

Suppression of production of mouse 28S ribosomal RNA in mouse-human hybrids segregating mouse chromosomes

(gene regulation/ribosomal RNA genes/human fibrosarcoma/cell hybrids)

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ABSTRACT Mouse-human somatic cell hybrids that lose (segregate) human chromosomes produce only mouse 28S ribosomal RNA even when they retain copies of the human chromosomes that contain the genes for 28S ribosomal RNA. In contrast, mouse-human hybrid cells that segregate mouse chromosomes produce only human 28S ribosomal RNA even when they have retained copies of mouse chromosomes that contain the 28S ribosomal RNA genes.

Somatic cell hybrids produced by fusing mouse cells, derived from continuous cell lines, and human cells preferentially lose human chromosomes (1). These hybrids produce mouse 28S ribosomal RNA (rRNA) but not human 28S rRNA (2). The genes for rRNA are located on human acrocentric chromosomes (3, 4) and even hybrids containing 2 to 11 human acrocentric chromosomes have been found to produce only mouse 28S rRNA (5).

Recently, Goodpasture and Bloom have shown that the Ag-AS silver staining method preferentially stains the nucleolus organizer regions of chromosomes (6), which are the regions that contain the genes for rRNA (7). Miller *et al.* (8) applied this method to a series of mouse-human hybrid cells that segregated human chromosomes and found that the nucleolus organizer regions of mouse, but not of human, chromosomes were stained in the hybrid cells. These results suggest that this staining method does not detect simply the rRNA gene sites but rather detects those rRNA gene sites that were active in the preceding interphase (8).

Somatic cell hybrids between either mouse peritoneal macrophages or primary mouse teratocarcinoma cells and HT-1080-6TG human fibrosarcoma cells retain the entire complement of human chromosomes and preferentially lose mouse chromosomes (9, 10). In these hybrids the nucleolus organizer regions of the human chromosomes are stained by the Ag-AS method while those of the mouse chromosomes are not, even though the mouse chromosomes that carry rRNA genes are present (10). We have now examined the expression of mouse and human 28S rRNA in these hybrids as well as in mouse-human hybrids that segregate human chromosomes. In each case the 28S rRNA produced reflects only the species whose chromosomes are retained.

MATERIALS AND METHODS

Cells. HT-1080-6TG cells, which were derived from the HT-1080 human fibrosarcoma cell line and are deficient in hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) (8, 9), were fused with either peritoneal macrophages from BALB/c mice or primary

mouse teratocarcinoma cells in the presence of β -propiolactone-inactivated Sendai virus at pH 8.0 (11). Hybrids were selected in hypoxanthine/aminopterin/thymidine medium (12) as described previously (9).

In addition, HT-1080 cells were fused with TH02 (13) mouse cells that were derived from 3T3 cells of BALB/c origin and are deficient in hypoxanthine phosphoribosyltransferase. The hybrid cells were selected in hypoxanthine/aminopterin/thymidine medium containing 1×10^{-4} M ouabain because the TH02 cells are resistant to 3×10^{-3} M ouabain (13) while the human cells are killed at concentrations of 1×10^{-6} to 1×10^{-7} M ouabain (14).

Chromosome Analysis. Chromosomes of parental and hybrid cells were identified by banding patterns after quinacrine mustard or Giemsa staining according to methods previously described (15, 16). In some cases, the chromosomes were also stained by the Ag-AS method (6). The results of the karyologic analysis after quinacrine mustard and Ag-AS staining of the HT-1080-6TG \times BALB/c macrophage and HT-1080-6TG \times OTT6050 mouse teratocarcinoma hybrids have been reported from our laboratories (10).

Isozyme Analysis. Three somatic cell hybrids between TH02 mouse cells and HT-1080 human fibrosarcoma cells that lost a few human chromosomes and retained human chromosomes 13, 14, 15, 21, and 22 were studied for the expression of human purine-nucleoside phosphorylase (purine-nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1), mannosephosphate isomerase (D-mannose-6-phosphate ketal-isomerase, EC 5.3.1.8), and superoxide dismutase-1 (superoxide:superoxide oxidoreductase, EC 1.15.1.1), which have been assigned to human chromosomes 14, 15, and 21, respectively (17), to confirm the results of the karyologic analysis. The methods of separation of the mouse and human enzymes by starch and cellulose acetate gel electrophoresis have been described elsewhere (18-20).

Purification of 28S rRNA from Hybrids. Subconfluent cultures were labeled with [3 H]uridine ($5 \mu\text{Ci}$, $2 \mu\text{g}\cdot\text{ml}^{-1}$) for 15 hr at 37° . Cells were washed twice with Tris-buffered saline (pH 7.4). The cytoplasmic fraction was prepared as described (21), extracted twice with phenol, and precipitated with 2 volumes of 95% ethanol at -20° . RNA was dissolved in 0.5 ml of sodium dodecyl sulfate (NaDodSO₄) buffer [0.1 M NaCl, 0.01 M Tris-HCl (pH 7.2), 0.5% NaDodSO₄] and layered on a 15-30% sucrose gradient in the same buffer (17 ml). Gradients were centrifuged for 18 hr at 20° (25,000 rpm in an SW27 Spinco rotor); 0.5-ml samples were collected and assayed for aliquots radioactivity. The fractions containing 28S rRNA were pooled and precipitated as before.

Electrophoretic Analysis of 28S rRNA. Gels (35 cm) were prepared by polymerization of a mixture of 2.6% (wt/wt)

Abbreviations: rRNA, ribosomal RNA; NaDodSO₄, sodium dodecyl sulfate.

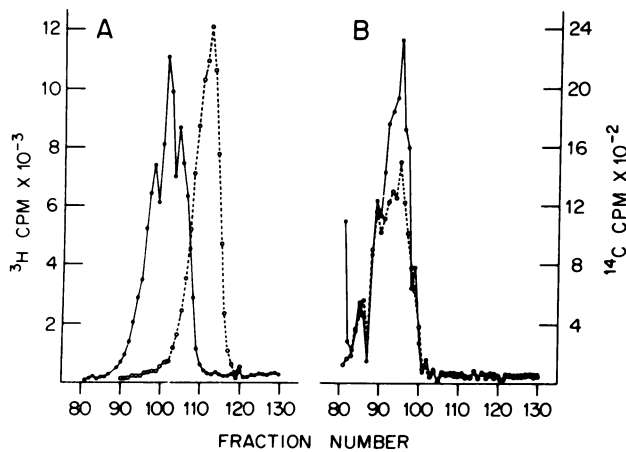


FIG. 1. ^3H -Labeled 28S rRNA derived from the hybrid 55-14 (solid line) was isolated after centrifugation in sucrose gradient (21) and was run with ^{14}C -labeled 28S rRNA derived from mouse TH02 cells (A) or human HeLa cells (B) (broken line). The RNAs were analyzed by polyacrylamide gel electrophoresis.

acrylamide and 0.13% (wt/vol) bisacrylamide in a buffer containing 20 mM Na acetate, 1 mM Na EDTA, 40 mM Tris (pH 7.2), 0.2% NaDodSO₄, and 10% glycerol. Previously purified 28S rRNA samples were dissolved in the same buffer and layered on the gels. Electrophoresis was for 24 hr in the same buffer without glycerol at 5 mA per gel. Gels were frozen and the portions containing 28S rRNA were cut into 2-mm slices, solubilized in 0.5 ml of NCS at 60° for at least 3 hr, and counted in a toluene-based scintillation fluid. Each electrophoresis run contained ^{14}C -labeled 28S rRNA markers prepared in the same way from TH02 mouse cells or HeLa cells.

RESULTS AND DISCUSSION

Three independent cell hybrid lines that segregated mouse chromosomes (9) were examined for the expression of mouse and human 28S rRNAs. Polyacrylamide gel electrophoresis gave a clear separation of mouse and human 28S rRNAs.

Hybrids 55-14 and 55-54 were derived from the fusion of BALB/c macrophages and HT-1080-6TG human fibrosarcoma cells and contained 19 and 18 different mouse chromosomes,

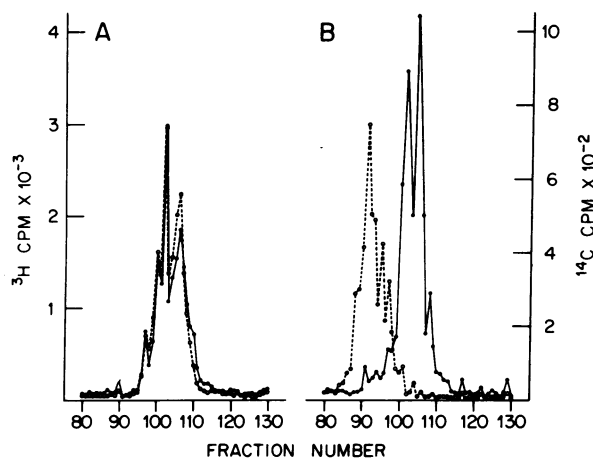


FIG. 2. ^3H -Labeled 28S rRNA derived from the hybrid 56-05F5 (solid line) that segregates human chromosomes was isolated after centrifugation in sucrose gradient and was run with ^{14}C -labeled 28S rRNA derived from mouse TH02 cells (A) or human HeLa cells (B) (broken line). The RNAs were analyzed by polyacrylamide gel electrophoresis.

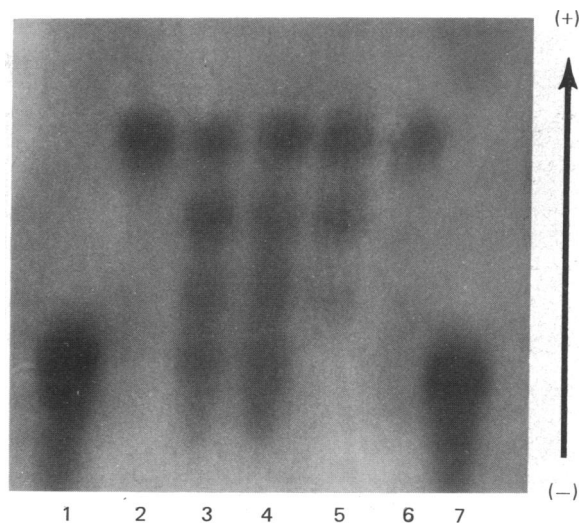


FIG. 3. Zymograms of nucleoside phosphorylase in starch gel. The extracts of HT-1080-6TG and HT-1080 cells are in lanes 1 and 7, respectively. The extract of TH02 mouse fibroblasts is in lanes 2 and 6. The extracts of three HT-1080 \times TH02 hybrid clones, 56-05F1, F4, and F5, are in lanes 3, 4, and 5, respectively. The two hybrids 56-05F1 and F4 express mouse and human nucleoside phosphorylase activities and two heteropolymers between the human and mouse enzymes. The hybrid 56-05F5 expresses mouse nucleoside phosphorylase activity and two heteropolymers between the mouse and human enzymes.

respectively, including mouse chromosomes 12, 15, and 18 where the rRNA genes are located (10). Hybrid 55-84 was derived from the fusion of OTT6050 mouse teratocarcinoma cells and HT-1080 cells and contained at least a copy of each mouse chromosome (10). Cells of hybrid 55-14 contained human but not mouse 28S rRNA (Fig. 1A). Similar results were obtained with the two other hybrid lines which had segregated mouse chromosomes. On the other hand, somatic cell hybrids between TH02 mouse cells and HT-1080-6TG human cells segregated human chromosomes (9) and produced only mouse and not human 28S rRNA (Fig. 2). The absence of human 28S rRNA was not due to the absence of the human chromosomes known to carry the 18S and 28S rRNA genes because these hybrid cells expressed the human forms of nucleoside phosphorylase (Fig. 3), mannosephosphate isomerase (Fig. 4), and superoxide dis-

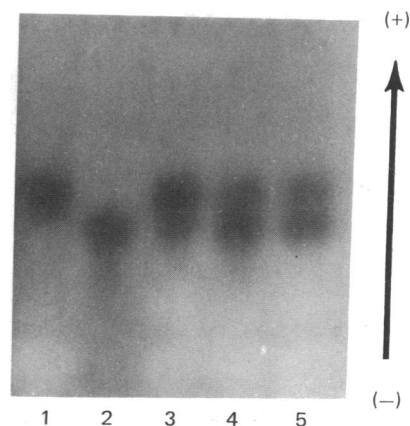


FIG. 4. Zymograms of mannosephosphate isomerase in starch gel (dark bands). The extract of TH02 cells is in lane 1. The extract of HT-1080 cells is in lane 2. The extracts of the hybrids 56-05F5, F4, and F1 are in lanes 3, 4, and 5, respectively. The hybrids express both mouse and human enzyme activities.

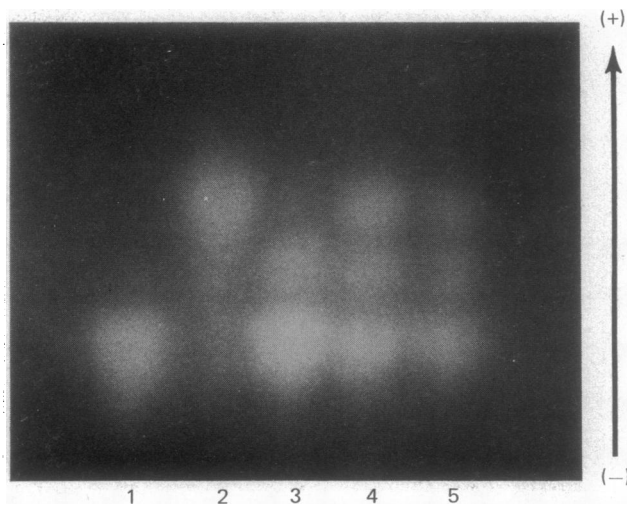


FIG. 5. Zymograms of superoxide dismutase-1 in starch gel. The extract of TH02 cells is in lane 1. The extract of HT-1080 cells is in lane 2. The extracts of hybrids 56-05F5, F4, and F1 are in lanes 3, 4, and 5, respectively. Hybrid 56-05F5 expresses the mouse enzyme and the heteropolymer between the mouse and human enzymes. Hybrids F4 and F1 express the mouse and human enzymes and a heteropolymer between them.

mutase-1 (Fig. 5) and retained the human chromosomes 13, 14, 15, 21, and 22 (Fig. 6) that contain the genes for rRNA.

The results indicate that mouse-human somatic cell hybrids

which segregate human chromosomes produce only mouse and not human 28S rRNA even though they have retained the human chromosomes containing the genes for rRNA. These results confirm previous data by Eliceiri and Green (2) and Marshall *et al.* (5) that the production of human 28S rRNA is suppressed in hybrid cells. Such results are in striking contrast to the results obtained with the mouse-human hybrids segregating mouse chromosomes, in which case the production of mouse 28S rRNA was suppressed. The absence of the mouse 28S rRNA is not due to the loss of mouse chromosomes that carry the structural genes since all three hybrids contained these mouse chromosomes at the time the analysis for 28S rRNA was conducted (10). It is also unlikely to be due to the specific loss of any other mouse chromosome, because the hybrid 55-84 appears to contain at least one copy of each mouse chromosome (10) but does not express mouse 28S rRNA. Deletion of a short segment of a chromosome could, however, have escaped detection. Because mouse-human heterokaryons produce both mouse and human 28S rRNAs (5), it appears likely that the suppression of the production of the 28S rRNA of one species occurs either at the synkaryon stage or after the formation of the daughter hybrid cells.

The mechanism for suppression of the production of the 28S rRNA of one of the two species is not yet known. Previous studies have demonstrated that human nucleolus organizer activity as detected by the Ag-AS silver staining method is suppressed in hybrids segregating human chromosomes (Fig. 6 and ref. 8) and that mouse nucleolus organizer activity is

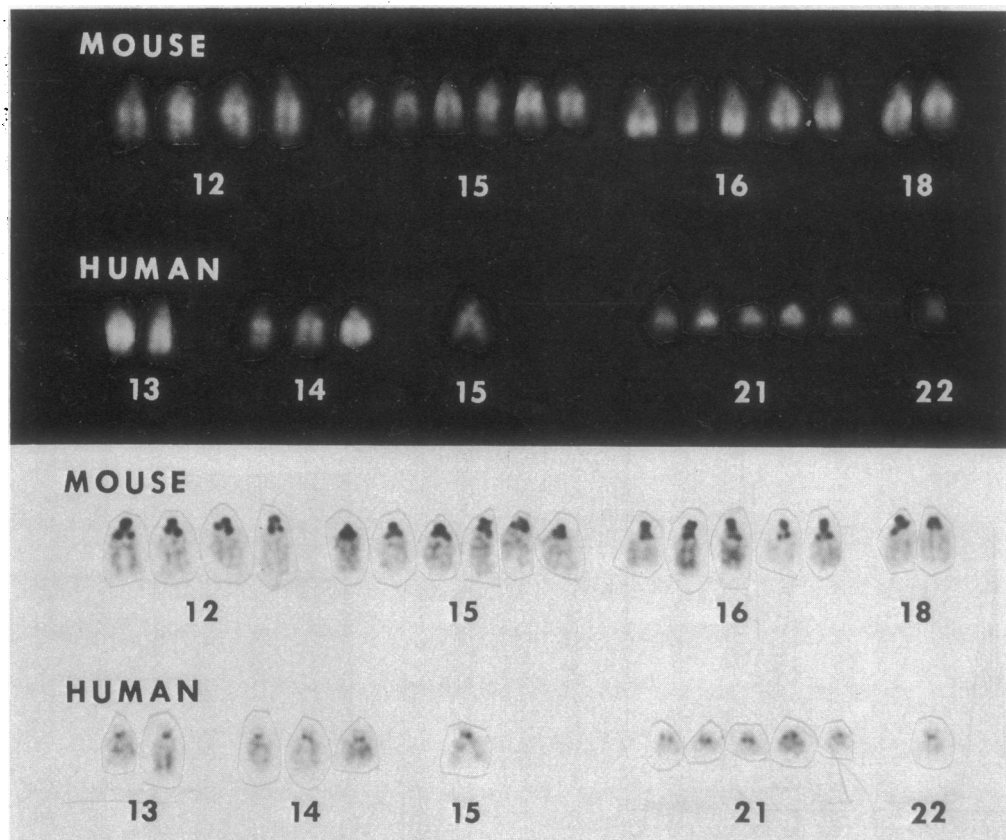


FIG. 6. Chromosomes from a cell of the HT-1080 \times TH02 hybrid clone 56-05F5, stained with quinacrine to identify the chromosomes and then by the Ag-AS method. The cell had 103 normal and 53 abnormal mouse chromosomes and 41 human chromosomes. Shown are mouse chromosomes 12, 15, 16, and 18 that have Ag-AS stained nucleolus organizer regions close to the centromeric heterochromatin. These are the same chromosomes that have Ag-AS stained nucleolus organizer regions in BALB/c mice (8), from which the TH02 line was derived. Also shown are all the human D and G group chromosomes in the cell. These show minimal staining of the centromeric heterochromatin but no Ag-AS stained nucleolus organizer region. Each of these chromosomes has an Ag-AS stained nucleolus organizer region in the human parental HT-1080 line (8).

suppressed in hybrids segregating mouse chromosomes (10). Thus, it seems likely that the suppression occurs at the transcriptional level. In interspecific *Xenopus* hybrids, in which there is a comparable suppression of nucleolus organizer activity (22) and rRNA (23) of one parental species, suppression has been shown to occur at a transcriptional level (23). Suppression might also be due to a failure to process the 45S precursor into 28S rRNA. However, the previous results (8, 10) make this hypothesis unlikely.

There is a point of interest regarding the relationship between chromosome segregation and suppression of rRNA production in mouse-human hybrids. The striking correlation between chromosome loss and suppression of rRNA production of the species whose chromosomes are segregated suggests a direct relationship between the two events, but we have no clues as yet as to what molecular mechanisms may be responsible for these phenomena. It is quite possible that suppression of rRNA genes actually precedes and determines the direction of chromosome segregation. It is also possible that this suppression involves other cellular functions.

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