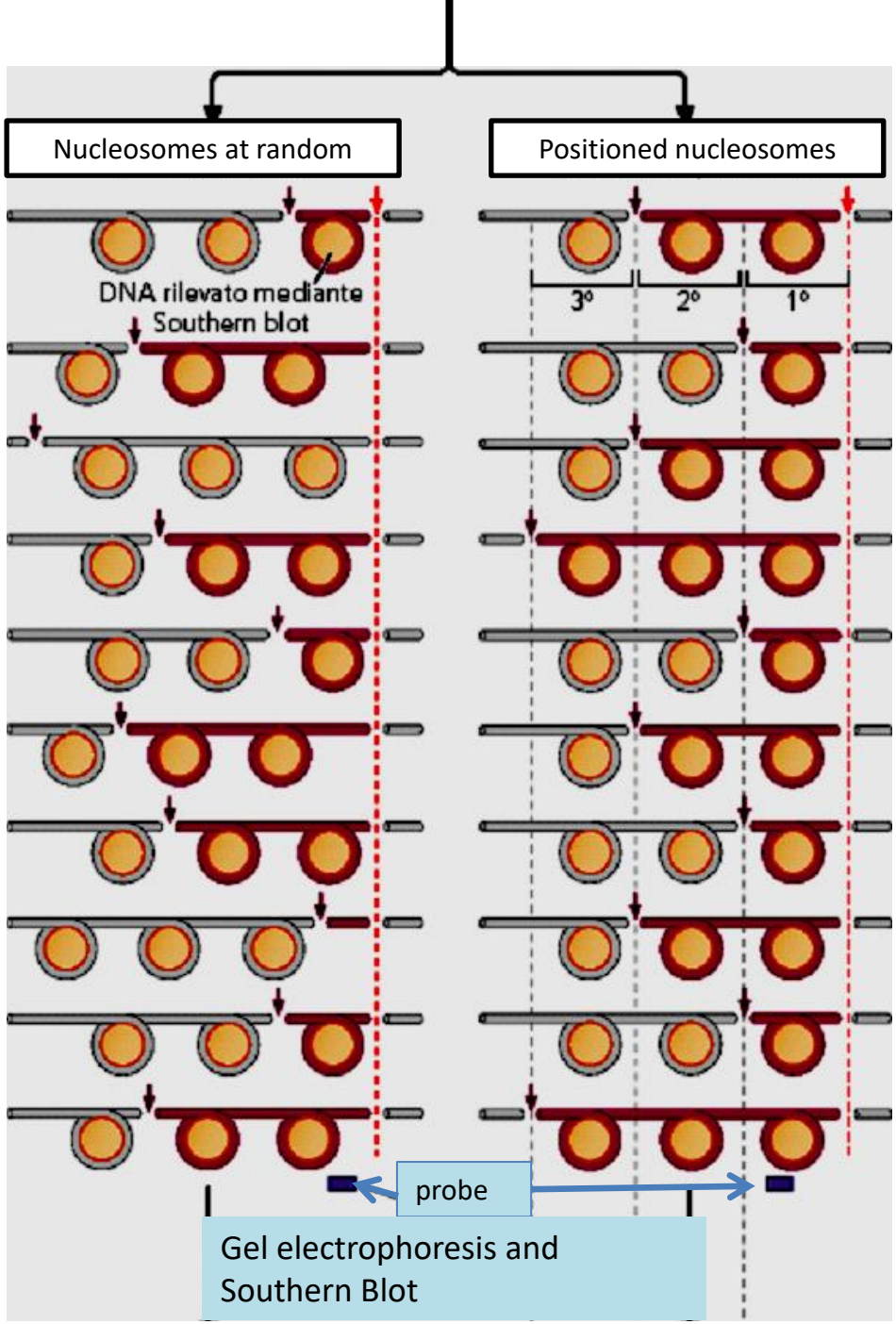


Micrococcal Nuclease I is a mixed endo-exonuclease.

MNase induces single-strand breaks and subsequently double stranded ones by cleaving the complementary strand in close proximity to the first break. MNase continues to digest the exposed DNA ends until it reaches an obstruction, such as a nucleosome or a very stably bound Transcription Factor.
In appropriate condition, then, MNase releases fragments of approximately one nucleosome length (~147 bp), which are typically selected for sequencing in MNase-Seq experiments.

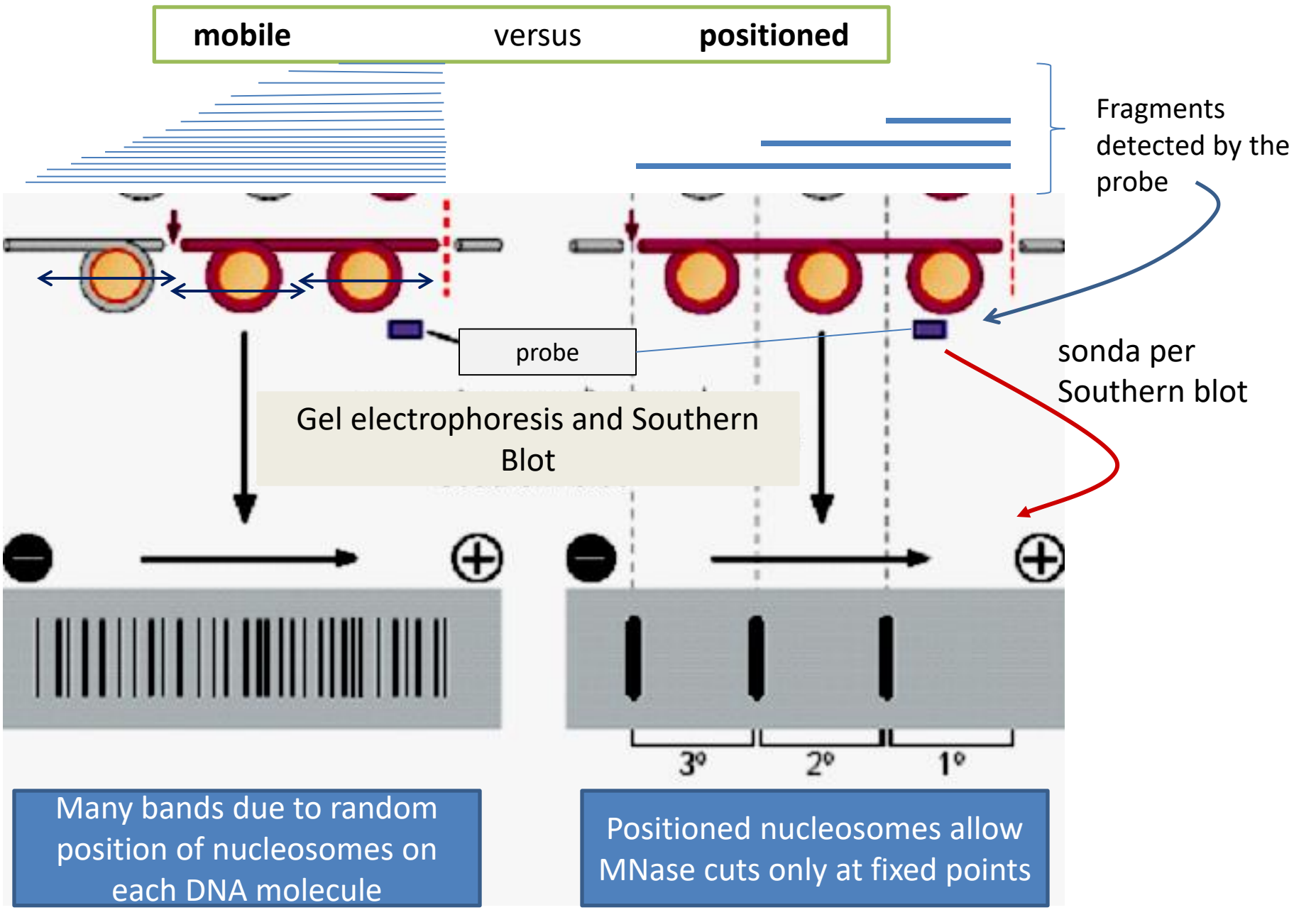
2 different cases:
mobile nucleosomes
versus
positioned nucleosomes

each row is one molecule of DNA extracted from cells



⬇ MNase cuts

⬇ R.E. cuts


mobile versus positioned
Fragments detected by the probe
probe
sonda per Southern blot
Gel electrophoresis and Southern Blot
3°
2°
1°
Many bands due to random position of nucleosomes on each DNA molecule
Positioned nucleosomes allow MNase cuts only at fixed points