What me worry?
Biosecurity in a time of biological revolution



David Relman, Stanford University

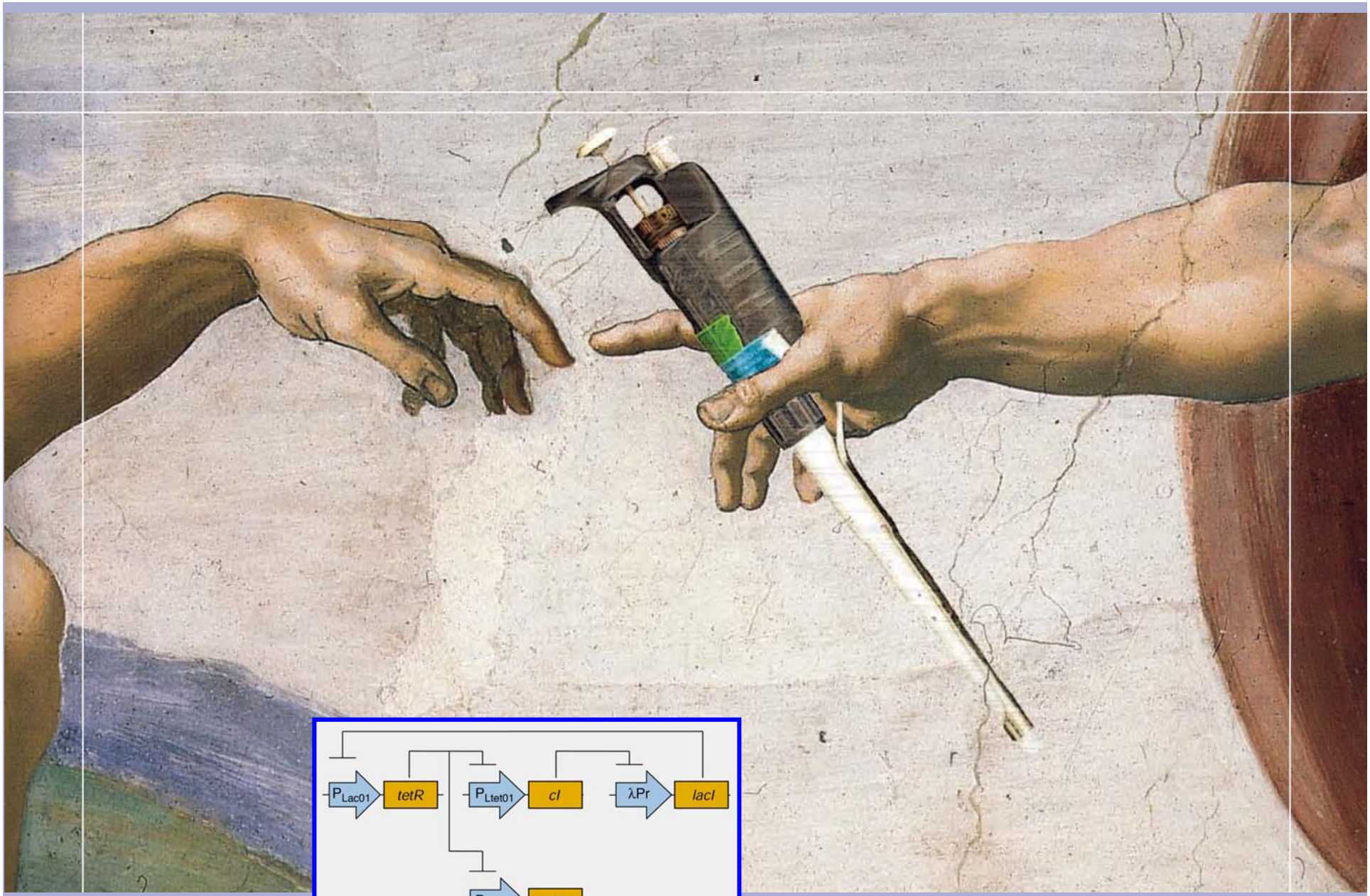
53rd Annual Biosafety Conference

American Biological Safety Association

Denver, October 4, 2010

Topics

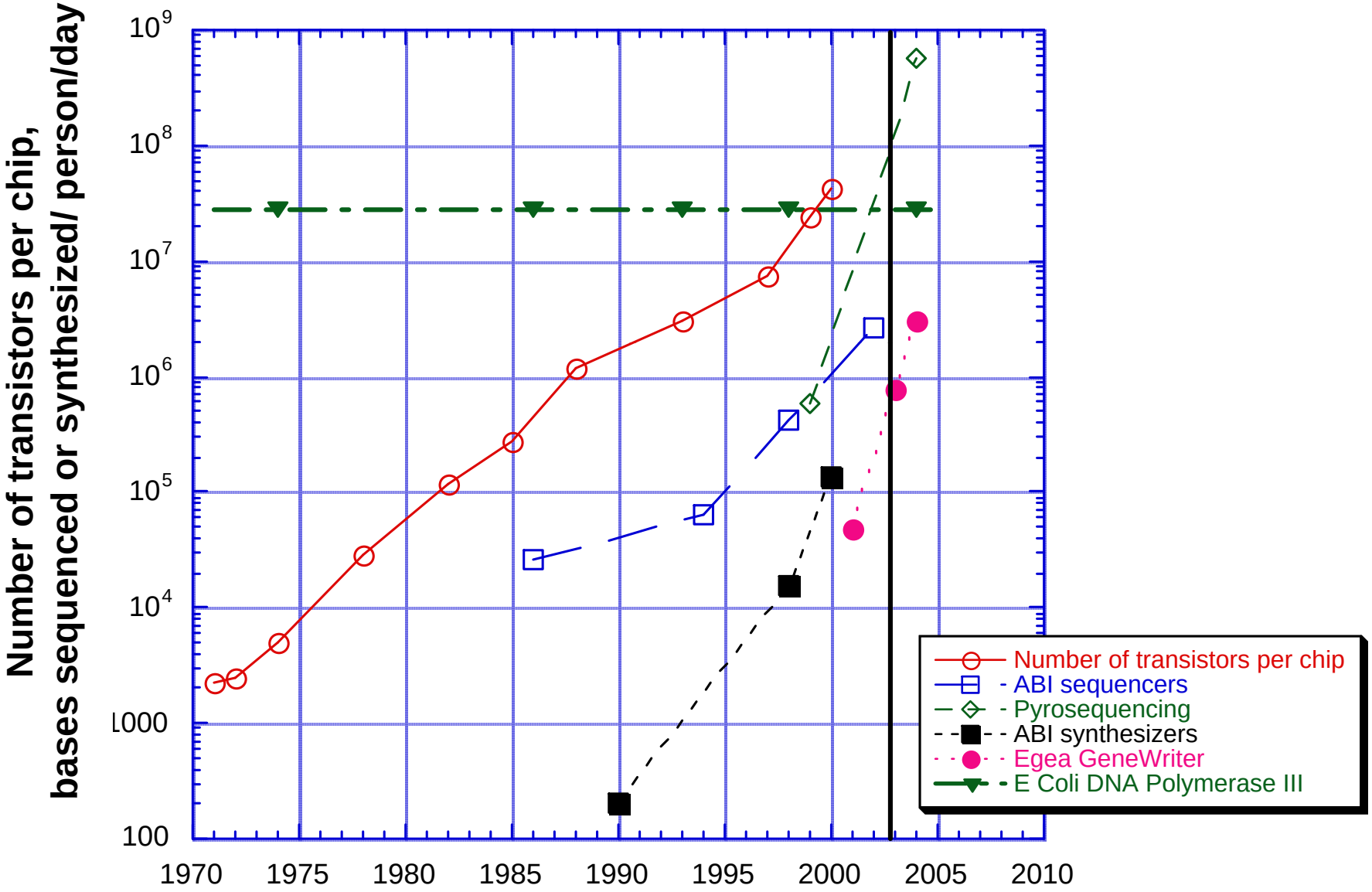
- Trajectories and advances in the life sciences
- Challenges and risks
- Approaches for mitigating risks



Alan Moses, Berkeley Science Review

From: Elowitz, Leibler; Nature 403:335, 2000

Comparing the pace of biological technologies and Moore's Law (Robert Carlson, 2003)



BIOSECURITY AND BIOTERRORISM: BIODEFENSE STRATEGY, PRACTICE, AND SCIENCE
Volume 1, Number 3, 2003

Commercial DNA Synthesis Foundries

Rob Carlson, University of Washington; Gerald Epstein and Anne Yu, CSIS

18 July 05. Method: Rough Google search. Thus not a thorough survey. No academic facilities.

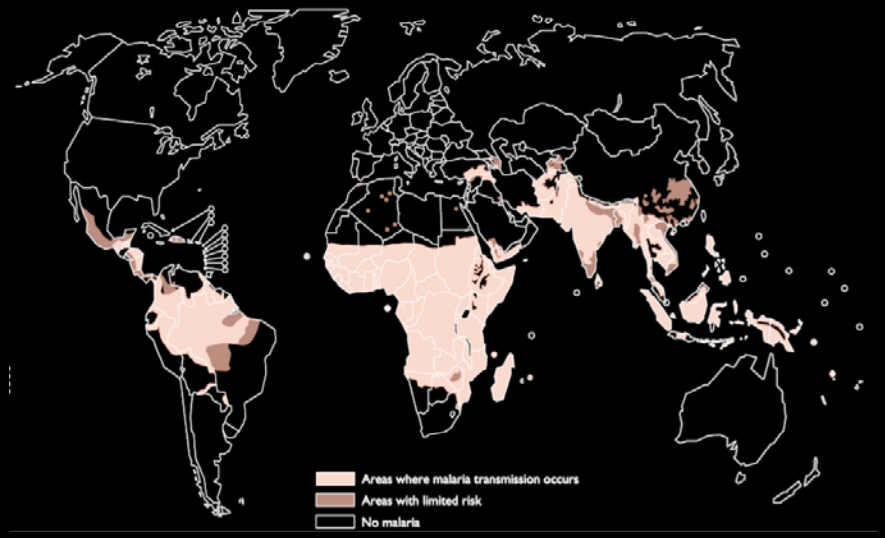
Data Source: Rob Carlson, U of W, Seattle
www.synthesis.cc, rob@synthesis.cc

nature Vol 440|13 April 2006|doi:10.1038/nature04640

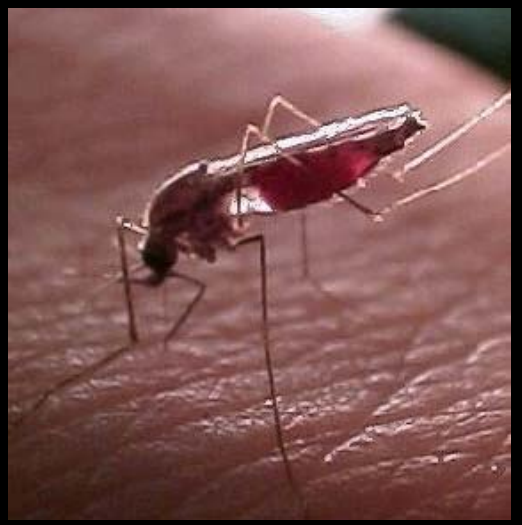
LETTERS

Production of the antimalarial drug precursor artemisinic acid in engineered yeast

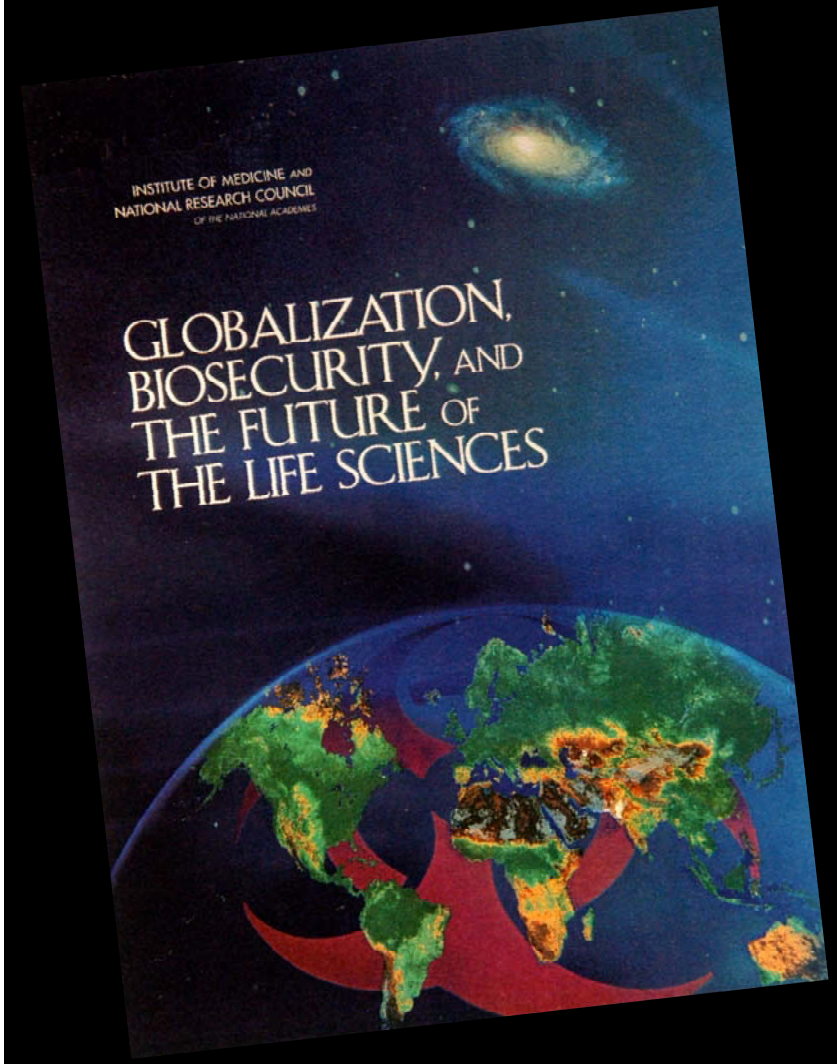
Dae-Kyun Ro[1]*, Eric M. Paradise[2]*, Mario Ouellet[1], Karl J. Fisher[6], Karyn L. Newman[1], John M. Ndungu[3], Kimberly A. Ho[1], Rachel A. Eachus[1], Timothy S. Ham[4], James Kirby[2], Michelle C. Y. Chang[1], Sydnor T. Withers[2], Yoichiro Shiba[2], Richmond Sarpong[3] & Jay D. Keasling[1,2,4,5]



www.who.int/malaria/ malariaendemiccountries.html



http://www.ncid.cdc.gov/vector/images/Anopheles.jpg



2006

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

Committee on Advances in Technology and the Prevention of their Application to Next Generation Bioterrorism and Biological Warfare Threats

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David A. Relman, co-chair, Stanford University
Roy Anderson, Imperial College London
Steven M. Block, Stanford University
Christopher F. Chyba, Stanford University and SETI Institute
Nancy Connell, University of Medicine and Dentistry of New Jersey
Freeman Dyson, Princeton University
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C. Kumar N. Patel, Pranalytica, Inc.
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George Poste, Arizona State University
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Julian Perry Robinson, University of Sussex
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Eileen Choffnes, Senior Program Officer
Stacey Knobler, Senior Program Officer
Leslie A. Pray, Science Writer
Kate Skoczdopole, Senior Program Assistant

Process-based classification of life sciences technologies

1. Acquisition of novel biological or molecular diversity (e.g., DNA synthesis, DNA shuffling, combinatorial chemistry)
2. Directed design (e.g., synthetic biology, reverse genetic engineering)
3. Understanding and manipulating biological systems (e.g., "systems biology", RNAi, modulators of homeostatic systems)
4. Production, packaging, delivery (e.g., microfluidics / microfabrication, nanotechnology, microencapsulation, gene therapy/targeting)

Globalization, biosecurity, and the future of the life sciences, NAS 2006

Optimized expression and specific activity of IL-12 by directed molecular evolution

Steven R. Leong, Jean C. C. Chang, Randal Ong, Glenn Dawes, Willem P. C. Stemmer, and Juha Punnonen*

The most improved evolved IL-12 (EvIL-12) derived from seven mammalian genes encoding both the p35 and p40 subunits of IL-12 showed a 128-fold improvement in human T cell proliferation compared with native hIL-12 during the initial screening of supernatants from transected cells. When purified hIL-12 and EvIL-12 proteins were compared *in vitro* in human T cell proliferation and Th1 differentiation assays, it was demonstrated that EvIL-12 exhibited a concomitant 10-fold increase in the specific activity of the protein compared with hIL-12. Furthermore, DNA shuffling improved the level of expression and homogeneity of the heterodimer synthesized by 293 human embryonic kidney cells transfected with EvIL-12 by at least 10-fold.

PNAS | February 4, 2003 | vol. 100 | no. 3 | 1163–1168

A myocardium tropic adeno-associated virus (AAV) evolved by DNA shuffling and in vivo selection

Lin Yang[a], Jiangang Jiang[a], Lauren M. Drouin[b], Mavis Agbandje-Mckenna[b], Chunlian Chen[a], Chunping Qiao[a], Dongqiuye Pu[a], Xiaoyun Hu[c], Da-Zhi Wang[c], Juan Li[a], and Xiao Xiao[a,1]

[a]Division of Molecular Pharmaceutics, University of North Carolina Eshelman School of Pharmacy, Chapel Hill, NC 27599; [b]Department of Biochemistry and Molecular Biology, College of Medicine, University of Florida, Gainesville, FL 32610; and [c]Carolina Cardiovascular Biology Center, University of North Carolina, Chapel Hill, NC 27599

Communicated by Yuet Wai Kan, University of California, San Francisco School of Medicine, San Francisco, CA, January 4, 2009 (received for review September 4, 2008)

3946–3951 | PNAS | March 10, 2009 | vol. 106 | no. 10

Breeding of retroviruses by DNA shuffling for improved stability and processing yields

Sharon K. Powell[1,2], Michele A. Kaloss[1], Anne Pinkstaff[1], Rebecca McKee[1], Irina Burimski[1], Michael Pensiero[1], Edward Otto[1]*, Willem P.C. Stemmer[3], and Nay-Wei Soong[3]

*[1]Genetic Therapy Inc., A Novartis Company, 9 W. Watkins Mill Road, Gaithersburg, MD 20878. [2]Present address: Avigen, 1201 Harbor Bay Parkway, Alameda, CA 94502. [3]Maxygen Inc., 515 Galveston Drive, Redwood City, CA 94063. *Corresponding author (ed.otto@pharma.novartis.com).*

NATURE BIOTECHNOLOGY VOL 18 DECEMBER 2000

The EMBO Journal vol.13 no.18 pp.4195–4203, 1994

Infectious rabies viruses from cloned cDNA

Matthias J.Schnell, Teshome Mebatsion and Karl-Klaus Conzelmann[1]

Institute of Clinical Virology, Federal Research Centre for Virus Diseases of Animals, Paul-Ehrlich-Strasse 28, D-72076 Tübingen, Germany

[1]Corresponding author

Communicated by R.Rott

The generation of infectious rabies virus (RV), a non-segmented negative-stranded RNA virus of the Rhabdoviridae family, entirely from cloned cDNA is described. Simultaneous intracellular expression of genetically marked full-length RV antigenome-like T7 RNA polymerase transcripts and RV N, P and L proteins from transfected plasmids resulted in formation of transcriptionally active nucleocapsids and subsequent assembly and budding of infectious rabies virions. In addition to authentic RV, two novel infectious RVs characterized by predicted transcription patterns were recovered from modified cDNA. Deletion of the entire non-translated pseudogene region, which is conserved in all naturally occurring RVs, did not impair propagation of the resulting virus in cell culture. This indicates that non-essential genetic material might be present in the genomes of non-segmented RNA viruses. The introduction of a functional extra cistron border into the genome of another virus resulted in the transcription of an additional polyadenylated mRNA containing pseudogene sequences. The possibility of manipulating the RV genome by recombinant DNA techniques using the described procedure—potentially applicable also for other negative-stranded viruses—greatly facilitates the investigation of RV genetics, virus–host interactions and rabies pathogenesis and provides a tool for the design of new generations of live vaccines.

Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice

Michelle M. Becker[a,1], Rachel L. Graham[b,1], Eric F. Donaldson[b], Barry Rockx[b], Amy C. Sims[b,c], Timothy Sheahan[b], Raymond J. Pickles[d,e], Davide Corti[f], Robert E. Johnston[c], Ralph S. Baric[b,c,d,2], and Mark R. Denison[a,g,2]

Departments of [a]Pediatrics and [g]Microbiology and Immunology, Vanderbilt University, Nashville, TN 37232; Departments of [b]Epidemiology and [d]Microbiology and Immunology, [e]Cystic Fibrosis/Pulmonary Research and Treatment Center and Department of Medicine, and [c]Carolina Vaccine Institute, University of North Carolina, Chapel Hill, NC 27599; and [f]Institute for Research in Biomedicine, CH-6500 Bellinzona, Switzerland

Edited by Peter Palese, Mount Sinai School of Medicine, New York, NY, and approved October 14, 2008 (received for review August 18, 2008)

Defining prospective pathways by which zoonoses evolve and emerge as human pathogens is critical for anticipating and controlling both natural and deliberate pandemics. However, predicting tenable pathways of animal-to-human movement has been hindered by challenges in identifying reservoir species, cultivating zoonotic organisms in culture, and isolating full-length genomes for cloning and genetic studies. The ability to design and recover pathogens reconstituted from synthesized cDNAs has the potential to overcome these obstacles by allowing studies of replication and pathogenesis without identification of reservoir species or cultivation of primary isolates. Here, we report the design, synthesis, and recovery of the largest synthetic replicating life form, a 29.7-kb bat severe acute respiratory syndrome (SARS)-like coronavirus (Bat-SCoV), a likely progenitor to the SARS-CoV epidemic. To test a possible route of emergence from the noncultivable Bat-SCoV to human SARS-CoV, we designed a consensus Bat-SCoV genome and replaced the Bat-SCoV Spike receptor-binding domain (RBD) with the SARS-CoV RBD (Bat-SRBD). Bat-SRBD was infectious in cell culture and in mice and was efficiently neutralized by antibodies specific for both bat and human CoV Spike proteins. Rational design, synthesis, and recovery of hypothetical recombinant viruses can be used to investigate mechanisms of transspecies movement of zoonoses and has great potential to aid in rapid public health responses to known or predicted emerging microbial threats.

The SARS-CoV genome is likely a mosaic of sequences derived from multiple recombination events, although this hypothesis is somewhat controversial (16). However, recombination within Spike has been described often (17), suggesting that the RBDs may be interchangeable between strains (18–20). During the SARS-CoV outbreak, evolution in the Spike RBD allowed for more efficient use of human angiotensin-converting enzyme 2 (hACE2) as a receptor for entry (21, 22). Because future zoonoses are likely, it is critical to identify strategies used by viruses to adapt in human populations. In this study, we have combined phylogenetic and bioinformatics analyses, large-scale cDNA synthesis, chimeric gene design, and reverse genetics to generate a consensus Bat-SCoV. Successful recovery of the infectious chimeric virus, Bat-SRBD, which includes the RBD within Spike from human SARS-CoV, demonstrates the plasticity of the CoV type I glycoprotein. The synthetic reconstruction and recovery of this novel chimeric virus identifies a necessary genetic element for CoV cross-species transmission, establishes a model system for testing experimental evolution of zoonotic CoVs, and allows for testing of vaccine and therapeutics against possible future zoonotic strains.

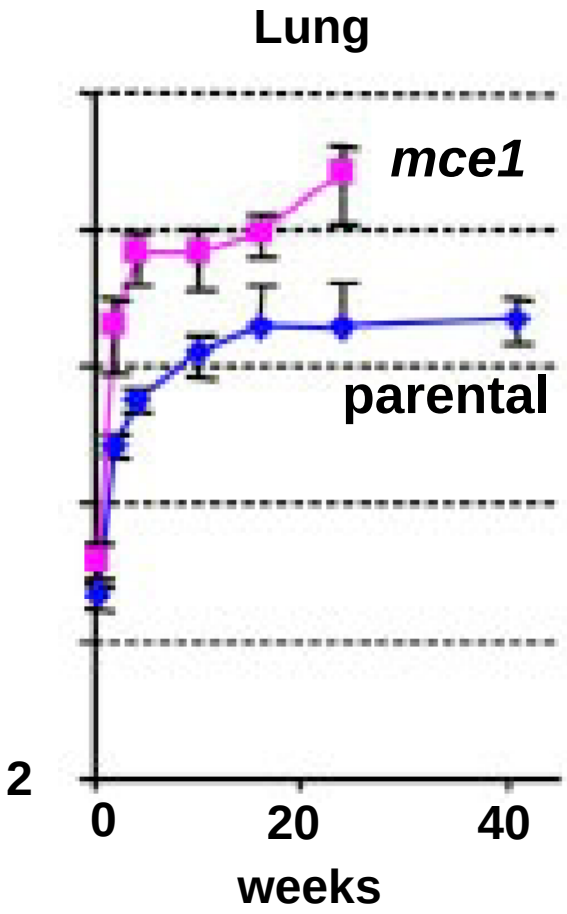
Results

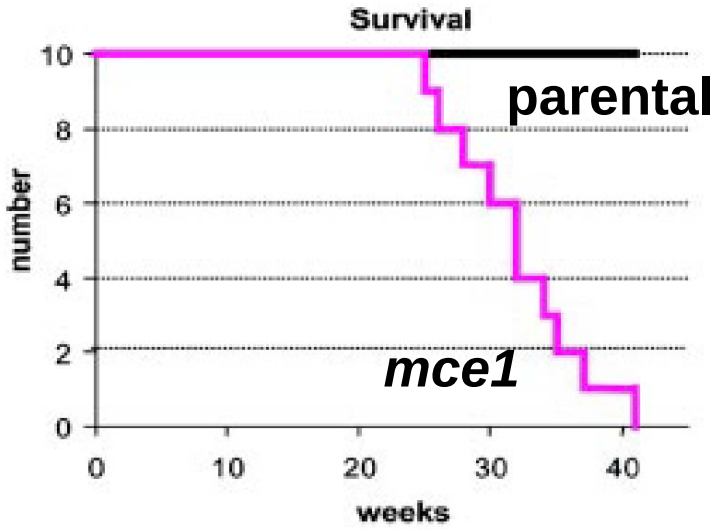
Consensus Bat-SCoV Sequence Design and Construction. When this study was initiated, 4 Bat-SCoVs had been identified (HKU3–1, HKU3–2, HKU3–3, and RP3) as the virus reservoir populations

Hypervirulent mutant of *Mycobacterium tuberculosis* resulting from disruption of the *mce1* operon

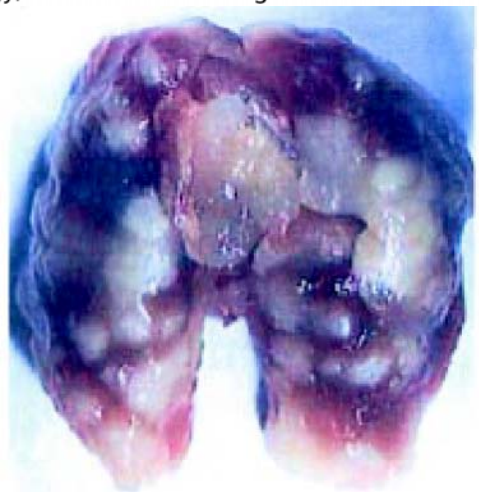
Nobuyuki Shimono*†‡, Lisa Morici*‡, Nicola Casali*, Sally Cantrell*, Ben Sidders*, Sabine Ehrt§, and Lee W. Riley*¶

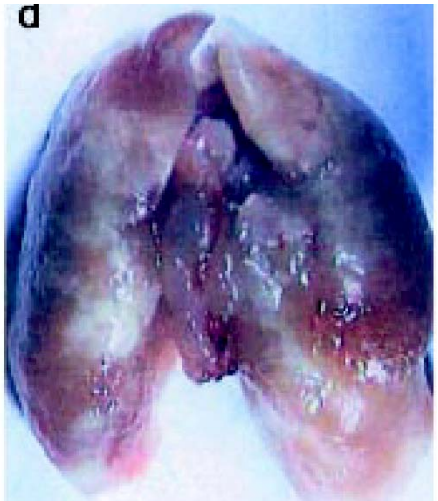
*Division of Infectious Diseases, School of Public Health, University of California, Berkeley, CA 94720; †Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka 812-8582, Japan; and §Department of Microbiology and Immunology, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021





diminished Th1 cytokine response





Engineering hypervirulence in a mycoherbicidal fungus for efficient weed control

Amsellem Z, Cohen BA, Gressel J

Nature Biotechnology, Oct 2002; 20:1035-1039

Nep1 (*Fusarium* phytotoxin)

+

***Colletotrichum coccodes* (fungal plant pathogen)**

***Abutilon theophrasti* (weed for cotton, maize)**

9x greater plant damage, more rapid effect, lethal for 3-leaf stage, less dependence on high humidity; unanticipated lethality for tomato and tobacco!!

"We consider it unwise to conduct uncontained experiments with such hypervirulent organisms before…fail-safe mechanisms are installed"

FEEDBACKLOOP

The Biohacking Hobbyist

Why does all biology happen in academic or industrial labs? Mac Cowell, cofounder of DIYbio, seeks to change that.

by **GREG BOUSTEAD** • Posted December 11, 2008 09:03 AM

When Mac Cowell says he wants to help people "do biology as a hobby," he's not talking about pinning insects to foamcore. He's talking about splicing DNA and reprogramming bacteria to create genetically engineered machinery.

The pushing of complex scientific information beyond the doors of hallowed institutions has been tagged with several modifiers: citizen, amateur, DIY, hobbyist. Call it what you will, but the "democratization of science" is flourishing. Nowhere is this trend arguably more evident than within synthetic biology, a field that applies engineering principles to the study and construction of biological systems. Through collaboration and an open-sourcing of genomic databases, Cowell and others hope that biohacking (with its etymological nod to the self-trained computer-programming movement) will provide nonscientists the opportunity to tinker with living machines.

Mac Cowell. Illustration by Bernd Schifferdecker.

RELATED

Novice Bioengineers Get Their Freak On
Recent iGEM judge, Jason Kelly, reflects on the future of synthetic biology.

http://seedmagazine.com/news/2008/12/the_biohacking_hobbyist.php

DIYbio

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Science without Scientists

Posted by Jason Bobe in DIYscience on 08 22nd, 2008 | 2 responses

As molecular tools get cheaper, and the know-how for using them more widely distributed, I think we're going to see a renaissance in science. The peculiar feature of this renaissance is that its going to take place outside of "science proper", away from the universities which dominate now, and funded out-of-pocket by enthusiasts without PhDs.

The democratization of technologies will enable more people to do their own science: make hypotheses, design experiments, collect large data sets, and apply a mixture of reasoning and cloud computing to make discoveries. Perhaps we'll see a multi-author journal article published written entirely by people without PhDs and no institutional affiliations. Although it sounds crazy, I'm not sure it is.

about us

DIYbio is an organization that aims to help make biology a worthwhile pursuit for citizen scientists, amateur biologists, and DIY biological engineers who value openness and safety. This will require mechanisms for amateurs to increase their knowledge and skills, access to a community of experts, the development of a code of ethics, responsible oversight, and leadership on issues that are unique to doing biology outside of traditional professional settings.

Lowering the bar to access: molecular biology in kits

Discovery DNA Explorer Kit
Other products by DISCOVERY CHANNEL
★★★★★ (2 customer reviews)

List Price: ~~$79.95~~
Price: **$39.98**
You Save: $39.97 (50%)

Availability: In Stock. Ships from and sold by **Discovery Channel Store**. Gift-wrap available.

- New Year Clearance--save up to 70% on toys and games

Related searches: biology science kits, forensic games csi

Product Features

- Extract, view and map real DNA (fabricated to mimic real DNA)
- Includes all the supplies needed for six fascinating DNA experiments
- Ideal for budding forensic-scientists or secret agents

The "Discovery DNA Explorer Kit"shouldn't be considered a toy. It really is way more than that, the educational value puts it over the top. If your child is under 9 or 10, they might need adult supervision. My son is ten and has successfully mapped DNA, used the centrifuge, and performed a number of experiments with some assistance from me.
The most important thing to remember is to read the instructions very carefully, timing is very critical. Some experiments need to be tended to after a certain number of hours so starting it at night could pose a problem.
I think it's an excellent product, well made, with lots of discovery potential.

http://www.amazon.com

Recovered from an al-Qaida training camp in Afghanistan...

society for applied sfam microbiology

(8) Therefore, keeping in view the above circumstances, a visit to [redacted] can be arranged for 10 days in the 1st week of [redacted]. *
This requir atleast the air ticket expanses.
For this visit, I should be informed as early as possible.

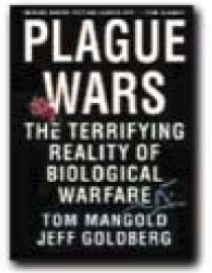
Yours Sincerely.
[redacted]

(9) The money with me is only for the purpose to buy strains or vaccines.

* There is a course in the 1st Week of [redacted] at

Society for Applied Microbiology • The Blore Tower • The Harpur Centre • Bedford MK40 1TQ
Telephone: 01234 326661 • Facsimile: 01234 326678
E-Mail: sfam@btinternet.com • Web site: www.blackwell-science.com/uk/society/sam/
Society for Applied Microbiology registered charity no. 326509 VAT registration no. 695 1572 03

(6) Unfortunately, I did not find the required culture of B. anthrax i.e. pathogenic. The culture available in [redacted] is non-pathogenic,



MORRIS, E. J. (1955). *J. gen. Microbiol.* **13**, 456–460

A Selective Medium for *Bacillus anthracis*

BY E. J. MORRIS

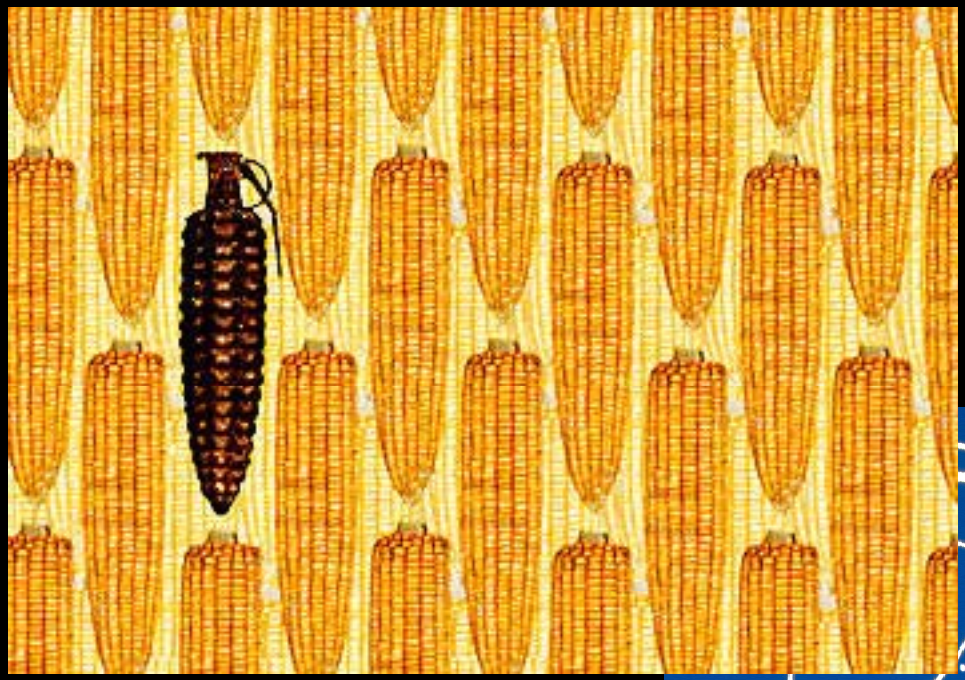
Microbiological Research Department, Ministry of Supply, Porton, Wiltshire

SUMMARY: A medium containing propamidine is described which has high selective activity for the species *Bacillus anthracis*. The spore form of the organism is essential as inoculum for the medium.

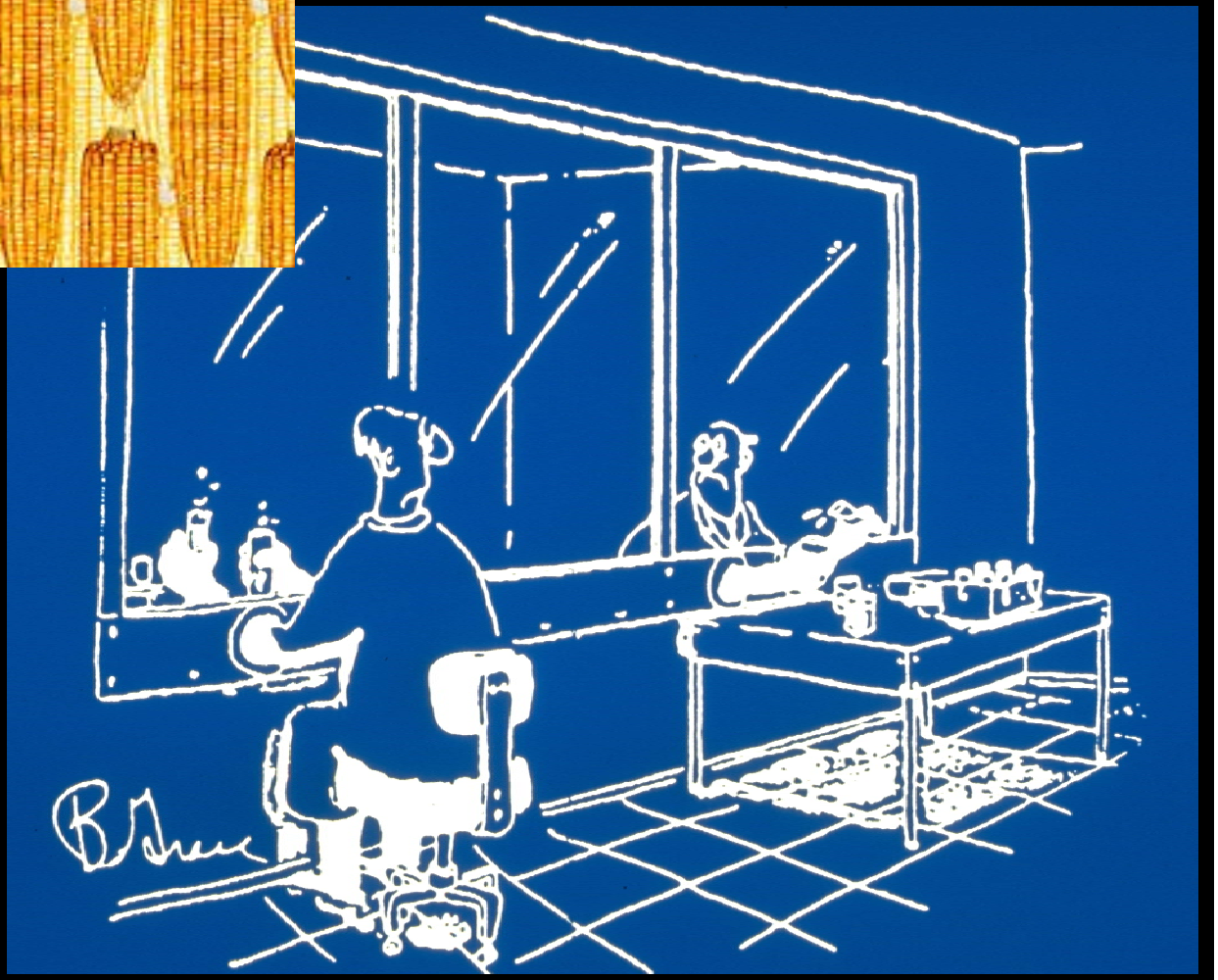
The isolation of *Bacillus anthracis* from material heavily contaminated by other organisms of the same genus can be difficult, particularly when small numbers of *B. anthracis* are present, or when quantitative examination is

JB Petro & DA Relman
Science 2003; 302:1898

Differing perceptions of risk



http://www.greenpeace.org.uk/
"Spot the GM crop"



General conclusions

- The life sciences will inevitably create new opportunities for misuse and potential for deliberate harm. These sciences and technologies are widely dispersed, easily accessible, and increasingly global.
- We can anticipate some developments, but not others. There will be a need for frequent re-assessment of the broad and changing threat spectrum. Attention should not be constrained by any list.

Globalization, biosecurity, and the future of the life sciences, NAS 2006

Topics

- Trajectories and advances in the life sciences
- Challenges and risks
- Approaches for mitigating risks

Life sciences vs. physical sciences

- requirements for entry; access
- breadth of research activities
- economic impact
- diversity and number of participants
- global nature of the enterprise

Mitigating the risks

- Regulate access to reagents, information?

Regulations, Code: recent history

1996 Antiterrorism & Effective Death Penalty Act

1997 "Select Agent Rule" Title 42 Part 72.6

2001 USA PATRIOT Act

2002 Public Health Security and Bioterrorism Preparedness Response Act

HHS AND USDA SELECT AGENTS AND TOXINS
7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

HHS SELECT AGENTS AND TOXINS
- Abrin
- Botulinum neurotoxins
- Botulinum neurotoxin producing species of *Clostridium*
- Cercopithecine herpesvirus 1 (Herpes B virus)
- *Clostridium perfringens* epsilon toxin
- *Coccidioides posadasii/Coccidioides immitis*
- Conotoxins
- *Coxiella burnetii*
- Crimean-Congo haemorrhagic fever virus
- Diacetoxyscirpenol
- Eastern Equine Encephalitis virus
- Ebola virus
- *Francisella tularensis*
- Lassa fever virus
- Marburg virus
- Monkeypox virus
- Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
- Ricin
- *Rickettsia prowazekii*
- *Rickettsia rickettsii*
- Saxitoxin
- Shiga-like ribosome inactivating proteins
- Shigatoxin
- South American Haemorrhagic Fever viruses
  - Flexal
  - Guanarito
  - Junin
  - Machupo
  - Sabia
- Staphylococcal enterotoxins
- T-2 toxin
- Tetrodotoxin
- Tick-borne encephalitis complex (flavi) viruses
  - Central European Tick-borne encephalitis
  - Far Eastern Tick-borne encephalitis
  - Kyasanur Forest disease
  - Omsk Hemorrhagic Fever
  - Russian Spring and Summer encephalitis
- Variola major virus (Smallpox virus)
- Variola minor virus (Alastrim)
- *Yersinia pestis*

OVERLAP SELECT AGENTS AND TOXINS
- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei* (formerly *Pseudomonas mallei*)
- *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*)
- Hendra virus
- Nipah virus
- Rift Valley fever virus
- Venezuelan Equine Encephalitis virus

USDA SELECT AGENTS AND TOXINS
- African horse sickness virus
- African swine fever virus
- Akabane virus
- Avian influenza virus (highly pathogenic)
- Bluetongue virus (exotic)
- Bovine spongiform encephalopathy agent
- Camel pox virus
- Classical swine fever virus
- *Ehrlichia ruminantium* (Heartwater)
- Foot-and-mouth disease virus
- Goat pox virus
- Japanese encephalitis virus
- Lumpy skin disease virus
- Malignant catarrhal fever virus (Alcelaphine herpesvirus type 1)
- Menangle virus
- *Mycoplasma capricolum* subspecies *capripneumoniae* (contagious caprine pleuropneumonia)
- *Mycoplasma mycoides* subspecies *mycoides* small colony (*MmmSC*) (contagious bovine pleuropneumonia)
- Peste des petits ruminants virus
- Rinderpest virus
- Sheep pox virus
- Swine vesicular disease virus
- Vesicular stomatitis virus (exotic): Indiana subtypes VSV-IN2, VSV-IN3
- Virulent Newcastle disease virus[1]

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS
- *Peronosclerospora philippinensis* (*Peronosclerospora sacchari*)
- *Phoma glycinicola* (formerly *Pyrenochaeta glycines*)
- *Ralstonia solanacearum* race 3, biovar 2
- *Rathayibacter toxicus*
- *Sclerophthora rayssiae var zeae*
- *Synchytrium endobioticum*
- *Xanthomonas oryzae*
- *Xylella fastidiosa* (citrus variegated chlorosis strain)

80 agents
11/17/2008

Subtitle J—Prevention of Terrorist Access to Destructive Weapons Act of 2004

"§ 175c. Variola virus

USC 175c

"(a) UNLAWFUL CONDUCT.—

"(1) IN GENERAL.—Except as provided in paragraph (2), it shall be unlawful for any person to knowingly produce, engineer, synthesize, acquire, transfer directly or indirectly, receive, possess, import, export, or use, or possess and threaten to use, variola virus.

"(2) EXCEPTION.—This subsection does not apply to conduct by, or under the authority of, the Secretary of Health and Human Services.

"(c) CRIMINAL PENALTIES.—

"(1) IN GENERAL.—Any person who violates, or attempts or conspires to violate, subsection (a) shall be fined not more than $2,000,000 and shall be sentenced to a term of imprisonment not less than 25 years or to imprisonment for life.

"(d) DEFINITION.—As used in this section, the term 'variola virus' means a virus that can cause human smallpox or any derivative of the variola major virus that contains more than 85 percent of the gene sequence of the variola major virus or the variola minor virus.".

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

NSABB: A USG-wide initiative

- To advise on strategies for mitigating the potential for misuse of dual use biological research
  - Consider both national security concerns and the needs of the research community
- Underpinned by MOA with 15 USG departments and agencies with a role/interest in life sciences research
  - Appoint *ex officio* member(s)
  - Consider recommendations of NSABB when developing and implementing life sciences research programs and policies

"Dual Use Research of Concern"

Research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment, or materiel

NSABB Charge (partial list)

Recommend:

- Criteria for identifying dual use research of concern
- National guidelines for oversight of dual use research at both local and federal levels, including
  - Local review and approval processes, e.g., Institutional Biosafety Committees (IBCs)
  - Criteria/processes for referral of issues to NSABB
- Strategies for oversight of new classes of experiments and technologies

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

Synthetic Genomics Working Group Charge

Phase 1: Examine the potential biosecurity concerns raised by synthesis of Select Agents (see Report of December 2006)

- --Assess the adequacy of the current regulatory and oversight framework
- --Recommend potential strategies to address any biosecurity concerns

Phase 2: Identify, assess, and recommend strategies to address potential dual use concerns that may arise from work being performed in the field of synthetic biology (see Report of April 2010)

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Synthetic Genomics Working Group Members

Voting members

David Relman (Chair)
Susan Ehrlich
Claire Fraser-Liggett
John Gordon
Mike Imperiale
Adel Mahmoud
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Tom Shenk

Ken Cole (DoD)
Dan Drell (DoE)
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Jenifer Smith (FBI)
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ADDRESSING BIOSECURITY CONCERNS RELATED TO THE SYNTHESIS OF SELECT AGENTS

DECEMBER 2006

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Office of the Secretary

Screening Framework Guidance for Synthetic Double-Stranded DNA Providers

AGENCY: Department of Health and Human Services, Office of the Secretary.

ACTION: Notice.

Authority: Public Health Service Act, 42 U.S.C. 241, Section 301; HSPD–10.

Virology **302,** 299–309 (2002)
doi:10.1006/viro.2002.1677

Recombinant Chimeric Western and Eastern Equine Encephalitis Viruses as Potential Vaccine Candidates

Randal J. Schoepp,*,†,[1] Jonathan F. Smith,* and Michael D. Parker*

**Virology Division and †Diagnostic Systems Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702*

Received October 30, 2001; returned to author for revision February 26, 2002; accepted July 8, 2002

Chimeric cDNA clones, pMWE1000 and pMWE2000, differing by five nucleotides at their 5′ termini, were constructed of the 5′ two-thirds of the western equine encephalitis (WEE) virus genome (encoding nonstructural proteins) and the 3′ one-third of the eastern equine encephalitis (EEE) virus genome (encoding structural proteins). The WEE virus sequences were derived from full-length cDNA clones, pWE1000 and pWE2000, which were isogenic except for five nucleotide differences at their 5′ termini and were responsible for significant differences in mouse virulence. Each cDNA clone was placed downstream from a T7 promoter to allow *in vitro* transcription of full-length RNA. Transfection of BHK-21 cells with the chimeric RNA by electroporation gave rise to high-titer infectious virus. The *in vitro* characteristics of each chimera virus were determined by electrophoretic analysis of its structural proteins, plaque morphology, neutralization characteristics, replication kinetics, and rate of viral RNA synthesis. With the exception of plaque morphology, the *in vitro* characteristics of MWE1000 and MWE2000 were indistinguishable from the parental EEE virus. Subcutaneous inoculation of 5-week-old C57BL/6 mice with varying doses of MWE1000 or MWE2000 virus demonstrated that both chimeric viruses were significantly attenuated compared to the parental WEE virus (Cba 87) and EEE virus (PE-6). Animals infected with 10^5 PFU or more of either MWE1000 or MWE2000 were completely protected from lethal challenge with the virulent EEE virus, FL91-4679, but were not protected from virulent WEE virus Cba 87 challenge. Construction of viable virus chimeras often results in attenuated viruses that may hold promise as genetically engineered alphavirus vaccine candidates (R. J. Kuhn, D. E. Griffin, K. E. Owen, H. G. M. Niesters, and J. H. Strauss, 1996, *J. Virol.* 70, 7900–7909).

Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax

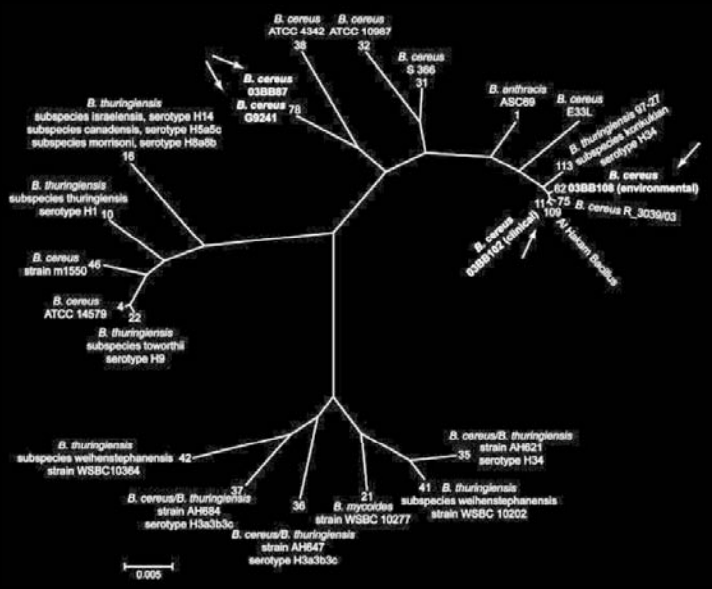
Alex R. Hoffmaster*†, Jacques Ravel†‡, David A. Rasko†‡, Gail D. Chapman§, Michael D. Chute§, Chung K. Marston*, Barun K. De*, Claudio T. Sacchi*, Collette Fitzgerald*, Leonard W. Mayer*, Martin C. J. Maiden¶, Fergus G. Priest‖, Margaret Barker‖, Lingxia Jiang‡, Regina Z. Cer‡, Jennifer Rilstone‡, Scott N. Peterson‡, Robbin S. Weyant*, Darrell R. Galloway§, Timothy D. Read†§, Tanja Popovic*†, and Claire M. Fraser†‡**

*Epidemiologic Investigations Laboratory, Meningitis and Special Pathogens Branch, Centers for Disease Control and Prevention, 1600 Clifton Road, MS G34, Atlanta, GA 30333; ‡Microbial Genomics and Pathogen Functional Genomic Resource Center, Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850; ¶The Peter Medawar Building for Pathogen Research and Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3SY, United Kingdom; ‖School of Life Sciences, Heriot Watt University, Edinburgh EH14 4AS, United Kingdom; and §Biological Defense Research Directorate, Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910

Communicated by John B. Robbins, National Institutes of Health, Bethesda, MD, April 5, 2004 (received for review March 24, 2004)

Bacillus anthracis **is the etiologic agent of anthrax, an acute fatal disease among mammals. It was thought to differ from *Bacillus cereus*, an opportunistic pathogen and cause of food poisoning, by the presence of plasmids pXO1 and pXO2, which encode the lethal toxin complex and the poly-γ-D-glutamic acid capsule, respectively. This work describes a non-*B. anthracis* isolate that possesses the anthrax toxin genes and is capable of causing a severe inhalation anthrax-like illness. Although initial phenotypic and 16S rRNA analysis identified this isolate as *B. cereus*, the rapid generation and analysis of a high-coverage draft genome sequence revealed the presence of a circular plasmid, named pBCXO1, with 99.6% similarity with the *B. anthracis* toxin-encoding plasmid, pXO1. Although homologues of the pXO2 encoded capsule genes were not found, a polysaccharide capsule cluster is encoded on a second, previously unidentified plasmid, pBC218. A/J mice challenged with *B. cereus* G9241 confirmed the virulence of this strain. These findings represent an example of how genomics could rapidly assist public health experts responding not only to clearly identified select agents but also to novel agents with similar pathogenic potentials. In this study, we combined a public health approach with genome analysis to provide insight into the correlation of phenotypic characteristics and their genetic basis.**

PNAS | June 1, 2004 | vol. 101 | no. 22 | 8449–8454



Hoffmaster AR et al., J Clin Microbiol 2006; 44:3352-60

Definitions: What is it?

- Problems with taxonomy-based definitions
  - Significant (and varying) degrees of natural variation (genetic and phenotypic) within taxon
  - Lack of correspondence between taxonomy, genotype, and phenotype
  - Increasing capability to create novel genotypes….with unclear, misleading taxonomy

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Recommendation 4

- 4.1 convene a group of experts from the scientific community to conduct an open and indepth examination of the Select Agent classification system to determine if it is possible to reconcile the current controls for Select Agents with the anticipated scientific advances enabled by synthetic genomics
- 4.2 assemble a group of experts from the scientific community to determine if an alternative framework based on predicted features and properties encoded by nucleic acids, such as virulence or pathogenicity, can be developed and utilized in lieu of the current finite list of specific agents and taxonomic definitions; and
- 4.3 consider the potential international implications…, foster an international dialogue and collaboration…

Sequence-Based Classification of Select Agents:

A Brighter Line

Committee on Scientific Milestones for the Development of a Gene-Sequence-Based Classification System for the Oversight of Select Agents

Board on Life Sciences
Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

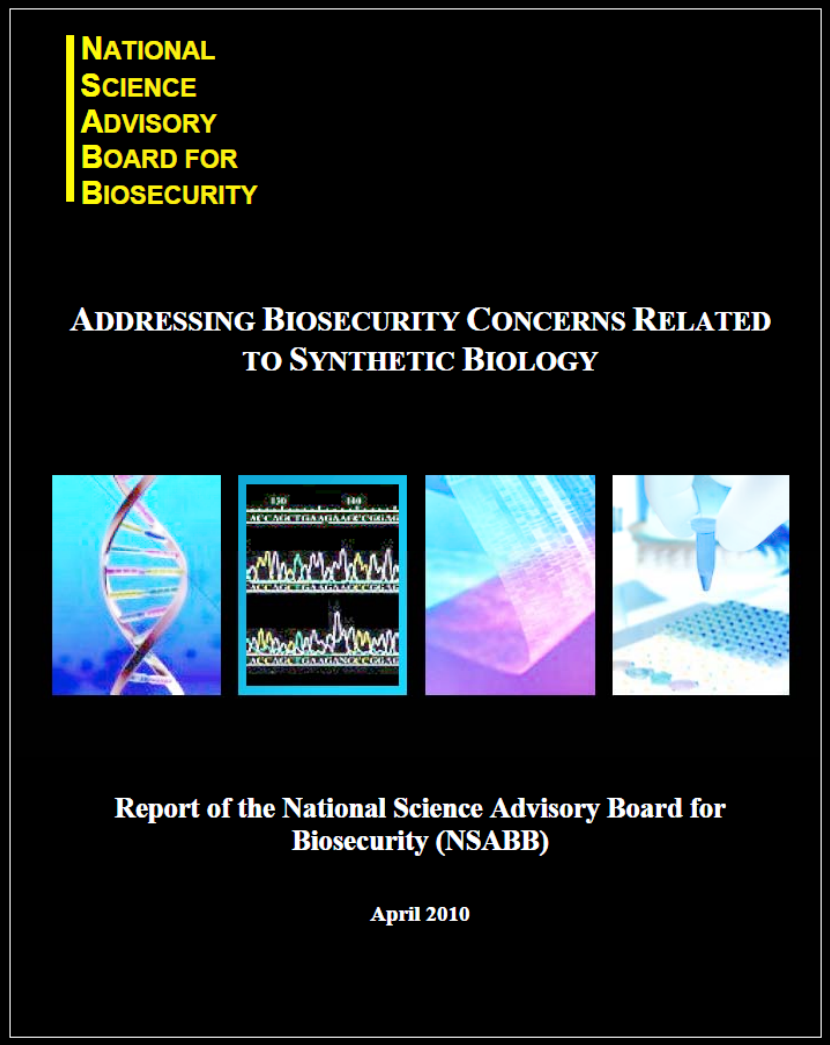
THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

Pre-publication Release: August 3, 2010

Findings

- A sequence-based *prediction* of Select Agent properties is *not* feasible, either now or in the foreseeable future
- A sequence based *classification system* for Select Agents focused on consideration of "sequences of concern" *could* be developed (but not sure if *should* be developed)

Sequence-based classification of Select Agents: A brighter line
NAS/NRC 2010



NSABB recommended:

- Synthetic biology should be subject to institutional review/oversight. NSABB has proposed an oversight paradigm that should adequately address dual use research issues associated with synthetic biology and strongly urges the federal government to develop and implement such policy*
- Oversight of dual use research should extend beyond the boundaries of life sciences and academia
- Outreach and education strategies should be developed to engage the diverse research communities
- The USG should include advances in synthetic biology in "tech-watch" endeavors

* Proposed framework for the oversight of dual use life sciences research: strategies for minimizing the potential misuse of research information, NSABB 2007

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

We report the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

2 JULY 2010 VOL 329 **SCIENCE**

THE DAILY TEXAN online
Serving the University of Texas at Austin since 1900

3/11/04

Tech professor gets 2 years for mishandling plague vials

By Betsy Blaney (Associated Press)

“Prosecutor Bob Webster said he was satisfied with the sentence. ‘It was a fair sentence that sent an appropriately strong message to the scientific and academic communities’, he said. ‘The government will do what it takes to ensure that the public is safe from the blatant disregard of laws intended to protect them from unwitting exposures to deadly agents.’”

VIEWPOINTS

Destroying the Life and Career of a Valued Physician-Scientist Who Tried to Protect Us from Plague: Was It Really Necessary?

Barbara E. Murray,[1,2] Karl E. Anderson,[3] Keith Arnold,[17,a] John G. Bartlett,[8] Charles C. Carpenter,[11,12] Stanley Falkow,[13] J. Ted Hartman,[4,b] Tom Lehman,[6] Ted W. Reid,[5] Frank M. Ryburn, Jr.,[7] R. Bradley Sack,[11] Marc J. Struelens,[16] Lowell S. Young,[14,15] and William B. Greenough III[9,10]

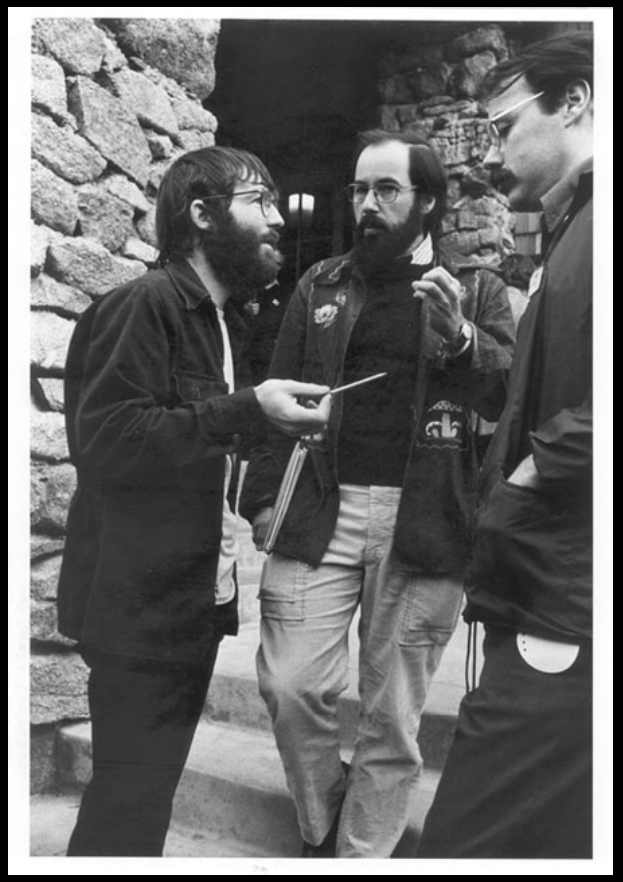
Clinical Infectious Diseases 2005; 40:1644–8

Mitigating the risks

- Regulate access to reagents, information?
- Promote awareness, sensitize relevant communities
  - self-governance
  - local (professional orgs, academia, industry)
  - national leadership (e.g., NAS, NSABB)
  - international organizations (e.g., UN, ICRC)

International Conference on Recombinant DNA Molecules
Asilomar Conference Center, Pacific Grove, California
February 1975

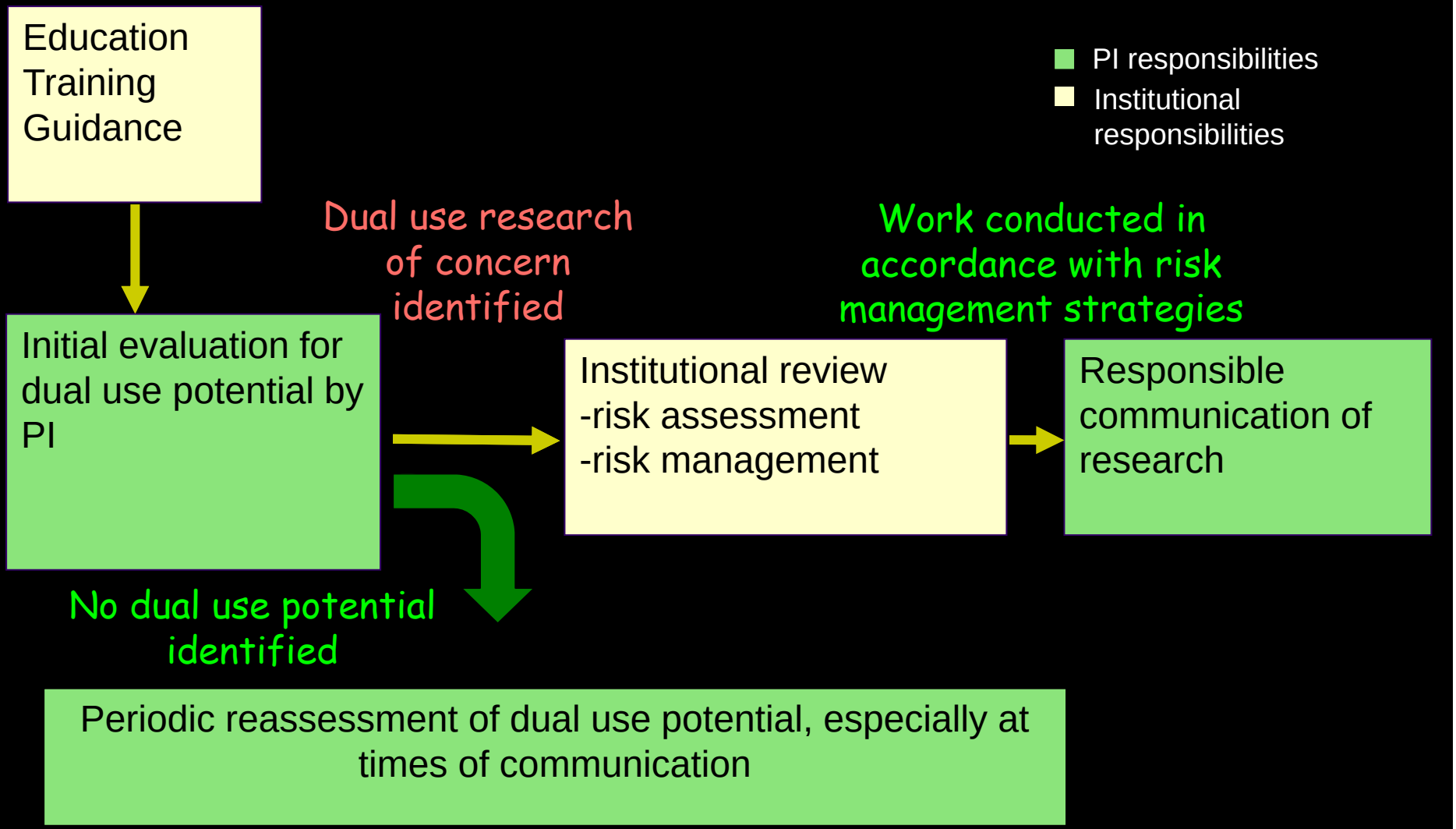
Maxine Singer, Norton Zinder, Sydney Brenner, Paul Berg



Philip Sharp, David Baltimore

http://libraries.mit.edu/archives/exhibits/asilomar/index.html

Steps in local oversight of dual use research



Mitigating the risks

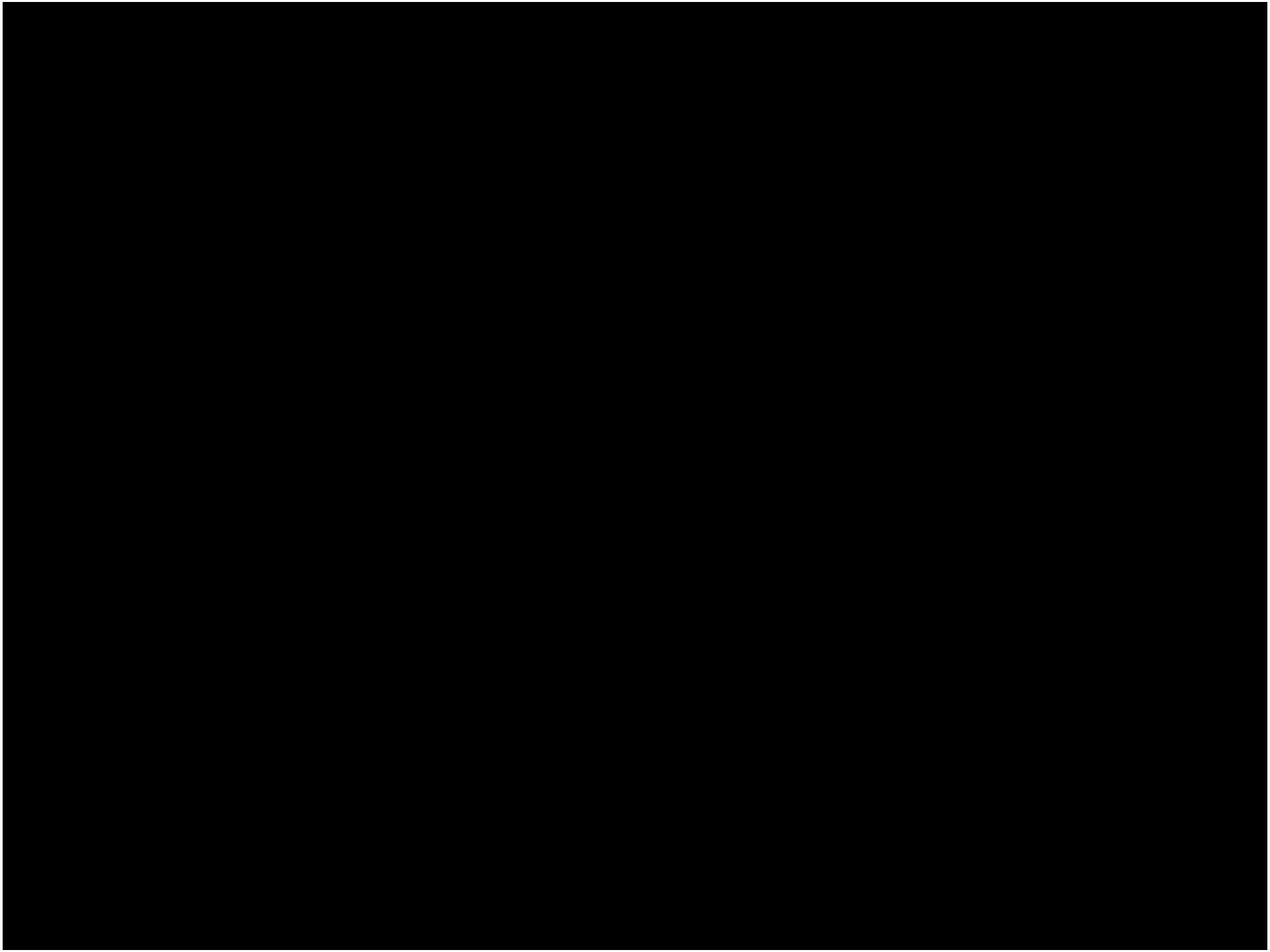
- Regulate access to reagents, information?
- Promote awareness, sensitize relevant communities
  - self-governance
  - local (professional orgs, academia, industry)
  - national leadership (e.g., NAS, NSABB)
  - international organizations (e.g., UN, ICRC)
- Anticipate, preempt threats
- Misuse, attack inevitable: strengthen defenses, public health infrastructure

Looking forward

- Outreach and education for scientific community
- Discussion (reality-check) with other stakeholders: public, policy-makers
- Rapidly evolving science may render today's guidelines and regulations moot
- Application of S&T for monitoring, prediction?
- Periodic re-review of risks, emerging S&T
  - NSABB "tech watch" activities, revisiting of synthetic biology (10/19/10)

- We are entering "The Biological Century"

  [Gregory Benford, 1992]
- Unimaginable capabilities, untold benefits, unforeseen issues, unavoidable risks
- Mitigating the risks: raise awareness, educate, communicate, norms, guidelines, anticipate threats, and promote flexible / agile / rapid / generic biodefense
- Be mindful of unintentional harm to a beneficial enterprise on which we depend



Is Mother Nature the best bioterrorist?

Considerations

- Nature has created a formidable array of pathogens, and will continue to do so.
- The ability to cause disease is a rare, and highly evolved trait.
- We have not yet encountered all that has been created in the natural world.

- At the same time, Nature has not "conceived" of all possibilities (various reasons). We have the ability to create and sample a supernatural "sequence space"…
- Success does not necessarily require long-term survival of the microbe; thus, our assessments may need revision.

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Recommendation 2 (continued)

Promote Screening

- Require federal grantees/contractors to order from providers that screen and retain information about requests for SA sequences
- Foster an international dialogue regarding best practices/standards for screening sequences

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Recommendation 1

Develop and Disseminate Harmonized Guidance

- Clarify what genetic elements or genomes are covered by Select Agent Rules (SAR)
- Increase awareness among investigators and providers about their responsibilities to know what they possess, manufacture and/or transfer in order to comply with the SAR

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Recommendation 2

Develop Standards & Practices

- Develop a process for determining the sequences for which to screen (SA or otherwise)
- Develop standards & practices (S&P) for screening orders and interpreting the results
- Draft "Points to Consider" for determining if genomic material is subject to the SAR
- Develop S&P for providers for retaining records of orders for gene-length or genome-length nucleic acids

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- Rob Weyant (CDC)

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Recommendation 4 (continued)

Consider Intl. Implications & Foster Intl. Collaboration

- Consider the potential international implications of any proposed changes to the current oversight framework for synthetic DNA and synthetic genomes
- Foster an international dialogue and collaboration on these issues

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Biosecurity Concerns

- Ease of acquisition of synthetic Select Agent nucleic acids [and ease of genetic manipulation]
- Need for additional regulatory clarity in specific areas
- Difficulty in developing a suitable regulatory framework

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Recommendation 3

Amend Current Laws/Regulations

- 3.1 Repeal 18 U.S.C. 175(c) because current scientific insight precludes meaningful definition of an agent based solely on sequence homology (arbitrariness…)
- 3.2 Examine current biosafety guidelines and regulations to ensure they provide adequate guidance for working with synthetically-derived DNA
- 3.3 Reconcile the genetic elements language in the CCL with that in the SAR

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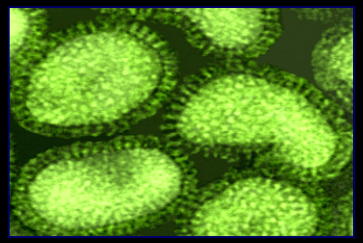
State of Science



- Methods are well-established for recovering/reconstructing certain Select Agents from DNA
- One can develop and produce agents that resemble, and have the attributes of specific Select Agent(s), without being clearly identifiable as SA based on their sequence. For example, researchers have created infectious viruses using combinations of genomic material from various SA; these novel organisms do not fit current taxonomic classification schemes.