Synthetic Biology: Anticipating New Hazards as We Pursue Biofuels

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Introduction

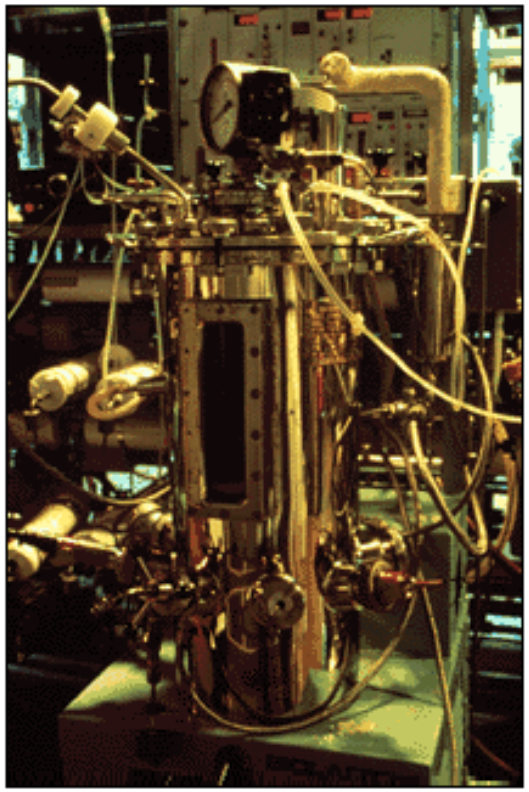
- Evolution from fermentation to ‘natural’ biotechnology to recombinant biotechnology to synthetic biology.
- Historical risks in biotechnology.
- What is synthetic biology, and how does it differ from prior biotechnology.?
- Forseeable hazards and risks of synthetic biology
  - —Biological hazards.
  - —Physical, chemical and process hazards
  - —Other risks and benefits
- Using biofuels as an example to predict potential risks and hazards.

Fermentation and Zymology

- Fermentation of alcoholic beverages back at least 6,000 years, also vinegar, yogurt, kimchi.
- 1914 acetone production by Chaim Weizmann during WW I, produced by fermentation, used to produce cordite.
- 1950's – the use of bacteria to produce vitamins, some medicines.
- 1962 – use of Bacillus Subtilis to produce detergent enzymes.
- Relied on screening and selection of native organisms
- Common use of 'fermentation' now includes both anaerobic and aerobic processes.

Fermentation Process

A Fermenter / Bioreactor And Its Parts



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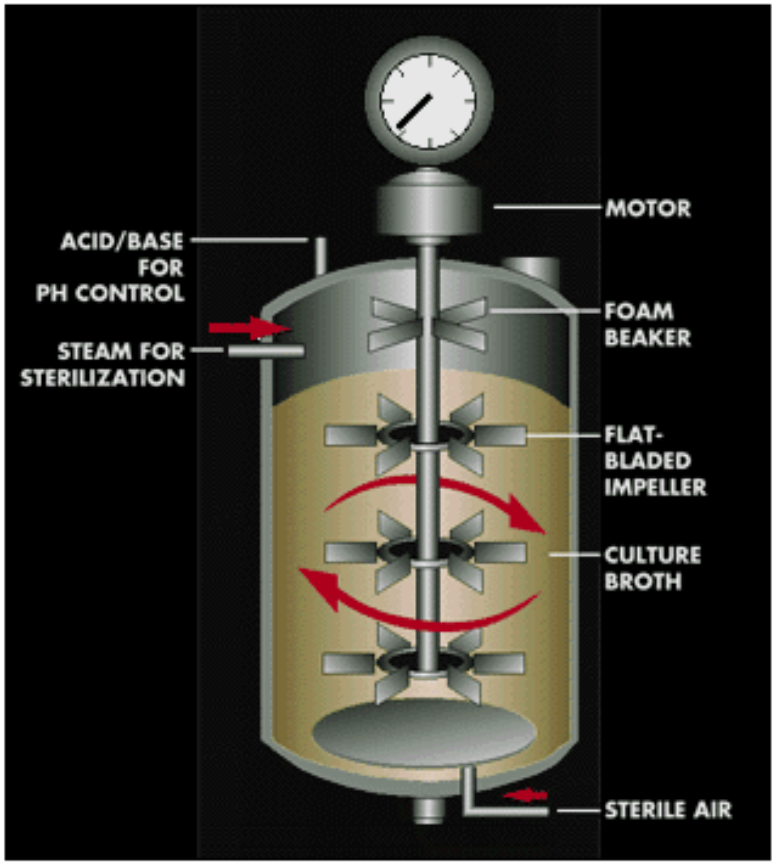

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Biological Product Examples

	Product	*Organism*	Significance
Metabolite	Ethanol	*Saccharomyces cerevisiae*	Alcoholic beverages
Metabolite	Vitamin B12	*Pseudomonas denitrificans*	Nutritional
Secretion	Cyclosporin A	*Tolypocladium inflatum*	Immuno-suppressant
Secretion	Penicillin	*Penicillium chrysogenum*	Antibiotic
Enzyme	Microbial rennet	*Mucor pusillus*	Cheese manufacture
Enzyme	Cellulase	*Trichoderma reesii*	Biofuels

Extremophiles – Tough Guys

Thermophiles and Hyperthermophiles	Applications
DNA polymerases	DNA amplification by PCR
Lipases, pullulanases and proteases	Detergents
Amylases	Baking and brewing
Xylanases	Paper bleaching
Halophiles	**Applications**
Bacteriorhodopsin	Optical switches and photocurrent generators
Lipids	Liposomes for drug delivery and cosmetics
Compatible solutes e.g. Ectoin	Protein, DNA and cell protectants
g-Linoleic acid, b-carotene and cell extracts,*e.g. Spirulina* and *Dunaliella*	Health foods, dietary supplements, food colouring and feedstock
Psychrophiles	**Applications**
Alkaline phosphatase	Molecular biology
Proteases, lipases, cellulases and amylases	Detergents
Polyunsaturated fatty acids	Food additives, dietary supplements
Ice nucleating proteins	Artificial snow, food industry e.g. ice cream
Alkaliphiles and Acidophiles	**Applications**
Proteases, cellulases, lipases and pullulanases	Detergents

‘Natural’ Biotechnology Hazards

- <u>Infection</u> with viable organisms
  - —Bacterial and viral bioaerosols – respiratory
  - —Skin contact, ingestion, contaminated injuries
  - —Bloodborne pathogens from mammalian cells
- <u>Allergic reactions</u> to nonviable biological material
  - —Wood dust
  - —Purified proteins, e.g. detergent enzymes
  - —Insect and animal allergies -- dander, urinary proteins
- <u>Toxic reactions</u> to biological material
  - —Endotoxins (LPS)
  - —Mycotoxins

Lab-Associated Infections

- **3,921 infections on record, <20% from known accidents (Sulkin and Pike, 1976)**

MOST FREQUENTLY DOCUMENTED REPORTS OF LABORATORY-ASSOCIATED INFECTIONS - 1930 to 1999

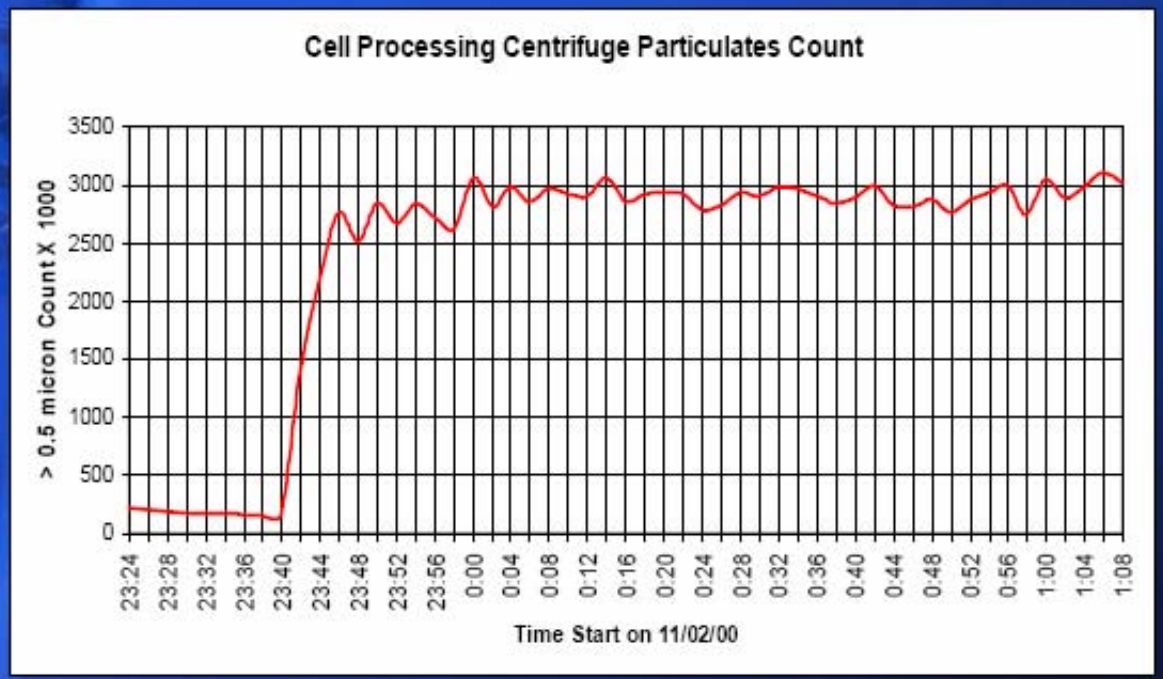
Agent	Laboratory-associated Infections
Brucella sp.	507
Coxiella burnetii	456
Mycobacterium tuberculosis	417
Hepatitis viruses	380
Salmonella sp.	324
Francisella tularensis	225
Hantavirus	169
Venezuelan equine encephalomyelitis virus	150
(Influenza A2 virus)	(15)

Endotoxin Effects

- Effects include chills, myalgia, fatigue, weakness, aches, fever, chest tightness and malaise.
- Adverse effects can be acute (I.e. cross-shift and cross-week) as well as chronic (e.g. accelerated lung function decline over extended time periods of exposure).
- Adverse effects following inhalation include fever, cough, nausea, reduced FEV1, reduced FVC, and reduced FVC/FEV1
- Can cause permanent decline in lung function in chronic exposures.

Endotoxin Aerosolization

Evaluation



Centrifuge was a major particulate contributor

Air Break to Drain

Control

The main culprit

Physical and Chemical Biotech Hazards

- **Heat, pressure during extraction**
  - **Taxol extraction from yew needles, 2,000 psi methanol and CO_2. 1994 death from pressure release.**
- **Confined spaces, anoxic environments**
- **Gentamycin allergy (culture medium component)**
- **Disinfection hazards (formaldehyde, hydrogen peroxide, ultraviolet light, photocatalytic oxidation with TiO_2)**
- **Solvent exposure during extraction**
- **Ergonomic hazards**

Recombinant Genetics -- Prologue

- Two major discoveries:
  - — DNA logic – lac operon discovery of gene control in 1961
  - —1973 creation of bacterial plasmids by Stanley Cohen and Herbert Boyer: “Construction of Biologically Functional Bacterial Plasmids in vitro”.
  - —E. Coli and yeast use plasmids.
- New ability to combine and insert DNA molecules that are not found together naturally
- 1975 Asilomar conference
- 1977 NIH Recombinant DNA Authority

Asilomar 1975

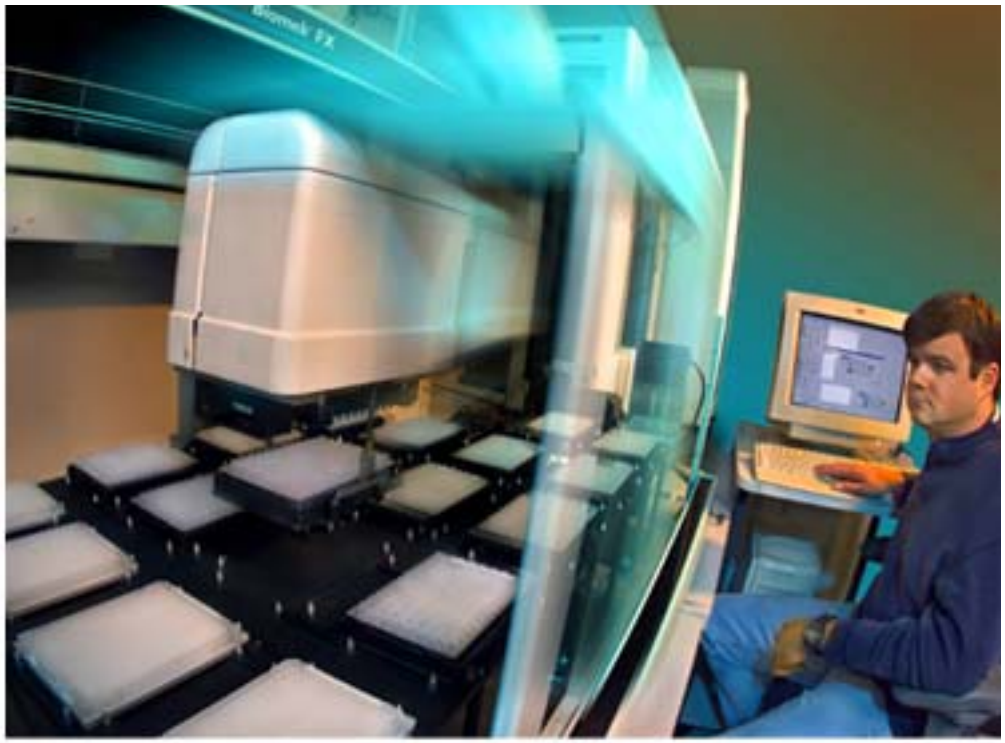
- Initiated by Paul Berg, biochemist, who created an bacteriophage containing Simian virus DNA for insertion into E. Coli.
- Biologists, lawyers, physicians attended.
- Organism containment should be designed into experiments.
- Containment level should correspond to the risk of the organism to animals, humans and the environment.
- Increased pathogenicity, drug resistance led to higher containment.
- Viral vector-host systems should have impaired reproduction.

Proteins intended for therapeutic use

- Humulin by Genentech 1982
- Epogen by Amgen 1989
- Monoclonal antibodies for in vivo use.
- Cytokines (e.g., interferons),
- Enzymes (e.g. thrombolytics)
- Coagulation factors (recombinant and human-plasma derived).
- Immunomodulators (intended to treat disease by inhibiting or down-regulating a pre-existing, pathological immune response).
- Growth factors, cytokines, and monoclonal antibodies intended to mobilize, stimulate, decrease or otherwise alter the production of hematopoietic cells in vivo. (AKA biological response modifiers).

DNA Sequencing Improvements

- **Automated DNA sequencing**
  - **—Robotics and computerization**
  - **—Supercomputing for analysis**



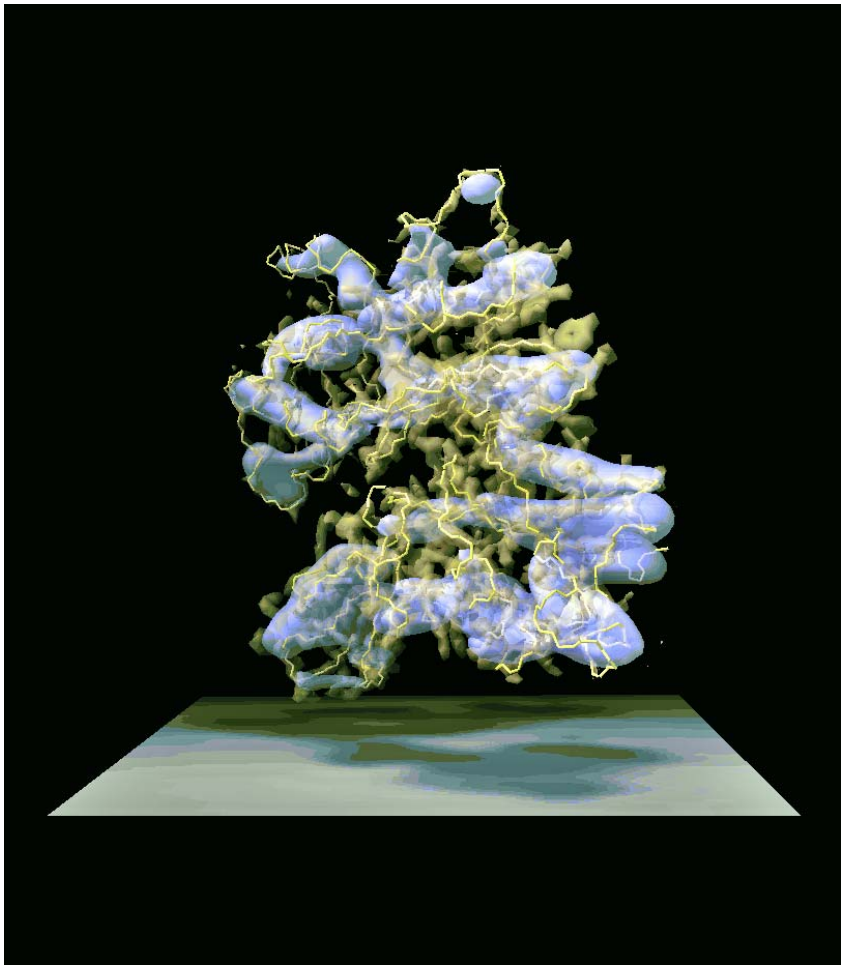
MegaBace 4000 Tasks

DNA Synthesis Progress

- **Price of synthesizing DNA now about a dollar per base pair.**
- **Synthesizer below available $2,150.**

Reshaping Proteins

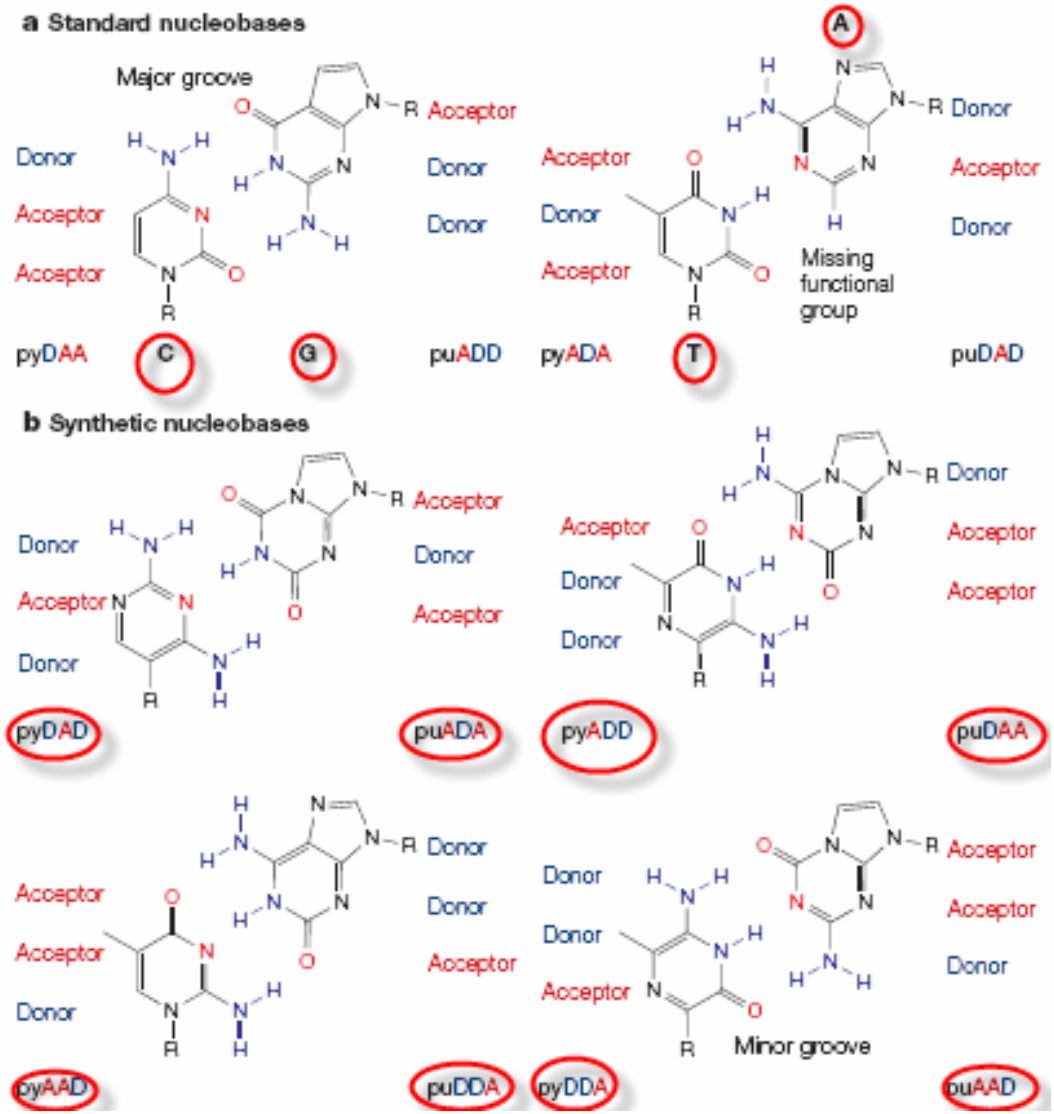
- High resolution x-ray crystallography helps predict the structural changes resulting from genetic manipulation of genes.
- Process now highly robotic
- Limited by ability to crystallize proteins, especially membrane-associated proteins.



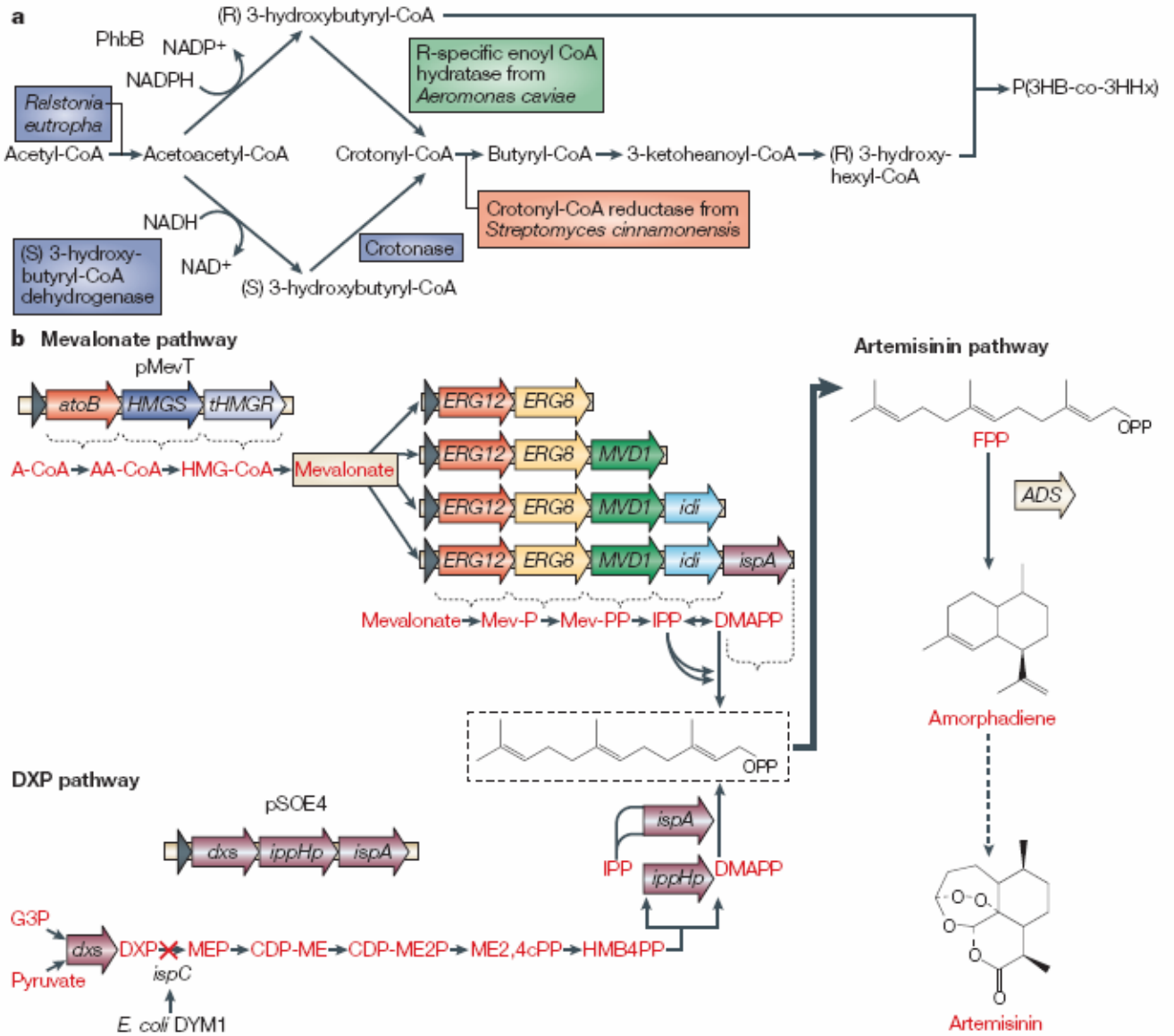
Two Types of Synthetic Biology

- 1. "Unnatural Molecules" or Biomimetic Chemistry
  - —New nucleosides
  - —Tapping unused codons
    - 64 codons, only 20 amino acids
    - Requires new tRNA
- 2. "Gene Circuitry"
  - —Switches, oscillators, proximity detectors
  - —Interchangeable parts from different species
- Term coined in 1980 by Barbara Hobom, referring to recombinant, not synthetic gene.
- New organisms exhibit inheritance, genetics and evolution

New Letters in the DNA Alphabet

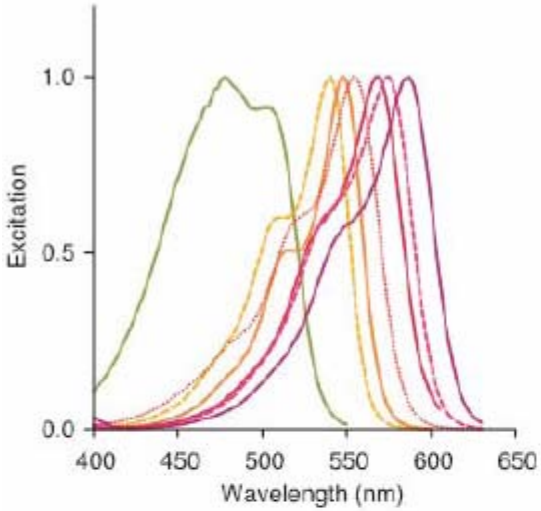


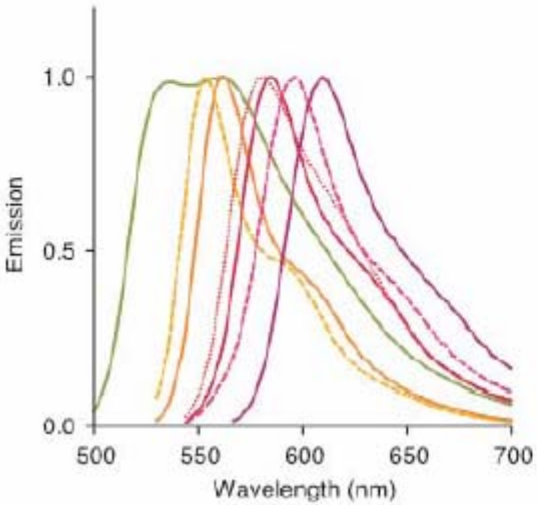
New Synthetic Pathways

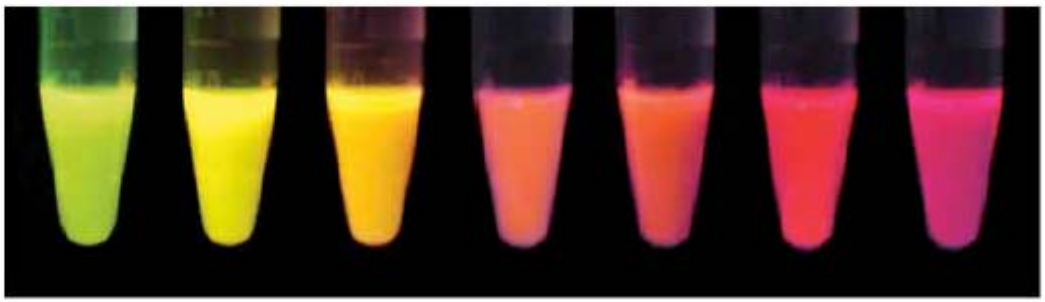


Black –
Ralstonia eutropha
Red –
Streptomyces Cinnamonensis
Green –
Areomonas caviae
Host –
E. Coli

Reporting Fluorescent Proteins







Fluorescent Animals

Guess which ones got the foreign DNA.

So, What Are the New Hazards?

- Infection
  - —Garden variety infections from ‘innocuous organisms’.
  - —Increased pathogenicity or resistance.
- Colonization
  - —Bacterial protein factories in your gut.
- Toxins
- Allergies
  - —New or unfamiliar allergens (kiwi example)
  - —Allergies to high levels of protein products
  - —Transfer of allergenic proteins to new organisms (soybeans and brasil nuts).
- Susceptible individuals – atopic, immunosuppressed

Synthetic Biology Process Hazards

- R&D
  - —Infection, allergic sensitization, vector safety, modified organisms, disinfection
- Scale-up
  - —Concentrated proteins and allergic/toxic effects
  - —Spills and releases
  - —Process failures and machine maintenance
- Routine use
  - —Population allergic sensitivity profile
  - —High-volume spills,
  - —Transportation accidents

Screening and Surveillance Options

- **Preplacement Evaluation**
  - —History of allergies, atopy, asthma, skin disease, immunosuppression, medications, organ transplantation.
  - —Exam for asthma, skin disease, allergies.
- **Surveillance**
  - —History of new onset allergies, skin disorders, interim medical history. Injury reporting, hazards encountered, process failures.
  - —Exam for skin disorders, PFT alterations.
  - —Possible allergen-specific RAST testing.

Phenol-Chloroform Burn – DNA Extraction

Warning Signs

- Species extinction
- Invasive species
- Failure to contain genetically modified foods
- Lack of surveillance of lab-associated infections.
- Mixed authority for safety review
  - —FDA
  - —Dept of Agriculture
  - —NIH
  - —CDC/NIOSH
  - —Dept of Labor
- Randomness of evolution

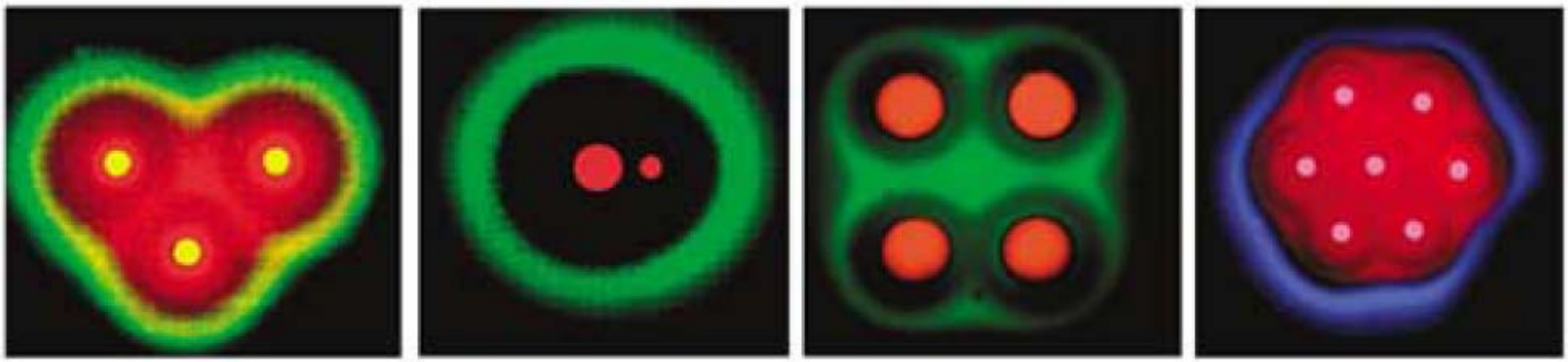
Escaped Organisms -- Biosafety Net?

- New organisms have not evolved to compete and survive in the wild.
- Unique nucleosides may not exist in the wild.
- Added genetic circuits are excess baggage and can be ejected.
- Evolution is conservative, proceeds in small steps (that's why extinction occurs, why avian flu pandemic hasn't occurred yet).

- Steven A. Benner and Michael Sismour "Synthetic Biology" Nature Reviews: Genetics Vol 6, pp. 533-543 July 2006.

Imagine this!

- Bacterial colony synchronization
- Proximity detection
- Synthetic DNA probes – Bayer Versant™
- Protein restructuring to improve activity
- Biodetectors sensitive below 50 ppb
- Biofilm photodetectors.
- Vaccination with recombinant commensal organisms



Bio-Photodetector

100,000 ‘pixels’ per inch

The Dark Side - Biosecurity

- **Enhance the harmful consequences of a biological agent or toxin;**
- **Disrupt immunity or the effectiveness of an immunization without a clinical and/or agricultural justification;**
- **Confer to a biological agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin, or facilitate their ability to evade detection methodologies;**
- **Increase the stability, transmissibility, or ability to disseminate a biological agent or toxin;**
- **Alter the host range or tropism of a biological agent or toxin;**
- **Enhance the susceptibility of a host population; or**
- **Generate a novel pathogenic agent or toxin or reconstitute an eradicated or extinct biological agent.**

NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY
July 13, 2006 National Institutes of Health Campus

Biofuels

- Goal: sustainable, regenerating, carbon-neutral liquid transportation fuel
  - 385 million gallons of gasoline are consumed per day, US 2005
  - Energy density gasoline>ethanol, hydrogen
- Ethanol
  - Blending at loading racks due to water affinity.
  - No use of floating-top tanks.
  - Risk to groundwater – double walled underground storage tanks
- Biodiesel
  - Possible incompatibility with jet fuel.
  - Biodiesel safer for groundwater, but congeals at low temperatures.

Biological Reactions That Yield Potential Fuels

sugar --> ethanol + carbon dioxide	*Yeast*
hydrogen + carbon dioxide --> methane + water	*Methanococcus jannaschii*
sugar + water --> hydrogen + acetic acid + carbon dioxide	*Clostridium burtyricum*
acetic acid --> methane + carbon dioxide	*Methanosarcina*
carbon monoxide + water --> hydrogen + carbon dioxide	*Carboxydothermus hydrogenoforma*
lactic acid + water --> hydrogen + carbon dioxide	Purple bacteria

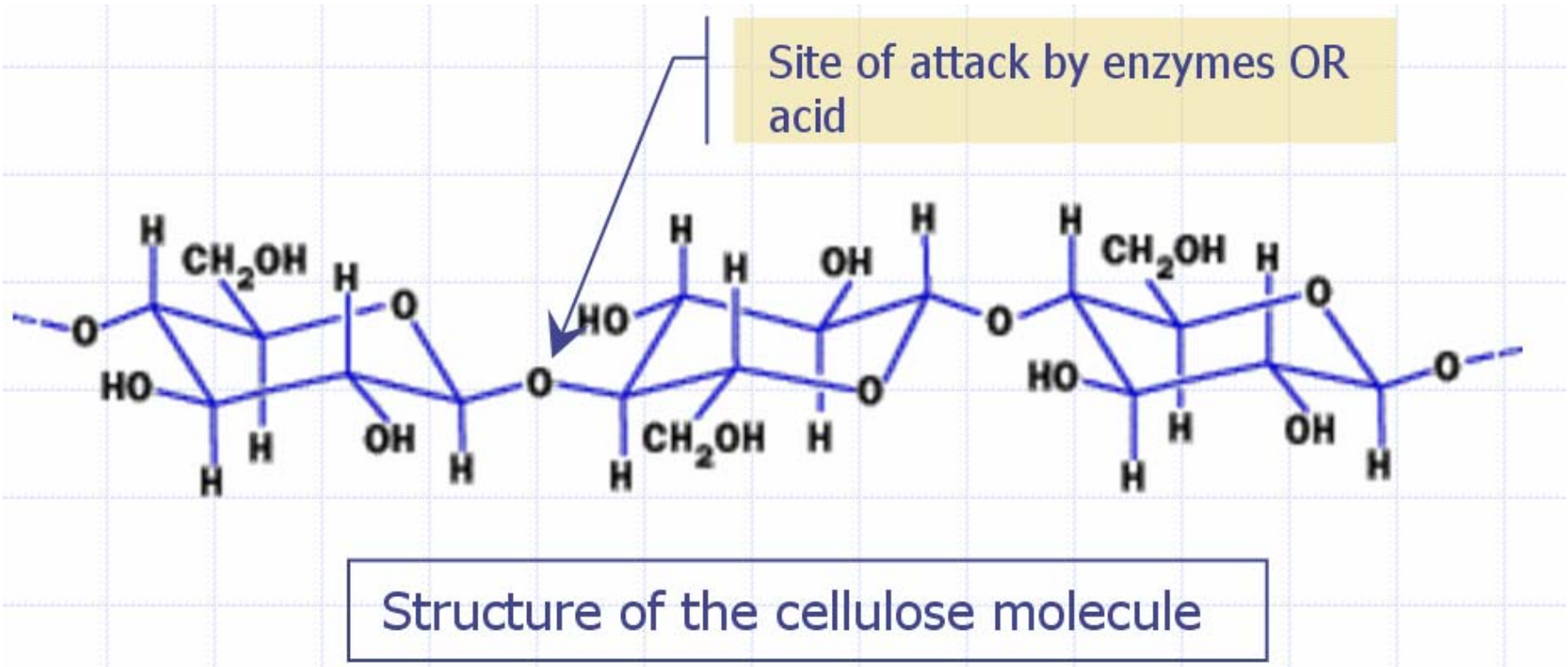
Biological Solutions to Renewable Energy Hamilton O. Smith, Robert Friedman, and J. Craig Venter
Volume 33, Number 2 - Summer 2003

Carbohydrate/Starch Versus Cellulose

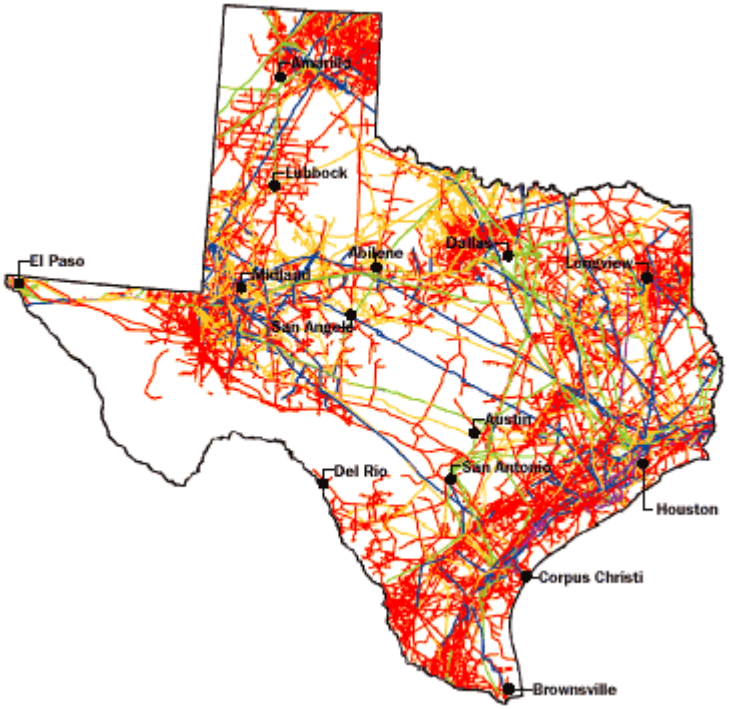
- Both yield ethanol
- Cellulose is more difficult
  - —Needs physical pretreatment to break up structure
  - —Hydrolysis yields pentoses as well as hexoses, which are less digestible
- Sources of biomass differ dramatically
  - —Corn and sugar cane - starch
  - —Poplar, switchgrass - cellulose
  - —Fertile versus barren soil – diversion of cropland
- Competition with people for food.

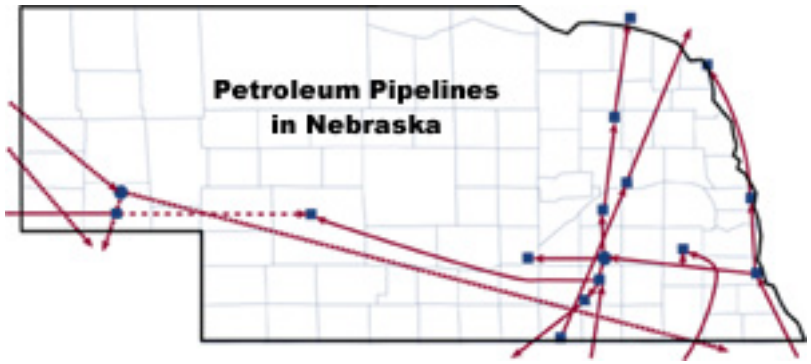
Cellulosic Biofuel

- **Thermochemical versus enzymatic hydrolysis**
- **Plants can be genetically engineered, possibly even to self-digest.**
- **Biomass handling**



Other issues - Transportation





Pipelines are in the wrong place, may not be able to carry multiple products.

Increased Safety

- Reduced use of less toxic pesticides.
- Less farmworker exposure to chemicals.
- Reduced global warming.
- Reduced burden of human disease.
- Replacement of more hazardous industries, like coal mining.

Summary

- Increase in the pace, not necessarily the processes of biotechnology.
- Known hazards of infection, allergy, immunosuppression.
- New theoretical risk of colonization.
- Physical hazards of new biofuels industry
- Weaknesses in biosafety
- Biosecurity risks
- Unknown risk of ‘unnatural’ DNA
- De novo organism creation from DNA sequences.

Questions?