The C-Group Pachytene Bivalent with a Locus Characteristic for Parachromosomally Situated Particulate Bodies (Parameres): A Provisional Map in Human Males*

(autosomes/meiosis/stereoelectron microscopy)

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Communicated by Thomas F. Anderson, June 5, 1972

ABSTRACT During prophase stages of the first meiotic division in human males, an autosomal bivalent in the C group (autosomes 6–12) characteristically has associated with it, at a specific locus, small, DNA-containing bodies (parameres). A pachytene chromomere map is presented, as is evidence suggesting that the parameres are disposed in two lateral loops, each of which is coaxial with one of the homologs. Stereophotographs of stacks of plates from electron micrographs of serial ultrathin sections show the parameres in their in situ configuration to be composed of tightly compacted fibrils, 85–90 Å in diameter.

Germ cells are formed in eukaryotic organisms through two successive nuclear divisions (meiosis I and II). The prophase stage of meiosis I is complex, and can be classified into sub-stages, one of which is pachytene. At pachytene, homologous chromosomes are paired into bivalents, which exhibit a linear sequence of compacted regions of chromatin (chromomeres). Chromomeres vary in size and, typically, the sequence in which these sizes appear and the total number of chromomeres are different for each chromosome in the complement. Thus, pachytene chromomere maps have proved useful in cytogenetic studies of various species. We are now developing pachytene maps of the human chromosome complement.

We have earlier mentioned (1) the presence, in the pachytene chromosome complement of human males, of a bivalent that exhibits at a characteristic locus a cluster of small, particulate bodies (a convenient descriptive term for which might be “parameres”). Parameres are evident in our preparations at stages earlier than late pachytene; they are not seen at later stages. A configuration of this kind has not to our knowledge been described heretofore in meiotic cells from human or any other source.

In this report, we present a provisional map of this bivalent and some results from a continuing investigation of the small bodies associated with it. The chromosome has been recognized in every human male from whom we have obtained adequate cytological preparations (more than 20); therefore, this configuration is assumed to be a normal characteristic of the genome. Data from extensive light microscope studies of testicular samples from six of these individuals are given here. Tissue from one of these six individuals was also studied by electron microscopy, as was a sample from a seventh individual not included in the light microscope studies.

MATERIALS AND METHODS

Cells were prepared for cytogenetic study with light microscopy by methods described elsewhere (1).

For electron microscopy, fragments of tubules were fixed in 2% glutaraldehyde in phosphate buffer at neutral pH. They were subsequently stained with indium by the technique of Watson and Aldridge (3), and embedded in cross-linked butyl methacrylate. Serial sections were cut on an LKB Ultratome I fitted with a Dupont or Reichert diamond knife. The sections were examined and photographed in a Siemens Elmiskop IA. Stereophotographs were made from stacks of positive transparencies of the serial sections (2).

RESULTS

Under the visible-light microscope, the bivalent described here consists of 23 chromosomes of various sizes, arrayed in the manner shown in Fig. 1. The largest of these chromosomes, numbers 9 and 10, typically form a doublet, and the locus (arrow) with which the particulates are associated lies between this doublet and chromosome number 11.

On the basis of relative length alone, this element is clearly an autosome belonging to the C group (chromosome numbers 6–12) (1). From observations that chromatin at the centric regions compacts precociously during pachytene (1), we have inferred that the doublet formed by chromosomes 9 and 10 marks the region of the centromere.

In exceptionally favorable figures, the cluster of particulates

* This is paper no. V in the series, “Chromosome Structure and Function in Man.”
† The six patients studied by light microscopy are identified by our accession numbers 625H, 690H, 692H, 761H, 767H, and 786H. According to the Chicago system [Chicago Conference: “Standardization in Human Cytogenetics,” Birth Defects: Orig. Art. Ser. 2 (1966)] they are designated SD251292, GS502100, WS040909, HM200291, FW180954, and GH080198, respectively.
All underwent orchidectomy as a therapeutic measure for carcinoma of the prostate, except patient 786H, who was treated for a neura of a left inguinal hernia. Preparations from this patient were also studied by electron microscopy.

‡ Patient 790H [PC031196], who had carcinoma of the prostate, was studied by electron microscopy, but is not included in the group studied by light microscopy.

2165
can be seen to comprise two rows that lie in parallel. In Fig. 1 (top row, third and fourth bivalents from left) double loops are apparent; in Fig. 3, the bodies lie in two more or less parallel rows. From observations such as these, we draw the inference that the primary configuration comprises two lateral loops that can become coaxial with the two paired chromosomes when, during preparation, the latter are greatly stretched or broken at this locus.

As reported earlier (1), the particulates are orcein-positive and RNase-resistant. We have since observed that they are also Feulgen-positive.

With the electron microscope, the particles are readily seen in stages of pachytene in which the chromosomes are relatively uncompacted; the adjacent large chromosomes serve as a marker. As the stereophotographs (Fig. 2 a–c) show, there are no obvious connections between the particles, although these are inferred from the light micrographs. They appear to be surrounded by a region different from the nuclear ground substance (Fig. 2b).

Application of the electron microscope in our human pachytene studies has not yet contributed to mapping. However, some new information has been derived concerning the

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**Fig. 1.** Representative examples of the pachytene bivalent with which the parameres are associated. Fifth from left in the top row is a diagrammatic chromomere map. Solid arrows indicate parameres; open arrowheads indicate points at which other bivalents overlap. Table at bottom of figure identifies patient source of each bivalent. Note that parameres form double loops in bivalents second and third from left in top row (see text for discussion). At the right is a single-stranded pachytene map. The 23 chromomeres recognized in this study are indicated. Dotted line indicates locus at which parameres are seen. Centromere may lie in the 9–10 doublet. (see text). Bar = 10 μm.
FIG. 2. (a) Stereophotograph of stack of seven electronmicrograph plates showing parameres. The large mass directly to the left of the arrangement is part of the XY bivalent. It is attached to the nuclear membrane, which can be seen at the lower left of the micrographs. Three parameres in the bottom plates are partly obscured by the others in the figure. One paramere at the left of the arrangement is close to the XY bivalent. Pachytene spermatocyte from accession number 786H. Indium stain ×17,000.

(b) Stereophotograph of stack of four plates showing sections 3–6 of the above figure at higher magnification. The individual parameres are composed of fibrils. The parameres at 5 and 11 o'clock appear to have unstained cores. The synaptonemal complex—unstained with indium—of the autosome with which the parameres are associated is indicated by arrows.

(c) Stereophotograph of stack of 11 plates of a pachytene figure from a different individual (790H). Only six bodies are clear. The regions of compacted chromosomal material at the top and bottom of the photograph are interpreted to be chromomeres 10 and 11, respectively (see map). ×20,000.
DISCUSSION

Maps of somatic metaphase chromosomes are now available from studies in which fluorescence labeling or Giemsa staining techniques are used (see for example ref. 4). It is appropriate to attempt to compare these less-detailed maps with pachytene chromomere maps. Thus, coarse features of the map shown in Fig. 1 appear to be present in the metaphase map identified as that of chromosome number twelve.

We feel that speculation concerning the function of this newly-described configuration should await acquisition of further data.

We are indebted to the following physicians for their cooperation in providing tissue specimens for this study: Drs. Joseph J. Blanche, Emmel F. Ciccone, David S. Cristol, Richard H. Dricoll, G. John Gislason, and Edward Whalen. Lorraine Massimillo provided essential technical assistance. David Gitlin participated in construction of the map. This investigation was supported in part by USPHS Research Grants CA-05903, GM-17551, and CA-06927 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania.