Notes on Code Breaker by Walter Isaacson

Walter Isaacson's book is an extraordinary work that takes the reader through the history of important scientific developments that led to the discovery of CRISPR biotechnology which can be applied to edit the human genome to cure diseases and to adjust human traits and characteristics for future generations. The book is so rich in detail that I found it impossible to write a comprehensive account of all aspects of the story. For example, I have left for another day a discussion of the end-of-book narrative on the utilization of CRISPR to expedite and simplify testing for Covid and to turn our cells into manufacturing plants for the spike protein that would stimulate our immunity to this deadly virus.

What follows is a summary regarding (1) the basic science, from our understanding of the composition and structure of DNA (deoxyribonucleic acid) to the focus on the critical functions of RNA (ribonucleic acid), (2) some of the people who played vital roles in the basic research and application of the scientific discoveries, and the emerging tension between collaboration and competition among these players as developments rapidly unfolded, and (3) the potential applied capabilities of CRISPR for curing disease and modifying the human genome for future generations, all clouded by the complex moral, ethical, sociological and political issues at stake for humanity.

Fortunately, for liberal arts-types like me, this work was produced by a journalist and a humanist author (recall, for example, his lengthy work, ***Leonardo da Vinci***) who, through a great deal of listening, reading, self-education and mingling among scientists and professors over a period of 7 to 8 years, was able to put together an objective and understandable story that those of us who are non-scientists can begin to comprehend. I tried reading Kevin Davies's highly-acclaimed account of the CRISPR story, entitled ***Editing Humanity***, and had to put it down as I became lost in a sea of highly technical analyses and equations. In contrast, Isaacson has produced a work that I believe is better suited to spread the knowledge and understanding of these vital scientific developments among a broader audience, including students who he hopes will become inspired to study and pursue the wonders of nature and the secrets of life.

The Science. The narrative throughout the book examines and explores various critical developments in the arena of natural science, starting in the mid-part of the 20th century and continuing to the present day. This path is not a one-way linear progression but an iterative dance that involves multiple different research projects and discoveries by world-class scientists who, sometimes in collaboration with each other and other times in fierce competition, are driven by the wonders of science, the enduring desire to search for better understanding of the origins of life, and the personal and professional satisfactions that derive from world-wide recognition and monetary awards associated with the Nobel and other prizes bestowed upon those who, by virtue of their extraordinary endeavors and insights, are deemed to have changed

the world. Over time, the collective fruits of these labors yielded dramatic enhancements in our understanding of the fundamental chemical and biological functions that drive life.

DNA. This story begins in the early 1950s with the work of James Watson and Francis Crick who, with the aid of x-ray data and photography produced by Rosalind Franklin, were able to piece together the three-dimensional structure of DNA: two sugar-phosphate strands that twisted and spiraled to form a double-helix. Protruding from these were the four bases in DNA: adenine, thymine, guanine, and cytosine (commonly known by the letters A, T, G, and C). When the two strands split apart, they could perfectly replicate, passing along the information encoded in the sequences. The structure was perfect for the molecule's function. It could carry a code that could replicate. The two men put together a paper in March 1953 that included the following sentence: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

Watson and Crick had discovered how instructions for building every cell in every form of life were encoded with the four-letter sequences of DNA. When the Human Genome Project (involving the sequencing of DNA in the human genome) was launched in 1990, Watson was anointed its first director. A decade later, after three billion dollars of investment, the definitive results were announced and characterized, with great fanfare, as "defining the hereditary script, the set of instructions that define humanity." However, in order to better understand human genes, scientists began to move their focus from DNA to its less famous sibling, RNA, which is a molecule in cells similar to DNA but it has one more oxygen atom in its sugar-phosphate back-bone and a difference in one of its four bases.

RNA. While DNA's primary activity is protecting the information that it encodes and occasionally replicating itself, RNA actually goes out and does real work, including the production of proteins. At the time of the Human Genome Project, RNA was seen mainly as a messenger molecule that carries instructions from the DNA that is nestled in the nucleus of the cells. A small segment of DNA that encodes a gene is transcribed into a snippet of RNA, which then travels to the manufacturing region of the cell. There this "messenger RNA" facilitates the assembly of the proper sequence of amino acids to make a specified protein. Proteins are varied, but among the most important are enzymes, which serve as catalysts. They spark and accelerate and modulate the chemical reactions in living cells. In particular, scientists discovered that RNA molecules that were enzymes could slice, splice and replicate themselves. This RNA became dubbed a "ribozyme."

Jennifer Doudna, a young scientist who was studying the structure of RNA, expressed the possible significance of her research: "We hope our discovery will provide clues as to how we might be able to modify the ribozyme so that it can repair defective genes." Thus began the

quest to translate basic science about RNA into a tool that could edit genes in order to cure or treat people who had genetic defects.

Clustered Repeats, or CRISPRs. In the ongoing study of the genetic make-up of many different bacteria and archea (organisms whose cells lack a defined nucleus and have distinct molecular characteristics that separate them from bacteria), researchers noted the presence of DNA segments that were identical to each other and that were repeated and sprinkled between normal-looking sequences of DNA. These became known as "clustered regularly interspaced short palindromic repeats" or the acronym "CRISPR." In most of the organisms that had CRISPRs, the repeated sequences were flanked by genes which encoded directions for making an enzyme, which in turn were named "CRISPR-associated," or *Cas*, enzymes.

A scientist named Francisco Mojica who was studying *E-Coli* bacteria was fascinated by what he observed as spacers, those regions of normal-looking DNA segments that were nestled in between the repeated CRISPR segments. When he ran tests to identify these spacer segments, he found that they matched the sequences that were in viruses that had attacked the *E-Coli*. He found the same results when he examined other bacteria: their spacer segments matched those of the viruses that had attacked them. This led him to conclude that bacteria have an immune system in that they are able to remember viruses that attack them. Those with CRISPR spacer sequences seemed to be immune from infection by a virus that had the same sequence, but bacteria without the spacer got infected. Moreover, the bacteria with the spacers appeared to be able to adapt to new threats. When new viruses came along, the bacteria that survived were able to incorporate some of that virus's DNA and thus create, in its progeny, an acquired immunity to that new virus.

What Mojica had stumbled upon was a battlefront in the long-running battle between bacteria and viruses, one that had been taking place for 3 billion years. Other scientists began to contribute papers evidencing similar discoveries.

Enzymes. Enzymes are a type of protein. They act as a catalyst that sparks chemical reactions in the cells of living organisms, including humans. These include breaking down starches and proteins in the digestive system, sending signals between cells, regulating metabolism and, importantly, cutting and splicing DNA and RNA. By 2008, scientists had discovered a handful of enzymes produced by genes that are adjacent to the CRISPR sequences in a bacteria's DNA. These CRISPR-associated (Cas) enzymes enable the system to cut and paste new memories of viruses that attack bacteria. They also create short segments of RNA, known as CRISPR RNA (crRNA), that can guide a scissors-like enzyme to a dangerous virus and cut up its genetic material; this process is the bacteria's adaptive immune system. The notation system for these enzymes became standardized into such names as Cas1, Cas9, Cas12 and Cas13. Scientists discovered that Cas1 has a distinct fold, indicating that it is the mechanism that bacteria use to

cleave a snippet of DNA from invading viruses and incorporate it into their CRISPR array, thus being the key to the memory-forming stage of the immune system.

With this discovery, scientists realized that, if the CRISPR-Cas system was aimed at the DNA of viruses, then it could possibly be turned into a gene-editing tool. CRISPR could be fundamentally transformative: if it could target and cut DNA, it would allow one to fix the cause of a genetic problem. But scientists didn't know precisely how the CRISPR enzyme cut DNA.

In order to understand the essential components of the system, they had to take the biochemical approach of isolating and studying molecules in a test tube, work that was led by scientists, Jennifer Doudna and Emmanuelle Charpentier. They formed a global team to solve the mystery. They coalesced around Cas9 because they determined that if one deactivated Cas9 in bacteria, the CRISPR system no longer cut up invading viruses. They also established the essential role of another part of the complex: CRISPR RNAs, known as crRNA. These are the small snippets of RNA that contain some genetic coding from a virus that had attacked the bacteria in the past. This crRNA guides the Cas enzymes to attack that virus when it tries to invade again. These two elements are the core of the CRISPR system: a small snippet of RNA that acts as a guide and an enzyme that acts as a scissors.

In addition, there was one other component, called "trans-activating CRISPR RNA," or tracrRNA, that played an essential role. It both facilitates the making of crRNA, the sequence that carries the memory of the virus that previously attacked the bacteria, and serves as a handle to latch on to the invading virus so that the crRNA can target the right spot for the Cas9 enzyme to chop.

Having completed this analysis, it soon became clear that the CRISPR system had a truly momentous application: the crRNA guide could be modified to target any DNA sequence one might wish to cut. It was programmable. It could become powerful tool for gene editing, a means to rewrite the code of life.

Engineering Human Genes. The 2012 discoveries by the Doudna-Charpentier team led to a furious sprint in many labs around the globe to determine if the CRISPR system, which worked in bacteria and archea (single-cell organisms with no nucleus), could work in human cells with nuclei. The answer, in the affirmative, came within six months. Multiple teams of scientists and researchers presented dueling presentations asserting that they had made the first and most significant discoveries, each asserting that they had, in their reports, demonstrated the key components of the CRISPR gene-editing system and its applicability to human cells and the advancement of medical technology.

They then formed companies (among them, CRISPR Therapeutics, Editas, and Intellia Therapeutics) for the purpose of channeling the application of science to applied medicine. In

furtherance of their product and business goals, these companies filed for patent protections (the patents typically being assigned to their associated academic institutions) in order to protect the monetary rewards arising from their novel techniques – a state of play that gave rise to years of major patent litigations, often driven by emotions and resentments, over coverage and priority issues.

Aside from the patent litigation, these new discoveries led to accelerating efforts by the medical community to apply the CRISPR system to treat diseases and conditions arising from genetic defects, including sickle cell anemia, cancer, blindness and other conditions. One of the approaches is the use of CRISPR to edit some, but not all, of the body (somatic) cells of a patient and make changes that will not be inherited. This can be done by taking the cells out of the patient, editing them, and returning them (*ex vivo*) or by delivering the CRISPR editing tool into cells inside of the patient (*in vivo*). Sickle cell anemia is one of the best candidates for *ex vivo* gene editing because it involves blood cells that can easily be extracted and returned. The disease is caused by a mutation of a single letter in a person's DNA, which causes a kink in the hemoglobin protein. A normal version of hemoglobin protein forms round and smooth blood cells, able to move easily though blood vessels and carry oxygen from the lungs to the rest of the body. But the kinked hemoglobin protein forms long fibers that contort the red blood cells, which causes them to clump together into the shape of a sickle. Oxygen does not get to the tissues and organs, causing severe pain and, in most cases, death by age fifty. Sickle cell disease affects more than four million people worldwide, about 80 percent of them in sub-Sahara Africa and about ninety thousand people in the U.S., mainly African-Americans.

The simplicity of the glitch and the severity of the syndrome make it a strong candidate for gene editing. This treatment in fact worked well on patient Victoria Gray. Doctors extracted stem cells from her own blood and edited them, using CRISPR, to activate a gene that produces a type of blood cell that is normally made only during the fetal stage of life. That fetal stage hemoglobin is healthy, so if the genetic modification works, patients can start producing their own good blood.

A few months after she was injected, Victoria had produced healthy blood cells constituting half of her blood, obviating the need for blood transfusions and enabling her to avoid attacks of pain. After nine months, healthy blood cells stood at over 80%, indicating that the transformation in her body via CRIPR edits was sustained. A remarkable achievement, though one that was expensive – in the range of $1 million. So, the prospect of doing great good came at a cost that, without further advancement, might break the bank.

In response, Jennifer Doudna decided that making sickle-cell treatments affordable should become a mission of her enterprise called Innovative Genomics Institute. She reached out to the Gates Foundation and the National Institutes of Health, which announced a partnership for

a Cure Sickle Cell Initiative funded with $200 million. The primary scientific goal is to find a method to edit the sickle-cell mutation inside of a patient without needing to extract bone marrow. One possibility is to inject into the patient's blood a gene-editing molecule with an address label that directs it right to the cells in the bone marrow.

If the initiative is successful, it will not only cure a lot of people; it will advance the cause of health justice for those who have historically been underserved by the medical community. The great promise of gene editing is that it will transform medicine. The peril is that it will widen the divide between rich and poor.

The book goes on to explore other potential applications of the CRISPR system to treat cancer, blindness, Alzheimer's and other diseases.

Rules of the Road. With the explosion of scientific discoveries on the use of the CRISPR system to edit human genes, the necessity for addressing ethical and moral issues and developing suitable guidelines and constraints designed to assure the prudent application of the technology became ever more apparent. One factor that complicated this challenge was the emerging corporate involvement in funding university research which created tensions between the goals of academia and industry, particularly in terms of the freedom to conduct pure basic research over a broad spectrum versus the desire of industry to focus on product development. Another major factor was the potential for genetic engineering to increase inequality across the globe. Given the high cost of many CRISPR procedures, people born into privilege would tend to receive the most benefits, a result that could widen and genetically encode existing inequalities, calling into question a central element of democratic political theory and practice: the commitment to equality of opportunity.

Seeking a Consensus. In 2001, the Kass Commission, which included many distinguished thinkers and philosophers, issued a report that warned of the dangers of using technology to go beyond the treatment of disease to using it to enhance human capabilities. Focusing on philosophical rather than safety concerns, the authors described what it meant to be human, to pursue happiness, to respect nature's gifts and to accept the given. Their report argued that going too far to alter what is "natural" was hubristic and endangered our individual essence.

Building on this work, and in light of the evolving CRISPR developments, top research scientists gathered for another conference in early 2015 to focus on the ethical considerations associated with effecting inheritable ("germline") genetic edits. Among other questions debated, the following ones formed the core of the group's focus:

1. Did the premium that America place on individual liberty require that decisions about the gene-editing of babies be left mainly to individual parents?

2. To what extent would the creation of gene-edited babies – abandoning the idea that our genetic endowments came from a random natural lottery – undermine our sense of moral empathy?
3. Is there a danger in decreasing the diversity of human species?
4. If the technology is available to make healthier and better babies, would it be ethically wrong *not* to use it?
5. Gene editing will be expensive; will only the wealthy have access?

The group agreed that the use of the CRISPR tools for non-inheritable editing in somatic cells in order to combat disease was a good thing, leading to beneficial drugs and treatments. But, in a move designed to ward off the threat of a government ban on human genome edits, they called for a "temporary halt" of germline editing of human cells until the safety and social issues could be better understood. These conclusions were incorporated into a paper prepared by Jennifer Doudna and others that was published in the March 2015 issue of *Science*.

The Experimental Twins. All of these issues boiled over when, in 2018, a rogue Chinese scientist, He Jiankui, undertook without authorization to make germline edits to viable human embryos to allow couples who suffered from AIDS to have babies who would be protected from the HIV virus. The work involved editing the *CCR5* gene in a way that would make a person less susceptible to HIV. Notably, there were simpler ways to prevent AIDS infection (through sperm washing and screening for healthy embryos) so the procedure was not medically necessary. Moreover, the procedure would not correct a clear genetic disorder as the *CCR5* gene is common and has multiple purposes, including aiding in the protection against West Nile Virus. However, Jiankui decided to proceed, apparently motivated by the desire to achieve personal fame and to enhance the global standing and reputation of Chinese science.

The results proved to be problematic. Twin girls, Lulu and Nana, who had undergone *CCR5* gene-editing at the embryonic stage, were born in November 2018. Nana's *CCR5* gene was edited successfully. However, in Lulu, only one of the two relevant chromosomes had been properly edited with the result that her system would still produce some of the *CCR5* protein. In addition, there was evidence that some unwanted off-target edits had been made and also that both embryos had been mosaics, meaning there had been enough cell division before the CRISPR editing was done that some of the resulting cells in the babies had been unedited. One scientist from the University of Pennsylvania described this first attempt to modify the human code of life as a "hack job."

The Hong Kong Summit. With shocking news of the birth of the genetically engineered twins, the world's leading scientists gathered at a Hong Kong Summit conference to discuss the implications of this development and determine a proper path forward. Their views on the issues associated with treatment of disease versus advancement of human characteristics

ranged widely from a call for extreme caution to urging wide-open application of the technology. The final consensus of the meeting was to steer a middle course: there was a need for more specific guidelines on when germline gene editing should be done, but it was also important to avoid rhetoric that would lead to national bans or moratoria. The decision was to move forward to address safety concerns and to develop additional criteria for the use of germline edits. Several international science commissions were created to deal with the issue. A lengthy report issued by the international academies of science commission in September 2020 noted that making inheritable gene edits was not yet safe and usually not medically necessary, but it came down in favor of "defining a responsible path for clinical use of inheritable genome editing."

Moral and Ethical Considerations. Despite all the scientific work and exploration of the humanistic considerations associated with CRISPR, the profound moral and ethical issues, particularly regarding germline editing, remain unresolved. If we can safely edit genes to make our children less susceptible to deadly diseases, debilitating abnormalities, HIV or coronaviruses, would it be wrong to do so? Probably not. But what about edits for other fixes and enhancements (better muscles, minds, memory, moods) that might be possible? If they turn out to be safe, should governments prevent us from using them?

Some argue that there is a distinction between "treatments," designed to fix dangerous genetic abnormalities, and "enhancements," designed to improve human capacities or traits. Yet these distinctions can become blurred and the issues quite complex. The author posits a series of "thought experiments," looking at Huntington's Disease, Sickle-Cell anemia, character, deafness, muscles, height, psychological disorders and intelligence, with his analyses producing no easy conclusions about most of these conditions. For example, people that get a copy of the bad gene that causes sickle-cell anemia from only one parent do not develop the disease, but they do develop immunity from malaria. So, in some places in the globe, such as sub-Saharan Africa, the gene has been useful. Now that there are treatments for malaria, it's less useful, but this example provides an important reminder, when we think about messing with Mother Nature, that genes may play multiple roles and have evolutionary reasons for existing.

Moreover, there are humanistic considerations. David Sanchez, who has sickle-cell disease and receives regular transfusions, marveled at the prospects of enabling children to be cleared of the risk of the disease via CRISPR edit, but, in a somewhat off-the-cuff ambivalent response when initially interviewed, questioned whether he would want the benefits of the edit: "There's a lot of things I learned having sickle-cell. Because I had it, I learned patience with everyone. I learned just how to be positive. Empathy is something that's really important to humans. That is something I learned from sickle-cell." Another example – take Miles Davis. The pain of sickle-cell probably drove him to drugs and drink. It may have even driven him to his death. It also, however, may have driven him to be the creative artist who could produce *Kind of Blue* and *Bitches Brew.* Would Miles David have been Miles Davis without sickle-cell?

This is not a new question. Think about Franklin Roosevelt, whose character was in many ways shaped by his experience with polio. And closer to home, think about Judge Myron Thompson who, in considering the twin challenges of being black and a victim of polio, stated that, of the two factors, his experience with polio was the more significant and transformative factor in shaping his life – his backbone, resilience, courage and humility. His success as a distinguished federal jurist in a southern state is partly because of his great depth of character.

A different example, even closer to home: A fellow by the name of Jory Fleming was born with severe autism as well as other challenging conditions. He couldn't cope in class so he was homeschooled. As he grew older, he taught himself how to deal with the fact that his internal world was different from those of other people. He ended up winning a Rhodes Scholarship to Oxford. In his memoir, ***How to Be Human,*** he reflects on whether gene editing should be used, if it becomes feasible to eliminate some of the causes of autism. "You'd be removing an aspect of the human experience," he writes, "but for what benefit exactly?" Autism, he argues, is a difficult condition to have, but the challenges largely come because the world is not good at accommodating people whose emotional lives are different. These differences can actually provide a useful perspective for the rest of us. He asks: "Should society change to recognize the benefits of autism instead of just the challenges? Certainly, my experience has been very challenging, and it has also been rewarding."

Overall, this is an interesting and challenging dilemma and, as one member of a family that has raised a child on the autism spectrum and continues diligently to address his daily concerns and needs in order to assure that he leads a fulfilling life, I can honestly, and without feeling of guilt, tell you that there are at least two sides to this complex issue. That said, I do love our son deeply and appreciate the diversity and value of the perspectives that his life has brought to us.

Conclusion. At the end of the day the author settles on a middle path: use CRISPR to treat or eradicate diseases and disabling abnormalities but avoid playing God by seeking ways to rig the natural lottery and design enhancements and perfections for our children. The latter path would undermine our sense of "there but for the grace of God go I" toward our fellow humans who are less lucky.

As he notes, "We can steer a course that avoids a Promethean quest for controlling our endowments while also avoiding complete submission to the vagaries of the lottery. Wisdom involves finding the right balance."

The book is a testament to the value of curiosity-driven basic research by a global community of gifted scientists. While Jennifer Doudna is clearly and deservedly the star of this book, many others, including Emmanuelle Charpentier, Feng Zhang, George Church and Francisco Mojica,

played vital roles in this dance. Each of their stories, while detailing their unique personalities, ambitions, quirks and foibles, provides compelling evidence of their collective concern for humanity and their strong desire to utilize science to improve the human condition.

After millions of centuries during which the evolution of organisms happened “naturally,” we humans now have the ability to hack the code of life and engineer our own genetic future. Where this leads the human species, nobody really knows. To guide us, we need not only scientists but humanists as well.