

THE RIBOSOMAL RNA OF HAMSTER-MOUSE HYBRID CELLS

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ABSTRACT

The ribosomal RNA (rRNA) of a series of hamster-mouse somatic cell hybrids was studied. Mouse 28S rRNA was separated from its hamster counterpart by a two-step procedure involving sucrose gradient centrifugation of ribosomes and polyacrylamide gel electrophoresis of rRNA. Both hamster and mouse types of rRNA were synthesized in the 11 hybrids tested, including hybrids containing only about one-half the haploid number of either mouse or hamster chromosomes. It appears that, for both hamster and mouse rRNA, when the chromosomes of one species constituted the majority of the chromosomes of a hybrid, a disproportionately higher percentage of rRNA of that species was present in the hybrid. Some hybrid clones, having a majority of mouse chromosomes, had a mouse rRNA cell concentration approximately four to five times higher than the concentration expected from linear extrapolation of the value found for the mouse parental cell line.

It has been shown that, in human-mouse hybrid cells, only mouse 28S ribosomal RNA (rRNA) can be detected, even in hybrids having up to 35 human chromosomes, including those believed to carry the rRNA genes (1, 2).

We have recently reported that in a hamster-mouse hybrid, T6aE-8A, both hamster and mouse rRNA were synthesized (3). The determination of hamster and mouse rRNA was based on two previous findings. First, Reader and Stanners (4) found that in the presence of 0.02 M Mg^{++} and 0.1 M K^+ the majority of the free ribosomes of hamster sediment in a sucrose gradient as dimers, while those of mouse sediment mostly as monomers. Second, we found that hamster 28S rRNA migrates faster than mouse rRNA on polyacrylamide gel electrophoresis (5).

In the present report, the rRNA of a series of hamster-mouse hybrids (T6aE) has been studied by combining these two procedures. The ratio of hamster to mouse chromosomes varied within a 50-fold range among the 11 hybrid clones studied,

and the chromosome content, for each parental type, varied from about one-half haploid to about six times the haploid number of that particular species.

MATERIALS AND METHODS

The mouse parental cell line, 3T3-4E (6), the hamster parental cell line, T6a (7), and the hamster-mouse hybrids, T6aE (8), have been previously described. All cells were grown in Petri dishes in Dulbecco's modification of Eagle's medium (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 10% bovine serum.

For determinations of hamster-mouse rRNA ratios, three semiconfluent 84 mm diameter plates of each cell type were incubated with uridine-5- 3H (about 25 Ci/mole, 20-30 $\mu Ci/ml$ of medium) for about 24 hr, or with carrier-free ^{32}P (about 15 $\mu Ci/ml$ of medium) for 14-16 hr. The cells were harvested and the ribosome content was analyzed by sucrose gradient centrifugation, according to Reader and Stanners (4). Briefly, the cells were removed from the plates by incubation with 0.1% trypsin for 5 min at 37°C and washed with phosphate-buffered

saline (0.14 M NaCl, 0.003 M KCl, 0.02 M MgCl₂, 0.01 M tris(hydroxymethylaminomethane (Tris)-HCl, pH 7.4); the pellet was resuspended in H buffer (0.1 M KCl, 0.02 M MgCl₂, 0.01 M Tris-HCl, pH 7.4) and incubated in ice for 20 min. Brij-58 (Atlas Chemical Industries, Inc., Wilmington, Del.) was then added to a final concentration of 0.5%, and the cells were broken with a Dounce glass homogenizer. After spinning down nuclei and heavy debris for 5 min at 500 g, the supernatant was layered on top of a linear gradient of 15–30% sucrose in H buffer. The gradient was then centrifuged for 5 hr at 24,500 rpm and 4°C, in a Spinco SW25.1 rotor (Spinco Div. of Beckman Instruments, Inc., Fullerton, Calif.). Fractions of 0.65 ml were collected and samples were precipitated with 5% trichloroacetic acid, filtered, and counted by liquid scintillation. The ribosomal fractions from sucrose gradients were precipitated by addition of 0.1 vol of 20% potassium acetate, pH 5.1, and 1 vol of isopropanol. The resulting ribosomal pellets were then resuspended in 0.5% sodium dodecyl sulfate (SDS) and analyzed by 35 cm long, 2.4% polyacrylamide gel electrophoresis. The polyacrylamide gel electrophoresis was carried out as described by Loening (9), with some modifications. Plexiglas tubing (Rohm and Haas Co., Philadelphia, Pa.) of 6–7 mm internal diameter was used, and the gels were 35 cm long. The composition of the gels was 2.4% acrylamide, 0.12% *N,N'*-methylenebisacrylamide, 0.04 M Tris-HCl, pH 7.2, 0.02 M sodium acetate, 0.001 M ethylenediaminetetraacetic acid (EDTA), 10% glycerol, 0.2% SDS, 0.08% *N,N,N',N'*-tetramethylethylenediamine, and 0.08% ammonium persulfate. The electrophoresis buffer, made up of Tris-HCl, pH 7.2, sodium acetate, EDTA, and SDS, in the same concentrations as in the gel, was recirculated throughout the run between the cathodic and anodic chambers with a pump. Electrophoresis was carried out at room temperature, at 5 ma/gel, for 50 hr. The desired portion of the gel was frozen with dry ice and fractionated with a Mickle gel slicer (Mickle Laboratory Engineering Co., England) into 1-mm thick slices. Each slice was incubated overnight at room temperature with 0.35 ml of toluene and 0.05 ml of NCS (Amersham-Searle Corp., Arlington Heights, Ill.). Each fraction was counted in a liquid scintillation counter after addition of 10 ml of scintillation mixture containing a 3½ ratio of toluene and ethylene glycol monomethyl ether. For each electrophoresis the unknown sample was mixed with a hamster or mouse 28S rRNA marker labeled either with ³H or with ¹⁴C, depending upon whether the unknown was labeled with ³²P or ³H, respectively.

Metaphase chromosome preparations were made by the method of Rothfels and Siminovitch (10). From the modal numbers of acrocentric, telocentric,

and bi-armed chromosomes in each hybrid, the hamster and mouse complements were estimated as described by Basilico et al. (8). The extreme chromosome numbers were usually within ± 15% of the modal numbers.

To determine the concentration of rRNA per cell, six semiconfluent 84 mm diameter plates of each cell type were grown for about 24 hr in the presence of uridine-5-³H (about 15 μCi/ml medium, about 25 mCi/μmole). The cells were then harvested in phosphate-buffered saline, counted in duplicate, and frozen in identical fractions. Some samples were then thawed, phenol extracted at 55°C (this was also done at room temperature), and their ³H cpm/OD 260 ratio was determined, before and after digestion with RNase-free DNase I (electrophoretically purified, Worthington Biochemical Corp., Freehold, N. J.), by polyacrylamide gel electrophoresis. The measurement of OD 260 in polyacrylamide gels was calibrated by quantitative addition of known samples of RNA to gels. Other samples were thawed and phenol extracted after being mixed with a series of identical aliquots of uridine-¹⁴C-labeled T6a whole cells, which had been grown and frozen in a similar way, independently. By measuring the ratio of ³H/¹⁴C trichloroacetic acid-precipitable counts, corrections could be made for losses during the phenol extraction of the ³H-labeled cells. Knowing the cell concentration, the specific activity of the RNA-³H, and correcting for losses during handling by the ³H/¹⁴C ratio in RNA, it was possible to determine the relative concentrations of total RNA per cell among various cell clones. These values are relative to the phenol extraction conditions used in these experiments. By polyacrylamide gel electrophoresis of these fractions, it was found that 4S RNA represented about 16% of the total cell RNA for these various cells. As these samples had been grown in the presence of the isotope for about 24 hr, it was assumed that most of the remaining 84%, heavier RNA, was ribosomal. The reproducibility of the determinations of RNA concentration per cell was within ± 10%.

RESULTS

Fig. 1 shows sucrose gradient centrifugations of ribosomes of T6a and 3T3-4E. The cells were grown and harvested, and their ribosomes were analyzed as described in Materials and Methods. With the cell types and conditions used in these experiments, no significant amounts of polysomes were found, at least by the time the sucrose gradients were collected. Therefore, in the rest of this report attention is focused on ribosomes not bound to polysomes, as they represented essentially all the ribosomes found in these cells. Under these high concentrations of Mg⁺⁺ and K⁺, most of

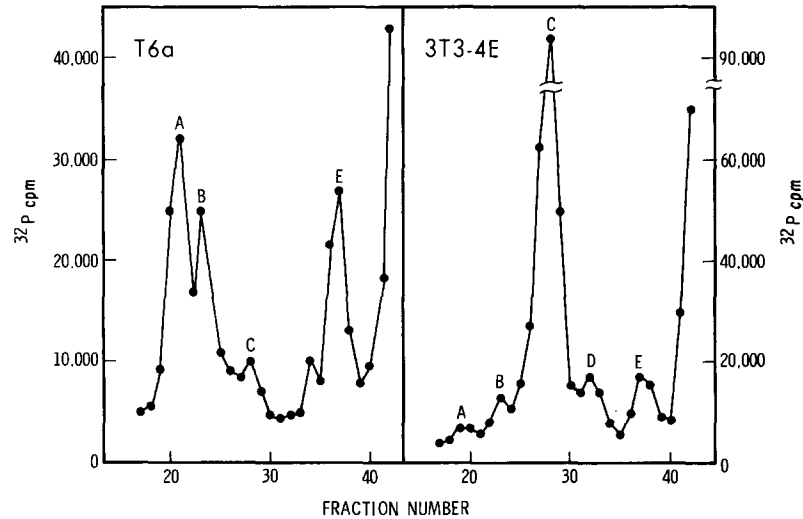


FIGURE 1 Sedimentation analysis of ribosomes from cultures of hamster parental cells (T6a) and mouse parental cells (3T3-4E). The conditions for isotope incorporation, preparation of cytoplasmic extracts, and sucrose gradient centrifugation were described in Materials and Methods. *A*, dimer; *B*, intermediate structure; *C*, monoribosome; *D*, 60S subribosomal particle; *E*, 40S subribosomal particle.

the ribosomes of the hamster parental cell line were present as dimers, a lesser amount as an intermediate, light structure, and a very small percentage as monomers. The amount of small ribosomal subunit (40S) was larger than that of 60S subunit. Fig. 1 also shows that most of the ribosomes of the mouse parent were present as monomers, with small amounts of the intermediate structure, dimers, and subunits. The intermediate structure of T6a in Fig. 1 seems to be made up of two 60S subunits and one 40S subunit, on the basis of the proportion of its 28S and 18S rRNA (G. L. Eliceiri, manuscript in preparation).

Figs. 2, 3, and 4 show the type of patterns obtained when the hybrid clones were analyzed by two successive steps, sucrose gradient centrifugation of ribosomes followed by polyacrylamide gel electrophoresis of rRNA, as described in Materials and Methods. In some hybrids, like 10A (Fig. 2) or 7A (Fig. 3), the majority of the ribosomes were present as monomers. The 28S rRNA of monomers consisted mostly of mouse rRNA, while hamster 28S rRNA could be found in dimer and intermediate peaks (Figs. 2 and 3). In other hybrids, like 3B, most of the ribosomes were present as dimers (Fig. 4). The dimer and intermediate peaks consisted mostly of hamster 28S rRNA, while mouse rRNA could be found in the monomer peak.

The ratio hamster/mouse 28S rRNA from dimer, intermediate, monomer, and 60S peaks was measured in 11 hybrids. These hybrid clones ranged between 16 and 92% of hamster chromosomes, with modal numbers between 12 and 131 for hamster chromosomes, and between 9 and 94 for mouse chromosomes.

During the course of these experiments it was noticed that some of the hybrids lost chromosomes, and that as they lost chromosomes of one species they also contained fewer ribosomes of that species. Therefore, in each experiment the chromosome composition of the hybrid was determined the day of cell harvest (see Materials and Methods).

Fig. 5 shows the relationship between the percentage of chromosomes of one species and the percentage of rRNA of the same species. The error in the determination of percentage of hamster and mouse rRNA was usually within $\pm 2\%$. It appears that when the majority of the chromosomes in a hybrid was of hamster type, a disproportionately higher percentage of hamster rRNA was present in that hybrid. Conversely, when hamster chromosomes were in the minority, a disproportionately lower percentage of hamster rRNA was present.

The total amount of rRNA per cell was determined in a third portion of cells, the same day of the preceding experiments, as described in Mate-

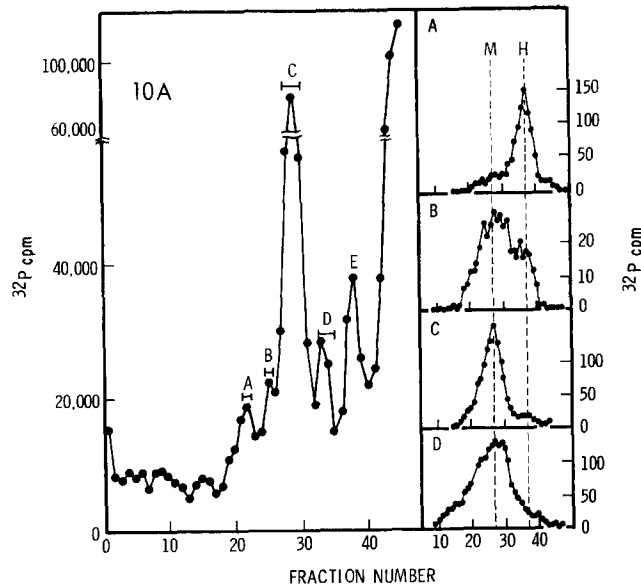


FIGURE 2 Sucrose gradient centrifugation of ribosomes from hybrid T6aE-10A (left panel) and electrophoretic analysis of 28S rRNA isolated from each sucrose gradient peak (right panels). The conditions of isotope incorporation, preparation of cytoplasmic extracts, sucrose gradient centrifugation, and polyacrylamide gel electrophoresis were described in Materials and Methods. *A*, dimer; *B*, intermediate structure; *C*, monoribosome; *D*, 60S subribosomal particle; *E*, 40S subribosomal particle. The locations of mouse (*M*) and hamster (*H*) 28S rRNA-³H markers, mixed with the unknown in the same electrophoresis gel, are indicated in the right panels (A-D).

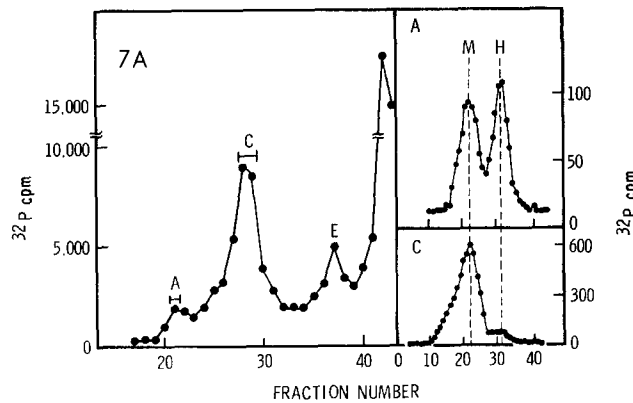


FIGURE 3 Sucrose gradient sedimentation of T6aE-7A (left panel) and electrophoretic analysis of its peaks (right panels, A and C). See legend of Fig. 2.

rials and Methods. The results are shown in Fig. 6, with linear extrapolations of the values found for the hamster parental cell (upper line) and for the mouse parental cell (lower line). The inset in Fig. 6 shows the same data expressed as micrograms of rRNA per milligram of total cell protein *versus* total number of chromosomes. Most hybrids have concentrations of rRNA per cell and rRNA

per total cell protein higher than could be expected by linear extrapolation of the rRNA concentration of 3T3-4E. The differences in content of rRNA per cell among various hybrids and T6a become negligible when the rRNA concentrations are expressed per total protein concentration.

Figs. 7 and 8 are obtained by combining the

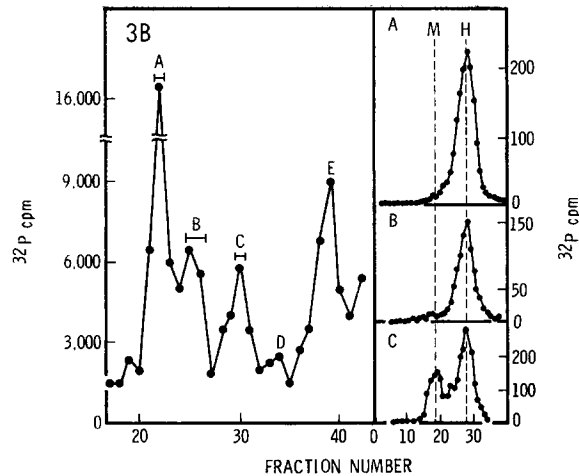


FIGURE 4 Sucrose gradient sedimentation of T6aE-3B (left panel) and electrophoretic analysis of its peaks (right panels, A-C). See legend of Fig. 2.

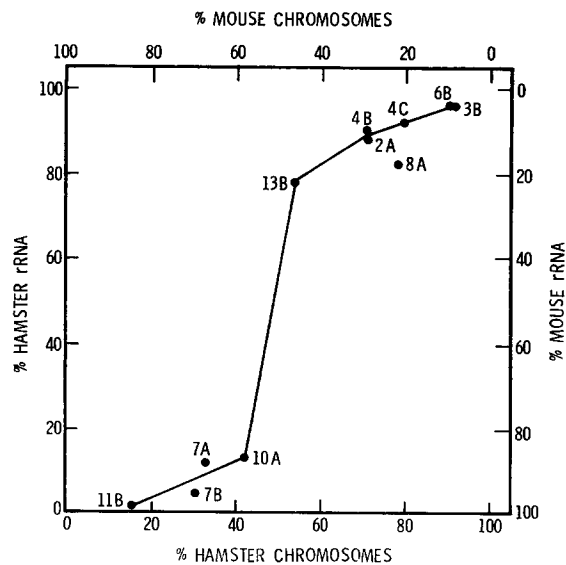


FIGURE 5 Comparison between the percentages of hamster and mouse rRNA per cell and the estimated percentages of hamster and mouse chromosomes in a series of T6aE hamster-mouse hybrids. The procedures to determine the ratio of hamster/mouse rRNA and the estimated modal numbers of hamster and mouse chromosomes were described in Materials and Methods and references 8 and 10.

data in Figs. 5 and 6. Fig. 7 shows the relationship between the hamster rRNA content and hamster number of chromosomes for each hybrid. Fig. 8 shows the equivalent comparison for the mouse species. In some hybrids the concentration of hamster rRNA per cell appears to be higher than the linear extrapolation of the T6a value (Fig. 7), but this difference disappears when the hamster rRNA concentrations are expressed per milligram

of total cell protein (inset of Fig. 7). There seems to be a sharp increase in the concentration of hamster rRNA per cell or per total protein when hybrids have over 70 hamster chromosomes.

In the case of mouse rRNA concentration, Fig. 8 shows that most hybrids parallel the linear extrapolation from the value of 3T3-4E. The exceptions are hybrids 7A, 7B, and 10A, in which the mouse rRNA concentration is approximately four

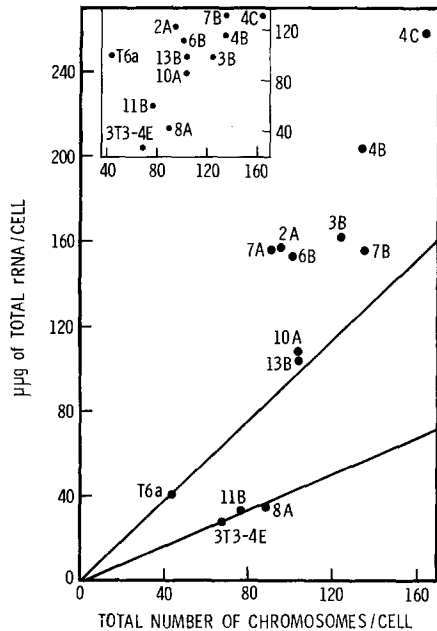


FIGURE 6 Comparison between the total concentration of rRNA per cell and the total modal number of chromosomes in a series of T6aE hybrids and their parental cells. The conditions to measure total rRNA concentration per cell and total number of chromosomes were described in Materials and Methods and reference 10. The *inset* shows the concentration of total rRNA per total cell protein (micrograms of rRNA per milligram protein in the ordinate) versus the total modal number of chromosomes (in the abscissa). The protein concentration of whole cells, broken with a Dounce glass homogenizer, was determined by the method of Lowry et al. (17), and the error limits were within $\pm 5\%$.

to five times higher than the concentration expected from that linear extrapolation. A relatively similar pattern is obtained when the data are expressed as mouse rRNA/total protein instead of mouse rRNA/cell (*inset* of Fig. 8). It is yet to be learned if these differences in concentrations of hamster or mouse rRNA are due to differences in the levels of RNA synthesis or degradation.

DISCUSSION

It has been shown that the cellular content of total RNA, rRNA, and ribosomes is two times higher in exponential growth phase than in stationary phase (11-14). As the present work was done with growing cultures, it would be interesting to study the rRNA content and hamster/mouse ratio of stationary cultures of these hybrids.

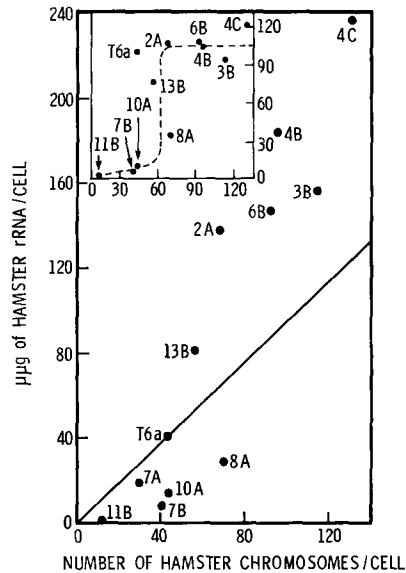


FIGURE 7 Comparison between the hamster rRNA concentration per cell and the estimated modal number of hamster chromosomes in a series of T6aE hybrids and the hamster parental cell line (T6a). This graph was made from data in Figs. 5 and 6. The linear extrapolation from the value for T6a is shown. In the *inset*, the ordinate is micrograms of hamster rRNA per milligram of total cell protein, and the abscissa is the estimated modal number of hamster chromosomes.

When the amount of rRNA of each species in hybrid clones is related to the number of chromosomes of that species, it is seen that the majority of the rRNA originates preponderantly from the genome contributing the majority of the chromosomes to the hybrid (Figs. 5, 7, 8). The result of this is that the minority genome does not contribute proportionately to the rRNA. This is shown for hamster chromosomes in Figs. 5 and 7, where there is an absolute depression of its contribution. In the case of mouse contribution there is also more than the proportional amount of mouse rRNA in clones possessing a large mouse genome (Figs. 5 and 8), and less than the proportional amount in clones possessing small complements (Fig. 5).

The mechanism of this effect can obviously not be explained until more is known about the means by which the synthesis of rRNA is regulated. One hypothesis would be as follows. The probability that any chromosome bearing ribosomal genes will form a nucleolus and transcribe rRNA would be about the same regardless of species. However,

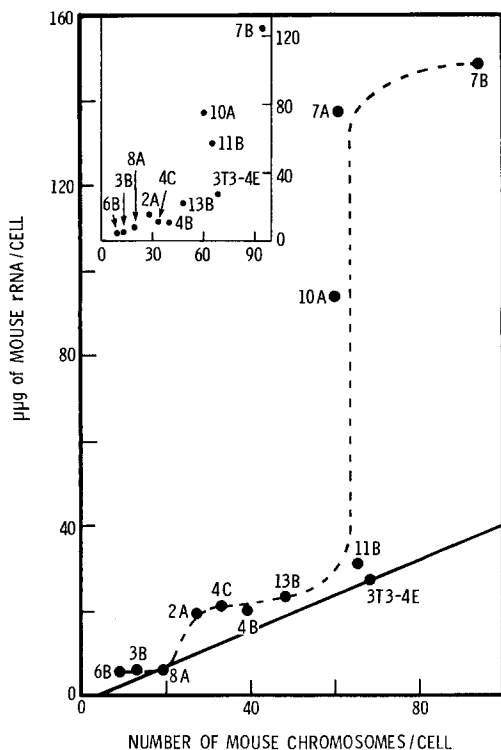


FIGURE 8 Comparison between the mouse rRNA concentration per cell and the estimated modal number of mouse chromosomes in a series of T6aE hybrids and the mouse parental cell line (3T3-4E). This graph was made from data in Figs. 5 and 6. The linear extrapolation from the value for 3T3-4E is shown. In the inset, the ordinate is microgram of mouse rRNA per milligram of total cell protein, and the abscissa is the estimated modal number of mouse chromosomes.

once a nucleolus is formed, other chromosomes of the same species can associate with it much more readily than chromosomes of the other species and transcribe their ribosomal genes. By this means the species contributing the majority of chromosomes will be more likely to initiate nucleolus formation (proportionate to chromosome ratio) and much more likely to synthesize rRNA (disproportionate to chromosome ratio). It is known that in certain plant hybrids the nucleolus-organizing capacity of chromosomes of one parental species is suppressed (Nawashin, cited by McClintock [15]). In gooseberry-blackcurrant hybrids, blackcurrant chromosomes cannot form nucleoli, but after loss of gooseberry chromosomes this function is regained (16).

The phenomenon described here may explain the observation of others that, in these hybrids, a

rather small difference in chromosome balance between the two species is associated with a large difference in the permissiveness of the hybrid to polyoma virus, which depends on the mouse genome. If the resulting viral DNA synthesis permitted by different hybrids is examined (8), it is clear from the present work that the hybrids which support viral DNA synthesis very well have over 80% mouse rRNA, while those which support it very poorly have less than 20% mouse rRNA. In both respects, therefore, the effects of imbalance of the two genomes are exaggerated at the phenotypic level. The question of whether the origin of the hybrid rRNA actually determines viral permissiveness by effects on translation of viral messenger RNA would be worth investigation.

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