

***PART 3 Written Work-up of your selected paper to CT***

***Submit the following by Mon Nov 26***

- a. Title of paper, author list (first and last only if there are more than 2 authors), name of journal, volume, page and year of publication
- b. A short summary (several sentences) of the paper including the biological process under examination, the model organism used, how the researchers screened or selected for phenotype of interest, the scale of the screen (how hard did they look for mutants?), the mutagen or other technique used to generate mutations, the number of mutants discovered and ***especially any interesting biology that the researchers uncovered.***

***PART 4: 10-12 minute in-class presentation of your paper (Dec 4 & 6)***

1. Structure your talk around the guidelines for the homework assignment -- in other words, be sure to convey the biological process under investigation, the model organism chosen (and why), the phenotype screened (or selected for), what the researchers learned that was interesting, the scale of the screen, etc.
2. *Work up a couple of slides WELL IN ADVANCE OF YOUR TALK and practice your talk on a friend or classmate to make sure it is coherent & of the appropriate length*
3. *On Tues and Thurs Nov 27 & 29, the labs will be on the short side giving you extra time to work on your presentation*

**Forward Genetics Part 3:**

- Submit info on Paper in the form of an *outline* for your presentation during the last week of classes
- Plan to present a few slides of text and figures (from the paper) during your talk
- In your outline indicate which figure or table from your paper you will present in your talk

Billy, Kenny, Nathan

## **Neuroscience – development and functioning of the nervous system**

**Isadora:** A Large-scale Mutagenesis Screen to Identify Seizure-resistant Zebrafish

**Kevin Hope:** A *C. elegans* p38 MAP kinase pathway mutant protects from dopamine, methamphetamine, and MDMA toxicity

**Miranda:** A forward genetic screen with a thalamocortical axon reporter mouse yields novel neurodevelopment mutants and a distinct *emx2* mutant phenotype

**Jonathan:** A Forward Genetics Screen in Mice Identifies Recessive Deafness Traits and Reveals That Pejvakin Is Essential for Outer Hair Cell Function

## **Host-Pathogen Interactions**

**Russell:** A Forward-Genetic Screen and Dynamic Analysis of Lambda Phage Host-Dependencies Reveals an Extensive Interaction Network and a New Anti-Viral Strategy

**Brianna:** Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication

**Greg K:** A High-Throughput Forward Genetic Screen Identifies Genes Required for Virulence of *Pseudomonas syringae* on *Arabidopsis*

## **Animal Developmental Biology & Physiology**

**Greg P:** Gravitaxis in *Drosophila melanogaster* : a forward genetic screen

**Katy:** A genetic screen in zebrafish identifies the mutants *vps18*, *nf2* and *foie gras* as models of liver disease

**Audriana:** Identification of Mutations that Delay Somatic or Reproductive Aging of *Caenorhabditis elegans*

**Jessica:** Zebrafish development

## **Plant Development & Physiology**

**Teresa:** Identification of plant stress-responsive determinants in *Arabidopsis* by large-scale forward genetic screens

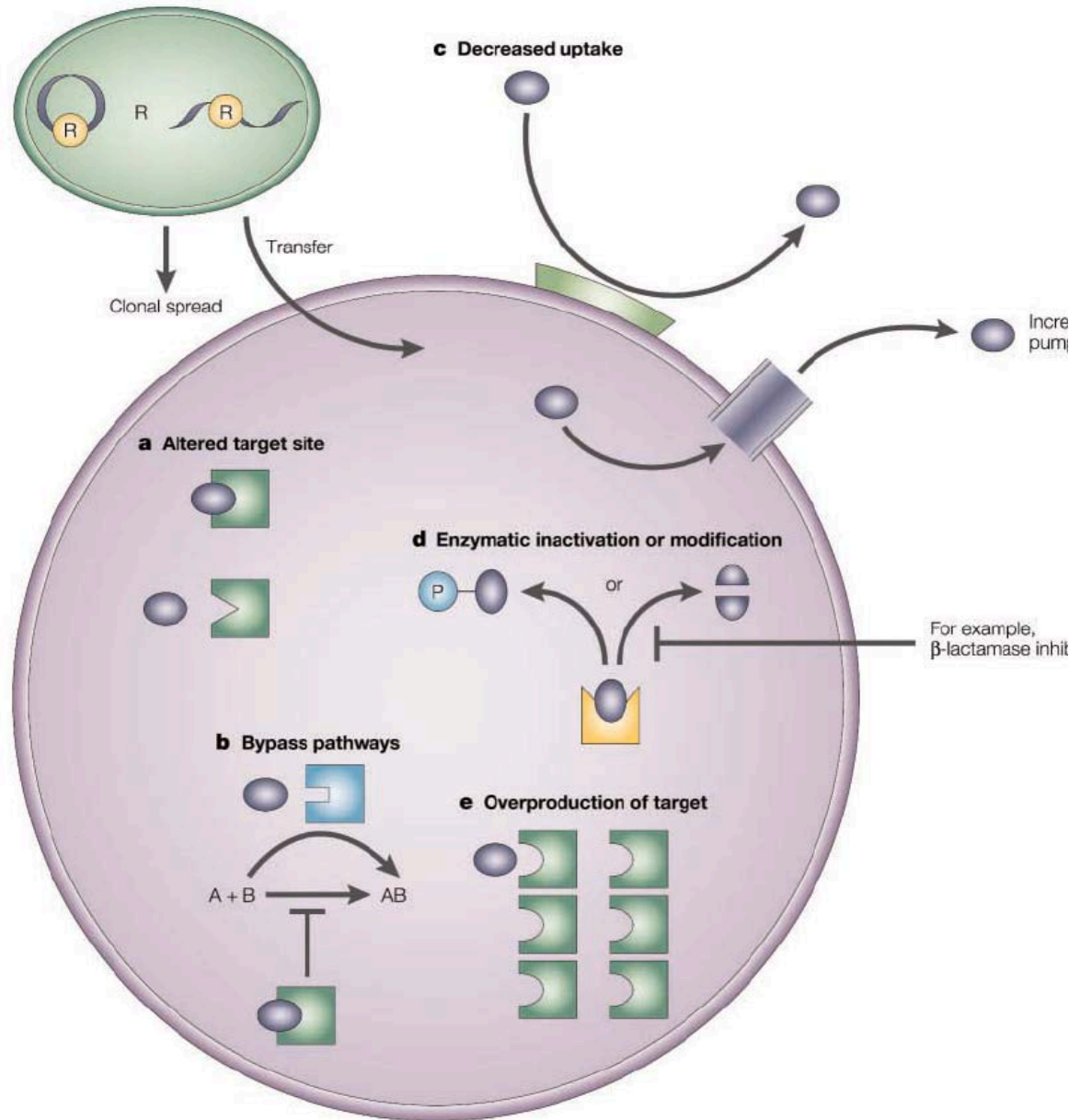
**Kevin Hager:** New Nodulation Mutants Responsible for Infection Thread Development in *Lotus japonicus*

# *Pulling together the threads of the Bacterial Genetics Experiments*

Don't forget about Google.doc assignment focusing on *Weapons of Microbial Drug Resistance Abound in Soil Flora*

<https://docs.google.com/spreadsheet/viewform?fromEmail=true&formkey=dEIZUk8tTHVTRDE0TVI5TXk2RIZKUYE6MQ>

## Mechanisms of genetic resistance to antimicrobial agents.



NATURE REVIEWS | DRUG  
DISCOVERY VOLUME 1 | 899  
| NOVEMBER 2002

Bacteria have developed, or will develop, genetic resistance to all known antimicrobial agents that are now in the marketplace.

*The five main mechanisms that bacteria use to resist antibacterial drugs are shown in the figure.*

a | The site of action (enzyme, ribosome or cell-wall precursor) can be altered. For example, acquiring a plasmid or transposon that codes for a resistant dihydrofolate reductase confers trimethoprim resistance to bacteria.

b | The inhibited steps can be bypassed.

c | Bacteria can reduce the intracellular concentration of the antimicrobial agent, either by reducing membrane permeability, or by active efflux of the agent.

d | They can inactivate the drug. For example, some bacteria produce  $\beta$ -lactamase, which destroys the penicillin  $\beta$ -lactam ring

e | The target enzyme can be overproduced by the bacteria.

## *Where do these antibiotic-resistance alleles/genes come from?*

Antibiotic resistance acquired by two fundamental mechanisms:

- *Sustain a mutation that confers resistance in a gene already present in the bacteria's genome: this mechanism explored in the mutagenesis lab*
- *Acquire a new gene from somebody else via horizontal gene transfer: this strategy mechanism explored the next bacterial genetics lab*

## *Weapons of Microbial Drug Resistance Abound in Soil Flora*

<http://fire.biol.wvu.edu/trent/trent/microbialresistance.pdf>

| Multidrug Resistance in <i>S. aureus</i> |             |             |                      |
|--|-------------|-------------|----------------------|
| Antibiotic                               | MSSA (1930) | MRSA (1994) | Resistance mechanism |
| Penicillin                               | S           | R           | + (1945)             |
| Streptomycin                             | S           | R           | + (1948)             |
| Tetracycline                             | S           | R           | + (1950)             |
| Methicillin                              | S           | R           | + (1961) <i>mecA</i> |
| Oxacillin                                | S           | R           | +                    |
| Cephalothin                              | S           | R           | +                    |
| Cefotaxime                               | S           | R           | +                    |
| Imipenem                                 | S           | R           | +                    |
| Chloramphenicol                          | S           | R           | +                    |
| Ciprofloxacin                            | S           | R           | A                    |
| Clindamycin                              | S           | R           | +                    |
| Erythromycin                             | S           | R           | +                    |
| Gentamycin                               | S           | R           | +                    |
| Rifampin                                 | S           | R           | A                    |
| Vancomycin                               | S           | S           | A (1997) <i>VISA</i> |
| Vancomycin                               | S           | S           | + (2002) <i>vanA</i> |
| Teichoplanin                             | S           | S           | +                    |
| Trimeth/Sulfa                            | S           | R           | A                    |

**Emergence of multidrug resistance in *Staphylococcus aureus*.**  
The Brazilian clone of methicillin-resistant *S. aureus* (MRSA), isolated in 1994 (2), was resistant (R) to nearly all the antibiotics listed. Most of the resistance mechanisms were not adaptive (A), but acquired (+) from an extraspecies source. In contrast, an invasive strain of *S. aureus* (MSSA), recovered in 1930, was susceptible (S) to all the agents.

(+) = acquired from an extraspecies source

What do we know about the transfer mechanisms?

What types of genetic elements are involved?

*How frequent are horizontal transfer events in natural settings?*

*Difficult to get a handle on frequency outside of the lab*

*But, consider first the scale of the microbial world & and then consider that even if horizontal gene transfer events are not all that common, we humans have created environmental conditions that would select very effectively for the products of such events*



*The world is awash in bacteria  
And  
Where there are bacteria, there are  
bacterial viruses (bacteriophage)*

## Prokaryotes the unseen majority

From PNAS 95: 6578 June 1998

### *Most of the earth's prokaryotes occur in*

- open ocean:  $1.2 \times 10^{29}$  cells
- soil:  $2.6 \times 10^{29}$  cells
- oceanic subsurfaces (marine sediments below 10cm):  $3.5 \times 10^{30}$  cells
- terrestrial subsurfaces (below 8 meters):  $0.25 - 2.5 \times 10^{30}$  cells

### *Other habitats*

- animals -- many vertebrate and invertebrate animals contain dense populations of prokaryotes that play important roles in nutrition and disease – see next page
- leaves
- air

# Microbes maketh man

**People are not just people. They are an awful lot of microbes, too**

Aug 18th 2012 | The Economist

POLITICAL revolutionaries turn the world upside down. Scientific ones more often turn it inside out. And that, almost literally, is happening to the idea of what, biologically speaking, a human being is.

The traditional view is that a human body is a collection of **10 trillion cells which are themselves the products of 23,000 genes**. If the revolutionaries are correct, these numbers radically underestimate the truth. For in the nooks and crannies of every human being, and especially in his or her guts, dwells the microbiome: **100 trillion bacteria of several hundred species bearing 3million non-human genes.....**humans are not single organisms, but superorganisms made up of lots of smaller organisms working together.

***We and other complex organisms are composed of an interconnected ecosystem of eukaryotic and prokaryotic cells:***

*Density of prokaryotes on human skin:*

$10^3$ - $10^4$  per  $\text{cm}^2$

[except in groin and axilla where it is  $10^6$  per  $\text{cm}^2$ ]

*Total estimated number of prokaryotes on the skin of an individual is  $3 \times 10^8$  cells*

*In the human colon:  $3.2 \times 10^{11}$  cells/g*

*(colon is about 220 g)*

***Compare to # of people on earth-- which is?***

ON NS

HOME UNFPA BBC INED: THE WORLD POPULATION AND ME

ABOUT 7

CURRENT WORLD POPULATION

**7,001,461,040**

How does the world's population reaching 7 BILLION affect you?

Get Started »

+1 Tweet Like 19k

<http://www.7billionandme.org/>

<http://www.census.gov/main/www/popclock.html>

United States  
**Census**  
Bureau

People | Business | Geography | Data | Research | News

you are here: Census.gov » U.S. & World Population Clocks

**U.S. & World Population Clocks**

Population Clocks Main

**Current Population Clock**

**U.S. 314,720,329**

**World 7,050,534,968**

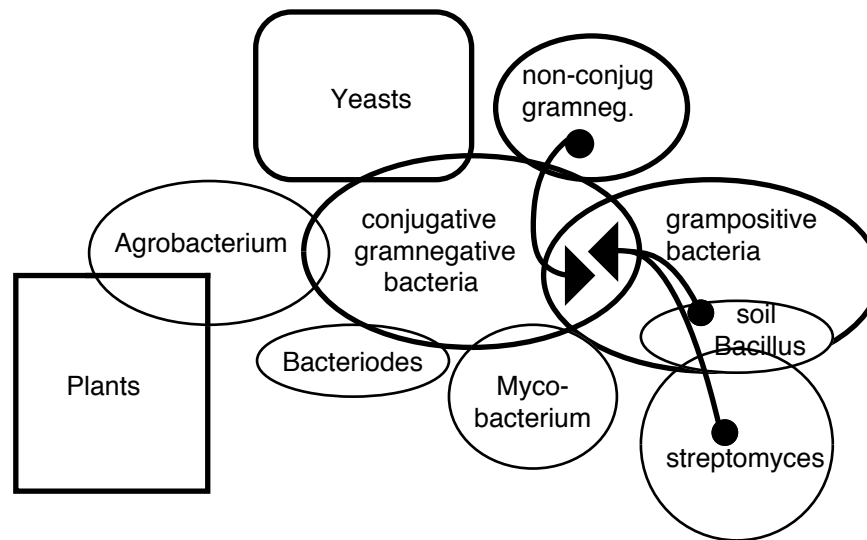
20:00 UTC (EST+5) Nov 06, 2012

***BOTTOM LINE: In natural settings there is a tremendous amount of promiscuous plasmid exchange via conjugation (most bacterial and some archeal species encode conjugation systems) and transformation***

- *This exchange can occur between distantly related bacterial species*
- *This sort of casual horizontal (lateral) transfer of genetic information (mostly on plasmids) has played a decisive role in the appearance and evolution of organisms resistant to various types of antibiotics*

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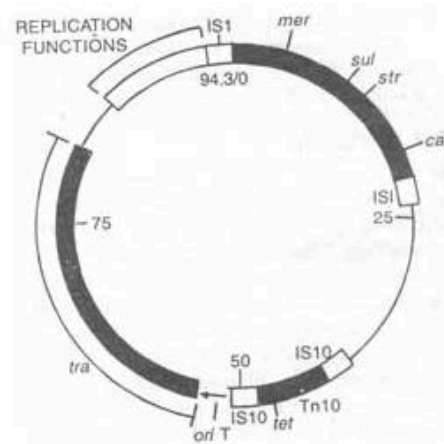
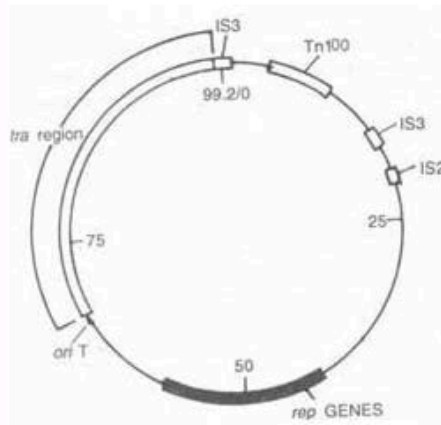
***Overlap of circles indicates documented gene exchange***



Range of Plasmid-Related Gene Flux .  
Regions of overlap between major genetically related organisms represent gene exchange. Almost all this network is based on plasmid self-mobilization, although other means of lateral transfer maybe involved. Arrows show the proposed origin and dispersion of different antibiotic resistance determinants. Some determinants arose from antibiotic-producing organisms and soil bacteria, others from mutant organisms exposed to drugs during the antibiotic era.

### (b) R-Plasmids

These are the most widespread and well studied group of plasmids conferring resistance (hence called resistant plasmids) to antibiotics and various other growth inhibitors. R-plasmids typically have genes that code for enzymes able to destroy and modify antibiotics.



Genetic Map of the F (Fertility Plasmid) of Escherichia coli. The tra region contains tra genes involved in conjugative transfer; Ori T sequences is the origin of transfer during conjugation; transposable element IS = insertion sequences. The rep genes regulate DNA replication.

Genetic map of the resistance plasmid R100. Cat = Chloramphenicol resistance gene; str = Streptomycin resistance gene; sul = sulfonamide resistance gene, mer = mercury ion resistance gene, IS = insertion sequences

They are not usually integrated into the host chromosome. Some R-plasmids possess only a single resistant gene whereas others can have as many as eight. Plasmid R 100, for example, is a 94.3 kilobase-pair plasmid that carries resistant genes for sulfonamides streptomycin and spectinomycin, chloramphenicol, tetracyclin etc. It also carries genes conferring resistance to mercury. Many R-plasmids are conjugative and possess drug-resistant genes as transposable elements, they play an important role in medical microbiology as their spread through natural populations can have profound consequences in the treatment of bacterial infections.

**Important implications of whole plasmid transfer:**

*This is a great way for a bacterial cell to acquire novel genes:* since no requirement for homologous crossing-over, the resident genome does not have to have homology to incoming genes in order for a stable recombinant to be formed

*It is well documented that many cases of AR acquisition involve transfer of plasmid-encoded genes between cells that are not necessarily closely related*

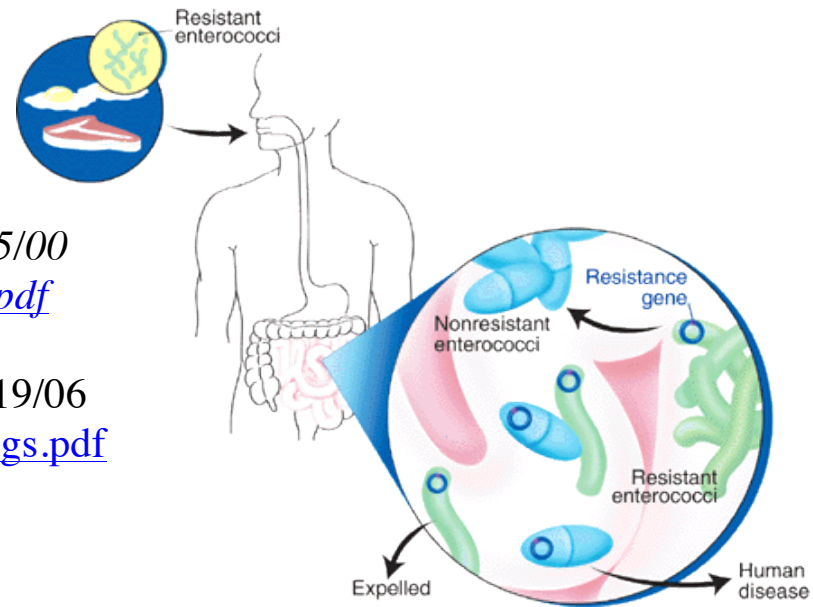
***Required Reading Assignments***

*Super bugs on the Hoof? Science 288: 792 5/5/00*

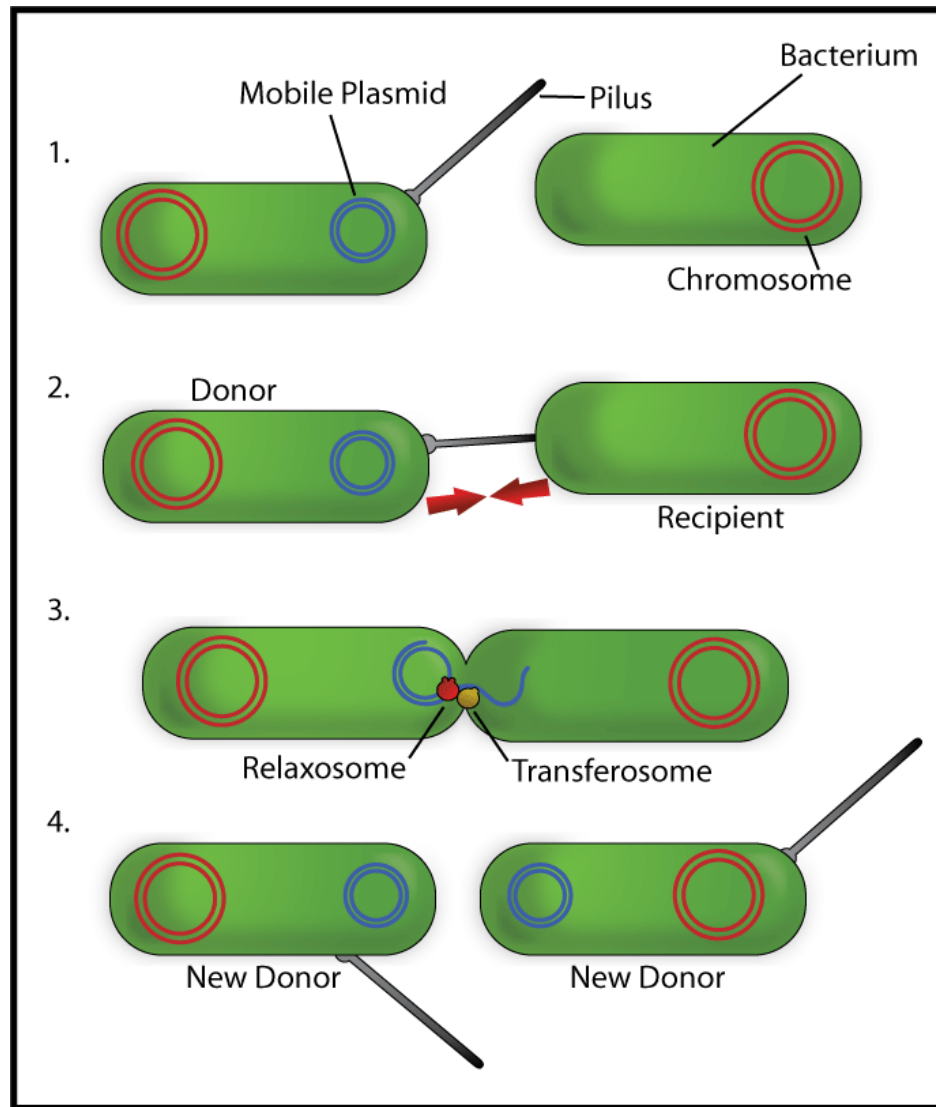
<http://fire.biol.wvu.edu/trent/trent/superbugs.pdf>

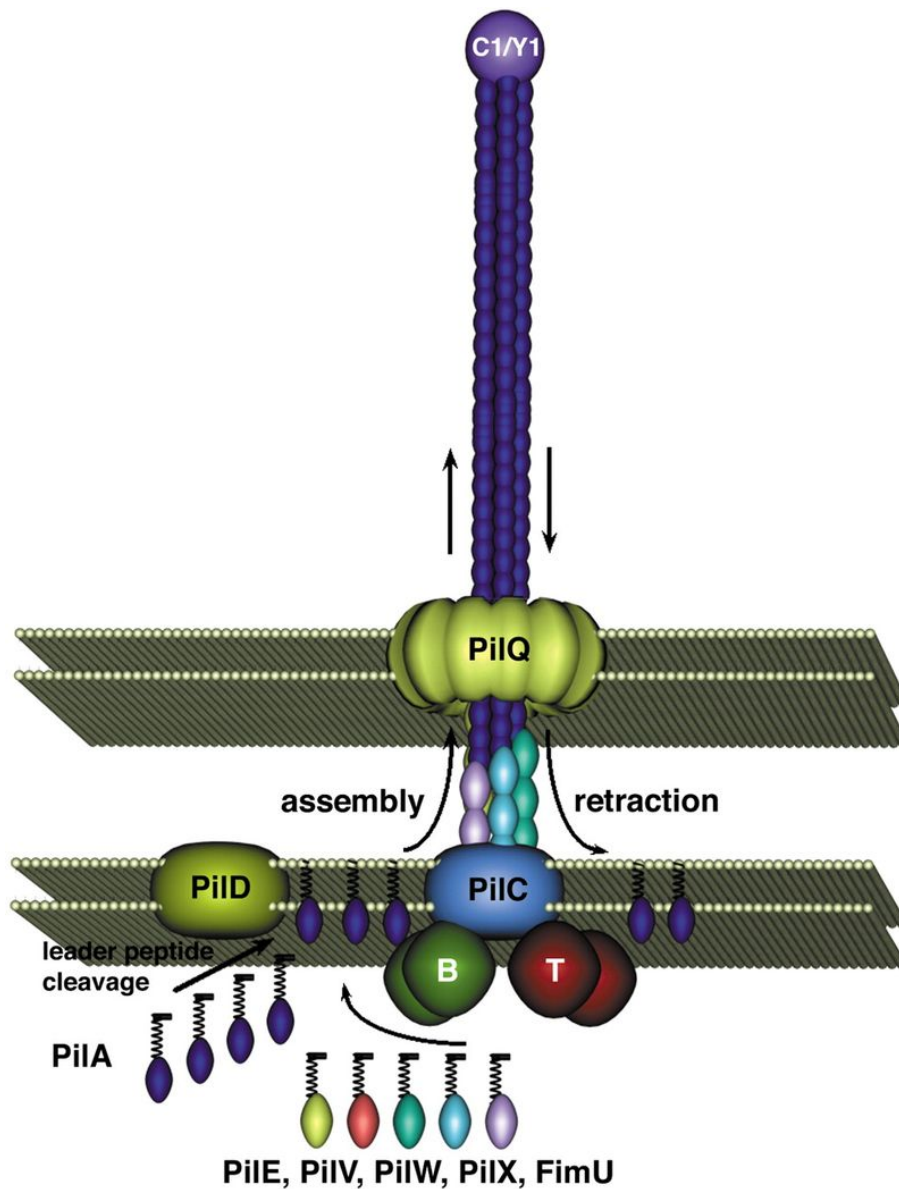
*SuperBugs Abound in Soil Nature News 1/19/06*

<http://fire.biol.wvu.edu/trent/trent/soilsuperbugs.pdf>



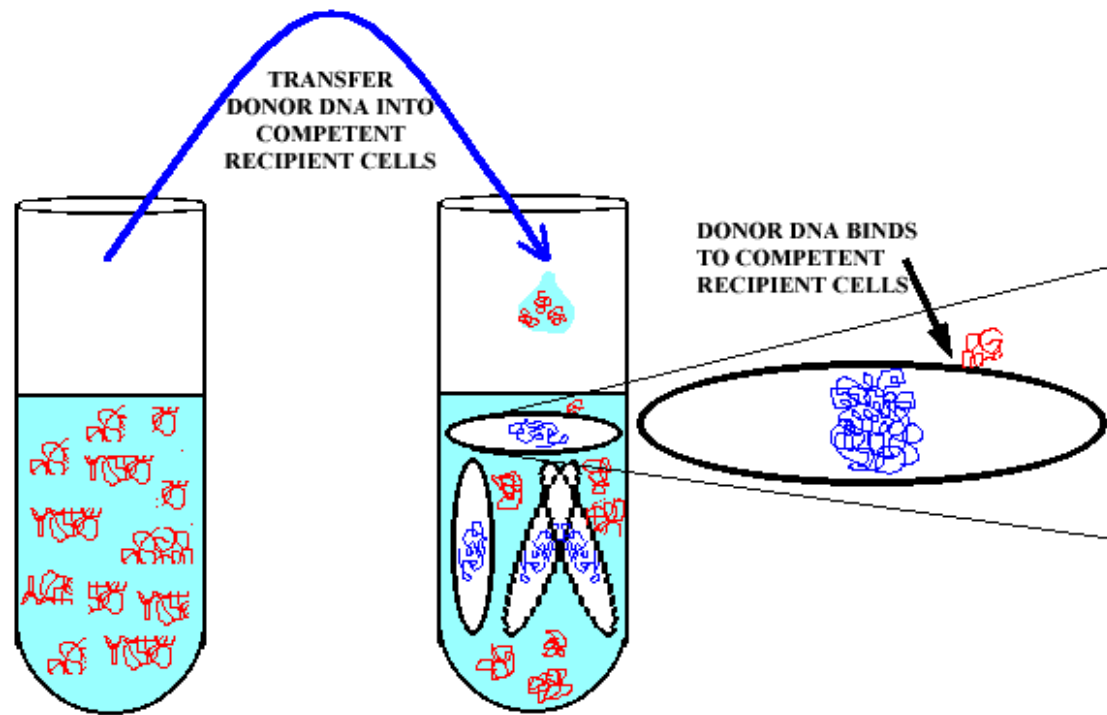






## The Microbial Grappling Hook

Model of type IV pili assembly and retraction. Prepilinleader sequences are cleaved by PilD. Processed PilA is assembled on a base of minor pilins (PiIE, V, W, X, and FimU) by the action of the cytoplasmic membrane protein PilC and the NTP-binding protein PilB. The pilus is extruded through the outer membrane via a pore of PilQ multi-mers. Pilus retraction is by the action of PilT.

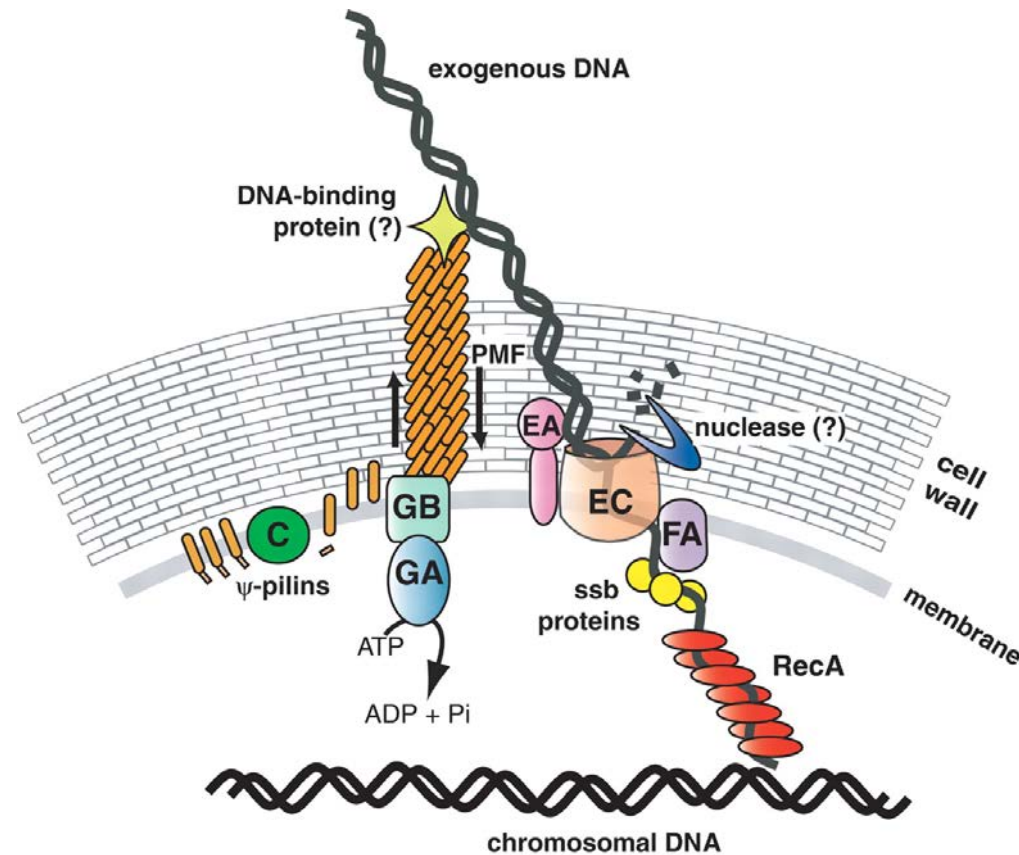


*Some bacteria (not including E. coli) have evolved specialized systems for taking up naked DNA from the environment*

*Includes Neisseria, Bacillus, Streptococcus*

***Transformation in B. subtilis -- note RecA “delivering” ssDNA to the chromosome***

**Fig. 2.** DNA uptake during transformation in *B. subtilis*. The uptake machinery is preferentially located at the cell poles. The  $\psi$ -prepilins are processed by the peptidase and translocate to the outer face of the membrane. With the aid of the other ComG proteins, the major  $\psi$ -pilin ComGC assembles into the  $\psi$ -pilus, which attaches exogenous DNA via a hypothetical DNA binding protein. Retraction of the  $\psi$ -pilus, driven by the proton motive force, and DNA binding to the receptor (ComEA) are required to transport one strand of DNA through the membrane channel (ComEC) while the other is degraded by an unidentified nuclease. The helicase/DNA translocase (ComFA) assists the process, along with ssDNA binding proteins that interact with the incoming DNA. RecA forms a filament around the ssDNA, and mediates a search for homology with chromosomal DNA. ADP, adenosine diphosphate; Pi, inorganic phosphate; PMF, proton motive force; ssb, single-stranded DNA binding protein. [View Larger Version of this Image (218K IPEG file)]



***Transformation:* active uptake of free DNA by bacterial cells and the heritable incorporation of its genetic information.**

***Transformation* occurs when DNA from a donor cell is present in the growth medium (or the environment) and is taken up by a recipient cell**

**For example, DNA released by dead bacterial cells can be taken up by a live bacterial cell and incorporated into its chromosome by *homologous recombination***

**WHY?**

- **maybe nutrition?**
  - as a way to acquire nucleic acids that can be broken down and recycled for the cell's use
- **maybe repair of damage DNA?**
  - use stretches of DNA to replace defective DNA in host chromosome  
-- a way to patch up deteriorating DNA
- **maybe to increase genetic variability?**
  - requires homologous recombination for the stable acquisition of new genetic material whereas plasmid transfer via conjugation does not

# The Maintenance of Sex in Bacteria Is Ensured by Its Potential to Reload Genes

Gergely J. Szöllsi\*, Imre Derényi\*<sup>1</sup> and Tibor Vellai

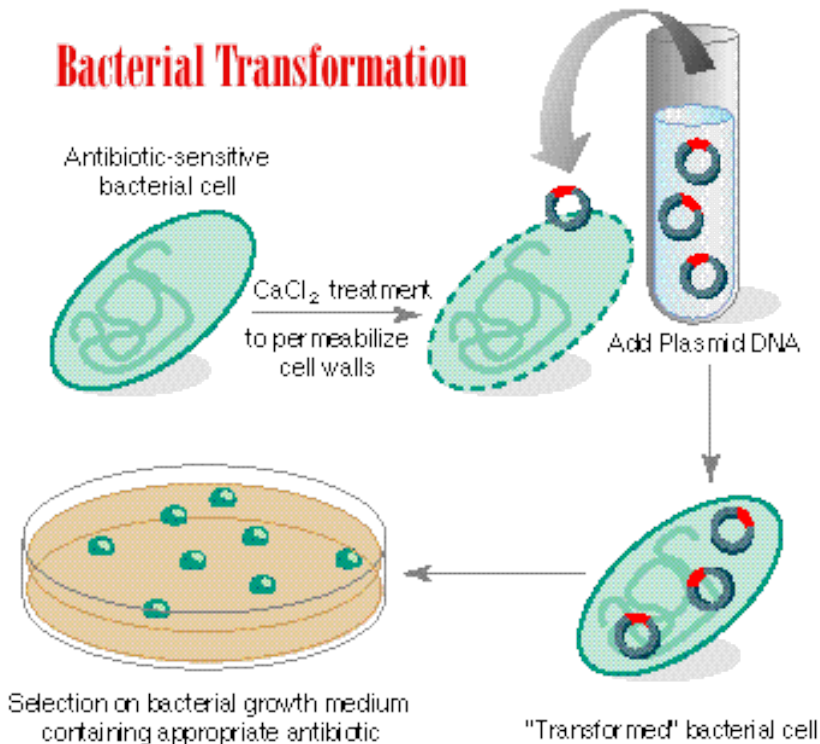
\* Department of Biological Physics and Department of Genetics, Eötvös University, H-1117 Budapest, Hungary

Why sex is maintained in nature is a fundamental question in biology. Natural genetic transformation (NGT) is a sexual process by which bacteria actively take up exogenous DNA and use it to replace homologous chromosomal sequences. As it has been demonstrated, the role of NGT in repairing deleterious mutations under constant selection is insufficient for its survival, and the lack of other viable explanations have left no alternative except that DNA uptake provides nucleotides for food. Here we develop a novel simulation approach for the long-term dynamics of genome organization (involving the loss and acquisition of genes) in a bacterial species consisting of a large number of spatially distinct populations subject to independently fluctuating ecological conditions. Our results show that in the presence of weak interpopulation migration NGT is able to subsist as a mechanism to reload locally lost, intermittently selected genes from the collective gene pool of the species through DNA uptake from migrants. Reloading genes and combining them with those in locally adapted genomes allow individual cells to readapt faster to environmental changes. The machinery of transformation survives under a wide range of model parameters readily encompassing real-world biological conditions. ***These findings imply that the primary role of NGT is not to serve the cell with food, but to provide homologous sequences for restoring genes that have disappeared from or become degraded in the local population.***



## Few species of bacteria can be “naturally” transformed

A routine step in gene cloning requires transformation of *E. coli* with plasmid DNA. The *E. coli* cells must be traumatized before they will take up the DNA



*The stable acquisition of a plasmid does not require any recombination events, since, by definition a plasmid can replicate independently of the bacterial chromosome*

*Standard cloning procedures in molecular lab involved the transformation of intact plasmids into *E. coli**



***Pathogenicity genes are often coded on plasmid or phage genes***

***Nature News 6/14/11: Phage on the Rampage***

<http://fire.biol.wvu.edu/trent/trent/PhagerampageNatureNews.pdf>

Phage encoded genes code for Shiga toxin in pathogenic *E. coli*

*Another example of fluid gene flow: Phage stole toxin genes from the bacterium Shigella and then proceeded to spread the genes to the genes to other bacteria*

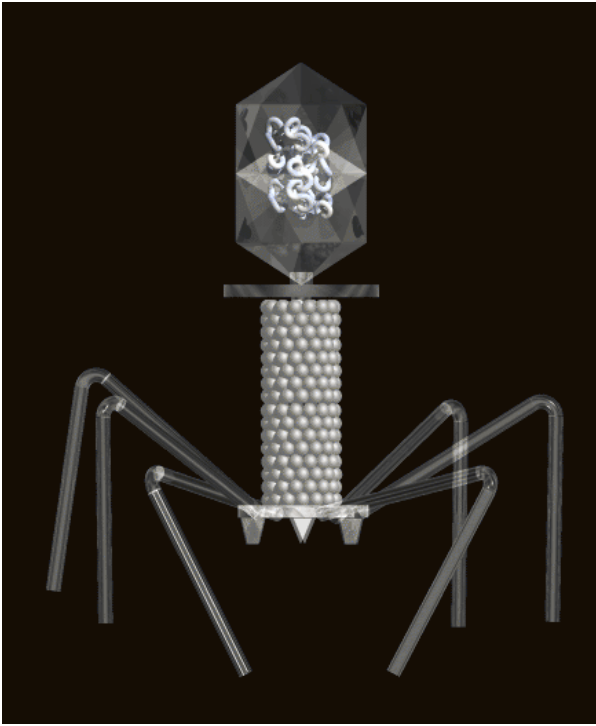
*...”Shiga toxins have been causing diarrheal disease in humans for centuries — the bacterial genus Shigella and the Shiga toxins were first named for Kiyoshi Shiga, a Japanese medical doctor who identified the bacterium during an outbreak of dysentery in Japan in 1897. According to Allison, Shiga-toxin producing phage probably picked up the genes encoding Shiga toxin from these bacteria, and since the 1980s have been spreading these virulent genes to other bacteria, including many strains of E. coli.”*

<http://micro.magnet.fsu.edu/primer/java/scienceopticsu/powersof10/>

## **SOME THOUGHTS:**

**Science 290: 673 2000**

- **There are at least  $10^{30}$  bacteria on the planet, and there are likely to be at least as many bacterial viruses or bacteriophages attacking them.**
- **Given this sheer weight of numbers, the predatory activities of phages should have global significance.**
- **Phages are also useful tools for gene transfer, and predicting their behavior under natural conditions is important**



**The first genomic analysis of human feces reveals that our guts are teeming with 1,200 different viruses -- more than half of which were previously unknown to science**

**Most of these viruses are bacteriophage (which prey on the 500 different types of bacteria that digest our food and regulate the health of our bowels)**

|   |  |  |   |
|---|--|--|---|
| <b>F<sup>-</sup></b>  | <b>NONE</b>  | <b>NOTHING</b>   | <b>NOTHING</b>                                |
| <b>F<sup>+</sup></b>  | <b>CYTOPLASMIC</b><br>(F factor integrated in 1/1,000 cells)                                 | <b>F FAC TOR</b>   | <b>CHROMOSOMAL GENES (VERY LOW FREQ)</b>      |
| <b>F<sup>'</sup></b>  | <b>CYTOPLASMIC CARRIES A BIT OF THE BACTERIAL CHROMOSOME</b>                                 | <b>F FACTOR AND BIT OF BACTERIAL CHROMOSOME</b>                | <b>MOST CHROMOSOMAL GENES (VERY LOW FREQ)</b> |
| <b>Hfr</b><br>→ <i>an Hfr strain is a laboratory artifact</i> | <b>integrated in all cells</b><br>(site of integration varies from Hfr strain to Hfr strain) | <b>CHROMOSOMAL GENES (ESPECIALLY NEAR SITE OF INTEGRATION)</b> | <b>F FACTOR (VERY LOW FREQ)</b>               |

**F<sup>+</sup> X F<sup>-</sup> cross:** Many F<sup>-</sup> cells converted to F<sup>+</sup>; rarely: some bacterial genes transferred to F<sup>-</sup> cells from rare cells with an integrated F factor

**F<sup>'</sup> X F<sup>-</sup> cross:** Many F<sup>-</sup> cells converted to F<sup>'+</sup>; rarely: some bacterial genes transferred to F<sup>-</sup> cells from rare cells with an integrated F factor

**Hfr X F<sup>-</sup> cross:** Bacterial genes transferred (from a fixed point in the chromosome in a fixed order) to F<sup>-</sup> cells at a high frequency. Order of gene transfer varies from strain to strain depending on where the F factor has integrated. Few F<sup>-</sup> converted to F<sup>+</sup> because the F factor genes are the last to be transferred in any Hfr strain and most mating pairs don't stay together long enough for the entire chromosome to be transferred.