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Progress Report on the Use of Molecular Hybridization to Explore Genetic Relationships

Primate rDNA

The limiting factor on this portion of the project has always been the arrangements for obtaining material. We were fortunate in getting lymphocyte cultures from the siamang, an aberrant gibbon in which the marker chromosome is replaced by an acrocentric superficially resembling a human D-group chromosome. This chromosome proved to carry the only site of rDNA in the chromosome complement. The banded karyotype was obtained, and surprisingly the acrocentric chromosome shows no obvious homology with the marker chromosome of the lar gibbon, nor with any rDNA-bearing chromosome we have observed in other primates. In preparations that had been treated with formamide all of the chromosomes of the siamang had prominent terminal knobs, a situation unique among the primate chromosomes we have studied.

The orangutan proved to have a multisite distribution of rDNA involving both the large and the small acrocentrics as in the chimpanzee and man, rather than only the smaller two pairs as in the gorilla.

Primate 5S DNA

All of the Pongids proved to have the major site of 5S genes at the end of the chromosome arm homologous to human 1q. The 5S gene site was also localized in a baboon, Papio cynocephalus, to the end of an arm that resembles human 1q by banding pattern. This resemblance had been overlooked until attention was focussed on it because of the position of the 5S site. Previously only the X chromosome was recognized as having a similar banding pattern in man and the Cercopithecine monkeys.

Satellite associations and rDNA

The correlation of rDNA with association frequency was studied in nine human subjects in which, for each chromosome pair, both the proportion of the total chromosomes in association and the proportion of the total grains at rDNA sites were determined. The 45 pairs of observations gave a correlation coefficient of +.81, where .38 would have been significant at the 1% level. Despite the highly significant correlation, the data include four "wild points" in which the relation of rDNA content to association frequency is obviously

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contrary to hypothesis. One of these is a chromosome 13 pair that accounts for 50% of the grains, but only 19% of the associations. In this instance the homologues are distinguishable by satellite morphology, and the homologue with most of the rDNA is seldom in association. The correlation and the exceptions to it make the working hypothesis more specific. First, chromosomes in the same nucleolar complex during the preceding interphase have the potential for remaining associated, and their probability of doing so is directly proportional to their rDNA content. Second, some chromosomes with very high rDNA content are seldom in a nucleolar complex with other chromosomes.

Mouse rDNA

Polymorphisms in rDNA content were found for all of the five chromosomes on which rDNA has been reported in the mouse.

Effect of prephotographing on hybridization

We have suspected for some time that prephotographing with atabrine fluorescent staining lowers the efficiency of hybridization in situ. This effect has now been systematically studied in five human subjects with distinctive differences in grains per rDNA site between D and G-group chromosomes (identifiable per se). Prephotographing always reduced specific labelling, which ranged from 20 to 60 per cent of the control values. The relative labelling of D and G-group chromosomes, however, was accurately maintained. This means that prephotographing can be used to identify chromosomes where the site contents are to be compared within the same experiment, but the results of different experiments are not comparable with prephotographed material. When this factor is taken into account it becomes evident that the chimpanzee has about the same total rDNA content as man; our previous report that the chimpanzee has less was based on pre-photographed chimpanzee chromosomes compared with untreated human chromosomes. When only untreated chromosomes are compared it appears that the increased site number in man and chimpanzee over that in gibbons and monkeys is accompanied by a proportional increase in total rDNA, the gorilla occupying an intermediate position.

Histone and immunoglobulin gene mapping

Hybridization of ostensible histone and immunoglobulin mRNA's to human

chromosomes gave the results mentioned in the Comprehensive Report. The human histone mRNA preparations met the three criteria of (1) being labelled only during the S period in synchronized cell cultures, (2) not having attached poly-A sequences, and (3) having an undegraded size corresponding to 10S. The presumed immunoglobulin light chain mRNA was obtained as 22S RNA from an IgG-secreting mouse cell line.

rDNA magnification in Drosophila

The major outlines of the project concerning the control of optimal rDNA multiplicity in Drosophila are set out in detail in the report of one year ago. Two new findings have come to light in the past year. First, external radiation produces magnification in ring chromosomes, and second, we have isolated an autosomal mutation that permits magnification in ring chromosomes.

The first of these results permits the timing of the elementary events of magnification by means of repeated matings of irradiated males at different times after irradiation. Chromosomes irradiated in mature sperm, spermatids, meiotic stages and gonial cells appear in that order in successive broods. The results of these experiments show that magnifying events take place in all of these stages. The surprising result was that magnification is facilitated in ring chromosomes irradiated in mature sperm (albeit not to so great an extent as in prior stages). This indicates that a magnifying event, i.e., a sister strand crossover in the rDNA, sometimes takes place after fertilization. This result is unexpected.

After having been kept for 31 generations under magnifying conditions without magnifying, a bobbed allele in a ring X chromosome suddenly began to magnify. The ring proved to have remained closed. A second-chromosome dominant that facilitates magnification of bobbed alleles in ring chromosomes was extracted from this stock, and some of its properties have been determined. Although a dominant the Ring magnifier, Rm, is viable, fertile when homozygous. In females the following effects are observed:

1) Homologous crossing-over is normal to the left of f, but is increased by 50% in the region from car to su-f.
2) Detachments of C(1)RM are caused to occur at a rate of 0.5%.
3) In ring-rod heterozygotes with a bb+ ring, double exchanges are recovered

five times more frequently in the rod than in the ring, but if the ring carries $\underline{bb}^{o}$ they are recovered with equal frequency in rod and ring. Similarly, where $\underline{y}$ and $\underline{bb}$ are adjacent in the ring, the crossover frequency between $\underline{y}$ and $\underline{f}$ is 0.6% if the ring carries $\underline{bb}^{o}$, but 4.8% if the ring carries $\underline{bb}^{+}$.

In males, the sex ratio of progeny is shifted in the direction of ring loss more in rings that carry $\underline{bb}^{o}$ than in rings that carry $\underline{bb}^{+}$.

These properties indicate that $\underline{Rm}$ increases both homologous and sister exchange preferentially in the right end of the X chromosome, both within and outside the rDNA. Its probable mode of action in facilitating bobbed magnification in rings is the production of extra sister exchanges outside of the rDNA.

In preparation for an attempt to produce magnifying conditions in $\underline{bb}^{+}$ flies, a study of the DNA-dependent RNA polymerases of Drosophila has been completed.