A POSSIBLE CASE OF POSITION EFFECT ON DNA REPLICATION IN *DROSOPHILA MELANOGASTER**

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ABSTRACT

The behavior of *T(3;4)10* provides evidence that the centromere of 4 is not in the doublet 101D3,4, and that most of what has been called the left arm of 4 should be called basal 4R. Basal 4R usually does not replicate as much as the tip of 4R when 4 is in its normal position in the chromocenter. Translocated to the tip of 3R, however, basal 4R attains a width equal to that of the rest of the arm—a possible position effect on DNA replication.

THE short left arm of chromosome 4 of *Drosophila melanogaster* has long been an enigma. It is as rarely seen by cytologists in its usual position in the chromocenter of salivary gland nuclei as is the planet Mercury by casual observers of the sky near the sun. Rare descriptions of the left arm of four by cytologists studying the microchromosome concur that 4L is usually invisible. If glimpsed at all in cells of wild-type flies, 4L is much thinner than 4R (HOCHMAN 1971). SLIZYNSKI (1941) for example, was able to see 4L in only 20 out of several hundred salivary nuclei; in these cases 4L was only 1/3 the diameter of 4R. Because the width of a salivary chromosome is at least roughly proportional to its degree of polyteny, it appears that 4L does not ordinarily replicate as much as 4R.

We wish to describe some observations of a strain of *Drosophila* in which chromosome 4, including much of what has been called 4L, has been translocated to the tip of the right arm of chromosome 3. In this new location, far from the chromocenter, 4L may be seen in all cells and is now consistently as wide as 4R. This novel ability of 4L to replicate in pace with 4R appears to be due to a position effect of DNA replication.

MATERIALS AND METHODS

The translocations described below were recovered by X-raying with 4000 R Canton S males, mating them to cubitus interruptus females (*ci/ci*, chromosome 4), and examining the F_1 progeny for the rare flies that were of the *ci* rather than the wild phenotype owing to position effect at the *ci* locus. Flies showing the *ci* phenotype were crossed to marker stocks to determine whether a translocation was present and, if so, which chromosomes were involved. Stocks were made of the putative translocations and the salivary chromosomes were examined to determine the location of the breaks. (A more complete description of these procedures may be found in ROBERTS 1972).

Colchicine metaphases of larval ganglion cells were prepared using the technique of LEWIS

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and RILES (1960). Autoradiography with ^3H -thymidine followed, essentially, the technique of PLAUT, NASH and FANNING (1966).

RESULTS AND DISCUSSION

The position of the centromere in chromosome 4: The existence of an "infinitely small, microscopically invisible second arm in chromosome 4 of *Drosophila melanogaster* was postulated by PANSIN and KHVOSTOVA (1938) from the appearance and behavior of $T(1;4)w^{m1}$. They interpreted this rearrangement as having resulted from a break at the tip of the X chromosome near the location of w followed by attachment of this acentric piece to a fourth chromosome that included all of the cytologically visible right arm together with its centromere. They concluded that 4 was broken in 4L although this arm was not visible in their drawings save for a heterochromatic smudge, possibly the tip of 4L, capping the proximal part of the X.

A similar rearrangement that apparently connected the tip of the X to 4L, $T(1;4)w^{mA}$, was described in 1940 by GRIFFEN and STONE. They were able to discern about 12 bands in 4L (five of which appear rather heavy), and characterized 101D of the polytene map as including a "distinct centromere between the arms". The suggestion that the very end of the basal region of 4R has a ring structure that may be interpreted as a spindle fiber organ was first made by BRIDGES in 1935, according to SLIZYNSKI (1941). Thus the ring-like bands at 101D3,4 came to be regarded as the centromere of 4 and they are represented as the spindle fiber attachment on SLIZYNSKI's (1944) map of chromosome 4 that appears in LINDSLEY and GRELL (1968). SLIZYNSKI 1941 pointed out that if this ring-like doublet is, in fact, the centromere of 4, it is the only centromere that has been observed in polytene chromosomes of *Drosophila*.

The appearance and behavior of $T(3;4)10$ suggest that localization of the centromere of 4 to the ring doublet, 101D3,4, is incorrect. The evidence for this is as follows:

1. Examination of polytene chromosomes of the translocation heterozygote reveals a simple reciprocal translocation. Chromosome 4, however, has been broken considerably to the left of 101D. The supposed centromere of 4 is now clearly visible in polytene chromosomes (Figure 1) near the tip of the translocated 3R that is capped by 4. If the original centromere of 3 has not been deleted by additional breaks, the translocated 3 should now be a dicentric. However, the translocation stock has been transferred for at least 70 generations; if $T(3;4)10$ is a dicentric, it is a stable dicentric! The other element of the translocation consists of the extreme tip of 3R that was broken off and replaced by 4. The only bands visible in this fragment are those missing from the other element of the translocation (100C-F, Figure 1 B). Because the translocation has been carried for so long with no tendency to lose the fragment, 100C-F is probably not an acentric fragment. Instead, the tip of 3R appears to have attached to a centromere with no detectable additional bands. The fragment tends to be found in the chromocenter (Figure 1 B) owing to the presence of a small amount of heterochromatin proximally, in all probability, the centromere of 4 together with the

small, microscopically invisible left arm of 4 and a very short segment of 4R.

2. $T(3;4)10$ shows no genetic evidence of involvement of regions other than the tip of 3R and the base of 4. Only the marker *e* on chromosome 3 behaves as if linked to *ey* on chromosome 4. There are no unusual pairing tendencies of polytene or mitotic chromosomes (see below) that would indicate involvement of regions other than the base of 4 and the tip of 3R in the translocation.

3. Bands 101D3,4, the supposed location of the centromere of 4 have also been translocated to the tips of other chromosomes recovered as rearrangements producing *ci* position effects. These translocations behave no more like unstable dicentrics than does $T(3;4)10$. The polytene configuration of one of these, $T(2;4)14$ has been illustrated in a previous report (ROBERTS 1972—Figure 3); another, $T(3;4)83$, is shown in Figure 1C. $T(3;4)83$ carries a puff in a region of chromosome 4 that ordinarily does not puff, the only rearrangement of some 90 cases of *ci* position effect in the wing of *Drosophila* in which there was a pronounced change in the activity of the *ci* region observed in the polytene 4 of the salivary glands (ROBERTS 1969). This region includes bands 101D3,4 which form much of the dark material visible when the puff is at its maximum (Figure 1C). When the puff has regressed, bands 101D3,4 usually fail to coalesce completely (Figure 1D); they may, judging from their increased width and dark staining, have undergone extra replications associated with puffing, as in the *Sciaridae* (PAVAN and DA CUNHA 1969), but we have not attempted to measure it.

4. Colchicine metaphases of chromosomes of ganglion cells from larvae heterozygous for $T(3;4)10$ show that the original centromere in the middle of chromosome 3 has *not* been deleted (Figure 2A-3⁴). The translocated chromosome 3 can be distinguished from its normal homologue by a consistent tendency for the tips of the chromatids of one arm to adhere, due, presumably, to the presence of the heterochromatin of the translocated 4. Many cells have been examined but in no case has a second centric constriction been seen at the tip of 3R at metaphase. The other element of the translocation, the tip of 3R (100C-F) attached to what is probably the centromere of 4, appears as a small dot, roughly the size of the normal 4.

Mitotic metaphase views of the translocation are, therefore, consistent with its polytene appearance: sections A-D of division 101 as well as division 102 of chromosome 4 are translocated to 100B, a point near the tip of 3R yet do not carry the centromere of 4 to this distal position; the other element of the translocation, the extreme tip of 3R (100C-F) is attached, in all probability, to a heterochromatic fragment consisting of the centromere, left arm and a small portion of the right arm of 4.

The appearance of 101A-D of the SLIZYNSKI 1944 map is heterochromatic and it appears to form the basal part of the *right* arm rather than the left arm of 4. Therefore, we shall, hereafter, refer to 101A-D as the basal heterochromatin of 4R rather than as 4L.

Differences in extent of replication of basal 4R: At least three different degrees of polyteny of basal 4R have been observed in the past 35 years. Ordinarily, when the chromosome is in its usual location in the centromeric regions of sali-

FIGURE 1.—A. *T(3;4)10* heterozygote lightly stained. Normal (3) and translocated third chromosomes are somatically paired proximal to the last heavy band in 100B. The translocated third chromosome (3^4) was broken distal to bands 100B4,5 and attached to a fourth chromosome that was broken considerably to the left of bands in 101D, the supposed location of the centromere. Note that the part of chromosome 4 that is mapped is as wide as the rest of chromosome 4 in A but not even visible in B. (The label, 102B, is at 102B1-2 of the standard map.) B. *T(3;4)10*

vary nuclei the basal part of 4R is not visible as anything but a wisp of heterochromatin, indistinguishable from the rest of the chromocenter (Figure 1B). In a very small percentage of nuclei examined, SLIZYNSKI (1941) has been able to find a thin "4L", looking somewhat like a vermiform appendix to the remainder of 4R three times its width. In nuclei of the translocation described and illustrated here, $T(3;4)10$, basal 4R is consistently as wide, and, in all probability, as polytene as the rest of 4R (Figure 1A, 2C).

Further study of the other translocation stocks discussed in the previous section might have provided additional clues to the behavior of this chromosome but unfortunately, these have passed away. No trace of the region in question is visible in drawings of $T(1;4)w^{m11}$ (PANSIN and KHVOSTOVA 1938) but GRIFFEN and STONE (1940) report that "the presence of the X fragment on this arm tends to pull 4L clear of the chromocenter and greatly facilitates its study." We know that centromeric heterochromatin of polytene chromosomes ordinarily does not replicate along with the euchromatin (RUDKIN 1965). This accounts for the lowest degree of replication of basal 4R when it is indistinguishable from the rest of the centromeric heterochromatin. It is possible that the rare cases of partial polytenization of basal 4R in wild-type flies depend on its lying free of the chromocenter to some extent and that the region was more visible to GRIFFEN and STONE (1940) because partial polytenization was more frequent in the translocation heterozygote. At any rate, there is no doubt that when basal 4R is translocated to the tip of 3R, a third level of polytenization, equal to that of the rest of the arm, is consistently attained.

Examination of the band pattern of proximal 4R is greatly facilitated by its new position. Unfortunately, homozygotes for $T(3;4)10$ do not survive, so the detail that may be photographed is limited by the narrowness of the single translocated chromosome. The number of bands (12) in basal 4R of $T(3;4)10$, (Figures 1A, 2C) is roughly the number described by GRIFFEN and STONE (1940) though not so many as are drawn in SLIZYNSKI's map. Bands of basal 4R are, for the most part, more darkly staining than the rest of the arm; for purposes of photography or mapping, the chromosomes must be lightly stained or basal 4R becomes a densely staining, heterochromatic blob. The most proximal bands visible in $T(3;4)10$ are a darkly stained doublet perhaps corresponding to 101 A in SLIZYNSKI's map. It would be imprudent to suppose that chromosome 4 was broken immediately distal to its centromere and translocated to the tip of 3R in

heterozygote. The normal 4 is in its usual position in this heterochromatic chromocenter. The basal section of 4 diagrammed in A is not visible proximal to 102B except, perhaps, as a thin strand that cannot be distinguished from the rest of the heterochromatin. The difference in polyteny between the region proximal to 101D in the translocated 4 (A-3⁺) and proximal to 102B in the normal 4 is attributed to position effect. The other element of the translocation, the tip of 3R (100C-F) attached, probably, to the centromere of 4, is also visible (4⁸). C. A different translocation, $T(3;4)83$, broken at 97D in 3R and to the left of 101D in 4 has a large puff that includes bands in 101D, the supposed location of the centromere of 4. D. After collapse of the puff in $T(3;4)83$, the width and density of staining of bands in 101D (compare with A) suggest that they may have undergone extra replications associated with puffing as in Sciarid flies. The appearance and behavior of 101D are not that expected of a centromere.

FIGURE 2.—A. Colchicine metaphase from ganglion cell of heterozygote for $T(3;4)10$. The longer metacentric third chromosomes may be readily distinguished from the metacentric second chromosomes and from the group of microchromosomes and sex chromosomes (possibly XYX in this case) at the top. The tips of the chromatids in the longer right arm of 3 that consistently adhere in one element probably do so because of translocated heterochromatin of 4 (3^4). Note that the centromere is still present in the middle of element 3^4 of the translocation as in 3. The

T(3;4)10. However, it is probable that most of 4R has been mapped and that 4 is acrocentric rather than submetacentric.

How can we account for these considerable variations in the extent of replication of basal 4R in salivary gland cells? As mentioned above, heterochromatic regions of polytene chromosomes apparently do not replicate as much as euchromatic (RUDKIN 1965). However, like euchromatin, heterochromatin transposed to a new position usually keeps the temporal pattern of replication that it had in its normal position (VALENCIA and PLAUT 1970). This is consistent with the observation of HUBERMANN and RIGGS (1968) that the chromosomes of higher organisms are composed of many tandemly joined replicating sections. Autoradiography of late 3rd instar salivary glands reveals that basal 4R in its distal position in *T(3;4)10* still incorporates ^3H -thymidine in synchrony with the centromeric heterochromatin, at a time when incorporation of label has ceased in distal 4R (Figure 2B). Although the degree of replication in basal 4R has increased in *T(3;4)10*, the timing of replication is, at least to some extent, retained.

Perhaps the simplest explanation for position effects on replication of basal 4R is a "neighborhood effect": too little of some metabolite required for regular DNA replication in centromeric heterochromatin or possibly too high a concentration of some inhibitor of replication in the chromocenter. But then, one must attempt to explain why, in most rearrangements, heterochromatin that is translocated or inverted into a distal position fails to replicate to the extent that basal 4R does in *T(3;4)10*. One remote possibility is that there is a controlling element for replication of most of 101 A-D that has been altered by position effect or by mutation at the time of chromosome breakage and translocation. Whatever the explanation, it is noteworthy that two of the properties of heterochromatin, underreplication and heavy staining are separable in *T(3;4)10*: basal 4R becomes more polytene while retaining its dense staining.

The regular replication of basal 4R but not other heterochromatic regions when removed from the chromocenter may be a consequence of its history. Genes homologous to those on chromosome 4 of *D. melanogaster* may be found attached to the X chromosome of *Drosophila busckii*, a species that lacks a microchromosome (KRIVSHENKO 1959). Basal 4R, then, may have been regularly polytene as part of another chromosome in somatic cells of an ancestral species. No genes have, as yet, been localized to basal 4R (HOCHMAN 1971) but *T(3;4)10* may permit a consistent return to the polytene condition of a heterochromatic region now relatively devoid of transcribed genes.

other short element of the translocation is not distinguishable from 4 in the group of chromosomes at the top but it may be seen in Figure 1B. Either 3* is a stable dicentric or the centromere of 4 is not 101D, as has been supposed (see text). B. Autoradiograph of *T(3;4)10* with ^3H thymidine. Incorporation at this stage is primarily over the centromeric heterochromatin. Label is found in basal 4R at a time when DNA synthesis in the rest of 4 has stopped. C. *T(3;4)10* heterozygote lightly stained and stretched to show bands in 101D and rest of basal 4R more clearly than in Figure 1A.

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