

Effects of Some *Trl* Mutations on Mitosis in Embryonic and Larval Tissues and Egg Chamber Morphology in *Drosophila melanogaster*

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Abstract—A study was made of three insertional mutations (*Trl*^{13C}, *Trl*^{s2325}, *Trl*^{EP(3)3184})

INTRODUCTION

The product of the *Trithorax-like* (*Trl*) gene, the GAGA factor (GAF), is a multifunctional transcription factor that binds to (GA/CT)_n sites in the promoter regions of many genes [1]. Since GAF is necessary for normal expression of several homeotic genes, its gene has been assigned to the *trithorax* (*trxGG*) group [2]. It has been shown that mutations of the *Trl* gene modify mosaic position effect variegation [2–4]. In addition, biochemical data have implicated GAF in regulating the activity of not only homeotic and segmentation genes, but also several housekeeping genes [1, 5]. On evidence of extensive studies, the major function of GAF should contribute to conformational changes in the chromatin structure [1, 5, 6].

As shown by studying the chromosomal location of GAF throughout the cell cycle, the factor is located at numerous dispersed euchromatic AG-rich sites during the interphase and in heterochromatic (predominantly pericentromeric) sites containing (AAGAG)_n and (AAGAGAG)_n satellites immediately before and during the mitosis [7, 8]. Genetic screening for new genes that regulate meiosis have revealed a role of GAF in meiotic chromosome segregation [9]. Chromatin remodeling and chromosome segregation are the key processes of mitosis and meiosis. With the *Trl*^{13C} mutation, GAF has been shown to affect these processes in *Drosophila melanogaster* embryos [10].

We thought it important to study in detail the effect of the *Trl* mutations on the oogenesis and mitosis in embryonic cells and in differentiating larval tissues. For this purpose, we analyzed the morphology of egg chambers and the dynamics of chromosome condensation and segregation at various developmental stages in three *D. melanogaster* strains carrying insertions of the *P* element in the second intron of the *Trl* gene.

MATERIALS AND METHODS

We used the wild-type *D. melanogaster* strains Hikone-AW and Oregon R and three strains carrying mutations in the *Trl* gene: *Trl*^{13C/TM3}, *Sb*¹, *Ser*¹, *y*⁺ (kindly provided by F. Carch, University of Geneva), *w*¹¹¹⁸; *P*_{w⁺mC = lacW}*l(3)s2325^{s2325}/TM3*, *Sb*¹ (obtained from the Bloomington *D. melanogaster* Stock Center, United States), and *w*¹¹¹⁸; *EP(3)3184/TM6B*, *Tb*¹ (this strain carrying an insertion of the *P*{*EP*} series [10] was obtained from the Szeged Stock Center, Hungary). Hereafter, we designate these *Trl* alleles and the corresponding balanced strains as *13C*, *l(3)*, and *3184*, respectively, and balancer chromosomes as TM3 and TM6. The strains were kept on the standard medium.

To establish the embryonic lethality stages, eggs layered in 1 h were collected and placed in a drop of halocarbonic oil on a slide. Slides with embryos were maintained in a moist chamber at 25°C. The preparations were viewed in transmitted light under a binocular

microscope, and developmental stages were identified according to the published criteria [11, 12].

To study the early embryonic divisions, females were placed in vials containing the medium for 2 h, eggs were collected from the medium surface, and preparations were obtained according to J. Puro and S. Nokkala [13].

To obtain preparations of metaphase chromosomes, the nervous ganglia and imaginal disks of third-instar larvae were treated with a hypotonic solution (0.1% sodium citrate) for 5 min and fixed with a methanol–acetic acid mixture (3 : 1) for 20 min. Then, the organs were dispersed with a dissecting needle in a drop of 60% propionic acid on a slide; the preparations were dried and stained with 4% Giemsa stain in the phosphate buffer (pH 6.8).

To study the oogenesis, the ovaries were isolated from females aged three to five days, fixed, and stained with Schiff's reagent according to Puro and Nokkala [13]. Statistical treatment employed Fisher's test (ϕ transformation) [14]. The data on morphological alterations observed in the *Trl* mutants at various oogenetic stages were compared with the corresponding data obtained for the control strain.

There are five stages commonly recognized in mitosis: prophase, metaphase, early anaphase, anaphase, and telophase. In addition, we distinguished one more stage—early prophase. The early prophase is a stage when compartments of four chromosome pairs are visualized. The next stage, when the filamentous chromosome structure is formed, is commonly termed the prophase; we defined this stage as middle and late (classical) prophase. The early anaphase is intermediate between the metaphase and anaphase and involves sister chromatid disjunction without the spindle [15]. In some cells, we observed more intense chromosome condensation in the interphase. To characterize their number, we used parameter *a* defined as the proportion of cells with hypercondensed chromosomes among the total number of dividing cells.

RESULTS

The Effect of the Trl Mutations on Embryonic Development

The mutations under study differed in their effects on viability: individuals homozygous for the *3184* mutation survived until the adult stage and could reproduce, *l(3)* homozygotes died at the third larval instar, and *13C* homozygotes died as embryos [16]. An appreciable decrease in viability was also observed in heterozygotes of all three strains (Table 1, rows 1–4); hence, the mutations were dominant (or semidominant) for this trait.

As is seen from Table 1, the survival of individuals heterozygous for these mutations was almost halved as compared with wild-type Hikone-AW individuals (rows 1–4). The proportion of individuals survived until the adult stage was seven times lower in homozygotes for the *3184* insertion (row 5) compared with the wild type (row 1). The maternal effect appeared to contribute little to lethality of the *3184* homozygotes, because the survival rate was more than three times higher in the progeny of homozygous females crossed with wild-type males (compare rows 5 and 6). It was difficult to analyze the survival of the homozygotes for the *l(3)* and *13C* mutations, because most of these mutants died in early ontogeny. To estimate the proportion of the homozygote lethality in the total lethality observed in the heterozygous strains, we studied the lethality stages in more detail.

As Table 2 shows, three embryonic periods of lethality could be distinguished in strains *l(3)* and *13C*:

- (1) nuclear cleavage divisions until the formation of the blastoderm (stages 1–5),
- (2) formation of mitotic domains (stages 8–12), and
- (3) late embryonic stages (16–17), when larvae were almost completely formed, but were still in the chorion.

The high lethality observed at the latest stages is explained by the fact that individuals homozygous for the balancer chromosome also die in this period (these eggs account for approximately 25% of all eggs produced by heterozygous females). To isolate the lethality of homozygotes for the mutation and lethality of

Table 1. Ontogeny in individuals carrying the *l(3)*, *13C*, and *3184* alleles

Experiment no.	Genotype	Total eggs	Lethality, %		Survived until the adult stage, %
			embryonic and larval	pupal	
1	<i>Hikone-AW</i>	987	9.79	0.54	89.67
2	<i>l(3)/TM3</i>	819	65.57	3.05	31.38
3	<i>13C/TM3</i>	933	72.56	2.36	25.08
4	<i>3184/TM6</i>	593	57.34	5.56	37.10
5					

Table 2. Lethality stages of individuals carrying the *l(3)* and *13C* alleles

Experiment no.	Allele	Strain	Embryonic lethality (%) at a given stage (stages [total duration])				
			1–5 [3 h]	6–7 [1 h]	8–12 [5 h]	13–15 [4 h]	16–17 [8–9 h]
1	<i>l(3)</i>	<i>l(3)/TM3</i> × <i>l(3)/TM3</i>	9.7	1.4	6.9	0	31.9
2		<i>l(3)/TM6</i> × <i>l(3)/TM3</i>	14.8	1.2	4.9	0	9.9
3	<i>13C</i>	<i>13C/TM3</i> × <i>13C/TM3</i>	6.6	0	9.2	0	51.3
4		<i>13C/TM6</i> × <i>13C/TM3</i>	16.7	0	1.4	0	18.2
5	+	<i>+/TM6</i> × <i>+/TM3</i>	2.7	1.4	0	0	7.0
6		<i>+/TM3</i> × <i>+/TM3</i>	0	0	0	0	14.5
7		<i>+/TM6</i> × <i>+/TM6</i>	0.7	0	0	1.0	23.9

homozygotes for the balancer chromosome from the total embryonic the lethality at the latest stages, we analyzed lethality of embryos obtained from crosses between males and females that combined the mutation with different balancer chromosomes (Table 2, rows 2 and 4). In these crosses, heterozygotes for both balancer chromosomes and heterozygotes for the mutation and a balancer chromosome were viable. The same three periods of embryonic lethality were observed (Table 2).

For an additional control, we studied the effect of the balancer chromosomes on embryonic survival (Table 2, rows 5–7). Our data demonstrated that the effect of the balancer chromosomes was seen at the latest (16–17) stages of embryonic development. Note that the lethality caused by the balancer chromosomes was always lower than the total lethality in the strains carrying the mutations under study (compare rows 1, 3, and 6 with rows 2, 4, and 5); therefore, the three lethality periods were determined by the mutations. Cytological examination revealed numerous defects in cleavage divisions, which varied in frequency. The *l(3)* and *13C* mutants displayed dramatic asynchrony of cleavage divisions, which was seen as simultaneous prophases and anaphases (data not shown) and simultaneous telophases and interphases (Fig. 1a). Normally, cleavage divisions are synchronous in all but sexual syncytial nuclei until the blastodermal stage [11, 17]. At cleavage divisions 10–14, after the migration of somatic nuclei to the embryo surface, minor modifications of the nuclear cycles can be observed. For instance, when central syncytial nuclei are in the anaphase, nuclei at the egg poles can be in the late metaphase; however, two distant stages are never observed in neighboring nuclei [11, 12].

Another clear indication to the effect of the *Trl* mutations on cleavage divisions was a changed ratio between the numbers of nuclei and centrosomes, which, in our opinion, suggests a mismatch between the nuclear and centrosomal cycles. In normal early embryonic development of *Drosophila*, each interphase nucleus is associated with two centrosomes, which then move apart and form the spindle. By the late telophase,

each centrosome is replicated and divided into two daughter centrosomes [18]. Thus, no more than two centrosomes occur in the vicinity of the nucleus throughout the cell cycle (Fig. 1b). However, we observed additional centrosomes close to some nuclei in the *l(3)* and *13C* mutants (Figs. 1c and 1d), which possibly resulted in an abnormal tripolar spindle and disturbed the chromosome segregation to the poles. As a result of this chromosome segregation defect, most chromosomes would remain in the central region of the spindle rather than move to the poles. This, in turn, might lead to the formation of triangular nuclei (Fig. 1e).

Another type of abnormal nuclei was nuclei with chromatin bridges, which were frequent in individuals of both mutant strains. A bridge connected two nuclei throughout the cell cycle, its chromatin structure corresponding to that in the connected nuclei (Fig. 1i). This a connection between two daughter nuclei is also indicative of disturbed chromosome segregation. Another evidence for this defect was asymmetric division in the anaphase, when unequal chromosome sets moved to the poles of the spindle (Fig. 1g), which resulted in telophase nuclei differing in size (Fig. 1h).

In addition, the mutations altered the chromatin structure in individuals of the *l(3)* and *13C* strains. A “granular” structure of the corresponding heteropicnotic nuclei is clearly seen in Figs. 1f, 1i. Since preparations were stained with a DNA-specific stain (Schiff’s reagent), this granular structure possibly reflected a nonuniformity of chromatin folding. We could not determine whether the granular structure was caused by premature and nonuniform chromatin condensation during the interphase–prophase or by late chromatin decondensation during the telophase–interphase. Possibly, both processes occurred.

Although caused by an insertion into one intron of the *Trl* gene, the three mutations markedly differed in the effect on embryo development in the corresponding strains. This primarily concerned the lethality associated with the maternal effect.

For instance, the survival increased threefold in the progeny obtained by crossing females homozygous for the *3184* mutation with wild-type males (Table 1, row 6).

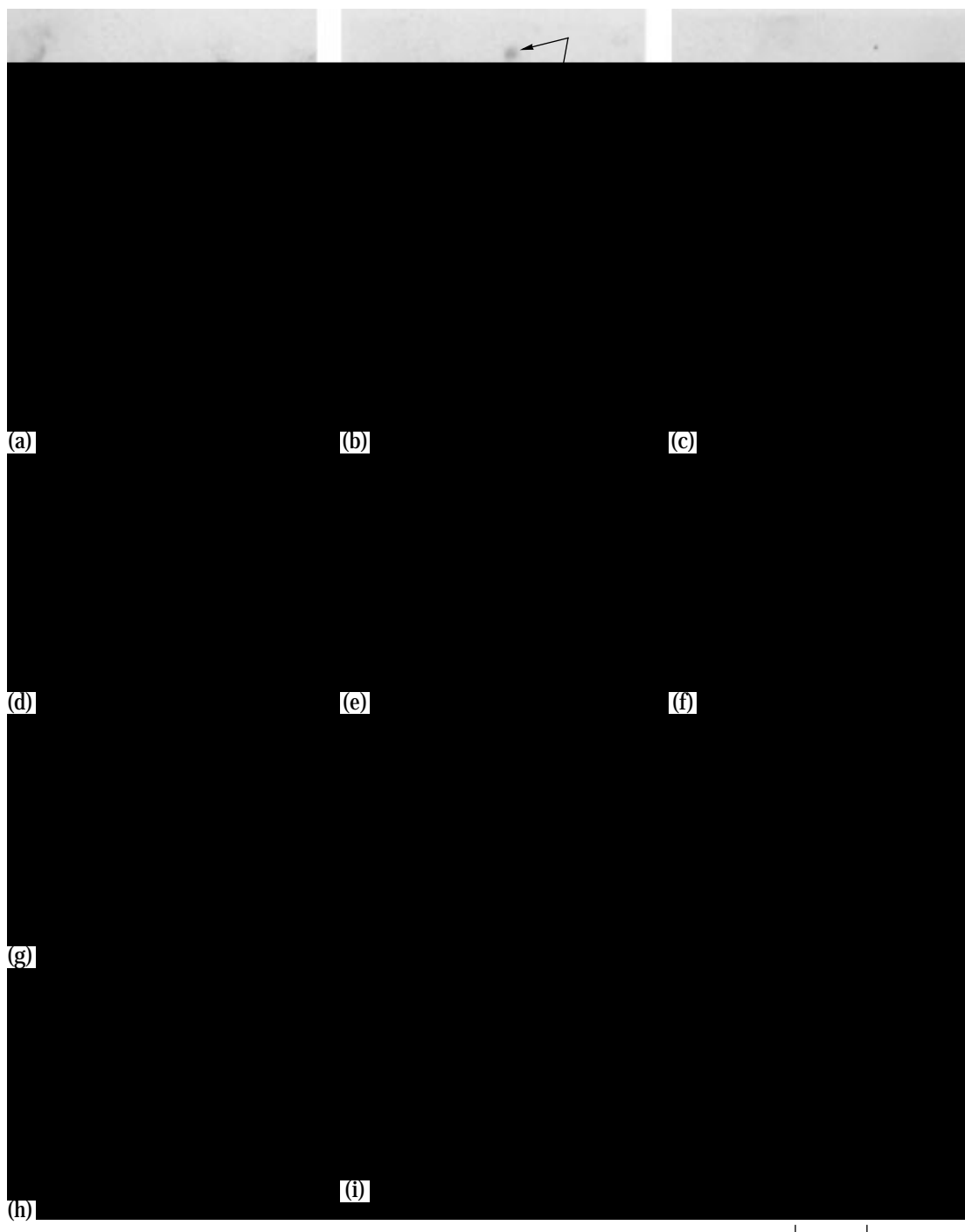


Fig. 1. Morphological defects of cleavage divisions in (d, i) *l3C/TM3* and (a, c, e–h) *l(3)/TM3* embryos. Preparations were stained according to Feulgen. Scale bar, 0.1 mm. (a) Asynchrony of nuclear division stages. Telophase and interphase nuclei are indicated with long and short arrows, respectively. (b) Telophase nuclei in wild-type Hikone-AW embryos. Arrows indicate the centrosomes. (c, d) Excessive centrosomes (indicated with arrows). (e) An abnormal triangular telophase nucleus. (f) Abnormal chromatin condensation in the telophase. (g) Asymmetrical anaphase. (h) Nonequivalent telophase nuclei (arrows), which result from asymmetrical anaphase (see panel g). (i) Granular chromatin structure, chromatin bridges (long arrows) between nuclei, and hyperploid nuclei (short arrows) in interphase. Normal morphology of interphase nuclei is seen in panel (a).

Possibly, the amount and the quality of the *Trl* gene product synthesized in females homozygous for the *3184* allele were sufficient for embryos to pass through the early developmental stages. Thus, strain *3184* did

not display an appreciable lethality determined by the maternal effect.

In strains *l(3)* and *l3C*, insertions were expressed as early lethals in homozygotes. The first wave of embry-

onic lethality occurred at stages 1–5 of embryonic development, when transcription of the embryo's own genes had not started and only the maternal products were translated in embryos (Table 2).

In addition to reduced survival, the *Trl* mutations were dominant for other traits studied, because various defects of the cell cycle and ontogeny were observed in heterozygotes. Probably, the maternal product of females heterozygous for the *l3C* and *l(3)* alleles was insufficient for normal development of their eggs. As a result, we observed the first wave of embryonic lethality, as well as numerous mitotic defects in differentiating cells of the heterozygous progeny that survived until the larval stage (see below).

The Effect of the Trl Gene Mutations on Mitosis in Imaginal Disks and Nervous Ganglia of Larvae

We studied the effect of the above mutations on the temporal and cytomorphological parameters of mitosis in cells of nervous ganglia and imaginal disks of larvae. In particular, we analyzed (1) the relative duration of the mitotic phases, which was determined from the frequency of cells at different division stages; (2) the formation of the mitotic chromosome structure, which was inferred from the chromosome length and morphology; and (3) chromosome segregation, including its synchronism and symmetry.

Proportions of cells at different mitotic phases are summarized in Table 3. As compared with the control strains, larvae of the mutant strains displayed a higher relative duration of the early prophase (P_1

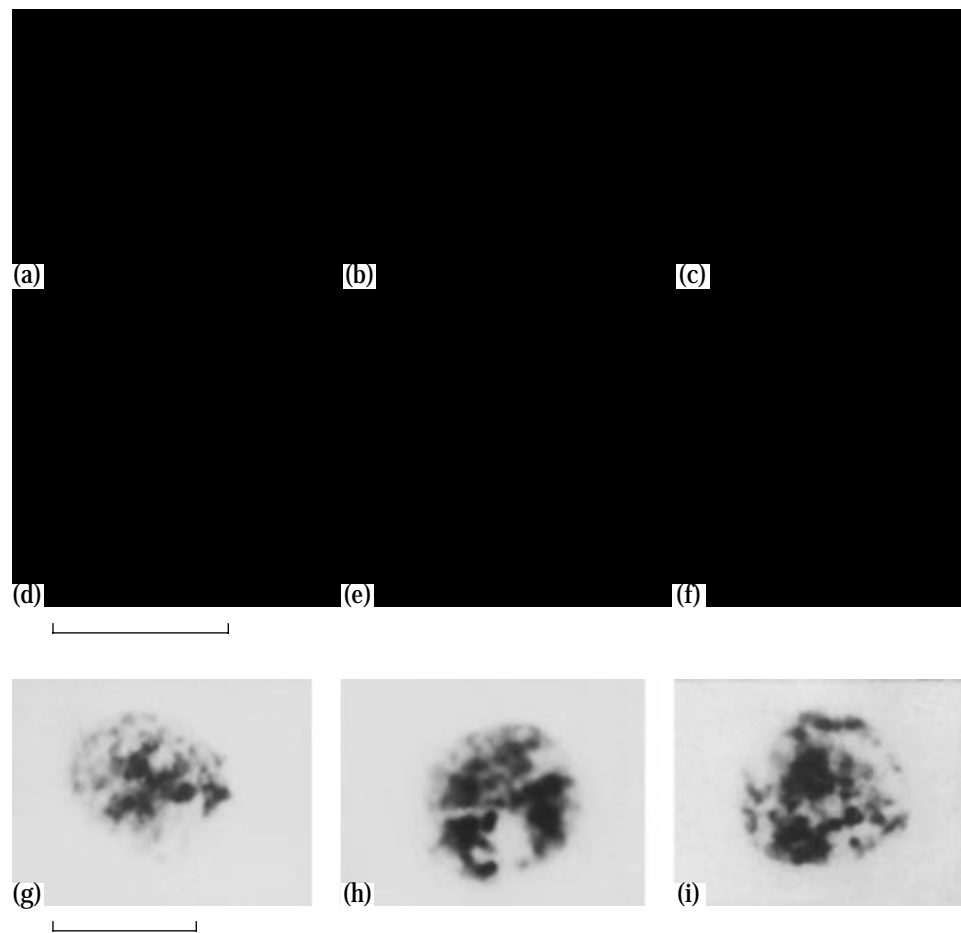


Fig. 2. Mitotic defects in cells of (c–f, h, i) nervous ganglia and (a, b, g) imaginal disks of *Trl* third-instar *D. melanogaster* larvae. Preparations were stained with Giemsa stain. Scale bars, 0.01 mm. (a) Asynchronous chromosome condensation in prophase in *3184/TM3* larvae, (b) granular structure of chromosomes in early anaphase in *13C/TM3* larvae, (c) weak staining of chromosome arms in metaphase in *13C/TM3* larvae, (d) unipolar anaphase in *l(3)/TM3* larvae, (e) nuclear cohesion in late telophase in *3184/TM3* larvae, (f) control interphase nuclei of Oregon R larvae, and premature chromatin condensation in late G₂ in (g, h) *13C/TM3* larvae and (i) homozygotes for *3184*.

quency distribution of cells differing in chromosome length in both tissues only slightly differed between heterozygous larvae of strain *13C* and wild-type larvae, suggesting the same extent of general chromosome condensation for these strains.

The major mitotic event, chromosome segregation in the anaphase, was also defective in mutant larvae. We observed asynchronous formation of the poles, agglomeration and lagging of chromosomes during their movement to the poles, asymmetric chromosome segregation, and unipolar orientation of chromosomes as a limiting case of their asymmetric segregation (Fig. 2d). Although some of these defects were also observed in the control strains (Table 4), they did not alter nuclear structures in the post-anaphase period and had no effect on the viability and fertility of flies in this case (our unpublished data). Hence, we believe that these were natural abnormalities of spindle formation and chromosome segregation, which could be repaired during mitosis.

Mitotic defects in larvae of the mutant strains differed from those in the control strains. In the control, retarded polar orientation of chromosomes did not impair the symmetry of chromosome segregation to the poles. In larvae carrying the *Trl* mutations, asynchronous formation of the poles often led to asymmetric or even unipolar chromosome orientation in the anaphase (Fig. 2d). In the mutants, chromosomes remained attached after the anaphase in some cases. This resulted in tetraploid cells with two nuclei, which were not completely isolated and either were in contact with each other to form an eight-shaped structure or were connected by a long chromatin bridge to form a dumbbell-like structure (Fig. 2e).

The Effect of the Trl Mutations on the Egg Chamber Morphology

We studied the effects of the three mutations in the *Trl* gene on the oogenesis and the morphology of egg

Table 4. Defects of cell division in the *Trl* mutants

Tissue	Strain	Metaphase				Anaphase				
		examined cells	defect frequency, %			examined cells	defect frequency, %			
			granular staining	chromosome length <5 µm	chromosome length >9 µm		laggard and agglomerated chromosomes	asynchronous formation of the poles	asymmetric segregation	unipolar mitosis
Nervous ganglia	<i>Hikone-AW</i>	211	–	7.1	16.6	11	0	45.5	0	0
	<i>Oregon R</i>	93	0	2.1	33.3	29	31.0	3.4	0	6.9
	<i>13C/TM3</i>	88	72.7	11.4	15.9	9	66.7	11.1	33.3	22.2
	<i>l(3)/TM3</i>	118	9.3	11.0	32.2	28	42.8	0	0	32.1
	<i>3184/TM6</i>	117	0	12.0	26.5	16	31.2	12.5	18.7	37.5
	<i>3184/3184</i>	134	2.2	21.6	14.9	28	28.6	0	0	10.7
Imaginal disks	<i>Hikone-AW</i>	310	–	1.6	32.2	24	12.5	20.8	0	0
	<i>Oregon R</i>	119	0	0	29.4	0	0	0	0	0
	<i>13C/TM3</i>	53	100.0	5.7	18.9	6	16.7	16.7	33.3	50.0
	<i>l(3)/TM3</i>	195	12.3	6.1	39.5	27	29.6	0	7.4	14.8
	<i>3184/TM6</i>	44	0	34.1	4.5	14	7.1	21.4	21.4	7.1
	<i>3184/3184</i>	119	0	0.8	36.1	0	0	0	0	0

Note: Length was measured for chromosome 3 in the strain *Hikone-AW* and for chromosome 2 in the other strains.

chambers. To exclude the possible effect of the balancer chromosome, only heterozygous females *l(3)/+* and *13C/+* were examined.

Normally, a 16-cell cyst embedded in a shell of follicular cells is formed in the germarium. Then, the egg chamber is separated from the germarium and passes through consecutive developmental stages (S1–S14 according to King *et al.* [19]) to form a mature egg (Figs. 3a–3d). The 16 cystic cells have large cytoplasmic circular channels between them [20]. One of the two cells that have four channels develops into an oocyte, and the other 15 cells become trophocytes. Trophocyte chromosomes undergo 10–12 cycles of endoreplication and, possibly, endomitosis [21, 22], which results in polyploid nuclei.

The proportion of egg chambers with abnormal number of trophocyte nuclei and disturbed chromatin condensation was increased in the *Trl* mutants (Figs. 3, 4). For instance, the number of trophocytes greatly varied (from 6 to 90) in the egg chambers of females homozygous for the *3184* insertion. Giant chambers (23–99 trophocytes) were arbitrarily assigned to stage S6/S7, because it was impossible to determine the actual stage of the chambers in the egg tube corresponded to these stages (Figs. 3j, 3l). The stages of chambers with six to eight trophocytes were also determined arbitrarily according to the shape and the size of the egg chamber and to the presence of the chambers at earlier or later

stages in the egg tube. Although the trophocyte number was insufficiently low in these chambers, the nuclei were several times larger in size compared with normal nuclei (Fig. 3f).

Trophocyte number reduced to 8–14 (Fig. 4a) and disturbed chromatin condensation in trophocytes (Fig. 3i) were characteristic of the *l(3)/+* and *13C/+* flies.

In total, the mutants displayed the following defects:

—hypercondensed chromatin in trophocytes at various stages of egg chamber development (Figs. 3h, 3i, 3k),

—an increased variation in the extent of chromatin condensation and in the size of trophocyte nuclei within one chamber as compared with the control (Figs. 3i, 3j), and

—an abnormal trophocyte number per egg chamber (Figs. 3e, 3f, 3j, 3l, 4).

The most plausible explanation of the above morphological alterations of egg chambers is that a defect arises when a cyst is formed in the germarium. Cystocyte divisions were either abnormal in number or asynchronous and asymmetric in the *Trl* mutants. If the number of cystocyte divisions was abnormal, the cell number per cyst would be a multiple of 2, i.e., the number of cysts with 2, 4, 8, 32, etc. cells would be elevated. After one of the cells becomes an oocyte in each abnormal cyst, there would be an increase in number of chambers with 1, 3, 7, 31, etc. trophocytes. However,

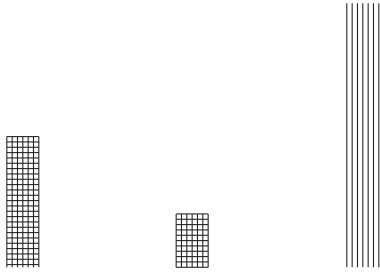
we did not observe an elevated proportion of chambers with $(2^n - 1)$ trophocytes in the *Trl* mutants.

In the case of asynchronous and asymmetric divisions, a defective cyst can initiate its abnormal enveloping by follicular cells, and cells from neighboring cysts might be trapped in the resulting egg chamber. Eventually, the egg chamber would also have an abnormal cell number. As has been shown with the *bam* and *fs(2)LYH* genes, a disturbed function of follicular cells in the germarium leads to the formation of egg chambers with an altered trophocyte number [21, 22]. Cyst enveloping might be disturbed in the case of the *3184* mutation, but we consider this only a secondary defect. If this had been the major defect, chambers with insufficient and excessive trophocyte numbers in the ovariole would have alternated without any regularity with respect to even or odd cell numbers. We did not observe this alternation in the strains studied.

Possibly, instead of entirely switching genetic programs, the mutations change the ability of genes to respond to various signals. Although dramatic morphological alterations occur in egg chambers, cells (trophocytes and follicular cells of various types) follow their predetermined differentiation pathway. Bhat *et al.* [10] drew the same conclusion from the effect of the *13C* mutation on the transcription of the *ftz* and *en* genes during the embryonic development.

In addition, we identified the stage of apoptotic cell death in the *Trl* mutants. According to the few pub-

lished data [23, 24], *Drosophila* oogenesis involves a protective mechanism that determines apoptotic degradation of abnormal or damaged egg chambers at stages S7/S8. Typical of apoptosis [24], this was associated with hypercondensation and fragmentation of chromatin in trophocytes (Figs. 3c, 3g, 3k), follicular cell nuclei remaining intact. Our data testify to the existence of this control stage in the wild-type strains (Fig. 5). In the *Trl* mutants, the total number of apoptotic chambers



—a disturbed ratio between the numbers of nuclei and centrosomes,

—hyperploid nuclei,

—altered chromatin structure and interphase nuclei with granular chromatin, and

—disturbed chromosome segregation.

(2) In nervous ganglia and imaginal disks of larvae, these were:

—cell division arrest in the early prophase,

—premature chromatin condensation in the interphase,

—disturbed structure of condensed chromatin (granular chromatin), and

—disturbed chromosome segregation.

(3) In developing egg chambers, these were:

—an altered trophocyte number,

—chromatin hypercondensation in trophocytes at various stages of the egg chamber development, and

—an increase in apoptotic death of egg chambers at stages S7/S8 and extension of the peak of cell death to S6/S9.

Two of our findings, the interphase chromatin condensation unusual for the cell-cycle stage and abnormal (granular) structure of metaphase chromosomes, suggest that the *Trl* mutations alter the dynamics and the mechanism of structural reorganization of chromatin during the mitotic cycle. Untimely chromatin condensation in the interphase can be explained by two mech-

anisms: (1) a lagging decondensation when the cell finishes mitosis and (2) premature condensation when the cell begins mitosis. In the former case, an elevated proportion can be expected for cells at the last mitotic stage, telophase, when chromatin decondensation starts. However, we did not observe this in our experiments, in which the proportion of telophase cells in the mutants was much the same as in the wild-type strains (Table 3). It is noteworthy in this connection that Bhat *et al.* [10] observed chromatin decondensation as early as in the late anaphase and telophase in embryos homozygous for the *Trl*^{13C} mutation. In addition, they detected a division arrest before prophase and found nuclei that contained replicated DNA but did not divide, which correlates with a lack of GAF [10]. We think that, taken together, these data and our finding of condensed interphase chromatin (Figs. 2f–2i) testify to the second explanation.

Thus, untimely chromatin condensation revealed in our experiments can be explained by a cell arrest in G₂, which was not accompanied by an adequate arrest in chromatin condensation. Possibly, the dynamics of chromatin condensation depends on the dynamics of GAF release from euchromatin. Since chromosomes are deficient in GAF or have incompetent GAF in the mutants, the suppressing effect of this factor on the condensation is impaired directly or indirectly, as its function of a transcription factor is disturbed.

The mutants displayed not only disturbed dynamics of condensation, but also an abnormal structure of condensed chromatin. In our experiments, this was seen as many nonstained regions in chromosomes (Fig. 2b) and was especially dramatic in strain 13C. The nature of these changes is still unclear. On evidence of staining according to Feulgen, DNA was possibly incompletely condensed in these regions. However, this would have resulted in an increase in the chromosome length. Since this increase was not detected, we think that the protein component is structurally abnormal in chromosomes with granular staining.

The post-synthetic period of the cell cycle involves two processes that set the stage for chromosome segregation; these are the chromosome condensation and the formation of the bipolar spindle. The two processes occur autonomously in individual cell-cycle phases. Their coordination, which is necessary for effective chromosome segregation, is provided by a specific

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