CHROMOSOME ASSIGNMENT OF THE TUMOR-SPECIFIC ANTIGEN OF A 3-METHYLCHOLANTHRENE-INDUCED MOUSE SARCOMA*

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The most compelling evidence for the existence of tumor-specific antigens (TSA) comes from the study of chemically induced tumors of inbred rodents. These tumors express antigens capable of immunizing syngeneic hosts against subsequent challenge with the same tumor. An intriguing feature of TSA of chemically induced tumors is their uniqueness, each tumor having a distinct antigen (1-3). No cross-reactions are observed between tumors induced by the same carcinogen, even if they are induced in the same animal (4). In vitro transformed cell populations, recently derived from a single clone, express TSA that are still non-cross-reactive (5, 6). TSA appear simultaneously with the neoplastic transformation of the carcinogen-treated tissue (5, 7, 8). Once expressed, TSA become a permanent trait of the tumor and remain stable through many transplantations in immunologically competent hosts or through serial passages in vitro (3). Tumors induced by different carcinogens differ in their immunogenicity. The strong TSA of 3-methylcholanthrene (MCA)-induced sarcomas made them preferred objects of immunologic studies and much of the present knowledge on TSA is based on the work with MCA-induced tumors.

Nothing is known about the location, the number, and the nature of genes coding for TSA. The assignment of TSA to particular chromosomes through somatic cell genetics techniques would be a step toward elucidating the genetic basis of tumor antigen diversity. More specifically, two questions could be addressed through this methodology: (a) are TSA coded by different, random loci, or are they coded by one or a few specific genes with high mutability or with antigenically diverse products? and (b) are TSA loci linked to genes (such as the H-2 complex) known to code for molecules with considerable antigenic variability?

To facilitate analysis of the TSA of chemically induced tumors, much effort has gone into developing serological reagents to detect these antigens. In most instances,
it has not been possible to demonstrate antibody with specificity for individual tumors in the serum of immunized mice, despite prolonged immunization and a high level of resistance to tumor transplants. Recently, however, DeLeo et al. (9) were able to produce syngeneic antisera that defined a cell surface antigen restricted to a single MCA-induced BALB/c sarcoma, Meth A. There is growing evidence that the serologically defined Meth A antigen is related to the strong, individually distinct transplantation antigen expressed by Meth A (10).

The present study was aimed at mapping the genetic locus of the serologically defined Meth A antigen through the use of somatic cell hybrids.

Materials and Methods

Cell Lines. Meth A is a strongly immunogenic sarcoma induced in 1960 by a subcutaneous injection of MCA in a BALB/c female mouse (3). An ascites variant of the tumor was derived in 1961. The two cell lines used in the present study, Meth A(a) and Meth A(s), were established in 1974 from the ascitic and solid forms of the tumor, respectively (9). E36 is an established line of Chinese hamster lung cells, derived from V79 (11). The clone used in the present study is a hypoxanthine phosphoribosyl transferase (HPRT-) mutant resistant to 30 µg/ml 6-thioguanine and to 3 × 10⁻² M ouabain. These cells show no density dependent inhibition of growth. THO is an HPRT⁺ clone of BALB/3T3 cells (12), resistant to 6 µg/ml 6-thioguanine. All cells were maintained in Dulbecco's modified Eagle medium with 10% fetal calf serum.

Typing Sera for Meth A Antigen. Antisera detecting the Meth A antigen were produced by immunization of BALB/c or (BALB/c × C57B16) F₁ mice as described (9). These antisera have cytotoxic antibody that defines a TSA on BALB/c Meth A sarcoma. The serologically defined Meth A antigen is stably expressed by the ascitic and solid forms of Meth A, as well as by cell lines derived from them. Absorption studies indicate that expression of this antigen is restricted to Meth A. The antigen has not been detected on a wide range of normal and malignant cells derived from BALB/c or from other strains of mice.

Serological Analysis. The hybrids were analyzed for Meth A antigen expression by absorption tests (9). The dilution of antiserum chosen for absorption tests was generally one doubling dilution below its endpoint (50% cytotoxicity), as determined in preliminary tests. 2 vol of diluted antiserum and 1 vol of packed, washed cells were incubated together, with frequent shaking, for 30 min at 4°C. After removal of the absorbing cells by centrifugation, the residual cytotoxic activity of the absorbed antiserum was tested.

Preparation of Microcells. Cells were micronucleated by exposure to colcemid (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) at a concentration of 0.05-0.075 µg/ml for 36-48 h (13, 14). Enucleation of the microcells was carried out on a discontinuous Ficoll (Ficoll 400; Pharmacia Fine Chemicals, Piscataway, N. J.) gradient after a modification of the protocol of Wigler and Weinstein (15).

Cell Hybridization. Whole cell and microcell Meth A(a) × E36 and Meth A(s) × E36 fusions were carried out with β-propiolactone-inactivated Sendai virus. The microcell hybrids were selected and maintained on medium containing 10⁻⁴ M hypoxanthine, 4 × 10⁻⁷ M aminopterin, and 1.6 × 10⁻⁶ M thymidine (HAT) (16). The whole cell hybrids were selected on HAT plus 10⁻³ M ouabain. The hybrid series ma and mAE were produced from two separate hybridizations of Meth A(a)-derived microcells with E36. Hybrid ms were isolated after fusion of microcells from Meth A(s) with E36. Only clones picked from separate flasks were considered independent.

Isozyme Analysis. The hybrids were analyzed for the presence of the mouse and Chinese hamster forms of the following enzymes: peptidase 3 (EC3.4.13), peptidase 1 (EC3.4.13), nucleoside phosphorylase (EC2.4.2.1), phosphoglucomutase 1 (EC2.7.5.1), phosphoglucomutase 2 (EC2.7.5.1), peptidase 2 (EC3.4.13), mannose phosphate isomerase (EC5.3.1.8), adenine phosphoribosyltransferase (EC2.4.2.7), uridine phosphorylase (EC2.4.2.3), guloalase 1 (EC4.4.1.5), galactokinase (EC2.7.1.6), malic enzyme (EC1.1.1.39), 6-phosphogluconate dehydrogenase (EC1.1.1.43), glucosohosphatase isomerase (EC5.3.1.9), glutathione reductase (EC1.6.4.2), triosephosphate isomerase (EC5.3.1.1), esterase 10 (Es10), adenosine kinase
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(EC2.7.1.20), acid phosphatase 1 (EC3.1.3.2), α-galactosidase (EC3.2.1.22), lactate dehydrogenase A (EC1.1.1.27), and enolase (EC2.4.1.11). The techniques for the enzyme assays have been described (17).

Chromosome Analysis. Chromosome preparations were G-banded after a modification of the method of Wang and Fedoroff (18). When necessary, additional C-banding techniques, barium hydroxide/saline/Giemsa banding (19) and Hoechst 33258 staining (20), were applied to distinguish between Chinese hamster and grossly rearranged mouse chromosomes in the hybrid cells and to determine the position of centromeric heterochromatin in rearranged chromosomes. A minimum of 30 banded metaphases of each hybrid were karyotyped. In some cases, up to 200 additional cells were scanned microscopically for the presence of a particular chromosome previously identified by karyotyping. The nomenclature of Nesbitt and Francke (21) is used for the description of rearrangements involving mouse chromosomes.

Results

Eight whole cell hybrids between one of the Meth A sarcoma-derived cell lines, Meth A(a), and the Chinese hamster cell line E36 and seven hybrids between the other Meth A-derived line, Meth A(s), and E36 were analyzed for Meth A antigen expression within the first five passages after their isolation. At that time, the hybrids had both the mouse and Chinese hamster forms of the 22 enzymes tested (assigned to 14 mouse chromosomes and to an unknown number of Chinese hamster chromosomes). By karyotype analysis, the hybrids had a complete set of mouse chromosomes and >90% of the cells also had the complete set of E36 Chinese hamster chromosomes. All these hybrids expressed Meth A antigens, as indicated by specific absorption of typing serum (Fig. 1A).

To assign the gene coding for Meth A antigen to a particular chromosome, it was necessary to produce hybrid lines retaining a few mouse chromosomes on a Chinese hamster background. The whole cell hybrids Meth A(a) × E36 and Meth A(s) × E36 slowly segregated Chinese hamster chromosomes and were thus unsuitable for mapping. To circumvent the species-determined direction of chromosome loss, we fused

![Fig. 1. Absorption analysis of hybrids between E36 and the sarcoma cell lines, Meth A(a) and Meth A(s).](image)
E36 with microcells derived from Meth A(a) and Meth A(s), each microcell carrying an incomplete set of mouse chromosomes.

A total of 48 independent clones was isolated after two separate hybridizations of Meth A(a) microcells with E36 (hybrids ma and mAE) and one hybridization of Meth A(s) microcells with E36 (hybrids ms). The hybrids were analyzed for their isozyme content at the earliest possible time (during the first four passages). 35 (73%) of the clones did not show any mouse isozymes. 21 of these lines were karyotyped and were found to contain single mouse chromosomes or mouse chromosome fragments in a small fraction (1–10%) of the population. Thus, rapid chromosome loss and fragmentation apparently had occurred in these lines. The mouse chromosome segment carrying the gene encoding HPRT, designated hprt*, which makes possible the survival of the cells in HAT, was probably integrated somewhere in the Chinese hamster genome. None of these hybrids expressed the Meth A antigen.

In 13 microcell hybrids, 1–4 mouse chromosomes were identified by karyotype and isozyme analysis. The results of the serologic and chromosome analysis of these hybrids are summarized in Table I.

### Table I

| Hybrids/controls | Mouse chromosome present | Percentage of cells with the chromosome | Meth A antigen expression* |
|------------------|--------------------------|----------------------------------------|---------------------------|
| ma 8c            | X¹²                      | 83                                     | ++                        |
| ma 12            | X¹²                      | 75                                     | ++                        |
| mAE 28           | X¹²                      | 50                                     | ++                        |
| mAE 18           | X¹²                      | 50                                     | +                         |
| mAE 6            | X¹²                      | 70                                     | ++                        |
| mAE 17           | 6, 10, 12, 14, X†         | 70–85                                  | +                         |
| mAE 12           | del (X) (A7/B)           | 76                                     | –                         |
| ms 5             | X                        | 55                                     | –                         |
| mAE 19           | 1                        | 68                                     | –                         |
| mAE 29           | 14                       | 56                                     | –                         |
| ma 4             | X¹⁲, del (12) (C1/C2)     | 54                                     | –                         |
| mAE 22           | X¹¹, del (12) (E/F1)      | 74                                     | –                         |
| mAE 32           | 16                       | 87                                     | +                         |
| Meth A(a)        |                          |                                        | ++                        |
| E36              |                          |                                        | –                         |

* As determined by the capacity to absorb cytotoxic activity from the Meth A antiserum. (+++) = absorption comparable to the parental Meth A(a) cells; (+) = partial absorption; (−) = absorption comparable to E36 (see Fig. 1 for examples of individual tests).
† The four mouse chromosomes in mAE 17 (M₁, M₂, M₃, and M₄) were rearranged and could not be identified by their banding. The numbers refer to chromosomes whose enzyme markers were present in the hybrid.
Microcell Hybrids Expressing Meth A Antigen

Hybrids ma 8c, ma 12, mAE 18, and mAE 28. All of these hybrid clones had the mouse forms of the enzymes α-galactosidase assigned to the X chromosome (22) and acid phosphatase 1, mapped on chromosome 12 (23). The karyotype analysis of these clones revealed the presence of a single rearranged mouse chromosome, consisting of an intact chromosome 12 attached (at its centromeric region) to the distal end of the mouse X (Fig. 2B and G). The marker was present in 50–85% of the cells (Table 1). (The remaining cells did not have any mouse chromosome material and probably represented segregants that had lost the mouse X chromosome, but could still survive in HAT by virtue of metabolic cooperation with the X-retaining cells. The high frequency of HPRT− cells in the population, as revealed by the ease with which back selectants were isolated and by the considerably lower plating efficiency of these cells in HAT as compared with regular medium, supports this interpretation.) The only missing segment of chromosome 12 was part of its paracentromeric region. The translocation resulting in the formation of the X12 chromosome had taken place in the parental Meth A(a) line, because 100% of these cells had one to two copies of the chromosome (Figs. 2A and 3). (Each Meth A(a) cell had in addition one to two copies of normal chromosome 12 and X.) No other mouse chromosome was found in the karyotyped hybrid cells or in 200 additional banded metaphases of each clone that were scanned microscopically.

In contrast to the absorption capacity of hybrids ma 8c, ma 12, and mAE 28 (Fig. 1A and B), hybrid mAE 18 consistently gave only partial absorption of Meth A typing serum. It is possible that this difference in antigen expression was due to the fact that in mAE 18 the X12 translocation was found as a single copy in a predominantly tetraploid population, whereas in mAE 28, ma 8c, and ma 12, one to four copies of the marker were present in mostly diploid cells.

Hybrids ma 8c and ma 12 were switched from HAT to medium containing 30 µg/ml 6-thioguanine, in which only cells that had lost the mouse X-linked hprt+ gene would survive. The back selectants were collected as a mass population from two T75
flasks (Corning Glass Works, Corning, N. Y.) within 10 d of the beginning of the experiment. These cells did not express the antigen.

**Hybrid mAE 6.** This clone had the mouse enzymes peptidase 3, assigned to chromosome 1 (24), α-galactosidase, and acid phosphatase 1. The karyotype analysis showed the presence of a mouse X1 and a 112 translocation in the hybrid cells (Table I and Fig. 2E). The rearranged 112 chromosome consisted of a mouse chromosome 1, to the distal end of which (at band H1) was attached chromosome 12 at its centromeric portion. The segment of chromosome 1 distal to H1 was attached to the telomeric end of X. The rearrangement had probably arisen soon after the formation of the hybrid as a reciprocal translocation between X12 and chromosome 1.

**Hybrid mAE 17.** This clone had four mouse chromosomes in 70–80% of the mostly tetraploid population and the mouse forms of the enzymes triosophosphate isomerase, assigned to chromosome 6 (25), acid phosphatase 1 (on chromosome 12), peptidase 2, assigned to chromosome 10 (26), nucleoside phosphorylase and adenosine kinase, both on chromosome 14 (26), and α-galactosidase (on X). All the mouse chromosomes in the hybrid were rearranged, and the origin of their material could not be determined cytologically. mAE 17 gave partial absorptions comparable to that of mAE 18.

**mAE 32.** Mouse chromosomes X and 16 were found in 87% of these cells (Table I; Fig. 1A).

**Microcell Hybrids Not Expressing Meth A Antigen**

**mAE 12.** 80% of the cells in this hybrid contained a small fragment with a banding pattern corresponding to the proximal half of the mouse X chromosome (Table I).

**mA 5.** 55% of these cells had a normal mouse X chromosome (and no other mouse chromosomes) (Table I).

**mAE 19.** In addition to the mouse X chromosome, this clone had mouse chromosome 1 (Table I).

**mAE 29.** This hybrid contained mouse chromosomes X and 14 (Table I).

**mAE 4.** A marker chromosome, derived from the X12 chromosome through deletion of the portion of chromosome 12 distal to band CI was found in 54% of the cells (Fig. 2D and G; Table I).

**mAE 22.** This clone (Fig. 1A) contained another chromosome marker derived from the X12 chromosome through terminal deletion. The breakpoint on chromosome 12 was at band E/F1 (Fig. 2C and G; Table I). The marker chromosome in mAE 22 differed from the X12 chromosome in hybrids mAE 8c, mA 12, mAE 28, and mAE 18, only in the absence of the terminal bands F1 and F1 from chromosome 12.

**Stability of Meth A Antigen Expression by the Microcell Hybrids.** After several weeks in culture, all of the microcell hybrids that expressed Meth A antigen began to show a gradual decline in antigenicity. The expression of Meth A antigen by one of the microcell hybrids, mAE 28, was followed for a period of 16 wk. During the first 6 wk, the antigen was expressed at a level sufficient to completely absorb the cytotoxic activity from the typing serum. After that, the absorptions became partial, and after 10 wk the hybrid was completely negative. At this point the cells were again karyotyped, and the X12 chromosome was found in 80% of the cells. The only difference from the earlier karyotype of mAE 28 was that the population had become predominantly tetraploid, whereas when karyotyped the first time, it was diploid.

Because the microcell hybrids tended to lose rapidly the mouse chromosomes, the
| 5 | 5 |
|---|---|
| 10 | 15 | X |
| 18 | 9 | 14 |
| 19 | 12 | 16 |
| 3 | 8 | 13 |
| 12 | 17 |
| 11 | 16 |
| 1 | 6 |
hybrid cell population was always a mixture of X- or X\textsuperscript{12}-containing cells and newly arisen segregants, whose proportion depended on the density at which the cells were cultured (a higher density facilitated metabolic cooperation and thus survival of the HPRT\textsuperscript{−} cells). To avoid the density-dependent fluctuation in the number of X\textsuperscript{12}-containing cells in the population, as well as to avoid the possible negative influence of the Chinese hamster genome on the expression of Meth A antigen by the microcell hybrids, the Meth A(a)-derived X\textsuperscript{12} chromosome was transferred from hybrid mAE 28 back into mouse cells. Microcells were prepared from mAE 28 at a time when this hybrid had ceased to express the Meth A antigen at a detectable level and were hybridized with an HPRT\textsuperscript{−} clone of BALB/3T3-THO. The X\textsuperscript{12} marker could be easily identified on the mouse 3T3 background by G-banding. Two of the microcell hybrids between mAE 28 and THO (18D/2 and 2T6/7), in which the X\textsuperscript{12} chromosome was found intact in nearly 100% of the cells completely absorbed the anti-Meth A cytotoxic activity from the serum (Fig. 1B). Back selection of 18D/2 on 6-thioguanine resulted in the loss of the X\textsuperscript{12} marker and Meth A antigen expression (18D/2 BS in Fig. 1B). Two additional mAE 28 × THO microcell hybrids, in which the X\textsuperscript{12} marker had undergone breakage and was seen intact in <5% of the cells, were negative for Meth A antigen.

Discussion

To assign the gene coding for the individually distinct Meth A antigen to a particular chromosome, we analyzed somatic cell hybrids of the Meth A sarcoma cell lines Meth A(a) and Meth A(s) with the Chinese hamster cell line E36.

Eight Meth A(a) × E36 and seven Meth A(s) × E36 whole cell hybrids expressed Meth A antigen (Fig. 1A), indicating that in whole cell hybrids, Meth A antigen expression was not detectably affected by the genome of the Chinese hamster parent.

In four microcell hybrids (ma 8c, ma 12, mAE 28, and mAE 18; Fig. 1 A and B), which expressed Meth A antigen, the only mouse chromosome present was an X\textsuperscript{12} translocation, which they had received from the parental Meth A(a) cells (Figs. 2 A and B, and 3). Hybrid mAE 6, which also expressed the antigen, had chromosomes X and 12 in a different arrangement, and in addition, chromosome 1 (Fig. 2 E). Because X and 12 are the chromosomes all these lines have in common, we concluded that one or both carry the genetic information necessary for the expression of Meth A antigen. As these five lines were independently isolated clones and were produced in two separate hybridizations, it was highly unlikely that Meth A antigen expression could be due to the presence of a cytologically undetected fragment of some other chromosome that had been co-transferred into and retained by the hybrid cells together with the X\textsuperscript{12} chromosome. This possibility was further ruled out by the concomitant loss of the X\textsuperscript{12} chromosome and Meth A antigen expression after back selection of hybrids ma 8c and ma 12. Another antigen expressing hybrid, mAE 17, had four rearranged mouse chromosomes and the enzyme markers for chromosome 6 (triosophosphate

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**FIG. 3.** Karyotype of a Meth(a) cell. The Meth A(a) cell line is subtetraploid, most cells having chromosome number between 65 and 71. Every cell has 2–4 normal copies of each chromosome; 6–10 rearranged telocentric chromosomes; 10–13 biarmed chromosomes, about half of which represent Robertsonian translocations between normal chromosomes (most often 3.3, 5.5, 9.9, 9.18, 14.15, 14.19, 17.17); 2–3 are the result of the joining of a normal chromosome (i.e., 10, 15) with a rearranged chromosome; and the rest are of unknown chromosome composition. The X\textsuperscript{12} and 12\textsuperscript{16} chromosomes are present in 100 and 90% of the cells, respectively.
isomerase), chromosome 10 (peptidase 2), chromosome 12 (acid phosphatase 1), part of chromosome 14 (nucleotide phosphorylase and adenosine kinase, but not esterase 10), and X (α-galactosidase). Thus, in agreement with the findings in the other Meth A antigen-positive lines, mAE 17 had some material derived from X and chromosome 12, although its size and location could not be determined.

Three lines containing a normal X either as a single chromosome (ms 5) or accompanied by chromosome 1 (mAE 19) or chromosome 14 (mAE 29), did not express Meth A antigen. mAE 12, which carried a fragment of X, including the hprt gene (27), was also negative. The absence of the antigen from the X-containing lines indicated that its expression requires the presence of chromosome 12 in the hybrid cells.

In two Meth A antigen-negative hybrids, the X\textsuperscript{12} chromosome had undergone deletions involving different portions of chromosome 12. In mAE 4, it lacked the part distal to band C1 (Fig. 2D and G). In mAE 22, the terminal bands F1 and F2 were missing from chromosome 12 (Fig. 2C and G). The fact that neither of these lines expressed Meth A antigen indicated that the distal portion of chromosome 12, and particularly bands F1 and F2, are essential for antigen expression by the hybrid cells, and thus the gene coding for Meth A antigen is probably located in that part of the chromosome. Because all antigen-positive lines had X in addition to chromosome 12, it is impossible to rule out some contribution of the X chromosome to Meth A antigen expression or requirement for the presence of both X and the terminal portion of chromosome 12 for a hybrid to be Meth A antigen positive.

The attachment of chromosome 12 to X in most of the Meth A antigen-expressing hybrids (ma 8c, ma 12, mAE 28, and mAE 18) made it possible to maintain this chromosome in a high percentage of the hybrid population for long period of time. Despite this, however, there was a gradual decline in Meth A antigen expression by the hybrid cells over a period of several months. Repeated karyotype analysis of the cells ruled out chromosome breakage or loss as the cause for this decline. The shift from diploidy to tetraploidy that occurred in the mAE 28 hybrid population paralleled antigen loss and may have contributed to this change through the increased dosage of some weak suppressive factors present in the Chinese hamster cells. As was mentioned in the previous section, hybrids mAE 17 and mAE 18, which were tetraploid when analyzed for the first time, had lower levels of the antigen compared with the diploid X\textsuperscript{12}-containing hybrids.

To eliminate the presumed negative influence of the Chinese hamster genome on the expression of Meth A antigen, we transferred the X\textsuperscript{12} chromosome from hybrid mAE 28 (when it no longer expressed Meth A antigen) into an HPRT\textsuperscript{-} BALB/3T3 clone, THO. Two microcell hybrids, which contained the X\textsuperscript{12} chromosome on a BALB/3T3 background, expressed the Meth A antigen (Fig. 1B). Although these results are consistent with the idea that factors adversely affecting Meth A antigen expression are present in the microcell hybrids, the nature and mechanism of action of these factors remain unclear.

Surprisingly, hybrid mAE 32, in which the karyotype analysis revealed mouse chromosomes X and 16 and no identifiable chromosome 12-derived material, also expressed the Meth A antigen (Fig. 1A). There are several ways to explain the discrepancy between this finding and the assignment of the Meth A gene to the distal portion of chromosome 12 on the basis of the data discussed above. First, the mouse
chromosomes in the microcell hybrids showed a pronounced tendency for fragmentation, as demonstrated by the deletions in mAE 22, mAE 4, and mAE 12, and by the rapid disappearance of any whole mouse chromosomes from most of the cells in 73% of the hybrids. Thus, it is conceivable that mAE 32 early in its history could have had, in addition to X and chromosome 16, a chromosome 12, a small piece of which remained integrated somewhere in the Chinese hamster chromosomes. The presence of a $12^{16}$ translocation (Figs. 2 F and 3) in 90% of the Meth A(a) cells increases the probability of the introduction of both chromosomes 12 and 16 in the hybrid (the apparently normal 16 being derived from the $12^{16}$ translocation). Second, molecules carrying Meth A determinants could be encoded by more than one locus, one of them at the terminal part of 12 and another one on 16. A third possibility, which we consider much less likely, is that the Meth A gene is located on X, the chromosome that ma 8c, ma 12, mAE 28, mAE 18, mAE 6, and mAE 32 have in common. The negative absorptions by five hybrid lines containing X either as a separate chromosome or as part of a deleted $X^{12}$ marker (ms 5, mAE 19, mAE 29, mAE 4, and mAE 22) would have to be explained by the rapid turning off of the antigen, which made it undetectable by the time these lines were first available for analysis. As discussed above, the antigen-positive lines showed a decrease in antigenicity unrelated to chromosome loss over a period of several months. Whatever the mechanisms of this decline, it would have to be assumed that for unknown reasons it had proceeded at a much faster rate in the five X-containing lines in order to explain their negativity in the first two to four passages (1-2 wk) after isolation.

We found no evidence for linkage between the Meth A coding gene and the H-2 complex. One of the current hypotheses regarding the nature of TSA is that they represent altered H-2 products or abnormally expressed alien H-2 specificities, on the basis of the model for the H-2 complex proposed by Bodmer (28). In some cases, abnormal expression of alien H-2 specificities by tumor cells has been documented (29, 30). However, the molecules carrying the alien H-2 specificities are distinct from the TSA of the tumor (31). None of our hybrids that expressed Meth A antigen had either a cytologically recognizable mouse chromosome 17 (which carries the H-2 complex) or expressed the mouse form of glyoxalase 1, assigned to chromosome 17 (32). This is in agreement with the results of Natori et al. (33), indicating that the solubilized Meth A-specific transplantation antigen (which seems to be related to the serologically defined Meth A TSA [10]) is distinct from the H-2 products. Klein and Klein (34) also found no correlation between the presence of chromosome 17 and the expression of the TSA by somatic cell hybrids of an MCA-induced mouse sarcoma.

The assignment of the Meth A gene to chromosome 12, and possibly 16, indicates linkage of this TSA with gene clusters encoding the immunoglobulin (Ig) heavy chains and Light chains. The heavy chain genes were assigned to chromosome 12 by Hengartner et al. (35) and D'Eustachio et al. (36). Two of the hybrids described in this paper (mAE 28 and mAE 4) were analyzed by Southern blotting for the presence of the mouse C $\gamma_2b$ and C $\mu$ DNA sequences (36). Mouse restriction fragments hybridizing with the cloned cDNA probes were present in mAE 28, but absent from mAE 4. On the basis of this, it was concluded that the mouse heavy chain gene cluster is located on the portion of chromosome 12 distal to band C1. Meo et al. (37) further localized the $\gamma_2a$ gene on the telomeric portion of chromosome 12, proximal to and closely linked to the break point in band 12 F1 in the (12,5) translocation studied by
them. Thus, the Ig heavy chain gene cluster is located in the same region of chromosome 12, which was found to be necessary for Meth A antigen expression by the hybrids (bands F1-F2). The light chain genes were recently mapped to chromosome 16 by DNA restriction analysis of a panel of somatic cell hybrids, including mAE 32 by D'Eustachio et al. (38).

On the basis of the close linkage of this TSA with the Ig heavy chain and possibly with the light chain genes, it is tempting to speculate that the genetic elements used to generate the enormous variety of the Ig idiotypes (the antigenic determinants on the variable portion of the molecule) are also involved in the generation of TSA diversity. The genes encoding the variable and constant region of the Ig heavy and light chains exist as separate elements that are recombined and thus made functional (with respect to Ig production) in the process of B cell development (39-42). The variable gene-encoded portion of the Ig molecule has the ability to recognize specific antigenic determinants, whereas the constant region carries an effector function common to a given class of Ig. The relative independence of recognition and effector parts is further demonstrated by the mounting evidence for the presence of variable gene-encoded antigenic determinants (idiotypes) on molecules other than immunoglobulins, such as receptors and antigen-specific regulatory products of T lymphocytes (43-48). One might speculate that the expression of the variable genes is not limited to the cells of the immune system, but has a much broader use by various somatic cells. Through the variable genes (possibly different subsets of them), the ability to recognize a specific structure could be transferred to other molecules, with different consequences of the recognition event for the cell. Some of these hypothetical molecules might be involved in the interactions controlling cell growth and differentiation.

If this is the case, TSA would be expected to be found in normal cells before the induction of malignant transformation. The fact that they remain unrecognized by the animal's immune system could be a result of (a) restricted occurrence in a small population of cells, (b) very low levels on normal cells, (c) predominantly intracellular localization, and (d) interactions with other molecules that mask its antigenic determinants, or any combination of these factors. Accordingly, malignant transformation would elicit immune reaction against TSA by significantly expanding a previously small population, by increasing the amount of the antigen per cell, by changing its distribution among the cellular compartments, and/or unmasking the TSA determinants. If it is further postulated that each cell is irreversibly committed to the expression of a single TSA, it would be difficult to reconcile this hypothesis with the observations of Basombrio and Prehn (5) and Embleton and Heidelberger (6), indicating that subpopulations of recently derived clones express non-cross-reacting TSA after in vitro transformation. However, if a cell can express several TSA at any given time of its life history (and a transformation event results in the increased amounts of one of them), or if the cells express different TSA sequentially as they continuously undergo differentiation and maturation, such a lack of cross-reacting is to be expected.

Summary
Chemically induced sarcomas of inbred mice express antigens that are distinct and specific for each individual tumor. Chromosome assignment of tumor-specific antigens
would help to elucidate the genetic basis of their diversity. Expression of the serologically defined Meth A sarcoma antigen is not suppressed in hybrid cells containing a complete foreign (Chinese hamster) genome. Chromosome and serologic analysis of microcell hybrids between Meth A sarcoma cells and Chinese hamster cells shows a clear correlation of Meth A antigen expression with the presence of the distal region of chromosome 12 (bands F1 and F2). Chromosome 16 may also be implicated. The significance of the close linkage of genes determining Meth A antigen expression with the immunoglobulin heavy chain gene cluster (on chromosome 12) and the λ light chain genes (on chromosome 16) is discussed.

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