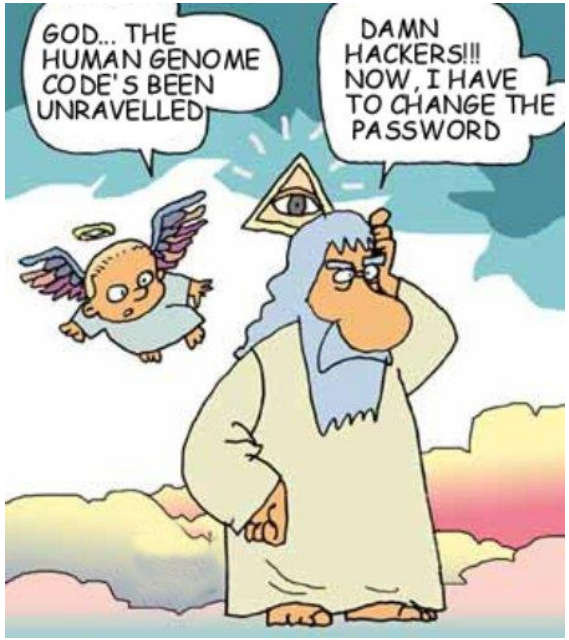
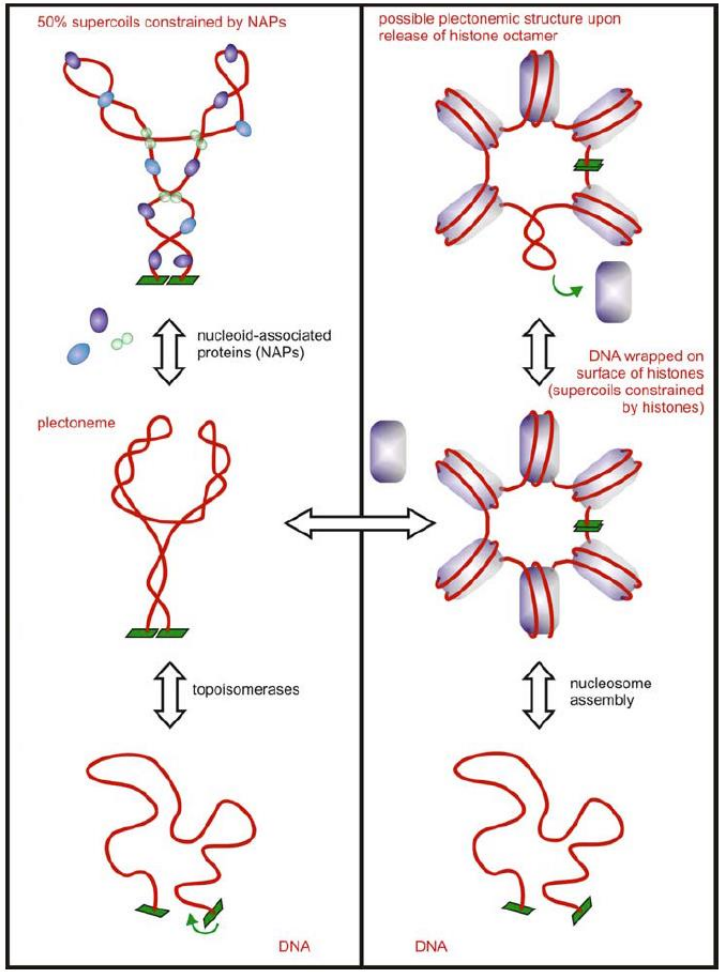
# PR4_Organizacija genoma v jedru in Kromosomi



# Organizacija genoma – vpliv arhitekturnih proteinov

The **genomic DNA** of all organisms across the three kingdoms of life needs to be **compacted and functionally organized**. Key players in these processes are **DNA supercoiling, macromolecular crowding and architectural proteins** that ***shape DNA by binding to it***. The **architectural proteins** in bacteria, archaea and eukaryotes generally **do not exhibit sequence or structural conservation** especially across kingdoms. Instead, we propose that **they are functionally conserved**. Most of these proteins ***can be classified according to their architectural mode of action: bending, wrapping or bridging DNA***. In order for DNA transactions to occur within a compact chromatin context, ***genome organization cannot be static***. Indeed chromosomes are subject to a whole range of ***remodeling mechanisms***. In this review, we discuss the role of ***(i) DNA supercoiling, (ii) macromolecular crowding and (iii) architectural proteins in genome organization, as well as (iv) mechanisms used to remodel chromosome structure and to modulate genomic activity***. We conclude that the underlying ***mechanisms that shape and remodel genomes are remarkably similar among bacteria, archaea and eukaryotes.***

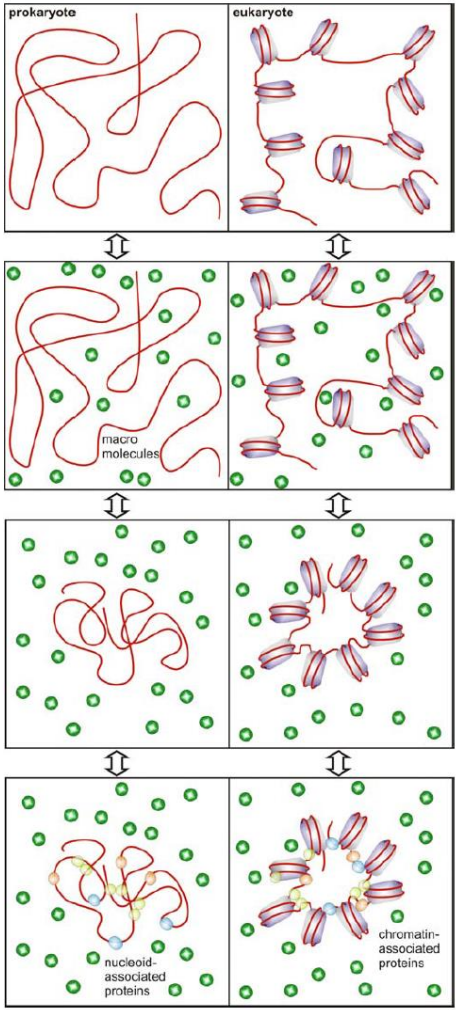
**The Major Architects of Chromatin: Architectural Proteins in Bacteria, Archaea and Eukaryotes (Luijsterburg et al. 2008)**




**DNA supercoiling in prokaryotes and eukaryotes.**

**Bacteria and some archaea** have enzymes that allow them to introduce supercoils into the DNA at the expense of ATP, which results in the formation of plectonemic structures (left panel). In bacteria, ***DNA gyrase*** introduces *negative supercoils*, while ***reverse gyrase*** leads to *positive supercoils* in some thermophilic organisms. The free superhelicity is partially constrained (about ~50%) by association of ***nucleoid-associated proteins (NAPs)***. The loss of the remaining free superhelicity by relaxation is prevented by the ***formation of topologically isolated domains***.

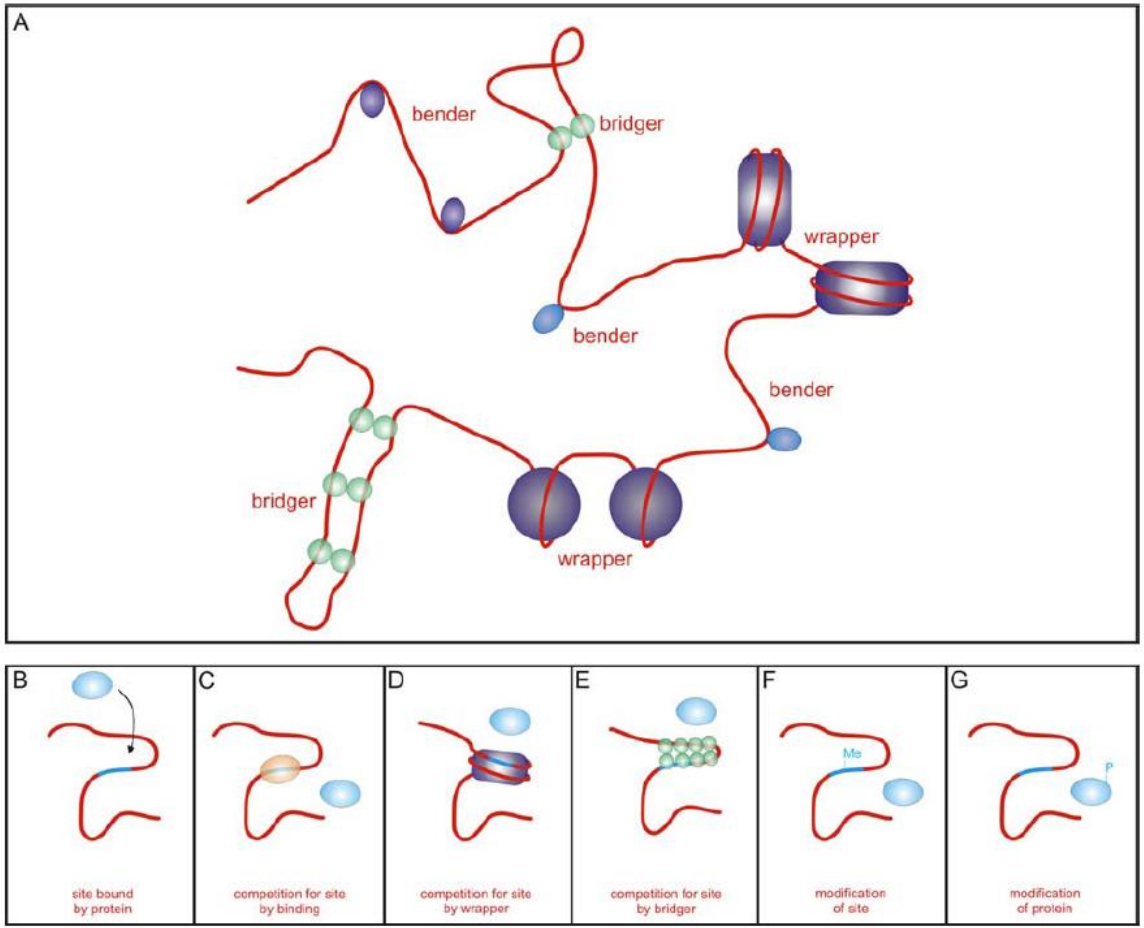
In **eukaryotes** (**and archaea** encoding histone proteins), which lack DNA gyrase, ***supercoils are introduced by wrapping of DNA around the nucleosome surface*** (right panel). These supercoils are constrained on the surface of histone proteins. Disassembly of a nucleosome (by remodelling) and subsequent unwrapping of the DNA, can release supercoils resulting in a topology similar to that in bacteria (i.e. plectonemic rather than toroidal supercoils). Since *free supercoils are only slowly removed by topoisomerases in eukaryotes*, this local free superhelicity could contribute to strand separation during transcription or result in recruitment of structure-sensitive regulatory proteins.




**Macromolecular crowding leads to strong compaction of genomes.**

The concentration of macromolecules (RNA and proteins) in the nucleus and nucleoid is about 100–400 mg/ml (indicated by the green spheres). Such a ***high macromolecular concentration results in entropy-driven compaction of genomic DNA*** (indicated in red, wrapped in nucleosomes depicted in purple) regardless whether DNA is wrapped in nucleosomes and shaped by other architectural proteins. For simplicity, DNA in prokaryotes is drawn as free of architectural proteins. It is important to note that it is not devoid of architectural proteins in general. In addition, ***such crowding conditions shift the equilibrium constant for many protein-DNA interactions, pushing the equilibrium towards binding of these proteins to DNA.*** Since ***crowding drives compaction of genomes non-specifically, it is expected to play an important role in genome compaction*** and to have only a ***minor role in regulation of genome functions*** by altering crowding conditions.

Macromolecular crowding = makromolekularno drenjanje




**Bending, wrapping and bridging of DNA by architectural proteins and mechanisms to modulate the occupancy of binding sites on the DNA.**

A) ***Chromatin*** in cells across all kingdoms is ***shaped*** by proteins that can be classified according to their architectural mode of action: 1) ***bending of DNA*** (depicted by the purple and blue ovals), 2) ***wrapping of DNA*** (depicted by the purple circle and rectangle that wrap DNA once or twice around their surface, respectively) and 3) ***bridging of two DNA duplexes*** (depicted by the green circles). B) Schematic representation of mechanisms to modulate binding site occupancy by C) direct competition for a binding site with another protein, D) modulating the binding of a protein by wrapping its target site around a protein surface (such as archaeal or eukaryotic nucleosomes, or Lrp-like proteins in bacteria, or E) modulating the binding of a protein by forming a bridged filament that includes the target site, F) modification of the binding site which alters the affinity of the protein binding to this site. This could be DNA methylation or histone methylation acting to lower or increase the affinity of a protein for such a modified site, G) modification of the DNA-binding protein (for instance by phosphorylation).

Bending: upogibanje/ukrivljanje,
wrapping: zavijanje;
bridging: premostitev.

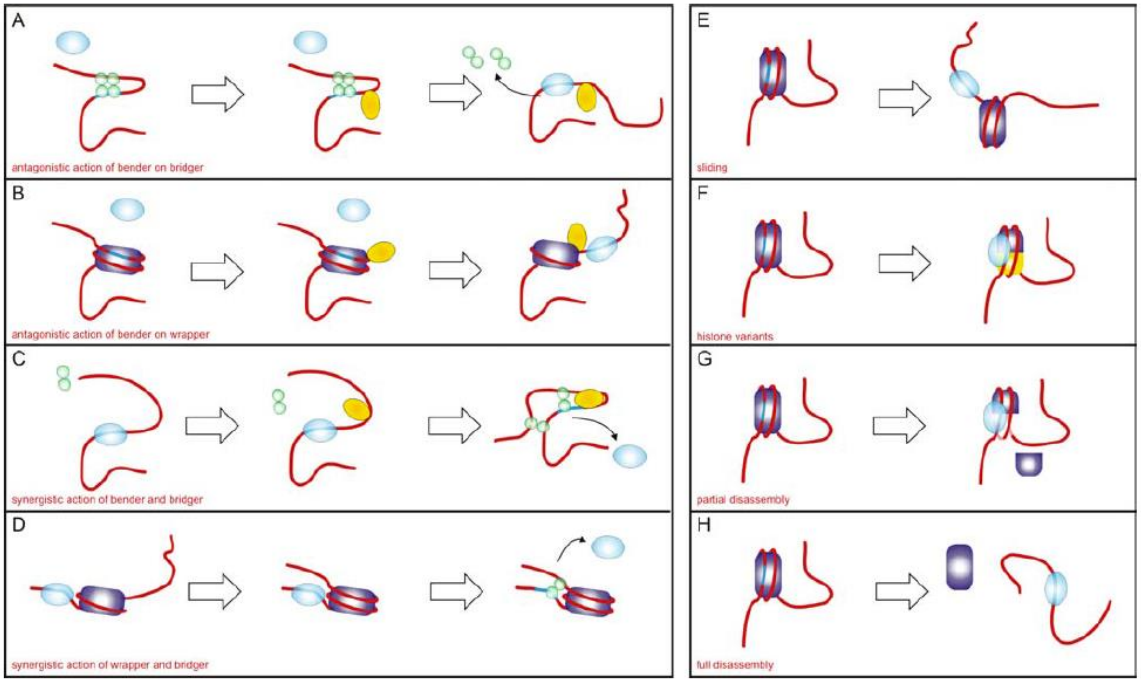
| | | *wrappers* | *benders* | *bridgers* |
|---|---|---|---|---|
| Eukaryotes | | H2A, H2B, H3, H4 (core histones) | HMG | H1 (linker histone) |
| | | | | BAF |
| | | | | SMC |
| Archaea | Crenarchaea | *Lrp** | Cren7 | Alba |
| | | | Sul7 | Lrp* |
| | | | | SMC |
| | Euryarchaea | HMfA and HMfB (histones) | MC1 | Alba |
| | | *Lrp** | HU | Lrp* |
| | | | | SMC |
| Bacteria | Gram positive | *Lrp** | HU | Lrp* |
| | | | | Lsr2 (H-NS-like) |
| | | | | SMC |
| | Gram negative | *HU** | HU* | H-NS |
| | | *Lrp** | IHF | Lrp* |
| | | | Fis | *Fis** |
| | | | | SMC |
| | | | | H1-like proteins |

*these proteins have been proposed to exhibit dual architectural properties, that are likely dependent on protein concentration or DNA binding sequence. The secondary binding mode is indicated in italic.

**Overview of different classes of architectural proteins (wrappers, benders or bridgers) and their distribution in Bacteria, Archaea and eukaryotes.**

Note that ***homologues of the eukaryotic histones are found in the other kingdoms***. H1 is absent in Archaea, but H1 homologues have been identified in Gram negative bacteria, whereas core histone proteins are absent from bacteria, but have been identified among Euryarchaea.

High-Mobility Group or **HMG** is a group of chromosomal proteins that are involved in the regulation of DNA-dependent processes such as transcription, replication, recombination, and DNA repair. **SMC** proteins represent a large family of ATPases that participate in many aspects of higher-order chromosome organization and dynamics. SMC stands for Structural Maintenance of Chromosomes. **BAF** (BRG1/brm-associated factor) complex in mammals, which is functionally related to SWI/SNF complex in S. cerevisiae and Drosophila; the latter is thought to facilitate transcriptional activation of specific genes by antagonizing chromatin-mediated transcriptional repression.




**Modulation of binding site accessibility by the concerted or opposing action of architectural proteins and energy-dependent displacement of DNA wrappers.**

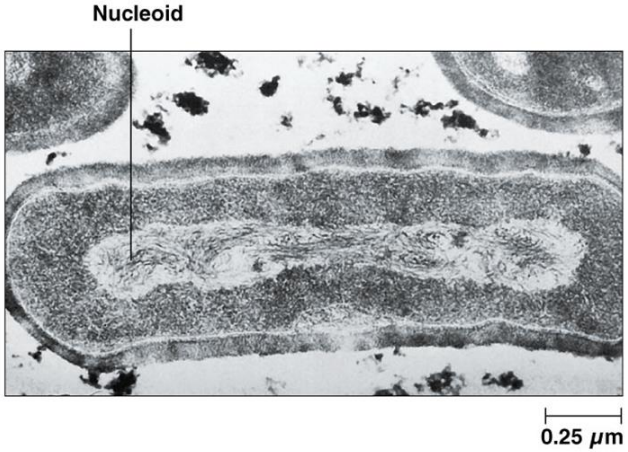
**A**) Opposing action of a bender and bridger. The binding of bridgers is often very cooperative, as binding depends on a high effective DNA concentration. A bender can destabilize a complex formed by bridging proteins by bending two DNA duplexes away from each other. **B**) Opposing action between a bender and wrapper: the bender bends the DNA away from the surface of the wrapper. For instance, HMG proteins facilitate unwrapping of DNA from nucleosomes by ATP-dependent remodelling complexes. **C**) Concerted action of a bender and bridger. This is analogous to (A) but with a different outcome. If a bender bends DNA duplexes towards each other this can create a site suited for binding of a bridger. **D**) Concerted action of wrapper and bridger. Wrapping DNA results in a local high concentration of DNA, for instance, where DNA enters and exits the nucleosome, which can facilitate bridging. A clear example is the binding (and bridging) of DNA that enters and exist the nucleosome by linker H1. **E**) A nucleosome can be "pushed" away from its binding site, which then becomes exposed. This type of "sliding" is mediated by remodelling complexes of the SWI/SNF (that randomize nucleosomes by sliding) and ISWI families (that promote equal spacing between nucleosomes by sliding). **F**) Incorporation of histone variants that alter the stability of nucleosomes (depicted in yellow). Several variants of H2A as well as of H3 exist that destabilize the nucleosomes into which they are incorporated to replace the "standard" H2A and H3 proteins. **G**) Partial disassembly of nucleosomes by release of H2A-H2B dimers. This mechanism results in partial unwrapping of the DNA and is often employed during transcription. **H**) Complete disassembly of nucleosomes, which can be mediated by ATP-dependent remodelling complexes of the SWI/SNF family, resulting in full unwrapping of the DNA. This may release supercoils constrained by that nucleosome.

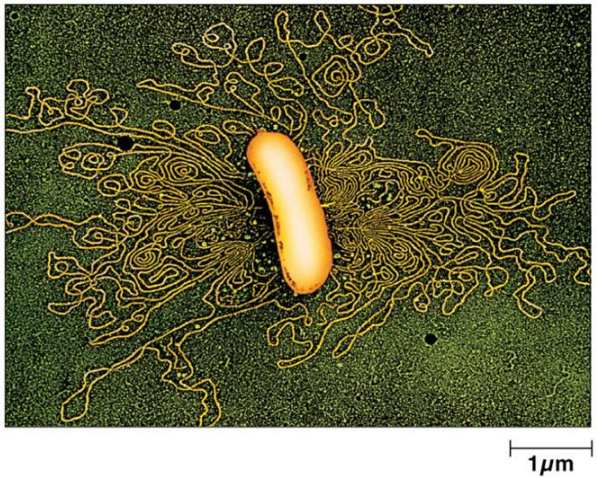
# Prokarionti - organizacija genoma in kromosomi

***Prokaryotic versus Eukaryotic Chromosomes***

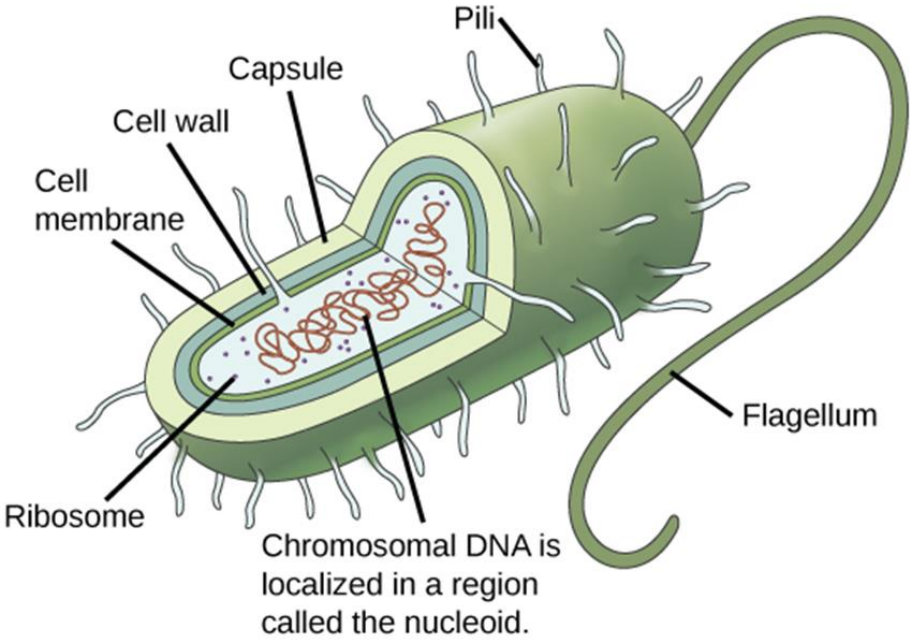
| Prokaryotic Chromosomes | Eukaryotic Chromosomes |
| --- | --- |
| • Many prokaryotes contain a single circular chromosome.<br>• Prokaryotic chromosomes are condensed in the nucleoid via DNA supercoiling and the binding of various architectural proteins.<br>• Because prokaryotic DNA can interact with the cytoplasm, transcription and translation occur simultaneously.<br>• Most prokaryotes contain only one copy of each gene (i.e., they are haploid).<br>• Nonessential prokaryotic genes are commonly encoded on extrachromosomal plasmids.<br>• Prokaryotic genomes are efficient and compact, containing little repetitive DNA. | • Eukaryotes contain multiple linear chromosomes.<br>• Eukaryotic chromosomes are condensed in a membrane-bound nucleus via histones.<br>• In eukaryotes, transcription occurs in the nucleus, and translation occurs in the cytoplasm.<br>• Most eukaryotes contain two copies of each gene (i.e., they are diploid).<br>• Some eukaryotic genomes are organized into operons, but most are not.<br>• Extrachromosomal plasmids are not commonly present in eukaryotes.<br>• Eukaryotes contain large amounts of noncoding and repetitive DNA. |

# Bakterijski kromosom

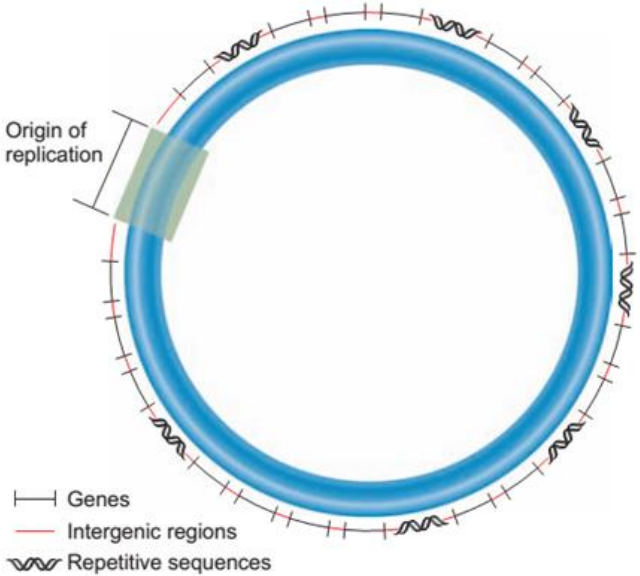




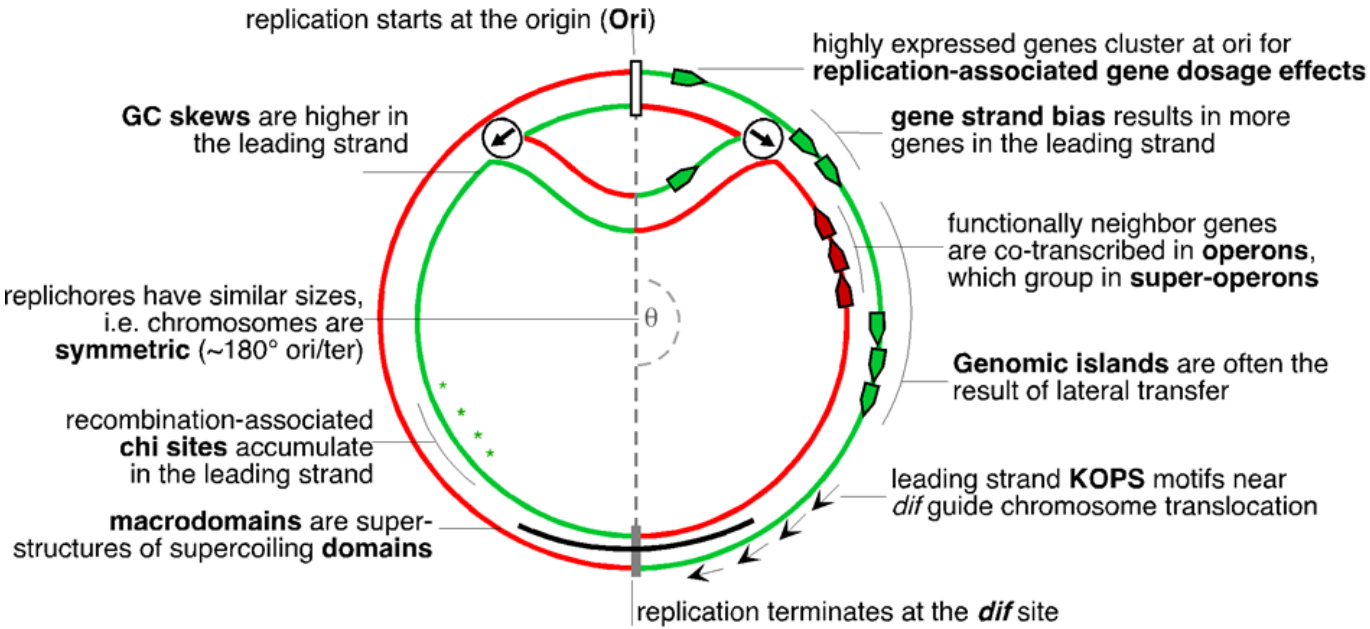



# Organization of sequences in bacterial chromosomal DNA



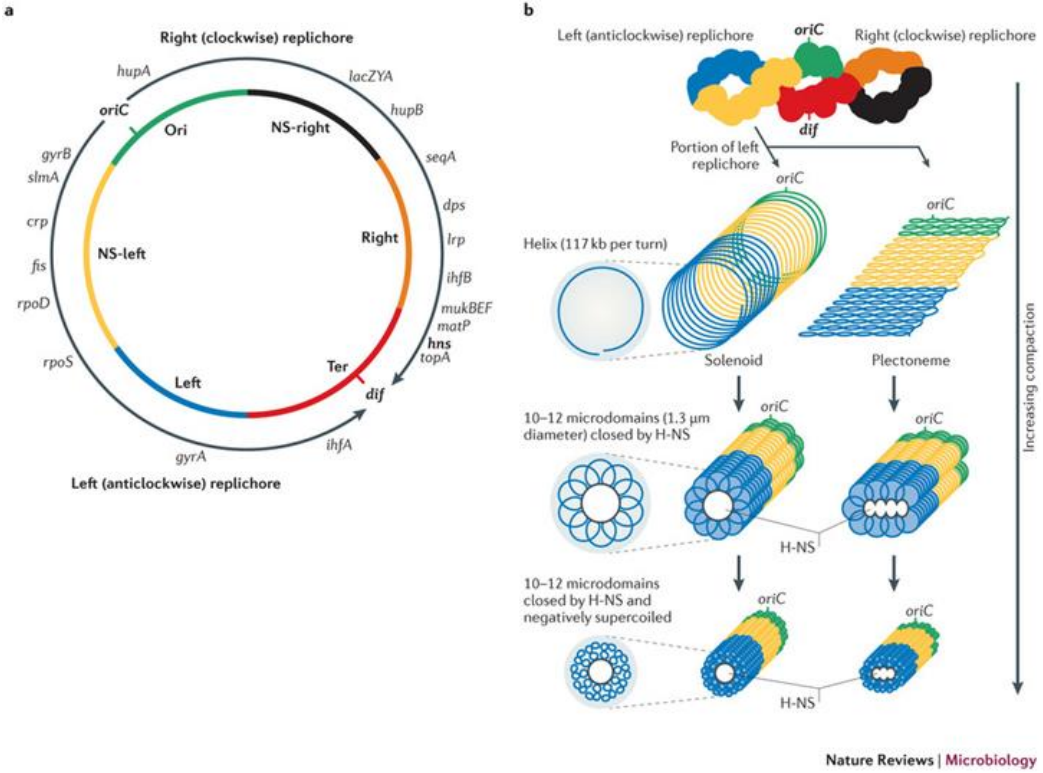

**Key features:**

- Most, but not all, bacterial species contain circular chromosomal DNA.
- A typical chromosome is a few million base pairs in length.
- Most bacterial species contain a single type of chromosome, but it may be present in multiple copies.
- Several thousand different genes are interspersed throughout the chromosome. The short regions between adjacent genes are called intergenic regions.
- One origin of replication is required to initiate DNA replication.
- Repetitive sequences may be interspersed throughout the chromosome.




**Elements associated with the organization of the bacterial chromosome.**

**Green** and **Red** distinguish between the ***leading and the lagging strands***. **Ori and Ter** identify the ***origin and terminus of replication***, encircled ***arrows indicate the direction of replication fork progression***. Besides these elements, several ***mutational biases*** related with replication have been described in bacterial genomes.

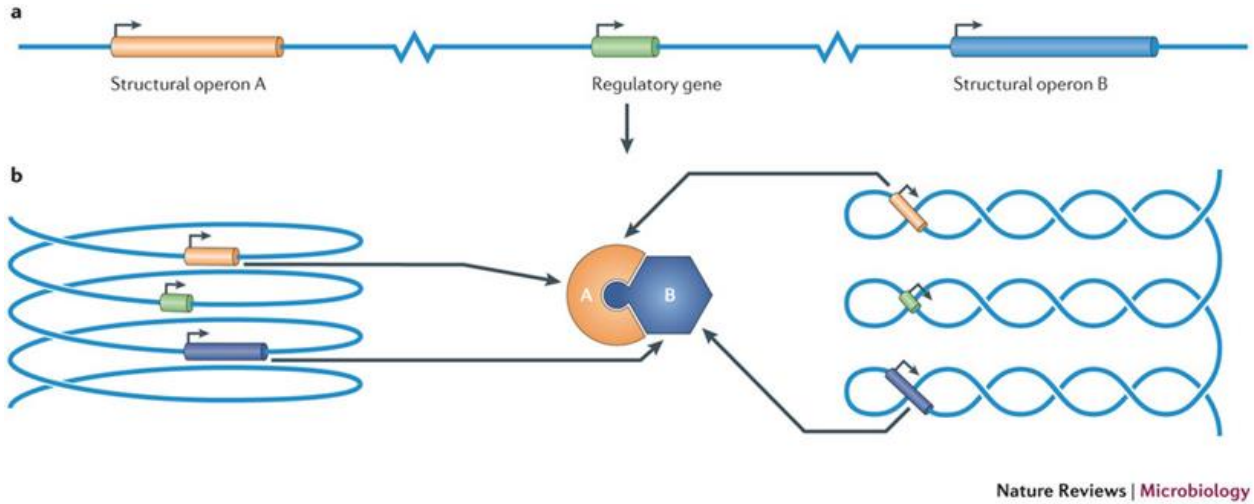



## Organization of the Escherichia coli nucleoid.

a| The **circular chromosome** is shown with its **macrodomains** indicated. The oriC locus is the origin of chromosome replication, and dif is the site where the XerC and XerD site-specific recombinases resolve chromosome dimers. The directions of DNA replication are shown by the black arrows, and these arrows constitute the right (clockwise) and left (anticlockwise) replichores.

b| The ***chromosome is shown as a writhed structure*** (top), reflecting imaging data which suggest that it adopts a conformation of this type, at least in rapidly growing bacteria. The thickness of this writhed DNA is indicative of the underlying layers of structure, as indicated below. A portion of the ***left replichore is illustrated as a solenoid*** and as a ***plectoneme***, both of periodicity 117 kb. The DNA is next compacted by introducing 10–12 ***microdomains*** into each of its 117 kb units. These microdomain circles (each of 10–12 kb) have a diameter of approximately 1.3 μm, giving the nucleoid a cross-section of about 2.6 μm. Supercoiling these small circles compacts them approximately twofold. The nucleoid-associated protein H-NS (histone-like, nucleoid-structuring protein) is thought to have a core role within the two replichores, as it holds together the ends of the microdomain loops.

crp, cyclic AMP receptor protein gene; dps, DNA protection during starvation; fis, factor-for-inversion stimulation; hup, HU subunit gene; ihf, integration host factor subunit; gyr, DNA gyrase subunit; topA, topoisomerase I.

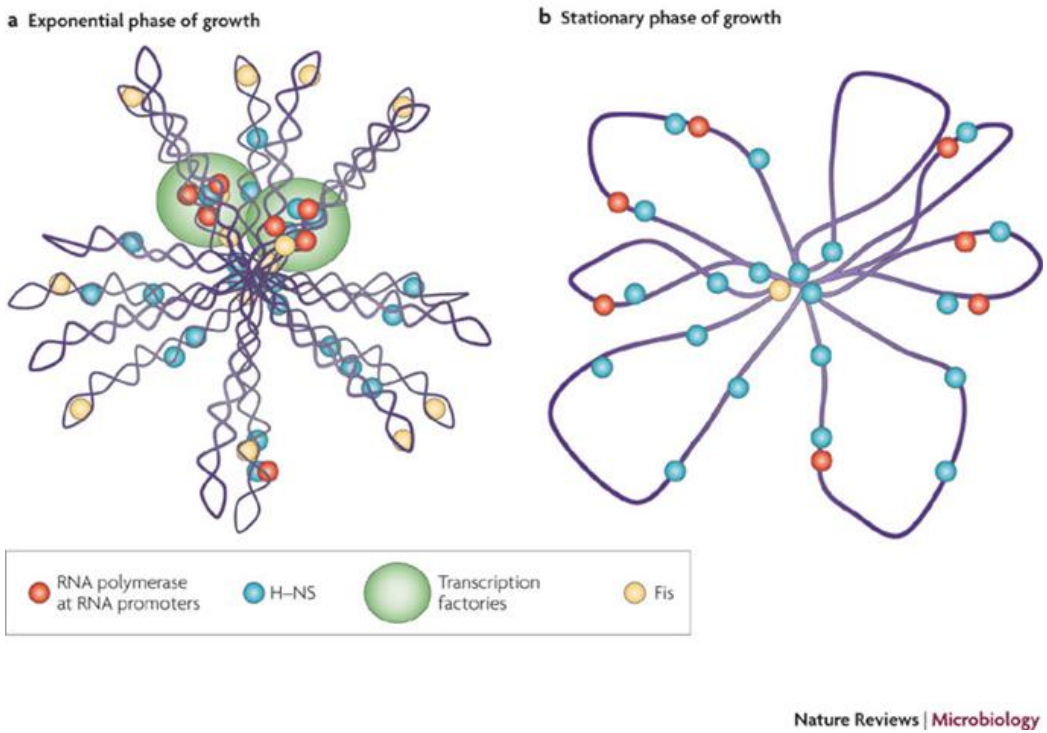



## Nucleoid folding and gene regulation.

A simple **regulon** consisting of a regulatory gene and two structural operons, A and B, is illustrated in various conformations.

a| When the chromosome is represented in a ***one-dimensional, linear form***, the three genetic loci are separated by large distances in space.

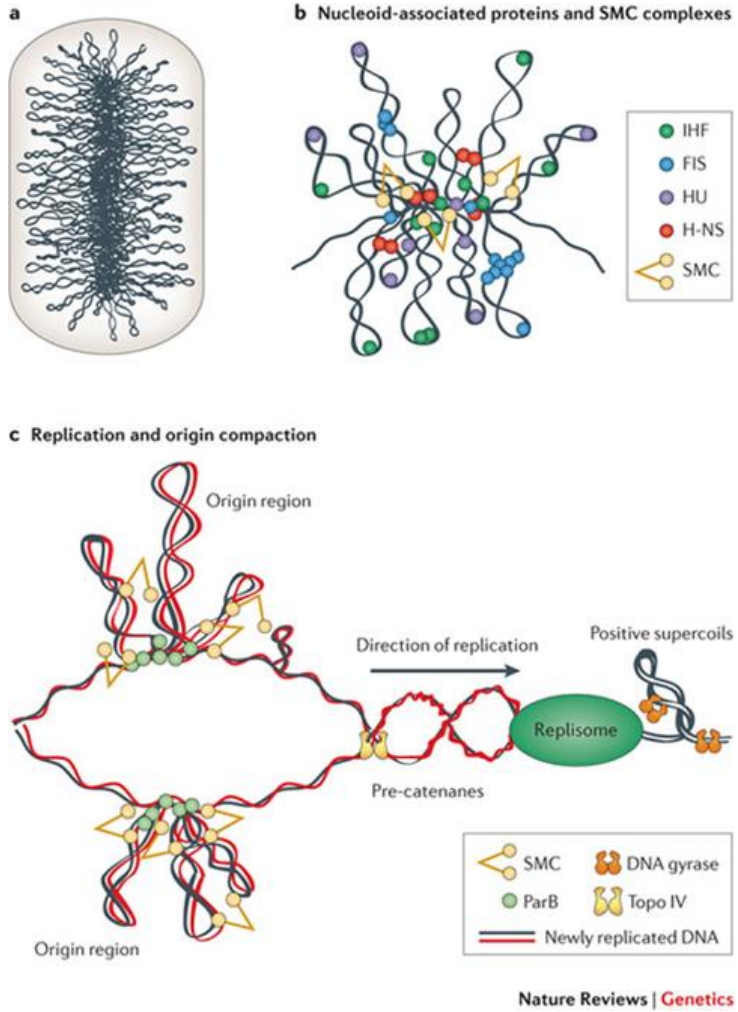
b| However, ***when the chromosome is reorganized as a solenoid (left) or as a plectoneme (=toroid) (right)***, the periodicity of these structures brings the three genes close together, facilitating communication between the regulatory gene and its two target operons. Moreover, the products of the A and B operons are produced in close proximity, favouring their interaction.




**Nucleoid-associated proteins and DNA supercoiling influence nucleoid structure.**

a| ***The folded chromosome is organized into looped domains that are negatively supercoiled during the exponential phase of growth.*** In this phase, the abundant nucleoid-associated proteins histone-like nucleoid-structuring protein (H-NS) and factor for inversion stimulation (Fis) bind throughout the nucleoid and are ***associated with the seven ribosomal RNA operons***. As shown here in two cases, ***these are organized*** into ***superstructures*** called ***transcription factories***.

b| ***In stationary phase the rRNA operons are quiescent*** and Fis is almost undetectable. The chromosome has ***fewer looped domains***, and those that are visible consist of ***relaxed DNA***.

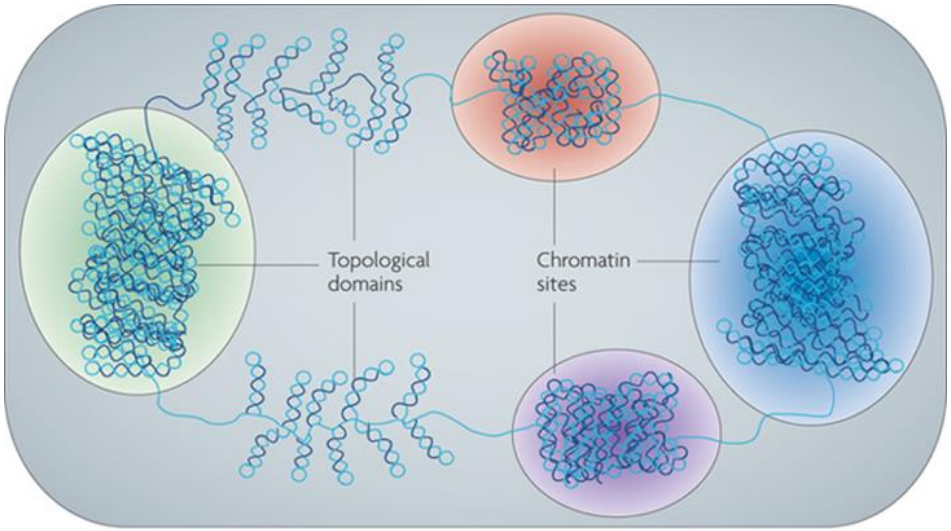



**Topological organization of the bacterial chromosome.**

a| Schematic representation of the ***bottlebrush model of the nucleoid***. This diagram depicts the ***interwound supercoiled loops*** emanating from a ***dense core***. The topologically isolated domains are on average 10 kb and therefore are likely to encompass several branched plectonemic loops.

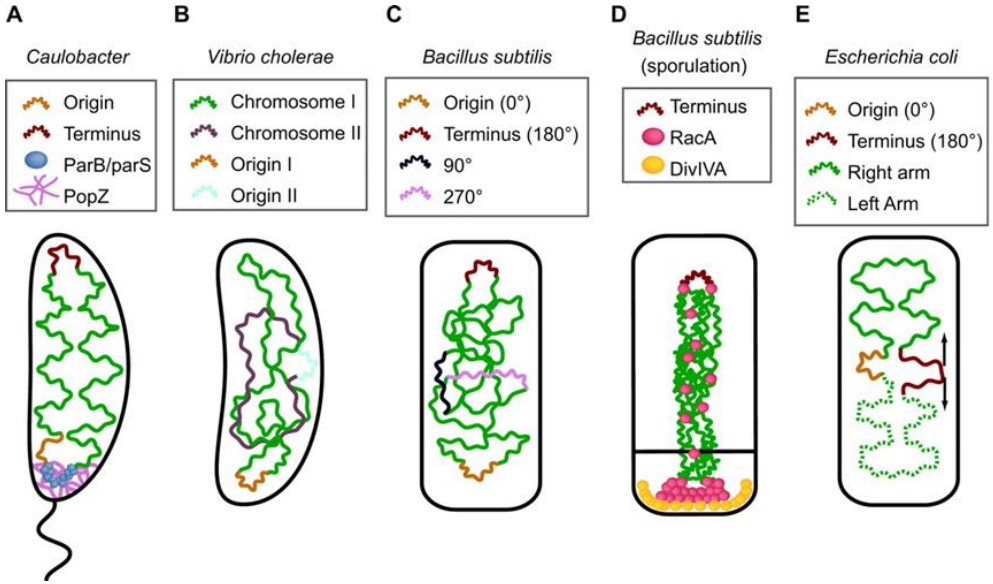
b| Schematic representation of the ***small nucleoid-associated proteins*** and the structural maintenance of chromosome ***(SMC) complexes***. These proteins ***introduce DNA bends and also function in bridging chromosomal loci.***

c| The diagram depicts ***replication fork progression and compaction of the origin region***. Replication generates positive supercoils ahead of the fork, which can diffuse behind the replisome, producing pre-catenanes. Positive supercoils are removed by DNA gyrase and topoisomerase IV (Topo IV), and pre-catenanes are unlinked by Topo IV. Newly replicated origin regions are thought to be compacted by the SMC complexes that are recruited to the origin by ParB and by the action of small nucleoid-associated proteins (not shown).




**Spatial organization of bacterial chromosomes.**

Recent studies indicate that the ***exquisite organization in eukaryotic nuclei is shared by bacterial nucleoids***. Assays assessing recombination efficiency in bacteria indicated that ***bacterial genomes consist of mutually inaccessible chromatin sites that are spatially confined by associations with stationary cellular components such as the cell membrane*** (see the figure). Notably, DNA motion is highly constrained even within a particular site. ***Each chromatin site comprises multiple topological domains, the average size of which in Escherichia coli is 10 kb***, substantially smaller than was thought initially. The resulting approx. ***500 domains were suggested to facilitate double-strand break repair by restricting DNA diffusion and therefore keeping severed ends in close and persistent proximity***. The most conclusive indication that ***prokaryotic chromosomes are characterized by a profoundly non-random organization***, reflected by a persistent localization of discrete chromosomal sites, was obtained from direct visualization of specific chromosomal sites. These cytological studies established the fact that ***the origin and terminus of replication are consistently positioned at particular locations in cells***. Subsequent analyses, in which numerous DNA loci were mapped in E. coli and Caulobacter crescentus, showed that, much ***like chromosome territories in higher eukaryotes, each site across bacterial chromosomes adopts a well-defined, species-specific address within the nucleoid***. ***As is the case in eukaryotes, constrained diffusion and confinement of chromatin sites are incompatible with extensive homology search processes.***




**Chromosome organization in model bacteria.**

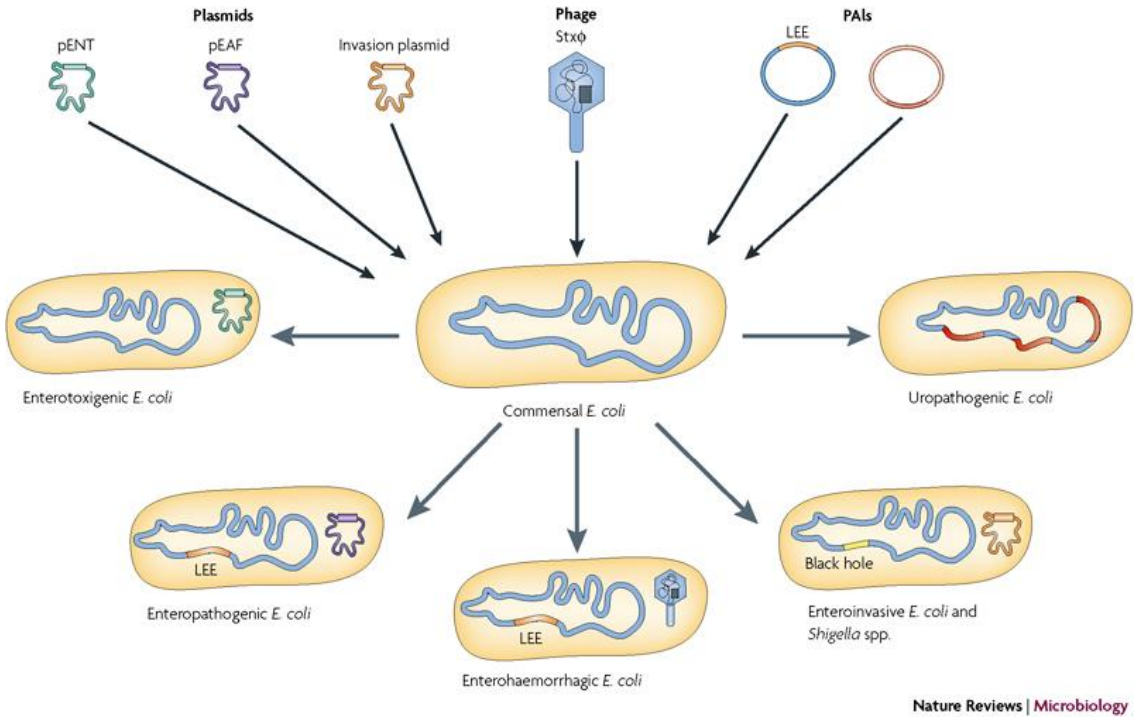
(A) The Caulobacter chromosome is ***linearly organized***, and anchored to the flagellated pole via parS/ParB/PopZ.

(B) In Vibrio cholerae, the origin region of the larger chromosome (chromosome I) is localized to the cell pole, whereas the origin of the smaller chromosome is localized to the cell center. The organization of the bulk of the chromosomes, as well as their separation or intermingling, are currently unknown.

(C) Four loci have been localized in vegetative cells of Bacillus subtilis, and their organization is reminiscent of the ***linear order*** seen in Caulobacter. Although the origin region is localized near to one pole, it appears not to be anchored to the cell membrane.

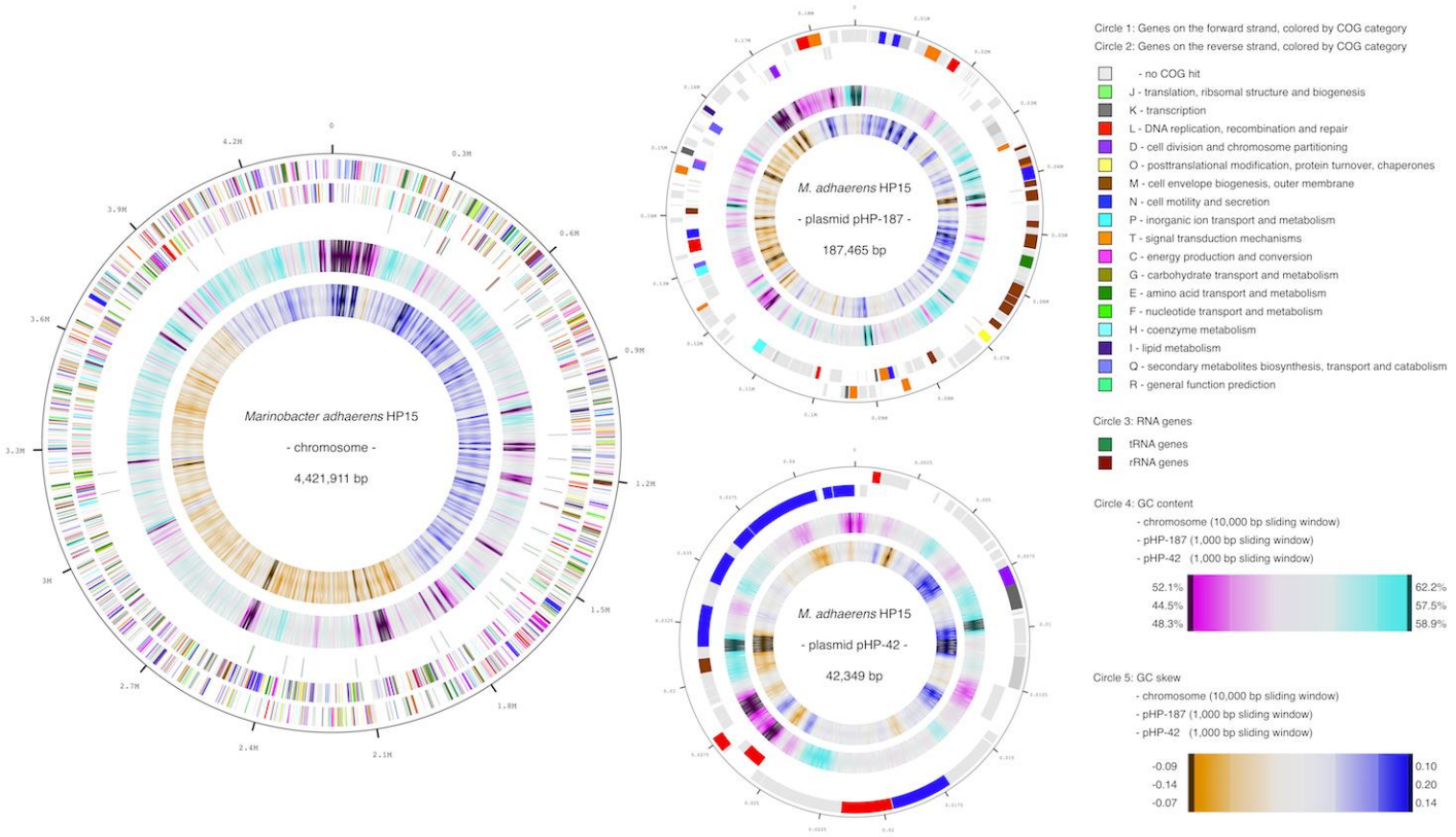
(D) Sporulating cells of B. subtilis, however, do anchor the origin region, through RacA/DivIVA, to the negatively curved membrane at the pole. RacA also binds all along the chromosome, compacting it into a long “axial filament” before sporulation.

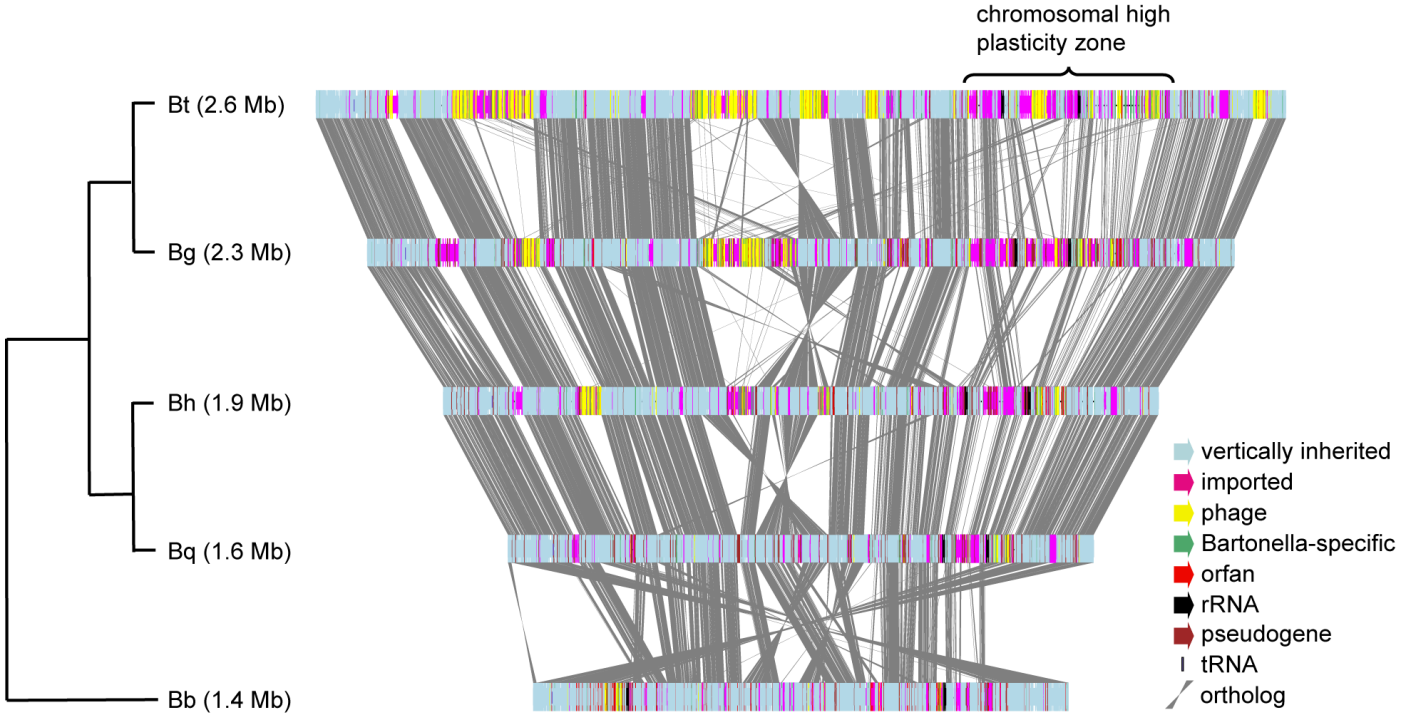
(E) The E. coli origin localizes to mid-cell, and the two replichores are separated into opposite cell halves. The terminus is broadly localized (arrows), and may be found on either side of the cell center.



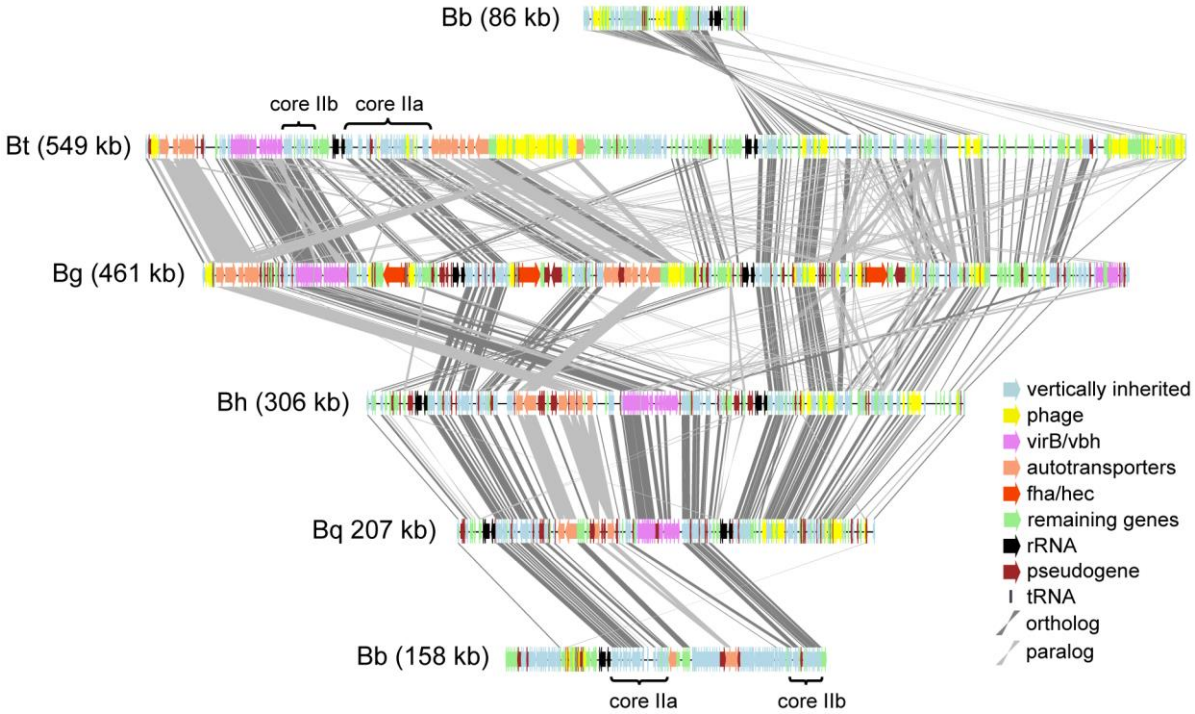

**Contribution of horizontal acquisition of mobile genetic elements to the evolution of Escherichia coli pathotypes.**
The uptake of mobile genetic elements (phages, virulence plasmids and pathogenicity islands), as well as the loss of chromosomal-DNA regions in different E. coli lineages, has enabled the evolution of separate clones, which belong to different E. coli pathotypes and are associated with specific disease symptoms. LEE, locus of enterocyte effacement; PAI, pathogenicity island; pEAF, enteropathogenic E. coli adhesion-factor plasmid; pENT, enterotoxin-encoding plasmids; Stx, Shiga-toxin-encoding bacteriophage.

# Prokarionti – kromosom in plazmidi



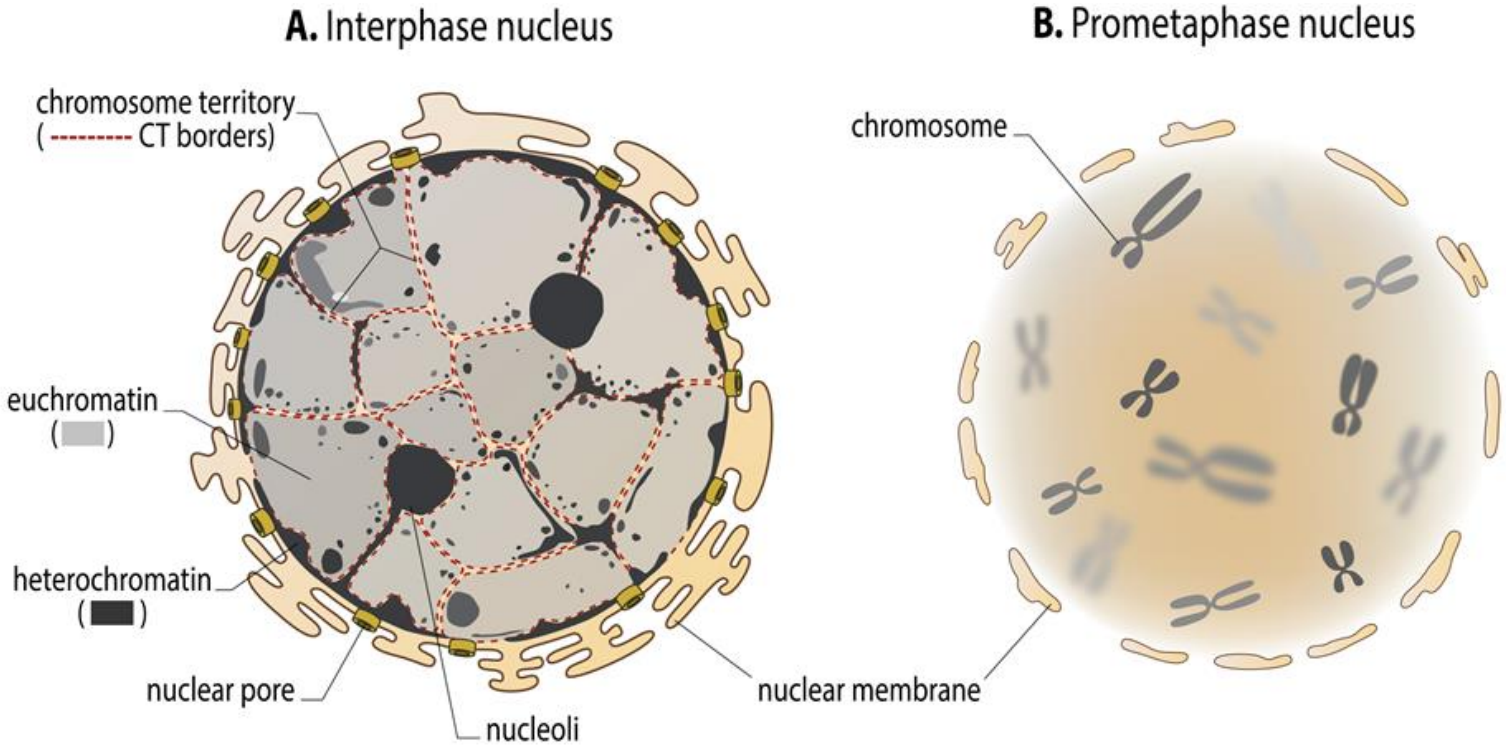


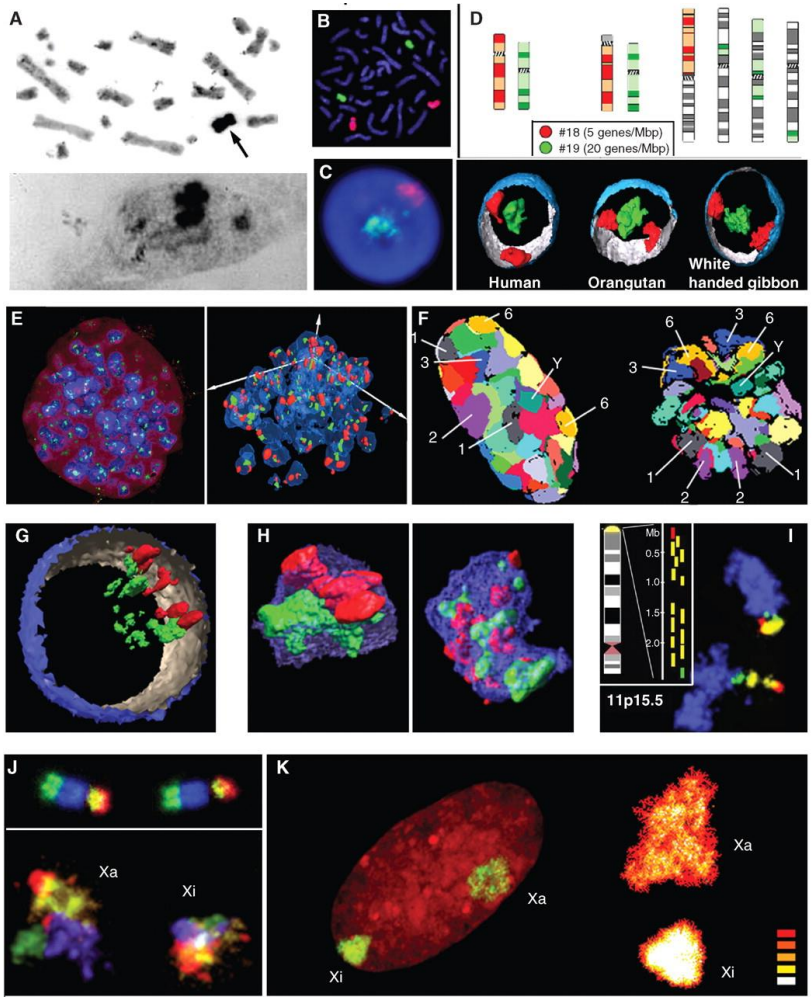

**Comparison of the structures of the Bartonella genomes.** A schematic illustration of the phylogenetic relationship of the five sequenced Bartonella species is shown to the left of a linear representation of their genomes. The total size of each genome is shown within parenthesis. Genes are color-coded based on phylogenetic classifications and annotation. Grey lines between genes indicate orthology. Bt: B. tribocorum, Bg: B. grahamii, Bh: B. henselae, Bq: B. quintana, Bb: B. bacilliformis.



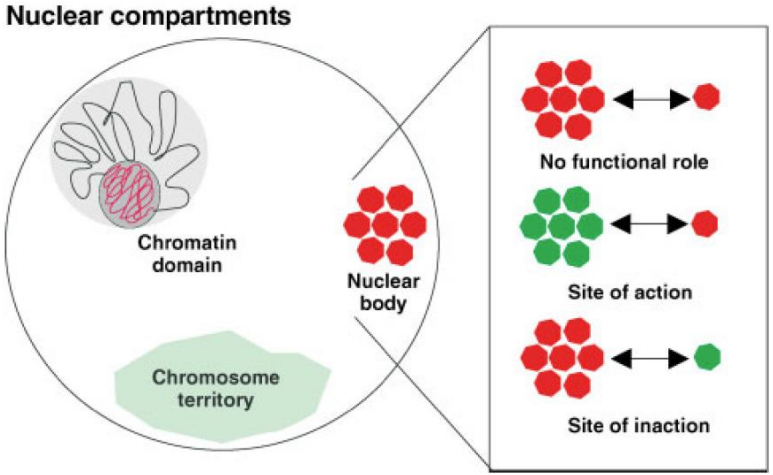

**Comparison of the chromosomal high plasticity zone in the Bartonella genomes.** Comparative gene map of the chromosomal high plasticity zone in the five sequenced Bartonella genomes. The locations of T4SS (virB/vbh) and T5SS (autotransporters, fha/hec) are shown. Species abbreviations are as in the legend to Figure 1. The total size of the region in each species is shown within parentheses. Due to the rearrangement around the replication origin in B. bacilliformis, the chromosomal high plasticity zone is divided into two parts, located on different sides of the origin.

# Eukarionti - organizacija genoma

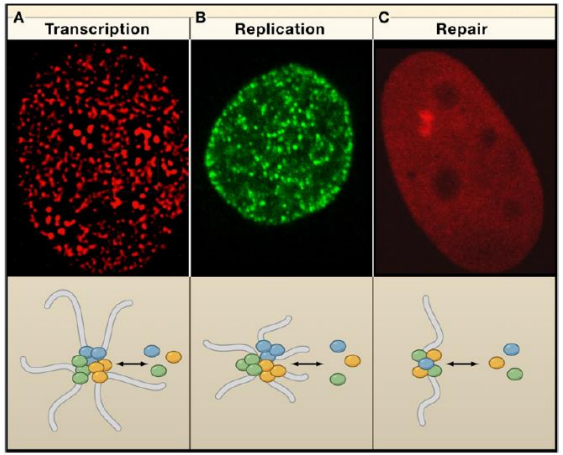

A. Interphase nucleus
B. Prometaphase nucleus
chromosome territory
( --------- CT borders)
euchromatin
( )
heterochromatin
( )
nuclear pore
nucleoli
chromosome
nuclear membrane



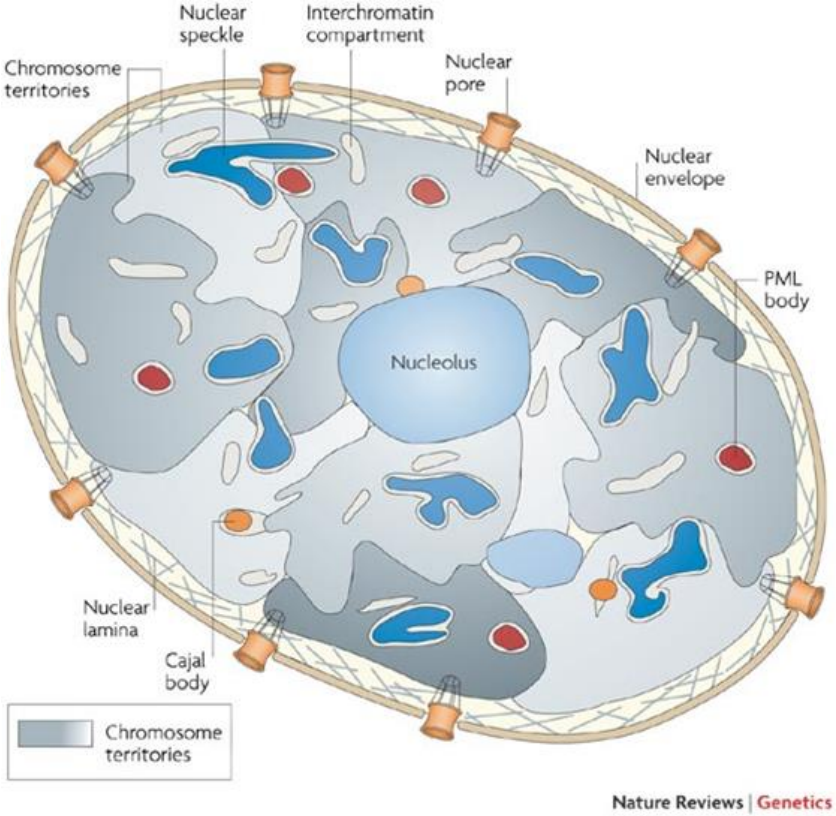

**Direct evidence for chromosome territories (CTs) by in situ hybridization experiments.** The territorial organization of chromosomes in interphase (chromosome territories, CTs) constitutes a basic feature of nuclear architecture.




**Compartmentalization in the mammalian nucleus.** The nucleus contains proteinaceous ***nuclear bodies***, ***chromatin domains*** including heterochromatin (dark grey) and euchromatin (light grey) and ***chromosome territories***. ***Nuclear bodies*** can either be ***non-specific aggregates***, ***sites of nuclear processes*** (*rRNA transcription in the nucleolus*; green) or ***sites of inaction*** (*storage of splicing components in splicing factor compartments*; red).




**Compartmentalization of Nuclear Processes.** Transcription, replication, and DNA repair are compartmentalized. (A) Transcription sites visualized by incorporation of bromo-UTP, (B) replication sites visualized by incorporation of bromo-dUTP, and (C) repair sites visualized by accumulation of repair factor 53BP1 at a double-strand break (DSB) are shown. In all cases, components are dynamically recruited from the nucleoplasm as single subunits or small preassembled subcomplexes.




**Organization of the mammalian cell nucleus.**

The nucleus is characterized by a ***compartmentalized distribution of functional components***. The ***nuclear envelope*** contains ***pores*** and rests on a meshwork of intermediate filaments, the ***nuclear lamina***. Nucleolar organizer regions cluster to form ***nucleoli***. Further ***topographical details*** that are shown in this schematic nuclear section are representative for the ***chromosome territory–interchromatin compartment (CT–IC) model***. ***Chromatin is organized in distinct CTs***.

Also depicted are ***nuclear speckles***, ***PML bodies*** and ***Cajal bodies*** located in wider IC lacunas (sections through smaller channels of the contiguous, three-dimensional IC network are not depicted). Nuclear topography remains a subject of debate, expecially with regard to the extent of ***chromatin loops*** expanding into the IC and intermingling between neighbouring CTs and chromosomal subdomains.

Compartment = predel

# Diversity of Nuclear Bodies (Jedrna telesca)

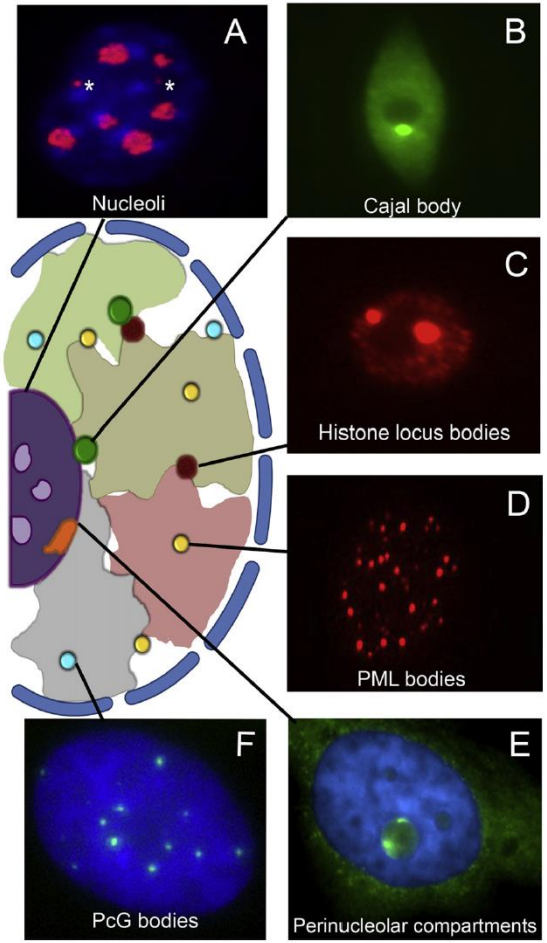
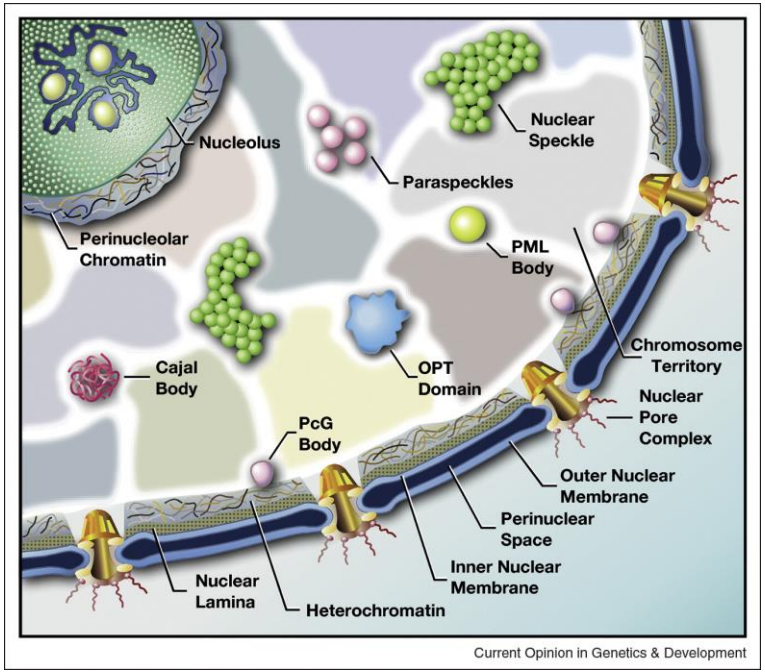
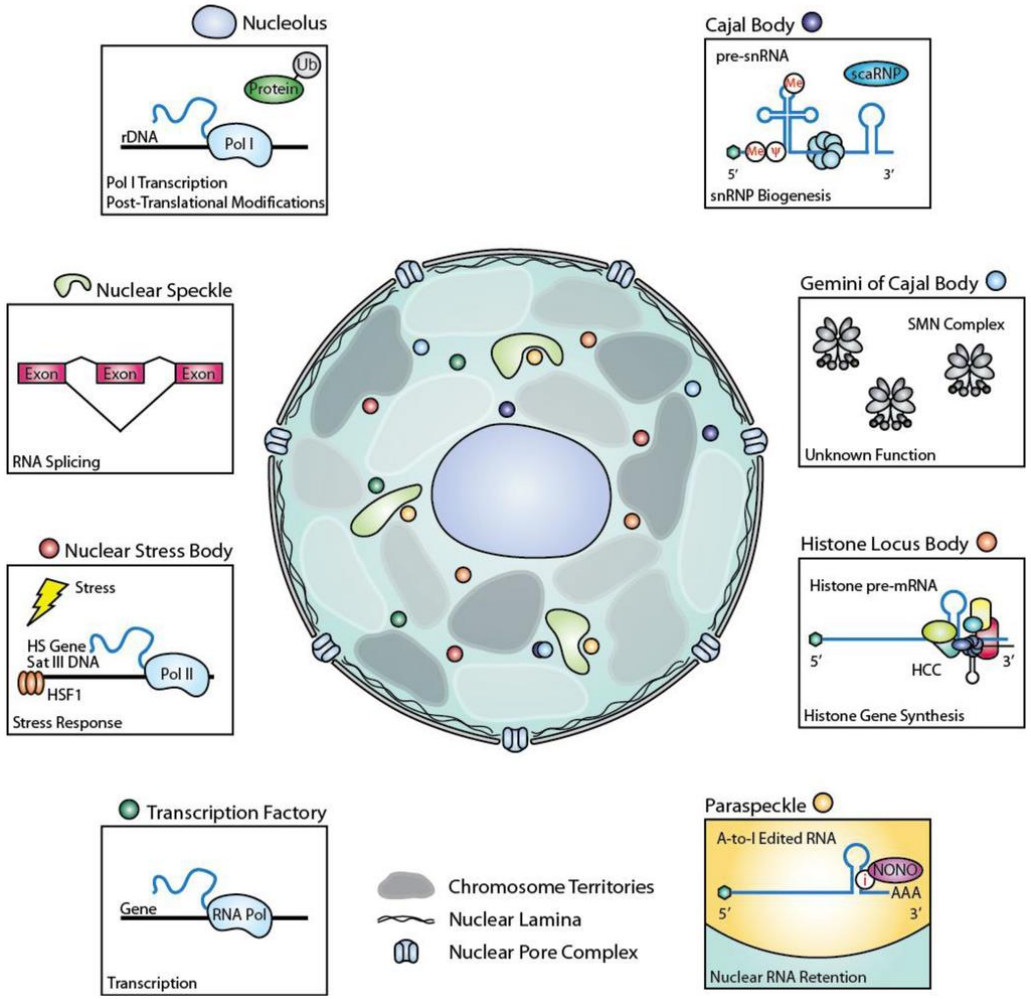


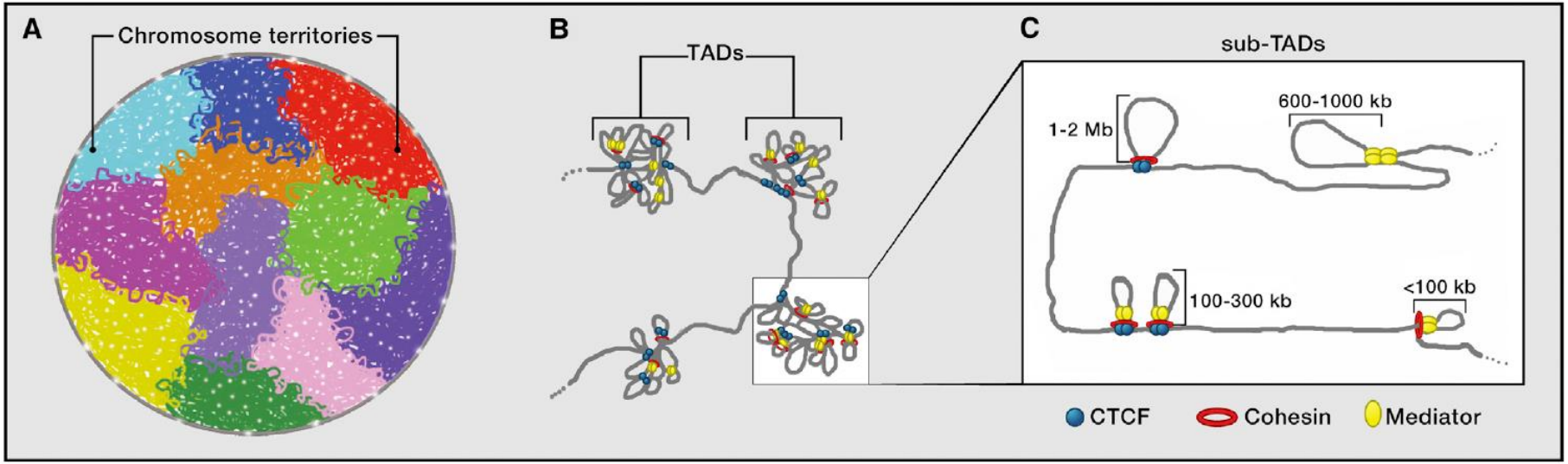

**Table 1. Basic characteristics of several nuclear bodies**

| Body name | Number per cell | Typical size (μm) | Defining components | (Putative) Functions |
|---|---|---|---|---|
| Cajal body | 0–10 | 0.1–2.0 | Coilin, SMN | Involved in snRNAs and snoRNAs modification, and assembly and trafficking of snRNPs and snoRNPs. Also plays a role in telomerase assembly and telomere length regulation. |
| Clastosome | 0–3 | 0.2–1.2 | 19S, 20S proteasome | Contains 20S and 19S proteasomes, ubiquitin conjugates, and protein substrates of the proteasome. Forms in response to stimuli that activate proteasome-dependent proteolysis. |
| Histone locus body | 2–4 | 0.2–1.2 | NPAT, FLASH | Involved in the transcription and processing of histone pre-mRNAs. |
| Nuclear speckle | 25–50 | 0.8–1.8 | SRSF2, SRSF1, Malat1 | Involved in the storage, assembly, and modification of pre-mRNA splicing factors. |
| Nuclear stress body | 2–10 | 0.3–3.0 | HSF1, HAP | Contains satellite III ncRNAs and is a part of the general response to stress. Precise function not yet determined. |
| Nucleolus | 1–4 | 0.5–8.0 | RNA Pol I machinery | Involved in the transcription and processing of rRNA and the assembly of ribosomal subunits. Plays roles in the modification and assembly of other nuclear RNAs and RNPs. Regulates cell cycle progression by sequestering and modifying many proteins. |
| Paraspeckle | 10–20 | 0.5 | PSP1, p54nrb, Men ε/β (Neat1) | Involved in nuclear retention of some A-to-I hyperedited mRNAs. |
| Perinucleolar compartment | 1–4 | 0.2–1.0 | PTB, CUGBP | Precise functions are unknown but its prevalence positively correlates with metastatic capacity. |
| PML-nuclear body | 10–30 | 0.3–1.0 | PML | Involved in response to many forms of stress, viral defense, and genome stability by the sequestration, modification, and degradation of many partner proteins. |
| Polycomb body | 12–16 | 0.3–1.0 | Bmi1, Pc2 | Involved in Polycomb proteins-mediated gene paring and silencing in *Drosophila*. Precise function in mammalian cells remains to be determined. |

# Diversity of Nuclear Bodies - functions



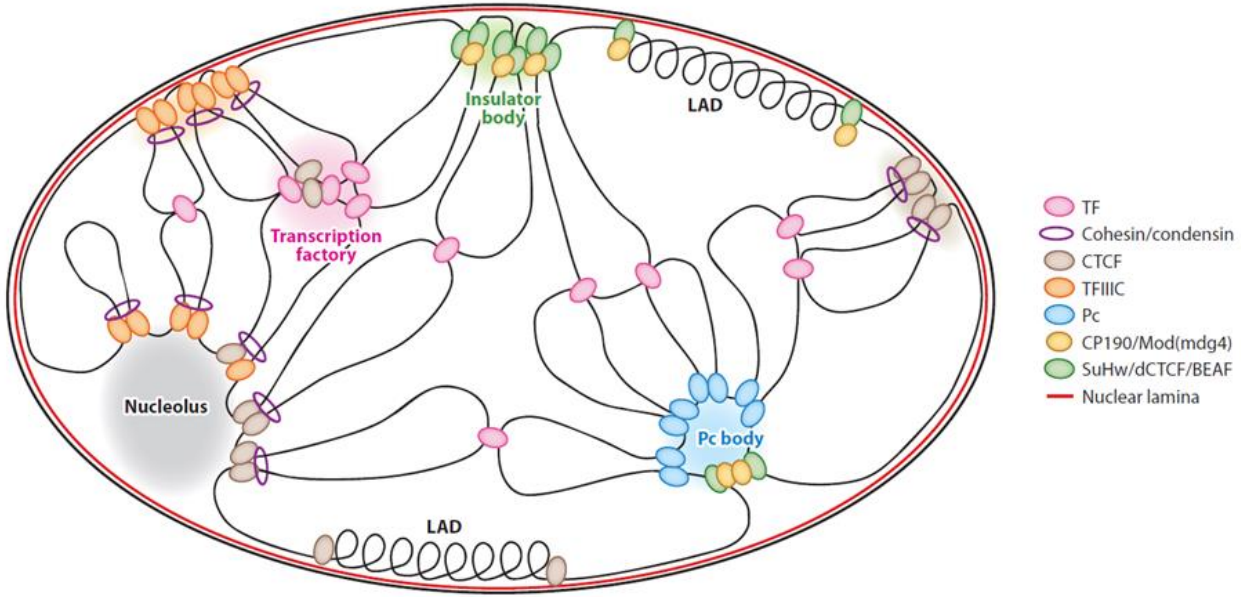



**Chromatin Organization in the Mammalian Nucleus**

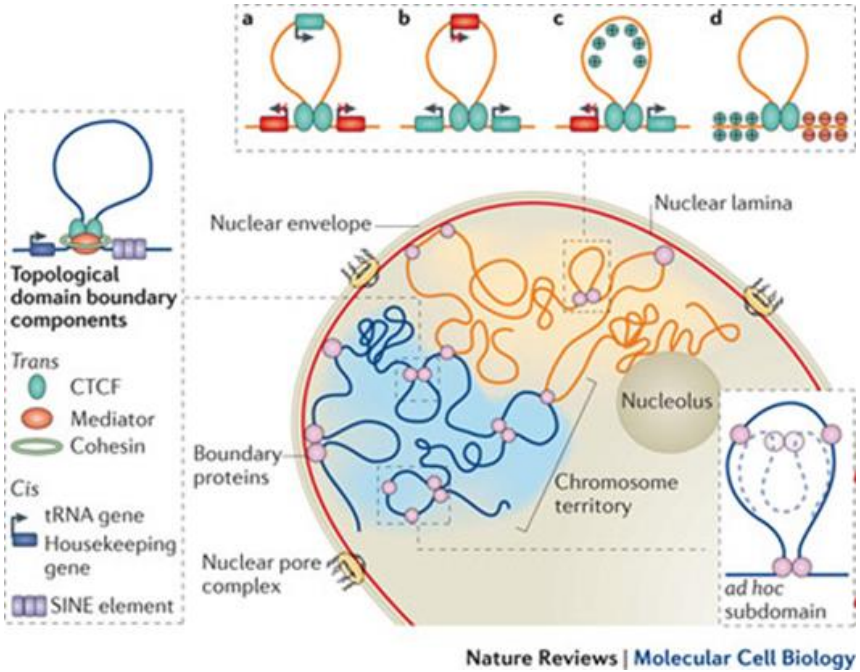
(A) Chromosomes are organized in chromosome territories. (Nonchromatin nuclear regions are not shown in this schematic drawing).

(B) Chromatin within chromosome territories is further organized into topologically associating domains (TADs).

(C) CTCF, Mediator, and cohesin cooperate to organize chromatin interactions at the megabase and submegabase level.

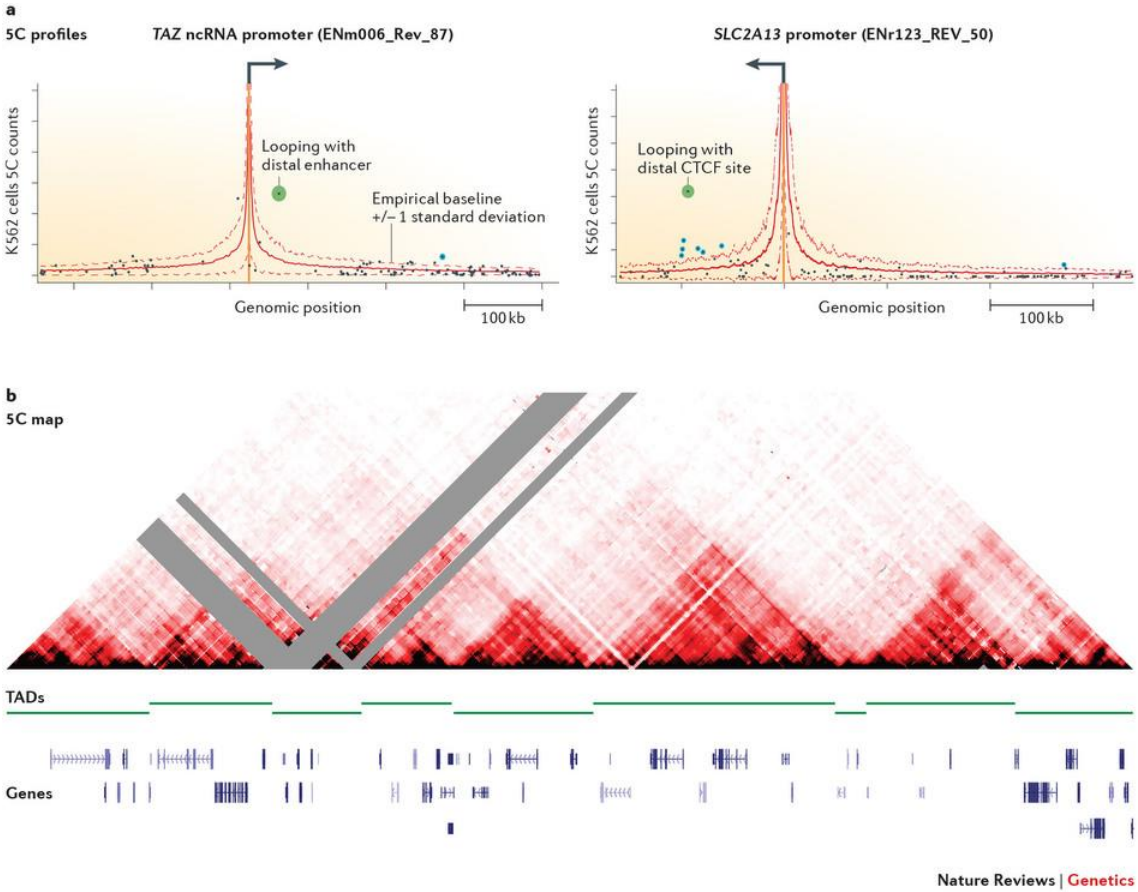



**Comprehensive model for the highly conserved role of insulators in nuclear organization.**
***Insulators*** in yeast (TFIIIC, orange), Drosophila [Su(Hw), dCTCF, BEAF, green; CP190, Mod(mdg4), yellow], and mammals (CTCF, brown; TFIIIC, orange) ***mediate long-range inter- and intrachromosomal interactions important for gene regulation and cluster into subnuclear foci called*** **insulator bodies**. Insulators underlie interactions necessary for **Polycomb (Pc) body** repression (blue) and localize with general transcription factors (TF, pink) to **transcription factories**. ***Insulators localize to subnuclear structures, including the nuclear lamina (red), where they are enriched at the borders of lamina-associated domains (LADs) and the nucleolus (gray)***. CTCF insulator activity in mammals requires **cohesin** (purple), and TFIIIC insulator sites are associated with both **cohesin and condensin** (purple). Insulator activity in Drosophila relies on recruitment of fly-specific proteins CP190 and Mod(mdg4).




## Model for the organization of topological domains in the genome.

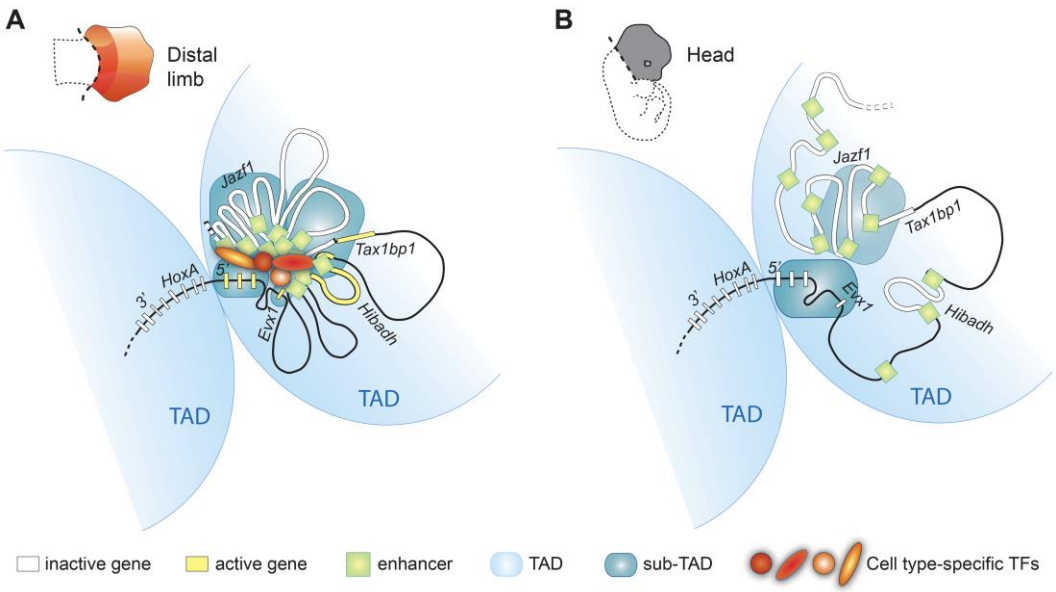
A conceptual schematic showing ***two chromosomes*** (shown as orange and blue lines) ***compartmentalized*** in their respective ***chromosome territories*** (shown as orange and blue shaded areas). **Chromatin boundary proteins partition the genome into topologically distinct domains by organizing intra- and inter-chromosomal interactions, as well as interactions between chromatin and the nuclear lamina**. The panel on the left shows a schematic summary of the ***cis and trans components enriched in topological domain boundaries*** as identified in recent studies. It is not necessary for a boundary to contain all components. ***CCCTC-binding factor (CTCF)*** is found in most boundaries of topological domains. The panel at the top depicts ***four types of CTCF-mediated topological domains***, grouped on the basis of ***histone modification profiles*** in the topological domains and its neighbouring regions. The observations suggest a ***common role of CTCF-mediated topological domains in ensuring regulatory independence, including independent transcription of neighbouring genes (a–c) and separation of open and repressive chromatin (d). Active and silent genes*** are depicted in ***green*** and ***red,*** respectively. ***Plus*** and ***minus signs*** represent ***open and repressive chromatin***, respectively. The panel on the right shows a model of an ***ad hoc subdomain*** that is organized by internal boundaries.




**Chromatin looping interactions and topologically associating domains (TADs).**

a | Examples of ***long-range interaction profiles in the human genome***, as determined by 5C. The ***orange vertical bar*** indicates the position of the **gene promoters**, the solid red line indicates the empirically estimated level of baseline interactions, and the dashed red lines indicate baseline plus or minus 1 standard deviation. The presence of a looping interaction is inferred when a pair of loci interact statistically more frequently than would be expected on the basis of the baseline frequency. The ***green data points represent significant looping interactions***.

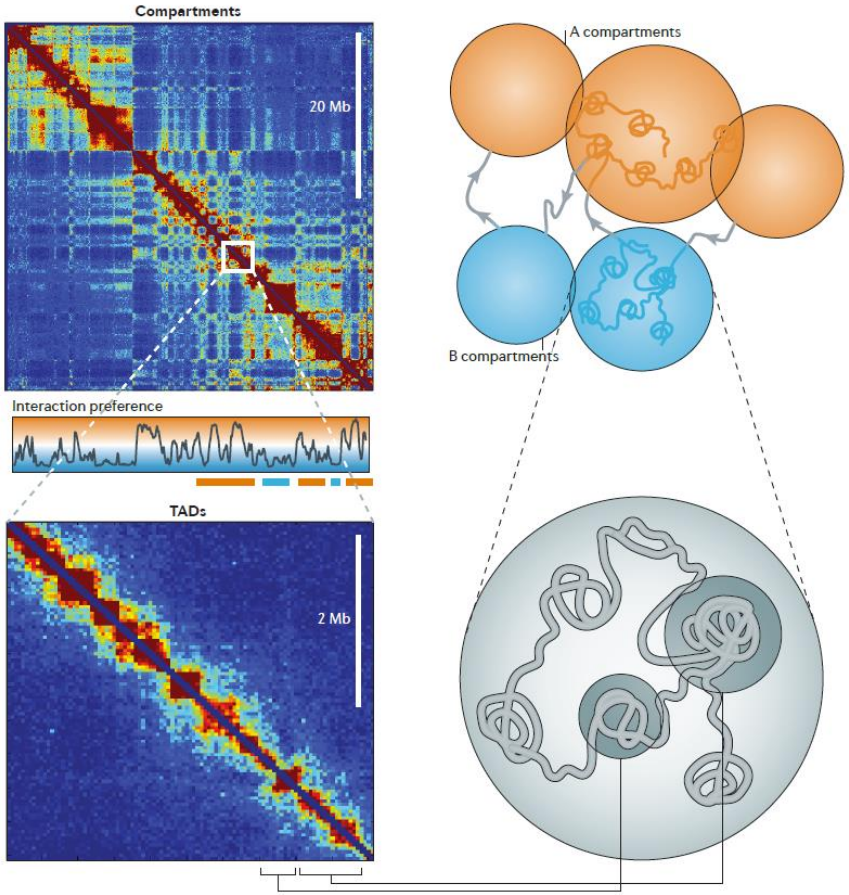
b | ***A dense 5C interaction map of a 4.5 Mb region on the mouse X chromosome containing the X-chromosome inactivation centre***. In red is the interaction frequency between pairs of loci, grey represents missing data due to low mappability. The interaction map is cut in half at the diagonal to facilitate alignment with genomic features. Visual inspection reveals the presence of ***triangles, which correspond to regions (topologically associating domains (TADs)) in which loci frequently interact with each other***. Loci located in different TADs do not interact frequently. TAD boundaries have been determined by computationally determining the asymmetry between up- and downstream interactions around them. ncRNA, non-coding RNA.




**Model illustrating how genome topology underlies the tissue-specific regulation of HoxA genes.**

The HoxA cluster is partitioned between two TADs (light blue), physically segregating 3′HoxA from 5′HoxA genes in a mostly cell-type independent manner. In contrast, the sub-TAD interaction pattern is drastically different in the limb (A) compared to the head (B). Limb enhancer sub-TADs (dark blue) interact with each other and with gene-sub-TADs in distal limb but not head tissue. Enhancer and gene interactions occur between sub-TADs from the same TAD (5′HoxA containing TAD) but not with 3′HoxA genes that are located in the adjacent TAD. The limb-specific sub-TAD interactions create a platform architecture controlling HoxA expression by the remote distal limb enhancers upon enhancer activation by transcription factors. The schemes of the chromatin conformation were designed assuming cellular homogeneity within each tissue.

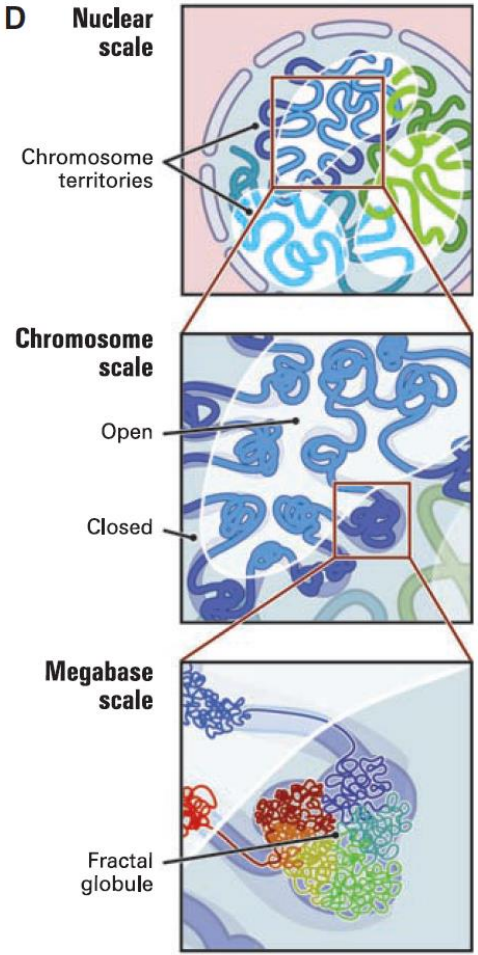
**TADs**: topologically associated domains.




## Genome compartments.

Inter- and intrachromosomal interaction maps for mammalian genomes have revealed a pattern of interactions that can be approximated by two compartments — A and B — that alternate along chromosomes and have a ***characteristic size of ~5 Mb each*** (as shown by the compartment graph below top heat map in the figure). A compartments (shown in orange) preferentially interact with other A compartments throughout the genome. Similarly, B compartments (shown in blue) associate with other B compartments. Compartment signal can be quantified by eigenvector expansion of the interaction map. The A or B compartment signal is not simply biphasic (representing just two states) but is continuous and correlates with indicators of transcriptional activity, such as DNA accessibility, gene density, replication timing, GC content and several histone marks. These indicators suggest that ***A compartments are largely euchromatic, transcriptionally active regions***.

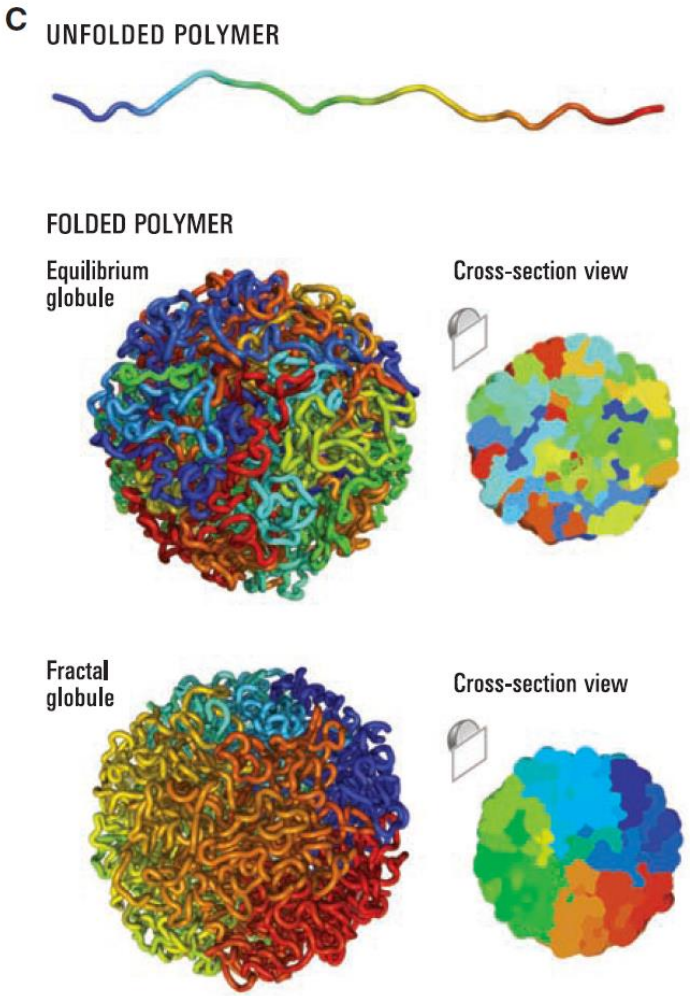
***Topologically associating domains (TADs)*** are distinct from the larger A and B compartments. First, analysis of embryonic stem cells, brain tissue and fibroblasts suggests that ***most, but not all, TADs are tissue-invariant, whereas A and B compartments are tissue-specific domains of active and inactive chromatin that are correlated with cell-type-specific gene expression patterns.*** Second, ***A and B compartments are large (often several megabases) and form an alternating pattern of active and inactive domains along chromosomes. By contrast, TADs are smaller (median size around 400–500 kb)***.




**Genome architecture at three scales.**
(Top) Two ***compartments***, corresponding to ***open and closed chromatin***, ***spatially partition the genome***. ***Chromosome***s (blue, cyan, green) occupy distinct ***territories***.

(Middle) Individual chromosomes weave back and forth between the ***open and closed chromatin compartments***.

(Bottom) At the ***scale of single megabases***, the chromosome consists of a series of ***fractal globules***.




# The fractal globule as a model of chromatin architecture in the cell

(Top) An **unfolded polymer chain**, 4000 monomers (4.8 Mb) long. Coloration corresponds to distance from one endpoint, ranging from blue to cyan, green, yellow, orange, and red.
(Middle) An **equilibrium globule**. The structure is highly entangled; loci that are nearby along the contour (similar color) need not be nearby in 3D.
(Bottom) A **fractal globule**. Nearby loci along the contour tend to be nearby in 3D, leading to ***monochromatic blocks both on the surface and in cross section***. The structure lacks knots.

The **fractal globule** is a compact polymer state that emerges during polymer condensation as a result of topological constraints which prevent one region of the chain from passing across another one. Recent characterization of human chromatin using a novel chromosome conformational capture technique brought the fractal globule into the spotlight as a structural model of human chromosome on the scale of up to 10 Mb (Lieberman-Aiden et al. 2009).

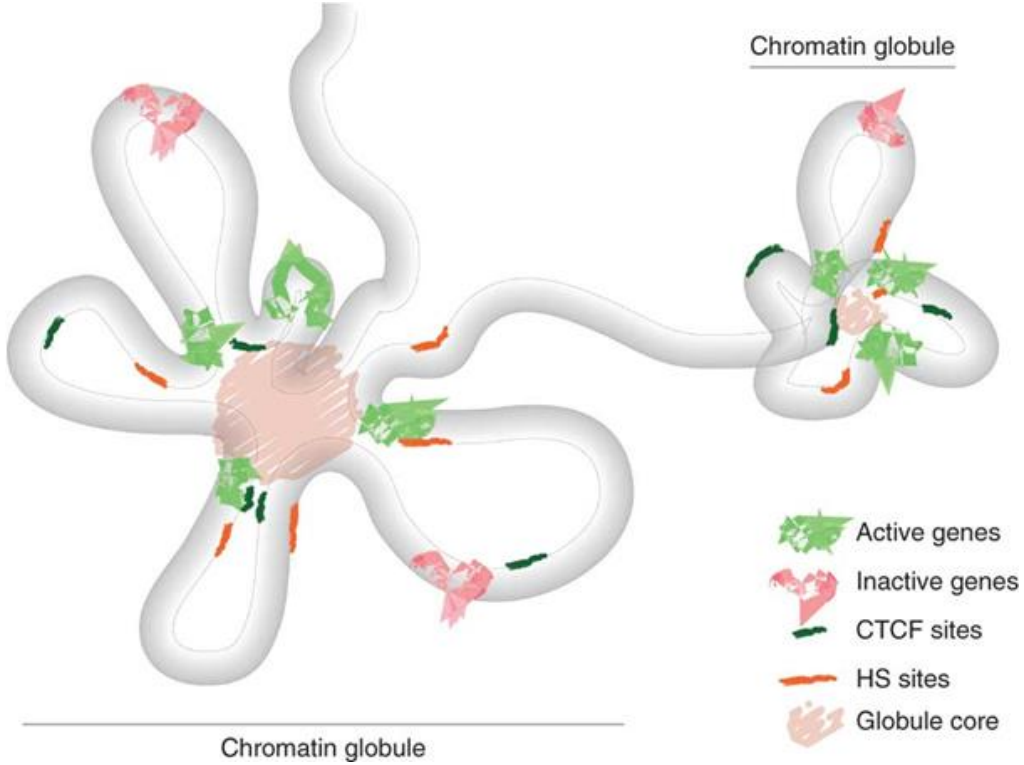
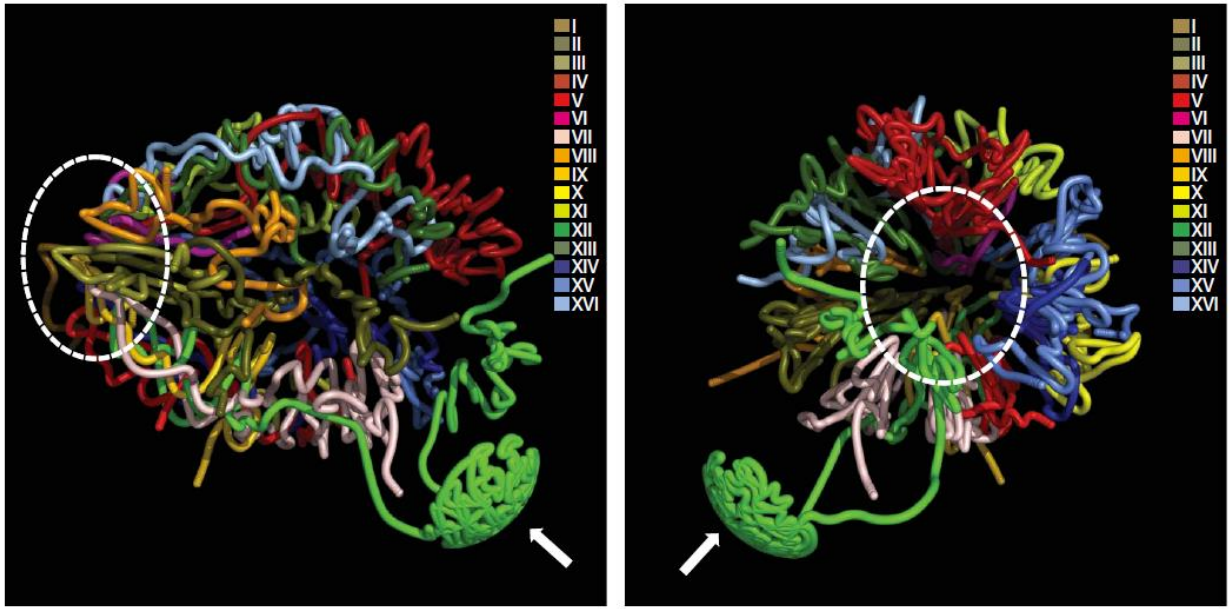
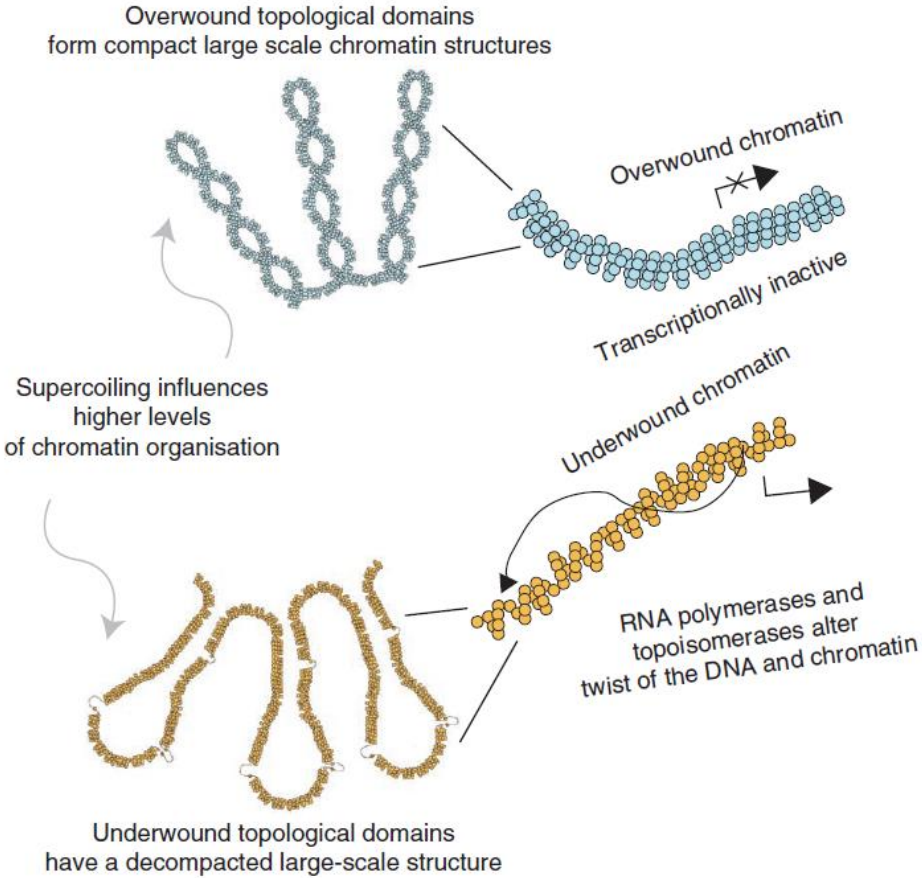



**Diagram of the proposed chromatin-globule model for higher-order chromatin folding of actively transcribed genomic regions.**

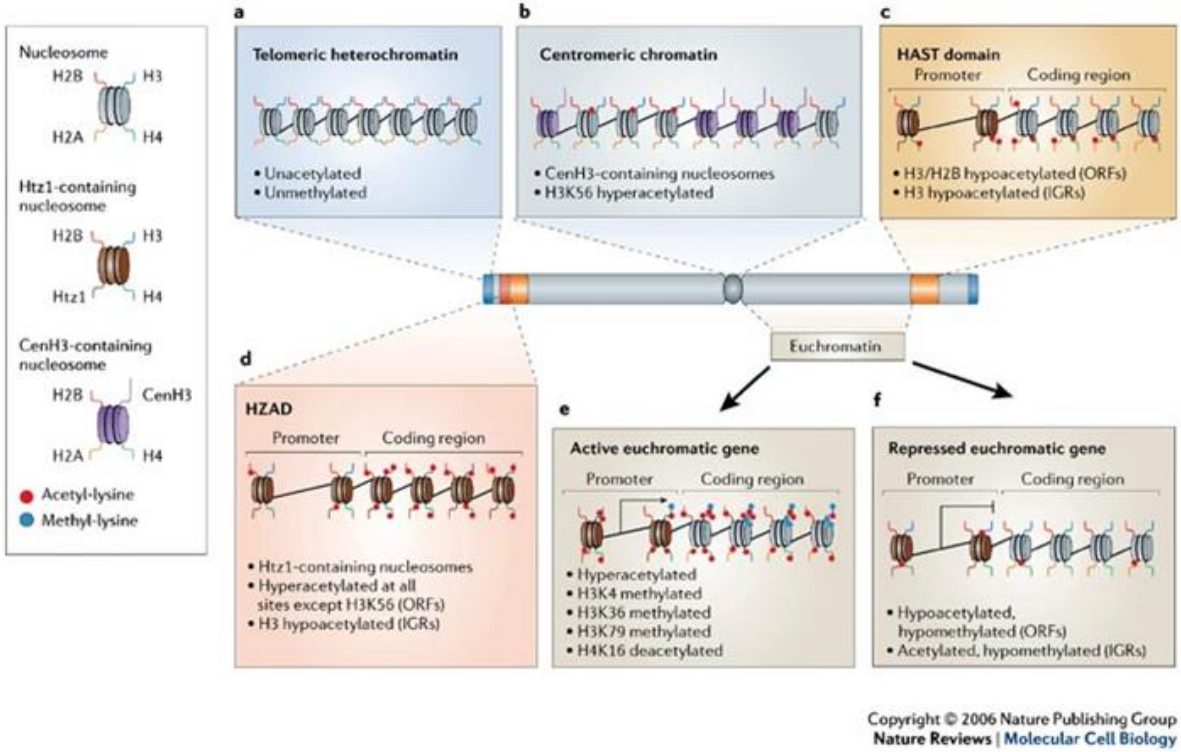



**Three-dimensional model of the yeast genome.**

Two views representing two different angles are provided. Chromosomes are coloured as indicated in the upper right. All chromosomes cluster via centromeres at one pole of the nucleus (the area within the dashed oval), while chromosome XII extends outward towards the nucleolus, which is occupied by rDNA repeats (indicated by the white arrow). After exiting the nucleolus, the remainder of chromosome XII interacts with the long arm of chromosome IV.



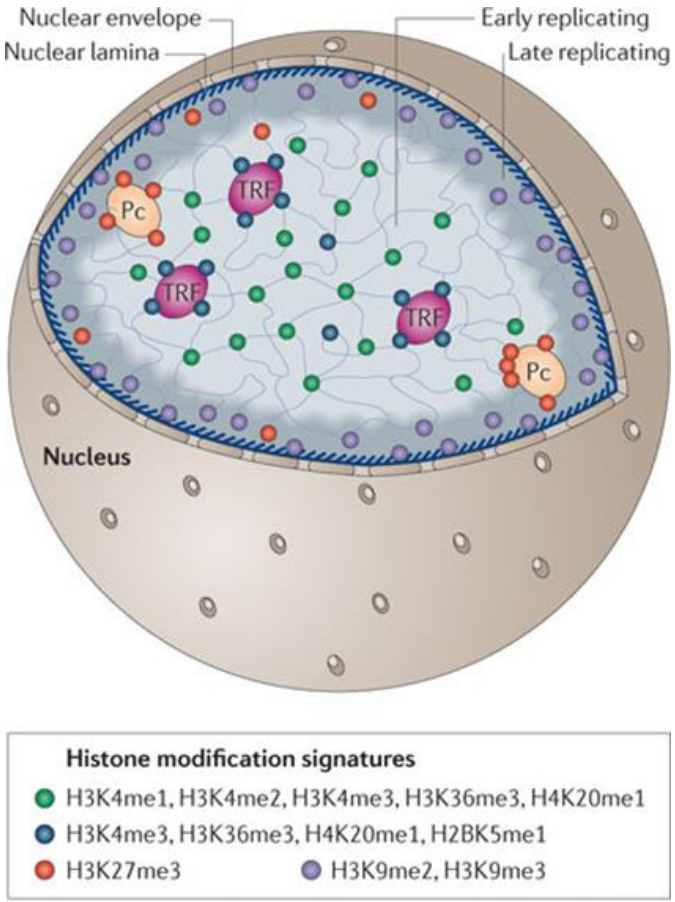

**Relationship among transcription, DNA supercoiling and large-scale chromatin structures.** Transcriptionally inactive chromatin is topologically ***overwound*** and has a cytologically ***compact large-scale chromatin structure***. In contrast, a transcriptionally active region or transcriptional activation ***alters DNA supercoiling, remodeling supercoiling domains***; this is accompanied by ***decompaction of large-scale chromatin structures***. Therefore, ***large structural domains***, for example as described by Hi-C31, are ***subdivided into smaller transcription-dependent supercoiling domains, providing an additional level of functional organization within the human genome***.



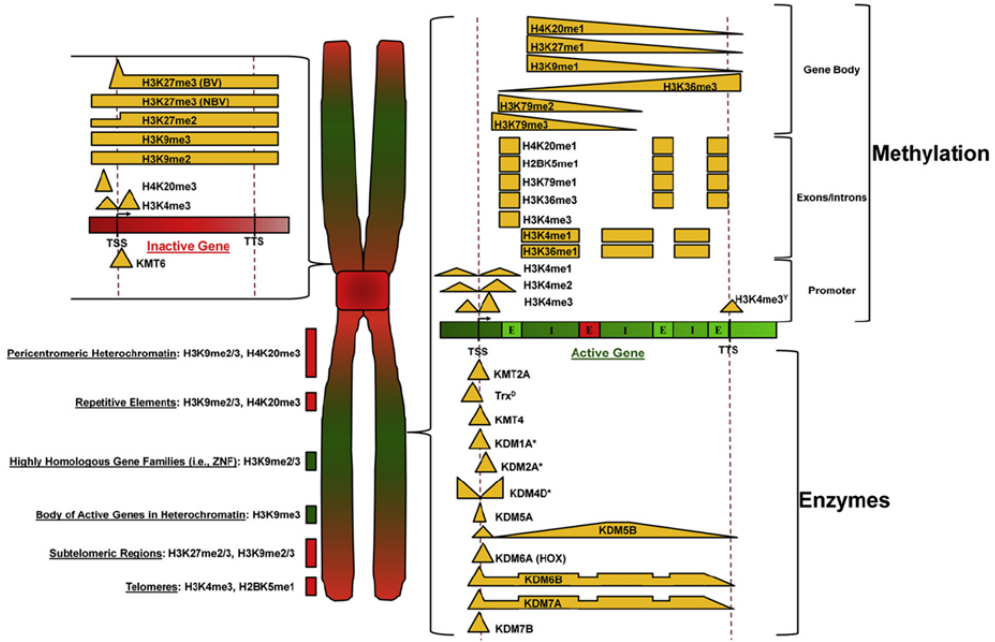



**Chromosomal domains with different combinations of histone modifications.**

Genome-wide patterns of histone modifications in yeast. A Saccharomyces cerevisiae chromosome is shown at the centre of the figure. ***HZAD domains***: Adjacent sub-telomeric genes, the expression of which is downregulated in the absence of the H2A variant Htz1. Hda1-affected subtelomeric (**HAST**) **domain:** Groups of contiguous genes that are deacetylated by the HDAC Hda1, and are located in the subtelomeric euchromatin.



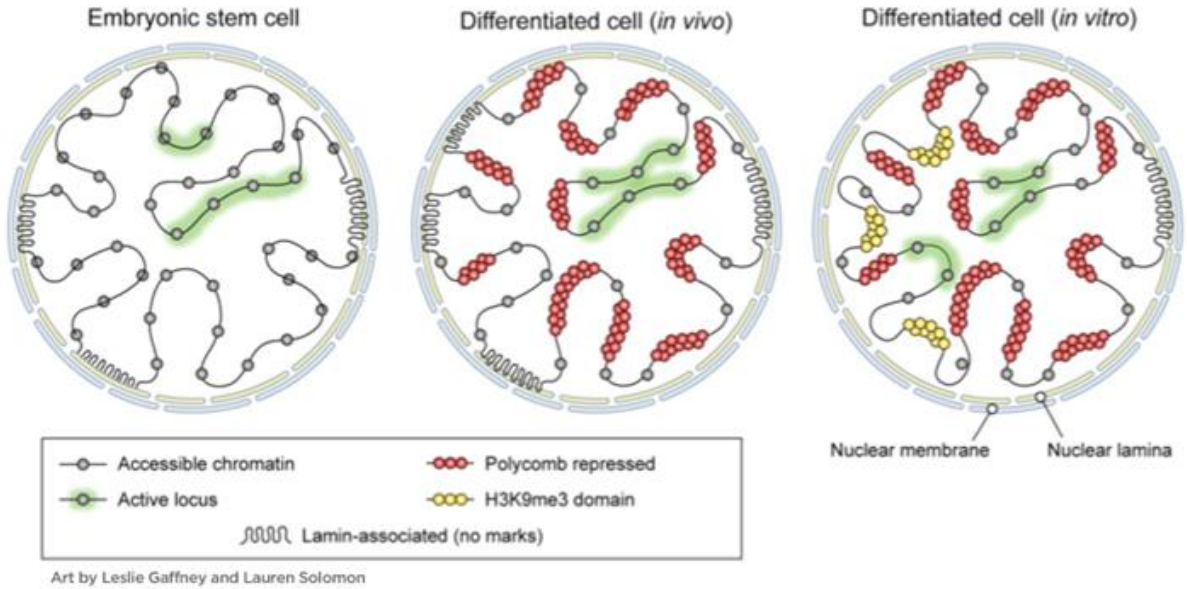

**Histone modification signatures associated with features in the mammalian cell nucleus.** Signature histone modifications correlate with various nuclear features, although the relationships might be indirect. Chromatin with ***modifications generally associated with active transcription (green dots) often replicates early, whereas chromatin with generally repressive modifications (purple dots) replicates late***. **Regions enriched for some sets of active modifications (blue dots) may converge into transcription factories (TRFs).** Blocks of histone H3 lysine 27 trimethylation (H3K27me3; red dots) may form ***Polycomb bodies (Pc)*** and diffuse domains marked by H3K9me2 or H3K9me3 (purple dots) may contact the nuclear lamina.




**Distribution of Histone Methylation, KMTs, and KDMs from Genome-wide Profiling Studies.** The ***distribution of methyl modifications is shown relative to chromosomal location, as well as in relationship to active and inactive genes.*** **Green** represents ***euchromatic regions***, while **red** coloring represents ***heterochromatic regions***. Distributions of modifications are represented by bars, and gradients were derived from metagene analysis published as part of the genome-wide data sets from the work of numerous labs. We have included plots from metagene analyses that included both a transcription start site (TSS) and a transcription termination site (TTS). Enzymes marked with an * indicate the distribution depicted from metagene analyses that did not include both a TSS and TTS or from distributions published as heatmaps centered on the TSS. In the ***active gene model, the green E represents expressed exons, while the red E represents a nonexpressed exon. I denotes introns.***

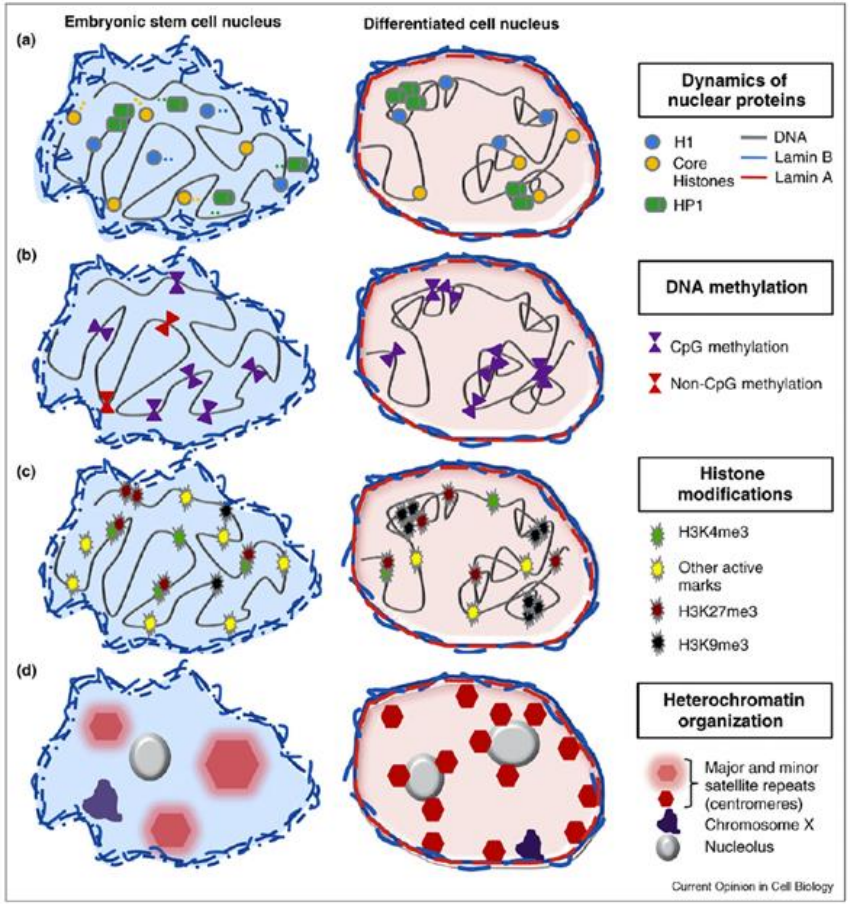
BV denotes bivalent genes, NBV denotes nonbivalent genes, HOX denotes patterns at Hox genes, and ZNF denotes zinc finger genes.

***Methylation of lysine residues within histones is tightly regulated by methyltransferases (KMTs) and demethylases (KDMs) to maintain cell fate and genomic stability.***




**Genome-wide chromatin state transitions associated with developmental and environmental cues.**

Although the amount of genome sequence engaged in gene regulatory activity is relatively consistent, the chromatin configurations of the inactive regions varies considerably between cell types. ***In ES cells (left), inactive regions are diffusely enriched for markers of chromatin exchange and accessibility. In differentiated cells acquired in vivo (middle), inactive loci instead tend to adopt a Polycomb-repressed chromatin state. In differentiated cells cultured in vitro (right), large domains enriched for the heterochromatin marker H3K9me3 arise in regions associated with the nuclear lamina***.



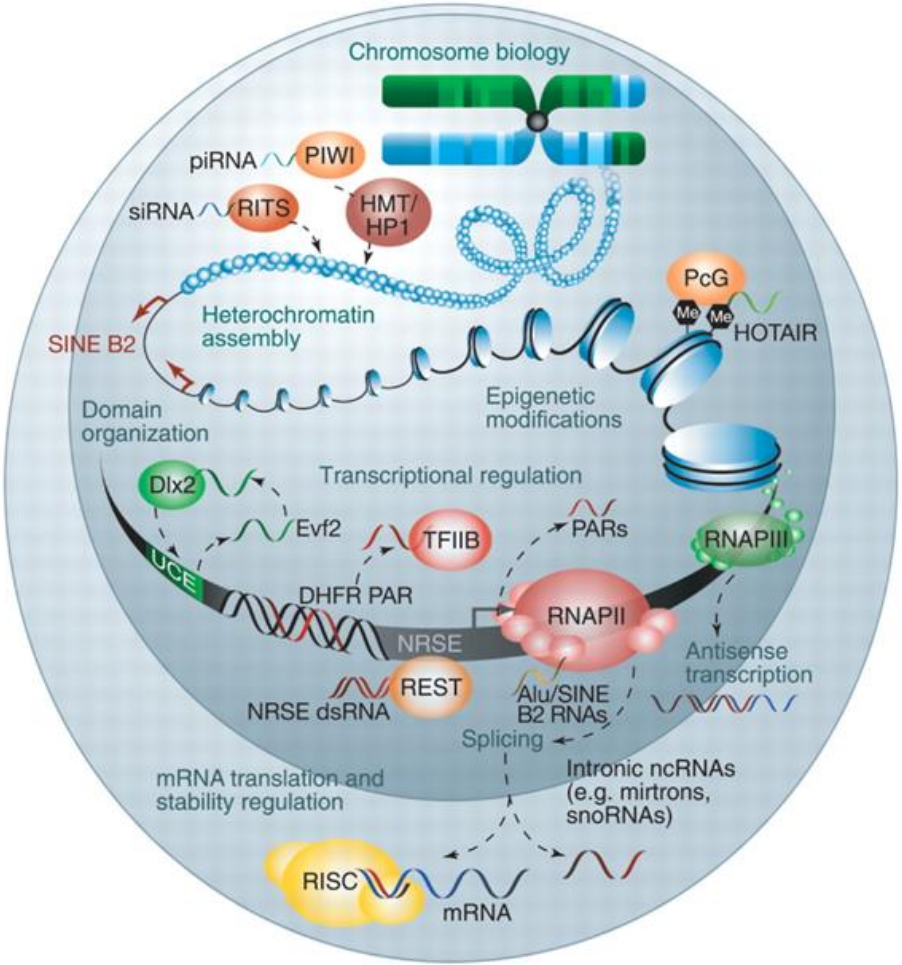

**A schematic view of chromatin and genome characteristics in ESCs and in early differentiation.**

(a) ***Chromatin protein dynamics***. Chromatin proteins such as the linker ***histone H1 and core histones are more dynamically associated with chromatin in ESCs*** than in differentiated cells. HP1a and lamin B nuclear proteins also bind more loosely in pluripotent cells. Lamin A expression and localization in the nuclear lamina occur during early differentiation.

(b) ***DNA methylation***. In mammalian somatic cells, DNA methylation is present on cytosines in a CpG context. In pluripotent ESCs, 25% of the cytosine methylation sites in the genome are found in a non-CpG context, suggesting that ***ESCs utilize a unique DNA methylation program***.

(c) ***Histone modifications***. The global levels of several histone modifications differ between ESCs and differentiated cells. This includes several active marks which are more abundant in ESCs, and repressive marks which are enriched in differentiated cells, and which accumulate in well-defined foci (H3K9me3). ***Bivalent marks (H3K4me3 together with H3K27me3) are found on promoters of developmentally regulated genes in ESCs***, some of which resolve into a single modification in differentiated cells.

(d) ***Centromeric heterochromatin***. ***Major and minor satellite DNA repeats*** which are normally found at the heterochromatic centric and pericentric regions, are ***dispersed in ESCs***, but form dense foci upon differentiation. Accordingly, centromeres are redistributed next to nucleoli and the nuclear periphery in differentiated cells. The inactive X chromosome in female somatic cells which is represented in the scheme is also repositioned next to the nuclear envelope. Telomeric chromatin is apparently not displaced in the nucleus, but in ESCs it has a more open structure.



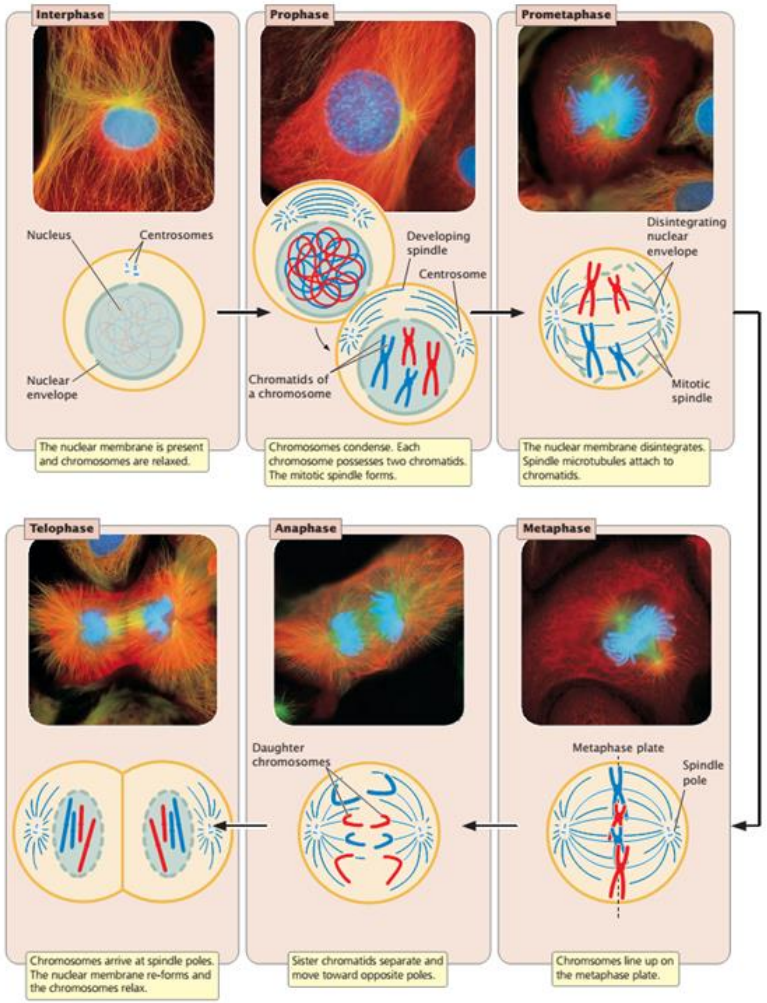

**The eukaryotic genome as an RNA machine.**

Recent examples of the various levels of regulation of eukaryotic gene expression and cell biology by ncRNAs. dsRNA, double-stranded RNA; HMT, histone methyltransferases; HP1, heterochromatin protein 1; PARs, promoter-associated RNAs; PcG, Polycomb group proteins; RISC, RNA-induced silencing complex; RITS, RNA-induced initiation of transcriptional gene silencing; siRNA, small interfering RNA; TFIIB, transcription factor IIB; and UCE, ultraconserved element.

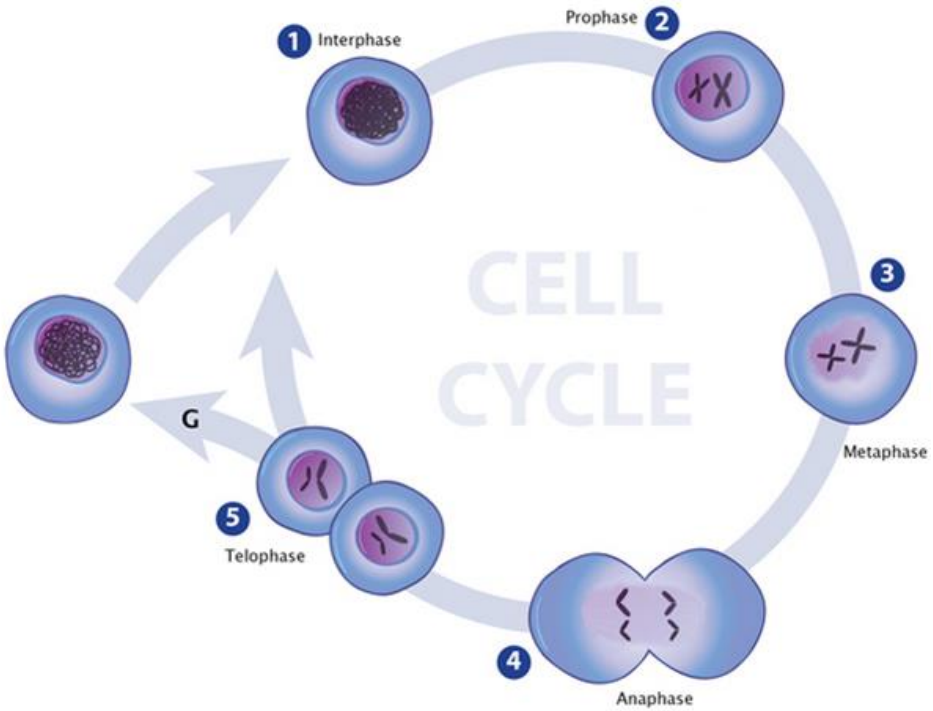
Higher-level nuclear organization and chromosome dynamics are also regulated by ncRNAs in a variety of systems.

**Regulation dominates the information content of complex systems.**

# Eukarionti - kromosomi

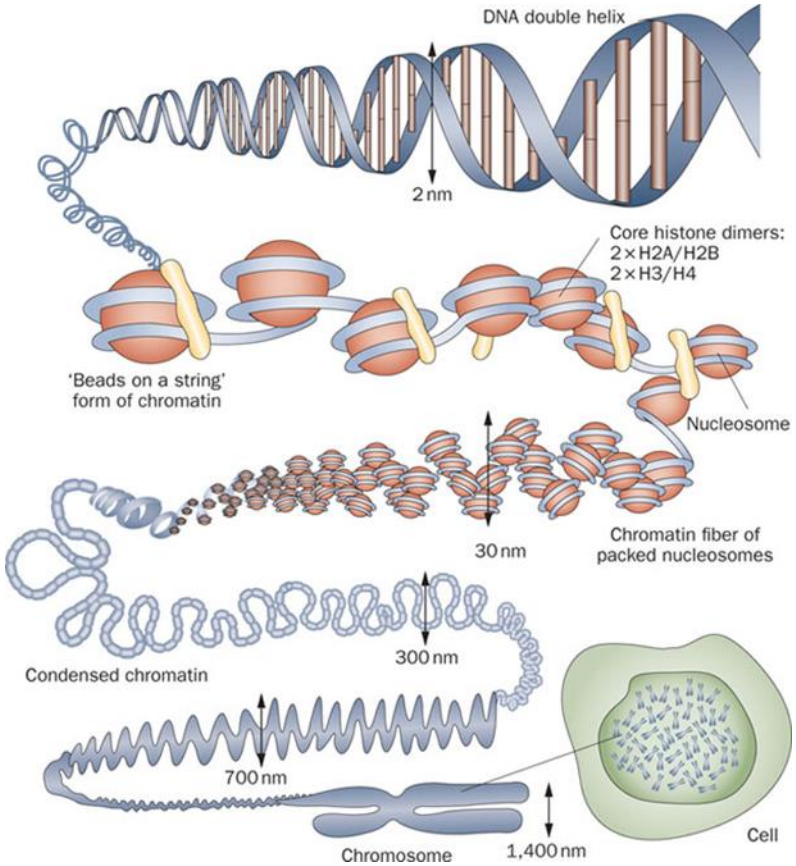



# The cell cycle is divided into stages

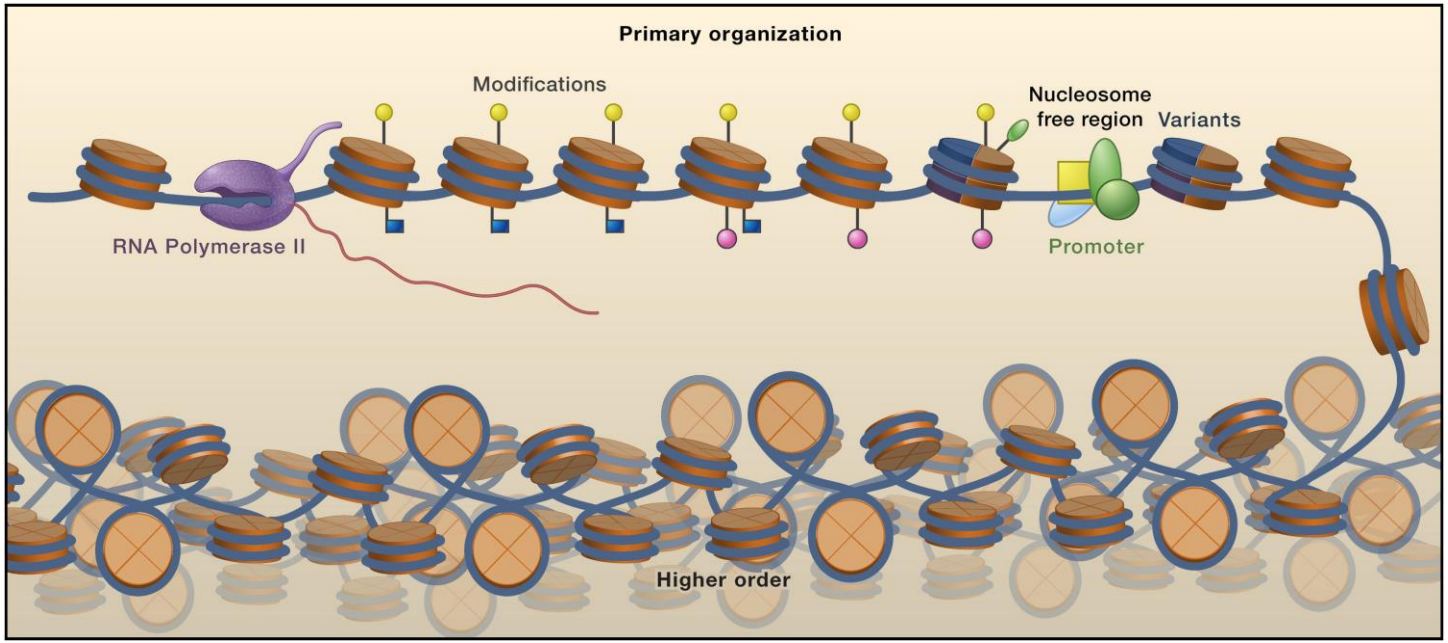



**Chromatin condensation changes during the cell cycle.**

During interphase (1), chromatin is in its least condensed state and appears loosely distributed throughout the nucleus. Chromatin condensation begins during prophase (2) and chromosomes become visible. Chromosomes remain condensed throughout the various stages of mitosis (2-5).

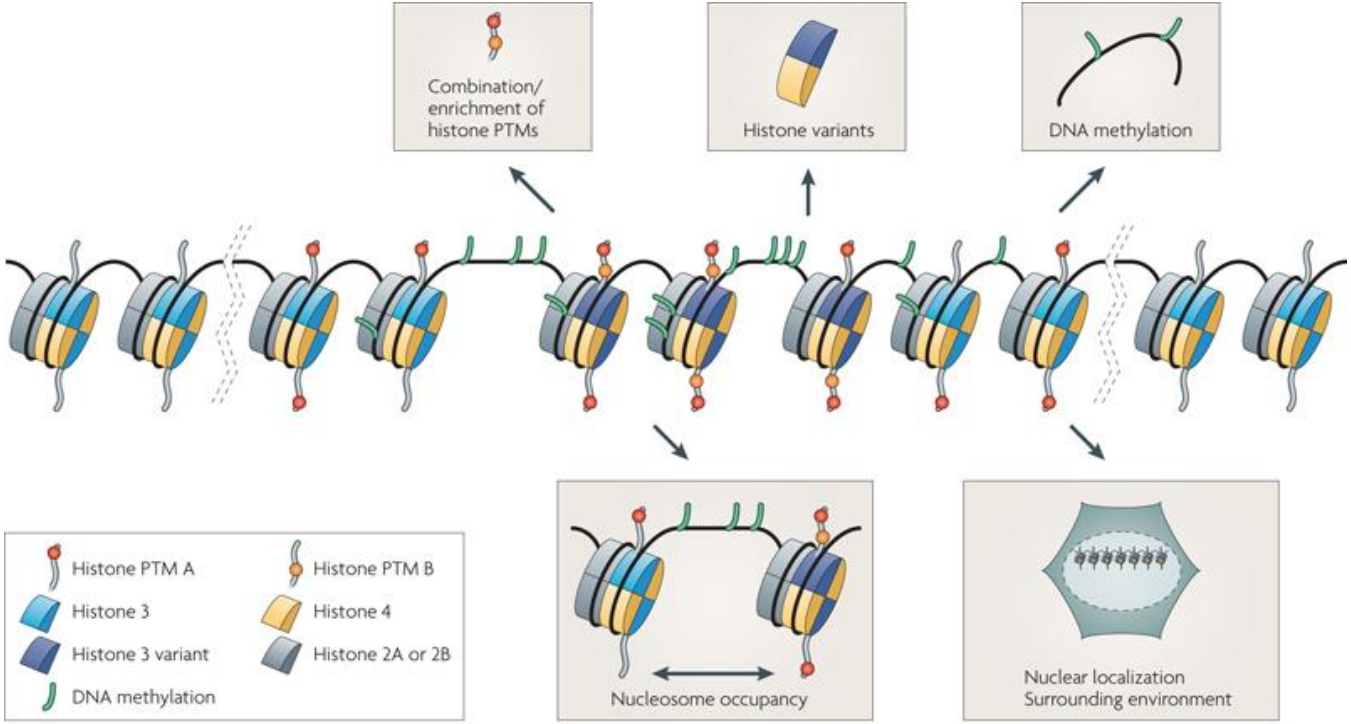



**Organizational network of chromatin in the cell.** Chromatin is DNA that is complexed to histone and nonhistone nuclear proteins and condenses to form a ***chromosome (approximately 1,400 nm in width)***. The ***condensed chromatin (approximately 700 nm in diameter)*** is composed of much finer ***chromatin (300 nm in diameter)*** and also ***nucleosomes (30 nm in diameter)*** that are used to package the genome into the cell nucleus. The ***core particle of the nucleosomes is composed of 147 bp of genomic DNA (2 nm in diameter)*** wrapped around a ***histone octamer*** that consists of ***two copies of the major types of histones (H2A, H2B, H3 and H4), which have varying functions.*** Recent work has demonstrated that hyperglycemia can induce a complex series of molecular events associated with gene-activating epigenetic changes.

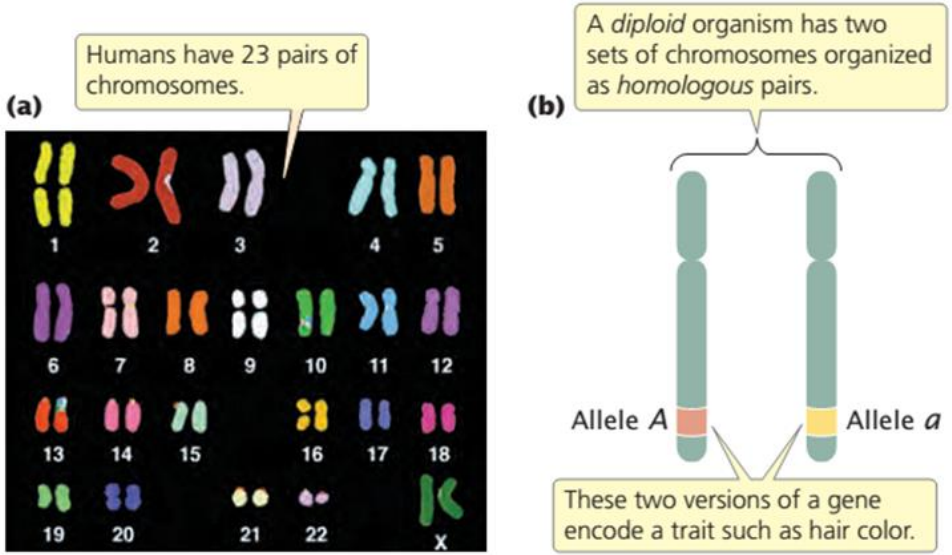



## Chromatin Architecture

The primary structure of chromatin can be thought of as “beads-on-a-string” with uniformly spaced arrays of nucleosomes at a fixed distance downstream of transcriptional start sites. With the exception of specific regulatory situations, intact nucleosomes generally avoid the core promoter region, where the transcription machinery assembles. These nucleosome-free regions provide an opportunity to regulate gene expression at steps beyond simple promoter access, for example through elongation control of RNA polymerase II. The protein core of nucleosomes is composed of histones, which often contain posttranslational modifications on specific amino acids and can be replaced by transcription-linked histone variants (dark blue and purple). As depicted, chromatin also folds into more compact structures aided by certain histone modifications.




**Characteristics of a chromatin domain.** Schematic depicting modifications that define different chromatin domains. The range of factors that can contribute to the characteristics of a domain are shown in the shaded boxes. The dashed lines represent the separation between two adjacent domains. PTM, post-translational modification.




**Diploid eukaryotic cells have two sets of chromosomes**

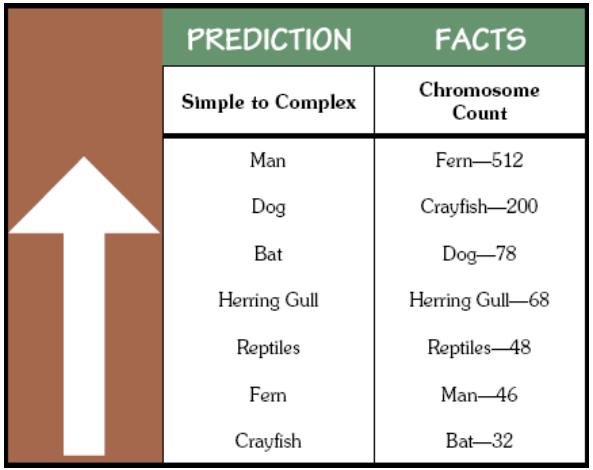
(a) A set of chromosomes from a female human cell. Each pair of chromosomes is hybridized to a uniquely colored probe, giving it a distinct color.

(b) The chromosomes are present in homologous pairs, which consist of chromosomes that are alike in size and structure and carry information for the same characteristics.

TABLE 2.1

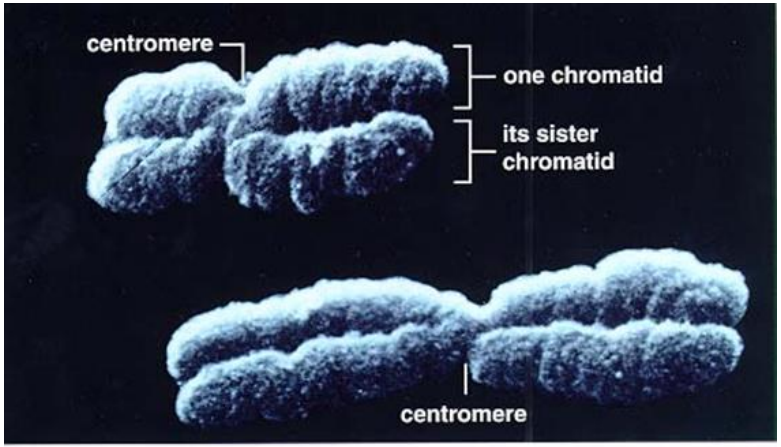
**The Haploid Number of Chromosomes for a Variety of Organisms**

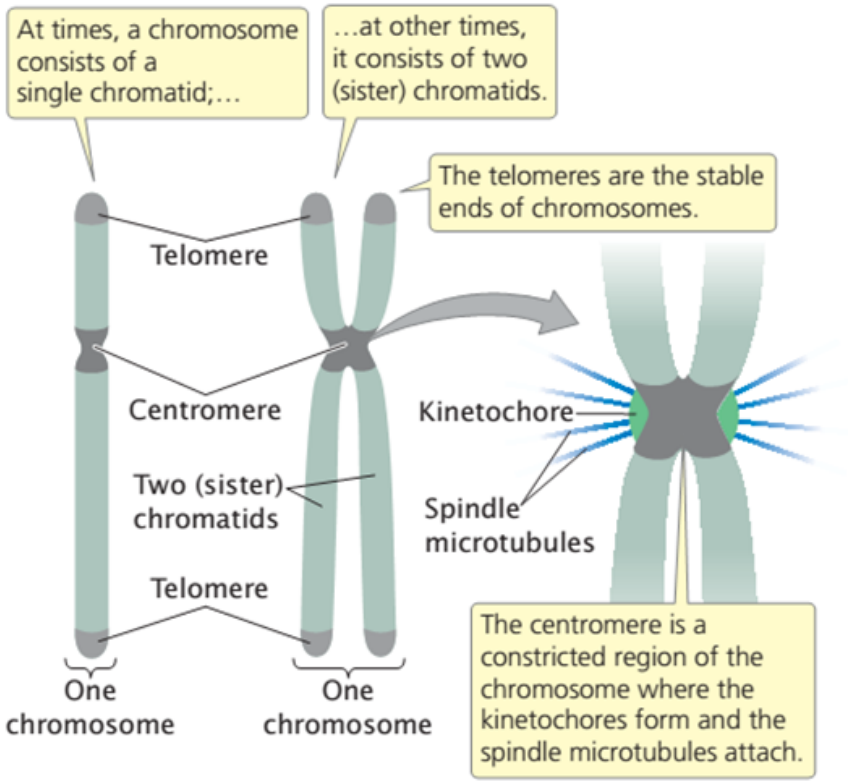
| Common Name | Scientific Name | Haploid Number |
|---|---|---|
| Black bread mold | *Aspergillus nidulans* | 8 |
| Broad bean | *Vicia faba* | 6 |
| Cat | *Felis domesticus* | 19 |
| Cattle | *Bos taurus* | 30 |
| Chicken | *Gallus domesticus* | 39 |
| Chimpanzee | *Pan troglodytes* | 24 |
| Corn | *Zea mays* | 10 |
| Cotton | *Gossypium hirsutum* | 26 |
| Dog | *Canis familiaris* | 39 |
| Evening primrose | *Oenothera biennis* | 7 |
| Frog | *Rana pipiens* | 13 |
| Fruit fly | *Drosophila melanogaster* | 4 |
| Garden onion | *Allium cepa* | 8 |
| Garden pea | *Pisum sativum* | 7 |
| Grasshopper | *Melanoplus differentialis* | 12 |
| Green alga | *Chlamydomonas reinhardtii* | 18 |
| Horse | *Equus caballus* | 32 |
| House fly | *Musca domestica* | 6 |
| House mouse | *Mus musculus* | 20 |
| Human | *Homo sapiens* | 23 |
| Jimson weed | *Datura stramonium* | 12 |
| Mosquito | *Culex pipiens* | 3 |
| Mustard plant | *Arabidopsis thaliana* | 5 |
| Pink bread mold | *Neurospora crassa* | 7 |
| Potato | *Solanum tuberosum* | 24 |
| Rhesus monkey | *Macaca mulatta* | 21 |
| Roundworm | *Caenorhabditis elegans* | 6 |
| Silkworm | *Bombyx mori* | 28 |
| Slime mold | *Dictyostelium discoideum* | 7 |
| Snapdragon | *Antirrhinum majus* | 8 |
| Tobacco | *Nicotiana tabacum* | 24 |
| Tomato | *Lycopersicon esculentum* | 12 |
| Water fly | *Nymphaea alba* | 80 |
| Wheat | *Triticum aestivum* | 21 |
| Yeast | *Saccharomyces cerevisiae* | 16 |
| Zebrafish | *Danio rerio* | 25 |



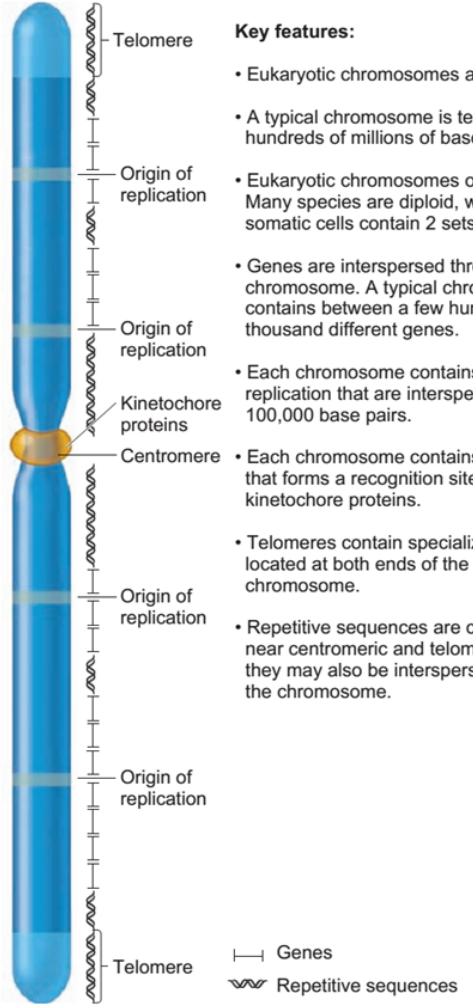
## Each eukaryotic chromosome has a centromere and telomeres

# Chromosome structure



# Organization of eukaryotic chromosomes




**Key features:**

- Eukaryotic chromosomes are usually linear.
- A typical chromosome is tens of millions to hundreds of millions of base pairs in length.
- Eukaryotic chromosomes occur in sets. Many species are diploid, which means that somatic cells contain 2 sets of chromosomes.
- Genes are interspersed throughout the chromosome. A typical chromosome contains between a few hundred and several thousand different genes.
- Each chromosome contains many origins of replication that are interspersed about every 100,000 base pairs.
- Each chromosome contains a centromere that forms a recognition site for the kinetochore proteins.
- Telomeres contain specialized sequences located at both ends of the linear chromosome.
- Repetitive sequences are commonly found near centromeric and telomeric regions, but they may also be interspersed throughout the chromosome.

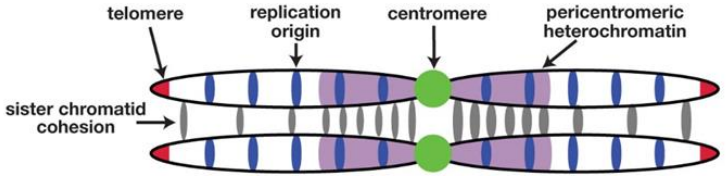
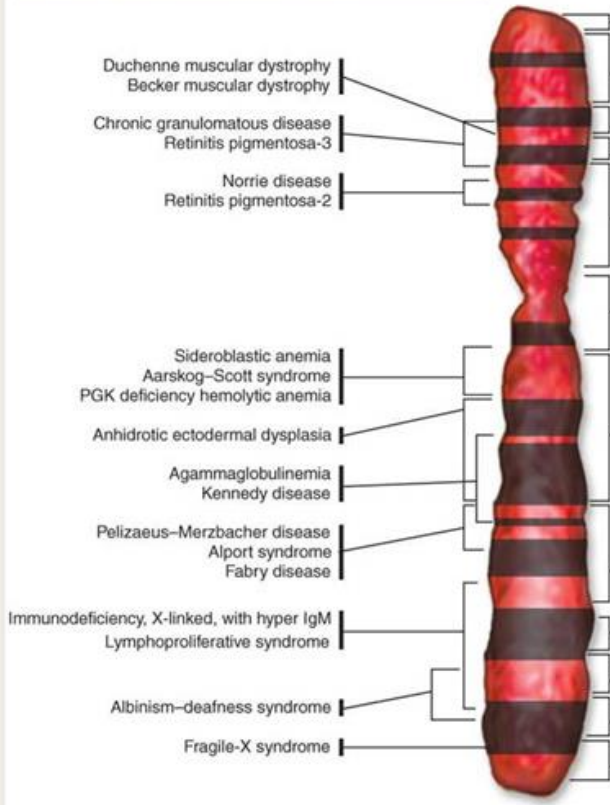



**Figure 2. Chromosome Inheritance Elements**

The diagram indicates the chromosomal elements essential for normal duplication (replication origins) and inheritance (centromeres, cohesion, telomeres) through mitosis and meiosis. Normal meiotic segregation also requires homolog pairing sites (not shown) and, in most cases, recombination.

# Location of some disease causing genes on the human X-chromosome



Duchenne muscular dystrophy
Becker muscular dystrophy

Chronic granulomatous disease
Retinitis pigmentosa-3

Norrie disease
Retinitis pigmentosa-2

Sideroblastic anemia
Aarskog–Scott syndrome
PGK deficiency hemolytic anemia

Anhidrotic ectodermal dysplasia

Agammaglobulinemia
Kennedy disease

Pelizaeus–Merzbacher disease
Alport syndrome
Fabry disease

Immunodeficiency, X-linked, with hyper IgM
Lymphoproliferative syndrome

Albinism–deafness syndrome

Fragile-X syndrome

Ichthyosis, X-linked
Placental steroid sulfatase deficiency
Kallmann syndrome
Chondrodysplasia punctata, X-linked recessive

Hypophosphatemia
Aicardi syndrome
Hypomagnesemia, X-linked
Ocular albinism
Retinoschisis

Adrenal hypoplasia
Glycerol kinase deficiency

Ornithine transcarbamylase deficiency

Incontinentia pigmenti
Wiskott–Aldrich syndrome
Menkes syndrome

Androgen insensitivity

Charcot–Marie–Tooth neuropathy
Choroideremia
Cleft palate, X-linked
Spastic paraplegia, X-linked, uncomplicated
Deafness with stapes fixation

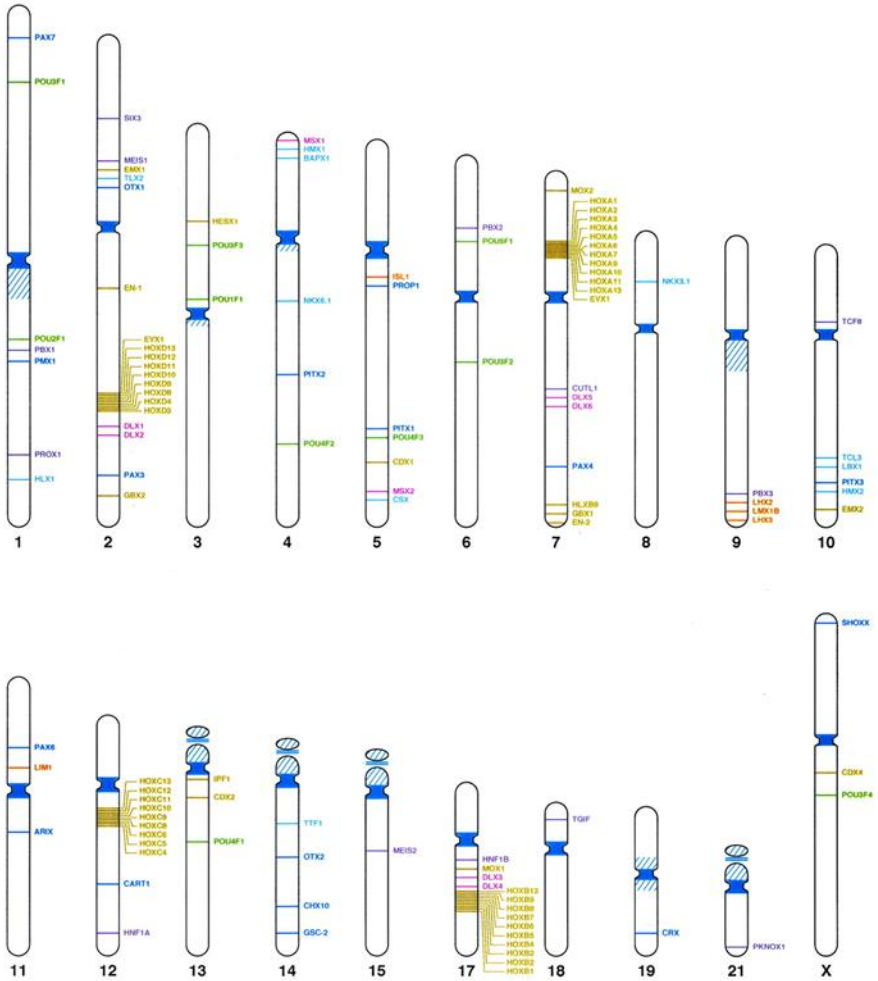
PRPS-related gout

Lowe syndrome

Lesch–Nyhan syndrome
HPRT-related gout

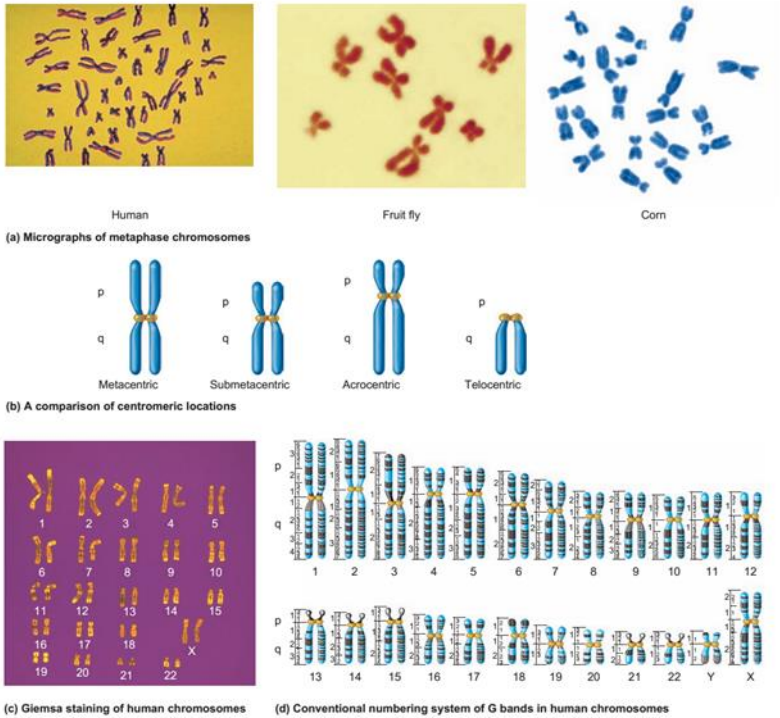
Hunter syndrome
Hemophilia B

Hemophilia A
G6PD deficiency: favism
Drug-sensitive anemia
Chronic hemolytic anemia
Manic–depressive illness, X-linked
Colorblindness, (several forms)
Dyskeratosis congenita
TKCR syndrome
Adrenoleukodystrophy
Adrenomyeloneuropathy
Emery–Dreifuss muscular dystrophy
Diabetes insipidus, renal
Myotubular myopathy, X-linked




**Chromosomal distribution of the human homeobox gene family**

The homeobox genes are color coded corresponding to the phylogenetic classes. The chromosomal map positions of the homeobox genes were obtained from LocusLink. The centromere is shown in dark blue. The four HOX clusters, HOXA, HOXB, HOXC and HOXD, are distributed on chromosomes 7, 17, 12 and 2, respectively.




## Features of normal chromosomes

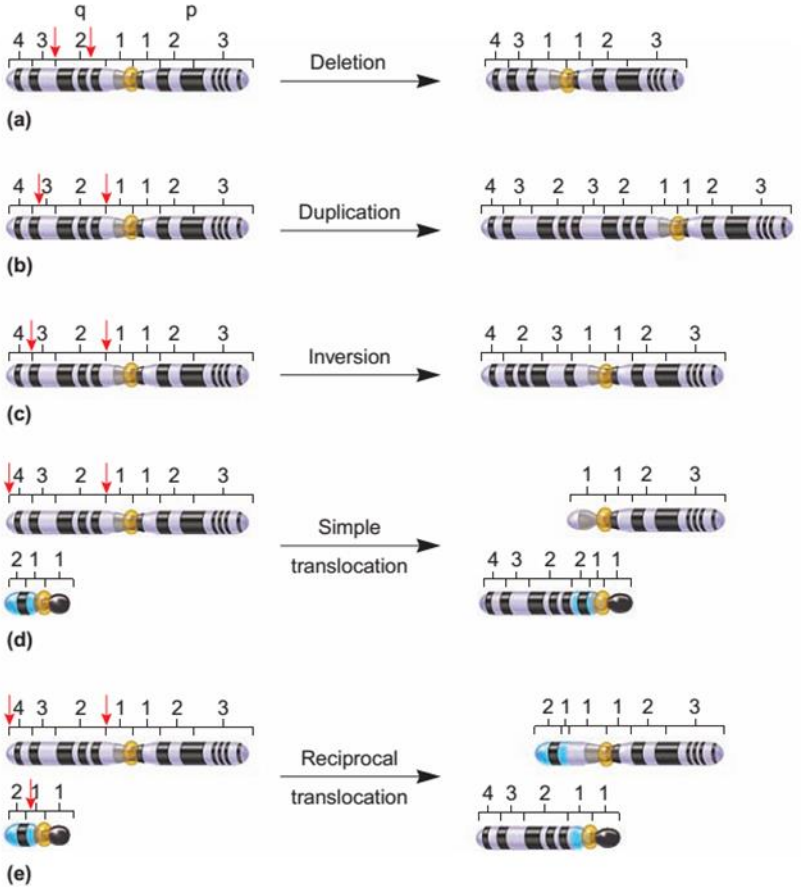
(a) Micrographs of chromosomes from a human, a fruit fly, and corn.

(b) A comparison of centromeric locations. Centromeres can be metacentric, submetacentric, acrocentric (near one end), or telocentric (at the end).

(c) Human chromosomes that have been stained with Giemsa.

(d) The conventional numbering of bands in Giemsa-stained human chromosomes. The numbering is divided into broad regions, which then are subdivided into smaller regions. The numbers increase as the region gets farther away from the centromere. For example, if you take a look at the left chromatid of chromosome 1, the uppermost dark band is at a location designated p35.The banding patterns of chromatids change as the chromatids condense. The left chromatid of each pair of sister chromatids shows the banding pattern of a chromatid in metaphase, and the right side shows the banding pattern as it would appear in prometaphase.

Note: In prometaphase, the chromatids are more extended than in metaphase.




## Types of changes in chromosome structure

The large chromosome shown throughout is human chromosome 1. The smaller chromosome seen in (d) and (e) is human chromosome 21.

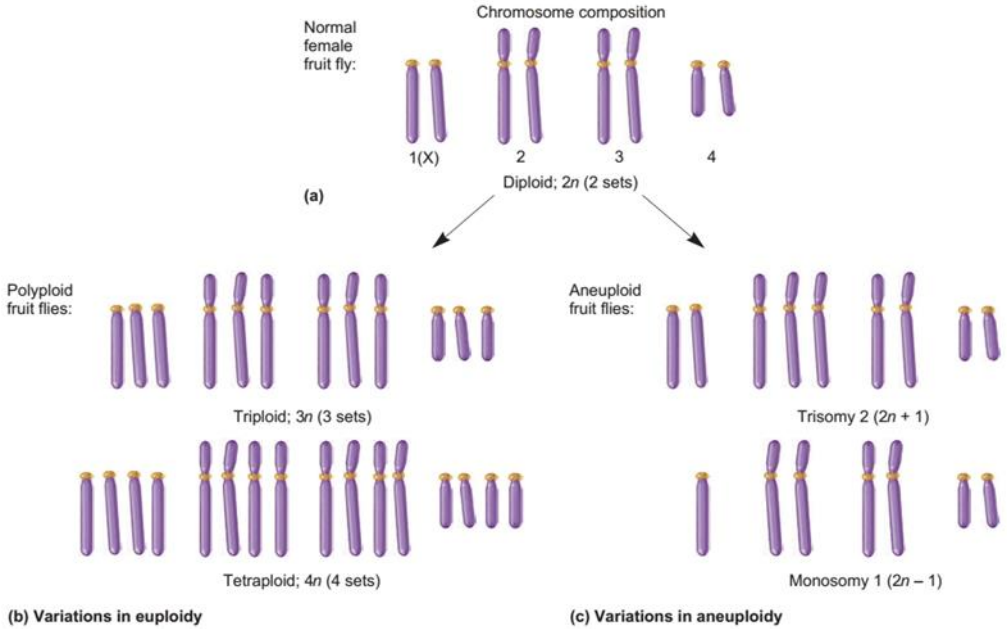
(a) A deletion occurs that removes a large portion of the q2 region, indicated by the red arrows.

(b) A duplication occurs that doubles the q2–q3 region.

(c) An inversion occurs that inverts the q2–q3 region.

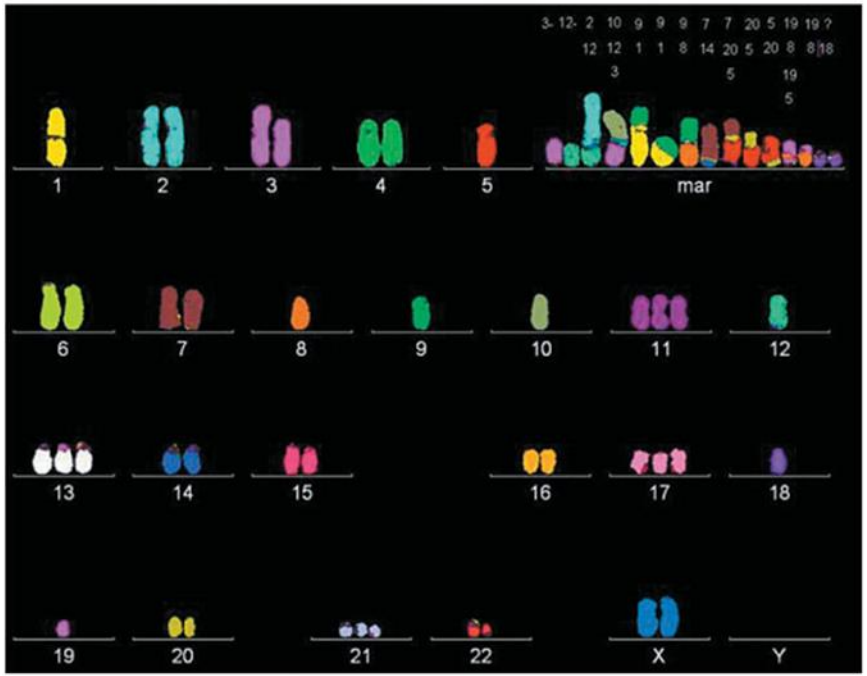
(d) The q2–q4 region of chromosome 1 is translocated to chromosome 21. A region of a chromosome cannot be inserted directly to the tip of another chromosome because telomeres at the tips of chromosomes prevent such an event. In this example, a small piece at the end of chromosome 21 must be removed for the q2–q4 region of chromosome 1 to be attached to chromosome 21.

(e) The q2–q4 region of chromosome 1 is exchanged with most of the q1–q2 region of chromosome 21.




**Types of variation in chromosome number**

(a) Depicts the normal diploid number of chromosomes in Drosophila. (b) Examples of polyploidy. (c) Examples of aneuploidy. Organisms with three or more sets of chromosomes are also called **polyploid**. A second way in which chromosome number can vary is by **aneuploidy**. Such variation involves an ***alteration in the number of particular chromosomes***, so the total number of chromosomes is not an exact multiple of a set. For example, an abnormal fruit fly could contain nine chromosomes instead of eight because it has three copies of chromosome 2 instead of the normal two copies.




**23.12 Cancer cells often possess chromosome abnormalities, including extra chromosomes, missing chromosomes, and chromosome rearrangements.** Shown here are chromosomes from a colon-cancer cell, which has numerous chromosome abnormalities. [Courtesy of Dr. Peter Duesberg, University of California at Berkeley.]

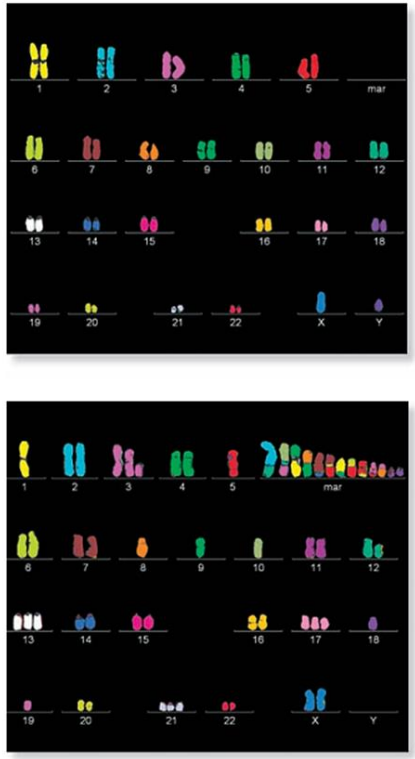
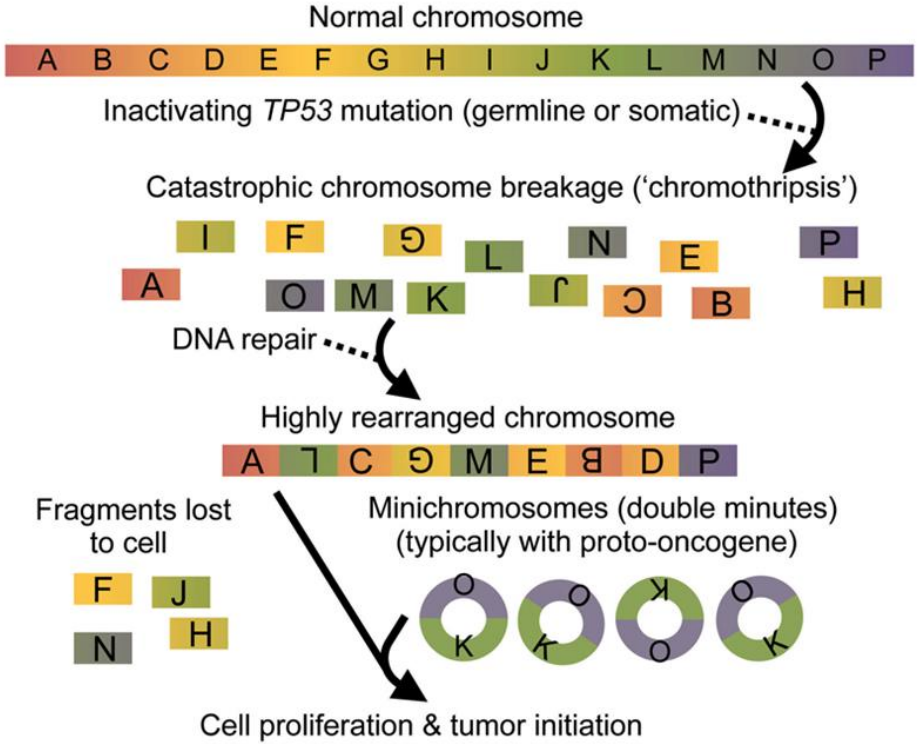



**FIGURE 23.18 A comparison between chromosomes found in a normal human cell and a cancer cell from the same person.** The bottom set found in a cancer cell is highly abnormal, with extra copies of some chromosomes and lost copies of others. Chromosomes made of fused pieces of chromosomes (designated mar in this figure) are also common in cancer cells.




**Catastrophic chromosome rearrangements resulting from chromothripsis.**

Human cancers commonly develop as a consequence of chromothripsis, a **structural rearrangement mechanism linked with predisposing TP53 mutations. Chromothripsis frequently leads to the formation of complex circular mini-chromosomes ('double minute chromosomes') harbouring proto-oncogenes** (Rausch et al., Cell 2012)