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Chapter 8

The Effect of *TBP-Related Factor 2* on Chromocenter Formation and Chromosome Segregation in *Drosophila Melanogaster*

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Additional information is available at the end of the chapter

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Abstract

Chromosome nondisjunction in meiosis causes the gene disbalance and a number of anomalies in development and fertility. Otherwise, genetically programmed sex-ratio meiotic drive occurs in a number of species. One of the forms of eukaryotic genome organization is a chromocenter evolutionally involved in the regulation of chromosome behavior in dividing cells among insects, plants, mammals, mollusks, and even yeast. In *Drosophila*, *TBP related factor 2 (Trf2)* belongs to a conservative *Tbp (TATA box-binding protein)* gene family and encodes a basic transcription factor. Recent data demonstrates that a decrease in TRF2 expression can result in the abnormalities of chromatin condensation; however, no details of this process have been studied. We demonstrated that a decrease in the TRF2 expression damaged proper chromocenter structure and abolished chromatin condensation and it was a reason for the chromosome nondisjunction. We found that compact chromocenter and correct homologue pairing were abolished in flies with a lower *Trf2* expression in germline and in somatic cells. We conclude that TRF2 can not only be involved in transcription activation, but also may perform structural function in pericentromeric heterochromatin organization. The possibility of TRF2 to regulate the evolutionary genetically programmed sex-ratio meiotic drive is discussed.

Keywords: chromocenter, chromosome nondisjunction, asinapsis, *TBP-related factor 2*, *Drosophila*

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1. Introduction

Chromosome nondisjunction during meiosis causes the gene disbalance and, consequently, a number of anomalies in development and fertility. On the other hand, genetically programmed sex-ratio meiotic drive occurs in a number of animal species when mainly males or females are born, which is normal within the given species [1]. The genetic regulation of these processes is actively being studied. There are many factors that can result in the incorrect chromosome segregation. The correct segregation of sister chromatids between daughter cells depends on the coordinated interaction of centrosomes, centromeres, kinetochores, spindle fibrils, topoisomerases, proteolytic processes, and motor proteins [2]. On the other hand, chromosomes must be "prepared" (or structurally organized) when they enter meiosis (or mitosis). Structural disorganization of chromosome or same their regions that control the correct pairing of homologs during meiosis frequently results in the incorrect chromosome segregation. The one way of eukaryotic genome organization is chromocenter, which is evolutionally involved in the regulation of chromosome behavior in dividing cells not only among insects but also among plants, mammals, mollusks, and even yeast [3–7]. This nuclear structure arises in differentiated somatic and germ cells during interphase and meiotic prophase. The chromocenter is generated by the association of pericentromeric regions of all or separate groups of chromosomes and plays an important role in spatial organization of chromosomes [8]. Studies on *Drosophila* have clearly demonstrated that its disorganization leads to genomic disbalance [9, 10]. The screening for genes that control the formation and reorganization of chromocenter is performed [11, 12]. The high frequency of chromosome nondisjunction in the progeny of mutant parents is a main characteristic of mutations in these genes.

In *Drosophila*, *TBP-related factor 2* (*Trf2*) encodes an alternative basic transcription factor that is homologous to vertebrate *Trf2* protein and belongs to a conservative *TATA box-binding protein* (*Tbp*) gene family [13]. It was shown that previously discovered $lawc^{p1}$ (*leg-arista-wing complex*) mutation [14] appeared to be the only viable mutation that decreases *Trf2* gene expression [15]. The high conservatism of the *Trf2* protein allows us to study its functions on *Drosophila*.

In the previous studies, we demonstrated that the $lawc^{p1}$ mutation suppresses the phenotype of mutations in genes that encode polycomb group (PcG) proteins, which are negative epigenetic regulators of transcription via chromatin modification [16]. At the same time, we have found that $lawc^{p1}$ increased the effect of transvection (or allelic complementation [17]) caused by disruptions of the homologous chromosome pairing at a number of loci.

Recent data demonstrated that a decrease in *Trf2* gene expression could result in the disruption of chromatin condensation [18]; however, almost no details of this process have been studied. At the same time, we have noted frequent cases of chromosome nondisjunction during genetic experiments with hypomorphic *Trf2* mutations in *Drosophila melanogaster*. The question is whether a decrease in the *Trf2* gene expression really increases the frequency of chromosome nondisjunction in the female meiosis and if it is so, is this anomaly associated with an abnormal chromatin packaging (and particularly with the disruption in the chromocenter structure)?

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Data of genetic experiments for the analysis of the frequency of X-chromosome nondisjunction in mutant lines and of cytogenetic experiments studding the structure of chromosomes in germ and somatic cells are presented below.

2. Analysis of frequency of X-chromosome nondisjunction in lines with lethal *Trf2* mutations

We calculated frequencies of X-chromosome nondisjunction in two groups of lines that contain lethal *Trf2* mutations. The lines of first group were obtained from Bloomington Drosophila Stock Centre: *l(1)G0039/FM7a*; *l(1)G0356/FM7a*; *l(1)G0424/FM7a*; *l(1)G0376/FM7a*; *l(1)G0425/FM7a*; *l(1)G0332/FM7a*; *l(1)G0152/FM7a*; *l(1)G0166/FM7a*; and *l(1)G0178/FM7a* [19]. Subsequently, we will call these lines "museum" lines. In museum lines, the lethality is caused by the integration of the *p{lacW}* transposon in the regulatory noncoding *Trf2* region (**Figure 1A**). Previously, we demonstrated that these lethal mutations did not complement $lawc^{p1}$ mutation suggesting that they are in the same gene region [15].

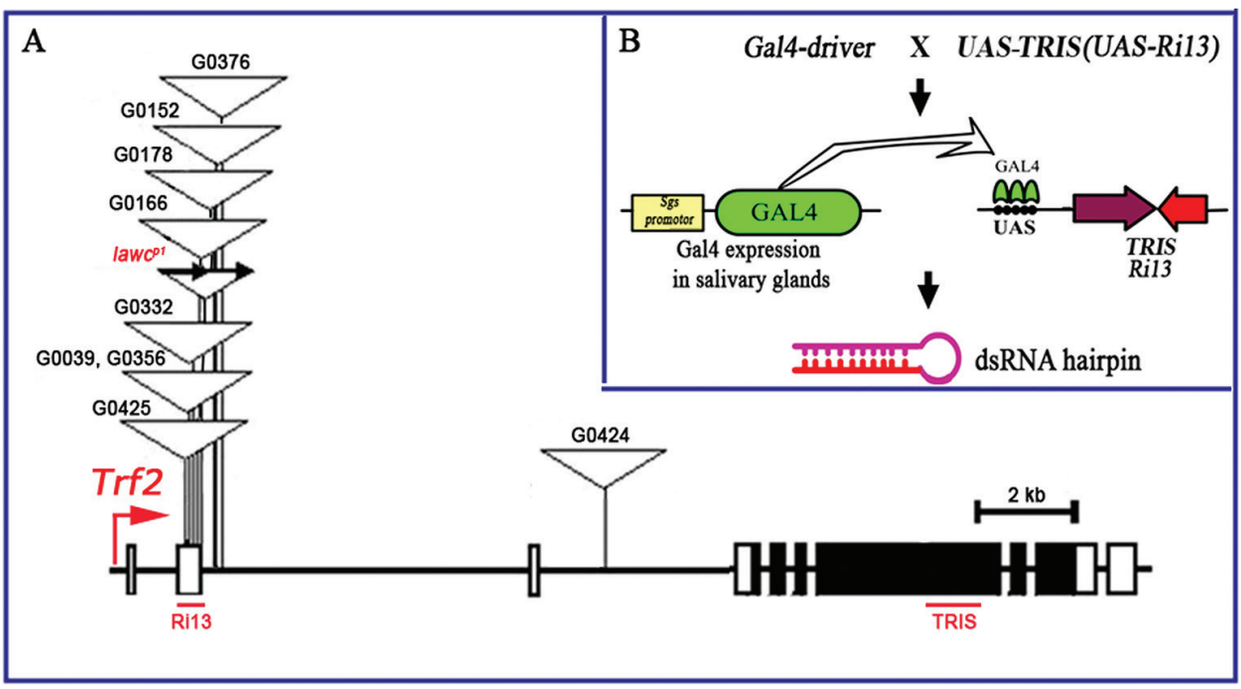

Figure 1. (A) Organization of the *Trf2* gene. Coding regions are shown as filled boxes, and noncoding ones are indicated with open boxes. Lethal insertions are marked with triangles. Double arrows mark the insertion of a double copy of the P element in the $lawc^{p1}$ mutation. Red lines indicate the regions for *UAS-Ri13* and *UAS-TRIS* constructs which express RNA hairpins under the control of inducible *UAS* yeast promoter. (B) The scheme of two component *GAL4-UAS* system. The system is composed of two independent parent transgenic lines, the *Gal4* driver line in which the yeast transcription activator *Gal4* gene is expressed in a tissue-specific manner and the *Upstream Activating Sequence* (*UAS*) responder line in which the gene of interest is under *UAS* control. Mating of the *UAS*-containing responder flies with the *Gal4* driver-containing flies results in progeny bearing the two components, in which the *UAS*-transgene expresses dsRNA hairpins in a transcriptional pattern that reflects that of the *Gal4* driver. In our experiment the *Gal4* driver is *Sgs3* which express in larvae salivary glands.

The lines of second group were obtained in our laboratory: *l(1)lawc*^{4}*/FM4*, *l(1)lawc*^{16}*/FM4*, *l(1)lawc*^{18}*/FM4*, *l(1)lawc*^{53}*/FM4*, *l(1)lawc*^{60}*/FM4*, *l(1)lawc*^{67}*/FM4*, *l(1)lawc*^{73}*/FM4*, *l(1)lawc*^{75}*/FM4*, and *l(1)lawc*^{90}*/FM4* [20]. We will further call these lines "laboratory" lines. They carry lethal *Trf2* mutations obtained after the destabilization of the mobile *P* element in the initial *lawc*^{p1} allele [20].

In lines with lethal mutation, the X chromosome is maintained on the *In(1)FM* balancer chromosome. This chromosome carries a dominant *Bar* (*B*) marker mutation (narrow eyes) and recessive allele of the *yellow* (*y*) gene (yellow body). We crossed *y*$^{+}$*l(1)/In(1)FM, yB* females with males that carried the X chromosome marked by the *y*^{1} mutation (*y*^{1}*/Y*) in order to identify exceptional classes of descendants and estimate the frequency of X-chromosome nondisjunction in these lines. Males and females of normal classes (the phenotype of which is easily identified) appeared in descendants of this crossing, including *In(1)FM, yB/Y* males with narrow eyes and yellow body and two classes of females including (1) *In(1)FM, yB/yB*$^{+}$ (yellow body and kidney-shaped eyes) and (2) *y*$^{+}$*l(1)/yB*$^{+}$ (grey body and normal oval eyes).

When X-chromosome nondisjunction occurred, males and females of exceptional classes (that always differ phenotypically) were detected in descendants. These were X/0 males with normal oval eyes and yellow bodies and XX/Y females with grey bodies and kidney-shaped eyes. Males of the normal class hemizygous for the X chromosome with a lethal allele—*l(1)/Y*—die. Exceptional classes of Y/0 males and XX/X super-females also die. Therefore, the frequency of X-chromosome nondisjunction (*Q*) was calculated according to the formula, $Q = 100\% \cdot \frac{2(X0 + XXY)}{XX + 2XY + 2X0 + 2XXY}$, where X0 and XXY are the number of flies of exceptional classes; XX and XY are the number of flies of normal classes. The sum of exceptional classes in the numerator was multiplied by two in order to take into account lethal classes with the XX/X and Y/0 genotype. The number of XY males in denominator was multiplied by two in order to take into account the class of lethal *l(1)/Y* males [21].

To estimate the influence of the *In(1)FM* balancer chromosome on the frequency of X-chromosome nondisjunction and compare it with the frequency of *Q* nondisjunction calculated for our lines, a control experiment was performed. For this, we crossed *In(1)FM, B/In(1)FM*, and *B* females with *y*^{1}*/Y* males. Females of normal class *In(1)FM* and *B/y*^{1} must have kidney-shaped eyes in the progeny of this crossing caused by a combination of one copy of the *Bar* mutant allele with one copy of the wild-type allele of this locus while *In(1)FM* and *B/Y* males must have narrow eyes caused by the presence of one copy of the *Bar* mutant allele. Exceptional females—*In(1)FM, B/In(1)FM, B/Y*—must have narrow eyes caused by two copies of the *Bar* mutant allele, while *y*^{1}*/0* exceptional males must have normal oval eyes and yellow bodies.

To determine the influence of *p{lacW}* transposon on X-chromosome nondisjunction in museum lines and to take into account the genetic background of laboratory lines, additional control experiments were performed. As a control for museum lines, we calculated the frequency of X-chromosome nondisjunction in *l(1)G0071* line with lethal mutation caused by the insertion of *p{lacW}* transposon not to *Trf2* gene region. As a control for laboratory line, we used line with *lawc*$^{p1+}$ reversion and unknown lethal mutation (complemented to *Trf2*), which we obtained after the destabilization of a mobile *P* element in the initial *lawc*^{p1} allele.

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All experiments were repeated three times, and the average frequency of X-chromosome nondisjunction ΔQ was calculated for each line. As a result of the experiment, it was found that the frequency of X-chromosome nondisjunction was increased in *lawc* mutants with decreased expression of the *Trf2* protein. The maximal frequency of X-chromosome nondisjunction was in the line *l(1)G0166* (31.2%), which increases the frequency of nondisjunction in the control line (1.4%) by approximately 22 times (**Table 1**).

Alleles	Normal classes		Exceptional classes		*Q* (%)
	♀♀ X/X	♂♂ X/Yx2	♂♂ X/0	♀♀ XX/Y	
Museum lines					
l(1)G0039	379	328	13	44	13.9
l(1)G0178	296	240	14	15	9.8
l(1)G0332	345	328	13	38	13.2
l(1)G0152	454	348	28	16	9.9
l(1)G0166	306	262	57	72	31.2
l(1)G0356	377	342	46	47	20.6
l(1)G0424	324	258	14	19	10.2
l(1)G0376	345	298	4	15	5.6
l(1)G0425	183	218	6	6	5.6
Laboratory lines					
$l(1)1awc^{4}$	450	402	10	52	12.7
$l(1)1awc^{16}$	478	530	32	6	7.0
$l(1)1awc^{18}$	290	268	8	14	7.3
$l(1)1awc^{53}$	220	224	26	54	26.5
$l(1)1awc^{60}$	361	292	6	8	4.1
$l(1)1awc^{67}$	450	306	20	14	8.3
$l(1)1awc^{73}$	427	304	34	34	15.7
$l(1)1awc^{75}$	439	334	12	8	4.9
$l(1)1awc^{90}$	466	360	6	20	5.9
Control lines					
In(1)FM	1242	1186	6	11	1.4
l(1)G0071	363	282	1	4	1.5
$lawc^{p1+}l(1)$	356	320	1	3	1.2

First column indicates *Trf2* alleles. Next two columns indicate the amount of viewed males and females of normal classes. X/X—total amount of females with $y^{+}l(1)B^{+}/yB^{+}$ and yB/yB^{+} genotypes; X/Yx2—doubled amount of males with *yB/Y* genotype. Next two columns indicate the amount of detected males and females of exceptional classes: X/0, males with $yB^{+}/0$; XX/Y, females with $y^{+}l(1)B^{+}/yB/yB^{+}$ genotype. *Q*—frequency of X-chromosome nondisjunction.

Table 1. The frequency of X-chromosome nondisjunction in females with lethal *Trf2* mutations.

3. Study of the origin of chromosome nondisjunction in *lawc* mutants

To identify the source of chromosome nondisjunction, we decided to study the meiosis of mutant females. We performed the cytological analysis of the oocyte nucleus in mutant $lawc^{p1}/l(1)EF520$ females (the frequency of X-chromosome nondisjunction is 5.2%) with low *Trf2* expression. Squash preparations of ovaries were prepared by modified Puro and Nokkala method [10, 22].

In germarium, the oocyte passes through the premeiotic DNA replication, meiosis prophase I, prometaphase I, and metaphase I. In mature oocyte of stage 14, division arrest usually occurs at the stage of metaphase I; chromosomes are collected in karyosome; and only achiasmatic chromosomes (IV and rarely X chromosome) are already oriented to opposite poles (**Figure 2A**).

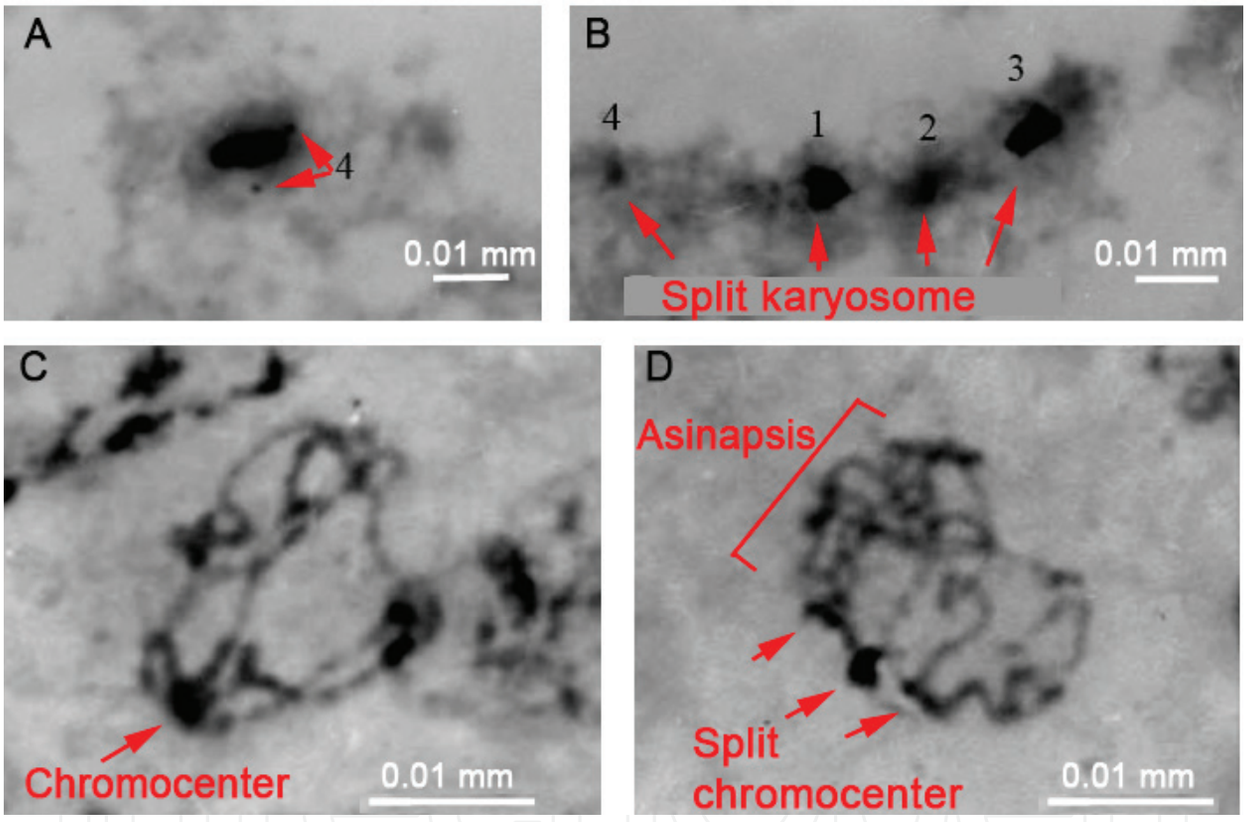

Figure 2. *Trf2* is necessary for chromatin condensation and chromocenter formation. (A–B) Late meiosis, the beginning of anaphase I, the oocyte nuclei of 13–14 stage. (A) Wild type; chromosomes of oocyte nucleus are assembled to karyosome with compact structure, while fourth chromosomes are oriented to opposite poles (arrows). (B) Mutant females; karyosome splitting. (C–D) Early meiosis, prophase I, the oocyte nuclei of 3rd stage. (C) Wild type; all chromosomes are attached by pericentromeric heterochromatin regions and thereby compact chromocenter is formed (arrow) following a correct pairing of homologous chromosomes. (D) Mutant females; chromocenter splitting occurs; chromosome compaction and homolog pairing are disturbed. Split chromocenter is indicated by arrows; failure of chromosome compaction is indicated by bracket.

We found that in anaphase I the chromocenter in mutant oocyte was often split and the compact karyosome structure was often broken (**Figure 2B**). The split karyosome assumes the disruption of the chromocenter; therefore, we performed an analysis of the early oocyte at

the stage of meiosis prophase I when oocyte chromosomes were held together by pericentromeric heterochromatin, and the compact chromocenter was easy to distinguish. As a result, we found that chromosome compaction and homolog pairing were disturbed in mutant females, and the splitting of the chromocenter was proved to exist (**Figure 2C** and **D**).

Thus, a decreasing of *Trf2* gene expression leads to failure of chromocenter formation and chromatin condensation required for proper homolog paring at premeiotic stages, and it is evidently a reason for the chromosome nondisjunction that we observe in genetic experiments.

4. *Trf2* participates in pericentromeric heterochromatin formation

Chromocenter splitting assumes the disruption of interchromosomal ectopic contacts in the pericentromeric heterochromatin region. We decided to examine *Trf2* influence the pericentromeric heterochromatin formation. We used the line with paracentric inversion on X chromosome $In(1)w^{m4}$ [23]. This inversion transfers the white locus next to the pericentromeric region, and as a result, w^{m4} mutants get a red-white mosaic colored eyes due to the position-effect variegation. To determine the ability of the $lawc^{p1}$ mutation to modify the position-effect variegation, we performed a genetic experiment using w^{m4} mutation as a sensitive test system. The combination of w^{m4} with the hypomorphic $lawc^{p1}$ mutation resulted the restoring of eye coloration in compound $w^{m4}lawc^{p1}$ flies (**Figure 3A**). This suggests that decrease in the concentration of *Trf2* protein causes the decompaction of normally tightly packed pericentromeric heterochromatin that results in *white* gene derepression. Thus, the *Trf2* is normally required for the formation of pericentromeric heterochromatin, which in turn participates in the chromocenter organization.

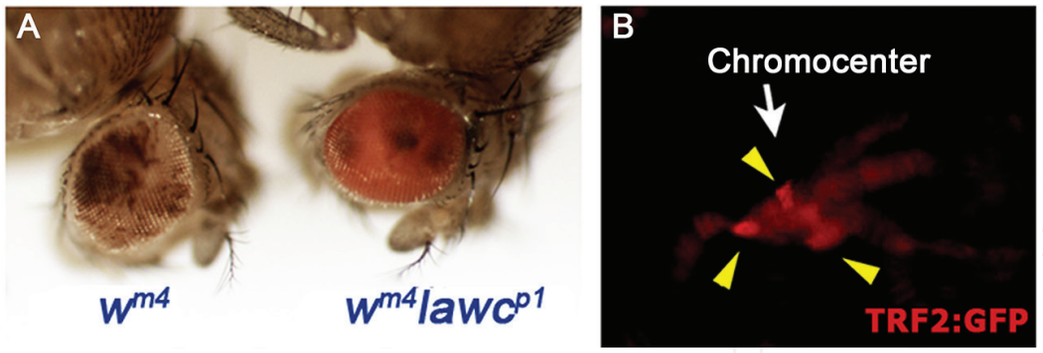

Figure 3. *Trf2* participates in pericentromeric heterochromatin formation. (A) The $lawc^{p1}$ mutation suppresses the position-effect variegation. Left: w^{m4} mutant with mosaic eye coloration. Right: double $w^{m4}lawc^{p1}$ mutant, the eye color of which is restored almost to wild type. (B) Localization of fusion *Trf2*:GFP protein in pericentromeric heterochromatin. Immunofluorescence staining of y^1w^1; *P{w⁺, [GFP~Trf2-1]}* larvae salivary gland polytene chromosomes by antibodies to GFP. Arrows indicate *Trf2* localization in the chromocenter region.

As the *Trf2* protein is a transcription factor and can indirectly influence the chromatin structure (through the activation of genes responsible for chromatin compaction), the question arises: whether *Trf2* protein can directly participate in chromocenter formation? To answer this question, we used the y^1w^1; *P{w⁺, [GFP~Trf2-1]}* flies express the hybrid GFP:*Trf2* protein

(short *Trf2* isoform fused with green fluorescent protein [GFP]) under the control of the constitutive *Hairy wing* (*Hw*) gene promoter. We performed the immunofluorescence staining of y^1w^1; *P{w*$^+$*, [GFP~Trf2-1]}* larvae salivary gland polytene chromosomes by antibodies to GFP and analyzed the distribution of fusion GFP:*Trf2* in chromocenter. As a result, we found sites of *Trf2* localization in pericentromeric heterochromatin regions (**Figure 3B**). These data allow us to confirm the direct participation of the *Trf2* in the chromocenter formation.

5. The effect of *Trf2* knockdown on salivary glands polytene chromosome morphology

We demonstrated in the above described experiments that the decrease in *Trf2* concentration influences proper chromatin compaction and chromocenter structure in germ cells. However, *Trf2* localization found in pericentromeric salivary glands heterochromatin region assumes the involvement of this protein in the compaction of chromosomes also in somatic cells.

As considered, polytene chromosomes are very favor objects for the analysis of numerous features of interphase chromosome organization and the genome as a whole [24]. To confirm our hypothesis, we decided to use *UAS-GAL4* two-component system [25] for specific RNA interference (RNAi)–mediated *Trf2* depletion in salivary gland. We obtained two *Drosophila* UAS-containing transgenic lines using P-element–mediated transformation. These lines contain constructs that express double-stranded RNA hairpins that are complementary to either 5′UTR *Trf2* regulatory (*P{w*$^+$*; UAS-Ri}13*) or encoding (*P{w*$^+$*; UAS-TRIS}*) *Trf2* gene region (**Figure 1A**). Both *UAS-Ri13* and *UAS-TRIS* constructs are able to express RNA hairpins under the control of inducible *UAS* yeast promoter element (**Figure 1B**).

For specific *Trf2* depletion in somatic cells, we used the *Sgs3-GAL4* driver (the line w^{1118}; *P{Sgs3-GAL4.PD}TP1*) that expresses yeast GAL4 activator in larvae salivary glands. After crossing *UAS*-containing flies with *Sgs3-GAL4* driver flies, the morphology of polytene chromosomes in descendant larvae *Sgs3>Ri13* and *Sgs3>TRIS* was analyzed. Larvae from *UAS*/+ lines and larvae from *Sgs3-GAL4*/+ line were used as the controls.

Normally, polytene chromosomes are present in salivary glands in singular due to the somatic synapsis occurs when two homologous chromosomes remain consistently conjugated. Polytene nonhomologous chromosomes in the nucleus are joined by their centromeres to form the most compact common region—chromocenter (**Figure 4A, C**, and **E)**. Studies of *Trf2*-depleted salivary gland polytene chromosomes show a number of structural aberrations in the polytene chromosomes morphology. Its banding patterns are changed, the pairing is significantly disturbed, and asynapsis frequently involves very extensive regions (almost the entire chromosome; **Figure 4B, D**, and **F)**. These defects were found approximately in 95% of analyzed nuclei (N = 100) in the experimental sample and approximately in 5% (N = 50) of analyzed nuclei in the control sample.

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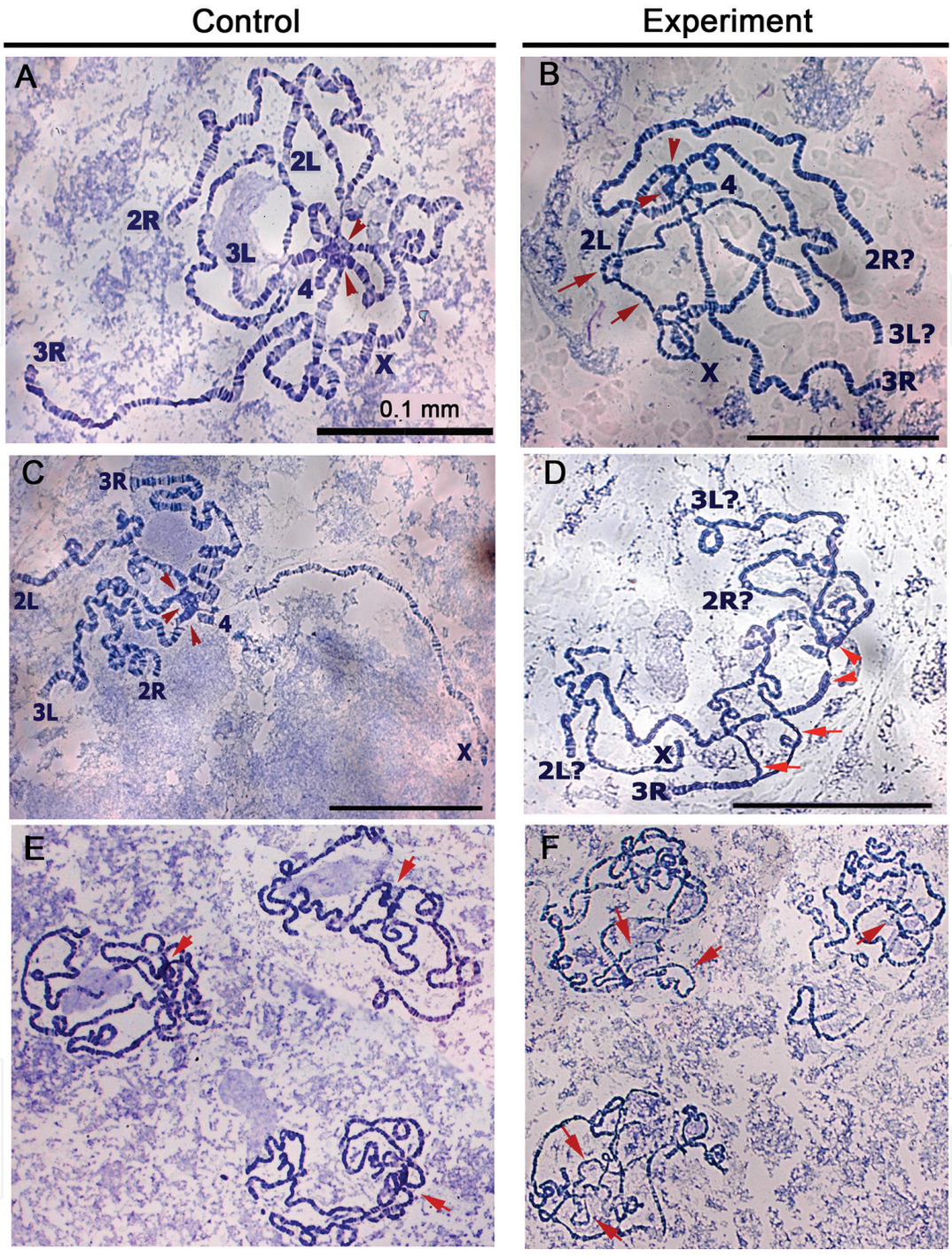

Figure 4. The effect of *Trf2* depletion on salivary glands polytene chromosomes morphology. Polytene chromosomes before and after *Trf2* depletion in salivary glands. Control—polytene chromosomes of larvae from *Sgs3-GAL4* driver (A) and from line with *UAS* transgene (C). Experiment—polytene chromosomes of *Sgs3>TRIS* (B) and *Sgs3>Ri13* (D) larvae containing activated constructions. Marks: X chromosome (X), left (2L) and right (2R) arms of the second autosome, left (3L) and right (3R) arms of the third autosome, and fourth (4) chromosome. Chromocenters and asynapses are indicated by arrowheads and arrows correspondently. Total view of polytene chromosomes in control (E) and experiment (F). Chromocenters are indicated by arrows on (E); homolog chromosome asynapses are indicated on (F). The regions of chromocenter on (F) are difficult to identify. Question marks mean that same chromosomes are hard to identify due to their abnormal morphology.

It is known that partial asynapsis is not a consequence of squashing of nuclei and variations in methods used to make preparations do not affect the frequencies of asynapsis [26]. So, we concluded that high frequency of chromosome asynapsis was induced by *Trf2* depletion. However, the main trait of nuclei in lines with depleted *Trf2* was the failure of chromocenter formation. Thus, the suppression of the *Trf2* expression in salivary glands reveals the involvement of *Trf2* gene in a chromocenter organization and in the correct pairing of homologous chromosomes not only in meiosis but also in somatic cells.

It is known that the chromocenter is responsible for the chromosome co-orientation during cell division and facilitates the paring of homologs [9, 27]. The disturbance of paring affects the transvection (or allelic complementation)—the phenomenon in which gene regulatory elements located in one of the homologs control a promoter of the same gene but located in another homolog [28, 29]. It is interesting to note that hypomorphic *lawc* mutations suppress transvection effect induced by *zeste* mutations [16]. This fact confirms the existence of abnormal homolog paring in lines with lower *Trf2* expression.

The study a set of mutations that cause chromosome nondisjunction allowed to conclude that the chromocenter is a genetically programmed structure, that is, there are genes that control its formation and reorganization [11]. For example, it was demonstrated that the recessive mutation of *crossover suppressor on 3 of Gowen* (*c*(*3*)*G*) gene influences the structure of the lateral element and the length of meiotic chromosomes [30–32].

The *Syntaxin 13* (*Syx13*) gene mutation (*ff16*) causes sterility and chromosome nondisjunction in males and females meiosis. Oocytes of mutant *ff16* females demonstrate a split karyosome and the disruption in the chromocenter formation. The product of this gene is homologous to the receptor of synaptosomal-associated protein of 25 kDa (SNAP-25) and is involved in cytokinesis [32, 33].

Another gene—*no distributive disjunction* (*nod*)—is involved in the organization and orientation of the spindle during mitosis and meiosis and is required for its binding to chromosomes in the *Drosophila* oocyte nucleus. This gene encodes the protein that contains DNA-binding domain and the conservative motor domain homologous to the Kinesin. *nod* mutations disrupt chromocenter formation in germ and somatic cells and cause achiasmatic chromosome nondisjunction in *Drosophila* females meiosis [30, 34]. All these proteins have distinct functions; however, a decrease in their activity leads to a similar result, that is, the disruption of chromocenter formation and chromosome nondisjunction.

It was shown that *Trf2* may be the part of the macromolecular chromatin-remodeling complex NUcleosome Remodeling Factor (NURF) which is correlated with transcriptional activation [35]. Nevertheless, the data we obtained have demonstrated that *Trf2* could not only be involved in transcription activation but also could perform structural functions in chromatin organization. This idea is supported by the observation that there is no proper chromatin condensation in early spermatids of mice with null *Trf2* mutation and, in particular, the chromocenter formation is disturbed [36]. Thus, we may conclude that the role of *Trf2* in the organization of chromocenter structure and chromatin condensation is evolutionarily conservative.

In yeast, it was demonstrated that kinetochores—large protein complexes assembled on the centromeric region of the chromosomes, to which spindle microtubule is attached during cell division—are formed by the epigenetic mechanism. This mechanism involves the generation of specialized nucleosomes in which a canonical histone H3 is replaced by its centromere-specific homologs—centromere protein A (CENP-A). This protein served as a landmark for kinetochore assembly to define the identity of centromeres [37, 38]. The high frequency of chromosome nondisjunction induced by decondensation of pericentric heterochromatin in *lawc* mutants allows us to assume that *Trf2* may be involved in the epigenetic regulation of kinetochores formation in *Drosophila*.

As it was mentioned above, the correct distribution of chromatids between daughter cells depends on the coordinated interaction of centrosomes, centromeres, kinetochores, spindle fibrils, topoisomerase, proteolytic processes, and motor proteins. The error of accurate spatiotemporal interactions between any of these factors results in a genomic disbalance. We cannot completely exclude the probability that *Trf2*, being transcription factor, can indirectly influence the process of cellular division through the regulation of genes that control mitosis and meiosis. In previous experiments, while looking for interactions between the *Trf2* and other genes, we performed genetic screening to detect cytological regions that are sensitive to a decreased level of *Trf2* expression [39]. **Table 2** shows genes of meiosis and mitosis localized in these regions. The genes involved in chromatin compaction are the largest group.

Process	Genes	
Chromatin compaction	*Top2* (*topoisomerase 2*) *Top3alpha* (*topoisomerase 3alpha*) *Mcm7* (*minichromosome maintenance 7*) *eIF-4E* (*eukaryotic initiation factor 4E*) *cid* (*centromere identifier*)	*kis* (*kismet*) *vls* (*valois*) *barr* *Bj1* *Df31* (*decondensation factor 31*)
Assembly of division spindle	*mad2* *cnn* (*centrosomin*)	*αTub67C*
Chromosome disjunction	*Sse* (*Separase*) *Gap1* (*GTPase-activating protein 1*)	*Dub* (*double or nothing*) *cdc23* (*cell-division-cycle 23*)
Organization of actin components of cytoskeleton	*spir* (*spire*) *dia* (*diaphanous*)	
Checkpoint	*mus304* (*mutagen-sensitive 304*) *Cdk8* (*Cyclin-dependent kinase 8*) *Myt1*	*hay* (*haywire*) *lok* (*loki*)
?	*Hs2st* (*heparan sulfate 2-O-sulfotransferase*) *I-2* (*Inhibitor-2*)	

Table 2. The classification of mitosis and meiosis genes that may interact with *Trf2*.

This does not mean that *Trf2* interacts with each of them; nevertheless, we cannot exclude the probability that decompaction of pericentric heterochromatin and defects in chromosome segregation in mitosis and meiosis in *lawc* mutant are induced by low expression of some of these genes. However, the localization of *Trf2* in the chromocenter supports the idea that this factor can be independently involved in the organization of chromatin structure.

6. Conclusion

We demonstrated that a decrease in the *Trf2* expression damages proper chromocenter structure and abolishes chromatin condensation required for correct homologs pairing at premeiotic stages and is evidently a reason for the chromosome nondisjunction that we observed in genetic experiments. Moreover, we found that compact chromocenter and correct homolog pairing were abolished in flies with a lower *Trf2* expression not only in germline but also in somatic cells. As *Trf2* is localized in pericentromeric regions, we conclude that *Trf2* can not only be involved in transcription activation but also may perform structural function in pericentromeric heterochromatin organization that is responsible for a chromocenter formation.

In conclusion, we would like to note that in the recent screening for genes that control the sex-ratio meiotic drive in *Drosophila simulans* (closely related species to *D. melanogaster*), the *Trf2* was suggested as the candidate for the factor responsible for this natural phenomenon typical for some animal species [40, 41]. It is interesting that in studied *D. simulans* population *Trf2* locus underwent the tandem duplication [41]. Thus, the function to control the specific X-chromosome nondisjunction may be adapted during the evolution by one of *Trf2* copies.

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