DNA Origami Windmill Tetramer Model

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Abstract:

Although the classic DNA double helix model proposed by Watson and Crick explains the static storage mechanism of genetic information, it fails to reasonably account for the physical driving force behind high-speed and high-fidelity DNA replication. Furthermore, the core features of Rosalind Franklin's X-ray diffraction Pattern 51—"alternating black and white stripes with a slight tilt"—have not been fully interpreted within the static framework. Based on the standard B-type DNA double helix as the basic unit, this hypothesis proposes an original DNA Origami Windmill Tetramer Model by drawing on MacKinnon's research on the tetrameric structure of potassium ion channels and the mechanical principles of the potassium ion channel origami windmill model. Four DNA double helices assemble into an inverted conical tetrameric functional unit at a non-90 ° oblique angle corresponding to the diffraction characteristics of Franklin's Pattern 51, forming an inverted conical ion channel at the center. Its dynamic drive relies on the electrostatic repulsion of intracellular cations such as K^+ and Na^+, without the need for ATP hydrolysis for energy supply. The core of the model follows the logic of whole-chain non-denaturing replication, realizing genetic transmission through pairing and recombination between double-helix units, thereby avoiding the mismatch risk caused by single-strand exposure[7]. This reasonably explains the replication phenomenon in minimalist systems such as archaea and φ29 bacteriophages that do not require helicases, and clarifies that the classic enzyme system is only an auxiliary regulatory factor in the complex chromatin environment. Combining the core laws of molecular theory and 2^n exponential logic, this study corrects the definition deviation between traditional DNA structural units and genetic functional units, confirming that the tetramer composed of 4 double helices is the optimal functional unit for complete DNA inheritance. Meanwhile, it is the first to reveal the direct correlation between the diffraction characteristics of Franklin's Pattern 51 and the folded stacking shape of the model's blades, breaking through the limitations of static cognition. This hypothesis provides a new and testable theoretical framework for dynamic DNA replication, whose predictions can be verified through five layers of decisive experiments. It is highly compatible with the classic double helix model and offers a testable theoretical perspective and experimental basis for research in related fields.

Keywords

DNA Origami Windmill Tetramer; Inverted Conical Ion Channel; Ion Repulsion-Driven; Whole-Chain Non-Denaturing Replication; ATP-Independent; Franklin's Pattern 51; Dynamic DNA Regulation; Molecular Theory; G-Quadruplex; Biophysics; Higher-Order DNA Structure; Genetic Functional Unit

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1 Introduction: From Anomalies in the "Enzyme-Centered" Paradigm to the Inevitability of a New Hypothesis

1.1 Consensus and Achievements of Classic Research

Since Watson and Crick proposed the DNA double helix model[1], the principle of base complementary pairing has become the core molecular basis for the storage and transmission of genetic information, laying the theoretical framework for modern molecular biology. On this basis, the "enzyme-centered" replication paradigm has gradually formed and become the mainstream academic cognition. This paradigm holds that the core driving force of DNA replication originates from the precise collaboration of protein factors such as helicases, topoisomerases, and DNA polymerases[2]: helicases unwind the double helix by hydrolyzing ATP to provide energy, topoisomerases relieve interchain torsional stress, and DNA polymerases synthesize complementary new strands using single strands as templates. This system is regarded as the classic framework for explaining the DNA replication process[3], and has achieved numerous breakthroughs in the reconstruction of in vitro replication systems and the analysis of enzymatic reaction mechanisms[4].

1.2 Core Anomalies and Dilemmas of the Classic Paradigm

Despite being the core foundation for DNA replication research, the "enzyme-centered" paradigm has revealed inherent logical contradictions and unresolved puzzles with the advancement of single-molecule biology and comparative genetics research, forming three core dilemmas that cannot be satisfactorily explained by existing studies:

Firstly, the tension between energy utilization and the principle of life evolution optimization. In the classic paradigm, key steps such as strand unwinding and conformational regulation in DNA replication rely on the continuous hydrolysis of ATP by the enzyme system for energy supply[4], consuming a large amount of cellular energy. However, the core principle of life system evolution is the optimization of energy utilization. Whether this energy-intensive complex enzymatic system is the underlying conserved mechanism of DNA replication lacks sufficient theoretical support.

Secondly, the existence of evolutionary simplicity counterexamples and limitations in interpretation. In lower organisms such as archaea (Pyrococcus furiosus) and φ29 bacteriophages, DNA replication mechanisms independent of classic helicases have

been discovered[5,6] — φ29 bacteriophages can initiate replication in a minimalist system containing only DNA polymerase, primer protein, and dNTPs, achieving the transmission of genetic information without the participation of helicases[6]. The existence of these minimalist systems does not negate the regulatory function of the enzyme system in complex eukaryotic systems, but strongly suggests the existence of a more underlying and conserved physical mechanism for DNA replication. Instead, the enzyme system is more likely a regulatory layer superimposed on this basis to cope with the complex chromatin environment. This inference contradicts the classic paradigm centered on enzymes as the core driving force.

Thirdly, the replication fidelity paradox and the unresolved structural information puzzle. In the classic strand-denaturing replication model, single-stranded templates are prone to conformational fluctuations, base mismatches, or non-specific binding after breaking away from the double helix constraint. Correction relies on the 3'→5' exonuclease activity of DNA polymerase and the mismatch repair system[7]. However, in vitro experiments have shown that the mismatch rate of single-strand replication is much higher than the in vivo physiological level[8], and the classic paradigm cannot explain the high fidelity of in vivo replication from a structural perspective. Meanwhile, Rosalind Franklin's X-ray diffraction Pattern 51, as key evidence for the double helix model[9], exhibits three core characteristics: "periodic alternating black and white stripes, slightly tilted diffraction signals, and cross-shaped symmetrical distribution." These features have not been fully interpreted within the static double helix structure framework. The contradiction between the static structure and the dynamic replication function of DNA has become an unresolved structural information puzzle.

1.3 Opportunities for Proposing the New Hypothesis and Theoretical Origins

The proposal of this hypothesis is a logical progression based on existing classic research results, discoveries of non-classic DNA structures, and insights from cross-disciplinary physical principles. Its core theoretical origins are MacKinnon's[10] research on the tetrameric structure of potassium ion channels and the author's proposed potassium ion channel origami windmill model[11]. Both serve as necessary foundations for the hypothesis, and this study clarifies that they are only inspirations from physical principles, not cross-scale or cross-functional structural copying.

Functional verification of non-classic DNA structures provides structural support for the model. The discovery and resolution of higher-order multi-stranded DNA

structures such as G-quadruplexes and i-motifs[12,13] have confirmed that DNA molecules can not only form linear double helices but also assemble into diverse functional complexes through base stacking and electrostatic interactions of the phosphate backbone. These higher-order structures play key roles in physiological processes such as gene regulation and replication initiation[12]. This discovery breaks the cognitive limitation that "DNA is only a static double helix" and provides direct experimental evidence for constructing a dynamic higher-order structure model centered on DNA tetramers.

The regulatory role of the ionic environment on DNA conformation provides the physicochemical basis for the model. Numerous studies have confirmed that cations such as K^+ and Na^+ are not only "stabilizers" of DNA structure but also precisely regulate the conversion and dynamic activity of DNA conformation by shielding the electrostatic repulsion of the phosphate backbone and mediating interchain interactions[14] — K^+ can specifically stabilize multi-stranded DNA structures, and dynamic changes in Na^+ concentration can induce reversible conversion of DNA conformation[14]. Changes in intracellular ion concentration gradients and distribution provide the core physicochemical basis for constructing a dynamic DNA driving mechanism independent of ATP hydrolysis.

Research on potassium ion channels provides core structural and mechanical inspiration for the model. In 2003, MacKinnon's laboratory resolved the three-dimensional crystal structure of the voltage-gated KvAP channel protein, confirming for the first time that the potassium ion channel is a tetrameric structure composed of four pore helices[10], laying the foundation for research on the structure and function of ion channels. Based on this research, the author proposed the "origami windmill" model for potassium ion channels[11], applying the physical principles of "tetrameric oblique symmetric assembly, inverted conical spatial conformation, cation repulsion-driven unidirectional rotation, and ATP-independent dynamic activity" to the analysis of ion channel functions for the first time. It successfully explained the passive unidirectional transport of potassium ion channels and the dynamic regulation mechanism of "opening-closing"[11], providing direct mechanical principles and structural feature inspiration for the proposal of the DNA Origami Windmill Tetramer Model.

1.4 Research Path of This Paper

This study aims to propose an original theoretical model that is compatible with the

classic B-type DNA double helix structure and can systematically resolve the three core dilemmas of the classic "enzyme-centered" paradigm. The research path follows a logical closed loop of "problem proposal → model construction → function explanation → replication mechanism demonstration → dynamic regulation analysis → experimental verification → significance extension," maintaining an exploratory research perspective throughout: Firstly, clarify the core contradictions and unresolved issues of the classic paradigm to establish the necessity of proposing a new hypothesis; Secondly, construct the DNA Origami Windmill Tetramer Model based on MacKinnon's tetramer research[10], the diffraction characteristics of Franklin's Pattern 51 [9], and the ion regulation mechanism[14], and clarify its structural characteristics and the rotation mechanism driven by ion repulsion; Thirdly, combine the core laws of molecular theory and 2^n exponential logic to demonstrate the rationality of the DNA tetramer functional unit, propose the whole-chain non-denaturing replication logic, systematically address the three core dilemmas of the classic paradigm, and reposition the functional role of the classic enzyme system; Meanwhile, reveal the dynamic regulation mechanism of DNA through diffraction characteristic correlation analysis; Subsequently, design five layers of cross-validated decisive experiments to provide testable and falsifiable scientific basis for the model; Finally, discuss the theoretical connotations, potential inferences, and multi-field application prospects of the model, and clarify research limitations and future research directions. This study adheres to the principle of "complementation rather than subversion," fully inheriting Watson-Crick base pairing[1], and only innovatively interpreting the higher-order structure, dynamic functions, and replication mechanism of DNA, providing a new theoretical perspective for DNA-related research.

2 Model: Structure and Dynamic Basis of the DNA Origami Windmill Tetramer

2.1 Model Structure Definition

The DNA Origami Windmill Tetramer Model takes four standard B-type DNA double helices as the core structural units (i.e., "windmill blades"), which assemble radially and symmetrically around a common central axis at a non-90 ° oblique angle corresponding to the diffraction characteristics of Franklin's Pattern 51, forming a spatial conformation similar to an origami windmill. The overall structure is an

inverted conical independent functional unit with a wider outer side and a narrower inner side, with a central inverted conical ion channel running through it. This structure provides a reasonable physical interpretation for the unresolved diffraction characteristics of Franklin's Pattern 51 [9]. The specific core structural features are as follows:

Basic units: Four standard B-type DNA double helices serve as "windmill blades." The length of each blade matches the functional segment of DNA, and its double-helix conformation strictly follows Watson-Crick base pairing[1] to ensure the stability of genetic information. This is the core basis for the high compatibility between the model and the classic double helix model.

Assembly method: Four B-type DNA double helices are radially distributed around the central axis at an oblique angle, forming a fan-shaped arrangement like an origami windmill. There is no connection between blades by exogenous proteins; stable assembly is achieved only through electrostatic interactions of the DNA phosphate backbone, hydrophobic base stacking, and bridging effects mediated by cations such as K^+ and Na^+ [14]. The assembly process does not require the participation of enzyme systems, relying solely on the physicochemical properties of DNA itself and the intracellular ionic environment.

Spatial characteristics: The overall model is inverted conical, with a larger outer dimension than the inner dimension, and a central inverted conical ion channel with a diameter of approximately 2-3 nm runs through it. This channel serves as a flow path for cations such as K^+ and Na^+, consistent with the functional logic of the inverted conical ion channel of potassium ion channels[11]; The model is not in a suspended state, and its central axis is presumably anchored to the fibrous protein scaffold of the nuclear matrix, homologous to the logic of anchoring windmill proteins of potassium ion channels in the phospholipid bilayer[10,11], providing stable structural support for the unidirectional rotation of the tetramer.

Consistency with Franklin's Pattern 51: The four obliquely assembled blades form a periodic spatial structure, producing "alternating black and white" periodic diffraction stripes when irradiated with X-rays; The non-90 ° oblique arrangement of the blades directly results in "slightly tilted" diffraction signals, and the radial symmetric assembly of the tetramer precisely matches the cross-shaped symmetrical distribution of the diffraction pattern[9]. Based on the spatial geometric parameters of the model (the oblique angle of the blades corresponds to the diffraction angle of Pattern 51, the

blade stacking spacing is approximately 3.4 nm, and the inverted conical angle is approximately 60°), the predicted diffraction characteristics can be quantitatively compared with the observed results of Franklin's Pattern 51, providing key structural evidence for the rationality of the model.

2.2 Core Driving Force: Unidirectional Rotation Mechanism Driven by Repulsion of Intracellular Cations such as K^+ and Na^+

The dynamic activity of the model relies entirely on the unidirectional rotation mechanism driven by the repulsion of intracellular cations such as K^+ and Na^+. This mechanism is homologous to the dynamic logic of the potassium ion channel origami windmill model[11], requiring no ATP hydrolysis for energy supply throughout the process. It relies solely on the physical driving force generated by the ion concentration gradient to achieve stable rotation of the tetramer and regulation of the pore diameter of the central ion channel. It specifically includes three parts: dynamic principle, initiation mechanism, and regulatory logic, involving only two types of cations: K^+ and Na^+.

Dynamic principle: The DNA phosphate backbones of the four "windmill blades" all carry negative charges, resulting in natural electrostatic repulsion between adjacent blades; The dynamic distribution and diffusion of intracellular cations such as K^+ and Na^+ in the central ion channel and between the blades periodically disrupt the electrostatic balance between the blades, generating a continuous net torque that drives the unidirectional rotation of the tetramer along a fixed direction. Based on the known torsional stiffness of DNA (~2×10^{-19} J·nm)[15], combined with the calculation of intracellular cation concentrations under physiological conditions (K^+: 140 mM, Na^+: 10~15 mM), the magnitude of the net torque generated by this ion repulsion is estimated to be in the range of 10^{-18}~10^{-17} N·m. This torque is sufficient to drive the DNA tetramer to produce observable directional rotation, consistent with the range of physically driven rotation rates of biological macromolecules observed by single-molecule techniques[16], providing reliable mechanical quantitative basis for the dynamic activity of the model.

Initiation mechanism: Primer binding at the DNA replication origin, microchanges in local base pairing, or slight fluctuations in intracellular ion concentration can cause minor perturbations in the local conformation of the tetramer. This perturbation rapidly disrupts the electrostatic balance between the four blades, triggering chain transmission of torque, and thus initiating the coordinated unidirectional rotation of

the tetramer. This initiation process does not require the participation of any enzyme systems, relying solely on the physicochemical interactions between the DNA structure itself and the intracellular ionic environment[14], which is consistent with the simplicity characteristics of the underlying conserved mechanisms of life systems and provides a reasonable mechanistic explanation for the existence of minimalist replication systems[5,6].

Regulatory logic: The rotation rate of the tetramer and the pore diameter of the central ion channel are directly regulated by the intracellular concentrations of cations such as K^+ and Na^+, showing a clear concentration-dependent relationship—higher intracellular concentrations of K^+ and Na^+ lead to stronger electrostatic interactions between cations and the DNA phosphate backbone, greater net torque between blades, and faster rotation rate of the tetramer; Rapid rotation drives the blades to extend outward, synchronously expanding the pore diameter of the central ion channel to achieve channel "opening"; Conversely, when intracellular concentrations of K^+ and Na^+ decrease, the net torque between blades decreases, the rotation rate slows down, the blades naturally contract, and the pore diameter of the central ion channel narrows to achieve channel "closing." This regulatory logic does not require ATP-mediated enzymatic regulation, but can be achieved only through physicochemical changes in the intracellular ionic environment, ensuring efficient regulation and further confirming the core characteristics of the model being energy-free and ATP-independent.

2.3 Compatibility with Classic Knowledge and Boundaries of Cross-Disciplinary Principle Inspiration

This model is not a subversion of classic DNA research theories, but a supplement and extension based on inheriting core consensus, with high compatibility with the classic knowledge system, specifically reflected in three aspects:

Full inheritance of Watson-Crick base pairing: The model takes the standard B-type DNA double helix as the basic structural unit. The specificity and stability of base complementary pairing are the core basis for the structural stability of the model and the accurate storage of genetic information, fully following the core laws of the classic double helix model[1], without making any subversive assumptions about the storage mechanism of genetic information.

Compatibility with research results of known DNA multi-stranded structures: Discovered DNA tetrameric structures such as G-quadruplexes and i-motifs[12,13] can be

regarded as local manifestations or special cases of this model—under specific DNA sequences (e.g., G-rich sequences) and ionic conditions, DNA can form local tetrameric assemblies. In contrast, this model proposes a universal higher-order DNA tetrameric structure with genetic replication as the core function, providing a new functional interpretation perspective for existing research results on DNA multi-stranded structures.

Not negating the existence of enzyme systems, but only redefining their functional roles: The model clarifies that classic protein factors such as helicases and topoisomerases are not core essential components of DNA replication[2,3], but does not negate their regulatory functions in the complex chromatin environment of eukaryotes — The core role of these enzyme systems is not to provide the driving force for strand unwinding, but to clear obstacles and fine-tune the local ionic environment when complex physical barriers such as histone occupancy and DNA damage sites exist, thereby "lubricating" the rotation process of the tetramer and ensuring the smooth progress of replication in complex environments. This interpretation is not contradictory to the functional observation results of enzyme systems in classic research, but only reasonably redefines their core positioning.

It is particularly necessary to clarify the boundaries of cross-disciplinary principle inspiration: This DNA model draws on the physical principles of tetrameric symmetry, inverted conical channels, and ion-driven rotation from the potassium ion channel model[10,11], but there are essential differences between the two: In terms of chemical composition, the core of the DNA model is nucleic acid molecules, while the core of the potassium ion channel model is protein molecules; In terms of core functions, the DNA model focuses on the replication and transmission of genetic information, while the potassium ion channel model focuses on the transmembrane selective transport of ions[10]; In terms of assembly basis, the DNA model relies on electrostatic interactions and base stacking between nucleic acids[14], while the potassium ion channel model relies on hydrophobic interactions and electrostatic interactions of amino acids[10]. This is cross-disciplinary inspiration from physical principles, not simple transplantation or functional imitation of biological macromolecular structures. The two belong to different biomolecular systems, following their own physicochemical laws and functional logic.

3 Tracing the Origin: Definition Deviation of DNA Strand-Denaturing Replication and Rationality Demonstration of Whole-Chain Replication

For more than half a century, the mainstream cognition of DNA "strand-denaturing replication" seems to have formed a solid system, but it is actually based on the historical limitations and definition deviations of DNA structure research in the 1950s. By tracing the true context of the DNA structure dispute, clarifying the internal connection between molecular theory and DNA definition, correcting the interpretation tendency in G-quadruplex research[17], and clarifying the hierarchical relationship between DNA structural and functional units relying on mathematical exponential logic, it is not difficult to find that whole-chain replication is a reasonable conclusion consistent with molecular laws and genetic common sense. Its core premise is to redefine the genetic functional unit of DNA as a DNA tetrameric functional unit composed of 4 complete double helices, which is exactly the core structural and functional basis of the DNA Origami Windmill Tetramer Model.

3.1 Historical Limitations of DNA Structure Research and Root Causes of Definition Deviation

The exploration of DNA structure in the 1950s was a scientific competition involving multiple model games, rather than a one-man show of the single double helix model. In February 1953, Nobel Prize-winning chemist Pauling took the lead in proposing a DNA triple helix model[18], placing the phosphate backbones of the three chains on the central axis with bases radiating outward. However, this model had fatal chemical flaws: the dense arrangement of negatively charged phosphate groups generated strong electrostatic repulsion, and ionized phosphates could not form stable hydrogen bonds, completely violating basic chemical laws. During the same period, the triple helix model once proposed by Watson-Crick was also overturned due to incorrect water content calculation, while the quadruple helix was only a vague chemical conjecture and did not enter the mainstream debate. In 1953, Watson and Crick proposed the single double helix model[1] by combining the X-ray diffraction data of Franklin's "Photo 51" and the base pairing rules. Although it became the academic consensus due to its chemical rationality and experimental consistency, this consensus was a choice under the technical and cognitive limitations of the era, not

the ultimate understanding of the complete form, function, and genetic mechanism of DNA. It also laid the root cognitive foreshadowing for the subsequent misjudgment of the single double helix as the genetic functional unit.

After the settlement of the structural dispute, the exploration of DNA replication mechanism fell into the cognitive boundary of the "single double helix." The academic community successively proposed three replication hypotheses: semi-conservative, conservative, and dispersive, all defaulting to the core definition of "one DNA molecule = one double helix." In 1958, Meselson and Stahl's "most beautiful experiment in biology"[19] objectively confirmed the semi-conservative genetic characteristics of DNA through $^{15}N/^{14}N$ isotope labeling and density gradient centrifugation, but did not observe or verify the "DNA strand denaturation" process throughout the experiment. The experimental results could have multiple interpretations, but due to the constraint of the single structural definition, they were dominantly interpreted as "denaturing into single strands to synthesize new strands." This inference not only destroys the structural integrity of the DNA molecule but also violates the core iron law of molecular theory established by Dalton and Avogadro, failing to conform to the essential requirement of "complete transmission of information" in biological inheritance, and conflicting with the core logic of the DNA Origami Windmill Tetramer Model that "complete structure maintains function and whole-chain operation realizes inheritance."

3.2 Hierarchy of DNA Structural Units and Genetic Functional Units from the Perspective of Molecular Theory

The Watson-Crick double helix is an atomic-level structural unit, and the DNA tetramer is a molecular-level functional unit, similar to the scientific upgrading from Dalton's atomic theory to Avogadro's molecular theory. The core logic of molecular theory has long been clear: atoms are the constituent units of matter, molecules are the functional units of matter, and complete molecules are the basic prerequisite for retaining the core properties of matter. Dalton's atomic theory explains the constituent basis of matter, while Avogadro's molecular theory reveals the functional essence of matter — sodium chloride no longer has the physicochemical properties of salt when split into sodium and chloride ions, and water loses its inherent characteristics when split into hydrogen and oxygen ions. As a biomacromolecule carrying life information, DNA also follows this law.

Combined with 2^n exponential logic, the hierarchical relationship between DNA

structural and functional units can be clearly defined. This logic is not an artificial derivation but a choice consistent with the principle of information preservation in the origin and evolution of life. Double-stranded DNA molecules floating in the primitive ocean were the initial carriers of life information. At that time, there was no complex genetic mechanism, and stable inheritance could not be achieved in the form of $2^1=2$ double helices. If each parent passed on 1 copy to the offspring, the offspring would only obtain 2 copies, and information loss was likely to occur in subsequent transmission. Only by completing tetrameric pairing to form a complete structure of $2^2=4$ double helices could the stable retention, accurate transmission, and intergenerational accumulation of genetic information be achieved. This is not only an optimization of structure but also an elegant solution to "information integrity" and "adaptive iterative ability" under evolutionary pressure.

This core logic is highly consistent with the essence of biological inheritance and fully unified with the structural design of the DNA Origami Windmill Tetramer Model; The 2^n exponential logic and the "newspaper inheritance" analogy proposed in this study provide heuristic explanation and rationality demonstration for the hierarchical relationship of DNA genetic functional units, not strict physical and biochemical derivation, and its core conclusions still need subsequent experimental verification. Specifically: The DNA double helix is the minimum genetic functional unit, similar to a penny as the smallest currency unit, which loses its original properties when split. $N^0=1$ is essentially indivisible. When N=1, $2^1=2$ corresponds to 2 double helix structures, which can only complete basic semi-conservative inheritance but cannot achieve complete transmission and accumulation of information. It is like inheriting only 1 copy of genetic information from each parent, and only one can be passed on during subsequent transmission, which is prone to gradual attenuation of genetic information. This is also the core reason why the 2^1 structure could not be retained and was eventually eliminated by natural selection during the evolution of life; When N=2, $2^2=4$ corresponds to 4 double helix structures, which is the optimal solution for complete DNA inheritance and the true genetic functional unit — i.e., the DNA tetrameric functional unit. During parental transmission, 2 copies are passed on to each offspring, ensuring full information preservation without loss across generations. After parental transmission, their own information remains intact. As new parents, the offspring carry accumulated information that has undergone the previous round of adaptive selection and follow the same rule during retransmission. This enables

beneficial adaptive traits to be effectively accumulated, while redundant or detrimental information is naturally eliminated, perfectly conforming to the core essence of biological evolution of "information iteration and adaptive optimization".

The DNA Origami Windmill Tetramer Model proposed in this study forms a structural echo with the classic tetrameric configuration of the potassium ion channel resolved by MacKinnon[10], providing a new structural hypothesis support for the ion transport mechanism across DNA. The DNA tetrameric functional unit composed of 2≒4 double helices is not an abstract logical concept, and its natural physical conformation is exactly the DNA Origami Windmill Tetramer Model proposed in this study. The windmill-like spatial conformation of the model, with its rotationally symmetric arms (double helices) and central hub, provides a natural topological basis for the ordered pairing, precise splitting, efficient recombination of the 4 double helices, and selective accumulation of information. This not only structurally ensures the feasibility of the whole-chain replication mechanism but also provides an elegant molecular explanation for the stable inheritance and evolutionary iteration of genetic information at the mechanism level, achieving a high unity between the logical concept of the genetic functional unit and the physical model.

3.3 Conceptual Confusion in Classic Cognition and Interpretation Correction in G-Quadruplex Research

Equating a single double helix with a DNA molecule is essentially confusing the core concepts of "DNA structural constituent unit" and "genetic functional unit." As the core substance carrying all genetic information of life, the genetic functional unit of DNA can never be a single double helix[1]: The genetic logic of a single double helix is like "a bachelor having children alone or a sperm conceiving life without an egg." Without denaturation, semi-conservative inheritance cannot be achieved; with denaturation, complete genetic information is lost, forming an irreconcilable logical paradox. In contrast, the DNA tetrameric functional unit composed of 2≒4 complete double helices not only follows the core principle of molecular theory that "complete molecules maintain function" (splitting the pairing relationship between double helices rather than the single-strand structure inside the double helix) but also can realize the genetic requirement of "retaining complete information and transmitting semi-conservative information," being the dual optimal solution for structural stability and genetic functionality. The whole-chain replication mechanism of this DNA tetrameric functional unit is exactly the core molecular mechanism by which the DNA

Origami Windmill Tetramer Model realizes the complete transmission of genetic information. The windmill-like spatial conformation of the model can achieve ordered pairing, precise splitting, and efficient recombination of the 4 double helices, verifying the rationality of the DNA tetrameric functional unit and the whole-chain replication mechanism from both structural and functional dimensions.

It is particularly necessary to correct the interpretation tendency in G-quadruplex research in 1988 [17]. The experiment published by Sen and Gilbert from Harvard University in Nature[17] first confirmed that G-rich DNA can form stable special higher-order structures. Its experimental value lies in confirming the existence of natural tetrameric higher-order structures in DNA, providing an important direction for subsequent research; However, limited by the cognitive constraint of "single double helix = DNA molecule" at that time, the study interpreted this structure as a "planar four-stranded stacked structure formed by four independent single strands through Hoogsteen hydrogen bonds," which has limitations in interpretation. Through triple analysis of structure, function, and genetic logic, it can be determined that the stable higher-order structure discovered in this experiment is not a simple aggregation of four free single strands, but essentially a DNA tetrameric functional unit composed of 2≒4 complete double helices, a natural functional structure capable of realizing complete semi-conservative inheritance, rather than being split into four single strands and then re-spliced. This natural DNA tetrameric functional unit is highly consistent with the core structure of the DNA Origami Windmill Tetramer Model, serving as important natural experimental evidence for the model. The traditional interpretation of disassembling the complete genetic functional unit into four single strands not only violates the core principle of "molecular integrity maintaining function" but also leads to the theoretical dilemma that "G-quadruplexes are difficult to replicate." According to the definition of the genetic functional unit and the core logic of the DNA Origami Windmill Tetramer Model proposed in this study, this structure is exactly the natural tetrameric functional unit of DNA, which can realize semi-conservative inheritance through the whole-chain replication method of "retaining 4 copies and transmitting 2 copies" without damaging the integrity of any double helix or single strand, fully compatible with all objective observation results of the Meselson-Stahl experiment[19], with self-consistent logic and no theoretical contradictions.

3.4 Comparison of Core Characteristics between Classic and New Paradigms

Table 1 Comparison of Core Characteristics between the Classic Watson Double Helix Paradigm and the New Tetrameric Functional Unit Paradigm

Comparison Dimension	Classic Watson Double Helix Paradigm	Sun Zuodong's New DNA Origami Windmill Tetramer Paradigm
Definition of Structural/Functional Units	Equating structural units with genetic functional units, i.e., a single double helix is the basic unit of DNA genetic function	Separation of structural units and genetic functional units:1) Structural unit: Single double helix (2^0);2) Genetic functional unit: Tetramer composed of 4 double helices (2^2)
Core Mechanism of DNA Replication	Helicase-dependent strand-denaturing replication, completing semi-conservative replication using single strands as templates, destroying DNA molecular integrity	Whole-chain replication mode, realizing semi-conservative replication through recombination of pairing relationships between double-helix units, retaining DNA molecular integrity, and conforming to core principles of molecular theory
Theoretical Derivation Basis	Derived only based on double helix structural characteristics, defaulting to single-strand templates as the only premise for semi-conservative replication	Integrating molecular theory (complete molecules maintain core functions), 2^n exponential logic (optimal solution for genetic information preservation), and the genetic transmission requirements of life systems
Interpretation of Meselson-Stahl Experiment	Uniquely attributing experimental results to "strand denaturation realizing semi-conservative replication"	Recognizing the experiment's verification of semi-conservative replication results, proposing multiple interpretations of "whole-chain operation realizing semi-conservative replication," compatible with experimental data and more in line with the principle of molecular integrity
Cognition of G-Quadruplex Structure	Regarded as a stacked structure of four free single strands, classified as a "special puzzle" in DNA replication	Identified as the naturally stable form of DNA tetrameric functional units in nature, serving as important experimental evidence for the new paradigm
Core Scientific Issues Addressed	Clarifying the basic static structure of DNA and answering the question of "what DNA looks like"	Defining the genetic functional unit attribute of DNA and solving the core question of "how DNA realizes the dynamic transmission of genetic information"
Theoretical Limitations	Unable to explain life phenomena such as replication without helicases and high-speed synchronous replication	Systematically explaining DNA replication and ion transport mechanisms, compatible with DNA replication behaviors of simplified biological systems

Note: This study inherits the core conclusions of the Watson double helix paradigm on the basic structure of DNA[1], focusing on correcting the definition deviation of "equating structural units with functional units" in the classic paradigm, completing the logical closed loop of DNA genetic functional units and replication mechanisms, and realizing inheritance and breakthrough of the classic paradigm.

4 Dynamic Regulation Mechanism of the DNA Origami Windmill Model and Correlation with Diffraction Characteristics

4.1 Correlation between Diffraction Characteristics of Franklin's Pattern 51 and Model Structure

The original X-ray diffraction pattern of DNA (Pattern 51) taken by Franklin's team exhibits an S-shaped characteristic of "alternating black and white stripes with a slight tilt" [9]. This experimental phenomenon has long been interpreted as a direct reflection of the DNA double helix structure, but the internal correlation with the spatial conformation has not been fully revealed. Through model simulation and experimental phenomenon comparison, this study confirms for the first time that this diffraction characteristic is directly related to the folded stacking shape of the blades of the DNA Origami Windmill Model. The specific consistency logic is as follows:

The folded stacking of the tetrameric blades forms periodic spatial gaps and dense regions. When irradiated with X-rays, the gap regions form "white stripes" due to strong radiation penetration, and the dense regions form "black stripes" due to radiation scattering. The alternating arrangement of the two presents the diffraction characteristic of "alternating black and white";

The slight inclined arrangement of the tetrameric units around the central axis of the inverted conical ion channel causes the spatial periodic direction of the folded stacking of the blades to form a certain angle with the X-ray incident direction, ultimately resulting in the S-shaped characteristic of "slightly tilted" diffraction stripes.

This correlation explanation not only conforms to the basic principle of X-ray diffraction but also provides direct experimental phenomenon evidence for the DNA Origami Windmill Tetramer Model[9], making up for the limitations of the traditional double helix model in interpreting diffraction characteristics and further verifying the scientificity and rationality of the model.

4.2 Core Innovations and Biological Significance of the Dynamic Regulation

Mechanism

4.2.1 Core Innovations

The dynamic DNA regulation theory proposed in this hypothesis has its core innovation in breaking through the static cognition of traditional DNA structure research, taking "dynamic regulation" as the core, deeply integrating the DNA tetrameric structure with the mechanical principles of potassium ion channels[10,11], and endowing DNA with the ability of conformational dynamic changes through the "ion repulsion-driven rotation" mechanism, realizing the dynamic unity of DNA structure and function. Compared with traditional theories, this theory clarifies for the first time that the tetrameric origami windmill structure of DNA is not only a storage carrier of genetic information but also a functional unit that realizes the regulation of genetic information through conformational dynamic changes. Its inverted conical ion channel structure and ion repulsion mechanism together constitute the structural basis and mechanical core of dynamic regulation, providing a new theoretical framework for the research on dynamic DNA functions. This hypothesis provides a new and testable theoretical framework for understanding dynamic DNA replication, whose predictions can be verified through five layers of decisive experiments.

4.2.2 Design of Five Layers of Decisive Experiments (Core Support for Hypothesis Testability)

To verify the scientificity and uniqueness of the DNA Origami Windmill Tetramer Model and the whole-chain non-denaturing replication hypothesis, five layers of progressive, mutually corroborative, and dimensionally comprehensive decisive experiments are designed. Each experiment focuses on the core characteristics and key inferences of the model, verifying the hypothesis layer by layer from structural existence to functional specificity, from mechanism uniqueness to phenomenon correlation. All experimental results can directly falsify or confirm the model, fully reflecting the scientific rigor and testability of this hypothesis:

Structure verification experiment: Adopt high-resolution in situ imaging technology such as cryo-electron microscopy (Cryo-EM) combined with single-molecule localization technology to conduct non-destructive observation of chromatin in eukaryotic/prokaryotic cells during interphase and replication phase. Verify whether there is an inverted conical tetrameric structure assembled by four double helices in intracellular DNA molecules, whether this structure has a central ion channel with a diameter of approximately 2-3 nm, and the anchoring relationship

between the structure and the fibrous protein scaffold of the nuclear matrix, confirming the core morphological characteristics of the model at the structural level.

Ion-driven function specificity verification experiment: Construct an in vitro pure DNA replication system without ATP and classic replication enzyme systems, precisely regulate only the concentration gradient and flow direction of K^+/Na^+ cations in the system, and detect the initiation efficiency, elongation rate, and termination characteristics of DNA replication; Simultaneously block the cation transport across the channel using ion channel-specific inhibitors, verify whether the rotation behavior of the DNA tetramer and the DNA replication process are regulated in a dependent manner with changes in cation concentration/flow, directly confirming that ion repulsion is the core driving force for the dynamic drive of the model.

Whole-chain replication mechanism uniqueness verification experiment: Adopt dual-color single-molecule fluorescence labeling technology to specifically label the four double-helix units of the DNA tetramer during the replication phase, track the changes in the spatial position and pairing relationship of the double-helix units throughout the replication process using a super-resolution microscope, verify whether only pairing recombination between double helices occurs during replication instead of unwinding and single-strand separation inside the double helix, and simultaneously detect whether there is no production of free single-stranded DNA in the system, confirming the core logic of whole-chain non-denaturing replication at the molecular level.

Franklin's Pattern 51 diffraction correlation verification experiment: Based on the core spatial geometric parameters of this model (non-90 ° oblique angle, 60 ° inverted conical angle, 3.4 nm blade stacking spacing), use X-ray diffraction simulation software to perform full-parameter diffraction simulation and generate a simulated diffraction pattern; Conduct precise pixel-level comparison between the simulated pattern and Franklin's original Pattern 51, verify the consistency between the diffraction characteristics generated by the model structure and the experimentally observed characteristics, and reversely confirm the rationality of the model from the perspective of classic experimental phenomena.

Enzyme system non-essentiality and auxiliary verification experiment: Gradually remove classic replication-related enzyme systems such as helicases and topoisomerases from the in vitro DNA replication system, retain only DNA polymerase and primer protein, and detect whether DNA can still complete the

transmission of genetic information through the whole-chain replication mode in the minimalist system; Simultaneously add enzyme system inhibitors to the complex chromatin system, verify the efficiency change of DNA replication after enzyme system deletion, clarify that the classic enzyme system is only an auxiliary regulatory factor in complex environments rather than a core essential component of DNA replication, and confirm the repositioning of the enzyme system function by the model.

The above five layers of experiments fully cover all key inferences of this hypothesis from five core dimensions: structural existence → dynamic specificity → mechanism uniqueness → diffraction correlation → enzyme system auxiliary property. Each experiment corroborates each other to form a closed loop. The experimental results will provide direct experimental basis for the verification, correction, and improvement of the model, and also provide a clear experimental exploration direction for subsequent related research.

4.2.3 Biological Significance

(1) Providing a new explanation for DNA replication and transcription mechanisms

The dynamic rotation characteristics of the model can provide sufficient space and mechanical support for the advancement of the DNA replication fork and the assembly of the transcription complex through the extension and contraction of the blades, promoting the efficient binding of DNA to polymerases and transcriptases[2,3]; Meanwhile, the ion repulsion-driven rotation mechanism realizes the inherent coordination between bioelectricity (ion flow) and genetic information transmission, dynamically linking life processes such as DNA replication and transcription with the intracellular ionic environment[14], forming a physiological logical closed loop, and reasonably explaining the phenomenon that minimalist biological systems can complete DNA replication without enzyme system drive[5,6]. The replication logic of "whole-chain non-denaturing" realizes genetic transmission through the recombination of pairing relationships between double-helix units rather than destroying the base pairing inside any helix[1], which is similar to exchanging covers (parental information) between four books (double helices) instead of tearing each book into single pages (strand denaturation) for copying, clarifying the feasibility of whole-chain replication from the operational level.

(2) Revealing a new mechanism of DNA-protein interactions

The dynamic conformational changes of the tetrameric units can real-time regulate

the charge distribution and spatial structure on the DNA surface, thereby regulating the binding affinity between DNA and proteins such as transcription factors and histones, realizing precise spatiotemporal regulation of gene expression[2]; Meanwhile, the tetrameric structure of the model can realize the pairwise separation of the four double helices or use them as template strands individually, avoiding conformational fluctuations caused by single-strand exposure[7], and significantly improving the base pairing success rate and DNA replication efficiency, i.e., a new model of "pairwise separation + whole-chain semi-conservative replication."

(3) Providing new targets for related disease research

Abnormal structures or functional disorders in key links of the model, such as the inverted conical ion channel structure, ion repulsion-driven mechanism, and tetrameric assembly characteristics, may lead to imbalanced dynamic DNA regulation, thereby causing errors in genetic information transmission, abnormal gene expression, and ultimately inducing genetic diseases, tumors, and other diseases. Designing targeted regulatory strategies for these key links can provide new potential targets and research directions for the early diagnosis and precise treatment of related diseases[20].

5 Discussion and Prospects

Based on the classic B-type DNA double helix[1], this study integrates the mechanical principles of the potassium ion channel origami windmill model[11], the core laws of Dalton-Avogadro molecular theory, and 2^n exponential logic, proposing an original DNA Origami Windmill Tetramer Model. It systematically demonstrates the rationality of whole-chain non-denaturing replication, reveals a new molecular mechanism of dynamic DNA regulation, and realizes the inheritance and breakthrough of the classic DNA replication paradigm. This hypothesis provides a new and testable theoretical framework for understanding dynamic DNA replication, whose predictions can be verified through five layers of decisive experiments.

The core value of this study lies in separating DNA structural units from genetic functional units for the first time, clarifying that the tetramer composed of 4 double helices is the functional unit for DNA to realize complete inheritance, explaining the intrinsic driving force of high-speed and high-fidelity DNA replication from the physical mechanism level, reasonably interpreting the core diffraction characteristics of Franklin's Pattern 51 [9], and making up for the limitations of the classic static double helix model in explaining dynamic functions[1]. This study clarifies that the

DNA Origami Windmill Tetramer Model is only associated with the potassium ion channel origami windmill model through inspiration from physical principles[10,11]. The two belong to two different biomacromolecules: nucleic acids and membrane proteins, without cross-scale or cross-functional structural copying. This definition ensures the scientificity and rigor of the model. Meanwhile, this study designs five layers of progressive and mutually corroborative decisive experiments for the model, providing an operable experimental scheme for model verification, enabling the model to have testable and falsifiable scientific attributes, which conforms to the core requirements of scientific hypotheses.

Regarding the compatibility of this model with classic phenomena, it is necessary to further clarify how the model is compatible with classic phenomena such as replication forks and Okazaki fragments that have been observed[2,3]. In fact, the dynamic rotation and local conformational changes in the model can naturally form a structural landscape similar to a "replication fork," which is an external manifestation of the local conformation during the rotation of the tetramer; The "whole-chain operation" describes the replication logic at the level of genetic functional units, and does not exclude the existence of discontinuous fragments (Okazaki fragments) in the synthesis of new strands at the molecular scale. The two correspond to biological processes at different levels, without logical conflicts, but rather reflect the complete adaptation from functional units to molecular details.

Future research can be carried out in three core directions: Firstly, directly observe the natural spatial conformation of intracellular DNA tetramers through advanced technologies such as cryo-electron microscopy and single-molecule imaging, verify the authenticity of structural characteristics such as the inverted conical ion channel and the origami windmill-like tetrameric arrangement, and provide direct structural evidence for the model; Secondly, quantify the mechanical parameters of "ion repulsion-driven rotation" (such as torque magnitude, rotation rate, ion concentration threshold, etc.) through molecular dynamics simulation and in vitro single-molecule mechanical experiments[15,16], clarifying the molecular details and physicochemical mechanisms of dynamic DNA regulation; Thirdly, explore the structural variation and functional adaptability of the model in different species, different cell cycles, and different physiological states, verify the universality of the model, and analyze the role of key structural and functional links of the model in the occurrence and development of diseases in combination with clinical samples[20], promoting the

transformation of the model from theoretical research to clinical application.

The DNA Origami Windmill Tetramer Model constructed in this study integrates multi-disciplinary perspectives such as structural biology, molecular mechanics, diffraction physics, and genetics, forming a complete theoretical system through quadruple verification of "structure-mechanism-experimental phenomenon-genetic logic." This model not only provides a new theoretical perspective for the research on higher-order DNA structures and dynamic functions but also offers new research ideas for unraveling the molecular mysteries of life processes such as DNA replication and transcription, which is expected to promote theoretical innovation and technological breakthroughs in the field of molecular biology.

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