Chapter 4
Epigenetic Regulation of Notch Signaling During *Drosophila* Development

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Abstract Notch signaling exerts multiple important functions in various developmental processes, including cell differentiation and cell proliferation, while mis-regulation of this pathway results in a variety of complex diseases, such as cancer and developmental defects. The simplicity of the Notch pathway in *Drosophila melanogaster*, in combination with the availability of powerful genetics, makes this an attractive model for studying the fundamental mechanisms of how Notch signaling is regulated and how it functions in various cellular contexts. Recently, increasing evidence for epigenetic control of Notch signaling reveals the intimate link between epigenetic regulators and Notch signaling pathway. In this chapter, we summarize the research advances of Notch and CAF-1 in *Drosophila* development and the epigenetic regulation mechanisms of Notch signaling activity by CAF-1 as well as other epigenetic modification machineries, which enables Notch to orchestrate different biological inputs and outputs in specific cellular contexts.

Keywords Notch · CAF-1 · Signal transduction · Gene expression · Epigenetic regulation · Chromatin assembly factors · Development · *Drosophila*

Introduction

The Notch pathway is an evolutionarily conserved signaling cascade present in most of multicellular organisms and plays important roles in development and physiology. Notch signaling regulates a variety of biological processes, including cell proliferation, differentiation, and apoptosis (Radtke et al. 2005). Mis-regulation of Notch signaling activity has been associated with various complex diseases, such as

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cancer and neurological disorders (Salazar and Yamamoto 2018). Understanding the mechanisms of signaling regulation, as well as the outcome of signaling in various tissues, is therefore of great importance. The existence of multiple paralogues of Notch receptor (Notch 1–4) and ligands (Delta 1–4 and Jagged 1–2) in mammals and other vertebrates makes Notch-related studies more complicated in those animals. However, the situation is much simpler in *Drosophila*, which has only one Notch receptor and two ligands, Delta (Dl) and Serrate (Ser). All of these three proteins share highly conserved sequences with their mammalian counterparts (Muskavitch 1994). The simplicity of the Notch signaling pathway in *Drosophila*, in combination with the availability of well-established powerful genetic tools and materials (Zacharioudaki and Bray 2014), makes *Drosophila* an extremely attractive system for studying Notch pathway. Recent studies in both *Drosophila* and mammals provide insights into the epigenetic regulation of Notch signaling, and this chapter summarizes the current understanding of how Notch signaling is epigenetically regulated, mainly by CAF-1.

Notch Signaling

A century ago, Notch was first described as a wing margin developmental defect phenotype in *Drosophila melanogaster* (Bray 2016; Ntziachristos et al. 2014). Notch locus was identified as a gene that is responsible for the notched wing phenotype (Welshons 1958a, b), which gives the name to the pathway. Notch gene encodes a single-pass type I transmembrane receptor, the extracellular domain of which includes a variable number of EGF-like repeats, with the functions of ligand binding (Fig. 4.1).

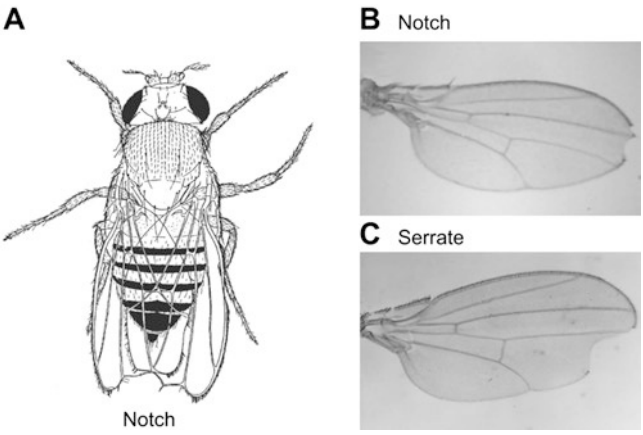
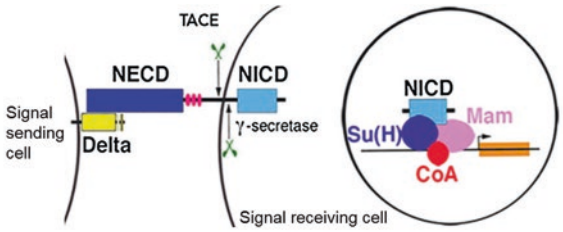


Fig. 4.1 Phenotype of *Drosophila* with Notch pathway mutations. (**a**) Drawing of a Notch receptor mutant fly with a notched wing tip. (**b**, **c**) Photo of a wing from a fly carrying a Notch mutation (**b**) and a mutation in Serrate (**c**). (Images are adapted from Alabi et al. (2018))



	Drosophila	Mammals
Ligand	Delta, Serrate	Delta 1-4, Jagged 1-2
Receptor	Notch	Notch 1-4
First cleavage	Kuzbanian, TACE	ADAM10, ADAM17
γ-secretase	Presenilin, Nicastrin, PEN2 and APH1	Presenilin1-2, Nicastrin, PEN2 and APH1
Effector	Notch intracellular domain (NICD)	Notch intracellular domain (NICD)
Transcriptional factor	Su(H)	CBF-1
Co-activator	Mastermind(Mam)	Mastermind(Mam) 1-3

Fig. 4.2 The core Notch signaling pathway and the main components of Notch pathway in *Drosophila* and mammals

Mutants with defects in other genes that are part of the Notch pathway were later identified because they had similar phenotypes and were named Delta (Dl) and Serrate (Ser) (Siren and Portin 1989; Shepard et al. 1989; Thomas et al. 1991). Dl and Ser are also transmembrane proteins and share the similar EGF repeats with Notch. Thus, in order for Notch signaling to occur, the ligand-expressing cells (or signal sending cells) have to be in intimate contact with the receptor-expressing cells (or signal receiving cells) (Fig. 4.2).

The canonical Notch signaling pathway is rather simple. While vertebrates have several Notch receptors and ligands, the *Drosophila* genome only contains one Notch receptor and two ligands, Delta (Dl) and Serrate (Ser) (Klueg and Muskavitch 1999). Like many other signaling pathways, Notch signaling is initiated by receptor-ligand interaction between neighboring cells with close contact or direct contact. The Notch receptor can be activated by binding with the ligands Dl or Ser that are expressed in adjacent cells. Upon activation by this intimate binding, the Notch receptor undergoes two consecutive cleavage events, which are catalyzed sequentially by an ADAM family metalloprotease (Kuz and Tace in *Drosophila*) (Alabi et al. 2018) and by the γ-secretase complex (containing Presenilin/Psn, Nicastrin/Nct, PEN2, and APH1) (De Strooper et al. 1999; Yang et al. 2019), resulting in the release of the intracellular portion of the protein, called the Notch intracellular domain (NICD), which then migrates into the nucleus and joins a protein complex directly bound to chromatin to initiate the transcription of target genes (Borggrefe and Oswald 2016). This complex includes the transcription factors Suppressor of Hairless (Su(H)), as well as other potential co-regulators, such as the transcriptional

coactivator Mastermind (Mam), thereby leading to the expression of Notch-dependent target genes (Borggrefe and Liefke 2012).

As we mentioned above, Notch signaling is an evolutionally conserved pathway throughout metazoans. Over time, it became clear that it is repeatedly employed in cell fate decisions, cell differentiation, cell proliferation, and cell survival in diverse contexts and at distinct stages of development (Bray 2016). In many developmental contexts, Notch specifies cell fate decisions. In the developing vertebrate eye, for example, Notch regulates which cells develop into glial cells and which develop into optic neurons (Genethliou et al. 2009). Not surprisingly, mutations leading to dysregulated Notch signaling have also been implicated in cancer, including hematological malignancies (Bugeon et al. 2011) and solid tumors (Mutvei et al. 2015). Mis-regulation of Notch signaling in ovarian follicle cells disturbs the balance between cell proliferation and cell differentiation in *Drosophila* oogenesis, leading to cell death and sterility (Deng et al. 2001; Lopez-Schier and St Johnston 2001; Palmer et al. 2014). In other developmental contexts, Notch regulates the survival of cells (Giraldez and Cohen 2003). For example, loss of Notch function results in increased cell death of neuron cells in the mouse nervous system (Mason et al. 2006). Notch signaling has also been associated with cell survival in B-cell malignancies, prostate cancer cells, and myeloma cells (Zhang et al. 2018; Nefedova et al. 2008; Zweidler-McKay et al. 2005).

Notch signaling must be under extremely tight control to keep its proper activity. Emerging evidence indicates that the regulation of Notch signaling seems to be considerately complicated; multiple levels of regulation are added to the pathway via receptor-ligand internalization, posttranslational modification, protein stability, and ligand availability (Kovall et al. 2017). Productive Notch ligand-receptor binding depends on the proper posttranslational modification, such as glycosylation of the receptor (Haines and Irvine 2003). The retention time of Notch and ligands on plasm membrane is determined by the endocytosis of the receptor and ligands (Kandachar and Roegiers 2012), mediated mainly by lysosomal degradation. Mutants that stabilize NICD can cause T-cell acute lymphoblastic leukemia in humans (Grabher et al. 2006). Polarity proteins, such as Numb (Song and Lu 2012) and Crumbs (Nemetschke and Knust 2016), are also required for local distribution of Notch in the plasm membrane, which results in region-specific Notch activity. Like Notch, Dl and Ser are also subject to transmembrane domain cleavage by the γ-secretase complex, with this process called ligand processing, which may be used to downregulate the activity of Notch pathway. Alternatively, ligand processing also could generate biologically soluble ligands that may act as antagonists of Notch signaling (Masuya et al. 2002). Although the mechanisms of signal transduction from the cell surface to the nucleus are relatively simple and clear, it is not fully understood how such a straightforward pathway can result in tremendously complex outcomes in different cellular contexts. Recent studies have revealed that epigenetic modifiers play important roles in regulating Notch activity and may provide a novel angle to explain how and why the various developmental outputs occur in different contexts by a single Notch signaling pathway.

The Advantages of *Drosophila* as Model Organism for Notch Signaling Study

Drosophila melanogaster is an ideal model organism and has been extensively used in scientific research for over 100 years since Professor Thomas H. Morgan (1866–1945), who won the Nobel Prize in Physiology or Medicine in 1933, started to use the *Drosophila* for genetic studies. Owing to several practical advantages that are suitable for laboratory study, *Drosophila* has significantly pushed forward the development of biological research in various fields, such as developmental biology, immunobiology, and metabolism (Mirth et al. 2019). First, *Drosophila* has a short life cycle (about 10 days in the laboratory conditions) and has high fecundity, which allow producing large number of progenies in a short time. Besides, it is relatively easy and cost-effective to maintain the stable *Drosophila* stocks. Second, *Drosophila* has a low number of chromosomes, which make it as one of the most studied organisms in biological research, particularly in genetics and developmental biology (Perrimon 2014; Tolwinski 2017). Notably, the genome sequencing of *Drosophila* reveals that approximately 75% of known human disease-associated genes have counterparts in *Drosophila* (McGurk et al. 2015; Chen and Crowther 2012). The high similarity and conservation in genomic features between *Drosophila* and human enables fly to benefit the biomedical studies of human diseases. Third, a large number of genetic tools are available for *Drosophila* researchers mostly through stock centers, such as the Bloomington Drosophila Stock Center (BDSC) and the Vienna Drosophila Resource Center (VDRC) (Zacharioudaki and Bray 2014; Housden et al. 2014). Large-scale mutagenesis and screen projects are easy to carry out to discover the novel components or novel regulators of a classic pathway. In particular, for those genes that are humongously lethal or semilethal when mutated, somatic or germline clonal analysis based on FRT recombination system would be a good choice. Other FRT system-derived methods, such as MARCM (mosaic analysis with a repressible cell marker) (Lee and Luo 2001), are further developed to create mutant cells in a wild-type background tissue, facilitating reduction of the inter-organismal variability when analyzing mutant versus wild-type tissues.

Notch and its ligands are broadly expressed in many tissues/organs in the *Drosophila*. Therefore, it is of great importance to develop tools to directly examine where the pathway is activated or inhibited. The current arsenal of genetic and biological tools makes *Drosophila* such a valuable model to study the fundamental principles of how Notch signaling transduces the signal and how it is regulated in different cellular contexts, which can deepen the understanding of its roles in physiological and pathological conditions in humans. Table 4.1 shows the commonly used biochemical (antibodies) and genetic (transgenic flies) tools for studying Notch signaling in vitro and in vivo.

Further genetic methods include (1) conditional gene expression and silencing with the Gal4-UAS system, (2) genome-scale bioinformatics analysis, (3) genomic tagging and disruption of genes using CRISPR/Cas9 genome editing for gene

Table 4.1 Commonly used tools for studying Notch signaling in *Drosophila*

Target gene reporters	
E(spl)mβ 1.5-lacZ	Enhancer trap of P-LacZ element in *E(spl)mβ*-locus
vg$^{[BE]}$-lacZ	Enhancer trap of P-LacZ element in *vg$^{[BE]}$-lacZ* locus
wg-lacZ	Enhancer trap of P-LacZ element in the *wg* locus
Cut lacZ	Enhancer trap of P-LacZ element in the *cut* locus
Antibodies	
Anti-Notch ICD	Recognizes amino acids 1791–2504 of Notch intracellular domain (C17.9C6, DSHB)
Anti-Delta	Recognizes amino acids 190–833 of Dl protein (C594.9B, DSHB)
Anti-Cut	Recognizes amino acids 1616–1836 of Cut protein (2B10, DSHB)
anti-Wg	Recognizes amino acids 3–468 of Wg protein (4D4, DSHB)
Anti-Hnt	Recognizes amino acids 824–1125 of Peb/Hnt (IG9,DSHB)
Tools to perturb or activate Notch pathway	
N^{1}	Loss of function of Notch
UAS-NICD	Express Notch intracellular domain under UAS control
UAS-Notch RNAi	RNAi targeting the Notch receptor
H^{1}	Loss of function of Hairless
Su(H)^{del47}	Loss of function of Su(H)
UAS-Dl	Expressing full length Dl under UAS control

All transgenic flies are available from Bloomington *Drosophila* Stock Center. DSHB indicates antibodies available from Developmental Studies Hybridoma Bank, University of Iowa

activation and inactivation (Yu et al. 2013a, 2014), and (4) advanced imaging technology, such as light-sheet microscopy (Lu et al. 2019). All these methods and tools are likely to further facilitate the use of this sophisticated model to better understand the Notch signaling.

It is worth mentioning that Guangzhou Drosophila Stock Center (GDSC), a newly established stock center for generating mutants through genome-wide gene targeting using CRISPR/Cas9 system, has generated more than 1000 mutant stocks. This resource would benefit a lot to those who use *Drosophila* as model for studying Notch signaling and other biological fields.

The Chromatin Assembly Factor CAF-1

Drosophila CAF-1 was first biochemically identified as a chromatin assembly factor about 30 years ago (Smith and Stillman 1989). *Drosophila* genome has three CAF-1-coding genes encoding three subunits, CAF-1 p180, CAF-1 p105, and CAF-1 p55, which correspond to human p150, p60, and p48, respectively (Ridgway and Almouzni 2000) (Table 4.2). Notably, there are two distinct CAF-1 complexes in *Drosophila*, each with three subunits of p180, p105, and p55 or p180, p75, and p55. Among them, p75 is a C-terminally truncated form of p105 in vivo.

Table 4.2 Evolutionarily conserved subunits of CAF-1 complex

Species	Large subunit	Medium subunit	Small subunit
Homo sapiens	p150	p60	p48
Mus musculus	p150	p60	p48
Drosophila melanogaster	p180	pl05	p55
Schmidtea mediterranea	p150	p60	p48
Caenorhabditis elegans	Chaf1	Chaf2	Rba1
Saccharomyces cerevisiae	Cac1	Cac2	Cac3
Arabidopsis thaliana	FAS1	FAS2	MSII

Though p105 and p75 have similar functions, p105 is predominantly expressed during embryogenesis, while p75 dominates after larval formation (Tyler et al. 2001).

CAF-1 has been biochemically well-characterized to be responsible for nucleosome assembly by guiding the histone trafficking and depositing them into chromatin by mediating H3 and H4 dimers onto newly synthesized DNA during DNA replication and DNA repair (Burgess and Zhang 2013). Reduction of CAF-1 activity in culture cells leads to reduced and delayed packaging of the DNA into chromatin, accompanied with DNA replication defects, S-phase arrest, checkpoint activation defects in cell cycle, and even cell death (Klapholz et al. 2009; Jiao et al. 2012; Krude 1995; Collins and Moon 2013). CAF-1 mutant mice exhibit developmental arrest at the embryonic stage with severe alterations in the nuclear organization of constitutive heterochromatin (Houlard et al. 2006). In *Drosophila*, knocking out any of the CAF-1 three subunits results in a similar lethal larval phenotype, and tissue-specific knockdown of CAF-1 p180 in the eye results in eye developmental defects, indicating the CAF-1 complex is also indispensable for the normal development in multicellular organism, including *Drosophila* (Jiao et al. 2012; Song et al. 2007; Huang et al. 2010; Yu et al. 2013b; Wen et al. 2012; Anderson et al. 2011).

However, genes encoding all three subunits CAF-1 are not essential in yeast (Kaufman et al. 1997; Monson et al. 1997; Enomoto and Berman 1998) and plants (Exner et al. 2006; Kirik et al. 2006; Endo et al. 2006; Schonrock et al. 2006), with the CAF-1 mutant in these species being viable, although mutants also exhibit some growth defects. The nonessential role of CAF-1 in unicellular eukaryotes (yeast) and plants appears to be inconsistent with the well-established role of CAF-1 in nucleosome assembly during DNA replication and DNA repair, an activity that might have been expected to be essential for all eukaryotic cells.

Interestingly, emerging evidence reported that CAF-1 p55, the small subunit of *Drosophila* CAF-1, not only functions in the CAF-1 complex but also is a component in several chromatin-modulating complexes, such as PRC1 (Jones et al. 1998) and NuRD (Campbell et al. 2018), indicating that CAF-1 may have multiple functional roles that are not restricted to acting as a histone chaperone. Moreover, it is reasonable to propose that CAF-1 may serve as a protein platform for chromatin metabolism that integrates epigenetic regulation cues of gene transcription by interacting with chromatin modification machinery or transcriptional factors (Yu et al. 2015).

Epigenetic Regulation of Notch Signaling by CAF-1

In a search for which signaling pathway is regulated by CAF-1 in *Drosophila* development, Yu et al. found that tissue-specific knockdown of the *Drosophila* CAF-1 p105, the medium subunit of CAF-1 complex, results in a notched wing phenotype, resembling that of Notch loss-of-function mutations (Yu et al. 2013b). Moreover, the notched wing phenotype could be enhanced by combination with loss of function of Notch, revealing a synergistic genetic interaction between CAF-1 p105 Notch signaling. This study also establishes a functional connection between CAF-1 complex and Notch signaling for the first time.

Similar to wing development, eye development defects caused by eye-specific knockdown of CAF-1 p105 are also significantly enhanced in heterozygous mutant backgrounds of several Notch-positive regulatory components such as Mam (Yu et al. 2013b). Altogether, these results suggest that CAF-1 p105 synergistically interacts with the Notch signaling pathway to regulate normal tissue development.

To further confirm that CAF-1 p105 is required for the normal activity of Notch pathway, Yu et.al generated a null allele of dCAF-1 p105, namely, *CAF-1 p105*^{36}, and performed clonal analyses in wing discs to investigate the effect of dCAF-1 p105 null mutation on Notch signaling, by examination of the developmental defects and the expression change of Notch target genes in the absence of CAF-1 p105. As expected, flies carrying *CAF-1 p105*^{36} clones exhibited a notched wing phenotype, and the protein expression level of *cut* and *wg*, two well-characterized Notch target genes, was also significantly decreased in *CAF-1 p105*^{36} clones, compared with control (Fig. 4.3). The transcriptional level of *cut* is also significantly downregulated when CAF-1 p105 is depleted. These results indicate that the activity of Notch signaling is compromised in the absence of CAF-1 p105 and thus CAF-1 p105 functions as a positive regulator of the Notch signaling pathway by promoting its target gene transcription.

As CAF-1 functions as a histone chaperone, it is likely that its reduction may trigger dilution of newly assembled nucleosomes at key enhancer elements and loosening of chromatin structure, resulting in a more accessible chromatin structure for efficient transcription factor binding to their target loci and activation of key target genes. However, the CAF-1 p105 specifically regulates the output of Notch signaling in the wing disc, since the Hedgehog (Hh) signaling is not affected in CAF-1 p105 mutant clone.

Further, it is found that CAF-1 forms a functional complex with NICD and Su(H), the core transcriptional factor for Notch target gene expression. And this complex directly binds to the enhancer region of one of the Notch target genes, *E(spl)mβ*. The occupancy of Su(H) at Notch target genes is highly increased to efficiently initiate gene transcription when Notch signaling is activated. In the absence of CAF-1 p105, the abundance of Su(H) at the *E(spl)mβ* enhancer region is dramatically decreased, proposing that CAF-1 probably regulates Notch target gene expression, at least in part, by controlling the accessibility and binding abundance of Su(H) to their enhancer regions.

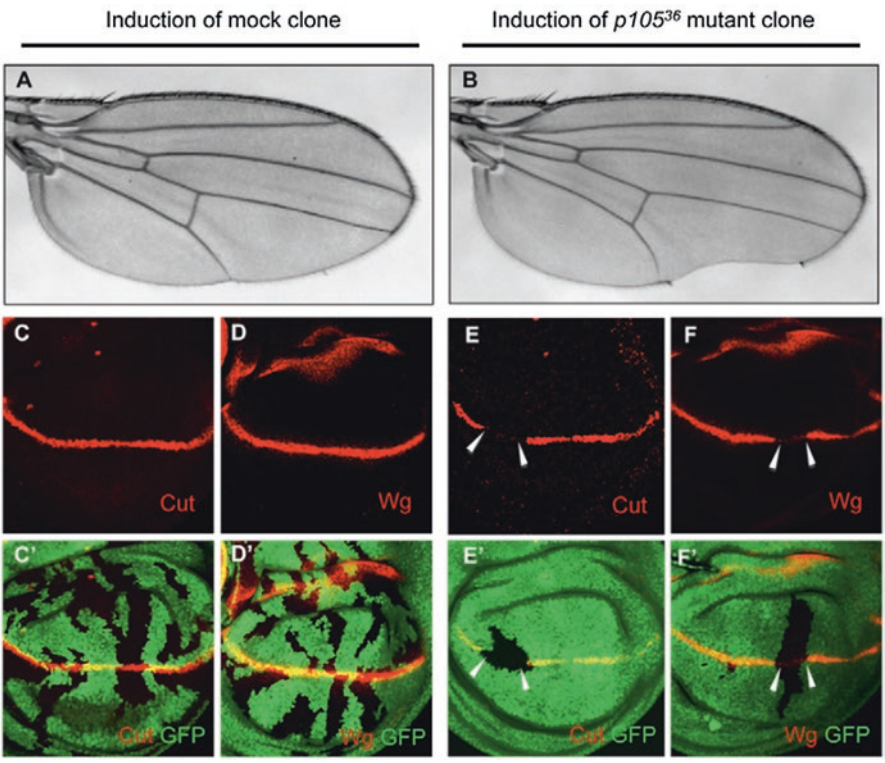


Fig. 4.3 CAF-1-p105 is required for the normal wing development and proper expression of Notch target genes cut and wg. (**a**, **b**) Induction of *CAF-1 p105*^{36} mutant clones leads to a notched wing (**b**), whereas induction of mock clones leads to wild-type wings (**a**). (**c**–**f**′) In *CAF-1 p105*^{36} mutant clones, the expression of Cut (**e**, **e**′, GFP-negative area, arrowheads) and Wg (**f**, **f**′, FP-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. (Images are adapted from Yu et al. (2013b))

It is reported that CAF-1 could act as a chromatin platform that is permissive for transcription by regulating histone modifications by forming complex with other chromatin remodeling complexes (Yu et al. 2015), and histone H4 acetylation is believed to be associated with active promoters of Notch target genes (Giaimo et al. 2018). As expected, H4ac level in the *E(spl)mβ* enhancer region is significantly reduced in CAF-1 p105 mutant flies. These results reveal that CAF-1 p105 promotes Notch target gene expression by maintaining a high level of histone H4 acetylation in the enhancer region of the Notch target genes to establish a local active chromatin structure. Interestingly, CAF-1 function in regulating Notch signaling is dependent on its integrity as a triple subunit complex. Knockdown of any component of CAF-1 complex causes the reduction of *cut* expression and the notched wing phenotype in *Drosophila* (Yu et al. 2013b). However, there are still open questions for how CAF-1 directs the H4 acetylation modification. One possibility is that

CAF-1 recruits histone acetylation machinery, such as p300/Nejire, directly or indirectly, to change the landscape of local chromatin modification, thus enhancing target gene transcription. CAF-1 has functions beyond its classic role in histone assembly and the newly established positive role in Notch signaling in wing development and plays an essential role in proliferating cells.

However, in contrast to the positive role of CAF-1 on Notch target gene expression in wing development, a recent study reported that CAF-1 play a negative role in regulating Notch signaling in *Drosophila* ovarian mitotic follicle cells (Lo et al. 2019). Loss of function of either CAF-1 p105 or CAF-1 p180 caused the increased activation of Notch signaling target genes in *Drosophila* ovarian follicle cells. Further, Notch is functionally responsible for these phenotypes observed in both the CAF-1 p105- and CAF-1 p180-deficient follicle cells. It is still unclear how CAF-1 p180 and CAF-1 p150 suppress Notch target gene expression in mitotic follicle cells. It is likely that CAF-1 have physical interaction with Su(H), which is known to be involved in maintaining the repressive chromatin status for inhibiting Notch target gene expression when it is associated with other repressive subunits, such as Hairless, Gro, or CtBP (Yu et al. 2015; Cheloufi and Hochedlinger 2017; Yuan et al. 2016). Thus, the molecular basis for that CAF-1 play a dual role to sustain cell proliferation positively (in imaginal discs) or negatively (in ovarian follicle cells) may lie in that CAF-1 recruits different histone modification machineries in imaginal discs and follicle cells to regulate *Drosophila* Notch signaling in a tissue context-dependent manner.

Two recent studies in mammals confirmed the negative role of CAF-1 in retrotransposon jumping and gene expression. Hatanaka et al. reported that CAF-1 mediates repressive histone modifications to protect preimplantation mouse embryos from endogenous retrotransposons (Hatanaka et al. 2015). Multiple classes of retrotransposons are derepressed in morula embryos when CAF-1 is depleted, likely through affecting the histone methylation status, thus influencing local chromatin accessibility. The other study found that the p150 and p60, two subunits of mammalian CAF-1 complex, are the most prominent chromatin-modulating factors during transcription factor-mediated reprogramming of mouse fibroblasts to induced pluripotent stem cells (iPS cells) (Cheloufi et al. 2015). Suppression of CAF-1 leads to a more accessible chromatin structure at enhancer elements and the increased binding of Sox2 to pluripotency-specific targets and activation of associated genes during reprogramming (Cheloufi et al. 2015).

Altogether, CAF-1 functions not only as histone chaperone for nucleosome assembly but also as an epigenetic regulation switch for regulating Notch signaling target gene expression in response to integrated proliferation and differentiation signals during animal development. However, CAF-1 does not harbor the histone modification enzyme activity; thus it is likely that CAF-1 works together with the histone modification machinery (histone methylation, histone acetylation, etc.) to regulate Notch signaling activity at the chromatin level through modifying the chromatin structure.

Epigenetic Regulation of Notch Signaling by Other Epigenetic Regulators

In addition to the epigenetic regulation of Notch signaling by the CAF-1 complex, this paragraph briefly summarizes the epigenetic regulation of Notch signaling by other epigenetic regulators. Several epigenetic regulators that are involved in Notch signaling are listed in Table 4.3. Among them, histone acetylation and methylation are main executors for epigenetic regulation of gene transcription (Tchasovnikarova and Kingston 2018).

Table 4.3 Several epigenetic regulators that are involved in Notch signaling

Epigenetic regulators	Molecular activity	Functions	References
UTX	H3K27me3 demethylase	Negatively regulate Notch signaling	Herz et al. (2010)
SIRT1	H4K16 deacetylase	Negatively regulates Notch signaling	Mulligan et al. (2011)
LSD1/KDM1A	H3K4 demethylase	Negatively regulates Notch signaling	Mulligan et al. (2011); Lopez et al. (2016)
CoREST	CoREST complex	Negatively regulates Notch signaling	Lopez et al. (2016)
CoREST	CoREST complex	Positively regulates Notch signaling	Domanitskaya and Schupbach (2012)
HDAC1	Histone deacetylase	Positively regulates Notch signaling	Wang et al. (2018); Mao et al. 2017)
HDAC1	Histone deacetylase	Negatively regulates Notch signaling	Kao et al. (1998); Cunliffe (2004); Yamaguchi et al. (2005); Wu et al. (2016)
Kdm5A	H3K4 demethylase	Negatively regulates Notch signaling	Liefke et al. (2010); Dreval et al. (2019)
Brms1	Histone deacetylase	Positively regulates Notch signaling	Zhang et al. (2014)
Tet2/3	Methylcytosine dioxygenases	Positively regulates Notch signaling	Li et al. (2015)
Nipped-A	SAGA and Tip60	Positively regulates Notch signaling	Gause et al. (2006)
BAP55	SWI/SNF Complex	Positively regulates Notch signaling	Pillidge and Bray (2019)
p300	Histone acetyltransferase	Positively regulates Notch signaling	Franz Oswald et al. (2001)
Tip60	Histone acetyltransferase	Positively regulates Notch signaling	Medgett and Langer (1984)
Nurf 301	NURF complex	Positively regulates Notch signaling	Kugler and Nagel (2010)
Pc	PRC1 complex	Positively regulates Notch signaling	Saj et al. (2010); Tolhuis et al. (2006)

Histone deacetylases are generally associated with transcriptional repressor complexes, such as Sin3 (Barnes et al. 2018), NuRD (Feng and Zhang 2003; Ahringer 2000), and CoREST (You et al. 2001; Domanitskaya and Schupbach 2012) complexes, and have regulatory functions in various signaling pathways. It is generally accepted that HDAC1 forms a transcriptional corepressor complex to modify chromatin structure for target gene silencing. For example, HDAC1 physically interacts with CBF1 (homolog of Su(H) in *Drosophila*), and treatment of HDAC1 inhibitor derepresses Notch target gene *ESR-1* expression in mammalian cells (Kao et al. 1998). In zebrafish, *her4* and *her6*, two of Notch target genes, are upregulated in HDAC1 mutant fish (Cunliffe 2004; Yamaguchi et al. 2005). Furthermore, overexpression of HDAC1 represses the expression of Notch target gene *Hey2* in mice (Wu et al. 2016). In these contexts, HDAC1 negatively regulates Notch signaling. Unexpectedly, opposite to the inhibitory role of HDAC1 in Notch signaling, knockdown of HDAC1 causes a notched wing phenotype and reduces Notch target gene expression in *Drosophila* (Wang et al. 2018), suggesting a positive role of HDAC1 in regulating the Notch pathway during *Drosophila* wing development, although the molecular mechanism behind this remains largely unknown. It is highly possible that HDAC1 directly regulates histone deacetylation status at the Notch target gene locus. Notably, a recent study reported that HDAC1 could activate the Notch signaling pathway to promote metastasis in a similar way (Mao et al. 2017).

The complicated regulation network by other epigenetic regulators, such as LSD1 (Mulligan et al. 2011; Lopez et al. 2016), Brms1 (Zhang et al. 2014), histone acetylase p300 (Franz Oswald et al. 2001), Brahma SWI/SNF chromatin remodeling complex (Pillidge and Bray 2019), UTX (Herz et al. 2010), H3K4 demethylase Kdm5A (Liefke et al. 2010; Dreval et al. 2019), methylcytosine dioxygenases Tet2/3 (Li et al. 2015), SAGA and Tip60 complex (Gause et al. 2006; Medgett and Langer 1984), PcG-TrxG complex (Saj et al. 2010; Tolhuis et al. 2006), Putzig-NURF complex (Kugler and Nagel 2010), and many others, may directly influence Notch-mediated gene transcription activity at the chromatin level and thus explain, at least in part, the pleiotropic effects of Notch in the complex biological processes that affect cell growth, differentiation, and cell death.

Conclusion

The Notch signaling pathway is a highly conserved molecular network that, depending on the cellular context, acts through the regulation of cell proliferation, differentiation, and apoptosis. In order to better control the expression of Notch target gene expression, the Notch signaling must be precisely regulated at different steps in a series of developmental events. Epigenetic regulation of Notch signaling by CAF-1 and other epigenetic regulators plays essential roles in fine-tuning the transcriptional output of Notch signaling to coordinate multicellular organism development. It remains an open question as to why and how different epigenetic regulators are

involved in mediating different histone modifications status, leading to different transcriptional outputs of either gene repression or gene activation in one specific signal transduction pathway.

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