Biotechnology

An Era of Hopes and Fears

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Abstract

Biotechnology capabilities continue to increase at a rapid pace. This increase in itself is not unexpected, unforeseen, or inherently good or bad. Increasing knowledge of genetics and cellular function, coupled with increases in computing power, is allowing development of novel, highly targeted treatments for all manners of disease and injury. The potential for breakthrough treatments is higher now than ever before. However, as knowledge and capability increase so does the ability to develop biological weapons with increasing lethality and precision. Every new treatment also represents a potential new weapon.

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In 1996 the world's DNA sequence repository, GenBank, had approximately 5×10^8 bases (bits) worth of sequence data in its database.[1] The human genome had yet to be sequenced, and cloning was still a theory. Now the world's genetic databases contain 1.3×10^{12} bases of data available for search within seconds.[2] Sequencing is no longer a task for graduate students but is now a commercial service provided by numerous companies offering sequencing and analysis of entire bacterial communities within days. The increase in computing power, combined with the ever growing amount of DNA and protein sequence data, allows deeper insights into the fundamental source of disease. These capabilities are allowing medical providers to identify specific disease characteristics for each individual patient, which allows for increasingly specific and effective treatment plans.

All life on earth is ultimately controlled by each organism's unique genetic code carried in its DNA, and many human disease states can be at-

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tributed to mutations in the chemical structure, and hence information content, of the DNA.[3] For example, noninfectious human disease states, such as cancer or sickle cell anemia, can be attributed to mutations. A mutation may be inherited, or it may arise spontaneously during an organism's lifetime. Any particular mutation may have no effect on a cell, while others can cause a change in cellular function that may increase or decrease the organism's ability to survive in the environment. Today, science has advanced our understanding of genetics and cellular processes to the point where we are developing the ability to identify mutations associated with disease, as well as developing the ability to treat disease by modifying DNA or targeting malformed proteins within a cell. With this information, doctors can design custom treatment plans with increased specificity and likelihood of success.

Researchers can even develop new treatments on a computer. Using molecular modeling software they can design and test existing chemical compounds or even design synthetic molecules capable of modifying the effects of a disease. Personalized DNA sequencing is also becoming a commercial commodity. For example, fitness companies are offering to test your DNA then develop exercise and nutrition plans tailored to your genetic makeup, while other companies offer to identify your genetic ancestry for under $100.[4] Today the cost of DNA sequencing is actually outpacing Moore's law, and to sequence one million bases of DNA literally costs a few pennies.[5]

As we develop our understanding of genetic diseases, we are also developing the ability to attack these diseases at their genetic roots. Again with increased knowledge of DNA sequence information and advances in computers, combined with advances in molecular manipulation of DNA, it is possible to construct certain molecules designed to knock out or modify the expression of a mutated gene.[6] This incredible increase in capability foreshadows the development of immensely effective medical treatments from diseases such as cancer, Alzheimer's, or diabetes and even the ability to edit the genetic errors associated with inherited diseases such as cystic fibrosis or sickle cell anemia.

The scientific understanding of cellular pathways is growing at an incredible pace, and with each advance, there is an opportunity for more potent therapies—and potentially more lethal uses. Unfortunately the ability to heal also opens the ability to harm, and current advances have an inherent ability to be used as biological weapons. Beneficial medical

treatments use biotechnology to manipulate cellular function, returning a diseased cell to a nondiseased "normal" state. The application of the same treatment to a healthy cell could result in modifying it to an abnormal state. The enormous capabilities being developed show great promise but have a dark side that cannot be ignored. The idea of advances in biotechnology increasing the biological weapons threat is not new. In 2003 an analysis of gene sequencing and synthesis capabilities found they were following Moore's law of computing power. The analysis also looked at the educational requirements associated with genetic manipulation and found it was no longer exclusive to PhD's but was becoming a global commodity powered by workers holding bachelor's degrees or even certificates of training.[7] In 2006 the National Science Advisory Board for Biosecurity (NSABB) found that commercial synthesis of "small" organic molecules was readily available and routine across the globe.[8] It also found that larger molecule synthesis, and even viral genome construction, was possible but limited to large institutions.[9] This article examines some of the hopes and fears of emerging biotechnology. It is an attempt to survey recent medical advances made possible by advances in biotechnology and at the same time remind the reader that these advances also carry a corresponding threat. Such advances will allow fine tuning of any cellular process associated with disease from cancer to metabolic imbalances but could also become extremely efficient, targeted biological weapons. Because it is not feasible to identify every possible technology or advance, this work focuses on a small sampling of the research published within the past three years.

The Hopes of Biotechnology

Much of the recent research concerns increasing knowledge about the human genome and the proteome, combined with an increasing ability to model and construct custom molecules.[10] This combination is allowing medicine to produce custom therapeutics designed to cure disease by modulating cellular action at the molecular level. The most-helpful, rapidly emerging biotechnologies highlighted here include computer modeling and genomic modification.

Computer Modeling and Analysis and Synthetic Drug Design

There are numerous ways to artificially interfere with the actions of proteins and cause a change in a cell's behavior.[11] For example, any process that changes the shape of a protein can have an impact on cellular function. As knowledge regarding the fundamental structure of proteins grows, researchers are increasingly able to apply that knowledge to engineer novel molecules (drugs) designed to modify the protein's activity, hence affecting cellular function and "curing" the patient of the associated disease condition. However, these advances in biotechnology are tied to advances in computer modeling capability, which is allowing greater understanding of protein activity within the cell.

While the idea of altering cellular communication using engineered molecules appears straightforward, it requires significant computational capabilities. The ability to visualize a protein and predict its actions requires information on its fundamental sequence (DNA and/or amino acid) coupled with the computing power to calculate the thousands of molecular interactions that drive the three-dimensional shape (and hence functionality) assumed by the protein. The model must then predict the multitude of chemical interactions among the protein of interest and other notional molecules with therapeutic potential. Today we have reached a point in sequence data and computer power where it is possible to model complex proteins and even protein/drug interactions without an actual laboratory.

Pharmacophore modeling is a process where a molecule is modeled in three dimensions. The model allows researchers to screen other molecules and select those that demonstrate (in the computer) the ability to interact with the target molecule. This allows a relatively quick and cost-effective method to screen hundreds to thousands of compounds without requiring individual cell cultures for each screen. By combining different models and programs, researchers are able to screen thousands of compounds and ultimately predict the molecular interactions of candidate molecules down to amino acid position, type of bonds, and even molecular distances.

This ability to model molecular structures and chemical interactions is fundamental to the idea of rational drug design, where researchers can create molecules designed for specific molecular interactions. This idea is not new, but its effectiveness has been limited by protein data and modeling capability. Today, computer models have improved to a

point where researchers are not only able to screen existing compounds for potential interactions but can also use models to reverse engineer synthetic molecules (drugs) designed to interact in specific ways with the target molecule.[12] The ultimate goal is to perfect modeling of therapeutic molecules to a state where "treatments are custom-designed and based upon the molecular genetic profile of normal versus cancerous tissues in patients."[13] In other words, each individual cancer patient will be screened and have a custom treatment optimized to match the genetic characteristics of their particular tumor.

Modeling capabilities can therefore be used to quickly identify the most likely candidates for drug development. Researchers can also use these models to examine how different molecules interact with the target, pulling out the most important molecular positions and orientations. This knowledge can then be used as the basis for the rational design of synthetic compounds with an optimal configuration to bind the target of interest. For example, researchers investigating cancer therapies based upon proteins that help maintain DNA structure were able to screen over two million compounds and identify four compounds with significant binding capability and potential utility as anticancer drugs.[14] Additional examples of current medical trials of compounds designed by pharmacophore modeling include compounds designed as modulators of cardiac action,[15] acetylcholinesterase inhibitors for treating Alzheimer's,[16] cell checkpoint modulators for cancer,[17] and enzyme blockers to treat Chagas disease.[18]

In addition to developing novel therapeutics, greater understanding of genetics and proteomics is uncovering previously unknown cellular communication pathways that can then be modulated to increase healing. For example, identification of existing genetic/cellular pathways previously unassociated with disk disease has identified many signal-modulating proteins as novel emerging treatments for disk degeneration.[19] The increased knowledge in signaling pathways is being directly translated into medical treatments, where signaling proteins or genes are being harnessed to construct cell-instructive biomaterials. These are synthetic materials supplemented with molecules known to enhance healing and regeneration within the graft or scaffold (for bone and tendon repair). The supplemental molecules mimic natural regenerative signals, controlling processes necessary for healing such as cellular adhesion, differentiation of cells, and growth of new blood vessels.[20] These pathways

can be modeled for each individual tissue type, and this knowledge is being used to fine tune the administration of growth factors and even to genetically modify stem cells that are injected into injury sites to control and enhance the repair process.[21]

Genomic Modification

The dream of genetic therapy—fixing genetic-based diseases by changing an individual's DNA sequence to reverse harmful mutations—has been around for many years. In theory, genetic modification is straightforward, but in practice, it requires an in-depth understanding of the organisms, normal versus mutated genetic sequence, the ability to predict which changes need to be introduced into the DNA to produce the desired result, and an ability to affect those changes without destroying the organism.

Twenty years ago, the total content of the human genome was unknown. To sequence the human genome, the US government funded the Human Genome Project, a groundbreaking program to read the approximately three billion bases of DNA contained in the human genome. The project ran for 13 years (1990–2003), with a total expenditure of $3 billion—which supported many biotechnology advances in addition to directly sequencing the human genome.[22] Today, sequencing capabilities have advanced to the point where commercial companies offer to completely sequence a human genome sample (with 30x coverage) for approximately $1,500 in about two weeks' time.[23] Armed with this vast amount of sequence data and an incredible increase in the ability to manipulate DNA, researchers are able to glean information about disease at the DNA and protein levels. Sequencing and computer analysis can also help researchers better understand the cellular and genetic processes that underlay "traditional" or even "ancient" homeopathic treatments. This knowledge then allows scientists to refine and tailor existing drug regimens.[24] Several different techniques for modifying DNA for medical purposes have advanced to the point where they are being tested on humans in clinical environments or approved as drugs. These include virus manipulation, genome editing, noncoding DNA, and epigenetics.

Viral Manipulation

Viruses are infectious particles that use host cells to replicate, and the idea of harnessing viruses as a mechanism to deliver engineered DNA

into a host cell is quite common. Viruses replicate by injecting their genetic material into a host cell, which then hijacks the host cell into producing progeny virus particles, and often target only specific subsets of cell types within an organism. While viruses present researchers a natural way to deliver therapeutic DNA, the need to understand the genetic code and an ability to precisely manipulate viral genetic material has historically worked against this approach. Today, as knowledge and techniques advance, the ability to use a virus to alter a target cell's DNA as a mechanism to combat disease at the genetic level is becoming a reality. A review of treatments for arthritis alone lists nine different examples of virus-based gene therapy being used to modulate inflammation.[25] Viral- and nonviral-delivery gene therapy are also being investigated for disk restoration, tendon repair, and bone repair.[26]

Another advance in the manipulation of viral genetics is the increasing use of chimeras—novel viruses constructed from the genetic material of at least two different "parent" viruses. In theory, it is possible to create novel viruses that combine desired traits from both parents. The idea of viral chimeras is not new and was pursued by the Soviet biological weapons program in an attempt to enhance the effectiveness of their weapons.[27] While it is unknown if the Soviets succeeded, viral chimeras are commonly used today as research tools. For example, researchers investigating the immune system may take a known disease-causing virus and modify it by adding novel genes from another virus that affects the host's immune system. They will then infect an animal with this chimeric virus to gain an understanding of how the immune system works.[28] One such experiment—which caused extreme concern in the biodefense community—was a mouse pox virus modified with genes to modulate the mouse immune system. When tested in the laboratory, the modified virus killed almost every infected mouse, including previously vaccinated mice and strains bred to be disease resistant.[29] The ability to manipulate the immune system is an important tool for researchers and offers potential for medical treatments but could have extreme implications if used to enhance the lethality of a biological agent.

While viral chimeras are a routine tool in laboratory practice, they are becoming common in therapeutic roles, for instance in vaccine production. A live, nonattenuated vaccine constructed from Eastern equine encephalitis (EEE) virus and Sindbis virus has demonstrated the ability to protect primates from EEE.[30] A small sample of some other chimeric

vaccines include Rift Valley fever/Moloney murine leukemia virus tested in mice,[31] a Japanese encephalitis/yellow fever vaccine virus in use in humans,[32] and a multistrain human papillomavirus has been tested in mice.[33] While viruses serve as one mechanism to modify the genetic code, the process suffers from many biological obstacles that are beyond the scope of this review.

Genome Editing

Genome editing refers to the ability to directly modify the DNA sequence of an organism without relying upon an intermediate mechanism, for example a virus or radiation, to induce genetic changes. With adequate sequence knowledge and the appropriate molecular tools, one could—in theory—modify any section of DNA. It would be possible to turn a gene off, turn a gene on, or alter the expression patterns or product of a particular gene. While several editing techniques have been available in the past, they were relatively inefficient and required a relatively high level of sophistication to employ.

Recently a revolutionary genetic editing tool referred to as CRISPR/Cas9 has been developed and commercialized.[34] This tool is so powerful it was specifically identified by retired USAF Lt Gen James R. Clapper, director of national intelligence, as a potential bioterrorism threat.[35] This molecular system allows researchers to design an experiment in which they can modify any region of DNA essentially at will. The technique has been perfected and commercialized to the point where reaction kits are available online for hundreds to thousands of dollars. A simple library database search for "CRISPR"—limited to the last two years—returned over 2,000 journal articles, which is a rate of almost three per day. Just a few examples of human CRISPR-related research areas include beta-thalassemia, retinal cell regeneration, generation of human organs from pigs, and generation of entire knockout libraries of the human genome. Chinese researchers have used this technique to increase muscle mass and hair production in dogs and goats and alter the neurological development in monkeys. They have even attempted to correct the genetic mutation responsible for beta-thalassemia in human embryos, although all attempts so far have failed.[36] This technique is moving into the commercial space as well. In 2015 Bayer HealthCare Pharmaceuticals announced a joint venture with CRISPR Therapeutics to "discover, develop

and commercialize new breakthrough therapeutics to cure blood disorders, blindness, and congenital heart disease."[37]

In addition to the potential to modify genes at will, the CRISPR also holds the potential to allow researchers to develop a "gene drive" system, where traditional Mendelian inheritance and Darwinian survival no longer dictate the prevalence of a gene within a population. Under normal conditions, the prevalence of a gene through a population is controlled by the number of parents with that gene in the population and the statistical likelihood that their offspring will inherit that gene (Mendelian inheritance). The spread of a mutation is also influenced by its contribution to the fitness of an individual; genes that cause disease or disadvantage will not spread rapidly, if at all, through a population, while those genes that offer an advantage will be more likely to spread (Darwinian survival).

Using CRISPR, researchers are able to construct mutations that drive the gene through a population much more rapid than predicted by Mendelian genetics and do so with no regard for the increase or decrease in fitness associated with the mutation. These drives offer the potential to insert and drive a mutation into a population within a few generations—even if detrimental to the offspring. A drive could be of great benefit if used to insert a beneficial trait quickly to a native population of insects or plants. Conversely, a drive could be used to weaken or even lead a population to extinction.[38] The use of genetic modification and drives to control insect populations is being commercialized by at least one company, which has proposed the use of genetically modified mosquitoes to control the current Zika virus outbreak.[39]

"Dark" or Noncoding DNA

As science learns more about the genetic code and its physical structure, the simple DNA → RNA → protein model for information flow becomes more complex.[40] It is known that the vast majority of the human genome does not contain sequences that directly result in proteins. Years ago, this noncoding DNA was seen as "junk" or evolutionary baggage that may or may not serve any practical purpose. As sequencing and computer analysis advance, researchers are identifying significant regions of DNA previously regarded as junk that demonstrate the ability to impact cellular function without coding for a functional protein, as would a traditional gene. Two examples of nonprotein-directed control

over DNA expression are noncoding RNA and physical alteration of the higher order structure of the DNA molecule itself.

Traditional thinking held that an RNA molecule needed to be translated into a protein in order to influence cellular function through the subsequent action of the protein, which has been found to be false. MicroRNAs (miRNA) are a class of RNA molecules that do not code for proteins but instead are produced for the express purpose of interfering with other message-carrying miRNA molecules, hence stopping protein production. MicroRNAs are believed to play a role in functions such as controlling tissue development or maintaining homeostasis.[41] Imbalances in miRNA expression have been implicated in diseases such as cancer, fatty acid metabolism, glucose metabolism, and pancreatic function and have been implicated in viral pathogenesis.[42] Therefore, it is not surprising that pharmacy and academia have explored the potential use of miRNAs to treat disease, for example developing miRNAs to target components of the inflammatory response implicated in arthritis.[43]

A fundamental understanding of the genetic component of disease also gives researchers the ability to mimic miRNA's behavior through the employment of antisense treatments. An antisense treatment or drug is a synthetically designed and constructed oligonucleotide—a short section of DNA or RNA, often single stranded—that has a genetic sequence capable of binding a cell's genetic material and interfering with the normal flow of genetic information. An antisense code is the negative image of the normal information contained within the cell. It can be used to block the message being produced by the cell, in essence 1 (sense) + (-1) (antisense) = 0 (no signal). To successfully develop an antisense treatment, two requirements exist: "silencing of specific genes in a defined population of cells which will produce therapeutic benefits" and "surface receptors expressed specifically on the cell population of interest that can deliver RNA ligands intracellularly."[44] In other words, one must know the specific gene or signal to target and have the ability to deliver the therapeutic molecule to the specific cells responsible for the disease.

Epigenetics

Epigenetics is another area of genetic regulation where gene expression is controlled by factors outside of the core DNA → RNA → protein construct. Specifically, epigenetics refers to the idea that factors external to the actual information contained within the gene sequence also affect

the physical appearance of an individual. An example of this phenomenon is the role the three-dimensional structure of DNA molecules play in genetic expression. Genes can be turned on or off based on changing the shape of the DNA molecule, regardless of the fundamental genetic sequence. In this case, a gene turned off by an epigenetic modification will not have a chance to influence the cell by producing a protein.[45] An understanding of epigenetic factors could allow researchers and therapists to selectively turn on or off copies of genes within an individual's genome by modifying the structure of the DNA molecule versus changing the genetic sequence as would be done in genetic engineering. A recent review of epigenetic research on cancer examined studies in which researchers have been able to modify the DNA structure to either alter cellular development or reprogram cancer cells (on or off). The review identified 17 significant studies during the last 10 years in which researchers developed the ability to reprogram cancer cells and judged that six of the techniques had commercial therapeutic potential.[46]

Epigenetic studies are also revealing that DNA has different characteristics within distinct human populations. One difference is DNA methylation, wherein the DNA molecule is chemically modified at specific sites. Methylation can turn off gene expression and is thought to be one mechanism used by the body to regulate the ability of different tissues to express different genes.[47] Methylation patterns can be inherited but also show changes within organisms as they transition through different phases of development and aging.[48] There is also evidence that methylation patterns differ within populations. A recent study comparing male versus female DNA found a significant difference in the methylation pattern between male and female genomes. The study found 1,184 regions with stable methylation differences and argues that "the differences between men and women are so substantial that they should be considered in design and analysis of future studies."[49] Other research has demonstrated that the methylation patterns of cancer-associated genes differ between ethnic populations.[50] Knowledge of unique methylation patterns may be used to enhance a particular treatment but, in theory, could also be used to design weapons that target individuals with specific methylation patterns, leaving other parts of the population untouched.

The Future of Biotechnology and Medicine

As shown by these few examples, the worlds of genetics, proteomics, and medicine are converging—aided by advances in computing power. This convergence has allowed researchers to dig deeper into the fundamental causes of disease states. The deeper we dig and the more we understand, the more we are able to develop treatments with increasingly narrow focus and much greater effective action. This has allowed us to go from chance observations of mold growth on a petri dish killing bacteria (penicillin) to systematic and deliberate design of compounds targeted against specific molecular links in the disease process. Analogously, medicine is moving from World War II's firebombing of entire cities toward today's GPS/laser-guided weapons that hit within feet of the target. We are developing the ability to cure disease by reaching into the genome or proteome and modifying single DNA bases or blocking specific molecular bonds, giving modern medicine unprecedented ability to restore healthy processes within the cell.

These advances give hope for a new era of medicine in which cellular imbalances can be treated and genetic disorders can be fixed at the tissue or even embryonic stage. Instead of using insulin injections to manage diabetes, it is possible to envision the ability to infect pancreatic cells with a virus that alters the genome of those cells, restoring normal insulin production. It may be possible to use custom-designed genes delivered to specific cells through an artificially constructed virus to regrow nerve tissue after spinal cord trauma or program the heart to regrow muscle tissue lost to a heart attack. In the future, every individual's cancer risk could be assessed at birth by screening for cancer-associated genetic mutations. Based upon that assessment, patients could be periodically monitored for abnormal levels of cancer-associated proteins. Those at risk will then be treated to downregulate the expression of risk-associated proteins, preventing cells from becoming cancerous. With our increased understanding of the differences within our DNA, these treatments might be further optimized to reflect methylation patterns based upon gender and even ethnicity. As these advances become routine medical practice, they will represent a new era of medicine dependent as much on modeling and synthesis as trial and observation.

The Fears of Biotechnology

With any scientific advance, there is always the possibility it will be used for harm. Historically, biological weapons have been developed by harnessing naturally occurring pathogens that were especially good at infecting humans and causing disease. The task of the early bioweaponeers was to take these natural agents and turn them into weapons by improving characteristics such as virulence, survivability, and ease of dissemination. Techniques that use genetic manipulation to increase virulence or convey antibiotic resistance have been evolving for decades but have been comparatively slow and labor intensive. Today's advanced techniques, such as CRISPR, will give bioweaponeers almost unlimited ability to modify any virus, bacteria, protein, prion, or parasite with any trait they desire. While there is no guarantee any singe modification would produce a viable "super" agent, the cost and time investments required to conduct a modification are low enough that many different combinations could be attempted with relative ease.

It is also important to remember that plants and animals can also be targets of biological weapons. The massive financial impact associated with natural diseases outbreaks such as foot-and-mouth or bird flu makes agriculture a serious target for an adversary seeking to inflict financial damage while not directly harming human life. This threat must be viewed with the realization that wholesale genetic modification of viable animals is already being performed in laboratories around the world and is being commercialized. The idea of genetic control over disease-vector insects could save millions from diseases such as dengue and malaria. However, what would be the impact of intentionally crashing the bee population, removing a predator from the ecosystem, or making a crop parasite resistant to insecticides?

Fortunately, most of the technologies discussed in this article remain experimental and require extremely sophisticated laboratories. Effective weaponization and large-scale employment of these new capabilities as a weapon would require a dedicated effort by a state sponsor. It is one thing for a medical provider to inject an experimental therapy into a patient but a much more difficult matter to deliver that substance simultaneously to thousands of people in a diverse environment. Traditionally, biological weapons require the agent be ingested, inhaled, or injected into the target—not trivial problems, and ones the US and Soviet pro-

grams spent many years and funds to overcome. Therefore, it is unlikely they present a near-term threat.

However, there is no reason to believe this will always be the case. Genetic techniques that took a 1990s-era graduate student months to master and days to accomplish are now sold as ready-made kits that perform the same process in hours. The commercialization of biotechnology consistently moves today's high-end techniques from sophisticated laboratories to common commercial kits.

As technology evolves—becoming accessible, cheaper, and easier to use—what are the associated threats? Compare the "cutting-edge" technology from 15 years ago—in flip phones, low-definition televisions, and dial-up modems for internet access—with the capabilities available today in a common smartphone, which offers high-definition, wireless internet access from almost any location in the country. The spectacular advances in biotechnology are no less amazing. Now think of the same rate of technological advance 5, 10, or 15 years in the future, and consider some hypothetical scenarios where today's cutting-edge, emerging biotechnologies are now commonplace and are used to produce biological weapons.[51]

One such scenario involves a "garage biologist," lone-wolf terrorist who seeks to create a "stealth" biological weapon to evade detection or medical treatment.[52] Many biological detection systems are based upon antibody recognition. These systems are able to "look" for unique molecules present on the surface of biological agents. To be effective, detection systems must look for markers present on the agent of concern and not present on other nonhazardous background bacteria. In an almost identical process, the body looks for molecular markers on invading organisms and targets them for destruction. Vaccines present the body's immune system with inert "training" targets that teach the body how to identify and eliminate invading organisms.

Both systems rely upon the ability to discern specific molecular patterns to identify bacteria or viruses. However, as has already been discussed, the nature of the surface molecules is related to the genetic information of the organism. An adversary who is aware of our detection techniques or our vaccine components could alter the surface molecules of a threat agent, rendering the detection or protective capability ineffective. To do this, an adversary could model the molecular interactions between the surface molecule on the threat agent and the detection molecule used by

the sensor. Once the reaction is understood, the adversary could model modifications to the agent's surface markers that would negate the recognition reaction. The changes in the surface molecule could then be reverse engineered to the source DNA sequence, which could be modified by a CRISPR-based replacement with a new sequence and resulting surface marker. Successful modification of a pathogen in this manner would essentially make it a "new" threat organism and would require the defensive community to develop new vaccines or new detection capabilities that could take months or years to implement.[53]

Another relatively easy scenario to envision is the development of a new and extremely fast-acting biological toxin. Traditional biological toxins rely upon molecules produced by other organisms that happen to be hazardous to humans. For example the toxin ricin is found in castor beans, and the botulinum toxin is produced by a bacteria. Countries seeking to weaponize these agents simply adapted what nature developed, resulting in a weaponized form capable of mass dissemination and entry into the target. As the agents were from nature, their relative toxicity and method of action were essentially "constants" within which the weaponeers had to work. Improvements in toxicity could come from scouring nature for more toxic versions of the same organism, or the organisms could be mutated in the lab, which was generally a haphazard and time-consuming process.

Emerging biotechnology and computing capabilities will remove the need to scour nature for toxins and will allow weaponeers to custom design their own toxins. The idea of pharmacophore modeling of drugs has already been discussed. One of the uses for this technology was to model the molecules responsible for cardiac polarization.[14] Polarization and depolarization of cells is a critical process utilized by nerve and muscle tissue to convey electrical signals. The polarization process relies upon molecular signals and receptors that open and close gates in the cell's membrane, allowing a change in the electrical potential. As the ability to model the receptors increases, it becomes possible to design, through computer simulation, molecules that will target and inactivate these receptors—hence, shutting the gates and preventing the electrical signal. Assuming an acceptable delivery system, a weaponized form of this type of molecule could shut down a victim's entire nervous system (brain) or muscular system (heart), causing rapid death with little chance of successful medical intervention.

In a more complex scenario using gene silencing techniques similar to those used in cancer treatments, it could be possible to design a RNA-based weapon capable of killing a specific tissue. It is possible to imagine a silencing system that is designed to target and kill only kidney cells. One possible delivery mechanism for such a device would be inserting the silencing genetic code within a viral chimera. A weaponeer could take a highly infectious but nonlethal virus—such as the common cold—and modify it to contain the silencing system. Upon infection, the silencing genes could be triggered and result in the death of the target tissue. Depending upon the dose, effects could range from a minor to total loss of kidney function. Victims would be dependent upon dialysis for survival, causing a massive strain on medical infrastructure and budgets. As science continues to refine the human genome and focuses on identifying the genes and proteins associated with tissue expression, the list of potential targets grows and is available to anyone with an internet connection.

A final scenario is the ability to eliminate a population from nature using the CRISPR-Cas9 system to construct a gene drive. Such a drive system is a reality and is currently in use to control mosquito populations.[54] Introducing a gene drive into a population eliminates the statistical and evolutionary factors used by nature to control the prevalence of mutations within a population. While current gene-drive systems are tightly controlled and designed to prevent spread in nature, a malicious gene-drive system could be used to eliminate an animal population from a large geographic region.

The idea of selective breeding predates knowledge of genetics, starting when the first farmers selectively bred plants or animals with the "best" traits to increase yield. Weak or disease-prone stocks were conversely not selected and reduced from the population. Today's scientists use knowledge of genetics to achieve the same goals as the early farmers. Traits such as size and disease resistance are, at their core, dependent upon the genetic makeup of the organism and hence can be manipulated in the laboratory. Honeybee hive collapse is a real problem in North America and the subject of ongoing research, seeking to discover the cause and at the same time identify genetic traits that convey resistance to the phenomenon.[55] It stands to reason that while some genetic traits will convey increased resistance, other traits will make the bees more susceptible to a particular condition. Instead of looking for a cure, a malicious research

program would focus on identifying genetic traits associated with the most-susceptible populations.

Once identified, a susceptibility gene could be incorporated into a gene-drive system, ensuring that close to 100 percent of the offspring from the engineered bees will carry the susceptibility gene. In this scenario, a large number of engineered bees could be raised in a protected environment; then a large population of engineered drones would be released to outbreed the natural population. Once introduced into the population, the hives in the area would become increasingly susceptible to collapse. The collapse of the bee population in an agriculturally intense geographic area could have enormous secondary effects, as crops that rely upon pollination would crash along with the bee population.

These are only a few hypothetical examples that, while they can be imagined, still require significant effort and resources to actualize. However as scientists continue to develop their understanding of cellular function, one can imagine an ability to interfere with any genetic or chemical reaction responsible for cellular function, essentially making any tissue or cell a potential target for a biological based weapon.

Epilogue

The goal of this work is to inform the defense community of the evolving power of biotechnology in the hope the United States will remain vigilant with its biodefense program. There is no easy answer to the dilemma of the hope and fear in biotechnology. Advances in biotechnology rapidly outpace the ability of governments to regulate. This work is often performed by commercial companies and not necessarily reliant upon government funding. It is also interesting to note that a retroactive review of journals cited in this article reveals less than 50 percent were written within the United States. While regulation or legislation may address some issues, it clearly cannot control the direction and pace of this research.

As a nation and as a military the United States tries to align its defensive programs to account for future threats. However, we have yet to develop a full line of defenses against biological weapons developed during the Cold War. US efforts to deal with flare-ups of diseases such as Ebola highlight our partial successes but also show how resource-intensive and time-consuming it can be to respond to even a known and somewhat expected threat. The issue is not unique to the United States,

as the whole world is facing these issues. The international community has yet to develop an effective enforcement mechanism for the Biological Weapons Convention. The physical detection of biological weapons programs remains extremely difficult, while covert offensive programs have been conducted under the cover of overt defensive or medical programs. In many ways, the world relies upon behavioral norms and moral behavior as much as any other mechanism to prevent a biological attack.

Will these restraints continue in the future, and if not, what can be done about it? The ability to imagine the biotechnology and medical capacities that will be available 10–15 years in the future is often limited by what we experience today. Likewise, it is impossible to predict how "low" today's cutting-edge biological techniques will be pushed by commercialization of laboratory practices. However, it is safe to say that the technology and knowledge will spread worldwide, and it will not be possible for the United States to exert total control over the process.

There is no magic bullet or novel approach for how to keep up with a rapidly evolving biological capability that is only one of many potential threats facing the nation. While "nimble" or "adaptive" responses may be cliché, they are needed and must be fed by the current threat assessment. What we cannot do is assume that these technologies will always be used for good; a strong sense of pessimism or red-team analysis must be practiced if we hope to anticipate the next biological threat before it is employed. SSQ

Notes

1. National Center for Biotechnology Information, "GenBank Release Notes," US National Library of Medicine web site, accessed 28 December 2015, http://www.ncbi.nlm.nih.gov/genbank/statistics.
2. Ibid.
3. DNA is a nucleic acid, a molecule made of pairs of chemical bases (adenosine, thymine, cytosine, and guanine) arranged in sequences that contain information, much in the way a computer code consists of ordered ones or zeroes. In this manner, each base could be thought of as one "bit" of information in a computer code. The cell converts the information within the DNA into another nucleic acid, RNA, in a process called *transcription*. The RNA is used by the cell as a blueprint to build proteins in a process called *translation*. RNA used to produce proteins is called messenger or mRNA. Not all RNA produced in the cell is mRNA, and RNA serves many other biological functions.

Proteins are constructed by creating a string of molecules known as amino acids. The sequence of amino acids is determined by the DNA → RNA sequence used as the blueprint to assemble the amino acids. There are 20 different amino acids that can be used at any one

position. The chemical properties of the amino acids and their relative sequence dictate how the string of amino acids will fold to create a unique shape that directly influences the protein's characteristics. Proteins are the most visible physical manifestation of one's genetic makeup—for example, blue or brown eyes, A- or B-type blood, and so forth. Proteins also perform a variety of less-visible biological functions, such as providing structure, moderating chemical reactions, sending and receiving signals, and moderating cellular growth patterns.

A *mutation* is a change in the information contained within the DNA. The change in information may change the production of a cellular product or mechanism. In and of themselves, mutations are neutral; the environment determines if the mutation is detrimental to the organism, beneficial to the organism, or is neutral toward the fitness of the organism.

4. Steven Moore, "Genetics Company Looking at What Can Make Athletes Better," *Las Vegas Review-Journal*, 14 November 2015; and "Discover the Family Story Your DNA Can Tell," Ancestry.com (web site), http://dna.ancestry.com/?s_kwcid=ancestry+dna&gclid=CLOhnOC43soCFceQHwodTC0GxQ&o_xid=58712&o_lid=58712&o_sch=Paid+Search+%e2%80%93+Brand.

5. Kris Wetterstrand, "DNA Sequencing Costs: Data from the NHGRI [National Human Genome Research Institute] Genome Sequencing Program," Large-Scale Genome Sequencing and Analysis Centers web site, 24 May 2016, http://www.genome.gov/sequencingcosts/.

6. This is most often accomplished by using oligonucleotides—short nucleic acid polymers used in research, genetic testing, and forensics. Oligonucleotides are usually comprised of 13 to 25 nucleotides and are designed to hybridize specifically to DNA or RNA sequences. By binding, they can block or modify the expression of the gene.

7. Robert Carlson, "The Pace and Proliferation of Biological Technologies," *Biosecurity and Bioterrorism* 1, no. 3 (2003): 203–14.

8. A reoccurring theme in the development of biotechnology is the ability to recreate biological process in the laboratory. Many original bioengineering techniques required researchers to harness bacteria and viruses to synthesize or modify molecules. As biotechnology advances, the technology to make DNA, RNA, and proteins from their raw materials using laboratory machines is available and common in all modern laboratories.

9. National Science Advisory Board for Biosecurity, *Addressing Biosecurity Concerns Related to the Synthesis of Select Agents* (Washington, DC: National Institutes of Health, December 2006), http://osp.od.nih.gov/sites/default/files/resources/Final_NSABB_Report_on_Synthetic_Genomics.pdf.

10. The term *genome* refers to the entire genetic component of a particular organism. While nearly every cell contains a complete genome, it only expresses a portion of the genome in the form of proteins. The protein content of a particular cell at a particular time is referred to as the *proteome*. The proteome varies from cell to cell, depending upon function, and can vary within cells over time as the cell responds to external stimuli.

11. Cellular actions, especially in complex organisms, are controlled by complex intracellular communication systems that rely heavily on proteins to function correctly. As with any communication system, for the system to function properly there must be a correct signal or message, plus it must be transmitted correctly, it must have a receiver, and it must be understood. In the case of a cell, a signaling protein is generated from the genetic code in the DNA then (in this example) secreted into the blood. It must then find only the intended target cells, which must recognize the presence of the signal protein and then alter their activity in the correct pattern. In many cases, the interaction between signaling proteins and target cells is mediated by receptors located on the surface of the target cell. The signal and receptor molecules interact through a combination of shape and charge recognition. Mutations in the

DNA can affect the shape of the signal or receptor molecules, altering the flow of information. It is possible to imagine a receptor that, through a mutation, becomes "blind" to the signal or, on the opposite extreme, the receptor behaves as if the signal is always present. In the first case, the target cell will never respond; in the second case, the target cell will be in the response state 100 percent of the time. It is also possible to introduce a third molecule (a drug) that can interact with the signal or receptor molecule, changing the flow of information and cellular action.

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13. Nirmal K. Prasad, Vishnupriya Kanakaveti, Siddhartha Eadlapalli, Ramakrishna Vadde, Angamba Potshangbam Meetei, and Vaibhav Vindal, "Ligand-Based Pharmacophore Modeling and Virtual Screening of RAD9 Inhibitors," *Journal of Chemistry* 2013, no. 15 (2013): 1–7.

14. Ibid.

15. Yuko Yamakawa, Kazuharu Furutani, Atsushi Inanobe, Yuko Ohno, and Yoshihisa Kurachi, "Pharmacophore Modeling for hERG Channel Facilitation," *Biochemical and Biophysical Research Communications* 418, no. 1 (February 2012): 161–66.

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17. Prasad et al., "Ligand-Based Pharmacophore Modeling."

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21. Martin J. Stoddart, Jennifer Bara, and Mauro Alini, "Cells and Secretome—Towards Endogenous Cell Re-activation for Cartilage Repair," *Advanced Drug Delivery Reviews* 84 (April 2015): 135–45.

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34. Clustered regularly interspaced short palindromic repeats (CRISPR, pronounced crisper) are segments of prokaryotic DNA containing short repetitions of base sequences. The CRISPR/Cas9 system is a prokaryotic "immune system" in that it is a genetic/protein combination which is able to "remember" previous viral infections and then cut specific sections of viral DNA, preventing successful infection of the bacteria. The bacteria does this by incorporating short, specific sections of the viral DNA into its own DNA. This DNA sequence then serves as a reference to guide the molecular complex to recognize invading viral genomic material.

When recognized, the complex guides the Cas9 protein to the specific DNA sequence, which is subsequently cut into smaller sections, destroying the virus. Scientists have been able to harness this complex and by reprograming the guide sequence with any desired sequence. In this way laboratories are able to design molecular "scissors" that can be used to cut and modify any sequence of DNA with extreme precision. For an in-depth review see Rodolphe Barrangou, "The Roles of CRISPR-Cas Systems in Adaptive Immunity and Beyond," *Current Opinion in Immunology* 32 (January 2015): 36–41.

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36. Christina Larson, "China's Bold Push into Genetically Customized Animals," *Nature*, November 2015.

37. Mike Orcutt, "Big Pharma Doubles Down on CRISPR for New Drugs," *MIT Technology Review*, 13 January 2016, accessed 22 January 2016, http://www.technologyreview.com/news/545366/big-pharma-doubles-down-on-crispr-for-new-drugs.

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39. Alanna Petroff, "Fighting the Zika Virus with Mutant Mosquitoes," *CNN Money*, 28 January 2016, http://money.cnn.com/2016/01/27/news/companies/zika-virus-oxitec-mosquito-brazil/index.html.

40. The central dogma of genetics is that information flows from DNA, is transcribed to RNA, and then is translated to proteins. The expressed proteins are then associated with physical traits (eye color) or metabolic processes (digestive enzymes). Interruption of the information flow at any point in this process can affect the overall physical nature of the organism. It is generally thought that this mechanism was the primary, and even only, mechanism for influencing the appearance and function of an organism. The identification of DNA sequences that were turned into RNA for the express purpose of modifying expression of a different genetic pathway significantly altered this view.

41. *Homeostasis* is the ability of a cell or organism to maintain a stable internal environment. This includes variables such as temperature, salt levels, pH, sugar content, and so forth. The inability of a cell or organism to maintain homeostasis will result in incorrect behavior or even death.

42. Scott M. Hammond, "An Overview of MicroRNAs," *Advanced Drug Delivery Reviews* 87 (June 2015): 3–14.

43. Venkatesha et al., "Cytokine-Modulating Strategies."

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45. An example of epigenetic modification and expression of appearance is a calico cat. In mammals, the female has two X chromosomes but only uses one to produce proteins. Early in embryonic development one X chromosome in each cell is shut down by tightly winding it, preventing gene expression and creating what is known as a Barr Body. This is a random process. The patches in coat color associated with a calico cat (which can only be female) are the expression of the coat color genes associated with the two different X chromosomes, only one of which can be expressed by any one cell.

46. Açelya Yilmazer, Irene de Lázaro, and Hadiseh Taheri, "Reprogramming Cancer Cells: A Novel Approach for Cancer Therapy or a Tool for Disease-modeling?," *Cancer Letters* 369, no. 1 (December 2015): 1–8.

47. As every cell in an organism contains the same DNA blueprint, it is essential that as the organism develops from a fertilized egg to a fully functional organism different genes be turned on and off at certain places and certain times in development. These requirements remain as a fully formed organism, where different tissues have distinctly different roles and must express only a subset of the full complement of genes. For example, intestinal tissues must produce digestive enzymes, which would not be appropriate for expression in another tissue, such as the skin.

48. Adrian Bird, "DNA Methylation Patterns and Epigenetic Memory," *Genes & Development* 16, no. 1 (2002): 6–21, doi: 10.1101/gad.947102.

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51. For the purpose of this paper, many of the technical complexities of these scenarios are assumed away. Pathogens (and all organisms) have evolved specific molecular structures and biological process to fit their specific life cycles. It is often the case that altering one trait causes follow-on effects in other biological processes. For example, increasing the ability of a natural pathogen to evade detection may also eliminate the ability of the new mutant strain to cause infection. Therefore, one is not able to simply modify one trait in an organism and magically create superweapons. Modification of biological agents takes significant time and effort. However, as our fundamental knowledge of biological pathways increases, it will be easier to target specific pathways while eliminating collateral effects in other areas of the organism.

Also ignored are many of the details that would require effective "weaponization" of a particular agent. Causing mass casualties with a biological agent requires an effective agent but also requires an effective delivery system. Affecting hundreds or thousands of individuals requires sophisticated delivery techniques. Assuring effective delivery mechanism and ensuring agent viability are often contradictory and represent a difficult balance to achieve.

However, while these scenarios are completely hypothetical and chosen arbitrarily, all of the technical capabilities described already exist and are "common" practice in high-end biological laboratories.

52. *Garage biology*, *DIY biology*, and *biohackers* are terms associated with a real community of amateur biologists who set up functioning microbiology laboratories in their homes. For only several thousand dollars, it is possible to acquire the equipment to do genetic manipulation of bacteria.

53. This scenario is not in itself revolutionary or novel, but the increase in knowledge and genetic engineering techniques make it easier, faster, and cheaper to do—while requiring less education.

54. Kevin M. Esvelt, Andrea L. Smidler, Flaminia Catteruccia, and George M. Church, "Concerning RNA-guided Gene Drives for the Alteration of Wild Populations," *eLife* 3 (July 2014), https://elifesciences.org/content/3/e03401; and Alanna Petroff, "Fighting the Zika Virus with Mutant Mosquitoes," *CNN Money*, 28 January 2016, http://money.cnn.com/2016/01/27/news/companies/zika-virus-oxitec-mosquito-brazil/index.html.

55. The cause of hive collapse is currently unknown, but it may be a combination of viral and fungal infections, mite infestations, and pesticide effects. Brian Dennis and William P. Kemp, "How Hives Collapse: Allee Effects, Ecological Resilience, and the Honey Bee," *PLoS One* 11, no. 2 (24 February 2016), e0150055, doi: 10.1371/journal.pone.0150055.

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