CHROMOSOMAL LOCALIZATION OF REPETITIVE DNA IN THE NEWT, TRITURUS

GIUSEPPINA BARSACCHI and JOSEPH G. GALL

From the Department of Biology, Yale University, New Haven, Connecticut 06520. Dr Barsacchi's present address is the Istituto di Zoologia e Anatomia Comparata, Università di Pisa, 56100 Pisa, Italy.

ABSTRACT

The repetitive DNA sequences of the newt, Triturus viridescens, have been studied by nucleic acid hybridization procedures. Complementary RNA was synthesized enzymatically from unfractionated newt DNA. This RNA hybridized strongly to the centromeric regions of both somatic and lampbrush chromosomes. It also bound to other loci scattered along the lengths of the chromosomes. The amplified ribosomal DNA in the multiple oocyte nucleoli was demonstrated by in situ hybridization.

INTRODUCTION

Hybridization of radioactive DNA or RNA with chromosomal DNA fixed in cytological preparations permits visualization and localization of those parts of the genome which are complementary to the radioactive nucleic acid employed in the assay (Gall, 1969; Gall and Pardue, 1969; John, Birnstiel, and Jones, 1969; Buongiorno-Nardelli and Amaldi, 1970).

This method is especially suitable for detecting repetitive DNA sequences. In particular, the localization of the ribosomal cistrons has been studied in the oogonia and oocytes of the toad Xenopus laevis (Gall and Pardue, 1969; Pardue and Gall, 1969) and in the polytene chromosomes of some Diptera (Pardue et al., 1970). Recently the 5S RNA genes have been located in the genome of Drosophila melanogaster (Wimber and Steffensen, 1970) and in Chinese hamster cells (Amaldi and Buongiorno-Nardelli, 1971).

The cytological localization of highly repeated DNA sequences has also been investigated by in situ hybridization. It has been shown that mouse satellite DNA is located in the centromeric heterochromatin (Pardue and Gall, 1969, 1970) and that centromeric regions are enriched in highly reiterated sequences in several species of Drosophila (Hennig, Hennig, and Stein, 1970; Jones and Roberston, 1970, Ra, 1970, Gall, Cohen, and Polan, 1971), in Rhynchosia albiroma (Eckhardt and Gall, 1971) and in Plectodon e cinereus (Macgregor and Kezer, 1971). In Muscosos argestis, repetitive DNA is located in the heterochromatin of the sex chromosomes (Arrighi et al., 1970).

It is of interest to investigate the localization of repetitive DNA in the genome of other organisms. Because of the occurrence of lampbrush chromosomes in the oocytes of salamanders, we chose to extend this study to these animals. The giant lampbrush chromosomes, because of their structural differentiations, represent an appropriate material for studies on the DNA constitution of chromosomes. This note reports experiments on the hybridization of radioactive complementary RNA, transcribed in vitro from the DNA of the newt, Triturus viridescens, with interphase nuclei, mitotic, and lampbrush chromosomes from the same species.
MATERIALS AND METHODS

Animals

All experiments were performed on specimens of the newt, *T. viridescens* (Amphibia, Urodela) collected in Massachusetts.

DNA Isolation

DNA was extracted by a detergent-phenol procedure. The tissues (liver, spleen, testes) were first homogenized in 1 X SSC, then incubated for 2-4 hr at 37°C in a solution containing 0.5% Sarkosyl (Na-lauroyl-sarcosinate, Geigy Chemical Corp., Ardsley, N.Y.), 0.1 M EDTA, 0.05 M Tris pH 8.4, 100 µg/ml of predigested pronase. The solution was extracted with an equal volume of water-saturated redistilled phenol. The nucleic acids were precipitated from the aqueous phase with two volumes of 95% ethanol. The precipitate was washed with 70% ethanol, redissolved in 0.1 X SSC and treated with RNase-T1 (330 units/ml), pancreatic RNase (100 µg/ml), and α-amylase (100 µg/ml) for 1 hr at 37°C to remove RNA and contaminating carbohydrates. The DNA was again extracted with phenol, precipitated with 95% ethanol, and then dissolved in a small volume of 0.1 X SSC.

DNA Centrifugation

DNA was centrifuged to equilibrium in preparative CsCl density gradients at 42,000 rpm and 15°C for 21-24 hr in a Spinco Al-50 or Ti-50 fixed angle rotor. Analytical CsCl gradient centrifugation was done for 24 hr at 44,770 rpm and 20°C in a Spinco model E centrifuge.

The initial density of the gradients was approximately 1.70 g/ml at 25°C. The volume of the preparative gradients was 4.5 ml, and each contained 25-50 µg of DNA. 30 fractions of 10 drops each were collected from the preparative gradients by puncturing the bottom of the centrifuge tubes. The optical density of each fraction was determined at 260 nm. The UV photographs taken in the analytical centrifuge were analyzed with a Joyce-Loebl microdensitometer. The buoyant density of the *T. viridescens* DNA was determined using *Monocentris lyssodactylus* DNA (buoyant density = 1.731 g/ml) as a marker. Heat-denatured DNA (5 min at 100°C in 0.1 X SSC) and renatured DNA (100 µg/ml for 2 hr at 66°C in 2 X SSC) as well as DNase-treated preparations (30 min at 37°C with DNase at 20 µg/ml) were also analyzed by both preparative and analytical centrifugations.

In Vitro Transcription of cRNA

The gradient fractions, except for those containing the cirrini coding for rRNA, were pooled and the DNA was recovered by ethanol precipitation. Highly radioactive RNA was transcribed from this DNA using RNA-polymerase prepared from frozen cells of *Escherichia coli*, strain A-19 (Burgess, 1969). The transcription and purification of the cRNA were carried out as previously described (Pardue et al., 1970). Approximately 2 µg of complementary polynucleotide was produced. It had a calculated specific activity of 1 X 10^6 dpm/µg (about 1 X 10^6 dpm/µg as counted on nitrocellulose filters in toluene-PPO-POP). Cytological Hybridization

Slides of interphase nuclei, mitotic chromosomes, and lampbrush chromosomes from *T. viridescens* were prepared. Whole larvae were fixed in ethanolic-acetic acid (3:1); small fragments of liver or tail tip were transferred into 45% acetic acid on a siliconized cover slip and then squashed. The slides had been previously coated with gelatin by dipping them into a solution of 0.1% gelatin in 0.01 M chrome alum. The preparations were frozen on dry ice, and the cover slips removed with a razor blade (Conger and Fairchild, 1953). After 5 min in 95% alcohol, the slides were air dried and used for cytological hybridization. Lampbrush chromosome preparations were made according to the procedure described by Gall (1966), fixed in acetic acid vapors, dehydrated in an alcohol series, and air dried. The cytological hybridization was carried out essentially as described by Gall and Pardue (1971). Each slide received 50 µl of 6 X SSC or 3 X SSC containing cRNA at concentrations from 1 X 10^6 to 3 X 10^6 cpmp/µl. The slides were coated with Kodak NTB-2 liquid emulsion diluted 1:1 with distilled water. They were developed after periods ranging from 3 days to 12 wk (2 min in Kodak D19, reared in 20% acetic acid, and 3 min in Kodak Fixer). After being rinsed several times in distilled water, slides were stained with Giemsa's and mounted in Permount.

Preparations of lampbrush chromosomes were also annealed with cRNA copied in vitro from the rDNA of *Xenopus*. The cRNA was used at concentrations

GIUSEPPE BARSACCHI AND JOSEPH G. GALL Repetitive DNA in Triturus Newt 581
from 1.7 to 7.0 × 10^6 cpm/ml in either 2X SSC or 6X SSC.

Control experiments were performed in which the cytological hybridization was carried out without the alkali denaturation step.

**Filter Hybridization**

Each fraction (0.14 ml) collected from the CsCl gradient was treated with 1 ml of 0.1 N NaOH and neutralized with 2 ml of 0.05 N HCl in 9X SSC. The samples were loaded onto nitrocellulose membrane filters and the hybridization was carried out essentially as described by Gillespie and Spiegelman (1965). The filters were incubated with different amounts of cRNA ranging from 0.0045 μg/ml (5 × 10^4 cpm/ml) to 0.045 μg/ml (5 × 10^5 cpm/ml) for 15–17 hr at 66°C. Filter hybridization of gradients of heat-denatured DNA (5 min at 100°C in 0.1X SSC) or renatured DNA (2 hr at 66°C in 2X SSC) was also carried out. In some instances the DNA was sheared before the centrifugation by passing it several times through a 27 gauge needle.

Gradient fractions were also annealed with cRNA copied in vitro from the rDNA of *Xenopus*. The cRNA was used at either 2.5 × 10^5 or 5 × 10^5 cpm/ml. Competition experiments were performed with a 3000-fold excess of nonradioactive *Xenopus* rRNA. The radioactivity of filters was counted in toluene-PPO-POPOP in a Liquid Scintillation Counter, Mark I (Nuclear-Chicago, Des Plaines, Ill.).

**Melting Profile**

The thermal dissociation of the double-stranded DNA was monitored in a Beckman DU spectrophotometer equipped with a Gilford model 2000 multiple sample absorbance recorder. The melting temperature determination was carried out as described by Mandel and Marmur (1968). The DNA (15.5 μg) had been collected from a CsCl gradient precipitated with ethanol and redissolved in 0.1X SSC. The fractions containing the rDNA cistrons had been discarded.

**RESULTS**

**Analytical Centrifugation of *T. viridescens* DNA**

The buoyant density of the main peak of *T. viridescens* DNA as calculated by CsCl centrifugation is 1.704 g/ml (Fig. 1 a).

Analytical centrifugation of a greater amount of DNA demonstrated three minor peaks on the lighter side of the main band and a shoulder on the heavy side (Fig. 1 b). The buoyant densities of the lighter peaks are 1.691, 1.688, and 1.681 g/ml. One of the minor bands (probably the one having a buoyant density of 1.688 g/ml) is resistant to DNase treatment. The heavier shoulder has a buoyant density of 1.717 g/ml. It may include the cistrons complementary to rRNA (see Fig. 16). Using the formula of Schildkraut, Marmur, and Doty (1962), we can estimate the G + C content of the main peak as 44.8%.

In some cases it is possible to reveal a rapidly renaturing fraction which has the same buoyant density as the main peak, or to accentuate the density difference between the main peak and a light satellite by equilibrium centrifugation of DNA which has been denatured and then renatured (Waring and Britten, 1966). Under such circumstances the rapidly renaturing sequences display the density characteristic of double-stranded sequences, whereas the main peak retains the heavier density of single-stranded material. Such an experiment with sheared, denatured and renatured *T. viridescens* DNA showed only a single peak at ρ = 1.716 g/ml in the analytical ultracentrifuge. This experiment is relatively insensitive and would not detect a small fraction of rapidly reassociating DNA, especially if it did not return completely to its native density.

**Hybridization of cRNA with CsCl DNA Gradients**

Hybridization experiments on nitrocellulose filters have been carried out in order to determine the buoyant density of the repetitive DNA sequence, i.e., those which hybridize with the cRNA at low C0t values (Britten and Kohne, 1966). Fig. 2 shows the result of one of these experiments: the major hybridization occurs with main peak sequences. After denaturation alone or denaturation followed by renaturation, the hybridizing sequences remain with the main peak and the hybridized gradients are similar to the one shown in Fig. 2. In the latter experiments the DNA was sheared to less than 5 × 10^6 daltons before denaturation.

Annealing gradient fractions with cRNA made from the rDNA of *Xenopus* enabled the position of the cistrons coding for rRNA to be visualized in the CsCl gradients (Fig. 3). After competition
FICHE 1 a Microdensitometer tracing of *T. viridescens* DNA centrifuged to equilibrium in neutral CsCl. 3.5 μg of native DNA was centrifuged at 44,770 rpm and 30°C for 44 hr. The marker DNA is from *M. lysodeikticus* and has a buoyant density of 1.731 g/ml. The buoyant density of the *T. viridescens* main peak DNA is 1.704 g/ml, corresponding to a G + C content of 44.8%.

**Figure 1 b** Microdensitometer tracing of *T. viridescens* DNA centrifuged to equilibrium in neutral CsCl. 85 μg of native DNA was centrifuged as in a. Several minor peaks are now visible. One of the low density peaks is probably carbohydrate.

*Giuseppina Barsacchi and Joseph G. Gall. Repetitive DNA in Triturus Neot.*
experiments, performed with a 3000-fold excess of nonradioactive rRNA from *Xenopus*, there was no significant radioactivity on the filters.

**Melting Profile**

The melting profile of *T. viridecens* DNA is shown in Fig. 4. The Tm is 72°C in 0 lx SSC corresponding to a calculated G + C content of 44.1% (Mandel and Marmur, 1968).

**Hybridization of cRNA with Cytological Preparations**

**INTERPHASE NUCLEI**. After *in situ* hybridization with cRNA copied from whole DNA the interphase...
Hybridization of radioactive cRNA copied in vitro from rDNA of *Xenopus* to a *T. viridescens* DNA gradient immobilized on nitrocellulose filters. A prominent peak of hybridization occurs on the denser side of the DNA peak. The cRNA was used at a concentration of $2.5 \times 10^5$ cpm/ml. The hybridization was carried out in $2 \times$ SSC for 12 hr at 66°C. After competition experiments, performed with a 8000-fold excess of nonradioactive rRNA from *Xenopus*, no significant radioactive hybrid was detected. Annealing to main peak sequences, as in this example, also occurs when the experiment is carried out with labeled rRNA.

Nuclei are more heavily labeled in the heterochromatic zones than in the euchromatic ones (Fig 5). Heterochromatin is easily recognizable not only by its condensed state but also because, after the hybridization procedure, it stains more intensely with the Giemsa dye than the euchromatin (Pardue and Gall, 1970). In each nucleus some of the heterochromatic portions are not heavily labeled. Nuclei in prophase show the same pattern of labeling, although the heterochromatin is separated into more numerous parts (Fig. 6).

Mitotic chromosomes: Mitotic chromosomes are labeled with cRNA throughout their lengths, but display a higher silver grain density near the centromeres, more precisely, the heterochromatin lying on each side of the centromeric constriction is the most heavily labeled part (Figs. 7 and 8). This chromatin, as well as that of the chromocenters of interphase nuclei and of the centromeres of lampbrush chromosomes, is the most intensely stained after Giemsa staining in slides treated for cytological hybridization. Occasionally the telomeres show a number of grains exceeding the average (Fig 8).

Lampbrush chromosomes: The lampbrush karyotype of *T. viridescens* consists of 11 bivalents identifiable on the basis of their length, centromere position, and morphological landmarks (Gall, 1954). Two chromosomes bear a sphere in a subterminal position (numbers 5 and 10); one of the long chromosomes (number 7) carries the nucleolus-organizing region, close to the right telomere, and two peculiar enlarged bodies at the other extremity. The centromere region is represented by conspicuous Feulgen-positive bars up to 10 μ long. These bars do not bear loops and so appear as short gaps in the generally fuzzy appearance of the chromosomes. A granule of varying size which stains blue with Giemsa's is often seen imbedded in the centromere bar or on one side of it.
After cytological hybridization with cRNA the lampbrush chromosomes show silver grains scattered along their axes. Some of the loops are labeled after long periods of exposure. The centromere regions show a preferential hybridization similar to that observed for the centromeric heterochromatin of mitotic chromosomes (Figs. 9 and 10). The granule inserted at the centromere bar is not labeled. In a few instances the telomeric regions and the chromosome axes on which the spheres are inserted show a number of grains exceeding the average (Figs. 11 and 12). Nucleoli, spheres, and globules are not labeled.

After annealing with RNA complementary to the rDNA from *Xenopus*, lampbrush chromosomes do not show any hybridization; however, nucleoli, both free in the nuclear sap or attached to the nucleolus-organizer region, are labeled. In some instances nucleoli exhibit a localized labeling (Figs. 13 and 14).

The control experiments, performed with cytological preparations not submitted to the DNA denaturation step, showed no detectable label.

**DISCUSSION**

The annealing of cRNA to the DNA of cytological preparations from *T. viridescens* resulted in a widely distributed hybridization; there was, however,
FIGURES 9–12 Radiautographs of lampbrush chromosome preparations from *T. viridescens* after hybridization with radioactive cRNA transcribed in vitro from *T. viridescens* DNA (rDNA excluded). Figs. 9 and 10. Silver grains are distributed along the chromosome axes; the centromeric heterochromatin (arrows) is intensely labeled. Nucleoli are not labeled. Figs. 11 and 12 show respectively the chromosome axes where the sphere is inserted and two homologous telomeric regions more heavily labeled than other areas of the chromosomes. Sphere (S) and nucleoli (N) are not labeled. The cRNA was used at a concentration of $3.1 \times 10^6$ cpm/ml in 6X SSC. The hybridization was carried out for 17 hr at 66°C. Exposure, 56 days (Figs. 9–11) and 70 days (Fig. 12). Giemsa stain. 10μ = 15.2 μm. × 1530.
Francés 18 and 14 Radioautographs of lampbrush chromosome preparations from *T. viridescens* after hybridization with radioactive RNA transcribed in vitro from *Xenopus* rDNA. Fig 13 Several labeled nucleoli and unlabeled segments of lampbrush chromosomes. Fig 14 Eccentrically labeled nucleoli. The cRNA was used at a concentration of $1.7 \times 10^9$ cpm/ml in 6X SSC. The hybridization was carried out for 15 hr at 66°C. Exposure, 70 days. Giemsa stain. $10 \mu = 15.2$ mm. $\times 1520$

preferential binding to the centromeric heterochromatin

Under our experimental conditions, only redundant DNA sequences should exhibit hybridization (Britten and Kohne, 1966, 1968). The labeling patterns of the mitotic and lampbrush chromosomes show, therefore, that repetitive DNA is widely distributed in the genome of *T. viridescens*.

Biochemical methods have demonstrated the presence of repetitive DNA in the genome of every eukaryotic species so far examined (Britten and Kohne, 1968). The amount of repeated sequences varies in different species from 20% to at least 80% of the total nuclear DNA under standard criteria of measurement. Variable numbers of identical or similar repeated sequences are included in different “families,” which are interspersed in the length of the genome. Some of these sequences are known to be transcribed, since in vivo-labeled RNA will hybridize to the DNA of higher organisms at low Cot values. Furthermore, different families of repeated sequences seem to be transcribed in different tissues and embryonic stages (see Britten and Kohne, 1970).

The hybridization of cRNA to mitotic and lampbrush chromosomes has also shown that binding occurs preferentially to the DNA of the centromeric heterochromatin. The labeling ob-
served in interphase nuclei demonstrates that the centromeric regions are associated in chromosomes. Since the rate of hybridization is proportional to the concentration of complementary sequences, the high level of hybridization indicates that the centromeric heterochromatin contains highly repeated sequences. This interpretation is supported by the situation observed in other organisms in which repetitious DNA is included in the centromeric heterochromatin (see Introduction). In particular, in the mouse the light satellite DNA (Waring and Britten, 1966), formed of a sequence between 8 and 13 nucleotide pairs long and repeated up to millions of times (Southern, 1970), is located in the centromeric heterochromatin (Pardue and Gall, 1969, 1970; Jones, 1970). These results raise questions about possible relationships between highly repetitive DNA and centromere structure and function (see Brinkley and Stubblefield, 1970): there is, in fact, evidence that the centromeric heterochromatin may affect the behavior of the centromere (Lindsley and Novitski, 1958; Crouse, 1960). Moreover, the presence of highly repetitive DNA in the centromeric heterochromatin could provide a biochemical basis for phenomena involving centromeres: in D. melanogaster, somatic crossing-over takes place preferentially in the centromere region (Stern, 1936), and in human lymphocytes it has been demonstrated that sister chromatid exchanges occur also at the centromeres (Cuevas-Sosa, 1967). Centromeric fusions have been observed in lampbrush chromosomes (Gall, 1954; Callan and Lloyd, 1960); and the chromocenters of polytene nuclei (Heitz, 1934) and of interphase nuclei of some species (for instance mouse: see Pardue and Gall, 1970) are examples of centromere associations. The hybridization of cRNA at the telomere regions indicates that they also contain repetitive DNA and suggests a biochemical similarity between centromeres and telomeres (see Eckhardt and Gall, 1971). These aspects, however, require further study to be clarified. 

Hybridization of lampbrush chromosomes showed that repetitive DNA is also located at the sphere loci. Although it is known that spheres probably contain acidic proteins (Gall, 1954) and incorporate radioactive amino acids but not RNA precursors (Mancino, Barsacchi, and Nardi, 1968), their functional meaning remains unknown. In lampbrush preparations made from oocytes undergoing maturation, after either a gonadotropic injection to the females or an in vitro treatment of oocytes with progesterone, the chromosomes lack spheres but display instead prominent loops at the sphere loci (G. Barsacchi and A. A. Humphries, Jr., unpublished observation). After cytological hybridization with cRNA these newly formed loops showed a moderate number of grains, indicating that they contain repetitive DNA. These observations may induce one to speculate about the possible relationships between the sphere loci and phenomena connected with oocyte maturation: if such a relationship exists, the repetitive DNA located at the sphere loci could be functionally related to the need for a large amount of a particular gene product.

The authors wish to thank Mrs. Cherry Barney for her technical assistance and Dr. R. A. Eckhardt for helping with the analytical centrifugations.

This work was supported in part by United States Public Health Service Grant GM-12427 from the National Institute of General Medical Sciences and was carried out while Dr. Barsacchi was the recipient of a NATO fellowship.

Received for publication 28 February 1972, and in revised form 19 May 1972.

REFERENCES

AMALDI, F., and M. BUONGIORNO-NARDELLI. 1971. Molecular hybridization of Chinese hamster 5S, 4S and “pulse-labelled” RNA in cytological preparations. Exp. Cell Res. 65:529.

ARRIGHI, F. E., T. C. Hsu, P. SAUNDERS, and G. F. SAUNDERS. 1970. Localization of repetitive DNA in the chromosomes of Mesocricetus auratus by means of in situ hybridization. Chromosoma. 32:224.

BRINKLEY, B. R., and E. STUbbLEFIELD. 1970. Ultrastructure and interaction of the kinetochore and centriole in mitosis and meiosis. In Advances in Cell Biology. D. M. Prescott, L. Goldstein, and E. McConkey, editors. North Holland Publishing Co., Amsterdam. 1:119.

BRITTEN, R. J., and D. E. KOHNE. 1966. Nucleotide sequence repetition in DNA. Carnegie Inst. Wash. Year Book. 65:78.

BRITTEN, R. J., and D. E. KOHNE. 1968. Repeated sequences in DNA. Science (Wash. D. C.). 161:529.

BRITTEN, R. J., and D. E. KOHNE. 1970. Repeated sequences of DNA. Sci. Am. 222:24.

BUONGIORNO-NARDELLI, M., and F. AMALDI. 1970. Autoradiographic detection of molecular hybrids between rRNA and DNA present in tissue sections. Nature (Lond.). 225:946.

BURGESS, R. R. 1969. A new method for the large
scale purification of Escherichia coli deoxyribonucleic acid-dependent ribonucleic acid polymerase. J. Biol. Chem. 244:6160.

Gallan, H. G., and L. Lloyd. 1960. Lampbrush chromosomes of crested newts Triturus cristatus (Laurenti). Phil. Trans. R. Soc. (London). Ser B Biol. Sci. 243:135.

Conger, A. D., and L. M. Fairchild. 1955. A quick-freeze method for making smear slides permanent. Stain Technol. 28:281.

Crouse, H. O. 1960. The controlling element in sex chromosome behavior in Serta. Genetics. 45:1429.

Cuevas-Sosa, H. 1967. Crossing-over and the centromere. Cytogenetics (Basel). 3:331.

Gall, J. G., and M. L. Pardue. 1971. Satellite DNA associated with heterochromatin in Rhyacochiara Chromosoma. 32:407.

Gall, J. G. 1954. Lampbrush chromosomes from oocyte nuclei of the newt. J. Morphol. 94:283.

Gall, J. G. 1966 Techniques for the study of lampbrush chromosomes. In Methods in Cell Physiology D. M. Prescott, editor. Academic Press Inc., New York. 2:37.

Gall, J. G. 1969. The genes for ribosomal RNA during oogenesis. Genetics. 61(Suppl.):121.

Gall, J. G., G. H. Cohen, and M. L. Polan. 1971. Repetitive DNA sequences in Drosophila. Chromosoma. 32:319.

Gall, J. G., and M. L. Pardue. 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Natl. Acad. Sci. U.S.A. 63:378.

Gall, J. G., and M. L. Pardue. 1971. Nucleic acid hybridization in cytological preparations. Methods Enzymol. 21:170.

Gillespie, D., and S. Spiegelman. 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. J. Mol. Biol. 12:829.

Hertz, E. 1934. Uber alpha- und beta-Heterochromatin sowie Konstanz und Baut der Chromosomen bei Drosophila. Biol. Zentralbl. 54:506.

Henning, W., I. Henning, and H. Stein. 1970. Repeated sequences in the DNA of Drosophila and their localization in giant chromosomes. Chromosoma. 32:31.

John, H. A., M. L. Brunsfeld, and K. W. Jones. 1969 RNA-DNA hybrids at the cytological level Nature (Lond.). 223:582.

Jones, K. W. 1970. Chromosomal and nuclear location of mouse satellite DNA in individual cells Nature (Lond.). 225:912.

Jones, K. W., and F. W. Robertson. 1970. Localization of reiterated nucleotide sequences in Drosophila and mouse by in situ hybridization of complementary RNA. Chromosoma 31:331.

Lindsay, D. L., and E. Novitski. 1958. Localization of the genetic factors responsible for the kinetic activity of the X chromosome of Drosophila melanogaster. Genetics. 43:790.

Macgregor, H. C., and J. Kezer. 1971. The chromosomal localization of a heavy satellite DNA in the testis of Plethodon c. cinereus. Chromosoma. 33:167.

Mancino, G., G. Barsacchi, and I. Nardi. 1968. Ulteriori ricerche autoradiografiche sull'in-corporazione di H³ eucinolammina nel lampbrush chromosomes di Triturus (Anfibii Urodelli). Atti Accad. Naz. Lincei. Ser. 8. 45:180.

Mandel, M., and J. Marmur. 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. Methods Enzymol. 12 B:195.

Pardue, M. L., and J. G. Gall. 1969. Molecular hybridization of radioactive DNA to the DNA of cytological preparations. Proc. Natl. Acad. Sci. U.S.A. 64:200.

Pardue, M. L., and J. G. Gall. 1970. Chromosomal localization of mouse DNA. Science (Wash. D.C.). 168:1356.

Pardue, M. L., S. A. Gerbi, R. A. Eckhardt, and J. G. Gall. 1970. Cytological localization of DNA complementary to ribosomal RNA in polytene chromosomes of Diptera Chromosoma. 29:268.

Rae, P. M. M. 1970. Chromosomal distribution of rapidly reannealing DNA in Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A. 67:1018.

SchilKraut, C. L., J. Marmur, and P. Doty. 1962 Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. 4:30.

Southern, E. M. 1970. Base sequence and evolution of guinea-pig α-satellite DNA. Nature (Lond). 227:794.

Stern, C. 1936. Somatic crossing-over and segregation in Drosophila melanogaster. Genetics. 21:625.

Waring, M., and R. J. Britten. 1966. Nucleotide sequence repetition: a rapidly reassociating fraction of mouse DNA. Science (Wash. D.C.). 154:791.

Wimber, D. E., and D. M. Steffenen. 1970. Localization of SS RNA genes on Drosophila chromosomes by RNA-DNA hybridization. Science (Wash. D.C.). 170:839.