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Expression of human and suppression of mouse nucleolus organizer activity in mouse–human somatic cell hybrids

(chromosomes/ribosomal RNA/gene regulation/histochemical stain)

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ABSTRACT **Most mouse–human somatic cell hybrids show preferential loss of human chromosomes, absence of human 28S ribosomal RNA, and suppression of human nucleolus organizer activity, as visualized by the Ag-AS silver histochemical stain. In contrast, the mouse–human hybrids studied here show preferential loss of mouse chromosomes. The hybrids were made by fusion of HT-1080-6TG human fibrosarcoma cells with BALB/c mouse peritoneal macrophages or strain 129 mouse teratocarcinoma cells. The Ag-AS staining method shows nucleolus organizer activity of chromosomes 13, 14, 15, 21 (rarely), and 22 in the human parent and chromosomes 12, 15, 16 (rarely), and 18 in the BALB/c mouse parent. In the hybrid cells the human nucleolus organizer regions are active, as shown by Ag-AS staining and involvement in "satellite association." The mouse nucleolus organizer regions are not stained by the Ag-AS method even though mouse chromosomes 12, 15, and 18 are present in the BALB/c hybrids and at least one copy of each mouse chromosome is present in the teratocarcinoma-derived hybrids. Thus, in these mouse–human hybrids, unlike those that lose human chromosomes, only human nucleolus organizer activity is expressed, and mouse nucleolus organizer activity is suppressed.**

Mouse–human somatic cell hybrids tend to retain mouse chromosomes and lose human chromosomes (1). Hybrids of this type produce only mouse, and not human, 28S ribosomal RNA (rRNA) (2–4), suggesting that the absence of the human rRNA may be related to the loss of human chromosomes. It is not due to the absence of the human acrocentric chromosomes, which carry the structural genes for rRNA (5, 6), because Marshall *et al.* (4) found no human 28S rRNA in a large series of mouse–human hybrids that contained 2 to 11 human acrocentrics.

The chromosome regions that carry the rRNA genes have been identified as the nucleolus organizer regions (NORs) (7), and these regions can be stained preferentially by the Ag-AS silver staining method (8). In human diploid cell cultures the Ag-AS method stains the short arm regions of most of the acrocentric chromosomes (9, 10). The NORs of the same human acrocentric chromosomes are not stained in a mouse–human hybrid that has lost some human chromosomes (11). There is no evidence to suggest that rRNA genes are deleted from the human acrocentrics in hybrid cells. Therefore the absence of Ag-AS stain suggests that this method detects only chromosome regions that functioned as nucleolus organizers in the preceding interphase, and, by implication, produced rRNA.

Somatic cell hybrids between either mouse peritoneal macrophages or mouse cells obtained from a teratocarcinoma and HT-1080 human fibrosarcoma cells retain human chromosomes and lose mouse chromosomes (12). If preferential chromosome elimination is closely correlated with preferential suppression of nucleolus organizer activity, these hybrids should express only human nucleolus organizer activity. This appears to be the case.

Abbreviation: NOR, nucleolus organizer region.

METHODS

BALB/c mouse peritoneal macrophages were obtained according to a modification of the procedure described by Cohn and Benson (13) and were fused with HT-1080-6TG human fibrosarcoma cells deficient in hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) (12) in the presence of β-propiolactone-inactivated Sendai virus at pH 8.0 (14). The fused cultures were maintained in hypoxanthine–aminopterin–thymidine (HAT) selective medium (15). Large colonies of hybrid cells were visible 3–4 weeks after fusion. The colonies were picked and, subsequently, grown. OTT6050 mouse teratocarcinoma cells were obtained from a solid teratocarcinoma of a strain 129 mouse (16, 17) by cutting in small fragments in trypsin/EDTA, resuspending in Eagle's minimal essential medium (MEM), and filtering through sterile gauze. The teratocarcinoma cells were fused with HT-1080-6TG cells in the presence of β-propiolactone-inactivated Sendai virus. Hybrid colonies were selected in hypoxanthine–aminopterin–thymidine medium.

Hybrid cells were maintained in Eagle's medium supplemented with 10% fetal calf serum. Mitotic cells were shaken from the culture flasks and transferred to a centrifuge tube containing 0.1 ml of colcemid (10 μg/ml) for every 10 ml of medium and the tubes were centrifuged immediately at 800 rpm in an IEC clinical centrifuge for 7 min. The cell pellet was resuspended in 75 mM KCl. After 10 min the suspension was centrifuged and the cells were fixed for an hour in freshly prepared 3:1 methanol:acetic acid. After two changes of fixative the cells were dropped onto cold wet slides, which were allowed to air dry.

Quinacrine staining was done by the method described by Miller *et al.* (18). Well-spread metaphases were photographed on H & W Control film. Silver staining was carried out by the Ag-AS method of Goodpasture and Bloom (8). In order to minimize staining of the C-band regions of the mouse chromosomes, some slides were stained by a modification of the Ag-AS method: two drops of 50% $AgNO_3$ solution were placed on a slide, which was then covered with a coverslip. The slide was placed on a moist paper in a petri dish and left overnight at 50°. The slide was washed in distilled water, developed in 3% (vol/vol) formalin at pH 4.5 for 10–15 min, rinsed in distilled water, and air dried. The metaphase spreads that had been photographed to show quinacrine banding patterns were relocated and photographed on Kodak high-contrast copy film.

Photographic prints of the same cell stained with quinacrine, to identify each chromosome, and with Ag-AS, for nucleolus organizer staining, were cut out simultaneously and double

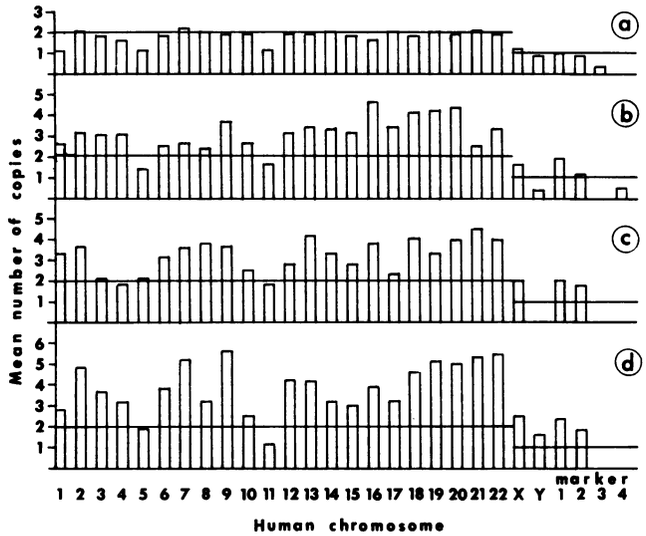

FIG. 1. Distribution of human chromosomes in (a) near diploid cells of the parent HT-1080-6TG and in three mouse–human hybrids derived from it: (b) 55-14, (c) 55-54, and (d) 55-84. The horizontal line indicates a diploid complement: two copies of each autosome and one of the X and Y. The hybrid cells have a greater than diploid complement. Single copies of numbers 11 and 5 are present in markers 1 and 2, respectively.

karyotypes were prepared. Ten cells from HT-1080-6TG and 10–12 from each hybrid were karyotyped and the number of copies of each kind of human and mouse chromosome was scored. The location of Ag-AS stained regions, which appeared as very dark areas, was also noted. Three cells from BALB/c were double karyotyped and an additional 13 were scored for the location of Ag-AS-stained regions.

RESULTS

The HT-1080 human fibrosarcoma cell line (19) and its 6-thioguanine-resistant HT-1080-6TG derivative are heteroploid. The latter has a modal number of 46 (41–48) chromosomes but

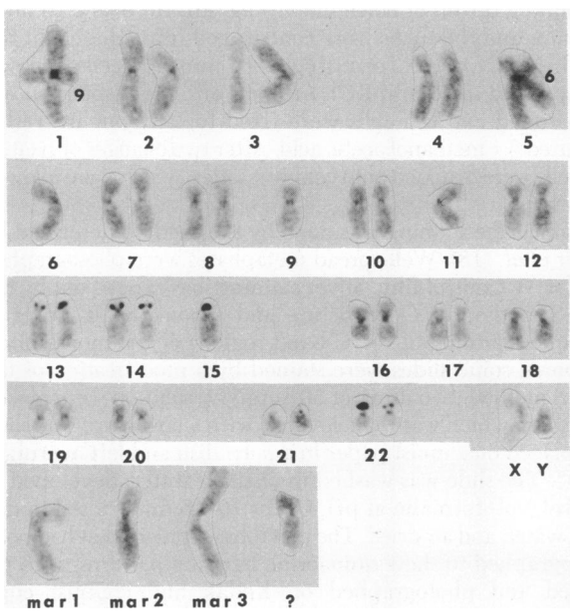

FIG. 2. Ag-AS-stained metaphase chromosomes of a human HT-1080-6TG fibrosarcoma cell. The chromosomes were identified by quinacrine banding of the same cell. Numbers 13, 14, 15, and 22 show Ag-AS stain.

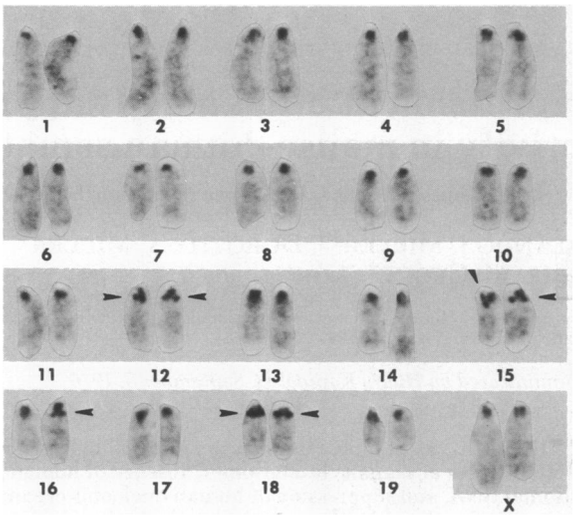

FIG. 3. Ag-AS-stained metaphase chromosomes from a diploid BALB/c cell. The chromosomes were identified by quinacrine banding of the same cell. Each chromosome is stained in the C-band region; three pairs, 12, 15, and 18, and a single 16, are stained by Ag-AS in the nucleolus organizer region.

with cells having as few as 21 and as many as 178 chromosomes. Most of the chromosomes appear normal, and the near-diploid cells have an average of two copies per cell of most chromosomes and a single copy of numbers 1, 5, 11, X, and Y (Fig. 1a). Also present are three marker chromosomes, probably derived from chromosomes 1 (mar 3), 5 (mar 2), and 11 (mar 1). Thus, the total chromosome complement approximates the diploid. Ag-AS staining shows active NORs on both members of the acrocentric chromosome pairs 13, 14, 15, and 22, and rarely on a chromosome 21 (Fig. 2). The relative amount of Ag-AS stain varies from chromosome to chromosome in any metaphase spread.

Ag-AS staining was carried out on a diploid BALB/c metaphase preparation. All the mouse chromosomes show some staining in the C-band region. The mouse NORs, which are very darkly stained by Ag-AS, are located just distal to the C-band region. If the C-band is darkly stained it is therefore difficult to distinguish the Ag-AS -positive region. In a sample of 17 BALB/c cells that show Ag-AS-positive regions, the chromosomes involved are number 15 (about two copies per cell), numbers 12 and 18 (about one copy per cell), and rarely number 16 (about one copy per three cells) (Fig. 3).

All three hybrids are of the type in which mouse chromosomes are reduced in number. The BALB/c peritoneal macrophage × HT-1080-6TG hybrid 55-14, for example, has a mean of 76 (range 61–87) human and 18 (11–23) mouse chromosomes. Copies of every human chromosome are present (Fig. 1b). There are about three to four copies of each human autosome, except for numbers 5 and 11, and about 1.5 copies of the X chromosome, suggesting a 2:1 fusion with a double input of human chromosomes in the original hybrid. Two copies of mar 1 and one copy of mar 2 are present. Of all the human chromosomes only the Y could be underrepresented, although mar 4 appears to be derived by translocation of at least part of the Y to the short arm of human chromosome 7 (Fig. 4a). A very small number of otherwise unidentifiable chromosomes of presumptive human origin are also present. The NORs on human acrocentric chromosomes 13, 14, 15, and 22 and in some cells 21 are stained by the Ag-AS method (Figs. 4b and 5a).

The mouse chromosome complement of hybrid 55-14 is greatly reduced, with less than a haploid genome present (Fig.

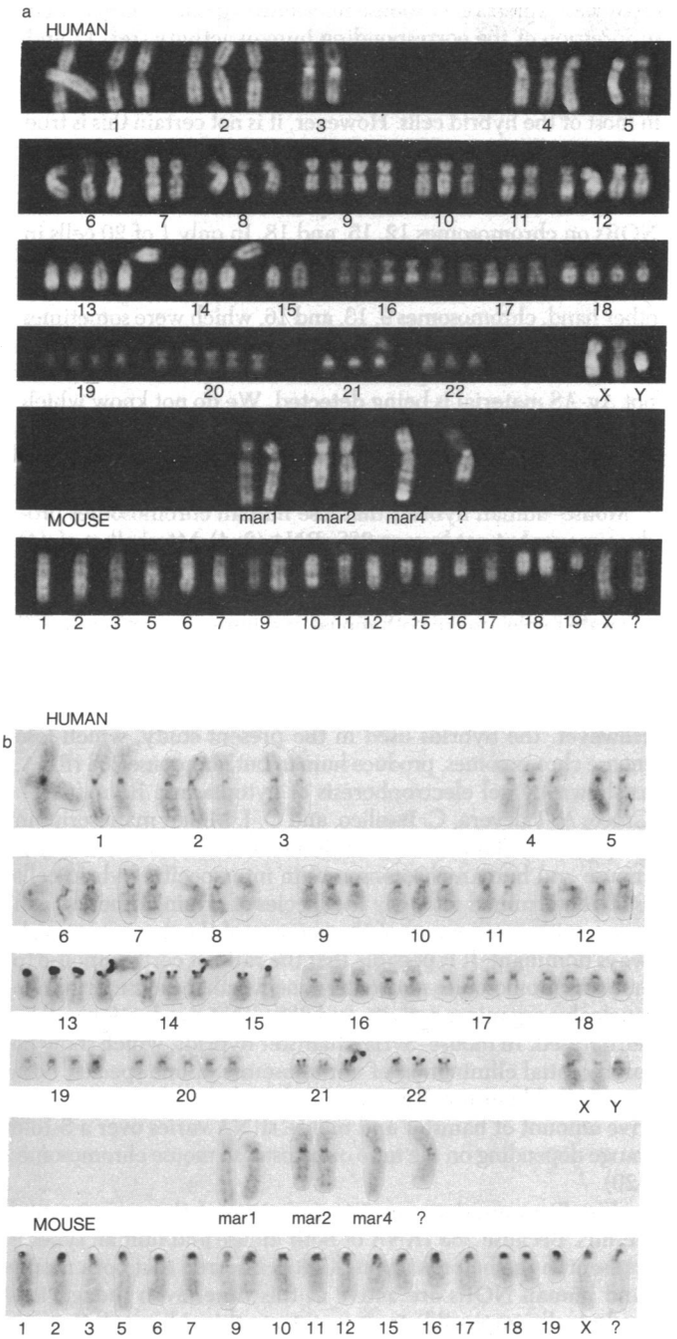

FIG. 4. (a) Quinacrine band karyotype of a hybrid 55-14 cell which has 84 human and 20 mouse chromosomes. The probable derivations of the human marker chromosomes are: mar 1 from number 11, mar 2 from 5, and mar 4 from 7 and the Y. (b) Ag-AS karyotype of the cell shown in (a). Ag-AS stain is seen on human chromosomes 13, 14, 15, 21, and 22. Three satellite associations (13–14, 14–15, and 21–22) are present. Small amounts of Ag-AS stain are also found in the centromeric region of each human chromosome, and in the C-band region of each mouse chromosome. No mouse nucleolus organizer region is stained.

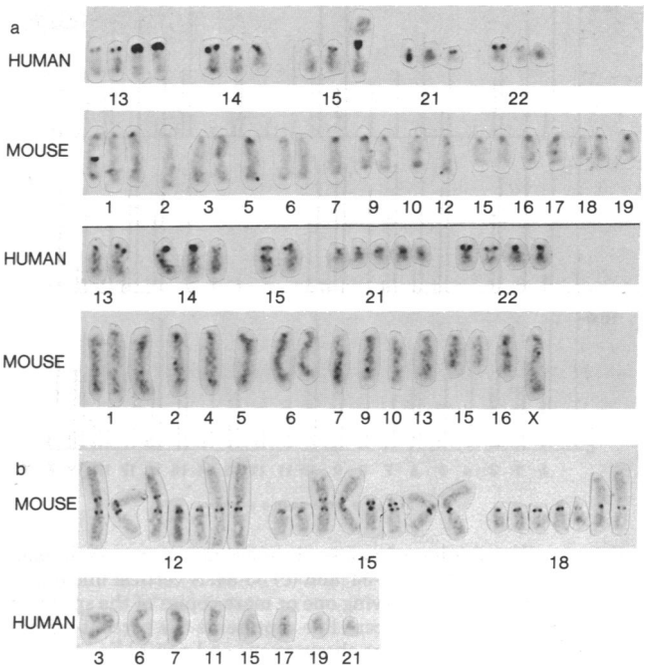

FIG. 5. (a) Partial karyotypes from two cells of hybrid 55-14 stained with Ag-AS under conditions that minimize staining of mouse C-band regions. The nucleolus organizer regions of most of the human acrocentric chromosomes are stained, while those of the mouse chromosomes are not. (b) Partial karyotype from a cell of a RAG × human hybrid that has lost most of its human chromosomes, stained as in (a). All the chromosomes which are Ag-AS stained and all the identifiable human chromosomes are included. RAG is derived from BALB/c and the chromosomes that have nucleolus organizer regions stained are all derived from mouse chromosomes 12, 15, and 18. The human chromosomes are not stained.

4a). Mouse chromosome 14 was not seen in any of the 10 cells scored, and chromosomes 4 and 12 were seen only once (Fig. 6a). (In a different subclone of this hybrid, mouse chromosome 12 is present but numbers 4, 8, 13, and 14 are absent, or present in only one of 10 cells.) Most of the mouse chromosomes are present in a single copy per cell, with zero and two copies per cell occasionally present. A small number of unidentifiable chromosomes of presumptive mouse origin are also present; their species of origin was decided on the basis of the C-banding. In any one cell, all the mouse chromosomes are stained to about the same extent in the C-band region. In seven of the cells it is clear that no mouse NOR is stained, since the mouse chromosomes are faintly stained even though the human chromosomes in the same cell show dark Ag-AS stain in NORs (Figs. 4b and 5a). In the remaining three cells, chromosomes 10, 16, and 13, plus 16 respectively, have dark Ag-AS staining in what might be an NOR, as well as in the C-band region.

In order to show the appearance in a hybrid cell of mouse chromosomes that have NORs stained by Ag-AS, we have included a partial karyotype from a mouse–human hybrid that lost human chromosomes (Fig. 5b). The mouse parent is the RAG cell line, which is of BALB/c origin, and the chromosomes that are Ag-AS stained are all derived from mouse chromosomes 12, 15, and 18. The NORs of the human acrocentric chromosomes in this hybrid are not stained.

The BALB/c peritoneal macrophage × HT-1080-6TG hybrid 55-54 is similar to 55-14, having 78 (50-84) human and 20 (12-25) mouse chromosomes per cell, but a greater number of mouse chromosomes (4, 11, 12, 13, and 14) are absent or present rarely (Fig. 6b). The human Y chromosome and mar 4 are both absent (Fig. 1c). As in 55-14, human acrocentric chromosomes are stained by Ag-AS. Six of the cells have no mouse NOR stained. In the remaining four cells the mouse chromosomes that may have NOR staining as well as C-band staining are numbers 9 (three cells) and 15, 16, and 19 (one cell each).

Hybrid 55-84, which has the same human parent but a different mouse parent (OTT6050) than hybrids 55-14 and 55-54,

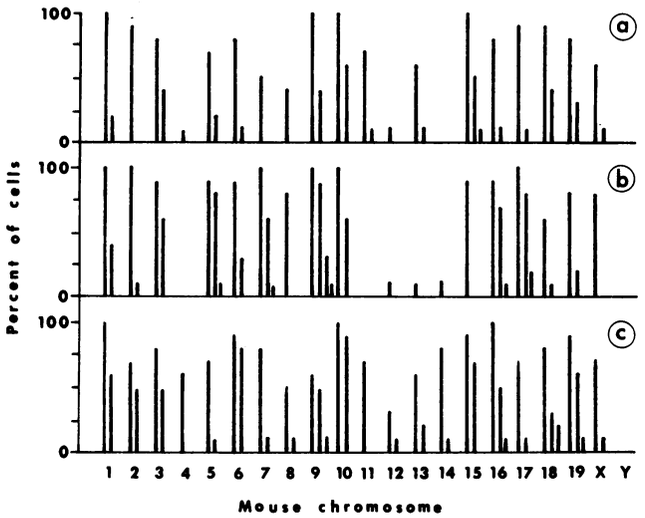

FIG. 6. Distribution of mouse chromosomes in mouse–human hybrid cells (a) 55-14, (b) 55-54, and (c) 55-84. A vertical line represents the percent of cells having one or more copies of the specified chromosome; a second adjacent line represents the percent of cells with two or more copies of the chromosome and a third line three or more copies.

has a larger number of chromosomes, with a mean of 97 (67-114) human and 23 (6-30) mouse chromosomes. Each human chromosome is present about half the time in three to four copies per cell, about a quarter of the time in five to six copies, and sometimes even in seven to eight copies (Fig. 1d). Every mouse chromosome is present in at least 30% of the cells (Fig. 6c). As in the other two hybrids, Ag-AS staining of the NOR is seen on the human acrocentric chromosomes. In seven of the cells no mouse chromosome has an Ag-AS-positive region. In the three remaining cells, chromosomes that could have Ag-AS staining are 9, 13, and 15 (two cells).

In all three hybrid cell lines fusion of the silver-stained regions of two or more human acrocentric chromosomes is sometimes seen (Fig. 4b and 5a). This corresponds to what is usually called satellite association.

DISCUSSION

The secondary constriction regions of the human acrocentric chromosomes are stained by the Ag-AS method in the mouse–human hybrids that have lost some of their mouse chromosomes. This finding, and their involvement in satellite associations, indicates that these human chromosomes are functioning as nucleolus organizers. In most of the cells it is clear that no mouse NORs are stained by the Ag-AS method. This is not due to the loss of the mouse chromosomes that carry the structural genes for rRNA (numbers 12, 15, 18, and sometimes 16 in BALB/c) because chromosomes 15 and 18 are present in high frequency in the hybrids (55-14 and 55-44) that were derived from BALB/c. Furthermore, it is unlikely to be due to the loss of a specific mouse chromosome that does not have an NOR because hybrid 55-84 has copies of every mouse chromosome. All these chromosomes appear normal, although very small chromosome changes might not be detected. Although no 55-84 cell has every type of mouse chromosome, no specific chromosome is absent from all the Ag-AS-negative cells. Therefore, our findings suggest that the mouse rRNA genes are present but are suppressed. These results are in striking contrast to our earlier findings in mouse–human hybrids from which *human* chromosomes were preferentially eliminated. In those cases, there was expression of mouse nucleolus organizer activity and suppression of the corresponding human activity (ref. 11 and Fig. 5b).

It is clear that the activity of the mouse NORs is suppressed in most of the hybrid cells. However, it is not certain this is true in every cell because in some cells it is difficult to distinguish mouse C-band from Ag-AS-positive material. Hybrids 55-14 and 55-54 should resemble the parental BALB/c, which has NORs on chromosomes 12, 15, and 18. In only 1 of 20 cells in these two hybrids is a chromosome 15 stained in the region that contains the NOR and in no cell is a 12 or 18 stained. On the other hand, chromosomes 9, 13, and 16, which were sometimes scored as Ag-AS-positive, are chromosomes which have large C-bands, and it seems probable that in every case C-band and not Ag-AS material is being detected. We do not know which chromosomes carry NORs in strain 129, from which the OTT6050 line was derived, but there is no evidence that any specific mouse chromosome is Ag-AS stained in the hybrid.

Mouse–human hybrids that lose human chromosomes produce mouse but not human 28S rRNA (2–4). Marshall *et al.* (4) showed that mouse–human heterokaryons, which provide an environment in which the mouse and human genomes are in separate nuclei, make both mouse and human cytoplasmic 28S rRNA. They suggested that suppression of human 28S rRNA production in these hybrid cells is due to a macromolecular mouse gene product that is unable to enter the human nucleus. However, the hybrids used in the present study, which lose mouse chromosomes, produce human but not mouse 28S rRNA, as shown by gel electrophoresis of cytoplasmic RNA (C. M. Croce, A. Talevera, C. Basilico, and O. J. Miller, manuscript in preparation). Thus, our results indicate that the interaction of mouse and human chromosomes in interspecific hybrid cells is not determined solely by the species of origin of the two cell types, with components of the genome of the same species always dominant. It is possible that the ratio of certain mouse to human chromosomes may determine whether mouse or human nucleolus organizer activity and 28S rRNA production will be suppressed. In mouse–Syrian hamster hybrids, which show no preferential elimination of chromosomes of one species, both mouse and hamster rRNA are produced (20, 21), but the relative amount of hamster and mouse rRNA varies over a 5-fold range depending on the ratio of hamster to mouse chromosomes (20).

Fig. 7 summarizes our interpretation of the experimental results. Because 28S rRNA of both mouse and human types is present in the heterokaryon (4), it is inferred that both mouse and human NORs are active at this time, even though it is technically impossible to demonstrate this. Although the heterokaryon data indicate that suppression must take place after the formation of a common nucleus containing both mouse and human genomes, it is not known whether this occurs during the synkaryon stage or only after chromosomes have been lost from either the human or mouse set. Nor is it clear whether the loss of chromosomes is the cause or the result of the suppression of nucleolus organizer activity by one genome. In maize the nucleolus organizer activity of one chromosome may be prevented by deletion of a locus on a different chromosome (22). This does not appear to be the explanation in somatic cell hybrids, since mouse NORs were suppressed in hybrid 55-84, in which a copy of every mouse chromosome is present in a large proportion of cells. Conversely, human NORs were suppressed in two mouse–human hybrids that had lost some human chromosomes, but in which every type of human chromosome was still present in a large proportion of the cells (11).

One of the most interesting conclusions to be drawn from these studies is that the Ag-AS method detects sites of rRNA

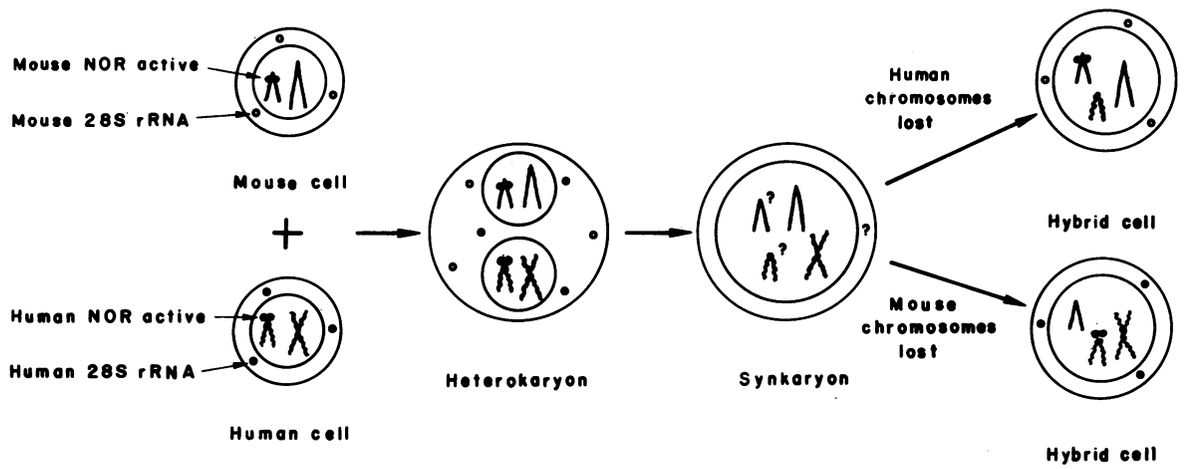

FIG. 7. Diagram showing the correlated suppression of activity of mouse nucleolus organizer regions (NORs) and suppression of production of mouse cytoplasmic 28S ribosomal RNA (○) in mouse–human hybrids that have lost some mouse chromosomes, and the correlated suppression of activity of human NOR and suppression of human 28S RNA (●) in hybrids that have lost some human chromosomes.

gene activity and can therefore be used to study the regulation of rRNA. Presumably, the same kind of regulation of ribosomal genes and nucleolus organizer activity that is seen in interspecific hybrids also occurs in diploid organisms (23). In the human, rRNA genes have been found on all five pairs of acrocentric chromosomes (5, 6) but Ag-AS staining is usually present on only a fraction of the 10 chromosomes (9, 10). Furthermore, the maximum number of nucleoli observed per human diploid cell, even in fetal liver, is six (24). It seems likely that not all rRNA genes in the average human complement are expressed. This may explain why a small amount of silver stain is seen on a chromosome 21 in only some cells of the HT-1080 parent and of the hybrids in the present study. The results obtained in interspecific hybrids suggest that the regulation operates across species barriers and the availability of mouse–human hybrids that have lost either mouse or human chromosomes provides a tool for further investigation of the genetic basis of the regulation of rRNA production.

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