Telomere Loss in Somatic Cells of Drosophila Causes Cell Cycle Arrest and Apoptosis

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ABSTRACT

Checkpoint mechanisms that respond to DNA damage in the mitotic cell cycle are necessary to maintain the fidelity of chromosome transmission. These mechanisms must be able to distinguish the normal telomeres of linear chromosomes from double-strand break damage. However, on several occasions, Drosophila chromosomes that lack their normal telomeric DNA have been recovered, raising the issue of whether Drosophila is able to distinguish telomeric termini from nontelomeric breaks. We used site-specific recombination on a dispensable chromosome to induce the formation of a dicentric chromosome and an acentric, telomere-bearing, chromosome fragment in somatic cells of *Drosophila melanogaster*. The acentric fragment is lost when cells divide and the dicentric breaks, transmitting a chromosome that has lost a telomere to each daughter cell. In the eye imaginal disc, cells with a newly broken chromosome initially experience mitotic arrest and then undergo apoptosis when cells are induced to divide as the eye differentiates. Therefore, Drosophila cells can detect and respond to a single broken chromosome. It follows that transmissible chromosomes lacking normal telomeric DNA nonetheless must possess functional telomeres. We conclude that Drosophila telomeres can be established and maintained by a mechanism that does not rely on the terminal DNA sequence.

DOUBLE-STRAND breaks in chromosomes are a common and particularly dangerous form of DNA damage. If a cell with a chromosome break divides, chromosome fragments will be lost from daughter cells, producing aneuploidy that is detrimental for viability and differentiation. Checkpoint mechanisms prevent this by sensing DNA damage and blocking cell cycle progression to allow time for DNA repair. The operation of checkpoints that respond to DNA damage in the mitotic cell cycle was first demonstrated in *Saccharomyces cerevisiae* (Weinert and Hartwell 1988). Irradiated yeast cells arrest at specific stages in the cell cycle. Some mutations with a heightened sensitivity to irradiation abolish the arrest response of cells, thereby identifying gene products that mediate checkpoint responses (Weinert and Hartwell 1988; Weinert *et al.* 1994). The *RAD9* gene is required for cells to arrest in the gap phases of the cell cycle after irradiation (Weinert and Hartwell 1988; Siede *et al.* 1993). Irradiated *rad9* mutant cells do not arrest, but undergo mitosis and die, presumably because the progeny cells lose essential genes when acentric fragments fail to segregate. The G_2 checkpoint defined by the *RAD9* gene is exquisitely sensitive to double-strand DNA damage: a single break will cause arrest of wild-type cells (Bennett *et al.* 1993; Sandell and Zakian 1993). Because of this sensitivity, it is critical that the cell does not see the ends of linear chromosomes as breaks. One of the functions of the telomere is to prevent cells from recognizing normal chromosome termini as double-strand breaks.

The mechanisms of detecting DNA damage and controlling the cell cycle may be conserved between many organisms. A family of genes with checkpoint-related functions has been uncovered in Saccharomyces, Schizosaccharomyces, Drosophila, and human cells (Greenwell *et al.* 1995; Hari *et al.* 1995; Hartley *et al.* 1995; Morrow *et al.* 1995; Paulovich and Hartwell 1995; Savitsky *et al.* 1995). The induction of DNA damage in mammalian cells results in a G_1 cell cycle arrest or in apoptosis. While arrest may allow time to repair damage, cell death is also an effective method to control damage in a multicelled organism: in many cases other undamaged cells can proliferate and replace those that were lost.

To analyze the response to chromosome breakage in a metazoan, we developed a method for the controlled generation of a single broken chromosome end in cells of Drosophila. The FLP site-specific recombinase was used to catalyze the formation of a dicentric chromosome and an acentric fragment. The acentric fragment does not segregate at mitosis and is lost, while the dicentric breaks, and the two daughter cells each receive a chromosome with a newly broken end (Ahmad and Golic 1998). These events can be induced at a very high rate—up to ~90% of cells exhibit dicentric chromosomes. When dicentric formation is induced during

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development, the resulting adults exhibit striking morphological defects. In our initial experiments, loss of the acentric fragment resulted in considerable aneuploidy, and we were not able to determine whether these morphological defects were a result of aneuploidy or of chromosome breakage (Golic 1994). To separate the two possible causes, we constructed chromosomes that are dispensable for viability and can be induced to form dicentric chromosomes. We now show that some of the morphological defects result from a specific response to chromosome breakage, and not from aneuploidy. We examined cells of the eye imaginal disc and found that the mitotic cycle is arrested after dicentric chromosomes are formed. This arrest can last several days, but when the eye begins to differentiate, arrested cells undergo programmed cell death. We further show that this response can be genetically manipulated and is subject to cell cycle control. Thus, in Drosophila, we have identified the existence of a mechanism that can detect and respond to a single broken chromosome end.

MATERIALS AND METHODS

Mutations and chromosomes not discussed here are described by Lindsley and Zimm (1992). The *mei41*^{D1} and *mei41*^{195} alleles were obtained from R. S. Hawley. All flies were raised at 25° on standard cornmeal medium.

***P*-element lines:** The FLP construct *P*[*ry*$^{+}$, *70FLP*] is a heat-inducible *FLP* gene (Golic *et al.* 1997). *70FLP*3A is a line with this construct inserted on chromosome *2*. The construction and transformation of the *P* elements *P*[>*w*^{hs}>] and *P*[*RS5*] have been described (Golic and Lindquist 1989; Golic and Golic 1996). *P*[>*w*^{hs}>] carries two *FRT*s in direct relative orientation flanking a *w*^{hs} selectable marker. *P*[*RS5*] carries two *FRT*s in direct relative orientation and a *w*^{hs} selectable marker. One *FRT* is in the first intron of *w*^{hs} and the second is downstream of the gene.

To produce aneuploid cells after mitotic recombination, we used allelic insertions of *P*[*RS5*] at 54A in flies heterozygous for the translocation *T(2;3)bw*^{v5} and a normal order chromosome (Beumer *et al.* 1998).

The insertion line *P*[*GMR-p35*]3 was obtained from B. Hay. The insertion line *P*[*GMR-p21*]B was obtained from I. Hariharan.

Lines that form dicentric chromosomes: The *Dc* designation is used to indicate that a chromosome carries inverted *FRT*-bearing elements and will form a dicentric chromosome upon FLP induction. *DcY* is a *Y* chromosome derivative that carries inverted copies of *P*[*RS5*] insertions in the *Dp(4;Y)*E portion on *YL* (Ahmad and Golic 1998). *DcX* is an *X* chromosome with inverted copies of *P*[>*w*^{hs}>]105 at 8F (Golic 1994). We also constructed a derivative of the *DcY* chromosome in which the *YS* arm is marked with *y*$^{+}$ (Ahmad 1997).

Heat-shock regimens: FLP expression was induced at various times in development by heat shock. Heat shocks were performed in a circulating water bath as described by Golic and Lindquist (1989).

X-irradiation of larvae: Second instar larvae were collected, placed in an open plastic petri dish, and irradiated with either 2 or 4 kRads in a Torrex 120D X-ray machine.

Examination of imaginal discs: The size of dissected larval eye discs was measured dorsoventrally across the widest part of the disc. Genotypes were compared using a randomization test (performed by the P-stat program provided by W. R. Engels). For detecting apoptosis, larvae were dissected in 5 μg/ml acridine orange (AO; Sigma, St. Louis) in PBS and imaginal discs were mounted in PBS on a ProbeOn Plus slide (Fisher Scientific, Pittsburgh, PA). Staining was observed by differential interference contrast (DIC) and epifluorescence with UV excitation on a Zeiss (Thornwood, NY) Axioplan microscope, using either 10× or 20× Plan-NEOFLUAR objectives and a G365, FT395, LP420 filter set. Discs from at least 10 larvae were examined for each of the genotypes discussed in the text.

Examination of adults: Wings were dissected and mounted in Gary's Magic Mountant (Lawrence *et al.* 1986). Adult heads were prepared for scanning electron microscopy as described by Ashburner (1989), except that 4% paraformaldehyde was used as a fixative.

RESULTS

Dicentric forming chromosomes: In this work we used two chromosomes that can form dicentric and acentric chromosomes by FLP-mediated site-specific recombination (Golic 1994; Ahmad and Golic 1998). *DcX* is a normal *X* chromosome that carries inverted *FRT*s inserted at 8F. *DcY* consists of a *Y* chromosome with a duplication of chromosome *4* appended to the end of the long arm. The chromosome *4* segment carries inverted *FRT*s. *DcY* is entirely dispensable in somatic cells.

When FLP catalyzes unequal sister-chromatid exchange between inverted *FRT*s on *DcX* or *DcY*, the sister chromatids fuse (Figure 1). A dicentric chromosome bridge is formed when the two centromeres segregate to opposite poles at the next mitotic division. Dicentric formation is very efficient with both *DcX* and *DcY*. Cytological examination showed that dicentrics were formed in almost 90% of cells with *DcX* (Golic 1994) and 70% of cells with *DcY* (Ahmad and Golic 1998) after a 1-hr heat shock was used to induce FLP expression from a heat-inducible *FLP* transgene (*70FLP*). In the germlines of *DcY*-bearing males, the frequency of dicentric formation and bridge breakage approaches 90% (Ahmad and Golic 1998).

Phenotypic consequences of dicentric chromosome formation: When the *70FLP* gene is induced to cause *DcX* to form a dicentric during the larval or early pupal stages, a characteristic set of developmental defects is seen in the adults that eclose. These include roughened eyes with fused ommatidial facets, scalloped wing margins, etched abdominal tergites, and missing and Minute bristles (Golic 1994). Occasionally, flies that had been heat shocked early in development (2 days after egg laying) also had small eyes that were somewhat disorganized. None of these defects were seen when offspring lacking the *70FLP* gene or *DcX* were heat shocked and therefore are not caused simply by heat shock.

To see whether these defects could be caused solely by chromosome breakage, we induced FLP synthesis during development in flies that carried *DcY*. We ob-

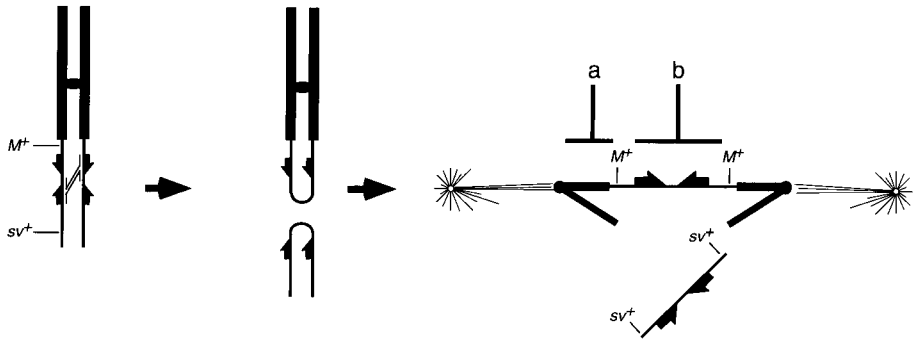

Figure 1.—FLP-mediated dicentric chromosome formation and breakage on *DcY*. The dicentric is formed by unequal sister-chromatid exchange between *FRT*s, which are then stretched between the poles of division. The acentric fragment (carrying sv^+) is lost, while the chromosome bridge breaks. Breakage may occur either (a) to one side, producing a fragment chromosome that lacks M^+, or (b) between the two M^+ genes, producing two fragment chromosomes each carrying M^+ (see text for details). The *FRT*s are indicated as half-arrows, the circles represent the centromeres, heterochromatic *Y* segments are thick lines, and chromosome *4* segments are thin lines.

served developmental defects in the eye and in the wings (Figure 2) similar to those observed with *DcX*. The defects observed with *DcY* were most frequent in flies that had been heat shocked 2 days after egg laying—80% of flies showed eye defects ($N = 44$). In these crosses, occasional *DcY*-bearing females were produced by nondisjunction (Bridges 1916). These females showed the same eye and wing margin defects that we observed in males with *DcY*, indicating that the defects are not specific to males. We conclude that the cause for the eye and wing defects can be traced specifically to the presence of the single broken chromosome end (see "The fate of dicentric chromosomes in somatic cells," below).

No other defects were observed when dicentrics were formed with *DcY*. Thus, the additional aspects of the phenotype produced by dicentric formation with *DcX* may be traceable to aneuploidy. To test this idea we generated aneuploid cells by inducing mitotic recombination in translocation heterozygotes. Some of the cells produced in this experiment will be deficient for a large portion of a chromosome. We observed severely Minute bristles, an expected consequence of aneuploidy. However, we did not see roughened eyes or notched wings, confirming that the cause for these phenotypes can be separated from aneuploidy.

The cellular consequences of dicentric chromosomes: We then wished to identify the cellular response to chromosome breakage that led to the visible defects in adult pattern formation. We first asked whether the growth of eye discs was inhibited after dicentric formation. Larvae that carried *70FLP* were heat shocked in second instar and the size of eye discs of *X/X* and *X/DcY* larvae were compared 2 days later. The eye discs from *X/X* larvae grew to an average width of 284 μm ± 11 during this time, but eye discs from *X/DcY* larvae were smaller, with an average width of 193 μm ± 9 ($P = 0.001$) (Figure 3). This provides an explanation for the small eyes of adults after dicentric formation: they were small because cells did not proliferate.

It seemed likely that broken chromosomes either caused cell cycle arrest or apoptosis. To distinguish these possibilities we incubated imaginal discs with an acridine orange (AO) solution to visualize apoptotic cell death in the eye disc. Apoptotic cells fluoresce brightly by staining with AO (Wolff and Ready 1991b). The eye discs of normal second instar larvae show essentially no AO staining. We examined eye discs from second instar larvae 4, 10, and 24 hr after heat shock was used to induce *DcY* dicentric formation. We observed no increase in staining of second instar discs after induction of dicentric chromosome formation (Figure 3). We confirmed that cell death can be detected by AO in early eye discs by irradiating cells to induce cell death. Larvae were irradiated with 2–4 kRad of X rays, and their eye discs were dissected and stained with AO. X-ray-induced apoptosis in early discs did result in punctate staining (Figure 4). Therefore, the growth inhibition of eye discs from *DcY*-bearing larvae results from mitotic arrest or delay, not cell death.

We also used AO staining to follow the fate of cells in eye discs after dicentric chromosome formation was induced in first instar. No cell death was observed until third instar: at this stage we observed a significant increase in the degree of cell death relative to eye discs from w^{1118} larvae (not shown). In third instar, apoptosis occurred in a specific double-striped pattern. The AO staining observed at this time most likely represents the death of cells that have been in a state of mitotic arrest since shortly after the heat shock in first instar. Thus, there is a delay of several days between the time of dicentric formation and cell death.

The eye discs from larvae heat shocked in first instar are quite small. To obtain a more precise picture of the pattern of cell death after dicentric chromosome formation, we examined eye discs from *DcY*-bearing larvae after FLP synthesis had been induced in late third instar. At this stage of development substantial cell death was observed in every eye disc from all larvae that made dicentric chromosomes. AO staining was first observed ~9 hr after heat shock, when it appeared in the same double-striped pattern (Figure 5c). In third instar a wave of differentiation proceeds from posterior to anterior, accompanied by a visible apical indentation termed the morphogenetic furrow (Ready *et al.* 1976). Cells in front of the furrow are undifferentiated; cells posterior to the furrow are recruited into ommatidial clusters and begin

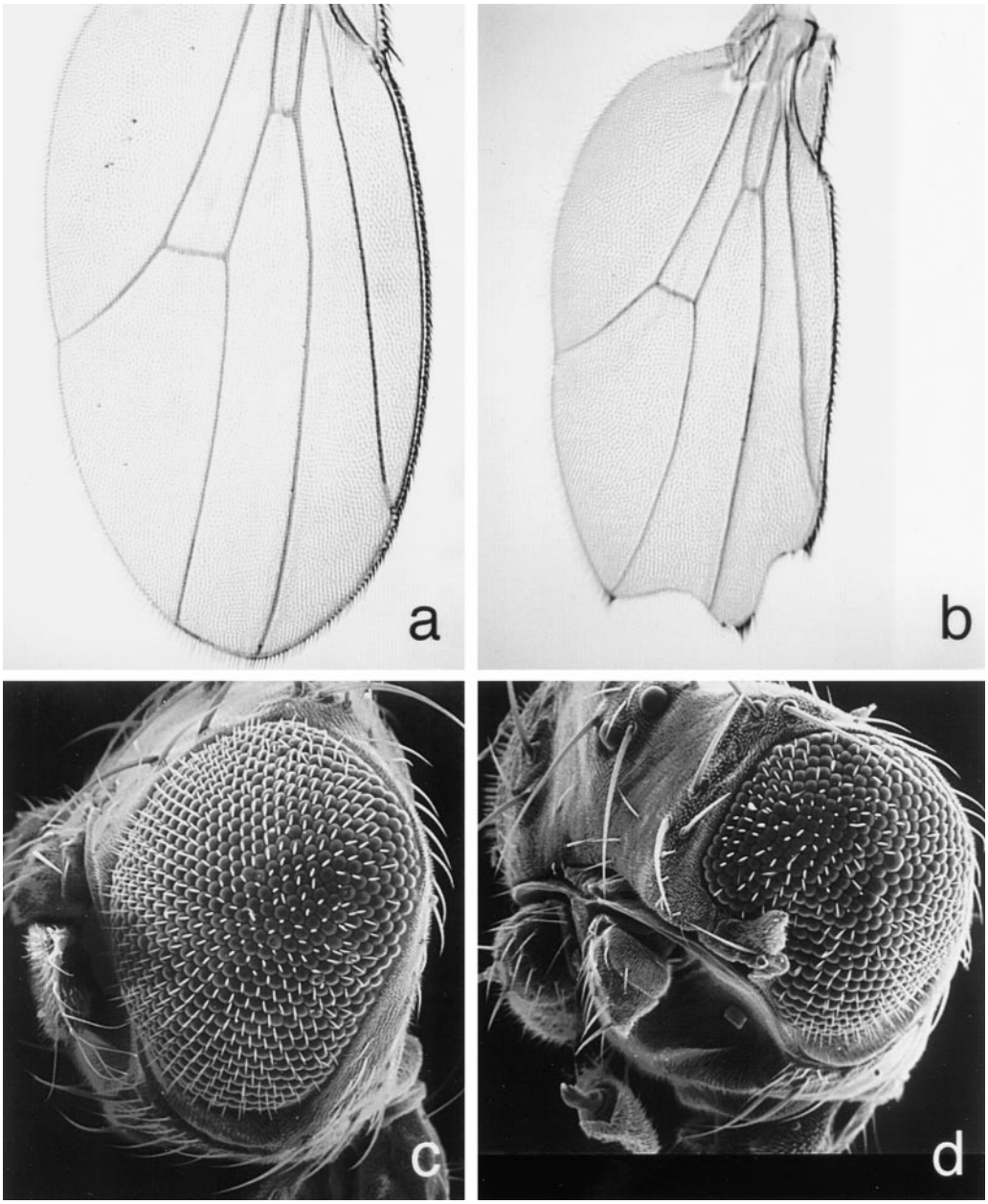

Figure 2.—Morphological defects after *DcY* dicentric formation. (a and b) Wings and (c and d) eyes of *X*/*DcY; 70FLP*3A/+ males. (a and c) no heat shock (no HS); (b) HS 6 days after egg laying (AEL); (d) HS 4 days AEL.

to differentiate (Wolff and Ready 1991a). In these discs it was clear that one stripe of apoptosis was immediately in front of the morphogenetic furrow, and the second was spaced a short distance behind the furrow. Thus, the pattern of cell death caused by chromosome breakage is correlated to the pattern of differentiation. We observed similar patterns of cell death in eye discs from *DcX*-bearing larvae after dicentric induction (not shown). There was little cell death at this stage in the controls that lacked either *70FLP* or *DcY*.

The 9-hr delay before the appearance of AO-staining cells is not limited to eye discs. After dicentric formation, we also observed a large increase in the number of cells undergoing cell death in wing and leg discs (Figure 5, h and j). From 8 to 9 hr elapsed between the time of heat shock and the appearance of apoptotic cells in these discs. This delay is not caused by a lag in the production of the broken chromosome end. Dicentric chromosomes are rapidly formed after heat shock, and cell division is not significantly inhibited by the heat shocks used in these experiments (our unpublished data and Richardson *et al.* 1995). Therefore, mitosis with dicentric bridges begins shortly after the induction of FLP. Because 8–10 hr is sufficient time for one cell cycle in imaginal disc cells (Postlethwait 1978; Wolff and Ready 1991a), it appears that cells begin to die around the time of their second mitosis after dicentric induction.

The observed delay in staining is not simply a result of a delay in uptake of AO. AO staining occurs rapidly when cells commit to apoptosis and is visible within 1 hr (Abrams *et al.* 1993). We confirmed this by staining irradiated discs with AO (not shown). X-ray-induced apoptosis in the disc was detectable 1 hr after irradiation.

Cell death is not the exclusive fate of cells with a

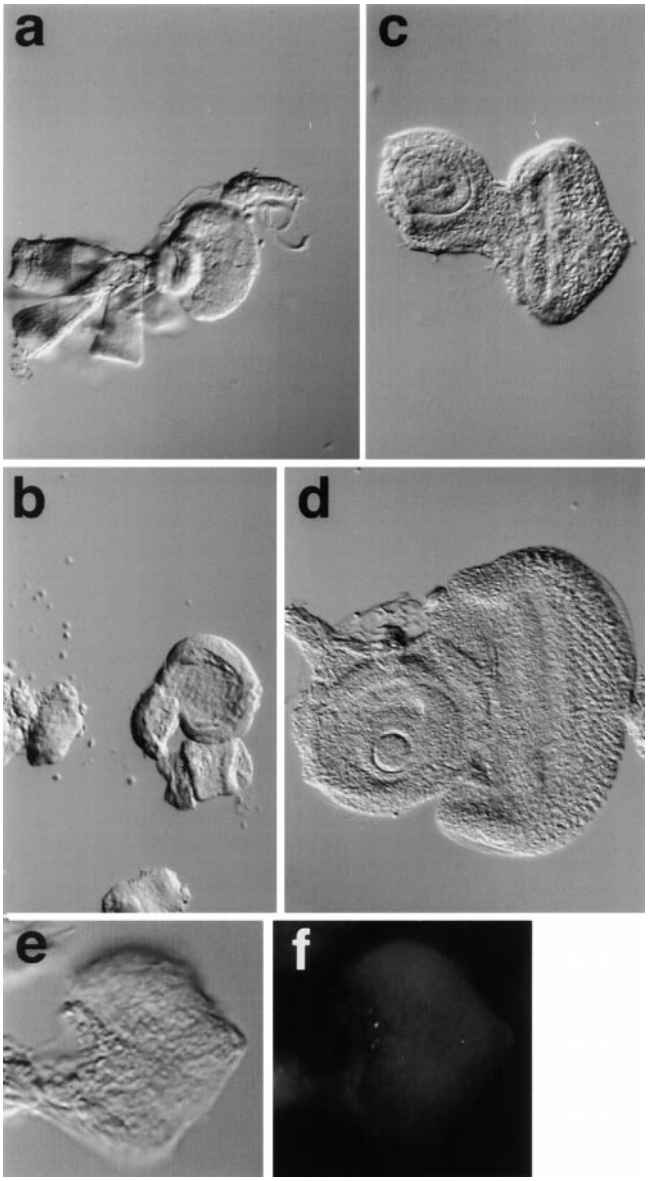

Figure 3.—Growth and apoptosis in the early eye imaginal disc. All discs are arranged with the anterior to the left. (a) w^{1118}/*DcY*; *70FLP*3A/+ L2 larva; (b) w^{1118}; 70FLP3A/+ L2 larva; (c) w^{1118}/*DcY*; *70FLP*3A/+ L3 larva 44 hr after HS; (d) w^{1118}; 70FLP3A/+ L3 44 hr after HS; (e and f) eye disc from a w^{1118}/*DcY*; *70FLP*3A/+ L2 larva 24 hr after HS, stained with AO to detect apoptosis. (a–e) DIC images; (f) epifluorescence. All images are shown at the same magnification.

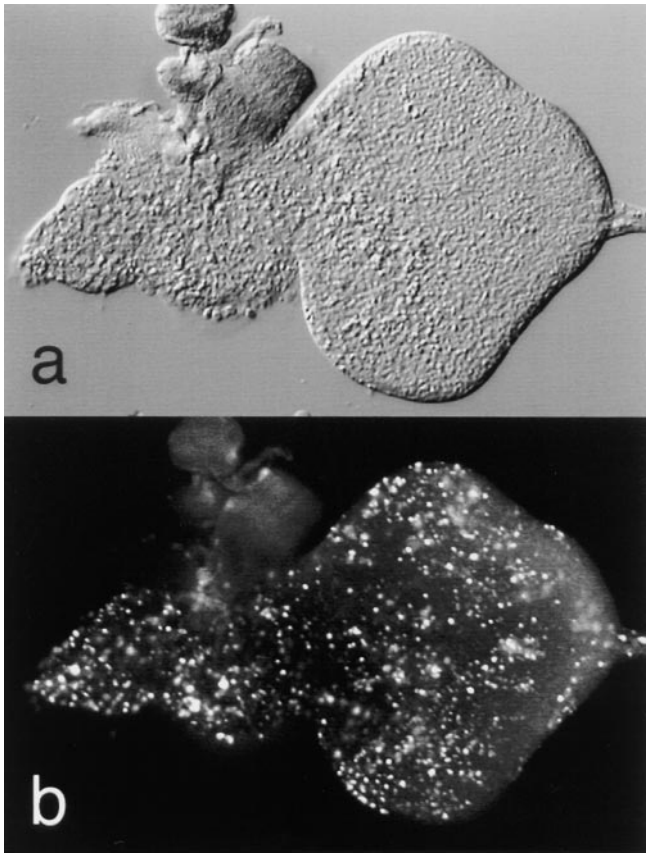

Figure 4.—Apoptosis after X-irradiation. w^{1118}/*Y* L2 larvae were dissected 24 hr after X-irradiation and the eye discs were stained with AO to visualize apoptosis. (a) DIC image of eye disc; (b) epifluorescence image.

broken chromosome. Although AO staining reveals the fate of the bulk of cells with a broken chromosome, it is also clear that some cells do survive. Cells that have experienced dicentric chromosome formation can differentiate as adult wing hairs or bristles (Golic 1994). These may be cells that experienced mitotic arrest, but did not die and were still able to differentiate, or may be cells that escape mitotic arrest and cell death.

To test whether any cells with newly broken chromosomes are capable of further division, we induced FLP early in larval development and examined the adults for bristles formed by cells with broken chromosomes. The progenitor of each adult bristle is a sensory organ precursor (SOP) cell that becomes determined during the third instar larval stage. Adult bristles are derived from an SOP by two mitotic divisions, with the earliest first division occurring 8 hr before pupariation (Hartenstein and Posakony 1989). The two daughter cells divide again during pupal development to give the four cells that form a single bristle complex. If a dicentric chromosome is formed in a cell early in development, and if its two daughter cells do not divide, it should not be possible for those cells to produce a bristle. The *DcY* chromosome carries the dominant wild-type allele of the *shaven* gene (sv^+) distal to the site of *FRT*s. Flies that are homozygous sv^n/sv^n on chromosome *4* have very small and fine bristles. In such flies, loss of the sv^+ allele of *DcY* will produce bristles with the shaven phenotype.

Shaven$^+$ and shaven macrochaetae on the dorsal thorax can be unambiguously distinguished, and we examined these bristles after *DcY* dicentric formation had been induced at different times in development. A shaven bristle might arise from cells that divided only once after dicentric formation if the dicentric was formed immediately prior to final mitotic division of the cells. However, by inducing *70FLP* long before this

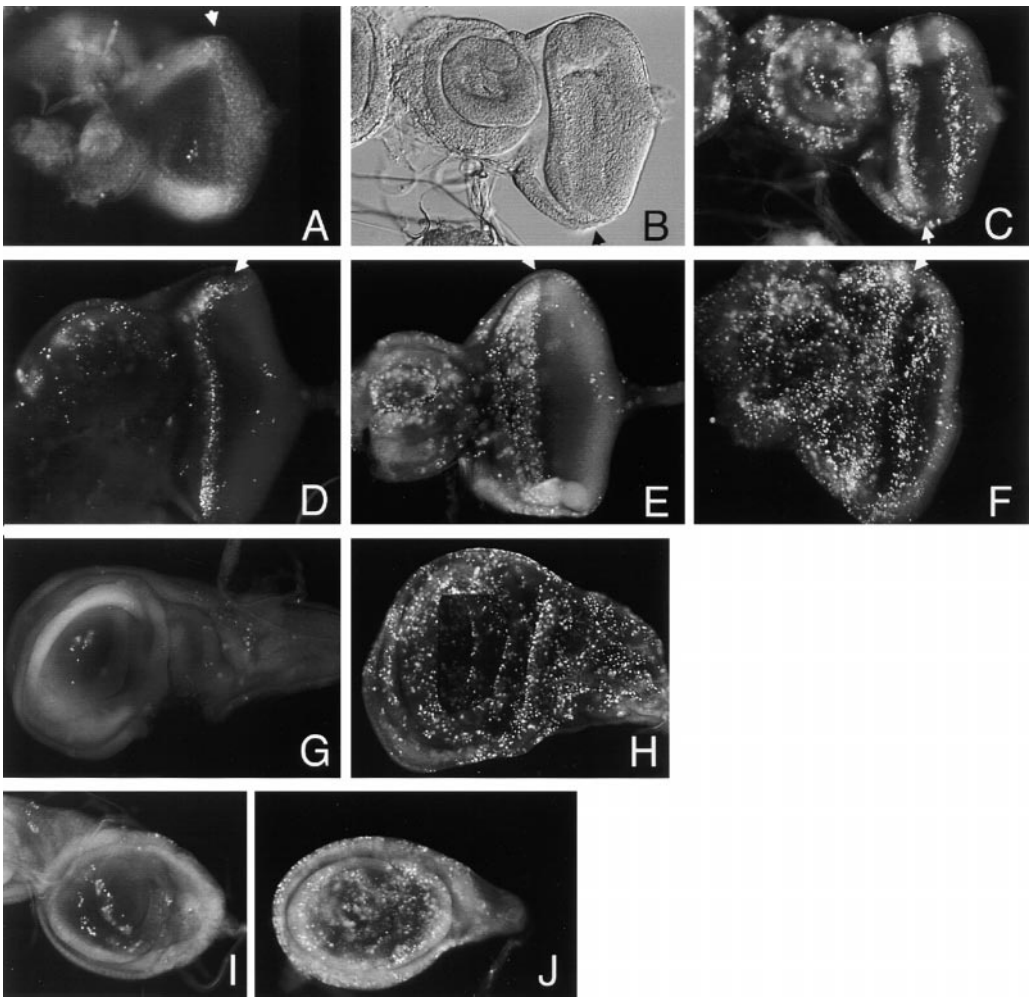

Figure 5.—Apoptosis in L3 imaginal discs. All males carried *DcY* and were heterozygous for *70FLP*3A. Except as specified, all larvae were heat shocked (HS'ed) 12–14 hr before dissection. Discs were stained with AO to visualize apoptosis. (a–f) Eye discs: (a) no HS control; (b) DIC image of eye disc from HS'ed larva; (c) epifluoresence image of same disc as in (b); (d) AO-stained disc from HS'ed larva that also carried *P*[*GMR-p35*]; (3) AO-stained disc from HS'ed larva that also carried *P*[*GMR-p21*]; (f) AO-stained disc from HS'ed larva that also carried $mei\text{-}41^{D1}$ on his *X* chromosome ($mei41^{195}$ was also tested and gave similar results); (g) wing disc, no HS control; (h) wing disc from HS'ed larva; (i) leg disc, no HS control; (j) leg disc from HS'ed larva. Arrowheads indicate the positions of the morphogenetic furrow in eye discs.

stage, we expect that dicentric formation will occur before the first mitotic division of determined SOP cells. FLP appears to be relatively unstable in Drosophila (Margolis and Spradling 1995), making it unlikely that it will persist long enough to produce a dicentric chromosome days after *70FLP* is induced.

When larvae with *DcY* and *70FLP* were heat shocked in the first 4 days after egg laying (AEL), most adults had shaven bristles. Heat shocking once on all 4 days resulted in all adults showing shaven bristles. These bristles are almost certainly produced by cells that divided more than once with a broken chromosome. Large clones were not observed after single heat shocks 1 or 2 days AEL, suggesting that, while cells with a broken chromosome can sometimes divide more than once, they proliferate poorly. All bristles of the no-heat-shock control flies were $shaven^{+}$.

Genetic modification of the cellular response to chromosome breakage: The baculovirus p35 protein is a potent inhibitor of apoptosis (Clem *et al.* 1991). Developmentally programmed and X-ray-induced apoptotic cell deaths can be suppressed by the *P*[*GMR-p35*] construct, which expresses p35 in the cells posterior to the morphogenetic furrow (Hay *et al.* 1994). When we generated dicentrics in flies that carried this construct, the posterior stripe of cell death was abolished in all discs examined (Figure 5d), and the pattern disruption in adult eyes caused by dicentric formation was also partly alleviated. The anterior stripe of cell death also seemed to be slightly reduced, possibly as a result of some expression from *P*[*GMR-p35*] anterior to the morphogenetic furrow (Hay *et al.* 1994). The p35 protein inhibits the cysteine proteases required for apoptosis (Xue and Horvitz 1995). The observation that it suppresses cell death that occurs after dicentric chromosome formation supports a conclusion that a broken chromosome end is not intrinsically toxic. Instead, the broken end acts as a signal whose ultimate consequence, at least in eye, wing, and leg discs, is cell death.

Our results also show that the response to a broken chromosome end depends on the cell's developmental context. The pattern of apoptosis is tightly linked to the patten of development in the eye. In particular, the two stripes of cell death coincide with the locations of the final mitotic divisions in the eye. As the eye differentiates, cells just ahead of the furrow are induced to undergo mitosis by extracellular signals. It is likely that the mitotic wave behind the furrow also occurs in response

to extrinsic developmental signals (Wolff and Ready 1991a; Heberlein *et al.* 1995).

We can conceive of two alternative explanations for the observed cell death. First, cells may die because they fail to differentiate. Perhaps a mitotically arrested cell is unable to respond to developmental cues that would normally determine its fate. Its failure to differentiate may provoke apoptosis. Alternatively, death may result if cells receive an extrinsic mitogenic signal that conflicts with a checkpoint arrest (Clarke *et al.* 1993), or if the extrinsic mitogen overrides the intrinsic arrest, but mitosis with a broken chromosome is lethal. If apoptosis only occurs in cells that are mitotically active, it should be possible to eliminate that death by altering the cell's response to a mitogenic signal. The human p21 protein is an inhibitor of the S-phase-specific cyclin-dependent kinase (Gu *et al.* 1993; Xiong *et al.* 1993). Larvae that carry a *P*[*GMR-p21*] construct express p21 in all cells posterior to the morphogenetic furrow, and this prevents the second mitotic wave (deNooij and Hariharan 1995). We found that *P*[*GMR-p21*] also abolished the posterior stripe of cell death associated with *DcY* dicentric formation in the eye discs of all larvae examined ($N = 19$; Figure 5e). This argues that dicentric-associated cell death requires resumption of the cell cycle in an arrested cell and is not simply the result of developmental failure.

The effect of *mei-41*: Because *DcY* carries no essential genes for somatic development, the developmental defects produced by dicentric formation with *DcY* can be attributed solely to the cellular response to dicentric breakage. If this response could be eliminated, the cells with the broken chromosomes should continue to divide and differentiate normally, and the developmental defects should be ameliorated. In *S. cerevisiae*, some mutations that confer sensitivity to mutagens identify checkpoint genes. By analogy, some mutagen-sensitive mutations in Drosophila may also identify genes required for responses to DNA damage and its repair (Boyd *et al.* 1983). Hari *et al.* (1995) proposed that the *mei-41* gene acts in a checkpoint that responds to induced DNA damage, because mitosis is not suppressed after X-irradiation of *mei-41* mutant larvae. Therefore, it seemed possible that *mei-41* mutations might reduce the severity of the developmental defects produced by *DcY* dicentric formation. We tested two alleles of *mei-41* for such an effect. The hemizygous *mei-41* adults exhibited wing scalloping and small roughened eyes. Cell death in the eye disc also occurred in the hemizygous *mei-41* background (Figure 5f) and was qualitatively similar to that seen in *mei-41*$^+$ animals. If *mei-41* is involved in the response to chromosomes that are broken as a result of dicentric formation, its role in the apoptotic response appears to be minor.

The fate of dicentric chromosomes in somatic cells: We previously showed that *DcY* dicentrics do break in the mitotic divisions of the male germline (Ahmad and Golic 1998). We have assumed that dicentric chromosomes also break in somatic mitoses, but we wished to demonstrate this directly. If breakage of a dicentric *DcY* does occur in the soma, then it should be detectable by loss of the wild-type alleles that are carried by the chromosome *4* duplication of *DcY*. We used the *M(4)101*$^+$ gene on *DcY* to determine whether dicentric bridges break in somatic mitoses. This gene lies proximal to the site of *FRT* insertion where sister-chromatid fusion occurs; thus, loss of the acentric fragment is not sufficient to cause loss of this gene. However, if a dicentric bridge breaks proximal to *M(4)101*$^+$ in mitosis, it will produce a daughter cell that lacks this marker (Figure 1). Such cells can be detected by Minute bristles when the flies are also heterozygous for *Df(4)M101-1* on the regular fourth chromosomes. The majority of such flies did show one or more Minute thoracic bristle when *70FLP*4A was induced during larval development.

These Minute bristles could be produced by chromosome breakage or by complete chromosome loss. To distinguish these possibilities we constructed a derivative of *DcY* that carries y^+ as a marker on the tip of the short arm (*DcYy*$^+$). In flies that carry the y^1 mutation, cells that lose the entire *DcYy*$^+$ chromosome can be detected by their yellow phenotype. We examined the thoracic and abdominal bristles of *y w*/*DcYy*$^+$; *70FLP*3A/+ males in which FLP synthesis had been induced 1, 3, or 5 days after egg laying (at least 20 males from each induction). These males showed many of the phenotypes that typify dicentric chromosome formation, but no yellow bristles were observed. We conclude that chromosome loss does not occur, or is very rare, following dicentric chromosome formation with *DcYy*$^+$. Because we saw no evidence of dicentric chromosome loss, the Minute bristles that appeared in the prior experiment must be produced by cells that carry broken fragments of *DcY*. It is therefore apparent that dicentric chromosomes do break in somatic mitosis.

We cannot absolutely exclude the possibility that some dicentric bridges stretch at anaphase but do not break, and this may contribute to the phenotypes that dicentric chromosomes produce. However, this would require that identical dicentric bridges experience different fates in different mitoses within the same tissue. In addition, breakage is the predominant if not sole fate of dicentric bridges in the mitotic divisions of male germline stem cells (Ahmad and Golic 1998). We consider it most likely that dicentric bridges break in all, or nearly all, mitoses.

DISCUSSION

The response to chromosome breakage: In mammals, irradiation causes cell cycle arrest and apoptosis. These responses are largely dependent on the *p53* tumor suppressor gene, implicating *p53* in the control of a checkpoint response to irradiation (Kuerbitz *et al.* 1992;

Clarke *et al.* 1993; Lowe *et al.* 1993). In contrast, some observations in Drosophila have suggested that radiation-induced responses result from detrimental effects of aneuploidy and not a cell cycle checkpoint, raising the possibility that Drosophila relies on aneuploid effects to eliminate cells with damaged chromosomes. Ripoll (1980) has demonstrated that aneuploidy can reduce cell growth and viability. Haynie and Bryant (1977) observed clones where cells had divided only once or twice after X-irradiation, and they suggested that these cells became aneuploid after division with a broken chromosome. Fryxell and Kumar (1993) reported that cells in irradiated eye imaginal discs died only after division. In both these cases, division implies that these cells were not subject to a DNA damage checkpoint. In addition, animals with broken chromosomes have been recovered after irradiation of *mu2* mutant females, after the expression of *P* transposase, and after dicentric chromosome breakage. These chromosomes evidently did not stimulate cell cycle arrest or apoptosis (Mason *et al.* 1984; Levis 1989; Ahmad and Golic 1998).

On the other hand, there is substantial evidence that DNA damage checkpoints do exist in the Drosophila cell cycle. Baker *et al.* (1987) have argued that the absence of aneuploid metaphase figures in mutants that regularly produce chromosome breakage indicates that cells with broken chromosomes never proceed through mitosis. Hari *et al.* (1995) demonstrated that *mei-41*$^{+}$ is required for cell cycle arrest immediately after X-irradiation. The *mei-41* gene shares homology with the *ATM* family of genes, implicating it in a checkpoint control that may act similarly to that of mammalian cells. Finally, Fogarty *et al.* (1994) provided evidence of a checkpoint response to radiation-induced DNA damage in early Drosophila embryos.

Our results demonstrate that Drosophila cells do detect and respond to a broken chromosome, entirely apart from aneuploidy. We previously showed that dicentric chromosomes break during mitotic division in the male germline, and chromosome fragments are recovered (Ahmad and Golic 1998). Here, we show that dicentric bridges formed in somatic divisions with the *DcY* chromosome also break. Because the *DcY* chromosome is not required for growth or viability, the developmental defects that are produced must be triggered by that double-strand break, and not by aneuploidy. We conclude that a single newly broken chromosome end is sufficient to activate a cell cycle checkpoint. The ultimate cellular consequence of that checkpoint varies according to the developmental context of the cell.

Developmental and genetic modulation of a DNA damage-induced checkpoint: In our experiments, the consequences of checkpoint activation depend on the developmental state of cells. Mitotic arrest occurs in early eye imaginal discs, but in late discs apoptosis occurs. We suggest that the primary consequence of the checkpoint is a cell cycle arrest, as seen in early discs. Apoptosis in the late eye disc coincides with the two waves of mitoses that are cued by extrinsic signals around the morphogenetic furrow. We showed that these deaths were suppressed by expression of the cell cycle inhibitor p21. This is consistent with the notion that apoptosis occurs because an intrinsic checkpoint arrest is incompatible with extrinsically instructed mitotic progression in these cells. The eye imaginal disc appears to present us with a tissue in which the two consequences of triggering the chromosome breakage checkpoint can be readily distinguished, because only cell cycles in the late eye disc are subject to precise extrinsic control. In the leg and wing discs we also observed apoptosis after dicentric chromosome formation. These cell deaths may indicate locations in those discs where mitoses are driven by external signals.

The consequences of checkpoint activation in other tissues may differ according to how cell division is regulated. Tissues may also differ with respect to their ability to accommodate and compensate for cell death. The morphological defects that were produced by dicentric breakage may be limited to certain tissues because of such differences.

Mitotic arrest and apoptosis also occur after the induction of DNA damage in mammalian cells, with variation observed in different cell types and environmental conditions. One model for this choice considers that p53 always induces a cell cycle arrest, but in some cells this conflicts with signals to divide and the conflict provokes apoptosis (Bates and Vousden 1996). Expression of p21 in mammalian cells also inhibits the cell cycle and suppresses apoptosis of irradiated cells (Wang and Walsh 1996). Our results from Drosophila coincide very closely with results from mammalian systems, suggesting that the response to chromosome damage is conserved across metazoans.

Dicentric breakage as a model for telomere loss: When FLP catalyzes the formation of dicentric and acentric chromosomes, the two sister telomeres are both found on the acentric molecule. These acentric chromosomes do not segregate at mitosis (Golic 1994; Williams *et al.* 1998) and the telomeres are lost. The dicentric breaks, exposing a single nontelomeric end in each of the two daughter cells (Ahmad and Golic 1998; results presented in this article). Because of the very specific nature of the damage and the high frequency with which it can be generated, dicentric breakage is highly suited as a model for studying the cellular response to telomere loss. In contrast, X-irradiation damages cells in a variety of ways. X rays cause double-strand breakage and each break generates two broken ends in a single cell. X rays also generate single-strand nicks in DNA, as well as damage to nongenetic components of the cell. Our results show that somatic cells respond differently to X-irradiation than they do to breakage of a dicentric chromosome. A single broken chromosome end triggers apoptosis only during the developmentally induced

mitoses in the differentiating eye disc, but after X-irradiation apoptosis occurs in both early and late eye discs. The amount and variety of damage produced by X rays, at least in the doses typically used, might be sufficiently overwhelming that apoptosis is the inevitable and immediate result in most cells.

This hypothesis provides a possible explanation for the failure of *mei-41* alleles to suppress cellular effects of dicentric breakage. The *mei-41* product is required for cell cycle arrest after X-irradiation but may not be involved in the response to a single broken chromosome. Perhaps other types of damage, or a minimal threshold of damage, are needed to stimulate the *mei-41* checkpoint. Alternatively, *mei-41* may function specifically in cell cycle arrest, but not in apoptosis. In support of this latter possibility, X-ray-induced apoptosis in the eye disc is also unaffected by mutation of the *mei-41* gene, although cell cycle arrest in this tissue requires it (M. Brodsky and G. M. Rubin, personal communication). In mammals, a homolog of *mei-41*, *ATM*, is not required for p53-dependent apoptosis and is only partially required for complete p53-dependent cell arrest, implying that there are other mechanisms that couple DNA damage to p53 activity (Barlow *et al.* 1997). In Drosophila, the mechanisms that mediate the cellular responses to a nontelomeric chromosome end may be similarly partitioned.

Telomere establishment and maintenance: Drosophila telomeres do not possess the simple-sequence repeats that constitute a telomere in most other organisms, but instead carry retrotransposon sequences. Chromosome length is maintained by occasional transpositions to chromosome ends (Levis 1989). Because chromosome ends are steadily eroded by incomplete replication, new sequences are continually exposed at the ends of Drosophila chromosomes. It follows that no specific sequence need be at the very end of the chromosome to confer telomere function. This view is reinforced by the findings that it is possible to recover flies that carry chromosomes with an end that completely lacks any vestige of the sequences normally found at telomeres (Mason *et al.* 1984; Levis 1989; Karpen and Spradling 1992; Laurenti *et al.* 1995; Ahmad and Golic 1998). It is difficult to escape the conclusion that telomere function is not encoded by DNA sequence and may be entirely attributable to proteins bound to the chromosome end (Biessmann *et al.* 1990).

In this work we provide the first demonstration that Drosophila cells are capable of recognizing and responding to a single newly broken chromosome: a freshly generated nontelomeric end causes cell cycle arrest and apoptosis in somatic cells of Drosophila. Thus, in the cases where stable chromosomes that lack normal telomeric DNA at an end have been recovered, those chromosomes must possess at least that portion of telomere function that prevents a cell from recognizing the end of a chromosome as a double-strand break. We conclude that Drosophila telomeres can be established *de novo*, by a non-sequence-dependent mechanism, and that once established they are maintained without particular regard for the sequence at the end of that chromosome. This is not to say that all sequences are equally susceptible to *de novo* telomere establishment—it is certainly possible that some sequences are incompatible with telomere function (Ahmad and Golic 1998). The parallel between the two major structural elements of Drosophila chromosomes is striking: other workers have proposed that the centromere is not encoded by a specific DNA sequence (Karpen and Allshire 1997; Williams *et al.* 1998), and it appears the same is true for telomeres.

Adaptation to a broken end: Our previous results showed that *DcY* can be induced to form a dicentric chromosome in 70–90% of cells. However, because cells with broken chromosomes produced very few clones, and no large clones, the proliferative potential of such cells must be greatly reduced. Mitotic arrest followed by apoptosis appears to be the predominant fate of cells with a broken chromosome, but it is not the exclusive fate. Occasionally, cells with a broken chromosome did divide at least once. These may be cells in which a checkpoint was not triggered by the broken chromosome. Alternatively, a checkpoint-mediated arrest may occur, but division eventually resumes. Such a cell may be subject to a checkpoint arrest again in the next division, leading to a severe limitation in growth potential. Similarly, in the yeast Saccharomyces, a broken chromosome triggers a G2/M checkpoint arrest that is not always permanent. Some cells eventually adapt to the presence of DNA damage and resume proliferation, a process that has been termed adaptation (Sandell and Zakian 1993; Toczyski *et al.* 1997).

Toczyski *et al.* (1997) suggested that adaptation is the result of DNA-damage response pathways becoming desensitized to a continually active checkpoint kinase, ultimately allowing cell cycle progression. However, in the Drosophila germline a broken end can gain a functional telomere, despite the lack of normally telomeric DNA. This process may also occur to some extent in the soma, allowing some cells with a broken chromosome to divide. Perhaps partial telomere function can also be established on newly broken ends in organisms that normally have simple-sequence DNA at their telomeres, providing cells an escape from checkpoint arrest. Drosophila may differ from these organisms in the frequency with which newly broken ends acquire telomere function, or in the stability of such an acquired telomere.

De novo telomere acquisition may be a normal event in the Drosophila germline, necessitated by Drosophila's use of retrotransposition to extend a chromosome end. While DNA damage produces two broken ends in a cell, retrotransposition uniquely generates only one new end. The number of newly exposed chromosome ends may be critical to distinguish damage from chromosome

end extension. This explanation could account for our previous demonstration that chromosomes broken after dicentric formation are readily recovered through the male germline (Ahmad and Golic 1998).

Adaptation to a checkpoint is a reasonable strategy for unicellular organisms such as Saccharomyces, where the propagation of each cell has utmost evolutionary importance. It is less obvious why this would be advantageous in a metazoan, where cells could be eliminated and then replaced by proliferation of neighboring cells. Adaptation may be necessitated by the syncytial development of germline cells. If a checkpoint eliminated one nucleus with damaged DNA, it might doom an entire syncytium. Thus, the cost of checkpoint-induced arrest or apoptosis may be significantly greater in the germline than in the soma. In this situation, adaptation could allow the other nuclei of a syncytium to survive, while the damaged nucleus will result in death of the zygote that receives it. Adaptation in somatic cells may be an unavoidable consequence of allowing adaptation in the germline.

Drosophila provides a model system that can bring powerful genetic approaches to bear in the examination of how cells respond to telomere loss. In human somatic cells, it is thought that the absence of telomerase activity and the resultant continual loss of telomeric sequences through rounds of replication plays a primary role in the senescence of such cells (Olovnikov 1996). This may provide a natural protection against the uncontrolled growth of cells. One method of defeating this control is by reactivating telomerase (Kim *et al.* 1994; Bodnar *et al.* 1998; Vaziri and Benchimol 1998). Another way of bypassing this control might be by the acquisition of a new telomere without regard to the underlying sequence, as we have suggested here. Such an event may serve to explain human tumor cells that do not show telomerase activity or telomere extension (Bryan *et al.* 1997). Identifying and understanding the mechanisms by which cells can escape this control on division is directly relevant to a complete understanding of the mechanisms of carcinogenesis.

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LITERATURE CITED

Abrams, J. M., K. White, L. I. Fessler and H. Steller, 1993 Programmed cell death during Drosophila embryogenesis. Development **117:** 29–43.

Ahmad, K., 1997 The tension is killing me: dicentric chromosomes in Drosophila. Ph.D. Thesis, University of Utah, Salt Lake City.

Ahmad, K., and K. G. Golic, 1998 The transmission of fragmented chromosomes in *Drosophila melanogaster*. Genetics **148:** 775–792.

Ashburner, M., 1989 *Drosophila: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Baker, B. S., A. T. C. Carpenter and M. Gatti, 1987 On the biological effects of mutants producing aneuploidy in Drosophila, pp. 273–296 in *Aneuploidy*, edited by B. K. Vig and A. A. Sandberg. Alan R. Liss, New York.

Barlow, C., K. D. Brown, C. X. Deng, A. A. Tagle and A. Wynshaw-Boris, 1997 Atm selectively regulates distinct p53-dependent cell-cycle checkpoint and apoptotic pathways. Nature Genetics **17:** 453–456.

Bates, S., and K. H. Vousden, 1996 p53 in signalling checkpoint arrest or apoptosis. Curr. Opin. Genet. Dev. **6:** 12–18.

Bennett, C. B., A. L. Lewis, K. K. Baldwin and M. A. Resnick, 1993 Lethality induced by a single site-specific double-strand break in a dispensable yeast plasmid. Proc. Natl. Acad. Sci. USA **90:** 5613–5617.

Beumer, K. J., S. Pimpinelli and K. G. Golic, 1998 Induced chromosomal exchange directs the segregation of recombinant chromatids in mitosis of Drosophila. Genetics **150:** 173–188.

Biessmann, H., S. B. Carter and J. M. Mason, 1990 Chromosome ends in Drosophila without telomeric DNA sequences. Proc. Natl. Acad. Sci. USA **87:** 1759–1761.

Bodnar, A. G., M. Ouellette, M. Frolkis, S. E. Holt, C.-P. Chiu *et al.*, 1998 Extension of life-span by introduction of telomerase into normal human cells. Science **279:** 349–352.

Boyd, J. B., P. V. Harris, J. M. Presley and M. Narachi, 1993 *Drosophila melanogaster*: a model eucaryote for the study of DNA repair, pp. 107–123 in *Cellular Responses to DNA Damage*, edited by E. C. Friedberg and B. A. Bridges. Alan R. Liss, New York.

Bridges, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1:** 1–54.

Bryan, T. M., A. Englezou, L. Dalla-Pozza, M. A. Dunham and R. R. Reddel, 1997 Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. Nature Medicine **3:** 1271–1274.

Clarke, A. R., C. A. Purdie, D. J. Harrison, R. G. Morris, C. C. Bird *et al.*, 1993 Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature **362:** 849–852.

Clem, R. J., M. Fecheimer and L. K. Miller, 1991 Prevention of apoptosis by a baculovirus gene during infection of insect cells. Science **254:** 1388–1390.

deNooij, J. C., and I. K. Hariharan, 1995 Uncoupling cell fate determination from patterned cell division in the Drosophila eye. Science **270:** 983–985.

Fogarty, P., R. F. Kalpin and W. Sullivan, 1994 The Drosophila maternal-effect mutation *grapes* causes a metaphase arrest at nuclear cycle 13. Development **120:** 2131–2142.

Fryxell, K. J., and J. P. Kumar, 1993 Characterization of the radiation-sensitive stage in the development of the compound eye of Drosophila. Mutation Research **285:** 181–189.

Golic, K. G., 1994 Local transposition of *P* elements in *Drosophila melanogaster* and recombination between duplicated elements using a site-specific recombinase. Genetics **137:** 551–563.

Golic, M. M., and K. G. Golic, 1996 A quantitative measure of the mitotic pairing of alleles in *Drosophila melanogaster* and the influence of structural heterozygosity. Genetics **143:** 385–400.

Golic, K. G., and S. Lindquist, 1989 The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. Cell **59:** 499–509.

Golic, M. M., Y. S. Rong, R. B. Petersen, S. L. Lindquist and K. G. Golic, 1997 FLP-mediated DNA mobilization to specific target sites in Drosophila chromosomes. Nucleic Acids Research **25:** 3665–3671.

Greenwell, P. W., S. L. Kronmal, S. E. Porter, J. Gassenhuber, B. Obermaier *et al.*, 1995 *TEL1*, a gene involved in controlling telomere length in *S. cerevisiae*, is homologous to the human ataxia telangiectasia gene. Cell **82:** 823–829.

Gu, Y., C. W. Turck and D. O. Morgan, 1993 Inhibition of CDK2 activity *in vivo* by an associated 20K regulatory subunit. Nature **366:** 707–720.

Hari, K. L., A. Santerre, J. J. Sekelsky, K. S. McKim, J. B. Boyd *et al.*, 1995 The *mei-41* gene of *D. melanogaster* is a structural homolog of the human ataxia telangiectasia gene. Cell **82:** 815–821.

Hartenstein, V., and J. W. Posakony, 1989 Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development **107:** 389–405.

Hartley, K. O., D. Gell, G. C. M. Smith, H. Zhang, N. Divecha *et al.*, 1995 DNA-dependent protein kinase catalytic subunit: a

relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. Cell **82:** 849–856.

Hay, B. A., T. Wolff and G. M. Rubin, 1994 Expression of baculovirus P35 prevents cell death in Drosophila. Development **120:** 2121–2129.

Haynie, J. L., and P. J. Bryant, 1977 The effects of X-rays on the proliferation dynamics of cells in the imaginal wing disc of *Drosophila melanogaster*. Wilhelm Roux's Arch. **183:** 85–100.

Heberlein, U., C. M. Singh, A. Y. Luk and T. J. Donohoe, 1995 Growth and differentiation in the Drosophila eye coordinated by *hedgehog*. Nature **373:** 709–711.

Karpen, G. H., and R. C. Allshire, 1997 The case for epigenetic effects on centromere function and identity. Trends Genet. **13:** 489–496.

Karpen, G. H., and A. C. Spradling, 1992 Analysis of subtelomeric heterochromatin in the Drosophila minichromosome *Dp1187* by single *P* element insertional mutagenesis. Genetics **132:** 737–753.

Kim, N. W., M. A. Piatyszek, K. R. Prowse, C. B. Harley, M. D. West *et al.*, 1994 Specific association of human telomerase activity with immortal cells and cancer. Science **266:** 2011–2015.

Kuerbitz, S. J., B. S. Plunkett, W. V. Walsh and M. B. Kastan, 1992 Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. USA **89:** 7491–7495.

Laurenti, P., Y. Graba, R. Rosset and J. Pradel, 1995 Genetic and molecular analysis of terminal deletions of chromosome *3R* of *Drosophila melanogaster*. Gene **154:** 177–181.

Lawrence, P. A., P. Johnston and G. Morata, 1986 Methods of marking cells, pp. 229–242 in *Drosophila: A Practical Approach*, edited by D. G. Roberts. IRL Press, Oxford.

Levis, R. W. 1989 Viable deletions of a telomere from a Drosophila chromosome. Cell **58:** 791–801.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila*. Academic Press, San Diego.

Lowe, S. W., E. M. Schmitt, S. W. Smith, B. A. Osborne and T. Jacks, 1993 p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature **362:** 347–349.

Margolis, J., and A. Spradling, 1995 Identification and behavior of epithelial stem cells in the Drosophila ovary. Development **121:** 3797–3807.

Mason, J. M., E. Strobel and M. M. Green, 1984 *mu-2*: Mutator gene in Drosophila that potentiates the induction of terminal deficiencies. Proc. Natl. Acad. Sci. USA **81:** 6090–6094.

Morrow, D. M., D. A. Tagle, Y. Shiloh, F. S. Collins and P. Hieter, 1995 *TEL1*, an *S. cerevisiae* homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene *MEC1*. Cell **82:** 831–840.

Olovnikov, A. M., 1996 Telomeres, telomerase, and aging: origin of the theory. Exp. Gerontol. **31:** 443–448.

Paulovich, A. G., and L. H. Hartwell, 1995 A checkpoint regulates the rate of progression through S phase in *S. cerevisiae* in response to DNA damage. Cell **82:** 841–847.

Postlethwait, J. H., 1978 Clonal analysis of Drosophila cuticular patterns, pp. 359–441 in *The Genetics and Biology of Drosophila*, edited by M. Ashburner and T. R. F. Wright. Academic Press, New York.

Ready, D. F., T. E. Hanson and S. Benzer, 1976 Development of the Drosophila retina, a neurocrystalline lattice. Dev. Biol. **53:** 217–240.

Richardson, H., L. V. O'Keefe, T. Marty and R. Saint, 1995 Ectopic cyclin E expression induces premature entry into S phase and disrupts pattern formation in the Drosophila eye imaginal disc. Development **121:** 3371–3379.

Ripoll, P., 1980 Effect of terminal aneuploidy on epidermal cell viability in *Drosophila melanogaster*. Genetics **94:** 135–152.

Sandell, L. L., and V. A. Zakian, 1993 Loss of a yeast telomere: arrest, recovery and chromosome loss. Cell **75:** 729–739.

Savitsky, K., A. Bar-Shira, S. Gilad, G. Rotman, Y. Ziv *et al.*, 1995 A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science **268:** 1749–1753.

Siede, W., A. S. Friedberg and E. C. Friedberg, 1993 *RAD9*-dependent G1 arrest defines a second checkpoint for damaged DNA in the cell cycle of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **90:** 7985–7989.

Toczyski, D. P., D. J. Galgoczy and L. H. Hartwell, 1997 *CDC5* and CKII control adaptation to the yeast DNA damage checkpoint. Cell **90:** 1097–1106.

Vaziri, H., and S. Benchimol, 1998 Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. Curr. Biol. **8:** 279–282.

Wang, J., and K. Walsh, 1996 Resistance to apoptosis conferred by Cdk inhibitors during myocyte differentiation. Science **273:** 359–361.

Weinert, T. A., and L. H. Hartwell, 1988 The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. Science **241:** 317–322.

Weinert, T. A., G. L. Kiser and L. H. Hartwell, 1994 Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev. **8:** 652–665.

Williams, B. C., T. D. Murphy, M. L. Goldberg and G. H. Karpen, 1998 Neocentromere activity of structurally acentric mini-chromosomes in Drosophila. Nature Genetics **18:** 30–37.

Wolff, T., and D. F. Ready, 1991a The beginning of pattern formation in the Drosophila compound eye: the morphogenetic furrow and the second mitotic wave. Development **113:** 841–850.

Wolff, T., and D. F. Ready, 1991b Cell death in normal and rough eye mutants of Drosophila. Development **113:** 825–839.

Xiong, Y., G. J. Hannon, H. Zhang, D. Casso, R. Kobayashi *et al.*, 1993 *p21* is a universal inhibitor of cyclin kinases. Nature **366:** 701–704.

Xue, D., and H. R. Horvitz, 1995 Inhibition of the Caenorhabditis elegans cell-death protease CED-3 by a CED-3 cleavage site in baculovirus p35 protein. Nature **377:** 248–251.

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