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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Recruitment to Chromatin of (GA)n-Associated Factors GAF and Psq in the Transgenic Model System Depends on the Presence of Architectural Protein Binding Sites

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Abstract—Polycomb group (PcG) repressors and Trithorax group (TrxG) activators of transcription are essential for the proper development and maintenance of gene expression profiles in multicellular organisms. In *Drosophila*, PcG/TrxG proteins interact with DNA elements called PRE (Polycomb response elements). We have previously shown that the repressive activity of inactive PRE in transgenes can be induced by architectural protein-binding sites. It was shown that the induction of repression is associated with the recruitment of PcG/TrxG proteins, including the DNA-binding factors Pho and Combgap. In the present study, we tested the association of the two other PRE DNA-binding factors, GAF and Psq, with *bxd*PRE in the presence and absence of sites for architectural proteins. As a result, it was shown that both factors can be efficiently recruited to the *bxd*PRE only in the presence of adjacent binding sites for architectural proteins Su(Hw), CTCF, or Pita.

Keywords: *Drosophila*, Polycomb, PRE, repression of transcription, Su(Hw), CTCF, Pita, GAF, Psq
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Epigenetic control of gene expression is required for the proper development and functioning of multicellular organisms. Proteins of Polycomb (PcG) and Trithorax (TrxG) groups, which are responsible for the repression and activation of transcription, respectively, are important epigenetic regulators of transcription. Disturbances in the function of the systems of transcription regulation by PcG/TrxG proteins are associated with many pathological conditions, such as cancer, which makes it necessary to understand the mechanism of their action, including the principles of recruitment to chromatin [1–5].

In *Drosophila*, PcG/TrxG proteins interact with specialized PRE (Polycomb Response Elements) DNA elements, which are also called silencers [6–8]. Experiments with transgenic constructs showed that a single PRE element can recruit PcG/TrxG proteins and induce the reporter gene repression. However, the functional activity of PRE strongly depends on the genomic environment, and repression is observed in approximately half of the transgenes, whereas in the rest of the cases PRE can either be in a neutral state (do not affect the reporter gene activity) or activate transcription. Importantly, PcG/TrxG proteins can be recruited to the DNA sequence of PRE regardless of its state; however, the level of their association with chromatin may change [9]. The issue of how PRE activity is controlled is currently the subject of active research.

We have previously shown that the insertion of binding sites for architectural proteins (Su(Hw), CTCF, or Pita) next to PRE can enhance or even induce the PRE-dependent repression of the target gene [10]. In this case, the induction of repression is associated with the recruitment of PcG (Ph, Sfmbt) and TrxG (Trx, CBP) proteins, as well as two PRE-associated DNA-binding factors (Pho and Combgap). However, it remains unknown whether other DNA-associated factors bind to the DNA sequence of PRE in the inactive state. There are potentially two variants. In the first case, it can be expected that, in the absence of sites for architectural proteins, the PRE region is strongly associated with nucleosomes and is inaccessible for any contacts with the DNA-binding proteins. In the second variant, a limited number of transcription factors can interact with the PRE region; however, they are insufficient to recruit a functional PcG/TrxG protein complex.

In this work, we tested the association of two DNA-binding proteins, GAF and Psq, with PRE in the neutral state and upon induction of repression.

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Both factors bind to $(GA)_n$ repeats (GAGA sites), recognizing the same motifs in PRE, and are direct physical partners interacting with each other through BTB domains [11]. Moreover, it was demonstrated for GAF that it is a pioneer transcription factor and, at least at a number of genomic positions, can cause the recruitment of chromatin remodelers and facilitate the binding of various functional complexes to DNA [11]. Thus, the aim of this study was to test the ability of the pioneer factor GAF and its partner Psq to interact with inactive *bxd*PRE in the absence of adjacent binding sites for architectural proteins.

The dependence of the recruitment of GAF and Psq proteins on the presence of sites for architectural proteins was tested using the previously developed model system [10]. All transgenes used in this study were inserted into the 96E region of the genome on the third chromosome using the PhiC31 site-specific integration system by recombination between the attB site, contained in the plasmid, and the attP site, previously inserted into the genome using the Mariner transposon [12]. The well-characterized 656-bp *bxd*PRE element from the regulatory region of the *Ubx* gene was used in this work in all transgenic constructs as a model PRE [9]. Figure 1a shows the *bxd*PRE sequence and the sites for the DNA-binding proteins in PRE: GAF/Psq—GAGAG [13, 14]; Pho—GCCAT [15, 16]; Zeste—YGAGYG (Y = C or T) [17, 18]; Sp1/Klf—RRGGYG (R = A or G) [19]; Comgbap (Cg)—GTGT [20].

The basic transgene, named bxd (Fig. 1b), contains the following functional elements: the attB site, the *bxd*PRE silencer, the *white* reporter gene, and the tissue-specific enhancer of the *white* gene (*E*). In addition, bxdPRE in the transgene is surrounded by approximately 1-kb DNA spacers, derived from the coding regions of the eGFP and RFP genes, as well as by transcription terminators (the SV40 terminator is inserted upstream of bxdPRE, and the yellow gene terminator is inserted downstream of bxdPRE).

The second transgene, named Su-bxd (Fig. 1b), contains all the elements of the *bxd* transgene and additionally the four binding sites for the architectural protein Su(Hw) (4xSu) inserted next to *bxd*PRE at the 5' side.

The *white* gene is responsible for eye pigmentation in *Drosophila* and is required for evaluating the repressor activity of the *bxd*PRE silencer. The tissue-specific enhancer is used to increase the *white* gene expression level and is necessary for work with transgenes containing repressor elements that effectively suppress the transcription of reporter genes. The eye phenotype of the transgenic flies directly correlates with the level of *white* gene transcription [9]. In the absence of repression, the eye phenotype of the transgenic flies will be red; in the case of repression, eye pigmentation will be reduced down towards the white color, depending on the degree of repression.

We have previously shown that the *bxd*PRE element in the bxd transgene at 96E is in a neutral state: it does not repress transcription (the eye phenotype of the transgenic flies in hemi- and homozygotes is red) and does not recruit proteins of the PcG/TrxG groups and the PRE-associated DNA-binding factors Pho and Combgap. At the same time, in the Su-bxd transgene, the insertion of four binding sites for the architectural protein Su(Hw) leads to effective repression the *white* gene by *bxd*PRE (the eye phenotype of the transgenic flies is brown in the hemizygote and light yellow in the homozygote). The repression is accompanied by recruitment of PcG/TrxG proteins, including two PRE-associated DNA-binding proteins Pho and Combgap. In addition, it was experimentally confirmed that, in the Su-bxd construct, Su(Hw) is effectively recruited to 4xSu sites [10].

In this work, using immunoprecipitation of chromatin (X-ChIP) isolated from the heads of homozygous transgenic flies, we tested the association of GAF and Psq proteins with *bxd*PRE in the bxd and Su-bxd transgenes at adult stage of development. We evaluated the enrichment of the studied proteins at five region of the transgenes: (1) the distal end of the spacer sequence upstream of *bxd*PRE, (2) *bxd*PRE, (3) the distal end of the spacer sequence downstream of *bxd*PRE, (4) the coding region of the *white* gene, and (5) the promoter of the *white* gene (Fig. 1b). A fragment of the coding region of the Ras64B gene (ras) was used as a negative internal control, and the *bxd*-PRE-Genome region, which binds GAF/Psq proteins next to *bxd*PRE in the genome but is not part of *bxd*-PRE in transgenes, was used as a positive internal control. The analysis showed that the GAF and Psq proteins do not interact with the inactive *bxd*PRE within the bxd transgene (Fig. 1c). At the same time, these proteins are effectively recruited to the *bxd*PRE silencer in the repressed state within the Su-bxd transgene in the presence of binding sites for the architectural protein Su(Hw).

At the next step, we studied the interaction of the GAF and Psq proteins with the *bxd*PRE silencer upon its induction by alternative architectural proteins, CTCF or Pita. For this purpose, either four binding sites for the architectural protein CTCF (CTCF-bxd transgene, Fig. 2a) or five binding sites for the architectural protein Pita (Pita-bxd transgene, Fig. 2a) were inserted next to the *bxd*PRE. In the previous study, we showed that the binding sites for these architectural proteins functionally act in the same way as the binding sites for the Su(Hw) protein: in their presence, the repressor activity of *bxd*PRE is activated. As in the case of the Su-bxd transgene, the enrichment of GAF and Psq proteins was tested using X-ChIP on five similar regions of the CTCF-bxd and Pita-bxd transgenes. As before, chromatin for immunoprecipitation was isolated from the heads of adult flies homozygous for the construct. As a control, all experiments were performed in parallel with the isolation and immunopre-

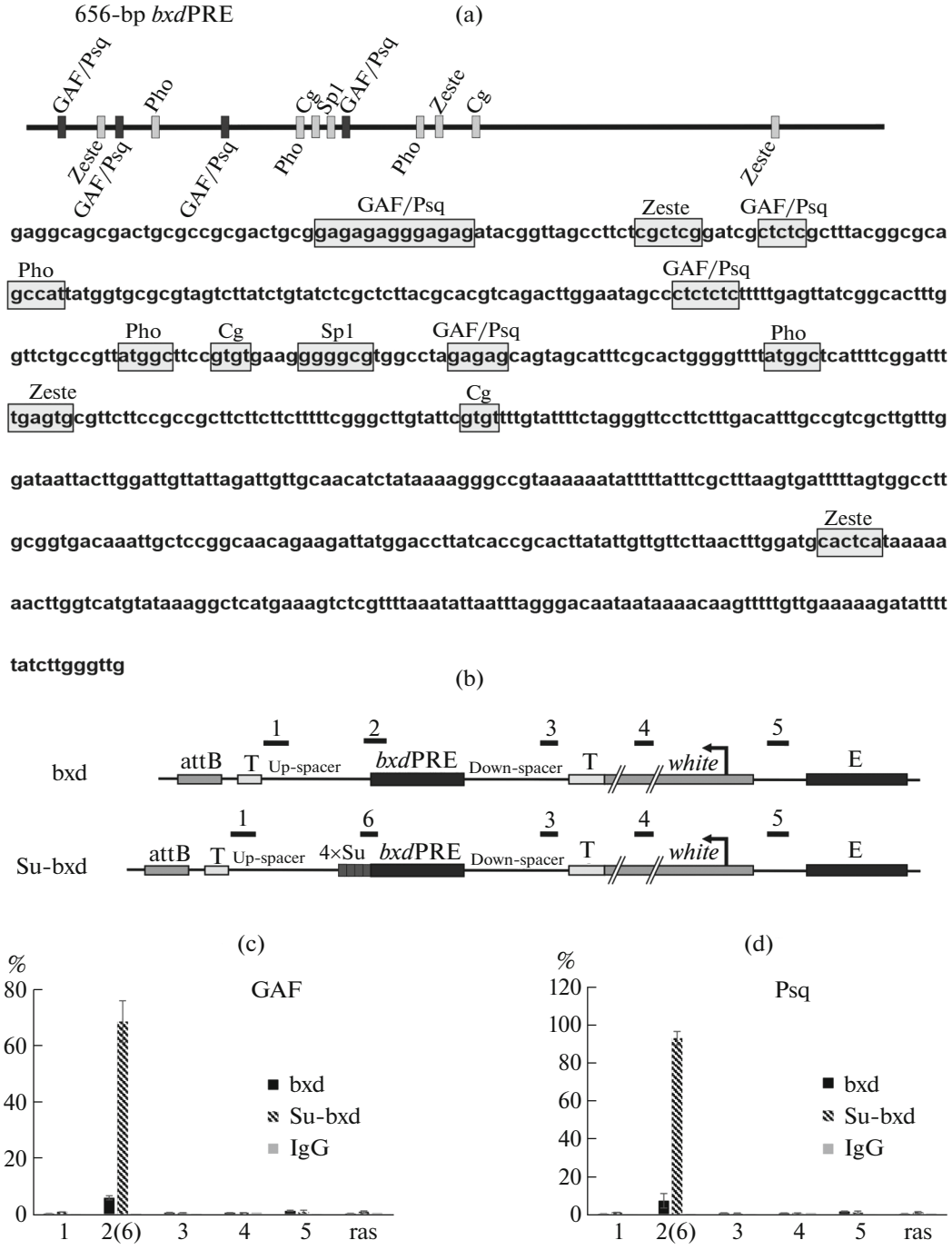

Fig. 1. (a) Predicted binding sites of known PRE-associated DNA-binding proteins of the 656 *bxd*PRE silencer. (b) Schematic representation of transgenes. Designations: attB, attB site required for transgene integration; *bxd*PRE, *bxd*PRE silencer; T, transcription terminators; *white*, reporter gene; E, *white* gene enhancer; 4xSu, four binding sites for the Su(Hw) protein. The numbers above the transgene schemes (1, 2 or 6, 3, 4, 5) denote the regions amplified by qPCR in X-ChIP experiments. (c, d) X-ChIP-qPCR analyzes performed using chromatin isolated from the heads of adult flies homozygous for the bxd or Su-bxd transgenes. The experiments were carried out using antibodies against GAF (c) and Psq (d) proteins, or with non-specific IgG of non-immunized animal (IgG). The ordinate axis indicates the percentage of enrichment of each region in the immunoprecipitated material relative to the Input DNA, normalized to the endogenous positive control—the region near 656-bp *bxd*PRE in the genome (*bxd*PRE-Genome). The abscissa axis indicates the regions analyzed by qPCR in the transgene and the negative control (*ras* is the coding region of the *Ras64B* gene). The bars indicate standard deviations.

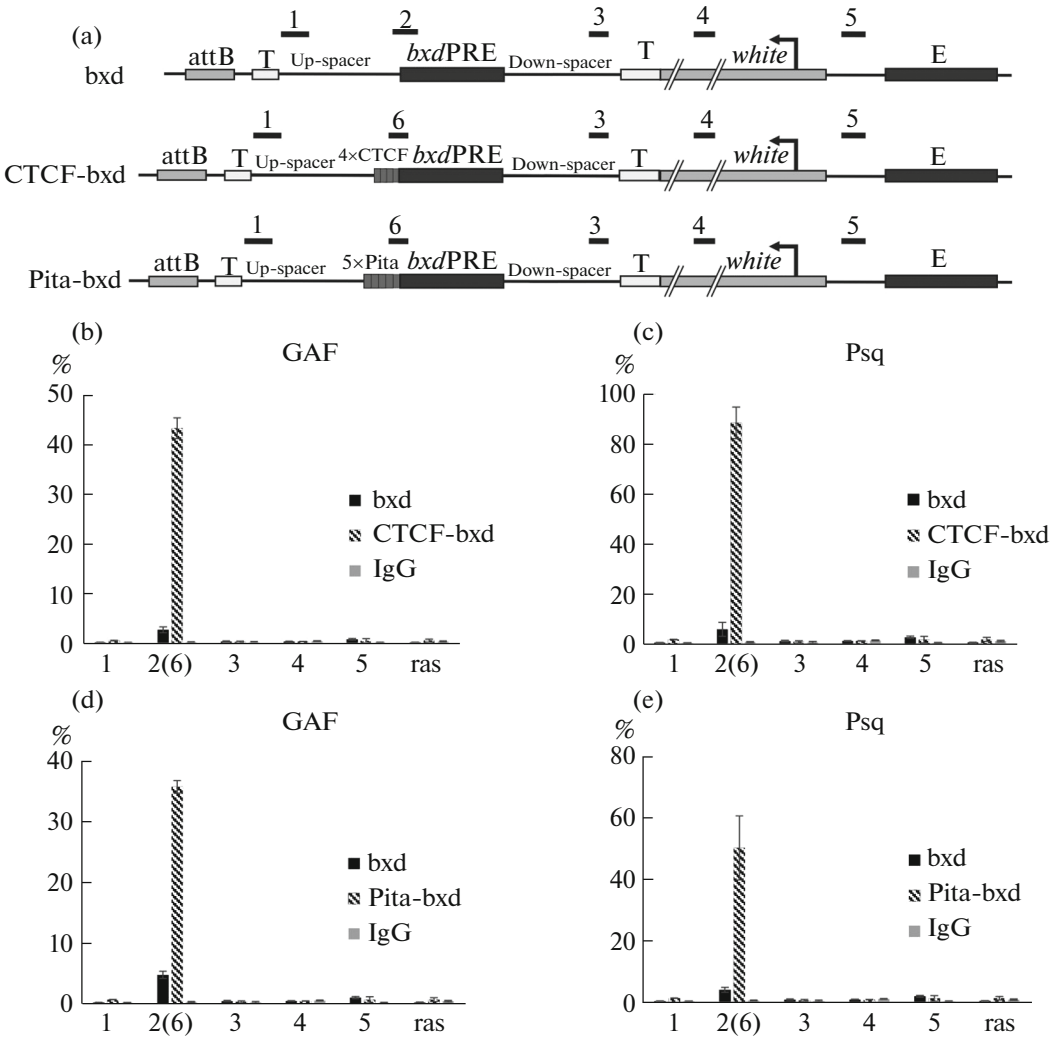

Fig. 2. (a) Schematic representation of transgenes. Designations: 4xCTCF, four binding sites for CTCF protein; 5xPita, five binding sites for Pita protein. (b, c) X-ChIP-qPCR analyzes performed using chromatin isolated from the heads of adult flies homozygous for the bxd and CTCF-bxd transgenes. Antibodies specific to GAF (b) and Psq (c) proteins were used. (d, e) X-ChIP-qPCR analyzes performed using chromatin isolated from the heads of adult flies homozygous for the bxd or Pita-bxd transgenes. Antibodies specific to GAF (d) or Psq (e) proteins were used. For other designations, see the legend to Fig. 1.

cipitation of chromatin isolated from flies carrying the bxd transgene. As a result of the analysis, we showed that the GAF and Psq proteins are recruited to the *bxd*PRE element only in the repressed state in the presence of binding sites for the architectural proteins CTCF or Pita (Figs. 2b–2e).

Thus, we have shown that, in the model system used, the GAF pioneer factor, as well as the Psq protein, do not interact with the inactive *bxd*PRE. The activation of *bxd*PRE-mediated repression of the marker gene in the case of the insertion of the binding sites for architectural proteins is accompanied by the recruitment of GAF and Psq. The obtained data show that, at least in some situations, GAGA sites are insufficient to recruit the GAGA-associated proteins and, as a consequence, the transcription complexes interacting with them to chromatin. Interestingly, the use of the binding sites for each of the three architectural factors tested in this study resulted in approximately the same enhancement of GAF and Psq binding to *bxd*PRE. Despite the fact that approximately ten PRE-associated DNA-binding factors are known today [7, 8], the question of the detailed mechanism of the recruitment of PcG/TrxG proteins to chromatin remains open. The results of this study demonstrate that, besides the known DNA-binding proteins associated with PRE, various architectural factors that determine the 3D structure of chromatin in the nucleus may play an important role in the control of these processes.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

1. Piunti, A. and Shilatifard, A., *Nat. Rev. Mol. Cell Biol.*, 2021, vol. 22, no. 5, pp. 326–345.
2. Chetverina, D.A., Lomaev, D.V., and Erokhin, M.M., *Acta Nat.*, 2020, vol. 12, pp. 66–85.
3. Parreno, V., Martinez, A.M., and Cavalli, G., *Cell Res.*, 2022, vol. 32, no. 3, pp. 231–253.
4. Cavalli, G. and Heard, E., *Nature*, 2019, vol. 571, no. 7766, pp. 489–499.
5. Erokhin, M., Chetverina, O., Gyorffy, B., et al., *Cancers* (Basel), 2021, vol. 13, no. 13, p. 3155.
6. Kassis, J.A., Kennison, J.A., and Tamkun, J.W., *Genetics*, 2017, vol. 206, no. 4, pp. 1699–1725.
7. Erokhin, M., Georgiev, P., and Chetverina, D., *Epigenomes*, 2018, vol. 2, no. 1, pp. 1–24.
8. Kassis, J.A. and Brown, J.L., *Adv. Genet.*, 2013, vol. 81, pp. 83–118.
9. Erokhin, M., Elizar'ev, P., Parshikov, A., et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2015, vol. 112, no. 48, pp. 14930–14935.
10. Erokhin, M., Gorbenko, F., Lomaev, D., et al., *BMC Biol.*, 2021, vol. 19, no. 1, p. 113.
11. Chetverina, D., Erokhin, M., Schedl, P., *Cell Mol. Life Sci.*, 2021, vol. 78, no. 9, pp. 4125–4141.
12. Bischof, J., Maeda, R.K., Hediger, M., et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2007, vol. 104, no. 9, pp. 3312–3317.
13. van Steensel, B., Delrow, J., and Bussemaker, H.J., *Proc. Natl. Acad. Sci. U. S. A.*, 2003, vol. 100, no. 5, pp. 2580–2585.
14. Lehmann, M., Siegmund, T., Lintermann, K.G., et al., *J. Biol. Chem.*, 1998, vol. 273, no. 43, pp. 28504–28509.
15. Fritsch, C., Brown, J.L., Kassis, J.A., et al., *Development*, 1999, vol. 126, no. 17, pp. 3905–3913.
16. Brown, J.L. and Kassis, J.A., *Genetics*, 2013, vol. 195, no. 2, pp. 407–419.
17. Biggin, M.D., Bickel, S., Benson, M., et al., *Cell*, 1988, vol. 53, no. 5, pp. 713–722.
18. Moses, A.M., Pollard, D.A., Nix, D.A., et al., *PLoS Comput. Biol.*, 2006, vol. 2, no. 10, p. e130.
19. Brown, J.L., Grau, D.J., DeVido, S.K., et al., *Nucleic Acids Res.*, 2005, vol. 33, no. 16, pp. 5181–5189.
20. Ray, P., De, S., Mitra, A., et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2016, vol. 113, no. 14, pp. 3826–3831.

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