











"How restriction enzymes became the workhorses of molecular biology"

A significant breakthrough came in 1970 when the first of two papers from Smith's laboratory described an enzyme, endonuclease R, that was able to cleave bacteriophage 177 DNA into specific fragments (2). This was the first type II restriction enzyme, the sort that now populates our freezers, because it recognize specific sequences and also gives rise to very specific cleavage. Smith had been looking for an enzyme that might be involved in site-specific recombination in *Haemophilus influenzae* and thought at first that endonuclease R might be his long-sought quary. With Tom Kelly, he went on to determine the DNA sequence recognized by endonuclease R and reported it as GTY RAC (<u>11</u>). This sequence seemed too short for a recombination enzyme, and during correspondence with his close friend Nathans, who ran the neighboring laboratory but was away on sabbatical, it became clear that this enzyme might have very practical uses for the analysis of DNA.

R. Roberts – PNAS 2005











 $\mathit{Science}, \mathit{September 1973}:$ letter to the NAS from participants at Gordon conference on nucleic acids.

"We are writing ... on behalf of a number of scientists to communicate a matter of deep concern. We presently have the technical ability to join together, covalently, DNA molecules from diverse sources... This technique could be used, for example, to combine DNA from animal viruses with bacterial DNA... In this way, new kinds of hybrid plasmids or viruses, with biological activity of unpredictable nature, may eventually be created. These experiments offer exciting and interesting potential, both for advancing knowledge of fundamental biological processes, and for alleviation of human health problems. Certain such hybrid molecules may prove hazardous to laboratory workers and to the public. Although no hazard has yet been established, prudence suggests that the potential hazard be seriously considered."

Maxine Singer (Carnegie)

Die



September 1974 – a moratorium?

New plasmids: novel antibiotic resistance markers into E. coli; *Xenopus* and *Drosophila* genomic DNA into E. coli.

Norton Zinder: "If we had any guts at all, we'd tell people to not do these experiments!"

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> GENETIC EXCHANGE IN SALMONELLA¹ NORTON D. ZINDER AND JOSHUA LEDERBERG Department of Genetics, University of Wisconsin, Madison, Wisconsin

Received for publication April 18, 1955

Genetic investigations with many different bacteria have revealed parallelisms and some contrasts with the biology of higher forms. The successful application of selective enrichment techniques to the study of gene recombination in *Escherichia coli* (Tatum and Lederberg, 1947; Lederberg *et al.*, 1951) suggested that a similar approach should be applied to other bacteria. This paper presents the results of such experiments with *Sathmonella typhimmrium* and other *Sathmonella* serotypes. The mechanism of genetic exchange found in these experiments differs from sexual recombination in *E. coli* in many respects so as to warrant a now descriptive term, transduction.



Sept. '74 – Feb' 75

"Until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring the following experiments" – cloning new antibiotic resistance genes in currently naïve hosts and cloning fragments of oncoviral genomes into bacterial plasmids.

"an epidemic of cancer"?

Asilomar conference, February 1975

Paul Berg, David Baltimore, Sydney Brenner, Mike Bishop, Don Brown, Ron Davis, James Watson, Phil Sharp, Herb Boyer, Joshua Lederberg + 150 more people

+ Nature, Science, NY Times, Washington Post, Boston Globe, SF Chronicle, etc





(Left to right) Maxine Singer, Norton Zinder, Sydney Brenner, and Paul Berg

MCB 140 09

The measures

- 1. Moratorium lifted.
- Experiments will proceed under two levels of containment, biological (use weakened host cell), and physical, the level of which will be gauged by the level of risk presented by the organism created (minimal, low, moderate, high):
 - 1. Stringent, commonsense cleanliness (P1)
 - 2. Similar to surgical operating theatre (P2)
 - 3. Giant isolator (P3)
 - 4. Comparable to what one uses in biological warfare research (P4)

Where to draw the cutoff line, idea #1

invertebrates (including flies)

vertebrates

iuling nies)

Similar to surgical operating theatre (P2)

Stringent, commonsense cleanliness (P1)

Giant isolator (P3)

Comparable to what one uses in biological warfare research (P4)







RNA splicing removes introns Exons – sequences found in a gene's DNA and mature mRNA (expressed regions) Introns – sequences found in DNA but not in mRNA (intervening regions) Some eukaryotic genes have many introns

MCB 140 09-2

















"What seems to have been disregarded completely is that we are dealing here much more with an ethical problem than one in public health, and that the principal question to be answered is whether we have the right to put an additional fearful load on generations that are not yet born... You can stop splitting the atom; you can stop visiting the moon... But you cannot recall a new form of life. Once you have constructed a viable *E. coli* cell carrying a plasmid DNA into which a piece of eukaryotic DNA has been spliced, it will survive you and your children and your children. An irreversible attack on the biosphere is something so unheard of, so unthinkable to previous generations, that I

unheard of, so unthinkable to previous generations, that I could only wish that mine had not been guilty of. The hybridization of Prometheus with Herostratus is bound to give evil results. ... My generation ... has been the first to engage, under the leadership of exact sciences, in a destructive colonial war against nature. The future will curse us for it.

Chargaff E, Simring FR. "On the dangers of genetic meddling." Science. (1976) 192:938



"Frankenstein monsters from Harvard Yard"

- Mayor Alfred Vellucci: "They may come up with a disease that can't be cured – even a monster! Is this the answer to Dr. Frankenstein's dream?"
- → an open meeting of the Cambridge City Council, June 13, 1976
- "Why didn't someone advise the mayor of Cambridge and the CCC what was going on at Harvard?"
- Resolution: ban all recombinant DNA research in the city, whether publicly or privately financed, for two years.
- Establish Cambridge Experimentation Review Board. City Manager Sullivan chose to name an ERB consisting entirely of nonscientists.

John Lear Recombinant DNA: the Untold Story

Not only Cambridge

- City ordinances requiring uniform compliance with city-specified controls on working with recombinant DNA:
- Princeton, Emeryville, Berkeley (Sept. 77), Amherst, etc etc.

Enter the public, part 2

The opening session of a three-day forum in March 1977, sponsored by the National Academy of Sciences, ... was disrupted by a citizens' group, The People's Business Commission, led by activist Jeremy Rifkin, whose members, chanting "We Will Not Be Cloned," draped across the platform a banner with the caption, "We Will Create the Perfect Race."

S. Wright Molecular Politics

The severe adverse events that have occurred in the subsequent 30 years of nonstop cloning of all imaginable sorts of DNA into E. coli vectors

Genetically modified plants

It's what's for dinner

"Evidently, most Americans are unaware of the extent to which crops derived from biotechnology have entered the marketplace. When consumers were asked how much processed food has genetically modified (GM) ingredients, only 14% responded with the correct answer (over 50% of processed foods have GM ingredients). Similarly, when asked whether or not they have eaten GM foods, 62% said no, they had not, while only 19% said that they had. With more than 60% of processed foods containing GM ingredients, it is highly probable that most people have eaten them at some time."

J. Nutr. 132:1384-1390, 2002

"Yes": US 75% Austria 58% Denmark 90% Greece 50% Spain 46%

"Yes": US 45% Canada 52% Denmark 44% Holland 51%

"By eating a genetically modified fruit, a person's genes can be changed"

"Yes":

US 61% Canada 62% Holland 74% Italy 58%











Three fundamental differences between "engineering" plants by traditional breeding and transgenic technology

- 1. Breeding is a rendez-vouz within the same species, or closely related species.
- 2. Meiosis (HR) is an ordered exchange of genetic material, not random shuffling.
- 3. GM accesses portions of the genome that are not subject to Darwinian selection. "A plant is no more likely to acquire the ability of secrete a bacterial toxin, than a breed of dogs is likely to grow an extra eye, or a goat is likely to sprout an elephant's trunk. Yet these are just the kinds of monstrosities that today's genetic engineers would aspire to create."

B. Tokar Redesigning life? p. 5

Bt: the non-pesticide pesticide

- A bacterial disease of silkworms discovered in 1902 was eventually attributed to a bacterium, *Bacillus thuringiensis*
- The insecticidal properties of the bacterium are due to its synthesis of crystal inclusion (Cry) proteins, aka insecticidal crystal protein
- The bacteria themselves have been used as a spray insecticide for over 40 years

A bad gut feeling

- 1. In the bacterial spore, the ICP crystals are inactive and, therefore, inocuous
- 2. A butterfly larva, a beetle, or a fly larva eats some of the bacterial spores.
- The crystals denature into endotoxins in the alkaline pH (> 8.0) of the insect midgut. In the acidic midgut of invertebrates, or of mammals, this either does not happen, or the endotoxins are rapidly degraded – this accounts for tier 1 of the safety of Bt ICPs.
- The denatured, activated endotoxins, then bind to receptors on insect midgut epithelial cells, enter the cells, form a pore, and the cells lyse. The endotoxins do not bind to mammalian gastrointestinal epithelium – this accounts for tier 2 of their safety.

Safe

"The information from the acute toxicity/pathogenicity studies on Bt strains has supported the assessment that the Bt microorganisms are not pathogenic to, or toxic to, mammals. The lack of adverse effects in toxicity tests for the entire preparation suggests that the protein components of the microorganism and fermentation residues are not toxic to mammals. It is reasonable to conclude from these findings that the ICPs, the major source of the insecticidal activity in Bt preparations, have not been implicated in mammalian toxicity." John Kough, US EPA, Office of Pesticide Programs, 2003

What do you think you just ate?

Microbial Bt use on US crops, 2000-01:Crop% treated with BtAlmonds18Cabbage60Celery40Bell peppers42Strawberries41















The cotton bollworm, *Helicoverpa zea,* and Bollgard

Total acreage planted with Bt-transgenic cotton in US in 2001: 5,800,000 acres







Le roi est mort, vive le roi: poor monarch butterflies?

- Losey (1999) Nature 399: 214.
- Hellmich (2001) PNAS 98: 11925.
- Sears ibid 11937



"If a single plant species were to be voted the most popular by scientists and laymen alike, it would be *Oryza sativa*. Rice, the world's most important cereal crop for human consumption, is the food staple of more than 3 billion people, many of them desperately poor."

Science, Vol 296, Issue 5565, 53 , 5 April 2002

World population: 6,379,157,361

Rice is the major staple food for hundreds of millions of people. It is generally consumed in its milled form with outer layers (pericarp, tegmen and aleurone layers) removed. The main reason for milling is to remove the oil-rich aleurone layer, which turns rancid upon storage, especially in tropical and subtropical areas. As a result, the edible part of rice grains consists of the endosperm, filled with starch granules and protein bodies, but it lacks several essential nutrients for the maintenance of health, such as carotenoids exhibiting provitamin A-activity. Thus, reliance on rice as a primary food staple contributes to vitamin A deficiency, a serious public health problem in at least 26 countries including highly populated areas of Asia, Africa and Latin America

J. Nutr. 132:506S-510S, 2002









"Vitamin A deficiency causes symptoms ranging from night blindness to those of xerophthalmia and keratomalacia, leading to total blindness." *Science*, Vol 287, 303-305

"Globally, 3 million preschool-aged children have visible eye damage due to a vitamin A deficiency. Annually, an estimated 250,000 to 500,000 preschool children go blind from this deficiency and approximately two-thirds of these children die within months of going blind. The estimates of the subclinical prevalence of vitamin A deficiency range between 100 and 250 million."

J. Nutr. 132:491S-494S, 2002

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J. Nutr. 132:491S-494S, 2002



The Problem ctd.

"Milled rice is normally white, being devoid of β carotene and other colored phytochemicals. Plant breeders have searched various rice collections for colored grains, **but no germ plasm has been found that makes carotenoids in its endosperm** (the edible part of the grain that is left after milling). Rice plants do have the genetic machinery to make carotenoids, as these are found in leaves, but several of the carotenoid pathway enzymes are not expressed in rice endosperm."

Grusak (2005) Nat. Biotech. 23: 429.

*Collection of seeds, or other cell material, from naturally occurring, or otherwise interesting variants of a particular plant species. For example, in England, at the John Innes Centre, there is a germplasm collection for wheat: Triticum - 9513 accessions. UK holdings 520 Cultivars and 782 breeders lines, 3658 landraces or selections from landraces. Durum types 200 accessions including 197 named varieties, 2135 breeders lines, 3658 landraces or selections from landraces. Durum types 200 accessions including 89 named cultivars, 49 breeders lines and 45 landraces or selections from landraces.

Farewell, alleles

"Genetic erosion has accelerated since the introduction of high-yielding varieties during the Green Revolution of the 1960s. In 1959, farmers in Sri Lanka grew over 2,000 traditional varieties of rice. Today, that number has been reduced to 5."

Levetin and McMahon Plants and Society

MCB 140 09-22













"Genetically Engineered 'Golden Rice' is Fool's Gold" Greenpeace Statement February 9, 2001

Genetically engineered "Golden Rice" containing provitamin A will not solve the problem of mainutition in developing countries according to Greenpeace. The Genetic Engineering (GE) industry claims vitamin A rice could save thousands of children from blindness and millions of mainourished people from vitamin A deficiency (VAD) related diseases. But a simple calculation based on the product developers' own figures show an adult would have to east at least twelve times the normal intake of 300 grams to get the daily recommended amount of provitamin A (1). Syngenta, one of the world's leading genetic engineering companies and pesticide producers, which owns many patents on the "Golden Rice", claims a single month of marketing delay of "Golden Rice" would cause 50.000 children to go blind.(2). Greenpeace calculations show however, that an adult would have to east at the

Greenpeace calculations show however, that an adult would have to eat at least 3.7 kilos of dry weight rice, i.e. around 9 kilos of cooked rice, to satisfy his/her daily need of vitamin A from "Golden Rice". In other words, a normal daily intake of 300 gram of rice would, at best, provide 8% percent of the vitamin A needed daily. A breast-feeding woman would have to eat at least 6.3 kilos in dry weight, converting to nearly 18 kilos of cooked rice per day.

http://www.biotech-info.net/fools_gold.html











The Golden Rice 2 reported here has up to 37 **G**/g carotenoid of which 31 **G**/g is **B**-carotene. This increase in total carotenoid and proportion of **B**-carotene over the original Golden Rice promises a greater impact on vitamin A deficiency and related health issues. A value of 30 **G**/g (25 **G**/g **C**-carotene), however, is used here for calculations of impact for vitamin A deficiency, this number being chosen at this early stage as a moderate prediction of future performance. Definitive statements on the benefit of Golden Rice for the alleviation of vitamin A deficiency cannot be made. The vitamin A delivered and its impact on the body depends on several unquantified factors, including **G**-carotene uptake and conversion to vitamin A, as well as the amount of rice consumed by the individual. These factors are under rigorous investigation at present but for the time being only estimates are available. The symptoms and effects of vitamin A deficiency are most dramatic in children. Therefore, for the purpose of this estimate we have used the US recommended dietary allowance (RDA) for 1- to 3-year-old children (300 **G**) vitamin A³. It would also be reasonable to assume that an individual receives at least some vitamin A from the current diet. Based on a retinol equivalency ratio for **G**-carotene of 12:1 (ref. 23), 50% of the children's RDA is delivered by 72 g of dry Golden Rice 2. This Is likely to be an underestimate because **G**-carotene may prove to be more bioavailable from rice, a comparatively simple food matrix, than it Is from furties and vegetables upon which the equivalency ratio is based²⁴. A typical child's portion is about 60 g of rice and more than one portion is frequently consumed per day in regions where rice is a staple food.

Paine et al. Nature Biotechnology 23, 482 - 487 (2005)

"'Mirage' of GM's golden promise" Alex Kirby, BBC News, Feb. 04

- "Golden rice may prove part of the answer to vitamin A deficiency, though not the comprehensive solution it seemed to be.
- But it would be an answer that came with a hefty price-tag: the persistent concerns about the safety of GM technology to human health and to wild species."