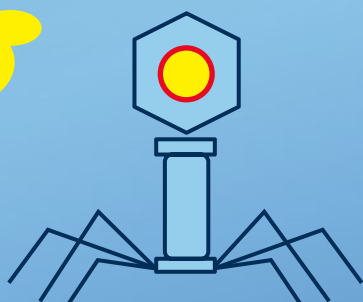
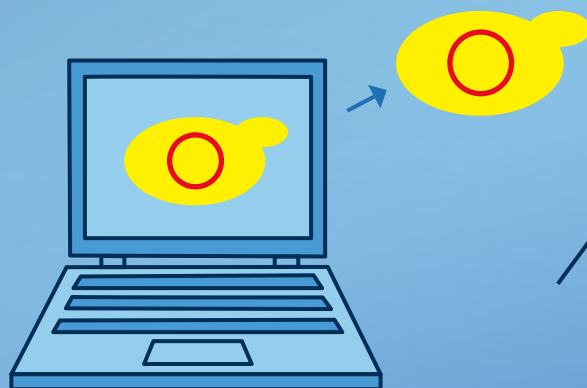
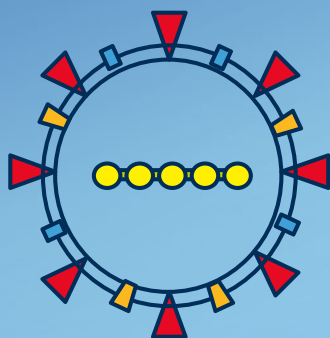
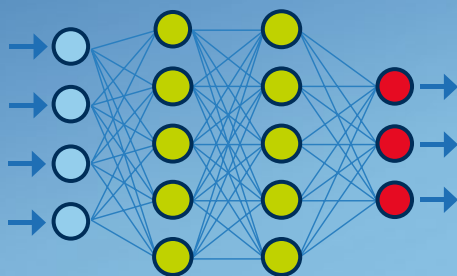


Mario Juhas

Brief Lessons in Microbiology

From the Origin of Life to
Artificial Intelligence



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Intelligence



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*I dedicate this book to my mother Helena. She always believed
in me and my dreams.*

Preface

My colleague was explaining his research project to my grandfather. Grandfather, who has no background in microbiology whatsoever, listened politely to the whole lecture, but once my colleague left he told: “I appreciate his effort, but I did not understand a single word of what he was saying.”

This illustrates that recent discoveries are beyond the grasp of those unfamiliar with the scientific jargon and the complex research data can be overwhelming even to those working in other research areas.

This book aims to address this issue by highlighting the most interesting findings and current trends in microbiology in a form that is easy to understand.

Fribourg, Switzerland

Mario Juhas

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About the Author



Mario Juhas Dr. Mario Juhas worked at a number of universities, including the University of Oxford and the University of Cambridge in the UK, the University of Hannover in Germany, and the University of Zurich and the University of Fribourg in Switzerland. His work spans microbiology and synthetic biology, and he has direct research expertise in each of the research topics discussed in this book. He has been travelling extensively around the world and visited more than a hundred countries. His hobbies include science, travelling, photography, swimming, diving, and history.

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1

The World of Microorganisms

Abstract We live in the world, which is teeming with microorganisms. Our bodies are full of microbes and they are crucial for our survival. Origin of life as we know it is tightly connected with microorganisms. First life on Earth was microbial. Without them, there wouldn't be us. Remnants of bacteria reside in our cells and generate energy for important biological processes. Although microbes are small and relatively simple, they are a very diverse group of organisms. Microorganisms can be found in all three domains of life: Bacteria, Archaea, and Eukarya. Other microscopic entities, which do not belong to any of the three domains of life, but are nevertheless fascinating are viruses, viroids, and prions.

We live in the world, which is teeming with microorganisms.

They are tiny, but what they lack in size, they more than make up in their numbers. A gram of soil contains up to ten billions of microorganisms (Torsvik et al. 1990; Raynaud and Nunan 2014). The vast majority of microorganisms live in the oceans and it's them and not the fish that fill up most of the space there. It has been estimated that microorganisms make up over two thirds of biomass in the oceans (Bar-On and Milo 2019; Durham 2021). According to some studies, around 90% of all biomass in the oceans is composed of microorganisms (Sogin et al. 2006).

Microorganisms inhabited our planet billions of years before the dawn of humankind. Let's return therefore together to the very beginning of our Universe and reconstitute what we know about their first appearance.

It all started with the Big Bang about 13.8 billion years ago. Then, the first stars were formed and in those first stars were generated heavier elements, including carbon (Henning and Salama 1998).

Carbon is required for life as we know it (Pace 2001). Some researchers suggested that instead of carbon, life on other planets could be silicon-based (Bains 2004; Benner et al. 2004; Schulze-Makuch and Irwin 2006; Petkowski et al. 2020). Silicon is chemically similar to carbon and could be also used as a backbone of large biological molecules (Pace 2001). However, the ability to form strong and simultaneously flexible bonds with other carbons and with a number of other elements makes carbon the best suited basic building block from all known elements. Carbon can be used to form a broad spectrum of different molecules. Consequently, important biological molecules and chemical reactions essential for life as we know it are carbon-based.

First generation of stars, which made heavier elements, including carbon, burned out and exploded, spewing heavy metal elements into the universe. Then, about 4.5 billion years ago our Solar system and Earth were formed from the dust and gas of the previous stars.

For some time after its formation, Earth was not an ideal place for life.

It was likely very hot and bombarded frequently by meteorites. After the conditions calmed down a little bit, Earth already had water and basic chemical compounds required for life. From these chemical compounds were formed the first organic molecules, such as amino acids and nucleotides. First organic molecules were the building blocks of more complex molecules, including proteins, nucleic acids, and lipids. Formation of these complex organic molecules was an important step toward emergence of the first living systems.

It is not entirely clear where exactly life on Earth first appeared.

It was probably near deep-sea hydrothermal vents. Life as we know it requires water, a source of energy, and presence of carbon and other elements. Deep-sea hydrothermal vents are therefore logical candidates for the location of the cradle of life, because they meet all these requirements. And traces of fossilized microorganisms, which are between 3.8 and 4.3 billion years old, have been found in the rocks from seafloor hydrothermal vents (Dodd et al. 2017).

Alternatively, life might have originated in hydrothermal ponds on dry land, which might be similar to hot springs of Yellowstone National Park. Like deep-sea hydrothermal vents, dry land's hydrothermal ponds also contain basic ingredients required for life (Mulkidjanian et al. 2012; Damer and Deamer 2015; Omran and Pasek 2020). Although some scientists argue that Sun's ultraviolet radiation and meteorite bombardment of Earth might have

posed a risk to the early life in dry land's hot springs and hydrothermal ponds, others think that these conditions might have also kick-start life. Traces of ancient microbial life, which are around 3.5 billion years old, have been found in the deposits from the dry land's hot springs (Djokic et al. 2017).

According to some scientists, life was imported to Earth from space on comets and asteroids. Comets and asteroids brought water to Earth. Could these long-distance space travelers bring also early life?

It is well documented that comets can carry water in the form of ice. This has been confirmed also by the European Space Agency's mission, which used Rosetta spacecraft to orbit and investigate comet 67P/Churyumov-Gerasimenko and which landed Philae probe on the comet's surface (De Sanctis et al. 2015; Filacchione et al. 2016; Fornasier et al. 2016). Ice has been also detected on the surface of asteroids (Rivkin and Emery 2010).

Organic molecules, including amino acids, which are the basic building blocks of proteins, have been also detected in comets and asteroids. Best known examples include successful detection of amino acids in the Murchison meteorite, which fell in Australia in 1969 and discovery of amino acids in Tagish Lake meteorite, which fell in Canada in 2000 (Kvenvolden et al. 1970, 1971; Alexander et al. 2017; Edalati et al. 2022).

If life was indeed brought to Earth by comets and asteroids, chances are high, that they did not spark life only on our planet. Who knows on how many other planets they landed? And to what forms did life evolve there, shaped by the unique environmental conditions on those other worlds?

Although many scientists now agree that water was brought to Earth on comets and asteroids, the idea that life itself arrived to Earth on these space travelers is far less widely accepted. Most widely accepted theory is that life originated near deep-sea hydrothermal vents.

However, it is not easy to pinpoint one singular event, which led to the emergence of life, because...what is life?

When trying to find the answer to this question, we are leaving the borders of biology and stepping into an area where several research fields, biology, chemistry, physics, and even philosophy merge.

Definitions of life differ. For this reason it is also not easy to say, which of the early organic systems on Earth were not yet alive and which of those that came after them can be already considered living.

In most cases we know either intuitively, from experience or from what we learned at school, what is alive and what is not on our own planet. For instance, we know that tree and deer are definitely alive, while rock and fire are not. But wouldn't fire look very much alive to an extraterrestrial? And once we land on other planets, will we be able to recognize life, which might have

evolved differently there? And what about viruses, are they alive or not? For that matter, what about computer viruses? They behave like traditional viruses, only instead of replicating their genetic information, they replicate their computer program code (Fig. 1.1). We will come back to viruses later.

Most scientists would agree that to be considered alive, a system should be able to replicate itself. But this definition would also include robots building their own replicas. Based only on this definition, can self-replicating robots then be considered alive? To an extraterrestrial, even a fire might seem to be capable of growth and self-replication, after all.

Besides self-replication, life should also be able to generate genetic variability in order to evolve and adapt to the changing environment. It should be also able to maintain that genetic variability by transferring it into the next generations (Pace 2001).

Based on this extended definition of life, cellular systems fulfill all requirements to be considered first living organisms on Earth. They are isolated from their environment by biological membrane, they are capable of self-replication and can generate genetic variability, which is transferred to next generations.

Therefore, although we don't know for sure what the very first life on Earth looked like, we can say that cellular organisms were already alive.

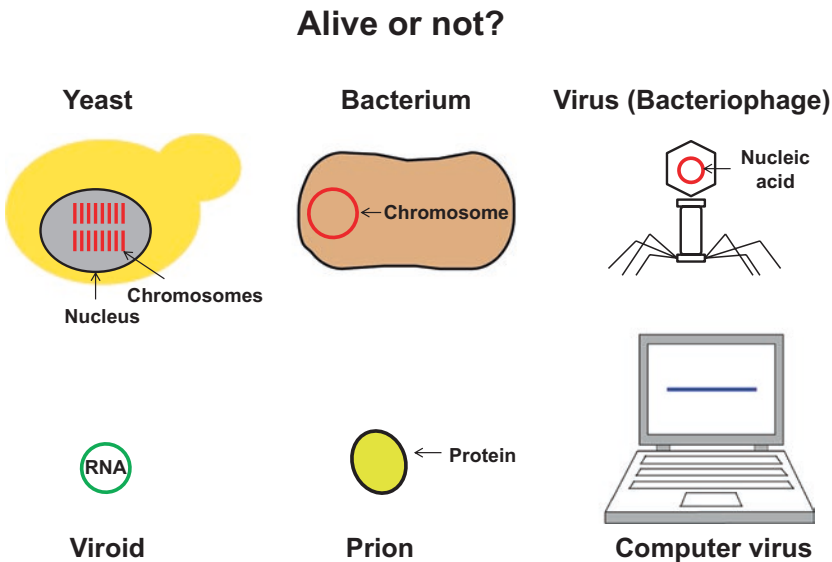


Fig. 1.1 Alive or not? In most cases we know what is alive and what is not. For instance, we know that yeast and bacteria are alive, but in some cases it is less than clear. Viruses, viroids, prions, and computer viruses are considered to be not alive, although some might argue that they have some features of living entities

So how did the first cellular organisms appear on the scene?

First cells were generated most likely by some form of “cooperation” between the complex organic molecules, proteins, nucleic acids, and lipids. Nucleic acids provided first cells with the ability to reproduce. Some proteins became catalysts enhancing chemical reactions and lipids formed a protective barrier between this precious cargo and often harsh environment, which was surrounding it.

These early cells have later become more complex, acquired a variety of functions and evolved into a broad spectrum of shapes. Cells are the basic building and functional units of living organisms, including humans.

But on the early Earth, all cells were microbial.

Three domains of life as we know it are Bacteria, Archaea, and Eukarya.

Bacteria and archaea, together referred to as prokaryotes were the first microorganisms on Earth, so let's have a look together at these two groups of microbes first.

Bacteria are better known to the general public than archaea. Bacteria are single-celled organisms, which come in a wide variety of shapes. Some bacteria might be spherical and look like orbs, while others might be rod-shaped and resemble a wizard's staff. They are usually large enough to be observed under a standard microscope.

Bacterial genes are carried on a circular DNA molecule (Wu et al. 2019; Dame et al. 2020). Bacterial chromosomal DNA, unlike chromosomal DNA of eukaryotes, is not located in a separate membrane-enveloped structure called nucleus, but is floating freely in the cell. It encodes functions essential for survival of a bacterium. Some bacteria have a linear chromosomal DNA, instead of circular (Hinnebusch and Tilly 1993; Yang et al. 2002; Lee et al. 2011).

In addition to chromosomal DNA, bacteria often carry smaller DNA molecules called plasmids (del Solar et al. 1998; Orlek et al. 2017; Rodríguez-Beltrán et al. 2021; Billane et al. 2022). Plasmids can encode a broad spectrum of accessory functions, including resistance to antibiotics and the ability to cause disease.

Unlike eukaryotes, bacteria lack membrane-bound organelles. Bacterial cells are enveloped by a cytoplasmic membrane and in most cases also by a rigid cell wall (Meroueh et al. 2006; Vollmer et al. 2008; Strahl and Errington 2017; Chen et al. 2021). Cell wall is made of peptidoglycan and determines the shape of bacterial cells.

Bacteria usually reproduce by a process called binary fission. Binary fission is the division of the parent bacterial cell and parent DNA into two daughter cells. Parent cell's DNA is replicated before division and both daughter cells

therefore carry the same genetic information as the parent cell (Casiraghi et al. 2020).

Bacteria can inhabit a broad spectrum of different environments, ranging from deep-sea hydrothermal vents to a human body (Hasan et al. 2015; Forster et al. 2019; Zykwiniska et al. 2019).

Some bacteria can form dormant endospores. Endospores are very stable and resistant against harsh environments, including UV irradiation. Best known representative of the endospore-forming bacterium is *Bacillus anthracis*, which causes anthrax (Nicholson et al. 2000; McKenney et al. 2013).

We will look at bacteria in detail in the next chapters.

Archaea form the second domain of life.

Similar to bacteria, archaea are single-celled microorganisms that come in different shapes. Their chromosomal DNA, like chromosomal DNA of bacteria, is not located in a nucleus but floating freely in the cell. And like bacteria, archaea don't have membrane-bound organelles.

Similarities between these two domains of life are so many that archaea were put into the same basket as bacteria and were not recognized as a separate group for a long time. This has changed in 1977 when based on the comparison of genetic sequences it was suggested that archaea are as distantly related to bacteria as bacteria are to eukaryotes. Numerous genome comparison studies performed since then by laboratories worldwide confirmed that archaea are evolutionary distant from bacteria (Woese and Fox 1977; Koonin et al. 1997; Makarova et al. 1999; Martinez-Gutierrez and Aylward 2022).

In addition, archaea differ from bacteria in a number of other features, including composition of cell envelope and mechanisms by which they replicate and repair their DNA (Barry and Bell 2006; Albers and Meyer 2011; Kelman and Kelman 2014; Pohlschroder et al. 2018; White and Allers 2018; Zatopek et al. 2018; Marshall and Santangelo 2020).

Archaea inhabit a wide range of environments on Earth. Many of them belong among extremophiles, organisms able to thrive in extremely harsh environments. They can be often found in the high temperature and pressure areas of the deep-sea hydrothermal vents, in the hot springs, in salt lakes, in Arctic and Antarctic oceans, and in other extreme environmental conditions. For instance, extremely thermophilic species of archaea called *Methanopyrus kandleri*, which was isolated from the deep-sea hydrothermal vent in California, can live at 122 °C. Extremely acidophilic archaea *Picrophilus torridus* isolated from a dry, hot solfataric field on Japan's Hokkaido island can survive around pH 0 (Slesarev et al. 2002, Fütterer et al. 2004, Stan-Lotter and Fendrihan 2015, Wang, Wang et al. 2021).

There is an abundance of planets with extreme environments in the universe, including in our own solar system. Therefore if we sometimes find life on other planets, it will not be surprising if it is similar to archaea.

Due to high tolerance of archaea to extreme environments we could use them to terraform other planets with harsh environments. We could bioengineer archaea by incorporating genes into their genomes whose products can modify the planets' extreme environments. We could then send these bioengineered archaea to other planets even on an unmanned spacecraft. It would take time until the effect is seen on a planetary scale, but because microbes can divide fast, archaea might gradually terraform these planets and prepare them for human habitation in the future.

Eukarya or eukaryotes form the third domain of life.

Eukaryotic cells are the basic building and functional units of the more complex living organisms, including humans. However, many eukaryotes are single-celled microorganisms. Best known examples of eukaryotic microorganisms include green algae and yeast.

Eukaryotic cells share some of the features of both bacteria and archaea. But one of the main differences is the way they store their genetic information. While DNA of bacteria and archaea is floating freely in the cell, in eukaryotes it is located in the separated specialized membrane-enveloped compartment called nucleus.

Genome of eukaryotes is also usually much larger than that of bacteria and archaea. There are some exceptions, such as parasitic eukaryotic microorganism *Encephalitozoon cuniculi*, whose genome is quite short and comparable in size (only approximately 2.9-megabases) (Katinka et al. 2001). But generally, eukaryotic genomes are larger and more complex than genomes of bacteria and archaea.

In contrast to bacteria and archaea, eukaryotes harbor also other organelles enclosed by membranes, such as mitochondria, chloroplasts, Golgi apparatus, endoplasmic reticulum, lysosomes, endosomes, and peroxisomes.

Mitochondria, and chloroplasts in plants, are the energy powerhouses of eukaryotic cells. They generate molecules of ATP that provide energy required for various processes in cells. Besides ATP synthesis, mitochondria are also involved in a number of other essential biological processes, including synthesis of amino acids (McBride et al. 2006; Jensen and Leister 2014; Spinelli and Haigis 2018; Annesley and Fisher 2019; Kummer and Ban 2021; Fish et al. 2022).

Mitochondria and chloroplasts were once separate living microorganisms, which were engulfed by other microbes and over time developed a mutually beneficial relationship with their hosts. First eukaryotic cells were therefore

generated by symbiosis of two simpler prokaryotic cells. It is widely accepted by the scientific community that the cell which was engulfed was a bacterium, while the host cell belonged among archaea. Discovery of DNA in mitochondria and chloroplasts related to bacterial DNA supports the idea about endosymbiotic origin of these organelles. Engulfed bacterium started to specialize on energy production, thus giving the early eukaryotic cells an edge over the simpler bacteria and archaea. It also facilitated evolution of more complex life forms.

Other membrane-enclosed organelles also have important functions in eukaryotic cells. Endoplasmic reticulum is essential for synthesis of proteins and metabolism of lipids in the cell (Preuss et al. 1991; Schwarz and Blower 2016; Kim et al. 2018). Golgi apparatus is involved in the processing and transport of proteins and lipids produced by endoplasmic reticulum (Noda and Yoda 2013; Tao et al. 2020). Lysosomes are important for degradation of unwanted cellular components or material taken in by the cell from the environment. Endosomes transport material for degradation to lysosomes (Wong et al. 2017; Naslavsky and Caplan 2018; Trivedi et al. 2020; Laidlaw et al. 2022). Peroxisomes are involved in cell metabolism and oxidative processes (Sibirny 2016; Ludewig-Klingner et al. 2018).

Other microscopic entities, which do not belong to any of the above three domains of life are viruses, viroids, and prions.

Viruses, such as SARS-CoV-2 which caused COVID-19 pandemic, are usually very small. There are some exceptions, such as the giant Mimivirus which is large enough to be viewed by standard microscopes (Sharma et al. 2016). But to observe most viruses you need a powerful electron microscope.

Viruses are also simpler than bacteria, archaea, and eukaryotes. They are only composed of DNA or RNA enclosed in the protein coat called capsid, which protects the viral genome. Some, but not all, viruses have an additional protective layer made of lipids, called envelope (Poltronieri et al. 2015; Fang et al. 2019; Dos Santos Oliveira et al. 2021; Jones et al. 2021a).

Viruses do not have independent metabolism and cannot reproduce without a host cell. In order to reproduce, virus must infect a host cell. After penetrating the host cell barriers, virus uses the cell's machinery to replicate its own viral genetic material and produce viral proteins. These viral components are then assembled into new viruses, which exit the host cell by budding or lysis (Dou et al. 2018; Jones et al. 2021a, b).

As we have learned above, independent replication is one of the essential functions of living organisms. Viruses cannot replicate independently without a host cell and are therefore generally not considered to be alive (Fig. 1.1).

Although, some might still argue that viruses are just very simple living entities, which are specialized on hijacking other living organisms for their own reproduction.

Because even some eukaryotic microorganisms, called obligate intracellular parasites, need a host cell to replicate. And because eukaryotes are usually more complex than bacteria and bacteria are alive, these eukaryotic microorganisms are therefore also considered to be alive.

But if eukaryotic obligate intracellular parasites are alive, why are viruses also not considered alive? And if viruses can be considered alive, why not also computer viruses? They replicate like traditional viruses, but instead of replicating genetic information they replicate their computer program code (Fig. 1.1).

What's more, viroids are even simpler than regular viruses.

Unlike viruses, viroids do not have a protective protein capsid. These simple infectious agents consist of only short molecule of RNA. They infect plants and replicate in either the nuclei or the chloroplasts of the host plant cells (Flores et al. 2020; Navarro et al. 2021; Lee et al. 2022).

Viroids or RNA molecules similar to viroids are the ancestors of viruses and therefore constitute an important step in the emergence and evolution of life on Earth. RNA likely emerged on Earth before DNA and proteins. Viroids are considered to be the remnants of this ancient RNA world, which lost some of their functions and nowadays can survive and reproduce only by infecting plant cells (Flores et al. 2014, 2022; Müller et al. 2022).

Early virus-like entities, which evolved from viroids likely existed before the first cells and were capable of independent replication without host cells (Koonin and Dolja 2014; Krupovic et al. 2020; Moelling and Broecker 2021).

Prions are another type of infectious agents.

Unlike viruses and viroids, prions do not have any genetic material. They consist of only proteins. Misfolded prion proteins induce abnormal folding of some normal proteins in the brain (Kraus et al. 2021; Sanz-Hernández et al. 2021).

Prions are transmissible and can enter an organism by eating infected food. They cause bovine spongiform encephalopathy (BSE) dubbed mad cow disease in cows and scrapie in goats and sheep. In humans, prions can cause fatal Creutzfeldt-Jakob disease (Scheckel and Aguzzi 2018; Frontzek et al. 2022).

As shown above, even if they are tiny and relatively simple, microbes are a very diverse group.

New species of microorganisms are being discovered on a regular basis. According to some estimates approximately one trillion microbial species live on our planet (Locey and Lennon 2016; Lennon and Locey 2020).

It is good to learn more about microorganisms for a number of reasons.

For instance, many patients are still asking for antibiotics to treat viral infections although antibiotics do not work against viruses. These two groups of microbes are in fact more different than humans and elephants.

Origin of life as we know it is tightly connected with microorganisms. First life on Earth was microbial. Without them starting the path of evolution, there wouldn't be us.

Remnants of bacteria are still present in the cells of our bodies in the form of mitochondria, which generate energy for important biological processes.

Our bodies are full of microorganisms and they are crucial for our survival. They are important for our immunity and metabolism and defend us against pathogens.

We live in the world teeming with microorganisms.

We cannot live without them, therefore we have to understand them and learn how to coexist. That is also one of the main aims of this book.

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2

The Dawn of Microbiology

Abstract Microorganisms remained hidden from the sight of microbiologists until the late seventeenth century when Antonie van Leeuwenhoek discovered a whole new world. World of microorganisms, which nobody before him had any idea existed. Another notable leap in microbiology, particularly in the light of the ongoing COVID-19 pandemic, was vaccination. Important contributions to vaccination were made by Edward Jenner and Benjamin Jesty. Other heroes from the dawn of microbiology include Louis Pasteur and Robert Koch. Numerous discoveries in microbiology were made possible thanks to the pioneering works of these microbiologists from the dawn of microbiology.

Microorganisms remained hidden from the sight of microbiologists until the late seventeenth century.

They remained hidden quite literally, because microscopes with magnifications high enough to see them did not even exist before. For that matter, nor did microbiologists.

But everything has changed with Antonie van Leeuwenhoek (1632–1723).

Antonie van Leeuwenhoek was born in Delft in the Netherlands in 1632. He has never received a formal education in science. Instead, he was an apprentice to a linen draper and later himself became a draper and textile merchant.

Although not formally educated as a scientist, what Antonie lacked in theoretical preparation, he more than made up with his open mind, curiosity, and persistence. Furthermore, his favorite hobby was grinding lenses into

magnifying glasses, which certainly gave him an edge against any competition in this area.

Although the concept of magnifying lenses was not new, his lenses were far better than any others available those days. He likely developed enthusiasm for making lenses during his apprenticeship by a linen draper as drapers used magnifying lenses to inspect the quality of textiles. Later Antonie started making his own lenses from small spheres of glass and assembling them into simple microscopes.

He made several hundreds of these simple microscopes but only a few of them survived and made it into museums. I saw one of them and it looks very different from the microscopes used today. It is an instrument, which looks more like a tiny metallic paddle. You could easily imagine a similar instrument in the hands of a draper inspecting the quality of textiles.

Antonie spent hours looking through these simple microscopes at different objects and materials. Then one day he used a microscope to look at droplets of water from a pond overgrown with green algae.

And I would imagine that what he saw made his jaw drop. Hundreds of tiny organisms were swimming chaotically around in those droplets of water. Organisms, which we now know are very common protozoan microorganisms, but to him must have looked like something out of this world.

Antonie discovered a whole new world. World of microorganisms, which nobody before him had any idea existed.

In his letters to the Royal Society Antonie described his observations of the myriads of small organisms, which were so tiny that they could only be seen through magnifying lenses. He called them animalcules or little animals because the word microorganisms didn't even exist at that time.

Besides protozoan microorganisms, Antonie was also first to observe bacteria, spermatozoa, red blood cells, and a number of other microscopic objects.

In his era, many scientists were still quite skeptical about the existence of Antonie's animalcules. But history gave Antonie the recognition he deserved and he is now considered to be the world's first microbiologist.

His observations have paved the way for a new research field of microbiology. Antonie van Leeuwenhoek is therefore usually referred to as the "Father of Microbiology" (Porter 1976; Anderson 2014; Lane 2015; Robertson 2015; Zuidervaart and Anderson 2016).

Another interesting leap in microbiology, particularly in the light of the ongoing COVID-19 pandemic, was vaccination.

Credit for this feat has been traditionally given to a Gloucestershire physician Edward Jenner (1749–1823). But it seems that a Dorset farmer Benjamin

Jesty (1737–1816) played an equally significant, if not more important, role in this achievement.

But I will come back to that later.

Let's first briefly discuss what vaccines are and how they work.

When a pathogenic microorganism enters our body, our immune system is activated in order to get rid of it. Important part of an immune system is composed of cells involved in adaptive immunity, which fight invading pathogens and can even remember previous encounters with the same pathogens in order to fight them off more efficiently during future infections.

Vaccines stimulate cells involved in adaptive immunity so that when they are faced with the same pathogen in the future they already know how to deal with it.

First type of vaccine, called attenuated vaccine, contains live pathogen, which was weakened so much that it can no longer cause serious infection.

Second type of vaccine, called inactivated vaccine, also contains the whole pathogen like attenuated vaccine but in this vaccine the pathogen is killed.

Third type of vaccine, called subunit vaccine, contains only parts of the pathogen called antigens, which activate the immune system.

These three traditional types of vaccines are nowadays often substituted by modern vaccines, such as DNA vaccines and mRNA vaccines. DNA vaccines and mRNA vaccines contain only the genetic information of the pathogen's antigen. DNA and mRNA vaccines have been developed against a number of pathogens recently, including SARS-CoV-2 (Iwasaki and Omer 2020; Arunachalam et al. 2021; Krammer et al. 2021, 2022; Lucas et al. 2021; Oberhardt et al. 2021; Payne et al. 2021; Tebas et al. 2021; Wang et al. 2021; Arevalo et al. 2022; Goel et al. 2022; Hayashi et al. 2022; Lee et al. 2022; Qu et al. 2022; Tauzin et al. 2022).

Edward Jenner and Benjamin Jesty are praised for their contributions to vaccination. Illness against which they vaccinated is called smallpox.

Smallpox used to be one of the deadliest diseases before WHO declared it eradicated in 1980. First known cases of smallpox were discovered by archeologists who revealed that even the Egyptian mummies carried signs of smallpox infection. Smallpox has later spread from ancient Egypt to different parts of the world and played an important role in the downfall of whole civilizations. It has been introduced into the Americas by conquistadors, where it has wreaked havoc among native populations.

In the time of Edward Jenner in the eighteenth century Europe smallpox was one of the most devastating diseases with an approximately 50% fatality rate.

The traditional story about the discovery of vaccination against smallpox goes that Edward Jenner noticed that milkmaids who were infected with

cowpox never suffered from smallpox. Cowpox is a mild disease and he therefore hypothesized that infecting a person with cowpox gives a protection against much more dangerous smallpox.

Edward tested his hypothesis in 1796 by infecting an 8-year-old boy first with the material from milkmaid's cowpox lesions. Then, several months later he infected the same boy with smallpox.

I always had a problem with this part of the story.

Shouldn't the hero in a good story test the deadly virus on himself rather than using a child as a guinea pig? Anyway, the vaccination worked and Edward Jenner is traditionally considered to be the "Father of Vaccination."

However, recent studies suggest that a similar process has been performed several years before the Edward's experiment by Benjamin Jesty.

Benjamin Jesty was a farmer from Dorset who inoculated his family with the material from cows, which suffered from cowpox. Benjamin did it to protect his family from smallpox during the outbreak in England in 1774 and they never suffered from smallpox.

Benjamin's successful vaccination was performed 22 years before Edward's and he should be therefore also given appropriate credit.

Furthermore, a similar technique called variolation has been used around the world centuries before Benjamin Jesty and Edward Jenner. The main difference was that traditional variolation used material from smallpox lesions for inoculation and was therefore more dangerous than inoculation with material from the mild cowpox disease (Hammarsten et al. 1979; Lakhani 1991; Gross and Sepkowitz 1998; Pead 2003, 2014; Riedel 2005; Boylston 2013; Iwasaki and Omer 2020).

Another hero from the dawn of microbiology is Louis Pasteur (1822–1895).

Born into the family of a leather tanner, Louis Pasteur became one of the most influential figures of his era.

Unlike, for instance, Antonie van Leeuwenhoek before him, Louis Pasteur was a well-educated man. And that's an understatement considering the education level of the majority of the population in those times. Following primary and secondary school, bachelor of Arts, and bachelor of science degree, Louis attended École Normale Supérieure in Paris and earned master of science and doctorate in science degrees.

Contributions of Louis Pasteur to chemistry, microbiology, and biology in general are too numerous to count.

For instance, he showed that the fermentation of food and drinks, such as beer, wine, and milk, is caused by microorganisms. Once Louis knew that, he also realized that wine, beer, and milk could be protected from harmful microorganisms by eradicating all microbes in them. This led to the discovery of the

nowadays popular pasteurization, process of sterilization, and killing of microorganisms by heat. Sterilization is crucial not only to protect milk but also to sterilize medical instruments to prevent infections of patients.

This knowledge Louis later applied also in an experiment where he showed that sterilization of air by heat or using filters prevents contamination by microorganisms. Demonstrating that microorganisms in the air were the cause of contaminations Louis proved that living microorganisms, in this case those causing contaminations, cannot emerge spontaneously from the nonliving matter as previously thought (Fig. 2.1).

Louis Pasteur is also one of the scientists who contributed to the idea that microbes are the cause of infectious diseases.

Louis also made a contribution to vaccination. Although successful vaccinations have been performed before him, for instance by Benjamin Jesty and Edward Jenner, Louis made significant improvements of the whole process. His vaccines were attenuated in the laboratory and were not just a biological material transferred directly between infected and uninfected organisms.

He inoculated chicken with attenuated bacterium causing chicken cholera, thus protecting them against the disease.

Refutation of the Spontaneous Generation theory

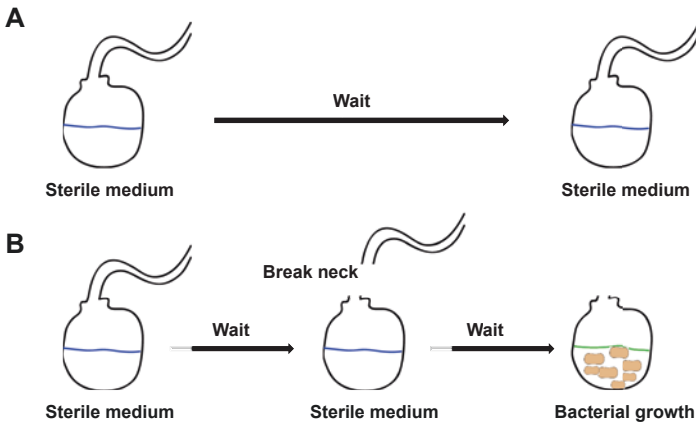


Fig. 2.1 Refutation of the Spontaneous Generation theory. (a) No bacterial growth as long neck prevents contamination of the sterile medium in the flask (b) Medium remains sterile until the flask's long neck remains unbroken. When the neck is broken, bacteria in the air contaminate the medium in the flask. With this experiment Louis Pasteur proved that microorganisms cannot emerge spontaneously from nonliving matter

Louis also developed laboratory-made vaccines against anthrax. In an experiment widely covered by the newspapers at that time he infected several farm animals with anthrax. Only those which previously received his vaccine remained healthy, while the others have either died or became severely ill.

Louis Pasteur also developed an attenuated vaccine against rabies. He used it first to save the life of a boy bitten by a rabid dog and then to save lives of thousands of others (Manchester 1995; Schwartz 2001; Berche 2012; Smith 2012; Cavaillon and Legout 2022).

Another hero from the dawn of microbiology is Robert Koch (1843–1910).

Born into family of a mining engineer, Robert Koch studied medicine and later worked as a physician.

He pioneered methods of growing bacterial species in pure cultures. This allowed him, for instance, to isolate a bacterium causing anthrax, observe it under the microscope, and describe its whole life cycle. Thanks to these experiments he was also the first to describe spores of the anthrax bacterium and to show that spores can also cause disease.

Robert proved that specific diseases are caused by specific microorganisms. He identified bacteria causing many of the most dangerous diseases of that time, including cholera, typhoid, and tuberculosis. For his work, particularly for discovering the causative agent of tuberculosis, bacterium *Mycobacterium tuberculosis*, he was awarded the Nobel Prize in 1905.

Later he spent too much time and effort developing and advertising tuberculin, a remedy for tuberculosis, which turned out to be ineffective. This shows that Nobel Prize laureates also make mistakes.

Robert Koch's enthusiasm for travel allowed him to see firsthand the outbreaks of infectious diseases in various parts of the world. He studied malaria in a number of different countries and recommended preventive measures against it. He was in Egypt and India during the cholera outbreak and recommended filtering the water to get rid of pathogens to prevent spread of cholera.

For his numerous contributions to medical microbiology and bacteriology in particular, Robert Koch is often referred to as the “Father of Medical Bacteriology” (Burke 1993; Ligon 2002; Kaufmann and Schaible 2005; Gradmann 2006; Tan and Berman 2008; Blevins and Bronze 2010; Cambau and Drancourt 2014).

“If I have seen further, it is by standing upon the shoulders of giants,” said the famous physicist and the author of the theory of gravity Isaac Newton.

In microbiology, numerous discoveries of the future generations of scientists were made possible thanks to the pioneering works of these microbiologists from the dawn of microbiology.

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3

Communication Between Microorganisms

Abstract The ability to communicate and coordinate group behavior has been traditionally attributed only to the higher organisms. Microbes have been considered too simple to be capable of such complex social interactions. This view has changed with the discovery of cell-to-cell communication, called quorum sensing. By quorum sensing, bacteria coordinate their actions with other members of the microbial community. This allows them to complete tasks that would be otherwise hard or completely impossible to accomplish for a single bacterium. Quorum sensing is important for intra-species, inter-species as well as for inter-kingdom communication. Communication between pathogenic bacteria is important for establishing and maintaining an infection. Development of novel antimicrobial drugs blocking communication between microorganisms is therefore crucial for the ongoing fight against current and emerging pathogens.

The ability to communicate and coordinate group behavior has been traditionally attributed only to the higher organisms. Microbes have been considered too simple to be capable of such complex social interactions.

This view has changed with the research involving bacterium *Vibrio fischeri*.

V. fischeri lives in marine environments in the symbiotic relationship with squids. It is a bioluminescent bacterium and the ability to produce light is of great value to its nocturnal squid host. Therefore, it keeps *V. fischeri* in the specialized light-organ.

Bioluminescence produced by *V. fischeri* at night allows squid to match the light of the moon and stars in the shallow waters. This prevents squid casting shadows and acts as a camouflage, which provides protection from predators.

Scientists curious about the mechanisms behind *V. fischeri* bioluminescence isolated this bacterium and grew it in the laboratory.

When grown solitarily in a jar *V. fischeri* behaved quite normally. It was growing and multiplying in the liquid culture, like any other bacterium would. But at first it did not produce any light, which was quite disappointing because bioluminescence was the reason why it was studied in the first place.

However, after a while something interesting happened.

The jar started to glow.

As if *V. fischeri* suddenly decided to turn on the light. And that decision was made not only by a few individual *V. fischeri* cells. It looked as if all bacteria in the jar suddenly made a deal and agreed to start producing light at the same time.

How did they do that?

Research revealed that bacteria communicated to each other the intention to turn on the light via chemical signals called autoinducers (Fig. 3.1).

At the beginning, when there was only a small number of bacteria in the jar, the concentration of autoinducers in the liquid in which bacteria were swimming was too low to trigger light production. But as the number of bacteria producing autoinducers grew, the concentration of these chemical signals in the jar was also gradually increasing. After reaching a certain critical threshold concentration, autoinducers induced light production in bacteria.

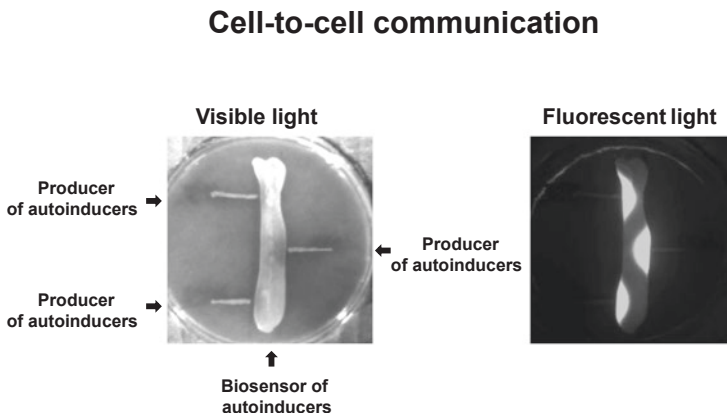


Fig. 3.1 Cell-to-cell communication using autoinducers. Autoinducer signaling molecules produced by bacteria induce light production in the bacterial biosensor strain in the middle

Today we know that autoinducers did trigger light production by binding to specific promoter regions in front of the bacterial genes, which are involved in light production. These include genes, which encode enzyme luciferase which generates light via a chemical reaction. Binding of autoinducers triggered the expression of the light-producing genes and therefore the jar started to glow.

This coordinated behavior using chemical signals was named quorum sensing, because it requires a certain number, or quorum, of bacteria to be initiated (Fig. 3.2).

Exactly as illustrated by the example of the light-producing *V. fischeri* above. The jar was dark, while only a few solitary *V. fischeri* cells were swimming in it, and started glowing only after enough bacteria have accumulated to trigger the light production (Nealson et al. 1970; Lupp et al. 2003; Xavier and Bassler 2003; Antunes et al. 2007; Verma and Miyashiro 2013; Kimura et al. 2016, 2020; Brodl et al. 2018; Nyholm and McFall-Ngai 2021).

Discovery of quorum sensing initiated a plethora of research projects aimed at elucidating the exact mechanisms of bacterial communication. This period is sometimes likened to a gold rush among microbiologists.

Quorum sensing

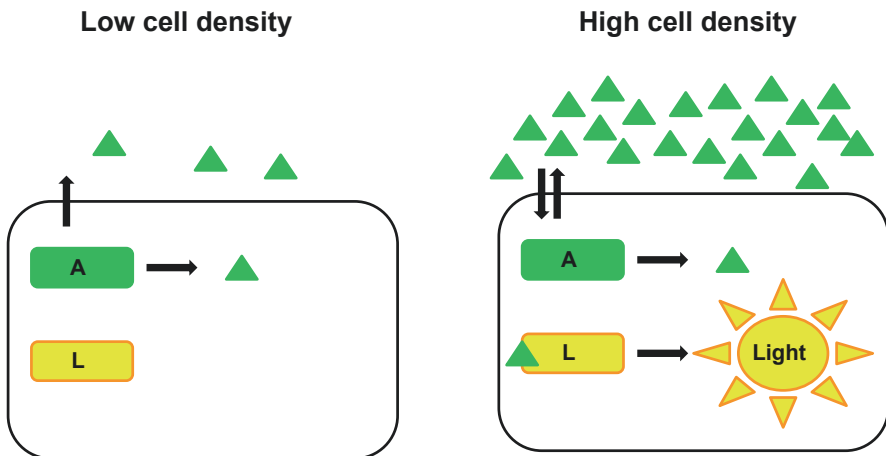


Fig. 3.2 Quorum sensing. At low cell density, concentration of autoinducers produced by bacteria is not high enough to trigger expression of the luciferase gene responsible for light production. At high cell density, after reaching a critical threshold concentration, or quorum, of bacteria, autoinducers induce light production. A autoinducer; L luciferase

These research studies by a number of laboratories around the world revealed that quorum sensing is not limited to *V. fischeri*.

Quite the contrary, quorum sensing is a very common process.

By quorum sensing bacteria coordinate their actions with other members of the microbial community. This allows them to complete tasks that would be otherwise hard or completely impossible to accomplish for a single bacterium.

Since the discovery of the quorum sensing-driven light production in *V. fischeri*, cell-to-cell communication was shown to play an important role in a wide variety of different tasks by a broad spectrum of microbes.

A good example of quorum sensing with far-reaching implications for healthcare is the cell-to-cell communication between pathogenic bacteria.

Pathogenic bacteria use quorum sensing to coordinate their attack on the host.

At the onset of infection, the number of bacteria in the host's body is quite low. It would not make any sense if bacteria started production of virulence factors in this phase of infection. Not only would it be inefficient, but it would also make them more vulnerable. Because once they start production of virulence factors, it also activates the host's immune system, which will try to eradicate them.

But what if the host's immune system could not detect them?

Therefore, at the onset of infection, while their numbers are still low, bacteria keep a low profile. They do that to amass high enough numbers for a better chance to overwhelm the host's immune system. Only when their numbers reach a critical threshold and there are enough invading bacteria in the host's body, they start coordinated expression of genes encoding a battery of virulence factors.

As if the invading pathogenic bacteria communicated to each other: "There are enough of us here. Let's take him down."

Such coordinated production of toxins and other virulence factors harmful to the host is crucial for establishing and maintaining an infection (de Kievit and Iglewski 2000; Juhas et al. 2005).

One of the best described quorum sensing systems among pathogenic bacteria is that of *Pseudomonas aeruginosa*.

P. aeruginosa is an opportunistic human pathogen, which can cause serious infections in certain susceptible individuals, such as immuno-compromised patients with AIDS, cancer, and severe burn wounds. Patients suffering from the genetic disorder called cystic fibrosis are also particularly susceptible to *P. aeruginosa*. This bacterium is one of the leading causes of nosocomial

infections, infections you might get when visiting a hospital. *P. aeruginosa* is also well-known for its resistance to many antibiotics.

P. aeruginosa can therefore be a very nasty and hard to eradicate pathogen once it establishes a successful infection.

Quorum sensing is one of the main reasons why *P. aeruginosa* causes so many problems. This is because it regulates expression of a broad spectrum of virulence factors required to establish and maintain an infection. Quorum sensing is also involved in the formation of *P. aeruginosa* biofilms, which contributes greatly to resistance of this bacterium to antibiotics.

Unlike the relatively simple quorum sensing system of *V. fischeri*, quorum sensing of *P. aeruginosa* is much more complex. It consists of several inter-linked hierarchically arranged quorum sensing systems and a number of additional regulators. Regulators add more complexity to *P. aeruginosa* quorum sensing by increasing or decreasing expression of different quorum sensing genes.

DNA microarrays have been used to compare the expression profiles of *P. aeruginosa* wild-type strains with quorum sensing mutants. These studies revealed that several hundreds of *P. aeruginosa* genes are regulated by quorum sensing. Expression of the subsets of genes was triggered by quorum sensing only in specific environmental conditions, for instance when *P. aeruginosa* was growing in biofilms.

This suggests that *P. aeruginosa* quorum sensing system is so complex because it is required for a very accurate regulation of specific genes in specific environments. Complexity of *P. aeruginosa* quorum sensing circuitry allows fine-tuning of cell-to-cell communication in response to different environmental stimuli (Chugani et al. 2001; Diggle et al. 2003; Greenberg 2003; Hentzer et al. 2003; Schuster et al. 2003; Wagner et al. 2003; Juhas et al. 2004, 2005; Schuster et al. 2004; Ahator and Zhang 2019; Groleau et al. 2020; Vrla et al. 2020; Asfahl et al. 2022; Borgert et al. 2022; Chadha et al. 2022; Li et al. 2022).

In some bacterial species quorum sensing system was shown to be involved in the regulation of antibiotics production (Yang et al. 2009; Morohoshi et al. 2013; Wilf et al. 2013; Quintero-Yanes et al. 2019, 2020).

In other bacteria quorum sensing plays an important role in the regulation of swimming, swarming, and twitching motility (Atkinson et al. 2006a, b; Trimble and McCarter 2011; Wang et al. 2014; Nickzad et al. 2015; Butt et al. 2016; Wang et al. 2016; Ng et al. 2018; Feng et al. 2019; Zhu et al. 2019; Robitaille et al. 2020).

Examples above show that cell-to-cell communication is important for a wide variety of coordinated group behaviors in bacteria.

Interestingly, not only similar microbes, but also those belonging to completely different species can communicate to each other.

Take, for instance, biofilms, structures formed by bacteria, which are so complex, that they have been compared to cities. Under the microscope, biofilms of some bacteria resemble thick lawns of tall mushrooms or futuristic cities, with channels for transporting water, food, and waste.

In biofilms, microbes are very close to each other and in sufficient numbers. This allows quorum sensing regulated expression of genes based on the needs of the whole community in the biofilm. Bacteria in biofilms are therefore more resilient against the environment and also more resistant against antibiotics. They are able to perform tasks, which would be hard to achieve for individual bacteria.

Different species of pathogenic bacteria living in the mixed biofilms can communicate. A simple experiment revealed that signaling molecules from one bacterial species can trigger gene expression in another. This confirmed that different pathogenic bacteria can communicate and co-operate to increase their chances to cause a real havoc during infection.

It also paved the way for the discovery of the bacterial Esperanto, bacterial language composed of signaling molecules that are widely understood and used for communication by different bacterial species.

A good example of inter-species cell-to-cell communication is that between *P. aeruginosa* and *Burkholderia cepacia*.

Burkholderia cepacia is a complex of opportunistic pathogens, which can cause infections in certain individuals, such as immuno-compromised patients or those suffering from cystic fibrosis. Like *P. aeruginosa* it can infect patients in the hospitals and is resistant to many antibiotics.

P. aeruginosa and *B. cepacia* can be found together in mixed biofilms in the lungs of patients suffering from cystic fibrosis. It has been demonstrated that these two bacterial species can communicate with chemical signals. Based on this observation it has been suggested that *P. aeruginosa* and *B. cepacia* use quorum sensing to coordinate their behavior during infection in the lungs of cystic fibrosis patients (Gotschlich et al. 2001; Riedel et al. 2001; Eberl and Tümmeler 2004; Leinweber et al. 2017).

In addition to communication between *P. aeruginosa* and *B. cepacia*, inter-species communication has been demonstrated in a number of studies and in a broad spectrum of diverse bacteria (Moons et al. 2006; Cook et al. 2013; Marchand and Collins 2013; Trejo-Hernández et al. 2014; Leinweber et al. 2017; Silvester et al. 2017; Puri et al. 2019; Banerji et al. 2020; Boopathi et al. 2022; Cui et al. 2022; Gomes-Fernandes et al. 2022; Liu et al. 2022).

What's more, several research studies revealed the existence of the so-called inter-kingdom communication.

A good example of inter-kingdom communication is that between pathogenic bacteria and the cells of their host. It has been hypothesized that this type of communication should exist, before it was actually proved, for several reasons. Bacteria and eukaryotic cells have lived together on Earth for a long time. Signaling molecules used in bacterial quorum sensing resemble eukaryotic hormones. Pathogens have very close relationships with their hosts. Our bodies are full of microbes. Some kind of interaction was therefore expected.

In inter-kingdom communication between pathogenic bacteria and host, the signaling molecules of bacteria enter the host's cells. Inside the cells they trigger expression of a broad spectrum of the host's genes, such as those leading to cell death or to the activation of the host's immune system. Cell death is obviously bad, but over-activation of the immune system is also not good, because it leads to inflammation and tissue damage. This in turn increases the chance of the invading bacteria to establish and maintain a successful infection.

P. aeruginosa quorum sensing signals have been shown to trigger a variety of changes in host cells, including activation of the host's immune system, inflammation, and cell death. Furthermore, host cells have been shown to listen to *P. aeruginosa* quorum sensing and adjust expression of certain genes to mount the most efficient counterattack against the invading bacteria.

And vice versa, because the host–pathogen communication is not flowing only in one direction. Molecules produced by host cells have been demonstrated to influence the expression of many *P. aeruginosa* genes (DiMango et al. 1995; Smith et al. 2002; Tateda et al. 2003; Chun et al. 2004; Hastings 2004; Li et al. 2004; Williams et al. 2004; Shiner et al. 2005; Wu et al. 2005; Wagner et al. 2006; Moura-Alves et al. 2019; Song et al. 2019).

Besides interactions between *P. aeruginosa* and its host, inter-kingdom communication has been documented between a broad spectrum of bacteria and eukaryotes, including plants, insects, and fish (Hughes and Sperandio 2008; Lee et al. 2015; Bernard et al. 2020; Joshi et al. 2021; Ma et al. 2021; Fan et al. 2022; He et al. 2022; Zhu et al. 2022).

The traditional view that microbes are too simple to be capable of communication was first challenged by the research involving the marine bioluminescent bacterium *Vibrio*. But we learned since then that communication plays a far more important role in the world of microorganisms than previously thought.

What started as a purely academic research of bioluminescence has far-reaching clinical consequences. If communication between pathogenic

bacteria is important for establishing and maintaining an infection, imagine what we could achieve, if we disrupt it?

And exactly this important question is the focus of investigation in a number of laboratories around the world. These research efforts revealed that communication between microorganisms can be indeed disrupted by drugs.

Drugs can inhibit quorum sensing by several mechanisms.

First is interfering with the production of the quorum sensing signaling molecules. This can be achieved by molecules, which are similar but not identical to the building blocks from which signaling molecules are made. And without functional signaling molecules, there is no cell-to-cell communication. It's as simple as that.

The second strategy is aimed at inactivation of signaling molecules, for instance, by their degradation with enzymes. Many bacteria produce such enzymes to inactivate signaling molecules of the competing bacteria.

The third strategy is blocking the signaling molecule receptor. Bacteria have receptors on their cells, which bind the signaling molecules once their concentration in the environment reaches critical threshold concentration. If the receptor is blocked by a drug, bacteria are fooled that there are no other bacteria around to initiate quorum sensing behavior.

Disruption of communication between microorganisms has tremendous consequences.

For instance, it prevents formation of the complex bacterial biofilms. As microbes in biofilms are more resistant to antimicrobial drugs, inhibiting biofilm formation makes them easier to eradicate by antibiotics.

Development of novel antimicrobial drugs blocking communication between microorganisms is therefore crucial for the ongoing fight against current and emerging pathogens (Carlier et al. [2003](#); Park et al. [2003](#); Uroz et al. [2003](#); Grandclément et al. [2016](#); Singh et al. [2017](#); Alexa Oniciuc et al. [2020](#); Hemmati et al. [2020](#)).

Now that we have an idea how cell-to-cell communication between bacteria and bacteria and their hosts works, we will have a look at the diverse world of microbial communities in the next chapter.

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4

Microbial Communities

Abstract Microorganisms are very social and can form complex communities. Microbial communities are often organized into elaborate structures, called biofilms, which have been compared to futuristic human cities. Besides protection from the surrounding environment, biofilms allow microbes to specialize on specific tasks similar to cells in the bodies of the higher organisms. Close contact between microbes in biofilms allows easier cell-to-cell communication and exchange of genetic information. Biofilms are often composed of not only single microbial species, but also microorganisms from all domains of life. Biofilms of pathogenic microbes represent a particular problem in healthcare because of their increased resistance against the immune system and antibiotics. Novel drugs targeting biofilms can therefore aid the ongoing fight against pathogens.

In the previous chapters, we have learned that microbes are a very diverse and widespread group of organisms. They are not only all around us, but also on us and in us.

We have also learned that microbes are capable of complex social interactions, which have been traditionally attributed only to the higher organisms. They can coordinate their group behavior and communicate to each other by quorum sensing. Pathogenic bacteria can even exchange signals with the cells of their hosts.

All of this points to the highly social nature of microbial life.

Therefore, it is perhaps not surprising that microorganisms form complex communities. Microbial communities are often organized into elaborate structures, which have been compared to futuristic human cities.

These microbial cities are called biofilms.

Biofilms are formed from the scores of microbial cells enveloped by a glue-like material called extracellular matrix. Extracellular matrix holds microbes in biofilm together and keeps them attached to the surface. It also provides protection from the surrounding environment.

Some microorganisms can form only flat and somewhat dull biofilms, which look like a lawn of grass. But others such as bacterium *Pseudomonas aeruginosa* form very complex structures. Under the microscope, *P. aeruginosa* biofilm looks like a forest of tall mushrooms. Some have compared *P. aeruginosa* biofilm to a futuristic city and it stuck.

Biofilms are therefore often referred to as microbial cities. Under the microscope biofilms really resemble cities. They even have water channels for the transport of water, food, and waste between individual city blocks.

Like many human cities, microbial cities also have certain blocks, neighborhoods, and areas, which are safer for its tiny inhabitants than others. Microorganisms living closer to the center of biofilm are better protected from the environmental insults, while those residing on the rim are more exposed to a broad spectrum of dangers. These dangers include, for instance, predatory microorganisms, bacteriophages, toxic chemicals and antibiotics, which might be present in the environment surrounding biofilm.

In addition to better protection from environmental insults, living in biofilms has a number of advantages for microorganisms.

For instance, it allows microbes to specialize on specific tasks. Microorganisms in biofilms can therefore perform functions, which are very different from those they did when they were living as individual cells.

For example, microbes on the rim of biofilm can specialize on the production of extracellular material, which envelops and protects the whole microbial community. This allows microorganisms in biofilms to survive in harsh environments that would easily eradicate individual microbial cells. Meanwhile, other microbes in the biofilm don't have to waste resources on the production of this protective envelope and can therefore specialize on other tasks, for instance on degradation of organic compounds in the environment. Compounds degraded by these specialized bacteria can then be used as a food source for the whole microbial community.

The underlying mechanisms for this task specialization in biofilms are genetic.

Microorganisms located in different areas of biofilms can express different sets of genes. Products of these genes are then used for different tasks and functions. Consequently, this allows microbes in biofilm to specialize on different tasks for the benefit of the whole microbial community. In some way it resembles multicellular organisms in which cells are also specialized to carry out different functions.

Interestingly, not only the distribution of tasks in biofilms, but also the formation of microbial cities is genetically regulated. Microbes were shown to express specific sets of genes at all stages of the biofilm formation. One set of genes might be therefore used for the initial attachment of the first microbial cells to the surface and another set of genes might be expressed for the formation of the complex structures in mature biofilms.

Close contact between individual microbial cells in biofilms allows easier cell-to-cell communication. Furthermore, it has been shown that cell-to-cell communication is crucial also for the formation of biofilms. Biofilms are therefore a great place for microorganisms to interact, communicate, and help each other with a wide variety of tasks.

Close contact between microbes in biofilms also makes it easier to exchange genetic information. Bacteria often exchange their genes by a horizontal gene transfer process called conjugation, which is described in more detail in the next chapter. During conjugation genes are transferred from one bacterium into another via a bridge-like connection. As this process requires close contact between bacteria, biofilms are an ideal place for gene exchange by conjugation.

All kinds of genes are exchanged between microbes living in biofilms, including virulence and antibiotic resistance genes. Biofilms are therefore dangerous reservoirs of antibiotic resistance genes and hotspots for emergence of resistant pathogens.

Besides cooperation to achieve difficult tasks and gene exchange, microbes in biofilms can also engage in various competitive interactions. For instance, they can produce antibiotics or toxins to kill microbes competing for the same food source or for the same space (Anderson et al. 2014; Balcázar et al. 2015; Dumitrache et al. 2017; Dragoš et al. 2018; Kobayashi 2021; Bhatt et al. 2022; Flemming et al. 2022; Ramakrishnan et al. 2022; Sauer et al. 2022; Yadav et al. 2022).

Biofilms of pathogenic bacteria represent a significant problem in health-care because of their increased resistance against a whole range of antibiotics and against eradication by the human immune system.

Good example of such a problematic biofilm is that formed by *P. aeruginosa*.

P. aeruginosa biofilms are those quintessential complex structures, which under the microscope resemble cities composed of mushroom-shaped buildings. Growth in biofilms provides *P. aeruginosa* with better protection against the host immune system and antibiotics. Increased antibiotic resistance poses problems particularly in patients suffering from cystic fibrosis as it makes it difficult to eradicate fully formed *P. aeruginosa* biofilms from the lungs of cystic fibrosis patients with antibiotics.

Numerous studies have shown the importance of cell-to-cell communication in *P. aeruginosa* biofilms. Quorum sensing is involved in the formation of *P. aeruginosa* biofilms. Besides biofilm formation, quorum sensing also plays an important role in the expression of a whole battery of virulence factors by *P. aeruginosa* biofilms (Bové et al. 2021; Cendra and Torrents 2021; Dostert et al. 2021; Warriier et al. 2021; Gheorghita et al. 2022; Valentin et al. 2022).

Biofilms are often composed of not only single microbial species, but also different species of microorganisms.

P. aeruginosa often forms mixed biofilms with other pathogenic microbes and that makes them even more difficult to eradicate. In cystic fibrosis lungs *P. aeruginosa* forms mixed biofilms with other bacteria, particularly with *Burkholderia cepacia*, *Streptococcus* and *Staphylococcus aureus*. Bacteria in mixed biofilms were shown to interact with each other and use each other's products to maintain infection and provide better protection against antibiotics. *P. aeruginosa* was also shown to communicate with other bacterial species in mixed biofilms using signaling molecules.

This inter-species communication in mixed biofilms has important medical implications. It can, for instance, enhance production of virulence factors to increase the severity of infection (Gotschlich et al. 2001; Riedel et al. 2001; Eberl and Tümmeler 2004; Korgaonkar et al. 2013; Armbruster et al. 2016; Leinweber et al. 2017; Scofield et al. 2017; Trizna et al. 2020; Cendra and Torrents 2021; Chadha et al. 2022; Park and Sauer 2022).

What's more, it has been revealed that not only bacteria from different species, but also microorganisms from all domains of life Bacteria, Archaea, and Eukarya can form mixed biofilms.

Good example of such mixed inter-kingdom biofilm is, for instance, the microbial community composed of bacteria and microscopic fungi. Microbes of inter-kingdom biofilms can also interact with each other in different ways. For instance, signaling molecules produced by a bacterium can increase or decrease expression of certain genes in fungi.

These types of biofilms are in fact very common in nature. But can pose a serious problem when they form on medical devices, such as prosthetic joints. Infections, which involve inter-kingdom biofilm, are often more resistant

against antibiotics and require longer treatments (Adam et al. 2002; Harriott and Noverr 2011; Kong et al. 2016; Sadiq et al. 2022).

Increased resistance toward antibiotics is among the most worrying features of biofilms of pathogenic microorganisms. Novel drugs targeting biofilms can therefore aid the ongoing fight against pathogens.

Antimicrobial compounds, which can disrupt biofilms of a number of human pathogens, including *P. aeruginosa* have been developed. Some of these novel drugs act at the very beginning, by disrupting the process of the biofilm formation. Because cell-to-cell communication plays an important role in several stages of biofilm formation, targeting bacterial quorum sensing is a promising strategy for the development of novel drugs.

Others promising antimicrobial compounds are those, which are targeting genes that regulate essential biofilm-specific pathways. Interfering with the expression of these genes breaks up biofilm into individual bacterial cells, which are easier eradicated by antibiotics (Herrmann et al. 2010; Römling and Balsalobre 2012; Ghorbani et al. 2017; González et al. 2018; Crabbé et al. 2019; Abdulkareem et al. 2021; Arnaouteli et al. 2021; Dostert et al. 2021; Lv et al. 2022; Ramakrishnan et al. 2022).

As shown in this chapter, microorganisms are very social and can form complex communities. Besides exciting basic research, investigation of these microbial communities has also many practical applications, including better treatment of infectious diseases.

Now that we have learned about complex social interactions in microbial communities, we will have a look at how microorganisms exchange their genes in the next chapter.

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5

Gene Transfer

Abstract In addition to vertical gene transfer between generations, microbes often transfer their genes horizontally. Horizontal gene transfer contributes greatly to evolution of microorganisms and their ability to adapt to changing environment. Mechanisms of horizontal gene transfer include transformation, conjugation, and transduction. Horizontal gene transfer is facilitated by a diverse group of mobile genetic elements, such as plasmids, transposons, and integrative and conjugative elements. Horizontal gene transfer has important implications in healthcare as it facilitates transfer of antibiotic resistance and virulence genes between microbes.

Most of us are probably already aware that genes are somehow transferred between generations.

Parents can see it in their children, which, more often yes than no, resemble them or grandparents. This type of gene transfer is called vertical because genes in it are transferred vertically, between generations.

Vertical gene transfer is not exclusive to the higher organisms. Microbes also pass genes between generations and it is important for maintenance of the identity of individual microbial species over time.

Good example of vertical gene transfer in the world of microorganisms is replication and transfer of the genetic material from the parent yeast cells into smaller daughter cells in a process called budding. Another example is replication and transfer of bacterial chromosome from the dividing bacterial cell into two daughter cells (Duina et al. 2014; Wagner et al. 2017; Misra et al. 2018).

But could you imagine transferring genes within the same generation? For instance, that if you did not like the color of your eyes, you could simply take genes from a friend whose eyes you fancy and instantly switch to the desired color?

That is what bacteria are doing in the process called horizontal gene transfer. And they are doing it frequently because it has been estimated that around a quarter of all genes in bacterial genomes have been acquired by horizontal gene transfer (Ochman et al. 2000; Nakamura et al. 2004; Ambur et al. 2016).

Although bacteria were suspected of this ability for some time before it was proven experimentally, it was only at the beginning of the twentieth century that the basic principles of horizontal gene transfer started to unravel.

In 1928 Frederick Griffith published the research manuscript describing the first recorded successful gene transfer and transformation of bacteria.

Frederick worked with two bacterial strains, which differed in their physical appearance and in their ability to cause disease. The first strain looked rough under the microscope and was harmless. The second strain looked smooth and was very virulent.

In this pivotal experiment, Griffith changed rough-looking bacteria into smooth-looking. What's more, the newly transformed smooth-looking bacteria, which were rough and mild before, were also virulent and caused disease.

At that time the exact reason for the change in the shape and virulence of bacteria was unknown, but the follow-up studies showed that transformation was caused by the transfer of genes between bacteria. More specifically, DNA-encoding genes responsible for the smooth-appearance and increased virulence were released from the smooth-looking bacteria into the environment. This DNA was then taken up from the environment by the rough-looking bacteria. Incorporation of the newly acquired genes into the genome of rough-looking bacteria caused their change into smooth-looking and their increased ability to cause disease.

This type of horizontal gene transfer is called transformation.

In transformation, a bacterium takes up foreign DNA from the environment and incorporates it into its own genome (Fig. 5.1).

Transformation requires engagement of massive cellular molecular machinery and genes encoding this transformation machinery are located on bacterial chromosome. Cells which are able to take up foreign DNA from the environment by natural transformation are called competent cells.

Some bacteria are naturally competent, others are competent only under certain conditions. For instance, competence in *Vibrio* is induced by quorum sensing described in one of the previous chapters. Thanks to quorum sensing *Vibrio* knows when there is a high enough number of other bacteria in the

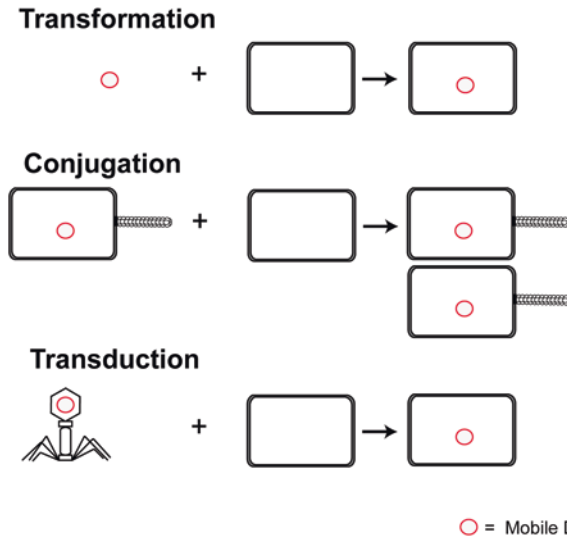


Fig. 5.1 Mechanisms of horizontal gene transfer. By transformation bacteria take up foreign DNA from the environment. By conjugation bacteria transfer DNA via a bridge-like connection mediated by pilus. By transduction genes are transferred between bacteria by bacteriophages

environment to become competent. Competence can be even induced in the laboratory to facilitate horizontal gene transfer in bacteria (Treangen et al. 2008; Ambur et al. 2009; Stingl et al. 2010; Wylie et al. 2010; Krüger and Stingl 2011; Suckow et al. 2011; Borgeaud et al. 2015).

Transformation was the first described process of horizontal gene transfer in bacteria, but discoveries of other mechanisms soon followed (Fig. 5.1).

The second mechanism of horizontal gene transfer in bacteria is called conjugation (Fig. 5.1).

Conjugation was discovered by Joshua Lederberg and Edward Tatum in 1946. In the process of conjugation bacteria do not take up DNA from their environment as they do during transformation. Genes are instead transferred from one bacterium into another via a bridge-like connection, which is mediated by conjugative pilus. Conjugative pilus resembles a needle or flagellum used by donor bacterium to spear and pull the recipient cell closer for DNA transfer (Fig. 5.2). Conjugation therefore requires very close contact between bacteria.

Pilus and molecular machinery required for conjugative gene transfer are encoded by genes of the so-called type IV secretion systems. Type IV secretion systems are large protein complexes, which traverse the membranes of bacteria and allow transfer of genes (Fig. 5.3).

Conjugative pilus

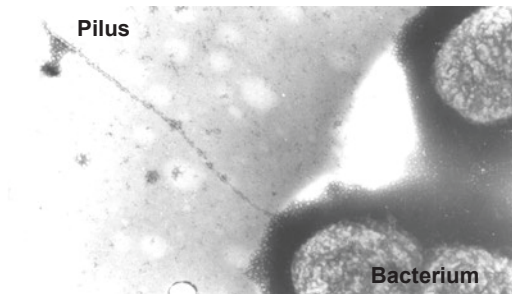


Fig. 5.2 Conjugative pilus. Photo of conjugative pilus taken with electron microscope

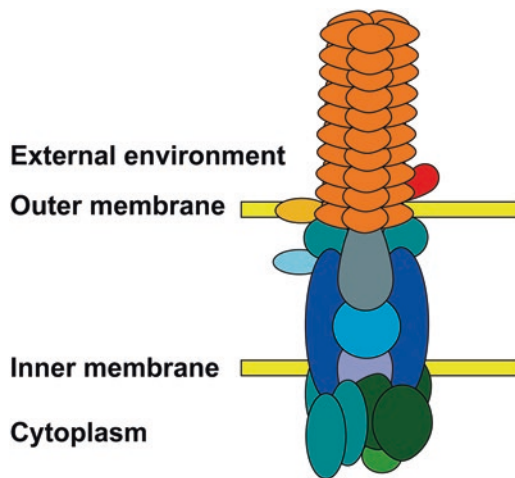


Fig. 5.3 Conjugative gene transfer machinery. Pilus and machinery required for conjugative gene transfer are large protein complexes, which traverse the membranes of bacteria

Genes encoding type IV secretion systems are not located among the core genes on the bacterial chromosome. They are instead located on mobile genetic elements, such as plasmids and transposons (Juhas et al. 2007a, 2008; Alvarez-Martinez and Christie 2009; Wallden et al. 2010; Dostál et al. 2011; Wong et al. 2012; Juhas 2015; Bragagnolo et al. 2020).

The third mechanism of horizontal gene transfer in bacteria is called transduction.

Transduction was discovered by Norton Zinder and Joshua Lederberg in 1952. In the process of transduction, genes are transferred between bacteria by bacteriophages, which are viruses that infect bacteria (Fig. 5.1). Bacteriophages can integrate between core genes on the bacterial chromosome and lay there inactive for longer time periods while waiting for the best conditions for transfer to the new host.

When these conditions are met, bacteriophages can excise from the chromosome and leave the original host bacterium. In the process they can even take some host's genes with them and transfer them to another bacterium. Genes transferred between bacteria by transduction can include virulence genes. Bacteriophages therefore contribute to the emergence of new pathogens (Coleman et al. 2006; Scott et al. 2008; Novick et al. 2010; Chiang et al. 2019).

More recently, other mechanisms of gene transfer between bacteria were discovered, which differ from all previously described.

In one of these novel mechanisms bacteria use nanotubes to transfer DNA between cells. In another, bacteria pack their genes into balloon-like structures made from their membranes and send them to other bacteria. After receiving the package, bacteria incorporate the sent genes into their genome. How often bacteria use these novel ways of gene transfer and how important it is for them will require further investigation (Dubey and Ben-Yehuda 2011; Fulsundar et al. 2014; Emamalipour et al. 2020).

Horizontal transfer is facilitated by a diverse group of mobile genetic elements, such as plasmids, transposons, and integrative and conjugative elements (Fig. 5.4).

Plasmids are circular extrachromosomal molecules of DNA, which can replicate and transfer independently of the chromosome of the host cell in which they reside (Fig. 5.4). They usually carry a broad spectrum of genes, which can be divided into two categories: core and accessory genes. Core genes are essential for the life cycle of plasmid, such as replication and transfer. Accessory genes encode functions, which might come in handy in specific situations, such as resistance to antibiotics and heavy metals and ability to degrade and utilize certain compounds in the environment (Juhas et al. 2009; Toleman and Walsh 2011; Lorenzo-Díaz et al. 2017).

Transposons are mobile repetitive pieces of DNA, which normally reside in the chromosome but can excise and jump between genomes (Fig. 5.4). By transporting into a new host cell and integrating into its chromosome, transposons can disrupt some of the host's genes or impact their expression or rearrange parts of the host's genome. Because transposons often carry antibiotic

Mobile genetic elements

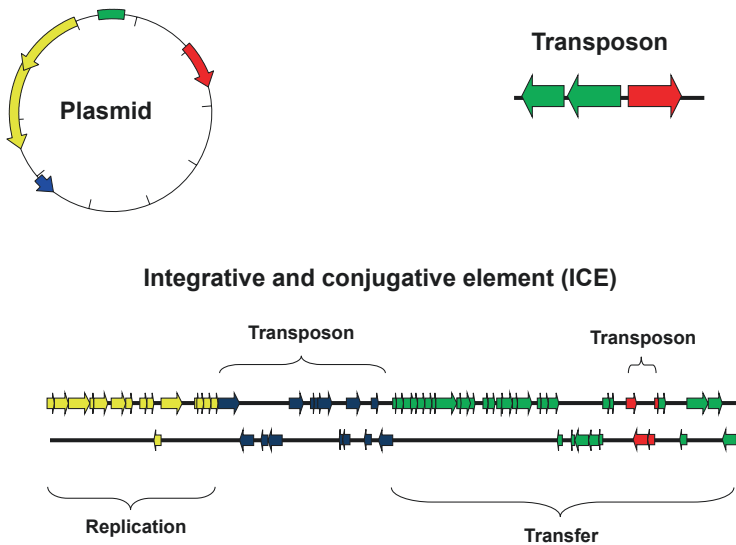


Fig. 5.4 Mobile genetic elements. Figure shows different types of mobile genetic elements: plasmid, transposon, and integrative and conjugative elements (ICE)

resistance genes, they contribute greatly to the spread of antibiotic resistance among microbes (Chuong et al. 2017; Kumar 2020).

Interesting mobile genetic elements are integrative and conjugative elements (Fig. 5.4). These are modular, large pieces of DNA, which combine traits of previous two types of mobile genetic elements.

Integrative and conjugative elements are often integrated into the host chromosome like transposons. However, they are usually much larger than transposons and carry a wide variety of genes. They even carry all genes encoding the whole machinery and pilus required for conjugation like plasmids. Like plasmids, integrative and conjugative elements are capable of excision from the chromosome and transfer into the new host cell by conjugation. Unlike plasmids however, integrative and conjugative elements usually reintegrate into the chromosome of the new host (Fig. 5.5) (van der Meer et al. 2001; Larbig et al. 2002; Juhas et al. 2007b, c, 2008, 2009; Klockgether et al. 2007; Ubeda et al. 2007; Sentchilo et al. 2009; Novick et al. 2010; Wozniak and Waldor 2010; Roy Chowdhury et al. 2016, 2017; Carraro et al. 2017; Botelho et al. 2018).

Life cycle of integrative and conjugative elements (ICE)

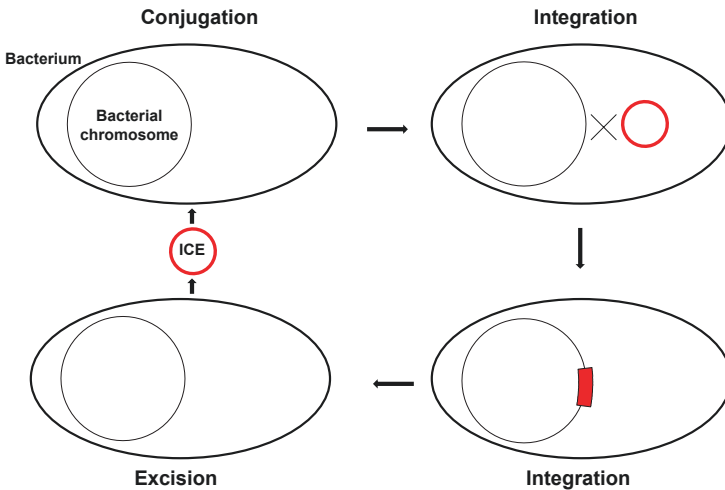


Fig. 5.5 Life cycle of integrative and conjugative elements (ICE). Following horizontal transfer into a new host by conjugation, ICEs can integrate into the chromosome. ICEs can also excise from the chromosome and transfer into a new host

This illustrates that bacteria have a number of interesting ways how to transfer their genes. But why do they do it?

In simple words: to adapt and to survive.

Horizontal gene transfer contributes greatly to evolution of microorganisms and their ability to adapt to changing environment.

Is there a new tasty organic material in the environment, which bacterium could potentially use as a food source? Then bacterium can acquire genes from other bacteria, which allow it to eat this material.

Is the environment toxic? No problem again because bacterium can acquire genes, which allow it to degrade the toxic stuff to harmless components. And maybe even use it as a yummy new food source.

Horizontal gene transfer allows microbes not only to survive but also thrive under different environmental conditions. By horizontal acquisition of new genes bacteria can rapidly evolve and adapt to the constantly changing environment. Modern whole genome sequence analyses revealed that a big percentage of genes in the bacterial genomes was acquired from other microbes by horizontal gene transfer.

Horizontal gene transfer has important implications in healthcare. Among the main negative effects is the transfer of antibiotic resistance and virulence genes between microbes.

For instance, gene transfer contributes greatly to the emergence and spread of multidrug-resistant bacterium *Staphylococcus aureus*, which is among the main causes of morbidity and mortality worldwide. *S. aureus* owes big part of its increased virulence and antibiotic resistance to a number of mobile genetic elements (Weigel et al. 2003; Bloemendaal et al. 2010; Smyth et al. 2011; Damle et al. 2012; Ram et al. 2012; Ubeda et al. 2012; Boundy et al. 2013; Fluit et al. 2013; Ray et al. 2016; Xue et al. 2017; Hosseinkhani et al. 2018).

Gene transfer contributed greatly also to the outbreak of the highly pathogenic strain of *Escherichia coli* which led to nearly 5000 cases of severe infections and 82 deaths in 2011 (Konczy et al. 2008; Asadulghani et al. 2009; Touchon et al. 2009; Bielaszewska et al. 2011; Mellmann et al. 2011; Rasko et al. 2011; Grad et al. 2013).

Now that we have an idea how microorganisms transfer their genes, we will have a look at how this gene transfer contributes to the emergence of antibiotic-resistant superbugs in the next chapter.

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6

Multidrug-Resistant Bacteria

Abstract Not long after the discovery of penicillin, bacteria resistant against penicillin emerged and spread across the globe. The race has begun. Discovery of each new antibiotic was always followed by the emergence of bacteria resistant against it. Besides natural antibiotic resistances of some bacterial species, horizontal gene transfer also contributes greatly to the emergence and spread of antibiotic resistance and evolution of multidrug-resistant bacteria. Transfer of virulence and antibiotic resistance genes between bacteria played an important role in the outbreak of the highly pathogenic strain of *Escherichia coli* in 2011 and in the emergence of many multidrug-resistant bacteria. New drugs are required to cope with the rapidly emerging resistant bacteria.

It is interesting how many major scientific discoveries in the history of humankind happened seemingly by accident or when someone's mind was occupied by something completely different.

This was also the case by the discovery of the first antibiotic, penicillin.

Although, penicillin probably was not the first antimicrobial in history used by people, as there are other documented cases even from ancient times. But I will come back to that.

We owe the discovery of penicillin in great extent to holidays and readiness of one mind to recognize the significance of what happened.

In 1928 Alexander Fleming, who was at that time working in London, took a well-deserved break and went on a two weeks long holiday. Before leaving, he inoculated the culture plates with bacteria and left them growing in the laboratory.

When Alexander returned from holiday, he found that while he was gone a mold has developed in one of the culture plates. He also noticed that bacteria were not growing in the area around the mold. This suggested to him that the mold must have produced some kind of substance that prevented bacterial growth around it.

The mysterious substance turned out to be the first antibiotic, penicillin.

Although as mentioned above, people have long before the discovery of penicillin used antimicrobial substances, but in a less scientific manner. There are many documented cases of using molds and plant extracts in ancient times.

These can be considered the first known successful applications of antibiotics when compound produced by a microorganism kills other microbes. Even if those who were using these natural treatments did not know the exact mechanism, they were often successful because of the antimicrobial substances they involved.

Such treatment has been used, for instance, already in ancient Egypt and China. Traces of the antibiotic called tetracycline have been found even in the bones of skeletons from ancient Sudanese Nubia.

Molds have been later used by John Parkinson and a number of other researchers to treat infections. Rudolf Emmerich and Paul Ehrlich also employed various biological and chemical agents to treat infections long before Alexander Fleming. By the end of the nineteenth century the existence of antibiosis between competing microorganisms was widely accepted (Bassett et al. 1980; Fleming 1980; Schwartz 2004; Kong et al. 2010; Gould 2016).

Alexander Fleming realized the great potential of penicillin for treatment of bacterial infections, but for some time it remained more or less just a small laboratory-based project.

Team at the University of Oxford started purification and isolation of penicillin from mold in larger volume in 1939. This novel, miraculous drug has later become crucial for the treatment of infections during World War II.

What came after Alexander Fleming's discovery of penicillin is often called the antibiotic era.

Before the wide application of antibiotics in medicine, the death caused by bacterial infections was common. A simple scratch wound could have led to a life-threatening infection. Infections with the more dangerous pathogenic bacteria, such as *Yersinia pestis*, which caused the Black Death pandemic of plague in the fourteenth century, were virtually untreatable.

Mass production of penicillin started a completely new era. It saved countless lives during World War II. Plague and other bacterial diseases could be suddenly successfully treated with antibiotics.

A number of other antibiotics are, like Alexander Fleming's penicillin, also naturally produced by various species of fungi and bacteria. They serve these microorganisms as weapons to eliminate competing microbes.

In the years following the discovery and mass production of penicillin a wide array of other antibiotics has been discovered and isolated from their natural producers. Other antibiotics have been generated in the laboratory by chemical modification of the previously known antimicrobial compounds.

It seemed that humankind has won the battle against pathogenic bacteria.

However, not long after penicillin was discovered and started to be widely used, bacteria resistant against this antibiotic emerged and spread rapidly across the globe. Diseases caused by the bacteria resistant to penicillin could no longer be successfully treated with penicillin and other antibiotics were required to eliminate the resistant microbes.

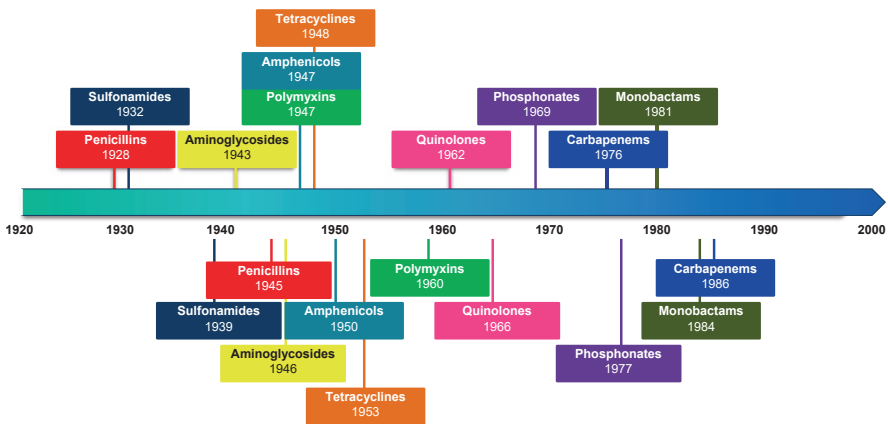
The race has begun. Discovery of each new antibiotic was always followed by the emergence of bacteria, which were resistant against it (Fig. 6.1).

How do bacteria become resistant to antibiotics?

There are several mechanisms contributing to antimicrobial resistance. Some bacterial species are naturally more resistant to antibiotics than others because of the composition of their cellular membranes. They might be also

Antibiotics: Discovery and Resistance

Antibiotics discovered



Antibiotic resistance

Fig. 6.1 Discovery of antibiotics and emerging resistances. Graph shows that discovery of new antibiotic is usually soon followed by the emergence of bacteria, which are resistant against it

more resistant due to their ability to form complex biofilm structures, which are harder to penetrate by antibiotics and therefore also more difficult to eradicate (Mah et al. 2003; Walters et al. 2003; Liao et al. 2013; Zhang et al. 2013).

Besides natural antibiotic resistance of some bacterial species, horizontal gene transfer of resistance genes between bacteria also contributes greatly to the emergence and spread of antibiotic resistance.

Antibiotic resistance genes acquired by bacteria in the process of horizontal gene transfer can function in different ways.

For instance, they can degrade antibiotics or modify them in a way that the modified antibiotics can no longer bind to their targets in bacteria. Or, instead of modifying antibiotics themselves, resistance genes can modify the antibiotic targets in bacteria. Such target modification prevents binding of antibiotics and therefore also their activity.

Another antibiotic resistance mechanism involves resistance genes, which encode cellular pumps. These pumps located in bacterial membranes can be used by bacteria to pump out the antibiotics from the bacterial cell into the environment (Hächler et al. 1996; Sobel et al. 2003; Linares et al. 2010; Srinivas et al. 2010; Tomás et al. 2010; Breidenstein et al. 2011; Krahn et al. 2012; Werneburg et al. 2012; Faure et al. 2013; Balibar and Grabowicz 2016; Morita et al. 2016; Zincke et al. 2016; Hall and Mah 2017; Nguyen et al. 2018).

Horizontal gene transfer plays an important role in the rapid spread of antibiotic resistance genes between bacteria. What's more, it is also involved in the emergence of bacteria resistant against multiple antibiotics simultaneously, which are sometimes dubbed superbugs.

How do bacteria become multidrug-resistant superbugs?

If a bacterium carries resistance gene against one antibiotic, and another bacterium carries resistance gene against another antibiotic, it only takes a simple horizontal gene transfer between these two for a bacterium resistant against both antibiotics to emerge. If the acquired resistant genes provide a selective evolutionary advantage, then both resistance genes can be kept and passed on to the next generations. Doubling time of bacteria usually takes only a few minutes. Therefore, in favorable conditions when having both resistance genes provides an advantage, bacteria with both genes soon out-compete those with only a single resistance gene. This way bacteria can over time accumulate resistance genes against multiple antibiotics and become multidrug-resistant superbugs.

In addition to acquiring resistance genes one by one, bacteria can become resistant against multiple drugs much faster. This rapid process of superbug

evolution involves large mobile modular pieces of DNA called integrative and conjugative elements, whose life cycle was described in the previous chapter.

Integrative and conjugative elements usually have several modules, each consisting of multiple genes. In addition to core modules required for their life cycle, integrative and conjugative elements often harbor accessory modules with genes providing selective advantage under certain environmental conditions. This can include modules, which harbor many resistance genes simultaneously (Fig. 6.2) (van der Meer et al. 2001; Larbig et al. 2002; Juhas et al. 2007a, b, 2008; 2009; Klockgether et al. 2007; Ubeda et al. 2007; Sentchilo et al. 2009; Novick et al. 2010; Wozniak and Waldor 2010; Roy Chowdhury et al. 2016, 2017; Carraro et al. 2017; Peng et al. 2017; Botelho et al. 2018).

By acquiring such large DNA segments with multiple resistance genes, bacterium can become resistant against a broad spectrum of antibiotics in a single step. This rapid process of evolution of superbugs by acquiring large DNA fragments with many resistant genes, such as integrative and conjugative elements, is therefore sometimes called evolution by quantum leaps.

Transfer of virulence and antibiotic resistance genes between bacteria played an important role in the outbreak of the highly pathogenic strain of bacterium *Escherichia coli* in 2011 (Fig. 6.3).

Integrative and conjugative elements (ICE)

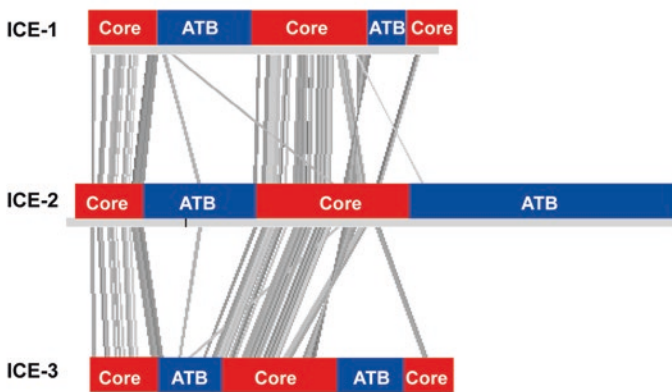


Fig. 6.2 Integrative and conjugative elements (ICE). ICEs are composed of modules, which can be divided into core and accessory. Core modules are often similar among ICEs and are involved in the life cycle of ICEs. Accessory modules provide selective advantage in specific environments and might encode, for instance, antibiotic resistances (ATB). Vertical lines show highly similar (core) regions

Horizontal gene transfer in *Escherichia coli* 2011 outbreak strain

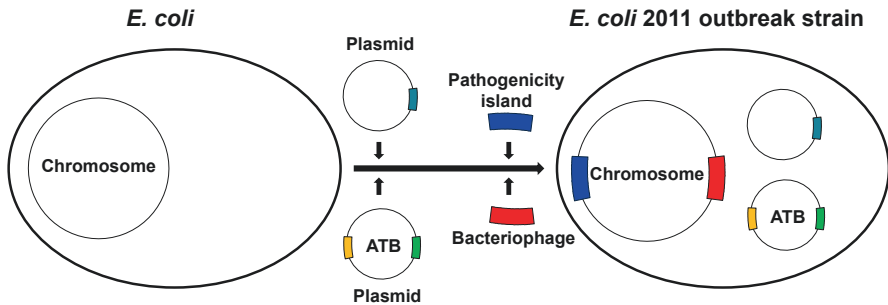


Fig. 6.3 Horizontal gene transfer in *Escherichia coli* 2011 outbreak strain. Horizontal transfer of virulence and antibiotic resistance genes played an important role in the outbreak of highly pathogenic *E. coli* strain in 2011. This bacterium has acquired a number of mobile genetic elements, including plasmid with antibiotic resistance genes (ATB)

The infections were caused by eating bean sprouts contaminated with this pathogenic bacterium and resulted in nearly 5000 cases of severe infections and 82 deaths (Bielaszewska et al. 2011, Mellmann et al. 2011).

Increased pathogenicity of the bacterium responsible for this outbreak was caused by horizontal acquisition of a battery of genes. Horizontally acquired genes were responsible for a number of clinically important traits of the highly pathogenic *E. coli*, including increased production and absorption of a toxin and resistance to multiple antibiotics.

Resistance against multiple antibiotics led to limited treatment options with the available antibiotic. Furthermore, multidrug resistance allowed the highly pathogenic *E. coli* to outcompete other bacteria in the guts of patients undergoing antibiotic treatment. This is because the normal gut bacteria, which were sensitive to antibiotics were likely eradicated by antibiotics (Konczy et al. 2008; Asadulghani et al. 2009; Touchon et al. 2009; Bielaszewska et al. 2011; Mellmann et al. 2011; Rasko et al. 2011; Grad et al. 2013).

Besides *E. coli*, other bacterial species resistant against multiple antibiotics started to emerge and spread around the world. Examples of these multidrug-resistant bacteria include well-known hospital superbugs methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* and the multidrug-resistant *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas*

aeruginosa (Weigel et al. 2003; Bloemendaal et al. 2010; Smyth et al. 2011; Damle et al. 2012; Ram et al. 2012; Ubeda et al. 2012; Boundy et al. 2013; Fluit et al. 2013; Ray et al. 2016; Martin et al. 2017; Xue et al. 2017; Hosseinkhani et al. 2018; Butler et al. 2019; Kunz Coyne et al. 2022).

These multidrug-resistant species of bacteria are considered to be among the biggest threats to public health nowadays.

But numbers of these dangerous bacteria might soon increase. Horizontal gene transfer can easily break inter-species boundaries. Antibiotic resistance genes carried by these bacteria can be transferred to other bacterial species and give rise to new superbugs. Therefore, there is a constant race between the spread of antimicrobial resistance and development of novel antibiotics.

Development of novel drugs involves close interplay between chemistry and biology and requires highly trained scientists in these research areas. Scientists have to know how to synthesize and chemically modify drugs and they have to also understand how these potential new drugs might impact the lifestyle of bacteria. In addition, they have to understand what effect their drugs might have on the human cells to avoid negative side effects.

However, only a few drugs with a truly novel mechanism of action were developed in the last years and superbugs resistant even against the last resort drugs are emerging and spreading across the globe.

In addition to drugs against traditional targets in bacteria, compounds targeting cell-to-cell communication described in one of the previous chapters show a good potential. Cell-to-cell communication plays an important role in the virulence of bacteria. Drugs interfering with cell-to-cell communication could therefore severely impact the ability of pathogenic bacteria to establish and maintain an infection (de Kievit and Iglewski 2000; Carlier et al. 2003; Park et al. 2003; Uroz et al. 2003; Juhas et al. 2005; Grandclément et al. 2016; Singh et al. 2017; Alexa Oniciuc et al. 2020; Hemmati et al. 2020).

Furthermore, to identify a novel antibiotic or test vast libraries of chemical compounds in order to find one with the best antimicrobial properties requires a lot of time and effort and highly skilled personnel. Tools provided by artificial intelligence and synthetic biology could make the drug discovery and development process more efficient.

Now that we have learned about multidrug-resistant bacteria, we will have a look at how synthetic biology can be used to build better bugs and how artificial intelligence can help in drug discovery in the next two chapters.

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7

Synthetic Biology in Microbiology

Abstract Synthetic biology is an emerging interdisciplinary research field, which brings engineering principles into biology. Objective of synthetic biology is to build novel biological systems and redesign the existing ones to make them useful to humankind. Progress in synthetic biology has been made possible thanks to a number of research and technology advances, most notably DNA sequencing, DNA synthesis, DNA assembly, genome transplantation, and CRISPR-Cas9 system. Synthetic biologists were capable of engineering only simple genetic circuits at first. But thanks to technology advances, they now aim to build whole synthetic organisms. Projects in this area include the first organism controlled solely by the chemically synthesized DNA called Synthia, the first synthetic eukaryotic microorganism Yeast 2.0 and the first minimal synthetic cell. Synthetic biology provides us with new tools, which can be used to improve our healthcare, agriculture, and other important areas. But the decision how to apply these tools is up to us.

As shown in the previous chapters, the world of microorganisms is very diverse. It encompasses a plethora of harmful microbes, such as multidrug-resistant bacteria. But it also includes beneficial microorganisms such as bacteria in guts that increase our immunity and protect us against other microorganisms.

Engineering microorganisms, which are useful to us, is one of the main goals of the emerging field of synthetic biology.

“What I cannot create, I do not understand,” said once the Nobel prize-winning physicist Richard Feynman.

His words took synthetic biologists close to their hearts.

Synthetic biology is an emerging interdisciplinary research field, which brings engineering principles into biology. Synthetic biology combines discoveries and methods of a wide variety of research areas, including bioengineering, microbiology, genetic engineering, molecular biology, computer sciences, and biophysics.

As an emerging field, synthetic biology is still only in the process of defining its own goals and potential practical applications. But the overarching main objective is to build novel biological systems and redesign the existing ones to make them useful to humankind.

Progress in synthetic biology has been accelerated thanks to a number of research and technology advances, most notably DNA sequencing, DNA synthesis, DNA assembly, genome transplantation, and CRISPR-Cas9 system.

DNA sequencing is used to read the letters of the genetic code, which is determined by the sequence of four letters called nucleotides or bases, namely adenine, cytosine, guanine, and thymine.

DNA synthesis allows writing of the genetic code by chemically synthesizing DNA from these four bases. Although DNA sequencing and synthesis technologies have been developed some time ago, they were not cheap. But the costs of DNA sequencing and synthesis have been decreasing rapidly and they are more affordable and efficient today than they were a few years ago. Such reliable, efficient, and cheap writing and reading of genetic code was one of the prerequisites of the recent explosion of advances in synthetic biology (Kosuri and Church 2014; Hughes and Ellington 2017).

The third technology fueling the progress in synthetic biology is DNA assembly.

This is because even though DNA synthesis has become cheaper and more efficient over the years, it is used usually for synthesis of shorter DNA fragments. DNA assembly is therefore required to stitch these shorter DNA fragments together to generate longer DNA pieces. DNA assembly can be used to generate even whole synthetic genomes.

Assembly of shorter DNA fragments is done in the test tube. But longer DNA fragments are difficult to handle in the test tube and are therefore usually assembled in the host cells by the process called homologous recombination. Homologous recombination can be used to assemble DNA fragments, which contain overlapping DNA sequences at their ends. These overlapping sequences can be designed by synthetic biology in the computer and then incorporated into DNA fragments during DNA synthesis. Most frequently used host cells for the assembly of large DNA are bacteria *Escherichia coli* and *Bacillus subtilis* and yeast *Saccharomyces cerevisiae* (Gibson et al. 2009;

Kuhlman and Cox 2010; Matzas et al. 2010; Blount et al. 2012; Merryman and Gibson 2012; Muller et al. 2012; Zhang et al. 2012; Jakočiūnas et al. 2015; Lin et al. 2015; Storch et al. 2015; Juhas and Ajioka 2016).

The fourth key technology of synthetic biology is genome transplantation. Once an entire synthetic genome has been assembled, it needs to be “booted up” to express its genes. This is achieved by transplanting synthetic genome into host cell. You can think of the synthetic genome as computer software and the host cell as hardware, which is needed to run it. Following successful transplantation, the transplanted synthetic genome is expressed by the translation machinery of the host cell and effectively takes it over. By expressing synthetic genes and translating them into proteins, which form cellular structures, the host cell becomes a different, synthetic cell (Lartigue et al. 2007; Lartigue et al. 2009; Gibson et al. 2010; Juhas 2015a, b).

Another technology frequently used by synthetic biologists is CRISPR-Cas9 system. Although it led to many practical applications, CRISPR-Cas9 was discovered in a basic science project aimed at investigating defense mechanisms of bacteria against invading viruses.

This is how CRISPR-Cas9 works.

When a bacterium encounters a virus, it stores part of viral DNA in its own chromosome. This location on the bacterial chromosome, which stores pieces of viral DNA, is called clustered regularly interspaced short palindromic repeats or CRISPR. Pieces of viral DNA from CRISPR are transcribed into RNA molecules and form a complex with Cas9 protein, which acts as molecular scissors. This complex of RNA and Cas9 is screening DNA in the cell until it encounters an invading virus whose DNA is complementary to RNA of the complex. RNA then binds to viral DNA and Cas9 cuts it.

Because CRISPR-Cas9 system is so good at locating and cutting specific DNA sequences it has been adopted by synthetic biologists.

Synthetic biologists can program CRISPR-Cas9 to cut specific genes at precise locations. What’s more, they can even insert new pieces of synthetic DNA into the cut location. This is possible because when the cell finds a cut in its DNA it attempts to repair it. If synthetic biologists add synthetic DNA into the cell, the cell repairs the cut by inserting synthetic DNA into the cut location.

Research on CRISPR gene editing technology was awarded a Nobel Prize in Chemistry in 2020 (Deltcheva et al. 2011; Jinek et al. 2012; O’Connell et al. 2014; Ledford and Callaway 2020; Al-Shayeb et al. 2022; Cofsky et al. 2022; Rubin et al. 2022).

Genes and their elements, such as promoters and terminators, are treated by synthetic biologists as construction bricks.

They have even been named Biobricks.

Biobricks, such as genes, promoters, and terminators, are standardized biological parts with well-known and quantified functions (Fig. 7.1). That allows their interchangeability between different biological systems and prediction what effect will a specific Biobrick have in a new system.

Promoters are DNA sequences located in front of the genes and are important for the start of the gene transcription. Terminators are not the killer robots from the movies, but different DNA sequences located at the ends of genes, which do exactly as their name suggests and terminate the gene transcription.

To design and engineer novel biological systems or redesign the existing ones, synthetic biologists are combining these Biobricks as if building a wall or a LEGO system. First, they design the desired biological system in the computer and then they build it in the laboratory (Shetty et al. 2008; Sleight et al. 2010; Venkatesh et al. 2015; Popp et al. 2017; Yamazaki et al. 2017; Wang et al. 2022).

Synthetic biologists were first capable of engineering only rather simple biological systems, referred to as genetic circuits (Torella et al. 2014; Barone et al. 2017; Yang et al. 2017; Ba et al. 2022).

Biobricks and Genetic circuits

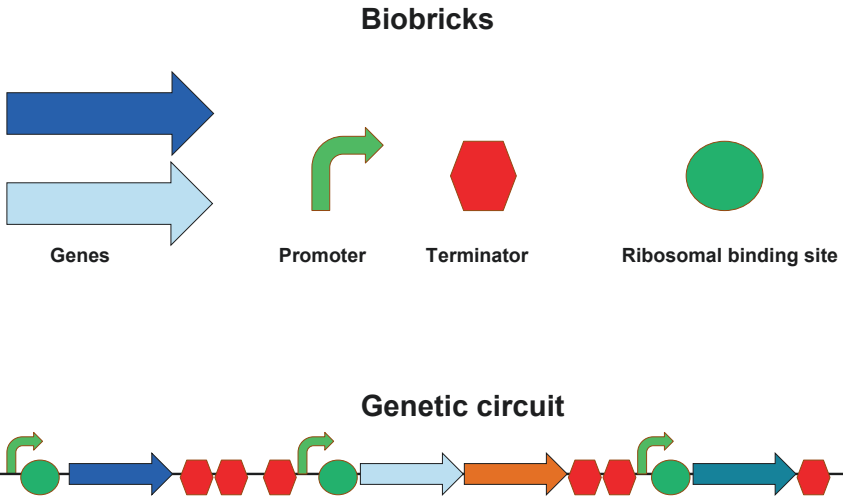


Fig. 7.1 Biobricks and genetic circuits. Biobricks are standardized biological parts, such as genes, promoters, terminators, and ribosomal binding sites, which are used by synthetic biologists to build genetic circuits

Genetic circuits are usually composed of only a few Biobricks. An example of a typical genetic circuit is a metabolic pathway in which genes, promoters, terminators, and other genetic elements were reshuffled to increase the yield and efficiency of the production of useful proteins, enzymes, or antimicrobials (Fig. 7.1).

But thanks to advances in DNA sequencing, synthesis, and assembly, in genome transplantation, and in DNA editing techniques, such as CRISPR-Cas9, the focus of synthetic biology has changed gradually over time.

Synthetic biologists now aim to build the whole synthetic organisms. What a few years ago might seem like science fiction has become a reality.

The first organism controlled solely by chemically synthesized DNA was built in 2010 by the researchers at the J. Craig Venter Institute.

It was a bacterium and considering its synthetic origin, was given an appropriately chosen name, Synthia (Fig. 7.2). In this pivotal experiment, the whole genome of one *Mycobacterium* species, *M. mycoides* was built from the basic chemical components. Shorter DNA fragments were chemically synthesized in the test tube from the four bases that make up the genetic code and the whole genome was assembled in yeast. Synthetic genome was then transplanted into the empty cell shell of different *Mycobacterium* species, *M. capricolum*. After the genome transplantation, the synthetic genome was booted up in the host cell, analogically to booting up a computer. The result of this experiment was a bacterium, which was controlled solely by the transplanted

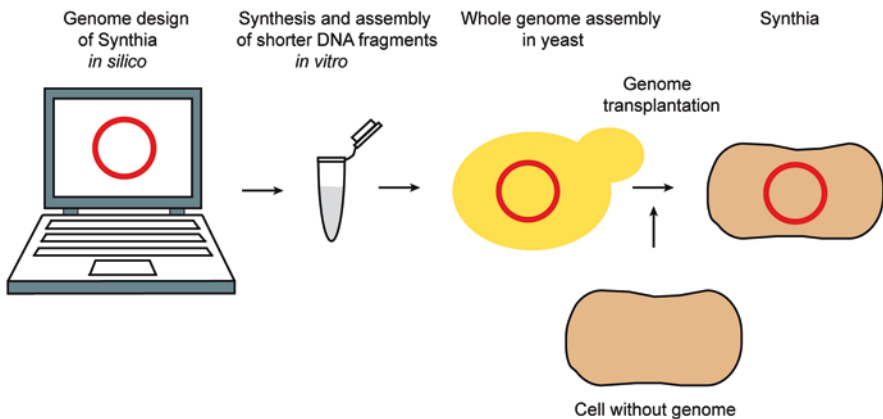


Fig. 7.2 Synthia: First organism controlled by a synthetic genome. Synthia's whole genome was designed in the computer (in silico), and chemically synthesized in the test tube (in vitro) and assembled in yeast (in vivo). Synthetic genome was then transplanted into a cell without genome, thus generating Synthia

synthetic genome and therefore resembling in every way the donor *M. mycoides* species (Fig. 7.2) (Gibson et al. 2010; Itaya 2010).

Building the first synthetic eukaryotic microorganism is the aim of another interesting synthetic biology project dubbed Yeast 2.0.

Yeast 2.0 project involves collaboration of many research laboratories worldwide and aims to redesign and build from scratch the whole genome of yeast, single-celled eukaryotic microorganism which is used to brew beer. This project seems more challenging than the one above as the genome of yeast is more than ten times bigger than that of Synthia.

What's more, synthetic biologists working on Yeast 2.0 are incorporating unique DNA sequences into the synthetic yeast genome, which can be used for rapid reorganization of the genome in the future. This system incorporated into the synthetic yeast genome called SCRaMbLE can be used for the directed evolution of the synthetic yeast by simply changing the growth conditions in the environment. By exposing it to different growth conditions, the SCRaMbLE system incorporated into the synthetic yeast genome can automatically, without human intervention, rearrange the genome in order to allow survival of synthetic yeast in a new environment (Dymond et al. 2011; Annaluru et al. 2014; Shen et al. 2016; Blount et al. 2018; Liu et al. 2018; Pretorius and Boeke 2018).

While some synthetic biologists are trying to build bigger and more complex synthetic organisms, others are aiming to do just the opposite. The goal of these synthetic biology projects is to build a truly minimal cell with the genome stripped to its bare essentials.

Minimal cell can be engineered using two different approaches. It can be built either by deleting all the non-essential genes and non-essential parts from the existing microorganisms. Or alternatively, thanks to the decreasing costs of DNA synthesis and novel and efficient methods of DNA assembly, it can be built from the scratch from the basic chemical components.

While methods, which could be used to engineer minimal cell have been developed, it is less than clear what exactly should be built. This is mainly because there is no complete agreement on which genes and cell components are really essential.

In 2016, researchers at the J. Craig Venter Institute, the same that built Synthia, did succeed in building the smallest synthetic free-living microorganism yet, dubbed JCVI-syn3.0 (Fig. 7.3).

To achieve that, they first disrupted genes of one *Mycobacterium* species, *M. mycoides*, to identify those genes, which were essential for the survival of this bacterium. New, minimized genome, which contained only genes identified as essential, was then synthesized from the scratch, transplanted and

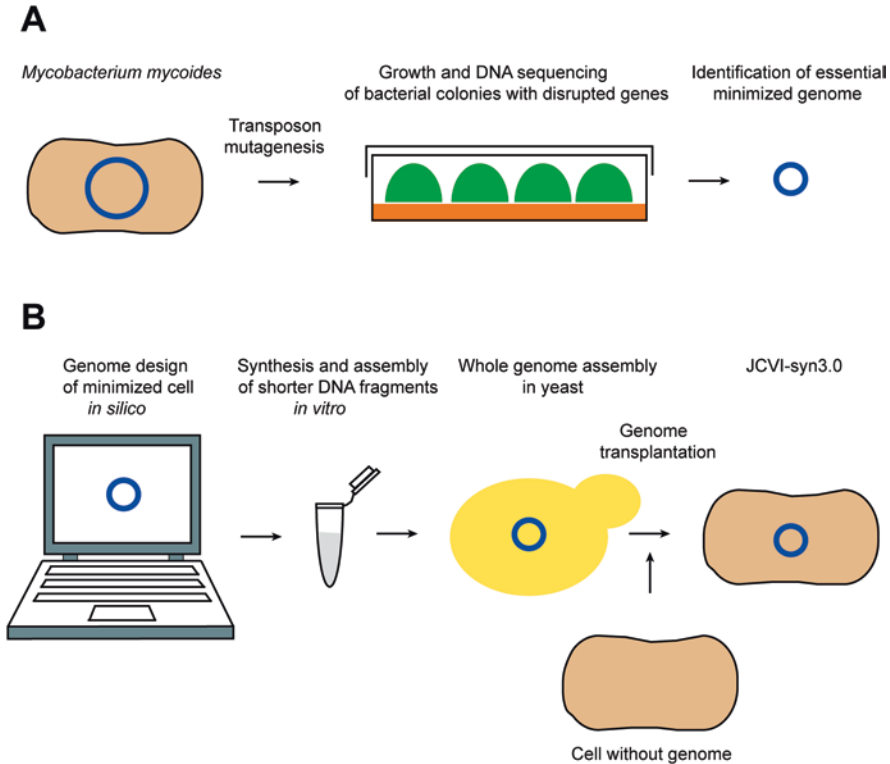


Fig. 7.3 JCVI-syn3.0: Minimized synthetic cell. (a) To identify genes essential for life, genes of *Mycobacterium mycoides* were first disrupted by transposon mutagenesis. If the bacterium grew with the disrupted specific gene, that gene was considered non-essential and deleted. (b) Minimized synthetic genome (without non-essential genes) was synthesized, assembled, and transplanted into a cell without genome, thus generating JCVI-syn3.0

booted up in the host cell. The resulting synthetic cell dubbed JCVI-syn3.0 has a genome of only approximately half of Synthia and harbors less than 500 genes (Fig. 7.3) (Hutchison et al. 2016; Venter et al. 2022).

But this might still not be a truly minimal cell.

JCVI-syn3.0 likely still harbors a set of *Mycobacterium* genes, which are essential for this bacterial species but not essential in general.

It is yet not clear whether such a thing as the universal minimal cell really exists. It is possible that the set of essential genes is rather quite specific for individual cell types and differs between microorganisms. Truly universal minimal cell composed of only the bare minimum of universally essential genes and parts will probably be built eventually, but it will require more time and research effort.

Efforts of synthetic biologists to build minimal cell contribute greatly to our understanding of the basic principles of life. They also help to clarify which genes are universally essential and which are essential only in specific environments and organisms (Juhas et al. 2011, 2012a, b; 2014; Juhas 2015a, b; Juhas and Ajioka 2016).

In addition to answering these fundamental biological questions, synthetic microorganisms and biological systems designed by synthetic biologists have also a plethora of practical applications.

In healthcare, engineered bacteria can be used to deliver drugs directly into tumors.

Traditional cancer treatment approaches such as chemotherapy are not very specific and have side effects. Tumors are also often resistant to traditional therapies.

This is where synthetic microorganisms come in. Bacteria can be engineered to specifically target tumors. Synthetic biologists can introduce synthetic circuits encoding cancer drugs into bacterial genomes, whose expression is triggered only by the specific conditions in tumors. This specificity makes bacterial cancer therapy more efficient and without side effects of the traditional approaches (Din et al. 2016; Wu et al. 2019; Gurbatri et al. 2022; Lim et al. 2022).

Synthetic biology can be used to engineer catabolic microorganisms.

Synthetic microorganisms with catabolic genes and whole synthetic catabolic pathways incorporated into their genomes can be used for degradation of toxic pollutants. These catabolic microbes can then be used, for instance, for terraforming planets with toxic environments to make them habitable by humans.

Besides terraforming other planets, catabolic microorganisms have a plethora of practical applications on Earth as we have also no shortage of toxic pollutants here.

Synthetic biology can also be used to engineer microorganisms, which can produce biomaterials, antimicrobial drugs, and vaccines. Renewable energy sources can be more efficiently obtained from the synthetic bugs engineered for the increased biofuel production. Biocomputing is another practical application of the synthetic microorganisms where the engineered microbes can be used as biological computers.

Synthetic biology has been recently used to engineer bacteria resistant to viruses.

This has been achieved by replacing some codons throughout the entire genome of *Escherichia coli* with synonymous codons. Codons encode the synthesis of amino acids, which make up the proteins. Removal of these codons

allowed also removal of some elements of the system, which translates genetic information into the structure of proteins.

Invading viruses use the same host cell translation machinery to make their own copies. Because some elements of this translation machinery were missing, the engineered cells were resistant against viruses.

Besides making virus-resistant cells, synthetic genetic information engineered in the same way has a number of advantages. For instance, it cannot escape into the natural environment because it is not compatible with natural life forms. This gives synthetic biologists an option to engineer biological systems, which can be better controlled and cannot escape from the laboratory (Dunkelmann et al. 2021; Robertson et al. 2021; Zürcher et al. 2022).

Similar approach can be used to engineer crops resistant to viruses.

Some argue that using genetically modified crops is irresponsible and might have far-reaching consequences for the environment. The counter-argument from the proponents of these techniques is that crops have been cultivated for thousands of years and in the process have been genetically modified anyway. They argue that it took a long time and now we have the technology to speed up the process.

We have to consider ethical and safety issues carefully and discuss how to use synthetic biology to transform our lives for the better.

Synthetic biology provides us with new tools, which can be used to improve our healthcare, agriculture, and many other important areas. But the decision how to apply these tools is up to us.

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8

Artificial Intelligence in Microbiology

Abstract When people hear the term artificial intelligence they often think about killer robots and supercomputer overlord. There are certainly risks associated with artificial intelligence, such as autonomous weapons and some features of social media. But artificial intelligence can do better than that. Artificial intelligence has already permeated many aspects of our lives, including internet, self-driving cars, and healthcare. In microbiology, artificial intelligence has also been successfully applied for a variety of tasks, including drug discovery and imaging and diagnostics of microorganisms. As with many previous inventions, artificial intelligence can be applied to do bad or good. It can free us from tedious and repetitive tasks, improve the quality of healthcare, and transform our lives for the better.

When people hear the term artificial intelligence they often think about killer robots and movies such as Terminator and Matrix where humans are hunted and enslaved by an intelligent supercomputer. Or they remember movies like Alien and 2001: A Space Odyssey, where the robot on the spaceship goes rogue and decides to give priority to the mission at the expense of lives of human crewmates.

But artificial intelligence can do better than that.

Don't take me wrong, there are certainly risks associated with artificial intelligence and I will come to them later. But reducing artificial intelligence to killer robots and supercomputer overlord is just not fair.

In fact, artificial intelligence is predicted to positively impact our lives more than any other research and technology advancement since the last Industrial Revolution.

There have been three Industrial Revolutions so far.

The First Industrial Revolution began in Great Britain in the eighteenth century. It was kick-started by technological innovations such as the steam engine, which greatly reduced the need for the power of human arms and replaced it with the power of machines. This had tremendous impact on society and on people's daily lives.

The Second Industrial Revolution from the late nineteenth century until the early twentieth century was augmented by inventions such as electrical power, telegraph, and telephone. As in previous Industrial Revolution, this led to profound changes in the way how people lived, worked, communicated, and spent their free time.

The Third Industrial Revolution began in the second half of the twentieth century. It was powered by inventions such as computers and digital cell phones, which became widely used by the general public. Internet, which most of us cannot imagine live without nowadays, was also invented during this period.

We live in the time of the Fourth Industrial Revolution. It is expected that it will lead to equally profound, if not greater, changes to our lives as the previous three Industrial Revolutions. And this time it is artificial intelligence, which is among the key research and technology advances fueling the fire of change.

Recent progress in the field of artificial intelligence has been accelerated thanks to the increasing computing power of modern computers and research on neural networks.

Increasing computing power is clearly visible all around us.

Long gone are times, when owning the Commodore 64 with a whopping 64 kilobytes (KB) of operational memory (RAM) and later the first Pentium-powered computer, I was the coolest kid on the block. Personal computers and mobile devices nowadays pack more computing power than the fastest supercomputers of that era. For illustration, most personal computers in use today have RAM of several gigabytes (GB), which is a unit million times larger than KB used in Commodore 64.

Groundwork for the second pillar of artificial intelligence, neural networks, has been laid out in the middle of the twentieth century.

In essence, neural networks are programs, which consist of a set of nodes wired together and resembling neurons in the human brain. Figures 8.1 and 8.2 show simplified views of a single artificial neuron and of the whole neural network, respectively.

Artificial neuron

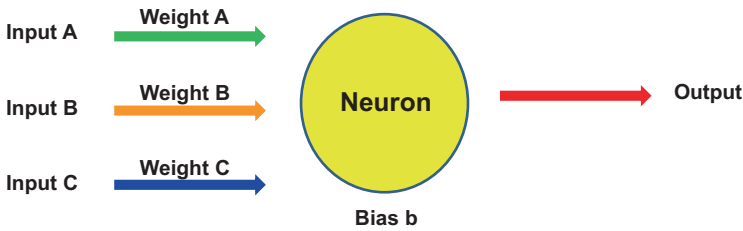


Fig. 8.1 Artificial neuron. Schematic view of artificial neuron. Output is calculated from the weights of each input and bias of the artificial neuron

Neural Network

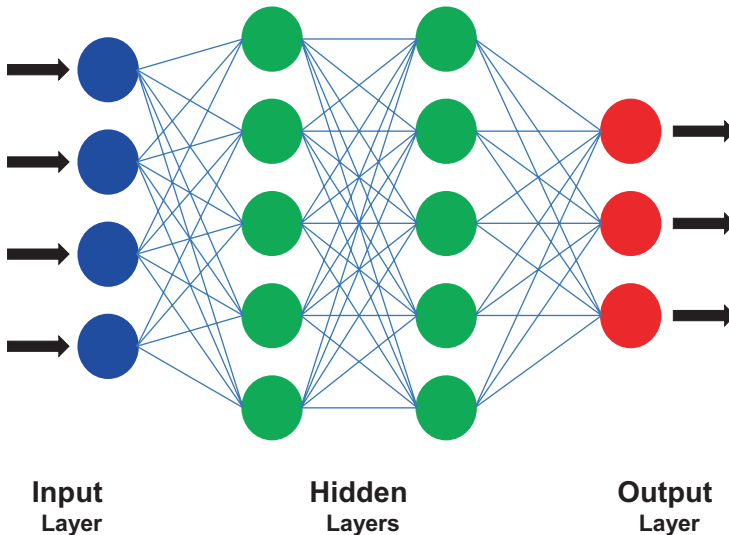


Fig. 8.2 Neural network. Schematic view of a neural network composed of input, hidden, and output layers of artificial neurons

Let's look first at individual artificial neurons. Into one end of this single-neuron system is fed the input information and each input has its own weight. Artificial neuron processes this information and based on the weights of each input and bias of the artificial neuron calculates the output (Fig. 8.1).

Neural networks are composed of layers of such artificial neurons, namely an input layer, one or more hidden layers, and an output layer. In neural networks the weights of connections between artificial neurons determine the final output (Fig. 8.2).

Neural networks have to learn from the big data fed to them to perform complicated tasks, such as predictions. Learning aspects of artificial intelligence are the focus of the subfields of artificial intelligence called machine learning and deep learning.

Learning methods of machine learning include supervised, unsupervised, and semisupervised learning (Botvinick et al. 2020; Choi et al. 2020a; Lo Vercio et al. 2020; Glielmo et al. 2021; Roscow et al. 2021; Eckardt et al. 2022).

Supervised learning, as the name already suggests, requires a lot of supervision and input. It also needs annotated data to train for specific tasks. As annotated data is often not easy to obtain, supervised learning requires trained personnel for data annotation.

Unsupervised learning doesn't require that much input and data annotation. It allows computers to detect patterns in datasets and to solve even such situations, for which they haven't been trained.

Semisupervised learning combines features of both supervised and unsupervised learning. It is most suitable for datasets, which harbor annotated as well as non-annotated data.

All these machine learning methods have their advantages and disadvantages, which makes them suitable for different tasks. Building better artificial intelligence systems might therefore require a combination of different learning approaches.

Machine learning is a subfield of artificial intelligence. Branch of machine learning called deep learning is particularly suitable for identification of patterns in large and high-dimensional datasets. For clarity and to make it less confusing to non-specialists without background in computer sciences, I will refer to machine learning and deep learning as artificial intelligence throughout this chapter.

Several notable advances have been made after the pioneering works on neural networks in the middle of the twentieth century (Fukushima 1980; Hopfield 1982; Fodor and Pylyshyn 1988; Eberhart and Dobbins 1990; McCulloch and Pitts 1990; Hinton et al. 1995; Hochreiter and Schmidhuber 1997; Wang and Terman 1995, 1997; Wang and Brown 1999; Hinton et al. 2006; Soman et al. 2018).

But progress in this area has been rather slow. Research on neural networks was even nearly abandoned for some time. One of the reasons for that was that computers at that time were lagging behind the theoretical research on neural networks.

Increasing computing power has led to an explosion of advances in artificial intelligence.

In 1997 IBM's supercomputer Deep Blue won a chess match against the reigning world chess champion Garry Kasparov.

In 2016 Google's DeepMind AlphaGo program powered by artificial intelligence won the board game Go against the world's best player Lee Sedol. Go, which was invented in China and is popular mainly in Asia, is a very complex game, much more complex than chess. To win it computer had to use what in human terms can be described as intuition.

In 2017 something arguably even more astounding happened. AlphaGo Zero, an updated version of AlphaGo program has beaten AlphaGo. This is interesting particularly because while AlphaGo was trained by learning from previous human matches, AlphaGo Zero was not given data from games played by humans. Provided only with the set of game's rules it trained by playing against itself and then won against AlphaGo 100 games to 0.

In 2018 Alpha Zero was developed employing generalized approach used previously in AlphaGo Zero and achieved superhuman performance in several games, namely in chess, shogi, and Go (Kasparov 2005; Silver et al. 2016, 2017, 2018).

And the progress in this area did not stop with games. Artificial intelligence has permeated many aspects of our lives.

When you go on YouTube, YouTube videos are recommended to you by artificial intelligence. When you search for something on Google, search results with the best matches on the top are organized by artificial intelligence. Articles in newsfeeds of social networks such as Facebook are recommended to you by artificial intelligence.

Artificial intelligence is driving autonomous mining trucks in mines and is increasingly used also in self-driving cars on our highways. It is predicted that artificial intelligence-powered personal robots will soon be as widespread as personal computers are nowadays.

In healthcare artificial intelligence saves doctors' time by freeing them from tedious and repetitive tasks, thus leaving them with more time for decision making and human approach to patients. This also allows doctors to treat more patients and consequently makes healthcare more effective and efficient.

A good example of the use of artificial intelligence in healthcare is personalized medicine. Artificial intelligence can be trained on large datasets, such as X-ray images, various test results, and genetic profiles of previous patients. Based on learned knowledge it can then predict and diagnose various diseases and recommend treatment, which is most likely going to work for each individual patient.

Artificial intelligence can recommend personalized treatment options for a variety of disorders, such as high blood pressure, diabetes, and cancer (Chaikijurajai et al. 2020; Subramanian et al. 2020).

Traditional cancer therapy often uses a similar approach for every patient and therefore might be not the most efficient in all cases. Artificial intelligence could recommend custom-made cancer treatment based on the genetic profile of each patient (Rafique et al. 2021; Esteva et al. 2022; Hwang et al. 2022).

Artificial intelligence has been successfully used for diagnosis and prediction of the best treatment options for breast cancer (Corti et al. 2022; Dang et al. 2022; Fong et al. 2022; Marinovich et al. 2022; Shamai et al. 2022; Taylor-Phillips et al. 2022; Wu et al. 2022).

Artificial intelligence has been also used for the diagnosis, treatment, and prognosis of lung cancer (Choi et al. 2022; Churchill et al. 2022; Hosny et al. 2022; Kim et al. 2022; Pei et al. 2022; Wang et al. 2022).

Artificial intelligence can be also used to identify genes and risk factors involved in diseases, which are caused by combinations of many genes and risk factors. Examples of such disorders, where artificial intelligence could play an important role include, for instance, Alzheimer's disease, heart conditions, schizophrenia, aging, and a wide spectrum of metabolic disorders (Wang et al. 2018; Wu et al. 2019; Li et al. 2020b; Ghazal et al. 2022; Rahman et al. 2022).

But this is just a fraction of artificial intelligence applications in healthcare. Artificial intelligence has been successfully used also in a number of other biomedical applications (Cao et al. 2018; Levine et al. 2019; Liu et al. 2019).

In microbiology, artificial intelligence has been also applied in a variety of tasks, including drug discovery and imaging and diagnostics of microorganisms (Riordon et al. 2019; Draz et al. 2020; Stokes et al. 2020).

Traditional drug discovery approaches take a lot of time and effort. Consequently, they often cannot keep up with the fast emerging pathogenic microorganisms and their novel mutations.

Compared to traditional methods, artificial intelligence can quickly sift through the large datasets of high-throughput test results and vast libraries of potential antimicrobial compounds and their biological and chemical features. Based on information learned from these datasets, artificial intelligence can highlight those drugs, which are likely to be most effective against specific diseases and microorganisms (Bender et al. 2007; Stephenson et al. 2019; Neves et al. 2020; Stokes et al. 2020; Zahradník et al. 2021).

Artificial intelligence has been used for the successful identification of drugs against a broad spectrum of microorganisms.

Example of a drug against bacteria identified by artificial intelligence is Halicin. It has been selected by artificial intelligence from the database, which contains more than 200 million compounds, as a promising drug against antibiotic-resistant bacteria. Laboratory tests confirmed antibacterial activity of Halicin against many antibiotic-resistant bacteria (Stokes et al. 2020; Zhang et al. 2021b).

Screening another database of nearly two million compounds, artificial intelligence selected several potential drugs against malaria with predicted strong activity and low cytotoxicity. Subsequent tests revealed that the selected compounds indeed have strong antimalarial activity and low cytotoxicity (Neves et al. 2020).

In addition to bacteria and parasites, artificial intelligence has also identified a number of potential antiviral drugs from millions of compounds in databases. These include, for instance, antiviral peptides and potential drugs against HIV (Andrianov et al. 2022).

Artificial intelligence has been also successfully employed to identify promising drugs against SARS-CoV-2.

Screening of vast databases of compounds by artificial intelligence has led to identification of a number of compounds with the potential to be used against SARS-CoV-2. It has also highlighted several drugs with known antiviral properties against other viruses, which can be re-purposed for use against SARS-CoV-2.

These compounds and drugs were selected by artificial intelligence either due to their known antiviral activity against other viruses, anti-inflammatory activity, and inhibition of viral replication or combination of these functions. Some were also selected for their potential to interact with SARS-CoV-2 proteins and host cell proteins, which are involved in the cell entry, such as ACE2 receptor and TMPRSS2 (Beck et al. 2020; Choi et al. 2020b; Nand et al. 2020; Ton et al. 2020; Zeng et al. 2020; Bai et al. 2021; Zahradník et al. 2021).

Despite many successes of artificial intelligence in drug discovery, some challenges still remain.

For instance, artificial intelligence-powered searches are often focused only on one type of data. They sift only through chemoinformatics data but do not associate it with clinical, molecular biology and cellular biology data or data obtained from microscopic images (Zhang et al. 2021b). Future artificial intelligence-based drug discovery searches could be therefore designed to combine these different forms of data in order to find the best possible candidates against specific pathogenic microorganisms.

Another challenge lies in the black box nature of artificial intelligence-powered systems.

We know what input was fed into the system and we know the output but what happened in between is not always perfectly clear. One of the challenges for future research in this area is therefore designing artificial intelligence-powered systems that are easier to understand. Better interpretability of the system's logic would be beneficial not only for general research, but also for design of more efficient and reliable drug discovery searches and for easier analysis of the potential drugs selected by artificial intelligence (Zhang et al. 2021b).

By speeding up the process of drug discovery, artificial intelligence can help stop the spread of pathogenic microorganisms.

Artificial intelligence has also been successfully applied for imaging and diagnostics of microorganisms.

Microbiology research generates huge quantities of microscopic images. These are used in clinical diagnostics for identification of pathogenic microorganisms. However, to generate high quality microscopic images and to analyze them requires trained personnel and a lot of time. It is a costly and challenging task, particularly in developing countries, which often do not have microscopes, let alone well-trained specialists who could operate them. Vast amount of accumulated microscopic images makes it difficult to analyze them even by traditional computational methods (Goldsmith and Miller 2009, Das et al. 2015, Stévenin and Enninga 2019, Li et al. 2020a, c, Zhang et al. 2021a).

This is where artificial intelligence comes in.

Artificial intelligence-powered microscopes can greatly enhance the speed and precision of diagnostics of different microorganisms.

Artificial intelligence has been used for identification of viruses in transmission electron microscopy images. Artificial intelligence in these studies detected human cytomegalovirus, feline calicivirus, adeno-associated virus, and human enterovirus 71 with better results than trained microbiologists (Devan et al. 2019; Matuszewski and Sintorn 2019; Xiao et al. 2020; Shaga Devan et al. 2021).

Artificial intelligence also showed good results in detection of different bacteria in microscopic images. Bacterial species successfully identified in these studies include *Escherichia coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Vibrio*, *Pseudomonas*, and many others. Notably, artificial intelligence in these studies was used to analyze not only standard microscopic images but also blood culture gram stains and 3D microscopic images (Zieliński et al. 2017; Hay and Parthasarathy 2018; Smith et al. 2018; Lugagne et al. 2020; Zhang et al. 2021a).

In addition to viruses and bacteria, artificial intelligence also showed good performance in identification of microscopic fungi, including yeast, in microscopic images. Interestingly, not only fungal cells were detected in these analyses by artificial intelligence but also different types of fungal spores, including those of *Aspergillus*, *Cladosporium*, and others (Tahir et al. 2018; Zieliński et al. 2020).

Artificial intelligence has been also used for the successful identification of different parasites in microscopic images. Parasites detected in these studies include *Plasmodium*, *Toxoplasma*, and *Babesia*.

Plasmodium, the causative agent of malaria, has been the subject of numerous artificial intelligence-based identification studies. *Plasmodium* was successfully detected with artificial intelligence-controlled application in blood smear microscopy images, including those analyzed by smartphones (Quan et al. 2020; Yang et al. 2020; Abdurahman et al. 2021; Kassim et al. 2021). Such smartphone-based identification of *Plasmodium* could solve the problem of insufficient number of well-trained microbiologists in developing countries.

Artificial intelligence systems in other studies used transferred knowledge about the specific morphologies and geometric shapes of these parasites to detect them in microscopic images. For instance, it is well known that *Plasmodium* looks like a ring under the microscope, while *Toxoplasma* and *Babesia* resemble banana and pear, respectively. Similar approach, based on geometrical features could be in the future applied also to other types of microorganisms, such as fungi, bacteria, and viruses (Jiang et al. 2020, Li et al. 2020a, c, Zhang et al. 2021a, 2022a, b).

Despite successful applications of artificial intelligence in imaging and detection of different types of microorganisms, some challenges still remain.

For instance, instead of standard microscopy images and transmission electron microscopy images used in the above studies, artificial intelligence could be also used for identification of microorganisms in fluorescence microscopy images.

Future artificial intelligence-powered microscopes and systems could be used for diagnostics of various types of microorganisms from different types of microscopic images and provide doctors with information about the disease development.

But with rapid advances in artificial intelligence come also risks.

Most obvious risks come from military applications. Although artificial intelligence is already used to some extent in military systems, real danger in this area comes from fully autonomous weapons. The decision to fire a weapon or drop a bomb from the artificial intelligence-controlled drone still comes

from a human operator. And we should ensure that this decision is not left to artificial intelligence in the future.

Less lethal danger but with far-reaching consequences is the improper use of artificial intelligence in social media. When not used correctly, artificial intelligence in social media can be exploited by politicians to steer masses in the desired direction. It can also isolate individuals into harmful social bubbles and trigger behavior and actions that might otherwise not happen.

Artificial intelligence, which is currently available, still fails sometimes. For instance, it can misidentify objects in an image. It is therefore better, for safety reasons, to keep the human in the loop in the decision-making process, particularly for cases when artificial intelligence is not sure about the best course of action.

In the process of discovery of new drugs against microbial diseases, artificial intelligence can quickly go through vast databases of chemical compounds to identify the most promising candidates. But safety checks and final approval of the drug for use are done by humans.

In diagnostics, artificial intelligence can quickly go through huge numbers of microscopic images and flag those, which require close attention from doctors.

Cooperation between humans and artificial intelligence therefore seems to be the best way forward. By cooperation, humans and artificial intelligence can augment each other and leverage their strengths to achieve the best overall outcome.

Artificial intelligence, which is currently used in many systems around us, is a narrow and very specialized artificial intelligence. It has mastered speech and language recognition, diagnosis, treatment, and prognosis of diseases and can even beat us at our most complex games. It can reach our level or even surpass it, but only in specific tasks.

In the next stage we will likely see the emergence and spread of artificial general intelligence.

Advantages of artificial general intelligence systems are numerous. For instance, currently used narrow artificial intelligence systems have to be developed by specialists and then extensively trained for specific tasks. Furthermore, they can then be used only for that task, which they were trained for and for nothing else.

Artificial general intelligence, on the other hand, can generalize and pursue new lines of thought. It is therefore adaptable to a broad spectrum of different tasks. Artificial general intelligence can process many different inputs, such as video, images, sound, and text, at the same time. It can be used even by non-specialists without education in computer sciences. Non-specialists can easily train and adapt it for specific tasks by feeding data into the system.

With repeated interactions with humans, artificial general intelligence would likely become smarter and incorporate more of our values into decisions.

In the next stage we could see the emergence of artificial super intelligence.

Although we are still not there yet, many scientists and technology experts are convinced that artificial super intelligence is coming. Unconstrained by biological limitations, once artificial intelligence reaches our levels, it will likely surpass us very fast. Therefore, we better make sure that it is aligned with our values.

As with many previous inventions, artificial intelligence can be applied to do bad or good things. It can free us from tedious and repetitive tasks, thus leaving us with more time to be creative. Artificial intelligence does not equal supercomputer overlord and killer robots. It can improve the quality of health-care and transform our lives for the better.

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9

Emerging and Zoonotic Diseases

Abstract Horizontal gene transfer and global climatic changes contribute to emergence, re-emergence, and spread of infectious diseases. Zoonotic diseases emerge when microorganisms break inter-species barriers and “jump” from their animal hosts to humans. Broad spectrum of microbes, including viruses, bacteria, and eukaryotic microorganisms, can cross inter-species barriers and infect humans. Zoonotic diseases include acquired immunodeficiency syndrome (AIDS), Ebola virus disease, Marburg virus disease, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), Zika virus disease, plague, anthrax, brucellosis, and toxoplasmosis.

As described in the previous chapters, microorganisms of different species can exchange genetic information by horizontal gene transfer. This gene exchange can lead to the emergence of more pathogenic microbes resistant to antibiotics.

In addition to emergence of novel superbugs, horizontal acquisition of virulence and resistance genes can also lead to re-emergence of old microbial threats. Microbes previously “tamed” by antibiotics and other therapeutics can return, augmented by new microbial weapons against our drugs.

Global climatic changes can also lead to emergence, reemergence, and spread of infectious diseases. Good examples are diseases transmitted by mosquitoes, such as Zika virus disease, malaria, and dengue fever. Because of global climatic changes, these mosquito-borne diseases are now occurring also in those parts of the world where they were not detected previously. Some studies indicate that more than 50% of all human pathogenic diseases known to date can be negatively affected and become more problematic due to

climate changes (Patz et al. 1996; Zell 2004; Dash et al. 2021; Mora et al. 2022; Neta et al. 2022; Semenza et al. 2022).

Novel diseases often emerge when microorganisms break inter-species barriers and “jump” from their animal hosts to humans. Microbes which can do that and the diseases which they cause are therefore called zoonotic (Fig. 9.1) (Jones et al. 2008; Morens et al. 2008; Young et al. 2014).

Zoonotic diseases form a big part of emerging infectious diseases. Crossing barriers between animals and humans by microbes was involved in the most serious outbreaks of infectious diseases in human history.

Due to the ongoing coronavirus disease 2019 (COVID-19) pandemic, this topic resonates strongly with the public so let’s look at the main zoonotic diseases and pathogens, which are causing them.

Acquired immunodeficiency syndrome (AIDS) is a chronic and potentially life-threatening disease caused by human immunodeficiency virus (HIV).

HIV belongs among the most widely known zoonotic pathogens due to its global spread and impact on human lives. Since the first cases of infections with HIV were reported in the 1980s, this virus claimed the lives of more than 30 million people worldwide.

Transmission routes of zoonotic pathogens

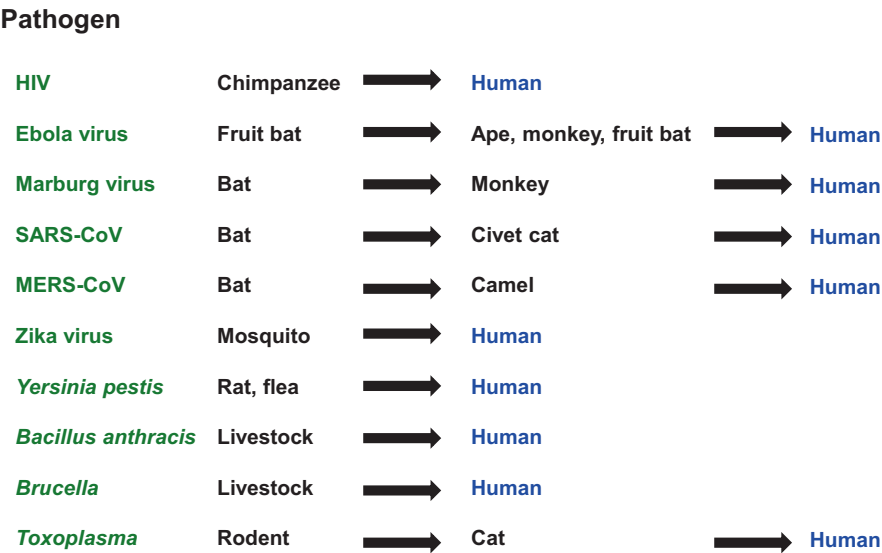


Fig. 9.1 Transmission routes of zoonotic pathogens. Figure shows transmission routes of the main zoonotic pathogens from their animal hosts to humans

Chimpanzees living in the jungles of Cameroon in Central Africa were the native hosts from which HIV jumped to humans (Fig. 9.1). HIV was initially thought to be harmless or causing only a mild illness in their native hosts; however, later research revealed that HIV can cause serious disease also in chimpanzees. How exactly HIV jumped from chimpanzees to humans is not entirely clear; however, it has been linked to bushmeat hunting of chimpanzees by humans.

After HIV crossed inter-species barriers and infected humans, viral genes were modified by adaptive mutations, which led to amino acid substitutions in key proteins. These adaptive modifications increased the ability of HIV to counteract the human immune system.

Immune system weakened by HIV leaves the door open for infections with opportunistic pathogens, which usually do not cause problems in the healthy people. Although a number of effective treatments have been developed over the years, the HIV pandemic is still ongoing (Van Heuverswyn and Peeters 2007; Wain et al. 2007; Sharp and Hahn 2010; Sharp et al. 2013; Deeks et al. 2021; Board et al. 2022; Ndashimye et al. 2022).

Ebola virus disease, caused by Ebola virus, is one of the deadliest infectious zoonotic diseases known to date.

Ebola virus disease is lethal in approximately 50% of the infected people. This virus was discovered in the jungles of the Democratic Republic of Congo in Central Africa and named after the river Ebola, which flows in the area.

Ebola virus probably jumps to humans via direct contact with apes, monkeys, or fruit bats, but the reservoir of virus are most likely fruit bats (Fig. 9.1). Bushmeat hunting and human encroachment on animal habitats played a key role in the emergence of Ebola virus in the Democratic Republic of Congo.

Like HIV, Ebola virus is also capable of human to human transmission once it crosses inter-species barriers between animals and humans. Specific adaptive amino acid substitutions in the Ebola virus protein likely contribute to increased human to human transmission rate; however, this will require further investigation.

Ebola virus infections affect the human immune system like HIV, but in a different way. Although some parts of the immune system are repressed by the virus, others are up-regulated. Infections with Ebola virus lead to hemorrhaging, damage of body tissues, and organ failure.

Most of the early Ebola virus outbreaks were limited only to remote areas of Central Africa. Some of the later outbreaks, such as the 2014–2016 West Africa outbreak, occurred in large urban areas in other parts of Africa but luckily did not spread globally (Leroy et al. 2004; Pourrut et al. 2007; Hayman et al. 2012; Gire et al. 2014; Dudas et al. 2017; Jacob et al. 2020).

Marburg virus disease is caused by Marburg virus, which belongs to the same class of viruses as Ebola virus.

The first infections with Marburg virus were detected in the 1960s in Germany and Yugoslavia and were associated with monkeys imported from Uganda. But similar to Ebola virus, the most likely reservoir of Marburg virus is bats (Fig. 9.1). Like Ebola virus, Marburg virus also has high mortality rates. Since the first recorder outbreak in the 1960s, several other outbreaks occurred with mortality rates ranging from 25% in modern hospitals to almost 90% in rural parts of Africa (Weidmann et al. 2007; Pourrut et al. 2009; Towner et al. 2009; Amman et al. 2012; Bozhanova et al. 2020; Guito et al. 2021; Cross et al. 2022; Koundouno et al. 2022).

Severe acute respiratory syndrome (SARS) is a viral respiratory disease caused by severe acute respiratory syndrome coronavirus (SARS-CoV).

SARS-CoV has emerged only quite recently. This zoonotic pathogen caused a large disease outbreak in 2002–2003, when it spread into nearly thirty countries around the world and infected more than 8000 people. Approximately 10% of SARS-CoV infections led to the death of the infected people.

Although the original reservoir host of SARS-CoV has not been yet conclusively determined, this virus most likely also originated in bats and crossed at least two inter-species barriers to infect humans. The first inter-species barrier crossed by SARS-CoV was that between bats and intermediate animal host, most likely a civet cat. The second barrier was that between this intermediate animal host and humans (Fig. 9.1).

Coronaviruses, which normally circulate in bats, are well known for their ability to mutate with relative ease. Due to their relatively high mutation rates, coronaviruses of bats have increased potential to cross inter-species barriers and to infect humans. Furthermore, coronaviruses closely related to SARS-CoV were identified in bats living in a remote cave. This study also suggested that similar disease can occur in the future if coronaviruses from bats cross inter-species barriers and jump to humans again (Li et al. 2005; Ge et al. 2013; Yang et al. 2015; Cyranoski 2017; Hu et al. 2017).

Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by Middle East respiratory syndrome coronavirus (MERS-CoV).

MERS-CoV is another zoonotic pathogen, which has jumped from animals to humans. The first outbreak of MERS was reported in Saudi Arabia in 2012. While the exact route of transmission is still debated, MERS-CoV probably jumped to humans from an intermediate animal host, which in this case was an infected dromedary camel (Fig. 9.1).

Latest studies indicate that besides SARS-CoV, bats are also the original reservoir hosts of MERS-CoV. MERS-CoV therefore likely also crossed two

inter-species barriers to infect humans. The first barrier was that between bats and camels and the second barrier was that between camels and humans (Fig. 9.1). MERS-CoV is capable of person to person transmission, thus after crossing inter-species barriers it could spread in the human population.

Although MERS-CoV infected less than one third of people, which were infected by SARS-CoV, infections were more lethal. More than 30% of the reported cases of MERS-CoV infections led to the deaths of the infected people (Breban et al. 2013; Kupferschmidt 2013; Anthony et al. 2017; Alnuqaydan et al. 2021).

Zika virus disease is caused by Zika virus, which is transmitted to humans usually by mosquitoes (Fig. 9.1).

The first cases of infection were identified in Africa in the 1940s but the virus has spread into different parts of the world since then. While the majority of cases occur in Americas and other endemic regions, infections transmitted by mosquitoes were reported also in Europe recently.

Zika virus can cause potentially life-threatening illness in adults, but the main danger lies in its ability to cause serious complications during pregnancy (de Araújo et al. 2018; Cardona-Ospina et al. 2019; Counotte et al. 2019; Leonhard et al. 2020; Halani et al. 2021).

All zoonotic diseases above are caused by viruses, but other microorganisms can also cross inter-species barriers and cause serious infections in humans (Fig. 9.1).

The plague, one of the best known zoonotic diseases, is caused by a bacterium *Yersinia pestis*.

Pandemic of plague called the Black Death, which ravaged Europe and Asia in the fourteenth century, caused the deaths of millions of people. As a consequence of the Black Death pandemic, the population of Europe and Asia dropped by approximately 50%.

The causative agent of plague, bacterium *Y. pestis*, typically resides in small animals such as rats and their fleas. But it is able to cross inter-species barriers and infect humans and then transfer from person to person (Fig. 9.1).

During the Black Death, the bacterium causing this disease was completely unknown. In fact, none of the microbes were known at that time. Antonie van Leeuwenhoek constructed microscopes, which allowed him to observe first “animalcules” a whole three centuries later. To make the matter worse, there was no effective treatment available against the Black Death in the fourteenth century. Antibiotics were discovered only at the beginning of the twentieth century.

Luckily, progress in microbiology led to the discovery of a microorganism causing this disease and to efficient treatments. Although cases of plague still

appear occasionally across the globe each year, *Y. pestis* infections can be treated with modern antibiotics. However, as discussed in the previous chapters, we should not take our ability to treat microbial infections with the available antibiotics for granted (Pechous et al. 2016; Demeure et al. 2019; Rosenzweig et al. 2021; Izdebski et al. 2022; Klunk et al. 2022; Spyrou et al. 2022).

Anthrax is a zoonotic disease caused by bacterium *Bacillus anthracis*.

B. anthracis infects mainly livestock, such as cattle, goat, and sheep, but it can cross inter-species barriers and jump into the human population (Fig. 9.1). *B. anthracis* transmissions from person to person are very rare and people usually get sick by close contact with an infected animal or with the contaminated animal products. *B. anthracis* infections can cause serious complications.

B. anthracis is also causing headaches due to its ability to form tough spores, which are highly resistant against harsh environments and treatment. Spores are therefore hard to eradicate and can survive in the environment without losing their pathogenicity and ability to cause infection for years.

Unfortunately, these features combined with the high mortality rates made *B. anthracis* one of the prime subjects of the biological weapons research programs of countries around the world. Luckily, biological weapons are now banned (Leendertz et al. 2004; Katharios-Lanwermyer et al. 2016; Pilo and Frey 2018).

Brucellosis is a zoonotic disease caused by different species of *Brucella* bacterium.

Brucella typically infects animals but can occasionally cross inter-species barriers and infect humans (Fig. 9.1). Person to person transmissions of *Brucella* are very rare. People usually get infected by close contact with an infected animal or alternatively by eating or drinking contaminated animal products, such as unpasteurized milk (Amjadi et al. 2019; Ghanbari et al. 2020; Heidary et al. 2022).

Toxoplasmosis is a zoonotic disease which is caused neither by viruses nor by bacteria but by a eukaryotic parasitic microorganism *Toxoplasma gondii*.

T. gondii can also cross inter-species barriers and infect humans (Fig. 9.1). What's more, studies indicate that crossing of inter-species barriers by *T. gondii* is occurring quite often. It has been estimated that in some countries more than 50% of the population are infected. Luckily, a vast majority of those infected do not show any serious symptoms. However, *T. gondii* can cause serious health complications in unborn children and in people with weakened immune system (Durieux et al. 2022; Milne et al. 2022).

As the above examples illustrate, a broad spectrum of microbes can cross inter-species barriers and cause serious infections in humans. Viruses, bacteria, and eukaryotic microorganisms can jump from animals to the human population and cause disease (Fig. 9.1). Horizontal transfer of genes between microbes and global climatic changes also contribute to emergence of novel superbugs and re-emergence of old microbial threats.

Now that we have learned about the emerging and zoonotic diseases, we will have a look at COVID-19 in the next chapter.

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10

COVID-19

Abstract The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become the largest public health emergency of the past decades. This chapter discusses some of the most interesting topics related to SARS-CoV-2, including its origin, how it infects human cells, detection of SARS-CoV-2 mutations, biosensors, aptamers, and treatments.

The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become the largest public health emergency of the past decades. As of December 2022, more than 650 million cases of infections have been detected worldwide and more than 6.5 million people have died as a result of infection with SARS-CoV-2.

At the time of writing this book some unknowns still remain, but many questions about COVID-19 have already been addressed in a number of studies by different laboratories around the world.

One of the topics drawing the attention of researchers is the origin of SARS-CoV-2.

Based on the recent articles published in the most respected peer-reviewed scientific journals, SARS-CoV-2 is another member of the large group of zoonotic pathogens, which originated in bats. As we have learned in the previous chapter, zoonotic pathogens can cross inter-species barriers and can be transmitted between animals and humans.

The exact transmission route of SARS-CoV-2 through the inter-species barriers is currently still debated in the scientific community. But most scientists believe that the reservoir hosts of SARS-CoV-2 are bats.

Bats are also the suspected hosts of the related coronavirus, SARS-CoV, which caused the large disease outbreak in 2002–2003. Coronaviruses closely related to SARS-CoV were identified in bats and it has been predicted that similar disease outbreak can occur if bat coronaviruses cross inter-species barriers and jump from bats to humans (Li et al. 2005; Ge et al. 2013; Yang et al. 2015; Cyranoski 2017; Hu et al. 2017).

SARS-CoV-2 most likely jumped from bats to humans via a second, intermediate animal host. In the previous epidemics caused by SARS-CoV and MERS-CoV, these intermediate animal hosts were likely civet cats and dromedary camels.

Latest findings indicate that in the case of SARS-CoV-2 this intermediate animal host was most likely a pangolin or a raccoon dog.

To avoid similar pandemics in the future the exact transmission route of SARS-CoV-2 across inter-species barriers from bat via pangolin, raccoon dog or another intermediate animal host to human will require further analyses (Andersen et al. 2020; Holmes et al. 2021; Lytras et al. 2021; Gupta et al. 2022; Shi et al. 2022; Worobey et al. 2022).

Another interesting topic deals with how SARS-CoV-2 infects human cells.

Numerous research analyses showed that SARS-CoV-2 enters human cells by binding to specific receptor protein located on the cell's surface. This receptor is called angiotensin-converting enzyme (ACE2).

SARS-CoV-2 is an RNA virus with four structural proteins called nucleocapsid (N) protein, membrane (M) protein, envelope (E) protein, and spike (S) protein (Fig. 10.1). SARS-CoV-2 uses one of its proteins, S protein, to bind to ACE2 and to mediate attachment and fusion with the host cell. This generates an opening in the host cell's membrane through which viral genome can enter (Fig. 10.2) (Lan et al. 2020; Shang et al. 2020; Walls et al. 2020; Wang et al. 2020; Jackson et al. 2022).

One of the concerns was that SARS-CoV-2 might mutate.

Because mutations are quite common in viruses, SARS-CoV-2 mutations were actually expected by researchers. More concerning was therefore that SARS-CoV-2 might mutate in a way that would make it more lethal, transmissible, and resistant against vaccines and antibodies at the same time.

SARS-CoV-2

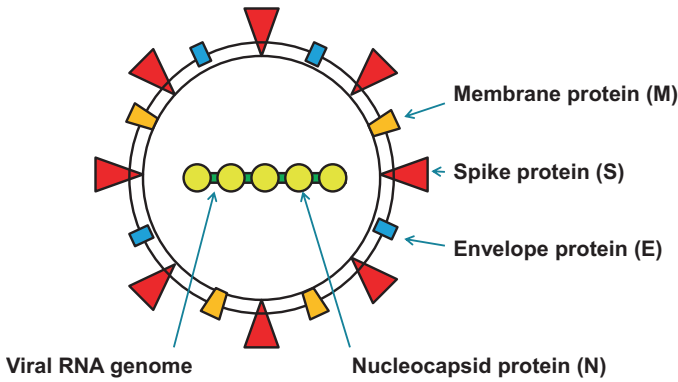


Fig. 10.1 SARS-CoV-2. SARS-CoV-2 is an RNA virus composed of four structural proteins: nucleocapsid (N) protein, membrane (M) protein, envelope (E) protein, and spike (S) protein

SARS-CoV-2 entry into cells

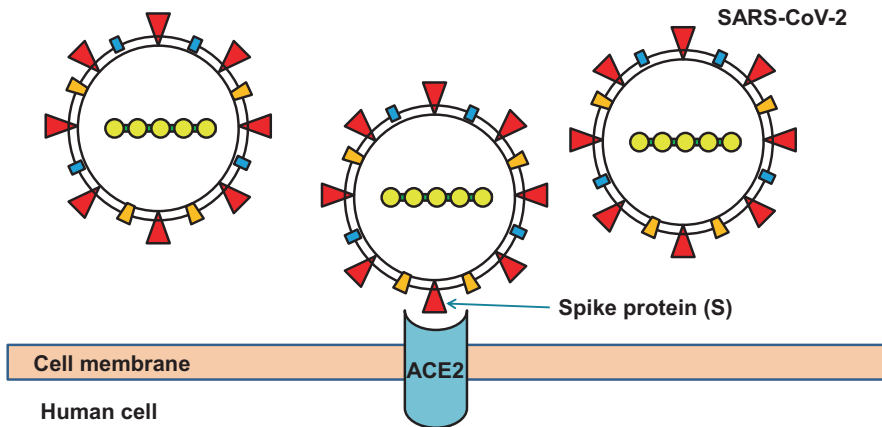


Fig. 10.2 SARS-CoV-2 entry into cells. SARS-CoV-2 enters cells by binding to the receptor protein called angiotensin-converting enzyme (ACE2) on the cell's surface

Luckily, SARS-CoV-2 has an enzyme, which proofreads its own genetic information and that results in overall fewer mutations compared to some other viruses. Despite the terribly high death toll of SARS-CoV-2, many scientists believe that we were quite lucky that the pandemic was caused by this type of virus. If it was caused by a virus without proofreading activity, there would most likely have been many more deaths.

But a number of concerning SARS-CoV-2 mutations occurred anyway.

Majority of SARS-CoV-2 mutations were silent and did not cause any problems. Most problematic mutations were predicted to be those which affect S protein. Due to the importance of S protein for viral entry, S protein is the target of many vaccines and antibodies. It was therefore predicted that mutations of S protein might affect efficiency of vaccines and treatments (Grubaugh et al. 2020; Korber et al. 2020; Li et al. 2020b; Zhang et al. 2020b; Faria et al. 2021; Leung et al. 2021; Meng et al. 2021; Thomson et al. 2021).

SARS-CoV-2 and its mutants can be detected by a number of methods.

However, traditional methods are often time-consuming, costly, and require well-trained professionals. Furthermore, they often suffer from the lack of specificity for emerging SARS-CoV-2 mutants. To overcome these limitations, SARS-CoV-2 and its mutants could be detected by biosensors.

Biosensors have been used previously to detect a broad spectrum of viruses, including HIV and Zika virus. Some progress has been achieved also in the application of biosensors for the detection of SARS-CoV-2. In the future, biosensor technology can be used for the fast and reliable detection of multiple SARS-CoV-2 mutants simultaneously (Gerasimov and Lai 2010; Gootenberg et al. 2018; Michelson et al. 2019; Broughton et al. 2020; Xi et al. 2020; Fozouni et al. 2021; Zhang et al. 2021).

A promising novel approach for detection and inhibition of SARS-CoV-2 and its mutants involves aptamer-based technologies.

Aptamers are short single-stranded nucleic acids, which can bind to their targets with high specificity by folding into a 3D structure. They have been used previously for detection and inhibition of a number of other viruses, including HIV, MERS-CoV, and SARS-CoV.

Aptamers specific to S protein and N protein of SARS-CoV-2 have also been developed. Some of these aptamers are universal and can recognize S protein of different SARS-CoV-2 variants, including Alpha, Beta, Gamma, Epsilon, Delta, and Omicron. Aptamers can be potentially applied in biosensors for the detection of SARS-CoV-2 and its mutants (Minunni et al. 2004; Tombelli et al. 2005; Cho et al. 2011; Li et al. 2020a, 2021; Song et al. 2020; Sun et al. 2021a; Zhang et al. 2020a, b).

Furthermore, aptamers with the potential to inhibit SARS-CoV-2 have been also developed. Some of them act by binding to S protein of SARS-CoV-2. This blocks binding of S protein to ACE2 receptor on the surface of host cells, thus preventing infection. Aptamers can be therefore potentially also used as therapeutics against SARS-CoV-2 (Alves Ferreira-Bravo and DeStefano 2021; Sun et al. 2021b, 2022; Valero et al. 2021; Zhang et al. 2022a).

COVID-19 pandemic has also led to fast development and approval of vaccines against SARS-CoV-2.

Vaccines stimulate cells involved in adaptive immunity to better deal with the same pathogens in the future. Different types of vaccines were developed against SARS-CoV-2, ranging from those more traditional to modern DNA and mRNA vaccines (Iwasaki and Omer 2020; Krammer et al. 2021, 2022; Lucas et al. 2021; Payne et al. 2021; Wang et al. 2021; Goel et al. 2022; Hayashi et al. 2022; Lee et al. 2022; Qu et al. 2022; Tauzin et al. 2022).

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11

Future Pandemics

Abstract This chapter discusses the main reasons why outbreaks of infectious diseases, similar to or worse than the ongoing COVID-19 pandemic, will likely happen in the future. Zoonotic diseases often emerge when people encroach on natural animal habitats, take wild animals from their natural habitats, sell them on markets, or hunt them for meat. Horizontal gene transfer leads to acquisition of virulence and antibiotic resistance genes by microbes. Global climatic changes also play an important role in the emergence and spread of infectious diseases, including malaria, dengue, and Zika virus disease.

The outbreaks of infectious diseases, similar to or worse than the ongoing COVID-19 pandemic, will likely happen in the future.

Here are some of the main reasons why (Fig. 11.1).

As shown in the previous chapters, a wide variety of microorganisms, which normally reside in animals, can infect humans. SARS-CoV-2 virus, which caused COVID-19 pandemic, is, based on recent articles published in the most respected scientific journals, another from the large group of zoonotic pathogens.

Zoonotic pathogens are considered to be the main cause of emerging infectious diseases. It has been estimated that approximately 75% of emerging infections are caused by zoonotic pathogens.

Zoonotic microorganisms can break inter-species barriers and jump from their animal hosts into the human population, which can lead to emergence of novel infectious diseases. Crossing of inter-species barriers by zoonotic pathogens was responsible for a number of deadly pandemics in the course of



Fig. 11.1 Future pandemics. Figure shows the main reasons why pandemics, similar to or worse than COVID-19 pandemic, will likely happen in the future

human history (Taylor et al. 2001; Jones et al. 2008; Morens et al. 2008; Young et al. 2014; Schmeller et al. 2020; Brindle et al. 2022).

Bats are the suspected reservoir hosts of not only SARS-CoV-2 but also of the related coronavirus, SARS-CoV, which caused a large disease outbreak in 2002–2003.

A number of other coronaviruses are circulating in the bats. Genes of these coronaviruses often mix together by passing from one bat host to another, which might give rise to a virus with the increased ability to cause serious infections in humans. The possibility of emergence of another coronavirus similar to SARS-CoV from the bat reservoir has even been predicted several years ago.

In this analysis researchers collected samples from bats living in a remote cave and analyzed the whole genomes of coronaviruses identified in those samples. They found that genomes of different coronavirus strains from that particular cave contained all the genetic components, which were present also in SARS-CoV.

Considering the frequent genetic recombination between coronavirus strains, they suggested that SARS-CoV has emerged as one of the genetic combinations of such genetic mixing of coronaviruses in their bat hosts. Authors of this study also highlighted the risk of emergence of similar SARS-like diseases arising from genetic recombination between bat coronaviruses in the future (Li et al. 2005; Ge et al. 2013; Yang et al. 2015; Cyranoski 2017; Hu et al. 2017).

Based on this and other studies we know that there are a number of coronavirus strains circulating in bats, they recombine frequently, and they might give rise to other viruses with the potential to cause worldwide pandemic.

Zoonotic diseases also often emerge when people encroach on natural animal habitats, take wild animals from their habitats, sell them on markets, or hunt them for meat (Fig. 11.1).

Infections with Ebola virus, one of the most lethal zoonotic viruses known to date, usually occur in the remote forested regions of Africa when people come into close contact with the wild animals living there.

Marburg virus, which belongs to the same class of viruses as Ebola virus and also has high mortality rates, was first detected in monkeys imported from Uganda.

The first known cases of infections with human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), are associated with the bushmeat hunting of chimpanzees by humans.

Civet cats from wildlife markets were the likely intermediate hosts in the transmission route of SARS-CoV to humans. Furthermore, wildlife markets are also linked to the first recorded cases of SARS-CoV-2 (Lau et al. 2005; Wang and Eaton 2007; Karesh et al. 2012; Dudas et al. 2017; Wilkinson et al. 2018; Jacob et al. 2020; Deeks et al. 2021; Holmes et al. 2021; Lytras et al. 2021; Cross et al. 2022; Gupta et al. 2022; Koundouno et al. 2022; Shi et al. 2022; Worobey et al. 2022).

Human encroachment on habitats of wild animals and practices shown above does not seem to have a declining tendency. Such frequent contact with wild animals and microorganisms they carry also increases the risk of pandemics in the future.

Global climatic changes also play an important role in the emergence and spread of infectious diseases (Fig. 11.1).

Let's take malaria as an example. Malaria is a serious disease, which can sometimes lead to death of the infected person. It is caused by a parasitic eukaryotic microorganism called *Plasmodium*. People get infected with *Plasmodium* through mosquito bites.

Mosquitoes spreading *Plasmodium* parasites have been traditionally found mainly in tropical and subtropical areas. Consequently, these areas used to be also endemic for malaria. This is because climates with higher temperature are important for the life cycles of both *Plasmodium* and mosquitoes that spread it.

Global warming therefore contributes greatly to the increasing number of malaria cases in other parts of the world. Malaria cases have occurred in several European countries, including Germany, the Netherlands, and the UK (Krüger et al. 2001; Arends et al. 2013; Fischer et al. 2020).

Dengue is another disease spread by mosquitoes, which are found mainly in tropical and subtropical regions. In some cases, this disease can lead to dengue fever and dengue shock syndrome and death of the infected person. Unlike malaria, dengue is not caused by a eukaryotic microbe but by a virus. Thanks to global warming, cases of dengue have been detected in regions,

which were previously not endemic for the disease, like malaria (Struchiner et al. 2015; Jia et al. 2017).

The same goes for Zika virus disease, which is also spread by mosquitoes.

Thanks to global warming, these mosquitoes and consequently also the diseases they spread have been occurring recently also in areas where they have not been detected before (Patz et al. 1996; Zell 2004; Dash et al. 2021; Mora et al. 2022; Neta et al. 2022; Semenza et al. 2022).

Horizontal transfer of genes is another reason for emerging diseases.

Microorganisms are constantly evolving and adapting to their environment. Genetic code of microbes undergoes frequent adaptive mutations. Furthermore, as shown in one of the previous chapters, microorganisms can mix their genetic information by horizontal gene transfer. This gene transfer can lead to acquisition of virulence and antibiotic resistance genes and consequently to emergence of more pathogenic and resistant microbes (Fig. 11.1).

Horizontal gene transfer contributes not only to emergence of novel microbial diseases, but can also lead to reemergence of those, which have been eradicated or do not pose a critical global threat nowadays. For instance, plague, which once killed approximately half of the population of Europe and Asia in the fourteenth century, is not considered an acute threat to public health these days. Bacterium *Yersinia pestis*, which is causing plague, can be treated with antibiotics. However, this might change in the future if this bacterium becomes resistant to antibiotics.

Furthermore, microbes resistant to a number of antibiotics or all available antibiotics simultaneously are emerging and spreading across the globe. Therefore, we might soon be back to the pre-antibiotic era when any cut could lead to life-threatening blood poisoning and death or when any operation could end in a fatal infection.

Antibiotic-resistant microbes are emerging and spreading fast, while the discovery of novel drugs is a lengthy and costly process. Consequently, there have not been many antibiotics developed in the last years with the novel mechanism of action.

Probability of infectious diseases outbreaks caused by resistant bacteria in the future is therefore also increasing.

As mentioned at the beginning of this chapter, pandemics similar to or worse than COVID-19 will likely happen in the future.

Almost precisely hundred years have passed between the currently ongoing COVID-19 pandemic and the deadly Spanish flu pandemic in 1918. But that is no guarantee that the next pandemic will wait until the next century. After all, there were numerous recent outbreaks. These included, for instance,

SARS-Co-V epidemic in 2002–2003 and the recurrent Ebola virus outbreaks in Africa.

Other viruses, potentially more dangerous than SARS-CoV-2, are waiting for their chance. Microorganisms are constantly evolving and adapting to their environment by mutations and acquisition of pathogenic and antibiotic resistance genes. Emerging multidrug-resistant bacteria, resistant even against the last resort antibiotics, are spreading across the globe.

It's just a matter of time until another deadly disease starts to spread globally. If nothing changes, the next large disease outbreak is not a question of if, but when it happens.

Now that we know that pandemics will likely happen in the future, we will have a look at what we can do to make our future brighter in the next chapter.

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12

Into a Brighter Future

Abstract Limiting certain practices, such as human encroachment on animal habitats, markets selling wild animals and bushmeat hunting can reduce the risk of emerging zoonotic diseases. Dealing with global climatic changes would also reduce the risk of disease outbreaks. Giving more emphasis on the discovery of novel antibiotics would provide us with more tools against resistant superbugs. Antimicrobial drugs blocking quorum sensing and artificial intelligence can be also used against emerging pathogens. Dealing with pandemics and other important issues would benefit from global cooperation, strong public health infrastructure, and reliable and efficient systems for early prediction, detection, and monitoring of emerging pathogens. To stop gambling with our future, we should establish permanent human presence on other planets.

As discussed in the previous chapter, due to frequent contact with wild animals and zoonotic pathogens they might carry, global climatic changes, and the spread of antibiotic resistance, there is a high risk of future pandemics.

Analysis of the transmission routes of zoonotic pathogens indicates that many previous outbreaks could have been prevented by limiting certain practices, such as human encroachment on animal habitats, wild animal markets, and bushmeat hunting (Lau et al. 2005; Wang and Eaton 2007; Karesh et al. 2012; Dudas et al. 2017; Wilkinson et al. 2018; Jacob et al. 2020; Deeks, Archin et al. 2021; Holmes et al. 2021; Lytras et al. 2021; Cross et al. 2022; Gupta et al. 2022; Koundouno et al. 2022; Shi et al. 2022; Worobey et al. 2022).

Limiting these practices would lead to less frequent contact with wild animals. Consequently, it would result in less frequent exposure to zoonotic pathogens circulating in wild animals and reduce the risk of jumping of zoonotic pathogens from animals to humans (Fig. 12.1).

The same goes for global climatic changes, which also contribute to outbreaks of infectious diseases, for instance, by aiding the spread of mosquito-borne pathogens (Patz et al. 1996; Zell 2004; Dash et al. 2021; Mora et al. 2022; Neta et al. 2022; Semenza et al. 2022).

Steps toward solving the problem of global climatic changes would also reduce the risk of disease outbreaks (Fig. 12.1).

Antibiotics still remain a powerful tool against diseases caused by pathogenic bacteria, but this might change in the future.

Microorganisms are constantly evolving and adapting to their environment by mutations and acquisition of genes, including those encoding resistances to antibiotics (Juhas 2015; Juhas et al. 2007, 2009, 2019; Evans et al. 2020; Matlock et al. 2021).

Giving more emphasis on the discovery and development of novel antimicrobial drugs would therefore provide us with more tools against antibiotic-resistant superbugs (Fig. 12.1).

Besides traditional drugs and drug targets, antimicrobials with novel mechanisms of action should be developed (Fig. 12.1).

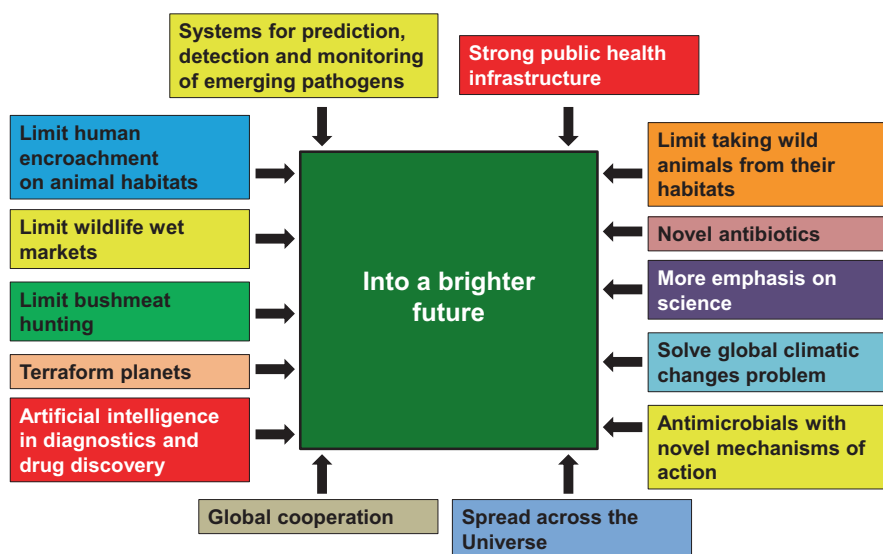


Fig. 12.1 Into a brighter future. Figure shows what steps can be done to reduce the probability of pandemics and to make our future brighter

Quorum sensing described in one of the previous chapters plays an important role in pathogenicity of microbes. Development of novel antimicrobial drugs blocking communication between microorganisms is therefore a promising strategy against microbial pathogens (de Kievit and Iglewski 2000; Juhas et al. 2005; Grandclément et al. 2016; Hemmati et al. 2020).

Artificial intelligence is another promising tool against pathogenic microorganisms.

Besides applications in various areas of biomedicine, artificial intelligence has also been successfully applied in drug discovery. Because traditional drug discovery approaches take a lot of time and effort, they often cannot keep up with the fast emerging pathogens.

Artificial intelligence can significantly speed up the drug discovery process by analyzing large datasets and libraries of chemical compounds and select those potential drugs, which should be given more attention by the researchers (Bender et al. 2007; Stephenson et al. 2019; Neves et al. 2020; Stokes et al. 2020; Zhang et al. 2021).

Artificial intelligence-powered computer systems can be used not only for the prioritization of potential antimicrobial drugs but also for early detection of pathogens and identification and monitoring of other life-threatening diseases, such as cancer. With the advances in artificial intelligence and increased computational capacity, deep learning systems can “watch over” patients and advise the healthcare personnel about the best course of treatment (Fig. 12.1).

Dealing successfully with pandemics and other important issues in our interconnected world would benefit from global cooperation and strong public health infrastructure. It would also help to have reliable and efficient systems for early prediction, detection, and monitoring of emerging pathogens, to prevent overwhelming of the future hospitals.

The importance of science should be emphasized (Fig. 12.1). Without advances in microbiology we would be blind in the fight against the pathogenic microorganisms. Not only would we not have antibiotics and vaccines to treat infectious diseases, but we would not even know what is causing them.

Thanks to science we have a fighting chance.

But other research fields should be also given more attention as the discoveries in one research area usually fuel the advancements in another.

I will finish by looking a little bit further into the future.

To stop gambling with our future, we should establish permanent human presence on other planets (Fig. 12.1). Spreading across the universe will decrease the threat caused by global pandemics, natural disasters, climate change, and “black swan” events.

To this end, microorganisms such as Archaea able to survive in extreme environments can be engineered by scientists using, for instance, synthetic biology tools described in one of the previous chapters. Such engineered microbes can be then deployed on other planets to terraform and prepare them for human habitation in the future (Fig. 12.1).

To reach for the stars and spread across the Universe will require collaboration across scientific disciplines and nations. But I believe that this task, together with many others, is within our reach.

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Glossary

ACE2 Angiotensin-converting enzyme 2, which was identified as the key receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Acidophilic Organism living in acidic conditions.

Acinetobacter baumannii Opportunistic human pathogenic bacterium, which is often multidrug resistant. One of the main causes of hospital acquired infections.

Adaptive immunity Immunity involving cells, which fight the invading pathogens and which can remember pathogens to fight them off more efficiently in the future.

Adeno-associated virus Small DNA virus that can infect human cells and is often used as a vector in gene therapy.

Algae Simple eukaryotic photosynthetic organisms found usually in water or near water.

Alpha Zero Updated version of AlphaGo Zero, which has trained itself without data from human games to master games Go, shogi, and chess.

AlphaGo Zero Updated version of AlphaGo trained without data from previous games played by humans. AlphaGo Zero defeated its previous version, AlphaGo at the game Go.

AlphaGo Google's DeepMind computer program powered by artificial intelligence, which defeated the best human player of the game Go.

Amino acid substitution Replacement of amino acid in a protein with another amino acid.

Amino acids Organic molecules, which are the basic building blocks of proteins.

Anthrax Serious bacterial infectious disease. It is caused by *Bacillus anthracis*, which can generate durable, hard to eradicate spores.

Antibiosis Competing association between microbes, when compound produced by one of the microbes is harmful to the other.

Antibiotic Medicine or chemical that can kill microorganisms or inhibit their growth.

Antibody Protein with high specificity for an antigen.

Antigen Part of pathogen, which stimulates host's immune system.

- Anti-inflammatory activity** Activity that reduces inflammation in the body.
- Antimalarial activity** Activity against *Plasmodium*, the causative agent of malaria.
- Antimicrobial compound** Substance that can kill microorganisms or inhibit their growth.
- Antimicrobial drug** Substance that can kill microorganisms or inhibit their growth.
- Antiviral activity** Activity that kills viruses or inhibits their growth.
- Aptamers** Short single-stranded nucleic acids which can specifically bind to their targets by folding into a 3D structure.
- Archaea** Single-celled microorganisms similar to but evolutionary distant from bacteria. Archaea often live in extreme environments.
- Artificial intelligence** Program which simulates human thinking.
- Aspergillus** Common type of fungus.
- ATP** Adenosine triphosphate. Organic molecule, which provides energy for important processes in the cell.
- Attenuated vaccine** Vaccine harboring live pathogen, which was weakened to make it unable to cause serious infection.
- Autoinducer** Signaling molecule, which bacteria use to communicate to each other by quorum sensing in response to changes in the number of bacterial cells in the surrounding environment.
- Autonomous mining trucks** Self-driving artificial intelligence-controlled heavy vehicles used in mining operations.
- Babesia** Parasitic microorganism spread by ticks that infects red blood cells.
- Bacillus subtilis** Common spore-forming Gram-positive bacterium.
- Bacteria** Single-celled microorganisms, which do not have a separate, distinct nucleus and other membrane-bound organelles.
- Bacteriophage** Type of virus, which infects bacteria.
- Big Bang** Huge explosion that marked the beginning of the universe and its expansion from a single point to the present state.
- Biobricks** DNA sequences of standardized biological parts, such as genes, promoters, and terminators, used by synthetic biologists to engineer larger DNA fragments, such as genetic circuits.
- Biocomputing** Biological computing, which uses biological molecules, such as DNA or proteins.
- Bioengineering** Biological engineering. Application of engineering principles into biology and medicine.
- Biofilm** Layer of microbial cells attached to the surface. Biofilms often form complex structures and are enclosed by a glue-like protective substance.
- Biofuel** Renewable fuel produced from biomass and not from fossils.
- Bioluminescence** Production of light by a living organism.
- Biophysics** Interdisciplinary field at the intersection of biology and physics, which applies approaches and principles used in physics to biology.
- Biosensor** Analytical device composed of a biological part and physical transducer. It generates a measurable signal.

- Budding yeast** Asymmetric division of yeast when the parent cell forms a smaller bud, which becomes the daughter cell.
- Budding** One of the methods how virus exits host cells. By budding virus acquires its lipid envelope from the host cell membrane.
- Burkholderia cepacia** Complex of opportunistic pathogens resistant to many antibiotics, which can cause infections in certain individuals, such as cystic fibrosis patients.
- Capsid** Protein coat of viruses.
- Cas9** RNA-guided protein, which cuts complementary target DNA. Part of CRISPR-Cas9 system.
- Catabolic pathway** Pathway involved in chemical degradation of more complex molecules into simpler ones.
- Cell wall** Outer layer of many cells outside of a cytoplasmic membrane. It provides shape and structure to the cell and protection from the external environment.
- Cellular pump** Transmembrane protein structures, which are used for transport across membranes.
- Chemotherapy** Cancer therapy, which uses drugs to kill cancer cells.
- Chloroplast** Membrane-enclosed organelle inside of some eukaryotic cells, such as green algae and plant cells, which generates chemical energy through photosynthesis.
- Cholera** Bacterial disease caused by bacterium *Vibrio cholerae*.
- Chromosomal DNA** DNA molecule which contains all or most of the genetic information of a cell. In eukaryotes it is localized in a specialized protective nucleus compartment. In prokaryotic microorganisms it is floating freely in the cell.
- Cladosporium** Common type of fungus.
- Clostridium difficile** Human pathogenic anaerobic bacterium, which is often multi-drug resistant.
- Codon** Triplet of nucleotides, which encodes certain amino acid.
- Commodore 64** One of the first popular personal computers.
- Competence** Physiological state of bacterial cells, which enables them to take up foreign DNA from the environment by transformation. Some cells are naturally competent, while competence in others can be induced.
- Conjugation** Gene transfer between bacteria by cell-to-cell contact, which is mediated via conjugative pili.
- Conjugative pilus** Tubular structure, which allows transfer of DNA between bacteria in the process of conjugation.
- Core gene** Strongly conserved gene at the nucleotide sequence level, which usually encodes an essential function.
- Coronaviruses** Type of RNA viruses with similar structure, which can cause disease also in humans.
- COVID-19** Coronavirus disease 2019. Infectious disease caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2).
- Cowpox** Mild viral disease of cows resembling smallpox.

- CRISPR-Cas9** System of clustered regularly interspaced short palindromic repeats and Cas9 protein, which acts as molecular scissors. Cas9 cuts viral DNA complementary to CRISPR sequence.
- Culture plate** A flat dish with growth medium, which is used to grow microorganisms.
- Cystic fibrosis** Rare genetic disorder that causes buildup of mucus in the lungs and digestive system. Patients with cystic fibrosis are particularly susceptible to infections, for instance, by *Pseudomonas aeruginosa*.
- Cytoplasmic membrane** Biological membrane barrier, which separates the cell's interior from the external environment.
- Cytotoxicity** Quality of a substance of being toxic to the cell by damaging or destroying them.
- Deep Blue** IBM's artificial intelligence-powered supercomputer, which has defeated the reigning world champion Grandmaster Garry Kasparov in chess.
- Deep learning** Subfield of machine learning based on neural networks, which is particularly suitable for identification of patterns in large and high-dimensional datasets.
- Directed evolution** Laboratory-based evolution of biological systems through rounds of selection of specific traits.
- DNA microarray** Often referred to as a DNA chip contains thousands of nucleic acid sequences bound to a surface. Used for the simultaneous measurement of expression of many genes.
- DNA sequencing** Process used for determination of the order of four nucleotides in nucleic acids.
- DNA synthesis** Process of generating deoxyribonucleic acid (DNA).
- DNA vaccine** Vaccine composed of a deoxyribonucleic acid sequence of the pathogen's antigen.
- DNA** Deoxyribonucleic acid. Organic molecule, which contains genetic information of an organism.
- Dormancy** Part of a life cycle of a microorganism usually induced by harsh environmental conditions. It is characterized by reduced metabolism, growth and replication and formation of protective spores to survive rough conditions.
- Electron microscope** Microscope which utilizes a beam of electrons for magnification. It can be used for observation of much smaller objects than the standard light microscope.
- Endemic disease** Disease typical for a particular region or population.
- Endoplasmic reticulum** Organelle of eukaryotic cells resembling a labyrinth of membrane tubules and sacks, which is involved in the synthesis of proteins and metabolism of lipids.
- Endosome** Membrane-enclosed organelle of eukaryotic cells involved in transport of material for degradation to other organelles called lysosomes.
- Endospore** Stable and resistant dormant form of some bacteria, which allows survival in harsh environmental conditions.

- Endosymbiosis** Mutually beneficial relationship between organisms, when one organism lives inside another.
- Enzyme** Protein which catalyzes biochemical reactions.
- Escherichia coli** Common rod-shaped Gram-negative bacterium, frequently used as laboratory model microorganism, which is also present in the human intestine.
- Essential gene** Gene indispensable for survival of an organism.
- Eukarya** Organisms whose cells' genetic material is localized in a specialized distinct membrane-enclosed organelle, called nucleus.
- Eukaryote** Organism whose cells' genetic material is localized in a specialized distinct membrane-enclosed organelle, called nucleus.
- Eukaryotic microorganism** Microorganism whose cells' genetic material is localized in a specialized distinct membrane-enclosed organelle, called nucleus.
- Expression profile** Expression of a large number of genes, for instance all genes in a bacterium. Can be measured simultaneously using DNA microarrays.
- Extracellular matrix** Glue-like substance produced by microbes in biofilm, which holds them together and protects them from environment.
- Extraterrestrial** Originating from outside the planet Earth.
- Extremophile** Organism that can survive and even thrive in extreme environmental conditions, such as high or low temperature, acidity, salinity, pH level, or radiation.
- Feline calicivirus** Virus that can cause respiratory and oral infections in cats.
- GB** Gigabyte is a unit of data storage capacity, which equals to one million bytes.
- Gene** Region of nucleic acids, which constitutes basic physical and functional unit of heredity.
- Genetic circuit** DNA fragment designed by synthetic biologists to perform specific functions.
- Genetic code** Order of four nucleotides in nucleic acids, which are the basic building blocks of nucleic acids.
- Genetic engineering** Engineering biological systems by altering their genetic information.
- Genetic profile** Specific DNA pattern.
- Genetic recombination** Rearrangement of DNA sequences.
- Genetic sequence** Order of four nucleotides in nucleic acids, which are the basic building blocks of nucleic acids.
- Genome transplantation** Replacement of the cell's genome with another genome (for instance, synthetic genome).
- Genome** Complete set of genes, whole genetic material of a cell or organism.
- Golgi apparatus** Membrane-enclosed organelle of eukaryotic cells involved in the processing and transport of proteins and lipids produced by endoplasmic reticulum.
- Habitat** Natural environment or area where an organism usually lives.
- Hemorrhaging** Extensive bleeding, which leads to acute loss of blood.
- High throughput process** Process, which is often automated and scaled up for rapid analysis of many samples.

- HIV** Human immunodeficiency virus, which negatively impacts the host's immune system.
- Homologous recombination** Process of exchange of genetic information between two similar DNA sequences.
- Horizontal gene transfer** Transfer of genetic material between unrelated organisms of the same generation and not between parent and offspring.
- Hormone** Molecule used for signaling between cells, tissues, and organs of eukaryotes.
- Human cytomegalovirus** Common herpesvirus, which infects a large portion of the human population.
- Human enterovirus 71** Small RNA virus that can cause disease particularly in children.
- Hydrothermal vent** Fissure on the ocean floor, which discharges superheated mineral-rich water. Hydrothermal vents are among the most inhospitable environments on Earth. Hydrothermal vents on land form hydrothermal ponds, hot springs, and geysers like those in Yellowstone National Park.
- Immune system** System composed of cells and processes, which protects host from foreign antigens, such as microbes.
- Immuno-compromised** Individual with a weakened immune system, for instance, due to HIV infection.
- Inactivated vaccine** Vaccine, which contains killed pathogen.
- Integrative and conjugative elements** Large modular mobile genetic elements. They are usually integrated into a chromosome, but are capable of excision, transfer into the new host cell, and reintegration into the chromosome of the new host.
- KB** Kilobyte is a unit of data storage capacity, which equals to one thousand bytes.
- Klebsiella aerogenes** Rod-shaped Gram-negative bacterium.
- Klebsiella pneumoniae** Rod-shaped Gram-negative bacterium, which can cause opportunistic infections and is often resistant against many antibiotics.
- Last resort antibiotic** Antibiotic, which is used only when all others do not work.
- Last resort drug** Drug, which is used only when all other drugs do not work.
- Luciferase** Enzyme which catalyzes production of light by living organisms.
- Lysis** Disruption of cellular membranes. It is also one of the methods how virus exits host cells.
- Lysosome** Membrane-enclosed organelle of eukaryotic cells, which contains enzymes for degradation of unwanted cellular components or material taken in by the cell from the environment.
- Machine learning** Subfield of artificial intelligence research, which specializes on learning.
- Metabolic pathway** Sequence of interconnected chemical reactions for conversion of certain substrate.
- Minimal cell** Cell, which harbors only the minimal set of essential genes indispensable for survival.
- Misfolded protein** Protein with incorrectly folded three-dimensional structure, which is usually nonfunctional.

- Mitochondria** Membrane-enclosed organelle of eukaryotic cells with many essential functions, including generation of ATP molecules that provide energy for various cellular processes.
- mRNA vaccine** Vaccine composed of a messenger RNA sequence of the pathogen's antigen.
- MRSA** Methicillin-resistant *Staphylococcus aureus*, which is difficult to treat because of its antibiotic resistance.
- Mutation** Modification of the nucleotide sequence in genes.
- Mycobacterium tuberculosis** Pathogenic bacterium causing tuberculosis.
- Nanotubes** Tubular structures, which allow transfer of genes and other molecules between bacteria.
- Neural network** Network of artificial nodes resembling neurons in the human brain.
- Nocturnal organism** Organism, which is active at night.
- Non-essential gene** Gene, which is not required for survival of an organism.
- Nosocomial infection** Infection acquired in the hospital setting.
- Nucleotide** Organic molecule, which is the basic building block of nucleic acids.
- Nucleus** Membrane-enclosed organelle of eukaryotic cells, which contains chromosomal DNA.
- Opportunistic pathogen** Microorganism, which is usually harmless but can cause infections under certain conditions, for instance in individuals with weakened immune system.
- Organelle** Membrane-enclosed compartment within eukaryotic cells, which is specialized on performing specific functions.
- Outbreak** Sudden increase of the number of the disease cases.
- Pasteurization** Process of sterilization and killing of pathogenic microorganisms by heat. It is used, for instance, to kill pathogens in milk.
- Pathogenicity** Ability of the pathogen to cause disease.
- Pentium** Popular microprocessor from Intel, which is often used in personal computers.
- Peptidoglycan** Polymer composed of sugars and peptides, which is an essential component of the bacterial cell wall.
- Peroxisome** Small membrane-enclosed organelle of eukaryotic cells involved in cell metabolism and oxidative processes.
- Photosynthesis** Process by which plants and green algae convert light energy into chemical energy that can be used for various cellular processes.
- Plasmid** Small molecule of DNA, which is distinct from chromosomal DNA and can replicate independently from chromosomal DNA.
- Plasmodium** Parasitic microorganism, which is the causative agent of malaria.
- Prion** Infectious agent, which consists only of proteins and can cause fatal disease in animals and humans.
- Promoter** DNA sequence in front of a gene, where RNA polymerase binds to initiate gene's transcription.
- Protozoa** Single-celled eukaryotic microorganism.

- Pseudomonas aeruginosa** Opportunistic bacterium resistant to many antibiotics, which can cause infections in certain individuals, such as cystic fibrosis patients.
- Pseudomonas** Rod-shaped Gram-negative bacterium, which can cause opportunistic infections in susceptible individuals.
- Quorum sensing** Type of cell-to-cell communication, which regulates gene expression in response to changes in the number of bacterial cells in the surrounding environment.
- RAM** Random access memory is a computer's short-term memory. It is often referred to as operational memory.
- Receptor** Protein on the surface of the host cell recognized by the virus. Virus binds to receptor to enter the cell.
- Reinforcement learning** Type of machine learning, which uses trial and error to achieve the desired outcome and is most similar to human learning.
- Re-purposed drug** Previously discovered drug for which has been found new use, for instance against different microorganisms.
- Reservoir host** Organism, which is usually chronically infected by a pathogen and can infect other organisms.
- RNA** Ribonucleic acid. Organic molecule, structurally similar to DNA, which is involved in conversion of genetic information stored in DNA into the structure of proteins.
- Saccharomyces cerevisiae** Common species of yeast used as a model organism in molecular biology and other scientific disciplines.
- SARS-CoV-2** Severe acute respiratory syndrome coronavirus 2. Causative agent of Coronavirus disease 2019 (COVID-19).
- SCRaMbLE** System integrated into the synthetic genome of Yeast 2.0, which allows rapid genome rearrangements and modifications.
- Semisupervised learning** Type of machine learning, which combines features of supervised and unsupervised learning.
- Signal receptors** Proteins on the cell's surface, which bind to signaling molecules in the environment and transmit the signal to the interior of the cell.
- Smallpox** One of the most deadly viral diseases. Smallpox was completely eradicated thanks to vaccination.
- Solfataric** Volcanic steam vent containing sulfur gasses.
- Spermatozoa** Motile male sex cells that carry genetic material.
- Staphylococcus aureus** Bacterium, which causes a wide range of infections and is well known for its resistance against a broad spectrum of antibiotics.
- Sterilization** Destruction of living microorganisms.
- Streptococcus** Non-motile bacterium, which causes a variety of infections.
- Subunit vaccine** Vaccine harboring only parts of the pathogen called antigens, which activate the immune system.
- Superbug** Microbe resistant against a number of antibiotics simultaneously.
- Supervised learning** Type of machine learning, which requires a lot of input and annotated data for specific tasks.

- Susceptible individual** Individual who is at risk of becoming infected, for instance due to weakened immune system.
- Swarming** Movement of microorganisms across a semi-solid surface, which usually requires a flagellum.
- Symbiosis** Mutually beneficial relationship between organisms.
- Synthetic biology** Emerging discipline, which applies engineering principles into construction of biological systems.
- Terminator** DNA sequence at the end of a gene, where transcription stops.
- Terraforming** Engineering of planets to make them and environmental conditions on them more similar to Earth and habitable by humans.
- Thermophilic** Organism living at a high temperature.
- TMPRSS2** Transmembrane serine protease 2. Key host cell factor for viral entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
- Toxin** Substance, which is harmful to living organisms.
- Toxoplasma** Parasitic microorganism, which lives inside host cells and can cause disease called toxoplasmosis.
- Transcription** Process of transcribing the information stored in the sequence of DNA into the sequence of mRNA.
- Transduction** Horizontal gene transfer by bacteriophages.
- Transformation** Horizontal gene transfer, which involves taking up foreign DNA from the environment.
- Translation** Process of translating the information stored in the sequence of mRNA into the sequence of amino acids in proteins.
- Transmissible** Able to transfer from one organism to another.
- Transmission electron microscopy** Type of microscopy, which uses a beam of electrons to visualize observed samples.
- Transmission route** Route of pathogen from one infected host to another.
- Transposon** Mobile repetitive pieces of DNA, which normally reside in the chromosome but can excise and jump between genomes.
- Tuberculosis** Serious infectious diseases caused by bacterium *Mycobacterium tuberculosis*.
- Twitching** Movement of microorganisms over moist and semi-solid surface, which doesn't require a flagellum but is mediated by type IV pili.
- Type IV secretion system** Large protein complexes, which traverse the membranes of bacteria and allow horizontal gene transfer by conjugation.
- Typhoid** Bacterial diseases caused by *Salmonella enterica* serovar Typhi.
- Unsupervised learning** Type of machine learning, which doesn't require much input and data annotation.
- Vaccination** Stimulation of cells involved in adaptive immunity so that when they are faced with the same pathogen in the future they already know how to deal with it.
- Vaccine** Substance, which induces production of antibodies in the organism into which it was injected.

- Variolation** Technique used in the past to protect against smallpox infection by prior infection with smallpox.
- Vertical gene transfer** Transfer of genetic material between generations, from parent to offspring.
- Vibrio** Gram-negative, motile, rod-shaped bacterium, which can cause serious disease in humans and animals.
- Viroid** Infectious agent of plants, which consist of only short molecule of RNA.
- Virulence factor** Molecule, which increases the ability of a pathogen to establish and maintain an infection.
- Virus transmission** Process by which virus spreads between hosts.
- Virus** Infectious agent composed of DNA or RNA, protein capsid, and in some cases also lipid envelope. Virus cannot replicate without a host cell.
- Wild-type strain** Standard strain with defined characteristics.
- Yeast 2.0** Project aimed at engineering yeast with a chemically synthesized genome.
- Yeast** Single-celled eukaryotic microorganisms, which belong among fungi.
- Yersinia pestis** Bacterium, which is the causative agent of plague.
- Zoonotic** Disease or pathogen, which can cross inter-species barriers and can be transmitted between animals and humans.