Antagonistic Interactions Between Alleles of the RpII215 Locus in Drosophila melanogaster

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Manuscript received January 8, 1988
Accepted April 19, 1988

ABSTRACT

The RpII215 locus encodes the large subunit of RNA polymerase II (polII). Three of 22 RpII215 alleles cause a synergistic enhancement of the mutant phenotype elicited by mutations in the Ultrabithorax (Ubx) locus. We have recovered and analyzed three new mutations that suppress this enhancement. All three mutations map to the RpII215 locus. In addition to suppressing the Ubx enhancement of other RpII215 alleles, two of the new mutations, JH1 and WJK2, themselves enhance Ubx. RpII215 alleles can be placed into three classes based on their ability to enhance Ubx. Class I alleles, including Ubl, C4, C11, JH1, and WJK2, enhance Ubx when heterozygous with class II alleles, which include wild-type RpII215. Class III alleles, which include amorphic alleles, do not enhance Ubx. The third new mutation, WJK1, is a conditional amorphic allele, which behaves like a class III allele at 29° but like a class II allele at 19°C. Another mutant phenotype is caused by certain RpII215 alleles, including all class I alleles. This phenotype is a synergistic enhancement of a mutant phenotype elicited by mutations at the Delta (Dl) locus. Unlike the enhancement of Ubx, the enhancement of Dl is not dependent upon antagonistic interactions between different classes of RpII215 alleles.

RNA polymerase II (polII), the enzyme responsible for the synthesis of messenger RNA (mRNA), is a multimeric enzyme composed of a minimum of nine subunits (reviewed by Roeder 1976). Low concentrations of α-amanitin specifically inhibit polII. This property was used to isolate one mutation, RpII215(C4) (C4; all alleles of RpII215 will hereafter be referred to by their superscript allelic designations), which conferred resistance to an otherwise lethal dose of α-amanitin (Greenleaf et al. 1979). The locus was cloned (Searles et al. 1982) and antibodies were used to identify the gene product as the large subunit of RNA polymerase II (Greenleaf 1983).

A class of RpII215 alleles causes a dominant homoeotic phenotype in flies such that the capitelium of the halter, an element of the third thoracic segment, is partially transformed into the analogous structure on the second thoracic segment: the wing blade (Mortin and Lefevre 1981). We will call this phenomenon the “Ubx effect” because the same phenotype is seen in flies heterozygous for the mutation Ultrabithorax (Ubx). Three out of 22 RpII215 alleles elicit this transformation even though none was initially selected for this visible mutant phenotype. The three alleles are Ultrabithorax-like (Ubl), C4 and C11 (Mortin and Lefevre 1981; Voelker et al. 1985; our unpublished data). Flies doubly heterozygous for one of these three RpII215 alleles and Ubx (e.g., Ubl+/+;Ubx+/+) display a synergistic enhancement such that there is a more complete, though still partial, transformation of halter into wing.

While C4/+ flies display a transformation of halter into wing, homozygous and hemizygous C4 flies are wild type (Voelker et al. 1985). Alleles that elicit the Ubx effect have been designated antimorphs as defined by Muller (1952). They are postulated to produce a product that competes with or inhibits the wild-type gene or gene product. This is particularly perplexing as only a single copy of the RpII215 gene product is thought to be present in the active RNA polymerase II multimeric enzyme (Vaisius and Werland 1982). As a consequence, negative complementation cannot explain this antagonistic interaction.

Embryos deficient for the RpII215 locus develop into pharate larvae solely on gene product supplied by a heterozygous RpII215+/RpII215− mother (Mortin and Kaufman 1984). These RpII215− embryos are morphologically normal at the time of their death. This is also true for homozygous or hemizygous Ubl embryos (Mortin 1983). In particular, no transformation of third thorax into second is observed. While maternal loading of the wild-type RpII215 gene product supports 24 hr of zygotic development at 22°, it is the zygotic genotype that determines whether a fly will exhibit the Ubx effect. This was demonstrated by generating gynandromorphs with patches of Ubl− tissue in flies developing from eggs maternally loaded...
with *Rpl215*" (MORTIN, PERRIMON and BONNER 1985). Patches of *Ubl*/- cuticle that include the capitellum do not show the Ubx effect. Clearly, the Ubx effect is the result of an antagonism between *Rpl215* alleles.

In the present study we characterize the antagonistic interaction between *Rpl215* alleles resulting in the Ubx effect. We find that *Rpl215* alleles can be placed into classes based on their ability to elicit the Ubx effect. Class I alleles including *Ubl*, *C4*, *C11*, and two new alleles, *JH1* and *WJK2*, cause the Ubx effect when heterozygous with class II alleles, which include wild-type *Rpl215*. Class III alleles include the amorphic allele *n* and do not elicit the Ubx effect. In other words, the Ubx effect is observed in flies trans-heterozygous for class I and II alleles but not I/I, I/III, II/II, II/III or III/III. Other mutant phenotypes elicited by *Rpl215* alleles are described, including a synergistic enhancement of the abnormal phenotype elicited by mutations in the *Delta (Dl)* locus. The "Dl effect," as we call this phenomenon, does not require the presence of both class I and II alleles in the same fly.

**MATERIALS AND METHODS**

Flies were maintained on standard cornmeal-sugar-agar media supplemented with live yeast. Experiments were conducted at room temperature (22–25°C), 19 ± 1°C, 25 ± 1°C or 29 ± 1°C.

**Halter and wing measurements:** A photonometer subdivided into units of 0.1 was used to measure the length and width of right halteres and wings. The length was measured as the distance from the base of the capitellum, where it attaches to the pedicel, to the tip. The width was measured as the greatest distance between the sides of the capitellum, measured perpendicular to the length. Wings were measured in a similar fashion. Sizes were calculated by multiplying the measured length and width. Note that halter and wing measurements are both reported in the same units—squared. Temperature and crowding conditions were found to affect overall fly size. In order to obtain more accurate comparisons of halter sizes of flies from different crosses, we adjusted the halter measurement to a "standard fly size." This was done by multiplying the measured capitellum size by an average of halter and wing size of *C4* flies. Temperature and crowding conditions were found to affect overall fly size.

**Statistical analysis:** Average halter and wing measurements are shown with their 95% confidence intervals. The t-test was used for comparisons, with a value of P < 0.05 considered significant.

**Strain construction:** A duplicated *X* chromosome, generated by T. C. KAUFMAN, was made by irradiation of yellow and forked (y f) males carrying the Y-linked duplication *Dp (1;Y)B*" v"*Y*" (LEFEVRE 1971). The duplication carries the wild-type alleles for *vermilion (v)*, *Rpl215* and *y*. Depending on the Y-linked duplication to the X chromosome created an X chromosome with two wild-type *Rpl215* alleles, in addition to *v*" and *y*". Three *Rpl215* alleles, *Ubl*, *C4* and *C11*, were individually recombined onto duplicated *X* chromosomes. Four chromosomes were utilized in this study: *Rpl215*" f: v: *Rpl215"* y", *Ubl* f: v: *Rpl215"* y", v: *C4* f: v: *Rpl215"* y", and *C11* f: v: *Rpl215"* y", abbreviated in the text as +: +, *Ubl*: +, *C4*: +, and *C11*: +. The presence of the *Rpl215* duplication was monitored by a tightly linked wild-type copy of the *y* gene. Hemizygous males with any of the four chromosomes were viable and fertile; however, homozigous females do not survive to adulthood. Stocks were balanced with *FM7* (MERRIAM 1968), which carried a wild-type copy of *Rpl215*, and resulted in female and male siblings with ratios of mutant to wild-type *Rpl215* of 1:2 and 1:1, respectively.

**Mutagenesis:** *C4* was the only *Rpl215* mutation that both caused the Ubx effect and was viable and fertile as a homozygous female. The mating scheme diagrammed in Figure 1 shows the crosses used to identify mutations that alter the Ubx effect. Males of the genotype +: Y: *Ubx*"/Di" were treated with ethyl methanesulfonate (EMS) according to the protocol of LEWIS and BACHER (1968) and mated at room temperature to v: *C4*: u: *C4*: *Ubx*"/Di" virgin females. Egg collections were made every 24 hr on 6 successive days. Eggs were shifted to 29°C and allowed to develop to the adult stage. Female progeny that displayed an altered halter size were mated to *FM7*: Y: *Ubx*"/Di" males and several lines were established from isochromosomes for each putative mutation. To avoid isolating the *C4* chro-

![Figure 1](image-url)

**Figure 1.—Mating scheme used to identify mutations that alter the Ubx effect of *C4*. F1, females were examined for altered expression of the Ubx effect. Progeny possessing putative mutations (as indicated by the asterisk) were mated to males carrying the balancer *X* chromosome, *FM7*, (F1 cross) and several lines were established from single female and/or male non-v F2 progeny. The various isolines were restaged with *C4* at 29°C and one line was saved from each putative mutation that restaged positive. New mutations were all distinguishable from the *C4* mutation, which causes a characteristic phenotype in *C4/FM7;Ubx*"/Di" flies.
mosome, we selected non-\(v\) progeny for our isolines. The \(v\) locus maps 2.7 \(\text{cM}\) from the \(Rpl1215\) locus (Mortin and Leefvre 1981; Greenleaf et al. 1980), so some of our non-\(v\) isolines were actually \(C4\). Further genetic tests unambiguously distinguished between \(C4\) and newly induced mutations. Three new mutations were recovered: \(JH1\), \(WJK1\) and \(WJK2\).

**Interactions between \(Rpl1215\) alleles:** Preliminary experiments had indicated that halter measurements of flies with a given genotype would vary from one experiment to the next, though this variability was less than that observed between flies with different genotypes. In order to reduce these differences we conducted crosses that would permit the comparison of sisters, where possible, or between genetically similar individuals whose mothers were also genetically similar. Females with one of the three genotypes, \(JH1/\text{FM7};\text{Ub}\bx^+/\text{D17}\), \(WJK2/\text{FM7};\text{Ub}\bx^+/\text{D17}\) or \(\nu;\text{C4}/\text{FM7};\text{Ub}\bx^+/\text{D17}\), were mated to \(y^+;\text{C11}/\text{Dp}(1;Y)^B\text{v}\text{v}Y^+\), \(y^+;\text{C4}/\text{Y}\), \(\text{UbI}\text{f}/\text{Dp}(1;Y)^B\text{v}\text{v}Y^+\) or \(\text{JH1}/\text{Y}\) males. Halter measurements were obtained from female progeny that inherited \(\text{Ub}\bx\).

**Quantitation of the DI effect:** Flies carrying specific mutations in the \(Rpl1215\) locus and mutations in the Delta \((\text{DI})\) locus displayed a mutant phenotype characterized by duplicated macrochaetae and microchaetae on their heads and thoraces. We quantitated this phenotype by counting the number of times 20 bristles on the thorax (anterior and posterior scutellars, anterior post-alar, anterior and posterior dorso-centrals, anterior and posterior supra-alar, anterior and posterior notopleurals, and presutural) and 14 on the head (ocellar, postvertical, anterior and posterior verticals, and the anterior, middle and posterior orbitals) were duplicated. Many bristles besides these 34 were duplicated in \(\text{DI}^+/\) flies; however, the number of these duplicated bristles provided an estimate of the severity of the mutant phenotype.

**Temperature studies:** \(WJK1/\text{FM7};\text{Ub}\bx^{130}/\text{D17}\) females were mated to \(y^+\) \(\text{C4}/\text{Y}\) males and placed at 19°, 25° or 29°. Eggs were collected and allowed to develop to the adult stage. The \(\text{Ub}\bx\) effect was measured in \(\text{C4}/\text{FM7};\text{Ub}\bx^+/\text{Y}\) and \(\text{C4}/\text{WJK1};\text{Ub}\bx^+/\text{Y}\) females and \(\text{DI}^+/\) females. Temperature shifts were conducted by collecting eggs for 24 hr from crosses placed at 19° and 29°. Zygotes were allowed to develop for 1 to 17 days at 19° and then shifted to 29° or for 1 to 7 days at 29° and then shifted to 19°. Female progeny were examined for \(\text{Ub}\bx\) and DI effects.

**Measurements of the Ubx effect:** A strong Ubx effect causes four distinct morphological changes in the capitellum: the appearance of three wing veins, the formation of marginal wing bristles, an increase in size and a change of shape. We initially wanted to find a method to compare more precisely the Ubx effect caused by the \(Rpl1215\) alleles, \(\text{UbI}, \text{C4}\) and \(\text{C11}\). We chose to investigate whether halter size might be an accurate measure of this effect. Transformation of halter into wing results in the flattening of the balloon-shaped capitellum into an essentially two-dimensional structure. Measuring the halter as if it were a two-dimensional structure provides an estimate of the degree of transformation.

To test if altered polymerase might affect overall fly size, leading to a bias in measurements of this transformation, wing measurements were made of flies possessing different \(Rpl1215\) alleles. Figure 2A shows that the size of flies, as determined by wing size, was unaffected by \(Rpl1215\) mutations. Differences due to sexual dimorphism were observed. Subsequent experiments showed fluctuations in wing size from one experiment to the next. We therefore chose to adjust halter measurements (as described in MATERIALS AND METHODS) to account for variability in overall fly size induced by such other parameters as food and crowding. These “standardized” values were used as measures of the transformation of halter to wing.

Standardized capitellae sizes of flies heterozygous for the \(\text{Ubx}^{P13}\) allele present on the balancer third chromosome, \(\text{TM6}\) (Lindsley and Grell 1968), were compared in Figure 2B. Note that the average adjusted capitellum size in males with two copies of the wild-type \(Rpl1215\) locus (1.20 ± 0.09) was not significantly different from halter measurements of females with three copies (1.14 ± 0.08). The largest average adjusted halter size was produced by \(\text{UbI}^+\text{/}Y;\text{TM6}/+\) flies and measured 5.92 ± 0.44. This halter size was reduced to 2.36 ± 0.07 in \(\text{UbI}^+\text{/}+/\text{TM6}/+\) flies, which had two wild-type copies of the \(Rpl1215\) locus. These results were consistent with previous studies of the morphology of the capitellum in flies with \(Rpl1215\) mutations (Mortin and Leefvre 1981). Halteres of \(\text{C4}\text{+/}Y;\text{TM6}/+\) (3.00 ± 0.21) and \(\text{C11}^+\text{/}Y;\text{TM6}/+(2.99 ± 0.10) flies were intermediate in size between those of \(\text{+.+/Y;TM6}/+\) and \(\text{UbI}^+\text{+/Y;TM6}/+\) flies (Figure 2B), which again was consistent with previous studies (Voelker et al. 1985). The average adjusted halter size of males lacking the \(\text{Ubx}^{P13}\) mutation \((\text{+.+/Y;Sb}/+)\), measuring 0.86 ± 0.04, was smaller than that of any fly with \(\text{Ubx}^{P13}\). We conclude that these halter measurements reflect an accurate measure of the Ubx effect.

The unadjusted average halter size of wild-type males (0.57 ± 0.02) was approximately 75 times smaller than the average wing size of the same flies (42.79 ± 1.19). However, the unadjusted average halter size of \(\text{UbI}^+\text{+/Y;TM6}/+\) males (4.28 ± 0.54) was only 11 times smaller than average size wings (46.47 ± 1.66).

Homozygous mutations at the \(\text{Ubx}\) locus can elicit complete transformation of the third thoracic segment into the second (Lewis 1978). Thus, we might have expected \(Rpl1215\) alleles to enhance the mutant phenotype of \(\text{Ubx}^{P13}/+\) flies in other tissues besides the capitellum. Careful examination of the flies used for Figure 2 failed to identify transformations of the third thorax other than in the capitellum. In other words, neither legs nor notum showed any sign of transformation. The Ubx effect is restricted to the same tissue.
Dose effects of the C4 allele: The addition of an extra copy of the wild-type Rpl215 locus to C4-/+;Y;TM6/+ flies resulted in a slight increase in the average halter size (Figure 2B). This result is opposite to the expected decrease of halter size if C4 were behaving like a typical antimorph. However, this comparison of males to females used a large correction factor to compensate for the smaller size of males. We therefore wanted to test the effect of both ratios of mutant and wild-type polymerases in females. The capitellae measurements of such females are shown in Figure 3. The largest halteres were observed in C4-.+;TM6/+ flies, 3.49 ± 0.17. Halteres of C4-/+;TM6/+ flies measured 2.58 ± 0.14 and C4-+/C4;TM6/+ flies had still smaller halteres, 2.25 ± 0.07. Homozygous C4/C4;TM6/+ flies had an average capitellum size of 1.13 ± 0.06, which was similar to that of TM6/+ flies homozygous for wild-type Rpl215. It therefore appears that one copy of the C4 allele “out competes” one copy of wild-type Rpl215.

We also conducted crosses to monitor the effect of a null allele of the Rpl215 locus, n (MORTIN and LEFEVRE 1981; GREENLEAF et al. 1980). Measurements of capitellae sizes from +/n;Ubx130/+ flies were not statistically different from those of +/FM7;Ubx130/+ flies. This confirmed the observation that flies heterozygous for a null allele of the Rpl215 locus fail to show the Ubx effect. In addition, average halter sizes of heterozygous C4/n;Ubx130/+ flies were similar to those of wild-type flies (Figure 3).

Identification of mutations that modify the Ubx effect: In order to understand the Ubx effect better we induced mutations that altered this effect. Figure 1 diagrams the protocol used to this end (also see MATERIALS AND METHODS). Approximately 6200 n C4/+;Ubx130/DL7 progeny of mutagenized males were examined for an altered Ubx effect. Three such mutations, JH1, WJK1 and WJK2, were identified. All three mutations were mapped by meiotic recombination to the region of the X chromosome between n and m, which contains the Rpl215 locus. Complementation
tests confirmed that these new mutations were alleles of the RpII1215 locus.

Two of the new mutations, JH1 and WJK2, were hemizygous and homozygous viable; the latter mutation was female sterile. Both mutations suppressed the Ubx effect at 19°, 25° or 29° when made heterozygous with C4. In addition, they caused the effect themselves when heterozygous with a wild-type allele of the RpII1215 locus. This brought to five the number of alleles that display the Ubx effect: UbZ, C4, CI1, JHI and WJK2.

The third new mutation, WJK1, was a temperature-sensitive recessive lethal allele of RpII1215. Flies with this mutation lived at 19° (permissive temperature), died at 29° (restrictive temperature) and appeared to completely suppress the Ubx effect when heterozygous with C4 but only if raised at 29°. Unlike the previous two new mutations, WJK1/+ flies did not display the Ubx effect at 19°, 25° or 29°.

We did not recover null alleles of the RpII1215 locus in this experiment, nor were mutations in other loci recovered.

**Effect of interallelic interactions on the Ubx effect:** We compared the Ubx effects of flies possessing trans-heterozygous combinations of six RpII1215 alleles. These included the five alleles known to cause the Ubx effect and a wild-type RpII1215 allele present on a chromosome that also carries the visible mutations yf and f. The average halter sizes of Ubx+/+ female progeny are shown graphically in Figure 4, A–C. The six RpII1215 alleles can be ranked by the degree of Ubx effect they elicited when heterozygous with a wild-type allele. The order is yf (no effect) < JH1 < CI1 < WJK2 < C4 < Ubl (Figure 5). As was the case with Ubx+/+, the Ubx effect observed in the presence of Ubx+/+ is restricted to the capitelium.

Flies that were trans-heterozygotes of JH1, CI1, WJK2 and C4 with each other, failed to elicit the Ubx effect. Flies trans-heterozygous for only three of the four weaker alleles and Ubl lived. Each combination showed a reduced Ubx effect compared to Ubl/+; Ubx+/+, though the reduction seen in JH1/Ubl; Ubx+/+ flies was not significant (Figures 4, A–C, and 5).

**Enhancement of Dl:** The crosses used to examine the enhancement of Ubx+/+ by different RpII1215 alleles resulted in Dl+/+ siblings with the same polymerase combinations. Mutations at the Dl locus are usually haplo-insufficient and can cause mutant phenotypes in adults, including thickened wing veins, fusion of ommatidia producing a rough eye of reduced size, and additional bristles (VASSIN and CAMPOS-ORTEGA 1987). Unexpectedly, flies that inherited the Dl' chromosome and RpII1215 mutations displayed an increase in one of these mutant phenotypes. Many macrochaetae and microchaetae on the thoraces and heads of these flies were duplicated. The duplicate macrochaetae from these flies, formed in individual sockets, were the same size and had the same orientation (i.e., did not have mirror image symmetry) as the duplicated bristles. The same mutant phenotype was seen in flies with mutant RpII1215 alleles and heterozygous for either of two other Dl alleles, Dl' or Df(3R)DlM2 (data not shown). This discovery of a second autosomal locus enhanced by RpII1215 mutations permitted the first direct comparison of two different mutant phenotypes elicited by RpII1215 alleles.

We quantitated the duplicated bristles, as described in MATERIALS AND METHODS, of flies with various polymerase combinations (Figure 5). Flies heterozygous for the Dl' chromosome but wild type for the
The wild-type allele can be ranked as follows: 

<table>
<thead>
<tr>
<th>Allele</th>
<th>F1</th>
<th>C11</th>
<th>WJK2</th>
<th>C4</th>
<th>Ubl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JH1</td>
<td>1.02</td>
<td>1.31</td>
<td>2.2</td>
<td>4.6</td>
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<tr>
<td>C11</td>
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<td>1.06</td>
<td>3.1</td>
<td>N.D.</td>
<td>Lethal</td>
</tr>
<tr>
<td>WJK2</td>
<td>1.47</td>
<td>0.94</td>
<td>0.88</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>C4</td>
<td>2.2</td>
<td>N.D.</td>
<td>1.8</td>
<td>4.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Ubl</td>
<td>2.2</td>
<td>1.09</td>
<td>0.88</td>
<td>1.04</td>
<td>1.16</td>
</tr>
</tbody>
</table>

**Figure 5.—The Ubx and Dl effects were tabulated for flies heterozygous for either Ubx$^{130}$ or Dl$^7$.**

The crosses used to generate flies homozygous or trans-heterozygous for six different Rpl1215 alleles were the same as in Figure 4. The vertical axis represents one X chromosome, the horizontal axis represents the other. The magnitude of the Ubx (top number) and the Dl (bottom number) effects were tabulated for flies heterozygous for Ubx$^{130}$ and Dl$^7$, respectively.

**Rpl1215 locus averaged 1.0 duplicated bristle per fly.** The same number of duplicated bristles was observed on flies heterozygous for an amorphic allele of Rpl1215, +/n;Dl$^7$/+ (data not shown). The five Rpl1215 alleles that displayed the Ubx effect also increased the number of duplicated bristles in Dl$^7$/+ flies. Flies heterozygous for different Rpl1215 alleles and the wild-type allele can be ranked as follows: γf (no effect) < WJK2 < JH1 = C11 < C4 < Ubl (Figure 5).

Surprisingly, all of the Rpl1215 alleles that elicited the DI effect in flies that were heterozygous with a wild-type allele continued to do so as homozygotes or trans-heterozygotes. This contrasted with the suppression of the Ubx effect in flies with these same Rpl1215 combinations. It should be noted, however, that some of the stronger DI effects were reduced in homozygous and trans-heterozygous flies (Figure 5).

**Temperature effects on C4/WJK1 flies:** We tested the ability of the conditional-lethal allele, WJK1, to alter the Ubx and DI effects of C4 in flies raised at different temperatures. Halter sizes from sibling C4/FM7;Ubx$^{130}$/+ and C4/WJK1;Ubx$^{130}$/+ flies raised at 19°, 25° and 29° were compared in Figure 6A. Flies with the two genotypes had similar capitellae sizes when raised at 19°. However, the halteres of C4/WJK1;Ubx$^{130}$/+ flies raised at 25° and 29° were significantly reduced compared to their siblings. The Ubx effect was completely suppressed in C4/WJK1;Ubx$^{130}$/+ flies raised at 29°, as they had an average capitellum size similar to Ubx$^{130}$/+ flies.

We also noticed a reduction in the size of halteres in C4/FM7;Ubx$^{130}$/+ flies upon increasing the temperature at which they were reared from 19° to 25° or 29°. This change in halter size was reproducible and statistically significant. Previous reports had shown that the C4 mutation did not display temperature dependent effects on viability (Voelker et al. 1985; Mortin and Kaufman 1982); however, the Ubx effect caused by C4 is temperature dependent. Also, note that the viability of C4/WJK1;Ubx$^{130}$/+ flies reared at 19° was greatly reduced. Only ten flies were recovered from a cross that yielded more than 100 each of C4/FM7;Ubx$^{130}$/+ and C4/WJK1;Dl$^7$/+ siblings. This reduced viability most likely results from the strong Ubx effect elicited at this temperature.

The DI effect of sibling C4/FM7;Dl$^7$/+ and C4/WJK1;Dl$^7$/+ flies raised at the same temperatures described above were also compared. The result of bristle counts are displayed in Figure 6B. Surprisingly, the DI effect of C4 was increased by WJK1 when flies were raised at restrictive temperature. In addition to having an increased number of duplicated bristles, the delta wing vein and rough eye phenotypes were also greatly increased, with eyes being less than half their normal size. Flies with the strongest mutant phenotype had greatly reduced viability as only ten flies eclosed compared to more than 100 each of C4/FM7;Dl$^7$/+ and C4/WJK1;Ubx$^{130}$/+ siblings.

**Temperature shift experiments:** We used the large difference in halter size resulting from rearing C4/WJK1;Ubx$^{130}$/+ flies at permissive or restrictive temperatures to determine the stage in development required for flies to be at permissive temperature in order to cause the maximum Ubx effect. The data for this experiment are summarized in Figure 7A. Flies raised at either temperature until the middle or late third instar larval stage had halter sizes similar to flies reared at the temperature to which they were shifted. Flies shifted during late third instar, white pupal or middle pupal stages had intermediate phenotypes, and those shifted after the middle pupal stages had the phenotype of flies reared at the starting temperature. In other words, the phenotypic critical period is between the stages middle-to-late third instar larva and middle pupa.

We also used the differential effect of temperature on the DI effect of C4/WJK1;Dl$^7$/+ flies to examine the effect of temperature shifts on this phenotype (Figure 7B). The phenotypic critical period for the expression of the maximum DI effect was determined to be between the second larval instar stage and the middle of the pupal stage.

Other mutant phenotypes caused by Rpl1215 al-
leles: Three other mutant phenotypes were readily observed in flies possessing different RpII215 alleles. The series of crosses shown in Table 1 were conducted to investigate the cause of these phenotypes.

First, 80% of Ubl.+/Y;TM6/+ males exhibited complete or near complete pigmentation of their fourth abdominal segments. Males with other combinations of RpII215 mutations looked like wild-type males. They had a dark stripe of pigment at the posterior margin of abdominal segments 2–4; only segments 5 and 6 were fully pigmented. The appearance of pigment on the fourth tergite of Ubl.+/Y;TM6/+ flies suggests that it was transformed into the fifth or sixth segment. This phenotype is also caused by the mutation Miscadestral pigmentation or Mcp (LEWIS 1978).

A second mutant phenotype was observed in 65% of C11.+/Y male progeny. These flies displayed from one to six sex comb teeth on second tarsal segments of their first legs. The normal complement of teeth was still present on the basitarsal segment of each first leg. No other combination of RpII215 alleles elicited this mutant phenotype.

A third mutant phenotype was observed in the presence of the dominant mutation Stubbled (Sb). This mutation by itself results in a shortening and thickening of bristles (LINDSLEY and GRELL 1968). Most combinations of polymerase mutations had no apparent effect on this phenotype. However, approximately 20% of the Ubl.+/Y;Sh/+ and ras v Ubl/ Dp(1;Y)B5-v*Yy;Sh/+ progeny generated in the C and B series of crosses (Table 1) displayed wings that were shorter and wider than wild-type wings. Wing veins often failed to reach the margins of these abnormal wings.

The series of crosses shown in Table 1 were conducted to further investigate the newly observed mu-
TABLE 1

Analysis of mutant phenotypes caused by RpII215 mutations

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Mutant phenotypes</th>
<th>Mcp-1&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Sex&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SB&lt;sup&gt;3&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td><strong>Original crosses</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>y Ubl f.+/FM7;TM6/Sb</td>
<td>y Ubl f.+/Y;TM6/+</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>y v C4.+/FM7;TM6/Sb</td>
<td>y v C4.+/Y;TM6/+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>y C11.+/FM7;TM6/Sb</td>
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* Miscadestral pigmentation like phenotype.
† Sex comb teeth on the second tarsal segment of the leg.
‡ Stubble interaction affecting wing shape and size.
− = No effect, + = mild effect, and ++ = strong effect.

M. A. Mortin, W. J. Kim and J. Huang

**DISCUSSION**

The present study had three goals: 1) To determine whether or not the mechanisms by which different
Rpi215 alleles cause the Ubx effect are the same or related. 2) to determine the extent of the Ubx enhancement, and 3) to examine whether the Ubx effect can best be explained by a general reduction in transcription.

**Do Rpi215 alleles elicit the Ubx effect by a common mechanism?** This question was addressed by inducing three new mutations that block the transformation of halter into wing by the C4 allele. Two of these new mutations, jH1 and WJK2, themselves elicit the Ubx effect, bringing to five the number of such alleles. The third mutation, WJK1, behaves like a conditional null allele of the Rpi215 locus and does not elicit the Ubx effect. The Rpi215 alleles we studied can be placed into groups with respect to the ability to elicit the Ubx effect. Class I alleles include Ub1, C4, C11, jH1 and WJK2. These alleles most strongly elicit the Ubx effect in flies heterozygous for a wild-type Rpi215 allele, which we place in class II. A third class is defined by the alleles, n and WJK1. These alleles behave like deficiencies for the Rpi215 locus, as they fail to eliciting the Ubx effect when heterozygous with either class I or II alleles. Note that WJK1 acts like a class III allele at restrictive temperature but like a class II allele at permissive temperature.

The four weakest class I alleles, C4, C11, jH1 and WJK2, each causes the Ubx effect to a different degree when heterozygous with class II alleles. However, in all cases tested, flies homozygous or hemizygous for these alleles failed to show the Ubx effect. Flies trans-heterozygous for these four class I alleles also failed to display the Ubx effect (Figure 5). The similar behavior of these four class I alleles indicates that they elicit the Ubx effect by a common mechanism.

Flies trans-heterozygous for Ub1 and any of the other class I alleles never returned to the wild-type phenotype, although the magnitude of the Ubx effect was reduced in all cases tested. This is in sharp contrast to the four weaker class I alleles (Figure 5). At least two possibilities could explain this difference. All five class I alleles may actually elicit the Ubx effect by the same mechanism, with observed differences representing a series of strengths. The Ub1 allele elicits a much stronger Ubx effect than the four weaker class I alleles. The weakness of these four class I alleles may be unable to counter the effect of Ub1. This is supported by the observation that three class I alleles, jH1, WJK2, and C4, can be ranked in order by the degree to which they elicit the Ubx effect, whether heterozygous with a class II allele or trans-heterozygous with Ub1 (Figure 5). Alternatively, there may be two lesions caused by Ub1. One of the lesions might be shared with other class I alleles, resulting in partial complementation by these alleles. The second lesion could be responsible for eliciting the residual mutant phenotype.

The same rank order that exists for class I alleles exhibiting the Ubx effect does not exist for viability. Two of the alleles cause recessive lethality, Ub1, the strongest and C11, one of the weakest, though both live as hemizygous males under special conditions (Voelker et al. 1985). We have been unable to separate the recessive lethality and the Ubx effect caused by these mutations (Morton and LeFevre 1981; our unpublished data). Other recessive-lethal alleles (e.g., the apparent amorphic allele, n) do not cause the Ubx effect. The intermediate class I alleles, C4 and WJK2, are homozygous viable. Thus, if enzymatic activity can be correlated with viability, it is not the activity of a given gene product per se that determines its ability to elicit the Ubx effect.

The C4 allele does not behave like a typical antimorph. The addition of an extra wild-type Rpi215 allele increases the Ubx effect in C4/+ flies. The class I allele, C4, out competes Rpi215*. This is in contrast to another class I allele, Ub1, which behaves like a classic antimorph (Morton and LeFevre 1981). The balance between class I and II gene products may in part determine the strength of the Ubx effect. This could occur by class I alleles encoding mutant proteins with different affinities for DNA or other macromolecules. The antagonistic interaction between class I and II alleles might then result from a competition for a binding site(s) by their different protein products. Alternatively, the stability of mutant Rpi215 gene products might vary, so that in order to maintain a balance that would elicit the maximum Ubx effect, more of one product would have to be synthesized.

**What is the nature of the Ubx enhancement?** The temperature-sensitive period of the Ubx effect starts in the middle of the third larval instar stage and lasts until the middle of the pupal stage (Figure 7A). This is unexpected as wild-type expression of the Ubx gene is not required for normal development later than 8–16 hr prepuparium formation (Morata and Garcia-Bellido 1976). It is difficult to reconcile these results with the hypothesis that Rpi215 alleles cause the Ubx effect by reducing the transcription of Ubx. The developmental timing of the Ubx effect is consistent with an effect caused by the abnormal functioning of Ubx protein(s). This is particularly interesting because the Ubx protein is thought to be a DNA binding protein (Laughon and Scott 1984) and might itself interact with polymerase. In addition, the restriction of the Ubx effect to the same tissue, the capitelum, as the initial mutant phenotype elicited by Ubx suggests that Rpi215 alleles may be responding to a defect caused by Ubx and not simply reducing overall expression of Ubx.

Alternatively, the unusual timing of the Ubx effect may be due to the perdurance of the Ubx protein. Wild-type Ubx expression is normally not required
after late larval stages; however, it can be demonstrated to affect differentiation of cultured halter discs (Adler 1981). The late temperature-sensitive period observed for the Ubx effect may reflect the last time in development when wild-type Ubx product can be made to ameliorate an earlier defect caused by RpII215 mutations.

The mutation Ubl also interacts with Ubx to result in the transformation of the fourth abdominal segment into the fifth or sixth. This mutant phenotype resembles one caused by a dominant gain-of-function mutation in the bithorax-complex, Mef (Lewis 1978). The Mef-like phenotype was not observed in flies mutant for C4 or C11. The phenotype caused by Mef is thought to result from the misexpression of another gene of the bithorax complex, identified as Abd-B (Sanchez-Herrero et al. 1985; Tiong, Bone and Whittle 1985), in a segment more anterior than it is normally expressed. The transformation elicited by the interaction between Ubl and Ubx might indicate that wild-type Ubx can act to negatively regulate expression of Abd-B. Another possibility is that the mutant phenotype is the result of reduced expression of other genes that regulate the bithorax complex, for example Polycomb (Lewis 1978).

Do polymerase mutations elicit mutant phenotypes by a general reduction in transcription? While we have described several mutant phenotypes elicited by RpII215 mutations, the best characterized in this study are the enhancement of mutations in the Dl and Ubx loci. Two similarities were evident in comparing the Dl and Ubx effects. First, class I alleles cause both effects, though the Dl effect may also be caused by other RpII215 alleles. Second, the class I alleles that elicit the strongest Ubx effect (Ubl and C4) also cause the strongest Dl effect. However, unlike the Ubx effect, negative interactions between class I and II alleles are not required to elicit the Dl effect. The Dl effect is the result of a dominant action of class I alleles and is observed in homozygotes and hemizygotes as well as trans-heterozygotes involving different class I alleles. A mechanism different from that responsible for eliciting the Ubx effect seems responsible for the Dl effect. This is also evident from examining the effects of temperature on trans-heterozygotes of C4 and the conditional null allele, WJK1. Figures 6A and 6B show that at restrictive temperature, while the conditional null allele suppresses the Ubx effect of C4, it enhances the Dl effect.

The occurrence of multiple alleles of the RpII215 locus eliciting similar mutant phenotypes suggests that different lesions may result in the same altered function of RNA polymerase II. The simplest explanation for this is that class I alleles exhibit a global reduction in the efficacy of transcription. Mutant phenotypes are only observed in cases where normal gene expression is initially near a threshold necessary for wild-type function, but which have been lowered below this threshold by polII mutations. It is difficult to reconcile such a general effect with the specific and nonparallel defects shown in Table 1. In addition, the apparent similarity of the Ubx and DI effects caused by the same class I alleles have been shown to have different genetic requirements for mutant expression (Figures 5 and 6, A and B). In order to explain these specific phenotypic differences in terms of global effects on transcription, we must postulate that the general effects alter the expression of a series of positive and negative regulators, which in turn affect the threshold of genes required for normal Dl and Ubx function.

An alternative explanation is also suggested by these data. The large subunit of RNA polymerase II may directly interact with specific promoters. Mutations in RpII215 could alter recognition in a promoter-specific manner. This explanation draws support from the observations of Suguita et al. (1977) that mutations in the b' subunit of Escherichia coli RNA polymerase alter the recognition of only some promoters. Given the high degree of amino acid positional identity between the large subunit of Drosophila melanogaster RNA polymerase II and the b' subunit of E. coli RNA polymerase (Biggs, Searles and Greenleaf 1985), it is possible that specific RpII215 alleles could elicit a discrete set of mutant phenotypes by a similar mechanism.

We thank J. Birchler, D. A. Hursh and B. J. Rutledge for comments on this manuscript. The research was supported by grants from the National Institutes of Health awarded to M. Meiselson. M.A.M. is supported by a grant from the Medical Foundation.

LITERATURE CITED


Communicating editor: W. M. GELBART