Lecture 3.
Mitosis. Mitotic chromosomes

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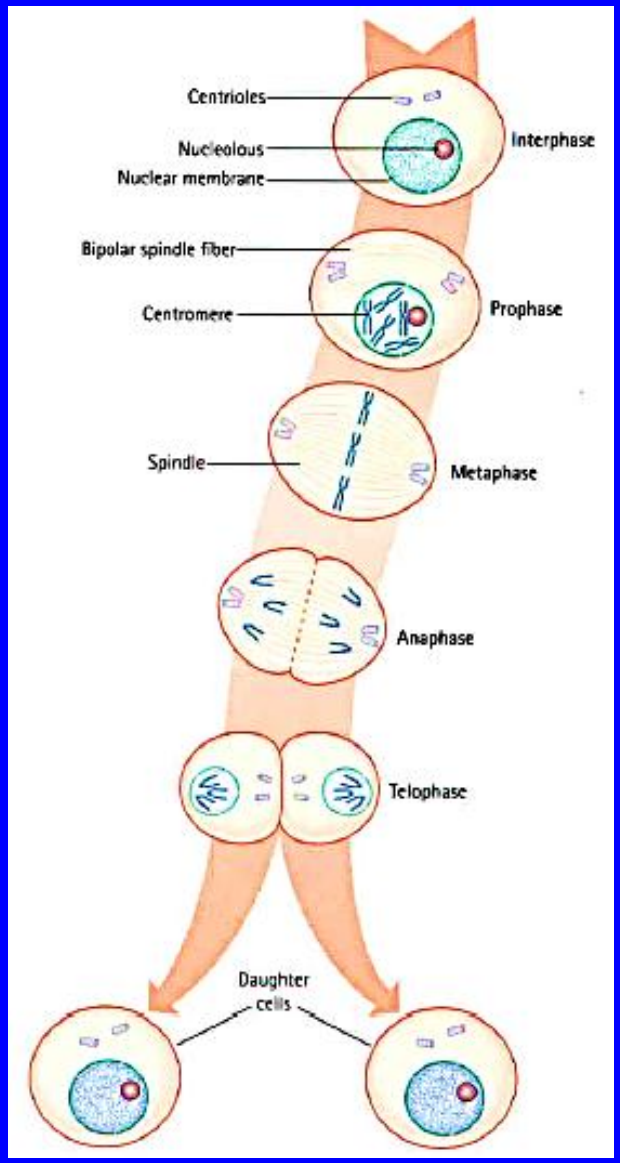
The Mitosis



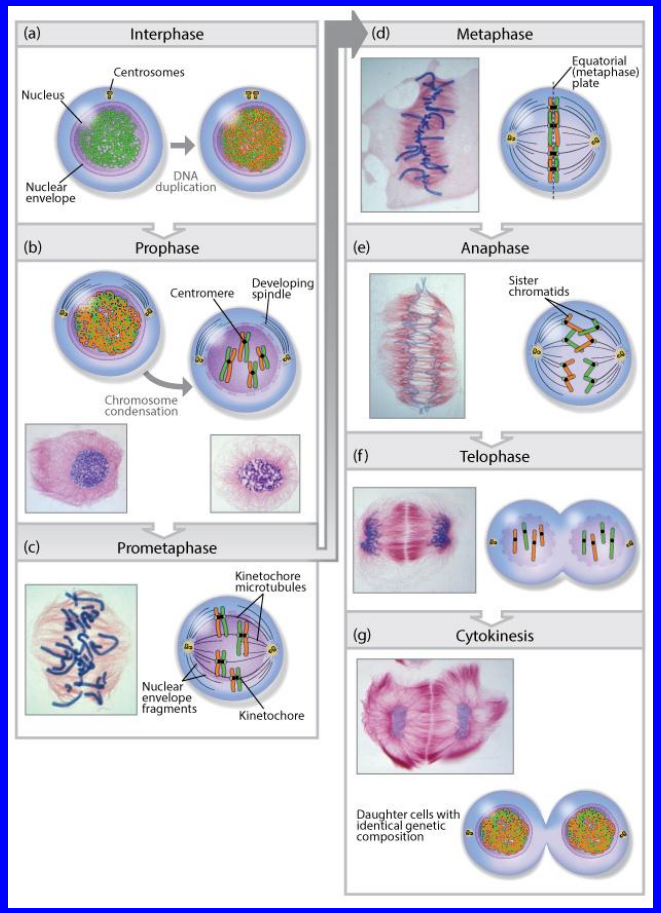
The mitosis lasts only 1–2 hours in most mammalian cells.

During the ***prophase***, chromosomes begin to coil, become more condensed, and begin to become visible as discrete structures. Nucleoli are visible early in the prophase, but disappear as the stage progresses.

Prometaphase is a short period between the prophase and the metaphase during which the nuclear membrane disappears and the spindle fibers begin to appear. Chromosomes attach to the spindle fibers at their kinetochores.

During ***metaphase***, the mitotic spindle is completed, the centrioles divide and move to opposite poles, and chromosomes line up on the equatorial plate. Chromosomes reach their maximum state of contraction during this phase. It is metaphase chromosomes that are traditionally studied in cytogenetics.

The Mitosis

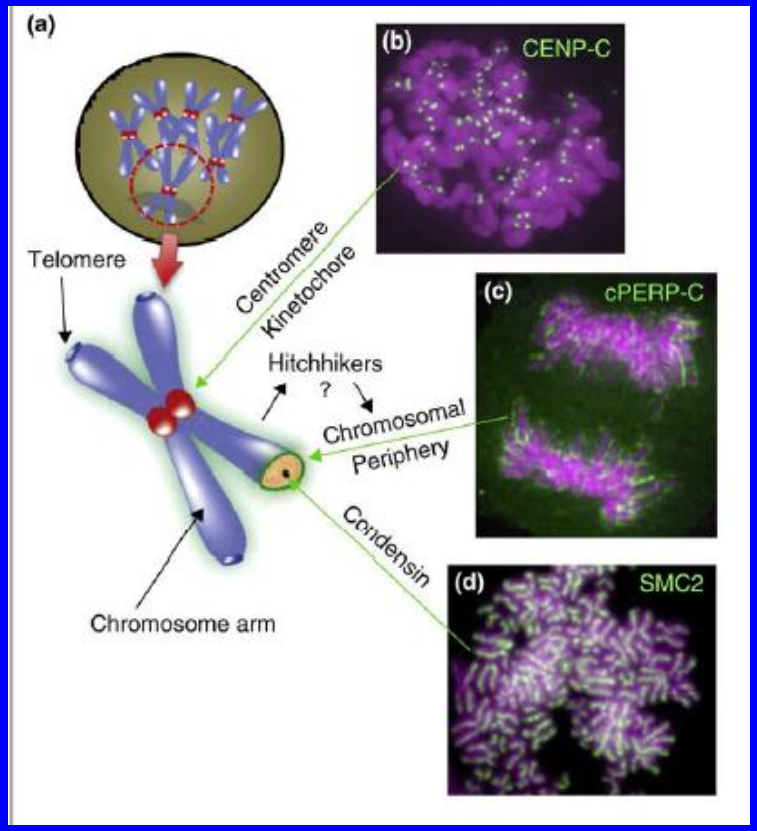


During ***anaphase***, centromeres divide longitudinally and the chromatids separate. Sister chromatids migrate to opposite poles as anaphase progresses.

The final stage of the mitosis is ***telophase***. The chromosomes uncoil and become indistinguishable again, the nucleoli reform, and the nuclear membrane is reconstructed. Telophase is usually followed by cytokinesis, or cytoplasmic division.

The products of mitosis are two genetically identical daughter cells, each of which contains the complete set of genetic material that was present in the parent cell. The two daughter cells enter interphase and the cycle is repeated.

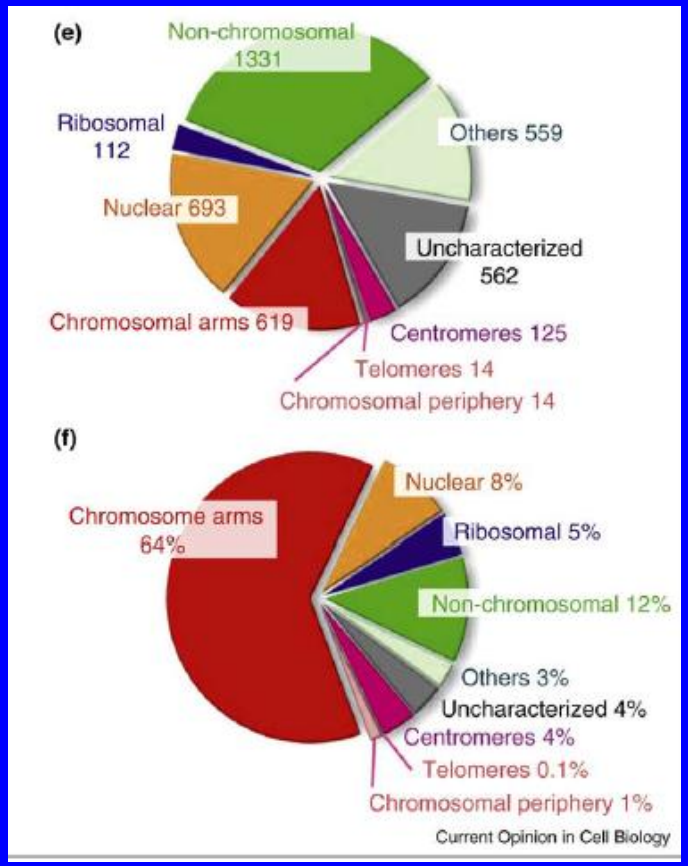
Chromosome anatomy and formation



Functional subdomains in mitotic chromosomes (a), include, (b) Centromeres, Telomeres, (c) the Chromosome periphery and (d) chromosome arms.

- ✓ Mitotic chromosomes have four structural/functional domains: ***centromeres, telomeres, the periphery, and arm chromatin***.
- ✓ Each domain has a characteristic protein composition.
- ✓ The ***centromere*** and its associated kinetochore together comprise an elaborate structure, with over 120 constituents described to date. They bind spindle microtubules and direct chromosome segregation in mitosis.
- ✓ ***Telomeres*** play an essential role in protecting chromosome ends and preventing chromosome fusion events. The protein composition of telomeres is relatively simpler.

Chromosome anatomy and formation



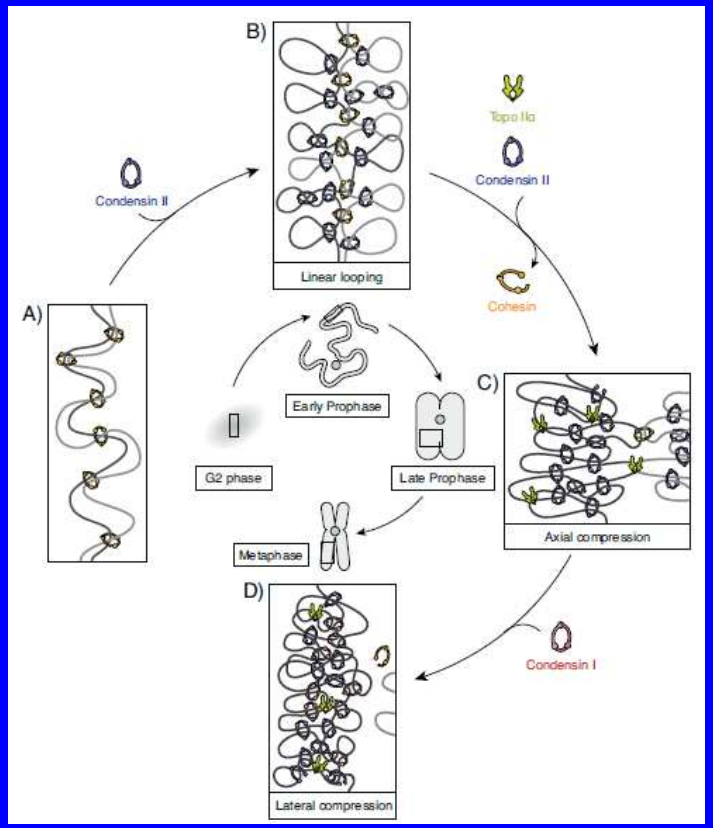
(e) The 9 classes of proteins found in chromosomes.
(f) Estimated percentages of total chromosomal protein mass in the major classes of proteins

✓ The ***chromosome periphery*** (perichromosomal layer) may act like a skin protecting the chromosome surface. Its components are enriched in ribosomal and nucleolar proteins.

✓ Many may simply be '***hitchhikers***'—proteins that bind to chromosomes in the cytoplasm following nuclear envelope breakdown and serve no essential function during mitosis. Others appear to function during chromosome segregation, as discussed below.

✓ The *condensin* complex is a factor essential for mitotic chromosome formation. This complex is distributed along the axial region of the ***chromosome arms***.

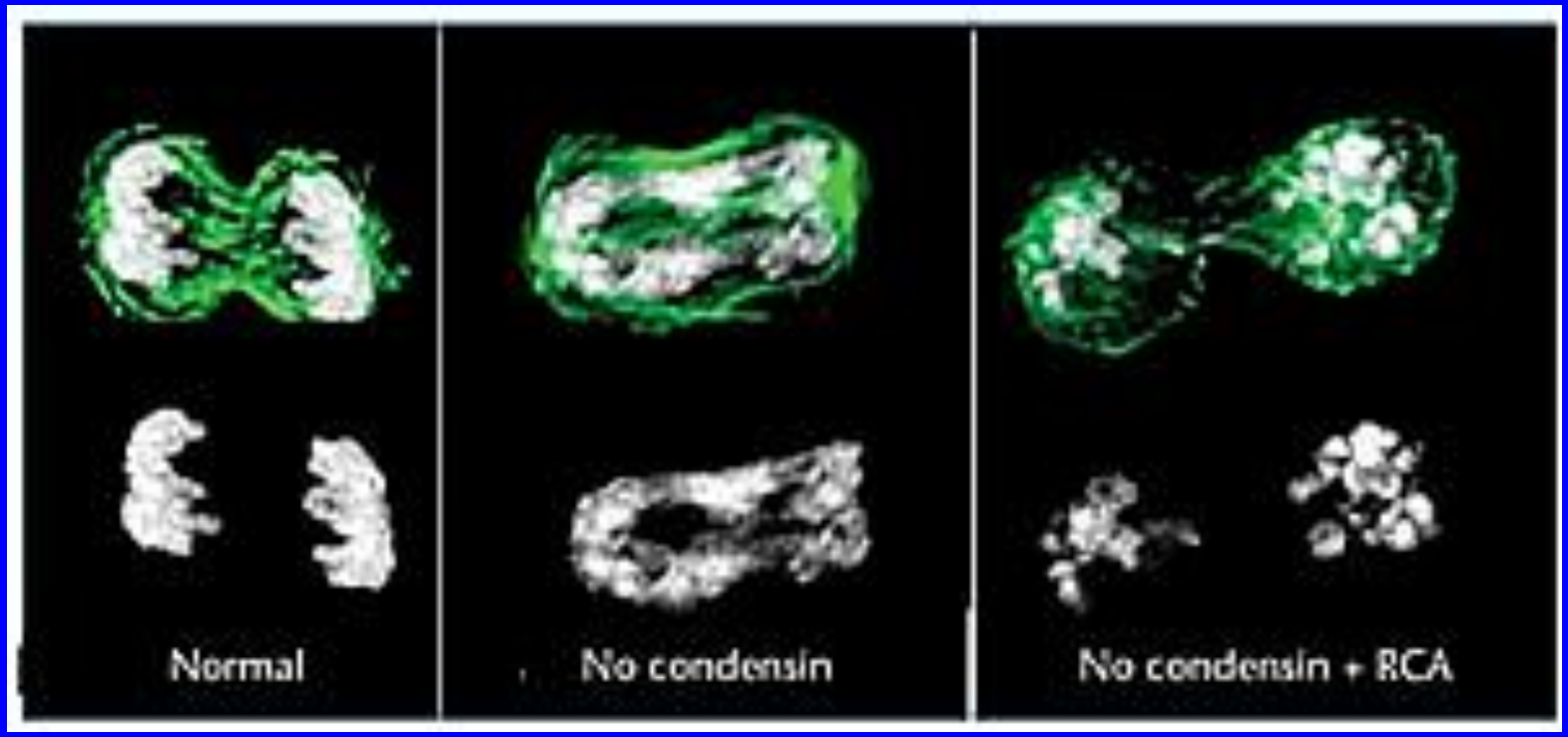
Chromosome anatomy and formation



✓ ***Condensin*** is required for successful completion of mitosis, but not for mitotic chromosome formation *in vivo*. Condensin is important for the timing of chromosome condensation, the elastic properties of chromosomes and centromeres, the segregation of rDNA in yeast, dosage compensation in *C. elegans*, and chromosome integrity during anaphase. Key condensin components are the SMC proteins, which have roles in many types of chromosome transactions.

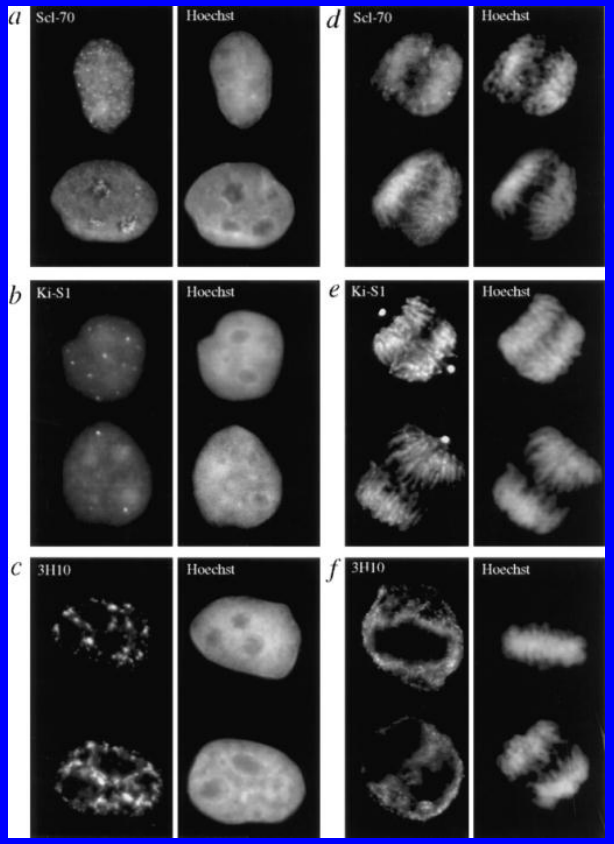
Chromosome anatomy and formation

✓ Dephosphorylation of a target termed RCA (regulator of chromosome architecture) by the Repo-Man:PP1 (protein phosphatase 1) holoenzyme correlates with a dramatic loss of chromosome organization during anaphase in cells lacking condensing. RCA could be a specific non-histone protein, or a combination of histone post-translational modifications. A recent study identified H3T3phK4me2R8me2 (termed the PMM mark) as specific for mitotic chromosomes.



Chromosome anatomy and formation

✓ Another protein previously linked with mitotic chromosome formation is DNA topoisomerase II (topo II), one of the most abundant non-histone proteins of mitotic chromosomes. However, topo II is dispensable for mitotic chromosome formation. Topo II could have an important influence on the behavior of chromosomes as they respond to forces within the mitotic spindle.



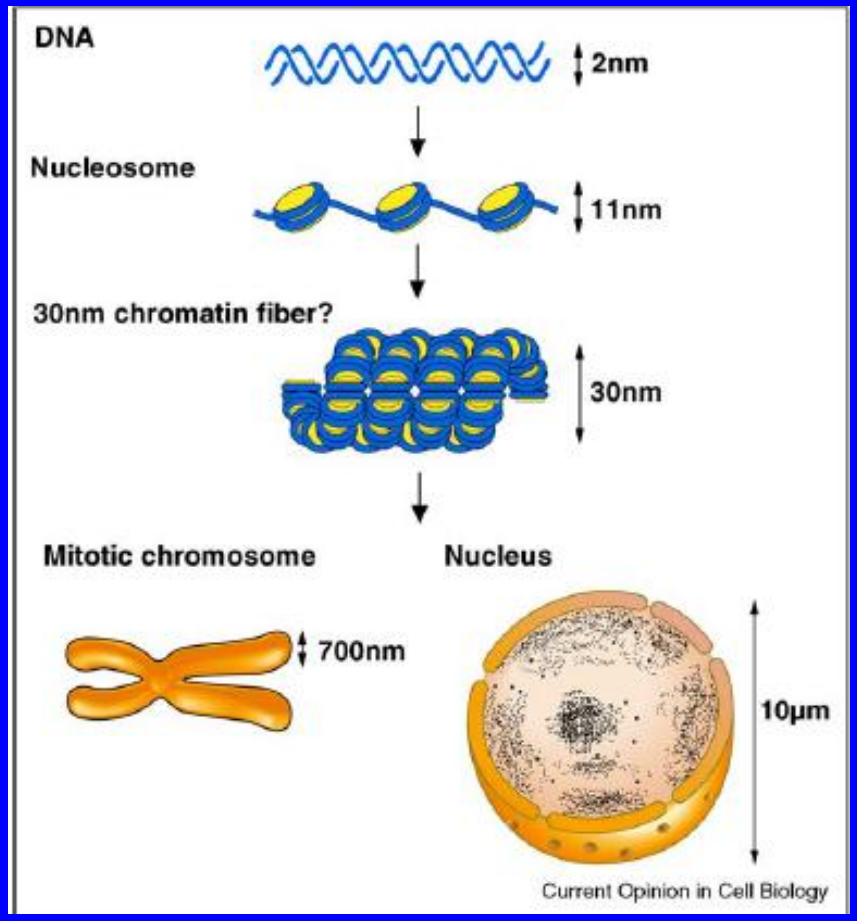
Localization of topoisomerases in interphase and mitosis. Close-up pictures of representative cells in interphase (a–c) or mitosis (d–f) immunostained for topoisomerase I (a and d), topoisomerase IIα (b and e), or topoisomerase IIβ (c and f). The left of each pair of images represents immunostaining. The right shows the corresponding DNA pattern (Hoechst).

Chromosome composition

✓ Isolated mitotic chromosomes are roughly 2:1 protein to nucleic acid on a mass basis. About half of this protein is histone, but the remainder is often lumped together under the not-very informative term ‘nonhistone proteins’.

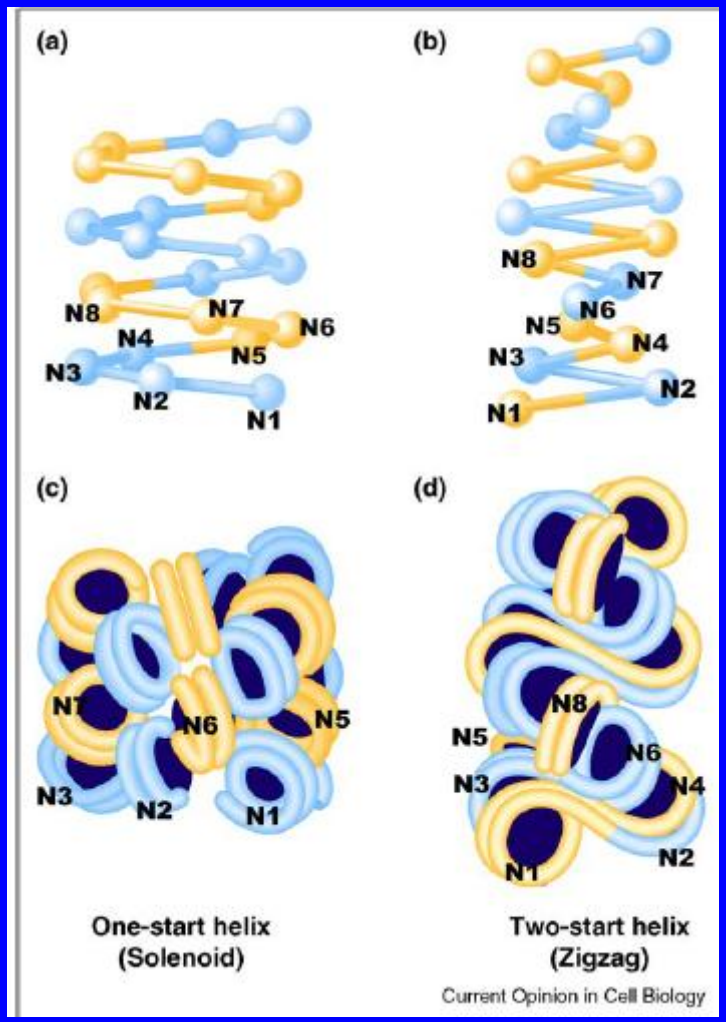
✓ A particularly thorough set of studies of the mitotic chromosome proteome has been carried out by the Fukui laboratory. They identified ≈250 proteins in isolated mitotic chromosomes, ≈100 of which are likely to be specific chromosomal proteins. The functional analysis of several proteins found at the chromosome periphery: nucleophosmin, nucleolin and regulator of ribosome synthesis 1 (RRS1) showed that they were found to be necessary for timely and efficient alignment of the chromosomes during prometaphase.

Chromosome topology



A long DNA molecule with a diameter of 2 nm is wrapped around a core histone octamer that consists of H2A, H2B, H3 and H4 histone proteins, and forms a 'nucleosome' with a diameter of 11 nm. The nucleosome has long been assumed to be folded into 30-nm chromatin fibres before the higher order organisation of mitotic chromosomes or interphase nuclei occurs.

Models of a 30-nm chromatin fibre



There are two well-known structural models for 30-nm chromatin fibres: one-start helix (solenoid) (a) and two-start helix (zigzag) (b).

(c) In the one-start helix proposed by Robinson and Rhodes, the 30-nm chromatin fibre is an interdigitated solenoid. Essentially, a nucleosome in the fibre interacts with its fifth and sixth neighbours.

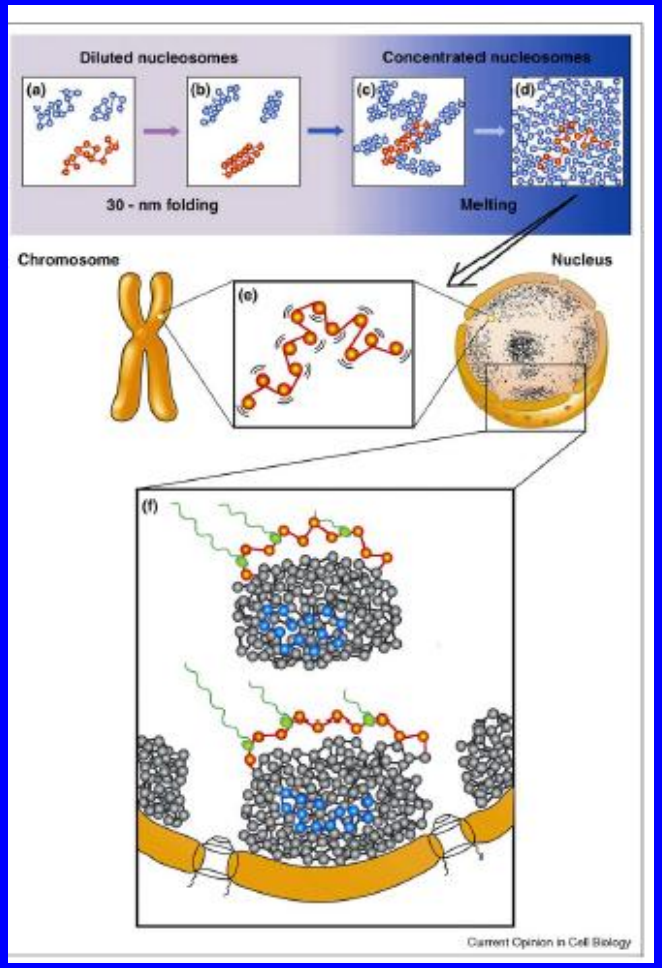
(d) In the two-start model proposed by Richmond and co-workers, nucleosomes are essentially arranged in a zigzag manner such that alternate nucleosomes form interacting partners. That is, a nucleosome in the fibre binds to the second neighbour nucleosome.

The concept of the polymer melt (Maeshima et al., 2010)

(a, b) Under diluted conditions, the flexible nucleosome fibres may compact through selective close neighbour associations, forming the 30-nm chromatin fibres. An increase in nucleosome concentration results in inter-fibre nucleosomal contacts, which interfere with the intra-fibre bonds (c).

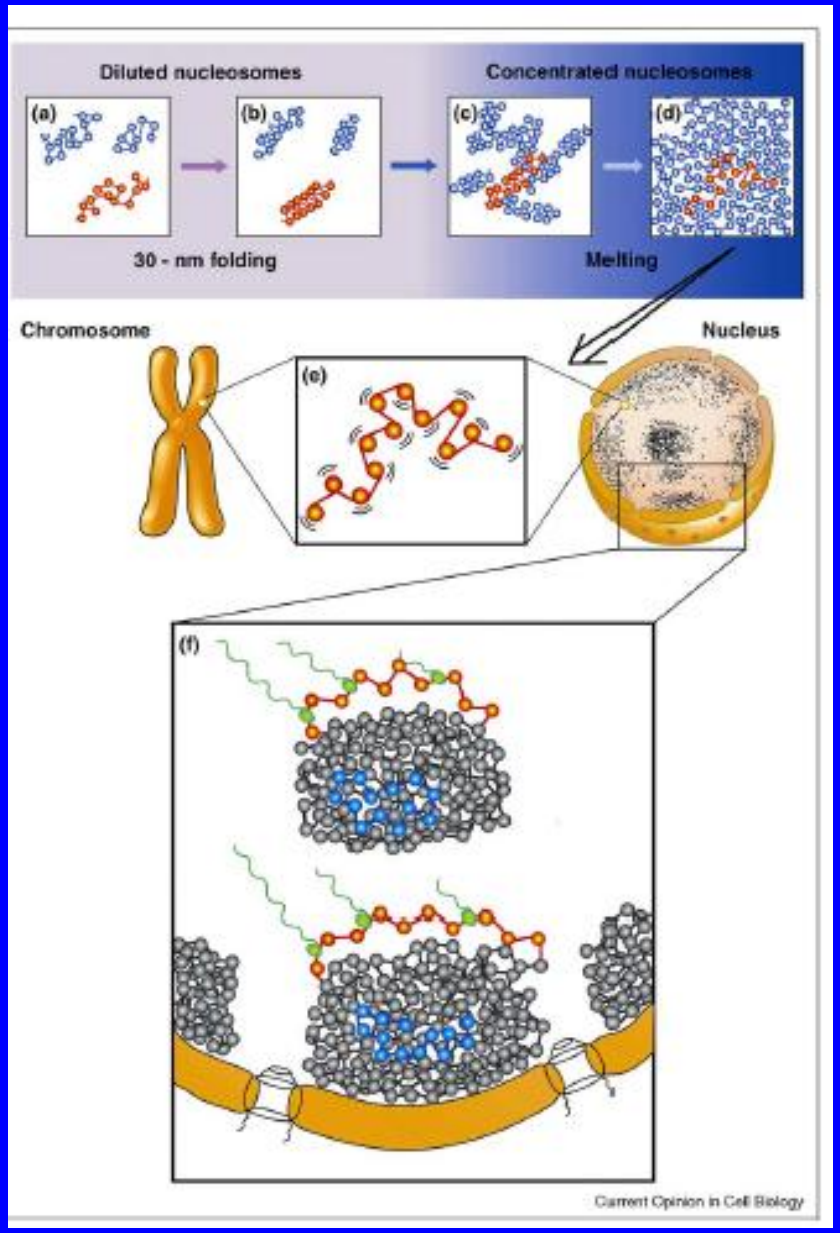
The nucleosomes of adjacent fibres interdigitate and intermix. This disrupts the 30-nm folding and the nucleosomal fibres progress to a state of 'polymer melt' (d).

(e) The concept of polymer melt implies dynamic polymer chains, that is, nucleosome fibres may be moving and rearranging constantly. This may have several advantages in chromosome condensation and segregation during mitosis and the transcription and DNA replication processes during interphase.



The concept of the polymer melt (Maeshima et al., 2010)

(f) ‘Chromatin liquid drop’: The transcriptional silencing can be established through a dynamic capturing of transcriptional regions inside compact chromatin melt domains. These domains can be considered as drops of viscous liquid, which could be formed by the nucleosome–nucleosome interaction and macromolecular crowding effect. Active and inactive chromatins are shown in orange and blue, respectively. Active chromatin regions are transcribed on the surfaces of the drops (shown in green).



Thank you for attention!