general are the causes of abnormal development, while the smaller the missing piece the more specific the effects. Thus in the region at the left end of the *X*-chromosome Muller⁴ has found "a viable two gene deficiency," and Demerec and Hoover⁵ have found a deficiency which survives in homozygous condition. In this same region many small lethal deficiencies are known, one of which (Deficiency scute-8) has been found to cause death very late in the egg stage just before hatching (Poulson, unpublished). Also small lethal deficiencies are found scattered throughout the chromosome complement. Hence it would appear that genes which play very definite parts in the embryonic developmental processes are distributed quite as much at random throughout the *X*-chromosome as are genes which affect only the most superficial characters.

A more detailed account of the behavior of these deficiencies, together with some further data, will be presented elsewhere.

¹ Work carried out at the Wm. G. Kerckhoff Laboratories, California Institute of Technology, Pasadena.

² Bridges, C. B., *Genetics*, **2**, 445 (1917).

³ Li, J. C., *Genetics*, **12**, 1 (1927).

⁴ Muller, H. J., *Jour. Hered.*, **26**, 469 (1935).

⁵ Demerec, M., and Hoover, Margaret E., *Jour. Hered.*, **27**, 206 (1936).

⁶ Huettner, A. F., and Rabinowitz, Morris, *Science*, **78**, 367 (1933).

⁷ Huettner, A. F., *Jour. Morph.*, **37**, 385 (1923).

⁸ Howland, R. B., and Child, G. P., *Jour. Exp. Zool.*, **73**, 109 (1935).

⁹ Poulson, D. F., "The Embryonic Development of *Drosophila melanogaster*," *Actualités Scientifique et Industrielle*, Paris. (In press.)

¹⁰ Mohr, O. L., *Z. f. indukt. Abst. Vererbl.*, **32**, 108 (1923).

¹¹ Mackensen, O., *Jour. Hered.*, **26**, 163 (1935).

DEFICIENCIES AND STRUCTURAL VARIATIONS WITHIN THE GIANT CHROMOSOMES IN RELATION TO THE PROBLEM OF GENE STRUCTURE

BY C. W. METZ

DEPARTMENT OF EMBRYOLOGY, CARNEGIE INSTITUTION OF WASHINGTON, AND JOHNS HOPKINS UNIVERSITY

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The giant chromosomes in salivary and certain other glands of dipteran larvae are characterized by transverse chromatic discs or "bands," separated from one another by intervening achromatic material. Since one of the primary aims of studies on these chromosomes is to identify the genic as distinguished from non-genic materials, and to interpret the visible structures in terms of individual genes, attention naturally becomes focused

on the two types of constituents and on the visible components of each. If one type of material is genic and the other non-genic it seems probable, on general grounds, that it is the chromatic which is genic,¹ although the opposite view is also supported.² In the present state of the evidence it seems best to consider that either or both may be genic. For this reason we have devoted much attention to the morphological characteristics of each. Some of the results of these observations are considered below.

In *Sciara*, as indicated previously,³ it may readily be seen that, in fixed preparations at least, the achromatic material is in the form of droplets or "vacuoles" and is essentially discontinuous, while the chromatic material is not thus organized and is in this sense essentially continuous. Each two successive chromatic discs are separated by a layer or plate of achromatic droplets, and correspondingly each two successive transverse layers of achromatic droplets are separated by a disc. The thickness and conspicuousness of the disc depends on how much chromatin it contains. The thinner ones range down almost to the point of invisibility and may appear merely as the boundaries between layers or plates of droplets. Although confined mainly to the transverse discs, the chromatic material may, and apparently does, extend between and around the achromatic droplets separating one disc from the next. It may be considered to be continuous both longitudinally and transversely, forming the groundwork of an essentially honey-comb type of organization. Many detailed features are not yet understood, but it is believed that the general aspects of visible organization in these chromosomes may all be interpreted in terms of the distribution of the two types of materials just mentioned.

One of the puzzling aspects of the present problem has resulted from the fact that the detailed structural characteristics of individual chromosomes vary greatly. In different preparations, or even different cells in the same gland, wide differences in detail are found in a given region of any chromosome (see published photographs).⁴ In some cases the transverse chromatic discs are clear cut, relatively smooth in outline and apparently dense (deeply staining), while the achromatic material between them is likewise sharply outlined and is almost transparent after ordinary staining methods. In other cases, however, the chromatic material is much more widely dispersed, and the achromatic droplets are very conspicuous because of the chromatin surrounding them. The chromosome is "vesiculated."

In vesiculated chromosomes, discs which are apparently single in the banded type often appear double, exhibiting a layer or plate of achromatic droplets between the two faces. This accounts in part for the frequent occurrence of paired discs. In extreme form the structures thus formed may appear inflated and be biscuit-like or capsule-like. Some discs tend to exhibit this characteristic under almost all conditions, while others show it

only when extreme vesiculation occurs.⁵ At first it was thought that the vesiculated condition was abnormal and due to injury or methods of treatment; but the evidence now indicates that, although treatment may cause modifications, the entire range of conditions may occur and even be reversible in living, normal chromosomes. In larvae from a culture at one stage the chromosomes may be vesiculated, while in sisters taken at a later period they may be in a sharply "banded" condition. Sometimes both conditions are found simultaneously in one gland.

Study of individual discs and regions of a chromosome in the vesiculated and non-vesiculated conditions indicates that with an increase of achromatic material "within" the (now double) discs there is a decrease of achromatic material between the discs, and vice versa. In all cases the achromatic material appears in the form of droplets in fixed preparations. Thus it appears that the exact distribution of the two types of material is not fixed, and also that the achromatic droplets do not have special significance as individual structures.⁶ The fundamental pattern represented by the chromatic discs appears to be fairly definite and regular, but many of the discs may appear as either single or double "bands," as indicated above, and their structural details, as well as those of the achromatic materials, exhibit wide variation. This variability applies also to the so-called "granules" of chromatic material which, like some of the heavy-walled droplets of achromatic material, have been considered by some observers to represent genes.⁷

As noted previously, the relationship between these two constituents indicates that the shape, size, number and position of the granules are determined by the droplets. The variability of the granules is illustrated by the fact that a disc which appears in the form of a single transverse row of granules under one condition (non-vesiculated) may exhibit two distinct rows under other conditions (vesiculated), even in the same gland. Likewise, the evidence indicates that some rows of granules are formed from materials derived from two separate discs and that a single disc may contribute to two separate rows of granules.⁸ The granules appear to represent thickenings in the discs (where the latter are distorted by pressure of adjacent achromatic droplets) or accumulations of chromatin in spaces between the droplets.

In considering the distribution of the achromatic materials two questions immediately present themselves. If an apparently single disc may be transformed into a double disc by the acquisition of achromatic material between its two faces, it may be asked whether the original disc was really single or double. No criterion is at present available for distinguishing between single and double discs except appearance. So far as known any disc, even the thinnest, may become double, or form a "capsule," in this manner. This consideration raises the second question, namely, is there

any difference between achromatic materials inside a double disc or "capsule" and those outside or between successive discs or "capsules?" Special attention has been given to this feature. No distinction has been detected. The achromatic materials inside the paired discs or capsules apparently exhibit the same range of characteristics as do those outside or between them. In either case the droplets may be small or large, thin walled or thick walled, depending on the particular region in which they lie. Likewise, capsules may be formed between two dissimilar discs, or between two pairs of discs, as well as between the two faces of a double disc or the two members of a pair—i.e., capsules may be formed by achromatic materials in either type of position.

In the search for the essential unit of structure here especial interest attaches to small deficiencies. Several cases of what appear to be single disc deficiencies have been studied in *Sciara ocellaris*. In each a pair of discs or a capsule is present in one homologue, and a single disc, apparently corresponding to one member of the pair, is present in the other homologue. The achromatic material on either side of the single disc appears to correspond to that on the corresponding sides of the pair of discs in the other homologue. The deficient member thus appears to lack one disc and the achromatic material between the two discs of the other member. Apparently, therefore, the achromatic material on one side of a disc is associated with it, and acquired or lost with it, or else a regulating factor of some kind determines the general nature or amount of achromatic material on either side of a given disc. General considerations would make the latter interpretation appear the more probable.

As noted previously the achromatic droplets differ greatly in size, number and appearance in different regions of a given chromosome, and equally great differences are exhibited by the chromatic "granules." Since both types of structures have been considered by some authors to represent genes, brief consideration may be given here to their size characteristics and to certain relationships between them and the structures visible in chromosomes of ordinary embryonic cells such as the salivary gland cells are before their enormous enlargement takes place.

The view which regards the granules or heavy-walled droplets as representing individual genes is based on the stated or implied assumption that the genes and the chromonemata (individual series of chromomeres) have definite size limits, and that growth automatically results in duplication. On this view a giant chromosome is large because the original chromonema which gave rise to it has undergone repeated divisions, giving a multiple structure like a cable. The number of "granules" in a transverse disc, then, indicates the degree of multiplication, and each granule (or droplet, as the case may be) identifies an individual chromonema. One of the difficulties inherent in such a view is illustrated by the smaller cells

of the mature salivary gland. Some of these possess chromosomes which, although enlarged like the cells to many times their original size, nevertheless appear single. In a purely descriptive sense such a structure may be designated a single chromonema, but it bears little resemblance to a chromonema in an "ordinary" cell.

Two aspects of the problem may be emphasized here, one dealing with size and the other with the shape and general appearance of the structures under consideration. The apparently single chromonema in these smaller salivary gland cells is many times as large as a single chromonema in ordinary embryonic cells. Likewise, the individual droplets or granules, which form a single linear row here, are many times as large as any units (chromomeres) visible in chromonemata of ordinary cells. It seems evident, therefore, either that the components of a chromonema (including genes?) do not have fixed size limits corresponding to those seen in an ordinary cell, or else that the visible structures under consideration are not single.

The same two alternatives are met in any attempt to interpret the droplets and granules in fully developed giant chromosomes. If either a droplet or a granule is considered to represent an individual gene the gene evidently does not have a fixed size limit that is reached in an ordinary cell. If either is considered to represent a gene with surrounding accessory material, the size limits of chromonemata and chromomeres similarly are not fixed and size is no necessary indication of the constitution of a chromosome in terms of chromonemata. Likewise there is no fixed relation between number of chromonemata or chromosomes and size of cell or nucleus, and it must be assumed that the bulk of a chromonema is composed of non-genic material produced by the genes or having the power of independent growth. Size of components in a chromonema, then, would have no obvious significance in relation to the problem of the physical nature of genes. There would be little theoretical difference between such a view and one which assumed the entire giant chromosome to be a single, enlarged chromonema.

On the other hand, if each droplet or granule is considered to be compound and to represent a bundle of sister genes, derived by multiplication of the original one, then the group of droplets or granules at any one locus (disc or band) presumably represents a group of sisters derived by multiplication of the original droplet or granule—which was itself compound. Thus a multiple would itself become a unit and undergo division when it reached a certain size. Unsupported by direct evidence such an assumption appears merely to complicate the situation.

Concerning the shape and general appearance of the structures under consideration it is to be observed that visible evidence from the smaller salivary gland cells, as well as general considerations, indicate that a chro-

monema is not to be regarded as a series of chromomeres on a thread, like a string of beads, but as a structure of more uniform diameter and more like a slender cylinder. This consideration seems important, because, on the view which regards the giant chromosome as a cable composed of visible chromonemata, the granulated striations seen when the chromosome is stretched are considered to represent these chromonemata. In the striations the granules or heavy-walled droplets appear like beads on a string. Likewise, the longitudinal lines between successive chromatic discs in the undistorted chromosome are considered on this view to represent the threads connecting successive granules or chromomeres. On our view these lines represent the interfaces, together with more or less chromatic material, between the achromatic droplets separating one chromatic disc from the next, and the striations in stretched chromosomes represent distortions of this fundamental honey-comb-like structure.⁹ If numerous chromonemata are present in the giant chromosomes we would not expect them to show the characteristics of these lines or striations, but rather to appear as slender cylinders, more like the fibrils of a striated muscle fibre. And, as indicated in earlier papers, if chromonemata of the type found in ordinary embryonic cells are present here their number should be very large and they should be practically invisible as units.

¹ See, e.g., Caspersson, T., *Skandinav. Arch. f. Physiol.*, Supplement to 73, 1-151 (1936).

² Koltzoff, N., *Science*, 80, 312 (1934).

³ Metz and Gay, these PROCEEDINGS, 20, 617-621 (1934); Metz, *Jour. Hered.*, 26, 177-188 (1935 a); Metz, *Jour. Hered.*, 26, 491-501 (1935 b); Metz, *Biol. Bull.*, 71, 238-248 (1936).

⁴ Figures 4, 5, 6, Metz, 1935 a, loc. cit.

⁵ In certain regions of some chromosomes the materials regularly tend to appear in the form of heavy-walled droplets or as clusters of bubbles. These apparently represent two or more independent discs, with intervening achromatic materials—the chromatic material in this case being dispersed around the periphery of the droplets. Examples are shown by Bauer (*Zoöl. Jahrb.*, 56, 239, 1936, figure 6) and by the writer (1935 a, loc. cit., figure 5B, at end of chromosome).

⁶ According to observations of Dr. D. F. Poulson and the writer an excess of achromatic material in a given region may result in the formation of a nucleolus. The chromatic discs may extend out into the nucleolus in the form of a branching root-like system. The expanded regions or "puffs" (see, e.g., photographs in figure 5B, C, Metz 1935 b, loc. cit.) are thus to be regarded as essentially nucleolar in nature.

⁷ See, e.g., Koltzoff, N., loc. cit.; Bridges, C. B., *Amer. Nat.*, 69, 59 (1935); Koller, P. C., *Proc. Roy. Soc. B.*, 118, 371-397 (1935); Bauer, H., *Zeit. Zellforsch.*, 23, 280-313 (1935) and loc. cit.

⁸ Metz, 1935 b, loc. cit., and unpublished observations.

⁹ As noted elsewhere, our observations on *Sciara*, *Chironomus* and *Drosophila* indicate that these striations or lines are not continuous or thread-like in nature, but form a branching meshwork or basketwork at all levels unless the chromosome is so severely distorted as to disorganize it. In other respects also conditions in *Chironomus* and *Drosophila* agree with those in *Sciara*.