농 학 석 사 학 위 논 문

초파리 *eggless* mutant에서 과발현되는 Dpp에 의한 난소 내 세포사멸

Depletion of Eggless may induce ovarian apoptosis through Dpp overexpression during early oogenesis in *Drosophila*.

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최 유 림

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Depletion of Eggless may induce ovarian apoptosis through Dpp overexpression during early oogenesis in *Drosophila*.

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A thesis submitted to the faculty of the Seoul National University Graduate School in partial fulfillment of the requirement for the degree of Master of Science in the Department of Agricultural Biotechnology.

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ABSTRACT

Depletion of Eggless may induce ovarian apoptosis through Dpp overexpression during early oogenesis in *Drosophila*.

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Fate of germline stem cell (GSC) is determined by precise and exquisite balance between self-renewal and differentiation. In *Drosophila* ovarian niche, Decapentaplegic (Dpp) plays an essential role in GSC self-renewal and proliferation by suppressing the transcription of differentiation factor *bag of marbles* (*bam*). Despite its role in maintaining GSC population, recent studies have revealed that excessive Dpp activity triggers apoptosis in certain developmental contexts. In this study, I show that inordinate *dpp* expression exacerbate defects in ovarian development by inducing apoptosis in *eggless*

mutants. Mutant females lacking Eggless function exhibited early oogenesis arrest, which led to ovary degeneration. Overall expression of apoptotic genes was increased in *eggless* mutant ovary. Interestingly, expression of key BMP signaling factor *dpp* was also drastically increased in the mutant ovary. Moreover, in ovarian somatic cells (OSCs), *dpp* overexpression stimulated expression of a pro-apoptotic gene *reaper* and reduced the cell viability. These results suggest that aberrant *dpp* activation is partly responsible for oogenesis arrest by promoting apoptosis in *eggless* mutant ovary, which leads to defective ovary development.

Key words: Eggless, dSETDB1, Decapentaplegic, Reaper, Ovarian apoptosis, Ovary development, Ovarian somatic cell

***Student number*: 2012-21188**

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INTRODUCTION

In *Drosophila* ovary, germline stem cells (GSCs) divide asymmetrically to generate two different types of daughter cells, GSC and cystoblast [1]. The self-renewing property of GSCs ensures female flies to continuously produce mature eggs and thereby to be fertile throughout life. Fate of GSCs is controlled by precise and exquisite balance between self-renewal and differentiation. Numbers of factors establish complex regulatory network for GSC fate determination. In the center of this network, the stem cell niche plays an instructive role by offering bone morphogenetic protein (BMP) signals [2-4].

The *Drosophila* ovary provides a principal model system for studying stem cell biology. *Drosophila* ovary consists of progressively developing eggs [1, 5]. An ovary has ~15 ovarioles and each ovarioles hold a germarium at the foremost region. Germarium is a functional unit of the ovary where the earliest phase of oogenesis takes place. At the apical side of the germarium, cap cells and escort stem cells (ESCs) surround GSCs, and thereby form niche [6]. This specialized regulatory microenvironment produces extracellular signals participating in stem cell maintenance [2, 3].

Decapentaplegic (Dpp) is a key signaling molecule secreted from the niche. Dpp is a member of bone morphogenetic proteins which belongs to the

mammalian transforming growth factor-β (TGF-β) superfamily [7]. As a morphogen, Dpp functions in various developmental processes. It governs polarization of dorsal-ventral [8, 9] and distinction of boundary between segmental compartments [10-12]. As a niche signaling factor, Dpp is essential for GSC maintenance [6, 13]. Once Dpp binds to its heterodimeric receptor Tkv and Punt, a signal transduction cascade initiates within GSC, leading to phosphorylation of Mad [14]. Phosphorylated Mad moves into nucleus along with Medea. Then this complex suppresses the transcription of *bag of marbles* (*bam*), a decisive differentiation factor [15, 16]. In this way, Dpp ensures GSC self-renewal and proliferation rather than differentiation.

Control of GSC identity is crucial for maintaining ovary homeostasis. As part of GSC regulatory system, Dpp signaling is spatially restricted over a short range within the niche [17, 18], unlike other Dpp-dependent developmental processes [19]. In ovarian niche, JAK-STAT signaling permits cap cells and ESCs to produce and secrete Dpp [20, 21]. Vkg and Dally capture this extracellular ligand, thereby restrict Dpp distribution to GSCs. Then Dpp binds to its receptor Tkv and Punt [22], activating GSC self-renewal system. Since Dpp signaling acts only one-cell diameter away from the niche, cystoblasts outside the niche receive low Dpp signal and initiate differentiation [23].

While the absence of Dpp leads to cell death in various developmental

processes [6, 24], recent studies have shown that ectopic Dpp activity also induces apoptosis in particular developmental processes. For example, excessive Dpp signaling results in dTAK1- and dJNK-dependent cardial apoptosis in *raw* mutant flies [25]. *Drosophila Lsd1* mutants also show ovarian apoptosis accompanied by increased *dpp* expression [26]. Further, sharp rise of Dpp level triggers local cell death both during wing development [27] and leg morphogenesis [12].

Drosophila eggless encodes an H3K9 methyltransferase required for maintenance of trimethylated H3K9 in GSCs [28]. Eggless is conserved in human protein SETDB1 and its mouse ortholog ESET (Fig. 1A) [29, 30]. The Eggless-dependent histone methylation has been identified to be important in ovary development by controlling GSC self-renewal and differentiation [31].

In the present study, I show that absence of Eggless caused developmental defects in ovary which is attributable to an oogenesis arrest. Further results indicate that *dpp* expression was activated and apoptotic genes were derepressed in *eggless* mutant ovary. Based on the findings that *dpp* overexpression led to derepression of pro-apoptotic gene *reaper* and induction of cell death in OSCs, I conclude that aberrant *dpp* activation is partly responsible for oogenesis arrest by promoting apoptosis in *eggless* mutant ovary, which results in defective ovary development.

MATERIALS AND METHODS

Drosophila strains

The strains used in this study include a wild-type w^{1118} and an *eggless* mutant egg^{2138}/*Df(2R)Dll-Mp*. All fly strains were provided from Bloomington *Drosophila* Stock Center. Flies were maintained at 24℃ on molasses-based media supplied with live yeast.

Preparation of *Drosophila* total RNA and cDNA synthesis

Ovaries were dissected from 2- to 3-day-old *Drosophila* females and frozen in liquid nitrogen. Total RNAs were extracted using TRIzol® reagent (Invitrogen). Isolated RNAs were treated with DNAse I (Takara) and further purified by acid phenol chloroform.

cDNAs were synthesized from 1ug of DNAse-treated total RNAs by reverse transcription (RT) reaction with SuperScript Ⅲ (Invitrogen). 2.5 μM oligo-$(dT)_{20}$ primers, 2.5 μM random hexamers, 0.5 mM each of dNTP, 10X reverse transcription buffer, 5 mM $MgCl_2$, 0.01 M DTT, 40 units/μl RNase-OUT™ and 200 units/μl SuperScript® III reverse transcriptase were used in 20 μl of RT reaction for 60 min at 50℃, 10 min at 25℃ and 5 min at 85℃.

Quantative Real-time PCR

Relative mRNA levels were quantified by LightCycler® 480 Real-Time PCR System (Roche). Each PCR reactions were carried out with 10 µl of PCR reaction: 5 µl of the LightCycler® 480 SYBR Green I Master (Roche), 0.25 µM of gene specific primers and 20 ng cDNA were used for each reaction. qRT-PCR was run at 95℃ for 5 min followed by 45 cycles at 95℃ for 10 sec, 58℃ for 10 sec and 72℃ for 10 sec. Results were normalized to *Drosophila* reference gene *ribosomal protein 49* (*rp49*). Gene expression levels were calculated using the comparative Ct method [32]. All experiments were carried out using three biological replicates with three replicates each. The gene specific primers used for qRT-PCR are as follows:

rp49 forward: 5'-CCGCTTCAAGGGACAGTATCTG

rp49 reverse: 5'-ATCTCGCCGCAGTAAACGC

eggless forward: 5'-GGCAACCTTGGACGCTATTT

eggless reverse: 5'-AACGCAGGTCGTGAGTATCC

dpp forward: 5'-GGCTTCTACTCCTCGCAGTG

dpp reverse: 5'-TGCTTTTGCTAATGCTGTGC

dally forward: 5'-ACCGAAATCATGAACCATCTG

dally reverse: 5’-CAAAGAAGTGCCTGACAGCCT

bam forward : 5’-GACCGAAAGCCACAAGTCG

bam reverse: 5’-GGACCTCCCAGTTTGTATCTCTG

p53 forward: 5’-CTTCAAGTTCGTCTGCCAAAA

p53 reverse: 5’-GCGCTTCTTGCTATTGAGCTG

chk-2 forward: 5’-GCTGCTGATCAACCAAATGCT

chk-2 reverse: 5’-GTGGCTCAAGGAAGTTCTCCT

reaper forward: 5’-GAGCAGAAGGAGCAGCAGAT

reaper reverse: 5’-GGACTTTCTTCCGGTCTTCG

hid forward: 5’-TCATCTTCGTCCTCCGCATC

hid reverse: 5’-CATCCTGACCCACTCGTAGA

Ovarian somatic cell (OSC) culture

OSC cell line was a kind gift from Saito, K. Cells were grown at 26℃ in Shields and Sang M3 Insect Medium (SIGMA) supplied with 0.6 mg/ml glutathione, 10% FBS, 10mU/ml insulin and 10% fly extract. Fly extract was prepared as described previously [33].

***dpp* overexpression in OSCs**

The full length of *dpp* cDNA was PCR-amplified from *Drosophila* ovarian total cDNAs with gene specific primers. This *dpp* cDNA was tagged by Flag

epitope (MDYKDDDDK) at the N-terminus and was inserted between EcoRI and XhoI site of pAc5.1/V5-HisA (Invitrogen), which generated pAc5.1-Dpp, a Dpp expression vector. Primers used are as follows:

dpp cloning forward: 5'-GAAGAATTCATGGACTACAAAGACGATGAC GATAAAATGCGCGCATGGCTTCTACTCCT

dpp cloning reverse: 5'-TTCCTCGAGCTATCGACAGCCACAGCCCACC AC

For *dpp* overexprssion in OSCs, 3×10^6 trypsinized OSCs were resuspended in 100 μl of Solution V of the Cell Line Nucleofector Kit V (Amaxa Biosystems) and mixed with 5 μg of pAc5.1/V5-HisA or pAc5.1-Dpp. Transfection was conducted in an electroporation cuvette using Nucleofector instrument (Amaxa Biosystems). The transfected cells were transferred to fresh OSC medium and incubated at 26 °C. After 48hr incubation, the cells were treated with TRIzol and total RNAs were extracted as described above.

Cell viability assay (MTS assay)

Cell viability was measured by using CellTiter 96 Aqueous One Solution kit (Promega). 3×10^6 trypsinized OSCs were transfected with 5 μg of pAc5.1/V5-HisA or pAc5.1-Dpp as described above. The transfected cells were seeded in

96-well plates and incubated at 26 °C. After 48hr incubation, the medium were changed with 10% M3/FBS medium. Then 20 μl of MTS solution was added to each well and cells were incubated at 26'C for 3 hrs. Absorbance at 490 nm was measured by Microplate Reader (Tecan). All experiments were carried out using three biological replicates.

RESULTS

Loss of Eggless causes developmental defects in the ovary

Eggless gene is located on the right arm of *Drosophila* chromosome 2 at position 60E1. *Eggless* gene encodes a H3K9 methyltransferase consisting of two tudor- like domains, a methyl DNA binding domain, a pair of SET domains, a Pre-SET domain and a Post-SET domain (Fig. 1A) [34]. In this study, two *eggless* alleles, egg^{2138} and *Df(2R)Dll-Mp* were used. The egg^{2138} encodes a truncated protein resulted from deletion of the SET domains and part of the second tudor domain (Fig. 1B) [31]. *Df(2R)Dll-Mp* is a 9 kb deletion that removes the entire *eggless* gene (Fig. 1C) [28]. Two types of *eggless* heterozygous mutants, egg^{2138}*/GCyO* and *Df(2R)Dll-Mp/GCyO*, were crossed with each other. From this crossing, I obtained two heterozygous mutant combinations (egg^{2138}*/GCyO* and *Df(2R)Dll-Mp/GCyO*), as well as one transheterozygous mutant combination (egg^{2138}*/Df(2R)Dll-Mp*) (Fig. 2A). I refer to egg^{2138}*/Df(2R)Dll-Mp* transheterozygote as *egg* mutant for brevity.

While *egg* heterozygous mutants were fertile, *egg* mutant females were unable to lay eggs (Fig. 2A). Necessarily, this inability is accompanied by sterile phenotype. As expected, these mutant phenotypes have a close correlation with *eggless* transcript level (Fig. 2C). To further investigate the contribution of

Eggless to fecundity of female flies, I examined morphological characteristics of ovaries dissected from 2-3 day old female flies. *Egg* heterozygous mutants exhibited normal progression of ovary development like as wild-type (Fig. 2B). In contrast, *egg* mutant showed a severe phenotype that the ovary was markedly reduced in size. Egg chambers also degenerated in *egg* mutant, which implies oogenesis arrested in germarium at stage 2 (Fig. 2B) [28]. Taken together, these observations indicate that disruption of Eggless leads to defects in ovary development via oogenesis arrest.

Loss of Eggless increases *dpp* expression

BMP signaling plays a central role in GSC self-renewal and proliferation [4, 6, 13, 16]. The failure of GSC maintenance causes germ cell loss and thereby leads to oogenesis arrest [6]. Thus, the oogenesis arrest observed in *egg* mutant provides evidence that BMP signaling is disturbed in this mutant. Recently, it has been shown that GSCs are unable to differentiate in soma-specific *eggless* knockdown mutants [35], indicating that oogenesis defects arise in non-autonomous manner in *egg* ovary.

Dpp is a niche-associated extracellular signaling factor which is responsible for GSC self-renewal and proliferation as a key BMP ligand. Since defective phenotype identified from *egg* mutants shows a correlation between Eggless and

GSC regulation, Dpp emerged as a leading candidate to investigate a link between them. For this purpose, BMP signaling was analyzed in a molecular level by examining the expression of relevant genes using high-throughput sequencing based transcriptome analysis. Among nine target genes, seven genes (*stat92E*, *notch*, *dpp*, *dally*, *tkv*, *punt*, and *bam*) are major members of BMP signaling: Stat92E and Notch positively regulate Dpp production [18]. Dally, Tkv and Punt restrict Dpp transport only into GSC. In addition, Mei-p26 and Brat activates *bam* expression in Dpp-independent manner via GSC intrinsic pathway.

Transcriptome analysis reveals that *dpp, dally* and *bam* mRNA level changed drastically in *egg* ovary (Fig. 3A). Interestingly, *dpp* mRNA level was elevated (15-fold) and *bam* mRNA level was reduced (9-fold), suggesting that GSC self-renewal and proliferation is enhanced in *egg* ovary. To confirm this result, qRT-PCR assay was performed on *dpp*, *dally* and *bam* whose expression was significantly changed in transcriptome analysis. Consistent with the result from transcriptome analysis, qRT-PCR analysis reveals that the loss of Eggless leads to increased *dpp* expression (18-fold) and decreased *bam* expression (10-fold) (Fig. 3B).

Pro-apoptotic genes are derepressed in *eggless* mutant

Genetic study shows degenerative phenotype of *egg* ovary, suggesting failure of GSC maintenance. In contrast, molecular studies show elevated *dpp* expression and resultantly decreased *bam* expression, providing evidence of enhanced GSC self-renewal and proliferation. Although hyperactivated BMP signaling leads to tumorous GSC accumulation and germarium enlargement [6, 36], it has been identified that somatic cell death arises in *eggless* mutant germarium, which leads to ovary degeneration [28]. From these results, it might be expected that abundant apoptosis occurs in *egg* germarium. To prove this assumption, expression of apoptotic genes in *egg* ovary was examined using transcriptome analysis. As depicted in Fig. 4A, overall expression of apoptotic genes was derepressed in the mutant ovary, showing a strong "apoptosis" signature. Especially, the expression of pro-apoptotic gene *reaper* (16-fold) and *head involution defective* (*hid*) (3-fold) was remarkably increased. Pro-apoptotic genes induce apoptosis by inactivating Drosophila Inhibitor of Apoptosis (DIAP) which is a negative regulator of apoptosis [37, 38]. Interestingly, however, the expression of *p53,* a marker gene of apoptosis showed no difference and expression of *chk-2* rather decreased (3.6-fold) in the mutant (Fig. 4A). To confirm this result, I performed qRT-PCR assay on *reaper*, *hid*, *p53* and *chk-2*. Consistent with the result from transcriptome analysis, qRT-PCR analysis

reveals that expression of pro-apoptotic genes was significantly elevated in *egg* ovary (Fig. 4B). Given that the oogenesis arrests in *egg* ovary where GSC proliferation signal is enhanced by excessive *dpp* expression, these results favor the idea that oogenesis arrest in *egg* germarium is facilitated by apoptosis.

Ectopic *dpp* overexpression promotes *reaper* expression

Increasing evidences have suggested that excessive Dpp signal triggers cell death and causes developmental defects in certain biological contexts. For example, hyperactivated Dpp signaling results in cardial apoptosis in *raw* mutant flies[25] and ovarian apoptosis in *Lsd1* mutant flies [26].

Ovarian somatic cells (OSCs) are mitotically active follicle cells derived from fGS/OSS cell line, comprising of GSCs and sheets of somatic cells [33]. As the *eggless* is expressed in OSCs (Fig. 5A), it provides excellent opportunity to study whether the ectopically overexpressed *dpp* itself could induce cell death. For *dpp* overexpression experiment, a Dpp expression vector, pAc5.1-Dpp was constructed from pAc5.1-V5/HisA vector system. pAc5.1-Dpp or pAc5.1-V5/HisA was transfected into OSCs by electroporation for *dpp* overexpression and control, respectively (Fig. 5B). The qRT-PCR assay was performed on two BMP signaling genes (*dpp* and *bam*) and four apoptotic genes (*p53, chk-2, reaper* and *hid*). qRT-PCR analysis reveals that only *reaper* expression was drastically elevated (6-fold) (Fig. 5D) when *dpp* was overexpressed about 1000-

fold (Fig. 5C). Although *hid* also showed remarkable increase in expression (2.6-fold), it is considered as an invalid result derived from too low absolute expression level. Given that ectopic expression of *reaper* is sufficient to trigger apoptosis [39], this result suggests that ectopic *dpp* overexpression has a potential to induce cell death by facilitating *reaper* expression.

Ectopic *dpp* overexpression induces cell death

In parallel with the molecular analysis, I tested whether ectopically overexpressed *dpp* actually triggers cell death in OSCs. MTS assay was performed to measure viability of OSCs with elevated *dpp* expression. Consistent with the result from qRT-PCR analysis, MTS assay reveals that *dpp* overexpression in OSCs caused striking cell death (Fig. 6A). Combined with the finding that *dpp* overexpression facilitated *reaper* expression, this result suggests that excessive *dpp* activation results in apoptotic cell death in OSCs.

DISCUSSION

Dpp is necessary for ovary homeostasis as it regulates GSC self-renewal and differentiation. It already has been demonstrated that Dpp accomplishes its role in GSC regulation by encouraging GSC maintenance [6, 13]. However, recent studies have identified that excessive Dpp activity rather causes germ cell loss by inducing apoptosis [12, 25-27]. In this study, I revealed a potential role of Dpp in ovarian apoptotic cell death. The *egg* ovaries exhibited degenerative phenotype derived from oogenesis arrest. In *egg* ovary, expression of apoptotic genes was significantly derepressed. Interestingly, BMP signaling was also activated in *egg* ovary, which is verified from the result that *dpp* expression was increased and *bam* was reduced. In addition, *dpp* overexpression in OSCs leads to activation of pro-apoptotic *reaper* gene expression and decrease of cell viability. Taken together, this study suggests that the oogenesis arrest in *egg* ovary may be a consequence of apoptotic cell death caused by aberrant *dpp* activation (Fig. 6B).

The findings from this study support the idea that Dpp serves as an important control system for ovary homeostasis in a dual way: Elimination of incorrectly destined cells is stimulated by excessive activation of Dpp as well as lack of Dpp. Limiting the level of survival signals is important to maintain growth homeostasis by matching the proper number of cells in a tissue [40]. Along with

the survival signal-dependent regulation, pro-apoptotic gene-mediated apoptosis also contributes to tissue homeostasis by eliminating aberrant cells [41, 42] and thus selecting the fittest cells to optimize organ performance [43].

In *Drosophila*, diverse signaling pathways converge to a common cell death program initiated by pro-apoptotic *reaper*, *hid* and *grim* (RHG) genes. Most of these pathways regulates RHG genes at the transcriptional level [44]. *Reaper* has a large transcriptional control region which is targeted by numerous transcription factors involved in these signalings [45-49]. As Dpp regulates multiple transcription factors [50], results presented here imply transcriptional regulatory interaction between Dpp and *reaper*. Indeed, recent study has shown that sharp discontinuity of Dpp signaling stimulates *reaper* activation [12], but the molecular mechanism of their interaction is not yet uncovered. So manifesting the mechanism by which Dpp regulates *reaper* expression and controls apoptosis would be an outstanding subject for the further study.

Because of their self-renewing potential, stem cells are at persistent risk of tumorigenesis derived from differentiation defects. Appropriate balance between self-renewal and differentiation is more important for GSCs, since their imbalance prevent organism from producing offspring. In this study, I propose that Dpp controls GSCs in a non-canonical pathway: Excessive *dpp* signal causes apoptotic cell death in incorrectly destined GSC, thereby preventing

tumorigenesis in ovary. I hope my study could provide useful insight into understanding stem cell biology as well as GSC regulation.

FIGURES AND LEGENDS

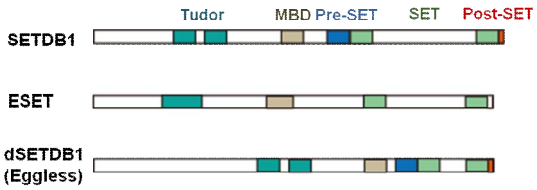
Figure 1. The *Drosophila eggless* encodes a SET domain protein.

(A) The predicted protein structures of SETDB1, ESET and Eggless, showing identified protein domains within each protein.

(B) egg^{2138} allele contains single nucleotide substitution (shown as asterisk) introducing a premature stop codon.

(C) Genomic locus showing the extent of deletion in *Df(2R)Dll-Mp*. Red box indicates the locus of *eggless*.

A


Tudor
MBD Pre-SET
SET
Post-SET
SETDB1
ESET
dSETDB1
(Eggless)

B


Egg2138
W1118:
CAA TGC ACC CGG GGA CAA AGT ATG ACC ACT GAA
Q C T R G Q S M T T E
egg2138:
*
CAA TGC ACC CGG GGA TAA AGT ATG ACC ACT GAA
Q C T R G stop

C

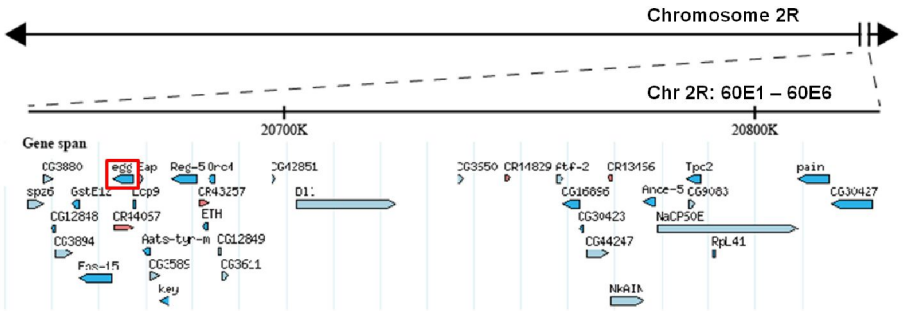

Chromosome 2R
Chr 2R: 60E1 – 60E6
20700K
20800K
Gene span

Figure 2. Loss of Eggless causes severe developmental defects in the ovary.

(A) A crossing scheme for *egg* transheterozygous mutant flies. Female fecundity is indicated under each genetype.

(B) Morphology of ovaries from each genotype of *eggless* mutants (egg^{2138}/*GCyO*, *Df(2R)Dll-Mp/GCyO* and egg^{2138}/*Df(2R)Dll-Mp*) and wild-type (W^{1118}). Scale bars: 0.2mm.

(C) Quantative RT-PCR shows that *eggless* expression is rarely detected in *egg* ovary. Error bars represent S.D. over three biological replicates.

A

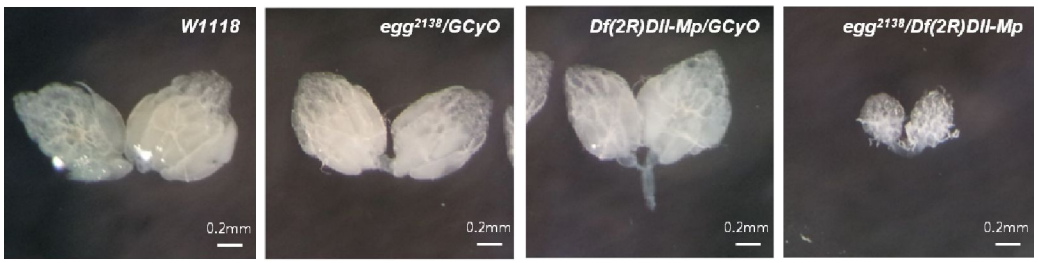
$\varphi \frac{egg2138}{GCyO} \times \sigma \frac{Df(2R)Dll-Mp}{GCyO}$ or $\varphi \frac{Df(2R)Dll-Mp}{GCyO} \times \sigma \frac{egg2138}{GCyO}$

↓

eggless mutant line Progeny classes : $\frac{egg2138}{GCyO}$ or $\frac{Df(2R)Dll-Mp}{GCyO}$ or $\frac{egg2138}{Df(2R)Dll-Mp}$

Fertile Fertile Sterile

B



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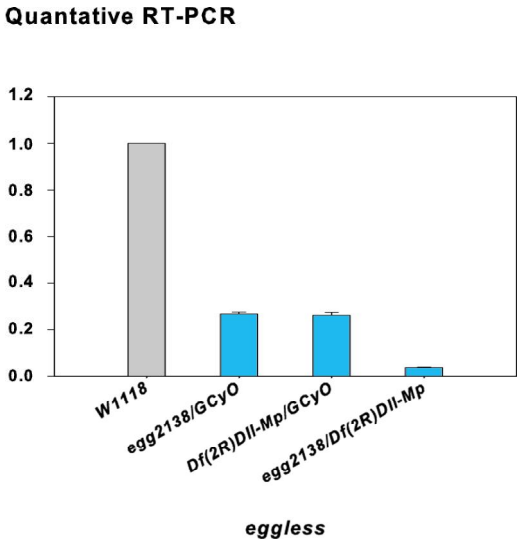
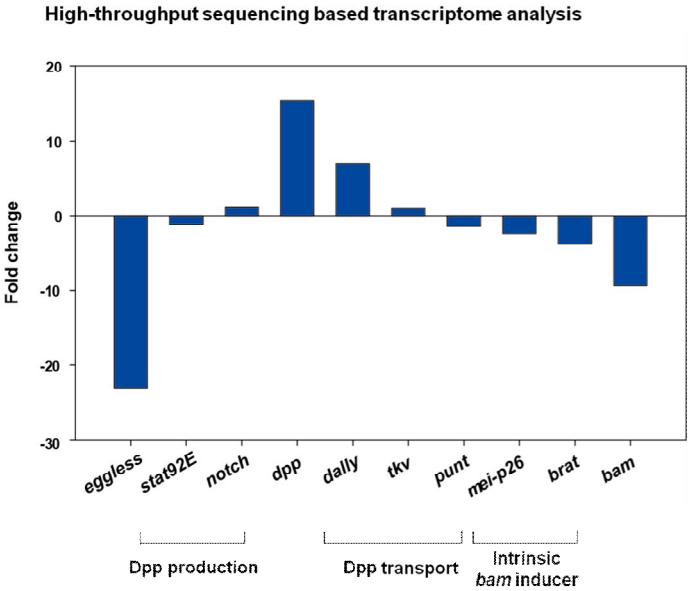


Figure 3. Loss of Eggless increases *dpp* expression

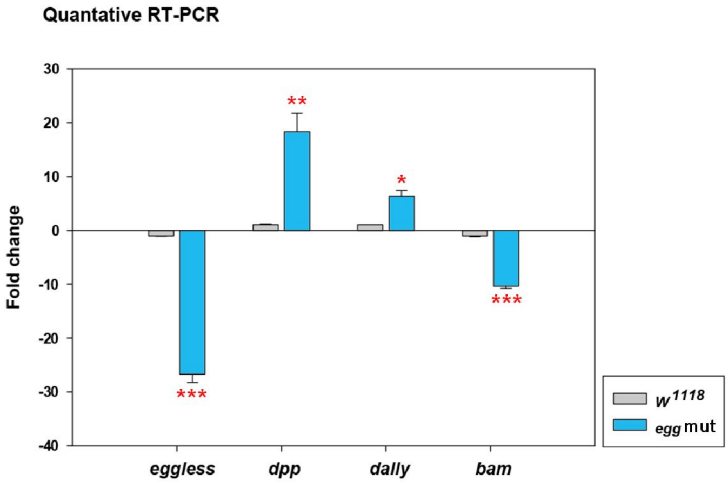
(A) High-throughput sequencing based transcriptome analysis shows relative expression of genes regulating GSC self-renewal and differentiation in *egg* ovary.

(B) Quantative RT-PCR shows that *dpp*, *dally* and *bam* expression signficantly changed in *eggless* ovary. Error bars represent S.D. over three biological replicates (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Plots of representative raw Ct values for each target gene are presented below. Ct values were normalized to *rp49* (grey line) and gene expression levels were calcuated using the comparative Ct method.

A


High-throughput sequencing based transcriptome analysis
Fold change
20
10
0
-10
-20
-30
eggless
stat92E
notch
dpp
dally
tkv
punt
mei-p26
brat
bam
Dpp production
Dpp transport
Intrinsic
bam inducer

B


Quantative RT-PCR
Fold change
30
20
10
0
-10
-20
-30
-40
**
*
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***
eggless
dpp
dally
bam
w1118
egg mut

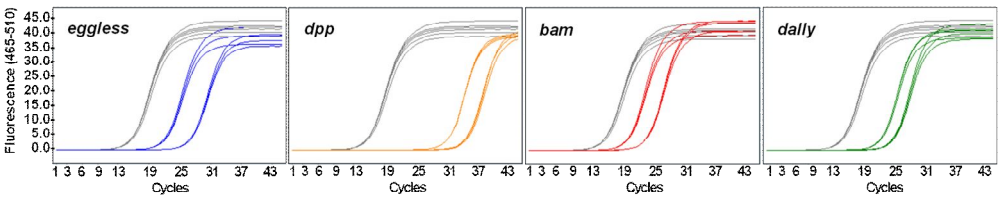

Fluorescence (465-510)
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
eggless
dpp
bam
dally
1 3 6 9 13 19 25 31 37 43
Cycles

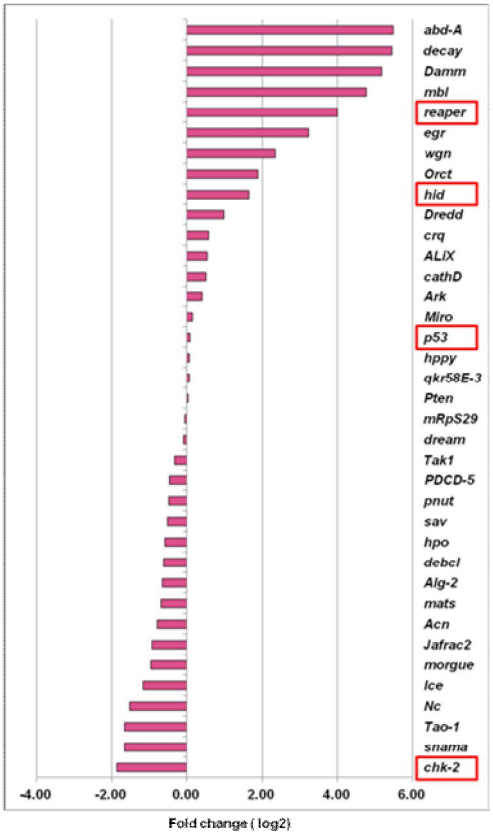
Figure 4. Pro-apoptotic genes are derepressed in *egg* ovary

(A) High-throughput sequencing based transcriptome analysis shows relative expression of apoptotic genes in *egg* ovary. Fold changes are shown on log2 scale. Red boxes indicates representative apoptotic genes whose expression was further analyzed by qRT-PCR.

(B) Quantative RT-PCR shows that *reaper* and *hid* expression signficantly increased in *egg* ovary. Error bars represent S.D. over three biological replicates (* $P < 0.05$, ** $P < 0.01$). Raw Ct values were processed as described above to calculate gene expression level.

A

High-throughput sequencing based transcriptome analysis



B

Quantative RT-PCR

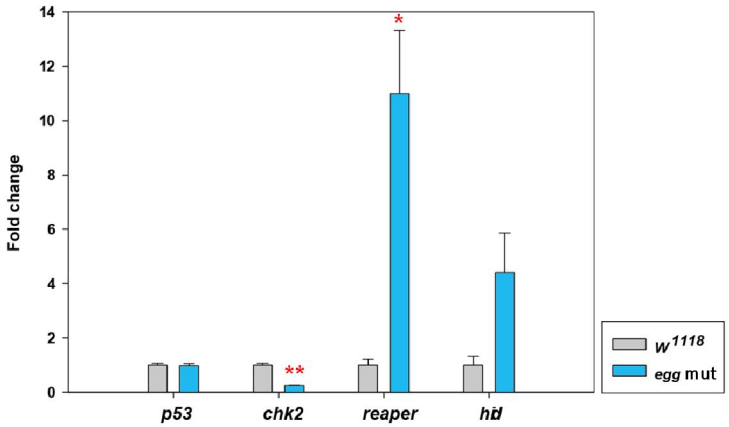


Figure 5. Ectopic *dpp* overexpression activates *reaper* expression in OSCs

(A) Ethidium bromide-stained agarose gel showing *eggless* expression in *Drosophila* ovaries and OSC cell line. *Rp49* was amplified as a loading control.

(B) Scheme of Dpp overexpression experiment in OSCs.

(C) The efficiency of *dpp* overexpression in OSC was determined by qRT-PCR. Error bars represent S.D. over three biological replicates. Raw Ct values were processed as described above to calculate gene expression level.

(D) Quantative RT-PCR shows the expression level of two BMP signaling genes (*bam* and *dally*) and four apoptotic genes (*p53*, *chk-2*, *reaper* and *hid*) in *egg* ovary. Error bars represent S.D. over three biological replicates. Raw Ct values were processed as described above to calculate gene expression level.

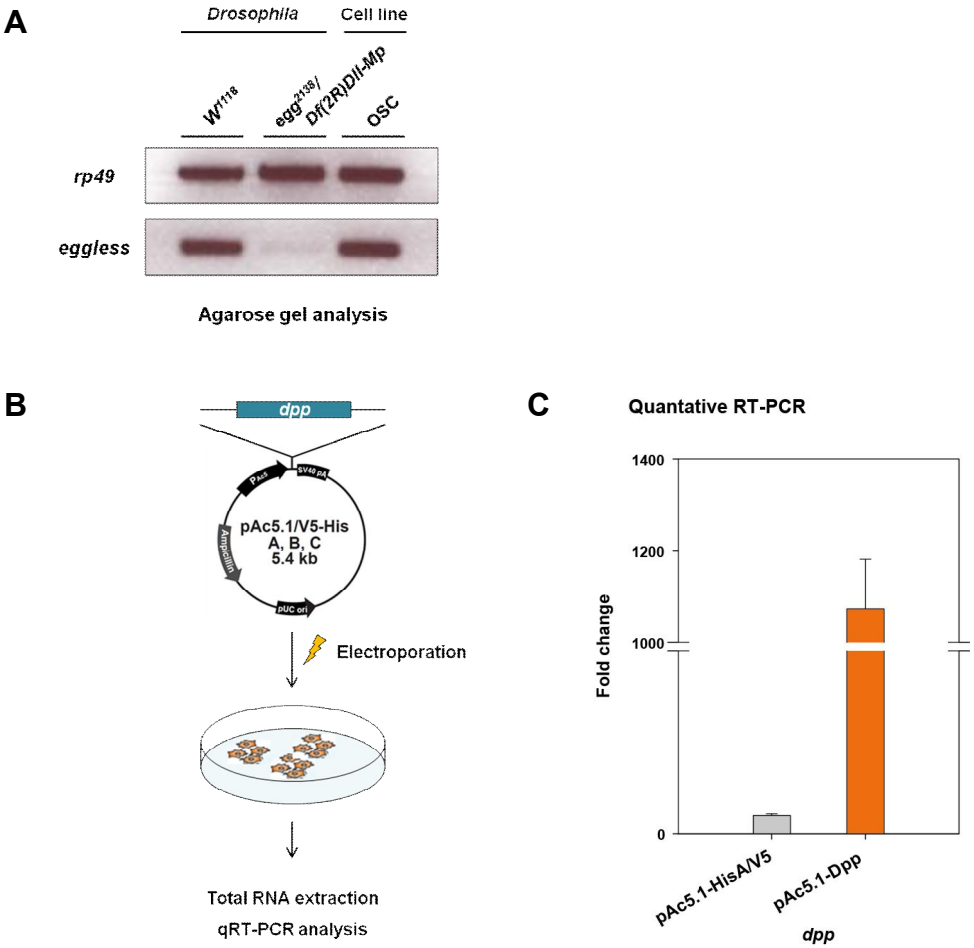
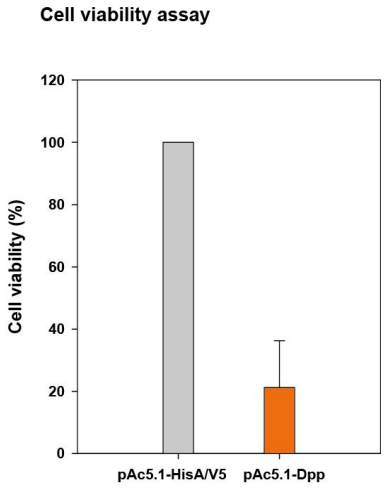

A
Drosophila
Cell line
W1118
egg2138/ Df(2R)DiI-Mp
OSC
rp49
eggless
Agarose gel analysis
B
dpp
pAc5.1/V5-His
A, B, C
5.4 kb
Ampicillin
pUC ori
Electroporation
Total RNA extraction
qRT-PCR analysis
C
Quantative RT-PCR
Fold change
1400
1200
1000
0
pAc5.1-HisA/V5
pAc5.1-Dpp
dpp
D
Quantative RT-PCR
Fold change
8
6
4
2
0
eggless
bam
dally
p53
chk2
reaper
hid
pAc5.1-HisA/V5
pAc5.1-Dpp

Figure 6. Ectopic *dpp* overexpression induces cell death in OSCs.

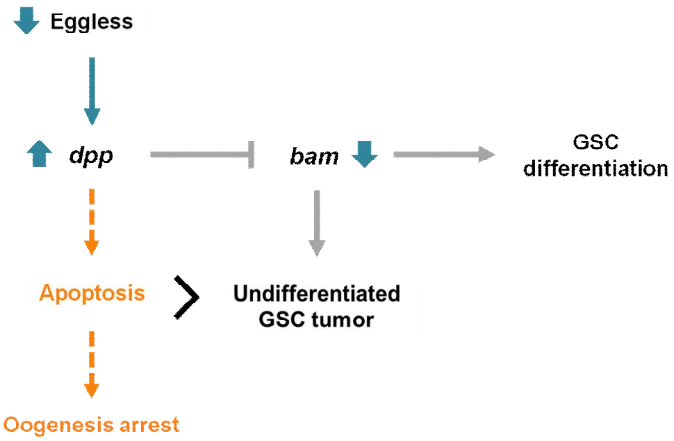
(A) MTS assay shows that the viability of *dpp* overexpressing OSCs is severely dereased. Error bar represents S.D. over three biological replicates.

(B) Proposed model in which depletion of Eggless activates *dpp* expression, where overexpressed *dpp* stimulates the ovarian apoptosis. Apoptotic activity overcomes BMP signaling-derived GSC proliferative activity and as a result, oogenesis arrest takes place in *egg* ovary.

A


Cell viability assay
Cell viability (%)
120
100
80
60
40
20
0
pAc5.1-HisA/V5
pAc5.1-Dpp

B


Eggless
dpp
bam
GSC differentiation
Apoptosis
Undifferentiated GSC tumor
Oogenesis arrest

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ABSTRACT IN KOREAN

초파리 *eggless* mutant에서 과발현되는 Dpp에 의한 난소 내 세포사멸

Decapentaplegic(Dpp)는 germline stem cell(GSC)의 증식 및 분화를 조절하는 niche signaling의 핵심인자로서, 초파리의 발달과정에 다양한 방식으로 참여한다. Dpp는 GSC의 분화인자인 *bam*의 발현을 억제함으로써 GSC의 증식을 촉진하는 것으로 알려져 있으나, 최근의 연구들에 따르면 *dpp* 과발현이 세포사멸을 유도하는 signal로 기능할 수 있음이 밝혀지고 있다.

본 연구에서는 초파리 *eggless* mutant의 난소에서 oogenesis arrest로 인한 developmental defect가 발생함을 확인하였다. High-throughput sequencing을 이용한 *eggless* mutant 난소의 transcriptome 분석을 토대로 qRT-PCR을 수행하여 mutant에서 *dpp*의 발현이 크게 증가함을 알아냈다. 이와 함께 mutant에서 apoptosis 촉진 유전자들의 발현이 전반적으로 크게 증가함을 확인했다. 한편, 초파리 OSC cell에서 *dpp*가 과발현될 경우, pro-apoptotic 유전자인 *reaper*의 발현이 증가하고, 이와 더불어

cell viability가 낮아지는 현상을 관찰하였다. 이러한 결과들을 종합하여, 본 연구는 초파리 *eggless* mutant에서 과발현되는 *dpp*에 의해 난소 내 세포사멸이 일어날 수 있음을 밝혔다.

주요어: Eggless, dSETDB1, Decapentaplegic, Reaper, Ovarian apoptosis, Ovary development, Ovarian somatic cell

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