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Mutation in Ontogene and Emergence of Secondary Chromosome Damages in Drosophila Germline Cells

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Abstract

The conditional mutations in drosophila were obtained by γ-irradiation and selected using the test for dominant lethality. The conditional mutations survive under permissive genetic conditions and, additionally, commence to display novel properties. One of such properties is a recessive lethality. Ten conditional mutations that displayed recessive lethality were mapped with the help of a standard set of deletions. Half mutations contained two and more lethal defects. The fact that a large number of the lethal defects are associated with one mutation suggests that γ-irradiation is the most unlikely cause of the defects. One of the conditional mutations carried four lethal regions and had a Small barrel (Smba) visual phenotype. The Smba phenotype in the Smba/In(2LR) Cy strain is inherited according to a parental type and disappears in the Smba/In(2LR) Pm strain. Lethality in two of the four lethal regions also disappears in this strain. A separate experiment was conducted to clarify how these regions lost a lethal manifestation after the In(2LR) Cy chromosome in the Smba/In(2LR) Cy strain was replaced with the In(2LR) Pm chromosome. The process of disappearance of the Smba phenotype was also observed in three Smba/In(2LR) Cy substocks. These data suggest that the regions of multiple recessive lethality emerge in a secondary manner under the effect of the earlier formed radiation-induced mutation in ontogene. It is assumed that the recessive lethal regions are the ontogenes with an altered DNA conformation. The conformation in ontogenes is changed in the germline cells during a regular "editing" of the individual development program.

Keywords

Cell, Morphogenesis, Ontogene, Ontogenesis, Electromagnetic Field, Drosophila

1. Introduction

The mutations referred to as conditional mutation manifest themselves differently under different genotypic conditions. Characteristic of a conditional mutation is under what particular genotypic conditions and how it manifests itself [1]. The genes responsible for emergence of conditional mutations are referred to as ontogenes [2] [3] [4]. Under permissive genetic conditions, mutations in ontogenes survive and, moreover, acquire most unusual manifestations [1], including a parental type of inheritance. The parental inheritance consists in that the mutant phenotype is inherited not only by the progenies that acquired the mutation, but also by the progenies that have not got it. The main point is that the mutation (mutational damage) was present in a parent [1] [5].

The parental inheritance of conditional mutations means that the ontogenes are active in the germline and that the "factors" they produce lose a physical link with the ontogenes. The segregation of ontogenes and the factor between the poles becomes independent in meiosis. The nature of the factors that vector the mutant phenotype is vague; however, it is known that this is neither an RNA nor a protein [6]. Presumably, the mutant ontogene in germline cells initiates local epigenetic changes in chromosomes. These changes are transferred to the zygote with the chromosome set and induce a mutant phenotype in the progeny [6].

Epigenetics [7] [8] has considerably expanded the boundaries of traditional genetics. This has brought into light the genetic events that are eventually based on DNA but implemented according to a scheme different from the customary DNA-mRNA-protein pattern (Crick's dogma). According to Riggs, epigenetics is "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" [7]. The main epigenetic mechanisms underlying the regulation of gene activity known so far are DNA methylation, acetylation of histones, noncoding RNA, and chromatin remodeling [8].

The research into conditional mutations quite unexpectedly outlined the association between epigenetics and ontogenes, the source of conditional mutations. The manifestation of conditional mutations [1] [5] [9] is typically "epigenetic" in many aspects. We assume that the epigenetic (non-Mendelian) phenomenology is nothing else but manifestation of the ontogenes, which constitute the main part of the genome [10] [11]. In this regard, the research into the specific features of ontogene manifestation assumes great importance for the area of genetic knowledge that is currently referred to as epigenetics.

The epigenetic inheritance directly contradicts the rules of Mendelian inheritance [10] [11] [12]. This contradiction is the "stumbling stone" of the modern genetic theory. An increase in the new epigenetic traits and the cases of epigenetic inheritance ever more frequently brings about the question on how "in heaven's name" the Mendelian genes and Mendelian inheritance continue to exist. The problem is resolved with the discovery of a new category of genes, onto-

genes. Providing that the epigenetic phenomenology refers to ontogenes and the classical Mendelian phenomenology, to coding genes, the contradiction in the existence of two types of traits and two types of inheritance disappears.

Here we report the experimental data on emergence of secondary damages in the mutants for ontogenes. These data have been obtained by cytogenetic mapping of conditional mutations in Drosophila melanogaster. According to these data, 1) the mutant ontogenes induce secondary local changes in chromosomes, appearing as recessive lethal mutations; 2) these changes take place in mitotically dividing germline cells; 3) these changes are local alterations in the conformation of DNA regions rather than changes in DNA primary structure, as is characteristic of the Mendelian mutations; and 4) the particular changes in the conformation of the region is vague; however, the available data suggest that genome remodeling takes place in the germline on a regular basis. The essence of this process is the changes in the conformation of ontogenes.

2. Materials and Methods

The presence of a genetic mutation can be verified according to an altered phenotype and its inheritance over several generations in terms of genetics; according to appearance of the phenotype in the heterozygote with deleted region of mutation in terms of cytogenetics; and according to the changes in DNA nucleotide sequence in terms of molecular biology. In this work, conditional mutations have been studied cytogenetically intending to confirm the presence of a DNA defect in the case of a conditional mutation and locate it on the map of polytene chromosomes.

Ten D. melanogaster strains carrying conditional mutations in chromosome 2 were examined. The mutations were earlier obtained by γ-irradiation of drosophila males and subsequent selection [9] [13] [14]. The mutations in nine strains lacked any visible phenotype and manifested themselves as recessive lethals. They were maintained in a compound with chromosome 2 balancers [9].

Maintenance of drosophila stocks carrying conditional mutations in chromosome 2. A conditional mutation was in wild-type (+) chromosome 2 in both females and males, while the other chromosome 2 carried a complex inversion, In(2LR) SM1, Cy, with a visible mutation, Cy (Curly) [15]. The homozygotes for the In(2LR) SM1, Cy inversion and the homozygotes for the conditional mutation died; correspondingly, the living progeny was represented by the heterozygotes l(2)/In(2LR)SM1, Cy with a Curly phenotype. The absence of the progeny with a normal phenotype in the stock means that the conditional mutation in chromosome 2 is a recessive lethal.

The drosophila stocks were kept and the strains were crossed in glass tubes filled with a standard agar-yeast medium used for drosophila cultivation. The flies developed in a thermostat at a temperature of 24˚C.

The mutation Smba (Small barrel) is phenotypically visible (**Figure 1**). The Smba females and males have shortened and slightly thickened body [9]. Smba pupae are also shortened. The shortening of pupae is demonstrated by their

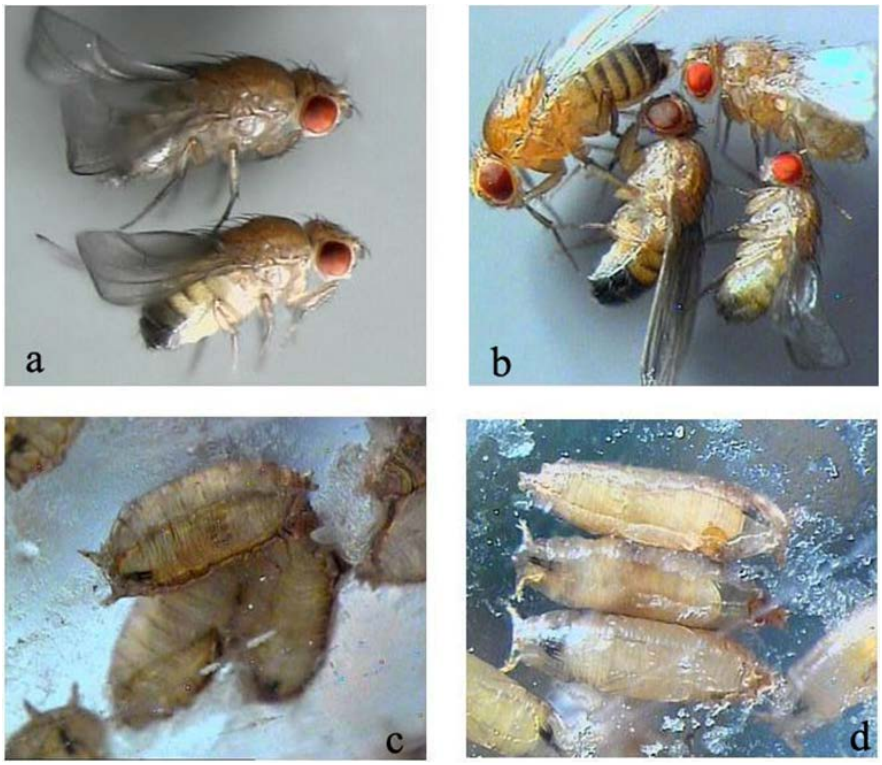

Figure 1. Small barrel (Smba) mutation: (a) general appearance of a mutant female and a male of the Smba/In(2LR)Cy stock; (b) left, a female and a male of the Smba/In(2LR)Pm stock (normal body size) and right, a female and a male of the Smba/In(2LR)Cy stock (reduced body size); (c) short pupae of the Smba/In(2LR)Cy stock; and (d) pupae of the normal size of the Smba/In(2LR)Pm stock [9].

length to width ratio (l/w). This ratio for the pupae of normal strains and Pm/Smba is l/w = 14.0/4.0 = 3.5 versus l/w = 6.7/3.1 = 2.2 for the Cy/Smba pupae [16]. The Smba pupae are easily distinguishable from the normal ones looking through the glass when they reside on the tube walls.

The Smba mutation was obtained by the technique of morphoses [16]. Males of wild-type drosophila laboratory strain were γ-irradiated and mated to females of the initial strain. A small-sized male was observed in F1 and mated to Cy/Pm; D/Sb females. A stock giving only Cy/+ progenies was derived from Cy/+ progenies. This means that the second chromosome 2 contains a recessive lethal. Another stock giving only Pm/+ was obtained from Pm/+ progenies. This means that it also carriers a chromosome 2 with a recessive lethal. According to the conditions used for obtaining mutations, the Cy/+ and Pm/+ stocks must have the same lethal mutation. The cross of Cy/+ and Pm/+ individuals confirmed this. The progeny lacked any +/+ individuals, demonstrating the allelism of the generated lethals. However, the visible manifestation of the mutation in Smba/ In(2LR) Cy and Smba/In(2LR) Pm was different despite the allelism of the lethal manifestation. The Smba/In(2LR) Cy strain displayed a Small barrel phenotype with shortened pupae and imagoes (**Figure 1**), whereas the Smba/In(2LR) Pm strain retained a normal phenotype [9] [16].

Deletion mapping of lethal mutations. All 10 strains carrying a conditional mutation were analyzed using a standard procedure of deletion mapping. For this purpose, the females carrying a set of the chromosome 2 deletions were crossed with the males representing 10 mutant strains. The set of deletions was obtained from the Bloomington Drosophila Stock Center (USA) and comprised 107 deletions covering the whole chromosome 2 without any gaps. The stocks with chromosome 2 deletions are the heterozygotes for Df(2)/In(2LR) Cy where one chromosome 2 carries a deletion and the other, the inversion complex In(2LR) Cy with the visible dominant mutation Cy (Curly). In the deletion stocks, the homozygotes for deletion and the homozygotes for inversion die before the stage of imago.

The event of mapping of a lethal mutation consisted in crossing of a l(2)/ In(2LR)SM1, Cy mutant male with the Df(2)/In(2LR)Cy females of each of the 107 strains. In the case the region of a lethal mismatched the region of deletion, the progeny comprised two phenotypic classes, Cy and Cy+, and in the case these regions coincided, only one class, Cy. The boundaries of the deletion the progeny of which in the crosses consisted of only Cy individuals, whereas the Cy+ individuals (wild-type phenotype) were absent were regarded as the location of the lethal. The cases of a positive response (the absence of Cy+ progenies) were double-checked in reciprocal crosses.

The Smba mutation of the Smba/In(2LR) Cy strain demonstrated the presence of four lethal regions and of the Smba/In(2LR) Pm strain, only two of them. The observed cytological difference of the mutation in Smba/In(2LR) Cy and Smba/ In(2LR) Pm strains demanded to study the inheritance of Smba phenotype in these strains. This study was performed in three stages. At the first stage, the Smba/In(2LR) Cy females (Smba phenotype) were crossed with Smba/In(2LR) Pm males (Smba+ phenotype). The progeny comprised three phenotypic classes: Cy, Pm, and Cy/Pm. The phenotypic class Cy contained the Smba/In(2LR) Cy flies that received the Smba chromosome with two lethal regions. The phenotypic class Pm contained the Smba/In(2LR) Pm flies that received the Smba chromosome with four lethal regions. Finally, the phenotypic class Cy/Pm contained the flies that lacked the Smba chromosome. Thus, we had to find out the effect of the change in the In(2LR) Cy and In(2LR) Pm opposite homologs on the manifestation of Smba.

At the second stage, the progenies of three phenotypic classes (Cy, Pm, and Cy/Pm) were sib-mated (two tubes with five pairs of parents in each). We planned to find out the Smba inheritance pattern in each substock over 13 generations. The inheritance was assessed according to the number of pupae (Smba and Smba+) on the tube walls and their phenotype.

After 13 generations, the progenies with a Smba phenotype almost disappeared in each of the three substocks. At the third stage in examining the Smba inheritance, the Smba/In(2LR) Cy individuals that lost the Smba phenotype after 13 generations were crossed with the Smba/In(2LR) Pm individuals that also lost the Smba phenotype. The progenies in this cross restored the initial structure of

strain Smba. The Smba/In(2LR) Cy progenies got back their Smba chromosome carrying four lethal regions and the Smba/In(2LR) Pm progenies, the Smba chromosome with two lethal regions. Thus, we had to find out whether this substitution would restore the Smba manifestation after it had almost disappeared over 13 generations of sib mating.

3. Results

1) The phenomenon of "multifocal" recessive lethality

The chromosome 2 regions with a lethal effect (the absence of (+) progenies in the crosses with deletions) are listed in Table 1. One might consider that the defects of DNA primary structure reside in these particular regions if it was not for an unexpected fact. Of the ten studied mutations, five (nos. 9, 14, 36, 37, and 44) had more than one region in question each, namely mutation no. 36 had two regions; no. 37, three regions; and mutations nos. 9, 14, and 44, more than three regions.

In addition to the regions that did not give any heterozygotes with a (+) phenotype, the regions that gave most rare (+) individuals, which additionally displayed weak deviations from the normal genotype were detected for mutation no. 44. The regions also differed in that the abundance of the (+) class varied depending on the cross direction. These regions as well as the regions with a complete absence of the (+) progenies were regarded as damaged (Table 1, column "replacement of Cy+ phenotype by another phenotype"). Finally, five of the ten studied mutations contained two to six lethal regions. It cannot be excluded that the number of individual lethal regions can be higher if the maximally possible set of overlapping deletions is used rather than the minimal set.

Mutation no. 14 (Smba), which had a lethal manifestation with four different deletions (Table 1), was studied in more detail. Table 2 lists the comprehensive data on its localization, including not only the deletions with a positive response to the presence of the mutation (the absence of Cy+ progeny), but also some deletions with a negative response (the presence of Cy+ progeny), which is important to find the boundaries of lethal regions. In addition, Table 2 gives the molecular boundaries of lethal regions.

A large share of conditional mutations with more than one defect as well as high numbers of defects in each mutation raises doubts that the detected DNA defects were caused by γ-irradiation, used to generate the mutations. As a rule, a damage in a single region is detectable in the Mendelian mutations generated by low-power γ-irradiation at a dose of 30 Gy. That is why the procedure of deletion mapping in classical genetics serves as a standard for localization of gene mutations.

The rate of conditional mutations in the X chromosome generated by the exposure to γ-radiation (at a dose of 30 Gy) was 0.04 [13] [14], which agrees well with the relevant data [17]. The probability to discover the second lethal in the chromosome with one lethal amount to the same 0.04, which is by one order of

Table 1. Deletion mapping of conditional lethal mutations in chromosome 2.

Mutation no.	Deletion that interacts with mutation		Type of response to deletion		Putative region of localization**
	Number of deletion in chromosome 2*	Boundaries of deletion	Absence of Cy+ progeny	Replacement of Cy+ phenotype by another phenotype	
U-6	6609	56F12; 57A4	+		56F12-57A4
7	7445	53D9; 54B10	+		53D9-54B1
24	3079	32F1; 33F2	+		32F3-33F2
14 (*Smba*)	3138	34C1; 35C1	+		35B9-35B10
	6241	35B2; 35B10	+		
	3588	35B4; 35E2	+		
	23683	35B8; 35C1	+		
	23154	35B6; 35C1	+		
	7445	53D9; 54B10	+		54B1-54B10 54B16-54B16
	7414	54B1; 54B10	+		
	9596	54B2; 54B17	+		
	5574	54B16; 54B16	+		
	5680	54B16; 54B16	+		
	6780	54E5; 55B7	+		54F4-55A1
	5426	54F2; 56A1	+		
	1547	55A1; 55F2	+		
36	490	25F3; 26D11	+		26D2-26D3 42B3-42E
	1007	42[a]1; 42F1	+		
	1888	42B3; 43E18	+		
9	7144	22D1; 22F2	+		22E1-22F1 37B10-38A6 42A1-42B3 49B6-49C1 58D1-59A1 60E8-60E11
	567	37B2; 38D5	+		
	1007	42A1; 42F2	+		
	754	49B2; 49E2	+		
	282	58D1; 59A1	+		
	2471	60E6; 60E11		+	
37	420	36C2; 37B10	+		36D-37B2 49B6-49C1 58D1-59A1
	754	49B2; 49E2	+		
	282	58D1; 59A1	+		
44	3366	31B1; 32A2		+	31B1-31B1 31D1-32A1 h35; 40A1 58D1; 59A1 60E6; 60E11
	4959	h35; 40A1		+	
	282	58D1; 59A1		+	
	2471	60E6; 60E11		+	

Continued

28	2471	60E8; 60E11	+	60E8-60E11
34	2471	60E8; 60E11	+	60E8-60E11

*Deletion numbers according to Bloomington Drosophila Stock Center. **In order to identify the boundaries of lethal regions, we took into account the deletions with positive (the absence of *Cy*+ progeny) and negative (the presence of *Cy*+ progeny) responses to the presence of the mutation. However, the latter are omitted in the table for space saving although some of them served for a more precise localization of the mutation.

Table 2. Deletion mapping of conditional lethal mutation no.14 (*Smba*).

Deletion that interacts with mutation			Positive (absence of Cy+ progeny) and negative deletion response	Putative region of localization	Molecular map
Deletion number	Deletion name	Boundaries			
3138	b87e25	34C1; 35C1	+	35B9-35B10	15057848-15061074
3588	TE35BC-24	35B4; 35E2	+		
23683	BSC299	35B8; 35C1	+		
23154	BSC254	34B6; 35C1	+		
6241	TE35BC-7	35B2; 35B10	+		
25136	k08808-rv70	35B8; 35B8	-		
24112	ED1054	35B10; 35D4	-		
25132	PZ07130-mr9	35B8; 35B9	-		
7445	BSC49	53D9; 54B10	+	54B1-54B10	17227748-17438696
7414	BSC44	54B1; 54B10	+		
9213	ED3181	53C9; 53F10	-		
24356	BSC331	53D14; 54A1	-		
9596	BSC161	54B2; 54B17	+	54B16-54B16	17448011-17462347
5680	robl-C	54B16; 54B16	+		
5574	k10-408	54B16; 54B16	+		
24379	BSC355	54B16; 54C3	-		
6780	14H10W-35	54E5; 55B7	+	54F4-55A1	17766984-17891154
5426	RM2-1	54F2; 56A1	+		
1547	PC4	55A1; 55F2	+		
6778	02B10W-08	54E8-54F4	-		
24987	BSC483	55A1; 55B7	-		

magnitude lower as compared with the rate of 0.5, observed in our sample of 10 mutations. For a frequency of 0.04 corresponding to a single lethal, the probabilities to get the chromosomes with three and more lethals are vanishingly small (0.0016); however, we got four such variants in our sample. It is clear that the

high rates of the chromosomes carrying more than one damage are stochastically unexplainable. The localization of conditional mutations in this experiment failed but discovered a novel property of mutations in ontogenes, namely, the ability to induce secondary damages. Thus, a mutation in an ontogene acts as a mutator gene [18] [19].

2) Smba, a conditional mutation with visible manifestation. Parental inheritance of Smba phenotype

The conditional mutations with multiple damages include Smba, the mutation with a visible manifestation. This mutation is maintained in two stocks: in compound with the In(2LR) Cy inversion and in compound with the In(2LR) Pm inversion. The Smba phenotype is observed only in the former.

The Smba phenotype is inherited in a parental manner. All crosses of the Smba/In(2LR) Cy males with Df(2)/In(2LR) Cy females gave all progeny with a Smba phenotype independently of the type of deletion. As early as the pupal stage, it is evident that all pupae are short. Thus, the Smba phenotype is transferred by both the sperm cell carrying Smba and the Cy sperm cell lacking the chromosome with Smba,

In the process of Smba deletion mapping, the (+) progeny, indicating the absence of defect in the tested region, is present in most crosses. The fact that the (+) progeny also has a Smba phenotype suggests that the presence of In(2LR) Cy in the opposite chromosome is not necessary for Smba manifestation. Thus, the prohibition on the Smba manifestation in Smba/In(2LR) Pm strain is determined by the presence of In(2LR) Pm in the opposite chromosome rather than by the absence of In(2LR) Cy.

3) The lethality pattern of Smba mutation depends on the structure of opposite chromosome 2 (a comparison of the Smba/In(2LR) Cy and Smba/In(2LR) Pm strains)

After mutation no. 14 (Smba; strain Smba/In(2LR) Cy) was localized according to 13 deletions that gave a positive response with the mutation (**Table 2**), a narrower set of nine deletions was composed. The set contained deletions of each of the four regions of Smba localization. The interaction of Smba with deletions depending on whether this mutation is maintained in the Smba/In(2LR) Cy or Smba/In(2LR) Pm strain was tested. As is mentioned above, the Smba mutation in the latter case has no phenotypic manifestation.

The regions of lethality of the Smba from Smba/In(2LR) Cy strain (**Table 3**) emerged to be the same as earlier found (**Table 1** and **Table 2**) but were different for the Smba from Smba/In(2LR) Pm strain (**Table 4**). Lethality was observable in the two outermost regions but disappeared in the two middle regions. The significance of these results is doubtless because the inference on lethality was based on the coincidence of responses of several deletions of the region and the coincidence of responses in reciprocal crosses. Thus, the conclusion states that the disappearance of Smba phenotype in strain Smba/In(2LR) Pm is accompanied by the disappearance of lethality in two closely adjacent regions in the chromosome 2 right arm.

Table 3. Local recessive lethality of the *Smba* mutation from the *Smba/In*(2*LR*) *Cy* strain (four lethal regions).

Lethal regions of *Smba* mutation		Progeny of direct cross ♀ *Smba*/*In*(2*LR*) *Cy* × ♂ *Df*(2)/*Cy*		Progeny of reverse cross ♀ *Df*(2)/*Cy* × ♂ *Smba*/*In*(2*LR*)*Cy*	
Lethal region	Number of deletion in region	*Cy*	(+)	*Cy*	(+)
35B4 - 35C1	Df(2)3138	79	-	223	-
	Df(2)3588	75	-	241	-
54B1 - 54B10	Df(2)7445	113	-	179	-
	Df(2)7414	158	1	169	-
54B16 - 54B16	Df(2)9596	125	1	255	-
	Df(2)5574	111	-	147	-
	Df(2)5680	97	-	188	-
55A1 - 55B7	Df(2)1547	141	-	174	-
	Df(2)6780	119	1	46	-

Table 4. Local recessive lethality of the *Smba* mutation from the *Smba/In*(2*LR*) *Pm* strain (two lethal regions).

Lethal regions of *Smba* mutation		Progeny of direct cross ♀ *Smba*/ *In*(2*LR*) *Pm* × ♂ *Df*(2)/*Cy*		Progeny of reverse cross ♀ *Df*(2)/*Cy* × ♂ *Smba*/*In*(2*LR*)*Pm*	
Lethal region	Number of deletion in region	*Cy*, *Pm*, and *Cy*/*Pm*	(+)	*Cy*, *Pm*, and *Cy*/*Pm*	(+)
35B4 - 35C1	Df(2)3138	97	–	171	–
	Df(2)3588	121	–	162	–
54B1 - 54B10	Df(2)7445	150	50	147	48
	Df(2)7414	82	21	104	35
54B16 - 54B16	Df(2)9596	77	49	114	93
	Df(2)5574	81	37	216	28
	Df(2)5680	83	40	461	33
55A1 - 55B7	Df(2)1547	90	–	205	–
	Df(2)6780	153	–	116	–

The disappearance of a lethal effect in two regions caused by In(2LR) Pm suggests the important conclusion that the local recessive lethality in conditional mutations is not always caused by a change in the nucleotide sequence in the region of the lethal. In this particular case, the change in the sequence caused by

the action of inversion in the opposite homolog is most unlikely. The disappearance of recessive lethality in two of the lethal regions that was caused by the impact of In(2LR) Pm suggests an important inference: The phenomenon of local recessive lethality in conditional mutations is not associated with any change in the nucleotide sequence in the region of the lethal. A tremendous volume of genetic data demonstrates that the primary nucleotide sequence is a stable and fundamental feature of genetic material. The nucleotide sequence in one homolog in principle cannot change depending on the structure of the opposite homolog, as is observed in the experiment with the In(2LR) Pm inversion. Otherwise, neither genetic code nor genetic variants could exist.

4) Specific features in the inheritance of Smba phenotype

In the initial cross ♀ Smba (four lethals)/In(2LR) Cy × ♂ Smba (two lethals)/In(2LR) Pm, the Cy progenies receive chromosome 2, Smba (two lethals) from the father with a normal long body, while the Pm progenies receive chromosome 2, Smba (four lethals) from the mother with a short body. The Cy/Pm progenies do not get chromosome 2, Smba at all. The overall examined F1 individuals of the initial cross (131 pupae and 199 eclosed imagoes) were short. Thus, a parental inheritance of Smba (four lethals) is evident. Another illustrative demonstration of the parental inheritance was that the Cy/Pm individuals that had no chromosome 2, Smba at all had a Smba phenotype. A possible cause underlying appearance of a Smba phenotype in the Cy/Pm progenies is that the In(2LR) Cy chromosome acquired the secondary damages that lead to a Smba phenotype. This damage could have taken place when the In(2LR) Cy chromosome was in the genome of a Smba (four lethals)/In(2LR) Cy mother. As is evident, the presence of In(2LR) Cy inversion in chromosome 2 does not prevent a secondary lethal damage in a radiation mutant for the Smba ontogene.

The rate of short pupae in substocks Cy, Pm, and Cy/Pm (**Table 5**), initially containing 100% of short individuals, decreased from generation to generation; however, the dynamics of this process was different. The share of short pupae uniformly decreased in the Cy/Pm substock, which led to their almost complete disappearance in F12 - F13. The Smba (two lethals)/In(2LR) Cy substock also displayed the trend of a decrease in the number of short pupae; however, the ratio of short to long pupae varied in different generations. As for the Smba (four lethals)/In(2LR) Pm, the ratio of short to long pupae sharply varied, including polar alterations, so that either short or normal individuals were prevalent; by F13, long pupae became prevalent.

The final cross between the Smba (two lethals)/In(2LR) Cy and Smba (four lethals)/In(2LR) Pm stocks after 13 rounds of sib mating gave the progenies with the initial genotypes: the Cy progenies got the In(2LR) Cy chromosome and the Smba chromosome with four lethal regions, while the Pm progenies got the In(2LR) Pm and the Smba chromosome carrying two lethal regions. Correspondingly, the Cy progenies were expected to be shortened and the Pm progenies, to be normal independently of the cross direction. However, the expectations were not met. The overall progeny of the final cross ♀ Smba (two lethals)/In(2LR)

Table 5. Change in the length of pupae in three substocks generated in the cross of *Smba/In*(2*LR*) *Cy* females (*Smba* phenotype) with *Smba/In*(2*LR*) *Pm* males (*Smba*$^+$ phenotype).

Generation	*Cy/Smba* stock		*Pm/Smba* stock		*Cy/Pm* stock	
	Short pupae	Long pupae	Short pupae	Long pupae	Short pupae	Long pupae
F_1	57	60	39	25	94	104
F_2	51	176	49	21	93	340
F_3	18	76	55	43	39	116
F_4	63	108	84	32	15	125
F_6	6	40	5	18	5	59
F_8	1	143	65	83	6	108
F_9	6	108	105	59	5	93
F_{10}	17	211	122	117	2	204
F_{11}	17	277	15	111	5	223
F_{12}	7	144	4	149	0	115
F_{13}	13	159	2	136	1	122

Cy × ♂ Smba (four lethals/In(2LR) Pm (432 individuals at the pupal stage) was represented by long pupae. However, 65 of the 262 counted pupae in the other reverse cross were short. The phenotypes of imagoes were not examined.

5) Dynamics of recessive lethality in the 54B1; 54B16 region after In(2LR) Cy replacement with In(2LR) Pm in opposite chromosome 2

The results suggested that the Smba phenotype is determined by the combination of the Smba chromosome carrying four lethals and the In(2LR) Cy chromosome, while the Smba+ phenotype, by the Smba chromosome carrying two lethals and the In(2LR) Pm chromosome. It was reasonable to find out whether it was possible to convert the Smba (four lethals) chromosome into Smba (two lethals) chromosome by replacing the In(2LR) Cy chromosome with the In(2LR) Pm chromosome and, if it was possible, how this process would go on in successive generations after the event of replacement.

No changes in lethality were observable in the F1 Smba (four lethals)/In(2LR) Pm flies. The (+) progenies were almost absent in the crosses with deleted middle region 54B1; 54B16 (Table 6), suggesting a 100% lethality of the region. The progeny was also absent in F2. Individual (+) progenies start to appear only in F4 and only in combination with two deletions of the five ones. The (+) progenies became more numerous in F6 and all five deletions were involved in the response. Thus, the presence of In(2LR) Pm in the opposite chromosome with each meiotic restoration of the Smba (four lethals/In(2LR)) Pm genome withdraws a lethal effect of the 54B1; 54B16 locus, residing in the middle of the Smba (four lethals) chromosome, in a discrete (quantum) manner.

Table 6. The loss of lethality by the 54B1; 54B16 region in successive generations of the *Smba/In(2LR) Pm* stock.

Generation of *Smba/In(2LR) Pm* stock	Deletions in 54B1; 54B16 region	Cross *Df*(2)*/SM1*, *Cy* × *Smba/In*(2*LR*)*Pm*	
		Total number of progenies	Progeny (+)
F_1	Df(2)5574	97	-
	Df(2)5680	51	1
	Df(2)7414	85	-
	Df(2)7445	62	-
F_2	Df(2)5574	65	-
	Df(2)5680	49	-
	Df(2)7414	70	-
	Df(2)7445	86	-
	Df(2)9596	80	-
F_4	Df(2)5574	55	-
	Df(2)5680	51	1
	Df(2)7414	91	8
	Df(2)7445	25	-
	Df(2)9596	90	-
F_6	Df(2)5574	68	1
	Df(2)5680	160	4
	Df(2)7414	144	1
	Df(2)7445	162	3
	Df(2)9596	145	11

4. Discussion

The main test for the presence of a conditional mutation is dominant lethality. Under restrictive genetic conditions, the heterozygotes for a conditional mutation die but survive under permissive conditions [9] [13] [14] [20]. However, the permissive conditions fail to provide the complete recovery to the norm: new manifestations are detectable in mutants, including genetic instability, secondary mutagenesis, disturbance of the basal metabolism, and emergence of monstrosities (morphoses) [1] [9]. Recessive lethality is among these novel manifestations. This property is used to maintain laboratory stocks of conditional mutations.

The mutations in the form of recessive lethals are widely known; however, the recessive lethality in the case of conditional mutations is peculiar. For example, the recessive lethality of the conditional mutation in the X chromosome is sex-dependent. In a homozygote, it manifests itself in the female genome but does

not appear in the male genome [1]. Common recessive lethals in the X chromosome manifest themselves in both sexes. Characteristic of the conditional mutations is a "withdrawal" of lethality [21] [22], which is untypical of ordinary lethals. The mapping of lethals, performed in this work, continues the "parade of peculiarities" associated with the lethal manifestation of conditional mutations: the recessive lethality appears to be multifocal. Half examined mutations display several regions with a lethal manifestation rather than a typical case of a single one (**Table 1**).

Because of its random nature, γ-irradiation is able to generate mutants with several lethal damages; however, emergence of the mutants with two and more lethals is a most rare event for the used exposure dose and rate. Conspicuous are not only numerous mutations with more than one defect, but also multiple defects for each of such mutations, namely, four defects for mutation no. 14, five defects for mutation no. 44, and six defects for mutation no. 9. Most likely, the multifocality of recessive lethality is a secondary rather than a primary effect. It results from the presence of a mutant ontogene and the events that take place in germline cells. Unlike the transit of a Mendelian gene through the germline "in a sealed railroad car", a mutant ontogene in the germline is active and generates secondary damages.

1) Multiplicity of recessive lethality and parental inheritance of conditional mutations represent two consequences of the same process, namely, the activity of ontogenes in germline cells

The association between the phenomenon of multiple lethality and another phenomenon characteristic of the conditional mutations, a parental inheritance, becomes evident. The latter is characteristic of most different manifestations of mutations in ontogenes [5] [9] [23] [24]. The cause underlying parental inheritance in general is in that the informational elements appear in meiotic cell, the distribution of which is independent of chromosomes. Another possible consequence resulting from generation of such elements is a large number of lethal defects (**Figure 2**). The chromosome damages act as new elements. If the damages reside within the same chromosome, this gives a multiple lethality. If the damages cover both homologs, this gives a parental inheritance of the phenotypes created by these damages. A mutant phenotype of a heterozygote for a mutation will be inherited with each gamete although the mutation itself may be carried by half of the gametes. The hypothesis on two consequences is confirmed by that both phenomena (multiple lethality and parental inheritance) are discovered for one and the same mutation, the Smba mutation.

The Smba phenotype follows a parental inheritance pattern in all crosses of Smba males with the females carrying deletion as well as in the crosses of Smba/In(2LR)Cy with Smba/In(2LR)Pm. If the Smba phenotype were the result of a primary radiation defect in DNA, it would be inherited only with the Smba chromosome; however, this phenotype is also inherited with the chromosome lacking Smba. This means that the mutant Smba phenotype has at least two sources. Thus, the data on Smba favor the assumption on a secondary nature of

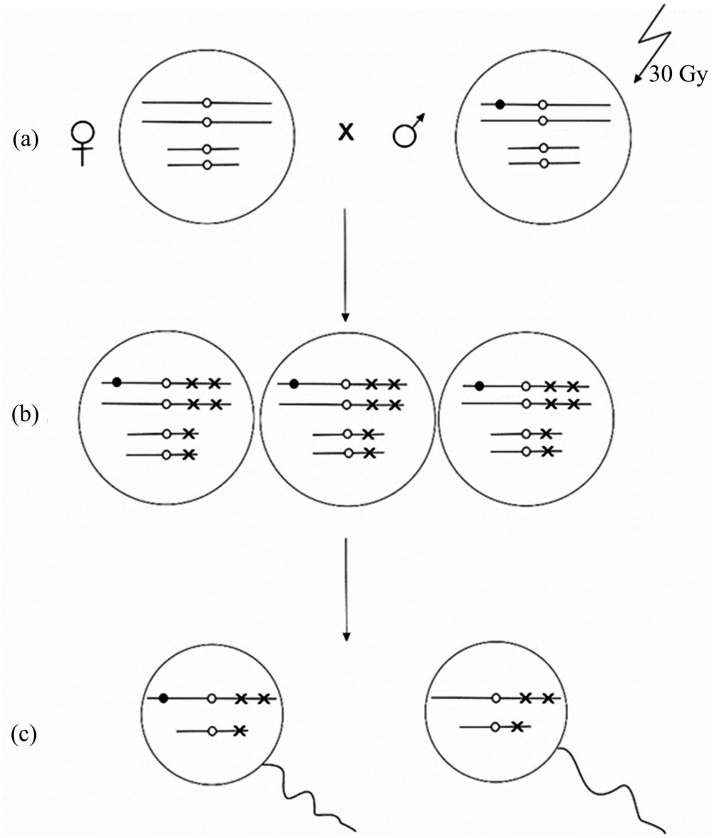

Figure 2. Formation of secondary damages caused by a radiation-induced mutation in ontogene and phenomenological manifestation of this process. (a) A male exposed to radiation (right) acquires a mutation in an ontogene (black circle). Chromosome pairs are shown as lines with centromeres (empty circles). The male carrying the mutation is crossed with a female without mutations. (b) Mitotically dividing germ line cells of a progeny. The presence of mutation in the ontogene induces formation of a set of secondary damages in other ontogenes (recessive lethals). The damages are located in the very chromosome carrying the mutation, in its homolog, and in the chromosomes of other pairs. (c) The gametes produced by a progeny. Both gametes carry the chromosomes with multiple (two) damages (the ph enomenon of multiple recessive lethals). Both gametes contain secondary damages although only one of them (left) carries the primary mutation (the phenomenon of parental inheritance of mutant phenotype).

the recessive lethal mutations. The hypothesis on the generation of secondary damages in germline cells appeared for the first time when considering the phenomenon of zygotic selection in the individuals with mutant ontogenes. A lethal effect of the conditional mutation of the father is modified by chromosome inversion of the mother [6] [7] [20] [21]. Amazing is the fact that the very presence of inversion in the maternal genome has a modifying effect. The effect is transferred to the zygote together with the opposite maternal chromosome lacking the inversion [6] [7] [25] [26].

2) Secondary genetic damages are similar to genetic mutations but still not the same

The discovered damages in chromosome 2 are similar to genetic mutations.

They are local and manifest themselves as ordinary recessive lethal mutations; however, they are not true genetic mutations, which are represented by changes in DNA nucleotide composition. The multiple lethality and parental inheritance, untypical of genetic mutations, are mentioned above. Another distinction is instability—the dependence on genetic factors, which have no effect on the true genetic mutations. The cases of such dependence are listed below:

1) The In(2LR) Pm inversion in the opposite homolog causes a loss of the Smba phenotype in the Smba/In(2LR) Pm strain and a loss of lethality in the 54B1; 54B16 region;

2) The lethality in the 54B1; 54B16 is lost in a discrete (quantum) manner as an increase in the number of the progenies without lethality in each generation (**Table 6**); and

3) The Smba phenotype in the laboratory strains displays drastic variations in penetrance down to a residual level (**Table 5**).

The observed cases of dependence suggest that the ontogenes in addition to the constancy, characteristic of the Mendelian genes, possess an opposite property, variability. Presumably, *the constancy is determined by a stable nucleotide sequence of a particular DNA region*, *whereas variability results from a changing conformation* (*condensation*) *of the corresponding region* [27] [28].

3) "*Natural editing*" of the genome in the drosophila germline cells

The Mendelian genes pass through the germline "in transit" in an inactive state. The activity of Mendelian genes appears only after fertilization with the commencement of somatogenesis. As for the ontogenes, they are active as early as in the germline. The parental inheritance of the manifestation of ontogenes is a sign of their activity [5] [9] [23] [24]. The fact of a common lethal phase for all conditional mutations suggests the existence of specific process of ontogene activity in the germline developing in time [23] [24]. The phenomenon of multiple lethality is another argument favoring the activity of ontogenes in germline cells. The lability of damages, mentioned above, also suggests the activity of ontogenes. The very process of a change in the labile component of an ontogene is also unusual. It is of a discrete (quantum) character. The changes take place in individual progenies despite that the cause for the change was not present in each of them. A quantum character of events resembles the classical penetrance [29], individual variation, and specific inheritance of quantitative traits [30].

Our data suggest that each gamete in the germline passes the stage of genome editing. Currently, the genome editing is understood as an artificial change in DNA sequence [31] [32] [33]. However, by the editing in the germline cells, we mean the editing of basically different type. First, this editing is a regular process of preparing the gametes and it takes place without any human interference. Second, ontogenes are involved in the process of editing but their nucleotide composition remains intact. The DNA conformation of an ontogene is edited (is changed or retained). Finally, the editing leaves the Mendelian genes untouched. As early as 1992, I.B. Panshin, the pioneer in studying the drosophila heteroch-

romatin, postulated the regulation of gene function with the help of heterochromatin operators [34].

In terms of biology, the genomes within a species are checked twice: first, when the gametes are prepared before meiosis and second, in the zygote after fertilization. The first check is described above as a regular process, which may be referred to as a "natural genome editing". This editing is completely different from the known artificial genome editing. The second check, taking place in the zygote after fertilization [6] [7], is the test of a pair of genomes of new parents for matching each other and the species standard. In the case of a mismatch, the pair of genomes is eliminated as a result of the so-called zygotic selection [25] [26].

5. Conclusions

Once the genetic coding of proteins was discovered, it seemed that the problem of building of a living organism was solved. Some genes synthesize structural proteins and the others, the regulatory proteins that control the former ones. The inconsistency of this concept was discussed [35]; however, a different ideology appeared just recently accompanied by the experimental studies. The decisive factor there was the discovery of a special category of genes (ontogenes) that control the regulation of individual development [13] [14] and the discovery that the larger part of the genome has a noncoding status [36]. The new ideology consists in the recognition that the genome comprises two components, namely, the traditional coding genes and ontogenes, which do not code for any proteins [3] [4] [37] [38].

The novelty here is the activity of ontogenes in the drosophila germline cells. The data described here and obtained earlier suggest that the program of individual development is "edited" in germline cells. This process was named "editing" because the occurring changes involve only the ontogenes rather than the overall genome and, moreover, not the entire ontogene but only the conformation of its DNA. The earlier data demonstrate that this process is stretched in time, implemented in the line of mitotically dividing gonial cells, and has its own pattern [23] [24]. The editing consists in: 1) activation of the ontogenes with involvement of nuclear RNAs [3] and 2) conferring of specific conformation of the DNA molecule in the region of an ontogene. *In addition to the information contained in the DNA sequence*, *the ontogene acquires additional information in the form of conformation* (*spiralization*, *coiling*, *or condensation*) *of this DNA sequence.* Here we discuss this particular stage of editing of the individual development program. As is postulated, the mutant ontogene (a conditional mutation) with a changed DNA sequence alters in the course of editing the normal conformation of other ontogenes residing downstream to certain a "not normal" conformation. The editing is referred to as natural since it takes place on a regular basis and differs from artificial correction of DNA nucleotide defects, widely discussed in the literature [33].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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