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Mutations in the Second-Largest Subunit of Drosophila RNA Polymerase II Interact with Ubx

Mark A. Mortin,*[,1] Richard Zuerner,*[,2] Shelley Berger*[,3] and Barbara J. Hamilton†[,4]

**Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138, and †Department of Biochemistry, Duke University Medical School, Durham, North Carolina 27710*

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ABSTRACT

Specific mutations in the gene encoding the largest subunit of RNA polymerase II (*RpII215*) cause a partial transformation of a structure of the third thoracic segment, the capitellum, into the analogous structure of the second thoracic segment, the wing. This mutant phenotype is also caused by genetically reducing the cellular concentration of the transcription factor Ultrabithorax (Ubx). To recover mutations in the 140,000-D second-largest subunit of RNA polymerase II (*RpII140*) and determine whether any can cause a mutant phenotype similar to *Ubx* we attempted to identify all recessive-lethal mutable loci in a 340-kilobase deletion including this and other loci. One of the 13 complementation groups in this region encodes RpII140. Three *RpII140* alleles cause a transformation of capitellum to wing but unlike *RpII215* alleles, only when the concentration of Ubx protein is reduced by mutations in *Ubx*.

SOME mutations in the largest subunit of *RNA polymerase II* (*215*,000 D), encoded by the *RpII215* locus (GREENLEAF 1983) cause discrete developmental defects (MORTIN and LEFEVRE 1981; VOELKER *et al.* 1985; MORTIN, KIM and HUANG 1988). The most striking of these is a partial transformation of the capitellum, a part of the halter structure of the third thoracic segment, into a wing, a structure of the second thoracic segment. The same mutant phenotype is caused by the loss of one wild-type copy of the *Ultrabithorax* (*Ubx*) locus, which encodes a transcriptional regulatory protein (JOHNSON and KRASNOW 1990) required for proper development of the entire third thoracic segment (LEWIS 1978).

Five *RpII215* alleles, *Ubl*, *4*, *7*, *H1* and *K2*, cause a *Ubx*-like phenotype, even when two wild-type copies of *Ubx* are present (MORTIN, KIM and HUANG 1988; Note that allele designations have been changed to correspond to Lindsley and ZIMM 1992). This transformation of capitellum to wing is synergistically increased by deleting one of the wild-type copies of *Ubx*, though it is still restricted to the capitellum. A detailed description of these interactions, which are called the "Ubx effect," can be found elsewhere (MORTIN, KIM and HUANG 1988).

The transformation of capitellum into wing caused by *Ubx* mutations is a consequence of the reduction of the cellular concentration of Ubx protein since deletions (and null alleles) that cause the most complete transformation show a loss of Ubx protein (WHITE and WILCOX 1985; BEACHY, HELFAND and HOGNESS 1985). *RpII215* mutations that cause the Ubx effect alter the functioning of RNA polymerase II as deletions do not cause the mutant phenotype. These observations lead to the formulation of two hypotheses to explain the Ubx effect. First, RNA polymerase II mutations might reduce the level of Ubx protein by lowering transcription of *Ubx*. RNA polymerase II transcribes *Ubx* (BIGGIN and TJIAN 1988) and the defect seen as the Ubx effect is phenotypically equivalent to a reduction in the expression of *Ubx*$^+$. Support for this hypothesis comes from the report that precursor cells for the adult capitellum in *Ubl/+;Ubx/+* late third instar larval halter discs have reduced levels of Ubx protein (BOTAS, CABRERA and GARCIA-BELLIDO 1988).

The second hypothesis is based on three experiments. (1) Clonal analysis suggests that transcription of *Ubx* is required from the cellular blastoderm stage until 16 hr prior to pupation (MORATA and GARCIA-BELLIDO 1976). (2) *RpII215*^{4} acts from the middle of the third larval instar stage until the middle of the pupal stage to partially transform capitellum into wing (MORTIN, KIM and HUANG 1988). (3) Purified Ubx protein can activate and repress specific promoters in

We dedicate this paper to the memory of GEORGE LEFEVRE, JR.

[1] Corresponding author's current address: Laboratory of Biochemistry, NIH/NCI, Building 37, Room 4C-19, 9000 Rockville Pike, Bethesda, Maryland 20892-0037.
[2] Current address: National Animal Disease Center, 2300 Dayton Road, Ames, Iowa 50010.
[3] Current address: Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.
[4] Current address: Howard Hughes Institute, Department of Biology, Indiana University, Bloomington, Indiana 47405.

an *in vitro* transcription assay, suggesting that it physically contacts or directly signals RNA polymerase II (JOHNSON and KRASNOW 1990). The Ubx effect may result from mutationally altered RNA polymerase II being unable to respond correctly to a given level of Ubx protein, thus altering the expression of genes regulated by *Ubx*.

The two aforementioned hypotheses are not mutually exclusive. Different *RpII215* alleles were used by BOTAS, CABRERA and GARCIA-BELLIDO (1988) and MORTIN, KIM and HUANG (1988), *Ubl* and *4*, respectively. The latter study postulated that the stronger effect elicited by *Ubl* could result from disruption of two steps during transcription, while only one of these is affected by *4*. Clearly more studies are required to determine the molecular mechanism behind the genetic interaction of *Ubx* and RNA polymerase II.

RpII215 encodes one subunit of a multimeric enzyme composed of approximately 11 unique polypeptides (YOUNG 1991). Our approach to determining the role of individual subunits is to mutate them and characterize the resulting developmental and molecular effects caused by the altered functioning of the enzyme. Of special interest is the question of whether mutations in other subunits of RNA polymerase II can cause the Ubx effect.

The second-largest subunit of *R*NA *p*olymerase *II* (*140*,000 D), encoded by the *RpII140* locus, has been cloned and mapped by chromosomal hybridization *in situ* to polytene interval 88A/B (FAUST *et al.* 1986). We describe in this paper an attempt to genetically saturate the region around *RpII140* with recessive-lethal mutations. We show that three of 31 alleles of one locus, *A5*, cause the Ubx effect. Molecular confirmation that the *A5* locus encodes the second-largest subunit will be presented elsewhere (HAMILTON 1990; Y. CHEN and A. GREENLEAF, personal communication).

MATERIALS AND METHODS

Strains and culture conditions: Isogenic third chromosomes carrying *red e* (red eyes and ebony body color) were used in the mutation screens described below. The *red e* stock was obtained from Indiana University (SCOTT *et al.* 1983). Deficiencies for the 88A/B interval were obtained from the Bowling Green State University and California Institute of Technology stock centers and from R. KELLEY (PARKHURST *et al.* 1988). Most are described in LINDSLEY and ZIMM (1992). The multiply marked *rucuca* third chromosome was used for meiotic mapping.

Stocks were maintained on cornmeal-agar-sugar-yeast food and raised at 19°, 23°, 25° or 29° all ±1°.

Mutagenesis and crosses: The crosses used to identify and recover recessive-lethal mutations are diagrammed in Figure 1. Three-day-old virgin *red e* males were mutagenized either by exposure to ^{137}Cs to a dose of 4,000 r (experiments A and B) or by treatment with 0.024 M ethyl methanesulfonate (EMS, experiments Z and M) according to LEWIS and BACHER (1968). These males were then mated to females with two different third chromosome balancers, *TM6B/TM3*. The F_1 progeny were individually mated to $Df(3R)red^{P52}/TM6B$ flies at 25° (A and B), 29° (Z) or 19° (M). Newly induced recessive-lethal mutations were identified by the failure of red eyed flies to eclose. Siblings carrying the putative recessive-lethal mutation and *TM6B* were identified by their ebony, Tubby and Humeral mutant phenotypes and used to establish stocks.

Quantitation of the Ubx effect: Capitellum size was measured as described elsewhere (MORTIN, KIM and HUANG 1988). Measurements were compared to that of an average capitellum on a wild-type fly, which is defined as one unit squared. The *t*-test was used to calculate 95% confidence intervals for the measurements. The temperature-sensitive period of $RpII140^{Z43}$ was determined as described elsewhere (MORTIN, KIM and HUANG 1988).

RESULTS

To study the role of individual subunits of RNA polymerase II on the expression of developmentally regulated genes we set out to: (1) identify mutations in the locus encoding the second largest subunit, and (2) compare their effects *in vivo* to existing mutations in the largest subunit.

Recovery of recessive-lethal mutations: A Southern blot analysis demonstrated that $Df(3R)red^{P52}$, which deletes cytological region 88A8;88B4, removes the *RpII140* locus (data not shown). The second-largest subunit of RNA polymerase II is encoded by a single copy gene (FAUST *et al.* 1986), is essential for viability in yeast (SWEETSER, NONET and YOUNG 1987) and is therefore also likely to be required for Drosophila viability. To identify mutations in the *RpII140* locus we first attempted to saturate the region removed by $Df(3R)red^{P52}$ with recessive-lethal mutations. The results of four mutagenesis experiments are presented in Table 1. Three recessive-lethal mutations were induced with γ-rays (experiments A and B) and 95 were induced with EMS (experiments Z and M).

Mapping complementation groups in the $Df(3R)red^{P52}$ region: Overlapping deficiencies were used in complementation tests to subdivide the recessive-lethal mutations into cytologically distinct regions. They were then placed into 11 complementation groups and ordered along the chromosome as shown in Figure 2. This was accomplished by crossing all recessive-lethal alleles to representative alleles (the first identified allele) for each complementation group and scoring for viability. All 98 mutations were placed into single complementation groups except *M36* and *M49*, which were placed in *l(3)88Ac* and *l(3)88Ae*, respectively, but which also fail to complement each other. We will tentatively assign this locus the designation *l(3)88Ad*. This may: (1) suggest that *l(3)88Ac*, *l(3)88Ad* and *l(3)88Ae* actually identify a single gene complex, (2) identify an additional locus within this region, or (3) result from a chance induction (or the prior existence) of second recessive-lethal mutations in a locus mapping outside $Df(3R)red^{P52}$. All three

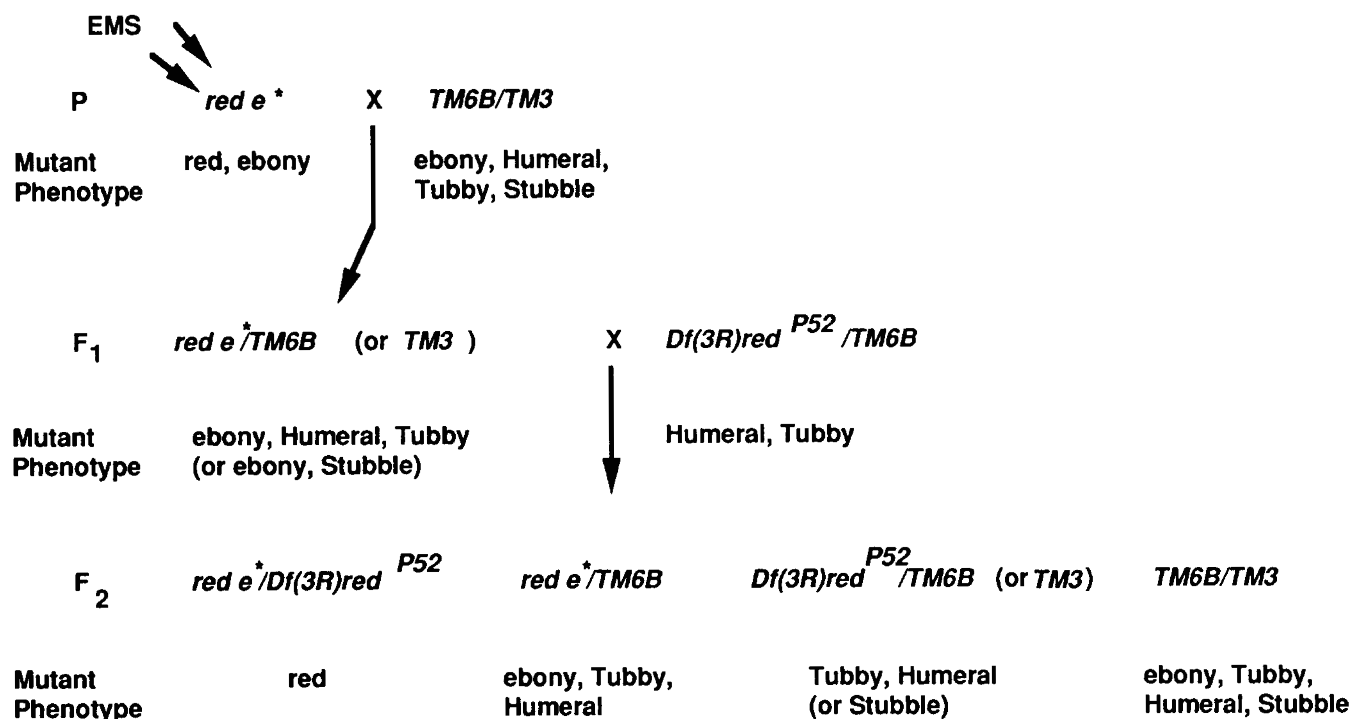

FIGURE 1.—Scheme for recovering mutations in the region deleted by $Df(3R)red^{P52}$. Mutagenized (indicated by the asterisk) isogenic *red e* flies were crossed to flies doubly balanced for the third chromosome balancers *TM6B/TM3* (P generation). Single males were mated to 1 or 2 females carrying the $Df(3R)red^{P52}$ chromosome (F_1 generation) and their progeny scored for the absence of red eyed flies in the F_2 generation. Stocks were established from ebony, Tubby, Humeral flies recovered in the F_2 generation.

TABLE 1

Mutagenesis results

Mutagen	No. matings	No. sterile	No. lethal	Frequency of lethals[a]
Gamma rays				
A and B[b]	4,254	838	3	0.0009
EMS				
Z[c]	12,382	4,925	52	0.007
M[d]	10,613	3,881	43	0.006

[a] Recessive-lethal mutations as a frequency of fertile matings.
[b] Flies were reared at 25°.
[c] Flies were reared at 29°.
[d] Flies were reared at 19°.

complementation groups affect activity of the enzyme dipeptidase B (J. COLLETT, personal communication), which HALL (1986) mapped to the region around $Df(3R)red^{P52}$, suggesting that the first hypothesis may be correct.

Complementation groups were tested for allelism with previously identified loci in the $Df(3R)red^{P52}$ region. *Z33* and *A7* alleles fail to complement *l(3)K43* and *trx alleles*, respectively. Molecular confirmation of the identity of *A7* as *trx* comes from the finding that the *B11* allele is an internal deletion resulting in a frame shift of the *trx* protein coding region (MAZO *et al.* 1990). The remaining nine complementation groups are not allelic with previously defined loci. No alleles of *su(Hw)* were recovered suggesting that it cannot be mutated to cause recessive lethality. Allelism with the *red* locus cannot be determined because the parent chromosome already possesses a recessive allele of this locus. HARRISON, MORTIN and CORCES (1992) showed that the *Z23* mutation is a 174-base pair internal deletion of a 15-kD protein with sequence identity to the ninth largest subunit of yeast RNA polymerase II (WOYCHIK, LANE and YOUNG 1991). This locus will henceforth be referred to as *RpII15*.

The order of *A5* and *Z6* cannot be determined from existing mutations. The order of *trx*, *red* and *su(Hw)* was determined by BREEN and HARTE (1991). The *su(Hw)* and *RpII15* loci are ordered by their molecular cloning and inclusion in the cytologically invisible deletion $Df(3R)su(Hw)^{V}$ (PARKHURST *et al.* 1988; HARRISON, MORTIN and CORCES 1992). The order of *M1* and *M41* was not determined with respect to *trx*, *red*, *su(Hw)* and *RpII15*. All complementation groups were mapped with respect to the map positions of mutations on the *rucuca* third chromosome. All map between *cu* (86D) and *sr* (90D/F), including the 11 *A5* alleles tested. This is the same interval that contains $Df(3R)red^{P52}$ (88A8;88B4).

Hybridizations *in situ* have shown that the *RpII140* locus maps within the cytological region deleted by $Df(3R)red^{P1}$ but not $Df(3R)red^{P93}$ (HAMILTON 1990). Only the *A5* and *Z6* complementation groups map to this interval (Figure 2). Molecular confirmation that the *A5* locus is *RpII140* comes from rescue of *A5* with

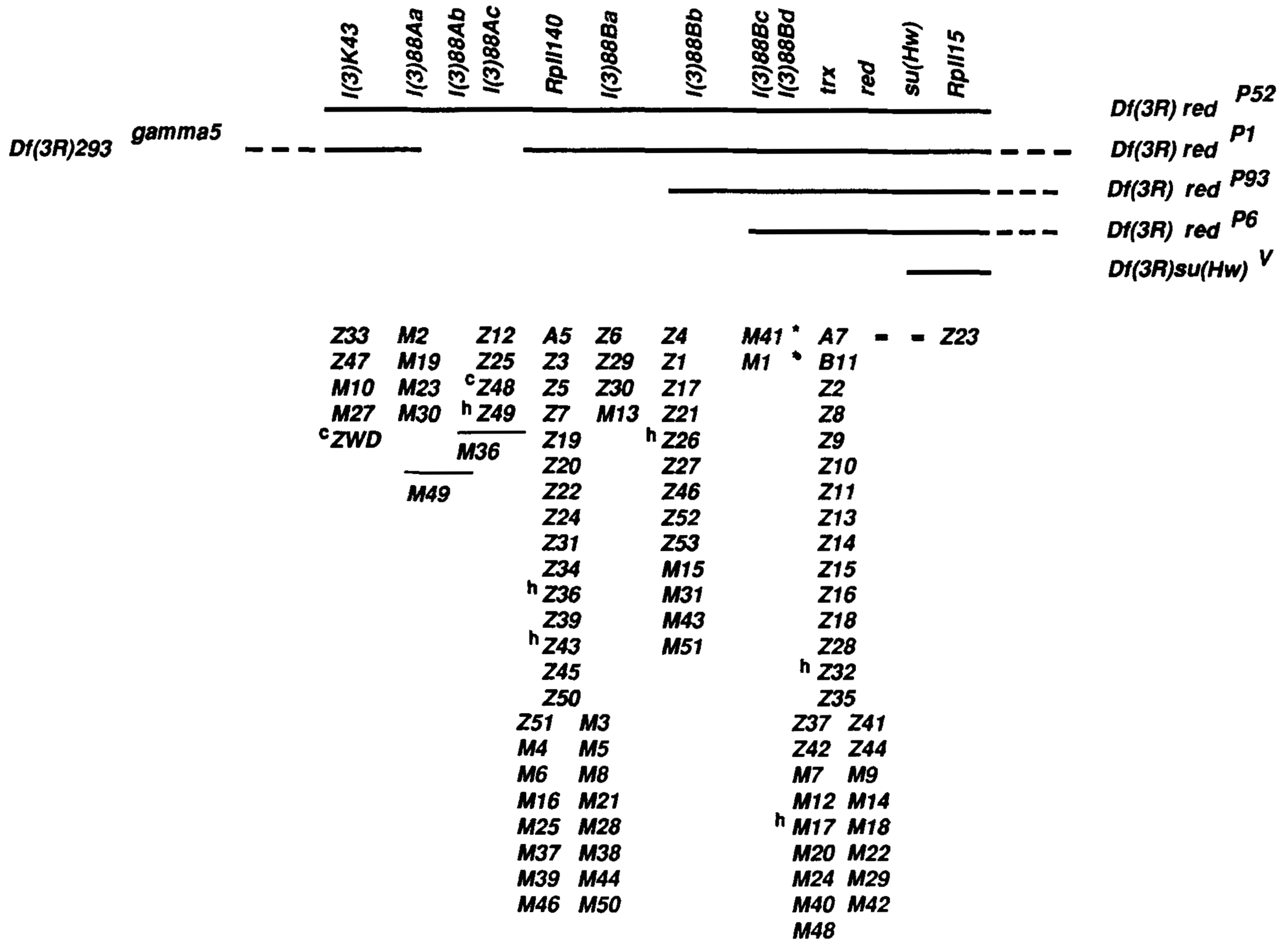

FIGURE 2.—Genetics of the *RpII140* region. Recessive-lethal mutations from Table 1 were placed into 11 of the 13 complementation groups identified in this region. Their order was determined using overlapping deficiencies (solid lines). The order of *RpII140* (*A5*) and *l(3)88Ba* (*Z6*) was not determined. Also, the relative position of $l(3)88Bc^{M1}$ and $l(3)88Bd^{M41}$ (*) was not determined with respect to *trx*, *red*, and *su(Hw)-RpII15*. The map positions of *trx*, *red* and *su(Hw)* were provided by BREEN and HARTE (1991). Heat sensitive (h) and cold sensitive (c) alleles are indicated.

the cloned *RpII140* gene (HAMILTON 1990; B. J. HAMILTON and A. GREENLEAF, unpublished data) and the molecular mapping of a lesion in the *A5* allele (Y. CHEN and A. GREENLEAF, personal communication). Henceforth we will refer to the *A5* locus as *RpII140*.

Identification of conditional lethal alleles: We determined whether flies heterozygous for each of the 98 recessive-lethal alleles and $Df(3R)red^{P52}$ are viable at 19° and 29°. Flies heterozygous for six different recessive-lethal mutations and $Df(3R)red^{P52}$ die at 29° but survive at 19°, indicated by a superscript h in Figure 2. These include 2 *RpII140* alleles. Flies heterozygous for two additional mutations and $Df(3R)red^{P52}$ live at 29° but die at 19°, indicated by a superscript c in Figure 2.

Do *RpII140* alleles cause the Ubx effect? Unlike some *RpII215* alleles, none of the 31 *RpII140* alleles display a dominant *Ubx*-like phenotype (*i.e.*, transformation of capitellum into wing) in the presence of two wild-type copies of Ubx^+. However, three alleles cause a dominant enhancement of the Ubx effect in flies where one of the normal copies of Ubx^+ is removed, for example in flies heterozygous for $Df(3R)Ubx^{109}$. It is important to note that a deficiency of *RpII140* does not cause this dominant enhancement.

One explanation for the enhancement of *Ubx* by three *RpII140* alleles is that the latter may actually carry weakly expressive *Ubx* mutations on the same chromosomes. This was tested by recombining either of the *Ubx* null mutations $Ubx^{9.22}$ or $Df(3R)Ubx^{109}$ onto the chromosomes carrying *RpII140* alleles, thus removing the Ubx^+ allele present on the mutagenized chromosome. The same transformation of capitellum to wing is observed whether *Ubx* mutations are in *cis* or *trans* to *RpII140* alleles (*RpII140 Ubx*/+ + *vs. RpII140* +/+ *Ubx*). We conclude that the transformation of capitellum to wing is a property of specific *RpII140* alleles and therefore they must cause the Ubx effect.

Measurement of the Ubx effect caused by RNA polymerase II mutations: To better characterize the

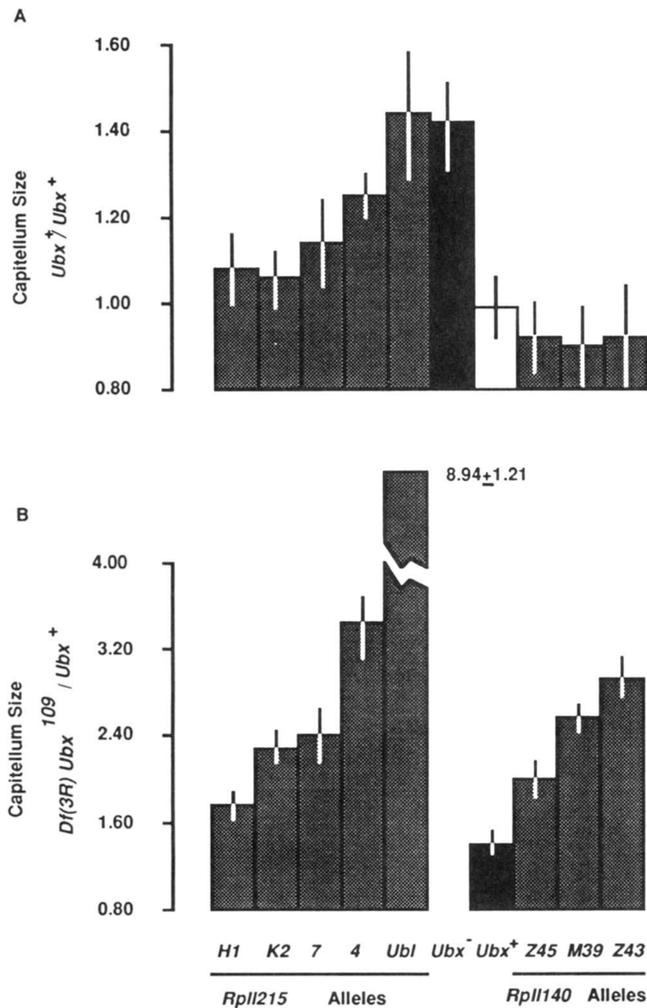

FIGURE 3.—Effect of *RpII215* or *RpII140* alleles on capitellum size. Capitellum size was measured as described in MORTIN, KIM and HUANG (1988), except that the measurements are not adjusted to a standard fly size. We define the size of a capitellum on a wild-type fly as equal to 1 unit squared (white bar). Black bars show the size of a capitellum on flies lacking RNA polymerase II mutations but carrying only one copy of Ubx^+. Gray bars show the effect of RNA polymerase II mutations on capitellum size in the presence of two (A) or one (B) copy of Ubx^+ (n = 10, vertical lines indicate 95% confidence intervals).

Ubx effect caused by *RpII140* alleles and to directly compare them to *RpII215* alleles, we crossed flies carrying the three *RpII140* and five *RpII215* alleles that cause the Ubx effect to flies heterozygous for the deficiency $Df(3R)Ubx^{109}$. Capitellum size was measured for flies with either two or one wild-type copy(ies) of Ubx^+ (Figure 3, A and B, respectively). We previously showed that an increase in capitellum size is a good indicator of the degree of transformation of capitellum to wing (MORTIN, KIM and HUANG 1988). Here we define a wild-type capitellum size as 1.00 unit^2. Flies with only one copy of Ubx^+ have a capitellum size of 1.42 (Figure 3).

All five *RpII215* alleles cause a dominant partial transformation of capitellum to wing even in the presence of two wild-type copies of Ubx^+ (Figure 3A). In fact, the capitellum size of heterozygous $RpII215^{Ubl}/+$ flies is indistinguishable from that of heterozygous $Df(3R)Ubx^{109}/+$ flies. In contrast, when two copies of Ubx^+ are present, the capitellum size of flies mutant for any *RpII140* allele is not different from that of wild-type flies (Figure 3A). The partial transformation of capitellum to wing caused by five *RpII215* alleles is dramatically increased by deleting one wild-type copy of Ubx^+. Three *RpII140* alleles also greatly increase the capitellum to wing transformation seen in flies with only one copy of Ubx^+ even though they have no effect in the presence of two copies of Ubx^+ (Figure 3, A and B).

***RpII140* alleles do not enhance the Ubx effect caused by *RpII215* alleles:** The capitellum size of flies heterozygous for either *RpII215* allele *Ubl* or *4* and $RpII215^+$ was examined in a $RpII140^+$ or heterozygous $RpII140/RpII140^+$ background. Heterozygotes for any of the three *RpII140* alleles that cause the Ubx effect were compared with $RpII140^+$ flies (Figure 4). None of the three *RpII140* alleles increases the transformation of capitellum to wing caused by *RpII215* alleles in the presence of two wild-type copies of Ubx^+ (Figure 4A). Thus, even though the transformation of capitellum to wing observed in $RpII215^{Ubl}/+$ flies is phenotypically indistinguishable from that of $Df(3R)Ubx^{109}/+$ flies, three *RpII140* alleles increase the transformation in the latter (Figure 3B) but not the former (Figure 4A).

The same comparison described above was carried out in the presence of only a single copy of wild-type Ubx^+. Figure 4B shows the capitellum size of $RpII215^{Ubl}/+;Df(3R)Ubx^{109}/+$ or $RpII215^4/+;Df(3R)Ubx^{109}/+$ flies in the presence of wild-type or heterozygous mutant *RpII140*. In flies missing one wild-type copy of Ubx^+ and mutant for *RpII215*, the capitellum size is increased when a *RpII140* mutation that causes the Ubx effect is crossed into the background. We consistently see small increases in capitellum size except for the $RpII215^4$ and $RpII140^{M39}$ combination, which shows a dramatic increase in size. We conclude that the Ubx effects elicited by most pairs of *RpII140* and *RpII215* alleles tested appear to be additive. This result suggests that the two subunits are acting independently of one another with respect to the Ubx effect. The large increase in the Ubx effect seen with the combination of $RpII215^4$ and $RpII140^{M39}$ may either represent a statistical aberration or an example of synergism between the two largest subunits of RNA polymerase II with respect to their ability to cause the Ubx effect.

The Ubx effect of $RpII140^{Z43}$ depends upon temperature and heterozygosity: The *RpII140* allele *Z43* causes the Ubx effect as a heterozygote reared at 25° and engenders temperature-dependent lethality as a homozygote. We therefore wanted to test whether

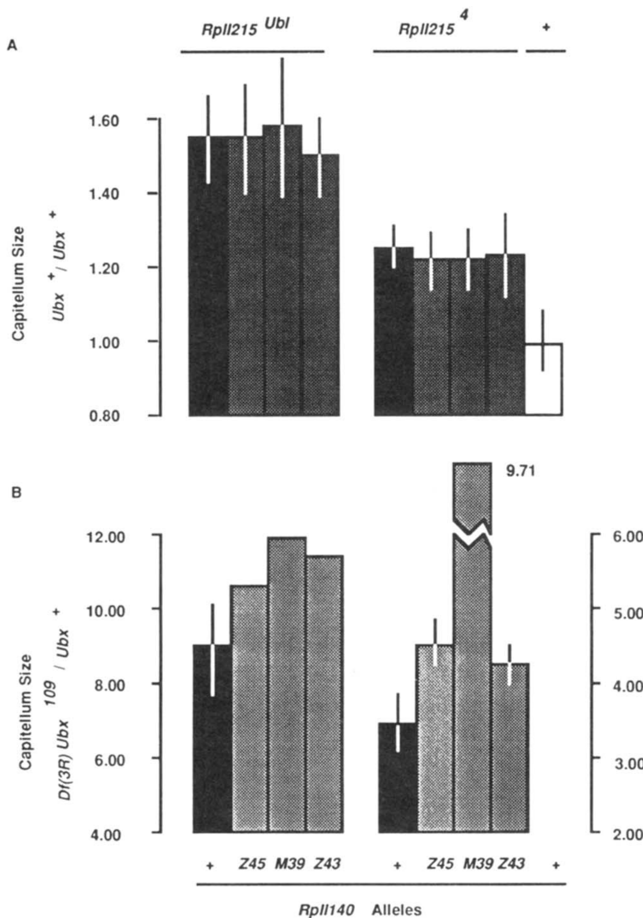

FIGURE 4.—Ubx effect in flies mutant for both *RpII215* and *RpII140*. Capitellum size was measured as described for Figure 3. Capitellum size of flies carrying two (A) or one (B) copy of Ubx^+ are shown for wild-type flies (white bar), flies mutant only for *RpII215* (black bars), and those mutant for both *RpII215* and *RpII140* (gray bars) ($n = 10$, vertical lines represent 95% confidence intervals, $n < 10$ for genotypes with greatly reduced viability, indicated by the absence of 95% confidence intervals).

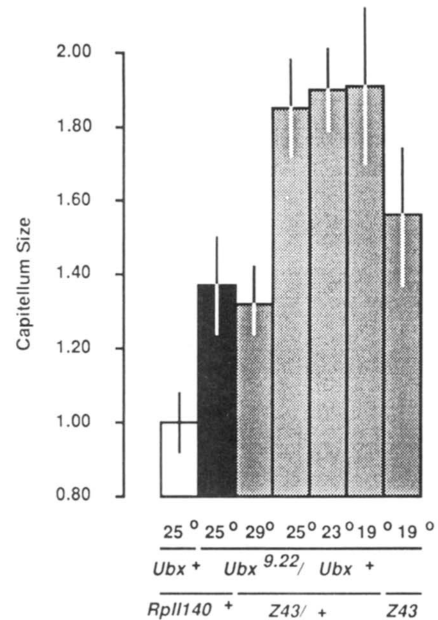

FIGURE 5.—Effects of temperature and heterozygosity on the Ubx effect caused by $RpII140^{Z43}$. Capitellum size was measured as described in Figure 3. Capitellum size is shown of wild-type (white bar), $Ubx^{9.22}/+$ (black bar), $RpII140^{Z43}$ $Ubx^{9.22}/+$ (gray bars) and $RpII140^{Z43}Ubx^{9.22}/RpII140^{Z43}$ + (gray bar) flies reared at the indicated temperatures ($n = 10$, vertical lines represent 95% confidence intervals).

temperature would affect the degree to which *Z43* elicits the Ubx effect. The capitellum size of wild-type and heterozygous $Ubx^{9.22}$ flies was compared to flies heterozygous for $Ubx^{9.22}$ and $RpII140^{Z43}$ reared at four different temperatures. Heterozygous $RpII140^{Z43}$ $Ubx^{9.22}/RpII140^+$ Ubx^+ flies reared at 29° have a capitellum size that is indistinguishable from $RpII140^+$ $Ubx^{9.22}/RpII140^+$ Ubx^+ flies reared at 25°; in other words they do not display the Ubx effect. At all three lower temperatures tested, heterozygous $RpII140^{Z43}$ $Ubx^{9.22}/RpII140^+$ Ubx^+ flies display the Ubx effect (Figure 5).

RpII215 alleles do not cause the Ubx effect when homozygous, hemizygous or *trans*-heterozygous for other alleles that also cause the Ubx effect (VOELKER *et al.* 1985; MORTIN, PERRIMON and BONNER 1985; MORTIN, KIM and HUANG 1988). They only elicit the Ubx effect when heterozygous with other *RpII215* alleles that do not cause this effect (MORTIN, KIM and HUANG 1988). We therefore wanted to test the effect of homozygous *Z43* on capitellum size in the presence of only one copy of Ubx^+. Homozygotes survive to the adult stage at 19° and show a significant reduction in the transformation of capitellum to wing compared to heterozygotes reared at all temperatures tested except 29° (Figure 5). Thus, like the *RpII215* alleles that cause the Ubx effect, $RpII140^{Z43}$ must be heterozygous with other *RpII140* alleles that do not cause the effect in order to elicit the strongest Ubx effect. We were unable to test the other heteroallelic combinations with the *RpII140* alleles *Z45* and *M39* because even at 19° they cause lethality when heterozygous with *Z43*.

Temperature-sensitive period of the Ubx effect caused by $RpII140^{Z43}$: We used the large difference in capitellum size of $RpII140^{Z43}$ $Df(3R)Ubx^{109}/RpII140^+$ Ubx^+ flies reared at 19° verses 29° to determine the temperature-sensitive period for the Ubx effect. Twenty-four-hour egg collections were taken at 19° and 29°, the resulting progeny were allowed to develop for different lengths of time and then shifted to the other temperature to complete development. The capitellum is transformed toward a wing when flies are reared throughout development at 19° but not at 29° (Figure 6). Flies that develop at one temperature and are shifted to the other, from the earliest embryonic stage until the middle of the third larval instar stage, display a capitellum size consistent with their new temperature. Flies shifted after the middle of the pupal stage display a capitellum size

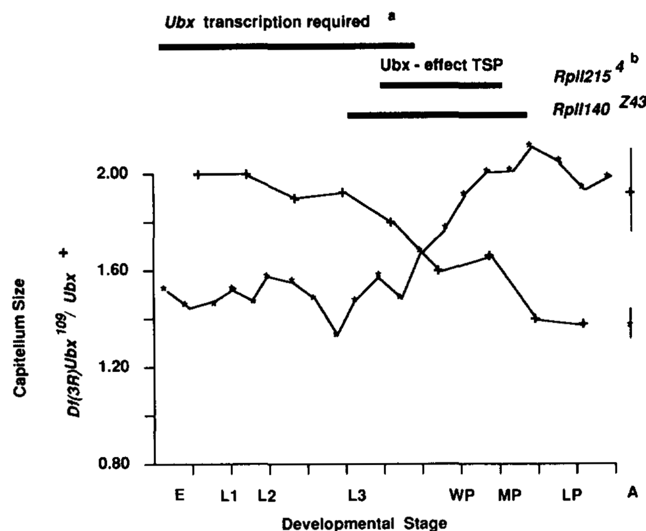

FIGURE 6.—Temperature-sensitive period for the Ubx effect elicited by $RpII140^{Z43}$. Eggs were collected at 19° or 29°, allowed to develop for from 1–21 days or 1–9 days, respectively, shifted to the other temperature and allowed to complete development. Capitellum size of resulting adult $RpII140^{Z43}$ $Df(3R)Ubx^{109}/+$ flies was measured as described in Figure 3. Thick black horizontal lines represent the temperature-sensitive periods for *Ubx*, ([a]MORATA and GARCIA-BELLIDO 1976; KAUFMAN, TASAKA and SUZUKI 1973), $RpII215^4$ ([b]MORTIN, KIM and HUANG 1988) and $RpII140^{Z43}$ ($n = 5$, E = embryo, L1 = first instar larva, L2 = second instar larva, L3 = third instar larva, WP = white pupa, MP = middle pupa, LP = late pupa, A = adult not shifted, * = flies completed development at 29°, + = flies completed development at 19°, vertical lines with the unshifted flies, A, represent 95% confidence intervals).

consistent with their previous temperature. Therefore, the middle of the third larval instar stage until the middle of the pupal stage define the TSP for the Ubx effect caused by $RpII140^{Z43}$ (Figure 6). Note that most of the TSP for $RpII140^{Z43}$ occurs after what is thought to be the end of the requirement for *Ubx* transcription to form a normal capitellum (MORATA and GARCIA-BELLIDO 1976; KAUFMAN, TASAKA and SUZUKI 1973; Figure 6).

DISCUSSION

The second largest subunit of RNA polymerase II, encoded by *RpII140*, maps to the cytological region 88A8;B4 removed by $Df(3R)red^{P52}$. In order to identify mutations in *RpII140*, we attempted to saturate this region for recessive-lethal mutations, assuming that *RpII140* is an essential gene. Two molecular genetic experiments confirm that the *A5* complementation group is *RpII140*. First, the cloned *RpII140* gene rescues *A5* lethality (HAMILTON 1990; B. J. HAMILTON and A. GREENLEAF, unpublished data), and second, a lesion has been mapped in the RpII140 coding region of the allele *A5* (Y. CHEN and A. GREENLEAF, personal communication).

The *RpII140* locus is not allelic to any of the four genes previously mapped to the 88A8;88B4 interval. (1) *l(3)K43*, whose product regulates cell proliferation in larval diploid tissue (SZABAD and BRYANT 1982). (2) *trx*, a gene that regulates the expression of other homeotic genes (GARCIA-BELLIDO and CAPDEVILA 1978; INGHAM 1983). (3) *red*, which reduces the pigmentation of the eye and malpighian tubules (LINDSLEY and ZIMM 1992). (4) *suppressor of Hairy wing* [*su(Hw)*], whose gene product, when deleted, corrects the mutant phenotype caused by the insertion of the retrotransposon gypsy into other loci (MODOLELL, BENDER and MESELSON 1983).

Two groups previously described alleles of the *RpII140* locus. Seven *RpII140* alleles were identified as dominant suppressors of the conditional lethality caused by a mutation in the largest subunit of RNA polymerase II (MORTIN 1990; M. A. MORTIN, unpublished data). In addition, one allele of *RpII140* called *wimp* acts as a dominant maternal enhancer of mutations in other genes acting early in Drosophila development, for example the transcription factor *hairy* (*h*) (PARKHURST and ISH-HOROWICZ 1991). Note that $RpII140^{wimp}$ does not interact with *Ubx* (PARKHURST and ISH-HOROWICZ 1991) and the three *RpII140* alleles that cause the Ubx effect do not interact with *h* (M. A. MORTIN, unpublished data).

Similarities between the genes encoding the two largest subunits of RNA polymerase II: The genes encoding the 215,000- and 140,000-D subunits of RNA polymerase II share a high degree of conservation even with the β and β' subunits, respectively, of *Escherichia coli* (ALLISON *et al.* 1985; BIGGS, SEARLES and GREENLEAF 1985; SWEETSER, NONET and YOUNG 1987; FALKENBURG *et al.* 1987). As single copy genes, both *RpII215* and *RpII140* are readily mutable to recessive lethality by treatment with EMS, a chemical that often results in DNA changes that give rise to single amino acid changes in protein. Specific mutations in the two largest subunits of RNA polymerase II, *RpII215* and *RpII140*, cause partial transformation of capitellum into a wing, referred to as the Ubx effect.

Properties of *RpII215* and *RpII140* alleles that elicit the Ubx effect are similar. Loss-of-function alleles of either locus do not cause the Ubx effect; therefore, alleles that elicit the effect display an altered function. Alleles of both loci that cause the Ubx effect require heterozygosity with other alleles of the same loci to elicit the strongest effect. Approximately one in ten EMS-induced mutations of either locus display the Ubx effect (MORTIN, KIM and HUANG 1988; this report). Assuming an average rate of mutation for both loci of 10^{-3}, alleles that display the Ubx effect are recovered at a frequency of approximately 10^{-4}.

The sole difference between the two loci with respect to their ability to cause the Ubx effect is that *RpII140* alleles only cause the transformation when one copy of the wild-type Ubx^+ locus is deleted. In

contrast, *RpII215* alleles also cause the Ubx effect when two wild-type copies of Ubx^+ are present in the genome (Figure 3). This difference cannot be explained by postulating that all three *RpII140* alleles cause a weaker Ubx effect than the *RpII215* alleles, because in the presence of a single copy of wild-type Ubx^+, *RpII140* alleles cause as strong or stronger an effect than three *RpII215* alleles (Figure 3B).

Do RNA polymerase II mutations cause the Ubx effect by altering expression of genes downstream from *Ubx*? Two lines of evidence suggest that RNA polymerase II mutations might cause the Ubx effect by altering the expression of genes regulated by *Ubx*. First, RNA polymerase II mutations cause the Ubx effect after the time in development during which *Ubx* transcription is thought to be required to form a normal third thoracic segment (Figure 6). The temperature-sensitive period of the Ubx effect elicited by $RpII215^4$ and $RpII140^{Z43}$ starts in the middle to late third larval instar stage and persists until the middle of the pupal stage (Figure 6). The small difference in its duration observed between the two mutations (Figure 6) is probably not significant. Most of this developmental period is thought not to require transcription of the Ubx^+ locus for normal capitellum development (MORATA and GARCIA-BELLIDO 1976; KAUFMAN, TASAKA and SUZUKI 1973). Second, we were unable to duplicate the results reported by BOTAS, CABRERA and GARCIA-BELLIDO (1988) from an experiment that suggests a reduction in the amount of Ubx protein in $RpII215^{Ubl}/+$; $Df(3R)Ubx^{109}/+$ halter discs is caused by $RpII215^{Ubl}$ (M. A. MORTIN, data not shown).

Further studies are required to conclusively demonstrate the mechanism for the interaction between RNA polymerase II mutations and Ubx. However, our results are consistent with the hypothesis that RNA polymerase II mutations cause the Ubx effect by incorrect transcription of genes that are regulated by the transcription factor Ubx. Since purified Ubx protein can both positively and negatively regulate transcription in an *in vitro* transcription system (JOHNSON and KRASNOW 1990), the Ubx effect may result from an increase in transcription of genes that are negatively regulated and/or the decrease in transcription of genes that are positively regulated by Ubx. It is intriguing to speculate that this proposed interaction between the Ubx and RNA polymerase II proteins might be direct.

Genetics of the Ubx effect does not recapitulate RNA polymerase II biochemistry: RNA polymerase II consists of approximately 11 different polypeptides (YOUNG 1991). The stoichiometry of RNA polymerase II suggests that only a single copy each of the two largest subunits are present in a functioning enzyme (SAWADOGO and SENTENAC 1990; YOUNG 1991). Our current understanding of RNA polymerase II function is that it recognizes and binds to promoter regions in response to general transcription factors (BURATOWSKI *et al.* 1989). RNA polymerase II can then be stimulated or repressed by spatially and temporally specific transcription factors (*e.g.*, Ubx).

It is difficult to reconcile this view of RNA polymerase II with the phenomenology described for the Ubx effect. Two observations warrant further discussion, the apparent requirement for heterozygosity within and independent action between mutations in the two largest subunits. First, specific mutations of either *RpII215* or *RpII140* must be heterozygous with other *RpII215* or *RpII140* alleles, respectively, in order to elicit the strongest transformation of capitellum to wing (MORTIN, KIM and HUANG 1988; Figure 5). Since the stoichiometry suggests that only a single copy of either of the largest two subunits are present in active RNA polymerase II, the requirement for heterozygosity might reflect the cooperative interaction between functioning multimeric RNA polymerase II enzymes.

Our results also suggest that mutations in *RpII215* and *RpII140* act independently to cause the Ubx effect. Evidence for this includes the observation that *RpII215* mutations cause the Ubx effect even when the concentration of Ubx protein is normal; however, *RpII140* mutations require a reduction in the concentration of Ubx protein before they elicit the Ubx effect. Furthermore, while the transformation of capitellum to wing observed in $RpII215^{Ubl}/+$ flies is indistinguishable from that of $Df(3R)Ubx^{109}/+$ flies, *RpII140* alleles increase the transformation in the latter but not the former flies (Figures 3B and 4A). Clearly the changes of gene expression responsible for the Ubx effect in $RpII215^{Ubl}/+$ and $Df(3R)Ubx^{109}/+$ flies are different even though they result in identical mutant phenotypes.

Finally, with the possible exception of $RpII215^4$ and $RpII140^{M39}$ the Ubx effect observed in flies mutant for both *RpII215* and *RpII140* appears to be additive (Figure 4). This again suggests that the two largest subunits of RNA polymerase II are eliciting the Ubx effect independently of one another. Thus, the interaction between Ubx and RNA polymerase II might identify a two step process, one mediated by the largest subunit and the other by the second largest subunit.

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