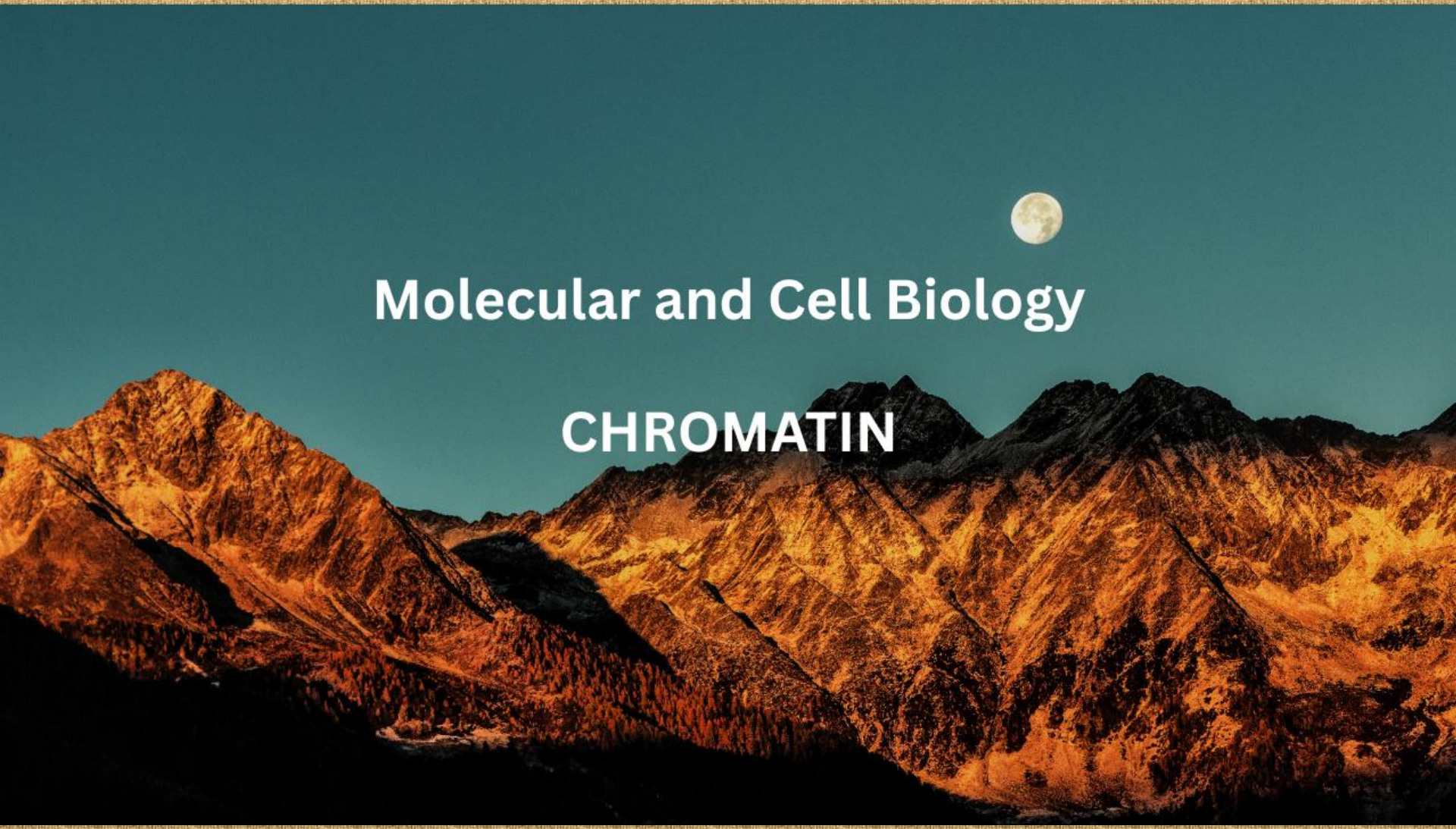
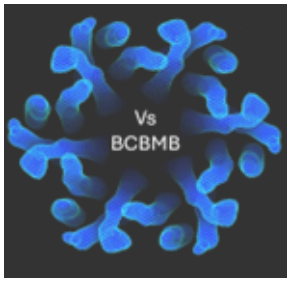

Molecular and Cell Biology
CHROMATIN



RECAP

The previous chapter about "Genomes", revealed first level answers to the following points:

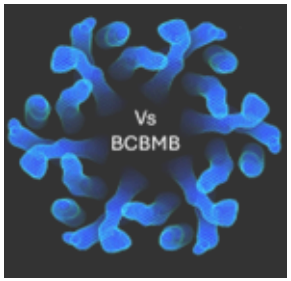
- **What** a genome is: *minimum complement of DNA that defines an organism (haploid set of "chromosomes")*
- **Why** genome size does not scale linearly with complexity of organism: *lots of non-coding DNA, some of which consists of mobile elements (transposons) that keep making copies of themselves*
- **Why** "non-coding" is not a synonym for "non-functional": *while some of the functions of non-coding DNA remain to be discovered, many functions have ALREADY emerged – eg.* ***centromeres, telomeres, pseudogenes, introns****, protection against mutation, error reduction during recombination events, alternate splicing, regulatory sequences*
- **Why** large genomes require subcellular confinement: *[conc] of reactants for DNA dependent processes, physical separation, regulation of access/read out*

At the end of the previous chapter we were left with the question how the enormous length of the genome – 2m in a diploid human cell – can physically be accommodated within the space available in the cell nucleus.

We discovered that breaking up the DNA into discrete pieces – chromosomes – helps but still requires an ~10,000-fold shortening of the end-to-end length of the chromosomes (down from 150,000-fold if were one piece).

Pondering how that shortening is possible, we were left with the perspective that it can be accomplished by exploiting the idiosyncratic elastic properties of double stranded DNA … which -with the help of proteins - allow the formation of a structure known as "chromatin"

This chapter is focused on an exploration of chromatin structure



GOALS of this CHAPTER

adding to the answers we already found, you should be able to answer the following three questions by the end of this chapter:

How genetic material is compacted (difference between ”nucleoid” and “chromatin”)

How compaction affects the structure of eukaryotic cell nuclei

How eukaryotic cell nuclei exchange matter/information with the cytoplasm

TIP: if you can get your hands on a pair of "Red-Cyan Glasses"…keep them handy…
(to use … make sure the red window is over your **left** eye)

Chromatin Overture

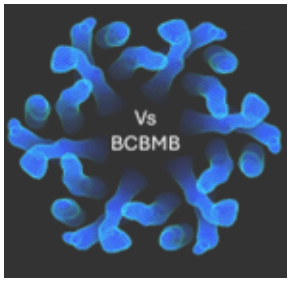
starting at 50 sec into it, watching this Video
Will Change the Way **How You Think About DNA and Chromatin**

Cirque De Soleil Luzia: Alebrije

https://www.youtube.com/watch?v=mTPe6fwOGP4

If you are curious – you can watch a short BBC documentary about Aleksei here:

https://www.youtube.com/watch?v=b4WS_wts1Ug



Setting the Stage

I hope you watched and enjoyed the video of Aleksei's performance.
Watching it, you may have asked yourself – what on Earth has that to do with DNA and/or Chromatin?

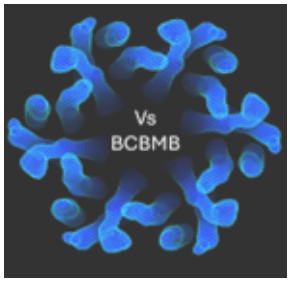
As we go, I hope you will see what the answer is

Lets start with summarizing what you saw …..

….how would you describe it?

Aleksei is a ……. ?

He can do these things because his body is extremely …….. ?



Setting the Stage

Aleksei is a …… **contortionist** (so is DNA)

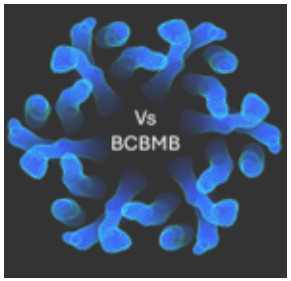
He can do these things because his body is extremely …….. **flexible** (so is DNA)

if you went beyond the call of duty and watched the short documentary about Aleksei, you may have found it "wild" that he will watch TV in this pose …

…and there you go … this starts to look a lot like a "supercoil" ….

a What…..?

…supercoil – a topological winding state of DNA … lets explore….



Supercoiling

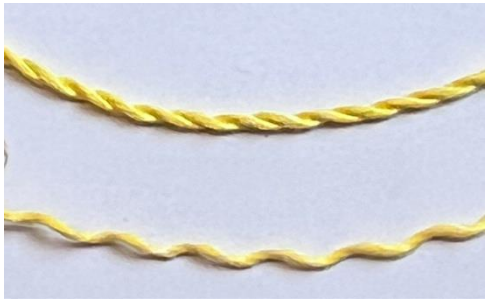
To understand some of DNA's mechanical properties – lets start with an exercise….

…..if you can find/get it somewhere ... take a piece of nylon rope like the one shown in the picture (Step 0)

while the details of the rope's structure are different from actual DNA, it's a reasonable approximation for our purposes and behaves qualitatively the same as actual DNA would.

students shared with me that doing this exercise helpd them a lot with understanding the material, but if you cannot (or do not want to) do this ...then the linked short videos will show you what you would have observed.

Step 0: remove one of the three strands of the rope to make it more closely "match" DNA structure (2 strands; top)

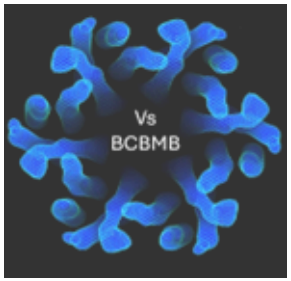


Step 1: like DNA the two strands of the rope form a right-handed helix → convince yourself that the rope has a right handed twist **CLIP1** (if you need help with that)

Step 2: hold one end of the rope with left hand – stretch tight extend your right thumb towards the left hand and take note of the direction your fingers are pointing.

→ what do you expect to happen when you twist the rope in the direction opposite of the direction that your right hand fingers are pointing? (= against intrinsic twist)
→ do it
→ what do you observe?
→ now bring your hands closer together….what do you "feel" and "see"?
→ let the system relax

CLIP2 (if you want to just see or confirm what to do)



Supercoiling

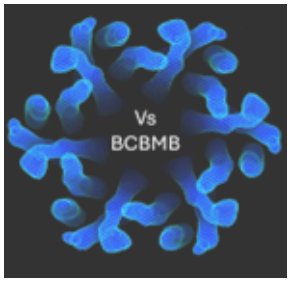
Step 3: repeat step 2 but this time twist in the direction of the rope's intrinsic twist

→ what do you expect to happen?
→ do it
→ what do you observe?
→ now bring your hands together….what do you "feel" and "see"?
→ let the system relax

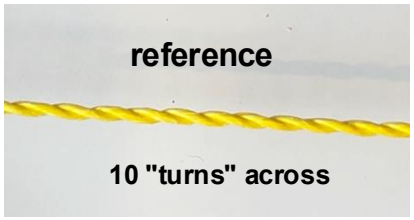
CLIP3 (if you just want to see or confirm what to do)

Step 4: repeat step 2 but after closing the rope into a circle → what do you feel/observe?

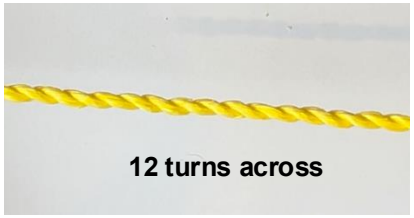
CLIP4 (if you just want to see or confirm what to do)



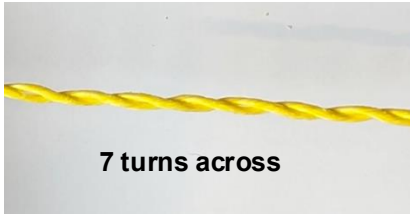
Supercoiling



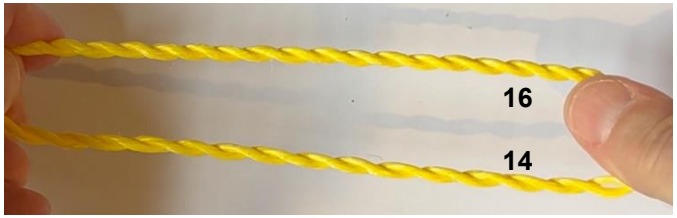
STEP 2



STEP 3



STEP 4

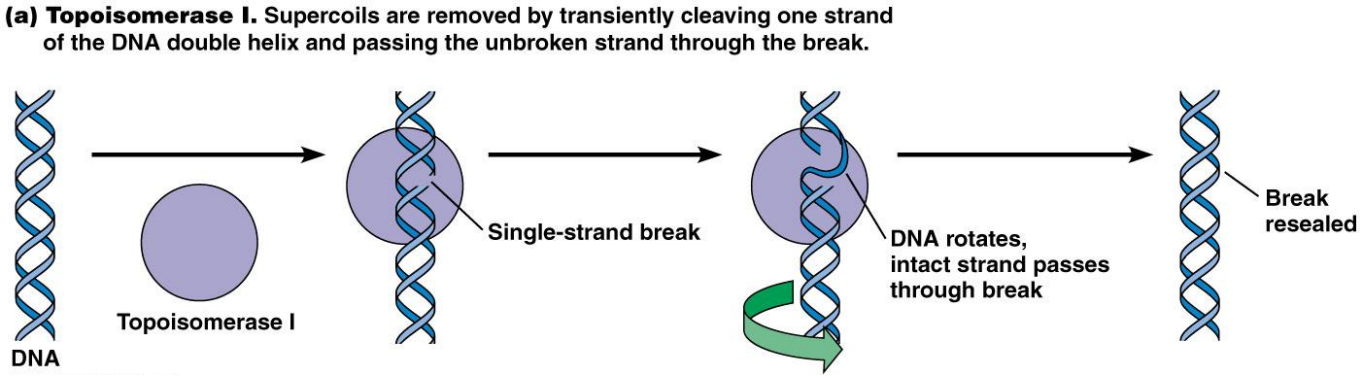


- ➔ Turning **against the direction of twist (Step2) = overwinding** = more tightly packed (at DNA level = more bp/turn than normal)
- ➔ Turning **in the direction of twist (Step3) = underwinding** = strands begin to and eventually separate (at DNA level = fewer bp/turn than normal)
- ➔ Both actions strain the backbone → mechanic strain can be released by snapping polymer into a "supercoil" ➔ supercoiling shortens the length! (you hands come closer together) **<u>and</u>** normalizes the twist ➔ **supercoiling is an essential aspect of how cells manage to compact DNA to fit into the available space.**

Supercoiling and Proteins

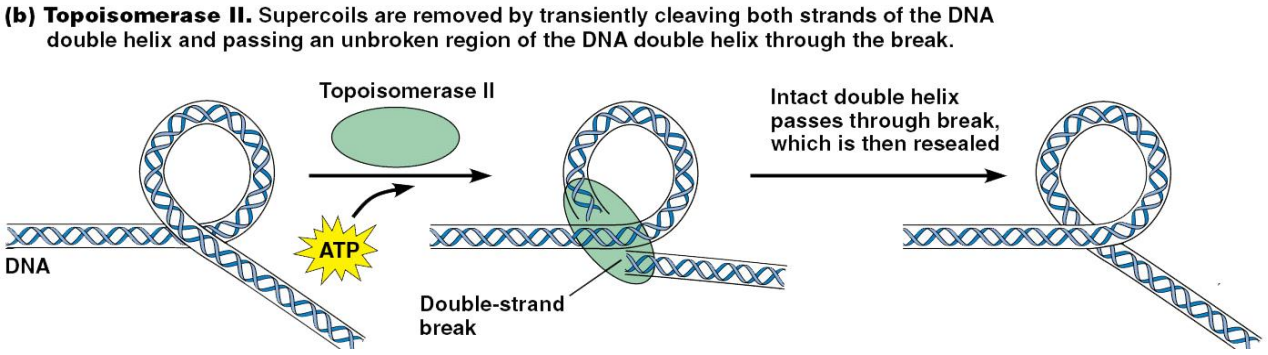
Cells **cannot** create supercoils in DNA the same way we did with the ropes because it would require two "clamps" to twist one relative to the other while holding on to DNA **until after** the supercoil has formed. Such a mechanism does not exist.

→Fortunately, over-/underwinding can be accomplished through alternate routes that are easy to do, are quite clever and use .....**proteins**



Topoisomerases = **several co-exist in same organism**

Topo I: **only relax supercoils** and require no energy beyond what release of backbone stress provides.



Topo II: **also relax supercoils but require energy (ATP)**; remove two supercoils at once (here: cross in front to cross in back).

Single exception in bacteria: gyrase B; this special topisomerase II **can make supercoils by underwinding** the DNA (=reducing the number of bp/turn)

right-handed supercoil
(like in STEP 2 of exercise)

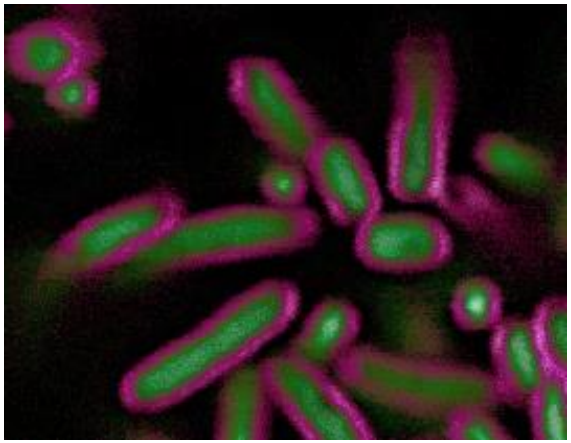
left-handed supercoil
(like in STEP3 of exercise)

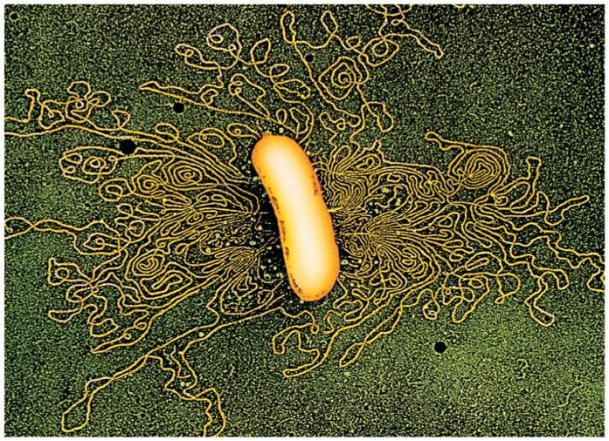
Supercoiling and Proteins

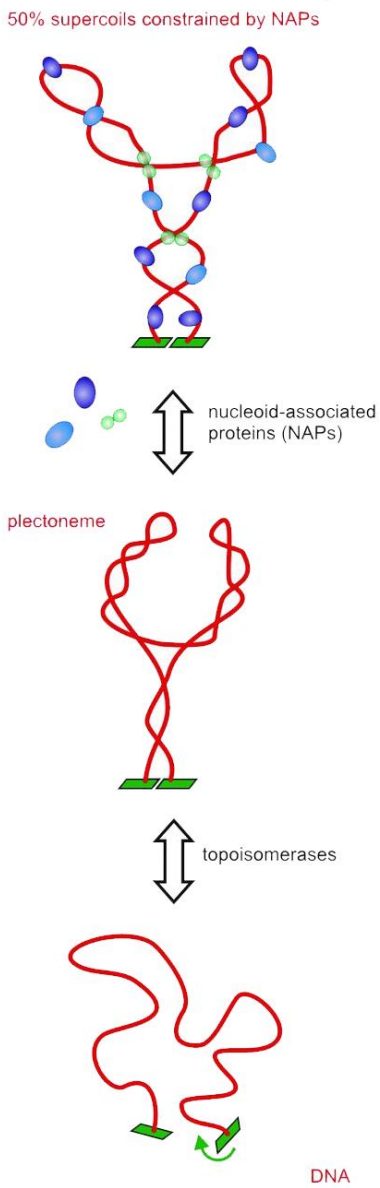
Introduction of supercoils by underwinding leads to a compaction of the bacterial chromosome. The supercoiled state is then further stabilized by scaffolding proteins and RNA molecules.

→ Final nucleoid resides in the center of the cell → length of nucleoid is 1000-fold shorter than total length of the DNA (= ~1.6mm → 1.6µm)

Gentle lysis of bacterial cells disrupts some of the chromosome structure and allows less compressed "DNA" loops to "spill out"





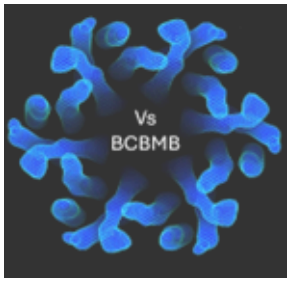


Luijsterburg M.S. et al (2008), ***Crit Rev Biochem Mol Biol*** *43:393-418*

Now that we are warmed up by looking at the "easy" challenge that bacteria face, lets look at how human cells can (and need to) shorten their DNA by another order of magnitude ….10,000-fold shortening!

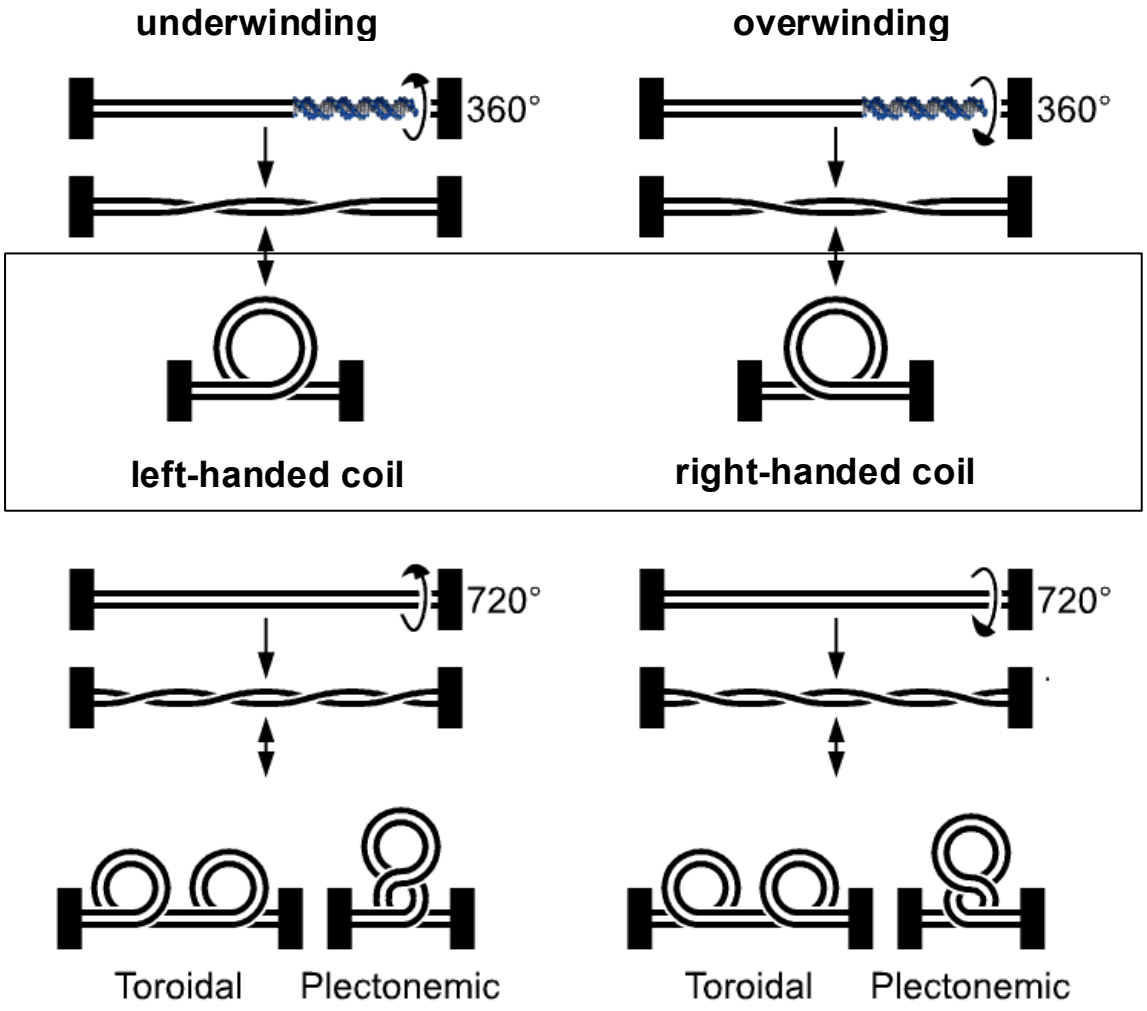
→Looking into this wonder, we start with an upsetting surprise:

Eukayotes have **<u>no</u> gyrase B** = cannot introduce supercoils with enzymes
➔ what now??



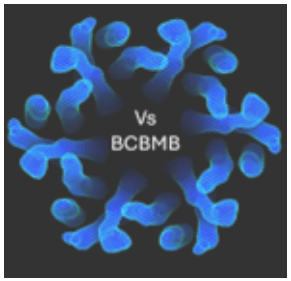
Supercoiling Without Gyrase B

To understand how eukaryotes achieve compaction without gyrase B...lets take a closer look at supercoiling of linear DNA segments (like human chromosomes).



Looking at this shape…does it give you any idea how we could get DNA supercoiled **without** using gyrase B?

....try to think of something .....



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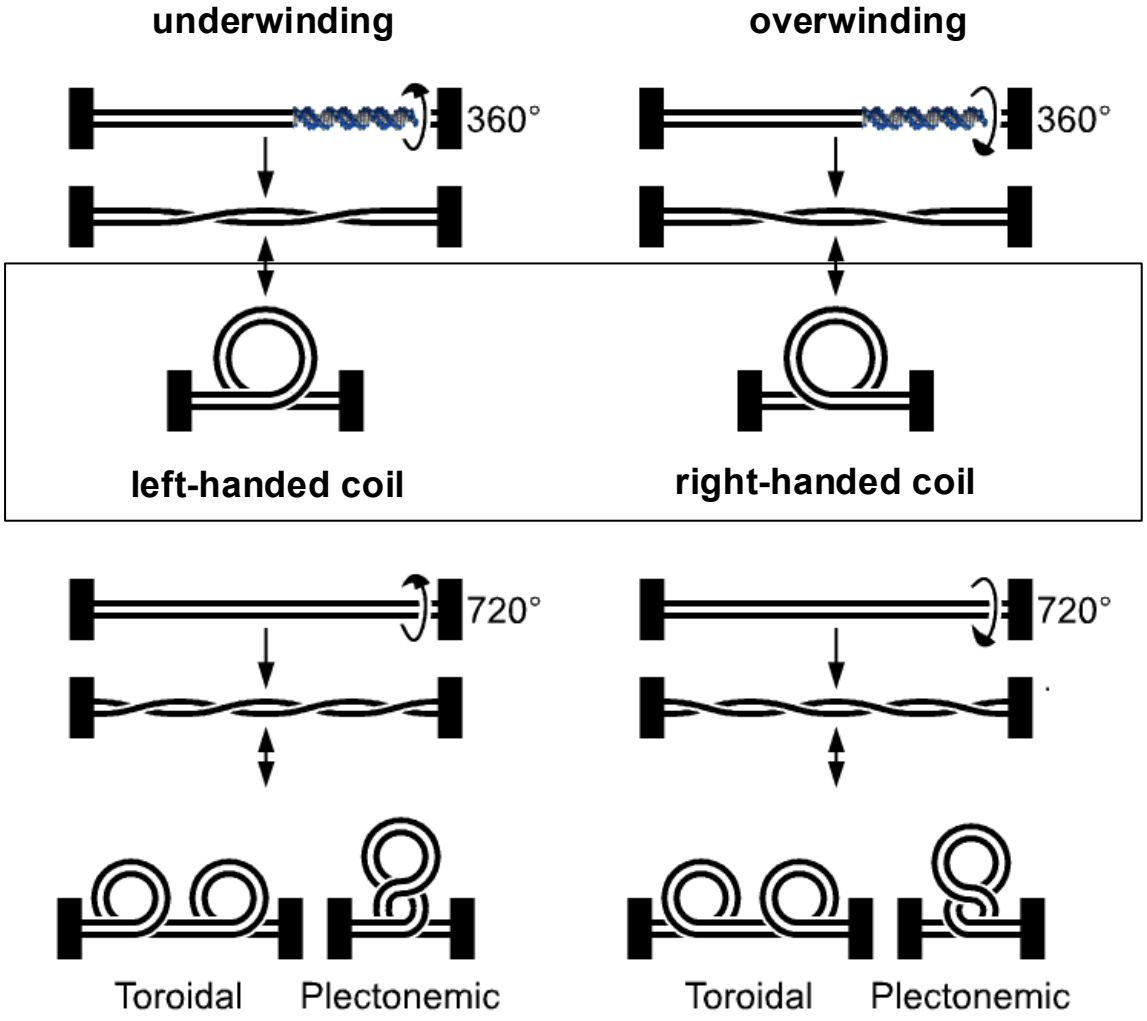
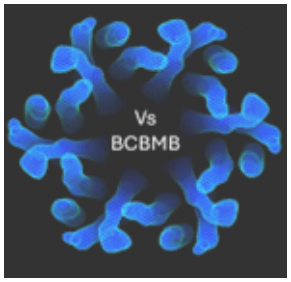


Looking at this shape…does it give you any idea how we could get DNA supercoiled **without** using gyrase B?

Answer: you could exploit the flexibility of the double strand to wrap it around some other molecule(s) like a ..??...(guess)

….yep, protein/protein complex



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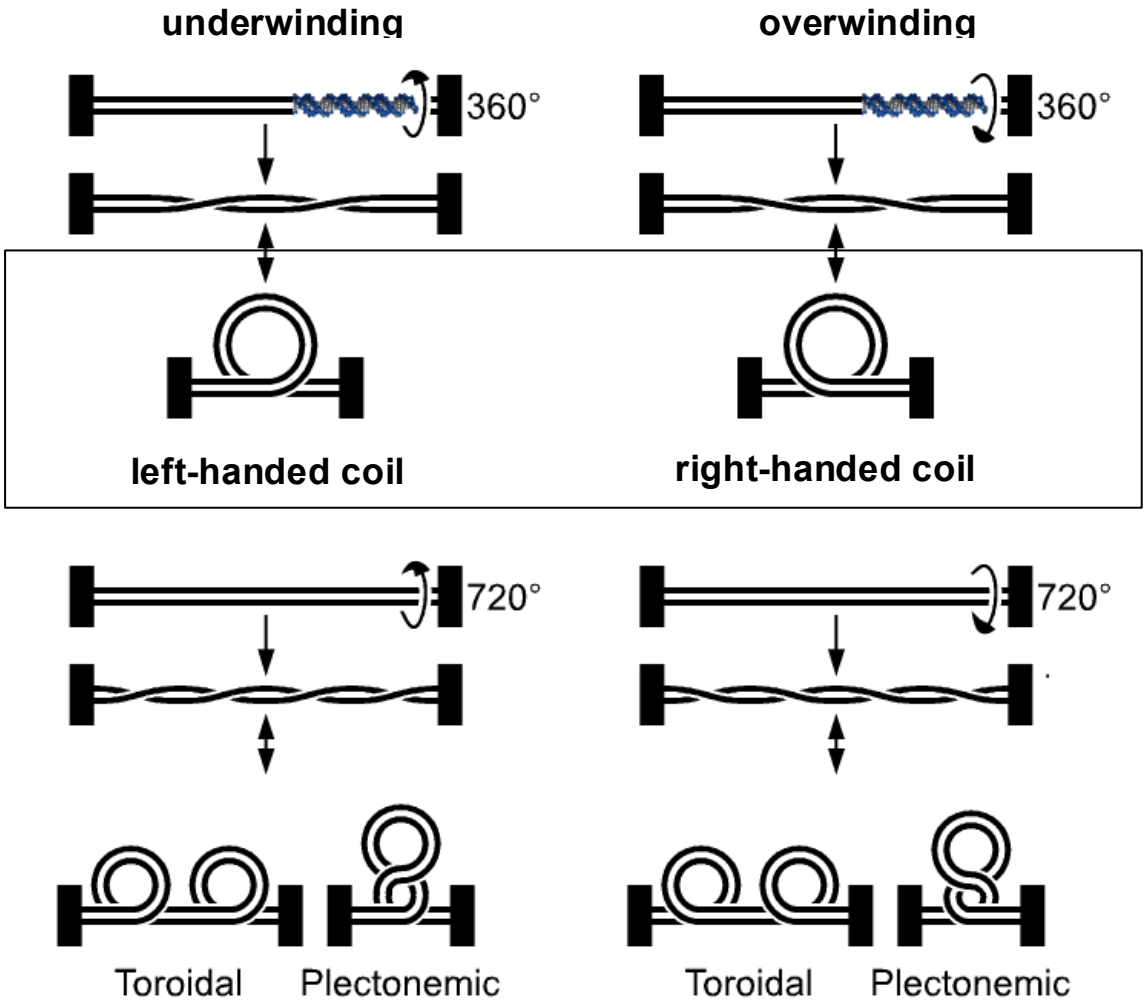
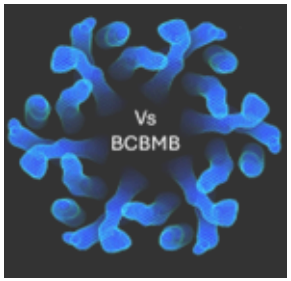


If you were to do that – which sense of winding would you use?

*…..**really** try to get engaged here and think about it for a moment…..*



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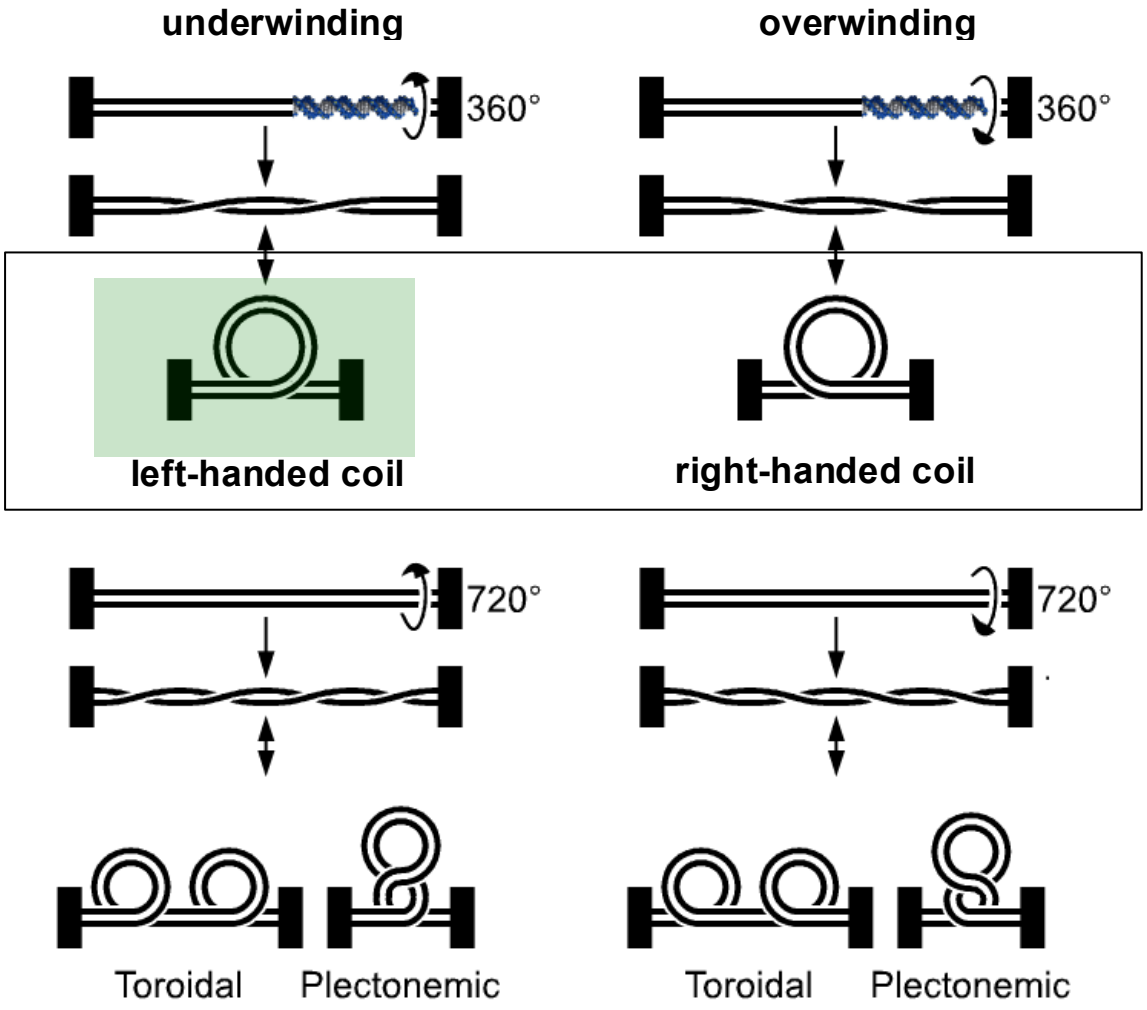
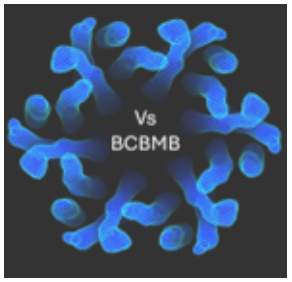


If you were to do that – which sense of winding would you use?

Answer: left one because underwinding DNA will make it easier to separate strands if you want to manipulate it (eg replication, transcription).

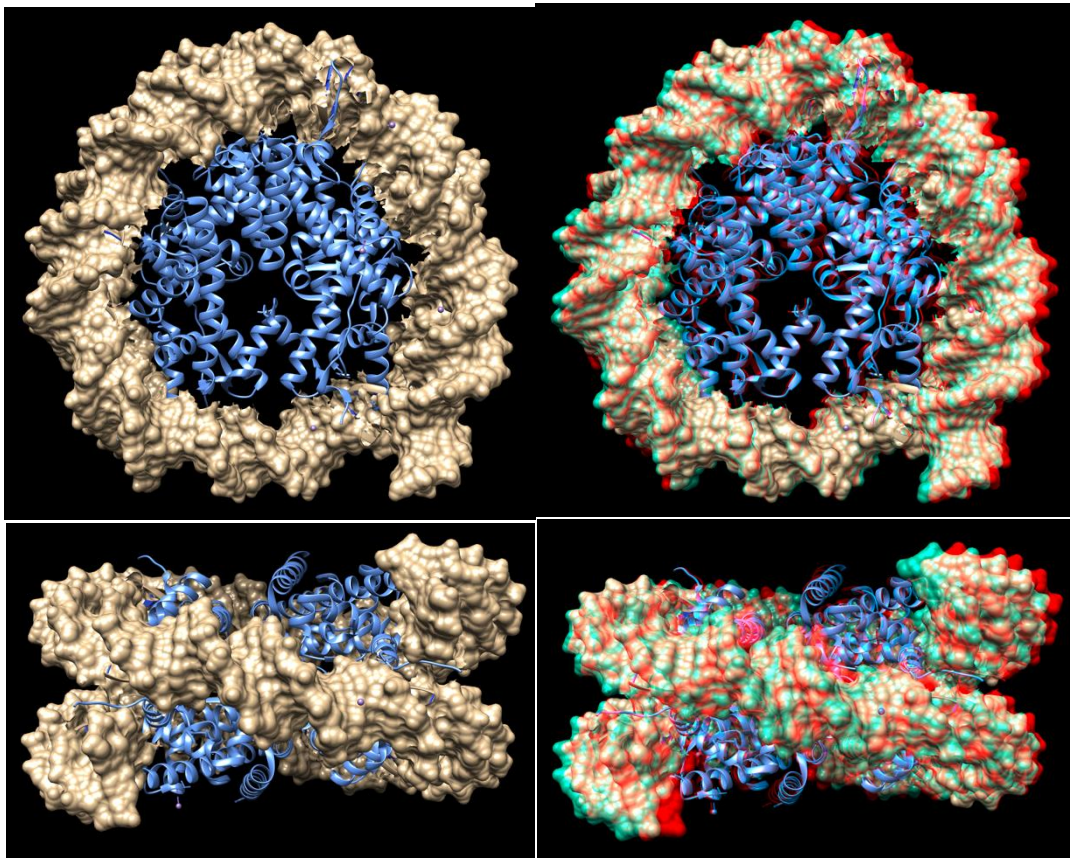
If you played with the rope, find a friend to twist it one way + hold it … then try to use your fingers to pry it open …..next: repeat, but ask your friend to twist it up the other way…. ==> you will find that the underwound rope allows strand separation more easily

→ is that the answer?



Supercoiling With Histones

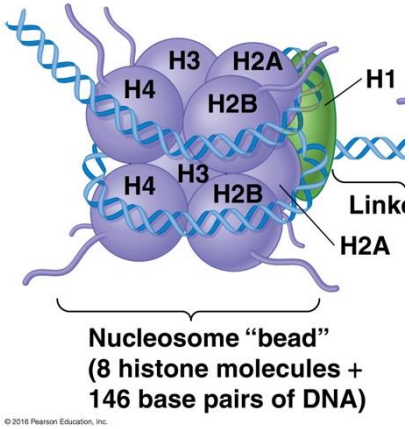
- Yes – that is the answer. Eukaryotes solved the problem of compaction by evolving a structure that is called “Chromatin” – a complex structure that is build from DNA, proteins (and RNA in a few cases)
- The basic unit that is used to construct all higher order chromatin structural elements are build from is called a **nucleosome.** Nucleosomes are a quaternary structure formed between DNA and an octameric complex of proteins called histones.



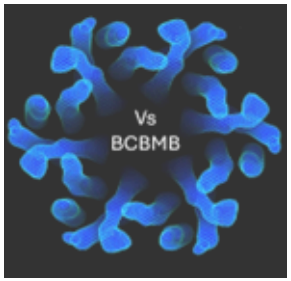
Mono view

Stereo view (red-cyan glasses)
cross-eyed version (next slide)

A nucleosome holds 146 bp of DNA that are wrapped around the core histone octamer completing 1.6 turns of a **lefthanded** superhelix (= underwinding!). *[Try to see this by following the instructions on the next slide]*

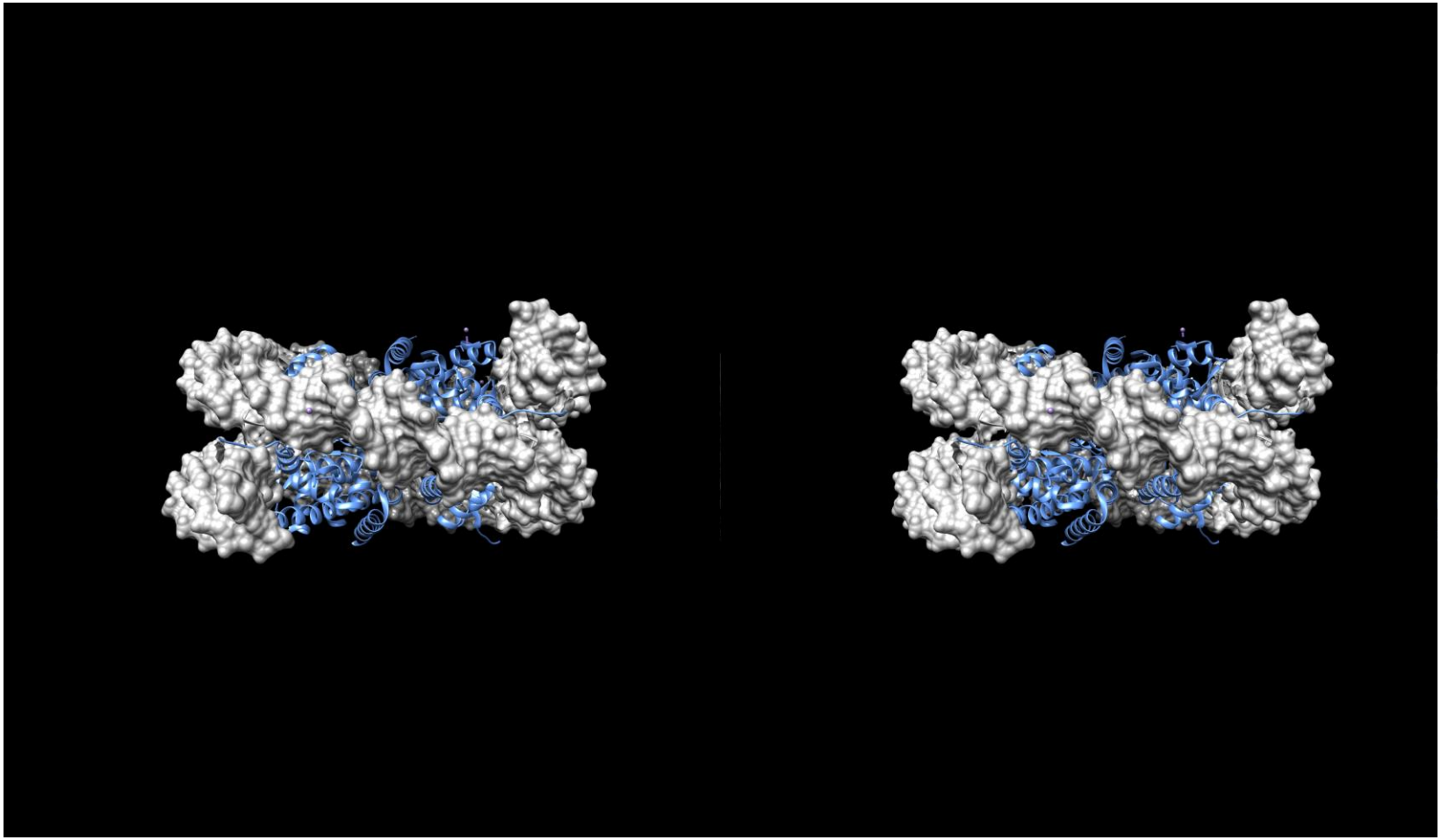


Winding DNA around histone octamers will shorten the effective length, but only by a factor of ~7-fold … = have a long way to go .....

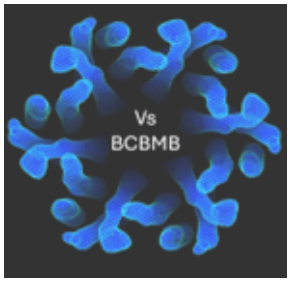


Supercoiling With Histones

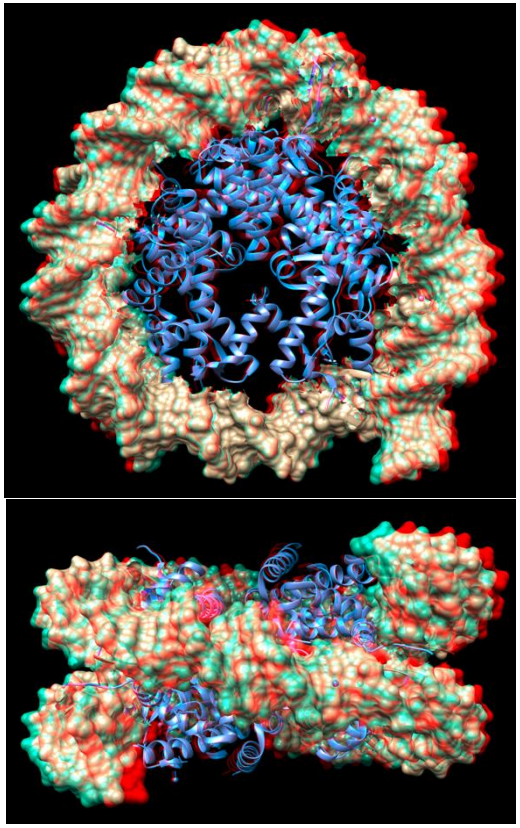
Look at the picture …. cross your eyes and keep adjusting your eyes until you see the 3D-effect. Once you have that, point the thumb of your left hand up, fingers curled towards you … bring it in the path of sight and convince yourself that the path of the DNA ascends (left lower corner → right top corner) in a lefthanded manner (following the direction of your thumb)



Cross-Eyed Stereoview of Nucleosome



Nucleosomes …Remember the Overture?



Stereo view (red-cyan glasses)

Aleksei is "overwinding" though…

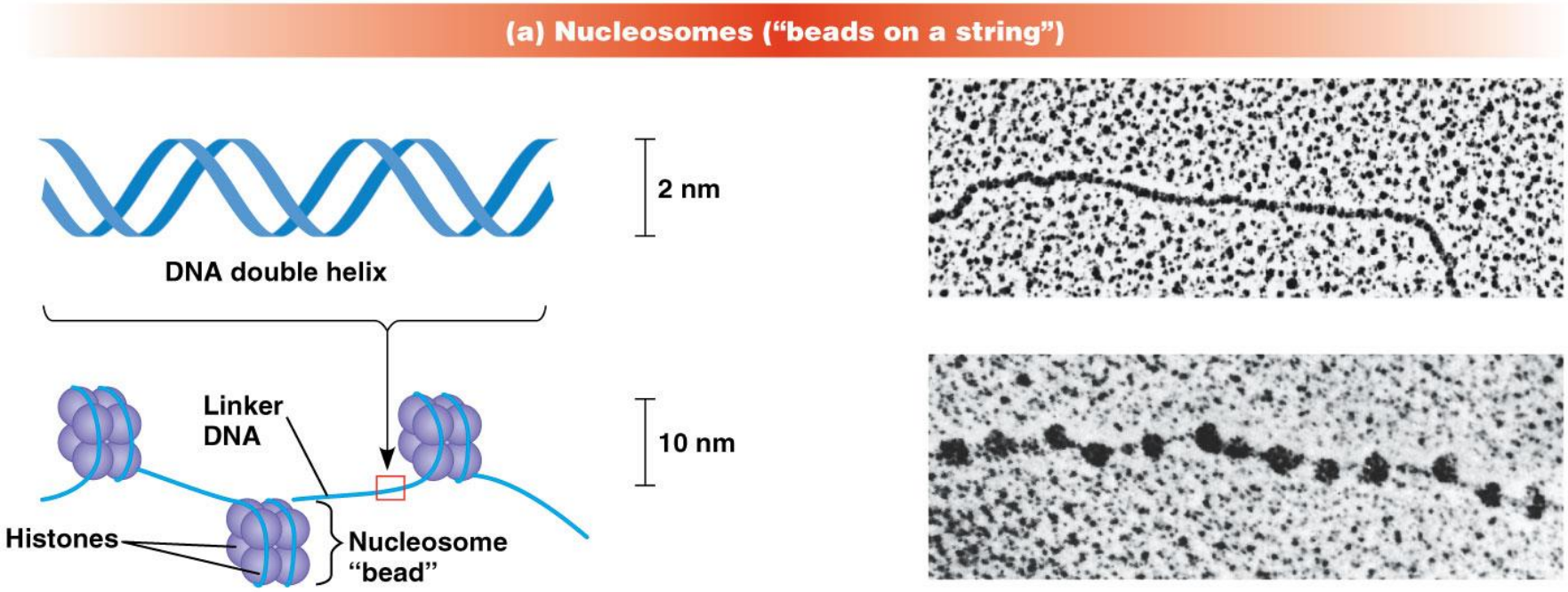
(point thumb of right hand at you and follow his body from knee → head
= sense of winding indicated by your curled fingers)

Winding DNA around histone octamers will shorten the effective length, but only by a factor of ~7-fold

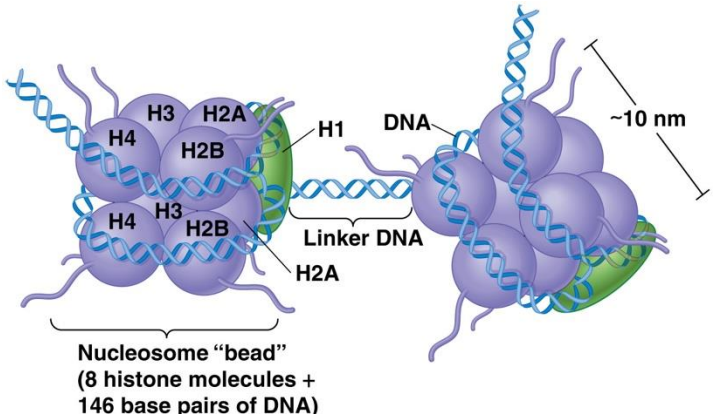
… = have a long way to go .....

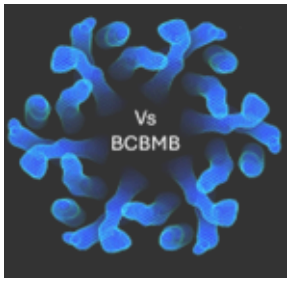
Compaction of Chromatin Exploits Entropy Effects

- Going beyond a single nucleosome....how does that look like?



The linker DNA is ~80bp long, and partially covered by another histone, called the "linker histone H1"





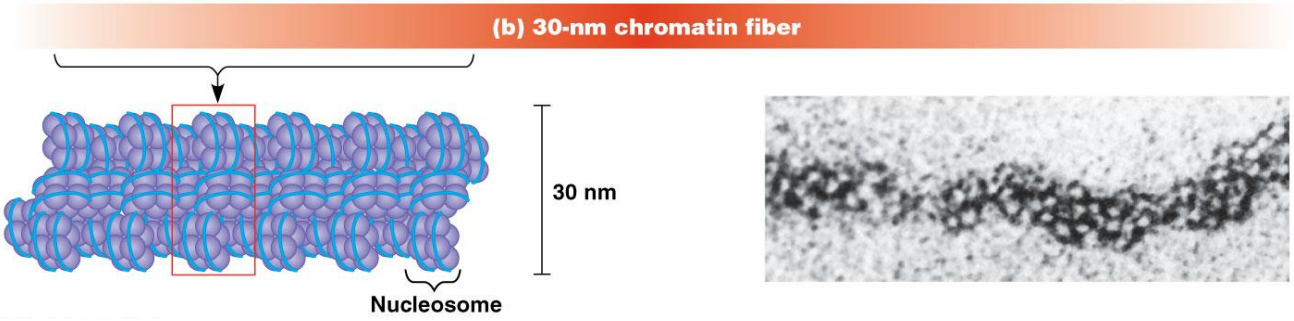
Compaction of Chromatin Exploits Entropy Effects

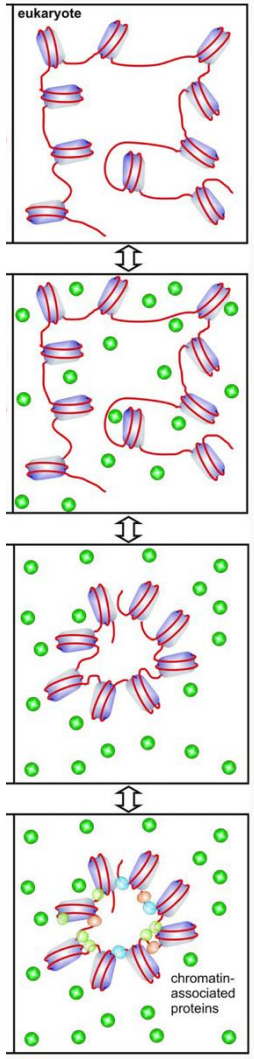
The “bead on the string” structure has very interesting properties that allow exploitation of a thermodynamic effect called “**colloid osmotic pressure**”. The basic idea of this phenomenon is that large macromolecular assemblies tend to aggregate in crowded environments …like a cell where the concentrations of nucleic acids and proteins is so high that it actually forms a “gel”.

This mechanism may appear counterintuitive because the increase in fold organization seems to carry a big entropic penalty.
It does carry a penalty ...but that penalty is paid for by the increase in entropy of the other macromolecules (just like liberation of water molecules in clathrates drives lipid aggregation, and early protein folding), and once the solenoid forms ....it is further stabilized by additional proteins.

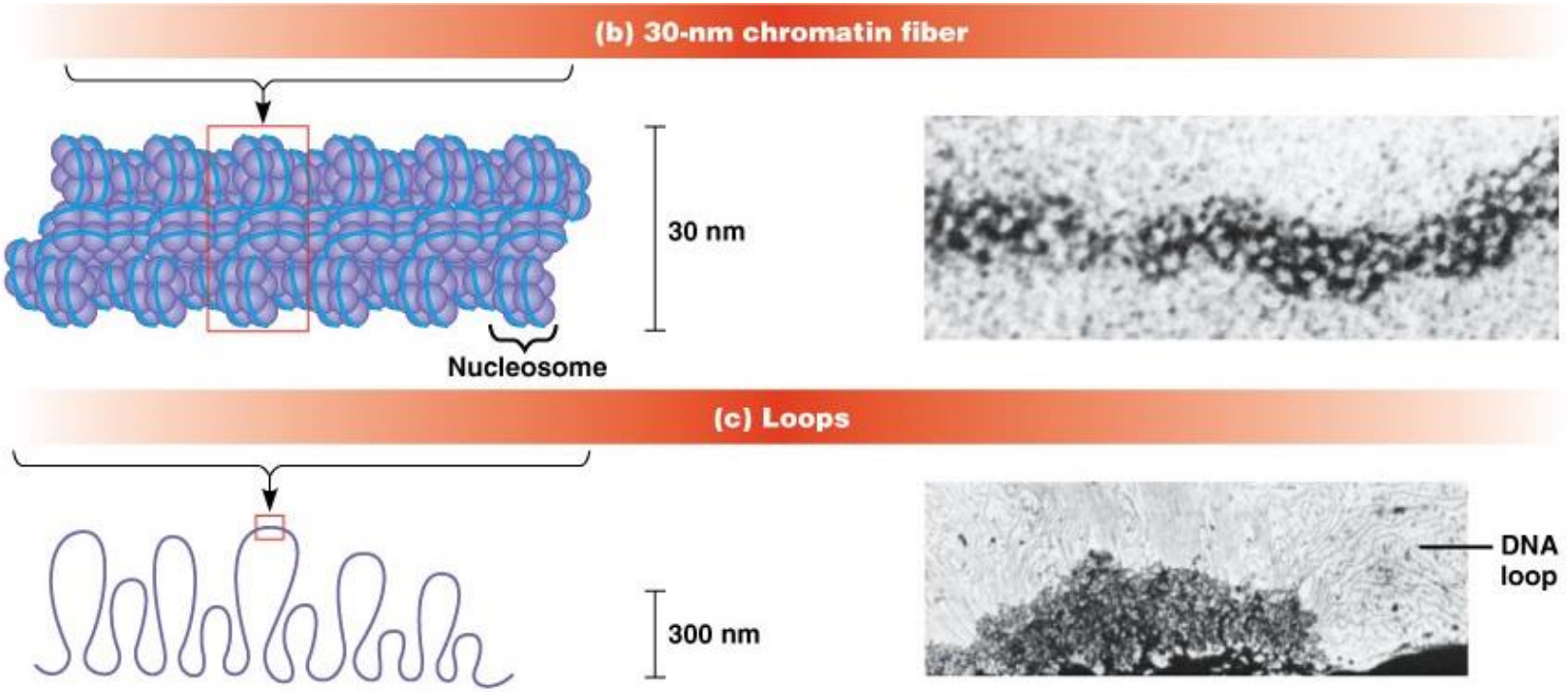
The picture illustrates how the entropy of other macromolecules (green dots) will increase if the “bead on a string” compacts itself into a solenoid = “superhelix” of nucleosomes (= “superhelix of nucleosome superhelix = another 6-fold shortening of naked DNA segment (→ 1 → 42-fold).

The resulting structure is called the “30-nm fiber”

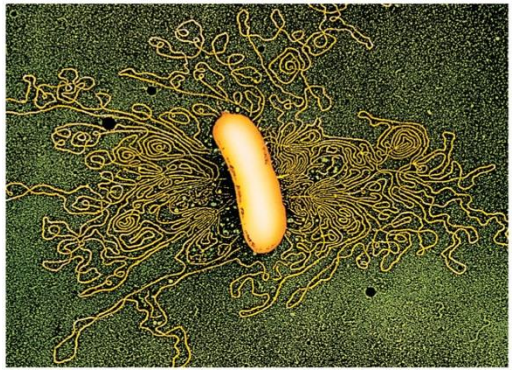




Going Further than the 30nm Fiber Requires More Protein

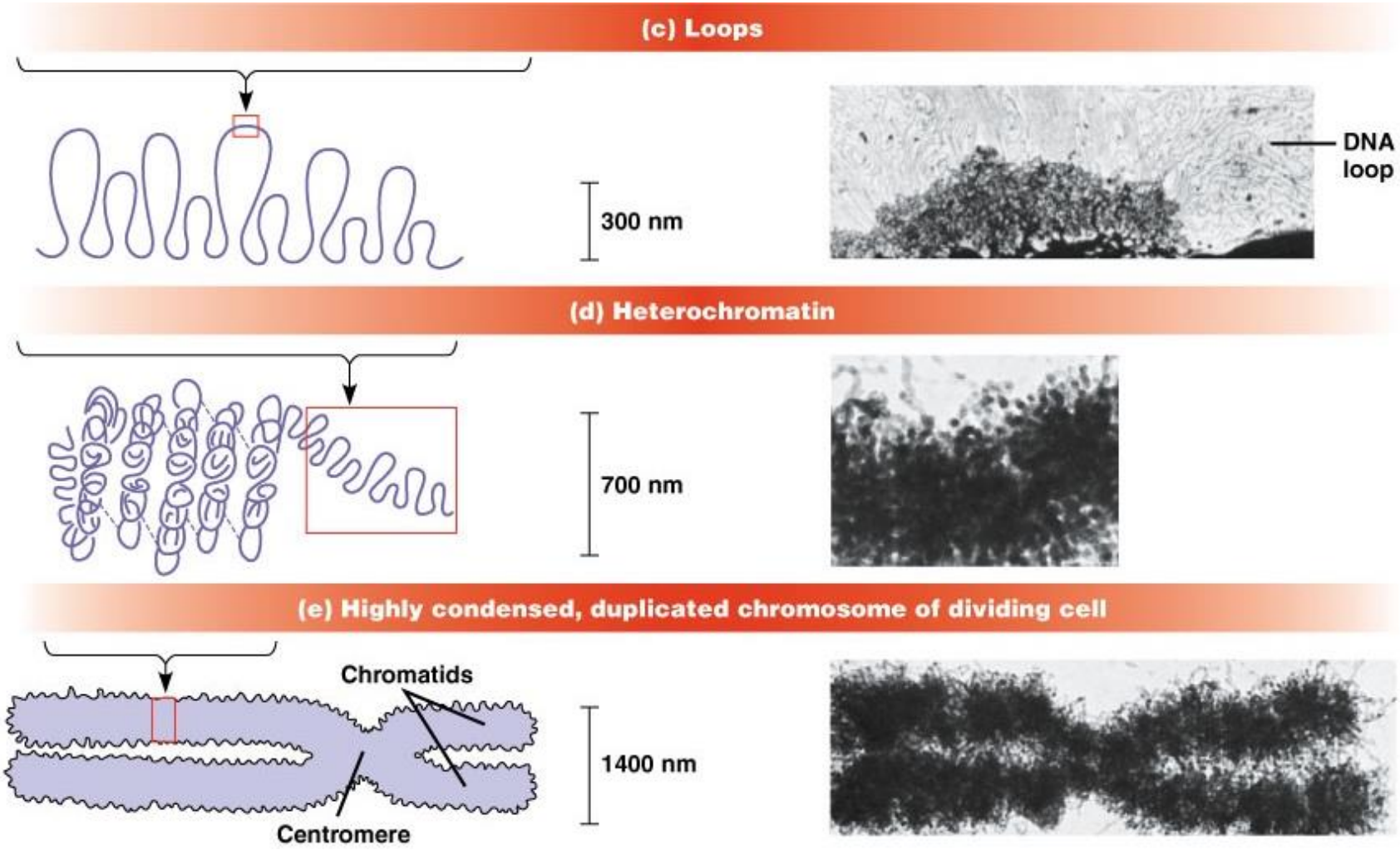


- The loop structures are stabilized by many different types of ...??..(guess). **Curiously**: a good number of these proteins **are not structural scaffolds, but** play important roles in regulating gene expression (= what is read out, and when)
- **Take note:** ...these loops look very similar to the "spilled E coli genome" ...and not too surprisingly – folding the 30nm fiber into loops results in shortening from ~42fold → ~750-fold overall.
- **Also note:** this state "LOOP state" of compaction is THE LAST EXIT before folding gets too tight to access info for read out.

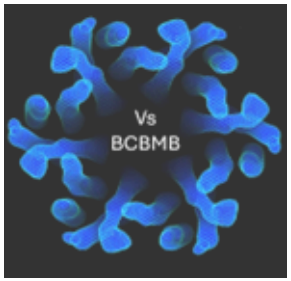


→ Anything beyond this state creates what is called "heterochromatin" because it appears optically dense in light microscopy.

Compaction Beyond Loops



Needless to say ...more .... ?? (guess ...yep, proteins).... are needed to reach the final two states where shortening finally reaches a packing ratio of >10,000:1 (eg 75**mm** long chromosome → 4-5**μm** long). Keeping track of all the twists, turns, supercoils, compactions is dizzing (to me)....how can something like this come together....and work reproducibly? One of Nature's many wonders ....



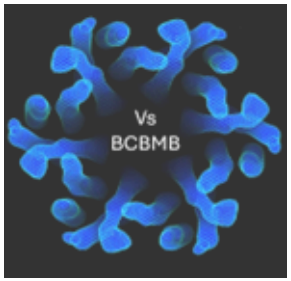
Nucleus

Lets finish with a **brief** look at the compartment that contains the genetic information in eukaryotes:

- **Reminder:** said at outset that partitioning the total length of human DNA into 23 separate pieces (called chromosomes) necessitates that they be constrained in a separate compartment for mostly two reasons:
    - **(a)** allow high enough [concentrations] of small chemicals (like building blocks of for nucleic acid synthesis, or protein components that are involved in chromatin dynamics) for reactions to happen at sufficient speed (reminder: $v_{reaction}$ ~ [concentration]), and
    - **(b)** to provide means for **regulation** of chromatin dynamics

→ **How can you implement a compartment inside the cells?**

…try to think of an answer ….



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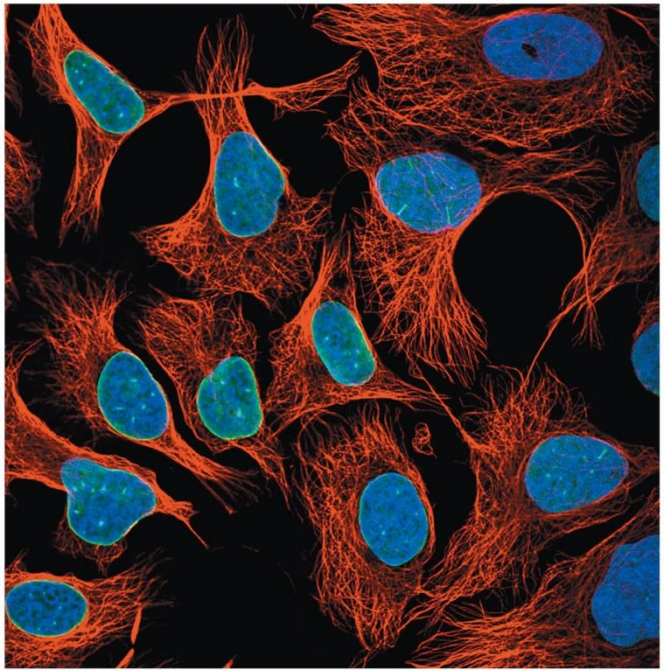
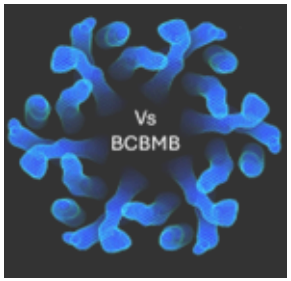
→ **How can you implement a compartment inside the cells?**

Answer: simplest = exploit lipid bilayers to make a membrane bound intracellular organelle.

→ At a fluorescence microscopy level (DNA: blue fluorescence), the cell nucleus appears cleanly separated from the cytosol
→ **Curiously though:** the blue stain is not even …but seems a little speckeled.
→ **If the fluorescence dye binds to DNA...what could "speckels" mean?**





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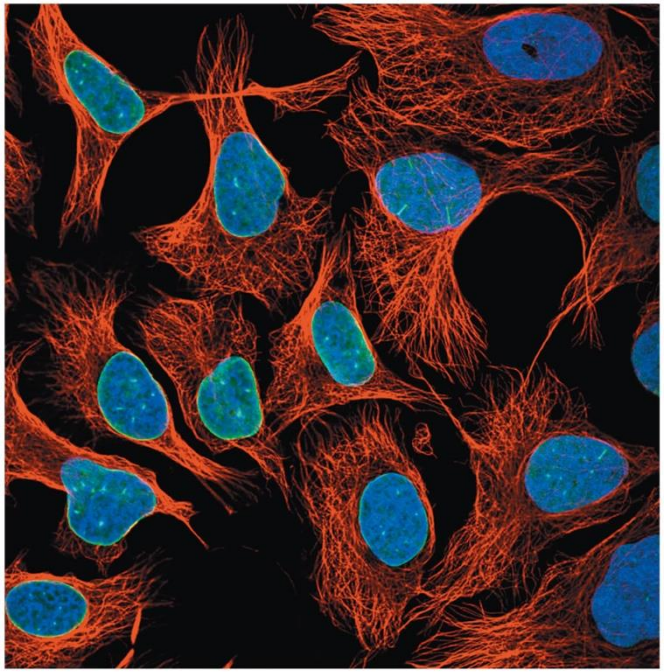
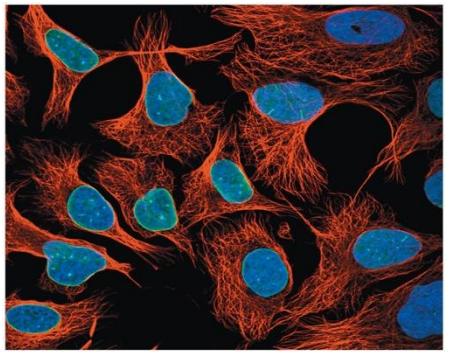
Answer: uneven "density" of DNA = even at this level it seems that the nucleus is further compartmentalized into even smaller regions. → more membranes? That'd be CRAZY!



Subnuclear Compartmentalization

How could you find out if the speckles are surrounded by membranes?

…try to think of an answer…..



5µm

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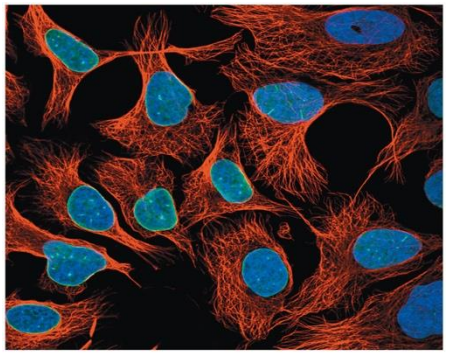
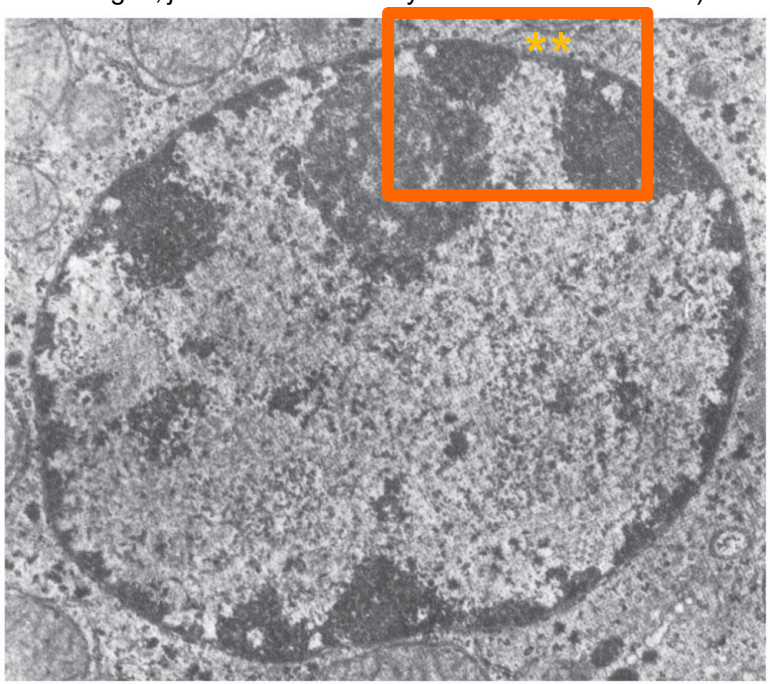


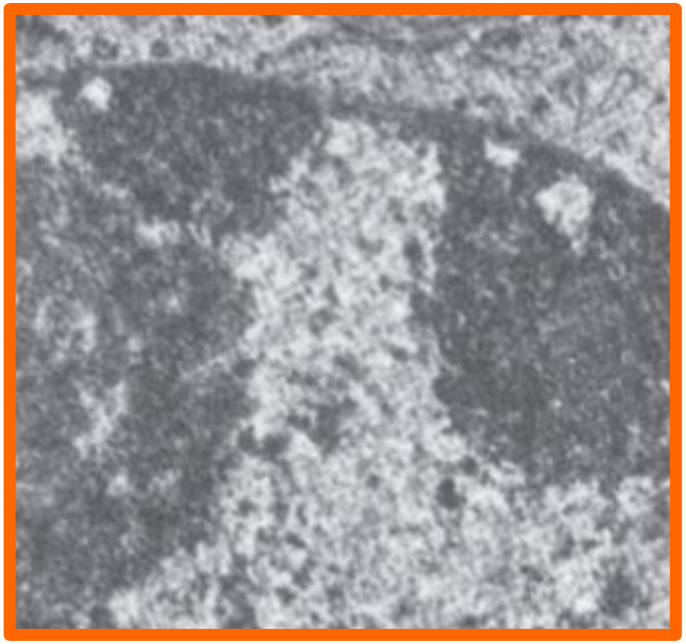
5µm

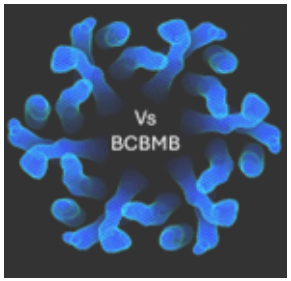
Answer: increase …??.. (resolution) by going from light microscopy to .....?? ... (electron microscopy)

verdict…? Looking at the smaller subarea by digitally increasing zoom ...it looks like inside the nucleus the "chunky" dark regions **<u>are not</u>** enveloped by separate membranes (if they were, it would look smooth along their edges, just like the boundary around the nucleus **)..... → what are they then?



1µm



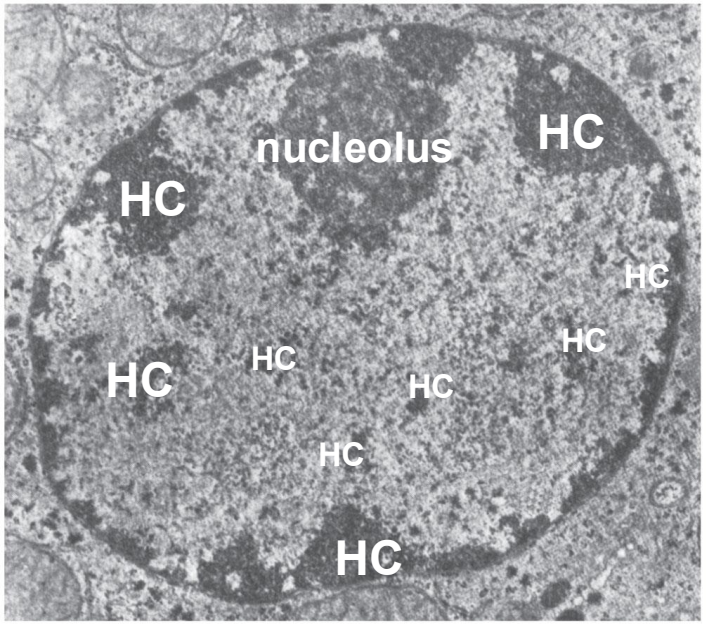


Subnuclear Compartmentalization

The dark regions seen in electron micrographs correspond to two different types of structures:

(a) subnuclear "organelle(s)" called the **nucleolus/nucleoli.** *A typical nucleus has 2-6 nucleoli. Nucleoli appear dark (even in light microscopy!!) because of an insane density of macromolecular complexes that actively produce ribosomal RNAs to make ribosomes that are needed for protein synthesis (Chapter: TRANSLATION)*

(b) additional dark regions and speckles are called "**heterochromatin**" (labeled "HC") = *any type of chromatin that is **more** compact than the "loop" stage of DNA compaction.*
Examples for heterochromatin are: centromeres, telomeres, "silenced" regions of the genome that are not expressed in a given cell, regions of chromosomes bordering the nucleoli, …lots more ...

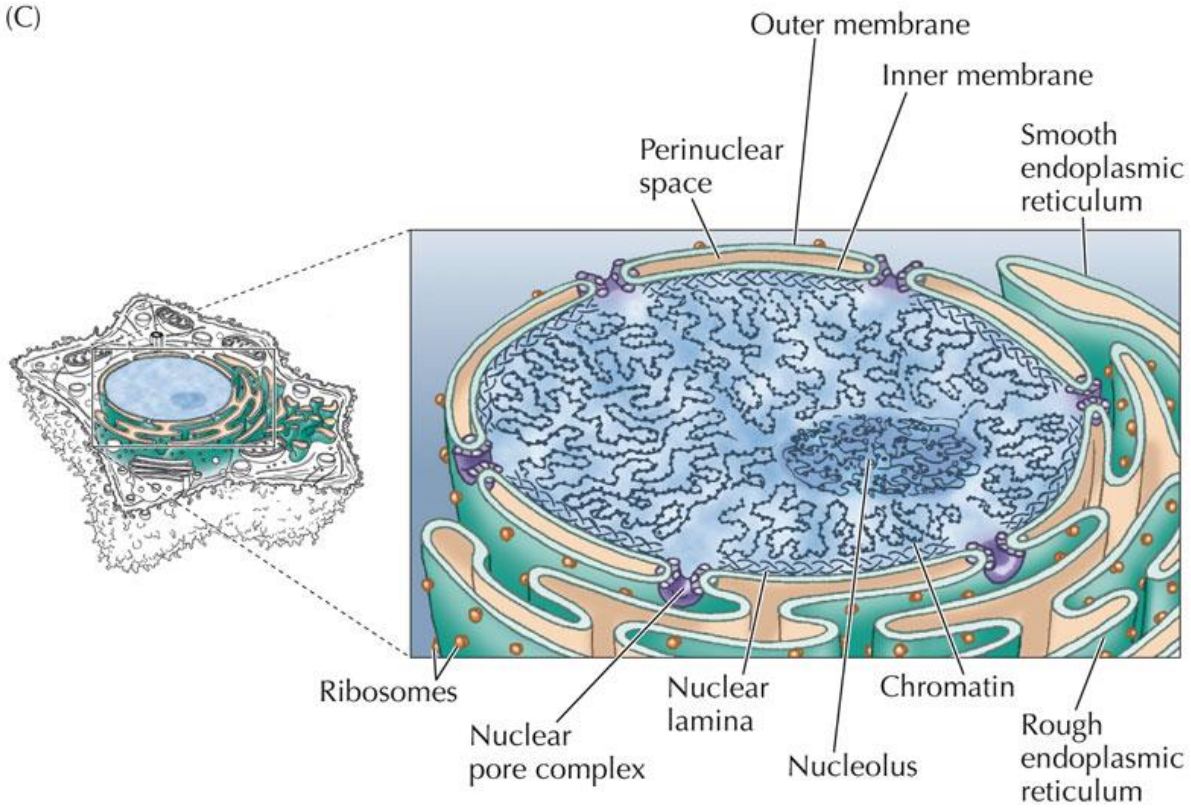


- The **"lighter" regions** that occupy space **between heterochromatin** are called **"euchromatin"**.
- Euchromatin is much less compacted ("loop stage" or less) than heterochromatin → this allows access for read out or replication = euchromatin is what is called "transcriptionally active".

→ Now that we "understand" what those dark and light regions are, we still need to deal with the nuclear membrane envelope…because it being a membrane raises questions how things get in and out of the nucleus.

Nuclear Envelope

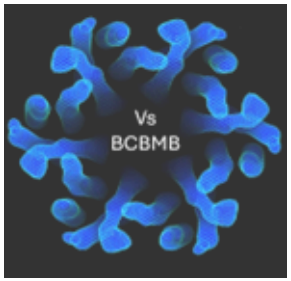
zooming in on the membrane that surround the nucleus – we find something unexpected ….



The nucleus is surrounded by TWO bilayers and the space between the two membranes is continuous with another cell organelle, the “rough endoplasmic reticulum”.

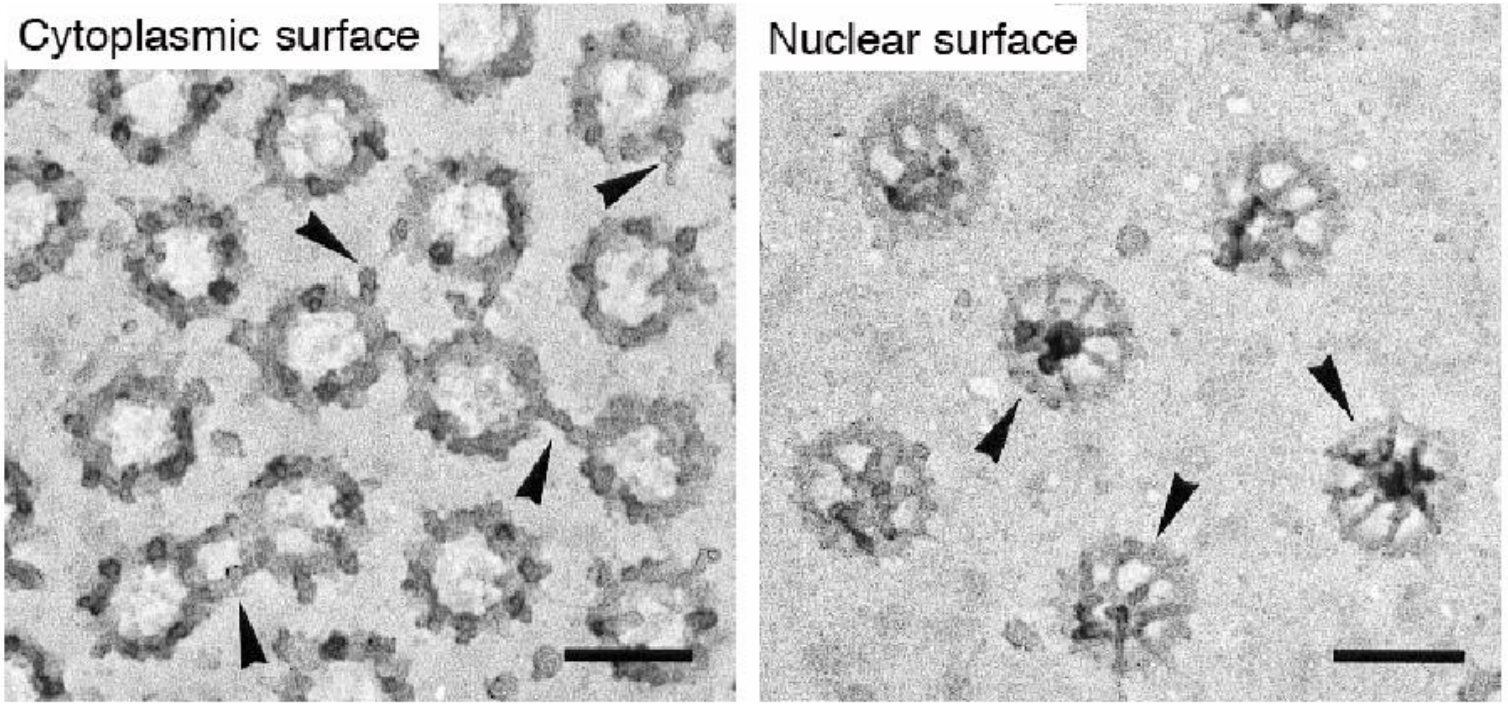
Inside the nucleus, the inner membrane is attached to a protein mesh called the “nuclear lamina”.

Spanning the two membrane, “nuclear pore complexes” provide gateways for materials to enter and exit the nucleus.

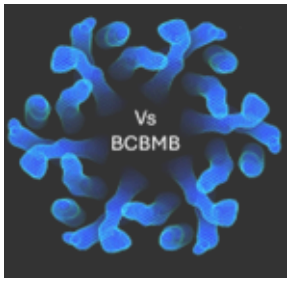


Gateways to the Nucleus

What can you observe in these electron micrographic images of nuclear pore complexes?



Scale Bar is 100nm



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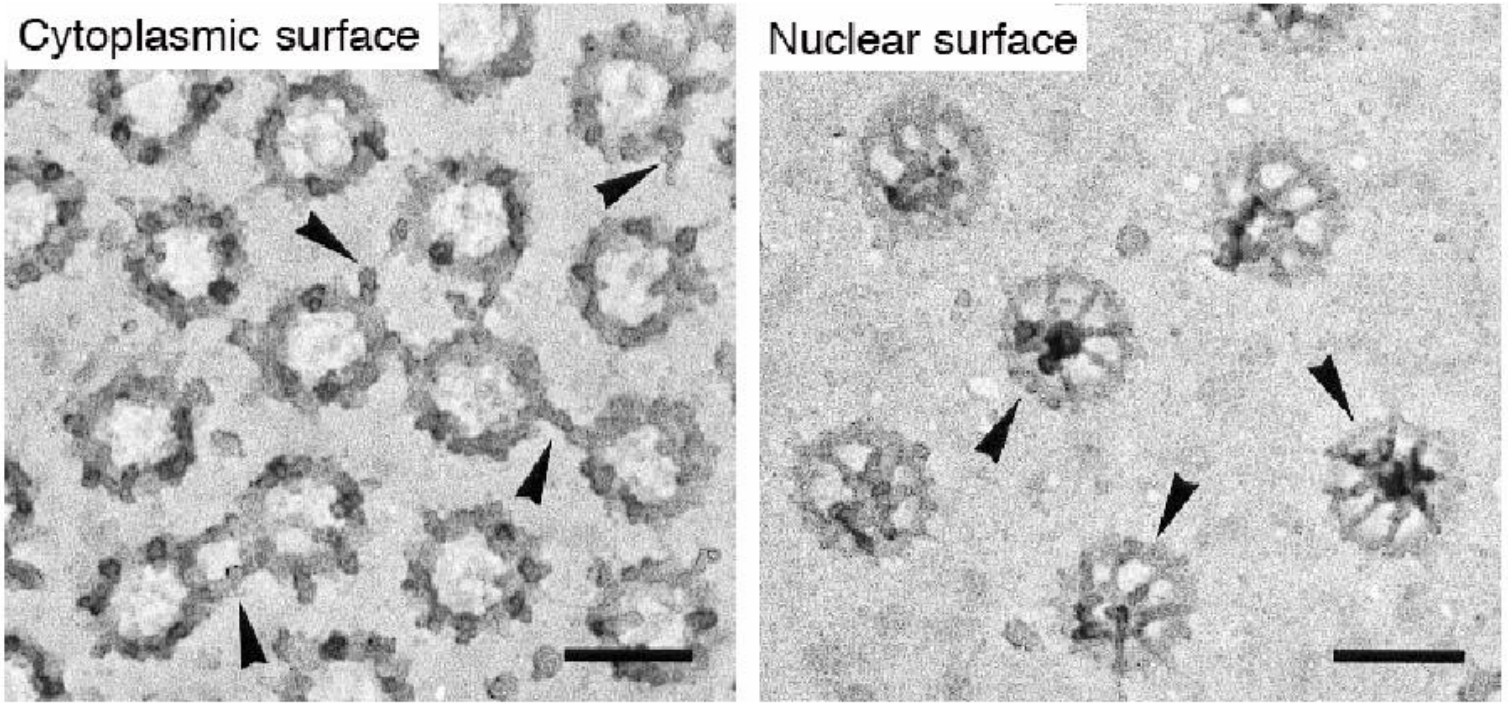
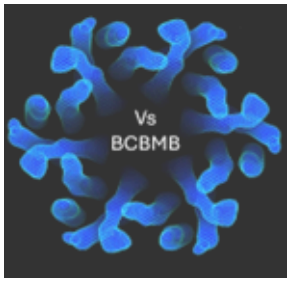


Scale Bar is 100nm

Answer:

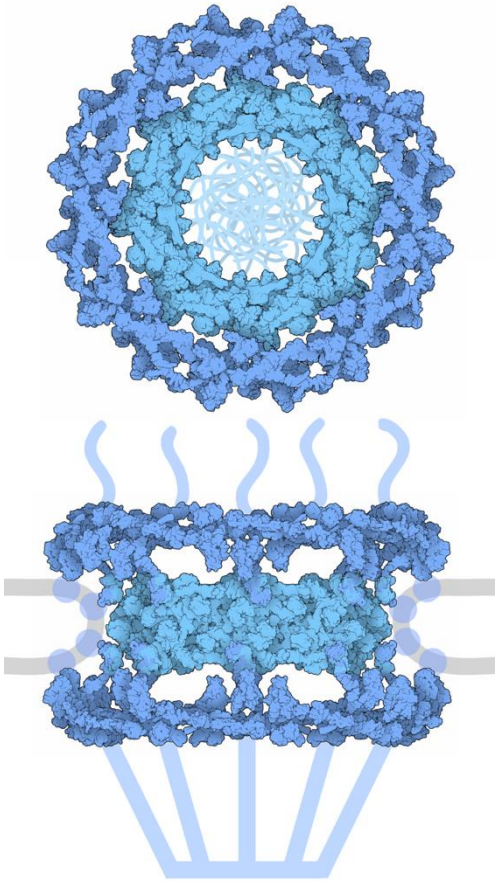
The gateways that allow molecules to enter/exit the nucleus are HUGE!
The gateways look like giant pores
The structure of the gateways is very different on the cytoplasmic and nuclear side (asymmetry)
The structure shows some sort of "regular pattern" around the circumference → this is a quaternary structure (= complex made from different components)



Gateway to the Nucleus

Images like the one shown on the previous slides and structural work on individual components resulted in this simplified (and still incomplete) model of the nuclear pore complex

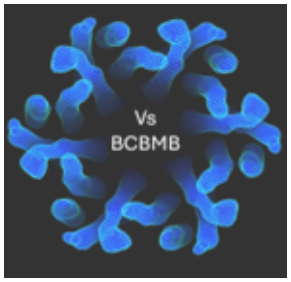
Top view cytoplasmic



Sideview
Sideview

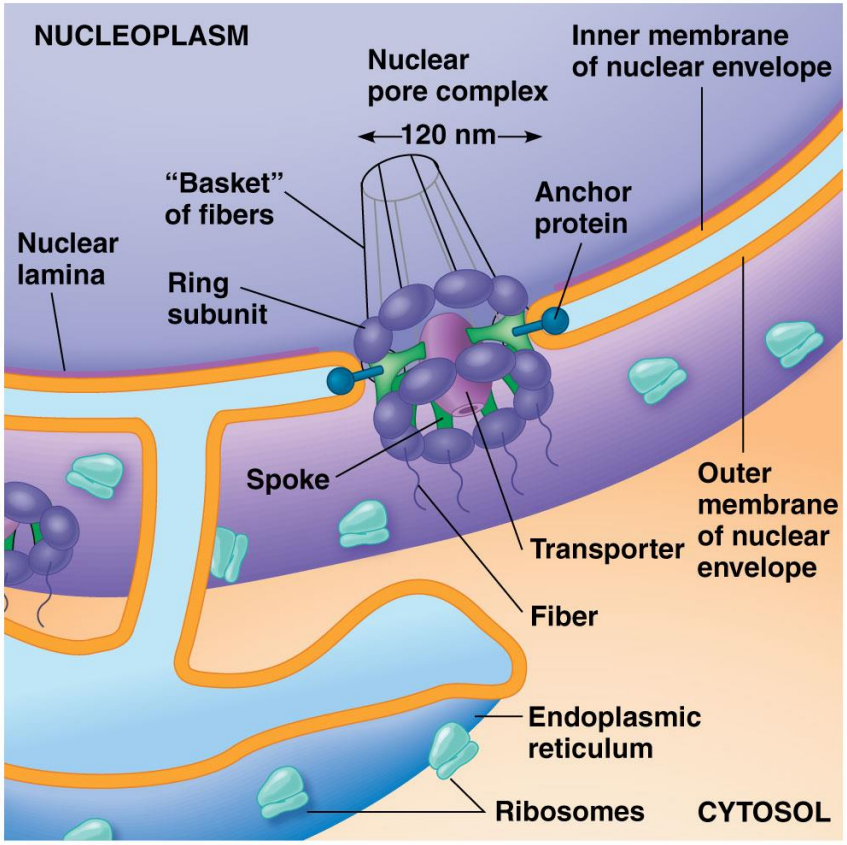
Nuclear envelope
(grey lines represent the two bilayers)

core infrastructure of the pore, including the outer ring from PDB entry 5a9q and the inner channel ring from PDB entry 5ijn.



Nuclear Pore Complexes Are Large

Typical nucleus has 3,000-4,000 nuclear pores that are built from 30 different proteins called nucleoporins, weighing a total of 128 **M**Da (compared to ~45**k**Da for average protein) – this is enormous!



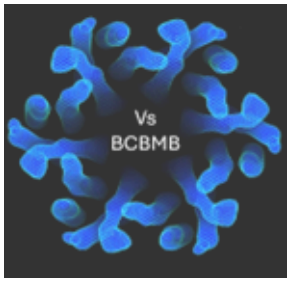
(b) Location of nuclear pores in nuclear membrane

Nuclear Pores are the most versatile of all transport machines – here are a few facts about them:

- Can freely passage anything from water/ions to small molecules like "nucleobases" and macromolecules up to ~30kDa
- >30kDa the pore switches to a different mechanism of solute movement that is deliberate, requiring specific import/export signals
- Perhaps the most impressive feat…the pore can pass entire ribosomal subunits (>1 MDa!)

Where it gets scary is when it comes to speed, for instance:

- can pass 100 histones/min per pore
- passes 5-6 entire ribosomal subunits per minute (these are HUGE) per pore
- passing an mRNA takes about 0.2s
- all in addition to the small(er) molecules that pass at the same time, going in both directions.



Summary Chromatin

Here is a repeat of the questions we put out in the beginning along with very terse summary answers

- **How** genetic material is compacted: *(difference between "nucleoid" [bacteria] and "chromatin" [eukaryotes]):*
  - ***supercoiling*** *(prokaryotes = gyrase B; eukaryotes = histones);*
  - *supercoiled structures are further condensed by exploiting thermodynamic properties of crowded environments ("colloid osmotic pressure")*
  - *and additional proteins (scaffolds, transcription factors, …);*
  - *bacterial nucleoids are not enclosed by membranes and overall simpler in structure than chromatin*

- **How** compaction affects the structure of eukaryotic cell nuclei
  - nuclei are subdivided into subregions - *euchromatin ("loosely packed") and heterochromatin (nucleoli, centrosomes, other…)*

- **How** eukaryotic cell nuclei exchange matter/information with the cytoplasm:
  - *nuclear pore complexes …very large multi protein structures that allow rapid exchange of both small (eg ions, amino acids, nucleotides) and very large molecules (eg ribosomal subunits)*