2

Replication Demands an Amendment of the Double Helix

You Cheng Xu
UT Southwestern Medical Center at Dallas
USA

1. Introduction

DNA is a very important macromolecule in biology. It carries the genetic code for every living creature.

The finding of the double helix is undoubtedly one of the most significant discoveries in the twentieth century (Watson & Crick 1953a). It inspired many important discoveries in biology and medicine. Now, the double helix has become an icon of molecular biology (Olby, 2003).

Presently, DNA is widely accepted as a right-handed double helix taught in almost all textbooks of biochemistry and molecular biology. The knowledge of DNA is widely applied in scientific research, industry, agriculture and medicine. The information of DNA has been successfully used in many fields previously unimaginable: archaeology, drug design, forensic science, nanometer technology, etc.

After more than 50 years of intensive investigation, the basic idea of the Watson- Crick Model is still considered to be correct (Crick et al. 1979; Arnott 2006). Innumerable sequencing data proved that the two anti-parallel strands of the DNA are held by hydrogen bonds between A•T and G•C base pairs. The secondary structure of it was additionally supported by the X-ray crystallography from the double stranded oligo-deoxyribonucleotides. Ironically, prior to the right-handed B-DNA, the detailed molecular structure of main atoms in a left-handed Z-DNA was determined by X-ray crystallography (Wang, et al., 1979). Nevertheless, Z-DNA is generally assumed as a special form and seldom found in native DNA, since its presence needs alternative purine-pyrimidine sequence and some special conditions.

The helical nature of the double helix involves a topological problem for its replication. Watson and Crick (1953 b) were aware of the problem right after their discovery. They stated: "Since the two chains in our model are intertwined, it is essential for them to untwist if they are to separate…. Although it is difficult at the moment to see how these processes occur without everything getting tangled, we do not feel that this objection will be insuperable."

To avoid this objection, many side-by-side models were proposed. (Cyriax & Gäth, 1978; Rodley et al., 1976; Sasisekharan & Pattahireman 1978). Unfortunately, no substantial evidence was available for solving the puzzle of double helix (Yagil, 1991; Schvartzman & Stasiak, 2004).

2. The accumulated facts against the right-handed double helix

In 1958, Meselson and Stahl reported their classic experiment which convincingly proved that the two parental strands of *E.coli* were completely separated after each round of replication. That kind of semi-conservative mechanism becomes a basic rule in molecular biology. However, according to the Watson-Crick Model, there are 10 base pair per turn which raises a serious problem from the purely right-handed DNA duplex.

Let's focus our attention on the replication of DNA in *E.coli* which is the best known prokaryotic cell. It is well known that the DNA replication in *E.coli* is a very fast process. In rich medium, the doubling time of *E.coli* is only 20 minutes at 37^{o}C. Each of the replication fork advances at 1 kb per second. According to the classical double helix model, the two parental strands have to untwist at the speed of 100 rounds per second or 6000 rounds per minute. The question is how can such a quick unwinding movement of the double helix proceed in the viscous cytosol where the friction is expected to be very high?

At first, the findings of the gyrase and other topoisomerases lead many scientists to believe that untwisting of DNA is no more a problem. Further investigation revealed that the only two enzymes responsible for untwisting DNA during *E.coli* replication are gyrase and topoisomerase IV. The reaction mechanisms of both enzymes are very complicated (Berger, et al 1996); and they catalyze an inter- or intra-molecular strand passing reaction respectively. And only 2 linking numbers were changed in each reaction.

Gyrase is the main operator for unwinding the DNA duplex, and its reaction rate is only 6 times per minute (Ullsperger & Cozzarelli, 1996). Whereas, topoisomerase IV is responsible for the separation of two mature chromosomal DNA molecules generated at the end of replication. Both enzymes were vital for the survival of *E.coli.*

The chromosomal DNA of *E.coli* is very long and circular. The base pair number in one of the sequenced *E.coli* DNA is 4,639,221 (Blattner et al. 1997). Hence, the linking number of *E.coli* chromosomal DNA should be around 4 X 10^5. The replication requires the two strands to be completely separated and distributed into two daughter cells. That means that the gyrase has to reduce the linking number from 4X 10^5 to exactly zero within a very short period of time (40 minutes in a fast growing *E.coli* cell). The slow reaction rate of gyrase definitely makes it unable to accomplish this task.

Digging deeper, more problems would be encountered: a) It is generally assumed that the DNA duplex can transfer the supercoiling from one region to another region like a car speedometer cable. In a rapidly growing *E.coli*, there are more than 6 replication forks (Skarstad et al. 1986). The positive supercoiling generated during the synthesis of new DNA is very difficult to transfer to the terminal along the highly twisted chromosomal DNA confined in the nucleoid (Zimmerman, 2004). b) At the same time, many tRNAs, ribosomal RNAs and mRNAs were being actively transcribed from the same chromosomal DNA. Hence many sites of the chromosomal DNA were occupied by various enzymes and nucleic acids. These macromolecules attached to the chromosomal DNA would physically block the advancement of DNA replication. In addition, the positive supercoiling generated in front of several replication forks and many transcription sites are also very difficult to pass through these regions. c) Each gyrase binds to around 150 base pairs, a toposite, on the chromosomal DNA (Bates & Maxwell, 1989; Condemine & Smith, 1990). Only the gyrase located in front of the replication fork is effective for the separation of parent strands. The effective toposites would be less and less as the bi-directional replication forks advance to their unique terminal. The rate of DNA replication would greatly slow down due to the less available toposites and consequently less effective gyrase. This imaginative effect has never been

found. d) The structure of chromosome itself can cause additional trouble in DNA replication. Inside the bacteria, the chromosomal DNA is composed of many supercoiled domains; each of them containing abundant amounts of proteins, (Travers & Muskhelishvili, 2007). Although the detailed structure of these domains is not clear, they are topologically independent of each other. Besides, the binding of chromosomal DNA to the cell membrane may prevent the rotation of speedometer cable-like-DNA (Bravo et al. 2005).
In brief, these theoretical considerations or arguments are almost no use for solving the problem in reality. They just provide something for us to remember while investigating the mechanisms of DNA replication or RNA transcription.
From the view point of topology, how the linking number drops from $4x10^5$ to zero is an unavoidable and difficult question for any biochemist.
Scientists are not easily to be swayed by eloquence. To solve these topological problems, solid evidence is badly needed.

3. The disproof of the classical double helix

The topological problem involved in DNA replication is evident to many scientists. It greatly agitates the curiosity and interest of many scientists. Common sense tells us that the high speed unwinding is unlikely the answer for the quick DNA replication. It is our understanding that all biochemical processes can be deciphered by chemistry and physics. The complicated process of DNA replication should not violate the basic laws of chemistry and physics.
After the initial literature searching and inspired by the results of experimental exploration, a hypothesis was proposed that the two strands may not wind as strictly as announced in the Watson-Crick Model (Xu et al. 1982; Xu & Qian, 1983).
However, the evidence in these papers was unable to convince many scientists to believe that the native DNA may differ from the classical double helix model. Some experts in the field did not think the suggested idea worthy following. It is true that inspiration or intuition cannot be judged by logic reasoning for arguments in science. The author has to find some other concrete evidence to support this new hypothesis. An effective way is to find the illegitimacy of the assumption—"**All DNA duplex is right-handed double helix**", an assumption that is deeply rooted in the minds of many scientists.
When trying to argue with a prevalently accepted scientific doctrine, such as the idea of the Watson-Crick Model, disproof may be the only way of choice. Just as psychologist Csikszentmihalyi (1996) once stated: "What I try to do is to disprove certain widespread assumptions. The advantage of disproof over proof in science is that whereas a single case can disprove a generalization, even all the cases in the world are not enough for a conclusive positive proof. If I could find just one white raven that would be enough to disprove the statement: ‹**All ravens are black.**›"
Fortunately, such disproof was found after many years of investigation. The finding of a zero linking number topoisomer is similar to the finding of Achilles' heel. Except in Z-DNA, the presence of a zero linking number topoisomer is unexplained by the classical double helix model.

3.1 Finding of a zero linking number topoisomer

The most straight forward test is to measure the linking number of a set of pure topoisomers by electro-microscopy. The method was found serendipitously from relaxed plasmids (Xu, 2009).

pBR322, a head to tail dimer circular double stranded DNA containing 2 X 4361 base pairs, was chosen in this experiment (Watson, N. 1988). According to the double helix model, 10 base pair per turn, the linking number of this plasmid is estimated to be around L ≈ 2 X 4361/10 = 872.

In solution, the double helix is in B-DNA form, and the helical repeat is 10.4 base pair per turn. (Wang, 1979; Rhodes & Klug, 1980). So, the adjusted linking number of dimer pBR322 should be around L ≈ 2 X 4361/10.4 = 838.

Supercoiled DNA was prepared from an *E.coli* strain HB101 harboring the dimer pBR322. The pure plasmid was converted into relaxed form. Each relaxed DNA topoisomer was collected from a preparative agarose gel with great precaution. Each relaxed DNA was carefully denatured by glyoxal which effectively prevents the formation of hydrogen bonds between the complementary strands. After appropriate EM procedurals, the paired SSC DNAs of the individual topoisomer can be visualized by EM as shown in Figure 1. These EM pictures are just enlarged images of the tiny DNA molecules projected on a two dimensional plan.

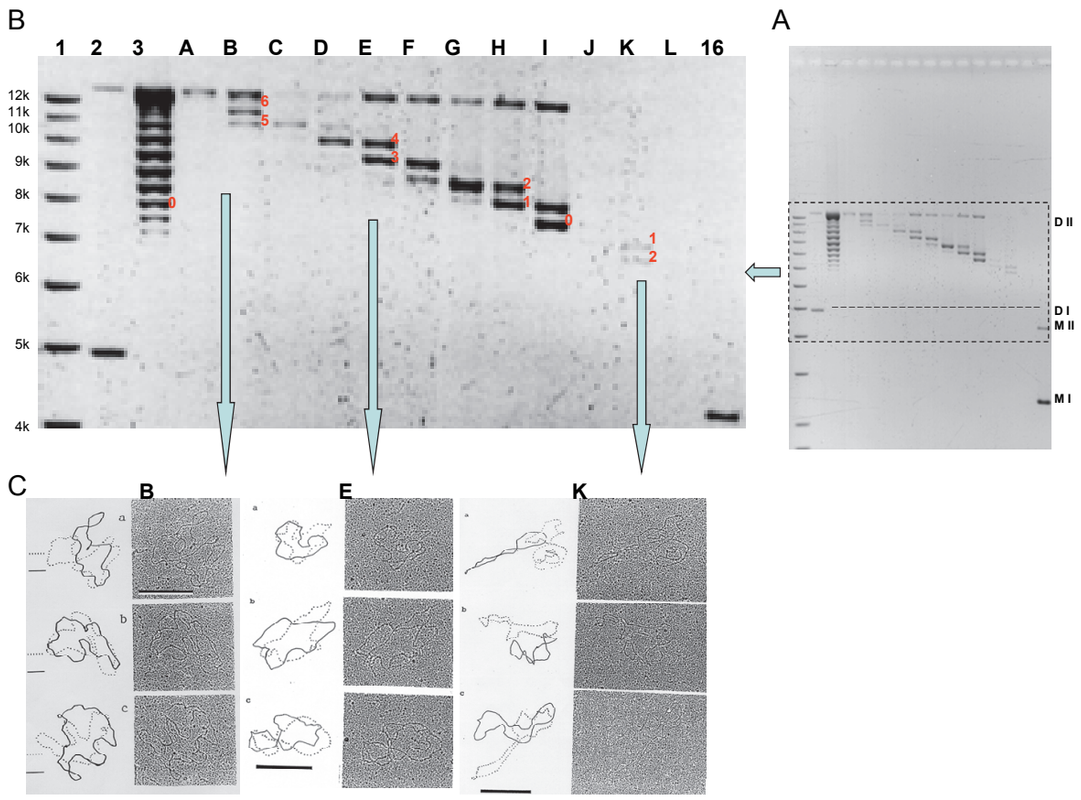

Fig. 1. The AGE purified pBR322 topoisomers were denatured and examined by EM. A) Relaxed pBR322 DNA dimers were purified by AGE and checked again on AGE. The electrophoresis buffer containing 1 μg chloroquine/ml. B) Lane 1, 1 kb DNA marker; Lane 2, supercoiled dimer; Lane 3, relaxed dimer; Lane A, B, C, D, E, F, G, H, I, J, K, L, are purified fractions; Lane 16, supercoiled monomer. (Only the nicked monomer appears in this picture). C) The fractions B, E, K were denatured by glyoxal and checked by EM. The bar represents 0.5μm. The red numbers represent absolute topological number, | Lk | (Xu, 2009).

Under optimal conditions, each linkage of the two rings generates two crossings on their two dimensional projection. The linking number of the denatured topoisomer molecule can be obtained by counting the crossing number of the two SSC DNAs on EM picture and divide it by two.
Surprisingly, the observed linking number greatly deviated from the value expected from the Watson-Crick Model.
Many reasons indicate that this result is not an artifact or an occasional occurance. Two of the cardinal reasons are that each topoisomer has the same measured linking number and that all the data is consistent with the established notion that the linking number differs by one between neighboring bands on the agarose gel (Crick et al. 1979). The individual EM pictures were thus fused into cohesive evidence.
The tricky part is that the zero linking number topoisomer itself could not be convincingly proved by checking the image of this denatured topoisomer. The reason is that an image of SSC DNA found under the EM may either come from a denatured zero linking number topoisomer or from the dissociated nicked DNA.
However, a zero linking number topoisomer can be definitely located on the agarose gel from the measured linking numbers of three different topoisomers.
A reasonable deduction is that the absolute linking number of supercoiled DNA is higher than that of relaxed DNA. This deduced result is remarkably different from the contemporary theory of DNA supercoiling.
An additional test was carried out to compare the EM pictures of supercoiled and relaxed monomer pBR322 DNA in their denatured form. Figure 2 clearly indicates that the absolute linking number of supercoiled DNA is higher than that of relaxed DNA. On the EM picture, the two SSC DNAs of relaxed pBR322 in relief exclude the possible overlapping of two independent SSC DNAs. Since the relaxed DNA samples were prepared from monomer pBR322 which is pure without any dimer as shown in figure 1 A and B, it excludes the presence of any catenated double stranded DNA.
It should be noted that similar EM pictures of denatured supercoiled PM2 DNA molecules were first published in 1975 (Brack et al.). Although the authors of the paper did not give their explanation to this phenomenon, their finding should be considered as extra evidence supporting the above results. PM2 DNA comes from a big bacterial virus carrying 10079 base pairs. Although its linking number cannot be clearly obtained from their EM pictures, its crossing number is estimated to be much less than $2x\ 10079/10.4 \approx 1938$.
With the observations from dimer or monomer pBR322 and PM2 DNA, it is appropriate to say that the linking numbers of covalently closed circular DNA are much less than that expected from the Watson - Crick Model.
Seeing is believing. Although the detailed winding direction of the two strands in the double helix was unable to be seen, the combination of EM and topological knowledge of circular DNA helps us to know that they could not always winding in one direction. That can be assumed as a disproof of the assertion that all DNA is right-handed double helix.

3.2 Annealing of two complementary SSC DNAs

The EM evidence has often worried some scientists who were not confident in the results obtained from this method. Further evidence is required to make sure that this eccentric idea is worth considering. To find out if the zero linking number topoisomer can be assembled by two independent complementary SSC DNAs, an experiment was performed as shown in Figure 3.

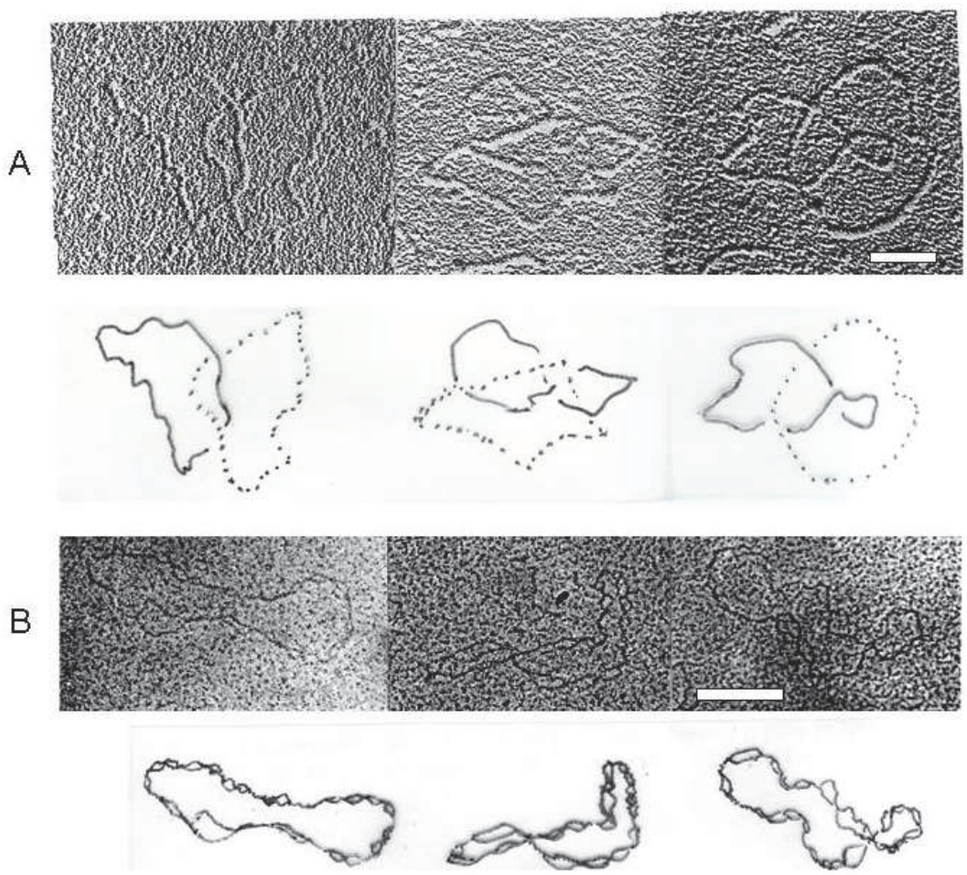

Fig. 2. EM pictures of pBR322 DNA molecules denatured by glyoxal. The bar represents 0.5μm. A) Relaxed DNA in relief, the EM samples were additionally shadowed in one direction. (B) Supercoiled DNA.

After singly nicked pBluescript DNA was obtained, a mixture of SSC DNA can be collected from alkaline sucrose gradient centrifugation. Under appropriate conditions, the annealing product of this SSC DNA was examined by a two dimensional AGE. A special topoisomer band appeared on the agarose gel, which is neither DNA II, DNA III nor DNA V (Stettler et al., 1979), but similar to one of the native topoisomers. According to topology, it strongly indicates that the linking number of this annealing product is zero, since the annealing solution contains nothing but the two complementary SSC DNAs together with a few chemical reagents.

Thus, the zero linking number topoisomer was proved to be the case by two different ways, i.e., disassembling and assembling with EM and AGE respectively.

Biegeleisen (2002) mentioned an interesting story about the assembling of complementary SSC DNA test conducted by Dr. Robert Chambers: "After becoming aware of the publication of the Stettler paper, Chambers retired his painstakingly isolated preparation of complementary single-stranded circular DNA to the refrigerator. Three months later, a significant portion of it had turned into Form I. Chambers, a staunch 'traditionalist' was unwilling to challenge the Watson-Crick theory, and, perhaps because he was unable to

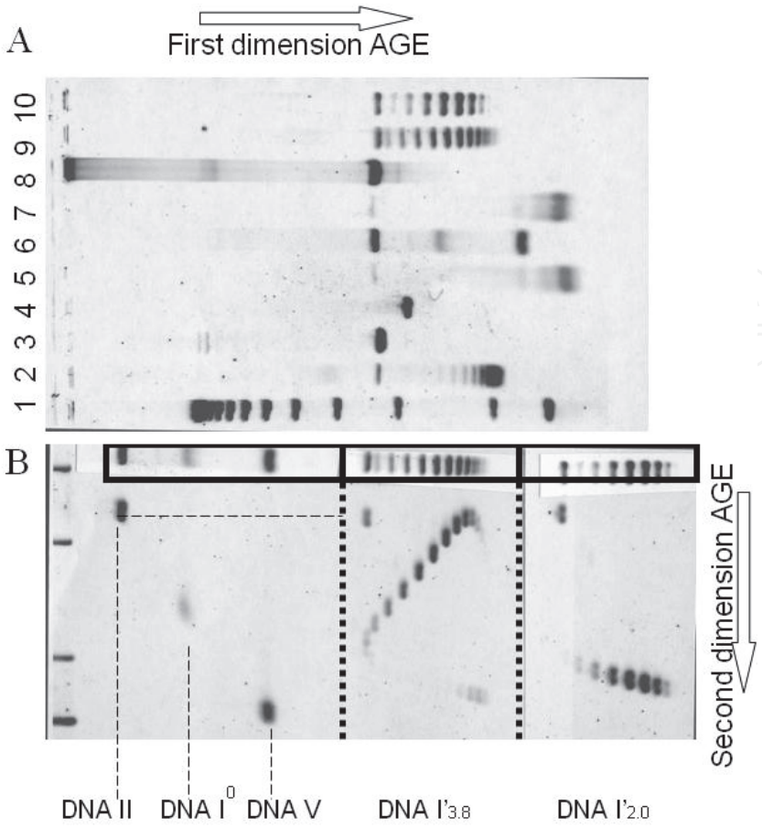

Fig. 3. The assembling products of pBluescript SSC-DNA. A) First dimension AGE in TBE buffer containing 1 µg chloroquine / ml. Lane 1, 1 kb molecular marker; Lane 2, supercoiled DNA; Lane 3, singly nicked DNA; Lane 4, linear DNA; Lane 5, SSC DNA; Lane 6, annealed SSC DNA; Lane 7, SSL DNA; Lane 8, annealed SSL DNA; Lane 9, DNA relaxed in the presence of 3.8 µg EthBr/ml; Lane 10, DNA relaxed in the presence of 2.0 µg EthBr/ml. B) Second dimension AGE in the TBE buffer containing 5µg EthBr/ml. Three slides of the sample in the first dimension were turned 90˚ for second dimension AGE. In the 3 square boxes, the 3 samples were electrophoresised in first dimension only and pasted in the way that keeps the nicked DNA alined with the corresponding nicked DNA in second dimension gel (Xu, 2009).

provide a satisfactory explanation for his discovery in terms of 'traditional' theory, he chose not to publish it (R.W. Chambers, personal communication, 1978)."

The finding of the zero linking number topoisomer is directly against the rule of DNA topology written in most textbooks. It is also a disproof of the idea that the two strands of DNA are always winding plectonemically in the right-handed direction.

3.3 Figure eight test

DNA structure is such an important molecule; that when trying to make even a slight modification one should be very careful and cautious. However, the finding of zero linking number questions the validity of the traditional double helix model. Advised by Wang, (A Mallinckrodt Professor of the department of Biochemistry and Molecular Biology at Harvard University), a figure eight test is designed to check whether DNA is really a right-handed duplex.

A 2 kb fragment of Hind III cleaved λDNA (from 23100 to 25157) was inserted into M13mp19 in opposite directions. The two SSC DNAs, each containing a 2 kb fragment complementary to the other one, can be prepared from two kinds of phage separately. It would be interesting to see the shape of the annealing product of these two SSC DNAs. According to the Watson-Crick Model, the annealed 2 kb fragment should have 200 right turns, that would force the rest part of the single stranded M13 turning 200 times left-handedly or turn the whole molecule into highly supercoiled form, so as to keep the linking number unchanged, i.e., $T_{right\text{-}handed} + T_{left\text{-}handed} + Wr = 0$. The experimental result is quite unexpected as shown in Figure 4.

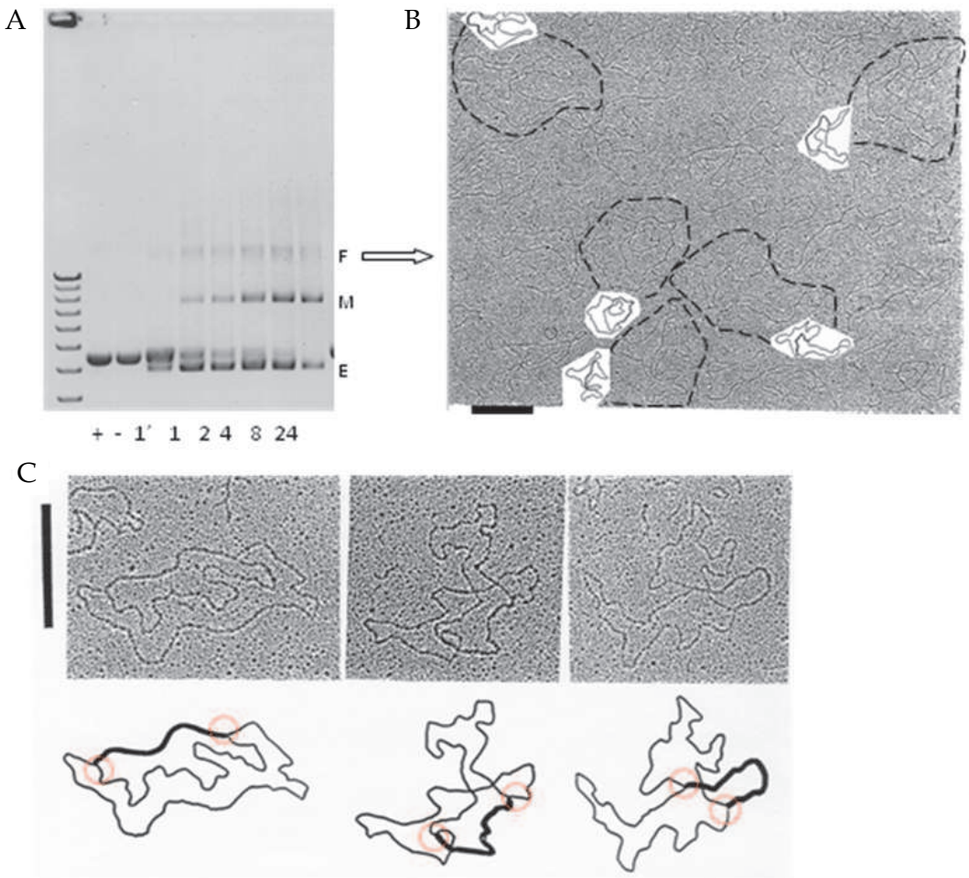

Fig. 4. Figure eight structures obtained from two SSC-DNAs with a 2 kb fragment inserted in opposite orientation. A) AGE of annealing products at different times. Lane 1, 1 kb DNA marker; Lane 2, SSC DNA+; Lane 3, SSC DNA—; Lane 4, 5, 6, 7, 8, 9, annealing of the two SSC DNA after 1 minute, 1, 2, 4, 8, 24 hours. B) The EM of annealing product from fraction F. A half sized image is pasted at the vicinity of each figure eight molecule. The bar represents 0.5μm. C) The three typical figure 8 molecules under EM. The bar represents 0.5μm (Xu, 2009).

The annealing product is just like figure Θ with two forks connecting the double stranded DNA with single-stranded DNA. Whereas, no product was found that resembles anything that could be expected from the double helix model.

The test clearly indicated that the 2 kb fragment of λHind III contains both right-handed and left-handed DNA. Due to the cancellation of the opposite twists, its net twist is close to zero. This result is consistent with other experimental findings showing that in native DNA, the two strands may wind in both directions. It constitutes one more piece of disproof of the right-handed double helix.

3.4 Denaturing singly nicked DNA

A much simpler experiment is also helpful to the understanding of the double helix. When singly nicked plasmid was denatured with alkaline, the fast denaturation process reflects the two strands of pBR322 DNA are unlikely winding 431 times. As shown in Figure 5, the singly nicked DNA can be denatured quickly within 10 minutes, one minute and even 1 second respectively.

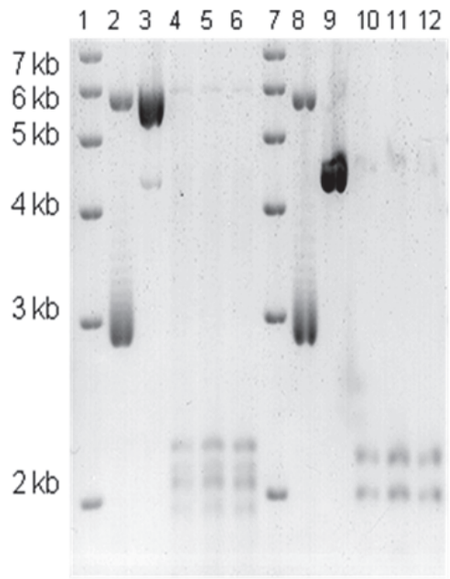

Fig. 5. AGE of pBR322 DNA II and DNA III denatured by NaOH. Lane 1 and 7, 1 kb DNA marker; Lane 2 and 8, supercoiled DNA; Lane 3, 0.5 μg of singly nicked DNA; Lane 4, 5, 6, 0.5 μg singly nicked DNA denatured by equal volume of 0.5 N NaOH after 10, 1 minute and 1 second; Lane 9, 0.5 μg linear DNA; Lane 10, 11, 12, 0.5 μg linear DNA denatured by equal volume of 0.5 N NaOH after 10, 1 minute and 1 second (Xu, 2009).

The powerful AGE separates the two kinds of SSC DNA and SSL DNA into 4 bands. It takes more thinking to figure out the denaturing process of this singly nicked DNA. In solution, the nicked DNA is moving in a three dimensional solution. Alkaline is supposed to destroy the hydrogen bond instantly that makes the SSL DNA departing from its complementary SSC DNA. According to the double helix model, a quick unwinding of the SSL DNA is required, that means that the two ends of the SSL DNA have to rotate in opposite directions. The question is that the rotating strand would cause tangling or knotting, which prevents the quick separation of the SSL DNA from SSC DNA. Besides, the SSC DNA is not always expanded at its extreme waiting for the two ends of SSL DNA to pass through. For better understanding of the process, a simplified cartoon is shown in Figure 6.

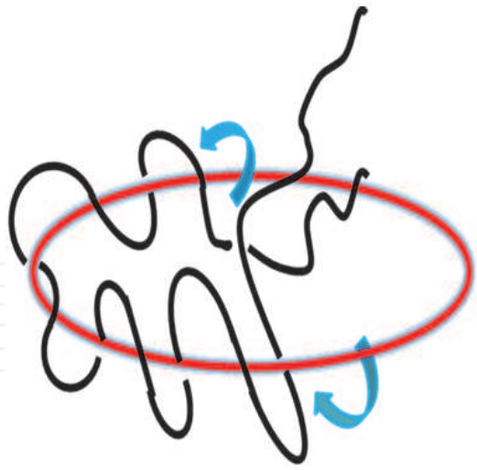

Fig. 6. The two ends of SSL DNA unwinding from SSC DNA in a nicked DNA

The observed phenomenon reflects that the twist number of singly nicked pBR322 DNA is probably very low, so that the two strands can be separated quickly without much topological impediment. However, this explanation is inconsistent with the right-handed double helix.

It is a real challenge to our wisdom as we have to explore the detailed winding directions of the two strands inside the double helix. There is no available protocol to follow. The present few tests were obtained after 30 years of trial-and-error experimentation. Here, only routine biochemical methods were used, combined with the topological knowledge of circular DNA duplex, a set of consistent evidences were obtained. They are supposed to provide a significant supplementary to the double helix model. Some of the tests are rather simple, but these experimental results need open-minded thinking.

3.5 Mobility of denatured topoisomers varies with their supercoiling

An additional test is useful in revealing the topological properties of the plasmid as shown in Figure 7.

Equal amounts of various pBR322 DNA samples, differing in their supercoiling, were denatured by alkaline first. The denatured products were examined by AGE. The mobility of denatured relaxed DNA moves the fastest. This phenomenon is difficult to explain by the Watson-Crick Model that assumes that the linking number of all these plasmids should be very big (from 430 of relaxed DNA to approximately 350 of highly supercoiled DNA); evidently these differences are relatively small and should not cause much difference in their denatured form.

On the other hand, suppose the linking number of relaxed pBR322 DNA is close to zero, after alkaline treatment, its two SSC DNAs should have more freedom to move in the alkaline solution. As soon as entering into the agarose gel under the electric force, they will renature instantly. A rigid entity is thus formed due to the formation of many illegitimate inter and intra-strand base pairs. The entity is so tight that it moves faster than the undenatured supercoiled DNA. Whereas, after denaturing, the mobility of those highly supercoiled DNA displays differently. This reflects that their linking number is higher; and the two strands are more topologically constrained and have much less freedom in alkaline solution. After entering the gel, they should have more chance, though not always, to find

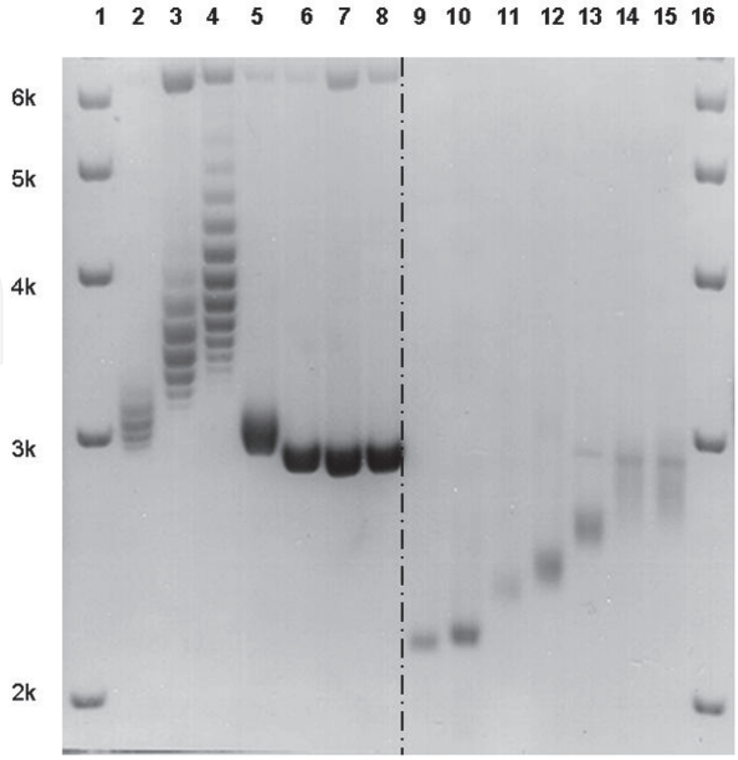

Fig. 7. Comparison of the pBR322 DNA with different supercoiling before and after alkaline denaturation. The electrophoresis buffer containing 2μg chloroquine/ml. Lane 1 & 16, 1 kb ladder; Lane 2, DNA I'_0; Lane 3, DNA $I'_{0.4}$; Lane 4, DNA I; Lane 5, DNA I'_3; Lane 6, DNA I'_5; Lane 7, DNA I'_{10}; Lane 8, DNA I'_{20}; Lane 9—15, same as 2—8 together with (1v/1v) 0.5 N NaOH.

their partners. Just as the thin band, seen in figure 7, moving with the same mobility as those untreated supercoiled DNA. However, most of them were renatured differently from those renatured relaxed counterparts due to their higher topological constrain.

All of these experiments afore-mentioned were designed to test a hypothesis that the two strands of the double helix are not restricted to wind right-handedly. Each experiment was conducted with a purely objective attitude, i.e., with no prejudgment. Just let the observed phenomena reflect the feature of DNA itself. The observed phenomena were explained independently of the prevailing theory. In order to assure the experimental results were reproducible, pure plasmids were used and the experiments were carried out under well defined conditions.

Although the five experiments have been independently carried out, the results were amazingly consistent with each other. Hence their combination makes a chain of evidence indicating that the two strands in the double helix cannot always wind right-handedly.

The provided evidence was designed on a hypothesis that is different from the canonical double helix model. It is by no means a challenge to anybody. It is not the intension of the author to commit blasphemy against authorities or leading scientists. The argument is totally in the field of science; hence no personal conflict is involved. However, our view point has to be declared for the sake of truth.

4. The ambidextrous model of the double helix

DNA generally presents as a uniform double helix, it can also adopt various different forms, such as left-handed Z-DNA, cruciform structures, three-stranded H-DNA, four stranded G-quartets or another four stranded PX DNA with reciprocal strand exchange (Mirkin, 2008; Wang, X. et al., 2010). Each of these DNA structures is important in its respective biological function. They are still rare structures that can seldom be found in native DNA. Even though their presence greatly expands our knowledge on DNA structure, they won't affect our understanding of the double helix. On the other hand, this ambidextrous model is different. It carries a conceptually different idea that may lead to some profound implications.

All our experiments, described above are consistent with each other and cannot be explained by the canonical double helix model. These results suggest that the two strands in native DNA must be wound bi-directionally. In other words, the two strands of DNA are winding ambidextrously, rather than plectonemically.

The meaning of this ambidextrous model is somehow similar to side-by-side DNA, which may cause some confusion and perplexity. Whereas, ambidextrous DNA implies that the two strands are mainly winding right-handedly or left-handedly at the same time in a native DNA duplex, which is an amendment to the classical double helix model.

The zero linking number topoisomer found in relaxed DNA indicates that there is a lot of left-handed DNA coexisting with right-handed DNA. It should be pointed out that the left-handed DNA found in these native DNAs is unlikely to be Z-DNA, because Z-DNA requires an alternative purine and pyrimidine sequence. It is plausible that Z-DNA is just a member of the left-handed DNA family.

An interesting finding is that the absolute linking number of relaxed DNA is less than that of supercoiled DNA which is contrary to the concept of traditional DNA topology.

Most native plasmids are negatively supercoiled DNA. The superhelical density of different plasmids has been measured to be similar, i.e., $\sigma \approx -0.05$. In a plasmid with N base pairs, its supercoiling is generally supposed to be around $Wr \approx \sigma N/10.4$, and the linking number should be $L_{w\text{-}c} \approx N/10.4$. Whereas, according to the ambidextrous model, the total twist number $T \approx 0$, so $L_{amb} = T + Wr \approx Wr$. It means that the two strands of native plasmid are still topologically inseparable. Hence, the ratio of $L_{w\text{-}c} / L_{amb} = 20$. It implies the linking number of a plasmid should be about 20 times less than the estimated value based on Watson-Crick Model.

An additional deduction is that the absolute linking number of positively supercoiled DNA is also higher than that of relaxed DNA. It further leads to the recognition that positively supercoiled DNA contains more left-handed DNA than right-handed DNA. This deduction has a significant implication on the understanding of heat resistance of DNA in hyperthermophilic strains.

5. The consequences of the amendment

Since the Watson - Crick Model is so widely accepted by the science community, it is likely that many scientists are unaware that some of their experimental phenomena may have alternative explanations.

The suggested ambidextrous double helix model is topologically different from the Watson-Crick Model. Consequently, many experimental results published previously could be

explained differently with the ambidextrous DNA model. A few examples are presented here that may be of interest to the authors and other scientists.

5.1 The two strands of λDNA can be progressively separated by SS-DNA binding protein

Dalius et al. (1972) published an interesting picture of λDNA, which was completely denatured by SS-binding protein gene 32 as cited here (Figure 8).

The gene 32 binds the SSDNA in a cooperative way, and each gene 32 protein binds to 10 nucleotides.

Considering the dynamic nature of long λDNA in solution, the two strands of the DNA not only move together constantly, but also have transient impairing or "breathing" at some regions, especially at those AT rich regions and at two single stranded terminals. These SS

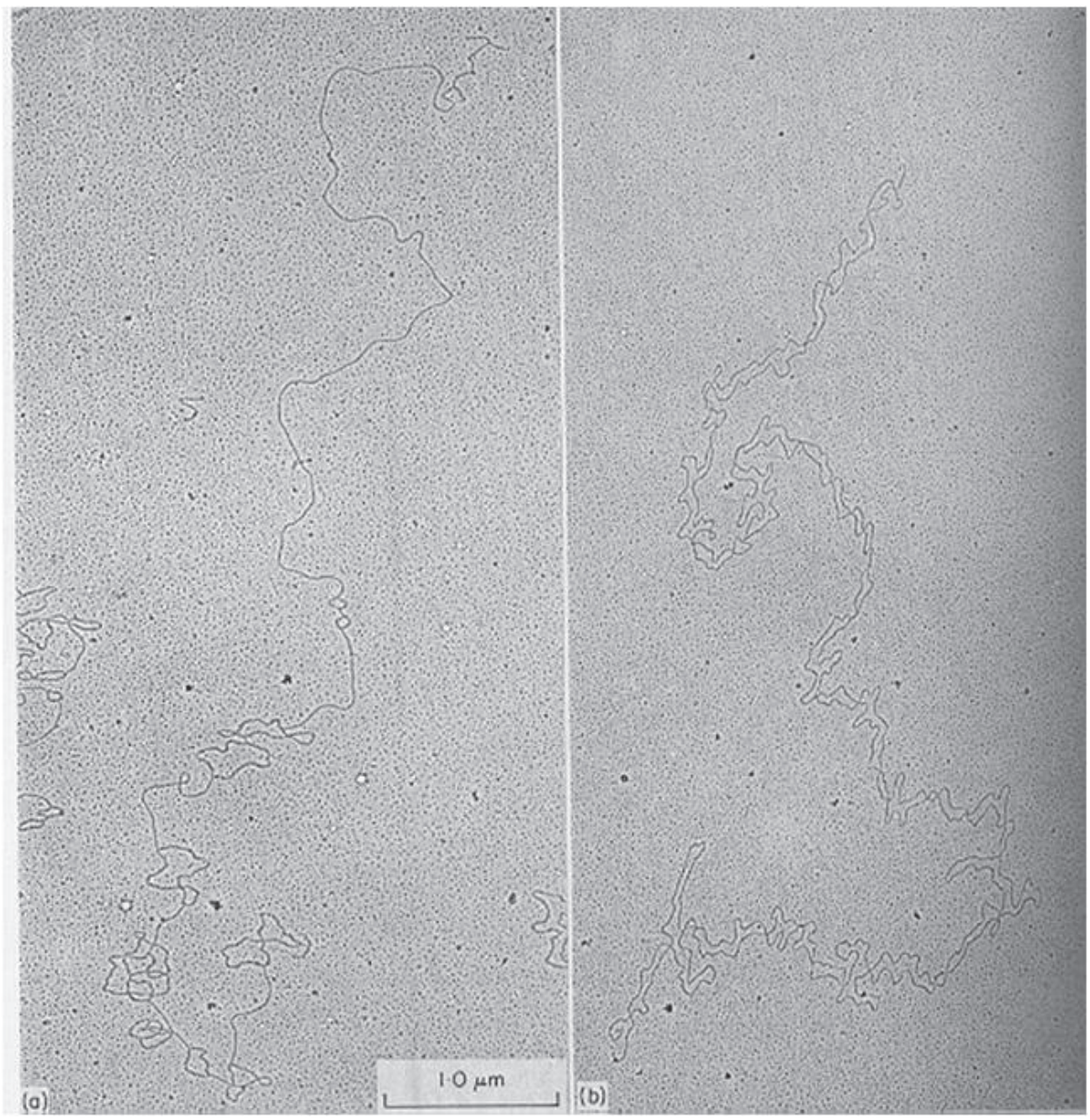

Fig. 8. The long λDNA can be partially or completely denatured by gene 32 (Dalius et al. 1972).

DNA regions were supposed to be preferentially occupied by gene 32 which has a molecular weight of 35,000Da. The gene 32 cooperatively occupied regions would be very clumsy and lumbering which would prevent their rotation in solutions as required by the Watson-Crick Model. As seen in Figure 8, the two strands of long λDNA were almost parallel as two side-by-side threads with no tangling. The complete separation implies that the two strands were not tightly winding in right-handed direction. Hence, the rotation of the gene 32 bound DNA is unlikely to happen.

5.2 The λDNA can be stretched to twice its normal size

Bensimon et al. (1995) proved that the λDNA can be stretched to twice its normal length. Lebrun and Lavery (1996) gave an instructive drawing as shown in Figure 9.

Only suppose the twist angle close to zero (θ= 0), it would be possible letting the two strands of λDNA to be stretched to almost parallel. It is well known that R = 3.4 Å (double helix pitch) and L = 7Å (supposed average distance between two adjacent phosphate atoms in stretched ss-DNA). The arithmetic tells us that the limit of a λDNA molecule could be stretched before rupture is let all the phosphate atoms line straight, i.e. let R ≈ L. The meaning of this experiment is much easier to be explained by the ambidextrous model, i.e., the net twist number in λDNA is close to zero.

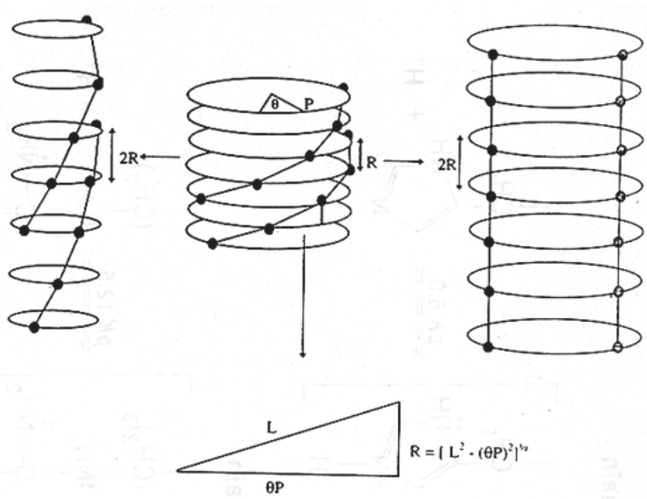

Fig. 9. Schematic model of DNA stretching: maintaining a constant inter-phosphate distance L within each strand of duplex, stretching to twice the normal rise R can be achieved by reducing the twist angle or by reducing the radius P of the duplex (Lebrun and Lavery 1996).

5.3 Sedimentation coefficient indicates the complete separation of T7 DNA

Freifelder & Davidson (1968) found the sedimentation coefficient of the denatured T7 DNA drops sharply at 54 ºC as shown in Figure 10.

Freifelder and Davidson explained their observation this way: "We therefore interpret the change as a halving of the molecular weight of the DNA resulting from the physical separation of the strands." However, how could the two strands be physically separated?

T7 DNA is a long linear DNA duplex with 39936 base pairs. According to the canonical double helix model, the two strands should twist almost 4000 times. In solution, after the

treatment of formaldehyde, the two ss DNA strands should be detached but still tangled with each other. Since the long strands of DNAs are very thin and curved, they are unlikely able to unwind quickly in solution. It would be almost impossible for the two strands of T7 DNA, highly tangled with each other as the classical double helix required, having such a dramatic change. Whereas, this observed phenomenon can be easily explained by the ambidextrous model.

Many other sedimentation experiments provided similar evidences indicating that the two strands are not tightly tangled with each other (Freifelder, 1983).

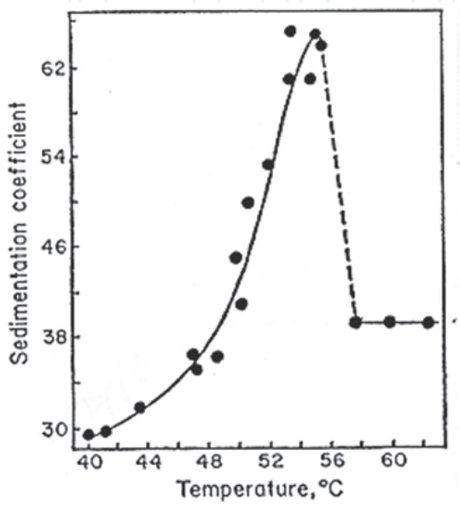

Fig. 10. Sedimentation coefficient of the sharp boundary of T7 DNA fully or partly denatured by heating for 10 minutes at the indicated temperatures in 12% HCHO, 0.1 M phosphate, pH 7.8 (Freifelder & Davidson 1968)

5.4 Point mutation shows the PCR reaction can be conducted on a plasmid

Point mutation is a clever method used in molecular biology. Stratagene Co. has successfully developed the method and a kit is ready for any users. In this method, DNA synthesis was started from two primers, differ in one specific site, on a supercoiled plasmid with Taq enzyme. The two new DNA were synthesized in opposite directions along the two strands of the same plasmid. It would be difficult for the two enzyme molecules to finish their job since two bulky enzymes would inevitably meet on their way and stop there. Whereas, if the two strands were not tightly wound, there would be enough space for both of them to pass through. This simplified explanation is based on an old idea explaining DNA synthesis. According to the presently accepted idea, the huge DNA polymerase is static, the template DNA moves in and the new DNA moves out like a movie projector. Nevertheless, it is still much easier to explain this phenomenon by the ambidextrous model.

5.5 The heat resistance of DNA in hyperthermophilic strains is difficult to explain

Hyperthermophilic strains live stably at high temperatures. An amazing strain even can duplicate at autoclaving temperature. The doubling time of strain 121 is 7 hr or 24 hr at 115ºC or 115ºC respectively (Kashefi & Lovley 2003). The strain is still alive after staying at 115ºC for 2 hrs.

How can the double stranded DNA in an hyperthermophilic strain resist the high temperature without any damage or denaturation? It is suspected that both positive supercoiling and reverse gyrase are protecting DNA against high temperatures (Forterre & Elie, 1993; Kikuchi, 1990).
When the unique single gene of reverse gyrase was removed from a hyperthermophilic strain, the strain is still viable at high temperatures (Atomi et al. 2004). It strongly indicates that positive supercoiling is responsible for the heat resistance of double helix.
How does DNA gets positive supercoiling in the hyperthermophilic strain without reverse gyrase?
Several scientists revealed that the two strands of DNA are regularly turning left-handedly with elevated temperatures. They found the double helix unwinding angle for each base pair is $\Omega = -0.01° / {}^{o}C/$ bp. (Depew & Wang, 1975; Duguel, 1993). Hence at high temperatures, the chromosomal DNA of hyperthermophilic strain would turn left-handedly. As mentioned before, this left-handed chromosomal DNA would stay in positively supercoiled form which is supposed to be more stable at high temperatures. It is plausible that the positively supercoiled DNA is stable at high temperatures just as the negatively supercoiled DNA can. It is well known that negatively supercoiled DNA is stable at boiling temperatures, since a routine plasmid preparation protocols is the "boiling method".
Our finding of the relationship between left-handed DNA and positive supercoiling is critical for the understanding of the heat resistance of DNA at high temperatures. However, this connection can not be derived from the canonical double helix model.

5.6 The catabolite gene active protein (CAP) binds to left-handed DNA

McKay & Steitz (1981) determined the structure of catabolite gene activator protein (CAP) at 2.9 Å resolution by x-ray crystallography. They found this protein fitting quite well with the left-handed DNA rather than right-handed DNA as shown in Figure 11. Although no direct evidence of the left-handed B-DNA is obtained by their model building method, its significance in understanding the double helix should not be neglected.

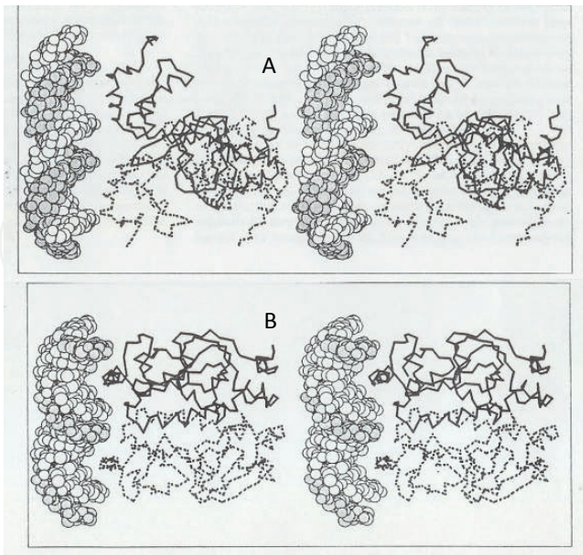

Fig. 11. Stereo drawing of the α–carbon backbone of the CAP dimer interacting with two kinds of DNA. One CAP subunit is drawn with dashed lines, the other subunit with solid lines. A) Right handed B-DNA; B) left-handed B-DNA (McKay & Steitz 1981).

All these evidences found in other labs imply that the two strands in the double helix are inconsistent to the right-handed B-DNA

Nowadays, nobody has the time or energy to read all the papers related to DNA. The listed six cases were just randomly found by the author. It is very likely that more interesting cases are hiding in the literature which may be explained differently. As seen in the above examples, except the last one, these papers did not suggest anything about the left-handed DNA. The hidden meaning on the shown facts has to be figured out after scrutinizing each paper.

Armed with the most advanced technique and instruments, the detailed information inside the native double helix is still unable to clearly figure out. The x-ray crystallography can only deal with short DNA fragments; the AEM can see some parts of DNA, but the image is not clear enough (Kato et al., 2009).

A special property of DNA is in its self-replicating function. Theoretically, the structure of this molecule enables its self- reproduction. It seems mysterious and inexplicable that after more than 50 years of research by many talented scientists, the topological problem is still unanswered.

6. The ambidextrous DNA model may be useful for understanding important biological mechanisms

Since the proposal of the central dogma (Crick 1970), great progress has been achieved; the framework of molecular biology was filled with abundant knowledge and evidence. It made modern biochemistry a reliable and valuable source of knowledge for young students. At the top of the central dogma, DNA plays a leading role in molecular biology. Any amendment of the double helix would have great implications in many aspects of molecular biology.

6.1 The mechanisms of DNA replication

Great progress accumulated over many years helps us gain a much better understanding of the mechanism of DNA replication. Nowadays, almost all elements involved in replication have been discovered and evaluated at the molecular level. Their individual function in the complicated replication process is known (Alberts, et al 2002). However, the topological problem involved in DNA replication is still largely unanswered.

Most of the knowledge on DNA replication is obtained from simple systems, i.e., in plasmids and prokaryotes, especially in *E. coli*. In principle, such knowledge is applicable to eukaryotes.

DNA replication needs a lot of proteins including DNA polymerase, helicase, ligase, primase, gyrase, single strand DNA binding proteins, etc. The huge DNA replicating machine which executes synthesizing DNA is called the replisome. It is believed that the replisome keeps stationary at the replication fork. The parental DNA rolled into the replisome; after processing, two new daughter DNAs were rolled out. The *in vitro* DNA synthesis system was proved to be valuable in elucidating the detailed replication process. The replication of leading strand and lagging strand was beautifully explained by the trombone model (Chastain et al. 2003). Now a simplified animation video of the DNA replication is available on internet. Albeit it does not mean we know the mechanism completely, especially how the double helix was untwisted. As mentioned above, the slow reaction rate of gyrase can not catch up the fast pace of DNA replication.

It should be noted that helicase, an active enzyme in DNA replication, can quickly open the double helix but is unable to cut and rebind DNA. It separates the energetically stable duplex DNA with the energy from NTP hydrolysis (Tuteja and Tuteja, 2004). The presence of this ubiquitous molecular motor protein implies that the two strands in the DNA duplex could not wind tightly as in the classical double helix model. According to the ambidextrous model, the function of helicase is reasonable and rational. The occasionally appeared topological problems would be easily solved by gyrase. However, if the DNA is really in the Watson-Crick Model, the accumulated positive supercoiling in front of the replication fork would be a big obstacle, because, the positive supercoiling is unable to be removed quickly by gyrase or to be transferred to the terminal.

The replication of DNA in eukaryotes is more complicated due to the presence of nucleosome structure and perhaps some other unknown problems. Anyone trying to uncover the mechanism of their DNA replication would encounter more topological problems. It is believed that the ambidextrous model may relieve that burden.

6.2 The mechanisms of RNA transcription

Accumulated evidence indicates that the DNA dependent RNA polymerase (RNAP) can read the information from the template strand through a relatively small "transcription bubble". At first, the finding of highly positively supercoiled pBR322 from a novobiocin treated *E.coli* strain (Lockshon & Morris, 1983) was unexpected. Later, a clever twin-supercoiled domain model was proposed, which nicely solved a difficult topological problem involved in transcription (Liu & Wang, 1987). In brief, the positive supercoiling generated in front of the RNAP is removed by gyrase, and the negative supercoiling behind the RNAP is removed by DNA topoisomerases I. When the activity of gyrase was inhibited by novobiocin or other inhibitors, the positive supercoiling were accumulated, which causes the yield of highly positively supercoiled DNA. It also explains why in a topoisomerase I mutant, the negatively supercoiled pBR322 is so unusually high (Pruss, 1985). The model was further proved by many excellent experiments (Wu et al. 1988; Tsao et al., 1989)

On the other hand, in a small extra-chromosomal DNA, the local positive supercoiling can be cancelled by the negative supercoiling concomitantly generated behind the RNAP through the speedometer like DNA. The question is how fast the diffusion of opposite supercoiling waves along the DNA could be within a topologically closed domain.

The devil is in the details.

Taking pBR322 as an example, the plasmid has 5 genes as shown in Figure 12. For simplicity, let's consider the two main genes first. According to the Watson - Crick Model, about 1182 /10.4 = 114 positive supercoilings should be generated from the transcription of tetracycline resistance (Tet) gene (86-1268), and 788/10.4 =76 positive supercoilings from β-lactamase gene (4084-3296),. Their transcriptions are oriented in opposite directions. If each gene initiated only once, their positive supercoilings generated should be additive, i.e., 76+114 = 190, which is much higher than that found in experiment. According to the result of Lockshon and Morris (1983), the positive supercoiling is estimated to be around +25, which is comparable to that of negative supercoiling (-25). The additional transcriptions from Rop and RNA II would make the situation even worse. Considering the transcriptions in a real pBR322 DNA, two successive positive supercoiling waves generated from β-lactamase and RNA II genes in one direction would clash with the other two successive positive supercoiling waves generated from Tet and Rop genes. The RNA I gene is relatively small; its contribution should be relatively small. What is the result of the clashing waves on

pBR332 in a novobiocin treated living *E.coli* strain? The positive supercoiling should be more than that found experimentally.

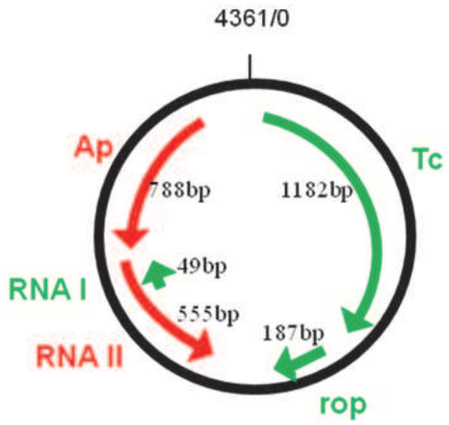

Fig. 12. The transcription orientations of 5 genes in pBR322 DNA

This remarkable discrepancy is favorable to the ambidextrous double helix model. Since the twist number is not necessarily proportional to the length of each gene, the positive or negative supercoiling generated in front of the RNAP should be much less than the expected value based on classical double helix model. It would greatly reduce the gap between experimental results and theoretical expectation. Thus, the mechanism of transcription can be understood more easily with less topological trouble.

In principle, the rules of transcription found from plasmids are applicable to the chromosomal DNA. As mentioned above, in the transcription of many genes from the chromosomal DNA, the positive supercoiling actually would not to cause any problem if the two strands are winding ambidextrously.

Transcription in eukaryotes is more complicated. However, the ambidextrous model would provide a good reason to believe that there is no topological problem during RNA transcription.

7. More problems waiting for answers

The suggested ambidextrous model seems to overcome a major topological obstacle in understanding the mechanism of DNA replication. However, it is still a hypothesis based on several topological evidences. We are not sure whether the left-handed DNA is determined or related to its sequence or not. The real nature of the two strands inside the double helix is largely unknown. Although the junction between B-DNA and Z-DNA in a 15 mer oligonucleotides was found by X-ray crystallography, in which two bases were extruded, it does not mean that similar junction could always be found in native DNA (Ha, et al., 2005).

Each restriction enzyme is sensitive to specific DNA sequence. This fact implies that secondary structure may not affect the activity of restriction enzymes. However, it is not clear that if the special sequence determines the secondary structure, and hence the activity of restriction enzymes is also affected. This is a question for future scientists to answer.

Crick et al. (1979) once stated: "DNA is such an important molecule that it is almost impossible to learn too much about it." Presently, our knowledge about the double helix has advanced much better than fifty years ago. However, many new findings remind us that

probably there are still some secrets hidden in the double helix. For example: a) In dilute DNA solutions, some kind of short double stranded DNA can generate electromagnetic signal and the DNA can communicate with other DNA (Montagnier et al.2009) ; b) Concentrated solution of oligo- deoxyribonucleotide DNA duplex behave the character of both right-handed and left-handed DNA (Zanchetta et al. 2010); c) Alberts (2010) pointed out that the frontier of science is endless and "a total of about two-third of our genetic information –'our dark genome'- is needed for processes whose nature mostly remains a mystery".

8. It is time to make a conceptual change

In the history of discovery, similar stories incredibly repeated again and again. The garden pea experiment of Mendel was ignored by his contemporary scientists for 35 years; proteins were assumed to be the carriers of heredity for a very long period of time; the long stories in discovering Krebs cycle, transposon, prion, ribozyme happened in different scenarios (Grinnell, 2011). Almost all of these cases occurred due to an analogous reason — old minds die hard. The prevalently accepted dogma is always believed to be true and correct, and the new concept is believed to be bizarre and weird. The famous notion of "chance favors the prepared mind" is routinely displayed in an alternative way: "novel new concept is always being neglected, rejected or even hated by unprepared mind".

The basic idea of the Watson - Crick Model is correct and was proved by numerous experimental findings afterwards. Its contribution to molecular biology is highly evaluated. However, in native DNA, the winding direction of the two strands inside the double helix is very difficult to detect. Available evidence is scarce, obscure and questionable. The only source comes from the x-ray analysis of DNA fiber, which could not rule out the presence of left-handed DNA.

Currently, most people take the double helix as a scientific doctrine, but in 1953 it was merely an untested hypothesis as Watson and Crick recognized themselves. Even in a textbook of 1958, the double helix model was described as "an ingenious speculation". (Fruton & Simmonds, 1958)

Epistemology tells us that no theory is perfect. Even a theory as sound as Newtonian physics, is not unassailable. No matter how a theory survived the most rigorous tests, it does not mean it can pass all future tests.

The experimental results mentioned above strongly support another hypothesis that the two strands in native DNA are winding ambidextrously rather than plectonemically, a hypothesis which differs from the old hypothesis.

This amendment has been demanded for years by many facts found by various investigators. The author just weaves these findings together. The double helix is now inspected from a new viewpoint, i.e. the topological viewpoint and a new facet of the double helix is appeared.

It seems critical that while handling the problems of a long DNA molecule, we have to take it as a three dimensional structure. The negligence of the topology would lead to misinterpretation of the facts. An evident example is the extremely high unwinding rate of DNA derived from the classical double helix. The unwinding rate was assessed to be $12X10^7$ molecular weight per second at 37 ^oC (Freifeleder, 1983). It is equivalent to $1.8x\ 10^5$ base pair per second, or $1.08\ X\ 10^7$ rpm. It is plausible that a calculation based on a wrong premise could not tell the truth.

It is well known that DNA in solution is highly hydrated. The water content was 0.339g H_2O/g ds-DNA and 0.434g H_2O/g ss-DNA that is equivalent to 20 water molecules per base pair in ds-DNA and 7.8 water molecules per nucleotide in ss-DNA (Bastos et al 2004). These hydrated water molecules are closely contacting with more water molecules nearby. It makes the DNA strands very sluggish. While denaturing a stretch of pure DNA duplex in solution, once the unwinding of the double helix starts, the two SSL DNAs would have to unwind in opposite directions. The questions are: a) Where does the energy for the rotation of DNA strands come from; b) How can the rotation rate of delicate thin SSL DNA in solution reach the assessed value? According to our common sense, this value is intuitively unacceptable. As we know that when a car is running at its top speed, the rotation rate of its engine seldom reaches 6,000 rpm, which is made from steel and rotating in air. Although nobody can see how the two strands inside the double helix untwist while the hydrogen bonds were abruptly destroyed chemically or physically, it is unlikely that the delicate thin strands of DNA can rotate at 10 million rounds per minute in solution.

The suggested amendment is actually a minor change on the winding direction of the two strands inside the double helix. However, the concept of ambidextrous winding of the two strands is difficult for many scientists to accept. Just as everybody has a blind spot in one's vision, the conceptual blind spot of the double helix appears in some scientists' minds.

Perhaps the extreme success of the double helix in teaching and mentoring young scientists prevents them from thinking differently. According to psychology, the first impression makes a deep mark in the mind of everybody. And this impression is very difficult to be changed in an adult. It is possible that there were a few students who were skeptical on the accuracy or correctness of the double helix. However, their discrete voices were unable to be noticed by the science community.

Things are not always as they seem. It is well known that scientific knowledge is universal, objective and provisional. Except in the field of mathematics, all scientific knowledge has to be modified or improved by new findings or discoveries.

In exploring scientific truth, not artifact or illogical reasoning, but the incomplete fact or partially correct notion confuses people the most. All our knowledge about the double helix is gained from evidence achieved by many scientists and experts in the field. The various experimental phenomena provide the basis for us to have the vision below the surface, to figure out how the two strands should be. However, each scientist makes his/her own conclusion or assumption upon one's knowledge, skill, experience, wisdom, imagination and vision. Even the same evidence can lead to different conclusions or assumptions by different scientists. That is why further exploration is often necessary to verify the validity of various conclusions. Just like a jigsaw puzzle, the picture will never be perfect if some parts were missing or misplaced. However, there is no standard answer to an appropriate question asked either by a curious pupil or a scientist who is trying to know the reason of a phenomenon. In the objective world, each scientific result has to fit into the pre-existing framework, or on rare occasions modifies the theoretical framework. Scientists are not completely free of explaining their results.

As mentioned in section 3 and 5, many observed phenomena and new experimental findings found in different laboratories strongly suggest that the native DNA cannot always be a right-handed double helix. The function of DNA in replication especially demands an amendment of the double helix. Now it is the right time for making a conceptual change. Perhaps more time is needed for more people to realize this.

Through discussion, debate, refutation, re-examination, etc, our scientific knowledge gains momentum. In the arena of science, there is no discrimination of gender, race, ethnicity, age, social class, nationality, disability, political beliefs, religion, sexual orientation or other personal characteristics to any player. Nobody can act as a judge since nobody is perfect and nobody knows everything. Hence democracy is not applicable in determining which conclusion is correct. Perhaps only time can make the final verdict. Although the present peer reviewing system normally works well for the science community, it should be noted that it is by no means a best system for the promotion of science.

Different from material wealth, scientific truth is extremely precious and priceless. It is the mental product of many people who dare to explore the secrets of nature. It has no smell, no shape, no weight, and can not be physically felt. Once it is produced, it can be banned, neglected, rejected or even hated, but it can never be dismantled, burned or destroyed which is somehow different from some kind of art work. One valuable trait of scientific truth is that it can correctly predict something never happened before (under appropriate conditions) that is also the test of the correctness of any theory.

9. A double helix conjecture and some other predictions

Based on the hypothesis of the ambidextrous double helix model, it is reasonable to predict that a zero linking number topoisomer should be found among a mixture of relaxed topoisomers.

An idealized test is suggested for the validity of the ambidextrous DNA model as shown in Figure 13. This suggested experiment may be assumed as a test of double helix conjecture.

The test seems to be very simple — by manipulating a set of pure relaxed topoisomers and letting the zero linking number topoisomer disappear.

According to topology, the two complementary SSC DNAs of all topoisomers can not be completely separated except the zero linking number topoisomer. Hence, after the appropriate denaturing and renaturing treatment, all those non-zero linking number topoisomers should reappear, acting as ideal internal controls and only the zero linking number topoisomer is expected to be invisible on the agarose gel after AGE.

If such an expected experimental result could be obtained, that would be very strong evidence to prove the presence of a zero linking number topoisomer. It could be easily understood even by people with no knowledge of DNA topology. However, up to now, nobody has succeeded such an experimental demonstration. Therefore, the expected result is temporarily just a conjecture and must be verified.

The author believes this conjecture is feasible and attainable. It is hoped that readers of this paper will find the way to prove this conjecture.

Actually, as many conjectures, this conjecture will be very difficult to verify. One reason is that when destroying all the hydrogen bonds between the complementary strands of suitable topoisomer preparations, the long back bone of the plasmid is prone to be broken. Even if only a single nick occurred in a plasmid, the topological properties of the plasmid would be greatly changed and no meaningful information could be collected.

A conjecture is generally used in mathematics for a statement which is probably wise and true but has not been proven yet. According to Karl Popper's opinion, all scientific theories are provisional conjectures and subject to re-examination. Therefore, the new discoveries, the refutation of old theory and the proper conjectures or hypotheses presented at the right time and right place are helpful for the advancement of science.

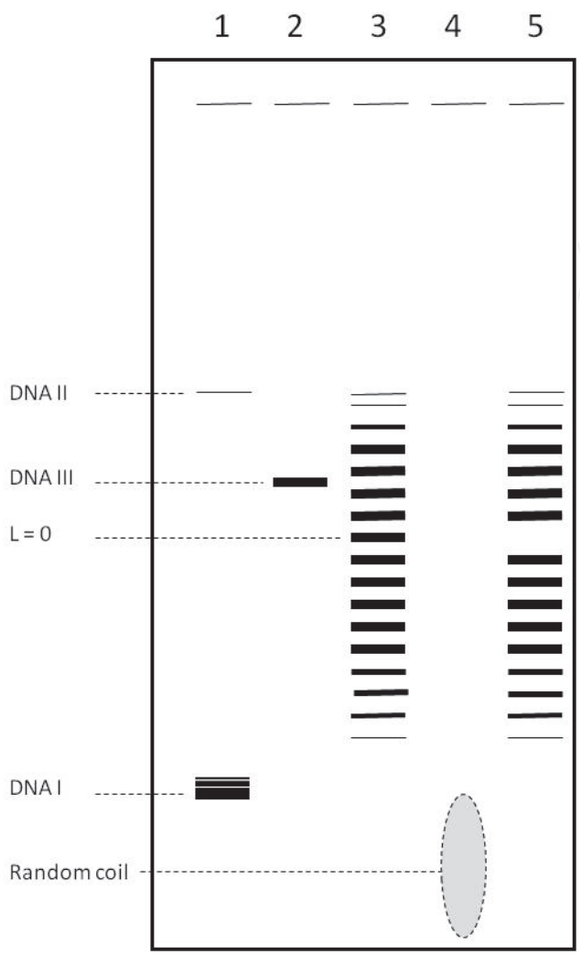

Fig. 13. The expected result of the double helix conjecture. Lane 1. Supercoiled DNA; Lane 2, Linear DNA; Lane 3, Relaxed DNA; Lane 4, Denatured relaxed DNA; Lane 5, Annealing product of samples from lane 4.

The proof of the famous Fermat conjecture took more than 300 years. (Now named as Fermat's last theorem: If an integer n is greater than 2, then the equation $a^n + b^n = c^n$ has no solutions in non-zero integers a, b and c). However, the quick advancement of modern biochemistry can not wait such a long period of time for the verification of this conjecture.

It is possible that when this conjecture is proven, the study of DNA and its various functions would be a new hot spot in biochemistry and molecular biology.

In addition to the double helix conjecture, according to the ambidextrous DNA model, the following assumptions or predictions are not difficult to be derived. It is also believed that they can be proven experimentally.

a. There is no quick rotation of the double helix during replication.
b. In any plasmid, the melting temperature of a zero linking number topoisomer should be very close to that of nicked DNA.

c. It is possible to make highly positively supercoiled DNA with superhelical density $\sigma > +0.1$.

10. Conclusion

Is DNA really a double helix? The question has been asked by some scientists for many years. There is no doubt about the two anti-parallel strands and base pairing. The only question is about the winding direction of the two strands inside the double helix.

In his book ⟨**What mad pursuit**⟩, Crick (1988) wrote: "The double-helical structure of DNA was thus finally confirmed only in the early 1980s. It took over twenty-five years for our model of DNA to go from being rather plausible, to being very plausible (as a result of the detailed work on DNA fibers), and from there to being virtually certainly correct." Presently, most people believe that there are no problems in the double-helical structure of DNA.

There is no doubt that the structures of oligonucleotides determined by x-ray crystallography are correct and important. However, selectively choosing evidences favorable to right-handed DNA is not the best way in preventing imperfect conclusions. Besides, extrapolating the results obtained from short DNA fragments to long native DNA leaves room for error.

Instead of providing thousands of evidence favorable to the Watson-Crick Model, this chapter shows just a few examples that cannot be explained by that prevalently accepted theory. Based on topological evidence and many supporting facts, it is plausible that the two strands are winding ambidextrously, rather than plectonemically. This conceptually different idea can reasonably explain many experimental results that the classical double helix cannot.

The tough topological problems involved in clarifying the mechanisms of DNA replication and RNA transcription may be dissolved in the ambidextrous double helix model.

Surely, the DNA cannot speak for itself. Limited by personal knowledge, vision and time, our present hypothesis for the observed phenomena may be imperfect. As Horrobin (1975) pointed out: "Many and probably most of the hypotheses published in the journal will turn out in some way to be wrong. But if they stimulate determined experimental testing, progress is inevitable whether they are wrong or right. The history has repeatedly shown that when hypotheses are proposed it is impossible to predict which will turn out to be revolutionary and which ridiculous. The only safe approach is to let all see the light and to let all be discussed, experimented upon, vindicated or destroyed." It is hopeful that more investigation would help us understand the double helix deeper and better.

Perhaps the publication of this chapter would cause furious argument and refutation, because the subject is just at the center of molecular biology. It is evident that every scientist has his/her own experience and idea on the double helix. Only one thing the author can guarantee is that all his experimental results were reproducible. The author is responsible for every sentence written in this paper.

11. Acknowledgement

The author is grateful to the advice, support and help of Dr. J.C. Wang, H. Bremer, D. Lang, and T. Lance. Thanks to Drs. Z. X. Shen, A. Lazinica and Ms. V. Poffenberger for their careful examining of the manuscript. I am indebted to publishers of Nature and Elsevier for

allowing me to cite the figures published in their journals. I owe special thanks to Dr. X.Y. Liu at the Shanghai Institute of Biochemistry and Cell Biology for allowing me working in his laboratory.

12. References

Alberts, et al. (2002) *Molecular biology of the cell*, 5 Th. Edition, Garland science.

Alberts, B. (2010). Is the frontier really endless? *Science,* 330, 1587.

Arnott. S. (2006). Historical article: DNA polymorphism and the early history of the double helix. *Trends Biochem. Sci.* 31, 349-354.

Atomi,H.; Matsumi, R. & Imanaka, T. (2004). Reverse gyrase is not a prerequisite for hyperthermophilic life. *J. Bacteriology,* 186 , 4829-4833.

Bates, A.D. & Maxwell, A. (1989). DNA gyrase can supercoil DNA circles as small as 174 base pairs. *EMBO*, 8, 1861-1866.

Bastos M et al. (2004) Hydration of ds-DNA and ss-DNA by neutron quasielastic scattering. Biophysical Journal 86, 3822-3827

Bensimon, D. et al. (1995). Stretching DNA with a receiding meniscus: Experiments and results. *Phys. Rev. Lett.* 74, 4754-4757.

Berger,J.M. et al. (1996). Structure and mechanism of DNA topoisomerases II. *Nature.*379, 225-232.

Biegeleisen, K. (2002). Topologically non-linked circular duplex DNA. *Bull. Math. Biol.* 64, 589-609.

Blattner et al., (1997). The complete genome sequence of *Escherichia coli* K-12, *Science*, 277, 1453-1562.

Brack, C; Bickle, T.A. & Yuan, B. (1975). The relation of single-stranded regions in bacteriophage PM2 supercoiled DNA to the early melting sequences. *J. Mol. Biol*. 96, 693-702.

Bravo, A.; Serrano-Heras, G. & Salas, M. (2005) Compartmentalization of prokaryotic DNA replication. *FEMS Microbiology Reviews* 29, 25-47.

Chastain, P.D. et al. (2003). Architecture of the replication complex and DNA loops at the fork generated by the bacteriophage T4 proteins. *J. Biol. Chem*. 278, 21276-21285.

Condemine, G. & Smith, C.L. (1990). Transcription regulates oxolinic acid-induced DNA gyrase cleavage at specific sites on the *E.coli* chromosome. *Nucleic acids Res.*18, 7389-7396.

Crick, F.H.C. (1970). Central dogma of molecular biology, *Nature*, 227, 161-163.

Crick, F.H.C. (1988). *What mad pursuit, A personal view of scientific discovery*, New York Basic Books.

Crick, F.H.C; Wang, J.C. & Bauer, W.R. (1979). Is DNA really a double helix ? *J. Mol. Biol.* 129, 449-461

Csikszentmihalyi, M. (1996). *Creativity*, Harper Collins publishers.

Cyriax , B, & Gäth R(1978). The conformation of double-stranded DNA. *Naturwissenschaften* 65, 106-108

Dalius, H., Mantell, N.J. & Alberts, B. (1972) Characterization by electron microscopy of the complex formed between T4 bacteriophage gene 32-protein and DNA. *J. Mol. Biol.*, 67, 341-350.

Depew,D.E. & Wang, J.C. (1975) Conformation fluctuations of DNA helix. *Proc. Natl. Acad. Sci. USA,*72, 4275-4279.

Duguet, M. (1993). The helical repeat of DNA at high temperature. *Nucleic Acids Res.* 21, 463-468.

Forterre, P & Elie, C. (1993) Chromosome structure, DNA topoisomerases and DNA polymerases in archaebacteria. In Kates, M. Kushnes, D. & Matheson, A. (Eds) *The biochemistry of archaea.* 26, 325-361. Elsevier Science Publisher.

Ha, S.C. et al., Crystal structure of a junction between B-DNA and Z-DNA reveals two extruded bases. *Nature,*437, 1183-1186.

Kates, M. Kushnes,D. & Matheson, A. (Eds) *The biochemistry of archaea.* 26, 325-361.

Freifelder, D. & Davidson, P.F. (1963). Physicochemical studies on the reaction between formaldehyde and DNA. *Biophysical J.* 3, 49-63.

Freifelder, D. (1983). *Molecular biology*. p.117, Science books International.

Fruton, J.S. and Simmonds, S. (1958) *General Biochemistry*, p.200, Wiley.

Grinell, F. (2011). Book excerpt from Everyday practice of science. *The scientist,* 25, 76-77.

Ha. S.c. et al., (2005). Crystal structure of a junction between B-DNA and Z-DNA reveals two extruded bases. *Nature,*437, 1183-1186.

Horrobin, D.F. (1975). Ideas in biomedical science: reasons for the foundation of Medical Hypotheses. *Medical Hypothesis*, 1, 1-2.

Kashefi, K & Lovley, D.R.(2003). Extending the upper temperature limit for life. *Science* 301, 934.

Kato t. et al. (2009) High resolution structural analysis of a DNA nanostructure by cryoEM. *Nano Lett,* 9, 2747-2750.

Kikuchi, K. (1990) Reverse gyrase and other Archaebacterial topoisomerases. In N.R.Cozzarelli & J.C. Wang (eds) *DNA topology and its biological effects,* Cold spring Harbor Laboratory Press, NY. 285—298.

Lebrun and Lavery (1996). Modelling extreme stretching of DNA. *Nucleic Acids Res.* 24, 2260-2267

Liu, L.F. & Wang, J.C. (1987). Supercoiling of the DNA template during transcription. *Proc. Natl. Acad. Sci. USA,* 84, 7024-7027.

Lockshon, D. & Morris, D.R. (1983). Positively supercoiled DNA is produced by treatment of *Escherichia coli* with DNA gyrase inhibitor. *Nucleic Acids Res.* 11,2999-3017.

McKay, D.B. & Steitz, T.A. (1981). Structure of catabolite gene activator protein at 2.9A resolution suggests binding to left-handed B-DNA. *Nature*, 290, 744-749.

Mirkin, S.M. (2008). Discovery of alternative DNA structure: a heroic decade. (1979—1989). *Front Biosci.* 13, 1064—1071.

Meselson, M. & Stahl, F.W. (1958). The replication of DNA in *Escherichia coli. Proc. Natl. Acad. Sci, U.S.A.*44, 671-.682.

Montagnier, L. et al. (2009). Electromagmetic signals are produced by aqueous nanostructures derived from bacterial DNA sequences. *Interdiscip. Sci. Comput life Sci.*1, 81-90. DOI: 10.1007/s12539-009-0036-7

Olby, R. (2003). Quiet debut for the double helix. *Nature*, 421, 401-405.

Pruss, G.J. (1985). DNA topoisomerase I mutants. Increased heterogeneity inn linking number and other replicon-dependent changes in DNA supercoiling. *J. Mol. Biol.* 185, 51-63.

Sasisekharan V, & Pattahireman N (1978). Some implications of an alternative structure of DNA. *Proc. Natl. Acad. Sci. USA,* 75: 4092-4096

Schvartzman, J.B. & Stasiak, A. (2004). A topological view of the replicon. *EMBO reports.* 5, 256-261.

Skarstad, K; Boye, S & Steen, H.B. (1986). Timing of initiation of chromosome replication in individual *Escherchia coli* cells. *EMBO journal,* 5, 1711-1717.

Stettler,U. H. et al., (1979). Preparation and characterization of DNA V, the duplex DNA resulting from association of complementary circular single-stranded DNA. 131, 21-40.

Rhodes, D. & Klug, A. (1980). Helical periodicity of DNA determined by enzyme digestion. *Nature.* 286, 573-578.

Rodley G.A. et al. (1976). A possible conformation for double stranded polynucleotides. *Proc. Natl. Acad. Sci. USA,* 73, 2959-2963

Travers, A. & Muskhelishvili, G. (2007) A common topology for bacterial and eukaryotic transcription initiation? *EMBO reports,* 8, 147151.

Tsao, Y.P; Wu, H.Y. & Liu, L.F. (1989). Transcription-driven supercoiling of DNA: direct biochemical evidence from in vitro studies. *Cell*, 56, 111-118.

Tuteja, N & Tuteja R, (2004) Unraveling DNA helicases. *Eur. J. Biochem.* 271, 1849-1863.

Ullsperger, C & Cozzarelli, N.R. (1996). Contrasting enzymatic activities of topoisomerases IV and gyrase from *Escherichia coli. J. Biol. Chem.,* 271, 31549-31555.

Wang, A.H.J. et al., (1979) Molecular structure of a left-handed double helical DNA fragment at atomic resolution., *Nature,* 282, 680-686.

Wang, J.C. (1979). Helical repeat in solution. *Proc. Natl. Acad. Sci, U.S.A.*76, 200-203.

Wang, X. et al. (2010). Double-stranded DNA homology produces a physical signature. *Proc. Natl. Acad. Sci, U.S.A.107*, 12547-12552..

Watson, N. (1988). A new revision of the sequence of plasmid pBR322. *Gene*, 70, 399-403.

Watson, J.D. & Crick, F.H.C. (1953a). A structure for deoxyribose nucleic acids. *Nature.* 171, 737-738.

Watson, J.D. & Crick, F.H.C. (1953b). Genetical implications of the deoxyribonucleic acid. *Nature.* 171, 964-967.

Wu, H.Y. Shyy, S. Wang, J. C. & Liu, L. F. (1988). Transcription generates positively and negatively supercoiled domains in the template. *Cell*, 53, 433-440.

Xu, Y.C.; Li Qian &Tao, Z.J. (1982). A hypothesis of DNA structure.—Inspiration from the topological transformation of supercoiled DNA. *Scientia Sinica.* 25B, 827-836.

Xu, Y.C. & Li Qian (1983). Determination of linking number of pBR322 DNA. *Scientia Sinica.* 26B, 602-613

Xu, Y.C. (2009). Finding of a zero linking number topoisomer. *B.B.A.* 1790, 126-133

Yagil, G. (1991). Paranamic structure of DNA and their in DNA unwinding. *Critical review in Biochem. and Mol. Biol*. 26, 475-559.

Zanchetta, G. et al. (2010). Right-handed double-helix ultreshort DNA yields chiral nematic phases with both right- and left-handed director twist. *Proc. Natl. Acad. Sci, U.S.A.*107, 17492-17502.

Zimmerman, S.B. (2004). Studies on the compaction of isolated nucleoids from *Escherichia coli. J. structural Biol.*147, 145-158

DNA Replication-Current Advances

Edited by Dr Herve Seligmann

ISBN 978-953-307-593-8
Hard cover, 694 pages
Publisher InTech
Published online 01, August, 2011
Published in print edition August, 2011

The study of DNA advanced human knowledge in a way comparable to the major theories in physics, surpassed only by discoveries such as fire or the number zero. However, it also created conceptual shortcuts, beliefs and misunderstandings that obscure the natural phenomena, hindering its better understanding. The deep conviction that no human knowledge is perfect, but only perfectible, should function as a fair safeguard against scientific dogmatism and enable open discussion. With this aim, this book will offer to its readers 30 chapters on current trends in the field of DNA replication. As several contributions in this book show, the study of DNA will continue for a while to be a leading front of scientific activities.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

You Cheng Xu (2011). Replication Demands an Amendment of the Double Helix, DNA Replication-Current Advances, Dr Herve Seligmann (Ed.), ISBN: 978-953-307-593-8, InTech, Available from: http://www.intechopen.com/books/dna-replication-current-advances/replication-demands-an-amendment-of-the-double-helix

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen