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Functional properties of the Su(Hw) complex are determined by its regulatory environment and multiple interactions on the Su(Hw) protein platform

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The Su(Hw) protein was first identified as a DNA-binding component of an insulator complex in *Drosophila*. Insulators are regulatory elements that can block the enhancer-promoter communication and exhibit boundary activity. Some insulator complexes contribute to the higher-order organization of chromatin in topologically associated domains that are fundamental elements of the eukaryotic genomic structure. The Su(Hw)-dependent protein complex is a unique model for studying the insulator, since its basic structural components affecting its activity are already known. However, the mechanisms involving this complex in various regulatory processes and the precise interaction between the components of the Su(Hw) insulators remain poorly understood. Our recent studies reveal the fine mechanism of formation and function of the Su(Hw) insulator. Our results provide, for the first time, an example of a high complexity of interactions between the insulator proteins that are required to form the (Su(Hw)/Mod(mdg4)-67.2/CP190) complex. All interactions between the proteins are to a greater or lesser extent redundant, which increases the reliability of the complex formation. We conclude that both association with CP190 and Mod(mdg4)-67.2 partners and the proper organization of the DNA binding site are essential for the efficient recruitment of the Su(Hw) complex to chromatin insulators. In this review, we demonstrate the role of multiple interactions between the major components of the Su(Hw) insulator complex (Su(Hw)/Mod(mdg4)-67.2/CP190) in its activity. It was shown that Su(Hw) may regulate the enhancer–promoter communication via the newly described insulator neutralization mechanism. Moreover, Su(Hw) participates in direct regulation of activity of vicinity promoters. Finally, we demonstrate the mechanism of organization of "insulator bodies" and suggest a model describing their role in proper binding of the Su(Hw) complex to chromatin.
Key words: Su(Hw); Mod(mdg4)-67.2; CP190; transcription regulation; insulation; insulator bodies; protein-protein interactions.

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Функциональные свойства Su(Hw)-зависимого комплекса определяются его регуляторным окружением и множественными взаимодействиями на белковой платформе Su(Hw)

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Белок Su(Hw) был впервые идентифицирован как ДНК-связывающий компонент инсуляторного комплекса. Инсуляторы представляют собой регуляторные элементы, которые могут блокировать энхансер-промоторные взаимодействия и работать как границы между активным и репрессивным хроматином. Su(Hw)-зависимый белковый комплекс – уникальная модель для изучения инсуляторов, поскольку основные структурные компоненты, влияющие на его активность, уже известны. Однако механизмы, вовлекающие этот комплекс в различные регуляторные процессы, и детали взаимодействий между компонентами Su(Hw) инсуляторов остаются недостаточно изученными. Наши недавние работы выявили детальный механизм формирования и функционирования инсулятора Su(Hw). В представленном обзоре мы демонстрируем, как множественные взаимодействия между основными компонентами Su(Hw)-зависимого комплекса (Su(Hw)/Mod(mdg4)-67.2/CP190) влияют на его активность. Показываем, что Su(Hw) может регулировать энхансер-промоторные взаимодействия через новый механизм нейтрализации инсулятора и, кроме того, участвует в прямой регуляции активности близлежащих промоторов. Наконец, мы описываем механизм формирования

«инсуляторных телец» и предлагаем модель, объясняющую их роль в рекрутировании Su(Hw)-зависимого комплекса на хроматин.
Ключевые слова: Su(Hw); Mod(mdg4)-67.2; CP190; регуляция транскрипции; инсуляция; инсуляторные тельца; белок-белковые взаимодействия.

Multiple interactions between the components of the Su(Hw) complex

The best-studied *Drosophila* insulator complex consists of two proteins, the Mod(mdg4)-67.2 and CP190, which are recruited to chromatin through interactions with the DNA-binding Su(Hw) protein. The Su(Hw) protein contains the N-terminal region, an array of 12 C_2H_2-type zinc finger domains (ZF), and the C-terminal region (aa 716–892) responsible for enhancer blocking activity (Harrison et al., 1993). Thus far, little is known about the precise mechanism of organization of the Su(Hw) complex. Mod(mdg4)-67.2 is one of the isoforms produced by the *mod(mdg4)* locus, which encodes a large set of proteins with common N- and different C-terminal parts (Dorn, Krauss, 2003). The common part contains the BTB domain that functions as a protein interaction domain facilitating oligomer formation (Golovnin et al., 2007; Bonchuk et al., 2011). In its specific part, each Mod(mdg4) isoform includes the degenerative FLYWCH domain presumably employed in protein-protein interaction. Mod(mdg4)-67.2 has an additional C-terminal acidic domain (SID), which interacts with the region of the Su(Hw) protein responsible for the enhancer-blocking effect (Golovnin et al., 2007). Another Su(Hw) binding protein CP190 also contains the N-terminal BTB/POZ domain, which forms stable homodimers that may be involved in protein-protein interactions (Bonchuk et al., 2011, 2015). The CP190 is a shuttle protein, since it localizes to centrioles via its M domain during the mitotic stage (Plevock et al., 2015), while being recruited to chromatin via its interaction with the Su(Hw) and many other insulator proteins during the interphase (Melnikova et al., 2018a). An array of four ZF domains is probably involved in protein-protein interactions, since no interaction between DNA and CP190 protein has been identified thus far despite the large number of studies conducted.The available data have been expanded and summarized in our recent studies. CP190 was shown to interact with the N-terminal part of the Su(Hw) protein located between aa 88 and 202 via its BTB domain (Melnikova et al., 2018a). Interestingly, some other known architectural/insulator proteins, such as dCTCF and Pita, also interact with CP190 via its BTB domain (Bonchuk et al., 2015; Maksimenko et al., 2015). Therefore, it is possible that specificity of CP190 interactions with DNA-binding proteins is determined by additional components. In the case of Su(Hw) insulator, this role can be played by Mod(mdg4)-67.2 as we have identified that the BTB domain of Mod(mdg4)-67.2 directly interacts with the M domain of CP190 (Golovnin et al., 2007). The results of yeast two-hybrid assay show that the FLYWCH domain improves the interaction between the BTB domain of Mod(mdg4)-67.2 and the M domain of CP190. Both CP190 and Mod(mdg4)-67.2 participate in recruitment of Su(Hw) to chromatin as the knock-down of each of them reduces Su(Hw) binding to the genomic sites (Melnikova et al., 2017a, 2018a). According to our results, it appears that the specificity of organization of the Su(Hw) complex and its recruitment to chromatin is achieved by complex interactions of the Mod(mdg4)-67.2 SID, FLYWCH, and BTB domains with CP190/Su(Hw) proteins. It seems likely that Mod(mdg4)-67.2 is recruited to the Su(Hw) sites in complex with CP190 (Fig. 1).

Identification of the first endogenous Su(Hw)-dependent insulators

The *Drosophila gypsy* insulator including a set of twelve degenerative binding sites for Su(Hw) protein was found in the 5′ region of the *gypsy* retrotransposon (Parkhurst et al., 1988). This insulator exhibiting enhancer blocking and boundary activities. For example, the *gypsy* insulator blocks enhancer-promoter interactions when placed between the enhancers and a promoter in the model system of the *yellow* gene.

However, many endogenous Su(Hw) binding sites observed in polytene chromosomes are not associated with *gypsy* retrotransposon. The functional Su(Hw) insulator between the *yellow* gene and AS-C, which was able to block the enhancer-promotor communication of the *scute* gene over significant distances was first described using *in vivo* and *in vitro* assays (Golovnin et al., 1999, 2003; Parnell et al., 2003). This insulator site (later named the 1A2 insulator), unlike the *gypsy* retrotransposone insulator, includes only two Su(Hw) binding sites. Interestingly, in transgenic lines, the small 126 bp fragment that includes only two Su(Hw) binding sites can only

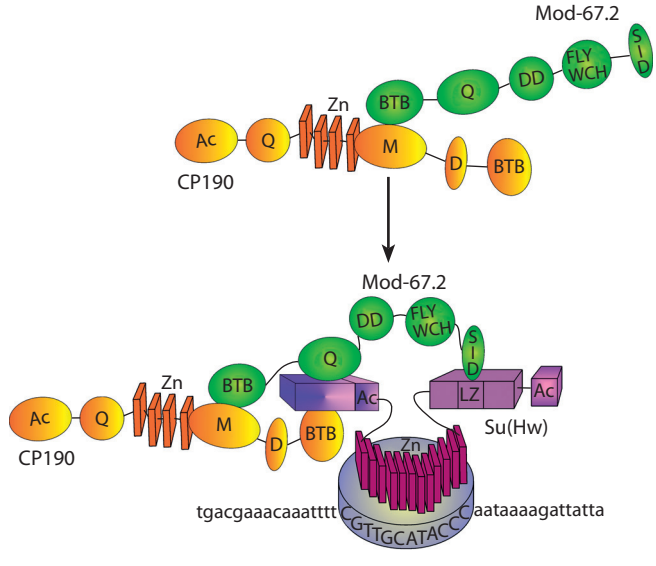

Fig. 1. Scheme of protein-protein interactions involved in formation of the (Su(Hw)/Mod(mdg4)-67.2/CP190) complex.

The CP190 domains are shown as yellow ovals and four zinc fingers, as yellow parallelepipeds; the Mod(mdg4)-67.2 domains are shown as green ovals; the Su(Hw) domains, as purple parallelepipeds. Bold capital letters indicate the Su(Hw) binding site. Domain abbreviations: Ac – acidic domains; Zn – zinc-finger domains; LZ – leucine zipper motif; BTB – BTB/POZ domain; Q – glutamine-rich (Q-rich) region; DD – dimerization domain; FLYWCH – FLYWCH-type zinc finger domain; SID – Su(Hw) interaction domain; D – aspartic acid-rich (D-rich) domain; M– centrosomal targeting domain.

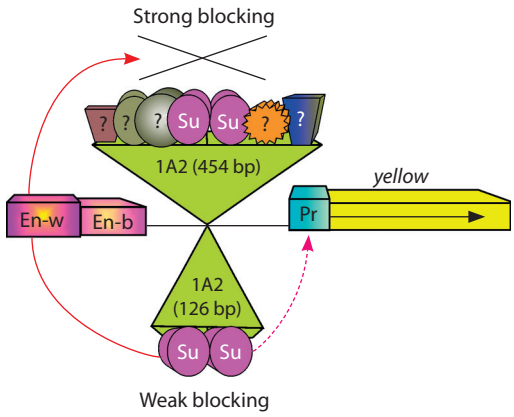

Fig. 2. The scheme of blocking the enhancer-promoter interactions with different fragments of 1A2 insulator in the model system of the *yellow* gene.

The boxes indicate the *yellow* promoter (Pr) and the wing (En-w) and body (En-b) enhancers. The 1A2 insertions are shown as triangles in which the purple ovals (Su) are the binding sites of the Su(Hw) protein and different figures with the question mark in them are unidentified proteins. The arrow indicates the direction of *yellow* gene transcription.

partially block the strong *yellow* enhancer, while the larger 454 bp fragment that includes the same Su(Hw) sites and neighboring sequences, completely blocks *yellow* enhancers. Thus, additional proteins binding to these sequences are required for strong insulator activity of the 1A2 insulator (Fig. 2).

It seems possible that in endogenous insulators, Su(Hw) cooperates with additional DNA-binding proteins to support insulator activity. Many endogenous Su(Hw)-dependent insulators were later identified and described (Parnell et al., 2006; Soshnev et al., 2011; Schwartz et al., 2012), suggesting that they play a significant role in gene regulation. Most of them have 2–4 binding sites for the Su(Hw) protein. They show different blocking activities, which do not directly correlate with the number of Su(Hw)-binding sites (Kuhn-Parnell et al., 2008). This fact is in line with the hypothesis that functionality of the Su(Hw) complex depends on the regulatory environment of Su(Hw) binding sites.

The mechanism of recruiting the Su(Hw) complexes to chromatin

As it follows from the limited experimental data, only some of the C_2H_2 domains of the Su(Hw) protein are usually involved in DNA binding, while the rest are involved in interactions with proteins or RNAs (Kim et al., 1996; Lei, Corces, 2006). Most of the genomic Su(Hw) binding regions have 2–4 consensus sites, while studies on transgenic lines have shown that only four reiterated binding sites for Su(Hw) can function effectively (Scott et al., 1999). Recent investigations suggest that the affinity of Su(Hw) for DNA and organization of the Su(Hw) complex may be dependent on the nature of the consensus site (Baxley et al., 2017). This model was described as the "Su(Hw) code", which assumed that Su(Hw) binds to a compound consensus sequence (approximately 26 nucleotide-long) consisting of three modules: the ZF6–ZF9 cluster binds to the main, central module; the ZF2–ZF4 cluster, to the downstream CG-rich module; and the ZF10–ZF12 cluster, to the upstream AT-rich module. The "Su(Hw) code" model predicts that the consensus site determines which of the Su(Hw) ZF domains are not involved in DNA binding and, hence, are free to interact with other proteins and/or complexes. In our recent study, we compared the influence of two mutations affecting the same tenth ZF (ZF10) of Su(Hw) protein on properties of *gypsy* insulator (Melnikova et al., 2018b). According to the "Su(Hw) code" model, *gypsy* insulator was mainly organized by the upstream and central modules (Baxley et al., 2017). We used the *su(Hw)^f* mutation involving a point substitution that affects the critical Zn-coordinating residue and thereby dramatically alters the structure of the C_2H_2 domain and leads to full deletion of ZF10 (Su(Hw)$^{\Delta 10}$). It was found that Su(Hw)^f becomes unable to interact with CP190 in the absence of DNA, while Su(Hw)$^{\Delta 10}$ restores the CP190–Su(Hw) interaction. These results suggest that the point mutation in ZF10 influencing the Su(Hw)^f conformation results in loss of interaction with CP190. The Su(Hw)$^{\Delta 10}$ mutant binds to the *gypsy* insulator better than Su(Hw)^f does and partially restores its enhancer-blocking activity but, in contrast to the wild-type "Su(Hw) protein", fails to interact with the *gypsy* insulator in the absence of Mod(mdg4)-67.2 protein *(mod(mdg4)^{u1}* mutant background) (Melnikova et al., 2018b). These results suggest that CP190 and Mod(mdg4)-67.2 are critical for binding of the mutant Su(Hw)$^{\Delta 10}$ to the *gypsy* insulator. Based on these findings we can draw a conclusion that both association with CP190 and Mod(mdg4)-67.2 partners and proper organization of DNA binding site are essential for efficient recruitment of the Su(Hw) complex.

Looping model describes the neutralization effect

However, in the transgenic experiments action of Su(Hw) insulator failed when two insulator sites were introduced at a distance from one another in the region between the enhancer and the promoter (Muravyova et al., 2001). The loss of insulator activity results from pairing between the two insulator complexes. "Looping out" of the sequences between the insulators brings the enhancer and promoter closer and may stimulate expression (Fig 3, *a*).

The insulator neutralization effects were observed only for the same type of insulators (Su(Hw)-Su(Hw)) but not for heterologous pairs (dCTCF-Su(Hw)) (Kyrchanova et al., 2008; Krivega et al., 2010). Such selectivity in insulator interaction implicates the existence of exclusive protein complexes organized on different insulator types. It is likely that these different complexes (despite the fact that all of them share the CP190 protein) are unable to interact with each other. Thus, restriction of interaction between the insulator elements may determinate the specificity of particular enhancer-promoter interaction.

The neutralization effect partially depends on tandem or opposite orientation of the tested insulator sites (Kyrchanova et al., 2008). Testing of the composite insulator fragment containing both dCTCF and Su(Hw) binding sites displayed strong dependence on orientation. Strong promoter stimulation was observed in the case of opposite orientation, while enhancer blocking was preserved in tandem orientation. These results demonstrate that loops having two different configurations and thus exhibiting different effects on enhancer activity can be formed depending on orientation of the insulator site (see Fig. 3, *b*). However, functional interaction between the

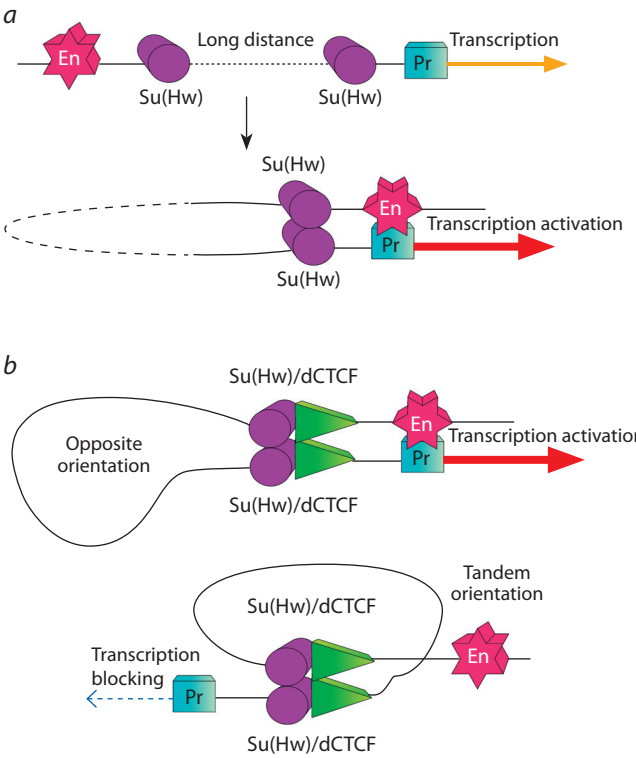

Fig. 3. The models of pairing between the insulators.

a – pairing of insulators (Su(Hw)) can provide the long-distance enhancer (En)–promoter (Pr) interactions; *b* – two models of pairing between the composed insulators (Su(Hw)/dCTCF) having either an opposite or the same orientation with respect to each other.

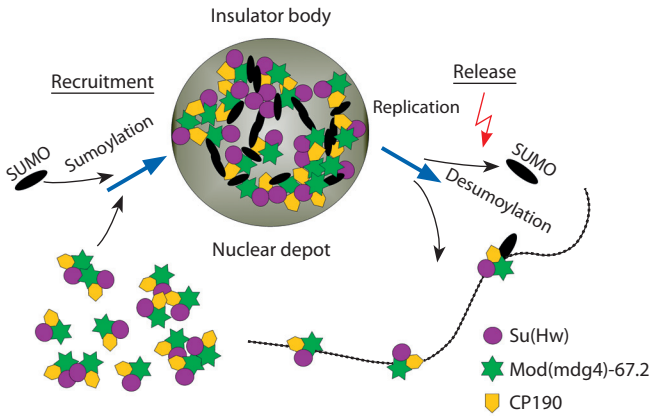

Fig. 4. The model describing the organization of insulator bodies (reproduced from (Golovnin et al., 2012)).

The circle, asterisk, and pentagon represent Mod(mdg4)-67.2, Su(Hw), and CP190 proteins, respectively; the black ovals are SUMO molecules; and the curve shows a chromatin fibril with insulators.

fragments containing only the Su(Hw) binding sites is less dependent on their relative orientation. It seems likely that the complex that can be formed on the Su(Hw) protein by Mod(mdg4)-67.2 and CP190 proteins is not sufficient to determine the long-range loop configuration. Thereby, in the context of endogenous Su(Hw) insulators, additional proteins located in the vicinity of the insulator site may determine the orientation-dependent interaction between insulators. Thus, the action of a particular insulator cannot be considered apart from its regulatory environment.

Direct regulation of gene expression by Su(Hw) insulators

Besides spatial organization of local enhancer-promoter communication, Su(Hw) insulator protein can behave as either a direct activator or repressor of transcription. It was shown that the *yellow* promoter weakened by deletion of the upstream regulatory region can be stimulated by the Su(Hw) protein in a distance-dependent manner. (Golovnin et al., 2005). Introduction of *su(Hw)*$^{-}$ background (*su(Hw)*^{v}/*su(Hw)*^{f}) completely abolishes the stimulation effect. This effect depends neither on the repression activity of neighboring regulatory elements nor on the boundary activity of Su(Hw) insulator as promoter stimulation was observed when the insulator was placed either upstream or downstream from the *yellow* promoter. This finding is in line with further studies that reported the stimulator effect of Su(Hw) on alcohol dehydrogenase promoter (Wei, Brennan, 2001) and weak *gypsy* promoter (Parkhurst, Corces, 1986; Smith, Corces, 1995). On the other hand, Su(Hw) may repress transcription of neural genes in ovaries in a tissue-specific manner (Soshnev et al., 2013). Hence, Su(Hw) protein may represent a platform that organizes the specific regulatory complex to provide particular activities.

"Maturation" of the insulator complex in the nucleus and it targeting to chromatin

The Su(Hw), Mod(mdg4)-67.2, and CP190 proteins in the interphase cell nucleus co-localize in discrete foci/speckles named "the insulator bodies" (Golovnin et al., 2007, 2008, 2012). It has been assumed that the "insulator bodies" arise via association of individual Su(Hw)-containing nucleoprotein complexes located at distant chromosomal sites, owing to interactions between the BTB domains of insulator proteins Mod(mdg4)-67.2 and CP190. Thus, it was supposed that the "insulator bodies" are responsible for insulator activity (Gerasimova et al., 1995). However, our results show that insulator bodies are rather aggregates of insulator proteins that comprise many unrelated proteins (Golovnin et al., 2008, 2012, 2015). Moreover, it was shown that the assembly of "insulator bodies" is determined by CP190 protein and sumolyation of Mod(mdg4)-67.2 (Golovnin et al., 2012). Sumolyation of Mod(mdg4)-67.2 is essential for incorporation of this protein and Su(Hw) into the "insulator bodies" but is dispensable for the stability of CP190-dependent speckles (Golovnin et al., 2012). The sumoylated Mod(mdg4)-67.2 and CP190 proteins interact with Su(Hw) and recruit it to the "insulator bodies". This protects the insulator complex from degradation. Moreover, the "insulator bodies" possibly facilitate the formation of complexes between Su(Hw)/Mod(mdg4)-67.2/CP190 and other transcription factors. "Mature" Su(Hw)-dependenent complexes may then transiently interact with chromatin fibril and be detached from the "insulator bodies" by means of desumoylation (Golovnin et al., 2008) (Fig. 4). Thus, the formation of "insulator bodies" does not directly correlate with insulator activity.

This model was confirmed by additional experiments with the EAST protein, which may serve as a structural basis for the

nuclear extrachromosomal compartment (Wasser, Chia, 2000). Altering EAST expression (overexpression or inactivation) affects the enhancer-blocking activity of the *gypsy* insulator, which, in its turn, directly correlates with the efficiency of Mod(mdg4)-67.2 and CP190 binding to the insulators (Melnikova et al., 2017b). There is no evidence that EAST directly binds to chromatin and to Su(Hw) insulators in particular under normal physiological conditions. However, by direct interacting with Mod(mdg4)-67.2 and CP190, EAST may also be directly involved in nucleation of "insulator bodies". The overexpression of EAST leads to segregation of the CP190 protein in independent speckles, thus inhibiting formation of "mature" insulator complexes (Golovnin et al., 2015). As a result, reduction of Mod(mdg4)-67.2 and CP190 proteins on the Su(Hw) binding sites and an increase in repression activity of Su(Hw) insulators were observed.

Conclusions

This review has summarized the results of the recent studies aimed to identify the mechanism of assembly and function of the Su(Hw)-dependent complex, one of the insulator/architectural complexes in *Drosophila*. We have demonstrated that activity of the Su(Hw) complex strongly depends regulatory elements in its vicinity and complex protein organization (tissues and stage of development in particular).

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