Protein acetylation as a metabolic switch for *de novo* lipogenesis in *Drosophila* development

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Abstract

Lipogenesis is tightly regulated during animal growth and development. Despite the well-established transcriptional regulation of lipogenic genes (e.g., fatty acid synthase, FASN), it remains largely unclear how post-translational modifications (PTMs) of these lipogenic enzymes modulate lipogenesis and contribute to the regulation of metabolic homeostasis. With a FLAG-tag knock-in fly line generated using CRISPR-Cas9 system, we observed an intriguing dynamics of the acetylation of endogenous *Drosophila* FASN1 protein, the rate-limiting enzyme in *de novo* lipogenesis, during larval development. The acetylation of FASN1 proteins varies with developmental stages and peaks at 96h after egg laying (AEL), which is positively correlated to the FASN1 enzymatic activity and fast larval growth. Mass-spectrometry analysis identified two evolutionarily conserved lysine residues that are acetylated in both fly and human FASN proteins. One of the lysine residues, K813, is located nearby the active site of the malonyl/acetyltransferase (MAT) domain. Acetylation of K813 is predicted to enlarge the binding pocket of MAT domain to allow fast substrate loading. Indeed, lysine to arginine substitution mutants (K813R, acetylation deficiency mutants) show decreased lipogenesis, reduced body weight, and delayed pupariation. Lastly, we identified a deacetylase, Sirt1, as the key negative regulator of FASN1 acetylation and *de novo* lipogenesis. Our epistasis analysis suggests that the repression of Sirt1-mediated deacetylation at 3rd instar stage might be responsible for the increased FASN1 acetylation and *de novo* lipogenesis in fast growing *Drosophila* larvae. Taken together, our results reveal a novel role of lysine acetylation in modulating pocket conformation of MAT domain of FASN1 protein and promotes its catalytic activity by enhancing substrate loading. Since acetyl-CoA is the key metabolite linking lipogenesis and acetylation, our findings further indicate that lysine acetylation may act as a fine tune mechanism to modulate enzymatic activity in responding to fluctuated metabolite availability during animal growth and development.

Results

▶ Generation of FASN1 knock-in flies (*FASN1*$^{FLAG\#54}$)

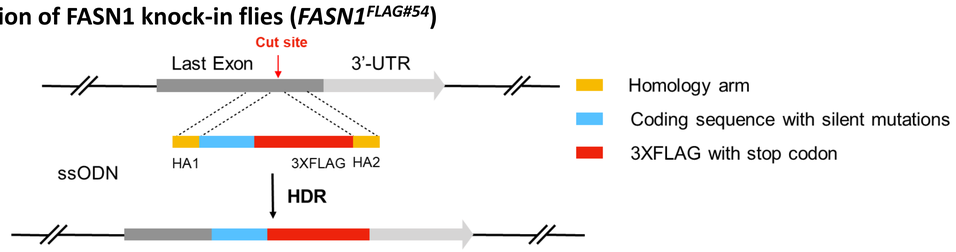


Figure 1 Diagram showing the generation of FASN1 knock-in flies using CRISPR/Cas9-mediated HDR and ssODN. The Cas9 cut site targeted the last exon and was close to the stop codon.

▶ Acetylation of FASN1 peaks at L3 and wandering stage, which is positively correlated to the FASN1 enzymatic activity and triglyceride level; while FASN1 protein level does not change significantly in larval development

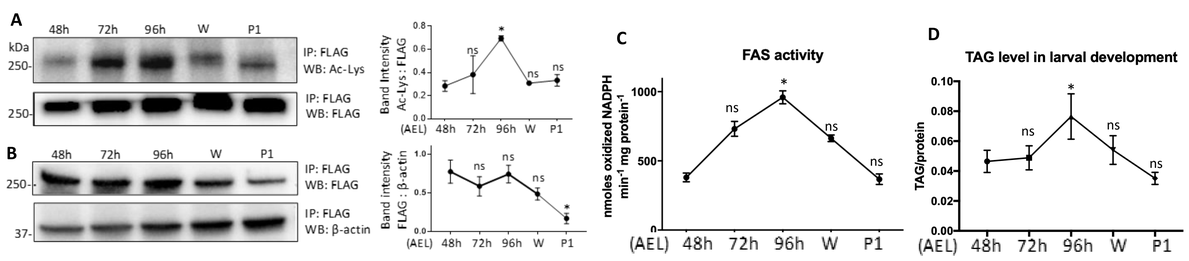
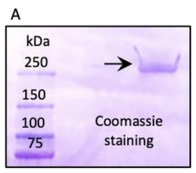


Figure 2 Western blots showing the levels of acetylated FASN1 **(A)** and total FASN1 protein **(B)** at different developmental stages. Quantification of band intensity is shown in the right panels. N=2. Fatty acid synthase activity **(C)** and total triglyceride (TAG) level **(D)** at different development stages. N=6. W: Wandering larvae. P1: day 1 pupa. One-way ANOVA. Multiple comparisons with 48h. * p<0.05, ns: not significant.

▶ Identification of lysine acetylation sites in FASN1

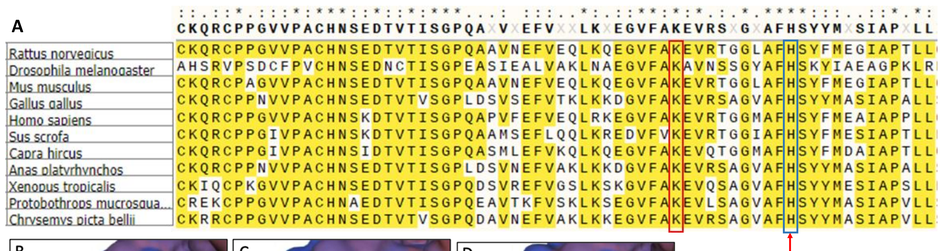


B

Acetylated Peptides	Domain	Posision
K.LK#DSDLENFDQQFFGVHQK.Q	KS	K193
K.VDNALVAGAGLILK#PTM*SLQFK.R	KS	K333
K.LNAEGVFAK#AVNSSGYAFHSK.Y	MAT	K813
R.ALGPDATNLSLVK#R.G	MAT	K926
K.K#LELADGYQPTLK.L	TE	K2466
R.FLEIGK#FDLSNNSPLGM*SVFLK.N	ER	K1800
K.DLISNVLK#NGAWGTFR.H	DH-ER	K1535
R.TNTDGFKEQGITYPIGK#M*QNR.L	KS	K407

Figure 3 **(A)** Coomassie staining of immunoprecipitated FASN1-FLAG protein. Arrow indicates the gel band used in mass-spec analysis. **(B)** The position of the FASN1 acetylated lysine residues by mass-spec.

▶ A conserved acetylation site K813 in MAT domain is hypothesized to alter the opening of malonyl-CoA binding pocket



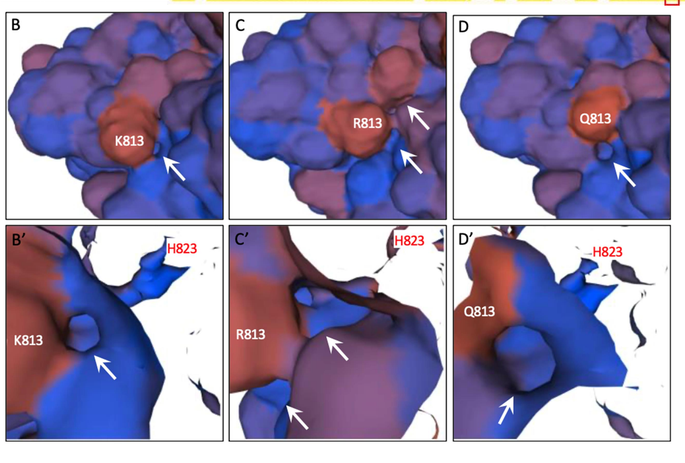


Figure 4 **(A)** Alignment showing the conserved lysine acetylation site K813 (*Drosophila*) in MAT domain. The asterisk indicates the active site H823 (*Drosophila*) of MAT domain. **(B-D)** *In silico* modeling (SwissDock, surface view) of the malonyl-CoA binding pocket (white arrow) in the MAT domain of wild-type (K813), acetylation dead mutant (R813) and acetylation mimic mutant (Q813). Amino acids at 813 position are indicated in orange. **(B'-D')** Zoom-in view of the pockets in Panels B-D.

▶ Acetylation dead mutants *FASN1*^{K813R} show delayed pupation, declined TAG level, reduced pupal size and decreased FASN1 enzyme activity

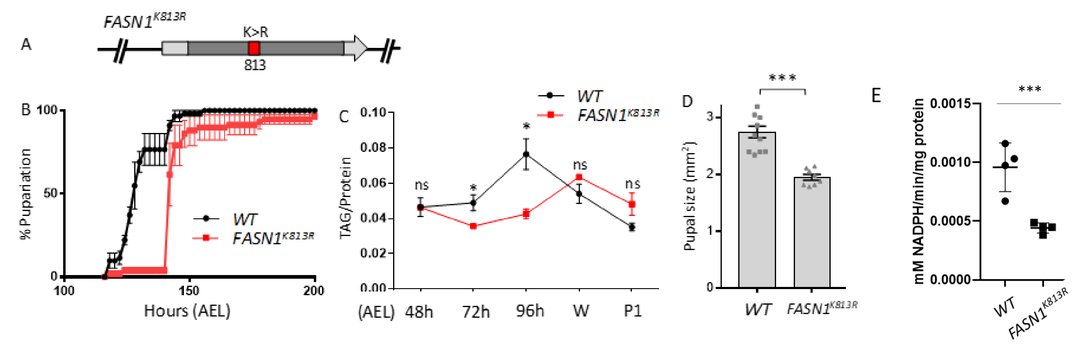


Figure 5 **(A)** Diagram showing Drosophila *FASN1*^{K813R} allele. **(B)** Developmental timing of WT and *FASN1*^{K813R} mutants. **(C)** Stage-specific TAG levels in both WT and *FASN1*^{K813R}. N=3. **(D)** Pupal size of WT and *FASN1*^{K813R}. N=10. **(E)** Fatty acid synthase activity of WT and *FASN1*^{K813R}. N=3. T-test, *p<0.05, *** p<0.001, ns: not significant.

▶ FASN1 acetylation is regulated by deacetylase *Sirt1*

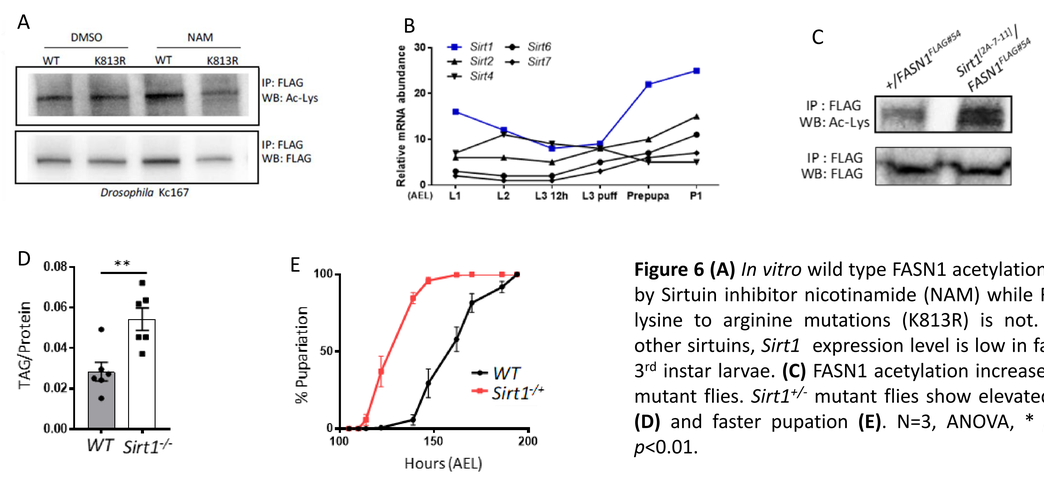


Figure 6 **(A)** *In vitro* wild type FASN1 acetylation is induced by Sirtuin inhibitor nicotinamide (NAM) while FASN1 with lysine to arginine mutations (K813R) is not. **(B)** Unlike other sirtuins, *Sirt1* expression level is low in fast growing 3rd instar larvae. **(C)** FASN1 acetylation increases in *Sirt1*$^{+/-}$ mutant flies. *Sirt1*$^{+/-}$ mutant flies show elevated TAG level **(D)** and faster pupation **(E)**. N=3, ANOVA, * p<0.05, ** p<0.01.

▶ *Sirt1* may regulate site-specific lysine acetylation of *Drosophila* FASN1 at K813

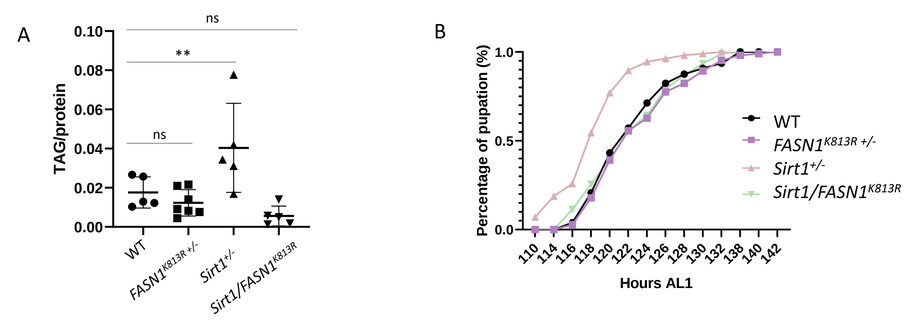


Figure 7 Epistasis analysis show rescue of TAG level **(A)** and developmental timing **(B)** in *Sirt1/FASN1*^{K813R} double mutants. N=3, ANOVA, * p<0.05, ** p<0.01, ns: not significant. AL1: after first instar larvae L1.

▶ Working model

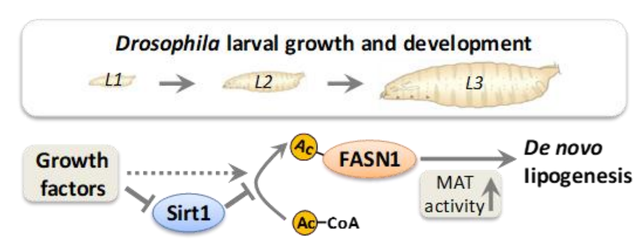


Figure 8 Proposed model for acetylation-regulated developmental *de novo* lipogenesis in *Drosophila*. Decreased expression of *Sirt1* at 3rd instar larval stage results in elevated acetylation at specific lysine residues of FASN1, which alters pocket structure of the MAT domain and promotes its catalytic activity through enhancing substrate loading. Ac: acetyl group. L1-L3: 1st to 3rd larval instar stage.

▶ Future plans

- To examine MAT domain activity and malonyl-CoA loading of both wild type FASN1 and FASN1 with lysine to arginine mutations (K<R and K<Q);
- To determine whether Sirt1 physically binds to FASN1
- …

Acknowledgements and references

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References:

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