PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 31 October 15, 1945 Number 10

STRUCTURE OF THE SALIVARY GLAND CHROMOSOMES OF DIPTERA

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Communicated September 4, 1945

The giant chromosomes of the Diptera constitute ideal material for studies on chromosome structure and chemistry and for cytogenetic analysis because of their tremendous size and obvious pattern of longitudinal differentiation. So far it has been impossible to take full advantage of them, however, because they have not been fully understood in terms of the structure of mitotic chromosomes.

In the literature we find three types of interpretation of the banded appearance of the giant chromosomes: (1) The chromosome is composed of several helically coiled threads. The gyres of this coil appear as bands.[1-3] (2) The chromosome is formed of a bundle of completely relaxed chromonemata which originated endomitotically; because of somatic synapsis homologous chromomeres join to form bands.[4-7] (3) The chromosome consists of a large number of chromonemata which are submicroscopic and therefore invisible. The visible structures do not correspond to chromomeres or chromonemata but originate in a different manner.[8]

None of these hypotheses is wholly satisfactory. The first one has been discredited because it is impossible to interpret the bands of the mature giant chromosomes as gyres of a simple large coil. On the other hand, many investigators have seen coils in the giant chromosomes, and bands and coils have even been found to occur simultaneously in the same nucleus.[1, 2, 9-11] A satisfactory interpretation of the giant chromosomes must take this evidence into account.

The second hypothesis has been adequately criticized by Metz. It cannot account for the vesiculated appearance which the giant chromosomes often show, nor for the length of the chromonemata unless some growth of

the chromosome is assumed in addition to uncoiling. This theory, nevertheless, is accepted today by most cytologists because it allows a uniform interpretation of chromosome structure based on the chromomere hypothesis. Recent work on plant and animal chromosomes, however, has shown that the chromonema is uniform and not a series of chromatic granules on an achromatic thread.[12-16] The chromomeres are misinterpretations of coiled structures or points of overlap of chromonemata. Therefore, since mitotic chromosomes are not composed of true chromomeres, it seems doubtful that bands can be interpreted as aggregates of chromomeres.

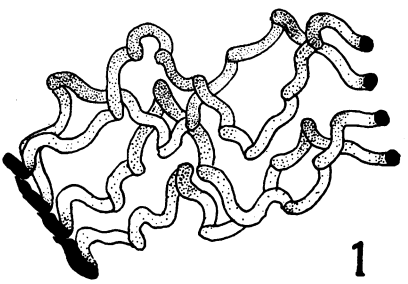
The third hypothesis, assuming submicroscopic chromonemata, places the giant chromosomes in a class by themselves since the component chromonemata are visible in all other types of chromosomes.

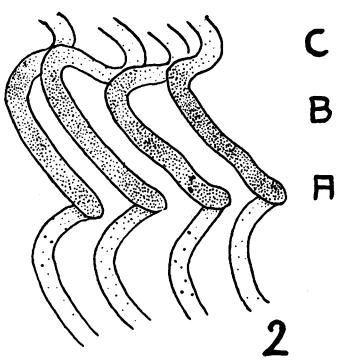
The purpose of this paper is to suggest an interpretation of the structure of giant chromosomes which is in harmony with the latest knowledge of chromosome structure in general (meiotic chromosomes of Tradescantia and the grasshopper, lamp-brush chromosomes of the frog oöcyte[15, 16]) and which can account for the apparently conflicting observations of different investigators. Proof of our hypothesis rests on a detailed study of the development of the giant chromosomes. This work is in progress.

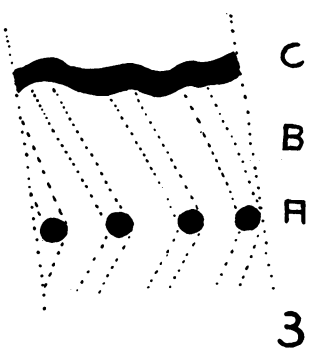
According to our hypothesis the salivary chromosome (Sciara) consists of a definite number of chromonemata which are coiled in a complicated fashion. The coiled threads can be seen most clearly in regions where the banded organization has been disrupted (so-called puffed regions). These regions occur at definite loci along the chromosomes of the larva and become increasingly prominent in the pupa as histolysis sets in. Figure 1 illustrates our interpretation of such a puffed region. Along each chromonema the gyres of a narrowly pitched helix ("minor coil") can be seen. The chromonemata do not run parallel to the length of the chromosome but proceed in an irregular manner, weaving back and forth across the width of the chromosome. Chemical agents which are known to uncoil the mitotic chromosome (KCN, $NaHCO_3$, hot water) can transform normally banded material into a similar mass of threads. These observations suggest that the banded chromosome is composed of coiled chromonemata. It will now be demonstrated how the coiled chromonemata can form bands.

Chromosomes from untreated medium aged Sciara larvae are best suited for this demonstration. At this stage of development four chromonemata can be followed in each homologue. Figure 2 represents our interpretation of two successive bands in terms of coiled chromonemata. It can be seen that the chromonemata are thrown into wide gyres ("major coil") as they proceed along the length of the chromosome. In region *A* they are running vertically, and therefore in optical cross section give the appearance of a granular band (Fig. 3). From here each chromonema can be traced as it

runs horizontally across an interband region B. Because of the major coil, the chromonemata of the interband region always run diagonally as most investigators have observed. Only in greatly stretched areas do they run parallel to the long axis of the chromosome. The much lighter appearance of the interband regions we explain as follows: (1) the chromo-







FIGURES 1-3

Diagrammatic representation of the structure of salivary gland chromosomes in a medium-aged larva (Sciara). Only one homologue is drawn.

Fig. 1. Puffed region with bands disrupted.

Fig. 2. The course of the chromonemata through two consecutive bands. (The minor coil is omitted.)

Fig. 3. Appearance of the same region at a medium focal level.

nemata run vertically in the band regions and horizontally in the interbands; consequently, much more light is absorbed in the bands; (2) one usually focuses on a longitudinal optical section of the chromosome, for here the bands are clearest. In such a section, however, more chromo-

nemata are in focus in the band than interband regions; (3) the salivary chromosomes are usually examined in smear preparations. Smearing stretches the chromonemata disproportionately in the interband regions, for the bands, where the chromonemata run transverse to the chromosome axis, resist stretching. In sectioned material stained by the Feulgen reaction we find that chromonemata are much more conspicuous in the interbands. Region *C* in figure 2 illustrates how a solid (i.e., non-granular) band could arise. Here we follow the chromonemata into the next gyre of the major coil. As they dip down, running from left to right across the chromosome, the impression of a continuous line rather than separate granules is produced (Fig. 3). The wavy border of such bands is caused by the minor coil of the chromonemata. Because the individual threads of region *C* proceed at different levels, filling out most of the cross section of the chromosome, this region looks like a solid disc (i.e., threads at all levels). Sometimes in chromosomes of medium aged larvae several gyres of a two-stranded helix can be seen within one homologue.[1-3, 9-11] This appearance can best be understood if we assume that the four chromonemata are closely appressed in pairs, forming the wide gyres of the major coil. When these threads have separated laterally, a banded structure is produced as has been shown above. Such an interpretation makes it possible to understand how different regions on the same chromosome may appear as gyres of a helix or as typical bands.

Several authors have observed that the bands often look like rings in cross section through the chromosome instead of solid discs. Our hypothesis could account for the apparently contradictory observations. When the chromonemata are closely appressed and proceed in a common helix, the gyres of the major coil appear in optical cross section as rings. When the chromonemata have come apart, however, the gyres of the major coil fill the entire cross section of the chromosome, and the bands now look like solid discs.

In old larvae the banded chromosomes have the same structural characteristics, the only difference apparently being the greater number of threads brought about by endomitosis.

If the chromonemata in these giant chromosomes have both minor and major coils, as appears to be the case, they must have grown enormously in length. Such a growth of chromonemata is, however, not restricted to the banded chromosomes. A comparable increase in length occurs, for instance, in the "lamp-brush" chromosomes of certain oöcytes where recent studies[16] have shown that the characteristic loops are actually the gyres of the major coil of the chromonema and contain in addition the minor coil. In contrast to this great increase in length, the chromonema seems to remain approximately constant in diameter. Growth is thus restricted to the long axis of the chromosome. Some authors have suggested that the

longitudinal growth occurs mainly in "nongenic" material of the chromonema. Since giant chromosomes are always found in cells which are especially active physiologically, and since no visible differentiation into alternate "genic" and "inert" regions is visible, it seems to us more likely that the genes themselves increase in mass. Heterochromatic regions which have been shown to contain few genes (Drosophila) do not exhibit in the salivary chromosomes longitudinal growth comparable to that of euchromatic regions. This is in agreement with the view that the genes themselves grow rather than the inert, non-genic material.

The extensive cytogenetic work on Drosophila has shown that the bands in the giant chromosomes can be correlated with definite gene loci. If the bands are due to specific coiling, an interesting relationship between the coiling and the specific molecular structure of the chromonema becomes apparent. This complex coiling pattern of the giant chromosomes could be an expression of the longitudinal differentiation in the gene string. The detailed correspondence of coiling and gene specificity poses an interesting problem for investigation.

Cooper[17] has pointed out that the increase in number of strands in a helix causes it to uncoil. This, however, is true only as long as the component threads remain closely appressed in a common helix. It no longer holds if the chromonemata can separate laterally, as appears to be the case in the giant chromosomes.

It is generally believed that somatic as well as meiotic synapsis is the consequence of a complete uncoiling of the chromosomes. If this were true, it would constitute a serious objection to our assumption that the somatically synapsed giant chromosomes consist of coiled chromonemata. However, the demonstration of a typical helix in synapsing meiotic chromosomes of Tradescantia and the grasshopper[15, 16] invalidates this objection. The chromosomes are coiled when they synapse in meiosis, and they are coiled when they synapse somatically in young dipteran larvae.

It is generally assumed that the chromonemata within one chromosome are held together by the same forces that result in synapsis of homologues. This assumption is unjustified. Rather, it appears that the chromonemata within a chromosome are held together in the same fashion as the coiled chromatids of ordinary prophase chromosomes. It has been suggested[18] that achromatic material binds the chromatids together. Experiments with hypo- and hypertonic solutions on salivary chromosomes[19] seem to confirm this hypothesis. In hypotonic solutions the chromonemata separate, and bands disappear. This can be reversed in isotonic medium. With hypertonic Ringer the chromosome shrinks in diameter, and the bands appear more distinct. All these facts can best be explained by a reversible swelling and shrinkage of an achromatic substance between the coiled chromonemata.

The most puzzling appearance of the salivary chromosome is the so-called vesiculated condition. This can occur at localized regions in different developmental stages of the Sciara larva. In certain physiological states all the giant chromosomes of larvae in the same culture may be vesiculated. Under other conditions the banding pattern completely disappears and the chromosomes look like masses of faintly staining coiled threads (ghost chromosomes). Appearances similar to these can be induced experimentally in normally banded material. Immersion for a few seconds in 1 *M* NaCl causes the chromosomes to become vesiculated. Prolonged treatment with the salt solution produces typical ghost chromosomes. We interpret these phenomena in the following manner. Around each chromonema there is a Feulgen-positive substance, which, under certain conditions, can come off the threads and form chromatic connections between chromonemata and between chromosomes (chromatic coating[18]). Such connections between the coiled chromonemata of the giant chromosomes give the appearance of vesiculation. As was shown by Mirsky and Pollister,[20] nucleoproteins can be dissolved from chromosomes with 1 *M* NaCl. The vesicles observed in the giant chromosomes after short treatment with the salt solution we believe to be caused by the initial dissolution of this substance. Prolonged treatment completely dissolves these nucleoproteins; ghost chromosomes and a basophilic cytoplasm result. This basophily of the cytoplasm is likewise characteristic of untreated ghost cultures.

Summary.—On the interpretation presented here the giant chromosomes of dipteran larvae consist, like mitotic chromosomes, of a number of helically coiled chromonemata. The chromomeres in the giant chromosomes are misinterpretations of coiled structures, as has been demonstrated for meiotic and mitotic chromosomes. It is shown here how the appearance of bands and interbands may be caused by complex coiling of a bundle of chromonemata. The chromonema itself is uniformly Feulgen-positive. The giant size of these chromosomes would then be due to: (1) great increase in length of the chromonema (longitudinal growth of individual genes); (2) increase in the number of chromonemata by endomitosis; (3) lateral separation of the coiled chromonemata.

* Aided by a grant from the Rockefeller Foundation to Dr. C. W. Metz.

[1] v. Herwerden, M. A., *Anat. Anz.*, **36**, 193–207 (1910).
[2] Kaufmann, B. P., *Am. Nat.*, **65**, 555–558 (1931).
[3] Sinotô, Y., and Yuasa, A., *Jap. Jour. of Gen.*, **10**, 245–248 (1935).
[4] Koltzoff, N., *Science*, **80**, 312–313 (1934).
[5] Bridges, C. B., *Jour. of Hered.*, **26**, 60–64 (1935).
[6] Painter, T., *Am. Nat.*, **73**, 315–330 (1939).
[7] Bauer, H., *Zeit. Zellf.*, **23**, 280–313 (1935).
[8] Metz, C. W., *Cold Spring Harbor Symposia on Quantitative Biology*, **9**, 23–36 (1941).
[9] Alverdes, F., *Arch. Zellf.*, **9**, 168–204 (1912).

[10] Tänzer, E., *Zeit. wiss. Zool.*, **119**, 114–153 (1921).
[11] Dawydov, W., *Zeit. Zellf.*, **10**, 625-641 (1930).
[12] Kaufmann, B. P., *Am. Nat.*, **65**, 280–282 (1931).
[13] Koshy, T. K., *Jour. Roy. Microsc. Soc.*, **54**, 104–120 (1934).
[14] Naithani, S. P., *Ann. Bot. N. S.*, **1**, 257–276 (1937).
[15] Swanson, C. P., *Am. Jour. Bot.*, **30**, 422–428 (1943).
[16] Ris, H., (in press).
[17] Cooper, K. W., these PROCEEDINGS, **24**, 452–458 (1938).
[18] Ris, H., *Jour. Exp. Zool.*, **90**, 267–322 (1942).
[19] Doyle, W. L., and Metz, C. W., *Biol. Bull.*, **69**, 126–135 (1935).
[20] Mirsky, A. E., and Pollister, A. W., these PROCEEDINGS, **28**, 344–352 (1942).

THE SPREAD OF AN EPIDEMIC

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Communicated September 11, 1945

The Frost-Soper theory of the rise and fall of an epidemic in a population[1] can at best represent only a part of the phenomenon of epidemics, for they spread from population to population as well as from individual infectious cases to susceptible individuals within a population. The fundamental hypothesis or law, namely,

$$C(t) = rS(t)C(t - \tau) \tag{1}$$

where C is the new-case rate, S the number of susceptibles, r a factor of proportionality and τ the time lag between infection and infectiousness may be very naturally extended to represent what hypothetically might happen between two populations S and S' of susceptibles by writing

$$C(t) = rS(t)C(t - \tau) + r''S(T)C'(t - \tau), \tag{2}$$

$$C'(t) = r'S'(t)C'(t - \tau) + r'''S'(t)C(t - \tau). \tag{2'}$$

It would indeed be only natural to argue that the new-case rate C among the susceptibles S should be the sum of what it would be due to the infectious rate within S and of a similar term due to the infectious rate in the other population of which a number of infectious individuals proportional to the total number within S' come in contact with the susceptibles S.

It may first be observed that if the two susceptible populations S and S' were merely ideal subdivisions of one and the same population S_0 so that $S = pS_0$, $S' = qS_0$, $p + q = 1$, and $C = pC_0$, $C' = qC_0$, the rate of intermixture of the infectious $C(t - \tau)$ with the susceptibles S or S' being the same as the rate of intermixture of the infectious $C(t - \tau)$ with S_0, and the