COMPLEMENTATION OF GENETIC ACTIVITY IN TRANSLOCATED FRAGMENTS OF THE Y CHROMOSOME IN *DROSOPHILA HYDEI*

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IN *Drosophila* a number of sites in the Y chromosome may form lampbrush loops. These "functional structures" indicate the existence of local, phase-specific genetic activities (HESS and MEYER 1963a; MEYER and HESS 1965; HESS 1965b, 1966a). The lampbrush loops of the Y chromosome have been investigated mainly in *D. hydei*, because this species exhibits a number of favorable qualities for this kind of study. However, there is good reason to assume that in principle the situation is similar in all or most of these species of the genus *Drosophila* (see also review article: HESS and MEYER 1967).

The loop forming sites in the Y chromosome of *D. hydei* appear to be active exclusively in male germ line cells and only during the growth phase of the primary spermatocytes. The physiological significance of the genetic material in these loops is not yet known with certainty. It is also not known whether or not this material is related to the previously found fertility factors of *D. melanogaster* (STERN 1929; BROSSAU 1960). However, it has been established that deficiencies of the Y chromosome which include loop forming sites are associated with male sterility (HESS 1965b, 1967). All of these deficiencies have arisen as X-Y translocations. They therefore invariably lack at least the distal regions of either the long or the short arm of the Y chromosome, and the association with loss of male fertility could in theory be considered as being due to the loss of "distal" fertility factors unrelated to the loop forming sites. However, different deficiencies produce different morphogenetic effects on spermiogenesis, and this is an argument against this possibility. In order to decide whether or not all of the loop forming sites of the Y chromosome are essential for normal spermiogenesis, it would be necessary to examine the morphogenetic effects of interstitial Y deficiencies. For technical reasons, such deficiencies are difficult to obtain directly. However, they can be constructed by combination of different translocated Y fragments. The results of such combinations are reported here. The experiments also contribute additional information about the number and loci of the loop forming sites in the Y chromosome of *D. hydei*.

MATERIALS AND METHODS

The approximate loci of the various loop forming sites in the Y chromosome of *D. hydei* have been previously mapped (HESS 1965b) (Figure 1). Often the chromatids between neighbouring loops separate. Therefore, the structure of different loops may appear as parts of the same

FIGURE 1.—Diagram of the Y chromosome of *D. hydei* in the state of an univalent lampbrush chromosome in spermatocytes, with loci of the loop forming sites.—Explanations: C, clubs; Co, cones of pseudonucleolus; N₁, N₂, nooses; P, pseudonucleolus; T₁, T₂, tubular ribbons; Thdd, Thpc, distal diffuse, and proximal compact sections of the threads; Y^L, Y^S, long and short arm of the Y chromosome.

FIGURE 2.—Y-autosome translocation. Normal chromosome set of male (left) with breakage points and the two translocation chromosomes Y.A₁ and A₁.Y.—Explanations: white, euchromatin of autosomes; black, heterochromatin of X chromosome and autosomes; dotted, euchromatin of X chromosome; thin vertical hatching, euchromatin of the involved autosome A₁; thick oblique hatching: heterochromatin of the Y chromosome; A₁–A₅, the autosome pairs; C, clubs; Co, cones of pseudonucleolus; N₁, nooses; P, pseudonucleolus; T, tubular ribbons; Th, threads.

giant "compound" loop. This is, for instance, the case for threads and pseudonucleolus, or tubular ribbons (T_2) and clubs.

For the combination crosses one reciprocal Y-autosome translocation and a large number of X-Y translocations were available. The reciprocal Y-autosome translocation used in these experiments (see also HESS 1965b) consists of two elements (Figure 2), the first of which, $Y.A_1$, is made up of the short arm, the kinetochore, the proximal three to four tenths of the long arm of the Y chromosome, and the entire euchromatin of one of the rod shaped autosomes. The Y fragment of that chromosome includes the sites for the "nooses", "clubs", and "tubular ribbons". The second translocation chromosome in this system ($A_1.Y$) consists of the kinetochore and a very short, completely heterochromatic proximal region of the involved autosome together with the distal six or seven tenths of the long arm of the Y chromosome. The Y fragment of this element carries the sites of the "threads" and the "pseudonucleolus". Because of the favorable location of the autosomal breakage point these two translocation chromosomes segregate independently from one another without causing lethal autosomal duplications or deficiencies. In the stock which contains this reciprocal translocation, there are no free Y chromosomes. Only the two (complementary) Y fragments of the translocation are present.

The X-Y translocations used in these experiments have been recovered by a method which involves the X-ray induction of detachments in attached-X females (HESS 1965b). Two types of translocation chromosomes are found (Figure 3). The first type of detachment chromosomes contains the short arm, the kinetochore, and a proximal fragment of the long arm of the Y chromosome together with a complete euchromatic arm of an X chromosome ("captured X", see MULLER and HERSKOWITZ 1954). The translocation chromosomes of this type which have so far been found carry the sites for the nooses only, for nooses and clubs, or these two loops together

FIGURE 3.—(a) Construction of attached-X chromosome. (b) Construction of X-Y translocations by X-ray induced detachments of attached-X chromosomes.—Explanations: white, euchromatin of autosomes; black, heterochromatin of X chromosome and autosomes; dotted, euchromatin of X chromosome; thick oblique hatching: heterochromatin of the Y chromosome; A_1 – A_5 , the autosome pairs; Kin_X , Kin_Y , kinetochore of X or Y chromosome; XX , attached-X chromosome; Y^L , Y^S , long and short arm of the Y chromosome; Y^{Ld} , Y^{Lp} , distal and proximal fragment of the long Y arm.

with the tubular ribbons, depending upon the length of the proximal part of the long Y arm. A case with nooses, clubs, tubular ribbons, and pseudonucleolus has also been found, but this stock was lost before combination experiments could be executed.

The second type of X-Y translocation chromosomes consists of the euchromatic arm and the kinetochore of an X chromosome together with a distal fragment of the long Y arm ("capped X"). Several translocations of this type have been recovered. They may carry sites for the threads only, for threads and pseudonucleolus, for threads, pseudonucleolus and tubular ribbons, or these three together with the clubs. In addition, the threads in the translocated Y fragments may be either of a "wild type" shape, or of the shape produced by one of the known mutants, *tube-proximal* or *tube-distal* (Hess 1965a, 1966a). Such translocations have been obtained from detachments which were induced in attached-X females bearing a mutant Y chromosome.

Detachment-bearing males are sterile unless they also carry a normal Y chromosome. Therefore, the translocations are maintained in an attached-X system in which the males constantly possess a normal free Y chromosome in addition to their translocation chromosome. In all cases in which males without this free Y chromosome are needed, males carrying the translocation chromosome (which is marked with the sex-linked recessives white (*w*) and light (*lt*)) are crossed to normal wild-type females. The patroclinous exceptions which occur after primary nondisjunction of the X chromosomes in the P females are males of the wanted constitution.

The combination crosses: With the available stocks of *D. hydei*, combination crosses can be made in such a way that one of the existing X-Y translocations is combined with one of the two elements of the reciprocal Y-autosome translocation.

In the first cross, attached-X females which were marked with white (*w*) light (*lt*) and had a free Y chromosome were crossed in mass cultures with males from the $T(Y;A)$ stock. These males were marked with the sex-linked recessive vermilion-1 (v_1). They possessed one pair of translocation elements and, in addition, sometimes either the first ($Y.A_1$), the second ($A_1.Y$), or both elements in duplicate.

$$(1) \quad P \quad \underline{w \, lt/Y;A_1/A_1} \times v_1/Y.A_1/A_1.Y/A_1 \\
\qquad \qquad \qquad Y.A_1/Y.A_1/A_1.Y \\
\qquad \qquad \qquad Y.A_1/A_1.Y/A_1.Y/A_1 \\
\qquad \qquad \qquad Y.A_1/Y.A_1/A_1.Y/A_1.Y$$

The F_1 females were attached-X females without a normal free Y chromosome, but they had either $Y.A_1$, $A_1.Y$, or both elements of the translocation. These females were subsequently crossed with males from one of the X-Y translocation stocks. It is not possible to determine the genetic constitution of the F_1 females directly. Therefore, the second cross was made in single matings.

$$(2) \quad F_1 \quad \underline{w \, lt/Y.A_1/A_1} \\
\qquad \qquad \qquad A_1.Y;A_1/A_1 \\
\qquad \qquad \qquad Y.A_1/A_1.Y/A_1 \times T(X,Y)/Y$$

From each mating some F_2 males were used to study spermatocyte nuclei. The loops of the Y chromosome which are developed in the spermatocytes made it possible to see which Y material was inherited from the mother. Thus, it is not only possible to determine the genetic constitution of the F_2 males with respect to Y-autosome translocation elements, but also to find out indirectly the constitution of their mother. In addition, morphogenetic effects on spermatid differentiation could also be studied in these males.

Ten other males of each mating were pair mated with females of a wild stock. This third cross was performed in order to check the fertility of the various combinations of translocation chromosomes in the F_2 males. In addition, this cross served as a proof for the spermatocyte studies. F_3 males, namely, under these conditions do not receive a free Y chromosome, but only elements of the Y-autosome translocation. Thus, one expects that the F_3 males from each mating are of the same constitution possessing either $Y.A_1$ or $A_1.Y$. Some X0 males also occur. In those cases in which both elements of the Y-autosome translocation were present in F_2 males, the F_3 males are not homogeneous and possess partly the first, the second, or both elements of the Y-autosome translocation.

- (3) F_2 $+/+; A_1/A_1 \times T(X,Y)/Y.A_1/A_1$ (a)
 $A_1.Y; A_1/A_1$ (b)
 $Y.A_1/A_1.Y/A_1$ (c)

- F_3 males: (a) $+/Y.A_1/A_1$
 (b) $+/A_1.Y; A_1/A_1$
 (c) $+/Y.A_1/A_1, +/A_1.Y; A_1/A_1, +/Y.A_1/A_1.Y/A_1$

During the investigations it turned out that the two translocation chromosomes of the Y-autosome system do not segregate at random. In cross (2) half of the male progeny of those F_1 attached-X females which had received only one of the translocation chromosomes are expected to receive this translocation chromosome, the second half should not get it. The findings are in accordance with the expectations. In contrast to this, the frequencies of different male classes in the progeny of F_1 females which had received both translocation chromosomes did not correspond with the expectations. Males with either $Y.A_1$, $A_1.Y$, or both translocation chromosomes should occur at equal frequencies. However, one finds either males with the constitution $Y.A_1/A_1.Y$ or only A_1/Y in about the same proportions, but males with only $Y.A_1$ are very infrequent. This segregation behaviour of the two translocation chromosomes requires further study.

RESULTS OF THE COMBINATION TESTS

The combination crosses may be divided into two series. In the first series X-Y translocations bearing a proximal fragment of the Y chromosome were tested in combination with the $A_1.Y$ element of the Y-autosome translocation. This element carries the sites for the threads and the pseudonucleolus (Figure 4). The shortest X-Y translocation used in this series (290/1, see Figure 4) carries only the loops of the short Y arm (nooses). This combination results in males which have nooses, threads, and pseudonucleolus and lack clubs and tubular ribbons. In these males, spermiogenesis proceeds until a comparatively late spermatid stage and is then arrested. The males remain completely sterile.

Previous investigations on the morphogenetic effects of Y deficiencies have shown that deficiencies in which only the threads and pseudonucleolus are present have very similar effects as compared with deficiencies in which only the nooses are present (MEYER, unpublished; HESS 1967). In both cases spermiogenesis is blocked at a rather early stage of spermatid differentiation (early effect). In con-

FIGURE 4.—Combination of the "distal" fragment (above) of the Y-autosome translocation with different "proximal" X-Y translocations (lower row).—Explanations: black, heterochromatin of the X chromosome and autosomes; dotted, euchromatin of the X chromosome; thick oblique hatching, heterochromatin of the Y chromosome; C, clubs; Noo, nooses; P, pseudonucleolus; T₁, T₂, tubular ribbons; Th, threads.

trast to this observation, it is clear from the light microscopical investigations of the combination males that a combination of nooses with threads and pseudo-nucleolus results in a considerable improvement of the differentiation capacities of the spermatids. This conclusion requires further specification from electron microscopical investigations which will be the subject of another publication (MEYER, in preparation).

The second translocation used in this series (340/2, see Figure 4) carries together with the nooses the most proximal loop of the long Y arm, the clubs. Thus, the males in the combination test have a deficiency for the tubular ribbons only. These males are also sterile and spermiogenesis shows a behaviour similar to that of the males described in the first experiment. If there are differences in differentiation capacity attributable to the addition of the clubs, they are too small to be detected with the light microscope. Similar results were obtained with a second translocation of this type (697/12).

In the next test an X-Y translocation was used which carries nooses, clubs, and tubular ribbons. This translocation (290/2, see Figures 4, 5) combined with the distal fragment of the Y-autosome translocation results in a genetic constitution in which all known spermatocyte loops are present. It turned out that this

FIGURE 5.—X-Y translocation 290/2. (a) Spermatocyte nucleus of a male without a free Y chromosome; only tubular ribbons (T), clubs (C), and nooses (Noo) are present; N, nucleolus; phase contrast photograph of a living nucleus, ca. 1200 $\times$.—(b,c) Neuroblast mitosis from larva of the same stock.—Explanations: A₁–A₅, the autosome pairs, showing somatic pairing; T(X,Y), translocation chromosome; Y, free Y chromosome. (b) phase contrast photograph of lactoorcein squash preparation, ca. 1800 $\times$.

combination is perfectly complementary both with respect to male fertility and spermiogenesis. Similar results were obtained with a second X-Y translocation of this type (697/32).

In contrast to this observation, a third translocation of the same type (340/7, see Figure 4) gives different results. Although the spermatocyte nuclei of the males in the combination test are not easily distinguishable from those of normal animals, all males in this experiment appeared to be completely sterile. Spermiogenesis is blocked at a late stage of spermatid differentiation, that is to say, the elongation of spermatids is incomplete. It turned out that the mitotic shape of the three translocation chromosomes used in the two groups of experiments shows important differences: Whereas the two translocations which give complete complementation (290/2, 697/32) have fragments of the long Y arm which include about six to seven tenths of the total arm length, the translocation with incomplete complementation (340/7) has a translocation chromosome in which the fragment of the long Y arm includes only two to three tenths.

In earlier investigations which were undertaken in order to localize the loop forming sites in the Y chromosome (HESS 1965b), this fact had been interpreted with the assumption that the site for the tubular ribbons is located in region 2 or 3, but not distally beyond 3. The results of the combination tests here can be interpreted in three ways: (1) The tubular ribbons are not developed by a single loop-forming site, but by two or more sites. One site would be located within region 2 or 3. The second and all possible additional ones would be located more distally in regions 3 to 7. The genetic material within these tubular loops is of more than one kind and, therefore, the function performed by the proximal part of the chromosome alone is not sufficient for normal spermiogenesis. (2) In translocation 340/7 the breakage point goes through the loop forming site of the tubular ribbons. This loop can be considered to be redundant similar to the situation realized in nucleolar organizers (RITOSSA and SPIEGELMAN 1965). Therefore, the translocated fragment of the site is able to form tubular ribbons, but the loop is so defective that its function is disturbed. (3) There might also be a fertility factor in region 3-5 which is not accompanied by the formation of a recognizable functional structure. There is no possibility at the moment to distinguish between these alternatives. However, cytological observations as well as theoretical considerations of the organization of the Y chromosome chromomeres (see BEERMANN 1965; HESS 1966b) are in favour of the first possibility.

The second series of studies involved tests in which X-Y translocations with "distal" Y fragments are combined with the "proximal" fragment of the Y-autosome translocation which carries nooses, clubs, and tubular ribbons (Figure 6).

In the first experiments of this series an X-Y translocation (689/2, see Figure 6) was used which carries all loop sites of the long Y arm, namely threads, pseudo-nucleolus, tubular ribbons, and clubs. Thus, the combination males possess a duplication of those regions of the Y chromosome which include the sites for clubs and tubular ribbons. As expected, the combination of these two Y fragments is complementary, and the males are perfectly fertile. The same results were obtained with another X-Y translocation of the same type (689/6).

FIGURE 6.—Combination of the “proximal” fragment (above) of the Y-autosome translocation with different “distal” X-Y translocations (lower row).—Explanations: black, heterochromatin of the X chromosome and autosomes; dotted, euchromatin of the X chromosome; thin vertical hatching, euchromatin of translocated autosome; thick oblique hatching, heterochromatin of the Y chromosome; C, clubs; Noo, nooses; P, pseudonucleolus; T, tubular ribbons; Th, threads.

Next, an X-Y translocation (689/12, see Figures 6, 7) was used which carries loop forming sites for the threads and the pseudonucleolus. The males in the combination test possess a normal set of spermatocyte structures. It is also found that they are of normal fertility. Thus, the two combined fragments of the Y

FIGURE 7.—X-Y translocation 689/12. (a) Spermatocyte nucleus of a male without a free Y chromosome; only threads (Th) of the mutant *tube-proximal* and the pseudonucleolus (P) are present; N, nucleolus; phase contrast photograph of a living nucleus, ca. 1200 $\times$.—(b,c) Neuroblast mitosis from larva of the same stock.—Explanations: A₁–A₄, the autosome pairs (the fifth pair not visible); T(X,Y), translocation chromosome; Y, free Y chromosome. (b) phase contrast photograph of lacto-orcein squash preparation, ca. 1800 $\times$.

chromosome are complementary. As spermatocyte nuclei of males with only the X-Y translocation chromosome do not possess tubular ribbons, one is able to conclude from the results of the combination in this experiment that the proximal fragment of the Y-autosome translocation carries a complete set of tubular ribbons. Otherwise one would expect on the basis of the results in the third experiment in the first series that the combination males would be sterile.

Similar results were obtained with several other X-Y translocations which also carry sites for threads and pseudonucleolus (689/21, 697/16, 697/100, 697/120). Two of these translocations have threads of the shape caused by the mutant *tube-proximal*, the others threads of the shape of the mutant *tube-distal*. Obviously, the mutants do not have any influence on the morphogenetic activity of the loops.

In a third group of experiments in this series X-Y translocations were used which had only a rather short fragment from the distal tip of the long Y arm. This fragment includes only the site for the threads (see Figure 6).

Spermatocyte nuclei of the combination males consequently exhibit an absence of the pseudonucleolus. In spite of this, spermiogenesis seems to be quite normal and the testes contain a large number of apparently typical sperm bundles. In fresh preparations of testes squashed in *Drosophila* Ringer's solution one can always find some motile spermatozoa. However, only single spermatozoa are found to move, and the motility is always very weak as compared to sperm of genetically normal males. A large number of combination males (about 40) with such a Y deficiency for the pseudonucleolus were raised and mated to normal females under conditions which normally produce progeny. However, no progeny have as yet been obtained. This indicates that the Y deficient males, although they possess some slightly motile spermatozoa, are sterile or at least very much reduced in fertility.

Results of the same type have been obtained with three similar X-Y translocations; the first (340/10) carrying the site for the threads of the normal "wild type" shape, and two others (689/19, 689/22) carrying the site for threads of the mutant *tube-proximal*.

Finally, a detachment (689/9) was used in which only a small dot of heterochromatin is translocated to the X euchromatin. Males possessing only this translocation chromosome have spermatocyte nuclei which do not contain any of the Y chromosomal loops. A small structure which has not been identified further is regularly seen near the nucleolus. In X0 males such a structure has never been found. In the combination test, the males have only the loops of the fragment from the corresponding "proximal" Y-autosome translocation chromosome, namely tubular ribbons, clubs, and nooses. Such males are completely sterile. Their testes show the typical picture of males lacking threads and pseudonucleolus, namely "late effects" with a number of incompletely elongated spermatids.

In all combination crosses, males also occurred which had received together with the X-Y translocation chromosome the "wrong" Y fragment of the Y-autosome translocation, i.e. in the crosses of series I cases in which "proximal" X-Y translocations were combined with the "proximal" Y-autosome fragment, and

correspondingly, in series II "distal" X-Y translocations with the "distal" Y-autosome fragment. Such males have duplications for one region of the Y chromosome, and at the same time a deficiency for another Y region. In none of these cases could any sign of complementary effects be detected. This demonstrates clearly that the different regions of the Y chromosome which are marked by sites for different spermatocyte loops, have specific functions.

Conclusions: The combination experiments reported here demonstrate clearly that the fertility factors of *D. hydei* can not all be located in the distal regions of the two arms of the Y chromosome. On the contrary, interstitial deficiencies of certain regions of the Y chromosome cause also male sterility. In all so far investigated cases, males are invariably sterile when they bear deficiencies of regions of the Y chromosome that include loop forming sites. This is another argument in favour of the hypothesis that genetic material in the loops is identical with the fertility factors.

The real mode of function of this genetic material is not yet clear. Specifically, it is not known whether or not these factors are conventional genes, i.e. structural genes carrying the information for the amino acid sequence of proteins, or operational genes which are involved in the regulation of the activity of other genes. Earlier doubts in this respect have come mainly from cytogenetic observations. The Y chromosome is seen to be entirely heterochromatic and, in addition, it has been shown to be largely "inert" with respect to Mendelian factors. However, exceptions to this rule are known, for instance, position effects of the variegated type (see LEWIS 1950), or Y suppressed lethals (LINDSLEY, EDINGTON, and VON HALLE 1960; HESS 1963). In addition, there is now experimental evidence which demonstrates that a chromosome, or a part thereof, is not irreversibly determined heterochromatic or euchromatic (e.g. LYON 1966; RUSSELL 1964, and many others). Thus, the possibility suggests itself that the Y chromosome appears heterochromatic in all cells excepting those in which the fertility factors are actually involved in the genetic activity, i.e. the spermatocytes. On the other hand, the Y chromosome could also be viewed as a generally heterochromatic chromosome with only a few "euchromatic" genes inserted. Assuming that in the spermatocyte loops the DNA is completely unfolded, then each loop forming site (chromomere?) would contain an amount of DNA which corresponds to about 50μ which is the average length of the fully developed loops in the spermatocytes. RITOSSA and SPIEGELMAN (1965) have reported that the total content of DNA per genome in *D. melanogaster* equals a length of 15–75 mm. BERENDES and KEYL (1967), on the other hand, have shown that in *D. hydei* 16% of the total DNA is found in the Y chromosome. Thus, the Y chromosome contains DNA corresponding to a length of about 2.5–12.5 mm and each loop forming site in that chromosome would contain only $1/50$ – $1/250$ of the DNA of the entire chromosome. Thus, by their DNA content these loci are expected to be too small to be visible cytologically. They are, however, in the relative size range of the chromomeres forming the bands of polytene chromosomes. The loop forming sites in the Y chromosome may, therefore, be compared to typical "chromomeres" also from this point of view.

However, the actually observed morphogenetic effects of these "genes" may be interpreted in quite a different manner. In *D. hydei*, spermatogenesis never proceeds beyond the stage of the primary spermatocytes in cases where the entire Y chromosome is absent (X0 males). However, already after the addition of only one loop pair, namely either the threads or the nooses, the meiotic divisions take place and spermatids are formed. So far it has not been possible to test other single loops. In different Y deficiencies spermiogenesis proceeds further to various stages, depending upon the nature of the loops present. In addition, all major structural components of the developing sperm, such as acroblast, nebenkern and its derivatives, or the flagellum with its numerous subunits are essentially formed in at least a few spermatids (MEYER, unpublished). This indicates that the genetic material of the loop forming sites possibly does not code for proteins or enzymes which are necessary for the differentiation of those structures.

Therefore, one is again led to the possibility (see the previous discussions on this point by BEERMANN 1965; HESS 1966b) that we are dealing with regulatory genes of a specific type: The loops might be structural devices for packaging and storing gene products which are synthesized during the spermatocyte stage but used only later during spermiogenesis. As the X and Y chromosomes segregate during the meiotic divisions, the spermatid nuclei are genetically incomplete. Moreover, spermatid nuclei are synthetically inactive (OLIVIERI and OLIVIERI 1965). Therefore, a mechanism for storage and stabilisation of gene products seems to be an absolute requirement in these cells. In addition, the effect of the Y chromosome material on sperm length could also be explained in this way: Males with two Y chromosomes have sperm of twice the normal length (in *D. hydei*, up to 13 mm (!) as compared to the normal length of 6.5 mm). Y deficient males always have immotile sperm which are shorter than normal sperm. Finally, males with partial Y duplications develop sperm of a length between those of males with one and two Y chromosomes with possible differences of the growth promoting capacity of different loops of the Y chromosome (HESS and MEYER 1963b; HESS 1967). If the loops in the Y chromosome are in fact involved in the storage of substances which are needed to develop the spermatozoa with their complicated structure, one would expect that an increase of the number of storage structures could result in an accumulation of a larger amount of morphogenetic substances. These, in turn, would increase the growth capacities of the spermatids. This is exactly what is found.

Thus, the study of the Y chromosomal loops may provide insight into presently unknown mechanisms by which higher organisms programme and regulate cell differentiation and growth processes.

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SUMMARY

The physiological significance of different regions of the Y chromosome of *D.*

hydei was studied by testing Y chromosomal fragments of different origin for complementation. Combinations of proximal and distal fragments were complementary with respect to morphogenetic effects in spermiogenesis. However, fertile males are recovered only in cases in which the combinations include a complete set of spermatocyte loops.—One of the loops, the tubular ribbons, is possibly complex in nature.—Males with a Y chromosome deficiency that includes only the pseudonucleolus have sperm of apparently normal shape. Single spermatozoa may even exhibit a weak motility. However, progeny have not yet been obtained from such males.—The results support the conclusion that the genetic material of the loops may be identical with the long known fertility factors in the Y chromosome. There is also evidence that the genetic material in the loop forming sites does not code for proteins or enzymes which are necessary for the formation of the major organelles of the differentiating spermatid. The physiological significance of the loops might be to function as structural devices for packaging and storing gene products.

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