Osa, a subunit of the BAP chromatin-remodelling complex, participates in the regulation of gene expression in response to EGFR signalling in the Drosophila wing

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Gene expression is regulated in part by protein complexes containing ATP-dependent chromatin-remodelling factors of the SWI/SNF family. In Drosophila there is only one SWI/SNF protein, named Brahma, which forms the catalytic subunit of two complexes composed of different proteins. The protein Osa defines the BAP complex, and the proteins Polybromo and Bap170 are only present in the complex named PBAP. In this work we have analysed the functional requirements of Osa during Drosophila wing development, and found that osa is needed for cell growth and survival in the wing imaginal disc, and for the correct patterning of sensory organs, veins and the wing margin. Other members of the BAP complex, such as Snr1, Bap55, Mor and Brm, also share these functions of Osa. We focused on the requirement of Osa during the formation of the wing veins. Genetic interactions between osa alleles and mutations affecting the activity of the EGFR pathway suggest that one aspect of Osa is intimately related to the response to EGFR activity. Thus, loss of osa and EGFR signalling results in similar wing vein phenotypes, and osa alleles enhance the loss of veins caused by reduced EGFR activity. In addition, Osa is required for the expression of several targets of EGFR signalling, such as Delta, rhomboid and argos. We suggest that one role of Osa and Brm in the wing is to establish a chromatin environment in the regulatory regions of EGFR target genes, making them available for both activators and repressors and facilitating transcription in response to EGFR signalling.

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Introduction

The patterning of the Drosophila wing involves the activities of several signalling pathways that act in collaboration with sequence-specific transcription factors to define alternative fates, the vein and the intervein (de Celis, 2003). The longitudinal veins (L2–L5) are specified as stripes of cells that run along the proximo-distal axis of the wing blade, extending from the wing hinge to the dorso-ventral boundary. The formation of each longitudinal vein depends on the activity of vein-specific transcription factors, such as Knirps (L2), Abrupt (L5) and the Iroquois complex (L3 and L5), whose expression is restricted to individual veins (Gómez-Skarmeta et al., 1996; Lunde et al., 1998; de Celis and Barrio, 2000, 2003; Cook et al., 2004). Adjacent longitudinal veins are separated by broader “intervein” regions that correspond in the wing disc to the domain of expression of the transcription factor Blistered (Bs) (Fristrom et al., 1994; Montagne et al., 1996; Roch et al., 1998). The localized expression of vein-specific transcription factors in the veins and Bs in the interveins is set by the activity of the Decapentaplegic (Dpp) and Hedgehog (Hh) signalling pathways, among others (Nussbaumer et al., 2000).

The longitudinal veins share a common program of cell differentiation, which is initiated in the wing disc epithelium by the activation of the EGFR signalling pathway (Sturtevant et al., 1993; Guichard et al., 1999). EGFR activity is maximal in the future veins, where it represses bs expression and confers on these cells the capacity to differentiate as veins during pupal development (Gabay et al., 1997; Roch et al., 1998). In addition, EGFR signalling in the veins is required for the activation of Delta (Dl), the ligand of the Notch receptor, and for the expression of several members of the EGFR pathway, such as rhomboid (rho), argos (aos) and dMKP3 (Sturtevant et al., 1993; Celenko et al., 1996; de Celis et al., 1997; Gabay et al., 1997; Ruiz-Gomez et al., 2005). These elements contribute to prevent vein differentiation in cells adjacent to the vein (Dl) and to maintain correct levels of EGFR activation in the vein (rho, aos and dMKP3). Very little is known about the proteins and mechanisms involved in regulating gene expression in response to EGFR signalling in the wing. In other developmental systems, EGFR regulates the activity of the ETS transcription factors Pointed-P2 and Yan/Aop (Lai and Rubin, 1992; Scholz et al., 1993; Klamt, 1993; O’Neill et al., 1994; Rebay and Rubin, 1995; Dickson, 1995), but these genes are not required for the development of the veins (our unpublished data). Only the activity of the repressors Capicua (Roch et al., 2002; Tseng et al., 2007), Atrophin (Charroux et al., 2006) and Groucho (Hasson et al., 2005) has been linked to transcription downstream of EGFR signalling in the wing.
veins, but the nature of putative transcriptional activators mediating the activity of the pathway is still unknown.

In a mutagenesis screen designed to identify genes affecting vein formation (Molnar et al., 2006), we found several P-UAS insertions in the osa gene. The increased expression of osa in the wing disc causes the transformation of intervein into vein tissue, a phenotype characteristic of higher than normal levels of EGFR activity in the interveins. Osa/Eyed encodes a protein containing an AT-rich interaction domain (ARID) that binds DNA in polenitric chromosomes at multiple sites (Collins et al., 1999; Mohrman et al., 2004). Osa forms part of the Brahma (Brm) complex, which is a chromatin-remodelling complex of 2MDa conserved from S. cerevisiae to humans (Tamkun et al., 1992; Tamkun, 1995). There are two human proteins related to Brm, BRM and BRG1, which form part of different protein complexes required for tissue-specific gene expression and cell cycle progression (Roberts and Orkin, 2004). In Drosophila there is only one Brm homolog that is present in two complexes (Mohrman et al., 2004). The BAP complex is characterised by the presence of Osa and the absence of Polybromo and Bap170, and the PBAP complex contains Polybromo and Bap170, but not Osa (Mohrman et al., 2004; Moshkin et al., 2007). The proteins Moira, (Mor), Brm and Osa are members of the Trithorax group, and they regulate positively the expression of Hox genes during development (Kennison and Tamkun, 1988; Papoulas et al., 1998; Vazquez et al., 1999; Collins et al., 1999). The Brm complex is also required for regulation of gene expression in response to the activity of signalling pathways. Thus, in the absence of Osa, targets of Wingless (Wg) signalling are inappropriately expressed, and the contrary occurs in the presence of excess of Osa, suggesting that the chromatin-remodelling activity mediated by the BAP complex is required to maintain the chromatin at the promoters of Wg-responsive genes in a repressed conformation (Treisman et al., 1997; Collins and Treisman, 2000). In addition, the expression of a dominant-negative form of Brm defective for ATP hydrolysis shows effects on wing vein formation, causing the loss of distal structures of the L5 vein (Elfring et al., 1998), and enhances the loss of vein phenotype caused by the viable mutation rho(+) (Marena et al., 2004). All together, these data indicate that Brm is involved in vein patterning, although the mechanisms by which it influences vein formation are not known.

In this work we have taken a genetic approach to analyze the role of osa during wing development, with the aim of determining its specific requirements and to identify candidate Osa target genes that might link Osa activity to transcriptional regulation in response to signalling pathways. We show that loss of osa in the wing causes the lack of veins, that osa and EGFR variants display gene-dose interactions and that Osa is required to activate the expression of EGFR targets in vein regions. We confirm that Osa is related to Wg signalling (Collins and Treisman, 2000), and also needed to activate wg expression in the wing margin. Although Osa might affect the expression of a large set of genes, our data suggest that a key component of its function during wing development is the regulation of EGFR target genes, perhaps by making their regulatory regions available for responding to direct nuclear effectors of the pathway. Other members of the BAP complex, such as Brm, SmR1, Bap55 and Mor share with Osa this function to regulate the response to EGFR signalling.

Materials and methods

Drosophila melanogaster strains and phenotypic analysis

We used the following Drosophila UAS lines: UAS-RasV12 and UAS-RasNT (Lee et al., 1996), UAS-ERK-expressing (Brunner et al., 1994), UAS-FLP (Duffy et al., 1998), UAS-MPK3 (Ruiz-Gomez et al., 2005), UAS-net (Brentrup et al., 2000), UAS-osa(+) (Collins et al., 1999), EP-osa (Mohrman et al., 2006) and UAS-EGF (Ito et al., 1997). We also used the Gal4 lines 638-Gal4, nub-Gal4, ill-Gal4 and sal-Gal4 (Calleja et al., 1996) and 765-Gal4 (de Celis et al., 1996), the alleles bs(2), Df(2L)Pc(2), P(1)net, rho(2), vn(2), osa(208) (Treisman et al., 1997), brm(2) (Kennison and Tamkun, 1988), and the UAS-RNAi lines UAS-iosa, UAS-birm, UAS-ism1, UAS-bap170 and UAS-imor from the Vienna Drosophila Resource Center. All mutants not described in the text can be found in Flybase (Gelbart et al., 1997).

All phenotypes were analysed at 25 °C, and flies were mounted for microscopic examination in lactic acid–ethanol (1:1). Pictures were taken in a Zeiss Axioplan2 microscope with a Spot digital camera and processed in Adobe Photoshop.

Clonal analysis

Mitotic recombination clones were generated using FRT/FLP (Xu and Rubin, 1993) in larvae of 48–72 h after egg laying (AEI). We used the following genotypes:

- hsFLP1.22 f(1)69a; FRT82B M(3)w P[f+] P[ubi-GFP]/FRT82B osa(208) (Minute(+) clones)
- hsFLP1.22 f(1)69a; FRT82B M(3)w P[f+] P[arm-lacZ]/FRT82B osa(208) (Minute(+) clones)
- hsFLP1.22; FRT82B ubi-GFP/FRT82B osa(208) (Twin clones)
- hsFLP1.22; P[act-stop-Gal4]/ UAS-GFP/+; UAS-osa/+ (Gain of expression clones)

Mosaic adults and discs were also made by driving FLP expression in particular regions of the wing in the following genetic combinations: osa(208)Gal4 f(1)69a; UAS-FLP/+; FRT82B M(3)w P[f+] P[ubi-GFP]/FRT82B osa(208) 638-Gal4 f(1)69a; UAS-FLP/+; FRT82B M(3)w P[f+] P[ubi-GFP]/FRT82B osa(208)

osa(208)Gal4 is a construct in which regulatory regions of the spalt gene were fused to Gal4 (J.F.d.C., unpublished).

Drosophila immunocytochemistry and in situ hybridization

We used mouse anti-phosphorylated ERK (Sigma), mouse monoclonals anti-DI, anti-Osa, anti-Wg (Hybridoma bank), mouse anti-jγ-galactosidase (Promega) and rabbit anti-jγ-galactosidase (Cappel), rabbit anti-DII, rat anti-Vvl (U. Llimargas and Casanova, 1997), rat anti-Caup (Gómez-Skarmeta and Modolell, 1996), Rabbit anti-Brm (a gift from J.W. Tamkun), Chinese pig anti-Kni (a gift from D. Kosman), and mouse anti-Bs (Montagne et al., 1996). We used the secondary antibodies from Invitrogen Alexa 488, Alexa 555 and Alexa 647 (used at 1:200 dilution). Third instar imaginal discs were dissected, fixed and stained as described in (de Celis, 1997). Confocal images were captured using a BioRad/Zeiss confocal microscopy. In situ hybridization in imaginal discs was carried out as described in (de Celis, 1997).

Results

Loss of function phenotypes of osa

We first studied the phenotypes in the wing of cells mutant for the strong loss-of-function allele osa(208), aiming to determine the requirements of osa during imaginal development. Clones of osa(208) induced in a non-Minute background proliferate poorly in the wing disc compared to their sister twins (Fig. 1B; 40 twins analysed), and could not be recovered in the adult wing (not shown). The growth of osa(208) cells was rescued with the growth advantage conferred by the Minute(+) condition in a Minute mutant background, where we found large osa(208)Minute(+) clones whose cells do not express Osa (Figs. 1C–F; > 70 osa(208)Minute(+) clones analysed). We found expression of activated Caspase 3 associated with these clones (Figs. 1D–F), indicating that a fraction of osa(208)Minute(+) cells undergo apoptosis. Cells positive for activated Caspase 3 are localized in the basal side of the wing blade epithelium, indicating that these cells dissociate from the epithelium and are
Fig. 1. Osa loss-of-function phenotypes. (A) Wild type wing showing the position of the longitudinal veins L2 to L5. (B) Twin clones induced in hsFLP1.22; FRT82B osa308/FRT82B P[ubi-GFP] wing discs. Osa mutant clones are labelled by the absence of GFP (white arrowheads), and twin clones are bright green. (C–C′) Expression of Osa (red in C and C′) is lost in osa308 clones induced in hsFLP1.22; FRT82B osa308/FRT82B M(3)w P[ubi-GFP] discs. Osa308 clones are labelled by the absence of GFP (green in C). (D–D′) Minute+ clones homozygous for osa308 labelled by the absence of GFP (green in D) show activated Cas3 (shown in red in D and D′). (E) Expression of GFP in the wing blade of 638-Gal4/UAS-GFP discs (green). (F) Wing disc of 638-Gal4/+; UAS-FLP/+; FRT82B/FRT82B M(3)w P[ubi-GFP] genotype showing that homoyzgous M+ cells occupy most of the wing blade (absence of green staining). Only the wing blade region of the wing disc is shown in panels B to F and H to I. (G) Wing of 638-Gal4/+; UAS-FLP/+; FRT82B osa308/FRT82B M(3)w P[ubi-GFP] flies. In these wings FLP-induced mitotic recombination generates Minute+ cells homozygous for the osa308 allele. The wing is reduced in size and lacks all longitudinal veins and the wing margin. The black arrowhead indicates the remnants of the wing margin. (H) Expression of GFP (green) in the central region of the wing blade of sal-Gal4/UAS-GFP wing discs. (I–I′) Expression of activated Cas3 (red in I and I′) in osa308 cells induced in sal-Gal4/+; UAS-FLP/+; FRT82B osa308/FRT82B M(3)w P[ubi-GFP] discs. Yellow arrowheads show the approximated position of the proximal L2 vein. (J) Wing of sal-Gal4 FRT82B/+; UAS-FLP/+; FRT82B osa308/FRT82B P[ubi-GFP] M(3)w flies, showing loss of veins and the formation of ectopic sensory organs and hairs in the wing blade. The inset is a higher magnification of a mutant posterior territory showing ectopic hairs. (K–K′) Higher magnification of the dorsal (K) and ventral (K′) region of a sal-Gal4 FRT82B/+; UAS-FLP/+; FRT82B osa308/FRT82B P[ubi-GFP] M(3)w wing. Wild type cells are enclosed in the black line. Osa mutant cells (labelled by flpGFP) do not differentiate veins, but cause ectopic vein tissue (black arrows) close to the position normally occupied by the vein. (L, M) High magnification of sal-Gal4 FRT82B/+; UAS-FLP/+; FRT82B osa308/FRT82B P[ubi-GFP] M(3)w wings showing loss of distal L2 vein with wild type cells forming a misplaced stretch of vein tissue (arrow in L), and cell autonomous loss of dorsal L3 vein (M). Non-mutant cells (J′) are circled in black.
(Diaz-Benjumea and Garcia-Bellido, 1990), although it has also been observed associated to clones of cells mutant for the Dpp receptor thick veins (Burke and Basler, 1996). We also studied osa requirements in the legs by inducing osa^{308} clones in dll-Gal4/UAS-FLP; FRT82B osa^{308}/FRT82B M(3)w flies. The expression of dll-G4 occurs in the tarsal segments (Supplementary Fig. 1A), and when osa^{308} cells occupy this region the adult tarsal segments are fused to each other, and the leg lacks its distal-most elements (claws; Supplementary Fig. 1B; compare with control leg in 1C). Loss of distal structures and failed tarsal segmentation are also caused when EGFR signalling is reduced in the leg (Galindo et al., 2002). The phenotypes caused by osa^{308} cells in clones indicate that osa is required for cell growth and viability (Figs. 1B, D, I), for the formation of the wing margin (Fig. 1G) and the differentiation of wing veins (Figs. 1G, J and K–M), to suppress the formation of sensory organs and hairs in the wing blade (Fig. 1), and also for the patterning of tarsal segments (Supplementary Fig. 1). In what follows we will focus in the wing vein phenotypes caused by loss of Osa, aiming to identify which aspects of vein formation are regulated by Osa.

Gain of function phenotype of osa in the wing

Previous studies have shown that higher than normal levels of Osa expression cause severe phenotypes in the wing and wing disc, indicating that the amount of Osa protein is critical to achieve the normal functional outcome of BAP complexes (Collins et al., 1999; Collins and Treisman, 2000). To further analyse Osa requirements during vein development, we studied the phenotypes caused by increased Osa expression in several genetic combinations between UAS-osa lines and Gal4 drivers expressed in different wing territories during imaginal and pupal development. Because Osa is expressed in all wing cells (see Fig. 2B), in these experiments there is increased amount of Osa in cells where the protein is normally present in wild type discs. We obtain a variety of phenotypes depending on the Gal4 driver used, but in all cases the Gal4/UAS-osa wings display ectopic vein tissue or fusion of adjacent veins (Fig. 2). Thus, the combination between 765-Gal4 (expressed at low levels in all wing disc cells (de Celis, 1997)) and the UAS-osa shows ectopic veins in a spatial pattern characteristic of mutations in the interveins genes blister (bs), net (net) and plexus (px) (Fig. 2C). The combination between 638-Gal4 (expressed in all wing blade cells during imaginal development) and UAS-osa results in the formation of large territories of vein differentiation in distal wing regions (638-Gal4/++; UAS-osa/+ females, Fig. 2D), and in the formation of smaller wings with ectopic veins (638-Gal4; UAS-osa/+ males, Fig. 2E). Finally, the combination between nub-Gal4 (expressed in all wing imaginal cells) and UAS-osa displays ectopic vein tissue in most of the wing (nub-Gal4/UAS-osa, Fig. 2F). In these flies, only the region located between the L3 and L4 veins and the region posterior to the L5 vein differentiate intervein tissue (Fig. 2F). The phenotypes of ectopic vein differentiation caused by increased Osa expression are similar to those caused by the over-expression of rho or EGFR (Bier et al., 1990; Sturtevant et al., 1993) (see Supplementary Fig. 2), and are much stronger than those produced by mutations in negative modulators of the EGFR pathway, such as sprouty (Casci et al., 1999), gap1 (Gaul et al., 1992), or MKP3 (Ruiz-Gomez et al., 2005) alleles. The over-expression of Osa has strong dose-sensitive effects. Thus, when Osa levels are progressively augmented using different copies of the sal-Gal4 driver or the UAS-osa, the wings show severe reductions in size, increase in extra vein tissue and loss of intervein territories (Figs. 2G–I). These phenotypes do not seem to be caused by changes in Dpp signalling, because P-Mad and Spalt are normally expressed in sal-Gal4; UAS-osa and in nub-Gal4/UAS-osa discs (Supplementary Fig. 3). In addition to changes in wing vein patterning and wing size, increased Osa expression also promotes the formation of ectopic sensory organs and hairs in the wing (Figs. 2E–F). In this manner, loss and gain of Osa affects the wing veins and the interveins in a coherent manner, because loss of osa eliminates the veins and excess of Osa promotes the differentiation of ectopic veins in the interveins. In contrast, both loss- and gain-of-function conditions for osa cause the appearance of ectopic sensory elements and wing margin hairs in the wing blade. Similarly, loss and gain of Osa reduce wing size, although only in the loss of osa could we detect expression of activated Caspase 3.

Fig. 2. Phenotypes of Osa over-expression. (A) Wild type control wing. (B) Expression of Osa (green) in the nucleus of all wing disc cells. (C) Phenotype of Osa over-expression in 765-Gal4/UAS-osa females, showing ectopic vein tissue (arrowheads). (D) Over-expression of Osa in 638-Gal4/++; UAS-osa/+ females, causing extra veins in the distal region of the L2/L3 and L4/L5 interveins. (E) Over-expression of Osa in 638-Gal4; UAS-osa/+ males, causing a reduction in the size of the wing, the formation of ectopic vein tissue and extra sensory organs. (F) Ectopic vein differentiation and formation of extra sensory organs and hairs in nub-Gal4/UAS-osa wings. (G–I) Dose effects of Osa on wing patterning generated by changing the dose of sal-Gal4 and UAS-osa constructs. sal-Gal4/++; EP-osa/+ (G), sal-Gal4/sal-Gal4; EP-osa/+ (H) and sal-Gal4/sal-Gal4; EP-osa/EP-osa (I). The wings show a progressive severity in the fusion of L2/L3 and L4/L5 veins correlated to the increase in Osa over-expression. The wing in panel E is shown at double magnification than the wings in all other panels.
heterozygosis of osa and bs, and Bier, 1995; Matakatsu et al., 1999; Brentrup et al., 2000). Thus, the phenotype by the heterozygosis of bs in all interveins during larval development (Nussbaumer et al., 2000). In addition, the formation of veins in the interveins, such as net and px (Fig. 3). Dpp and Hh signalling activate the expression of bs in all interveins during larval development (Nussbaumer et al., 2000). In addition, bs is repressed in the veins by EGFR signalling (Roch et al., 1998). A reduction in the dose of osa (not shown), or the double heterozygosis of osa and a brm mutation, correct the phenotype of loss of bs (bs2; Fig. 3B compare to bs2; osa308/brm2 Fig. 3C). Complementarily, excess of Osa strongly enhances the ectopic veins characteristic of a reduction in bs (638-Gal4/+; bs2; OSA-osa/+ females Fig. 3D, compare with Fig. 3B and with Fig. 2D). The effect of osa doses on the bs phenotype suggests that the formation of extra veins in bs mutants requires Osa function. Mutations in osa also affect significantly the phenotype of other mutations that cause the formation of veins in the interveins, such as px and net (Sturtevant and Bier, 1995; Matakatsu et al., 1999; Brentrup et al., 2000). Thus, the extra veins of the net px combination (net1 px72, Fig. 3E) are eliminated in flies double heterozygous for osa and brm (net1 px72; osa308/brm2, Fig. 3F), and the phenotype of loss of veins typical of net ectopic expression (nub-Gal4/+; UAS-net/+; Fig. 3G) (Brentrup et al., 2000) is enhanced in heterozygosis for osa (nub-Gal4/+; UAS-net/osa308, Fig. 3H). From these data we conclude that: 1) osa and the intervein genes net, px and bs affect the formation of veins in opposite manners, and 2) the functions of Osa and Net/Px/Brat could stimulate or antagonise, respectively, the activity of a common vein-promoting factor. Because the activity of intervein genes is required to restrict EGFR signalling to the veins (Sturtevant and Bier, 1995; Roch et al., 1998), the interactions between osa with net, px and bs alleles could be explained if they exert opposite effects on EGFR activity.

Genetic interactions between osa and the intervein-promoting genes bs, net, and px

We explored the possible interactions between osa and other genes required for the development of the interveins such as bs, net and px (Fig. 3). Dpp and Hh signalling activate the expression of bs in all interveins during larval development (Nussbaumer et al., 2000). In addition, bs is repressed in the veins by EGFR signalling (Roch et al., 1998). A reduction in the dose of osa (not shown), or the double heterozygosis of osa and a brm mutation, correct the phenotype of loss of bs (bs2; Fig. 3B compare to bs2; osa308/brm2 Fig. 3C). Complementarily, excess of Osa strongly enhances the ectopic veins characteristic of a reduction in bs (638-Gal4/+; bs2; OSA-osa/+ females Fig. 3D, compare with Fig. 3B and with Fig. 2D). The effect of osa doses on the bs phenotype suggests that the formation of extra veins in bs mutants requires Osa function. Mutations in osa also affect significantly the phenotype of other mutations that cause the formation of veins in the interveins, such as px and net (Sturtevant and Bier, 1995; Matakatsu et al., 1999; Brentrup et al., 2000). Thus, the extra veins of the net px combination (net1 px72, Fig. 3E) are eliminated in flies double heterozygous for osa and brm (net1 px72; osa308/brm2, Fig. 3F), and the phenotype of loss of veins typical of net ectopic expression (nub-Gal4/+; UAS-net/+; Fig. 3G) (Brentrup et al., 2000) is enhanced in heterozygosis for osa (nub-Gal4/+; UAS-net/osa308, Fig. 3H). From these data we conclude that: 1) osa and the intervein genes net, px and bs affect the formation of veins in opposite manners, and 2) the functions of Osa and Net/Px/Brat could stimulate or antagonise, respectively, the activity of a common vein-promoting factor. Because the activity of intervein genes is required to restrict EGFR signalling to the veins (Sturtevant and Bier, 1995; Roch et al., 1998), the interactions between osa with net, px and bs alleles could be explained if they exert opposite effects on EGFR activity.

Genetic interactions between osa and the EGFR pathway

To explore the possibility of a relationship between Osa and EGFR signalling, we analysed the consequences of changing the level of Osa in genetic backgrounds with altered EGFR activity. The expression of a

Fig. 3. Genetic interactions between osa and genes regulating the formation of the interveins. (A) Control wild type wing. (B) Homozygous bs2 female wing, showing ectopic wing tissue in the distal region of the L3, L4 and mostly L5 veins. (C) Homozygous bs2 wing that is also double heterozygous for the mutations osa308 and brm2, showing the suppression of the bs phenotype. (D) Enhancement of the bs phenotype upon the over-expression of Osa in 638-Gal4/+; bs2; EP-osa/+ female wings. The control 638-Gal4/+; UAS-osa/+ is shown in Fig. 2D. (E) Adult net1 px72 wing, showing the formation of ectopic veins in the L2/L3 and L4/L5 interveins. (F) Suppression of the net1 px72 phenotype by the double heterozygosis of osa308 and brm2 (net1 px72; osa308/brm2). (G) Loss of veins caused by ectopic net in nub-Gal4/+; UAS-net/+ females. (H) Enhancement of the nub-Gal4/UAS-net phenotype by the heterozygosis of osa308.

Fig. 4. Interactions between osa and several EGFR components. (A) 638-Gal4/+; UAS-Ras117/+ female wing. The vein L4 is not entirely formed and the distal ends of L2 and L5 are missing. (B) 638-Gal4/+; UAS-Ras117/+ ; osa/+ female wing showing an enhancement of the loss of veins phenotype characteristic of Ras117 mis-expression. (C) Phenotype resulting from generalised expression of Ras117 in combination with osa308 in heterozygosis (638-Gal4/+; UAS-Ras117/+ ; osa308/+, C). This phenotype is reduced compared with its control 638-Gal4/+; UAS-Ras117/+ (F), which is pupal lethal and the wing has to be dissected out of the puparium from a pharate adult. (D and H) The extra veins differentiated in wings expressing the ERK activated form sevenmaker (ERKSem) depend on the dose of Osa, being much weaker in sal-Gal4/sal-Gal4; UAS-ERKSem/osa308 (UAS-ERKSem/+; osa308, D) compared to sal-Gal4/sal-Gal4; UAS-ERKSem/EP-osa wings (UAS-ERKSem/+; UAS-osa/+; H). (E and I) Expression of RNAi against Ras85D in the sal domain prevents the formation of the L2, L3 and L4 veins (sal-Gal4/+; UAS-ras85D, E). The loss of veins caused by Ras RNAi is not modified by Osa ectopic expression (sal-Gal4/+; UAS-ras85D/EP-osa, I). (G and J) Increased expression of Osa cannot induce vein formation in the ve1 vn1 mutant background (638-Gal4/+; UAS-osa/+; rho vn, G). The rho.vn control wing is shown in J (rho vn).
Ras dominant-negative form (Ras\textsuperscript{N17}) in the wing blade eliminates middle stretches of the L4 vein (638-Gal4/+/UAS-Ras\textsuperscript{N17}/+, Fig. 4A), and this phenotype is enhanced when the dose of osa is reduced (638-Gal4/+/UAS-Ras\textsuperscript{N17}/+; osa\textsuperscript{108}/+, Fig. 4B). The expression of a constitutively active form of Ras (Ras\textsuperscript{V12}) in the wing blade (638-Gal4/++; UAS-Ras\textsuperscript{V12}/+) causes pupal lethality, and the wings dissected from pharate adults are severely malformed and display excess of vein differentiation (638-Gal4/++; UAS-Ras\textsuperscript{V12}/+, Fig. 4F). The heterozygosity for osa in this combination allows adult viability, and the effects on wing shape and size of Ras\textsuperscript{V12} are partially rescued, although these wings still differentiate excess of vein tissue (638-Gal4/++; UAS-Ras\textsuperscript{V12}/+; osa\textsuperscript{108}/+, Fig. 4C). The level of Osa also modifies the phenotype of gain of EGFR activity caused by mis-expression of ERK\textsuperscript{sem}, an activated form of ERK that mimics the non-dephosphorilable allele ERK\textsuperscript{sem} (sal-Gal4/++; UAS-ERK\textsuperscript{sem}/osa\textsuperscript{108}, Fig. 4D) compared to sal-Gal4/UAS-osal; UAS-ERK\textsuperscript{sem}/+, Fig. 4H). Taken together, these results suggest that Osa and EGFR signalling cooperate to establish vein fates in the wing, because osa mutations enhance the phenotypes caused by increased EGFR activity and correct the phenotype caused by constitutively active EGFR signalling, and because Osa over-expression enhances the phenotype caused by increased EGFR activity. We also studied the dependence of the extra veins caused by increased Osa on EGFR activity. The reduction of Ras expression by driving Ras RNAi in the centre of the wing (sal-Gal4/++; UAS-irras, Fig. 4E) eliminates the veins L2, L3 and L4, and this phenotype does not change in the presence of excess of Osa (sal-Gal4/++; UAS-osa/ UAS-irras, Fig. 4F). The rho\textsuperscript{V1}vn\textsuperscript{1} combination shows a severe loss-of-vein phenotype caused by the simultaneous reduction in the EGFR ligand Vn and the ligand-processing protease Rho (rho\textsuperscript{V1}vn\textsuperscript{1}, Fig. 4J). This phenotype is not modified when Osa is over-expressed in rho\textsuperscript{V1}vn\textsuperscript{1} wings (nub-Gal4/UAS-osa; rho\textsuperscript{V1}vn\textsuperscript{1}, Fig. 4C). These data indicate that the veins differentiated by increased Osa expression require EGFR activity, and consequently, Osa, as a member of the Brg complex, could function during vein development to facilitate the transcription of genes regulated by EGFR signalling.

Expression of provein and intervein genes in osa mutant backgrounds

To further analyse the developmental basis of osa requirements during vein formation, we studied the expression of several genes related to intervein and vein development in osa mutant clones and in UAS-osa wing discs. We included in this analysis the intervein-promoting gene bs and the vein-specific genes knirps (kni) and caupolican (caup). The expression of bs is activated by the Dpp and Hh pathways in the interveins (Nussbaumer et al., 2000), and repressed by the EGFR pathway in the proveins (Roch et al., 1998). Consequently, bs is expressed at high levels only in the interveins in wild type third instar discs (Fig. 5A) (Fristrom et al., 1994; Montagne et al., 1996). Increased expression of Osa reduces bs expression in the interveins, and only the L3/L4 intervein retains normal levels of Bs (Fig. 5B). The reduction of Bs in cells expressing higher than normal levels of Osa is cell-autonomous (Figs. 5C–C’). Loss of bs in the interveins is generally associated with the formation of ectopic veins (Roch et al., 1998), and therefore the reduction of Bs expression by increasing Osa could explain the extra vein phenotype observed when Osa is over-expressed (see Fig. 2). Surprisingly, clones of osa mutant cells induced in a Minute background also lose Bs expression in the interveins in a cell-autonomous manner (Figs. 5D–D’). The loss of Bs observed in osa mutant cells is unexpected because these cells fail to differentiate veins (see Fig. 1). To reconcile these observations, we propose that Osa is required for bs activation, perhaps collaborating with known bs activators such as Ash2, Dpp and Hh (Nussbaumer et al., 2000; Angulo et al., 2004), and that Osa also participates in bs repression, which in wild type discs is mediated in the veins by EGFR signalling. Interestingly, Osa is not required for the expression of other Hh or Dpp targets, such as patched (Supplementary Fig. 3A), vestigial (Supplementary Fig. 3B) and spalt (Supplementary Fig. 3E).

Because osa mutant cells fail to differentiate the veins independently of Bs expression, the provein genes are strong candidates to be OsA targets. We studied the expression of the provein genes kni and caup in osa mutant clones and in wing discs where the level of Osa is reduced by expressing osa interference RNA (UAS-osa). The expression of Kni and Caup is restricted to the L2 and the L3 and L5 veins, respectively (Fig. 5E). These two genes are still expressed in osa mutant clones (Figs. 5F and G, respectively). The expression of UAS-iosa in the centre of the wing (sal-Gal4/++; UAS-iosa/+) strongly reduces the level of Osa protein (Fig. 5H). In these discs, the expression of Kni and Caup is not modified (Fig. 5I). These data indicate that Osa does not affect the establishment of provein territories, and therefore

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Fig. 5. Changes in provein and intervein gene expression observed in osa mutant cells. (A) Expression of Bs in a third instar wild type disc (red). (B) Increased expression of Osa (nub-Gal4/UAS-osal) reduces Bs expression in all regions except the L3/L4 intervein (white arrowhead). (C–C’) The reduction of Bs expression (Bs is shown in red) in cells expressing higher levels of Osa is cell autonomous (–UAS-osal; the clones of cells expressing higher levels of Osa were induced in hsFLP1.22; P[act-stop-Gal4] UAS-GFP/++; UAS-osal+/+ larvae and are labelled in green). (D–D’) Minute clones of osa mutant cells (marked by the absence of green in D) lose Bs expression (red). In this clone, the L3/L4 intervein domain is absent in the ventral compartment (white arrowheads in D and D’). (E) Expression of the vein-specific transcription factors Kni (red) and Caup (green) in a wild type third instar wing disc. (F) Expression of Osa (green) in sal-Gal4/++; UAS-iosa/+ discs is reduced in the sal dorsal domain of expression. (G) The expression of Kni (red) and Caup (green) in the L2 and L3 proveins, respectively, is not affected in sal-Gal4/++; UAS-iosa/+ discs. (H, I) The expression of Kni (red in H) and Caup (red in I) in the L2 and L3 proveins, respectively, is not affected in osa\textsuperscript{108} mutant clones (labelled by the absence of green in H and I). The inset in (H) corresponds to Kni expression in the ventral compartment. All discs are oriented with the ventral side up and the anterior region to the left.
Fig. 6. Expression of dP-ERK in osa and RasV12 wing discs. (A) Expression of dP-ERK in a third instar wild type disc (red). The position of the dorsal L3 and L4 veins is labelled. (B–B′) Expression of dP-ERK (red) in a third instar disc carrying osa308 mutant clones (labelled by the absence of green) induced in hsFLP1.22; FRT82B osa308/FRT82B M(3)w P[ubi-GFP] larvae. (C) Higher magnification of an osa308 clone showing loss of dP-ERK in its distal-most region (white arrowhead). (D) Expanded expression of dP-ERK (red) in a sal-Gal4/UAS-RasV12 wing disc. (E–E′) The expansion of the domain of dP-ERK expression caused by RasV12 is not affected in osa mutant cells (labelled by loss of green staining) induced in sal-Gal4; UAS-FLP/UAS-RasV12; FRT82B osa308/FRT82B M(3)w P[ubi-GFP] larvae. All discs are oriented with the ventral side up and the anterior region to the left.

Fig. 7. Expression of EGFR target genes in osa mutant backgrounds. (A–C) Expression of argos mRNA (aos) in wild type (A), sal-Gal4/+; UAS-iosa/+ (B) and nub-Gal4/UAS-osa (C). (D–F) Expression of rhomboid mRNA (rho) in wild type (D), sal-G4; UAS-iosa (E) and nub-Gal4/UAS-osa (F). The expression of aos and rho is only detected in the L5 vein (L5 in B and E), which is not included in the domain of sal-Gal4 expression, in discs with reduced expression of Osa. aos and rho are expressed in broader domains in discs with overexpression of Osa, but not expressed at the dorso-ventral boundary (arrows in C and F). (G) Expression of Di (red) in the apical region of the wing blade in a third instar wild type discs. The L3, L4 and L5 proveins are indicated in the ventral compartment. (H) The expression of Di (red) is only detected at normal levels in the L5 vein of sal-Gal4/+; UAS-iosa/+ discs. (I–I′) Expanded expression of Di (red) is detected in sal-Gal4/UAS-osa third instar wing discs. The pictures show the anterior (I) and posterior (I′) parts of the same disc. (J–J′) Expression of Di (red in J and J′) in a third instar wing disc carrying osa304 mutant clones induced in sal-Gal4; UAS-FLP/+; FRT82B osa304/FRT82B M(3)w P[ubi-GFP] larvae. osa mutant cells (labelled by the absence of green in J and J′) lose Di expression in all regions except around the dorsal ventral boundary (white arrowhead), where the effects are variable. All discs are third instar discs from wandering larvae and are oriented with the ventral side up and the anterior region to the left.
its requirements appear restricted to the implementation of the vein differentiation program after the proveins are specified.

Expression of EGFR target genes in osa genetic backgrounds

We have shown that osa is required for vein formation in the wing, and the interactions observed between osa and EGFR variants are compatible with a function of Osa to facilitate the transcription of EGFR target genes. To monitor directly the activity of the pathway and analyse whether osa is required for some step in the pathway upstream of ERK phosphorylation, we studied the expression of dP-ERK in wing discs carrying osa mutant clones. The expression of dP-ERK in wild type discs is detected at higher levels in L3 and L4 veins (Gabay et al., 1997) (Fig. 6A). In osa mutant clones we observed variable effects on dP-ERK accumulation. Thus, most osa clones contain cells expressing and cells not expressing dP-ERK (Figs. 6B–B′ and C). In this manner, it seems that Osa is required for ERK phosphorylation, because in its absence the expression of dP-ERK cannot be sustained. Expression of activated Ras (RasV12) causes a robust increase in dP-ERK accumulation (Fig. 6D). This effect is, as expected, not modified when cells expressing RasV12 are simultaneously mutant for osa (Figs. 6E–E′), indicating that upon a constitutive activation of the EGFR pathway Osa is not needed for ERK phosphorylation. The maintenance of EGFR activity, and therefore the expression of dP-ERK, relies in part on the ability of EGFR signalling to regulate the expression of several genes required for EGFR activation. Thus, the pathway activates the expression of its positive regulator rho in the veins (Roch et al., 1998), and also the expression of aos, which antagonises EGFR activity (Golemb et al., 1996). We find that the expression of these targets is reduced from the longitudinal veins L3 and L4 in sal-Gal4/+; UAS-iosa/+ discs (aos in Fig. 7B and rho in Fig. 7E, compare with control discs in Figs. 7A and D, respectively). Only the expression of aos and rho in the L5 vein, the place where sal-Gal4 is not expressed, can be detected at normal levels in sal-Gal4/UAS-iosa discs (L5 in Figs. 7B and E). In contrast, the expression of aos and rho is detected at higher than normal levels through the wing blade in wing discs over-expressing Osa (aos in Fig. 7C and rho in Fig. 7F). In this manner Osa is required to regulate the expression of EGFR target genes, and this activity could explain the partial loss of ERK phosphorylation observed in osa mutant cells. We also noticed a loss of aos and rho expression in the dorsal and ventral stripes abutting the dorso-ventral boundary in discs over-expressing Osa (nub-Gal4/UAS-osai; Figs. 7C and F, respectively), an effect that might be related to ineffective Wg signalling by cells expressing higher than normal levels of Osa (see below).

We also studied the expression of the Notch ligand Dl in different osa mutant backgrounds. In wild type discs, DI is detected at higher levels in the developing veins, and this expression depends on EGFR signalling (de Celis et al., 1997; Huppert et al., 1997). The expression of osa iRNA in the centre of the wing disc (sal-Gal4/+; UAS-iosa/) strongly reduces the level of DI in the veins L3 and L4 (Fig. 7H, compare with Fig. 7G). Similarly, clones of osa mutant cells lose DI expression in the longitudinal veins (Fig. 7I). The remnants of DI expression detected in these clones (induced in sal-Gal4/+; UAS-FLP/+; FRT82osalos/FRT M(3)w) P[ubi-GFP] discs) are localized close to the dorso-ventral boundary (Figs. 7J–J′). Conversely, ectopic expression of Osa increases the expression of DI in the wing blade (nub-Gal4/UAS-osai; Figs. 7I–I′). Taken together, these data indicate

![Fig. 8. RasV12 expression overcomes Osa requirement to activate DI and aos expression. (A) Expression of DI (red) in sal-Gal4/+; UAS-iosa/+ discs. (B) Loss of DI is not corrected by the expression of activated ERK in i-os discs (sal-Gal4/+; UAS-iosa/UAS-ERK120). (C) Expression of RasV12 in discs with reduced Osa still show increased levels of DI expression (sal-Gal4/UAS-RasV12; UAS-iosa/+). (D–D′) Cell autonomous induction of DI (red) in clones of cells expressing RasV12 (RasV12–; labelled in green in D). (E–E′) The activation of DI expression by RasV12 is not affected by the loss of aos. The disc shows expression of DI (red in E) in sal-Gal4/UAS-RasV12/UAS-FLP; FRT82osalos/FRT M(3)w discs P[ubi-GFP]. aos mutant cells are labelled by the absence of green staining in E and E′. All discs are third instar discs oriented with the ventral side up and the anterior region to the left. (F–H) Expression of aos in wild type discs (F), in sal-Gal4/UAS-RasV12 discs (G) and in sal-Gal4/UAS-RasV12; UAS-iosa/+ (H).]
that Osa is also required to promote DI expression in vein territories. The expression of Notch is not affected in osa mutant cells (Supplementary Fig. 3F).

We next studied whether the effect of loss of Osa on DI and aos expression can be corrected by increasing EGFR signalling in the veins. The reduction in DI expression observed in wing discs expressing i-osa (sal-Gal4/+; UAS-iosa/+; Fig. 8A) is not modified in combination with a moderate increase in EGFR activity (sal-Gal4/+; UAS-osai/UAS-ERK+C; Fig. 8B), but it is rescued when i-osa is expressed in cells with constitutive activation of Ras (sal-Gal4/UAS-RasV12; UAS-iosa/+; Fig. 8C). Similarly, the strong induction of DI expression by RasV12 expression (Figs. 8D-D′) is not modified when cells expressing RasV12 are simultaneously mutant for aos (sal-Gal4/UAS-RasV12; UAS-iosa/+; Fig. 8C). We found similar results in the case of aos, as the induction of its expression by RasV12 (sal-Gal4/UAS-RasV12; Fig. 8G) is not modified when aos is reduced (sal-Gal4/UAS-RasV12; UAS-osai/+; Fig. 8H). In this manner, Osa is required to activate the expression of the EGFR targets DI, rho, and aos, but upon a strong constitutive activation of the pathway (RasV12 expression) the function of Osa is dispensable at least for the expression of DI and aos.

Functions of Osa in wing margin formation

So far, we have shown that a subset of Osa requirements in the wing disc is related to the establishment and/or maintenance of EGFR target gene expression in the veins. The analysis of osa loss of function also uncovered a variety of phenotypes that cannot be accounted by a relation between Osa and EGF signalling. Thus, osa mutant wings lose most structures of the wing margin, and in both loss- and gain-of-function conditions there is differentiation of ectopic sensory organs and hairs in the wing blade. These phenotypes might be in part related to the previously observed antagonism between Osa and Wg signalling (Treisman et al., 1997; Collins and Treisman, 2000; Collins et al., 1999). To further qualify the relationship between Osa and Wg in the wing, we studied the expression of several known Wg target genes and of wg itself in wing discs carrying osa mutant clones. We found that osa cells located at the dorsal ventral boundary, where wg is normally expressed (Supplementary Fig. 4A), fail to express normal levels of Wg (Supplementary Fig. 4B). The faint signal of Wg protein detected in osa cells likely corresponds to Wg produced by wild type surrounding cells (Supplementary Figs. 4B–B′). In fact, the transcription of wg mRNA is lost from the dorso-ventral boundary in wing discs where the level of Osa is lower than normal (nub-Gal4/+; UAS-iosa/+; Supplementary Fig. 4D) in comparison to wild type disc in Supplementary Fig. 4C. We also found Wg protein in osa mutant cells located at longer distances from the dorsal ventral boundary that in wild type cells (Supplementary Figs. 4B–B′), suggesting that Wg diffuses very efficiently through osa mutant clones. In addition, we found changes in the expression of the Wg target genes venal veils (vvl) (de Celis et al., 1995) and distalless (dll) that were consistent with a role of Osa

Fig. 9. Wing phenotypes of loss of several components of the BAP complex. (A–H) Wing phenotypes resulting from the expression at different levels (by changing the temperature) of interference RNA directed against aos (A and B, sal-Gal4/+; UAS-iosa/+; at 17 °C and 25 °C, respectively), moira (C and D, sal-Gal4/+; UAS-imor/) at 17 °C and 25 °C, respectively), brahma (E, G38-Gal4/+; UAS-ibrm/+), sort1 in the combinations sal-Gal4/+; UAS-iosa/+ (F) and G38-Gal4/+; UAS-iosa/+ (G, shown at double magnification), and bap55 (H, sal-Gal4/UAS-bap55). (I–L) Expression of Brm in wing discs of the following genotypes: wt (I), nub-Gal4/+; UAS-ibrm/+ (J), sal-Gal4/+; UAS-iosa/+ (K), and sal-Gal4/+; UAS-iosa/+ (L). (M–P) Expression of Osa in wing discs of the following genotypes: sal-Gal4/+; UAS-iosa/+ (M) sal-Gal4/+; UAS-ibrm/+ (N), sal-Gal4/+; UAS-imor/+ (O) and sal-Gal4/+; UAS-imor/+ (P). Note that i-osa expression affects the levels of Osa and Brm proteins (L and P), and that i-mor affects the expression of Osa (N). i-ibrm and i-osa do not modify the expression of Osa (N) and Brm (K), respectively, but reduce Brm (J) and Osa (M) expression.
in antagonising Wg signalling and/or spreading. Thus, the domain of vvl repression and dll expression is extended away from the dorso-ventral boundary (Vvl, Supplementary Figs. 4E–E′ and Dll, Supplementary Figs. 4F–F′), suggesting that the effectiveness of Wg signalling and/or the range of Wg diffusion are extended in osa mutant cells.

**Wing phenotypes of other members of the BAP complex**

The functional requirements revealed by the analysis of osa loss and gain of function conditions are likely a consequence of Osa acting in the BAP complex. To confirm the involvement of BAP during wing development, we studied the consequences of the loss of function in other subunits belonging to the complex such as the products of the Snr1, Bap55, Mor and Brm genes. The expression of iRNA targeting these genes in the wing blade results in phenotypes similar to those of osa clones or osa-i expressing discs, including loss of wing veins and margin, reduced wing size and the formation of ectopic sensory organs and hairs (Fig. 9). These phenotypes vary in severity depending on each iRNA used, but this is likely a consequence of the different effectiveness of each UAS-iRNA construct. The similar consequences of loss in Osa (Figs. 9A, B), Mor (Figs. 9C, D), Brm (Figs. 9E), Snr1 (Figs. 9F, G) and Bap55 (Fig. 9H) indicate that the activity of the BAP complex, and not a putative Osa function independent of BAP, is required to regulate gene expression during wing development. The effectiveness of different iRNA constructs, and the possibility that a reduction of one member of the BAP complex affects in vivo the accumulation of other components were analysed by looking at the expression of Osa and Brm in wing discs expressing iRNAs directed against osa, brm, snr1 and mor. We find that loss of Osa (Fig. 9M) does not affect Brm expression (Fig. 9K), and, similarly, reduced Brm expression (Fig. 9J) has no consequences on Osa accumulation (Fig. 9N). In contrast, iRNA directed against snr1 causes a strong reduction in the expression of both Osa and Brm proteins (Figs. 9L and P, respectively). In a similar way, mor iRNA reduces the accumulation of Osa (Fig. 9O). These results might explain why isnr1 and imor have the strongest effects on wing patterning. Interestingly, the expression of iRNA directed against bap170, a member of the PBAP complex not present in BAP, causes the formation of extra veins (Supplementary Fig. 5 and see also Carrera et al., 2008), suggesting that BAP and PBAP have opposite effects during vein formation. The expression of irna against bap170 does not affect Osa expression (data not shown).

**Discussion**

Chromatin structure is critical to modulate gene expression during development, and is affected by a variety of alterations such as histone modification, DNA methylation and changes in conformation (Eberharter and Becker, 2004). Proteins related to *Drosophila* Brm, such as yeast SNF2 modify chromatin in an ATP-dependent manner, causing repositioning of nucleosomes along the DNA and re-distribution of histone proteins between nucleosomes (Flaus and Owen-Hughes, 2004). The SWI/SNF complexes are conserved in all eukaryotes (Peterson and Workman, 2000), and display specificity of interactions with distinct transcription factors to regulate different subsets of genes. There are several examples where sequence-specific transcription factors interact specifically with SWI/SNF complexes. For example, the ATPase BRG1 binds Zn-finger proteins and hBRM interacts specifically with CBF-1/Su(H), which recruits hBRM to Notch target promoters such as those of HES1 and HESS (Kadam and Emerson, 2003).

A key aspect in the analysis of Brm function is the identification of targets accounting for the functions of the complex. A necessary step in this analysis is the description of its functional requirements using genetic approaches; which helps to identify the specific processes affected by loss of BAP function. Our data indicate that Osa is required during wing disc development for cell viability, cell proliferation, and for the formation of wing veins and the wing margin. Interestingly, increased expression of Osa in the wing also causes phenotypes related to wing growth and patterning, such as reduced wing size, ectopic sensory organs and hairs and the formation of extra vein tissue in most interveins. We have focused our analysis mostly on Osa, and this raises the question of whether its requirement reflects the function of the BAP complex. We think this is the most likely scenario, because the preliminary analysis of other BAP members, such as Snr1, Bap55, Mor and Brm uncovers similar phenotypes in the wing. Thus, lowering Snr1, Bap55 or Mor levels reduces wing size, disrupts the wing epithelium and causes the differentiation of ectopic sensory organs and hairs. These wings also display loss of veins, and in general the overall phenotypes are similar to those of loss of Osa. The phenotype of iRNA expression directed against brm is much milder, perhaps due to a lower efficiency of this construct, but still these wings show a loss of veins phenotype. The reduction of Bap170, a member of the PBAP complex, causes the formation of ectopic veins (see Supplementary Fig. 5 and Carrera et al., 2008), which is the opposite phenotype to loss of function in osa and in other members that are present in both the BAP and PBAP complexes. Thus, although Brm is the catalytic subunit in both BAP and PBAP, these complexes could act in opposite manners on the same target genes at least during wing vein formation.

Some Osa requirements can be explained by modifications in the transcriptional response to the activity of the Wg signalling pathway (Collins and Treisman, 2000) and by effects on wg expression. The function of Wg is required for the formation of the wing margin, including the development of sensory organs and veins along the anterior wing margin (Phillips and Whittle, 1993; Couso et al., 1994). In the absence of Wg signalling the wing margin does not form, and when Wg signalling is inappropriately activated ectopic sensory organs and hairs differentiate throughout the wing blade (Simpson et al., 1988; Couso et al., 1994). In addition to affecting the response to Wg signalling, Osa is also required for the expression of wg along the dorso-ventral boundary. This requirement might be related to Notch signalling in these cells, and explains why the remnants of wing tissue formed in osa mutant wings do not form the wing margin or ectopic sensory organs.

We have focused on the characterisation of Osa during the formation of the longitudinal wing veins. This process is independent of Wg signalling, and requires the activities of the Notch, Dpp and EGFR signalling pathways (de Celis, 2003). We find that Osa is needed for the expression of bs in the interveins, because bs is not expressed in cells mutant for osa. The regulation of bs expression involves the activity of Ash2 and the function of the Hh and Dpp pathways (Nussbaumer et al., 2000; Angulo et al., 2004). We suggest that Osa participates in the activation of bs facilitating the availability of its regulatory regions to these activators. This aspect of Osa function does not explain the phenotype of loss of veins characteristic of osa mutant cells, because the loss of Bs expression is normally associated with the differentiation of ectopic veins (Fristrom et al., 1994; Montagne et al., 1996; Roch et al., 1998). The only context where bs mutant cells differentiate as interveins is when the activity of the EGFR pathway is reduced (Roch et al., 1998). Therefore, we suggest that loss of bs expression is accompanied in osa mutant cells by a failure in the response to EGFR activity, leading to the differentiation of intervein tissue. Interestingly, the expression of bs is also severely reduced when Osa is present at higher than normal levels, and in this case loss of Bs is accompanied, as expected, by the formation of ectopic veins. The effects of increased Osa on bs expression can also be explained if Osa facilitates EGFR activity, because this pathway mediates the repression of bs in the proveins (Roch et al., 1998). In both cases, the common aspect mediated by Osa might be to regulate bs expression in collaboration with its transcriptional activators and repressors.
Because the failure of osa mutant cells to differentiate the veins is not due to changes in bs expression, nor to changes in the expression of provein genes such as kni and caup, we narrowed the search for Osa candidate targets to the EGFR pathway. Several results suggest a close relationship between Osa and EGFR signalling in the wing. First, the phenotypes of changing osa expression in the veins are very similar to those resulting from the same manipulation in EGFR activity. Thus, a reduction in any core component of the EGFR pathway eliminates the veins, whereas the increase in EGFR signalling activity causes the formation of extra veins in intervein territories (Guichard et al., 1999; Sotillo and de Celis, 2005). Second, we observed genetic interactions between osa and several components of the EGFR pathway compatible with a function of Osa promoting EGFR activity in the veins. Finally, the extra veins caused by excess of Osa are suppressed when the activity of EGFR is reduced, indicating that Osa cannot substitute for EGFR activity. The changes in vein and intervein expression patterns are already detected in the wing disc, before other signalling pathways, such as Dpp, act to promote vein formation (de Celis, 1997). Taken together, these observations suggest that Osa facilitates the response to EGFR activity in the wing disc, but cannot promote the transcription of EGFR targets in the absence of EGFR signalling.

The changes in the expression of EGFR target genes observed in osa mutant cells or in osa gain-of-function experiments are compatible with a direct function of Osa/BAP is the transcriptional regulation of EGFR targets such as Dl, rho and aos. How Osa and the BAP complex are targeted to specific genomic regions is not entirely clear, although it is likely that sequence-specific transcription factors are involved in this process (review in Flaus and Owen-Hughes, 2004). Transcription in response to EGFR signalling is mediated by proteins belonging to the ETS family, such as Pointed-P2, Pointed-P1 and Yan in Drosophila. However, these genes are not required during wing vein formation (our unpublished data), suggesting that other ETS proteins or uncharacterised transcription factors bring about interactions between the regulatory regions of EGFR target genes and the BAP complex.

It is unlikely that Osa participates in any step of the EGFR pathway previous to the transcription of its target genes. We noticed, however, that the expression of dp-ERK, a direct read-out of the pathway activity, is also affected in osa mutant cells. Thus, these cells frequently fail to express normal levels of dp-ERK, a result indicating that EGFR activity is reduced. We observed this effect in the wing, the EGFR pathway is engaged in a positive feedback loop mediated by the activation of rho expression, which maintains EGFR activity in cells where it has already been activated (Roch et al., 1998). Thus, loss of osa leads to a failure to express rho and subsequently to a reduction in the activity of the pathway detected as a loss of dp-ERK expression. There is one experimental situation in which Osa function appears to be dispensable for the expression of EGFR target genes. Thus, when a constitutive active form of Ras, RasV12, is driven in the wing, the augmented expression of DI and aos, and the accumulation of dp-ERK are not affected by a reduction in Osa levels. It is possible that in this situation of strong and constitutive activity of the pathway, the possible modifications to chromatin structure brought about by Osa/BAP on EGFR target genes are not necessary, perhaps because at this level of EGFR activation the transcriptional repressors antagonising EGFR target gene transcription, such as Cic and Gro, are inactivated by the pathway (Hasson et al., 2005; Astigarraga et al., 2007), and this might make dispensable the function of Osa.

It is not entirely clear to what extent the link we observe between BAP function and EGFR signalling during wing disc development is conserved in other developmental systems and in other organisms. Some phenotypes of osa and brm alleles described in the eye disc, such as the loss of photoreceptor cells (Treisman et al., 1997), are also observed upon a reduction in EGFR activity (Freeman, 1996). Similarly, the loss of distal growth in the legs is also characteristic of reduced EGFR activity (Galindo et al., 2002). These data are indicative of a general requirement for Osa in the expression of EGFR target genes at least in imaginal discs. The genetic approach we used identifies transcription downstream of EGFR signalling as a relevant in vivo function of BAP complexes. Subsequent biochemical analysis should determine whether the functional interactions we observed are mediated by direct binding of BAP to the regulatory regions of bs and other EGFR target genes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2009.03.010.

References


