Somatic Cells as Indicators of Germinal Mutations in the Mouse

by Liane B. Russell*

In attempts to find a prescreen for mutagens that may induce heritable mutations in mammals, an in vivo somatic mutation test has been developed in the mouse that uses a localized gene product (hair pigment), is relatively fast and cheap, and gives results that have some predictive value for point mutation induction in spermatogonia. Embryos heterozygous at specific coat color loci are exposed to the presumptive mutagen, and 3 weeks later the fur is observed for spots of altered color. It is possible to distinguish spots resulting from expression of the recessive (RS's) from spots having various other causes.

In tests with seven compounds, mutation rates per locus and unit dose have been calculated on the assumption that 175 cells are at risk per 10½-day embryo (a number derived from distribution of spot proportions). These rates are found to be roughly parallel to, but uniformly higher than spermatogonial point-mutation rates for the same seven compounds. The higher somatic rates are presumably due to the fact that RS's can result from several genetic mechanisms besides point mutations. The spot test, which has not to date given any false negatives, may thus be considered a useful in vivo prescreen for heritable germinal mutations in mammals.

Despite the recent proliferation in development of submammalian and in vitro test systems for mutagenic studies, there is general agreement that in vivo studies on heritable genetic effects in mammals must, at some stage, enter into the risk evaluation for compounds that show a potential for affecting human populations. Since such testing can, however, be relatively time- and space-consuming, it must be reserved for only selected possible mutagens.

For this reason, some effort has been devoted to the perfection of an in vivo mammalian somatic-mutation test that might be capable of acting as a prescreen for germinal mutations of various kinds. This test was first developed by us in an x-ray experiment about 20 years ago and, at that time, explored for its various complexities (1). The usefulness of the method in chemical mutagenesis has been tested more recently (2–9). We have elsewhere presented some of our recent results in some detail (3), have discussed some of the more basic aspects of the method (9), and have compared the interpretations various investigators have made of their data (9). For the purposes of this symposium, some of the findings will be summarized, and the relation between somatic and germinal mutation rates explored.

In a germinal mutation experiment, each individual examined for presence or absence of a whole-body mutation scores one cell that was at risk to the mutagen. In an in vivo somatic mutation experiment, an individual is examined, instead, for clones of mutant cells (Fig. 1). While each clone again represents just one cell at risk, the whole individual represents a population of cells at risk—an obvious advantage in numbers. It should be noted, however, that the altered clone (consisting, as it does, of somatic cells) cannot ordinarily be involved in breeding tests, so that the nature of the genetic event that produced it cannot be genetically analyzed.

The method as originally developed (1) meets the following prerequisites of a successful somatic mutation prescreen: it uses a localized gene product (namely hair pigment) so that clones of exceptional cells can be directly identified; it involves an in vivo mammalian situation; and it is relatively fast and cheap. In addition, as more recently demonstrated, the results have predictive value for germinal point mutations (3, 9).

The in vivo somatic mutation test consists of treating embryos (i.e., populations of cells) that are

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heterozygous at specific coat color loci, allowing animals to be born and to grow a coat, and examining this for mosaic patches, indicative of clones of mutant cells. From the time of mating the animals to the final result, this process takes only 5 weeks.

Recessive mutations at the heterozygous loci do not, however, constitute the only cause of spots. This feature of the results, if properly used, can lend additional resolving power to the method. It can, however, also lead to misinterpretation in inexperienced hands. Our interpretation of the various causes of spots was derived from a comparison of results from heterozygous embryos with those from homozygous wild-type embryos (having mothers of the same strain): types of spots found exclusively in the former population are presumably due to an "uncovering" of the recessive, while types of spots found in both populations presumably have other causes (1). The various causes of spots are summarized in Table 1.

In order to determine whether there was any relation between the frequency of recessive spots (RS) and the frequency of germinal point mutations, we carried out in vivo somatic-mutation tests with compounds for which specific-locus mutation-rate data are available for spermatogonia. [Spermatogonia are the male cell stage of importance for risk evaluations (10).] These results, an extension of some published earlier (3), are summarized in Table 2. White near-midline ventral spots (WMVS), which are scored independently of RS's, are found with elevated frequencies in some of the treatment groups, generally those yielding also appreciable frequencies of malformed animals (observed by external examination at birth). Both WMVS's and teratogenic effects have been ascribed to cell killing (3).

Doses that have been used in spermatogonial specific-locus experiments are generally near the highest compatible with continuous fertility of the males. In the spot test, however, the maximum tolerated doses are considerably lower, since the method requires the treatment of embryos during one of their most vulnerable stages, day 10½ (1). In general, we have found it possible to use arrays of exposures that straddle a dose one-third of the one used in spermatogonia. In such dose ranges, one

### Table 1. Causes and appearance of spots found in the in vivo somatic-mutation test.

| Cause of spot                                      | Color<sup>a</sup>                                      | Location                      | Symbol |
|---------------------------------------------------|------------------------------------------------------|-------------------------------|--------|
| Expression of the recessive at marked loci        |                                                      |                               |        |
| Somatic point mutation to the recessive           | Various shades of gray, brown, or tan                | Random                        | RS     |
| Deficiency of a chromosomal segment                |                                                      |                               |        |
| Whole chromosome loss (from nondisjunction or other causes) | Various shades of gray, brown, or tan; or near-white |                               |        |
| Somatic recombination                              |                                                      |                               |        |
| Other events                                       |                                                      |                               |        |
| Decrease in melanocyte precursor cells            | White                                                | Near midline, ventral         | WMVS   |
| Errors in differentiation                         | Near-agouti (yellowish-brown)                       | Nipple sites, genitals, snout, base of ears; forehead tufts. | DS     |
| Dominant mutations scattered through genome<sup>b</sup> | Various shades of gray, brown, tan, cream, yellow; white |                               |        |

<sup>a</sup> Colors listed are for the case in which a/a; +/b; +/c<sup>b</sup>p; +/d se; +/s embryos are treated.

<sup>b</sup> Probably rare.
obtains near-normal litter sizes and survival to 12 days (3), the age for initial scoring for spots.

Since it is not possible to compare the spermatogonial and somatic mutation results at the same dose points, mutation rates per unit dose have been computed for both sets of data. The induced spermatogonial mutation rate was calculated for seven loci from data of Russell, Ehling, and Cattanach (3) and from unpublished BP and DEN data of W. L. Russell, subtracting the overall control rate (11) of 28 in 531,500. To calculate mutation rates for the spot-test results, one must first estimate the number of precursor cells at risk at the time of treatment. A distribution was derived by estimating, for each spot induced on day 101/4, the proportion of total fur occupied, and assuming that the reciprocal of this proportion represents the number of precursor cells (1). Since the mode lies between 150 and 200, we have used a figure of 175 for the cell population at risk per embryo. The number of loci screened in the spot test was probably four (9).

The mutation rates per locus per unit dose for both sets of data are listed in Table 3. The induced spermatogonial rate is shown on line with the somatic rate per unit dose is, in each case, considerably higher than the spermatogonial rate (and somatic rate per unit dose is, in each case considerably higher than the spermatogonial rate (and higher even than the upper 95% confidence limit in cases where the spermatogonial rate was zero).

The relatively higher RS frequencies, and the fact that easily measurable, though low, RS rates are obtained for compounds that have yielded totally negative results in germinal (spermatogonial) experiments, is undoubtedly due to the fact that expression of the recessive, which is scored in the spot-test, can be brought about by several mechanisms besides point mutations (Table 1). There is evidence from certain known chromosome aberrations that relatively long autosomal deficiencies are tolerated if they occur in up to about one-half the somatic cells of a mouse (12). It is conceivable that even monosomies could survive in a small clone. By contrast, almost all spermatogonially induced mutations are either intragenic changes or minute deficiencies (13).

For these reasons, results from the in vivo somatic-mutation test—even if carefully restricted to true RS’s can, and do, yield positive results for compounds that are negative in spermatogonial specific-locus tests, i.e., they give false positives in a prescreen for germinal point mutations. Some of these positives are probably the result of genetic changes, other than point mutations, that represent certain types of hereditary risks. The spot test has

### Table 2. Spot incidence and morphology of offspring of a cross of C57BL females by T males, following injection with various agents on day 101/4 postconception.

| Agent | Dose, mg/kg | No. born | Abnormal morphology at birth, % | WMVS, %b | RS, %b |
|-------|-------------|----------|---------------------------------|-----------|--------|
| EMS   | 50          | 93       | 0                               | 3.7       | (1.2)? |
|       | 100         | 193      | 1.6                             | 2.5       | 0.6    |
| MMS   | 50          | 126      | 0.8                             | 1.7       | 0.9    |
|       | 100         | 69       | 16.4                            | 13.1      | 4.9    |
| TEM   | 0.5         | 149      | 47.7                            | 17.5      | 3.3    |
|       | 0.8         | 126      | 91.1                            | 22.0      | 2.4    |
| MC    | 2           | 91       | 5.5                             | 15.2      | 3.0    |
|       | 100         | 171      | 0.7?                            | 4.9       | 2.1    |
|       | 150         | 135      | 0.9                             | 3.6       | 4.5    |
| HC    | 20          | 57       | 0                               | 8.9       | (1.8)? |
|       | 30          | 228      | 0.4                             | 13.5      | 0.5    |
|       | 50          | 160      | 16.3                            | 8.1       | 1.5    |
| DEN   | 20          | 79       | 0                               | 2.9       | 0      |
|       | 30          | 148      | 0                               | 2.1       | 1.5    |
|       | 50          | 156      | 0                               | 3.9       | 1.6    |
| Control | —         | 562      | 0                               | 3.8       | 0      |

* Abbreviations: EMS, ethyl methanesulfonate; MMS, methyl methanesulfonate; TEM, triethylenemelamine; MC, mitomycin C; HC, hycanthone; BP, benzo[a]pyrene; DEN, diethylnitrosamine.

### Table 3. Comparison of mutation rates at specific loci induced by seven agents in spermatogonia or somatic cells.

| Agent | Spermatogonia | Melanocyte precursors |
|-------|---------------|-----------------------|
|       | Dose mg/kg    | Mutation ratea x 10^-7 | Dose mg/kg | Mutation ratea x 10^-7 |
| EMS   | 293.1°        | 0 (U95%CL, 0.8)a      | 50         | (0, or 3.5?) |
| MMS   | 101.3°        | 0.8                   | 50         | 2.5          |
|       | 100           | 2.5                   | 50         | (7.0)        |
| TEM   | 2.3°          | 128.8                 | 0.5        | 952.4        |
|       | 30            | 2.3                   | 0.8        | (435.5)      |
| MC    | 5.25          | 64.5                  | 2.0        | (216.5)      |
| HC    | 150           | 0 (U95%CL, 2.2)d      | 50         | 3.9          |
| BP    | 500           | 3.0                   | 100        | 4.3          |
| DEN   | 138°          | 0                     | 20         | (0)          |
|       |               | 30                    | 30         | 7.2          |

* For abbreviations, see Table 2.

a Per locus (7 in spermatogonial, 4 in somatic cells), and per unit dose. Rates based on fewer than 100 observations are shown in parentheses.

b Weighted average.

d Where no mutations were observed, the upper 95% confidence limits of zero-frequency is shown in parentheses.
not, to date, given any false negatives. Because of this, and because of the finding of a rough parallelism (i.e., a relatively higher frequency of RS's in the case of compounds that induce spermatogonial mutations), the spot test can be a useful in vivo prescreen for heritable germinal mutations in mammals.

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