

A microscopic view of various bacteria, including several red rod-shaped cells and a few green rod-shaped cells, set against a light blue background. The bacteria are shown in different orientations and depths, creating a sense of a three-dimensional space.

Sackler Forum 2015

Trends in synthetic biology
and gain of function and
regulatory implications

Sackler Forum 2015 *Trends in synthetic biology and gain of function and regulatory implications*
Issued: September 2016 DES4538

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Preface

Since 2008, the Raymond and Beverly Sackler US – UK Scientific Forums have sparked new excitement and enthusiasm for the exchange of ideas among thought leaders from the United States and United Kingdom on topics of worldwide scientific concern.

As Presidents of the Royal Society and National Academy of Sciences, we are profoundly grateful to the Sacklers for this far-sighted act of generosity. In the modern world, science and technology have become engines that drive not only economic growth but also social change. By establishing the Forum, the Sacklers have made it possible to examine forces that are creating our collective future. Previous Forums have examined the worldwide food supply, neuroscience and the law, earth modelling, climate change and cybersecurity.

The sixth Forum, which was entitled ‘Trends in Synthetic Biology and Gain of Function and Regulatory Implications’, took place on 15 – 17 November 2015 at the Kavli Royal Society International Centre in Buckinghamshire, UK. The Organising Committee which developed the Forum and reviewed the report, consisted of:

- Professor Jef Boeke, Director, Institute for Systems Genetics
- Professor Paul Freemont, Head of Structural Biology, Department of Medicine, Imperial College
- Diane Griffin, Vice President, National Academy of Sciences
- Professor Peter Kim, Department of Biochemistry, Stanford University School of Medicine
- Professor Robert Lamb, Northwestern University Medical School and Investigator of the Howard Hughes Medical Institute
- Sir John Skehel FRS FMedSci, Biological Secretary, Royal Society

The Forum was a great success, bringing together a distinguished group of speakers and participants to discuss the cutting edge of research in synthetic biology and gain of function. Presentations covered synthetic biology, gene drives and gain of function research. This was followed by robust and vigorous discussion of the implications of this research for society and questions about how we manage the risks in order to obtain the benefits, including how to regulate these technologies appropriately.

This summary of the Forum was written by Shaoni Bhattacharya, a freelance science writer, in consultation with the organising committee and the following staff: Elizabeth Bohm and Franck Fourniol, the Royal Society and Audrey Thevenon and Doug Friedman, the National Academy of Sciences. It captures the main points from the presentations and discussions between Forum participants.

As Presidents of the Royal Society and the National Academy of Sciences, we are pleased to introduce the latest piece of work supported by the Sacklers’ inspired generosity.

Venki Ramakrishnan
President, Royal Society

Ralph J Cicerone
President, National Academy of Sciences

Summary

This report summarises the high-level interdisciplinary discussions, facilitated by the Sackler Forum 2015, on two related areas of research: those of synthetic biology and gain of function.

It covers some of the latest cutting edge research and developments, including the newest and potentially transformative work on gene drives. It also highlights many of the challenges posed by these areas and the possible responses to them, as well as potential gaps in existing regulations.

This is not a consensus document and does not make recommendations. However, what is clear is the need for forums such as this which bring together scientists, social scientists and regulators, amongst other stakeholders, to reflect on major new technologies, their potential impacts (both positive and negative), and the regulatory landscape ahead of the time when such discussions may become urgent and reactive.

An ongoing scientific dialogue in order to think ahead on issues such as governance is far preferable to blunt-edged tools like moratoriums in the face of undesirable events or negative public relations. The hope with such technologies would be to develop good governance in advance by facilitating discussions like these.

The world faces major challenges such as climate change, poverty and infectious disease. New technologies may have a powerful and transformative role in tackling these. Science is advancing ever more rapidly and with it the promise of addressing some of the most pressing global challenges. Discussions from the Forum highlighted that in the future, regulators, scientists and ethicists may need to adjust their traditional models of thinking about risk, to models which also factor in uncertainty and potential benefits, in order to take full advantage of new and emerging technologies.

Though new technologies bring with them an entire set of new concerns or raise familiar concerns in a new context, they may also herald a new era of development and optimism, which with considered governance, best practice and appropriate regulations could facilitate their use for humanity's maximum benefit.

Introduction

The Raymond and Beverly Sackler Foundation supports an annual event that brings together leading scientists, policy and regulation experts from the United Kingdom and the United States to discuss pressing topics of potential global benefit and concern. The 2015 Sackler Forum was hosted at the Kavli Royal Society International Centre in Buckinghamshire, UK, on 15–17 November. The Forum focused on synthetic biology and gain of function research.

Nearly 50 leading experts and other stakeholders attended the Forum in the inspiring setting of Chicheley Hall – a Georgian manor house built in 1715, where the Kavli Royal Society International Centre is based – some 50 miles north of London. Delegates included a mix of experts from diverse academic and industry backgrounds such as virology, bioengineering, computer sciences, plant biology, social sciences and philosophy. There were also representatives from policy bodies and UK regulators.

In order to encourage open discussion and sharing of information, without prejudice, the discussions took place under the Chatham House Rule, which means that what was said is not attributed to any individual or institution. Instead, this report presents the overall discussions at the Forum, reflecting the work presented and the ensuing topics of conversation stimulated.

The Forum focused on the biological manipulation of living organisms and pathogens for scientific research, both the practical and theoretical applications. To this end, the agenda tied together two main areas that fall under this auspice: synthetic biology, including gene drives, and gain of function research.

Discussions covered how the field of synthetic biology is allowing the construction and design of metabolic pathways and the organisms housing them and how specific molecular biology tools can be employed to create ‘gene drives’; genetic changes that can drive desired traits through a natural population to replace or suppress it. The Forum also looked at gain of function research, where genetic alterations to gain or lose functions are made to pathogens (currently mostly viruses) to understand transmission, virulence and resistance.

Leading scientists in these fields discussed their current research and future advances and implications. The Forum also provided an opportunity to showcase some of the most cutting-edge developments in these fields; the selection was by no means exhaustive but rather provided a window on what is possible using the latest technology.

Current legislation and governance issues raised by the emerging biotechnologies were also discussed, and again this report will reflect these talks rather than presenting a comprehensive overview of these concerns.

The potential worldwide benefits of emerging biotechnologies such as better disease prevention and malaria control and eradication, as well as issues of global concern and safety, regulation and governance were discussed. Unlike the long established debates about gain of function research and the recent concerns around the use of gene drive modified organisms in the wild, some felt that the synthetic biology field has not yet reached a point where governance is an immediate issue, but that such a time would come soon. Given previous experiences with public perceptions of emerging technologies, in particular genetic modification of organisms, it was felt that such issues might need some thought ahead of synthetic biology and its potential products and benefits becoming more mainstream.

However, it is important to note that the aim of the Forum was not to reach a consensus. This report is not a consensus document and does not make recommendations. Rather it draws on the discussions, presentations and debates that took place at Chicheley Hall over the two days to illustrate the current and future state of these fields, and some of the strong feelings and debates that they invoke – both in terms of their potential game-changing benefits, and the anticipated pitfalls and challenges which must be met.

This report outlines some of the huge advances that have been made. The fruits of this work have already been implemented in many cases, and discussions at the Forum gave a sense that innovative synthetic biology technologies, like gene drives, are nearing application making discussion of the issues they raise an important and timely one.

Across the Forum the main issues discussed centered on how to:

- Make such technologies safe?
- Regulate them and develop good governance?
- Develop the research agenda?
- Stop such technologies being used maleficently?
- Reap their potential benefits in the best ways?
- Best present these issues to the public?

Experts gathered for this two day Forum agreed that trust and dialogue are key. An ongoing and open discussion within the field and beyond may be crucial in realising the benefits of these exciting new technologies.

Chapter one

Synthetic biology

Synthetic biology

The concept of biologically manipulating the machinery of nature for the betterment of human society is not new. It started rapidly expanding with recombinant DNA technology in the 1970s; hopes rose with gene therapy and genetic engineering in the 1990s; and in the 21st century we have seen a plethora of new advances and approaches to the harnessing of biological systems for human ends. In particular the ability to read DNA through sequencing, including whole human genomes, is now being driven forward by the ability to write DNA through synthesis.

Chief amongst these is synthetic biology, defined by the UK Synthetic Biology Roadmap in 2012 as: "...the design and engineering of biologically based parts, novel devices and systems as well as the redesign of existing, natural biological systems." This chapter focusses on how synthetic biology works, what can be achieved, and current and future work in this field.

Synthetic biology builds on traditional genetic engineering in that it designs biological systems using engineering principles to make specific and often wholesale changes to an organism's genome. Taking an engineering approach to living systems, together with advances in technology, means scientists can currently engineer 'top-down' organisms by editing existing genetic code, but many researchers are also closing in on 'bottom-up' designs on life.

Furthermore, new tools mean the field can push the limits on life and our understanding of complex biology for more theoretical research. Plant genomes can be mined to find and trigger ancient defense systems against disease. Whole genomes can be 'recoded' and efforts are currently ongoing to build an entire yeast genome from scratch. In addition, engineering living systems can operate in non-living environments, for example that are cell-free¹ or in vitro.

New biological vistas may be opened up through understanding the defining characteristics of a yeast, or a cell's components, or by swapping life's building blocks for a new non-standard kit. The basic building blocks can themselves be remade – with the twenty-plus natural amino acids of life augmented with new unnatural or 'non-standard' amino acids. Even DNA itself can be replaced by a human-made nucleic acid – XNA (xeno nucleic acid).

Many argue that the field could be a 'game-changer' in tackling global medicine or food supply challenges, alleviating disease and poverty and boosting human quality of life. Already, vital medicinal products on the market are produced by engineered living systems. Future synthetic biology may offer improvements for medicine, agriculture, food production, materials production, energy production and storage and cleaner industrial processing.

1. A cell-free system is a tool used to study biological reactions within cells while reducing the complex interactions found in a whole cell. Parts of the internal cell structure are separated out to provide molecular machinery that can be used in reactions in the absence of many of the other cellular components.

It has been suggested that synthetic biology might offer solutions to the urgent challenge of climate change – potentially offering microbes that sequester carbon dioxide, better energy storage and greener chemical and industrial processes. For example, the manufacture of materials from petrochemicals could potentially be replaced by harnessing synthetic biology to make products using biology on an industrial scale.²

Such systems have the potential to be used to produce a range of vital compounds cheaply and sustainably compared with traditional methods, including also the extraction of high value molecules from nature. Products made using synthetic biology processes are already on the market and the global market value of such industrial biotechnology is predicted to increase to \$10.8 billion by 2016, up from \$1.6 billion in 2011.³

Applying an engineering approach to biology

Tools are fundamental to synthetic biology, reflecting the field's multidisciplinary origins. Synthetic biology applies the standard engineering model of 'design, build and test' to living systems at the genetic level to iteratively alter their genetic code ('operating systems'), redesign or replace genetic parts, synthesise new metabolic pathways or modify host cells like bacteria with ones that better suit a bio-production purpose. Genes or the proteins they code for, in the synthetic biology 'parts' list can also be further altered to achieve specific design objectives.

Scientists may focus on a specific objective, for example to engineer a bacterium into a 'living foundry' to produce a high value chemical, and then design the organism from characterised 'parts' to do so.

Computer-aided design and the availability of chemically synthesised DNA, including genomic libraries, can now make designing a bespoke genome possible. Computer simulations can be used to predict which designs would be best, and then these designs can be constructed and tested in the laboratory, with the best designs scaled up for bio-production applications.

The Forum heard presentations on such work and moves towards establishing the vision of desktop genome design and manufacture – Combinatorial DNA at your Desk. The language of DNA, with its triplet codons for amino acids or stop sequences also lends itself to computer coding, with a standardised computational way to share synthetic biology parts in a design framework already developed, the Synthetic Biology Open Language,⁴ and work beginning to develop a programming language for living cells.⁵

Computing power and harnessing robotics can also make this design stage more efficient than in the past, allowing multiple designs to be built and tested using feedback to adjust initial designs to achieve the desired outcome.

2. *Biology is technology*, R. Carlson Harvard University Press (2011)

3. <http://www.bccresearch.com/market-research/biotechnology/global-synthetic-biology-markets-bio066b.html>

4. <http://sbolstandard.org/>

5. *Genetic Circuit design automation*, A. A. K. Nielsen et al., Science. 2016 Apr 1;352(6281):aac7341. doi: 10.1126/science.aac7341.

Genetically designed biochemical feedback loops can also help meet design challenges, such as the trade-off between the productivity of a microbe engineered to be a living foundry and the needs of the host cell to survive. A major design challenge for synthetic living foundries and other synthetic biology engineered organisms is the balance between the robustness and performance of the engineered cell, and the cellular burden.

The tools of synthetic biology are relatively new, and crucially they allow specific and widespread changes to be made to a genome in a living cell, rather than the often non-selective tweaks and touches made by more conventional genetic engineering or genetic modification in vitro. Such techniques include whole genome editing using Clustered regularly interspaced short palindromic repeats (CRISPR) with CRISPR associated protein 9 (CRISPR/Cas9), Multiplex Automated Genome Engineering (MAGE) and conjugative assembly genome engineering (CAGE) methods.

The CRISPR/Cas9 technique has provided a transformative leap in what is technologically possible as it provides a precise method of editing the genome; homing in on the target DNA sequence using a complementary 'guide RNA', which directs the Cas9 protein to cut the DNA at the defined location.

As with any engineering production challenge, the outcome using synthetic biology would be to achieve the desired product in a reliable, predictable, low-cost, controllable and safe way. The predictability of biotechnological solutions potentially offered by synthetic biology is one of the major advantages of this approach over conventional genetic modification or gene therapy.

Conversely, synthetic biology research may not just focus on an end product, but rather probe the biological effects of experimenting with genetic operating systems and parts of living organisms. For example, scientific questions being explored include: how much of an organism's genome can be pared down and leave it still functioning as that organism? Whole genomes can be recoded or re-written, and biological systems can be explored to test inherent properties.

Living systems can also be engineered to contain their own biosafety mechanism; their own biological kill switches, or multi-lock systems amongst other measures which can be used to prevent accidental release or control organisms designed for release (see Chapter 3).

FIGURE 1

Price per base of DNA Sequencing and Synthesis March 2016.

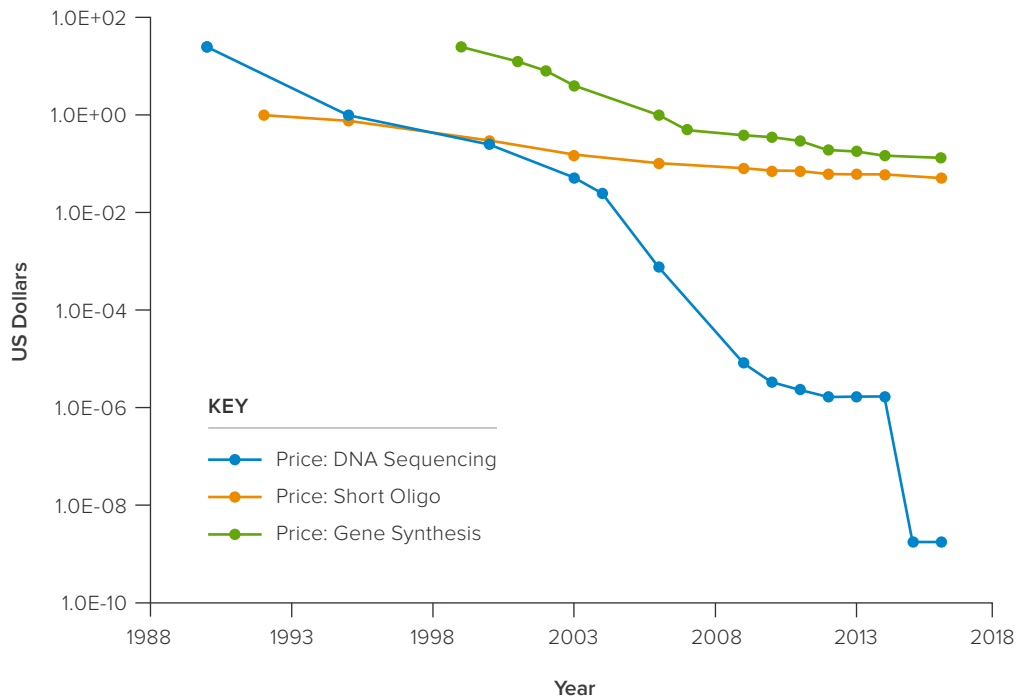


Figure reproduced with permission of Rob Carlson www.synthesis.cc

Technological advances enabling progress in synthetic biology

A variety of technological leaps in recent decades means that harnessing synthetic biology for real-world applications is achievable, with drugs and products made by manipulating biological systems already on the market. This section will explore the changing technological landscape, and the toolkit available for synthetic biology today.

‘Traditional’ biotechnology uses recombinant DNA technology to make use of living organisms. For example, insulin has been produced for medical use by *Escherichia coli* (*E. coli*) since 1978 by introducing DNA into the bacterium using a plasmid carrier.

Since then, there has been a massive increase in computing power, and a precipitous drop in the cost and time needed to sequence or read DNA. While in the 1980s it cost around \$6400 to synthesise a base pair (bp) of DNA, the price nowadays has fallen to \$0.03 to \$0.10 per bp.

FIGURE 2

Timeline of the production of synthetic genes and genomes.

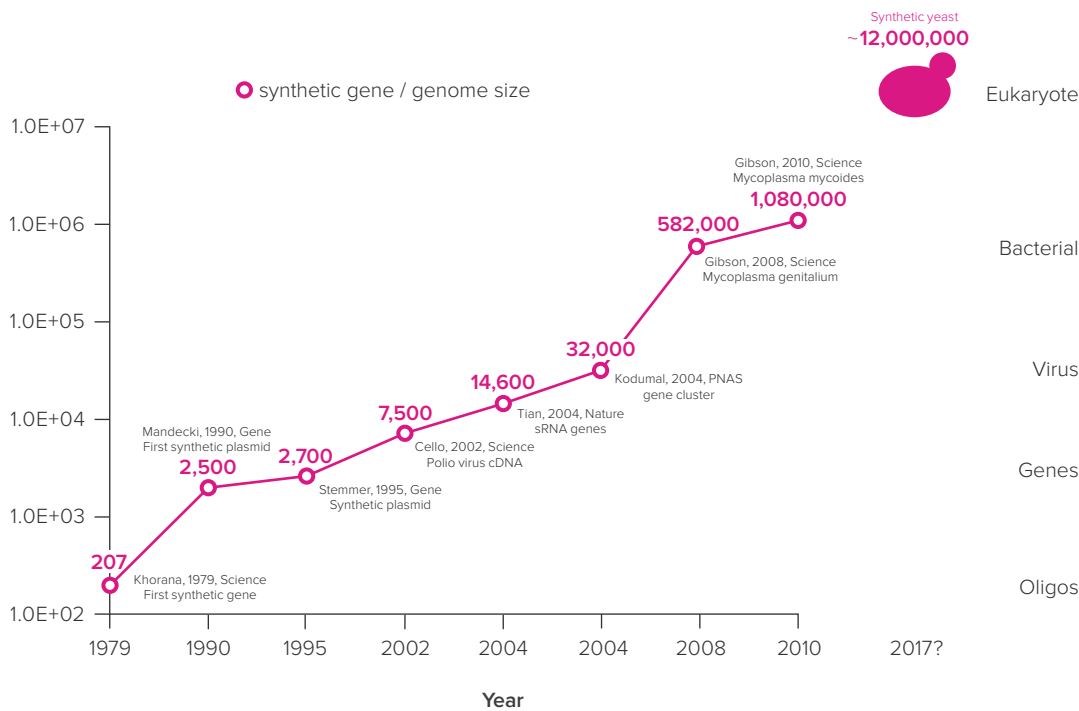


Figure reproduced with permission of Patrick Cai.

The advent of next generation high-throughput DNA sequencing (NGS), whole genome sequencing, genome libraries and new gene-editing techniques has meant that scientists can move from reading DNA sequences to potentially being able to re-write sequences or whole genomes driven also by the decreasing costs of chemically synthesised DNA.

Our understanding of whole genomes has increased vastly with genomic sequencing and genome libraries, giving insights into complex biology and functional genetic ‘parts’ which might be harnessed for human use. Computer design software such as GeneGenie for DNA oligomer design and RetroPath for whole metabolic pathway design can make the recoding and rewriting of genomes possible to probe and test in simulations.

In 2010, scientists managed to replace a bacterial genome with the first whole artificial genome that was able to self-replicate, and efforts are now underway to construct a whole yeast genome from scratch.⁶

The naturally occurring range of 20 or so amino acids has also been extended with completely new synthetic ones, opening up new chemical possibilities. Synthetic amino acids may also offer an inbuilt safety system – for biocontainment, but also protecting engineered microbes from viral contamination.

Wholesale recoding of the genome is possible with the advent of MAGE. Unlike traditional genetic engineering, this system allows multiple changes to be made to a genome at multiple points. This is achieved by introducing a library of synthetic DNAs to be combined with a microbe's natural DNA, and then selecting the resultant combinatorial strains over many cycles for the desired phenotype.

Using this method – an artificial and speeded up evolutionary process – researchers were able to engineer *E. coli* to produce lycopene (the red antioxidant abundant in tomatoes) at six times its natural abundance in four days. They did this by simultaneously targeting 24 genes in an *E. coli* population and generating 15 billion genomic variants over 35 cycles of MAGE, from which they were able to select the best new strains with the desired phenotype.

Integral to recoding genomes are two basic biological principles. One is the redundancy of nature's genetic code – that one amino acid, or a stop codon may have more than one triplet code assigned to it. The other is the conservation of the genetic code across organisms.

Genomes can be recoded in such a way as to 'free' up redundant codons, so that they may be used for something other than their original amino acid instruction enabling new non-natural amino acids to be introduced into proteins.

Current and future research applications

The products of synthetic biology are already becoming a reality. This section will explore what is available today, and the synthetic biology derived products and developments we may see in the future. It will draw on presentations of cutting-edge research given at Chicheley to give a snapshot of the field, giving a glimpse of some of the vast range of work ongoing rather than a comprehensive overview.

Synthetically derived, high-value compounds that are hard to extract from nature have been in the global market place for the last few years, and there is scope for synthetic biology to produce many more. This involves creating 'foundries' of synthetic biology microbes which can produce compounds through biological processes. These biological systems may be able to create compounds without the need for high temperature or high pressure systems that traditional industrial systems require which are often derived from synthetic chemistry and petrochemicals.

6. Science 02 Jul 2010, Vol. 329, Issue 5987, pp. 52-56, DOI: 10.1126/science.1190719

High value natural products which could be produced by harnessing synthetic biology systems include the malaria drug artemisinin, derived from the sweet wormwood plant; the chemotherapy drug paclitaxel (Taxol) that requires six 100-year-old Pacific yew trees to derive enough for the treatment of one patient; perfumier's ingredient ambergris expelled from the intestines of sperm whales onto the ocean floor; and food flavouring vanillin from the vanilla orchid.

Already on the market is a semisynthetic artemisinin, launched by Amyris and Sanofi Aventis in 2013. The drug precursor, artemisinic acid is produced by engineered yeast. The global market of this synthetic antimalarial drug is about \$90 million each year, and it bolsters artemisinin derived from botanicals, the supply of which is unpredictable and subject to fluctuation.

Developing a synthetic biology system to produce artemisinic acid using the older techniques available at the time was slow, arduous and expensive, taking 11 years and around 130 R&D person years to produce, and costing over \$100 million. The current new technologies available such as CRISPR and MAGE, may offer faster future synthetic biology solutions.

Other drugs are also synthetically produced by living organisms, including orphan drugs by Genzyme for rare or orphan diseases, the cholesterol-lowering heart drug, lovastatin, produced by fungi, and a vast array of antibiotics produced in bacteria and fungi.

On a non-medical front, a synthetic biology vanillin is in development. Currently the market provenance of this food flavouring is only 1% from the natural plant extract, 99% being made as artificial flavouring is chemically synthesised from lignin or coal tar. The cost of natural vanillin is exorbitant at \$1,500 per kilogram compared with the artificial one priced at about \$10-20/kg. The hope is that synthetic biology may provide a biological, sustainable and cheap source of the food compound.

Other commercial products are available at the nanoscale. These include light-weight batteries currently used by US military, nanowires and other nanoscale components made by using living systems (See: From Nanowires to Ovarian Cancer).

One of the major projects presented to the Forum was an international, multicenter endeavour to build the first eukaryotic genome from scratch. The Sc 2.0 project aims to build a synthetic yeast genome which is of high fitness but can also be tweaked, pared down and added to ('scrambled') in order to enhance our understanding of eukaryotic biology and genome organisation and regulation.

The project, which has been running for ten years, harnesses LoxP sites to 'SCRaMbLE'⁷ the genome, removing non-essential genes and completing re-organising the genome.⁸ Hundreds of undergraduates had also been enlisted via 'DNA bootcamps' as an army of gene builders.

7. SCRaMbLE: Synthetic chromosome rearrangement and modification by loxP-mediated evolution

8. <http://syntheticyeast.org/sc2-0/related-technology/evolution-experiments/>

So far scientists have found that at least 10% of the yeast genome can be deleted ‘up front’ without impairing its survival fitness. However, this is just the beginning, as substantially more can be deleted by SCRaMbLEing. They are also adding ‘neochromosomes’ using plasmids to the yeast. These custom-made chromosomes carry a few genes in a pathway, for example, to produce carotenoids. Yeast strains expressing these neochromosomes can then be selected out. By understanding the complex biology of the eukaryotic genome, researchers hope to gain insights into how yeast genome engineering might be harnessed, for example, to optimise the manufacture of the chemotherapy drug Taxol and range of other products.

The Forum also heard presentations on how synthetic biology in plant systems may be opening up new avenues for exploring disease resistance. This approach may also make it possible to produce plant compounds like those of the triterpene group for applications varying from antifungals to anti-cancer agents and sweeteners. Such compounds were said to be so “horribly complex” that they are almost impossible to synthesise by conventional synthetic chemistry means.

Furthermore, the organism that is chosen to start with (the ‘chassis’) can be designed so these ‘microbial factories’ can be used in different environments. For example, microbes that grow at high temperatures, so called ‘extremophile’ microbes, can be harnessed to facilitate chemical reactions at high temperatures – potentially removing the need for catalysts or expensive high pressure facilities.

The Forum heard from one project using *Geobacillus thermoglucosidasius* to provide a thermophilic chassis. This extremophile is found in the hot springs of Yellowstone National Park in the US, and thrives at temperatures of 55 – 60 degrees Celsius. More commonly, its spores can be found in most garden compost heaps.

Its ability to grow at such high temperatures may be useful for synthetic biofuels production as enzymatic conversions will be faster at these temperatures, making production more efficient.

G. thermoglucosidasius can be used as a custom-made chassis to house modular engineered plasmid ‘parts’ for specific reactions at high temperatures.

This engineered microbe may also offer an inbuilt biocontainment system, in that it will not grow in normal environmental temperatures and so is unlikely to spread with any accidental escape.

Another example of a custom-made chassis presented to the Forum, was that of a cellulose-producing chassis from the bacterium *Gluconacetobacter* where bacterial cellulose can be harvested for various applications including water filters, paper and wound dressings.

While product-manufacturing by synthetic biology is well underway, the field may offer even greater benefits for the future. The type of applications mooted at the Forum include designer mammalian chromosomes, unlimited supplies of organs for transplant, microbes which consume atmospheric CO₂, revolutionary new antibiotics and designer microbiomes as preventative medicines.

BOX 1

From nanowires to ovarian cancer

Synthetic biology is being used commercially to produce a variety of materials. Leading materials research using the M13 phage was presented at the Forum. The team working with this bacteriophage is using it both as a discovery tool at the nanoscale, and as an intrinsic part of some of the products developed.

Inspired by some of the super-tough, geometric nanoscale materials produced by nature such as the abalone shell, or the silica lattices of diatoms, scientists have explored using M13's biological properties to bind various inorganic materials to a template.

In this way, the team hoped – and succeeded – in opening up the parts of the Periodic Table not usually used as natural substrates; that is elements besides calcium, silica, iron and the transition metals usually used in biological systems.

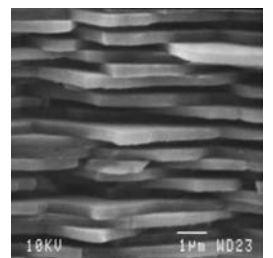
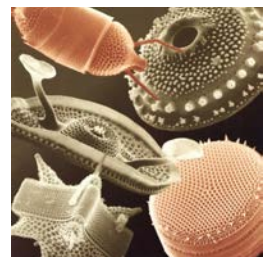
Harnessing biology to build inorganic materials also has the benefit of using soft, biological templates offering flexibility, and overcoming physical materials problems such as lattice mismatch issues.

The approach has been used to build nanoscale biological devices and circuits, nanowires, materials for better energy storage, touchscreens, fuel cells and batteries, catalysts, lightweight and strong materials, and to target ovarian tumor cells, amongst other applications.

For ovarian cancer, M13 has been engineered to pick up carbon nanotube probes targeted to tiny ovarian tumors of around 0.5 millimeters. Once attached to the tumor, these can be removed surgically guided by a bespoke imaging system. Mouse models have shown a 40% increase in life expectancy and the team expects the technology to trial in humans within the next year.

Phage-based synthetic biology has also been applied to biofuels. The Forum heard how M13-phage was used to derive ten inorganic catalysts for use in making ethylene. This precursor of gasoline and diesel fuel is usually extracted from natural gas by breaking down carbon chains, but the researchers decided to try the opposite approach of building up carbon units to make ethylene using the phage-derived catalysts.

A commercial company has since been spun off, with four sites; one site producing a tonne of ethylene a day. Crucially, this method has a 60% lower carbon footprint compared with traditional production methods for ethylene.



Top
Abalone shell.

Middle
Diatoms.

Bottom
Silica lattice.

Gene drive technology

Gene drive technology is a cutting edge synthetic biology technique that has taken off in the last two years, enabled by the emergence of genome editing technologies, most notably the CRISPR/Cas9 technique which has become much more available recently.

As yet, no gene drive modified organisms have left the lab. But this newest area of research is already provoking much heated debate since unlike most synthetic biology products, organisms altered with gene drives are intended for release into our shared environment. Gene drives are different to the way conventional genetic modification techniques are characterised as they are designed to alter wild populations and be self-sustaining.

This section will explore this new technique; how it works; how it differs from previous genetic engineering technologies; its potential benefits, including a case study on malaria prevention; current research; and some of the issues arising from this technology. Gene drives also raises various biocontainment, regulatory and governance issues which are discussed in more detail in Chapter 3.

A gene drive uses genetic changes to drive desired traits through a natural population, or undesirable traits out of a population, or to control and suppress a population itself.

Natural inheritance following Mendelian genetics means that a gene has a 50:50 chance of being inherited by the next generation. The skewing of inheritance, which underpins the goal of gene drive, occurs naturally in many biological systems, including transposons, gamete killers like T-haplotype in mice and homing endonuclease genes in microbes. Using an engineered gene drive, inheritance can be altered to the desired skew, for example, to produce 70:30 or 95:5 inheritances (see Figure 3).

Altered patterns of gene transmission from one generation to the next can mean that a modified gene can spread quickly through a population. For example, if an altered gene is introduced into 1% of a study population, using a gene drive with a 70:30 split inheritance, it can be spread into 99% of the population in about 20 generations.

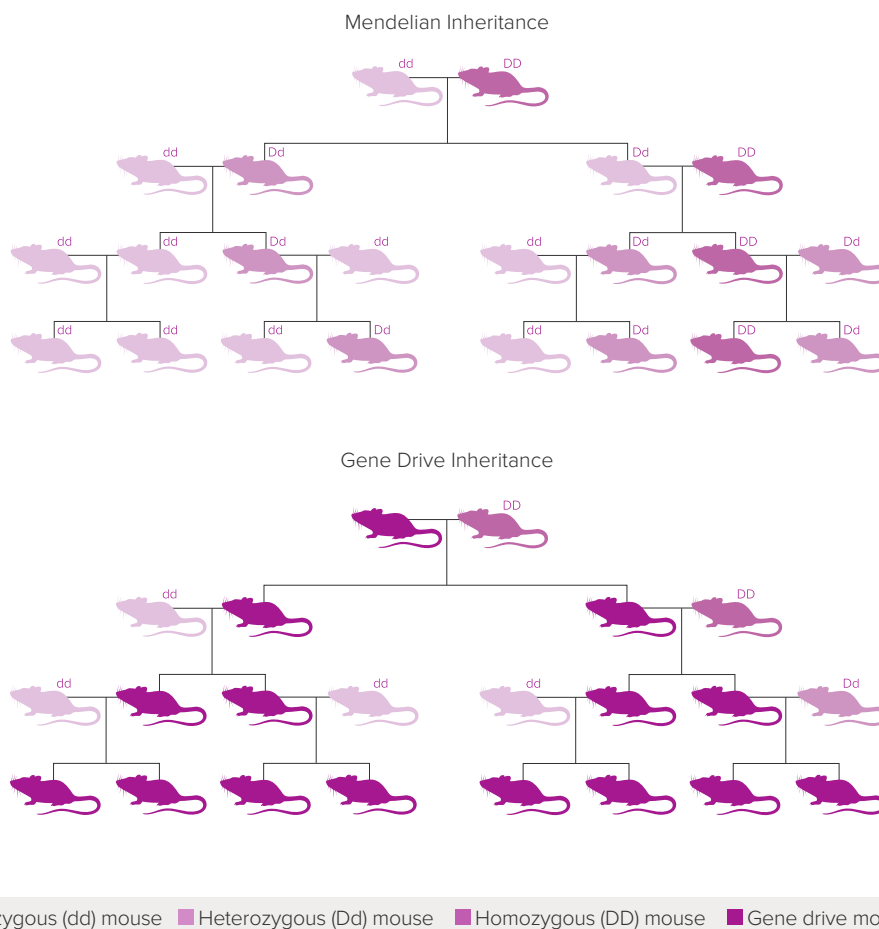
In the wild, natural selection means that genes conferring a survival advantage and increase fitness may spread through a population, while an engineered gene drive can make genes that harm an organism spread through a population. This makes it an attractive technology for pest or disease control. From a population genetics point of view, gene drive may provide a 'fifth force', adding to mutation, migration, drift and selection.

Gene drives work by adding to an organism altered DNA, which contains code for molecular scissors like endonucleases that cut the organism's original DNA at a specific point in a target gene. The technique harnesses recombination to copy the genetic construct across into the second strand of DNA, so turning a heterozygous genome into a homozygous one, and passing itself onto the next generation (see Figure 4).

FIGURE 3

An idealised illustration of Mendelian inheritance versus gene drive.

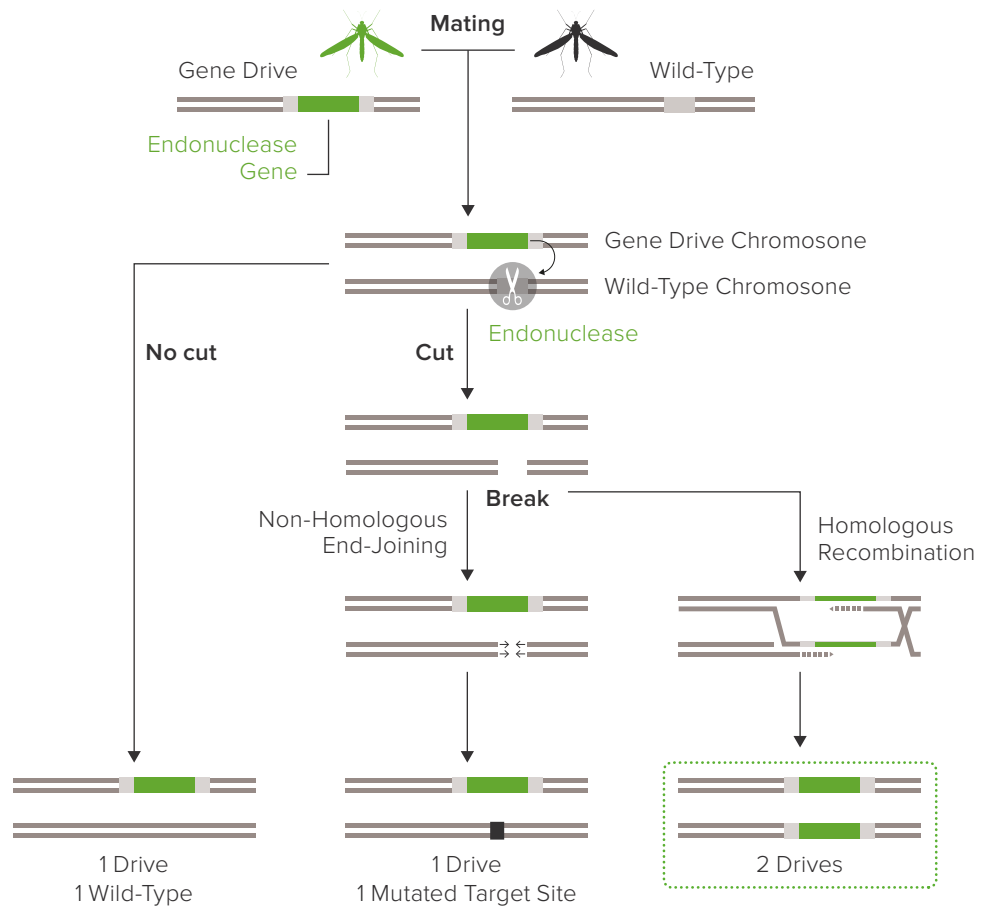
Under normal Mendelian inheritance, offspring have a 50% chance of inheriting a gene. Mating between a mouse homozygous for dominant gene (DD) and a mouse homozygous for recessive gene (dd), produces two heterozygous offspring (Dd). The frequency of the dominant gene (D) does not increase above 50% in any generation of mice. With a gene drive, the offspring will almost always receive the targeted genetic element (shown in dark purple), the end result of which is preferential increase of a specific genotype. The different shades of purple color correspond to the different genotypes (dd, Dd, DD or gene drive) of the mice. In this idealised illustration, the targeted genetic element is present in 100% of the population. However, note that the number of generations and amount of time for a selfish genetic element to spread throughout a population will vary depending on the drive mechanism, the species, and a variety of environmental conditions.⁹



9. National Academies of Sciences, Engineering, and Medicine. 2016. *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values*. Washington, DC: The National Academies Press.

FIGURE 4

Endonuclease gene drives are preferentially inherited because the endonuclease cuts the homologous wild-type chromosome. When the cell repairs the break using homologous recombination, it must use the gene drive chromosome as a repair template, thereby copying the drive onto the wild-type chromosome. If the endonuclease fails to cut or the cell uses the competing non-homologous end-joining repair pathway, the drive is not copied, so efficient gene drives must reliably cut when homology-directed repair is most likely.¹⁰



10. Esvelt KM, Smidler AL, Catteruccia F, Church GM, Concerning RNA-guided gene drives for the alteration of wild populations. *eLife*. 2014; 3: e03401. doi: 10.7554/eLife.03401.

This has been done previously using homing endonucleases to cut DNA, but gene drives have become a much more tangible prospect with the use of CRISPR/Cas9 in recent years. A gene drive system using this typically contains the cargo gene, namely Cas9 and a guide RNA to take the construct to the exact desired location within a genome.

The ubiquity of CRISPR/Cas9 amenability across many species and advances in genome sequencing make the creation of gene drives attractive. For a gene drive to work an organism has to be susceptible to being made transgenic in the first place, that is, its genome must be susceptible to Cas9. It must also be an obligate sexual reproducer and ideally it should have a short generation time.

One gene drive can also be used to overwrite or block a previous gene drive, called a reversal drive, making its effects theoretically reversible, which is one of the major safeguarding precepts of this technology currently explored (see Chapter 3). However, any changes to an ecosystem may not be able to be undone although this needs to be considered on a case-by-case basis.

The current state of gene drive research

No gene drives for wild populations have been developed as yet, though research projects are underway for gene drives in *Anopheles* mosquito to fight malaria (see Box 2) and in mice to tackle Lyme disease spread in North America¹¹.

However, many different research groups worldwide are working on gene drives. Proof-of-principle experiments have been completed in yeast and *Drosophila*. Scientists were able to show that copying of a gene drive construct with the CRISPR/Cas9 system was over 99.5% efficient in yeast, and 97% efficient for one gene in fruit flies.

Research on intrinsic safeguards and the population impacts of gene drive on wild type organisms is also underway.

Nematode worms are being used as a model population system in the lab as billions of individuals and over 100 generations can be cycled through in a year. Long-term studies are using this model to test whether gene drives can spread into a related species and whether drive resistance will evolve.

Confinement strategies are also being tested in yeast. Risks, safeguards, and appropriate regulation and governance are critically important issues for gene drives, and will be discussed in Chapter 3.

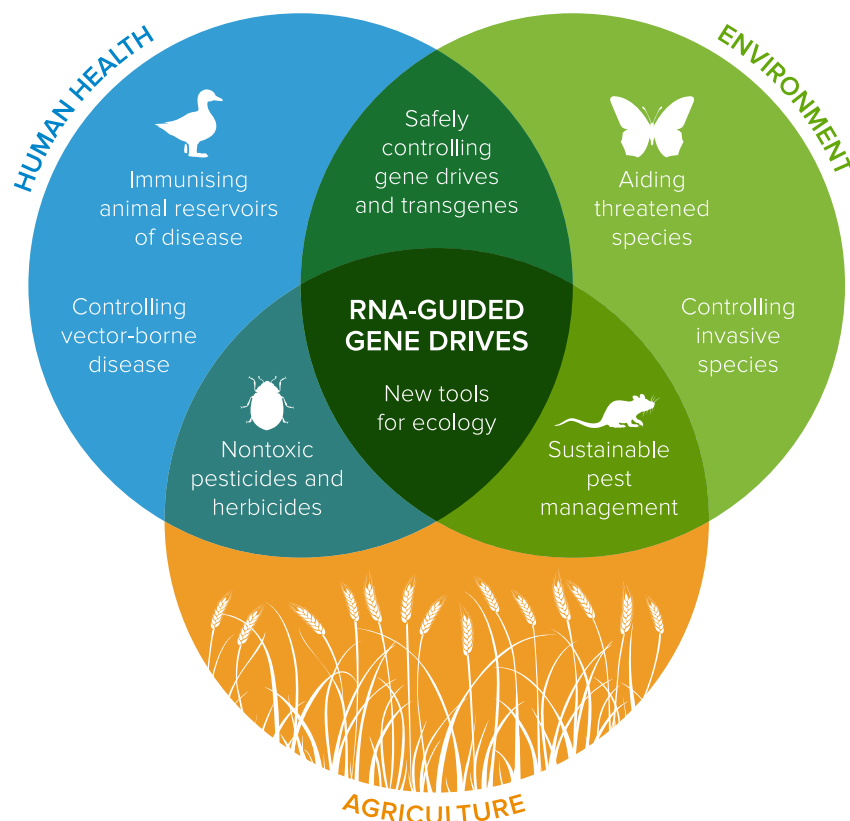
Gene drives represent a technique which offers for the first time, the ability to “rewrite the code of life in the wild”. The potential of this for human and ecological benefit is enormous (see Figure 5), however, scientists in the field acknowledge that it is also a ‘Pandora’s box’ with significant risks which will need to be addressed (see Chapter 3).

11. Since the experts gathered in November 2015 at the Forum, two proof-of-concepts on gene-drive modified *Anopheles* mosquitoes have been published.

FIGURE 5

Potential applications of RNA-guided gene drives.

Clockwise from left. Disease vectors such as malarial mosquitoes might be engineered to resist pathogen acquisition or eliminated with a suppression drive. Wild populations that serve as reservoirs for human viruses could be immunised using Cas9, RNAi machinery, or elite controller antibodies carried by a gene drive. Reversal and immunisation drives could help ensure that all transgenes are safe and controlled. Drives might quickly spread protective genes through threatened or soon-to-be-threatened species such as amphibians facing the expansion of chytrid fungus (Rosenblum et al., 2010). Invasive species might be locally controlled or eradicated without directly affecting others. Sensitising drives could improve the sustainability and safety of pesticides and herbicides. Gene drives could test ecological hypotheses concerning gene flow, sex ratios, speciation, and evolution.¹²



12. Esvelt KM, Smidler AL, Catteruccia F, Church GM, Concerning RNA-guided gene drives for the alteration of wild populations. *eLife*. 2014; 3: e03401. doi: 10.7554/eLife.03401.

BOX 2

The malaria case

Gene drive technology may offer a new avenue for malaria prevention by curbing transmission. In spite of current efforts to control malaria using bed nets and treatments like artemisinin, there are still 200 million cases of infection and half a million deaths each year from the disease, 90% of which are in Africa. Even in the best possible future scenarios, with a three to four-fold increase in malaria control funding, 62 countries will still be stricken by the disease in 2030, according to the World Health Organisation.

One solution that has been considered is to control the spread of the malarial parasite *Plasmodium* by the *Anopheles* mosquito. About five species of mosquito carry and spread malaria in Africa. There are over 3,500 species of mosquito worldwide which would not be targeted by gene drive in the malaria-carrying species.

The Forum heard about two gene drive approaches which could be used in this situation. The first would be to modify the target mosquito population in some way that curbs spread of the malaria parasite. This could be done using knock-out or knock-in genes to create alternative phenotypes. For example, the age profile of the population could be altered as only older female mosquitos spread malaria, or a 'death-on-infection' trait could be added, or the mosquitos' feeding preferences could be altered so humans are unaffected.

The second approach using a gene drive would be to directly target the population of vectors; that is, to suppress the mosquitos' populations in some way. This could be done by affecting the mosquito's survival or its reproduction, either via its fertility or the sex ratio.

It is this population suppression approach that is currently being developed with gene drive. Homing endonucleases could be used to render females infertile by knocking out a female fertility gene in the germ line.

Or the sex ratio of the next generation can be distorted by giving the Y-chromosome a gene for recognising a sequence only found on the X-chromosome. When making sperm, this can be used to shred up all the X chromosomes at meiosis, so that only Y-bearing chromosomes are produced (see Figure 6).

The idea of using gene drives to tackle malaria in this way is to potentially produce a widely-applicable and long-lasting reduction in malaria transmission that is not dependent on human behaviour such as the use of bed nets, or indeed the wealth and stability of national states or healthcare systems.

Chapter two

Gain of function

Gain of function

Much of the second day of discussions at Chicheley Hall focused on gain of function research. This chapter explores some of the background context of the field and research presented at the Forum. Some projects discussed the natural acquisition by organisms of certain functions, which may give insights into assessing evolution, and the role of retroviruses in imbuing organisms with gain of function.

This scientific area takes pathogens (currently mostly viruses) and endows them with specific genetic alterations to gain or lose functions with the intention that this will help scientists better understand the processes of transmission, acquisition of virulence and resistance to counter-measures such as vaccines or therapeutics.

This work explores the relationship between genotype and phenotype in the fundamental biological properties of pathogens. Gain of function research has been used for studies of the influenza virus and was a major point in the discussion at the Forum. Researchers have focused on engineering parts of the flu virus to understand the tenants of its transmissibility between species and how a pandemic strain might evolve naturally.

This particular type of manipulation of biological systems is not new, dating back a quarter of a century to 1990 when reverse genetics was first used to change the genome of the influenza virus.

While this report notes some of the regulatory and safety issues raised at the Forum on gain of function research, these areas will not be covered in depth as other recent reports provide thorough overviews.^{13, 14, 15}

Gain or loss of function research's dual use aspect – the idea that research could inadvertently or deliberately, if in the wrong hands, unleash pandemic strains – has meant that federally-funded work on certain viruses and for a subset of gain of function research is currently under a moratorium in the US,¹⁶ but U.S. government policy regarding gain of function studies is being developed and oversight mechanisms are currently reassessed.

As covered in a 2015 report by the European Academies Science Advisory Council, in Europe, there is no specific gain of function legislation or moratorium, rather a system of self-regulation using the GM regulations of individual European Union member states.¹⁷

13. http://www.easac.eu/fileadmin/PDF_s/reports_statements/Gain_of_Function/EASAC_GOF_Web_complete_centred.pdf

14. <http://www.nap.edu/catalog/21666/potential-risks-and-benefits-of-gain-of-function-research-summary>

15. <http://www.nap.edu/catalog/23484/gain-of-function-research-summary-of-the-second-symposium-march>

16. <http://www.nih.gov/about-nih/who-we-are/nih-director/statements/statement-funding-pause-certain-types-gain-function-research>

17. http://www.easac.eu/fileadmin/PDF_s/reports_statements/Gain_of_Function/EASAC_GOF_Web_complete_centred.pdf

What can gain of function research do?

Gain of function work can potentially answer some vital questions on viruses that have a huge impact on human health. This section aims to illustrate some of the scope of gain of function research, drawing on work presented at the Forum. However, it is noted that strong feelings were expressed both for and against gain of function work in discussing the potential benefits and risks.

Experiments on gain or loss of function could help inform the development of therapeutics and vaccines against pathogens with a large human health burden, or that may pose a significant threat in the future. These include influenza, measles, smallpox and diseases emerging from animal reservoirs like Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) coronaviruses.

For example, research with influenza in mice has shown that modern antivirals are effective against the reconstructed 1918 pandemic flu strain that killed more people than the Great War around the world.

Gain of function studies to illuminate aspects of vaccine response by flu and measles virus strains were presented at the Forum. The influenza virus is constantly changing its surface glycoproteins, which is why the flu vaccine composition must change every year to be effective. On the other hand, we have had the same reliable measles vaccine for over 50 years.

More recent studies have used mutagenesis and deep sequencing of influenza and measles viruses to further elucidate the reasons for their differences in vaccine response.

By changing one nucleotide at a time in genes encoding the main flu surface proteins of neuraminidase and hemagglutinin, researchers were able to show that influenza can tolerate significant genetic manipulation and still be viable. Measles virus in contrast does not.

Another current aim of research is to develop a universal influenza vaccine which could give long-term immunity (20 years) against all flu strains, negating the need for a different vaccine each year. Novel antigenic constructs, produced by reverse genetics, have been tested in immunised mice to help develop a more broad-effect vaccine. This has not yet been tested in humans.

Research on other viruses such as SARS and MERS coronavirus strains was presented, and prompted strong feelings at the Forum.

SARS and MERS emerged very recently from animal reservoirs to cause human outbreaks, and the public health concern is that they may acquire characteristics that make them more likely to become pandemic strains. The human SARS strain, known as Urbani, emerged in 2003 and is now thought to be functionally extinct, although a nearly identical SARS-like bat coronavirus (WIV16) exists in bats (96% nucleotide identity/genome) which is very efficient at using the human ace2 receptor for entry.¹⁸

In fact, a large and genetically varied animal pool of SARS-like viruses remains, which already have the capacity to use the human ACE2 receptor for entry and which replicate efficiently in human cells. Thus, these viruses might easily acquire the potential to cross over to humans again.

18. J Virol. 2015 Dec 30;90(6):3253-6

SARS evolves by RNA recombination rather than mutation. Unusually for a virus, it appears to have an inbuilt proofreading system, which means that its natural effective mutation rate is low, except under conditions of environmental stress.

Rather, several SARS strains are often present in animal hosts which could act as mixing vessels for recombination to form new strains.

Work conducted prior to the US moratorium on gain of function studies and a subsequent funding pause focused on generating strains of SARS-like coronaviruses in order to determine their capacity to replicate in humans and to develop key reagents that are essential for the development of a broad-effect live, vectored or attenuated vaccine. It also probed which strains might pose a potential threat to humans in the future.

As some SARS-like bat coronaviruses could efficiently use human, but not mouse ACE2 receptors, transgenic mouse models were developed that expressed the human ACE2 receptor in lung epithelial cells. Some SARS-like bat coronaviruses replicated more efficiently and caused more serious disease in these models, consistent with the hypothesis that several bat SARS-like viruses were high risk and poised for human emergence. The transgenic mouse models will be valuable for vaccine testing and understanding the evolution of virulence.¹⁹

Gain of function experiments suggest that WIV1, WIV16 and SHC014 are all high risk viruses that can use the human ACE2 receptor, replicate efficiently in primary human airway epithelial cells (natural target cell population in humans) and cause disease in transgenic mice.

Likewise, MERS surfaced in 2012 in Saudi Arabia, and is thought to have emerged from a bat virus, with camels as its intermediate host. There is currently no vaccine for MERS either.

Work to build a MERS humanised mouse model (as MERS does not naturally infect mice) by passaging MERS strains was discussed at the Forum. This work was originally halted under the US government's moratorium on gain of function research and then lifted in December 2014 for MERS mouse studies. This came after a call from researchers for an exemption to allow them to continue their work "urgently necessary to protect the public health."

This research group has panels of highly variant strains that capture the family tree of SARS-like and MERS-like viruses that are being used to develop broadly active therapeutics in conjunction with a pharma company, and also to test vaccines.

19. Proc Natl Acad Sci U S A. 2016 Mar 15;113(11):3048-53.

Predicting Evolutionary Risk, Natural Gain of Function

Part of the risk-assessment in doing gain of function research – or indeed any research which changes functionality – involves predicting what might happen if an altered microbe gets into the general population.

Before specific concerns about gain of function research are discussed, the concepts of risk and uncertainty raised during discussions at the Forum must be noted.

Risk formed a major topic of discussion. Bioethicists noted that risk is usually quantified or measured for assessment purposes on known risks, which cannot be done for emerging technologies. Instead, it was proposed that for governance purposes the field could be examined from three perspectives of: uncertainty, ambiguity and transformative potential, the idea being that governance could be developed going beyond risk, and rather recognising uncertainty.²⁰

Work on naturally acquired gain of function by pathogens was presented to the Forum to explore issues of evolutionary risk and the implications for gain of function work.

Pathogens can gain function such as increased virulence naturally through evolution within their host species. Researchers have looked at whether vaccination under certain conditions could actually drive the evolution of more virulent strains.

This concept was backed by studies of Marek's disease virulence with vaccination in chickens and by looking back at a previous experience of biological control – the use of myxomatosis in the 1950s to curb rabbit populations in Australia.

Experiments that exposed naive laboratory rabbits to myxomatosis strains from wild rabbit populations in Australia showed that current strains are far more deadly than the most virulent strain used in the 1950s – killing lab rabbits faster. However, their effect on wild rabbits is not more deadly, as the host has also co-evolved immunity.

Notably, in these cases, nothing predictive could be found in the genome to suggest how and which strains would evolve. It has been found that vaccines which completely stopped onward transmission could stop natural gain of function from evolving, while vaccines giving incomplete protection could not.

According to the conclusions of these studies, evolutionary risk is extremely hard to assess, especially in quantitative terms, and carries with it huge levels of uncertainty. Many reasons can contribute to this problem, such as the fact that many different genetic routes can lead to a particular phenotype, or that there may be complex pathogen-host interactions exacerbated by the fact that lab models and strains are only a subset of genetic variants available.

Other issues that may lead to uncertainty can be that identifying one evolutionary trajectory means only that it is biologically plausible – not that it will actually happen – and there may be a range of possible evolutionary paths.

The imprecise nature of predicting evolutionary risk may therefore be relevant when assessing the costs and benefits of doing gain of function research.

20. also see: Nuffield Council on Bioethics Working Party on Emerging Technologies report <http://nuffieldbioethics.org/report/chapter-downloads/threefold-challenge/>

Retroviruses and possible gain of function issues

Endogenous retroviruses were also discussed in relation to gain of function. These ancient RNA elements are widespread throughout nature, having integrated into the genomes of many species including humans from exogenous retroviruses embedding in the germline. Some 6 to 8% of our genomes are of retroviral origin, and in many cases they may have conferred useful functions such as promoting amylase gene activity in saliva so we can digest starch, or across mammals in promoting cell fusion in placental development.

But they also cause disease, and their effects are unpredictable: what may be harmless in one species may be extremely damaging in another if the retrovirus element transfers horizontally.

Human cells have various defense mechanisms against retroviruses, a key one is known as Anti-gal, an antibody against the alpha-gal epitope. Alpha-gal is absent in apes and Old World monkeys²¹ but present on the surface lipids and proteins of most other non-primate mammals. Anti-gal has been considered as a knock-out candidate for xenotransplantation to prevent organ rejection from pigs to humans.

Many lab studies, such as the passaging of human tumour lines through nude mice have inadvertently generated retroviruses, which may affect study results but as yet are not known to have caused any harm to humans.

HIV-1 has an accessory protein Vif which helps it overcome another major defense that human cells have against retroviral infection, called APOBEC. Other retroviruses lack this protein and research could in theory examine, or even inadvertently produce strains, which can overpower this line of defense.

Therefore, some argue that retroviruses should also be included in discussions of gain of function research.

21. <http://www.nature.com/icb/journal/v83/n6/full/icb200593a.html>

Chapter three

Challenges, regulation and governance

Challenges, regulation and governance

Synthetic biology (including gene drive technology) and gain of function research have potential for improving human existence and safeguarding our health and environment.

Technologies developed within the last couple of years, such as the CRISPR/Cas9 system for genome editing, or the MAGE system for recoding and evolving entire genomes at a greatly accelerated speed compared with nature, promise remarkable advances such as eliminating malaria, developing new antibiotics to beat drug resistance, universal vaccines, greener agriculture and a shift away from our reliance on polluting petrochemicals for materials production.

With potential benefits, however, come issues of potential risks that can lead to a negative public perception of the technologies and challenge current regulations and governance. This chapter will delineate these issues for both synthetic biology (including gene drives) and gain of function research, highlighting concerns and possible responses and solutions.

Researchers at the Forum were mindful that these potentially world-changing biotechnologies could have applications which address challenges that public want solved. To this end, most agreed on the need for a close assessment of the field's potential impacts; not just scientifically but in a much broader sense encompassing societal impacts, public perception and acceptance, ecological risks and unpredicted effects, biosafety and biosecurity issues, as well as how regulation and governance may manage these.

The discussion at the Forum was broad and some example questions were raised to help frame the debate on emerging technologies, particularly on environmental impacts of gene drives and biosafety concerns or dual use research of concern that may arise from gain of function research. These were:

- 1) Is there some principled objection to doing this? What is the principle at stake?
- 2) There may be no such principle, in which case is the work important enough? That is, is there a non-trivial use or purpose for doing the research?
- 3) Can the research be done safely on an operational level? For example, is the enhanced-BSL3/BSL4 system of laboratories satisfactory for this purpose, and who would ensure that work is done safely? What is the regulatory force? Is there money for oversight?
- 4) Are there forces in our society now that might make this not the right time, or a difficult time, for emergent technologies? For example, with huge inequalities globally and anxieties over dual use. Even if there is no principled objection and the work has a good purpose, there may be social, ethical or cultural reasons why it might be difficult.

In addition to the biological systems themselves, concerns were raised over biology as an information science. That is, how the publication and availability of information from research might be used by others – without the same precepts of justification, high safety standards and regulation, to alter the shared environment.

Public Perception

Issues:

Multidisciplinary scientists, social scientists and ethicists were conscious and mindful of how the public's perception of the emerging technologies discussed at the Forum might shape the field, particularly synthetic biology.

This section explores discussions at the Forum on public perception as it relates to synthetic biology research, including gene drives, and gain of function research. It also notes the historical context for the scientific community's concern about how new emerging biotechnologies are perceived by the general public.

General public relations questions were raised – such as how the naming and framing of the emerging biotechnologies might affect public perception. For example, the idea was raised that the name synthetic biology triggers a negative perception in the public.

Notably the way that the public engagement and debate on genetically modified organisms (GMOs) evolved over the course of the twentieth century was considered as informative for currently emerging technologies and that lessons could be learnt. In particular looking at ways that the scientific community, government and regulators could engage more effectively and earlier to ensure there was public input and understanding into the use and direction of emerging technologies.

Some felt that the new emerging biotechnologies were better placed at trying to address challenges that the public wanted solved, such as mass-producing cancer drugs cheaply or eliminating malaria, and the willingness of the community to conduct early discussions was positive and essential, incorporating a diverse range of funders to support the research.

Issues of the public perception of funders also provoked much discussion. In 2014, two-thirds of US government funded synthetic biology projects were backed by the US Department of Defense, and DARPA.²² Though their applications and premises for research may not have been military, the public's perception of this was reflected upon.

The public perception of private and commercial ventures in synthetic biology were also mentioned particularly in relation to the GM debate that focused on the private monopoly of plant seeds globally.

In some quarters, a public resistance has already started. One presentation highlighted a Friends of the Earth poster about synthetic vanillin, which encourages consumers to reject synbio vanilla.

Potential responses

Most agreed that engaging with and listening to the public was crucial.

The issue of transparency from the outset of research was discussed, with particular respect to the newer areas such as genome editing and gene drives.

22. <http://www.synbioproject.org/publications/u.s-trends-in-synthetic-biology-research-funding>

Discussions with the public should be started before some research projects even start, suggested some scientists. But it was acknowledged that this would require a major shift in the culture of science and curiosity driven research.

The current model of incentivising research, with an emphasis on publishing results in high-ranking journals and consequent negative knock-on effects on transparency, were highlighted by some as unhelpful to disclosure.²³ Complex intellectual property issues were also noted as being counter to transparency.

For the newest area of gene drives, it was suggested that regulations could help scientists to be more transparent and engaged with the public as this discipline of synthetic biology may require ‘informed consent’ from the public in order to release modified organisms into the shared environment in the future.

‘Town hall’ meetings with the public were also suggested as a way of discussing gene drive proposals in particular, before research might even be started. However, some were mindful that these forums can privilege some voices over others and any public engagement should be designed to hear from a broad range of people and stakeholders.

Others raised the prospect that transparency, if not done properly, could actually increase public mistrust of science. For example, terminologies or language such as the use of ‘natural’ versus ‘synthetic’ may carry certain connotations for the public, for example that ‘natural’ is always good, which are not the same as when these terms are used by the scientific community. Also, concerns were raised that most scientists are untrained in conveying the complexity of their work, and scientific hubris can erode public trust (as was suggested with the case of early HIV research).

Many felt that positive examples such as the use of genome editing in leukaemia could demonstrate the tangible benefits.

To maintain public trust, potential benefits of the emerging technologies should be properly highlighted along with issues such as biosafety and risk, which also need to be addressed effectively. Any accidental release caused by lapses in lab safety could undermine public support for continuing research on these technologies.

The idea of an interdisciplinary research agenda, bringing together synthetic biologists, ecologists, evolutionary biologists and environmental scientists amongst others, to examine potential risks from the outset, was also mooted as important for public trust. A current lack of funding was noted – with a recent US analysis of federal funding showing that work on the environmental and ecological impacts on synthetic biology has no investment currently.²⁴

23. The Royal Society had a conference on these issues – ‘The Future of Scholarly Scientific Communication’ the recordings and report are available here: <https://royalsociety.org/events/2015/04/future-of-scholarly-scientific-communication-part-1/>

24. <http://www.synbioproject.org/publications/u.s-trends-in-synthetic-biology-research-funding/>

It was suggested that synthetic biologists could support interdisciplinary grant applications or include such collaborative, interdisciplinary assessments within their grant applications.

It was noted that the European Commission was doing work on risk assessment in synthetic biology and the last of three 'Opinion' reports was expected in November 2015.²⁵

Containment and unintentional release

Issues:

One of the main safety issues common to both synthetic biology and gain of function fields was that of escape from a laboratory or bioreactor.

This section addresses biosafety issues which are concerns for all the research areas covered at the Forum – that is synthetic biology in general; gene drives; and gain of function research.

Organisms not intended for release

The biosafety issues that were raised in relation to organisms within controlled environments will be considered first and those intended for release second.

In terms of the accidental escape of genetically-modified organisms, concerns vary from human and animal safety, to impacts on the ecosystem and environment and contamination of the natural gene pool for example, by recombination with related species or horizontal gene transfer to unrelated species.

Many scientists agreed that to some extent, particularly with synthetically engineered microbes in bioreactors, that some accidental escape into the wild might be inevitable. The question then raised was: does it matter?

The answer may depend on the individual case. For example, escaped synthetic yeast producing insulin would be unlikely to cause concern, while an escaped pathogenic virus with enhanced transmissibility using gain of function techniques, would garner an entirely different level of anxiety.

In most cases organisms used in synthetic biology are unlikely to survive in the natural environment, as they are optimised for artificial environments and have impaired fitness meaning they are unlikely to survive and compete against natural counterparts.

However, there are cases of increased fitness, for example, some engineered bacteria have been altered to make them immune to phages.²⁶ As phages may keep many natural organisms in check, phage-resistant synthetic bacteria could be considered as having a gain of function. Organisms like this would have an advantage over natural counterparts and additional safety measures might be required to prevent escape.

Particular concerns in general remain over gain of function research where a pathogen may be more deadly than the original and an accidental escape may result in an outbreak.

25. This report has now been published: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenih_r_o_050.pdf

26. 'Phage' is short for bacteriophage, which are viruses that infect and replicate in bacterial cells.

The growing ease and accessibility of newer, cheaper and powerful genetic manipulation techniques were also raised as potential biosafety issues. As these technologies become more accessible to laboratories that may not comply with the required level of safety, it is important that new regulations are considered and implemented so that the biosafety concerns are fully addressed.

This may be of particular importance for non-professional enthusiast laboratories, sometimes described as 'biohacker labs' or 'DIY biologists', and for laboratories in different parts of the world, which may have varying standards of safety and security. Although it was noted that the DIYBIO community that run biohacker labs has been very proactive about educating their members and setting safety and ethical standards for their community.

Organisms intended for release

Some synthetic biology organisms are intended for release into the environment, complicating the biosafety situation further. For example, some synthetic biology microbes are being developed for bioremediation, biomining or as probiotics and are destined for potential spread in the environment.

Gene drive organisms are designed to be released directly into the environment. A gene drive project discussed at the Forum was the population-suppression of *Anopheles* mosquitoes for curbing malaria, which would be designed to be released into the shared environment.

Gene drive technology is advanced enough that some laboratories could, in theory, release gene drives into the wild but this technology may not be covered by current regulations and the governance structures in countries where the gene drive modified organisms are to be released may not be adequate.

Potential responses:

Multiple layers of enhanced biosecurity would be the major response to curbing any ill effects of accidental escape. This takes two forms: extrinsic biosecurity, that is physical containment measures, and intrinsic biosecurity, that is biologically engineered safety mechanisms.

Good practice suggests that such biocontainment safeguards should be multiple and redundant, so that if one fails, another one should stop any potential escapee from taking hold in the natural environment and spreading.

Intrinsic safeguards could include measures such as multiple 'kill switches' in essential genes that would be activated in a natural environment or be set off by a lack of a laboratory input. In this way, synthetic organisms can be enslaved to chemistry by altering them so they can only use non-natural amino acids and therefore survive only in synthetic environments.

There was some discussion on the need for such multi-lock systems for relatively benign applications of synthetic biology. Some scientists were concerned that current efforts to automatically include multi-lock intrinsic safety systems for everything, might send an incorrect message to the public – that the technology was inherently unsafe and be a waste of resources where they are not necessary. Intrinsic biocontainment is also subject to the powers of evolution, which must be considered in the overall safety design.

It was noted that it is important that such systems are developed before they are actually needed and that safeguards are already being worked on in anticipation of future applications. Testing such systems in safe, already contained organisms would be preferable to using them for the first time in 'open system' organisms, it was argued.

The prospect that this might run counter to safety was also raised, as engineering genetic safety systems before they are needed could lead to the evolution of resistance ahead of time in a similar fashion to the problem of antibiotic resistance.

With gene drives, as well as the use of multi-lock biological systems and precision gene drives to target sequences unique to a species or population, the idea of reversibility as a safety measure invoked much discussion. In theory, gene drives can be rewritten or reversed by introducing a new gene drive into a population. However, even if an original gene drive is reversed, the CRISPR/Cas9 component previously added will always remain in the population genome. In this sense, some argue it is not entirely reversible and as previously discussed the effects on the ecosystem would not be reversible.

To address biosafety concerns it will be important to develop best practice and good governance for the future, which should include community or biohacker laboratories to ensure that new technologies are developed responsibly.

Current regulations and legislation may be used to curb some of the biosecurity issues, but as these emerging biotechnologies, particularly gene drives, are so new there are gaps in the current framework (this will be discussed in detail in the section on Regulation and Governance).

There are also international treaties that cover biological weapons and it is important that these Forums keep up to date with the science in order to implement appropriate measures to address biosecurity risks that arise from new technologies.²⁷

Environmental and Ecological Impacts

Issues:

The other spectre of laboratory escape of engineering organisms is that of environmental or ecological damage, which may be difficult if not impossible to reverse. This concern applies mainly to synthetic biology so this section will focus on the possible impacts of synthetic biology.

While intrinsic safeguards might be helpful in stopping environmental and ecological impacts, the feeling amongst evolutionary biologists and social scientists was that “Nature finds a way”.

A major worry under discussion was the threat of synthetic biology organisms becoming invasive species. Though current engineered organisms would have significant fitness disadvantages if they escaped into the wild, there was concern that they might eventually evolve to acquire fitness. For example, the possibility of synthetic biology organisms that are dependent on synthetic environments due to the need for unnatural amino acids, might adapt to be able to use natural amino acids, was raised.

Biology’s complexity and unpredictability were also cited as difficult issues to mitigate against in terms of foreseeing future safety issues and designing components against them.

It was postulated that escaped or deliberately engineered microbes for purposes like bioremediation, could have unanticipated knock-on effects on systems such as geological, geophysical and atmospheric systems.

27. Also see: InterAcademies Panel, ‘Biological and Toxin Weapons Convention Implications of advances in science and technology’ <https://royalsociety.org/topics-policy/projects/biological-toxin-weapons-convention>

Others noted that previous technologies that had massive human benefits – such as the use of synthetic nitrogen fertilisers in agriculture, incurred adverse environmental effects not seen for decades or even centuries. Therefore, impact studies may be limited by duration in predicting possible negative outcomes of emerging biotechnologies.

The concept of ‘improbable pathogenic events’ occurring in synthetic biology products, was also raised with examples such as that of pathogenic passengers hitchhiking in live vaccines for chickens.

The potential for horizontal gene transfer and recombination of gene drive populations with natural populations was also raised from an ecosystem perspective.

Potential responses:

For concerns over ecological spread of synthesised genes or gene drives, intrinsic multi-lock systems such as kill-switches and adaptation to non-natural synthetic substrates were mooted as potential safeguards.

The principle of engineered organisms having an inherent low fitness in the natural world is an important one.

Where organisms are intended to be in open systems, such as those being developed for activities such as biomining, inherent safety systems to stop genetic spread are essential.

For gene drives, proponents suggest that organisms should be assessed on a case-by-case basis for risk, based on lessons learned from non-driving engineered organisms released in nature.

There are no guarantees that hybridisation will not occur with closely related species, and therefore if organisms engineered with gene drive are released we must be comfortable with the genes used possibly crossing to related species.

One of the simplest barriers to ecological contamination from gene drive research might be geographical or ecological confinement, with the use of off-shore labs or offshore field trials. In the case of the malaria gene drive work, the research is being conducted in the UK in contained labs with *Anopheles gambiae* mosquitoes, in the unlikely event of escape from the lab they should not be able to survive due to the cool climate.

A paucity of funding for research on the environmental impacts of synthetic biology was highlighted. In many cases, the answers are unknown. The idea of developing a research agenda to address some of these concerns and questions was suggested.

Good governance and regulation may also be key in harnessing these new technologies whilst safeguarding the environment, but current systems may not be fit-for-purpose for these emerging technologies as discussed in the section on regulation and governance.

Regulation and governance

Developing appropriate and effective governance and regulations for emerging technologies will be crucial in advancing the fields of synthetic biology and gain of function safely and with public trust and support in order to fulfil the transformative promises they may offer.

Some of the regulatory challenges are different for synthetic biology and gain of function, though commonalities underlie both.

This section will focus on synthetic biology as the regulation of gain of function research has already been discussed in detail in other reports.

Currently in the US, government funded gain of function research is under a moratorium but the deliberative process initiated by the U.S. government to assess the potential risk and benefits associated with a subset of gain of function studies is underway.²⁸ This process involves the National Science Advisory Board for Biosecurity (NSABB) that recently provided draft recommendations to federal entities²⁹ and the U.S National Academy of Sciences that organised two symposia over the past years to discuss with the broader life-science community and other stakeholders potential risks and benefits of certain gain of function studies.^{30,31}

The situation in Europe has recently been reviewed by the European Academies Science Advisory Council, identifying that there is no specific gain of function legislation or moratorium, rather a system of self-regulation is in place. Individual European Union member states may also have their own national regulations,³² combined with existing GMO regulations.

Lessons may be learnt from the research community's experience with the field of gain of function. For some researchers the current moratorium in the US represents a blunt tool, and it was espoused that self-governance might be the most subtle and productive way of meeting synthetic biology's challenges.

Gene drives have their own additional regulatory issues as they are intended to be released into the environment, and indeed alter shared ecosystems.

While some synthetic biology research may fall under regulations for GMOs, this is not comprehensive or tailored for this new industrialisation of biology.

Other international treaties including the Biological Weapons Convention, or US government policy on Dual Use Research of Concern may have some bearing on some synthetic biology and gain of function research, but again this is not comprehensive. There may also be impacts for other international treaties, like the Convention on Biological Diversity.³³

In the US, a review of the biotechnologies regulatory systems for commercial products is currently underway with the White House Office of Science and Technology seeking to clarify the roles and develop long-term strategies within the responsible regulatory agencies: the Environmental Protection Agency (EPA), US Department of Agriculture (USDA) and the Food and Drugs Administration (FDA)³⁴ and the U.S. NAS is currently doing a study on Future Biotechnology Products and Opportunities to Enhance Capabilities of the Biotechnology Regulatory System.³⁵

28. <http://www.phe.gov/s3/dualuse/Documents/gain-of-function.pdf>

29. <http://osp.od.nih.gov/office-biotechnology-activities/event/2016-05-24-143000-2016-05-24-200000/national-science-advisory-board-biosecurity-nsabb-meeting>

30. http://dels.nas.edu/Workshop-Summary/Gain-Function-Research-Summary/23484?bname=bls_bls

31. <http://dels.nas.edu/Workshop-Summary/Potential-Risks-Benefits-Gain/21666?bname=>

32. http://www.easac.eu/fileadmin/PDF_s/reports_statements/Gain_of_Function/EASAC_GOF_Web_complete_centred.pdf

33. Synthetic biology and gene drives were both reviewed by ad hoc technical expert groups which reported to the Convention; <https://www.cbd.int/doc/meetings/synbio/synbioahateg-2015-01/official/synbioahateg-2015-01-03-en.pdf>; <https://www.cbd.int/doc/meetings/bs/bsrarm-ahateg-2015-01/official/bsrarm-ahateg-2015-01-04-en.pdf>

34. <https://www.whitehouse.gov/blog/2015/07/02/improving-transparency-and-ensuring-continued-safety-biotechnology>

35. www.nas.edu/biotech

The current US framework dates back to 1986, with an update in 1992, so the review aims to inform a future regulatory landscape for new and emerging biotechnologies.

A key issue for regulation in the US, that the US EPA has considered, is how to regulate synthetically engineered organisms that are so far removed from nature that they have no natural wild counterparts to use as reference points.³⁶ Though this situation has not yet been reached, such an ‘inflection point’ might occur in the future. For example, where XNA (xeno nucleic acid) might replace natural DNA in a synthetic organism.

Under the EPA’s current rules, only ‘intergeneric’ organisms are regulated, so while synthetic organisms with genes from different genera may fall under them – an entirely synthetic organism such as a pared-down synthetic yeast will not.³⁷

The core concepts of regulation are also being examined in the context of emerging biotechnologies. In the US, as in Europe, much of the regulation around GM and the environment are predicated on the concept of risk. This may be changing, with some consideration now also being given to the idea that magnitude of benefit should also be weighed up when making assessments and authorisations.

The European GM Regulatory Framework is looking at different ways of incorporating risk versus benefits in its assessment of new products. Currently, European regulations only allow risk to be considered in the authorisation procedure.

Where some countries already have some legislation that could deal with the potential risks of the emerging biotechnologies, other countries do not, or have loopholes, it was noted. For example, in the UK, health and safety legislation covers all research regardless of how the research is funded, whereas in the US current restrictions will often only apply to work funded by the NIH.

Some concerns were raised over burdensome regulation slowing down the innovation process and raising costs to levels that would not support commercialisation. The idea that synthetic biology might be able to harness elements of the pharma model for drug development (that is, with phase I-IV trials) was suggested.

In terms of safety and regulation, collective scrutiny and self-governance by the scientific community were also discussed, with transparency and communication being a key element to this.

No conclusions were made about the best way to regulate synthetic biology. It was clear that an important discussion needed to occur to ensure that potential technical safeguards were deployed when appropriate, that the regulation remained flexible to deal with changes and advances in the science and that any regulation was proportionate so that innovation could continue.

36. This is because the assessment system in the US for genetically modified organisms in the US requires comparison with ‘substantially equivalent’ non-genetically modified species. This is not the case in Europe.

37. http://sites.nationalacademies.org/cs/groups/pgasite/documents/webpage/pga_086096.pdf



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