Centromere separation: existence of sequences

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Summary. The centromeres of chromosomes from human lymphocytes as well as root tips of *Vicia faba* and *Crepis capillaris* release the sister-chromatids in nonrandom, genetically controlled sequences. In man chromosomes 18 and 17 are amongst the earliest and 13, 14, 15 the last. The sequences may be a universal phenomenon among eukaryotes.

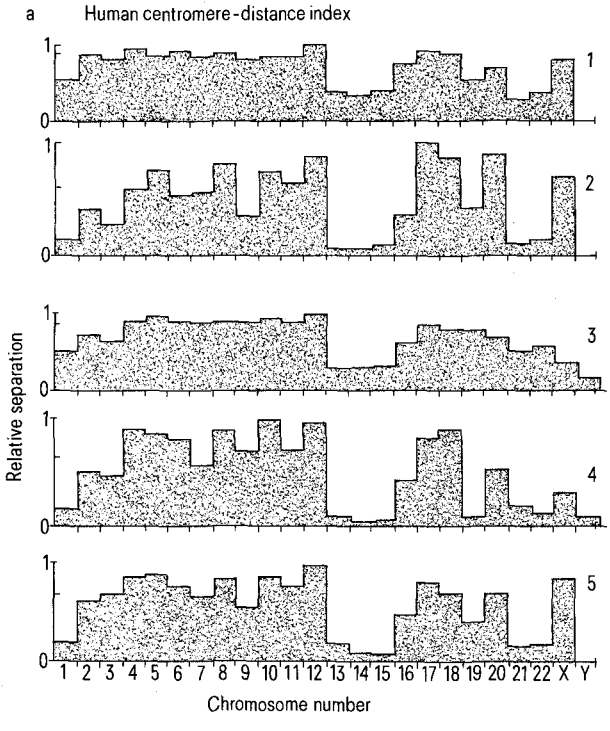
The centromeres in a genome are considered to separate simultaneously or at random just before separation of chromatids in mitosis or meiosis II. Fitzgerald[2,3] has alluded to 'premature' separation of the human X chromosome, and occasional asynchrony has been also observed in some other species[4]. We analyzed the entire genome in man[4] and Chinese hamster[5] and found nonrandom sequences in which the centromeres separate. Also, Méhes has found early separation of chromosome 18 in man[6].

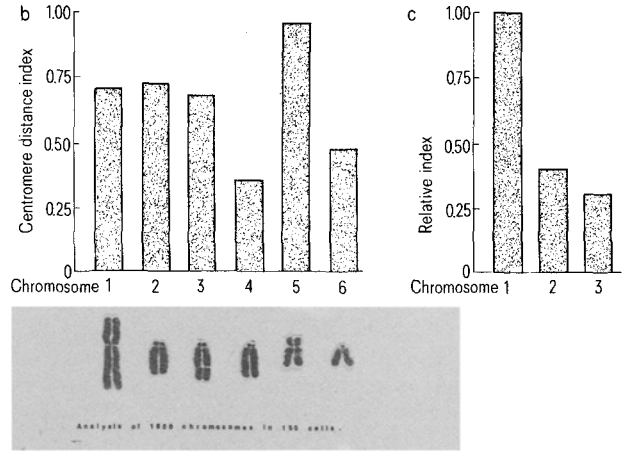
We have now studied the genomes of man, *Vicia faba* and *Crepis capillaris* to establish detailed sequence in which the centromeres separate. There are definite, apparently genetically controlled, nonrandom sequences which, though somewhat quantitative in nature, are neither influenced by the length of the chromosome, nor the position of the centromere in a given chromosome.

To obtain cells at late metaphase-anaphase, one has to rely on chance for obtaining cells at appropriate stages for analysis. The sample in the present study included cells with at least one centromere having had separated to as many as all but one having had separated. The centromeres which had not separated were assigned a score of 0, those which had just begun separation with a clear tendency of loosening of the structure, were assigned a numerical score of 1, and those which showed clear separation were rated as 2. The scores obtained from a random sample were pooled for individual pairs of chromosomes. These totals divided by the largest number in the series provided the relative distances (centromere distance index or CDI) between daughter centromeres in a given genome. The lower the CDI for a chromosome, the later it is in the sequence of separation.

The human lymphocytes. The lymphocyte microcultures from 3 females and 2 males were treated with colcemid (0.05 µg/ml) for 2–6 h. The slides were first stained with giemsa, selected cells photographed, then restrained with quinacrine dihydrochloride and rephotographed with fluorescent microscope. (Trypsin treatment obliterated the distances between sister centromeres due to swelling.) Even then, identification of the last separating chromosomes was not always unequivocal because of their very compact nature.

The data on centromere distance index obtained from 100 cells per individual are depicted in figure a. A few generalizations emerge: a) several chromosomes initiate separation at the centromere almost simultaneously or in rapid succession; b) a distinct group of chromosomes D and G, are the last ones to separate; c) the size of chromosomes or position of centromeres may have no bearing on earliness or lateness of separation (notice that acrocentrics and No. 1 are late separating); d) the average of indexes is only a rough





Graphic representation of the relative frequency of the degree of centromere separation - Centromere Distance Index (CDI) - as discussed in the text. The abscisse show chromosome number, and the ordinates refere to CDI. The CDI of the chromosome pair showing maximal separation is scored at 1 and all others are calculated relative to this unit value.

a The 5 human genomes represent studies with 3 female subjects (1, 2 and 5) and 2 males (3 and 4). Notice the uniformly low CDI for chromosomes No. 13, 14, and 15 in all subjects. In males, Y also has a low CDI. The X in males shows low value because of a single copy in comparison to the female complement. The variations observed in CDI's of genomes from different subjects may represent sampling variations in these limited studies or point to the existence of a polymorphism for this trait. Reproduced from Human Genetics, with permission.

b The CDI of *V. faba* karyotype ACB. Chromosome pair No. 5 shows the earliest separation whereas No. 4 is the last one to separate. Chromosome No. 6 is the next to last to separate. The data indicate that pairs No. 1 and 2 separate almost simultaneously, or in rapid succession. Pair No. 3 is only slightly behind No. 1 in its time of separation.

c This graph shows that of the 140 cells of *C. capillaris* analyzed for CDI, pair No. 1 starts separation ahead of No. 2 which is slightly ahead of No. 3 in initiation of separation of its centromeres.

quantitative measure (e.g. to find out the earliest separating chromosomes one needs cells with only 2 to 3 separated chromosomes); e) there might exist polymorphism for the sequence in our species and f) constitutive heterochromatin or some satellite DNA might control sequential separation. Fraction IV of SAT-DNA[7] is common to all late separating acrocentric chromosomes. Also, with the exception of Y, the NOR regions are located on these chromosomes.

Vicia faba (broad bean) root tips. In ACB karyotype of *Vicia faba*[8] all pairs of chromosomes can be identified without banding. The secondary roots treated with 0.05% colchicine for 4 h were kept in running tap water for 30 min to 1 h to release the metaphase block. A sample of 1800 chromosomes (150 cells) stained with acetocarmine showed chromosome No. 5 to be the most frequently separated. No. 5 constituted 98% of the population (100 cells) with only 1 or 2 chromosomes showing separation, and the 2 homologues showed 80% synchrony of separation. Figure b gives the sequence of separation. It also shows that: a) chromosome No. 4 is the last one to separate, b) No. 6 is the next to last and c) chromosome Nos 1, 2, and 3 all separate almost simultaneously.

The mitotic cells of crepis capillaris. Crepis capillaris (compositae) has 3 pairs of identifiable chromosomes. Preparation was made as for *Vicia faba.* In a sample of 140 cells analyzed, the most frequently separated chromosome was No. 1. No. 2, was second in separation and in close proximity to No. 3. Relative centromere distances index for the genome is shown in figure c.

Possible existence of synchrony and polymorphism. If one takes a sample of 2 pairs of chromosomes some distance apart on the spectrum of centromere separation, one observes that homologues almost always separate closer to each other in time than non homologues. This has been observed for 100 pairs of chromosome No. 2 vs any pair belonging to group D in man. Thus, homologues may have a common genetic element controlling the time of separation; this suggests the existence of synchrony. However, in one individual showing heteromorphism for the amount of constitutive heterochromatin in pair No. 1, the one with large C-chromatin block expressed a somewhat delayed separation in 44 chromosomes compared to only 6 in the one with small C-chromatin block. These data point to the existence of polymorphism; but are not sufficient to elucidate the role of centromeric-heterochromatin in centromere separation. Similarly, earlier separation of chromosome 2 in Méhes' studies[6] (compared to ours) may indicate possible polymorphism for this, and possibly other, chromosomes.

The degree of apparent synchronous separation (same value for the homologues) in *C. capillaris* was lower (40%) for the earliest separating chromosome (No. 1) than for the earliest separating chromosome (No. 5) in *V. faba* (81%). Such differences might be useful in study of control of separation. Since the data were compiled for several root tips without regard to their genetic homogeneity, possiblity exists that in crossfertilized *C. capillaris* the genetic heterogeneity for the element(s) controlling centromere separation is far greater than that for selfertilized *V. faba.*

Possible significance of sequential centromere separation. The idea that sister chromatids in a metaphase-anaphase cell separate at the centromere in a non-random, predetermined fashion has limited literature available. We do not know if the phenomenon is genetically controlled, if centromeric heterochromatin or any fraction of it is involved in the control, if sequences exhibit polymorphism and if it has any bearing on mitotic and meiotic II non-disjunction. It is of interest that in trisomic 18 individuals and their mothers chromosome 18 is not the earliest to separate as seen in normal population[6].

1 I wish to thank Drs Rieger and J. Van't Hoff for supply of seed.
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Soluble hepatic lectin in regenerating liver

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Summary. Similar activity of a soluble hepatic lectin was measured in regenerating rat liver 12 h following partial hepatectomy or gentle manipulation. Lectin response seems to be related to trauma rather than cell proliferation.

Of the hepatic lectins described, several are membrane bound, but one seems to be primarily intracellular. The membrane bound hepatic binding glycoprotein of Ashwell and Morell requires calcium for binding and carries a specificity directed towards B-galactose[1]. The lectin, composed of 2 subunits with mol. wts of 40,000 and 48,000 daltons, may function in the clearance at desialyated glycoproteins[2]. A 2nd carbohydrate binding lectin, isolated from bovine liver plasma membrane, has not yet been fully characterized[3] although like the previous glycoprotein, it is solubilized with detergents. Several other hepatic lectins have been described mainly by their saccharide binding properties[4,5]. We chose to study an intracellular hepatic lectin, with binding specificity to lactose or thiodigalactoside. It has been isolated previously from neonatal rat brain[6], and embryonic muscle[7]. This lectin is soluble and does not require calcium ions for agglutination. A role in embryonic development has been postulated for the lectin in brain and muscle muscle. However, studies with embryonic liver shows this lectin is not involved in hepatic differentiation[8,9].

Methods. 43 male Sprague-Dawley rats (200 mg) were subjected to 70% hepatectomy[10]. 23 rats underwent a sham operation with gentle manipulation of the liver. After sacrifice at timed intervals, lectin extract was prepared as described by Nowak et al.[7]. Livers were homogenized in phosphate buffered saline with 4 mM-mercaptoethanol, 2 mM EDTA and 0.3 M lactose, pH 7.2. Following centrifugation $\times 35{,}000$ for 3 h 15 min, supernates were dialyzed exhaustively against a lactose free extraction buffer. Lectin activity was determined by the hemagglutination method of Nowak et al.[7] using trypsin-treated, gluteraldehyde fixed