Research article

Corto and DSP1 interact and bind to a maintenance element of the Scr Hox gene: understanding the role of Enhancers of trithorax and Polycomb

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Abstract

Background: Polycomb-group genes (PcG) encode proteins that maintain homeotic (Hox) gene repression throughout development. Conversely, trithorax-group (trxG) genes encode positive factors required for maintenance of long term Hox gene activation. Both kinds of factors bind chromatin regions called maintenance elements (ME). Our previous work has shown that corto, which codes for a chromodomain protein, and dsp1, which codes for an HMGB protein, belong to a class of genes called the Enhancers of trithorax and Polycomb (ETP) that interact with both PcG and trxG. Moreover, dsp1 interacts with the Hox gene Scr, the DSP1 protein is present on a Scr ME in S2 cells but not in embryos. To understand better the role of ETP, we addressed genetic and molecular interactions between corto and dsp1.

Results: We show that Corto and DSP1 proteins co-localize at 91 sites on polytene chromosomes and co-immunoprecipitate in embryos. They interact directly through the DSP1 HMG-boxes and the amino-part of Corto, which contains a chromodomain. In order to search for a common target, we performed a genetic interaction analysis. We observed that corto mutants suppressed dsp1 sex comb phenotypes and enhanced AntpScx phenotypes, suggesting that corto and dsp1 are simultaneously involved in the regulation of Scr. Using chromatin immunoprecipitation of the Scr ME, we found that Corto was present on this ME both in Drosophila S2 cells and in embryos, whereas DSP1 was present only in S2 cells.

Conclusion: Our results reveal that the proteins Corto and DSP1 are differently recruited to a Scr ME depending on whether the ME is active, as seen in S2 cells, or inactive, as in most embryonic cells. The presence of a given combination of ETPs on an ME would control the recruitment of either PcG or TrxG complexes, propagating the silenced or active state.
Background

Many transcription factors are expressed only transiently during development. After they have disappeared, the patterns of gene expression they have induced must be inherited by daughter cells. In eukaryotes, two groups of proteins, the Polycomb-group (PcG) and the Trithorax-group (TrxG), fulfill this memory function. Their existence was first revealed in *Drosophila melanogaster* where homeotic gene (Hox) expression is established in early embryos by the transient Gap and Pair-Rule transcription factors and controlled by PcG and TrxG proteins during the rest of development [1-3]. The PcG and TrxG proteins combine into several heteromic complexes that bind chromatin. PcG complexes maintain Hox gene silencing whereas TrxG complexes counteract the action of PcG complexes (reviewed in [4]). These proteins regulate many other genes such as *engrailed* [5,6], *ph* [7], *fork head* [8] and the *iroquouis*-complex [9].

In *Drosophila*, at least two PcG complexes called PRC2 and PRC1 (Polycomb Repressive Complex) and two TrxG complexes (TAC1 and BRM) have been biochemically purified (reviewed in [10]). The PRC1 complex contains the PcG proteins Polycomb (PC), Polyhomeotic (PH), Posterior Sex Combs (PSC) and dRING1, and additional polypeptides such as the DNA binding protein Zeste [11,12]. The PRC2 complex contains the PcG proteins Extra Sex Combs (ESC), SU(Z)12 and Enhancer of Zeste (E(Z)), a histone methyltransferase that methylates lysine 27 of histone H3 (H3K27me3), and the histone binding protein p55 [13,14]. PC is also found in another complex that contains the DNA binding protein Pipsqueak [15], ESC and E(Z) are found in a larger complex that contains Polycomb-like (PC1), the histone deacetylase RPD3 and p55 [16], and E(Z) is found in a complex that contains the deacetylase SIR2 [17]. The TrxG complex TAC1 contains CBP, a member of the CBP/p300 histone acetyl transferase (HAT) family, the anti-phosphatase SBF1, and the TrxG protein TRX, which is homologous to mammalian MLL/ALL and methylates histone H3 on lysine 4 (H3K4me) [18-20]. Lastly, the BRM complex contains the TrxG proteins BRM, Osa and Moira. It is related to the yeast Swi/Snf ATP-dependent chromatin remodeling complex, sharing four polypeptides with it, among which is the ATPase BRM [21]. It also contains BAP111, an HMGB protein that binds nonspecifically in the minor groove of the double-helix, thus bending the DNA [22,23].

In *Drosophila*, the PcG and TrxG proteins bind overlapping sequences called Polycomb/Trithorax Response Elements (PRE/TREs) (reviewed in [4]). Two PRE/TREs (*Fab-7* and the Hedgehog PRE/TRE) have been demonstrated to be true maintenance elements (ME), i.e. to control the maintenance of activation or repression of target loci through cell division [24,25]. A major issue is to understand how PcG and TrxG complexes are specifically recruited to ME. On the one hand, the specific recruitment of these complexes could be achieved by recognition of posttranslationally modified histone tails. For example, PC recognizes K27 methylation of H3 via its chromodomain. Hence, PRC1 may be recruited to chromatin by the recognition of the H3K27me3 mark laid down by PRC2 through E(Z) [26]. Furthermore, the methyl-transferase activity of E(Z) is required for correct repression of Hox genes, suggesting that this histone modification is actually related to gene regulation [14]. On the other hand, DNA binding proteins such as Zeste, GAF (GAGA factor), PHO (Pleiohomeotic), the HMG-box protein DSP1 (Dorsal Switch Protein 1) and Pipsqueak, which are present at some ME, also seem to be involved in the specific recruitment of PcG and TrxG [15,27-31]. Indeed, their various combinations and associations with co-factors could make complex binding more specific.

In a large screen to identify modifiers of *trxG* mutations, 6 genes previously identified as PcG genes (*Asx, E(z), E(Pc), Psc, Scm and Su(z)2*) were isolated as enhancers of *trxG* phenotypes [32]. The authors suggested that the corresponding genes be renamed "Enhancers of trithorax and Polycomb* (ETP) to account for their role both in repression and activation of Hox genes. Further studies identified other ETP proteins, among which is GAF [29,33], or PHO, which directly binds a PC-containing complex as well as the BRM complex [34]. The chromodomain protein Corto [35,36] and the HMGB protein DSP1 [37] also behave as ETP. Indeed, a loss-of-function allele of *corto* enhances the macrochaete phenotype of the *trxG* gene *osa* as well as the *Polycomb* phenotype of the *PcG* genes *mxc, Pc, Pcl, ph* and the ETP genes *E(z)* and *Scm* [35,38]. On the other hand, a *dsp1* null allele enhances the haltere to wing transformation of several *trxG* mutants *ash1, brm, osa, trx*, whereas male hemizygotes for this allele exhibit transformation of the A4 segment into a more posterior one, which is a *Polycomb* phenotype [37]. Thus, *dsp1* also behaves like an *ETP*. To date, little information regarding the mode of action of ETP has become available. No ETP has yet been found in TrxG complexes. Some of them belong to PcG complexes (such as *E(z), Psc* and *SCM*) but most of them (such as *ASX, Corto, GAF* and *SU(Z)2*) have not been found in PcG complexes to date. However, in embryonic extracts, Corto co-immunoprecipitates with ESC and PC, while SU(Z)2 and GAF co-immunoprecipitate with PC, suggesting that these ETPs can transiently interact with PcG complexes [29,36,39]. How ETPs promote either the activation or the repression of a defined target gene is an open question.

To understand better the role of ETP, we analyzed the interaction between Corto and DSP1. We show that both proteins co-localize at 91 sites on *Oregon-R* polytene chro-
mosomes. These include 84B, which corresponds to the distal part of the ANTP-complex. In addition, Corto and DSP1 co-immunoprecipitate in embryonic extracts and directly interact through the HMG-boxes of DSP1. Moreover, we show that the corto and dsp1 genes interact and that corto, like dsp1, is directly involved in the regulation of the Hox gene Scr. Chromatin immunoprecipitation experiments indicate that Corto, like DSP1, localizes on a 10 kb-XbaI ME of the Scr cis-regulatory sequences. On the basis of our results, we propose that both proteins interact on

Figure 1
Immunofluorescence detection of Corto and DSP1 on polytene chromosomes. (A-C) Simultaneous detection of Corto (green) and DSP1 (red) on Oregon-R polytene chromosomes stained with DAPI (blue). (D, E) Magnification of chromosome 2L end of Oregon-R (D) or dsp1 (E) labeled with anti-Corto (green) and anti-DSP1 (red). Sites shared by DSP1 and Corto in Oregon-R are yellow.
chromatin to regulate a subset of common targets including Scr.

Results

**DSP1 and Corto co-localize on polytene chromosomes**

To address the genomic targets shared by Corto and DSP1, we first examined their binding on polytene chromosomes. Corto was previously shown to bind polytene chromosomes at many discrete euchromatic sites and to share these binding sites partially with PcG proteins such as PC and PH, or with ETPs such as E(Z), SCM and GAF [36]. Similarly, DSP1 binds polytene chromosomes at multiple loci and partially co-localizes with PH [31]. Simultaneous detection of Corto and DSP1 on chromosomes enabled us to reveal many overlapping sites (Figure 1A–C). Precise localization of Corto and DSP1 on the polytene chromosomes of Oregon-R flies revealed 270 euchromatic sites of antibody staining for Corto and 173 for DSP1. These sites are listed in Figure 2 and compared with the PC sites previously determined using the same strain and staining conditions [40]. On Oregon-R polytene chromosomes, 91 sites are shared by Corto and DSP1. Moreover, PC shares 40 sites with Corto and 42 sites with DSP1. Lastly, 24 sites are simultaneously occupied by the three proteins, notably 84B, which corresponds to the distal part of the ANTP-complex.

To examine whether DSP1 plays a role in Corto recruitment, we looked at the localization of Corto in the dsp11 homozygote mutant strain, which is devoid of DSP1 protein [37]. No modification of Corto binding sites was observed in this strain, indicating that the DNA-binding
protein DSP1 is dispensable for Corto recruitment to chromatin (Figure 1D–E).

**DSP1 and Corto are parts of a common complex in embryos and interact directly**

We also investigated whether Corto and DSP1 belong to a same molecular complex in vivo by performing co-immunoprecipitation assays using extracts from 0–14 hour-old embryos. As shown in Figure 3, Corto co-immunoprecipitated with DSP1, indicating that DSP1 and Corto are physically associated in embryos.

Next, we asked whether DSP1 and Corto interact directly. First, DSP1 was submitted to far-western analysis using full-length radiolabeled Corto as a probe. The 386 amino acid DSP1 protein exhibits two polyglutamine series located in the 1–155 region, two HMG-boxes (HMG-A box: amino acids 171 to 246; HMG-B box: amino acids 258 to 336) and an acidic tail. As shown in Figure 4A, Corto was retained on full-length DSP1. In order to identify the domain(s) of interaction in the DSP1 protein, we used several truncated forms of DSP1. Neither deletion of the NH2-terminal polyglutamine regions nor that of the COOH-terminal acidic tail impaired Corto binding (see B22, C8, D16, E5, F33, J11, M2). In contrast, Corto did not bind to G81, L5 or N4. Our results allowed us to conclude that the minimal DSP1 sequence needed for Corto binding was an intact HMG-box preceded by twelve amino acids (F33 and J11 rather than G81 or L5).

The reverse experiment, i. e. migration and transfer of the GST-Corto fusion proteins incubated with radiolabeled DSP1, gave no conclusive results owing to the difficulty in renaturing Corto. We showed previously that the only noticeable domain of the 550 amino acid Corto protein is a chromodomain located in the NH2-terminal half (aminoacids 107 to 203) [36]. In order to characterize the Corto domains interacting with DSP1, we performed GST pull-down experiments using full-length or truncated forms of Corto fused to GST and radiolabeled full-length DSP1. The results are shown in Figure 4B. DSP1 was retained by the full-length Corto protein, corroborating the far-western results with DSP1. Moreover, DSP1 was retained by a GST fusion protein containing the NH2-terminal half of Corto (GST-C1/324), but was not detectably retained on the COOH-terminal half (GST-C325/550 and GST-C440/550). Interestingly, DSP1 was not retained on the Corto chromodomain (GST-C127/203).

Taken together, these results indicate that, in vitro, DSP1 and Corto directly interact through either the HMG-box A or the HMG-box B of DSP1 on the one hand, and the amino-terminal half of Corto on the other.

**corto and dsp1 interact genetically and participate in Sex comb reduced (Scr) regulation**

To determine whether *corto* and *dsp1* are involved in common functions, we analyzed their genetic interactions. *dsp1*1 hemizygous males present several homeotic transformations, notably a partial transformation of T1 to T2 leg as shown by a reduced sex comb (average size 6 teeth, rather than the 10 to 11 in a wild-type strain), and a partial transformation of A6 to A5 as shown by the presence of bristles on the A6 sternite (25% of *dsp1*1 males exhibit this phenotype) [37]. These phenotypes are related to a role of *dsp1* in the regulation of *Scr* and *AbdB*, respectively. We observed that loss of *corto* strongly suppresses both homeotic phenotypes (Table 1). Together, these data sug-
Figure 4  
Corto and DSP1 interact in vitro. (A) Far-western assays. Left, top: Coomassie-stained SDS-PAGE of the MBP-DSP1 fusion proteins. Left, bottom: Phosphorimager scan of the membrane after transfer of the proteins and incubation with radiolabeled Corto. Right: Schematic representation of DSP1 and DSP1 truncated forms (orange: HMG-A and HMG-B boxes; yellow: polyglutamine series and acidic tail). Corto is retained on MBP-DSP1 and on B22, C8, D16, E5, F33, J11 and M2 MBP-DSP1 truncated forms but not on G81, L5 and N4 MBP-DSP1 truncated forms. (B) GST pull-down assays Left, top: Coomassie blue staining of GST and GST-Corto fusion proteins (labeled with asteriks). Left, bottom: Autoradiography. 35S-labeled DSP1 was retained on GST-Corto and GST-C1/324 proteins and not on GST-C325/550, GST-C127/203 or GST-C440/550. Input: 1/5 of the total radioactivity was loaded. Note that the full-length DSP1 protein as well as the truncated forms (degradation products or abortive translations) are retained on GST-Corto and GST-C1/324. Right: Schematic representation of Corto (blue: chromodomain).
suggest that corto is involved with dsp1 in Hox gene regulation. Notably, they suggest that both corto and dsp1 regulate Scr in the T1 leg imaginal disc.

Interestingly, dsp1 was previously shown to cause partial suppression of the gain-of-function allele Scr3 [37]. Better to understand the relationship between corto and dsp1 in the regulation of Scr, we analyzed genetic interactions between corto and Scr. We first used two loss-of-function alleles of Scr, the EMS-induced Scr allele and Df(3R)Scr4, a deficiency of the distal end of the Scr cis-regulatory regions. Males heterozygous for either allele (Scr+/+ or Df(3R)Scr4/+) exhibit a reduction in the size of the sex comb on the first leg with an average number of 6.5 teeth per comb. No modification of this kind was observed in Scr/corto or Df(3R)Scr4/corto males (data not shown). We next checked the interactions between corto and the gain-of-function allele Scr Antp (Table 2). In this allele, the insertion of a transposable element near the Antp P1 promoter disturbs Scr silencing in the T2 and T3 leg imaginal discs [3]. Indeed, 39% of AntpScs/ males exhibited an ectopic sex comb on the T2 leg, i.e. a transformation of T2 leg into T1 leg. This percentage increased to 83%, 95% or 100%, depending on the corto allele, in AntpScs/corto males (Table 2). These results show that corto, like Pcg genes, participates in the maintenance of Scr repression in the T2 and T3 leg imaginal discs and corroborates our previous results showing that corto interacts with some Pcg genes for this ectopic sex comb phenotype [35,38].

### Table 1: Genetic interactions between dsp1 and corto

| Genotype          | Legs observed | average sex comb teeth on the T1 leg | Males observed | A6 to A5 (%) |
|-------------------|---------------|--------------------------------------|----------------|--------------|
| +/Y               | 52            | 11.1                                 | 36             | 0            |
| dsp1+/Y ; +/+     | 40            | 6.0                                  | 46             | 25           |
| +/Y, corto420/+   | 61            | 10.5                                 | 37             | 0            |
| +/Y, corto07128/+ | 48            | 11.2                                 | 34             | 0            |
| dsp1+/Y, corto420+ | 113          | 9.0                                  | 68             | 0            |
| dsp1+/Y, corto07128+ | 31         | 9.0                                  | 69             | 3            |

Homologous dsp1 females were crossed with either corto+_TM3 or corto+07128_TM3 males, or with Oregon-R males as controls. The resulting male progeny was scored for two homeotic transformations previously observed in dsp1 mutants: (i) transformation of T1 leg into T2 leg monitored by the size of the sex comb, (ii) transformation of segment A6 into segment A5 as revealed by the presence of bristles on A6 sternites.

### Discussion

We report in the present work that the two ETPs corto and dsp1 interact genetically and that the proteins they encode (i) directly interact in vitro, (ii) co-immunoprecipitate in embryos and (iii) co-localize on 91 sites in salivary gland polytene chromosomes. These results suggest that the proteins are simultaneously involved in the regulation of several target genes. DSP1 can bind Corto through one of the two HMG-boxes that also mediate DNA binding. It has been suggested that during nucleoprotein complex formation, the HMG-box B of HMGB bends DNA whereas the

### Table 2: Genetic interactions between corto and Scr

| Genotype          | Males observed | T2 to T1 (%) |
|-------------------|----------------|-------------|
| Antp/+/+          | 163            | 38.6        |
| Antp/corto420     | 177            | 83.0        |
| Antp/corto07128   | 179            | 94.9        |
| Antp/corto1       | 173            | 100.0       |

Antp/+/+ males were crossed with corto+420/+/+, corto+07128/+/+, or corto1/+/+ males as controls. Reciprocal crosses were also performed. As similar results were obtained, they were pooled. The resulting male progeny was scored for transformation of T2 into T1 leg as revealed by the presence of ectopic sex comb teeth.
HMG-box A mediates interaction with transcription factors, thus promoting their contact with targets [22]. DSP1 seems to follow that scheme to enhance the binding of transcription factors as Dorsal or Bicoid to DNA [42,43]. What therefore could be the role of the DSP1-Corto interaction in the regulation of common targets? First, DSP1 could bring Corto to the chromatin, where it could further interact with other partners. These partners could be PcG factors or GAF, which have previously been shown to interact with Corto [36]. Nevertheless, this hypothesis is unlikely since we observed no modification of Corto binding to polytene chromosomes in the dsp11 strain. Second, DSP1 could inhibit the interaction between Corto and PcG factors or GAF, thus preventing the silencing of targets that bind both proteins. Third, Corto could modify the DNA bending ability of DSP1 and thus modulate its targets. Indeed, the interaction with other factors, for example TrxG complexes, thus promoting their contact with targets [22]. DSP1 could inhibit the interaction between Corto and PcG factors or GAF, thus preventing the silencing of targets that bind both proteins. Third, Corto could modify the DNA bending ability of DSP1 and thus modulate its interaction with other factors, for example TrxG complexes. Indeed, the dsp1 gene was previously shown to interact with the TrxG genes trx and brm [37] Our results do not allow us to discriminate between these last two, non-exclusive possibilities.

We also report that the Hox gene Scr is a common target of Corto and DSP1. Both proteins bind a 10-kb Xbal fragment located 37-kb upstream of the Scr transcription start. Genetic studies have shown that this fragment is required for Scr function in the embryo and in the imaginal disc [44-46]. In embryos, it restricts the expression of a Scr-lacZ fusion gene to the labial and prothoracic segments [44], whereas in larvae it is required for Scr expression in the first leg imaginal disc and for Scr silencing in the second and third leg imaginal discs [45]. Interestingly, the function of the 10-kb Xbal fragment is sensitive to a subset of PcG and TrxG mutations [47] and has been genetically characterized as an upstream maintenance element of Scr [46]. At the end of embryogenesis, the Scr expression domain is restricted to the labial and prothoracic segments. In consequence, the mean state of this ME in the whole embryo would be silenced. We can thus assume that the global situation in embryos mimics that of the T2 and T3 leg imaginal discs. Conversely, since Scr is expressed in S2 cells (data not shown), we propose that the situation in S2 cells rather mimics that of T1 leg imaginal disc cells. Hence, Corto, which is present on the Scr ME whether active (S2 cells) or silenced (embryos), could be present on this ME in all three leg imaginal discs. On the other hand, DSP1, which is present on the ME in S2 cells but not in embryos, could bind the ME only in cells where this element is active, hence in T1 leg imaginal disc cells. We thus propose that both Corto and DSP1 proteins localize on this Scr ME in the first leg imaginal disc.

Some trxG mutants as well as the dsp1 null mutant exhibit a reduced sex comb and our previous work has shown that dsp1 interacts with certain trxG genes and regulates Scr expression in T1 discs [2,37]. HMGB, the vertebrate homologue of DSP1, has been reported to activate and stabilize the TFIID-TFIIA-promoter complex in vitro [48] and some TrxG factors have been shown to interact with the RNA polymerase II complex, thus facilitating transcriptional elongation [49,50]. This leads us to propose that in the T1 leg imaginal disc, DSP1 facilitates the interaction between a TrxG complex and the transcription machinery, thus maintaining Scr activation. Moreover, Corto has been shown to interact with PcG complexes [36]. The binding of Corto to DSP1 could then impede the interaction between Corto and PcG complexes, thus limiting their recruitment. Therefore, the interaction between the two proteins on the ME would lead to a level of Scr transcription compatible with T1 identity. Conversely, in the T2 and T3 leg imaginal discs, since DSP1 does not bind the ME, Corto would be able to interact with PcG complexes, thus enhancing the silencing of Scr.

Conclusion

In summary, we have shown that the two ETPs Corto and DSP1 directly interact and are simultaneously found on a Scr ME when active, whereas Corto alone is found on the same ME when inactive. Our data suggest that different combinations of ETP favor the recruitment of either PcG or TrxG complexes, participating in the maintenance of the silenced or active state of ME.

Methods

Drosophila strains and genetics

Flies were raised on standard medium at 25°C except for the dsp1 strain, which was maintained at 22°C. All mutations and chromosome aberrations used are described in Flybase [51]. Oregon-R or w1118 were used as wild-type reference strains. dsp11 is a null allele of dsp1, which is maintained as a homozygous strain [37]. corto420, corto07128 and corto11 are loss-of-function alleles: a deficiency, a P-element insertion and an uncharacterized mutation obtained by EMS mutagenesis, respectively. These alleles were balanced over TM3.

Localization of proteins on polytene chromosomes

Co-immunostaining of Oregon-R or dsp11 polytene chromosomes was performed as previously described using rabbit affinity purified anti-DSP1 (1:150) and rat anti-Corto (1:40) as primary antibodies [36]. Secondary antibodies (Alexa Fluor® 594 goat anti-rabbit IgG and Alexa Fluor® 488 goat anti-rat IgG, Molecular Probes) were used at a 1:1000 dilution. To determine the precise localization of Corto and DSP1, immunostainings were performed separately on squashes of Oregon-R chromosomes using rabbit affinity-purified anti-DSP1 (1:150) or rabbit anti-Corto (1:40) as described [40].
Co-immunoprecipitation assays

Embryos (0–14 hour-old w^{118}, 2 g) were crushed in 4 ml of 50 mM Tris pH 7.5, 150 mM NaCl, 1% NP40, 0.1% SDS, 1 mM PMSF supplemented with protease inhibitors (Roche). After sonication and high-speed centrifugation, the extracts were pre-cleared with protein A-agarose beads and incubated overnight at 4°C with 10 µg of anti-DSP1 affinity-purified antibodies or 10 µl of rabbit serum. Following extensive washing, the beads were resuspended in Laemmli buffer and analyzed by SDS-PAGE and western blotting.

Far-western and GST pull-down assays

Most of the vectors expressing full-length or truncated forms of MBP-DSP1 or GST-Corto fusion proteins have been described previously [36,37]. pGEX-C1/324 was obtained by digestion of pEG-Corto with EcoRI and PvuII and sub-cloning the resulting 1-kb fragment into pGEX4T-1. pGEX-C325/550 was obtained by PCR amplification of a 0.7 kb DNA fragment from pBS-Corto using oligonucleotides 5'-CCG GAA TTC CGG GCT GCG GCC CAG GCC TCG ATA GCC-3' and 5'-CCG CTC GAG CGG CAC GGT GTA GCA GGA GAT CTG CGG-3', digestion with EcoRI and XhoI, and sub-cloning into pGEX4T-1. In vitro synthesis of radiolabeled proteins was performed using the Tnt® coupled reticulocyte lysate system (Promega) and ^35^S methionine. Far-western and GST pull-down assays were performed as previously described [36,37].
**Immunoprecipitation of crosslinked chromatin (XChIP) and semi-quantitative PCR analysis**

Formaldehyde cross-linking of *Drosophila* S2 cell or 0–14 hour-old w1118 embryo chromatin and chromatin immunoprecipitation were performed as described [52]. Immunoprecipitation of purified chromatin was performed either with rabbit Corto antibodies (1:20), affinity-purified DSP1 antibodies (5 µg) or rabbit serum (1:20) in 250 µl final volumes. One percent of the co-immunoprecipitated DNA was used for the PCR reactions. Nine primer pairs amplifying 500–700 bp fragments in the cis-regulatory sequences of Scu (NCBI accession number: NT_033777) were designed: SL forward 5′-AAATCGGACATTTGAGG-3′, reverse 5′-AATCAAATTGGTACCAGCC-3′, S2 forward 5′- CGCAGCATGAGGATAACCATGGG-3′, S3 forward 5′- GTCCCGAATTTCTCGTTCTG-3′, S4 forward 5′- GCAAGCTCTCTCAAGCT-3′, reverse 5′- CGGATAAGGAAATTCCTCCTG-3′, S5 forward 5′- CGTTCTCGTACCCAAACG-3′, S6 forward 5′- GAGAGGAGGAGAATCTATGAGG-3′, S7 forward 5′- GAGAGGAGGAGAATCTATGAGG-3′, reverse 5′- CCAGGATGATGTTCTGCTG-3′, reverse 5′- TGCCAGTTTCTTCTCCAGCC-3′, S8 forward 5′- GAGAGGAGGAGAATCTATGAGG-3′, S9 forward 5′- GAGAGGAGGAGAATCTATGAGG-3′, reverse 5′- TGCCAGTTTCTTCTCCAGCC-3′, S10 forward 5′- GAGAGGAGGAGAATCTATGAGG-3′, reverse 5′- TGCCAGTTTCTTCTCCAGCC-3′, S11 forward 5′- GAGAGGAGGAGAATCTATGAGG-3′, reverse 5′- TGCCAGTTTCTTCTCCAGCC-3′, S12 forward 5′- GAGAGGAGGAGAATCTATGAGG-3′, reverse 5′- TGCCAGTTTCTTCTCCAGCC-3′.

**Authors’ contributions**

JS performed the fluorescent immunodetection of DSP1 and Corto on polytene chromosomes and chromatin immunoprecipitation assays together with MB. MD and DL performed the genetic interactions between corto and dsp1. EMV performed the GST pull-down assays. AD performed the far-western experiments. LB and IZ performed the fluorescent immunodetection of DSP1 on polytene chromosomes and determined the Corto and DSP1 binding sites. FP performed the genetic interaction between corto and Scu. The manuscript was written by FP; all the co-authors reviewed and approved the final manuscript.

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