

THE NUCLEOLUS & DNA PACKAGING

Structure, Function, Levels of Chromatin Organisation, and End-Semester Questions

Part A — The Nucleolus

1. Introduction

The **nucleolus** is the most prominent structure inside the eukaryotic nucleus, visible even under the light microscope as a dense, rounded body. It is not membrane-bound — it forms around chromosomal regions called **Nucleolar Organizer Regions (NORs)** that carry the tandemly repeated genes for ribosomal RNA. The nucleolus is the site where rRNA is synthesised and processed and where ribosomal subunits are assembled, making it the cell's "ribosome factory".

- First described by the Italian scientist Felice Fontana in 1774, well before the nucleus itself was formally defined.
- Number and size vary with cell activity: cells that synthesise large amounts of protein (e.g. secretory or growing cells) have large, prominent nucleoli; the nucleolus shrinks or disappears in metabolically quiescent cells.
- A cell may contain one or several nucleoli, depending on the number of active NOR-bearing chromosomes.

2. Structure of the Nucleolus

Under the electron microscope, an active nucleolus typically shows three distinguishable sub-regions, reflecting the sequence of steps in ribosome biogenesis:

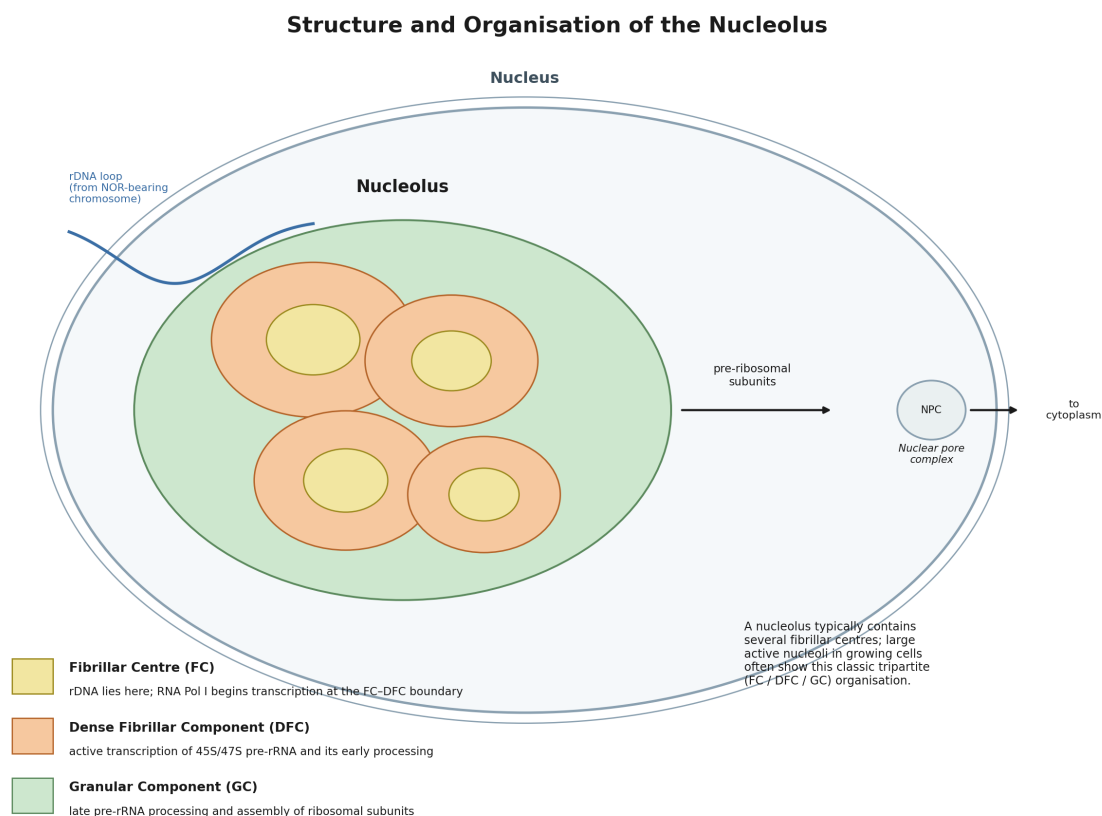


Fig. 1 — Tripartite organisation of the nucleolus (Fibrillar Centre, Dense Fibrillar Component, Granular Component) and export of pre-ribosomal subunits.

- **Fibrillar Centre (FC)** — pale, low-density zone containing the rDNA (NOR) itself, largely non-transcribed rDNA and RNA polymerase I molecules.
- **Dense Fibrillar Component (DFC)** — surrounds the FC; this is where active rDNA transcription occurs and where the 45S/47S pre-rRNA undergoes its earliest processing.
- **Granular Component (GC)** — the outermost and often largest zone, packed with granules representing pre-ribosomal particles in the later stages of maturation and subunit assembly.
- A fibrous **nucleolar matrix (nucleolonema)** and surrounding **perinucleolar chromatin** (often heterochromatic) help organise and anchor the structure.

3. Functions of the Nucleolus

- **Ribosome biogenesis** — the primary role: transcription of rDNA by RNA polymerase I, processing of the pre-rRNA transcript, and assembly of rRNA with ribosomal proteins (imported from the cytoplasm) to form the pre-40S and pre-60S ribosomal subunits, which are then exported through nuclear pores.
- **5S rRNA integration** — 5S rRNA, transcribed elsewhere by RNA polymerase III, is imported into the nucleolus to be incorporated into the large subunit.
- **snRNP and other RNP assembly** — the nucleolus also participates in the maturation of small nucleolar RNAs (snoRNAs, which guide chemical modification of rRNA), and contributes to assembly of some other ribonucleoprotein particles (e.g. signal recognition particle components, telomerase RNA in some organisms).
- **Cell-cycle and stress regulation** — the nucleolus acts as a stress sensor: various forms of cellular stress disrupt nucleolar structure and release nucleolar proteins (e.g. that regulate p53), linking ribosome biogenesis to cell-cycle checkpoints and apoptosis.
- **Sequestration of regulatory proteins** — certain regulatory proteins are temporarily stored/inactivated in the nucleolus and released when needed.

4. The Nucleolar Cycle

- **Interphase** — the nucleolus is fully formed and metabolically active, carrying out rRNA transcription and ribosome assembly.
- **Prophase** — as chromosomes condense, the nucleolus gradually disperses and disappears; rDNA transcription is switched off.
- **Metaphase/Anaphase** — no nucleolus is visible; NORs are simply part of the condensed chromosomes.
- **Telophase** — as the nuclear envelope re-forms around the decondensing chromosomes, nucleoli reassemble around the NOR-bearing chromosomes, and rRNA transcription resumes.

Part B — DNA Packaging (Chromatin Organisation)

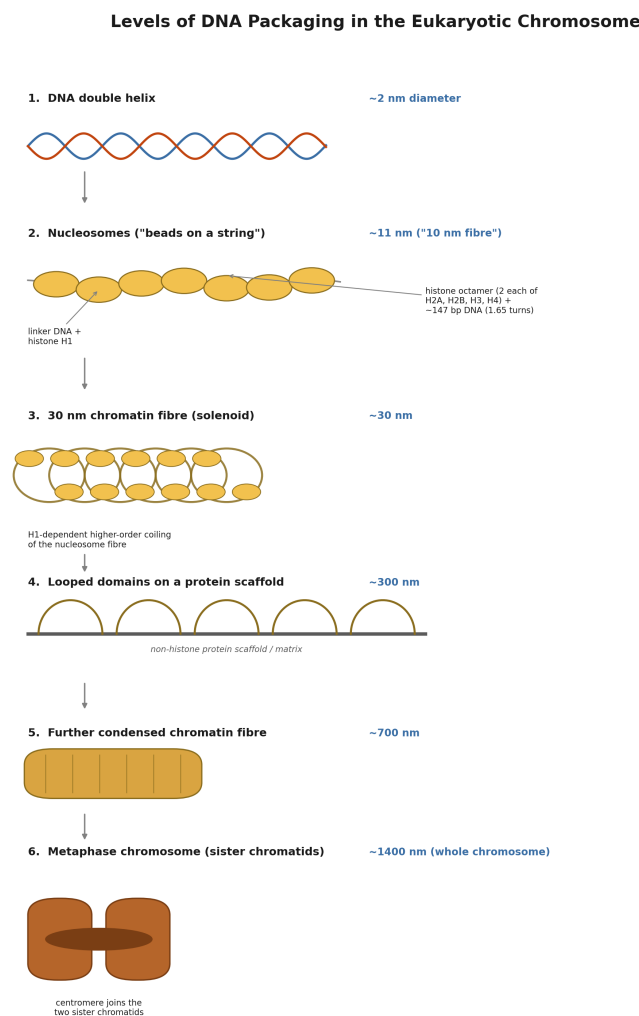
5. Why DNA Must Be Packaged

A single human diploid cell contains about 2 metres of DNA in total, yet this must fit inside a nucleus only 5–10 micrometres across — a compaction on the order of 10,000-fold. This is achieved by wrapping and coiling DNA around proteins (chiefly **histones**) in a hierarchy of increasingly compact levels, forming the nucleoprotein complex called **chromatin**. Packaging is not just space-saving — it also regulates which genes are accessible for transcription.

- **Euchromatin** — loosely packed, lightly staining chromatin; generally transcriptionally active.
- **Heterochromatin** — densely packed, darkly staining chromatin; generally transcriptionally inactive. **Constitutive heterochromatin** (e.g. centromeric, telomeric regions) is permanently condensed in all cells; **facultative heterochromatin** is condensed only in certain cells or at certain times (e.g. one inactivated X chromosome, forming the Barr body).

6. Levels of DNA Packaging

DNA packaging proceeds through several well-defined levels of organisation, each achieved with the help of specific proteins:



Overall compaction: the ~2 nm double helix is packaged roughly 10,000-fold into a metaphase chromosome, allowing about 2 m of human DNA to fit inside a nucleus only ~5–10 µm across.

Fig. 2 — The hierarchy of DNA packaging, from the naked double helix to the fully condensed metaphase chromosome.

Level	Structure	Approx. diameter
1	DNA double helix	~2 nm
2	Nucleosome ("beads on a string") — DNA wound ~1.65 turns around a histone octamer (H2A, H2B, H3, H4 × 2)	~11 nm
3	30 nm chromatin fibre — nucleosomes coiled with the help of linker histone H1 (solenoid/zig-zag model)	~30 nm
4	Looped domains anchored to a non-histone protein scaffold/matrix	~300 nm
5	Further condensed chromatin fibre of the mitotic chromosome	~700 nm
6	Fully condensed metaphase chromosome (two sister chromatids)	~1400 nm

7. The Nucleosome and Histones

- The **nucleosome core particle** is the fundamental repeating unit of chromatin: ~147 base pairs of DNA wrapped around a histone octamer made of two molecules each of the core histones **H2A**, **H2B**, **H3**, and **H4**.
- Adjacent nucleosomes are joined by short stretches of **linker DNA** (roughly 20–80 bp), giving chromatin its classic "beads on a string" appearance under the electron microscope.
- The linker histone **H1** binds at the point where DNA enters/exits the nucleosome and helps draw nucleosomes together into the more compact 30 nm fibre.
- Histones are small, highly basic (arginine/lysine-rich) proteins that bind tightly to the negatively charged DNA backbone; N-terminal histone "tails" undergo chemical modifications (acetylation, methylation, phosphorylation) that regulate chromatin compaction and gene expression (the "histone code").

Note — the scaffold/loop model

At the 300 nm level, chromatin loops (each roughly 20,000–100,000 bp) are anchored at their bases to a non-histone protein scaffold, seen clearly in histone-depleted mitotic chromosomes. Repeated coiling of this looped fibre produces the further condensed chromatin of the mitotic chromosome and, finally, the fully condensed metaphase chromosome.

8. Quick Revision Summary

Aspect	Key point
Nucleolus forms at	Nucleolar Organizer Regions (NORs) — the chromosomal rDNA loci
Nucleolar sub-regions	Fibrillar Centre (FC) → Dense Fibrillar Component (DFC) → Granular Component (GC)
Main nucleolar function	rRNA transcription (Pol I), processing, and ribosomal subunit assembly
Nucleolar cycle	Disperses in prophase; reforms around NORs in telophase

Aspect	Key point
Chromatin states	Euchromatin (active, loose) vs heterochromatin (inactive, condensed)
Nucleosome	~147 bp DNA + histone octamer (2×H2A, H2B, H3, H4); linker DNA + H1 between beads
Packaging hierarchy	2 nm helix → 11 nm nucleosome fibre → 30 nm solenoid → 300 nm loops → 700 nm → 1400 nm metaphase chromosome
Overall compaction	~10,000-fold — packs ~2 m of DNA into a nucleus only 5-10 micrometres across

END-SEMESTER EXAMINATION QUESTIONS

Topic: The Nucleolus and DNA Packaging — Full Marks: 50 | Time: 2 Hours

Section A — Multiple Choice Questions (1 mark each)

- 1 The Nucleolar Organizer Region (NOR) is associated with the gene(s) for: (a) tRNA (b) rRNA (c) mRNA (d) snRNA
- 2 Which RNA polymerase transcribes the major rDNA transcription unit inside the nucleolus? (a) RNA Pol I (b) RNA Pol II (c) RNA Pol III (d) Primase
- 3 The sub-nucleolar region where active rRNA transcription first occurs is the: (a) Granular Component (b) Fibrillar Centre (c) Dense Fibrillar Component (d) Nucleolar matrix
- 4 5S rRNA is transcribed by: (a) RNA Pol I, inside the nucleolus (b) RNA Pol II, in the cytoplasm (c) RNA Pol III, outside the nucleolus (d) RNA Pol III, inside the nucleolus
- 5 The basic repeating unit of chromatin is the: (a) chromomere (b) nucleosome (c) solenoid (d) kinetochore
- 6 How many histone molecules make up the core of a nucleosome (the histone octamer)? (a) 4 (b) 6 (c) 8 (d) 10
- 7 Which histone is NOT part of the nucleosome core particle but instead binds linker DNA? (a) H2A (b) H2B (c) H3 (d) H1
- 8 Approximately how many base pairs of DNA are wrapped around one histone octamer? (a) 74 (b) 147 (c) 300 (d) 1400
- 9 The diameter of the "30 nm chromatin fibre" corresponds to which level of packaging? (a) Nucleosome (b) Solenoid (c) Looped domain (d) Metaphase chromosome
- 10 Darkly staining, transcriptionally inactive, permanently condensed chromatin (e.g. at centromeres) is termed: (a) Euchromatin (b) Facultative heterochromatin (c) Constitutive heterochromatin (d) Chromomere

Section B — Short Answer Questions (2–3 marks each; answer any six)

- 1 Define the nucleolus and name the chromosomal region around which it is organised.
- 2 List the three sub-regions of the nucleolus and state the main event occurring in each.
- 3 What is the nucleolar cycle? Briefly describe its relationship to mitosis.
- 4 Distinguish between euchromatin and heterochromatin, giving one example of each.
- 5 What is a nucleosome? State its composition.
- 6 What is the role of histone H1 in chromatin organisation?
- 7 Why must DNA be packaged inside the nucleus? Give an approximate figure for the degree of compaction.
- 8 Differentiate between constitutive and facultative heterochromatin with an example of each.

Section C — Long Answer / Essay Questions (8–10 marks each; answer any three)

- 1 Describe in detail the structure of the nucleolus and explain how each sub-region contributes to ribosome biogenesis. Support your answer with a labelled diagram.
- 2 Discuss the various functions of the nucleolus, beyond ribosome synthesis, in maintaining normal cell physiology.
- 3 Describe, with the help of labelled diagrams, the successive levels of DNA packaging from the double helix to the metaphase chromosome.
- 4 Explain the structure of the nucleosome and discuss the role of histones and their modifications in regulating chromatin compaction and gene expression.
- 5 "Chromatin organisation is central to both the compaction and the regulated expression of the eukaryotic genome." Discuss this statement with reference to euchromatin, heterochromatin, and the hierarchy of DNA packaging.

Answer key — Section A (MCQs)

1-(b) 2-(a) 3-(c) 4-(d) 5-(b) 6-(c) 7-(d) 8-(b) 9-(b) 10-(c)

Compiled study note — cell biology / molecular biology reference, with a model end-semester question set. Figures are original schematic diagrams prepared for this note and are not to scale.