MOLECULAR PRINCIPLES UNDERLYING THE Ames SALMONELLA/MICROSOME TEST:
ELEMENTS AND DESIGN OF SHORT-TERM MUTAGENESIS TESTS

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ABSTRACT

The drug resistance plasmid pKM101 has played a critical role in the success of the Ames Salmonella tester strains in detecting carcinogens as mutagens. pKM101 increases the susceptibility of bacterial cells to both point and frameshift mutagenesis by a variety of chemical mutagens and makes them more resistant to killing by ultraviolet light. It does so in a recA+lexA+-dependent fashion and suppresses the mutagenesis and repair deficiencies of Escherichia coli umuC mutants, suggesting that the plasmid codes for protein(s) which participate in the process of "error-prone repair." In the absence of pKM101, Salmonella typhimurium appears to be less proficient than Escherichia coli in its capacity to carry out "error-prone repair"; but if pKM101 is present, both have similar capacities. In addition, much of this capacity to process chemical damage and give mutations is constitutively expressed in pKM101-containing cells. pKM101 is able to increase the frequencies of both transitions and transversions in response to chemical mutagens. Point mutants of pKM101 have been isolated which have concomitantly lost the ability to increase base-pair substitution and frameshift mutagenesis and to protect cells against killing by UV. Recently, we have isolated pKM101 mutants with similar properties by insertion of the translocatable drug resistance element Tn5. All such insertions were shown to map within a ca. 1500 bp region of pKM101. Genetic experiments suggest that at least two pKM101-coded functions are involved in the enhancement of chemical mutagenesis.
INTRODUCTION

The Salmonella/mammalian microsome test developed by Bruce N. Ames and his colleagues at Berkeley (Ames et al., 1975) is currently one of the most widely used, extensively evaluated (McCann et al., 1975; Ames and McCann, 1976), and successful (McCann et al., 1978; H. Bartsch et al., these proceedings) of the short-term tests used to detect potential carcinogens and mutagens. Since this meeting is directed at assessing the current and future possibilities of short-term tests, I feel that it would be appropriate to examine the principles underlying the Ames test with the aims of: (1) delineating the crucial molecular mechanisms playing a role in the success of this system; and (2) focusing attention on some general design features that should be kept in mind in modifying current short-term mutagenesis tests or in constructing new tests (Walker, 1979).

BASIS OF THE AMES TEST

The biological endpoint examined in the Ames test is the reversion of a his\(^-\) mutation in Salmonella typhimurium LT2 to His\(^+\) (Ames, 1971). A histidine auxotroph of S. typhimurium LT2, i.e., a mutant unable to grow in the absence of histidine because of a defect in one of the histidine biosynthetic enzymes, is grown in rich medium and then about 10\(^8\) cells are plated on minimal medium containing just a trace of histidine. There is sufficient histidine in the medium to allow growth to 10\(^9\) cells. At this point, the histidine is exhausted and the bacteria stop growing unless they have reverted to His\(^+\), in which case they grow and form a visible colony. The mutation causing the reversion to His\(^+\) can occur either at the site of the original mutation or it can be a suppressor mutation occurring at a second site. A chemical is mutagenic if it increases the frequency of reversion to His\(^+\). The substance to be tested is either placed in the center of the plate or incorporated into the top agar along with the bacteria, in which case linear dose-response relationships can usually be obtained. Thus the basis of the test is extremely simple. However, its success at detecting carcinogens as mutagens is the result of a series of other features that Ames et al. incorporated into the testing system. These features are discussed below.

Generally speaking, the ultimate success of a short-term mutagenicity test is not necessarily determined as much by the initial choice of a system as by a subsequent series of refinements of that system. If the Ames test consisted simply of examining the reversion of a his mutant of a normal S. typhimurium