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The Nobel Collection, Volume 3

Edited by
Idan Segev and Robert Knight

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Frontiers for Young Minds believes that the best way to make cutting-edge science discoveries available to younger audiences is to enable young people and scientists to work together to create articles that are both accurate and exciting. That is why distinguished scientists are invited to write about their cutting-edge discoveries in a language that is accessible for young readers, and it is then up to the kids themselves – with the help of a science mentor – to provide feedback and explain to the authors how to best improve the articles before publication. As a result, Frontiers for Young Minds provides a collection of freely available scientific articles by distinguished scientists that are shaped for younger audiences by the input of their own young peers.

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The Nobel Collection, Volume 3

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About this collection

This third Volume of our unique Nobel Collection brings you new, fascinating articles by Nobel Prize winners (called Laureates), written specifically for young minds. These amazing scientists explain their ground-breaking discoveries and how they achieved them, and also share their insights on how to make your own path in a science career in a way that leads to a happy future. Like everything Frontiers for Young Minds publishes, these articles have been reviewed and approved by young students like you!

What Are the Nobel Prizes?

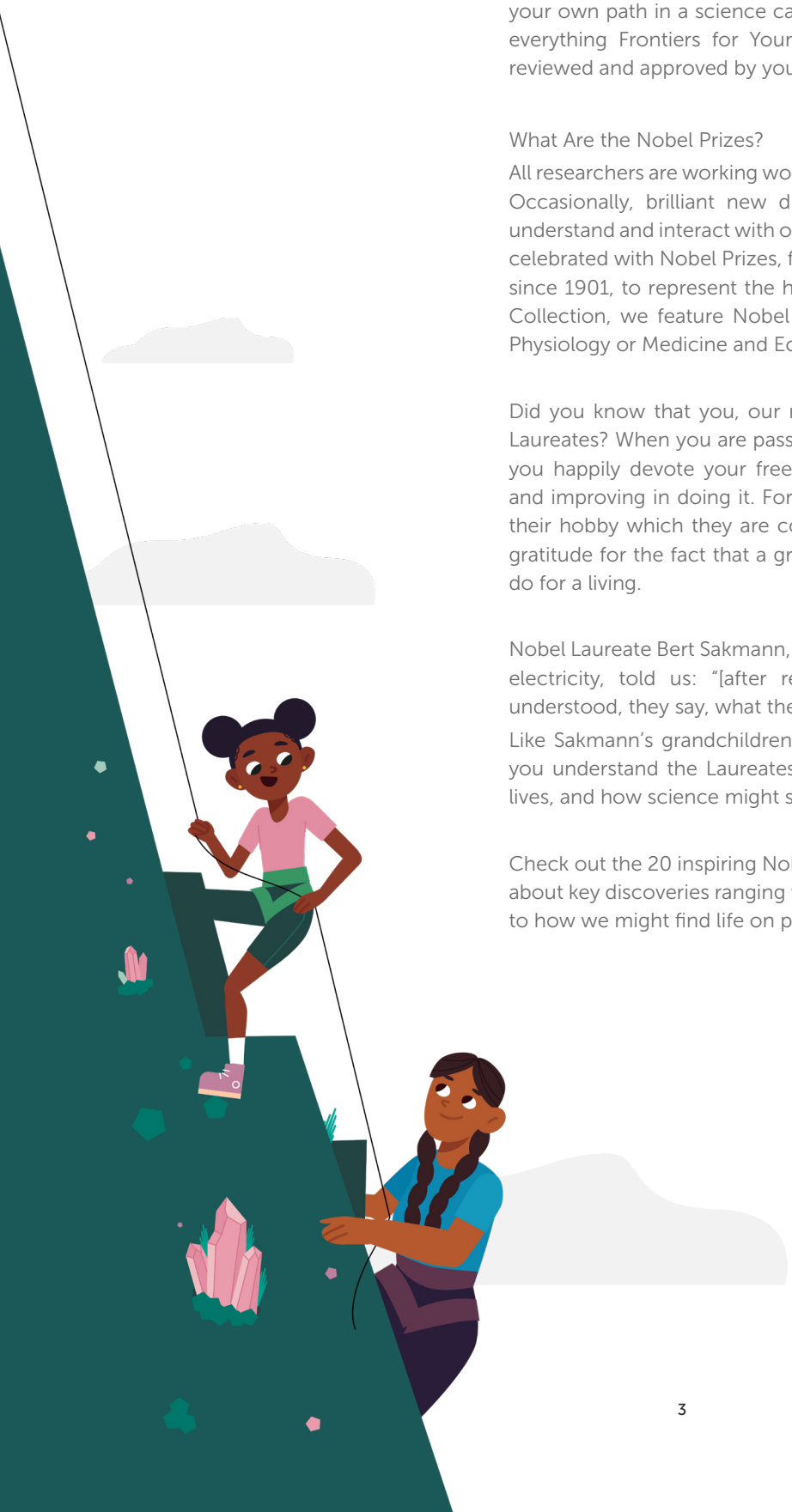
All researchers are working worldwide to add to the sum of human knowledge. Occasionally, brilliant new discoveries can totally transform the way we understand and interact with our universe and ourselves. These discoveries are celebrated with Nobel Prizes, founded by Alfred Nobel in his will and awarded since 1901, to represent the highest level of recognition for research. In our Collection, we feature Nobel Laureates in the fields of Chemistry, Physics, Physiology or Medicine and Economics.

Did you know that you, our readers, share important traits with our Nobel Laureates? When you are passionate about something, like a hobby or a skill, you happily devote your free time to it and enjoy the process of learning and improving in doing it. For many Nobel Laureates, their scientific work is their hobby which they are continuously curious about. They often express gratitude for the fact that a great interest or skill of theirs became what they do for a living.

Nobel Laureate Bert Sakmann, who discovered how cells in the brain generate electricity, told us: "[after reading my article] my grandchildren, finally understood, they say, what their grandfather was doing for a living!".

Like Sakmann's grandchildren, let the articles published in this volume help you understand the Laureates' work, how their discoveries are shaping our lives, and how science might shape your future too!

Check out the 20 inspiring Nobel articles in Volume 1 and Volume 2– find out about key discoveries ranging from how we can live longer and healthier lives, to how we might find life on planets beyond our solar system!



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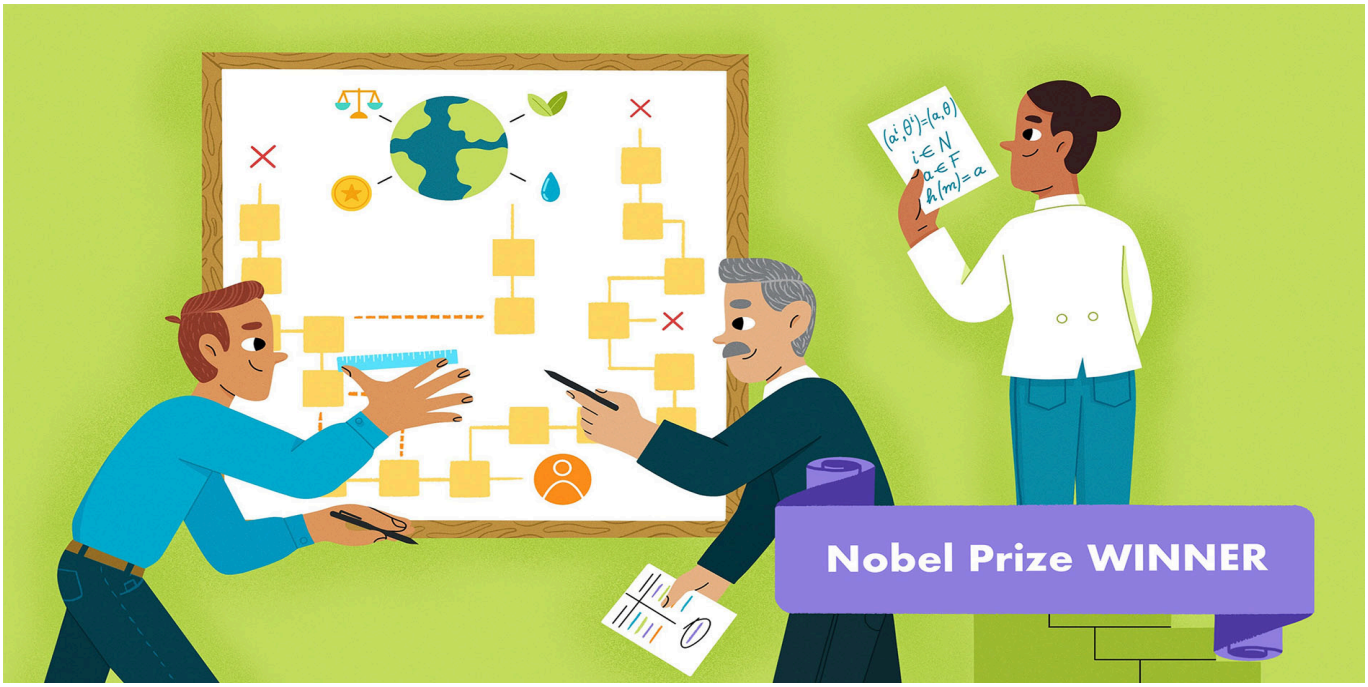

CAN WE USE MATH TO DESIGN A BRIGHTER FUTURE?

Eric Maskin *

Department of Economics, Harvard University, Cambridge, MA, United States

YOUNG REVIEWERS:

DIVYA
AGE: 13

LI
AGE: 11

Did you know that math can be used to help improve society? Well, believe it or not, it does so on a daily basis. Math not only helps us develop new technologies and engineering techniques, but also enables us to design modifications of our society that achieve desirable social goals—goals like reducing pollution and allocating resources to the people who value them the most. In this article, I will tell you about a branch of economics called mechanism design which helps economists do exactly that. Using mechanism design, we can reach important social goals that could not be attained without the modifications it points to. Keep reading to join me on a journey describing an economic theory that can help design a brighter future for us all.

Professor Maskin won the Nobel Prize in Economics in 2007, jointly with Profs. Leonid Hurwicz and Roger Myerson, for having laid the foundations of mechanism design theory.

CAN WE DESIGN MODIFICATIONS TO THE ECONOMY?

ECONOMY

A system in which goods and services are created and distributed.

A modern **economy** is a complex phenomenon that no one understands completely. When we think about the economy, we usually think of elements such as buyers and sellers, companies and consumers. These elements typically interact with one another pretty freely. That is, the interactions are largely not under the control of any supervisor such as the government. Nonetheless, using economic principles, laws, and regulations, we can often make modifications to the economy that improve people's lives.

EXTERNALITIES

By-products of an economic activity that affect others but are not taken into account by the one pursuing the activity.

For example, modification can help in an economy with important **externalities** [1]. An externality is the effect that a person or company has on others but that the person or company has no reason to take responsibility for. Take air pollution, for instance. If a steel manufacturing factory emits smoke into the air, that smoke will harm other people and damage the environment. But unless there is some sort of intervention, there is usually nothing stopping the manufacturer from continuing to pollute the air.

You might think that designing a modification to control air pollution is very easy—we can just prohibit everyone from releasing smoke into the air. But that would be "overkill." Such a strict regulation would cause many businesses to shut down, and that would be bad for society. Alternatively, we can take a more sophisticated approach to reducing air pollution, while still allowing businesses to flourish. For example, we could require companies that emit smoke to pay a tax proportional to the amount of smoke they emit (Figure 1). Maybe for every metric ton of smoke emitted they must pay, say, $100. Then, if a company emits 10 metric tons of smoke, they would have to pay $1,000. The idea here is that the polluter should pay an amount equal to the damage they cause by emitting the smoke. This is a

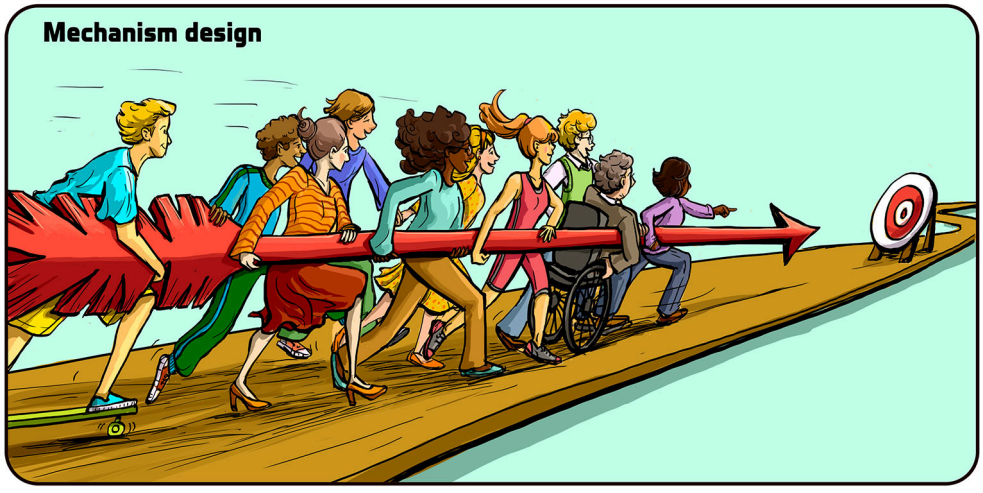

Figure 1

Designing the modifications to deal with externalities. Air pollution is a common example of an externality. Polluters do not generally have an incentive to pollute less, unless the government intervenes in some way. One effective way is to tax polluters based on how much pollution they create. Illustration by: Iris Gat.

clever trick that gives businesspeople the incentive to do the right thing [2]. It ensures that they will take pollution into account when conducting their businesses. The trick is an idea from **mechanism design** [3–6], a fascinating part of economics that has been developing since the 1960's.

MECHANISM DESIGN

An economic theory that studies how to build institutions and procedures to achieve desired economic goals.

As you might expect, in certain situations it might be rather complicated to design the very best intervention to benefit society. Sometimes it may be hard to quantify the harm that a certain activity causes over time; at other times, many parties with different interests are involved, so an intervention needs to take account of all these parties; and often, additional issues such as fairness come into play. For example, to stop global warming, one potential solution is to tax countries according to how much carbon dioxide they release (since carbon dioxide in the air is responsible for the warming). But poor countries may have a harder time paying such taxes than rich countries, and a successful global-warming treaty must factor this in. Fortunately, even for complex problems, mechanism design can be very helpful.

MECHANISM DESIGN—MY LOVE AT FIRST SIGHT

I like to think of mechanism design as the "engineering" part of economics. Usually, in economics, we start by looking at existing economic institutions and try to understand what social outcomes they will lead to. However, in mechanism design, we reverse the direction and begin by deciding what social outcomes we would like to have and then ask how we might intervene in the economy by building institutions or procedures that will give rise to these outcomes (Figure 2). Mechanism design is often used to implement important social

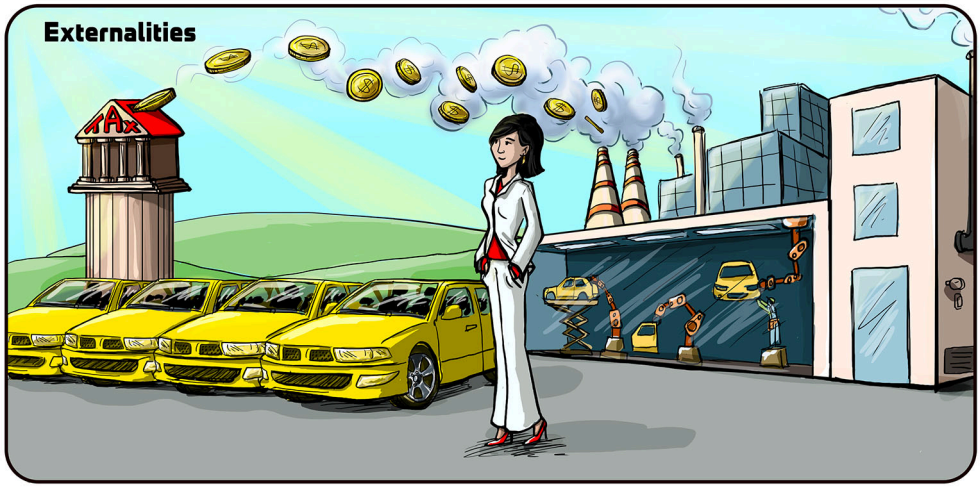

Figure 2

Figure 2

Mechanism design. In mechanism design, we begin by deciding on our desired outcome (represented by the red target), such as a pollution-free environment. Then, we design and build the necessary institutions or procedures (represented by the red arrow) that will generate this outcome. Illustration by: Iris Gat.

goals, such as protecting the environment and establishing fair and effective voting systems.

I first encountered mechanism design theory as an undergraduate at university, where I started out studying mathematics—a subject I had really liked since high school. In my last year at university, I took a course called Economics of Information, taught by Kenneth Arrow—a very well-known figure in the field who won the Nobel Prize in Economics in 1972. One of the topics that Arrow taught us was what would later be called mechanism design. It offered ways that math could be used to make improvements to society. This was a great revelation to me, as I did not know that math could be used this way, and it appealed to me. Like many young people, I felt drawn to do something good for society. Soon after I fell in love with mechanism design, I decided to earn a Ph.D. with Kenneth Arrow as my advisor. And my love for mechanism design is still alive today! More than 50 years later, I am still working in this field and trying leverage it for the benefit of society.

USING MECHANISM DESIGN: MY NOBEL DISCOVERY

To use mechanism design intelligently, we must first determine which social outcomes can and cannot be achieved. There is a part of mechanism design called **implementation theory** [7] that helps us answer this question. Implementation theory allows us to characterize, in mathematical terms, the achievable social goals—the goals that can be reached by some procedure.

IMPLEMENTATION THEORY

A subfield of mechanism design that studies which goals are achievable, and which are not.

For example, imagine a situation in which society has four possible energy sources—natural gas, oil, solar energy, and nuclear energy—to choose from and must select just one of them. Each citizen has a personal ranking of the four options. We might ask: Can society design a procedure to reach the goal of selecting an energy source all citizens will be reasonably happy with—one that makes a good compromise between the rankings of different citizens?

My research on implementation theory implies that the answer to this question is yes provided that the rule for determining the compromise satisfies a condition called **monotonicity** [8]. Roughly speaking, monotonicity means that if, say, solar energy makes a good compromise given a particular configuration of citizens' rankings and we now look at a different configuration in which citizens like solar no less than before (so that if, for example, a citizen previously ranked solar above oil, she continues to rank solar above oil), then solar must continue to make a good compromise for the new configuration.

MONOTONICITY

A key requirement for a goal to be achievable. It demands that if a certain outcome is the goal in one situation and is not ranked lower by anyone in a different situation, then it must also be the goal in the second situation as well.

This discovery about monotonicity was the work singled out by the Prize committee when they awarded me the 2007 Nobel

Prize in Economics, jointly with my colleagues Leonid Hurwicz and Roger Myerson.

AN EXAMPLE OF MECHANISM DESIGN IN ACTION

One important feature of mechanism design is that it allows us to implement goals in situations where we initially lack crucial information. Here is an example: Suppose you have a valuable item that you cannot use yourself and so want one of your friends to have. It can be anything worthwhile—maybe an old guitar, a rare book, or a concert ticket. Since the item is valuable, you want the friend who values it the most to get it. The problem is that you do not *know* how much each friend values the item. What can you do?

You might try setting up a bidding competition between your friends. Each friend makes a bid (an amount of money she is willing to pay for the item) and the winner is the one with the highest bid. However, if the winner actually pays her bid, she will have an incentive to underbid—to bid less than her value for the item. To see this, imagine the value the friend places on the item is $10. If she bids $10 and wins, then she will be getting something worth $10 but paying $10—so, her net gain is $0. That is, the only chance she has for a positive payoff is to bid less than $10. But if all your friends are bidding less than their actual values, there is no guarantee that the one who values the item the most will have the highest bid. In other words, the wrong bidder might win.

Mechanism design suggests how to modify the competition to solve this problem. The winner of the competition will still be the person who bids highest, but you tell your friends that the winner will only pay the second *highest* bid that was offered [9]. For instance, if the highest bid was $10 and the second highest was $9, then the person who offered $10 gets the item for $9 (Figure 3). This simple yet clever procedure ensures that each bidder will bid exactly the amount that the item is worth to her. This is because no one will be motivated to underbid any longer since they do not pay their bids anyway and so do not reduce their payments by underbidding. Moreover, if they do underbid, they could well-regret doing so. If the item is worth $10 to me and I bid only $8, I will lose to someone who offers $9. And that will be too bad for me because if I had bid my true value, I would have won and earned a net payoff of $1 ($10–$9).

Since all your friends will bid their actual values in this modified mechanism, the winner will indeed be the one with the highest value—and so your problem is solved. This bidding procedure—or variations of it—is often used in real-world situations, such as when the government wants to sell radio spectrum to telecom companies.

This is just one example of how mechanism design helps designers (e.g., governments or organizations) to achieve their goals, even when

Figure 3

Mechanism design in action. A bidding procedure that ensures each bidder bids the exact amount that the item is actually worth to them. In this procedure, the highest bidder is the winner, but that person only pays the amount of the second-highest bid. Illustration by: Iris Gat.

Figure 3

they lack important information (in the example, you did not know your friends' values). As I mentioned earlier, mechanism design can also be used for creating international agreements between countries (to reduce greenhouse gas emissions, for example), and for calculating the right taxes on smoke pollution. Over the years, mechanism design has been very successful, and I am certain that it will continue to be helpful for years to come.

RECOMMENDATIONS FOR YOUNG MINDS

What you do in life is very much a personal choice. People have different tastes and preferences about how they want to spend their lives, and many possible choices are valid. Based on my personal experience, however, I want to make a pitch for scientific research as a career. There are very few other jobs in which you will have so much control over what you are doing. In scientific research, you get to decide on the questions that you want to answer (Figure 4A). No one tells you what you should study—you get to choose the topic. This gives you a great sense of freedom and independence that is rarely found in other lines of work.

Additionally, human beings are curious creatures by nature, and they want to know the answers to lots of questions. Currently, science is one of the best means we have for satisfying curiosity. Sometimes science can be frustrating because you can work for a long time and not feel as though you are getting anywhere. So, you have to be patient. But personally, I can think of few occasions in my life when I was more excited or gratified than when I answered a scientific question I had been struggling with for a while. It is a wonderful thrill, and if that kind of reward appeals to you, I definitely recommend a career in research.

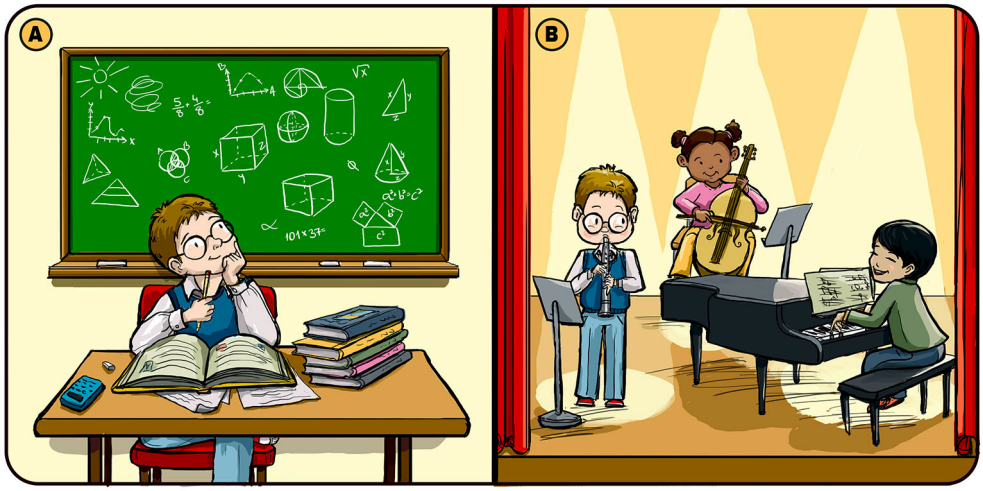

Figure 4

Figure 4

Recommendations for Young Minds. **(A)** For me, one of the biggest thrills in life is answering a scientific question I have been struggling with for a while. If you share this thrill, I advise you to pursue a career in research. **(B)** If you do choose research as your career, I recommend that you find a hobby or activity to enjoy in your leisure time—one that will balance your scientific work by providing an emotional and social outlet. Illustration by: Iris Gat.

If you do choose research as your career, I recommend finding an activity to balance your work. For me, this is playing music (Figure 4B). I play the clarinet, and I have a trio with a cellist and a pianist. Music is a wonderful complement to my professional life. Though doing research is a lot of fun, it is often a somewhat lonely activity and one in which you cannot readily express your feelings. In contrast, making music is highly social and there is a lot of opportunity to put your emotions into the music, so it is a very good outlet. If you are not attracted to music, you can choose some other activity that allows you to freely express yourself and connect with others in beautiful ways. This, together with your work, will give you some balance in life.

ACKNOWLEDGMENTS

I wish to thank Noa Segev for conducting the interview which served as the basis for this paper, and for co-authoring the paper, and Iris Gat for providing the figures.

ADDITIONAL MATERIALS

Mechanism design theory—Eric Maskin—YouTube.

Games and how math can help us win them—Frontiers for Young Minds.

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YOUNG REVIEWERS

DIVYA, AGE: 13
I find the intersection of science and creative writing fascinating. My other passions include classical vocal music and learning languages. In my free time, I enjoy reading, as well as creating and solving puzzles.

LI, AGE: 11
My name is Li. I love reading books, especially mysteries. I am very interested in math, science, and history. I enjoy arts, writing, and swimming. During my spare time, I also play flute and guzheng.

AUTHORS

ERIC MASKIN

Prof. Eric Maskin is an American economist and mathematician. He is the Adams University Professor and a professor of economics and mathematics at Harvard University (United States) and a Chief Research Fellow at the Higher School of Economics (Russia). Prof. Maskin earned his A.B. in mathematics and his Ph.D. in applied mathematics at Harvard University. During his Ph.D., Prof. Maskin took several courses in economics and met Roger Myerson, his co-laureate. Prof. Maskin's research dealt with finding the conditions under which a mathematical function called the social welfare function is solvable. After his Ph.D., Prof. Maskin served as a postdoctoral fellow at Jesus College, Cambridge University (England), where he studied the circumstance under which a mechanism to implement a given social goal could be designed, and he developed what is now called Maskin monotonicity. In 1977, Prof. Maskin joined the Massachusetts Institute of Technology (United States) as a faculty member. Between 1985 and 2000, Prof. Maskin was a faculty member at Harvard University, where he rejoined in 2012 after serving as a visiting professor in the Institute for Advanced Study (New Jersey, United States) between 2000 and 2011. In 2007, Prof. Maskin was awarded the Nobel Prize in Economics, jointly with Leonid Hurwicz and Roger Myerson, for work on what is called mechanism design theory, a theory that attempts to maximize gains for economical players within economic markets. *emaskin@fas.harvard.edu

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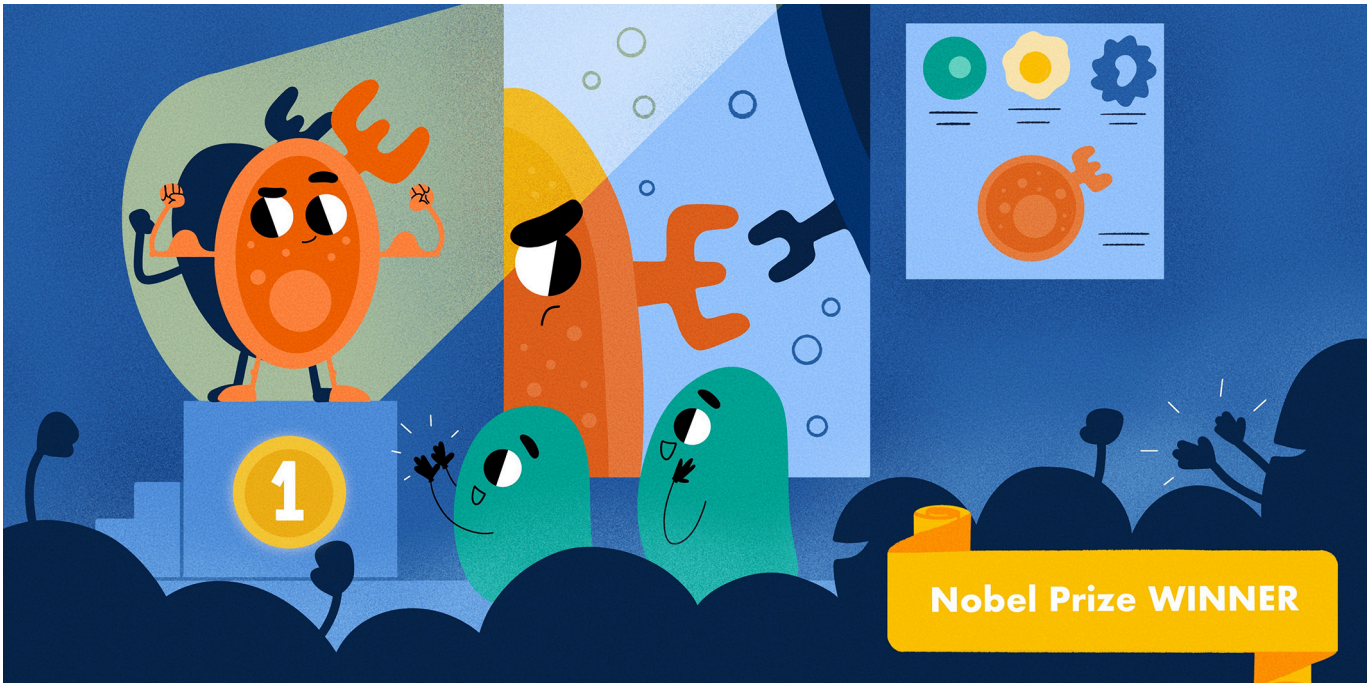

KILLER T CELLS: IMMUNE SYSTEM HEROES

Peter Doherty [1,2*] ***and Noa Segev*** [3*]

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AGE: 11

LOYOLA ELEMENTARY SCHOOL, MRS. RUBINSTEIN'S CLASS
AGES: 10–11

This article is based on an interview between the two authors.

The human body is like a jungle, with trillions of fascinating things happening inside it every second, most of which occur without our awareness. In this article, we will zoom in on a very interesting part of this jungle of human-body activities—the immune system. The immune system protects the body from intruders with its experts in martial arts, called killer T cells. Killer T cells are responsible for eliminating virus-infected cells so that the virus cannot reproduce itself and spread throughout the body. Join me for an adventurous journey into the immune system, in which we will find out how killer T cells know which cells to attack and which to leave alone.

Professor Peter Doherty won the Nobel Prize in Physiology or Medicine in 1996, jointly with Prof. Rolf Zinkernagel, for their discoveries concerning the specificity of cell-mediated immune defense.

THE BODY'S GUARDIAN CELLS

The **immune system** is one of the body's vital systems. It is made up of **white blood cells** and the substances they produce. The immune system is responsible for keeping us healthy by successfully fighting off infections (To learn more about the immune system, see the Additional Materials.). Viruses are one type of intruder that can invade the body and make people sick. Viruses are tiny particles that infect the cells of an organism, which is called the host. Viruses "trick" the host cells into making more copies of the virus. The infected cells usually die, releasing many newly made viruses that can spread throughout the body and infect more cells (To learn more about viruses, see this Nobel Collection article.).

The immune system has two main ways of dealing with viral infections. One strategy involves the production of **antibodies** [1]. Certain **lymphocytes**, called B cells, are responsible for antibody production. Antibodies bind to the virus and inactivate it before it can infect additional cells (Figure 1A). You can think of antibodies like a bunch of football players jumping on the opponent runner (the virus) to prevent the opponent from advancing with the ball.

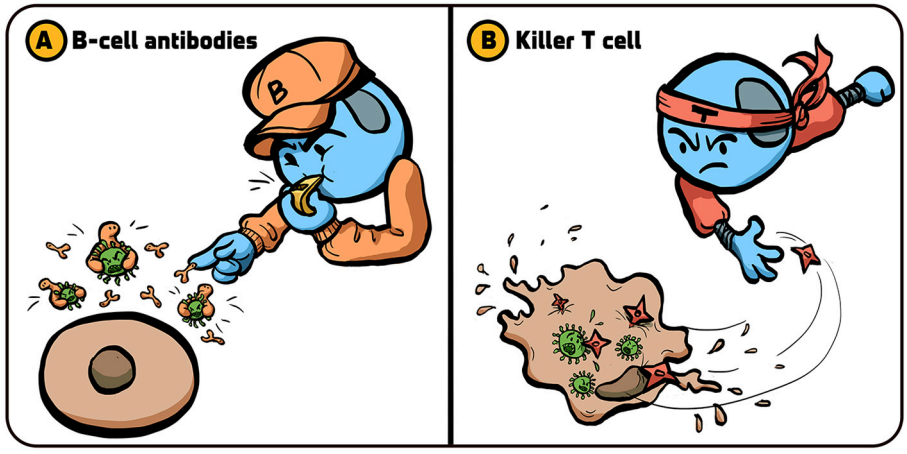

Figure 1

As another strategy, the immune system also kills cells that are infected with viruses (Figure 1B). This prevents infected cells from producing many copies of the virus. The lymphocytes that kill virus-infected cells are called **cytotoxic T lymphocytes**, or killer T cells for short [2]. When killer T cells encounter a virus-infected cell, they kill it by making channels in the cell membrane and triggering the infected cell to self-destruct, through a process called **apoptosis** (To learn more about apoptosis, read this article.).

IMMUNE SYSTEM

The defense system of the body that protects us against infections and certain diseases.

WHITE BLOOD CELLS

the main players in the immune system that help the body fight infections.

ANTIBODIES

Proteins that attach to viruses and inactivate them.

LYMPHOCYTES

White blood cells responsible for producing antibodies and killing infected cells. B cells and T cells are lymphocytes.

Figure 1

Strategies used by the immune system to fight viruses. The immune system has several strategies for dealing with viral infections. **(A)** B cells release proteins called antibodies (small "pliers") that attach to viruses (green) and prevent them from penetrating into the body's cells. **(B)** Killer T cells kill cells that are infected by viruses, preventing the production of new viruses that can infect additional cells. Illustration by: Iris Gat.

CYTOTOXIC T LYMPHOCYTES

Also called killer T cells; white blood cells that kill virus- infected cells.

APOPTOSIS

A "suicide" process of cells that gets rid of damaged or infected cells.

IS IT ME OR IS IT NOT ME?

The immune system uses extreme measures, like killing cells, to deal with potential hazards. How does the immune system know which structures to make antibodies against or which cells to kill? In other words, how does it eliminate infections without harming the body itself? Research has shown that this is a fine balance, and at the heart of it lies a fundamental question: what is me, or "self" (my own cells and molecules) vs. not-me, or "non-self" (intruders)? It is critical that the immune system answers this question correctly. If, on the one hand, the immune system does not recognize intruders well-enough, then the host remains unprotected against those threats. On the other hand, if the immune system mistakenly recognizes *the body's own cells* as intruders, it might attack the body and cause serious damage. Diseases resulting from a misguided immune system attacking the body are called **autoimmune diseases**, and many of them do not have effective treatments (Researchers are currently checking the possibility that the SARS-CoV-2 virus causing the COVID disease is triggering some rare form of autoimmunity in infected people. Such autoimmunity could be one possible cause of the long-COVID manifestations we are seeing.).

AUTOIMMUNE DISEASES

Diseases where the body is being attacked by its own immune system.

TWO RECEPTORS VERSUS ONE?

When I started working on killer T cells in the early 1970s, scientists did not yet understand how these lymphocytes could tell "self" from "non-self." After we discovered that killer T cells are in some way targeting "self" molecules (the transplant proteins recognized in organ graft rejections), most scientists thought that killer T cells had two different types of **receptors** (proteins that recognize specific molecules) on their surfaces: one type of receptor that could recognize "self" molecule and another type that could recognize foreign (virus), "non-self" molecules (Figure 2A). The idea was that that "self" molecules and "non-self" molecules are two distinctly different types of elements in the body, that require two different types of receptors to be recognized.

RECEPTOR

A protein on the surface of a cell that binds to a specific molecule that matches its shape, triggering a cellular response.

My colleague Rolf Zinkernagel and I argued that this was not the case—there is actually only *one* type of receptor on the killer T cell changes in "self" molecules (Figure 2B). This idea allowed a different interpretation of **immunology** which, as the technical approaches to answering the question improved, turned out to be right. Before I tell you how the recognition of dangerous invaders happens using only one receptor, let me tell you about some of the milestones that led us to this finding.

IMMUNOLOGY

The study of how the immune system functions.

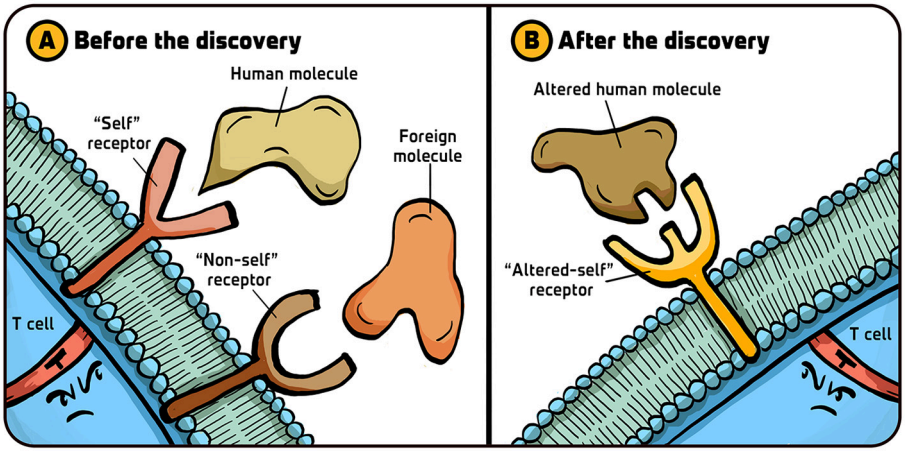

Figure 2

Figure 2

Killer T-cell receptor hypotheses. **(A)** Before our Nobel discovery linking killer T cell recognition to "self," the view that dominated the thinking of those working with "helper" $CD4^+$T cells was that these lymphocytes have two types of receptors on their surfaces—one that recognizes the body's molecules ("self") and another that recognizes foreign molecules ("non-self"). **(B)** After our discovery, it eventually became clear that all T cells have only one immunologically specific receptor, which recognizes changes in the body's molecules ("altered-self"). Illustration by: Iris Gat.

MY PERSONAL JOURNEY: FROM FEEDING THE WORLD TO DECIPHERING IMMUNE SECRETS

When I was 17 years old, I decided to study veterinary medicine. It was a strange decision because I was a city kid in Queensland, Australia. But I thought that learning veterinary medicine would be a great adventure, and that I could contribute to feeding the world by improving animal production. Today, due to climate change, we know that we will be better off, at least in the advanced countries, by focusing on plant products, but back then we had different ideas. During my Ph.D. studies, I became very interested in how infections cause diseases. So, I went on to study infections in sheep and cattle.

One of my early discoveries was made when I was studying a virus infection of the brains of sheep. At that time, scientists were just starting to understand the role of lymphocytes, but they did not yet understand the specific mechanisms by which those cells operate. In Edinburgh, Hugh Reid and I showed that antibody-producing B cells were present in the brains of the virus-infected sheep, and that those B cells were producing antibodies to fight the virus [3]. This was the first direct evidence that antibody-producing cells could be found in an infected tissue. This discovery inspired me very much, and I wanted to know more about what immune cells were doing in tissues.

While working on sheep in Edinburgh, I realized I need to know more about T cells, and arranged to spend a couple of years at the Australian National University in Canberra where there was a very active group of researchers in this area. The experimental system I chose was lymphocytic choriomeningitis (LCMV) infection of mice. From earlier studies, I adapted a technique to tap the cerebrospinal fluid compartment that bathes the brain to obtain the inflammatory WBCs, which were largely T cells that invade the brain and its surrounding membranes (the meninges) following LCMV infection. Then, a medical graduate from Basel University named Rolf Zinkernagel arrived in the

lab. Together, we conducted the experiments that, some 20 year later, won us the Nobel Prize in Physiology or Medicine.

ONE RECEPTOR RECOGNIZING ALTERED-SELF

Rolf and I did the following experiment: we infected laboratory mice with LCMV and, after a week (at the peak of their immune response), we collected the killer T cells the mice produced against LCMV (Figure 3). We placed these killer T cells in little tubes containing LCMV-infected mouse cells. The killer T cells came from LCMV-infected mouse strains that did, or did not, share the same transplantation molecules (called H2 in mice, HLA in humans) as the LCMV-infected 'target' cells. We found that the H2 identical, LCMV-immune T cells did a fantastic job of killing the H2 identical targets, while the H2 different killers totally ignored them. Based on these results, we hypothesized that a killer T cell has a *single receptor that recognizes altered-self (infected cells)* and developed a new theory of T cell immunology around that.

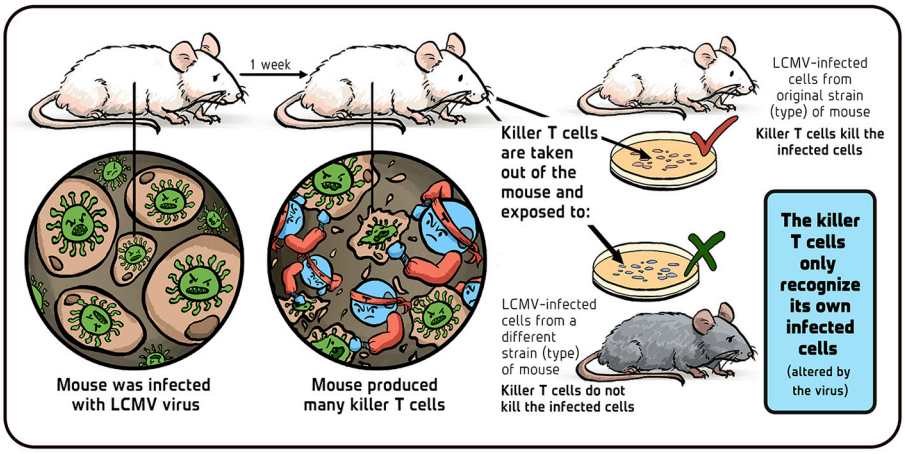

Figure 3

Do Killer T cells attack cells from different mouse strains? Illustration by: Iris Gat.

In other words, we assumed that the virus changes one of the normal self-molecules—molecules of non-infected cells, which are similar in genetically identical mice and different in genetically different mice. These self-molecules are always present on the surface of host cells and we figured that killer T cells spot this virus-induced change as a signal of infection and respond by killing the infected cell [4].

At that time, we did not have the experimental techniques to prove that our hypothesis was correct. But technological advances that happened in the next two decades allowed others to prove that we were right [5–8]. Using these new techniques, self- molecules called **major histocompatibility complex (MHC)** molecules were found on the surface of cells. When a virus invades a cell, the viral proteins get chopped up, and small virus fragments are displayed on the surface of

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

Self-molecules found on the surface of cells.

the cell, "held" by MHC molecules (Figure 4). Holding the piece of virus changes the shape of the MHC molecules, and this change in shape is what the killer T cells "see" and react to [9]. In other words, T cells recognize an infected, "altered-self" cell by the modified structure of its MHC molecules.

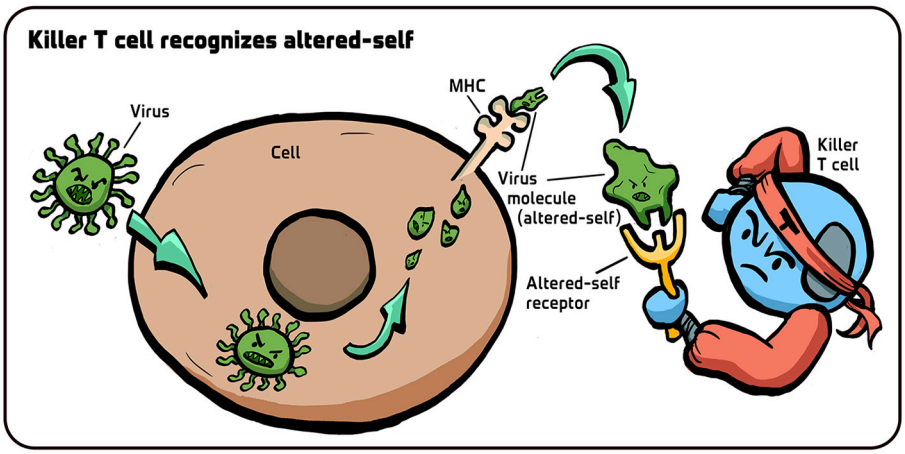

Figure 4

Figure 4

How do killer t cells recognize "altered-self"? When a virus infects a cell, its proteins are chopped up. Small pieces of the virus (small green structures) are then brought to the surface of the cell, held by MHC molecules. The presence of these virus chunks on MHC molecules changes the structure of the MHC molecule, and the receptors on killer T cells recognize this shape change ("altered-self"), thereby realizing that the cell is infected. Illustration by: Iris Gat.

When our theory proved to be correct, it caused a fundamental shift in our understanding of how T cells—one of the most important parts of the immune system—operate.

CAN OUR FINDINGS IMPROVE HUMAN HEALTH?

Our understanding of killer T cells could be helpful in several areas of medicine. First, this knowledge could help us to improve vaccines. Now that we know that the immune system recognizes pieces of viruses "held" by MHC molecules, we can hopefully make better vaccines by including a wide variety of pieces from the virus. The more different pieces a vaccine contains, the easier it is to trigger a strong killer T-cell response. COVID-19 vaccines, for example, only contain pieces of one protein (called the spike protein), while the virus has more than 20 different proteins that could potentially trigger killer T-cell responses.

I also focused a lot of my effort on understanding the nature of the protective, 'resting' memory T cells that are 'recalled' to being killer T cells on reinfection. Others worked out how to 'wake up' killer T cells that had localized to cancers but had apparently 'gone to sleep' (This is how certain anti-cancer treatments work, which you can learn more about here [10]). Other possible uses for this knowledge could involve new treatments for autoimmune diseases, in which we might find ways to restore balance to T cells and B cells that have gone astray and are attacking the body's own cells.

RECOMMENDATIONS FOR YOUNG MINDS

I think that scientific training is quite useful to have, even if you do not eventually become an active scientist. The reason is that, as a trained scientist, you learn how to think through a problem (Figure 5), how to deal with data, how to formulate an argument, and how to clearly write and convey your thoughts and ideas. These are important skills that can be helpful in many situations in life.

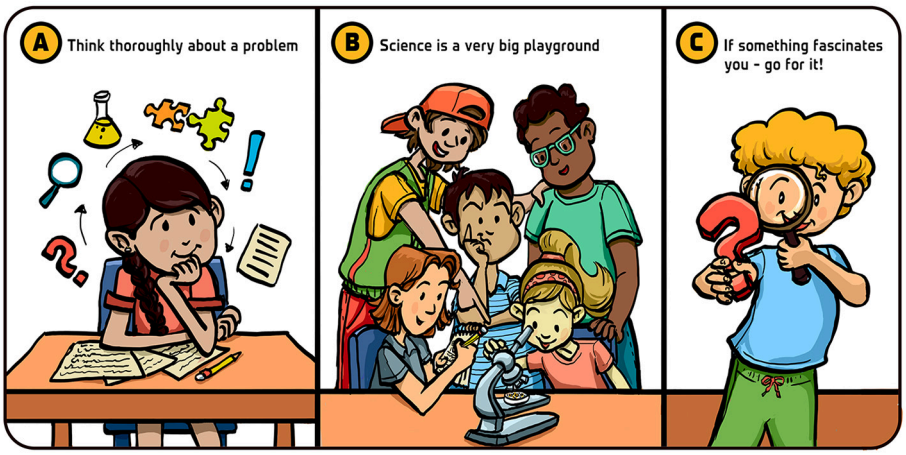

Figure 5

Figure 5

Three recommendations for Young Minds. **(A)** Think thoroughly about a problem. **(B)** Science is a very big playground. **(C)** If something fascinates you - go for it! Illustration by: Iris Gat.

If you *do* wish to become a scientist, and specifically a biologist, then I have some good news for you. First, there is still much more to discover in this field. Second, biology— especially biomedical research—offers great opportunities for many types of people with various skill sets and approaches. Some might have computer skills and want to set up systems that analyze and link data. Others might like to perform thought experiments and formulate new ideas and theories. People with good management or product-development skills can also flourish in biomedical research. It is a very big "playground" that holds space for many types of people. Those of you who enjoy connecting the dots in some way might especially enjoy a career in science.

The last thing I will tell you is: if something fascinates you—go for it! Dive into it as deeply as you can and do everything possible to pursue it. You will never know where your interests might lead you. During this voyage, you will gain unique ways of thinking that will serve you wherever you end up. So, instead of being discouraged by the uncertainty that life brings, be confident in your skills and trust that you will get a chance to use them in exciting ways.

ADDITIONAL MATERIALS

1. How the Innate Immune System Fights for Your Health

2. The Immune System, in Sickness & in Health—Part 1: Microbes and Vaccines
3. Types of immune responses: Innate and adaptive, humoral vs. cell-mediated | NCLEX-RN | Khan Academy
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YOUNG REVIEWERS

ARMAAN, AGE: 11
Armaan is a 6th grader who is a wrestler. His hobbies are video gaming, baking, and wrestling. He also plays basketball. He loves reading and exploring concepts behind science, especially health and disease-related which incites his love for reading more about immune cells.

LOYOLA ELEMENTARY SCHOOL, MRS. RUBINSTEIN'S CLASS, AGES: 10–11
5th graders at Loyola Elementary School never let an opportunity pass them by when it comes to gaining knowledge. Our class took the challenge to read and review an article for Frontiers for Young Minds, which was exciting for all of us to participate in. The article was unique in its timeliness as it was about Killer T-cells and we are just moving past the COVID pandemic. Our school is situated in a little pocket of Silicon Valley with beautiful vistas of the nearby foothills.

AUTHORS

PETER DOHERTY
Prof. Doherty is an Australian immunologist. He studied veterinary medicine and earned his bachelor's and master's degrees at the University of Queensland (Queensland, Australia). During his doctoral studies at the University of Edinburgh (Edinburgh, Scotland), Prof. Doherty studied pathology. He then conducted research at the John Curtin School of Medical Research in Canberra (Australia), where he met

Rolf Zinkernagel. Together, they studied the role of T cells in mice infected with a certain type of virus. This collaborative study led Profs. Doherty and Zinkernagel to discover how T cells distinguish between normal, healthy cells and virus-infected cells. This was a great breakthrough in understanding how the immune system functions, and for this they were awarded the Nobel Prize in Physiology or Medicine (1996). Prof. Doherty is the head of the Peter Doherty Institute for Infection and Immunity, a joint venture between the University of Melbourne and the Royal Melbourne Hospital. Prof. Doherty has won numerous prestigious awards, including the Paul Ehrlich Prize (1983), the Gairdner Foundation International Award (1986), and the Albert Lasker Basic Medical Research Award (1995). He has been awarded more than 20 honorary doctorates and has published some 500 research papers and reviews. He received a Companion of the Order of Australia in 1997, is listed as a living National Treasure and his face appears on a postage stamp. Alongside his scientific career, Prof. Doherty has been deeply involved in science communication and has written several popular science books, including *The Beginners Guide to Winning the Nobel Prize* (2005); *Pandemics: What Everyone Needs to Know* (2013); *The Knowledge Wars* (2015); and *An Insider's Plague Year* (2021). In recent years, Prof. Doherty has also been interested in the science of climate change. He currently lives with his wife Penny in Melbourne, Australia. They have two sons, Michael and James, and six grandchildren. *pcd@unimelb.edu.au

NOA SEGEV

Noa Segev is a scientific writer and project coordinator at Frontiers for Young Minds. She earned her B.Sc. in physics at The Hebrew University of Jerusalem and her M.E. in renewable energy engineering at the Technion-Israel Institute of Technology. Since 2019, she has been interviewing Nobel Prize winners and co-authoring articles for the Nobel Collection at Frontiers for Young Minds. Noa aims to make the science behind Nobel Prize-winning discoveries accessible to all, and to share valuable insights from the vast professional and personal experience of Nobel Laureates. *noasegev@gmail.com

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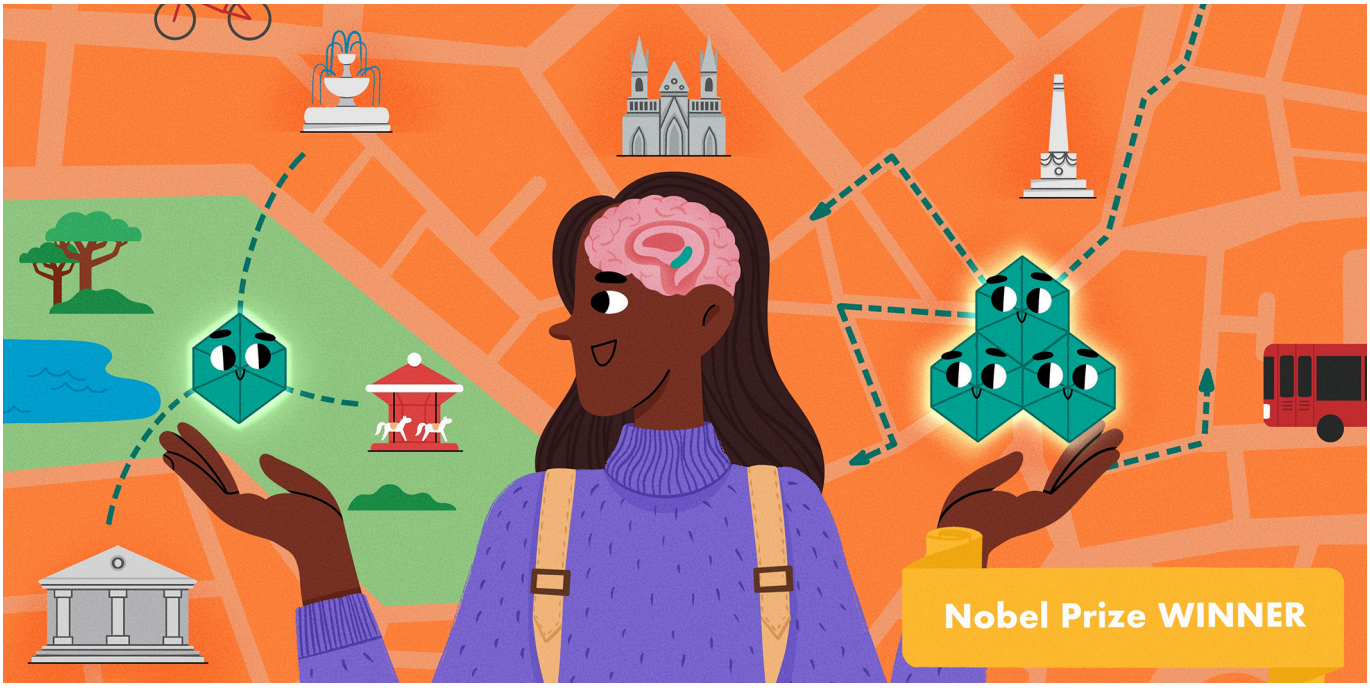

CAN GRID CELLS HELP US UNDERSTAND THE BRAIN?

Edvard I. Moser*[1]* *and Noa Segev*[2]

[1] *Kavli Institute for Systems Neuroscience and Centre for Algorithms in the Cortex, Norwegian University of Science and Technology, Trondheim, Norway*

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YOUNG REVIEWERS:

DEERFIELD ELEMENTARY
AGES: 9–10

OZZY
AGE: 11

THEO
AGE: 11

This article is based on an interview between the two authors.

Grid cells are special brain cells that play a key role in the brain's navigation system. Research on these cells is one of the most interesting and rapidly advancing topics in brain science today. Much has changed since my colleagues and I discovered grid cells in 2005, and even since we were awarded the Nobel Prize in 2014. In this article, I will describe the advancements that transformed the field of grid cell research and tell you about the way that we study grid cells today. Finally, I will give you a peek into how we hope to use our understanding of grid cells as a "window" to understanding the brain as a whole.

This article assumes readers have the basic knowledge shared in previous articles on grid cells and place cells.

Professor Edvard Moser won the Nobel Prize in Physiology or Medicine in 2014, jointly with Prof. May-Britt Moser and Prof. John

O'Keefe, for their discoveries of cells that constitute a positioning system in the brain.

GRID CELLS—A SHORT RECAP

Have you ever wondered how your brain maps the world so you can navigate successfully in it? As you might have read in a previous Nobel Collection article, **grid cells** are a specific type of **neurons** (brain cells) that are an essential part of the brain's navigation system. Grid cells were found by my colleagues and I in 2005 [1]. They are located in a brain region called the **entorhinal cortex**. Each grid cell responds to a specific hexagonal pattern of locations (Figure 1). The joint activity of many grid cells creates an internal "coordinate system" in the brain that helps an animal know where it is in the environment, how to get from one location to another, and how to estimate the distance between points (to learn more about grid cells, you can watch this video).

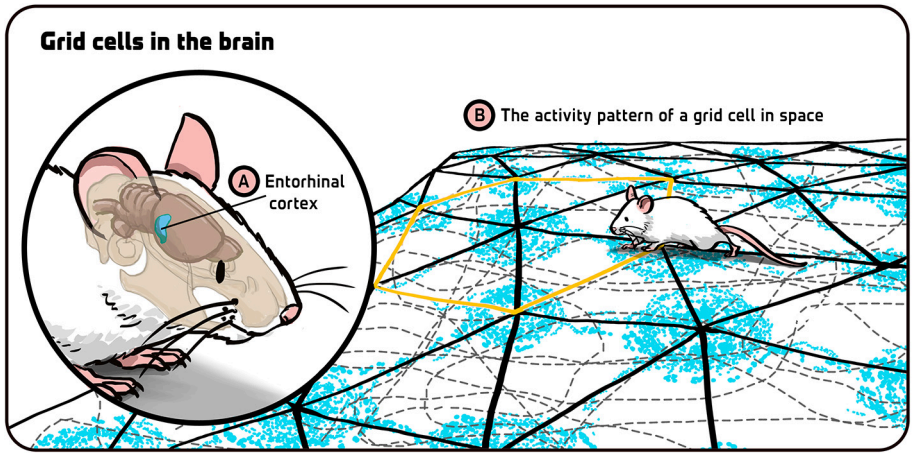

Figure 1

Grid cells work in coordination with other types of navigation cells in the entorhinal cortex, including head-direction cells, object-vector cells and border cells. Grid cells also work in coordination with place cells, which are located in another region of the brain called the hippocampus [2]. Our aim is to understand how groups of grid cells work within themselves and how they work with those other cells as well.

CURRENT RESEARCH IN GRID CELLS

Since our discovery, important and rapid changes have taken place in the field of grid cell research. In 2005, the activity of grid cells was studied by examining the electrical activity of one grid cell at a time, which allowed us to discover the unique hexagonal activity pattern of grid cells. In the past few years, however, the single-cell approach was

GRID CELLS

Neurons in a part of the brain called the entorhinal cortex, whose firing locations form a system of coordinates in the brain that helps an animal navigate through its environment.

NEURONS

Nerve cells; cells in the brain that produce electrical impulses to send signals to the body, brain, or other neurons.

Figure 1

Grid cells in the brain. **(A)** Grid cells are found in a part of the brain called the entorhinal cortex. **(B)** When an animal moves around in its environment (gray dotted lines representing the animal's movement in space), each of its grid cells is active at specific locations (blue dots, representing the activity of one specific grid cell). The activity pattern of each grid cell creates a hexagonal, grid-like pattern in space (yellow hexagon highlighting one such hexagon within the grid pattern). Illustration by: Iris Gat.

ENTORHINAL CORTEX

A brain region containing navigation cells, including grid cells, head-direction cells, object-vector cells and border cells.

ELECTRODES

Measuring devices used to record the electrical activity of neurons.

NEURAL POPULATION RECORDING

Recording of the electrical activity of many neurons simultaneously.

replaced by the study of the activity of many grid cells at the same time. These days, scientists who study grid cells examine the activity of *networks* of grid cells and how these networks represent an animal's environment. But how can we keep track of many grid cells that are active simultaneously?

This new approach was enabled by two technologies. The first was the development of new **electrodes** called Neuropixels probes that enable us to "listen to" neurons by recording the electrical activity of many of them simultaneously (Figure 2A) [3, 4]. The most recent Neuropixels probes contain more than 5,000 recording sites that can pick up electrical signals created by individual neurons. Using Neuropixels probes, scientists can record the electrical activity of whole groups, or populations, of neurons—that is why this type of recording is called a **neural population recording**. Currently, we can record from about 380 sites on a microchip at once, giving us access to the activity of more than a thousand cells at the same time. I expect that by 2025 we will be able to record from most or all possible sites at once, which will increase the cell numbers by another order of magnitude.

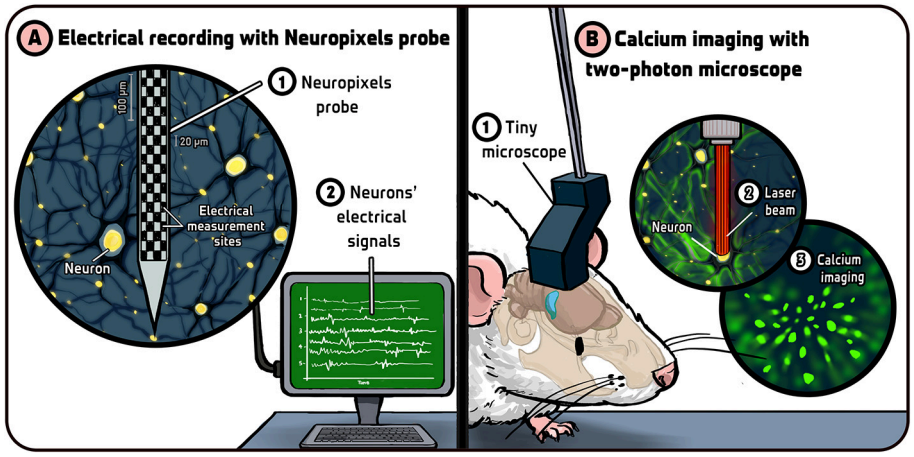

Figure 2

Technological developments in grid cell research. **(A)** Recently, a new device for measuring the electrical activity of many neurons simultaneously was developed, called a Neuropixels probe **(1)**. This device is essentially a small computer chip with many recording sites (black squares) from which it can measure brain activity **(2)**. **(B)** A tiny two-photon microscope is placed on the animal's head **(1)**. It shines a laser beam on neurons **(2)**. The calcium inside the neurons then fluoresces in green, allowing scientists to see active neurons in a specific brain area **(3)**. Illustration by: Iris Gat.

VIDEO 1

Calcium imaging using a two-photon microscope. Using a tiny two-photon microscope, we can track the calcium activity in the brain of an animal (bright spots on the left side of the screen) while it moves freely in its environment (right side of the screen). This tracking allows us to see which grid cells in the entorhinal cortex are active at any given moment [Video adapted from [6]].

The second recent technological advancement was the development of exceptionally light microscopes that can be placed over the animal's head [5]. These are miniature two-photon microscopes that can pick up changes in the flow of calcium (Ca^{2+}) ions within neurons (Figure 2B) and tell us which cells are active at any given moment. Using calcium imaging, we can "see" grid cells by having a visual image of the active cells in the network, and combine different images to create a video of active cells over time (Video 1) [6].

My lab in Norway applies the advanced technologies described above to study grid cell networks. We use Neuropixels probes to record the activity of many cells in the entorhinal cortex simultaneously. Using our knowledge of grid cells, we are able to isolate the grid cells from our recordings and study them in small groups. We then ask whether

certain groups of grid cells act together in a synchronized way, or whether certain groups of cells tend to be active in a specific order or arrangement when the animal moves around in its environment. If we do find such "rules" in our data, we can use them to better understand the activity of the entire network of grid cells (using advanced statistical methods and machine learning techniques). We can compare our new data to existing theoretical models of grid cell activity [7]. If we find that a certain model is accurate, we can then plan which future experiments to perform, which new experimental data to collect, and how to better analyze our data. This is the ideal scenario in science—when experiments and theory go hand-in-hand.

THE FUTURE OF GRID CELLS RESEARCH

There are many unanswered questions in grid cell research, such as: How do grid cells produce the activity patterns that they have? How do sub-networks (modules) of grid cells work together? And how do networks of grid cells work with networks of other types of navigation-related cells to create the brain's complete internal map? I believe that, as we improve our understating of how various cell types operate, we will gradually be able to address the bigger question of how all these cells work together. Our next challenge will then be to understand how this collective activity leads to an animal's perception of space—how does the activity of all these cells create the experience of navigating successfully in the world?

Another question that grid cell researchers are interested in is whether grid cells are involved in navigation planning. In other words, can grid cells predict or plan the next location(s) of the animal? And if so, from how far away? There is some indication that grid cells contain information about the immediate future location of the animal. But, if an animal is navigating a maze, then it needs to make a plan of how to get from the starting point to the end of the maze (where it gets a tasty treat). Does it make that plan right from the beginning and remember a trajectory through the maze (Figure 3A) or does it navigate "on the fly" and decide locally where to turn in each junction (Figure 3B)?

Another direction that grid cell researchers are currently studying is whether the brain uses grid cells for other things that it needs to measure, which we call **metrics** [8]. Scientists speculate that grid cells are used for many types of metrics, like keeping track of social networks and understanding the "social distances" within them. In general, we think of grid cells as a neural network that performs computations in the brain. We hope to use this network, and its relations with other networks of cells in the navigation system, to understand general principles of how the brain performs computations and processes information. We believe that the grid cell system can serve as a "window" for understanding how large neural

METRICS

Measures that supply quantitative information about some parameter (distance, in our case).

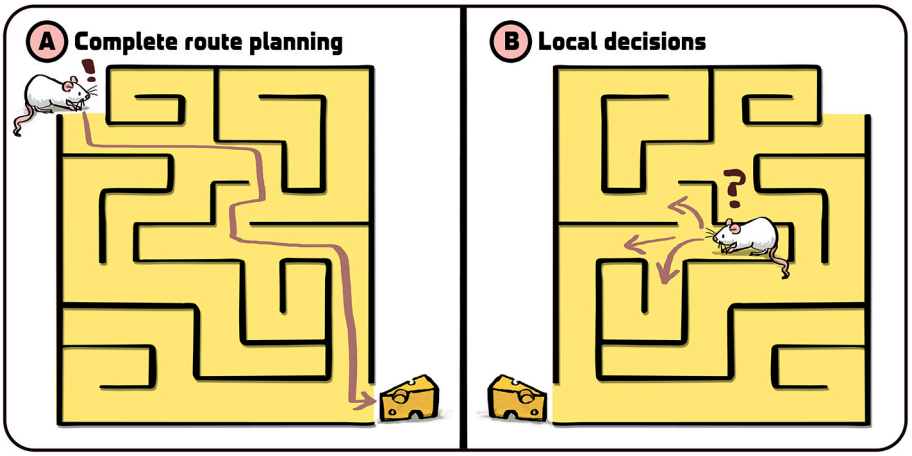

Figure 3

How do grid cells "plan"? By studying grid cells, scientists hope to find out whether animals navigate by **(A)** planning their whole route from point A to point B, or **(B)** whether they make local decisions "on the fly" at every junction along the route. Illustration by: Iris Gat.

networks operate in the brain and eventually explain how **cognition** works—which I find very exciting!

COGNITION

Mental processes like thinking and remembering used to acquire knowledge and understanding.

RECOMMENDATIONS FOR YOUNG MINDS

To be a good scientist these days, the most important quality is curiosity (Figure 4). If you feel inspired to understand something, follow your curiosity and your passion—then do your best to figure it out. Do not worry about things like earning a lot of money or trying to figure out in advance what will be useful in your future. I also think it is important to be ambitious, to have high goals, and to be motivated to really make a difference for humanity. Aspire to achieve something big and, if you find it is not currently possible, break the question down into smaller steps and pursue them individually. I think that curiosity and ambition can very much be cultivated, so it is important to train yourself by facing meaningful challenges and problems.

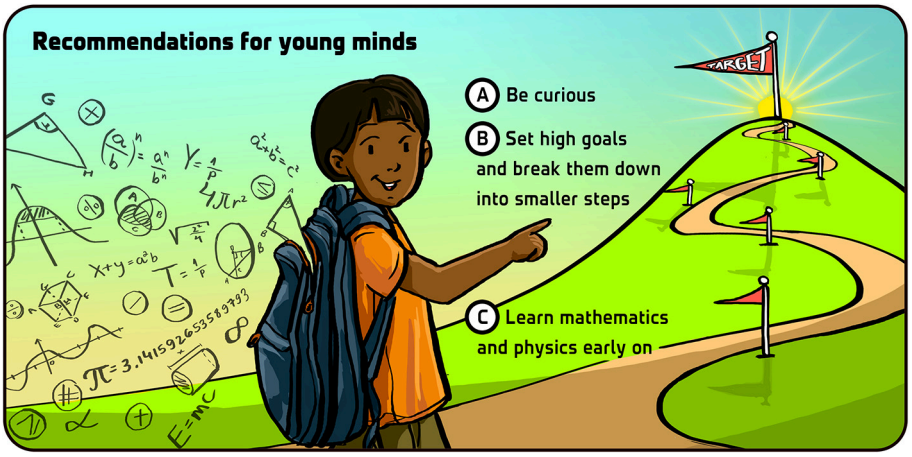

Figure 4

Three recommendations for young minds. Illustration by: Iris Gat.

If you would like to go into neuroscience, I recommend getting a solid foundation in physics and mathematics. With time, these disciplines are becoming increasingly essential for neuroscience, and they are much easier to learn at a young age than later on in your career. You will also need to know about biology, psychology, and other topics, but these are easier to learn at a later stage. Much has changed since I started my career in neuroscience in the 1980s, and I think that much more change will happen during the next 40 years. This will likely be true not only for neuroscience, but for *all* scientific fields. I think this makes a career in science a really exciting and rewarding adventure.

ADDITIONAL MATERIALS

1. The Brain's GPS Tells You Where You Are and Where You've Come from–Scientific American
2. How the Mosers discovered grid cells–Kavli Institute for Systems Neuroscience

ACKNOWLEDGMENTS

We wish to thank Iris Gat for providing the figures. The work is supported by a Synergy Grant to E.I.M. and Yoram Burak from the European Research Council ('KILONEURONS', Grant Agreement N° 951319), a Centre of Excellence scheme grant to May-Britt Moser and E.I.M. from the Research Council of Norway (grant number 223262), the Kavli Foundation (May-Britt Moser and E.I.M.), and a direct contribution to May-Britt Moser and E.I.M. from the Ministry of Education and Research of Norway.

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YOUNG REVIEWERS

DEERFIELD ELEMENTARY, AGES: 9–10
Our class is a group of fourth-grade students from Irvine, California. We love critical thinking, competition, collaborating, creating, and playing games with each other. Our class is a very tight-knit group of 33 and we are like a large, cohesive family.

OZZY, AGE: 11
I am Ozzy. I really like geography and transit. I love to ride on and watch trains and airplanes. I enjoy learning just a bit of a bunch of languages (not to fluency). The world is really interesting, and to learn as much as possible about it is my goal. I am also a swimmer and a musician (piano and clarinet).

THEO, AGE: 11

My name is Theo. I am 11 years old and in fifth grade. I love reading, math, opportunities to help people and animals, and I hope to become a professional chess player. I go to a homeschool in Boston, MA, USA that has very few kids which means each kid gets a lot of learning attention. My hobbies are: chess, reading books, math, programming, and playing video games.

AUTHORS

EDVARD I. MOSER

Prof. Edvard Moser is a Norwegian neuroscientist. He grew up in a small, remote town on the coast of Norway. He studied mathematic and statistics at the University of Oslo and earned a degree in psychology in 1990. Prof. Moser then continued his graduate studies in Oslo, under Prof. Per Andersen. Prof. Moser studied brain activity in the hippocampus—a brain region involved with the perception of space—and completed his doctorate in neurophysiology in 1995. The following year, after a brief period as a postdoctoral researcher with British neuroscientist Richard Morris at the University of Edinburgh, and with Prof. John O'Keefe, at University College London, he returned to Norway to serve as a professor of biological psychology at the Norwegian University of Science and Technology in Trondheim (NTNU), Norway. Together with Prof. May-Britt Moser, he discovered grid cells in the entorhinal cortex, along with other cell types known as border cells and head direction cells. Prof. Moser was a founding codirector of the Kavli Institute for Systems Neuroscience at NTNU in 2007, and of the Centre for Neural Computation in 2013. Currently, Edvard Moser and May-Britt Moser are the scientific directors of the Kavli Institute, where their lab studies the navigation system in the brain. Over the course of his career, Prof. Moser has won numerous prestigious awards, including a prize for young scientists awarded by the Royal Norwegian Academy for Sciences (1999), the Louis-Jeantet Prize for Medicine (2011), the Perl-UNC Neuroscience Prize (2012), and the Nobel Prize in Physiology or Medicine (2014). Prof. Moser has two daughters, Isabel Maria and Ailin Marlene. *edvard.moser@ntnu.no

NOA SEGEV

Noa Segev is a scientific writer and project coordinator at Frontiers for Young Minds. She earned her B.Sc. in physics at The Hebrew University of Jerusalem and her M.E. in renewable energy engineering at the Technion-Israel Institute of Technology. Since 2019, she has been interviewing Nobel Prize winners and co-authoring articles for the Nobel Collection at Frontiers for Young Minds. Noa aims to make the science behind Nobel Prize-winning discoveries accessible to all, and to share valuable insights from the vast professional and personal experience of Nobel Laureates. *noasegev@gmail.com

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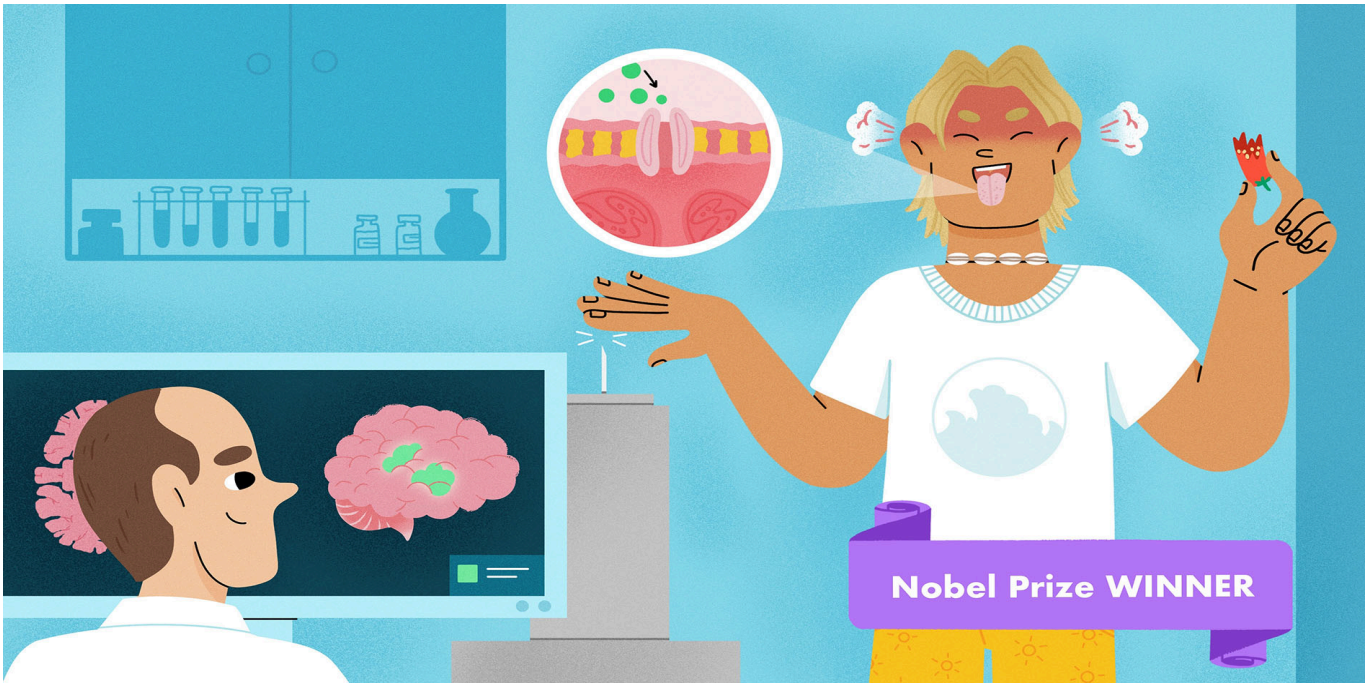

HOT CHILI PEPPERS HELP UNCOVER THE SECRETS OF PAIN

David Julius *

Department of Physiology, University of California, San Francisco, San Francisco, CA, United States

YOUNG REVIEWERS:

ALEXIA
AGE: 13

GLEESON COLLEGE STRETCH PROGRAM
AGES: 13–15

Our life experience is shaped by our senses. We see, hear, smell, touch, and taste the things around us, and this is how we get to know the world. In my research, I study receptors—small sensing structures present on cell membranes that react to stimuli from the environment or from within the body. The receptors I tell you about in this article are sensitive to pain and heat, and they respond both to high temperatures and to spicy substances, such as hot chili peppers. We believe that, by studying the structures of these receptors and gaining a better understanding of how they operate, we will be able to develop new drugs to treat long-lasting pain more effectively and safely. Read this article to learn how the chili pepper has opened the door for us to uncover some great mysteries of the sensation of pain, paving the way toward future pain treatments.

As background materials for this article, it is recommended to view two previously published articles on the patch clamp technique and on cryogenic electron microscopy.

Professor David Julius won the Nobel Prize in Physiology or Medicine in 2021, jointly with Prof. Ardem Patapoutian, for their discoveries of receptors for temperature and touch.

DOES IT HURT FOR A REASON?

For all animals, including humans, pain is a very important sensation. Pain tells us when we are injured and need to take care of ourselves, and when we should stop or avoid a potentially harmful activity. In scientific terms, pain is part of **somatosensation**—the ability to sense touch, temperature, pain, and our limbs position and movement in the space around us. Somatosensation is performed by small sensing structures that are spread throughout the body, which we call **receptors**. Receptors located on the membrane of nerve cells respond to stimuli from the environment (e.g., hot liquid in a cup) and, consequently, these somatosensory nerve cells send signals to the relevant brain regions, where these signals are processed and deciphered (Figure 1).

SOMATOSENSATION

The ability to sense touch, temperature, pain, and the position and movement of our bodies in space.

RECEPTORS

Small sensing units, typically proteins in the cell's membrane, that respond to stimuli (such as specific chemicals or temperature).

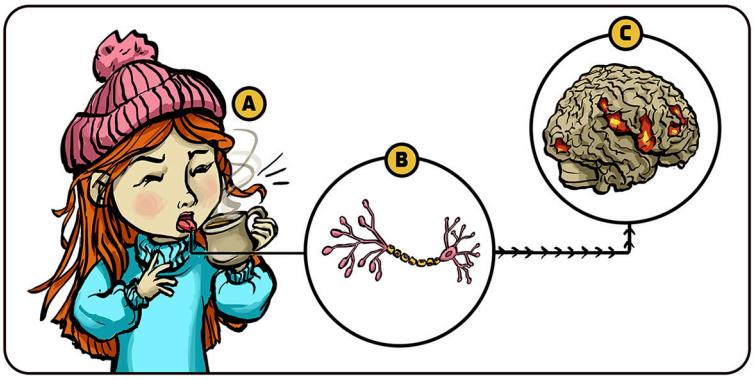

Figure 1

Somatosensation. **(A)** Stimuli like temperature and pain are sensed by tiny structures called receptors that are located on the membrane surface of special nerve cells (located in our tongue in this example). **(B)** When these receptors sense the relevant stimulus, they generate electrical signals in the nerve cell which is then sent to the brain. **(C)** Specific regions in the brain interpret these signals—in this example, letting the girl know that the liquid she drinks is hot.

In this article, we will focus on one type of receptors called **ion channel receptors**. These receptors use ion channels—small "tunnels" or "gates" present in cell membranes—to create electrical signals in response to stimuli from the environment (to learn more about ion channels, read this Nobel Collection article). Ion channel receptors are very complicated and fascinating signaling machines that lie at the heart of our ability to sense our internal and external environments. Over the years, scientists developed advanced tools for studying the structures and functions of ion channel receptors. For example, we can use a special imaging method called cryogenic electron microscopy, where we send electrons through frozen specimens, to take highly detailed pictures of these receptors, and can build three-dimensional models of them. We can also use a sophisticated method called patch clamp, to measure electrical charges that flow through these ion channels and this enables us to study the electrical behavior of ion channel receptors.

ION CHANNEL RECEPTORS

Receptors that create electrical signals in response to stimuli, by allowing ions (charged particles) to flow into and out of nerve cells.

These methods help us to answer basic scientific questions about how people sense pain, which can further help us to develop new drugs for pain relief. When pain-sensitive ion channel receptors work properly, they help people protect themselves against physical harm. However, occasionally something goes wrong with this protective mechanism, and it gets out of control. Then people might experience **chronic pain**—pain that is persistent and does not seem to have a useful protective function. So far, conventional pain-relief medications, called **analgesics** (or painkillers, such as Aspirin), have been very efficient at treating **acute pain**, but not chronic pain. In addition, common analgesics (painkillers) like morphine are addictive and can have undesired side effects, including dizziness, nausea, and vomiting [1].

CHRONIC PAIN

Pain that lasts for long periods of time (more than 12 weeks), even after the original cause is gone. Examples: chronic abdominal pain and chronic pain in the joints.

ANALGESICS

Drugs that are designed to relief pain ("painkillers").

ACUTE PAIN

Sudden pain that lasts for a relatively short time and passes when its original cause is gone (for example, the pain you feel when you cut your finger).

Overuse of addictive analgesics has contributed to what is known as the opioid crisis, during which many people have become addicted to analgesics, abusing them in ways that harm their health. We hope that a better understanding of pain receptors—particularly ion channel pain receptors—will help us to develop new analgesics that are more effective at treating chronic pain. Such new pain medicines will likely help us address the opioid crisis. Before we look into the new medicines that might be developed from pain receptor research, I will first tell you a little about the family of pain receptors that my colleagues and I discovered.

HOT CHILI PEPPERS AND PAIN RECEPTORS

I was always fascinated by how the senses operate and by the ways that certain chemicals, especially natural products, affect the brain and the body. Through my research, I could combine these two fascinations to better understand how somatosensation works. I specifically wanted to understand how humans detect stimuli that might be harmful to the body—through a process called **nociception**.

NOCICEPTION

The process in the sensory system that enables to detect harmful (noxious) stimuli.

CAPSAICIN

The spicy substance in hot chili peppers that causes the burning sensation.

During the 1970s, scientists found that **capsaicin**—the ingredient in hot chili peppers that causes the burning sensation—evokes a sensation of pain in the eye of rats by activating nociception receptors in specific sensory nerve endings [2]. A decade later, scientists suggested that capsaicin evokes pain by allowing ions, including calcium ions, to flow into the sensory neurons that are involved in nociception [3]. These finding gave me and my students the hints we needed to tackle the following questions: Is there a specific receptor for capsaicin in sensory nerve cells? If so, what does it look like, and what role does it have in the ability to sense pain?

To answer this question, my students and I decided to search for the gene that encoded the specific protein of the capsaicin receptor. Because we did not know what that receptor—or its respective gene—looked like, we had to search for them using only of few clues

to guide us. We knew (according to the central dogma of molecular biology) that the production of *any* protein, including pain receptors, involves the generation of a molecule called mRNA, which carries the instructions from the DNA that are needed to make that protein. Our strategy was to take nerve cells that respond to pain (Figure 2A), isolate the mRNA that codes for the pain receptors (Figure 2B), and then transform this mRNA back into the DNA instructions (the gene) that codes for the pain receptor (Figure 2C) [4] (to learn more about mRNA, read this Nobel Collection article; and to learn more about how mRNA can be transformed into DNA, read this Nobel Collection article).

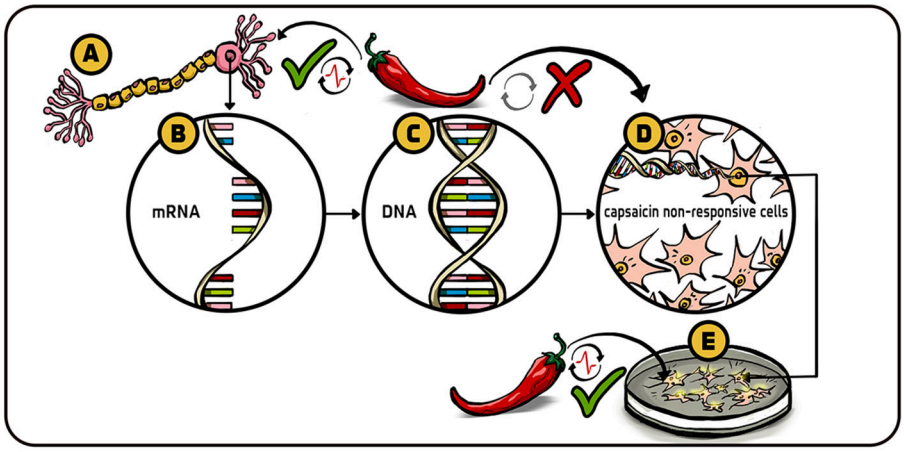

Figure 2

Looking for the capsaicin receptor gene. **(A)** Receptor neuron, called nociceptor, that responds to capsaicin and eventually transmits pain information to the brain. **(B)** We removed from these cells the mRNA that we suspected to be involved with coding for the receptor-sensitive to capscaicin. **(C)** We then turned each of the many mRNA molecules that we screened back into the DNA (genes) that they were initially made from. This DNA library should include a DNA fragment or several fragments encoding the protein capable of reacting to capsaicin. **(D)** The DNA fragments were introduced into cells that normally did not respond to capsaicin. **(E)** The cells that did respond to capsaicin after the DNA was introduced to them were then known to contain the capsaicin gene. After a lot of screening, we identified the single gene that codes for the capsaicin receptor.

Toward this goal we created a library of millions of DNA fragments corresponding to genes that are expressed in the sensory neurons which can react to pain, heat, and touch. To find the *specific* gene we were looking for, we had to screen hundreds of thousands of DNA pieces produced from many different mRNAs collected from the pain-responsive nerve cells. This was a laborious process that involved sophisticated ways of dividing the enormous number of DNA pieces that we created into smaller and smaller groups, until we could finally detect the exact gene we were interested in.

To check whether we found the right gene, we put our DNA pieces that we suspected code for the pain receptor into cells that originally did not respond to capsaicin (Figure 2D). We then checked whether these cells became sensitive to capsaicin by using a method invented by a late Nobel Laureate named Roger Tsien, with which we could actually see a glowing flash of light from the cells every time calcium ions entered into the cell (Figure 2E). We then used the patch clamp technique to record current flow across small patches of membrane from these glowing cells and confirmed that they responded to capsaicin by enabling the flow of ions into the cell—bingo!

Our results told us that there *is* a capsaicin receptor, and that it is an ion channel. We named it the TRPV1 receptor (Figure 3A), as it is

a member of a group of ion channels called the transient receptor potential (TRP) channels. Later, we found that this receptor responds not only to capsaicin (Figure 3B) but also to harmful heat—it becomes activated at temperatures above 43°C (Figure 3C) [4].

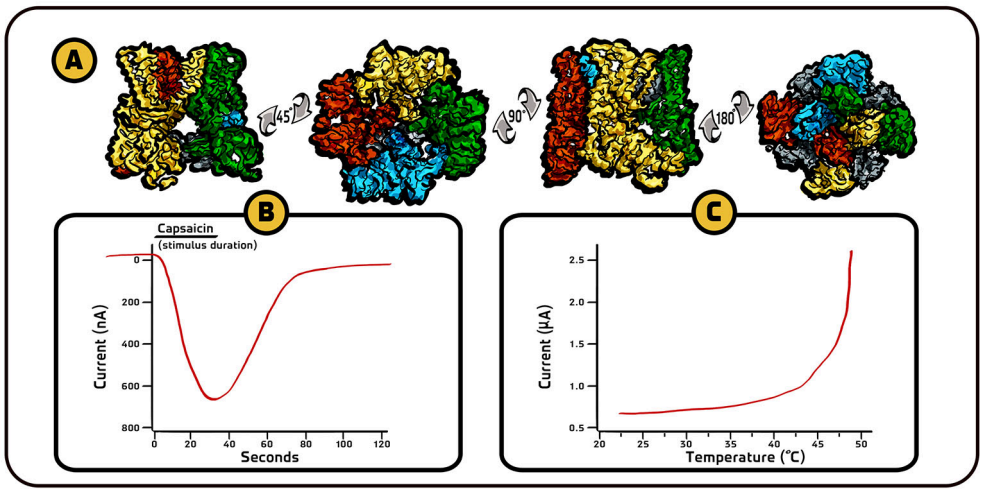

Figure 3

Figure 3

Structure and behavior of TRPV1 receptor. **(A)** High-resolution three-dimensional drawings in four different views of TRPV1 receptor channel, created using images obtained from cryogenic electron microscopy (images adapted from [5]). **(B)** In response to capsaicin, TRPV1 receptors allow calcium ions (which are positively charged) to enter the cell, as expressed by a change in electrical current. When a positive charge flows into the cell, the convention is that the current is displayed in the downward direction (graph adapted from [4]). **(C)** TRPV1 receptors also respond to high temperatures by allowing calcium and sodium ions (positive charge) to flow into the cell. These results tell us that the same receptors respond both to chemicals (capsaicin in this case) *and* to hot temperatures which can be harmful for the body.

After finding the TRPV1 receptor, it was easier for us to discover additional receptors from the TRP family, such as TRPM8, which responds to menthol and cold temperatures [6], and TRPA1 (sometimes called the wasabi receptor), which responds to pungent agents from mustard and garlic, and agents produced during inflammation, among other stimuli [7]. TRP channels are found in a lot of sensory tissues, including the eyes, tongue, and skin. Many of them are involved in detecting external signals that are related to our senses, and some of them detect signals from within the body (such as signals generated by internal organs). Our understanding of their structures and functions have greatly advanced, but there are still some riddles yet to be solved.

THE FUTURE OF PAIN RESEARCH

One of the main riddles we are still trying to solve is how heat and cold activate TRP receptors. Currently, we do not understand how TRP receptors "sense" temperature. We think that, as opposed to chemicals like capsaicin, temperature does not act on one specific part of the TRP receptor but instead acts on multiple parts simultaneously. To study this, we are trying to freeze and take images of receptors (using cryogenic electron microscopy) as they respond to hot or cold temperatures, and then try to understand what those temperatures are doing to the receptors.

To develop better drugs for acute and chronic pain, which could begin to address the opioid crisis, I believe we need to understand the various

types of pain, including the molecules, cell types, and pathways that are most involved in each type (for example, we know that pain that is perceived in the skin, called cutaneous pain, is very different from pain that is generated by internal organs, called visceral pain). I do not think we can solve every type of pain using the same approach, so we will probably have to develop specific approaches for each type of pain. We must also develop better ways to measure pain, since everyone feels pain differently. Accurate pain measurements will allow us to better assess the performance of new drugs.

I am also interested in diving deeper into the atomic structure of TRP receptors, and in using this knowledge to better understand how they operate. An enhanced understanding of how TRP receptors work will allow us to develop new drugs that target only specific parts or mechanisms of these receptors. Many analgesics used today block pain receptors completely and therefore interfere with the receptors' ability to warn people against harm. For example, some TRPV1 drugs diminish people's ability to detect painful heat, and as a result patients could unknowingly burn themselves when touching something hot or drinking hot liquids [8].

Is it possible to develop drugs that do not shut TRP receptors down, but instead change the way the receptors interact with a specific stimulus? In the case of TRPV1, we are looking for ways to block the ability of inflammation-causing substances to increase the sensitivity of the receptor—without blocking its ability detect heat under normal conditions. This is a challenging task, and a lot of work is still ahead of us—but the great potential of helping many people that suffer from chronic pain makes the effort worthwhile.

RECOMMENDATIONS FOR YOUNG MINDS

Science is like any kind of creative endeavor—you have the freedom to follow your curiosity, but there is also a lot of unpredictability along the way. You can forge your own path and get great enjoyment from your work, but there are also times of frustration and anxiety, when you do not understand your results or what you should do next. If you choose a career in science, you must be driven by curiosity and passion, enjoy understanding how things work, and have real motivation for solving puzzles. Often you will have to simply put one foot in front of the other and pay attention to the short term, enjoying coming into the lab and doing your experiments.

I often tell my students that persistence pays off. You must find a way to keep advancing toward a scientific goal that you find exciting, interesting, and significant. In my career, I focused on big questions that had been unanswered for many years, that would be very exciting to solve, and which could open up new possibilities in my field of research. When things were not working for me, I tried to figure out

why and I searched for a new approach. As long as I could think of something new to try, I had the energy to go back to the lab and look at the problem differently. This path was challenging, but my persistence paid off.

Finally, I want to highlight another unusual and exciting aspect of science: scientists are part of an international community (Figure 4). I know people from all over the world and I visit them frequently. I think this is something very special and unique and it has really broadened my horizons, making my life even more interesting.

Figure 4

Scientists are part of an international community. One of the things I love about being a scientist is that I am part of an international community of many scientists. Knowing people from all over the world enriches me and makes my life more interesting.

Figure 4

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YOUNG REVIEWERS

ALEXIA, AGE: 13
Alexia enjoys writing poetry, playing golf, and traveling. She is interested in engineering, inventions, and research in the sciences.

GLEESON COLLEGE STRETCH PROGRAM, AGES: 13–15
The Gleeson College Stretch Program is a co-curricular enrichment program. We meet and participate in fun challenges which help us to develop skills like being creative and thinking outside of the box. We have a wide variety of interests and therefore across the year we get to partake in different activities including code-breaking, problem solving, engineering challenges, VR, baking competitions, and creativity tasks. We enjoy trying to challenge our thinking in the Stretch Program.

AUTHORS

DAVID JULIUS

David Julius is an American physiologist and a professor at the University of California, San Francisco. Julius received his B.S. in life sciences from the Massachusetts Institute of Technology (MIT), where he worked at the laboratory of Prof. Alexander Rich studying tRNA. He then earned his Ph.D. in biochemistry at the University of California, Berkeley, where he worked with professors Jeremy Thorner and Randy Schekman (Nobel Laureate in Physiology or Medicine, 2013) on hormone processing and secretion in yeast. Julius did his postdoctoral studies in the laboratory of Prof. Richard Axel (Nobel Laureate in Physiology or Medicine, 2004) at Columbia University, New York, where he studied receptor genes. Julius joined the University of California, San Francisco where he started studying ion channel receptors. He and colleagues used the chemical properties of natural products to discover a family of pain- and temperature-sensing receptors called TRP channels, including the TRPV1 receptor that responds to hot chili peppers and heat. Julius is currently studying the atomic structure of TRP receptors, trying to understand how they respond to temperature and interact with inflammatory agents, which is relevant to developing new drugs to treat chronic pain. Julius is married to Holly Ingraham, Professor of Physiology at the University of California, San Francisco, and they have a son named Philip. *David.Julius@ucsf.edu

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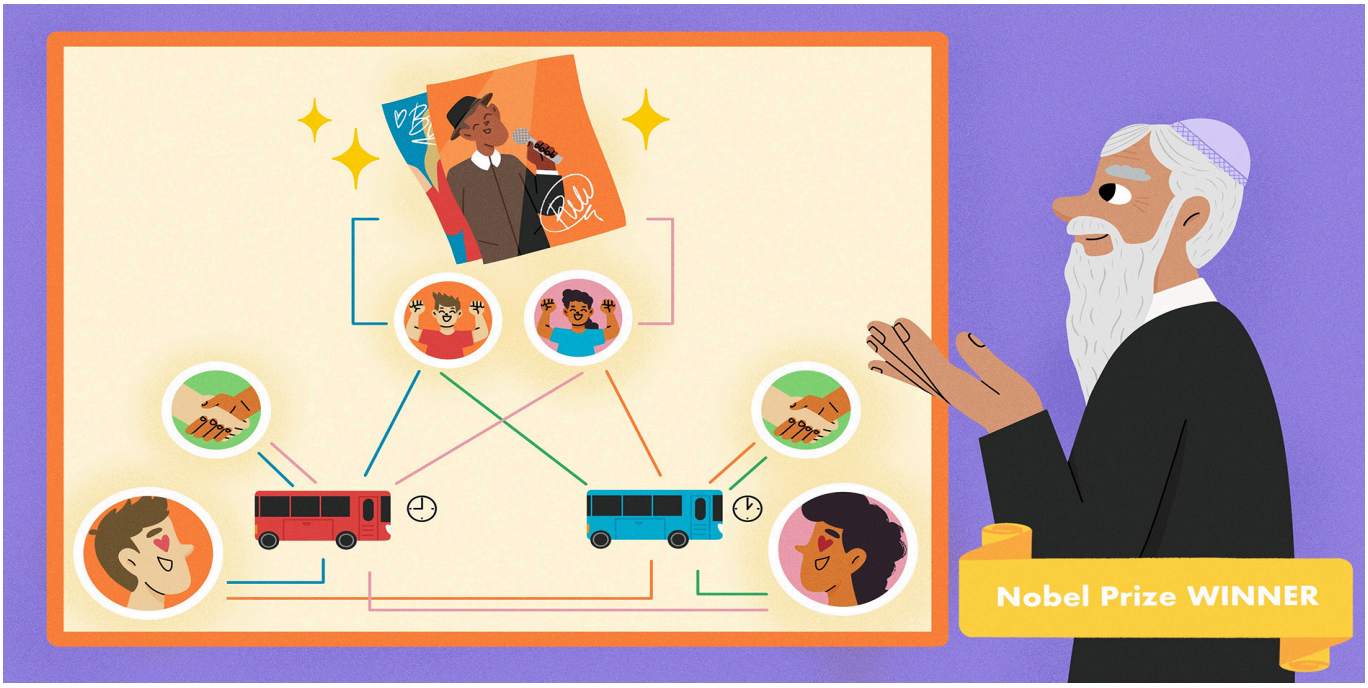

GAME THEORY—MORE THAN JUST GAMES

Robert Aumann *

Department of Mathematics and Federmann Center for the Study of Rationality, The Hebrew University of Jerusalem, Jerusalem, Israel

YOUNG REVIEWERS:


ELIORA
AGE: 13


ESTELLE
AGE: 12


JULIA
AGE: 12


SHARON
AGE: 12

With Yonatan Aumann, Department of Computer Science, Bar Ilan University, Ramat Gan, Israel.

Game theory is *not* about games. It is a scientific field that uses logic to understand how decisions should be made, taking into account the choices of others. Despite its name, it deals with serious real-life situations like business, politics or even war. This article explains what game theory is about, and how it can be used in many real-world settings. I will explain how game theory uncovers the roots of cooperation, and why its study is important for reducing hostility. We will conclude with an elegant game theory method which you can apply in your everyday life.

Professor Robert Aumann won the Nobel Prize in Economics in 2005, jointly with Prof. Thomas Schelling, for having enhanced our understanding of conflict and cooperation through game theory analysis.

EARLY BUS OR LATE BUS?

I study the scientific field called "game theory." Game theory is really not about games. To explain what game theory *is* about, let us start with some examples.

Suppose your hobby is collecting celebrity autographs. One day, you get the following message from Chris, a legendary autograph collector from a nearby town: *"At the age of 85, I have decided to end my autograph-collecting hobby. I will give away my entire collection to whoever comes to my house first, starting at midnight tonight. If several people arrive at the same time, I will split the collection equally among them all."*

You know that no one in Chris's town is interested in autographs, but there is another collector, Beth, who lives in your town. Two buses run from your town to Chris's, one leaving at 5 AM and one at 9 AM. Which bus should you ride?

If you think about it, you should most definitely ride the 5 AM bus. The reasoning is as follows.

- If Beth rides the 9 AM bus, then you should ride the 5 AM bus—thus winning the entire collection.
- If, on the other hand, Beth decides to ride the 5 AM bus, then you should do the same, in order to get at least half the collection (with the 9 AM bus you would end up with nothing).

So, regardless of what Beth does, you should ride the 5 AM bus.

But the same reasoning is also true for Beth. So you both meet, red eyed, on the 5 AM bus, on the way to splitting the collection. Evidently, you would both be better off if both of you took the 9 AM bus—you would get more sleep, *and* the same number of autographs. But that desirable outcome is not possible! If each of you behaves *rationally*—that is, does what is best for him/her—then riding the 5 AM bus and splitting the collection is the *only* reasonable thing to do.

Now, suppose there is yet another bus, leaving at 2 AM. Which bus should you take? Unlike in the previous case, your best choice depends on what Beth does:

a) If Beth takes the 9 AM bus, then your best choice is to take the 5 AM bus.
b) If Beth takes either the 5 or 2 AM bus, then your best choice is to take the 2 AM bus.

Is it reasonable to suppose that Beth will take the 9 AM bus? Well, you know that Beth is a clever girl, so you expect her to follow the same

reasoning as yours. So, she will not take the 9 AM bus—just as *you* would not. So, Case (b) holds, and your best choice is the 2 AM bus. The same is true for Beth. So again, you and Beth meet sleepless on the 2 AM bus, getting the exact same number of autographs you would have gotten if you had both taken the 9 AM bus!

In this example, did you notice how *logical reasoning* was used to figure out the best decision? This type of reasoning is what game theory is all about. Game theory is the logical analysis of decision making, specifically in situations involving multiple parties with possibly conflicting interests. Game theory uses logical reasoning to analyze such situations.

ANOTHER EXAMPLE OF LOGICAL REASONING

Here is another example. Suppose you and Beth play a game with the following rules: First, each of you secretly writes either the number 1 or the number 2 on a piece of paper. Then, you both reveal your choices. If Beth wrote 1, then *she* pays *you* the amount that you wrote (in dollars). If Beth wrote 2, then *you* pay *her* the amount that you wrote. What should you do? Here is a table of the possible choices and outcomes (Table 1):

Table 1

Logical reasoning game rules.

		Beth	
		1	2
You	1	Beth pays you 1	You pay Beth 1
	2	Beth pays you 2	You pay Beth 2

Table 1

Choosing 2 is better if Beth chooses 1, while choosing 1 is better if Beth chooses 2. So, in order to determine your best move, you must try to figure out what Beth will do—but this is easy. For Beth, choosing 2 is clearly the better choice, regardless of what you do. If Beth is smart, she will always play 2, which means you should play 1.

As with the bus example, logical reasoning was used to analyze the situation and determine the best play. We employed "game theory"—the logical analysis of decision making in situations involving multiple parties. Importantly, game theory analyzes such situations from a purely logical perspective, without involving emotions and psychology. It tries to determine the most *logical* decision.

IF NOT GAMES, THEN WHAT?

The name "game theory" is misleading. Game theory is mostly *not* about games (although it can also be applied to games). Rather, game theory considers any interaction among multiple parties with

conflicting interests, and uses logical reasoning (and mathematics) to analyze such interactions.

Game theory can be applied to situations much more serious than getting an autograph collection, such as business, politics, and even war.

Consider two neighboring countries that are hostile to each other. We will call them Astan and Beestan. Both countries keep armies along the shared border. The armies are stationed next to each other, on opposite sides of the border. Come night, soldiers of both armies enter their barracks, hoping for a good night's sleep, except for a couple of soldiers standing guard. But now, the Astan commander says to his deputy, "I have an idea. Since Beestan's army is going to sleep, this is our chance to attack them. Using all our troops, we can easily overcome their guards, and surprise attack the others in their sleep. Wake up all our soldiers. We are heading to the border!"

The deputy, wanting to sleep, replies, "Sir, but what if Beestan's army does not go to sleep? Then we will not be able to defeat them." To which the commander replies, "If that is the case, we surely need to head to the border, or else they will attack us!" With this fully rational argument, Astan's entire army heads to the border, ready for battle.

But the *exact same* rational argument is also true for Beestan's unit. So, they also head to the border, where the two units meet—for an unavoidable brutal clash. What a waste!

Sadly, the same logic seems to hold every night... so are the two units destined to endless clashes, night after night? Does game theory tell us that endless fighting is the only rational behavior?

Fortunately, the answer is no. In fact, advanced game theory analysis tells us the opposite: in such situations, logical reasoning leads to increased cooperation, not confrontation.

How can that be? Is there a flaw in the commander's logic? Yes and no. The commander's logic is indeed irrefutable if each night is considered *on its own*. But, when considering the repeated nature of the conflict, night after night, the logic changes. How so?

Consider again the conversation between the commander and his deputy. The commander wants to attack, but now the deputy says, "Sir, yesterday I was walking along the border and spotted the deputy of Beestan's army. He shouted over the fence that they plan on going to sleep tonight; and he warned me that if we attack them in their sleep, they will retaliate and attack us *every night from now on*! I shouted back that the same is true for us."

Is attacking tonight still the most logical move? Probably not. Even though an attack would give the attacker some short-term advantage, this advantage will be overshadowed by the overall loss created by fruitless battles that will then take place night after night. This is true for both sides! So, both units choose not to attack and to enjoy a good night's sleep. The same thing happens the night after, and the night after that...

You can see that repeated interaction completely changes the situation! While *confrontation* was the logical move in the one-time case, *cooperation* is the logical behavior when interactions will happen again and again. Game theory teaches us that cooperation is frequently the most logical behavior in repeated interactions, even between opposing and hostile parties! This means that, according to game theory, cooperation is frequently the best possible action—even if all that you care about is your own good.

MAKING THE WORLD A BETTER PLACE

Why am I stressing this point? Because that is what I got my Nobel prize for!

In the words of the prize committee:

> Robert Aumann was awarded the Prize in Economic Sciences for his theory of repeated games, which enhances our understanding of the prerequisites for cooperation.

So, the Nobel prize committee thinks that this idea—that cooperation is a result of repeated interaction—is sufficiently important to deserve a Nobel prize!

If you think about it, the idea itself is actually quite simple; If you will meet someone again, you may be better off cooperating. But although it is simple, it is extremely important. Our world is full of hostility, wars, and conflicts. We all want this situation to change. How can we bring about such change? Some people work or volunteer in peace organizations, while others use their political or financial power. All these are worthy and important undertakings; but as a scientist, I believe that a systematic and scientific study of the topic is no less important. I believe that *understanding* the causes of conflict and cooperation is a necessary first step toward promoting peace. You cannot build an airplane without understanding the laws of physics, and you cannot find a cure for cancer without understanding how cancer cells function. Similarly, I believe that you cannot promote cooperation without understanding its true sources; and you cannot promote peace if you do not understand its true roots—as well as those of war. Understanding why people fight or cooperate and what makes nations go to war or live in peace is necessary if we wish to try to

change human behavior for the better. Game theory provides us with such an understanding. Therefore, I see my study of game theory as my small contribution to improving the world.

I will end this article with a clever and practical game theory idea that you can use in your everyday life for reducing envy and conflict. Suppose that your mother got you and your brother a tasty doughnut: vanilla-flavored dough covered with chocolate and candies. Unfortunately, she only got one—so you will need to share. What is the best way to split the doughnut? One solution is for your mother to cut the doughnut in half and give one piece to each of you. But, as your mother probably knows all too well, this could easily result in one of you being unhappy and envious of the other. Regardless of how fairly your mother tries to split the doughnut, one of you will probably think that their piece is smaller, has less chocolate or candies, or has some other flaw. Letting you or your sibling split the doughnut is probably not a good idea either! So, how should the doughnut be split?

Game theory has an elegant solution. It suggests using the following procedure (Figure 1):

1. Your brother cuts the doughnut into two pieces, in any way he sees fit.
2. You choose one of the pieces for yourself.
3. Your brother gets the remaining piece.

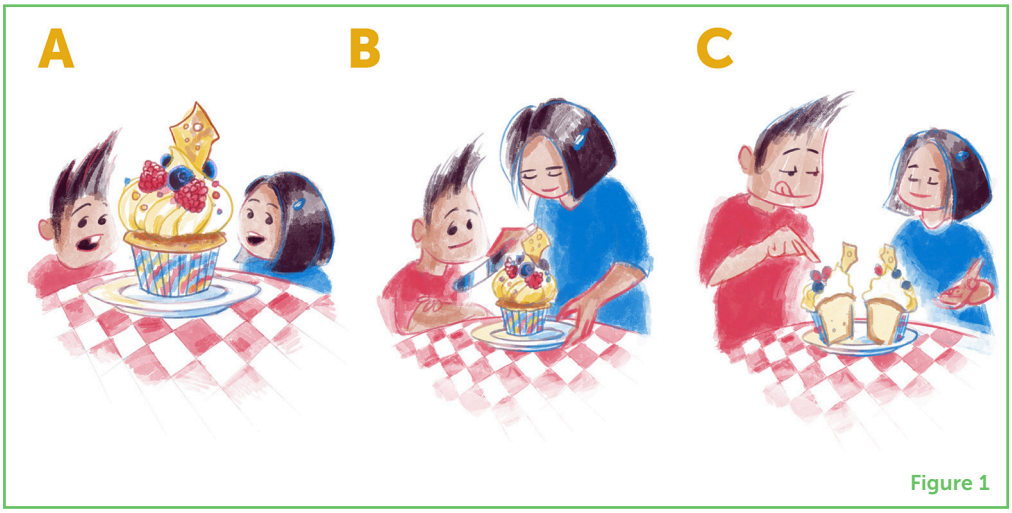

Figure 1

A clever procedure for splitting a treat. **(A)** Suppose your mother buys you and your brother a tasty treat. How should she split it between you and your brother so that both of you will be satisfied? **(B)** Game theory suggests that, first, your brother cuts the treat into two pieces. **(C)** Next, you choose which piece you want to eat and then your brother gets the other piece.

Here is why with this procedure, neither you nor your brother have any cause for complaint or grumble.

You surely cannot complain, because you got to choose your piece. But what about your brother? Since you get to choose, is he not getting the lesser piece? The answer is no. Remember that he gets to cut the

doughnut however he wants. When doing so, his reasoning should be as follows: "After I cut the doughnut in two, my sibling gets to choose their piece. If I create two uneven pieces, then my sibling will take the better piece, and I will get the lesser one. So it is best for me to split the doughnut into two *exactly even* parts. This guarantees that I get my fair share."

In this way, both you and your brother get equally desirable shares, and neither of you can grumble! Is that not a clever solution? I frequently used it with my kids.

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YOUNG REVIEWERS

ELIORA, AGE: 13
My name is Eliora and I am 13 years old. My interests include swimming, computer programming, and crocheting. I chose to become a Frontiers' Young Reviewer due to my interest in the scientific content presented within the Frontiers articles. I hope to 1 day venture into the medical field and become a doctor. Some things I love to do in my spare time are drawing, crocheting, and coding. My favorite books are the Maximum Ride Series and Front Desk.

ESTELLE, AGE: 12

Estelle is 12 and goes to middle school. She plays basketball, and she loves to dance and ice skate. When Estelle grows up, she will be a sport broadcaster. Estelle knows Julia since kindergarten.

JULIA, AGE: 12

Julia is 12 and goes to middle school, she loves engineering and music. She plays many instruments and she wants to be a race car mechanic. Julia know Estelle since kindergarten.

SHARON, AGE: 12

My name is Sharon. I love to draw, read, swim, crochet, and play tennis. My favorite activity is to teach kids how to code. My favorite subject in school is math and I play the violin and the piano. My favorite books are Maximum Ride and Diary of a Wimpy Kid.

AUTHORS

ROBERT AUMANN

Robert (Yisrael) Aumann was born in Frankfurt am Main, Germany, in 1930. When he was 8 years old, his family fled Nazi Germany and settled in New York. Aumann attended Yeshiva Elementary and High Schools, got a bachelor's degree from the City College of New York in 1950, and a Ph.D. in Mathematics from the Massachusetts Institute of Technology in 1955. The following year he emigrated to Israel, joining the Mathematics Department of the Hebrew University of Jerusalem, where he has been ever since. He is a member of the National Academies of Science of both Israel and the United States. In 2005, he was awarded the Nobel Memorial Prize in Economic Sciences. He has five children, 21 grandchildren, and 37 great-grandchildren (as of this writing). *aumann@mail.huji.ac.il

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THE RIBOSOME—THE FACTORY FOR PROTEIN PRODUCTION ACCORDING TO THE GENETIC CODE

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[2] *Frontiers for Young Minds, Lausanne, Switzerland*

YOUNG REVIEWERS:


ANSHUL
AGE: 12


JULIANA
AGE: 11


SUHAS
AGE: 16

Proteins are small biological machines that work in our bodies as well as in the bodies of all animals, plants, viruses, and bacteria. They are responsible for a wide range of vital activities. Proteins are synthesized based on instructions contained in the genetic code, within their DNA, by a cellular organelle called ribosomes. The ribosome assembles proteins very quickly and accurately. Most of my scientific research is devoted to understanding the function of the ribosome, based on its structure, as we determined it. In this article, I describe the major findings of my research, some important applications of these findings, and the challenges I have faced along the way.

Professor Ada Yonath was awarded the Nobel Prize in Chemistry in 2009, jointly with Prof. Venkatraman Ramakrishnan and Prof. Thomas A. Steitz, for studies of the structure and function of the ribosome.

DNA

The genetic material passed from parents to their offspring. It contains the instructions to produce the cell components, called genes. DNA consists of four building blocks called nucleic acids.

PROTEINS

The "workers" of the cell—molecules that perform most actions in the cell, and in the body in general. These include, for example, producing movement (muscle activation), transporting oxygen and removing carbon dioxide from the blood (respiration), building the body's skin and connective tissues (structure), and protecting the body from invaders (immune system).

AMINO ACIDS

The building blocks of proteins. There are about 20 different amino acids. Within the proteins, the amino acids are linked together by a peptide bond. These bonds connect the amino acids into chains, and this is how the protein is formed. An average sized protein consists of 150–300 amino acids.

PEPTIDE BOND

The chemical bond that links amino acids together in a protein.

Figure 1

The structure of proteins. Proteins are made up of 20 building blocks called amino acids. When the amino acids are joined together, they form a chain called the primary structure of the protein. Each amino

FROM DNA TO PROTEINS

Each living cell contains a genetic code, which is the instructions for the creation of the cell components and the entire living organism. This substance is inherited from parents to their offspring. The genetic material is called **DNA** and can be considered a cookbook that contains all the information necessary for the creation and functioning of the living organism. One of the most important components of DNA are genes, which contain instructions for the synthesis of the **proteins**—the "machinery" of the cells and of the entire living body. Proteins perform many actions that are important for the functioning of the body.

All natural proteins consist of a combination of about 20 building blocks called **amino acids**. To form a protein, the amino acids are joined together like beads on a chain. This connection is called a **peptide bond**, and a medium-sized protein contains between 150 and 500 amino acids linked together. Each such long chain of amino acids folds into a unique, functional three-dimensional structure determined by the sequence of the amino acids that make it up. The protein structure is precisely tailored to execute the function that the protein is intended to perform (Figure 1).

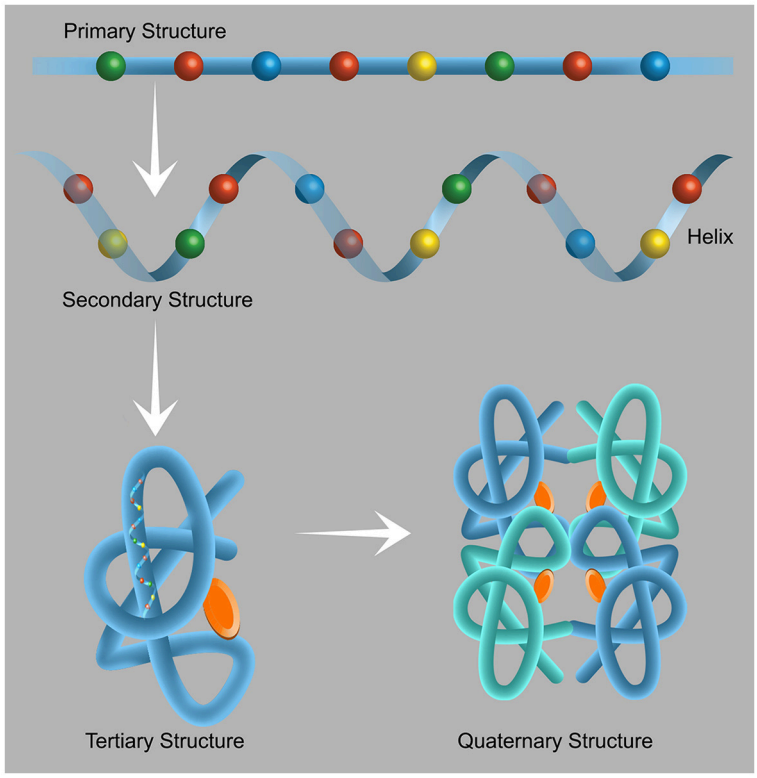

Figure 1

Protein production in cells occurs in several stages (Figure 2). In the first stage, a part of the DNA which contains the genetic material, is

acid has its unique chemical properties that are necessary for the protein to function. Therefore, the sequence in which the amino acids are ordered is extremely important. Each of the protein chains folds according to the properties of its components to form the secondary structure, which can take a variety of forms, including that of a helix. The tertiary structure is the three-dimensional structure of the protein, i.e., the arrangement of the helices and the chains connecting the helices that enables the specific execution of the specific functions of that protein. Some proteins are active in the form of clusters—the arrangement of proteins in a cluster is called a quaternary structure. These clusters consist of at least two protein components bound together in a way that enables their activity. Figure adapted from here.

Figure 2

Stages of protein production in cells. **(1)** In the first stage, the part of the DNA containing nucleotides with the sequence of the protein **(left)** is transcribed to generate the messenger RNA. **(2)** In the second stage, the mRNA and the amino acids reach the ribosome and create the amino acid chain (namely the protein) that leaves the ribosome through a protected tunnel. **(3)** After exiting from the ribosome, the chain folds into the three-dimensional

copied into **messenger RNA (mRNA)** in a process called **transcription**. An mRNA molecule is a "copy" of the genetic information that allows the genetic material to be transferred inside the cell without "endangering" the original code. In the next stage, the mRNA reaches the **ribosome**, the cell's "factory" for protein production. The ribosome "reads" the instructions from the mRNA and prepares the desired protein by forming a chain of amino acids joined to each other by peptide bonds. After exiting from the ribosome, the protein chains are folded into the proper three-dimensional structure required for the protein to function. This process of protein production in the ribosome is called **translation**. The rest of this article will focus on how the translation process occurs in the ribosome.

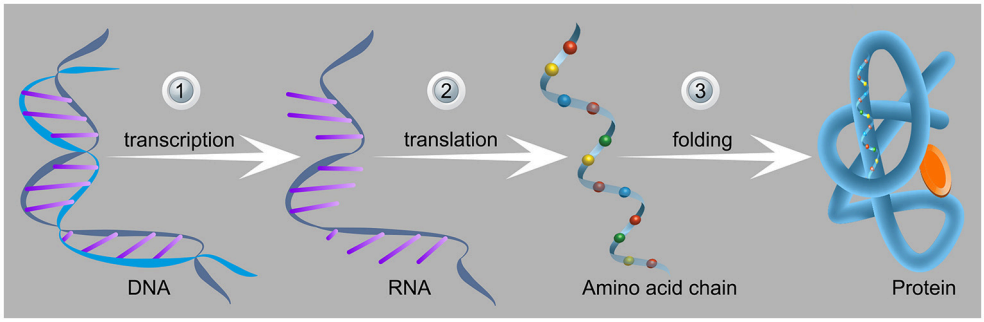

Figure 2

THE RIBOSOME

The translation of a new protein is a complicated process. The "recipe" for proteins is encoded in the genetic code (DNA and mRNA), in a genetic "language" of four letters that is translated into a "language" of amino acids that contains about 20 different building blocks—the amino acids. You are probably wondering, "How is this possible?" The process is called translation, and the name suggests the process. The solution to this problem is that the ribosome reads the genetic code as a series of three-letter combinations of nucleotides called codons. Each codon corresponds to a particular amino acid (some amino acids have more than one codon. Sometimes there are up to four different codons that are translated into the same amino acid) or a stop signal. The ribosome is responsible for the translation, namely creates the connections between the amino acids into the nascent protein.

The ribosome is a cellular organelle composed of two subunits: the small ribosomal subunit and the large ribosomal subunit (Figure 3), each of which has a specific function. The small ribosomal subunit is responsible for reading the genetic code from the mRNA and matches between the bound mRNA codon and the corresponding anticodon on the **transfer RNA (tRNA)**. The site where the genetic code is read is called the decoding center. The large ribosomal subunit is responsible for catalyzing the new peptide bond between the amino acids and

structure of the protein **(right)**. This unique structure is appropriate for the function of the protein produced (one of the many different functions of the various proteins in the body). Image adapted from here.

Figure 3

The structure of the ribosome. The ribosome consists of a few dozen proteins (purple and magenta) and chains of ribosomal RNA (turquoise and gray), organized into two subunits: the small ribosomal subunit (turquoise and purple) and the large ribosomal subunit (magenta and gray). The small subunit contains the site where the genetic material is read, called the decoding center, into which the mRNA molecule enters and in which it binds to the transfer RNA (tRNA) according to the genetic code, represented by three letters (see Figure 4). The large ribosomal subunit contains a site called the peptidyl transferase center (PTC), where the amino acids, carried by tRNA, bind to each other. The two subunits of the ribosome come together during the assembly of a new protein and are separated at the end of protein assembly (Figure credit: Ada Yonath).

MESSENGER RNA (mRNA)

A type of RNA that copies the genetic information from DNA to the ribosome, where it serves as a template for protein synthesis. During the process of

for passing the synthesized protein through the ribosome into the Nascent Peptide Exit Tunnel (NPET). In the catalytic center of the ribosome, the peptidyl transferase center (PTC), the amino acids bind to each other, and a new peptide bond is formed. The PTC allows the bonding of the two amino acids as within it, they are situated in close proximity and in the proper orientation to create a new peptide bond. After the new bond is formed, the tRNA holding the nascent protein moves together with the mRNA to the "next station" across the ribosome, ready to form a new peptide bond. The nascent protein chain passes through the large ribosomal subunit within the NPET and emerges at the other end of the ribosome.

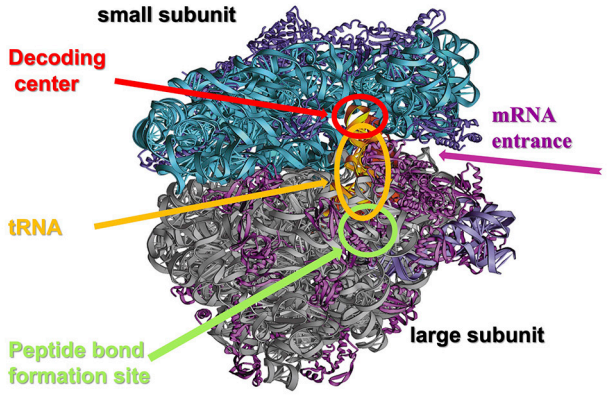

Figure 3

The small and large ribosomal subunits are bound to each other during protein assembly and detach from each other when protein translation is complete. Each ribosomal subunit consists of proteins and very long RNA chains called **ribosomal RNA (rRNA)** (Figure 3) [1]. There are many ribosomes in each living cell—from tens of thousands in bacteria, for example, to millions in human cells. The work of ribosomes requires a lot of energy—more than 60% of the total energy used by the cell [2]! This highlights the importance of the ribosome within the cell, as it is the factory that produces the proteins required for all cellular activities. The ribosome works at an amazing speed: a bacterial ribosome can produce a protein of average size within a few seconds to a minute.

Ribosome translation of an mRNA molecule occurs in three stages: Initiation, Elongation, and Termination: during initiation, an mRNA binds to the small ribosomal subunit of the ribosome. Then a tRNA carrying the amino acid methionine binds to the start codon of the mRNA sequence. The start codon in all mRNA molecules has the sequence AUG, which codes for methionine. Initiation factors join the small ribosomal subunit, mRNA, and tRNA complex, and the initiation complex is formed. Next, the large ribosomal subunit binds and forms the translation complex, i.e., the active ribosome. During the elongation stage, the ribosome translates every codon in turn. For

protein synthesis, the mRNA is read by the ribosome and used to assemble a chain of amino acids in the correct order according to the genetic code. The mRNA molecule produced by this process serves as an accessible and safe copy to be translated to proteins, and disintegrates once it is translated.

Figure 4

Protein production in the ribosome. The ribosome is composed of two distinct and separate subunits: the small ribosomal subunit **(bottom)** and the large ribosomal subunit **(top)**. The small ribosomal subunit decodes the mRNA (multicolored chain) that contains the instructions for assembling the protein. The large ribosomal subunit catalyzes the formation of peptide bonds and synthesizes the nascent protein chain according to the instructions of the mRNA as follows: the first transfer RNA (tRNA) loaded with the corresponding amino acid (colored circle) binds to the peptidyl site (P site), then the next loaded tRNA binds to the aminoacyl site (A site) of the ribosome. A new peptide bond is formed between the amino acids bound to the tRNA at the peptidyl site (P site) and the A site (pale blue and orange circles in the A and P sites). The tRNA from the P site is transferred to the exit site (E site) (dark red), the tRNA from the A site is transferred to the P site with the nascent protein chain bound to it, and a new tRNA

each amino acid, there is a separate and unique tRNA that binds to the ribosome and carries the amino acid with it. As the ribosome moves across the mRNA, new tRNA molecules loaded with the corresponding amino acid keep binding and moving between the three active sites of the ribosome: the Aminoacyl site (A site), Peptidyl site (P site), and the Exit site (E site) (Figure 4). Then the next tRNA carries an amino acid, enters the ribosome, binds to the A site, a peptide bond is being formed between the amino acids bound to the A site and the P site, and the nascent protein chain moves on within the NPET. Later, the tRNA from the P site moves to the E site and is released from the ribosome. The tRNA from the A site moves to the P site, and a new tRNA binds to the A site. Each time a new amino acid arrives, it binds to the previous amino acid by a peptide bond. In this way, the nascent protein chain is formed [3]. The growing peptide chain passes through the ribosome through a Nascent Peptide Exit Tunnel (NPET) and emerges from the ribosome. Elongation continues as long as the mRNA advances in the ribosome and presents new codons. Termination occurs when the ribosome reaches a stop codon. A stop codon is a codon on the mRNA for which there are no tRNA molecules that can recognize it. When the translation is complete, the two subunits of the ribosome are separated and the nascent protein exits and folds into its three-dimensional structure, alone or with helpers (chaperones). In this way, the new protein is released from the ribosome and can perform its function in the cell (a video describing this process, can be found here).

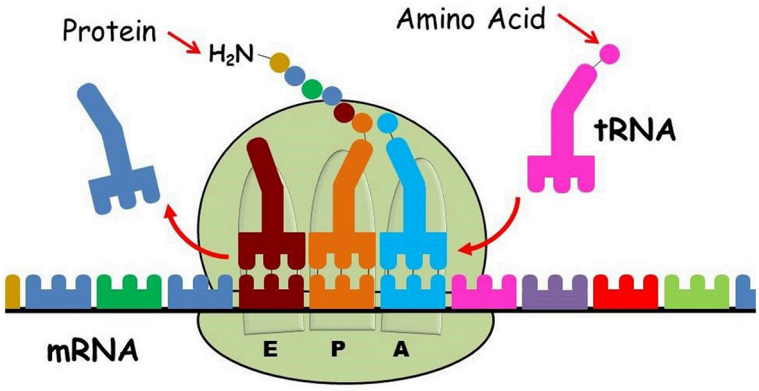

Figure 4

How did we discover the structure of the ribosome? We developed a unique method to do this, which we will discuss in the next section.

CRYSTALLOGRAPHY INSPIRED BY POLAR BEARS

We have deciphered the structure and function of the ribosome using a method called **Crystallography**. This method enables us to study

loaded with the next amino acid can bind the A site. At the end of the process, the amino acid chain is released from the ribosome and folded into the functional protein (Image credit: Ada Yonath's modified Google figure).

Figure 5

Deciphering the structure of a crystal using crystallography. **(A)** An example of the atomic arrangement in a crystal known from our daily lives—table salt. Table salt consists of two ions: sodium cation (a positive ion—in green) and chloride anion (a negative ion—in blue), arranged periodically in unit cells that repeat in all directions of space. **(B)** Determining the structure of a crystal using crystallography: the crystal is irradiated with a uniform and concentrated X-ray beam. Due to the arrangement of the atoms that make up the crystal, the X-ray beam is diffracted after hitting the crystal. During the experiment, the crystal is irradiated from several angles, and the strength and position of the dots of the image appearing on the screen are measured (right). Using the image of the diffracted beam, we can decipher the three-dimensional structure of the crystal.

TRANSCRIPTION

The process by which a messenger RNA molecule is created based on the DNA molecule. DNA contains all the operating instructions for the production of proteins in the cell (the genes). Since DNA is the original copy and

crystallized materials. A **crystal** is a structure consisting of units with internal symmetry that repeat in space in an orderly fashion. The simplest and smallest repeating unit in a crystal is called a "unit cell". A unit cell of table salt is shown in Figure 5A. Each unit cell in a crystal has a fixed measure of length, height, width, and fixed angles between its feces. In a crystal, the unit cells are arranged periodically in three dimensions, side by side, like floor tiles. In crystallography, the crystal is irradiated with a uniform and very concentrated X-ray beam (Figure 5B). The atoms presented in each unit cell of the crystal diffract the X-ray beam to a few specific directions, determined by the physical law, called Bragg's law, and their properties and positions, once the X-ray beam hits them. During irradiation, images of the scattering of the beam are captured and collected on a screen (Figure 5B, screen at right) or detector. Using these diffraction patterns by mathematical procedures called Fourier equations, we can decipher the three-dimensional structure of the unit cell.

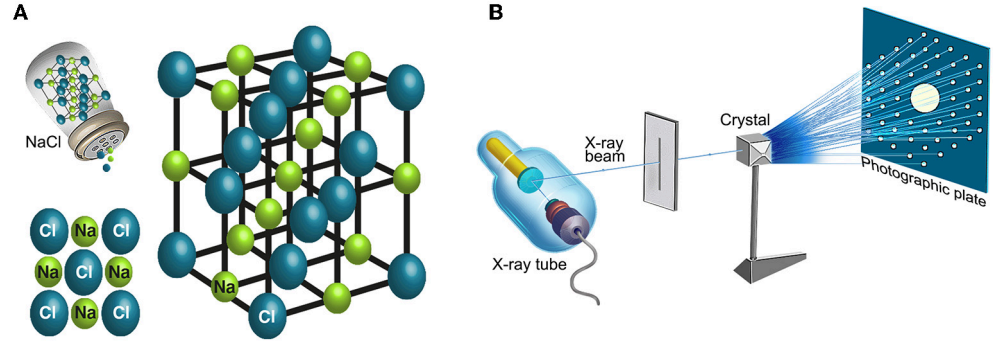

Figure 5

The first requirement for deciphering structure using X-rays is the preparation of crystals. Some materials, such as sugar, table salt and quartz stone, are natural crystals—these crystals are stable and solid. But most materials and structures in nature and in the body, such as proteins (e.g., hemoglobin) or their complexes, like ribosomes, are not naturally crystallized. Therefore, the first challenge in our research was to find conditions to crystallize the multi-components complicated and flexible ribosomes—a task previously considered impossible, since many famous scientists had already tried and failed. For a long time, we could not crystallize ribosomes because of their extremely complicated structure. Due to their functional requirements in the body, they have motor abilities—and tend to disassemble outside the cell, making their crystallization even more difficult.

One of the many important breakthroughs we made while trying to crystallize the ribosome was inspired by polar bears. It turned out that during their winter hibernation, a large portion of the bears' ribosomes are maintained in a dense, almost periodic structure throughout the winter, until the bears awaken from their sleep in the spring. We assumed that this happens so that the bears' ribosomes do not fall

cannot be replaced, the cell has several different mechanisms to protect it, including the transcription mechanism.

RIBOSOME

The organelle in the cell that produces proteins. The ribosome consists of two subunits, the small ribosomal subunit, and the large ribosomal subunit. Both subunits are composed of many ribosomal proteins and ribosomal RNA. The small ribosomal subunit is responsible for decoding the genetic code according to the messenger RNA, while the large ribosomal subunit is responsible for protein production according to the instructions of the genetic material.

TRANSLATION

This is the second phase of protein synthesis, in which the genetic information encoded in messenger RNA is used to assemble a chain of amino acids by the ribosome. As the amino acids are added to the growing chain, the protein begins to advance within its tunnel toward exiting the ribosome and folding into its final three-dimensional structure.

TRANSFER RNA (tRNA)

A family of unique RNA molecules each brings a specific amino acid to the ribosome during translation. Each tRNA is specific for a particular anticodon sequence that binds to a complementary codon on the mRNA and carries the amino acid decoded by the mRNA.

apart when they are inactive over the winter—so that they can still function in the spring. This told us that, under stress or extreme conditions, the ribosomes tend to arrange in an almost periodic density, and therefore we chose to produce crystals from ribosomes of bacteria living under extreme stress conditions in the dead sea. Indeed, through intense work and after trying countless different conditions, we managed to get stable crystals of active ribosomes.

After producing the crystals, we knew we would face another difficulty. The X-ray beam that we had to use to decipher the structure of the ribosome was of very high energy (produced in giant accelerators, e.g., un Grenoble, Stanford or APS) that affects the chemical bonds between the atoms of the ribosome crystals and causes migrating atoms. This means that as we irradiate the crystal to collect data about its structure, we are destroying it. In practice, therefore, we would not be able to measure more than a few percent of the information required to determine the ribosome's structure. We knew we had to find a way to collect enough data from the crystallized ribosome before it was destroyed. Hence, we tried to make crystals that would last longer so that we could make more measurements. We developed a method to reduce the damage from the X-ray radiation by rapidly freezing the crystals at very low temperatures (around −200 degrees Celsius, by using liquid nitrogen), under conditions that would prevent the formation of ice crystals in the liquid surrounding the crystallized ribosome, aiming at minimizing the ability of the ribosomes atoms to move around after their bonds are broken by the intense beam. By using special solvents mixtures, we developed a procedure, that enabled us to minimize the spread of damage caused by the radiation. That is, measuring the diffraction of small areas of cooled crystals, while minimizing the damage during measurements, and avoiding the spread of the broken atoms to all areas of the crystal that had not yet been exposed to the radiation. Using this method and some other experimental procedures developed years earlier at the Weizmann Institute of Science, we succeeded in maintaining the diffraction abilities of the ribosome's crystals for a long time, and eventually—after 20 years—also deciphering its structure and understanding how it functions.

Throughout our entire project, many scientists thought it was impossible to decipher the structure of the ribosome. Well-known scientists even made fun of us for the substantial challenge we were trying to tackle. But thanks to our determination and creativity, we succeeded to accomplish our mission by constantly searching for "out of the box" solutions. In fact, despite the mistrust of our ability in the scientific world, and the wide contempt for our deep cooling procedure, the entire bio-crystallography adopted it within a few months. Our remarkable success also led to an important medical application, which you will read about in the next section.

FIGHTING FOR HEALTH—HOW TO NEUTRALIZE BACTERIA

As mentioned earlier, the ribosome is a highly important component of every living cell. When the function of the ribosome is significantly impaired, the cell stops producing proteins and cannot continue to function. This is also true for bacteria. Bacterial ribosomes function similarly to human ribosomes but their structures are slightly, although significantly, different from that of human ribosomes. These differences allow us to use antibiotics that harm the bacteria without wounding the patients taking the antibiotics.

There are several antibiotic families. Natural antibiotics are produced by bacteria as weapons in the "wars" between different types of bacteria fighting for the same resources. They damage other bacteria by paralyzing an essential mechanism required for bacterial activity. One of these targets is the ribosome. Although the ribosome is huge compared to the size of the antibiotic, most antibiotics bind and block one of the active ribosomal sites: for example, they inactivate the PTC or block the NPET in the large ribosomal subunit, thus preventing the nascent protein chain growth (Figure 4) by discontinuing its elongation. Another ribosomal target is to block the movement of mRNA within the small ribosomal subunit. This blockade prevents the mRNA from being properly read, which is necessary for the production of a proper protein. Other antibiotics target the A site of the small ribosomal subunit and prevent the tRNA from binding to the ribosome. When this happens, the amino acid cannot bind to the next amino acid in the chain [4]. Click here to watch a video demonstrating how antibiotics can disrupt the function of bacterial ribosomes.

Indeed, the findings from our research have important applications in the field of medicine. But you may have heard that some disease-causing bacteria have developed resistance to certain antibiotics, making them even more dangerous. Understanding the structure and function of the ribosome has allowed us to better understand how different antibiotics work and how bacteria have developed resistance mechanisms to them, thus provided ideas of how to the fight against antibiotic resistance. This is an example of how the great perseverance and struggle we have invested over dozens of years in deciphering the structure and function of the ribosome have been justified and have borne fruit. In this context, I would like to share with you some recommendations that I have formulated as a result of this process.

RECOMMENDATION FOR YOUNG MINDS

My main recommendation is to follow your curiosity. If you try to contribute new scientific information to a scientific field you are extremely passionate about, you may face problems in searching for

RIBOSOMAL RNA (rRNA)

A unique RNA sequence that forms the structural and catalytic core of the ribosome. About two-thirds of the mass of the ribosome is rRNA. The last third of its mass is ribosomal proteins.

CRYSTALLOGRAPHY

A research technique used to determine the three-dimensional structure of molecules, including proteins and nucleic acids. It involves growing crystals of the molecule and radiating them with an X-ray beam to produce a diffraction pattern that can be used to calculate the positions of the atoms in the molecules within the unit cell. This technique is used to understand protein function and interactions.

CRYSTAL

A solid material with a repeating pattern of atoms or molecules. This pattern gives crystals special properties, such as the ability to diffract light and generate electricity. Scientists use crystals to study the shape and internal bonds of molecules such as metals, organic compounds, proteins and DNA that are too small to see by a light microscope.

support—especially in the beginning. Some might say you have no chance of success. Others might say that your research is misleading, and they might even claim that you are wasting the public's money. I recommend that you focus on your curiosity—be strong and believe in yourself even if you do not yet achieve the goal you wished for. If you delve deeper into the problem that interests you and its logical basis, you will increase the chances of its solution. My goal was to decipher the structure of the ribosome. Throughout the process, I believed that even if I did not succeed in deciphering the entire structure, I would make significant scientific progress along the way. Even before the structure of the ribosome was deciphered, my research led to the development of new methods in crystallography. These methods led to the solution of tens of thousands of previously unknown structures that helped improve our understanding of many biological processes.

Another aspect I would like to share with you is that the more challenging the path, the greater the satisfaction at its end. So be determined and work to overcome the difficulties that stand in your way, trusting that great satisfaction awaits you around the next corner.

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YOUNG REVIEWERS

ANSHUL, AGE: 12

Hello! My name is Anshul and I am a seventh grader at Germantown Academy, which is close to Port Washington. I am very interested in Biology and Entomology. I am an active member of the Johns Hopkins CTY program, and my favorite hobbies are reading and observing the environment.

JULIANA, AGE: 11

Juliana is a 5th-grader with a passion for Science and Math. She has always had an innate curiosity about the world around her, and has learned about the scientific method and hypothesis testing. She recently attended an experiment-based science camp focusing on genetics and molecular biology. Juliana also enjoys solving problems in algebra and geometry, particularly in the coordinate plane. She is delighted to work alongside the team and review manuscripts for Frontiers for Young Minds.

SUHAS, AGE: 16

My name is Suhas and my favorite subjects are Physics and Biology. I am passionate about learning how life across the planet survives and how diverse it is. My favorite domain of animal life is marine biology. It is fascinating to me how complex the life processes are, yet possible to unravel them. I am greatly inspired by Nobel laureates like Ada Yonath who dedicate their lifetime on great scientific discoveries! I enjoy playing sports like Basketball and Football in my free time.

AUTHORS

ADA YONATH

Ada Yonath is a professor of chemistry at the Weizmann Institute of Science in Israel. She received her bachelor's degree in chemistry and her master's degree in biochemistry from The Hebrew University of Jerusalem (Israel). She then, received her Ph.D. from the Weizmann Institute of Science, where she worked on deciphering the high-resolution structure of collagen. In 1968, Prof. Yonath began a short postdoctoral fellowship at the Carnegie Mellon University in Pittsburgh, Pennsylvania, United States, where she worked on deciphering the structure of muscles. During her second postdoctoral fellowship, at the Massachusetts Institute of Technology (MIT), she studied the structure of enzymes involved in staphylococcal a bacterial nuclease, namely a naturally-secreted nucleic acid degrading enzyme using X-ray crystallography. In 1970, Prof. Yonath returned to the Weizmann Institute as a faculty member and established the first laboratory for biological crystallography in Israel. During her time at the Weizmann Institute, Prof. Yonath held serval important leadership positions including Director of the Joseph and Ceil Mazer Center for Structural Biology, Director of The Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly, Head of the Department of Structural Chemistry, and the Head of the Department of Structural Biology. In parallel, for 25 years she led the Center for Ribosome Research in DESY Hamburg. Over the years, Prof. Yonath has received numerous prizes and awards, including the Il-Pis Prize for Extraordinary Achievements in Advanced Studies (1967), the first European Prize for Crystallography (2000), a Kilby International Award (2000), an Honorary Fellowship from the Israeli Crystallographic Society (2001), the Israel Prize for Chemistry Research (2002), the L'Oréal-UNESCO prize (2008), and the Nobel Prize in Chemistry for deciphering the structure of the ribosome (2009). *Ada.Yonath@weizmann.ac.il

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NOA SEGEV

Noa Segev is a scientific writer and project coordinator at Frontiers for Young Minds. She earned her B.Sc. in physics at The Hebrew University of Jerusalem and her M.E. in renewable energy engineering at the Technion—Israel Institute of Technology. Since 2019, she has been interviewing Nobel Prize winners and co-authoring articles for the Nobel Collection at Frontiers for Young Minds. Noa aims to make the science behind Nobel Prize-winning discoveries accessible to all, and to share valuable insights from the vast professional and personal experience of Nobel Laureates. *noasegev@gmail.com

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THE SECRETS OF SECRETION: PROTEIN TRANSPORT IN CELLS

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YOUNG REVIEWERS:


ANJISHNU
AGE: 15


ELISA
AGE: 11


JOONSAH
AGE: 10

Secretion is a fundamental process in which cells release substances to their external environments. Secretion is essential for many body functions, including growth, digestion, and cell communication. To be secreted, proteins must pass through various stations inside cells, which together form what is called the secretory pathway. In this article, I will tell you about the secretory pathway and the stages that proteins go through from their production up until their secretion. Then, I will present the original work that we did in our lab to identify genes related to the secretory pathway in yeast cells. Finally, I will explain the importance of our work to the overall study of the secretory pathway, both in yeast and mammalian cells.

Professor Randy Schekman won the Nobel Prize in Physiology or Medicine 2013, jointly with Prof. James Rothman and Prof. Thomas Südhof, for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells.

THE SECRETS OF SECRETION

Our complex bodies perform various functions, many of which require the transportation of substances from one place to another. One key process involved in moving substances around in the body is called **secretion** [1]. Secretion is the controlled release of substances from inside a cell to the blood or to other cells. For example, cells in the digestive tract secret digestive enzymes that help break down the foods that we eat, and cells in our glands secrete hormones that support our growth and development. Another type of secretion occurs in the brain, where nerve cells secrete messengers called neurotransmitters to communicate with other nerve cells.

SECRETION

A critical process by which cells release substances, such as hormones and enzymes, in a controlled way.

Before a cell secretes a substance, the substance must first be produced within the cell and then transferred from inside the cell across a membrane to be released outside the cell. Sounds simple, right? Well, this process is actually quite complex. As you might already know, the cells of all **eukaryotes** including humans are built in a compartment-like structure and contain various "organs", called organelles (Figure 1A). Each organelle performs specific functions and requires its own special environment to function optimally. This environment can be very different, and often compete with the environments of other organelles or other areas within the cell. Therefore, every organelle is enclosed by a membrane that separates it from the rest of the cell. This membrane is like a barrier that prevents the free movement of substances into and out of the organelle, in the same way that the cell's membrane prevents the free movement of substances into and out of the cell. So, it seems that we have a problem—how do substances move through those membranes to be secreted, if the membrane's job is to prevent the substances from passing (Figure 1B)?

EUKARYOTES

Organisms whose cells contain a nucleus.

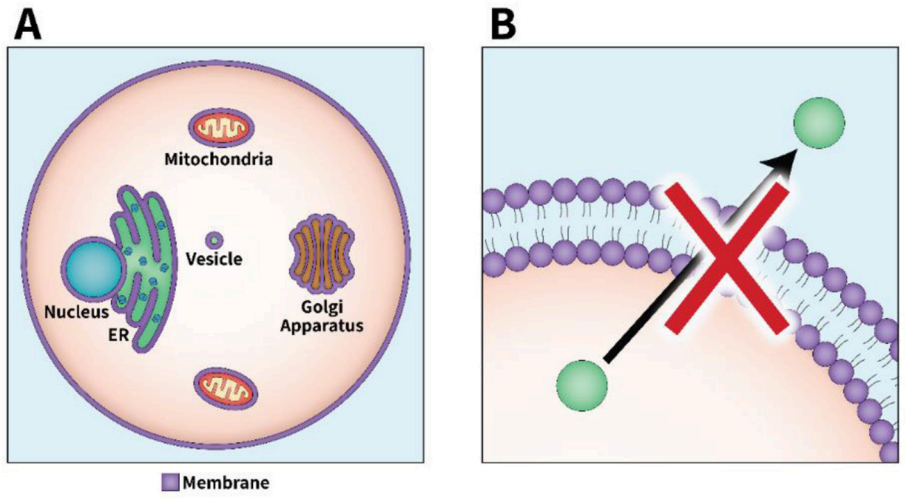

Figure 1

Figure 1

A cell's organelles are important for transporting materials. **(A)** A eukaryotic cell is defined as a cell with a nucleus and multiple organelles that are separated from one another by membranes. Cells also have a plasma membrane that separates the contents of the cell from the outside environment. A cell's membranes block many substances from passing through them. **(B)** The process of secretion requires materials to be passed between organelles and to the outside of the cell. But most materials cannot freely pass through the membranes covering the organelles and the cell, so cells must have a special process for secretion.

It turns out that there is a special mechanism that allows the passing of substances between organelles and out of the cell. This mechanism involves small carriers called **vesicles**, which are like little cars that take a passenger substance to its destination [1, 2]. In the next section, I will tell you about the production and action of the vesicles that take part in the secretion of molecules called proteins, which are the "workers" that drive many processes in the body.

VESICLES

Membranous sacs that carry substances inside the cell.

THE SECRETORY PATHWAY FOR PROTEINS

About 30% of the proteins made in the human body are secretory proteins, which are proteins designed to be secreted from cells. Like all proteins, secretory proteins are produced in sewing machine-like particles called ribosomes (the Nobel Prize in Chemistry in 2009 was awarded to three researchers who studied the structure and function of ribosomes). The ribosomes that produce secretory proteins are located on a special channel in the membrane of an organelle called the **endoplasmic reticulum (ER)**. After secretory proteins are produced, they pass through a channel embedded in the ER membrane into a canal-like network of ER tubules that are spread around the cell. There, other proteins called coat proteins form a special structure on a small surface of the ER membrane, kind of like a dome. This dome collects the secretory proteins and then pinches off small spheres of the membrane—the vesicles—which contain the secretory protein cargo produced in the ER membrane. This process of vesicle formation, in which part of the ER membrane is pinched off, is called **budding** [1, 2]. After budding, coat proteins fall off the vesicles, and the naked vesicles containing the secretory proteins are passed on to an organelle called the Golgi apparatus. The Golgi sorts and directs the secretory cargo vesicles to their final destination—the cell membrane (also called the plasma membrane). In the plasma membrane, with the help of additional proteins (such as SNARE and RAB), the vesicles go through the last two stages before protein secretion—docking and fusion [3]. In these stages, the vesicles come very close to the membrane in a specific alignment, then finger-like structures on the vesicles and the membrane intertwine and pull the vesicles and the membrane toward each other, kind of like Velcro. When a vesicle and the membrane are close enough together, they fuse spontaneously, somewhat like two soap bubbles merging. Then, the secretory protein cargo is released to the other side of the membrane, outside of the cell—a process called **exocytosis**.

ENDOPLASMIC RETICULUM (ER)

The organelle in which secretory proteins are produced and placed into vesicles.

BUDDING

A process where part of the ER membrane is pinched off and form a vesicle.

EXOCYTOSIS

A process where a substance is released from within the cell to the outside environment by the fusion of a vesicle with the cell's plasma membrane.

SECRETORY PATHWAY

The pathway used to secrete proteins from inside the cell to the outside environment. It includes organelles such as the ER and Golgi apparatus, as well as the cell membrane and vesicles.

As you can see, the process of producing, transporting, and secreting proteins is quite complex. You can think of it like an automobile assembly plant, in which the automobile is put together piece-by-piece along an assembly line before it can be sent out of the plant. The "assembly line" for secretory proteins is called the **secretory pathway** [4], and the process by which vesicles carry substances between compartments within the cell and out of the cell

VESICLE TRAFFICKING

The process in which vesicle carry substances between compartments in the cell and between the cell and its outer environment.

is called **vesicle trafficking** (Figure 2). You might be surprised to know that many of the mechanisms that control vesicle trafficking in the secretory pathway have existed in cells for more than 2 billion years! This means that cells in our bodies, including the nerve cells in our brains, share basic secretion machinery with simpler organisms, such as yeasts [5, 6]. In fact, the first discoveries about the machinery that operates the secretory pathway were made using yeast [7–9]. In the next section, I will tell you about the early discoveries that were made in yeast in my lab, which earned me the Nobel Prize in Physiology or Medicine.

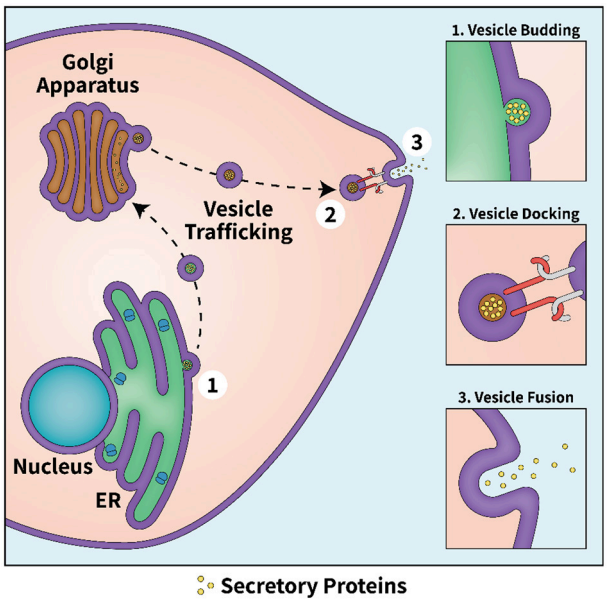

Figure 2

The secretory pathway. The secretory pathway in cells involves several steps. First, secretory proteins are produced by ribosomes on the membrane of the ER. Vesicles containing the proteins bud off from the ER membrane (**step 1**) and travel to the Golgi apparatus, which directs them to their final destination: the plasma membrane. The vesicle's membrane and the plasma membrane hook together like Velcro, in a step called docking (**step 2**), and then fuse like two soap bubbles, releasing the vesicle's contents to the outside of the cell (**step 3**).

SECRETORY PATHWAY IN YEAST

When I started studying the secretory pathway with my students, there were some hypotheses about the machinery controlling vesicle trafficking, but no one had identified the genes involved in this machinery. We believed that studying the genes involved in the secretory pathway would be a powerful way to learn about vesicle trafficking. We chose to work on baker's yeast, a simple microorganism that is used to make bread and beer. Baker's yeast was attractive to us because we could easily grow it in the laboratory, and we had simple techniques for accurately analyzing its genes.

To identify genes related to the secretory pathway in yeast, we exposed the yeast cells to chemicals that cause random changes in the DNA, called mutations. Random mutations affect one gene randomly, out of the approximately 5,000 genes in yeasts. How, then, did we manage to identify one specific secretory pathway gene in this haystack of genes? First of all, we knew we were looking for essential genes, which

are genes that cells need to survive. We knew that if we introduced a mutation into an essential gene that inactivated that gene completely, the cell would die and we would have no cells to work with. Therefore, we needed to somehow introduce a mutation that would allow the cell to stay alive long enough for us to study it. Among the many mutations that we introduced randomly, some by chance change the protein encoded by that gene to make it unstable at human body temperature. Normal yeast cells are perfectly happy at human body temperature but these particular mutations can only function at room temperature. These are called **temperature-sensitive lethal mutations** [10]. So, using temperature-sensitive lethal mutations, we could narrow our search down to about 1,500 essential genes out of the 5,000 total genes of yeast. This is still a large number, so we needed another parameter to help us find the genes responsible for vesicle trafficking. For that, we based our search on a hypothesis made by George Palade, a Nobel Prize winner who pioneered the study of the secretory pathway. Palade hypothesized that secretion is linked to cell growth through vesicle fusion. That is a logical assumption because when vesicles fuse with the plasma membrane, the membrane should get bigger. This allowed us to use another search parameter in our study—we looked for mutations that prevented the yeast cells from getting bigger, and that also caused secretory proteins to build up inside the cells.

TEMPERATURE-SENSITIVE LETHAL MUTATIONS

Cells that act normally at low temperatures but die at higher temperatures.

Specifically, we were searching for the piling up of a protein called invertase, which is the enzyme that breaks sucrose (table sugar) into two smaller sugars—fructose and glucose. Normally, invertase is found in the **cell wall** of yeast cells, so when the secretion process works properly, we expect to see the same amount of fructose and glucose products when we incubate *whole* cells in sucrose as we do when we break the cells open and incubate the pieces in sucrose. However, when the secretion process does *not* function properly and invertase piles up inside the cells, we should get a larger amount of fructose and glucose when we incubate broken cells compared to whole cells. That is the exact result we got in an experiment led by my brilliant student Peter Novick, which we published in 1979 [7]. We called the gene that was changed by this mutation sec1. When we examined cells with mutated sec1 using a powerful microscope called an electron microscope, we saw that many vesicles containing invertase had accumulated inside the cell (Figure 3A). This was very exciting—it was the first time that vesicle accumulation inside a cell was ever seen. This finding also supplied strong evidence to support George Palade's hypothesis that secretion and cell growth are related. From there, we had to keep working to understand the specific role that the sec1 protein (produced from the instructions in the sec1 gene) played in vesicle trafficking.

CELL WALL

The outermost layer of the cell that covers the cell's membrane.

Eventually, we found out that the sec1 protein is involved in the final step of the secretion process, namely docking and fusion. Soon after we identified the sec1 mutant, we identified more mutants involved

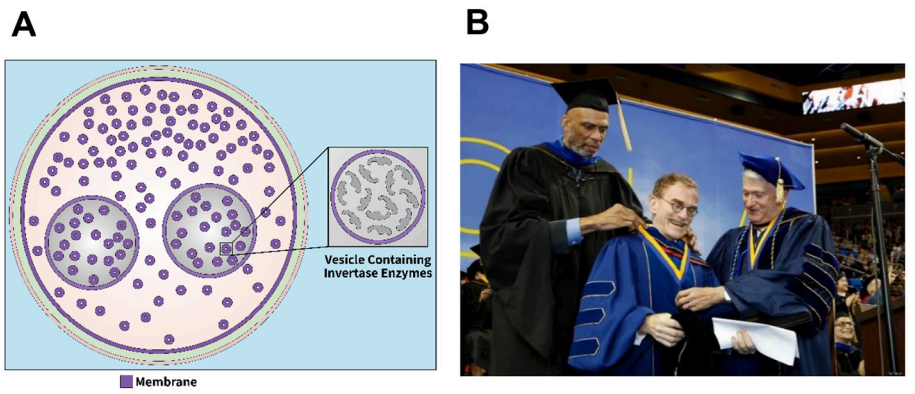

Figure 3

Figure 3

The Nobel Prize-winning discovery of Sec1 in Baker's yeast. **(A)** The discovery that led to the Nobel Prize involved the piling up of vesicles containing a protein called invertase, inside a baker's yeast cell with a mutation in a gene called sec1 (image adapted from [7]). **(B)** After I won the Nobel Prize, I was invited by my colleagues at UCLA, where I received my undergraduate degree, to give the commencement address. I arrived at the very same pavilion where my basketball idol, Kareem Abdul Jabbar (previously named Lewis Alcindor), used to play. He introduced me in front of 20,000 people, and then placed an honorary medal around my neck. This was a surreal moment that I will never forget (Image credit: UCLA).

in the yeast's secretion process [8], and we began to understand the order of the events that occur in the secretory pathway of yeast [9]. Our discoveries of genes involved in the yeast secretory pathway also advanced the understanding of how this process occurs in mammals, including humans [6]. More specifically, many of the genes and proteins we discovered were found to have counterparts in mammalian cells. One of the leading scientists who found some of the counterparts of the genes we discovered is Prof. James Rothman, with whom I shared the Nobel Prize in Physiology or Medicine in 2013. After winning the Nobel Prize, I even got to meet my basketball idol Kareem Abdul Jabbar! (Figure 3B).

Our work was pioneering for two reasons: first, due to our specific findings of genes involved in the secretory pathway; but also due to our novel, successful use of microbial genetics to study the secretory pathway. Our findings opened a new line of research, which many of my students and *their* students have followed over the years. Once the secretory pathway in yeast was well-understood, people started using that knowledge for medical applications. One important example in which I took part is the use of the yeast secretion pathway to produce human insulin to treat diabetes [10]. As secretion is such a fundamental process in the human body, there are many more examples of medical conditions that could be treated based on our increasing understanding of the secretory pathway—such as heart diseases and digestion problems. Though I never intended to be involved in the development of medical applications, I am glad that our work contributes to treatments that help people all over the world.

RECOMMENDATIONS FOR YOUNG MINDS

I think that one of the most fundamental traits that a scientist should have and try to develop is the excitement of discovery. In my view,

science is the process of discovery—not just a list of facts about what is already known and established. If you have a passion for science, you should find an independent way of exploring—do not limit yourself to the material you learn in class. When it comes to expanding your knowledge, I believe that independent study such as in a laboratory is just as important as reading from textbooks. To understand whether you can be a good research scientist, you must experience research yourself. Research is frequently very frustrating—lots of things fail and you must learn to persevere in the face of constant failure. You can only equip yourself for that by experiencing laboratory research firsthand. This is also how you can develop faith in yourself and in your skills, which will allow you to believe that things can work out and that you can reach your goal, even when the process is very complicated. That is why I advise every young student to start working in the lab or in some form of independent study straight away.

Another aspect of a good scientist is the ability to identify an exciting problem to study and to fully commit to it. This means that you must keep focusing on that specific problem and think independently about the design and execution of experiments, observation or theory that can help you solve it. Even if you experience challenges along the way, you should keep thinking about the problem, turning it over in your head until you find a new way to approach it. I know that focus can be hard, especially for creative people who have many new ideas all the time. But each of us has only a certain amount of time and energy, and if we do not focus, we are not able to make good progress. Therefore, my advice is to keep your eyes on the prize and persevere until you find a solution to one important problem to which you have decided to dedicate yourself.

ADDITIONAL MATERIALS

1. A pathway of a hundred genes starts with a single mutant: Isolation of sec1-1.
2. Publishing important work in the life sciences: Randy Schekman at TEDxBerkeley.
3. Rothman and Schekman: Uncovering the Secretory Pathway - Youtube.

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CONFLICT OF INTEREST: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

ANJISHNU, AGE: 15

Hello, my name is Anjishnu and I am in tenth grade. I have a passion for writing, reading, and science. I enjoy playing tennis. I want to study biology when I grow up so that I can pursue a career in science.

ELISA, AGE: 11

Hi! My name is Elisa. I am 11 years old, and am in 6th grade. I have played the violin since I was 5 years old, and also have played tennis since I was 5 years old. I love doing many things, such as being outside with friends, reading, being with animals, playing the violin and tennis, and playing games. My favorite animals are dogs (DOGS RULE).

JOONSAH, AGE: 10

Hi, my name is Joonsah. I am 10 years old. I like to play sports, such as soccer, tennis, and skiing. My favorite animal is a snake. I play three instruments: piano, viola, and trumpet. I also speak four languages: English, Korean, Chinese and some German. I really like science too.

AUTHORS

RANDY SCHEKMAN

Prof. Randy Schekman is a professor of molecular and cell biology at the University of California, Berkeley, United States. Prof. Schekman completed a bachelor's degree at the University of California, Los Angeles, where he performed a research project on the replication of bacteriophages. He then went on to perform his graduate research in the laboratory of the Nobel laureate Prof. Arthur Kornberg at Stanford University, in the field of DNA replication. After his graduation in 1974, Prof. Schekman continued to the University of California, San Diego, where he studied the cell membrane. In 1976, Prof. Schekman became a faculty member at the University of California in Berkeley, where he still works today. During his years there, Prof. Schekman studied the secretory pathway in yeast, work for which he was awarded the Nobel Prize in Physiology or Medicine in 2013. Prof. Schekman won numerous other prizes and awards throughout his career, including the Gairdner Foundation International Award (1996), Lasker Award (2002), Louisa Gross Horwitz Prize (2002), Massry Prize (2010), and E. B. Wilson Medal (2010). Prof. Schekman is an avid supporter of open science publication, a topic he has been promoting for decades. After the death of his wife from Parkinson's disease a few years ago, Prof. Schekman organized a worldwide effort to engage scientists in a collaborative program of basic science to uncover the mechanism of Parkinson's disease. This effort continues today. *schekman@berkeley.edu

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SEEING BEYOND THE LIMITS WITH SUPER-RESOLUTION MICROSCOPY

Eric Betzig [1,2,3,4*]

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[3] *Department of Physics, University of California, Berkeley, Berkeley, CA, United States*
[4] *Howard Hughes Medical Institute, Chevy Chase, MD, United States*

YOUNG REVIEWERS:


AMY
AGE: 14


BREANNA
AGE: 13


EDWARD
AGE: 15

When I was young, I wanted to be an astronaut and I was a hard-core science fiction fan. I was always drawn to heroic characters that invented something new that resulted in an amazing breakthrough. When I started my scientific career, I did not want to do something small—I wanted to do something that would be really different and impactful. This is why I chose to work on one of the most difficult problems in the field of optical (light-using) microscopy—how to see objects that are smaller than the wavelength of visible light. This meant challenging a limit that was long believed to be unbreakable. In my research, I developed a method called photoactivated localization microscopy (PALM), which enabled us to break that limit with the help of glowing (fluorescent) molecules. Using the PALM method, and other methods based on glowing molecules, scientists can learn new things about living cells and single molecules and significantly advance our understanding of life.

Professor Eric Betzig won the Nobel Prize in Chemistry in 2014, jointly with Prof. Stefan Hell and Prof. William Moerner, for the development of super-resolved fluorescence microscopy.

HOW CAN WE SEE SMALL THINGS?

When we look at any object, we are actually detecting the light that bounces back from the object and reaches our eyes. Think, for example, about a submarine using sonar. When a submarine is navigating in the sea, it sends out sound waves and detects the waves that bounce back to it from underwater objects, such as rocks and sea animals (Figure 1A). In this way, the crew of the submarine knows how to navigate underwater. The same principle applies when we want to see things in the laboratory, such as cells or tiny organisms. We shine light (or some other form of **radiation**, such as electrons) on the tiny object, and we look at what bounces back from the object. The type of radiation is determined by a measure called the **wavelength**. As you might know, waves have a repeating pattern of high peaks and low troughs. The wavelength is the distance between two peaks (Figure 1B).

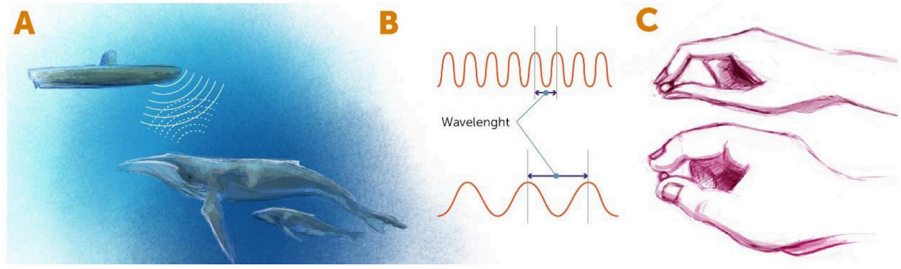

Figure 1

In terms of looking at tiny objects, the wavelength of energy bouncing off that object determines the resolution with which we can see that object. The shorter the wavelength is, the smaller the objects we can see. You can think about it like fingers that are trying to "feel" an object (Figure 1C). If the fingers are thick (meaning long wavelengths), we cannot sense the fine details of the object—it is like trying to feel millimeter-wide features with chubby fingers. So, if we want to see the tiny details of an object, we have two choices. First, we could use a form of radiation with a very small wavelength, such as x-rays. The problem is that prolonged exposure to the high energy of short wavelengths can kill living things, so we cannot study living cells or organisms using such short wavelengths. The other choice is to find some trick that will enable us to use longer wavelengths, which are less energetic, while somehow managing to see beyond the limits of those wavelengths. This is the idea behind **super-resolution microscopy**, which is the term for any method of microscopy that allow us to see beyond the limits of the wavelengths that it uses.

RADIATION

Energy, in the form of waves or particles, that is emitted from a source.

WAVELENGTH

A measure of the distance between two adjacent peaks of a wave.

Figure 1

Detecting objects using radiation. **(A)** In the sonar systems of submarines, radiation of sound waves is used to detect nearby objects. The sound waves are emitted from the submarine, bounce off nearby objects, and return to the detector in the sonar system. Similarly, when we see something with our eyes, light waves hit the object and bounce back to our detectors (our eyes). **(B)** Forms of radiation can be described by their wavelengths. Wavelength is defined as the distance between two adjacent peaks of the radiation wave. **(C)** A short wavelength (top) is like small fingers that help us "see" the small details of an object, whereas a long wavelength (bottom) is like chubby fingers, with which we can "see" only obvious details.

SUPER-RESOLUTION MICROSCOPY

Any method of microscopy which enables us to overcome the limits of the wavelengths it uses and therefore see objects with greater resolution.

Before the development of super-resolution microscopy, we could see living things as small as about 200 nanometers (or 0.0002 millimeters)—such as the larger compartments within the cells of animals, and even one-celled organisms like bacteria. We could not see smaller organisms such as viruses, or smaller parts of cells like individual proteins or other small molecules (Figures 2A, B). The ability to see living things at such high resolution was a huge leap forward! It opened up a whole new area of research and gave researchers the potential to better understand the most fundamental processes of life. Details that were previously undetectable using conventional microscopy techniques were exposed right before our eyes, and generated great excitement for studying the mysteries of life (Figures 3A, B).

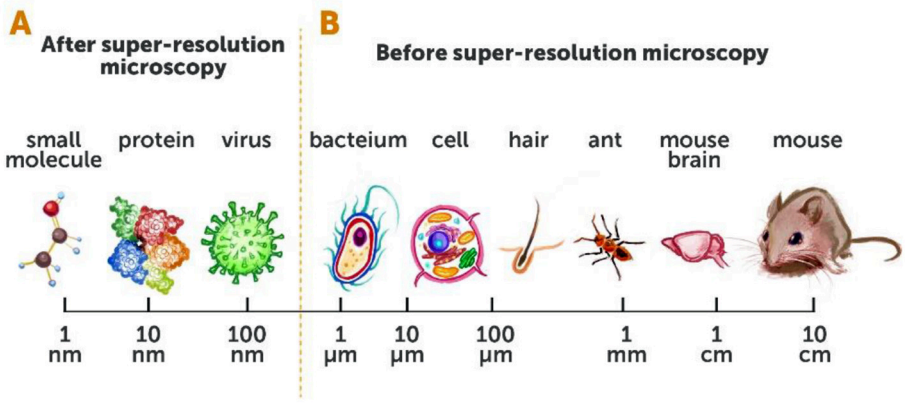

Figure 2

Conventional microscopy vs. super-resolution microscopy. **(A)** Super-resolution microscopy has opened up our ability to see a whole range of objects, down to ~10 nanometers [1 nanometer (nm) = one billionth (0.000000001) of a meter]. **(B)** Before super-resolution microscopy, we could only view objects that were 200 nm in size (about the size of a bacterium) or larger.

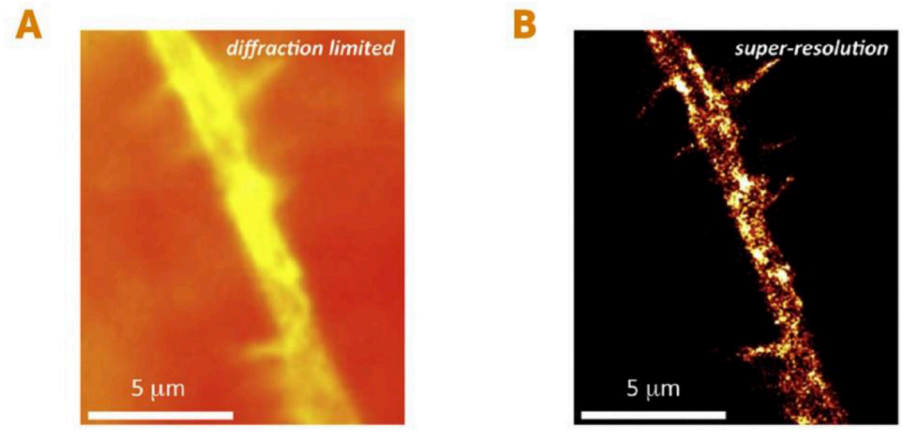

Figure 3

Seeing individual proteins in living cells using super-resolution microscopy. **(A)** An image of a small branch of a living nerve cell, taken using conventional microscopy. **(B)** The same nerve cell branch using super-resolution microscopy. This method allows us to see small details that were previously unobservable. In this case, we can see small ion channels, which are proteins in the nerve cell's membrane (bright yellow spots) responsible for conducting electricity in nerve cells [figure adapted from [1]]. Scale: one micrometer (µm) is 1,000 nanometers.

THE BEGINNING: NEAR-FIELD MICROSCOPY

When I started my advanced studies at university in 1983, two of my professors—Mike Isaacson and Aaron Lewis—had the crazy idea of trying to break what was called the **Abbe's diffraction limit**. This

was the concept that the smallest thing we can see using light waves must be at least half the wavelength of that light. For example, if the wavelength is 1 mm, we could see objects that are at least 0.5 mm long. My professors thought they could break this limit by manipulating light in a specific way. Their idea was based on the first demonstration of breaking the Abbe's diffraction limit, which was performed back in 1972 [2]. The basic idea was to drill a tiny hole, much smaller than the wavelength of light, in a small black plate. When the plate is placed very close to the object to be examined, and a light is shone through the hole, it illuminates a very tiny spot of the object—much smaller than the wavelength of light. The object can then be "scanned" by moving the illuminated plate around, point by point, across the object. Using this trick, we can see the object with resolution higher than the "natural" resolution of the incoming light. Today, this method is called **near-field scanning optical microscopy** [3, 4].

ABBE'S DIFFRACTION LIMIT

The physical limit of light microscopes whereby we can distinguish between two points in an object only if the distance between them is not smaller than half the wavelength of the imaging light.

NEAR-FIELD SCANNING OPTICAL MICROSCOPY

The first super-resolution microscopy method that was developed during the 1980s.

This was the first super-resolution microscopy method that I worked on. The main problem with this method is that the light that passes through the small hole spreads out very quickly on the other side. To get high resolution, we must work extremely close to the object we are imaging. In the case of cells, for example, this is challenging because cells are not flat, so it is difficult to control the plate on top of them. After working on this method for several years and taking it as far as I thought possible, I decided to quit this work, and science altogether. Little did I know that, a few years later, a big breakthrough in the field of biochemistry would bring me rushing back to science and microscopy.

SUPER-RESOLUTION FLUORESCENCE MICROSCOPY

In 1994, a pioneering study was published [5] showing that, using genetic engineering, we can attach a glowing handle, or "marker," called a fluorescent protein, to any protein in living cells. This is a special protein that glows when one specific wavelength of light is shone on it. I immediately realized that this work was a complete game changer in the field of microscopy, because it could help us to see the tiny structures of inside of cells. A year later, in 1995, I published a paper that laid out the foundations for a new method of microscopy [6]. But it was only in the early 2000s that additional advancements in the field of fluorescent molecules allowed me to follow through on my idea. The advancement created molecules that were not always fluorescent, but could be "activated" to glow when a certain wavelength of light was shone on them [7]. This meant that we could attach glowing markers to certain proteins inside living cells and intentionally activate them, to study cellular structures and processes. That was the start of the method of super-resolution fluorescence microscopy that I helped to develop, which was originally called **photoactivated localization microscopy (PALM)** [8, 9]. In 2014, I received the Nobel Prize in Chemistry for this method.

PHOTOACTIVATED LOCALIZATION MICROSCOPY (PALM)

A super-resolution microscopy method that I developed, that uses fluorescent molecules to break Abbe's diffraction limit.

The idea behind PALM is as follows: each cell contains about 20,000 different kinds of proteins, and often many thousands of each kind. We want to understand how they work together. Using a conventional microscope like you have in biology class at school, all you can see when you look at these proteins in a cell is a big glowing blob. The proteins are so close together that you cannot tell them apart. In PALM, we attach special fluorescent markers to proteins—markers that can be turned on by a low-power (violet light) laser (Figure 4, step 1) and then glow and become detectable when a separate, higher-power (blue light) laser is shone on them (Figure 4, step 2).

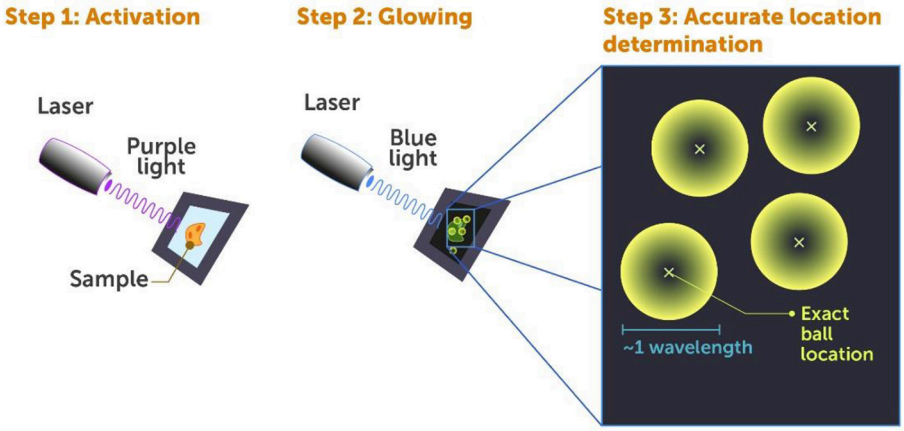

Figure 4

Figure 4

Photoactivated localization microscopy (PALM). Super-resolution microscopy of fluorescently marked cells using the PALM method includes three steps: **Step 1**: A laser beam of weak violet light is shone on the cell in brief pulses, to activate the fluorescent markers on only some of the proteins, making them ready to glow. **Step 2**: A blue laser beam is shone on the cell, which makes the activated proteins glow so they can be detected. **Step 3**: The location of individual proteins is determined by a computer, by finding the center (small x) of each "ball of light" generated by each fluorescent protein.

If we were to activate *all* the markers at once, they would all glow at the same time and this would create a big mess—that is where the big glowing blob comes from. This is why we use pulses of very low energy violet light to activate the markers—only a few at a time with each pulse. These few are randomly activated and are most likely well-separated from one another within the cell. When we then use blue light to detect these activated markers, they look like small glowing balls (Figure 4, step 2) ~1 wavelength of light in size, since that is the smallest anything can appear in the conventional diffraction-limited microscope we use to look at them (Figure 4, step 3).

Now here is the trick—we can use a computer to process the image we get from the microscope and accurately find the center of each of these "balls". You can think of it like a basketball which had a spherical shape with a certain diameter. You are able to point to the very center of the basketball with much better precision than estimating its diameter, even if you do not directly see the center. The same is true for these molecular balls—we can find their centers with very high precision, much closer to their true size than the size of the glowing balls. This means that, each time we pulse the cells with light, we can find the positions of a small set of proteins within the cell (Figure 4, step 3). The fluorescence of these proteins turns off naturally; then we

can illuminate another set of proteins and find their location. Typically, it takes tens of thousands of rounds of activation to map a whole cell. But the effort is worth it, as we get a very high-resolution image of the cell or any other object we are studying (see Figure 3B and images in Betzig et al. [9]).

CHALLENGES AND POTENTIALS OF SUPER-RESOLUTION MICROSCOPY

As you have seen, PALM is quite simple—all you need is a laser to shine a beam of light on the object, a camera, and a relatively simple software program to find the centers of the glowing proteins. This equipment is cheap and simple. In fact, my friend Prof. Harald Hess and I built the first model of PALM in his living room, with equipment that we bought with our own money while we were both unemployed! The difficult part involves working with the biological sample. There are many challenges including difficulties preparing living cells for our experiments, causing damage to the cells in response to the light, and figuring out the best way to detect and analyze the light emitted by the molecules we are interested in.

In terms preparing the cells, it turns out that many of the markers we can activate using light do not actually attach to the proteins that we want to see, but rather to other objects that happen to be nearby. This means that often the markers we use do not actually point us toward the locations of the proteins we are interested in. Additionally, even if we manage to label the correct proteins, we label only a small percentage of them—which is often not enough to give us a full image of the cell at the highest possible resolution. Even if we manage to label enough of the correct proteins, cells do not like to have intense light shone on them. Yet, the more intense the light we shine on the cells, the more information we can get. Therefore, we are always trying to find the balance between extracting as much information as possible information while avoiding damage to the cells.

The last challenge that I will mention here is a phenomenon called **photobleaching**. Photobleaching describes the fact that a marker can only glow a certain number of times. In other words, only a limited amount of light can radiate from a specific marker before the marker is destroyed or becomes permanently dark. Sometimes, this amount of light is not enough for us to extract the information we need to find the marker's exact location.

PHOTOBLEACHING

A phenomenon in fluorescent materials, in which they become permanently unable to shine light after they have done so a certain number of times.

As I mentioned previously, super-resolution fluorescence microscopy is unique because it enables us to image *live* cells and organisms. Using this technique, we do more than simply determine the structure of living things—we can also track processes happening within a cell, such as the movement of proteins, over time (see this video) [10, 11]. Using what is called single-molecule tracking, we can see into

the deepest mysteries of living cells and have a peek into the most fundamental processes of life. For example, single-molecule tracking has helped us to understand how RNA copies are made from the DNA inside a cell's nucleus—a process called transcription).

Tracking single molecules and figuring out how they move around within cells might be very important for the development of new drugs. In my view, the information we can learn about cellular mechanisms that were previously invisible to us might lead to a whole new paradigm of drug discovery and to important new treatments for various diseases, such as Alzheimer's and Parkinson's diseases. I think this might be the greatest thing that will come out of super-resolution microscopy, and it is why my colleagues and I started a drug-discovery company called Eikon Therapeutics©.

RECOMMENDATIONS FOR YOUNG MINDS

As I mentioned earlier, when I was a kid, heroic fantasy characters and astronauts inspired me. They represented the chance of having an impact on the world, by significantly improving people's lives. For me, that is the highest goal a person can choose in life. Therefore, I recommend that, whatever you do in your career, do something impactful that provides a meaningful contribution. It does not have to be big thing—raising children is impactful, and packing groceries is also impactful. Try to find something that is a good mixture of your own interests and that also has the potential to positively impact the people around you or society as a whole (Figure 5). If you do choose to become a scientist, do not get stuck on the idea of becoming a professor. This should not be a goal in itself, since there are many other ways to make meaningful contributions and great discoveries outside of working at a university.

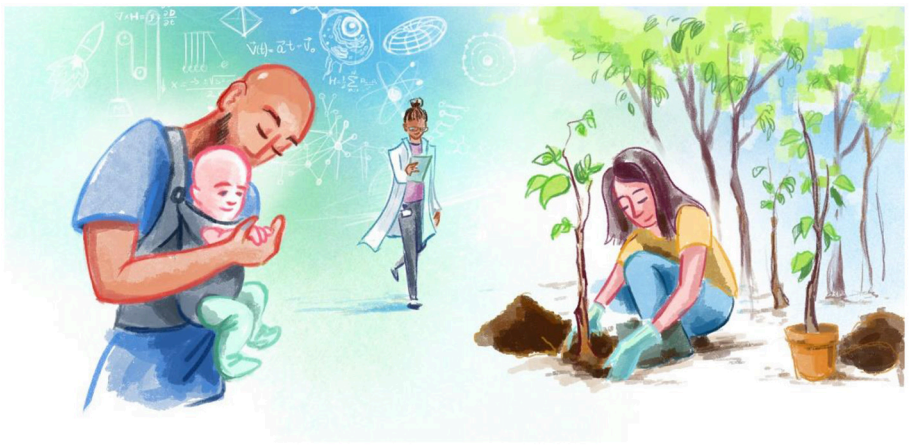

Figure 5

Recommendations for Young Minds. As you build your future, try to do something that you like and that also provides a meaningful contribution to society.

Personally, I find many advantages to doing the kind of research that I do. First, I am my own boss. I enjoy that because I like to make decisions myself rather than being told what to do. Second, in the type of science that I do, I try to invent new tools for people who are trying to answer scientific questions that are outside my expertise. This means I must learn lots of new things and become a "jack of all trades". I know a little bit about many things in many areas—from which materials work best in different machines, to biology and physics, to designing new research tools. This broad knowledge spills over into my day-to-day life, and I now understand things I see around me and enjoy the beauty and complexity in my everyday world.

The last thing I would like to discuss is the attitude you choose in whatever you do. First, you should never forget to think critically about any question that you are faced with. Do not be satisfied with superficial, automatic thinking—really try to see into the depths of the things you encounter. Second, do not be afraid to take risks. In my opinion, society has become too risk-adverse, which limits our ability to innovate and advance—both as individuals and as a society. Last, hard work is important! No matter what you do, at any age, try to push yourself while also having fun. Do not beat yourself up if things are difficult, but make serious efforts to get what you want. If you do not excel that is OK, because everybody is good at different things—nobody is good at everything. But push hard in everything that you do—hard work will get you anywhere. Find the thing that you love to do, work hard and become good at it, and then run with that ball, make good use of it, and enjoy the process, too.

ACKNOWLEDGMENTS

I wish to thank Noa Segev for conducting the interview which served as the basis for this paper, and for co-authoring the paper, and Alex Bernstein for providing the figures.

ADDITIONAL MATERIALS

1. Eric Betzig and Harald Hess (Janelia Farm/HHMI): Developing PALM Microscopy.
2. Prof. Betzig Nobel Lecture.

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CONFLICT OF INTEREST: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

AMY, AGE: 14

Hi, my name is Amy, I am 14 years old and I live in England. I am studying for my GCSEs, and whilst I am doing all three sciences, my favorite subject is English. In my spare time I play netball for a local club and I enjoy spending time with my friends and family.

BREANNA, AGE: 13

Hello! I am a seventh grader! I love drawing, figure skating, dancing, and tennis! I like watching movies and reading books when I am bored. I have a brother, a helpful mom and dad, and my best friend dog. My house has a surrounding of many trees and beautiful birds. My favorite foods are ice-cream, banana bread, and pasta. I like being myself!

EDWARD, AGE: 15

My name is Edward, I am 15 years old and I am from England. In my free time I like to code, solve puzzles (sudokus, Rubik's cubes, etc.), and play video games. I also do trampolining and trampoline coaching/mentoring and have competed in a couple of regional competitions. My favorite TV show is "Sherlock" and my favorite book series is "Harry Potter."

AUTHORS

ERIC BETZIG

Eric Betzig is a professor of physics and molecular and cell biology at the University of California, Berkeley, and a senior fellow at the Howard Hughes Medical Institute, Janelia Farm Research Campus, United States. Prof. Betzig studied physics at the California Institute of Technology and earned his doctorate in applied physics at Cornell University, focusing on near field microscopy as means for breaking Abbe's limit of resolution in classical optical microscopy. He then went on to work on near field microscopy at Bell Labs for several years. After exhausting that route of research, and as a result of advancements in the field of fluorescent molecules, Prof. Betzig and his friend Harald Hess started developing a high-resolution optical imaging method called photoactivated localization microscopy (PALM), for which he won the Nobel Prize in chemistry in 2014. In 2005, Prof. Betzig joined the Howard Hughes Medical Institute, where he continued to work on super high-resolution fluorescence microscopy. In 2017, Prof. Betzig joined the faculty of the University of California, Berkeley, where he is currently working on other microscopes to reveal the detailed workings of living cells. *ebcal@berkeley.edu

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THE QUIRKY LIVES OF QUARKS: A CLOSE LOOK INTO MATTER

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YOUNG REVIEWERS:

EDOARDO
AGE: 8

ILYAN
AGE: 8

MATTIA
AGE: 9

WHIT-CHURCH PRIMARY SCHOOL
AGES: 10–11

In the field of particle physics, we try to understand what our universe is made of. We study the basic properties of matter, describe the elementary particles that compose matter, and try to understand how the various particles that build our world work together. In this article, we will dive into the heart of atoms—the building blocks of matter—and try to answer some intriguing, basic questions about the universe such as: "What are protons and neutrons, the particles that make up the nucleus, made of?" and, "Can those components be divided into even smaller particles?"

Professor David Gross was awarded the Nobel Prize in Physics in 2004, jointly with Prof. Hugh David Politzer and Prof. Frank Wilczek, for the discovery of asymptotic freedom in the theory of the strong interaction.

THEORETICAL PHYSICS—MY MIDDLE SCHOOL SWEETHEART

I was curious about science from a young age. When I was in middle school, I was enormously interested in reading popular science books, such as *One Two Three...Infinity* by George Gamow, which explores concepts in mathematics and physics. For my 13th birthday, I got a very special book signed by no other than Albert Einstein himself. It was given to me by a relative of Einstein's collaborator, Leopold Infeld, who co-authored the book. That was when I fell in love with **theoretical physics**. I was fascinated by the idea of using math and my own mind to understand the universe. From then on, I knew I wanted to be a theoretical physicist, and indeed I became one—and still am. My love for theoretical physics has matured with the years, but it is basically the same love I felt as a teen. I am still excited to understand the basic puzzles of our universe, one of them being: What is the universe made of?

THEORETICAL PHYSICS

A branch of physics that uses mathematical equations to build models that help to describe the world.

STUDYING WHAT MATTERS WITH PARTICLE PHYSICS

What exactly is matter? What is it composed of? These are the kinds of questions we try to address in **particle physics**. Nowadays, it is common knowledge that all matter—from the stars to our own bodies—is made of atoms, which are themselves made of protons, neutrons, and electrons. Up until the early 1900s, the structure of the atom was not understood. Between 1908 and 1913, a physicist from New Zealand named Ernest Rutherford and his students performed a series of experiments to explore the structure of the atom [1]. They took tiny particles called alpha particles and smashed them into the atoms in a sheet of gold foil (To read more about Ernest Rutherford and this experiment, see here and here). Some of the alpha particles passed through the foil uninterrupted, while others seemed to have bumped into something stiff and scattered off in various directions (Figure 1A). The results of these famous experiments revealed that most of the volume of the atom is empty space, while most of its mass and all of its positive charge are concentrated in a very small volume in its center, called the nucleus. This was a great discovery, and it marked the beginning of particle physics.

PARTICLE PHYSICS

A branch of physics that studies the building blocks of matter.

A few years later, Rutherford discovered that the positive charge of the atom is created by particles called protons, and that the number of protons in an atom is equal to the number of electrons circling the nucleus [2]. Then, it took more than 10 years to make the next step toward understanding the structure of the nucleus. In 1932, a renowned physicist named James Chadwick discovered that the nucleus contains protons and neutrons [3]. Chadwick's discovery completed our basic understanding of the classic "solar system" model of the atom, in which electrons orbit around the nucleus (Figure 1B). But what are protons and neutrons made of?

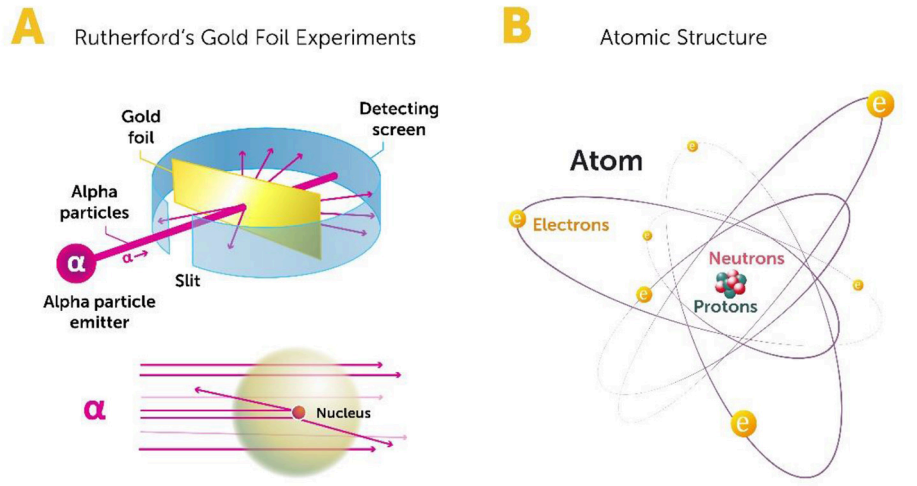

Figure 1

Figure 1

Early studies of the atom's structure. **(A)** Rutherford's gold foil experiment in 1919 revealed that the atom has a solid nucleus at its center. When alpha particles were used to probe the atoms in a sheet of gold foil, some of them passed right through while others bounced off something that changed their path. This obstacle turned out to be the nucleus (Image adapted from: https://flexbooks.ck12.org/cbook/ck-12-chemistry-flexbook-2.0/section/4.14/primary/lesson/rutherfords-atomic-model-chem/). **(B)** Studies in the 1930s revealed that the atom has a solar system-like structure, in which electrons orbit around the nucleus.

SPEEDING UP OUR UNDERSTANDING WITH ACCELERATORS

Since Rutherford's pioneering gold foil experiment, particle physicists have done many similar experiments, scattering one particle, called a probing particle, off a different particle, called a target particle, to learn about the properties of the target particle. This process is called probing. We measure the scattering of the probing particle (for example, an electron) off the target particle (for example, a proton), when the probing particle is sent at various angles and with various amounts of energy. This information allows us to infer the structure of the target particle. To understand probing, we can use the following analogy: Imagine a room full of people. You want to know where the people are, but you cannot get into the room. What you *can* do is throw balls into the room. So, you throw one ball in and nothing happens. Then you throw another ball, at a slightly different angle, and you hear "Ouch!", so you know there is a person in that direction. If you throw many balls in many different directions and at various speeds, you can eventually get a sense of how the people are organized in the room. The same principle applies when we scatter one particle off another—we learn about the structure of the target particle from the feedback we get from the scattering of probing particles.

To probe the structure of the nucleus, Rutherford scattered alpha particles off it, which he could do in his lab. If we want to zoom even deeper into matter, probing the structure of **subatomic particles** like protons and neutrons, or if we want to discover *new* subatomic particles, we must use probing particles with much higher energies than Rutherford used. The reason is that subatomic particles are held together by strong forces, so we must use great energies to break them apart so we can study their structures. To do this, we use

SUBATOMIC PARTICLES

Particles that make up atoms and are therefore smaller than the size of an atom.

ACCELERATORS

Devices that accelerate particles to very high speeds and collide them with target particles, to study the structure of the target particles.

accelerators—devices that accelerate the probing particles to very high speeds.

At the beginning of my scientific career in the 1960s and 1970s, new accelerators were being built and used. Two accelerators that influenced my career were the Bevatron at the University of California, Berkeley, where I was a graduate student, and the linear accelerator at the Stanford Linear Accelerator Center (SLAC) at Stanford University (Figure 2). Compared to the strongest accelerators we have today (notably the Large Hadron Collider at CERN), the Bevatron and the linear accelerator could not accelerate the probing particles to very high velocities. In fact, they managed to produce energies of about six giga electron volts (GeV; one electron volt equals the energy that one electron has when it is accelerated by a voltage of one volt), which is about a thousand times less than the energies of today's accelerators. Yet, those accelerators were strong enough to allow scientists to discover new particles on a weekly basis. This was an extremely exciting time in particle physics, and I knew I wanted to be where the great action was. Even though experiments were flourishing, we had very little theoretical understanding of the structures of subatomic particles and the interactions that govern their behavior. I chose to focus on a very basic problem: what is the proton made of?

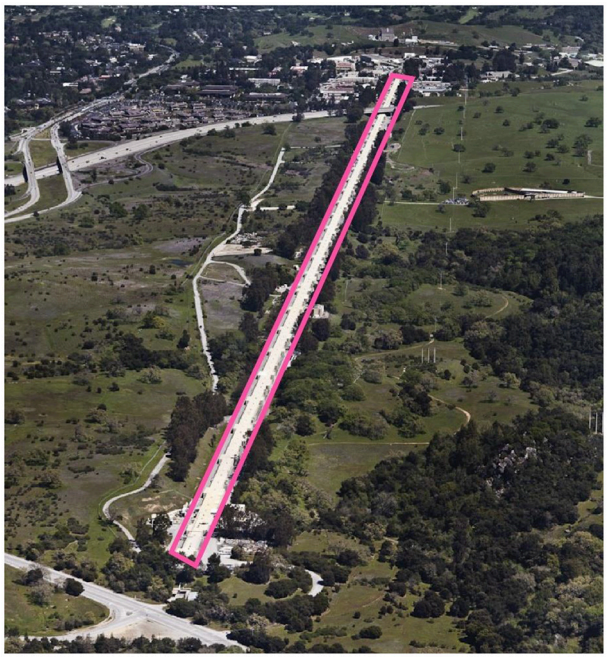

Figure 2

The SLAC linear accelerator at Stanford University. The SLAC accelerator (highlighted in pink) was constructed in 1966. It is 2 miles (3.2 kilometers) long and is capable of accelerating electrons to energies of 50 giga electron volts (Image source: https://upload.wikimedia.org/wikipedia/commons/e/ef/SLAC_National_Accelerator_Laboratory_Aerial.jpg).

THE STRUCTURE OF PROTONS

At SLAC's linear accelerator, electrons, which are simple point particles (meaning particles concentrated into an extremely small volume) were used to probe protons to study their structure. At that time, no one

knew what protons were made of. One hypothesis was that the proton, like the electron, was a point particle, and that it was not composed of any smaller parts. Another hypothesis was that the proton was made of an evenly distributed, unknown material. Surprisingly, the results from the SLAC accelerator did not fit either hypothesis, but seemed to indicate that the proton was made out of other point particles. However, no one had ever seen the point particles that supposedly composed the proton, and no matter how hard we hit the proton with the probing particles, those point particles did not come out (In contrast, when we smash atoms together, their electrons fly off.). Additionally, probing results seemed to indicate that the point particles in the proton were bouncing around as if no force was acting between them. That was very strange because we knew that the point particles were bound tightly within protons—but we could not explain what was keeping them bound there.

In my search for an explanation of these surprising findings, I was assisted by two previous theories. One theory was proposed by physicists Murray Gell-Mann and George Zweig in the early 1960s [4, 5]. Gell-Mann and Zweig realized that the data emerging from the measurements of strongly interacting subatomic particles could be explained mathematically, by assuming that they were made of three kinds of more basic particles that Gell-Mann called quarks (Figure 3A). At first, this assumption was thought to be only a mathematical "trick" that had nothing to do with reality. But, as time went on, some of the predictions of this model turned out to be pretty accurate, and together with new results from accelerator experiments the idea of quarks seemed to be worth exploring in greater detail. The other theory I used was generalizations of Maxwell's theory of electromagnetism. Maxwell's theory explains the force of electricity and magnetism based on the existence of a single type of charge, called the electrical charge.

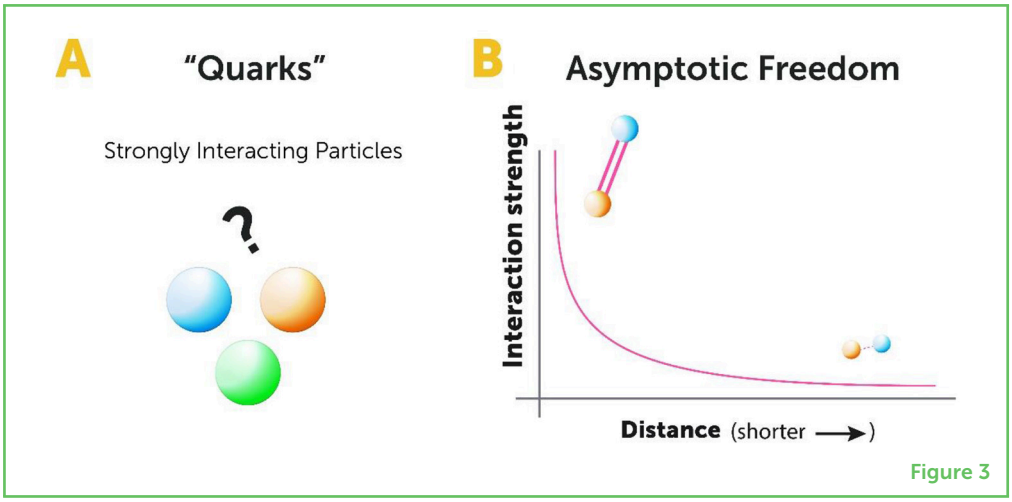

Figure 3

Figure 3

Studying subatomic particles. **(A)** In the 1960s, physicists Murray Gell-Mann and George Zweig suggested that subatomic particles, like protons and neutrons, are made of basic particles called quarks. Quarks are called strongly interacting particles because they are bound by the strong force. **(B)** In the 1970s, my colleagues and I found a mathematical explanation of the behavior of quarks—a phenomenon called asymptotic freedom. Asymptotic freedom describes how the interaction between quarks becomes weaker as they come closer together and stronger as they move further apart. This explains why quarks stay "locked" within protons, which is called confinement.

Generalizations can be made from Maxwell's theory, such that other kinds of charges can be used to explain other natural forces. In

physics, there are four basic forces: the electromagnetic force, which is responsible for electricity and magnetism; the weak force, which is responsible for **radioactivity** (to learn more about radioactivity, see here); the **strong force**, which binds protons and neutrons in the nuclei of atoms; and gravity, which pulls massive objects toward one another. I was trying to explain the strong force holding protons and neutrons together.

RADIOACTIVITY

A process by which particles and/or energy are emitted from an unstable atom.

STRONG FORCE

The force that binds protons and neutrons in the nuclei of atoms.

After doing some complicated math based on these two theories, I developed a new theory called quantum chromodynamics (QCD). QCD explains the properties of quarks and the forces that act on them. In QCD, there are three types of charges (opposed to only one type—electrical—in classical electromagnetism) and eight types of forces that act between these charges. One of QCD's greatest achievements is its ability to explain the surprising finding that quarks behave like free particles inside the proton. My colleagues and I found a mathematical explanation for why the strong force that acts between quarks becomes weaker and weaker as the quarks get closer together—a phenomenon called **asymptotic freedom** [6, 7] (Figure 3B). The discovery of asymptotic freedom led to a great advancement in particle physics, for which I was awarded the Nobel Prize in Physics in 2014, jointly with Hugh David Politzer and Frank Wilczek. Asymptotic freedom was a surprising discovery because other forces in nature get *weaker* as the particles move further apart. Asymptotic freedom explains why we do not see free quarks—the further we try to separate them, the stronger the force pulling them together becomes. This means that quarks stay "locked" inside protons—a phenomenon called **confinement**.

ASYMPTOTIC FREEDOM

A phenomena whereby quarks act like free particles when they are short distances apart, but oppose being separated from one another as the distance increases.

CONFINEMENT

The property of quarks to stay "locked" inside protons (or other particles), even when great forces are applied to separate them.

QUIRKY QUARKS: SURPRISING BUILDING BLOCKS OF MATTER

We currently believe that quarks are point particles that make up protons, neutrons, and a whole class of strongly interacting particles called **hadrons**. As we have seen, quarks are confined within the particles they constitute, and they move like small balls bouncing around inside a larger ball. Quarks have three types of charges: electric charge, flavor, and color (not the same "flavor" and "color" in the traditional sense that we detect with the tongue or eyes). In terms of electric charge, quarks can have a charge that is some fraction of the charge of an electron or proton: either $-\frac{1}{3}$ ($\frac{1}{3}$ of the negative charge carried by an electron) or $+\frac{2}{3}$ ($\frac{2}{3}$ of the positive charge carried by a proton). Quarks come in six flavors, which we call up, down, strange, charm, top, and bottom (Figure 4A). Quark flavors are related to the weak force responsible for radioactivity. Protons are made of two "up" quarks and one "down" quark; neutrons are made of two "down" quarks and one "up" quark (Figure 4B). In addition to electric charge and flavor, quarks can come in one of three colors:

HADRONS

Particles that are built from quarks and interact via the strong force.

red, white, and blue. Colors are the source of the strong force that binds quarks together, and this is also the source of the name chromodynamics—"chromos" means "color" in Greek.

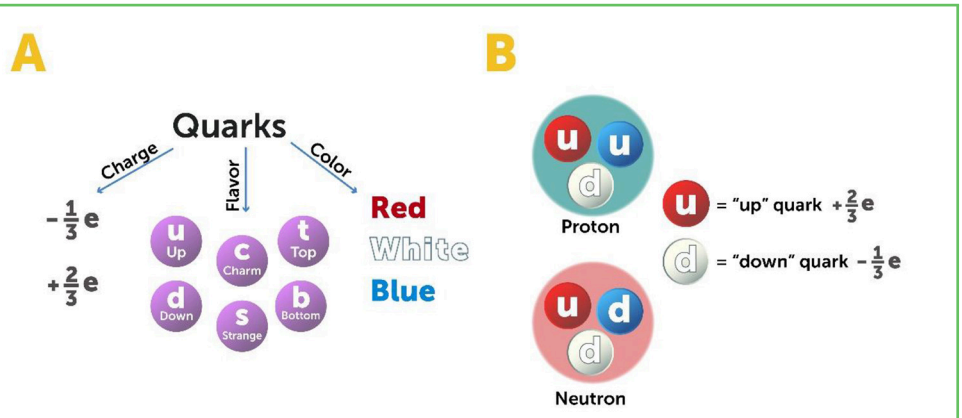

Figure 4

Figure 4

Quarks. **(A)** Quarks come in various electric charges, flavors, and colors. Their charge is some fraction of the charge of an electron or proton. They come in one of three colors, which are the source of the strong force that binds them together, and in one of six flavors which are related to the weak force. **(B)** Protons are made of two "up" quarks and one "down" quark, while neutrons are made of two "down" quarks and one "up" quark.

At this stage, you might be wondering whether quarks themselves are made up of even smaller particles. In the history of physics, we have progressively found smaller and smaller particles that make up matter: atoms are made of electrons and nuclei, nuclei are made of protons and neutrons, protons and neutrons are made of quarks...maybe quarks are made up of something else? So far, there is no experimental evidence to indicate that quarks are made of sub-quarks. To get definite experimental proof that quarks are truly indivisible, our accelerators will need extraordinarily high energies—of the order of 100 trillion trillion eV, which is about a trillion (or 10^{12}) times higher than the energies we currently use. This is very difficult and costly, and the path to getting there is still unclear. On the theoretical side, we can extrapolate our current knowledge of the basic forces of nature to very short distances. When we do so, we find that gravity should play an important role at these short distances, equivalent to that of the strong force. With our current models, we do not understand this interplay between gravity and the strong force, so something unexpected might be occurring at very short distances. Experiments indicate that something is still missing in our theory, and study of quarks might help us overcome some of the current gaps in our understanding of the basic building blocks of the universe.

STRING THEORY: AN ALTERNATIVE PERSPECTIVE ON MATTER

String theory, which many scientists (myself included) have been working on for several decades, is another interesting theory that could offer us a new way of understand the universe. String theory is extremely complex mathematically, and its details exceed what can be described in this article. But, in principle, string theory is an attempt to understand how all the elementary particles, including quarks, could

STRING THEORY

A theory of physics that describes the basic particles of nature in terms of a string that vibrates in different ways.

be made of one type of object, called a string. This string can vibrate in many different ways, and each vibrational pattern corresponds to a specific elementary particle. For example, if the string vibrates in one specific pattern we get a quark, if it vibrates in a different pattern we get an electron, and so on (Figure 5). If string theory is correct, then quarks and other elementary particles do not contain additional sub-particles but are instead made of vibrating strings. Testing string theory experimentally will require energies much more powerful than we currently have—so it might take a while, but I think this is an exciting development to look forward to.

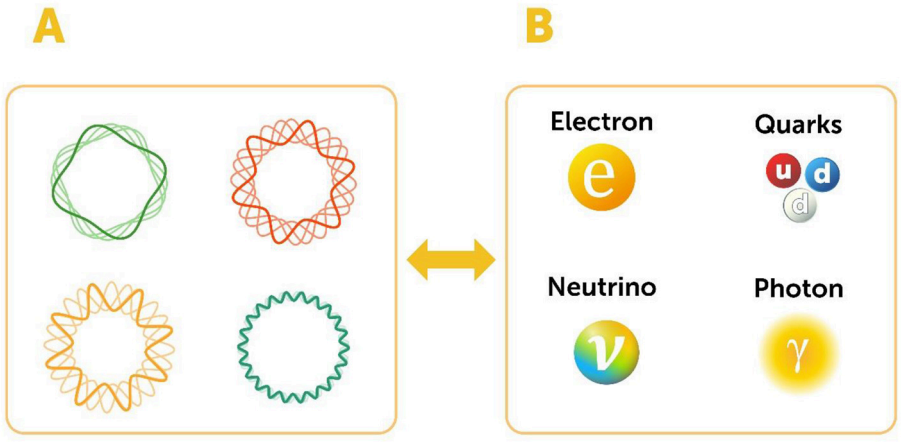

Figure 5

Figure 5

String theory. **(A)** String theory suggests that all elementary particles are composed of an object called a string. **(B)** When this string vibrates in different ways, specific particles are produced. We do not currently have the technology to test whether string theory is true, but hopefully we will be able to do so in the future.

RECOMMENDATIONS FOR YOUNG MINDS

In physics, as in all of science, it is important for scientists to be creative. Unfortunately, we do not know how to teach people to be creative, but we *can* serve as examples of creativity and be inspiring mentors for our students. The best thing students can do is observe how creative and successful people work and learn their "tricks." I see my students as colleagues, and I like to work with them as equal contributors. That might be challenging for some students, but others flourish in that type of relationship. Prof. Frank Wilczek, my first official student, won the Nobel prize with me for our shared work on asymptotic freedom—he is a good example of a student who flourished by being treated like an equal.

To become good scientists, it is also important to start doing research as soon as possible. Research is very different from taking classes and studying. In physics class, problem sets are the best method we have to prepare students to do research. We give students lots of problems, and they must solve those problems and usually gain insights while doing so. But even the best problems students get in class are made up. In contrast, research is based on real, unresolved problems that no one knows in advance how to solve. The biggest challenge in

research is to come up with the *right questions*. Before you even tackle a scientific question, you must first make sure that the question is a good one (Figure 6). Asking good questions is also a skill we cannot teach directly—we can only supply examples and model productive ways of thinking. Once you manage to ask a good question, you might not be able to answer it right away, but at least you can make progress toward an answer.

Figure 6

Figure 6

Recommendations for Young Minds. One of the most important skills needed for doing good research is coming up with good research questions.

In my view, there are many reasons why being a scientist can lead to a happy life. For one, society generally respects the talent that it takes to be a scientist, because science is so important to our lives. Therefore, science allows you to both be supported and respected by society and to make a living, which is a great pleasure. It feels good to be in a field where your work is your play, your passion, and your interest. Another advantage of being a scientist is that you belong to a world-wide, international community of people who share the same passions and interests. You can go anywhere in the world and find people who are interested in the same topics you are, and have interesting discussions with them. Science is a kind of family that you choose to join.

I think that people should do what they love doing. It does not have to be science—it could be anything. If you can spend your life doing what you really enjoy, you are very lucky. This is my best advice about how to decide what to do with your life—find out what you enjoy and what you are good at. Then, be ambitious—try hard and be willing to fail. If you have chosen a path that you enjoy, the joy that you receive is worth the chance of failure.

ACKNOWLEDGMENTS

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ADDITIONAL MATERIALS

1. Quantum chromodynamics - David Gross.
2. Quantum Chromodynamics (QCD) - Professor Dave Explains.

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CONFLICT OF INTEREST: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

EDOARDO, AGE: 8

Hi, my name is Edoardo and I really like Pokémon. I am 8 years old, I live in Monte Porzio Catone in the neighborhood of Rome and I have a very pretty little sister. I am interested in constellations, especially Sagittarius because it is my zodiac sign. I really like jumping, climbing and running so I practice parkour.

ILYAN, AGE: 8

Hi, my name is Ilyan, I am eight years old and I come from Borghesiana, a town close to Roma. My school is GermogliAmo. I like to play soccer, tap dance, piano and love singing., I like gifts and surprises. My favorite animal is the gorilla. My best friends are Edoardo, Yuri, Leonardo and Flavio.

MATTIA, AGE: 9

Hi, my name is Mattia, I like skateboard, basketball and draw comic strips. I think that scientific articles could be more interesting if they were written in cartoon bubbles. Maybe one day I will be a scientific cartoonist!

WHITCHURCH PRIMARY SCHOOL, AGES: 10–11

We are a group of 16 year six students in Cardiff, Wales, who love exploring the world around us. We have loved learning about all sorts of science as part of this project, and discovering what is at the cutting edge of human knowledge, and we have encountered so many new words! The experience is going to be really valuable as we move up to High School!

AUTHORS

DAVID GROSS

Prof. David Gross is an American physicist. Prof. Gross earned his B.Sc. in physics and mathematics at the Hebrew University of Jerusalem, Israel. He earned his Ph.D. in physics studying strong interactions at the University of California, Berkeley, under the supervision of Geoffrey Chew. After graduation in 1966, Prof. Gross joined the Harvard Society of Fellows and, in 1969, became an assistant professor at Princeton University, where he stayed for 27 years. At Princeton, Prof. Gross worked with his first graduate student, Frank Wilczek and, in 1973, they discovered asymptotic freedom. This finding led to the development of the quantum chromodynamics theory and later earned Gross and Wilczek a Nobel Prize in Physics, in 2004. In 1984, Prof. Gross started working on string theory, which has been his main research focus ever since. After his years at Princeton, Prof. Gross moved to the Kavli Institute for Theoretical Physics at the University of California, Santa Barbara, where he served as the director from 1997 to 2012. Prof. Gross has two children with his first wife (Shulamith Toaff), Ariela Gross and Elisheva Gross, and two grandchildren.

He currently lives with his second wife, Jacquelyn Savani, with whom he has a stepdaughter, Miranda Savani. *gross@kitp.ucsb.edu

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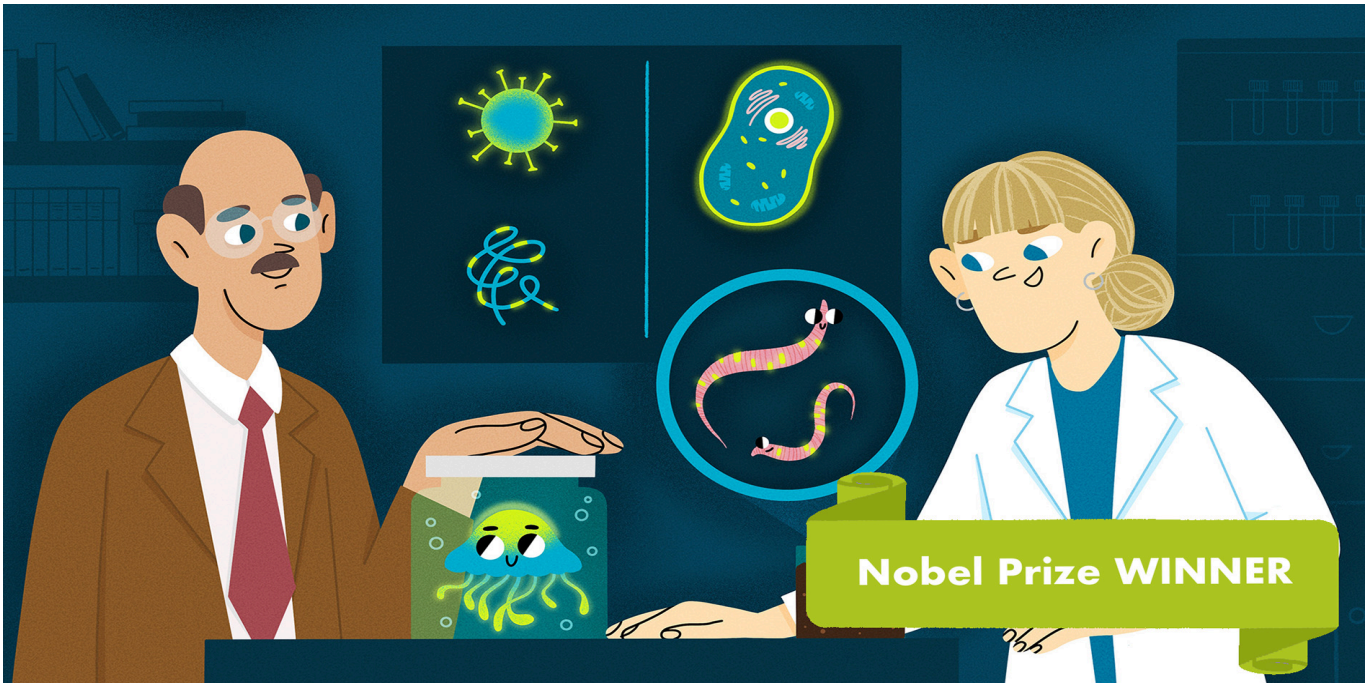

MOLECULAR FLASHLIGHTS THAT LIGHT UP SCIENCE

Martin Chalfie *

Department of Biological Sciences, Columbia University, New York, NY, United States

YOUNG REVIEWERS:


ALI
AGE: 13


BILAL
AGE: 15


LAURENT
AGE: 10


YA'EL
AGE: 11

This article is based on an interview between Prof. Martin Chalfie and Noa Segev.

Scientists investigate things by observation. They look at a phenomenon that interests them and try to understand it, using the most advanced tools they have. It is often challenging for scientists to see and measure what they want to study, often because they want to go beyond what had been previously seen. The development of modern imaging techniques has allowed scientists to see things that they could not see before. In this article, I will tell you about one of those breakthroughs in imaging, based on a wonderful glowing protein called green fluorescent protein (GFP). GFP not only changed my life, but the lives of many other scientists, and ultimately of many non-scientists as well. Among other things, GFP allows us to detect and observe the activity of proteins and whole cells in living animals, and to detect the activity of genes that code for specific proteins. By the end of this article, I hope you will understand much more about GFP and how it lights up science.

Professor Martin Chalfie won the Nobel Prize in Chemistry in 2008, jointly with Prof. Osamu Shimomura and Prof. Roger Tsien, for the discovery and development of the green fluorescent protein, GFP.

GLOWING JELLYFISH AND A MIRACULOUS MISTAKE

Have you ever been lucky enough to see the astoundingly beautiful phenomenon of a glowing firefly lighting up a dark night? Fireflies are part of a fascinating group of organisms that can produce light—a phenomenon that is called **bioluminescence**. Other bioluminescent organisms include glow-worms, some types of bacteria, and certain types of fish. Our story begins with a bioluminescent jellyfish called *Aequorea victoria*, or *A. victoria* for short (Figure 1).

BIOLUMINESCENCE

The production of light by living organisms.

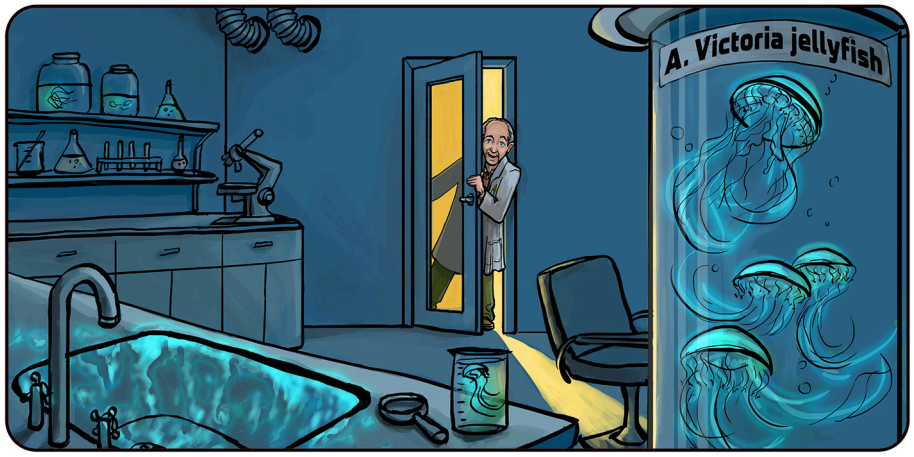

Figure 1

Osamu Shimomura and the glowing *Aequorea victoria* jellyfish. After a frustrating day in the lab, Prof. Shimomura turned off the lights and was about to go home for dinner. He suddenly saw that the sink, which had samples of *A. victoria* cells and sea water in it, was glowing in blue. That led him to the discovery of the glowing protein called aequorin. Illustration by: Iris Gat.

In the 1960s, a Japanese scientist named Osamu Shimomura wanted to understand how bioluminescent organisms produce light. He decided to work on *A. victoria*, which produces green light. Osamu worked for a whole summer, trying to make cells from *A. victoria* glow (as happens naturally in the sea) but none of his experiments succeeded. One night, when it was already dark outside and Osamu wanted to go home for dinner, he threw his samples of *A. victoria* proteins from another failed experiment into the lab's sink and turned off the light to close up the lab. But just as he was about to leave, he noticed that the sink was glowing blue. Since the sink also contained sea water, Osamu reasoned that something in the sea water must have trigger the production of light. He soon realized that calcium in the seawater made the jellyfish proteins glow. He named the blue-glowing protein **aequorin**, after the name of the jellyfish [1, 2].

AEQUORIN

A blue glowing protein that was discovered in the *Aequorea victoria* jellyfish by Prof. Osamu Shimomura.

After this great breakthrough, Osamu had to answer another burning question: why did aequorin generate blue light, when the jellyfish it came from glow green? While purifying the aequorin protein,

Osamu searched for something else that could create the green light emitted by *A. victoria*. Eventually, he found another protein that was a **fluorescent molecule** because it took the blue light, whether made from aequorin or from a hand-held lamp, and converted it into green light. This was another astonishing discovery, as no one at that time had any idea that proteins could be fluorescent. Although Osamu originally called this the green protein, we now call it the **green fluorescent protein**, or GFP [3]. This story is also a wonderful example of how many scientific discoveries are rather accidental. The role of the scientist, as in Osamu's case, was to notice, wonder, and investigate these accidental discoveries.

FLUORESCENT MOLECULES

Molecules that convert one color of light (blue, for example) to another color (green, for example).

GREEN FLUORESCENT PROTEIN (GFP)

A protein that was first identified in *A. victoria* jellyfish. GFP absorbs blue light and converts it to green light.

NERVE CELLS

Cells of the nervous system that receive information from the environment, process it using electrical and chemical signals, and generate an output, such as movement.

BIOLOGICAL MARKER

A biological molecule used by researchers to indicate a biological process or state.

HOW GFP CHANGED MY LIFE

On Tuesday, April 25th, 1989, I heard a lunch-time lecture at my university about Osamu Shimomura's work on GFP. As soon as I heard about GFP, I was fascinated. My lab was working on a tiny (about 1 mm long) transparent worm called *Caenorhabditis elegans*, or *C. elegans*. We were studying a group of the worm's **nerve cells** that respond to physical stimuli, such as touch and sound, and convert it into electrical and chemical signals. I wondered whether we could find a way to make those nerve cells in *C. elegans* produce GFP, so we could actually see and study them in a completely new way. GFP could then be used as a **biological marker**, which is a biological molecule that scientists can observe to learn what is going on inside cells or organisms. At the time, the method we were using to see specific cell types in *C. elegans* was cumbersome and did not allow us to work with living tissue. These were serious limitations. We first had to "fix" the worm with chemicals to preserve the cell structure, but that process also killed the animal. That meant that our current method gave us only a "snapshot" picture of what was happening inside the worm—one "frame" at a time. I thought that GFP might allow us to view the nerve cells of *C. elegans* while it was alive and interacting with its environment.

I was so excited that I could not even listen to the rest of the lecture. All I could think about for the next few days was GFP and its potential for my research. I contacted a researcher named Douglas Prasher, who was working on producing and copying the genetic instructions (DNA) that coded for the GFP protein. We were both excited about the possibility of using GFP in *C. elegans* and other organisms, so we decided to collaborate. After losing contact for a few years due to an unfortunate misunderstanding, we reconnected in September 1992, when a student named Ghia Euskirchen came to my lab and was interested in the project. She had a good background in working with fluorescence, and this reminded me of my idea to use GFP to mark living cells. As we studied scientific papers published on GFP, we discovered that Douglas had recently published a paper on GFP [4] and contacted him again. Soon after, he sent us the DNA coding for GFP and Ghia started working with it.

MAKING ORGANISMS GLOW WITH GFP

When Ghia started her GFP experiments, she wanted to see if bacteria containing the DNA that codes for GPF would become fluorescent. We did not know at that time whether production of the GFP protein from GFP DNA was enough to make a cell fluorescent (to learn more about how proteins are made from DNA, see this article). It could be that something else was needed—either something that the cell itself produces and adds to the GFP protein to make it fluoresce, or something that must be added externally to make the cells fluoresce.

The first decision we had to make at the start of the experiment involved what to do with the GFP DNA that we got from Douglas. The DNA that Douglas sent us contained extra sequences in addition to the section that coded for GFP (Figure 2A). We knew we had to make lots of copies of the GFP DNA for our experiments, and the question was whether to use Douglas's GFP DNA as-is (with the extra pieces) or work only with the part that coded for GFP. The tricky part was that the method was used to get only the coding DNA, called **polymerase chain reaction** (or PCR—which you might know from PCR tests for COVID-19) [5], often introduced mistakes in the copied DNA. Despite this limitation, I decided that we would use PCR, since we were going to be looking at millions of bacteria and some of them would have GFP DNA without any mistakes.

POLYMERASE CHAIN REACTION (PCR)

A laboratory method used for creating many copies of a specific segment of DNA using a DNA-copying enzyme called DNA polymerase.

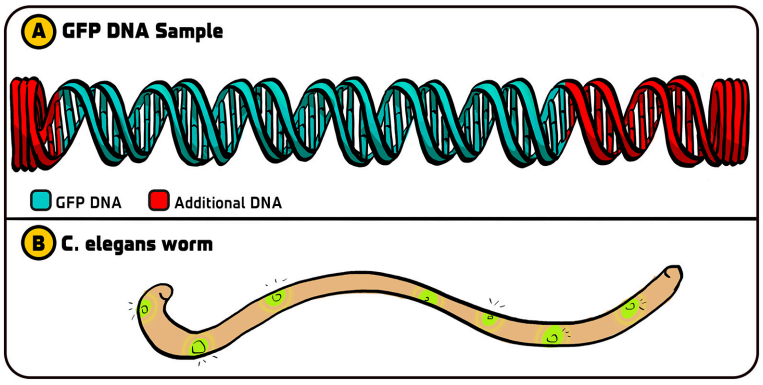

Figure 2

Initial experiments with GFP. **(A)** The original GFP DNA that we got from Douglas Prasher contained the DNA that coded for GFP (green) along with additional DNA bits on each side (red). **(B)** After we made copies of just the GFP DNA using PCR, we could use that DNA in *C. elegans* so that certain cells of the worm fluoresced with a green light. Illustration by: Iris Gat.

As it turned out, we chose the right strategy. Ghia used PCR to copy the GFP DNA. She then incorporated this GFP DNA into a DNA molecule called a plasmid, which bacteria "swallow" and integrate as their own DNA. After shining blue light on the *E. coli* containing GFP DNA, they fluoresced [6]. (In contrast, the other groups that used the original GFP DNA *with* the additional DNA did not see fluorescence. Something in the extra DNA interfered with the production of GFP. If we had not used PCR, our experiment would not have worked). Since our method worked in bacteria, we next used it to incorporate GFP DNA into *C.*

elegans, as I originally dreamt of doing—and we made these worms glow as well (Figure 2B). This paved the way for incorporating GFP into all sorts of organisms and using it for various applications.

HOW GPF CHANGED SCIENCE

Any new scientific development can give us a better understanding of basic biological or physical principles, and can also help in the creation of new technologies. Many times, the original discovery develops in surprising and unexpected directions over time. The discovery of the laser is a good example. Charles Townes, whose work led to the laser, never imagined it would be useful in grocery stores for scanning the prices of products, or in the record industry for making compact disks, or in the movie business for making DVDs, or in medicine for performing laser surgeries. The same holds true for the discovery of GFP—it has evolved, and will continue to evolve, in many different directions that advance both fundamental scientific knowledge and various technological applications.

GENE

A segment of DNA that carries the instructions for making a specific protein.

In scientific research, GFP can be used as a biological marker to tell us about the action of **genes** and their products. Genes can be thought of as having two parts: the coding part, which indicates what product should be made (the RNA and protein) and the regulatory part that says where, when, and how much of the product should be made. If the GFP DNA sequence added to just the regulatory part, GFP will be made and fluoresce whenever the normal gene is "turned on." Alternatively, if the GFP DNA sequence is added to the coding sequencing, the normal protein will have GFP protein attached to it, and it will glow when we shine blue light on it [7]. We can see where the proteins reside in cells (e.g., in the nucleus, at the cell membrane) and also watch them as they move through living cells, a process that was not possible with earlier methods. Importantly, once the GFP DNA is inserted into the DNA of an organism, it can be passed on to that organism's offspring—this makes the experimental use of GFP very convenient.

I would like to briefly mention two discoveries made using GFP that I personally admire. The first was made by Clifford Brangwynne and Anthony Hyman in 2009 [8]. They were looking at proteins within the cytoplasm—the liquid that fills up the space within cells. Until then, people thought that the cytoplasm was quite uniform, but when these scientists looked at a particular cytoplasmic protein labeled with GFP, it appeared to be contained in particles that were separated from the rest of the cytoplasm. These particles acted like tiny drops of oil in water—sometimes they would come together and fuse, and other times they would split into two. These structures did not mix with the rest of the cytoplasm, they were a separate phase (they are often called phase-separated particles (Figure 3A). This discovery turned out to be exceptionally important for our understanding of the structure and function of cells, and it opened up a very active area of research.

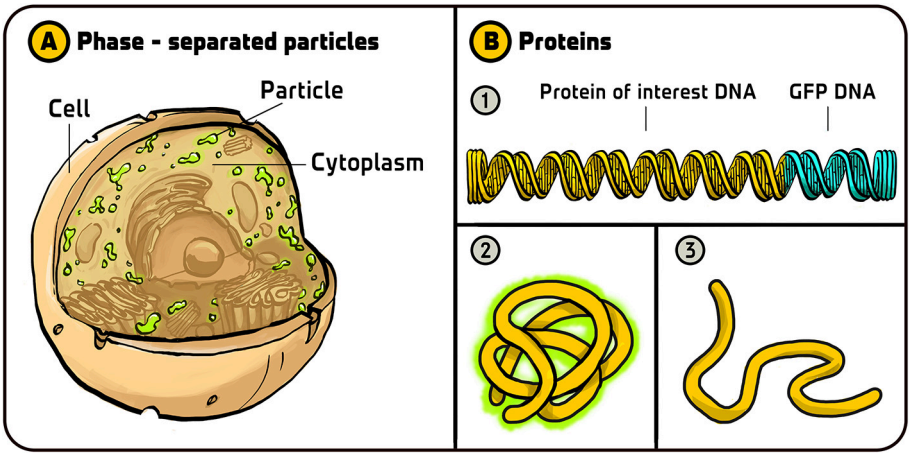

Figure 3

Figure 3

Uses of GFP. **(A)** Using GFP, Clifford Brangwynne and Anthony Hyman found that the cytoplasm of cells contains two separate phases—the well-known liquid phase and another phase that behaves like oil particles in water. **(B)** Geoff Waldo used GFP to monitor the folding of proteins. (1) He added GFP DNA to the end of the DNA coding for a protein he was interested in. (2) If the protein (including the GFP part) folded correctly, it would fluoresce in green. (3) If the protein folded incorrectly, it would not fluoresce. Illustration by: Iris Gat.

Another use of GFP that I particularly like was done by Geoff Waldo [9]. Geoff found a clever way of using GFP to monitor the folding of proteins; he called it a "folding reporter." After a protein is produced as a long chain of building blocks called amino acids, it must fold into a specific three-dimensional structure to perform its function. If a protein folds incorrectly, it will not work properly. When we study proteins by making them in bacteria, for example, we want to make sure that they are folded properly. Geoff constructed a DNA that encoded both the protein he was interested in *and* GFP. He reasoned that if the protein of interest did not fold properly, then the GFP would also not fold properly and this would not glow. So, if he saw fluorescent cells, he could conclude that the protein of interest folded correctly, and if there were no fluorescent cells, he could conclude that the protein folded incorrectly. This is a very nice way for scientists to ensure that they are working with properly folded proteins (Figure 3B).

There are many other uses for GFP, so I will touch on just a few more. Some people use GFP to study how viruses infect cells. One notable study used GFP to color HIV, the virus that causes AIDS, to study how the virus spreads from one cell to another [10]. In this study it was found that HIV viruses can move between cells without destroying the cells (as many other viruses do). This discovery has implication as to how the transfer of the virus from one cell to another could be controlled. Other groups are studying how to use GFP to detect land mines and the residues of explosives [11]. In Japan, people even use GFP to produce silk that glows green [12]. There are many more interesting uses of GFP, and of other fluorescent proteins with other colors that scientists have found or have generated. These examples give you an initial glimpse of just how useful fluorescent proteins can be.

RECOMMENDATIONS FOR YOUNG MINDS

I do not think that there is a "recipe" for doing important scientific research, but there *are* things that I believe to be important. One of the most important abilities of a successful scientist is the ability to ask questions. Many great questions are sitting right under our noses, waiting to be asked, but if we are not in the habit of asking questions, we might miss them. Asking question is an excellent way to understand things. When you learn something new, ask yourself how the new thing you learned might apply to other topics you are interested in. This is an important approach that I use in my work all the time. Another important aspect of asking questions is questioning your assumptions (Figure 4) — why do you believe what you believe? Quite often, when we investigate our assumptions in this way, we find errors in our assumptions. Correcting these errors by updating our assumptions can help us to advance our knowledge and understanding.

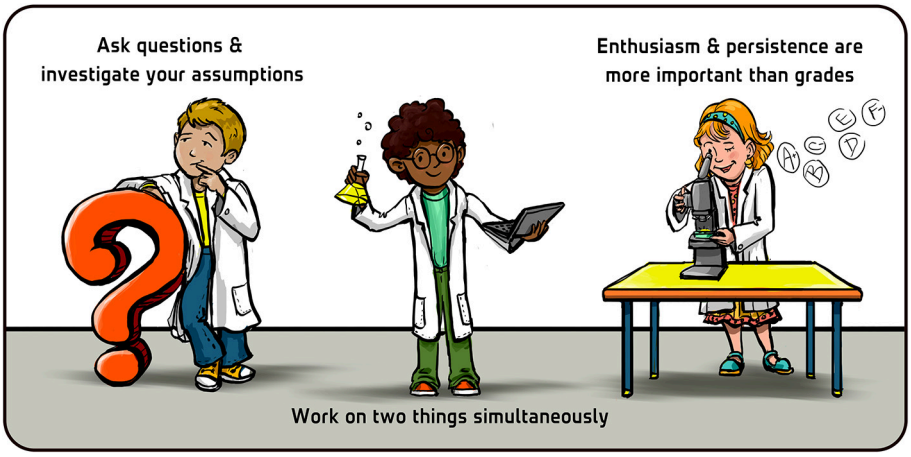

Figure 4

Three recommendations for young minds. Illustration by: Iris Gat.

I often recommend that students work on two things at the same time. This way, if one project is not working, they still have the other to draw success and motivation from. Science is a journey of grappling with the unknown. It is not always easy and may be frustrating, and we make lots of errors along the way. Our ideas do not always work, but making headway against the unknown is an exciting part of being a scientist. Occasionally, this challenging journey brings great rewards—like discovering something that no one else has known before, and then sharing that discovery with others.

Finally, do not worry too much about your grades. Grades can have absolutely nothing to do with success in science. I think that enthusiasm and persistence are far more important than grades. I got average grades (C's) in chemistry when I was in university, and then, somehow, was awarded a Nobel Prize in Chemistry some 30 years later (I enjoy the irony of that). If you are interested in science, one of the best ways to become good at it is to do it. Try doing experiments

and see what it is like—this is the most reliable way to know if science is the right choice for you.

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ADDITIONAL MATERIALS

- The five myths about scientists according to Nobel Laureate Martin Chalfie
- Martin Chalfie, Nobel Laureate, on Failed and Useless

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CONFLICT OF INTEREST: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

ALI, AGE: 13
My name is Ali, I am 13 years old, and I like video games and sports, although I love science but I dislike school. I am in 7th grade, and I am doing well in my grades because that is the only way I can keep my video game privileges.

BILAL, AGE: 15
My name is Bilal; I am a 15 years old high school sophomore. I am interested in engineering and medicine as I prepare to go to college in next few years. I love long distance running.

LAURENT, AGE: 10
My name is Laurent. I love spending time with friends from my neighborhood and with classmates. I love reading and writing, and I have won already prices for my poems! I am French and also speak English. I play basketball and have played Judo and Football. I like reading fiction-stories, manga's, "Enquettes Policières" horror stories, myths and histories, and I like watching scientific documentaries. My favorite season is spring when you can play outdoor and ride a bicycle.

YA'EL, AGE: 11

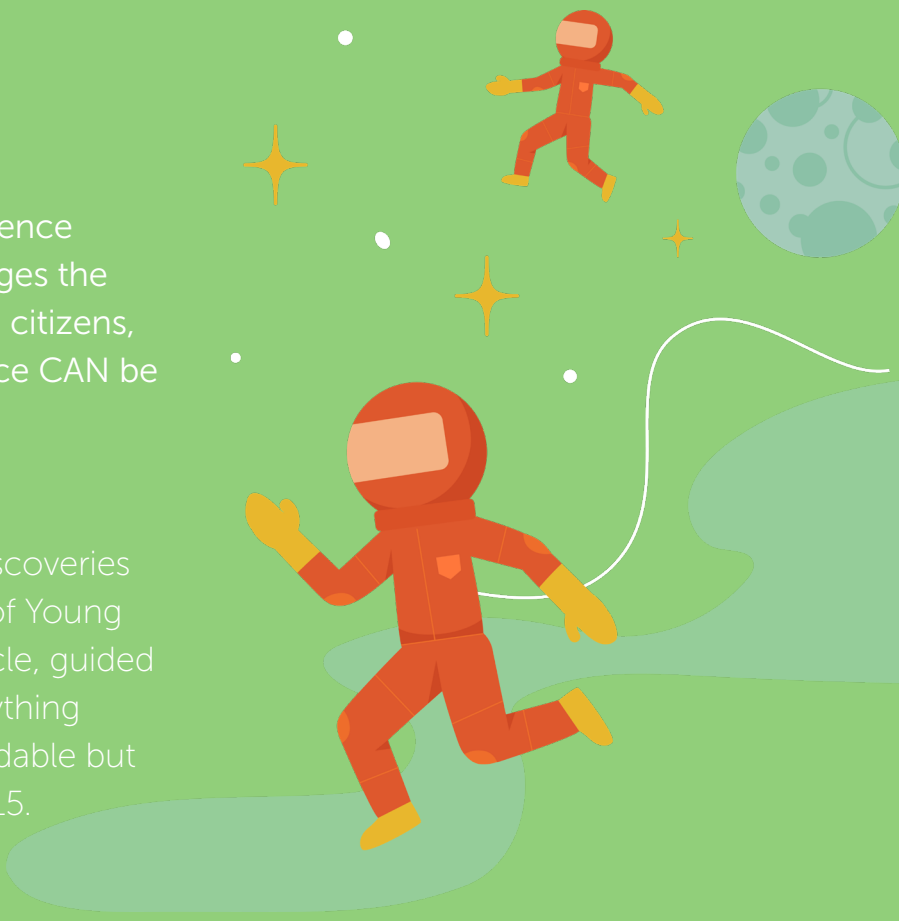
I like Minetest, computer programming, maths, science, running, playing with dolls, drawing, baking, reading, playing with lego, watching videos, arts and crafts, and thinking.

AUTHORS

MARTIN CHALFIE

Prof. Martin Chalfie is an American geneticist and professor at Columbia University (New York, United States). Prof. Chalfie studied biochemistry at Harvard University (Massachusetts, United States). After a negative experience in scientific research one summer, he decided to drop out of science after graduation. Prof. Chalfie then worked at several temporary jobs, including selling dresses for his parents' business in Chicago, and he taught chemistry at a high school in Connecticut. In 1971, Prof. Chalfie was looking for a temporary job during summer vacation. One of his friends connected him with a professor at Yale University, and he conducted a successful research project in that lab—this reignited his interest in science. Prof. Chalfie earned his Ph.D. in physiology at Harvard University in 1977. In 1982, he became a professor of biological sciences at Columbia University (New York, United States), studying the nervous system of a worm called *C. elegans*. In 1989, following a lecture at Columbia, he started working on GFP with his collaborator, Douglas Prasher. In 1994, Prof. Chalfie, Douglas Prasher, Chalfie's graduate student Ghia Euskirchen, and others published a paper describing how they added the DNA for GFP to the DNA of the bacterium *E. coli* and the nematode *C. elegans* and showed that each organism could produce the fluorescent protein. Prof. Chalfie lives with his wife, Tulle Hazelrigg, a professor of biology at Columbia University. They have a daughter named Sarah. *mc21@columbia.edu

Frontiers for Young Minds



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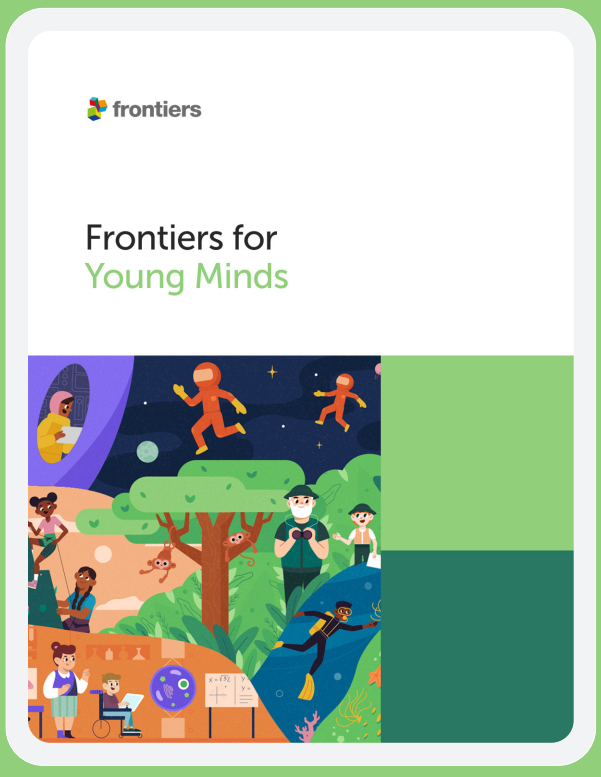

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