Lesson 4: Tick tock...Broken clock

I. Overview
In this lesson, students learn about the role played by DNA and proteins in the circadian cycle. Students also explore some of the scientific and diagnostic techniques used by doctors and researchers to diagnose and study circadian rhythm disorders. Using a case study format, students investigate the source of a fictional character’s sleeping problems. Students are presented with information that they must utilize to progress through four “checkpoints” throughout the course of the lesson. Each checkpoint gives student groups access to additional information based on current research regarding the nature of the patient’s sleep difficulties. The students, who act as case investigators, review records collected by a hospital case investigation team to help solve the young man’s sleeping problems.

After completing all of the checkpoints in the order of their choosing, each group will regroup as possible “diagnoses” teams, where each group member gains in-depth knowledge of a possible diagnosis for the patient. Following this jigsaw, students return to their investigation teams to come to a final consensus on the patient’s diagnosis.

Connection to the Driving Question
In this lesson, students address what happens when someone’s “clock” is disrupted due to a genetic disorder. Thus, students use data that has been generated about circadian rhythms to determine how the processes that make us “tick...tock” can be altered and cause problems with our circadian rhythms.

Connection to the Previous Lesson
Lesson 4 builds upon the core concepts established in Lesson 3. Lesson 4 requires students to take their new knowledge of the PER2 gene and apply it interpret the scientific information presented in the case study. In Lesson 4, students compare the DNA sequences of a mutated and non-mutated per gene and then transcribe and translate the sequences to verify any changes to the amino acid sequence. Students also utilize their knowledge of protein structure to aid them in interpreting Western blot results to insight into the cyclical nature of the PER2 gene.

II. Standards/Benchmarks

National Science Education Standards

Content Standard C: The Cell
• Cells store and use information to guide their functions. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires. (9-12 C: 1/3)
• Cell functions are regulated. Regulation occurs both through changes in the activity of the functions performed by proteins and through the selective expression of individual genes. This regulation allows cells to respond to their environment and to control and coordinate cell growth and division. (9-12 C: 1/4)

Content Standard C: The Molecular Basis of Heredity
• Changes in DNA (mutations) occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can change cells and organisms. Only mutations in germ cells can create the variation that changes an organism’s offspring. (9-12 C: 2/3)

Content Standard C: The Behavior of Organisms
• Multicellular animals have nervous systems that generate behavior. Nervous systems are formed from specialized cells that conduct signals rapidly through the long cell extensions that make up nerves. The nerve cells communicate with each other by secreting specific excitatory and inhibitory molecules. In sense organs, specialized cells detect light, sound, and specific chemicals and enable animals to monitor what is going on in the world around them. (9-12 C: 6/1)

AAAS Benchmarks for Science Literacy

The Nature of Science: The Scientific Enterprise
• In doing science, it is often helpful to work with a team and to share findings with others. All team members should reach their own individual conclusions, however, about what the findings mean. 1C/P2

The Living Environment: Cells
• The work of the cell is carried out by the many different types of molecules it assembles, mostly proteins. Protein molecules are long, usually folded chains made from 20 different kinds of amino acid molecules. The function of each protein molecule depends on its specific sequence of amino acids and its shape. The shape of the chain is a consequence of attractions between its parts. 5C/H3
• The genetic information encoded in DNA molecules provides instructions for assembling protein molecules. 5C/H4a

The Human Organism: Physical Health
• Faulty genes can cause body parts or systems to work poorly. Some genetic diseases appear only when an individual has inherited a certain faulty gene from both parents. 6E/H2

III. Learning Objectives
<table>
<thead>
<tr>
<th>Learning Objectives</th>
<th>Assessment Criteria</th>
<th>Location in Lesson</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explain the basic principles behind key techniques that scientists utilize when conducting circadian research</td>
<td>Analysis of Western Blot data reveals darker bands as higher levels of protein expression, with a specific protein probed via antibody detection. Polymerase Chain Reaction (PCR) amplifies a specific segment of DNA, in this case a segment of the per2 gene. Then, using DNA sequencing, Sandman’s per2 sequence is decoded and compared against another’s code at the same section of the gene, in this case the father’s code. The actogram measures the activity level of Sandman across a 24-hour period, with taller peaks indicating a higher level of activity.</td>
<td>Activities 2 and 3</td>
</tr>
<tr>
<td>Use sample data to explain a circadian anomaly, from phenotypic expression to the underlying transcriptional processes.</td>
<td>John Sandman’s irregular sleep patterns, as well as family history and pedigree chart denoting the phenotypic expression of the circadian anomaly, correlate to the PCR data on Sandman’s genetic code. His father does not suffer from the circadian disorder, and when comparing his per2 gene to his son’s, there is a specific point mutation that causes a mutation protein to be expressed, leading to John Sandman’s circadian disorder symptoms.</td>
<td>Activities 1-3</td>
</tr>
<tr>
<td>Explore and explain how a genetic mutation can modify normal circadian functioning in humans and model organisms (mice)</td>
<td>Checkpoint B: The protein levels of the mouse with the mutated PER2 protein were significantly slower in amount overall, and peaked at different times, from those in the mouse with the wild type PER2 protein. The mutation also affected the mouse’s actograms, showing the mutated mouse more likely to be active during the “lights on” as well as having a free-running schedule when placed in constant darkness. Checkpoint C: In humans, the mutation in the per2 gene of John Sandman confirmed the link between the genetic mutation and the behavioral effects on circadian rhythms. The DNA mutation must have led to a protein structure change, causing the circadian disorder.</td>
<td>Activities 2 and 3</td>
</tr>
<tr>
<td>Describe the interconnectedness between the various levels at which circadian rhythms can be studied</td>
<td>If someone is suspected of having a circadian rhythm disorder based on behavior, his/her protein expression levels and genetic code can be analyzed to discover whether or not the behavior correlates to a change in these molecular processes.</td>
<td>Activity 4</td>
</tr>
</tbody>
</table>
Conversely, a mutation in a gene and/or protein expression can lead to a behavioral change, or it may not due to the redundancy of the genetic code. Other environmental factors may also be at play that can change one’s circadian rhythm, not just genetics.

**IV. Adaptations/Accommodations**

This lesson builds upon the concepts introduced in Lessons 1 through 3 of the circadian unit, including: explanation of circadian rhythms and their diversity, the process of DNA -> RNA -> Protein, the nature of mutations and their effect on an amino acid sequence, protein size and structure, and the role of actograms in studying circadian rhythms. If this lesson is being done without this previous knowledge, time should be set-aside during each checkpoint to explain these concepts where they are applied in the lesson. This will require significant additional time and should be considered when planning the sequence of the case.

This lesson is designed to be completed in groups of four with each group completing all four checkpoints in sequence. While this is ideal, modifications to this lesson flow can be made to shorten the amount of class time and resources needed to complete the lesson. If the introduction document to each checkpoint is modified the lesson can be split into a large jigsaw activity. Each checkpoint would stand alone as a set of resources and the included student checkpoint sheet. In this way the first three checkpoints can be completed in one class period, with a student becoming an “expert” on one checkpoint. To replace the jigsaw conclusion the class would then be divided into diagnosis groups with one member from each checkpoint (groups of three) and they would collectively share their knowledge, presenting their evidence and the conclusions they reached to complete the final conclusion, checkpoint D.

If the jigsaw activity for Checkpoint D documents are considered too technical for students without excessive scaffolding, the conclusion activity can be turned into a class discussion about the group’s progress through the case materials and the major findings uncovered along the way. The students can be given the supplemental “circadian disorders overview” sheet and the class can reach a consensus on the appropriate diagnosis.

**V. Timeframe for activity**

**Opening of Lesson**

- Introduce patient case and structure of “checkpoint system” – 15 minutes

**Main Part of Lesson**
• Activity 1: Checkpoint A – 30 minutes
• Activity 2: Checkpoint B – 30 minutes
• Activity 3: Checkpoint C – 30 minutes
• Activity 4: Checkpoint D – 30 minutes
  o Introduce sequence for conclusion
  o Break into jig-saw groups by disorder
  o Reassemble in investigation teams for final decision

Conclusion of Lesson
• Class diagnosis consensus and discussion of role of research – 5-10 minutes

VI. Advance prep and materials

Activity 1: Checkpoint A

Materials:
• “Checkpoint A” Folder
• Checkpoint A Case Files, U3_L4_CheckpointA_CaseFiles.pdf, which contains:
  o Case A Review: Checkpoint A Contents
  o Central Midwest Hospital Email Records
  o Doctor-Patient Interview Transcript
  o Epworth sleepiness survey
• Checkpoint Student Sheet, U3_L4_CheckpointA_StudentSheet.docx (one per student)

Preparation:
• Print out one Checkpoint A Student Sheet for each student
• Print out enough copies of the Checkpoint A case files document so that there is one copy of the folder per group. Put case files in “Checkpoint A” folder for each student group to use.

Activity 2: Checkpoint B

Materials:
• “Checkpoint B” Folder
• Checkpoint B Case Files, U3_L4_CheckpointB_CaseFiles.pdf, which contains:
  o Case Review: Checkpoint B Contents
  o PER2 Mutant Data Analysis and Actograms
  o Sleep Log
  o Hospital Info Sheet: Western Blots
    ▪ The file U3_L4_CheckpointB_HospitalInfoSheetWesternBlots.pdf can be substituted here as an abbreviated reading
• Checkpoint Student Sheet, U3_L4_CheckpointB_StudentSheet.docx (one per student)
Preparation:
• Print out one Checkpoint sheet B worksheet for each student
• Print out enough copies of the Checkpoint B case files document so that there is one copy per group. Put case files in “Checkpoint B” folder for each student group to use.

Activity 3: Checkpoint C

Materials:
• “Checkpoint C” Folder
• Checkpoint C Case Files, U3_L4_CheckpointC_CaseFiles.pdf (one per group), which contains:
  o Case Review: Checkpoint C Contents
  o Guidelines for Review of Chronotype Survey
  o Family Survey Interview Responses
  o Sleep Assessment- Personal Accounts
  o Family History Tree For Jonathan Sandman (blank)
  o DNA Cheek Swab Results
  o Hospital Info Sheet: PCR and DNA Sequencing
    ▪ The file U3_L4_CheckpointC_HospitalInfoSheetPCR&DNA.pdf can be substituted here as an abbreviated reading
• Checkpoint Student Sheet, U3_L4_CheckpointC_StudentSheet.docx (one per student)

Preparation:
• Print out one Checkpoint sheet C worksheet for each student
• Print out enough copies of the Checkpoint C case files document so that there is one copy per group. Put case files in “Checkpoint C” folder for each student group to use.

Activity 4: Checkpoint D

Materials:
• “Checkpoint D” Folder
• Diagnostic Criteria for the different sleep disorders
  o Non 24 Hour (Free Running) Disorder, U3_L4_CheckpointD_Non24HourDisorder.docx
  o Advanced Sleep Phase Syndrome,
    U3_L4_CheckpointD_AdvancedSleepPhaseSyndrome.docx
  o Delayed Sleep Phase Syndrome, U3_L4_DelayedSleepPhaseSyndrome.docx
  o Irregular Sleep Wake Pattern, U3_L4_IrregularSleepWakePattern.docx
• Circadian Disorders Overview, U3_L4_CheckpointD_Circadian DisorderOverview.docx (Optional)
• Checkpoint Student Sheet, U3_L4_CheckpointD_StudentSheet.docx

Preparation:
• Print out one Checkpoint sheet D worksheet for each student
• Print out enough copies of the diagnostic criteria so that there are enough for each jigsaw group. Put case files in “Checkpoint D” folder for each student group to use.

Homework and Assessments
• Checkpoint A Student Sheet, U3_L4_CheckpointA_StudentSheet.docx
• Checkpoint B Student Sheet, U3_L4_CheckpointB_StudentSheet.docx
• Checkpoint C Student Sheet, U3_L4_CheckpointC_StudentSheet.docx
• Checkpoint D Student Sheet, U3_L4_CheckpointD_StudentSheet.docx

VII. Resources and References
The format for this case investigation consists of a series of checkpoints. Each of these checkpoints provides a set of related resources (case files) for students to examine along with a checkpoint sheet students complete. The resources in each checkpoint are based off of related circadian research, and the original documents that inspired the case materials can be found below:

VIII. Lesson Implementation

Opening of Lesson:
Begin the lesson by asking students:

- Would you go to the doctor if you had trouble sleeping for one night?

Asking this question will get students thinking about sleep disruption as a medical problem. Make certain that students understand that sleeping poorly for one night does not warrant the attention of a doctor.

Continue the discussion by asking students:

- What if you had trouble sleeping for a week? Two weeks? Would you go to the doctor then?

Through this discussion, make certain students are thinking about where they would draw the line between an inconvenient loss of sleep and a possible medical problem. Tell students that in this lesson they are going to get the chance to explore the story of one patient who went to the doctor because of problems with their sleep pattern.

Let students know that they will be building upon what they have learned so far in Lessons 1, 2, and 3 and applying it to help solve a medical mystery. Students will be taking on the role of medical case investigators to interpret hospital documents and scientific research to help diagnose the patient.

Teacher Content Knowledge
While the items in this case are presented in the form of a medical diagnosis, the characters and documents for the case are purely fictional. Documents will contain things such as the name of a hospital, email addresses, and other personal information. All of this is fictional and has been designed with the intent that it not reflect a real hospital or real email address (email addresses in the case materials are designed to not function). Additionally, resources such as doctor-patient transcripts are not normally recorded, as they would violate patient confidentiality. They are presented here as a way to communicate the patients interactions with their doctor.

If a student asks about this, let them know that while things such as the name of the hospital, personal details of the character, and other information have been created to help tell a story, other parts of the case are based on real research data and diagnostic techniques. Students will be exposed to real circadian research, with the fictional story acting as a guide through the material.

Main Part of Lesson

Activity 1: Checkpoint A
To begin the activity split the class into groups of four students. Tell students that these groups will represent their case investigation teams, and they will be responsible for working together to examine all of the materials as they move through the case files.

**Teacher Pedagogical Knowledge**

If there is an odd number of students a group of three can be used, but some modifications will need to be done to the final jigsaw activity to ensure that each group gets information on the final four potential circadian disorders they will be diagnosing. One way to do this would be to have a group of three combine with another group for the final diagnosis after the jigsaw activity. This would ensure that a smaller group does not miss out on any information.

Once students are in their groups, introduce the overall sequence of the case. Students will be given a file of documents and an associated worksheet for each checkpoint. To complete the worksheet students need to search through the documents for the answers. This means that it will be important for the group members to split the work and communicate in order to efficiently find the information they need. Make sure the students are aware that while they are working in groups to explore the materials, they must each hand in a completed worksheet for each checkpoint at the end of the lesson.

**Teacher Pedagogical Knowledge**

When a group has completed their worksheet they must return the case file for Checkpoint A in order receive the next set of materials (Checkpoint B). Make sure the students know that while each group may move through the materials at a different pace, they must all reach the goal set for the class period (i.e. finish Checkpoint A before the end of class). The checkpoint system is designed so that the student groups are not flooded with materials as they investigate the case. Each checkpoint is designed so that the students can complete it, return the case materials and retain their worksheet as their notes. They then get access to a new set of materials and a new worksheet. By the end of the case, their set of completed checkpoint worksheets will act as a summary of their materials.

Hand out a set of Checkpoint A case materials and worksheets for each group. As the materials are being passed out tell the students that to begin the investigation they are going to explore the reasons behind the patient’s first trip to the doctor. Once the students have the materials and worksheets they should begin their investigation. Let the students know that while they are working in groups, they can still raise their hand if they have questions about the materials.
Teacher Pedagogical Knowledge

While the student groups should guide their own progress through the resources, the teacher should still play an active role as an expert advisor. Move through the groups and ensure that they are all utilizing their time efficiently. If any groups have questions, act as an advisor to their group and assist in their interpretation of the materials. Do not make any conclusions for the group. In this case the teacher is acting as the liaison between the hospital and the student investigators, delivering materials and assisting in the diagnosis.

At the conclusion of the class period make sure that the students either bring back their checkpoint sheets to the next class, or collect them for redistribution. If enough time remains at the end of the period begin a discussion with the students about what questions they were attempting to answer through the case materials. Ask students:

- What if the patient’s mother hadn’t noticed her son’s difficulty sleeping? What do you think the consequences might be if the patient hid his difficulty sleeping and never went to the doctor?

Have students come up with reasons why the patient may hide his sleep difficulties. Make sure that the discussion addresses the consequences of inadequate sleep (refer to some of the patient’s problems in school as examples.)

Activity 2: Checkpoint B

Teacher Content Knowledge

The actogram used in this checkpoint has been fictionalized for the case. The western blot information and images are all based on current circadian research on the effects of PER2 mutations in mice. Current research shows that cyclical changes in the level of PER2 protein within timekeeping cells are one of the major components for maintaining a particular circadian rhythm. PER2 protein is produced up to a threshold level, and then production stops. Over the course of the day the PER2 protein that was produced slowly breaks down. The time needed for the protein to break down is one of the major determinants of the length a circadian cycle. Mutations to the PER2 protein can cause it to degrade faster or slower within the cell, changing the circadian rhythm in the organism. For more information on the research behind the case materials for Checkpoint B see the “teacher resources” folder.

Have students return to their case investigation groups and, before handing out the materials for the next checkpoint, ask students:

- How does scientific research help a doctor make a diagnosis?

This question is designed to initiate a short discussion about how scientific research often influences medical treatments and diagnosis. Students can address any number of relationships, including:
pharmaceutical research, research into hereditary disease, virology, physiology, biochemistry, and many more. While there is no need to have students address all of these areas, make sure that the discussion gets to the main concept that, while doctors diagnose and treat a patient, the information they use to make a diagnosis and the treatments they prescribe are all based on scientific research.

Hand out the case files for Checkpoint B while explaining to the students that the next two checkpoints will be utilizing research to help gain more insight into the patient’s sleep difficulties.

Allow students to work through Checkpoint B.

Activity 3: Checkpoint C

<table>
<thead>
<tr>
<th>Teacher Content Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td>The family history information that the students will be utilizing during this case are based off of real circadian research into a PER2 gene that causes familial advanced sleep phase syndrome, the ultimate cause of the patient’s sleep disruptions. Checkpoint C includes a family tree that shows a similar rate of occurrence of the disorder, and collects data via survey in the same manner as the real research. Discussions of the PER2 gene itself are not introduced until the students examine the DNA-PCR results during this checkpoint. For more information on the research behind the case materials for Checkpoint B see the “teacher resources” folder.</td>
</tr>
</tbody>
</table>

Checkpoint C continues the theme of research in medicine with another set of resources based on current circadian research. Students will transition directly into Checkpoint C from B and will continue their investigation in their groups.

After students have completed Checkpoint C, ask them:

- Are we ready to make a diagnosis? What else do we need?

Have each group contribute to this question. This can aid in gauging the progress of the groups and help in planning the final conclusion discussion.

<table>
<thead>
<tr>
<th>Scientific Practices: Analyzing and Interpreting Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>In both Checkpoints B and C, students are asked to analyze data modified from primary research literature to eventually draw a conclusion on the type of sleep disorder John Sandman has. To support students in this analysis, a deeper discussion about Western Blot and PCR techniques may be helpful for students to fully understand what the data means. However, this discussion will prove helpful, as it will improve the students’ ability to analyze data, a key scientific practice and useful life skill.</td>
</tr>
</tbody>
</table>

While students have been exposed to a wide array of circadian-related data, they still do not have detailed information about the various ways in which circadian rhythm disorders are classified and
diagnosed. Let the students know that for the final wrap-up they will be applying all of the information they have collected to make a final diagnosis for the patient.

**Activity 4: Checkpoint D**
Have the class return to their investigation groups. This checkpoint will be completed in two parts, the first being a jigsaw activity in new groups, and the second being the final diagnosis with their investigation group. Before dividing up the class for the jigsaw reading, ask the students:

- Last time we talked about what we would need for a final diagnosis, where might we be able to get that information?

Tell students that an organization exists that can help them make a diagnosis: The American Academy of Sleep Medicine. The AASM specializes in studying sleep disorders (circadian and others) and also creates diagnostic criteria to help doctors diagnose and treat patients. One of the major documents the AASM released in 2007 was a major review of circadian sleep disorder research. Let students know that for the first part of the diagnosis they will be splitting up into new groups to study a portion of this review document.

Have the members of each investigation group number off by four and assign each number to one of the four circadian disorder documents: delayed sleep phase syndrome, advanced sleep phase syndrome, irregular sleep wake rhythm, and free-running disorder. Have students regroup using these new numbers and begin passing out the AASM diagnostic criteria to each appropriate group. While the documents are being handed out make sure students are aware that these documents will contain circadian-based terminology and research information, and they will need to work together to draw out the information they need to complete the first part of their checkpoint sheet. When the students have completed Part one, they will be viewed as the expert on that disorder for the rest of their case group, and will need to be able to discuss that disorder to them.

The four samples of the AASM diagnostic criteria are presented in one word document. Each group should receive only the summary for their disorder. If needed, the diagnostic criteria can be edited and shortened in order to facilitate student interpretation of the materials. Additionally, the shorter *U3_L4_CheckpointD_CircadianDisorderOverview.docx* can be used to help communicate the “big picture” of the disorder to the students.

### Scientific Practices: Constructing Explanations

In Checkpoint D, the students are asked to consolidate and synthesize the data they have collected in Checkpoints A-C to make a final diagnosis. For their diagnosis, the students must provide evidence to support their decision based on the data in the previous Checkpoints. The explanation can also include a “scientific reasoning” component that solidifies the link between the diagnosis and the evidence the students are using to support the diagnosis. To extend this further, their explanations could also contain a section on why it could not be another disorder based on the evidence provided, strengthening the students’ arguments for their accepted diagnosis.
As students examine their diagnostic criteria and complete Part One of the worksheet, move throughout the groups and make sure students are making progress on their interpretation of the document. Students may have questions about terminology or research in the documents, and it is important to be familiar with each document to help guide the students.

Once students have completed Part One, have the jigsaw groups break up and return to their investigations groups to begin Part Two.

In Part Two, students will use their checkpoint to confer with their group about their disorder and make a final diagnosis. Each expert will need to present their disorder to the other students in the group so that all of the investigation team members understand each of the disorders. In completing Part Two, students will use their checkpoint worksheets to identify one major resource from each checkpoint and explain how it supported their diagnosis.

**Conclusion of Lesson:**

**Teacher Content Knowledge**

While all four of the disorders examined are related, the major cause of the patient’s sleep disruption is Advanced Sleep Phase Syndrome. Specifically, the patient has a heritable form of this disorder, which is very rare. If a student group made a different diagnosis, see where their interpretation of the materials differed from the other groups, and have them present evidence to support their decision.

When all the groups have decided on their final diagnosis, have each group share their conclusion, and the resources they found most helpful in making their decision. To wrap up the discussion, ask the students:

- Did any of the AASM diagnostic criteria mention the PER2 gene? Why not?
- Could you have made a diagnosis just based on the doctor interview and emails? Would it have fully explained the patient’s sleep disruption?
- What extra information did the research provide?

Discuss how the AASM diagnostic criteria and the research into PER2’s role in circadian rhythms were occurring at around the same time. Using the information presented by the AASM, the students were able to connect the sleep disruptions observed in circadian research to the characteristics of an existing disorder with an unknown cause. Make sure students understand that this research is still only a piece of the complete puzzle, and while it has provided new insight into disorders such as Advanced Sleep Phase Syndrome, there is still a lot that is unknown. This case presents one example of how research into circadian rhythms can help change the way we understand and diagnose circadian disorders.
Assessments
Evidence of learning and application of knowledge will be tracked through the student’s completion of the worksheet for each checkpoint. Each checkpoint sheet has been designed to guide the student through the materials and allow them to synthesize and represent the information they are gathering. Also, active participation and discussion during the jigsaw conclusion can be monitored and evaluated as the class presents their groups final decision. In justifying their group’s diagnosis, students will be asked to apply their knowledge of the case materials to novel situations.