Issues in the Use of Genetic Technologies in Bioterrorism

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Secretary's Advisory Committee on Genetics, Health, and Society

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Current Vulnerabilities

Inadequate systems for detection and recognition of an actual attack or the specific agent

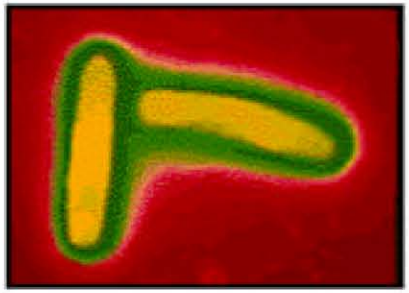
Lack of rapid and accurate diagnostic tests

Lack of basic knowledge regarding pathogenesis of most potential biowarfare agents

Lack of adequate forensic methods for the purposes of attribution

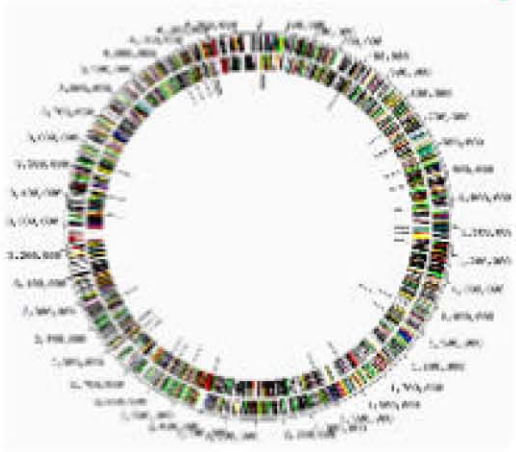
Lack of adequate vaccines, anti-microbial and anti-viral drugs

Pathogen Genome Sequencing Leads to New Tools

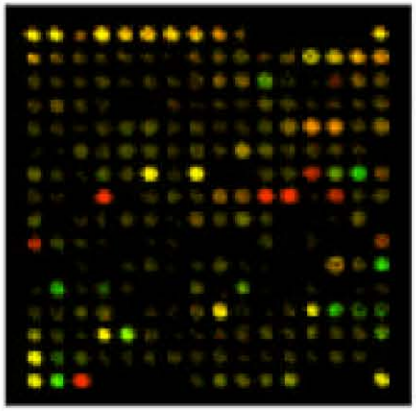


Pathogen

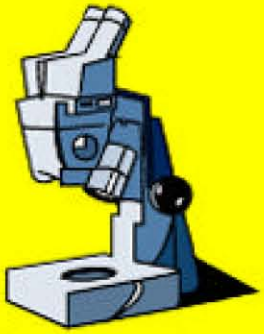
Cloned genomic DNA



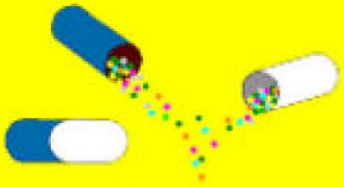
Whole genome sequence



Analysis



Diagnostics



Drugs

Vaccines

Bioterrorism: Category A Agents*

Variola major	Smallpox
Bacillus anthracis	Anthrax
Yersinia pestis	Plague
Clostridium botulinum	Botulism
Francisella tularensis	Tularemia
Filo- and Arenaviruses	Viral hemorrhagic fevers

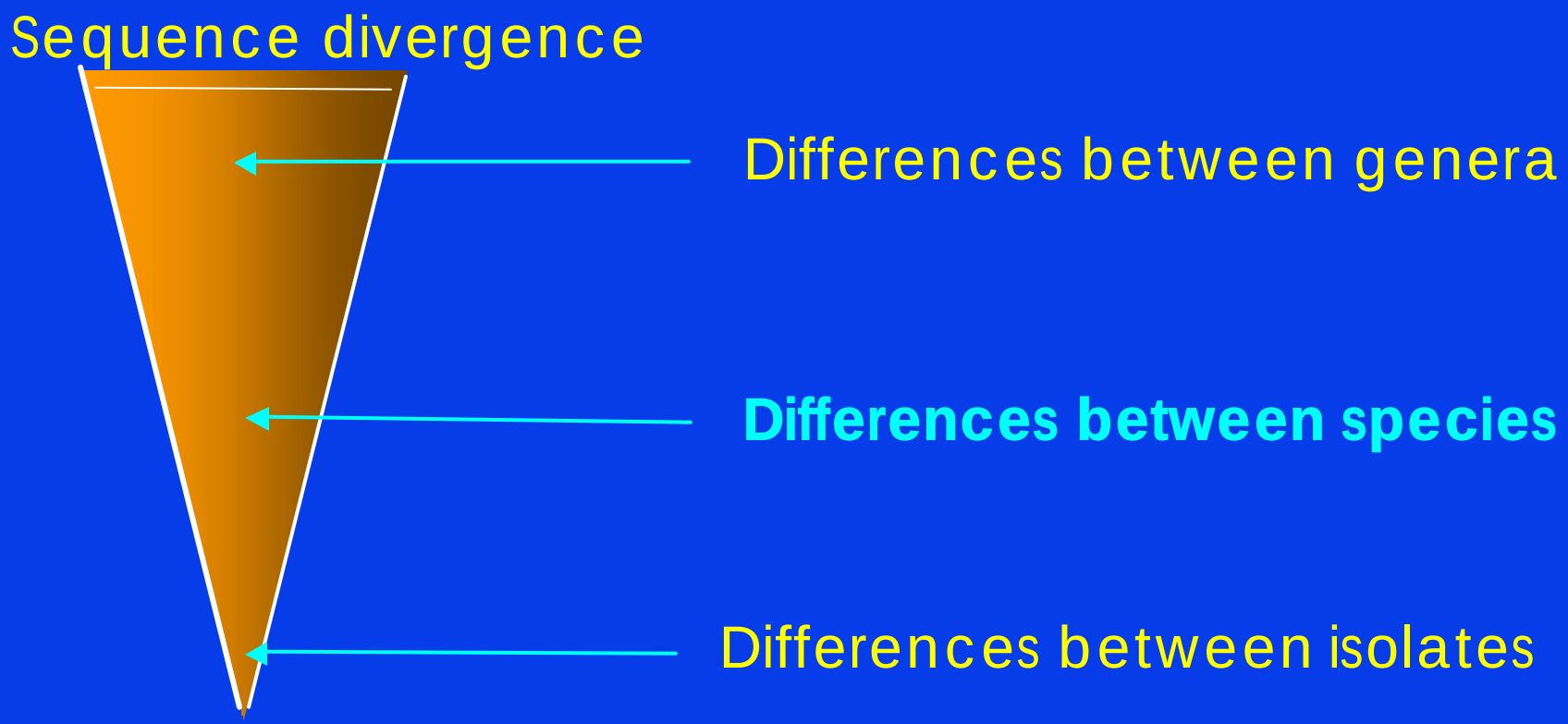
*Category A agents represent the highest risk for national security because they are (1) easily disseminated or transmitted person to person, (2) cause high mortality, (3) might cause public panic, and (4) require special public health preparedness.

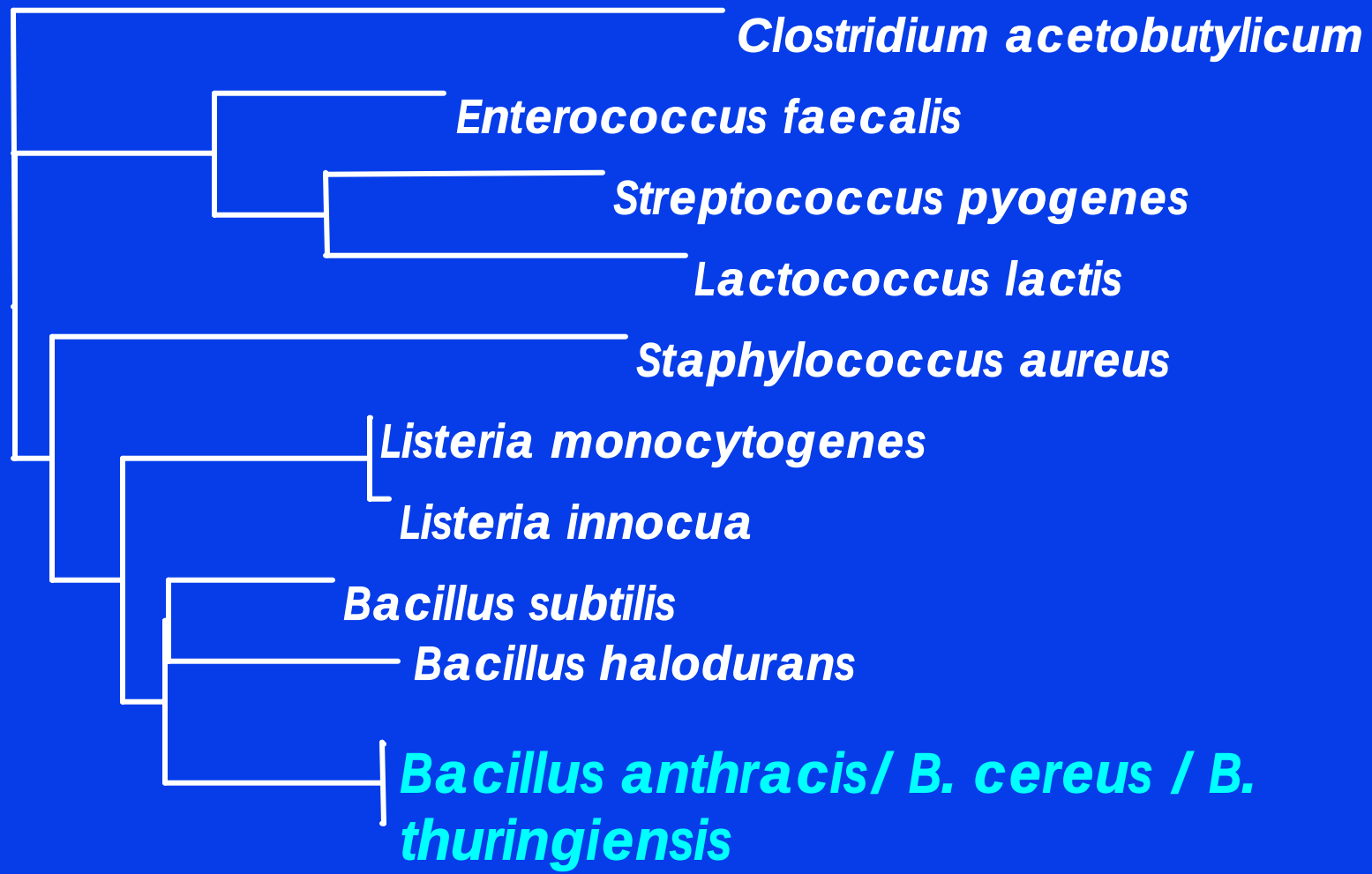
Bioterrorism: Category B Agents*

Coxiella burnetii	Q fever
Brucella species	Brucellosis
Burkholderia mallei	Glanders
Burkholderia pseudomallei	Melioidosis
Ricin toxin	
C. perfringens epsilon toxin	
Staphylococcus enterotoxin	

*Category B agents represent a lower risk for national security because they are (1) able to be disseminated, (2) cause moderate morbidity and mortality, and (3) require enhancements to CDC's diagnostic capabilities and surveillance.

Comparative Genomics Questions





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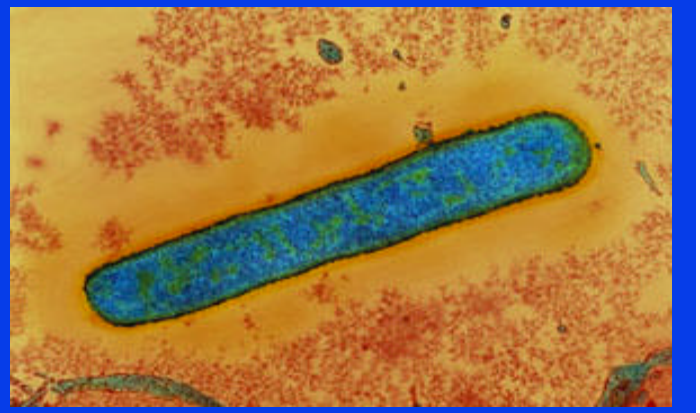
Bacillus anthracis (Read et al., 2003)



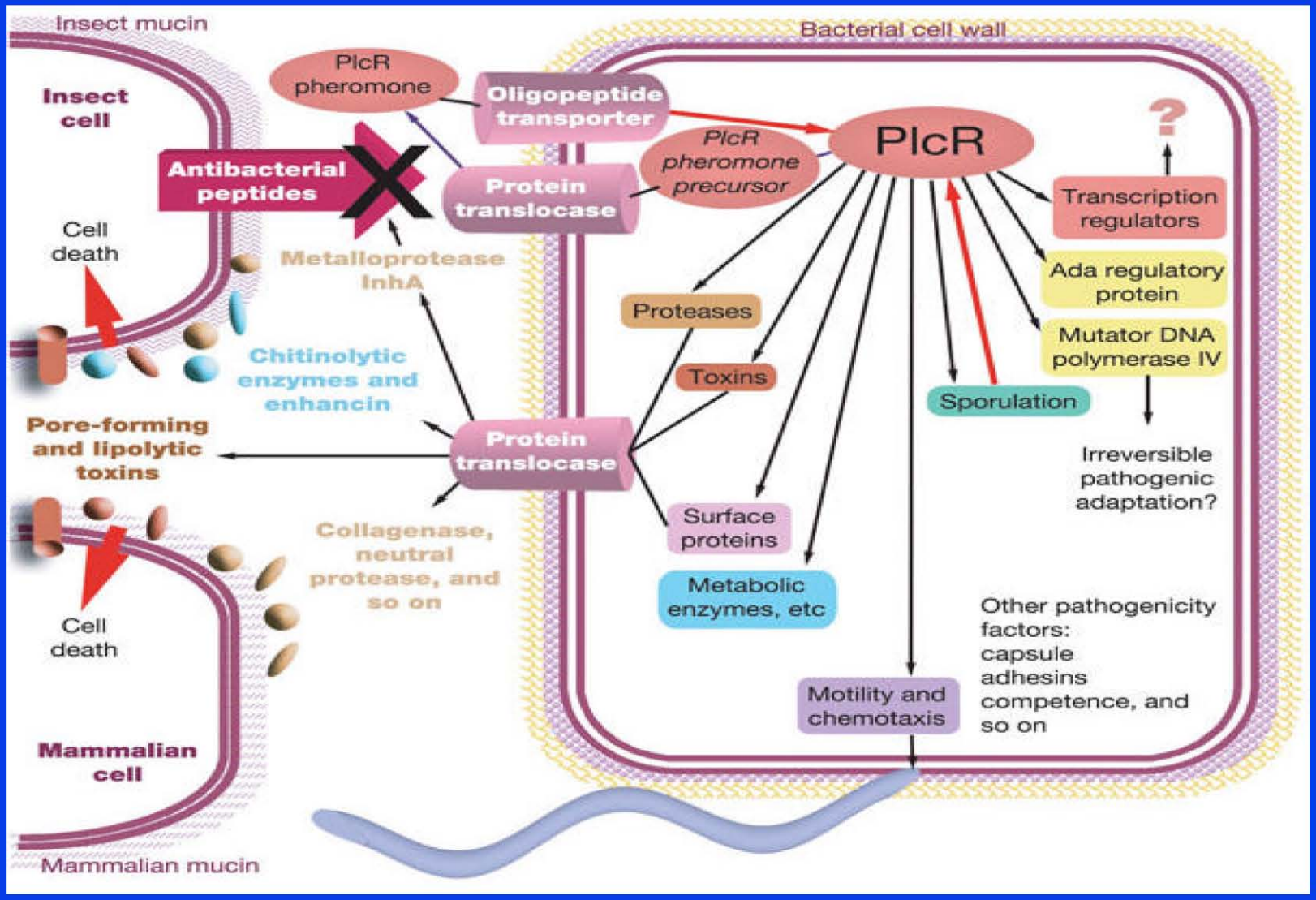
- Sporulating, low G+C Gram+
- 5.37 Mb chromosome, 182 kb pXO1, 96 kb pXO2
- Toxins, capsule genes on pXO1, pXO2
- Causative agent of anthrax

Bacillus cereus (Ivanova et al., 2003)

- Sporulating, low G+C Gram+
- 5.26 Mb chromosome, 15 kb linear plasmid
- No orthologs of toxin and capsule genes seen in *B. anthracis*
- Soil borne, opportunistic pathogen

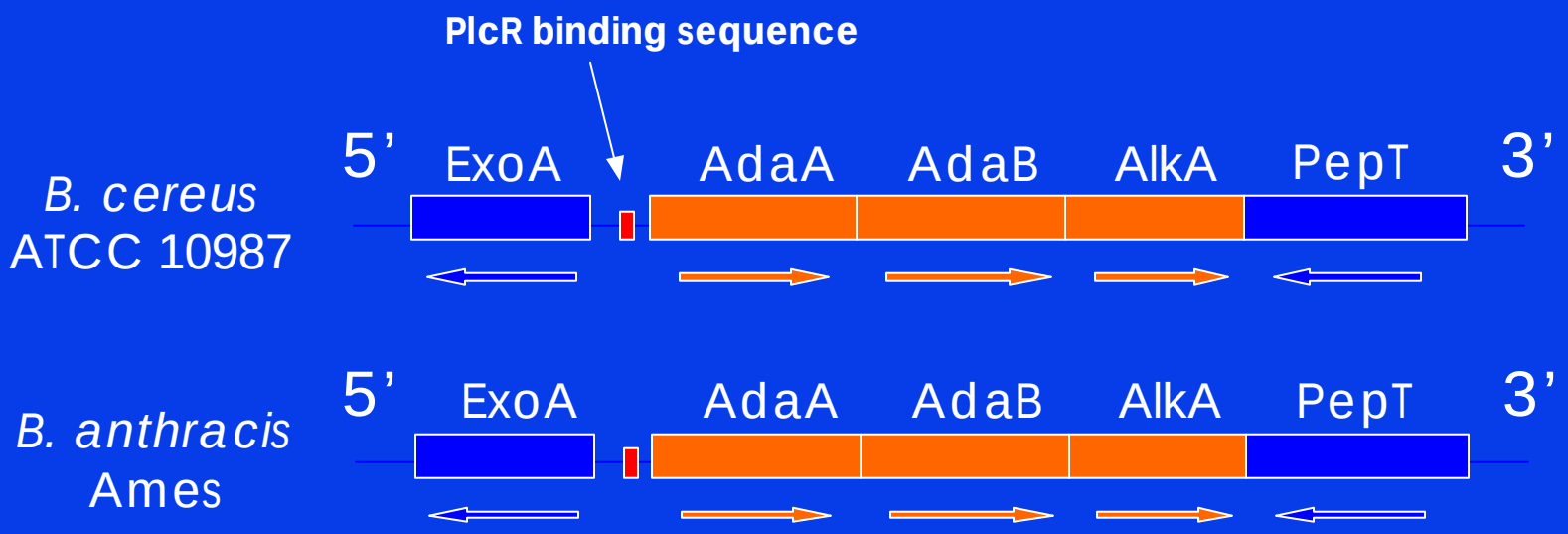
Approximately 75% of CDSs have reciprocal best hits
Approximately 85% of shared CDSs are found in conserved clusters
Approximately 15% have no orthologs – many are prophage genes

PlcR is a pleiotropic regulator of extracellular virulence factors in *Bacillus* species



From Ivanova et al., Nature 423: 87-91 (2003)

Gene organization in *B. anthracis* and *B. cereus*



PlcR binding sequence:

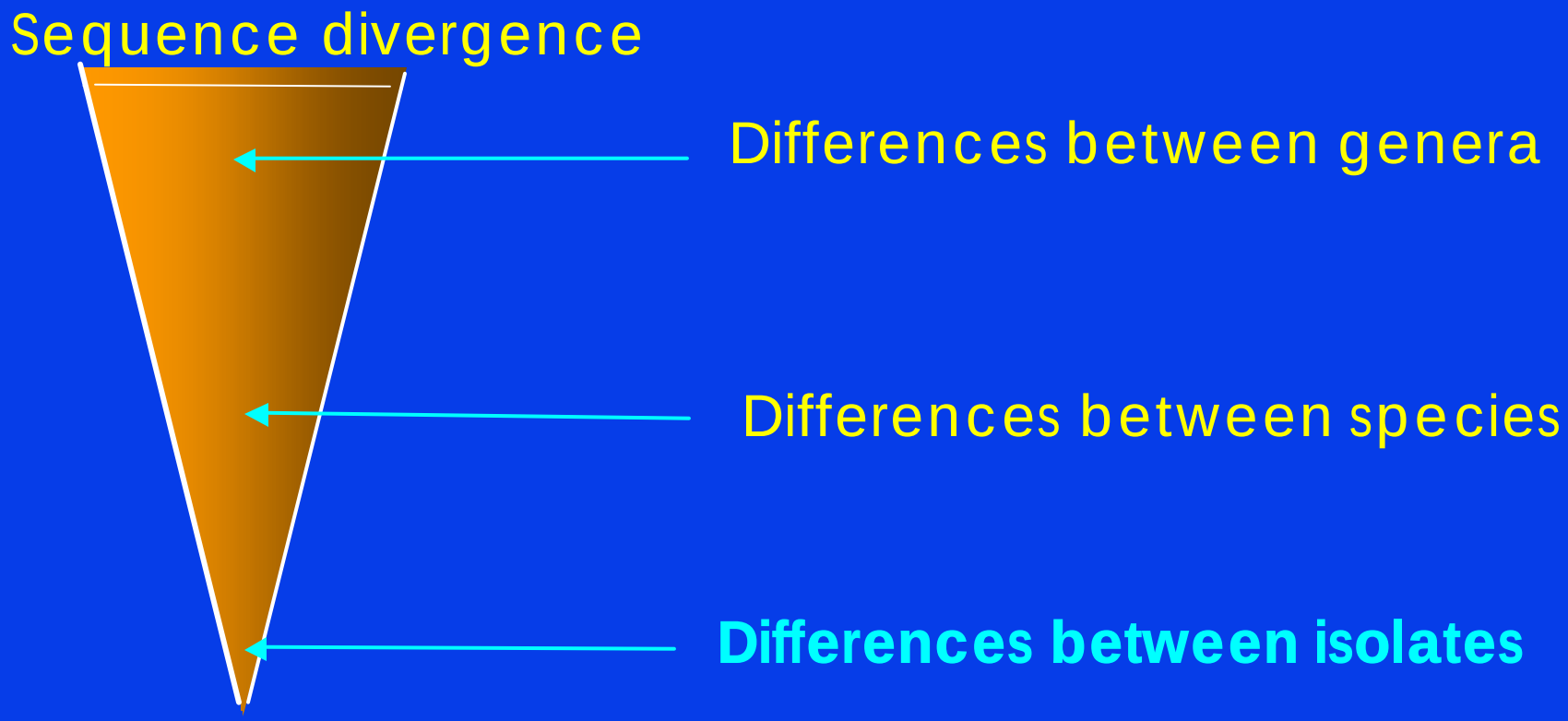
B. cereus	TATGAATACATACATA
B. anthracis	TAT**A**AATACATACATA

Virulence genes on *B. anthracis* chromosome

- Iron acquisition genes (dihydroxybenzoic acid, aerobactin-like siderophore)
  - Host iron scavenging
- Hemolysins
  - Pathogenic factors of *B. cereus* and *B. thuringiensis*
- *Listeria* –like virulence genes (internalins, enterotoxins, etc)
  - Reflecting similar pathways of intracellular escape?
- Superoxide dismutases, peroxidases etc
  - Detoxification functions, mitigate damage by free-oxygen radicals
  - Assist survival in the macrophage

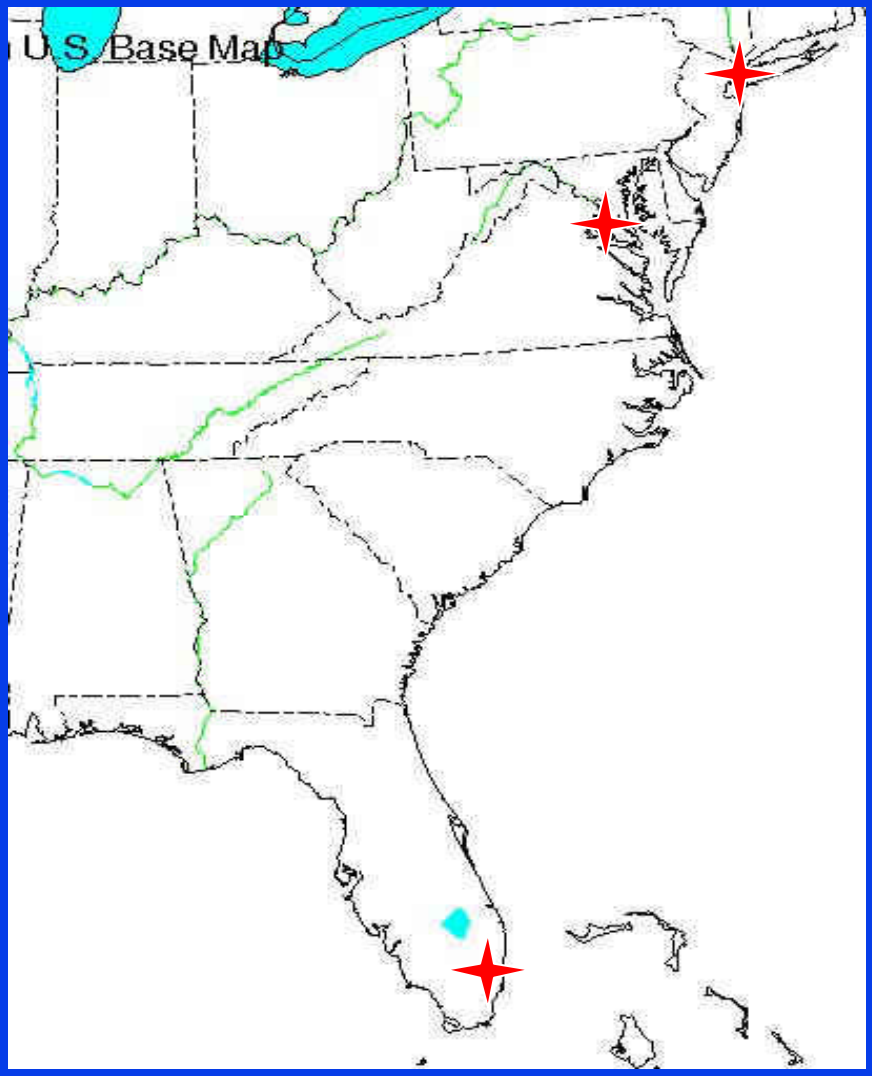
Virtually all putative chromosomal virulence genes have homologs in *B. cereus*

Comparative Genomics Questions



The anthrax attacks of 2001

- 22 human cases of anthrax
- 5 deaths
- All transmitted by U.S. mail
- Billions of dollars in economic damage



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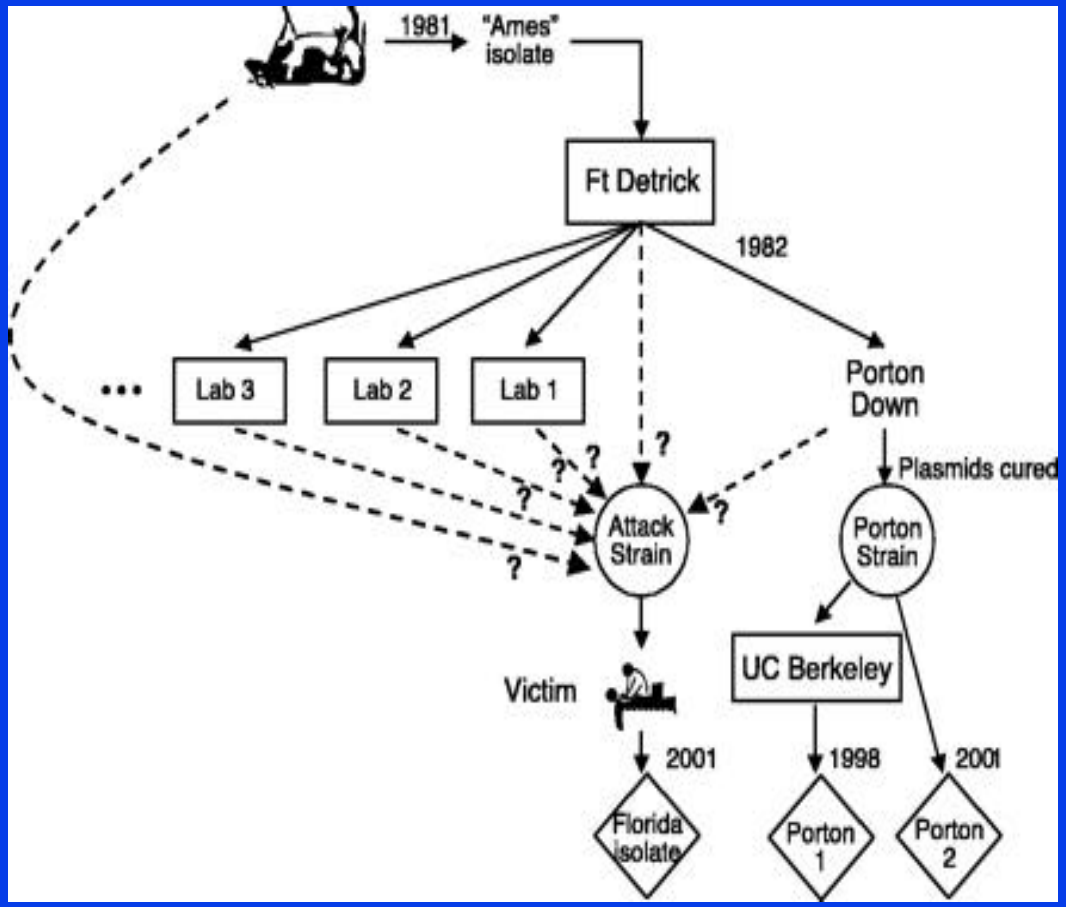
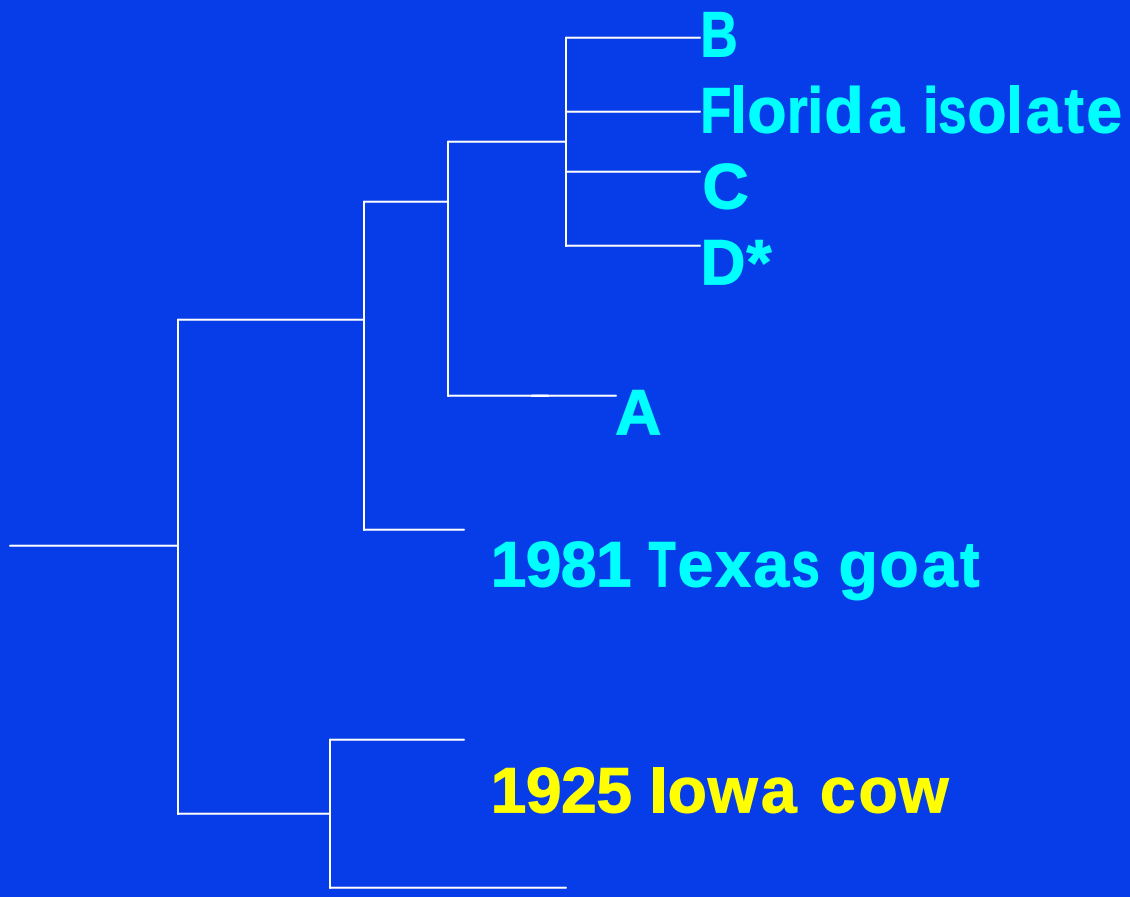
Comparative genome sequencing: leveraging genome data

- VNTR analysis showed bioterror isolate to be the Ames strain
- TIGR received NSF funding to rapidly sequence an isolate related to the attack



Results of *B. anthracis* strain comparison

- Chromosome (FL Ames vs Index Ames) (~5.4 Mbp)
  - 2 SNPs
  - 2 Indels
- Plasmids
  - pXO1 (182 kbp) (FL Ames vs Sterne)
    - 32 SNPs
    - 2 VNTRs
    - 2 Indels
    - 2 large inversions mediated by IS elements
  - pXO2 (96 kbp) (FL Ames vs Pasteur)
    - 22 SNPs
    - 6 VNTRs
    - 2 Indels (1bp)
    - 1 large Indel (1.4 kbp)



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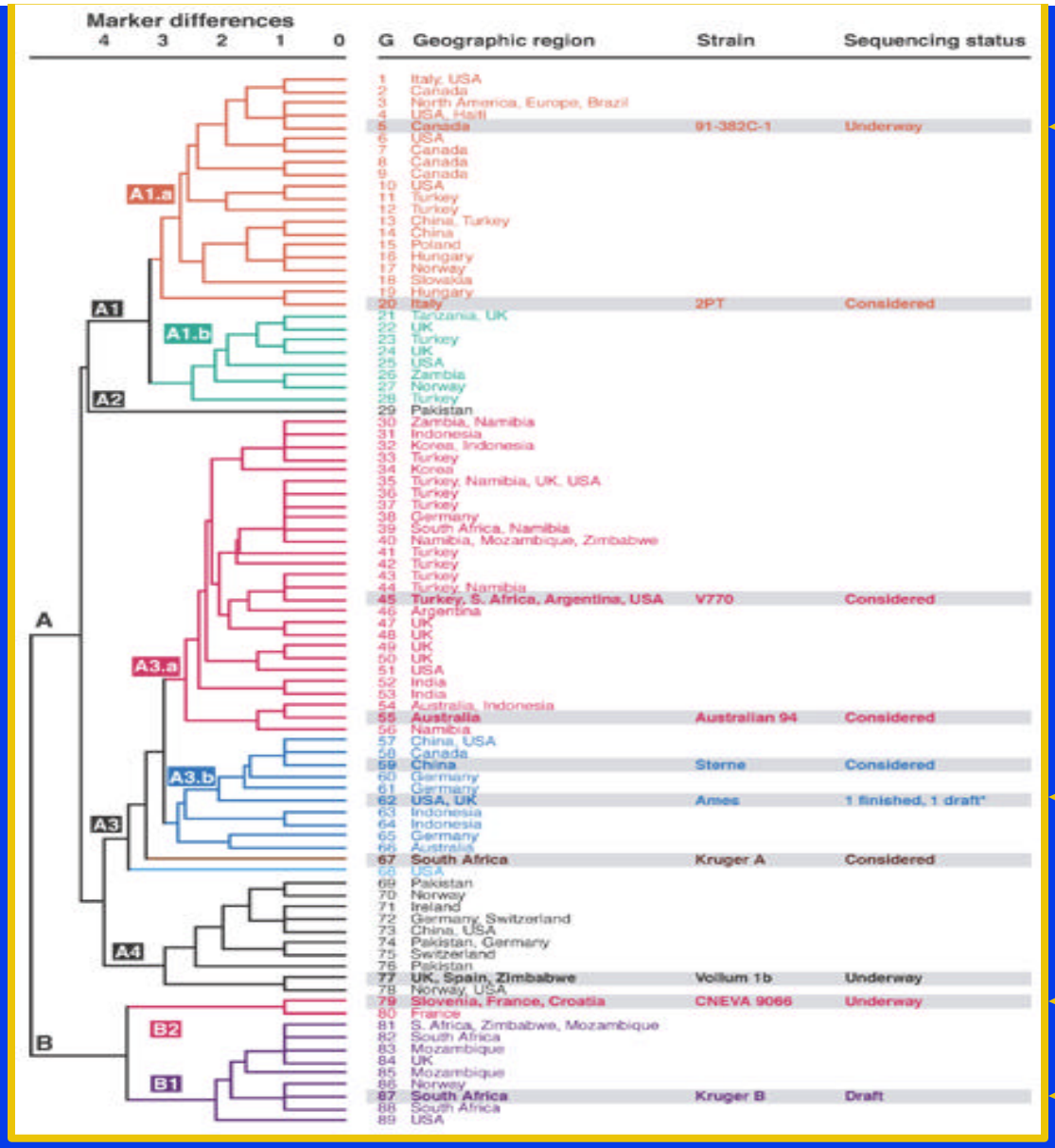
Goals of comparative *B. anthracis* project

Development of new tools to analyze closely related genomes/clonal strains

Automated polymorphism discovery/analysis

***B. anthracis* a model system for comparative genomics**



Western North America
GT5, A1a

Ames
GT62, A3b

French CNEVA 9066
GT79, B2

Kruger B
GT87, B1

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TIGR *B. anthracis* Genome Sequencing Status

Name	Genotype	Status	SNPs	Total asmbl
Ames	GT62, A3b	Closed		
Kruger B	GT87, B1	8x	1351	320 (187)
Western North America	GT3, A1a	8x	428	320 (150)
France CNEVA-9066	GT79, B2	8x	1169	237 (150)
Ancestor, Ames 0581	GT62, A3b	8x	11	395 (198)
Florida, Ames	GT62, A3b	8x	11	416 (236)
A01055	Group C	8x	2090	206 (131)

+ an additional 6 Ames strains

B. cereus 10987 closed genome and plasmid (205 kb)

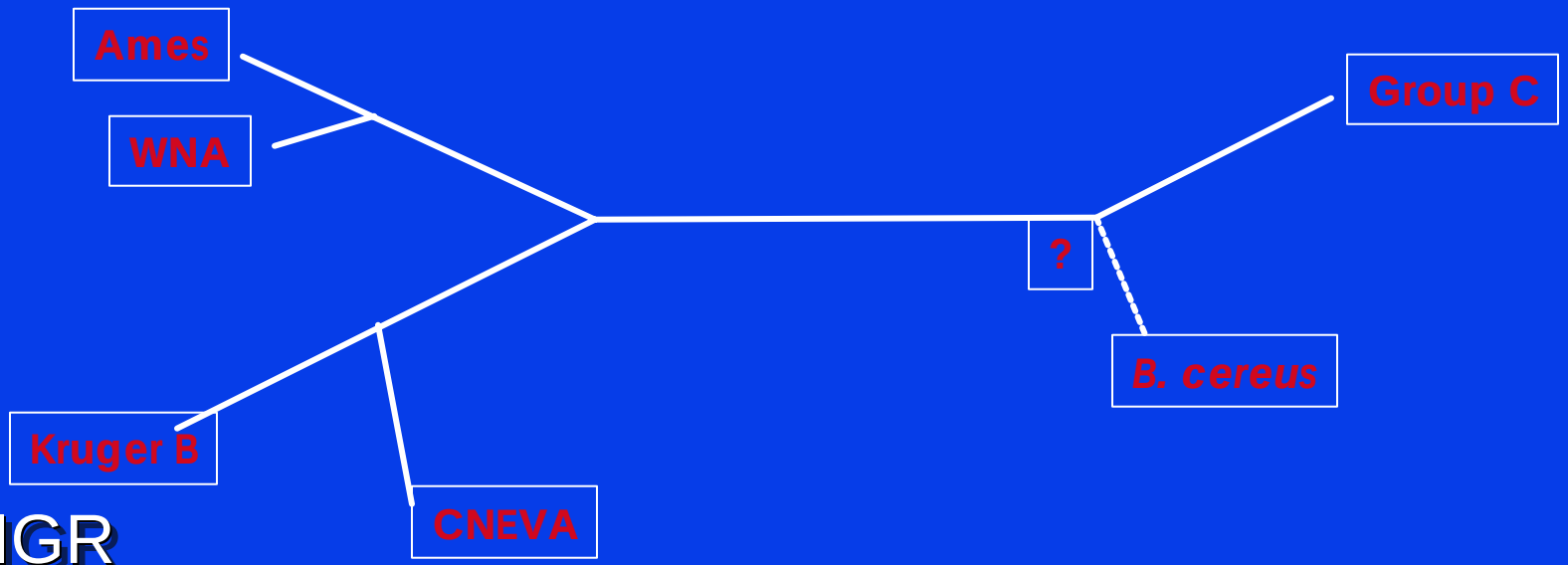
B. anthracis A01055: A new *B. anthracis* taxonomic group

- *B. anthracis* A01055 pXO1^{-}, pXO2^{+}: Louisiana (2 strains)
- Discovered by SNPs analysis/typing (Paul Keim)
- Whole genome sequence analysis confirmed novel Group C
- Flexibility in comparative genome program

B. Anthracis SNP Analysis

	Ames	WNA	CNEVA	Kruger B	Group C
Ames	-				
WNA	428	-	258[b]	228[b]	254[b]
CNEVA	1169	1081[a]	-	875[b]	649[b]
Kruger B	1351	1323[a]	770	-	571[b]
Group C	2090	2009[a]	1960[a]	2299[a]	-

[a] Total SNP genome 1 + Total SNP genome 2 – 2x (Shared SNPs).
[b] Shared SNPs



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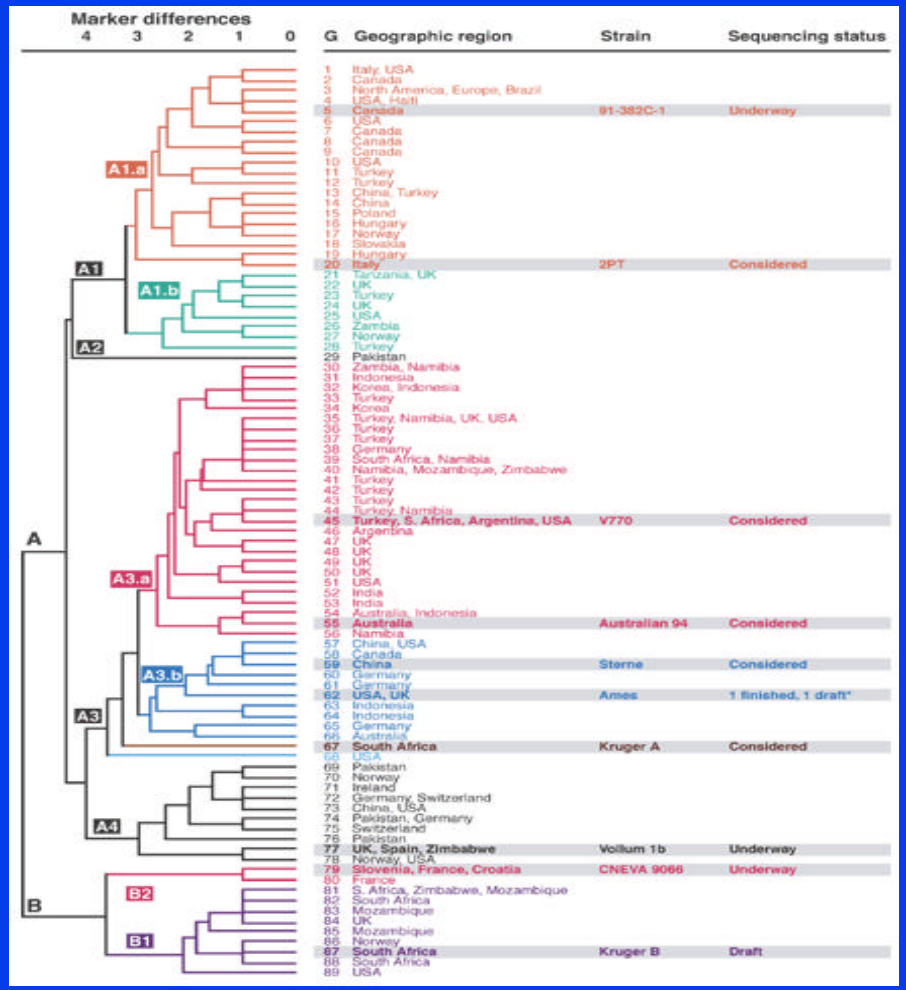
A comprehensive *B. anthracis* database…

- 14 additional genomes
- MLST
- Affymetrix chips



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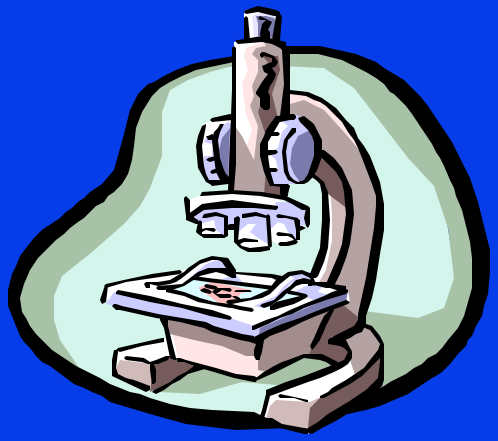
How might we benefit from a better understanding of DNA variation among bacterial strains/isolates?

- Epidemiological studies
- Microbial forensics
- Prediction of clinical outcomes
- Detection of genetically modified strains
- Implications for vaccine development
- Implications for therapeutic strategies

The Future of Biodetection/Diagnosis

Genome-based, information-rich

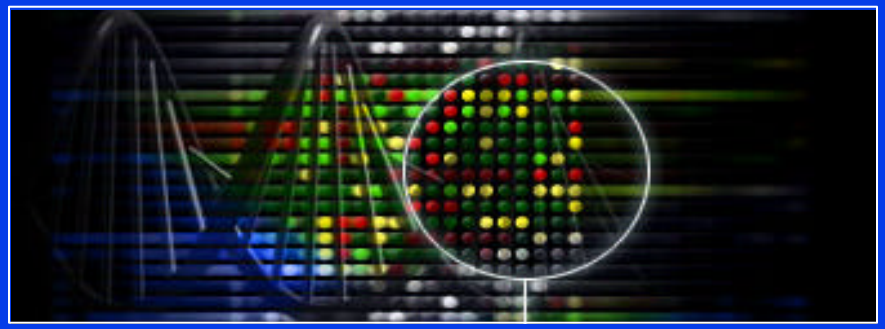
Today



Anthrax

Ames strain

Tomorrow



Anthrax

Type 73526

Source: Univ of XYZ Lab 1

Genotype: 138 SNPs, 3 insertions, 2 rearrangements

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Newsday

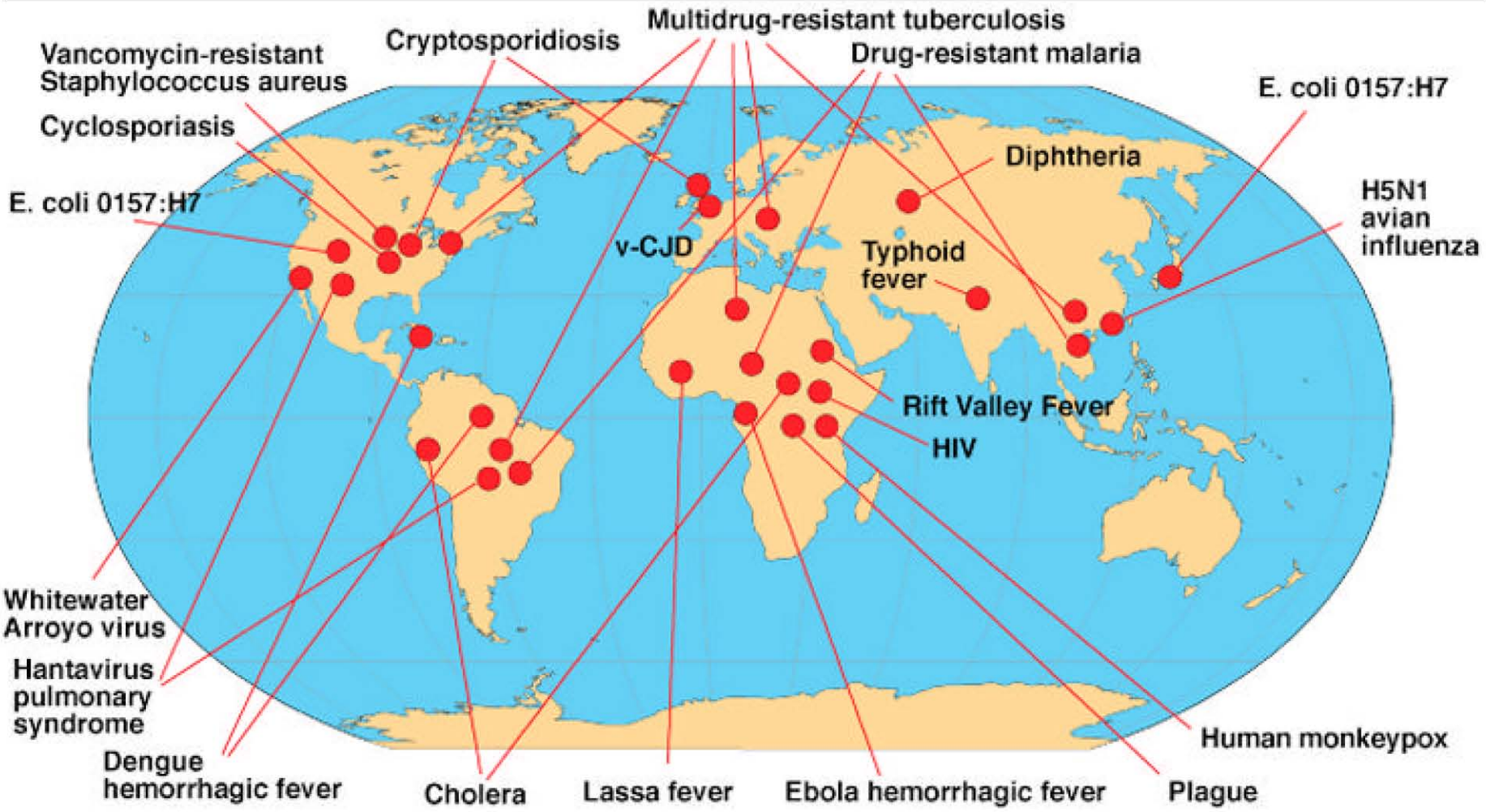
November 16, 2001

The Worst Bioterrorist May Be Nature Itself

By Frederick M. Cohan

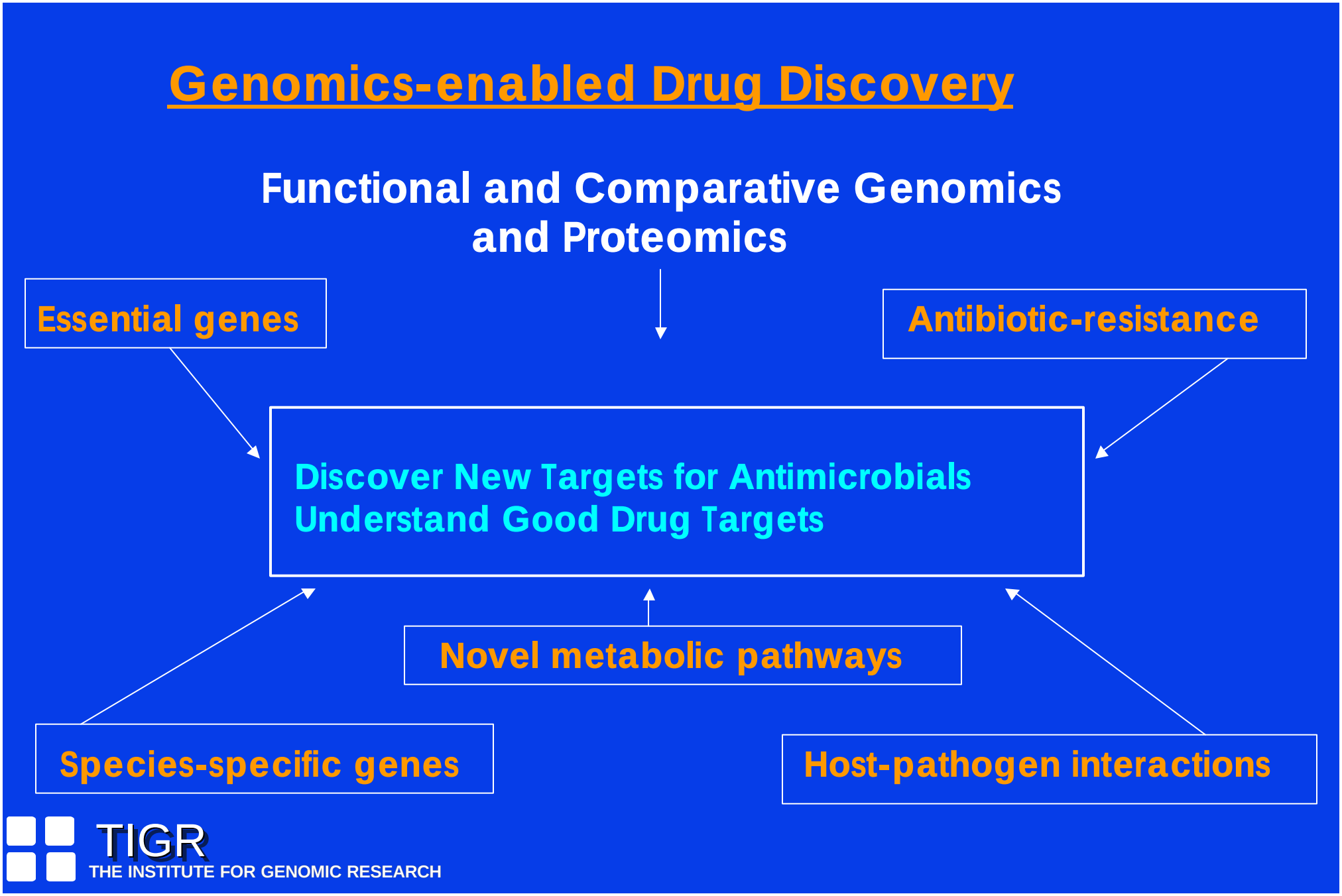
While we are under siege by microbes spread by terrorists, we face what is probably a worse threat from pathogens that we encounter naturally as part of our human ecology. This important fact is getting overlooked in the national panic over anthrax.

Examples of Emerging and Re-Emerging Diseases



Genomic Approaches to Understanding and Treatment of Infectious Disease

Genomics-enabled Drug Discovery



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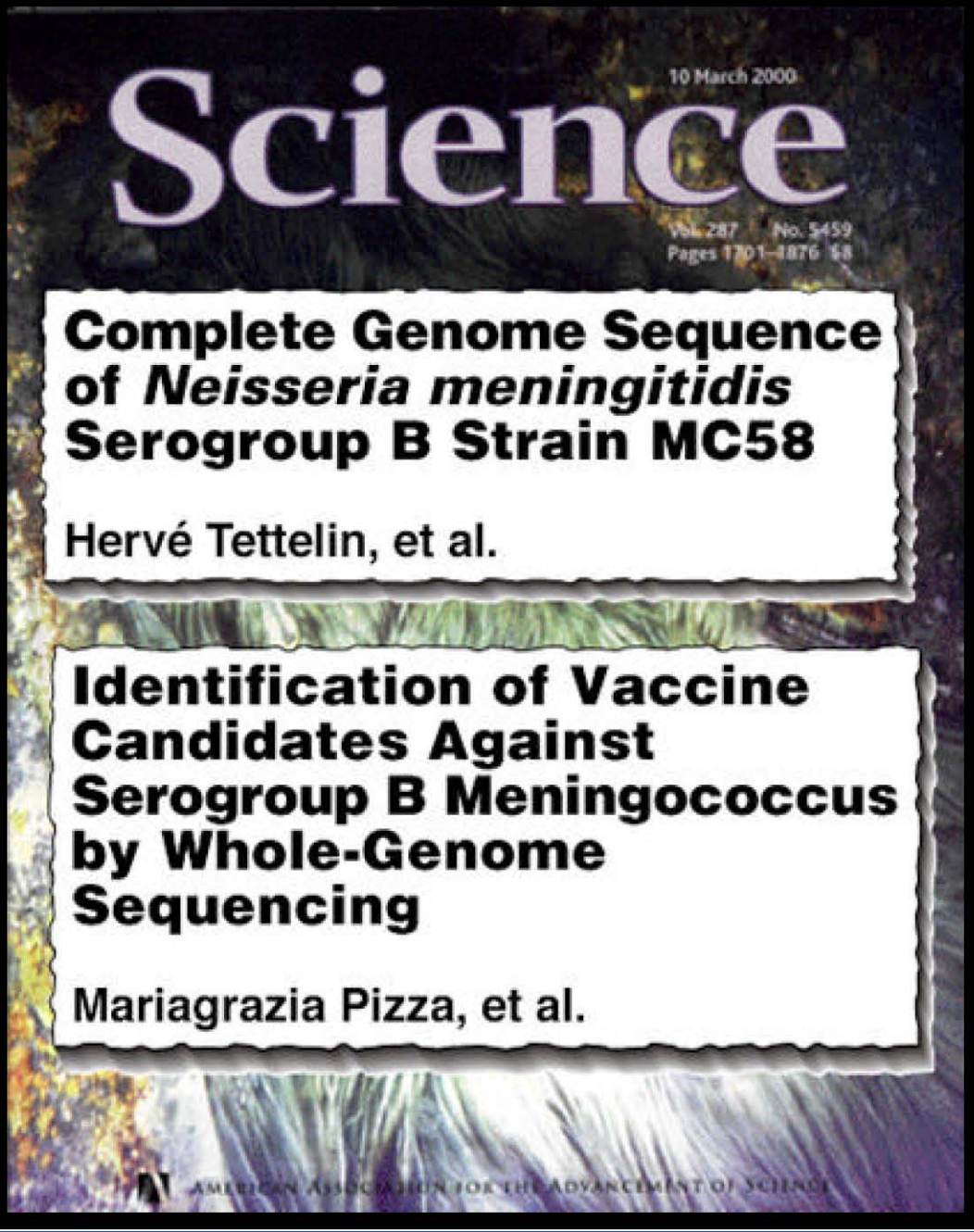
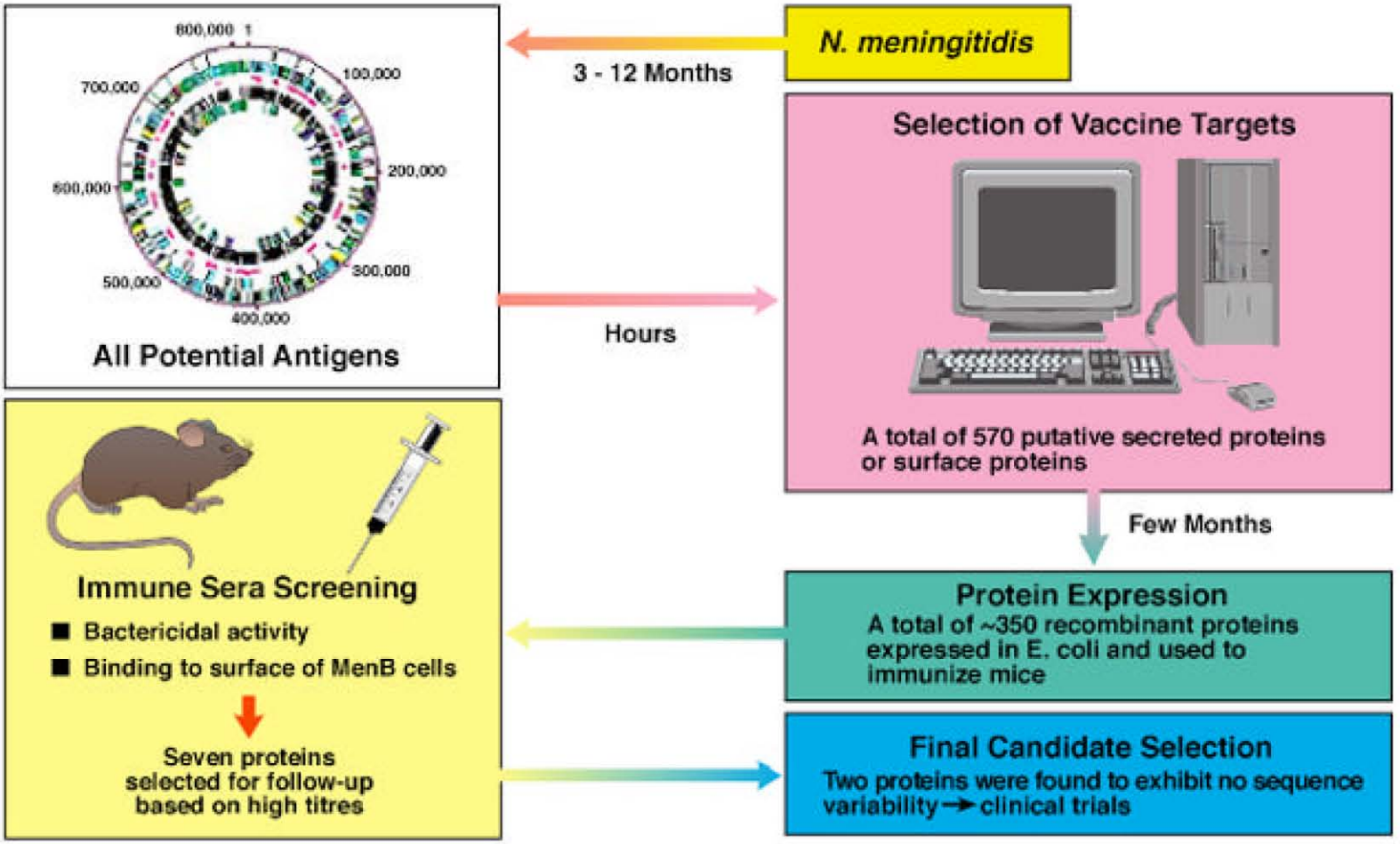
Bioterrorism Vaccine Research

- Protect all groups of civilians
- Develop improved vaccines against microbes for which vaccines currently exist
- Develop novel vaccines against microbes for which none currently exist

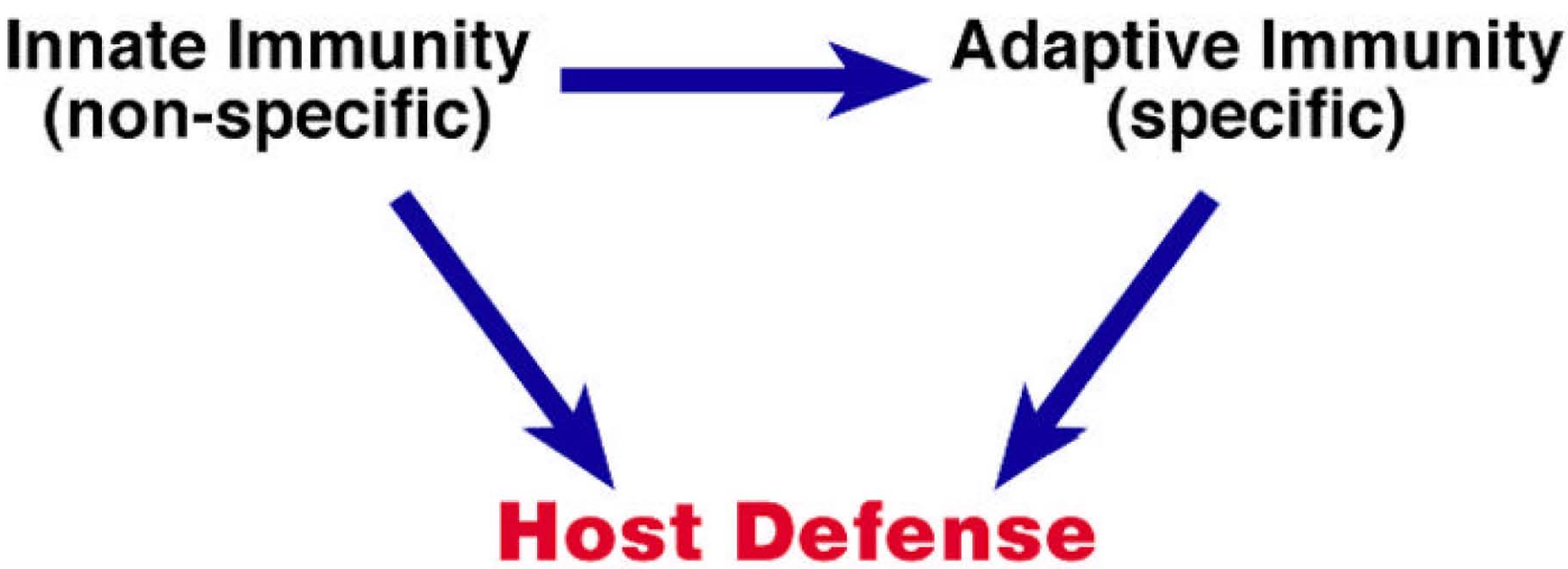

Science
10 March 2000
Vol. 287 No. 5459
Pages 1701–1876 $8
Complete Genome Sequence of Neisseria meningitidis Serogroup B Strain MC58
Hervé Tettelin, et al.
Identification of Vaccine Candidates Against Serogroup B Meningococcus by Whole-Genome Sequencing
Mariagrazia Pizza, et al.
AAAS
American Association for the Advancement of Science

Complete Microbial Sequence Data Can Accelerate Vaccine Development



Source: Fraser C, et al. *Nature* 406:799, 2000

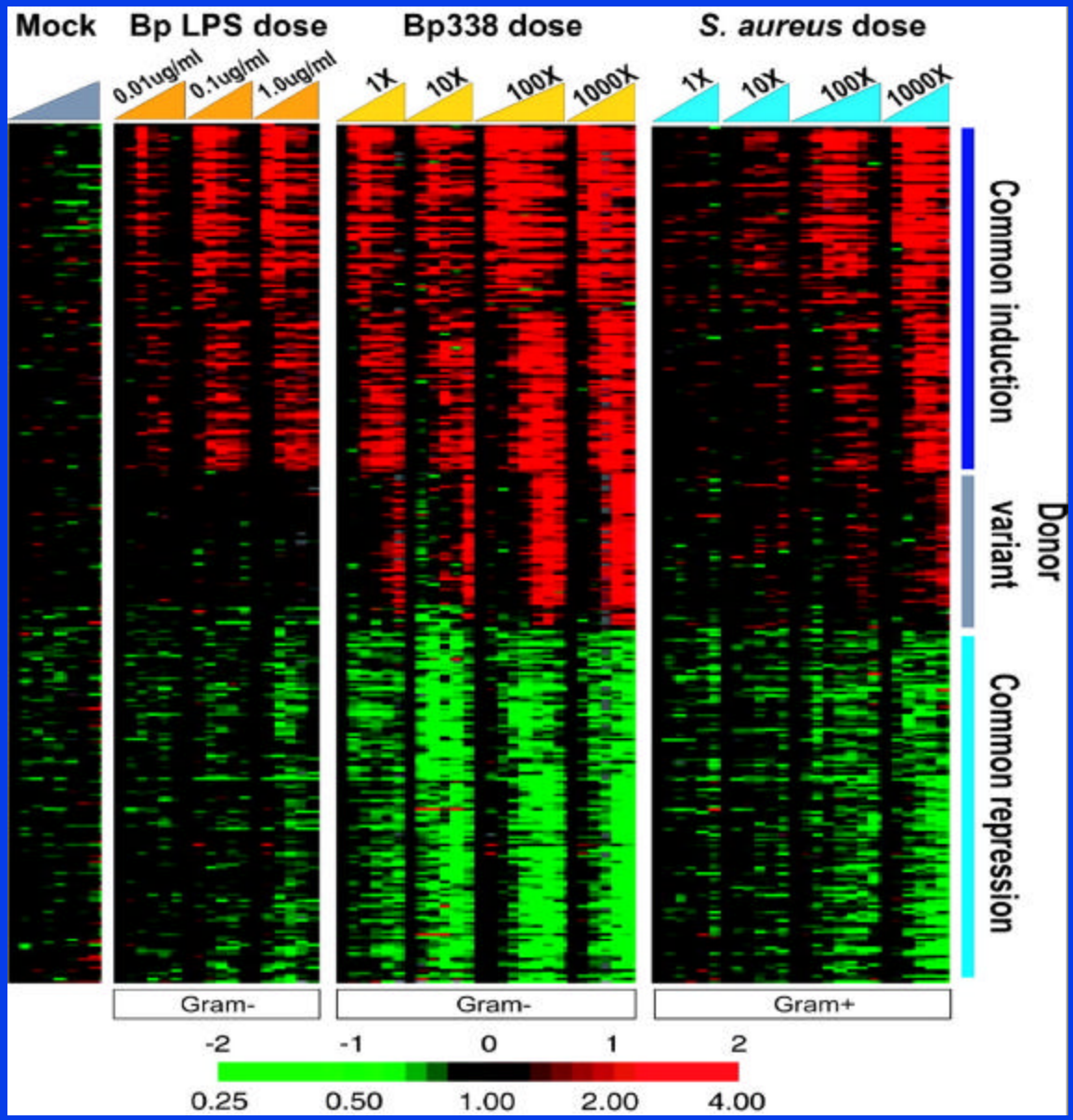
Host Defense Against Microbial Infection



Stereotyped and specific gene expression programs in human innate immune responses to bacteria.

Boldrick JC, Alizadek AA, Diehn M, Dudoit S, Lui CL, Belcher CE, Botstein D, Staudt LM, Brow PO, and Relman DA.

Proc. Natl. Acad. Sci USA 99: 972-977 (2002).



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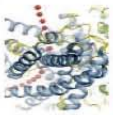
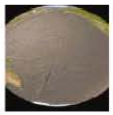


Protein plea
UK structural biologists call for more automation
p822

Live samples
Human lung tissue back in the fray for tuberculosis research
p823



Global view
Institute has designs on the Earth's interior
p824

On target
Instrument-rich satellite set for launch
p825

Biologists apprehensive over US moves to censor information flow

Erika Check, Washington

Fears are growing among biologists that the US government will impose new restrictions on the publication of scientific research.

Such a move has looked increasingly likely in the aftermath of last autumn's bioterrorism attacks in the United States (see *Nature* **415**, 237; 2001).

But it has now emerged that some biologists with government funding are being encouraged to rein in the full publication of their own work. And some agencies, including the National Institutes of Health (NIH), are for the first time considering the support of classified research.

The American Society for Microbiology (ASM) says that some researchers have asked to omit certain information from the methods sections of papers to be submitted to its 11 journals. "We are in a phase of discussion that could lead to fundamental changes in the way we do science," says Ron Atlas, president-elect of the society.

Atlas says that the ASM does not intend to comply with the researchers' requests. He also says that the society is concerned about the implementation of an order signed last October by President Bush allowing the health department — including the NIH — to fund classified projects.

Anthony Fauci, head of the National Institute of Allergy and Infectious Diseases, the NIH institute most involved in research related to bioterrorism, says that his agency has not so far asked any of its researchers to keep their work secret. He adds that although most NIH-funded research should remain transparent, restricted access to some of it cannot be ruled out.

"As we move into more research on counter-bioterrorism, we should examine this issue on a case-by-case basis," Fauci says.

The possibility of restrictions riles many researchers. "Censorship would not accomplish anything but stifling beneficial work that will better prepare us to face a bioterrorism attack in the future," says Claire Fraser, director of The Institute for Genomic Research in Rockville, Maryland, which has been investigating the genome of different strains of the anthrax bacterium for the government.

The New York Times has reported that the White House will issue new guidelines on information security within the a few weeks.

US scientists are not the only ones fretting about new restrictions on their work. Some British researchers say that new export control laws under consideration in the United Kingdom include the export of information and will in theory allow government vetting of scientific material before publication.

David King, the British government's chief scientific adviser, is consulting with scientists on a response to the threat of bioterrorism, but a spokesman for the Cabinet Office declined to elaborate on any plan to restrict access to research findings. ■

Anthony Fauci: refuses to rule out restrictions.

A. WONG/GETTY

Bush plan deepens divide over Kyoto Protocol

Tony Reichhardt, Washington

Further distancing his administration from the Kyoto Protocol on climate change, President George W. Bush last week rejected the idea of reducing US greenhouse-gas emissions to below current levels.

The president's long-awaited alternative to the Kyoto plan effectively calls for no new action on the part of the United States. The Kyoto signatories pledged to cut greenhouse-gas emissions to below 1990 levels by 2012. Bush instead envisions reducing the "emissions intensity" — the ratio of emissions to a nation's economic output — by 18% over the same period. Using this measure, US emissions intensity dropped by about 15% in the 1990s, although actual emissions went up by 15%.

Any reductions in industrial emissions would be strictly voluntary, as mandatory caps would harm the economy, he added. Bush said that the country should reconsider this course of action in 2012 based on progress in reducing emissions and improved scientific understanding of global warming.

The new policy deepens the divide between the United States and other industrial nations, which have been more supportive of the Kyoto agreement, at least in their rhetoric. No major economy has yet ratified the protocol, but Japan may soon become the first to do so (see page 822).

US advocates of action on global warming are now set to shift their attention to the Congress, where their first objective is legislation to force corporations to report their greenhouse-gas emissions publicly. Such reporting is voluntary under the Bush plan.

Bush's statement on 14 February drew fire from environmental groups and from some in Congress. Senator Jim Jeffords (Independent, Vermont), who chairs the Senate's Environment and Public Works Committee, says the policy is "divorced from the reality of global warming". ■

Acknowledgements

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