

Enhancer–promoter communication mediated by Chip during Pannier-driven proneural patterning is regulated by Osa

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The GATA factor Pannier activates proneural *achaete/scute* (*ac/sc*) expression during development of the sensory organs of *Drosophila* through enhancer binding. Chip bridges Pannier with the (Ac/Sc)–Daughterless heterodimers bound to the promoter and facilitates the enhancer–promoter communication required for proneural development. We show here that this communication is regulated by Osa, which is recruited by Pannier and Chip. Osa belongs to Brahma chromatin remodeling complexes and we show that Osa negatively regulates *ac/sc*. Consequently, Pannier and Chip also play an essential role during repression of proneural gene expression. Our study suggests that altering chromatin structure is essential for regulation of enhancer–promoter communication.

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Transcription factors must gain access to their target sites in vivo to regulate gene expression and this implies alteration of chromatin structure around regulatory regions by chromatin remodeling complexes. In *Drosophila*, the *polycomb* group of genes maintains repression of homeotic genes like *Ultrabithorax*, presumably by inducing a repressive chromatin structure (Pirrotta 1997). In contrast, some members of the *trithorax* group of genes were identified by their ability to suppress dominant *polycomb* phenotypes (Kennison and Tamkun 1998). The *osa trithorax* group gene encodes a large ubiquitously expressed protein with an ARID domain (A/T-Rich Interaction Domain; Treisman et al. 1997; Collins et al. 1999) that binds AT-rich DNA sequences, and a C-terminal domain, the *eyelid* homologous domain (EHD) whose function is not known. The EHD is conserved in multicellular organisms including *Caenorhabditis elegans* and humans but is not present in yeast. Osa is a component of Brahma (Brm) chromatin remodeling

complexes (Treisman et al. 1997; Collins et al. 1999), the *Drosophila* homologs of the yeast SWI/SNF. These Brm complexes are believed to play a crucial role during gene expression by regulating chromatin structure. However, how these complexes are recruited to specific genes remains poorly understood.

The patterning of the large sensory bristles (macrochaetae) of the thorax of *Drosophila* is a classical model to study how a specific pattern is generated during development. There are only 11 sensory organs on each heminotum and they occupy stereotyped positions. The development of the bristle precursor depends on expression of the *achaete/scute* (*ac/sc*) proneural genes (Modolell 1997). Genes of the *ac/sc* complex encode basic helix–loop–helix proteins that heterodimerize with Daughterless (Da) to activate downstream genes required for neural fate. Transcription of *ac* and *sc* appears to be initiated by enhancers (Gomez-Skarmeta et al. 1995; Garcia-Garcia et al. 1999) and the expression is maintained throughout development by autoregulation mediated by (Ac/Sc)–Da heterodimers binding to E boxes within the *ac/sc* promoters (Martinez and Modolell 1991; Van Doren et al. 1991). Each enhancer interacts with specific transcription factors and promotes proneural expression in only one or a few proneural clusters. These factors, expressed in broader domains than the proneural clusters, define the bristle prepatterning. Pannier (Pnr) belongs to the GATA-1 family of transcription factors (Romain et al. 1993; Heitzler et al. 1996) and activates proneural expression required for development of the dorsocentral (DC) sensory bristles through binding to the DC enhancer (Garcia-Garcia et al. 1999) located at 4 and 30 kb from *ac* and *sc*, respectively. Consequently, the loss of function *pnr* alleles fail to activate *ac/sc* and lack DC sensory bristles. Chip is a ubiquitous nuclear protein required for maximal activation by diverse remote enhancers (Morcillo et al. 1996, 1997; Torigoi et al. 2000). It was shown that it physically interacts both with Pnr and the (Ac/Sc)–Da heterodimers and facilitates enhancer–promoter communication (Bulger and Groudine 1999; Dorsett 1999) during Pannier-driven neural development (Romain et al. 2000).

In our current study, we present genetic interactions between *osa*, *pnr*, and *Chip* that reflect direct interactions between Osa and Pnr and between Osa and Chip. We show that Osa negatively regulates neural expression because loss of *osa* function exhibits increased expression of *ac/sc* and an excess of DC sensory bristles. Pnr and Chip have been previously identified as essential proteins of a proneural complex that activates *ac/sc* during neural development. Our study reveals that Pnr and Chip are also essential in recruiting Osa during neural repression. Hence, our study provides insights into how chromatin remodeling activity might be targeted to specific promoter sequences to regulate enhancer–promoter communication during development.

Results and Discussion

Chip^E is a viable allele of *Chip* that is associated with a point mutation in the LIM-interacting domain (LID), which specifically reduces interaction with the bHLH proteins Ac, Sc, and Da. As a consequence, the *Chip^E* mutation disrupts the functioning of the proneural com-

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plex encompassing Chip, Pnr, Ac/Sc, and Da (Romain et al. 2000). A homozygous *Chip^E* mutant shows thoracic cleft and loss of the DC bristles, similar to loss of function *pnr* alleles (Fig. 1B).

To identify new factors that regulate this proneural complex, we screened for second-site modifiers of the *Chip^E* phenotypes (I. Biryukova and P. Heitzler, unpubl.). We found one allele of *osa* (*osa^{E17}*) among the putative mutants. *Osa^{E17}* corresponds to a loss-of-function allele, and homozygous embryos die with normal cuticle patterning. Both *osa^{E17}* and null alleles of *osa* (*osa⁶¹⁶* or *osa¹⁴⁰⁶⁰*) enhance the cleft but suppress the loss of DC bristle phenotypes of *Chip^E* flies. Indeed, *Chip^E* flies with only one copy of *osa⁺* (*Chip^E;osa⁶¹⁶/+*) are weak and sterile but show wild-type DC bristle pattern (Fig. 1C).

These genetic interactions suggest that Osa can antagonize the function of Pnr. Moreover, overexpressed Osa (+/*UAS-osa*; *Gal4-pnr^{MD237}/+*) induces a thoracic cleft and the loss of DC bristles (Collins et al. 1999; our current study) similar to the loss-of-function *pnr* alleles (Heitzler et al. 1996; Garcia-Garcia et al. 1999). In contrast, loss-of-function *osa* alleles display an excess of DC bristles similar to overexpressed Pnr (Haenlin et al. 1997). For example, (*osa¹⁴⁰⁶⁰/+*), (*osa⁶¹⁶/+*), and (*osa^{E17}/+*) flies exhibit respectively 2.35 ± 0.12 , 2.38 ± 0.12 , and

2.43 ± 0.17 DC bristles per heminotum (Oregon wild-type flies have 2.00 DC bristles/heminotum). Furthermore, transallelic combination of *osa¹⁴⁰⁶⁰* with the hypomorphic *osa^{4H}* (*osa^{4H}/osa¹⁴⁰⁶⁰*) accentuates the excess of DC bristles (Fig. 1D) compared with (*osa¹⁴⁰⁶⁰/+*). (*osa^{4H}/osa¹⁴⁰⁶⁰*) flies display 4.17 ± 0.19 DC bristles per heminotum. On the other hand, (*osa^{4H}/osa^{4H}*) flies display 2.50 ± 0.11 DC bristles per hemithorax. The development of the extra DC bristles revealed by phenotypic analysis was compared with the positions of the DC bristle precursors detected with a *LacZ* insert, A101, in the *neuralized* gene (Boulianne et al. 1991) that exhibits staining in all sensory organs (Huang et al. 1991). In (*osa¹⁴⁰⁶⁰/osa^{4H}*) discs, additional DC precursors are observed that lead to the excess of DC bristles (Fig. 1E). The *pnr^D* alleles encode Pnr proteins carrying a single amino acid substitution in the DNA binding domain that disrupts interaction with the U-shaped (Ush) antagonist (Cubadda et al. 1997; Haenlin et al. 1997). Consequently, *pnr^D* constitutively activates *ac/sc*, leading to an excess of DC bristles. We found that this excess is accentuated when *osa* function is simultaneously reduced (*pnr^{D1}/osa⁶¹⁶* in Fig. 1F).

As *osa* shows genetic interactions with *trithorax* group genes encoding components of the Brm complex like *moira* (*mor*) and *brm* (Collins et al. 1999; Crosby et al. 1999; Vazquez et al. 1999), we investigated whether mutations in *mor* and *brm* suppress the *Chip^E* phenotype. We found that loss of one copy of *brm⁺* in (*Chip^E;brm²/+*) flies suppresses the lack of DC bristles observed in *Chip^E* flies (Fig. 1G), similar to loss of one copy of *osa⁺* (Fig. 1C). This shows that *brm* and *osa* both act during Pnr-dependent patterning, in agreement with the fact that they have been shown to be associated in the Brm complex. In contrast, reducing the amount of *Mor* by half [(*Chip^E;mor¹/+*) flies] is not sufficient to modify the *Chip^E* phenotype (data not shown). This does not definitely exclude the possibility that *mor* is directly or indirectly involved, via the Brm complex, in Pnr-dependent patterning.

The complete *osa* open reading frame of 2715 amino acids and the intronic splicing signals were PCR amplified from genomic DNA prepared from homozygous embryos (*osa^{E17}* and *osa¹⁴⁰⁶⁰*) and homozygous first instar larvae (*osa^{4H}*). For *osa¹⁴⁰⁶⁰* and *osa^{4H}*, the sequence analysis revealed a single mutation in the N terminus that causes a glutamine to stop codon substitution (Q n°251 for *osa¹⁴⁰⁶⁰*; Q n°1281 for *osa^{4H}*; Fig. 1H). The conceptual translation of *osa¹⁴⁰⁶⁰* leads to a truncated Osa protein lacking both functional domains, whereas *Osa^{4H}* retains the ARID domain but lacks the C-terminal EHD (Fig. 1H). Wild-type *osa* function is necessary for patterning of the DC bristles. Although *osa^{E17}* behaves as a stronger allele than *osa¹⁴⁰⁶⁰* and *osa^{4H}*, we were unable to molecularly identify the mutation. Hence, the *osa^{E17}* phenotype may result from a mutation in regulatory sequences that affects *osa* expression.

We have previously shown that a complex containing Pnr, Chip, and the (Ac/Sc)–Da heterodimer activates proneural expression in the DC proneural cluster and promotes development of the DC macrochaetae (Romain et al. 2000). Osa and Pnr/Chip have antagonistic activities during development because loss of *osa* function (*osa^{4H}* and *osa¹⁴⁰⁶⁰*) displays additional DC bristles. However, as our current study reveals that *osa* genetically interacts with *pnr* and *Chip*, we asked whether Osa physically

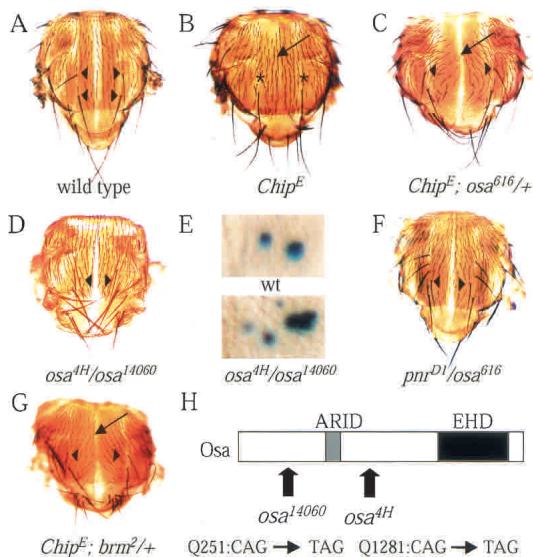


Figure 1. *osa* mutants genetically interact with *pnr* and *Chip* and display extra DC bristles. (A–D,F,G) Dorsal thoraces of flies. (A) Wild-type flies have four DC bristles (arrowhead). (B) *Chip^E* flies resemble hypomorphic *pnr* mutants lacking DC bristles (asterisks) and bearing a thoracic cleft (arrow). (C) Null alleles of *osa* enhance the cleft (arrow) but suppress the lack of DC bristles (arrowhead) in *Chip^E* flies (*Chip^E/Chip^E; +/osa⁶¹⁶*). (D,E) Hypomorphic transallelic combination of *osa* (*osa^{4H}/osa¹⁴⁰⁶⁰*) exhibits extra DC bristles (D, arrowhead) associated with extra DC precursors (E) as revealed by A101 *lacZ* staining in comparison with staining of wild-type disc. (F) *osa⁶¹⁶* behaves as dominant enhancer of the excess of DC bristles associated with the *pnr^D* encoding constitutive activators of *ac/sc* (*pnr^{D1}/osa⁶¹⁶*). (G) Reducing the amount of *brm* also enhances the cleft (arrow) but suppresses the lack of DC bristles (arrowhead) in *Chip^E* flies (*Chip^E/Chip^E; +/brm²*). (H) Molecular characterization of the *osa¹⁴⁰⁶⁰* and *osa^{4H}* alleles. *osa* encodes a protein carrying an ARID DNA binding domain (gray box) and an EHD (black box) conserved in human and *Caenorhabditis elegans* homologs. *osa¹⁴⁰⁶⁰* and *osa^{4H}* encode truncated Osa protein where the EHD is lacking. The glutamine mutated to a stop codon in *osa¹⁴⁰⁶⁰* (Q n°251) and *osa^{4H}* (Q n°1281) is indicated by an arrow.

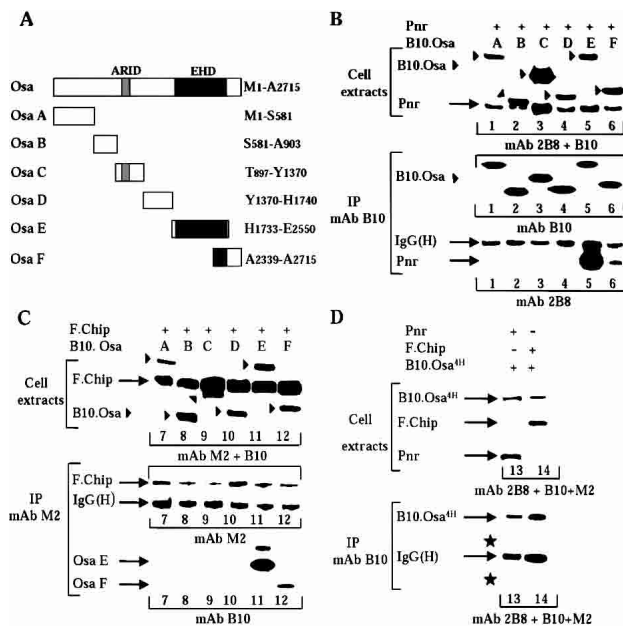


Figure 2. Osa physically interacts with Pnr and Chip through its C-terminal EHD. Osa^{4H} consequently no longer interacts with Pnr and Chip. (A) Schematic drawing of the Osa domains used throughout the current study. They are as follows: Osa A, Meth1/Ser581; Osa B, Ser581/Ala903; Osa C, Thr897/Tyr1370; Osa D, Tyr1370/His1740; Osa E, His1733/Glu2550; Osa F, Ala2339/Ala2715. (B,C) Osa interacts with Pnr (B) and with Chip (C). In each case, an immunoblot of a representative set of transfected cells extracts is shown in the upper part of the panels. Immunoprecipitations of transfected cell extracts are shown in the lower part of the panels. The transfected expression vectors are shown at the top of the panels. The B10 and M2 mouse antibodies used to immunoprecipitate the extracts are shown in the left of the panels and the antibodies used to reveal the blots are indicated at the bottom. Pnr is recognized by the 2B8 monoclonal antibody, the B10-tagged Osa domains are detected with the B10 antibody, and the full-length flagged Chip is detected with the M2 antibody. The locations of the Osa domains are indicated by arrowheads. The locations of the flagged Chip and Pnr proteins as well as those of the immunoglobulin heavy chain [IgG(H)] are indicated at the sides. (D) Osa^{4H} no longer interacts with Pnr or Chip. The layout is as in B and C. The stars highlight the absence of Pnr (lane 13) and F-Chip (lane 14) coimmunoprecipitating with immunoprecipitated B10.Osa^{4H}.

interacts with the Pnr and Chip proteins. We performed immunoprecipitations of protein extracts made from Cos cells cotransfected with expression vectors for tagged Osa and either Pnr or tagged Chip (Fig. 2B,C). Because Osa is a large protein, we used several expression vectors encoding contiguous domains of Osa (Fig. 2A, domains A–F). Osa coimmunoprecipitates with Pnr and Chip (Fig. 2B,C) and can be detected on Western blots with appropriate antibodies. The interactions appear to require the overlapping domains Osa E (His1733/Glu2550) and Osa F (Ala2339/Ala2715; Fig. 2B,C) corresponding to the EHD. Enhancer-promoter communication during proneural activation and development of the DC bristles requires regulatory sequences scattered over large distances and appears to be negatively regulated by interaction of Pnr and Chip with Osa through the EHD. Interestingly, the EHD is not conserved in yeast. In yeast, the UAS sequences are generally close to the promoter and there is no requirement for long-distance interactions. This observation could support the idea that

the EHD is essential for long-distance enhancer-promoter communication. Alternatively, yeast may just lack proteins like Chip or Pnr.

The DNA-binding domain and the C-terminal region are essential for the function of Pnr during development of the DC sensory organs (Ramain et al. 1993). The *pnr^{VX1}* and *pnr^{VX4}* alleles (collectively *pnr^{VX1/4}*) are characterized by frameshift deletions that remove two C-terminal α -helices and result in reduced proneural expression and loss of DC bristles.

We thus investigated the molecular interactions between Osa and Pnr^{D1} and between Osa and Pnr^{VX1}. We found that the Pnr^{D1} protein interacts with the EHD as efficiently as wild-type Pnr (Fig. 3A,B, lanes 1,2). In contrast, the physical interaction is disrupted when the C terminus of Pnr encompassing the α -helices is removed (Fig. 3A,B, lanes 1,3). Because the C terminus of Pnr is required for the Pnr–Osa interaction in transfected cells extracts, we tested the abilities of in vitro translated ³⁵S-labeled Osa domains to bind to GST–C^TPnr attached to glutathione-bearing beads (Fig. 3D, lane 4). We found that only Osa E and Osa F interact with the C terminus of Pnr (Fig. 3D; data not shown). As expected, GST–C^T–

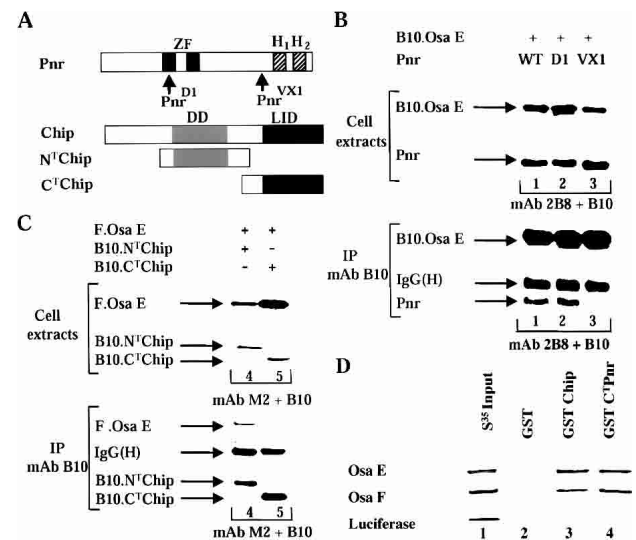


Figure 3. The C-terminal domain of Pnr encompassing the two helical structures and the N-terminal homodimerization domain of Chip mediate interactions with Osa. (A) Structural features of the Pnr and Chip proteins used in the present study. The two zinc fingers (black boxes; ZF) and two amphipathic α -helices (hatched boxes; H1 and H2) of Pnr are shown. The mutations associated with the proteins of the alleles *pnr^{D1}* and *pnr^{VX1}* are localized within the first zinc finger and in the N terminus of the helices, respectively. The N-terminal homodimerization domain of Chip (N^T Chip; gray box; DD) and the C-terminal LIM-interacting domain (C^T Chip; black box; LID) are also shown. (B,C) The C-terminal domain of Pnr containing the α helices (B) and the N-terminal homodimerization domain of Chip (C) mediate the interactions with Osa. Immunoprecipitations of transfected cell extracts are shown. The layout is as described in Figure 2B–D. The B10 Chip C^T comigrates with the immunoglobulin light chain and was detected in B (lane 5) using a peroxidase-conjugated protein A-Sepharose. (D) Autoradiographs of SDS-PAGE gels from representative affinity chromatography experiments with the GST–C^TPnr (lane 3), the GST–C^TPnr (lane 4), or the GST control beads (lane 2) and in vitro translated ³⁵S proteins are indicated on the left. One-tenth of the ³⁵S input is shown in lane 1. Luciferase is used as a negative input. Experiments were performed three times and, with all proteins except luciferase, 50-fold more protein bound to GST–C^TPnr and GST Chip than to GST control.

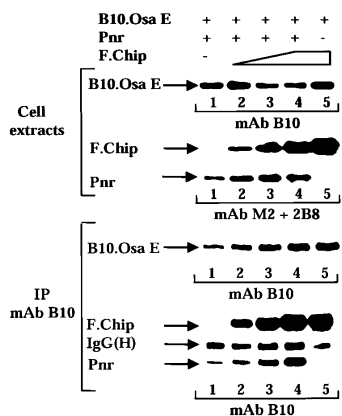


Figure 4. Chip and Osa do not compete for binding to Pnr. The immunoprecipitation of transfected cell extracts is shown. The layout is as described in Figures 2B–D and 3B–C. The *top* part of the figure shows the immunoblot of a representative set of transfected cell extracts where a constant amount of Pnr and a constant amount of the tagged Osa E have been expressed in the presence of increasing amounts of F.Chip. The *bottom* part displays the immunoprecipitation of the protein extracts with a constant amount of immunoprecipitated Osa E and an increasing amount of coimmunoprecipitating Pnr when the amount of coimmunoprecipitating F Chip is increasing.

Pnr did not bind the luciferase input and none of the ³⁵S proteins bound GST control beads (Fig. 3D, lanes 4,2). We also further investigated the interaction between Chip and Osa and we found that Osa associates with the N-terminal homodimerization domain of Chip (Fig. 3A,C, lane 4), also required for the interaction between Chip and Pnr. Furthermore, Osa E and Osa F bound also to immobilized GST-Chip (Fig. 3D, lane 3). We found that deletion of the α helix H1 disrupts the interactions between Pnr and Osa (data not shown). Interestingly, the same deletion also disrupts the interaction with Chip (Ramain et al. 2000). Therefore, the functional antagonism between Chip and Osa during neural development may result from a competition between these proteins for association with Pnr. Alternatively, the deletion of H1 may affect the overall structure of the C terminus of Pnr and disrupt the physical interactions with Chip and Osa. To discriminate between these hypotheses, we performed immunoprecipitations of protein extracts containing a constant amount of Pnr, a constant amount of the tagged Osa E domain, and increasing concentrations of Chip (Fig. 4). Pnr immunoprecipitates with immunoprecipitated tagged Osa E and the amount of Pnr immunoprecipitated increases in the presence of increasing concentrations of Chip. The presence of increasing amounts of Chip does not inhibit the Osa–Pnr interaction as would be expected if Osa and Chip were to compete for binding to Pnr. In contrast, it suggests that Chip and Pnr act together to recruit Osa and to target its activity and possibly the activity of the Brm complex to the *ac/sc* promoter sequences.

Using expression vectors encoding contiguous domains of Osa, we showed that the EHD of Osa mediates interactions with Pnr and Chip. Because the EHD is lacking in the truncated Osa¹⁴⁰⁶⁰ and Osa^{4H}, we hypothesized that the loss of interaction with Pnr and Chip are responsible for the excess of DC bristles observed in *osa*^{4H} and *osa*¹⁴⁰⁶⁰. However, it is possible that we disrupted an additional N-terminal interaction domain in

constructing the expression vectors. To exclude this possibility, we performed immunoprecipitations of protein extracts made from Cos cells cotransfected with expression vectors for a tagged Osa^{4H} and either Pnr or a tagged Chip. We found that the truncated Osa^{4H} does not interact either with Pnr or with Chip, indicating that there is no dimerization domain in the N terminus of Osa (Fig. 2D, lanes 13,14).

To investigate whether these interactions between Osa, Pnr, and Chip function *in vivo* during DC bristle development, we have examined the effects of both loss of function and overexpression of *osa* on the activity of a *LacZ* reporter whose expression is driven by a minimal promoter sequence of *ac* fused to the DC enhancer (transgenic line DC:*ac-LacZ*; Fig. 5A; Garcia-Garcia et al. 1999; Ramain et al. 2000). We found that expression of the *LacZ* transgene is increased in *osa*¹⁴⁰⁶⁰/*osa*^{4H} wing discs when compared with the wild-type control (Fig. 5A, a,b). For overexpression experiments, we used the UAS/GAL4 system (Brand and Perrimon 1993) using as a driver the *pnr*^{MD237} strain that carries a GAL4-containing transposon inserted in the *pnr* locus (driver: *pnr-Gal4*). This insert gives an expression pattern of Gal4 indistinguishable from that of *pnr* (Calleja et al. 1996; Heitzler et al. 1996). We found that overexpressed Osa leads to a strong reduction of *LacZ* staining in the DC

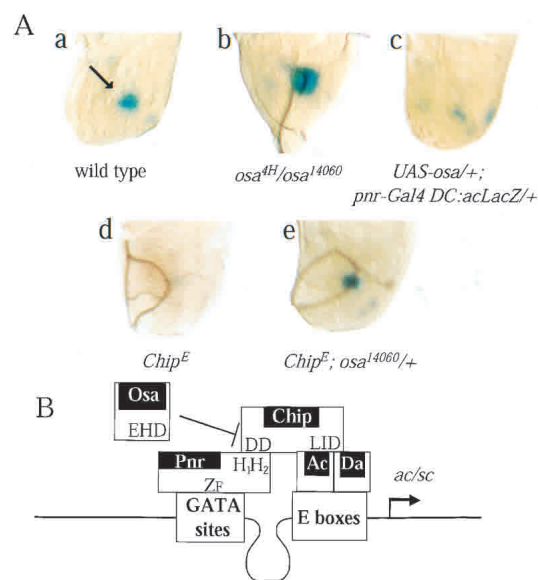


Figure 5. Osa regulates proneural expression through the DC-specific enhancer of the *ac/sc* complex. Late-third instar thoracic discs show *lacZ* expression in the DC area (Gomez-Skarmeta et al. 1995). In each case, the reaction was left for 1 h at 22°C. (A,) Wild-type pattern (*w*¹¹¹⁸/+; DC:*ac-LacZ*/+) with DC expression (arrow). (b) Hypomorphic transallelic combination of *osa* (DC:*ac-LacZ* *osa*^{4H}/*osa*¹⁴⁰⁶⁰) promotes overexpression of *LacZ*. (c) Overexpressed Osa mimics the effects of *Chip*^E [*UAS-Osa*^{+/+}; +/DC:*ac-LacZ* *pnr*^{MD237}(*Gal4*)]. (d) In *Chip*^E flies (*Chip*^E/*Chip*^E; DC:*ac-LacZ*/+), the *lacZ* expression driven by the enhancer is strongly reduced. (e) Lowering the dosage of *osa*⁺ suppresses the effects of *Chip*^E (*Chip*^E/*Chip*^E; DC:*ac-LacZ*/*osa*¹⁴⁰⁶⁰) and restores the activity of the promoter sequences (cf. a,d). (B) A model on how the proneural complex encompassing Pnr, Chip, and the (Ac/Sc)–Da heterodimer is regulated by Osa. The proneural complex activates *ac/sc* and is negatively regulated by interactions of Pnr and Chip with Osa. These interactions are mediated by the C-terminal domain of Pnr containing two α helices (H1H2), the N-terminal homodimerization domain (DD) of Chip, and the C-terminal EHD of Osa.

area (Fig. 5A, a,c), consistent with the lack of DC bristles (Collins et al. 1999; data not shown). Thus, overexpressed Osa represses activity of the *ac* promoter sequences required for DC *ac/sc* expression and development of the DC bristles. It has been previously reported that *wingless* expression is also required for patterning of the DC bristles (Phillips and Whittle 1993). However, the repressing effect of Osa on development of the DC bristles is unlikely to be the result of an effect of Osa on *wingless* expression because overexpressed Osa driven by *pnr^{MD237}* has no effect on the expression of a *LacZ* reporter inserted into the *wingless* locus (data not shown; Kassis et al. 1992; Collins and Treisman 2000). Thus, Osa acts through the DC enhancer of the *ac/sc* promoter sequences to repress *ac/sc* and neural development.

Chip^E disrupts the enhancer–promoter communication and strongly affects expression of the *LacZ* reporter driven by the *ac* promoter linked to the DC enhancer (Fig. 5A, d; Romain et al. 2000). Because null alleles of *osa* suppress the loss of DC bristles displayed by *Chip^E*, we investigated the consequences of reducing the dosage of *osa* in *Chip^E* flies. We found that the expression of the *LacZ* reporter is not affected in *Chip^E* flies when Osa concentration is simultaneously reduced (*Chip^E/Chip^E; DC:ac-LacZ/osa¹⁴⁰⁶⁰*; Fig. 5A, e).

In conclusion, we have previously shown that Pnr function during proneural patterning is regulated by interaction with several transcription factors (Fig. 5B). Pnr function is negatively regulated by Ush that interacts with its DNA-binding domain (Haenlin et al. 1997). Chip associates with the C terminus of Pnr, bridging Pnr at the DC enhancer with the AC/Sc–Da heterodimers bound at the proneural promoters, thus activating proneural gene expression (Romain et al. 2000). Our current study reveals that Pnr function can also be regulated by interaction with Osa. Thus, Osa activity is specifically targeted to *ac/sc* promoter sequences and the binding of Osa therefore has a negative effect on Pnr function, leading to reduced expression of the proneural *ac/sc* genes. Osa belongs to Brm complexes, which are believed to play an essential role during chromatin remodeling necessary for gene expression. For example, in vitro transcription experiments with nucleosome assembled human β -globin promoters have shown that the BRG1 and BAF155 subunits of the mammalian SWI/SNF homolog are essential to target chromatin remodeling and promote transcription initiation mediated by GATA-1 (Kadam et al. 2000). In contrast to what was observed in vitro, our results suggest that in vivo the SWI/SNF complexes can also act to remodel chromatin in a way that represses transcription. Alternatively, the observed repression of proneural genes may simply define a novel function of Osa, independent of chromatin remodeling.

Materials and methods

All recombinant DNA work was performed according to standard procedures (Sambrook et al. 1989). Details concerning plasmid constructions are available on request.

Fly stocks and genetics

Alleles used were *osa^{4H}* (Kaminker et al. 2001), *osa⁶¹⁶* (Treisman et al. 1997), *osa¹⁴⁰⁶⁰* (gift of M. Hoch), *mor¹*, and *brm²* (FlyBase). The original *osa^{4H}* chromosome leads to embryonic lethality. We found that this lethality is associated with a separable and distal mutation. Thirty hemithoraces were examined for each of the combinations presented in Figure

1, but for each genotype the phenotype was found to be remarkably similar from fly to fly.

PCR analysis of the *osa* mutants

Genomic DNA for PCR analysis was extracted from homozygous *osa^{E17}*, *osa¹⁴⁰⁶⁰* embryos, and homozygous *osa^{4H}* first instar larvae.

Plasmid constructions

The expression vectors pXJ Pnr⁺, pXJ Pnr^{D1} and pXJ Pnr^{VX1}, encoding truncated versions of wild-type Pnr, Pnr^{D1}, and Pnr^{VX1}, are described in Haenlin et al. (1997). The sequences encoding the domains of Osa (Osa A, Meth1/Ser581; Osa B, Ser581/Ala903; Osa C, Thr897/Tyr1370; Osa D, Tyr1370/His1740; Osa E, His1733/Glu2550; Osa F, Ala2339/Ala2715) and Osa^{4H} were amplified by PCR and inserted into the vectors pXJB, pXJF for transient transfections in Cos cells (Romain et al. 2000). pXJB and pXJF encode fusion proteins carrying at their N terminus the B epitope of the estrogen receptor (pXJB: RPNSDNRRQGGRELR) and the Flag epitope (pXJF: DYKDDDDK), respectively. The vectors encoding the Flag-tagged Achaete, the B-tagged N-terminal, and the B-tagged C-terminal domain of Chip are described in Romain et al. (2000).

DNA transfections, immunoprecipitations, Western blot analysis, and GST pull-down assays

Cos cell transfections, protein extract preparations, immunoprecipitations, and Western blot analysis were performed as described in Haenlin et al. (1997). The protein extracts were immunoprecipitated with the B10 or M2 antibodies that recognize the B and Flag epitopes encoded by the pXJB and pXJF vectors. GST pull-down assays were performed as in Torigoi et al. (2000).

Staining for β -galactosidase activity

Wing discs were stained as described in Cubadda et al. (1997). A101 contains a *LacZ* gene insert at the *neuralized* locus (Boulianne et al. 1991) and exhibits staining in all sensory organ precursors (Huang et al. 1991). The transgenic strain *DC:ac-LacZ* is described in Romain et al. (2000).

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