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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

PRE/TRE Elements Act as Transcription Activators in *Drosophila* S2 Cells

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Abstract—The *Drosophila* PRE/TRE elements are DNA targets for Polycomb and Trithorax group proteins, which control repression and activation of gene transcription, respectively. In this study, we show that, in transiently transfected *Drosophila* S2 cells, *bxd*PRE activates transcription driven from different promoters. Using *CG32795* gene promoter, we demonstrate that other PRE/TRE elements—*Fab7, en, eve,* and *CycB*—also act as transcription activators in this model system.

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Transcription regulation is the key step required for a proper gene expression. The maintenance of the correct pattern of gene transcription in metazoans is ensured by Polycomb (PcG) and Trithorax (TrxG) group proteins [1–4]. PcG proteins act as transcriptional repressors, whereasTrxG factors act as activators. In *Drosophila melanogaster*, these factors interact with the DNA sequences that are called PRE/TRE elements (Polycomb Response Element/Trithorax Response Element) [5, 6]. In transgenic *Drosophila* lines, PRE/TRE elements usually repress the transcription of marker genes via recruitment of the PcG factors. Conversely, earlier in our laboratory it was shown [7] that the *bxd*PRE PRE/TRE element transiently transfected into *Drosophila* S2 embryonic cell culture functions as a transcriptional activator.

In this study, we investigated the ability of different transiently transfected PRE/TRE elements to activate transcription.

The activity of PRE/TRE elements was studied using the constructs containing luciferase reporter genes *FLuc* and *RLuc*. These constructs were transiently transfected into *Drosophila* S2 (Schneider 2) cells. The plasmid vector carrying the luciferase gene *FLuc* without promoter was used to test the regulatory elements. To control the transfection efficiency, the cells were cotransfected with a plasmid vector carrying the luciferase gene *RLuc* under the control of the actin promoter. The specific signal intensity was determined by the ratio of activities of luciferases Fluc/Rluc.

At the first step, we tested in detail the effect of the *bxd*PRE element (FB2016_04 3R:16764122-16764782, 661 bp) on the transcription initiated from the CG32795 gene promoter (FB2016_04 X:2789300..2790035 minus). The previously studied [7] construct carrying the *bxd*PRE element upstream of the *CG32795* gene promoter in the forward orientation was used as a positive control. Neutral DNA fragments of the coding regions of *eGFP* and *LacZ* genes ≈700 and 1800 bp long, respectively, inserted upstream of the promoter, were used as negative controls. The presence of the *bxd*PRE element, but not the neutral linkers, led to a threefold activation of the *Fluc* gene expression (Fig. 1a). The increased expression level of the *Fluc* gene can be explained either by the activation of transcription from the *CG32795* gene promoter by the *bxd*PRE element or by a stronger transcription that could potentially be initiated at the *bxd*PRE.

To confirm the ability of the *bxd*PRE element to activate transcription, we created several control constructs. In the first construct, the *bxd*PRE element was inserted in the reverse orientation, and in other two constructs is was separated from the *CG32795* promoter and the *FLuc* gene by the differently oriented 702-bp SV40 transcription terminator (from the pUAST plasmid vector) [8, 9]. This terminator is bilateral, i.e., can terminate transcription going from different DNA strands. As a result of analysis it was found that the *bxd*PRE element is also capable of activating transcription in the reverse orientation and that the presence of the SV40 terminator does not prevent the increase in the expression of the reporter *FLuc* gene. Thus, *bxd*PRE directly activates the *CG32795* gene promoter.

Promoters of protein-coding genes of higher eukaryotes, whose activity is associated with RNA polymerase II, have different structures [10]. To show that the ability of *bxd*PRE to activate transcription is not specific to the *CG32795* gene promoter, at the next

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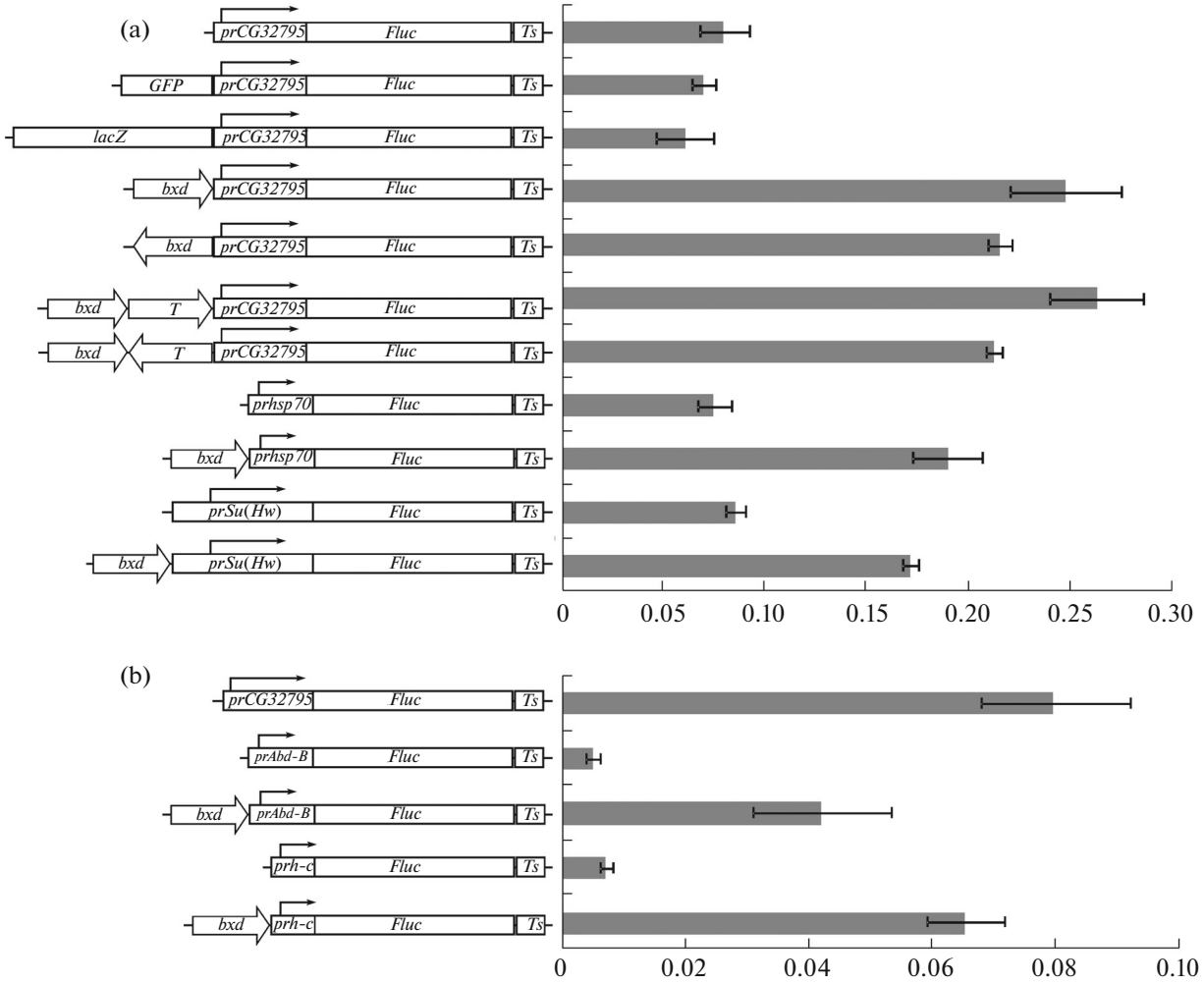

Fig. 1. Testing the functional activity of the PRE/TRE element *bxd*PRE in *Drosophila melanogaster* S2 cells. The abscissa axis shows the *FLuc* luciferase activity normalized to the *RLuc* luciferase activity (*Fluc*/*Rluc*). The ordinate axis shows the structure of the constructs used for transient transfection. All experiments were performed at least in triplicate. Designations: *bxd*—*bxd*PRE element (661 bp) from the regulatory region of the *Ubx* gene (the arrow shows the element orientation); *prCG32795*, *prSu(Hw)*, *prhsp70*, and *prAbd*-B—promoters of *CG32795*, *Su(Hw)*, *hsp70*, and *Abd-B* genes, respectively, controlling the *Fluc* gene expression; *prh-c*—core fragment of the *hsp70* gene promoter. Neutral linkers: the coding region of the *GFP* gene (≈700 bp) and a fragment of the coding region of the *lacZ* gene (1.8 kb). *T*—SV40 transcription terminator 702 bp long (the arrow shows the element orientation relative to the location in the pUAST plasmid vector). *Ts*—core fragment of the SV40 transcription terminator 222 bp long, whose orientation corresponds to the plasmid vector pGL3-Basic (Promega). Here and in Fig. 2, data are represented as $M \pm SD$.

step of the work we tested the ability of *bxd*PRE to stimulate different promoters. We used two "strong" promoters of the *hsp70* heat shock gene (FB2016_04 3R:11958298..11958743) and the *Su(Hw)* gene (FB2016_04 3R:14308539..14309849 minus), which set a high transcription level, and two "weak" promoters of the *Abd-B* (*B3*) gene (FB2016_04 3R:16960646..16960181 minus) and the core fragment of *hsp70* gene (*h-c*, FB2016_04 3R:12505755..12505996), which set a lower transcription level. As a result of analysis, it was found that the *bxd*PRE element activates transcription from all promoters tested (Figs. 1a, 1b). Similar to the *CG32795* gene promoter, the transcription from the *hsp70* and *Su(Hw)* gene promoters increased by 2.5 and 2 times, respectively. In the case of "weak" promoters *Abd-B* and *h-c*, the level of transcription increased by 8 and 9 times, respectively. In addition, the *bxd*PRE element was able to stimulate transcription from the core fragment of the *hsp70* gene promoter. This fact indicates that the PRE/TRE element acts via the main transcription factors and does not require the presence of binding sites for additional transcriptional activators on the promoter.

At the next step of the study, we tested the activity of other known PRE/TRE elements in transient transfection: *Fab7* (FB2016_04 3R:16898915..16900043, 1129 bp), *en* (FB2016_04 2R:11529835..11528272, 1564 bp), *CycB* (FB2016_04 2R:22806630..22806308

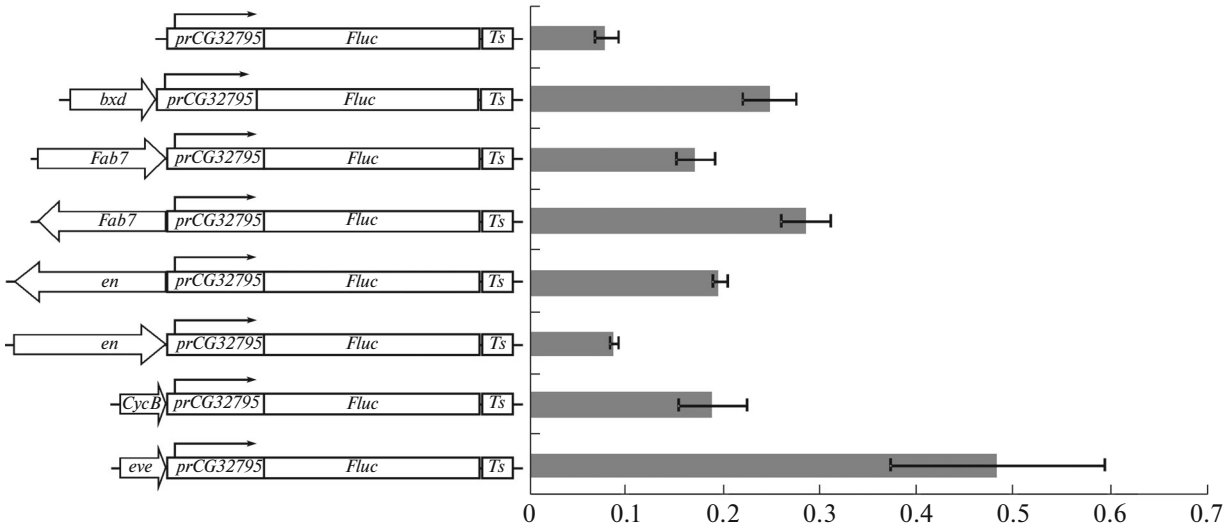

Fig. 2. Testing of functional activity of PRE/TRE elements *Fab7*, *en*, *CycB*, and *eve* in the *Drosophila* S2 cells. For designations, see the legend to Fig. 1.

minus, 323 bp), and *eve* (FB2016_04 2R:9988256..9988599, 344 bp).

As a result of analysis it was found that all tested elements activated transcription from the *CG32795* promoter (Fig. 2). The expression level of the reporter gene *FLuc* increased, on average, 2.5 times (6 times in the case of the PRE/TRE element *eve*).

It was found that the ability of the *Fab7* element, as well as *bxd*PRE, to stimulate transcription does not depend on its orientation: activation in the forward and reverse orientation increased 2.1 and 3.6 times, respectively. At the same time, the PRE/TRE element *en* activated transcription only in the reverse orientation.

Thus, our study showed that five known PRE/TRE elements of the *Drosophila* genome can activate the reporter gene transcription being transiently transfected into S2 embryonic cell culture. Importantly, in the constructs that are inserted into genome, PRE/TRE elements usually function as repressors rather than activators of transcription. Both TrxG activators and PcG repressors bind to PRE/TRE elements [11]. The resulting effect of these factors may depend on many characteristics, including the ratio of the PcG/TrxG binding. In the created system, the balance between PcG/TrxG factors is apparently shifted towards the TrxG activators, which determine the ability of PRE/TRE elements to activate the promoter. However, further studies are required to elucidate the molecular mechanisms that determine the status of PRE/TRE elements.

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REFERENCES

1. Beisel, C. and Paro, R., *Nat. Rev. Genet.*, 2011, vol. 12, pp. 123–135.
2. Muller, J. and Verrijzer, P., *Curr. Opin. Genet. Develop.*, 2009, vol. 19, pp. 150–158.
3. Di Croce, L. and Helin, K., *Nat. Struct. Mol. Biol.*, 2013, vol. 20, pp. 1147–1155.
4. Schuettengruber, B., Martinez, A.M., Iovino, N., and Cavalli, G., *Nature Revs. Mol. Cell Biol.*, 2011, vol. 12, pp. 799–814.
5. Kassis, J.A. and Brown, J.L., *Adv. Genet.*, 2013, vol. 81, pp. 83–118.
6. McElroy, K.A., Kang, H., and Kuroda, M.I., *Open. Biol.*, 2014, vol. 4, p. 140006.
7. Erokhin, M.M., Georgiev, P.G., and Chetverina, D.A., *Dokl. Biol. Sci.*, 2009, vol. 428, pp. 229–231.
8. Erokhin, M., Elizar'ev, P., Parshikov, A., Schedl, P., Georgiev, P., and Chetverina, D., *Proc. Natl. Acad. Sci. U. S. A.*, 2015, vol. 112, pp. 14930–14935.
9. Brand, A.H. and Perrimon, N., *Development*, 1993, vol. 118, pp. 401–415.
10. Kadonaga, J.T., *Wiley Interdiscip. Rev. Develop. Biol.*, 2012, vol. 1, pp. 40–51.
11. Geisler, S.J. and Paro, R., *Development*, 2015, vol. 142, pp. 2876–2887.

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