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The Gene and the Pseudogene for Mouse p53 Cellular Tumor Antigen Are Located on Different Chromosomes

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The chromosomal assignments of the two genes encoding the murine p53 cellular tumor antigen were determined by using a panel of mouse-Chinese hamster somatic cell hybrid clones and a mouse p53-specific cDNA clone. One gene, probably the functional member of the family, was found to be on chromosome 11. The other gene, which is probably a processed pseudogene, was assigned to chromosome 14. The potential relevance of these findings to documented cases of chromosome 11 trisomy are also discussed.

The cellular tumor antigen p53 is a phosphoprotein found in elevated levels in a variety of transformed cells (8). It has been suggested that p53 is a cell cycle protein (19) and that it may be involved in the control of cell proliferation (1, 11, 17). Recently, it has been demonstrated that the mouse genome contains two p53-specific genes (14). When *Eco*RI-digested mouse DNA is annealed with p53-specific DNA probes, one of the genes is represented by a 16-kilobase (kb) fragment and the other one by a 3.3-kb fragment; for the sake of simplicity, we shall refer to them here as the 16-kb gene and the 3.3-kb gene, respectively. Nucleotide sequence analysis of those genes revealed that the 3.3-kb member displays several structural features characteristic of a processed pseudogene, whereas the 16-kb one is probably the functional, p53-encoding gene (20).

To clarify the relationship between the two p53-specific genes, we attempted to assign them to particular mouse chromosomes. To that end, we took advantage of a series of mouse-Chinese hamster somatic cell hybrid clones, each containing the whole hamster genome and various combinations of mouse chromosomes. The somatic cell hybrids used in this study, their growth and karyotype analysis, and the isolation of DNA have been described elsewhere (6, 7). The chromosome composition of these hybrids was determined by both karyotype and isozyme analysis (13).

High-molecular-weight DNA was extracted from each hybrid cell clone as well as from parental mouse and hamster cells. The DNA was digested with the restriction endonuclease *Eco*RI, separated electrophoretically on a 1% agarose gel, transferred to nitrocellulose (18), and hybridized with radiolabeled (15) DNA of pp53-176, a mouse p53-specific cDNA clone (18). Typical results of such experiments, utilizing *Eco*RI-digested cellular DNA, are displayed in Fig. 1.

The data clearly indicate that the two mouse p53-specific genes are unlinked. Hybridization of the probe with mouse DNA detected two DNA fragments possessing sizes of 16 and 3.3 kb (14, 20). Hybridization of the same probe with hamster DNA also revealed two bands, both of which are easily distinguishable from their murine counterparts. The mouse 16-kb fragment, corresponding to the functional gene, was detectable only in DNA extracted from the hybrid cell line 22. Since this is the only cell line containing mouse chromosome 11 (Fig. 2), we conclude that the gene encoding the p53 polypeptide must be located on mouse chromosome 11. The 3.3-kb *Eco*RI fragment, containing the pseudogene, was detected in numerous cell hybrids; the distribution pattern of this fragment among the hybrids is compatible with its being derived from mouse chromosome 14 (Fig. 2). Similar results were obtained with identical cellular DNAs digested with *Bam*HI (data not shown).

The results presented here clearly demonstrate that the two p53-specific genes are located on different mouse chromosomes. This is consistent with the notion that the 3.3-kb gene is in fact generated via the integration of a naturally occurring p53 cDNA molecule into a new, probably random, chromosomal site. This further confirms the conclusion, drawn from nucleotide sequence data (20), that it is indeed a processed pseudogene.

Rotter et al. (16) have recently demonstrated that the ability to produce detectable p53, as assayed with monoclonal antibodies, correlates with the presence of mouse chromosome 11. Taken together with those findings, our data establish that the structural gene for p53 is in fact located on chromosome 11. Furthermore, at least in the somatic cell hybrids studied by Rotter et al. (16), the 3.3-kb gene does not appear to direct the production of any detectable p53 or even any shorter polypeptide recognizable by the monoclonal antibodies used in that study.

Detectable alterations involving mouse chromosome 11 have not been among those regularly detected in specific types of malignancies (12). However, trisomy 11 has indeed been reported in at least two cases, suggesting that a function residing in this chromosome may in fact be associated with some tumor-related processes. Thus, Croce et al. (5) detected the presence of trisomy 11 in a tumor generated by injection of nude mice with somatic cell hybrids formed between simian virus 40 (SV40)-transformed human cells and mouse cells. The appearance of trisomy 11 in SV40-induced tumors brings to mind the recent findings of Chen et al. (3). These investigators demonstrated that the ability of normal mouse cells to be efficiently transformed by SV40 is correlated with the presence of higher p53 levels in those cells, as quantitated by metabolic labeling. p53 has long been

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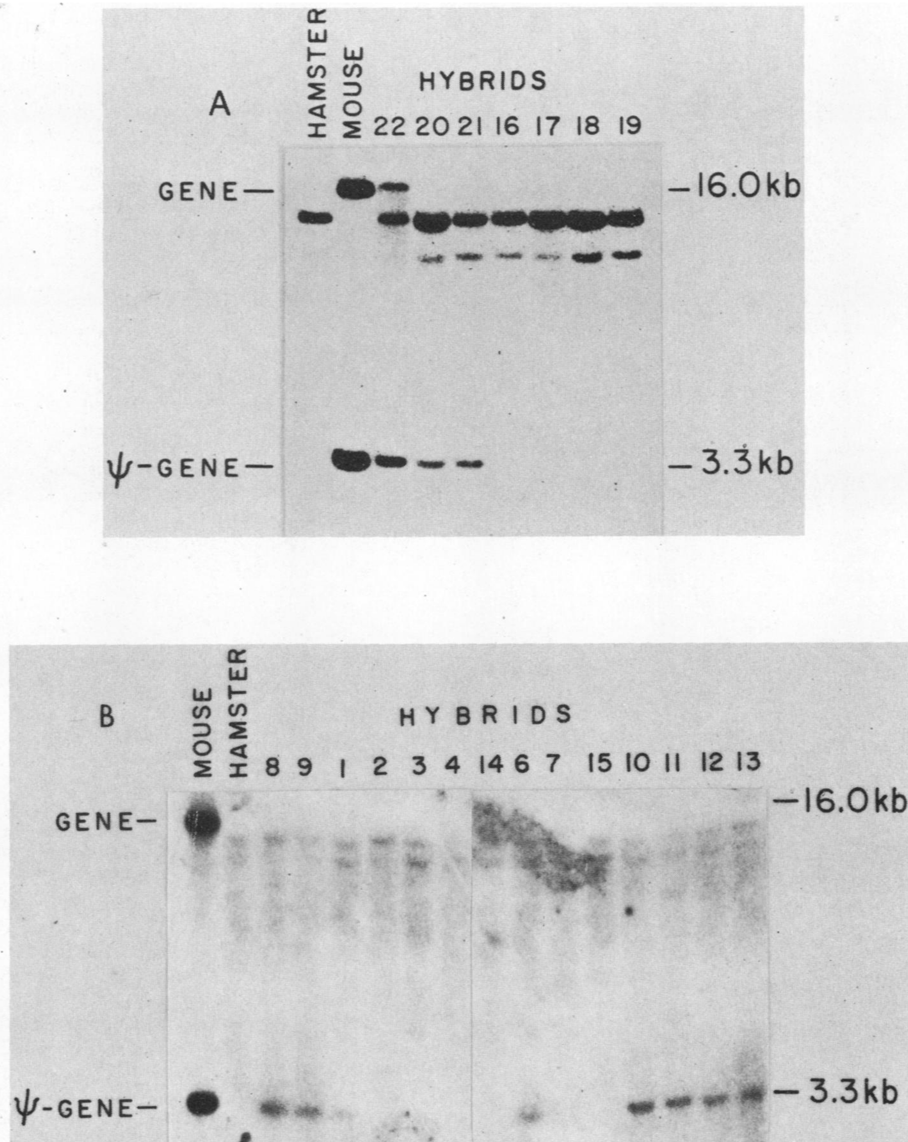

FIG. 1. Presence of mouse p53-specific DNA in mouse-Chinese hamster hybrid cell lines as determined by Southern blot analysis. DNA (10 μg) isolated from parental and hybrid cell lines (described in the legend to Fig. 2) was digested with *Eco*RI, electrophoresed on a 1% agarose gel in either Tris-phosphate (A) or Tris-acetate (B) buffer, transferred to nitrocellulose paper (18), and hybridized to nick-translated (15) DNA of clone pp53-176 (20). Hybridization conditions were as described by Carmon et al. (2). The filters were washed in 150 mM NaCl–15 mM Na-citrate at 65°C for 1 h and subjected to autoradiography. The figure shows the hybridization patterns obtained with DNA from the parental cell lines and from some of the hybrid cell lines (see Fig. 2).

known to form a specific complex with the SV40 large T antigen which is particularly tight in mouse cells (4, 9, 10). If the formation of this complex plays a role in the generation of the transformed phenotype, it is likely that the presence of higher p53 levels could make SV40-induced transformation more efficient. The possible relevance of trisomy 11 to such a process still remains to be determined.

More recently, trisomy 11 has been described in a very different system (S. Ohno, S. Migita, F. Wiener, M. Babonits, G. Klein, J. F. Muchinski, and M. Potter, submitted for publication). Those authors have demonstrated that in plasmacytomas induced by Abelson virus and pristane, many tumors had a chromosome 11 trisomy, whereas all the other chromosomes were present in the normal diploid number. It still remains to be seen whether such tumors make increased amounts of p53 and whether this may have anything to do with the ability of the chromosome 11 trisomic cells to form tumors.

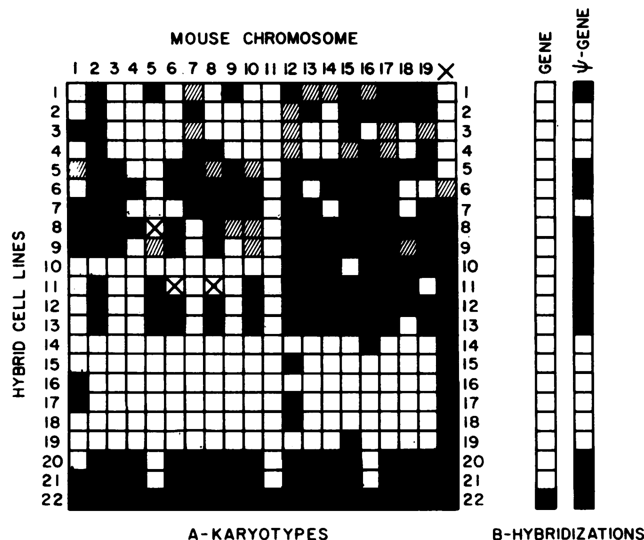

FIG. 2. Chromosomal assignment of the mouse p53 gene and pseudogene with a panel of mouse-Chinese hamster hybrid cell clones. (A) Distribution of mouse chromosomes in the mouse-Chinese hamster cell lines. ■, The hybrid cell line (numbered vertically) contains the mouse chromosomes (marked horizontally) in ≥30% of the cells analyzed. ▨, The hybrid contains a given chromosome in 15 to 29% of the cells. □, The hybrid contains the mouse chromosome in <15% of the cells analyzed or has lost it altogether. ☒, A given chromosome was detected by isozyme analysis only. The presence of a mouse-hybridizable DNA fragment is consistently detectable only if the carrier chromosome is present in >15% of the cells in a given hybrid (6). (B) Hybridization of pp53-176 DNA with DNA from the hybrid cell lines. The vertical columns indicate the hybrid cell lines tested for the presence of the mouse p53 gene and pseudogene, as marked above each column. The presence of a positively hybridizing mouse fragment in a given hybrid cell line is indicated by a solid square, and its absence by an open square. Chromosomal assignment was done by comparing the pattern of hybridization in (B) with the chromosome distribution in (A). The hybrid cell lines were formed between the Chinese hamster fibroblast cell line E36 and A/HeJ mouse peritoneal macrophages (MACH series), BALB/c fetal mouse fibroblasts (BEM series), BALB/c CMS4 tumor (TuCE), or BALB/c Meth A fibrosarcoma (MAE and mFE series). Rows: 1, MACH 7A13-3B3; 2, MACH 4A63; 3, MACH 4A64-A1; 4, MACH 4B31AZ3; 5, MACH 2A2; 6, MACH 2A2-B1; 7, MACH 2A2-C2; 8, BEM1-6; 9, BEM 1-4; 10, TuCE 12G/8; 11, TuCE 12G/5; 12, TuCE 12G/3; 13, TuCE 12G/9; 14, MAE 32; 15, MAE 4; 16, MAE 19A; 17, MAE 6A; 18, MAE 8C; 19, MAE 29; 20, mFE 2-1-2; 21, mFE 2-1-1; 22, mFE 11.

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