Title
THE DNA CONTENT OF FIVE CHINESE HAMSTER METAPHASE CHROMOSOME GROUPS

Authors
Regimbal, T.J.
Mel, H.C.

Publication Date
1971-07-01

Submitted to Experimental
Cell Research

THE DNA CONTENT OF FIVE CHINESE HAMSTER METAPHASE CHROMOSOME GROUPS

H. John Burki, T. J. Regimbal, Jr. and H. C. Mel
DONNER LABORATORY

July 1971

AEC Contract W-7405-eng-48

DISCLAIMER

THE DNA CONTENT OF FIVE CHINESE HAMSTER METAPHASE CHROMOSOME GROUPS

H. JOHN BURKI, T. J. REGIMBAL, JR.[1] and H. C. MEL

Donner Laboratory, University of California,

Berkeley, Calif. 94720, USA

Proofs and reprints should be sent to: Dr. H. John Burki,

Donner Laboratory, University of California, Berkeley, Calif. 94720

Number of Tables: 4

Number of Figures: 9

Abbreviated running title: Hamster Chromosome DNA

[1] Present address: University of Washington School of Medicine,

Pediatric Department, Seattle, Wash. 98105.

THE DNA CONTENT OF FIVE CHINESE HAMSTER METAPHASE CHROMOSOME GROUPS

H. JOHN BURKI, T. J. REGIMBAL, JR.[1] and H. C. MEL

Donner Laboratory, University of California,

Berkeley, Calif. 94720, USA

SUMMARY

Chinese hamster cells in culture were synchronized, collected at metaphase, homogenized to release the chromosomes, and the chromosomes fractionated in a sucrose gradient using a zonal centrifuge with an A12 zonal rotor. Chromosomes in the separated fractions as well as in control metaphase spreads were quantitatively classified into five easily distinguished groups, according to individual measurements of length and centromeric index. For each zonal fraction, chemical determinations were made of the amount of DNA per average chromosome. Using the group compositional data for each fraction, the amount of DNA per average chromosome in each of the groups was then calculated to be: Group I (chromosomes 1, 2) = 1.00 $\pm$ 0.14 pgm/chromatid; Group II (chromosomes 4, X, 5) = 0.39 $\pm$ 0.05 pgm/chromatid; Group III (chromosomes Y, 6, 7, 8) = 0.24 $\pm$ 0.04 pgm/chromatid; Group IV (chromosomes 9, 10, 11) = 0.13 $\pm$ 0.004 pgm/chromatid; and Group V (a small marker in this cell line) = 0.06 pgm/chromatid. These values are in good agreement

with the literature values for relative chromosomal DNA content derived from cytospectrophotometric measurements of fuelgen stained hamster metaphase spreads. They indicate that unlike the case for human chromatids the amount of DNA found in hamster chromatids is not directly proportional to the chromatid length.

Estimates of the DNA content are also given for individual chromatid types.

A major purpose in preparative fractionation of mammalian cell chromosome populations is to obtain chromosomal specific DNA for use in a variety of investigations of the genetic organization of the mammalian cell genome. Such studies include organization of DNA replication, inter- and intrachromosomal DNA-DNA hybridization, DNA-RNA hybridization including viral RNA localization, and "in vitro" production of chromosome specific RNA. Such chromosomal DNA separation and ancillary DNA studies could also be applied to the field of human cytogenetics to investigate disease conditions known or suspected to be associated with specific chromosomal imbalances.

Recent work on fractionation of human, mouse, and Chinese hamster chromosomes has been reported by Maio and Schildkraut [21, 22] and Mendelsohn et al. [23], based on swinging bucket centrifugation in steep sucrose gradients and demonstrating substantial size separations of the metaphase chromosomes. We have reported size fractionation of Chinese hamster chromosomes using zonal centrifugation with an A12 rotor [27, 28]. A current report by Schneider and Salzman [32] indicates that fractionation

of human chromosome populations from phytohaemoglutin-stimulated primary lymphocyte cultures is now possible. We report here on the analysis of the chromosomes in zonal centrifuge separated fractions of the Chinese hamster metaphase complement. The approach is first in terms of a classification into five Groups, defined according to combined parameters of measured length and centromeric index, and second by measurements of DNA per average chromosome in each fraction. From these a calculation is made of the DNA per average chromosome in each Group, and the results compared with literature reports of relative DNA differences between different hamster chromosome types.

MATERIALS AND METHODS

Cell culture and synchrony methods

The M3-1 cell line was originally isolated from the bone marrow of a male Chinese hamster obtained from an inbred line from Yerganian. It is grown in medium containing 10% fetal calf serum, and is recultured at 6-month or shorter time intervals by thawing from a frozen stock. Slow freezing with DMSO and rapid thawing are employed [4]. In this study the cells were grown in 20 cm diameter glass dishes, blocked with 10^{-2} M thymidine for 8 hr, and washed to remove the thymidine. They were then grown in medium containing colchecine (10^{-4} M), and the mitotic cells dislodged by blowing medium over the blocked cultures, a variation of the method described by Terasima and Tolmach [36]. A typical harvest is about 2×10^8 metaphase cells, yielding about 4×10^9 chromosomes.

Homogenization of metaphase cells and size fractionation of metaphase chromosomes

The washed metaphase cells were added to a solution containing 0.3% triton-X 100 in a hypotonic formate buffer (10^{-3} M $MgCl_2 \cdot 6H_2O$; 10^{-3} M $CaCl_2$; 10^{-3} N KOH; .02 N Formic Acid) and homogenized on a Virtis 23 homogenizer run at maximum speed for 30 minutes. The mixed suspension containing chromosomes, nuclei, and debris was allowed to stand for a week or more before using to concentrate the chromosomes. Ten ml of mixed chromosome sample was layered onto a 5 to 30% sucrose gradient and loaded with a Beckman Model No. 141 high capacity gradient pump in an A12 zonal centrifuge rotor (International Equipment Co.). The sample was sedimented at 1000 rpm for 30 min and the rotor then unloaded at speed into 17-ml fractions in a fraction collector, using a 40% sucrose expelling solution.

Preparation of slides

Metaphase controls. Well-spread metaphases were obtained by blocking with thymidine and adding colcemid as described above. The cells were swollen in hypotonic solution, fixed with acetic alcohol (ethyl alcohol: acetic acid solution [3:1]), and stained with Giemsa using MacIlvain buffer at pH 7.6.

Crude zonal fractions. Chromosomes in their respective sugar solution fractions were centrifuged for 2 hr at 100 g, and the supernatant sugar solution discarded. The chromosome pellet was then washed once in the formate buffer plus Triton-X 100, and the pellet resuspended in the formate buffer alone. The chromosomes were resuspended in the

buffer and centrifuged down onto 15 mm diameter round coverslips on size 00 rubber stopper "platforms" through a depth of 1 cm of acetic alcohol fixative. The coverslips were air dried, mounted on slides, and the chromosomes stained with Giemsa as above.

Measurement of chromosomal length and centromeric index

Individual Giemsa-stained metaphase figures, and mixed isolated or size fractionated chromosomes were projected to 3100 X using a Leitz microscope with projection prism, and the "backbone" of the chromosomes traced. The tracing was then photographed and the chromosomes measured using a high speed scanning and measuring program, "Chromo" [13]. For comparison, several thousand tracings were measured by hand using a precision map measure (Keuffel and Esser No. 620300). From the measurements of each of the two chromatids per metaphase chromosome the average total arm length is divided by the average short-arm length; this ratio is known as the centromeric index.

Chromosome counting and collection for chemical analysis

Aliquots from the collected fractions were counted in a hemocytometer. The concentrations were repeatable within a 10% standard deviation. The remainder of the fraction (or the complete crude fraction in some experiments) was filtered through a Nucleopore filter of pore diameter 0.5 μ (General Electric Livermore) and thoroughly washed, first with the formate buffer then with cold 10% TCA. This method of chromosome collection was more reproducible in our hands than other centrifugal methods that were tried [Burki and Benz, unpublished observations].

Chemical determination of DNA in chromosomal fractions

From control experiments it was determined that the optimum DNA hydrolysis conditions in 5% TCA at 93°C was 30 min. The DNA was determined colorimetrically using the diphenylamine reagent, by the method of Dische [10]. Thirty minutes in the diphenylamine reagent at 93°C was found to give the most reproducible results. Calf thymus DNA was used to make DNA standard solutions. The concentration of these calf thymus DNA standards was normalized to twice phenol extracted, pronase treated, RNase treated, calf thymus DNA, O.D. ratios (260/280)= 1.81 and (260/230)= 2.01. This DNA was kindly supplied to us by Drs. W. Blackman and W. Taylor at The Pennsylvania State University (Department of Biophysics).

RESULTS

Construction of the idiogram for the M3-1 Chinese hamster cell line

In order to decide on the particular linkage groups of isolated chromosomes in the fractions one must have available an "absolute idiogram" based on measurements of the chromosome arm lengths in metaphase spreads. Fig. 1a is a photomicrograph of an M3-1 Chinese hamster spread. Fig. 1b shows a tracing of another metaphase spread. A distribution diagram of centromeric indexes (irrespective of total chromosome lengths) measured for 19 such metaphase chromosome complements is given in Fig. 2. On this diagram a natural division exists between metacentrics and submetacentrics, and acrocentric chromosomes, at a centromeric index 0.35. The apparent telocentric chromosome group (C.I. < 0.05) is included in the acrocentric group because of uncertainties in sketching and measuring

the short arm lengths. Some of the chromosomes measured in this cell line, however, appear to be truly telocentric.

Distribution diagrams for chromosome length are given in Fig. 3a (for acrocentrics) and Fig. 3b (for meta- and submetacentrics). The chromosome number designations are those proposed for the Chinese hamster by Hsu and Zenzes [15]. From Figs. 2 and 3 it is evident that, despite the variations between different metaphase figures, major Groups of chromosomes can be naturally defined according to measures of size and centromeric index. Specifications for the five Groups seen in Figs. 3a and 3b are summarized in idiogram form in Table 1. With suitable precaution (correcting for length contraction in isolated chromosomes), this same kind of classification can also be used for isolated chromosome fractions to obtain 5 major chromosome Groups.

Classification of chromosomes in the homogenate and crude zonal fractions

Attention was usually concentrated on zonal fractions 8 through 18, 20, 22, 25, 28 and 32. (The residual from the original sample was in fraction 7.) Beyond fraction 32 were found mostly clumps. Backbone traces for 3 slides in a typical zonal run are given in Figs. 4a, b and c. (Photomicrographs of various chromosome fractions will be given elsewhere [29]). Typically, at least 50 chromosomes are sketched and measured from each fraction, or approximately 800 total chromosomes per each zonal run.

Summary data for chromatid lengths in each zonal fraction, from three different experiments, are given in Fig. 5. The average of these chromatid lengths is seen to vary from 2 to 6 microns. This measured

size and size range is less than expected from the metaphase spread idiogram (Table 1), for two reasons: (1) these zonal fractions contain mixtures of different size Groups of chromosomes and thus the average chromatid length per fraction is reduced due to the presence of small chromosomes in zonal fractions enriched in large chromosomes, and (2) the absolute lengths for all of the fractions, however, are somewhat smaller than those expected from measurements of metaphase spreads, as indicated for one experiment in Fig. 6, wherein the average contraction is seen to be about 40%.

To correct for this absolute length contraction the following procedure was adopted. A normalization factor (to multiply all zonal fraction chromatid lengths) was computed as the ratio of the average length for Group IV chromosomes in the metaphase spreads (Fig. 4) to the average chromatid length in zonal fraction 11. This method was chosen because crude fraction 11 was generally enriched to at least 90% in Group IV chromosomes. The data corrected in this way, for 2800 chromosome measurements in three different experiments, are given in Fig. 7.

Measurement of chromosomal DNA and calculation of linear density of DNA per chromosome

The DNA per average chromosome in each zonal fraction, i, D(i), was calculated by measuring the total amount of DNA in the fraction $D_t(i)$ using the diphenylamine method and determining the total number of chromosomes in the fraction $N_t(i)$ using a hemocytometer. Thus, $D(i) = D_t(i)/N_t(i)$. The average amount of DNA per chromatid in a fraction is then $D(i)/2$. The values of D(i) determined in five different experiments

are given in Fig. 8. They are seen to range from about 0.2 pgm to about 1.1 pgm per average isolated chromosome, a range of about 5.5-fold.

We can now determine the average "linear density" of chromosomal DNA by dividing each D(i) by twice the average chromatid length for that fraction, $\ell(i)$ (see Fig. 5), with the results given in Fig. 9. There appear to be at least two discreet values for the linear densities, i.e. those for fractions 8-13: 0.059 pgm/micron of chromatid, and for fractions 14-18: 0.075 pgm/micron. The difference between the SE values of linear density for fractions 8-13 and for fractions 14-18 is significant at the .07% level. The difference between the values of linear density for fraction 14-18 and fraction 20-32 is probably not significant.

DNA content of the various chromosomal types

The classification of chromosomes in the homogenate and crude zonal fractions permits calculating compositional data, in terms of each of the 5 size-form Groups, for each of the 16 zonal fractions. Combining these with the DNA data for each fraction we can set up and solve 16 simultaneous equations in 5 unknowns and arrive at figures for the DNA content per average chromosome in each Group.

For example, in a typical experiment we have 16 equations of the following type:

$$D(i) = f_I \cdot D_I + f_{II} \cdot D_{II} + f_{III} \cdot D_{III} + f_{IV} \cdot D_{IV} + f_V \cdot D_V \qquad (1)$$

where D_I, D_{II}, D_{III}, D_{IV}, D_V = the estimated DNA content of an average metaphase chromosome (2 chromatids) in each

defined chromosome Group,

and, f_I, f_{II}, f_{III}, f_{IV}, f_V = the fraction of the total chromosomes in zonal fraction i which are from a specific Group.

Initial guesses of D_I, D_{II}, etc., are made and equation (1) is solved. This will usually result in a D(i) value for each zonal fraction differing from the measured value by an amount $\pm \Delta D(i)$. This procedure is iterated using values of D_I, D_{II}, etc., chosen to minimize the mean square deviations, $\sum_{m=1}^{16} (\Delta D(i))^2$, by a least squares program kindly supplied by Mr. Fred Gey of the Lawrence Radiation Laboratory. For each experiment a best estimate of D_I, D_{II}, D_{III}, D_{IV}, and D_V can then be made.

The results from three different experiments are shown in Table 2. The DNA content expressed in molecular weight units is seen to vary from 7.8×10^{10} daltons for Group IV chromosomes to 60×10^{10} daltons for Group I chromosomes. From these values one may estimate the average amount of DNA per metaphase cell;

$$\bar{D}_{meta.} = 4 \cdot \bar{D}_I + 5 \cdot \bar{D}_{II} + 7 \cdot \bar{D}_{III} + 6 \cdot \bar{D}_{IV} + 1 \cdot \bar{D}_V$$

where $\bar{D}_I$, $\bar{D}_{II}$, $\bar{D}_{III}$, $\bar{D}_{IV}$, and $\bar{D}_V$ are the average DNA/chromosome Group values from several experiments.

The result is $\bar{D}_{meta} = 16.9 \pm 2.8$ pgm; the diploid amount of DNA is thus 8.5 ± 1.4 pgm. This value may be compared with those reported by Stubblefield *et al.* [34] for diploid Chinese hamster DON cells, of 9 pgm DNA per interphase cell and 16 pgm per cell in a population containing 90% metaphases. Lett *et al.* [20] have found a value of 8.5 pgm per cell for Chinese hamster CHO cells in logarithmic growth conditions. The amount of DNA/cell measured in the latter two works is a function of

the life cycle states in their cell populations. Our results with logarithmic cells average to 8.4 ± 0.6 pgm DNA per average cell. Taking into account life cycle parameters of our own cultures of M3-1 Chinese hamster cells and assuming a constant rate of DNA synthesis during the S period, the DNA estimate is 6 ± 1 pgm for G1 cells or 12 ± 2 pgm for metaphase cells.

Standardization of linear DNA density for hamster metaphase chromosome spreads

The linear density of DNA can also be determined from our average metaphase-spread chromatid length data which are similar to those of Hsu and Zenzes [15], Table 1. The total metaphase spread chromatid length for an individual spread may be normalized to some number which is near the average metaphase spread chromatid length. These results are given in column 5 of Table 3. The absolute values of the linear densities differ from those values seen in Fig. 9 because of the previously discussed contraction effect in isolated chromosomes. In order to compare the available literature data, relative linear densities (based on normalization to Group IV chromosomes taken as 1.0) are also included in Table 3 (columns 4 and 6).

DISCUSSION

Chromosome separation and scoring

The yields of the different chromosomal Groups in our zonal fractions demonstrate the suitability of this system for large scale preparative separations of defined chromosomes. With refractionation one may obtain

much greater purity, in some cases almost pure fractions of one particular chromosome type.

An important adjunct of any separation procedure is the availability of an objective method of scoring the results. In our case, having chosen size and Group type I-V as primary assays, we needed measurements on at least 50 chromosomes per fraction, for 16 fractions per run. Although hand measurements on the backbone sketches of individual chromosomes are feasible, with large numbers this method is impractical. Thus the automation provided by the "Chromo" scanning and pattern recognition program [13], originally established for measuring chromosomes in human cell metaphase spreads, was invaluable for quickly processing our voluminous data, giving the composition of each fraction in every experiment.

Some additional problems are found in scoring the size and centromeric index of isolated mammalian metaphase chromosomes. For different metaphase spreads, lengths of individual chromosomes may be normalized to some arbitrary total cellular chromatid length, thus correcting for chromosome size variability due to differing contractions (resulting in part from differing times the cells are held in mitosis by colchecine). Such a length normalization obviously cannot be carried out directly for chromosomes in isolated fractions, however. Another contraction problem may arise from the use of our acid (pH 3.7) isolation buffer [5]. As mentioned above we dealt with this problem by normalizing fraction 11, which was virtually always quite pure in the smallest Group IV chromosomes, to the average size of the chromosomes 9, 10, and 11 obtained from metaphase spread measurements. Size variation is a problem even in neutral pH, as in Maio and Schildkraut [22], in which some of the

isolated chromosomes are larger than the largest metaphase chromosomes, perhaps due to uncoiling. These authors chose to normalize their measurements to the mitotic figures "in which the length of the longest chromosome approximated that of the longest of the isolated chromosomes."

All normalizations, including the one used in this report, assume a linearity of contraction or expansion for all chromosomes. If not correct this could lead to an error in identifying certain chromosomes. It was for this reason that the hamster karyotype was scored only in terms of the most obvious five size Groups. Future more positive identification procedures for individual chromatids, e.g. by characteristic fluorescence patterns [8] or by specific denaturation [Hsu, personal communication], would be of great value.

The DNA linear density

General comparison with other chromosomal DNA data. Table 3 includes two sets of data on chromosomal DNA linear density. Our data are seen to agree quite well with those of Carlson et al. [6] whereas other previous data for Chinese hamster metaphase chromosomes appear to be contrary to these [23].

From the cytospectrophotometric data of Carlson et al. [6] we calculate the ratio of 1.5 for the linear DNA density between chromosomes in combined Groups I, II and III, and those in Group IV. The data of Mendelsohn et al. [23] give a ratio of 2.4 for the same quantities. This latter estimate depends on the constancy of average cross sectional area of chromosomes, since the Mendelsohn et al. [23] data are given in

terms of relative DNA mass per chromosome Group. Very likely, however, the chromosomes in the larger Groups have a larger average cross sectional area [9] which would lead to an even larger figure than 2.4 for this ratio.

One possible reason why the data of Mendelsohn et al. [23] differ from the other two sets is that in their isolated fractions the smaller chromosomes appear to be contaminated with RNA [37] and perhaps even with adsorbed protein coating and debris. Thus their measurements of relative DNA content may be quite different from absolute DNA measurements, in small chromosomes.

A possible source of error in absolute DNA measurement would be incomplete hydrolysis of the DNA. If occurring preferentially for the smaller chromosomes this would lead to an increased ratio of DNA content of large to small chromosomes. To consider this possibility we looked at the hydrolysis kinetics of tritiated thymidine labelled DNA in different size chromosomes, but found them apparently to be identical [Burki and Benz, unpublished observations]. We therefore believe the absolute DNA measurements reported here, which agree with data obtained in a quite different fashion by Carlson et al. [6] to be the more reliable.

Variations in DNA linear density. From Caspersson et al. [7] (which appeared during preparation of this manuscript), it is possible to make a further analysis of the 'linear density of DNA' data. These authors give the relative DNA distribution patterns along the length of the hamster chromosomes. The data in the Caspersson et al. [7] report are consistent with our data and with the following: Group IV chromosomes

and the short arms of Group III chromosomes have a relatively lower DNA linear density, while the long arms of Group III as well as both arms of Group II and Group I chromosomes have a different and higher average linear density.

Comparison with human chromosomes. The linear density values reported here indicate a qualitative difference between hamster and human chromosome DNA organization, for in human chromosomes the average DNA per unit length appears to be constant [11, 30]. This raises interesting questions: (1) What is the genetic significance of the greater DNA 'linear density' in certain hamster chromosomes, and (2) do the larger hamster chromosomes have a reduced susceptibility to breakage per unit length than do Group IV chromosomes?

Estimate of DNA per individual chromosome type. In columns 2 and 3 in Table 4 are presented comparative data on the DNA content of the different chromosome Groups. It is evident that the method of Carlson et al. [6] and our method complement each other in the sense that the spectrophotometric measurements are most precise for the largest chromosomes, whereas ours are most precise for the smaller (Group IV) chromosomes.

Calculated amounts of DNA per individual chromosome type, based on the Carlson et al. [6] data, are given in columns 5 and 6 in Table 4. Chemical verification of these values will come when "pure" individual types of chromosomes are available in sufficient quantity to measure the DNA, using the diphenylamine or other suitable method.

ACKNOWLEDGEMENTS

The authors are indebted to John Gofman, Robert Tandy, Stuart Stone, Jay Minkler, and Dolores Piluzo at the Bio-Medical Section of the Lawrence Radiation Laboratory Livermore for help in setting up a chromosome sketching program for hamster chromosomes and use of the "Chromo" system and high speed scanner for measuring the chromosome parameters.

The competent technical support of Mrs. Katherine Lathrop, the late Mrs. W. M. Jackson, and Miss Patricia Leslie is especially appreciated.

Mr. Fred Gey is thanked for his assistance with the Linear Least Squares computer data processing program.

Support for this research was received from the United States National Aeronautics and Space Administration (grant no. W12 792 Task 6) and the California Division of the American Cancer Society.

FIGURE LEGENDS

Fig. 1a. Typical metaphase spread of the M3-1 cell line. This spread contains 23 chromosomes, the average for this cell line (50% of the metaphases). Other spreads contain 22 chromosomes (23%) and 24 chromosomes (23%), and 4% have other chromosome numbers. (Based on 80 metaphase figures.)

Fig. 1b. "Backbone" tracing of another M3-1 metaphase spread. (The number at the bottom is the tracing number for reference to tape storage of computer measurements.)

Fig. 2. The percentage of the total chromosomes from 19 metaphases as a function of centromeric index.

Fig. 3a. The percentage of the acrocentric metaphase chromosomes from 19 metaphases as a function of the measured length of the chromosomes.

Fig. 3b. Percentage of the meta- and submetacentric chromosomes from 19 metaphases as a function of the measured lengths of the chromosomes. (Recent analysis of 100 metaphases yields essentially the same distributions.)

Fig. 4. Backgone chromosome tracings for zonal separation 445; (a) zonal fraction 11; (b) zonal fraction 15; (c) zonal fraction 28.

Fig. 5. Average lengths of chromatids within the different 17 ml zonal fractions.

Fig. 6. Cumulative percent of the chromosomes as a function of their absolute lengths for metaphase cells (unfilled dots) and one zonal separation E 450 (black dots). The isolated chromosomes are about 40% shorter in this experiment than those measured in metaphase spreads.

Fig. 7. The distribution of the various chromosomal Groups within the zonal fractions. (a) Group I: containing chromosomes 1 and 2; (b) Group II: chromosomes 4, X, 5; (c) Group III: chromosomes 6, 7, 8, and Y; (d) Group IV: chromosomes containing 9, 10, 11; (e) Group V: acrocentric marker in this M3-1 cell line; and (f) unclassified chromosomes.

Fig. 8. The DNA per average metaphase chromosome (2 chromatids) found for each zonal fraction. The mean and standard errors are based on five experiments starting with different initial chromosome homogenates.

Fig. 9. The DNA per micron of average chromosome for each zonal fraction. This graph is obtained from the data in Fig. 8 and Fig. 5, and is not normalized to metaphase chromosomal length.

REFERENCES

1. Britten, R J & Davidson, E H, Science 165 (1969) 349.

2. Britten, R J & Kohne, D E, Science 161 (1968) 529.

3. Buongeorno, M, Nardelli, M & Amaldi, F, Nature 225 (1970) 946.

4. Burki, H J & Okada, S, Proc soc exptl biol & med 127 (1968) 794.

5. Cantor, K P & Hearst, J E, Proc nat acad sci US 55 (1966) 642.

6. Carlson, L, Caspersson, T, Foley, G E, Kudynowski, J, Lomakka, G, Simonsson, E & Saren, L, Exptl cell res 31 (1963) 589.

7. Caspersson, T, Simonsson, E & Zech, L, Exptl cell res 63 (1970a) 243.

8. Caspersson, T, Zech, L, Johansson, C & Modest, E J, Chromosoma (Berlin) 30 (1970b) 215.

9. Cole, A, Theoretical and experimental biophysics, vol. 1, M. Dekker, New York (1967).

10. Dische, Z, The nucleic acids (ed E Chargoff & J N Davidson) vol. I, p. 67. Academic Press, New York (1955).

11. DuPraw, E J, Cell and molecular biology, p. 522. Academic Press, New York (1968).

12. Gall, J G & Pardue, M L, Proc nat acad sci US 63 (1969) 378.

13. Gofman, J W, Minkler, J L & Tandy, R K, UCRL-50356, Lawrence Radiation Laboratory Livermore (1967).

14. Hearst, J E & Botchan, M, Ann rev biochem 39 (1970) 151.

15. Hsu, T C & Zenzes, M, J natl cancer inst 32 (1964) 857.

16. Huberman, J A & Attardi, G, J cell biol 31 (1966) 95.

17. Huberman, J A & Attardi, G, J molec biol 29 (1967) 487.

18. John, H, Birnsteil, M & Jones, K, Nature 223 (1969) 582.

19. Jones, K W, Nature 225 (1970) 912.

20. Lett, J T, Klucis, E S & Sun, C, Biophys j 10 (1970) 277.

21. Maio, J J & Schildkraut, C L, J molec biol 24 (1967) 29.

22. Maio, J J & Schildkraut, C L, J molec biol 40 (1969) 203.

23. Mendelsohn, J, Moore, D E, & Salzman, N, J molec biol 32 (1968) 101.

24. Nitsch, B, Murken, J & Brück, H, Histochemie 23 (1970) 254.

25. Pardue, M & Gall, J, Proc nat acad sci US 64 (1969) 600.

26. Pardue, M & Gall, J, Science 168 (1970) 1356.

27. Regimbal, T J & Mel, H C (Abstract) 13th Bioph. Soc. Meeting (Feb. 26-Mar. 1, 1969) Los Angeles, Calif.

28. Regimbal, T J, Mel, H C & Burki, H J (Abstract) 14th Bioph. Soc. Meeting (Feb. 25-27, 1970) Baltimore, Md.

29. Regimbal, T J, Ph.D. thesis (1971), Univ. of Calif., Berkeley.

30. Rudkin, G T, The chromosome (ed G Yerganian) p. 12. Williams & Wilkins, Baltimore (1967).

31. Salzman, N, Moore, D & Mendelsohn, J, Proc nat acad sci US 56 (1966) 1449.

32. Schneider, E L & Salzman, N P, Science 167 (1970) 1141.

33. Stone, S & Littlepage, J, UCRL-71493, Lawrence Radiation Laboratory Livermore (1969).

34. Stubblefield, E, Klevecz, L & Deaven, L, J cell physiol 69 (1967) 345.

35. Studier, F W, J molec biol 11 (1965) 373.

36. Terasima, T & Tolmach, L J, Biophys j 3 (1963) 11.

37. Wray, W & Stubblefield, E, Exptl cell res 59 (1970) 469.

38. Yerganian, G, J nat cancer inst 20 (1958) 705.

Table 1

CHINESE HAMSTER
METAPHASE CHROMOSOME GROUPS

GROUP	CHROMOSOMES	NO.	CHROMATID LENGTH ±S.E.	CENTROMERIC INDEX	DIAGRAM
I	1, 2	4	9.8 ± 0.10	0.47	
II	4, X, 5	5	5.8 ±0.07	0.45	
III	Y, 6, 7, 8	7	4.0 ±0.05	0.20	
IV	9, 10, 11	6	2.7 ±0.04	0.45	
V	Marker 1	1	1.5 ±0.09	0.00	

DBL 704 5659

Table 2. Average DNA Content Per Chinese Hamster Cell Chromatid[a]

Group	Chromosomes	DNA/Chromatid (pgm)	DNA/Chromatid[b] (cm)	DNA/Chromatid (daltons)
I	1,2	1.00 ±.18	31 ±6	60 ±11 x 10^{10}
II	4,X,5	0.39 ±.06	12 ±1.9	23 ±3.6 x 10^{10}
III	Y,6,7,8	0.24 ±.05	7.4 ±1.6	14.4 ±3.0 x 10^{10}
IV	9,10,11	0.13 ±.005	4.0 ±0.2	7.8 ±0.3 x 10^{10}
V	Marker 1	0.06	1.9	3.6 x 10^{10}

[a]The results are the means of three different experiments ± the standard error.

[b]Assuming 31 cm double stranded DNA/picogram DNA.

Table 3. A Comparison of Data on DNA Content in Chromosomes

Group	Chromosomes	Carlson et al. (1963)			Burki et al. (1970)		
		E.C.[a]/micron	Relative Linear Density[b]		DNA/micron	Relative Linear Density[b]	
I	1,2	1.02 ±.08[c]	1.7 ±.2	1.5	0.10 ±0.014	2.1 ±0.3	1.6
II	4,X,5	1.03 ±.14	1.7 ±.2		0.067 ±0.009	1.4 ±0.2	
III	Y,6,7,8	0.74 ±.10	1.2 ±.2		0.060 ±0.01	1.25 ±0.2	
IV	9,10,11	0.61 ±.08	1.0		0.048 ±0.002	1.0	
V	Marker 1	----	----		0.04	0.9	

[a]Extinction coefficient.

[b]The relative linear density is with respect to chromosomes in group IV.

[c]Standard deviation.

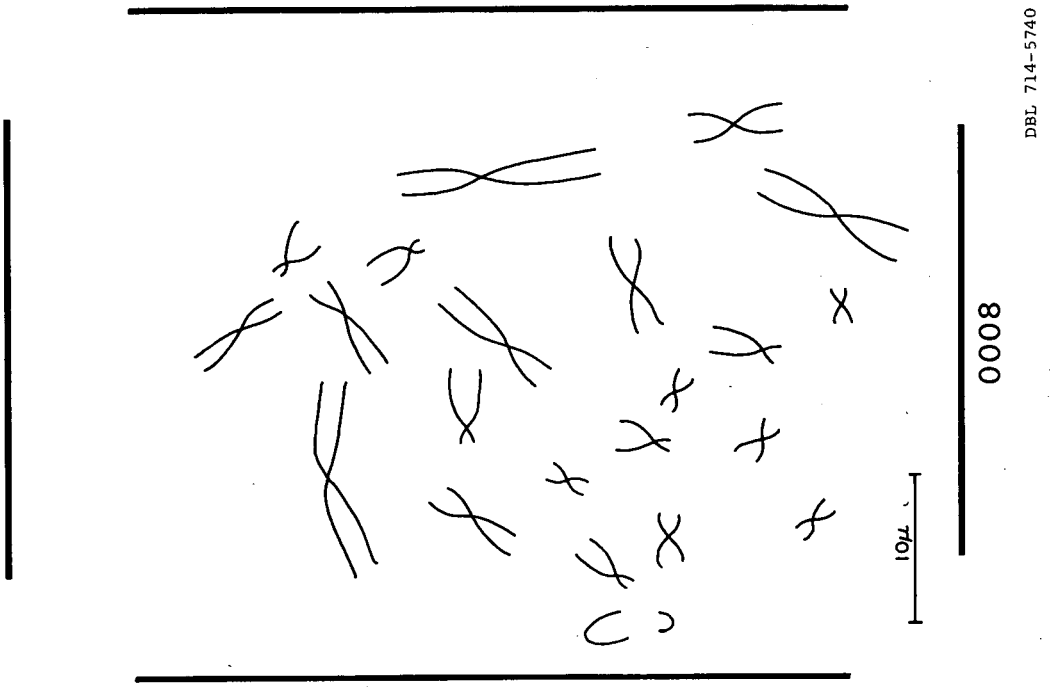
Table 4. Amount of DNA in Chinese Hamster Chromosomes[a]

Group	DNA/Chrom (pgm) this report	DNA/Chrom (pgm) based on Carlson et al. data	Ave. DNA/ Chrom (pgm)	Individual chromosomal DNA[b] (pgm)	Chromosomes
I	1.00 ± .14	0.79 ± .07	0.90	0.98 0.82	1 2
II	0.39 ± .05	0.40 ± .05	0.40	0.45 0.41 0.34	4 X 5
		----[c]	----	----	Y
III	0.24 ± .04	0.27 ± .03	0.26	0.28 0.26 0.24	6 7 8
IV	0.13 ± .004	0.13 ± .02	0.13	0.16 0.13 0.10	9 10 11

[a]Assuming diploid amount of DNA per cell is 8.0 picograms.

[b]Calculation is based on the assumption that the DNA content for individual chromosomes within a group reflects the total extinction coefficient measured by Carlson et al. (1963).

[c]The Carlson et al. (1963) data was based on female cells.



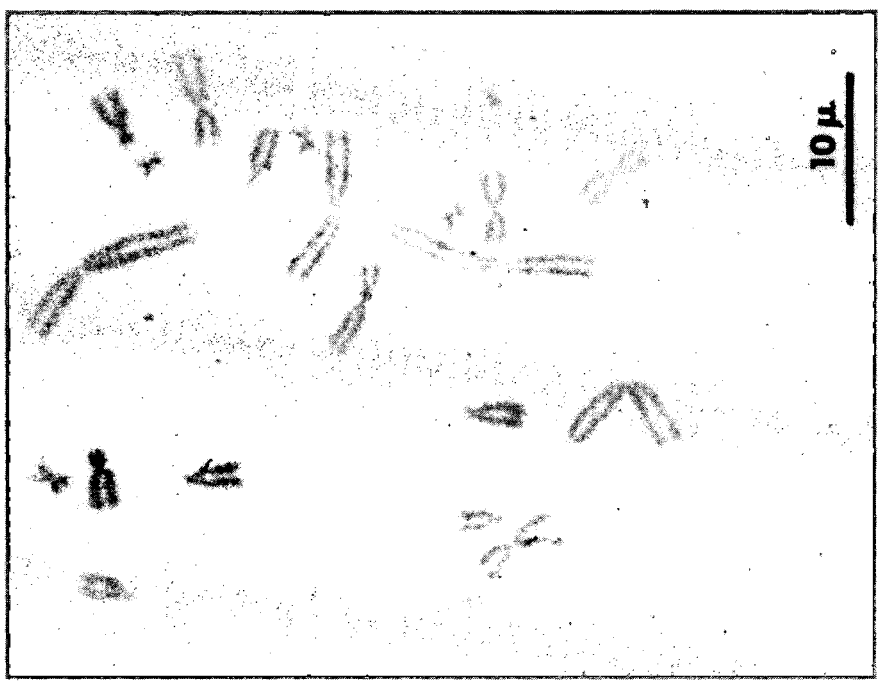
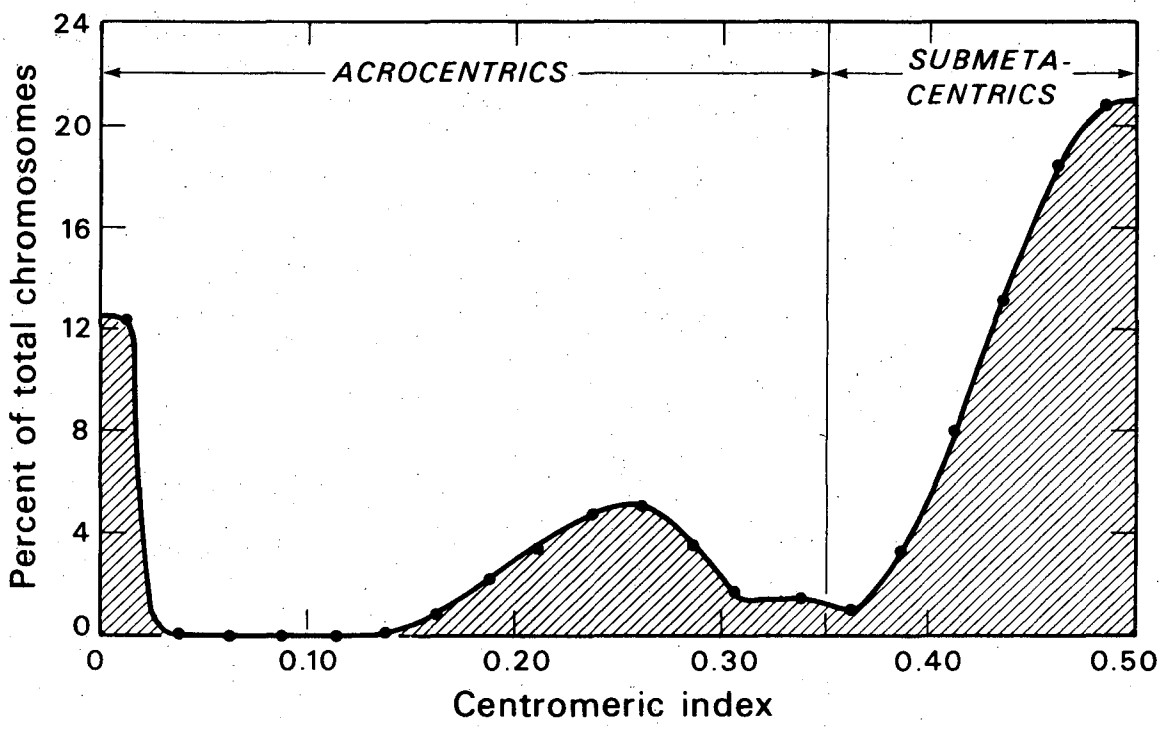
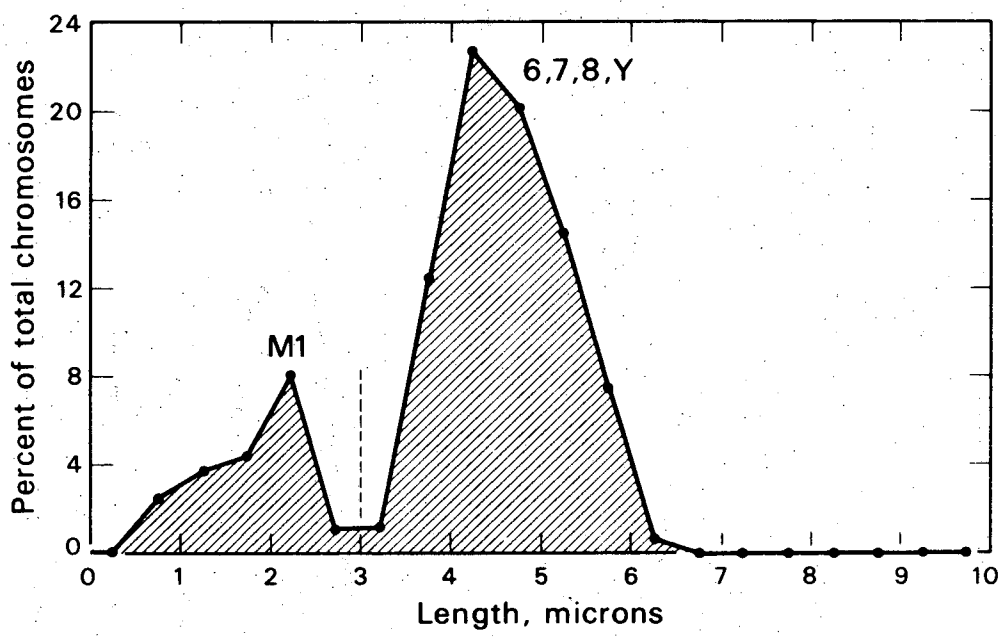


Fig. 1

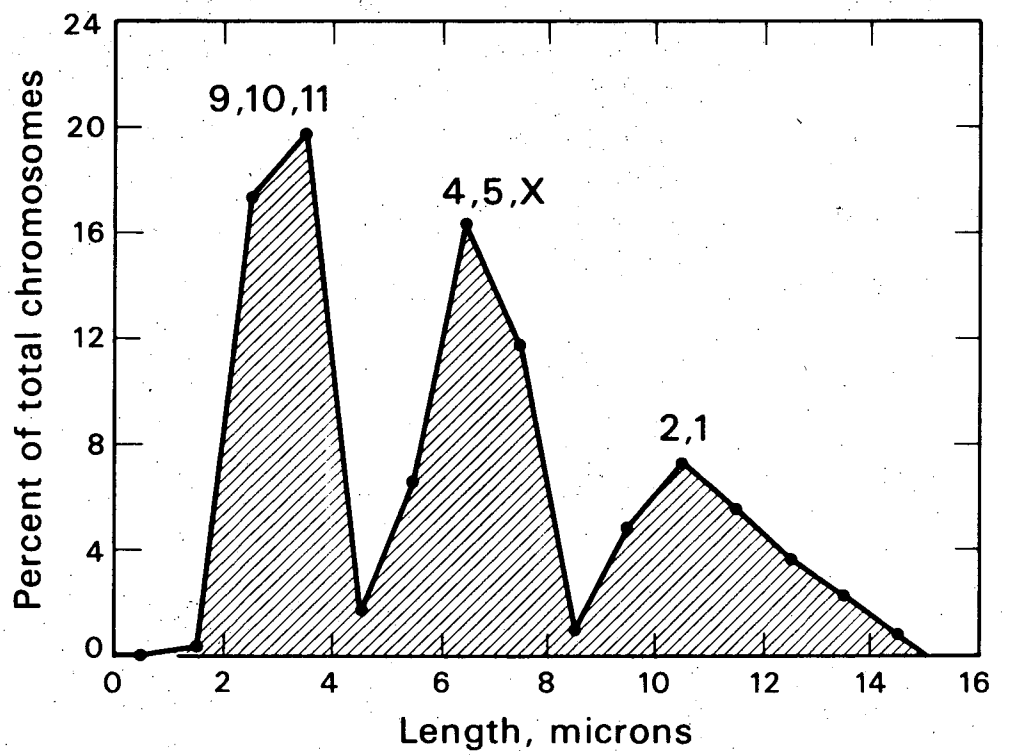


DBL 704 5654

Fig. 2



DBL 704 5653



DBL 704 5655

Fig. 3

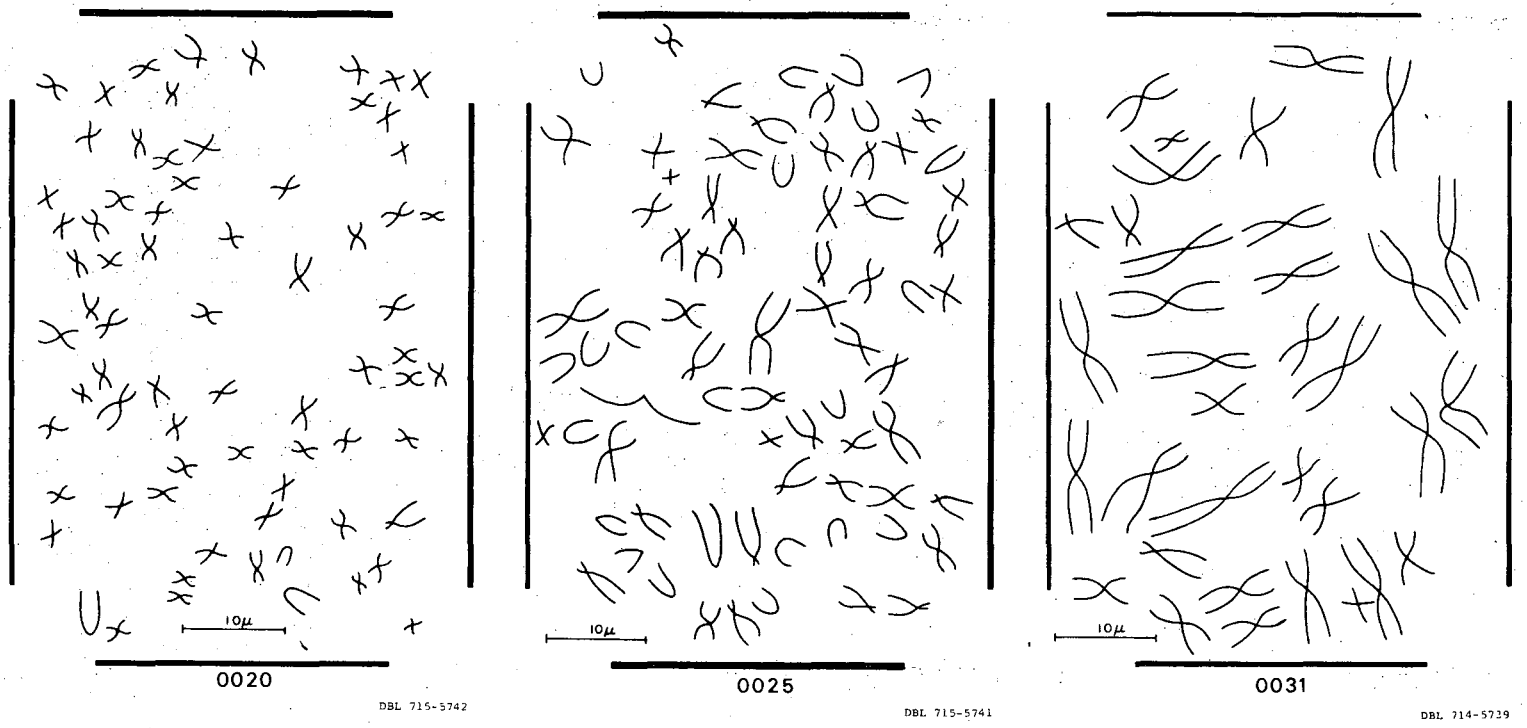

10μ
0020
DBL 715-5742
10μ
0025
DBL 715-5741
10μ
0031
DBL 714-5739

Fig. 4

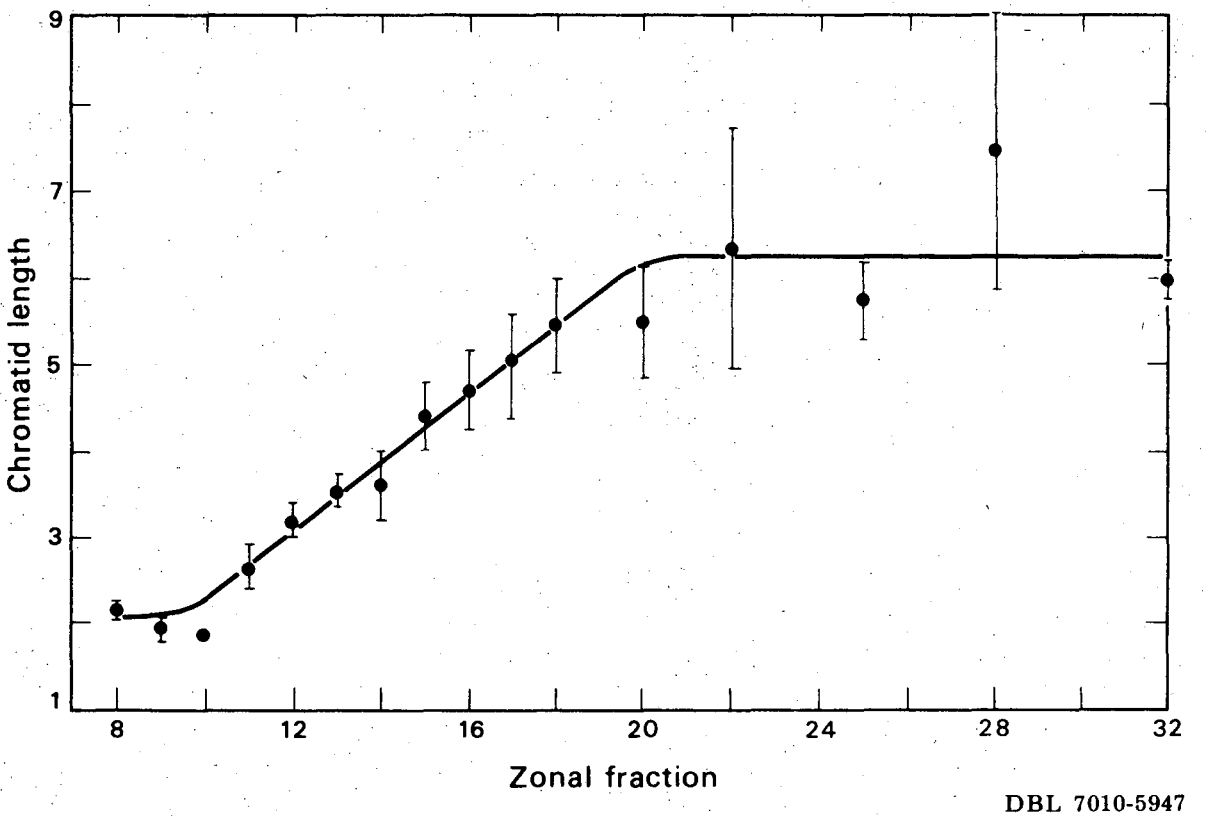
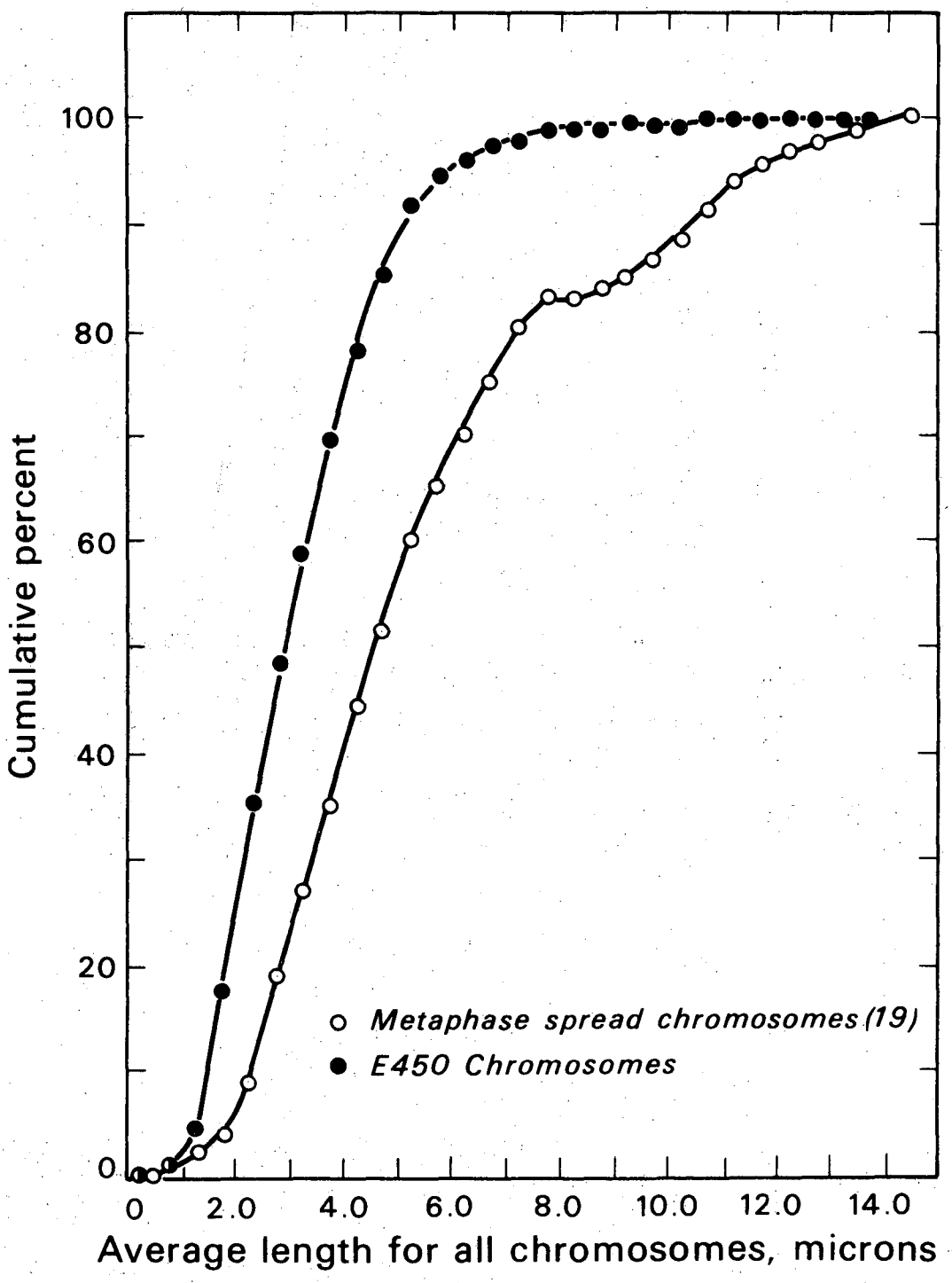
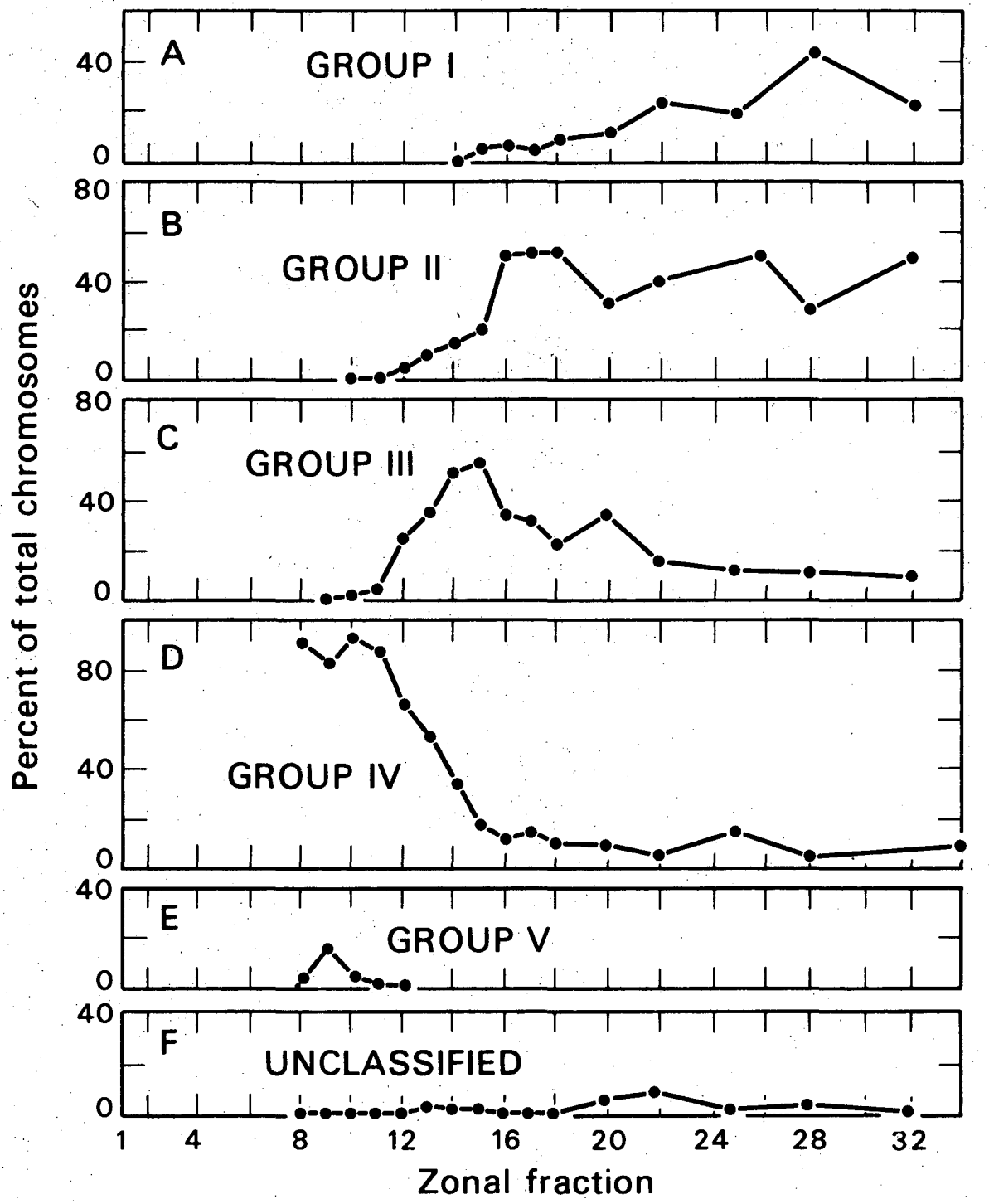

9
7
5
3
1
Chromatid length
8
12
16
20
24
28
32
Zonal fraction
DBL 7010-5947

Fig. 5



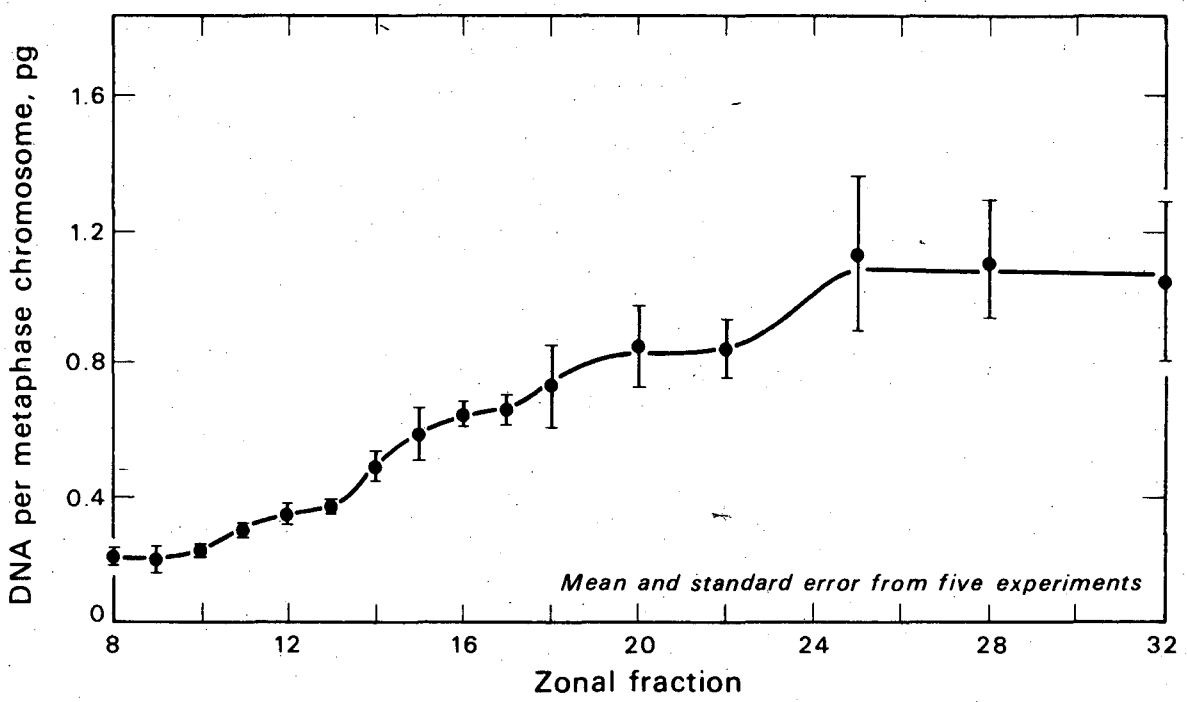
DBL 704 5660

Fig. 6



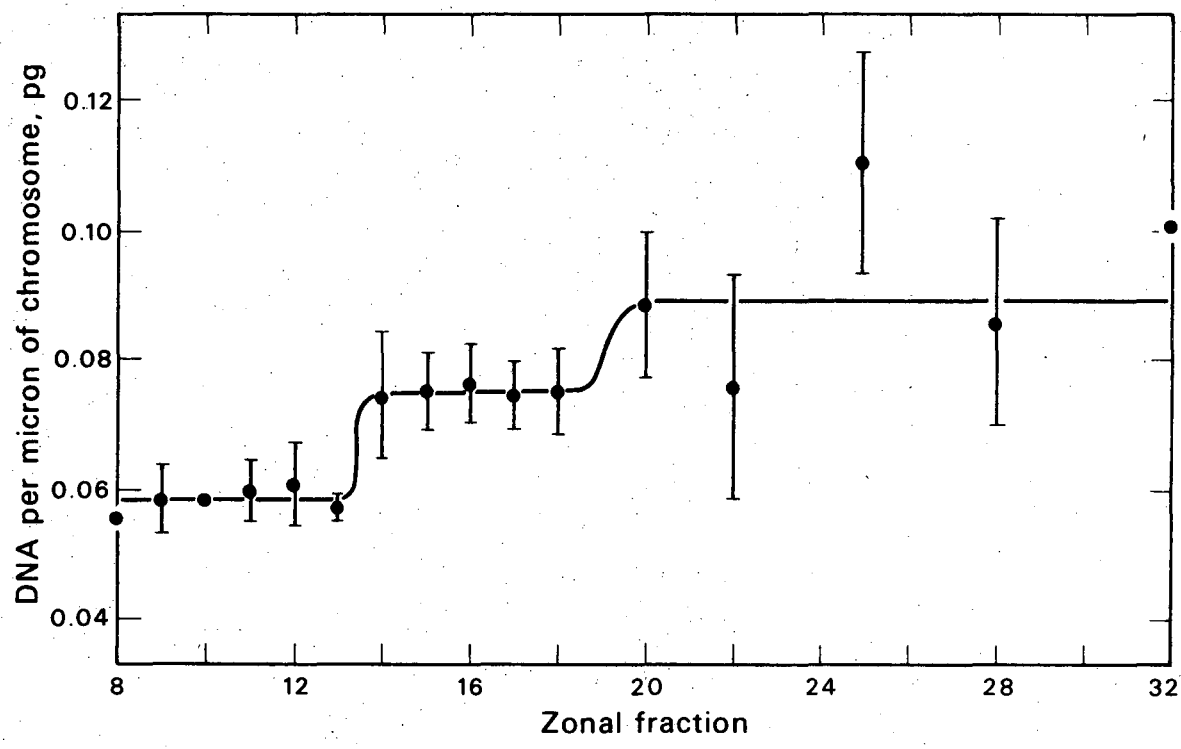
DBL 712 5671

Fig. 7



DBL 703 5630

Fig. 8



DBL 703 5632

Fig. 9

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720