

EXTRA VIEW

Trans-inactivation: Repression in a wrong place

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ABSTRACT

Trans-inactivation is the repression of genes on a normal chromosome under the influence of a rearranged homologous chromosome demonstrating the position effect variegation (PEV). This phenomenon was studied in detail on the example of *brown*^{Dominant} allele causing the repression of wild-type *brown* gene on the opposite chromosome. We have investigated another trans-inactivation-inducing chromosome rearrangement, *In(2)A4* inversion. In both cases, *brown*^{Dominant} and *In(2)A4*, the repression seems to be the result of dragging of the euchromatic region of the normal chromosome into the heterochromatic environment. It was found that cis-inactivation (classical PEV) and trans-inactivation show different patterns of distribution along the chromosome and respond differently to PEV modifying genes. It appears that the causative mechanism of trans-inactivation is *de novo* heterochromatin assembly on euchromatic sequences dragged into the heterochromatic nuclear compartment. Trans-inactivation turns out to be the result of a combination of heterochromatin-induced position effect and the somatic interphase chromosome pairing that is widespread in Diptera.

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Position effect variegation (PEV) was first discovered by Muller¹ who observed patched pigmentation of the fly eye owing to the heritable repression of the *white* gene in a subset of the eye precursor cells. Further, it was established that PEV is an epigenetic phenomenon caused by the displacement of a gene from its normal chromosomal environment close to the heterochromatin by rearrangement or transposition.^{2,3} The epigenetic nature of PEV means that DNA sequences of the affected genes are not disturbed. Instead, euchromatic genes are repressed by acquiring heterochromatic marks including specific histone modifications (mainly H3K9me and H3K27me), proteins like HP1a and condensed nucleosome package. It has been shown that this heterochromatin structure can spread from the new eu-heterochromatin border into the euchromatin by self-assembly and propagation of the multiprotein complex encompassing histone methyltransferase (HKMT) Su(var)3–9, H3K9me2/3-binding HP1a protein and Su(var)3–7 protein. The JIL-1 kinase and the H3S10 histone modification counteract heterochromatin spreading.^{4–10}

According to the current model of heterochromatin formation, Su(var)3–9 HKMT methylates the lysine 9 residue of histone H3. HP1a binds to H3K9me and then recruits another molecule of Su(var)3–9 for further methylation of H3 in the adjacent nucleosome.^{10,11} This methylation/HP1a binding loop repeats until it reaches a boundary element¹² or ceases due to the action of histone code modifiers like JIL-1 kinase⁹. This model of heterochromatin propagation assumes the assembly of protein complexes via the short-range interactions between protein domains along the chromatin fiber, thus providing a molecular basis for the classic concept of linear heterochromatin spreading.¹³ This self-assembly model suggests cis-action of PEV – i.e. it can affect genes near the eu-heterochromatic border only in the rearranged chromosome. The model of linear heterochromatinization is applicable for heterochromatin extension at small distances,^{10,14} but cannot adequately explain interrupted propagation of PEV, long range PEV spreading (hundreds of thousands of kilobases) and the repression of genes in a non-rearranged homologous chromosome (trans-inactivation).

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Only a few cases of trans-inactivation have been described in detail. These include rearrangements with breakpoints near or inside the *brown* gene, such as the *brown*^{Dominant} (*bw*^D) allele,^{15,16} *mini-white*-containing transgenes over the PEV-inducing rearrangements¹⁷ and the *In(2)A4* inversion.^{18–20} The best-studied trans-inactivation-inducing rearrangement is *bw*^D allele, which emerged as a result of insertion of 1.6 Mb of AAGAG satellite into the coding region of the *brown* gene.²¹ This allele can repress the wild-type *brown* gene on the opposite chromosome, as well as reporter genes inserted near the *brown*.²² The results of *bw*^D-induced trans-inactivation studies are presented in several papers, and the general scheme of the process was elucidated.^{14,22–26}

It was found that satellite DNA inserted into the *brown* locus promotes the dragging of the *bw*^D and homologous regions of the wild-type chromosome toward the pericentromeric heterochromatic nuclear compartment.^{26,27} Nuclear compartments are defined as distinct intranuclear volumes with specific molecular composition.^{28,29} Examples of nuclear compartments are the nucleolus, the histone locus body (HLB) and the pericentromeric heterochromatin. The pericentromeric heterochromatin compartment assembles in the interphase and can be revealed by immunostaining for heterochromatin proteins like HP1a. Cell division disrupts the compartment, and it takes some time (hours) to reestablish it.

The dragging of *bw*^D into heterochromatin occurs due to the sequence-independent stickiness of heterochromatin blocks.³⁰ The process of dragging lasts for at least 5 h during the G0/G1 phase of the cell cycle and the association disrupts during the S-phase.³¹ Association of the *bw*^D with the pericentromeric region appears to proceed by confinement of the allele in heterochromatin after an accidental contact in the course of random movements in the nucleoplasm. Normal euchromatic genes are excluded from the heterochromatic compartment and do not enter it.³² However, since the normal and the *bw*^D-bearing chromosome are somatically paired in a more than 75% of nuclei, the sticking of satellite block in *bw*^D to the main bulk of the pericentromeric heterochromatin also causes the dragging of the homologous region of the normal chromosome into the heterochromatin. The dragging into the heterochromatic compartment leads to the repression of the wild-type *brown* gene and the reporter genes located near it (at a distance of

up to 86 kb).^{22,25,27} The repressed reporter genes acquire the specific chromatin composition including HP1a enrichment and compact nucleosome package, but not H3K9me2 histone modification.¹⁴ The absence of the H3K9me2 mark at the trans-inactivated genes correlates with the insensitivity of trans-inactivation process to the loss of the *Su(var)3–9* HKMT, which is responsible for the writing of this histone modification.

In(2)A4 is an inversion with breakpoints in the euchromatin at 39B and at *h37* in the pericentromeric heterochromatin of 2L, encompassing approximately 1 Mb of euchromatin and heterochromatin blocks *h35–h37*. *In(2)A4* induces robust and widespread trans-inactivation of *mini-white* reporter genes located on the homologous, non-rearranged chromosome as well as of genes in cis, located near the new eu-heterochromatin border.^{18–20} Trans-inactivation effects in *In(2)A4* and *bw*^D are similar in many aspects, apparently reflecting common properties of trans-inactivation process in *Drosophila*. FISH data show that, like in the *bw*^D case, the normal chromosome and the inversion are somatically paired, and the region of the inversion near the new eu-heterochromatin boundary and the homologous region of the normal chromosome are dragged into the heterochromatic nuclear compartment.²⁰ The dragging of a reporter gene into the heterochromatin correlates with its repression at the single-cell level, pointing to the dragging process as the direct cause of trans-inactivation.¹⁹ The effects of genetic modifiers of PEV on *In(2)A4*-induced trans-inactivation resemble that at the *bw*^D locus: *Su(var)2–5* (*HP1a*) and *Su(var)3–7* strongly suppress the inactivation while *Su(var)3–9* HKMT mutations produce no effect. It appears that the HP1a participates in the repression independently of *Su(var)3–9*-mediated H3K9me2 modification.¹⁸

In contrast to *bw*^D transposition of satellite sequences into the otherwise unrearranged chromosome, *In(2)A4* affects the linear order of genes in a region of more than one megabase. This indicates that somatic pairing between the *In(2)A4* carrying chromosome and the normal chromosome proceeds via the formation of loop-like structure (Fig. 1). The loop formed by rearrangement and the normal chromosome is visible on polytene chromosome spreads (our unpublished observations) and FISH signals from the putatively paired regions in transheterozygous *In(2)A4/+* individuals revealed a single spot in a majority

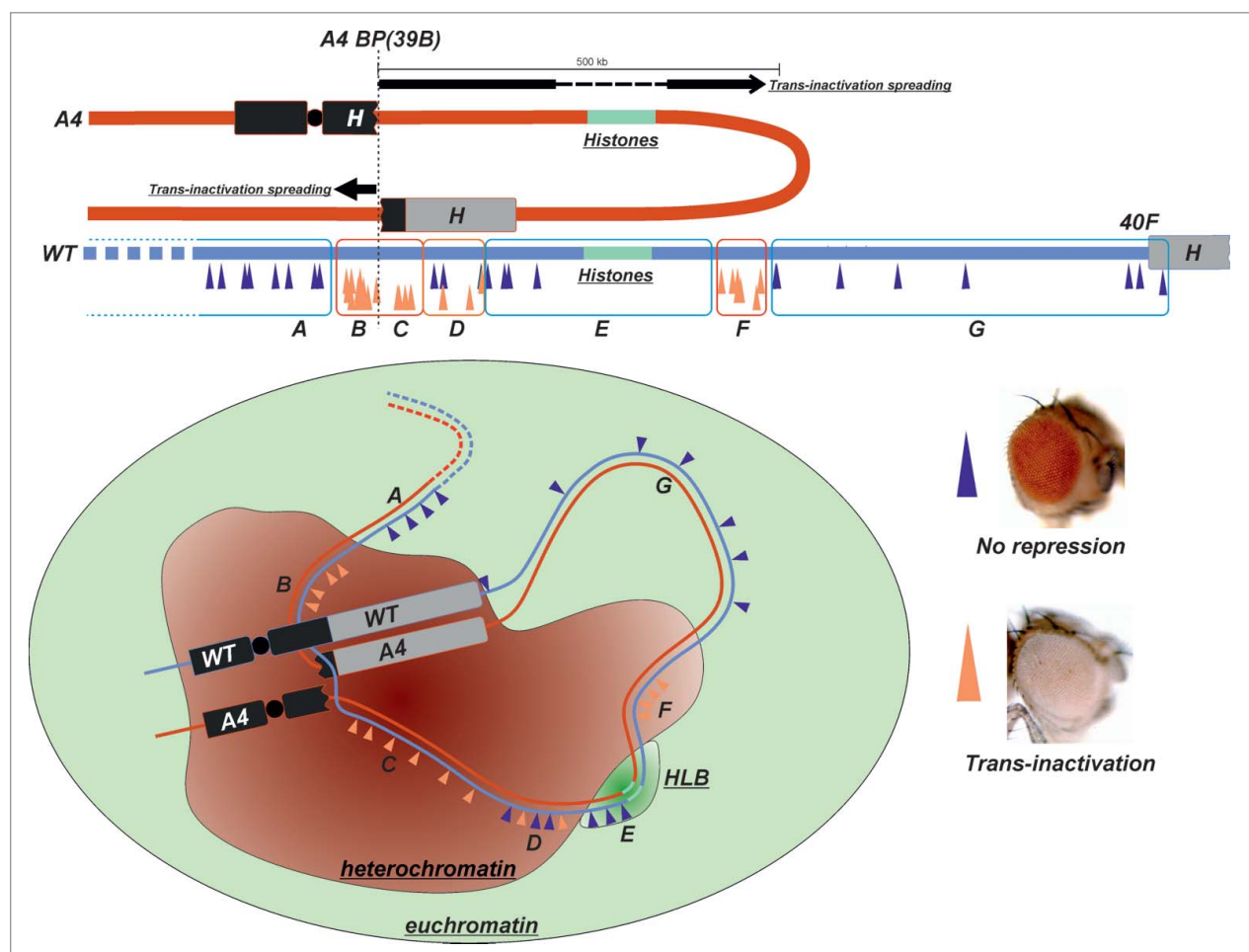


Figure 1. The possible configuration of the loop between *In(2)A4* inversion and the wild type chromosome relative to the euchromatic and heterochromatic nuclear compartments in interphase nuclei. On the top of the drawing, the linear map of the trans-inactivation distribution in the wild-type chromosome (dodger blue) paired with *In(2)A4* (red) is shown. Blue triangles mark the position of non-inactivated transgenes, orange ones – trans-inactivated, the position of the histone genes cluster is shown in green, and pericentromeric heterochromatin is gray boxes. The vertical dotted line represents the *In(2)A4* breakpoint location (39B). Checked transgenes could be grouped into 7 areas marked A, B, C, D, E, F and G, where A and G represent the regions outside the trans-inactivation spreading zone, B and C are the regions of approximately 50 kb in size with complete repression, D is the region where some transgenes are inactivated while the others are not. E is the region near the histone genes cluster, no repression observed here, and F is the “island” of trans-inactivation after the histone genes cluster. Below the linear map, the putative organization of the loop of paired chromosomes in the nuclear space is shown according to a combination of FISH data¹⁸⁻²⁰ and polytene chromosomes arrangement. The light green is euchromatin; the brown gradient zone is HP1a-stained pericentromeric heterochromatin compartment. The green area is the histone genes cluster (HLB – histones locus body), which was shown to locate on the border of HP1a-enriched volume¹⁸. The positions of the areas of trans-inactivation spreading (A, B, C, D, E, F and G) correspond to those on the linear map. According to the model, the regions near the breakpoint (B and C) are dragged deepest into heterochromatin and fully repressed, while the region near the histone genes cluster (E) is transferred outside the heterochromatin due to the specific properties of HLB, and the transgenes in this region are not inactivated.

of the cells, confirming strong somatic pairing.¹⁸ We suppose that the pairing is preserved due to a relatively small size of the inverted euchromatin block (approximately 1 Mb of DNA) and a large size of the separated heterochromatin block (sections *h35-h37* of 2Lh), sufficient to promote effective sticking to the main bulk of the pericentromeric heterochromatin. These peculiarities of the *In(2)A4* structure should stabilize the loop formed by the paired chromosomes.

Inactivation caused by *In(2)A4* is powerful and was detected at a distance of up to 470 kb from the position of the breakpoint in the rearranged chromosome. In contrast, the farthest repressed transgene in *bw^D* is located at 86 kb from the satellite insertion, and no repression was detected at a distance of 200 kb.²² We have checked more than 70 transgenic insertions of several types scattered in the region of 1.5 Mb in size around the position of the euchromatic breakpoint in

In(2)A4 for the ability to be trans-inactivated. The repression of the transgenes was essentially continuous in an area of approximately 100 kb nearby the breakpoint position. Interrupted silencing was observed in more distant regions, where the repressed transgenes in some cases are located several hundred base pairs away from the active transgenes. Differences in the sensitivity of closely placed transgenes to trans-inactivation were also detected in the case of *bw^D*.^{22,25}

Mechanisms responsible for the different sensitivity of transgenes to repression are still enigmatic. No obvious correlation between the sensitivity to repression and the transgene type or orientation was detected.¹⁸ We propose that each transgene insertion event creates a specific and unique arrangement of regulatory elements. The closely located *P[lacW]* transgenes *SH0764* and *11019* show different responses to inactivation, *SH0764* is repressed while *11019* is not. Both transgenes are located in the 5' UTR of the *CG8671* gene but in regions with different chromatin composition. The *SH0764* disrupts a potential insulator (BEAF-binding site, according to Modencode profile) while the *11019* is inserted between the BEAF-binding sites. In the second case of 2 transgenes (*20102* and *12400*) located only 1 kb from each other, but responding differently to transactivation, the reason for the insensitivity of the reporter to trans-silencing may be the location of the *20102* transgene in the strong GAGA factor binding site. It seems that no common rule or feature determines the sensitivity of the transgene insertion to trans-inactivation. Instead, there are different mechanisms in each case, and transgenes may react individually to potential modifiers of trans-repression, like mutations in genes of insulator proteins.

Interestingly, we found a gap in *In(2)A4*-induced trans-inactivation spreading in the region near the histone genes cluster. The transgenes in this region are resistant to repression. The cluster of histone genes is known to form a distinct nuclear subcompartment (histone locus body, HLB) in the early G1 phase of the cell cycle.^{33,34} It is possible that specific properties of the HLB could override the heterochromatic compartment influence on the transgenes located nearby. Taking into account that trans-inactivation is a result of heterochromatic compartment dragging, we have checked whether the histones cluster is dragged into the heterochromatin in *In(2)A4*. Confocal imaging shows that the histone genes cluster tends to be

excluded from the nuclear volume enriched in HP1a and usually locates at the border between heterochromatic and euchromatic compartments.¹⁸ Thus, specific nuclear compartmentalization of the histones cluster appears to be the reason for the absence of trans-inactivation in its vicinity.

The *bw^D*- and the *In(2)A4*-caused trans-inactivation examples are similar in their reaction to classical modifiers of PEV. A remarkable common feature is an insensitivity to mutations in *Su(var)3-9*. *Su(var)3-9*, which encodes a histone lysine methyltransferase (HKMT), is recruited to chromatin by HP1a and introduces H3K9me2 mark, which in turn, is recognized by HP1a. In this way, the self-assembly and cis-spreading of heterochromatin into the euchromatin occurs.^{10,11,35,36} The dispensability of *Su(var)3-9* for trans-inactivation points to the existence of a mechanism of heterochromatin formation different from the cis-spreading. Another HKMT, dSETDB1 (also known as *eggless*) could promote the heterochromatinization of transgenes since the *eggless* mutations suppress the trans-inactivation in *In(2)A4*. The *Su(var)3-9* and the *eggless* have partially overlapping targets of activity, and *eggless* appears to act through the whole lifespan, while *Su(var)3-9* is mainly active at the embryo stage.³⁷ The activity of *eggless* during the late larval and pupal stages (a period when the expression of the *mini-white* reporter occurs) could be the reason for the sensitivity of trans-inactivation caused by *In(2)A4* to the *eggless* mutations.

Mutations in an *e(y)3* (SAYP) gene cause a strong suppression of trans-inactivation caused by *In(2)A4*,¹⁸ pointing to the role of *e(y)3* product in transgene heterochromatinization. SAYP is a component of SWI/SNF-class chromatin remodeling complex PBAP, which is involved in transcriptional regulation of some *Drosophila* genes.^{38,39} SAYP is also known as a factor associated with heterochromatin on the fourth chromosome and as a dominant suppressor of PEV.³⁸ The participation of SAYP in trans-inactivation points to a link between the heterochromatin establishment and chromatin remodeling processes. It appears that in the case of trans-inactivation, the heterochromatinization of the transgene occurs *de novo* upon its dragging into the nuclear volume enriched in protein components of heterochromatin, and some remodeling event may be necessary for the initiation of binding of HP1a protein to the target sequence. According to published data, HP1a is capable of binding to the

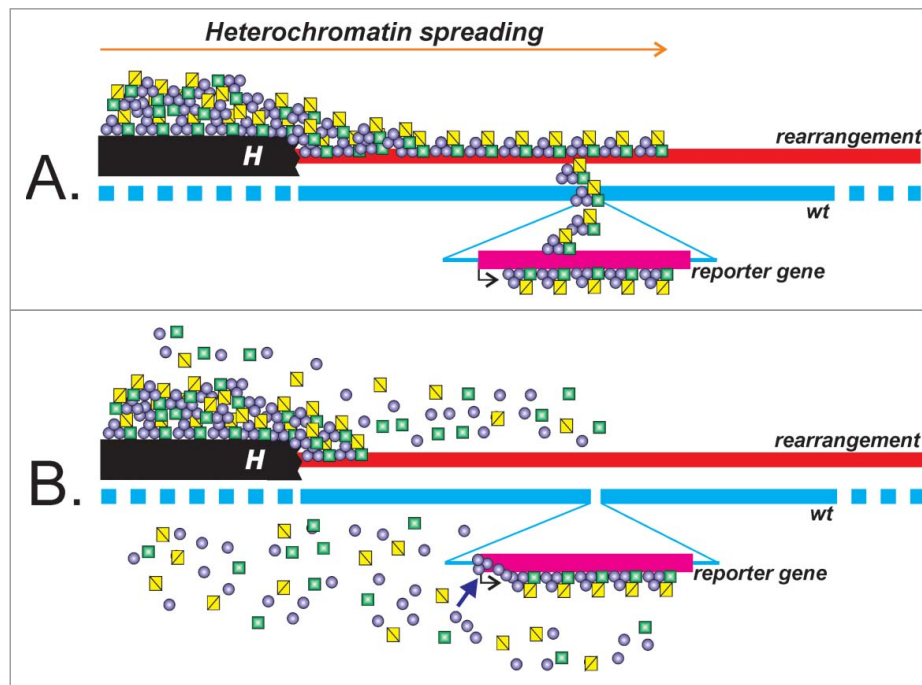


Figure 2. Two models of trans-inactivation establishment. A. Heterochromatin proteins and the histone modifications form a self-assembled complex propagating *in cis* from the new eu-heterochromatin border deep into the euchromatin. Upon reaching the trans-inactivated reporter gene position, the heterochromatin complexes either jump to paired chromosome or interact with regulatory elements of a reporter gene (like in transvection), causing the repression. B. The reporter gene is dragged into the heterochromatin due to pairing with the rearranged chromosome. The high local concentration of heterochromatin components like HP1a facilitates *de novo* formation of the heterochromatin on the reporter gene sequence. The peculiarities of *In(2)A4*-caused trans-inactivation (a vast distance of spreading, the lack of correlation between cis- and trans-inactivation, HP1a binding to reporter transgene but not to the same region on the opposite rearranged chromosome) favor the model B of the trans-inactivation establishment.

open chromatin promoter regions in the absence of specific histone modifications,⁴⁰ and the chromatin remodeling process may be required to maintain an open chromatin state.

The trans-inactivation induced by both *In(2)A4* and *bw^D* is the result of dragging of a region of the normal chromosome, somatically paired with the rearrangement, into the heterochromatic nuclear compartment. Two roles of the rearranged chromosome in trans-inactivation establishment could be considered. It is possible that the rearranged chromosome only drags the euchromatic region into the heterochromatin environment, where the heterochromatin-specific structure is assembled on it *de novo*. Alternatively, the rearranged chromosome, which has a newly formed eu-heterochromatin boundary, may itself have a more active role in trans-inactivation and permits cis-spreading of heterochromatin into the euchromatin via self-assembly. Heterochromatinized euchromatin regions could, in turn, induce the same process on the paired normal chromosome, similarly to the transvection phenomenon. If the last assumption is true, a

positive correlation between the cis-inactivation of genes on the rearranged chromosome and the trans-inactivation of transgenes on the normal homologous chromosome would be expected.

We have measured cis-effects of *In(2)A4* rearrangement by ChIP and RNAseq and compared the distribution of cis- and trans-effects along the chromosome length. Only three out of 20 genes near the new eu-heterochromatin borders demonstrate the changes in transcription levels and HP1a occupancy in *In(2)A4*. Inactivation does not spread linearly; some genes immediately near the breakpoints are not repressed while distant genes show inactivation. In addition, we found non-inactivated genes flanked by inactivated ones. HP1a distribution was shown to be discontinuous, and HP1a tends to associate only with inactivated genes. Surprisingly, no correlation between cis- and trans-inactivation was revealed: some reporter transgenes demonstrate trans-inactivation while genes located at the same position on the inverted chromosome show no cis-repression. To directly check whether the region on the normal chromosome could

acquire the heterochromatin configuration independently of the paired rearranged chromosome, we measured HP1a enrichment in trans-inactivated transgene on the normal chromosome and at the place of transgene insertion on the rearranged chromosome. It was found that the trans-inactivated transgene is enriched in HP1a while no accumulation of HP1a was detected on the homologous rearranged chromosome, thus demonstrating *de novo* formation of heterochromatin on the normal chromosome, dragged into the heterochromatic compartment. This observation points to the model that proposes a passive role of paired rearranged chromosome in trans-inactivation. It seems that dragging of the euchromatic region into the heterochromatic compartment *per se* is enough for its heterochromatinization and heterochromatin assembles *de novo* on euchromatic sequences without propagation from the neighbor heterochromatin block (Fig. 2).

In the *In(2)A4* rearrangement, the trans-inactivation is observable in a region of over 500 kb in size and affects multiple transgenic insertions, while the cis-repression affects just several genes at a distance of up to 60 kb. The transgenic reporter constructs appear to be more sensitive to the influence of the heterochromatin environment compared to endogenous genes at their normal location. One reason for this might be the lack of a full set of regulatory elements (like insulators) in these transgenes. The reporter *mini-white* gene is quite sensitive to the position effects of different types, including trans-repression, since it lacks the tissue-specific enhancers and some upstream regulatory region. We note that *SUPor-P* transgene carrying *mini-white* under the control of eye enhancer and flanked by Su(Hw) binding sites is resistant to the *In(2)A4*-induced trans-inactivation (our unpublished observations).

In summary, trans-inactivation seems to be a suitable model for studying the initial steps of heterochromatin formation since it allows monitoring *de novo* heterochromatin assembly using artificial transgenic constructions containing desired regulatory elements and reporters.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

- [1] Muller HJ. Types of visible variations induced by X-rays in *Drosophila*. *J Genet* 1930; 22:299; <http://dx.doi.org/10.1007/BF02984195>
- [2] Girton JR, Johansen KM. Chromatin structure and the regulation of gene expression: the lessons of PEV in *Drosophila*. *Adv Genet* 2008; 61:1-43; PMID:18282501; [http://dx.doi.org/10.1016/S0065-2660\(07\)00001-6](http://dx.doi.org/10.1016/S0065-2660(07)00001-6)
- [3] Spofford JB. Position-effect variegation in *Drosophila*. In: Ashburner M, Novitski E (eds.), *The genetics and biology of Drosophila*, vol 1C. Academic Press, New York, pp 955–1018.
- [4] Deng H, Cai W, Wang C, Lerach S, Delattre M, Girton J, Johansen J, Johansen KM. JIL-1 and Su(var)3–7 interact genetically and counteract each other's effect on position-effect variegation in *Drosophila*. *Genetics* 2010; 185:1183–92; PMID:20457875; <http://dx.doi.org/10.1534/genetics.110.117150>
- [5] Ebert A, Lein S, Schotta G, Reuter G. Histone modification and the control of heterochromatic gene silencing in *Drosophila*. *Chromosome Res* 2006; 14:377–92; PMID:16821134; <http://dx.doi.org/10.1007/s10577-006-1066-1>
- [6] Jaquet Y, Delattre M, Montoya-Burgos J, Spierer A, Spierer P. Conserved domains control heterochromatin localization and silencing properties of SU(VAR)3–7. *Chromosoma* 2006; 115:139–50; PMID:16463146; <http://dx.doi.org/10.1007/s00412-005-0036-2>
- [7] Schotta G, Ebert A, Krauss V, Fischer A, Hoffmann J, Rea S, Jenuwein T, Dorn R, Reuter G. Central role of *Drosophila* SU(VAR)3–9 in histone H3-K9 methylation and heterochromatic gene silencing. *Embo J* 2002; 21:1121–31; PMID:11867540; <http://dx.doi.org/10.1093/emboj/21.5.1121>
- [8] Wang C, Cai W, Li Y, Deng H, Bao X, Girton J, Johansen J, Johansen KM. The epigenetic H3S10 phosphorylation mark is required for counteracting heterochromatic spreading and gene silencing in *Drosophila melanogaster*. *J Cell Sci* 2011; 124:4309–17; PMID:22247192; <http://dx.doi.org/10.1242/jcs.092585>
- [9] Wang C, Girton J, Johansen J, Johansen KM. A balance between euchromatic (JIL-1) and heterochromatic [SU(var)2–5 and SU(var)3–9] factors regulates position-effect variegation in *Drosophila*. *Genetics* 2011; 188:745–8; PMID:21515582; <http://dx.doi.org/10.1534/genetics.111.129353>
- [10] Hines KA, Cryderman DE, Flannery KM, Yang H, Vitalini MW, Hazelrigg T, Mizzen CA, Wallrath LL. Domains of Heterochromatin Protein 1 Required for

- Drosophila melanogaster* Heterochromatin Spreading. *Genetics* 2009; 182:967-77; PMID:19487560; <http://dx.doi.org/10.1534/genetics.109.105338>
- [11] Schotta G, Ebert A, Reuter G. SU(VAR)3-9 is a conserved key function in heterochromatic gene silencing. *Genetica* 2003; 117:149-58; PMID:12723694; <http://dx.doi.org/10.1023/A:1022923508198>
 - [12] van der Vlag J, den Blaauwen JL, Sewalt RG, van Driel R, Otte AP. Transcriptional repression mediated by polycomb group proteins and other chromatin-associated repressors is selectively blocked by insulators. *J Biol Chem* 2000; 275:697-704; PMID:10617669; <http://dx.doi.org/10.1074/jbc.275.1.697>
 - [13] Talbert PB, Henikoff S. Spreading of silent chromatin: inactivation at a distance. *Nat Rev Genetics* 2006; 7:793-803; PMID:16983375; <http://dx.doi.org/10.1038/nrg1920>
 - [14] Nisha P, Plank JL, Csink AK. Analysis of chromatin structure of genes silenced by heterochromatin in trans. *Genetics* 2008; 179:359-73; PMID:18493059; <http://dx.doi.org/10.1534/genetics.107.084004>
 - [15] Slatis HM. Position Effects at the Brown Locus in *Drosophila Melanogaster*. *Genetics* 1955; 40:5-23; PMID:17247537
 - [16] Henikoff S, Dreesen TD. Trans-inactivation of the *Drosophila* brown gene: evidence for transcriptional repression and somatic pairing dependence. *Proc Natl Acad Sci U S A* 1989; 86:6704-8; PMID:2505257; <http://dx.doi.org/10.1073/pnas.86.17.6704>
 - [17] Martin-Morris LE, Csink AK, Dorer DR, Talbert PB, Henikoff S. Heterochromatic trans-inactivation of *Drosophila* white transgenes. *Genetics* 1997; 147:671-7; PMID:9335603
 - [18] Abramov YA, Shatskikh AS, Maksimenko OG, Bonaccorsi S, Gvozdev VA, Lavrov SA. The Differences Between Cis- and Trans-Gene Inactivation Caused by Heterochromatin in *Drosophila*. *Genetics* 2016; 202:93-106; PMID:26500261; <http://dx.doi.org/10.1534/genetics.115.181693>
 - [19] Lavrov SA, Shatskikh AS, Kibanov MV, Gvozdev VA. [Correlation on a cellular level of gene transcriptional silencing and heterochromatin compartment dragging in case of PEV-producing eu-heterochromatin rearrangement in *Drosophila melanogaster*]. *Mol Biol (Mosk)* 2013; 47:286-91; PMID:23808163
 - [20] Abramov YA, Kibanov MV, Gvozdev VA, Lavrov SA. Genetic and molecular analysis of gene trans-inactivation caused by homologous eu-heterochromatic chromosome rearrangement in *Drosophila melanogaster*. *Dokl Biochem Biophys* 2011; 437:72-6; PMID:21590379; <http://dx.doi.org/10.1134/S1607672911020050>
 - [21] Platero JS, Csink AK, Quintanilla A, Henikoff S. Changes in chromosomal localization of heterochromatin-binding proteins during the cell cycle in *Drosophila*. *J Cell Biol* 1998; 140:1297-306; PMID:9508764; <http://dx.doi.org/10.1083/jcb.140.6.1297>
 - [22] Csink AK, Bounoutas A, Griffith ML, Sabl JF, Sage BT. Differential gene silencing by trans-heterochromatin in *Drosophila melanogaster*. *Genetics* 2002; 160:257-69; PMID:11805061
 - [23] Sage BT, Wu MD, Csink AK. Interplay of developmentally regulated gene expression and heterochromatic silencing in trans in *Drosophila*. *Genetics* 2008; 178:749-59; PMID:18245337; <http://dx.doi.org/10.1534/genetics.107.083105>
 - [24] Thakar R, Csink AK. Changing chromatin dynamics and nuclear organization during differentiation in *Drosophila* larval tissue. *J Cell Sci* 2005; 118:951-60; PMID:15731005; <http://dx.doi.org/10.1242/jcs.01684>
 - [25] Sage BT, Jones JL, Holmes AL, Wu MD, Csink AK. Sequence elements in cis influence heterochromatic silencing in trans. *Mol Cell Biol* 2005; 25:377-88; PMID:15601858; <http://dx.doi.org/10.1128/MCB.25.1.377-388.2005>
 - [26] Csink AK, Henikoff S. Genetic modification of heterochromatic association and nuclear organization in *Drosophila*. *Nature* 1996; 381:529-31; PMID:8632827; <http://dx.doi.org/10.1038/381529a0>
 - [27] Harmon B, Sedat J. Cell-by-cell dissection of gene expression and chromosomal interactions reveals consequences of nuclear reorganization. *PLoS biology* 2005; 3:e67; PMID:15737020; <http://dx.doi.org/10.1371/journal.pbio.0030067>
 - [28] Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, Hoichman M, Parrinello H, Tanay A, Cavalli G. Three-dimensional folding and functional organization principles of the *Drosophila* genome. *Cell* 2012; 148:458-72; PMID:22265598; <http://dx.doi.org/10.1016/j.cell.2012.01.010>
 - [29] Bickmore WA, van Steensel B. Genome architecture: domain organization of interphase chromosomes. *Cell* 2013; 152:1270-84; PMID:23498936; <http://dx.doi.org/10.1016/j.cell.2013.02.001>
 - [30] Sage BT, Csink AK. Heterochromatic self-association, a determinant of nuclear organization, does not require sequence homology in *Drosophila*. *Genetics* 2003; 165:1183-93; PMID:14668374
 - [31] Csink AK, Henikoff S. Large-scale chromosomal movements during interphase progression in *Drosophila*. *J Cell Biol* 1998; 143:13-22; PMID:9763417; <http://dx.doi.org/10.1083/jcb.143.1.13>
 - [32] Thakar R, Gordon G, Csink AK. Dynamics and anchoring of heterochromatic loci during development. *J Cell Sci* 2006; 119:4165-75; PMID:16984972; <http://dx.doi.org/10.1242/jcs.03183>
 - [33] White AE, Leslie ME, Calvi BR, Marzluff WF, Duronio RJ. Developmental and cell cycle regulation of the *Drosophila* histone locus body. *Mol Biol Cell* 2007; 18:2491-502; PMID:17442888; <http://dx.doi.org/10.1091/mbc.E06-11-1033>
 - [34] Liu JL, Murphy C, Buszczak M, Clatterbuck S, Goodman R, Gall JG. The *Drosophila melanogaster* Cajal body. *J Cell Biol* 2006; 172:875-84; PMID:16533947; <http://dx.doi.org/10.1083/jcb.200511038>
 - [35] Grewal SI, Elgin SC. Heterochromatin: new possibilities for the inheritance of structure. *Curr Opin Genet Dev*

- 2002; 12:178-87; PMID:11893491; [http://dx.doi.org/10.1016/S0959-437X\(02\)00284-8](http://dx.doi.org/10.1016/S0959-437X(02)00284-8)
- [36] Elgin SC, Reuter G. Position-effect variegation, heterochromatin formation, and gene silencing in *Drosophila*. Cold Spring Harb Perspect Biol 2013; 5:a017780; PMID:23906716; <http://dx.doi.org/10.1101/cshperspect.a017780>
- [37] Brower-Toland B, Riddle NC, Jiang H, Huisinga KL, Elgin SC. Multiple SET methyltransferases are required to maintain normal heterochromatin domains in the genome of *Drosophila melanogaster*. Genetics 2009; 181:1303-19; PMID:19189944; <http://dx.doi.org/10.1534/genetics.108.100271>
- [38] Shidlovskii YV, Krasnov AN, Nikolenko JV, Lebedeva LA, Kopantseva M, Ermolaeva MA, Ilyin YV, Nabirochkina EN, Georgiev PG, Georgieva SG. A novel multidomain transcription coactivator SAYP can also repress transcription in heterochromatin. EMBO J 2005; 24:97-107; PMID:15616585; <http://dx.doi.org/10.1038/sj.emboj.7600508>
- [39] Chalkley GE, Moshkin YM, Langenberg K, Bezstarosti K, Blastyak A, Gyurkovics H, Demmers JAA, Verrijzer CP. The transcriptional coactivator SAYP is a trithorax group signature subunit of the PBAP chromatin remodeling complex. Mol Cell Biol 2008; 28:2920-9; PMID:18299390; <http://dx.doi.org/10.1128/MCB.02217-07>
- [40] Figueiredo ML, Philip P, Stenberg P, Larsson J. HP1a recruitment to promoters is independent of H3K9 methylation in *Drosophila melanogaster*. PLoS Genet 2012; 8:e1003061; PMID:23166515; <http://dx.doi.org/10.1371/journal.pgen.1003061>