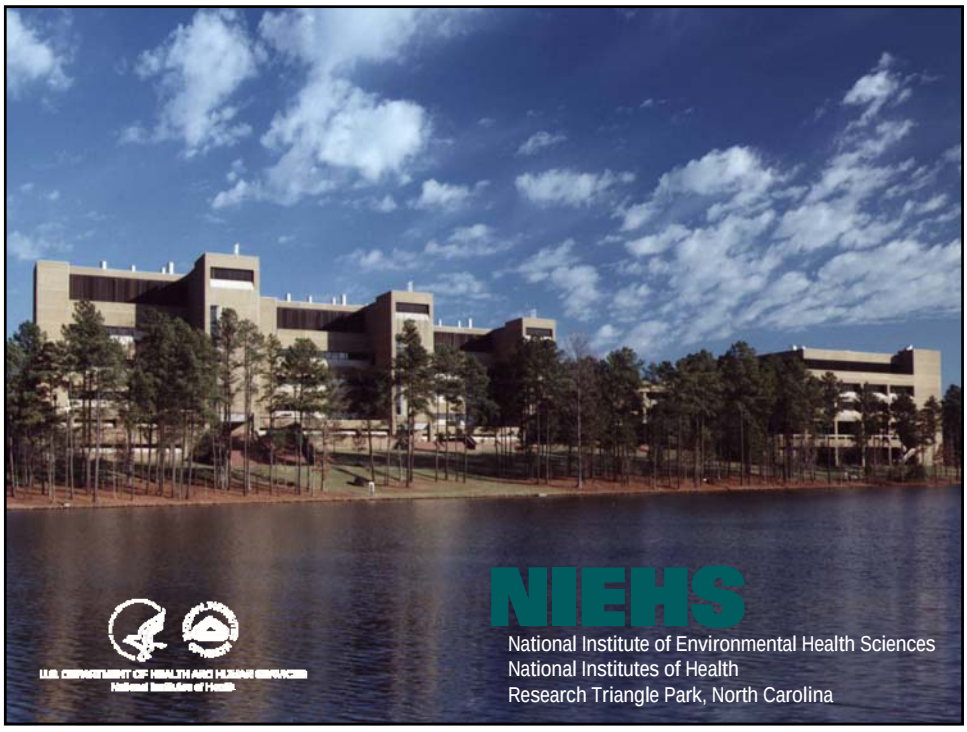
DNA REPAIR INTEREST GROUP VIDEOCONFERENCE

Tues May 15, 2012

Dr. Jan Drake . NIEHS .

History of DNA Repair - Anecdotal Observations on the Origins of Mutation Research

[Origin: NIEHS]



This NIEHS portrait conceals two limits to an otherwise often idyllic house of science:

1. The water in the plains of NC is *not* blue,

2. We are in much the same funding situation as the rest of the NIH, with debilitating cuts even to outstanding programs.

These anecdotes are based largely on my happenchance personal experiences.

More historical and anecdotal stories are due to appear this fall in the autobiographical sketches now appearing monthly in the journal *DNA Repair*.

In a book entitled *Eugenics, Genetics, and the Family*,
Herman Muller contemplates
the state of mutation research in 1923:

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Herman Muller contemplates
the state of mutation research in 1923:

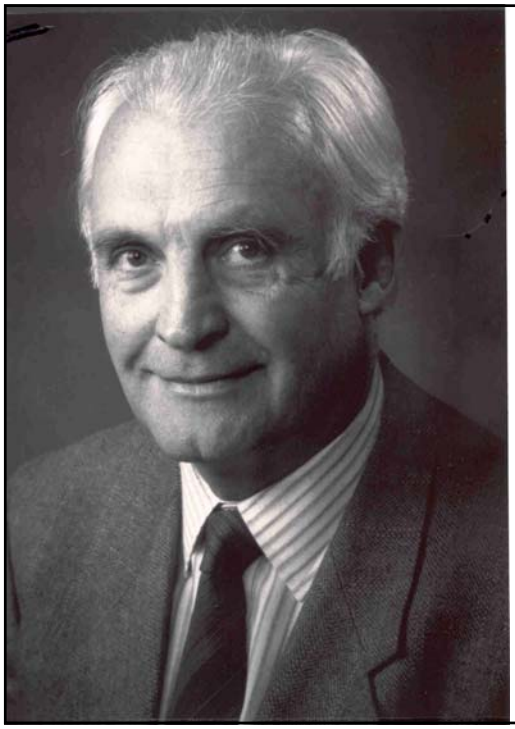
"Beneath the imposing structure
called Heredity, there has been
a dingy basement called Mutation."

The way it used to be:

I was due to take up graduate studies in embryology at a Midwestern university when Don Poulson, who had taught my undergraduate genetics course at Yale, stopped me in the hall one day to tell me that he had phoned up George Beadle and arranged for me to attend Caltech instead.

I asked, "what's Caltech"?

This soon led me partway into the young world of molecular biology.



From: *Ernst Freese (1925-1990)*.
Mutation Research 251: 165-169.

Soon Ernst Freese made his appearance at Caltech.
Ernst was a large, gentle man whose obit I wrote many years later. One of his interests was 5-bromouracil mutagenesis in phage T4, that had been recently discovered by Rose Litman. He noticed that T4 mutants induced by the base analogues 5-bromouracil and 2-aminopurine were generally also reverted by the same agents, whereas many spontaneous and most acridine-induced mutants were not reverted by base analogues.

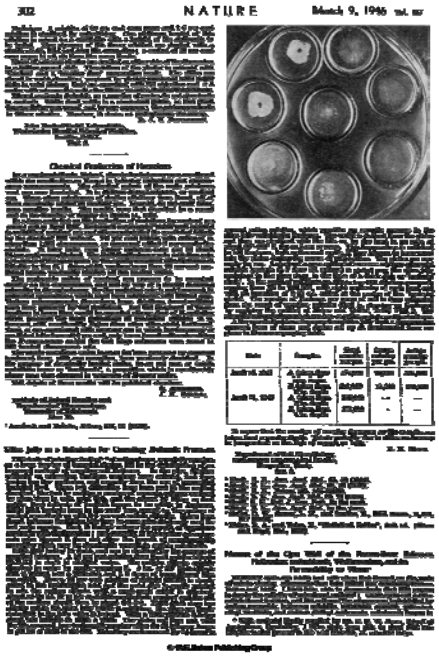
• He proposed that the molecular pathway promoted by these base-analogues involved purine-pyrimidine mispairs, whereas the other pathway involved purine-purine or pyrimidine-pyrimidine mispairs. Having been recruited from the physics community by Max Delbrück, Ernst chose the names "transitions" and "transversions" for these two kinds of base substitutions.

• Soon, however, Sydney Brenner, Francis Crick, Leslie Barnett and Leslie Orgel realized that the second pathway probably consisted of skipped or doubled bases, which led them directly to their proof of the triplet nature of the genetic code. Even today, no agent is known that *specifically* induces transversions.

From:
Charlotte Auerbach (1899-1994).
GENETICS 141: 1-5.

Next, a little about Charlotte Auerbach.
Until the early 1940s, there had been no convincing demonstration of chemical mutagenesis despite many reports. Lotte had fled Germany and managed to settle in Edinburgh, where Herman Muller visited and suggested to her that mustard gas be checked using his Drosophila sex-linked recessive method.

Lotte had excellent success but her papers were embargoed until after the war.


302 NATURE March 9, 1946 vol. 157

Auerbach, C., and J. M. Robson (1946)

1946: Chemical production of mutations. Nature 157:302.

1947: The production of mutations by chemical substances. Proc. Roy. Soc. Edinburgh B: 62: 271-283.

Lotte was also the first person to resolve a long-standing debate about whether the "resting" gene could mutate spontaneously.

Auerbach, C. (1959)
Spontaneous mutations in dry spores of *Neurospora crassa*.
Zeitsch. Vererbungslehre 90: 335-346.

She did this using dry spores of *Neurospora crassa*, and I followed a few years later using phage T4.

Auerbach, C. (1959)
Spontaneous mutations in dry spores of *Neurospora crassa*.
Zeitsch. Vererbungslehre 90: 335-346.

Drake, J. W. (1966)
Spontaneous mutations accumulating in bacteriophage T4
in the complete absence of DNA replication.
Proc. Natl. Acad. Sci. USA 55: 738-743.

I came to know Lotte both at meetings in the 1960s and during a sabbatical year in Edinburgh. She was passionate about keeping in mind non-conforming or opaque phenomena,
• She knew the entire classical literature on mutagenesis, and
• She disliked what she viewed as oversimplifications by molecular biologists.
• I countered that early steps in exploring virgin territory needed to concentrate on central questions and that paradoxes and nonconforming results often had to be put on hold for later clarification.

To that end, I stressed how

• François Jacob, André Lwoff and Jacques Monod had at first not even mentioned several mutants whose properties seemed at odds with their operon model. In another example,

François Jacob, David Perrin, Carmen Sanchez, and Jacques Monod (1960)

The operon: a group of genes whose expression is coordinated by an operator.

Comptes Rendus des Séances de l'Academie des Sciences 250: 1727–1729.

In another example,

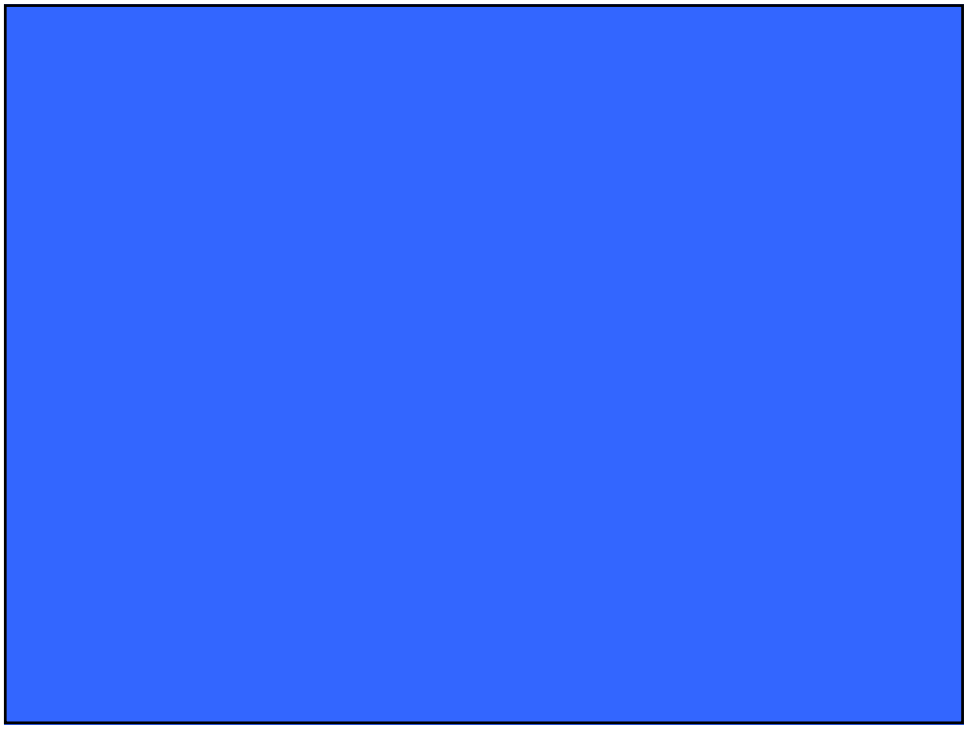
• Francis Crick and Sydney Brenner, in their first triplet-codon paper,
said hardly anything about anomalies such as T4 *rII* frameshift mutants that could be reverted by base analogues, although they explained these in detail in a later paper.

F. H. C. Crick, L. Barnett, S. Brenner, and R. J. Watts-Tobin (1961)
General nature of the genetic code for proteins.
Nature 192: 1227-1232.

In both cases, when the anomalies were clarified, they and were found to enhance rather than to detract from the original conclusions.

F. H. C. Crick, L. Barnett, S. Brenner, and R. J. Watts-Tobin (1961)
General nature of the genetic code for proteins.
Nature 192: 1227-1232.

L. Barnett, S. Brenner, F. H. C. Crick, R. G. Shulman and R. J. Watts-Tobin (1967)
Phase-shift and other mutants in the first part of the *r*II B cistron of bacteriophage T4
Phil. Trans. Roy. Soc. London, Ser. B 252: 487-560.



Because of her passion for unexpected or bizarre results, Lotte once listed a number of instances in which mutation experiments produced misleading and/or mystifying results.

Here I will show some examples from work by me or colleagues. The most frequent problem is altered mutant detectibility resulting from unrecognized environmental aberrations or from epistatic interactions with background mutations.

•The first example comes from spontaneous mutation in extracellular T4 particles, which turned out to be driven by two different mechanisms, both acting on G:C base pairs.

The first generated transition mutations by converting T4 5-hydroxy-methyl-cytosines to hydroxy-thymines and was simply an early example of the now famous cytosine-deamination pathway.

Baltz, R. H., P. M. Bingham and J. W. Drake (1976)
Heat mutagenesis in bacteriophage T4: the transition pathway.
Proc. Natl. Acad. Sci. USA 73: 1269-1273.

•The second process was deduced to involve a modification of guanine leading to G·C → T·A transversions.

Baltz, R. H., P. M. Bingham and J. W. Drake (1976)
Heat mutagenesis in bacteriophage T4: the transition pathway.
Proc. Natl. Acad. Sci. USA 73: 1269-1273.

Bingham, P. M., R. H. Baltz, L. S. Ripley and J. W. Drake (1976)
Heat mutagenesis in bacteriophage T4: the transversion pathway.
Proc. Natl. Acad. Sci. USA 73: 4159-4163.

Kricker, M. C., and J. W. Drake (1990)
Heat mutagenesis: another walk down the transversion pathway.
J. Bacteriol. 172: 3037-3039.

• Curiously, the rate of this reaction dropped sharply in the late 1970s when we moved from Illinois to North Carolina.

One day I was back in Urbana to give a seminar when the new department head called me in for a chat. He mentioned that the excellent director of the glassware and media center had just retired and had come in on his last day with a confession (glass still versus metal still). Unfortunately, the metal still had been decommissioned some time ago . . .

Baltz, R. H., P. M. Bingham and J. W. Drake (1976)
Heat mutagenesis in bacteriophage T4: the transition pathway.
Proc. Natl. Acad. Sci. USA 73: 1269-1273.

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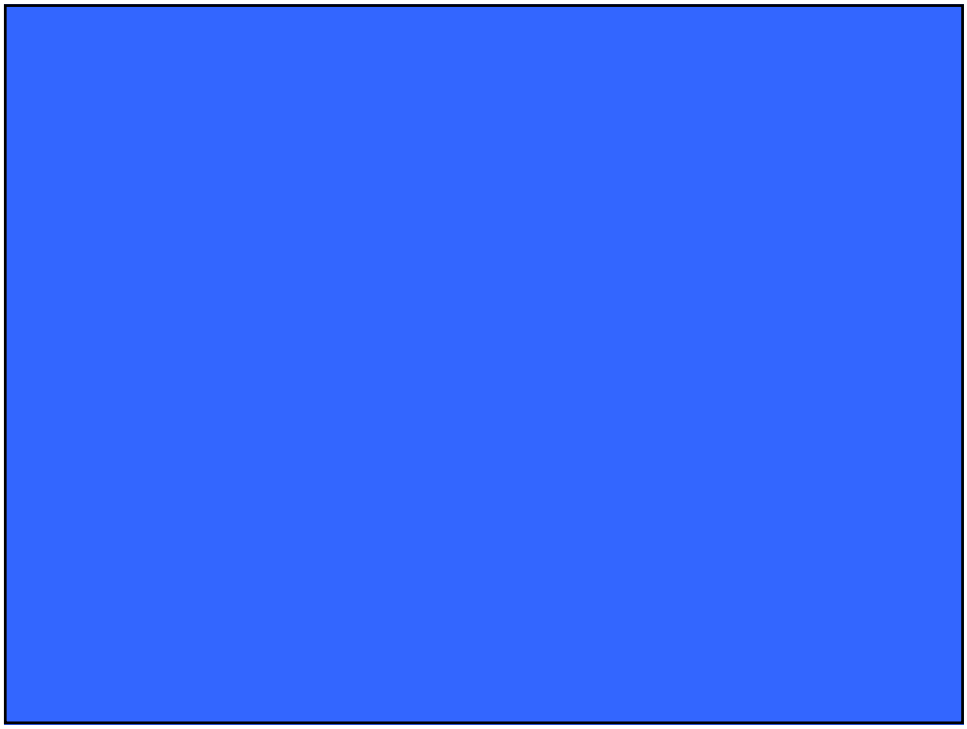
Drake, J. W., and L. A. Smith (1999)
Some puzzling observations on heat-induced
transversion mutagenesis in bacteriophage T4.
J. Genet. 78: 3-5.

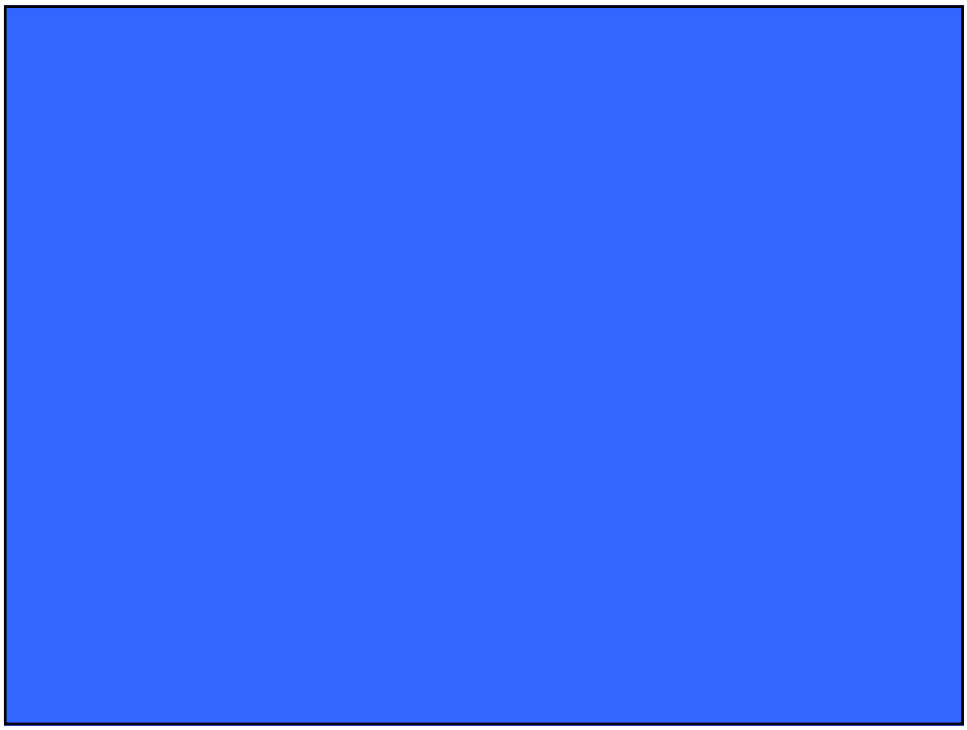


•**Nancy Nossal described the phenotypes of phage T4 RNaseH null mutations. Because a defective cognate gene in yeast results in a curious mutator phenotype, we asked whether T4 *rnh* mutants were mutators. We scored total T4 *r* mutants, a roughly 4-kb mutational target. We were amazed to find a three-fold *decreased* mutant frequency. The reason was soon found to be that *rII* mutants, about 2/3 of all T4 *r* mutants, simply lost their dramatic large-plaque phenotype in the presence of the *rnh* mutation.**

Bebenek, A., L. A. Smith and J. W. Drake (1999)

Bacteriophage T4 *rnh* (RNase H) null mutations: effects on spontaneous mutation and epistatic interaction with *rII* mutations.

J. Bacteriol. 181: 3123-3128.

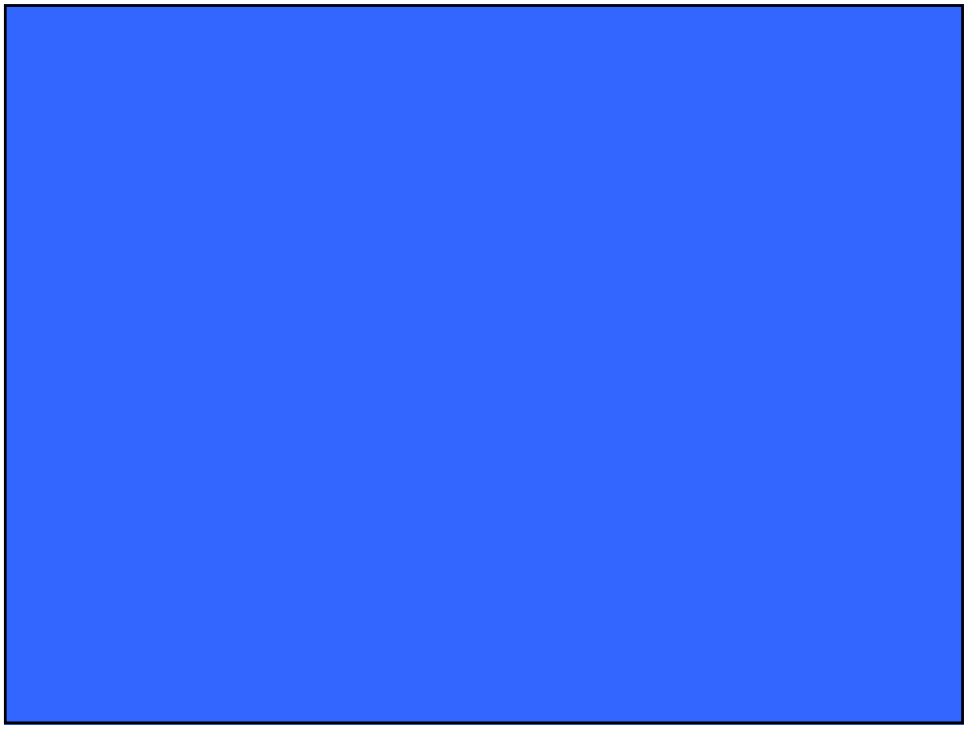


• Roel Schaaper has often studied *E. coli* antimutator mutants. Such a mutation described by Joe Speyer displayed a very large decrease in mutations to valine resistance. This was a crucial result because valine resistance is a very large mutational target. However, Roel found that the decrease simply reflected the one-day delayed growth of the mutants on the selection plates.

Schaaper, R. M., and R. L. Dunn, 2001

The antimutator phenotype of *E. coli mud* is only apparent and results from delayed appearance of mutants.

Mutat. Res. 480-481: 71-75.



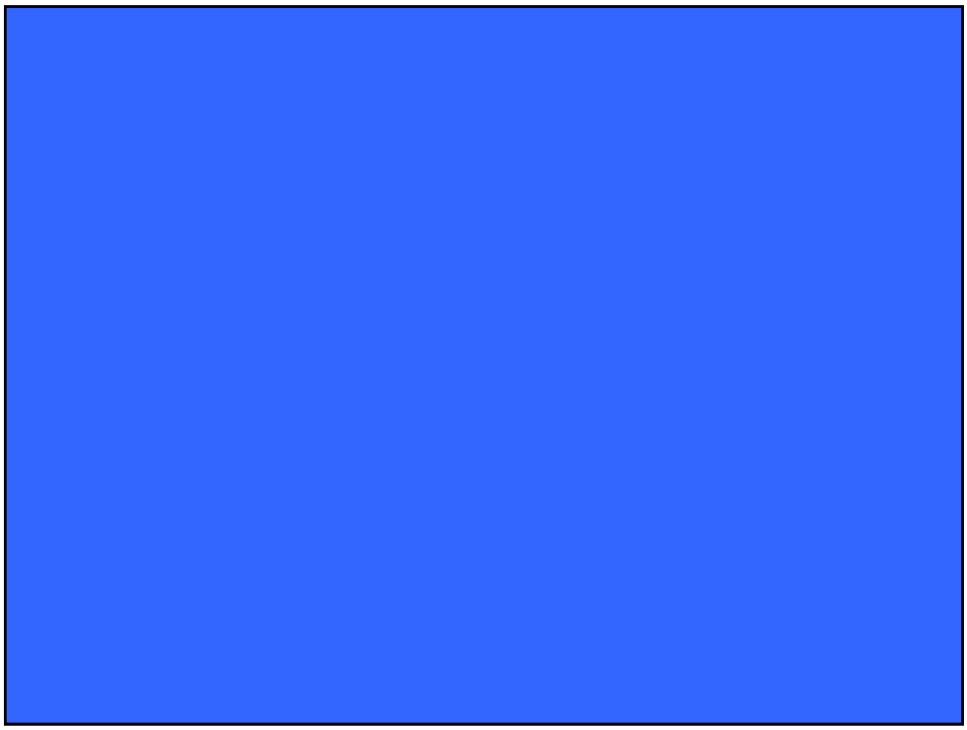
Hoping to find a mutator DNA polymerase that preferentially produced transversions, we screened many phage T4 ts DNA polymerase mutants for their frequencies of *r* mutants. However, the first thing we noticed was that a number of the TS mutants displayed *reduced* frequencies of r mutants. When we switched to reversion tests, however, only a few of these polymerase mutants were real antimutators, and antimutators were also found among mutants with *normal* frequencies of *r* mutants. The mutants with lower frequencies of *r* mutants just made small plaques, among which *r* mutants are difficult to see.

• Although our mutants that *did* score as antimutators in reversion tests, the effect was very pathway-specific: transitions at A·T base pairs could be sharply reduced with little effect on other pathways. When we did very careful forward-mutation tests, none of the mutants were general antimutators, and some were even weak mutators. We argued that general antimutators were mechanistically improbable and would in any case carry a large fitness cost.

Drake, J. W. (1993)

General antimutators are improbable.

J. Mol. Biol. 229: 8-13.



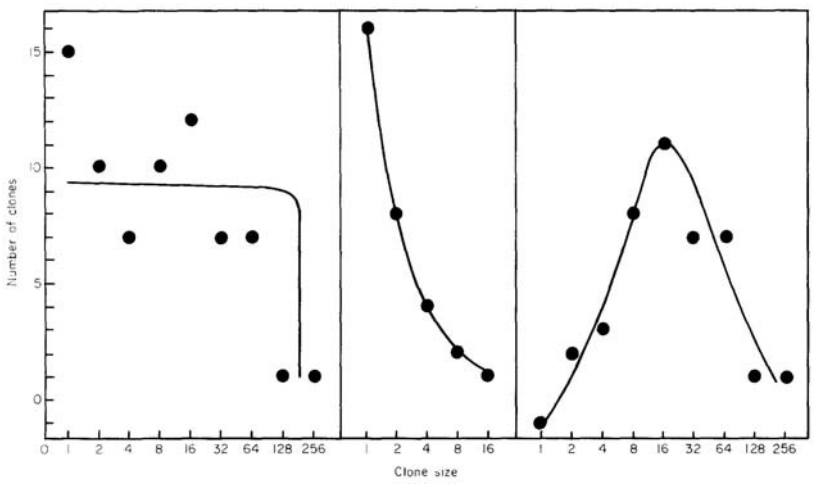
In the early days of chemical mutagenesis, the chemists said that EMS methylated the N-7 position of guanine, making an adduct that mostly blocked DNA synthesis but sometimes mispaired. In the early 1960s, a PNAS paper described the distribution of mutant clone sizes from EMS-treated T4 particles. Frequent mispairing should produce clones clustering around half of the burst size, whereas rare mispairing should produce a flat distribution. The latter was observed. However, the chemists soon changed their minds, saying that the mutagenic culprit was alkylation of guanine O-6. For years I wondered how the T4 experiment went wrong. Although many small problems could be found, none were lethal.

• One summer I was doing a Drosophila mutation experiment at Sussex University and living in an otherwise empty student dorm. A free day arrived, so I decided to have another go at the problem, this time with the help of an adjuvant. I bought a bottle of an excellent single-malt Scotch and started sipping at about noon.

Suddenly, at about 2 pm, the elephant in the living room trumpeted. The authors had used an EMS dose that increased the mutant frequency hugely, so that the spontaneous background could be ignored. Wrong! The induced mutant clones were large, while most spontaneous clones were small. Thus, the factor of increase in clones was only several-fold.

An hour or so of regenerating the expected clone-size distribution for the spontaneous mutations yielded a plot that, when subtracted from the observed data, yielded the observed distribution.

However, I have forgotten the name of the specific adjuvant.



Drake, J. W. (1988) Clone size distributions of mutations induced by ethyl methanesulfonate in bacteriophage T4. J. Mol. Biol. 202: 11-16.

My favorite running title

Bebenek, A., H. K. Dressman, G. T. Carver, S. Ng, V. Petrov,
G. Yang, W. H. Konigsberg, J. D. Karam and J. W. Drake (2001)

Interacting fidelity defects in the replicative
DNA polymerase of bacteriophage RB69.

J. Biol. Chem. 276: 10387-10397.

Bebenek, A., H. K. Dressman, G. T. Carver, S. Ng, V. Petrov, G. Yang, W. H. Konigsberg, J. D. Karam and J. W. Drake (2001)

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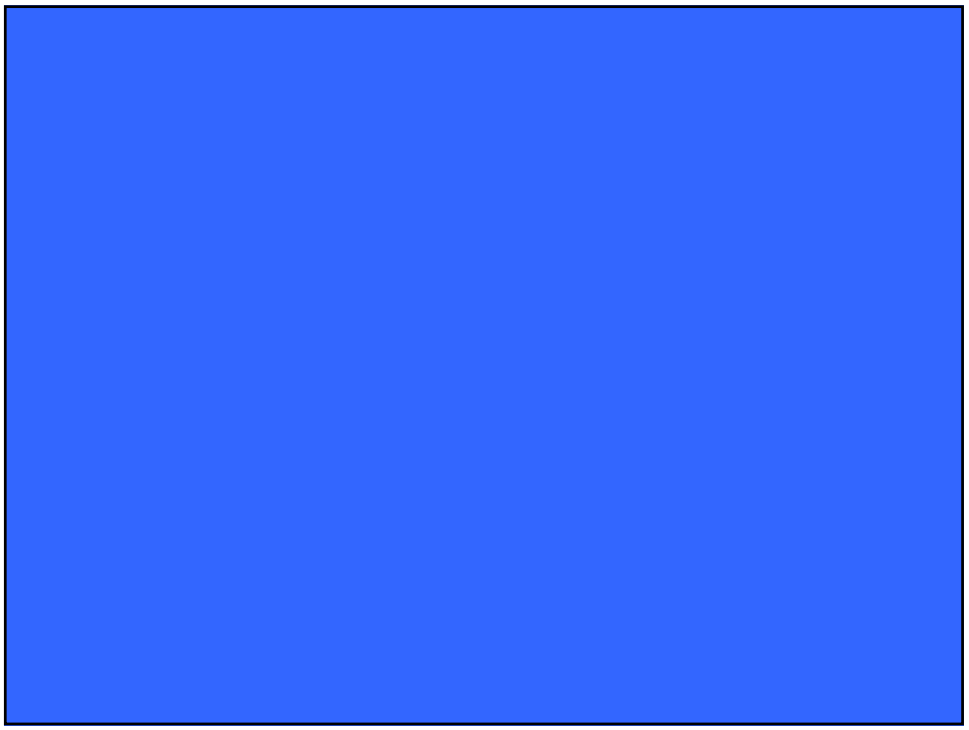
In Vivo Veritas



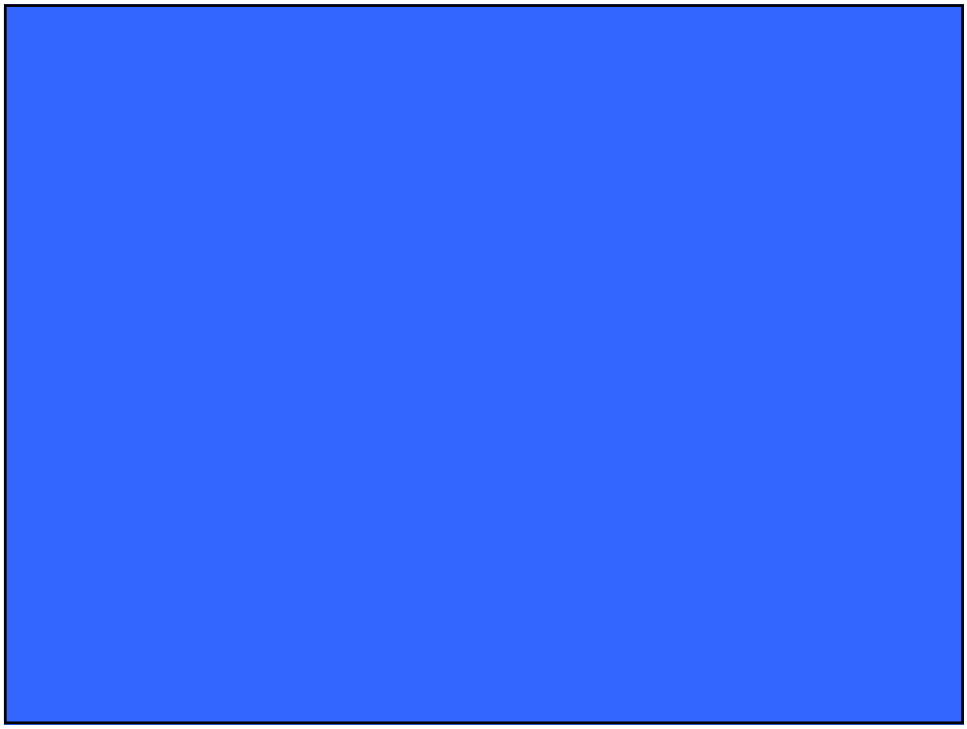
Next, two little stories from Madison and its environs

• Seymour Abrahamson worked for several decades on spontaneous and induced mutation in Drosophila. He also taught a heavily attended course on genetics for non-scientists. One semester, a very pregnant woman in the front row seemed to look more and more puzzled as some detail of meiosis were laid out. Finally she raised her hand and asked a question that revealed to Seymour that she had completely missed a vital point. He responded immediately and without forethought, exclaiming that he "was afraid that you are laboring under a misconception"

The resulting chaos ended the lecture for that day.

"I'm afraid you are laboring

under a misconception."



• Seymour and I, and especially Jim Crow, frequently worked together in the formative years of genetic toxicology. One day, my excellent secretary in North Carolina, a warm and caring black lady, received a phone call asking for me. She asked who was calling and received the response, “Jim Crow”. “Oh, yes?” she replied coolly.

Some years later, she and her mother served up a traditional North Carolina soul dinner to a committee chaired by Jim, and they shared mutual admirations.

•That same committee usually met in a very formal room in the National Academy of Sciences, with about 15 people sitting around a long table. Jim would lean back in his chair at the end of the table and let the jabbering go on for a while, and then summarize and move us along. One day, after a rather long jabber, Jim leaned too far and the chair flipped backwards. Jim followed smoothly with a backwards summersault, landed in a crouched position, rose smoothly to a standing position, and righted and reoccupied his chair. He was 66 years old at the time. The committee's already high respect for him instantly doubled.

I have sometimes wondered whether his expertise with the viola played some role in this athleticism.

Finally, two mysterious segregation patterns

• Early in her career, Evelyn Witkin tried to confirm the implications of the Watson-Crick scheme in the segregation of new mutations in *E. coli*. She grew the cells in the presence of 5-BU for one generation and then plated on dye-indicator plates to score Lac$^-$ mutants as white sectors. She also determined the distribution of nuclear bodies. *All* of the mutant sectors could be *fully* explained by the number of nuclear bodies. Thus, the mutants seemed to appear almost immediately as homoduplexes.

Witkin, E. M., and N. A. Sicurella (1964)

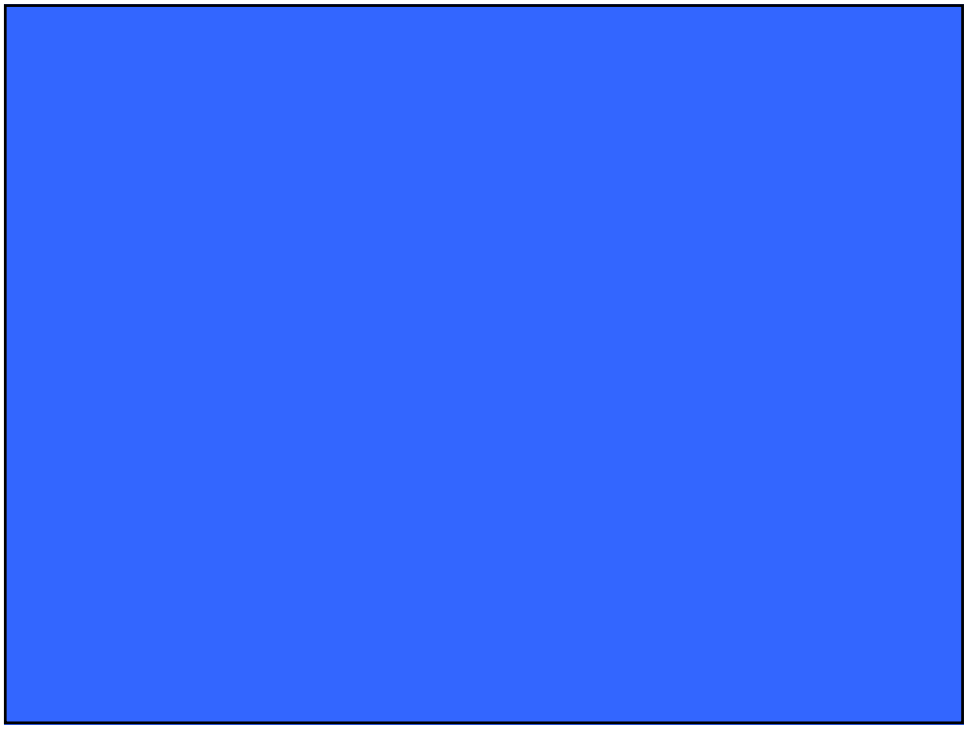
Pure clones of lactose-negative mutants obtained in *Escherichia coli*

after treatment with 5-bromouracil.

J. Mol. Biol. 8: 610-613.

Evelyn postulated a repair process and repeated the experiment with a strain lacking excision repair, but with the same result.

Nowadays, this work suggests that many newly detected mutations were mispairs that were incorrectly processed by MMR. This result would introduce an extra factor of 2 into the classical mathematical analysis of rates of spontaneous mutation. Perhaps one of you will now do the right experiment.



Azotobacter vinelandii **was a favorite model organism before it was overtaken by *E. coli*, but it is still studied because it is an important nitrogen-fixing soil bacterium. Its genome is an unremarkable 5.364 mbp circle**

Setubal, J. C., and 43 others (2009)
Genome sequence of *Azotobacter vinelandii*, an obligate aerobe specialized to support diverse anaerobic metabolic processes.
J. Bacteriol. 191: 4534-4545.

but it seems to carry a remarkable 40-80 genomes per cell.

Setubal, J. C., and 43 others (2009)
Genome sequence of *Azotobacter vinelandii*, an obligate aerobe specialized to support diverse anaerobic metabolic processes.
J. Bacteriol. 191: 4534-4545.

Nagpal, P., S. Jafri, M. A. Reddy and H. K. Das (1989)
Multiple chromosomes of *Azotobacter vinelandii*.
J. Bacteriol. 171: 3133-3138.

But see Pulakat, L., S.-H. Lee and N. Gavini (2002)
Genome of *Azotobacter vinelandii*: counting of chromosomes by utilizing copy number of a selectable genetic marker.
Genetica 115: 147-158.

• However, a number of experiments indicate that it segregates new mutations and it is easy to recover new mutations in an apparently homozygous state, so that it behaves more as an almost monogenomic organism.

Maldonado, R., A. Garzon, D. R. Dean and J. Casadesús (1992)
Gene dosage analysis in *Azotobacter vinelandii*.
Genetics 132: 869-878.

Pulakat, L., E. T. Efuet and N. Gavini (1998)
Segregation pattern of kanamycin resistance marker in *Azotobacter vinelandii*
did not show the constraints expected in a polyploid bacterium.
FEMS Microbiol. Lett. 160: 247-252.

Suh, M.-H., L. Pulakat and N. Gavini (2001)
Isolation and characterization of *nifDK*::kanamycin and nitrogen fixation proficient
Azotobacter vinelandii strain, and its implication on the status
of multiple chromosomes in *Azotobacter*.
Genetica 110: 101-107.

Now, if you are going to tote around 60 or so genomes, you will almost never be able to profit from an adaptive recessive mutation, and purifying selection may be very inefficient.

There is an obvious solution to this problem provided you can sense when a new mutation has just appeared. If this solution is used here, it may well be used elsewhere when only several genomes are present Such a mechanism would certainly roil the waters of many analyses of mutation rates and prokaryotic evolution.

"The capacity to blunder slightly is the real marvel of DNA. Without this special attribute, we would still be anaerobic bacteria and there would be no music."

Lewis Thomas (1974)
The Lives of a Cell: Notes of a Biology Watcher