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 physical 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-accepted six axioms about signs, shapes and handedness of DNA supercoils, and (3) our three new prepositions about correlations between DNA supercoils and chromosomal structures, we formulate new theories and models of eukaryotic chromosomal structures in the current report. It is our conclusion that all levels of chromosomal structures in eukaryotic cells are governed mainly by negative supercoils that are present in their naked linker DNA regions.

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 owned DNA reverse gyrase.[10, 11] These[6-11] and immense other evidence [12-18] have demonstrated that DNA supercoils play vital roles in cellular functions in each cell of every single organism 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, 2, 19, 20] This topologically closed DNA could either appear as a covalently closed circular entity or 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 had 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 four 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 discoveries[29-32], previously well-established principles about

supercoiling properties of DNA[1, 2, 33, 34] and our newly presented propositions, we (i) formulate a new supercoiling theory (Conclusion 1 to Conclusion 21) and (ii) originate supercoil-driven three-dimensional structural models of interphase and mitotic/meiotic chromosomes (Fig. 2 and Fig. 13). Our new supercoiling theory and models (1) clarify the physical forces that drive chromosomes to adopt their highly ordered hierarchal architectures and (2) justify why and how chromosomes and their sublevel architectures are capable of accomplishing their innate biological actions inside eukaryotic cells in highly ordered and well organized manners.

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

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) toroid-shaped positive supercoils, which is the cause of generation of negative supercoils in their adjacent naked linker DNA regions.[30]

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]

Discovery 4. From the quantitative point of view, binding of ~11.5 histone H1 proteins leads to changes of linking number by -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]

To briefly sum up, we discovered in the past four years that naked linker DNA segments in polynucleosomes are negatively supercoiled (Fig. 1). In the presence of histone H1, linking number change (ΔLk)[1, 2] in naked linker DNA segments between two neighboring nucleosomes is about -0.09 (Fig. 1A) while in the absence of histone H1, linking number change (ΔLk) in naked linker DNA segments between two neighboring nucleosome core particles is between 0 to -0.09 (Fig. 1B). The negative supercoils in naked linker DNA segments of polynucleosomes are produced by right-handed toroidal shapes adopted by 10-base pair arm DNA segments when they bind to surfaces of histone octamers (Fig. 1C).

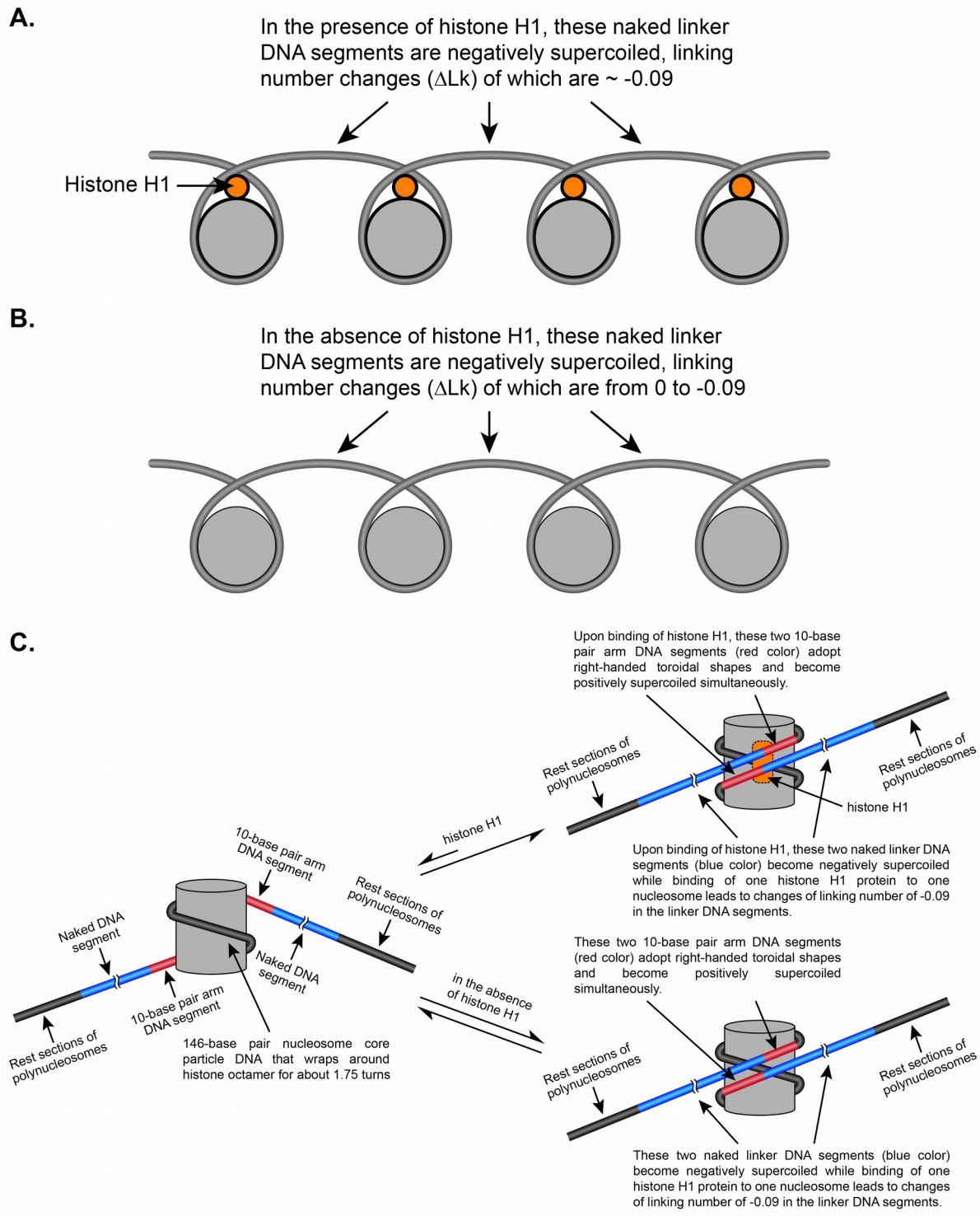

Fig. 1. Illustrative summaries of our four recent discoveries. [29-32] (A) In the presence of histone H1, negative supercoils exist in naked linker DNA segments of polynucleosomes, linking number change (ΔLk) of which is ca. -0.09; (B) in the absence of histone H1, negative supercoils exist in naked linker DNA segments of polynucleosomes as well, linking number

change (ΔLk) of which is between 0 and -0.09; and (C) alignment of 10-base pair arm DNA segments along surfaces of histone octamers in right-handed toroidal shapes causes generation of negative supercoils in naked linker DNA segments of polynucleosomes.

2.2 Six 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.[33, 35, 36] Commonly accepted rules about correlations among the aforementioned shapes, signs, and handedness of DNA supercoils can be summarized into six 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;

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;

Axiom 5: In a physically steady environment, linking number in a topologically closed DNA structure remains the same if there is absence of chemical alterations in its structure. In other words, in a physically steady environment under which no chemical reaction takes place, once negative linking number is introduced into a topologically closed DNA structure, the same magnitude of positive linking number must be produced in the DNA structure simultaneously, and vice versa; and

Axiom 6. In a topologically closed DNA structure (*e.g.* plasmid DNA), magnitudes of superhelical density are correlated with degrees of backbone curvatures of DNA (*e.g.* increase of superhelical density in plasmid DNA result in increase of backbone curvatures of the DNA).

2.3 Our three new propositions

Proposition 1. Genomic DNA can be divided into two structural categories: (1) naked linker DNA (DNA segments that are not bound by any other type of biomolecules) and (2) biomolecule-bound DNA while (1) naked linker DNA segments are the only conformation-alterable elements in chromosomal structures and (2) ratios of naked linker DNA segments to biomolecule-bound DNA as well as superhelical densities in naked linker DNA segments alter dynamically in chromosomal structures in eukaryotic cells;

Proposition 2. Supercoils that are present in naked linker DNA segments drive chromosomal structures at all hierarchical levels (1) to behave as supercoiled structural entities and (2) to display all supercoiling characteristics that protein-free plasmid DNA possesses, which are describable by Călugăreanu–White–Fuller Theorem and the aforementioned six axioms (Section 2.1 and Table 1); and

Proposition 3. When viewed on the scale of polynucleosome backbones, there are only two types of structural components in chromosomes: (1) jointer polynucleosomes and (2) insulated neighborhoods/plectoroids.

Table 1. Illustration of axioms 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 lines in these drawings represent backbones of duplex DNA.

3. Our New Supercoiling Theory and Model of Chromosomal Structures in Interphases of Eukaryotic Cells

On the basis of our recent four discoveries[29-32] and our analyses from the DNA supercoiling viewpoints, we classify interphase chromosomes into five hierarchical ranks in the current report, as depicted in Table 2 and Fig. 2.

Table 2. Our new supercoiling view of hierarchical ranks of interphase chromosomal structures.

Entry	Different-level architectures of interphase chromosomes	Their hierarchical ranks
1.	DNA and DNA-interacting biomolecules	Primary structures
2.	Nucleosomes	Secondary structures
3.	Polynucleosomes	Tertiary structures
4.	Insulated neighbourhoods and jointer polynucleosomes	Quaternary structures
5.	Poly(insulated neighbourhoods/jointer polynucleosomes) (Overall structures of interphase chromosomes)	Quinary structures

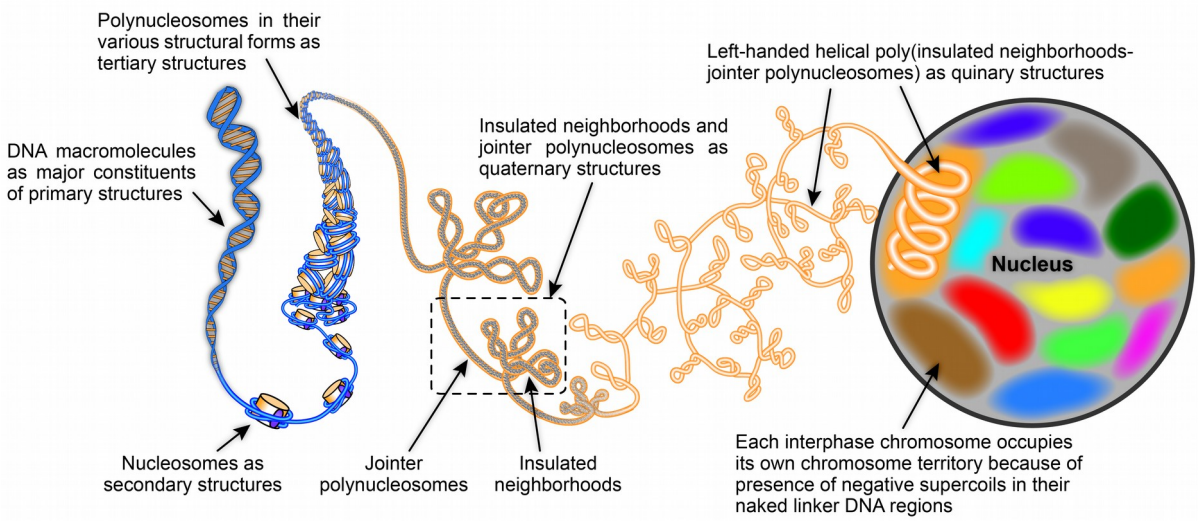

Fig. 2. Our new supercoil-driven models of chromosomal structures in interphase of eukaryotic cells.

3.1. DNA and DNA-interacting molecules as primary structures of interphase chromosomes

In view of the fact that supercoils are uniquely owned by DNA macromolecules, we define DNA macromolecules along with their interacting histone proteins, non-histone proteins (*e.g.* cohesions, condensins and topoisomerases) and other types of biomolecules (*e.g.* RNA) as the constituents of primary structures of interphase chromosomes. From the supercoiling standpoint, when DNA macromolecules exist in eukaryotic cells in the forms of double helical structures, they could adopt the forms of (1) relaxed structures, (2) negative supercoils, and (3) positive supercoils respectively. Examples of these three forms of double helices of DNA are (1) structures of newly formed leading and lagging strands on their templates during DNA replication (relaxed forms), (2) structures generated in front of transcription bubbles (positive supercoils) as well as (3) those left behind transcription bubbles (negative supercoils). When negative or positive supercoils are introduced to topologically closed double helical structures of DNA[1, 2], these DNA macromolecules could display either toroidal shapes or plectonemic shapes[1, 2], as illustrated in the last column in Table 1. Besides their existence in duplex forms, DNA macromolecules can emerge in their single-stranded forms as well in eukaryotic cells. Examples of such single-stranded DNA include those generated during transcription, replication, recombination and DNA repairs.[1, 2] In addition, when supercoiled duplex DNA possesses (i) excessively higher superhelical density and (ii) high adenine/thymine contents, their single-stranded forms could arise as well, a process that releases constraints in supercoiled duplex DNA backbones.[2, 37, 38]

3.2. Nucleosomes as secondary structures of chromosomes

Conventionally, DNA macromolecules within the structures of nucleosomes are classified into two categories: (1) nucleosome core particle DNA[39] and (2) linker DNA[40] while nucleosomes are often taken as the structural combinations of (1) nucleosome core particles and (2) linker DNA[41, 42]. On the grounds of our analyses from the supercoiling and structural viewpoints, we categorize DNA segments within the assembly of nucleosomes into three structural types in the current report: (1) nucleosome core particle DNA, (2) 10-base pair arm DNA segments, (3) naked linker DNA (Fig. 1C). This categorization is made based on the fact that these three types of DNA segments display different supercoiling properties as pictorially illustrated in Fig. 1C.[29-32] In addition, we define nucleosomes as the secondary structures of interphase chromosomes, which are the structural combinations of (1) nucleosome core particles, (2) 10-base pair arm DNA segments, (3) naked linker DNA (Fig. 1C).

3.3 Polynucleosomes as tertiary structures of interphase chromosomes

3.3.1 Polynucleosomes are constituted by mixtures of diverse types of structural entities. In the current report, we define polynucleosomes as long linear structures of massive alternating repeating units of (1) nucleosome core particles, (2) 10-base pair arm DNA segments and (3) naked linker DNA, which are either entirely bound, or partially bound or unbound by histone H1. On the basis of our new analyses, we infer that polynucleosomes in interphase chromosomes are composed of mixtures of diverse types of structural entities (*e.g.* Type 1 to Type 8 as listed in Table 3) while conventionally defined 30-nm chromatin fibers[43-45] should be taken as only one particular type of miscellaneous structures of polynucleosomes (Type 1 in Table 2). We further reason that this structural diversity of polynucleosomes is attributed to the fact that (1) non-uniform lengths of linker DNA segments occur in polynucleosomes of eukaryotic chromosomes[46], (2) not all histone H1 proteins bind to nucleosome core particles in polynucleosomes at a given instant because equilibrium exist between free and bound histone H1 proteins[47] and (3) in some circumstances, insufficient amount of histone H1 proteins occur in the surroundings of polynucleosomes in eukaryotic cells' nuclei[48]. On the basis of our new discoveries (Discoveries 1 to 4) as well as Axioms 1 and 2, we draw the following conclusion for describing supercoiling characteristics of polynucleosomal structures:

Conclusion 1: (1) Polynucleosomes are the tertiary structures of interphase chromosomes, which are constituted by diverse structural types (*e.g.* Type 1 to Type 8 as listed in Table 3), (2) overall shapes and handedness of polynucleosomal segments in interphase chromosomes are largely determined by negative supercoils present in their naked linker DNA regions, and (3) Type 1, Type 2, Type 4 and Type 5 of polynucleosomal segments display left-handed toroidal shapes on the whole because of their possession of relatively short lengths of linker DNA segments while Type 3, Type 6, Type 7 and Type 8 of polynucleosomal segments are capable of displaying either left-handed toroidal shapes or right-handed plectonemic shapes because of their possession of relatively long lengths of linker DNA segments.

Table 3. Our new classifications and supercoiling view of polynucleosomes as tertiary structures of interphase chromosomes

Categories of polynucleosome segments	**Lengths of linker DNA in polynucleosome segments**	**Nucleosome core particles in polynucleosome segments are entirely bound by histone H1**	**Nucleosome core particles in polynucleosome segments are partly bound by histone H1 or unbound by histone H1 at all**	**Handedness and shapes of polynucleosome segments**
Type 1 (30-nm	< 30 base pairs	yes	---	left-handed toroid

chromatin fibers)				
Type 2	≥ 30 bp and ≤ 70 bp	yes	---	left-handed toroid
Type 3	> 70 bp	yes	---	left-handed toroid/right-handed plectoneme
Type 4	< 30 base pairs	---	yes	left-handed toroid
Type 5	≥ 30 bp and ≤ 70 bp	---	yes	left-handed toroid
Type 6	> 70 bp	---	yes	left-handed toroid/right-handed plectoneme
Type 7	mixed lengths	yes	---	left-handed toroid/right-handed plectoneme
Type 8	mixed lengths	---	yes	left-handed toroid/right-handed plectoneme

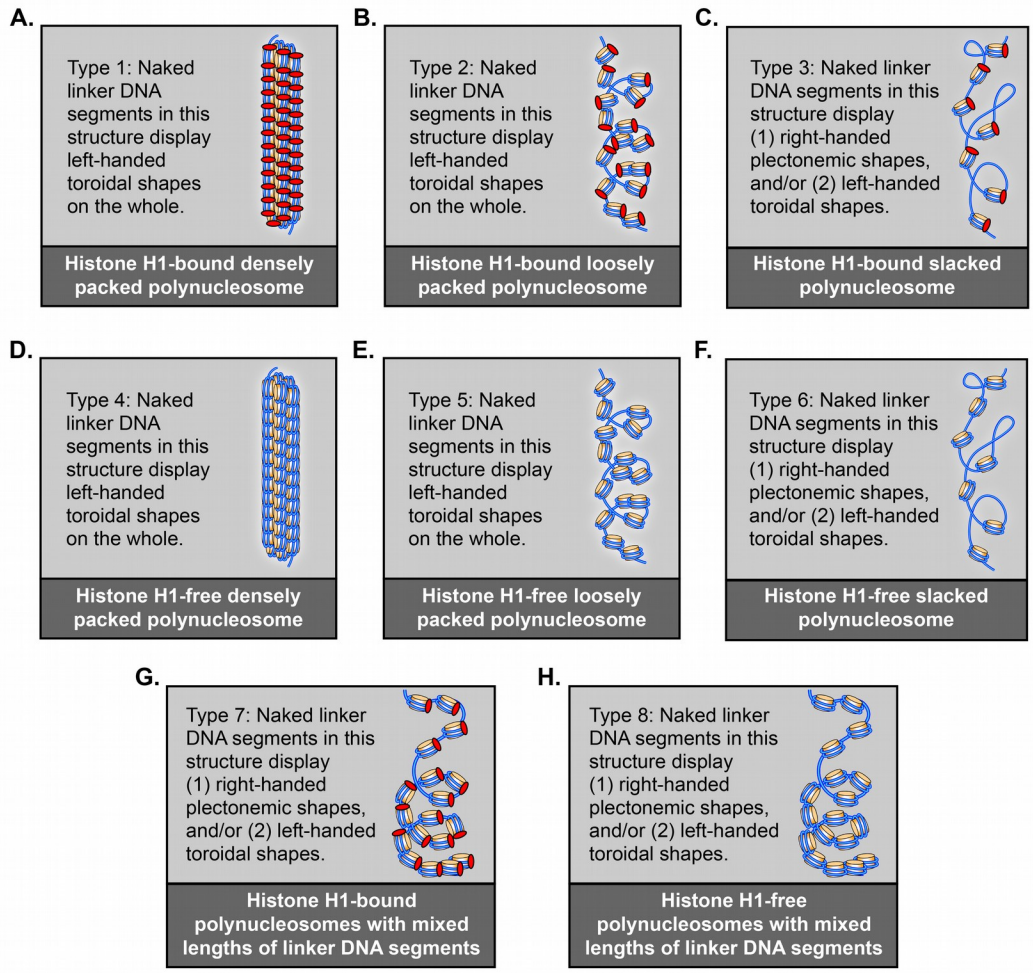

Fig. 3. Our new supercoil-driven models of (A) Type 1, (B) Type 2, (C) Type 3, (D) Type 4, (E) Type 5, (F) Type 6, (G) Type 7 and (H) Type 8 of polynucleosomal structures.

3.3.2. 30-nm chromatin fiber is only one of the eight types of polynucleosomal structures and should displays supercoiling characteristics as other types of polynucleosomal structures do. In the preceding section (Section 3.3.1), we infer that 30-nm chromatin fiber is only one of the eight structural forms that supercoil-driven polynucleosomes are able to adopt. This particular form of polynucleosomes have been extensively studied in the past[43-45], from which three exquisite models were suggested for describing packaging patterns of nucleosome core particles in the fabric structures, namely (1) Rhodes one start model[43], (2) Richmond two start model[44] and (3) Li and Zhu two start tetra-nucleosomes model[45]. Because naked linker DNA segments in polynucleosomes are negatively supercoiled (Discoveries 1 to 4 and Fig. 1), we infer in the current report that naked linker DNA regions in the aforementioned three models[43-45] must display left-handed toroidal shapes. In order to verify this left handedness issue, we singled out naked linker DNA segments from each of the aforementioned three models and replaced them in three-dimensional spaces separately (Figs. 4A, 4B and 4C). We accordingly uncover newly that naked linker DNA segments in all of

these three models display indeed left-handed toroidal shapes on the whole (Fig. 4), as we inferred. In other words, even though negative DNA supercoils have not been recognized in Rhodes one start model, Richmond two start model and Li and Zhu two start tetra-nucleosomes model since they were initially proposed[43-45], our four recent discoveries validate that negative supercoils must (i) be present in naked linker DNA regions of 30-nm chromatin fibers and (ii) be the driving forces that compel naked linker DNA segments as well as nucleosome core particles in 30-nm chromatin fibers to adopt left-handed toroidal shapes on the whole (Fig. 4).

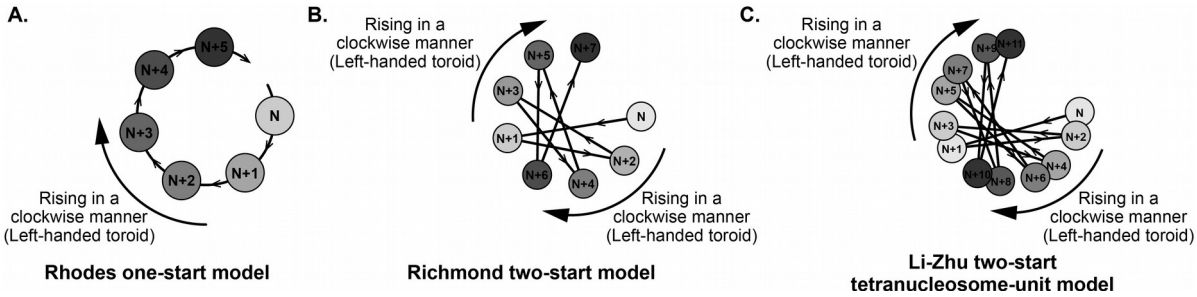

Fig. 4. Our newly identified toroidal shapes and left handedness of naked linker DNA segments from (A) Rhodes one-start model[43], (B) Richmond two-start model[44], and (C) Li-Zhu two start tetranucleosome-unit model[45].

3.4 Insulated neighborhoods and jointer polynucleosomes as quaternary structures of interphase chromosomes

In the current report, we introduce the term "jointer polynucleosome" for the first time to describe structural components that reside between two insulated neighborhoods and infer that interphase chromosomes are made of repeating units of only two types of constituents: (1) insulated neighborhoods and (2) jointer polynucleosomes. Our new analyses of insulated neighborhoods and jointer polynucleosomes from the DNA supercoiling perspective are presented in the following twelve sections.

3.4.1 CTCF-clasped root regions of insulated neighborhoods prevent mutual transmission of negative supercoils between insulated neighborhoods and jointer polynucleosomes. Insulated neighborhoods are conventionally defined as spatial organizations of polynucleosomal segments in interphase chromosomes, two ends of which are bound together by CTCF and co-bound by cohesins. [49, 50] 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[51, 52]. For the purpose of simplifying our further discussions, we now define "roots" in the current report as the regions in insulated neighborhoods where two CTCF-binding DNA sequences[49, 50] are bound by CTCF proteins and cohesins. When viewed from the supercoiling perspective, once root regions are clasped, (1) sizes of insulated neighborhoods are fixed and

(2) DNA supercoils within insulated neighborhoods cannot be leaked out any longer. We therefore infer in the current report that (1) each insulated neighborhood is a factual "insulated" supercoiling domain, and (2) there is no mutual transmission of DNA supercoils between insulated neighborhoods and jointer polynucleosomes.

3.4.2 Polynucleosome backbones within structures of insulated neighborhoods display left-handed plectonemic shapes and/or right-handed toroidal shapes. Negatively supercoiled protein-free plasmid DNA intrinsically exhibits either left-handed toroidal or right-handed plectonemic shapes (Table 1) in its structure.[53, 54] In view of the fact that negative supercoils are present in naked linker DNA regions of polynucleosomes (Discoveries 1 to 4), we deduce in the current report that polynucleosome backbones within structures of insulated neighborhoods display left-handed plectonemic and/or right-handed toroidal shapes (Fig. 5B) as negatively supercoiled protein-free plasmid DNA[53, 54] does. In other words, even though shapes of polynucleosome backbones within structures of insulated neighborhoods have frequently been observed as multiple irregular loops under microscopic examinations[55-57] (Fig. 5A), we predict that left-handed toroidal/right-handed plectonimic shapes of polynucleosome backbones in insulated neighborhoods (Fig. 5B) will be experimentally verifiable in the near future through using advanced microscopic techniques.

3.4.3 Crossover points of polynucleosome backbones are affiliated at all times with insulated neighborhoods. One of the distinctive characteristics of supercoil-driven plectonemic and toroidal shapes of protein-free plasmid DNA is the existence of crossover points within their backbone structures[53, 54], which reflects non-zero writhe number of supercoiled DNA as defined in Călugăreanu–White–Fuller Theorem[22]. Because naked linker DNA segments of polynucleosomes are negatively supercoiled, we infer in the current report that crossover points of polynucleosome backbones are intrinsically affiliated with insulated neighborhoods (Fig. 5B) as it happens to supercoiled protein-free plasmid DNA[53, 54].

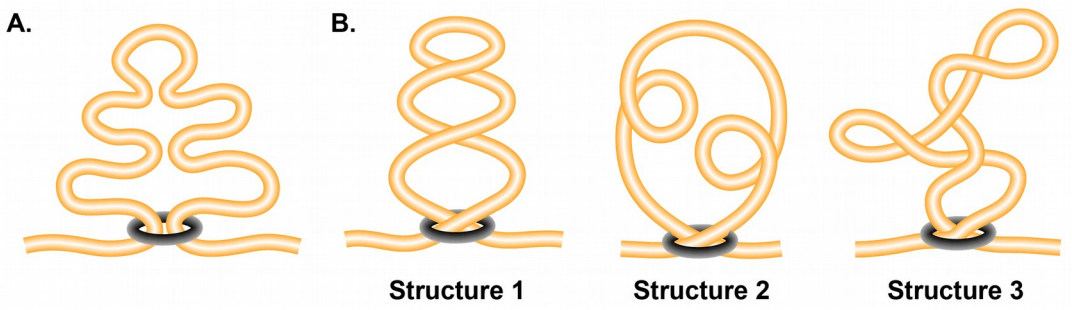

Fig. 5. (A) Conventional models of structures of insulated neighborhoods[55-57], in which supercoils and supercoil-affiliated crossover points of polynucleosome backbones are not present. (B) Our new models of supercoil-driven insulated neighborhoods, which are

composed of crossover point-affiliated right-handed plectonemic shapes (Structure 1), left-handed toroidal shapes (Structure 2), and combination of right-handed plectonemic shapes and left-handed toroidal shapes (Structure 3).

3.4.4. Co-emergence of enhancers and promoters at crossover points of polynucleosome backbones within insulated neighborhoods make gene expression permissible. Existences of spatial proximities between two remote pairs of DNA segments in insulated neighborhoods have been well documented nowadays, examples of which are two distal pairs of enhancers and promoters[58] and two distal pairs of silencers and promoters[58]. Since these distal pairs are often separated by up to a million base pairs along their linear polynucleosomal backbones[59, 60], driving forces that bring these two far-off DNA segments precisely into spatial closeness have been in debate up until now[61]. In view of the fact that two DNA segments at crossover points of supercoil-driven structures are in proximity in space, we draw the following conclusion for describing correlation of crossover points of polynucleosome backbones with spatial closeness between two distal DNA segments along polynucleosome backbones:

Conclusion 2: Crossover points of polynucleosome backbones are intrinsically affiliated within all types of insulated neighborhoods in interphase chromosomes, which are manifestations of negative writhe numbers of supercoiled polynucleosomal backbones in the insulated neighborhoods. It is the crossover points of supercoil-driven polynucleosome backbones that sustain proximities between pairs of two distal DNA segments within insulated neighborhoods, which include (1) pairs of promoters and enhancers[58], (2) pairs of promoters and silencers[58], (3) pairs of insulators and insulators[58] and (4) pairs of two CTCF-binding DNA sequences[49].

3.4.5 Active, inactive and silent insulated neighborhoods are three substructural types of insulated neighborhoods. Based on our analyses of their gene expression capacities, we classify insulated neighborhoods in the current report 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 in the current report. According to our inference, active insulated neighborhoods (Fig. 6A) contain mainly (1) Type 3, Type 6, Type 7 and Type 8 polynucleosomes (Table. 3) while inactive insulated neighborhoods (Fig. 6B) mainly contain Type 2 and Type 5 polynucleosomes (Table. 3). Different from active insulated neighborhoods and inactive insulated neighborhoods, silent insulated neighborhoods (Fig. 6C), however, consist of mostly compact polynucleosomes such as Type 1 and Type 4 polynucleosomes (Table. 3). In addition, based on our analyses from the supercoiling standpoint, we deduce that 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.

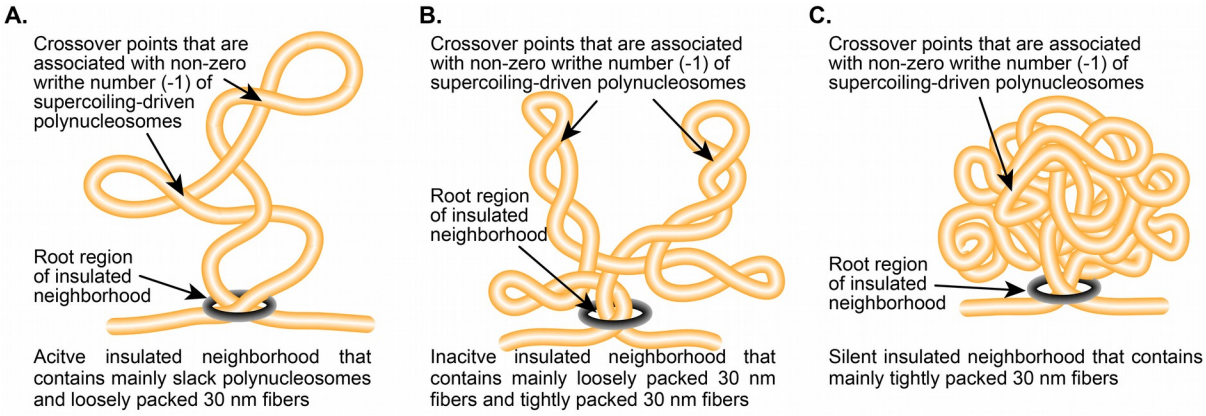

Fig. 6. Our new structural models of (A) active insulated neighborhoods, (B) inactive insulated neighborhoods, and (C) silent insulated neighborhoods, which possess different magnitudes of superhelical densities in their naked linker DNA segments.

3.4.6 Insulated neighborhoods are stable conformers of local polynucleosome backbones that are clasped by CTCF proteins and cohesions at crossover points. In comparison with relaxed forms of plasmid DNA, supercoiled plasmid DNA is known to possess relatively high energy in its overall structure as well as high tensions and constraints in its backbones.[1, 2] These high tensions and constraints in DNA backbones will often make supercoiled plasmid DNA capable of adopting various conformational forms known as topological isomers.[62] In view of the fact that naked linker segments in polynucleosomes are negatively supercoiled (Discoveries 1 to 4), we infer in the current report that local polynucleosome backbones are able to adopt various conformations as protein-free plasmid DNA does[53, 54]. For the convenience of our further discussion, we define "conformations of polynucleosomes" as any possible spatial organizations that polynucleosomal backbones may be able to adopt in an insulated neighborhood. This definition of conformation of polynucleosome backbones is analogous to those of conformations of organic molecules that are resulted from rotations of sigma bonds. In addition, "stable conformers" or "conformers" are defined in the current report as any spatial conformations of polynucleosomal backbones that correspond to local minimal potential energy. In view of the facts that polynucleosome backbones are negatively supercoiled structural entities, we infer the following conclusion for describing the sequential steps for forming insulated neighborhoods from stable conformers of local polynucleosome backbones:

Conclusion 3. At steady states, local polynucleosome backbones of interphase eukaryotic chromosomes exist in the forms of stable conformers (Structure 1 in Fig. 7). If two CTCF DNA sequences[49] emerge at crossover points of polynucleosome backbones in stable

conformers of polynucleosome backbones, CTCF proteins will clasp the two CTCF DNA sequences, which will turn the local stable conformers of polynucleosomes into insulated neighborhoods (Structure 2 in Fig. 7).

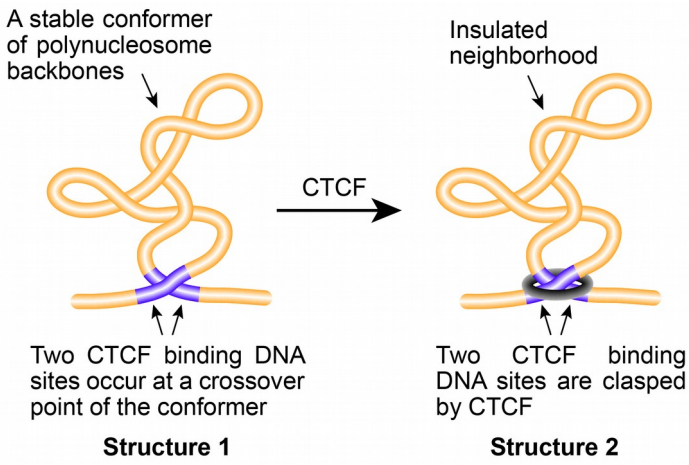

Fig. 7. Our new models for forming structures of insulated neighborhoods (Structure 2) from stable conformers of local polynucleosome backbones (Structure 1) in interphase chromosomes.

3.4.7 Fate of gene expressions within insulated neighborhoods are determined by whether and what pairable elements emerge at crossover points of polynucleosome backbones.

On the basis of our estimation using the currently available data, there are 2 to 20 crossover points of polynucleosomes in each insulated neighborhoods. On the basis of our analyses we infer that within silent and inactive insulated neighborhoods (Figs. 6B and 6C), crossover points of polynucleosome backbones do not play any roles in regulating gene expressions because they do not contain actively expressed genes. In addition, we deduce that even within an active insulated neighborhood, only a very limited number of crossover points play roles in regulating gene expressions since only 1 to 10 genes[50] are present in each active insulated neighborhood. In order to simplify our further discussions, we define "pairable DNA elements" in the current report as two DNA segments in active 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) silencers and silencers, (4) enhancers and insulators as well as (5) two CTCF DNA sequences. In view of the fact that regulations of gene expressions largely rely on spatial closeness of pairable elements[58-60], we derive the following conclusion for describing correlation between fate of gene expressions and relative positions of pairable elements within insulated neighborhoods:

Conclusion 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 polynucleosomal backbones and are further clasped by DNA binding proteins and other molecules (Insulated Neighborhood 1 in Fig. 8), (2) Gene expressions in insulated neighborhoods are suppressed if (1) silencer DNA sequences and gene promoter DNA sequences occur at crossover points of polynucleosomal backbones and are further clasped by DNA binding proteins and other molecules (Insulated Neighborhood 2 in Fig. 8), or (2) neither promoter DNA sequences nor enhancer DNA sequences emerge at crossover points of polynucleosomal backbones, (3) When two CTCF-binding DNA sequences occur at the crossover points of polynucleosomal backbones and are clasped by CFCT proteins and cohesions, they mainly serve as root regions of insulated neighborhoods (Fig. 8).

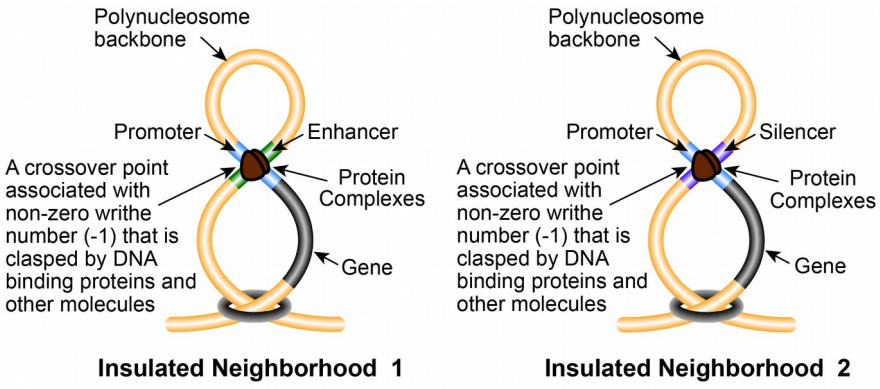

Fig. 8. Our new models of emergence of pairable elements at crossover points in correlation with fate of gene expressions within an insulated neighborhood. In Insulated Neighborhood 1, gene expression is permissible because a pair of promoter and enhancer are clasped at crossover point. In Insulated Neighborhood 2, gene expression is not permissible because a pair of promoter and silencer are clasped at crossover point.

3.4.8 Jointer polynucleosomes are one of the two essential structural and functional components of interphase chromosomes. In the current report, we define "jointers" as segments of polynucleosomes that reside between two neighboring insulated neighborhoods and infer that jointer polynucleosomes and insulated neighborhoods constitute interphase chromosomes. Unlike structures of insulated neighborhoods whose root regions are clasped together, the two ends of jointer polynucleosomes stay far apart from each other (Fig. 9). On the grounds of our analyses and deduction from the structural and supercoiling standpoint, we infer that jointer polynucleosomes play the following roles within chromosomal structures:

(a) Integrating massive amounts of insulated neighborhoods in a cell's nucleus into a limited number of chromosomes. Taking into account of the fact that eukaryotes adopt

insulated neighborhoods as structural units of their chromosomes as well as for regulating their gene expressions in their cells (Fig. 12), we infer that one of the innate roles of jointer polynucleosomes is to integrate massive amounts of gene-containing and non-gene-containing insulated neighborhoods into single structural entities to warrant these neighborhoods to reside highly orderly in a cell's nucleus in the forms of a limited number of chromosomes (*e.g.* only 23 pairs of chromosomes in human cells).

(b) Facilitating structural transformation of chromosomes during cell division cycles. As discussed in Section 4.2.3 and illustrated in Fig. 16, transformation chromosomal structures during cell division cycles depend heavily on (i) alteration of superhelical densities of jointer polynucleosomes and (ii) binding interaction of other biomolecules to polynucleosomes. We accordingly infer in the current report that alterations of superhelical densities and structures in jointer polynucleosomes dominate structural transformation of chromosomes from interphase to prophase and to metaphase as well as from anaphase to telophase and to interphase; and

(c) Accommodating housekeeping genes. In view of the fact that unlike the structures in insulated neighborhoods, There is lack of crossover points of polynucleosome backbones in jointer polynucleosomes, we deduce in the current report that jointer polynucleosomes are ideal residing places of genes[63] whose expression do not rely on formation of bound pairs of promoters and enhancers (*e.g.* many housekeeping genes).

3.4.9 Topologically associating domains are particular combinations of insulated neighborhoods and jointer polynucleosomes, and should belong to quaternary structures of interphase chromosomes. A topologically associating domains is conventionally defined as a genomic region in interphase chromosomes, within which DNA sequences physically interact with each other more frequently than those beyond itself[64, 65]. Because (1) insulated neighborhoods and jointer polynucleosomes are defined as quaternary structures of interphase chromosomes, and (2) topologically associating domains are virtually a particular combination of insulated neighborhoods and jointer polynucleosomes, we suggest in the current report that these structural domains should be categorized as quaternary structures of interphase chromosomes as well. On the grounds of our analyses from the supercoiling perspective, we infer that relatively long spans of jointer polynucleosomes make two neighboring topologically associating domains act less responsively, as outlined as follow:

(a) Two neighboring topologically associating domains are connected by long jointer polynucleosomes while neighboring insulated neighborhoods within a topologically associating domain are connected by short jointer polynucleosomes. In the current report, we classify jointer polynucleosomes into short jointers and long jointers respectively. Long

jointers are defined as those that connect two neighboring topologically associating domains while short jointers are those that connect two neighboring insulated neighborhoods within a topologically associating domain. We accordingly infer that the short lengths of short jointer polynucleosomes make motions and actions of neighboring insulated neighborhoods within a topologically associating domain mutually responsive, which cause insulated neighborhoods in a topologically associating domain to behave as self-interacting entities. Similarly, we deduce that relatively long spans of jointer polynucleosomes make motions and actions of two topologically associating domain mutually less responsive, which drive two neighboring topologically associating domain to behave as relatively independent structural entities.

(b) Topologically associating domains can be classified into active, inactive and silent topologically associating domains respectively. On the basis of their gene expression capacity and structural denseness, we classify topologically associating domains into three categories in the current report: (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. 9). In addition, from our analyses from the supercoiling standpoint, we conclude that magnitudes of absolute values of negative 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.

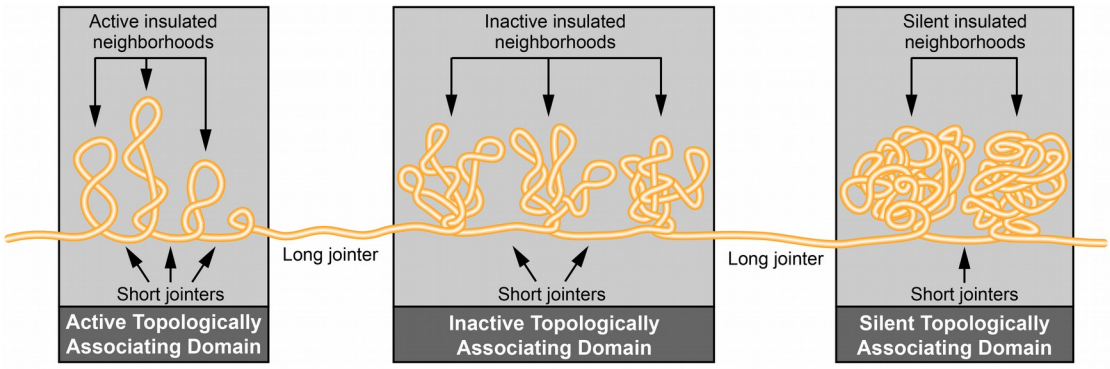

Fig. 9. Illustration of our new classification of active, inactive and silent topologically associating domains.

3.4.10 Transposition of transposons and viral insertion are capable of affecting patterns of gene expressions within insulated neighborhoods. Mobile genetic elements are DNA sequences that are able to relocate or to 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 inserting itself into genomic DNA.[68-70] In view of the fact that transposition of transposons and viral insertion are capable of increasing spans of DNA sequences, we draw the following conclusion in the current report for describing likely consequences of their actions on gene expressions:

Conclusion 5: Transpositions of transposons or viral insertions are able to interrupt preexisting spatial proximity between two pairable DNA elements (Fig. 10A) or to establish spatial proximity between new pairable elements within insulated neighborhoods (Fig. 10B), which could in turn change patterns of gene expressions in the insulated neighborhoods (Fig. 10).

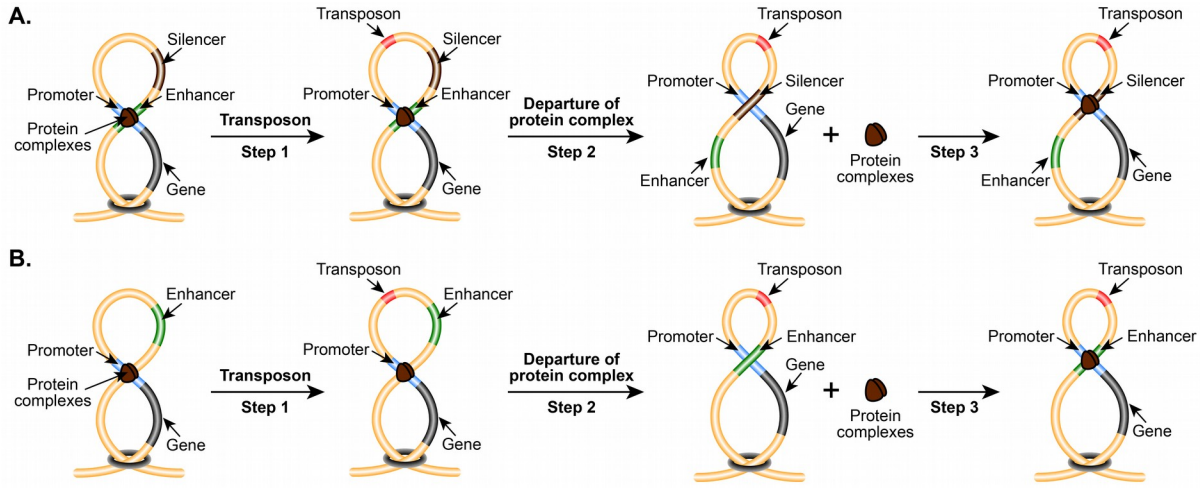

Fig. 10. Our new supercoil-driven models of alterations of gene expressions in insulated neighborhoods by actions of transposons. (A) Action of transposon-caused interruptions of gene expression. Structure 1: A preexisting insulated neighborhood, in which the two pairable elements of promoter and enhancer are clasped at its crossover point. Step 1: Insertion of a simple/complex transposon leads to generation of unstable conformations of polynucleosome backbones in the preexisting insulated neighborhoods, which will cause departure of originally bound proteins; Step 2: Departure of DNA-binding proteins leads to (i) release of proceeding backbone constraints of polynucleosomes and (ii) reestablishment of a new crossover point of polynucleosome backbones; and Step 3: DNA-binding proteins clasp the pair of promoter and silencer at new crossover point. (B) Action of transposon-caused expressions of new genes. Structure 1: A preexisting insulated neighborhood, in which the two pairable elements of promoter and enhancer are not located at its crossover point. Step 1: Insertion of a simple/complex transposon that leads to generation of constraints of polynucleosome backbones in the preexisting insulated neighborhoods, which will cause departure of originally bound proteins; Step 2: Departure of DNA-binding proteins that leads to (i) release of backbone constraints of polynucleosomes and (ii) reestablishment of a new

crossover point of polynucleosome backbones; and Step 3: DNA-binding proteins that clasp promoter and enhancer at crossover points.

3.4.11 Transposition of transposons and viral insertion are capable of affecting structures and boundaries of insulated neighborhoods and jointer polynucleosomes. When transpositions of transposons or viral insertions take place, spans of original polynucleosome backbones will be altered. Because such alterations are capable of changing conformation and superhelical densities of polynucleosome backbones, we infer in the current report that transpositions of transposons or viral insertions are able to alter shapes, sizes and boundaries of insulated neighborhoods and jointer polynucleosomes as illustrated in Fig. 11.

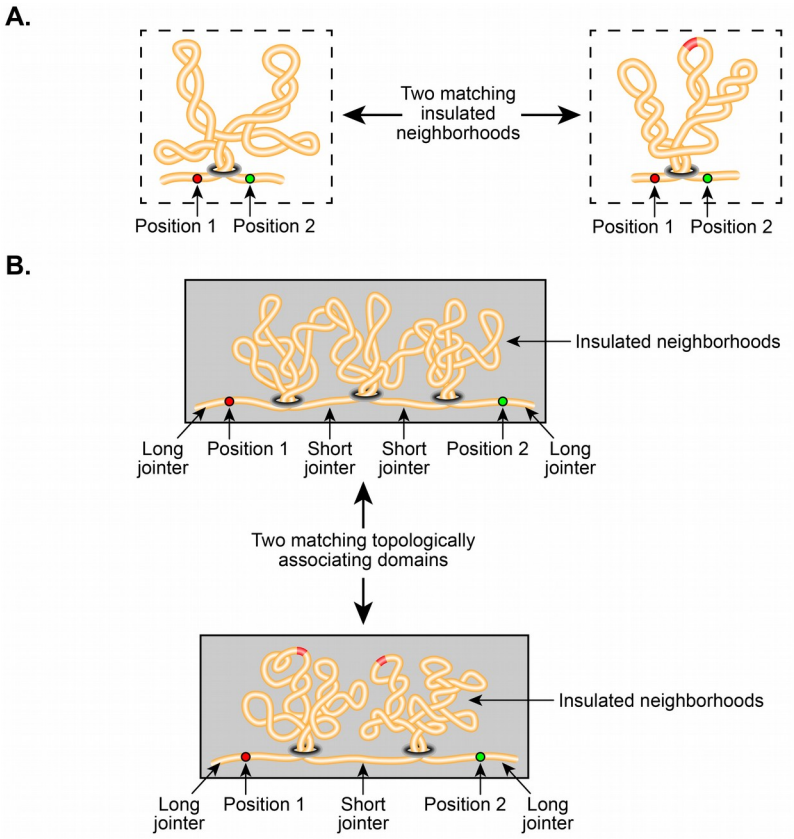

Fig. 11. Our new models of alterations of quaternary structures caused by actions of transposons. (A) transposon-caused alterations of shapes of an insulated neighborhood without changing its root regions, and (B) transposon-caused alterations of shapes and boundary of insulated neighborhoods.

3.4.12 Alterations of crossover points of polynucleosome backbones in insulated neighborhoods by transpositions of transposons are correlated with (1) different cell types, (2) genetic diversity, (3) diverse variants of chromosomal structures in cells in the same brain and (4) adaptive roles of transposable elements. For the convenience of our further

discussions, we define “matching insulated neighborhoods” in the current report as the insulated neighborhoods that occur at the same loci of homologous chromosomes (1) in different or identical cell types in the same organism, or (2) in different or identical cell types of organisms in the same species. Because transposition of transposons on these matching insulated neighborhoods could possibly lead to alterations of (1) crossover points of polynucleosome backbones as well as (2) shapes and boundaries of insulated neighborhoods (Figs. 10 and 11), we infer in the current report that these transposon-caused alterations of gene expressions (Fig. 10) are held responsible for (1) different cell types, (2) genetic diversity, (3) diverse variants of polynucleosomal structures in cells in the same brain and (4) adaptive roles of transposable elements as outlined as follows:

(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] We infer in the current report that (1) certain amount of matching insulated neighborhoods between two distinct cell types of a eukaryotic organism hold different positions of crossover points of polynucleosome backbones, which leads to distinct patterns of gene expressions in distinct cell types, and (2) these different positions of crossover points of polynucleosome backbones can be caused by transposition of transposons (Conclusion 6).

(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] On the basis of our analyses from the DNA supercoiling standpoint, we infer in the current report that shapes and sizes of some matching insulated neighborhoods at the same loci on chromosomes between different individuals in a species population are different, which leads to district patterns of crossover points of polynucleosome backbones as well as distinct patterns of gene expression profiles. These differences of shapes and sizes of matching insulated neighborhoods at the same loci on chromosomes between different individuals in a species population are mainly caused by transposition of transposons as well as viral insertion (Conclusion 6).

(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 the 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 drastically structural changes of an entire insulated neighborhood (Figs. 10 and 11). We therefore infer that it is the (1) exceptionally

high activity of transposons in brain cells[76] and (2) high structural vulnerability of supercoiling-driven insulated neighborhoods domains to actions of transposons that are accountable for emergence of wide-ranging variants of genomic structures in cells in the same brains.

(4) Adaptive transposable elements. It has been known that adaptive transposable elements are widespread in nature and their transpositions enable organisms to adapt gene expressions to environmental changes.[77-79] Because actions of transposable elements are able to alter shapes and sizes of DNA supercoiling-driven insulated neighborhoods, we infer in the current report that these structural alterations could (1) introduce new adjacent pairable elements to insulated neighborhoods as well as (2) alter the distances between preceding adjacent pairable elements. It therefore is our notion that transposon-affiliated structural changes of insulated neighborhoods are accountable for the fundamental mechanisms that underlie transposable element–induced adaptation[78, 79] in organisms.

In view that spatial organizations of supercoiling-driven insulated neighborhoods are highly susceptible to length changes of their constituent polynucleosomes, Conclusion 7 is drawn in the current report for describing consequences of actions of transposons and viral insertions on functions of eukaryotic cells:

Conclusion 6: Transposition of transposon and virus insertion are capable of generating differences (1) in shapes and sizes between matching insulated neighborhoods, as well as (2) in 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 (Figs. 10 and 11).

3.5 Left-handed toroid-shaped poly(insulated neighborhoods/jointer polynucleosomes) as quinary structures of interphase chromosomes

In the current report, we use the term "poly(insulated neighborhoods/jointer polynucleosomes)" to describe a structural entity of massive assembly of alternating repeating units of insulated neighborhoods and jointer polynucleosomes, which constitutes a single interphase chromosome. Our new inferences on supercoiling characteristics of quinary structures of interphase chromosomes are outlined in the two sections below.

3.5.1 Backbones of jointer polynucleosomes in an interphase chromosome display left-handed toroidal shapes on the whole. Hypothetically speaking, if an interphase chromosome is mechanically stretched out, length of the chromosome will be roughly equal to that of its entire jointer polynucleosomes. This happens because root regions of insulated

neighborhoods are clasped, which are separated from jointer polynucleosomes. In view of the fact that insulated neighborhoods are segregated out from jointer polynucleosomal backbones and, we deduce that overall shapes of interphase chromosomes reflect largely the overall shapes of their constituent jointer polynucleosomes and draw Conclusion 8 for describing the causes of their shape and handedness (Fig. 12).

3.5.2 Negative supercoils in naked linker DNA regions drive each individual interphase chromosome to behave as an elastic and self-aggregating structural entity. It has been known that structures of supercoiled protein-free plasmid DNA are elastic and self-aggregating because backbones of supercoiled DNA are curved, tensile and constrained.[80, 81] Taking into account that naked linker DNA segments in polynucleosomes are supercoiled (Discoveries 1 to 4), we infer that quinary structures of interphase chromosomes will display the same supercoiling characteristics as supercoiled protein-free plasmid DNA,[53, 54] and accordingly draw the following conclusion for describing structural characteristics of quinary structures of interphase chromosomes:

Conclusion 7: (1) Overall structures of jointer polynucleosomes in each interphase chromosomes display the shapes of loose and uneven left-handed toroids (Fig. 12), which are governed (1) mainly by negative supercoils of low-degree superhelical density in their naked linker DNA segments, and (2) partly by mutual physical interactions among insulated neighborhoods and jointer polynucleosomes in three-dimensional space, (2) Negative supercoils in naked linker DNA regions of polynucleosomes at this stage cannot be relaxed by DNA topoisomerases because their relatively low superhelical densities, and (3) Self-aggregating and elastic behaviors are constantly affiliated with overall structures of interphase chromosomes, which are manifestations of curved, tensile, and constrained backbones of negative supercoils in naked linker DNA segments of their constituent polynucleosomes (Fig. 12).

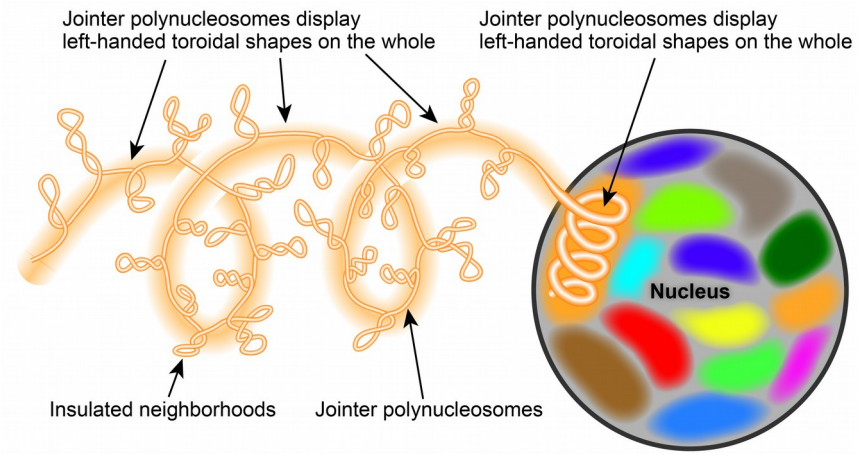

Fig. 12. Our new supercoil-driven models of quinary structures of interphase chromosomes in a eukaryotic cell's nucleus.

4. Our New Supercoiling Theory and Model of Chromosomal Structures in Mitotic and Meiotic Phases of Eukaryotic Cells

Chromosomes in mitotic phase and meiotic phase of eukaryotic cells are highly condensed structural entities of nucleic acids along with other biomolecules, in which compactions of DNA can be up to ~250-fold higher than interphase chromosomes.[82, 83] On the grounds of our analyses of supercoiling and structural characteristics, we divide mitotic and meiotic chromosomal structures into six hierarchical ranks in the current report as depicted in Table 4 and Fig. 13, and infer that negative supercoils present in their naked linker DNA regions play predominant roles in determining three-dimensional structures at all these six hierarchical levels (Propositions 1 to 3). Because supercoiling characteristics of (1) DNA and DNA-interacting biomolecules as primary structures, (2) nucleosomes as secondary structures and (3) polynucleosomes as tertiary structures have been discussed in the proceeding Section 3.1 to Section 3.3 in the current report, focus of our discussions in the current section will only be on (1) plectoroids and jointer polynucleosomes as quaternary structures, (2) poly(plectoroids/jointer polynucleosomes) as quinary structures, and (3) bivalents as senary structure respectively.

Table 4. Our new supercoiling views of chromosomal structures in mitotic and meiotic phases.

Entry	Different-level architectures of mitotic/meiotic chromosomes	Their hierarchical ranks
1.	DNA and DNA-interacting molecules	Primary structures
2.	Nucleosomes	Secondary structures
3.	Polynucleosomes	Tertiary structures
4.	Plectoroids and jointer polynucleosomes ("loop domains")	Quaternary structures
5.	Poly(plectoroids/jointer polynucleosomes)	Quinary structures
6.	Bivalents	Senary structures

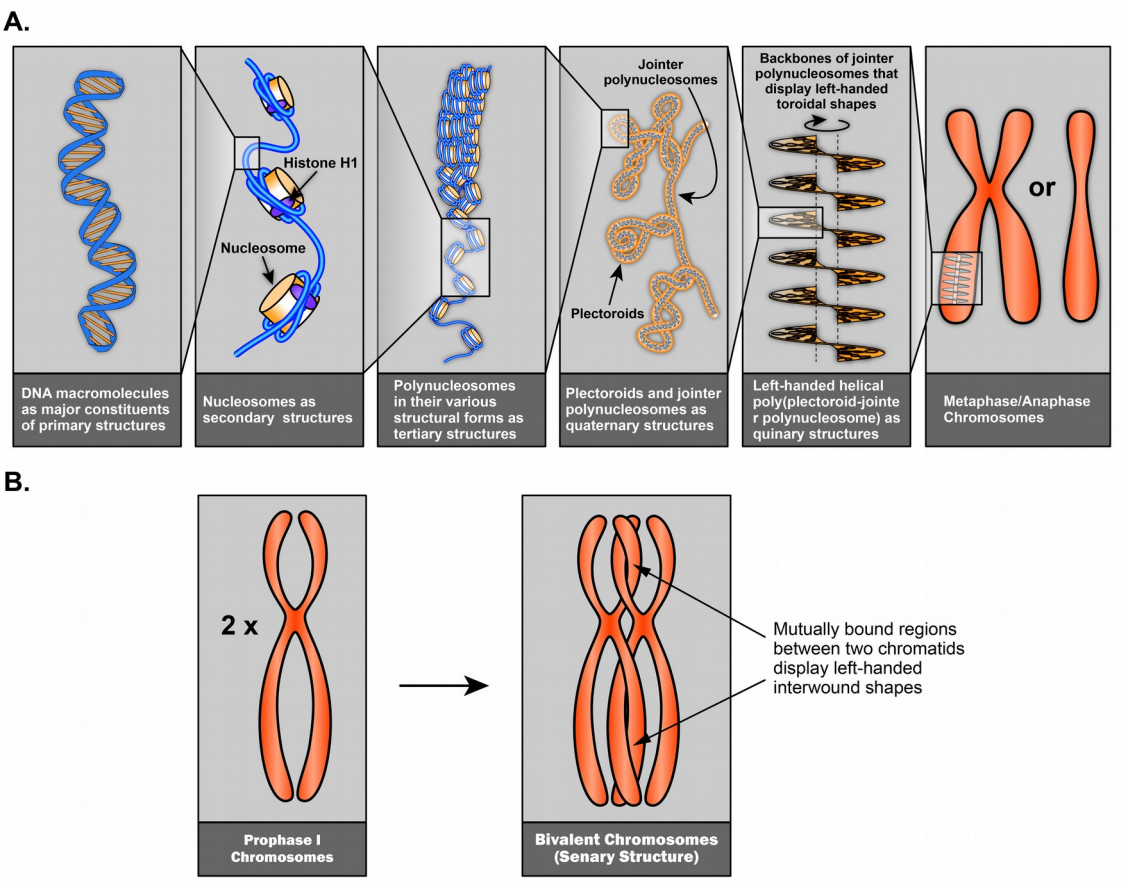

Fig. 13. Our new supercoil-driven models of chromosomal structures in (A) mitotic and (B) meiotic phases of eukaryotic cells.

4.1 Plectoroids and jointer polynucleosomes as quaternary structures of mitotic and meiotic chromosomes

The term “loop domain” has commonly been used in the past for describing a subcategorical structure of metaphase chromosomes, namely 300-nm fibers[84-87] (Fig. 14A), which is one hierarchical level higher than 30-nm fibers[43-45]. On the basis of our new analysis from the supercoiling and structural perspective, we infer in the current report that there are in effect two different types of structural entities within the conventionally defined 300-nm fibers: (1) left-handed toroidal and/or right-handed plectonemic shapes of polynucleosome backbones whose root regions are clasped by CTCF dimers , and (2) jointer polynucleosomes (Fig. 14B). In order to simplify our further discussions, we introduce a new term “plectoroid” for substituting the phrase “left-handed toroidal and/or right-handed plectonemic shapes of polynucleosome backbones whose root regions are clasped by CTCF dimers” in the current report. That is to say, when viewed from the perspective of polynucleosome backbones, we infer that conventionally defined 300-nm fibers are constituted by two types of structural

units: plectoroids and jointer polynucleosomes (Fig. 14B). Even though components of 300-nm fibers were often observed in the past as "loop domains" under microscopic examinations[84-87], on the other hand, we envisage that presence of supercoil-driven plectoroids and jointer polynucleosomes as two constituents of 300-nm fibers (Fig. 14B) will be experimentally provable in the near future through using advanced microscopic techniques. In addition, in view of the fact that naked linker DNA segments in polynucleosome backbones are negatively supercoiled (Fig. 1), we deduce the following conclusion for describing supercoiling characteristics of quaternary structures of mitotic and meiotic chromosomes:

Conclusion 8. Quaternary structures of mitotic and meiotic chromosomes are composed of two types of structural units of (1) plectoroids and (2) jointer polynucleosomes (Fig. 14B), three-dimensional structures of which are mainly sustained by negative supercoils present in their naked linker DNA regions.

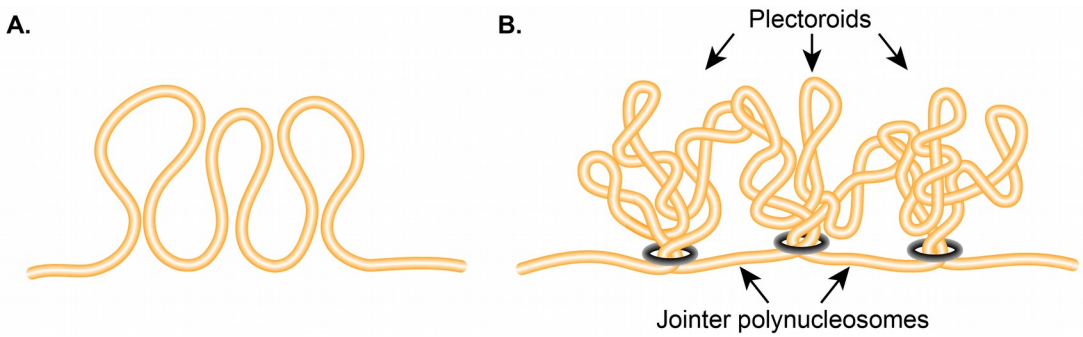

Fig. 14. (A) Conventional view of loop domains as components of 300-nm fiber[84-87], and (B) Our new supercoil-driven models of quaternary structures of mitotic and meiotic chromosomes, which are composed of two types of structural units: plectoroids and jointer polynucleosomes.

4.2 Left-handed toroid-shaped poly(plectoroids/jointer polynucleosomes) as quinary structures of mitotic/meiotic chromosomes

In the current report, we use "left-handed toroid-shaped poly(plectoroids/jointer polynucleosomes)" (or "poly(plectoroids/jointer polynucleosomes)") for describing structural entities of massive alternating repeating units of plectoroids and jointer polynucleosomes, in which jointer polynucleosomes display left-handed toroidal shapes on the whole (Fig. 15). We accordingly define "left-handed toroid-shaped poly(plectoroids/jointer polynucleosomes)" as quinary structures of mitotic/meiotic chromosomes and further classify them into (1) loose poly(plectoroids/jointer polynucleosomes) (Section 4.2.1) and (2) compact poly(plectoroids/jointer polynucleosomes) (Section 4.2.2) respectively. Our new

analyses on supercoiling characteristics of these quinary structures of mitotic/meiotic chromosomes are presented in the three sections below.

4.2.1 Telophase chromosomes and prophase chromosomes are "loose left-handed toroid-shaped poly(plectoroids/jointer polynucleosomes)" and "interconnected loose left-handed toroid-shaped poly(plectoroids-jointer polynucleosomes)". Under microscopic examinations, telophase chromosomes and prophase chromosomes were often observed as linear thin columnar and X-shape thin columnar subjects respectively.[88] On the basis of our analyses from the perspective of supercoiling and polynucleosome backbones, we infer in the current report that the experimentally observed (1) thin columnar structures of telophase are in fact loose poly(plectoroids/jointer polynucleosomes) and (2) X-shape thin columnar structures of prophase chromosomes are in effect two loose poly(plectoroids/jointer polynucleosomes) that are joined together in their centromere regions (Fig. 15A). Since naked linker DNA segments in polynucleosomes are negatively supercoiled, we draw the following conclusion for describing supercoiling characteristics of telophase and prophase chromosomes:

Conclusion 9: (1) Jointer polynucleosomes in loose poly(plectoroids/jointer polynucleosomes) (telophase chromosomes) and interconnected loose poly(plectoroids/jointer polynucleosomes) (prophase chromosomes) (Figs. 15A and 15D) display the shapes of left-handed toroids on the whole, which are mainly governed by negative supercoils present in their naked linker DNA segments, and (2) individual plectoroids in three-dimensional structures of poly(plectoroids/jointer polynucleosomes) are oriented upward, downward and straight outward respectively (Fig. 15).

4.2.2 Anaphase and metaphase chromosomes are "compact left-handed toroid-shaped poly(plectoroids/jointer polynucleosomes)" and "interconnected compact left-handed poly(plectoroids/jointer polynucleosomes)". Under microscopic examinations, anaphase chromosomes and metaphase chromosomes were often observed as linear thick columnar and X-shaped thick columnar subjects respectively.[88] Based on our analyses from the perspective of supercoiling and polynucleosome backbones, we infer that the experimentally observed (1) thick columnar structures of telophase are in fact compact left-handed poly(plectoroids/jointer polynucleosomes) and (2) X-shape thick columnar structures of prophase chromosomes are in effect two compact left-handed poly(plectoroids/jointer polynucleosomes) that are joined together in their centromere regions (Fig. 15B).

At the end of prophase of mitosis and prophase I of meiosis, on the other hand, nuclear envelope is broken down,[89] which allows cytoplasmic condensin I to interact with prophase chromosomes to form metaphase chromosomes. Condensin I is known to be a type of protein complexes that generate positive supercoils in the DNA segment that it binds to.[90, 91] On the

basis of Axiom 5, we deduce that once bindings of condensin I to DNA macromolecules take place, negative supercoils must be produced simultaneously in their adjacent naked linker DNA regions in polynucleosome backbones in metaphase chromosomal structures. In addition, because condensin I is a highly abundant protein in metaphase[92], we further infer that remarkably high negative superhelical densities will be generated in the naked linker DNA regions all over entire metaphase chromosomal structures, which will simultaneously lead to generation of unusually high degrees of backbone curvatures in naked linker DNA regions at these stages. On the grounds of our analyses from the supercoiling and structural standpoint, we reason that the remarkably high negative superhelical density-affiliated backbone curvatures of naked linker DNA segments along with binding actions of cohesions, topoisomerase II, histone H1, condensins and other molecules are accountable for extraordinarily high structural compactness of metaphase (Fig. 15B) and anaphase chromosomes (Fig. 15C). We accordingly draw the following conclusion in the current report for describing supercoiling characteristics of metaphase and anaphase chromosomes:

Conclusion 10: (1) Metaphase chromosomes (Fig. 15B) possess remarkably high negative superhelical densities in their naked linker DNA regions, which are generated (i) mainly by binding actions of condensin I to DNA macromolecules, and (ii) partly by binding of 10-base pair arm DNA segments to histone octamers (Discoveries 2 and 3); (2) these remarkably high superhelical densities lead to generation of exceptionally high degrees of backbone curvatures in naked linker DNA segments, which are the leading causes of structural compactness of metaphase and anaphase chromosomes; (3) superhelical densities in naked linker DNA regions in metaphase and anaphase chromosomes (Figs. 15B and 15C) are much higher than those in prophase and telophase chromosomes (Figs. 15A and 15D); and (4) cellular DNA topoisomerases are not capable of relaxing DNA supercoils in the chromosomal structures in metaphase and anaphase because (i) high structural compactness of chromosomes and (ii) short lengths of their naked linker DNA segments at these stages prevent supercoil-relaxing catalytic actions of DNA topoisomerases from taking place.

In addition, on the grounds of our new analyses from the structural standpoint, we infer that (1) magnitudes of vertical distances between one consecutive helical turn in prophase and telophase chromosomes (Pitch 1 in Fig. 15A) are greater than those of metaphase and anaphase chromosomes (Pitch 2 in Fig. 15B), and (2) these differences are caused mainly by binding actions of cohesins.[93, 94]

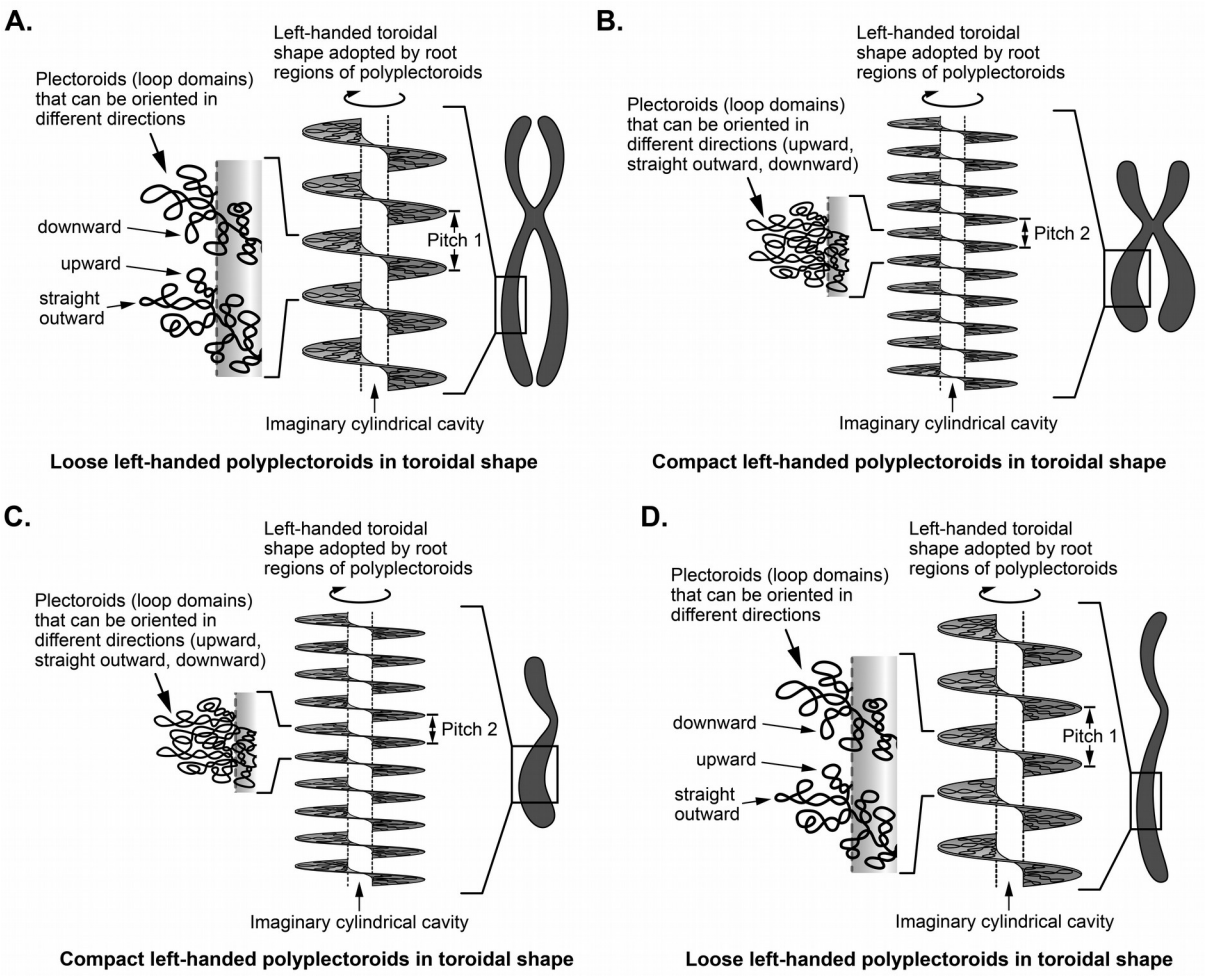

Fig. 15. Our new supercoiling-driven models of quinary structures of mitotic/meiotic chromosomes (left-handed toroid-shaped poly(plectoroids/jointer polynucleosomes)). (A) prophase chromosomes, (B) metaphase chromosomes, (C) anaphase chromosomes, and (D) telophase chromosomes. Jointer polynucleosomes in chromosomal structures in all prophase, metaphase, anaphase and telophase display left-handed toroidal shapes on the whole owing to presence of negative supercoils in their naked linker DNA regions.

4.2.3 Mitotic phase quaternary structures and interphase quaternary structures are correlated with each other. Eukaryotic cell division cycles can be divided into two major distinct phases: (1) interphase and (2) mitotic phase.[95] In conjunction with the progression of cell cycles, transformations of eukaryotic chromosomal structures take place as well.[95] In the proceeding sections of the current report, we have defined (1) poly(insulated neighborhoods/jointer polynucleosomes) as quaternary structural forms of interphase chromosomes (Table 2 and Fig. 2) and (2) poly(plectoroids/jointer polynucleosomes) as quaternary structural forms of mitotic/meiotic phase chromosomes (Table 4 and Fig. 13A) respectively. On the grounds of our analyses on their supercoiling and structural

characteristics, we draw the following conclusion for describing correlations between mitotic quaternary structures and interphase quaternary structures:

Conclusion 11. When eukaryotic cells progress from interphase (G2 phase) to metaphase in cell cycles, vast majority of insulated neighborhoods and jointer polynucleosomes in interphase chromosomes are converted directly into plectoroids and jointer polynucleosomes of prophase/metaphase chromosomes without alterations of their sizes (Fig. 16). When eukaryotic cells progress from anaphase to interphase (G1 phase) in cell cycles, vast majority of plectoroids and jointer polynucleosomes are converted directly into insulated neighborhoods and jointer polynucleosomes without alterations of their sizes.

In addition, taking into account of the fact that (1) supercoil-generating condensin I complexes reach chromosomes exclusively in metaphase[96, 97] and (2) metaphase and prophase chromosomes possess more bound protein molecules in their structures than interphase chromosomes[98], we deduce the following conclusion for describing characteristics of superhelical densities in chromosomal structures in cell cycles:

Conclusion 12. Magnitudes of superhelical densities of naked linker DNA regions in chromosomal structures in different phases of cell cycles are in the following order:

$$\text{Metaphase} \approx \text{Anaphase} > \text{Prophase} \approx \text{Telophase} > \text{Interphase}$$

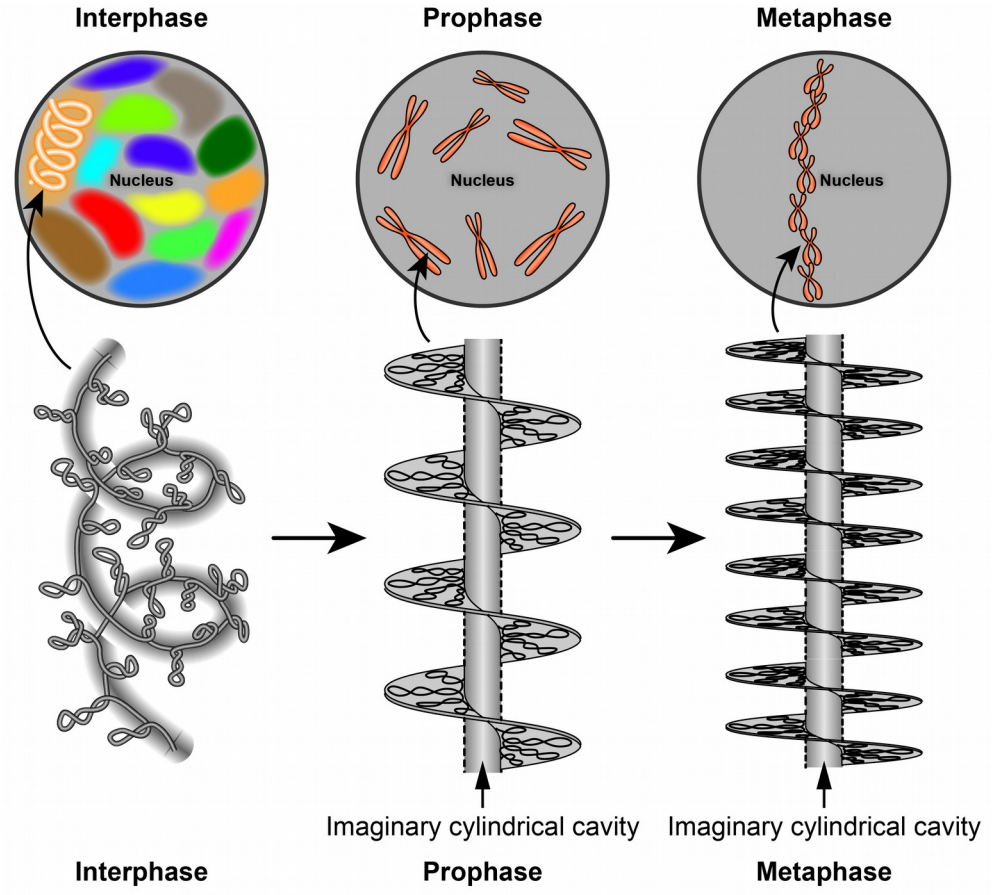

Fig. 16. Our new supercoil-driven models of transformations of chromosomal structures among interphase, prophase and metaphase (jointer polynucleosomes of chromosomal structures in all phases display left-handed toroidal shapes).

4.4 Bivalents as senary structures of meiotic chromosomes

The term bivalents have commonly been used nowadays for describing structural entities of two pairs of homologous chromosomes that are formed in prophase I and metaphase I in meiosis.[99] Based on their structural denseness, we define the bivalents in prophase I as loose bivalents and those in metaphase I as compact bivalents (Fig. 17) in the current report, and take both loose bivalents and compact bivalents as senary structures of meiotic chromosomes.

4.4.1 Mutually bound regions between two pairs of homologous chromosomes in loose bivalents preferably adopt left-handed interwound shapes. Because (1) poly(plectoroids/jointer polynucleosomes) are packed in prophase chromosomes in left-handed toroidal shapes (Fig. 16), and (2) surface structures of homologous chromosomes could affect formation of bivalent structures (Conclusion 14 in Section 4.4.2), we deduce the following conclusion to describe structural relationship between two pairs of homologous chromosomes loose in bivalents:

Conclusion 13: During formation of loose bivalent structures at zygotene phase[99], preexisting left-handed toroid-shaped packing patterns of poly(pectoroids/jointer polynucleosomes) are capable of facilitating two chromatids from each of two homologous pairs of chromosomes to adopt left-handed interwound structures in their mutually bound regions (Fig. 17). Such left-handed interwound structures will remain during the transition from loose bivalents in prophase I to compact bivalents in metaphase I (Step 2 in Fig. 17).

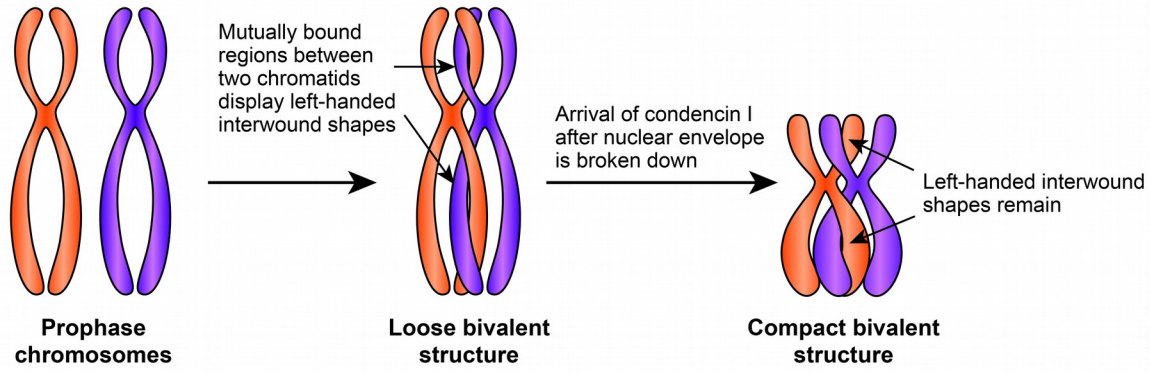

Fig. 17. Our new supercoil-driven models of spatial organizations of loose bivalents and compact bivalents, in which mutually bound regions between two pairs of homologous chromosomes preferably display left-handed interwound shapes.

4.4.2 Homologous chromosome-matching zones on surfaces of loose bivalent structures are accountable for specific recognitions between pairs of homologous chromosomes. Most of plant and animal cells are known to possess two or more pairs of chromosomes[100], such as 23 pairs of chromosomes in human cells[101] and 17 pairs of chromosomes in the cells of sunflowers[102]. Accurate recognition between pairs of homologous chromosomes within a cell's nucleus to form correct bivalent structures is therefore imperative for the subsequent events of chromosomal crossover and segregations.[71] In the current report, we infer that "homologous chromosome-matching zones" (or "matching zones") exist on the surfaces of homologous chromosomes, which are composed of different-dimensioned (1) interacting zones and (2) spacer zones respectively, which align along columnar structures of prophase chromosomes in alternating manners (Fig. 18A). In addition, we deduce that (1) 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. 18A), and (2) physical interactions between interacting zones are mainly responsible for physically holding two pairs of homologous chromosomes together. We accordingly derive the following conclusion for describing roles of homologous chromosome-matching zones in the formation of bivalents:

Conclusion 14. Homologous chromosome-matching zones are present on surfaces of homologous chromosomes, which are constituted by alternating interacting zones and spacer

zones. Because two pairs of homologous chromosomes possess identical patterns of homologous chromosome-matching zones, they are capable of forming correct bivalent structures in prophase I during meiosis (Fig. 18B). The chances for non-homologous chromosomes to form bivalent structures are significantly low because they possess different patterns of homologous chromosome-matching zones on their surfaces (Fig. 18C).

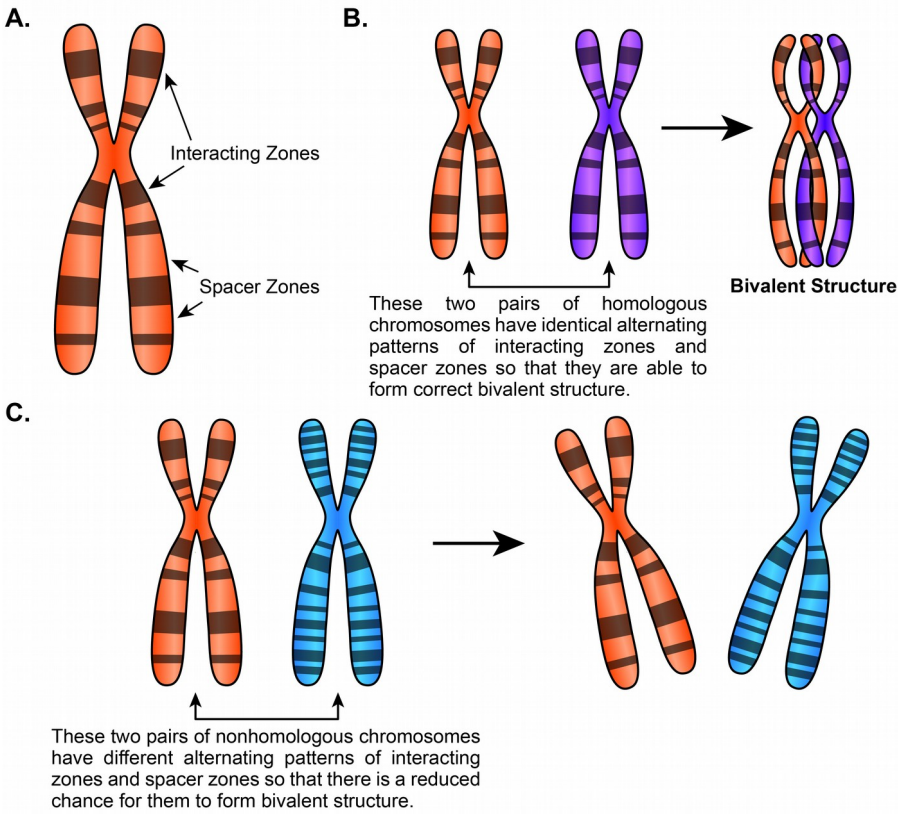

Fig. 18. (A) Our new notions of possessions of homologous chromosome-matching zones on surfaces of homologous chromosomes, (B) presence of identical homologous chromosome-matching zones that allows formations of correct loose bivalents between two pairs of homologous chromosomes, and (C) absence of identical homologous chromosome-matching zones that reduce chances of non-homologous chromosomes to form loose bivalent structures.

In view of the facts, on the other hand, that insulated neighborhoods and jointer polynucleosomes at the same loci of two chromatids from separate pairs of sister chromatids possess identical DNA sequences in general[103], we infer that at the molecular scales, interacting zones of two chromatids from separate pairs of sister chromatids are able to recognize and interact with each other through the following two sequential steps of events: (1) generation of single-stranded DNA from duplex DNA, a process that is driven by preexisting negative supercoils[2, 37, 38] in their naked linker DNA regions; and (2) formation of duplex DNA by two single-stranded DNA from two separate sister chromatids, a process that

is assisted by condensin I as this protein complex is able to facilitate generation of duplex DNA structures from their single-stranded precursors[104].

5. Our new supercoiling theory and model of chromosomal structures in sperm cells

Because DNA-bound proteins in post anaphase chromosomes in spermiogenesis are made of protamines[105] rather than histones, these chromosomal structures are fundamentally different from those in interphase (Fig. 2) and mitotic phase (Fig. 13). Consequently, we discuss supercoiling characteristics of physical forms of post anaphase chromosomes in sperm cells as a separate topic in the current section. In conjunction with transformation of secondary spermatocytes to haploid spermatids during meiosis II in spermiogenesis, sister chromatids turn into separated chromatids.[103] In the subsequent Golgi phase of spermiogenesis, most parts of DNA macromolecules in these separated chromatids form complexes with transition proteins upon their dissociation from histone proteins while a certain amount of folded histone solenoids from chromatids still remains.[106, 107] Protamines will then emerge to selectively replace transition proteins to generate DNA-protamine toroids.[106-109] Taking into account that (i) linker DNA regions in folded histone solenoids left from original chromatids are negatively supercoiled[29-32] and (ii) DNA-protamine toroids and histone solenoids coexist in harmony as a single structural entity, we infer in the current report that protein-unbound DNA regions in DNA-protamine toroids are negatively supercoiled. In accordance with Axiom 1, we further infer that once protein-unbound DNA in DNA-protamine toroids in sperm cells bears negative supercoils, it must be left-handed, and therefore draw the following conclusion for describing correlation among shapes, handedness and supercoils in chromosomal structures in sperm cells:

Conclusion 15: DNA-protamine toroids in sperm cells (1) possess negative supercoils in their protein-unbound DNA regions and (2) display left-handed helical shapes on the whole whereas DNA supercoils in the toroid-shaped structures cannot be relaxed by DNA topoisomerases because of their possession of comparably low superhelical densities. Besides DNA-protamine toroids[106-109], overall spatial organizations of (1) side-by-side stacked protamine toroids[110] and (2) bulky assemblies of side-by-side stacked protamine toroids[110] display left-handed toroidal shapes as well, handedness of which is governed largely by negative DNA supercoils present in their protein-unbound DNA regions.

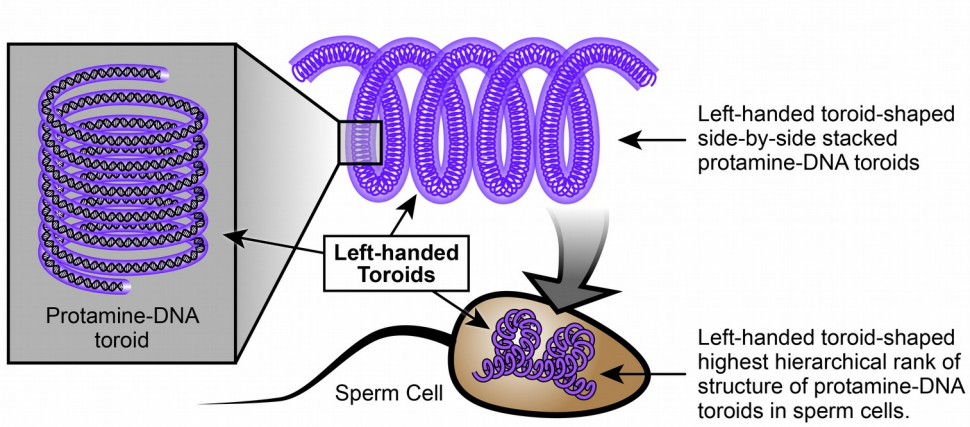

Fig. 19. Our new supercoil-driven model of chromosomal structures in sperm cells whose shapes, sign and handedness are sustained mainly by negative supercoils present in their protein-unbound DNA regions.

6. Our New Supercoiling Theory about Necessity 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.[111, 112] Even though protein translation is not advanced on these nucleic acids, various cellular roles of noncoding DNA have been recognized in the past [113-115], which include (1) their transcriptions into functional noncoding RNA such as ribosomal RNA, transfer RNA and microRNA[116, 117], and (2) their functions as telomeres[118, 119], centromeres and origins of DNA replication[118, 120]. On the grounds of our analyses from the DNA supercoiling standpoint, we infer in the current report that noncoding DNA sequences are essential constituents for sustaining structures and functions of supercoil-driven eukaryotic chromosomal structures as outlined in the six sections below.

6.1. Noncoding DNA sequences are main structural components of insulated neighborhoods.

We inferred in Section 3.4 in the current report that crossover points of polynucleosomal backbones occur in insulated neighborhoods, which are the manifestation of non-zero writhe number of supercoiling-affiliated structures (Conclusion 3). Once pairable DNA elements (*e.g.* enhancers, silencers, promoters, insulators as well as pairs of CTCF) emerge and are clasped at crossover points of polynucleosomes, they are correlated with regulation of gene expressions in their host insulated neighborhoods (Conclusion 4). We accordingly draw the following conclusion for describing essentialness of noncoding DNA in regulations of gene expressions:

Conclusion 16: Presence of noncoding DNA sequences in insulated neighborhoods is essential for sustaining the rise and absence of pairable elements (*e.g.* enhancers, silencers, promoters, insulators as well as pairs of CTCF) at crossover points of polynucleosome backbones, which are accountable for regulation of gene expressions within the insulated neighborhoods.

6.2 Noncoding DNA sequences are major constituents of jointer polynucleosomes.

We inferred in Section 3.4 in the current report that jointer polynucleosomes and insulated neighborhoods/plectonemes are the two components of interphase and mitotic phase chromosomes. Taking into account of the fact that jointer polynucleosomes are composed of predominantly noncoding DNA sequences, we draw the following conclusion for describing necessity of noncoding DNA for sustaining innate cellular roles of jointer polynucleosomes:

Conclusion 17. Noncoding DNA sequences as major constituents of jointer polynucleosomes are essential constituents for facilitating (i) integration of massive amount of insulated neighborhoods/plectonemes into a limited number of chromosomes in a eukaryotic cell's nucleus (*e.g.* 23 pairs of chromosomes in human cells), and (ii) transformations of chromosomal structures in cell division cycles (Fig. 16).

6.3 Transposons as noncoding DNA are closely correlated with diverse structures, functions and activities of eukaryotic cells

It has been known that both inactive transposons and active transposons belong to the category of noncoding DNA sequences.[121, 122] 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[122], and has been considered to be genetic fossils[123, 124]. Unlike inactive transposons, on the other hand, active transposons are capable of rearranging themselves alongside genomic DNA nowadays.[125, 126] As discussed in Section of 3.4.12 in the current report, transpositions of transposons are closely affiliated with diverse structures, functions and activities of eukaryotic cells. We accordingly draw the following conclusion for describing necessity of transposons as noncoding DNA sequences in eukaryotic cells:

Conclusion 18: Because their actions are able to alter three-dimensional structures of insulated neighborhoods as well as 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 organisms to adapt gene expressions to environment changes through mechanisms of transposon-induced adaptation (Section 3.4.12).

6.4 Noncoding DNA sequences are major components of spacer zones of homologous chromosome.

We inferred in Section 4.4 in the current report that homologous chromosome-matching zones are associated with columnar structures of prophase I chromosomes. In addition, within the homologous chromosome-matching zones, spacer zones are composed mostly of noncoding DNA whose quantities and dimensions are critically important for facilitating correct recognition and formation between two pairs of homologous chromosomes (Conclusion 14). We accordingly draw the following conclusion for describing essentialness of noncoding DNA in accurate recognition between pairs of homologous chromosomes:

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

6.5 Noncoding DNA sequences are major constituents of lamina-associated domains and nucleolus-associated domains

Based on their capacity for gene expressions, we infer in the current report that nucleic acid components in both lamina-associated domains[127] and nucleolus-associated domains[128, 129] consist of inactive and silent insulated neighborhoods, which belong to noncoding DNA sequences. Lamina-associated domains are particular genomic regions that physically interact with nuclear lamina and constitute ~40% of the human genome.[130, 131] 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.[127] Different from lamina-associated domains, on the other hand, nucleolus-associated domains bind to nucleolus instead.[128] These domains make up ~4% of the genome[132], and are known to be affiliated with nucleolus functions.[129] We accordingly draw the following conclusion for describing necessity of noncoding DNA as parts of lamina-associated domains and nucleolus-associated domains:

Conclusion 20. Since noncoding DNA sequences are the main constituents of lamina-associated domains and nucleolus-associated domains, they are essential for sustaining structures and functions of these two types of domains in nuclei of eukaryotic cells.

6.6 Centromeres as noncoding DNA sequences are essential for preventing structural distortion of centromeric regions in sister-chromatid pairs during transition from metaphase to anaphase in cell cycles.

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 materials between two daughter cells occurs, defective cells will be resulted.[133, 134] Centromere, on the other hand, is a type of noncoding DNA that makes up of up to 5% of entire eukaryotic genomic DNA sequences[135-137], through which a sister-chromatid pair are joined together. On the grounds of our analyses from supercoiling and structural perspective, we draw the following conclusion to describe necessity of noncoding DNA as components of centromere in cell cycles:

Conclusion 21: Noncoding DNA sequences in regional centromeric regions of sister-chromatid pairs are essential (1) for maintaining high absolute values of negative superhelical density in naked linker DNA regions of centromeric polynucleosomes and (2) for generating high structural compactness of centromeric 30-nm fibers, which prevent sister-chromatid pairs from structural distortions caused by pulling force-affiliated physical tensions at kinetochore-microtubule interfaces during separations of the pairs in early anaphase of cell division cycles.

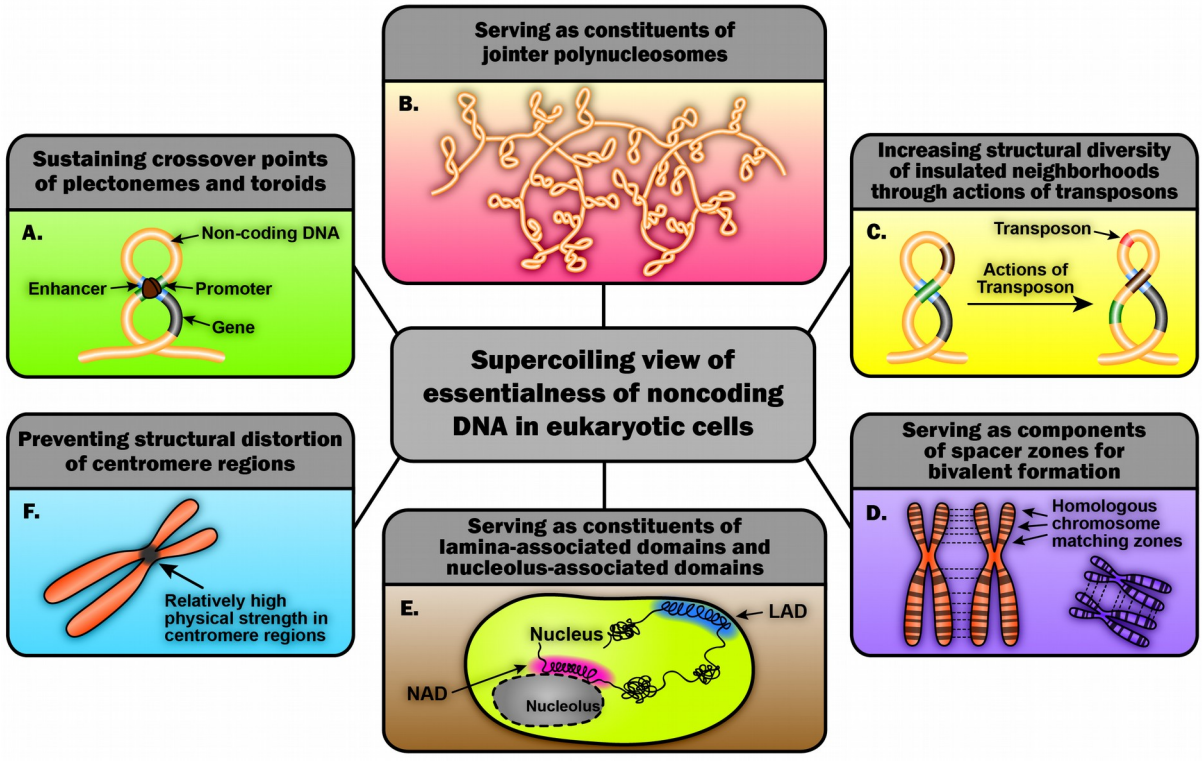

Fig. 20. Our new supercoiling theory of necessity of noncoding DNA sequences (A) as structural components of plectonemes/toroids of polynucleosomes, (B) as constituents of jointer polynucleosomes for maintaining structural integrity of chromosomes and for facilitating structural transformations of chromosomes in cell division cycles, (C) in the forms of transposons for altering shapes, sizes and crossover points of insulated neighborhoods, (D) as constituents of spacer zones for correct recognition between pairs of

homogenous chromosomes, and (E) as constituents of LADs and NADs for maintaining cellular functions of interphase chromosomes, and (F) as structural components to prevent distortion of regional centromeric regions.

7. Our new supercoil-driven models of nucleosome-like structures and chromatins in hyperthermophilic archaea

In human cells, DNA macromolecules wrap around histone octamers to form nucleosomes and display left-handed toroidal shapes.[1, 2] As opposed to those in human cells, DNA in nucleosome-like structures in hyperthermophilic archaea exhibits right-handed toroidal shapes instead[138] and are known to be positively supercoiled[139-141]. Different from negative DNA supercoils, positive DNA supercoils are tightly overwound structures and highly compact.[1, 2] In view of the fact that physical changes of positive DNA supercoils in their structures insensitive to temperature variations in high temperature ranges[142, 143], we infer in the current report that naked linker DNA of chromatins in hyperthermophilic archaea should display positive supercoils as well in order to avoid undesirable structural distortions. In addition, it is our deduction that once naked linker DNA in hyperthermophilic archaea adopt positive supercoils, shapes and handedness of their chromatin structures and higher hieratical architectures should obey Axiom 3 and Axiom 4 (Table 1). We accordingly draw the following conclusion for describing shapes and handedness of chromatin structures in hyperthermophilic archaea:

Conclusion 22: Naked linker DNA segments in nucleosome-like structures in hyperthermophilic archaea are positively supercoiled, which drive hyperthermophilic archaeal chromatins and their higher hieratical structures in hyperthermophilic archaea to adopt the shapes of either right-handed toroids or left-handed plectonemes (Fig. 21).

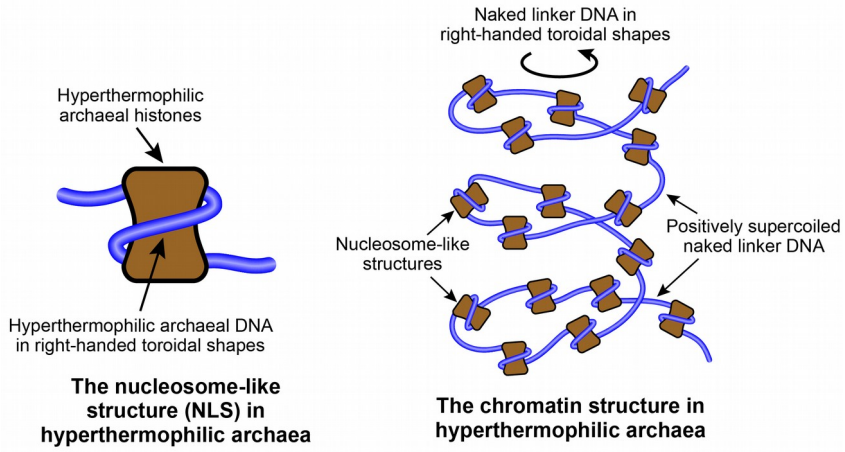

Fig. 21. Our new supercoil-driven models of nucleosome-like and chromatin structures in hyperthermophilic archaea.

8. Conclusion

It is our notion that similar to long linear assemblies of repeating units of nucleotides that act as molecular frameworks of nucleic acids, polynucleosomes are the backbone structures of eukaryotic chromosomes. When viewed on the scale of polynucleosomes, we envisage that eukaryotic chromosomes are constituted by two types of structural units: (1) jointer polynucleosomes and (2) insulated neighborhoods/plectoroids. Because naked linker DNA segments are negatively supercoiled and the only conformation-alterable elements in polynucleosomes, we deduce that distinct characteristics of DNA supercoils that occur at the molecular level of protein-free plasmid DNA[53] ought to manifest at the scale of polynucleosomal backbones of eukaryotic chromosomes as well, such as (1) toroidal and plectonemic shapes of polynucleosome backbones structures, (2) backbone crossover points, (3) alterations of superhelical density and (4) tensile and elastic behaviors of backbone structures. It is our conception that these distinct characteristics of supercoils play imperative roles in sustaining cellular structures and functions of eukaryotic chromosomes such as (1) regulations of gene expressions, (2) transposon-mediated cell type diversity and genetic diversity, (3) structural transformations of chromosomes in cell division cycles, (4) centripetal and elastic mechanical behaviors of chromosomes[144-146] and (5) highly ordered homologous chromosome recognition, crossover and segregation. We therefore conclude that (1) spatial organizations of eukaryotic chromosomal structures at all hierarchical levels in all phases of cell division cycles, as well as (2) their cellular activities are mostly manifestation of distinct characteristics of negative supercoils that are present in naked linker DNA segments of their constituent polynucleosomes.

Acknowledgments

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