Note

Healing of Euchromatic Chromosome Breaks by Efficient *de novo* Telomere Addition in *Drosophila melanogaster*

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ABSTRACT

Previously, we observed that heterochromatic 4 and Y chromosomes that had experienced breakage in the male germline were frequently transmitted to progeny. Their behavior suggested that they carried functional telomeres. Here we show that efficient healing by *de novo* telomere addition is not unique to heterochromatic breaks.

WHEN H. J. Muller observed that terminal deficiencies could not be recovered following mutagenesis with ionizing radiation, he was led to conclude that a special organelle existed at the end of a linear eukaryotic chromosome, which he called a telomere (MULLER 1940). However, it is now clear that *Drosophila* chromosomes with terminal deficiencies, or terminally deficient (TD) chromosomes, can be recovered in at least four circumstances: (1) when *mu2* females are irradiated (MASON *et al.* 1984) and, to a lesser degree, after irradiation of *mu2*⁺ females, although not after irradiation of *mu2* males (MASON *et al.* 1997); (2) when *mu2*⁺ males carrying the *Dp(1;f)1187* mini-chromosomes are irradiated (LE *et al.* 1995); (3) when transposition of a *P* element located near a chromosome end is induced (LEVIS 1989; ZHANG and SPRADLING 1993); and (4) after breakage of a dicentric chromosome (AHMAD and GOLIC 1998).

Dicentric breakage produces TD chromosomes at a much higher rate than the other mechanisms. In the *mu-2* and *P* transposase experiments, terminal deficiencies were recovered on the X or the major autosomes at rates of ~0.5% (MASON *et al.* 1984; LEVIS 1989) or up to ~3% in the case of terminal deficiencies induced on a mini-chromosome (TOWER *et al.* 1993). However, when site-specific recombination was used to produce dicentric chromosomes, which then break during division (Figure 1), TD chromosomes 4 or Y were transmitted through the male germline at rates of up to ~50% (AHMAD and GOLIC 1998). In fact, we now find that if

the combination of *nanos-Gal4* and *UAS-FLP* transgenes is used to express the FLP recombinase in the germline of a male with a Y chromosome carrying inverted FLP recombination targets (*FRTs*), then TD chromosomes constitute 100% of the Y chromosomes transmitted by such a male (Table 1A). Because the Y chromosome is entirely heterochromatic and chromosome 4 has characteristics of heterochromatin dispersed throughout its length, these results raise the question of whether new chromosome termini are more readily formed on heterochromatin of Y and 4 than on the euchromatin of major autosomes, which possibly accounts for the differences in TD chromosome recovery rates between our experiments and those from other labs.

To determine whether the production of TD chromosomes can occur at a high rate on chromosomes that are not largely or entirely heterochromatic, we generated chromosomes 3 carrying inverted *FRTs* near the left or right ends (Figure 2). One of these dicentric inducible chromosomes, *Dc3(FrTr61A5)1A*, carries a single copy of the *P{FrTr}* element located at 61A5. The *P{FrTr}* element carries a *white*⁺ gene (*w*^{hs}) adjacent to its inverted *FRTs*. The other chromosome, *Dc3(FrTr100E3)1A*, carries a single copy of *P{FrTr}* and a remnant of the *P{RS5}* element 5-HA-1852 with a single *FRT*; therefore, this chromosome carries three *FRTs*: two from the *P{FrTr}* element and one from the *P{RS5}* remnant such that *FRTs* flank *w*^{hs} on this chromosome (Figure 2C). We employed the *nanos-GAL4 UAS-FLP* combination to express FLP recombinase and to generate TD chromosomes.

The *white*⁺ gene is distal to the inverted *FRTs* on the *Dc3(FrTr61A5)1A* insertion and will be located on the acentric fragment formed after site-specific recombina-

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FIGURE 1.—FLP-induced dicentric bridge formation and breakage in anaphase (TITEN and GOLIC 2008).

tion; therefore, TD chromosomes may be recognized by the gene's loss. Males that carried this chromosome marked by *Stubble* (*Sb*) and *nanos-Gal4 UASFLP* transmitted *Sb* chromosomes that had lost w^+ at a rate of 21.7% (Table 1B). In control crosses without *UASFLP*, no w *Sb* progeny were recovered (data not shown).

We used an *eyFLP* test to determine whether these chromosomes still carried inverted *FRTs*. Flies carrying *eyFLP* (NEWSOME *et al.* 2000) and a chromosome with adjacent inverted *FRTs*, including the *Dc3(FrTr61A5)1A* and *Dc3(FrTr100E3)1A* chromosomes, produce dicentric chromosomes in the cells of the developing eye-antennal disc, resulting in a reduced rate of eclosion (because many die as pharate adults) and adults with small rough eyes (data not shown). This is likely a consequence of the cell death that follows dicentric chromosome breakage (AHMAD and GOLIC 1999; TITEN and GOLIC 2008). When we tested 49 of the w *Sb* putative TD chromosomes with *eyFLP*, all progeny had eyes with a wild-type phenotype, confirming that they no longer carried inverted *FRTs*.

Polytene chromosome analyses further confirmed that these w *Sb* chromosomes were terminally deficient. Fifteen putative TD chromosomes isolated from *Dc3(FrTr61A5)1A* were examined and found to have varying degrees of terminal chromatin loss (data not

shown). We conclude that the loss of w^+ and the absence of inverted *FRTs* is indicative of a TD, and therefore the frequency of fragment transmission for the *Dc3(FrTr61A5)1A* *Sb* chromosome is 21.7%.

Scoring for loss of w^+ is not a reliable way to determine TD chromosome transmission with the *Dc3(FrTr100E3)1A* *Sb* chromosome because the w^+ gene on this chromosome is flanked by directly repeated *FRTs* and w^+ may be lost without terminal loss. In fact, 100% of progeny from this experiment were white owing to these two means of w^+ loss (data not shown). To determine how many of these flies carried TD chromosomes, we crossed 11 lines that had lost w^+ to *eyFLP* females and found that four produced adults of the informative genotype with normal eyes, five produced adults with small rough eyes, and two produced no adults of the informative genotype. This suggests that approximately one-third of *Dc3(FrTr100E3)1A* lines (4/11) that have lost w^+ carry TD chromosomes.

As an alternative measure of TD formation, we crossed *Dc3(FrTr100E3)1A* *Sb* males expressing FLP in their germlines directly to *eyFLP* females. In this experiment, 67% of the *Sb* progeny had normal eyes (Table 1C). The apparent increased frequency of progeny lacking inverted *FRTs* (67% *vs.* 36% in the previous experiment) is likely, in part, a result of the lethality associated with

TABLE 1
Frequency of TD chromosome transmission

A. <i>yw/y+ · DcY(H1)B^s; UAS-FLP/+; nos-GAL4/+</i> ♂ × <i>yw</i> ♀											
♂			♀	<i>n</i>	TD frequency						
<i>B^s</i>	+										
0	1116		1385	30	1.00						
B. <i>yw; UAS-FLP/+; Dc3(FrTr61A5)1A Sb/nos-GAL4</i> ♂ × <i>yw</i> ♀											
♂				♀							
<i>Sb</i>				<i>Sb</i>							
+	<i>w</i>		+	+	<i>w</i>	+	<i>n</i>	TD frequency			
381	105		543	393	110	488	30	0.217			
C. <i>yw; UASFLP/+; Sb Dc3(FrTr100E3)1A/nos-GAL4</i> ♂ × <i>w1118; ey-FLP/Tm6(Ubx)</i> ♀											
♂					♀						
<i>Sb/+</i>					<i>Sb/+</i>						
Wild-type eyes	Small rough eyes	+/ <i>Ubx</i>	<i>Sb/Ubx</i>	+	Wild-type eyes	Small rough eyes	+/ <i>Ubx</i>	<i>Sb/Ubx</i>	+	<i>n</i>	TD frequency
95	67	214	140	246	140	51	213	142	287	31	0.666

Three dicentric inducible chromosomes were used to analyze the transmission frequency of TD chromosomes through the male germline. TD chromosomes are identified by loss of a distal marker: (A) B^s from $DcY(H1)$; (B) w^+ from $Dc3L$; or (C) by lack of a dicentric inducible small rough-eye phenotype when crossed to a $P\{ey4x-FLP.Exel\}1$ located on chromosome 3.

inverted-*FRT*-bearing chromosomes in the presence of *eyFLP*. Polytene chromosome analysis of six independent lines isolated from this experiment showed that all carried abnormal tips indicative of a TD (Figure 3, A, C, E, and G).

One of the TD lines on 3R carried an inverted terminal duplication (Figure 3G). Breakage of the dicentric bridge at a noncentral location should produce one short fragment and one long fragment with just such an inverted terminal duplication. However, only 1 of the 21 TD chromosomes examined in polytenes was, on visible inspection, a long fragment. The unequal recovery of long and short fragments may indicate that long fragments are associated with reduced viability, or it may be due to the fact that long fragments are subject to further cycles of bridge and breakage by virtue of the retention of inverted *FRT*s. However, this partic-

ular long fragment appears not to carry inverted *FRT*s, as judged by the lack of an abnormal eye phenotype when crossed to *eyFLP*. Such a long fragment could arise if a cell with a short broken chromosome arrives in G_2 of the cell cycle without prior end-healing and the replicated broken ends can then fuse (see TITEN and GOLIC 2008).

Flies with the TD chromosomes appear normal, apart from somewhat reduced viability that may be attributable to aneuploidy. They show none of the abnormalities that are produced when dicentric formation is induced during normal development (GOLIC 1994). This is strong evidence that the TD chromosomes have been healed by *de novo* telomere addition. To confirm that the TD chromosomes have telomeres, we examined five of the 3L terminal deficiencies and six of the 3R deficiencies for the presence of HOAP, a vital component of functional telomeres (CENCI *et al.* 2003). All had

or the tip of 3R. The $DcY(H1)$ chromosome was made by mobilizing the $P\{iw\}$ element using transposase and then by screening for linkage to the $B^s Y^+$ chromosome.

FIGURE 2.—Schematic of dicentric inducible chromosomes. The $Dc3(FrTr)$ chromosomes were produced from two $P\{RS\}$ insertion lines obtained from the DrosDel collection (RYDER *et al.* 2004), $CB-0127-03$ located at $61A5$ and $5-HA-1852$ located at $100E3$. First, we used FLP recombinase to remove part of the *white* gene, rendering the gene nonfunctional. The method of *P*-element substitution (reviewed by ASHBURNER *et al.* 2005) was then used to move $P\{FrTr\}$, which carries a functional *white*⁺ gene adjacent to inverted *FRT*s, from the second chromosome to the site of the $P\{RS\}$ remnant at the tip of 3L

FIGURE 3.—Analysis of chromosomes 3 with terminal deficiencies. (A, C, E, and G) Orcein-stained polytene chromosomes from four different lines deficient for the tip of $3R$ distal to the $P\{FrTr\}$ insertion. (B, D, F, and H) The localization of the HOAP protein to the new tip of TD chromosomes.

HOAP at the new chromosome terminus, including the long-fragment chromosome (Figure 3, B, D, F, and H; chromosome $3L$ TD results not shown).

We conclude that euchromatic sequences are compatible with efficient healing by *de novo* telomere addition and that our earlier results were not unique simply because they occurred on heterochromatic chromosomes.

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