CHAPTER 1

An Insuperable Problem

"I am willing to bet that the plectonemic coiling of the chains in your structure is radically wrong…"

Max Delbrück to James D. Watson, May 12, 1953

FEW PUBLICATIONS OF THE 20TH CENTURY can match the brevity and impact of the April 25, 1953 paper on DNA, the genetic material of all living organisms on Earth. Written by James D. Watson and Francis H.C. Crick (1916–2004), the one-page paper, published in the journal *Nature*, described a unique structure for DNA. Watson, then only 23 years old, had received his Ph.D. a little more than 2 years earlier; Crick, although almost 37, had yet to earn his doctoral degree because of his long years at the British Admiralty before embarking on graduate studies.[1] Their structure of DNA, later termed the double helix or the Watson–Crick structure, was to become one of the most celebrated images in science books and the medical literature, as well as in boardroom brochures and the news media.

Numerous pictorial renditions of this structure of DNA have since appeared, including two by the eminent Spanish surrealist painter Salvador Dali (1904–1989). However, a simple hand-drawn sketch in the original paper by Watson and Crick (Fig. 1-1a)[2] nicely captures the essential features of the double helix. In this depiction, the DNA molecule is shown to possess two separate strands, with the backbone

1. Crick thought that his late start had been a blessing in disguise. He wrote in his autobiography *What Mad Pursuit* (1988. Basic Books, Inc., New York, p. 16): "By the time most scientists have reached age thirty they are trapped by their own expertise. They have invested so much effort in one particular field that it is often extremely difficult, at that time in their careers, to make a radical change. I, on the other hand, knew nothing, except for a basic training in somewhat old-fashioned physics and mathematics and an ability to turn my hand to new things." Watson also remarked on several occasions that the mediocre work of his Ph.D. thesis had actually helped him, because it left him very little to chew on and forced him to move in a new direction.

2. Drawing done by Odile Crick, an artist and Francis Crick's wife.

Figure 1-1. The DNA double helix. (*a*) In the double helix model of DNA proposed by James D. Watson and Francis H.C. Crick in 1953, the ribbons represent the sugar–phosphate backbone chains of the two strands of the DNA molecule, each following a right-handed helical path about a common central axis indicated by the vertical line. The chains run in opposite directions, as indicated by the two arrows. The horizontal bars represent hydrogen-bonded base pairs (bp), each of which lies in a plane perpendicular to the vertical axis. See the text for additional details. (Redrawn, by permission of Macmillan Publishers Ltd., from Watson J.D. and Crick F.H.C. 1953. *Nature* **171:** 964–967.) (*b*) The arrangement of atoms in each of the two backbone strands of DNA is shown in this three-nucleotide stretch of a strand. The numbering system for the pentose ring, the five-carbon sugar moiety, is shown for the topmost nucleotide; the five carbon atoms in the ring are numbered 1′ through 5′. The lines in the structure represent covalent bonds with the two adjoining atoms sharing one or more pairs of electrons to create the bond. The single and double lines represent, respectively, the sharing of one pair of electrons (a single bond) and the sharing of two pairs of electrons (a double bond). The oxygen (O), phosphorus (P), and carbon (C) atoms normally form two, five, and four bonds, respectively, with their neighboring atoms (counting each double bond as two bonds), whereas the hydrogen (H) atom forms only one bond with a neighboring atom. To avoid the overclustering of a diagram, the H atoms attached to a carbon atom are often omitted. For simplicity, the four carbon atoms (1′–4′) and the O atom in each sugar moiety are shown as lying in a flat plane; in its actual structure, the five-membered pentose ring is puckered, with the 2′ carbon atom of the ring projecting upward, in the same direction as the base attached to the ring. Each phosphate moiety (PO_4^-) in the backbone strand of the DNA is shown as carrying a negative charge (–), but such negative charges on the backbone chains are usually shielded by positively charged "counterions" (not shown). The arrow to the left of the sketch indicates the polarity of the backbone strand of the DNA in the 5′ to 3′ direction.

With all the accolades bestowed on DNA in the decades since Watson and Crick's 1953 paper, its humble earlier history has been all but forgotten. Thus, for example, the name guanine was derived from the word guano, in which this base was initially identified. DNA itself was first isolated in 1869, by the young Swiss physician Johann Friedrich Miescher (1844–1895), from excrements of wounds that he extracted from discarded surgical wound bandages that he obtained from a local clinic.

of each strand represented by a narrow ribbon. The two ribbons coil around a common axis resembling the side rails in a twisted ladder. Chemically, each strand of the helix is a chain of alternating sugar (deoxyribose, the "D" in DNA) and phosphate moieties. Attached to each sugar moiety is a substance known as a heterocyclic "base," which consists of atoms of carbon, nitrogen, oxygen, and hydrogen. The four different kinds of DNA bases are adenine, cytosine, guanine, and thymine, represented, respectively, by the single letters A, C, G, and T.[3]

In chemical terms, DNA is a polymer of building blocks called nucleotides, each of which consists of a deoxyribose sugar moiety, a phosphate moiety, and a base—A, C, G, or T—attached to the sugar (see Fig. 1-1b). How the sugar, phosphate, and base components are arranged in a nucleotide, and the way in which the nucleotides are linked together to form a DNA strand, had been elucidated by 1953, mainly through the efforts of the chemists Albrecht Kossel (1853–1927), Phoebus Levine (1869–1940), and Alexander Todd (1907–1997). The Watson–Crick structure specifically addressed the way in which the two strands are arranged in space.

A key feature of the DNA double helix is that its two separate strands are held together by the pairing of bases via the formation of hydrogen bonds (H-bonds). In the Watson–Crick structure, pairing of the bases follows a particular scheme: The base A specifically pairs with the base T (and vice versa) through the formation of two hydrogen bonds between them, and the base G pairs with the base C (and vice versa) through the formation of three hydrogen bonds.[4] This pairing scheme allows the different base pairs to assume a nearly uniform geometry throughout the DNA molecule (Fig. 1-2). In the illustration shown in Figure 1-1a, the hydrogen-bonded base pairs are represented as rods connecting the two backbone strands of the DNA molecule. All of the rods are of the same length because the overall shape of an A–T pair is about the same as that of a C–G pair (Fig. 1-2). Be-

3. The four bases present in DNA are sometimes modified with additional chemical groups; such modifications serve important biological functions, but these subjects are outside the scope of our discussion here.

4. In the original Watson–Crick structure of DNA, both A–T and G–C pairs were assumed to have two H-bonds per pair; it was Linus Pauling (1901–1994) who correctly noted that in a G–C pair, three H-bonds are formed between the two bases.

In the biological molecules, the formation of a hydrogen bond often involves a hydrogen (H) atom that is covalently bound either to a nitrogen (N) or an oxygen (O) atom. Atoms like N or O have a high "electronegativity," or tendency to attract and hold tightly the pair of electrons that join it to an H atom. As a consequence, an H atom that is covalently bound to an N or O atom becomes electron deficient. This deficiency in turn favors the interaction of the H atom with another, relatively electron-rich N or O atom. An H-bond is commonly represented by a dotted line, as

—N—H···N— or —O—H···O— or —O—H···N—

in which the solid lines represent the regular covalent bonds between atoms. The strength of a hydrogen bond is only a fraction of that of a typical covalent bond, such as the bond between two hydrogen atoms in a molecule of hydrogen (H—H), or that of the bonds between hydrogen and oxygen in a molecule of water (H—O—H). The presence of a large number of hydrogen bonds between two macromolecules, such as the two strands of the DNA double helix, often contributes very significantly to their association with one another.

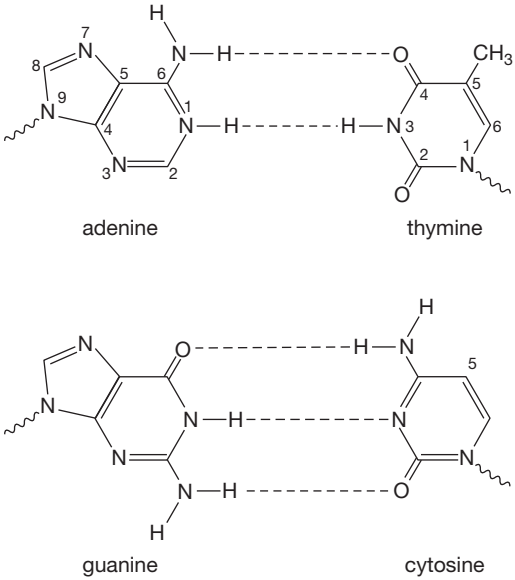

Figure 1-2. Schematic representations of an adenine–thymine (AT) base pair (*top*) and a guanine–cytosine (GC) base pair (*bottom*). Covalent bonds are represented by continuous lines and H-bonds by dotted lines; the wiggly line in each base indicates the position at which the 1′ carbon atom (C1′) of the sugar moiety is linked to the nitrogen atom of the base. Many of the H atoms are omitted in these sketches. The numbering systems for the bases A and T are shown in the *top* diagram. The atoms in G, in the *bottom* diagram, are numbered as in A in the *top* diagram (G and A belong to the class of bases known as purines), and the atoms in T (*top* diagram) are numbered as in C (*bottom* diagram) (T and C belong to the class of bases known as pyrimidines). In the *bottom* diagram, only the numbering of the C5 atom is indicated; this position is sometimes modified, for example, by methylation. The geometries of the base pairs in both the *top* and *bottom* diagrams are drawn according to the Watson–Crick model. It is significant that the overall shapes of the A–T and G–C pairs are very similar; this similarity allows the base pairs to be vertically "stacked" in an ordered way, linking the two strands of the DNA so as to form a smooth double helix. It is now known that there are significant variations in the geometry of a base pair, depending on its type and the types of its neighboring base pairs; consequently, the structure of a typical DNA molecule is not the evenly regular double helix depicted in the idealized Watson–Crick model.

cause of the asymmetric structure of a nucleotide (see Fig. 1–1b), there is a built-in polarity when the nucleotides are linked together to form one DNA strand. In the Watson–Crick double helix, the two strands run in opposite directions and are thus termed "antiparallel" as is shown in Figure 1-1a.

The discovery that base pairing holds the two strands of a DNA molecule together in the double helix structure electrified the scientific world: The Watson–Crick scheme immediately suggested a way of passing genetic information from one generation of an organism to the next. It was clear that the strands or backbones of DNA molecules, composed of an invariant and rather monotonous alteration of sugar and phosphate groups, could not possibly code for the genetic information that distinguishes one organism from another. But, different sequences of the four bases A, C, G, and T along a DNA backbone could spell out different genetic blueprints. In other words, the genetic blueprints in all forms of life could be determined by the "language" created by varying arrangements of the four letters A, G, C, and T. And, because the Watson–Crick pairing scheme specifies a one-to-one correspondence between bases on the two antiparallel strands, a sequence of 5′·····AATGCCTTA·····3′ in one strand (in which the symbols 5′ and 3′ at the ends of the sequence specify the polarity of the strand[5]) requires that the sequence of the other strand must be 3′·····TTACGGAAT·····5′. The two strands in the DNA double helix are thus not identical; rather they are "complementary" to each other.

The concept of complementarity immediately suggested that a parent DNA molecule could be duplicated by copying each strand in accordance with the same Watson–Crick scheme base-pairing: Each strand of the pair of parental strands in the duplex could thus acquire a newly synthesized complementary strand to yield two identical double helices.

Another key feature of the Watson–Crick double helix is that both strands follow a right-handed helical path around a central axis, owing to the orderly "stacking" of the planar base pairs, each of which is rotated 36° counterclockwise relative to the base pair below it. It is this very feature that makes DNA a "double helix." It is also easy to see, in the helical structure depicted in Figure 1-1a, that the two strands revolve around each other. Structures with intertwined lines are termed *plectonemic*, whereas multistranded structures in which the strands do not intertwine are called *paranemic*—terms first used in 1941 by C.L. Huskins (1897–1953) in a discussion of chromosome structures. Although many who read the 1953 Watson and Crick paper were awestruck by the principle of complementarity and its implication in passing genetic information from one generation to the next, others were skeptical about the plectonemic nature of the double-helix structure. Among the doubters was Max Delbrück (1906–1981).

5. The numbers 5′ and 3′ refer to the particular carbon atoms in the sugar moiety (see Fig. 1-1b). The notation 5′·····AATGCCTTA·····3′ specifies that the sequence of nucleotides runs in the 5′ to 3′ direction.

A physicist by training, and in his later years often referred to as the "father of molecular biology," Delbrück was in 1953 already a towering figure. The youthful Watson was in close correspondence with Delbrück both before and after the publication of the DNA double-helix paper. Delbrück was never known for mincing words. In a letter to Watson written on May 12, 1953—a month after the double-helix paper appeared—he was characteristically blunt, saying "I am willing to bet that the plectonemic coiling of the chains in your structure is radically wrong, because (1) The difficulties of untangling the chains do seem, after all, insuperable to me. (2) The X-ray data suggest only coiling but not specifically your kind of coiling." [6]

Interpretation of the DNA X-ray data, then only available for oriented DNA fibers pulled from a concentrated DNA solution, was not as straightforward as is usually perceived. As late as 1979 there were still those who argued that the famous X-ray photograph of the DNA helix could be interpreted in terms of a paranemic structure, in which two side-by-side strands were not intertwined.[7] Because the X-ray data available in 1953 could not really "prove" a particular structure for DNA, Delbrück's objection to the double-helix structure, stemming from his concern about the "untangling problem," was justified. But what exactly was this problem?

THE UNTANGLEMENT PROBLEM

Delbrück envisioned that the scheme of semiconservative replication of DNA—so designated because one-half of the progeny duplex was inherited and the other half was newly synthesized—would convert a pair of intertwined strands in the parent DNA to a pair of intertwined DNA double helices (Fig. 1-3). Unless the pair of intertwined

6. In addressing the potential problem of coiling and uncoiling of chromosomes during mitosis, Watson and Crick had written earlier: "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*" (1953. *Nature* **171:** 964–967). Delbrück's choice of the adjective "*insuperable*" was therefore likely deliberate. The X-ray data to which Delbrück referred was that of Rosalind E. Franklin (1920–1958), who first obtained the X-ray diffraction patterns of ordered bundles of DNA fibers.

7. In 1976, two groups of researchers had proposed DNA structures in which two antiparallel chains of nucleotides alternate in 5-bp segments of left-handed and right-handed twists. These and additional concepts favoring a *paranemic* "side-by-side" double-stranded DNA, as well as fiery advocacy by a noted mathematician William Pohl (1938–2000), had sufficiently alarmed a no less distinguished figure than Francis Crick. Crick came to the defense of the plectonemic double-helix structure, and with colleagues published a summary of the arguments for the plectonemic structure, based on the properties of DNA rings with intact strands (Crick, F.H.C., et al. 1979. *J. Mol. Biol.* **129:** 449–461). Even after that retort, however, the champions of the side-by-side model stuck to their guns. A.G. Rodley and his collaborators in New Zealand made detailed calculations to show that their paranemic side-by-side model could fit the X-ray diffraction data for DNA fibers as well as, if not better than, could the Watson–Crick structure itself. Rodley and colleagues' results were circulated to many of those interested in DNA structure, but by then their cause was already lost. Decades after the 1979 debate, a few still remained skeptical that the double helix was the biologically important DNA structure within cells (see, e.g., the review by Delmonte, C.S. and Mann, L.R.B. 2003. *Curr. Sci.* **85:** 1564–1570).

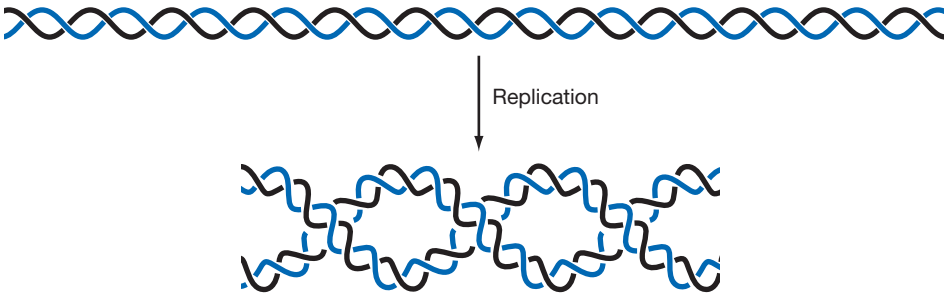

Figure 1-3. The formation of a pair of intertwined DNA double helices following the replication of a parental DNA double helix, as envisioned by Max Delbrück. Delbrück reasoned that, because of the right-handed intertwining of the two strands in a DNA molecule (*top* diagram), upon duplication of the strands, the pair of progeny DNA double helices would also be intertwined in a right-handed way (*bottom* diagram). If the two parental DNA strands did not uncoil at all as they replicated, then as the Watson–Crick double-helix model suggests, the two progeny double helices would revolve about each other approximately once for every 10 base pairs; the progeny double helices shown here are not nearly as tightly intertwined as that.

progeny helices could be completely untangled, it would be impossible for them to separate when a cell divided. Improper segregation of newly replicated DNA into two progeny cells would have disastrous consequences, because the faithful passage of genetic information from one parent cell to two progeny cells would be impaired.

In principle, the conversion of intertwined parental strands of DNA to intertwined progeny double helices could be avoided by proper rotational motions of the double helices of the parent and progeny DNA molecules around their respective axes, as illustrated in Figure 1-4.[8] In the 1950s there was little information about the sizes of DNA molecules inside a cell. Later, however, it would become clear that a DNA molecule inside a cell can be very long, and that an entire chromosome consists of a single unbroken DNA molecule that may contain huge numbers of nucleotides. The largest chromosome in a human cell, for example, contains about a quarter of a billion base pairs, and if it were extended from one end to the other, would span a length of 8.5 cm. This is an enormous length relative to the dimension of a cell nucleus, which is only about several micrometers in diameter. If a nucleus were to be enlarged to the size of a basketball, an 8.5-cm-long chromosome would become 5 kilometers or about 3 miles long! Once the length of a DNA molecule inside a cell became known,

8. Watson and Crick recognized the advantage of directly untwisting the unreplicated DNA double helix to separate it into two side-by-side strands to allow replication to occur (1953. *Cold Spring Harb. Symp. Quant. Biol.* **18:** 123–131). Others also pointed out that if a DNA replication fork is represented by a Y, its vertical stem representing the unreplicated parental portion and the two arms of the Y the growing progeny duplexes, then rotating each part of this DNA "trio" around its respective helical axis would shorten the stem and lengthen the arms, without untanglement of the arms (see, e.g., Levinthal, C. and Crane, H.R. 1956. *Proc. Natl. Acad. Sci.* **42:** 436–438).

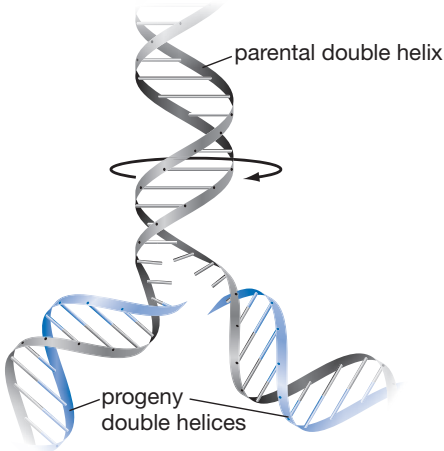

Figure 1-4. Avoidance of the intertwining of a pair of progeny DNA double helices by rotation of the unreplicated parental DNA double helix. The drawing represents a replication fork, as described in Footnote 8, proceeding in the upward direction. Intertwining of the progeny DNA molecules with one another could be avoided by rotation of the parental double helix in the direction indicated by the arrow.

the prospect of solving the untanglement problem by rotating a molecule of DNA from one end of a chromosome to the other end did not seem very plausible.

THE PROBLEM WORSENED: THE DISCOVERY OF DNA RINGS

The discovery of ring-shaped DNA molecules in the 1960s changed the nature of the untanglement problem. These molecules, often called "circular DNA" or "cyclic DNA," were initially found in virus particles.[9] Of particular significance was the 1963 finding, made in the laboratories of Renato Dulbecco and Jerome Vinograd (1913–1976), that the DNA of a polyoma virus that infects animals is a double-stranded ring with intact strands (Fig. 1-5).[10] This observation, the existence of ring-shaped DNA molecules, underscored the conceptual as well as the actual difficulty of unentangling DNA.

9. In 1962, a single-stranded DNA from the *E. coli* virus ϕX174 became the first known ring-shaped DNA, through the work of Walter Fiers and Robert L. Sinsheimer (1962. *J. Mol. Biol.* **5:** 424–434).

10. Two papers, written by researchers at two laboratories at the same institution and published in the same journal 2 mo apart, reported nearly identical studies (Dulbecco, R. and Vogt, M. 1963. *Proc. Natl. Acad. Sci.* **50:** 236–243; Weil, R. and Vinograd, J. 1963. *Proc. Natl. Acad. Sci.* 730–738). In the first of these papers, Renato Dulbecco and Marguerite Vogt stated that "This work will be supplemented by studies with the analytical ultracentrifuge," citing the Weil and Vinograd paper as being "*in preparation.*" Weil and Vinograd, in their turn, acknowledged Dulbecco for providing the polyoma virus for their study, and for providing them with a copy of the Dulbecco and Vogt manuscript before its publication.

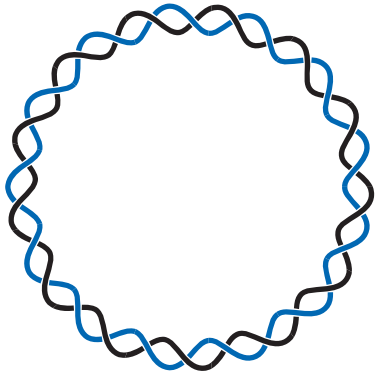

Figure 1-5. Representation of a DNA ring in which both strands are intact (a "covalently closed DNA ring"). Because of the double-helical structure of DNA, such a ring can be viewed as containing a pair of multiply linked, single-stranded rings of complementary nucleotide sequences. Polyoma virus DNA contains ~5000 base pairs in a double-stranded ring; if this DNA assumes the classical Watson–Crick structure, the two strands revolve around each other about 500 times in the viral DNA ring.

A decade before the discovery of ring-shaped DNA, Watson and Crick had admitted in their second and third publications on the DNA double helix that the "difficulty of untwisting is a formidable one." But they did not think that uncoiling the strands of a double-helix structure would pose any fundamental problem.[11] If, however, a plectonemic double-stranded DNA is in the form of a ring (as shown in Fig. 1-5), then its two component single-stranded rings are linked and cannot possibly come apart without breaking at least one of the rings. The very conclusion that the DNA of the polyoma virus is in the form of a ring with two intact circular strands was based on the finding that the two strands of polyoma DNA did not come apart when the DNA was exposed to conditions that disrupt base pairing.[10]

In the same year, 1963, several striking images of the DNA of the bacterium *Escherichia coli* also appeared. John Cairns used a clever strategy to obtain images of very long DNA molecules embedded in an overlaid film of photographic emulsion on a coated microscope slide. Cairns did this by first incorporating tritium into *E. coli* DNA. (The nucleus of the usual hydrogen atom, ^{1}H, has just one proton, whereas the nucleus of its radioactive isotope tritium, ^{3}H, contains one proton and two neutrons.) When the tritium atoms incorporated into the DNA of *E. coli* subsequently decayed, they emitted β particles (high-speed electrons) that caused the deposition of silver grains in the photographic emulsion near the sites of these radioactive decays, thereby tracing out the shape of the DNA and yielding an "autoradiogram" of it. From such images, Cairns deduced that the entire chromosome of *E. coli*, several million base pairs long, likely existed in the form of a ring that replicates semiconservatively from a unique region of the parent DNA ring.[12]

11. Watson, J.D. and Crick, F.H.C. 1953. *Nature* **171:** 964–967; 1953. *Cold Spring Harb. Symp. Quant. Biol.* **18:** 123–131.

12. Cairns, J. 1963. *J. Mol. Biol.* **6:** 208–213.

A QUIESCENT PERIOD BEFORE THE SHIFTING TIDES

As initially conceived by Delbrück, the untanglement problem arose from the semiconservative replication of a plectonemically wound two-stranded DNA structure. Any hope that replication might not require a separation of the strands was dashed in 1958, when Matthew Meselson and Frank Stahl published their celebrated results, which showed unequivocally that *E. coli* DNA replicated semiconservatively.[13] The entanglement problem was further heightened by the 1963 work of Dulbecco and Vinograd on polyoma DNA, which had shown convincingly that the two strands in a DNA double helix must be intertwined to some extent, if not exactly to the extent required by the Watson–Crick structure. Looking back, it seems surprising that the DNA untanglement issue, so forcefully promoted by Delbrück at the birth of the concept of a plectonemic DNA double helix, was not tackled more aggressively in the following years. Several attempts, however, were made in the mid- to late 1950s. Delbrück himself had suggested one solution, which turned out to be off track. George Gamow (1904–1968), the physicist and cosmologist who posited the "Big Bang" as the source of the universe, proposed another solution for the problem, but it too was doomed.

By the 1960s, however, it seemed that Delbrück's objection to the double-helix structure had largely been forgotten, and even Delbrück himself had apparently turned his attention away from this problem.[14] Moreover, neither Dulbecco nor Vinograd, then working at the California Institute of Technology, where Delbrück was also residing, elaborated on the problem of unlinking the parental strands in a polyoma DNA ring in their 1963 publications. Similarly, although Cairns' spectacular images of ring-shaped *E. coli* DNA molecules were spreading like wildfire in the molecular biology community, there was very little discussion of the problem of how the strands of a ring-shaped chromosome became untwined during DNA replication.

Toward the end of the 1960s, however, a subtle shift occurred in the approach to the problem of DNA untanglement. Before this, the problem had mainly attracted the attention of physicists and chemists who tended to focus on a structural solution to the problem. But by the late 1960s, biochemists were beginning to consider solutions that rely on the actions of enzymes—protein catalysts known to promote a variety of reactions in the biological world.[15]

13. A detailed account of the experiment can be found in the late F.L. Holmes's 2001 book *Meselson, Stahl, and the replication of DNA: A history of "the most beautiful experiment in biology"* (New Haven, Yale University Press).

14. There was apparently a $5 bet between Crick and Delbrück on the plectonemic nature of the DNA structure (see Watson, J.D. 2002. *Genes, girls, and Gamow*, p. 43. Alfred A. Knopf, New York). It is not known whether Delbrück ever paid up, and if so, whether it was the data or circumstance that caused him to concede.

15. It was found in the 1980s that certain ribonucleic acids (RNAs) could also act as biological catalysts; such RNAs are termed ribozymes.

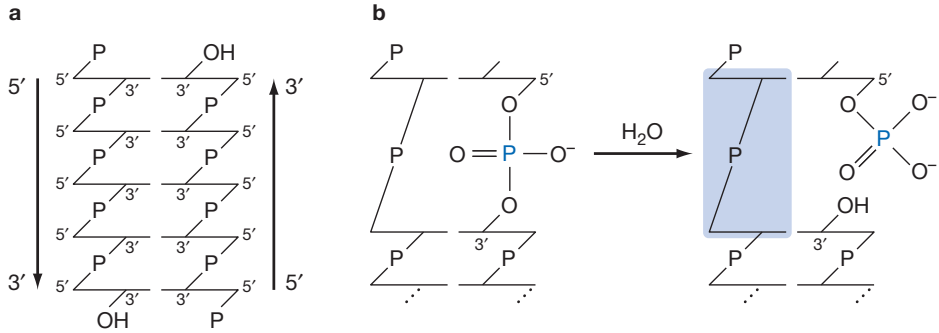

Figure 1-6. Solving the DNA untanglement problem during replication by nicking one of the two DNA strands. (*a*) A simplified representation of a double-stranded DNA. Each zigzag line represents a DNA backbone strand consisting of phosphate moieties (P) linked to the 5′ carbon atoms of ribose moieties, whose 3′ carbon atoms are linked to other phosphate moieties (·····P-5′– 3′- P-5′– 3′- P····) (compare with Fig. 1-1b). The horizontal lines connecting the two zigzag lines represent the base pairs joined by hydrogen (H) bonds at the gaps between the horizontal lines. (*b*) Expanded section of the DNA representation in *a*, to show the cleavage of the bond linking one phosphoryl moiety to the 3′ carbon of an adjacent ribose group. DNase catalyzes the attack of the phosphorous atom by the oxygen atom of a water molecule (H_2O), to break a P–O bond in the DNA backbone, yielding either a 5′- or a 3′-phosphate group that carries a double-negative electronic charge ($–PO_4^{=}$), depending on the particular DNase, and a 3′- or 5′-OH group (in the figure, a 5′-phosphate and a 3′-OH group are produced). The shaded area opposite the nick is shown in greater detail in Fig. 1-7.

One class of enzymes that stood out as attractive candidates in solving the DNA untanglement problem was the so-called deoxyribonucleases, or DNases. These enzymes, which were known long before the double-helix structure was proposed, catalyze the hydrolysis of the chemical bonds that link the components in the backbone strands of a DNA molecule. In this hydrolysis, a water molecule attacks a phosphorous, breaking a bond in the DNA backbone between the phosphorous and the sugar moiety to which it is attached (Fig. 1-6). There are two major categories of these enzymes: Those that "nibble" from the ends of DNA strands are called DNA exonucleases, and those that introduce breaks within DNA strands are called DNA endonucleases. Among the endonucleases, those that can break a single strand of the DNA double helix, rather than breaking both strands at once, appeared to be the most attractive candidates for resolving the DNA untanglement problem. In a replicating double-stranded DNA, a few strategically placed breaks ("nicks") in one of the strands could in principle solve the problem of how to separate the interwound strands. Chemists had long known that there is very little resistance to the rotation of two parts of a molecule separated by a single bond, like the bond between the two CH_3 groups in ethane, $H_3C—CH_3$. Accordingly, if a nick is made on either side of a DNA segment within a long DNA molecule, then each of several single bonds on the strand opposite each nick can serve as a swivel for rotation of the DNA segment

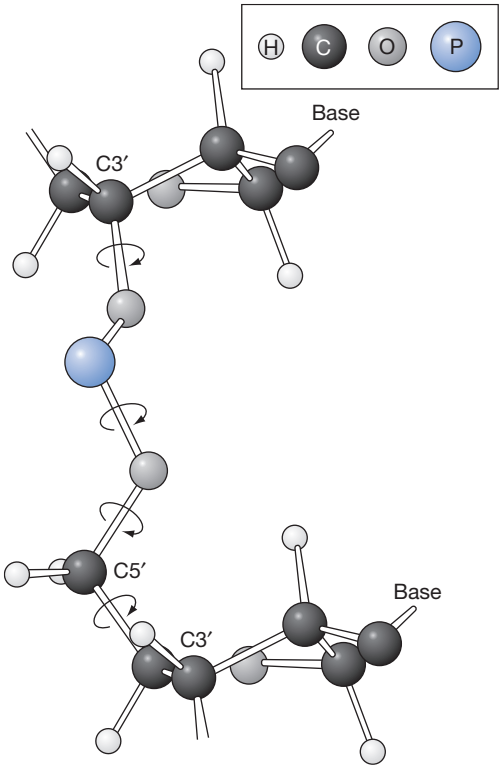

Figure 1-7. A structural model depicting the backbone bonds that link two neighboring bases in the strand directly across from the nick in its complementary strand (the shaded area in Fig. 1-6b). The carbon atoms are represented by black spheres, the hydrogen atoms by white spheres, the oxygen atoms by gray spheres, and the phosphorus atoms by blue spheres. The sugar rings are shown in the puckered conformation in which they exist in a typical DNA molecule (see legend to Fig. 1-1b). The curved arrows are placed around single bonds in the DNA backbone that connect two adjacent bases in the DNA strand. Rotation around these single bonds can take place in either direction and does not necessarily have to occur in the direction indicated by the arrows.

relative to the rest of the molecule (Fig. 1-7). In other words, in the presence of a few nicks, the untwining of one part of a long DNA could proceed without rotating the entire DNA.

When in 1968 Ju-ichi Tomizawa and Tomoko Ogawa attempted to find such strand breaks in a replicating bacteriophage λ DNA, they found that fewer than one, and probably none, could be detected in either of the two strands of this DNA, each of which comprised 50,000 nucleotides.[16] Nevertheless, their findings could not rule out the possibility that breaks in DNA strands are indeed the means that solve the DNA untanglement problem. The breaks, or nicks, might be of a mobile type—that is, they might be created at one site, disappear upon rejoining of the ends of the nicked or broken DNA strand by an enzyme called DNA ligase, and occur again somewhere else in the DNA molecule ahead of a replication fork (Fig. 1-4). It would indeed be a daunting task to prove or disprove the presence of such mobile replication swivels.

16. Tomizawa, J.-I. and Ogawa, T. 1968. *Cold Spring Harb. Symp. Quant. Biol.* **33:** 533–551.

"Fortunately, science" wrote the English chemist Humphrey Davy (1778–1829) "…is neither limited by time nor by space." By 1968, when Tomizawa and Ogawa showed that very few if any strand breaks were present in the DNA of a replicating phage λ, hints about Nature's way of solving the DNA untanglement problem were about to emerge. It would soon be revealed that the idea of mobile replication swivels was close to the mark, but the particular kinds of mobile swivels found would be entirely unexpected.