THE USE OF MOLECULAR HYBRIDIZATION WITH RIBOSOMAL RNA TO EXPLORE DISTANT
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64

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Abstract

Heterologous rRNA-DNA hybridizations and competition experiments indicated that homology among distantly related eukaryotes is not the residual of random divergence, but resides in special conserved sequences. In situ hybridization of rRNA to human metaphase plates mapped the rDNA in the five satellited chromosomes. Similar studies in the mouse indicated that no more than two pairs are involved. An efficient screening system was constructed in *Drosophila* for detecting X-ray induced reactivation of inactive rDNA. In *E. coli* the number of large rRNA genes in certain episomes did not equal the number of 5S RNA genes, showing that the 5S RNA genes do not occupy the same transcriptional units as those for 16S and 23S rRNA.

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Heterologous rRNA-DNA hybridizations

Experiments described in last year's report were extended to 11 further sources of vertebrate DNA and two of invertebrate. In addition to mouse and Drosophila, HeLa cells were employed as a source of ^3H -rRNA. These studies added nothing important to the conclusions previously drawn. Vertebrate DNA sources not represented in the previous report (comprising mammals, birds, reptiles and amphibians) clearly distinguished between mammalian and Drosophila rRNA, in contrast to DNA from sources more distantly related to either Drosophila or mammals. A manuscript is in preparation embodying our evidence that the residual homology among very distantly related eukaryotes results from sequences conserved in common, rather than from incomplete random divergence from the rRNA sequence in a common ancestor.

rRNA-DNA hybridizations in mouse t locus heterozygotes

The contaminant that prevents saturation plateaus in hybridizations with rRNA from L1548Y cells was isolated by means of reiterated annealing and melting from filter-bound DNA. At a concentration of 40 $\mu\text{g}/\text{ml}$ of ^3H -RNA, filters with 100 μg of DNA bind about .05 μg of RNA, which was recovered upon heating in the same volume as the original reaction mixture. The product anneals to fresh DNA at concentrations below 0.1 $\mu\text{g}/\text{ml}$, whereas the original RNA preparation is not detectably bound to DNA at this concentration. This confirms our suspicion that the contaminant is complementary to repetitious DNA of much higher multiplicity than rDNA.

In view of the success achieved with in situ hybridization of rRNA

to human chromosomes (see subsequent section); it was decided to locate the rDNA in the mouse chromosome complement by the same means. Two intermediate sized chromosomes are apparently involved, suggesting that the t locus may not coincide with the rDNA. Slides were prepared of cells bearing a translocation between chromosomes 17 (LG IX) and 16, to permit identification of chromosome 17, which carries the t locus, on the autoradiographs. These are now being exposed.

Chromosomal location of human rDNA

By means of in situ hybridization and autoradiography the sites of human rDNA were identified as the satellite regions of chromosomes 13, 14, 15, 21 and 22, and the presence of rDNA elsewhere in the chromosome complement was excluded. The manuscript in press (Proc. Nat. Acad. Sci.) is appended to this report.

During these investigations another phenomenon was noted, i.e., linear arrays of grains connecting the satellites of different acrocentric chromosomes. On this basis we propose the hypothesis that human satellite associations are caused by rDNA connectives, usually subvisible, and that individual variations in the patterns of satellite association reflect variations in the relative amounts of rDNA among the ten acrocentric chromosomes. The rDNA content of a given chromosome is expected to be a heritable property, thus the association patterns are expected to be inherited in a complex way. One easily testable prediction is that the relative frequencies with which the different satellited chromosomes participate in associations should be concordant in monozygotic twins. A paper advancing this hypothesis is in preparation.

We have succeeded in preparing human 5S RNA with sufficient specific activity of ^3H to map the human 5S loci by means of in situ hybridization. The slides are being exposed.

X-ray induced reactivation of inactive rDNA in Drosophila

Irradiation of the detachment chromosome described in the previous report, $y^+XY^S \cdot Y^LBS$, resulted according to plan in a deletion of the X euchromatin to yield a doubly marked Y^{bb-} chromosome. The rDNA content of this chromosome was estimated by means of ^3H -rRNA hybridization to DNA of $y \ v \ bb \ C(1)RM$; $y^+Y^{bb-}B^S$ females, the results indicating the Y^{bb-} is not deficient in rDNA, although the rDNA is completely inactive. Males with bb^+ and lethal $(1)J1$ on the X are viable with $y^+Y^{bb-}B^S$ since the y^+ segment includes $1(1)J1^+$. When males of this constitution were crossed with $y \ w \ f \ C(1)DX$; w^+Y the result, as expected, was no viable progeny other than nondisjunctional exceptions. Reactivations will appear as $w \ B$ females. Screening for X-ray induced reactivations is in progress.

The rRNA genes in E. coli

It has been suggested that the genes for 5S RNA are in the same transcriptional units as those for 16S and 23S in E. coli (Doolittle and Pace, 1971, Proc. Nat. Acad. Sci. 68:1786-1790). As noted in the previous report, the genes for 5S RNA are in the same episomes as those for large rRNA, i.e., KLF-11 and F-14. All isolates of F-14, however, contain only two or three copies of the combined large rRNA genes, whereas KLF-11 usually contains about five copies. If the genes for 5S RNA are in the same transcriptional units, their multiplicity in different episomes

should parallel that of the large rRNA genes. We find that F-14 with a reduced number of 16S and 23S genes has a normal number (about six) of 5S RNA genes, contrary to the foregoing hypothesis.