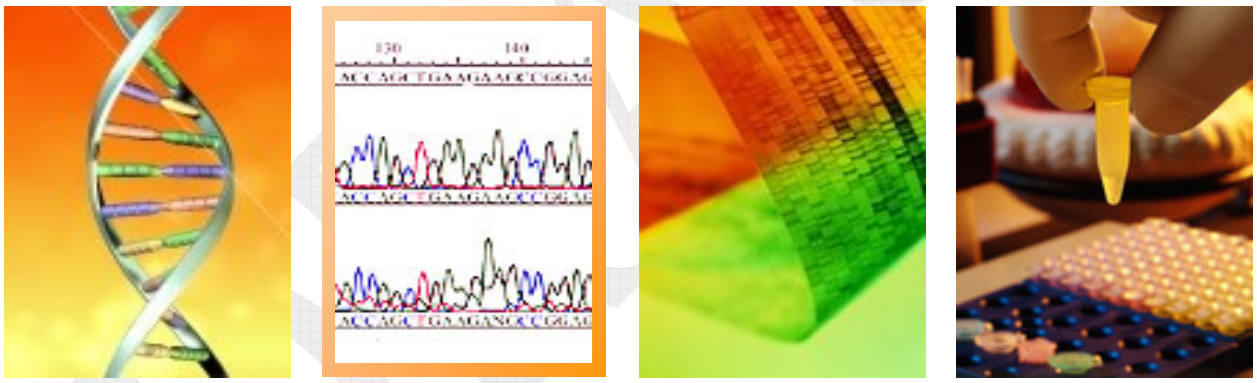
NATIONAL
SCIENCE
ADVISORY
BOARD FOR
BIOSECURITY

ADDRESSING BIOSECURITY CONCERNS RELATED TO SYNTHETIC BIOLOGY



DRAFT Report of the National Science Advisory Board for Biosecurity (NSABB)

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ABBREVIATIONS AND ACRONYMS

BSO	Biological Safety Officer
IBC	Institutional Biosafety Committee
NIH	National Institutes of Health
NIH Guidelines	*NIH Guidelines for Research Involving Recombinant DNA Molecules*
RAC	NIH Recombinant DNA Advisory Committee
USG	United States Government

EXECUTIVE SUMMARY

The National Science Advisory Board for Biosecurity (NSABB) was established by the U.S. Government (USG) to provide advice, guidance, and leadership regarding the oversight of dual use life sciences research – that is, research with a legitimate scientific purpose that yields information or technologies that may be misused to pose a threat to public health or other aspects of national security. In this report, the NSABB addresses the biosecurity and dual use research concerns that may arise from work being conducted in the nascent field of synthetic biology. The report considers synthetic biology in the context of the NSABB's proposed oversight framework for dual use research[1] as well as the biosafety guidelines that are described in the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. Specifically, it describes the assessment of any biosecurity concerns presented by the ability to synthesize new genes, biochemical pathways, and biological components with specified or novel properties, and ultimately by the design of genetic systems, devices, and organisms with specified functions. As part of this assessment, the report examines whether all such biosecurity concerns would be adequately addressed by current and proposed oversight frameworks.

The term "synthetic biology" is used and defined in a variety of ways within the scientific community. Indeed, the dynamic nature of science all but ensures that a precise definition of "synthetic biology" will remain elusive and will evolve over time. The power of synthetic biology, as more broadly envisioned, reflects the possibility of synthetic biological systems that are programmable, self-referential, and modular. The ability to reformat the modules (e.g., the use of altered genetic codes or unnatural amino acids) leads to increased information content and vastly expanded possibilities for new function. Functionally, two disparate experimental approaches can be used to describe synthetic biology: "top down" and "bottom up." The goal of both is to create novel biological structures with predictable properties and functions. The top down approach is related to classical recombinant DNA approaches and involves the re-engineering of existing organisms or genomes for a defined purpose. Increasingly powerful methods for DNA synthesis and assembly have significantly accelerated re-engineering capabilities, however. The bottom up approach entails the assembly of biological components in a variety of novel ways. This approach attempts to assemble systems (both living and non-living) that perform desired functions in a predictable manner. Additionally, synthetic biology can be viewed even more broadly to include several emerging areas of study (e.g., nanotechnology, biocomputation), and we note that the biosecurity risks and dual use potential of new technologies and scientific information, regardless of their fields of origin, will remain important considerations for all areas of science.

Synthetic biology is a rapidly evolving field, and, given its potential benefits to public health and national and economic security, research in these disciplines should be encouraged and maintained. There are still several uncertainties surrounding the relatively nascent field, however. For example, one aspect of synthetic biology that differentiates it from other scientific disciplines is the degree to which it relies and depends on the ability to predict biological characteristics from nucleic acid or protein sequences or structures. But considerable scientific

[1] National Science Advisory Board for Biosecurity, *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information* (Washington, DC: June 2007), http://oba.od.nih.gov/biosecurity/biosecurity_documents.html.

advancement still is needed for a sound understanding of how sequence, structure, and biological context contribute to biological properties, underscoring the difficulty in predicting how new biological materials will function.

Compounding this uncertainty is the pace at which synthetic biology is evolving, the sheer amount of new information the field is producing, and the growing diversity and number of practitioners. New approaches that enable genomes to be manipulated, rearranged and engineered on a large scale provide the ability to generate novel organisms whose properties are unknown. As with all scientific fields, it is often impossible to predict which novel discoveries will be made, but with synthetic biology this seems particularly challenging as technologies and new applications are being rapidly developed by especially varied practitioners.

This issue of diverse practitioners is particularly relevant with respect to synthetic biology because the field itself is somewhat of an amalgamation of several different disciplines. Synthetic biology has been described as "engineering biology"[2] and includes traditional life scientists as well as engineers, chemists, materials scientists, computer modelers and others. Moreover, the allure of synthetic biology has attracted the private sector, students at all levels (including high school), and amateur scientists who may lack formal institutional affiliations. Together, these practitioners include individuals of different ages and dissimilar social and educational backgrounds who may not have been sensitized to ethical, social, and legal norms and expectations of the traditional life sciences research communities.

Diversity among researchers is valuable for the scientific enterprise. But, since current biosafety and biosecurity paradigms address life sciences research conducted at research institutions, there well may be gaps in oversight resulting from the large numbers of synthetic biology practitioners who come from backgrounds that are not traditionally considered life sciences or who lack formal institutional affiliations. This highlights the importance, and the challenge, of raising awareness about dual use research issues and biosecurity concerns among individuals outside the life sciences, within the private sector and unaffiliated with research institutions.

Given these considerations for biosafety, biosecurity, and the dual use aspects of synthetic biology, the NSABB has developed the following recommendations:

1. **Synthetic biology should be subject to institutional review and oversight since some aspects of this field pose biosecurity risks.** Biosafety concerns can be adequately addressed by the application of current biosafety practices and procedures. If the proposed amendments to the *NIH Guidelines* are implemented, they will address research with synthetic nucleic acids in a more explicit fashion, including the expansion of guidance on risk assessment and risk management to address unique aspects of synthetic biology. To the extent that synthetic biology may present biosecurity or dual use research concerns, the NSABB has proposed an oversight paradigm that should adequately address such issues, but there is currently no federal policy in place for the review and conduct of dual use research of concern. The NSABB strongly urges the federal government to develop and implement such policy.

[2] Drew Endy, "Foundations for engineering biology," *Nature* 438 (2005): 449-453.

2. **Oversight of dual use research should extend beyond the boundaries of life sciences and academia**. While it is likely that current and proposed oversight paradigms will more than adequately address the biosafety and biosecurity concerns presented by synthetic biology, gaps in oversight remain, primarily due to the large numbers of synthetic biology practitioners who come from backgrounds that are not traditionally considered life sciences or who lack formal institutional affiliations. Moreover, synthetic biology is just one example of an area of science that may pose some dual use research concerns and whose practitioners span multiple scientific disciplines. In other instances, research that is highly relevant to the life sciences or that has implications for public health may be conducted outside the life sciences. Finally, dual use research of concern is as likely to be conducted in the private and voluntary sectors as it is in academia and government laboratories, so oversight should be uniform and comprehensive.

3. **Outreach and education strategies should be developed that address dual use research issues and engage the research communities that are most likely to undertake work under the umbrella of synthetic biology.** A critical first step in extending the oversight of dual use research of concern will be raising awareness of the dual use issue among synthetic biology's diverse practitioners, especially among those that have not been participants in recent discussions on this topic. The focus should be raising awareness about the potential biological, dual use, and public health implications of their work as well as the need for considering and addressing any biosecurity risks during the conduct of the research. Education efforts should be developed that target synthetic biology researchers who are a) not subject to federal biosafety and biosecurity requirements (e.g., private sector), b) not formally affiliated with universities or research institutions, and c) students (at all levels). This may present significant challenges, particularly in reaching individuals who lack formal affiliations, but this is important nonetheless.

4. **The US Government should include advances in synthetic biology and in our understanding of virulence/pathogenicity in "tech-watch" or "science-watch" endeavors.** It is appropriate for tech-watch or science-watch activities to identify emerging dual use technologies and new knowledge that could change the calculus about dual use risks and biosecurity concerns. As necessary, the USG should convene workshops to assess or re-assess our ability to create novel or unanticipated types of pathogens and to assess the biosecurity risks or dual use aspects of new technologies and whether they are adequately addressed by the extant biosecurity/dual use research oversight system.

INTRODUCTION

Purpose of this document

The National Science Advisory Board for Biosecurity (NSABB) was established to advise the United States Government on strategies for the biosecurity oversight of dual use life sciences research. At its first meeting,[3] the NSABB was given a two-part charge concerning synthetic genomics and synthetic biology. The first part of the charge was to identify any potential biosecurity or dual use research concerns presented by the rapidly advancing ability to synthesize nucleic acids, especially the genomes of select agents.[4] Within that context, the NSABB was asked to assess the adequacy and applicability of the current regulatory and oversight framework for synthetic select agents. The findings and recommendations of the NSABB on this issue are set forth in a 2006 report, *Addressing Biosecurity Concerns Related to the Synthesis of Select Agents*.[5]

The second part of the charge, and the subject of this report, was to identify, assess and recommend strategies to address any biosecurity or dual use research concerns that may arise from work being conducted in the nascent field of synthetic biology. In accordance with its charter, the NSABB was to consider the potential that information and/or technology stemming from legitimate scientific research could be misused to threaten elements of national security. This report considers synthetic biology in the context of the NSABB's proposed oversight framework for dual use research[6] as well as the biosafety guidelines that are described in the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*). Specifically, it describes the assessment of any biosecurity concerns presented by the ability to synthesize new genes, metabolic pathways, proteins, and ultimately to design genetic systems and organisms with specified functions, and analyzes whether all such biosecurity concerns would be adequately addressed by current and proposed oversight frameworks.

What is synthetic biology?

The term "synthetic biology" is used and defined in a variety of ways within the scientific community. For the purposes of this report, we use the term to encompass a variety of different goals and approaches, including:

- the design and construction of new biological parts and devices[7]—including computational devices, and other functional nucleic acid-based structures;
- the re-design of existing, natural biological systems for specific purposes;[8] as well as

[3] Meeting of the National Science Advisory Board for Biosecurity, June 30 – July 1, 2005 http://oba.od.nih.gov/biosecurity/nsabb_past_meetings.html.
[4] Select Agents are biological agents and toxins regulated by the Select Agent Rules (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73) that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products. A list of Select Agents and Toxins can be found at http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html (accessed 10/29/09).
[5] National Science Advisory Board for Biosecurity, *Addressing Biosecurity Concerns Related to the Synthesis of Select Agents* (Washington, DC: December 2006), http://oba.od.nih.gov/biosecurity/biosecurity_documents.html.
[6] National Science Advisory Board for Biosecurity, *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information* (Washington, DC: June 2007), http://oba.od.nih.gov/biosecurity/biosecurity_documents.html.
[7] http://syntheticbiology.org/ (accessed 9/29/09).
[8] *Ibid*.

- the synthesis of self replicating entities from scratch.[9]

The power of synthetic biology, as more broadly envisioned, reflects the possibility of synthetic biological systems that are programmable, self-referential, and modular. The ability to reformat the modules (e.g., the use of altered genetic codes or unnatural amino acids) leads to increased information content and vastly expanded possibilities for new function. Two disparate experimental approaches are employed in synthetic biology. The goal of both is to create novel biological structures with predictable properties and functions. One is a "top down" approach that involves the re-engineering of existing organisms or genomes for a defined purpose. This might involve the metabolic engineering of microbes to produce useful products such as biofuels (or their precursors[10]) or synthetic molecules tailored for therapeutic purposes. This would also include synthesizing a life form that consists only of a minimal number of functional elements by removing redundant or non-essential genetic material, or that employs a modified genetic code. This so-called "chassis organism" would contain a genome that might be more easily engineered.[11, 12, 13, 14] Another top down approach is to replace a portion of an existing genome with one or more standardized DNA modules that encode single, simple functions.[15] These approaches have made possible the creation of organisms with novel collections of properties. In fact, top down approaches for the re-engineering of microbes first appeared with the emergence of recombinant DNA techniques in the 1970s. Today, "classical" recombinant techniques and synthetic approaches are interwoven in the day-to-day practice of biological engineering. Yet the increasingly powerful methods for DNA synthesis and assembly have significantly accelerated re-engineering capabilities. Laborious, complex, site-directed mutagenesis and ligation procedures are now obviated by directed synthesis of the desired sequence, followed by relatively simple cloning or splicing steps. We use the term "synthetic biology" broadly and view practitioners as those who use classical recombinant molecular biology techniques as well as synthetic DNA constructs as we consider the impact and biosecurity implications of this discipline.

The other experimental approach in synthetic biology is to design and synthesize life forms, materials, or structures from the "bottom up" by taking non-living biological components and putting them together in a variety of novel ways. This approach assembles systems (both self-replicating and non-self-replicating) that perform desired functions in a predictable manner. Bottom up synthetic biology might involve creating artificial cells from nonliving materials with the goal of creating an entity that can regenerate, replicate, and evolve or that could be more easily engineered at the metabolic level. Bottom up synthetic biology may also include biofabrication – the synthesis of new materials or structures by assembling biological components such as nucleic acids, proteins or polysaccharides, perhaps in combination with non-

[9] Steen Rasmussen et al., "Evolution: Transitions from Nonliving to Living Matter," *Science* 303, no. 5660 (2004): 963-965.
[10] Travis S. Bayer et al., "Synthesis of Methyl Halides from Biomass Using Engineered Microbes," *J. Am. Chem. Soc*. 131 (2009): 6508–6515.
[11] Andres Moya et al., "Toward minimal bacterial cells: evolution vs. design," *FEMS Microbiol. Rev*. 33, (2009): 225-235.
[12] Daniel G. Gibson et al., "Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium genome*," Science 319, no. 5867 (2008): 1215-1220.
[13] John I. Glass et al., "Essential genes of a minimal bacterium," *Proc. Natl. Acad. Sci*. 103, no. 2 (2006): 425-430.
[14] C. Lartigue et al., "Creating Bacterial Strains from Genomes That Have Been Cloned and Engineered in Yeast," *Science* 325, no. 5948 (2009): 1693-1696.
[15] The BioBricks Foundation; http://bbf.openwetware.org/ (accessed 10/1/09).

biological components, into novel configurations. As envisioned, the synthetic or artificial life forms synthesized using this bottom up approach will not necessarily resemble or function like extant living cells. Likewise, when assembled, novel biomaterials may function in a different manner than would the material's individual components in their native biological context. The goal is to understand the fundamental nature of living organisms or biological materials and develop technology based on the same principles as those found in living systems.

With both approaches, the synthetic biologist seeks to understand the form and function of living organisms or their products (e.g., metabolites, enzymes, toxins) and utilize them in a predictable and controlled manner. Some describe this endeavor as being more akin to engineering than to biology because it involves taking parts of natural biological systems, characterizing and simplifying them, and using them as components of an unnatural, engineered, biological system.[16] Because of this, synthetic biology is sometimes referred to as engineering biology.[17]

One of the hallmarks of synthetic biology is that it is interdisciplinary in nature. In addition to biologists, practitioners also include engineers, chemists, and computer modelers. In a review article,[18] one of those practitioners, Drew Endy, describes the allure of synthetic biology for different types of scientists:

> …for biologists, the ability to design and construct synthetic biological systems provides a direct and compelling method for testing our current understanding of natural biological systems; disagreements between expected and observed system behavior can serve to highlight the science that is worth doing. For chemists … synthetic biology is an extension of synthetic chemistry; the ability to create novel molecules and molecular systems allows the development of useful diagnostic assays and drugs, expansion of genetically encoded systems, study of the origins of life, and so on. For "re-writers," the designs of natural biological systems may not be optimized for human intentions (for example, scientific understanding, health, and medicine); synthetic biology provides an opportunity to test the hypothesis that the genomes encoding natural biological systems can be "re-written," producing engineered surrogates that might usefully supplant some natural biological systems. Finally, for engineers, biology is a technology; building upon past work in genetic engineering, synthetic biology seeks to combine a broad expansion of biotechnology applications with … an emphasis on the development of foundational technologies that make the design and construction of engineered biological systems easier.

In part, it is this interdisciplinary nature that makes synthetic biology such a promising field because individuals with related interests and diverse expertise can converge on a problem or challenge to produce rapid and profound results.

Finally, "synthetic biology" can be viewed even more broadly to include several emerging areas of study. For example, certain areas often cited as "nanotechnology" aim to engineer viral particles to serve as delivery devices for therapeutics.[19] This type of research clearly incorporates engineering and biological aspects and may be considered synthetic biology by some. Electrical and computer engineers are beginning to develop DNA-based nanoelectronics and "biocomputation" applications[20, 21] that further merge engineering and biotechnology and

[16] http://syntheticbiology.org (accessed 9/29/09).
[17] Drew Endy, "Foundations for engineering biology," *Nature* 438 (2005): 449-453.
[18] *Ibid*.
[19] Ravi Singh and Kostas Kostarelos., "Designer adenoviruses for nanomedicine and nanodiagnostics," *Trends in Biotechnology* 27, no. 4 (2009): 220-229.
[20] Robert D. Barish et al., "An information-bearing seed for nucleating algorithmic self-assembly," *Proc. Natl. Acad. Sci.* 106 (2009) 6054-6059.
[21] Pengcheng Fu, "Biomolecular computing: Is it ready for take off?" *Biotechnology Journal* 2 (2007): 91-101.

expand the boundaries of what is considered "synthetic biology." The dynamic nature of science all but ensures that a precise definition of "synthetic biology" will remain elusive and evolve over time. However, the biosecurity risks and dual use potential of new technologies and scientific information, regardless of their fields of origin, will remain important considerations for all areas of science.

NSABB approach

To examine the biosecurity concerns posed by synthetic biology and to determine whether they are adequately addressed by current oversight paradigms, the NSABB formed a Working Group on Synthetic Biology. The NSABB also hosted a scientific roundtable[22] to explore the state of the science, current capabilities, and future directions. (See Appendix B). Presenters were asked to describe their area of research and also to speak to following:

- how they define synthetic biology and what makes it unique among scientific approaches;
- the goals and experimental approaches in synthetic biology;
- the current capabilities and applications associated with synthetic biology;
- how these capabilities go beyond what is achievable using recombinant DNA or other related technologies;
- the major milestones to date in synthetic biology and the current challenges;
- future directions and goals;
- how close we are to predicting the detailed behavior of a cell based on its component parts;
- how close we are to designing biological systems and novel organisms with predictable functions; and
- whether their synthetic biology research routinely undergoes and biosafety review.

The roundtable was co-hosted by the NIH Recombinant DNA Advisory Committee (RAC).[23] The topic of synthetic biology was of interest to this group as well because the RAC was tasked with assessing the applicability of the *NIH Guidelines* to synthetic genomics and synthetic biology. This tasking was the result of the US government acting on one of the recommendations from the NSABB regarding the need to ensure that biosafety guidance and requirements adequately address work with synthetic nucleic acids and are understood by all those working in areas subject to the biosafety guidance and requirements.[24] Towards this end, one session of the roundtable was focused on current understanding of the relationship between biological properties and sequence and structure, the current ability to predict biological properties, and the tools that are available for predicting function.

The Working Group also received a briefing in September 2009 by the Executive Director of the RAC, Dr. Jacqueline Corrigan-Curay, on proposed updates to the *NIH Guidelines* that address synthetic nucleotides and organisms.

[22] Meeting of the National Science Advisory Board for Biosecurity, October 11, 2007, http://oba.od.nih.gov/biosecurity/nsabb_past_meetings.html.
[23] Recombinant DNA Advisory Committee website: http://oba.od.nih.gov/rdna/rdna.html.
[24] National Science Advisory Board for Biosecurity, *Addressing Biosecurity Concerns Related to the Synthesis of Select Agents* (Washington, DC: December 2006), http://oba.od.nih.gov/biosecurity/biosecurity_documents.html.

In addition, the Working Group received a briefing by Special Agent Edward You regarding the recent outreach efforts of the Federal Bureau of Investigation (FBI) aimed at the synthetic biology community and a 2009 FBI-sponsored conference titled "Building Bridges Around Building Genomes."

Throughout the course of the Working Group's deliberations on this topic, NSABB members provided updates to the group after informally attending meetings (including the FBI conference above) and symposia. NSABB members also informally consulted with subject-matter experts during the process to help inform the group's findings and recommendations.

Because the use of synthetic oligonucleotides is often combined with the use of naturally occurring genetic material and involves recombinant DNA methods and approaches, this report is directed at a broad range of science and scientists and not just those who work exclusively with synthetic DNA.

FINDINGS

The promise of synthetic biology: early days in a rapidly evolving field

During the course of the NSABB's deliberations, it became evident that synthetic biology is both a relatively nascent field, but also a rapidly developing one. Although the term "synthetic biology" was coined in 1912 by Stéphane Leduc in reference to the synthesis of artificial life,[25] the field of synthetic biology as a discipline within the life sciences remains in its relative infancy. However, in recent decades, synthetic biology has benefited from advances in related disciplines, allowing this burgeoning field to develop into one that holds great promise to improve human, animal, plant, and environmental health Advances in molecular biology have allowed genetic material to be readily manipulated, enabling the synthetic biologist to splice virtually any piece of DNA into another. High-throughput DNA sequencing has provided the synthetic biologist with access to the complete genome sequences of more than 980 microorganisms[26] and advances in DNA synthesis technology allows the rapid chemical synthesis and assembly of suites of genes (e.g., the genes encoding a metabolic pathway) *de novo*. A deeper understanding of the specific types and functions of RNA molecules has allowed the synthetic biologist to construct, and program, biological devices.[27] Moreover, advances in other fields such as materials science and protein engineering are allowing the fabrication of new biomaterials.

While many of the ambitious goals of synthetic biology have yet to be achieved (e.g., *de novo* synthesis of a wholly new organism or the ability to reliably assemble biological components into complex systems with predictable outcomes), in recent years the science has matured to a point where some of this field's promise is being realized. Synthetic approaches have resulted in the more efficient production of therapeutics that are less expensive and more environmentally responsible,[28, 29] as well as the development of new materials for tissue and organ growth and

[25] Juli Peretó and Jesús Català, "The renaissance of synthetic biology," *Biological Theory*, 2, no. 2 (2007): 128-130.
[26] http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi?view=1 (accessed 10/7/09).
[27] Maung Nyan Win et al., "Frameworks for programming biological function through RNA parts and devices," *Chemistry and Biology* 16 (2009): 298-310.
[28] Jesse W.-H. Li and John C. Vederas, "Drug discovery and natural products: End of an era or an endless frontier?" *Science* 325 no. 5937 (2009): 161-165.

drug delivery.[30, 31, 32] More efficient means of producing energy are being developed that utilize metabolically engineered microbes that can convert plentiful, renewable resources into hydrogen, ethanol, diesel, or biofuel precursors.[33, 34] In an approach referred to as "synthetic metagenomics," automated chemical synthesis and computational optimization of genes from environmental metagenomic libraries have been used to create symbiotic consortia of engineered organisms that produce significant amounts of precursor molecules for important industrial chemicals and fuels.[35] Plants and microbes also are being engineered for biological remediation applications that offer low-cost, environmentally friendly solutions to pollution and contamination.[36, 37] In addition, synthetic approaches have been employed toward the detection and neutralization of, and defense against chemical and biological threats. Given the great number of potential benefits offered by synthetic biology, encouraging and maintaining a strong research program in this discipline is crucial to the nation's physical and economic security and public-health efforts.

Significant uncertainties

Presentations and discussion at the NSABB-RAC roundtable were particularly helpful for understanding the multiplicity of goals and approaches in synthetic biology, the state of the science of systems biology, and the ability to predict key biological properties such as virulence from nucleic acid sequence. One aspect of synthetic biology that differentiates it from other disciplines is the degree to which it relies and depends on the ability to predict biological characteristics from nucleic acid or protein sequences or structures. This is especially true for the bottom up approach where new organisms and/or structures are assembled using more simple starting materials. However, with all aspects of synthetic biology, there are varying degrees of uncertainty regarding the predictability of biological properties of partially or completely synthetic agents or materials. This is due in large part to the fact that the biological context or environment of a gene or gene product is absolutely key to its function. Biological environment may in fact be as important a determinant of the function of a biological component of that environment, as is the sequence or structure of the component.

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[29] Michael A. Fischbach and Christopher T. Walsh, "Antibiotics for emerging pathogens," *Science* 325 no. 5944 (2009): 1089-1093.
[30] Samuel K. Sia et al., "Synthetic tissue biology: Tissue engineering meets synthetic biology," *Birth Defects Research* 81 (2008): 354-361.
[31] M. P. Lutolf and J. A. Hubbell, "Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering," *Nature Biotechnology* 23 (2005): 47-55.
[32] Stuart Kyle et al., "Production of self-assembling biomaterials for tissue engineering," *Trends in Biotechnology* 27, issue 7 (2009): 423-433.
[33] Stephen Picataggio, "Potential impact of synthetic biology on the development of microbial systems for the production of renewable fuels and chemicals," *Curr. Opin. Biotechnol.* 20, issue 3 (2009): 325-329.
[34] Sung Kuk Lee et al., "Metabolic engineering of microorganisms for biofuels production: from bugs to synthetic biology to fuels," *Curr. Opin. Biotechnol.* 19 issue 6 (2008): 556-563.
[35] Travis S. Bayer et al., "Synthesis of Methyl Halides from Biomass Using Engineered Microbes," *J. Am. Chem. Soc*. 131 (2009): 6508–6515.
[36] Victor de Lorenzo, "Systems biology approaches to bioremediation," *Curr. Opin. Biotechnol*. 19 issue 6 (2008): 579-589.
[37] Elizabeth L. Rylott and Neil C. Bruce, "Plants disarm soil: engineering plants for the phytoremediation of explosives," *Trends in Biotechnology* 27 issue 2 (2008): 73-81.

As a follow-up to a recommendation made by the NSABB in its 2006 report on synthetic genomics,[38] the US Government commissioned the National Research Council to examine the uncertainties surrounding the ability to predict biological function from sequence in a project titled *Scientific Milestones for the Development of a Gene-Sequence-Based Classification System for Oversight of Select Agents*. An aim of this study is "to identify the scientific advances that would be necessary to permit serious consideration of developing and implementing an oversight system for select agents that is based on predicted features and properties encoded by nucleic acids rather than a relatively static list of specific agents and taxonomic definitions."[39] While this project focuses on the scientific advancements that will be required to develop a predictive oversight framework for select agents, it underscores the desirability of and current limitations to accurately predicting biological characteristics from DNA sequences. Clearly, the Council's findings will be highly relevant to the field of synthetic biology.

However, considerable scientific advancement is still needed for a sound understanding of how sequence, structure, and biological context contribute to biological properties. Until those scientific milestones are realized, it will continue to be difficult to predict with any reasonable certainty the biological risk of a synthetic entity, especially one that bears little resemblance to natural organisms. This inability to predict function from sequence calls for greater awareness of potential biosafety and biosecurity risks, screening and characterizing the properties of DNA constructs and engineered proteins and organisms, and greater sharing of practices among the scientific community. Of note, a recurring theme in the NSABB's discussions with experts was that, although synthetic biology poses potential risks, there remain significant limitations to our current ability to custom design and produce novel organisms, especially pathogens, either by *de novo* synthesis or by engineering extant organisms in predictable ways.

Compounding this uncertainty is the pace at which synthetic biology is evolving and the sheer amount of new information the field is producing. Massively parallel DNA sequencing and synthesis approaches are increasing the speed and decreasing the cost of synthesizing virtually any custom DNA sequences *de novo*.[40] New approaches have been developed to engineer entire microbial genomes as well using synthetic oligonucleotides that integrate into multiple chromosomal locations in a rapid, continuous manner, generating combinatorial genomic diversity and accelerated evolution.[41] In a demonstration of this approach, investigators produced up to 15 billion genetic variants, and of this mutagenized population, cells were selected and characterized with enhanced efficiency in expressing the products of a particular metabolic pathway. Other approaches have been developed to drive chromosomal evolution and engineer biosynthetic pathways in microbes.[42] The ability to mutagenize microbial populations is not new and has been employed routinely by researchers. However, new approaches that enable genomes to be manipulated and rearranged on a large scale provide the ability to generate novel organisms whose properties are unknown. The possibility that, during the research

[38] National Science Advisory Board for Biosecurity, *Addressing Biosecurity Concerns Related to the Synthesis of Select Agents* (Washington, DC: December 2006), http://oba.od.nih.gov/biosecurity/biosecurity_documents.html.
[39] http://www8.nationalacademies.org/cp/projectview.aspx?key=49063 (accessed 10/16/09).
[40] David S. Kong et al., "Parallel gene synthesis in a microfluidic device," *Nuc. Acids Res*. 35 no. 8 (2007).
[41] Harris H. Wang et al., "Programming cells by multiplex genome engineering and accelerated evolution," *Nature* 460 (2009): 894-898.
[42] Keith E.J. Tyo et al., "Stabilized gene duplication enables long-term selection-free heterologous pathway expression," *Nature Biotechnology* 27 no. 8 (2009): 760-765.

process, certain selective pressures might lead to the identification of mutants with enhanced virulence presents potential biosafety, biosecurity and/or dual use concerns.

Finally, the uncertainties surrounding synthetic biology are amplified further by the myriad of potential applications being developed by a growing number of practitioners. Synthetic biology is a rapidly developing field, and, as noted above, it is conducted not only by biologists but also by engineers, chemists, material scientists and others, often through interdisciplinary collaborations. It is impossible to predict the information, technologies, and new applications that will be developed by or applied to this relatively new field that is attracting especially diverse practitioners.

In light of this, it is necessary to conduct synthetic biology research under appropriate biosafety and biosecurity oversight. This may require a risk assessment, which may be challenging depending on the nature of the work. For instance, the degree of novelty of the synthetic agent and how closely its biological properties might track to a parental agent(s) may need to be considered. Of particular concern, in terms of a safety and security risk assessment, are experimental techniques, such as directed molecular evolution ("DNA shuffling") and shotgun synthesis that generate very large numbers of recombinants and sequence variants.[43] While it is likely that only a very small fraction of resulting new organisms might be viable, it would be very difficult, if not impossible, to predict biological properties, including virulence.

Current oversight paradigms

The NSABB considered how potential biosafety and biosecurity concerns posed by synthetic biology would fit within current or proposed oversight paradigms and whether any gaps might exist. Currently, basic or clinical research involving recombinant DNA being conducted at NIH-funded institutions is subject to the *NIH Guidelines*. The *NIH Guidelines* outline principles for safe research practices with respect to recombinant DNA molecules. They detail sound practices, including procedures for the containment of various forms of recombinant DNA research, including research involving genetically modified microorganisms, plants and animals, as well as human gene-transfer experiments. Institutions must also establish an Institutional Biosafety Committee (IBC) to review research involving recombinant DNA. Under certain circumstances the *NIH Guidelines* require that institutions appoint a Biological Safety Officer (BSO) to oversee the management of biosafety risks. In addition, the Recombinant DNA Advisory Committee (RAC) was established to advise the NIH Director on the content and implementation of the *NIH Guidelines* and to provide public, in-depth review of the scientific, safety, and ethical dimensions of research and clinical trials that involve the transfer of recombinant DNA to humans.[44]

In 2009, the RAC proposed expanding the scope of the *NIH Guidelines* to include nucleic acid molecules made solely through synthetic means.[45] In general, the RAC has found that, in most cases, research with synthetic nucleic acids presents biosafety risks that are comparable to

[43] Joseph R. Warner et al., "Genomics enabled approaches in strain engineering," *Curr. Opin. Microbiol*. 12, no. 3 (2009): 223-230.
[44] The *NIH Guidelines* and more information about the Recombinant DNA Program, the RAC, and IBCs are available at http://oba.od.nih.gov/rdna/rdna.html.
[45] Federal Register notice of consideration of a proposed action under the *NIH Guidelines*: http://oba.od.nih.gov/oba/RAC/meetings/jun2009/Final%20Published%20FRN.pdf.

recombinant DNA research and that certain work with synthetic nucleic acids in a non-replicating form may not require oversight under the *NIH Guidelines* (although other biosafety standards would apply). It also found that the current risk assessment framework described in the *NIH Guidelines* can be used to evaluate synthetically produced nucleic acids with attention to the unique aspects of this technology. The RAC also has noted how rapidly the field is growing and concluded that safety issues surrounding synthetic nucleic acids will likely need to be revisited in the near future as proposed amendments to the *NIH Guidelines* can only address what is presently known about the science.

Previously, the NSABB has proposed an oversight framework for addressing dual use research concerns for life sciences research.[46] The framework is based on a proposed criterion for identifying research that constitutes "dual use research of concern", and describes principles that should underpin the oversight of dual use life sciences research, key features of such an oversight system, and strategies for assessing and managing the risks posed by dual use research of concern. The NSABB's proposed framework relies primarily on the local oversight of dual use research. Briefly, the NSABB envisions a system wherein principal investigators make the initial evaluation (and periodic re-evaluation) of their work for its dual use potential. Research identified as being potentially of dual use concern would receive additional institutional review and risk-management plans would be developed as appropriate (and would include plans for the responsible communication of research findings). Researchers are the most critical element in the NSABB's oversight framework because they are in the best position to anticipate the types of information or technologies that might be generated by their work. As such, the NSABB emphasized that awareness of the dual use issue must be the foundation of such a system, and accordingly has developed outreach and educations strategies that target relevant communities.[47] There is currently no federal policy for the oversight of dual use research; the NSABB's oversight framework is being considered by the US Government, however, and the recommendations contained in this report assume that a federal policy is forthcoming.

Biosafety and biosecurity concerns

The *NIH Guidelines* and the NSABB's proposed framework for dual use research oversight focus on individuals participating in life sciences research and operating within a university or institutional setting. Not all biologists operate within these settings, however, and this is especially true of practitioners of synthetic biology. In addition, despite the term synthetic "biology," not all practitioners consider that their work is biological in nature; rather, they may view their work as a type of engineering, an extension of synthetic chemistry, or materials science.[48] As such, they may not be considering the biological and public health implications of their work. In addition, many practitioners have backgrounds that are not rooted in the life sciences. Consequently, their training may not have included or emphasized principles and practices of biological risk assessment and biocontainment. Thus, raising awareness within the disparate scientific communities that engage in synthetic biology about the possible biosafety risks and the need for the responsible conduct of research will be critical.

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[46] National Science Advisory Board for Biosecurity, *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information* (Washington, DC: June 2007), http://oba.od.nih.gov/biosecurity/biosecurity_documents.html.
[47] National Science Advisory Board for Biosecurity, *Strategic Plan for Outreach and Education on Dual Use Research Issues* (Washington, DC: December 2008), http://oba.od.nih.gov/biosecurity/biosecurity_documents.html.
[48] Drew Endy, "Foundations for engineering biology," *Nature* 438 (2005): 449-453.

We note that biosafety and biosecurity are two distinct but related concepts.[49] Biosafety typically refers to the policies, practices, equipment, facilities, and medical treatments designed to protect workers and the environment from the accidental exposure to hazardous laboratory agents and materials. Biosecurity refers to the protection, control of, and accountability for high-consequence biological agents and toxins, and critical relevant biological materials and information, to prevent unauthorized possession, loss, theft, misuse, diversion, or intentional release. The concept of 'dual use research of concern' is an aspect of biosecurity because it refers to the potential for misuse of scientific information to threaten public health, animal or plant populations, or other aspects of national security. While the focus of the NSABB is biosecurity, biosafety and biosecurity often converge because both require the assessment and management of laboratory risks.

The biosecurity or dual use research concerns potentially associated with synthetic biology might be considered according to experimental goal and approach. The bottom up synthetic biology research that aims to synthesize artificial life forms from scratch is still in its infancy and likely does not pose any significant biosecurity or dual use research concerns at the present time that would not be addressed, in theory, by the proposed oversight system of dual use research. Biosafety concerns regarding recombinant techniques typically utilized in this type of research would be adequately covered by the *NIH Guidelines*. However, since bottom up approaches toward developing synthetic organisms may be conducted by individuals without a background in the life sciences, or outside the formal settings subject to biosafety and dual use research oversight, the proposed outreach and education programs, from a practical perspective, may not reach a significant number of practitioners.

Well-developed and ongoing bottom up approaches also include the design, construction, and use of new biological parts, devices, and systems. Biological devices, switches, and engineered gene circuits that can respond to stimuli and behave in a predicted manner have been developed using simple biological materials.[50, 51] Moreover, efforts to standardize biological parts are beginning to enable interchangeability. For example, standardized DNA sequences with defined structures and functions are being developed such that they share a common interface and can be readily spliced together with the aim of designing and/or programming new, living biological systems. A "parts-type" sequence might encode basic biological functions such as encoding a certain protein or providing a promoter to let RNA polymerase bind and initiate transcription of downstream sequences. "Device-type" sequences are collections of parts that implement some human-defined function such as producing a fluorescent protein whenever the environment contains a certain chemical. "Systems-type" sequences perform high-level tasks such as oscillating between two colors at a predefined frequency or serving as a toggle switch. A registry of several hundred of these standardized parts is maintained in the public domain to facilitate the open sharing of these interchangeable pieces of DNA.[52] Whereas the standardization of biological parts itself does not present biosecurity concerns, the particular applications of them could. For example, it might be possible to design an organism that

[49] J.H. Kuhn. "Filoviruses, A Compendium of 40 Years of Epidemiological, Clinical, and Laboratory Studies," SpringerWienNew York 2008: 37-58.
[50] Maung Nyan Win et al., "Frameworks for programming biological function through RNA parts and devices," *Chemistry and Biology* 16 (2009): 298-310.
[51] Jeffrey J. Tabor et al., "A Synthetic Genetic Edge Detection Program," *Cell* 137 (2009): 1272–1281.
[52] BioBricks Foundation: http://bbf.openwetware.org/ (accessed 10/1/09).

contains a biological switch for the production of a toxin. Although this could be considered dual use research of concern, it also should be adequately addressed by researchers and institutions while they assess their recombinant research for safety risks and for dual use potential.

Top down approaches, namely the re-design of existing, natural biological systems for specific purposes, could in some circumstances pose dual use or biosecurity risks. Obvious examples are the deliberate creation of novel pathogens, enhancement of the pathogenicity of a naturally occurring pathogen, and the re-design of a non-pathogen into a pathogen using synthetic biology technologies. Again, however, we anticipate that the safety concerns presented by these types of experiments would be adequately addressed under the *NIH Guidelines* and the dual use risks would be addressed by the oversight paradigm previously proposed by the NSABB (aside from the issue of adequate outreach to these practitioners). Indeed, the NSABB anticipated these types of experiments, and others of potential dual use concern, in the broader context of life sciences research when developing the proposed oversight framework for dual use research.[53]

In all of these cases, as the science and technology progresses, it will be necessary to reconsider both the biosecurity and the biosafety issues. It also should be emphasized that the current systems of oversight are aimed at life sciences practitioners within universities or institutional settings; those individuals conducting synthetic biology research without a life sciences background or formal affiliations may present a gap in the current oversight systems.

Implications of diverse practitioners

Another potential concern—that perhaps falls somewhere between biosafety and biosecurity—involves the diversity of the individuals conducting synthetic biology research. As synthetic biology techniques become easier and less expensive and the applications become more widely relevant, the range of practitioners expands to include scientists from a variety of disciplines; students at all levels, including high school; and amateur scientists and hobbyists who may lack any formal affiliations with universities or research institutions. The diversity of practitioners will also include individuals of different ages and varied social and educational backgrounds who may not have been sensitized to the ethical, social and legal norms of the traditional life sciences research communities. However, these communities have a longstanding engagement with such concerns. For example, in 1975, experts convened to discuss the risks and benefits of the newly emerging field of recombinant DNA research at the Asilomar Conference on Recombinant DNA Molecules. This landmark conference illustrates these communities' commitment to the responsible conduct of research. The importance of its legacy should be impressed on the new generation of researchers, including those not affiliated with the life sciences.

Certainly, students at all levels who are utilizing synthetic biology technology need to be educated about the importance of working responsibly. The importance of educating students about safety has been recognized within some components of the synthetic biology community, and, as one example, this year, participants in the International Genetically Engineered Machine

[53] National Science Advisory Board for Biosecurity, *Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information* (Washington, DC: June 2007), http://oba.od.nih.gov/biosecurity/biosecurity_documents.html.

(iGEM) competition were required to address biosafety issues as part of their projects.[54] At the same time, to hold educational activities to the same biosafety and biosecurity oversight paradigm as academic and industry research probably is unrealistic and may have the unintended effect of stifling some excellent opportunities for getting young people excited about science. Thus, education efforts and oversight requirements should be tailored to the audience. For the practitioners of synthetic biology who are from non-biological disciplines, outreach and education efforts will need to be targeted and designed differently from those directed at scientists who already are familiar with biosafety and biosecurity principles and practices. Effectively engaging amateur scientists and hobbyists—in terms of both education and oversight—is even more challenging but nonetheless needs to be addressed, even as these "citizen scientists" are forming communities that are engaged with biosafety issues and responsible oversight and developing codes of ethics.[55] In fact, for those with little history of discussion of these issues, biosafety may become confused with or a surrogate for biosecurity, and prevent proper consideration of the latter. Sensitivity about the appropriate physical management of biological materials is not the same thing as sensitivity about the information generated from the experimental use of the same materials.

Although the variety of synthetic biology practitioners presents a challenge to current oversight systems, this diversity is good for the scientific enterprise. Such diversity enables the convergence of expertise and perspectives that leads to fruitful collaborations and exciting new findings and technologies. Synthetic biology, as a discipline, is poised to grow, and the increased participation of individuals with many different interests is inevitable. This highlights the importance, and the challenge, of raising awareness about dual use research and biosecurity issues among individuals outside the life sciences, within the private sector and unaffiliated with research institutions.

The importance of engagement about synthetic biology

The goals, potential benefits and risks, and current limitations of synthetic biology are not uniformly understood within the scientific community or the general public. More effective and extensive dialogue within and across the disparate scientific communities engaged in synthetic biology is necessary in this regard. Certainly, more effective outreach to the general public is needed to foster a more realistic understanding of synthetic biology. Public engagement would shed light on the goals, as well as the current limitations of synthetic biology, and would not only generate excitement about this research but help to dispel some misconceptions and concerns that designer pathogens are readily available.

RECOMMENDATIONS

Synthetic biology is an emerging field with tremendous potential to impact in a positive manner the overall health and environment of not only the US, but the planet. As such, it is important to encourage the further development of this scientific endeavor and not place undue restrictions on the ability to move the research forward. At the same time, there are some potential biosecurity risks associated with synthetic biology, and in particular, the concern that synthetic biology offers an opportunity for the synthesis or re-design of harmful pathogens that could be used to

[54] iGEM: http://2009.igem.org/Main_Page.
[55] DIYbio: http://diybio.org (accessed 10/1/09).

threaten public, plant or animal health. Another, potentially larger, risk is the development of information or technology through the normal scientific process that could be misused to threaten aspects of national security. With these considerations in mind, we recommend the following:

1. **Synthetic biology should be subject to institutional review and oversight since some aspects of this field pose biosecurity risks.** Biosafety concerns can be adequately addressed by the application of current biosafety practices and procedures. If the proposed amendments to the *NIH Guidelines* are implemented, they will address research with synthetic nucleic acids in a more explicit fashion, including the expansion of guidance on risk assessment and risk management to address unique aspects of synthetic biology. To the extent that synthetic biology may present biosecurity or dual use research concerns, the NSABB has proposed an oversight paradigm that should adequately address such issues, but there is currently no federal policy in place for the review and conduct of dual use research of concern. The NSABB strongly urges the federal government to develop and implement such policy.

2. **Oversight of dual use research should extend beyond the boundaries of life sciences and academia**. While it is likely that current and proposed oversight paradigms will more than adequately address the biosafety and biosecurity concerns presented by synthetic biology, gaps in oversight remain, primarily due to the large numbers of synthetic biology practitioners who come from backgrounds that are not traditionally considered life sciences or who lack formal institutional affiliations. Moreover, synthetic biology is just one example of an area of science that may pose some dual use research concerns and whose practitioners span multiple scientific disciplines. In other instances, research that is highly relevant to the life sciences or that has implications for public health may be conducted outside the life sciences. Finally, dual use research of concern is as likely to be conducted in the private and voluntary sectors as it is in academia and government laboratories, so oversight should be uniform and comprehensive.

3. **Outreach and education strategies should be developed that address dual use research issues and engage the research communities that are most likely to undertake work under the umbrella of synthetic biology.** A critical first step in extending the oversight of dual use research of concern will be raising awareness of the dual use issue among synthetic biology's diverse practitioners, especially among those that have not been participants in recent discussions on this topic. The focus should be raising awareness about the potential biological, dual use, and public health implications of their work as well as the need for considering and addressing any biosecurity risks during the conduct of the research. Education efforts should be developed that target synthetic biology researchers who are a) not subject to federal biosafety and biosecurity requirements (e.g., private sector), b) not formally affiliated with universities or research institutions, and c) students (at all levels). This may present significant challenges, particularly in reaching individuals who lack formal affiliations, but this is important nonetheless.

4. **The US Government should include advances in synthetic biology and in our understanding of virulence/pathogenicity in "tech-watch" or "science-watch" endeavors.** It is appropriate for tech-watch or science-watch activities to identify emerging dual use technologies and new knowledge that could change the calculus about dual use risks and biosecurity concerns. As necessary, the USG should convene workshops to assess or re-assess our ability to create novel or unanticipated types of pathogens and to assess the biosecurity risks or dual use aspects of new technologies and whether they are adequately addressed by the extant biosecurity/dual use research oversight system.

Appendix A

National Science Advisory Board for Biosecurity Roster

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Appendix B

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

NIH RECOMBINANT DNA ADVISORY COMMITTEE (RAC)

AGENDA
Roundtable on Synthetic Biology
October 11, 2007

NIH Campus
9000 Rockville Pike, Bethesda, MD
Building 31, C Wing, 6th Floor, Conference Room 10

8:00 a.m. **Welcome and Opening Remarks**

Paul Keim, PhD
NSABB Member
Northern Arizona University

Howard Federoff, M.D., Ph.D.
Chair, NIH RAC
Georgetown University Medical Center

8:10 a.m. **Overview of Roundtable**

David Relman, M.D.
Chair, NSABB Working Group on Synthetic Genomics
Stanford University

8:20 a.m. **Session 1: State of the Science of Synthetic Biology**

This session will provide a broad understanding of the field of synthetic biology—the various research approaches, current capabilities, short and long-term goals. Questions to be addressed during the presentations and/or discussion period are listed below.

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 & Immunology and of Oncology
University of Rochester

Questions for Consideration:

How would you define the field of work to which various parties refer under the rubric of "synthetic biology"? How is this field of scientific activity related to, and distinguished from other fields? What are the goals of synthetic biology?
What are the principal lines of research in this area?
What are the capabilities and applications associated with the field of synthetic biology at present? What capabilities do the approaches used in synthetic biology provide that are beyond those that can be achieved using recombinant DNA or other related technologies?
What are some of the more important accomplishments to date by those that might be labeled as synthetic biologists? What are the current major hurdles or challenges in synthetic biology?
Where do you see the field of synthetic biology headed in the near and long terms?
How close are we to predicting the detailed behavior of a cell based upon its component parts? How close are we to designing biological systems and novel organisms with predictable functions?
Does your research within the realm of synthetic biology routinely undergo any biosafety review? If so, please describe.

8:25 a.m. **A Bird's Eye View of Synthetic Biology**

Roger Brent, Ph.D.
Director and President
The Molecular Sciences Institute
Berkeley, CA

8:50 a.m. **Synthetic Biology As A Twenty Year Old Field**

Steven A. Benner, Ph.D.
Distinguished Fellow
Foundation for Applied Molecular Evolution
Gainesville, FL

9:15 a.m. **Synthetic Biology: From Bacteria to Stem Cells**

Ron Weiss, Ph.D.
Professor of Electrical Engineering
Princeton University

9:40 a.m. **Synthetic Organisms**

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

10:05 a.m. ***Break***

10:20 a.m. **Discussion**

11:40 a.m. Break for Working Lunch

12:00 noon **Session 2: Predicting Function**

This session will address our 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. Questions to be addressed during the presentations and/or discussion period are listed below.

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

Questions for Consideration:

- To what degree are we able to explain virulence of the more common bacterial and viral pathogens, based on their genome sequences? 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?
- In general, what kinds of virulence factors (and how many, in relative terms) are sufficient in establishing virulence in a heterologous, a virulent organism?
- What kinds of factors determine the evolutionary distance across which virulence might be genetically transferred? How does our current understanding of lateral gene transfer (its prevalence and role) in nature help us predict the degree to which virulence and other relevant phenotypes can be deliberately manipulated or created *de novo*?
- What are the major challenges and unmet needs that hinder recognition and prediction of virulence? What would be the most effective strategies for addressing these challenges and needs?
- What are the considerations for predicting function within systems?
- What kinds of properties of a biological system are most successfully predicted from its genetic composition?
- To what degree, and in what ways do current scientific knowledge allow construction of entirely new systems or organisms with predictable behaviors? How will this capability change as continued efforts increase our understanding of the function of individual proteins and macromolecular complexes?
- What kinds of tools or approaches are currently most effective for predicting function from sequence? What are their capabilities? What are their limitations? How are they currently being used?
- What are the major challenges and goals for developing more accurate predictive tools?

12:05 p.m. **Form and Context in Predicting Biological Function**

William Goldman, Ph.D.
Professor of Molecular Microbiology
Washington University School of Medicine

12:25 p.m. **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

12:45 p.m. **Design Considerations for Robustness and Vulnerability in Biological Systems**

Marc W. Kirschner, Ph.D.
Chair, Department of Systems Biology
Harvard Medical School

1:05 p.m. **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

1:25 p.m. **Discussion**

2:45 p.m. ***Break***

3:00 p.m. **Session 3: Risk Assessment and Risk Management in a Context of Uncertainty**

This session will explore 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. Discussion questions are listed below.

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

Panelists:

Rocco Casagrande, Ph.D.
Managing Director
Gryphon Scientific

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

3:30 p.m. **Discussion**

Are there novel or distinct biosafety risks or challenges associated with synthetic biology?

In general, to what degree are the biosafety risks of synthetic biology currently being adequately addressed?

For recombinant DNA research, the initial risk assessment is based on the risk group of the parental agent. However, for the more novel synthetic agents, a parental agent may not be obvious and/or the biological properties of the new organism may be largely unknown. How can risk assessment be conducted in such situations? What is the most appropriate risk management approach in such cases?

What kinds of efforts have been, or are being taken to engineer biological containment into synthetic systems/organisms (e.g, use of unnatural genetic code or amino acids, self-destruct mechanisms, other safeguards)? Are there other biological containment practices that could be considered?

Are there any existing risk assessment tools that would be applicable to the biosafety risk assessment process in the context of synthetic biology?

Once the difficult and inherently imprecise process of risk assessment has been completed and a risk management plan has been defined, are there ways to assess the efficacy of the management plan as the project goes forward?

4:50 p.m. **Closing Remarks**

5:00 p.m. ***Adjourn***

Summary: 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.