*THE KARYOTYPE OF THE MOUSE, WITH IDENTIFICATION OF A TRANSLOCATION**

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The metaphase chromosomes of the house mouse have a well-deserved reputation of being refractory to detailed analysis because of their small size, gradual gradation in length, and effectively terminal centromeres. Nevertheless, because of the wide use of the mouse in experimental biology and genetics, continued and productive efforts have been made to define the normal chromosome number[1, 2] and to analyze the normal karyotype.[3–5] Cytological identification of male and female somatic cells has proved possible.[6] Numerous analyses of complicated and unstable abnormalities of chromosome number and morphology in tissue culture and tumor cells have been carried out (see Hsu[7] for review). Aneuploidy for both sex and somatic chromosomes has been described (see Russell[8] for review), but there has been only a limited number of reports of consistent and stable abnormalities of structure visible in somatic metaphase chromosomes. Painter[9] described a deficiency of one of the autosomes which was associated with waltzing behavior. The T_6 translocation, produced by irradiation and genetically identified as involving linkage group III by Carter, Lyon, and Phillips,[10] was described by C. E. Ford *et al.*[11] as consisting of an unequal reciprocal translocation between two of the smallest members of the chromosome set, resulting in one of the two rearranged chromosomes being smaller than any in the normal complement. Russell and Bangham[12] detected an extraordinarily long chromosome in heterozygotes for a reciprocal translocation between linkage groups VIII and the X chromosome. Ohno and Cattanach[13] detected a nonreciprocal translocation of a portion of the chromosome-bearing linkage group I to the X, again because of the existence of an abnormally long chromosome. None of the above cases were subjected to detailed karyotype analysis.

The purpose of this paper is to present a cytological analysis of two more translocations: T_{190} which is clearly demonstrable cytologically and T_{138} which is undetectable; and to offer additional observations on the normal karyotype of the mouse.

Materials and Methods.—These studies were initially undertaken with the possibility in mind of detecting, in the chromosomes of somatic cells, abnormalities of size or structure associated with recessive lethal alleles at the *T*-locus in linkage group IX. Genetic data (see Dunn, Bennett, and Beasley[14] for review) and cytological evidence[15] suggest that these mutants are not point mutations but deficiencies. There is also some evidence[16] that one *T*-locus mutant, t^{12}, interferes with RNA synthesis; this could be taken as inferential evidence for the possibility of a defective nucleolus organizing region. Therefore, the first cells that were examined were from $+/t^{12}$ and $+/t^{0}$ embryos, with $+/T$ as controls. No deviation from normal was detected, however, either in chromosome length or in the appearance of chromosomes which showed secondary constrictions and could therefore be presumed to be involved in nucleolus organization.[17] In an attempt at finer discrimination, chromosomes were examined from mice heterozygous for two different reciprocal translocations (T_{190} and T_{138}), produced by irradiation in the same series as T_6,[10] and known to involve linkage group IX, and each marked with t^6 (equivalent to the allele t^0).[10] The translocation stocks were kindly supplied by Dr. Mary F. Lyon.

Air-dried chromosome preparations were made according to the method of E. H. R. Ford and Woollam.[18] Liver cells from embryos of 14–17 days were used. About 100 well-spread cells in metaphase were selected from all embryos for photography and printing at a final magnification of about 4500 ×. The criteria for selection, in addition to adequate separation, were that chromosomes should be well extended and that clear secondary constrictions should be apparent on rapid inspection. Of these cells, 26 were selected for karyotyping. The distribution by sex and genotype was as follows:

	Embryos	Cells	♀	♂
$+/T$	2	4	3	1
$+/t^{12}$	3	3		3
$+/t^{9}$	1	1		1
$+/T_{190}$	9	12	7	5
$+/T_{138}$	3	6	2	4

Results.—Karyotypes were prepared by first arranging chromosomes roughly in order of size. It then became evident that in each cell there were six chromosomes with clearly visible "satellites." The "satellites" are set off from the rest of the chromosome by a negatively heteropycnotic region. This is considered to be a secondary constriction because, although in favorable preparations the satellite region is distinguishably split longitudinally, there is never any falling apart of the two halves which would lead one to consider them short arms. It seems therefore that in the satellited chromosomes the centromere is also effectively terminal and distal to the secondary constriction. The six satellited chromosomes could be classified roughly as two large, two medium-sized, and two small ones. Since there was always an even number of such satellited chromosomes, it was concluded that these represented three pairs, even though the members of the two longest pairs did not always rank exactly together by size. The remaining chromosomes were also arranged in pairs, with length, degree of contraction, degree of separation of the arms, and especially morphological differentiation of the centromere region being used as criteria. It is recognized that many of these pairs may be spurious and not composed of homologous chromosomes, but examination of the figures will show that many of the elements arranged as pairs show similarities which appear to distinguish them morphologically from other pairs of essentially similar size. Thus, with the reservation always in mind that some "pairs" will be mismatched, it was thought that preparing karyotypes in this way might serve to provide material which could be more closely analyzed than karyotypes prepared simply by ranking by size alone, especially since it is obvious that purely mechanical variations in degree of stretching will produce at least minor variations in length.

The karyotypes thus prepared fall, exclusive of sex, into two sharp groups: cells from T_{190} heterozygotes showed two chromosomes of abnormal length; cells from embryos of all other genotypes had chromosome complements which were indistinguishable from one another. Although the T_{190} translocation was detectable, no abnormalities which could be considered as associated with the T-locus were found. Cells from all genotypes other than T_{190} were lumped into one group, taken as being homogeneous, and used as controls. As expected,[6] the cells could be immediately classified as male or female (Figs. 1 and 2). In confirmation of the report of Ford and Woollam[4] the following morphologically distinct pairs could be recognized: (1) a pair distinctly longer than any other; (2) a pair distinctly shorter than any other, and usually, but not always, longer than the Y (this pair always

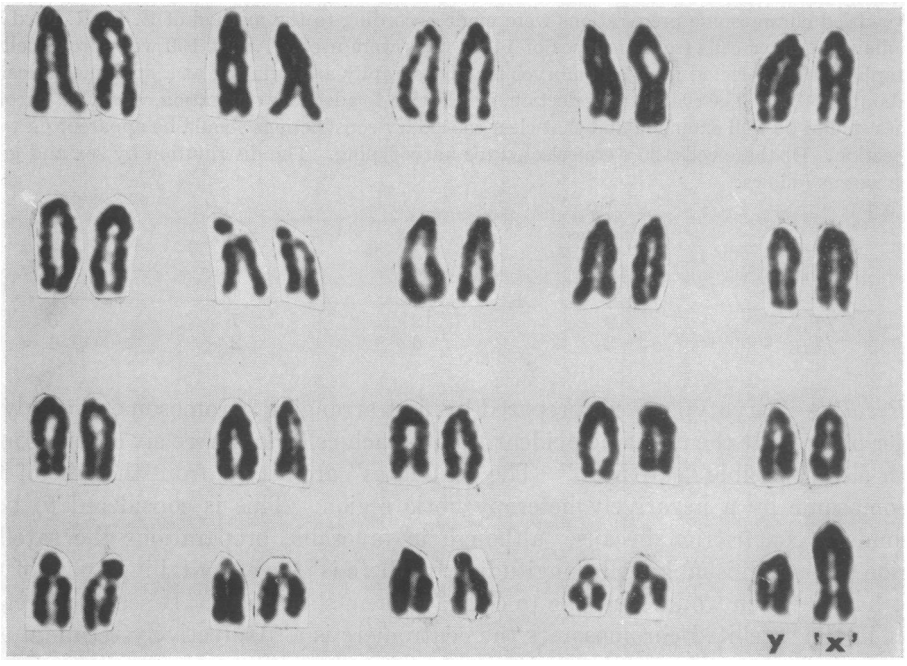

FIG. 1.—Karyotype of a normal male cell, genotype $^{+}/t^{0}$. Pairs 7, 16, and 19 show pronounced secondary constrictions. Pair 17 has negatively heteropycnotic centromere region. The Y and putative X chromosome are shown in lower right corner.

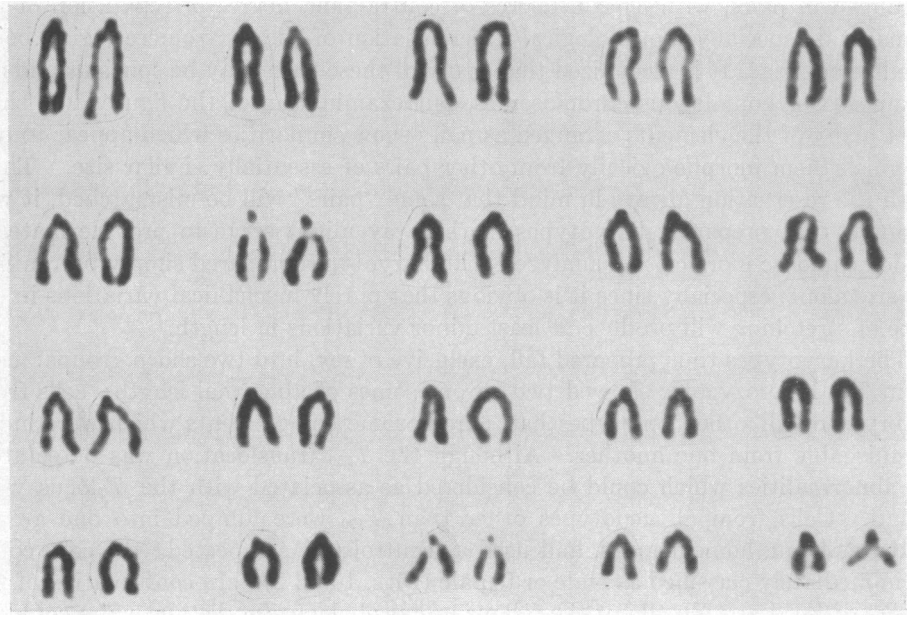

FIG. 2.—Karyotype of a normal female cell, genotype $^{+}/T$. Pairs 7, 18, and 20 show secondary constrictions.

TABLE 1

POSITION OF TWO PAIRS OF CHROMOSOMES WITH SATELLITES IN THE KARYOTYPIC SERIES

	Position in karyotype	I T_{190} No. cells	I T_{190} Sex ♀	I T_{190} Sex ♂	II Control No. cells	II Control Sex ♀	II Control Sex ♂	Combined I and II No. cells	Combined I and II Sex ♀	Combined I and II Sex ♂
First pair (longest)	6	1	1		1		1	2	1	1
	7	4	1	3	5	2	3	9	3	6
	8	2	2		2	1	1	4	3	1
	9	2		2	3	1	2	5	1	4
	10	2	2		—			2	2	
	11	—			—			—		
	12	1	1		—			1	1	
	13	—			2		2	2		2
	Totals	12	7	5	13	4	9	25	11	14
Second pair (medium length)	14	1		1	—			1		1
	15	1		1	2		2	3		3
	16	2		2	6	1	5	8	1	7
	17	6	5	1	4	3	1	10	7	3
	18	1	1		1	1		2	2	
	Totals	11	6	5	13	5	8	24	10	14

showed secondary constrictions, and could therefore be differentiated from the Y chromosome in male cells); and (3) two additional pairs with marked secondary constrictions. In the material studied here, the longer pair of this set ranked in length about half the time as pair number 7, with a range from the 6th to the 13th pair depending on the individual cells. The shorter pair ranked in length from 14th to 18th, most often being found in the 17th position (see Table 1 for exact figures).

In addition, in 5 of the 6 cells studied from T_{138} heterozygotes, an extra satellited pair was present which ranked in length between the 13th and 16th pair (Fig. 3). Since the cells were all heterozygous for the translocation, this extra satellited pair

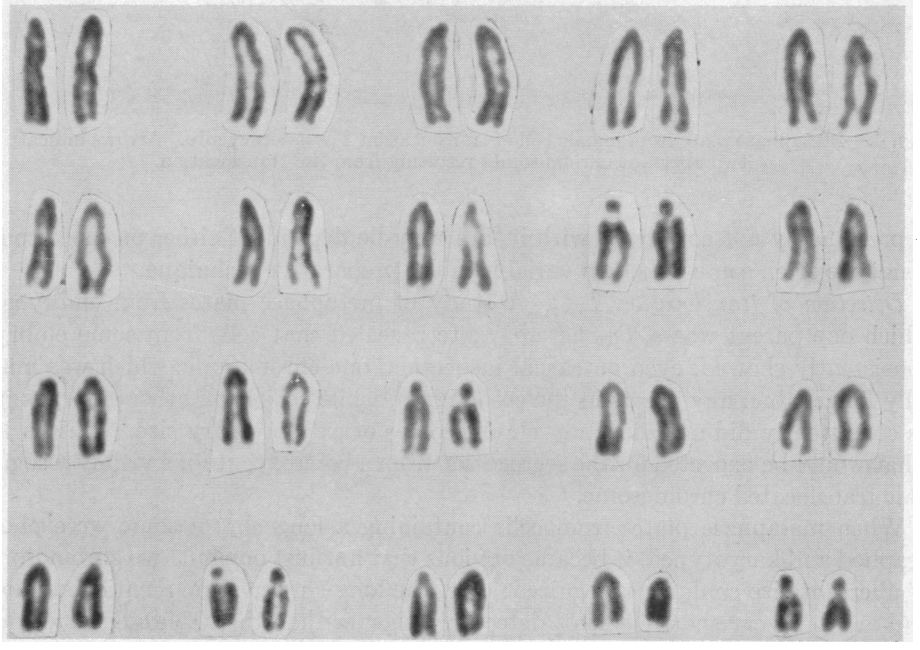

FIG. 3.—Karyotype of female cell from T_{138} heterozygote. Four pairs (9, 13, 17, 20) show secondary constrictions.

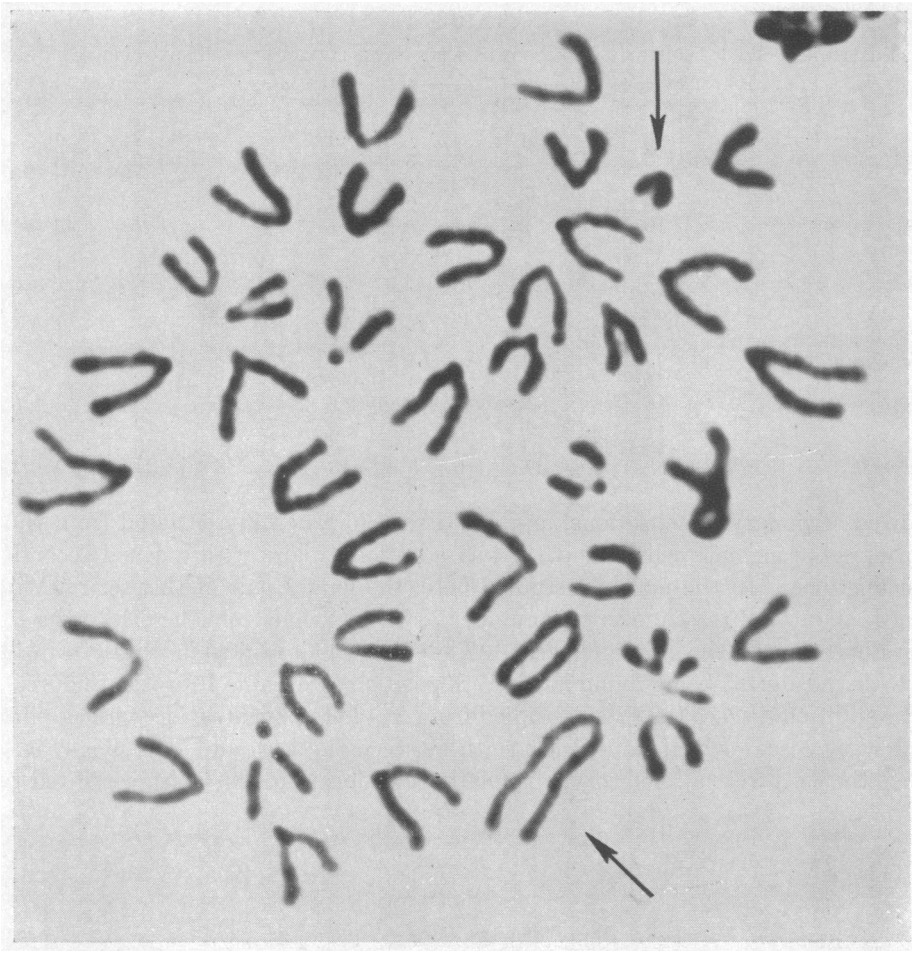

FIG. 4.—Metaphase plate from female cell of translocation T_{190} heterozygote. Arrows indicate the two aberrant chromosomes resulting from the translocation.

is presumably not connected with it, but may be dependent either on background genotype or on some unknown variable in the preparative technique.

Detection of translocation T_{190}: A study of metaphase plates from embryos of which one parent was a T_{190} heterozygote revealed that cells from some embryos consistently showed, even on casual inspection, one chromosome which was markedly longer than any others in the set (Fig. 4), while cells from other embryos just as consistently did not show any chromosomes of extraordinary size. This is just what would be expected for the segregation from a heterozygote of a visibly recognizable translocated chromosome.

When metaphase plates from cells containing a long chromosome were photographed and karyotyped, it became obvious that not just one but two chromosomes of aberrant size could be recognized: one, the long chromosome mentioned above, and another very short element, detectably shorter than the Y chromosome which is normally the smallest in the set (Figs. 5 and 6). Because of the similarity in size and morphology of mouse chromosomes, the homologues of the chromosomes in-

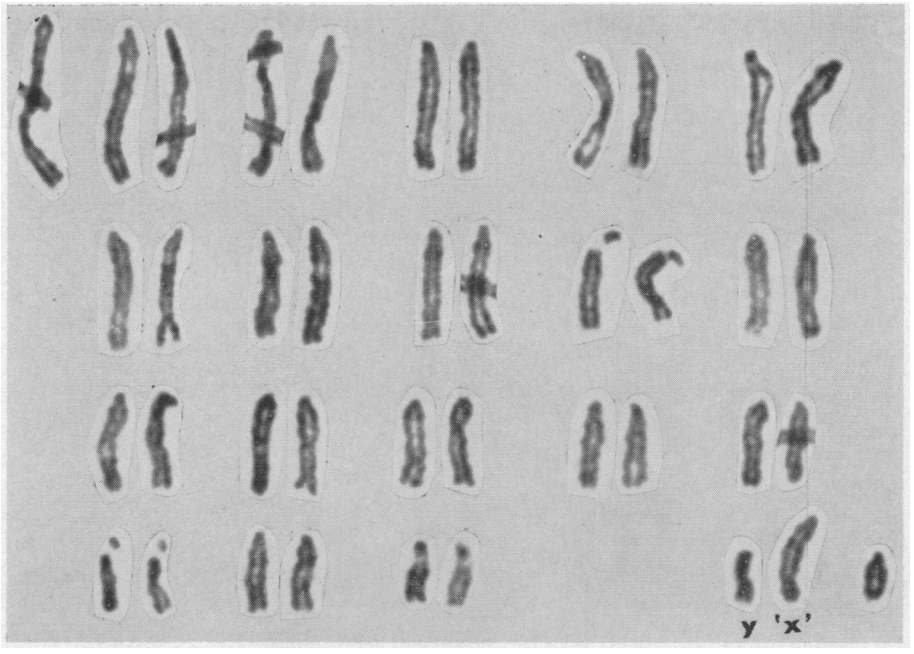

FIG. 5.—Karyotype of male cell heterozygous for T_{190}. The extraordinarily long chromosome is at top left; the small one at bottom right. Pairs 9, 16, and 18 show secondary constrictions.

volved in the translocation cannot be recognized in these preparations. Since, however, a first pair which was identifiably longer than any others could still usually be recognized, and since no members of the 3 consistent pairs with secondary constrictions were detectably unequal in size, it can be said with certainty that none of these four pairs is involved in the translocation.

In an attempt to define the position of the two pairs involved in the translocation, an analysis was made of the size distribution of the two large pairs of satellited chromosomes, with the assumption, for example, that if both pairs were long ones and normally ranked in size before the first satellited pair, the position of that pair would tend to shift to the left. However, the distribution of lengths, as seen in Table 1, of satellited pairs does not differ between T_{190} cells and controls. The position and lengths of the two pairs involved is therefore undetermined.

Attempts to evaluate size of X chromosome: The X chromosome of the mouse is usually considered to be one of the longest ones, as pair no. 3 in the female or as pair 4 or 5. Since there is no clear heteropycnosis of one X at metaphase in females, these estimates were made in the first cases[3, 4] by length measurements at male mitotic metaphase and the finding of an uneven number of the longest chromosomes, or in the second case[19] by measurements of a chromosome assumed to be X because of heteropycnosis at 2nd meiotic metaphase. Accordingly, in the karyotypes presented here, a chromosome of that size range was arbitrarily designated as X, and removed from its rank order and placed with the Y chromosome in male cells. It seemed then that in these karotypes, where the satellited chromosomes appear as relatively constant landmarks, their relative position in males and females might serve as an indicator of the size of the X; that is, if the X

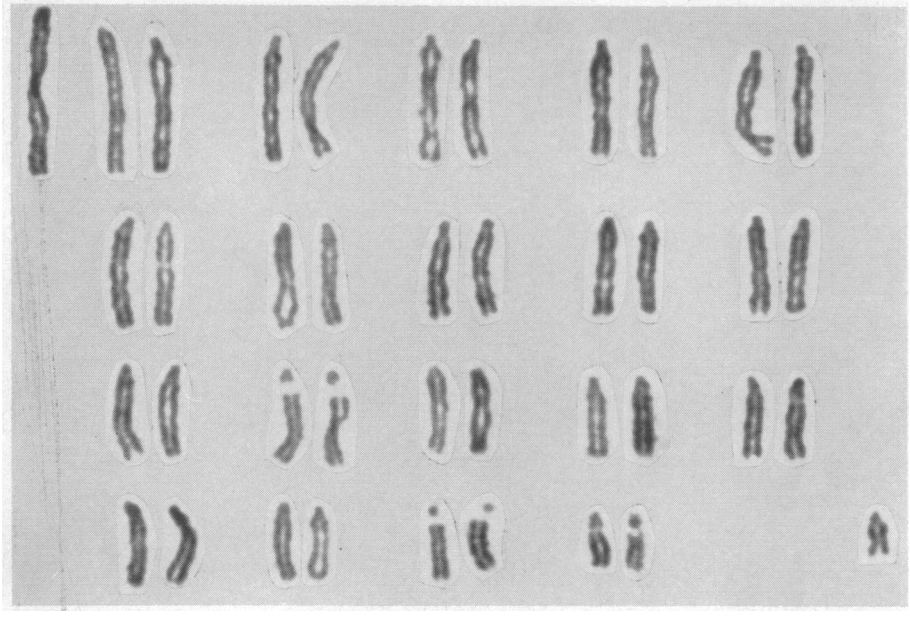

FIG. 6.—Karyotype of female cell heterozygous for T_{190}, arrangement as in Fig. 5. Pairs 12, 18, and 19 show pronounced secondary constrictions; note also differentiation of centromere region in most of these chromosomes.

chromosome is indeed one of the largest ones, it would be expected that since a long pair would be present in female karyotypes that was not present in males, the position of the longest satellited pair should be shifted to the right in females. Examination of Table 1, column 3, will show, however, that this is not the case. However, the same table shows that the position of the second satellited pair does clearly show a shift to the right in females. It seems possible, therefore, that the X chromosome in the mouse is not in fact one of the longest, but rather of medium size.

Summary and Conclusions.—By using the methods described by Ford and Woollam[4] and selecting only the most favorable cells for analysis, it is possible to identify consistently in normal mouse cells four autosomal pairs, three of which show secondary constrictions and differences in size which make them useful as landmarks, and the Y chromosome. Furthermore, even though mouse chromosomes are difficult to classify, it is felt that an approach to a workable karyotype can be made by arranging chromosomes in sequence roughly by size but also in pairs based on morphological similarity. This method seemed to be more realistic and also to lend itself more readily to the detection of differences among different karyotypes than methods based on size alone. The possibility is offered, based on karyotype analysis and the use of satellited chromosomes as landmarks, that the X chromosome may not be, as currently thought, one of the largest chromosomes, but one of medium size instead.

The cytological detection of a translocated chromosome is presented; heterozygotes for translocation T_{190} have one chromosome far longer than any in the normal set, and one smaller. This difference can be detected without karyotyping and thus would serve as a cell marker.

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*LOCALIZATION OF DNA COMPLEMENTARY TO RIBOSOMAL RNA IN THE NUCLEOLUS ORGANIZER REGION OF DROSOPHILA MELANOGASTER**

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The use of hybrid formation between DNA and isotopically labeled RNA[1] combined with RNAase treatment to eliminate unpaired RNA permitted the detection[2–4] in bacteria of sequences in DNA complementary to the two (16S and 23S) homologous ribosomal RNA components. The methods developed with microorganisms sufficed to establish that a similar situation exists in higher plants,[5] mammals,[6, 7] and insects.[8]

The proportion (0.3%) of the total genome involved was constant in the bacteria examined and indicated[3, 4, 9] a multiplicity of sites for each of the two ribosomal components. The densities of the DNA-RNA hybrids suggested[3] that the multiple sites were clustered rather than scattered throughout the genome. However, the bacteria were not convenient material for a more detailed attempt at illuminating the relation of these cistrons to each other and to the rest of the genome.

It seemed likely that higher organisms would furnish a better opportunity by permitting the correlation of cytogenetic and cytochemical information with data derived from molecular hybridization. Thus, diverse observations implicate the nucleolus with protein synthesis,[10–12] ribosomes[13–16] and ribosomal RNA formation,[17–19] the most striking being the absence of ribosomal RNA synthesis in a lethal anucleolate mutant of the aquatic toad, *Xenopus laevis*.[20]