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Drosophila stem cells share a common requirement for the histone H2B ubiquitin protease Scrawny

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Abstract

Stem cells within diverse tissues share the need for a chromatin configuration that promotes self-renewal, yet few chromatin proteins are known to regulate multiple types of stem cells. We describe a *Drosophila* gene, *scrawny* (*scny*), encoding a ubiquitin protease, which is required in germline, epithelial and intestinal stem cells. Like its yeast relative UBP10, Scrawny deubiquitylates histone H2B and functions in gene silencing. Consistent with previous studies of this conserved pathway of chromatin regulation, *scny* mutant cells have elevated levels of ubiquitylated H2B and trimethylated H3K4. Our findings suggest that inhibiting H2B ubiquitylation via *scny* represents a common mechanism within stem cells that is used to repress the premature expression of key differentiation genes, including Notch target genes.

Keywords

Stem cell; Ubiquitin specific protease; Histone H2B; gene silencing

Stem cells are maintained in an undifferentiated state by signals they receive within the niche and are subsequently guided toward particular fates upon niche exit (1). Within ES cells and during differentiation, cell state changes are controlled at the level of chromatin by alterations involving higher order nucleosome packaging and histone tail modifications (2). Polycomb group (PcG) and Trithorax group (trxG) genes influence key histone methylation events at the promoters of target genes, including H3K27 and H3K4 modifications associated with gene repression and activation, respectively, but few other genes with a specific role in stem cells are known.

Histone H2A and H2B mono-ubiquitylation play fundamental roles in chromatin regulation, and H2A ubiquitylation has been linked to PcG-mediated gene repression and stem cell maintenance. The mammalian Polycomb repressive complex 1 (PRC1) component RING1B is a H2A ubiquitin ligase that is required to block the elongation of poised RNA polymerase II on bivalent genes in ES cells (3). Mutations in the PRC1 component, BMI-1, which complexes with RING1B, causes multiple types of adult stem cells to be prematurely lost (4).

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Materials and Methods

Figs. S1 to S10

Table S1

References

The role of H2B ubiquitylation in stem cells is unclear, however. In yeast, ubiquitylation of Histone H2B by the RAD6 and BRE1 ligases controls H3K4 methylation (H3K4me3) (5,6), a process that requires the polymerase accessory factor PAF1 (7,8). Conversely, H2B deubiquitylation by the ubiquitin-specific protease (USP) family member UBP10 is required for silencing telomeres, rDNA and other loci (9). The *Drosophila* homolog of BRE1, *dBRE1*, also is needed for H3K4 methylation, suggesting that this pathway is conserved (10). Furthermore, the *Drosophila* ubiquitin-specific protease USP7 is part of a complex that selectively deubiquitylates H2B and genetically interacts with PcG mutations (11). Mutations in another USP family member, *Nonstop*, increase H2B ubiquitylation and cause axon targeting defects in the eye (12).

In order to gain further insight into the role of H2B ubiquitylation in stem cells, we characterized a novel *Drosophila* gene, *scrawny* (*scny*) (CG5505), whose encoded USP family protein shares homology with human USP36 and among yeast USPs closely matches UBP10 within the core protease domain (13, Fig. 1A). Strains bearing *scny* insertions (Fig. 1B), except for a viable GFP protein trap (CA06690), were female sterile or lethal, and proved to be allelic (13, Table S1). Transposon excision or expression of a *scny*-RB cDNA reverts the phenotype of tested alleles. An anti-SCNY antibody raised against a domain common to all SCNY isoforms recognizes wild type and SCNY-GFP on a Western blot (Fig. 1C). SCNY protein levels in homozygous third instar larvae are greatly reduced in lethal mutants (Fig. 1C), and SCNY expression is also lower in stem cell-enriched ovarian tissue from adults homozygous for the sterile d06513 allele (Fig. 1D). Consistent with a role in gene silencing, several *scny* mutations act as dominant suppressors of position effect variegation (Fig. 1E, Fig. S1).

Further studies strongly suggested that SCNY functions in vivo as an H2B-ubiquitin protease. Recombinant full-length SCNY protein, but not a version bearing a point mutation in the protease domain, efficiently deubiquitylates histone H2B in vitro (Fig. 2A). *scny*^{f01742} homozygous tissue contains levels of Ub-H2B that are elevated at least twofold compared to wild type (Fig 2B, Fig. S2). As expected if Ub-H2B is required for H3K4 methylation, clones of homozygous *scny*^{e00340} mutant cells stain more strongly for H3K4me3 than heterozygous cells (Fig. 2C, C'). Consistent with a direct rather than an indirect action on Ub-H2B levels, anti-SCNY antibodies but not pre-immune serum co-immunoprecipitated H2B from *Drosophila* embryonic nuclear extracts (Fig. 2D). Moreover, epitope-tagged SCNY co-immunoprecipitated *Drosophila* PAF1, but not Cyclin T (or several other tested chromatin proteins) when co-expressed in S2 tissue culture cells (Fig. 2E). Together, these data support the view that SCNY participates in a conserved pathway of chromatin regulation linking H2B ubiquitylation with H3K4me3 methylation. Because the effects of *scny* mutation on Ub-H2B and H3K4me3 are opposite to those of *dBRE1* mutation (10), SCNY likely opposes *dBRE1* action on H2B, just as UBP10 opposes BRE1 action on H2B in yeast.

Drosophila male and female gonads contain well characterized germline stem cells (GSCs) that allow the effects of genes on stem cell maintenance to be quantitatively analyzed (14). High levels of *scny* expression were observed in female and male GSCs using SCNY-GFP (Fig. 3A, B) and identical staining was observed using anti-SCNY immunofluorescence (Fig. S3). SCNY protein resides in cell nuclei and is enriched in nucleoli (Fig. S3). In sterile or semi-fertile *scny* mutant adults, the numbers of germline stem cells surrounding the testis hub (Fig. 3C, Fig. S4) and within germaria (Fig. S5) were clearly reduced. The half-lives of female GSCs bearing clones of three different *scny* alleles were all sharply reduced (Fig. 3D). Later follicular development was also abnormal suggesting that *scny* continues to function after the stem cell stage. However, previous studies indicate that accelerated GSC loss is a specific phenotype, and hence that *scny* has a preferential requirement in GSCs (1,15).

A known mechanism of increased GSC loss is the premature activation of differentiation genes. Staining germaria with an antibody specific for multiple sites of histone H3 acetylation (H3-Ac) suggested that *scny* mutation affects the global chromatin organization of GSCs (Fig. 3E, F). Wild type GSCs (arrow) contain lower levels of H3-Ac than slightly older germ cells within cysts (Fig. 3E, arrowhead). Presumptive GSCs located in the GSC niche in *scny* mutants frequently stained more strongly (Fig. 3F), suggesting that they have begun to upregulate general transcription. Some *scny* GSC-like cells also expressed *bag-of-marbles* (*bam*) (Fig. S6), a key cystoblast differentiation gene (*1*), and GSC-like cells in *scny^{d06513}; bam^{Δ86}* mutant females persist in the germarium (Fig. S7). However, we could not completely rule out that the observed increases in H3-Ac levels and *bam* expression were a result rather than a cause of the premature differentiation and loss of *scny* GSCs.

To determine if *scny* is also required in a very different type of stem cell, the epithelial FSC (16), we quantitatively analyzed the persistence of individual *scny* mutant FSCs. The half-life of FSCs mutant for *scny^{l(3)02331}* was reduced more than 10-fold, while the *scny^{f01742}* mutation also caused a sharp decline (Fig. 4A). However, mutant follicle cells continued to develop normally at later stages (Fig. S8). Thus, *scny* is preferentially required to maintain FSCs as well as GSCs.

The largest population of *Drosophila* stem cells are the hundreds of multipotent intestinal stem cells (ISCs) that maintain the adult posterior midgut (17,18). ISCs signal to their daughters via Delta-Notch signaling to specify enterocyte vs. enteroendocrine cell fate, but the pathway must remain inactive in the ISCs themselves to avoid differentiation (19). Most ISCs (those about to produce enterocytes) express high levels of the Notch ligand Delta, allowing them to be specifically distinguished from other diploid gut cells (19). We found that SCNY-GFP is expressed in ISCs (Fig. 4B, arrow) suggesting that SCNY plays a role in these stem cells as well. While 7-day old normal adult midguts contain a high density of ISCs, as revealed by Delta staining (Fig. 4C), we found that corresponding tissue from 7-day-old *scny^{f01742}* (Fig. S9) or *scny^{f01742/scny^{l(3)02331}}* (Fig. 4D) escaper adults possess very few Delta-positive cells. ISCs are present in near normal numbers at eclosion, but are rapidly lost in the mutant adults, indicating that *scny* is required for ISC maintenance (Fig. 4F).

We suspected that inappropriate Notch pathway activation was responsible for the premature ISC loss in *scny* mutants. *dbp1* mutations strongly reduce Notch signaling, suggesting that Notch target genes are particularly dependent on H2B mono-ubiquitylation and H3K4 methylation (10). Consequently, *scny* mutations, which have the opposite effects on Ub-H2B and H3K4me3 levels, might upregulate Notch target genes, stimulating ISCs to differentiate prematurely. We tested this idea by supplementing the food of newly eclosed *scny^{f01742/scny^{l(3)02331}}* adults with 8 mM DAPT, a gamma-secretase inhibitor that blocks Notch signaling and phenocopies *Notch* mutation when fed to wild type animals (17). *scny^{f01742/scny^{l(3)02331}}* DAPT-treated adults remained healthy and the guts of 7-day old animals still contained many ISCs (Fig 4E), although not as many as wild type (Fig. 4F). Tumors like those produced in wild type animals fed DAPT (17,18) were not observed. Thus, in these animals endogenous stem cell loss can be slowed by drug treatment.

Our experiments provide strong evidence that a pathway involving the ubiquitin protease Scrawny and the ubiquitin ligase dBRE1 controls the levels of Ub-H2B, and H3K4me3 at multiple target sites in the *Drosophila* genome. Although, other ubiquitin proteases also act on Ub-H2B in *Drosophila* (11,12), the direct interaction between SCNY and H2B, and the strong effects of *scny* mutations argue that it plays an essential, direct role in silencing genomic regions critical for cellular differentiation, including Notch target genes. SCNY interacts with the RNA polymerase accessory factor complex component, PAF1. Upregulation of H2B ubiquitylation and H3 methylation in yeast is mediated by the PAF1 complex and is

associated with elongating RNA Pol II (7,8,20). *Drosophila* PAF1 is required for normal levels of H3K4me3 at the *hsp70* gene (21), and another PAF1 complex member, RTF1, is needed for H3K4 methylation and Notch target gene expression (22). Indeed, the pathway connecting Ub-H2B, H3K4me3 and gene silencing appears to be conserved in organisms as distant as *Arabidopsis* (23). A human protein closely related to SCNY, USP36, is overexpressed in ovarian cancer cells (24), and our results suggest it may act as an oncogene by suppressing differentiation.

Above all, our experiments indicate that SCNY-mediated H2B deubiquitylation is required to maintain multiple *Drosophila* stem cells, including progenitors of germline, epithelial and endodermal lineages. In ES cells and presumably in adult stem cells, many differentiation genes contain promoter-bound, arrested RNA Pol II and are associated with Polycomb group proteins (2,3). We envision that in the niche environment SCNY activity overrides that of dBRE1, keeping levels of Ub-H2B (and hence H3K4me3) low at key differentiation genes (Fig. S10). Upon exit from the niche, the balance of signals shifts to favor H2B ubiquitylation, H3K4 trimethylation, and target gene activation. Thus, the control of H2B ubiquitylation, like H2A ubiquitylation, plays a fundamental interactive role in maintaining the chromatin environment of the stem cell state.

Supplementary Material

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Figure 1.

scny encodes a ubiquitin protease family member and functions in gene silencing. (A) Schematic of SCNY protein shows similarity to human USP36 and yeast UBP10 in the conserved protease (C19E) domain (red boxes). Blue bar indicates region used for antibody production. (B) *scny* locus structure showing transposon insertion sites (triangles), including the GFP protein trap CA06690 (green). *scny* transcripts identified by cDNA sequencing are shown below. (C–D) Western blot showing predicted size increase of SCNY in CA06690, and reduced SCNY levels in extracts from third instar larvae bearing lethal alleles: *l(3)02331*, *e00340* and *f01742* (C); and in ovarian tissue from *d06513* homozygous adults that were also mutant for *bam* to enrich for germline stem cells (D). (E) *scny^{l(3)02331}* dominantly suppresses *In(1)^{w^{m4h}}*-induced position-effect variegation in eye pigmentation. (see also Fig. S1)

Figure 2.

SCNY deubiquitylates H2B *in vitro*, affects chromatin modification *in vivo*, and interacts with H2B and PAF1. (A) Wild-type SCNY protein (SCNY) but not SCNY protein bearing a protease domain mutant (SCNY^{ut}) deubiquitylates *Drosophila* embryonic histone H2B *in vitro*, as visualized by Western blot using anti-H2B and anti-SCNY antibodies. Reaction mix without SCNY (load). (B) Ub-H2B is elevated *in vivo* in L3 *scny*^{f01742} larvae compared to the wild type control as shown by a Western blot probed with anti-Ub-H2B antibodies (upper panel) normalized using anti-H2B antibody (lower panel). (See also Fig. S2). (C) H3K4me3 (green) is elevated in follicle cell clone mutant for *scny*^{e00340} (dotted line) marked by the absence of LacZ (red). DNA (blue). (C') H3mK4 channel alone. bar = 10 μm. (D) Co-immunoprecipitation

of histone H2B from nuclear extracts of *Drosophila* embryos (load) with anti-SCNY antibodies (SCNY) but not pre-immune serum (pre). (E) Co-immunoprecipitation with SCNY of PAF1, but not of the CyclinT control, and co-immunoprecipitation with PAF1 of SCNY but not of the CyclinT control from S2 cell extracts over-expressing these proteins. Starting extract: (load).

Figure 3.

SCNY maintains germline stem cells. Wide expression of *scny*-GFP (green) during early oogenesis (A) and spermatogenesis (B), including GSCs (arrows). SCNY protein localizes to the nucleus and is highly enriched in the nucleolus. HTS (red). Hub (dashed line). (C) Reduced GSC number in 1–2-day old adult *scny^{d06513}* testes (red bars) compared to wild type (blue bars). (D) Reduced half-life ($t_{1/2}$) of female GSCs mutant for *scny*. (E) GSCs (arrow) have lower global levels of acetylated histone H3 (green) than cyst cells (arrowhead). Vasa (red). Inset: Ac-H3 channel. (F) GSC-like anterior germ cells in *scny^{d06513}* germaria have global Ac-H3 (green) levels more comparable to cyst cells. VASA (red). Inset: Ac-H3 channel. bar in A, B, E and F = 10 μ m.

Figure 4.

SCNY maintains FSCs and ISCs. (A) Reduced halflife ($t_{1/2}$) of FSCs mutant for *scny*. (B) Expression of SCNY-GFP (green) in nuclei and nucleoli of ISCs (arrow, contains cytoplasmic Delta), and developing enterocytes (lacks Delta), but is low in enteroendocrine cells (contain nuclear Prospero). (C–F) ISCs (red, cytoplasmic) in 7-day old wild type adult midguts (C) are lost prematurely in *scny*^{f01742}/*scny*^{l(3)02331} flies (D) but not in *scny*^{f01742}/*scny*^{l(3)02331} adults whose food was supplemented with the Notch inhibitor DAPT (8mM) (E). (F) Quantitation of the midgut ISC density as a function of time in wild type (blue), *scny*^{f01742}/*scny*^{l(3)02331} (red) and *scny*^{f01742}/*scny*^{l(3)02331} + 8mM DAPT (yellow). In (B–E) Prospero (red, nuclear) marks enteroendocrine cells, and Delta (red, cytoplasmic) mark ISCs. DNA (blue) bar in B–E = 10 μ m.