New Supercoiling Theory and Model of Chromosomal Structures in Eukaryotic Cells

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Abstract:

About six billion base pairs of DNA reside highly orderly in each human cell's nucleus through their manifestation as twenty-three pairs of chromosomes. Delicate patterns of spatial organizations of DNA macromolecules in these eukaryotic chromosomes as well as their associated driving forces have, however, not been fully understood thus far. On the basis of (1) our four recent discoveries about supercoiling properties of histone H1, nucleosomes, linker DNA and polynucleosomes, (2) well-established axioms about sign, shapes and handedness of DNA supercoils, as well as (3) the fact that alterations of DNA supercoils are affiliated with every single steps of cellular genetic events, we analyze effects of DNA supercoils on eukaryotic chromosomal structures in systematical and comprehensive manners in the current report, and present new theory and models of eukaryotic chromosomal structures from the DNA supercoiling perspective. It is our hope that our current presentation of new supercoiling theory and models could provoke future new efforts to unravel exquisite eukaryotic chromosomal architectures in an all-inclusive manner.

Key words:

Chromosomes, DNA supercoiling, meiosis and mitosis, interphase and metaphase DNA, chromatin, nucleosomes.

1. Introduction

Supercoiling and its alterations are affiliated at all times with cellular DNA at all levels of life, from prokaryotes, to archaea, and to eukaryotes.[1-5] During DNA replication and chromosome packaging in eukaryotic cells, for example, histones and topoisomerase II as two of the most abundant supercoiling-associating proteins act on DNA to adjust superhelical densities of genomic DNA in their host cells.[6-7] In addition, after a transcription process starts in prokaryotic cells, DNA gyrase will emerge to relax positive DNA supercoils that are built up in front of transcription bubbles.[8-9] Furthermore, DNA macromolecules in all hyperthermophilic archaea exist in their positively supercoiled forms, which are resulted from action of their uniquely own DNA reverse gyrase.[10-11] These[6-11] and immense other evidence [12-18] have demonstrated that DNA supercoiling plays vital roles in the functions of cells in all types of organisms on Earth.

From the structural standpoint, on the other hand, supercoiling of DNA is a physical arrangement of topologically closed double helical structure of nucleic acids that exists in space in an underwound or overwound fashions.[1,19-20] This topologically closed DNA could (1) either appear as a covalently closed circular entity or (2) possesses non-rotatable terminuses in its linear duplex structures[21]. With the purpose of mathematically describing supercoiling features of DNA, Călugăreanu–White–Fuller Theorem[22-25] ("DNA Topological Conservation Law")[26-27] was suggested in the 1960s and 1970s, which is expressed in form of the following equation:

$$\text{Linking number} = \text{Twist number} + \text{Writhe number} \qquad \text{(Equation 1)}$$

In view of the fact that vast new knowledge on DNA structures has been acquired since 1960s,[22-25] our research group reformulated Călugăreanu–White–Fuller Theorem and presented a new "General Topological Conservation Law of DNA" in 2011 based on experimental data newly obtained in our lab,[28] in which effects of non-canonical structures of DNA were taken into account:

$$Lk - Tw + Nb = Wb + Wn = Wr \qquad \text{(Equation 2)}$$

In addition, with the purpose of gaining new information about the perplexed spatial organizations of eukaryotic chromosomes, our research group had conducted a series of particularly designed studies in the past two years, from which four new discoveries (Discovery 1 to Discovery 4 as discussed in Section 2.1 below) about supercoiling properties of histone H1, linker DNA, nucleosomes and polynucleosomes were made.[29-32] In the current report, on the basis of our recent four discoveries[29-32] and previously well-established principles about supercoiling properties of DNA,[1-2,33-35] we present supercoiling theory and views on eukaryotic chromosomal structures from the conceptual and deductive standpoints. It is our hope that our new analyses and deductions could serve as incentives for developing new comprehensive structural models in the future that will unveil secrecy of subtle and sophisticated architectures of eukaryotic chromosomes.

2. Our Recent Four Discoveries, Previously Established Axioms about Supercoiling Properties of DNA and Our Three Propositions

2.1 Our recent four discoveries

Discovery 1. Binding of histone H1 to nucleosomes leads to generation of negative supercoils in naked linker DNA regions of polynucleosomes.[29]

It had been known in the past that upon their mixing-up with histone H1, loose circular polynucleosomes turned instantaneously into aggregate structures.[36-37] Our recent studies demonstrated that these aggregate structures were relaxable by *E. coli*. topoisomerase I (Fig. 1A).[29] Because the only substrate of *E. coli*. topoisomerase I is negatively supercoiled DNA, our new observations[29] signified that negative supercoils were produced in the naked DNA regions of polynucleosomes once they were bound by histone H1. In addition, since backbones of supercoiled DNA is forcibly curved,[38-39] it is evident that backbone curvatures of negatively supercoiled DNA cause polynucleosomes to aggregate.[29]

Discovery 2. Upon binding of histone H1, two 10-base pair arm DNA segments at ends of chromatosome DNA form (1) right handed and (2) toroidal shaped positive supercoils, which is the cause of generation of negative supercoils in their adjacent naked linker DNA regions.[30]

Our recent FRET studies demonstrated that when two 10-base pair arm DNA segments exist in their histone H1-enforced arm-closed forms, the two arms were oriented toward each other[30] (Fig. 1B). These orientations denote that two 10-base pair arm DNA segments in chromatosomes are right-handed, toroidal shaped positive supercoils.[30] As compensation of positive supercoils produced in these two 10-base pair arm DNA segments, negative supercoils are generated simultaneously in their adjacent naked linker DNA regions.[30] In theory, the two 10-base pair arm DNA segments in chromatosomes are able to emerge in their relaxed forms (**Structure 1** in Fig. 1B), in their overwound forms (**Structure 2** in Fig. 1B) or in their underwound forms (**Structure 3** in Fig. 1B). If two 10-base pair arm DNA segments adopt its relaxed form (**Structure 1** in Fig. 1B), right-handed orientations of the DNA segments alone will be able to cause generation of negative supercoils in naked linker DNA regions. If two 10-base pair arm DNA segments adopt its overwound form (**Structure 2** in Fig. 1B), both right-handed orientations of the DNA segments and overwound structures will cause accumulation of negative supercoils in naked linker DNA regions. If (1) two 10-base pair arm DNA segments adopt its underwound form (**Structure 3** in Fig. 1B) and (2) effects of right-handed orientation of two 10-base pair arm DNA segments is greater than those produced by underwound form of DNA, negative supercoils will still be produced in naked linker DNA regions.

Discovery 3. In the absence of histone H1, two 10-base pair DNA segments in nucleosomes (1) exist in their arm-closed form if ATP (polyanions) is present and (2) exist in their arm-open form if spermidine (polycations) is present.[30]

Our recent studies revealed that in the absence of histone H1, ATP as polyanions facilitated two 10-base pair DNA segments to adopt their arm-closed forms in histone H1-free chromatosomes (Fig. 1C).[30] Different from ATP, spermidine as polycations, however, interacted with negatively charged DNA backbones,[40] which causes two 10-base pair DNA segments to adopt their arm-open forms in nucleosomes[30].

Discovery 4. From the quantitative viewpoint, binding of ~11.5 histone H1 proteins leads to changes of linking number of -1 in naked linker DNA regions of polynucleosomes, which is equivalent to that binding of one histone H1 protein to one nucleosome leads to changes of linking number of -0.09 in linker DNA regions of polynucleosomes.[31-32]

Comparison studies of linking number differences between histone-free and histone-bound circular polynucleosomes were conducted by our group previously on the basis of chloroquine-assisted gel electrophoretic analysis.[31-32] These studies allowed us to quantitatively determine magnitudes of negative DNA supercoils caused by binding of histone H1 to nucleosomes (Fig. 1D).[31-32]

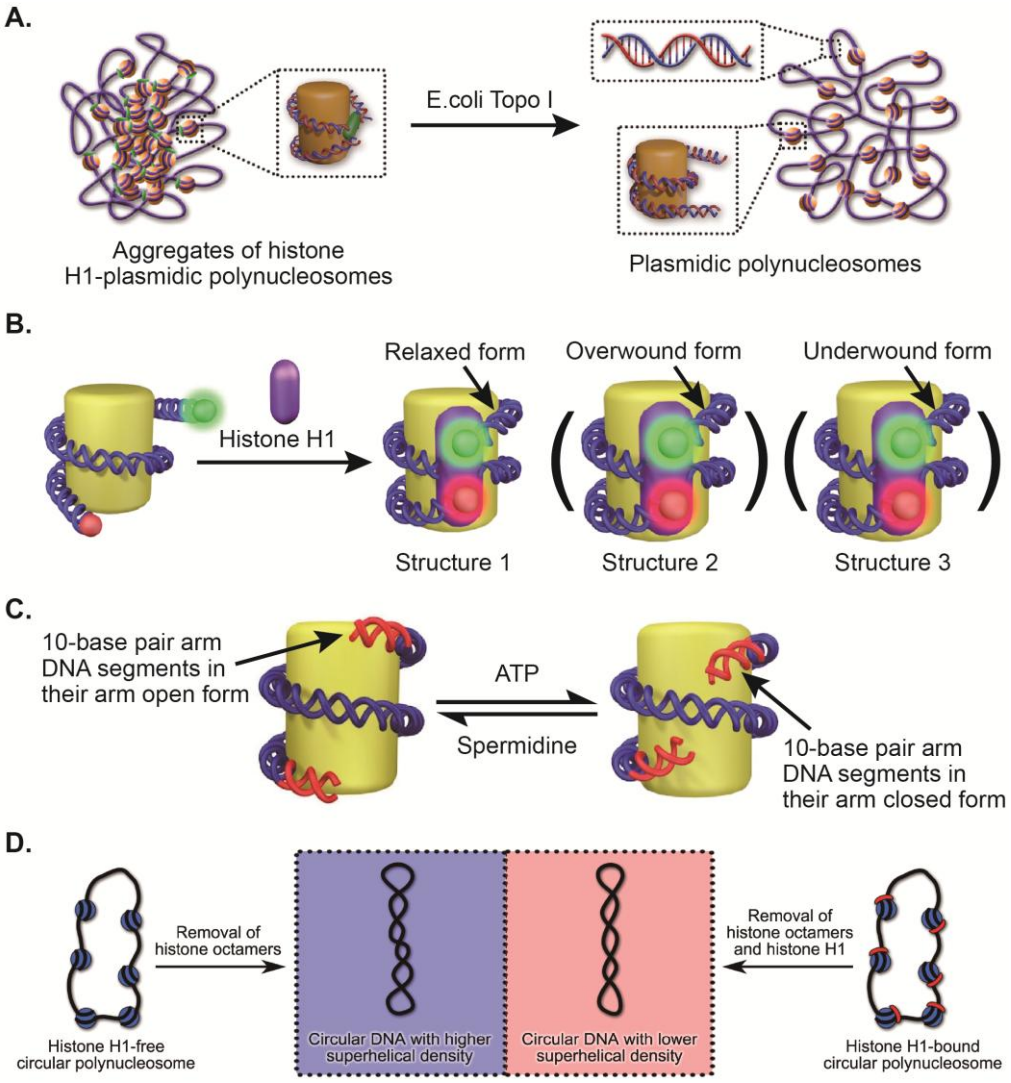


Fig. 1. Pictorial illustration of Discovery 1 (A), Discovery 2 (B), Discovery 3 (C) and Discovery 4 (D) that were made by our group in the recent years.[29-32] Adapted from (1)

Bioorganic & Medicinal Chemistry Letters, 27, Hao Zhang and Tianhu Li, Presence of negative supercoiling in aggregates of histone H1-plasmidic polynucleosome complexes, 168-170, Copyright (2018), (2) Bioorganic & Medicinal Chemistry Letters, 27, Hao Zhang and Tianhu Li, Effects of spermidine and ATP on stabilities of chromatosomes and histone H1-depleted chromatosomes, 1149-1153, Copyright (2018), and (3) Bioorganic & Medicinal Chemistry Letters, 28, Hao Zhang and Tianhu Li, Quantitative determination of linking number differences between circular polynucleosomes and histone H1-bound circular polynucleosomes, 537-540, Copyright (2018), with permission from Elsevier.

2.2 Four axioms about correlations among shapes, signs and handedness of DNA supercoils

From the supercoiling standpoint, (1) covalently closed circular DNA and (2) linear DNA with non-rotatable terminuses can exist in their (i) underwound form, (ii) overwound forms, and (iii) relaxed forms, whose signs are designated as (i) negative (-), (ii) positive (+), and (iii) zero respectively.[1-2] Different from relaxed form of DNA, negative and positive DNA supercoils are capable of adopting one of the following four types of shapes: (1) right-handed toroids, (2) left-handed toroids, (3) right-handed plectonemes, and (4) left-handed plectonemes.[1-2,33-34] Commonly accepted rules about correlations among the aforementioned shapes, signs, and handedness of DNA supercoils can be summarized into four axioms (Table 1) as outlined as follows:

Axiom 1. If sign of a DNA supercoil is negative and it holds a toroidal shape, this DNA toroid is left-handed, and vice versa;

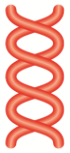
Axiom 2. If sign of a DNA supercoil is negative and it holds a plectonemic shape, this DNA plectoneme is right-handed, and vice versa;

Axiom 3. If sign of a DNA supercoil is positive and it holds a toroidal shape, this DNA toroid is right-handed and, vice versa; and

Axiom 4. If sign of a DNA supercoil is positive and it holds a plectonemic shape, this DNA plectoneme is left-handed, and vice versa.

Table 1. Illustration of commonly accepted rules about correlations among signs, shapes, and handedness of DNA supercoils.

Axioms	Signs of DNA supercoils	Shapes of DNA supercoils	Handedness of structures of DNA supercoils	Pictorial illustrations of DNA supercoils*
Axiom 1	Negative	Toroid	Left-handed	

Axiom 2	Negative	Plectoneme	Right-handed	
Axiom 3	Positive	Toroid	Right-handed	
Axiom 4	Positive	Plectoneme	Left handed	

* Single coiled heavy line in these drawings represents backbones of duplex DNA.

2.3 Our three propositions

Proposition 1. Genomic DNA macromolecules can be classified into two structural categories: (1) protein-bound DNA and (2) naked linker DNA (protein-free DNA) while (1) naked linker DNA segments are the conformation-alterable elements in chromatins and chromosomes and (2) the ratios of naked linker DNA to protein-bound DNA vary dynamically in chromosomes in eukaryotic cells;

Proposition 2: Three dimensional structures of chromatins and chromosomes at a given instant are govern by (1) superhelical densities of their naked linker DNA segments, (2) backbone rigidness of naked linker DNA segments and (3) abundance and distribution patterns of nucleosome core particles and chromatosomes along their genomic DNA macromolecules; and

Proposition 3: Because conformation-alterable naked linker DNA regions of chromatins and chromosomes are supercoiled,[30] spatial organizations and handedness of chromosomes and their sublevel architectures can be predicted using the aforementioned four axioms (Section 2.1 and Table 1).

3. Supercoiling Views of Interphase Chromosomal Structures

From the standard points of DNA supercoiling and structural organizations, interphase chromosomes can be classified into six hierarchical ranks as depicted in Table 2 and Fig. 2. Because topological properties of double helical structures of DNA (Primary structures) and nucleosomes (Secondary structures) were discussed previously by our group[28] and others[22,35,41-44], focus of our discussion in this section of the current report will be on polynucleosomes (Tertiary structures), insulated neighborhoods (Quaternary structures), topologically associating domains (Quinary structures), and interphase chromosomes (Senary structures) respectively.

Table 2. DNA supercoiling views of hierarchical ranks of structural organizations in interphase chromosomes.

Entry	Sublevel structures of interphase chromosomes	Level of hierarchical ranks
1	Double helices of DNA	Primary structures
2	Nucleosomes	Secondary structures
3	Polynucleosomes	Tertiary structures
4	Insulated neighborhoods	Quaternary structures
5	Topologically associating domains	Quinary structures
6	Interphase chromosomes	Senary structures

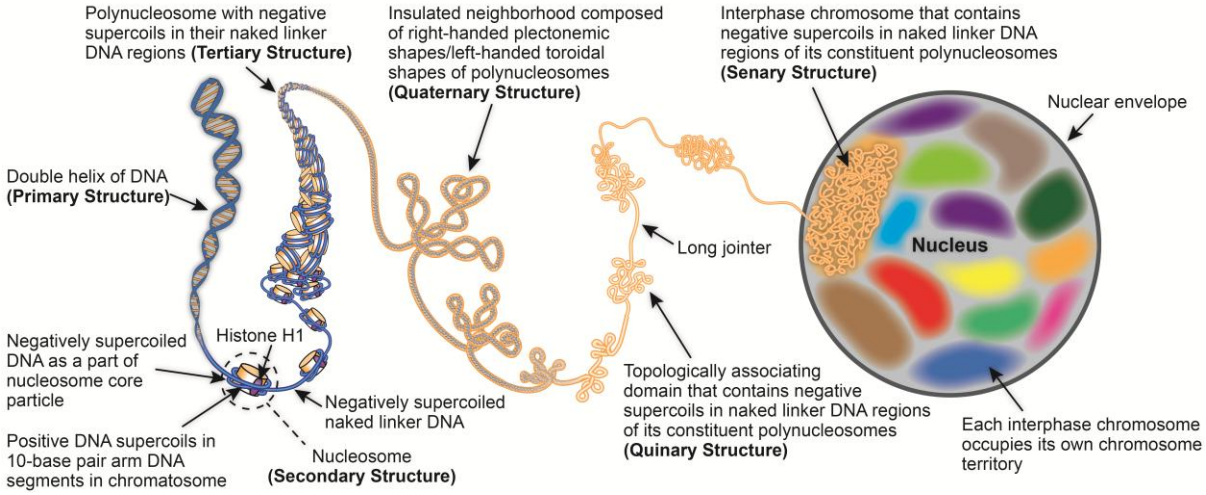


Fig. 2. DNA supercoiling views of spatial organizations of interphase chromosomes and their sublevel architectures.

3.1 Polynucleosomes as tertiary structures of interphase chromosomes

A linker DNA is often defined as duplex DNA segments that connect two adjacent nucleosome core particles in polynucleosomes[45] whereas average length of such DNA segments are ~55 base pairs[46-47]. On the basis of this aforementioned information[45-47], (1) densely packed 30 nm fibers, (2) loosely packed 30 nm fibers, and (3) slack polynucleosomes are defined in the current report as the polynucleosomal structures that possess their linker DNA segments (1) less than 30 base pairs, (2) around 50 base pairs and (3) longer than 70 base pairs in length respectively (Fig. 3).

(a) Densely packed 30 nm fibers and loosely packed 30 nm fibers. Because naked linker DNA segments in 30 nm fibers are negatively supercoiled (Discovery 1), these negative supercoils will force naked linker DNA segments in 30 nm fibers to adopt left-handed shapes on the whole once 30 nm fibers form toroid-like columnar structures (Axiom 1 in Table 1). In addition, because DNA segments in nucleosome core particles are in close physical contact with histone octamers, their conformations are not alterable. Instead, it is the conformation-alterable naked linker DNA segments that dominate three-dimensional organizations of nucleosome core particles in 30 nm fibers (Propositions 2 to 3). Deduction 1 is accordingly

presented in the current report for describing correlation of overall shapes of naked linker DNA segments with their handedness in 30 nm fibers.

Deduction 1. Naked linker DNA segments as well as three-dimensional arrangements of nucleosome core particles in densely and loosely packed 30 nm fibers display left-handed toroidal shapes on the whole (Fig. 3A and Fig. 3B), handedness of which is determined by negative supercoils present in their naked linker DNA regions. In addition, negative DNA supercoils in 30 nm fibers are irresolvable by topoisomerases in eukaryotic cells because of their (1) low magnitudes of superhelical density, (2) structural compactness and (3) dynamic transitions of 10-base pair arm segments between their arm-open and arm-closed forms.

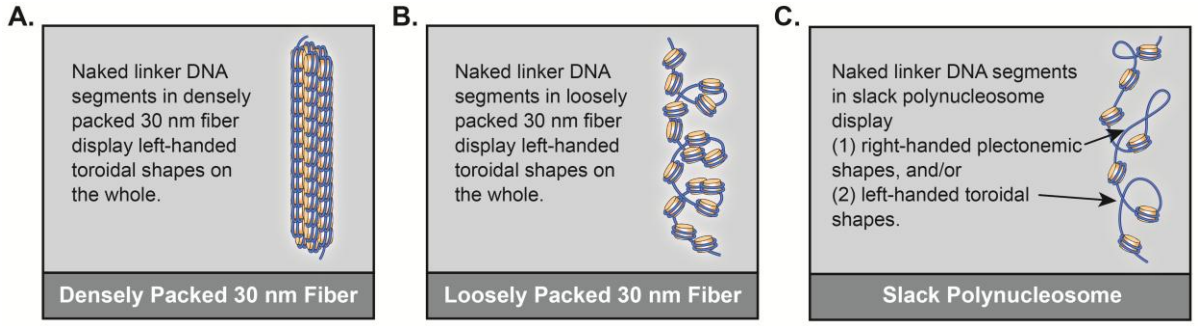


Fig. 3. Pictorial illustration of structures of supercoiling-driven densely packed 30 nm fibers (A), loosely packed 30 nm fibers (B) and slack polynucleosomes (C).

Three exquisite models, on the other hand, were introduced in the past for describing spatial packaging patterns of nucleosome core particles in 30 nm fibers, namely (1) Rhodes one start model[48], (2) Richmond two start model[49] and (3) Li and Zhu two start tetra-nucleosomes model[50] respectively. Even though these three models portray different spatial arrangements of nucleosome core particles, overall stacking patterns of naked linker DNAs in their suggested structures are all left-handed[48-50] (Fig. 4). This left handedness is coherent with the description specified in Deduction 1.

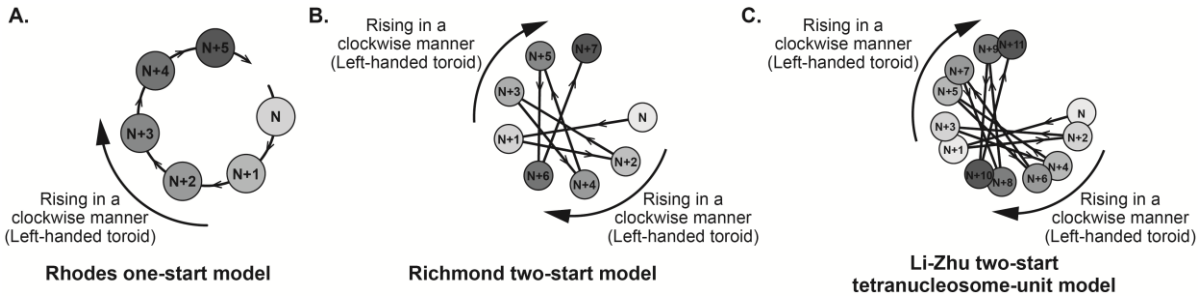


Fig. 4. Illustration of left handedness of naked linker DNA segments in Rhodes one-start model[48] (A), Richmond two-start model[49] (B), and Li-Zhu two start tetranucleosome-unit model[50] (C).

(b) Slack polynucleosomes. Different from (1) densely packed 30 nm fibers and (2) loosely packed 30 nm fibers (Fig. 3A and Fig. 3B), slack polynucleosomes as a third form of polynucleosomes possess relatively long naked linker DNA segments between two adjacent

nucleosome core particles (Fig. 3C). These long linker DNA-holding polynucleosomes are capable of adopting either left-handed toroidal structures or right handed plectonemic structures, which resemble the behaviors of protein-unbound plasmid DNA[51]. In addition, from the supercoiling viewpoint, magnitudes of superhelical densities of naked linker DNA regions in the abovementioned three types of polynucleosomal structures are in the following order: densely packed 30 nm fibers > loosely packed 30 nm fibers > slack poly-nucleosomes. Deduction 2 is accordingly presented in the current report for describing correlations of shapes of slack polynucleosomes with negative DNA supercoils in their naked linker DNA regions:

Deduction 2. Overall backbone structures of slack polynucleosomes as well as their constituent naked linker DNA segments can exist in the shapes of left-handed toroids or right-handed plectonemes (Fig. 3C), which are sustained by negative supercoils present in their naked linker DNA regions.

3.2 Insulated neighborhood as quaternary structures of interphase chromosomes

(a) Active, inactive and silent insulated neighborhoods as well as their correlations with plectonemic and toroidal structures of polynucleosomal backbones. An insulated neighborhood refers commonly to a structural assembly of multiple loops of polynucleosomes in interphase chromosomes whose root regions are (1) bound together by CTCF homodimers and (2) co-bound by cohesions[52-53]. It has been estimated that ~13,000 insulated neighborhoods are present in a eukaryotic cell, each of which contains ~90 kbp in size on average[52,54]. From the DNA supercoiling and topological standpoint, overall shapes of insulated neighborhoods are govern by the negative supercoils because (1) negative supercoils are present in naked linker DNA regions of their constituent polynucleosomes and (2) naked linker DNA segments are the only conformation-alterable elements in polynucleosomes. In other words, even though shapes of insulated neighborhoods have been frequently observed under microscopes as multiple irregular loops[55-57] (Fig. 5A), they are in effect assemblies of supercoiling-driven (1) left-handed plectonemic shapes (Fig. 5B), (2) right-handed toroidal shapes (Fig. 5C) or (3) combination of left-handed plectonemic and right-handed toroidal shapes of polynucleosomes.

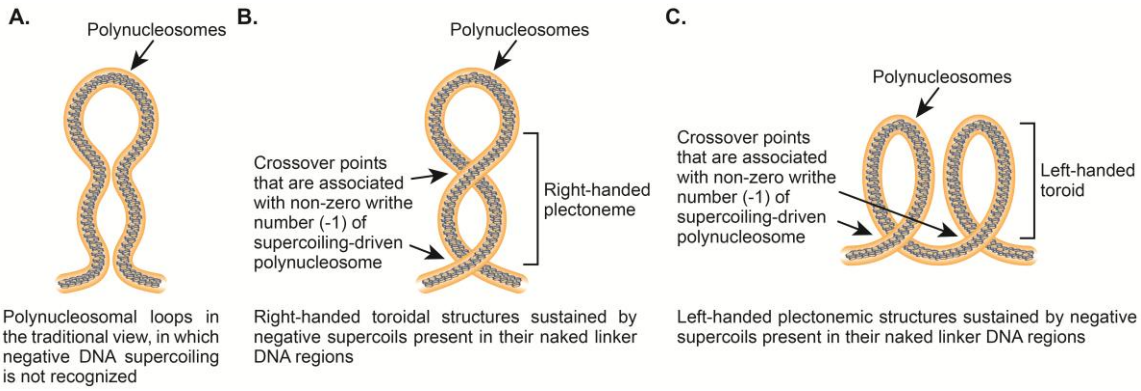


Fig. 5. Illustration of differences between traditional view[55-57] (A) and supercoiling view (B and C) on long spans of backbone structures of polynucleosomes as components of insulated neighborhoods.

One of the spatial characteristics of supercoil-driven plectonemic and toroidal shapes of DNA, on the other hand, are occurrence of crossover points within their backbone structures,[1,38,58] which reflects non-zero writhe number as defined in Călugăreanu–White–Fuller Theorem[22]. More specifically, proximities between two pairs of DNA segments (*e.g.* enhancers and promoters) were often observed experimentally within insulated neighborhoods even though these pairs are in fact separated by up to a million base pairs along their linear polynucleosomal backbones.[59-62] When viewed from the supercoiling perspective, these proximities between two DNA segments (*e.g.* two distal enhancers and promoters) are in fact the crossover points of polynucleosomal backbones associated with supercoiling-driven plectonemic and toroidal structures (Fig. 5B and Fig. 5C). Deduction 3 is accordingly presented in the current report for describing correlation of structural characteristics of insulated neighborhoods with negative supercoils in their naked linker DNA regions:

Deduction 3. (1) Insulated neighborhoods are assemblies of (i) left-handed plectonemic shapes (Fig. 5B), (ii) right-handed toroidal shapes (Fig. 5C) or (iii) combination of left-handed plectonemic shapes and right-handed toroidal shapes of polynucleosomes (Fig. 6), spatial organizations of which are sustained by negative supercoils present in their constituent naked linker DNA segments, and (2) it is the non-zero writhe number-affiliated crossover points of polynucleosomal backbones (Fig. 5B and Fig. 5C) that bring distal DNA segments (*e.g.* enhancers, silencers, promoters, insulators as well as pairs of CTCF) into proximity in insulated neighborhoods.

In addition, based on their gene expression capacities, insulated neighborhoods can be classified into (1) active insulated neighborhoods, (2) inactive insulated neighborhoods, and (3) silent insulated neighborhoods, which accommodate predominantly (1) active genes, (2) inactive genes and (3) no gene respectively. Active insulated neighborhoods contain mainly loosely packed 30 nm fibers and slack polynucleosomes (Fig. 6A) while inactive insulated neighborhoods mainly contain tightly and loosely packed insulated neighborhoods (Fig. 6B). Different from active insulated neighborhoods and inactive insulated neighborhoods, silent insulated neighborhoods, however, consist of mostly tightly packed 30 nm fibers (Fig. 6C). From the supercoiling standpoint, magnitudes of superhelical densities in naked linker DNA regions in the aforementioned three types of structural organizations are in the following order: silent insulated neighborhoods > inactive insulated neighborhoods > active insulated neighborhoods.

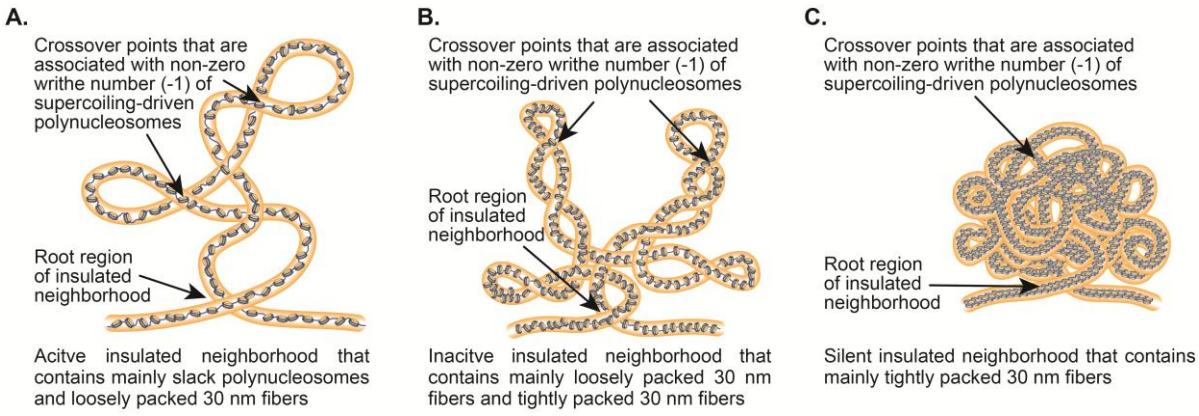


Fig. 6. Illustration of active insulated neighborhoods (A), inactive insulated neighborhoods (B), and silent insulated neighborhoods (C), (i) which are composed of left-handed plectonemes and/or right-handed toroids of polynucleosomes, and (ii) three-dimensional structures of which are sustained by negative supercoils present in their linker DNA regions.

(b) Correlation of regulations of gene expressions with plectonemic and toroidal structures in active insulated neighborhoods. For the convenience of our further discussion, "conformations of polynucleosomes" are defined in the current report as any spatial organizations that polynucleosomes may be able to adopt in an insulated neighborhood. In addition, "stable conformers" are defined as any spatial conformations of polynucleosomal backbones that correspond to local minimal potential energy. Furthermore, "pairable DNA elements" are named as two DNA segments in insulated neighborhoods that are capable of being bound together by protein and other molecules. Examples of such pairable DNA elements are (1) enhancers and promoters, (2) silencers and promoters, (3) insulators and insulators, (4) enhancers and insulators as well as (5) two CTCF DNA sequences[61]. When two pairable DNA segments occur within close ranges at crossover points of supercoiling-driven plectonemic and toroidal structures, they are named "adjacent pairable DNA segments". Once protein and other molecules clasp two adjacent pairable DNA segments together, the resultant plectonemic and toroidal structures in insulated neighborhoods are called "clasped stable conformers" or "clasped conformers". From the viewpoint of supercoiling-driven structures, it is the clasped conformers that determine patterns of gene expressions in insulated neighborhoods (Fig. 7). Deduction 4 is accordingly presented in the current report for describing correlations between regulation of gene expressions and clasped conformers:

Deduction 4. (1) Gene expressions (*e.g.* facultative and inducible gene expressions) in insulated neighborhoods are permissible if enhancer DNA sequences and promoter DNA sequences occur at crossover points of plectonemes/toroids and are further clasped by DNA binding proteins and other molecules; (2) gene expressions in insulated neighborhoods are suppressed if (i) silencer DNA sequences and gene promoter DNA sequences occur at crossover points of plectonemes/toroids and are further clasped by DNA binding proteins and other molecules, or (ii) either promoter DNA sequences or enhancer DNA sequences do not emerge at crossover points; and (3) when two CTCF DNA sequences occur at the crossover points of plectonemic/toroidal structures and clasped by DNA binding proteins and other molecules, they (i) serve as root regions of insulated neighborhoods or (ii) sustain overall structures of insulated neighborhoods (Fig. 7).

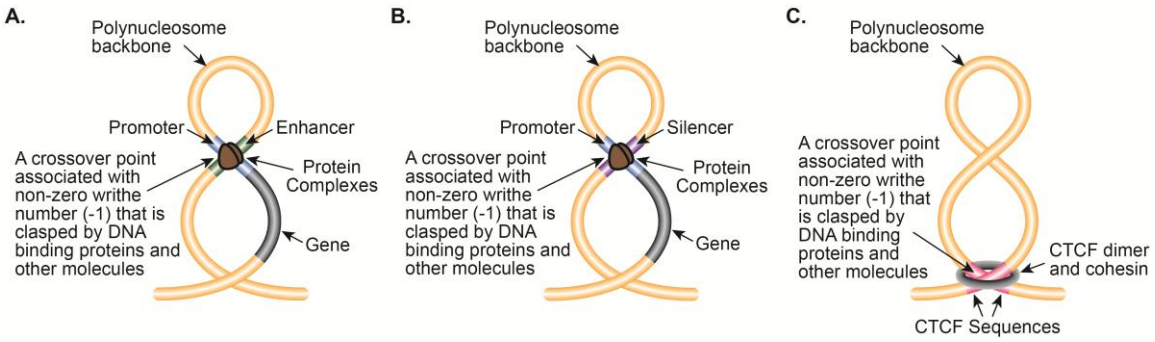


Fig. 7. Illustration of correlations between clasped conformers in insulated neighborhoods and regulations of gene expressions. (A) An enhancer and a promoter are clasped by proteins at a crossover point of supercoiling-driven plectonemes, in which gene expression is permissible, (B) a promoter and a silencer are clasped by proteins at a crossover point of plectonemes, in which gene expression is suppressed, and (C) two CTCF DNA sequences are clasped by proteins at a crossover point of plectonemes, which serves as the root or supporting structural element of an insulated neighborhood.

3.3 Topologically associating domains as quinary structures of interphase chromosomes

(a) Active, inactive and silent topologically associating domains. A topologically associating domain is a genomic region in interphase chromosomes, within which DNA sequences physically interact with each other more frequently than those beyond itself.[63-64] Based on their gene expression capacity and structural denseness, these self-interacting domains can be further classified into (1) active topologically associating domains, (2) inactive topologically associating domains, and (3) silent topologically associating domains, which contain mainly (1) active insulated neighborhoods, (2) inactive insulated neighborhoods, and (3) silent insulated neighborhoods respectively (Fig. 8). In addition, from the supercoiling standpoint, magnitudes of superhelical densities in naked linker DNA regions in these three types of topologically domains are in the following order: silent topologically associating domains > inactive topologically associating domains > active topologically associating domains.

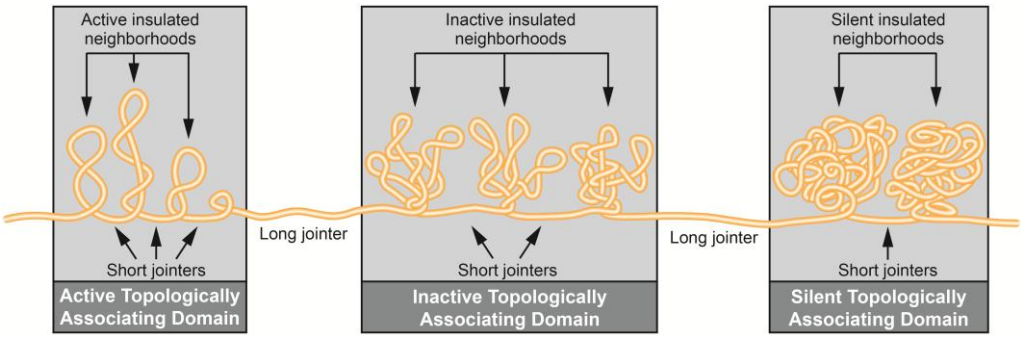


Fig. 8. Illustration of structures of three types of topologically associating domains, which contain mainly (i) active insulated neighborhoods, (ii) inactive insulated neighborhoods, and (iii) silent insulated neighborhoods respectively.

(b) Jointer sequences. The term jointer sequence is defined in the current report as DNA sequences that reside (1) between two insulated neighborhoods and (2) between two topologically associating domains in interphase chromosomes (Fig. 8). Within a topologically associating domain, jointer sequences between two adjacent insulated neighborhoods are comparably short, which are named "short jointers" (Fig. 8). These short lengths of jointers allow negative DNA supercoils to be spread out among adjacent insulated neighborhoods, which in turn makes a topologically associating domain act a self-interacting entity. Conversely, the jointer sequences between two topologically associating domains are relatively longer, which are named "long jointers" in the current report. These longer jointers will prevent propagation of negative DNA supercoils from spreading out of each individual

topologically associating domain owing to their extended lengths. In addition, since a long jointer region does not contain abundant nucleosome structures, this region could be one of the preferable places for constitutive gene to reside in.[65] Deduction 5 is accordingly presented below for describing correlations of characteristics of topologically associating domains with negative supercoils in naked linker DNA regions of their constituent polynucleosomes:

Deduction 5. It is the (1) negative supercoils in linker DNA regions of polynucleosomes and (2) short lengths of jointer sequences between insulated neighborhoods that drive each topologically associating domain to act as a self-gathering and self-interacting structural entity (Fig. 8).

(c) Correlation of transposition of transposons and viral insertion with sizes and shapes of insulated neighborhoods and topologically associating domains. Mobile genetic elements are DNA sequences that are capable of relocating or can be copied from one location to another in organismal genomes.[66] In eukaryotic cells, these mobile genetic elements are mainly transposons (transposable elements), which include retrotransposons and DNA transposons.[66-67] Similar to transposons, virus, on the other hand, is capable of increasing spans of DNA sequences as well through its insertion actions.[68-70] From DNA supercoiling standpoint, transposition of mobile genetic elements and viral insertion could lead to alteration of (i) shapes, (ii) crossover points of polynucleosome backbones and (iii) sizes of insulated neighborhoods and topologically associating domains (Fig. 9 and Fig. 10). Deduction 6 is accordingly presented in the current report for describing consequence of transposition of mobile genetic elements and insertion of virus on plectonemic and toroidal structures of polynucleosomes:

Deduction 6. Transposition of transposons and viral insertion (1) are capable of altering shapes, crossover points of polynucleosome backbones and sizes and boundaries of insulated neighborhoods and topologically associating domains, which could in turn change original relative positions of pairable DNA elements and affect patterns of gene expressions in the neighborhoods and domains (Fig. 9 and Fig. 10), and (2) can position the DNA sequences of promoters, enhancers, silencers and CTCF that transposons and viruses carry at crossover points or away from crossover points of polynucleosome backbones in insulated neighborhoods and topologically associating domains.

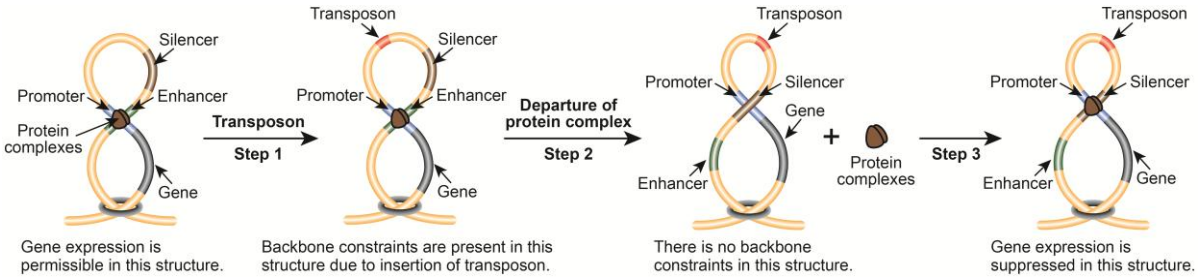


Fig. 9. Illustration of actions of transposons on gene expressions within an insulated neighborhood. **Step 1**: Insertion of a transposon that leads to generation of backbone constraints in clasped polynucleosomal structures; **Step 2**: departure of DNA binding protein complexes that leads to release of backbone constraints; and **Step 3**: association of complexes that re-clasps newly established stable conformer.

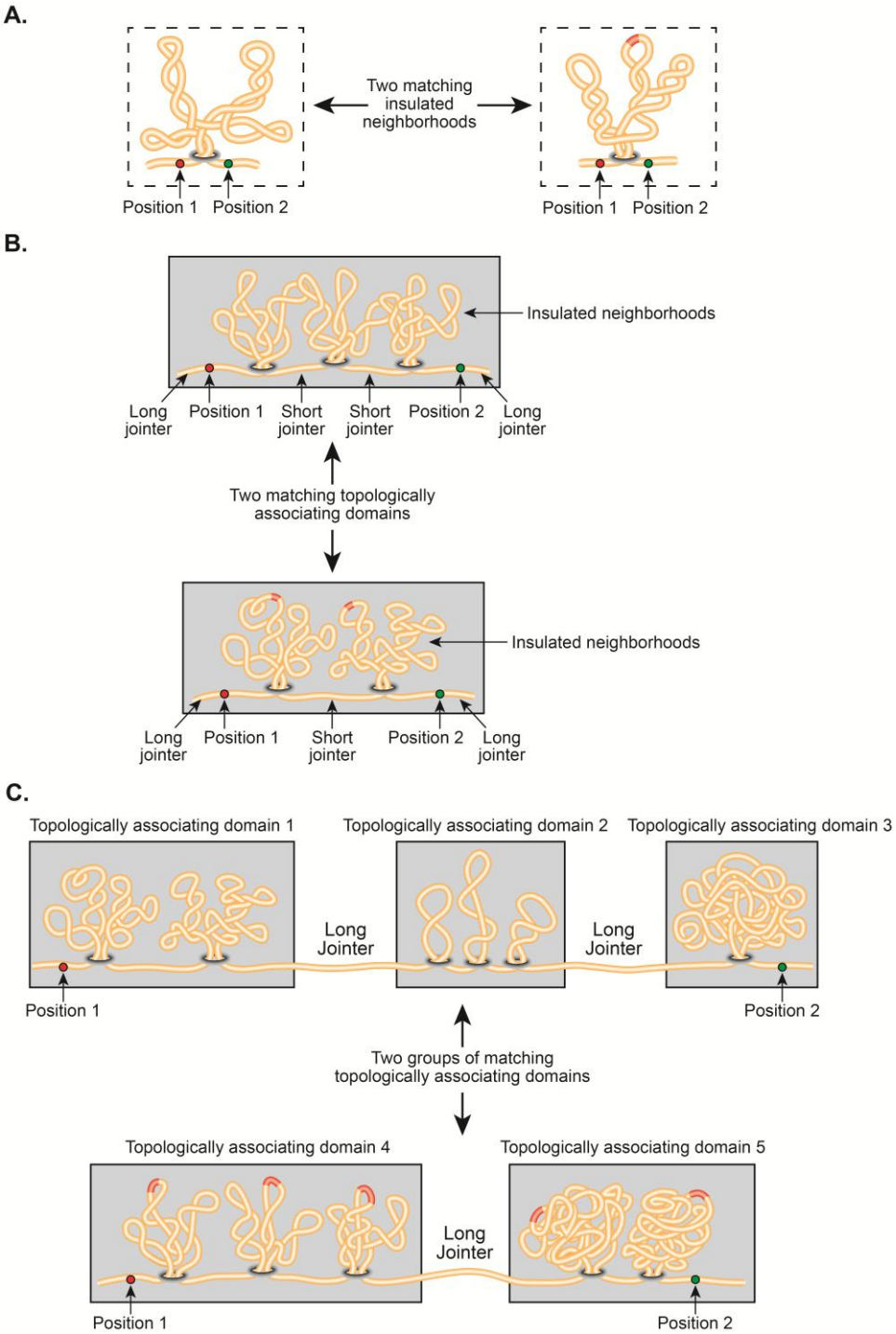


Fig. 10. Illustration of transpositions of transposon-caused changes of matching insulated neighborhoods/topologically associating domains. (A) Alteration of shapes of matching insulated neighborhoods without changes in root regions. (B) (i) Alteration of sizes of matching insulated neighborhoods and (ii) alteration of shapes of two matching topologically associating domains without changes of boundaries of the domains. (C) Alterations of size and shape of matching topologically associating domains.

(d) Correlations of supercoiling-driven insulated neighborhoods/topologically associating domains with (1) cell types, (2) genetic density, (3) diverse variants of polynucleosomal structures in cells in the same brain and (4) adaptive roles of transposable elements. For

the convenience of our further discussions, matching insulated neighborhoods and matching topologically associating domains are defined in the current report as the insulated neighborhoods and topologically associating domains that occur at the same loci of homologous chromosomes (1) in different or identical cell types, or (2) in the cells of organisms of the same species.

(1) Cell types. An individual cell in a multicellular organism possesses an identical set of genomic DNA to that of every other cell in the organism.[71] In spite of this genetic equality, a multicellular organism possesses various specialized cell types (*e.g.* liver cells and lung cells in human) for their diverse cellular functions.[71] It has been commonly acceptable nowadays that distinct patterns of gene expressions make genetically identical cells turn out to be different cell types.[72-73] Transposition of transposons[66] and viral insertions[68], on the other hand, are capable of altering shapes and sizes of supercoiling-driven insulated neighborhoods and topologically associating domains as well as crossover points of their constituent polynucleosomes (Deduction 6). From the DNA supercoiling viewpoint, it is the differences of crossover points of plectonemic and toroidal structures caused by activity of transposons in matching insulated neighborhoods/topologically associating domains that lead to different profiles of gene expressions in different cell types.

(2) Genetic diversity. Variations of alleles are present in chromosomes within a species population, number of which is often used as a measure of genetic diversity.[74-75] From the DNA supercoiling standpoint, one of the main structural bases of such variations in alleles and genetic diversity is that shapes and sizes of matching insulated neighborhoods/topologically associating domains as well as positions of crossover points at the same loci on chromosomes in individuals of identical species are different while such differences can be resulted from actions of transpositions of transposons and/or insertion of virus.

(3) Wide-ranging variants of genomic structures in cells in the same brain. It has been known that unlike those in any other organs of human body, cells in the same brain are widely different from one another in their genomic structures.[76] From the DNA supercoiling viewpoint, a single action of transposon could cause drastical structural changes of an entire insulated neighborhood and an entire topologically associating domain (Fig. 9 and Fig. 10). Therefore, it is the (1) high structural vulnerability of supercoiling-driven insulated neighborhoods and topologically associating domains to actions of transposons and (2) exceptional high activity of transposons in brain cells[76] that is accountable for emergence of wide-ranging variants of genomic structures in cells in the same brain.

(4) Adaptive transposable elements. It has been known that adaptive transposable elements are widespread in nature and their transpositions enable organisms to adapt gene expression to environmental changes.[77-79] Because actions of transposable elements are able to alter shapes and sizes of DNA supercoiling-driven insulated neighborhoods and topologically associating domains, these structural alterations could (1) introduce new adjacent pairable elements to insulated neighborhoods and topologically associating domains as well as (2) alter the distances between preceding adjacent pairable elements. It is therefore anticipated

that transposon-affiliated structural changes of insulated neighborhoods and topologically associating domains are accountable for the fundamental mechanisms that underlie transposable element–induced adaptation[78-79] in organisms.

In view that spatial structures of supercoiling-driven insulated neighborhoods and topologically associating domains are highly susceptible to length changes of their constituent DNA, Deduction 7 is accordingly presented in the current report for describing consequences of activity of transposons and viral insertions on properties of eukaryotic cells:

Deduction 7. Transposition of transposon alone, virus insertion alone or combination of the aforementioned two types of actions are capable of leading to differences in (1) shapes and sizes between matching insulated neighborhoods and matching topologically associating domains, as well as (2) relative spatial positions of pairable elements, which are accountable for (i) different gene expression profiles by different cell types, (ii) genetic diversity of a species' population, (iii) wide-ranging variants of genomic structures in cells in the same brains, and (iv) implementation of adaptable roles of transposable elements in organisms (Fig. 9 and Fig. 10).

3.4 Overall spatial organizations of interphase chromosomes as their own senary structures

It has been known that (1) interphase chromosomes in nuclei of eukaryotic cells display nearly spheroidic shapes on the whole[80], and (2) individual chromosomes perform their cellular actions in their own chromosome territories[81-83]. From the DNA supercoiling standpoint, these self-cohesive behaviors of interphase chromosomes are caused by immense accumulation of negative supercoil-affiliated backbone curvatures in their naked linker DNA regions (Fig. 2). In addition, on the basis of their interactions with membrane structures, interphase chromosomes can be classified into (1) lamina-associated domains[84], (2) nucleolus-associated domains[85-86], and (3) non-membrane-associated domains. Lamina-associated domains and nucleolus-associated domains bind to inner membrane of the nucleus and surround highly transcribed region of nucleolus respectively, and are structurally composed of silent and inactive topologically associating domains. Non-membrane-associated domains, on the other hand, are defined in the current report as polynucleosomal structures that are free from binding to lamina and nucleolus. When viewed from the DNA supercoiling perspective, magnitudes of superhelical densities of the aforementioned three types of domains are in the following order: lamina-associated domains = nucleolus-associated domains > non-membrane-associated domains. Deduction 8 is accordingly presented in the current report for describing correlation of structural characteristics of interphase chromosomes with negative supercoils present in their naked linker DNA regions:

Deduction 8: Centripetal and elastic characteristics of overall structures of interphase chromosomes in eukaryotic cells are determined by negative supercoil-enforced backbone curvatures in naked linker DNA regions of their constituent polynucleosomes, which drives each interphase chromosome to stay in its own chromosome territory in a eukaryotic nucleus (Fig. 2).

4. Supercoiling Views of Chromosomal Structures in Mitotic Phase and Meiotic Phase of Cell Divisions

Chromosomes in mitotic phase and meiotic phase of eukaryotic cells are highly condensed structural entities of nucleic acids and proteins, in which compactions of DNA could be up to ~250-fold higher than those in interphase chromosomes.[87-88] Based on their supercoiling and structural characteristics, these mitotic phase and meiotic phase chromosomal structures can be further classified into six hierarchical ranks as depicted in Table 3 and Fig. 11. Because (1) topological features of double helical structures of DNA (Primary structures as listed in Table 3) were reviewed previously[2,22,41] and (2) supercoiling properties of nucleosomes and polynucleosomes (Secondary and Tertiary structures as listed in Table 3) were discussed in Section 2.1 and Section 3.1 in the current report, focus of our discussions in the current section will be on (1) plectoroids (Quaternary structures), (2) left-handed helical polyplectoroids (Quinary structures), and (3) bivalent chromosomes (Senary structures) respectively.

Table 3. DNA supercoiling views of hierarchical ranks of structural organizations of chromosomes in mitotic phase and meiotic phase.

Entry	Sublevel architectures of mitotic phase and meiotic chromosomes	Hierarchical ranks of architectures in mitotic phase and meiotic chromosomes
1	Double helical DNA	Primary structures
2	Nucleosomes	Secondary structures
3	Polynucleosomes	Tertiary structures
4	Plectoroids (Loop domains)	Quaternary structures
5	Left-handed helical polyplectoroids	Quinary structures
6	Bivalents	Senary structures

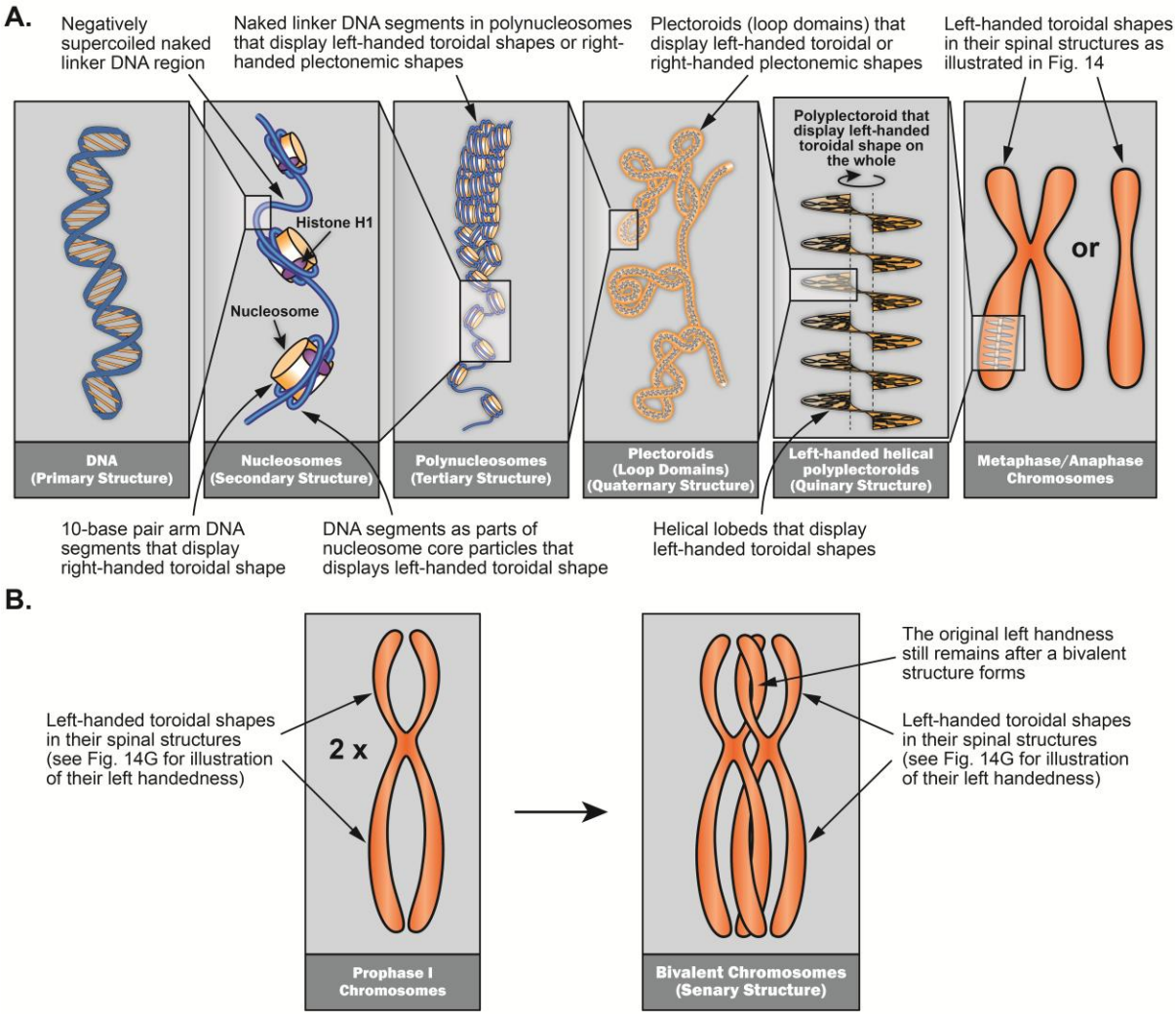


Fig. 11. Supercoiling models of hierarchical architectures of metaphase and anaphase chromosomes in mitosis and meiosis (A), and in meiotic synapsis in Prophase I (B).

4.1 Plectoroids ("Loop domains") as quaternary structures of metaphase chromosomes

The term "loop domain" has been used in the past for describing a spatial organization of metaphase chromosomes that is one hierarchical level higher than 30 nm fibers.[89-90] When viewed from the DNA supercoiling perspective, loop domains are in fact closely correlated with insulated neighborhoods even though these two terms have been used for portraying substructural organizations of chromosomes in metaphase and interphase in cell division cycles separately[53,89-92]. In other words, both loop domains and insulated neighborhoods are assemblies of supercoiling-driven polynucleosomal structures (Fig. 12), whose root regions are clasped by CTCF homodimers and cohesins while structural denseness and superhelical densities in these two types of structures are different. In addition, similar to insulated neighborhoods, loop domains are not simply loop-like structures as they were portrayed in the past.[89-90] From the DNA supercoiling standpoint, they are (1) left-handed toroidal shapes, (2) right-handed plectonemic shapes and/or (3) combination of left-handed toroidal shapes and right-handed plectonemic shapes of polynucleosomes. In order to accurately describe quaternary structures of chromosomes in prophase, metaphase, anaphase and telophase, the

word “plectoroid” (Fig. 11) as a combination of two words, plectoneme and toroid, is suggested in the current report for replacing the term “loop domain”. Deduction 9 is consequently presented in the current report for describing correlation of insulated neighborhoods with plectoroids in eukaryotic cells:

Deduction 9: Insulated neighborhoods and plectoroids (loop domains) are the basic functional units in interphase chromosomes and mitotic/meiotic phase chromosomes respectively, both of which are assemblies of supercoiling-driven polynucleosomes. Insulated neighborhoods of interphase chromosomes will turn into plectoroids (loop domains) of prophase and metaphase chromosomes, and plectoroids (loop domains) in anaphase and telophase chromosomes will become insulated neighborhoods in interphase chromosomes in cell division cycles whereas such conversions are affiliated with superhelical densities change of naked linker DNA segments in their constituent polynucleosomes as well as their association/dissociation of proteins and other molecules.

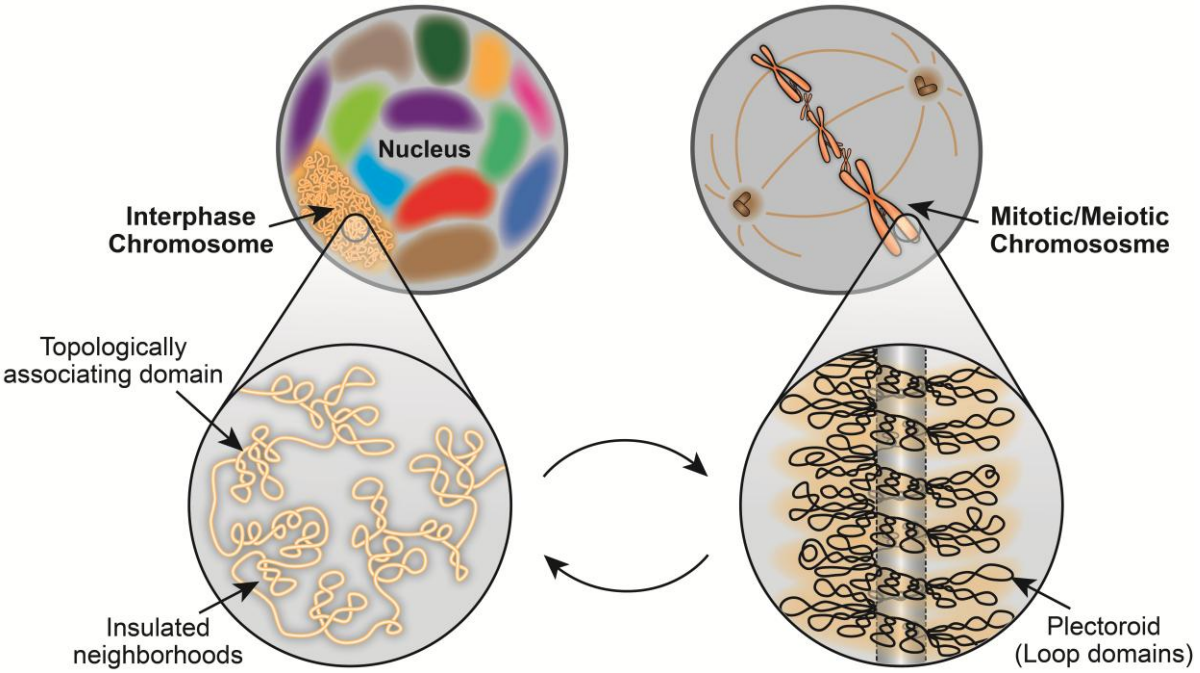


Fig. 12. Illustration of correlations of insulated neighborhoods of interphase chromosomes with plectoroids (loop domains) of mitotic/meiotic phase chromosomes.

4.2 Left-handed helical polyplectoroids as quinary structures of mitotic/meiotic phase chromosomes

The term “left handed helical polyplectoroid” is designed in the current report for describing bulky stacks of supercoiling-driven plectoroids, which is the quinary structure of mitotic/meiotic phase chromosomes. Based on their overall spatial denseness and pairing relationship, these left-handed structural entities can be categorized into (1) loose left-handed helical polyplectoroids, (2) interconnected loose left-handed helical polyplectoroids, (3) compact left-handed helical polyplectoroids, and (4) interconnected compact left-handed helical polyplectoroids respectively.

(a) Loose left-handed helical polyplectoroids and interconnected loose left-handed helical polyplectoroids. Loose left-handed helical polyplectoroids (Fig. 14G) and interconnected loose left-handed helical polyplectoroids (Fig. 14A) are the forms of chromosomes that occur in prophase and telophase in cell division cycles in mitosis and meiosis respectively. Within these chromosomal structures in prophase and telophase (Fig. 14G and Fig. 14A), root regions of polyplectoroids form helical structures while the rest portions of polyplectoroids are oriented upward, downward and straight outward respectively (Fig. 13A). The overall shapes and left handedness of these polyplectoroids are determined by negative supercoils in their naked DNA regions, which are affiliated with (1) equilibrium between their arm-open and arm-close forms of 10-base pair arm DNA segments in the absence of histone H1 (Discovery 3) and (2) histone H1-enforced formation of arm-close forms (Discovery 2) respectively. Because (1) these loose helical polyplectoroids display toroid-like structures on the whole, and (2) sign of supercoils in their naked linker DNA regions is negative (-), these toroidal structures are left-handed (Axiom 1). Deduction 10 is accordingly formulated in the current report for describing correlation of handedness of loose helical polyplectoroids with negative supercoils present in their naked linker DNA regions:

Deduction 10. Prophase chromosomes (Fig. 14A) and telophase chromosomes (Fig. 14G) adopt the structures of loose left-handed helical polyplectoroids and interconnected loose left-handed helical polyplectoroids (Fig. 13A), which are sustained by negative supercoils present in naked linker DNA regions of their constituent polynucleosomes. The negative supercoils in these loose helical polyplectoroids are produced mainly by binding of 10-base pair arm DNA to histone octamers (Discovery 2 and Discovery 3 as illustrated in Fig. 1B and Fig. 1C), which cannot be relaxed by topoisomerases owing to their (1) low magnitudes of superhelical density, (2) structural compactness and (3) dynamic transitions of 10-base pair arm segments between their arm-open and arm-closed forms.

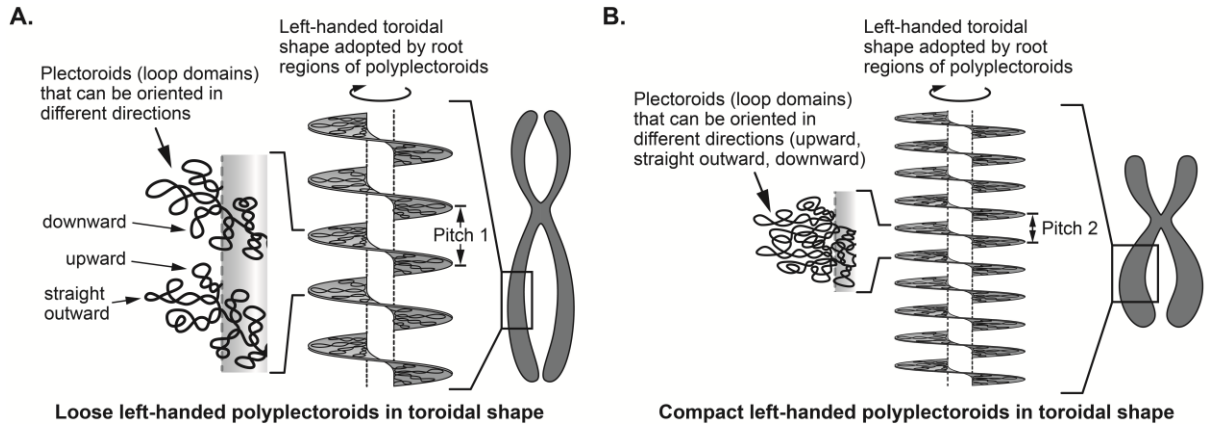


Fig. 13. Illustration of spatial structures of loose left-handed helical polyplectoroids/interconnected loose left-handed helical polyplectoroids (A), and compact left-handed helical polyplectoroids/ interconnected compact helical polyplectoroids (B).

(b) Compact left-handed helical polyplectoroids and interconnected compact left-handed helical polyplectoroids. At the end of prophase of mitosis and prophase I of meiosis, nuclear envelope is broken down,[93] which allows cytoplasmic condensin I to interact with

interconnected loose helical polyplectoroids (prophase chromosomes) (Fig. 13A) to form interconnected compact left-handed plectoroids (metaphase chromosomes) (Fig. 13B and Fig. 13E). Condensin I is a protein complex that generates positive supercoils in the DNA segment that it binds to.[94-95] From the DNA supercoiling viewpoint, negative supercoils must be produced simultaneously in its adjacent DNA regions in order to compensate positive supercoils of DNA segments that are in close contact with condensin I. In addition, because condensin I is a highly abundant chromosome-associated protein in metaphase[96], remarkably high negative superhelical densities should be generated in the naked linker DNA regions of entire chromosomes at this stage. These condensin I-affiliated high negative superhelical densities in linker DNA regions along with (1) pre-existing 10 base pair arm DNA-affiliated negative supercoiling (Discovery 1 and Discovery 2) as well as (2) actions of cohesions, topoisomerase II, histone H1, condensin II and other related proteins[36,97-100] lead to extraordinarily high denseness of interconnected compact left-handed helical polyplectoroids (metaphase chromosomes) and compact left-handed helical polyplectoroids (anaphase chromosomes) (Fig. 13B, Fig. 14B and Fig. 14F). In addition, magnitude of vertical distance between one consecutive helical turn in compact left-handed polyplectoroids (Pitch 1 in Fig. 13 A) is greater than that of loose left-handed polyplectoroids (Pitch 2 in Fig. 13B). Deduction 11 is accordingly presented in the current report for describing correlation of high denseness of compact helical polyplectoroids with negative supercoils in naked linker DNA regions of their constituent polynucleosomes:

Deduction 11. Binding of Condensin I to DNA macromolecules will cause tremendous enhancement of magnitudes of negative superhelical densities in naked linker DNA regions, which cannot be relaxed by topoisomerases owing to (1) high structural compactness of chromosomes and (2) short lengths of naked linker DNA segments at this stage.

Deduction 12. Interconnected compact left-handed helical polyplectoroids (metaphase chromosomes) (Fig. 14B) possess remarkable high negative superhelical densities in their naked linker DNA regions, which (1) are produced (i) by binding of Condensin I to DNA macromolecules (Deduction 11), and (ii) by binding of 10-base pair arm DNA segments to histone octamers (Discovery 2 and Discovery 3 as illustrated in Fig. 1B and Fig. 1C). These high negative superhelical densities in naked linker DNA regions at this stage along with binding actions of cohesions, topoisomerase II, histone H1, condensins and other molecules are accountable for extraordinarily high structural compactness of metaphase chromosomes (Fig. 14B) and anaphase chromosomes (Fig. 14 F).

4.3 Bivalents as senary structures of chromosomes that emerge in prophase I of meiosis

(a) Loose bivalent structures. From the supercoiling standpoint, even though they were often portrayed as long column-like entities in the past[71], the spines of (1) chromosomal structures in telophase and (2) each chromatid within homologous chromosomes in prophase do not display uniform columnar shape. Instead, they display the features of left-handed toroids along their spinal columns (Fig. 14F and Fig. 14 G) because negative supercoils are present in naked linker regions of their constituent polynucleosomes (Axiom 1). Bivalents, on the

other hand, are spatial organizations of two pairs of homologous chromosomes that are formed prior to chromosomal crossover in prophase I of synapses in meiosis.[71,101] The bivalent structures formed at this stage are defined in the current report as loose bivalents (Fig. 14C) in reference to the bivalent structures formed in metaphase I in meiosis, which are named compact bivalents[102] (Fig. 14D). Within a loose bivalent structure, two chromatids from each of the two homologous pairs of chromosomes are held together in the forms of synaptonemal complexes.[103] As a result, each of the two chromatids in the mutually bound portions of loose bivalents remain their left-handed toroidal shapes (Fig. 14C).

(b) Compact bivalent structures (Fig. 14 D)**.** After chromosomal crossover completes and before metaphase I starts, a bivalent structure still displays its left-handed loose form (Fig. 14 C). Once nuclear envelope is disintegrated at the beginning of metaphase I, cytoplasmic condensin I along with other pre-existing molecules (*e.g.* condensin II and cohesin) convert prophase I loose bivalent structures into metaphase I compact bivalents (Fig. 14E). Owing to the presence of negative supercoils in their constituent DNA regions, spinal columns of metaphase I compact bivalent structures still remain their left-handed toroidal shapes even though the overall length of columnar structures of chromatids become shorter at this stage. Deduction 13 is accordingly presented in the current report for describing alignments of two pairs of homologous chromosomes in bivalent structures:

Deduction 13. Within a loose bivalent structure, sister chromatids wind around each other and hold left-handed toroidal shapes along their spinal columns (Fig. 14C) whereas this left handedness still remains when loose bivalent structures are converted into compact bivalent structures (Fig. 14 D and Fig. 14 E) upon broken down of nuclear envelops.

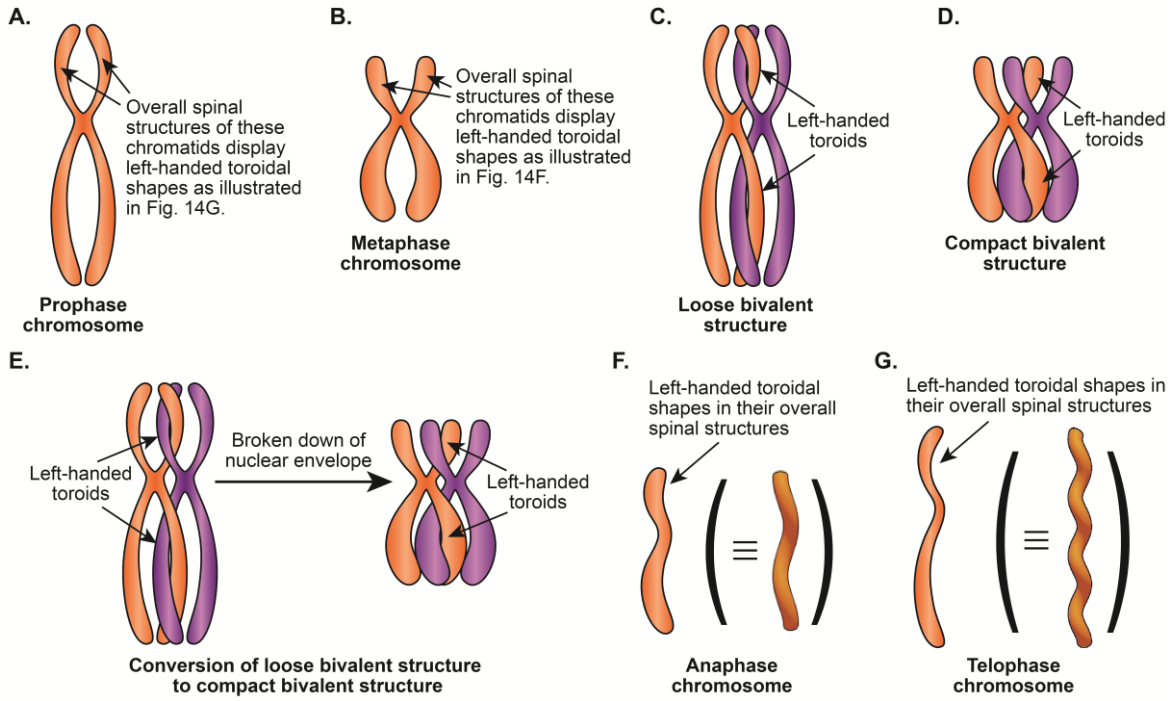


Fig. 14. Illustration of structures of handedness of prophase chromosomes (A), metaphase chromosomes (B), loose bivalent structure (C), compact bivalent structure (D), conversion of

loose bivalent structure to compact bivalent structure (E), anaphase chromosome and its toroidal spine (F), and telophase chromosome and its toroidal spine (G).

(c) Homologous chromosome-matching zones. It has been known that most of plant and animal cells possess two or more pairs of chromosomes[104], such as 23 pairs of chromosomes in human cells[105] and 17 pairs of chromosomes in the cells of sunflowers[106]. Accurate recognition between pairs of homologous chromosomes to form correct bivalent structures is therefore imperative for the subsequent events of chromosomal crossover.[71] In the current report, existence of "homologous chromosome-matching zones" (or "matching zones") on the surfaces of interconnected loose left-handed helical polyplectoroids (prophase I chromosomes) is suggested, which are responsible (1) for correct recognition between homologous chromosomes and (2) for reducing chances of association between two non-homologous chromosomes in prophase I of meiosis. These matching zones are composed of different-dimensioned (1) interacting zones and (2) spacer zones that align along columnar structures of prophase chromosomes in alternating manners (Fig. 15A). Interacting zones are the sectors in columnar structures of chromatids that consist predominantly of active insulated neighborhoods while spacer zones are mainly composed of salient and inactive insulated neighborhoods (Fig. 15A). The physical interactions between spacer zones from homologous chromosomes in loose bivalent structures are insignificant owing to their possessions of salient and inactive insulated neighborhoods. Instead, it is the physical interactions of interacting zones between two chromatids from separate pairs of homologous chromosomes that are mainly responsible for holding loose bivalent structures together. Because homologous chromosomes share the same patterns of alternating interacting zones and spacer zones, they are pairable to form bivalent structures (Fig. 15B). As opposed to homologous chromosomes, non-homologous chromosomes, however, possess (1) different alternating patterns of interacting zones and spacer zones and (2) different DNA sequences in interacting zones in their columnar structures. These differences reduce likelihoods of formation of bivalents between non-homologous chromosomes (Fig. 15C).

Even though homologous chromosome-matching zones exist, repulsion effects of enormous amount of negative charges in genomic DNA prevent pairs of homologous chromosomes from spontaneously approaching each other to form bivalent structures. To overcome these electric repulsions, particular membrane proteins emerge concurrently that will bind to sister chromatids and bring them in a close proximity.[107-110] Once pairs of homologous chromosomes are brought together by these membrane proteins[111-112], they will be able to form bivalents because they possess identical homologous chromosome-matching zones between them. In contrast to homologous chromosomes, pairs of non-homologous chromosomes have diminishing chances to form bivalent structures because they possess non-identical homologous chromosome-matching zones in their structures.

On the molecular scales, on the other hand, interacting zones at the same loci of two chromatids from separate pairs of sister chromatids recognize and interact with each other by following two sequential steps of events: (1) generation of single stranded DNA from duplex DNA, a process that is driven by pre-existing negative supercoils in their naked linker DNA

regions; and (2) formation of duplex DNA by two single stranded DNA from two separate sister chromatids, which is assisted by condensin I as this protein complex is known to be able facilitate the generation of duplex DNA structures from their single stranded precursors [113]. Because the same loci of homologous chromosomes are known to possess identical DNA sequences in general [114], this identicalness in sequence makes it possible to form duplex DNA structures by separate pairs of sister chromatids. Deduction 14 is accordingly presented in the current report for describing roles of homologous chromosome-matching zones in formation of bivalent structures:

Deduction 14. Homologous chromosome-matching zones are present in interconnected left-handed loose helical polyplectoroids, which are accountable for (1) correct formation of loose bivalent structures by two pairs of homologous chromosomes (Fig. 15B). Electric repulsions between pairs of homologous chromosomes during formation of loose bivalents are overcome by actions of membrane-binding proteins and other molecules that characteristically emerge during meiosis.

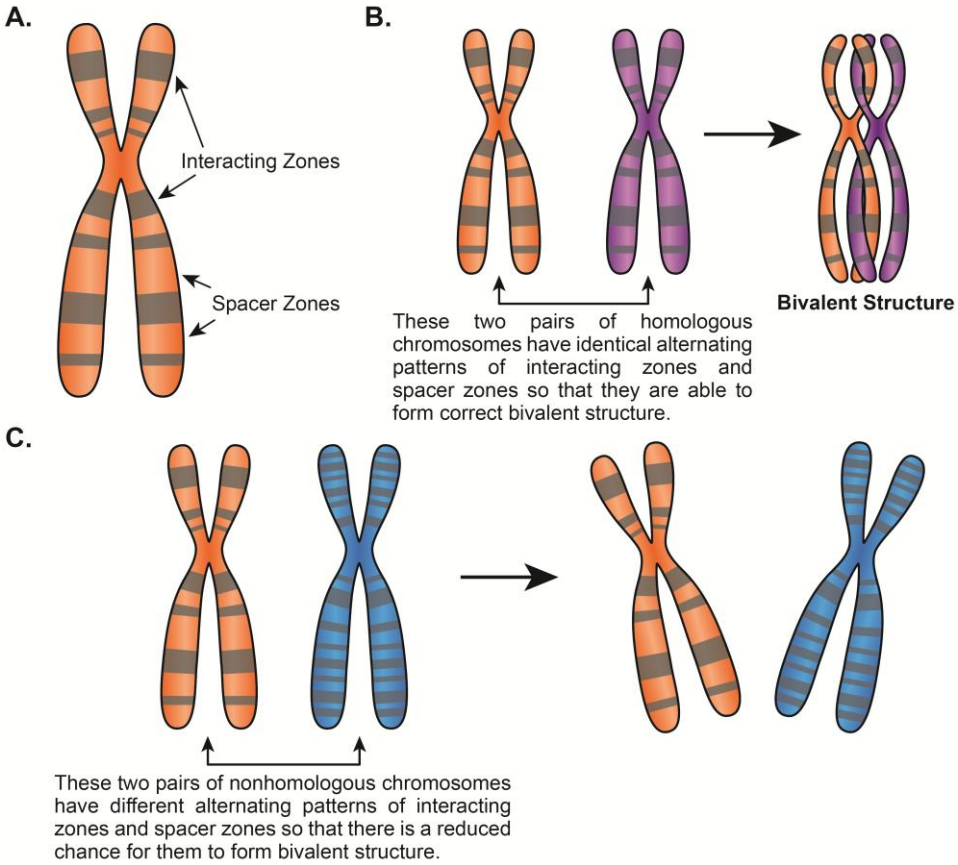


Fig. 15. Illustration of presence of homologous chromosome-matching zones on prophase I chromosomes (interconnected loose left-handed helical polyplectoroids) (A), formation of a correct bivalent structure between two pairs of homologous chromosomes through recognition of identical patterns of matching zones (B), and reduced chances for pair of non-homologous chromosomes to form bivalent structures because they do not possess identical matching zones (C).

4.4 Left-handed toroidal structures of protamine-bound DNA and their higher hierarchical architectures as physical forms of post-metaphase chromosomes in sperm cells

In conjunction with transformation of secondary spermatocytes to haploid spermatids during meiosis II in spermiogenesis, sister chromatids turned into separated chromatids.[114] In the subsequent Golgi phase of spermiogenesis, DNA in these separated chromatids dissociate from histone proteins to form complexes with transition proteins.[115-116] Protamines will emerge then to replace transition proteins to generate DNA-protamine toroids.[115-118] From the supercoiling standard point, protein-unbound DNA regions in protamine toroids of sperm cells should be negatively supercoiled as well. According to Axiom 1, DNA-protamine toroids in sperm cells should display left-handedness because of presence of negative supercoils in their protein-free DNA regions. Deduction 15 is accordingly presented in the current report for describing correlation between shapes, handedness and supercoils in DNA-protamine toroids in sperm cells:

Deduction 15: DNA-protamine toroids in sperm cells (1) are left handed and (2) possess negative supercoils in their protein-unbound DNA regions whereas DNA supercoils in the toroidal structures cannot be resolved by topoisomerase mainly because they possess comparably low superhelical densities. Besides DNA-protamine toroids[115-118], overall spatial organizations of (a) side-by-side stacked protamine toroids[119] and (b) bulky assemblies of side-by-side stacked protamine toroids[119] are determined by negative DNA supercoils that are present in their protein-free DNA regions.

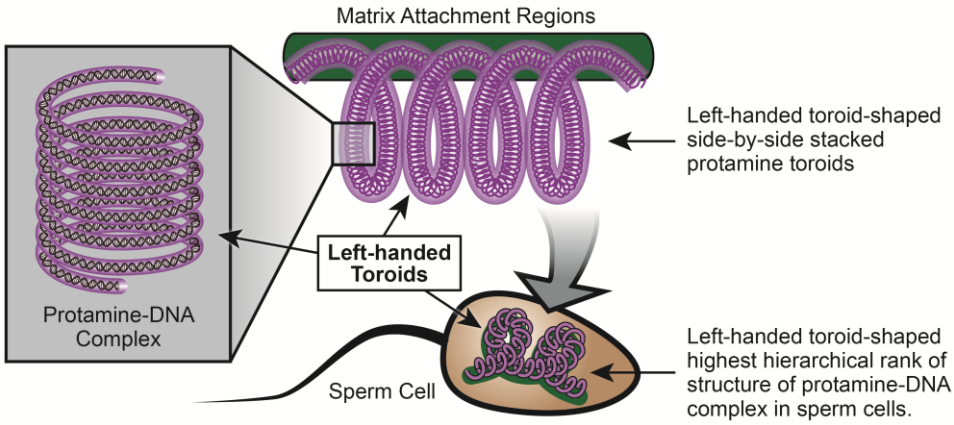


Fig. 16. Supercoiling views of shapes, sign and handedness of protamine-DNA toroids and their higher hierarchical architectures in the sperm cells.

5. Supercoiling View of Roles of Noncoding DNA in Maintaining Structures and Functions of Eukaryotic Chromosomes

Noncoding DNA refers commonly to DNA sequences in an organism that do not encode information of proteins [120-121]. Even though protein translation is not advanced on these nucleic acids, various cellular roles of noncoding DNA have been recognized in the past [122-125], which include (1) their transcriptions into functional noncoding RNA such as ribosomal

RNA, transfer RNA and microRNA[122,126-127], and (2) their functions as telomeres[128-129], centromeres and origins of DNA replication[128,130]. From the DNA supercoiling standpoint, noncoding DNA sequences are essential for maintaining supercoil-driven plectonemic and toroidal structures of polynucleosomes in chromosomes of eukaryotic cells as discussed in the five sections below.

5.1. Noncoding DNA as components of plectonemic and toroidal structures of polynucleosomes for maintaining proper positions of crossover points in insulated neighborhoods.

Crossover points of polynucleosomal backbones occur in insulated neighborhoods, which are the manifestation of non-zero writhe number of supercoiling-affiliated structures (Deduction 3). Once pairable DNA elements (*e.g.* enhancers, silencers, promoters, insulators as well as pairs of CTCF) emerge and clasped at these crossover points, they contribute to regulation of gene expressions in their host insulated neighborhoods (Deduction 4). Deduction 16 is accordingly presented below for describing essentialness of noncoding DNA in maintaining spatial organizations of plectonemic and toroidal structures:

Deduction 16. Presence of noncoding DNA sequences is essential for sustaining the rise of pairable elements (*e.g.* enhancers, silencers, promoters, insulators as well as pairs of CTCF) at crossover points of plectonemic and toroidal structures of polynucleosomes, which are accountable for regulation of gene expressions in their host insulated neighborhoods.

5.2 Vital correlation of transposons with (i) cell types, (ii) genetic diversity, (iii) diverse structural variants in brain cells and (iv) adaptive roles of transposable elements.

It has been known that both inactive transposons and active transposons belong to the category of noncoding DNA sequences.[131-132] Inactive transposons are incapable of jumping from one place to another in genomic DNA in our time due to the loss of their corresponding transposase gene[132], and has been considered to be genetic fossils[133-134]. Unlike inactive transposons, on the other hand, active transposons are capable of rearranging themselves alongside genomic DNA nowadays.[135-136] As discussed in Section of 3.3(d) in the current report, transpositions of these transposons are critically important to eukaryotic cells for their innate functions. Therefore, essentialness of active transposons as noncoding DNA in chromosomes in eukaryotic cells is accordingly presented as follows:

Deduction 17: Because their actions are able to alter three-dimensional structures of insulated neighborhoods and crossover points of polynucleosome backbones, transposons as noncoding DNA are essential (1) for sustaining distinct cell types, (2) for making up genetic diversity in a species' population, (3) for producing diverse structural variants in brain cells, and (4) for enabling organism to adapt gene expressions to environment changes through mechanisms of transposon-induced adaptation.

5.3 Centromeres as noncoding DNA sequences for preventing structural distortion of centromeric regions in sister chromatid pairs.

When a cell divides, each of its two daughter cells must receive a full and intact copy of genetic material. If any unequal division of genetic material between two daughter cells occurs, defective cells will be resulted.[137-138] Centromere, on the other hand, is a type of noncoding DNA that makes up of up to 5% of entire eukaryotic genomic DNA sequences[139-141], and is believed to play vital roles in cell division cycles[141-143]. From the DNA supercoiling and structural standpoints, there are three characteristics in the spatial organizations of centromere regions:

***(1) High stability of centromeric nucleosomal structures*.** DNA sequences in the portions of regional centromeres are made of repetitive alpha satellite DNA, which is composed of 171-base paired particular DNA segments.[130,144-145] It has been known that nucleosome structures formed by these alpha satellite DNA repeats resemble those generated between 601 elements and histone octamers[146], which signifies that the nucleosomal structures in the eukaryotic centromeric regions are enormously stable.

***(2) High compactness of centromeric 30 nm fibers*.** Length of a linker DNA is often defined as the number of base pairs of a DNA segment that resides between two adjacent nucleosome core particles[147], whose average length in eukaryotic cells is ~55 base pairs[148-149]. Centromeric polynucleosomes, on the other hand, possess ~25 base pairs (171 base pairs – 146 base pairs = 25 base pairs) in their linker DNA, which is much shorter than overall average length of linker DNA in eukaryotic chromosomes. In other words, the 30 nm fibers in centromeric regions belong to the category of extraordinarily compact 30 nm fibers, whose compactness is much higher than those in non-centromeric regions in eukaryotic chromosomes.

(3) High negative superhelical density in naked linker DNA regions in centromeric 30 nm fibers. It is known that eukaryotic centromeres can be classified into (1) point centromeres and (2) regional centromeres respectively.[150] Certain previous studies unveiled that DNA segments within nucleosomes formed by point centromeres were positively supercoiled, which was affiliated with the presence of centromeric histone H3 in their constituent histone octamers.[151-153] From the DNA supercoiling viewpoint, in affiliation with these positive supercoils of DNA in point centromere nucleosomes, extra negative supercoils must be generated simultaneously in naked DNA linker segments in centromere regions. Besides point centromeres, if regional centromeres held positive supercoils in their centromeric nucleosomes as well as point centromeres do[151-153], higher negative superhelical densities would in theory be accumulated in naked linker DNA regions across the entire centromere regions in regional centromere-holding cells. These higher negative superhelical densities will in principle greatly enhance structural firmness of left-handed helical polyplectoroids in centromeric regions in sister chromatid pairs.

In view of the aforementioned structural characteristics of regional centromeres, Deduction 18 is presented in the current report for describing the essentialness of noncoding DNA as components of centromeres during cell division cycles:

Deduction 18. The major roles of noncoding DNA in centromeric regions of sister

chromatid pairs are (1) to form highly stable structures of centromeric nucleosomes, (2) to generate high structural compactness of centromeric 30 nm fibers, and (3) to produce high negative superhelical density in naked linker DNA regions of centromeric poly-nucleosomes, which prevent sister chromatid pairs from structural distortions caused by pulling force-affiliated physical tensions at kinetochore-microtubule interface during separations of the pairs in early anaphase of cell division cycles.

5.4 Noncoding DNA sequences as components of spacer zones in prophase I chromosomes for correct formation of bivalent structures between homologous chromosomes.

As discussed in Section 4.3(C) in the current report, homologous chromosome matching zones are associated with columnar structures of prophase and metaphase chromosomes. Within the homologous chromosome matching zones, spacer zones are composed of noncoding DNA whose quantities and dimensions are critically important for achieving correct recognition between two pairs of homologous chromosomes (Deduction 14). Deduction 19 are therefore presented below for describing essentialness of noncoding DNA as parts of homologous chromosome matching zones:

Deduction 19. Presence of noncoding DNA sequences as components of spacer zones in chromosomes in prophase I are imperative for specific recognitions between two pairs of homologous chromosomes to form correct bivalent structures in the pachynema stage of meiosis during cell division cycles.

5.5. Noncoding DNA sequences as constituents of lamina-associated domains and nucleolus-associated domains for maintaining cellular functions of interphase chromosomes

The nucleic acid components in both (1) lamina-associated domains[84] and (2) nucleolus-associated domains[85-86] consist of inactive and silent topologically associating domains, which are in the category of noncoding DNA. Lamina-associated domains are particular genomic regions that physically interact with nuclear lamina and constitute ~40% of the human genome.[154-155] These domains are known to be vital for directing spatial folding of chromosomes in the interphase nucleuses and for regulating DNA replication and gene expressions.[84] Different from lamina-associated domains, on the other hand, nucleolus-associated domains bind to nucleolus instead.[85] These domains make up ~4% of the genome[156], and are known to be affiliated with nucleolus functions.[86] Necessity of noncoding DNA as components of lamina-associated domains and nucleolus-associated domains is therefore summarized as follows:

Deduction 20. Noncoding DNA sequences are constituents of lamina-associated domains and nucleolus-associated domains, which are essential for sustaining structures and cellular functions of interphase chromosomes on the whole in eukaryotic cells.

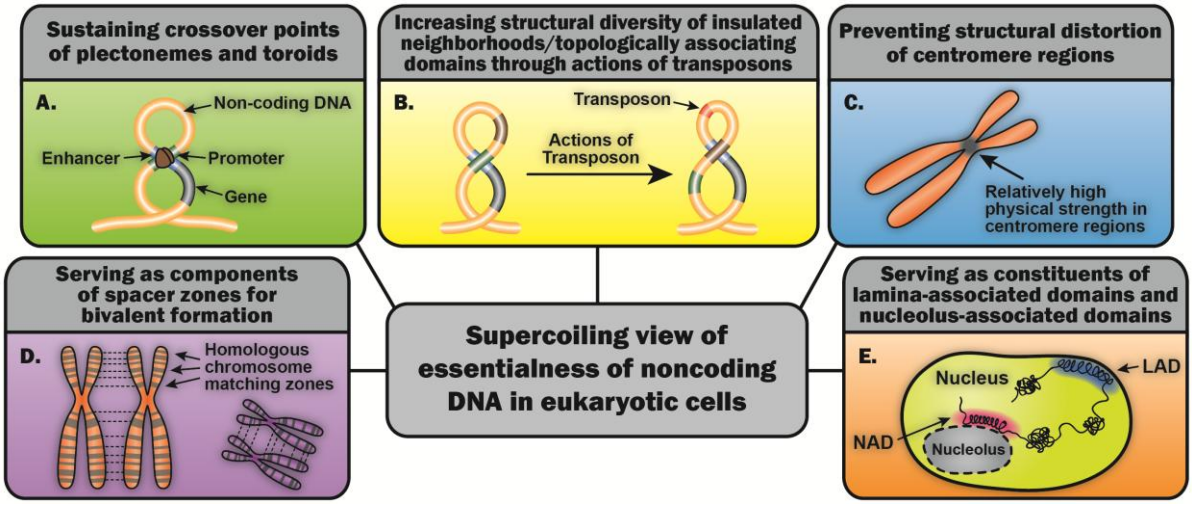


Fig. 17. Illustration of essentialness of noncoding DNA sequences (A) as structural components of plectonemes/toroids of polynucleosomes, (B) in the forms of transposons to alternate shapes, sizes and crossover points of insulated neighborhoods, (C) as structural components to prevent distortion of centromeric regions, (D) as constituents of spacer zones for specific recognition between pairs of homogenous chromosomes, and (E) as constituents of LADs and NADs for maintaining cellular functions of interphase chromosomes.

6. Conclusion

With the aim of acquiring new knowledge of mystifying spatial features of eukaryotic chromosomes, a series of experimental examinations on sublevel architectures of chromosomes have been carried out by our group in the past two years.[29-32] From these studies, four types of previously unknown supercoiling properties of linker DNA, histone H1, nucleosomal structures and polynucleosomes were discovered.[29-32] Because the only conformation-alterable elements in structures of eukaryotic chromosomes are their naked linker DNA, it is our conviction that these protein-free DNA segments play decisive roles in determining spatial organizations of chromosomes. Consequently, on the foundation of our previous four discoveries[29-32] and commonly accepted axioms[1-2,33-34], eukaryotic chromosomal architectures are analyzed from the viewpoints of shapes, signs and handedness of DNA supercoils in the current report. Our intention in presenting the analyses and deductions is to inspire future in-depth endeavors to systematically elucidate sophisticated and subtle molecular architectures of eukaryotic chromosomes from both theoretical and experimental perspectives.

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