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REVIEWS
AND THEORETICAL ARTICLES

Control of the Gene Activity by Polycomb and Trithorax Group Proteins in *Drosophila*

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Abstract—Combinatorial expression of the genes in multicellular organisms leads to the development of different cell types. The important epigenetic regulators of higher eukaryotes are the Polycomb group (PcG) and Trithorax group (TrxG) proteins. These factors control the transcription of a large number of genes involved in various cellular processes. Dysregulation of PcG and TrxG systems leads to developmental abnormalities and cancer. This review focuses on the main characteristics and properties of the *Drosophila* PRE elements. Furthermore, we summarize the information on the protein components of the PcG and TrxG groups and their functional activities and discuss the main aspects of competition between the proteins of these classes as well as their possible mechanisms of action.

Keywords: Polycomb, Trithorax, epigenetic memory, transcription regulation, chromatin, *Drosophila*

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INTRODUCTION

Proper development of multicellular organisms requires the individual gene expression patterns to be established in all cell types and to be stably transmitted through multiple cell divisions. Epigenetic control of gene expression in multicellular organisms is carried out by Polycomb group (PcG) and Trithorax group (TrxG) proteins [1–5]. Dysregulation of PcG and TrxG systems leads to developmental abnormalities and cancer [6–8].

PcG and TrxG proteins were initially identified in *Drosophila* as regulators of the HOX gene expression [9–12]. HOX genes encode transcription factors that control proper body segmentation. Subsequent studies showed that PcG and TrxG proteins target many genes involved in various cellular processes [13–18].

PcG and TrxG proteins act antagonistically: PcG proteins repress, while TrxG proteins activate gene transcription [2–5, 19].

It was demonstrated that, in *Drosophila*, PcG and TrxG proteins communicate with specialized DNA elements termed PREs (Polycomb Response Elements) [20–22]. PREs were shown to be involved in maintaining proper spatial and temporal gene expression patterns during development. Moreover, for some PREs, the ability to switch the activity from silencing to activation was demonstrated in the transgenic *Drosophila* model systems. However, at present the molecular mechanism of the PRE activity switch is not established [22].

Detailed analysis of PcG and TrxG proteins showed that many of them function together in multisubunit complexes [1, 5, 23]. It was demonstrated that the activities of PcG and TrxG proteins are opposite suggesting the presence of direct competition between the proteins of these two groups [22]. However, many aspects of the interactions between PcG and TrxG factors, as well as their functioning, remains unclear.

This review is devoted to analysis of functional activities of proteins PcG/TrxG and their role in the maintenance of the gene expression profile in *Drosophila*.

IDENTIFICATION OF PcG AND TrxG FACTORS

PcG factors were identified by mutations that caused characteristic phenotypes indicating derepression of HOX genes. In *Drosophila*, HOX genes are organized in two complexes: *Antennapedia* (ANT-C) (the *labial* (*lab*), *proboscipedia* (*pb*), *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*) genes) and *Bithorax* (BX-C) (the *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), *Abdominal-B* (*Abd-B*) genes) (Fig. 1a).

The combination of transcription factors encoded by maternal, gap, pair-ruled, and segment polarity groups of genes subdivides the embryo into 14

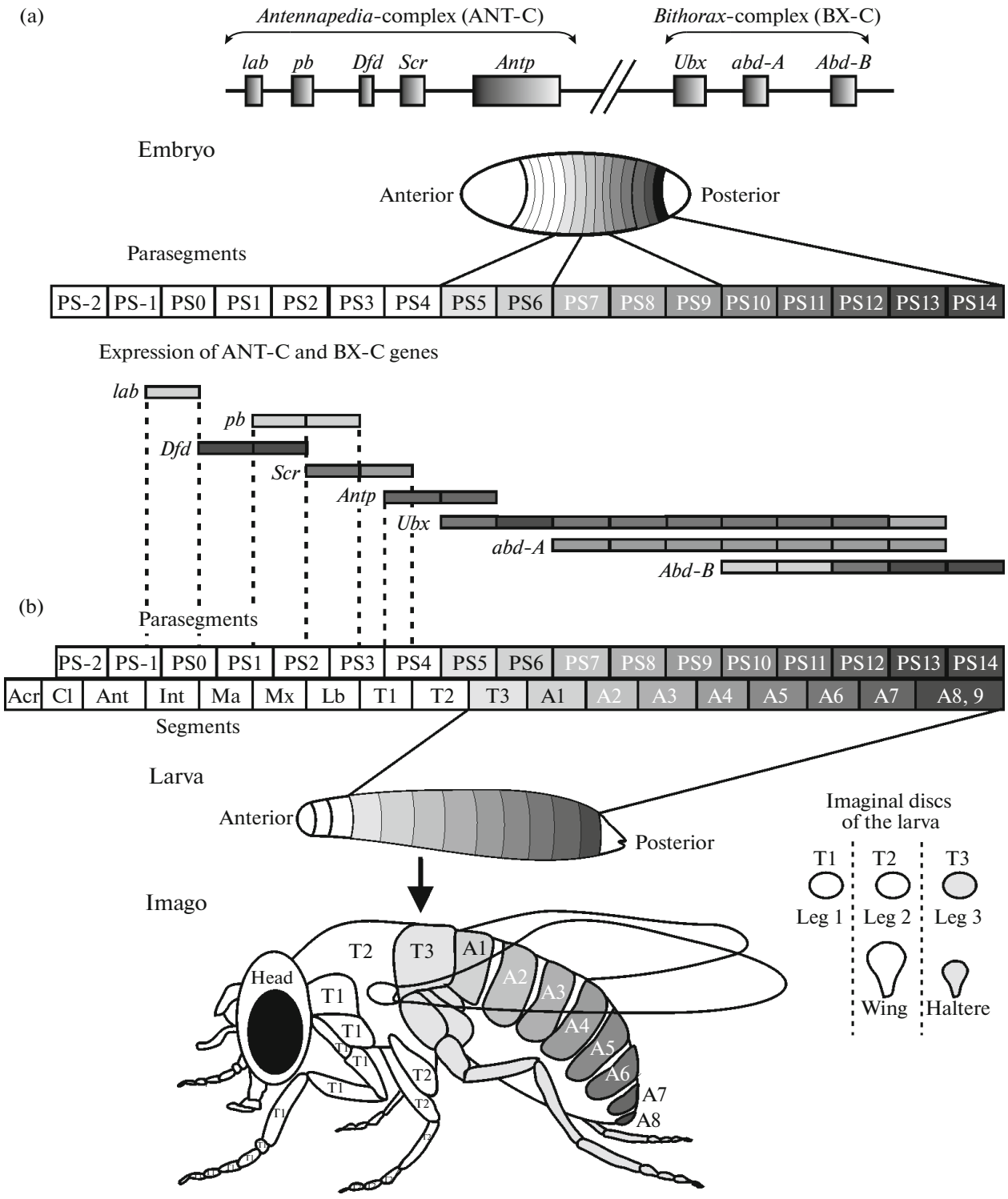


Fig. 1. HOX genes of *Drosophila* regulate proper segmentation. (a) Structure of HOX loci in *Drosophila*. ANT-C complex (*lab*, *pb*, *Dfd*, *Scr*, and *Antp* genes); BX-C complex (*Ubx*, *abd-A*, and *Abd-B* genes). Gene expression in epidermis relative to the parasegments of the embryo is demonstrated. (b) Correspondence of the embryo parasegments to the segments of the larva and imago. Each segment is formed from the parts of two parasegments. Right panel, the correspondence of some imaginal discs to the segments. PS, parasegment; Acr, acron; Cl, clypeus; Ant, antennae; Int, intercalary segment; Ma, mandible; Mx, maxilla; Lb, labrum, T, thorax; A, abdomen.

parasegments and sets the initial HOX gene transcription pattern [24, 25]. At later stages, the established HOX gene expression pattern is maintained by PcG and TrxG proteins [1–5].

In the epidermis, the genes of the ANT-C complex are expressed in anterior parasegments (PS) up to PS5 (PS-1–PS5); genes of the BX-C complex are expressed in parasegments 5 to 14 (PS5–PS14). The *lab* gene is active in PS-1–PS0; *pb*, in PS1–PS3; *Dfd*, in PS0–PS2; *Scr*, in PS2–PS4; *Antp*, in PS4–PS5 [26, 27]; the *Ubx* gene is active in PS5–PS13; the *abd-A* gene, in PS7–PS13; the *Abd-B* gene, in PS10–PS14 [28–31] (Fig. 1a).

The parasegments of embryos give rise to the segments of imago that partially correspond to parasegments. Each segment is formed from the parts of two parasegments (Fig. 1b). Thus, ANT-C is necessary for proper development of the anterior part of the body, and BX-C, of the posterior part.

Classical PcG phenotype arising from the derepression of the BX-C genes is the transformation of anterior abdominal segments toward posterior ones, which is identified by the analysis of lethal mutations at the embryonic stage of development [10]. In addition, PcG-dependent developmental defects can be detected by weak homeotic transformations in heterozygous adult flies that may be enhanced by a combination of different PcG gene mutations. Classical phenotype is partial transformation of the second and third pairs of legs into the first one resulting from the derepression of the ANT-C *Scr* gene [32]. The *Polycomb* (*Pc*) gene was identified first [11], and the whole group was named after it. At present, more than 20 PcG genes have been characterized (table), the mutations in which either lead to PcG phenotypes or enhance the *Pc* mutation or the mutations in other PcG genes [9–12, 32–49]. Most of these mutations were confirmed to cause derepression of HOX genes [34–36, 41, 44, 48, 50–55].

Simultaneously with the PcG factors, a number of genes, mutations in which led to the phenotypes indicating the loss of HOX genes activity and behaved opposite to the PcG mutations or suppressed the phenotypes associated with the mutations in the PcG factors, were identified. These factors were assigned to the TrxG group [32, 56–60] (table). Classical TrxG phenotypes include partial transformation of the first pair of legs into the second ones due to the decrease in the *Scr* gene expression and the transformation of posterior abdominal segments into anterior ones due to the decrease in the BX-C gene expression. For some of the factors, it was demonstrated that they were involved in the activity of both groups (table).

PREs ARE THE TARGETS OF PcG AND TrxG FACTORS AND PROVIDE THE MEMORY OF INACTIVE GENE STATE

Analysis of functional activities of the *Drosophila* PcG and TrxG gene products showed that they are associated with the chromatin and have targets in the regulatory regions of HOX genes. These target elements were called PREs [20–22].

Most of the data on the PRE function were obtained by the analysis of the BX-C locus [61]. BX-C is divided into nine regulatory domains, in eight of which the presence of functionally active PREs was confirmed in the transgenes [62–70] (Fig. 2a). In addition to PREs, all domains contain enhancers required for the gene activity. Enhancers are DNA regulatory elements that activate transcription [71]. The *abx/bx* and *bxd/pbx* domains contain the *Ubx* gene enhancers; the *iab-2*, *iab-3*, and *iab-4* domains include the *abd-A* gene enhancers; the *iab-5*, *iab-6*, *iab-7*, and *iab-8, 9* domains control the *Abd-B* gene expression [72].

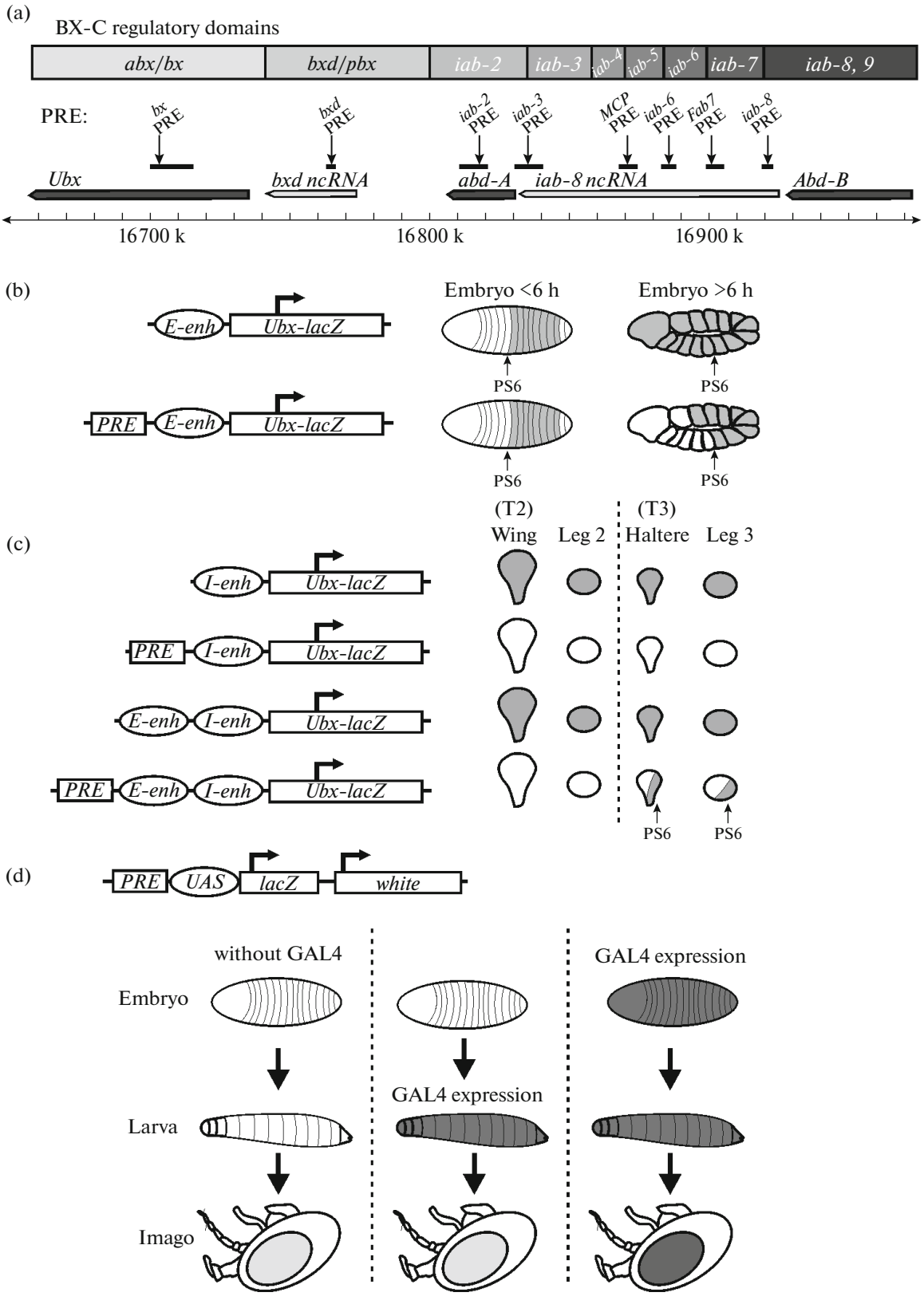
Analysis of the embryonic enhancers in transgenic constructs showed that they exhibit parasegment-specific activity only at the early stages of embryogenesis (between 0 and 6 h of development) (Fig. 2b). After six hours, these enhancers become activated also in the parasegments where they are normally silent. However, if an enhancer in the transgene is supplemented with PRE, the correct pattern of the enhancer activity will be maintained until late stages of embryogenesis (Fig. 2b). Thus, PREs are memory elements ensuring gene silencing in the appropriate cells.

The property to maintain the pattern of gene expression was demonstrated for the PRE from the *Ubx* regulatory region [66]. Later, this property was shown for other PREs of HOX genes from the BX-C [63, 65, 69, 70] and ANT-C [73] regulatory domains.

By replacing PREs and enhancers in the transgenes, it was demonstrated that enhancer determines the region of gene expression. PREs lack predetermined tissue specificity, but provides the memory of the segments in which enhancer was inactive [64, 65, 69, 70]. In addition, PRE from the *engrailed* regulatory region was able to maintain the pattern set by the enhancer from the *Ubx* regulatory region [74], and *bxdPRE* from the *Ubx* regulatory region maintained the pattern of *engrailed* enhancer [75].

The PRE-mediated control of activity was also demonstrated for the imaginal disc enhancers of the BX-C. It was shown that PREs are able to transfer the proper activity pattern from embryonic enhancers to the imaginal disc enhancers [64, 76, 77] (Fig. 2c).

In the transgenes, imaginal disc enhancers isolated from other regulatory elements are not active during embryogenesis, while they uncontrolledly activate expression of the reporter gene at the larval stage. At the same time, these enhancers do not determine the pattern of reporter gene activity and in the presence of


(a)
BX-C regulatory domains
abx/bx
bxd/pbx
iab-2
iab-3
iab-4
iab-5
iab-6
iab-7
iab-8, 9
PRE:
bx PRE
bxd PRE
iab-2 PRE
iab-3 PRE
MCP PRE
iab-6 PRE
Fab7 PRE
iab-8 PRE
Ubx
bxd ncRNA
abd-A
iab-8 ncRNA
Abd-B
16700 k
16800 k
16900 k
(b)
Embryo <6 h
Embryo >6 h
E-enh
Ubx-lacZ
PRE
PS6
(c)
(T2)
(T3)
Wing
Leg 2
Haltere
Leg 3
I-enh
(d)
PRE
UAS
lacZ
white
without GAL4
GAL4 expression
Embryo
Larva
Imago

PREs they are inactive. Only in the presence of embryonic enhancers and PREs the proper spatial activation of transcription by imaginal disc enhancers is established [64, 76, 77].

Thus, PREs provide the memory of inactive gene state silencing the enhancers in the required cells.

PREs PROVIDE THE EPIGENETIC MEMORY OF ACTIVE GENE STATE

The further studies using transgenic model systems showed that the influence of a number of PREs on the target genes could be switched from silencing to transcriptional activation. This was achieved by a combination of PREs within the transgene either with enhancers or with the binding sites for the yeast GAL4 activator [78–84].

In the GAL4 system, binding sites for this factor are placed near PRE upstream of the reporter genes. In the absence of GAL4 activator, PRE silences the reporter expression. However, silencing is relived when GAL4 is expressed. When GAL4 expression is shutoff, PRE-mediated silencing is reestablished.

The functional properties of some tested PREs were different. Specifically, the activation did not disappear when GAL4 expression was shutoff (Fig. 2d). First this property was demonstrated for the 3.6-kb *Fab7*PRE of the BX-C [78]. The *Fab7*PRE maintained activation of the reporter genes in the transgene that was epigenetically transmitted through mitosis (during fly development) and meiosis (in the next generation). This effect was observed upon the induction of GAL4 expression at the embryonic, but not at the larval stage of development (Fig. 2d). When GAL4 expression was induced at the larval stage, only temporal switch of the PRE activity occurred, which was not preserved until the adult stage. Thus, PRE is characterized by higher plasticity at the embryonic stage of development. The *Fab7*PRE was named "cellular memory module" (CMM). The CMM activity was demonstrated for other PREs, including different fragments of *Fab7*, 4.5 kb *MCP*, 2.3 kb of the *bxd* BX-C, and 3.4 kb *hh*PRE of the *hedgehog* locus [80, 82, 84].

However, not all PREs and/or not all PRE fragments able to repress transcription could possess CMM activity [81, 84, 85]. Probably, the CMM activity of PRE requires the presence of certain regulatory modules [81, 84]. Potentially DNA regulatory modules required for the CMM activity of PRE can be represented by enhancers and insulators. Insulators are DNA regulatory elements capable of modulating the activity of both enhancers and PREs through spatial interactions [72, 86, 87]. It was shown that enhancers and insulators are present in at least three PREs of BX-C, including 3.6 kb *Fab7PRE*, 4.5 kb *MCP*, and 2.3 kb *bxd*PRE [76, 78, 88].

Furthermore, the presence of CMM activity depends on the exact PRE DNA fragment taken in the analysis. For instance, the 219-bp *Fab7*PRE, exhibiting the CMM activity [80], is part of the 1.8-kb *Fab7*PRE, which doesn't have CMM [84]. At the same time, both of these elements are able to repress the reporter gene expression [80, 84]. This fact may point to the role of the PcG and TrxG protein concentrations and their competition with each other in the CMM effect.

Thus, PREs coupled to other sequences are switchable from silencing to activation and can maintain both silencing and activation of target genes through cell divisions.

THE IMPACT OF ENDOGENOUS REGULATORY DNA ELEMENTS ON PRE ACTIVITY IN THE TRANSGENES

In addition to the interplay between the regulatory elements in the transgenes, PREs are heavily influenced by the surrounding chromatin, and the resultant activity of PREs and memory of inactive state depends on the genome integration site of the construct [69, 70, 76]. Depending on the integration site, PRE can both repress and activate transcription [77, 85, 89]. Analysis of the transgene *en*PRE from the *engrailed* locus demonstrated that this PRE functionally interacted with endogenous enhancers and transferred their activity pattern on the reporter gene [85]. In

Fig. 2. Functional analysis of the BX-C PREs. (a) Structure of BX-C. Black arrows indicate the *Ubx*, *abd-A*, and *Abd-B* genes; gray arrows, long noncoding transcripts of *bxd ncRNA* and *iab-8 ncRNA*. On the top are the regulatory domains of the locus (*abx/bx*, *bxd/pbx*, *iab-2*–*iab-9*) and PREs, functionally confirmed in the transgenes (*bx*PRE, *bxd*PRE, *iab-2*PRE, *iab-3*PRE, *MCP*-PRE, *iab-6*PRE, *Fab7*PRE, *iab-8*PRE). (b) Epigenetic memory of repression at the stage of embryo development [66]. Left panel, schematic representation of the transgenic constructs used in the study. Rectangle *Ubx-lacZ*, the *lacZ* gene under control of *Ubx* promoter; the arrow at the top designates the direction of transcription. E-enh, embryo enhancer. In the absence of PRE, *Ubx-lacZ* at the late embryo stage of development is expressed in all segments; in the presence of PRE, the expression segments (PS6–PS13) correspond to those set at the early stage of embryo development. (c) Epigenetic memory of repression at larval stage [76]. In the absence of PRE, imaginal enhancer (I-enh) is active in all discs. In the presence of PRE, imaginal enhancer is inactive. The combination of PRE, I-enh, and E-enh leads to the required pattern of the *Ubx-lacZ* activity. (d) Epigenetic memory of activation (CMM). In this system [78], in addition to *lacZ* (under control of minimal promoter of *hsp70* gene), the second reporter, *white* gene controlling eye pigmentation, was used. UAS, binding sites of the yeast GAL4 activator protein. In the case of PRE in repressing state (without GAL4), the reporter genes are inactive. Expression of GAL4 activator in transgene at the larval stage is accompanied by temporal switch in PRE activity state, which is inherited untill the imago. The expression of GAL4 at embryo stage can mediate the heritable switch in the activity of some PREs that leads to activation of reporter genes at adult stage of development.

PcG and TrxG factors confirmed in the genetic tests by the presence of the homeotic transformations and/or by direct analysis of HOX gene transcription

Gene	Group	Reference	Gene	Group	Reference
E(z) (*Enhancer of zeste, pco*)	PcG	44, 46, 54	*trx* (*trithorax*)	TrxG	32, 60, 168
esc (*extra sex combs*)	PcG	12, 54, 55	*osa*	TrxG	32
Su(z)12 (*Suppressor of zeste 12*)	PcG	34	*mor* (*moira*)	TrxG	32
Caf-1 (*Chromatin assembly factor 1*)	PcG/TrxG	107	*brm* (*brahma*)	TrxG	32
Pcl(*Polycomblike*)	PcG	9, 10, 32, 37, 50, 54, 111	*Snr1* (*Snf5-related 1*)	TrxG	56
Rpd3 (*HDAC1, Histone deacetylase 1*)	PcG	36	*e(y)3* (*enhancer of yellow 3, SAYP*)	TrxG	154
Sir2 (*Sirt1, Sirtuin 1*)	PcG	40	*nej* (*nejire, dCBP*)	TrxG	168
escl (*esc-like*)	PcG	48	*Dll* (*Distal-less, Brista*)	TrxG	32
Pc (*Polycomb*)	PcG	11, 32, 42, 50, 54	*btl* (*breathless, devenir*)	TrxG	32
ph (*polyhomeotic*)	PcG	38, 42, 50, 54	*vtd* (*verthandi, Scc1*)	TrxG	32
Sce/dRing (*Sex combs extra*)	PcG	9, 42, 50, 54	*Vha55* (*l(3)87Ca*)	TrxG	32
Psc (*Posterior sex combs*)	PcG	10, 41, 42, 45, 50, 54	*sls* (*sallimus*)	TrxG	32
Su(z)2 (*Suppressor 2 of zeste*)	PcG	42, 50	*Bre1*	TrxG	39
Scm (*Sex comb on midleg*)	PcG	9, 10, 32, 50, 54	*lid* (*little imaginal discs*)	TrxG	57
Kdm2 (*Lysine* (*K*)*-specific demethylase 2*)	PcG	39	*tna* (*tonalli*)	TrxG	58
jing	PcG	133	*kis* (*kismet*)	TrxG	32
Asx (*Additional sex combs*)	PcG	9, 10, 41, 45, 50, 54	*ash1* (*absent, small, or homeotic discs 1*)	TrxG	60
Calypso	PcG	41	*ash2* (*absent, small, or homeotic discs 2*)	TrxG	60
crm (*cramped*)	PcG	49	*lolal* (*lola like*)	PcG/TrxG	153
sxc/Ogt (*super sex combs*)	PcG	43, 52	*siren1/kto/med12*	PcG/TrxG	32, 41
mxc (*multi-sex combs*)	PcG	47	*siren9/skd/med13*	PcG/TrxG	32, 41
dom (*domino*)	PcG	149	*RYBP* (*Ring and YY1 Binding Protein*)	PcG/TrxG	33, 39
dSfmbt (*Scm-related gene containing four mbt domains*)	PcG	53	*corto*	PcG/TrxG	150
			E(Pc) (*Enhancer of Polycomb*)	PcG/TrxG	57, 152
PcG and TrxG factors with DNA-binding activity					
Trl (*Trithorax-like, GAF*)	PcG/TrxG	180, 181			
Psq (*pipsqueak*)	PcG	181, 182			
pho (*pleiohomeotic, l(4)29*)	PcG	9, 51, 54			
phol (*pho-like*)	PcG	35			
Dsp1 (*Dorsal switch protein 1*)	PcG/TrxG	192			
fs(1)h (*female sterile* (*1*) *homeotic*)	TrxG	60			
Adf1 (*Adh transcription factor 1*)	PcG	91			
Spps (*Sp1-like factor for pairing sensitive-silencing*)	PcG	199			

addition to the interaction of PREs with enhancers, the activity of PREs is affected by their ability to interact with other PREs and with the promoters. The presence of direct contacts between PREs and the target gene promoters is evident by copurification of the promoter factors and PcG factors [90]; the presence of the promoter factors on PREs [91, 92]; and the direct detection of the contacts using the methods of chromatin architecture analysis [93–95].

One of the most demonstrative examples of functional interactions between PREs is the PSS effect (pairing sensitive silencing). The PSS effect is the ability of PRE copies in homozygote to interact with each other, which leads to the increased repression of the reporter genes in transgens [96]. The PSS effect is a characteristic feature of most, if not all, PREs [20]. Increased repression can also be observed as the result of long-distance interactions between PREs (3.6-kb *Fab*7PRE) placed at different genomic locations [97].

The ability of DNA elements to interact underlies the "homing" effect: the high frequency of the integration into the genome region near the endogenous copy of the element present in construct [98]. Homing was shown for PREs containing the DNA fragments from the *engrailed*, *even-skipped*, and BX-C loci [99, 100]. It is suggested that microinjection of plasmid DNA into embryonic nuclei is accompanied by the assembly of protein complexes on transgenic DNA. The assembled protein factors are proposed to interact with similar complexes in the genome and to be physically recruited to a particular locus, near which the integration then takes place. It should be noted that, at least in some cases, homing might depend on the presence of insulators in the transgenes [101].

Nuclear staining with antibodies against PcG proteins detects individual speckles of these proteins, called Pc bodies [102]. It is suggested that the genes repressed by the PcG proteins spatially move into the Pc bodies, and under derepression, on the contrary, they leave these structures. Thus, PREs could be actively involved in the formation of spatial interactions in the nucleus, which can facilitate the process of transcriptional repression.

Thus, PREs and/or other DNA regulatory modules within them interact with genome regulatory elements, which may also affect the result of functional analysis of PREs in the transgenes, integrated at random positions into the genome. Further analysis of the PREs properties and CMM using the insertion systems based on the attB/attP, which makes it possible to integrate different construct variants in the same genome sites, as well as the analysis of the PRE properties at the endogenous level using local deletions and substitutions of the DNA fragments, seems to be reasonable.

THE POLYCOMB GROUP PROTEINS

Currently, it is demonstrated that most of the PcG factors identified genetically act together in multisubunit protein complexes. The main characterized complexes are PRC2, PRC1, dRAF, PR-DUB, and PhoRC [1, 2, 4, 23] (Fig. 3).

The PRC2 (Polycomb repressive complex 2) with the size of about 650 kDa contains E(z), Esc, Su(z)12, and Caf1 core subunits [103–106]. The E(z), Esc, and Su(z)12 function as PcG factors (table). The phenotypes of flies carrying the *Caf1* mutation correspond to the derepression of HOX genes; at the same time, the *Caf1* mutation suppresses *Pc*, indicating the role of Caf1 also in the activation [107]. The PRC2 trimethylates histone H3 at lysine 27 (H3K27me3) via the SET domain of the E(z) protein [103–106]. The H3K27me3 modification is a characteristic mark of the chromatin regions repressed by the PcG [17, 92]. The E(z) protein alone has only weak methylation activity, which greatly increases (by approximately 1000 fold) in association with other PRC2 components [106, 108, 109]. The inactivation of the E(z) leads both to the gene derepression [44, 54, 106] (table) and to disruption of the H3K27me3 modification [110, 111]. Similar, the absence of the H3K27me3 modification upon the substitution of lysine for arginine at position 27 of the third histone also leads to derepression of the PcG targets in the imaginal discs [112].

In addition to core components, noncanonical subunits Pcl [111, 113, 114], Sir2 [40], Escl [115], and Rpd3 [104, 114], which act as PcG in the genetic tests (see table), could be purified together with PRC2. Escl is a homolog of Esc; it can functionally replace Esc within PRC2 [48, 115–117].

The results of chromatography and immunoprecipitation demonstrate that, at the early stage of embryo development (0–16 h), Pcl together with the core subunits of PRC2 form the 1-MDa complex, which also includes Rpd3 [114]. It was demonstrated that, in the genome, Pcl is colocalized with Rpd3 [114] and with the PRC2 components [92]. Moreover, it was found that Pcl is essential for the repression of HOX genes (table) and for the high level of H3K27me3 in vivo [111]. This suggests that the Pcl-containing PRC2 is the main complex, necessary for the repression at the early stage of development. Rpd3 participates in the repression of HOX genes (table). Functionally, this factor is a histone deacetylase and deacetylates H3K27ac, removing the modification, which is characteristic mark of active chromatin [118]. At embryo stage a PRC2 complex purified using antibodies directly against the Pcl contains Su(z)12, E(z), Caf1, and Esc. This complex is named Pcl-PRC2. Rpd3 is not detected upon purification, indicating weaker association of the histone deacetylase with other PcG factors [111].

At the larval stage, Pcl forms a 1.5-MDa complex; however, its subunit composition is still unknown

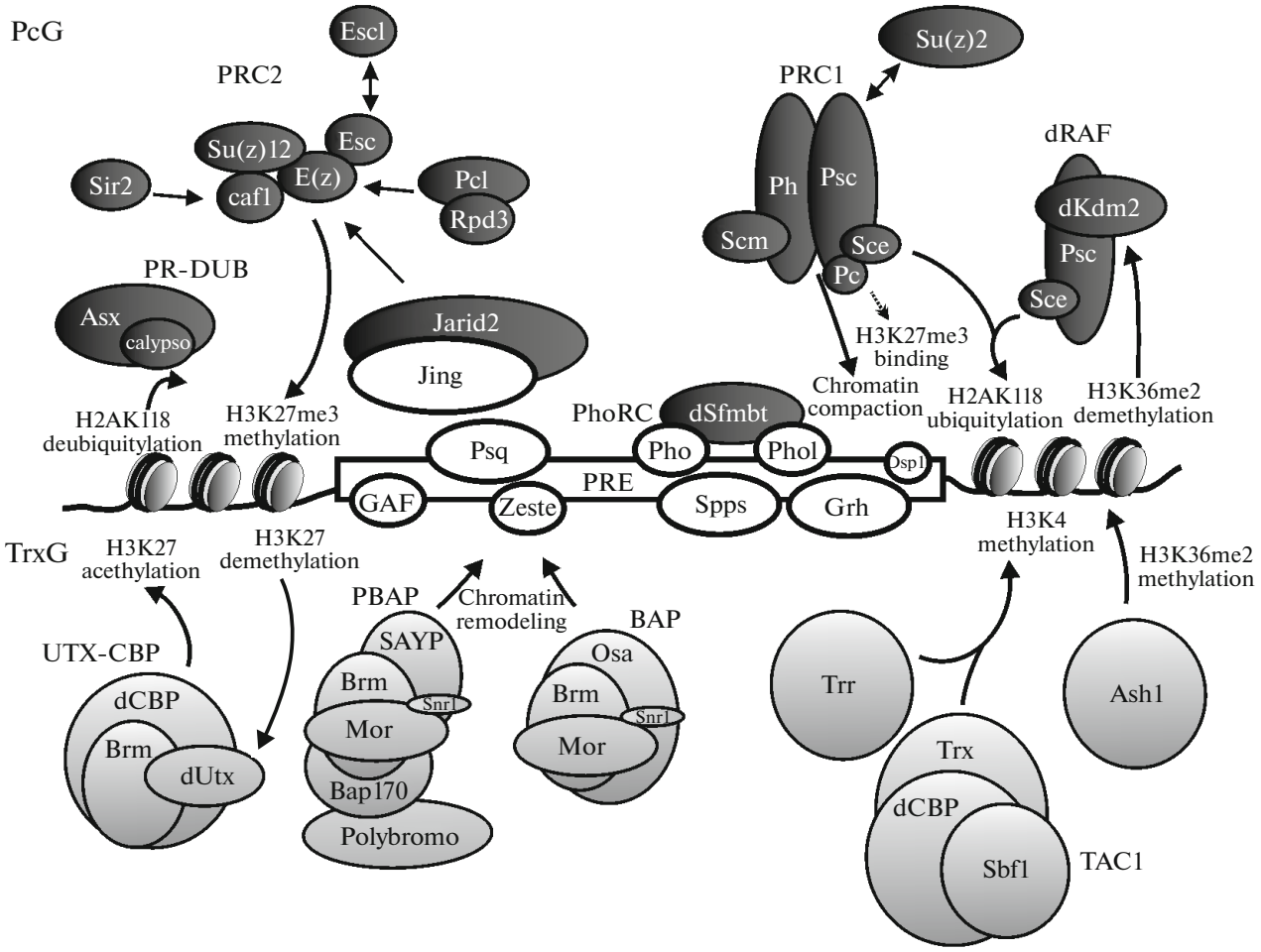


Fig. 3. Schematic representation of the Polycomb group and Trithorax group factors and their activities. PRE (white rectangle), the DNA sequence depleted of nucleosomes (gray tilted ovals) and serving as a landing platform for the DNA-binding proteins (marked in white): Psq, GAF, Zeste, Pho, Phol, Spps, Grh, Dsp1, and, presumably, Jing. Pho and dSfmbt form the PhoRC PcG complex. In addition, Phol, independently of Pho, can interact with dSfmbt. Other PcG complexes: PRC1, PR-DUB, dRAF, PRC2. An arrow pointing to the complex shows the components of alternative variants of complex. The factors Su(z)2 (homolog of Psc) and Escl (homolog of Esc) can functionally replace Psc and Esc, respectively, which is indicated by double-headed arrow. The arrows leading to and from the nucleosomes indicate the main activities of PcG complexes: deubiquitination of H2AK118 by PR-DUB complex; H3K27-trimethylation by PRC2 complex; interaction with H3K27me3; compaction of nucleosomes and blocking of chromatin remodeling by PRC1 complex; H2AK118 ubiquitination by PRC1 and dRAF complexes (this activity is significantly higher in dRAF complex); H3K36me2 demethylation by dRAF complex. At the bottom of the scheme in gray color are the main representatives of Trithorax group and their activities. Complexes: PBAP, BAP, UTX-CBP, TAC1; factors: Trx and Ash1. Functional activities: chromatin remodeling by PBAP and BAP complexes; H3K27 acetylation by dCBP factor; demethylation of H3K27 by dUtx factor; H3K4 methylation by Trr and Trx factors; H3K36me2 methylation by Ash1 factor.

[113]. In the larvae, the PcG system functions with participation of histone deacetylase Sir2, which forms a 3-MDa complex with E(z) [40]. However, unlike Rpd3, Sir2 does not affect the H3K27ac level, and elucidation of its functions needs further studies [118].

The 2-MDa PRC1 (Polycomb repressive complex 1) includes core subunits Pc, Ph, Sce/dRing, and Psc [90, 119, 120]. Under certain purification conditions, PRC1 is co-isolated with Su(z)2, which is the homolog of Psc [121, 122]. In functional tests, all these factors behave as PcG (table).

The PRC1 core complex is responsible for chromatin compaction, inhibition of nucleosome remodeling, and transcription silencing [119, 120, 123–125]; the main role in these processes is played by Psc and Su(z)2 [123–126].

The Pc protein of PRC1 interacts with the H3K27me3 histone modification via chromodomain [127, 128]. It was previously suggested that the PRC2-dependent recruitment of PRC1 could take place. Specifically, PRC1 was proposed to be recruited via recognition of H3K27me3 modification mediated by PRC2 [129]. However, it was further shown that PRC1 may be recruited to chromatin also in the absence of H3K27me3 [116, 130]. Thus, at least in some cases, this order of recruitment is not followed and

H3K27me3 is not the determining factor responsible for the PRC1 binding.

The Sce protein catalyzes ubiquitination of histone H2A at position 118 (H2AK118ub). This activity of Sce is much higher not within PRC1, but within alternative complex, named dRAF (dRing Associated Factors). The dRAF complex also contains Psc and the Kdm2 demethylase. Kdm2 demethylates histone H3 at lysine 36 (H3K36me2), removing the modification associated with the active gene state [131].

It was demonstrated that the H2AK118ub modification is recognized by the alternative PRC2 complex, JARID2–AEBP2–PRC2 [132], which contains the Jarid2 and Jing/AEBP2 proteins [133]. It was shown that this complex interacts with H2AK118ub and stimulats the formation of H3K27me3 on the H2AK118ub nucleosomes in vitro. The existence of a positive feedback (PRC1 recognizes H3K27me3 and forms H2AK118ub; H2AK118ub stimulates H3K27me3, which facilitates the PRC2 binding; PRC2 forms H3K27me3) was proposed [132]. However, the *Jarid2* mutation has little effect on the level of H3K27me3 in vivo [134]. Moreover, the removal of Sce and H2AK118ub does not affect the H3K27me3 level in vivo [42] and the PcG-dependent repression of HOX genes [135]. Furthermore, although Jarid2 colocalizes with the PRC2 components, no recruitment of Jarid2 is observed on many of PREs enriched in PRC2 [134], suggesting that JARID2–AEBP2–PRC2 can participate in the activity of only a limited number of PcG/TrxG targets.

The PR-DUB complex (Polycomb repressive deubiquitinase) contains the Calypso and Asx proteins [136]. Both factors were genetically approved to act as PcG (table). The Calypso protein is the histone H2AK118 deubiquitinase. The catalytic activity of this enzyme greatly increases in the presence of the Asx protein [136]. Thus, this complex is able to remove H2AK118ub, the modification catalyzed by the Sce protein. The role of the simultaneous presence of ubiquitinase and deubiquitinase activities in the PcG system remains unknown. It is suggested that cyclic introduction and removal of the H2AK118ub modification is necessary for effective regulation of PcG-mediated repression.

The PhoRC (PHO recognition complex) includes the dSfmbt and Pho proteins [53]. dSfmbt contains four MBT domains capable of interacting with the methylated lysines in the N-termini of histones H3 and H4 [53, 137]. Pho is the best studied PcG DNA-binding protein [51, 138]. The Pho binding sites are found in many PREs and are necessary for their functioning in transgenic constructs [35, 51, 68, 139–142]. It was demonstrated that, in the genome, dSfmbt and Pho are colocalized with PRC1 and PRC2 [15, 53, 92]. Moreover, Pho is detected in many binding peaks of Pc and Ph (from 50 to 96%) [13, 16]. The dSfmbt and Pho proteins are required for proper expression of HOX genes (table).

In *Drosophila*, a homolog of Pho protein, Phol, was found. Pho and Phol bind to the same DNA site [35]. The *phol* mutants are alive in the homozygote and have no homeotic transformations. However, simultaneous inactivation of *pho/phol* enhances the *pho* phenotypes and the HOX gene derepression (table). Both factors, Pho and Phol, directly interact with dSfmbt; however, these interactions are mutually exclusive [53]. Moreover, Pho/Phol do not always work together since their genomic distribution differs. Phol to a lesser extent colocalizes with PcG, as only 21% of the Ph/Pc binding sites contain Phol [16].

Pho/Phol directly interact with the components of other PcG complexes. Pho is able to interact with E(z) [53, 129], Esc [129], Ph, and Pc [143], while Phol interacts with Esc [129]. It is demonstrated that Phol and Pho participate in the recruitment of E(z) and Pc to *bxdPRE* of the BX-C [129]. At the same time, simultaneous inactivation of *pho/phol* leads to elimination Pc, Psc, Scm, E(z), and Ph binding only at few sites of polytene chromosomes, suggesting the presence of additional PcG recruiters [35].

The PhoRC [121] and PRC1 [90, 121] complexes can be copurified together with the Scm protein. In the in vitro experiments, Scm is able to interact directly with Ph [144] and dSfmbt [137]. Scm contains two MBT domains that, as is the case with dSfmbt, are able to interact with the methylated lysines at the N-termini of histones [145]. At the same time, Scm doesn't belong to the core components of PRC1, PRC2, or PhoRC in vivo [53, 90, 146, 147] and is recruited to the chromatin regardless of them [148]. Factors recruiting Scm to chromatin are not found.

In addition to the described above, a number of PcG proteins are known to be involved in the repression of homeotic genes, including Sxc/Ogt [52], Mxc [47], Dom [149], and Crm [49]. It is known that Sxc/Ogt is the O-GlcNAc transferase that can modify Ph [52].

Several factors appear to be closely involved in the functioning of both the PcG and TrxG systems, including RYBP [33, 39], Corto [150, 151], E(Pc) [57, 152], Lolal [153], Kto/Med12, and Skd/Med13 [32, 41]. It is shown that artificial recruitment of RYBP to the transgene leads to the reporter gene repression, that depends on the Pc, Sce, and Pho factors. Moreover, overexpression of RYBP leads to the *Ubx* repression in a part of the haltere discs, where it is normally active [33]. In another study, it is demonstrated that RYBP copurifies with the Sce and Kdm2 proteins, but at the same time, RYBP interacts with the Bre1 activator and in the genetic tests acts also as the TrxG factor [39]. For the Corto factor the interaction with E(z), Esc, Ph, Scm, GAF proteins and colocalization with them at some sites of the polythene chromosomes, is demonstrated [151]. In the genetic tests, Corto acts as

both PcG and TrxG factors [150]. Kto/Med12 and Skd/Med13 were originally discovered as the suppressors of the *Pc* mutation [32]. However, at the same time, mutations in these factors cause the *Ubx* derepression [41]. Kto/Med12 and Skd/Med13 are the components of the Med12-Med13-Cdk8-CycC repressor submodule of the Mediator multisubunit complex. Due to the fact that Cdk8 and CycC do not genetically act as PcG factors, it is suggested that participation of Kto/Med12 and Skd/Med13 in the activity of the PcG system is independent of their association with the Cdk8-CycC components of Mediator [41].

THE TRITHORAX GROUP PROTEINS

A number of genes the mutations in which lead to the suppression of the PcG phenotypes were identified in the genetic tests. These genes were assigned to the Trithorax group (TrxG), named after the founding member of this group. Further studies demonstrated that TrxG proteins are a heterogeneous group (Fig. 3), which includes the components of the chromatin remodeling (Brm, Osa, Mor, Snr1, Kis) and histone methyltransferase (Trx, Ash1) complexes, the subunits of the Mediator complex (Kto, Skd), a subunit of the cohesin complex (Vdt), etc. [19].

It was demonstrated that TrxG proteins identified genetically, Osa, Brm, Mor, and Snr1, are subunits of BAP (Brahma-associated proteins) and/or PBAP (polybromo-associated BAP) [154, 155] chromatin remodeling complexes. Mor [32, 156], Snr1 [56, 154], and ATPase Brm [32, 157–159] are members of both complexes. The Osa protein is a specific component of the BAP complex [32, 160]. The Polybromo, BAP170, and SAYP TrxG factors are subunits specific to PBAP complex [154].

Trithorax (Trx) methylates the fourth lysine of the third histone, being responsible for the H3K4me1 modification. In addition to Trx, the H3K4me1 modification is mediated by the Trx-related factor (Trr) [161]. Trx via Taspase 1 is cleaved into N- and C-terminal fragments [162], because of which the catalytic SET domain required for introduction of H3K4me1 remains in Trx-C [161]. The Trx-N and Trx-C show different genome distribution. The Trx-N localization is well correlated with active chromatin, whereas Trx-C colocalize with both active chromatin and PcG factors in inactive loci [16, 163]. Association of the Trx-C catalytic fragment with inactive loci is unclear. It is likely that Trx is functionally active only in association with other factors activating transcription.

For instance, it was demonstrated that Trx in co-IP and in vitro interacts with the SET domain of the Ash1 TrxG protein [164]. Ash1 is the lysine methyltransferase which methylates histone H3 at lysine 36 (H3K36me1/2) [165, 166]. It is demonstrated that Ash1 colocalizes with Trx in active loci [163].

Trx and Trr factors are the subunits of different dCompass-like complexes; the common subunit of which is the Ash2 TrxG protein [167].

Trx is also a component of the TAC1 complex containing the dCBP and Sbf1 proteins [168]. The dCBP protein is acetyltransferase, which, in particular, modifies histone H3 at Lys27 (H3K27ac) [118] and marks active enhancers and promoters [169]. It was demonstrated that Trx directly interacts with dCBP via the region near the SET domain [161]. These factors (Trx and dCBP), H3K4me1 and H3K27ac modifications were found to colocalize at many genomic sites [161, 163]. It was demonstrated that effective acetylation of H3K27ac requires Trx, Trr, and H3K4me1 [118, 161, 170], and the recruitment of Trx depends on dCBP [171]. In addition to Trx, dCBP interacts with other TrxG factors, among which are dUtx, Brm (UTX-CBP complex) [172], and Ash1 [173]. The dUtx protein is the H3K27me2/3 demethylase [174].

It was demonstrated that the Kismet (Kis) TrxG factor is necessary for recruitment of Trx and Ash1 [175]. Kis stimulates methylation activity of the Ash1 factor with respect to H3K36 modification [165]. The loss of Kis, as well as of Trx and Ash1, leads to the increase in the H3K27me3 level without affecting the recruitment of Pc [175].

It was demonstrated the Lid factor acts as the TrxG in genetic test [57]. However, further analysis showed that the Lid is H3K4me3 demethylase, associated with active transcription [176–178]. The detailed role of this factor in the activity of TrxG/PcG system is unknown.

Thus, TrxG factors represent a heterogeneous group of proteins, many of which can functionally and physically interact with each other.

PRE DNA BINDING FACTORS

Most of the functional tests used the PRE DNA fragments of several kb in size. However, further analysis showed that the core PRE fragments of several hundred bp are sufficient for repression.

The PRE core DNA fragments are depleted of nucleosomes and hypersensitive to nucleases [92, 142, 179]. These DNA fragments contain binding sites for various transcription factors [20, 21]. In *Drosophila*, in addition to the described above Pho/Phol, a number of factors bound to PREs were characterized: GAF, Psq, Grh, Dsp1, Zeste, Adf1, Spps and possibly other members of Sp1/Klf family (Fig. 3).

GAF (GAGA factor) is encoded by the *Trithorax-like* (*Trl*) gene, and in genetic tests, it has been first characterized as the TrxG factor [180]. At the same time, mutations in *Trl* enhance the homeotic phenotype of *ph* [181], and GAF is necessary for the activity of PRE [65]. Psq is the PcG factor. Mutations in Psq enhance the phenotype of *Pc* and *ph* [181, 182]. GAF and Psq proteins bind to the same DNA sequence,

GAGAG [183]. These factors are colocalized on the polythene chromosomes [184]. It was demonstrated that GAF/Psq sites are essential for the activity of PRE [139, 181]. GAF could be found at about 50% of Ph sites [14, 16] and co-immunoprecipitates with Ph and Pc [185, 186]. At the same time, GAF was also isolated with the components of the complexes involved in transcriptional activation: PBAP (TrxG complex) [187], FACT [188], NURF [189]. Furthermore, mutations in the bin1/SAP18 (Sin3-HDAC complex), Dre4/Spt16 (FACT complex), and bip2/TAF3 (TFIID complex) factors enhance the TrxG phenotype of *Trl* [188, 190, 191], suggesting that GAF together with these proteins is implicated in the activity of TrxG system.

Dsp1 affects the activity of both PcG and TrxG. The *Dsp1* mutation enhances the TrxG phenotypes, while overexpression enhances the PcG phenotypes [192]. It was confirmed that Dsp1 is required for repression mediated by some PREs [193]. In the genome, Dsp1 is present at approximately 50% of Ph sites [16]. Grh (Grainyhead) was discovered as a factor bound to the *iab*-7PRE; the *grh* mutation enhances *pho*-mediated derepression of transgene [194].

The Zeste protein is a minor component of PRC1 [90]. In the genome, Zeste is present at about 25% of Ph sites [16]. Phenotypically, Zeste mutations do not show PcG/TrxG phenotypes [195]; however, this factor is involved in the activity of both enhancers [196] and PREs [65]. It was demonstrated that Zeste could directly interact with Ph [197].

The Spps binding sites were found in the analysis of 181-bp PRE from the *engrailed* locus. It was demonstrated that Spps and most other representatives of the Sp1/Klf family could interact with these sites in vitro [198]. It was demonstrated that Spps is involved the PRE activity; moreover, it was found that the Spps mutations enhances the homeotic phenotype of *pho* [199].

Adf1 was originally identified as an activator associated with the gene promoters [200]. However, it was recently demonstrated that Adf1 is present at different PREs and interacts with the Pc protein in the co-immunoprecipitation experiments. Furthermore, Adf1 acts as the PcG factor, since the *Adf1* mutation enhances the phenotype of *Pc* [91].

Some other proteins with DNA-binding domains were copurified with PcG complexes: Br140, Fs(1)h [121, 122], Jing [121, 134], CG9932 [121]. Probably, these factors may also be involved in the recruitment of PcG/TrxG complexes to PREs. The gene encoding Fs(1)h was identified as the TrxG factor in genetic screening (table). It was demonstrated that Fs(1)h interacts with Zeste sites in the *Ubx* promoter and activates transcription of this gene [201]. The recruitment of this factor to PREs was not tested. The mammalian homolog of Jing, AEBP2, interacts with DNA at the sites of PRC2 association and is a cofactor of this complex [202–204].

Thus, many DNA-binding factors are involved in PRE activity. However, at present, the mechanism of PcG/TrxG complexes recruitment to DNA via the action of these factors remains unknown. Moreover, for many DNA-binding factors, the direct partners of the PcG/TrxG system are not established. It is noteworthy that none of the factors is associated with all of the predicted PREs [13–16, 91]. It is suggested that the cooperative interaction of the different DNA-binding factors with PREs is required for recruitment of the PcG/TrxG protein complexes; moreover, the exact combination of DNA-binding factors at different PREs may vary [193, 194, 205, 206].

INTERACTION OF PcG AND TrxG FACTORS WITH PRE IN DIFFERENT ACTIVITY STATES

One of the key issues is to determine the factors that control the resulting PRE activity. It was suggested that the switch of PRE activity is accompanied by complete replacement of PcG/TrxG proteins on PREs: the PcG proteins are bound in the case of repression, TrxG proteins are bound in the case of activation. However, most of the studies suggest that the core components of PhoRC, PRC1, and PRC2 PcG complexes may be present on PRE regardless of its state and the target gene expression [13, 16, 81, 163, 207–209]. Moreover, many identified factors affect the activity of both systems (table).

The difference in the distribution of histone modifications is more obvious. It is demonstrated that H3K27me3 and H3K27ac modifications in the case of repression and activation, respectively, cover extended DNA regions, exceeding the limits of PRE [16, 163, 208, 210]. However, even in the case of active domain, H3K27me3 may be present at the PREs [81, 92, 163].

Apparently, different in the distribution component is the Ash1 TrxG factor, which in the active state is associated with transcribed regions of the target genes [92, 163]. It is possible that whole-genome analysis will reveale the similar differences in the distribution of Kis factor. Specifically, in the *Ubx* regulatory region, Kis binds to promoter only in the case of active transcription, while its association with *bx*PRE and *bxd*PRE does not depend on the *Ubx* activity state [92].

It is possible that the ratio of PcG and TrxG proteins is crucial in determining the PRE activity state. In addition, the presence of chemical modifications of PcG and TrxG proteins, which may influence their activity or the dynamics of interaction with PREs, as well as the presence of other, not analyzed or not yet identified, factors influencing the PcG/TrxG system, cannot be excluded. The importance of a balance between PcG and TrxG and their competition is supported by the fact that the repression does not always mean a complete absence the target gene transcription [163].

COMPETITION BETWEEN PcG AND TrxG FACTORS

According to the generally accepted model, a considerable role in activation/repression of transcription is played by specific histone modifications that can either increase or decrease the recruitment of the complexes determining the level of transcription. For instance, many characterized activities of the TrxG proteins are functionally opposite to the activities of PcG proteins.

PcG repressors promote H3K27me3 methylation [103–106], deacetylation of H3K27ac [118], and demethylation of H3K36me2 [131]. H3K27me3 provokes binding of PRC1 [127, 128], which modifies H2AK118ub [131]. TrxG activators, in turn, promote H3K4me1 [161] and H3K36me1/2 [165, 166, 211] methylation, acetylation of H3K27 [118, 161], and demethylation of H3K27me2/3 [174].

Histone modifications associated with active chromatin inhibit histone modifications mediated by PcG. For example, histone methylation H3K4me3 [212], H3K36me2/3 [165, 212], and acetylation of H3K27 inhibit H3K27me3 [118].

Competition between PcG and TrxG proteins is also visible at the level of chromatin structure. Specifically, PRC1 is capable of chromatin compaction [123], and TrxG complexes BAP and PBAP, on the contrary, are capable of chromatin decompaction [154, 155]. Furthermore, acetylation of nucleosomes (H3K27ac), promoted by TrxG proteins, potentially must disrupt the interaction of histones with DNA, neutralizing the positive charge on lysine [213].

As mentioned above, PREs are able to interact with the target gene promoters. Therefore, it is likely that competition is realized at the stage of initiation and/or transcription elongation. Consistent with this, in *Drosophila*, general transcription factors are recruited to the promoters in a state of repression [92, 214, 215], and the TrxG factor Kis stimulates transcription elongation [216]. In addition, the PcG and TrxG complexes may directly affect the RNAPII (RNA polymerase II), as it was confirmed in vertebrates [217–219]. It is likely that the escape from pausing and stimulation of elongation can be the key events in derepression of the PcG targets.

THE ROLE OF NON-CODING TRANSCRIPTS IN PRE ACTIVITY

The currently available data shows that big part of the noncoding genome regions is transcribed into lncRNAs (long noncoding RNAs) [220, 221]. The same is true for the BX-C complex. The fact that the direct link between the PcG repression system and noncoding RNAs was reported in mammals increases the interest in the studies of the ncRNAs in *Drosophila* as well [222].

Several studies indirectly indicate the role of noncoding transcription in inheritable PRE activity switch from repression to activation [84, 223, 224]. At the same time, the use of highly sensitive multiplex in situ hybridization demonstrated that transcription of the *bxd* ncRNA through the *Ubx* regulatory region (Fig. 2a) correlated with the inactive state of the *Ubx* gene. Moreover, the absence of the *bxd* ncRNA leads to ectopic *Ubx* expression [225]. A similar result was obtained in a detailed study of the *iab-8* ncRNA passing through the *abd-A* regulatory region [226]. It was demonstrated that the *iab-8* ncRNA is involved in the *abd-A* repression. In both studies, the authors suggest that the main repression factor is the transcriptional interference of the ncRNA with the target gene promoters. At the same time, transcription can negatively affect the activity of other regulatory elements within these domains. For instance, we demonstrated that transcription passing through the *white* and *yellow* enhancers suppresses their ability to activate the target gene promoters in transgenic systems [227, 228]. It cannot as well be excluded that long ncRNAs of BX-C can physically interact with the PcG factors, promoting their recruitment to PREs.

It is possible that the resulting effect of transcription may depend on the direction of transcription through PRE. For instance, transcription through *vg*PRE of the *vestigial* gene can be induced by both forward and reverse DNA strands, which correlates with either repression or activation of the target gene transcription [223].

However, noncoding RNAs were not detected in the PRE regions of some loci (*invected*, *engrailed*), suggesting that transcription is not the key factor controlling the PRE activity [209].

A number of studies were focused on the role of transcription in epigenetically inheritable switch in PRE activity from silencing to activation. Rank et al. [84] showed that 3.6-kb *Fab7*PRE, 4.5-kb *MCP*, and 2.3-kb *bxd*PRE are CMMs: their activity could be epigenetically heritable switched from silencing to activation by GAL4. At the same time, smaller PRE fragments were able to medaite repression, but lacked the CMM function. The inheritable switch in the activity of larger PRE fragments from repression to activation correlated with the presence of transcripts in the PRE regions. The authors suggested that these PREs could contain a promoter that in the presence of GAL4 drives the transcription through PRE and is responsible for its activity switch. According to this model the transcription physically displaces PcG proteins, promoting the recruitment of TrxG proteins and the switch in PRE activity [84]. However, this hypothesis conflicts with the fact that PcG proteins bind to the *Ubx* and *abd-A* introns in the cells in which these genes are transcribed, indicating that transcription passing through PREs does not lead to a complete loss of the PcG/TrxG ability to bind DNA [13, 92, 208]. More-

over, 219-bp *Fab7*PRE could be heritably switched in the absence of transcription [80].

In the Erokhin et al. study [81], we directly tested the effect of passing transcription on the *bxd*PRE activity. For these purposes, we used the 660-bp *bxd*-PRE, which is a part of the 2.3-kb *bxd*PRE, but lacks DNA fragments of the regulatory elements of other classes. To generate a pulse of transcription through PRE, we used a minimal promoter of the *hsp70* gene under control of GAL4. As in previous studies, the binding of GAL4 to the construct caused inactivation of PRE silencing activity. We showed that transcription through 660-bp *bxd*PRE in the absence of other elements is not able to induce the epigenetically inheritable switch in the activity of this element. Once GAL4 and activated transcription through PRE disappeared from the system, silencing of the reporter gene was reestablished. Thus, transcription itself cannot provide the CMM function, and presumably additional regulatory elements present in 2.3-kb *bxd*PRE (possibly enhancers or insulators) are needed for CMM.

Analysis of the protein complexes assembled on PRE using chromatin immunoprecipitation assay provides a possible explanation for the observed effects. Even in the presence of a high level of continuous transcription, PcG and TrxG proteins (Ph, dSfmbt, Pc, Trx-N, and GAF) remain associated with *bxd*-PRE. In addition, it doesn't completely eliminate the H3K27me3 histone modification. Thus, transcription is insufficient for substitution of one class of proteins by another one. At the same time, in the presence of GAL4 activator we observed the changes in the levels of these factors enrichment on PRE, regardless of transcription through *bxd*PRE. Specifically, binding of PcG factors decreases, while binding of TrxG factors increases. Thus, maintaining the active/inactive state can depend on the relative ratio between these factors on PRE rather than on mutually exclusive binding of activators/repressors.

We suggest that in the discussed above study with GAL4 [84], as well as in the native genomic environment, the direct interaction between activators (enhancers) and PREs take place. This possibly could lead to the activation of either cryptic or weak promoters near enhancers and PRE, resulting in the formation of noncoding transcripts. Association of the promoter factors with PRE [91, 92] may not only indicate interaction with the target gene promoter but also reflect the direct recruitment of these proteins on the PRE DNA.

CONCLUSIONS

Over the past decade, a large body of data on the activity of the PcG/TrxG system was accumulated. Specifically, protein components of the core complexes, as well as a number of DNA-binding factors, were characterized. Moreover, functional activities of many core components, as well as the properties of PREs in transgenic systems, were investigated. These studies provide visualization of the regulatory mechanism of gene expression as a result of gracefully regulated competition between the PcG and TrxG factors. To date, there are still many unresolved questions. How do the PRE-binding factors recruit the PcG and TrxG complexes to DNA? What are the mechanisms regulating the competition between the PcG and TrxG systems? What are the protein factors and modifications that determine the resulting PRE activity? What is the mechanism for changing the PRE activity state and epigenetic inheritance?

Resolution of these and many other questions related to the activity of the PcG/TrxG system is necessary for the understanding of the mechanisms governing the development, functioning of multicellular organisms, and the role of PcG/TrxG in cancer and other pathologies.

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