DNA in Motion: A Student Exercise for Modeling Key Molecular Events

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Abstract
Students often struggle to understand the full implications of some basic chemical concepts of DNA structure and function, especially how DNA's directionality and antiparallel nature determine key functional features of replication and molecular recombination. Visualizing the complexities of these processes requires a working knowledge of how DNA's nucleotides are assembled and how these components interact. This article describes a simple activity that can be used to visualize how nucleotides join together, how base pairs form, and, most importantly, how the active processes of replication and recombination are related to DNA chemistry. In this activity, students model DNA structure, with each student representing a single nucleotide, then join together to form a polynucleotide with 5' to 3' directionality. Two chains then pair to form the antiparallel DNA duplex. The activity not only illustrates the basic chemistry of DNA but also allows students to participate in active modeling of leading-strand and lagging-strand replication and in the formation of the Holliday junction molecule, the basic intermediate of recombination events including crossing over and gene conversion. The demonstrations can be videotaped from above to make a permanent copy of these events for teaching and study purposes. Example illustrations and links to videos are included.

Key Words: antiparallel DNA; DNA polymerase I; DNA polymerase III; DNA replication; heteroduplex; Holliday junction molecule; lagging-strand replication; leading-strand replication; ligase; Okazaki fragment; primase.

Introduction
Understanding basic biological concepts requires a knowledge of the nature of the molecules involved. In teaching these concepts at the secondary school or introductory college level, many useful models of biochemical mechanisms have been developed. These include models that compare biochemical mechanisms to everyday objects, like the lock-and-key model of enzyme activity (Fischer, 1894; Schneider, 2015), the ratchet-like activity of myosin's activity in muscle fibers (Gebhardt, 2006), and the rotary engine-like activity of the ATP-producing mitochondrial enzyme ATPase (Yoshida et al., 2001). Similarly, many helpful models of DNA structure and action have been published, including a clear, simple paper model (Sigismondi, 1989); models using pop beads (e.g., “DNA in Action–Super Value Kit,” item no. FB1223, Flinn Scientific, https://www.flinnsci.com; “DNA Simulation 4-Way Beads,” item no. 171043, Carolina Biological Supply, https://carolina.com); and other commercially available tabletop models (e.g., “Flow of Genetic Information Kit,” item no. 211130, and “DNA, Replication and Transcription Set,” item no. 211119, Carolina Biological Supply; “DNA Structure and Replication Molecular Model,” item no. 470014-600, and “DNA and Its Replication Model Kit,” item no. 470123-044, Ward’s Science, https://wardsci.com). Although some of these models are structurally sound, they do not all illustrate the directionality of a polynucleotide or the double helix's antiparallel nature. Also, on occasion, I prefer to use a model that can be part of a full-class-participation activity.

While modeling biochemical mechanisms at one’s desk using the kits mentioned above has the advantage of requiring the students to understand the entire process involved, my experience has been that a whole-class activity has the advantage of promoting a degree of involvement and excitement that enhances the learning activity, partially related only to the fact that it requires students to get out of their desks and, in this case, go outside. But it also forces students to think about the molecular interactions that occur between chemical components. This modeling activity not only illustrates the importance of DNA's directionality and antiparallel structure in its function, but also requires students to take an active role in the dynamics of DNA function. I emphasize to students that they should think of DNA not as just a stationary blueprint – rather, DNA should be seen as a molecule in motion.
models propose, DNA should be seen as a molecule in motion. For example, during replication, it is not a stationary molecule that DNA polymerase travels down, but rather it is moving through a large replication complex, emerging on the other side as two identical helices (HHMI: BioInteractive, https://www.biointeractive.org/classroom-resources/dna-replication-basic-detail). While the replication exercise described here does not model the multi-enzyme complex through which DNA passes during replication, it does, along with the recombination exercise, illustrate two aspects of the DNA dance (Saintillan et al., 2018; Sumner, 2018) involved in cellular processes.

○ An Activity to Illustrate DNA Structure

In this modeling exercise, a DNA nucleotide is portrayed by a single student wearing a red top who is viewed from directly overhead (Figure 1). In this view, the body/head represents the deoxyribose sugar; the student’s right arm, extended straight out to the right, is the nitrogen base, which is attached to the deoxyribose at position 1’ (the right shoulder); and the student’s left arm, extended straight forward, is the 5’ carbon side chain with a phosphate, the left hand, at its end.

To construct a DNA polynucleotide, students line up with arms outstretched as above, placing their left hands on the left shoulders of the students in front, creating a chain of students facing the same direction (Figure 2A). This polynucleotide demonstrates the directionality of DNA, running 5’ to 3’ as shown in Figure 2A, and demonstrates the sugar-phosphate backbone of each strand, with a phosphate (left hand) attached to the 5’ carbon (left elbow) of the deoxyribose. The phosphate is also attached to the 3’ carbon (left shoulder blade) of the adjacent nucleotide (the next student).

The double-stranded DNA molecule is constructed by adding a second, identical line of students standing on the right-hand side of the first line, but facing in the opposite direction (Figure 2B). Now, the two lines of students “shake hands” with their right hands, forming hydrogen bonds (base pairing) between the complementary bases.

○ An Activity to Illustrate Leading-Strand & Lagging-Strand Replication

The events of DNA replication described below are demonstrated in the DNA replication video accessible at https://youtu.be/DsGVSv0bnno.

Figure 1. Modeling a single nucleotide. In this DNA simulation, a student, viewed from directly overhead, represents a single DNA nucleotide. The body/head is deoxyribose, with the region of the left shoulder blade being where the 3’ carbon is located. The left arm extended straight forward is the chain that includes the 5’ carbon (the position of the elbow) with a phosphate attached at its terminus (the hand). The right arm extended to the right is the nitrogen base, which is attached to the 1’ carbon (the right shoulder).

Figure 2. Modeling single- and double-stranded DNA. The DNA polynucleotide (A, upper frame) is made by students placing their left hands (5’ phosphate) on the left shoulders (3’ carbon) of the students in front of them, thus illustrating the sugar-phosphate backbone as well as the 5’ to 3’ directionality of the polynucleotide. The double-stranded molecule (B, upper frame) is made by forming an identical line of students facing in the opposite direction and “shaking hands” (base pairing) with the complementary line, illustrating duplex DNA. Panel B, middle frame, is an overhead photo of the double-stranded molecule. The lower frame of panel B shows the molecular details of that duplex.
In modeling DNA replication (Figure 3 and the DNA replication video), I assume the events and enzymes as they occur in *E. coli* and as summarized in most college-level introductory genetics textbooks (e.g., Klug et al., 2018, chapter 11; see also the review by Xu & Dixon, 2018). That is, replication requires only DNA polymerase III on the leading strand (once replication is under way), but four enzymes, primase, DNA polymerase III, DNA polymerase I, and ligase, are needed on the lagging strand – replication being continuous on the leading strand but discontinuous with the joining of Okazaki fragments on the lagging strand. These concepts were first covered in class, and then one class session was devoted to an explanation of the model and practice modeling in the classroom. Since the class size was insufficient to provide enough nucleotides for the replication exercise, students from other classes were recruited. These students were used in parts of the model not requiring much active participation, such as the outer parental strands (upper and lower strands in Figure 3). Other than being told what color tops to wear, they were not given advance instruction. On the day of the event, with the help of the course’s students, they were instructed what to do. In fact, since the demonstration would benefit from having even more students than I used, in order to more accurately model these mechanisms, it might always be advisable to recruit outside students. The course’s students could always be placed in the active positions and therefore maximize their learning experience. After a few practice run-throughs, a drone was launched and the demonstration was videotaped from overhead.

The video shows the process involved in all stages of replication, while Figure 3 illustrates just two of them: the unwinding of DNA by helicase (Figure 3A) and a midway stage (Figure 3B) in which DNA polymerase III has nearly completed replication on the leading strand while primase has begun the synthesis of the RNA primer. The beginning point shown in the video demonstrates leading- and lagging-strand replication and shows students forming a replication fork with a partial Okazaki fragment (which began with RNA nucleotides) on the lagging strand and a few nucleotides of the nascent leading strand nucleotides in place. This RNA-containing Okazaki fragment is visible as the bases paired to the right end of the upper strand in Figure 3A. As already noted, students wear red tops if they

**Figure 3.** Modeling leading-strand and lagging-strand replication. See text for a detailed description. DNA nucleotides are in red, RNA nucleotides in blue. Above each photograph is the molecular interpretation of the student model. (A) Helicase’s action. (B) Leading-strand replication nearly finished on the bottom strand and, on the top, lagging-strand replication beginning with primase’s action. The time stamp at the bottom of each photo is the time on the DNA replication video.
are DNA nucleotides, but blue tops if they are RNA nucleotides. In Figure 3A, the upper lagging strand has a short RNA primer (four nucleotides long). These students are wearing blue tops. Also present is just the first base of the DNA polynucleotide of that Okazaki fragment at the extreme right end, 5’ to 3’ synthesis on this strand will proceed left to right. Each Okazaki fragment would actually extend for a thousand or more DNA nucleotides to the right in *E. coli*, whereas it would extend for only hundreds of nucleotides in eukaryotes. The true length of the RNA primers in both prokaryotes and eukaryotes is <10 nt (Okazaki et al., 1968, Balakrishnan & Bambara, 2013). On the nascent leading strand, which is base paired to the bottom strand, replication proceeds right to left, 5’ to 3’.

Figure 3A shows the breaking of the hydrogen bonds holding the two strands together by the enzyme helicase, which is modeled by a student walking down the center of the duplex breaking the handshakes as the two denatured chains move apart from each other.

As shown in the video, denaturation of the duplex DNA then proceeds to near the left end. Beginning near the right end of the bottom (leading) strand, DNA polymerase III, modeled by another student, adds DNA nucleotides (students with red tops) onto the 3’ end of the DNA primer on the leading strand, ensuring that the proper base pairing (handshake) forms and catalyzing the formation of the phosphodiester bond (left hand contacts left shoulder), thus extending the leading strand at each addition by one nucleotide. This process continues moving from right to left, 5’ to 3’.

In Figure 3B, on the bottom leading strand, DNA polymerase III has nearly completed synthesizing the complementary strand up through the last exposed template nucleotide. Now on the upper lagging strand, the enzyme primase, modeled by another student, has begun synthesizing the short RNA primer (made from students with blue tops), left to right, 5’ to 3’. The first RNA nucleotide added does not need a primer (a characteristic of all RNA polymerases including primase), but the additional RNA nucleotides are added onto the 3’ hydroxyl (left shoulder blade) of the most recently added nucleotide.

Once this short RNA primer (in this case, four nucleotides long) has been completed, DNA polymerase III first adds a complementary DNA nucleotide (red) to the 3’ hydroxyl of the RNA primer, then a second one onto that new DNA nucleotide’s 3’ hydroxyl end, then repeating this process for the entire Okazaki fragment. As mentioned above, in reality this segment made by DNA polymerase III, which constitutes most of the Okazaki fragment, is thousands of nucleotides long in *E. coli*. For practicality, we modeled only five nucleotides (red-topped students) being added by DNA polymerase III.

Once DNA polymerase III completes the Okazaki fragment— that is, when it comes to the blue RNA primer of the previously made Okazaki fragment— DNA polymerase I, modeled by yet another student, then takes over. DNA polymerase I will both remove the blue 5’ RNA nucleotide from the complex, breaking its phosphodiester linkage with the adjacent RNA nucleotide, and replace that RNA nucleotide with a red DNA nucleotide, forming the 5’ to 3’ linkage with the DNA nucleotide to the left.

In this way, the RNA nucleotides of the primer of the previous Okazaki fragment are completely replaced with DNA nucleotides. However, the final nucleotide added by DNA polymerase I is not bonded to the DNA nucleotide to the right. In the final step of the video, a student modeler represents the enzyme ligase, which forms the phosphodiester linkage between two adjacent DNA polynucleotides. With this, the entire stretch of DNA that was uncoiled by helicase has been faithfully replicated.

The time stamps from the DNA replication video are indicated at the bottom in Figure 3A and 3B.

○ **An Activity to Illustrate Molecular Recombination & the Holliday Junction Molecule**

The events of molecular recombination described below are demonstrated in the Molecular Recombination video accessible at https://youtu.be/UJ9ZQOtc2A4.

The proposed DNA student model can also be used to illustrate the molecular events involved in recombination, as originally proposed by Holliday (1964). His model, with various modifications, has been confirmed to be an accurate portrayal of the basic molecular events involved in the initiation of recombination both in prokaryotes and eukaryotes—the events responsible for recombination with outside marker exchange (crossing over) as well as those that do not result in outside marker exchange (gene conversion). To model recombination, four chains of student nucleotides in two duplexes are needed. The events of molecular recombination form an intermediate complex, the Holliday junction molecule, as shown in Figure 4 (Holliday, 1964). As with DNA replication, these concepts were first covered in class, then a partial class session was devoted to explanation and modeling in the classroom before moving outside. Since fewer nucleotides were needed for the recombination demonstration, recruiting other students was not necessary. The modeling of these steps using chains of students is shown in the video and summarized below.

Students begin by forming two polynucleotide chains, but these two duplexes line up in such a way that two chains of the same directionality are next to each other. That is, in Figure 4, going from top to bottom, the second and third chains both have their 5’ ends on the right and their 3’ ends on the left. (Of course, when two double helices lie next to each other, both are coils, so this would not be necessary if we were modeling double helical DNA. However, requiring students to twist around the DNA axis one 360° turn for every 10 base pairs would present a contortion nightmare. But, according to Holliday’s model, it is two chains of the same directionality that will interact with each other, so it is easiest in this non-helical duplex to have the two duplexes line up as shown in Figure 4.) This necessitates that one of the two student-formed duplexes, in this case the upper one, be formed by students placing their right hands (not left hands) on the shoulders of the student in front and they must make a left-handed handshake with the complementary strand (see details in Figure 4). Notice that one duplex (the lower one) wore light tops and the other duplex wore dark tops.

As shown in the video, recombination begins with a single-strand nick occurring in one of the two strands and that nicked strand moving toward the other duplex.

Next, the 5’ end of that nicked strand displaces the identical strand of the other duplex, forming base pairs with the homologous molecule in a process called strand invasion or strand displacement. A nick occurs in that displaced strand at the same position where the nick in the other strand occurred and that displaced strand then pairs with the available single-stranded region of the first molecule. These two steps require a bit of acrobatics on the part of the two student chains that are swapping places. If you watch the video carefully, you will see that the strand from the bottom duplex squats down and the strand from the top duplex, beginning at the right 5’...
end, steps over the other strand. Also, while the individuals from the bottom strand were using their left arms to form the phosphodiester linkage with the nucleotide in front, they now switched and use their right arms. This frees their left hands to now form the handshake (base pairs) with the uppermost strand in Figure 4. While DNA does not exactly behave this way, the polynucleotides essentially do the same thing by rotating around the phosphodiester bond (which our human chain cannot do easily).

Finally, the 5’ ends that were created by the nicking are now ligated with the exposed 3′ ends of the other duplex. This creates the recombination intermediate known as the Holliday junction molecule (Figure 4).

One important feature of Holliday’s model is that it predicts that recombination will produce a region of duplex, the “heteroduplex region,” in which one strand comes from one homologue and the other strand comes from the other homologue. This region is visible in Figure 4 as the zone with one dark and one light strand and is labeled on the upper part of the figure. The time stamp from the molecular recombination video is indicated at the bottom of Figure 4.

The actual events involved in recombination are more complex, but since this demonstration is designed as an introduction to the beginning steps of molecular recombination, adding more complexity might obscure the basic structural elements that the students need to learn.

Overhead videos of modeling exercises, accessible at https://youtu.be/DsGVsvbnnno and https://youtu.be/UJ9ZQOtc2A4, were filmed using a Phantom 3 Standard drone (DJI, Shenzhen, China). Videos can also be made from a window of an upper floor of a building. Videos were edited using iMovie and Pages (Apple Computer, Cupertino, California) and Graphic Converter 11 (Lemke Software, Peine, Germany).

○ Conclusions

The active modeling of DNA replication and molecular recombination described here has proven effective in my classroom teaching of a college-level introductory genetics course. Students were excited to take part in this activity. Since the DNA replication was posted on YouTube, it has received almost 20,000 views, with all very strongly positive comments.

The use of unmanned aerial vehicles (UAVs or drones) in basic research (Nowak et al., 2019) and learning (Schaffhauser, 2018) has soared, partially due to the availability of high-quality devices at reasonable prices. The Mavic Mini (DJI) and the FIMI X8 SE (Xiaomi) are available for $399 and $499, respectively. The use of drone videography in this modeling exercise optimizes the experience for the students, providing the best overall view of the processes involved, and yields a product easily distributable to students for learning. However, drone use is not mandatory for this exercise, since videos of nearly the same quality could be taken from an upper-floor window of a nearby building. Additionally, my experience has been that the physical process of modeling DNA and RNA has instructional value even without filming, as students were made to consider what “directionality” and “antiparallel” really meant, rather than just memorizing a definition.

Of course, this model has limitations, as do all models. Concerning the chemical representation of DNA and RNA, the moieties of each nucleotide are not to scale and do not have the correct shapes. Viewed from overhead, the head/torso represents the pentose, while the left outstretched arm is the sugar’s 5′ carbon side chain, and the left hand is a too-small representation of the phosphate attached to the 5′ carbon. Similarly, the extended right arm represents two different-sized moieties: either a double-ring purine or a single-ring pyrimidine. Also, the replication enzymes, each represented by a student, do not reflect their true comparative sizes, as their diameters should be greater than that of the double helix. Another obvious limitation of the model is that it completely ignores the helical nature of DNA. In fact, this model must be thought of as an overhead snapshot of unwound DNA. That is, the modeled DNA is reduced to a two-dimensional structure. The other body parts (torso, legs) that are not visible from above are ignored—they are assumed to not exist. This reveals one more limitation of the model. The rotation of a single-stranded polynucleotide is possible around the phosphodiester linkages holding one nucleotide to the adjacent one. The angles of these bonds in a double helix are fixed due to the constraints of base pairing with the other strand, but when the base pairs connecting the two strand are disrupted by denaturation, they are now free to rotate. Imagine that each person (nucleotide) who has a left hand from the adjacent nucleotide resting on their left shoulder blade could rotate like a wheel, using that arm as an

Figure 4. Modeling molecular recombination and the formation of the Holliday junction. See text for a detailed description. This figure shows the Holliday junction molecule completed. Above the photograph is the molecular interpretation of the student model. The time stamp at the bottom of the photo is the time on the molecular recombination video.
axle. That would be a truer representation of the fact that single-stranded DNA is actually free to rotate around the phosphodiester linkage. However, this problem of the modeled polymolecule not being capable of rotation was addressed and partially resolved in the molecular recombination explanation above by having students switch the handshake hand from the right hand to the left hand. If it is imagined that a chain had rotated 180° and we were viewing only a silhouette of the nucleotide, it would then appear as if the person’s left hand was forming the handshake with the opposite strand.

After enacting these activities, several potential improvements became clear. The different colored tops students wore to distinguish DNA from RNA were not as clearly visible as desired. Since the activity is viewed from overhead, one improvement might be to have students wear wide-brimmed hats of clearly distinguishable colors or possibly with a large D or R on them, designating deoxyribonucleotides or ribonucleotides. Also, students could wear four different colored gloves to illustrate the specific A-T and G-C base pairs.

This DNA activity could be expanded to demonstrate other DNA processes. For example, one possible resolution of the Holliday junction molecule, recombination without outside marker exchange, could also be modeled easily. Although not illustrated in the video, by simply nicking both single strands at the point where they cross over each other and then ligating them together, a recombination product can be created that illustrates the recombination product of gene conversion. Also, illustrating branch migration might be possible. Branch migration (Panyutin & Hsieh, 1994) is the process in which the branch point (the X where the two single strands cross each other in Figure 4) migrates to the left and then to the right. This enables the recombination machinery to perform test pairing over an extensive area on either side of the exchange point in order to guarantee that the two duplexes are truly homologous over several thousand base pairs (Amit et al., 2004). This could be modeled, but it might best be done by a group that has already mastered these first steps. That is because modeling branch migration would involve one of the two chains of students stepping over the other migrating chain down a considerable length of the duplex. This modeling activity could also be used to demonstrate DNA mismatch repair (Fleck & Nielsen, 2004). If students used different-colored gloves to illustrate A-T versus G-C pairs as suggested above, the nucleotides of the segment with the mismatched colors would be identified and removed, and repair enzymes would then synthesize the correct segment. Besides mismatched pairs, it would also be possible to model thymine dimers by requiring adjacent students with thymine right arms, instead of shaking hands with the complementary strand base, to bend their arms such that each grabbed the other’s right arm. This would require considerable contortion on the part of those two students, but in fact, the DNA backbone is contorted where such a thymine dimer occurs.

My experience is that this modeling activity enhances students’ understanding of the chemical activity of DNA in the processes of replication and molecular recombination.

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