ON THE ANAPHASE MOVEMENT OF CHROMOSOMES

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The kinetic properties of chromosomes are controlled by the centromere or kinetochore. In somatic mitoses it is the centromeres which become oriented on the equatorial plate while the two arms of each chromosome may extrude from the spindle figure into the cytoplasm. At metaphase a thread-like structure, descriptively known as the spindle fiber, can be seen in properly fixed and stained material extending from the centromere to the pole. The centric region plays a decisive rôle in congression, orientation on the spindle and anaphase separation. Acentric fragments behave in an irregular fashion. The centric region becomes "attached" to the spindle and leads the way to the pole at anaphase with the arms of the chromosome apparently being moved as passive bodies. A subterminal centromere imparts a J-shaped appearance to an anaphase chromosome while a chromosome with a median centromere assumes a V-shape. In either case the centromere (i.e., centric region) is at the apex of the configuration.

At the first meiotic metaphase the bivalent chromosomes undergo congression and orientation. When the bivalent (tetrad) moves upon the spindle the two homologous centromeres become coöriented and lie symmetrically on either side of the equatorial plane and directed toward opposite poles. The two homologous centric regions lead the way to opposite poles in anaphase disjunction. Inasmuch as two chromatids are attached to each centromere, the disjoining dyads have the appearance of double V's or J's, depending upon the position of the centromere in the chromosome. The double V's or J's characterizing the first anaphase are later transformed into X-shaped configurations by the repulsion between the four constituent arms. The undivided centromere lies at the center of the X and holds the two chromatids together until the second anaphase. The dyads at MII have their undivided centromeres lying on the equatorial plate as in a somatic mitosis. The effective split of the centromere occurs and the two chromatids comprising each dyad pass to opposite poles, with the centric region advancing foremost. In maize there is no relational coiling at the second division to delay disjunction. This fact coupled with the marked contraction of the chromosome arms at MII leads to the two chromatids moving somewhat parallelly to the poles, and the pronounced V's and J's characteristic of other mitoses are not always seen, although it is clear that the centric region is in the front. The above outline is true for maize chromosomes, and with minor exceptions holds for those plants and animals possessing chromosomes with localized centromeres. Recently Hughes-Schrader and Ris[1] have shown that the hemipterous insects have a diffuse type of spindle attachment region and consequently in these insects the movement of the chromosomes follows another pattern. There is then for chromosomes no universality in type of spindle behavior. This is emphasized by the following preliminary report of an anomalous situation in maize.

The unorthodox behavior is limited to the two meiotic divisions. The somatic mitoses are normal. Studies of meiosis have been limited to microsporogenesis. The first meiotic division is normal up to metaphase, when the bivalents congress upon the spindle figure. The pachytene chromosomes are of normal appearance with clearly defined centromeres. The bivalents become oriented on the spindle in a regular manner. The first indication of an unusual behavior occurs when structures similar to the primary centric region arise from distal portions of the chromosomes before the beginning of anaphase. These newly arisen structures will be tentatively called secondary centric regions inasmuch as they, like the primary centric region, become attached to the spindle and affect anaphase movements of the chromosomes. These secondary centric regions move poleward more rapidly than does the primary centric region, so that the distal ends of the chromosome, instead of facing the center of the spindle as is commonly true at anaphase, are pulled ahead and, overtaking the true centric region, come to lead the way to the poles. These secondary centric regions may be formed by one or more of the four arms comprising each dyad. The activity of these regions superimposed on the anaphase movement due to the primary centric region produces great complexity in the types of configurations observed. At the end of AI ten dyads usually are found at each pole, there being a surprisingly high regularity in disjunction.

Cytological conditions in the second division are much more favorable for observation and diagrammatic configurations are often found. Second prophase stages do not noticeably differ from normal but the onset of metaphase occurs before the usual contraction of the chromosomes has taken place. However, the somewhat extended dyads usually become oriented on the metaphase plate with the undivided centromere lying on the equatorial plate. Before the centromere splits and a normal anaphase separation is initiated, secondary centric regions again arise from or near the distal ends of the chromosomes. These new centric regions become attached to the spindle and move rapidly poleward, with the result that the chromosome arms become greatly attenuated if their proximal portions were anchored on the metaphase plate by the still undivided centromere (see Fig. 1). The centromere eventually divides and the monads pass to the poles.

When both ends of the same chromosome form secondary centric regions the chromosome literally backs into the pole with the apex (representing the centromere) of the V- or J-shaped chromosome pointing toward the equatorial plate while the two distal ends lead the way. Often only the distal end of one of the two arms forms a centric region, in which case the chromosome becomes an extended rod-shaped element. Occasionally both arms of the same chromosome may form secondary centric regions which are directed toward opposite poles, thus forming a chromosome bridge. Usually one of the two opposing forces prevails and the chromosome passes to one of the poles but infrequently the chromosome becomes suspended on the spindle with a consequent breaking of the chromosome at late anaphase. It is somewhat surprising that in both the first and second meiotic divisions regular disjunction usually occurs and that plants exhibiting this decidedly aberrant behavior are very fertile.

The formation of these secondary centric regions is limited to those plants having an abnormal type of chromosome 10[2, 3] with extra chromatin near the distal end of the long arm. In plants homozygous for this abnormal tenth chromosome the frequency is high for the formation of these secondary centric regions, while it is much less in plants heterozygous for this chromosome. Sister plants homozygous for a normal chromosome 10 had a completely orthodox behavior.

It is obvious that the unparalleled behavior reported here is of great interest to current theories on cell mechanics, especially that of the kinetic movement of chromosomes. A detailed account will be published elsewhere.

FIGURE 1

Second metaphase showing precocious poleward movement of secondary centric regions. The functionally undivided centromeres of some of the dyads are oriented on the metaphase plate. In some cells the secondary centric regions reach the periphery of the cell before division of the centromere occurs resulting in an extreme attenuation of the chromosome arms.

Summary.—In maize the primary centric region representing the localized centromere is responsible for the kinetic movement of the chromosome in anaphase. The concentration of the kinetic forces produces J- or V-shaped configurations in anaphase. Plants carrying an abnormal type of chromosome 10 exhibited a unique behavior in that centric regions were formed by portions of the chromosome other than the centromere.

[1] Hughes-Schrader, S., and Ris, H., *Jour. Exp. Zoöl.*, **87**, 429–456 (1941).

[2] Rhoades, M. M., *Genetics*, **27**, 395–407 (1942).

[3] Longley, A. E., *Jour. Agric. Res.*, **56**, 177–195 (1938).

HEAT-INDUCED TRIPLOIDY IN THE NEWT, TRITURUS VIRIDESCENS

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Heat and cold have both been widely used to induce polyploidy in plants.[1, 2, 3, 4] Depending on whether the treatment is applied during meiosis or during the early divisions of the zygote, triploid or tetraploid plants are produced. In general, the abnormal temperature seems to disturb the formation of the spindle and the normal separation of the daughter chromosomes at anaphase, probably through changes in the viscosity of the cytoplasm. Exposure during early prophase of meiosis may also affect the behavior of the chromosomes directly and prevent pachytene pairing,[3, 4] or completely suppress both meiotic divisions so that a diplotene nucleus changes directly into a pollen-grain nucleus.[5]

Similar effects of temperature have been described in some invertebrate animals. Refrigeration of normally parthenogenetic eggs of the brine shrimp, *Artemia salina*, may double the chromosome number from diploid to tetraploid through inhibition of the single, equational maturation division.[6, 7] Heat treatment of unfertilized eggs of the silkmoth, *Bombyx mori*, induces parthenogenesis and also causes retention of the diploid chromosome number, sometimes with subsequent fusion of diploid cleavage nuclei to form tetraploid or partially tetraploid animals.[8, 9, 10]

Among vertebrates, spontaneous and experimentally induced polyploidy have been studied extensively in several species of salamanders, because of the ease with which the chromosome number of living young larvae may be determined in whole-mounts of the amputated tailtip.[11] Spontaneous deviations from the normal, diploid chromosome number occur rather frequently.[12] Among 1878 larvae of the newt, *Triturus viridescens*, which were examined from November, 1937, to August, 1942, 38 were found to possess various deviating chromosome numbers; the majority of these, 25 (1.33% of the total), were triploid.

Experimental triploidy was first induced in salamanders by refrigeration