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Clonal analysis of two mutations in the large subunit of RNA polymerase II of *Drosophila*

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Summary. Two mutations in the gene, *RpII215*, were analyzed to determine their effects on cell differentiation and proliferation. The mutations differ in that one, $RpII215^{ts}$ (*ts*), only displays a conditional recessive lethality, while the other, $RpII215^{Ubl}$ (*Ubl*), is a recessive lethal mutation that also displays a dominant mutant phenotype similar to that caused by the mutation *Ultrabithorax* (*Ubx*). *Ubl* causes a partial transformation of the haltere into a wing; however, this transformation is more complete in flies carrying both *Ubl* and *Ubx*. The present study shows that patches of *Ubl*/− tissue in gynandromorphs are morphologically normal. Cuticle that has lost the wild-type copy of the *RpII215* locus fails to show a haltere to wing transformation, nor does it show the synergistic enhancement of *Ubx* by *Ubl*. We conclude that an interaction between the two *RpII215* alleles, *Ubl* and $RpII215^{+}$, is responsible for the mutant phenotype. Gynandromorphs carrying the *ts* allele, when raised at permissive temperature, display larger patches of *ts*/− cuticle than expected, possibly indicating that the proliferation of *ts*/+ cells is reduced. This might result from an antagonistic interaction between different *RpII215* alleles. Classical negative complementation does not appear to be the cause of the antagonistic interactions described above, as only one *RpII215* subunit is thought to be present in an active multimeric polymerase enzyme. We have therefore coined the term 'negative heterosis' to describe the aforementioned interactions.

We also observed that the effects of mutationally altered RNA polymerase II on somatic cells are different from its effects on germ cells. Mutant somatic cells (either *Ubl*/− or *ts*/−, the latter shifted to restrictive temperature) reduce cell proliferation, but otherwise do not appear to disrupt cell differentiation. However, mutant germ cells often differentiate into morphologically abnormal oocytes.

Introduction

Classical and molecular genetic analyses have provided much information on the subunit structure and function of prokaryotic RNA polymerase (see Yura and Ishihama 1979, for a review). Similar analyses in eukaryotes such as mammalian tissue culture cells (Chan et al. 1972; Amati et al. 1975; Lobban et al. 1976; Bryant et al. 1977), yeast (Young and Davis 1983; Windsor et al. 1979) and *Drosophila* (Greenleaf et al. 1979, 1980; Mortin and Lefevre 1981; Mortin and Kaufman 1982) have proved more complex. While prokaryotes have a single form of RNA polymerase (reviewed by Polya 1973), eukaryotes have three types of functionally distinct nuclear RNA polymerases, each consisting of eight or more subunits (Chambon 1975; Roeder 1976). We describe here an analysis in *Drosophila melanogaster* of two mutations in the gene *RpII215*, which encodes the large subunit (Greenleaf 1983) of the enzyme responsible for synthesizing messenger RNA, RNA polymerase II (RNA nucleotidyltransferase; EC 2.7.7.6).

The first mutation, $RpII215^{ts}$ (*ts*), is viable at 22° C, lethal at 29° C, and does not display a dominant mutant phenotype mimicking that of the mutation *Ultrabithorax* (*Ubx*) (Mortin and Kaufman 1982, 1984). The second mutation, $RpII215^{Ubl}$ (*Ubl*), produces two phenotypes: lethality when homozygous, and when heterozygous, a dominant *Ubx*-like effect on segment identity whereby the third thoracic segment is partially transformed into the second. Thus, the haltere develops wing-like characteristics; 1–4 marginal wing bristles develop at the base of a slightly enlarged haltere (the phenotype is identical to that of the haltere shown in Fig. 1B).

When a fly is constructed carrying both *Ubl* and *Ubx*, there is a synergistic effect changing the haltere more completely into a wing (Fig. 1A). The magnitude of this homeotic transformation is reduced but not eliminated by adding an additional copy of the wild-type gene to *Ubl*/+ flies (Mortin and Lefevre 1981). This led to the hypothesis, tested below, that both the dominant mutant phenotype and the enhancement of *Ubx* by *Ubl* are caused by an antagonistic interaction between the *Ubl* and $RpII215^{+}$ (wild type) gene products.

Although flies homozygous for either the *Ubl* or *ts* (at 29° C) mutation die, mosaic flies possessing patches of mutant cells in a heterozygous wild-type background survive (Mortin 1983). Since the protein product of the *RpII215* locus is restricted to the cell in which it is synthesized (Mortin and Kaufman 1982), it is therefore possible to examine the effects of mutationally altered polymerase on the proliferation and differentiation of *Ubl*/− or *ts*/− cells (− = loss of the X chromosome carrying the wild-type allele, + will be used to indicate its presence).

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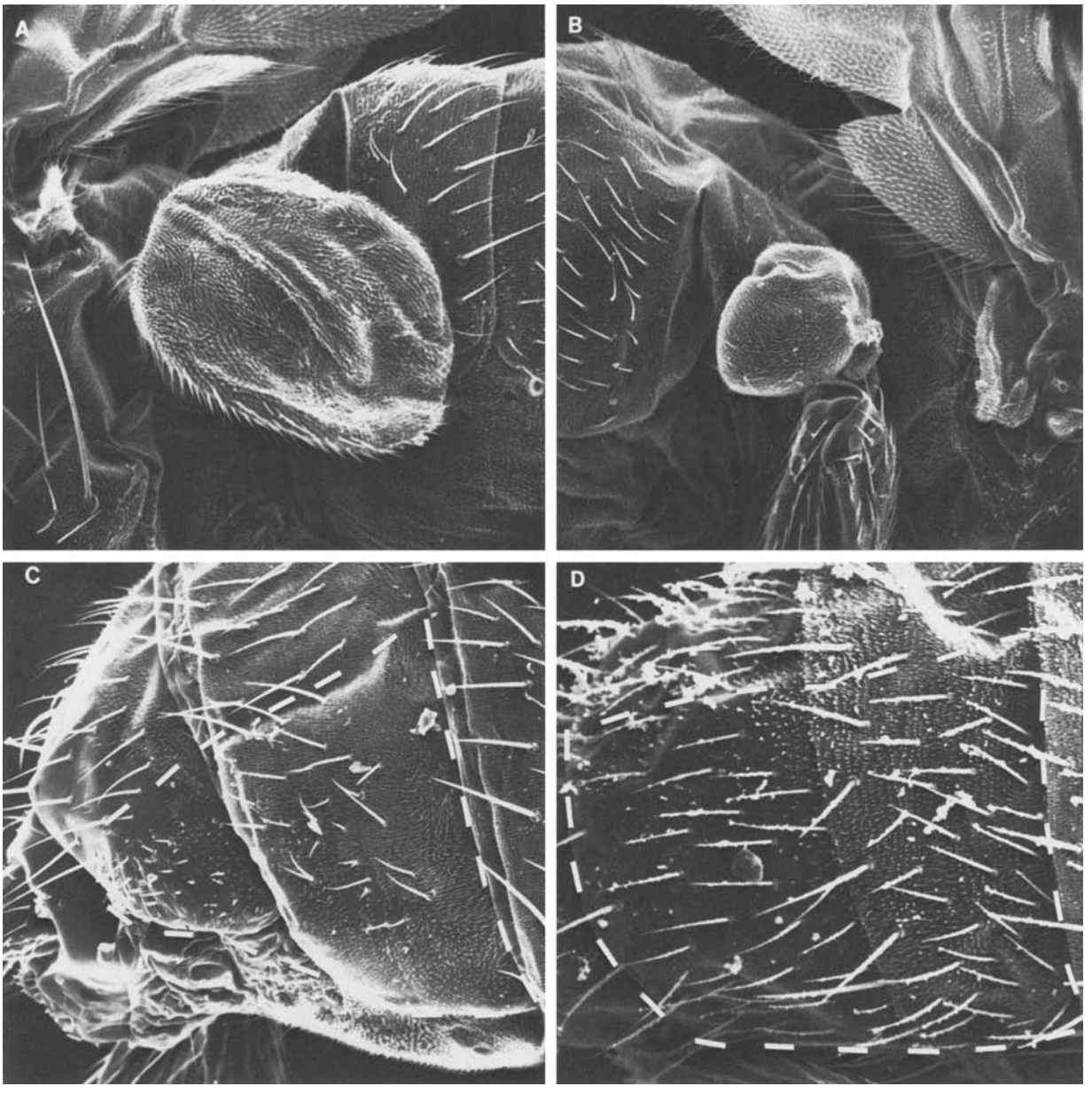

Fig. 1 A–D. Scanning electron micrographs of gynandromorphs (**A**) *y Ubl f/R(1)2,In(1)w^{vC}*; *Ubx^{130}/+* fly showing a typical transformation of haltere toward wing caused by the enhancement of *Ubx* by *Ubl*. ×90. **B** The same fly as shown in **A**, only the right haltere of the fly has lost the wild-type copy of the *RpII215* locus located on the ring-X chromosome. The haltere shows only the mutant effect of *Ubx^{130}*. ×90. **C** *y ts f/R(1)2,In(1)w^{vC}* fly raised at 29° C. The dashed line outlines cuticle that has lost the ring-X chromosome. ×140. **D** A fly raised at 22° C but with the same genotype and showing loss of the ring-X chromosome from the same cells as those shown in **C**. ×140

Materials and methods

Lines of *y ts f* ('mutant') and *y f* ('control') flies were maintained on a standard food source at 22±1° C. Both stocks were derived from a common Oregon-R strain of wild-type flies and are equally viable at 22° C (permissive temperature). All *ts* embryos shifted to 29° C (restrictive temperature) die while *y f* embryos are unaffected by this treatment (Mortin and Kaufman 1982). The mutant chromosome *y Ubl f* was derived from an M56i Amherst wild-type strain (Mortin and Lefevre 1981) and therefore may possess differences in genetic background from the *y f* stock.

Flies were prepared for scanning electron microscopy by ethanol fixation, critical point drying, and coating with a gold/platinum alloy (Turner and Mahowald 1976).

Gynandromorph study. The unstable ring-X chromosome, *R(1)2,In(1)w^{vC}* (kindly provided by Dr. Jeffrey Hall) is occasionally lost from cleavage stage nuclei (Catcheside and Lea 1945; Zalokar et al. 1980). To maintain a high rate of loss of the ring chromosome, it was necessary to select and save only those flies showing loss of the chromosome. Over the course of a year, this led to an increase in the rate of loss of the ring-X chromosome, as is reflected in the variability of the control data in Table 1. The experimental design was therefore to select virgin females carrying the ring-X

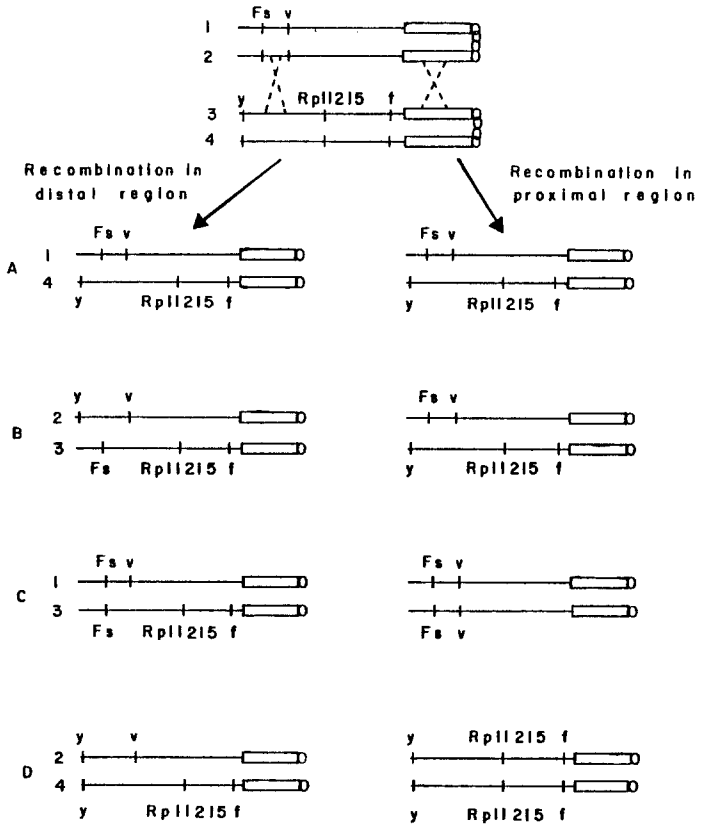

Fig. 2A–D. Diagram of the method of induction of germ line clones homozygous for a mutation in the *RpII215* locus. A single recombination event (dashed lines) occurring in either the proximal or distal region can give rise to four types of daughter cells following segregation of the four chromatids. Only one of these events (*D*) might produce postvitellogenic oocytes. Box = heterochromatin thin line = euchromatin, circles = centromere

chromosome, divide them into two groups and then mate one group to control and another to mutant flies. Thus, only paired groups can be compared.

Germ line clones. *y f/Fs(1)K1237 v*^{24} (control), *y ts f/Fs(1)K1237 v*^{24} and *y Ubl f/Fs(1)K1237 v*^{24} (mutant) flies were given a 1,000 r dose of X-rays with a Philips MG102 X-ray machine (100 KV, 4 ma, 1.5 mm aluminum filter). X-rays induce high rates of recombination in mitotic cells (Garcia-Bellido 1972). Four types of daughter cells can result from a single recombination event (Fig. 2A–D), the consequences of which differ depending upon the location of recombination (proximal or distal). Most recombination events are in heterochromatin and therefore will occur in the proximal region of the chromosome.

Fs(1)K1237 is a mutation that when heterozygous or homozygous in germ cells blocks oogenesis prior to the vitellogenic stage (Perrimon and Gans 1983). However, a mitotic recombination event in a heterozygous *Fs(1)K1237 v/y RpII125 f* germ line can give rise to two classes of non-*Fs(1)K1237* cells, both of which may be able to complete oogenesis (Fig. 2D). Proximal recombination events can produce homozygous *y RpII215 f* germ cells in a heterozygous wild-type background. If oogenesis is normal in homozygous *y RpII215 f* germ cells, *y f* progeny can result. *RpII215* germ cells that arrest oogenesis after the vitellogenic stage may also be detected by dissecting and examining female reproductive organs. Rare recombination events will also occur in the euchromatic region of the chromosome (diagrammed as distal in Fig. 2). This event serves as an internal control as *y RpII215 f/y v* germ cells can complete oogenesis; resulting progeny may be distinguished from proximal recombination because both *y f* and *y v* (vermilion produces an orange eye color instead of the wild-type red color) progeny can be produced.

Results and conclusions

Gynandromorph study of Ubl

We examined gynandromorphs resulting from the loss of the wild-type copy of the polymerase gene located on an unstable ring-X chromosome. Heterozygous *Ubl/+* flies display a *Ubx*-like phenotype; however, patches of *Ubl/−* tissue appear morphologically normal even in the halteres (data not shown). This observation is shown more dramatically in *Ubl/−* tissue generated in the presence of the *Ubx* mutation. Fig. 1A and 1B show the same fly. The haltere pictures in Fig. 1A displays the phenotype of flies heterozygous for both *Ubl* and *Ubx* (i.e., the fly's genotype is *Ubl/+*; *Ubx/+*). However, the haltere shown in Fig. 1B developed from *Ubl/−* tissue generated by the loss of the ring-X chromosome (i.e., the genotype of the haltere cells is *Ubl/−*; *Ubx/+*). The phenotype resembles that of *Ubx/+* flies, that is, the mutant phenotype is not enhanced by the *Ubl* mutation. Thus, both the dominant *Ubx*-like phenotype and the synergistic enhancement of *Ubx* by *Ubl* require that both wild-type and *Ubl* alleles are present in the same cell.

Although the morphology of *Ubl/−* cells is normal, there is the additional mutant phenotype in which the proliferation of *Ubl/−* cells is reduced compared to control cells. This is shown in Table 1 as a reduction in the observed number of flies with large patches of *Ubl/−* tissue. There is also a reduced frequency of recovery of all gynandromorphs most likely resulting from the lethality of some gynandromorphs possessing large patches of mutant tissue. An alternative hypothesis is that *Ubl/−* tissue undergoes limited cell death which gives rise to gynandromorphs with smaller mutant patches of cuticle. This hypothesis seems unlikely because other mutations that cause cell death also result in pattern deficiencies and duplications (Arking 1975; Simpson and Schneiderman 1975); these defects are not seen in *Ubl/−* gynandromorphs.

The distribution of patches of mutant cuticle is random; there is no evidence that certain tissues are more sensitive to abnormal polymerase than others. It is likely that the combined effects of *Ubl/−* defects in multiple tissues of a fly disrupt development to a degree proportional to the amount of mutant tissue.

Gynandromorph study of ts

We next examined gynandromorphs of the temperature-sensitive RNA polymerase II mutation, *ts*. The *ts* allele was selected for analysis because of its contrasting phenotype, following several different temperature regimes, to that of the *Ubl* allele; that is, it does not display a *Ubx*-like phenotype and it is a conditional recessive lethal mutation. One surprising result was that proliferation of *ts/−* cells at 22° C (permissive temperature) is significantly greater than *ts/+* cells, as patches of *ts/−* cells are larger than expected (Table 1). This suggests that proliferation of cells possessing

Table 1. Frequency of gynandromorphs

	Total flies examined		All gynandromorphs frequency		X^2	P	>3 Bristles frequency		X^2	P	>1/2 Region frequency		X^2	P
	Control	Mutant	Control	Mutant			Control	Mutant			Control	Mutant		
ts														
29° C	128	117	0.62	0.43	9.59	<0.005	0.50	0.20	23.09	<0.005	0.27	0.02	31.33	<0.005
22° C	122	86	0.65	0.65	0.00	N.S.	0.62	0.64	0.13	N.S.	0.19	0.30	9.78	<0.005
1st ↑	134	183	0.52	0.38	6.14	<0.025*	0.47	0.18	29.45	<0.005	0.19	0.01	32.21	<0.005
1st ↓	56	50	0.75	0.78	0.13	N.S.	0.64	0.70	0.39	N.S.	0.34	0.22	1.85	N.S.
3rd ↑	89	96	0.72	0.54	6.22	<0.025*	0.64	0.34	16.27	<0.005	0.22	0.08	7.19	<0.01
3rd ↓	80	97	0.78	0.56	9.25	<0.005	0.74	0.34	27.72	<0.005	0.52	0.06	47.58	<0.005
WP ↑	75	91	0.69	0.66	0.22	N.S.	0.65	0.55	1.84	N.S.	0.32	0.23	1.69	N.S.
WP ↓	78	91	0.78	0.58	7.62	<0.01	0.73	0.30	31.65	<0.005	0.54	0.02	58.18	<0.005
Ubl														
R.T.	60	105	0.85	0.58	12.68	<0.005	0.77	0.34	27.43	<0.005	0.48	0.10	31.86	<0.005

N.S. = Not Significant; * $P > 0.01$

y ts f/Y or *y Ubl f*; *Dp(1; Y)B*$^{S-}$ *Yy*$^{+}$ mutant males and *y f*/Y control males were mated to females [*R(1)2,In(1)w*^{vC}/*y w spl,In(1)d1-49*] possessing an unstable ring-X chromosome, *R(1)2,In(1)w*^{vC}. Cuticle of *y f*/*R(1)2,In(1)w*^{vC} (non-*y*ellow body color, non-*f*orked bristles) adult females was examined for patches of *y f*/− (*y*ellow and *f*orked) cells that also develop as male in sexually dimorphic tissue. Eggs were collected from the mated flies described above at either room temperature (R.T. = 22° C–25° C, *Ubl*), 22° C, or 29° C (*ts*). The resulting progeny were either left to develop at their respective temperature or shifted to the other temperature as first instar larvae (1st), third instar larvae (3rd), or white pupae (WP). The frequency of all gynandromorphs, those with mutant tissue encompassing more than three bristles, and those with mutant cuticle filling 1/2 a region (arbitrarily defined as either the right or left half of the head, thorax, or abdomen) are reported above. The frequency of detected gynandromorphs in each size class was then analyzed statistically using 2 × 2 contingency tables to compare similarly treated mutant and control progeny

ts and wild-type polymerase is reduced due to an antagonistic interaction between the gene products of the two different alleles. An alternative explanation is that the control (*y f*) chromosome carries a mutation that reduces the proliferation of *y f*/− cells at 22° C. This hypothesis cannot be ruled out, but seems unlikely because the *y ts f* and *y f* chromosomes were derived from the same parental stock and were shown to be equally viable at 22° C (see Materials and methods, Mortin and Kaufman 1982, 1984).

Shifting *ts*/− gynandromorphs to 29° C during different stages of development eliminates the increased proliferation observed in *ts*/− cells at 22° C (Table 1). This is most strikingly shown in gynandromorphs raised at 29° C throughout development. The flies displayed in Fig. 1C and 1D have patches of *ts*/− tissue encompassing their fifth and sixth abdominal tergites. The fly in Fig. 1C was raised at 29° C while the one in Fig. 1D was raised at 22° C. Note the reduced size of the segments and the abnormal bristle morphology of the fly raised at 29° C. The cuticular morphology of this fly is reminiscent of that caused by other mutations, which reduce cell proliferation (e.g., *Minutes*, Morata and Ripoll 1975; Simpson 1976). We conclude that *ts*/− cells raised at 29° C have reduced proliferation and that this reduction is much greater than that observed in *Ubl*/− or *ts*/+ (22° C) cells. While it is not surprising that RNA polymerase II mutations reduce cell proliferation, it was unexpected that reduced proliferation would be the primary defect observed upon generating hemizygous mutant cells.

Germ line clones

The fact that we could examine *Ubl*/− and *ts*/− cells in gynandromorphs suggested a homologous experiment in the germ line. Germ line clones could be induced in flies heterozygous for *RpII215* mutations in order to study the development of the germ line (i.e., nurse cells and oocytes) in the absence of wild-type polymerase and to determine whether eggs possessing only mutant maternally loaded polymerase can support embryonic development.

We first examined the production of germ line clones in *ts*/*Fs(1)K1237* females following their irradiation as first instar larvae. Females, possessing specific germ line clones and raised at 18°, 22° or 25° C, produced normal eggs, which developed into viable progeny (Table 2). However, only one germ line clone was recovered from flies raised at 29° C. This fly never laid eggs, and upon dissection was seen to have one ovariole filled with abnormally shaped eggs. Because distal clones (an internal control) were obtained at 29° C, it is likely that more germ line clones were induced but that their development was arrested prior to vitellogenesis.

We next shifted irradiated *ts*/*Fs(1)K1237* females from permissive to restrictive temperature at various stages of development. No postvitellogenic eggs were recovered if the shift was done prior to the late pupal stage (Table 2). However, late pupae shifted to 29° C did produce postvitellogenic eggs. These eggs were abnormally small and displayed fused filaments. Adults shifted to 29° C behaved exactly like homozygous *ts* adults (Mortin and Kaufman 1984), they produced normal eggs that underwent abnormal embryogenesis. Thus the defects observed in homozygous *ts* females shifted to 29° C can be attributed solely to the disruption of germ line development.

Germ line clones of *Ubl* were recovered with frequenies similar to control clones (Table 2); however, the postvitellogenic oocytes recovered had abnormal morphology. Mu-

Table 2. Number of germline clones recovered

Genotype	Temperature	Total	Proximal	Distal
y f/Fs(1)K1237 v^{24}	18° C	200	5	0
	25° C	200	7	0
	29° C	200	4	0
y ts f/Fs(1)K1237 v^{24}	18° C	400	21	1
	22° C	450	35	2
	25° C	250	7	0
	29° C	350	1	2
	1st ↑	350	0	1
	2nd ↑	350	0	1
	WP ↑	250	0	1
	LP ↑	220	8	1
	Adult ↑	150	4	0
y Ubl f/Fs(1)K1237 v^{24}	18° C	130	6	0
	25° C	320	10	1
	29° C	250	3	0

First instar larvae of the genotypes shown above were irradiated as described in Materials and methods and in the legend of Fig. 2. The larvae were then placed at the indicated temperature and allowed to develop to the adult stage or were shifted from 18° C or 22° C (permissive temperature) to 29° C (restrictive temperature) as first (1st ↑) or second (2nd ↑) instar larvae, as white pupae (WP ↑), three days after formation of white pupae (LP ↑) or as adults. Adults were examined for their ability to lay eggs and the location of their recombination event (proximal or distal). Ovaries were dissected from females that failed to lay eggs and examined for postvitellogenic oocytes

tant phenotypes were similar to those observed for *ts* clones in late pupae shifted to 29° C. It is interesting to note that while *Ubl/−* somatic tissue is morphologically normal, *Ubl/Ubl* germ lines produce abnormal oocytes.

Germ cells mutant for *Ubl* and *ts* (at restrictive temperature) do not undergo normal development even in a heterozygous wild-type somatic background. However, residual transcription must be sufficient to permit *Ubl* and *ts* germ cells to complete oogenesis (the latter following shifts of late pupae or adults to 29° C), though abnormal oocytes are usually produced. The residual transcription could result from the perdurance of wild-type gene product synthesized in a heterozygous wild-type parental cell that gave rise to the clonally derived cells. Alternatively, a lower efficiency of transcription by mutant polymerase might be sufficient to permit the completion of oogenesis, albeit abnormally.

Discussion

In contrast to the abnormal oogenesis of homozygous mutant germ cells, germ cells heterozygous for mutant and wild-type alleles of RNA polymerase complete oogenesis in an apparently normal fashion; however, in somatic cells the situation is reversed. The present study clearly demonstrates an antagonistic interaction between the gene products of two different *RpII215* alleles when both are present in the same somatic cell. Two examples of this effect have been documented above. They are the enhanced mutant *Ubx* phenotype observed in *Ubl/+* adult cuticle but not *Ubl/−* or *+/+* tissue (Fig. 1A, B) and possibly the decrease in *ts/+* cell proliferation at 22° C compared to *ts/−* cells (Table 1). Other examples of this phenomenon have also been observed (Robert Voelker, personal communication; Mortin and Kaufman 1982).

Interactions of the kind described above have been termed negative complementation. They are thought typically to result from the incorporation of different gene products, encoded by a single locus, into the same multimeric enzyme molecule such that one subunit disrupts the activity of the other. RNA polymerase II contains only one copy of the large subunit per active enzyme molecule (Cochet-Meilhac and Chambon 1974; Kramer and Bautz 1981; Vaisius and Wieland 1982); therefore, the negative interaction between *RpII215* alleles probably results from a different mechanism. We propose the term 'negative heterosis', or the opposite of hybrid vigor, to describe the phenomenon. Use of the word heterosis is meant to imply that the defects observed in heterozygous cells and individuals result from a pervasive effect, which disrupts metabolic pathways: In the case of *RpII215* alleles, this is postulated to occur by disrupting transcription.

There are at least two hypotheses which can be put forward to explain negative heterosis of *RpII215* alleles. The first postulates that the interaction occurs at the level of autoregulation of the amount of RNA polymerase II present in the cells. Cellular levels of RNA polymerase II are precisely regulated as shown in mammalian tissue culture cells possessing mutations in a single subunit of RNA polymerase II. Cells possessing both α-amanitin-sensitive and -resistant RNA polymerase II regulate their levels of RNA polymerase II. Following the addition of α-amanitin, the steady-state level of the resistant polymerase increases at the expense of the α-amanitin-sensitive polymerase (Guialis et al. 1977, 1979; Somers et al. 1975). RNA polymerase II must not only synthesize mRNA but must regulate the levels of its own component subunits. Cells heterozygous for the *RpII215* alleles described above might misread the amount of functional polymerase in a given cell, while mutant cells might gauge correctly their reduced levels of activity and compensate for this by synthesizing more polymerase.

Alternatively, negative heterosis could result from an interaction between different RNA polymerase II enzymes at some step in transcription. For example, the translocation of mutant RNA polymerase II along the DNA template might proceed more slowly than wild-type polymerase, resulting in a collision that could hinder the function of both polymerases. The *Ubx* transcript, which is unusually long (possibly 70 kb, Bender et al. 1983; Akam et al. 1984), might be more sensitive to disruption than genes with shorter transcripts. This possibility is supported by the observation that other genes thought to have long transcripts, such as *Notch* (40 kb, Artavanis-Tsakonas et al. 1983) and *Antennapedia* (105 kb, Scott et al. 1983) also interact with *Ubl* (Mortin and Lefevre 1981, unpublished data).

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