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

Xiankun Zeng1,*, Xinhua Lin2,3 and Steven X. Hou1,*

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 enteroendocrine cells by regulating the expression of Asense, 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 asense (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 ISCs 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 (Hochedlinger 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 (Bouazoune 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 (Bouazoune 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* (Shigeko Hayashi, Riken); *esg-lacZ* (Stephen DiNardo, University of Pennsylvania); *mirA-GFP* (Francois Schweisguth, CNRS); *DI-lacZ* (Bruce Edgar, University of Heidelberg); *UAS-N354* (Ken Irvine, Rutgers); *UAS-N39* (Mark Fortini, Thomas Jefferson University); *Su(H)G8E-lacZ* (Sarah Bray, University of Cambridge); *UAS-ase* (Yuh Nung Jan, UCSF); *ase-Gal4* (Tramien Lee, Janelia Farm); *FRT28-osa* (James Kennison, NIH); and *FRT28-Snp1* (Andrew Dingwall, Loyola University). *UAS-Di, act-Gal4, UAS-SC, UAS-2XENFP, tub-Gal80* and fly strains used for MARCM cloning (*FRT28-osa, pim, act->>Gal4, UAS-GFP, SM6, hs-fp; FRT28 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-osaRNAi (V7810 and BL31266), UAS-Snr1RNAi (V12645, V108599 and BL32372), UAS-bmnRNAi (V37721 and BL31712), UAS-mor1RNAi (V6969 and V110712) and UAS-aseRNAi (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 *FRT28-osa, pim* (as a wild-type control), *FRT628-osa*, *FRT628-Snp1* and *FRT628-DlRevF10*, we generated the following flies: *act->>Gal4, UAS-GFP/Sm6, hs-fp; FRT28 tub-Gal80/FRT28 mutant*. The genotype of flies used to generate *N* and *osa* double-mutant clones is *hs-fp, FRT01U tub-Gal80/FRT01U N* or *act->>Gal4, UAS-GFP; FRT628-osa*/FRT28 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.

**RNA interference (RNAi)-mediated gene depletion**

Four male *UAS-RNAi* transgenic flies were crossed with eight virgin *act-Gal4, esg-lacZ/Cyo; tub-Gal80* or *esg-Gal4, UAS-2XENFP/Tub; cyo; tub-Gal80* flies. One-week-old adult progenitors of the correct genotype were transferred to new vials at 29°C for 7 days before dissection.

**Quantitative PCR (qPCR) and chromatin immunoprecipitation (ChIP) assay**

Total RNA from adult guts was isolated using the RNeasy Mini Kit (Qiagen) with on-column DNase digestion to remove genomic DNA. cDNA was synthesized using the ThermoScript RT-PCR system (Invitrogen). Real-time PCR analysis was performed on the Mastercycler Realplex real-time PCR system (Eppendorf) using SYBR Green PCR Master Mix (Clontech). qPCR results are presented as mean ± s.e.m. of three biological replicates. Primer pairs for qPCR are listed in supplemental material Table S1.

Two hundred fly intestines were dissected for ChIP assays using the Magna ChIP G Tissue Kit (Millipore) with some modification. The primer pairs used to detect immunoprecipitated DNA are listed in supplemental material Table S1.

**Antibody production**

Polyclonal rabbit antisera were raised against 6×His fusion proteins containing amino acids 2-180 of the Snr1 protein. To produce the 6×His fusion protein, a 537 bp fragment of *Snr1* was amplified by PCR using primers 5'-AGTAACTCTCAGACAGATACAGGGGA-3' and 5'-AGTACGAGGCTCATACTCGTCCATCGCT-3' (restriction sites underlined). The amplified fragments were cloned into the EcoRI and NotI sites of PET-28a (+) (Novagen). 6×His-Snr1 fusion protein was expressed in *E. coli* BL21(DE3) pLysS, purified on Ni-NTA agarose columns (Qiagen) and used to immunize rabbits as described previously (Zeng et al., 2007).

**Histology and image capture**

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 (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 649 (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 µm^2 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 µm^2, *n*=31; Fig. 1B,B',F) compared with the wild-type (7.8 *esg*+ cells/5×10^3 µm^2, *n*=24; Fig. 1A,A,F) posterior midgut. To test whether Osa functions specifically in progenitors, we knocked down *osa* specifically in ISCs and EBs using *esg-Gal4, UAS-GFP; tub-Gal80* (referred to as *esg*). Compared with the wild-type control (8.6 *esg*+ cells/5×10^3 µm^2, *n*=30; Fig. 1C,C',G), knockdown of *osa* using *esg* also caused dramatic expansion of the *esg*+ cells (*V7810; 46.1 *esg*+ cells/5×10^3 µm^2, *n*=32; Fig. 1D,D,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 µm^2, *n*=42; Fig. 1E,E,F) generated from independent sequences (Ni et al., 2009). We further stained wild-type and *osa* RNAi midguts for phospho-Histone H3 (pH3), a specific marker for mitotic cells. More pH3+ cells were found in *osaRNAi* 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) (Bozuoaune 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
was expressed in midguts by esg<sup>+</sup> (supplementary material Fig. S3D,D'), which confirms the specificity of our new anti-Snr1 serum.

**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<sup>+</sup> cells in osa<sup>RNAi</sup> midguts, we analyzed the expression of Su(H)GBE-lacZ, a marker of EBs (Ohlstein and Spradling, 2007), in wild-type and osa<sup>RNAi</sup> posterior midguts. In wild-type midguts, only some esg<sup>+</sup> cells expressed Su(H)GBE-lacZ (Fig. 2A,A'), which is consistent with its reported expression in EBs (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2007). In osa<sup>RNAi</sup> posterior midguts (Fig. 2B,B'), Su(H)GBE-lacZ-expressing cells were missing, suggesting that the expanded esg<sup>+</sup> cells in osa<sup>RNAi</sup> 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<sup>+</sup> cells in osa<sup>RNAi</sup> posterior midguts (supplementary material Fig. S4A,A'), which suggests that these esg<sup>+</sup> cells might be ISC-like cells.

Dl and Sanpodo (Spdo) are markers of ISCs (Ohlstein and Spradling, 2007; Perdigoto et al., 2011). We first examined Dl expression in the wild-type (esg<sup>+</sup>/WT; Fig. 2C,C') and osa<sup>RNAi</sup> (esg<sup>+</sup>/osa<sup>RNAi</sup>; Fig. 2D,D') posterior midguts. In wild-type midguts, some of the esg<sup>+</sup> cells that are ISCs express cytoplasmic Dl (Fig. 2C,C'). Surprisingly, none of the esg<sup>+</sup> cells in the osa<sup>RNAi</sup> midguts expressed Dl (Fig. 2D,D'). We also found that Dl expression was undetectable in Snr1<sup>RNAi</sup> midguts (esg<sup>+</sup>/Snr1<sup>RNAi</sup>; supplementary material Fig. S4B,B'). In wild-type posterior midguts, some of the esg<sup>+</sup> cells that are ISCs expressed Spdo (Fig. 2E,E'). In the osa<sup>RNAi</sup> midguts (Fig. 2F,F'), all esg<sup>+</sup> cells expressed Spdo, further suggesting that the esg<sup>+</sup> cells are ISC-like cells.

**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<sup>2</sup> (Vázquez et al., 1999) clones using the mosaic analysis with a represesible cell marker (MARC) technique (Lee and Luo, 1999). Clones marked homozygous for wild type (Fig. 3A,A'), and osa<sup>2</sup> (Fig. 3B,B') were generated in the posterior midgut and identified by GFP expression. Eight days after clone induction (ACI) we observed that, in GFP-labeled wild-type clones (Fig. 3A,A'), there were differentiated EC cells with large nuclei (asterisk in Fig. 3A,A'), Pros<sup>+</sup> EE cells (arrowheads in Fig. 3A,A'), DI ISCs (arrows in Fig. 3A,A') and Spdo<sup>+</sup> ISCs (arrow in Fig. 3C). However, osa<sup>2</sup> mutant clones were almost devoid of EC and EE cells, and all cells were DI' and Spdo<sup>+</sup> ISC-like cells (Fig. 3B,B').

To more precisely assess the phenotypes, we counted the number of Spdo<sup>+</sup> ISC-like cells and Pros<sup>+</sup> EE cells in GFP-labeled MARCM clones of wild type, osa<sup>2</sup> and Dl<sup>RevF10</sup> (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 Spdo<sup>+</sup> ISCs at 4 and 8 days ACI (n=20, 21), respectively. However, 91% and 98% of cells were Spdo<sup>+</sup> ISC-like cells in osa<sup>2</sup> clones at 4 and 8 days ACI (n=20, 21), respectively; and 70% and 67% of cells were Spdo<sup>+</sup> ISC-like cells in Dl<sup>RevF10</sup> clones at 4 and 8 days ACI (n=20, 21), respectively. The Pros<sup>+</sup> EE cells were dramatically reduced in osa<sup>2</sup> 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\textsuperscript{-} clones, whereas 24.9% and 31.8% of cells were EE cells in 
Dl\textsubscript{RevF10} clones.

We similarly generated Snr\textsuperscript{R3} (Zraly et al., 2003) MARCM clones 
and found that Snr\textsuperscript{1} mutation also resulted in ISC expansion 
without differentiation (supplementary material Fig. S6). Together, 
these results suggest that Osa and Snr\textsuperscript{1} might regulate ISC self-
renewal and differentiation into both EC and EE cells.

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’). However, the expression of Dl-lacZ was 
dramatically reduced in osa\textsuperscript{RNAi} posterior midguts (osa\textsuperscript{RNAi}; 
fluorescence intensity of 200, n=58; Fig. 4B,B’). We also detected 
Dl mRNA expression by qPCR and found that the level of Dl 
mRNA was significantly reduced in the osa\textsuperscript{RNAi} 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 osa\textsuperscript{RNAi} 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 
(’esg\textsuperscript{>osalacZ}\textsuperscript{N534a}’), all ISCs differentiate into ECs (compare 
supplementary material Fig. S7B with S7A). In the osa\textsuperscript{RNAi} midgut 
(esg\textsuperscript{>osalacZ}; 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 (N\textsuperscript{osalacZ}) in the osa\textsuperscript{RNAi} midgut (esg\textsuperscript{>osalacZ}\textsuperscript{N534a}; 
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 osa\textsuperscript{RNAi} midgut as well, Osa might regulate ISC 
differentiation into ECs by controlling Dl expression. To test this, we 
expressed UAS-Dl in osa\textsuperscript{RNAi} posterior midguts (osa\textsuperscript{RNAi}+UAS-
Dl; Fig. 5C) or in GFP-marked wild-type and osa\textsuperscript{2} mutant MARCM 
clones (Fig. 5E; supplementary material Fig. S7E-F’). UAS-Dl expression 
using the esg\textsuperscript{>d} driver rescues the ISC tumor phenotype in 
osa\textsuperscript{RNAi} midguts to a phenotype resembling wild type (8.5, 43.5 and 
10.5 esg\textsuperscript{>d} cells/5×10\textsuperscript{3} \textmu m\textsuperscript{2}; n=23, 36 and 42, respectively; Fig. 5A-
C,F). In osa\textsuperscript{2} mutant MARCM clones, all GFP-marked cells were 
ISC-like diploid cells (Fig. 3B,B’;D, Fig. 5D). UAS-Dl expression in 
osa\textsuperscript{2} 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\textsuperscript{+} EE cells in the midguts 
of wild type (Fig. 5A), osa\textsuperscript{RNAi} (Fig. 5B) and osa\textsuperscript{RNAi}+UAS-Dl 
expression (Fig. 5C). Dl expression does not rescue the EE cell-loss 
phenotype in osa\textsuperscript{RNAi} midguts (3.8, 1.8 and 1.7 EE cells/5×10\textsuperscript{3} \textmu m\textsuperscript{2}; 
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 (esg\textsuperscript{>N}\textsuperscript{DN}; 50.9 EE 
cells/5×10\textsuperscript{3} \textmu m\textsuperscript{2}; n=33; Fig. 5H,K) resulted in the formation of
osa2

ACI. Almost all cells in the Spdo+ ISC-like cells in wild-type, (A) Analysis of the percentage of E cells in wild-type clones. Data are mean ± s.e.m. Scale bars: 10 μm. (B) The FRT82B-osaRNAi clone at 8 days ACI. There are ISCs labeled by Dl (arrow), whereas neighboring wild-type ISCs express Dl (arrow). (A, B) The FRT82B-osaRNAi clone is outlined. (D) In the wild-type clone (C), Dl expression is weakly expressed in ISCs (asterisk) in the clone. (A-D) For 24 hours at 29°C (supplementary material Fig. S8A-B); however, unlike in the NDN 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 (esgts>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 NDN 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'); nevertheless, Sc and Ase were readily detected in the midgut with ectopic sc and ase expression driven by esgts>UAS-sc and esgts>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 NDN midgut as assessed by qPCR (Fig. 6A,B) and antibody immunofluorescence (compare supplementary material Fig. S9G with S9A,A'). 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; esg>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 (esg>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
Snr1-specific antibodies or an unrelated IgG control (Fig. 7A–B’). We found that Osa and Snr1 associated with the DI and ase promoters at the chromatin level, whereas neither bound to the spdo promoter, which suggests that Osa and Snr1 might regulate DI and ase expression directly. However, we could not exclude the possibility that DI and ase are indirectly regulated by another transcriptional target of Osa.

In summary, these data suggest that Osa regulates ISC differentiation into ECs by controlling DI expression in ISCs, and regulates EE cell formation by controlling ase expression (Fig. 7C).

DISCUSSION

Previous studies have demonstrated that N signaling regulates ISC fate specification in the Drosophila posterior midgut (Ohlstein and Spradling, 2007). The DI ligand is specifically expressed in ISCs to activate N signaling in neighboring EBs to promote EB to EC or inhibit EB to EE cell differentiation. However, it is still not fully understood how ISCs are coordinately controlled to differentiate into EC or EE cells, thus maintaining tissue homeostasis. Here, we found that the commitment of stem cells to discrete differentiated cells is coordinately regulated by the Osa-containing SWI/SNF chromatin-remodeling complex. Osa regulates ISC differentiation into ECs by controlling the expression of DI to elaborate N signaling in EBs to promote EB to EC differentiation. Repressing DI expression by loss of function of Osa enhances ISC self-renewal and blocks ISC commitment to ECs, resulting in ISC expansion/tumors. DI 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 DI at the chromatin level. Consistently, genetic interaction between the SWI/SNF complex and DI 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 DI 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 DI expression. Another possibility is that some transcription factors recruit the complex to regulate DI 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 DI 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. Pronuclear 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 ([f]1sc) 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 NRNAi midgut. 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 NRNAi 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, 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 DI is essential for EB differentiation into ECs. Osa promotes the expression of DI in ISCs and maintains activation of the N signal in EBs to promote EB to EC differentiation. Repressing DI expression by loss of function of Osa enhances ISC self-renewal and blocks ISC commitment to ECs, resulting in ISC expansion/tumors. DI 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 DI at the chromatin level. Consistently, genetic interaction between the SWI/SNF complex and DI 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.

The role of the Osa-containing SWI/SNF complex in ISC self-renewal and differentiation into ECs

Our results suggest that Osa, a component of the SWI/SNF complex, is essential for ISC differentiation into EC and EE cells in the Drosophila posterior midgut. The SWI/SNF chromatin-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 DI is essential for EB differentiation into ECs. Osa promotes the expression of DI in ISCs and maintains activation of the N signal in EBs to promote EB to EC differentiation. Repressing DI expression by loss of function of Osa enhances ISC self-renewal and blocks ISC commitment to ECs, resulting in ISC expansion/tumors. DI 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 DI at the chromatin level. Consistently, genetic interaction between the SWI/SNF complex and DI 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 DI 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 DI expression. Another possibility is that some transcription factors recruit the complex to regulate DI expression, and these transcription factors exist only in ISCs.
inducing EB cells to differentiate into EE cells. Excess EE cells in the Ase 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, If(1)sc and ase (Bailey and Posakony, 1995; Lecitoisis and Schweisguth, 1995). In addition to sc and ase, the overexpression of ac and If(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, mutant phenotype and in some DNA-binding properties (Jarmen 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.

Acknowledgements
We thank Jiangsha Zhao, Chihi Chauhan and Ashley DeVine for critical reading of the manuscript; Shigen Hayashi, Stephen DiNardo, Francois Schweisguth, Bruce Edgar, Ken Irvine, Mark Fortini, Sarah Bray, James Kennison, Andrew Dingwall, Yuh Nung Jan, Tzumin Lee, VDRC, BDSC and TRIP at Harvard Medical School for fly stocks; Xiaohang Yang, Jim Skeath, Stephen Lockett, Bruce Edgar, Ken Irvine, Mark Fortini, Sarah Bray, James Kennison, Andrew Dingwall, Yuh Nung Jan, Tzumin Lee, VDRC, BDSC and TRIP at Harvard Medical School for fly stocks; Xiaohang Yang, Jim Skeath, Stephen Lockett for help with confocal microscopy.

Funding
This research was supported by the Intramural Research Program of the National Cancer Institute, National Institutes of Health. Work in the X.L. laboratory was supported by grants from the Strategic Priority Research Program of the Chinese Academy of Sciences Grant [XDA01010101] and the Nature Sciences Foundation of China [10303049] to X.L. Deposited in PMC for release after 12 months.

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.

Supplementary material
Supplementary material available online at http://dev.biologists.org/lookup/suppl; doi:10.1242/dev.096891//DC1


Vázquez, M., Moore, L. and Kennison, J. A. (1999). The trithorax group gene osa encodes an ARID-domain protein that genetically interacts with the
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 esgts. 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\(^{ts}\) (act-Gal4, esg-lacZ /+; tub-Gal80\(^{ts}\) /+) 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 5×10\(^3\) 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\(^{ts}\). 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 \( \text{osa}^{\text{RNAi}} \) cells. The wild-type control and \( \text{osa}^{\text{RNAi}} \) or \( \text{Snr1}^{\text{RNAi}} \) are expressed in the adult midgut using \( \text{esg}^{\text{ts}} \). (A,A') The ISC marker Mira-GFP is expressed in all expanded \( \text{esg}^{\text{ts}} \) cells in the \( \text{osa}^{\text{RNAi}} \) midgut, suggesting that these cells are ISC-like cells. (B,B') No Dl expression was detected in the expanded ISC-like cells in the \( \text{Snr1}^{\text{RNAi}} \) midgut. (C-D') In comparison to the wild-type control (C,C'), the expression of N is unchanged in the \( \text{osa}^{\text{RNAi}} \) midgut (D,D'). Scale bars: 10 μm.

Fig. S5. \( \text{Dl} \) mutant MARCM clone. (A-A') GFP\(^{\text{ts28-}}\)\( \text{FRT}^{\text{ts28}}\)\( \text{Dl}^{\text{RevF10}} \) 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 \( \text{Dl} \) 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+ 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+ cell clusters in the osaRNAi midgut. (D,D') nc82-positive EE cell clusters in the NDN midgut. All flies were driven by esgts 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 DI-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.
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