Development 140, 3635-3644 (2013) doi:10.1242/dev.094599

CAF-1 promotes Notch signaling through epigenetic control of target gene expression during *Drosophila* development

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SUMMARY

The histone chaperone CAF-1 is known for its role in DNA replication-coupled histone deposition. However, loss of function causes lethality only in higher multicellular organisms such as mice and flies, but not in unicellular organisms such as yeasts, suggesting that CAF-1 has other important functions than histone deposition during animal development. Emerging evidence indicates that CAF-1 also has a role in higher order chromatin organization and heterochromatin-mediated gene expression; it remains unclear whether CAF-1 has a role in specific signaling cascades to promote gene expression during development. Here, we report that knockdown of one of the subunits of *Drosophila* CAF-1, dCAF-1-p105 (Caf1-105), results in phenotypes that resemble those of, and are augmented synergistically by, mutations of Notch positive regulatory pathway components. Depletion of dCAF-1-p105 leads to abrogation of *cut* expression and to downregulation of other Notch target genes in wing imaginal discs. dCAF-1-p105 is associated with Suppressor of Hairless [Su(H)] and regulates its binding to the enhancer region of *E(spl)mβ*. The association of dCAF-1-p105 with Su(H) on chromatin establishes an active local chromatin status for transcription by maintaining a high level of histone H4 acetylation. In response to induced Notch activation, dCAF-1 associates with the Notch intracellular domain to activate the expression of Notch target genes in cultured S2 cells, manifesting the role of dCAF-1 in Notch signaling. Together, our results reveal a novel epigenetic function of dCAF-1 in promoting Notch pathway activity that regulates normal *Drosophila* development.

KEY WORDS: *Drosophila*, CAF-1, Notch, Epigenetic regulation, H4 acetylation

INTRODUCTION

Chromatin assembly factor 1 (CAF-1) is a highly conserved three-subunit histone chaperone in eukaryotes (Ridgway and Almouzni, 2000), which has been shown to facilitate chromatin assembly by depositing histone H3 and H4 onto newly synthesized DNA (Tyler et al., 2001; Groth et al., 2007). Increasing evidence suggests that CAF-1 has important functions in other cellular and developmental processes in addition to DNA replication (Ridgway and Almouzni, 2000; Groth et al., 2007; Quivy et al., 2008; Huang et al., 2010; Autran et al., 2011; Nakano et al., 2011). For example, it is involved in the restoration of chromatin structure after DNA repair (Groth et al., 2007; Chen et al., 2008; Li et al., 2008); CAF-1 also participates in the establishment of epigenetic information during heterochromatin formation (Song et al., 2007; Quivy et al., 2008; Huang et al., 2010); recent studies in *Arabidopsis* and *C. elegans* suggest that CAF-1 is involved in the regulation of gene expression as well as asymmetric cell division (Autran et al., 2011; Nakano et al., 2011). However, the molecular mechanisms by which CAF-1 regulates transcription and whether this function is coupled to specific signaling pathways that are essential for animal development remain unclear.

In metazoans, the highly conserved Notch signaling pathway plays essential roles in the control of cell proliferation and cell fate specification during animal development (Artavanis-Tsakonas and Muskavitch, 2010). Defects in the Notch pathway are associated with various types of human disorders, such as T-cell leukemia and several breast cancers (Ranganathan et al., 2011). In *Drosophila*, the core components of the Notch pathway consist of ligands Delta/Serrate (Dl/Ser), receptor Notch and the DNA-binding transcription factor Suppressor of Hairless [Su(H)] (Bray, 2006). The binding of Dl/Ser situated on one cell to Notch situated on the neighboring cell results in two proteolytic cleavages of Notch, which mediate the release of Notch intracellular domain (N^{ICD}) into the nucleus to activate Notch target gene transcription with the assistance of Su(H) and co-activator Mastermind (Mam) (Bray, 2006; Artavanis-Tsakonas and Muskavitch, 2010). Despite the relative simplicity of primary Notch signaling, the presence of a large number of fine-tuning regulators at different levels, from the outside of the cell membrane to the nucleus, dramatically increases the complexity of Notch pathway outputs and its cellular responses, thus controlling a wide range of developmental processes (Neumann and Cohen, 1998; Acar et al., 2008; Hautbergue et al., 2009; Kim et al., 2009; Xie et al., 2012). At the nuclear level, recent findings suggest that chromatin-associated regulatory mechanisms are important for proper Notch target gene expression (Bray et al., 2005; Kugler and Nagel, 2007; Moshkin et al., 2009; Duan et al., 2011; Mulligan et al., 2011; Domanitskaya and Schüpbach, 2012; Endo et al., 2012). Transcriptional silencing complexes have been identified by proteomic methods in the absence of Notch activation (Moshkin et al., 2009; Mulligan et al., 2011). In flies, LAF (LID-associated factor) and RLAF (RPD3-LID-associated factor) silencing complexes cooperate with histone chaperones Asf1 and Nap1 to mediate epigenetic silencing at the Notch target *Enhancer of Split* [*E(spl)*] cluster (Moshkin et al., 2009). In both mammals and flies, an SIRT1-LSD1 co-repressor complex represses Notch target genes in cultured cells (Mulligan et al., 2011). Epigenetic regulators and chromatin modifiers that execute positive regulatory roles in Notch signaling are now being identified (Bray, 2006). Specifically, in

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Accepted 25 June 2013

mammalian cells, the histone acetyl transferases (HATs) PCAF (KAT2B) and GCN5 (KAT2A) have been shown biochemically to function in RBPJ [the mammalian homolog of Su(H)]-mediated transactivation by N^{ICD} (Kurooka and Honjo, 2000). In flies, the RING-finger E3 ubiquitin ligase Bre1 and chromatin remodeling complex NURF have been shown to be involved in the epigenetic regulation of Notch target gene expression (Bray et al., 2005; Kugler and Nagel, 2010). Recently, an RNAi screen in cultured *Drosophila* cells revealed that a Notch pathway transcriptional reporter is sensitive to chromatin-modifying enzymes and remodelers (Mourikis et al., 2010). However, the precise mechanism of how Notch signaling is epigenetically regulated during development remains unclear.

In this report, we describe a novel function of *Drosophila* chromatin assembly factor 1 (dCAF-1) in regulating the expression of Notch target genes. dCAF-1 genetically interacted with the Notch pathway in both the eye and wing. The expression of two Notch target genes, *cut* and *wg*, was largely abolished in *dCAF-1-p105* (*Caf1-105* – FlyBase) mutant cells of the wing disc. Biochemical and chromatin immunoprecipitation experiments revealed that dCAF-1-p105 regulates the binding of Su(H) to the enhancer region of *E(spl)mβ* to establish a local active chromatin structure by maintaining a high level of histone H4 acetylation. Our results show that dCAF-1 functions specifically to regulate the Notch signaling pathway, promoting its target gene expression through epigenetic regulation, during *Drosophila* development.

MATERIALS AND METHODS

Fly strains and genetics

The *dCAF-1-p105* mutant line ($p105^{36}$) was generated by a standard P-element jump-out strategy (Song et al., 2007) (Fig. 2A). For clonal analyses, the mutation of $p105^{36}$ was recombined with FRT42D on the left arm of the second chromosome. To produce the *UAS-HA-dCAF-1-p105* transgenic flies, a modified pUAST-HA vector was used: an ATG start codon and HA tag sequence were inserted at the *Eco*RI site of the pUAST construct. PCR products of *dCAF-1-p105* were amplified using 5′-AAGTGCAAGATACCCGAGATTTCGT-3′ and 5′-GTTAAGTCTAATCTATTGCATTGTCTACTC-3′ from a w^{1118} genomic DNA template and cloned into the pUAST-HA vector. *pUAST-HA-dCAF-1-p105* construct of correct DNA sequence was used for microinjection following standard protocols (Xu et al., 2009). Transgenic lines were verified by their ability to rescue the $p105^{36}$ mutants.

The *UAS-dCAF-1-p105 RNAi* (*UAS-dCAF-1-p105^{IR}*) lines 12892R-1 (II) and 12892R-3 (III) were obtained from the National Institute of Genetics, Japan; the *UAS-dCAF-1-p55* RNAi line of v26456 was from the Vienna Drosophila RNAi Center; the *UAS-CAF-1-p180* RNAi line of *dCAF-1-p180^{HM05129}*, *smo^{3}/CyO*, *yki^{MB09079}/CyO*, *Egfr^{f2}/CyO*, *N^{1}/FM7c*, *H^{1}/TM6*, *Dl^{Rev10}/TM6B Tb*, *mam^{2}/CyO* was obtained from the Bloomington Stock Center; *tkv^{7}/CyO* was from the Kyoto Drosophila Genetic Resource Center; *cut-lacZ* and *E(spl)mβ-lacZ* (Jack et al., 1991; Cooper et al., 2000) were kindly provided by Dr Kenneth Irvine (Rutgers University); *eyg^{M3-12}/TM6B Tb* was a generous gift from Dr Y. H. Sun (Jang et al., 2003).

Immunohistochemistry and antibodies

Wandering third instar larvae of the correct genotype were collected and dissected in cold PBS. Wing imaginal discs were fixed in 4% paraformaldehyde in PBS for 20 minutes at room temperature. After four washes of 0.3% PBST (PBS containing 0.3% Triton X-100) and 15 minutes blocking in 10% goat serum in 0.3% PBST, wing discs were incubated with the following primary antibodies: mouse anti-Cut, mouse anti-Wg and mouse anti-β-Gal [1:50, Developmental Studies Hybridoma Bank (DSHB]; rabbit anti-dCAF-1-p180 (1:100, Abcam); rabbit anti-dCAF-1-p105 [1:100, Antiomics; for antigen production, a fragment of *Drosophila* CAF-1-p105 (amino acids 281-539) was cloned into the pGEX vector; expression, purification and subsequent immunization of the GST fusion protein were carried out by Antiomics]; and rabbit anti-dCAF-1-p55 [1:500, a gift of Jessica Tyler (Tyler et al., 2001)]. Fluorescent secondary antibodies were used for signal detection. Images were captured by a Leica SP5 confocal microscope as previously described (Chen et al., 2010).

S2 cell culture, transfection and RNA interference (RNAi) assays

Drosophila S2 cells were cultured at room temperature in Hyclone serum-free insect cell culture media (Roche). Transfection of S2 cells was performed using FuGENE HD transfection kit reagents (Roche) following the manufacturer's instruction. Constructs used for transfections are described in supplementary material Table S2. The S2 cells were normally harvested 48 hours after transfection for further experiments. Full-length Notch expression was induced by 500 μM $CuSO_4$ for 24 hours after pMT-Notch transfection (Fehon et al., 1990). Double-stranded (ds) RNA was prepared with the RiboMAX large-scale RNA production system-T7 kit (Promega) as previously described (Huang et al., 2011). Primers (forward and reverse, 5′-3′) were: *dCAF-1-p105*, gatcactaatacgactcactatagggCCGAGTCAGCAAATGTGTAC and gatcactaatacgactcactatagggACTGACACTGTGCGTTGATG; *GFP* control, ttaatacgactcactatagggagaATGGTGAGCAAGGGCGAGGAGCTG and ttaatacgactcactatagggagaCTTGTACAGCTCGTCCATGCCGAGAG (lowercase letters indicate T7 promoter sequences). For a 6-well plate, cells in 2 ml medium in each well were treated with 15 μg dsRNA for 4 days prior to plasmid transfection or $CuSO_4$ induction.

RNA isolation and quantification

Total RNA from animals of the correct genotype was isolated using Trizol reagent (Invitrogen) and further cleaned with TURBO DNase (Ambion). Then, 5 μg total RNA was used for reverse transcription with the SuperScript III reverse transcriptase kit (Invitrogen) according to the standard protocol (Liu et al., 2011). Quantitative PCR (qPCR) was performed on an MJ Research CHROMO4 real-time detector machine and the results were analyzed with Opticon Monitor 2 software. All qPCR values are the mean of three independent experiments after normalization and Student's *t*-tests were used to evaluate significance. Gene-specific primers used for qPCR are listed in supplementary material Table S3.

Co-immunoprecipitation (co-IP) assay

Total protein extracts from embryos or S2 cells were prepared in IP lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA pH 7.4, 1% Triton X-100, 0.1% SDS) in the presence of protease inhibitors [1 mM PMSF; protease inhibitor cocktail (Calbiochem)]. For co-IP assays, extracts were incubated with specific antibodies and protein A/G agarose beads (Abmart) at 4°C overnight before washes and elution. Immunoprecipitates were boiled in 2×SDS loading buffer for elution from the beads. Goat anti-Su(H) (1:100, Santa Cruz), rabbit anti-Flag (1:100, Sigma) and mouse anti-HA (1:100, Abmart) antibodies were used for co-IP experiments; mouse anti-HA (1:1000, Abmart), mouse anti-N^{ICD} (1:1000, DSHB), and rabbit anti-Myc (1:2000, Sigma) were used for western blots.

Chromatin immunoprecipitation (ChIP) assay

Second instar larvae were harvested and fixed in 1% paraformaldehyde in PBS at 37°C for 15 minutes with vortex. The cross-linked chromatin was resuspended in IP lysis buffer (see co-IP assay) followed by sonication to obtain DNA fragments of ~500-1000 bp. Anti-acetylated H4 (Upstate Biotechnology), anti-dCAF-1-p105 (Antiomics), anti-H3 (Abcam) or anti-Su(H) (Santa Cruz) together with protein A/G agarose beads were incubated with sonicated lysates at 4°C overnight. Following elution (Huang et al., 2010), cross-linking of the chromatin samples was reversed at 65°C for 6 hours. Subsequently, genomic DNA was extracted and used as PCR template for quantitative analyses. Primers used to detect the *E(spl)mβ* enhancer and *hh* enhancer are listed in supplementary material Table S3. The PCR values in all ChIP experiments are the mean of three independent experiments after normalization and Student's *t*-tests were used to evaluate significance.

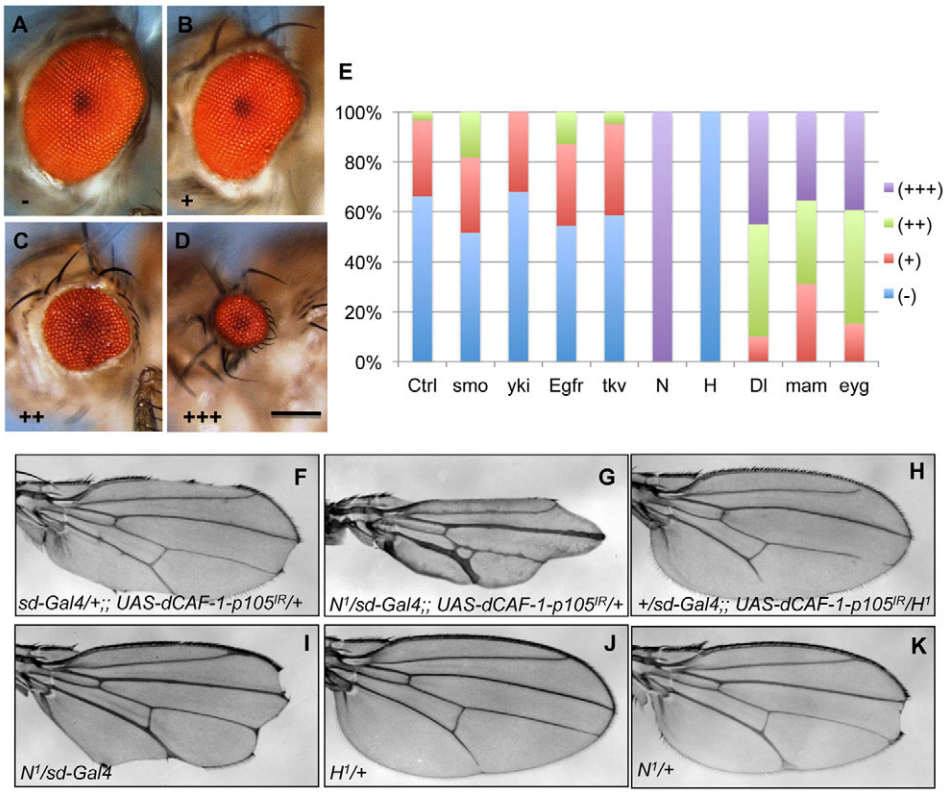

Fig. 1. *dCAF-1-p105* genetically interacts with the Notch pathway in the eye and wing. (**A-E**) Genetic interaction of *dCAF-1-p105* and the Notch pathway in the eye. Eyes of wild-type adult flies (A) and flies that express *dCAF-1-p105^{IR}* in a wild-type (B,C) or heterozygous *N^{1}* background (D) are classified into four classes according to eye size (E). Scale bar: 200 μm. (E) The penetrance and expressivity of small-eye phenotypes for flies of the different genotypes. Ctrl represents *ey>dCAF-1-p105^{IR}*. *smo*, *yki*, *Egfr*, *tkv*, *N*, *H*, *Dl*, *mam* and *eyg* represent flies of *ey>dCAF-1-p105^{IR}* in the background of one copy of the mutation for each indicated gene. (**F-K**) Genetic interaction of *dCAF-1-p105* and the Notch pathway in the wing. (F) dCAF-1-p105 knockdown under the control of *sd-Gal4* causes loss of wing margins (100%, *n*=50). (G) The loss of wing margin phenotype of *sd>dCAF-1-p105^{IR}* is enhanced in the presence of one copy of *N^{1}* (100%, *n*=50; removing nearly all of the wing margin). (H) The loss of wing margin phenotype of *sd>dCAF-1-p105^{IR}* is suppressed by a copy of the *H^{1}* mutation (66.1%, *n*=62). (J) *H^{1}*/+ shows a mild Notch gain-of-function phenotype with an interruption of longitudinal vein 5 (LV5) in the distal part (100%, *n*=50). (I,K) Note that the notched wings of *N^{1}*/+ can apparently also be enhanced in combination with *sd-Gal4* (greater loss of wing margins), but is further enhanced when *dCAF-1-p105^{IR}* is introduced (G). For I, the penetrance is 100%, referring to a phenotype that, on average, removes nearly 50% of the total wing margin (*n*=50), and for K the penetrance is 11.8%, referring to a weak phenotype that usually exhibits a single distal notch of the wing (*N*=110; supplementary material Fig. S1B).

RESULTS

Depletion of dCAF-1-p105 leads to developmental defects that resemble the effects of Notch signaling downregulation in the *Drosophila* eye and wing

In a search for signaling pathways that are regulated by CAF-1 in *Drosophila*, we performed a small-scale candidate screen using *dCAF-1-p105 RNAi* (*dCAF-1-p105^{IR}*) flies, in which the RNAi is specifically expressed in the eye under the control of *eyeless-Gal4* (*ey-Gal4*). *dCAF-1-p105^{IR}* flies exhibited a mild small-eye phenotype (Fig. 1A,C versus 1E, Ctrl). Two lines of evidence suggest that this represents a specific loss-of-function phenotype of *dCAF-1-p105*: (1) the small-eye phenotype was rescued by expression of *UAS-HA-dCAF-1-p105* under the control of the same Gal4 driver (supplementary material Table S1); (2) in *dCAF-1-p105^{IR}* flies, the transcription of *dCAF-1-p105* was specifically downregulated (supplementary material Fig. S1).

Next, we examined how the alteration of particular signaling pathways that are known to control cell proliferation and/or patterning during *Drosophila* eye development affected this small-eye phenotype. The pathways examined included the Hedgehog (Hh), Hippo, Epidermal growth factor receptor (Egfr), Decapentaplegic (Dpp) and Notch (N) signaling pathways (Fig. 1E). The small-eye phenotype was significantly enhanced in heterozygous mutant backgrounds of different Notch positive regulatory pathway components. The enhancement was judged by: (1) the fraction of flies with small eyes increased from 33.9% to 100% as compared with the control (*ey>dCAF-1-p105^{IR}*); (2) the eyes were also generally smaller than those of the control flies (Fig. 1C-E, *N*, *Dl*, *mam*, *eyg*; supplementary material Table S1). By contrast, the heterozygous mutation of *Hairless* (*H*), a negative regulator of Notch signaling, fully suppressed the small-eye phenotype (compare *H* and Ctrl in Fig. 1E). Mutations of components of other signaling pathways (e.g. *smo*, *Egfr*, *yki* and *tkv*) did not appear to affect the small-eye phenotype of *ey>dCAF-1-p105^{IR}* flies (Fig. 1E; supplementary material Table S1). These results suggest that *dCAF-1-p105* genetically interacts with the Notch pathway to control *Drosophila* eye development.

Next, we extended our investigation to determine whether dCAF-1-p105 also interacts with the Notch pathway during wing development, where Notch plays important roles in vein patterning and wing margin specification (Artavanis-Tsakonas and

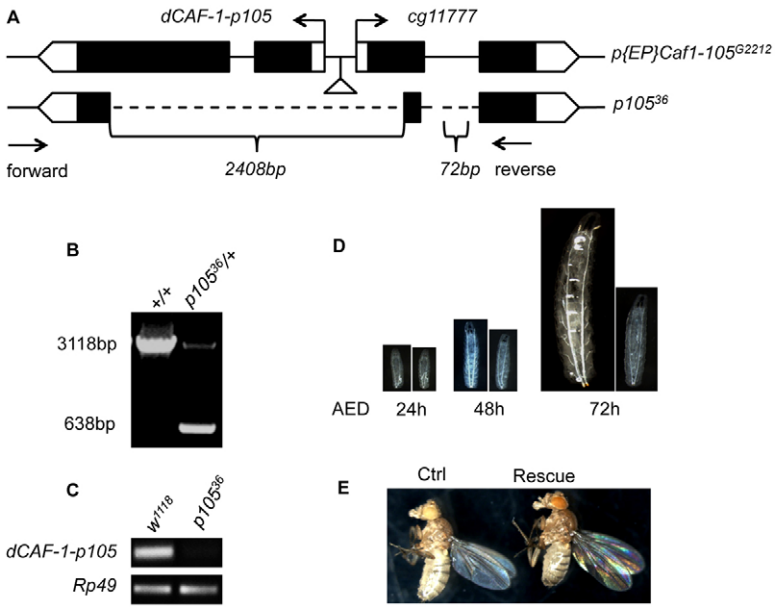

Fig. 2. Generation and molecular identification of the *dCAF-1-p105* mutant. (**A**) Genomic organization of the *dCAF-1-p105* locus and one of its neighboring genes, with the *p[EP]Caf1-105^{G2212}* P-element insertion site (white triangle) and the fragment deleted in *p105^{36}* (dashed line) indicated. Black bars indicate the coding regions of *dCAF-1-p105* and *CG11777*; white bars indicate the 5′ and 3′ UTRs. PCR primers used to amplify fragments containing the deletions are indicated (forward and reverse arrows). (**B**) PCR analysis of genomic DNA of *p105^{36}* heterozygous animals shows a short, 638 bp fragment that is not present in the wild type in addition to the 3118 bp wild-type fragment. Sequencing (not shown) indicated that the deletion encompasses two regions of 2408 bp and 72 bp (see A). (**C**) RT-PCR illustrating that no transcripts of *dCAF-1-p105* can be detected. *Rp49* (*RpL32*) provides an internal control. (**D**) Comparison of the larvae of *w^{1118}* and *p105^{36}* at 24, 48 and 72 hours after egg deposition (AED), showing a developmental delay in the *dCAF-1-p105* mutant. (**E**) Ubiquitous expression of the transgene *UAS-HA-dCAF-1-p105* under the control of *da-Gal4* rescues the lethality of *p105^{36}* mutants. The rescued flies did not exhibit any detectable defects compared with the wild type.

Muskavitch, 2010). Expression of *dCAF-p-105^{IR}* in the wing under the control of *scalloped-Gal4* (*sd-Gal4*) led to a notched wing phenotype (Fig. 1F) resembling that of *Notch* loss-of-function mutations (Fig. 1K). These wings typically exhibit notches at the wing margin of the anterior/posterior (A/P) boundary and mildly thickened veins at longitudinal vein (LV) 3 and 5 (Fig. 1K; supplementary material Fig. S1B,D). When the *N^{1}* heterozygous mutation was introduced into the *sd>dCAF-1-p105^{IR}* flies, the entire wing margin failed to develop, constituting a much stronger notched wing phenotype than *dCAF-1-p105^{IR}* alone (Fig. 1G,F). In addition, LV3 and LV5 were much more thickened than those of heterozygous *N^{1}* wings (Fig. 1G,K), suggesting a synergistic effect between *dCAF-1-p105^{IR}* and *N^{1}*. To show directly the genetic interaction between *dCAF-1-p105* and *Notch*, we examined whether loss of one copy of the *dCAF-1-p105* gene affected the *N^{1}* phenotype. One copy of *p105^{36}* enhanced the notched wing phenotype of *N^{1}*, with increased penetrance from 11.8% to 17.9% (supplementary material Fig. S1B-D). Importantly, the notched wing phenotype caused by *sd>dCAF-1-p105^{IR}* was suppressed by one copy of the *H^{1}* mutation, as expected (Fig. 1H). Together, these results suggest that dCAF-1-p105 synergistically interacts with the Notch pathway to regulate normal tissue development not only in the eye but also in the wing.

dCAF-1-p105 is required for proper expression of the Notch target genes *cut* and *wg*

To exclude any potential off-target artifacts in the RNAi experiments, and to ascertain that dCAF-1-p105 is required for the output of Notch signal transduction, we generated a null allele of *dCAF-1-p105* through P-element-mediated imprecise excision. Imprecise excision of the *EP^{G2212}* insertion resulted in a 2480 bp deletion, which covers most of the *dCAF-1-p105* coding region and part of the adjacent gene *CG11777*, yielding a predicted double mutant of *dCAF-1-p105* and *CG11777* designated *p105^{36}* (Fig. 2A,B). Further RT-PCR analysis showed that *dCAF-1-p105* mRNA was completely abolished in *p105^{36}* homozygous mutants (Fig. 2C). The majority of the *p105^{36}* mutant larvae did not survive longer than 48 hours after egg deposition (AED) and exhibited an apparent developmental delay and small body size when compared with wild-type larvae of the same age (Fig. 2D). A few escapees of the *p105^{36}* mutants lived longer than 72 hours, but never survived to the pupal stage (Fig. 2D). The *p105^{36}* phenotypes, including lethality, were completely rescued by ubiquitous expression of the *UAS-HA-dCAF-1-p105* transgene under the control of *daughterless-Gal4* (*da-Gal4*) (Fig. 2E; supplementary material Fig. S2A), suggesting that *p105^{36}* is a null allele for *dCAF-1-p105* and that the adjacent *CG11777* is a non-essential gene.

To test the effect of *dCAF-1-p105* null mutation on Notch signaling, we performed clonal analyses to monitor developmental defects and to detect the expression of Notch target genes in the absence of dCAF-1-p105. *p105^{36}* mutant clones were generated in the *Minute* background to overcome the growth disadvantage of the mutant cells. After clonal induction by heat shock, flies carrying *p105^{36}* clones showed a notched wing phenotype (Fig. 3B), whereas flies bearing mock clones showed no defects in the wing (Fig. 3A). The *Notch*-like phenotype in *p105^{36}* mosaic flies suggests that Notch signaling is compromised in the absence of dCAF-1-p105. To confirm this, we examined the expression of two well-characterized Notch target genes, *cut* and *wg*, in *p105^{36}* mosaic wing imaginal discs. In wild-type wing discs and discs with mock clones, Cut and Wg were both expressed along the dorsal/ventral (D/V) boundary (Fig. 3C,D). In *p105^{36}* mutant clones, their expression was significantly reduced to almost below the detection level (Fig. 3E-F′), indicating significant downregulation of Notch signaling in the absence of dCAF-1-p105. To verify the specificity of the effects of dCAF-1-p105 on Notch signaling, we examined the activity of Hh signaling in *p105^{36}* mosaic wing discs. Consistent with the genetic interaction results (Fig. 1), in *p105^{36}* mutant clones, the expression of two Hh signaling readouts, *hh-lacZ* and *dpp-lacZ*, was at about the same level as that in the wild-type region (supplementary material Fig. S3A,B). These results suggest that dCAF-1-p105 specifically regulates the output of Notch signaling in the wing disc.

Next, investigated the molecular mechanism(s) underlying how dCAF-1-p105 functions in Notch signaling. To test whether dCAF-1-p105 functions at the transcriptional level to regulate Notch target gene expression, we examined the transcription of two well-defined reporter genes of the Notch signaling pathway:

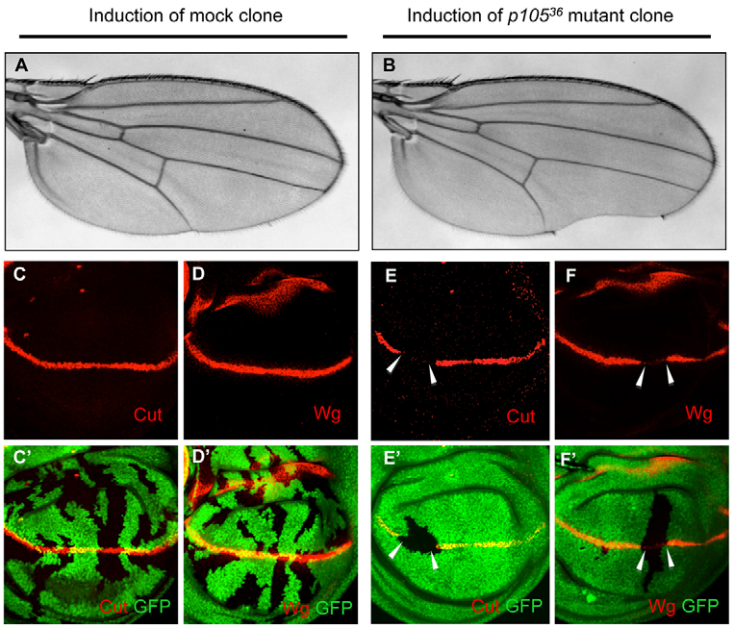

Fig. 3. dCAF-1-p105 is required for the proper expression of Notch target genes *cut* and *wg*. (**A**,**B**) Induction of *dCAF-1-p105* (*p105*^{36}) mutant clones leads to a wing notch, whereas induction of mock clones leads to wild-type wings. Mock clones and *p105*^{36} mutant clones were induced at the second instar larval stage at 38°C for 1 hour. The genotype of flies with mock clones is *hs-Flp/+; FRT42D, Minute, ubi-GFP/FRT42D*. The genotype of flies with *p105*^{36} mutant clones is *hs-Flp/+; FRT42D, Minute, ubi-GFP/FRT42D, p105*^{36}. (**C-F′**) In *dCAF-1-p105* mutant clones, the expression of Cut (E,E′, GFP-negative area, arrowheads) and Wg (F,F′, GFP-negative area, arrowheads) is abolished in a cell-autonomous manner, whereas in the mock clones the expression of both Cut (C,C′) and Wg (D,D′) is unaffected. Wing discs were dissected for immunostaining 3 days after clonal induction.

cut and the *E(spl)* complex component gene *E(spl)mβ* (Jack et al., 1991; Neumann and Cohen, 1996; Xie et al., 2012). The *cut-lacZ* (Jack et al., 1991) and *E(spl)mβ-lacZ* (Xie et al., 2012) transgenic reporters were used for this purpose. When dCAF-1-p105 was knocked down at the posterior region of the wing disc, *cut-lacZ* and *E(spl)mβ-lacZ* expression was significantly downregulated specifically in the posterior region (Fig. 4B,B′,D,D′). These results suggest that dCAF-1-p105 functions as a positive regulator of the Notch signaling pathway by promoting its target gene transcription.

dCAF-1-p105 associates with Su(H) to regulate its binding to the enhancer region of *E(spl)mβ*

Su(H) is the core transcription factor regulating Notch target gene expression (Bray, 2006). To determine whether dCAF-1-p105 physically associates with Su(H) *in vivo*, we expressed a *UAS-HA-dCAF-1-p105* transgene under the control of *da-Gal4* in developing *Drosophila* embryos, in which the total amount (non-tagged plus HA-tagged) of dCAF-1-p105 was 2.39-fold that in wild-type animals (supplementary material Fig. S2A). From the extracts of such embryos, HA-tagged dCAF-1-p105 was co-

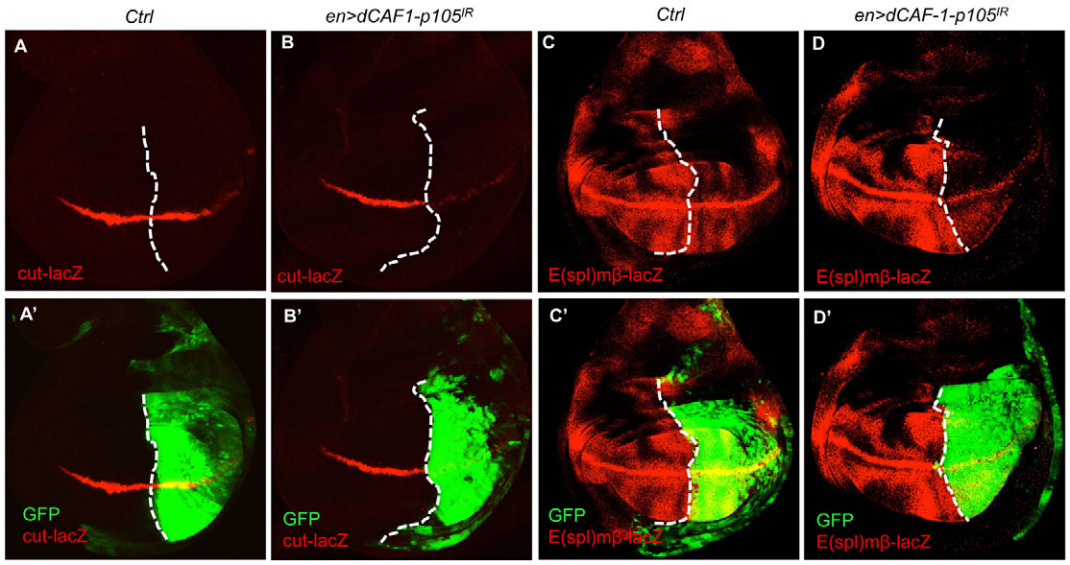

Fig. 4. Notch target genes are regulated by dCAF-1-p105 at the transcription level. (**A-B′**) The expression of *cut-lacZ* is downregulated upon depletion of *dCAF-1-p105*. In control discs (A,A′) *cut-lacZ* is expressed normally along the D/V boundary (dashed line) in both the anterior and posterior regions. Knocking down dCAF-1-p105 under the control of *en-Gal4* (B,B′) substantially reduces *cut-lacZ* expression in the posterior region. Genotypes: (A,A′) *UAS-Dicer2/+; en-Gal4, UAS-GFP/+; cut-lacZ/+*; (B,B′) *UAS-Dicer2/+; en-Gal4, UAS-GFP/UAS-dCAF-1-p105*^{IR}*; cut-lacZ/+*. *UAS-Dicer2* was used to enhance RNAi efficiency. (**C-D′**) The expression of *E(spl)mβ-lacZ* is downregulated in the dCAF-1-p105-depleted area. In control discs (C,C′) *E(spl)mβ-lacZ* is expressed normally in the entire wing disc. Knocking down of dCAF-1-p105 under the control of *en-Gal4* (D,D′) substantially reduces *E(spl)mβ-lacZ* expression. The genotype of Ctrl is *UAS-Dicer2/+; en-Gal4, UAS-GFP/E(spl)mβ-lacZ*, and *en>dCAF-1-p105*^{IR} is *UAS-Dicer2/+; en-Gal4, UAS-GFP/E(spl)mβ-lacZ, UAS-dCAF-1-p105*^{IR}.

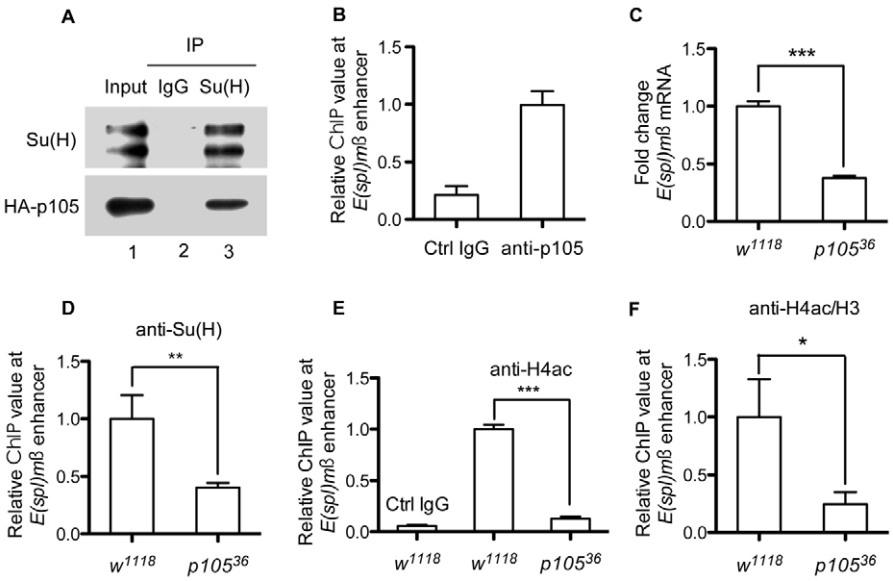

Fig. 5. dCAF-1-p105 associates with Su(H) and maintains the local histone H4 acetylation level. (**A**) Su(H) associates with HA-tagged dCAF-1-p105 *in vivo*. Total protein extracts were prepared from *Drosophila* embryos (0-12 hours AED) with ubiquitous expression of HA-tagged dCAF-1-p105 under the control of *da-Gal4*. Input (lane 1) represents 5% of the extracts that were used for immunoprecipitation (IP) with IgG (control) or Su(H) antibody. Antibodies used for western blot detection are indicated on the left. (**B**) ChIP assay shows that dCAF-1-p105 preferentially occupies the enhancer of the Notch target gene *E(spl)mβ*. The *y*-axis indicates relative protein occupancy values at the enhancer region as detected by qPCR after ChIP. (**C**) qPCR shows that the level of *E(spl)mβ* mRNA in $p105^{36}$ mutants is significantly lower than in w^{1118} animals. (**D**) ChIP assay showing a decrease in Su(H) abundance at the *E(spl)mβ* enhancer in the absence of dCAF-1-p105. (**E**,**F**) ChIP assay shows a decrease in acetylated histone H4 (H4ac) at the *E(spl)mβ* enhancer in the absence of dCAF-1-p105. H4ac at the *E(spl)mβ* enhancer region is maintained at a significantly higher level in the wild type than in $p105^{36}$ mutants. The abundance of H4ac relative to total histone H3 is higher in wild type than in $p105^{36}$ mutants. The mean of three independent experiments after normalization is shown; error bars indicate s.e.m. *P<0.05, **P<0.001, ***P<0.0001; Student's t-test.

immunoprecipitated with an anti-Su(H) antibody (Fig. 5A), but not with the control antibody (Fig. 5). More importantly, in the extracts of wild-type embryos the association of Su(H) with endogenous dCAF-1-p105 was also detected (supplementary material Fig. S2B), suggesting that dCAF-1-p105 is physically associated with Su(H) in *Drosophila*.

Next, we asked whether dCAF-1-p105 is specifically associated with the chromatin at the Su(H)-binding enhancer region of one of the Notch target genes, *E(spl)mβ*. Using a ChIP assay, we detected a significant increase in dCAF-1-p105-associated *E(spl)mβ* enhancer DNA as compared with the control IgG (Fig. 5B), suggesting an enrichment of dCAF-1-p105 at the *E(spl)mβ* enhancer region. To assess the biological effects of this enrichment in Notch signaling, we examined the expression of *E(spl)mβ* in the presence and absence of dCAF-1-p105. The expression level of *E(spl)mβ* was consistently and significantly reduced in the second instar larvae of *dCAF-1-p105* mutants compared with the wild type, whereas expression of target genes of the Hh pathway, such as *hh*, was unaffected (Fig. 5C; supplementary material Fig. S3C).

Following Notch induction, the occupancy of Su(H) at Notch target genes has been reported to be increased (Krejcí and Bray, 2007). We hypothesized that the local chromatin structure, as regulated by dCAF-1-p105, affects Su(H) occupancy of its target DNA sequence. To test this hypothesis, we assessed the binding efficiency of Su(H) at the *E(spl)mβ* enhancer region in a ChIP assay. Compared with the wild-type control, $p105^{36}$ mutant larvae exhibited a significant decrease in the abundance of Su(H) at the *E(spl)mβ* enhancer region, as judged by the decreased amount of chromatin immunoprecipitated by the Su(H) antibody (Fig. 5D). These data suggest that dCAF-1-p105 regulates Notch target gene expression, probably by controlling the accessibility of Su(H) to its target DNA sequence in the enhancer regions of Notch target genes.

dCAF-1-p105 maintains the acetylation level of histone H4 at the enhancer region of *E(spl)mβ*

To elucidate the precise mechanism of how dCAF-1-p105 promotes Notch target gene transcription, we examined the possibility that dCAF-1-p105 plays a role in providing a chromatin platform that is permissive for transcription by regulating histone modifications. Because histone H4 acetylation is associated with active promoters of Notch target genes (Krejcí and Bray, 2007), we monitored the level of active histone H4 acetylation in the *E(spl)mβ* enhancer region in both wild type and *dCAF-1-p105* mutants. Using acetylated H4 (H4ac)-specific antibodies that recognize H4K5ac, H4K8ac, H4K12ac and H4K16ac in a ChIP assay, we showed that, in wild-type flies, the amount of H4ac-associated *E(spl)mβ* enhancer DNA was significantly higher than that associated with control IgG, suggesting that H4ac was enriched at the enhancer region of *E(spl)mβ* (Fig. 5E). Interestingly, the amount of immunoprecipitated DNA from the *E(spl)mβ* enhancer region by anti-H4ac was significantly lower in the *dCAF-1-p105* mutant than in wild type (Fig. 5E), suggesting a decrease in H4ac associated with the absence of dCAF-1-p105. To exclude the possibility that the reduction of H4ac in *dCAF-1-p105* mutant larvae was caused by a decrease in overall histone abundance or nucleosome density at the *E(spl)mβ* enhancer region, an antibody that recognizes total histone H3 was used in the ChIP assay for normalization (Fig. 5F). Compared with the control, the normalized H4ac level in the *E(spl)mβ* enhancer region also appeared significantly reduced in *dCAF-1-p105* mutant larvae compared with that in wild-type animals (Fig. 5F). By

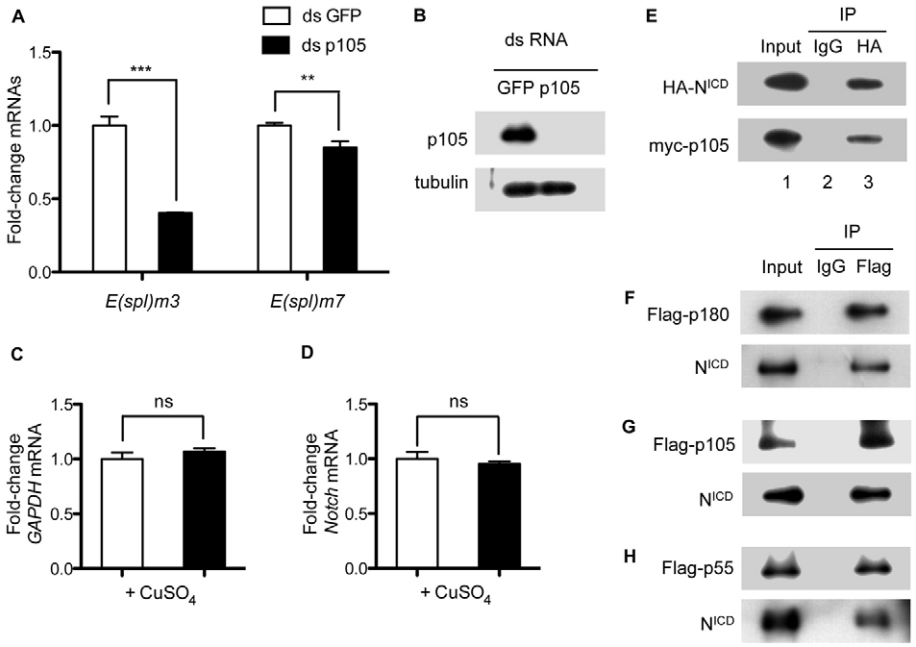

Fig. 6. dCAF-1 physically interacts with N^{ICD} and regulates Notch target gene expression in S2 cells upon artificial Notch signal induction. (**A**) qPCR to monitor mRNA levels of *E(spl)m3* and *E(spl)m7* when dCAF-1-p105 was knocked down in S2 cells with artificial induction of Notch signaling. *E(spl)m3* and *E(spl)m7* are highly expressed when Notch signaling is induced by $CuSO_4$ in normal S2 cells (supplementary material Fig. S4B,C). A reduction in *E(spl)m3* and *E(spl)m7* transcription was observed upon *dCAF-1-p105* RNAi manipulation (black bars in A). *GFP* dsRNA (white bars in A) was used to control for RNAi specificity. (**B**) *dCAF-1-p105* mRNA was abolished by *dsp105* treatment but not in the *dsGFP* treatment control. (**C**,**D**) In both groups of dsRNA treatment, *Notch* and *Gapdh* mRNA levels were not significantly altered. Error bars indicate s.e.m. ns, not significant; $^{**}P<0.001$, $^{***}P<0.0001$; Student's *t*-test. (**E**) HA-tagged N^{ICD} is associated with Myc-tagged dCAF-1-p105. HA-tagged N^{ICD} and Myc-tagged dCAF-1-p105 were co-transfected into cultured S2 cells and total cellular extracts prepared for the co-IP assay with IgG (control) or anti-HA antibody. Proteins detected by western blot are indicated to the left. (**F-H**) All three subunits of dCAF-1 are associated with N^{ICD} in response to the induction of Notch signaling in S2 cells. Each subunit of dCAF-1 and pMT-Notch were co-transfected into cultured S2 cells and $CuSO_4$ was added to induce full-length Notch expression. (B,E-H) Antibodies used for IP are indicated at the top, proteins detected by western blot after IP are indicated to the left. Input lanes represent 5% of the S2 cell extracts that were used for IP.

contrast, loss of *dCAF-1-p105* did not affect the H4ac level in the enhancer region of the *hh* gene (supplementary material Fig. S3D). Collectively, these results suggest that dCAF-1-p105 regulates the epigenetic modification mark of H4 acetylation in the enhancer region of the Notch target gene *E(spl)mβ* in order to maintain an active chromatin status.

dCAF-1-p105 is required for Notch target gene expression in S2 cells following Notch signal induction

To determine whether dCAF-1-p105 can promote Notch target gene transcription when Notch signaling is artificially induced, we made use of a transient Notch activation system in cultured S2 cells by transfecting a Notch-expressing plasmid, pMT-Notch (Krejcí and Bray, 2007). In this assay, the overexpressed full-length Notch can be processed to an active form of N^{ICD} that physically interacts with Su(H), mimicking the *in vivo* Notch activation process (supplementary material Fig. S4A). A significant upregulation of the *E(spl)* complex genes *E(spl)m3* and *E(spl)m7* (Krejcí and Bray, 2007; Endo et al., 2012) was detected when Notch signaling was induced (supplementary material Fig. S4B,C). In this transient Notch activation system, assuming that dCAF-1-p105 is required for regulating Notch target gene expression, the induced expression should be compromised by a perturbation of dCAF-1-p105. As expected, the introduction of interfering *dCAF-1-p105* dsRNAs (*dsp105*), but not *dsGFP*, into the Notch-activating S2 cells led to a significant reduction in expression of the primary endogenous Notch target genes *E(spl)m3* and *E(spl)m7* (Fig. 6A,B). No significant difference in transcription was detected for the control gene *Gapdh* (Fig. 6C). These results suggest that dCAF-1-p105 is also required for artificially induced Notch signaling in cultured S2 cells.

The N^{ICD}-Su(H)-Mam ternary complex has been reported in many organisms to regulate Notch target gene activation (Bray, 2006), and we considered whether dCAF-1 physically interacts with N^{ICD} to promote Notch activation. To test this, a construct that expresses Myc-tagged dCAF-1-p105 was co-transfected with HA-tagged N^{ICD} into S2 cells (Fig. 6E). In the co-IP assay, dCAF-1-p105 was detected in association with N^{ICD} (Fig. 6G). Interestingly, in addition to dCAF-1-p105, the other two subunits of dCAF-1 were also co-immunoprecipitated with N^{ICD} (Fig. 6F-H). Together, these results suggest that dCAF-1 might physically interact with N^{ICD} in the form of a complex in *Drosophila* cells, consequently promoting Notch target gene expression.

An integral dCAF-1 complex, rather than a subunit, is required for proper Notch signaling during *Drosophila* development

dCAF-1 has been identified biochemically as a heterotrimeric complex consisting of dCAF-1-p180, dCAF-1-p105 and dCAF-1-p55 (Caf1-180, Caf1-105 and Caf1 – FlyBase) (Tyler et al., 2001), which are evolutionarily conserved throughout the eukaryotes

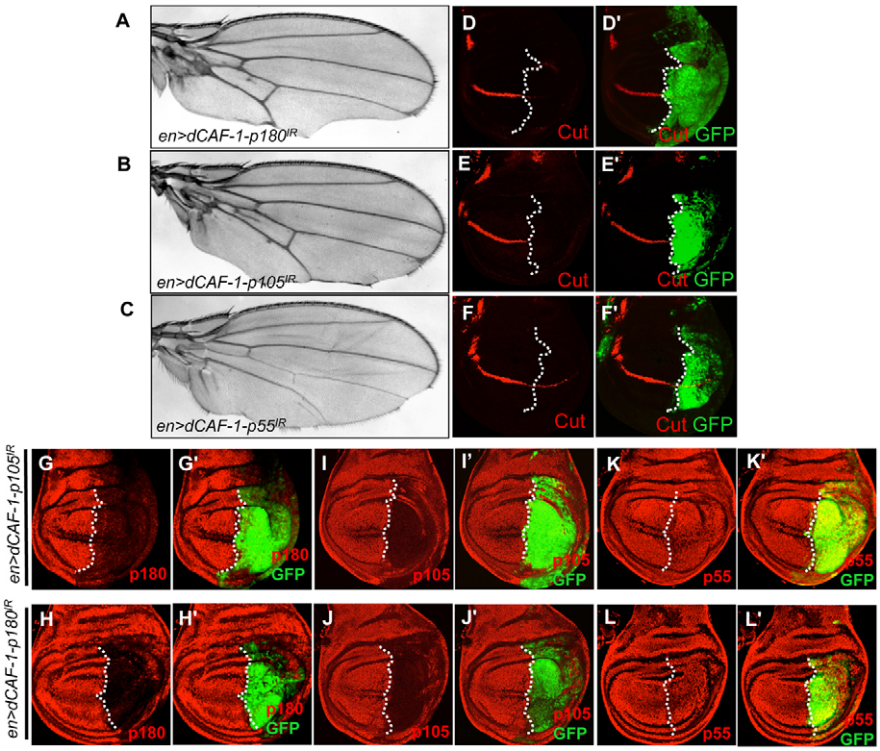

Fig. 7. The function of dCAF-1 in regulating Notch signaling is dependent on its integrity as a complex. (**A-C**) Depletion of any subunit of the dCAF-1 complex by RNAi under the control of *en-Gal4* is sufficient to generate the notched wing phenotype at the wing posteriors. (**D-F′**) Depletion of any subunit of the dCAF-1 complex under the control of *en-Gal4* is sufficient to downregulate *cut* expression (red). Complete genotypes: *en>dCAF-1-p180^{IR}* is *en-Gal4, UAS-GFP/+; UAS-dCAF-1-p180^{IR}*; *en>dCAF-1-p105^{IR}* is *en-Gal4, UAS-GFP/+; UAS-dCAF-1-p105^{IR}*; and *en>dCAF-1-p55^{IR}* is *en-Gal4, UAS-GFP/+; UAS-dCAF-1-p55^{IR}*. (**G-L′**) dCAF-1-p105 and dCAF-1-p180 are mutually dependent on the presence of each other. (G,I,K) Knockdown of dCAF-1-p105 by *en-Gal4* leads to downregulation of not only dCAF-1-p105 (I) but also dCAF-1-p180 (G), but not dCAF-1-p55 (K). (H,J,L) Knockdown of dCAF-1-p180 by *en-Gal4* leads to downregulation of not only dCAF-1-p180 (H) but also dCAF-1-p105 (J), but not dCAF-1-p55 (L).

(Ridgway and Almouzni, 2000; Loyola and Almouzni, 2004; Huang and Jiao, 2012). Our co-IP results suggested that all three subunits of dCAF-1 were associated with N^{ICD} during Notch activation in cultured S2 cells (Fig. 6F-H). We next asked whether these three subunits of dCAF-1 function as a complex in Notch signaling *in vivo*. First, we examined the effects of their individual depletion by RNAi on *cut* expression (the effectiveness and specificity of the RNAi were validated by testing independent RNAi lines, RT-PCR and rescue experiments; data not shown). Consistent with what was observed in the *p105^{36}* mutant clones (Fig. 3E), knockdown of dCAF-1-p105 at the posterior region of the wing disc led to a significant decrease in *cut* expression (Fig. 7E,E′), whereas adult flies that developed from these larvae exhibited the notched wing phenotype (Fig. 7B). Similarly, *en-Gal4*-driven knockdown of either dCAF-1-p180 or dCAF-1-p55 also resulted in reduced Cut expression at the DV boundary of the posterior wing disc and the notched wing phenotype (Fig. 7A,C-D′,F,F′), suggesting that the three subunits of dCAF-1 are all required for the activation of the Notch signaling pathway.

Given that all three subunits of dCAF-1 are associated with N^{ICD} (Fig. 6F-H), we hypothesized that dCAF-1 regulates Notch signaling as an integral protein complex. One possibility in maintaining a functional protein complex is that the subunits of this complex mutually stabilize each other. Indeed, knockdown of dCAF-1-p105 at the posterior region of the wing disc led to downregulation of dCAF-1-p180 cell-autonomously (Fig. 7G,I). The reverse situation was also true; when dCAF-1-p180 was knocked down, a significant downregulation of the dCAF-1-p105 level was detected (Fig. 7H,J). However, the smallest subunit, dCAF-1-p55, was evidently unaffected by knockdown of either dCAF-1-p105 or dCAF-1-p180 (Fig. 7K,L). Additionally, when dCAF-1-p55 was knocked down in the posterior region of the wing discs, neither dCAF-1-p180 nor dCAF-1-p105 was affected (supplementary material Fig. S5). When dCAF-1-p180 was knocked down by the ubiquitously expressed *da-Gal4*, *dCAF-1-p105* transcription was apparently unaltered (supplementary material Fig. S5). Similarly, when dCAF-1-p105 was knocked down, the transcription level of *dCAF-1-p180* remained unchanged (supplementary material Fig. S6), indicating that dCAF-1-p105 and dCAF-1-p180 mutually stabilize each other at the protein level, rather than the loss of one affecting the expression of the other. These results suggest that the function of dCAF-1 in regulating Notch signaling during *Drosophila* development is dependent on its integrity as a protein complex.

DISCUSSION

Signaling cascades are essential in instructing cell proliferation and differentiation during animal development and are often manifested by turning on or shutting off the expression of a group of genes. The specificity of different signaling pathways is usually determined by activating specific transcription factors that bind to the enhancers of their target genes. However, in eukaryotic cells the full transcription of a particular target gene is also dependent on the recruitment of chromatin remodelers and histone modifiers, in addition to transcription factors, in order to ensure a local chromatin environment that is permissive for the access of a complete set of regulatory proteins. Although CAF-1 was initially identified as a histone chaperone for DNA synthesis-coupled chromatin assembly (Smith and Stillman, 1989; Das et al., 2009), it is becoming increasingly evident that CAF-1 has functions in the regulation of other cellular and developmental processes, such as heterochromatin formation and asymmetric cell division (Quivy et al., 2008; Huang et al., 2010; Nakano et al., 2011). Recent studies in *C. elegans* and *Arabidopsis* imply that CAF-1 is involved in gene activation (Autran et al., 2011; Nakano et al., 2011); however, the precise mechanisms by which CAF-1 mediates gene activation and whether CAF-1 specifically regulates gene expression in association with a particular developmental pathway remain unclear. Here, we report

a novel function of dCAF-1 in epigenetically regulating the Notch signaling pathway. Our results show that, by interacting with Su(H) and N^{ICD}, dCAF-1 is enriched in the enhancer regions of Notch target genes, maintaining the local H4 acetylation level to provide an appropriate chromatin niche for gene activation.

Our results also show that depletion of dCAF-1-p105 affects the binding efficiency of Su(H) to its target DNA sequence (Fig. 4D). Therefore, the role of dCAF-1-p105 in regulating Notch target gene expression may be interpreted as follows: in response to Notch signaling, dCAF-1-p105 is recruited to the enhancer regions of Notch genes through its interaction with Su(H) and N^{ICD}; the recruited dCAF-1-p105 alters local histone H4 acetylation (Fig. 4E,F), establishing a chromatin structure that is more accessible to Su(H) binding. The mutual recruitment of dCAF-1-p105 and Su(H) eventually promotes the establishment of a microenvironment (chromatin niche) for the expression of Notch target genes. We have shown that, in the absence of dCAF-1-p105, the binding of Su(H) to the enhancer of Notch target genes is compromised (Fig. 4D); however, owing to technical difficulties we could not perform a reverse ChIP experiment to examine how dCAF-1-p105 binding to chromatin is affected in the absence of Su(H) because these mutants die at a very early stage. Our proposal that the binding affinity of Su(H) for the enhancer region of Notch genes depends not solely on the DNA sequence but also on an appropriate local chromatin structure is supported by findings from other laboratories (Krejcí and Bray, 2007; Endo et al., 2012). In cultured *Drosophila* cells, an increased occupancy of Su(H) and histone modifications (H4ac and H3K4me3) at Notch targets are found to be in proportion to Notch induction (Krejcí and Bray, 2007). Endo and colleagues reported that Hamlet acts at the chromatin level through altering histone modifications to change the accessibility of Su(H) at Notch target loci (Endo et al., 2012).

In *Drosophila*, LAF and RLAF silencing complexes cooperate with the histone chaperones Asf1 and Nap1, respectively, to mediate epigenetic silencing (Moshkin et al., 2009). Asf1 delivers histone H3/H4 heterodimers to CAF-1 during *de novo* DNA synthesis (Ridgway and Almouzni, 2000), but it is not known whether Asf1 is also a histone deliverer for CAF-1 during transcriptional silencing. Our findings do not support the notion that CAF-1 and Asf1 function in the same pathway to mediate gene silencing in *Drosophila*, at least not in the case of regulating Notch target gene expression. Instead of silencing Notch target genes, dCAF-1-p105 interacts with positive Notch pathway components during *Drosophila* tissue development, leading to the activation of Notch target gene expression. Our results appear to be consistent with the finding that the binding of Asf1 to LAF and CAF-1 is mutually exclusive (Moshkin et al., 2009), suggesting that Asf1 and CAF-1 might function synergistically in DNA synthesis, but distinctively in the process of regulating gene expression during developmental signaling.

CAF-1 is a conserved three-subunit protein complex in metazoans (Ridgway and Almouzni, 2000). Previous studies in mammals reported that loss of CAF-1-p60 (CHAF1B) results in phenotypes that are indistinguishable from that of loss of CAF-1-p150 (CHAF1A) in the chromatin assembly process during DNA replication and DNA repair (Ye et al., 2003; Polo et al., 2006). Expression of a dominant-negative form of CAF-1-p150, CAF-1-p150C, in human U2OS cells leads to a reduction of endogenous CAF-1-p150, suggesting that CAF-1-p150 is degraded when not assembled with CAF-1-p60 (Ye et al., 2003). These data indicate that the subunits of CAF-1 are stable only in the form of a complex during their functions in DNA synthesis-coupled chromatin assembly. However, whether the subunits of CAF-1 function independently during their roles in regulating gene expression is not known. Here, we show that downregulation of dCAF-1-p105 leads to a reduction in the protein level of dCAF-1-p180 and vice versa, but not of dCAF-1-p55. Given that depletion of any one of the three subunits of dCAF-1 leads to similar *Notch*-like phenotypes, and that all three subunits are associated with N^{ICD} in S2 cells, our data suggest that dCAF-1 is likely to function as a complex, rather than as individual subunits, during Notch signaling.

In conclusion, our study demonstrates that dCAF-1 plays an essential role during tissue development through regulating the Notch pathway by an epigenetic mechanism. For the first time, we have linked the function of CAF-1 to a specific developmental signaling pathway during *Drosophila* development. Our study provides clues as to why loss of function of CAF-1 leads to lethality only in higher multicellular organisms, such as *Drosophila*, but not in unicellular organisms, such as yeast; it is the role of CAF-1 in regulating developmental pathways, rather than its role in DNA replication, that leads to lethality upon its loss in multicellular animals.

Acknowledgements

We thank Drs Kenneth Irvine, Y. Henry Sun, Xinhua Lin and Zhaohui Wang for providing fly strains; Jessica K. Tyler and Jianming Chen for providing antibodies; Nick Contreras for critical reading of the manuscript; and the anonymous reviewers for their time and constructive suggestions.

Funding

This work was supported by grants from the National Natural Science Foundation of China (NSFC) [31228015, 31071087 and 31271573] and grants from the 973 Program [2009CB918702 and 2012CB825504]. W.-M.D. is supported by the National Institutes of Health and National Science Foundation. Deposited in PMC for release after 12 months.

Competing interests statement

The authors declare no competing financial interests.

Author contributions

R.J., W.-M.D. and Z.Y. conceived and designed the experiments. Z.Y., H.W., H.C. and R.W. performed the experiments. R.J., W.-M.D., Z.Y., H.W., H.C., C.L. and J.L. analyzed the data: X.L. contributed reagents/materials/analysis tools. Z.Y., W.-M.D. and R.J. wrote the paper.

Supplementary material

Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.094599/-/DC1

References

Acar, M., Jafar-Nejad, H., Takeuchi, H., Rajan, A., Ibrani, D., Rana, N. A., Pan, H., Haltiwanger, R. S. and Bellen, H. J. (2008). Rumi is a CAP10 domain glycosyltransferase that modifies Notch and is required for Notch signaling. *Cell* **132**, 247-258.

Artavanis-Tsakonas, S. and Muskavitch, M. A. (2010). Notch: the past, the present, and the future. *Curr. Top. Dev. Biol.* **92**, 1-29.

Autran, D., Baroux, C., Raissig, M. T., Lenormand, T., Wittig, M., Grob, S., Steimer, A., Barann, M., Klostermeier, U. C., Leblanc, O. et al. (2011). Maternal epigenetic pathways control parental contributions to Arabidopsis early embryogenesis. *Cell* **145**, 707-719.

Bray, S. J. (2006). Notch signalling: a simple pathway becomes complex. *Nat. Rev. Mol. Cell Biol.* **7**, 678-689.

Bray, S., Musisi, H. and Bienz, M. (2005). Bre1 is required for Notch signaling and histone modification. *Dev. Cell* **8**, 279-286.

Chen, C. C., Carson, J. J., Feser, J., Tamburini, B., Zabaronick, S., Linger, J. and Tyler, J. K. (2008). Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. *Cell* **134**, 231-243.

Chen, Y., Dui, W., Yu, Z., Li, C., Ma, J. and Jiao, R. (2010). Drosophila RecQ5 is required for efficient SSA repair and suppression of LOH in vivo. *Protein Cell* **1**, 478-490.

Cooper, M. T., Tyler, D. M., Furriols, M., Chalkiadaki, A., Delidakis, C. and Bray, S. (2000). Spatially restricted factors cooperate with notch in the regulation of Enhancer of split genes. *Dev. Biol.* **221**, 390-403.

Das, C., Lucia, M. S., Hansen, K. C. and Tyler, J. K. (2009). CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature* **459**, 113-117.
Domanitskaya, E. and Schüpbach, T. (2012). CoREST acts as a positive regulator of Notch signaling in the follicle cells of Drosophila melanogaster. *J. Cell Sci.* **125**, 399-410.
Duan, H., Dai, Q., Kavaler, J., Bejarano, F., Medranda, G., Nègre, N. and Lai, E. C. (2011). Insensitive is a corepressor for Suppressor of Hairless and regulates Notch signalling during neural development. *EMBO J.* **30**, 3120-3133.
Endo, K., Karim, M. R., Taniguchi, H., Krejci, A., Kinameri, E., Siebert, M., Ito, K., Bray, S. J. and Moore, A. W. (2012). Chromatin modification of Notch targets in olfactory receptor neuron diversification. *Nat. Neurosci.* **15**, 224-233.
Fehon, R. G., Kooh, P. J., Rebay, I., Regan, C. L., Xu, T., Muskavitch, M. A. and Artavanis-Tsakonas, S. (1990). Molecular interactions between the protein products of the neurogenic loci Notch and Delta, two EGF-homologous genes in Drosophila. *Cell* **61**, 523-534.
Groth, A., Rocha, W., Verreault, A. and Almouzni, G. (2007). Chromatin challenges during DNA replication and repair. *Cell* **128**, 721-733.
Hautbergue, G. M., Hung, M. L., Walsh, M. J., Snijders, A. P., Chang, C. T., Jones, R., Ponting, C. P., Dickman, M. J. and Wilson, S. A. (2009). UIF, a New mRNA export adaptor that works together with REF/ALY, requires FACT for recruitment to mRNA. *Curr. Biol.* **19**, 1918-1924.
Huang, H. and Jiao, R. (2012). Roles of chromatin assembly factor 1 in the epigenetic control of chromatin plasticity. *Sci. China Life Sci.* **55**, 15-19.
Huang, H., Yu, Z., Zhang, S., Liang, X., Chen, J., Li, C., Ma, J. and Jiao, R. (2010). Drosophila CAF-1 regulates HP1-mediated epigenetic silencing and pericentric heterochromatin stability. *J. Cell Sci.* **123**, 2853-2861.
Huang, H., Du, G., Chen, H., Liang, X., Li, C., Zhu, N., Xue, L., Ma, J. and Jiao, R. (2011). Drosophila Smt3 negatively regulates JNK signaling through sequestering Hipk in the nucleus. *Development* **138**, 2477-2485.
Jack, J., Dorsett, D., Delotto, Y. and Liu, S. (1991). Expression of the cut locus in the Drosophila wing margin is required for cell type specification and is regulated by a distant enhancer. *Development* **113**, 735-747.
Jang, C. C., Chao, J. L., Jones, N., Yao, L. C., Bessarab, D. A., Kuo, Y. M., Jun, S., Desplan, C., Beckendorf, S. K. and Sun, Y. H. (2003). Two Pax genes, eye gone and eyeless, act cooperatively in promoting Drosophila eye development. *Development* **130**, 2939-2951.
Kim, H. J., Seol, J. H. and Cho, E. J. (2009). Potential role of the histone chaperone, CAF-1, in transcription. *BMB Rep.* **42**, 227-231.
Krejcí, A. and Bray, S. (2007). Notch activation stimulates transient and selective binding of Su(H)/CSL to target enhancers. *Genes Dev.* **21**, 1322-1327.
Kugler, S. J. and Nagel, A. C. (2007). putzig is required for cell proliferation and regulates notch activity in Drosophila. *Mol. Biol. Cell* **18**, 3733-3740.
Kugler, S. J. and Nagel, A. C. (2010). A novel Pzg-NURF complex regulates Notch target gene activity. *Mol. Biol. Cell* **21**, 3443-3448.
Kurooka, H. and Honjo, T. (2000). Functional interaction between the mouse notch1 intracellular region and histone acetyltransferases PCAF and GCN5. *J. Biol. Chem.* **275**, 17211-17220.
Li, Q., Zhou, H., Wurtele, H., Davies, B., Horazdovsky, B., Verreault, A. and Zhang, Z. (2008). Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* **134**, 244-255.
Liu, J., Wu, Q., He, D., Ma, T., Du, L., Dui, W., Guo, X. and Jiao, R. (2011). Drosophila sbo regulates lifespan through its function in the synthesis of coenzyme Q in vivo. *J. Genet. Genomics* **38**, 225-234.
Loyola, A. and Almouzni, G. (2004). Histone chaperones, a supporting role in the limelight. *Biochim. Biophys. Acta* **1677**, 3-11.
Moshkin, Y. M., Kan, T. W., Goodfellow, H., Bezstarosti, K., Maeda, R. K., Pilyugin, M., Karch, F., Bray, S. J., Demmers, J. A. and Verrijzer, C. P. (2009). Histone chaperones ASF1 and NAP1 differentially modulate removal of active histone marks by LID-RPD3 complexes during NOTCH silencing. *Mol. Cell* **35**, 782-793.
Mourikis, P., Lake, R. J., Firnhaber, C. B. and DeDecker, B. S. (2010). Modifiers of notch transcriptional activity identified by genome-wide RNAi. *BMC Dev. Biol.* **10**, 107.
Mulligan, P., Yang, F., Di Stefano, L., Ji, J. Y., Ouyang, J., Nishikawa, J. L., Toiber, D., Kulkarni, M., Wang, Q., Najafi-Shoushtari, S. H. et al. (2011). A SIRT1-LSD1 corepressor complex regulates Notch target gene expression and development. *Mol. Cell* **42**, 689-699.
Nakano, S., Stillman, B. and Horvitz, H. R. (2011). Replication-coupled chromatin assembly generates a neuronal bilateral asymmetry in C. elegans. *Cell* **147**, 1525-1536.
Neumann, C. J. and Cohen, S. M. (1996). A hierarchy of cross-regulation involving Notch, wingless, vestigial and cut organizes the dorsal/ventral axis of the Drosophila wing. *Development* **122**, 3477-3485.
Neumann, C. J. and Cohen, S. M. (1998). Boundary formation in Drosophila wing: Notch activity attenuated by the POU protein Nubbin. *Science* **281**, 409-413.
Polo, S. E., Roche, D. and Almouzni, G. (2006). New histone incorporation marks sites of UV repair in human cells. *Cell* **127**, 481-493.
Quivy, J. P., Gérard, A., Cook, A. J., Roche, D. and Almouzni, G. (2008). The HP1-p150/CAF-1 interaction is required for pericentric heterochromatin replication and S-phase progression in mouse cells. *Nat. Struct. Mol. Biol.* **15**, 972-979.
Ranganathan, P., Weaver, K. L. and Capobianco, A. J. (2011). Notch signalling in solid tumours: a little bit of everything but not all the time. *Nat. Rev. Cancer* **11**, 338-351.
Ridgway, P. and Almouzni, G. (2000). CAF-1 and the inheritance of chromatin states: at the crossroads of DNA replication and repair. *J. Cell Sci.* **113**, 2647-2658.
Smith, S. and Stillman, B. (1989). Purification and characterization of CAF-I, a human cell factor required for chromatin assembly during DNA replication in vitro. *Cell* **58**, 15-25.
Song, Y., He, F., Xie, G., Guo, X., Xu, Y., Chen, Y., Liang, X., Stagljar, I., Egli, D., Ma, J. et al. (2007). CAF-1 is essential for Drosophila development and involved in the maintenance of epigenetic memory. *Dev. Biol.* **311**, 213-222.
Tyler, J. K., Collins, K. A., Prasad-Sinha, J., Amiott, E., Bulger, M., Harte, P. J., Kobayashi, R. and Kadonaga, J. T. (2001). Interaction between the Drosophila CAF-1 and ASF1 chromatin assembly factors. *Mol. Cell. Biol.* **21**, 6574-6584.
Xie, G., Zhang, H., Du, G., Huang, Q., Liang, X., Ma, J. and Jiao, R. (2012). Uif, a large transmembrane protein with EGF-like repeats, can antagonize Notch signaling in Drosophila. *PLoS ONE* **7**, e36362.
Xu, Y., Lei, Z., Huang, H., Dui, W., Liang, X., Ma, J. and Jiao, R. (2009). dRecQ4 is required for DNA synthesis and essential for cell proliferation in Drosophila. *PLoS ONE* **4**, e6107.
Ye, X., Franco, A. A., Santos, H., Nelson, D. M., Kaufman, P. D. and Adams, P. D. (2003). Defective S phase chromatin assembly causes DNA damage, activation of the S phase checkpoint, and S phase arrest. *Mol. Cell* **11**, 341-351.