Differential Induction of Altered Gene Expression by Carcinogens at Mutant Alleles of a Drosophila Locus with a Transposable Element

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ABSTRACT

Alterations in gene expression by carcinogens were analyzed on three unstable alleles of the white \( w^+ \) locus of Drosophila melanogaster: white-crimson \( w^c \); white-ivory 16 \( w^{16} \); and white-unstable 11 \( w^{11} \). Two of these alleles \( w^{16} \) and \( w^{11} \) were spontaneous mutant derivatives of \( w^+ \), which is known to harbor a transposable element. The compounds studied were dimethylnitrosamine, 7,12-dimethylbenz(a)anthracene, and aflatoxin B\(_2\). These carcinogens were topicaly applied on the early larval stages, and the genetic effects assayed were the alterations in eye color either to wild-type \( w^+ \) or to other \( w \) mutants, initiated both somatically and germinally, as well as the simultaneously induced X-chromosome recessive mutations.

The tested compounds influenced the different unstable \( w \) alleles in a highly selective manner, both as a function of the inducing agent and the organization of the genome in the target cells. The same treatments raised the somatic reversions to \( w^+ \) above the corresponding controls for \( w^c \) and \( w^{16} \) but not for \( w^{11} \), whereas the simultaneous induction of other \( w \) mutant phenotypes occurred appreciably only with \( w^c \). Furthermore, these treatments gave high and variable somatic reversions to \( w^+ \) with \( w^{16} \), whereas the simultaneously induced germinal events were uniformly very low.

The frequencies of altered expression at the unstable test loci, whether in the soma or germ line, were quantitatively uncorrelated with the mutagenic effects of the treatments in terms of the yield of X-chromosome recessive mutations assayed in the progeny of males emerging from the same treated larvae. There was also an association between the time of induction of these alterations by the tested carcinogens in the soma and the cellular stage in genomic differentiation. Reversions to \( w^+ \) were induced preferentially after the onset of genetic determination, whereas changes to the \( w \) mutant phenotypes occurred predominantly during the predetermination phases.

The genetic properties of transposable elements and the manner of their response to carcinogens supported the hypothesis that nonviral cancer might arise from molecular processes similar to those involved in the evolution of retroviruses.

INTRODUCTION

The genetic and epigenetic factors thought to be involved in cancer development and the experimental and epidemiological evidence for the relative importance of such factors to the initiation and progression of the disease in humans has recently been the subject of a stimulating, although speculative, review by Cairns (6). A critical examination of the available information on the subject led him to infer that most human cancers did not appear to be caused by minor changes in DNA base sequence of the type known to result in point mutations but were more likely to be an outcome of more drastic genetic transpositions. Cytogenetic evidence in favor of this conclusion was later presented in a review by Klein (26) on the possible significance of the specific chromosome rearrangements often observed in some human and animal tumors. It was argued that such rearrangements might be associated with the genetic transpositions which caused altered activity at the specific genes controlling normal cell growth and differentiation, thus leading to cancer.

An early step towards the understanding of gene regulation in eukaryotes came with the discovery in Drosophila melanogaster of mutationally unstable loci, such as white-crimson \( w^c \), and the realization that these might well be “insertion mutations” (17–22). It was further suggested that the instability of such genes might be due to the insertion within them of transposable elements, analogous to the DNA “insertion sequences” of bacteria (5, 32). Experimental confirmation of this suggestion was provided by the demonstration that a TE in association with the \( w^+ \) locus did in fact transpose spontaneously from its initial position on the X-chromosome to several new sites within the autosomes (24). Other TEs have more recently been discovered in Drosophila during recombinant DNA studies on the dispersed repeated gene families, exemplified by copia, 412, 297, and 225 (13, 23, 33, 36, 40, 45). A general feature of these gene families is that they form a collection of highly conserved DNA segments, occupying some 30 sites within the genome, which are capable of transpositions to new chromosomal sites (cf. Ref. 40).

Molecular studies on the effects of the structure and movements of TEs on the expression of specific genes in Drosophila have thus far been undertaken on the \( w^+ \) locus and a few of its alleles. Most significant in this connection were the results with the recombinant plasmid 61F\(_4\), which enabled the demonstration of a close linkage between the white-apricot \( w^c \) mutant allele and the dispersed repetitive element copia (14). This linkage was later shown to hold true even at the level of fine-scale genetic mapping as determined by intralocus recombination analysis (2). Further investigations by molecular cloning and sequencing techniques revealed that the copia element was in fact inserted within the \( w^c \) sequence and that it was excised during the reversion of at least some \( w^+ \) mutants.

Received June 15, 1981; accepted October 22, 1982.

1 This work was supported by grants to the Institute of Cancer Research (Chester Beatty Research Institute, Royal Cancer Hospital) from the Medical Research Council and the Cancer Research Campaign.

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3 The abbreviations used are: TE, transposable element; DMN, dimethylnitrosamine; DMBA, 7,12-dimethylbenz(a)anthracene; AFB\(_1\), aflatoxin B\(_1\).

4 The genetic symbols used are: \( w^+ \), wild-type white allele; \( w \), white; \( w^c \), white-crimson; \( w^{16} \), white-ivory 16; \( w^{11} \), white-unstable 11; \( w^c \), yellow 2.
to the w\(^+\) state (3, 16, 27). The molecular characterization of other w\(^-\) mutant alleles is still in progress, but it has already become apparent that w\(^-\) and some of its derivatives carry mobile repeated DNA insertions which can influence gene expression by alteration of the insert and its flanking regions (7).

The great diversity of TEs within the unstable loci of Drosophila and the feasibility of their study in this organism both by cytogenetic and molecular techniques promises to make this system ideally suited for the analysis of the mechanisms of genetic transpositions by carcinogens. Studies to this end have already been undertaken on a partially duplicated w\(^-\) locus with a TE, which enabled the demonstration that carcinogens were highly effective in the induction of regulatory alterations in gene expression during somatic cell differentiation (9-11).

The present communication describes experiments with other unstable w\(^+\) alleles with a view to elucidation of the role of TEs in the misregulation of gene activity by carcinogens.

**MATERIALS AND METHODS**

**Genetic Stocks.** Altered gene expression was examined in 3 unstable mutant derivatives of the w\(^+\) sex-linked eye color gene of D. melanogaster. The starting point was the w\(^-\) allele isolated and characterized genetically by Green (17, 18) and molecularly by Collins and Rubin (7). Two other phototypically distinguishable alleles were also used, w\(^-\)\(^{16}\) and w\(^+\)\(^{11}\), both arose spontaneously in our laboratory within a stock carrying y\(^2\) w\(^-\).

**Compounds and Administration.** Three potent carcinogens were used in the present investigation: DMN, representing the nitrosamines; DMBA, representing the hydrocarbons; and AFB\(_1\), representing the mycotoxins. The sources of the tested samples and their genetic activities in the soma and germ line of Drosophila have previously been reported (10).

The method of carcinogen administration has already been described in detail (10) and need only be mentioned here in outline. Newly emerged females and males with the test loci (w\(^-\), w\(^-\)\(^{16}\), w\(^+\)\(^{11}\)) were aged separately for 4 days and then allowed to mate and oviposit on the eggs and newly hatched larvae on the surface of the solution on the eggs and newly hatched larvae on the surface of the vehicle was used in the concomitant controls. The treated cultures were kept at 25\(^\circ\), and the carcinogens were left in them throughout development.

**Phenomena Assayed.** The effects of carcinogens on the soma were deduced from their efficiency in the induction of aberrantly colored sectors in the adults’ eyes during their development from the larval anlagen. The level of activity was calculated as the percentage frequencies of sectors with altered pigmentation among the emerging adults with the relevant genotypes. Samples of the sectors with the various carcinogens were examined at a higher magnification for the determination of their size in terms of the number of aberrantly colored ommatidia.

The germinal mutagenicity of the tested carcinogens was measured simultaneously with the somatic eye sectoring on the X-chromosomes from the treated larval gonads of the w\(^+\)\(^{10}\) stock. The sex-linked recessive mutations were assayed by the Muller-5 technique in the progeny of a sample of the emerging males (both sectored and unsectored). In these tests, the F\(_2\) cultures were scored for all visibles (complete and mosaic) and for complete lethals as indicated by the absence of males carrying the descendants of the treated X-chromosomes. The specific effects on the w\(^+\)\(^{10}\) locus were also analyzed by the mass inbreeding of both sectored and unsectored male and female adults which emerged from the treated larvae and scoring the progeny for altered eye color.

**RESULTS**

**Spontaneous TE Effects.** Alterations in gene expression through spontaneous changes initiated in the germ line at the 3 unstable test loci (w\(^-\), w\(^-\)\(^{16}\), and w\(^+\)\(^{11}\)) are summarized in Table 1. The aberrant phenotypes observed comprised complete reversion to the wild-type (w\(^+\)) eye color and changes to other w\(^-\) mutant colors. The effects were scored in both sexes, with the revertants on a single X-chromosome in the female showing complete dominance and the other w\(^-\) mutant alleles giving various interaction shades of eye pigmentation. The females heterozygous for w\(^-\) and the lighter alleles (w\(^-\) and w) gave a characteristic pale orange eye color, with the exact nature of the changed allele being identifiable on the basis of the altered eye color in one-half of their sons.

Among the loci analyzed, the greatest instability occurred with w\(^-\), where reversion to w\(^+\) occurred at a frequency of 1\% and changes to other w\(^-\) alleles at the still higher level of 1.8\%, both being inordinately higher than the spontaneous mutability rates for the stable w alleles (maximally of the order of 10\(^{-5}\)).

### Table 1

**Spontaneous germinal changes in unstable w loci**

The frequencies of altered gene expression initiated in the germ line of stocks homozygous for the unstable w loci: w\(^-\), w\(^-\)\(^{16}\), or w\(^+\)\(^{11}\). The effects were scored in both sexes as revertants to wild-type (red) eye color or changes to the phenotypes of other w\(^-\) mutant alleles. Females of the genotype w\(^-\)/w\(^-\) or w/w\(^+\) showed a pale orange eye color, and the exact nature of the changed allele (w or w\(^+\)) was identified on the basis of the eye colors in their sons.

<table>
<thead>
<tr>
<th>Genetic locus</th>
<th>Sample observed</th>
<th>Variants recovered in clusters of</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2-10</td>
<td>&gt;10</td>
<td>%</td>
</tr>
<tr>
<td>w(^-)</td>
<td>50,444</td>
<td>9</td>
<td>44</td>
<td>16</td>
<td>1.00 (\pm) 0.15(^a)</td>
</tr>
<tr>
<td>w(^-)(^{16})</td>
<td>65,755</td>
<td>4</td>
<td>2(^b)</td>
<td>0</td>
<td>0.01 (\pm) 0.005</td>
</tr>
<tr>
<td>w(^+)(^{11})</td>
<td>19,888</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.02 (\pm) 0.01</td>
</tr>
</tbody>
</table>

\(^a\) Mean \(\pm\) S.D. Percentage standard deviation was adjusted for clusters (31) on the basis that progeny with the same phenotypic change in a given culture might have a common origin from a single premeiotic event.

\(^b\) The cluster size for these events was minimal; each was represented by 2 w\(^+\) individuals/culture.
locus). The majority of these events were recovered as clusters, often of medium size (10 to 20 representatives/culture), but could reach much larger sizes (in excess of 50 representatives). Such clustering of the aberrant phenotypes would suggest that the underlying genetic events predominantly occurred in the mitotically active premeiotic stages, thus permitting their subsequent multiplication through repeated chromosome replications. In view of this phenomenon, the standard deviations of the frequencies of altered phenotypes were calculated by Müller’s formula (31).

The stability of the $w^{16}$ locus was decisively higher than the $w^{6}$ from which it was derived. The frequency of revertants to $w^{+}$ dropped by 2 orders of magnitude to a level of 0.01%, although this value was still significantly above zero (normal deviate, 2.0; $p = 0.02$). Furthermore, the locus did not yield other allelic states in some 66,000 loci. In contrast to the situation with $w^{16}$, $w^{111}$, which was also derived from $w^{6}$, appeared to maintain a greater degree of genetic instability. The $w^{111}$ locus gave both revertants and other mutant alleles, although the frequencies of both of these classes were considerably rarer than those for $w^{6}$. This rarity was further indicated by the lack of clusters with the revertants and their relatively smaller sizes with the mutant alleles.

The comparative data in Table 1 for $w^{6}$, $w^{16}$, and $w^{111}$ vividly illustrate the extent to which even closely related alleles of common origin could behave differently as a function of the exact molecular organization of the structural genes and their regulators.

**Carcinogen Effects in the Soma.** The somatic alterations in gene expression at the 3 unstable test loci after the treatment of larvae from stocks homozygous for unstable $w$ loci with various carcinogens.

<table>
<thead>
<tr>
<th>Genetic locus</th>
<th>Compounds</th>
<th>Dose (mm)</th>
<th>Sample observed</th>
<th>Aberrant eye color sectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>$w^{6}$</td>
<td>Control</td>
<td>15,562</td>
<td>4 0.03 ± 0.01</td>
<td>Wild-type $w^{+}$ 1 0.01 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>DMN 10.0</td>
<td>14,997</td>
<td>24 0.16 ± 0.03</td>
<td>9 0.06 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>DMBA 3.0</td>
<td>15,955</td>
<td>27 0.17 ± 0.03</td>
<td>44 0.28 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>AFB 0.05</td>
<td>2,536</td>
<td>17 0.67 ± 0.16</td>
<td>14 0.55 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>DMN 2.5</td>
<td>30,638</td>
<td>23 0.10 ± 0.02</td>
<td>0 0.0</td>
</tr>
<tr>
<td>$w^{16}$</td>
<td>Control</td>
<td>18,021</td>
<td>375 3.07 ± 0.21</td>
<td>16 0.89 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>DMN 10.0</td>
<td>14,997</td>
<td>24 0.16 ± 0.03</td>
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</tr>
<tr>
<td></td>
<td>DMN 2.5</td>
<td>30,638</td>
<td>23 0.10 ± 0.02</td>
<td>0 0.0</td>
</tr>
<tr>
<td>$w^{111}$</td>
<td>Control</td>
<td>18,021</td>
<td>375 3.07 ± 0.21</td>
<td>16 0.89 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>DMN 10.0</td>
<td>14,997</td>
<td>24 0.16 ± 0.03</td>
<td>9 0.06 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>DMBA 3.0</td>
<td>15,955</td>
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</tr>
<tr>
<td></td>
<td>DMN 2.5</td>
<td>30,638</td>
<td>23 0.10 ± 0.02</td>
<td>0 0.0</td>
</tr>
</tbody>
</table>

The carcinogens used in the present study were tested at the higher molar doses which were previously found to be effective in the induction of somatic eye sectoring on other stocks with unstable $w^{+}$ loci (9–11). All compounds at the tested doses proved active on the $w^{+}$ locus, raising the frequencies of both $w^{+}$ and $w$ mutant sectors very significantly above the corresponding control levels. These activities were particularly striking with AFB, which at a comparatively low molarity (0.05 mm) induced some 50-fold the spontaneous frequency of $w$ mutant sectors (Table 2, $w^{6}$, AFB).

The activity of the test carcinogens was also pronounced on the somatic $w^{16}$ locus, but only with respect to $w^{+}$ sectors, reaching some 40-fold the control level with 10 mm DMN and 0.04 mm AFB. In contrast to the positive results in all of the $w^{16}$ experiments with respect to the $w^{+}$ (red) sectors, only that with 3.0 mm DMBA also raised the frequency of the $w$ mutant sectors significantly above the control level ($p = 0.02$); experiments with other compounds were decisively negative (Table 2, $w^{16}$, Column #7). Somatic eye sectoring was also investigated on the $w^{111}$ using the same carcinogens and doses as with $w^{6}$ and $w^{16}$. All such treatments, however, proved ineffective on this locus (Table 2, $w^{111}$).

At the levels of carcinogen treatment used in the present studies on somatic eye color sectoring, the eyes of the adults emerging from the treated larvae showed no morphological defects as to size, shape, or ommatidial distribution. This would suggest that such treatments did not cause any major cytogenetic aberrations in the cells of the eye discs of the type that might be expected to result in cell lethality or abnormal development.

**Carcinogen Effects in the Germ Line.** The effects of the investigated carcinogens on the germ line were analyzed in detail on the $w^{16}$ stock, both as regards the specific effects on the mutant locus itself and the simultaneously induced overall X-chromosome mutations. Changes at the $w^{16}$ locus were detected by inbreeding the adults emerging from the treated larvae, which were scored for somatic eye sectoring (Table 2, $w^{16}$), Samples of the sectored and unsectored adults were inbred separately, and their progenies were scored for aberrant eye colors. There was no significant difference in the incidence of the aberrant individuals from the 2 parental classes, and the
combined data are detailed in Table 3. The specific locus effects with DMN could be undertaken only with the lower dose investigated somatically (2.5 mM), since higher doses caused complete sterility of the treated males. At the investigated doses, all 3 carcinogens increased the yield of w\(^+\) revertants to about 2- to 3-fold the control level but did not induce any changes from w\(^16\) to other w mutant alleles. However, even the maximal induction of w\(^+\) revertants, which occurred with DMBA, proved to be only on the verge of statistical significance (\(\chi^2_{(1)} = 3.6; p = 0.06;\) Table 3).

The mutagenically effective levels actually reaching the larval gonads with the carcinogen treatments in the w\(^16\) experiments were biologically assessed on the basis of the induction of the X-chromosome recessive mutations as determined by the Muller-5 technique. These assays were undertaken on individual males from the experiments on somatic eye sectoring detailed in Table 2. There were no significant differences between the mutation yields from the sectored and unsectored males, and the combined data are presented in Table 4. All carcinogens at the tested doses were decisively mutagenic, especially with respect to the induction of X-lethals, where the induced frequencies were invariably significantly above the controls. It should be noted, however, that the mutagenicities of the applied treatments here reported could well be underestimates because of the possible lack of recovery of some gonial hemizygous recessive mutations through germinal selection (12).

The relationship between the specific effects on the w\(^16\) locus and the general mutagenic potencies of carcinogens when applied to the same population of germ cells could be gathered from the data in Tables 3 and 4. As previously noted, reversions from w\(^16\) to w\(^+\) occurred at roughly the same low frequency with all 3 carcinogens (DMN, DMBA, and AFB\(_1\)), reaching no more than 3-fold the control level. In contrast, the simultaneously induced X-lethal frequencies exceeded 40-fold the control level with DMN and DMBA and about 10-fold this level with AFB\(_1\). Evidently, therefore, the poor yield of w\(^+\) revertants, especially with DMN and DMBA, cannot be attributed to the lack of the proximal mutagen in the gonial cells. Nor can this phenomenon be explained in terms of selection against the w\(^+\) revertants, since the genetic change involved a restoration of normal gene function and would be expected to confer a selective advantage to the harboring cells.

**Time of Altered Gene Expression.** The timing of the carcinogen-induced changes in the unstable w loci during embryonic eye development was deduced from the analysis of the size distributions of the different aberrantly colored eye sectors in the emerging adults. The biological basis for this approach and the required calculations have been described previously (10) and need only to be recalled here in outline. Embryological studies on induced eye color mosaicism enabled the conclusion that genetic determination during the development of this organ occurred late in the first larval instar, when its anlage consisted of about 32 cells, each contributing some 25 ommatidia of the 800 total in the adult's eye. A heritable change induced in any of the 32 cells of the eye anlage at genetic determination would thus yield an aberrant mosaic sector of about 25 ommatidia, with variations from this size in either direction according to the time of the genetic alteration relative to that of determination. In the present experiments, no disturbances in eye morphology were associated with the observed eye color mosaics. This would indicate that the applied treatments did not disturb normal eye development in a manner which differentially distorted the size of the aberrantly colored sectors. The timings for the somatic alterations obtained in the present study are shown in Table 5.

The w\(^+\) revertants with all carcinogens and test loci predominantly yielded aberrant (red) sectors of sizes below the 33 to 64 ommatidia class (Table 5A), which indicated that their

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**Table 3**

Carcinogen-induced germinal changes at the w\(^16\) locus

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (mM)</th>
<th>Sample observed</th>
<th>Progeny with wild-type (w(^+)) eyes(^a)</th>
<th>No. and cluster size</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.02</td>
<td>65,755</td>
<td>4 (1), 2 (2)(^b)</td>
<td>0.01 ± 0.005(^b)</td>
<td>100</td>
</tr>
<tr>
<td>DMN</td>
<td>2.5</td>
<td>89,248</td>
<td>12 (1), 2 (2), 1 (3)</td>
<td>0.02 ± 0.01</td>
<td>100</td>
</tr>
<tr>
<td>DMBA</td>
<td>3.0</td>
<td>30,268</td>
<td>3 (1), 3 (2), 1 (4)</td>
<td>0.03 ± 0.01</td>
<td>100</td>
</tr>
<tr>
<td>AFB(_1)</td>
<td>0.02</td>
<td>35,511</td>
<td>2 (1), 1 (2), 1 (4)</td>
<td>0.02 ± 0.01</td>
<td>100</td>
</tr>
<tr>
<td>AFB(_1)</td>
<td>0.04</td>
<td>4,285</td>
<td>0</td>
<td>0.00</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^{a}\) Changes to w mutant eye colors were not observed.

\(^{b}\) Numbers in parentheses, sizes of mutant clusters recovered within the same culture; thus, 2 (2) denotes 2 cultures each with a cluster of 2 identical mutants.

**Table 4**

Carcinogen-induced X-chromosome mutations in the w\(^16\) strain

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (mM)</th>
<th>Sample observed</th>
<th>Complete lethals</th>
<th>Total visibles(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.02</td>
<td>1872</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>DMN</td>
<td>2.5</td>
<td>559</td>
<td>6 (2), 1 (3)</td>
<td>5 (1), 1 (2)</td>
</tr>
<tr>
<td>DMBA</td>
<td>3.0</td>
<td>1386</td>
<td>6 (2), 1 (3)</td>
<td>7 (1), 1 (2), 1 (4)</td>
</tr>
<tr>
<td>AFB(_1)</td>
<td>0.02</td>
<td>1443</td>
<td>7 (1)</td>
<td>7 (1), 1 (2), 1 (4)</td>
</tr>
<tr>
<td>AFB(_1)</td>
<td>0.04</td>
<td>1435</td>
<td>4 (1), 1 (2), 1 (3)</td>
<td>3 (1), 0.2 ± 0.1</td>
</tr>
</tbody>
</table>

\(^{a}\) None of the recovered visibles involved the w\(^16\) locus.

\(^{b}\) Mean ± S.D. Percentage standard deviation was adjusted for mutant clusters (31).
induction occurred in eye cells at stages of genomic differentiation at or following determination. Within this period, however, some shifts in the timing occurred with the various genetic stocks and test carcinogens, which were reflected in the frequency distributions of the induced sector sizes and their statistical means. Thus, these distributions for revertants to \( w^+ \) from \( w^c \) were homogeneous for DMBA and AFB\(_1\) (\( \chi^2[6] = 7.02; p = 0.3 \)), giving a mean sector size of \( 33 \pm 4 \) ommatidia, but were different from that of DMN (\( \chi^2[7] = 18.8; p = 0.01 \)), which gave a mean sector size of \( 19 \pm 8 \) ommatidia (Table 5A). A comparable analysis of the data with \( w^{16} \) and \( w^{111} \) loci again revealed that the distributions of sector sizes were similar for DMBA and AFB\(_1\), but different for DMN, with means of \( 16 \pm 1 \) and \( 7 \pm 1 \) ommatidia, respectively (\( w^{16} \) and \( w^{111} \), Table 5A). It would thus appear that reversion to \( w^+ \) with the different loci and carcinogen treatments occurred within developing eye anlagen of varying sizes, being largest with DMN on \( w^{16} \) and smallest with DMBA or AFB\(_1\) on \( w^c \). These observed differences in anlage size were in multiples of 2, suggesting that the biological factor underlying the variability was the speed of cell replication in the treated eye discs.

The size distributions of sectors showing mutant \( w \) alleles were very different from those arising from reversion to \( w^+ \) for the same loci and carcinogen treatments (compare Sections A and B in Table 5). The mutant \( w \) sectors were of comparatively larger sizes, predominantly falling within the class limits of 33 to 64 ommatidia or above, which indicated that their induction mainly occurred early in eye development, at the predetermination phases of genomic differentiation. This is further indicated by the statistical homogeneity of the sector size distributions for these events for the different loci and carcinogens (\( \chi^2[12] = 15.15; p = 0.2 \)), with an overall mean of \( 77 \pm 7 \) ommatidia, corresponding to about 10 cells in the eye anlage at the time of induction.

**DISCUSSION**

Considerable attention has been directed recently to the molecular characterization of \( w^+ \) and some of its unstable mutant alleles, in the hope of obtaining an idea about the mechanisms involved in its expression, mutation, and regulation. The studies of particular interest to this communication are those on \( w^c \) and \( w^c \) by Rubin et al. (7, 25, 36, 37). A preliminary investigation by the technique of *in situ* hybridization of labeled DNA segments on the polytene salivary gland chromosomes indicated that both \( w^c \) and \( w^c \) contained “putative copia-like elements”, one (2180) being present in both alleles (37). More recently, it was reported that \( w^c \) was due to a 2.9-kilobase tandem duplication of the \( w^+ \) gene sequences, without DNA from outside the locus (25), and the \( w^c \) resulted from a 10-kilobase insertion of an element of the “fold-back” class into the \( w^c \) duplication (7, 36). The \( w^c \) allele used in the present study was a subline of the stock analyzed molecularly and almost certainly harbors the same TE. The other alleles tested with the carcinogens (\( w^{16} \) and \( w^{111} \)) were derivatives of \( w^c \) and have not, thus far, been analyzed by molecular techniques. Genetically, however, both maintained a level of instability compatible with the retention of at least part of the \( w^c \) TE or the acquisition of some other destabilizing element. The studies of particular interest to this communication are those on \( w^c \) and \( w^c \) by Rubin et al. (7, 25, 36, 37). A preliminary investigation by the technique of *in situ* hybridization of labeled DNA segments on the polytene salivary gland chromosomes indicated that both \( w^c \) and \( w^c \) contained “putative copia-like elements”, one (2180) being present in both alleles (37). More recently, it was reported that \( w^c \) was due to a 2.9-kilobase tandem duplication of the \( w^+ \) gene sequences, without DNA from outside the locus (25), and the \( w^c \) resulted from a 10-kilobase insertion of an element of the “fold-back” class into the \( w^c \) duplication (7, 36). The \( w^c \) allele used in the present study was a subline of the stock analyzed molecularly and almost certainly harbors the same TE. The other alleles tested with the carcinogens (\( w^{16} \) and \( w^{111} \)) were derivatives of \( w^c \) and have not, thus far, been analyzed by molecular techniques. Genetically, however, both maintained a level of instability compatible with the retention of at least part of the \( w^c \) TE or the acquisition of some other destabilizing DNA insertion. In particular, \( w^{16} \) gave a spontaneous frequency of the \( w^+ \) revertants in the germ line one order of magnitude above that obtained with the \( w^c \) allele investigated molecularly (7, 25, 36). More significant, \( w^{16} \) showed a higher level of somatic instability than \( w^c \) itself (see Table 2, controls). Carcinogens interacted with the unstable test alleles in a highly idiosyncratic manner. The induced effects depended to various degrees on the test compounds, the treated cells, and the structural organization of the genetic targets. The compound and target effects were conveyed by the data on somatic eye sectoring. The same carcinogen treatment affected the 3 test alleles differentially with respect to both reversion to \( w^+ \) and changes to other \( w \) alleles. Furthermore, each of these effects manifested different patterns of interlocus response after comparable treatments with the various test carcinogens. In spite of these variabilities, however, a common feature was discernible with regard to the induced \( w^+ \) revertants in the soma. The relative frequencies of these events between loci seemed to parallel those occurring spontaneously, being in the
sequence $w^{16} > w^* > w^{111}$. This would seem to suggest that the intrinsic stability of the target gene might play some role in its response to carcinogens.

The importance of cell type to the induction of $w^*$ revertants by carcinogens was most dramatically illustrated by the marked difference in the response of $w^{16}$ in the soma and germ line. As was stressed previously (see "Results"), the effects of the tested carcinogens in the induction of such events were far lower and less variable germinally than somatically. The germline data were also of relevance to the question as to the relationship between the phenomena of gene regulation and mutagenesis. Virtually the same frequencies of reversion from $w^{16}$ to $w^*$ (about $2 \times 10^{-4}$) occurred after treatment with the 3 carcinogens (DMN, DMBA, and AFB.), although these yielded very different X-recessive lethals (from 0.5% for AFB, to 2.5% for DMBA). Of further interest in this connection are the early results on the reversion of other $w'$ alleles to $w^*$ after comparatively high doses of X-irradiation. The earliest of these reports (29) set the frequency of such reversions at $2.4 \times 10^{-4}$ after the exposure of $w'$ female gametes to a dose of 4000 roentgens, while a later investigation (4) gave this frequency as $2.5 \times 10^{-4}$ after 5000 roentgens. These data clearly indicate that reversion among the $w'$ alleles was virtually constant, irrespective of the applied mutagenic treatments and their potencies in chromosome breakage. This would seem to suggest that reversion at this locus could well be an outcome of an intrinsic property of its molecular organization which did not appear to be greatly modifiable by the processes involved in mutagenesis. Interestingly, classical mutagens also failed to mobilize TEs in both Escherichia coli (38) and yeast (35).

The most outstanding feature of the effect of carcinogens on unstable loci relates to the dependence of the process on the genomic state during somatic cell differentiation. The analysis of the sector sizes for the aberrant eye color mosaics with the various carcinogens and test loci enabled the timing of the underlying genetic alterations relative to the onset of genetic determination during larval eye development. The induction of $w^*$ revertants by such agents was rather late in the development of the eye, generally during the postdetermination phases of genomic differentiation, but with some variations during this period as a function of compound and test locus. On the other hand, the $w$ mutant alleles recovered in the same experiments occurred early in eye disc development at the predetermination phases of genomic differentiation and did not show detectable variations between compounds or loci.

The possible role of genetic transpositions in carcinogenesis has been the subject of a conference, the proceedings of which were summarized by Sager (39). Particularly interesting were the views of McClintock (30) on the basis of her discoveries with the "controlling elements" of Zea mays, which spanned a period of over 3 decades. In McClintock's words: "Should a genome-disturbing event appear, as such as a cancer-inducing agent, the result could be induction of transposition of elements that can serve to control gene action. The insertion of such elements at given gene loci could initiate those genomic changes that lead to a cancerous state of the cell" (39). The central postulate in this proposal, concerning the effects of insertions at specific loci, received support from genetic and molecular work on Drosophila, which indicated that many unstable loci in this species were indeed associated with such elements (reviewed in Refs. 22 and 36). The further suggestion as to the possible induction of altered gene expression through interactions between carcinogens and TEs has also been demonstrated in the same species through studies on somatic alterations in gene expression at a variety of unstable $w$ loci (Refs. 9 to 11 and the present data). Lastly, the theme that interactions might occur between carcinogens and DNA sequences analogous to those in TEs has featured repeatedly in the writings of both oncologists and molecular biologists (6, 15, 26, 41).

The alterations in gene expression here described through interactions between unstable loci and carcinogens are of obvious practical value insofar as they could be exploited in the design of new screening techniques for studies on environmental carcinogenesis. Far more challenging is the question as to the role of these interactions in cancer development. The most significant evidence in this connection emerged from recent molecular studies on the TEs of Drosophila and some oncogenic viruses. Recombinant DNA and sequencing studies revealed common structural and organizational features between the mobile dispersed genetic elements of the TEs, such as copia (8, 28), and the long terminal repeats of integrated preretroviruses (1, 44). The interactions between TEs and carcinogens reported in our studies also appear to parallel the genomic changes thought to be mediated by preretroviruses during the generation of the oncogenic forms of mouse type C viruses (34). These structural and behavioral similarities between the TEs and preretroviruses lend support to Temin's protovirus hypothesis, including the postulate that nonviral cancer could result from processes similar to those involved in the evolution of retroviruses (41–43).

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46. Carcinogens and Altered Gene Expression
Differential Induction of Altered Gene Expression by Carcinogens at Mutant Alleles of a Drosophila Locus with a Transposable Element

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