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The recovery and preliminary examination of a temperature sensitive suppressor of the *cryptocephal* mutant of *Drosophila melanogaster*

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SUMMARY

The recovery of two EMS induced mutations which are dominant suppressors of the lethality of *cryptocephal* in *Drosophila melanogaster* are described. One of these mutations *Su(crc)1* is described in detail. It maps very close to *cryptocephal* at 54·7 on the second chromosome and its suppression of cryptocephal is temperature-sensitive. Temperature shift experiments show that the temperature-sensitive period is from before the pupariation until 12 h post pupariation. The temperature-sensitive period of *Su(crc)1* is discussed in relation to the expression of *l(2)crc*, head eversion and the timing of pupal chitin synthesis.

1. INTRODUCTION

The differentiation of the imaginal disks of *Drosophila melanogaster* results in the production of the adult cuticle and its associated structures. Despite the fact that this represents a major synthesis in the developing pupa, little is known of the biochemistry of cuticle synthesis and even less of its genetic control.

The insect cuticle consists mainly of protein and α-chitin (Hackman, 1976). Analysis of the lethal *cryptocephal* mutant (*l(2)crc*) in *D. melanogaster* led Fristrom (1964, 1965) to propose that this mutant affects chitin synthesis during the pupal stage of development. This recessive lethal mutant was first described in detail by Hadorn & Gloor (1943) and has a characteristic phenotype in which the cephalic complex fails to evert from the thorax during development. The mutant pupae continue to differentiate but fail to eclose. As the result of a number of experiments, including estimation of chitin content at the time of pupation, Fristrom (1964, 1965) claimed that *l(2)crc* causes synthesis of excess chitin prior to the time of normal head eversion. This results in increased cuticular rigidity and an inability of the pupae to evert their heads. Although his analysis showed excess chitin content Fristrom did not determine the enzyme affected by the mutation.

In this paper I report on selection experiments for autosomal dominant suppressors of *cryptocephal* which were conducted to identify other genes involved in chitin biosynthesis. The selection rationale is that if other genes coding for chitin

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biosynthetic enzymes are altered by mutation then *l(2)crc* homozygotes may survive. The fact that *l(2)crc* homozygotes do survive at a low frequency in the stocks used, seemed to imply that this would be a sensitive method for detecting changes in other genes involved in chitin biosynthesis.

2. MATERIALS AND METHODS

The *SM5/crc cn* stock was obtained from the Pasadena Stock Centre. The proximal 2L deficiency stocks were kindly provided by Professor T. R. F. Wright, University of Virginia. Details of their recovery are described in Wright, Hodgetts & Sherald (1976). *SM5* and *CyO* are second chromosome balancers. All other genes and gene symbols are as described in Lindsley and Grell (1968), with the following exceptions. The symbol *crc* is used for brevity in this section and the Results section in place of *l(2)crc*. The two *crc* suppressors recovered in this study and designated as *Su(crc)1* and *Su(crc)2* are similarly shortened to *Su1* and *Su2*, respectively.

Hadorn & Gloor (1943) showed that the expression of the cryptocephal mutant (*crc*) is influenced by the genetic background, and they isolated stocks which either rarely produced surviving homozygotes or produced considerable numbers. Fristrom (1964) was able to maintain a homozygous stock. The strain provided by the Pasadena Stock Centre for the current project never produces more than 0·1 % surviving homozygotes. In crosses using strains derived from outcrossing the *SM5/crc cn* stock, homozygous escapers never account for more than 1·0 % of the total eclosing progeny.

The low level of homozygote survival in this stock could be due to a second lethal or semi-lethal mutation in the *crc cn* chromosome. This possibility was investigated by recombinational analysis using the *al dp b pr c px sp* chromosome. The results showed that the *crc cn* chromosome carries a single lethal mutation mapping at $54{\cdot}9 \pm 0{\cdot}2$. All recombinant cultures with the lethal produce pupae with the *cryptocephal* phenotype, and it was therefore concluded that this lethal mutation at 54·9 is the lethal *cryptocephal* mutation described by Hadorn & Gloor (1943) with a map position of $55{\cdot}0 \pm 1{\cdot}0$.

Flies were cultured in third-pint milk bottles containing a yeasted agar-cornmeal-molasses-yeast food or in vials containing an agar-yeast-sucrose medium (Carpenter, 1950). Cultures were incubated at 25 °C unless otherwise stated.

For the egg-pick experiments eggs were collected and transferred 50 at a time to vials containing Carpenter's medium as previously described (Sparrow & Wright, 1974). Egg mortality and fertilization were assessed 36–48 h after the eggs were picked. Unhatched white eggs were scored as unfertilized eggs and brown eggs as those which had been fertilized but had died during embryogenesis. The number of larvae was estimated by the total number of eggs picked less the unhatched eggs. Dying or dead pupae were examined after removal from the vials and the dissection of the pupae from the pupal cases.

Mutants were selected by a one-generation screen (Fig. 1) for the survival of *crc* homozygotes following EMS treatment of the male parents as described by Lewis

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& Bacher (1968). Selection of the surviving *crn cn*/crc cn bw^D* progeny was facilitated by their straight wings and white eyes. (* will be used throughout this paper to denote the second or third chromosomes derived from the mutagenized male parent.) The other surviving progeny are *SM5/crc cn** and *SM5/crc cn bw^D* which both have curly wings and cinnabar or light brown eyes, respectively. The *bw^D* mutation was used in subsequent crosses to identify the unmutagenized *crc cn bw^D* chromosome. The *crc cn*/crc cn bw^D* class was expected to include escapers as well as new mutants. A second cross was made (Fig. 1) and from this cross only

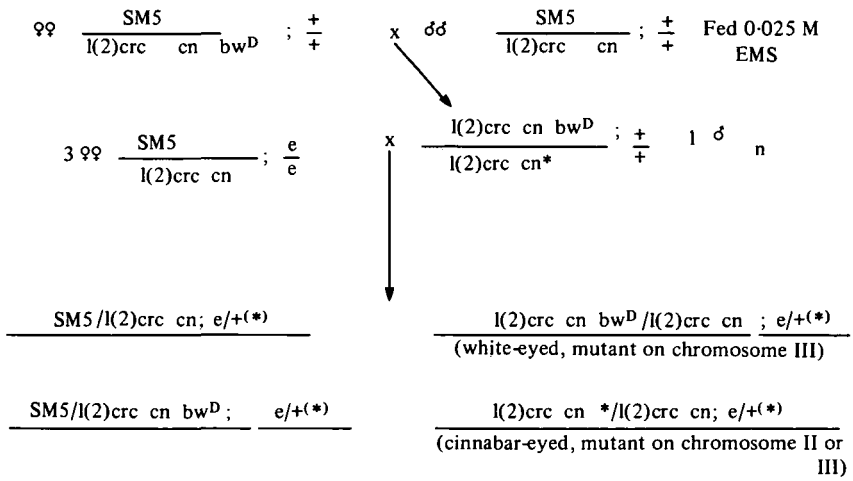

Fig. 1. Crosses for the recovery of revertants and autosomal suppressors of *cryptocephal*. *, denotes chromosomes from the mutagenized male parent in cross 1. (*), denotes chromosomes which may or may not be derived from the mutagenized male parent.

the true mutants should have produced cultures in which the frequency of *crc* homozygotes was significantly increased. In this cross *crc cn/crc cn bw^D* progeny could only arise in appreciable numbers if the newly induced suppressor mutation is on the second chromosome.

No attempt was made to deal with the problem of mosaics induced by the post-meiotic mutagenesis with EMS (Jenkins, 1967). A number of potential mutants will have been lost because the proportion of germ cells in a mosaic fly which carry the mutation may have been too low to significantly increase the frequency of *crc* homozygotes in the progeny.

3. RESULTS

In the selection experiment 15007 F_1 flies were scored and among these were 31 *crc cn*/crc cn bw^D* males and 23 females. The females were not kept, first because they may have had newly induced sex-linked suppressors which could not have been easily isolated from the strains used, and second because recombination in

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the females between the two second chromosomes would have caused difficulties in following the mutagenized chromosome. Only 19 of the males produced progeny in the cross with *SM5/crc cn; e/e* females and only two cultures labelled *Su1* and *Su2*, contained homozygotes at a greater frequency than 10% (Table 1). This figure was chosen as an arbitrary cut-off as it represents an approximate hundredfold increase in *crc* homozygote survival. In both the *Su* cultures the surviving homozygotes were *crc cn*/crc cn; e/+* (*). There were no

Table 1. *Frequency of* crc *homozygotes in the second cross of the selection procedure*

(Cross: ♀♀ *SM5/crc cn; e/e* × *crc cn bw^D/crc cn*; +/+** ♂♂.)

		Culture	
Progeny genotype		*Su1*	*Su2*
SM5crc cn; e/+*$^{(*)}$		16	70
SM5crc cn bw^D; e/+$^{(*)}$		14	25
crc cn/crc cn; e/+*$^{(*)}$		19	15
crc cn/crc cn bw^D; e/+$^{(*)}$		0	0
	Total	49	110
crc homozygotes (%)		38·8	13·6

crc cn/crc cn bw^D progeny. Since the latter class of progeny would only be produced by the action of a third chromosome suppressor it was believed that the two new mutants must be on the second chromosome. Single *crc cn*/crn cn; e/+* (*) males from the two cultures were mated in replicate vials to *SM5/crc cn bw^D* females. If the suppressor mutation is on the second chromosome then all replicate vials should have produced *crc cn*/crc cn bw^D* progeny; if it is on the third chromosome then only males carrying a third chromosome from the EMS treated male parent could produce such progeny, which would consequently only appear in some of the replicate vials. The *Su2* males produced *crc* homozygotes in only one of the eight replicate vials and this isolate must therefore be considered to contain a third chromosome suppressor. This was confirmed by crossing *crc cn*/crc cn; e/+*(*) males from the *Su2* culture to *SM5/+ ; TM3/+* females. Single male progeny from this cross which were respectively, *SM5/crc cn*$^{(*)}$*; TM3/+*$^{(*)}$ and *SM5/crc cn*$^{(*)}$*; TM3/e* were mated to *SM5/crc cn bw^D* females. None of the second group of males produced cultures containing *crc* homozygotes but five of the seven males tested from the first fathered *crc* homozygotes in excess of 10 % of the total progeny eclosing. This confirms that *Su2* is a third chromosome suppressor and is not on the second chromosome as preliminary results had suggested. The reason for these earlier results is not apparent but the difference lies in the non-survival of *crc cn/crc cn bw^D* in the earlier cross (Table 1).

The *Su1* males in the initial cross to *SM5/crc cn bw^D* females produced considerable numbers of *crc* homozygotes in every replicate vial. It can therefore be concluded that Su1 contains a second-chromosome suppressor. This was confirmed by crosses of *crc cn*/crc cn; e/+*$^{(*)}$ males from the Su1 culture to *SM5/crc cn;*

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e/e females. The progeny of this cross included *crc* homozygotes half of which were also ebony homozygotes and the suppressor mutation cannot therefore be on the third chromosome.

In this paper only the analysis of the second chromosome suppressor *Su1* will be considered further. The analysis of the third chromosome suppressor, *Su2* will be described in a subsequent paper.

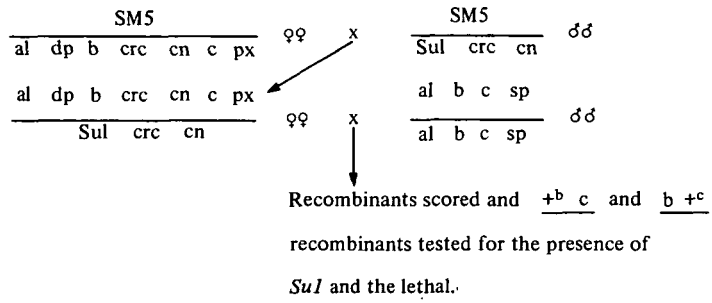

Fig. 2. Crosses for the mapping of the suppressor and lethal effects in the *Su1* chromosome.

The *Su1 crc cn* chromosome contained at least one recessive lethal mutation since homozygote survivors were very rare and it was kept balanced over *Cy0* or *SM5*. The lethality was mapped using an *al b c sp* chromosome. The results showed there was a single lethal gene mapping at $59{\cdot}2 \pm 3{\cdot}0$.

The suppressor effect was approximately mapped by crossing *SM5/Sul crc cn* females to *al dp b pr c px* males. Since any detection of the suppressor effect depends upon having homozygous *crc* progeny, only non-purple recombinants which were either *al dp b* or *c px* were test-crossed to *SM5/crc cn bw*^{D} females. The *pr* gene (54·5) is close to the *crc* gene (54·9) and the non-purple recombinants would therefore almost always carry *crc*. All the *c px* recombinants (6) and all but one of the *al dp b* recombinants (5) when tested produced viable *crc* progeny, suggesting that the suppressor mutation maps between *b* and *c*.

Since the suppressor mutation and the lethal mutation both map in the region between *b* and *c*, a part of the genome that includes *crc*, it was important to investigate the genetic relationships of these genes more closely.

The possible identity between the suppressor mutation and the new lethal mutation was resolved by mapping both effects in a single experiment (Fig. 2). Each of the males selected from the total male progeny (656) of the second cross on the basis of carrying the recombinant chromosomes $b+^c$(76) or $+^b c$(74) was individually mated to two *SM5/crc cn bw*^{D} females. Surviving *crc cn bw*^{D}*/crc cn* flies in the cultures indicate the presence of the suppressor mutation in the recombinant chromosome. From each of these vials SM5/recombinant males were crossed to *SM5/Su1 crc cn c px* females and the progeny scored for the presence of *crc* homozygotes. From 150 males initially selected only 108 produced sufficient progeny in both test-crosses to definitely identify the genotype of each recombinant

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(Table 2). The occurrence of some recombinants which showed suppressor activity or lethality but not both shows that the two effects are not due to a single mutation. The two mutants have been designated as *Su(crc)1* and *l(2)YOa*. From this experiment, using the known map distance between *b* (48·5) and *c* (75·0), *Su(crc)1* was mapped at 54·7 ± 0·9 and *l(2)YOa* at 57·6 ± 0·7.

Since the *crc* gene has been located cytologically to polytene chromosome bands 39C2–39D1 (Wright *et al.*, 1976) it was possible to use a Y : 2 translocation *T(Y : 2)G*

Table 2. *Results of mapping the suppressor effect and the lethal mutant in the* Su1 *chromosome*

Recombinants		Recombinants	
b Su + *c*	8	+ + + *c*	10
+ *Su l c*	37	+ + *l c*	0
b Su ++	3	*b* + *l* +	3
b Su l +	9	*b* + ++	38

Total male progeny scored for *b* and *c* 656.
Total chromosomes tested for *l(2)YOa* and *Su1* 108.
Total number of crossovers detected between *b* and *c* 131.
Map distances were calculated from $\frac{\text{crossovers}}{131} \times 27{\cdot}0$.

to produce a wild-type duplication of the *crc* region to determine if the *l(2)YOa* mutation is also in proximal 2L, or in 2R where the recombination analysis placed it. The *Dp(Y : 2)G* segregants are duplicated for 36B–5; 40F and therefore include the *crc* region (Lindsley & Grell, 1968). The results of cross 1 (Table 3) show that the male progeny homozygous for *crc* survived (10·7 % of total male progeny) confirming that *crc* is in the duplicated region. The maximum expected frequency of these males is 25 %. There are no *crc cn* females in these cultures or in those of cross 2 since the females do not have the Y : 2 duplication. Cross 3 produced no homozygous males despite the presence of the duplication. This observation and the lack of any differential survival of *l(2)YOa* homozygous males and females in crosses 3 and 4 show that this lethal is not included in the duplicated region. The *l(2)YOa* mutant must therefore have been a second mutation simultaneously induced with the *Su1* in the *crc* chromosome. The three males from cross 3 which were supposedly *crc* homozygotes because they were also *cn/cn* could have resulted from crossovers between *crc* and *cn*. The occurrence of homozygous males and females in crosses 3 and 4 may be explained in the same way but could have been *Sul crc l(2)YOa* homozygous survivors, which have been observed at a low frequency in the balanced stock. This experiment further shows that a wild-type allele is dominant to two doses of *crc*, since *Dp crc*$^{+}$*/crc/crc* males survive.

All subsequent experiments on *Su1* were performed with recombinant chromosomes which do not contain *l(2)YOa*.

The other possible identity is between *Su1* and *crc*. The results so far do not distinguish between two possibilities. First, that *Su1* is a revertant of *crc*; second,

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that *Su1* is a dominant suppressor of *crc* lethality in a separate gene in proximal 2L.

Reversion of a mutation occasionally results in the recovery of a temperature-sensitive allele (*ts*). The *Su1* chromosome was examined for temperature sensitivity at 30 °C (Table 4) and showed the presence of a recessive temperature-sensitive lethal. This lethal is expressed when present in *Su1 crc* homozygotes (cross 1) or in heterozygous combination with the *crc cn bw*^{D} chromosome (cross 2). However,

Table 3. *The use of the* T(Y:2)G *stock to locate the lethal present in the* Su1 *chromosome*

	Progeny							
	SM5/al b crc cn		*SM5/b pr*		*crc cn/b pr*		*al b crc cn/crc cn*	
Cross	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
(1)	46	23	43	37	65	48	0	13
(2)	72	71	79	64	102	104	0	3
	SM5/al Su1 crc cn		*SM5/b pr*		*Su1 crc cn cp/b pr*		*Su1 crc cn sp/ al Su1 crc cn*	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
(3)	45	12	37	12	71	446	1	0
(4)	47	22	55	52	56	54	2	4

Cross (1): + / + ; *SM5/crc cn* ♀♀ × + / *Y,DpG; al b crc cn/b pr* ♂♂.
Cross (2): + / + ; *al b crc cn/b pr* ♀♀ × + / *Y; SM5/crc cn* ♂♂.
Cross (3): + / + ; *SM5/Su1 crc cn sp* ♀♀ × + / *Y,DpG; al Su1 crc cn/b pr* ♂♂.
Cross (4): + / + ; *al Su1 crc cn/b pr* ♀♀ × + / *Y; Su1 crc cn sp/b pr* ♂♂.

the *ts* lethal is not always fully penetrant at 30 °C (see Table 5). This temperature-sensitive behaviour is found in all crosses between *Su1 crc* and *crc* chromosomes. The viability of *Su1 crc/crc* heterozygotes developing at 25 °C varies between different crosses and experiments.

The deficiencies recovered by Wright *et al.* (1976) locate *crc* to the four polytene chromosome bands 39C2–39D1 and are described in Fig. 3. In fact these investigators were unable to detect cytological differences between the proximal breakpoints of *Df(2)12* and *Df(2)1*. However, although *Df(2)12/crc* heterozygotes are lethal, *Df(2)1/crc* individuals are viable (Wright *et al.*, 1976: see also Table 5). These deficiencies were used to answer two questions. First, is the temperature-sensitive lethal effects of the *Su1* chromosome in proximal 2L? Second, does it behave in deficiency mapping as a revertant of *crc?* Crosses between *SM5/al dp b crc cn c* females and males from the deficiency stocks were performed at 25 and 30 °C as controls. The *ts* effect was mapped by crossing *Cy0/Su1 crc cn c* females to males from the deficiency stocks. These later crosses were made at 25 °C and left at this temperature for three days before half of the vials of each cross were moved to

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Table 4. *The temperature sensitivity of the* Su1 *chromosome*

Cross: *SM5/Su1 crc cn c* ♀♀ × *SM5/Su1 crc cn c* ♂♂

Temperature (°C)	*SM5/Su1 crc cn c*	*Su1 crc cn c*	P (%)
25	393	44	22·4
30	214	0	0·0

Cross: *SM5/Su1 crc cn* ♀♀ × *SM5/crc cn bw*^{D} ♂♂

Temperature (°C)	*SM5/crc cn bw*^{D}	*SM5/Su1 crc cn c*	*Su1 crc cn c/ crc cn bw*^{D}	P (%)
25	43	37	30	81·8
30	64	66	0	0·0

P, fraction (as %) of expected numbers of non-*SM5* genotypes eclosing.

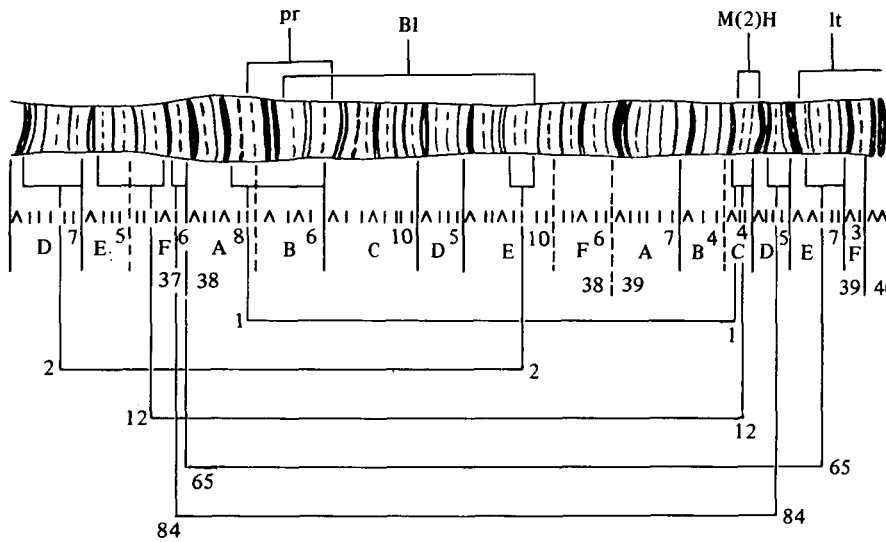

Fig. 3. Polytene chromosome map of proximal 2L showing the cytological map of the deficiency chromosomes used to locate the temperature sensitive effect. (Derived from Wright *et al.* 1976).

30 °C to complete their development. Under these conditions, large progeny numbers are produced and the *ts* lethal is almost completely penetrant. The results are presented in Table 5. The results of crosses between *Cy0/al dp b crc cn c* and *Cy0/Su1 crc cn c* strains are included for comparison. Note that the *Su1 crc/crc* heterozygotes are not as viable at 25 °C in these crosses as the *Cy0* heterozygotes but this is not always so. The results obtained in the crosses between *crc* and the deficiency stocks confirm the findings of Wright *et al.* (1976). The *crc* is lethal in combination with *Df(2)84* and *Df(2)12* but not with *Df(2)1* at both temperatures. The *Su1 crc cn c* chromosome is viable in all combinations with the Df(2) chromosomes at 25 °C. At the higher temperature *Su1 crc cn c* heterozygotes with Df(2)84 and Df(2)12 are lethal, as might be expected if *Su1* is a temperature-sensitive allele

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of *crc*. However, the lethality of *Df(2)1/Su1 crc cn c* heterozygotes at 30 °C suggests that the temperature-sensitive effect is due to a mutation in a gene other then *crc* which maps more distally in chromosome arm 2L within polytene chromosome bands 38A7–B1 (distal breakpoint of *Df(2)1*) and 39C2–D1 (proximal breakpoint of *Df(2)1*). Since *Su1 crc cn c /Df(2)2* heterozygotes survive at both

Table 5. *Deficiency mapping of* crc *and the temperature-sensitive effect in the* Su1 *chromosome*

Cross *Cy0/Su1 crc cn c* ♀♀ × *Cy0/Df(2)* ♂♂

Df(2)	Temperature (°C)	*Cy0/Su1 crc*	*Cy0/Df*	*Df/Su1 crc*
1	25	211	167	186
	30	191	81	0
12	25	196	183	154
	30	176	96	0
84	25	223	288	198
	30	236	7	0
2	25	99	78	66
	30	122	74	64

Cross *Cy0/al dp b crc cn* ♀♀ × *Cy0/Df(2)* ♂♂

		Cy0/crc	*Cy0Df*	*Df/crc*
1	25	166	154	139
	30	205	107	146
12	25	584	361	1
	30	274	52	0
84	25	548	358	0
	30	396	196	0
2	25	81	76	70
	30	49	25	29

Cross *Cy0/al dp b crc cn* ♀♀ × *Cy0Su1 crc cn c* ♂♂

	Cy0/crc	*Cy0/Su1 crc*	*crc/Su1 crc*
25	285 (214)	300 (247)	142 (131)
30	260 (249)	350 (241)	5 (8)

Figures in parentheses are the progeny from the reciprocal cross.

temperatures, the *ts* mutation is not included within *Df(2)2* and must be included in the region bounded by the proximal breakpoint of *Df(2)2*, 38E7–9 and the proximal breakpoint of *Df(2)1*, 39C2–D1. As can be seen from Fig. 3 this region contains approximately 30 polytene chromosome bands.

Temperature sensitivity has been used as a powerful tool with which to study gene expression in a number of organisms. For *D. melanogaster* Suzuki (1970) has described the use of temperature shifts at different stages of development to determine the temperature-sensitive period (TSP) of mutant gene expression. This

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technique of temperature shifts from 30 to 25 °C (shift-down) and from 25 to 30 °C (shift-up) has been used to determine the TSP of *Su1* (Fig. 4). Eggs were collected at 25 °C for 12 h and placed in vials which were then incubated at 25 or 30 °C. At different times after egg collection the cultures were shifted to the higher or lower temperature. The development times of the cultures incubated initially at 30 °C have been adjusted to correspond to the development times at 25 °C. The development times for the 30 °C cultures are taken from the mean time taken to eclosion of the two genotypes which survive at this temperature. The organisms develop more rapidly at the higher temperature and the times of shift-down were adjusted by the ratio of complete development at 30 °C to that at 25 °C.

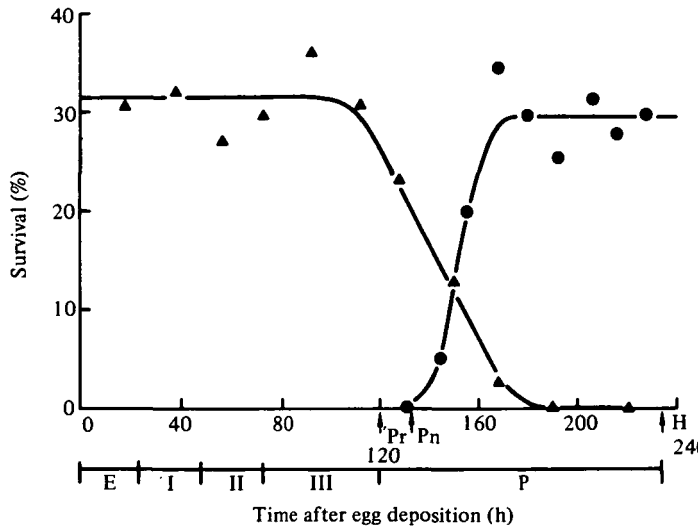

Fig. 4. Results of temperature shift experiment with cultures of the *Cy0/Su1 crc cn c* stock. ○, Shift-up from 25 to 30 °C. ▲, Shift-down from 30 to 25 °C. E, embryonic period; I, II, III, larval instars; P, pupal period; H, eclosion; Pn, pupation; Pr, pupariation. The ordinate (% survival) is *Su1* homozygote survival expressed as percent of the *Cy0/Su1 crc cn c* heterozygotes eclosing from the same cultures.

The results (Fig. 4) show that 112 h of development has no effect on *Su1 crc cn c* homozygote survival prior to shift down. After this time fewer homozygotes survive despite being shifted down, until at 190 h none survive. The first significant decrease in viability is at 128 h and this defines the beginning of the TSP. Cultures shifted up show no appreciable homozygote survival until 144 h, and survival increases with the lateness of the shift until at about 168 h survival reaches the level found in cultures maintained at 25 °C throughout development. This defines the end of the TSP. The temperature-sensitive period determined in this experiment thus extends from about 128 to 168 h. Similar data has been obtained from *crc cn* bw^D*/Su1 crc cn* and *al dp b crc cn/Su1 cn c px* heterozygotes (Sparrow unpublished). This appears to be the only TSP for *Su1/Su1* homozygotes and

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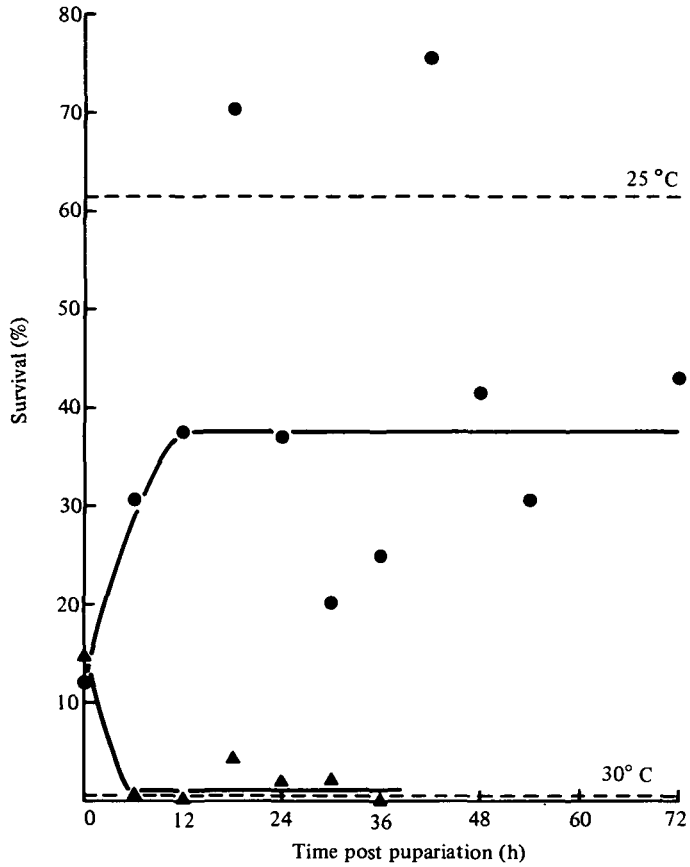

Fig. 5. Results of temperature shift experiment with pupae collected from a cross between *Cy0/al dp b crc cn* ♀♀ and *Cy0/Su1 crc cn c px* ♂♂ Symbols as in Fig. 4. The ordinate (% survival) is *Su1 crc/crc* eclosion expressed as percent of the total of the two *Cy0* genotypes eclosing. The dashed line is the *Su1 crc/crc* % survival for cultures grown and kept at 25 or 30 °C.

Su1crc heterozygotes during development. Collections of eggs from the parents used in these experiments on consecutive days at 25 and 30 °C show the same degree of egg hatching. Adult *Su1 crc cn* homozygotes for a number of days at 30 °C.

Individuals whose development is initially synchronous in short period egg collections will inevitably become increasingly asynchronous. The above experiment in which the TSP is some 5 days post egg lay is expected to have reduced synchrony of individual development by this time. A further experiment was performed in which the individuals were collected at pupariation. Adults from the cross *Cy0/al dp b crc cn* ♀♀ × *Cy0/Su1 cn c px* ♂♂ were allowed to lay eggs in vials for a 24-h period. Half of these vials then kept at 25 °C and the others placed at 30 °C. Pupae were collected at 2-h intervals from both sets of vials as they

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pupariated. The pupae were shifted up or down at various times after collection. Collections for each shift time were made from the vials both early and late in the total collection period. The results of these pupal shifts are presented in Fig. 5. It can be seen that the TSP begins prior to pupariation and from the shift-up data is apparently complete by 12 h post pupariation. This TSP is clearly not coincident with that obtained in the previous experiment (Fig. 4) in which cultures were shifted up and down. Casual observation suggested that early pupae collections contained few *Su1/crc* heterozygotes. During the pupal experiment described

Table 6. *Estimation of the genotype frequencies of pupae collected from the* Cy0/al dp b crc cn ♀♀ × Cy0/Su1 cn c px ♂♂.

Time since egg collection		Genotypes		
		Su1/crc	*Cy0/Su1* + *Cy0/crc*	% *Su1/cn**
Day 5	a.m.	4	68	5·9
	p.m.	14	34	41·2
Day 6	a.m.	70	133	52·5
	p.m.	49	41	119·5
Day 7	a.m.	142	147	96·6
	p.m.	11	12	91·6

* % *Su1/crc* is the percentage of eclosing *Su1/crc* flies as a percentage of the sum of *Cy0/Su1* and *Cy0/crc* flies

above a series of collections were made at 25 °C overnight (8 h) and for 4 h each evening throughout the total period when pupae were collected (3 days). These pupae were maintained for the rest of pupal development at 25 °C and the adults scored to estimate the proportion of the genotypes pupating at the collection times. Pupal collection times since egg lay are expressed in half days (a.m. and p.m.) because the egg collection period (24 h) does not permit greater accuracy. The results show that the development of *Su1/crc* heterozygotes is much slower at 25 °C than is that of other surviving genotypes. The same is probably also true at 30 °C although this is not directly testable. This slower development provides an explanation for the rather large spread of data points (Fig. 5) as due to each pupal collection not containing the genotypes in the expected ratios. The difference in TSP's between the two experiments (Fig. 5 and Table 6) is also resolved by these observations. Cultures contain all three genotypes in equal frequencies but the shift times correspond to earlier developmental stages of *Su1/crc* heterozygotes and *Su1/Su1* homozygotes compared to those stages reached by the *Cy0* and *SM5* heterozygotes. The more accurate TSP determination, although incomplete, is clearly that from pupal collections. The TSP for *Su1* suppression thus begins before pupariation and ends at about 12 h post pupariation just prior to or at the time of head eversion at 12 h.

Although the temperature-sensitive period of *Su1 crc* lethality occurs before and

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Table 7. *Results of an experiment to determine the effects of temperature on the survival patterns of* Su1 *and* crc *genotypes*

Cross	Temperature (°C)	(E)	(L)	(P)	Eggs	Eclosing genotypes		
(A)	25	11·2	25·7	1·6	187	*Cy0/Ore* 43·3	*Ore/Ore* 18·2	—
	30	14·7	25·8	9·5	190	27·9	22·1	—
						Ore+Ore/crc	*crc/crc*	
(B)	25	1·3	8·8	20·6	194	69·1	0·0	—
	30	2·0	10·2	20·8	197	67·0	0·0	—
						Cy0/Su1 crc	*Cy0/Df84*	*Df84/Su1 crc*
(C)	25	3·1	21·3	6·9	160	28·7	23·8	16·3
	30	6·0	39·0	16·8	167	22·8	24·6	0·0
						Cy0/Su1 crc	*Su1 crc/Su1 crc*	
(D)	25	15·1	15·1	5·9	186	43·6	20·4	—
	30	14·5	14·5	22·4	179	48·6	0·0	—
						Cy0/Su1 crc	*Cy0/crc*	*crc/crc Su1*
(E)	25	16·9	25·0	6·1	148	16·9	19·6	15·6
	30	13·8	20·8	17·6	159	23·9	22·6	1·3

All figures are expressed as percent fertilized eggs. *Eggs* is the total number of fertilized eggs. E, L and P are the egg, larval and pupal mortalities respectively.

Crosses: (A) *Cy0/Ore* ♀♀ × *Cy0/Ore* ♂♂
(B) *Ore/al dp b crc cn* ♀♀ × *Ore/al dp b crc cn* ♂♂
(C) *Cy0/al Su1 crc cn c px* ♀♀ × *Cy0/Df(2)84* ♂♂
(D) *Cy0/Su1 crc cn c* ♀♀ × *Cy0/al Su1 crc cn c px* ♂♂
(E) *Cy0/al dp b crc cn* ♀♀ × *Cy0/Su1 crc cn c* ♂♂

up to head eversion, casual observation showed that death at the higher temperature was primarily occurring late in the pupal period. The dead pupae have everted heads and are apparently fully differentiated. To examine this further, eggs were picked from a number of crosses raised at 25 or 30 °C to examine the time of death more closely. The results are shown in Table 7. Cross A was studied to determine the time of death of *Cy0* homozygotes, since these would be produced in all but one of the other crosses. The results of cross A show considerable embryonic and larval mortality. The survival of *Cy0/0re* and *0re* progeny is not as high as expected

Table 8. *Pupal mortality patterns of crosses described in Table 7*

Cross	Temperature (°C)	Early	Cryptocephal	Late	Total
(A)	25	0	0	3	3
	30	0	0	18	18
(B)	25	10	27	3	40
	30	8	18	15	41
(C)	25	0	0	11	11
	30	1	0	27	28
(D)	25	0	0	11	11
	30	1	1	38	40
(E)	25	0	0	9	9
	30	0	0	28	28

(50 and 25 % respectively). It is not clear from these data whether *Cy0* homozygotes die as embryos or larvae. Clearly however they do not survive to the pupal stage. In cross B the survival of the phenotypically indistinguishable *Ore* homozygotes and *crc* heterozygotes is not significantly reduced from the 75 % expected at either temperature. There was the typical pupal lethality associated with the *crc* homozygotes. Throughout these experiments dead pupae were classified into three distinct classes. They were classified as 'early' if the pupal case was formed and hardened but there was no fully formed pupa inside. Cryptocephal pupae were those with the characteristic phenotype: a fully differentiated pupa which had not everted the cephalic complex. The third class of dead pupae, classified as 'late', were fully formed and differentiated pupae which had everted their heads but had failed to eclose. In the case of cross B at 25 °C pupae of all three classes were observed although the majority showed the cryptocephal phenotype (Table 8). The presence of *crc* homozygous pupae with the phenotype designated 'late' in this paper have been previously described by Hadorn & Gloor (1943). The occurrence of 'early' dying pupae has been found in nearly all crosses producing *crc* homozygotes in the present study. At 30 °C, although there was no significant change in the survival pattern of progeny from cross B (Table 7), examination of the dead pupae show by comparison with the progeny at 25 °C a decrease in the proportion of pupae with 'cryptocephal' phenotype and an increase from 7·5 to 36·5 % in those pupae dying with the 'late' phenotype ($\chi^2 = 10{\cdot}0$, 2 D.F.). The

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results of crosses C, D and E (Table 7) show again the temperature sensitivity of *Su1 crc* homozygotes, hemizygotes and *Su1 crc/crc* heterozygotes. Examination of the pupal mortality patterns (Table 8) shows that the majority of all dying pupae in these three crosses died as 'late' pupae. In each case the number of 'late' pupae is much increased at the higher temperature, suggesting that the reduction of *Su1 crc* genotypes eclosing is due to to the death of the organisms at this stage.

Surviving *Su1 crc/crc* heterozygotes and *Su1 crc* homozygotes have a normal external appearance. However, the flies are invariably slow-moving, exhibit low spontaneous movement, never fly and have extreme difficulty in climbing the walls of the vials. Both males and females of these genotypes are only marginally fertile but the degree to which this is expressed is in some part dependent on the genotype of the mating partner.

4. DISCUSSION

Hadorn & Gloor (1943) found that typically most *l(2)crc* homozygous pupae fail to evert their cephalic complexes and have shorter legs than normal. Death of these pupae does not occur at the time of normal head eversion and the pupae continue to differentiate, producing most normal adult structures. One structure frequently underdeveloped is the abdomen. Death apparently occurs at the end of the pupal period. The phenotype is expressed to varying degrees. Some pupae have small everted or partially everted heads or manage to evert only one side of the head. A further small group appear normal in all external characteristics except for shortened legs, but fail to eclose.

The production of *l(2)crc* phenocopies by dietary glucosamine led to the suggestion that the mutant phenotype arises due to an alteration in chitin synthesis (Rizki, 1960), of which glucosamine is a precursor. Assays of the glucosamine released by acid hydrolysis of pupal cuticles (Firstrom 1964, 1965) showed that the phenotypically most extreme *l(2)crc* homozygotes have 50–60 % more chitin than wild type controls. This led to the hypothesis that excess chitin in the pupal cuticle stiffens the cuticle, causing greater resistance to the eversion of the cephalic complex. Since Fristrom's work no further reports have appeared to substantiate this hypothesis or determine the biochemical lesion produced by the mutation.

The genetic analysis of the Su1 chromosome identifies two or perhaps three genes in proximal 2L which are mutant in the *Su1* chromosome and produce a temperature-sensitive effect and a suppressor effect on one of these genes, *l(2)crc*. Alternative models are possible to explain the results of the genetic experiments (Fig. 6). In model 1 the temperature-sensitive effect and the suppressor activity are due to two new mutations in a chromosome which still retains the original *l(2)crc* mutant. The other two models assume that the suppressor activity is either temperature-sensitive (model 3) or due to a revertant of the original *l(2)crc* mutant (model 2). Since the deficiency experiments (Table 5) show that the *l(2)crc cn* chromosome does not carry the temperature-sensitive mutation present in the *Su1* chromosome, the temperature-sensitive mutation must have a dominant expres-

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sion in *Su(crc)1 l(2)crc/l(2)crc* combinations. However as *Su(crc)1 l(2)crc/+ +* genotypes are not temperature-sensitive (Tables 4, 5 and 7) the temperature sensitivity cannot be due to the expression of an independent dominant *ts* mutation but must be involved in the expression of the *l(2)crc* gene. As both the suppressor and temperature-sensitive effects are dominant and affect the expression of *l(2)crc*, it seems likely that they are both due to the same mutation and if so, since the *ts* effect is separable from *l(2)crc* this supports model 3. Models 1 and 2 depend on

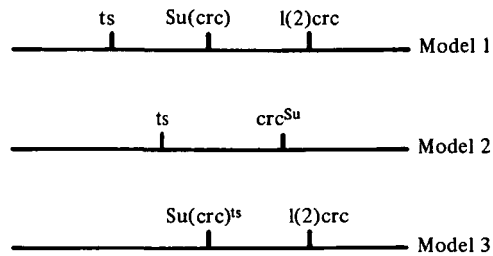

Fig. 6. Alternative models of the mutants in proximal 2L on the *Su1* chromosome.

two new mutations being involved in the expression of *l(2)crc*. A prediction of models 1 and 3 is that the original *l(2)crc* mutation should be recoverable by recombination from the *Su1* chromosome. The working hypothesis is that *Su(crc)1* is dominant, temperature-sensitive suppressor of *l(2)crc*. However it should be noted that in certain genotypes *Su(crc)1* may also be considered a recessive temperature-sensitive mutation.

The cytogenetic data show that the *1(2)crc* mutation is located at 39C2–D1, a chromosome segment of only four polytene chromosome bands (Wright *et al.* 1976). The deficiency chromosomes localise the temperature sensitive effect of *Su(crc)1* to the 30 polytene chromosome bands lying between 38A7–B1 and 39C2–D1 (Table 5). However the data also prove that *Su(crc)1* does not lie in the four band region to which *l(2)crc* has been localized, since *Su(crc)1 l(2)crc/Df(2)1* individuals are viable at the restrictive temperature (Table 5). These results do not preclude the possibility that the two genes, *l(2)crc* and *Su(crc)1* are very closely linked but separated by the proximal breakpoint of *Df(2)1*.

Attempts to recombine the *Su(crc)1* mutation from *l(2)crc* in the *Su1* chromosome have so far proved unsuccessful. In these experiments the design has depended on the assumption that the *Su(crc)1crc*$^+$ recombinant chromosome would be viable at 30 °C in combination with $+^{Su}$*l(2)crc* but temperature-sensitive as a hemizygote with all the deficiencies described here with the exception of *Df(2)1*, and as a homozygote. Despite the screening of many (> 200) recombinants obtained in the region between *pr* (54·5) and *cn* (57·0) no recombinant chromosome with the expected phenotype has yet been recovered. It could be that the temperature-sensitive behaviour of *Su(crc)1* depends upon having *l(2)crc* in the cis configuration and that the expected phenotype of *Su(crc)1 crc*$^+$ is in error. This can be countered in part by the observation that the reciprocal product of recombination,

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$+^{Su}$*l(2)crc*, has also not been recovered in these experiments. In the absence of a positive result one cannot estimate the distance between the two mutations. The failure to recover the recombinants reflects the close proximity of these mutations as demonstrated by deficiency mapping.

Fristrom (1965) found that in homozygous *l(2)crc* pupae there was an increased chitin content of 50–60 %. He postulated that this might be due to increased levels of the initial enzyme or to loss of feedback control in the chitin biosynthetic pathway. Although there is no further biochemical evidence on this point the gene dosage data on *l(2)crc* and its wild-type allele are relevant. One possible hypothesis is that the cryptocephal phenotype is due to increased activity of the *crc* gene product. On this model crc^+/crc^+, $crc^+/l(2)crc$ and $crc^+/Df\ crc$ genotypes are viable because they produce sufficient gene product that chitin is produced in amounts sufficient for a functional cuticle but not in excess. This permits the eversion of the pupal cephalic complex and eventual eclosion. However, this model fails to explain why Df *crc*/*l(2) crc* is lethal when the prediction would be that it should produce less gene product than $crc^+/l(2)crc$ but more than $crc^+/Df\ crc$ and thus be viable. It also fails to explain the suvival of $crc^+/l(2)\ crc/l(2)crc$ males from crosses involving *T(Y :2)G* (Table 3). The simplest explanation of the dosage data is that *l(2)crc* is a hypomorphic or amorphic allele of the crc^+ gene.

There are a number of ways that an allele which effectively reduces gene product levels could effect an increase in chitin synthesis, but a more simple hypothesis is that the crc^+ gene product is involved in a second pathway which competes for substrates with the chitin biosynthetic pathway. Mutation to an amorphic or hyphomorphic allele might then increase substrate availability for chitin biosynthesis.

The single temperature sensitive period of *Su(crc)1* expression extends from before pupariation up to 12 h post-pupariation (Fig. 5). Chitin deposition in the pupal cuticle of wild-type and *l(2)crc* pupae is initially detectable at 10–11 h post-pupariation and about 2 h before head eversion (Chadfield & Sparrow, in preparation). The TSP for *Su(crc)1* expression thus precedes the period of chitin deposition and head eversion.

Examination of the time of death of *Su(crc)1 l(2)crc* homozygotes, hemizygotes and *Su(crc)1 l(2)crc*/*l(2)crc* heterozygotes at 30 °C (Table 8) leads to the conclusion that the effective lethal phase occurs at the end of the pupal period. The dead pupae are morphologically normal. Clearly at the higher temperature suppression by *Su(crc)1 of l(2)crc* is only partial. Suppression permits the pupae to pass through the *l(2)crc* phenocritical stage of head eversion but they fail to survive a second late pupal *l(2)crc* phenocritical stage to eclose. Lethal cryptocephal homozygotes grown at 30 °C show an increased frequency of death just prior to eclosion with a normal morphology (Table 8). This observation and the description of some *l(2)crc* homozygotes which at 25 °C die with the same late phenotype (Table 8 and Hadorn & Gloor, 1943) identify this second phenocritical stage for *l(2)crc*. No chitin assays have yet been performed on such pupae at the end of the pupal period but *l(2)crc* pupae which have everted their heads by 15 h after pupariation have a chitin

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content intermediate between non-everted *l(2)crc* homozygotes and wild-type controls (Fristrom, 1965). It is not known if death at the second phenocritical stage is due to increased chitin deposition.

Even at 25 °C suppression of *l(2)crc* by *Su(crc)1* does not produce normal adult flies. The flies have a much reduced level of spontaneous activity and are only marginally fertile. It is possible that the reduced fertility is a product of the behavioural syndrome. Increased cuticular stiffness from excess chitin deposition could account for the behavioural abnormality, but chitin assays have not shown any significant differences between these flies and wild-type controls (Sparrow, unpublished).

The recovery of the *Su(crc)1* suppressor mutation identifies another gene involved in the expression of *l(2)crc*. The genetic data cannot be used to identify the form of suppression occurring in this system but it seems likely that both genes interact in the formation of an active enzyme or a biochemical pathway. Informational suppressors have not yet been identified in *Drosophila melanogaster*. The added information which a temperature-sensitive, dominant suppressor of *l(2)crc* can provide will hopefully lead to the determination of the molecular lesions produced by the *l(2)crc* mutant and the suppressor mutation itself.

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