ANALYSIS OF HUMAN CHROMOSOMAL VARIANTS BY
QUANTITATIVE ELECTRON MICROSCOPY
I. GROUP D CHROMOSOME WITH GIANT SATELLITES

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THE so-called "normal" variations in the human karyotype found in clinically normal individuals have been useful for linkage studies as chromosome markers (e.g., No. 1 and No. 16 chromosomes) (DONAHUE *et al.* 1968; MAGENIS, HECHT and LOVIEN 1970). The old order Amish in the United States are an inbred group that has been extensively studied genetically (McKUSICK, HOSTETLER and EGELAND 1964). During chromosome screening of Amish males for patrilinear variation in the length of the Y chromosome (BORGAONKAR *et al.* 1969), a D group chromosome was found to have a giant satellite knob (inset, Figure 1). Extensive cytogenetic studies are in progress, and the data collected so far indicate that this giant-satellite carrying marker D chromosome is present in about 10% of the Amish in Lancaster County, Pa. Autoradiographic studies indicate that the marker chromosome may be a number 14 chromosome since it is labeled more heavily in the region proximal to the centromere (McKUSICK 1969).

Karyotyping of single human chromosomes by determinations of dry mass from electron micrographs has recently become possible (BAHR and GOLOMB 1971) and allow for rather precise grouping and evaluation of individual marker chromosomes. This technique is applied to the giant-satellite carrying large acrocentric chromosome in this study.

Our study is the first in a series of detailed analyses of abnormal human chromosomes in which quantitative electron microscopy demonstrates the capacity of rendering new information in two orders of magnitude below the resolution of the light microscope.

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MATERIALS AND METHODS

Blood was obtained from a phenotypically normal patient, a male who was heterozygous for the marker chromosome. Following the usual commercial culture techniques, the cells were treated hypotonically for approximately 10 min with Hanks' solution diluted 1:1 with distilled water. They were then applied to the surface of distilled water in a modified Langmuir trough. When surface tension disrupted the cells, chromosomes were released and were picked up by touching Formvar-coated grids to the water surface and then immersed immediately into 30% ethanol. The specimens were dehydrated through ascending concentrations of ethanol in water, absolute ethanol, and finally in absolute amyl acetate. They were dried by the critical-point method of ANDERSON (1951). The unstained chromosomes were viewed and electrographed in a Siemens Elmiskop 1A operated at 100 kV under conditions required for quantitative densitometric evaluation of the negatives for determinations of dry mass according to BAHR and ZEITLER (1965). Total and partial chromosome mass was determined with the aid of the integrating photometer IPM-2 of Carl Zeiss, Oberkochen. Fiber mass and fiber diameter were measured in a scanning densitometer. Analogue density measurements were sampled at constant, short intervals and fed through an A/D converter onto computer-compatible magnetic tape for subsequent programmed data processing.

RESULTS

The total dry mass of the satellite chromosome (Figure 1) is 18.92×10^{-13} g, of which satellites and stalks contribute together 2.23×10^{-13} g. The long arms weigh 15.52×10^{-13} g, and the short arms 3.40×10^{-13} g (including satellites), giving a long/short arm ratio (L/S ratio) of 4.56. Taking the two parameters of total mass (with or without satellites and stalks) and the L/S ratio, we find that this chromosome fits into the proposed classification of BAHR and GOLOMB (1971) as a group D chromosome.

Together the two stalks weigh 0.18×10^{-13} g, i.e., the satellite-to-stalk dry mass ratio is 11.5:1. Of two other chromosomes in close proximity to the satellite chromosome, one weighs 29.40×10^{-13} g with an L/S mass ratio of 1.94, while the other weighs 31.90×10^{-13} g with an L/S mass ratio of 1.61. These chromosomes fit into the proposed classification as a B₄ and an A₃, respectively. The fact that normalized mass values for different karyotypes are normally distributed with respect to total and arm masses allows for unequivocal assignment of the satellite chromosome to Group D, if this chromosome consists of bumpy, tortuous fibers, as all other human chromosomes do (GOLOMB and BAHR 1971).

Twenty-five of such fibers were scanned with a photometer slit corresponding to 198Å of actual fiber length. The average diameter of fibers scanned was 383Å. The average fiber weight/micron was 35.57×10^{-16} g.

Scans were also made across selected portions of the long arms, short arms, the assumed centromere region, each stalk, and each satellite (Figure 2). Since the numerical data for the average fiber are known, the cross-sectional mass of a chromosomal part can be expressed as "fiber equivalents" (DUPRAW and BAHR 1969). This approach gives a rough value for the fiber mass in the long arms, but since they are unequal in diameter and length, the number of fiber equivalents in Arm A (92) is greater than in Arm B (83). The subtelomeric scans are similar to the scans of the individual long arms, with Arm A (99) again greater than Arm B (80). The subcentromeric scan revealed 125 fiber equivalents, while the "centromeric" one registered 84 fiber equivalents. Truly significant information

FIGURE 1.—As a result of either the squashing procedure or the considerable surface forces acting on chromosomes during air drying, preparation for light microscopy (inset, $\times 1,500$) favors presentation of the giant-satellited chromosome in its broadest (two-dimensional) aspects. The critical-point drying procedure renders a three-dimensional skein of chromatin (GOLOMB and BAHR, 1971). $\times 20,700$ (AFIP Neg. 70-10710-1.)

can be gained from measurements of the stalk, which show 10 chromatin fiber equivalents for A and 11 for B. Fiber equivalents in the satellites proper are very much alike (34 for A and 33 for B).

Total length of chromatin fiber per chromatid can be calculated by dividing the total mass of one chromatid by the fiber weight per micron. For our case the calculation yields 266μ of fiber per chromatid. This length is subsequently used as the denominator in calculating the so-called "DNA packing ratio", which is an expression of the length of DNA helix per chromatid as compared to the length of chromosome fiber per chromatid (DUPRAW and BAHR 1969). Using RUDKIN's (1965) data for the relative DNA content in a Group D chromosome of 1.79% (0.895% per chromatid) and using the value of 12.00×10^{-12} g DNA per metaphase plate (BAHR 1970), the amount of DNA per chromatid is calculated at 10.74×10^{-14} g.

Considering the naked DNA double helix to weigh 3.26×10^{-18} g/ μ (DUPRAW

FIGURE 2.—Schematic representation of fiber equivalents. The “centrometric scan” (84) probably measures both centromere and long-arm fibers, as the centromere is not well defined. (AFIP Neg. 70-10710-2.)

and BAHR 1969), one can calculate the total length of DNA helix per chromatid of the satellite carrying chromosome to be $32,945 \mu$. When one divides this DNA helix length by the fiber length per chromatid (266μ), one obtains a DNA packing ratio of 124:1, i.e., there are 124 lengths of DNA in one length of fiber.

DISCUSSION

Analysis of the giant satellites by quantitative electron microscopy enables one to evaluate the possible significance of these bodies as genetic carriers. When the tetraploid amount of DNA (12×10^{-12} g) contained in a metaphase plate is divided by the weight of an average metaphase plate of 83.59×10^{-12} g (BAHR and GOLOMB 1971), one arrives at a concentration of 14.4% DNA in chromosomal chromatin. This quantitative figure is in good agreement with biochemical data for the DNA content of metaphase chromosomes (HUBERMAN and ATTARDI 1966; SALZMAN, MOORE and MENDELSON 1966). Upon multiplying 14.4% by the weight of satellites and stalks together (2.23×10^{-13} g), a DNA content of 3.21×10^{-14} g for both satellites results. If we again divide this figure by the weight of DNA per micron of DNA helix (3.26×10^{-18} g), we obtain the value

of $9,844\ \mu$ as the length of DNA in the giant satellite material. Thus, in the active interphase period, there would be $4,922\ \mu$ of DNA per cell representing the giant satellite. A length of $4.96\ \mu$ of DNA is considered to be equivalent to 14,600 base pairs (SINCLAIR and STEVENS 1966). We therefore calculate the "extra" base pairs in a satellite to number 14.48×10^6 . For all base pairs, 4.96×10^6 amino acids in polypeptides of a total molecular weight of 5.79×10^8 can be specified; in other words, 28,950 polypeptides of average molecular weight 20,000.

This is a significant quantity of genetic material—approximately one-third of that involved in Down's syndrome, since an average G chromosome weighs 6.88×10^{-13} g (BAHR and GOLOMB 1971). We suggest that the giant-satellite carrying chromosome is probably not the result of a translocation, since it appears to behave in the expected fashion as far as segregation is concerned (McKUSICK 1969). Several members of the large kindred have been found to carry the chromosome with no ill effects.

Since additional autosomal material usually results in marked phenotypic abnormalities (e.g., trisomy 18, trisomy 21), the absence of clinical abnormalities in this donor suggests three possible explanations for the nature of the giant satellites.

The first is that the stalks are inheritable short arm secondary constrictions and the amount of genetic material is thus not quantitatively abnormal. This point is supported by the fact that the chromosome fits into the D group of our classification (BAHR and GOLOMB 1971), both with and without its satellites.

A pericentric inversion is the second possibility. An inversion is a euploid rearrangement and is of no phenotypic consequence in the present case.

That the genetic material contained in morphologic satellites is also biochemically satellite DNA is the third possibility. Mouse satellite DNA consists of repetitive sequences with an average length of about 300 nucleotides (BRITTEN and KOHNE 1968). This appears to mean that there could be approximately 4.83×10^4 repetitive sequences in a giant satellite. Large quantities of satellite DNA are also reported for calf DNA, in which it constitutes as much as 40% and is divided in sequences that are repeated between 10,000 and 1,000,000 times (BRITTEN and KOHNE 1968). It is likely that the recently devised technique of *in situ* hybridization by PARDUE and GALL (1969) will render an answer to this question.

SUMMARY

The electron microscope is used in a new approach to evaluate abnormalities of human chromosomes. The giant satellites on a Group D chromosome have been characterized in terms of their absolute dry mass and DNA content.

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