
Cell Biology

Weeks 4-7

Content focus: Cell biology

READI practice focus: Building a repertoire of literacy and discourse practices

Text is increasingly used as a way to deepen students’ understanding of scientific phenomena. Attention is given to the kind of evidence embedded in different text types (written and visual), the kinds of interpretations one can make given this evidence, and how this helps students construct explanations for scientific phenomena.

Students utilize a READI science module to build knowledge of the conventions of scientific models. The criteria for evaluating scientific models is the curricular foundation of these weeks. Students’ awareness, confidence and ownership of science reading and reasoning practices grows. To accomplish this, teachers and students will draw from these routines and practices:

<p>Build a repertoire of literacy and discourse practices by...</p>	<p>Resources in READI PD and Reading for Understanding (page numbers are from RfU except where noted)</p>
<p>Building a practice of previewing texts to set purpose and strategize ways to tackle challenges.</p>	<ul style="list-style-type: none"> • Predicting and previewing: 222-226 • READI Day 4: Questioning and handling roadblocks
<p>Investigating how to handle roadblocks in science reading; practice handling roadblocks; establish that confusion is cool.</p>	<ul style="list-style-type: none"> • Reading as problem solving: 192-205, 26-272 • Establishing an inquiry culture: 97-100
<p>Engaging students in questioning while reading science text; model and practice to extend the range of questioning (pushing beyond “I wonder about” or “What is...?” to “How do they know?”, “How does it work?” and “Why does that happen?”) and digging in to answer one’s own questions.</p>	<ul style="list-style-type: none"> • Questioning: 210-216, especially ReQuest (213) and Question-Answer Relationships (214-15) • Disciplinary focus for questions: 274-276 (knowledge for putting “generic” reading strategies to use in science reading)
<p>Using evidence and interpretation (E/I) charts to help make sense of text, to differentiate evidence and interpretation, and to build knowledge.</p>	<ul style="list-style-type: none"> • Metacognitive Double-Entry Journal: 110-118, 164-165

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<p>Looking at a variety of visual representations in science, including both examples and non-examples of scientific models, to establish purposes/processes for reading models and criteria for evaluating models.</p>	<ul style="list-style-type: none"> • Diagram Dialogues and visual texts: 208-210 • READI PD and READI Models Module
<p>Developing dispositions towards scientific models as critical texts used to engage in scientific practice – to raise questions, to make sense of one’s own understanding, and to provide an explanatory account.</p>	<ul style="list-style-type: none"> • Disciplinary reading practices: 274-276 • Integration of reading strategies: 225-232 • READI PD
<p>Utilizing texts with multiple levels of difficulty, supporting a single learning goal (in cell biology) including models.</p>	<ul style="list-style-type: none"> • Vertical text sets: 144, 147
<p>READI provides six recommended texts and the READI Reading Science Models Module</p>	<ul style="list-style-type: none"> • See next table for texts.

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This table offers a description of each text and recommendations for READI / Reading Apprenticeship routines and practices to support student reading development and learning.

Text	Content and Practice Learning Goals	READI / Reading Apprenticeship Practices and Routines
<p>Excerpts of “Inside the Cell” (pp. 5, 32-34) and a textbook diagram of cell</p> <p><i>National Institutes of Health (NIH) 2006</i></p>	<p>Comparing and contrasting form and function of specialized cells. Invites students to wonder and ask questions about the cell and the purpose of cell differentiation. Presents cells “in context” of the body.</p>	<ul style="list-style-type: none"> • Talk to the Text foregrounding: reading diagrams, especially how model represent / approximate phenomena (actual cells), questioning, schema connections and knowledge building • Begin Individual Evidence and Interpretation notes focused on Cell Structure(s) and Function(s) as well as poster* version. • Think-Pair-Share discussion of affordances of specialized vs. generic cell models • Metacognitive Conversation and begin a Reading Strategies List for science models • Update Reading Strategies List
<p>“Cell Size and Scale”</p> <p><i>Genetics Science Learning Center University of Utah Health Sciences</i> (online simulation)</p>	<p>Size and scale of various cells, organelles, and microorganisms; mathematical reasoning, microscopy.</p>	<ul style="list-style-type: none"> • Introduce Think Aloud foregrounding: reading and investigating with online simulations, questioning, predicting, comparing, reasoning, attending to scale (size) and measurement, connecting visual and written texts. • Metacognitive Conversation and update Reading Strategies List • Add to Evidence and Interpretation notes focused on cell structure(s) and function(s)
<p>“Science schisms” (pdf, pages 18-19)</p> <p><i>Inside the Cell. National Institutes of Health (NIH) 2006</i></p>	<p>Discussion of competing models of the organelles (Golgi); emphasis on evidence-based argument (and data) as a central feature of science models as ways of communicating science ideas.</p>	<ul style="list-style-type: none"> • Talk to the Text foregrounding: previewing, setting own reading purpose, connecting visuals and “text,” structure/function, role of data in advancing science knowledge, questioning focused on mechanism, explanation (not exclusively) • Metacognitive Conversation and RSL • Add to Evidence and Interpretation notes focused on cell structure(s) and function(s) • Discuss and write to consolidate understandings

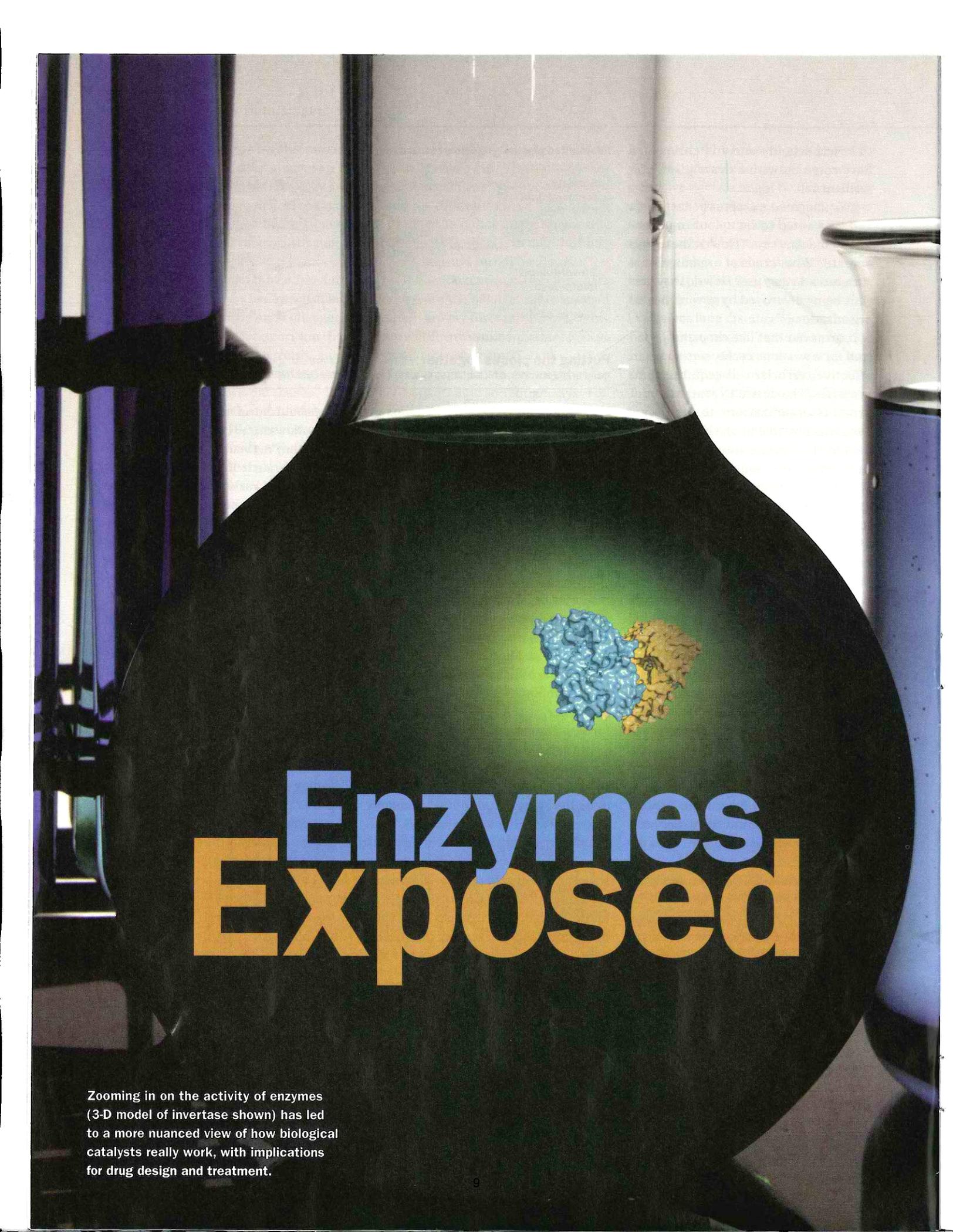
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<p>“When cell communication goes wrong.” <i>Genetics Science Learning Center University of Utah Health Sciences</i> 2 pages or online</p>	<p>Cellular communication and its role in preventing breakdown and disease. Cell signaling, differentiation, and interconnectedness of different systems in the human body.</p>	<ul style="list-style-type: none"> • Talk to the Text foregrounding: Previewing, setting own reading purpose/process, pairing visuals with written texts, making connections (text-to-schema, text-to-text, text-to-visual), asking questions • Metacognitive Conversation update Reading Strategies List • Add to Evidence and Interpretation notes focused on cell structure(s) and function(s) especially cell signaling
<p>“Enzymes Exposed” (pdf) <i>Science News Magazine 2010</i></p>	<p>Structure/function (enzymes/substrate interactions), biochemical reactions, role of new technology/methodology in generating new data and challenging existing science knowledge.</p>	<ul style="list-style-type: none"> • Talk to the Text foregrounding: Previewing, setting own purpose/process for reading, handling roadblocks, chunking texts, visualizing, questioning, predicting, reading models • Add to Evidence and Interpretation notes focused on cell structure(s) and function(s) especially cell signaling • Discuss and write to consolidate understandings
<p>“Transformation of Scientific Knowledge in Biology: Changes in our Understanding of the Living Cell through Microscopic Imaging” <i>Max Plank Institute for the History of Science 2010</i></p>	<p>Discussion of the relationship between technological advances in science and increased science understanding: issues of scale and how the size of cells puts limits on our understandings, history of cell biology, understanding how technology allows for revision of hypotheses and models about cells.</p>	<ul style="list-style-type: none"> • Talk to the Text foregrounding: Making connections between texts (e.g. Cell Size and Scale, Science Schisms) • Metacognitive conversations with partners, adding to RSL list (specific to tables or figures) • Engaging images, opportunity to discuss scale and limitations to new understandings, the role of technology (microscopes) in aiding scientific discovery • Add to Evidence and Interpretation notes focused on cell structure(s) and function(s), especially cell signaling • Discuss and write to consolidate understandings about cell structure and function, especially about cell signaling

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<u>Recommended excerpts:</u> Section 4.1.1 (Early cell biology, pp. 46-7); Figure 16 on page 30; Table 1 on page 14)		
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Note: When Talking to the Text is listed, Think Aloud and Metacognitive Reading Logs may be used as well. The goal is to extend students' thinking while reading, to make their thinking visible and available for metacognitive conversation of how and what they read.



Enzymes Exposed

Zooming in on the activity of enzymes (3-D model of invertase shown) has led to a more nuanced view of how biological catalysts really work, with implications for drug design and treatment.

Clearer views of the cell's movers and shakers threaten a century-old mainstay of biology

By Lisa Grossman

In some ways, cells are a lot like cities. Maps of a cell's innards depict thoroughfares linking factories that build large molecules to post offices where those molecules are packaged up and shipped out, for example. The cell's denizens — proteins and other molecules — shuttle around busy cellular byways like people on the street, meeting up, interacting and keeping the whole enterprise going.

But anyone who has ever been delayed on the way to an important meeting knows something about cities that biochemists are just beginning to learn about cells: Maps don't capture a lot of details — traffic, closed roads, a downed tree — that can drastically slow a journey.

For almost a century, biologists trying to describe cells' inner workings have assumed that the differences between map and street didn't matter. That has been especially true for studies of the cell's go-to, workhorse proteins called enzymes, which orchestrate the majority of the chemical reactions necessary for life. A revered textbook formula that describes how these crucial molecular catalysts speed up reactions, the Michaelis-Menten equation, assumes that enzymes don't usually get stuck in traffic. Enzymes are supposed to meet other molecules at regular intervals and do their transactions at a constant speed, more like workers on an assembly line than urban pedestrians.

In most laboratory experiments, researchers make sure that molecules can move freely and interact often, so the classic formula seems to work well. But lab experiments don't reflect the inner lives of cells. And the differences could dethrone the venerable equation.

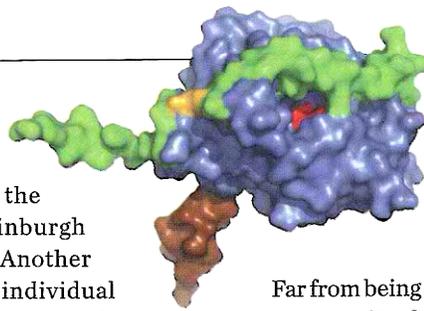
Because of the crowds, in a cell it's more difficult for enzymes to find their

partner molecules than it is in a test tube, biophysicist Ramon Grima of the University of Edinburgh recently showed. Another study noted that individual cells can have different numbers of enzymes — even when the cells are otherwise identical. Combined, the new results could mean models of enzyme kinetics based on the Michaelis-Menten equation are wrong.

"It's a system that people thought they had understood for 100 years," says enzymologist Kerstin Blank of Radboud University in Nijmegen, the Netherlands. "Now we get some new information that, a little bit, turns everything upside down."

When scientists model the chemistry in a cell, Michaelis-Menten is the default equation for enzymes. "It has a broad impact," Grima says. "Given any biochemical pathway, you'll always find that at the backbone of the pathway you will have a few enzymes. When you're modeling that enzyme, you will naturally assume a Michaelis-Menten equation for it."

By zooming in to the street view, scientists hope that they can draw a more accurate map of the cellular city. Experimental methods for watching enzymes in cells aren't yet good enough to see how important variations in these chemical reaction speeds actually are. But if it turns out that the Michaelis-Menten equation doesn't accurately predict how fast enzymes work in living cells, it could change everything from introductory biochemistry classes to strategies for cancer treatments.



Shape-shifting enzymes like CalB are revealing the role of form in reaction speeds.

Far from being discouraged about having to rewrite their textbooks, though, scientists are now dreaming about how to use this newfound knowledge to engineer new drugs or biofuels. "Ultimately," says Nathan Price of the University of Illinois at Urbana-Champaign, "you want to understand those processes so you can control them."

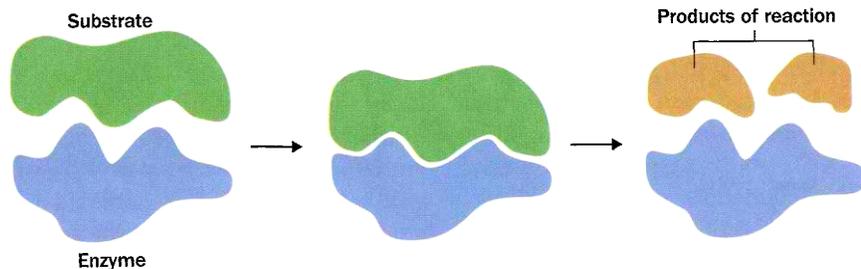
Shape-shifting enzymes

Enzymes make the cellular city run on time. Reactions that would take more than 300 years unassisted can take about a second when an enzyme steps in. By embracing a specific partner molecule, called the substrate, and morphing it into something new, enzymes enable everything from transcribing DNA to digesting food to generating light in fireflies. So understanding how enzymes work is crucial for understanding how cells work — and for manipulating them.

When Leonor Michaelis and Maud Menten published their now-famous paper in *Biochemische Zeitschrift* in 1913, watching an individual enzyme at work was impossible. To figure out how quickly enzymes help transform neighboring molecules from one form to another, the duo had to make do with analyzing test tubes full of billions of molecules.

Michaelis and Menten focused on the enzyme invertase, which helps break down sucrose, ordinary table sugar. If

Lowering the energy Enzymes make reactions in the body go, and go fast, by permitting them to proceed with less energy input than would otherwise be needed. The substrate molecule is held by weak forces, such as hydrogen bonds, in a suitably shaped active pocket in the enzyme. This facilitates the conversion of substrate into products, which are then released without using up the enzyme.



they could have somehow seen exactly what was happening in their test tubes, Michaelis and Menten would have seen the enzyme embrace a sugar molecule (fitting part of it neatly into a cleft) and then breaking it in two. The resulting simple sugars, fructose and glucose, go on to become energy sources for the cell, and the enzyme sits and waits for a new sucrose molecule to come around.

Michaelis and Menten found that the time it takes to transform a spoonful of sucrose to glucose and fructose depends on how much sucrose there was to begin with. The more sucrose, they showed, the faster the reaction — up to a point. After that, the reaction went at a steady pace.

Biologists explained this phenomenon by picturing enzymes and their partners fitting together like a lock and key. Each type of enzyme generally works with only one type of partner, and the two shape themselves to fit together perfectly. But each enzyme can couple with only one partner at a time. When all the enzymes are busy, new partners have to wait for an enzyme to free up.

Researchers were more or less satisfied with that picture for the next 85 years, and plugged in the Michaelis-Menten formula to determine reaction rates in cells. As far as most lab experiments went, it worked.

But in 1998, Sunney Xie, now of Harvard University, and colleagues used a fluorescent marker to watch a single molecule of the enzyme cholesterol oxidase as it met and morphed its partners one at a time. The researchers noticed something strange: The enzyme didn't always work at the same speed.

"If you had simple chemical reactions, you'd expect these times [between one reaction and the next] to be constant," Blank says of Xie's work. "These times are not constant."

The speeds didn't vary randomly, either.

The enzyme seemed to work quickly for several partner molecules in a row, slow down for the next several molecules, then speed up again. If one reaction took a particularly short time, the next one was more likely to go quickly as well, as if the enzyme could remember how long it spent on the last reaction it performed.

In 1998 in *Science*, Xie proposed that the enzyme was flip-flopping between many different shapes, each of which did the same job at a different speed.

"For many years we just thought that the substrate fits in the enzyme with this lock-and-key mechanism. That's what

we all learned at school," Blank says. "It's basically not true."

The leading hypothesis posits that one shape fits best with the partner molecule and so works more efficiently, but takes more energy to maintain, Blank says. Other shapes may not work as well,

but are more energetically relaxed. Scientists think that the enzyme chugs along in a high-energy shape for as long as it can, but inevitably slouches into a couch-potato shape. It stays slouched until a new burst of energy, perhaps from temperature

"We just thought that the substrate fits in the enzyme with this lock-and-key mechanism.... It's basically not true."

KERSTIN BLANK

changes in its environment or from random fluctuations, kicks it back into high-efficiency mode.

"The enzyme molecule, like us, works hard for a while and then slows down," Xie says.

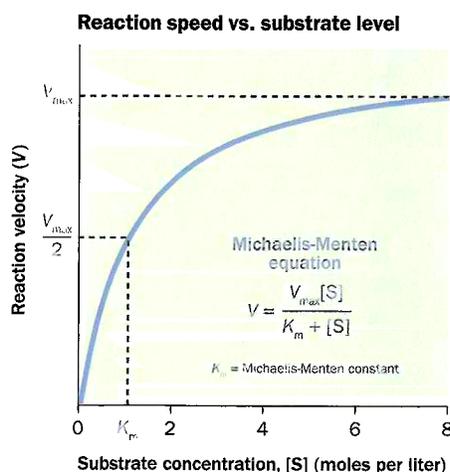
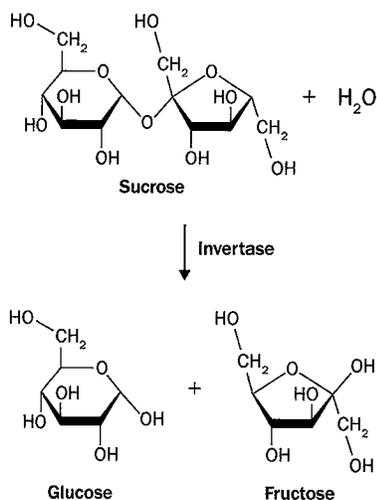
Microscopes still aren't sensitive enough to take snapshots of these shape-shifting enzymes in action, but a decade of research backs up Xie's idea. In one particularly illustrative case, Blank and her colleagues recently found that when they tug on part of the enzyme CalB using an atomic force microscope, the enzyme works faster. Pulling the enzyme may open it up, like pulling on a tab in a pop-up book, changing the enzyme's shape and ability to catalyze reactions.

The ease with which the body absorbs medication and digests food may depend on how much time an enzyme spends in each shape. Blank suggests that shape-shifting enzymes could even drive evolution, if a genetic mutation were to enable a helpful enzyme to stay in a more efficient shape for a longer time.

These shifting reaction rates should shift the outcome of the Michaelis-Menten equation, too. Initially, the scientific community reacted with confusion: If Michaelis-Menten was wrong, why had all the experiments so far worked?

"When Sunney began these researches in the late '90s, people said, 'Gee, if you have these fluctuating rates, how come we almost always see Michaelis-Menten

Sweet biochemistry Leonor Michaelis and Maud Menten's classic work on the speed of enzymatic reactions was based on studies of the conversion of table sugar, or sucrose, into two simpler sugars, glucose and fructose, by the invertase enzyme (left). Michaelis and Menten found that the reaction rate rises with the concentration of sucrose before leveling off at a maximum, shown in graph (right). These studies led to their long-used equation for determining reaction rates.



SOURCE: ENCYCLOPEDIA BRITANNICA, INC.

kinetics? Something must be wrong with your experiment,” recalls Attila Szabo of the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Md.

In 2005, Xie resolved the paradox. He and his colleagues found a partner molecule that let off a burst of fluorescence after reacting with an enzyme. The researchers watched the molecular fireworks show for roughly 20,000 reaction cycles, about 40 times more reactions than were captured in the 1998 studies.

The enzyme was still wiggling and shifting its efficiency every few reactions, the researchers found. But given enough reaction cycles, the differences averaged out. “Enzymes seem to have a changing personality,” Xie says. “But in spite of that, the Michaelis-Menten equation still holds.”

The biochemistry community seemed to breathe a sigh of relief. The 2006 issue of *Nature Chemical Biology* where Xie’s paper appeared also included a commentary titled, “Michaelis-Menten is dead; long live Michaelis-Menten!”

Back-alley trysts

Yet, as Xie and others predicted, still another challenge to the classic equation has been brewing. Recent studies of individual cells suggest that while some of the basic assumptions behind Michaelis-Menten may work in the lab, they don’t always work for real cells.

“In test tubes, you have a very artificial environment,” says Grima, who has explored the basic question of how reactions actually happen in cells.

Cells have a few obvious differences from test tubes. For one thing, cells are crowded. Just the largest molecules inside take up between 5 and 40 percent of the physical volume of a cell. What free space remains is found in tiny compartments that range from about 50 nanometers to just a few micrometers on a side. Enzymes themselves may be between a few and a hundred nanometers long. Some enzyme-assisted reactions can take place only inside the nucleus or other cellular organelles. Inside real cells, liaisons between enzymes and

their partners may be relegated to the back alleys, where only a few individual molecules can fit at a time.

This means that it’s not always easy for enzymes and their partners to find each other. Biologists have shown that cells have what are called active transport networks, filaments that molecules can slide along to travel between meeting places. If enzymes can’t meet locally, they have to take public transport.

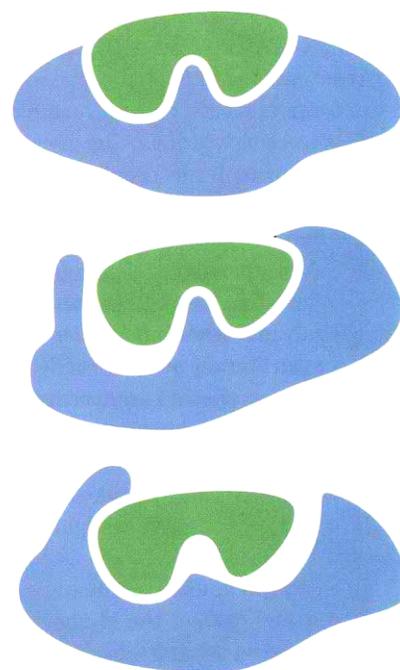
If reactions inside cells are like back-alley trysts, reactions inside test tubes are like square dances in a big hall. With such a large space to move around and researchers constantly mixing the solution, every enzyme is almost guaranteed to dance with every potential partner.

These differences ought to influence how quickly enzyme-aided reactions go, Grima reasoned. There should be some big departures from the Michaelis-Menten equation inside real cells.

In 2009, Grima used mathematical models and computer simulations to show that two basic assumptions behind the Michaelis-Menten equation throw its predictions off in real cells. First, he considered the number of molecules interacting. In a test tube, billions of molecules could come together. But in a cell, only 10 to 100 may meet at any given time.

Accounting for this and other “noise” in a cell, Grima’s model suggests that enzyme reactions in real cells proceed as much as 20 percent slower than Michaelis-Menten predicts. Next, he considered active transport. If partners must ride intracellular subway lines to meet up with their enzymes, Grima found, Michaelis-Menten may overshoot the real reaction rate in a cell by as much as several hundred percent.

A faster reaction rate translates into more reaction products from the same amount of enzyme. For drug designers, miscalculating the amount of product throws off the prediction of how much enzyme should be added to begin with. To explore such implications, Grima ran his simulations for a made-up drug that works by binding to an enzyme before the enzyme’s proper partner can



Flip-flopping forms As an enzyme (blue) shifts its shape, its fit with a substrate molecule (green) shifts too, changing the enzyme’s ability to drive a reaction. Frequent changes in form translate into more variability in reaction rate than the Michaelis-Menten equation takes into account. But the average reaction rate, it turns out, is in line with the equation.

reach it, a phenomenon called enzyme inhibition. In the case Grima studied, the amount of the drug needed to effectively combat the theoretical disease was seven times higher than the amount predicted by Michaelis-Menten.

“When I computed those estimates for drug dosages, that’s when I had the ‘aha!’ moment,” Grima says. “That’s when I thought, oh wow, these things may be actually important.”

Population effects

Price and Pan-Jun Kim of the University of Illinois at Urbana-Champaign think their results, like Grima’s, could have important implications for drug development. Even if Michaelis-Menten does work for one particular cell, variations between cells can pose another threat to the equation — and to the efficacy of drugs designed using it.

“Any enzyme in a chemical soup has a potential chance to catalyze substrates anywhere else in the chemical

soup,” Kim says. This was the case in Xie’s single-enzyme studies: Thousands of partner molecules floated past a solitary enzyme, and each one had an equal chance of reacting with it. But in real cells, each enzyme usually meets only with the partners that happen to live with it.

And different cells may make different numbers of any particular enzyme, even when the cells are genetically identical. A 2008 study in *Science* showed that this difference can literally mean life or death for a cell: Tumor cells that survived treatment with chemotherapy were shown to make more molecules of a particular enzyme than cells that succumbed to the drug, hinting that the enzyme might play a role in drug resistance.

This variation could also mean that even if one cell follows the Michaelis-Menten equation, large groups of cells taken all together might not, Kim says. And drugs designed using equations that ignore the differences between cells could therefore be less effective.

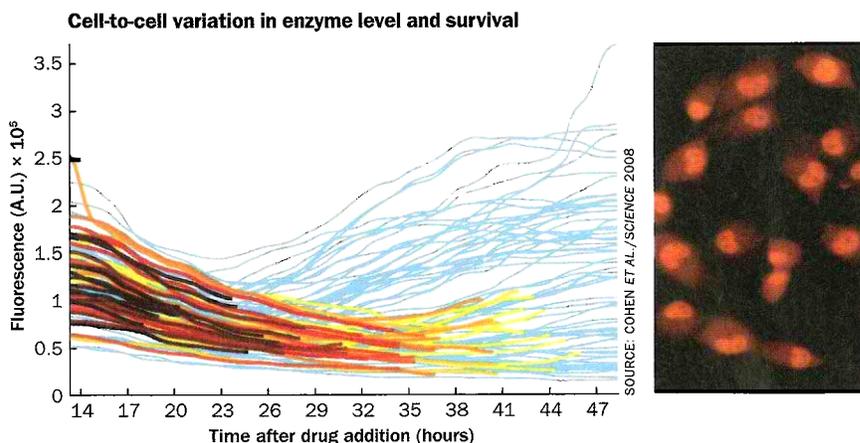
“Even in an ideal situation where the Michaelis-Menten equation might be working well inside a single cell, it is still unavoidable to witness its breakdown at a population of cells,” Kim says.

Kim and Price showed mathematically that using the Michaelis-Menten equation to calculate how fast a large group of cells will perform a reaction gives a different answer than averaging the reaction speeds of each individual cell. By comparing the old equation with new data on single cells, the researchers found that the standard predictions for how fast enzymes work can be off by about 25 percent.

“When we first started this we thought, oh, this looks interesting, but maybe it’s negligible. Turns out they have pretty huge effects,” Price says. “For any scenario where we know protein copy number varies between cells, which looks to be common, you’d be off.”

Enzyme engineering

Understanding how the differences within and among cells change reaction rates can also eventually let scientists engineer better enzymes. Most of Price’s



Life or death Differences in enzyme levels among individual cells were tracked using a red fluorescent marker (right). Human tumor cells (at left, each line represents one cell) that withstood a dose of the anticancer drug camptothecin made more of the DDX5 enzyme (as measured by fluorescence level), while enzyme levels dropped in the cells eventually killed by the treatment. Blue lines mark cells that survived treatment. Other colors show cells that died, with the darker colors corresponding to earlier cell death.

research focuses on building computational models of metabolic networks in cells, which means that he is concerned with what the cell eats and excretes. Ultimately, he says, better models will mean better control.

“You could have an organism that eats something toxic and spits out a biofuel, or something like this,” Price says. “You could convert compounds that are either cheap and abundant, or deleterious, and make them into something valuable and useful and good for the environment.”

Not everyone is convinced that the Michaelis-Menten equation is really doomed, however. When Grima presents his results at conferences, “a lot of people get enthusiastic, and a lot get defensive.”

A major problem is that the new models don’t have experimental data backing them up.

“I wouldn’t consider a few theoretical papers as a substantial claim unless it’s backed up by experiments,” Xie comments. “That’s my experimentalist prejudice.”

Grima acknowledges that this is a weakness of his argument. “At this point, what is limiting its more wide acceptance is combined theoretical and experimental studies,” he says. “Nobody has done a study in which they do a model, make

predictions and then test them, all at one go. This would be the killer.”

Such an experiment may be around the corner. At the moment, there are limited techniques for getting quantitative data on individual molecules inside a cell without killing the cell in the process. But several groups – including Xie’s – are developing more. A recent review paper in *Trends in Biotechnology* heralds single-cell analyses as a new frontier that will transform differences between cells “from a source of noise to a source of new discoveries.”

Even if future observations of the location and concentration of enzymes vindicate Michaelis and Menten, though, many scientists think that the emerging street-level details of the cellular city will continue to challenge traditional ideas.

“Any reaction occurring inside the cell will be impacted by these conditions, but we don’t know exactly how,” Grima says. “We’re probably sitting on top of the iceberg.” ■

Lisa Grossman is a writer in Seattle.

Explore more

- Daojing Wang and Steven Bodovitz. “Single cell analysis: the new frontier in ‘omics.” *Trends in Biotechnology*, June 2010.

COHEN ET AL./SCIENCE 2008

**Transformation of Scientific Knowledge in Biology:
Changes in our Understanding of the Living Cell
through Microscopic Imaging**

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4. Transformation of our knowledge of the cell and the cytoskeleton: From the static to a dynamic concept

Motility is one of the central criteria for life. Studies on movement of cells and cellular components are therefore a major field of study in biology. As discussed above, microscopic imaging techniques play a dominating role in studying cell motility, and with the rapid improvement of microscopy techniques dramatic transformations in our views have occurred.

Here we present a case study of cell science with a particular interest in the ways biological thought has changed over the decades and how these changes in thought may have affected scientific approaches. We have found that the history of research on the cytoskeleton and its role in intracellular motility provides a valuable example to examine the influence of technological innovations of the scientific toolkit on scientific reasoning. Since philosophy of science in the 20th century has focused mainly on physics, we want to analyze whether the specific biological episodes that we are giving an account of can also be made fruitful for philosophical reflections. One of our central questions is: How well do the common criteria of “scientific theories” or “predictions” work in cell biology? We will discuss whether there are such things as paradigms and scientific revolutions in cell biology and if this field functions by constant alternation of the two, as proposed by Kuhn for all of natural science.

4.1. A short history of cell biology

4.1.1 Early cell biology

It was the invention of light microscopy in the 17^h century that allowed the initial observations of the cell and channeled the interest of early naturalists into exploration of the new miniature world. Cell biology therefore started out as a science dealing mainly with structural and descriptive data, a status maintained perhaps until the end of the 19th century - as thorough observation and documentation of what the early optic apparatuses revealed to the previously naked eye. The function of the observed intracellular structures could be interpreted only in the light of the contemporary understanding of living systems until methods were invented to collect the necessary data by experimentation.

The wealth of observed structural detail grew rapidly with the establishment of selective staining procedures first introduced by Francois-Vincent Raspail (1794-1878), (reviewed in Schliwa 2002) and the development of microscopes based for the first time on optical knowledge by Joseph von Fraunhofer, Friedrich Adolph Nobert, Ernst Abbe and others, which provided the ability to resolve structures close to the diffraction limit (reviewed in Gerlach 2009, S399-462). Little however could be said of the function of the newly determined structures. As structures could be made visible only in chemically fixed cells, a debate on reality or artefact of the observed structures ensued (see for

example: Rumjantzew and Wermel 1925). The highly speculative character of functional interpretation posed a serious threat to objectivity before the advent of high resolution vital staining and high resolution microscopy of living cells. Scientists were well aware of this danger, as put by Henry Baker in 1866 (see above). Functional understanding of cellular substructures or mechanisms of cell motility remained a field of hypotheses and predictions but without empirical testing, since live observation with the necessary resolution was not possible and the technology for analytic experimental approaches not developed. Nevertheless, the pioneers of cell biology such as Matthias Jakob Schleiden, Theodor Schwann or Rudolph Virchow (for review see for example Marcello 1999) recognized the cell as living unit which possesses the ability to reproduce, to detect and to react to external stimuli, and with internal mechanisms of maintenance, distribution and translocation of molecules and organelles.

4.1.2 Discovery of the cytoskeleton

The cytoskeleton, as we know it today, describes a network made up of different types of filamentous protein polymers which are found in every living cell and represent part of the cytoplasm. The cytoskeletal fibers are highly dynamic, which is shown as constant elongation and shortening by polymerization and depolymerization. We know now that the fibers are important for maintaining the mechanical stability of the cell but also for cell motion, changes in cell shape and internal transport of organelles or smaller particles.

One of the first scientists to get a glimpse at the cytoskeleton was Robert Remak who observed cytoskeletal fibers in nervous tissue of the crayfish (1843, reviewed in Frixione 2000, Schliwa 2002). These observations were extended by Sigmund Freud (1856-1939) in his doctoral dissertation on vertebrate nervous tissue (Freud 1881). At the Institute of Physiology at the University of Vienna, Freud carried out an investigation on the internal structure of nerve fibers and cells. In pursuing the nature of the “neurofibrils” that formed the basis of the Golgi method, Freud was able to describe fine fibrils following straight courses in the nerve fibers, as well as loose loops surrounding the nuclei. He confirmed and extended the observations made by Remak almost 40 years earlier, which had remained controversial. Later, electron microscopy of the crustacean nervous system confirmed Freud's main points and in turn vindicated those of Remak. Freud was in this way probably the first to picture the intracellular framework that future cell biologists would call the cytoskeleton. However, the existence of these structures *in vivo* had to be defended against accusations of artifact caused by the chemical fixation procedure (see section 3.1).

This could be resolved with the first empirical support for the existence of an elastic intracellular scaffold. The support arose from experiments for which micromanipulation with fine dissection needles or centrifugation were used to actively displace organelles in the body of living cells. This work was carried out on single cells

2.5.3. Video-intensified fluorescence microscopy: Localizing molecules in the cell.

Video intensification is the procedure for making visible low light level objects and scenes generating too few photons to be seen by the naked eye (Fig. 10). Video-intensifier (VIM) or highly sensitive slow scan CCD cameras are needed which amplify low light signals so that extremely weak fluorescence and luminescence, not visible when looking down the microscope, can be visualized (see reviews by Weiss et al.1989, Lange et al. 1995). This is of utmost importance in biology because living specimens benefit from the sparing application of potentially hazardous vital dyes and phototoxic effects caused by excessive illumination. The localization in the living cell of a multitude of proteins under all kinds of different physiological or pathological conditions has led to the situation that we now know exactly which of thousands of proteins are located at which organelle, how they move to their target structures, which their neighbors or ligands are, and where the effectors and signalling molecules are located which cause changes under varying physiological conditions (Figs. 5 and 16).

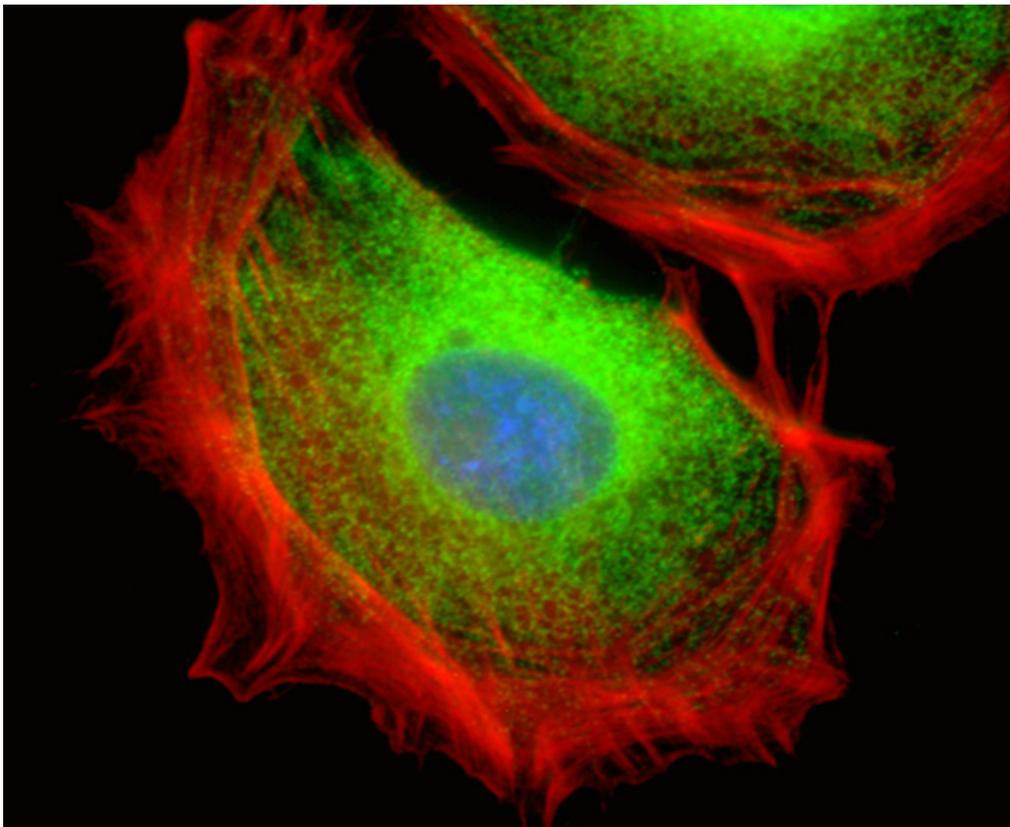


Figure 16. Video intensified fluorescence microscopy (VIM). Simultaneous staining of three cell components in fibroblast cells in culture: actin (red by immunofluorescence), a marker enzyme for the endoplasmic reticulum (green fluorescence caused by GFP-labeling) and DNA (stained with the dye DAPI, blue). Photo Live Cell Imaging Center Rostock, courtesy of Eik Hoffmann.

2. Development of microscopic techniques and their influence on the understanding of the cell

Table 1: Resolution limits of different viewing techniques shown in comparison to the size of cytoskeletal elements.

Limit of resolution and the size of cell components	
Naked eye	0,3mm
Light microscope	250nm (2,000-fold magnification)
Electron microscope	0.2nm (in biology: 400,000-fold magnification)
Cell components	
Actin filaments	7nm
Intermediate filaments	10nm
Microtubules	25nm
Organelles	40 - 2000nm

While in brightfield and darkfield microscopy objects such as the flea or a transparent, stained tissue section are seen in a similar way as macroscopic objects by visual experience, this changed with knowledge-driven microscopy design. Additional physical and material properties of the specimen were now used to create contrast by inserting specific optical elements in the light path. The object is, therefore, often not seen as a whole object, but only some of its optical properties such as birefringent or fluorescent regions are selectively depicted. An overview of contrasting methods and their underlying physical principle is shown in Table 2. (Table 2).

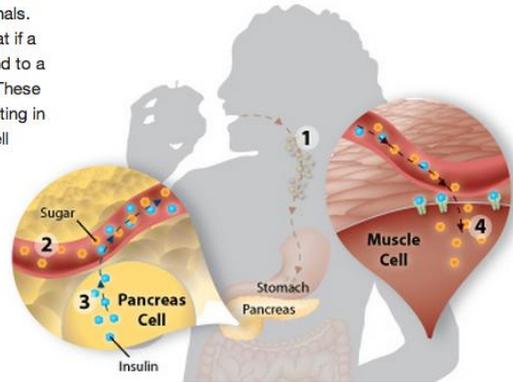
Frits Zernike discovered that differences in the velocity of a traveling light wave passing through materials of different refractive index can be used to generate contrast by inserting a phase retarding ring in a modified light path (Zernike 1935). When a light wave passes through a cell and a closely adjacent wave passes just outside the cell, they will exhibit a relative shift of phases. These, when interfering with each other, lead to constructive and destructive interference which causes a bright halo and dark ring around all objects. This means that the image contains information on the different velocities of travelling light waves passing materials of different refractive index. Zernikes phase contrast microscope creates images of cells without staining (Fig. 4). It is an elegant method to visualize completely transparent, non absorbing objects such as living cells. Phase contrast microscopes are today used in all cell culture laboratories around the world to check the growth of living cells.

WHEN CELL COMMUNICATION GOES WRONG

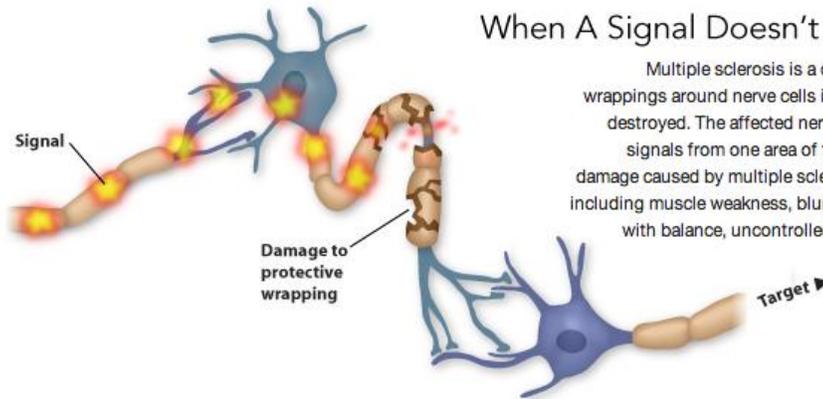
The cells in our bodies are constantly sending out and receiving signals. But what if a cell fails to send out a signal at the proper time? Or what if a signal doesn't reach its target? What if a target cell does not respond to a signal, or a cell responds even though it has not received a signal? These are just a few ways in which cell communication can go wrong, resulting in disease. In fact, most diseases involve at least one breakdown in cell communication.

Losing The Signal

The food that you eat is broken down into sugar, which enters the blood stream. Normally, cells in the pancreas release a signal, called insulin, that tells your liver, muscle and fat cells to store this sugar for later use. In type I diabetes, the pancreatic cells that produce insulin are lost. Consequently, the insulin signal is also lost. As a result, sugar accumulates to toxic levels in the blood. Without treatment, diabetes can lead to kidney failure, blindness and heart disease in later life.



Normal blood sugar regulation. After food enters the body (1), it is broken down and sugar enters the bloodstream (2). Sugar stimulates cells in the pancreas to release insulin (3). Insulin travels through the blood to other cells in the body and signals them to take up sugar (4).

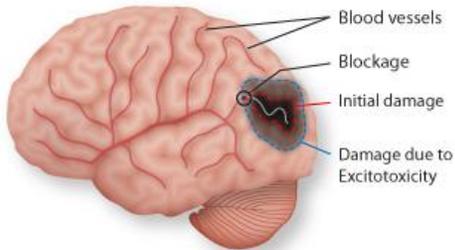
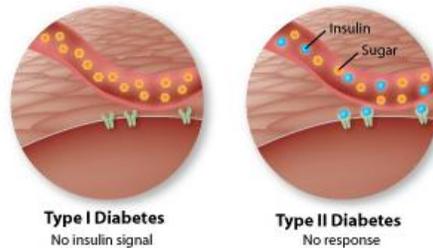


When A Signal Doesn't Reach Its Target

Multiple sclerosis is a disease in which the protective wrappings around nerve cells in the brain and spinal cord are destroyed. The affected nerve cells can no longer transmit signals from one area of the brain to another. The nerve damage caused by multiple sclerosis leads to many problems, including muscle weakness, blurred or double vision, difficulty with balance, uncontrolled movements, and depression.

When The Target Ignores The Signal

Type I and type II diabetes have very similar symptoms, but they have different causes. While people who have type I diabetes are unable to produce the insulin signal, those with type II diabetes do produce insulin. However, the cells of type II diabetics have lost the ability to respond to insulin. The end result is the same - blood sugar levels become dangerously high.



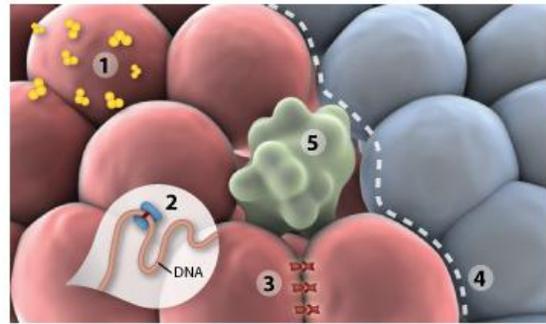
Excitotoxicity: After a brain injury such as a stroke, lethal amounts of signaling molecules leak out of dying nerve cells resulting in widespread damage to the brain.

Too Much Signal

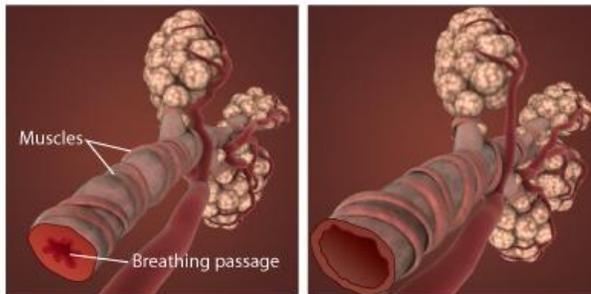
A stroke occurs when a blockage forms in a blood vessel, cutting off blood flow to part of the brain. The immediate result is the death of nearby brain cells. But the most catastrophic event comes later, when the dying cells release large amounts of the signaling molecule glutamate. Low concentrations of glutamate control many actions in the brain, but at high concentrations it is toxic to cells. Through a process called excitotoxicity, glutamate spreads through the brain and kills cells that were not affected by the blockage, often leading to widespread brain damage.

Multiple Breakdowns

Cell growth and division is such an important process that it is under tight control with many checks and balances. But even so, cell communication can break down. The result is uncontrolled cell growth, often leading to cancer. Cancer can occur in many ways, but it always requires multiple signaling breakdowns. Often, cancer begins when a cell gains the ability to grow and divide even in the absence of a signal. Ordinarily this unregulated growth triggers a signal for self-destruction. But when the cell also loses the ability to respond to death signals, it divides out of control, forming a tumor. Later cell communication events cause blood vessels to grow into the tumor, enabling it to grow larger. Additional signals allow the cancer to spread to other parts of the body.



Many mechanisms maintain appropriate cell growth: Cell division occurs in response to external signals (1). Enzymes repair damaged DNA (2). Cells make connections with their neighbors (3). If these connections suddenly change, neighboring cells send out an alert. Cells respect and stay within tissue boundaries (4). If a cell is beyond repair, it initiates its own death (5).



During an asthma attack, signaling molecules cause a narrowing of breathing passages in the lungs, making it difficult to breathe.

Many drugs that treat asthma mimic natural signals that tell muscle cells in the lungs to relax, allowing breathing passages to open.

Treatments

Just as cell communication can go wrong resulting in disease, many disease treatments rely on cell communication. If you think of disease as a roadblock in cell communication, treatment is an alternate route.

The first step is to locate the problem. The second step is to find a way around the problem. Sometimes it's easy. The treatment for type I diabetes is to inject insulin into the blood stream. Other times it's more difficult, especially in diseases such as cancer where cell communication has broken down in multiple places.

Source: The Learn Genetics Center, The University of Utah.

<http://learn.genetics.utah.edu/content/begin/cells/badcom/>