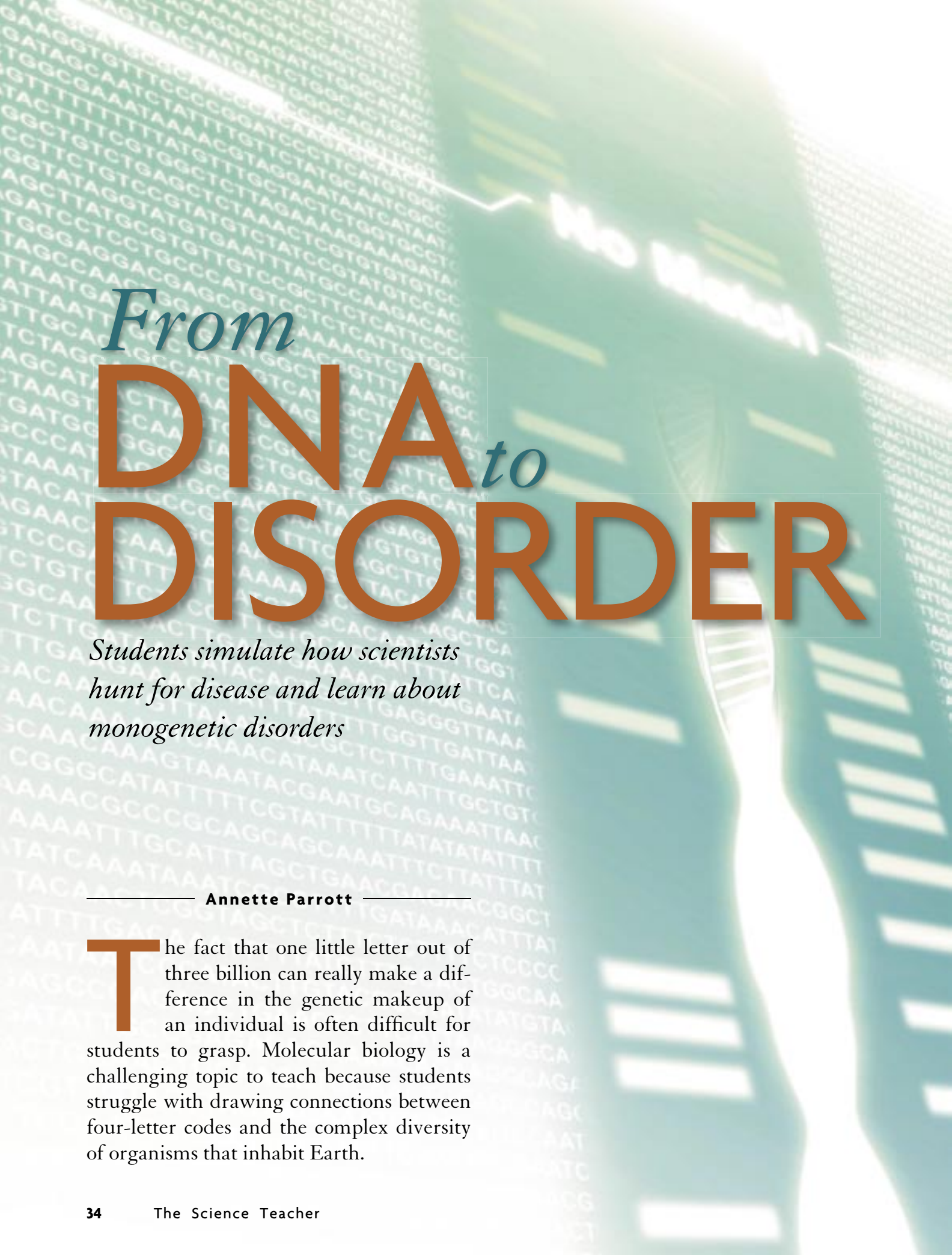


From DNA to DISORDER

Students simulate how scientists hunt for disease and learn about monogenetic disorders

Annette Parrott

The fact that one little letter out of three billion can really make a difference in the genetic makeup of an individual is often difficult for students to grasp. Molecular biology is a challenging topic to teach because students struggle with drawing connections between four-letter codes and the complex diversity of organisms that inhabit Earth.

Explaining to students the genetic blueprint that makes each of us unique is valuable, though, because it is the central dogma of molecular biology. The *National Science Education Standards* stress the importance of molecular biology:

> Students should understand the chemical basis of life not only for its own sake, but because of the need to take informed positions on some of the practical and ethical implications of humankind's capacity to manipulate living organisms (NRC 1996, p. 181).

The science content that educators are expected to cover grows in leaps and bounds, almost as quickly as the innovations in science itself. As a result, science educators are obliged to find creative ways of teaching and reinforcing multiple content areas concurrently. One creative way to teach about molecular biology is with the "From DNA to Disorder" activity, which focuses on monogenetic diseases and disorders. The activity offers teachers a way to link molecular biology to the functions of cells, systems, organisms, inheritance, ecology, and evolution.

Monogenetic disorders and diseases are caused by one identifiable gene. Scientists believe that approximately 6,000 monogenetic diseases and disorders exist, including achondroplasia, Angelman syndrome, Burkitt's lymphoma, congenital adrenal hyperplasia, cystic fibrosis, Duchenne muscular dystrophy, fragile X, hemophilia, Huntington's disease, Marfan syndrome, phenylketonuria, retinoblastoma, sickle-cell anemia, spinal muscular atrophy, and Tay-Sachs disease. For the activity described in Figures 1 and 2 (p. 36), students simulate how scientists hunt for genes. Students also create a paper three-dimensional model (Figures 3 and 4, pp. 37 and 39) of the protein called fibroblast growth factor receptor 3 (FGFR3), which, when mutated, causes the disorder achondroplasia.

Achondroplasia

Achondroplasia comes from Greek roots meaning "without cartilage formation" and is one of the most common forms of dwarfism, a genetic condition that usually results in an average adult height of about 130 cm. A person with achondroplasia has an average-sized trunk, disproportionately short arms and legs, a slightly enlarged head, and a prominent forehead. Achondroplasia only occurs in 1 per 16,000 to 40,000 births, but can cause death in individuals who inherit two copies of the gene and orthopedic impairments and other complications in individuals who inherit one copy of the gene.

Although achondroplasia can be inherited as a dominant trait, approximately 80% of the cases are due to new point mutations (Bellus et al. 1995) (see sidebar). Approximately 98% of all cases of achondroplasia are due to a G to A substitution at the 1138th nucleotide with the remaining 2% a result of a G to C substitution (Bellus et al. 1995; Shiang et al. 1994). Both mutations result in the replacement of the amino acid glycine by arginine, the 380th amino acid in the protein chain. This mutation involves the FGFR3 gene, which is located on the short arm of human chromosome 4 and codes for the fibroblast growth factor receptor 3. FGFR3 mutations in achondroplasia have been interpreted as gain-of-function mutations (Thompson et al. 1991). The mutation increases the activity of FGFR3, severely limiting bone growth.

Before beginning the activity in Figure 1, the teacher should lecture about achondroplasia and explain how scientists find prospective disorder genes by looking in regions of the genome suspected of being connected with the disorder. The teacher should also explain that scientists look for genetic similarities among people who have the disorder and that another way of finding disorder genes is by comparing gene sequences of people who do not express the disorder with people who do. In this lab, students are given six fragments of mRNA suspected of having some relevance to achondroplasia. Students compare and contrast the segments in hopes of finding the gene that causes achondroplasia.

More on achondroplasia.

Two types of achondroplasia exist, de novo and inherited. De novo mutations account for greater than 80% of achondroplasia cases, however, it can still be inherited. Over 80% of individuals with achondroplasia have parents with normal stature and have achondroplasia as the result of a de novo gene mutation. Such parents have a low probability of having another child with achondroplasia. An individual with achondroplasia who has a partner with normal stature has a 50% probability in each pregnancy of having a child with achondroplasia. When both parents have achondroplasia, the probability of their offspring having normal stature is 25%; of having achondroplasia, 50%; and of having homozygous achondroplasia (a lethal condition), 25% (Francomano 2003). Prenatal molecular genetic testing is available, but recommended only to detect the lethal homozygous form (LPA 2005).

Achondroplasia is inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to cause the disorder. Most people with achondroplasia have average-size parents; these cases result from a new mutation in the FGFR3 gene. Other people with achondroplasia inherited an altered FGFR3 gene from a parent who has the condition (NLM 2005).

FIGURE 1

From DNA to Disorder activity.

(Note: An answer key to this entire activity can be found with the online version of this article at *www.nsta.org/highschool#journal*.)

Directions

In this lab you will be given six fragments of mRNA suspected of having some relevance to achondroplasia. You will compare and contrast these segments in hopes of finding the mutation that causes achondroplasia. You will also create a three-dimensional (3-D) model of the normal and mutated FGFR3 protein.

Materials

- Tape
- mRNA codon chart (found in textbooks)
- Lab handouts—mRNA transcript fragments (Figures 2A and B) and amino acid squares (Figure 3)
- Scissors

Procedures

1. Using scissors, cut out the mRNA transcript fragments for achondroplasia (Figure 2A). Three strips (N1—N3) represent a segment (base pairs 1111–1158) from three individuals with the normal gene and three strips (A1—A3) represent the same segment from three individuals with the achondroplasia gene.

FIGURE 2A

A. Processed mRNA transcripts fragments (for base pairs 1111–1158) for achondroplasia. Cut into strips.

N = Normal A = Achondroplasia

N1: ...gcccgacugacc cugggcaagccc cuuggggagggc ugcuucggccag...

N2: ... gcccggcugacc cugggcaagccc cuuggggagggc ugcuucggccag...

N3: ... gcccgguugacc cugggcaagccc cuuggggagggc ugcuuuggccag...

A1: ...gcccggcugacc cugggcaagccc cuuagggagggc ugcuucggccag...

A2: ...gcccgguugacc cugggcaagccc cuaagggagggc ugcuucggccag...

A3: ...gcccggcugacc cugggcaagccc cuucgggagggc ugcuucggccag...

FIGURE 2B

mRNA transcript key for fragments in Figure 2A.

	1111–1122 (bp)	1123–1134 (bp)	1135–1146 (bp)	1147–1158 (bp)
N1	gcc cg**a** cug acc	cug ggc aag ccc	cuu ggg gag ggc	ugc uuc ggc cag
N2	gcc cgg cug acc	cug ggc aag ccc	cuu ggg gag ggc	ugc uuc ggc cag
N3	gcc cgg **u**ug acc	cug ggc aag ccc	cuu ggg gag ggc	ugc uu**u** ggc cag
A1	gcc cgg cug acc	cug ggc aag ccc	cuu **a**gg gag ggc	ugc uuc ggc cag
A2	gcc cgg **u**ug acc	cug ggc aag ccc	cua **a**gg gag ggc	ugc uuc ggc cag
A3	gcc cgg cug acc	cug ggc aag ccc	cuu **c**gg gag ggc	ugc uuc ggc cag

		Amino acid coded for:	Mutation location base number	
N1	cg**a**	arginine	1116	**N = Normal** **A = Achondroplasia** **Orange: mutated base with no consequence** **Teal: mutated base causing achondroplasia**
N2 N3 A1 A2 A3	cgg	arginine	1116	
N1 N2 A1 A3	**c**ug	leucine	1117	
N3 A2	uug	leucine	1117	
N1 N2 A1 A2 A3	uuc	phenylalanine	1152	
N3	uu**u**	phenylalanine	1152	
N1 N2 N3	ggg	glycine	1138	
A1 A2	**a**gg	arginine	1138	
A3	**c**gg	arginine	1138	

2. Compare the mRNA sequences within the normal and achondroplasia groups and then between the normal and achondroplasia groups. What did you find?
   - How many variations existed within each group?
   - How many variations existed between the groups?
   - Which base pair(s) do you think is responsible for the mutated protein? Explain:
3. Use your mRNA codon chart to translate each of the mRNA sequences into an amino acid chain. You may write the amino acids on the strips. Compare the amino acid sequences within the normal and achondrolpasia groups and between the normal and achondroplasia groups. What did you find?
   - How many variations existed within each group?
   - How many variations existed between the groups?
   - Which amino acid(s) do you think is responsible for the mutated protein? Explain:
   - Can different nucleotide sequences result in the expression of the same gene? Explain:
4. Using scissors, cut out the amino acid squares (Figure 3, p. 37). Twenty different amino acids are used to synthesize

FIGURE 3

Amino acid squares.

(Note: One amino acid sheet will make one amino acid chain. For students to make both the normal and the achondroplasia protein fragment, each student will need two amino acid sheets. Complete amino acid structures can be found in most advanced biology textbooks or on the web at sites such as *www.chemie.fu-berlin.de/chemistry/bio/amino-acids_en.html*.)

H +NH_3--Ser--COO- OH	H +NH_3--Arg--COO- H_2N NH	H +NH_3--Arg--COO- H_2N NH	H +NH_3--Leu--COO- H_3C CH_3	H +NH_3--Leu--COO- H_3C CH_3
H +NH_3--Leu--COO- H_3C CH_3	H +NH_3--Leu--COO- H_3C CH_3	H +NH_3—Thr--COO- OH	H +NH_3--Gly--COO- H	H +NH_3--Gly--COO- H
H +NH_3--Gly--COO- H	H +NH_3--Lys--COO- NH_2	H +NH_3--Pro--COO- CH_2	H +NH_3--Glu--COO- HO O	H +NH_3--Glu--COO- HO O
H +NH_3--Glu--COO- HO O	H +NH_3--Gly--COO- H	H +NH_3--Cys--COO- SH	H +NH_3--Phe--COO- 	H +NH_3—Gln--COO- H_2N O
H +NH_3--Val--COO- H_3C CH_3	H +NH_3--His--COO- 	H +NH_3--Ala--COO- CH_3	H +NH_3--Tyr--COO- OH	H +NH_3--Val--COO- H_3C CH_3

proteins. Every amino acid has a central carbon (not shown) and attached to the central carbon are four things: an amino terminus (NH2/NH3+), a carboxyl terminus (COOH/COO-), a hydrogen (H) atom, and one of the 20 different R groups. Only the last portion of each R group is shown on your amino acid sheet.

- Which of the amino acids on your sheet have R group portions that are polar? Nonpolar? Hydrophilic? Hydrophobic?

5. Use the amino acid sequence that you just translated to make a paper version of your amino acid chain (see Figure 4 as an example). Make one amino acid chain for a normal protein and one for an achondroplasia protein. Use tape to link the carboxyl terminus of one amino acid to the amino terminus of the next amino acid at the peptide bonds.

Proteins, like all organic compounds, are 3-D structures. Part of this shape is caused by hydrogen bonds. Hydrogen atoms can have partially positive charges. They are attracted to atoms with partially negative charges, such as oxygen (O), nitrogen (N), and sulfur (S). In a molecule, hydrogens bend toward these negatively charged atoms, changing the shape of the molecule. Please demonstrate this now.

Look at the first amino acid in your chain. From there, count over three amino acids and look at the R group of the fourth amino acid. If the R group is negative (because it has N, O, or S), bend your amino acids so that you can tape the hydrogen group of the first amino acid to the negative R group of the fourth amino acid. (Make sure the writing of both amino acids is facing up.) If the R group is non-polar, do nothing.

Now consider the hydrogen group on the fourth amino acid. From there, count over three amino acids and look at the R group of the seventh amino acid. If the R group is negative, bend your amino acids so that you can tape the hydrogen group of the fourth amino acid to the negative R group of the seventh amino acid.

Continue this pattern (checking every third amino acid) until you have reached the end of your amino acid chain.

- How does hydrogen-bonding change the shape of your protein chain? Although the actual folding of the protein is much more complex, you should now have the basic idea of how polar and non-polar interactions help shape a protein molecule.
- Is there a difference in shape between your normal protein and your achondroplasia protein?

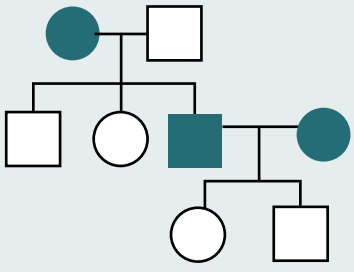
6. Sickle-cell anemia is a monogenetic disease. The mutation in the HBB gene causes hemoglobin (a protein that carries oxygen in the blood) to have a different 3-D shape. As a result, red blood cells form into curved sickle shape and become stuck in blood vessels. People with sickle-cell anemia often do not receive enough oxygen in their tissues, which can result in lack of energy and extreme pain.

- What are other ways that changes in protein shape might cause disease?
- Achondroplasia is an autosomal dominant trait. Sickle-cell anemia is an autosomal recessive trait. What is the minimum number of mutated alleles necessary for someone to express achondroplasia? Sickle-cell anemia?

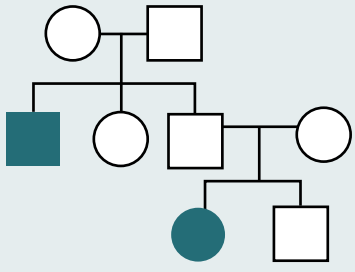
7. View the pedigrees below of inherited traits. A shaded circle represents a woman who phenotypically expresses the trait and a shaded square represents a man who phenotypically expresses the trait. Write possible genotypes below each individual.

- Which pedigree(s) could demonstrate inherited achondroplasia?
- Which pedigree(s) could demonstrate sickle-cell anemia (or another example of autosomal recessive inheritance)?
- Circle the individuals in the pedigree that best inform your decision.

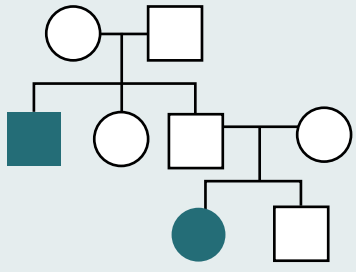
Pedigree 1



Pedigree 2



Pedigree 3



Extending the activity

After completing the activity, teachers should discuss with students what was learned, focusing primarily on the interconnection between molecular biology and protein synthesis, cellular regulation, tissue and organ system function (and malfunction), evolutionary significance, familiar inheritance, and population genetics.

One way to extend the activity is for students to explore bioinformatics technology—the use of computers in managing information in the life sciences. Due to bioinformatics, students have access to the nucleotide sequences that influence phenotypic expressions. Students and teachers can access extensive databases to find specific genes, chromosome maps, nucleotide (DNA, cDNA, mRNA) sequences, proteins, and other related information on genetic disorders and diseases. The National Center of Biotechnology Information (NCBI) houses a gene database online at *www.ncbi.nlm.nih.gov*.

Students can also illustrate, demonstrate, and research how the change in FGFR3 protein shape alters the lock-and-key mechanism necessary for receptor binding on the surface of the cell membrane. In addition, the "From DNA to Disorder" activity can be performed with an alternate set of nucleotides.

Advanced learners can also create a "DNA to Disorder" PowerPoint presentation. In it, students can trace the pathway of a genetically caused disorder. For example, students can explore the following 10 points in depth:

- What gene is involved? (length, arm, +/- strand and loci on chromosome, name, discovery information)
- What is the DNA code? (base sequence, introns/exons, cDNA, mRNA)
- What protein is affected? How are primary through quaternary structure affected? How is enzyme activity affected?
- How does the disorder affect cellular, tissue, and organ functions? How are pathways interrupted?
- How does it affect organ systems? What body system is affected? How is it affected? How is disordered function different than normal function?
- What symptoms exist?
- How can the disorder be treated or prevented? What preventative measures can be taken? What treatments are available? What treatments are presently being researched or are in clinical trials?
- What is the mode of inheritance? Include a Punnett square and pedigree.
- What are the ecological and evolutionary effects and significance? Is this trait found in different types or frequencies in different populations? Why?

FIGURE 4

Paper version of amino acid chain.

- Student choice or miscellaneous points. Students should choose an area not already mentioned to delve further into.

The understanding of the molecular basis of genetic disorders is one of the most exciting frontiers of science. The Standards recognize that "molecular biology will continue into the 21st century as a major frontier of science" (NRC 1996, p. 181). New discoveries are announced almost daily, discoveries that undoubtedly will revolutionize biology and medicine. "From DNA to Disorder" is a way to involve students in actively learning about the molecular basis of genetics. The activities described in this article can provide a way for students to visualize and understand these all-important molecular processes. ■

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On the web

An answer key for Figure 1 is available with the online version of this article at *www.nsta.org/highschool#journal.* "Your Genes Your Health," a multimedia guide to genetic disorders and diseases, can be accessed online at *www.ygyh.org.* The GeneTests website also provides summaries of various genetic disorders and diseases and is online at *www.geneclinics.org/profiles.* For more specific information on dwarfism and achondroplasia, visit *www.nlm.nih.gov/medlineplus/dwarfism.html.*