Interaction between the *Drosophila* heterochromatin proteins SUUR and HP1

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Summary

SUUR (Suppressor of Under-Replication) protein is responsible for late replication and, as a consequence, for DNA underreplication of intercalary and pericentric heterochromatin in *Drosophila melanogaster* polytene chromosomes. However, the mechanism by which SUUR slows down the replication process is not clear. To identify possible partners for SUUR we performed a yeast two-hybrid screen using full-length SUUR as bait. This identified HP1, the well-studied heterochromatin protein, as a strong SUUR interactor. Furthermore, we have determined that the central region of SUUR is necessary and sufficient for interaction with the C-terminal part of HP1, which contains the hinge and chromoshadow domains. In addition, recruitment of SUUR to ectopic HP1 sites on chromosomes provides evidence for their association in vivo. Indeed, we found that the distributions of SUUR and HP1 on polytene chromosomes are interdependent: both absence and overexpression of HP1 prevent SUUR from chromosomal binding, whereas SUUR overexpression causes redistribution of HP1 to numerous sites occupied by SUUR. Finally, HP1 binds to intercalary heterochromatin when histone methyltransferase activity of SU(VAR)3-9 is increased. We propose that interaction with HP1 is crucial for the association of SUUR with chromatin.

Supplementary material available online at
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Key words: Heterochromatin, HP1, SUUR, Polytene chromosomes, *Drosophila melanogaster*

Introduction

At least two pathways are involved in epigenetic maintenance of the repressed chromatin state: HP1-mediated silencing of pericentric heterochromatin and Polycomb group (PcG)-dependent silencing of intercalary heterochromatin. The best characterized proteins involved in the first pathway are HP1 (reviewed by Hiragami and Festenstein, 2005), SU(VAR)3-9 (reviewed by Schotta et al., 2003) and SU(VAR)3-7 (Delattre et al., 2004), which interact directly with each other (Delattre et al., 2000; Schotta et al., 2002). HP1 is encoded by the *Su(var)2-5* gene (also known as *Su(var)205*) and is a relatively small protein consisting of a conserved N-terminal chromodomain and a C-terminal chromoshadow domain, which are separated by a variable hinge region. All three domains of HP1 were shown to be involved in the interactions with HP1-associating proteins (reviewed by Hiragami and Festenstein, 2005). The second pathway is based on the PcG proteins that form at least three distinct multiprotein complexes: PRC1 (Saurin et al., 2001; Shao et al., 1999), PRC2 (Czermin et al., 2002; Müller et al., 2002; Tie et al., 2003) and PhoRC (Klymenko et al., 2006). There are differences between these two systems with regard to their components, mechanisms of assembly, spreading and maintenance. However, there are also notable common features. Both pathways involve methylation of histone tails and their subsequent stable association with other factors, which cause chromatin compaction and gene repression (reviewed by Brock and Fisher, 2005; Craig, 2005; Hediger and Gasser, 2006; Maison and Almouzni, 2004; Ringrose and Paro, 2004; Schwartz and Pirrotta, 2007). Another common feature of silent chromatin domains is late replication of DNA in the S-phase of cell cycle (Schübeler et al., 2002; Donaldson, 2005; Zhimulev and Belyaeva, 2003).

In *Drosophila* salivary gland polytene chromosomes, silent chromatin is cytologically observed as blocks of pericentric heterochromatin that fuse to form a common chromocenter and as a series of dense intercalary heterochromatin bands scattered throughout euchromatic chromosome arms. Although simple-sequence repeats and transposable elements make up pericentric heterochromatin (reviewed by Dillon and Festenstein, 2002; Maison and Almouzni, 2004), intercalary heterochromatin regions correspond to clusters of unique genes that are coordinately replicated and expressed (Belyakin et al., 2005). Heterochromatin regions are underrepresented in polytene chromosomes that appear on cytological preparations as chromosomal breaks (also called constrictions or weak points) (Zhimulev et al., 1982).

SUUR (Suppressor of Under-Replication) is a unique protein factor that contributes to the replication timing in *Drosophila*. When the *SuUR* gene is mutant, earlier completion of replication in heterochromatin regions is observed. This results in the suppression of DNA underreplication (UR), which manifests as the absence of breaks in polytene chromosomes (Belyaeva et al., 1998; Moshkin et al., 2001). Notably, the suppression of UR is absolute in intercalary heterochromatin and partial in pericentric heterochromatin. Conversely, extra doses of the *SuUR* gene enhance

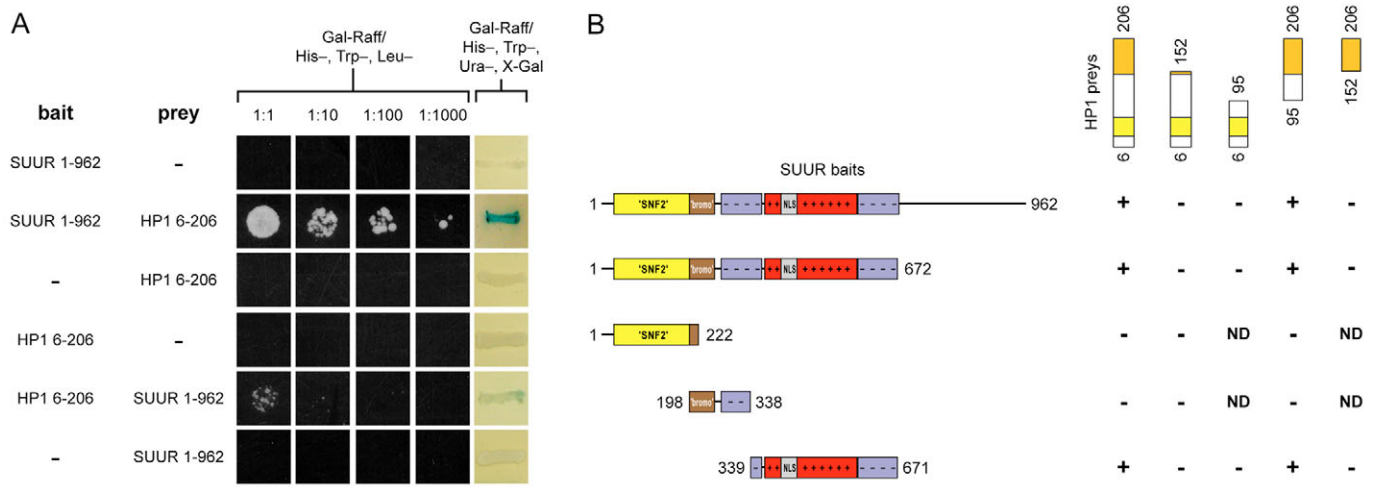

Fig. 1. Yeast two-hybrid analysis of interaction between SUUR and HP1. (A) Equal amounts of SKY191 cultures containing the constructs indicated in each case were planted at a density of 2×10^6 cells/ml (column 1) and serial tenfold dilutions (columns 2-4) on His–, Trp–, Leu– plates containing galactose and raffinose. Yeasts were grown for 7 days at 30°C. SKY191 cells containing pSH18-34 and indicated combinations of plasmids were streaked on His–, Trp–, Ura– plates containing galactose, raffinose and X-Gal (column 5) and incubated for 4 days at 30°C. (B) The SUUR bait deletion constructs were tested for interaction with the HP1 prey deletion constructs (Delattre et al., 2000). Minus and plus signs indicate the absence and presence of interaction between tested constructs, respectively; ND, no data. SUUR regions are shown: the homology region to the SNF2 domain is in yellow, the homology region to the bromodomain is in brown, the negatively charged amino acid clusters are in blue, the positively charged amino acid clusters are in red and the NLSs are in grey. In HP1 the chromodomain is indicated in yellow and the chromoshadow domain is in orange.

UR (Zhimulev et al., 2003a). Also, the *SuUR* mutation affects position effect variegation (PEV) silencing in a dose-dependent manner (Belyaeva et al., 2003). The *SuUR* gene is expressed throughout development, but peaks in embryos and in nurse cells of adult females. Previously, we demonstrated that a transient embryonic expression of a $SuUR^+$ transgene in *SuUR* homozygous mutants restores the frequency of chromosomal breaks to that of the wild type (Makunin et al., 2002). The deduced length of SUUR is 962 amino acids (AA); it contains homology regions to the SNF2 domain and bromodomain, positively and negatively charged AA clusters and presumptive nuclear localization signals (NLS; Fig. 1) (Makunin et al., 2002; Tchurikov et al., 2004). On polytene chromosomes, SUUR localizes both to pericentric and intercalary heterochromatin (Makunin et al., 2002) and to chromosome regions compacted as a result of PEV (Belyaeva et al., 2003), i.e. it is found in all late-replicating chromatin domains. More than 60% of the intercalary heterochromatin regions overlap with PcG binding sites (Zhimulev et al., 2003a), whereas pericentric heterochromatin represents the major localization territory for HP1 (James et al., 1989). Genome-wide analysis of SUUR targets in the *Drosophila* Kc cell line demonstrated the high correlation between SUUR and PcG binding and the medium correlation in SUUR and HP1 binding (Pindyurin et al., 2007).

The identification of SUUR partners could give a clue to the mechanism of its action. A comprehensive analysis of protein-protein interactions in *Drosophila* has previously been carried out by means of the yeast two-hybrid system. This detected over 20,000 interactions (Giot et al., 2003). The resulting constructed protein-interaction map indicated that SUUR only interacts with the product of the predicted *CG18563* gene (Giot et al., 2003). Here, we report that SUUR interact directly with HP1 in yeast two-hybrid and GST pull-down assays and that this interaction is mediated by the central region of SUUR, enriched in positively charged AA, and by the C-terminal part of HP1. The relevance of this interaction is strongly supported by the fact that ectopically localized HP1 can recruit SUUR. Moreover, association of SUUR with chromosomes depends on HP1: both absence and overexpression of HP1 prevent SUUR chromosome binding. Finally, complete colocalization of these proteins is observed upon overexpression of SUUR. Together, these data strongly suggest that the interaction between SUUR and HP1 is necessary for SUUR association with chromosomes and for its proper functioning.

Results

Identification of HP1 as an interaction partner of SUUR in a yeast two-hybrid system

We used a LexA-based yeast two-hybrid system (Golemis et al., 2002) to identify putative SUUR-interacting proteins. First, we found that the full-length SUUR (AA 1-962) bait construct self-activates the chromosomal *LEU2* reporter in the presence of six *lexA* operators in the promoter region of this gene in the commonly used yeast strain EGY48. This self-activation was significantly weaker with four *lexA* operators in the yeast strain SKY473 and negligible with two *lexA* operators in the yeast strain SKY191 (data not shown). Therefore, all subsequent experiments were performed in SKY191 cells. Using a cDNA library of 0-12-hour-old *Drosophila melanogaster* embryos, we screened approximately 300,000 yeast transformants and initially isolated 96 clones on Leu– plates. Out of them, 12 strongly activated the second reporter gene, *lacZ*. All the 12 positives interacted with the full-length SUUR bait in a galactose-dependent fashion upon retransformation and 11 turned out to be one single gene *Su(var)2-5*.

All *Su(var)2-5* clones contained almost the complete coding sequence for HP1 (AA 6-206, hereafter full-length HP1). Specificity of the interaction was confirmed by additional tests depicted on Fig. 1A; the growth on selective medium lacking leucine required both the SUUR bait and HP1 prey fusion proteins. Although the HP1 bait fusion protein inhibits yeast growth (data not shown), we could observe an activation of both reporters when co-expressing the full-length HP1 bait and SUUR prey fusions (Fig. 1A) in the reciprocal two-hybrid test.

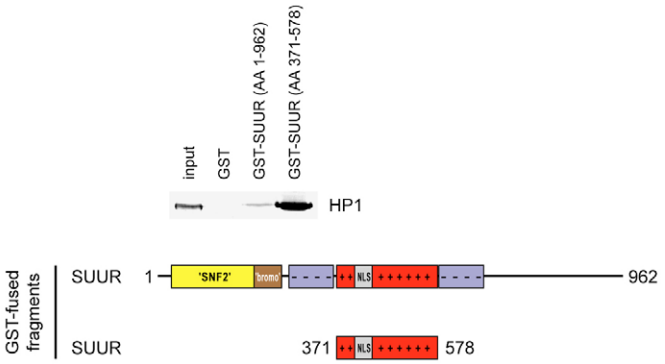

Fig. 2. SUUR interacts with HP1 in vitro. The ability of the full-length SUUR and its central portion (AA 371-578) to recruit HP1 from *Drosophila* embryo nuclear extracts was tested by GST pull-down assays. GST alone, GST-SUUR (AA 1-962) and GST-SUUR (AA 371-578) were produced in bacteria and immobilized on glutathione-Sepharose beads. The beads were then incubated with nuclear extracts of wild-type *Drosophila* embryos. Bound proteins were washed, resolved by SDS-PAGE, and transferred to nitrocellulose. The blot was probed with antibodies against HP1. The amount in the input column is 5% of the amount applied to beads. SUUR constructs employed in the experiment are schematically depicted below the blot; the homology region to the SNF2 domain is in yellow, the homology region to the bromodomain is in brown, the negatively charged amino acid clusters are in blue, the positively charged amino acid clusters are in red, the NLSs are in grey.

To delimit the domain of SUUR involved in the interaction with HP1, we generated and tested a number of SUUR deletion mutant baits (Fig. 1B). First, we found that the SUUR fragment encompassing AA 1-672 retains the ability to interact with HP1. Its subsequent dissection demonstrated that the binding activity is localized to the central portion of SUUR (AA 339-671) containing the clusters of the positively and negatively charged AA and presumptive NLSs (Fig. 1B, and supplementary material Fig. S1). The N-terminal part of the protein (AA 1-338), including the homology regions to the SNF2 domain (AA 28-198) (Makunin et al., 2002) and to the bromodomain (AA 199-255) (Tchurikov et al., 2004), seems not to be required for the interaction, since corresponding bait constructs (AA 1-222 and AA 198-338) do not interact with HP1 (Fig. 1B and supplementary material Fig. S1).

Next, we identified the HP1 portion required for interaction with SUUR using several previously described HP1 deletion mutant preys (Delattre et al., 2000). No interaction was observed between SUUR and the following HP1 fragments: AA 6-95, AA 6-152 and AA 152-206. The C-terminal part of HP1 (AA 95-206) containing most of the hinge region and the entire chromoshadow domain specifically interacted with SUUR constructs (Fig. 1B and supplementary material Fig. S1). Apparently, the central region of SUUR (AA 339-671) and the C-terminal part of HP1 (AA 95-205) are necessary and sufficient for the interaction between these proteins in yeast.

Since in any yeast two-hybrid library screen there is a possibility of obtaining 'false negatives', we examined the interactions of the full-length SUUR with some its putative partners [SU(VAR)3-7, SU(VAR)3-9, Polycomb (PC) and SUUR itself] in the additional direct yeast two-hybrid tests and, indeed, found no interaction (data not shown).

The central positively charged region of SUUR interacts with HP1 in vitro

To confirm that HP1 can be a partner of SUUR, in vitro pull-down assays were performed. Equal amounts of bacterially expressed GST-SUUR (AA 1-962) fusion protein or GST alone were immobilized on glutathione beads and later incubated with nuclear extracts prepared from 0-12-hour-old *Drosophila melanogaster* embryos. Bound proteins were then examined for the presence of HP1 by immunoblotting. HP1 was clearly detected on GST-SUUR (AA 1-962) beads, whereas no interaction of HP1 with GST alone was observed (Fig. 2). We next explored whether the central region of SUUR is involved in this interaction. Indeed, we found that the central positively charged region of SUUR (AA 371-578) is able to pull-down HP1 even more efficiently than the full-length SUUR (Fig. 2). However, it is important to note that the difference in the binding affinities of the full-length and the truncated SUUR GST-fused proteins for HP1 might be due to partial degradation of the former protein (data not shown). As such, these results confirm our data obtained using the yeast two-hybrid assay.

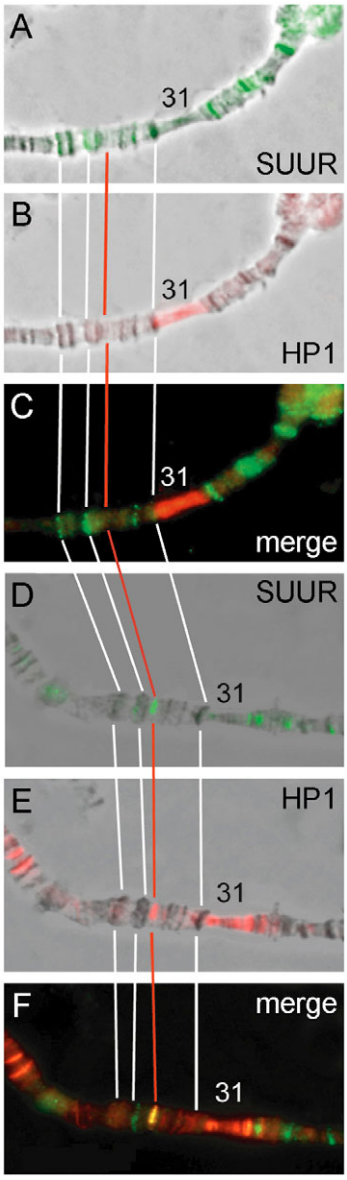

Fig. 3. Recruitment of SUUR to ectopically localized HP1. Immunolocalization of HP1 (red) and SUUR (green) in polytene chromosomes of *hs-GAL4-HP1*/*Wink-D* transheterozygotes before (A-C) and after (D-F) heat-shock-induced expression of GAL4-HP1.

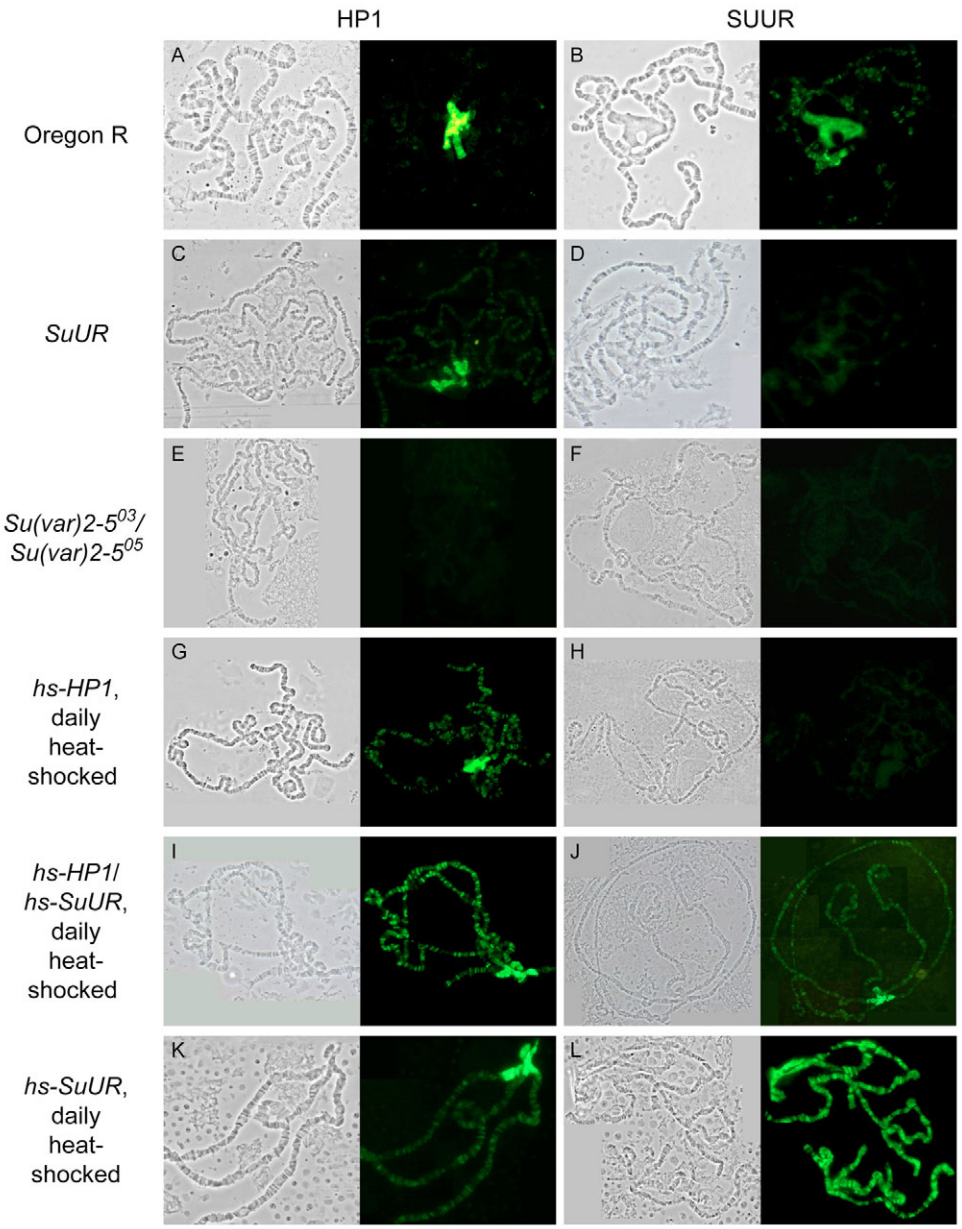

Fig. 4. Distributions of SUUR and HP1 on chromosomes are interdependent. Salivary gland polytene chromosomes from the indicated *Drosophila* strains were stained with antibodies against HP1 (column 2) and SUUR (column 4). Corresponding phase-contrast images are shown in columns 1 and 3.

Recruitment of SUUR to ectopically localized HP1

To address whether SUUR and HP1 can interact in vivo, we used a fusion gene *hs-GAL4-HP1* containing the HP1 sequence (AA 10-206) fused to the DNA-binding domain of yeast GAL4 protein and placed under the control of the heat-inducible *hsp70* promoter (Seum et al., 2000). The GAL4-HP1 fusion protein is targeted to an artificial chromosomal site by means of the *Winkelried* transposon, equipped with binding sites for the GAL4 DNA-binding domain (UAS) (Seum et al., 2000). In our experiments, we used the line *Wink-D* bearing insertion of the *Winkelried* transposon at the chromosome region 30B (Seum et al., 2000), where HP1 and SUUR are not normally detected. Also, neither HP1 nor SUUR was present at the chromosomal region 30B of *hs-GAL4-HP1*/*Wink-D* transheterozygous larvae reared at the non-permissive temperature (Fig. 3A-C). By contrast, after a single heat-shock treatment, we detected both proteins co-localizing at region 30B (Fig. 3D-F). Analogous results were obtained when the *Wink-A* line, with insertion of the *Winkelried* transposon in a different chromosome region, was used (data not shown). Thus, the recruitment of SUUR to the ectopic site upon expression of the GAL4-HP1 suggests that SUUR is either interacting directly with HP1 or is a part of an HP1-containing complex. The lack of the complete overlap between SUUR and HP1 could be due to insufficient availability of the GAL4-HP1 fusion protein in the endogenous HP1 binding sites. In addition, HP1 is known to be involved in the transcriptional activation of many genes in the genome (e.g. genes within region 31) (Cryderman et al., 2005; Hediger and Gasser, 2006) that might be incompatible with SUUR binding, since the latter protein is normally associated exclusively with the chromocenter and intercalary heterochromatin regions (Makunin et al., 2002).

SUUR does not bind to chromosomes in *Su(var)2-5* mutants

In order to evaluate the biological significance of this newly discovered interaction, we examined the effects of *Su(var)2-5* and *SuUR* mutations on the chromosome-wide distribution of SUUR and HP1 by immunofluorescent staining of the polytene chromosomes. As noted previously, in a wild-type background, HP1 shows predominant staining in pericentric heterochromatin, including a banded pattern on the fourth chromosome, and minor staining of several euchromatic sites (Fig. 4A) (James et al., 1989), whereas SUUR protein can be detected in pericentric heterochromatin, the nucleolus and intercalary heterochromatin regions (Fig. 4B) (Makunin et al., 2002). We found that HP1 distribution is unchanged in the *SuUR* mutant line (Fig. 4C), where SUUR is not detected on chromosomes (Fig. 4D). At the same time, SUUR was not detected in chromosomes of *Su(var)2-5*^{03}/*Su(var)2-5*^{05} heterozygotes (HP1 null mutants that die at the end of the third instar larval stage when the maternally supplied product is exhausted; Fig. 4E,F). However, the frequencies of chromosomal breaks, which reflect the degree of UR, observed in a number of marker intercalary heterochromatin regions in *Su(var)2-5*^{03}/*Su(var)2-5*^{05} heterozygotes were not significantly different from

Table. 1. Frequencies of breaks in the intercalary heterochromatin regions of salivary gland polytene chromosomes upon expression of different dosage of HP1 and PC

Experiment	Frequencies of breaks in the intercalary heterochromatin regions (%)							
	3C	11A	19E	39E	42B	64C	75C	89E
Oregon R 25°C	15±2	93±5	78±4	100	64±5	64±5	90±4	74±4
Oregon R daily heat shock	13±2	88±3	84±4	100	59±4	62±5	92±3	67±4
Su(var)2-5^{03}/ Su(var)2-5^{05} 25°C	14±4	89±4	81±5	100	44±3	62±4	86±3	69±4
hs-HP1/hs-HP1 25°C	18±4	91±3	71±5	100	55±5	69±6	92±3	74±5
hs-HP1/+ daily heat shock	0	35±4	25±4	79±4	32±4	2±1	21±3	3±1
hs-HP1/hs-HP1 daily heat shock	5±3	54±6	21±6	79±5	27±5	5±2	25±4	2±1
hs-GAL4-HP1/ hs-GAL4-HP1 daily heat shock	4±2	56±5	20±5	100	17±3	10±2	50±4	14±3
hs-Pc/hs-Pc daily heat shock	19±2	91±4	89±5	100	69±5	68±4	89±3	67±5

those in the wild-type Oregon R strain (Table 1). Given that the formation of chromosomal breaks is determined during the early embryonic development (Belyaeva et al., 2006; Makunin et al., 2002), it is likely that *Su(var)2-5^{03}/Su(var)2-5^{05}* heterozygotes embryos have enough maternally supplied HP1 to recruit SUUR to chromosomes. Thus, our data suggest that HP1 is required for SUUR association with chromosomes.

Overexpression of HP1 displaces SUUR from chromosomes

To further investigate the ability of HP1 to recruit SUUR, we examined the SUUR binding to chromosomes after HP1 overexpression. To this end, we used the *P[(neo^{r})HSHP1.83C]* (hereafter *hs-HP1*) transgene consisting of the complete HP1 coding sequence placed downstream of the heat-inducible *hsp70* promoter (Eissenberg and Hartnett, 1993). This transgene noticeably increases the level of HP1 upon heat-shock induction (Eissenberg and Hartnett, 1993). In the absence of heat-shock treatment, localization of SUUR on polytene chromosomes of the *hs-HP1* line was normal and indistinguishable from the wild-type pattern. When this line was subjected to daily heat-shock treatments starting from the embryo stage, HP1 was revealed in many chromosomal sites (Fig. 4G) including all previously described intercalary heterochromatin regions (Zhimulev et al., 2003a). Contrary to our expectations, we did not detect any association of SUUR with chromosomes under these experimental conditions (Fig. 4H). The same result was obtained after daily heat-shock treatments of *hs-GAL4-HP1/Wink-D* transheterozygotes (Fig. 5). For comparison, we analyzed the chromosomal distribution of PC and SU(VAR)3-7, proteins specific for intercalary and pericentric heterochromatin, respectively. No changes in the PC distribution along chromosomes were found, whereas SU(VAR)3-7 was delocalized to the ectopic sites in the euchromatic arms of chromosomes (data not shown) as was reported previously (Delattre et al., 2000).

To ensure that repeated induction of the *hs-HP1* transgene expression leads to the absence of SUUR on chromosomes starting from the early stages of development, we made use of the ability of the *SuUR* mutation to cause a suppression of UR in intercalary heterochromatin regions (Belyaeva et al., 1998; Belyakin et al., 2005; Moshkin et al., 2001). In the absence of heat shock, the frequencies of chromosomal breaks observed in homo- and heterozygous *hs-HP1* lines were not significantly different from those in the wild-type Oregon R strain. However, we found that daily heat-shock inductions of the *hs-HP1* expression cause a stable reduction of the break frequencies. In some regions, such as 89E, the breaks were no longer observed (Table 1). The same effect was observed upon daily heat-shock induced overexpression of GAL4-HP1 (Table 1). It should be mentioned that a daily heat-shock treatment per se neither alters the frequency of breaks in intercalary heterochromatin (Table 1) nor affects UR (Belyaeva et al., 2006). Using quantitative Southern blot hybridization as well as in-gel hybridization, we demonstrated that DNA polytenization of the *abd-A* gene located in the zone of UR in the region 89E (Belyakin et al., 2005; Moshkin et al., 2001) is increased 4.3-fold by daily heat-shock induction of the *hs-HP1* expression (Fig. 6 and data not shown). These data indicate that overexpression of HP1 starting early in development drives SUUR depletion from chromosomes and that, in turn, results in phenocopying of the *SuUR* mutation.

We have previously generated the transgenic stock *H7* (hereafter *hs-SuUR*) bearing the *hs-SuUR* construct in which *SuUR* expression is under the control of the *hsp70* promoter (Makunin et al., 2002). Surprisingly, combined overexpression of HP1 and SUUR in *hs-HP1/hs-SuUR* transheterozygotes restored SUUR binding to chromosomes (Fig. 4I,J). This suggests that SUUR is able to bind to chromosomes in the presence of large amounts of HP1, but this might require a certain ratio of HP1 to SUUR molecules.

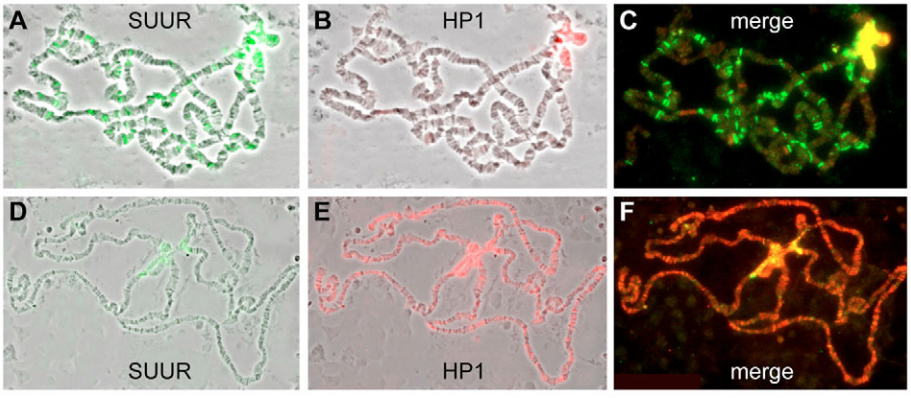

Fig. 5. Repeated overexpression of GAL4-HP1 results in the displacement of SUUR from chromosomes. SUUR (green) and HP1 (red) localization patterns were detected by immunofluorescence staining of polytene chromosomes from control (non-heat-shocked, A-C) and experimental (heat-shocked, D-F) *hs-GAL4-HP1/Wink-D* transheterozygous larvae.

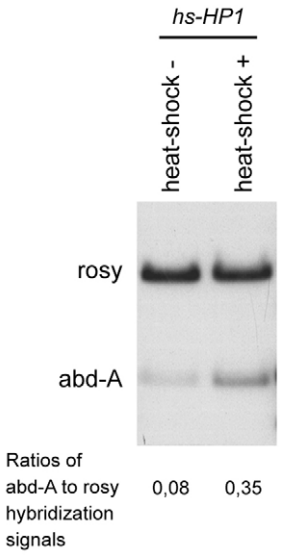

Fig. 6. Overexpression of HP1 leads to *SuUR* mutant phenotype. UR level of the region 89E in salivary glands polytene chromosomes of the *hs-HP1* homozygous was measured without (lane 1) and with (lane 2) heat-shock treatments. The Southern blot was hybridized with the abd-A and rosy (control) probes; the ratios of abd-A-specific hybridization signals to rosy-specific hybridization signals are given below the blot.

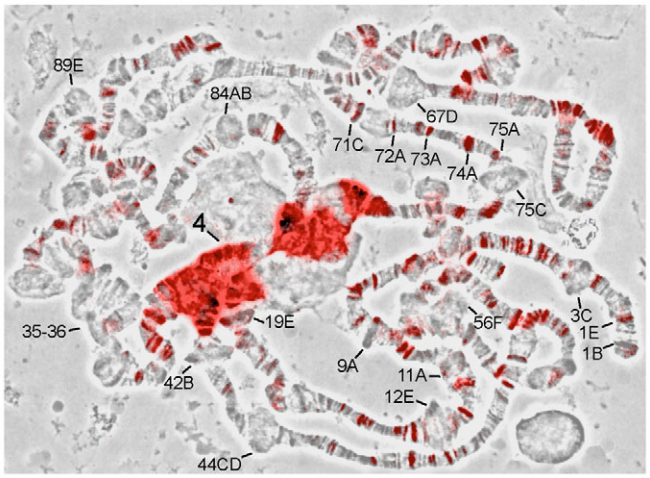

Fig. 7. Overexpression of full-length SUUR under the control of the *Sgs3*-GAL4 driver results in HP1 redistribution to the intercalary heterochromatin regions of polytene chromosomes. Sites of swellings (Zhimulev et al., 2003b) and the fourth chromosome are indicated.

HP1 is codistributed with overexpressed N-terminus of SUUR

Heat-shock treatment of the *hs-SuUR* line resulted in the appearance of SUUR and HP1 in numerous sites in the euchromatic arms of chromosomes (Fig. 4K,L). The same results were obtained when SUUR was overexpressed under the control of the *Sgs3*-GAL4 driver that becomes active exclusively in the salivary glands at the mid-third transition (Fig. 7). It should be emphasized that the total amount of HP1 in cells was the same (supplementary material Fig. S2A). By expressing SUUR under the control of the *arm*-GAL4 driver that provides mosaic expression in salivary glands, we observed direct correlation between the numbers of SUUR and HP1 sites (supplementary material Fig. S2B). Furthermore, these proteins demonstrated complete colocalization on chromosomes (supplementary material Fig. S2B). Thus, SUUR can influence HP1 association with chromosomes under certain conditions.

To delimit the portion of SUUR that is responsible for the redistribution of HP1 along chromosomes, we made use of recently characterized N- and C-terminal truncations of SUUR (Kolesnikova et al., 2005). In fact, three constructs, namely rs4 (AA 1-599), rs5 (AA 1-779) and C17 (AA 495-962) were investigated. As was shown before, the binding pattern of the first two SUUR truncations to chromosomes upon overexpression resembles that of the full-length protein under the same conditions. By contrast, the latter SUUR truncation shows non-specific continuous binding to chromosomes on overexpression (Kolesnikova et al., 2005). We expressed these constructs under the control of the *Sgs3*-GAL4 driver in the *SuUR* mutant background and established that both rs4 and rs5 were capable of driving HP1 redistribution in the same manner as full-length SUUR (Fig. 8A; data not shown for rs5), whereas C17 was not (Fig. 8B). Bearing in mind the yeast two-hybrid and GST pull-down data, these results strongly suggest that HP1-binding activity of SUUR resides in the middle part of the protein (AA 371-578).

Expression of *SuUR* gene is not regulated by HP1

Since the absence of SUUR on chromosomes of both *Su(var)2-5*^{03}*/Su(var)2-5*^{05} heterozygotes and the heat-shocked *hs-HP1* line might be conditioned by downregulation of the *SuUR* gene by HP1, we analysed *SuUR* expression in salivary glands in these particular conditions by non-quantitative RT-PCR analysis. The results demonstrated that *SuUR* expression is independent of the amount of the *Su(var)2-5* transcripts and vice versa (Fig. 9). This suggests that the SUUR protein is most probably present in these cells, but is unable to bind to chromosomes.

Colocalization of HP1 and SUUR in *Su(var)3-9*^{ptn} mutant

Normally, SU(VAR)3-9 is almost exclusively associated with the chromocenter and the fourth chromosome (Schotta et al., 2002). The hypermorphic *Su(var)3-9*^{ptn} mutation (Kuhfittig et al., 2001) induces defined ectopic binding of SU(VAR)3-9 and HP1 to a large number of euchromatic sites (reviewed by Schotta et al., 2003). We found that the SUUR binding pattern in the *Su(var)3-9*^{ptn} strain (Fig. 10A) is identical to that previously described for the stock carrying four *SuUR*$^{+}$ copies (Zhimulev et al., 2003a). At the same time, ectopic HP1 localization observed in the *Su(var)3-9*^{ptn} background coincides well with SUUR localization (Fig. 10B). Thus, intercalary heterochromatin regions seem to be attractive for HP1 binding when histone methyltransferase activity of SU(VAR)3-9 is increased.

SUUR binds to chromosomes independently of PC

It has previously been demonstrated that the *SuUR* mutation does not alter the localization pattern of PC (Zhimulev et al., 2003a). As we found, neither does *hs-SuUR* nor *hs-HP1* overexpression. Furthermore, no difference in SUUR binding and, therefore, the frequencies of chromosomal breaks were observed between the wild-type Oregon R strain and the *Pc-ORF F15* line, in which overexpression of PC had been induced by daily heat-shock treatments (Table 1). Taken together, these data indicate that bindings of PC and SUUR to chromosomes are not interconnected.

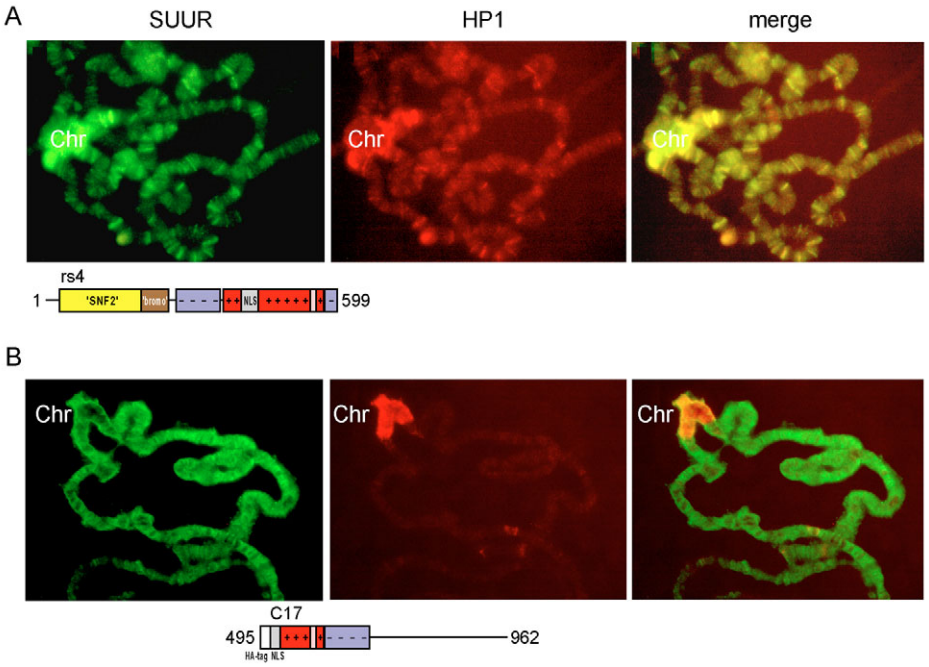

Fig. 8. Overexpressed N-terminal portion of SUUR recruits HP1 to ectopic sites. Immunofluorescent staining of polytene chromosomes with antibodies against SUUR (green) and HP1 (red) after overexpression of the N-terminal SUUR fragment rs4 (A) and of the C-terminal SUUR fragment C17 (B) under the control of the *Sgs3*-GAL4 driver in the *SuUR* mutant background. Schematics of the rs4 and C17 constructs are depicted below the corresponding panels; the homology region to the SNF2 domain is in yellow, the homology region to the bromodomain is in brown, the negatively charged amino acid clusters are in blue, the positively charged amino acid clusters are in red, the NLSs are in grey and the HA-tag is in white.

Discussion

Interaction between SUUR and HP1 in a yeast two-hybrid assay

The *Drosophila* HP1 (also known as HP1a) protein has been reported to self-dimerize (Badugu et al., 2005; Smothers and Henikoff, 2000; Zhao et al., 2001) and interact directly with a number of proteins including SU(VAR)3-7 (Delattre et al., 2000), SU(VAR)3-9 (Schotta et al., 2002), HP2 (Shaffer et al., 2002), HP3, HP4, HP5 (Giot et al., 2003; Greil et al., 2007), HP1/ORC-associated protein (HOAP) (Badugu et al., 2003) and components of the origin recognition complex (Pak et al., 1997). Here, we have identified HP1 as an interacting partner of SUUR in a yeast two-hybrid screen. Since the SUUR-binding activity of HP1 is located in its C-terminal portion (AA 95-206), which is also implicated in the binding to SU(VAR)3-7 (Delattre et al., 2000), SU(VAR)3-9 (Schotta et al., 2002), HP2 (Shaffer et al., 2002) and HOAP (Badugu et al., 2003), we can speculate that SUUR might compete with these proteins for the association with HP1 during heterochromatin formation. A consensus pentapeptide that specifically interacts with the HP1 chromoshadow domain has previously been defined by use of a phage display assay (Smothers and Henikoff, 2000). Subsequently, this pentapeptide was found in the HP1 chromoshadow domain itself as well as in several other HP1-interacting proteins. Point mutations in the HP1-interacting motif PRVSL of mouse TIF1β (substitution of RV with EE) completely abolish its interaction with HP1 (Ryan et al., 1999). Within the SUUR region shown to interact with HP1 (AA 371-578), we found the sequence LRVSL (AA 429-433) with a high similarity to the motif of mouse TIF1β and to the peptide LRVML detected in the phage display assay (Smothers and Henikoff, 2000). It would be interesting to examine the effect of such mutations in the SUUR protein on its ability to bind HP1. The interaction of SUUR with HP1 points to the possibility of SUUR interaction with the other proteins from the HP1 family [HP1b, HP1c (Smothers and Henikoff, 2001), HP1d (also referred to as Rhino) (Vermaak et al., 2005; Volpe et al., 2001) and HP1e (Vermaak et al., 2005)] and with HP6 consisting only of the chromoshadow domain (Greil et al., 2007). It is necessary to mention that we did not detect the previously reported interaction between the full-length SUUR and CG18563 gene product (Giot et al., 2003) in our yeast two-hybrid assay. This discrepancy might arise from differences between the GAL4- and LexA-based yeast two-hybrid systems that were employed (Stanyon et al., 2004). Therefore, additional experiments are required to shed light on this putative partner of SUUR.

Since two-hybrid interactions take place within the yeast nucleus, we cannot completely exclude the possibility that some endogenous yeast protein mediates the observed interaction between SUUR and HP1. Future in vivo FRET experiments will help in clarifying the nature of this interaction. At the same time, the lack of interaction between SUUR and PC in the yeast interaction trap system is consistent with our findings of their independent binding to polytene chromosomes. Therefore, the association of SUUR and PC with many common sites/genes on chromosomes (Pindyurin et al., 2007;

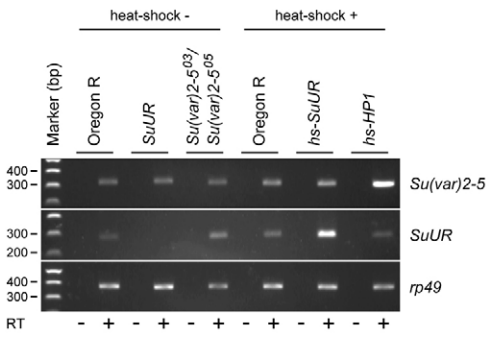

Fig. 9. *SuUR* and *Su(var)2-5* genes are expressed independently. RT-PCR analysis of *SuUR*, *Su(var)2-5* and *rp49* (control) expression levels in salivary glands of wild-type OregonR, *SuUR*, *Su(var)2-5*^{03}/*Su(var)2-5*^{05} heterozygotes (HP1 null mutants), *hs-SuUR* (SUUR overexpression) and *hs-HP1* (HP1 overexpression) strains. Minus and plus signs indicate the absence and presence of reverse transcriptase in RT-PCR reaction, respectively. Transcription of *Su(var)2-5* gene in *Su(var)2-5*^{03}/*Su(var)2-5*^{05} heterozygotes is not abolished because the *Su(var)2-5*^{05} mutation is caused by a dinucleotide deletion in the coding region (Eissenberg et al., 1992); the molecular lesion associated with the *Su(var)2-5*^{03} mutation is unknown.

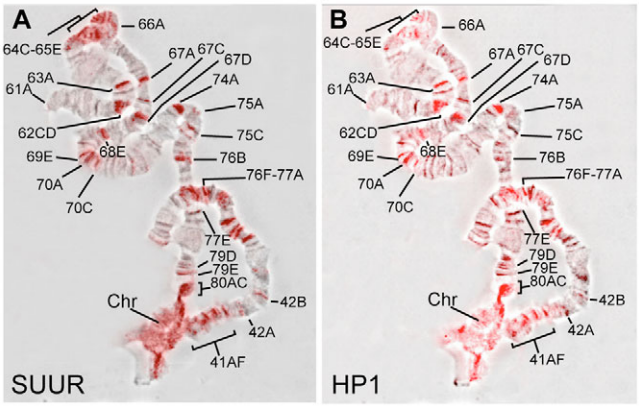

Fig. 10. High similarity between the localization patterns of SUUR (A) and HP1 (B) on chromosome arm 3L of a *Su(var)3-9*^{ptn}/+ heterozygous larva.

Zhimulev et al., 2003a) is still enigmatic. The simplest explanation of this phenomenon is a presence of a common partner, which recruits both proteins independently. This may be a particular histone modification or another chromatin protein, e.g. a member of the large Polycomb group.

HP1 is required for SUUR binding to chromosomes

In the majority of immunostaining experiments HP1 is found in the chromocenter, region 31, a few minor euchromatic sites and frequently at telomeres on wild-type salivary gland polytene chromosomes (Fig. 4A) (James et al., 1989; Shaffer et al., 2002). In addition to this, Pimpinelli and co-workers detected HP1 in about a further 200 sites, 30% of which correspond to intercalary heterochromatin regions (Fanti et al., 2003). Using a DamID assay, which is known to be much more sensitive to the amount of protein, HP1 was recently mapped to the multiple sites on the chromosomes of *Drosophila* Kc cells (Greil et al., 2007; Pindyurin et al., 2007) revealing a 46% overlap with SUUR targets (Pindyurin et al., 2007). Here, we show that HP1, when repeatedly overexpressed, occupies all intercalary heterochromatin regions and SUUR can no longer be observed on chromosomes. We also show that HP1 highly overlaps with SUUR in *Su(var)3-9*^{ptn} mutants, which display many ectopic sites for SU(VAR)3-9 (Kuhfittig et al., 2001) and enhanced methylation at H3-K9 (Ebert et al., 2004) in the arms of polytene chromosomes. It was proposed that this hypermorphic *Su(var)3-9*^{ptn} mutation allows the detection of weak native binding sites of SU(VAR)3-9 (Ebert et al., 2004). Taken together, these data indicate that marginal amounts of HP1, which directly binds to both the methylated H3-K9 and SU(VAR)3-9, might be present in most intercalary heterochromatin regions in wild-type chromosomes. Also, HP1 might be associated with a part of these regions only transiently during the initiation of silencing (Verschure et al., 2005) and therefore is not routinely detected there. Since we observed ectopic recruitment of both HP1 and SUUR to the *Winkelried* transposon, we speculate that HP1 could directly recruit SUUR to all binding sites of the latter. Possibly, transient interaction between these proteins during the cell cycle progression is enough to anchor SUUR in the chromatin. Consistent with this idea, in HP1 null mutants, SUUR is properly associated with chromosomes with the help of maternally supplied HP1 at embryonic stages that finally results in chromosomal breaks. Decreasing amounts of HP1 during development leads to the gradual loss of SUUR associated with chromosomes. This situation is aggravated with each cell cycle (or endo cycle in polytene cells) and finally SUUR is completely absent from polytene chromosomes of third instar *Su(var)2-5*^{03}/*Su(var)2-5*^{05} heterozygous larvae. However, SUUR is likely to still be present in the nuclei, since the transcription of its gene is not affected.

The HP1-dependent chromosomal binding of SUUR could also be caused by several other mechanisms. For example, HP1 might be necessary for some post-translational modification of SUUR, which in turn could be required for its binding to chromosomes. Indeed, SUUR migrated slower than expected on western blots (Makunin et al., 2002) indicating the possibility of some modification(s). It is interesting to speculate that SUUR is modified by some component of an HP1-containing complex. Intriguingly, mammalian TIF1α, TIF1β and Pim-1 kinases directly interact with and phosphorylate the HP1 family proteins (Koike et al., 2000; Nielsen et al., 1999). Alternatively, an indirect pathway might be involved, for example, participation of some mediator protein that is downregulated in HP1 null mutants. Finally, SUUR might recognize and bind to a repressed chromatin structures formed by other components of pericentric and intercalary heterochromatin in a topological manner.

The interactions of HP1 with HOAP (Badugu et al., 2003) and the components of the origin recognition complex (Pak et al., 1997) also raise the possibility of its role as a linker between SUUR and the DNA replication apparatus, the progression of which is believed to be slowed-down by SUUR in heterochromatin.

SUUR and HP1 behave differently in response to overexpression of each other

Given that SUUR is an HP1-interacting protein, its disappearance from chromosomes upon repeated HP1 overexpression appears very unusual. The observed concomitant *SuUR* mutant phenotype, namely the suppression of UR in intercalary heterochromatin regions, is most probably a consequence of SUUR removal from chromatin. It seems that SUUR leaves chromatin as soon as HP1 is overexpressed during embryogenesis. However, the underlying mechanism is unclear. One explanation of this phenomenon is that the amount of overexpressed HP1 might be so high that SUUR could be completely depleted by the pool of chromosome-unbound HP1. Apart from this, HP1 is known to form homodimers (Badugu et al., 2005; Zhao et al., 2001) via the PRMVI motif in its chromoshadow domain (Smothers and Henikoff, 2000), which is the candidate for interaction with SUUR. With an excess of HP1, the formation of the homodimers might dominate the interaction between HP1 and SUUR, therefore preventing SUUR binding to chromosomes. We assume that association of SU(VAR)3-7 with chromosomes upon overexpression of HP1 might be mediated by one of its HP1-interacting regions that does not contain the established pentapeptide consensus (Delattre et al., 2000). Both these ideas are supported by the fact that overexpression of both HP1 and SUUR partly restores chromosomal binding of SUUR, thereby emphasizing the importance of a certain proportion in numbers of these molecules. In view of the fact that hypo- and hyper-phosphorylated HP1 isoforms possess different binding activities (Badugu et al., 2005; Huang et al., 1998), it is also possible that the phosphorylation level of overexpressed HP1 is not suited for interaction with SUUR. Finally, one cannot exclude the possibility that SUUR might be degraded upon repeated HP1 overexpression.

Our observation that overexpression of SUUR causes ectopic binding of HP1 along the chromosomes supports the idea of the direct association between these proteins, since their colocalization

together with a constant amount of HP1 in the cells were detected. Moreover, using different SUUR truncations, we revealed that the N-terminal portion of SUUR (AA 1-599) is enough to induce this redistribution of HP1, which is consistent with the results of the yeast two-hybrid and GST pull-down experiments.

The *SuUR* mutation is known to have influence on PEV, although its effect is weaker than that of *Su(var)2-5* (Belyaeva et al., 2003). The data described in this study suggest that the effects of *Su(var)2-5* mutations on PEV might be partially conditioned by the absence of SUUR on chromosomes. By contrast, enhancement of PEV upon overexpression of SUUR (Belyaeva et al., 2003) might be slightly attenuated by the decrease of the local concentration of HP1 in pericentric heterochromatin after its redistribution to the euchromatic arms of chromosomes.

Materials and Methods

Yeast two-hybrid screen and interaction tests

All two-hybrid plasmid vectors and yeast strains used in this study are part of the LexA-based dual bait two-hybrid system (Golemis et al., 2002; Serebriiskii et al., 1999). Plasmids were introduced into yeast cells by lithium acetate transformation (Becker and Lundblad, 2002). The two-hybrid screen was performed in the yeast strain SKY191 using an embryonic *Drosophila* cDNA library RFLY1 (a gift from Pierre Spierer, University of Geneva, Geneva, Switzerland) (Finley, Jr et al., 1996). Activation of the chromosomal *LEU2* reporter was tested on medium lacking histidine, tryptophan and leucine, and supplemented with 2% galactose and 1% raffinose. Activation of a *lacZ* reporter from the pSH18-34 plasmid was tested on medium lacking histidine, tryptophan and uracil, and containing 80 μg/ml X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), 2% galactose and 1% raffinose. No growth was observed on the same medium supplemented with 2% glucose, which suppresses the expression of the prey fusion proteins.

Vectors pJK202 (kindly provided by Ilya Serebriiskii, Fox Chase Cancer Center, Philadelphia, USA) and pJG4-5 (kindly provided by Pierre Spierer, University of Geneva, Geneva, Switzerland) were slightly modified to introduce a unique *Sac*II restriction site in their polylinker regions: the sequences between *Eco*RI and *Xho*I sites of both vectors were replaced by double-stranded DNA fragments composed of oligonucleotides TH_MCSs (5′-aattcccgcggtctgagggcccc-3′) and TH_MCSa (5′-tcgaggggccctcagaccgcggg-3′). This yielded pJK202-S2 and pJG4-5-S2 plasmids, respectively. To obtain full-length SUUR bait and prey constructs, pJK202-SUUR and pJG4-5-SUUR, a 2896 bp *Sac*II-*Sac*II fragment bearing the *SuUR* open reading frame was excised from pBS-S2-SUUR-S2 (Pindyurin et al., 2007) and cloned correspondingly into pJK202-S2 and pJG4-5-S2 plasmids in the correct orientation.

To generate the pJK202-SUUR 1-222 construct, the pJK202-SUUR plasmid was digested with *Xho*I and then religated. To obtain the pJK202-SUUR 1-672 construct, the pJK202-SUUR plasmid was digested with *Bam*HI and *Sal*I, and ligated with double-stranded DNA linker composed of oligonucleotides BS672_s (5′-gatccatcgcagtgag-3′) and BS672_a (5′-tcgactcactgcgatg-3′). The pJK202-SUUR 198-338 construct was obtained as follows. A DNA fragment containing the appropriate region of *SuUR* open reading frame was PCR-amplified from a f40 cDNA clone (Makunin et al., 2002) using the primers SB198_s (5′-aagtcgacggtatcgat-aagctttgcgagcttc-3′) and SB338_a (5′-ttgatctgcatcaggttctactg-3′). After digestion with *Sal*I and *Bsa*BI (which recognize the sequences underlined in the primers), a 437 bp *Sal*I-*Bsa*BI fragment was ligated to the pJK202-S2 vector that had been digested with *Sal*I, filled in with Klenow fragment, and digested with *Xho*I. To obtain the pJK202-SUUR 339-671 construct, a 991 bp *Bsa*BI-*Bam*HI fragment from the f40 cDNA clone (Makunin et al., 2002) was ligated to the pJK202 vector that had been digested with *Eco*RI, filled in with Klenow fragment, and digested with *Bam*HI.

The full-length HP1 (AA 6-206) prey construct, pJG4-5-HP1, was isolated from the embryonic *Drosophila* RFLY1 cDNA library. To obtain the full-length HP1 bait construct, pJK202-HP1, an 859 bp *Eco*RI-*Xho*I fragment from pJG4-5-HP1 was inserted into the corresponding sites of the pJK202 vector. The SU(VAR)3-7 baits (AA 189-844 and AA 736-1169) (Delattre et al., 2000), SU(VAR)3-9 baits (AA 1-569 and AA 81-635) (Schotta et al., 2002) and HP1 deletion mutant preys (Delattre et al., 2000) were kindly provided by Pierre Spierer (University of Geneva, Geneva, Switzerland) and Gunter Reuter (Martin-Luther University Halle-Wittenberg, Halle, Germany).

The full-length *Pc* open reading frame was PCR-amplified from the appropriate template (kindly provided by Vincenzo Pirrotta, Rutgers University, Piscataway, USA) using the primers Pc_ATG (5′-ttgaattcatgactggtcgaggcaag-3′) and Pc_Stop (5′-ttctcgagtcaagctactggcgac-3′). After digestion with *Eco*RI and *Xho*I, the DNA fragment was inserted into the corresponding sites of the pJG4-5 vector to produce the pJG4-5-Pc prey construct.

The *CG18563* open reading frame (264 bp) was RT-PCR-amplified (see below) with specific primers SPshort_d (5′-ttgaattcatgaggaatccgattctgag-3′) and SPshort_r (5′-ttctcgagctaccacccaggaatatagac-3′) from a pool of total RNA isolated from all developmental stages of wild-type *Drosophila*. After digestion with *Eco*RI and *Xho*I, the DNA fragment was inserted into the corresponding sites of the pJG4-5 vector to produce the pJG4-5-CG18563 prey construct.

All plasmids were verified by sequencing. Also, all bait fusions were verified to be capable of binding LexA operator sequences by repression assay (Golemis et al., 2002).

GST pull-down assay and western blots

A DNA fragment containing the full-length *SuUR* open reading frame was amplified by PCR from the f40 clone (Makunin et al., 2002) using the primers Sma_ATG (5′-ttttcccgggctgcagcatg-3′) and Sal_Ad (5′-ttttgtcgactcacttgaacagttccaatcg-3′). The product was digested with *Sma*I and *Sal*I and cloned into the corresponding sites of the pGEX 4T-1 vector (Amersham Biosciences, Piscataway, USA) to produce the pGEX 4T-SUUR (AA 1-962) construct, which was sequenced to confirm the absence of mutations. The pGEX 4T-SUUR (AA 371-578) construct was described previously (Makunin et al., 2002). Recombinant proteins were expressed in *Escherichia coli* BL21-CodonPlus-RIL (Stratagene, La Jolla, USA) and purified by standard procedures.

The *Drosophila* nuclear extracts were prepared as described previously (Kadonaga, 1990). The GST pull-down experiments were performed as described previously (Jimenez et al., 1999) with the following modifications. Binding reactions were carried out in binding buffer [20 mM Hepes-KOH (pH 7.6), 2.5 mM $MgCl_2$, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT, 0.1% NP-40]. Unbound proteins were removed with a series of washes with wash buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 300 mM NaCl, 0.1% NP-40]. Bound proteins were resolved by SDS-PAGE.

Western blots were performed according to standard procedures using mouse monoclonal anti-HP1 (C1A9; 1:1000) (James and Elgin, 1986) and anti-tubulin antibodies (Bx69; 1:5000; a gift from Harold Saumweber, Humboldt University, Berlin, Germany).

Fly stocks

Flies were reared on standard medium at 18°C, 25°C or 29°C. Oregon R and yw^{67} stocks were used as wild-type controls. The *SuUR* mutation was described previously (Belyaeva et al., 1998; Makunin et al., 2002) and the *Su(var)2-5* alleles were obtained from Joel C. Eissenberg (Saint Louis University School of Medicine, St Louis, USA). The *H7* stock carries two *hs-SuUR* transposons on the *SuUR* mutant background (Makunin et al., 2002). The *hs-HP1* stock homozygous for the *P[(neo^r)HSHP1.83C]* construct (Eissenberg and Hartnett, 1993) was provided by Joel C. Eissenberg (Saint Louis University School of Medicine, St Louis, USA). The lines with the *hs-GAL4-HP1* and *Winkelried* transposons (Seum et al., 2000) were obtained from Pierre Spierer (University of Geneva, Geneva, Switzerland). The *Pc-ORF F15* line bearing the *hs-Pc* transgene, in which *Pc* expression is under the control of the *hsp70* promoter, was obtained from Renato Paro (University of Heidelberg, Heidelberg, Germany).

Induction of heat-shock-driven expression was performed in two ways. In the first regimen, a heat shock of 37°C for 40 minutes was given daily starting with 5-6-hour-old embryos and continuing until the late third instar larval stage. Alternatively, a single heat shock (37°C for 40 minutes) was applied to late third instar larvae. For immunostaining of salivary gland polytene chromosomes and other analyses, larvae were allowed to recover for 3 hours after the final heat-shock treatment, and then dissected. The control and experimental progeny were derived from the same parents.

To express the full-length SUUR and its fragments rs4, rs5 and C17 (Kolesnikova et al., 2005), the GAL4>UAS system (Brand and Perrimon, 1993) was used. *arm*-GAL4 activates variegated expression in salivary glands, whereas *Sgs3*-GAL4 drives strong expression in salivary glands starting from the mid-third instar larval stage (Kolesnikova et al., 2005). Larvae were kept at 18°C until mid-third instar larval stage when they were placed at 29°C to enhance GAL4 activity.

Immunofluorescent staining of polytene chromosomes

Indirect immunofluorescent staining of *Drosophila* polytene chromosomes was performed as described previously (Zhimulev et al., 2003a) with some modifications. Salivary glands were fixed in 0.1 M NaCl, 2 mM KCl, 10 mM NaH_2PO_4, 1% Tween 20, 1.7% formaldehyde for 1 minute, then in 45% acetic acid, 3.7% formaldehyde for 3 minutes and squashed. Slides were washed in PBS, 0.1% Tween 20 and incubated overnight at 4°C in a humid chamber with primary antibodies in PBS, 0.1% Tween 20, 0.1% bovine serum albumin. The following primary antibodies and dilutions were used: rabbit polyclonal anti-SUUR (1:50) (Makunin et al., 2002), mouse monoclonal anti-HP1 (C1A9; 1:100; a gift from Sarah Elgin, Washington University, St Louis, USA) (James and Elgin, 1986), rabbit polyclonal anti-SU(VAR)3-7 (1:100; a gift from Pierre Spierer, University of Geneva, Geneva, Switzerland) (Cléard et al., 1997), rabbit polyclonal anti-PC (1:600; a gift from Vincenzo Pirrotta, Rutgers University, Piscataway, USA) (Poux et al., 2001). After three washes in PBS, 0.1% Tween 20 (5 minutes each wash), the secondary antibodies were added: FITC- or Rhodamine-labeled goat anti-rabbit and/or anti-mouse IgG-specific conjugates (1:200; Abcam, UK). After 1 hour incubation at room temperature, slides were washed three times in PBS, 0.1% Tween 20 (5 minutes each wash) and mounted in Vectashield antifade mounting medium (Vector Laboratories, USA).

Quantitative Southern blot hybridization

Quantitative Southern blot hybridization and hybridization done directly within agarose gels (in-gel hybridization) were performed as described previously (Glaser and Spradling, 1994; Moshkin et al., 2001). Genomic DNA was purified from 100 pairs of salivary glands of third instar larvae and was digested with *Eco*RI. A 2.3 kb probe for the *abd-A* locus was PCR-amplified using the primers dem83t18 (5′-tggaagtgcgaaacaaat-3′) and dem83a18 (5′-cagcgaacggaatacaga-3′). A 1.1 kb *Kpn*I-*Bam*HI *rosy* fragment was used as control. Equal amounts of the probes were mixed before hybridization.

RT-PCR

The RT-PCR analysis was performed on total RNA from third instar larvae salivary glands using the AccessQuick™ RT-PCR System (Promega, UK) according to the manufacturer's directions. The following pairs of primers were used to detect the presence of transcripts: RtSuUR1 (5′-gaacgcaaccttcgaatgg-3′) and RtSuUR2 (5′-tcacttgaacagttccaatcgc-3′) for *SuUR*, HP1_1 (5′-aggaaactcaaggacgc-3′) and HP1_2 (5′-tcagagtaccaggataggc-3′) for *Su(var)2-5*, rp49d_2749 (5′-caggcccaagatcgtgaag-3′) and rp49r_2750 (5′-tgagaacgcaggcgacc-3′) for *rp49*.

We thank Ilya Serebriiskii and Pierre Spierer for supplying the yeast strains and the plasmid vectors of the yeast interaction trap system; Pierre Spierer for an embryonic *Drosophila* cDNA library RFLY1, the HP1 deletion prey constructs, SU(VAR)3-7 bait constructs, anti-SU(VAR)3-7 antibodies and the lines with *hs-GAL4-HP1* and *Winkelried* transposons; Gunter Reuter for SU(VAR)3-9 bait constructs; Joel Eissenberg for the *Su(var)2-5* mutants and the *hs-HP1* transgenic line; Vincenzo Pirrotta for the *Pc* cDNA clone and anti-PC antibodies; Sarah Elgin for anti-HP1 antibodies; Renato Paro for the *hs-Pc* transgenic line; Harold Saumweber for anti-tubulin antibodies; Fotis Kafatos, George Christophides and Peter Verrijzer for their gifts of reagents; Stepan Belyakin for valuable discussions; and Gillian Chalkley for correcting the English in the manuscript. The work was supported by the Russian Foundation for Basic Research Grants #06-04-49305-a and #06-04-48387-a, the Interdisciplinary Integration Project of SB RAS #45, the Molecular and Cellular Biology RAS Program #10.1, the Russian Federation President Grant #MK-540.2007.4 and the Russian Federation Government Contracts #02.512.11.2065 and 02.512.11.2145.

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