Roundtable on Synthetic Biology
October 11, 2007
Convened by the National Science Advisory Board for Biosecurity
and the NIH Recombinant DNA Advisory Committee
Bethesda, Maryland

Welcome and Opening Remarks

Paul Keim, Ph.D., Northern Arizona University and the Translational Genomics Research Institute in Phoenix, Arizona, and voting member of the National Science Advisory Board for Biodefense (NSABB) committee, opened the roundtable, which was hosted by the NSABB committee and the NIH Recombinant DNA Advisory Committee (RAC).

Dr. Keim explained that the NSABB was established to recommend strategies for biosecurity oversight of dual use research, taking into consideration both national security concerns and the needs of the research community. The NSABB was given a two-part charge at its first meeting concerning synthetic genomics: to identify the potential biosecurity concerns raised by advanced nucleic acid synthesis technologies and to assess the adequacy of the current regulatory and oversight framework for addressing those issues. The NSABB has completed this phase of its charge and issued its findings and recommendations in its report *Addressing Biosecurity Concerns Related to the Synthesis of Select Agents*. The NSABB Working Group on Synthetic Genomes, which was formed to address the first part of the charge, is now poised to address the second phase—to identify and assess potential dual use concerns that may arise from work being performed in the broader field of synthetic biology. The experts gathered at this roundtable will share their knowledge and insights regarding the state of the science in synthetic biology. What is learned today and in future Working Group discussions of dual use potential with synthetic biology will be used to determine whether the field represents any novel biosecurity risks that would not be adequately addressed by the current oversight paradigm that the NSABB recently recommended.

Howard Federoff, M.D., Ph.D., Georgetown University Medical Center, and Chair, RAC, presented an overview and history of the RAC, from its establishment in 1974, in response to concerns raised by the advent of recombinant DNA, to today. He said that this roundtable is an important step in this tradition, because synthetic biology has advanced to the point where it soon will be able to generate novel biological entities that go beyond what can be achieved through traditional recombinant DNA approaches, and current guidance was developed with more traditional recombinant technology in mind. In light of new and advancing technologies, NIH has asked the RAC to revisit the concept of what a recombinant biological entity is and how to ensure that all relevant research is conducted safely. Toward this end, the RAC Biosafety Working Group will be exploring the current and future capabilities of synthetic biology and the implications for biosafety risk assessment and risk management. What is learned today will be applied in developing new draft principles and procedures for the safe conduct involving research using synthetic biological entities. Some of the questions NIH has asked the roundtable to explore include the following:

- What capabilities does synthetic biology provide beyond those achieved by recombinant DNA technology?
- Are there novel or distinct biosafety risks associated with synthetic biology?
- Currently, how are the biosafety risks of synthetic biology being addressed?
- What should be the principles of risk assessment and management for synthetic biology?
- Are the oversight systems in place for recombinant DNA research applicable to synthetic biology research?

Overview of Roundtable

David Relman, M.D., Stanford University, and Chair, NSABB Working Group on Synthetic Genomics, discussed the two phases identified as part of the charge to the group—the first involving issues related to the adequacy of the current regulatory framework for oversight on work involving select agents, and the second constituting a more encompassing set of tasks and issues related to whether there are dual use concerns that arise from synthetic biology, what they are, and how they can be addressed. The USG already has adopted a number of recommendations related to the issue of the current regulatory framework and select agents, among them one recommending that a system for screening sequences be examined and the features of such a system be fleshed out in some detail.

Dr. Relman outlined the day's goals as exploring the state of the science of synthetic biology and how it may be distinguished from other, related fields; considering whether one can predict the biological properties of a DNA construct from sequence and focusing on how we can assess and manage risk in this area. He outlined a number of difficult challenges, one of which is how to define the field in a practical, working sense.

Session 1: State of the Science of Synthetic Biology

Moderators:

Harvey Rubin, M.D., Ph.D.
NSABB Member
Director, Institute for Strategic Threat Analysis and Response
Professor of Medicine, Microbiology, and Computer Science
University of Pennsylvania

Stephen Dewhurst, Ph.D.
RAC Member
Professor of Microbiology and Immunology and of Oncology
University of Rochester

This session provided a broad understanding of the field of synthetic biology—the various research approaches, current capabilities, short and long-term goals.

A Bird's Eye View of Synthetic Biology
Roger Brent, Ph.D., Director and President, The Molecular Sciences Institute

Dr. Brent presented an overview synthetic biology and the significance of the science involving the ability to predict biological function. He noted that biological function is not a term that is

precisely defined, because it operates on multiple levels. Biological function can be explored from the use of high-throughout data, but this has been a slow process, and at the atomic level of function, DNA sequences are by far the best predictor of function. He emphasized that most of the gains in the understanding of individual gene function have come from the study of comparative genomics.

He discussed how systems biologists work, emphasizing that to understand and describe the quantitative behavior of any biological system, one must understand the key phenomena that describe its function and the physics that describes those phenomena. Only with these abstractions, which arise from close, thoughtful observation and consideration of living systems, can one achieve real quantitative understanding: without such abstractions, the work cannot succeed. This, Dr. Brent said, is how biology has been successful in the past. He discussed some scientific studies in this area, including some involving the study of yeast.

Dr. Brent also reviewed the history of recombinant DNA and regulatory schemes over the past 35 years, including the Asilomar model, and he emphasized some noteworthy points on the timeline, including 1994 and the advent of the web browser, the availability of the DNA sequence, and the origins of the term *synthetic biology*.

In the area of policy, Dr. Brent discussed DNA hacking and problems with some of the claims of novelty, because there are many more technical paths to engineering biological systems than are found in the synthetic biology canon. Thus the emphasis on synthetic biology may be distorting focus and causing the policy establishment to miss more important technical developments. He noted that there is no need to assemble long pieces of DNA in laboratories or by direct chemical synthesis; it may be easier and cheaper to do so in coli and in yeast.

He cautioned about the continued democratization and deskilling that is taking place in the hacking of DNA and organisms and about the entry of a new class of DNA hacker—someone who thinks of himself as an engineer of sorts first, rather than a scientist or researcher. He said that even though universities are providing first-rate ethical training to synthetic biologists, our society needs at all costs to avoid the creation and glamorization of such a class of outlaw hackers. Rather, we need to devise workable licensing schemes and a regulatory framework such as Asilomar, which has kept the peace since 1975.

Synthetic Biology as a 20-Year-Old Field
Steven A. Benner, Ph.D.
Distinguished Fellow
Foundation for Applied Molecular Evolution

Dr. Benner began by noting that synthetic genetic biology is nearly 25 years old, no risks have emerged, it underpins therapies that affect about 400,000 patients annually (e.g., with HIV, Hepatitis B and C), and is still constrained by the realities of organic chemistry. He added that there are many approaches to understanding biology—observation, analysis, reductionism, and synthesis. Synthesis guides discovery and innovation in ways that analysis cannot. With the other three approaches, if the data contradict theory, the tendency is to discard the data. In contrast, with synthesis, if at some point there is no agreement between theory and reality, one knows to

stop—something is wrong and the investigator learns something about the system being constructed.

The power of chemistry comes from its ability to develop theory through synthesis of new matter. Several important molecular structures are associated with a major advance in structure theory in chemistry that came via synthesis, and which would not have occurred through analysis, for example development of the compound B12. Nonetheless, you cannot predict function based on structure—perhaps a naïve and overly optimistic hope of scientists involved in the Human Genome Project. Synthesis allows one to create structure to better understand function. The entry of synthesis into biology is a long tradition, dating back to biomimetic chemistry, the study of how enzymes work from serine proteases up to vitamin B12 using enzymes, recombinant DNA technology, and gene synthesis. Techniques such as codon optimization and strategic replacement of restriction sites are now routine in synthesis. In Benner's lab, synthesis has included total synthesis of genes, metabolic pathways, and bottom up synthesis of new proteins and new genetic systems.

Synthesis allows scientists to redesign a system to test theory against the reality of the model. Then one can determine whether the model, which is admittedly abstract, is able to support predictive chemistry. The question that challenges science, and philosophical debate—is whether one can or should create a self-sustaining artificial chemical system capable of Darwinian evolution. In addition, can these unnatural systems work in biological systems—which is the focus of "modern" synthetic biology. Benner stated that the future of synthetic biology is in self-avoiding genetic systems, i.e., DNA that binds to natural DNA, but not to other DNA. He also predicted that synthetic biology will produce: RNA molecules that catalyze template-directed RNA synthesis; understanding of how RNA emerged on prebiotic Earth; a 12-letter genetic system working in living cells; and a broad-based model of systems biology set in paleontological context.

He noted that biology has become more predictive by adopting chemistry's meta-language. It is conceivable that this could allow us to predict virulence, for example. A challenge to the biological paradigm, however, is the notion that we could produce interchangeable parts in biological systems, a framework with which chemists are more familiar.

Dr. Benner concluded by stating that hazard in synthetic biology requires standard biochemistry (parasitism), self-sustenance, and the ability to evolve. In discussion, he agreed with Dr. Relman that it is not just hazards of unnatural origin that cause concern. Others raised the issue of inorganic-to-organic conversions and whether that changes the nature of what we call biology. In addition, one synthetic step can alter numerous other aspects in the organism that might not have been predicted. The more complicated the system the more likely it is that a single atomic perturbation will produce multiplexed, multiple interactions, most of which we do not understand.

Synthetic Biology: From Bacteria to Stem Cells
Ron Weiss, Ph.D.
Professor of Electrical Engineering
Princeton University

Dr. Weiss related that he was a computer scientist by training and became fascinated with the notion that we might be able to program cells with the same ease and capability that we program computers. He noted that synthetic biology uses genetic engineering principles and techniques to figure out ways to design complex systems. To design DNA, we need the ability to synthesize long pieces of DNA very quickly, which requires the ability to understand the system and knowledge of a mechanism by which to take parts in a rational computer-assisted way and put them together to achieve a predetermined purpose. Existing tools facilitate the analysis of systems with hundreds or thousands of components but are not useful for design.

A bottom-up approach assumes, for example, that if you want to have a particular biological property embedded in a biological system, you can take an existing biological system that exhibits behavior that is somewhat close to what you are seeking, and through some mechanism (e.g., directed evolution or cross breeding), get closer to the function that you want. A bottom up approach would be similar to the development of software, in which several versions are tested before arriving at a successful one. Biological systems, however, unlike computer systems are not predictable. In addition, several issues in engineering biology have to be considered, such as:

- Device characterization
- Rules of composition
- Noise
- Cellular context
- Mutations
- Environmental conditions
- Rational design vs. directed evolution
- Crosstalk
- Impedance matching
- Cell death
- Chemical diffusion
- Motility
- Reliance on incomplete models

The systems do not have to be designed in the same way as evolution. The beauty of synthetic biology is that you one can make intermediate systems that are not very functional or highly optimized to work within a particular context. Those might allow us to think about how to make version 2.0 or version 3.0. The goal of synthetic biology is to set the foundation for building modules—for example, cascades, toggle switches, pulse generators, ultrasensitive switches, or oscillators. One can then modify certain attributes of the modules to imagine how the system would then be modified. Computational tools will be needed to understand how the modules will interact within the system, relying on digital logic to understand the potential cascade of events and why certain designs do or do not work.

Dr. Weiss provided an example of applying synthetic biology to stem cell research, which poses a fundamental question in tissue engineering—can we create large scale spatially predefined tissue patterns? He stated that we can use our experience with bacterial synthetic multicellular systems to implement sophisticated rules of interactions between mammalian stem cells that result in spatial patterns of differentiation. The goal would be programmed tissue (re)generation through differentiating stem cells in space and time into desired 3-D patterns. Once the system is understood, the challenges is making it work *in vitro.* The concepts of design and programming

could be extended to engineering mammalian cells to communicate with one another or to program multiple steps of interaction. He cited diabetes research in which a genetic network could be designed to drive beta cell development.

Dr. Weiss concluded by emphasizing that what synthetic biology needs is design principles, i.e., engineering rules. The construction of basic modules can then be tested in applications such as programmed tissue regeneration, artificial tissue homeostasis, and artificial immune systems.

Synthetic Organisms
Steen Rasmussen, Ph.D.
Team Leader for Self-Organizing Systems
Los Alamos National Laboratory

The "bottom-up" approach to synthetic biology attempts to assemble minimal living systems by taking nonliving components and putting them together in a variety of ways. The premise, said Dr. Rasmussen, is if we understand how to make living systems from scratch, we can probably make technology based on the same principles as living systems. That capability would be robust and autonomous, and have local intelligence and the ability to repair itself and evolve. Dr. Rasmussen discussed the difficulty in defining "minimal life," which is notoriously complex. One operational definition is based on three interconnected functionalities that can transform resources into building blocks that grow, divide, and undergo evolution.

Because building protein synthesis machinery is incredibly complicated, it can be sidestepped by designing a metabolic system where the efficiency is determined by the sequence; thus, if you can replicate the sequence you have a hope for selection because bad metabolism/good metabolism means that you can select the best outcome. Rasmussen's work focuses on vesicles—how they grow on a particular structure and how it grows. He is focused on representing, generating, analyzing, and controlling self-organizing and related systemic processes as they are manifested in natural and human-made systems.

Dr. Rasmussen described his work involving assembly of protocells. He emphasized that the question is not whether new simple life forms can be assembled, but under which conditions they can be assembled. He believes that eventually we can use this technology to build protocells that will be increasingly autonomous and able to be weaned off microfluidics support. This "living technology" could have a large socioeconomic impact in 20-25 years. But it will only be realized through a deep understanding of the nature of living processes, which can occur through making life from scratch. If successful, this approach could have applications in the development of self-healing materials, medical diagnostics and treatment, security (the ability to recognize and neutralize bioagents, or modify chemical composition of nuclear waste), environmental protection, and energy production.

Discussion

One of the biggest challenges for synthetic biology—as is the case for any new or evolving field—is arriving at a definition that everyone can agree on. This has implications for oversight and policy. For example, for oversight purposes, if synthetic biology includes designing an organism that is a potentially self-replicating, evolving entity with predictable properties and an

anticipated evolutionary or adaptive rate, does that require oversight? Many participants felt that a critical consideration is whether the new entity is self-sustaining. In fact, the goal of some current research is design for self-replication. The real challenge is building a system that is able to exhibit open-ended evolution. The view from the bottom-up experts was that the science is still very far away from achieving the goal of self-replication.

One more pragmatic goal would be not to predict function of a completely random sequence of DNA but rather to focus on being able to predict functions of a restricted set of DNA sequences. One might then be able to predict the behavior of a single base mutation or at least a small set of mutations from that given set of initial sequences. Function can be defined on many levels, however, a certain level of functional insight is sufficient to predict a given outcome. Thus, both bottom-up and top-down approaches are needed. There was some agreement that the more immediate concerns will arise in the top-down approaches, e.g., modifying mycoplasma through knowledge of certain polymorphisms and their significance.

There was some discussion of building in safety features to any design based on the statistical probability that a given failure will occur. However, biological systems work on different principles than do engineered or computational systems, for example, there is variation in selection, some of which is random and some of which is adaptive. What is needed is a biological programming language that includes all of the underlying features of biological systems.

The discussion ended with broad agreement that although there are no imminent risks raised by synthetic biology, the public, and even parts of the scientific community are misinformed about the goals and limitations of the field; thus, education is essential.

Session 2: Predicting Function

This session addressed current understanding of the relationship between biological properties and sequence and structure, our ability to predict biological function/properties, and the tools that are available or under development for predicting function.

Moderators:

Claire Fraser-Liggett, Ph.D.
NSABB Member
Director, Institute of Genome Sciences
University of Maryland School of Medicine, Baltimore

Nikunj Somia, Ph.D.
RAC Member
Assistant Professor
University of Minnesota, Twin Cities

Form and Context in Predicting Biological Function
William Goldman, Ph.D.
Professor of Molecular Microbiology
Washington University School of Medicine

As a microbiologist, Dr. Goldman studies pathogens, trying to define what is required for

virulence based on sequence—more of an analytical and reductionist approach than a synthetic one. Bacteria are the perfect example of how "form follows function" because the external design of these rather simple organisms reveals the exact architecture of the rigid peptidoglycan skeleton underneath.

Dr. Goldman described a study with *Bordetella pertussis*, the whooping cough agent, where a specific peptidoglycan fragment called "tracheal cytotoxin" was found to be responsible for much of the pathology in the disease. The current model of how this works is that the organisms attached to the ciliated cells in the respiratory tract release tracheal cytotoxin, along with endotoxin (LPS), and that triggers a series of events inside the neighboring cells resulting in production of a large amount of nitric oxide. It is the host cell production of nitric oxide that kills off the ciliated cells and forces their ejection from the epithelium. A few other organisms release little pieces of peptidoglycan for specific purposes, one of which is *Vibrio fischeri*; in this case, the peptidoglycan is important for light organ development in the Hawaiian bobtail squid, which demonstrates a symbiotic use of the same molecule. The significance of this is that a virulence factor in one system is not always a virulence factor in another—it is a matter of host interpretation—and that sometimes function follows form. The behavior of a molecule can be context-dependent, and sequence information will not tell you that.

Another example is *Histoplasma capsulatum*, a fungal pathogen that also causes a respiratory tract disease. It is a dimorphic fungus, existing as either a mold or yeast. It can be switched from mold to yeast just by raising the temperature *in vitro* to 37 degrees celsius. Thus, it changes lifestyle when encountering a mammalian host. The best-studied *Histoplasma* virulence factor is a small yeast phase-specific protein called CBP, and no hints about its function have come from sequence homologs or motifs. However, the 3-dimensional structure of CBP has structural homologs that provide major clues regarding function, even though the primary amino acid sequence did not. In this case, as with *Bordetella pertussis*, sometimes function follows form, and the only way to get at function is to actually look closely at the molecule and do the biochemistry.

Genotype to Phenotype
Jim Musser, M.D., Ph.D.
Co-Director and Executive Vice President
The Fondren Foundation Distinguished Endowed Chair
The Methodist Hospital Research Institute

Dr. Musser described his research involving molecular dissection of epidemic waves and strain genotype-infection phenotype in Group A *streptococcus*, the flesh eating bacteria. One ongoing project is a study of the molecular genetic processes contributing to epidemics and clone emergence (using serotype M1 and M3 strains). Dr. Musser's lab is attempting to understand to what extent they can get to a predictive model of epidemics and clone emergence. They also are attempting to develop a predictive model of what at the genetic level mediates disease specificity. These approaches involve an integrated strategy for studying bacterial pathogenesis, including genome sequencing, development of infection models in the mouse and nonhuman primates, iterative expression microarray analysis, bioinformatics, and human specimens and accompanying clinical data. To date they have 12 Group A strains chosen for their probe-specific

genotype-phenotype relationships, e.g., extremely high virulence or associated with post infectious sequelae like acute glomerulonephritis and acute rheumatic fever.

Dr. Musser's lab has learned that acquisition of bacteriophages expressing novel virulence factors is a crucial issue in clone emergence and disease specificity. Permutation of virulence factors is also an important effect, i.e., strep permutates its genome with mobile elements. In addition, genetic inactivation of one particular gene that results in up-regulation of virulence factors can be important in clone emergence and disease specificity. There are multiple permutations in Group A occurring during its daily activities in the human host. This is all complicated by the fact that Group A streptococci differ at up to 15 percent in chromosomal gene content. Thus, a sequence-based predictive model of behavior is currently not possible.

Other work involves developing a reasonable model system in the human to understand what molecular forces may be contributing to phenotypically distinct epidemic waves. Each distinct epidemic wave has very distinct nonrandom phenotypic traits. However, one clone caused significantly fewer cases of necrotizing fasciitis despite it being the most common genotype. It was subsequently found that a truncation mutation in the mtsR gene—thought to be important to growth and virulence *in vivo*—may have been the factor lowering the virulence of that clone. Dr. Musser noted that numerically speaking, most of the events that differentiate one strain of bacteria from another are single nucleotide polymorphisms. They are modest changes in the genome that contribute significantly to distinct disease specificity. It is critical in moving forward to have an integrative investigative approach in which strains carefully matched with patient phenotype are used. It is likely that in many infectious agents rare alleles are very important mediators of disease phenotype.

Design Considerations for Robustness and Vulnerability in Biological Systems
Marc W. Kirschner, Ph.D.
Chair, Department of Systems Biology
Harvard Medical School

Dr. Kirschner began by agreeing with previous speakers that you cannot really understand a biological entity until you can make it from scratch, a concept that chemists have long embraced. As for prediction, there are three major types of information to predict things from—structure, genes, and databases. Accurate prediction could short circuit very difficult experiments in toxicology and drug design, in terms of both efficacy and of side effects.

It is difficult in many system to predict function because the number of genes in a complex animal is surprisingly low; the number of types of signaling pathways is exceedingly low; and pathways adapt genetically and physiologically. There is also an unexpected paradox of conservation which makes it difficult to explain diversity. The groundbreaking work of Beadle and Tatum did not consider the role of context. Thus, their brilliant effort at the first genotype-phenotype map turned out to be overly simplistic and unworkable for multicellular organisms.

Systems biology aims to understand the versatility of these conserved core processes for the purpose of predicting function, which is very context dependent. Interestingly, it turns out that one of the features of biological system is that the components do not change all that much,

which is why there are so few genes. What does change is regulation. Systems biology needs to understand not only the structure of processes in terms of current use but also their modifiability in evolution. It needs to address on a higher level the robustness of processes; this can only be done on a level where the tradeoffs between constraint and deconstraint can be evaluated. It also needs to understand what is accessible in evolution—the range of each process must be considered not merely the range of the organism. Thus, we need to understand the adaptive nature of the engineered organisms and also the adaptive nature of the hosts that have not been engineered. Dr. Kirschner provided a detailed example of Wnt signaling as a real life circuit, whose structure is just being understood. It raises questions as to why conserved pathways do not change—perhaps form does follow function.

He concluded that we know very little about predicting function. The goal of synthetic biology is a more predictive and ultimately quantitative relationship of genotype to phenotype. Even for microorganisms with relatively small genomes, we have insufficient knowledge to make quantitative predictions. Though we can show genetic requirements, it is much harder to predict fitness for systems that operate far from steady state. Developments in this field should aid in the production of new drugs and in predicting the behavior of organisms in novel hosts.

Design and Use of Predictive Tools: State of the Art
Owen White, Ph.D.
Director of Bioinformatics
Institute for Genome Sciences
University of Maryland School of Medicine

Dr. White began by emphasizing that there is a range of risk in synthetic biology, with the bottom-up approaches—entirely synthesized cells—on one end the spectrum and modified living cells on the other end. He then proceeded to talk about the design and use of predictive tools, specifically, to what degree are we able to explain virulence of the more common bacterial and viral pathogens, based on their genome sequences?

He described a “genome property” as “an attribute of biological organisms that is rigorously defined such that assertion of its absence, presence, or quantitative extent can be made (either automatically or manually) in a self-consistent manner.” The property could be any type of biological processes, including metabolic pathways, observable phenotypes, and quantitative measures of genomic content. Dr. White’s lab employs a pattern recognition method to develop probability tables to determine how closely an unknown sequence matches known sequences (i.e., to determine conserved proteins). Hidden Markov Models (HMMs) allow automated assignment of sequences to homology families. The purpose is to detect families having the same function based on conserved peptide positions.

The properties of a biological system most successfully predicted from its genetic composition include: amino acid metabolism, polyketide and non-ribosomal peptides, and cofactor and vitamin metabolism. The ability to correctly predict pathways was successfully tested by running a new genome sequence for which sequencing had not yet been done. Other investigators are attempting similar predictions based on different approaches, for example, Ross Overbeek has developed something called “Subsystem,” and Eugene Koonin’s clusters of autologous groups.

The next questions are: How accurately can virulence or other pathogenic properties be predicted on the basis of sequence alone? Can the predictions be generalized or are they restricted to a particular pathogen-host system? Advances in this area have been accelerated through bioinformatics and resource centers funded by NIH. The focus of some of these activities is curating genomic sequences of pathogens and looking at different strains to identify products that might become vaccines, therapeutics, or diagnostics. The types of data being gathered could be rolled into a genome property system that could be used to predict virulence of new bacterial genome. As for predicting function from sequence, there is sufficient evidence that regulation might be as important, if not more so, than sequence.

Discussion

The fact that genes code for proteins is but a small piece of the puzzle in predicting function.

With regard to synthesizing a virulent pathogen, there was skepticism that all prediction of function could occur anytime soon since no one has figured out how to synthesize something that self replicates. If someone wanted to act maliciously, it would be far more expedient to modify a known pathogen, e.g., smallpox, to enhance virulence (the "top-down" approach). Loss of function seems to be a central featured of increased virulence, thus, that would be a logical phenomenon to try to understand. Another approach would be to modify the host in some way. There was some discussion about how to characterize risk, especially for dual use research.

A few simple rules for responsible research have already been in place in this area of research. For example, if you want to investigate self-reproducing programs and spread of those inside a computer, you have to simulate a computer inside a computer, that is, you cannot use the operation system itself. History has shown that it is difficult to predict the scenario in which an accident might happen because often it is a series of two or three sequential events. It is also difficult to regulate in a uniform way because the pathogens are so unique, requiring cell and animals models for study. What makes virulence so complex is that much of infectious disease pathology is driven by the immune response of the host, which is highly variable and dependent on immunogenetics. There was agreement that understanding specific organisms is incredibly complicated by their biological context.

Session 3: Risk Assessment and Risk Management in a Context of Uncertainty

This session explored the challenges of assessing biosafety risks in synthetic biology research when there is uncertainty about the biological properties of an agent, how biosafety risk assessment might be approached in such circumstances, and principles and strategies for risk management.

Moderators:

Claudia Mickelson, Ph.D.
RAC Biosafety WG Member
Biosafety Program Deputy Director,
Environment, Health & Safety
Massachusetts Institute of Technology

Michael Imperiale, Ph.D.

NSABB Member
Professor
University of Michigan Medical School

Dr. Imperiale began the session by saying that judgments need to be made regarding what types of risks certain synthetic biological experiments might pose and how they should be managed. He reminded participants that this discussion would go beyond covering human pathogens to elements that affect agriculture, animals, and the environment. Dr. Mickelson added that it is important when communicating to the public about this science to use language that is more descriptive and realistic about its goals. She emphasized the importance of having strong advocates to communicate with the public using paradigms that are easily explained in order to convey the seriousness with which scientists regard this research and their sense of responsibility regarding any risks that may be involved. She said she looked forward to hearing panelists' thoughts about how relevant and reasonable criteria can be developed to assess this research so that it moves forward as rapidly as possible.

Panelists:

Rocco Casagrande, Ph.D.
Managing Director
Gryphon Scientific

Lawrence McCray, Ph.D.
Research Associate, Program on Emerging Technologies
Massachusetts Institute of Technology

Dr. Casagrande focused his comments and observations on risk management and risk assessment as they relate to completely synthetic organisms, suggesting that such observations might help inform biosafety and biosecurity guidelines. He noted that scientists known as bioprospectors bring organisms with which we have no previous experience into the human realm every day, but these scientists are not required to wear extensive protective equipment, largely because they claim that there is no selective pressure on these organisms to be pathogenic. This same argument could be made for the results of DNA synthesis. He also discussed how hospitals and hospital laboratories handle risk from unknown microbes routinely without imposing extreme levels of bioprotection or isolation. Although there always is some risk that someone in a hospital may be infected with a pathogen that requires a higher level of containment, the cost of broad containment is so high that it would cause our infectious disease system to grind to a halt. He also emphasized the astounding diversity of the microbial world and yet the not-so-astounding diversity of emerging pathogens, which generally fall into predictable categories. And, he noted, even when a new virus such as SARS emerges, it is usually related to an existing animal pathogen, because pathogens that have experience with humans already know how to evade the human immune system and elbow out competing microbes. Something completely synthetic that has no experience with humans has a very little chance of doing that.

Dr. Casagrande suggested that it may be advisable to control selective pressure instead of trying to regulate the creation of organisms and that when adding pathogenic components to nonpathogenic organisms, the organism should be treated at the higher BSL level of the pathogen until information is available that indicates otherwise. He suggested that an important question to ask is whether the researcher or the PI is the right person to make the decision about

what is safe. He also said it would be important to ask if a higher level of scrutiny is needed when dealing with known pathogenic elements in nonpathogenic organisms.

Dr. McCray drew on his Washington policy experience at the NAS and in the Executive Office of the President to lay out three questions that the synthetic biology community may want give greater consideration.

[1] From a policy perspective, what is really new about synthetic biology; does it really present a new type of risk beyond what safeguards contemplate? In asking MIT researchers about this, he mentioned that one person responded that the risk wasn't new, but the reduced costs of doing synthetic biology may mean that new classes of people—well beyond academic researchers — may have access to it, and that today's safeguards will not automatically reach those people.

[2] Traditionally, those who are worried about the risks of emerging technologies have put their main effort into prevention. The record of experience, however, suggests that past technological predictions are rarely reliable. Can the research community help find adaptive mechanisms that will detect and respond to any future unexpected adverse effects of synthetic biology?

[3] Several attendees today have expressed concern about future "garage-level" work on synthetic biology—analogous to the garage software shops that are found in the "dot.com" sector. Is this a real possibility, and if so, on what time scale? Can the research community provide input on this question?

Discussion

Dr. Imperiale said that that both the NSABB and the RAC would be interested in hearing about whether there are new or different types of risks that are presented by synthetic biology. If it is believed that there are no new risks and that we can use an existing oversight structure, the situation would be very different from one in which new risks are apparent that will require new means of assessment and management.

There was agreement that what is new in the area of synthetic biology is that the cost is becoming lower and the technology is disseminating and that this is making it easier for hackers to enter the field. It would be difficult to stop them, because at any given time, a half million people have access to “how-to-clone” cookbooks in the United States alone. Some argued that the question is whether the Asilomar paradigm will survive when it becomes possible for those who are not biologists or university faculty to work on their own. It was suggested that the Asilomar paradigm could continue to work, so long as everyone who has access to the technology subscribes to it. The risk of acquisition by adversaries was discussed, as were possible risks involving media reports about a stolen organism that could cause panic and result in severe economic consequences.

It also was suggested that what is new is the ever-increasing enabling technologies of high-throughput sequencing and synthesis, which together create a new capability. The question is whether a new system of oversight is needed and whether our university systems are prepared to

deal with these technologies and their possibilities, such as making recombinant retroviruses that can infect human cells. In addition, it was pointed out that recently the House Energy Commerce Subcommittee held a hearing about the biosafety of high containment laboratories and that GAO questioned whether there has been adequate biosafety supervision and training—issues the scientific community must address.

In summarizing comments on this subject to this point, Dr. Federoff noted that there has been a confluence of new approaches that enable biology to move much faster and that will spawn may new insights that in time will be highly relevant to what the RAC currently considers. These approaches may be of value to us societally, but they also may carry associated risk and will require a high degree of vigilance. As the evidence comes forward, it needs to be evaluated prospectively and it needs to be assessed in the context of whether this represents an apparent risk or a real risk, and if it is a real risk, it needs to be attended to in a formal way.

He also outlined how the two subcommittees and ultimately the parent advisory committee bodies might be able to constructively interact and said that more could be accomplished at this gathering to codify an effort involving risk evaluation and mitigation that is based on biological context. The items that warrant the most discussion are those that lie in the gray area regarding their purview under the RAC, and it is the biological context that makes them relevant.

Further discussion involved how those present have been dealing with biosafety as it relates to the kind of work presented in the morning sessions. Most reported a perception of no or low risk in their activities, and participants described some of the systems used for analyzing and understanding risk. One comment was that although the current NIH Guidelines speak primarily to traditional recombinant techniques, they also speak to synthetic DNA, but not unequivocally.

It was suggested that developments in the field of risk assessment be monitored, and there was some discussion of broadening the charge to include the synthesis of infectious agents not just by infecting existing replicating cells but also by using in vitro methods. Participants agreed that most of what is under discussion already falls under existing guidelines, but that there are concerns involved in bridging the line between chemistry and life.

Dr. Weiss ended the session by proposing that it may be useful to envision a spectrum where on one end there are the existing definitions perhaps by the RAC that genes and organisms define risk and on the other end there are random DNA sequences for which we have no way of predicting what may happen. Somewhere in the middle may be the notion that we are trying to create new DNA sequences that are sufficiently different from what exists now. Because it would be difficult to establish well-specified guidelines for dealing with this middle area, it might be best in such cases to have the investigator who is familiar with a particular project conduct a risk assessment.

Closing Remarks

Dr. Imperiale and Dr. Relman thanked all participants for their contributions and emphasized the importance of involving the public in continued discussions.