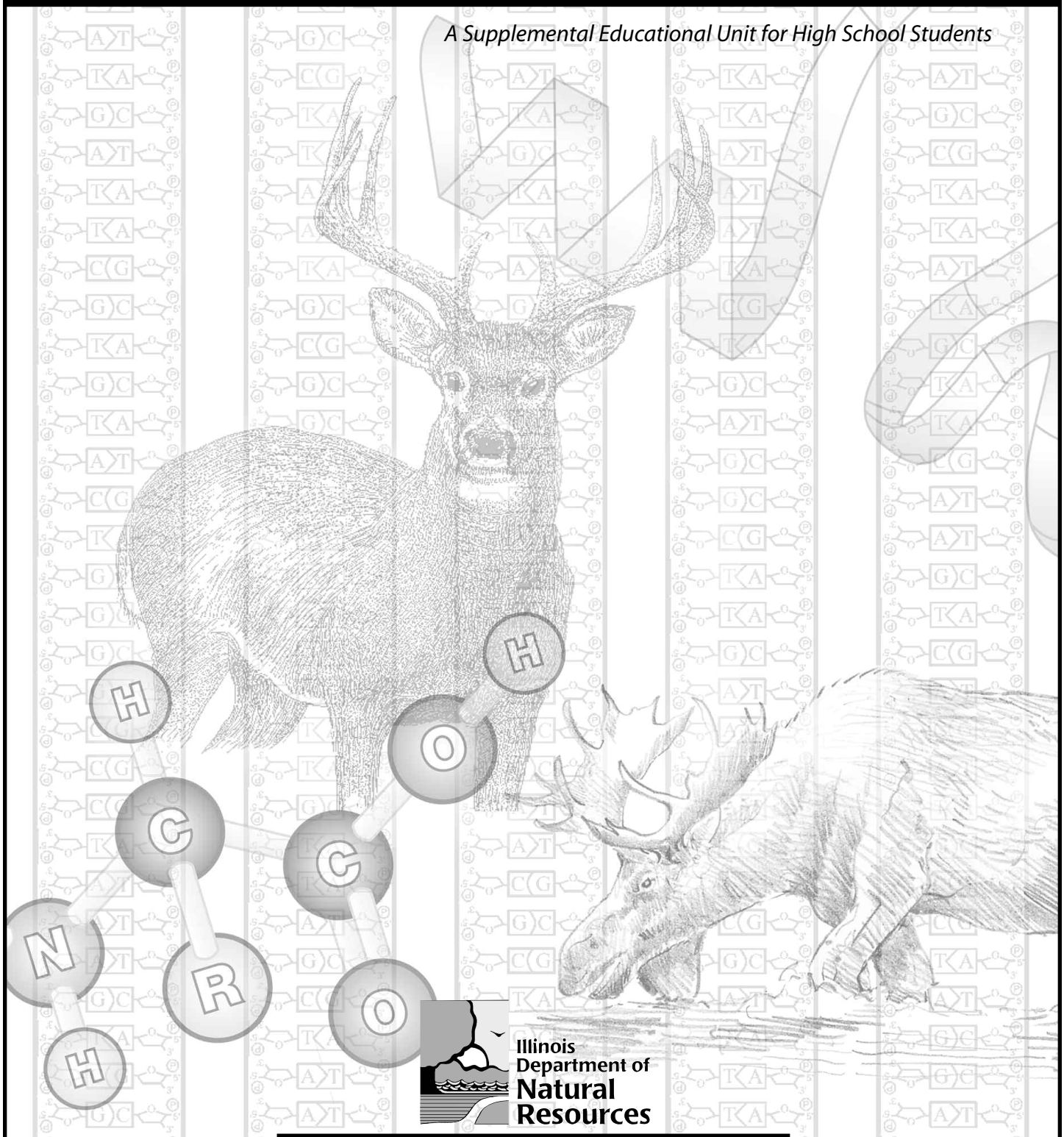
State of Illinois

Illinois Department of Natural Resources

Prying into Prions

Investigating Chronic Wasting Disease

A Supplemental Educational Unit for High School Students



Illinois Department of Natural Resources

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Colorado Division of Wildlife, 6060 Broadway, Denver, CO 80216

In Dedication to

Dr. Elizabeth ("Beth") Williams

Dr. Elizabeth ("Beth") Williams was a life-long student, contemplating problems she encountered with an open mind and viewing them as new opportunities to learn. Beth's innate curiosity, combined with her careful and thorough approach to diagnostic pathology, allowed her to succeed where others had previously failed in unraveling the cause of a mysterious but little-known disease of captive mule deer called "chronic wasting disease." The realization that the chronic wasting syndrome seen in captive deer was in fact one of a handful of diseases in the transmissible spongiform encephalopathy group opened the door to three decades of research and scientific progress toward a better understanding of chronic wasting disease, as well as other prion diseases of animals and humans. Although notable progress has been made, there is still much work to be done before our knowledge and technology is sufficient to control and manage these diseases with effectiveness comparable to our capacity to deal with diseases caused by more conventional pathogens. Because future advances likely will depend in no small part on the creativity and inquisitiveness of a new generation of scientists, it seems only fitting that the Colorado Division of Wildlife dedicates this high school science education module *Prying into Prions—Investigating Chronic Wasting Disease* to the memory of Dr. Beth Williams. We hope this tool will help stimulate growth and training, and will help foster a similar passion for life-long learning in our next generation of scientists.

We also recognize that no successful scientist accomplishes much of consequence in isolation, and thus we want to acknowledge and thank the many other researchers, biologists, pathologists, and technicians in Colorado, Wyoming, and elsewhere who have contributed to the body of knowledge on chronic wasting disease and other prion diseases. The challenges the Division of Wildlife and other management agencies have faced in addressing chronic wasting disease would have been far more daunting in the absence of the firm foundation of knowledge provided by their collective efforts.

Michael W. Miller
Senior Wildlife Veterinarian
Colorado Division of Wildlife

Acknowledgments

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Prying into Prions
Investigating Chronic Wasting Disease

What is CWD?

Chronic wasting disease (CWD) is a fatal neurological disease found in deer, elk and moose. It belongs to a family of diseases known as transmissible spongiform encephalopathies, or prion diseases. The disease attacks the brain of infected deer, elk and moose, causing the animals to become emaciated, display abnormal behavior and lose coordination, and eventually die.

First discovered in captive mule deer in 1967, CWD has been recognized in free-ranging deer in Colorado since 1985, but how long it has been present or how it arose are unknown. Unlike all infectious diseases known at the time, the pathogen for transmissible spongiform encephalopathies, or TSEs, was identified as a protein. Stanley Prusiner, the scientist who isolated the protein, named it a "prion," which is short for "proteinaceous infectious particle." The prion protein exists in two forms. The normal form is found in many types of cells, including those in the central nervous system. The pathological prion form has the same chemical composition, but a different shape. This abnormally shaped prion protein damages the brain tissue and converts other normal prion proteins in cells into the same abnormal shape!

Why Study CWD?

The idea that form dictates function is a central tenet of science and biology. There is probably no better group of diseases that illustrate this concept than TSEs such as CWD—diseases caused by a simple change in the form of a protein. To understand these diseases, students must first understand what proteins are, how they are made and how they fold and misfold.

Students are often unenthusiastic about studying the basic chemistry of life—the structure of proteins, lipids, carbohydrates, and nucleic acids. They can be equally apathetic learning about protein synthesis and gene expression. Prion diseases, unfortunately, are fear-provoking and seem to compel human interest, providing a relevant context to study life's molecules and processes.

Why is the Illinois Department of Natural Resources (IDNR) Providing this Module?

CWD was first noticed by researchers at Colorado's Foothills Wildlife Research Facility in 1967. Captive mule deer that were being maintained for nutritional studies began to lose weight. They became listless, started slobbering and drooling, and seemed constantly thirsty. Some stopped socializing with other deer. Most of the affected deer died within months. This new affliction was named "chronic wasting disease."

In 1977, Elizabeth Williams, studying for her doctorate in veterinary medicine at Colorado State University, identified CWD as a new TSE. In 1985, CWD was discovered in free-ranging deer and elk in Colorado. That same year, a handful of cattle from various areas in the United Kingdom began dying of a strange illness. Examination of the dead cattle's brains revealed abnormal, microscopic holes. The new disease was named bovine spongiform encephalopathy, or BSE.

In 1996, health officials in the United Kingdom discovered a new variant of a human TSE called Creutzfeldt-Jakob disease (CJD). CJD normally occurs in older people, but in 1996 a few teenagers started showing classic CJD symptoms. Biopsies of the brain tissue of these victims revealed that the new variant Creutzfeldt-Jakob disease, vCJD, involved the same prion strain as BSE. It became clear that all TSEs could potentially be a human health problem.

CWD was first found in Illinois in the fall of 2002 along the Boone-Winnebago county line northeast of Rockford. After receiving confirmation of CWD in a suspect white-tailed deer, the IDNR launched an effort to help control the spread of CWD in the state by decreasing white-tailed deer density in areas where CWD is present. There are two parts to this program. First, surveillance is used to identify the distribution and severity of the disease. Second, management programs are implemented to prevent the spread of the disease and possibly eliminate it. Full-scale efforts began during the 2002 firearm deer-hunting season in Illinois. More than 4,000 deer tissue samples were collected from 36 counties statewide with results showing that 14 of these deer were infected with CWD. Sampling during the 2003-2004 firearm deer-hunting season found 51 deer with CWD, all in the far northern part of the state (Boone, McHenry, Winnebago and De Kalb counties). Thirty-one harvested deer from the 2004-2005 hunting season tested positive for CWD. For the 2005-2006 hunting season, only deer harvested in seven northern Illinois counties were tested: Boone, De Kalb, McHenry, Winnebago, Ogle, Grundy and La Salle). Fifty-one deer with CWD were found, and Ogle County was added to the list where the disease was confirmed to be present. In 2006-2007, a deer harvested from La Salle County was found to have CWD along with 42 cases from the other confirmed counties in northern Illinois. The 2007-2008 hunting season and subsequent sharpshooting efforts yielded another 38 positive cases of CWD.

Several methods are now being used to combat the disease in Illinois. Following the close of the deer-hunting season in January, teams of sharpshooters from the IDNR and U.S. Department of Agriculture's Wildlife Services begin to cull deer from known CWD locations. Deer removals by trained sharpshooters are conducted four days per week from mid-January through the end of March. All deer removed are tested, and those negative for CWD are processed and donated to food pantries. To help increase testing in the seven "hot spot" counties in northern Illinois, hunters are eligible for additional deer-hunting permits for these counties at a reduced cost. Hunters, who purchase a special antlerless-only CWD permit for $5 and successfully harvest a deer, may obtain an additional either-sex permit, free of charge, if they allow testing or an attempt at testing for CWD on the harvested deer. The additional either-sex permit is issued by IDNR personnel at the check/testing station. The Illinois Department of Agriculture monitors captive deer and elk herds in the state for CWD and also obtains samples from captive deer and elk herds at slaughter plants.

Controlling CWD in Illinois is not an isolated project. Wisconsin also has CWD in their deer herd. Success or failure in controlling CWD in either state has major implications for the other state. Controlling any slowly evolving, chronic disease in wild animal populations requires a long-term commitment to the process.

The IDNR has many very good reasons to study CWD and share what is learned with the public. Wildlife biologists want to contain the disease, protect wild herds, and protect the public health.

What is this Module?

This six-lesson module, designed for approximately two weeks of classroom instruction, begins by presenting an interesting group of emerging diseases—transmissible spongiform encephalopathies (TSEs). After students discuss the variety of evidence that scientists must collect to determine the origin, infectious agent, and route of transmission of a

transmissible disease, they explore the role of proteins in organisms, the chemistry and behavior of proteins, and the genetic code that creates protein (DNA, transcription, and translation). Throughout the module, they look at and evaluate experimental design. The module is designed to supplement or replace the activities found in most high school biology textbooks that address the chemistry of life, DNA and the genetic code, and protein synthesis. Materials are inquiry based, develop critical thinking skills, supply evidence to support each concept, and include real data from recent research projects.

Using *Prying into Prions: Investigating Chronic Wasting Disease* in Your Classroom

The lessons in this module are designed to be taught in sequence. For each activity, students assume the role of researchers studying an emerging disease. As they evaluate and analyze information concerning CWD and other TSEs, they explore the larger role of proteins in living organisms.

Correlation to the Illinois Learning Standards

Prying into Prions: Investigating Chronic Wasting Disease supports teachers in their efforts to provide the knowledge and skills specified in the Illinois Learning Standards and the corresponding grade level assessment frameworks.

SCIENCE GOAL 11 Understand the processes of scientific inquiry and technological design to investigate questions, conduct experiments and solve problems.						
	Lesson 1 A Brain Wreck	Lesson 2 Pathological Proteins?	Lesson 3 We Moose Crack the Code	Lesson 4 Cannabalism, Forgetfulness, and Sleepless Nights	Lesson 5 Oh. . . Deer	Lesson 6 Here to Stay, Not Gone Tomorrow
11.A.4a Formulate hypotheses referencing prior research and knowledge.	X				X	
11.A.5a Formulate hypotheses referencing prior research and knowledge.	X				X	

SCIENCE GOAL 12 Understand the fundamental concepts, principles and interconnections of the life, physical and earth/space sciences.						
	Lesson 1 A Brain Wreck	Lesson 2 Pathological Proteins?	Lesson 3 We Moose Crack the Code	Lesson 4 Cannabalism, Forgetfulness, and Sleepless Nights	Lesson 5 Oh. . . Deer	Lesson 6 Here to Stay, Not Gone Tomorrow
12.A.4a Explain how genetic combinations produce visible effects and variations among physical features and cellular functions of organisms.			X	X	X	X
12.A.4b Describe the structures and organization of cells and tissues that underlie basic life functions.	X	X	X	X	X	X
12.A.4c Describe processes by which organisms change over time.			X	X	X	X
12.A.5a Explain changes within cells and organisms in response to stimuli and changing environmental conditions.	X		X	X	X	X
12.A.5b Analyze the transmission of genetic traits, diseases and defects.	X	X	X	X	X	X
12.B.4a Compare physical, ecological and behavioral factors that influence interactions and interdependence of organisms.					X	X
12.B.4b Simulate and analyze factors that influence the size and stability of populations within ecosystems.	X	X	X	X	X	X
12.B.5b Compare and predict how life forms can adapt to changes in the environment by applying concepts of change and constancy.			X	X	X	X
12.C.5b Analyze the properties of materials in relation to their physical and/or chemical structures.		X				

SCIENCE GOAL 13

Understand the relationships among science, technology and society in historical and contemporary contexts.

	Lesson 1 A Brain Wreck	Lesson 2 Pathological Proteins?	Lesson 3 We Moose Crack the Code	Lesson 4 Cannabalism, Forgetfulness, and Sleepless Nights	Lesson 5 Oh. . . Deer	Lesson 6 Here to Stay, Not Gone Tomorrow
13.A.4a Estimate and suggest ways to reduce the degree of risk involved in science activities.						X
13.A.5a Design procedures and policies to eliminate or reduce risk in potentially hazardous science activities.						X
13.A.4b Assess the validity of scientific data by analyzing the results, sample set, sample size, similar previous experimentation. possible misrepresentation of data presented and potential sources of error.						X
13.A.5b Explain criteria that scientists use to evaluate the validity of scientific claims and theories.						X
13.A.4c Describe how scientific knowledge, explanations and technological designs may change with new information over time.						X
13.A.5c Explain the strengths, weaknesses and uses of research methodologies including observational studies, controlled laboratory experiments, computer modeling and statistical studies.						X
13.A.4d Explain how peer review helps to assure the accurate use of data and improves the scientific process.						X
13.A.5d Explain, using a practical example, why experimental replication and peer review are essential to scientific claims.						X
13.B.4d Analyze local examples of resource use, technology use or conservation programs; document findings; and make recommendations for improvements.						X

Lesson 1

Educator's Overview

A Brain Wreck

Duration

One or two 45-minute class periods

Vocabulary

Bovine spongiform encephalopathy (BSE)

Creutzfeldt-Jakob disease (CJD)

Emerging disease

Infect

Pathogen

Sporadic disease

Transmissible spongiform encephalopathies (TSEs)

Variant Creutzfeldt-Jakob disease (vCJD)

Zoonotic

Illinois Learning Standards

science: 11.A.4a, 11.A.5a, 12.A.4b, 12.A.5a, 12.A.5b, 12.B.4b

Summary

Students read an article about an interesting group of emerging diseases—transmissible spongiform encephalopathies (TSEs). They reflect upon and discuss what they already know and understand about disease to prepare themselves to study TSEs.

Learning Objectives

After completing this activity, students will be able to:

- Explain why several diseases have been grouped together in a category called transmissible spongiform encephalopathies (TSEs).
- Explain why a state wildlife agency might devote considerable resources—time, money, and people—to study these diseases.
- Reflect upon and discuss their prior knowledge about the cause, transmission, and treatment of disease.
- Recognize the variety of evidence that scientists must collect to determine the origin, infectious agent, and route of transmission of a transmissible disease.

Background

This lesson is designed to engage your students' interest in an unusual group of diseases—transmissible spongiform encephalopathies (TSEs)—and prepare them for the activities in this module. The reading and questions are designed to make connections between their past and present learning experiences. Students will apply their knowledge about infectious or transmissible diseases to the scientific research of a new disease.

TSEs are a rare group of degenerative diseases that affect the brain and central nervous system. All are untreatable and fatal. These diseases are *transmissible* because they are capable of being transferred from one animal to another, *spongiform* because they cause the appearance of microscopic sponge-like holes in the brain of the affected animals, and *encephalopathic* because they are neurodegenerative diseases of the brain.

TSEs have been found in many mammals, including humans. The first known TSE, scrapie, was described in sheep in Great Britain and Western Europe over 250 years ago. The name scrapie comes from one of the symptoms of the disease.

In addition to excessive lip-smacking, strange walking gaits, and convulsions, sheep with this disorder appear to itch. The animals compulsively scrape off their fleece against rocks, fences, or trees.

Another TSE of livestock was discovered in Great Britain in the early 1980s—bovine spongiform encephalopathy (BSE)—commonly called "mad cow disease." Nearly 200,000 cattle in Great Britain and thousands in other countries have since succumbed to BSE.

Creutzfeldt-Jakob disease (CJD) is the most well-known of the human TSEs. It is a rare type of dementia that affects about one in every one million people worldwide each year. Other human TSEs include kuru, fatal familial insomnia (FFI), and Gerstmann-Straussler-Scheinker disease (GSS). Kuru was identified in people of an isolated tribe in Papua, New Guinea, and has now almost disappeared. FFI and GSS are extremely rare hereditary diseases, found in just a few families around the world.

A new type of CJD, called variant CJD (vCJD), was first described in 1996 and has been found in Great Britain and several other European countries. The initial symptoms of vCJD are different from those of classic CJD and the disorder typically occurs in younger patients. Research suggested that vCJD resulted from human infection with BSE.

Another TSE, chronic wasting disease or CWD, was first noted in Colorado in the late 1960s in captive deer. It has been widespread in free-ranging deer and elk in northcentral Colorado and southeastern Wyoming since at least the early 1980s. The disease has also been discovered in moose in Colorado. It was first found in white-tailed deer in Illinois in 2002.

CWD is an important wildlife health issue, and potentially a human health issue. It is being aggressively studied. This is a new—emerging—disease, and there is still much to learn.

Teaching Strategies

1. Thoroughly read the student materials for *Molecules and Cells*, and *A Brain Wreck*.

2. ***Optional:*** There are some fundamental concepts about the biochemical nature of life that your students must understand before beginning this module. Students' prior knowledge should include these ideas:

- Living things are made up of molecules, which are made of various atoms.
- Atoms join together to make molecules by sharing or giving up electrons.
- The chemical properties of molecules are determined by their ability to bond with other molecules.

High school students may have difficulty thinking about living organisms as chemical systems. They may confine the concept of molecules to things encountered in physics and chemistry. Many may believe that living organisms are not made up of molecules, only cells. Specific to proteins, students may believe

Materials and Preparation

- ***Optional:*** *Student Activity Page:* Molecules and Cells—*one photocopy per student*
- *Student Reading Pages:* A Brain Wreck—*one photocopy per student*
- *A large, visible writing surface (i.e. chalkboard, whiteboard, butcher paper)*

that proteins are made up of cells and that molecules of protein are bigger than the size of a cell. Or, they may only associate protein with diet—things that they eat—such as meats, cheeses, etc. If you suspect that your students may have these misconceptions, or are just curious, you may want to have your students do the activity page *Molecules and Cells* before beginning this module. Discuss the answers provided in the key and clarify any misconceptions. This activity could also be done prior to the second lesson in this module.

3. Give each student a copy of the student reading pages: *A Brain Wreck*.

4. After giving students sufficient time to read or after reading the material as a class, ask them to work in small groups to brainstorm answers to each question. Instruct each student in the group to record the group's answers. An alternate strategy would be to "jigsaw" the questions and ask each group to answer just one of the questions.

5. As a class, discuss the information that was recorded by each group. It may be helpful to ask each group to appoint a spokesperson and to allow each group to speak in turn until no more new ideas are added to the discussion. Record all answers in a visible place (i.e. chalkboard, whiteboard, butcher paper).

6. Keep in mind that this discussion is to elicit what students collectively know about disease and to get them thinking about the task that a scientist might face when confronted with a new disease. Keep the discussion open and encourage students to ask questions of each other. See the key below for possible responses to the questions.

7. Do not erase or throw away the responses to the questions. The students can look back on these responses as they move on to Lesson Two: *Pathological Proteins?*

Assessment

Student discussion about the causes, transmission, and treatment of disease serves as an assessment for this engagement activity.

Extensions

Students may want to discuss or review Koch's postulates, a four-step procedure for identifying specific pathogens (disease-causing agents):

1. The pathogen must be found in an animal with the disease and not in a healthy animal.
2. The pathogen must be isolated from the sick animal and grown in a laboratory culture.
3. When the isolated pathogen is injected into a healthy animal, the animal must develop the disease.
4. The pathogen should be taken from the second animal and grown in a laboratory culture. The cultured pathogen should be the same as the original pathogen.

Key

Student Activity Page: *Molecules and Cells*

All items on the list contain molecules. The following items contain cells: apple, mouse, bacteria, human, lettuce, liver, and donut. (Some components contain cells, such as the wheat flour. Other components are just molecules, such as salt).

Items that are part of the mouse include protein, fat, and liver. Bacteria could also be placed on the list, since most multicellular organisms contain bacteria. Mice eat apples, donuts, and lettuce to get nutrients such as vitamin C, fat, and protein that the body needs.

If students describe fat (lipid) or protein as containing cells, try to clarify that they are molecules contained in cells. Also, because students eat certain food items to get protein, they may think that some cells are just protein cells. In reality, all cells contain some amount of fat (lipid) and protein molecules.

Student Pages: *A Brain Wreck*

1. What is disease? ***A disease might be anything that prevents a cell, tissue, organ, or organ system from working normally.***

2. What causes disease? ***Most students will say that germs cause disease. Others will be more specific and mention bacteria, viruses, and hopefully fungi, parasites such as protozoa and "worms" such as ringworms, tapeworms, and flatworms. Hopefully students will mention genetic diseases such as cystic fibrosis or sickle cell anemia. Some students may mention aging and environmental toxins (including lifestyle choices such as diet or drug use) as sources of disease.***

3. Can you have an infection without having a disease? ***Most of us contain microbes and micro-organisms throughout our bodies. Some of these are beneficial—like the bacteria in our intestines that help us digest certain foods. If being infected—that is, having another organism invade or go inside our bodies—always caused disease, we'd be in big trouble.***

4. Are infectious diseases preventable? ***In a perfect world, where science could identify every pathogen and a way to defeat each of them, infectious diseases might be preventable.***

5. Are infectious diseases the same as transmissible diseases? ***This question can lead to interesting discussion. For example someone has a tapeworm infection, can he/she transmit it to another person?***

6. How can diseases be transmitted from one organism to another? ***Direct contact (bites, shaking hands, exchange of body fluids, etc.), air, water, food, etc.***

7. What makes an organism immune or susceptible to a disease? ***It is known that many organisms develop immunity to a disease once they recover from the disease. It is known that non-virulent strains of some viruses can be used to create vaccines to make organisms immune to that virus. There also seems to be a genetic predisposition to some diseases, and scientists are working on various "gene therapies" to prevent or treat these diseases. This question was included because it is one that has been looked at extensively by scientists investigating TSEs.***

8. The scientists who began studying TSEs had only the small amount of information about each disease that was presented in the reading. *How would you figure out what was going on if it was up to you to find the answer?* ***Answers will vary. Encourage as many ideas as possible. Do not dismiss any ideas. Ideally, write the class answers in a visible place to look back upon when students move to Lesson 2. Essentially, the scientists looking into TSEs acted as "detectives" to track down the cause or causes of the new diseases. Students may suggest looking for common locations where the disease appeared, to check to see if something in the environment caused the illness. Some might suggest looking for other things that the victims may have in common, from diet to genetic similarity.***

Student Page

Molecules and Cells

Circle items on this list that contain molecules and underline those items that contain cells.

Apple	Fat
Protein	Vitamin C
Mouse	Lettuce
Bacteria	Chlorophyll
Human	Liver
Donut	

Explain your choices.

Deer Mouse

Look at the list again. *What items on the list are parts of the mouse?* Explain.

What does the mouse eat? Why?

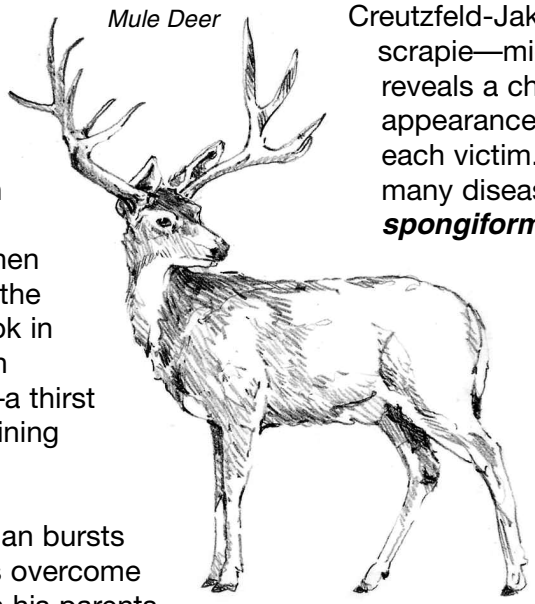
Student Pages

A Brain Wreck

A woman trembles as she stumbles forward in her thatched hut. Her unsteady gait seems strangely matched to her slurred speech. Anxious relatives sadly shake their heads, for they realize that this daughter of the Fore tribe of New Guinea will soon be dead.

In a high Colorado meadow, a mule deer doe trembles on her wide-legged stance as her herd moves on without her. Drooling, she lowers her ears then she stumbles toward the narrow mountain brook in an effort to quench an uncontrollable thirst—a thirst that will last the remaining months of her life.

Mule Deer



A young Englishman bursts out in laughter then is overcome by a bout of crying as his parents push his wheelchair from the doctor's office. Their physician has just explained that the most recent CAT scan shows continued deterioration in the young man's brain and that their son's illness is terminal.

In the Scottish Highlands, a ram's head trembles slightly. He obsessively scratches and rubs against a large tree, apparently to relieve itching. He smacks his lips and bites his feet. Alarmed by a sudden noise, the ram falls down in convulsions.

Can the illnesses of the New Guinea tribeswoman, the mule deer, the British teenager, and the Scottish sheep be related? In a broad sense, it appears so. All are degenerative disorders of the central nervous system. Although each illness has a different name—kuru, chronic wasting disease (CWD), variant Creutzfeld-Jakob disease (vCJD), or scrapie—microscopic examination reveals a characteristic sponge-like appearance in the brain tissue of each victim. They are just three of many diseases called ***transmissible spongiform encephalopathies*** or ***TSEs***. These diseases are ***transmissible*** because they are capable of being transferred from one animal to another, ***spongiform*** because they cause the brain to look like a hole-ridden sponge under a microscope, and ***encephalopathies*** because they are diseases of the brain (*pathy* is Greek for disease; *encephalo* is Greek for brain).

Why Learn about Transmissible Spongiform Encephalopathies?

Most people cannot repeat "transmissible spongiform encephalopathies" three times quickly. Some cannot even pronounce the words once slowly! So, how are TSEs

transmitted? Why should you care? Why should the Illinois Department of Natural Resources (IDNR) worry?

Let's start with that last question. The IDNR is the state agency responsible for protecting and managing wildlife and habitat, and providing wildlife-related recreation. The IDNR employs trained biologists and other specialists to "manage" wildlife—to use scientific knowledge to maintain healthy populations of wildlife and its habitat. Chronic wasting disease (CWD) was first recognized in Colorado in 1967, in a captive research herd of mule deer. It wasn't until 1977 that scientists suspected that CWD was a TSE, and that it might pose a significant threat to the health of deer and elk. CWD was first found in Illinois white-tailed deer in 2002.

In 1984, unusual behavior was noted among cattle on a farm in Sussex in the United Kingdom (UK). They wobbled and staggered and appeared fearful. Then they died. Brain samples collected from one of those cattle looked a bit like Swiss cheese. Scientists called this new disease ***bovine spongiform encephalopathy,*** or ***BSE***. Since the original discovery, BSE has been detected in more that 182,000 cattle in more than 35,000 different herds in the UK, and in more than 3,800 cattle in other countries throughout the world! This disease seemed highly contagious, though no one knew how it spread. Could CWD be spread as quickly?

Sporadic Creutzfeldt-Jakob disease (***CJD***) has been reported for centuries. This rare disorder affects about one in a million people. ***Sporadic diseases*** like CJD occur occasionally and at random intervals in a population. Most victims are between 63 and 66 years old and die of the disease within a year of diagnosis. Autopsies show that each victim's brain is riddled with microscopic holes.

In 1996, two British teenagers and one 29 year-old displayed many symptoms of classic CJD. Further research linked this new ***variant Creutzfeldt-Jakob disease (vCJD)*** of the young victims to infection with the BSE ***pathogen*** (disease-causing agent). The probable cause was consumption of BSE-contaminated meat products. The "Mad Cow" scare began. Countries worldwide began screening cattle for BSE, to insure a safe food supply.

Let's get back to that question about the IDNR. Deer are game animals. That means that licensed hunters can harvest and eat these animals. Also, many other people eat venison, too, such as from the Sportsmen Against Hunger program. Deer accidentally hit and killed by vehicles can be claimed and eaten, too. When it was evident that BSE was ***zoonotic***, that is, *the disease could be transmitted to humans* by eating meat from diseased cattle, similar concerns were raised about eating deer that might be infected with CWD. Elk raised in captivity could be affected, too.

The IDNR has three very good reasons to study CWD. Wildlife biologists want to contain the disease, protect wild herds, and inform the public about the disease.

Studying TSEs—Looking for the Cause and the Cure

Whenever a new or ***emerging disease*** appears, scientists try to figure out what causes the illness, if it is transmitted, how it is transmitted, and how it can be controlled or cured. If a ***pathogen*** is found, scientists then need to find out how it gets into—***infects***—the animal. Let's put

you in the role of one of these scientists. Consider each of these questions and write down your thoughts about each:

1. *What is disease?*

2. *What causes disease?*

3. *Can you have an infection without having a disease?*

4. *Are infectious diseases preventable?*

5. *Are infectious diseases the same as transmissible diseases?*

6. *How can diseases be transmitted from one organism to another?*

7. *What makes an organism immune or susceptible to a disease?*

8. The scientists who began studying TSEs had only the small amount of information about each disease that was presented in the reading. *How would you figure out what was going on if it was up to you to find the answer?*

Lesson 2

Duration

Two 45-minute class periods (A third class period may be necessary if students have little prior knowledge of chemical bonding.)

Vocabulary

Alpha helix (α-helix)
Amino acid
Amino group ($-NH_2$)
Amino-terminus (N-terminus)
Amyloid fibers
Assay
Astrocytes
Beta sheet (β-sheet)
Carboxyl group (-COOH)
Carboxyl-terminus (C-terminus)
Cross-linkage
Dehydration synthesis
Dipeptide
Disulfide bridge
Domain
Fold
Hydrogen group
Hydrophobic
Loop or turn
Pathogen
Peptide bond
Polymer
Polypeptide
Primary protein structure
Prion
Protein
Quaternary protein structure
R group
Secondary protein structure
Tertiary protein structure

Illinois Learning Standards

science: 12.A.4b, 12.A.5b, 12.B.4b, 12.C.5b

Educator's Overview

Pathological Proteins?

Summary

Students read about the discovery of protein as the pathogen for TSEs and about the important roles of protein in organisms. They construct paper models of α-helix and β-sheet secondary protein structures. Students then create Silly Putty®, a cross-linked polymer, and test its bounce under various temperatures. Students infer how temperature might affect the function of a protein, which is also a cross-linked polymer.

Learning Objectives

After completing this activity, students will be able to:

- Explain why an understanding of protein is essential for studying TSEs.
- Identify five important roles that proteins have in organisms.
- Identify the importance of a protein's three-dimensional shape to its role in an organism.
- Identify amino acids as the building blocks of proteins and describe how they attach to one another.
- Describe the four levels of protein structure or organization.
- Construct paper models of α-helix and β-sheet secondary protein structures.
- Create a cross-linked polymer and explore its physical properties.
- Infer the effect of temperature changes on the function of protein in the body.

Background

Unlike all infectious diseases known at the time, the pathogen for transmissible spongiform encephalopathies, or TSEs, was identified as a protein. Stanley Prusiner, the scientist who isolated the protein, named it a "prion," short for "proteinaceous infectious particle."

The prion protein (PrP) exists in two forms. There is a normal cellular form (PrP^c) that is found in many types of cells, including those in the central nervous system. No one knows the exact purpose of the normal prion protein, but it seems to have some role in long-term memory. Then there is a pathological form (PrP^{res}). The "res" in PrP^{res} stands for "resistant," because this form resists destruction! It resists "digestion" from protease enzymes in the cell and can survive temperatures of 600°C for more than 15 minutes. Further, it is not destroyed by some common disinfectants and is not quickly broken down by ionizing or ultraviolet radiation.

The structure of proteins has always tantalized biologists. Proteins have many different functions in organisms and each function is unquestionably linked to the molecule's three-dimensional shape. By studying the components and structures of proteins, scientists and students are better able to understand how they function normally and how some proteins with abnormal shapes can cause disease.

Teaching Strategies

1. Thoroughly read the student materials for *Pathological Proteins?* and *Silly, Silly Protein Structure*.

2. Give each student a copy of the student reading pages: *Pathological Proteins?*

3. Give students sufficient time to read the materials or read them together as a class.

4. Check for understanding. Ask students if they have any questions about the scientists' discovery. Have them look at their list of "causes of disease" from Lesson 1. Did they have protein on their list of items that caused disease? Make sure students understand the terms used in the reading. If possible, you may want to demonstrate the structure of an amino acid using a model made from Styrofoam™ balls. ***Note:*** *If your students have little knowledge of chemical structure or chemical bonding, you may need to back up a bit and spend a day making models of molecules and talking about how atoms share, gain, or lose electrons to bond with other atoms.*

5. Tell students they will be doing a lab activity to learn more about protein folding. Explain that is it necessary to know how and why proteins fold to understand how they might misfold and cause disease.

6. Assign students to groups of four. Give each student a copy of the laboratory activity *Silly, Silly Protein Structure* and the activity page *Polypeptide Chains*.

7. Instruct the groups to attempt to complete Activities 1 and 2 of *Silly, Silly Protein Structure* by the end of the class period. Tell them that they need to complete these activities at home if they are not able to complete them in class because you will need the next day's class time to do Activity 3.

8. Assist each group, if needed. The diagrams and pictures should make these activities fairly self-explanatory. Ask students to keep the models that they create. They may help the students' understanding of future lessons in the module.

9. Activity 3 is a simple and enjoyable lab activity, but it does present some hazards. Please review the safety issues and precautions with students. Students should not ingest any of the lab materials. Borax® should be kept away from eyes and goggles should be worn. Hands should be washed thoroughly at the end of the activity. Silly Putty® should not be put in a sink, on carpet, or in hair.

10. After students complete the activity, review their results as a class. Be sure to relate the behavior of the Silly Putty® polymer to protein polymers (see Key).

Materials and Preparation

- *Student Pages:* Pathological Proteins?—*one photocopy per student*
- *Student Activity Pages:* Silly, Silly Protein Structure—*one photocopy per student*
- *Student Activity Page:* Polypeptide Chains—*one photocopy per student*
- *Transparent tape—one roll for each group of four students*
- *Scissors—one to four for each group of four students, depending on availability*
- *Safety goggles—one per student*
- *Lab aprons (if available)—one per student*
- *Graduated cylinders or measuring cups with metric units—one per group of four students*
- *Rulers—one per group of four students*
- *Clear 8-oz. (or larger) plastic cups—5 for each group of four students*
- *All-purpose white glue such as Elmer's®—washable glue is not as effective*
- *Plastic spoons—5 for each group of four students*
- *Plastic Ziploc™ bags—one per student*
- *Borax®. Borax® can be found in stores as a laundry detergent. Place one tablespoon (much more than students will need) in a Ziploc™ bag for each group of four students.*
- *Permanent marker—one per group of four students*
- *Water*
- *Paper towels*
- *Newspaper (to protect the working surface)*
- *Access to a refrigerator/freezer or to a cold water and an ice water bath*

11. ***Optional:*** Most high school biology classes include a laboratory experiment to analyze the effect of pH and/or temperature on enzyme activity. You may wish to have students do the experiment to further reinforce the impact of protein shape on protein function.

Assessment

Ask students to draw the basic structure of an amino acid and label the four groups. Ask students to describe how these molecules join together to form a protein. Using diagrams and/or descriptors, ask students to depict the primary, secondary, tertiary, and quaternary structure of protein. Lastly, ask students to explain the importance of a protein's three-dimensional shape and why a change in that shape may be important.

Extensions

- Students can learn more about each individual amino acid and view microscopy photos of them at

  http://micro.magnet.fsu.edu/aminoacids/index.html

- Many protein shapes have been determined experimentally. When scientists decipher protein structures, they submit their findings to the Protein Data Bank. Students can view various protein structures at

  http://www.rcsb.org/

  Students can enter the protein that they are interested in looking at in the search box.

Key

Activity 3: *Silly Polymers*

10. Form the Silly Putty® into a ball. Using a ruler to measure, drop the ball from a height of 30 centimeters. How high does it bounce? ***Answers will vary.***

11. Now, place your ball in a refrigerator or ice bath for 10 minutes. Again, bounce the ball from a height of 30 centimeters. How high did it bounce this time? Why do you think this happened? ***Answers will vary, but the ball should bounce higher than the first drop at room temperature.***

12. Now place your ball about six inches from a light bulb for about five minutes and again check how high it will bounce when dropped from a height of 30 centimeters. How high did it bounce this time? Explain your results. ***This time, the ball will deform when dropped and may not bounce at all.***

13. Now put your Silly Putty® ball in the freezer or in an ice bath for 10–20 minutes. Check how high it will bounce when dropped from a height of 30 centimeters. How high did it bounce this time? Explain your results. ***If frozen, the ball is likely to shatter rather than bounce.***

14. Try to transfer your observations of the behavior of Silly Putty® to the behavior of protein. Remember that proteins are the workhorses of the cell or body. Why might a change in temperature, such as when a person is exposed to extreme heat or cold, cause problems? ***In extreme temperatures, the bonds that give a protein its shape may break and other bonds may form. The shape of the protein would change, and it would not be able to "do its job." It is possible that the organism could die or lose important functions.***

Lesson 2

Student Pages

Pathological Proteins?

Scientists who have been studying transmissible spongiform encephalopathies (TSEs) over the past few decades have learned things that have completely challenged commonly held beliefs about infectious diseases. Their findings will probably surprise you. Researchers looked for all the usual ***pathogens*** (disease-causing agents). In all of the animals with TSEs, including humans, they tried to find the bacterium, virus, fungus, protozoan, or helminth (parasitic worm) responsible for this group of diseases.

After painstaking and careful research, scientists did not find any of the usual causes of disease! They concluded that TSEs are not caused by bacteria or viruses. They aren't caused by parasites. Scientists discovered that in the brain cells of each TSE victim, a normal protein had changed its shape. This abnormally shaped protein damages the brain tissue and converts other normal proteins in cells into the same abnormal shape!

Prusiner's Amazing Discovery

Dr. Stanley Prusiner began his medical residency in July 1972 at the University of California San Francisco in the Department of Neurology. Two months later, he admitted a female patient who was experiencing memory loss and difficulty performing some routine tasks. She was dying of Creutzfeldt-Jakob disease (CJD), thought to be caused by a "slow virus" infection. He learned that scientists were unsure if a virus was really the cause of CJD since the pathogen had some unusual properties. The disease captivated Prusiner's imagination and he decided he wanted to research the disease. He began to read about CJD and the seemingly related diseases—kuru of the Fore people of New Guinea and scrapie of sheep.

He proposed methods to study the supposed "slow virus." Since scrapie-infected sheep were readily available, he focused his research on them. The task was daunting. The work was tedious, slow, and very expensive. He had to develop an ***assay***—a test or procedure to purify the scrapie pathogen and determine what it was made of.

Prusiner had anticipated that the purified scrapie agent would be a virus and was puzzled when the data kept telling him that the disease-causing agent contained protein but not nucleic acid. He named the agent a ***prion***, which is short for "proteinaceous infectious particle." The prion protein (PrP) can be found in two shapes. The normal form (PrP^{c}) is found in many types of cells, including those in the central nervous system. Then there is a pathological form (PrP^{res}). The "res" in PrP^{res} stands for "resistant," because this form resists destruction! It resists "digestion" from protease enzymes in the cell, it survives temperatures of 600°C for more than 15 minutes, it cannot be destroyed by some common disinfectants or even by ionizing or ultraviolet radiation.

Proteins were never thought to be infectious on their own. If you look back on your class discussion of disease, protein probably was not listed as a cause

of disease. It definitely wasn't on the list of the scientists when they began studying TSEs. The idea that proteins could be pathological (disease-causing) was counter to everything that scientists knew or believed at the time. How can proteins transmit disease? How can these diseases be treated or prevented? These questions are among the most challenging and interesting in medicine today.

What are Proteins?

Life would be impossible without proteins. As *enzymes*, proteins are the driving force behind all biochemical reactions in living things. As *structural elements*, they make up bones, muscles, hair, skin, and parts of all cells. As *antibodies*, proteins recognize invading elements and allow the immune system to get rid of the intruders. As *hormones*, they regulate growth and body processes. Other proteins *transport* nutrients into the cell and move waste out. You might say that proteins are the workhorses of life—they are how life gets things done.

Proteins are named after Proteus, an ancient Greek god of the sea, who could change himself into any shape he felt like. Proteus, also known as the "Old Man of the Sea," could foretell the future—but only if someone could catch him. He changed form to avoid capture. Proteus often turned himself into a sea lion to sleep on the beach at night. At other times, Proteus might be a sea serpent and sink a ship. Or—he could become a huge tidal wave and wipe out a small coastal village.

Just like Proteus, proteins are capable of assuming many forms. Why is the shape of a protein so important? *The shape of each protein gives it a unique function*. Hemoglobin's shape lets it carry oxygen. Collagen's shape makes it a good connective tissue. Insulin fits in spaces like a key, enabling it to regulate blood sugar.

Before any protein can carry out its function, it must take on its particular shape, known as a ***fold***. What happens if proteins don't fold correctly? Diseases such as bovine spongiform encephalopathy, scrapie, Creutzfeldt-Jakob disease, chronic wasting disease, Alzheimer's, an inherited form of emphysema, and even many cancers are believed to result from protein misfolding.

How are proteins made? How do they fold and misfold? Some of the world's most prominent scientists are studying these questions, and you are about to join them.

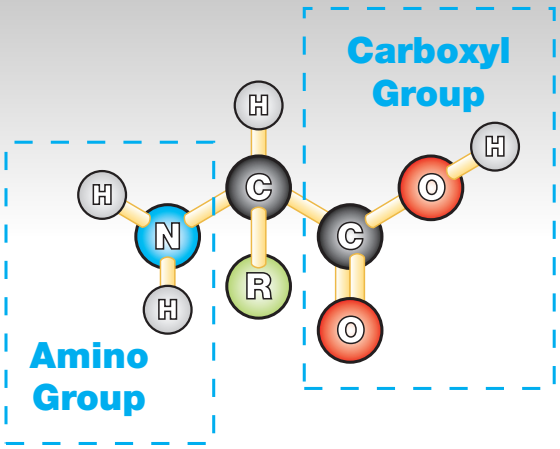
Proteins are Polymers

Proteins are ***polymers***, very large molecules made up of smaller molecules. In Greek, the word *poly* means "many" and *mer* means something like "unit, part, component, building-block or element." All proteins, no matter how complex, are built from hundreds or thousands of smaller molecules called ***amino acids***. There are only 20 different amino acids. Twenty seems like a small number to make the many types of proteins that exist, but there are only 26 letters in the alphabet, and millions of books, letters, poems and songs have been written with them!

Amino Acids

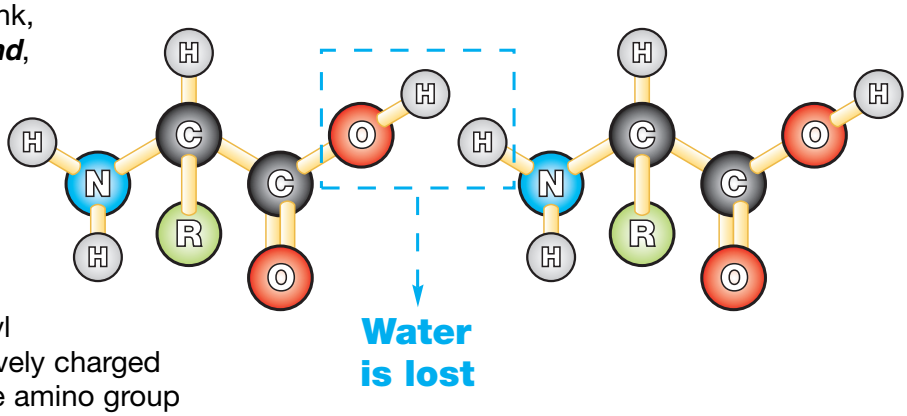
What do amino acids "look" like, and how do they link together? Like all molecules, amino acids are made of atoms. An atom of carbon is at the center

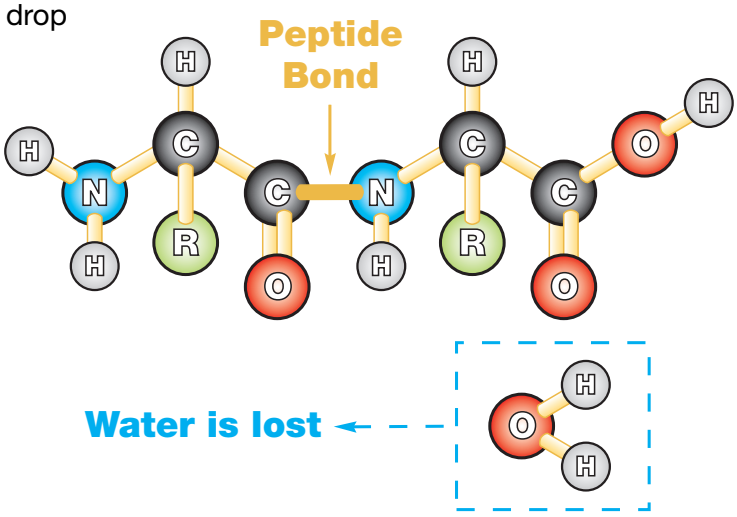
of each amino acid. Carbon atoms can share four of their electrons with other atoms or atom groups. In amino acids, the central carbon shares electrons with four groups of atoms. Each amino acid has an identical ***amino group*** ($-NH_2$), ***carboxyl group*** (-COOH) and a single ***hydrogen group***. It is the fourth group, the ***R group***, of the molecule that makes each of the 20 amino acids different from one another.



Two molecules linked by a peptide bond are called a ***dipeptide***. A chain of molecules linked by peptide bonds is called a ***polypeptide***. A ***protein*** is made up of one or more polypeptide chains.

Amino acids always link together in the same way. The link, called a ***peptide bond***, occurs between the carboxyl group of one amino acid and the amino group of another. When the two amino acids connect, the carboxyl group loses a negatively charged OH molecule and the amino group loses a positively charged H atom—they join by losing a drop of water! This is called ***dehydration synthesis***—making something while losing water. Other names for this process are dehydration reaction, dehydration linkage, and condensation reaction. Don't get confused if your teacher or textbook uses a different name.





Student Activity Pages

Silly, Silly Protein Structure

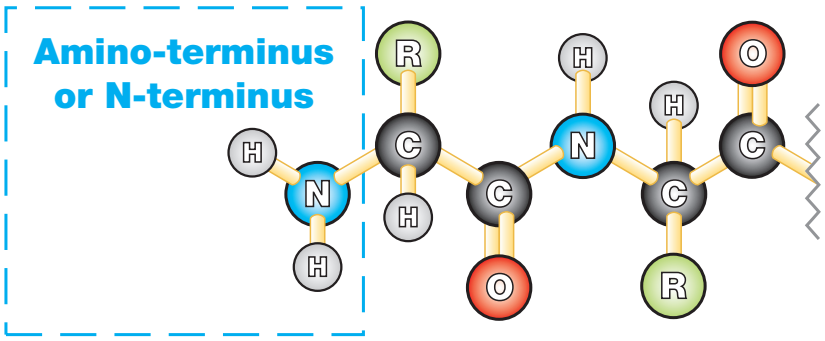
Proteins must fold into their correct 3-D shape before they can do anything useful. The structure of a protein as it folds can have as many as four levels, or steps, of organization. Most proteins have three.

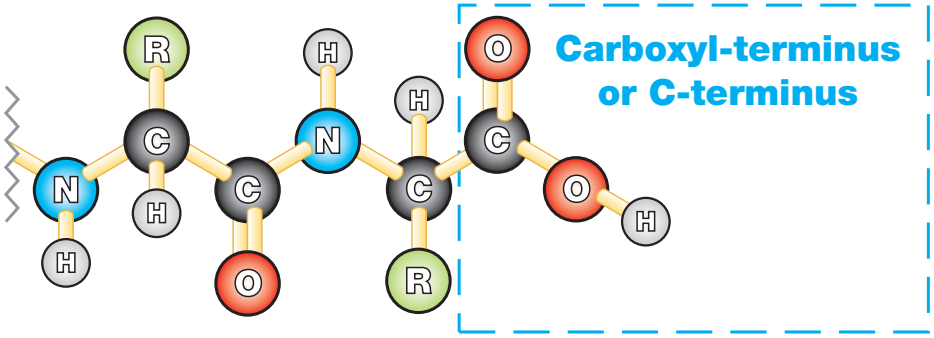
Primary Protein Structure

The first or ***primary structure*** is determined by the number, kind, and order of the amino acids joined together. The amino acid sequence is genetically determined (we'll talk more about that later). Think of this structure as a long bead necklace or a piece of spaghetti. When the protein is like this, it cannot do any work. It is just a long polypeptide chain.

Each end (terminus) of the polypeptide chain has a name. The first amino acid in the chain has its amino group (NH_2) and this end of the chain is called the ***amino-terminus*** or the ***N-terminus***. The last amino acid to be added still has its carboxyl group (-COOH) and is called the ***carboxyl-terminus*** or ***C-terminus***. We wouldn't bother you with all these "terms" about terminuses, but the front end and back end of a protein are important. The protein always starts folding at the front end.

Beginning of an amino acid chain…



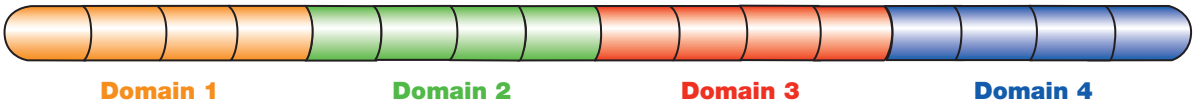


…end of an amino acid chain.

Secondary Protein Structure

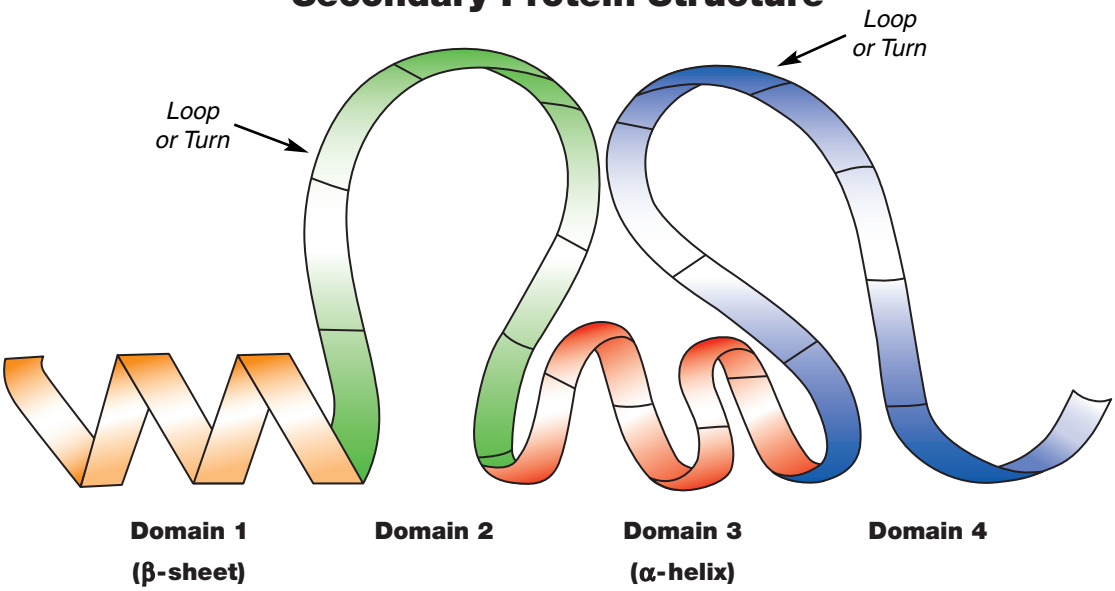
Once the amino acid chain forms, it doesn't just randomly wad up into a twisted mass. Short sections of the polypeptide chain, called ***domains***, fold into recognizable shapes. These shapes are the ***secondary structure***.

Primary Protein Structure



The two most common secondary structures are a coiling ***alpha (α) helix*** or a folded ***beta (β) sheet***. Sometimes a portion of the amino acid chain does not form a definite shape and looks just like a ***loop*** or ***turn*** in the chain. The shapes are caused by hydrogen bonding. ***Hydrogen bonds*** are weak attractions between the positively charged hydrogen of the amino group of one amino acid and the negatively charged oxygen of a carboxyl group of another. Whether a certain portion of the chain coils in an α-helix, folds into a β-sheet, or just loops or turns is determined by the R groups of the amino acids in the chain.

Secondary Protein Structure

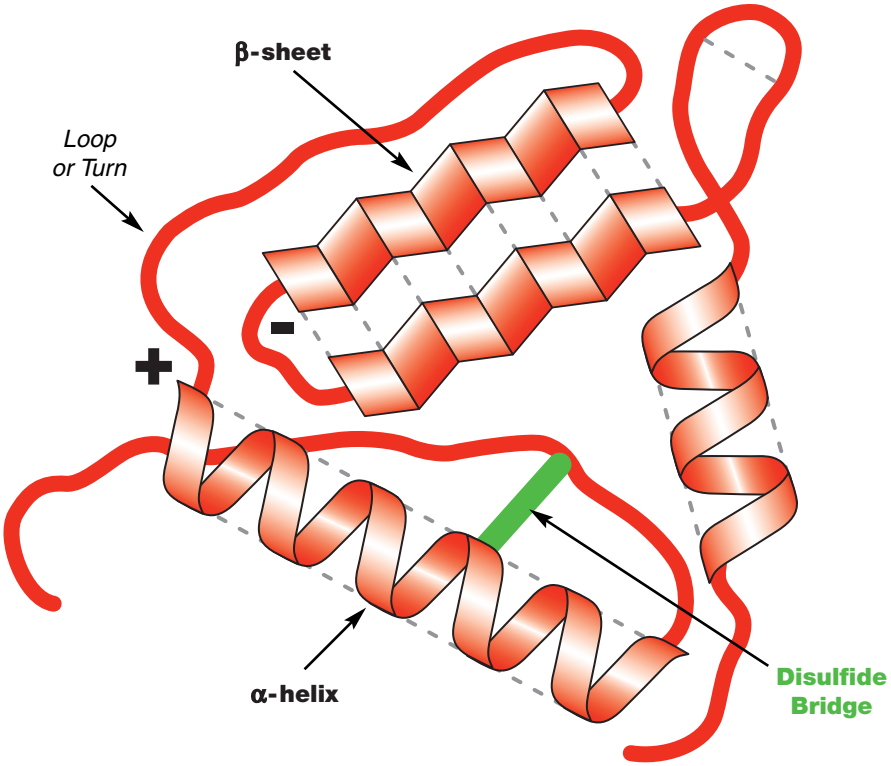


Tertiary Protein Structure

The ***tertiary structure*** determines a protein's function. More complex folding creates a tertiary structure. As with the secondary structure, this folding is determined by the properties of each different amino acid in the chain. Lots of different kinds of interactions happen between the R groups. Some of the R groups are positively charged and form ionic bonds with R groups that are negatively charged. Some R groups are ***hydrophobic***, they are not charged, and like oil, repel water. The parts of the amino acid chain that are hydrophobic squish into the center of the protein molecule to avoid the liquid in the cell environment.

One type of chemical bond between R groups is really special. *Cysteine is the only amino acid that has an R group that contains the element sulfur.* The cysteine R groups can link together to form strong bonds called ***disulfide bridges*** or ***cross-linkages***. These special bonds help form the ultimate shape of each protein. Most animals cannot live without cysteine in their diet.

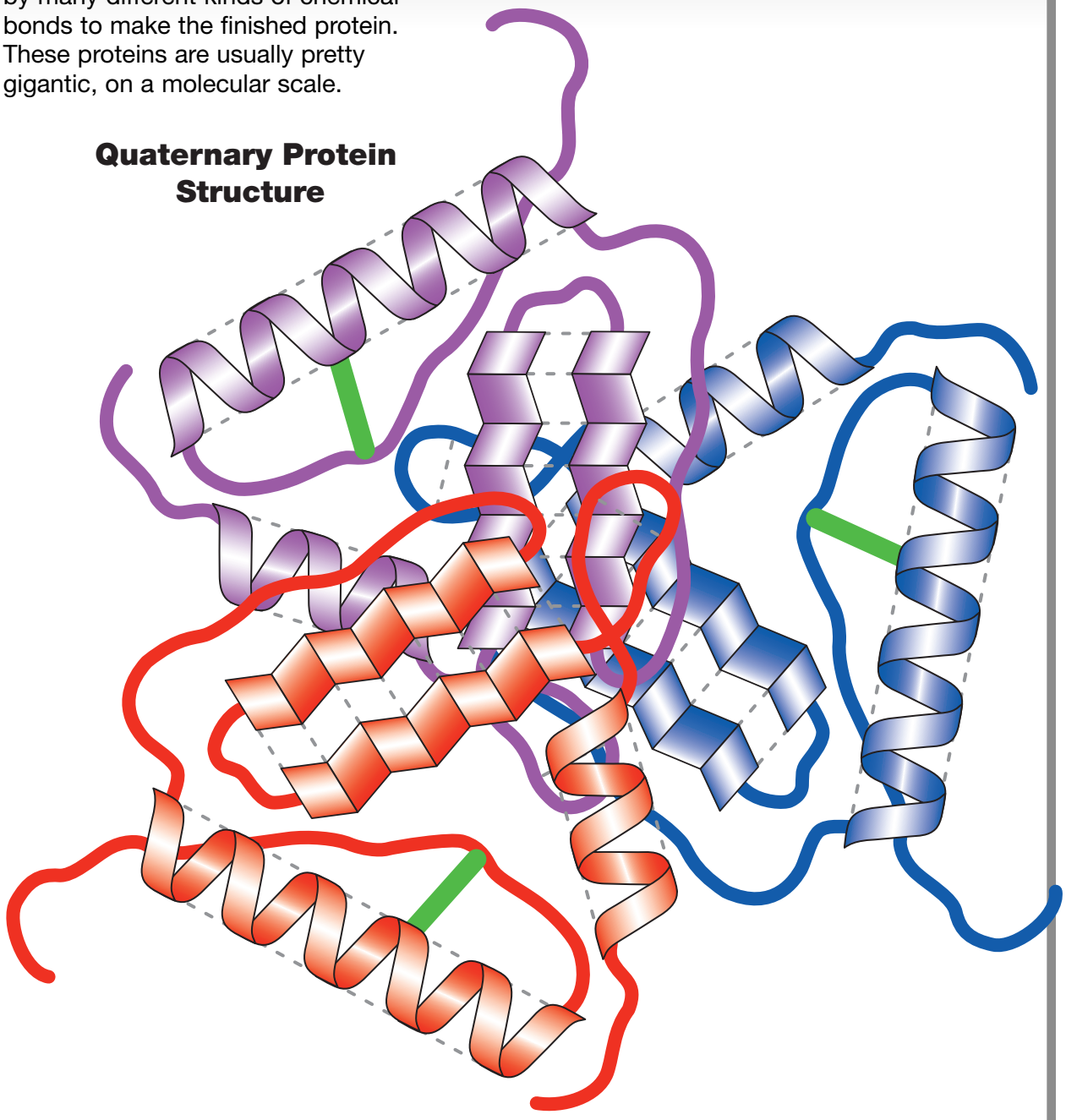
Tertiary Protein Structure



Quaternary Protein Structure

Not all proteins have ***quaternary*** or fourth-level structure. In this level, two or more polypeptides are joined together by many different kinds of chemical bonds to make the finished protein. These proteins are usually pretty gigantic, on a molecular scale.

Quaternary Protein Structure

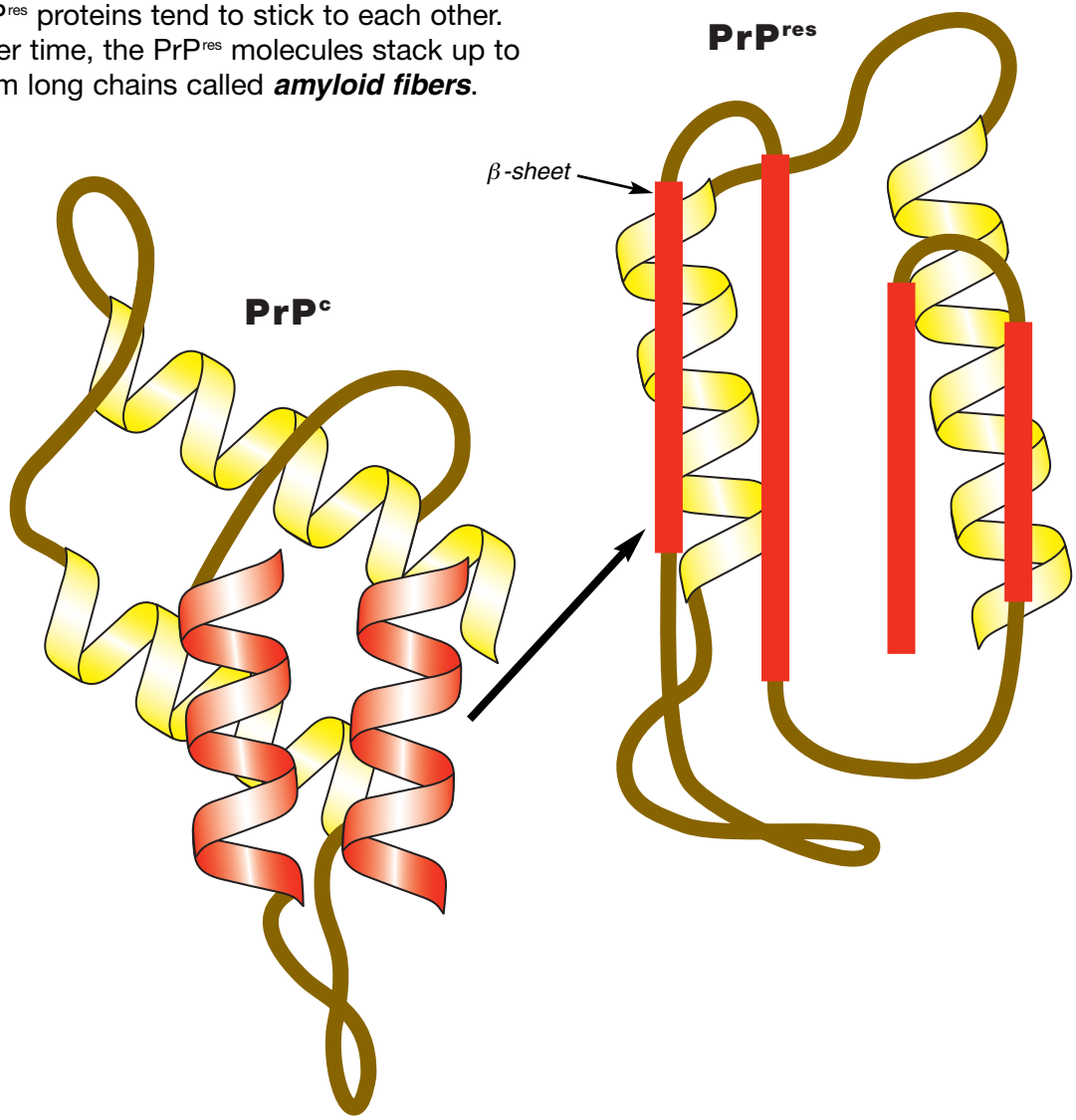


Protein Structure and Prion Diseases

As mentioned before, the prion protein exists in two forms, normal (PrP^c) and pathological (PrP^{res}). The difference between PrP^c and PrP^{res} is in their tertiary structure. The change is caused when many of the α-helices in the normal protein misfold into β-sheets.

Because of their abnormal shape, PrP^{res} proteins tend to stick to each other. Over time, the PrP^{res} molecules stack up to form long chains called ***amyloid fibers***.

Amyloid fibers are toxic to neurons (nerve cells) and ultimately kill the cells. Cells called ***astrocytes*** crawl through the brain digesting the dead neurons, leaving holes where neurons used to be. The amyloid fibers are left behind. When tissue from the brain of a victim of a TSE is examined under a microscope, one can see holes, clumps of amyloid fibers, and large numbers of astrocytes, all unique features of spongiform diseases.



Activity 1: Fold an α-helix

The α-helix, or right-handed coil shape, is caused by the hydrogen bonds that form in the carboxyl and amino groups of every fourth amino acid.

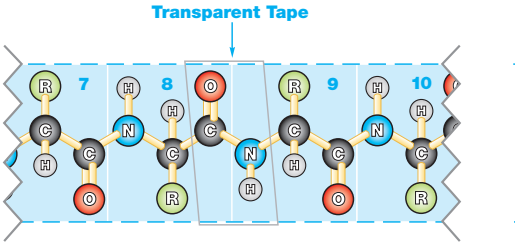
Materials Needed:

- Student Activity Page: *Polypeptide Chains*
- Scissors
- Transparent tape

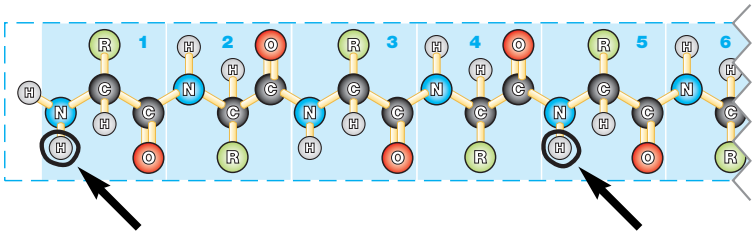
Procedure:

1. Cut out the strips labeled Polypeptide Chain 1a and Polypeptide Chain 1b along the dotted lines.

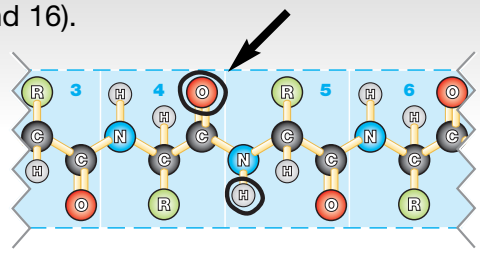
2. Using transparent tape, join the C-terminus of Polypeptide Chain 1a to the N-terminus of Polypeptide Chain 1b.



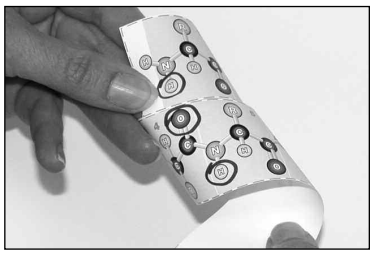
3. Circle the H atom on the amino group of every fourth amino acid beginning with amino acid #1 (amino acids #1, 5, 9, and 13).



4. Circle the O atom on the carboxyl group of every fourth amino acid beginning with amino acid #4 (amino acids #4, 8, 12, and 16).



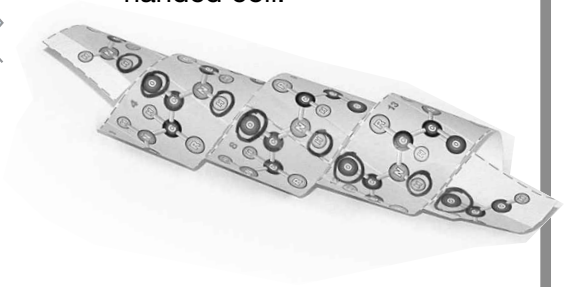
5. Hold the N-terminus end of the polypeptide chain in your left hand. Using your right hand, begin a right-handed coil.



Using transparent tape, join (with a hydrogen bond) the H atom of the amino group in amino acid #1 to the O atom in the carboxyl group of amino acid #5.

6. Continue to coil and hydrogen bond the following atoms of the polypeptide chain:
   a. H atom of amino group of amino acid #5 with O atom of carboxyl group of amino acid #8.
   b. H atom of amino group of amino acid #9 with O atom of carboxyl group of amino acid #12.
   c. H atom of amino group of amino acid #13 with O atom of carboxyl group of amino acid #16.

7. Congratulations, you have constructed an α-helix or right-handed coil.



Activity 2: Fold a β-sheet

The β-sheet also has hydrogen bonding between the amino and carboxyl groups but in this case the polypeptide chain is folded back on itself to give a flattish structure.

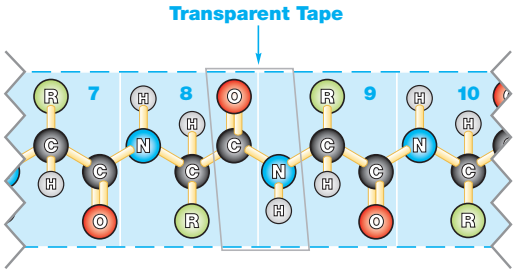
Materials Needed:

- Student Activity Page: *Polypeptide Chains*
- Scissors
- Transparent tape

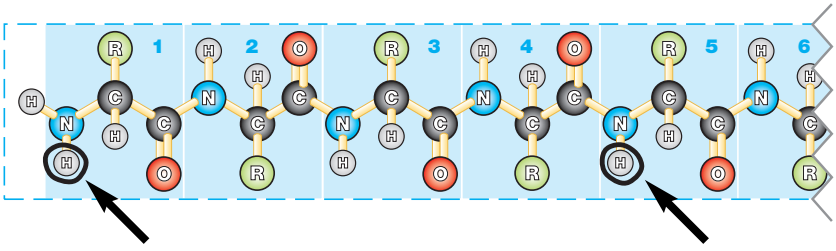
Procedure:

1. Cut out the strips labeled Polypeptide Chain 2a and Polypeptide Chain 2b along the dotted lines.

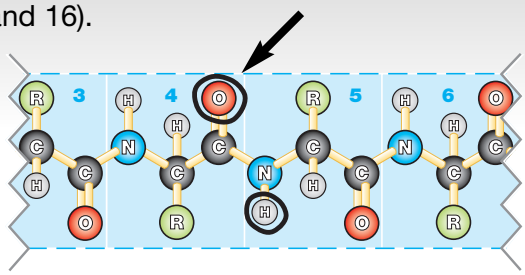
2. Using transparent tape, join the C-terminus of Polypeptide Chain 2a to the N-terminus of Polypeptide Chain 2b.



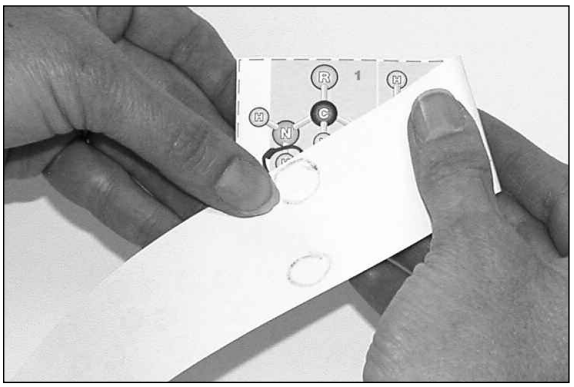
3. Circle the H atom on the amino-group of every fourth amino acid beginning with amino acid #1 (amino acids #1, 5, 9, and 13).



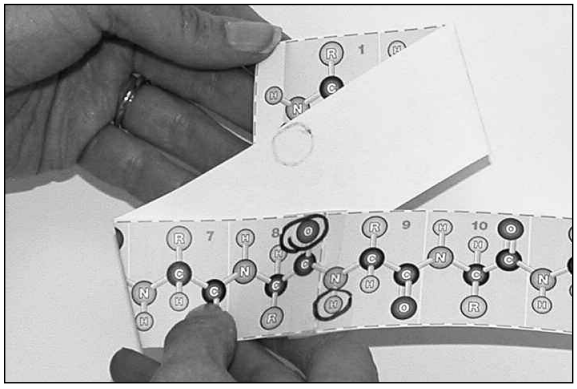
4. Circle the O atom on the carboxyl group of every fourth amino acid beginning with amino acid #4 (amino acids #4, 8, 12, and 16).



5. Hold the N-terminus end of the polypeptide chain in your left hand. Hold amino acid #4 in your right hand. Fold so that the O atom on the carboxyl group of amino acid #4 touches the H atom of the amino group of amino acid #1 (sideways H bond).



6. Continue to fold and hydrogen bond the following atoms of the polypeptide chain:
   a. Fold back in the opposite direction so that the O atom of carboxyl group of amino acid #8 is on top of the H atom of amino group of amino acid #5.



   b. Reverse the fold again so that the O atom of carboxyl group of amino acid #12 is on top of the H atom of amino group of amino acid #9.
   c. Fold back in the opposite direction so that the O atom of carboxyl group of amino acid #16 is on top of the H atom of amino group of amino acid #13.

7. Congratulations, you have constructed a β-sheet.

Activity 3: Silly Polymers

You've done a lot of brain work, so its time to get silly. You are going to cross-link a polymer to create Silly Putty®! Since there is no feasible cross-linking activity that you can do with proteins in your classroom, this silly substitute will give you an idea of how the shape and function of proteins might change when cross-linking occurs. The objective is to cross-link a polymer and observe the changes in the physical properties as a result of this cross-linking. You will also observe changes in the physical properties of your cross-linked polymer in different temperatures.

The glue contains a polymer called polyvinyl acetate resin. This polymer has a structure a bit like the secondary structure of protein, like long strands of spaghetti. When water is added, the glue becomes runny, and these strands separate from each other. The Borax® acts as a cross-linker that chemically "ties together" the long strands of the polyvinyl acetate. The strands can no longer slip and slide past one another. The Silly Putty® will be "stiffer." The Silly Putty® is held together by very weak intermolecular bonds, similar to the hydrogen bonds that join portions of the polypeptide chains in proteins. Like proteins, the Silly Putty® is not "rigid" or "solid." There is flexibility around the weak bonds and throughout the cross-linked polymer.

Materials Needed:

You will work in groups of four. Each *person* needs:

- 1 clear 8-oz. (or larger) plastic cup
- 1 plastic teaspoon
- 1 plastic Ziploc™ bag

Each *group of 4* needs:

- 1 clear 8-oz. (or larger) plastic cup (which the group should label "Borax®")
- 1 clear 8-oz. (or larger) plastic cup (which the group should label "Water")
- 1 graduated cylinder
- 1 ruler
- white all-purpose glue
- Borax®
- 1 plastic teaspoon (which the group should label "Borax®")
- 1 permanent marker
- water
- paper towels
- newspaper (put down first to protect the working surface)
- Access to a refrigerator/freezer or to a cold water and an ice water bath

General Safety Guidelines:

- Since solid Borax® is a bleaching agent and solution will burn the eyes, goggles and aprons should be worn.
- Some people are allergic to Borax® (may have skin reaction). Wash your hands after kneading the Silly Putty® and finishing the experiment
- It is important to label anything containing or used with the Borax®. Only reuse these things with Borax®. Borax® is very basic and will contaminate materials for a long time, even after cleaning.

Procedure:

1. Put newspaper on your work surface to protect it. Put on your goggles and lab apron.

2. Use the permanent marker to label one plastic cup "Borax®" and another plastic cup "Water" for the group. Label one plastic spoon "Borax®."

3. Use the permanent marker to write your name on your Ziploc™ bag, your individual plastic cup, and your teaspoon.

4. Fill the "Water" cup with water.

5. Use the graduated cylinder to measure 200 ml of water. Pour it into the cup labeled "Borax®."

6. In your individual clear cup, put six teaspoons of glue. Add four teaspoons of water, from the "Water" cup, to the glue. Stir.

7. *Using the "Borax®" spoon*, add 1 teaspoon of Borax® to the water in the cup labeled "Borax®." Stir until most of the Borax® is dissolved.

8. Add 4 teaspoons of the Borax® solution to your individual cup using the "Borax®" spoon. Count three seconds (one-one thousand, two one-thousand, three one-thousand). Then gently stir your cup using your spoon. Stir thoroughly so that all of the glue comes into contact with the Borax® solution.

9. Observe what is happening. Take out the Silly Putty® and play with it. Do not try to "dry" it on paper towels or newspaper because it will stick. The Silly Putty® will naturally dry out and become the correct consistency as you play with it.

10. Form the Silly Putty® into a ball. Using a ruler to measure, drop the ball from a height of 30 centimeters. *How high does it bounce?*

_____

11. Now, place your ball in a refrigerator or ice bath for 10 minutes. Again, bounce the ball from a height of 30 centimeters. *How high did it bounce this time? Explain your results.*

_____
_____
_____
_____

12. Now place your ball about six inches from a light bulb for about five minutes and again check how high it bounces when dropped from a height of 30 centimeters. *How high did it bounce this time? Explain your results.*

_____
_____
_____
_____

13. Put your Silly Putty® ball in the freezer or in an ice bath for 10 minutes. Check how high it bounces when dropped from a height of 30 centimeters. *How high did it bounce this time? Explain your results.*

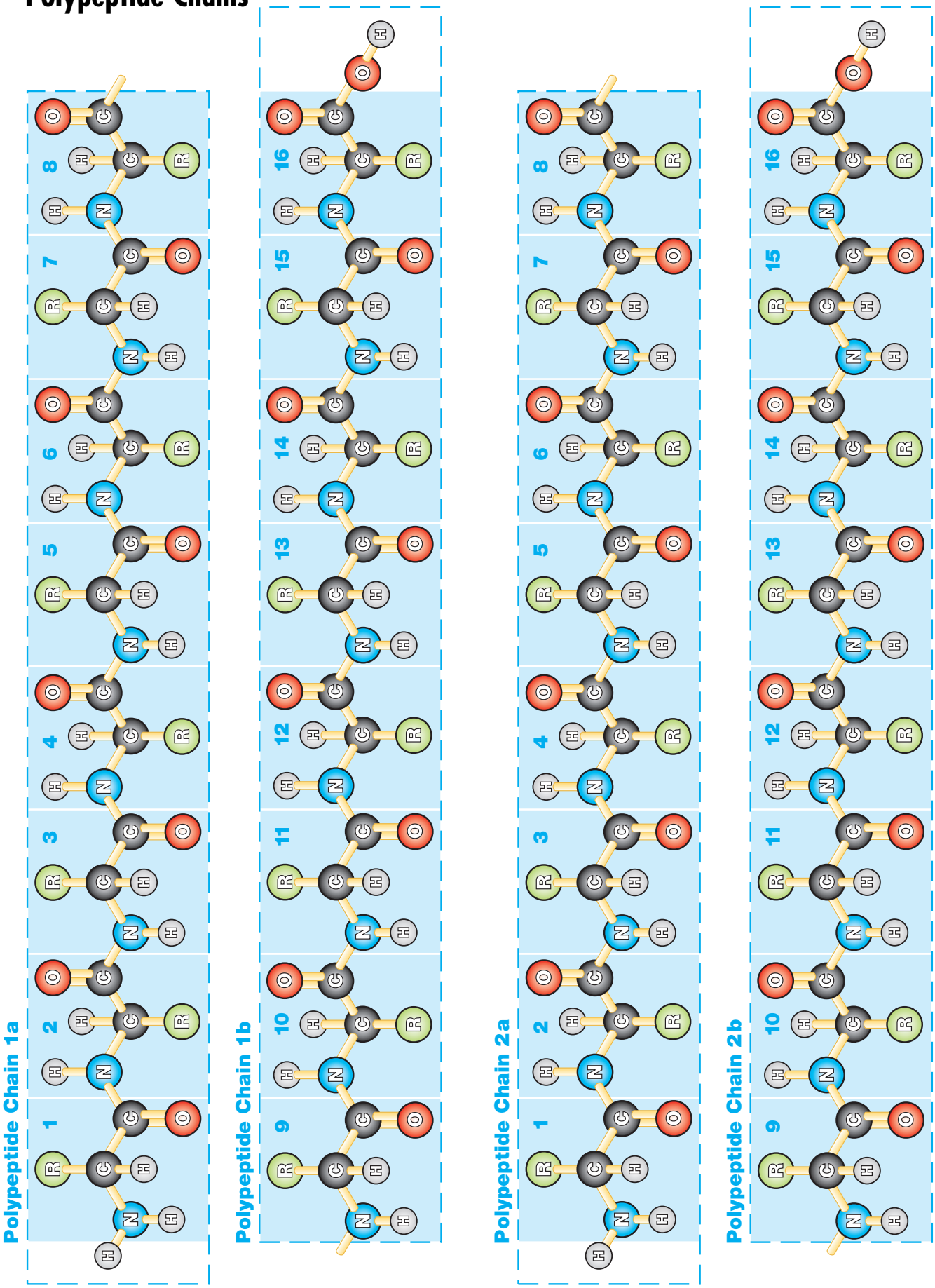
_____
_____
_____
_____

14. Try to transfer your observations of the behavior of Silly Putty® to the behavior of protein. Remember that proteins are the workhorses of the cell or body. *Why might a change in temperature, such as when a person is exposed to extreme heat or cold, cause problems?*

_____
_____
_____
_____
_____
_____
_____
_____

15. Wash your hands with soap and water when you have finished the experiment

Polypeptide Chains



Lesson 3

Duration

One or two 45-minute class periods

Vocabulary

Anticodon

Codon

Complementary base pairing

Deoxyribonucleic acid (DNA)

Eukaryotes

Gene

Genetic code

Genome

Messenger RNA (mRNA)

Nontranscribed strand (a.k.a Noncoding strand)

Nucleotide

Ribonucleic acid (RNA)

Ribosomal RNA (rRNA)

Ribosome

RNA polymerase

Promoter site

Protein folding problem

Protein synthesis

Transcribed Strand (a.k.a Coding Strand or Sense Strand)

Transcription

Transfer RNA (tRNA)

Translation

Triplet

Illinois Learning Standards

science: 12.A.4a, 12.A.4b, 12.A.4c, 12.A.5a, 12.A.5b, 12.B.4b, 12.B.5b

Educator's Overview

We Moose Crack the Code

Summary

Students read an article that explains how proteins are made from genetic information in the cell—that is, protein synthesis. They then complete an activity that simulates transcription and translation in order to decode a humorous message in a "gene."

Learning Objectives

After completing this activity, students will be able to:

- Describe the process of protein synthesis in five simple steps.
- Describe and distinguish between transcription and translation.
- Identify the roles of DNA, mRNA, rRNA and tRNA in protein synthesis.
- Transcribe and translate a DNA code.

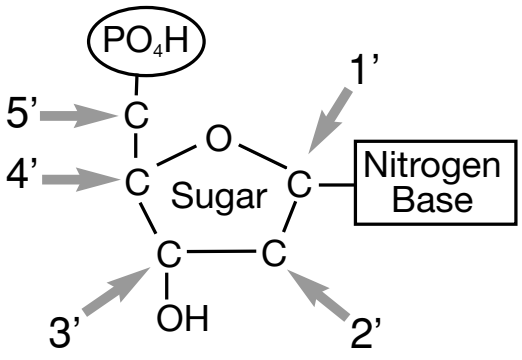
Background

Understanding how proteins are made in the cell and how they fold is essential to understanding how proteins might misfold and cause disease. This activity is designed to demonstrate how the genetic code contained in DNA determines the type of proteins that the organism produces. It is an overview of the process of protein synthesis.

Each protein is made using the genetic information stored in the genome, the entirety of hereditary information of an organism. Each gene—unit of heredity—in the genome is a sequence of DNA (deoxyribonucleic acid) that codes for one unique protein.

DNA is a polymer made up of four different nucleotides. Each nucleotide consists of a 5-carbon deoxyribose sugar, a phosphate group, and a nitrogen base. The sugar and phosphate group are identical for each nucleotide, but there are four different bases: adenine, thymine, guanine, and cytosine—abbreviated A, T, G, and C.

DNA Nucleotide



Each carbon of the sugar is numbered. The phosphate group is attached to the 5' carbon atom, and the nitrogen base is attached at the 1' carbon. The

phosphate of one nucleotide is then bonded to the 3' carbon of the next nucleotide to form a DNA strand. The complete DNA molecule has two complementary strands that coil anti-parallel to each other and form a double helix. In DNA molecules, C always pairs with G, and A always pairs with T.

The nucleic acid "language" of DNA is written in three letter (three nucleotide) sequences called triplets. To make a protein, this genetic information is transmitted in two stages. Each of these stages requires RNA, ribonucleic acid. RNA is very similar in chemical structure to DNA. The 5-carbon sugar in RNA is ribose instead of deoxyribose. While both DNA and RNA contain adenine (A), guanine (G), and cytosine (C) bases, the fourth nucleotide base in RNA is uracil (U). There are several different kinds of RNA, each with its own function.

The first step of making protein is transcription. In transcription, only one strand of the DNA double helix is transcribed. RNA polymerases are enzymes that attach to the DNA at specific promoter sequences and begin constructing messenger RNA (mRNA). RNA polymerase unwinds the double helix and moves in from 5' → 3' along the transcribed strand.
It forms the mRNA molecule by temporarily joining complementary RNA nucleotides to the DNA nitrogen bases in this fashion:

DNA base		***RNA base***
Cytosine (C)	joins with	Guanine (G)
Guanine (G)	joins with	Cytosine (C)
Thymine (T)	joins with	Adenine (A)
Adenine (A)	joins with	Uracil (U)

The resulting three letter or three nucleotides of the mRNA that carry the genetic code are called codons.

The second step of making protein is called translation. This is the step where nucleic acid "language" is converted to protein "language." After the RNA nucleotides are joined to form an mRNA molecule, the mRNA leaves the cell nucleus and connects to a ribosome (made up of ribosomal RNA, or rRNA) in the cytoplasm. Translation begins at the START codon of the mRNA. Since the codon or genetic code for each amino acid of a protein is three nitrogen bases long, molecules of transfer RNA (tRNA) with a complementary three-letter code (or anticodon) attach to the mRNA codon in sequence. One side of the tRNA molecule carries a specific amino acid. These amino acids link together in that sequence, making the protein. When a STOP codon is reached, the protein molecule is complete.

Teaching Strategies

1. Thoroughly read the student materials for *We Moose Crack the Code* and *Decode that Gene*.

2. Prior to class, make all photocopies. Separate the 'genes' on the sheets *Gene Sequences A through DD* so that you will be able to give one gene strip to each student. Cut apart the tRNA START molecule from the *tRNA "Word" Molecules* sheet so that you can give each student a tRNA START molecule.

3. Place the *tRNA "Word" Molecules* sheets and scissors at four stations around the room. Those starting with "A" can be placed at one station, those

Materials and Preparation

- *Student Pages:* We Moose Crack the Code—*one photocopy per student*
- *Student Activity Pages:* Decode that Gene—*one photocopy per student*
- Gene Sequences A through DD—*One photocopy per class. Each student needs one gene sequence for this activity, 30 are provided. Copy duplicated sequences as needed to accommodate class size.*
- tRNA "Word" Molecules—*one photocopy of each*
- *Four distinct "stations" in the classroom where* tRNA "Word" Molecules *can be placed. Those starting with "A" can be placed at one station, those starting with "C" at another, and so on.*
- *Scissors—several at each "tRNA station"*
- *Chalkboard, whiteboard, or some other visible writing surface*

starting with "C" at another, and so on. This arrangement will help alleviate crowding when students do their activity.

4. Give each student a copy of the Student Pages: *We Moose Crack the Code*. Allow students to read at their own pace, read as a group, or read aloud as a class.

5. After students have read *We Moose Crack the Code*, reinforce the following concepts:

- The building blocks of DNA and RNA are called nucleotides.
- Three of the nitrogen bases of the DNA and RNA nucleotides are the same: adenine (A), guanine (G), and cytosine (C). The fourth nucleotide base differs in the two molecules. RNA contains uracil (U) instead of thymine (T).
- The DNA molecule is a double helix. The strands are complementarily paired. A always pairs with T. C always pairs with G.
- Three bases of DNA will code for an amino acid. These three bases are called triplets.
- mRNA forms complementary pairs with DNA. A always pairs with U. T always pairs with A. C always pairs with G. The three mRNA nucleotides that pair with the DNA triplet are called a codon.
- tRNA carries an amino acid on one side and has three nucleotides called anticodons on the other to pair with the codon on mRNA
- DNA strands are "read" from the 5' carbon end of the strand to the 3' carbon end of the strand.

6. On the chalkboard or other visible place, review the example given in the Student Pages of base pairing and then provide additional examples and practice.

Molecule	***DNA***	***mRNA***	***tRNA***
3-Letter Code	Triplet	Codon	Anticodon
Complementary Bases	**C**	G	C
	G	C	G
	T	A	U
	A	U	A

7. Give each student a copy of the Student Activity Pages: *Decode that Gene*, one gene, and one tRNA START molecule. Tell each student that they have their own unique DNA gene, and they will need to transcribe and translate that gene to get their unique message.

8. Students may require assistance to begin the activity. It may be helpful to begin transcription with the DNA triplet TAC as a class. Be sure that all students have identified the correct start sequence and are working from top to bottom on the right side of their gene strip.

9. While each message is humorous, each message should make grammatical sense, so any nonsensical messages are due to errors in transcription and/or translation.

10. When students have completed the activity, emphasize the importance of having a START and STOP signal in protein synthesis. Remind students that chromosomes—the long strands of DNA in their cells—contain thousands of genes. Each cell has the organism's entire DNA, but not all of it needs to be "expressed." For example, liver cells do not need to produce the protein for eye color. Since not all proteins need to be made at once, and some not at all, there needs to be a signal to produce a specific protein.

11. Also emphasize the problem of mutation—the addition, deletion, or change of a DNA nucleotide. The end product, in this case, the student's message, may not make sense. In a cell or organism, the change may cause death or disease.

Assessment

Students should be able to write a few paragraphs describing protein synthesis and the processes of transcription and translation in their own words. They should be able to explain the role of DNA, mRNA, and tRNA in protein synthesis.

Extension

- You may wish to introduce students to the Human Genome Project, and discuss its importance to the study of biology.

Key

1. Your instructor will give you a "gene," a portion of the DNA molecule. Write down the letter(s) that identify your gene. ***Answers vary.***

2. If you look at your gene, what direction would the RNA polymerase enzyme move if it were reading the left side of your gene? ***Bottom to top.***

3. What direction would the RNA polymerase enzyme move if it were reading the right side of your gene? ***Top to bottom.***

4. What does your "message" say?

A. Start A moose is my best friend Stop
B. Start My large brain is my best feature Stop
C. Start My best friend has the biggest moose Stop
D. Start I have the biggest brain Stop
E. Start I have the smallest brain Stop
F. Start My best friend has the smallest brain Stop
G. Start My best feature is my large brain Stop
H. Start My brother has a large moose Stop
I. Start I have the brain of a moose Stop
J. Start My best friend is a large moose Stop
K. Start My moose has the biggest mouth Stop
L. Start A large moose is my best friend Stop
M. Start My friend has the brain of a moose Stop
N. Start My large mouth is my best feature Stop
O. Start I have the best feature of a moose Stop
P. Start My sister has the brain of a moose Stop
Q. Start My brother has the brain of a moose Stop
R. Start My sister has the best feature of a moose Stop
S. Start My moose has the biggest brain Stop
T. Start The friend of my sister is a moose Stop
U. Start The large moose has the smallest brain Stop
V. Start My sister has the smallest mouth Stop
W. Start My moose has the smallest mouth Stop
X. Start I have the biggest mouth Stop
Y. Start I have the smallest brother Stop
Z. Start I have the smallest mouth Stop
AA. Start My sister is the best friend of a moose Stop
BB. Start I have the smallest sister Stop
CC. Start My moose is the smallest Stop
DD. Start My brother is the friend of a moose Stop

5. What "word" did the final codon code for? Why is this codon necessary? ***STOP is necessary because the DNA strand has many genes and the RNA polymerase enzyme needs a signal to stop making a mRNA strand.***

6. How does your message compare to those of your classmates? ***It is different.***

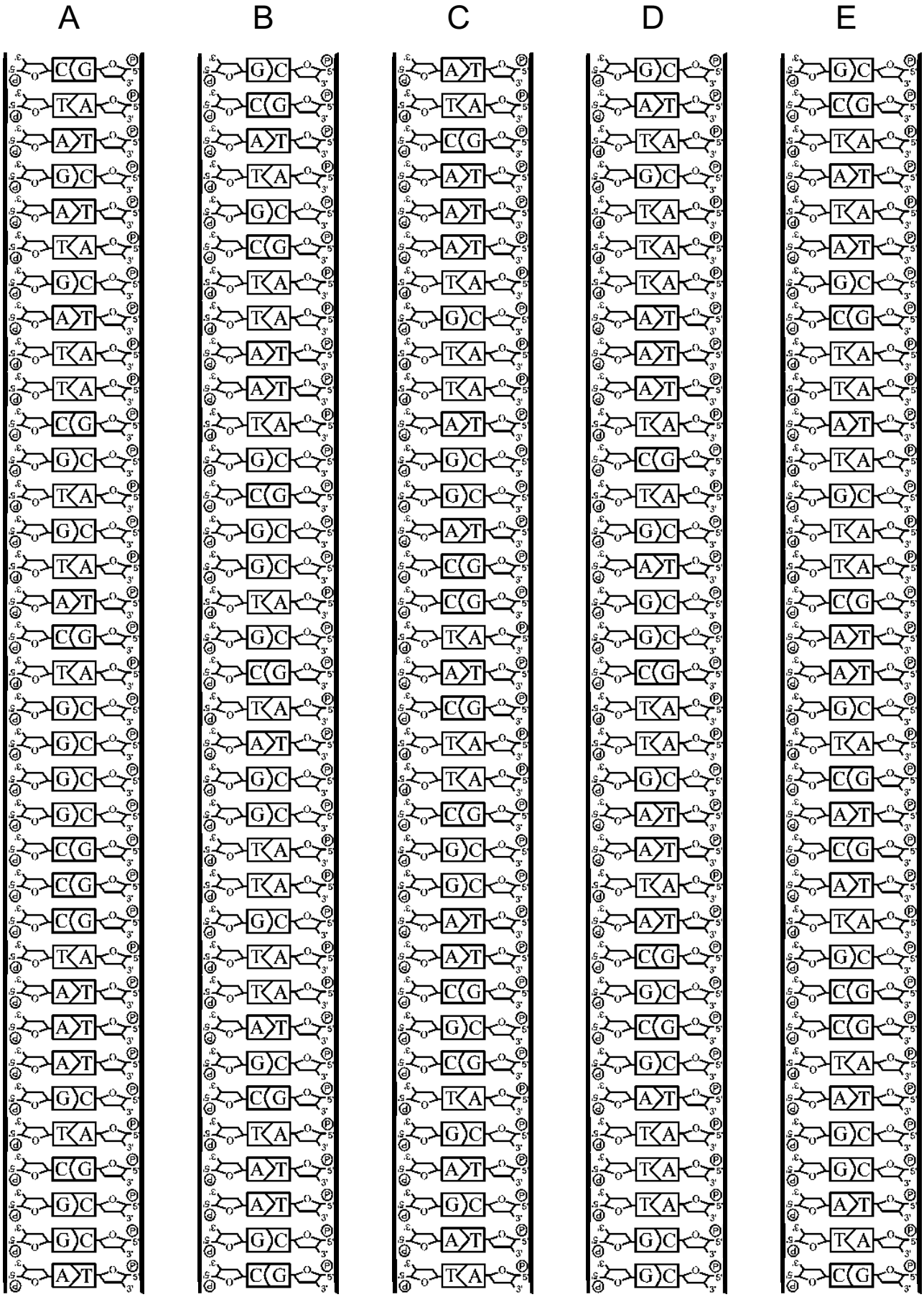
7. Looking at your assigned gene, how might your message change if the fifth nucleotide base of the DNA strand after the TAC START triplet were changed? ***A change in one letter may change the word that is coded for. The message might change, and it might not make any sense.***

8. Looking at your assigned gene, how might your message change if the fifth nucleotide base of the DNA strand after the TAC START triplet were deleted? ***A deletion of one letter may change the word that is coded for, as well as all the words that follow. The message might change, and it might not make any sense.***

9. In this activity, DNA coded for a message. In the actual genetic code, each triplet codes for a specific amino acid. If this DNA strand was coding for an amino acid chain, a protein, and a nucleotide base was changed or deleted, what might happen? ***Any change in the DNA might change the amino acid that is coded for, so the protein that is made may not be the same.***

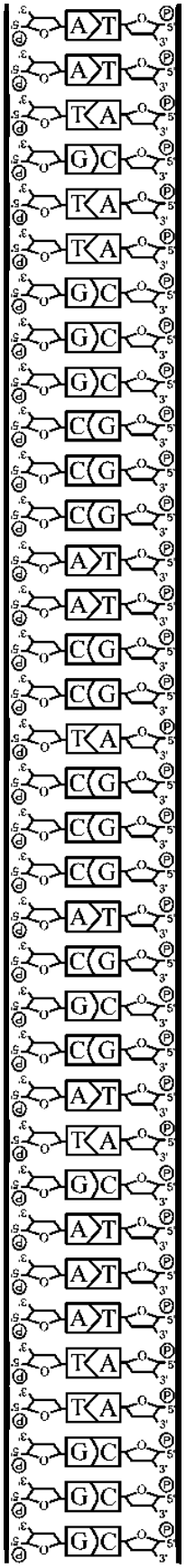
10. What might scientists call a change in the DNA strand (change, addition, or deletion of a nucleotide base)? ***They might call it a mutation.***

Gene Sequences



Gene Sequences

F



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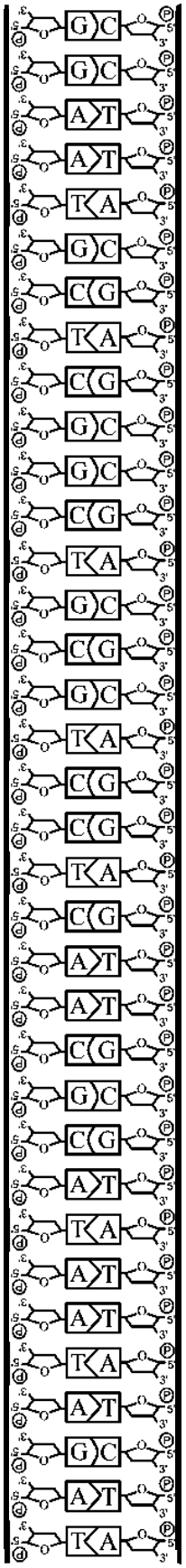
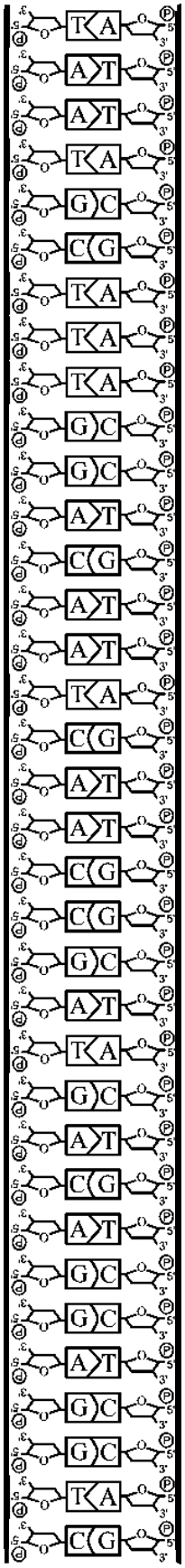
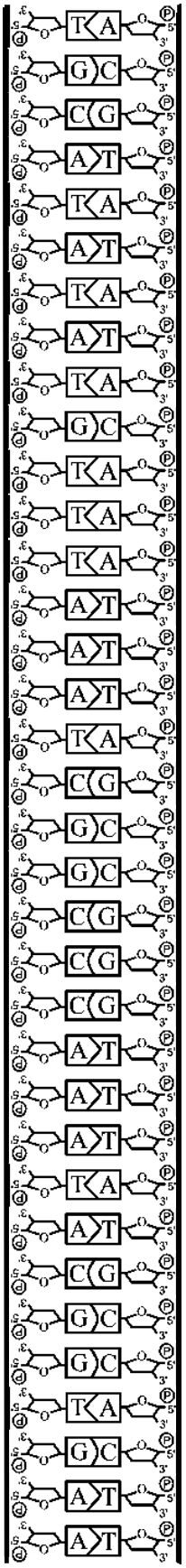


H



I



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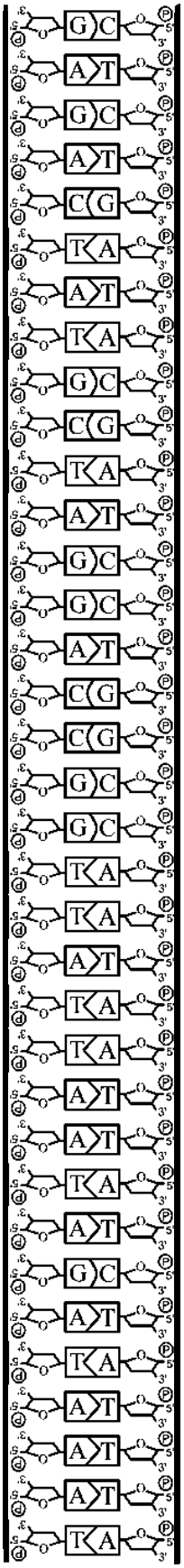
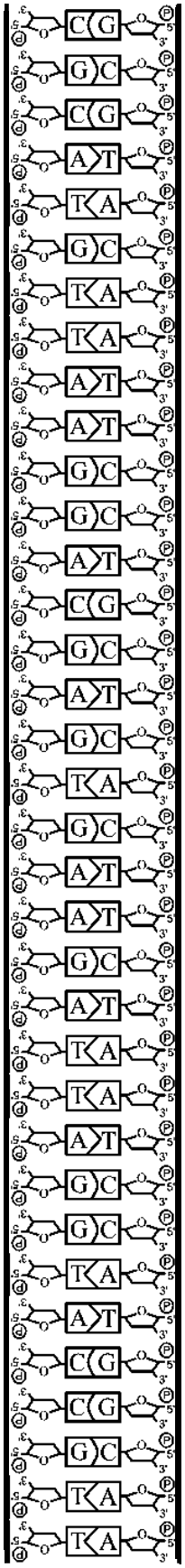
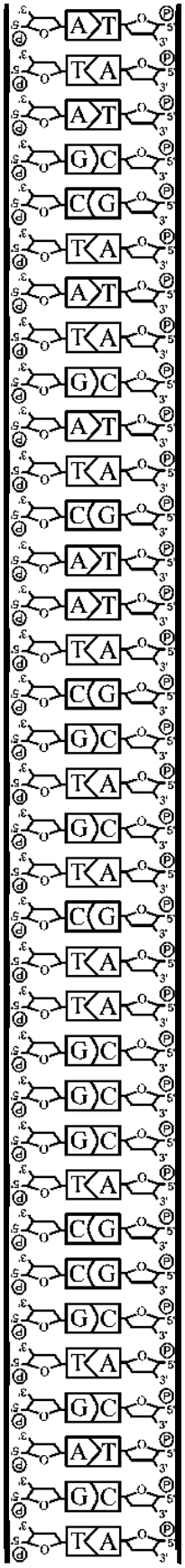


Gene Sequences

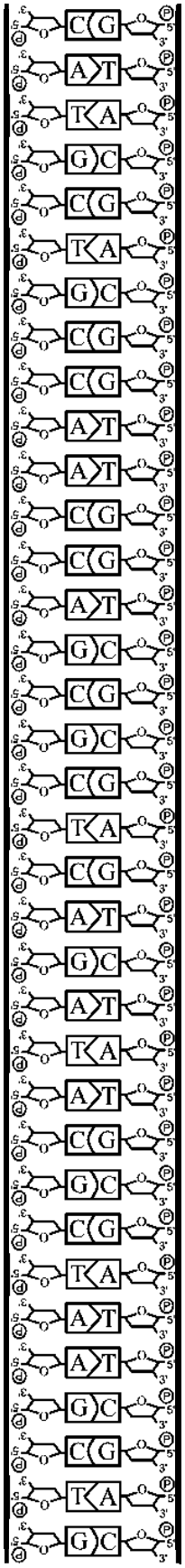
K



L



M



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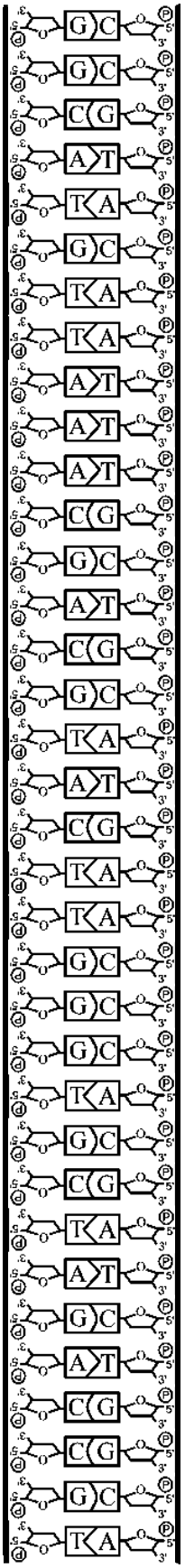

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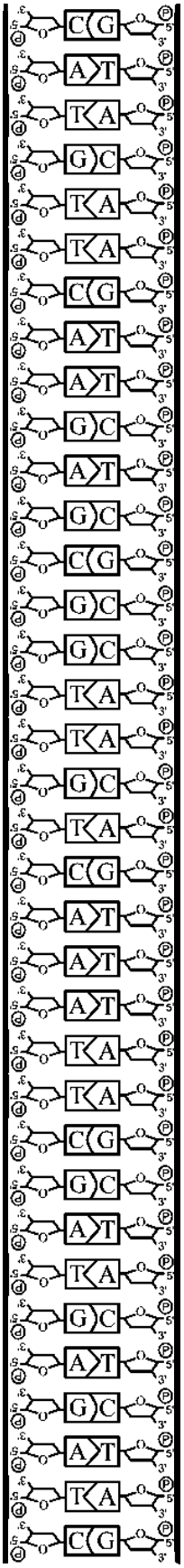
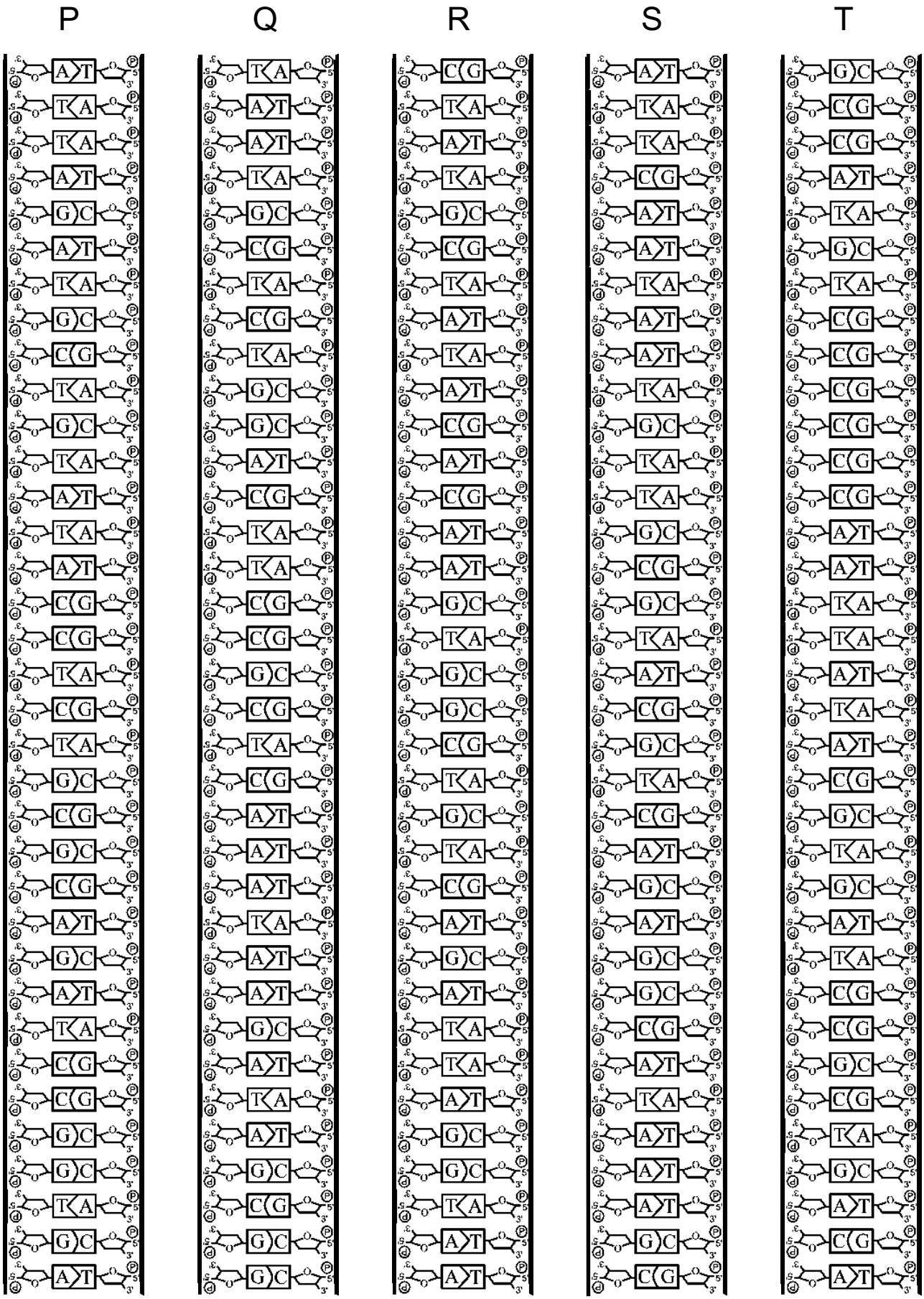
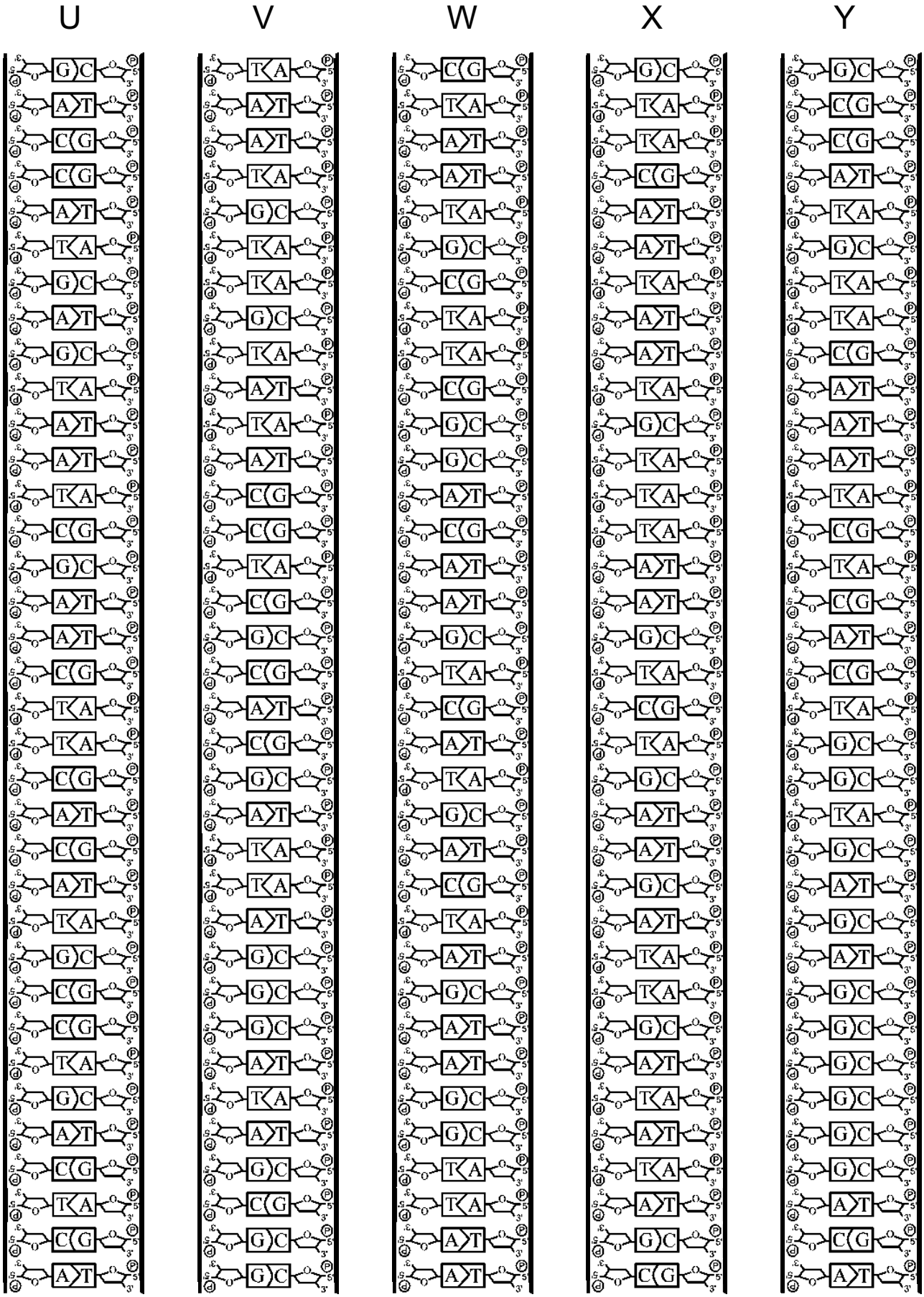


Gene Sequences

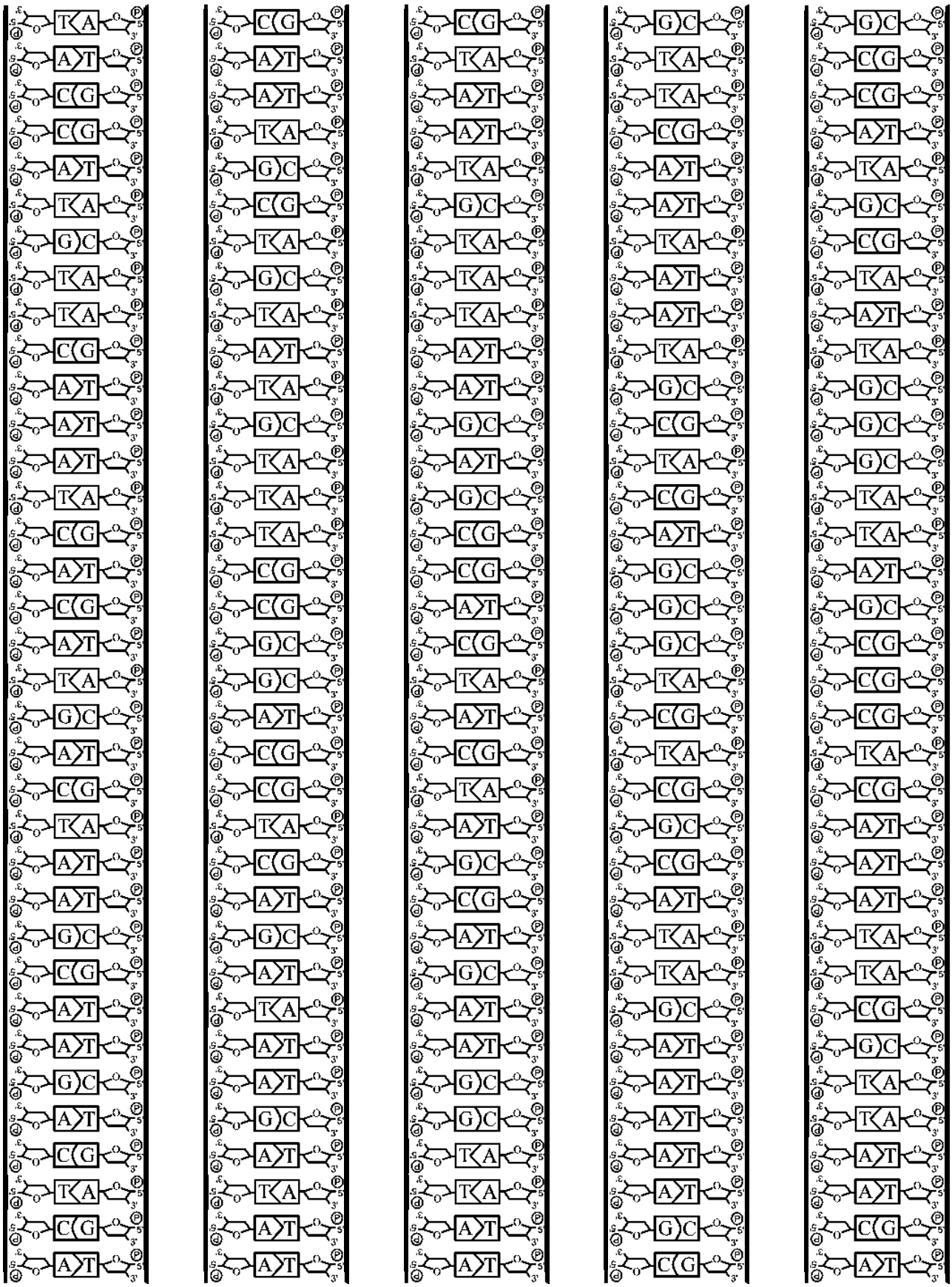


Gene Sequences



Gene Sequences

Z AA BB CC DD

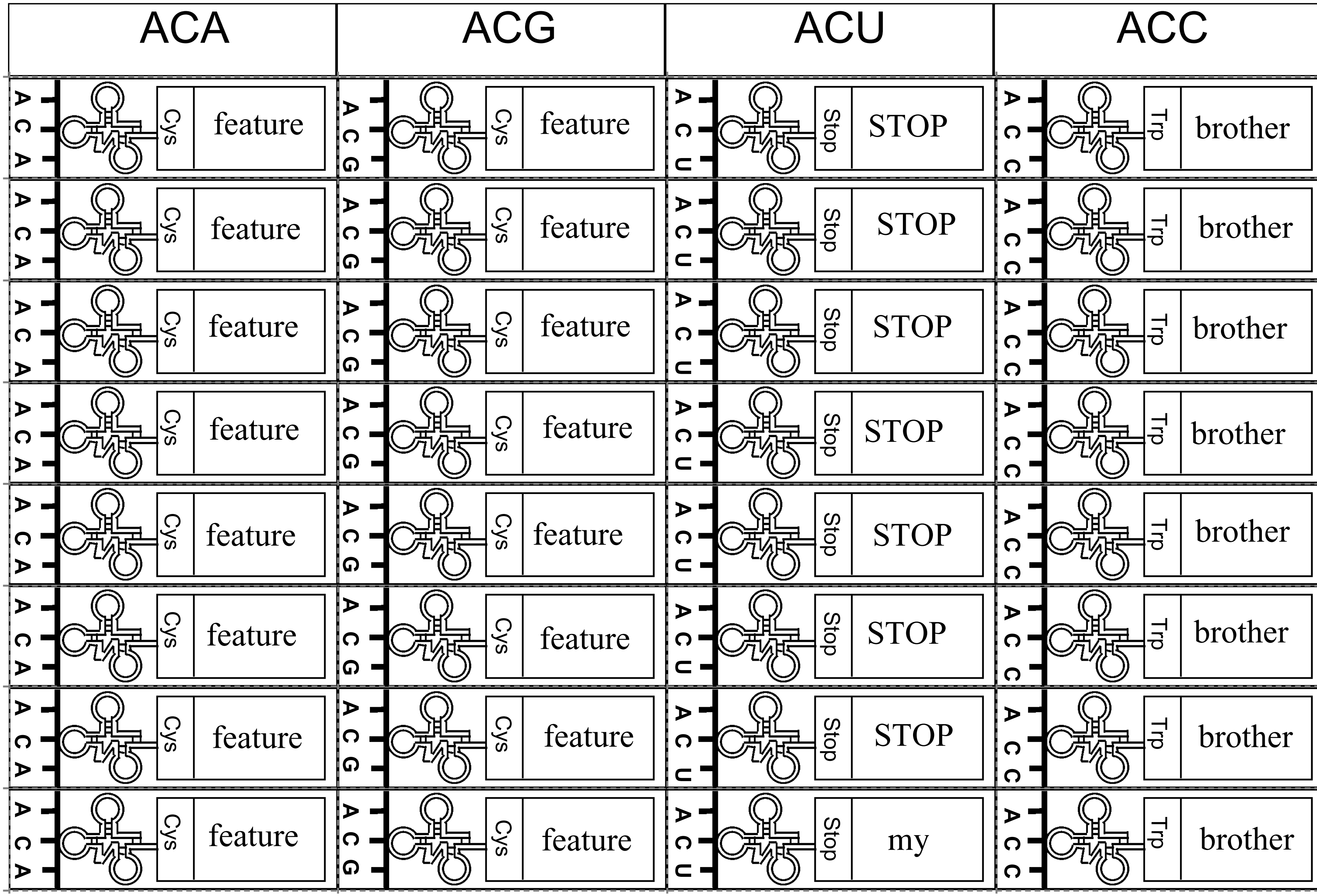


tRNA "Word" Molecules

Cut on dashed lines only

AAA	AAG	AAU	AAC
I	I	my	my
Phe	Phe	Leu	Leu
A A A	A A G	A A U	A A C

tRNA "Word" Molecules



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tRNA “Word” Molecules

AGA	AGG	AGU	AGC
A G A · Ser · the	A G G · Ser · the	A G U · Ser · the	A G C · Ser · the
A G A · Ser · the	A G G · Ser · the	A G U · Ser · the	A G C · Ser · the
A G A · Ser · the	A G G · Ser · the	A G U · Ser · the	A G C · Ser · the
A G A · Ser · the	A G G · Ser · the	A G U · Ser · the	A G C · Ser · the
A G A · Ser · the	A G G · Ser · the	A G U · Ser · the	A G C · Ser · the
A G A · Ser · the	A G G · Ser · the	A G U · Ser · the	A G C · Ser · the
A G A · Ser · the	A G G · Ser · the	A G U · Ser · the	A G C · Ser · the
A G A · Ser · the	A G G · Ser · the	A G U · Ser · my	A G C · Ser · the

Cut on dashed lines only

tRNA “Word” Molecules

AUA	AUG	AUU	AUC
A U A — Tyr — sister	A U G — Tyr — sister	A U U — Stop — STOP	A U C — Stop — STOP
A U A — Tyr — sister	A U G — Tyr — sister	A U U — Stop — STOP	A U C — Stop — STOP
A U A — Tyr — sister	A U G — Tyr — sister	A U U — Stop — STOP	A U C — Stop — STOP
A U A — Tyr — sister	A U G — Tyr — sister	A U U — Stop — STOP	A U C — Stop — STOP
A U A — Tyr — sister	A U G — Tyr — sister	A U U — Stop — STOP	A U C — Stop — STOP
A U A — Tyr — sister	A U G — Tyr — sister	A U U — Stop — STOP	A U C — Stop — STOP
A U A — Tyr — sister	A U G — Tyr — sister	A U U — Stop — STOP	A U C — Stop — STOP
A U A — Tyr — sister	A U G — Tyr — sister	A U U — Stop — my	A U C — Stop — STOP

Cut on dashed lines only

tRNA "Word" Molecules

Cut on dashed lines only

CAA	CAG	CAU	CAC
C A A	C A G	C A U	C A C
Val	Val	Val	Val
is	is	is	is
C A A	C A G	C A U	C A C
Val	Val	Val	Val
is	is	is	is
C A A	C A G	C A U	C A C
Val	Val	Val	Val
is	is	is	is
C A A	C A G	C A U	C A C
Val	Val	Val	Val
is	is	is	is
C A A	C A G	C A U	C A C
Val	Val	Val	Val
is	is	is	is
C A A	C A G	C A U	C A C
Val	Val	Val	Val
is	is	is	is
C A A	C A G	C A U	C A C
Val	Val	Val	Val
is	is	is	is
C A A	C A G	C A U	C A C
Val	Val	Val	Val
is	is	my	is

tRNA "Word" Molecules

CCA	CCG	CCU	CCC
C C A · Gly · best	C C G · Gly · best	C C U · Gly · best	C C C · Gly · best
C C A · Gly · best	C C G · Gly · best	C C U · Gly · best	C C C · Gly · best
C C A · Gly · best	C C G · Gly · best	C C U · Gly · best	C C C · Gly · best
C C A · Gly · best	C C G · Gly · best	C C U · Gly · best	C C C · Gly · best
C C A · Gly · best	C C G · Gly · best	C C U · Gly · best	C C C · Gly · best
C C A · Gly · best	C C G · Gly · best	C C U · Gly · best	C C C · Gly · best
C C A · Gly · best	C C G · Gly · best	C C U · Gly · best	C C C · Gly · best
C C A · Gly · best	C C G · Gly · best	C C U · Gly · my	C C C · Gly · best

Cut on dashed lines only

Cut on dashed lines only

tRNA "Word" Molecules

CGA	CGG	CGU	CGC
Ala brain C G A	Ala brain C G G	Ala brain C G U	Ala brain C G C
Ala brain C G A	Ala brain C G G	Ala brain C G U	Ala brain C G C
Ala brain C G A	Ala brain C G G	Ala brain C G U	Ala brain C G C
Ala brain C G A	Ala brain C G G	Ala brain C G U	Ala brain C G C
Ala brain C G A	Ala brain C G G	Ala brain C G U	Ala brain C G C
Ala brain C G A	Ala brain C G G	Ala brain C G U	Ala brain C G C
Ala brain C G A	Ala brain C G G	Ala brain C G U	Ala brain C G C
Ala brain C G A	Ala brain C G G	Ala my C G U	Ala brain C G C

tRNA "Word" Molecules

CUA	CUG	CUU	CUC
C U A — Asp — mouth	C U G — Asp — mouth	C U U — Glu — biggest	C U C — Glu — biggest
C U A — Asp — mouth	C U G — Asp — mouth	C U U — Glu — biggest	C U C — Glu — biggest
C U A — Asp — mouth	C U G — Asp — mouth	C U U — Glu — biggest	C U C — Glu — biggest
C U A — Asp — mouth	C U G — Asp — mouth	C U U — Glu — biggest	C U C — Glu — biggest
C U A — Asp — mouth	C U G — Asp — mouth	C U U — Glu — biggest	C U C — Glu — biggest
C U A — Asp — mouth	C U G — Asp — mouth	C U U — Glu — biggest	C U C — Glu — biggest
C U A — Asp — mouth	C U G — Asp — mouth	C U U — Glu — biggest	C U C — Glu — biggest
C U A — Asp — mouth	C U G — Asp — mouth	C U U — Glu — my	C U C — Glu — biggest

Cut on dashed lines only

tRNA "Word" Molecules

Cut on dashed lines only

GAA	GAG	GAU	GAC
my Leu G A A	my Leu G A G	my Leu G A U	my Leu G A C

tRNA "Word" Molecules

Cut on dashed lines only

GCA	GCG	GCU	GCC
Moose Arg G C A	Moose Arg G C G	Moose Arg G C U	Moose Arg G C C
Moose Arg G C A	Moose Arg G C G	Moose Arg G C U	Moose Arg G C C
Moose Arg G C A	Moose Arg G C G	Moose Arg G C U	Moose Arg G C C
Moose Arg G C A	Moose Arg G C G	Moose Arg G C U	Moose Arg G C C
Moose Arg G C A	Moose Arg G C G	Moose Arg G C U	Moose Arg G C C
Moose Arg G C A	Moose Arg G C G	Moose Arg G C U	Moose Arg G C C
Moose Arg G C A	Moose Arg G C G	Moose Arg G C U	Moose Arg G C C
Moose Arg G C A	Moose Arg G C G	my Arg G C U	Moose Arg G C C

tRNA "Word" Molecules

Cut on dashed lines only

GGA	GGG	GGU	GGC
G G A — Pro — friend	G G G — Pro — friend	G G U — Pro — friend	G G C — Pro — friend
G G A — Pro — friend	G G G — Pro — friend	G G U — Pro — friend	G G C — Pro — friend
G G A — Pro — friend	G G G — Pro — friend	G G U — Pro — friend	G G C — Pro — friend
G G A — Pro — friend	G G G — Pro — friend	G G U — Pro — friend	G G C — Pro — friend
G G A — Pro — friend	G G G — Pro — friend	G G U — Pro — friend	G G C — Pro — friend
G G A — Pro — friend	G G G — Pro — friend	G G U — Pro — friend	G G C — Pro — friend
G G A — Pro — friend	G G G — Pro — friend	G G U — Pro — friend	G G C — Pro — friend
G G A — Pro — friend	G G G — Pro — friend	G G U — Pro — my	G G C — Pro — friend

tRNA "Word" Molecules

GUA	GUG	GUU	GUC
GUA / His / smallest	GUG / His / smallest	GUU / Gln / of	GUC / Gln / of
GUA / His / smallest	GUG / His / smallest	GUU / Gln / of	GUC / Gln / of
GUA / His / smallest	GUG / His / smallest	GUU / Gln / of	GUC / Gln / of
GUA / His / smallest	GUG / His / smallest	GUU / Gln / of	GUC / Gln / of
GUA / His / smallest	GUG / His / smallest	GUU / Gln / of	GUC / Gln / of
GUA / His / smallest	GUG / His / smallest	GUU / Gln / of	GUC / Gln / of
GUA / His / smallest	GUG / His / smallest	GUU / Gln / of	GUC / Gln / of
GUA / His / smallest	GUG / His / smallest	GUU / Gln / my	GUC / Gln / of

Cut on dashed lines only

tRNA “Word” Molecules

UAA	UAG	UAU	UAC
U A A · Ile · a	U A G · Ile · a	U A U · Ile · a	U A C · Met · START
U A A · Ile · a	U A G · Ile · a	U A U · Ile · a	U A C · Met · START
U A A · Ile · a	U A G · Ile · a	U A U · Ile · a	U A C · Met · START
U A A · Ile · a	U A G · Ile · a	U A U · Ile · a	U A C · Met · START
U A A · Ile · a	U A G · Ile · a	U A U · Ile · a	U A C · Met · START
U A A · Ile · a	U A G · Ile · a	U A U · Ile · a	U A C · Met · START
U A A · Ile · a	U A G · Ile · a	U A U · Ile · a	U A C · Met · START
U A A · Ile · a	U A G · Ile · a	U A U · Ile · my	U A C · Met · START

Cut on dashed lines only

tRNA "Word" Molecules

UCA	UCG	UCU	UCC
U C A — Ser — the	U C G — Ser — the	U C U — Arg — Moose	U C C — Arg — Moose
U C A — Ser — the	U C G — Ser — the	U C U — Arg — Moose	U C C — Arg — Moose
U C A — Ser — the	U C G — Ser — the	U C U — Arg — Moose	U C C — Arg — Moose
U C A — Ser — the	U C G — Ser — the	U C U — Arg — Moose	U C C — Arg — Moose
U C A — Ser — the	U C G — Ser — the	U C U — Arg — Moose	U C C — Arg — Moose
U C A — Ser — the	U C G — Ser — the	U C U — Arg — Moose	U C C — Arg — Moose
U C A — Ser — the	U C G — Ser — the	U C U — Arg — Moose	U C C — Arg — Moose
U C A — Ser — the	U C G — Ser — the	U C U — Arg — my	U C C — Arg — Moose

Cut on dashed lines only

tRNA "Word" Molecules

UGA	UGG	UGU	UGC
U G A · Thr · has	U G G · Thr · has	U G U · Thr · has	U G C · Thr · has
U G A · Thr · has	U G G · Thr · has	U G U · Thr · has	U G C · Thr · has
U G A · Thr · has	U G G · Thr · has	U G U · Thr · has	U G C · Thr · has
U G A · Thr · has	U G G · Thr · has	U G U · Thr · has	U G C · Thr · has
U G A · Thr · has	U G G · Thr · has	U G U · Thr · has	U G C · Thr · has
U G A · Thr · has	U G G · Thr · has	U G U · Thr · has	U G C · Thr · has
U G A · Thr · has	U G G · Thr · has	U G U · Thr · has	U G C · Thr · has
U G A · Thr · has	U G G · Thr · has	U G U · Thr · my	U G C · Thr · has

tRNA "Word" Molecules

Cut on dashed lines only

UUA	UUG	UUU	UUC
U U A · Asp · large	U U G · Asp · large	U U U · Lys · have	U U C · Lys · have
U U A · Asp · large	U U G · Asp · large	U U U · Lys · have	U U C · Lys · have
U U A · Asp · large	U U G · Asp · large	U U U · Lys · have	U U C · Lys · have
U U A · Asp · large	U U G · Asp · large	U U U · Lys · have	U U C · Lys · have
U U A · Asp · large	U U G · Asp · large	U U U · Lys · have	U U C · Lys · have
U U A · Asp · large	U U G · Asp · large	U U U · Lys · have	U U C · Lys · have
U U A · Asp · large	U U G · Asp · large	U U U · Lys · have	U U C · Lys · have
U U A · Asp · large	U U G · Asp · large	U U U · Lys · my	U U C · Lys · have

tRNA “Word” Molecules

UAC	UAC	UAC	UAC
START Met U A C	START Met U A C	START Met U A C	START Met U A C
START Met U A C	START Met U A C	START Met U A C	START Met U A C
START Met U A C	START Met U A C	START Met U A C	START Met U A C
START Met U A C	START Met U A C	START Met U A C	START Met U A C
START Met U A C	START Met U A C	START Met U A C	START Met U A C
START Met U A C	START Met U A C	START Met U A C	START Met U A C
START Met U A C	START Met U A C	START Met U A C	START Met U A C
START Met U A C	START Met U A C	my Met U A C	START Met U A C

Cut on dashed lines only

tRNA "Word" Molecules

ACU	ACU	AUU	AUC
A C U — Stop — STOP	A C U — Stop — STOP	A U U — Stop — STOP	A U C — Stop — STOP
A C U — Stop — STOP	A C U — Stop — STOP	A U U — Stop — STOP	A U C — Stop — STOP
A C U — Stop — STOP	A C U — Stop — STOP	A U U — Stop — STOP	A U C — Stop — STOP
A C U — Stop — STOP	A C U — Stop — STOP	A U U — Stop — STOP	A U C — Stop — STOP
A C U — Stop — STOP	A C U — Stop — STOP	A U U — Stop — STOP	A U C — Stop — STOP
A C U — Stop — STOP	A C U — Stop — STOP	A U U — Stop — STOP	A U C — Stop — STOP
A C U — Stop — STOP	A C U — Stop — STOP	A U U — Stop — STOP	A U C — Stop — STOP
A C U — Stop — STOP	A C U — Stop — STOP	A U U — Stop — my	A U C — Stop — STOP

Cut on dashed lines only

Lesson 3

Student Pages

We Moose Crack the Code

All the transmissible spongiform encephalopathies (TSEs) (like bovine spongiform encephalopathy, scrapie, and chronic wasting disease), cystic fibrosis, Alzheimer's disease, and many cancers have something in common. All these apparently unrelated diseases result from protein folding gone wrong. If scientists could understand what directs a protein to fold a certain way, they might be able to treat or prevent disorders like TSEs that result from protein misfolding.

For more than 50 years researchers have tried—with little success—to figure out what directs protein folding. The ***protein folding problem*** remains one of the great challenges of biology. Each day, though, scientists come closer to understanding protein folding. They use information from another code, the genetic code, which they cracked in the mid-1960s.

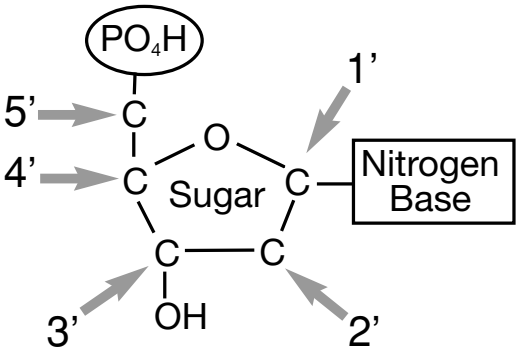
The ***genetic code*** is the set of rules by which information encoded in hereditary material in each cell of an organism makes all of the proteins needed by the organism. These proteins then determine, among other things, how the organism looks, how well its body metabolizes food or fights infection, and sometimes even how it behaves. Using the genetic code, scientists can determine a protein's exact amino acid sequence, which brings them closer to predicting the final structure of the protein.

Genes are units of genetic information and each gene provides the instructions for a single inherited property or characteristic of an organism—a protein. In other words, every gene makes a specific protein or amino acid chain. Genes are made of ***DNA*** or ***deoxyribonucleic acid***. DNA is a polymer made up of four similar chemicals called ***nucleotides***.

DNA Nucleotides—The Genetic Alphabet

Each DNA nucleotide has three parts: a phosphate group (PO_4H), a 5-carbon deoxyribose sugar, and a nitrogen-containing base. The phosphate group and the sugar are identical in every nucleotide. The nucleotides differ only in their four alternative bases: adenine, thymine, guanine, and cytosine—abbreviated A, T, G, and C.

DNA Nucleotide



These four "letters" are the genetic alphabet. They are repeated millions or billions of times throughout a genome. A ***genome*** is the entire DNA of an

organism. The particular order of these "letters" is extremely important. The order underlies all of life's diversity, dictating whether an organism is a human or another species such as an amoeba, bumblebee, or squirrel.

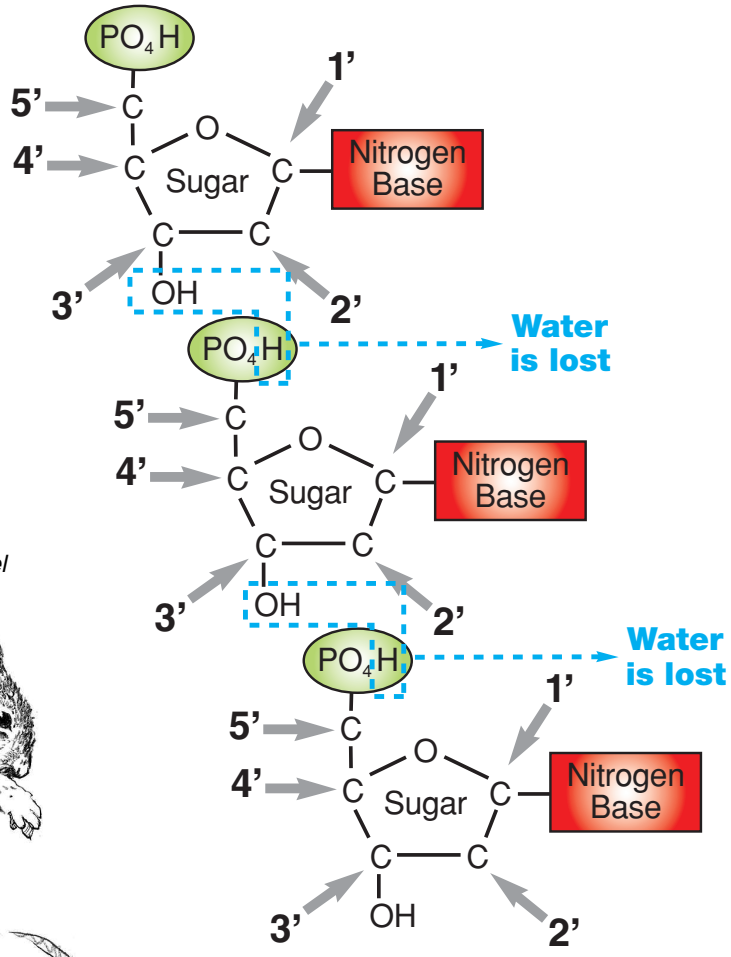
Bumblebee

Red Squirrel

DNA—The Double Helix

The phosphate groups and the 5-carbon sugars form the backbone of each strand of the DNA polymer. The bases are joined to the sugar and stick out sideways. Notice that each carbon atom (C) of the deoxyribose sugar is numbered.

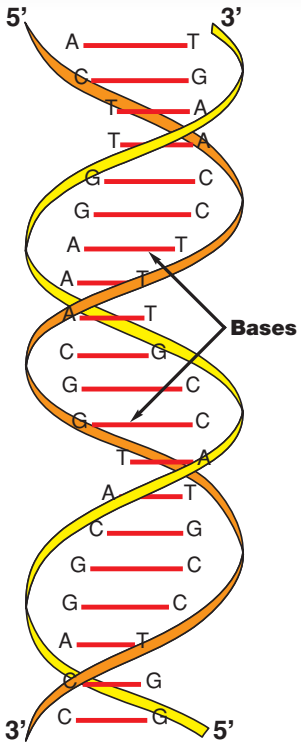
Nucleotides are joined by linking the phosphate on the 5' end of the deoxyribose sugar of one to the 3' carbon of the next as shown below. When the nucleotides link together, water is lost—dehydration synthesis again!



Notice how the nucleotides do not join in a straight line, but stagger as they join. This pattern causes the DNA strand to coil in a clockwise way and form a right-handed helix, just like the α-helix you made in the protein. In reality, DNA is normally found as a double-stranded molecule, and the two separate strands are wound around each other as a double helix.

In double-stranded DNA, the bases on one strand are paired with the bases on

the other strand. Adenine (A) in one strand is always paired with thymine (T) in the other and guanine (G) is always paired with cytosine (C). This kind of pairing is called ***complementary base pairing***. If you know the base sequence of either one of the strands of a DNA molecule, you can figure out the sequence of the other strand.



RNA—How DNA Makes Protein

How does the DNA that makes up a gene code for a protein? What are the instructions? How are the instructions used to determine the order of amino acids in proteins? It's a bit complicated.

When scientists began studying genes and DNA, they found something peculiar. In ***eukaryotes***, cells that have a nucleus, the DNA always stays in the nucleus. However, protein assembly happens in the cytoplasm! In fact, even the proteins found in the nucleus are made in the cytoplasm and then transported through the nuclear membrane. *How does DNA in the nucleus direct the construction of protein in the cytoplasm?* The answer is ***RNA—ribonucleic acid***.

RNA is a polymer of nucleotides that is somewhat similar to DNA, but is different in many ways:

1. The 5-carbon sugar in RNA is ribose instead of deoxyribose.
2. While both RNA and DNA contain adenine (A), guanine (G), and cytosine (C), the fourth nucleotide base differs in the two molecules. RNA contains uracil (U) instead of thymine (T).
3. RNA is a single stranded molecule and doesn't coil into a helix.
4. While there is only one type of DNA, *there are several kinds and sizes of RNA*. Each different kind of RNA has a different function.

Transcription: Making RNA

DNA instructions for protein are contained in three-base combinations called ***triplets***. In ***transcription***, the chemical instructions encoded in DNA are copied to make RNA. Only one strand of the double-stranded DNA is transcribed. Logically enough, this is called the ***transcribed strand*** (although some books may call it the ***sense strand*** or ***coding strand***). The other is called the ***nontranscribed*** strand, or ***noncoding*** strand, but strangely enough, is never called the nonsense strand!

To make a protein, the DNA code must first be transcribed into a ***messenger RNA (mRNA) strand***. This requires a special enzyme, ***RNA polymerase***, which unwinds the DNA. This giant enzyme attaches to the DNA and unwinds small portions of the double helix as *it moves down the transcribed strand in the 5' to 3' direction*. Once DNA bases on the transcribed strands are exposed, complementary bases of mRNA will pair with them like this:

DNA base		***RNA base***
C (Cytosine)	always pairs with	***G*** (Guanine)
G (Guanine)	always pairs with	***C*** (Cytosine)
T (Thymine)	always pairs with	***A*** (Adenine)
A (Adenine)	always pairs with	***U*** (Uracil)

Translation: Making Protein

After messenger RNA (mRNA) is formed, it leaves the nucleus and enters the cytoplasm of the cell. The next step uses the information carried by the mRNA to string together amino acids to make a specific protein. This step involves converting the nucleic acid "language," the genetic code, to protein "language," and that is why it's called ***translation***.

During translation, the bases of mRNA are read off in groups of three, which are known as ***codons***. Each codon represents a particular amino acid. Since there are four different bases, there are 64 possible groups of three bases, that is, 64 different codons in the genetic code.

Translation is carried out by a submicroscopic cellular machine called a ***ribosome***. Ribosomes are made of another kind of RNA, called ***ribosomal RNA*** or ***rRNA***. The ribosome attaches to the mRNA and moves along it and helps to put the amino acids in the correct order. However, something is needed to get the amino acids to the ribosome to read the mRNA! ***Transfer RNA*** (***tRNA***) carries amino acids to the ribosome. The transfer RNA molecule has an ***anticodon*** on one end that consists of three bases that are complementary to the three bases of the codon on messenger RNA. At its other end, each tRNA carries the amino acid corresponding to the codon it recognizes. In RNA molecules, G always pairs with C, and A always pairs with U.

Quick Summary of Protein Synthesis

Protein synthesis is the making of protein in the cell and was described at length above. We can summarize the process in five main steps:

1. *DNA provides the master genetic code (triplet) for the order of amino acids in a protein.* DNA does not leave the nucleus.

2. *The code is transcribed to mRNA.* The complementary base pairs to the DNA triplet are called codons.

3. *mRNA carries the genetic code for the protein to a ribosome.* The ribosome is made up of rRNA.

4. *tRNA brings the appropriate amino acid to the ribosome to assemble the protein. At the ribosome, tRNA translates the "genetic code" to "protein code."*

5. *Amino acids join together through dehydration synthesis to make the protein.*

Molecule	***DNA***		***mRNA***		***tRNA***
3-Letter Code	Triplet	⇨	Codon	⇨	Anticodon
Complementary Bases	C		G		C
	G		C		G
	T		A		U
	A		U		A

Student Activity Pages

Decode That Gene

Through the processes of transcription and translation, the genetic code found in DNA creates all the proteins found in organisms. It's amazing how much variety in life can come from just four simple molecules. You will now be given the task of transcribing and translating a DNA message. You won't be using the actual genetic code this time—you will work with the code for amino acids another day. Instead, you will end up with a message—words you string together using the DNA code.

Getting Started

1. Your instructor will give you a "gene," a portion of the DNA molecule. Write down the letter(s) that identify your gene:

_____

A strand of DNA can have millions of nucleotide bases. How can you tell where a gene starts? For that matter, how can you even figure out which strand is the transcribed strand? Remember that the RNA polymerase enzyme that transcribes *the DNA message into a mRNA molecule works in one direction, from the 5' carbon of one nucleotide to the 3' carbon of the next*.

2. *If you look at your gene, what direction would the RNA polymerase enzyme move if it were reading the left side of your gene?*

_____

3. *What direction would the RNA polymerase enzyme move if it were reading the right side of your gene?*

_____

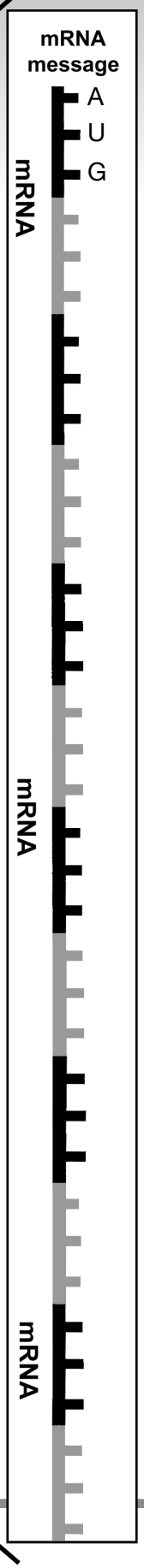
If this were a real gene, there would be a portion of nucleotide bases that would identify the strand to be transcribed. This portion is called the ***promoter site***. The RNA polymerase enzyme would attach here and the actual genetic message for a protein would start after this portion. For this exercise, you will transcribe the right side of your gene.

The DNA code is written in words consisting of three letters called a ***codon***. Once the RNA polymerase enzyme attaches to the DNA at the promoter site, it moves down along the DNA strand until it finds the START site. ***The START site always has the triplet of bases TAC.***

Carefully read your right DNA strand from the 5' carbon to 3' carbon direction and look for the DNA nucleotides TAC. Circle this start sequence on your gene!

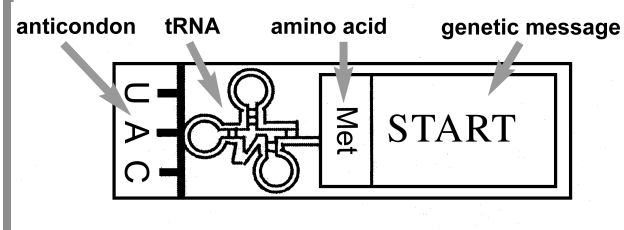
Decoding your DNA message is a two-step process. The first step, transcription, begins by pairing each DNA triplet on your gene to a complementary mRNA codon. Keeping DNA and RNA base pairing rules in mind, transcribe your mRNA molecule. Match your TAC triplet to the AUG codon of the mRNA and then complete the mRNA strand.

Attach DNA "Gene Sequence" here. Match your circled "TAC" triplet to the complementary mRNA codon "AUG" then, transcribe the rest of your DNA by writing the complementary mRNA base on the mRNA message.



Attach "tRNA 'Word' Molecules" here

Once your mRNA has been completely transcribed, it is time for the second step in protein synthesis—translation. You have been given sheets of tRNA molecules. Each tRNA molecule has an anticodon on one side, and a message on the other. Keeping in mind the proper way for mRNA and tRNA molecules to pair, cut out and tape each tRNA anticodon next to its complementary mRNA codon.



4. *What does your "message" say?*

_____

5. *What "word" did the final codon code for? Why is this codon necessary?*

_____
_____
_____
_____

6. *How does your message compare to those of your classmates?*

_____

7. *Looking at your assigned gene, how might your message change if the fifth nucleotide base of the DNA strand after the TAC START triplet were changed?*

_____
_____
_____
_____

8. *Looking at your assigned gene, how might your message change if the fifth nucleotide base of the DNA strand after the TAC START triplet were deleted?*

_____
_____
_____
_____

9. In this activity, DNA coded for a message. In the actual genetic code, each triplet codes for a specific amino acid. *If this DNA strand was coding for an amino acid chain, a protein, and a nucleotide base was changed or deleted, what might happen?*

_____
_____
_____
_____

10. *What might scientists call a change in the DNA strand (change, addition, or deletion of a nucleotide base)?*

_____

Lesson 4

Educator's Overview

Cannibalism, Forgetfulness, and Sleepless Nights

Duration

One 45-minute class period

Vocabulary

Allele

Dominant allele

Familial disease

Heritable

Heterozygous

Homozygous

Human Genome Project

Mutation

Polymorphism

Recessive allele

Silent (Neutral) mutation

Illinois Learning Standards

science: 12.A.4a, 12.A.4b, 12.A.4c. 12.A.5a, 12.A.5b, 12.B.4b, 12.B.5b

Summary

Students review the process of forming proteins from DNA. They read an article that links changes in DNA—mutations—to changes in protein shape and disease. Then, using information gained from the Human Genome Project, students identify some changes in the human prion gene, PRNP, associated with inherited human prion diseases.

Learning Objectives

After completing this activity, students will be able to:

- Identify and code for a mutation in the human prion gene PRNP.
- Describe the way a mutation might change the function of the PRNP gene.
- Describe and provide examples of a gene polymorphism.
- Predict the percent of a population that might be affected by a dominant genetic disorder.

Background

This activity focuses on human prion diseases and sets the stage for upcoming activities which will focus on chronic wasting disease (CWD). The reading connects the last lesson, in which students decoded a "message" from a DNA sequence, to the creation of a protein. An error in decoding, or a change in the code, can produce a protein that doesn't "make sense." The protein will not have the right shape to do its job. Since mutations in DNA are heritable, these altered forms of the gene can be passed to offspring. If the changed gene produces a protein that causes disease in the organism, the disease can also be passed to offspring.

Since many prion diseases were thought to be genetic or ***familial***, the human prion gene has been the focus of a lot of research. Information compiled by the Human Genome Project has enabled scientists to identify over 30 mutations in the human prion gene that are associated with human transmissible spongiform encephalopathies (TSEs)—10 of these mutations are linked to Creutzfeldt-Jakob disease (CJD).

Some of the human prion diseases, like Gerstmann-Sträussler-Scheinker syndrome

(GSS) and Familial Fatal Insomnia (FFI), are solely inherited genetic disorders. Some, like CJD, seem to occur either spontaneously or genetically (or, as we'll talk about in the next lesson, by exposure to abnormal prions). Another human TSE, kuru, is transmitted by eating infected tissue of a kuru victim. Students will learn how scientists apply the knowledge gained from these human prion diseases to the study of CWD in the next lesson.

Teaching Strategies

1. Thoroughly read the student materials for *Cannibalism, Forgetfulness, and Sleepless Nights*.

2. Give each student a copy of the Student Pages *Cannibalism, Forgetfulness, and Sleepless Nights*. Allow students to read the assignment and answer the questions silently on their own, in groups, or aloud as a class.

3. When the assignment is completed, again emphasize that a mutation—the addition, deletion, or change of a single DNA nucleotide—can have serious consequences.

4. Discuss the Human Genome Project and its value to the study of biology in general and to the study of prion diseases in particular. ***Optional:*** If possible, have students visit the Human Genome Project Web site at

http://genomics.energy.gov/.

5. Ask students to keep their copy of this lesson. They will need to refer to these pages when completing the next lesson.

Assessment

Ask students to describe these mutations, which are each associated with one or more inherited forms of Creutzfeldt-Jakob disease (CJD).

a. PRNP V180I
b. PRNP R208H
c. PRNP E211Q
d. PRNP M232R

Answers:

a. Isoleucine replaces valine as amino acid 180 in the prion protein. Isoleucine is hydrophobic, just like valine.
b. Histidine replaces arginine as amino acid 208 in the prion protein. Histidine is hydrophilic and acidic and has a negative charge. Arginine is hydrophilic and basic and has a positive charge.
c. Glutamine replaces glutamate as amino acid 211 in the prion protein. Glutamine is polar and moderately hydrophilic, glutamate is hydrophilic and acidic (negatively charged).
d. Arginine replaces methionine as amino acid 232 in the prion protein. Arginine is hydrophilic and basic (+ charge) while methionine is hydrophobic.

Materials and Preparation

- *Student Pages:* Cannibalism, Forgetfulness, and Sleepless Nights—*one photocopy per student*
- ***Optional:*** *Provide students with access to the Internet to explore the Web site of the Human Genome Project at http://genomics.energy.gov/.*

Extensions

- Students can research the human prion diseases: Creutzfeldt-Jakob disease (CJD), kuru, Gerstmann-Sträussler-Scheinker syndrome (GSS) and Familial Fatal Insomnia (FFI).
- Students can visit the Protein Data Bank Web site at

  http://www.rcsb.org/

  and view the structure of normal and mutated human prion proteins.

Key

1. What amino acid is encoded by PRNP E200? ***Glutamate***

2. What amino acid is encoded by PRNP E200K? ***Lysine***

3. How are the chemical properties of these two amino acids different? ***Glutamate has a negative charge and is acidic, and lysine has a positive charge and is basic.***

4. If 37 percent of the Caucasian population has two copies (are homozygous) for the PRNP M129 allele, and 12 percent are homozygous for PRNP M129V, what alleles do the other 51 percent of the Caucasian population have? ***The other 51 percent of the Caucasian population have one PRNP M129 allele and one PRNP M129V allele.***

5. If a TSE was linked to the PRNP M129V allele, what percent of the Caucasian population would be susceptible to the TSE? ***63 percent***

6. How can the same PRNP D178N mutation be linked to two different TSE diseases and cause two different sets of symptoms? ***The two different PRNP 129 polymorphisms probably have different effects on the final shape of the protein. The difference in the final protein shape may affect how the protein functions in different cells in different parts of the brain.***

Lesson 4

Student Pages

Cannibalism, Forgetfulness, and Sleepless Nights

You discovered in the last activity that a change in one nucleotide base in the DNA sequence can result in a changed message—one that might not make sense. In an organism, a change in one nucleotide base in the DNA sequence can result in a change in the sequence of amino acids that make a particular protein. Sometimes, the result is a protein that no longer makes sense—one that does not have the right shape or fold to do its job! Any change in DNA, which is a change in genetic information, is called a ***mutation***. The changes in the DNA are ***heritable***, that is, the mutated form of the gene is passed to the organism's offspring.

A mutation may or may not produce a noticeable change in the protein that is made. Here's why. Look at *Table 1. The Genetic Code*. There are 64 different codons in the genetic code, but only 20 different amino acids. So, some amino acids are encoded by more than one codon. For example, codons CUU, CUC, CUA, and CUG all code for the amino acid leucine! If one nucleotide in the DNA sequence changes, but the resulting triplet still matches a codon that makes the same amino acid, the protein will not be changed and the mutation will be ***silent*** or ***neutral***—it will do no harm.

Table 1. The Genetic Code

First Base (5' end)	Second Base: U	Second Base: C	Second Base: A	Second Base: G	Third Base (3' end)
U	UUU *Phe* UUC *Phe* UUA *Leu* UUG *Leu*	UCU *Ser* UCC *Ser* UCA *Ser* UCG *Ser*	UAU *Tyr* UAC *Tyr* UAA ***STOP*** UAG ***STOP***	UGU *Cys* UGC *Cys* UGA ***STOP*** UGG *Trp*	U C A G
C	CUU *Leu* CUC *Leu* CUA *Leu* CUG *Leu*	CCU *Pro* CCC *Pro* CCA *Pro* CCG *Pro*	CAU *His* CAC *His* CAA *Gln* CAG *Gln*	CGU *Arg* CGC *Arg* CGA *Arg* CGG *Arg*	U C A G
A	AUU *Ile* AUC *Ile* AUA *Ile* AUG *Met or Start*	ACU *Thr* ACC *Thr* ACA *Thr* ACG *Thr*	AAU *Asn* AAC *Asn* AAA *Lys* AAG *Lys*	AGU *Ser* AGC *Ser* AGA *Arg* AGG *Arg*	U C A G
G	GUU *Val* GUC *Val* GUA *Val* GUG *Val*	GCU *Ala* GCC *Ala* GCA *Ala* GCG *Ala*	GAU *Asp* GAC *Asp* GAA *Glu* GAG *Glu*	GGU *Gly* GGC *Gly* GGA *Gly* GGG *Gly*	U C A G

The Not So Silent Mutation

Mutations that alter a protein may cause disease in some organisms or increase the organism's susceptibility to certain illnesses. To determine the effects of any mutation, one must look at both the genetic code and the characteristics of the individual amino acids that result from the change. Take a look at both *Table 1. The Genetic Code* and *Table 2. Amino Acids and Their Chemical Properties*. Let's say that a mutation in a DNA strand resulted in an mRNA codon of CAU instead of CUU. That means that the amino acid histidine (coded for by CAU) would be placed in the amino acid chain instead of leucine (coded for by CUU). Histidine is a charged and hydrophilic amino acid that is usually found on the surface of a protein, while leucine is hydrophobic and would be found on the interior of a protein. This change would cause the protein to fold differently!

Table 2. Amino Acids and Their Chemical Properties

Amino Acid	*Abbreviation*	*One Letter Code*	*Chemical Properties*
Alanine	Ala	A (a)	Hydrophobic
Arginine	Arg	R (r)	Hydrophilic and basic (+ charge)
Asparagine	Asn	N (n)	Can link to a sugar; Hydrophilic
Aspartate	Asp	D (d)	Hydrophilic and acidic (- charge)
Cysteine	Cys	C (c)	Has a -SH group. Can strongly link to the -SH group of another cysteine, forming a disulfide bridge.
Glutamate	Glu	E (e)	Hydrophilic and acidic (- charge)
Glutamine	Gln	Q (q)	Moderately hydrophilic, polar
Glycine	Gly	G (g)	Small, can fit into places too small for larger amino acids
Histidine	His	H (h)	Hydrophilic and basic (+ charge)
Isoleucine	Ile	I (i)	Hydrophobic
Leucine	Leu	L (l)	Hydrophobic
Lysine	Lys	K (k)	Hydrophilic and basic (+ charge)
Methionine	Met	M (m)	Hydrophobic
Phenylalanine	Phe	F (f)	Hydrophobic
Proline	Pro	P(p)	Kinks or bends the amino acid chain
Serine	Ser	S (s)	Can link to a sugar; Hydrophilic
Threonine	Thr	T (t)	Can link to a sugar; Hydrophilic
Tryptophan	Trp	W (w)	Hydrophobic
Tyrosine	Tyr	Y (y)	Moderately hydrophilic, polar
Valine	Val	V (v)	Hydrophobic

Can a change in just one amino acid be that big of a problem? Yes! Sickle cell disease, which most often affects people of African descent, is caused by a single change in the gene for hemoglobin, the oxygen-carrying protein in red blood cells. Only one amino acid is changed at one position in the hemoglobin molecule, distorting the normally smooth, saucer-shaped red blood cells into jagged sickle shapes.

Another disease caused by a defect in one amino acid is cystic fibrosis. The deletion of a single amino acid in a protein called CFTR makes the protein fold incorrectly. The function of the CFTR protein is to allow chloride ions to pass through the outer membrane of cells. When this function is disrupted, glands that produce sweat and mucus are most affected. Thick, sticky mucus builds up in the lungs and digestive organs causing malnutrition, respiratory infections, and difficulties breathing.

Not all properties of amino acids would cause the resulting protein to change shape, but could be harmful anyway. For example, some amino acids are designed to link to sugar molecules.

These amino acids are an important part of immune system proteins. They help the cell identify foreign viruses or bacteria. Removing some of these amino acids from a protein may decrease an organism's immunity to foreign substances, while additions of them may cause the organism to wrongly attack its own cells as foreign.

Mutations and Prion Diseases

In order for researchers to determine whether mutations had any relationship to prion diseases, they first needed to know the sequence for a "normal" prion protein. Luckily, a lot of information was available through the ***Human Genome Project*** (HGP). In 1990, the government established the HGP to compile the entire genetic sequence of humans and other organisms. The goal of the project was to:

- *determine* the sequences of the 3 billion chemical base pairs that make up human DNA;
- *identify* all the approximately 20,000–25,000 genes in human DNA;
- *store* this information in databases;
- *improve* tools for data analysis.

Scientists from around the world worked together and completed the task in 13 years. The data from the human genome is still being analyzed, and researchers are comparing the structures of different proteins from different organisms.

The Human Prion Gene (PRNP)

Researchers identified the human prion gene (PRNP) and the entire sequence of amino acids that are

coded for by the gene. The translated portion of the gene has 253 codons, and so it codes for a protein with 253 amino acids. Scientists use a code to identify each amino acid in the sequence and also to identify any mutation in the complete prion protein. Every amino acid is numbered and identified by its single letter code. For example, all proteins, including the prion protein, begin with the amino acid methionine (met or M). So, the first amino acid in the prion protein is PRNP M1. The code PRNP D178 would mean that D, or aspartate, is the 178th amino acid in the polypeptide chain.

When a mutation occurs, the single letter code for the changed amino acid follows the code for the normal form of the amino acid. For example, PRNP D178N means that N, or asparagine, has replaced the usual D, or aspartate, as the 178th amino acid in the chain.

Approximately five to ten percent of all cases of human transmissible spongiform encephalopathies (TSEs) have been found to result from one or more mutations in the PRNP gene. One human TSE that has been extensively studied is Creutzfeldt-Jakob disease (CJD). Throughout the world, CJD affects about one person in a million. Some cases appear spontaneously in elderly persons and have no known cause. It has been discovered that some victims of CJD may have any of ten identified prion mutations, but the most common is PRNP E200K.

1. *What amino acid is encoded by PRNP E200?*

_____

2. *What amino acid is encoded by PRNP E200K?*

_____

3. *How are the chemical properties of these two amino acids different?*

_____

_____

_____

_____

Allele What?

Any one form of a gene is called an ***allele***. There are many forms of the PRNP gene. There are several forms of "normal" PRNP genes that are not associated with any prion disease. Other forms of the PRNP are linked with susceptibility to TSEs. Whether or not a person is susceptible to a TSE depends on which genes are expressed or made into proteins in the body. The ***dominant allele*** is the allele whose properties or characteristics are expressed in the organism. The ***recessive allele*** is the allele whose properties or characteristics are not expressed. It appears that alleles that are linked with susceptibility to TSEs are dominant, but there might not yet be enough data to say for sure.

Each person has two alleles for every gene, including the PRNP gene, one from each parent. A person could have two normal PRNP alleles, two PRNP alleles linked to a TSE, or one of each. When a person has two of the *same* PRNP alleles, they are ***homozygous*** for that allele. So, a person who has two "normal" PRNP alleles is homozygous for "normal" PRNP alleles. A person who has two TSE-associated PRNP alleles is homozygous for those alleles. A person who has one normal PRNP allele and one TSE-associated PRNP allele has two *different* alleles and is ***heterozygous*** for the PRNP gene.

4. *If 37 percent of the Caucasian population has two copies (are homozygous) for the PRNP M129 allele, and 12 percent are homozygous for PRNP M129V, what alleles do the other 51 percent of the Caucasian population have?*

5. *If a transmissible spongiform encephalopathy was linked to the PRNP M129V allele, what percent of the Caucasian population would be susceptible to the TSE?*

Poly Who?

Sometimes, there are two or more sequences of DNA (and the resulting protein) that are widespread in a population. For instance, 88 percent of the Japanese population has PRNP E219 (glutamate as amino acid 219) and the remaining 12 percent have PRNP K219 (lysine as amino acid 219). The existence within a population of two or more genetically different forms of a protein is known as a ***polymorphism***. Another common and normal polymorphism is the one described in question 4—the polymorphism of amino acid 129 in the PRNP gene. Interestingly, both the polymorphism of amino acid 219 and the polymorphism of amino acid 129 may influence susceptibility to human TSEs.

A Family Affair

Diseases that are inherited are called ***familial***—they are passed on in families. The forms of CJD disease that can be passed through families are called familial CJD disease or fCJD. There are two other closely related familial human prion diseases, Gerstmann-Sträussler-Scheinker syndrome (GSS) and Familial Fatal Insomnia (FFI). These two diseases have symptoms very similar to CJD. Like CJD, both FFI and GSS are dominant disorders—a person having one allele for the disease will get the symptoms.

FFI is a rare prion disease that has been found in only 28 families worldwide. FFI affects the thymus, a region of the brain responsible for sleep. As the disease progresses, the patient's insomnia worsens into complete sleeplessness. FFI is untreatable and ultimately fatal.

It appears that FFI only occurs in persons who have both the PRNP M129 polymorphism and a mutation at amino acid 178—PRNP D178N. N, or asparagine, has replaced the usual D or aspartate as the 178th amino acid in the chain.

Some individuals with CJD also have the PRNP D178N mutation. These CJD victims have the M129V polymorphism. CJD affects the cerebral part of the brain and causes memory loss, jerky movements, rigid posture, and seizures.

6. How can the same PRNP D178N mutation be linked to two different TSE diseases and cause two different sets of symptoms?

The "Family" Dinner

Sporadic CJD was first described by two German neurologists, Hans Gerhard Creutzfeldt and Alfons Maria Jakob in the early 1920s. More than a decade later, in 1936, Austrian neurologists Josef Gerstmann, Ernst Sträussler, and I. Scheinker, detailed their findings on Gerstmann-Sträussler-Scheinker syndrome (GSS). Family records of the victims of GSS showed a history of seemingly related deaths. It appeared to most doctors studying these unusual neurological diseases that most human TSE diseases were either sporadic (occurring at random) or inherited. The disease that changed this perception was kuru, the "laughing death" of the South Fore tribe in New Guinea. Kuru is a progressive, fatal brain malady that robs its victims of the ability to walk, talk, and even eat.

Kuru was first noticed in New Guinea in the early 1900s and the "laughing death epidemic" reached its peak in the early 1960s. Between 1957 and 1968, over 1,100 South Fore people died from kuru. The vast majority of the victims were women. Eight times more women than men contracted the disease! It seemed to affect small children and the elderly at a high rate as well.

Anthropologists who studied the disease in the 1950s first thought that it was a genetic disorder, because it had a tendency to occur among family members. Most scientists did not think kuru could be solely a genetic disorder, because kuru was too common and always fatal.

Then, veterinary pathologist William Hadlow observed similarities between kuru and scrapie. Both diseases caused trembling, loss of coordination and certain death. Like scrapie, kuru produced a microscopic "Swiss-cheesing" of the brain. Since scrapie was an infectious disease, Hadlow believed that kuru was also infectious. But how was the disease transmitted?

Shirley Lindenbaum, another researcher, pinpointed a probable cause. Female villagers honored the death of close relatives—even kuru victims—by eating them. When an individual died, the female kin were responsible for the dismemberment of the corpse. The women would feed portions of the brains and various organs to their children and the elderly.

In 1966, Carleton Gajdusek and Michael Alpers at the U.S. National Institutes of Health tested Lindenbaum's idea. They transmitted kuru to chimpanzees by injecting them with infected brain tissue, proving that the cause was not genetic. They thought it was a slow virus and began focusing attention on prevention. Ritual cannibalism was outlawed.

Although kuru was later found to be caused by prions, and not a slow virus, prevention has worked. Generations born after the ban are kuru-free. Older people in the region are still "getting" kuru. All of the current victims were born before 1950, meaning that the incubation period for some of the victims was over 40 years!

Kuru research has been important to the study of TSEs, because it demonstrated that prion diseases can be transmitted by eating infected tissues. It also demonstrated that the disease could be transmitted to genetically similar species. Today, researchers are using this knowledge and genetic research from the Human Genome Project to find answers to questions about CWD. Their work will be the topic of our next lesson.

Lesson 5

Educator's Overview

Oh…Deer

Summary

Students read an article that chronicles the discovery of chronic wasting disease and the disease's emergence as a public concern. They then compare the 14 domains of the prion protein sequence for nine mammalian species and predict the susceptibility of each species to different prion diseases.

Duration

One or two 45-minute class periods

Vocabulary

Biopsy

Prion or "protein only" hypothesis

Illinois Learning Standards

science: 11.A.4a, 11.A.5a, 12.A.4a, 12.A.4b, 12.A.4c, 12.A.5a, 12.A.5b, 12.B.4a, 12.B.4b, 12.B.5b

Learning Objectives

After completing this activity, students will be able to:

- Compare the prion protein sequences of nine mammalian species.
- Hypothesize the susceptibility of different species to different prion diseases and provide support for their prediction.
- Evaluate the relative importance of numbers of variations in prion sequence or specific polymorphisms to predicting susceptibility to prion diseases.

Background

Chronic wasting disease (CWD), a fatal disease of deer, elk, and moose, has been found in Colorado since the late 1960s. It didn't garner headlines until the mid-1990s, when British health officials linked eating food products from cattle affected with bovine spongiform encephalopathy (BSE) to a new human transmissible spongiform encephalopathy (TSE) called variant Creutzfeldt-Jakob disease (vCJD). The perception was that since both CWD and BSE were the same type of disease, humans could potentially acquire a TSE from eating meat from deer or elk affected with CWD.

This lesson examines the types of information that scientists review to predict transmission of prion diseases from one species to another. Students will compare the 14 domains of the prion protein sequence for nine mammalian species and predict the susceptibility of each species to different prion diseases. They will try to identify crucial portions of the prion protein to protein function, as well as identify domains that seem associated with susceptibility or resistance to prion disease. They will hypothesize whether the numbers of variations between species or specific polymorphisms are a more likely predictor of TSE transmission between species.

Teaching Strategies

1. Thoroughly read the student materials for *Oh…Deer*.

2. Give each student a copy of the Student Pages *Oh…Deer* and *Prion Amino Acid Sequences by Protein Domain*. Students may also need to refer back to the reading and tables in Lesson 4 *Cannibalism, Forgetfulness, and Sleepless Nights*.

3. Allow students to read the article silently on their own, in groups, or aloud as a class.

4. Review the concepts of domain and polymorphism. Make sure that students understand what these terms mean and how to identify them in a protein sequence.

5. This activity involves comparison of many gene sequences. You may want to assign the students to groups and have each group compare one to three of the domains, depending on the number of amino acids in each domain. Then, students can compile the data as a class. Because this comparison requires so much attention to detail, it would be wise to have at least two groups independently compare the prion sequences for each domain. Alternatively, you may want to allow additional class time or assign homework if you wish each student to complete the comparison individually.

6. Provide scissors. Tell students that the easiest way to compare prion sequences is to separate the species in each domain and compare them in the pairs requested in the table.

7. After each student has completed their assigned task, compile the data table.

8. Ask students to complete their questions individually or in pairs.

9. Discuss student answers in class. Allow time to probe students' scientific reasoning. Can students point to evidence that supports their answers? Can students think of counter examples? What assumptions are they making? What might they do to gather further evidence for their predictions?

Assessment

Students' ability to answer questions 1 through 12 using data that they have compiled from comparing prion protein sequences serves as the assessment for this lesson.

Extensions

- Using the same procedures used in *Oh…Deer*, students can compare the possibility of transmission of other mammalian spongiform encephalopathies to various species. They can examine the role of polymorphism in the transmission or susceptibility of animals to various prion diseases. There are prion protein sequences for 53 species listed by domain available at this Web site:

  http://www.cyber-dyne.com/~tom/protein_domains.html

  Students will need to research the scientific name of the species to use this Web site, but that information is readily available.

Materials and Preparation

- *Student Pages:* Oh…Deer—*one photocopy per student*
- *Student Activity Pages:* Prion Amino Acid Sequences by Protein Domain—*one photocopy per student*

Scissors—one pair per student

Three other mammalian transmissible encephalopathies have been linked with consuming feed containing either BSE-infected cattle or scrapie-infected sheep. They are transmissible mink encephalopathy (TME), feline spongiform encephalopathy (FSE), and exotic ungulate encephalopathy (EUE).

TME is rare, and has largely been confined to the United States, although incidents have also occurred in Canada, Finland, East Germany and Russia. TME takes the form of a rapidly evolving epidemic, usually involving single mink farms, and appears not to spread from mother to offspring. The disease is fatal and appears to be associated with the feeding of contaminated food (containing BSE-infected cattle or scrapie-infected sheep) and perpetuated through cannibalism among mink.

FSE was first reported at the Bristol Veterinary College in Great Britain. FSE has been found in domestic cats and captive wild cats, including tigers, puma, an ocelot and a cheetah; all ingested infected feedstuffs. Researchers are testing to see if mountain lions can contract FSE from eating meat from CWD-infected deer.

EUE has been seen in captive nyala, gemsbok, Arabian oryx, eland, kudu, scimitar-horned oryx, ankole, and bison. All of these animals are in the same taxonomic family as cattle. All of these animals were in zoos and were fed either BSE-infected cattle or scrapie-infected sheep.

BSE prions from cattle can be experimentally transmitted to cats, mink, mice, pigs, sheep, goats, marmosets and cynomolgus monkeys.

Students can also construct a timeline of events that chronicles the discovery and research findings of the various TSE diseases.

Table 1. Comparison of Prion Protein Domain for Select Species

Species Compared	Number of Potential Differences in Each Prion Protein Domain														Total Number of Differences
	Signal	Pre-repeat	Octa. repeat	Core	Beta 1	Loop 1	Alpha 1	Loop 2	Beta 2	Loop 3	Alpha 2	Asn	Alpha 3	Pre-GPI	
Odocoileus virginianus & Odocoileus hemionus hemionus															0
Odocoileus virginianus & Cervus elaphus nelsoni														1	1
Odocoileus virginianus & Alces alces shirasi															0
Odocoileus virginianus & Ovis aries		1		1						2			1		5
Odocoileus virginianus & Bos taurus						1		1		2	1				5
Odocoileus virginianus & Homo sapiens	9	1	1	2		2		1		3	1		1	4	25
Ovis aries & Homo sapiens	9	1	1	3		2		1		1	1		2	4	25
Ovis aries & Bos taurus		1		1		1		1			1		1		6
Bos taurus & Homo sapiens	9	1	1	2		1				1	2		1	4	22
Mus musculus & Homo sapiens	7		2	1		3	1	1		2			1	7	25
Pan troglodytes & Homo sapiens										1					1

Key

1. What prion domains seem to have the most changes or variations in amino acids among the species? ***The domains that have a lot of variation among species include signal, pre-repeat, octa-repeat, core, loop 1, loop 2, loop 3, alpha 2, alpha 3, and pre-GPI.***

2. Which prion domains seem to have the least changes or variations among species? ***Beta 1, alpha 1, beta 2, and asn domains have the least changes or variations among the species.***

3. Usually, when there are few changes in amino acids in the prion domains among species, it means that those portions of the protein are most essential for protein function. Which domains seem most important in prion protein function? ***Beta 1, alpha 1, beta 2, and asn domains seem to be the most important to prion protein function.***

4. Researchers hypothesize that species with similar prion proteins are more susceptible to each other's prion diseases. White-tailed deer and mule deer are very susceptible to CWD. What other species might be more susceptible to transmission of CWD from white-tailed deer? ***According to this hypothesis, the North American moose prion sequence is identical to the white-tailed deer prion sequence, and moose might be more susceptible to the transmission of CWD. The next most susceptible species might be the elk.***

5. Check the Colorado Division of Wildlife Web site.

http://wildlife.state.co.us

Has CWD been found in this species (your answer to question 4)? When? ***In September 2005, a male moose (Alces alces) harvested on the west side of the Never Summer Range in northcentral Colorado was diagnosed with CWD.***

6. People have reported scrapie in sheep for over 300 years. Not all sheep get scrapie. Veterinarians looked at the prion gene sequences of sheep with scrapie and sheep that were resistant to scrapie. They found many polymorphisms—different forms of genes—in sheep prions for amino acids number 171 and 136. Sheep that had polymorphisms that coded for the amino acid arginine (R) as the 171st amino acid in the protein, and for alanine (A) as the 136th amino acid in the protein chain, seemed resistant to getting scrapie. However, sheep that had prion genes that coded for glutamine (Q) or histidine (H) as the 171st amino acid in the prion protein chain, or that had valine (V) as the 136th amino acid in the chain, were likely to get scrapie. In what domains of the prion protein are these polymorphisms found? ***These polymorphisms are found on loop 1 and loop 3.***

7. So far, there is no recorded case of a human contracting a prion disease from eating meat products from domestic sheep. Comparing the prion proteins for humans (*Homo sapiens*) and sheep (*Ovis aries*), is this event likely? Explain your answer. ***There are 25 differences in the prion sequences of domestic sheep and humans, so this event does not seem likely.***

8. Some researchers believe that cattle (*Bos taurus*) contracted bovine spongiform encephalopathy (BSE) after consuming meat and bone meal from scrapie-infected sheep (*Ovis aries*). Other scientists believe that BSE is a sporadic disease that was amplified or made worse from consuming the scrapie-contaminated feed. Comparing the prion proteins data for the two species, are either of these events likely? Explain your answer. ***There are only six differences in the polymorphisms of these prion sequences of domestic sheep and cattle, so the events seem likely.***

9. Researchers also believe that humans (*Homo sapiens*) who have variant Creutzfeldt-Jakob disease (vCJD) acquired the disease from eating meat products from BSE-infected cattle (*Bos taurus*). Comparing the data prion proteins for the two species, is this event likely? Explain your answer. ***There are 22 differences in the prion protein sequences of humans and cattle, so this event seems unlikely.***

10. So far, all human vCJD patients have two copies of PRNP M129 (methionine as amino acid #129 in the protein). It appears that being heterozygous or homozygous for PRNP M129V (valine as amino acid 129 in the protein) provides some resistance to getting vCJD. *On what domain of the human prion does this polymorphism occur?* ***This polymorphism occurs on the beta 1 domain of the human prion protein.***

11. A recent study was conducted to determine the effect of a polymorphism on the appearance of clinical CWD symptoms in mule deer. The study compared mule deer that were homozygous for the serine (S) codon for amino acid 225 (that is the deer were 225SS) with mule deer that were heterozygous for serine (S) and

phenylalanine (F) at codon 225 (deer that were 225SF). The results of the study were that the 225SS mule deer showed clinical signs of CWD before the 225SF deer did. What results would you predict for mule deer that were homozygous for phenylalanine (F) at codon 225—the 225FF deer? How could you test your prediction? ***Students may suggest that the appearance of clinical CWD in 225FF deer is delayed even longer or that these deer are resistant to CWD. Any study suggested should include testing deer with all three genotypes (225SS, 225SF, and 225FF).***

12. Thinking about your answers to questions 6 through 11, which seems more important in predicting the transmission of a prion disease, the number of variations in the prion genes of the species or specific polymorphisms? Support your answer. ***Specific polymorphisms seem more important in predicting the transmission of a prion disease. There are 22 differences between cattle and human prion proteins, but there are some cases of humans getting vCJD from eating meat products from BSE cattle. On the other hand, humans with the PRNP M129V polymorphism seem to be resistant to vCJD. Specific polymorphisms (R171 and A136) seem to provide sheep with resistance to scrapie. So, individual polymorphisms seem to be more important.***

13. Human diseases are often studied using other animals, particularly mice (*Mus musculus*). According to your data in *Table 1. Comparison of Prion Protein Domain for Select Species*, which species has the closest prion protein sequence to humans? Why is the mouse used to study prion diseases instead? ***There is only one difference in the prion protein sequence of chimpanzees and humans, so that would be the best species to study human prion diseases. There are 25 differences in the prion protein sequences of humans and mice. However, using chimpanzees would be more expensive and more difficult than using mice for medical research because chimpanzees are larger, have a longer life span, fewer offspring, and are less numerous. Plus, many people would consider using chimpanzees unethical, because of their closeness to humans, their intelligence, and their rarity in the world.***

Deer Mouse

Lesson 5

Student Pages

Oh...Deer

No one knows for sure where or how chronic wasting disease (CWD) started. It was first noticed by researchers at a Colorado State University (CSU) research facility in 1967. Captive mule deer that were being maintained for nutritional studies began to lose weight as young adults. They later became listless, started slobbering and drooling, and seemed constantly thirsty. Some stopped socializing with other deer. The affected deer died within months of the appearance of these symptoms.

At first, researchers thought that the illness, named "chronic wasting disease" because of the symptoms, was caused from nutritional deficiencies, poisoning, or stress from confinement. However, CWD seemed highly contagious. In the 1970s, 90 percent of the deer that stayed more than two years in the research facility died or had to be euthanized.

In 1980, CWD was first noticed in deer at Wyoming's Sybille Research Unit, about 120 miles northwest of Colorado's facility. Wyoming had sent deer to Colorado for breeding purposes. To make matters worse, elk at both facilities contracted the disease. It was becoming clear that CWD was an infectious disease, but what caused it?

Student Discovers New Disease

In 1977, Dr. Elizabeth Williams, studying for her doctorate in veterinary pathology at CSU, made an extraordinary discovery. She decided to look at tissue samples from the brains of some of the "wasting" deer. She saw that the tissue was full of microscopic holes. She shared her findings with Dr. Stuart Young, a neuropathologist at CSU. The holes were unmistakably like scrapie, the sheep sickness that was the first documented spongiform encephalopathy. Elizabeth Williams and Stuart Young had discovered a new transmissible spongiform encephalopathy (TSE).

Scientist Proposes New Agent of Disease

Before 1982, most researchers believed that TSEs were some sort of genetic disorder, occurred spontaneously, or were caused by a slow-incubating virus. Then, Stanley Prusiner of the University of California at San Francisco proposed the ***prion*** or ***"protein only" hypothesis***—that a misfolded protein could become a pathogenic entity that kills. Prusiner's ideas set off a storm of criticism in the scientific community. At the time, no self-respecting biologist believed that a pathogen could replicate and pass on its traits without assistance from nucleic acids (DNA or RNA).

While it is only natural for scientists to be skeptical of new ideas that do not fit within the accepted realm of scientific knowledge, Prusiner became the target of vicious personal attacks in the media. Still,

he continued his research. It would take many years for Prusiner and others to gather enough evidence to convince the scientific community that some proteins could in fact copy themselves. By the early 1990s, the existence of prions was coming to be accepted in many quarters of the scientific community, and in 1997, Prusiner would receive the Nobel Prize in Physiology/Medicine. Once again, as throughout history, scientists who press on in spite of criticism, often contribute the most to our body of knowledge.

Meanwhile

Dr. Elizabeth Williams graduated with her doctorate in Veterinary Pathology and went to work for the University of Wyoming in Laramie. She continued researching CWD. Michael Miller, veterinarian for the Colorado Division of Wildlife, later joined in these efforts. Initially, the disease seemed to be confined to deer and elk at Colorado's Foothills Wildlife Research Facility and Wyoming's Sybille Research Unit. For over a decade, only veterinarians, infectious disease specialists, and neurologists knew anything about CWD and other prion diseases. The diseases didn't grab any headlines. That would eventually change.

1985

By 1985, a few cases of CWD had been diagnosed in free-ranging deer and elk. Researchers began to wonder whether the disease originated in the wild and spread to the captives or vice versa. The original stock for both research facilities came from the wild. The wild and captive populations had plenty of time to mingle. Wild deer and elk often lingered around captives, especially during the mating season. Either way, state wildlife officials were concerned about maintaining the health of the wild herds.

That same year, a handful of cattle from various areas in the United Kingdom began dying of a strange illness. Examination of the dead cattle's brains revealed abnormal, microscopic holes—the same spongy appearance of scrapie in sheep, kuru in the Fore people, and CWD in deer and elk. The new disease was named bovine spongiform encephalopathy, or BSE.

The "Mad Cow" Epidemic

BSE quickly ballooned into an epidemic that struck more that 37,000 cattle per year by 1992. Scientists studying the human genome had identified the human prion gene, and many prion genes for other species. They proposed a cause of the new illness. Feed producers in Britain used parts of dead animals to boost protein content in their products. Body parts of sheep that had died from scrapie were used for cattle feed.

By the time the full extent of the epidemic was known, many BSE-infected cattle entered the human food chain. In fact it has been estimated that 840,000 to 1.25 million infected cattle became hamburger or roast or other meat products. British health officials, concerned that the transmission of BSE to humans was a possibility, increased surveillance of Creutzfeldt-Jakob disease (CJD). CJD normally occurs in older people, but in 1996 a few teenagers in the United Kingdom started showing CJD symptoms. ***Biopsies*** (the taking of tissue for examination) of brain tissue of these victims revealed that a new variant, vCJD,

involved the same prion strain as seen in BSE cases in cattle.

Newspapers, television stations, and radio programs all carried the story. Panic set in worldwide. Hundreds of thousands of cattle were put down.

Oh Deer, Oh Deer, Oh Deer!

With the discovery of BSE transmission to humans, CWD and other prion diseases came under the microscope. Since both CWD and BSE are TSEs, and if cattle affected with BSE could cause new variant CJD, then why couldn't eating meat from deer or elk affected with CWD cause human disease? Could CWD spread to other wildlife species? Could CWD spread to livestock?

First Steps

The first step that scientists take when trying to find answers to new questions is to compile all the research that has ever been done on the topic. The researchers who were concerned that CWD might be transmissible to other species, especially to humans, gathered all known research on prion diseases in animals and humans. This is a brief list of some of the facts known about prion diseases in 1996:

A. *Some prion diseases seem to arise spontaneously.*

B. *Prion disease can be transmitted by eating infected tissue.* Kuru victims contracted the disease by eating tissue from deceased relatives. Cattle may have contracted BSE by eating tissue from scrapie-infected sheep. Humans probably contracted vCJD by eating tissue from BSE-infected cattle.

C. *Prion diseases can be transmitted to genetically similar species.* Chimpanzees were easily infected with kuru from human victims.

D. *Prion diseases can be transmitted to genetically-dissimilar species under certain circumstances.* Some cattle that ate feed containing sheep parts developed BSE; some humans that consumed BSE-contaminated meat developed vCJD.

E. *In the hundreds of years that scrapie has been infecting sheep, it appeared that no human had acquired a prion disease by eating sheep meat.*

F. *Sheep with scrapie were contagious to other sheep and seemed to transmit the disease through contact with each other.*

G. *Prions were hard to destroy. Normal sterilization methods that eradicate bacteria and viruses did not work.*

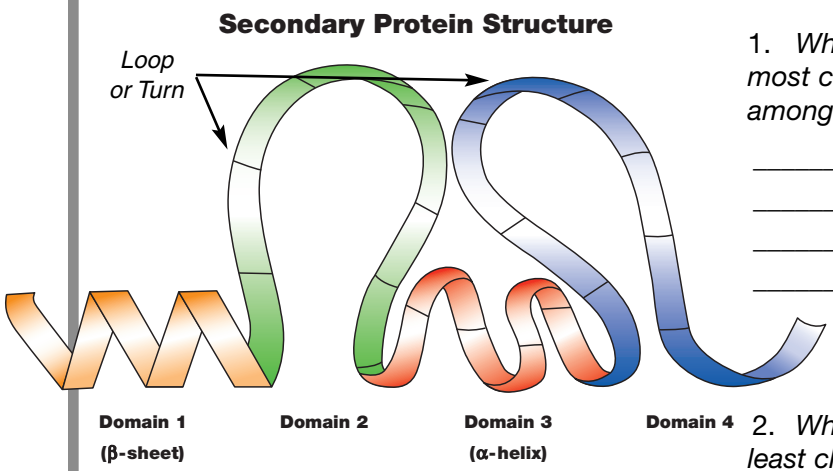
Comparing Prion Sequences

Later, researchers would compile and compare the amino acid sequences produced by the prion genes from as many species as possible. Each sequence was presented in a standard manner, according to the human prion (PRNP) sequence, so that researchers could easily compare the similarities and differences between species. By 2003, many sequences for the deer family had been sorted out. As in humans, many polymorphisms of the prion gene were found in each species.

The researchers compared the prions by protein domain. Remember that a domain is a segment of the polypeptide chain that folds into a recognizable shape.

The prion protein has 14 domains. The first domain, the signal sequence, contains the "start" code and also identifies the final destination of the prion protein in the cell. The last domain, the pre-GPI, adds a fat molecule to the prion protein to anchor it to the cell membrane.

You are now going to take on the role of the CWD researcher and compare the prion sequences from nine species. Keep in mind that you will only be comparing one of the prion polymorphisms of each species. You will be given *Prion Amino Acid Sequences by Protein Domain*. The amino acids in each sequence are represented by their one letter code. The 14 domains of the prion sequences are arranged in order, and the number in parentheses before each domain indicates the starting amino acid of the sequence. Some species have a greater or lesser number of amino acids in each domain. When a species doesn't have an amino acid in a certain place in the sequence, the space is marked with a dash (-) so that the domains of each species can be easily compared.



Your instructor will tell you how many prion domains you or your group will compare. Compare the prion domains for two species at a time. Count the number of differences in the amino acids in each domain and enter your answer in the correct row and column of *Table 1. Comparison of Prion Protein Domain for Select Species*.

Example: If you compare the "signal" domain for white-tailed deer and humans, you would find 9 differences:

Species	***(1) signal***
Odo.vir	m v k s h i g s w i l v l f v a m w s d v g l c
Hom.sap	m a n l - - g c w m l v l f v a t w s d l g l c

The answer "9" has been placed in the correct box in *Table 1. Comparison of Prion Protein Domain for Select Species* for you.

After you have completed the table, answer these questions:

1. *What prion domains seem to have the most changes or variations in amino acids among the species?*

2. *Which prion domains seem to have the least changes or variations among species?*

Table 1. Comparison of Prion Protein Domain for Select Species

Species Compared	Number of Potential Differences in Each Prion Protein Domain														Total Number of Differences
	Signal	Pre-repeat	Octa. repeat	Core	Beta 1	Loop 1	Alpha 1	Loop 2	Beta 2	Loop 3	Alpha 2	Asn	Alpha 3	Pre-GPI	
Odocoileus virginianus & Odocoileus hemionus hemionus															
Odocoileus virginianus & Cervus elaphus nelsoni															
Odocoileus virginianus & Alces alces shirasi															
Odocoileus virginianus & Ovis aries															
Odocoileus virginianus & Bos taurus															
Odocoileus virginianus & Homo sapiens	9														
Ovis aries & Homo sapiens															
Ovis aries & Bos taurus															
Bos taurus & Homo sapiens															
Mus musculus & Homo sapiens															
Pan troglodytes & Homo sapiens															

3. Usually, when there are few changes in amino acids in the prion domains among species, it means that those portions of the protein are most essential for protein function. *Which domains seem most important in prion protein function?*

4. Researchers hypothesize that species with similar prion proteins are more susceptible to each other's prion diseases. White-tailed deer and mule deer are very susceptible to CWD. *What species might be more susceptible to transmission of CWD from white-tailed deer?*

5. Check the Colorado Division of Wildlife Web site.

http://wildlife.state.co.us

Has CWD been found in this species (your answer to question 4)? When?

6. People have reported scrapie in sheep for over 300 years. Not all sheep get scrapie. Veterinarians looked at the prion gene sequences of sheep with scrapie and sheep that were resistant to scrapie. They found many polymorphisms—different forms of genes—in sheep prions for amino acids number 171 and 136. Sheep that had polymorphisms that coded for the amino acid arginine (R) as the 171st amino acid in the protein, and for alanine (A) as the 136th amino acid in the protein chain, seemed resistant to getting scrapie. However, sheep that had prion genes that coded for

glutamine (Q) or histidine (H) as the 171st amino acid in the prion protein chain, or that had valine (V) as the 136th amino acid in the chain, were likely to get scrapie. *In what domains of the prion protein are these polymorphisms found?*

7. So far, there is no recorded case of a human contracting a prion disease from eating meat products from domestic sheep. Comparing the prion proteins for humans (*Homo sapiens*) and sheep (*Ovis aries*), *is this event likely?* Explain your answer.

8. Some researchers believe that cattle (*Bos taurus*) contracted bovine spongiform encephalopathy (BSE) after consuming meat and bone meal from scrapie-infected sheep (*Ovis aries*). Other scientists believe that BSE is a sporadic disease that was amplified or made worse from consuming the scrapie-contaminated feed. Comparing the prion proteins data for the two species, *are either of these events likely?* Explain your answer.

9. Researchers also believe that humans (*Homo sapiens*) who have variant Creutzfeldt-Jakob disease (vCJD) acquired the disease from eating meat products from BSE-infected cattle (*Bos taurus*). Comparing the prion proteins data for the two species, *is this event likely?* Explain your answer.

10. So far, all human vCJD patients have two copies of PRNP M129 (methionine as amino acid #129 in the protein). It appears that being heterozygous or homozygous for PRNP M129V (valine as amino acid 129 in the protein) provides some resistance to getting vCJD. *On what domain of the human prion does this polymorphism occur?*

11. A recent study was conducted to determine the effect of a polymorphism on the appearance of clinical CWD symptoms in mule deer. The study compared mule deer that were homozygous for the serine (S) codon for amino acid 225 (that is the deer were 225SS) with mule deer that were heterozygous for serine (S) and phenylalanine (F) at codon 225 (deer that were 225SF). The results of the study were that the 225SS mule deer showed clinical signs of CWD before the 225SF deer did. *What results would you predict for mule deer that were homozygous for phenylalanine (F) at codon 225—the 225FF deer? How could you test your prediction?*

12. Thinking about your answers to questions 6 through 11, *which seems more important in predicting the transmission of a prion disease, the number of variations in the prion genes of the species or specific polymorphisms?* Support your answer.

13. Human diseases are often studied using other animals, particularly mice (*Mus musculus*). According to your data in *Table 1. Comparison of Prion Protein Domain for Select Species*, *which species has the closest prion protein sequence to humans? Why is the mouse used to study prion diseases instead?*

Key to Species

Abbreviation	***Scientific Name***	***Common Name***
Alc.alc	*Alces alces shirasi*	North American moose
Bos.tau	*Bos taurus*	domestic cow
Cer.ela	*Cervus elaphus nelsoni*	elk
Hom.sap	*Homo sapiens*	human
Mus.mus	*Mus musculus*	house mouse
Odo.hem	*Odocoileus hemionus hemionus*	mule deer
Odo.vir	*Odocoileus virginianus*	white-tailed deer
Ovi.ari	*Ovis aries*	domestic sheep
Pan.tro	*Pan troglodytes*	chimpanzee

Prion Amino Acid Sequence

Species	*(1) signal*
Alc.alc	m v k s h i g s w i l v l f v a m w s d v g l c
Bos.tau	m v k s h i g s w i l v l f v a m w s d v g l c
Cer.ela	m v k s h i g s w i l v l f v a m w s d v g l c
Hom.sap	m a n l - - g c w m l v l f v a t w s d l g l c
Mus.mus	m a n l - - g y w l l a l f v t m w t d v g l c
Odo.hem	m v k s h i g s w i l v l f v a m w s d v g l c
Odo.vir	m v k s h i g s w i l v l f v a m w s d v g l c
Ovi.ari	m v k s h i g s w i l v l f v a m w s d v g l c
Pan.tro	m a n l - - g c w m l v l f v a t w s d l g l c

Mule Deer

Species	*(23) pre repeat*
Alc.alc	k k r p k p g g g w n t g g s r y p g q g s p g g n r y p
Bos.tau	k k r p k p g g g w n t g g s r y p g q g s p g g n r y p
Cer.ela	k k r p k p g g g w n t g g s r y p g q g s p g g n r y p
Hom.sap	k k r p k p g g - w n t g g s r y p g q g s p g g n r y p
Mus.mus	k k r p k p g g - w n t g g s r y p g q g s p g g n r y p
Odo.hem	k k r p k p g g g w n t g g s r y p g q g s p g g n r y p
Odo.vir	k k r p k p g g g w n t g g s r y p g q g s p g g n r y p
Ovi.ari	k - r p k p g g g w n t g g s r y p g q g s p g g n r y p
Pan.tro	k k r p k p g g - w n t g g s r y p g q g s p g g n r y p

Species	*(51) octapeptide repeat*
Alc.alc	p q g g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g g w g q
Bos.tau	p q g g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g g w g q
Cer.ela	p q g g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g g w g q
Hom.sap	p q g g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g - w g q
Mus.mus	p q g g - t w g q . p h g g g w g q . p h g g s w g q . p h g g s w g q . p h g g g - w g q
Odo.hem	p q g g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g g w g q
Odo.vir	p q g g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g g w g q
Ovi.ari	p q g g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g g w g q
Pan.tro	p q g g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g w g q . p h g g g - w g q

Prion Amino Acid Sequence

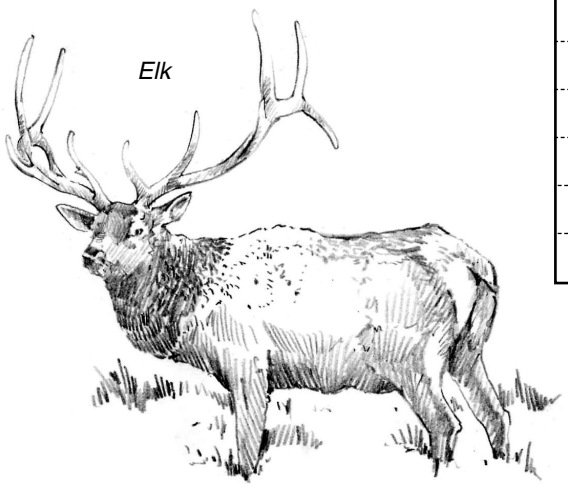
Species	(92) Core
Alc.alc	g g - t h s q w n k p s k p k t n m k h v a g a a a a g a v v g g l g g
Bos.tau	g g - t h s q w n k p s k p k t n m k h v a g a a a a g a v v g g l g g
Cer.ela	g g - t h s q w n k p s k p k t n m k h v a g a a a a g a v v g g l g g
Hom.sap	g g g t h s q w n k p s k p k t n m k h m a g a a a a g a v v g g l g g
Mus.mus	g g g t h n q w n k p s k p k t n m k h m a g a a a a g a v v g g l g g
Odo.hem	g g - t h s q w n k p s k p k t n m k h v a g a a a a g a v v g g l g g
Odo.vir	g g - t h s q w n k p s k p k t n m k h v a g a a a a g a v v g g l g g
Ovi.ari	g g - s h s q w n k p s k p k t n m k h v a g a a a a g a v v g g l g g
Pan.tro	g g g t h s q w n k p s k p k t n m k h m a g a a a a g a v v g g l g g

Species	(128) beta 1
Alc.alc	Y M L G
Bos.tau	Y M L G
Cer.ela	Y M L G
Hom.sap	Y M L G
Mus.mus	Y M L G
Odo.hem	Y M L G
Odo.vir	Y M L G
Ovi.ari	Y M L G
Pan.tro	Y M L G

Species	(132) loop 1
Alc.alc	s a m s r p l i h f g n
Bos.tau	s a m s r p l i h f g s
Cer.ela	s a m s r p l i h f g n
Hom.sap	s a m s r p i i h f g s
Mus.mus	s a m s r p m m h f g n
Odo.hem	s a m s r p l i h f g n
Odo.vir	s a m s r p l i h f g n
Ovi.ari	s a m s r p l i h f g n
Pan.tro	s a m s r p i i h f g s

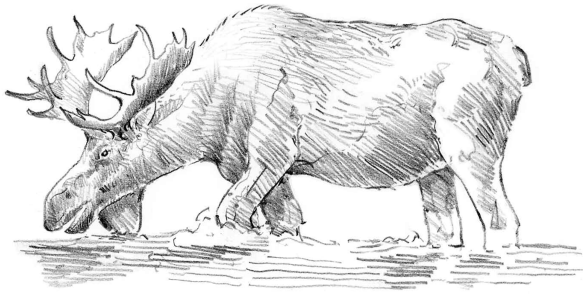
Species	(144) alpha 1
Alc.alc	D Y E D R Y Y R E N M
Bos.tau	D Y E D R Y Y R E N M
Cer.ela	D Y E D R Y Y R E N M
Hom.sap	D Y E D R Y Y R E N M
Mus.mus	D W E D R Y Y R E N M
Odo.hem	D Y E D R Y Y R E N M
Odo.vir	D Y E D R Y Y R E N M
Ovi.ari	D Y E D R Y Y R E N M
Pan.tro	D Y E D R Y Y R E N M

Species	(155) loop 2
Alc.alc	y r y p n q
Bos.tau	h r y p n q
Cer.ela	y r y p n q
Hom.sap	h r y p n q
Mus.mus	n r y p n q
Odo.hem	y r y p n q
Odo.vir	y r y p n q
Ovi.ari	y r y p n q
Pan.tro	h r y p n q



Elk

Prion Amino Acid Sequence



Moose

Species	(161) beta 2
Alc.alc	V Y Y R
Bos.tau	V Y Y R
Cer.ela	V Y Y R
Hom.sap	V Y Y R
Mus.mus	V Y Y R
Odo.hem	V Y Y R
Odo.vir	V Y Y R
Ovi.ari	V Y Y R
Pan.tro	V Y Y R

Species	(165) loop 3
Alc.alc	p v d q y n n q n t f v h d
Bos.tau	p v d q y s n q n n f v h d
Cer.ela	p v d q y n n q n t f v h d
Hom.sap	p m d q y s n q n n f v h d
Mus.mus	p v d q y n n q n n f v h d
Odo.hem	p v d q y n n q n t f v h d
Odo.vir	p v d q y n n q n t f v h d
Ovi.ari	p v d q y s n q n n f v h d
Pan.tro	p m d q y s s q n n f v h d

Species	(179) alpha 2
Alc.alc	C V N I T V K Q H T V T T T T
Bos.tau	C V N I T V K E H T V T T T T
Cer.ela	C V N I T V K Q H T V T T T T
Hom.sap	C V N I T I K Q H T V T T T T
Mus.mus	C V N I T I K Q H T V V T T T
Odo.hem	C V N I T V K Q H T V T T T T
Odo.vir	C V N I T V K Q H T V T T T T
Ovi.ari	C V N I T V K Q H T V T T T T
Pan.tro	C V N I T I K Q H T V T T T T

Species	(194) Asn
Alc.alc	k g e n f t
Bos.tau	k g e n f t
Cer.ela	k g e n f t
Hom.sap	k g e n f t
Mus.mus	k g e n f t
Odo.hem	k g e n f t
Odo.vir	k g e n f t
Ovi.ari	k g e n f t
Pan.tro	k g e n f t

Species	(200) alpha 3
Alc.alc	E T D I K - M M E R V V E Q M C I T Q
Bos.tau	E T D I K - M M E R V V E Q M C I T Q
Cer.ela	E T D I K - M M E R V V E Q M C I T Q
Hom.sap	E T D V K - M M E R V V E Q M C I T Q
Mus.mus	E T D V K - M M E R V V E Q M C V T Q
Odo.hem	E T D I K - M M E R V V E Q M C I T Q
Odo.vir	E T D I K - M M E R V V E Q M C I T Q
Ovi.ari	E T D I K - I M E R V V E Q M C I T Q
Pan.tro	E T D V K - M M E R V V E Q M C I T Q

Species	(218) pre GPI
Alc.alc	y q r e s q a y y q - r g a s - - v i l f
Bos.tau	y q r e s q a y y q - r g a s - - v i l f
Cer.ela	y q r e s e a y y q - r g a s - - v i l f
Hom.sap	y e r e s q a y y q - r g s s m - v - l f
Mus.mus	y q k e s q a y y d g r r s s s t v - l f
Odo.hem	y q r e s q a y y q - r g a s - - v i l f
Odo.vir	y q r e s q a y y q - r g a s - - v i l f
Ovi.ari	y q r e s q a y y q - r g a s - - v i l f
Pan.tro	y e r e s q a y y q - r g s s m - v - l f

Lesson 6

Educator's Overview

Here to Stay, Not Gone Tomorrow

Duration

One or two 45-minute class periods

Vocabulary

Autopsy

Cervids

Control group

Controlled experiment

Dependent variable

Epidemiologic studies

Epidemiologist

Euthanize

Excreta

Experiment

Immunology

Independent variable

Pharmacology

Wildlife ecology

Illinois Learning Standards

science: 12.A.4a, 12.A.4b, 12.A.4c, 12.A.5a, 12.A.5b, 12.B.4a, 12.B.4b, 12.B.5b, 13.A.4a, 13.A.5a, 13.A.4b, 13.A.5b, 13.A.4c, 13.A.5c, 13.A.4d, 13.A.5d, 13.B.4d

Summary

The first step in controlling and possibly eradicating chronic wasting disease (CWD) is to learn as much as possible about the transmission of the disease. Students read about three experiments that examine the transmission of CWD in deer, elk, and moose. They then consider how results from those studies influence wildlife managers in minimizing the disease's impact on wild, free-ranging deer, elk, and moose populations and protecting the public welfare. The activity focuses on efforts in Colorado because the disease has been studied longer there than in Illinois and affects at least three species. The information is applicable anywhere CWD is found.

Learning Objectives

After completing this activity, students will be able to:

- Identify the control group, independent variable, and dependent variable in three CWD research studies.
- Describe three or more actions that wildlife managers have taken to limit the spread of chronic wasting disease.
- Describe simple steps that a hunter can take to limit his or her exposure to prions from CWD-infected animals.

Background

Since the appearance of bovine spongiform encephalopathy (BSE) in the mid-1980s and, especially since the 1996 announcement of an apparent relationship between BSE and variant Creutzfeldt-Jakob disease (vCJD), there has been considerable media, public, and animal and human health agency interest in transmissible spongiform encephalopathies (TSEs) like CWD. Recent research demonstrates that CWD poses significant challenges for wildlife managers attempting to control or eradicate the disease. These challenges are the focus of this activity.

The three studies included in this activity show that: 1) CWD prions remain in the environment for a long time after infected deer, elk, and moose are gone; 2) prions from infected animals are transmissible to healthy animals through saliva and blood; 3) no tissue from CWD-infected cervids can be considered free of disease-causing prions; and 4) no one can predict with absolute certainty that CWD will never cause human disease.

Wildlife managers cannot completely control the spread of CWD and cannot entirely eradicate the disease. However, CWD can be managed. The second part of this activity details how the Colorado Division of Wildlife (DOW) has attempted to manage the disease to minimize its impact on wild, free-ranging deer, elk, and moose populations and also strives to help protect public welfare.

The spread of CWD can largely be prevented through several strategies used by the Colorado DOW. Managers selectively cull diseased animals to reduce the overall density of cervids (deer, elk, and moose) in the infected area and to slow the transmission of the disease. The agency also has an extensive surveillance program to examine hunter-harvested deer, elk, and moose and identify new areas of concern. Colorado has implemented regulations that only allow boned meat, quarters (without spinal column or head) or processed meat from hunter-harvested deer, elk or moose to be transported out of certain CWD areas.

To help protect the public, the DOW works with public health authorities to provide scientifically accurate information regarding CWD, as well as simple strategies to lower the risk of exposure to the disease. This information is continually updated and available on the DOW's Web site and in print materials. The agency informs all media outlets—print, radio, and television—of new discoveries or breakthroughs in management. Hunters are able to have their harvested animals tested for CWD, insuring that they can make informed decisions about consuming the meat.

Most importantly, DOW researchers and biologists are studying CWD on numerous fronts—addressing wildlife health issues and assisting public health experts and scientists with their ongoing research. It is anticipated that new research will provide greater insight into CWD and other TSEs, lead to the development of new diagnostic tests and possibly treatments, and continue to provide the basis for sound decision-making processes that protect animal and human health.

Teaching Strategies

1. Thoroughly read the student materials for *Here to Stay, Not Gone Tomorrow*. Visit the Colorado DOW Web site to become familiar with the information and materials related to CWD.

2. Decide whether you will be printing information from the DOW Web site or giving students access to the Internet to complete this activity. Prepare for the lesson accordingly.

3. Write these vocabulary words in a visible place: control group, dependent variable, and independent variable.

4. Ask students what the words pertain to and what they mean. Most students will recognize that these words have something to do with experimental design and the way scientists investigate questions and gain knowledge. Most scientific processes include collecting observations, asking questions about those observations, making predictions, designing experiments to test those predictions, and drawing some sort of conclusion from the results. The results often lead to still other questions.

5. Discuss the idea of a ***controlled experiment***—a planned procedure to test a hypothesis or prediction. A ***control group*** is a group in an experiment that receives no experimental treatment. The control and experimental groups are designed to be identical except for one factor or variable. The factor that is changed or is different in an experiment is called the ***independent variable***. The result, or the variable that is measured, is called the ***dependent variable***.

Materials and Preparation

- *Student Pages:* Here to Stay, Not Gone Tomorrow—*one photocopy per student*
- *Video:* Managing Chronic Wasting Disease
- *Access to the Internet*
- *Current* Colorado Big Game Regulations—*one copy per student of the 1–2 pages of information concerning Chronic Wasting Disease. A COPY can be downloaded from the DOW Web site at http://wildlife.state.co.us/RulesRegs/RegulationsBrochures/BigGame.htm.*

6. Tell students that the reading for this lesson will contain information from three experiments related to CWD. In addition to the questions that they will be asked in the activity, tell students that they should draw a chart like the one below on a sheet of paper, and identify the control group and the independent and dependent variables for each experiment.

7. Allow students to read the article silently on their own, fill in the chart, and answer the questions.

8. Discuss student answers in class.

	Control Group	***Independent Variable***	***Dependent Variable***
First Experiment			
Second Experiment			
Third Experiment			

9. Tell students that they will now see Colorado DOW researchers and wildlife managers talking about their work and demonstrating how they test for CWD. Inform students that many scenes on the video are graphic—they will see tissues being removed from animal heads and lab work as it is performed by DOW personnel. Most students will not feel uncomfortable about the presentation if they are not surprised by what they will see. Show the video: *Managing Chronic Wasting Disease* and then discuss it as a class.

10. Each year, about 10,000 deer, elk, and moose heads are submitted for CWD testing, about half the number of heads submitted when the program started. As more data

became available, researchers and hunters alike recognized that CWD incidence was very low to non-existent in most parts of the state. However, there are a few hot spots. You may want to ask students to visit the Colorado DOW Web site and identify areas of the state where there is a high prevalence of CWD.

Assessment

Students should be able identify the control group, independent variable, and dependent variable for each of the three experiments described in the text.

Extensions

- Colorado DOW staff members serve nationally as a source of information and resources concerning CWD research. Scientific publications of current CWD research are available at

  http://wildlife.state.co.us/Hunting/BigGame/CWD/CWDResearchArticles.htm.

You may want to choose several of the studies to review in depth. Students can discuss experimental design, assumptions made, questions raised by the results of the research, and other topics.

	Control Group	*Independent Variable*	*Dependent Variable*
First Experiment	***Pasture that had never had CWD infected animals***	***Pasture with decomposing carcass from a deer that had died from CWD, one with a live CWD-infected deer, and one that had been previously occupied by CWD-infected deer***	***Whether or not healthy animals placed in the pastures got CWD***
Second Experiment	***Deer exposed to a) saliva, b) feces and urine, c) blood, or d) brain tissue from healthy wild or captive deer***	***Deer exposed to a) saliva, b) feces and urine, c) blood, or d) brain tissue from wild or captive deer with CWD***	***Whether or not the exposed deer got CWD***
Third Experiment	***Researchers injected the extract of leg muscle from healthy deer into the brains of genetically-altered mice***	***Researchers injected the extract of leg muscle from CWD-infected deer into the brains of genetically-altered mice***	***Whether or not the mice got a TSE***

Key

1. Why would these researchers have a type "D" pasture in their experiment? ***Every experiment needs a control group, one that doesn't receive any experimental treatment. Each experimental treatment is an independent variable, and the result of each treatment—getting CWD or not—is the dependent variable.***

2. Why did researchers inoculate the fifth set of tame deer with a) saliva, b) feces and urine, c) blood, or d) brain tissue from healthy wild deer? ***The fifth set of deer was the control group.***

3. The urine- and feces-exposed deer did not get sick. Does that mean that deer, elk, and moose cannot get CWD from exposure to urine and feces from infected animals? ***No, it does not mean that. The sample size (number of deer tested) may have been too small, or the study might have been too short for the disease to emerge, or the genetic variety of the fawns might have influenced the results.***

4. Why would researchers be so interested in testing muscle tissue for CWD prions? ***Humans contract variant Creutzfeldt-Jakob disease (vCJD) by eating meat products, which are muscle tissue, from cattle with bovine spongiform encephalopathy.***

5. To test the infectivity of skeletal muscle, the researchers used genetically-altered mice that had normal cervid prion protein (CerPrP), which made them susceptible to CWD. Researchers injected the extract of leg muscle from CWD-infected deer into the brains of one group of these special mice. They injected a second group with leg muscle extract from CWD-free deer. Why did the researchers have two groups of mice? ***The second group was the control group.***

6. Regardless of where the animal is harvested, what portions of deer, elk, and moose should NOT be consumed? ***Research indicates that prions accumulate in certain parts of infected animals at early stages in the disease—the brain, eyes, spinal cord, lymph nodes, tonsils, pancreas and spleen. Those portions of the animal should not be consumed.***

7. Both the Web site and the Big Game Regulations provide maps and information that let hunters know where CWD-infected animals have been found. Why is this information important? ***Hunters will know to take extra precautions when handling the carcass, or, they may choose to hunt in a different location in the state.***

8. Simple precautions are advised when handling deer, elk, and moose killed in units where the disease has been detected. What are these simple precautions?

- ***Do not shoot, handle or consume any animal that appears sick.***
- ***Wear rubber gloves when field dressing and processing animals.***
- ***Bone out the meat from your animal. Minimize the handling of brain and spinal tissues.***
- ***Wash hands and instruments thoroughly after field dressing is completed.***
- ***Avoid consuming brain, spinal cord, eyes, spleen, tonsils, pancreas and lymph nodes of harvested animals. Normal field dressing, coupled with boning out a carcass, will remove most, if not all, of these body parts. Cutting away all fatty tissue will remove remaining lymph nodes.***
- ***Do not consume meat or organs from animals known to be infected with CWD.***
- ***Disinfect knives, saws and cutting table surfaces by soaking in a solution of 50 percent unscented household bleach and 50 percent water for an hour. Afterward, allow them to air dry.***

9. What portion of the animal is submitted for testing? ***The animal's head, which should be removed two to four inches below the point where the neck joins the skull (below the first vertebrae), should be submitted for testing. Lymph nodes in the upper throat are used for testing. The brain stem (not brain) can also be used, so it is important to submit the entire head for testing. Both lymph nodes and brain stem are used for moose testing. Whole brains or pieces of brain tissue cannot be used for testing.***

10. Why is it mandatory to submit moose tissue for CWD testing? ***CWD testing is mandatory for moose to provide more data on CWD in this species.***

11. What happens when an animal tests positive for CWD? ***Hunters whose deer or elk test positive are eligible for either a license fee refund, an antlerless license for the same species in a remaining season, or an antlerless license for the original unit and species during a season next year. Moose hunters whose animals test positive can get a license fee refund or a cow moose license for the same unit.***

12. Looking again at the DOW Web page, what are some of the research projects that DOW employees are involved in? ***Employees have been involved in the following CWD work:***

- ***The successful development of improved, more sensitive testing procedures to detect CWD in deer, elk, and moose.***
- ***An ongoing field study designed to measure the relationship between deer density and disease prevalence.***
- ***Ongoing research to track the progression of the disease through a deer's body to better understand how the disease is transmitted and how it can be better diagnosed.***
- ***Ongoing studies to determine if CWD can be passed to bighorn sheep, mountain lions and other animals.***
- ***Specific studies to determine the ability of CWD to infect cattle.***
- ***Epidemiological studies conducted by state and federal agencies to determine if a link between CWD and human neurological disorders exists.***
- ***Laboratory studies to assess the potential susceptibility of different animal species, including humans, to CWD.***
- ***Ongoing monitoring studies to determine geographic distribution and level of prevalence of CWD in the state.***
- ***Research into early detection methods to diagnose CWD in live, healthy-appearing animals.***
- ***Studies of deer movement patterns to determine if links between disease prevalence and deer movement exist.***

13. Visit the IDNR Web site at http://dnr.state.il.us. What are agencies in Illinois doing to stop and possibly eradicate the spread of CWD in the state?

- ***Teams of sharpshooters from the IDNR and U.S. Department of Agriculture's Wildlife Services are culling deer from known CWD locations.***
- ***To help increase testing in the seven "hot spot" counties in northern Illinois, hunters are eligible for additional deer-hunting permits for these counties at a reduced cost. Hunters, who purchase a special antlerless-only CWD permit for $5 and successfully harvest a deer, may obtain an additional either-sex permit, free of charge, if they allow testing or an attempt at testing for CWD on the harvested deer***
- ***Meat processors have been recruited to take deer samples for CWD testing.***
- ***The Illinois Department of Agriculture monitors captive deer and elk herds in the state for CWD and also obtains samples from captive deer and elk herds at slaughter plants***

14. Why is it important for wildlife managers in both Illinois and Wisconsin to work together to eradicate CWD? ***Controlling CWD in Illinois is not an isolated project. Wisconsin also has CWD in their deer herd. Success or failure in controlling CWD in either state has major implications for the other state. Controlling any slowly evolving, chronic disease in wild animal populations requires a long-term commitment to the process***

Lesson 6

Student Pages

Here to Stay, Not Gone Tomorrow

As of 2008, deer, elk, or moose with chronic wasting disease (CWD) have been found in 14 U.S. states and two Canadian provinces. It is clear that CWD spreads from animal to animal and place to place. But how?

Since CWD was first noticed in captive animals at Colorado's Foothills Wildlife Research Facility and Wyoming's Sybille Research Unit, it was reasonable to guess that the disease could only be transmitted between animals that were in close contact with each other. Both facilities tried hard to eradicate CWD. The Sybille center killed all the deer and elk in the affected area. They waited a year to bring new, disease-free animals into the area. Four years later, these healthy deer and elk started showing signs of CWD.

The Fort Collins Wildlife Research Facility took more drastic action. First, the affected deer and elk were destroyed. Then, researchers turned over several inches of soil and repeatedly sprayed the pastures and fences and other structures with swimming-pool chlorine, which would easily wipe out any bacteria or viruses. After waiting a year, they brought in 12 healthy elk calves. Three years later, one of these elk contracted CWD.

Permanent Environmental Contamination?

The idea that the infectious agent for CWD could permanently contaminate both research facilities was disturbing, to say the least. Historically, wildlife managers have controlled the spread of wildlife diseases one of three ways.

- If the disease is treatable, biologists can either put out medicated feed to treat herds, or trap and treat individual sick animals. For example, wildlife managers will often put out fenbendazole-medicated bait in winter to control lungworm disease in Rocky Mountain bighorn sheep.
- If a vaccine is available to prevent the disease, biologists can inoculate vulnerable animals.
- If the first two options are not possible, managers can cull ill animals to prevent the spread of the disease.

It was apparent that controlling and possibly eradicating CWD would be much more difficult if the pathogen—the prions—stayed in the environment years after the diseased animals were gone.

Identifying Prion Sources—The First Experiment

How did the prions get on the soil or pasture? No one knew. Colorado Division of Wildlife (DOW) veterinarians Michael Miller and Lisa Wolfe, and Elizabeth Williams and Thompson Hobbs of Colorado State University (CSU) set up an experiment to find out. They placed healthy deer in one of four types of pasture:

A. One type of pasture had not previously had deer or elk in it. One live CWD-infected deer was put in the pasture.

B. One type of pasture had not had live CWD-infected deer in it, but had a decomposed carcass from a deer that had died from CWD placed in it.

C. One type of pasture had been previously occupied by CWD-infected deer (more than two years earlier) and had what remained of the deer's ***excreta*** (urine, feces, and saliva).

D. One type of pasture had never been occupied by CWD-infected deer.

1. *Why would these researchers have a type "D" pasture in their experiment?*

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The veterinarians observed the deer closely. One observation was that the deer in pasture type B did not eat any of the decomposed carcass remains, but did eat the vegetation around it. Deer in all of the other types of pasture fed and interacted normally. After one year, they tested the deer for CWD. All of the deer in pasture type D were still healthy and CWD-free. Two of the ten deer in pasture type A had contracted CWD. Three of the 12 deer in pasture type B had the disease and one of the nine deer in pasture type C was infected with CWD.

All of the conditions that were mimicked in pasture types A, B, and C exist in the wild. Deer will often encounter the carcasses and excreta of other deer. Also, deer usually group together in the winter, so transmission between infected deer and healthy deer is likely. It seemed that CWD would be a tough disease to manage.

Identifying Prion Sources— The Second Experiment

More research was needed to determine what deer excreta contained the prions that contaminated pasture type C. Researchers from the DOW and CSU set up a second experiment. They tested four groups of six-month-old hand-raised deer, exposing them to a) saliva, b) feces and urine, c) blood, or d) brain tissue from wild or captive deer with CWD. A fifth set were exposed to each of those materials from wild deer without CWD. The fawns were not genetically identical.

2. *Why did researchers inoculate the fifth set of tame deer with a) saliva, b) feces and urine, c) blood, or d) brain tissue from healthy wild deer?*

_____

The deer were observed for 18 months and their tonsil tissue was regularly tested for signs of infection. After 18 months the deer were ***euthanized*** (killed humanely), and their brains were removed and examined after death (***autopsied***). All the deer exposed to saliva and to blood from CWD-infected deer were infected, while those exposed to urine and feces remained healthy.

The presence of CWD prions in saliva may explain why the disease spreads so easily. Deer are social animals that nuzzle, lick and groom one another. But direct deer-to-deer contact is apparently not necessary for transmission. Healthy deer entering areas that once held sick deer can contract CWD because the saliva from the diseased deer remains on the grass and other surfaces.

It was not surprising to researchers that healthy deer given a single large

transfusion of blood from CWD-infected deer would get the disease. Studies showed that scrapie could be transmitted between sheep that way. From human studies, it was already known that people who received blood or other tissues from victims of variant Creutzfeldt-Jakob disease (vCJD) contracted the illness. But, the findings suggest that it is possible that CWD could be spread to healthy deer, elk, or moose by blood-sucking insects like mosquitoes or ticks. There will need to be more research to find out for sure.

3. *The urine- and feces-exposed deer did not get sick. Does that mean that deer, elk, and moose cannot get CWD from exposure to urine and feces from infected animals?*

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Identifying Prion Sources—The Third Experiment

From the beginning, researchers were aware that CWD prions concentrate in certain tissues, such as the brain, spinal cord, lymph nodes, and spleen. They were not sure that CWD prions were present in other tissues or if they were present in concentrations too low to detect. As CWD-infected ***cervids*** (the family of animals that includes deer, elk, and moose) were found in an increasingly wide geographic area, and concern about potential zoonotic transmission of CWD increased, public health officials were especially interested in testing muscle tissue for prions.

4. *Why would researchers be so interested in testing muscle tissue for CWD prions?*

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5. To test the infectivity of skeletal muscle, the researchers used genetically-altered mice that had normal cervid prion protein (CerPrP), which made them susceptible to CWD. Researchers injected the extract of leg muscle from CWD-infected deer into the brains of one group of these special mice. They injected a second group with leg muscle extract from CWD-free deer. *Why did the researchers have two groups of mice?*

_____

The researchers found that the mice injected with muscle samples from infected deer showed the progressive mental dysfunction associated with transmissible spongiform encephalopathies (TSEs) within 18 months. The skeletal muscle from uninfected deer didn't cause disease when injected into the special mice.

A Public Health Problem?

The presence of infectious CWD prions in the muscle tissue did raise some issues. Were prions present in meat in enough quantity to be of public health significance? Would people who consumed CWD-contaminated meat from infected deer get some type of TSE? So far, this seems unlikely. ***Epidemiologic studies***, studies of the factors determining

and influencing the frequency and distribution of disease, injury, and disability in a population, suggest that the risk of transmission of CWD to humans is very low. Nevertheless, there is no way to prove that a human prion disease associated with CWD cannot appear in the future. Relatively few people in the United States and Canada eat meat from deer, elk, or moose. Most can now test the animals they have harvested for CWD. Since few people have been exposed to CWD-contaminated meat, and since individual genetics influence susceptibility to prion diseases, there may never be enough vulnerable people exposed to CWD to result in a clinically recognizable human disease. Since prion diseases have a long incubation period, ***epidemiologists*** (people who do epidemiologic studies) will need to continue surveillance for human prion diseases, particularly in areas where CWD has been detected.

The Big Picture

All of this research has led to several conclusions:

- CWD prions may remain in the environment for a long time after infected deer, elk, and moose have left the area.
- Wildlife managers probably cannot completely control the spread of CWD and cannot entirely eradicate the disease.
- No tissue from CWD-infected cervids can be considered free of disease-causing prions.
- No one can predict with absolute certainty that CWD will never cause human disease, although all research to date suggests this is impossible.

Even though CWD is serious and cannot be completely eliminated, state wildlife agencies like the Colorado DOW may still attempt to manage the disease and protect herds of deer, elk, and moose. The DOW's management efforts are focused on:

- Preventing the spread of CWD beyond historically infected areas.
- Reducing CWD prevalence within infected areas by removing deer and elk from diseased herds.
- Enforcing illegal feeding regulations. In Illinois it is unlawful to make available food, salt, mineral blocks or other products for ingestion by wild deer or other wildlife in areas where wild deer are present. See the *Illinois Digest of Hunting and Trapping Regulations* for exceptions to this law. When people feed deer, they do not help the animals. Instead, they increase the chances that unhealthy animals will spread disease.
- Enforcing transport laws that prevent people from moving deer, elk and moose from infected areas or from other states into disease-free areas. In Colorado, people often raise herds of these animals to supply restaurants and specialty grocers with meat. In the past, transporting infected animals from one game ranch to another has been a problem.
- Continuing research with other agencies and states to further knowledge about CWD and to improve management of affected deer, elk, and moose herds.

To inform the public, the DOW works with public health authorities to provide current CWD information and recommendations on its Web site, in its print publications, and to newspaper, radio and television agencies.

In Illinois, the Illinois Department of Natural Resources (IDNR) and the Illinois Department of Agriculture work to make

information about CWD available to the public through their Web sites, in print publications and in media releases.

Educating Hunters and Others Who Eat Deer, Elk, and Moose Meat

While studies do not show any relationship between CWD and human health problems, the Colorado DOW takes extra steps to ensure that people who eat deer, elk, and moose meat are informed. You will look at the Colorado DOW Web site to answer the following questions:

http://wildlife.state.co.us/Hunting/BigGame/CWD/

6. *Regardless of where the animal is harvested, what portions of deer, elk, and moose should probably NOT be consumed?*

7. Both the Web site and Colorado's Big Game Regulations provide maps and information that let hunters know where CWD-infected animals have been found. *Why is this information important?*

8. Simple precautions are advised when handling deer, elk, and moose killed in units where the disease has been detected. *What are these simple precautions?*

9. *What portion of the animal is submitted for testing?*

10. *Why is it mandatory to submit moose tissue for CWD testing?*

11. *What happens when an animal tests positive for CWD?*

The Future of CWD Research and Management in Colorado

While CWD is not going to go away anytime soon, scientists are learning more about CWD and other prion diseases every day. Amazing research in the areas of protein folding, genetics, ***immunology*** (study of the immune system), ***pharmacology*** (the use of drugs to prevent and treat disease) and ***wildlife ecology*** (the study of the interaction of wildlife and its environment) may someday allow wildlife managers to control this disease. DOW researchers and biologists are studying CWD on numerous fronts—addressing wildlife health issues and assisting public health experts and scientists with their ongoing research.

The work of some DOW researchers is internationally renowned. While some staff researchers and biologists conduct studies on the disease, others help to collect information from hunters and to keep the public informed. Hundreds of DOW employees and volunteers work every year to further efforts to gain knowledge about and prevent the spread of CWD. It is the hope of all these hard-working people that you—yes you—will stay informed about this important issue, and that possibly someday you might choose to join them in their work.

12. *Looking again at the DOW Web page, what are some of the research projects that DOW employees are involved in?*

13. *Visit the IDNR Web site at http://dnr.state.il.us. What are agencies in Illinois doing to stop and possibly eradicate the spread of CWD in the state?*

14. *Why is it important for wildlife managers in both Illinois and Wisconsin to work together to eradicate CWD?*

Glossary

allele: one of the alternative forms of a gene that governs a characteristic

alpha helix (α-helix): a right-handed coil, one possible secondary protein structure

amino acid: a molecule made up of a central carbon atom, an amino group, a carboxyl group, a single hydrogen group, and an R group

amino group: the molecule -NH_2, an essential component of all amino acids

amino-terminus (N-terminus): the end of a polypeptide chain that is made first and that has a free (unbonded) amino group

amyloid fibers: PrP^{res} prion molecules stacked up, forming long chains

anticodon: groups of three complementary bases on tRNA that recognize and bind to a codon on the mRNA

assay: a test or procedure to purify a substance and determine what it is made of

astrocytes: cells in the brain that digest dead neurons, leaving holes

autopsy: removal and examination of tissue, cells, or fluid from a dead organism

beta sheet (β-sheet): a sharp-angled, folded secondary protein structure

biopsy: the removal and examination of tissue, cells, or fluids from the living body

bovine spongiform encephalopathy (BSE): degenerative and fatal transmissible spongiform encephalopathy disease of the brain in cattle (see TSE)

carboxyl group: the molecule -COOH, an essential component of all amino acids

carboxyl-terminus (C-terminus): the end of a polypeptide chain that is made last and has a free (unbonded) carboxyl-group

cervids: the family of mammals that grow and shed antlers yearly that includes deer, elk, and moose

codon: three sequential bases of mRNA that code for a particular amino acid

complementary base pairing: nucleotide bases that are always paired together (in the DNA double helix, adenine pairs with thymine, guanine pairs with cytosine)

control group: the group used in an experiment that receives no experimental treatment and is used as a standard for comparison with the experimental groups

controlled experiment: a planned procedure to test a hypothesis or prediction

Creutzfeldt-Jakob disease (CJD): chronic, progressive, fatal disease of the central nervous system of humans; a transmissible spongiform encephalopathy found in humans

cross-linkage: *(see disulfide bridge)* strong bonds formed by cysteine R groups linked together

dehydration synthesis: bond that forms between two molecules by the removal of water (one oxygen and two hydrogen molecules); also known as a dehydration reaction, dehydration linkage, or condensation reaction

deoxyribonucleic acid (DNA): polymer made of four similar chemicals called nucleotides: adenine, thymine, guanine, cytosine

dependent variable: measurable result of an experiment; it changes with alteration of the independent variable

dipeptide: two molecules linked by a peptide bond

disulfide bridges or cross-linkages: strong bonds formed by cysteine R groups linked together

domain: short sections of the polypeptide chain that fold into recognizable shapes

dominant allele: the allele's traits that are physically expressed in the organism

emerging disease: a new disease

epidemiologic studies: studies of the factors that determine and influence the frequency and distribution of disease, injury, and disability in a population

epidemiologist: person who conducts epidemiologic studies

eukaryotes: complex cells that contain a nucleus

euthanize: to kill humanely

excreta: excretions from the body of an organism such as urine, feces, and saliva

experiment: a methodic test used to deduce information about the surrounding environment

familial disease: disease that is passed genetically from parent to offspring

fold: signature shape of a protein that defines its function

gene: unit of genetic information that provides the instructions for a single inherited property or characteristic of an organism—a protein

genetic code: set of rules by which information encoded in hereditary material in each cell of an organism makes all of the proteins needed by the organism

genome: the entire DNA of an organism

heritable: changes in DNA that can be passed on from parent to offspring

heterozygous: having two different forms of an allele

homozygous: having two of the same forms of an allele

Human Genome Project: a worldwide project started in 1990 with the mission of compiling the entire genetic sequence of humans and other organisms

hydrogen bond: weak attractions between the positively-charged hydrogen of the amino group of one amino acid and the negatively-charged oxygen of a carboxyl group of another

hydrogen group: a single hydrogen atom attached to the center carbon molecule of an amino acid

hydrophobic: portions of a molecule that are not charged and repel water

immunology: the study of the immune system

independent variable: factor in the experiment that is changed by the scientists

infect: action of a disease-causing pathogen entering the body

loop (or turn): one possible secondary structure in a polypeptide chain (protein)

messenger RNA (mRNA): RNA molecule that carries genetic information from the genes (DNA) to the ribosome

mutation: a change in the genetic information

nontranscribed strand (noncoding strand): strand of a double-stranded DNA that is not used in transcription

nucleotide: building blocks of DNA or RNA that contain a phosphate group (PO_4H), a sugar, and a nitrogen-containing base

pathogen: disease-producing agent

peptide bond: type of chemical linkage holding amino acids together, a bond between the carboxyl group of one amino acid and the amino group of another

pharmacology: study of the use of drugs in preventing and treating disease

polymer: large molecules made up of smaller molecules

polymorphism: existence within a population of two or more genetically different forms of a protein

polypeptide: chain of amino acid molecules linked by peptide bonds

primary protein structure: number, kind, and order of amino acids joined together in a polypeptide chain

prion: proteinaceous infectious particle that is responsible for transmissible spongiform encephalopathies such as chronic wasting disease

prion or "protein only" hypothesis: proposal that a misfolded prion protein could become a pathogenic entity that kills

promoter site: sequence of DNA bases that identifies the strand to be transcribed, the site where the RNA polymerase enzyme attaches

protein: large compounds made up of one or more polypeptide chains

protein folding problem: the struggle of trying to uncover the mechanism that directs protein folding

protein synthesis: making protein in the cell through the process of DNA transcription

quaternary or fourth-level protein structure: two or more polypeptides joined together by many different kinds of chemical bonds to make a large, finished protein

R group: The molecule that makes the 20 amino acids different from each other; attached to the central carbon atom

recessive allele: allele's traits are not physically expressed in the organism unless two copies of it are present

ribosome: organelle in the cell responsible for protein synthesis

ribonucleic acid (RNA): polymer of nucleic acids similar to DNA but containing the base uracil instead of thymine, and having a ribose sugar rather than a deoxyribose sugar. It is used in directing protein synthesis outside of the nucleus

ribosomal RNA (rRNA): central component of the ribosome that helps translate mRNA into amino acids

RNA polymerase: enzyme that unwinds DNA during transcription to make mRNA

secondary protein structure: shape of an amino acid after hydrogen bonding takes place; can be either a coiling alpha (α) helix or a folded beta (β) sheet; determined by the R groups of the amino acids in the chain

silent (neutral) mutation: mutation results in a change in the nucleotide sequence, but the resulting triplet still matches a codon that makes the same amino acid; a harmless mutation

sporadic disease: disease that occurs occasionally and at random intervals in a population

tertiary protein structure: complex folding of protein which determines the resulting function of the protein

transcribed strand (sense strand or coding strand): strand of double-stranded DNA that is used for transcription

transcription: process by which genetic information from DNA is converted into its RNA equivalent

transfer RNA (tRNA): RNA molecules that carry amino acids to the ribosome

translation: making a protein using the information provided by messenger RNA

transmissible spongiform encephalopathies (TSEs): transmissible, fatal diseases of the central nervous system that result in a sponge-like appearance of the brain tissue

triplet: sequence of three nucleotide bases of DNA that code for a specific amino acid

turn (or loop): one possible secondary structure in a polypeptide chain (protein)

Variant Creutzfeldt-Jakob disease (vCJD): form of Creutzfeldt-Jakob disease linked to consumption of bovine spongiform encephalopathy-infected meat products

wildlife ecology: study of the interaction of wildlife and its environment

zoonotic: disease that can be transferred to humans from other animals