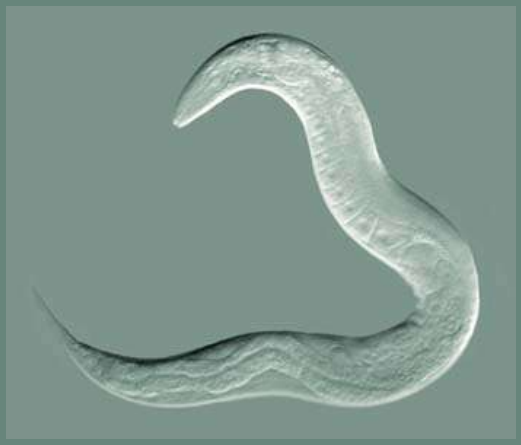
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

A Different Kind of Stress

Protein Trafficking and the Unfolded Protein Response



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Preparation

Before beginning the case:

1. Take the Protein Trafficking Quiz (*before* watching the following videos).
2. Watch the first 30 seconds of this video, which shows the normal movement of the organism *C. elegans:* **http://youtu.be/GgZHziFWR7M**
3. Watch this next video to help understand the traffic pattern: **http://vcell.ndsu.nodak.edu/animations/proteintrafficking/movie-flash.htm**

Part I – Sugar Rush?

"*Ugh!* This room always stinks," Rick commented as he and Claire entered the small lab room. "How can something so tiny smell so bad?"

"It's really the food that smells bad," Claire reminded him.

Claire and Rick were undergraduate students participating in a summer research program at their college. It was the start of the summer and they were learning about the organism they would be studying, *Caenorhabditis elegans,* a 1mm-long nematode (worm). They were learning about the growth and maintenance of this organism. *C. elegans* grows well in petri dishes, is easy to feed (typically using commonly found bacteria as a food source), and many individuals can grow in one petri dish. *C. elegans* has an average life span of approximately two to three weeks and a generation time of approximately four days, which means many individuals to study. They also have many of the same organ systems as other animals, including humans. The entire life cycle of this organism is known, including what happens to each cell from the time of fertilization of the egg to full adult form. It is often used as a model organism for studying genetics and disease.

Both Claire and Rick were working on a project exploring how long-term excess glucose in the worm diet may affect the worm's cellular function. It is known that many of the responses to environmental conditions of the worms are analogous to responses of humans. Claire, who was a nursing major, was interested in the research project because diabetes ran in her family; she was hoping to learn more about how too many sugars or carbohydrates can contribute to chronic disease. Rick was still unsure of his major. He had taken an introductory biology course, which he liked, and so was excited when he was offered the position in this lab as a work study student.

The worms they were working with were a strain of *C. elegans* that had been mutated so that the worms were unable to produce an essential protein needed when the worms were put into various stress conditions, such as excess sugars. Part of Claire's and Rick's responsibilities was to feed half of the mutated worms a normal diet and the other half a high glucose diet, and record worm responses. This meant keeping dozens of dishes for each condition.

"This is kind of odd. Look at the guys in this dish. Do you see how many of them are moving weird? All twitchy and slow." Rick picked up and observed one of the plates in which the worms had been fed an excess of glucose. Indeed, many of the dozens of worms in each petri dish were moving oddly. None of the worms in the petri dishes without extra glucose were moving oddly at all.

Claire came over to look. "That is weird," she said. "I thought that these guys would get really fat from all the calories in the sugar, not twitchy. Maybe it's like a sugar rush."

Dr. Eniz, the scientist in charge of the lab, came over to look. "This is interesting," she added. "That doesn't look like a sugar rush to me, more like some sort of movement disorder. We should take a look at some of these worms more closely."

Questions

1. What are model organisms?
2. What does the phrase "responses to environmental conditions of the worms are analogous to responses of humans" mean?
3. Thinking about the size, easy maintenance, and what scientists already know about the *C. elegans* life cycle, why is it a good choice as a model organism?
4. Define mutation and mutant. Explain why it is important to use mutants in research to understand how some part of life functions.
5. List four to five different chronic medical disorders that you are aware of already.

Part II – Cell Structure

"So here we have some normal cells from the guts of the control group worms." Dr. Eniz was peering through a light microscope.

"OK. Let's move on to some muscle cells from the control worms." Dr. Eniz switched the slides on the microscope. "Normal," she proclaimed. After a moment she added, "Note how they look very much like the classic textbook animal cells."

Claire and Rick glanced at each other when they saw the look on Dr. Eniz's face. They had worked with Dr. Eniz long enough to recognize this was turning into a review lesson.

"Can you point out the different parts of the cell structure to me?" Dr. Eniz asked. She opened the microscope view on her computer so that Claire and Rick could both see the cells more clearly. Claire and Rick focused on the cells, trying to recall what they had learned in their biology classes.

"Well, I see the cell membrane and the nucleus pretty clearly." Rick pointed to the parts on the screen.

"And here and here are what look like mitochondria," Claire added. "And these black lines all close together are the ER. These little ball-like things may be lysosomes or peroxisomes."

"Very good, both of you," Dr. Eniz said. "Now, can you tell me what these parts do?"

Questions

1. Draw and label a typical generic animal cell focusing on the following organelles and cell parts: *cell membrane, nucleus, nucleolus, nuclear membrane, free ribosomes, lysosomes/peroxisomes, mitochondria, cytoskeleton, centrioles, Golgi, endoplasmic reticulum (smooth and rough).* Don't crowd your drawing!

   Next to each label, add a brief description of the function of each organelle. If you need to review, you should use your textbook or a website such as: http://www.cellsalive.com/cells/cell_model.htm

2. Draw a second picture showing the path of proteins from the RER to Golgi to membrane/vesicles/exocytosis. You may review the following video to help understand the traffic pattern: **http://vcell.ndsu.nodak.edu/animations/proteintrafficking/movie-flash.htm**

3. Briefly describe the organelle modifications the following cell types have based on their function (i.e., the numbers of organelles):
   - Skeletal muscle cells
   - Cells in the small intestines that will be absorbing nutrients
   - Pancreas cells making insulin

STOP!

Take the Protein Trafficking Quiz a second time now that you have watched the videos and completed this section. Then continue on to the next section of the case.

Part III – Response

"Now let's look at some of the cells from the experimental group." As Dr. Eniz looked through the microscope to get a good view, Claire and Rick could see she was smiling. "This is what I thought we might see," Dr. Eniz explained. "Look." She showed the cells on the computer screen. "Tell me what you see that looks different in these cells from the control groups." Dr. Eniz looked expectantly at Claire and Rick.

They both looked at the cells carefully. "It looks like the lines in the cells aren't as organized or in regular lines like they were in the control cells. I think those lines were the endoplasmic reticulum, right?" Rick asked.

"Correct. Claire, what do you notice?" Dr. Eniz probed.

"Some of these cells look like they are breaking apart. I think they're dying," Claire responded.

"Apoptosis in action," Dr. Eniz explained. "Did you both learn about apoptosis?"

"Yes—that's when cells kill themselves because they're so damaged," Claire replied.

"Another correct answer," Dr. Eniz smiled proudly.

"But why are the cells dying or making weird ER?" Rick asked.

Dr. Eniz motioned for Claire and Rick to sit down while she answered. "You know that cells make many proteins, and that the structure of the protein—the correct 3-D structure—matters for proper protein function. And you know that proteins made for export out of the cell or use in the membrane or proteins in the lysosomes are made in the rough endoplasmic reticulum, then repackaged in the Golgi."

"While the proteins are being made in the rough ER they are folded into a particular shape, or at least that's what's supposed to happen. But sometimes things go wrong and the proteins don't fold right, or the cell gets stressed by something in the environment. No big deal; the cell can get rid of them. However, if too many proteins are made very quickly and are not folding correctly, this causes the proteins to pile up inside the RER. This really puts pressure on the RER to do something, which is called endoplasmic stress. This stress in turn triggers the cell to try to deal with the situation. Scientists call the response the unfolded protein response." Dr. Eniz paused to see if Claire and Rick were following. They both nodded, although hesitantly.

Dr. Eniz could see that there was still some confusion. "What happens in simple terms is that misfolded proteins cause a traffic jam in the rough ER. It is analogous to having a door in the rough ER that allows certain shaped molecules out, but if molecules are not the right shape, they jam up the door and no molecules can get out and they start to accumulate in the rough ER, causing stress in the cell." She paused to make sure Claire and Rick understood.

Dr. Eniz continued, "So there are a bunch of unfolded or misfolded proteins piling up in the rough ER—that is called endoplasmic reticulum stress. The cell knows it has to do something, so the cell responds—this is the unfolded protein response. The cell can try to refold the proteins or destroy them, the cell can try to stop the proteins from being made, and if these fail to fix the problem, the cell commits apoptosis. The mutant worms are unable to respond to endoplasmic stress and so are very sick."

Questions

View the PowerPoint animation **Normal Protein Trafficking and the Unfolded Protein Response**, and then answer the following questions:

1. Explain the normal role of the rough endoplasmic reticulum and Golgi in protein production and export in your own words.
2. Explain the basic idea and possible outcomes of the unfolded protein response in your own words.
3. If many cells commit apoptosis in any one organ, what may happen to that particular organ?
4. Besides response to unfolded proteins, under what other conditions might apoptosis be a positive thing in a cell's life?

Part IV – Endoplasmic Stress and Disease

"I get that now," Rick exclaimed. "But what caused these cells to do that? Was it all the sugar the worms were fed?"

"Maybe. That's what I'm studying," Dr. Eniz explained. "Scientists know that too much glucose or sugars can cause cells to overproduce insulin, which is a protein made by the RER. That basically can jam up the cells' protein export system, so no insulin gets out and the cells may die. This may be what happens in some people that develop diabetes. But it's not only insulin production that can get messed up. The backup may cause other proteins produced by the RER to get jammed up too. What is it that you noticed about the experimental worms that caught your attention in the first place?"

"Oh, yeah—the worms were twitchy."

"That's right. In addition to insulin not getting out of cells, other exported proteins don't get out. The protein I'm really interested in studying is found in the brain of humans, but is found in *C. elegans* too. When this protein, called alpha-synuclein, doesn't fold right, it leads to apoptosis of the cells, and in humans apoptosis of these cells in the brain is associated with both Alzheimer's and Parkinson's disease. It can even cause problems in the smooth ER too." Dr. Eniz stopped and looked at Claire and Rick for questions.

"So you think that stressing the worms somehow is what causes them to be twitchy?" Claire asked.

"That's one of the many questions that need to be answered. And if endoplasmic stress leads to diabetes. We are only at the start of this research. It may be years before we really know," Dr. Eniz responded. "So, we better get back to work."

Questions

1. In groups of four, have each person look up one of the following diseases and describe the symptoms of that disease. Relate what you have learned to how endoplasmic stress and cell death could cause these symptoms. Include sources of information.
   - Type II diabetes
   - Parkinson's
   - Alzheimer's
   - Fatty liver disease
2. Using an online resource such as *Science Daily* or *Science News,* search for a short news article using the term "endoplasmic stress response" or "unfolded protein response." Read and summarize the article in one paragraph. Relate it back to what you have learned in this case in a second paragraph.

References

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