

Carcinogens are Mutagens: Their Detection and Classification

by Bruce N. Ames*

We detect mutagens by using a special set of bacterial tester strains. We have described our test system for mutagen and carcinogen detection in a series of recent papers. I will give here only a summary of some of these results, omitting references to other work, which is credited in our original papers.

A simple procedure for combining human (or rat) liver for carcinogen activation and the bacteria for detection and classification is used. We show that a wide variety of carcinogens (aflatoxin, benzpyrene, acetylaminofluorene, etc.) are frame shift mutagens after activation and can be detected with these strains. We explain the structure of these carcinogens on the basis of the theory of frameshift mutagenesis. We postulate that carcinogens cause cancer by somatic mutation and suggest what the combined bacteria/liver system be used as a simple procedure for carcinogen detection.

Construction of Strains for Testing Mutagens

During the last several years we have developed a set a tester strains of *Salmonella typhimurium* for detecting mutagens (1-4). This system is supersensitive and simple to use. The principle of the tester strains is to use mutants caused by a known type of DNA damage (base-pair substitutions, and the various kinds of frameshift mutations)

for detecting mutagens by the highly sensitive and convenient back mutation test (reversion to prototrophy). We have introduced a deletion of one of the genes of the excision repair system in all of our tester strains, and this has made them hundreds of times more sensitive to most mutagens. We have made a great improvement in the sensitivity of the four tester strains by introducing into each strain a mutation which eliminates the lipopolysaccharide which coats the surface of these bacteria and acts as a partial barrier to the penetration of compounds to the bacterial membrane. This is especially important for large compounds.

A bacterial test system has many practical and theoretical advantages in the detection of mutagens, among which are the small genome (about 4×10^6 base pairs), the large number of organisms exposed (about 10^9 /plate), and the positive selection of the mutated organisms. The set of *Salmonella* tester strains has three additional advantages: the lack of excision repair, the loss of the lipopolysaccharide barrier, and the scoring of mutations in "hot spots" for frameshift mutagenesis (e.g., the CGCGCGCG sequence in tester strain TA1538). The scoring of reversion in easily mutated "hot spots" combined with the smallness of the genome aid the test by maximizing reversion relative to killing. Our set of tester strains for detecting frameshift mutagens is not yet complete, and we are attempting to complete the theoretically expected set of frameshift

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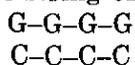
tester strains containing different repetitive sequences in DNA.

We have also developed new methodology (1) for testing mutagens by forward mutagenesis using strains lacking the excision repair system and the lipopolysaccharide as an adjunct to our set of tester strains. We also compare (1) the zones of inhibition on two strains both of which lack the lipopolysaccharide and one of which lack repair.

Use of These Strains for Detecting Carcinogens

Identification of Activated Carcinogens as Frameshift Mutagens

It has long been known that many simple alkylating agents, as well as radiation (ultraviolet, x-rays, etc.) are both carcinogenic and mutagenic, and we have shown that a variety of these agents can be detected with our tester strain that detects base pair substitutions (1-3). We have found that a large variety of chemical carcinogens can also be detected as mutagens with our frameshift mutation tester strains. We have identified these carcinogens as members of a special class of mutagens: reactive frameshift mutagens. An addition or deletion of a base pair from the DNA (a frameshift mutation) can occur when there is a shifted pairing in a repetitive string of nucleotides, e.g.,



during DNA replication. This process is enormously increased by the addition of an acridine type compound that intercalates in the DNA base pair stack and stabilizes the shifted pairing. A number of years ago we discovered that when an intercalating agent (an acridine) also has a side chain that can react with DNA (a nitrogen half mustard) it is a more potent mutagen for causing frameshifts by one to two orders of magnitude (4,5).

Polycyclic hydrocarbons intercalate in DNA, and it is thought that polycyclic hydrocarbons are metabolized in mammals to the epoxides, which are believed to be the pri-

mary carcinogens. We have shown that epoxides of the carcinogenic polycyclic hydrocarbons are extremely potent frameshift mutagens (6) of the reactive type.

In addition to these carcinogens, we have shown that metabolites of a variety of aromatic amine carcinogens are frameshift mutagens in our system (7). 2-Nitrosofluorene, a known metabolite of 2-aminofluorene in the rat (and a more effective carcinogen), is thousands of times more effective as a frameshift mutagen than the parent compound. The nitroso group is a reactive group, and we believe, by analogy with the acridine half mustards, that nitrosofluorene is both intercalating and reacting with DNA. Because of this we have tested the nitroso metabolites of a variety of aromatic amine carcinogens and have shown that they are very potent frameshift mutagens (7). Among these compounds are nitrosonaphthalene, nitrosobiphenyl, nitroso-*trans*-stilbene, nitrosoazobenzene, and nitrosophenanthrene. We have also repeated the work of Hartman and shown that 4-nitroquinoline-*N*-oxide, a well known carcinogen, is another member of this class (1).

Detection of Carcinogens and Mutagens: Mammalian Microsomal Hydroxylase

The true or primary carcinogen or mutagen is often a metabolic product of the secondary carcinogen or mutagen that was originally ingested. It is known that the mammalian liver microsomal hydroxylase systems are responsible for activating many classes of carcinogens and mutagens; among these are aflatoxin, aromatic amines, and polycyclic hydrocarbons.

We have now coupled a rat and human liver microsomal hydroxylase system to our tester strains in a simple manner (8). We make a mitochondria-free supernatant preparation from rat (or human) liver and have found that the preparation is stable for months in the freezer. We have worked out conditions for spreading this microsomal system on our Petri plates along with our tester bacteria (the enzyme systems are quite active for a long period of time on our

Petri plates), so that we can test the variety of metabolites that are made by the microsomal system from a putative carcinogen or mutagen. Using this coupled system, we have shown that a large number of carcinogens that are not directly mutagenic can now be detected as mutagens. Among the carcinogens that are metabolized by liver to active frameshift mutagens under these conditions are: aminobiphenyl, amino- and dimethylamino-*trans*-stilbene, 2-amino- and 2-acetylaminofluorene, aminoanthracene, benzidine, aminopyrene, aminochrysene, dimethylbenzanthracene, benzpyrene, and aflatoxin B₁. Malling (9) has shown that dimethylnitrosamine is activated to a product that causes base pair substitution. We believe we can detect all of the major classes or carcinogens by our method.

The *Salmonella* test system can detect carcinogens with great sensitivity. In any system for detecting mutagens one only scores mutations in a small part of the genome. Thus the revertant colonies we see represent only a tiny fraction (10^{-2} to 10^{-4}) of the bacteria mutated. Nevertheless, because of the sensitivity of the tester strains and the potency as mutagens of the activated carcinogens one can detect nanograms of carcinogen: e.g., 0.5 μ g of 2-aminoanthracene gives 11,000 revertant colonies per Petri plate, compared to a control of about 30 colonies (8).

Characterization of Tester Strains

We have made good progress in characterizing our frameshift tester strains (1). One of them has a repetitive G sequence at the site of the mutation, and another has been shown by Isono and Yourno (in preparation) to have a repetitive CGCGCGCG sequence. We are finding that the different carcinogens and mutagens have a great deal of specificity as to the repetitive DNA sequence that they cause mutations in, and we are constructing new frameshift "hot spots" to complete our set of frameshift tester strains.

Carcinogens are Somatic Mutagens

I believe that the simplest interpretation

of our own and other's work on carcinogens as mutagens is that carcinogens cause cancer because of their action as mutagens. I think that the principle of the mutagenesis for a large group of the carcinogens can be explained by the theory of frameshift mutagenesis.

A simple bacterial test can be used to see if a compound is likely to be a carcinogen for humans (mammalian testing is, of course, expensive and takes years). I also think that the prediction of what will be carcinogenic can be put on a more rational basis.

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