The Osa-containing SWI/SNF chromatin-remodeling complex regulates stem cell commitment in the adult Drosophila intestine

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SUMMARY
The proportion of stem cells versus differentiated progeny is well balanced to maintain tissue homeostasis, which in turn depends on the balance of the different signaling pathways involved in stem cell self-renewal versus lineage-specific differentiation. In a screen for genes that regulate cell lineage determination in the posterior midgut, we identified that the Osa-containing SWI/SNF (Brahma) chromatin-remodeling complex regulates Drosophila midgut homeostasis. Mutations in subunits of the Osa-containing complex result in intestinal stem cell (ISC) expansion as well as enteroendocrine (EE) cell reduction. We further demonstrated that Osa regulates ISC self-renewal and differentiation into enterocytes by elaborating Notch signaling, and ISC commitment to differentiation into EE cells by regulating the expression of A sense, an EE cell fate determinant. Our data uncover a unique mechanism whereby the commitment of stem cells to discrete lineages is coordinately regulated by chromatin-remodeling factors.

KEY WORDS: Chromatin-remodeling factor, SWI/SNF, Osa, Intestinal stem cells, Self-renewal, Differentiation, Drosophila

INTRODUCTION
Adult tissue homeostasis is maintained by adult stem cells, which are multipotent cells that can self-renew and differentiate into functional cell types throughout the lifetime of the organism. The differentiation into multiple mature cell types and the self-renewal of adult stem cells are well balanced, and alterations in this equilibrium may cause diseases such as premature aging and carcinogenic transformation.

Like its mammalian counterpart, the adult midgut of Drosophila is maintained by multipotent intestinal stem cells (ISCs). After an asymmetrical division (de Navascués et al., 2012; Goulas et al., 2012; O’Brien et al., 2011), ISCs give rise to one new ISC (self-renewal) and one immature daughter cell, an enteroblast (EB). The EB can further differentiate into either an absorptive enterocyte (EC) or a secretory enteroendocrine (EE) cell without mitotic division (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). Notch (N) signaling plays a major role in regulating ISC self-renewal and differentiation, and its loss leads to ISC expansion at the expense of ECs and to increased numbers of EE cells, probably because of the elevated expression of EE cell fate determinants scute (sc) and a sense (ase), whereas N overactivation results in ISC differentiation into ECs (Bardin et al., 2010; Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006; Ohlstein and Spradling, 2007). The ligand of the N pathway, Delta (DI), is specifically expressed in ISC s and unidirectionally switches the N signaling pathway on in neighboring EBs to promote differentiation toward ECs.

Stem cell fate is orchestrated by both intrinsic programs within the stem cells and extrinsic cues, namely the stem cell niche (Decotto and Spradling, 2005). Epigenetic programming, such as DNA methylation, histone modification and chromatin remodeling, which can generate variable patterns of gene expression from an invariant regulatory DNA sequence, has been identified as a major intrinsic mechanism for stem cell fate regulation (Hochdedinger and Plath, 2009; Juliandi et al., 2010). However, the epigenetic regulation of stem cell self-renewal and differentiation in vivo is not well understood.

SWI/SNF is an evolutionarily conserved and well characterized ATP-dependent chromatin-remodeling complex (Bouazaoune and Brehm, 2006). A growing body of evidence indicates that many counterparts of SWI/SNF in mammals have a widespread role in tumor suppression; a high frequency of mutations in several SWI/SNF subunits have been identified in various cancers (Clapier and Cairns, 2009; Wilson and Roberts, 2011). There are at least two subtypes of the SWI/SNF (Brahma) complex in Drosophila: BAP and PBAP (Bouazaoune and Brehm, 2006; Mohrmann et al., 2004). BAP and PBAP share common subunits including Brahma (Brm), Snr1 and Moira (Mor) but contain different signature proteins. Osa defines the BAP complex, which is required for normal embryonic segmentation and antagonizes Wingless signaling (Collins and Treisman, 2000; Treisman et al., 1997). The BAP complex also plays a role in the regulation of gene expression in response to Egfr signaling in the Drosophila wing (Terriente-Félix and de Celis, 2009). The mammalian homologs of Osa, BAF250a (ARID1A) and BAF250b (ARID1B), are required for maintaining the pluripotency of embryonic stem cells (Gao et al., 2008; Yan et al., 2008).

To further understand the molecular mechanisms of ISC self-renewal and differentiation, we carried out a transgenic RNAi screen and identified that the Osa-containing SWI/SNF complex regulates Drosophila ISC commitment to differentiation into discrete lineages. Loss-of-function mutations of subunits of the Osa-containing SWI/SNF complex resulted in ISC-like cell expansion at the expense of differentiated EC and EE cells. We demonstrated that Osa binds to the promoters of DI and ase to regulate their expression, thus controlling ISC self-renewal and commitment to differentiation into EC and EE cells.
MATERIALS AND METHODS

Fly strains
The following fly strains were used: esg-Gal4 (Shigeo Hayashi, Riken); esg-lacZ (Stephen Di Nardo, University of Pennsylvania); mira-GFP (Francois Schweisguth, CNRS); DI-lacZ (Bruce Edgar, University of Heidelberg); UAS-NFAT (Ken Irvine, Rutgers); UAS-NFD (Mark Fortini, Thomas Jefferson University); Su(H)GBE-lacZ (Sarah Bray, University of Cambridge); UAS-ase (Yuh Nung Jan, UCSF); ase-Gal4 (Trummee Lee, Janelia Farm); FRT52-osa (James Kennison, NIH); and FRT628-Snr1 (Andrew Dingwall, Loyola University). UAS-Di, act-Gal4, UAS-se, UAS-2XSYFP, tub-Gal80 and fly strains for MARCM clones (FRT628, pM, act>y-Gal4, UAS-GFP; SM6, hs-htp; FRT52 tub-Gal80) were obtained from the Bloomington Drosophila Stock Center (BDSC) at Indiana University. The following transgenic RNAi lines were obtained from BDSC or the Vienna Drosophila RNAi Center (VDRC): UAS-osRNAi (V7810 and BL31266), UAS-Snr1 RNAi (V12645, V108599 and BL32372), UAS-brm RNAi (V37721 and BL31712), UAS-mor RNAi (V6969 and V110712) and UAS-ase RNAi (V108511).

The UAS-Snr1 transgene was generated by cloning full-length cDNA of Snr1 into pUASt (Brand and Perrimon, 1993), and injecting purified DNA into the embryo using standard protocols.

MARCM clone assay
To induce MARCM clones of FRT628, pM (as a wild-type control), FRT52, osa, FRT52-Snr1 and FRT52-Dp none, we generated the following flies: act>y-Gal4, UAS-GFP/S6M, hs-htp; FRT52 tub-Gal80/FRT52 mutant. The genotype of flies used to generate N and osa double-mutant clones is hs-htp, FRT52i tub-Gal80/FRT52i N561, act>y-Gal4, UAS-GFP; FRT52i osa/FRT52i tub-Gal80. Three- or four-day-old adult female flies were heat shocked twice at an interval of 8-12 hours, at 37°C for 60 minutes. The flies were transferred to fresh food daily after the final heat shock, and their posterior midguts were processed for staining at the indicated times. The fly intestines were dissected in PBS and fixed in PBS containing 4% formaldehyde for 20 minutes. After three 5-minute rinses with PBT (PBS + 0.1% Triton X-100), the samples were blocked with PBT containing 5% normal goat serum overnight at 4°C. Then, the samples were incubated with primary antibody at room temperature for 2 hours and then with fluorescent secondary antibody for 1 hour at room temperature. Samples were mounted in Vectashield mounting medium with DAPI (Vector Laboratories). We used the following antibodies: mouse anti-β-Gal (1:200; Clontech); mouse anti-DI [1:50; Developmental Studies Hybridoma Bank (DSHB)]; mouse anti-Pros (1:50; DSHB); nc82 (1:20; DSHB); rabbit polyclonal anti-Pdm1 (1:1000; a gift from X. Yang, Zhejiang University); rabbit anti-Spd (1:1000; a gift from J. Skeath, Washington University in St Louis); mouse anti-Osa (1:20; DSHB); rabbit anti-Snr1 (1:1000; this study); rabbit anti-Ase (1:2000; a gift from Yuh Nung Jan); guinea pig anti-Sc (1:1000; a gift from S. Crews, UNC-Chapel Hill) and chicken anti-GFP (1:3000; Abcam). Secondary antibodies were goat anti-mouse, anti-chicken and anti-rabbit IgG conjugated to Alexa 488, Alexa 568 or Alexa 646 (1:400; Molecular Probes). Images were captured with a Zeiss LSM 510 confocal system and processed with LSM5 Image Browser (Zeiss) and Adobe Photoshop.

Quantification and statistical analysis
To quantify the number of escargot (esg) or Prospero (Pros) cells in Fig. 1 and Fig. 5, the esg or Pros cells were counted in a 5×10^3 µm² area of the field. In Fig. 4, all of the images were taken with the same confocal settings and the fluorescence intensity was measured using LSM5 Image Browser. All data were analyzed using Student’s t-test and sample size (n) is shown in the text.

RESULTS

Knockdown of the Osa-containing SWI/SNF chromatin-remodeling complex results in the expansion of esg-expressing cells
To identify new regulators of ISCs, we carried out a screen in which a collection of transgenic RNAi lines from the Vienna Drosophila RNAi Center and the Bloomington Stock Center (Dietzl et al., 2007; Ni et al., 2009) were crossed with act-Gal4, esg-lacZ, Tub-Gal80 (referred to as act, esg-lacZ) flies. One-week-old adult flies were shifted to the restrictive temperature (29°C) for 1 week, dissected and stained, and then examined for esg-lacZ-labeled progenitors.

One of the first genes identified in this screen was osa. Knockdown of osa by transgenic RNAi (osaRNAi; V7810) resulted in a dramatic expansion of esg cells (average of 49.5 esg cells/5×10^3 µm², n=31; Fig. 1B,G). Knockdown of genes by dsRNAs often produces false-positive phenotypes because of off-target effects (Kulkarni et al., 2006). We ruled out the possibility of false-positive effects and confirmed the osa phenotype with a second transgenic RNAi line (BL31266; 44.7 esg cells/5×10^3 µm², n=42; Fig. 1E,G) generated from independent sequences (Ni et al., 2009). We further stained wild-type and osaRNAi midguts for phospho-Histone H3 (pH3), a specific marker for mitotic cells. More pH3+ cells were found in osa RNAi posterior midguts than in wild type (supplementary material Fig. S1), indicating that these esg cells kept dividing to achieve the cell expansion.

Osa is a component of the BAP SWI/SNF complex (supplementary material Fig. S2E) (Bouazoune and Brehm, 2006; Clapier and Cairns, 2009; Collins et al., 1999; Vázquez et al., 1999). Knockdowns of three other components (Snr1, brm and mor) by RNAi also resulted in significant increases of esg-lacZ+ cells in the
expected, in GFP-labeled cells, which is consistent with their role in chromatin remodeling. As we compensate the loss of function of one of the other components in the complex, it might partially compensate the loss of function of Snr1. In the wild-type flies, Osa and Snr1 were expressed in all cells. We examined their expression patterns in the midgut using antibodies. In the wild-type midguts, Osa and Snr1 were expressed in all cells. The knockdown of esg resulted in the expansion of ISCs and EBs, which is consistent with its reported expression in ISCs (Ohlstein and Perrimon, 2006; Ohlstein and Spradling, 2007). In osaRNAi posterior midguts, we also found that Dl expression was undetectable in Snr1RNAi midguts (esgRNAi; Snr1RNAi), which suggests that the expanded esg RNAi cells that are ISCs express cytoplasmic Dl (Fig. 2C–C').

**Osa and Snr1 autonomously regulate ISC self-renewal and differentiation**

To further determine the function of Osa in regulating ISC self-renewal or differentiation, we generated wild-type and osa mutant clones using the mosaic analysis with a repressible cell marker (MARCM) technique (Lee and Luo, 1999). Clones marked homozygous for wild type (Fig. 3A–A'), Pros + EE cells (arrowheads in Fig. 3A–A'), Dl ISCs (arrows in Fig. 3A–A') and Spdo ISC-like cells (arrow in Fig. 3C). However, osa mutant clones were almost devoid of EC and EE cells, and all cells were Dl and Spdo ISC-like cells (Fig. 3B–B').

To more precisely assess the phenotypes, we counted the number of Spdo ISC-like-like cells in wild-type and Pros + EE cells in GFP-labeled MARCM clones of wild type, osa and DlRNAi (a null allele of Dl; supplementary material Fig. S5) (Heitzler and Simpson, 1991) midguts at 4 and 8 days ACI (Fig. 3E, F). In the wild-type control clones, 23% and 18% of cells were Pros + EC cells at 4 and 8 days ACI (n = 20, 21), respectively. However, 91% and 98% of cells were Pros + ISC-like cells in osa clones at 4 and 8 days ACI (n = 20, 21), respectively, and 70% and 67% of cells were Pros + ISC-like cells in DlRNAi clones at 4 and 8 days ACI (n = 20, 21), respectively. The Pros + EE cells were dramatically reduced in osa mutant clones. At 4 and 8 days ACI, respectively, 9.7% and 11% of cells were EE cells in wild-type clones and only 1.8% and 0.9% of cells were EE cells in osa mutant clones.

**Knockdown of Osa produces excess ISC-like cells**

Esg is a marker of both ISCs and EBs (Micchelli and Perrimon, 2006). To characterize expanded esg cells in osaRNAi midguts, we analyzed the expression of Su(H)GGE-lacZ, a marker of EBs (Ohlstein and Spradling, 2007), in wild-type and osaRNAi posterior midguts. In wild-type midguts, only some esg cells expressed Su(H)GGE-lacZ (Fig. 2A–A'), which is consistent with its reported expression in EBs (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2007). In osaRNAi posterior midguts (Fig. 2B–B'), Su(H)GGE-lacZ-expressing cells were missing, suggesting that the expanded esg cells in osaRNAi posterior midguts are not EBs. miranda promoter-GFP (Mira-GFP) is a target of Daughterless-dependent bHLH transcriptional activity and is specifically expressed in ISCs (Bardin et al., 2010). Interestingly, Mira-GFP is indeed expressed in all expanded esg cells in osaRNAi posterior midguts (supplementary material Fig. S4A–A').

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might specifically regulate N expression in the posterior midgut. Mutant MARCM clones rescued their phenotypes to that of wild-type MARCM clones, all GFP-marked cells were ISC-like diploid cells (Fig. 3B,B'). However, no Dl expression was detected in the wild-type midguts (arrows in C,C'). In the Dl mutant marcm clones, all GFP-marked cells were ISC-like cells. Scale bars: 10 μm.

**Osa regulates Dl expression in ISCs**

The undetectable level of Dl expression, as judged by antibody staining, encouraged us to further examine the expression of Dl using Dl-lacZ, an enhancer trap at the Dl locus (Beebe et al., 2010; Jiang et al., 2009; Zeng et al., 2010). In wild-type posterior midguts, Dl-lacZ was highly enriched in ISCs (fluorescence intensity of 3991, n=56; Fig. 4A,A',C). However, the expression of Dl-lacZ was dramatically reduced in osaRNAi posterior midguts (esaRNAi, fluorescence intensity of 200, n=58; Fig. 4B,B',C). We also detected Dl mRNA expression by qPCR and found that the level of Dl mRNA was significantly reduced in the osaRNAi midguts (Fig. 4D). These data suggest that Osa regulates Dl expression at the transcriptional level in the posterior midgut.

Expression of the Dl receptor N was unaffected, as N expression can be detected in the osaRNAi midguts as well as in the wild type (supplementary material Fig. S4C-D'). These data suggest that Osa might specifically regulate Dl expression at the transcriptional level to control ISC self-renewal and differentiation of ISCs into ECs.

**Dl expression is sufficient to rescue the ISC tumor phenotype but not the EE cell phenotype of osa mutants**

When an activated form of N is expressed in the posterior midgut (esaRNAi), all ISCs differentiate into ECs (compare supplementary material Fig. S7B with S7A). In the osaRNAi midgut (esaRNAi, supplementary material Fig. S7C), excess ISC-like cells were found at the expense of differentiated EC and EE cells. To determine the epistatic relationship between Osa and the N signaling pathway, we expressed the constitutively activate form of N (NΔ34a) in the osaRNAi midgut (esaRNAi,NΔ34a, supplementary material Fig. S7D). After shifting the adult flies to the restrictive temperature (29°C) for 7 days, all ISCs had differentiated into ECs. These results support the idea that Osa functions upstream of N in regulating EC fate.

Since Osa functions upstream of N signaling, and Dl expression is blocked in the osaRNAi midgut as well, Osa might regulate ISC differentiation into ECs by controlling Dl expression. To test this, we expressed UAS-Dl in osaRNAi posterior midguts (esaRNAi+UAS-Dl; Fig. 5C) or in GFP-marked wild-type and osaRNAi mutant MARCM clones (Fig. 5E; supplementary material Fig. S7E-F'). UAS-Dl expression using the esaRNAi driver rescues the ISC tumor phenotype in osaRNAi midguts to a phenotype resembling wild type (8.5, 43.5 and 10.5 esaRNAi cells/5×10³ μm²; n=23, 36 and 42, respectively; Fig. 5A-C,F). In osaRNAi mutant MARCM clones, all GFP-marked cells were ISC-like diploid cells (Fig. 3B,B',D, Fig. 5D). UAS-Dl expression in osaRNAi mutant MARCM clones rescued their phenotypes to that resembling wild-type clones including ISCs (or EBs) and polyploid ECs (Fig. 5E; supplementary material Fig. S7E-F').

We also quantified the number of Pros+ EE cells in the midguts of wild type (Fig. 5A), osaRNAi (Fig. 5B) and osaRNAi with UAS-Dl expression (Fig. 5C). Dl expression does not rescue the EE cell-loss phenotype in osaRNAi midguts (3.8, 1.8 and 1.7 EE cells/5×10³ μm²; n=23, 36 and 42, respectively; Fig. 5G).

These data suggest that Osa regulates ISC self-renewal and differentiation into ECs by controlling Dl expression and regulates EE cell formation by controlling other gene(s).

**The Osa-containing SWI/SNF complex functions downstream of N in regulating EE cell formation**

In addition to regulating Dl expression to control EC fates, the Osa-containing SWI/SNF complex might control EE cell formation by regulating other signal(s). We examined the epistatic relationship of N signaling and the SWI/SNF complex in regulating EE cell fate determination. Expressing a dominant-negative form of N in the posterior midgut (esaRNAi,NΔN; 50.9 EE cells/5×10³ μm², n=33; Fig. 5H,K) resulted in the formation of...
Fig. 3. Osa cell-autonomously regulates ISC self-renewal and differentiation into EE cells. GFP+ clones were generated in the posterior midguts of flies of the indicated genotypes using the MARCM technique. They were stained on the eighth day after clone induction (ACI) with the indicated antibodies. DAPI, blue or white. (A–A′) The FRT82B; pIIM wild-type clone at 8 days ACI. There are ISCs labeled by Dl (cytoplasmic red, arrows), EE cells labeled by Pros (nuclear red, arrowhead), and polyploid ECs (asterisk) in the clone. (B–B′) The FRT82B, osa2−/− clone at 8 days ACI. All cells in the clone are diploid and do not express Dl, whereas neighboring wild-type ISCs express Dl (arrow). (A′,B′) GFP-labeled MARCM clone is outlined. (C,D) In the wild-type clone (C), Spdo is only expressed in ISCs (arrow), whereas all cells in the osa2−/− clone (D) express the ISC-specific marker Spdo. (E) Analysis of the percentage of Spdo+ ISC-like cells in wild-type, osa2−/− and DI clones at 4 and 8 days ACI. Almost all cells in the osa2−/− clone are Spdo+ ISC-like cells. (F) Analysis of the percentage of Pros+ EE cells in wild-type, osa2−/− and DI clones at 4 and 8 days ACI. osa2−/− clones contain significantly fewer Pros+ EE cells than DI and wild-type clones. Data are mean ± s.e.m. Scale bars: 10 μm.

excess EE cells. The expression of osaRNAi (esg>.uaN=osaRNAi; 3.2 EE cells/5×10^3 μm^2, n=45; Fig. 5I,K) and Snr1RNAi (esg>uA^Dl=osaRNAi; 3.2 EE cells/5×10^3 μm^2, n=25; Fig. 5I,K) in N^{DN} midguts completely suppressed the phenotype of excess EE cells to wild-type midgut levels (3.7 EE cells/5×10^3 μm^2, n=30; Fig. 5A,K).

In N^{DN}null (N^{DN}N^{DN}) mutant MARCM clones, excess Pros+ EE cells were found (38% of the cells are Pros− EE cells, n=30; supplementary material Fig. S7G-I). As in osa RNAi mutant MARCM clones (Fig. 3B,B′,F), very few Pros− EE cells were detected in N^{DN}/osa−/− double-mutant MARCM clones (only 1.2% of the cells are Pros− EE cells, n=40; supplementary material Fig. S7I,1). Consistently, we also noted that osa2 repressed Dl expression in N^{DN} clones (supplementary material Fig. S7H). To exclude the possibility that Osa directly regulates expression of the EE cell marker Pros in contexts other than in EE cell formation, we identified another EE cell-specific marker, nc82 (Bruchpilot – FlyBase), which labels the synaptic active zone in the Drosophila neuromuscular junction (Wagh et al., 2006). nc82 expresses punctate staining at the membrane of Pros+ EE cells (supplementary material Fig. S8A–B); however, unlike in the N^{DN} midgut where there are many nc82+ EE cell clusters (supplementary material Fig. S8D–D′), we could not detect the expansion of any nc82+ EE cells in osaRNAi midguts (esg>uaN=osaRNAi; supplementary material Fig. S8C–C′). Together, these data suggest that the Osa-containing SWI/SNF complex functions downstream of N in regulating EE cell formation.

Osa regulates EE cell formation through Ase

Two transcription factors, Sc and Ase, have been shown to play a major role in EE cell fate determination and to be upregulated in the N^{DN} midgut by mRNA profiling (Bardin et al., 2010). Expression of Sc and Ase in the posterior midgut was below the detection level using specific antibodies (Brand et al., 1993; Stagg et al., 2011) (supplementary material Fig. S9A–A′,C–C′). Nevertheless, Sc and Ase were readily detected in the midgut with ectopic sc and ase expression driven by esg>uaN=UAS-sc and esg>uaN=UAS-ase) for 24 hours at 29°C (supplementary material Fig. S9B–B′,D–D′). In addition, consistent with previous observations by mRNA profiling (Bardin et al., 2010), both Sc and Ase were upregulated in the N^{DN} midgut midgut as assessed by qPCR (Fig. 6A,B) and antibody immunofluorescence (compare supplementary material Fig. S9G with S9A,A′/H11032). Using the more sensitive ase-Gal4 (ase-Gal4>UAS-mCD8-GFP) transgene (Zhu et al., 2006), we detected that ase is weakly expressed in ISCs and EBs but not in Pros− EE cells (supplementary material Fig. S9E–F′).
To examine the relationship of Osa to Sc and Ase, we first compared mRNA levels of sc and ase in wild-type and osaRNAi midguts by qPCR. Although sc mRNA levels were lower in osaRNAi than in NDN midgut, sc mRNA levels were upregulated in osaRNAi compared with wild-type midgut (Fig. 6A). However, unlike in the NDN midgut (Bardin et al., 2010), the ase mRNA levels were significantly lower in osaRNAi than in wild-type control midgut (Fig. 6B). We further confirmed the expression levels of Sc and Ase in osaRNAi midgut by antibody immunofluorescence. osaRNAi midguts had much higher expression of sc than wild-type midguts (compare supplementary material Fig. S9H with S9A, A′), whereas ase expression was undetectable in osaRNAi as in wild-type midguts (compare supplementary material Fig. S9J with S9C, C′).

These data suggest that the Osa-containing SWI/SNF complex might control EE cell formation through regulating the expression of ase. Indeed, expression of aseRNAi in the NDN midgut (Fig. 6D; espN>NDN+aseRNAi) suppressed the excess EE cell phenotype of NDN (compare Fig. 6D with 6C). When we tried to overexpress ase in the osaRNAi midgut (espN>osaRNAi+ase) it suppressed the EE cell-loss phenotype of osaRNAi (only 1.8 EE cells/5×10^3 μm^2 in osaRNAi midgut but 39.7 EE cells/5×10^3 μm^2 in the osaRNAi+ase midgut; n=36 and 33, respectively; Fig. 6E-G). These data together suggest that Osa regulates EE cell formation through Ase. Osa and Snr1 bind to the promoters of DI and ase

We further examined the interaction of Osa and Snr1 proteins with the promoters of DI, ase, and spdo in a ChIP assay using Osa- and

**Fig. 5. Repression of ISC expansion by expression of DI in osa mutants.** (A-C) The wild-type control (A), osaRNAi (B) and osaRNAi+UAS-Dl (C) are expressed in the posterior midgut using espN. The expression of osaRNAi results in ISC-like cell expansion (B), and the expression of UAS-Dl rescues the ISC-like cell expansion phenotype caused by osa depletion (compare C with B and A). (D,E) GFP+ clones were generated in the posterior midgut of flies of the indicated genotypes by the MARCM technique, and were stained with the indicated antibodies at 6 days ACI. (D) osaRNAi midguts by qPCR. Although scRNA levels were lower in osaRNAi, and in those expressing osaRNAi+UAS-Dl. (E) The expression of osaRNAi, whereas midguts (compare supplementary material Fig. S9H with S9A,A′). (F) Analysis of the percentage of espN EE cells in wild type, in those expressing osaRNAi, and in those expressing osaRNAi+UAS-Dl. (G) Analysis of the percentage of Pros+ EE cells in wild type, in those expressing osaRNAi, and in those expressing osaRNAi+UAS-Dl. (H) The expression of DI in osaRNAi midguts does not increase the number of Pros+ EE cells to the wild-type level. (I) Knockdown of esgRNAi expressed in the posterior midgut using UAS-esgts>NDN. (J) Knockdown of Snr1RNAi expressed in the posterior midgut (Fig. 6B). We further confirmed the expression levels of Sc and Ase in osaRNAi midgut by antibody immunofluorescence. osaRNAi midguts had much higher expression of sc than wild-type midguts (compare supplementary material Fig. S9H with S9A, A′), whereas ase expression was undetectable in osaRNAi as in wild-type midguts (compare supplementary material Fig. S9J with S9C, C′).
remodeling complex performs a crucial function in gene regulation by using energy derived from ATP hydrolysis to alter the contacts between histones and DNA in nucleosomes, resulting in increased DNA accessibility to transcription factors and other regulatory proteins. Activating the N signal in EBs with the ISC-originated N ligand Dl is essential for EB differentiation into ECs. Osa promotes the expression of Dl in ISCs and maintains activation of the N signal in EBs to promote EB to EC differentiation. Repressing Dl expression by loss of function of Osa enhances ISC self-renewal and blocks ISC commitment to ECs, resulting in ISC expansion/tumors. Dl expression alone is sufficient to rescue the ISC expansion/tumor phenotype in osa mutants. SWI/SNF components have been shown to frequently occupy transcription start sites, enhancers and CTCF-binding regions (Euskirchen et al., 2011). Indeed, Osa and Snr1 bind to the promoter region of Dl at the chromatin level. Consistently, genetic interaction between the SWI/SNF complex and Dl were found during a genetic screen (Armstrong et al., 2005), and the expression of a dominant-negative form of Brm, a core component of the SWI/SNF complex, in the adult sense organ precursor lineage causes phenotypes similar to those resulting from impaired DI-N signaling.

Why does the Osa-containing SWI/SNF complex regulate Dl expression only in ISCs and not in EBs? One possible explanation is that the complex in ISCs and EBs has different associated factors, and only that in ISCs is able to regulate Dl expression. Another possibility is that some transcription factors recruit the complex to regulate Dl expression, and these transcription factors exist only in ISCs.

The role of the Osa-containing SWI/SNF complex in ISC differentiation into EE cells

Disrupting the DI-N signal in ISCs results in both ISC and EE cell expansion. Given that Osa regulates Dl expression in ISCs, osa mutant ISCs should have phenotypes similar to those that result from impaired DI-N signaling; however, osa mutation only causes ISC expansion, and EE cell formation is repressed. Proneural bHLH factors of the achaete-scute complex (AS-C), Sc and Ase, have been reported to play a crucial role in EE cell fate specification (Bardin et al., 2010). sc or ase overexpression in midgut progenitors leads to dramatically increased EE cell numbers, whereas mutant ISC clones of Df(1)scB57, which lack all four AS-C genes, i.e. achaete (ac), sc, lethal of scute (l(1)sc) and ase, are devoid of EE cells (Bardin et al., 2010). sc and ase expression is upregulated when the N signal is blocked in the intestinal midgut. Similarly, we also found that the expression of sc is upregulated in the osaRNAi midgut compared with the wild-type control, whereas ase expression is significantly decreased in the osaRNAi midgut. Furthermore, knockdown of ase alone blocks EE cell formation in the N0Nmidgut. Thus, our data suggest that Osa controls EE cell formation by regulating the expression of ase. Terminal differentiation of EBs into EC or EE cells has been proposed to be regulated by differential N signaling, whereby a high level of N activity induces EBs to differentiate into ECs, whereas a low level of N activity promotes the EE cell fate (Ohlstein and Spradling, 2007). However, EE cells can still be generated even when N activity is suppressed by either the expression of N0N or the clonal deletion of DI or N, suggesting that the N signal is not required for EE cells at all. Both Sc and Ase may play important roles in regulating EE cell fate in the midgut. A high level of N activity might suppress the expression of sc and ase and thereby promote EC fate. By contrast, a low level of N activity might activate the expression of sc and ase.
inducing EB cells to differentiate into EE cells. Excess EE cells in the A^{Dp} midgut might be the result of upregulation of ase and sc caused by disruption of the N signal. Indeed, previous studies have shown that N signaling activates the expression of the Enhancer of split complex (E{spl}-C), which can, in turn, repress the expression of the proneural genes ac, sc, l(1)sc and ase (Bailey and Posakony, 1995; Lecourtois and Schweisguth, 1995). In addition to sc and ase, the overexpression of ac and l(1)sc in the midgut also results in dramatically increased EE cell numbers, but only Sc and Ase are required for EE cell formation (data not shown). Why does Osa regulate ase and not other AS-C members? Even though Ase belongs to the AS-C proneural family, it differs from the other AS-C members in its expression pattern, regulation, mutate phenotype and in some DNA-binding properties (Jarman et al., 1993). These characteristics suggest that Ase might be associated with some specific transcription factors that bind to ase DNA at particular chromatin states controlled by the Osa-containing SWI/SNF chromatin-remodeling complex.

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Competing interests statement
The authors declare no competing financial interests.

Author contributions
X.Z. and S.X.H. conceived the project, designed and performed the experiments and wrote the manuscript. X.L. provided reagents and edited the manuscript.

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brahma chromatin-remodeling factor to regulate transcription. Development 126, 733-742.


Fig. S1. Knockdown of *osa* increases cell division in posterior midgut. (A–C’) The wild-type control and *osa* RNAi were expressed in the adult midgut using *esg*<sup>ts</sup>. As compared with the wild-type control (A,A’), there were more pH3-positive dividing cells in *osa* RNAi posterior midguts (V7810 in B,B’ and BL31266 in C,C’). (D) Quantification of pH3-positive cells in wild-type and *osa* RNAi posterior midguts. Data are mean ± s.e.m.
Fig. S2. Knockdown of other components in the SWI/SNF complex results in the expansion of esg-expressing cells. (A-D) The indicated genes were depleted in the adult Drosophila intestine using the act\({s} >\) act-Gal4, esg-lacZ/; tub-Gal80ts/ + driver. (A) Wild-type control (WT). (B) UAS-Snr1\({RNAi}\) V12645. (C) UAS-brm\({RNAi}\) V37721. (D) UAS-mor\({RNAi}\) V6969. Scale bars: 10 μm. (E) Illustration of the Osa-containing SWI/SNF (Brm) complex. (F) Quantification of esg\(^+\) cells in wild-type, Snr1\({RNAi}\), brm\({RNAi}\) and mor\({RNAi}\) midguts. esg-lacZ-positive cells in \(5 \times 10^3 \) μm\(^2\) gut tissue number eight for wild type (\(n=25\)); 44 for Snr1 (\(n=22\)); 29 for brm (\(n=23\)); and 31 for mor (\(n=24\)). Data are mean ± s.e.m.

Fig. S3. Osa and Snr1 are expressed in all cell types in the posterior midgut. (A-B') The wild-type control and osa\({RNAi}\) were expressed in the adult midgut using esg\(^s\). Osa is expressed in all cell types, including ISCs and EBs (arrows), in the wild-type midgut (A,A'). Osa is undetectable in GFP-labeled osa\({RNAi}\) cells (B,B', arrows). (C-E') The wild-type control, UAS-Snr1 and Snr1\({RNAi}\) were expressed in the adult midgut. Snr1 is expressed in all cell types, including ISCs and EBs (arrows), in the wild-type midgut (C,C'). The higher expression of Snr1 was detected in GFP-labeled ISCs and EBs in the UAS-Snr1-overexpressing midgut (D,D', arrows), but the expression of Snr1 in GFP-labeled Snr1\({RNAi}\) cells (E,E', arrows) was undetectable. Scale bars: 10 μm.
**Fig. S4. The expression of Notch is unchanged in osaRNAi cells.** The wild-type control and osaRNAi or Snr1RNAi are expressed in the adult midgut using esg+. (A,A') The ISC marker Mira-GFP is expressed in all expanded esg' cells in the osaRNAi midgut, suggesting that these cells are ISC-like cells. (B,B') No Dl expression was detected in the expanded ISC-like cells in the Snr1RNAi midgut. (C-D') In comparison to the wild-type control (C,C'), the expression of N is unchanged in the osaRNAi midgut (D,D'). Scale bars: 10 μm.

**Fig. S5. Dl mutant MARCM clone.** (A-A') GFP+ FRT82B-DlRevF10 clones were generated in the posterior midguts of flies of the indicated genotypes using the MARCM technique, and the clones were stained at 8 days ACI with the indicated antibodies. In the Dl mutant clone, there were many Pros+ EEs in addition to Spdo+ ISC-like cells. Scale bars: 10 μm.
Fig. S6. Snr1 autonomously regulates ISC fate. GFP+ clones were generated in the posterior midguts of flies of the indicated genotypes using the MARCM technique and stained at 6 days ACI with the indicated antibodies. (A, A') FRT82B wild-type control clones. In wild-type clones there are Di-labeled ISCs (arrow), EBs, differentiated ECs (large nuclei, asterisk) and Pros-positive EE cells (arrowhead). (B, B') MARCM clones of Snr1R3 lead to ISC-like cell expansion.
Fig. S7. Osa functions upstream of N in regulating ISC differentiation into ECs but downstream of N in regulating EE cell formation. (A-D) Osa functions upstream of N in regulating ISC fate specification. (A) Wild-type control. (B) Overactivation of N by expressing UAS-NΔ34a leads to ISC differentiation into ECs. (C) The expression of osaRNAi results in ISC-like cell expansion. (D) The expression of osaRNAi plus UAS-NΔ34a results in ISC differentiation into ECs. (E-F') The expression of UAS-Dl in osa2 mutant MARCM clones rescues the ISC-like stem cell tumor phenotype (F,F'). The wild-type MARCM clones expressing UAS-Dl were used as a control (E,E'). (G-I) Osa functions downstream of N in regulating EE cell formation. GFP+ clones were generated in the posterior midguts of flies of the indicated genotypes using the MARCM technique, and the clones were stained at 6 days ACI with the indicated antibodies. The osa2 mutation suppresses the phenotype of excess EE cells associated with the N55e11 mutation. Arrows, Dl-positive ISCs; arrowheads, Pros-positive EE cells. (I) Analysis of the percentage of Pros+ EE cells in N or N; osa double-mutant clones at 6 days ACI. osa2 suppresses the excess Pros+ EE cells in the N55e11 mutant clones. Data are mean ± s.e.m. Scale bars: 10 μm.
Fig. S8. nc82, a synaptic marker, specifically labels EE cells in the midgut. (A,A’) nc82 specifically labels esg<sup>+</sup> diploid cells in wild-type flies. Membrane-associated punctate staining by nc82 (inset in A’). (B,B’) nc82 (arrow, punctate staining) labels Pros-positive (asterisk, nuclear staining) EE cells. (C,C’) No nc82-positive EE cells were found in esg<sup>+</sup> cell clusters in the osaRNA<sup>in</sup> midgut. (D,D’) nc82-positive EE cell clusters in the NDN<sup>in</sup> midgut. All flies were driven by esg<sup>+</sup> and stained with the indicated antibodies. Scale bars: 10 μm.
Fig. S9. Ase and Sc are expressed in the midgut at low levels. (A-B') Endogenous Sc is not detected above background levels by immunofluorescence (A,A'). Nevertheless, Sc is readily detected in the gut with ectopic UAS-sc expression driven by esgts for 24 hours at 29°C (B,B'). (C-D') Endogenous Ase is also not detected above background levels by immunofluorescence (C,C'). Nevertheless, Ase is readily detected in the gut with ectopic UAS-ase expression driven by esgts for 24 hours at 29°C (D,D'). There is non-specific staining in some portion of the EE cells by the anti-Ase serum, labeling the membrane and cytoplasm, which is not consistent with the nuclear localization of Ase (inset in C,C'). (E-F') Ase-Gal4 is weakly expressed in ISCs and EBs (E,E') but not in Pros+ EEs (asterisk). GFP is detected in doublet cells, one of which is a Dl-positive ISC (arrow in F,F') and the other an EB (arrowhead in F,F'). (G,H) The expression of Sc is upregulated in both NDN (G) and osaRNAi (H) midguts (compare with A,A'). (I,J) The expression of ase is upregulated in the NDN midgut (I) but not in the osaRNAi midgut (J) (compare with C,C'). The wild-type and osaRNAi were driven by esgts and stained by the indicated antibodies. Scale bars: 10 μm.
<table>
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Table S1. Primers