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## THE FUNCTION OF Socs GENES IN DROSOPHILA DEVELOPMENT AND SIGNALING PATHWAYS

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## ABSTRACT OF THESIS

### THE FUNCTION OF *Socs* GENES IN *DROSOPHILA* DEVELOPMENT AND SIGNALING PATHWAYS

The duration and intensity of the JAK/stat signaling must be tightly regulated to prevent excessive transcriptional response and to reset the pathway to receive additional signals. *Socs* are the largest class of these regulators in mammals. Eight *Socs* genes have been found in mammals. *CIS*, and *SOCS1-3*, the canonical *Socs*, are transcriptionally activated by and down-regulate the JAK signaling. *Socs4-7*, the non-canonical *Socs*, are less studied and their relationship with the JAK/STAT pathway has not been well established. The *Drosophila* genome encodes three non-canonical *Socs* homologues, *Socs16D*, *Socs36E*, and *Socs44A*. Expression of *Socs36E* is controlled by the JAK pathway and misexpression causes phenotypes similar to that from reduction of JAK in both ovary and wing, which may make it functionally more similar to the canonical *Socs*. Expression of *Socs44A* is not controlled by the JAK pathway and misexpression causes JAK mutant phenotypes in wing but not in ovary. Imprecise excision mutants of the three *Socs* genes have been generated by us and have no visible phenotypes. The mutants of *Socs36E* and *Socs44A* significantly enhance the tumor formation in *hop<sup>Tum-1</sup>* mutant, a gain-of-function mutation of the JAK/STAT pathway. The function of *Drosophila Soc*s will be further studied with different strategies.

KEY WORDS: JAK/STAT pathway, Suppressor of Cytokine Signaling, regulation of signal transduction, *Socs36E*, *Socs44A*

Qian Guo  
May 1, 2007

THE FUNCTION OF SOCS GENES  
IN *DROSOPHILA* DEVELOPMENT AND SIGNALING PATHWAYS

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May 2007



THESIS

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The Graduate School

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IN *DROSOPHILA* DEVELOPMENT AND SIGNALING PATHWAYS

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THESIS

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A thesis submitted in partial fulfillment of the  
requirements for the degree of Master of Science in the  
College of Arts and Science  
at the University of Kentucky

By

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Lexington, Kentucky

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2007

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QGuoThesis. Pdf (2.7MB)

# Chapter I

## Background

The **J**Anus **K**inase/**S**ignal **T**ransducer and **A**ctivator of **T**ranscription (JAK/STAT) signaling pathway plays essential roles in development, cell proliferation, differentiation, cell migration, immunity, hematopoiesis, and many other biological processes (Bach et al., 2006). The level and duration of this pathway must be tightly regulated to prevent excessive transcriptional response and to reset the pathway to receive additional signals (Rawlings et al., 2004). Ectopic activation of JAK/STAT pathway causes abnormal cell proliferation and differentiation and leads to many diseases, like a specific form of acute lymphocytic leukemia (ALL)(Lacronique et al., 1997), human hepatocellular carcinoma (HCC) (Calvisi et al., 2006), diabetic nephropathy (Marrero et al., 2006), and inflammatory diseases (O'Shea et al., 2005).

There are three main conserved protein families that can regulate JAK activation: the **S**uppressors **O**f **C**ytokine **S**ignaling (SOCS), the **P**rotein **I**nhibitors of **A**ctivated **S**TATs (PIAS), and the **S**H2-containing **P**hosphatases (SHP) (Rakesh et al., 2005). The SOCS are the largest class of negative regulators in mammalian JAK/STAT pathway. Eight *Socs* genes have been found in mammals, *CIS* and *Socs1-7*. *CIS*, and *Socs1-3*, the canonical *Socs*, have been well studied in vertebrates and function in a negative feedback loop. Canonical *Socs* are transcriptionally activated by and down-regulate the *JAK* signaling. *Socs4-7*, the non-canonical *Socs*, were identified by searching the GenBank and the institute of Genetic Research database of human ESTs for genes with the SOCS box and SH2 domain (Hilton, et al., 1998). The non-canonical *Socs* are less studied, the transcription is not regulated by the JAK/STAT pathway, and their relationship with the JAK/STAT pathway has not been established. All the SOCS proteins have an N-terminus with varied length and sequence, a central SH2 domain and a C-terminal SOCS box (Rakesh et al., 2005). The SH2 domain could interact with the phosphorylated tyrosine residue of target proteins like the components in JAK/STAT pathway; the carboxy-terminal SOCS box has been shown to interact with the elongin BC complex, which interacts with an E3 ubiquitin ligase (cullin-2) that could bind a ring finger protein-ROC1; ROC1 recruits the E2 Ubiquitin conjugating enzyme and targets the bound proteins for proteosomal degradation, which causes the inhibition of signaling pathways (Fig. 1-2).

Studies in mammals show *Socs* can inhibit JAK signaling by different mechanisms (Fig. 1-1). For example, SOCS1 can directly bind to the phosphorylated JAK2 kinase domain

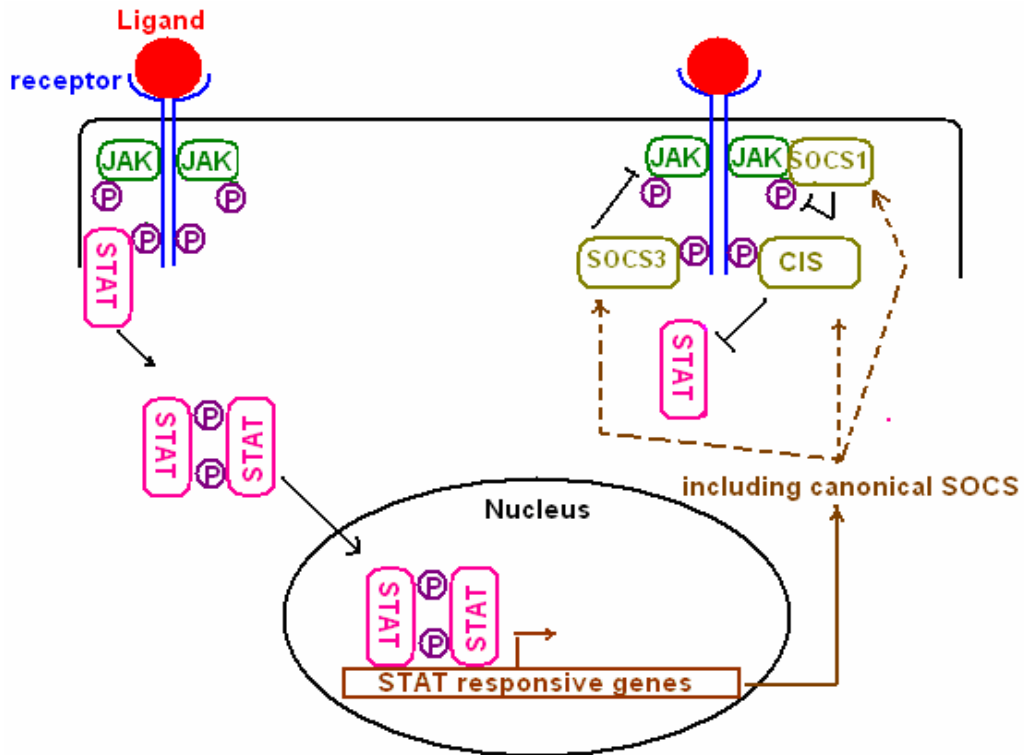
and inhibit its activation (Larsen et al., 2002); CIS and SOCS3 can inhibit the signaling by interacting with activated cytokine receptors (Cooney et al., 2002). There is also evidence that shows the non-canonical SOCS play roles in additional pathways: SOCS4 and SOCS5 are shown to negatively regulate the EGFR pathway in mammalian cell lines (Kario et al., 2005); SOCS6 and SOCS7 affect the insulin action (Mooney et al., 2001; Banks et al., 2005). Previous studies show the *Socs1* knockout mice grow slowly and die within 3 weeks of birth and the lethality can be delayed by *Stat1*<sup>-/-</sup> and *Stat6*<sup>-/-</sup> backgrounds (Tan and Rabkin, 2005; Cooney, 2002; Alexander, 2002), which indicates the functional role of *Socs1* in the JAK/STAT pathway; The *Socs2* knockout mice develop gigantism (Tan and Rabkin, 2005; Cooney, 2002; Alexander, 2002), which supports the regulatory function of *Socs2* in growth; The *Socs3* knockout mice show uncontrolled LIF signaling and embryonic lethality (Tan and Rabkin, 2005; Cooney, 2002; Alexander, 2002), which suggests its role in LIF signaling; The *CIS* knockout mice have no significant abnormalities (Cooney, 2002; Alexander, 2002), which indicates some functional redundancy; The *Socs6* knockout mice develop normally, but weigh less than wild-type mice (Krebs et al., 2002), so the *in vivo* function of *Socs6* is not defined; The *Socs7* knockout mice develop hydrocephalus and are hypersensitive to insulin (Krebs et al., 2004), suggesting an essential role of *Socs7* in the regulation of the insulin signaling; The *Socs5* knockout mice show no abnormalities (Brender et al., 2004), which suggests functional redundancy; The *Socs4* is the least studied and its knockout is not reported yet (Knisz and Rothman., 2007).

*Drosophila* has a complete JAK/STAT pathway that functions very similarly to mammalian JAK. The *Drosophila* JAK activity is also involved in many biological processes like embryonic patterning, sex determination, hematopoiesis, wing vein development, adult photoreceptor cluster orientation, oogenesis, innate immune response, and stem/germ cell development (Arbouzova and Zeidler, 2006; Harrison et al., 1995; Jinks et al., 2000; Luo et al., 1995; McGregor et al., 2002; Strutt and Strutt, 1999; Xi et al., 2003; Yan et al., 1996; Zeidler et al., 2000). Compared with the mammalian pathway, the *Drosophila* JAK/STAT pathway is much simpler with low redundancy but highly conserved, which makes *Drosophila* an ideal model organism to study JAK/STAT pathway and the *Socs* genes.

Based on the sequence similarities, there are three *Socs* homologues in the *Drosophila* genome, which are *Socs36E*, *Socs44A* and *Socs16D*. All of them contain a SOCS box at the carboxyl terminus, preceded by a SH2 domain, and are homologues of the non-canonical SOCS family. *Socs36E* is most similar to the mammalian *Socs4-5*, while *Socs44A* and *Socs16D* are most similar to the *Socs6-7* family (Rawlings et al., 2004). The expression of *Socs36E* is responsive to the JAK activity (Karsten et al., 2002; Rawlings et al., 2004), and misexpression of *Socs36E* causes phenotypes similar to that from reduction of JAK in both the ovary and the wing (Rawlings et al., 2004), which may

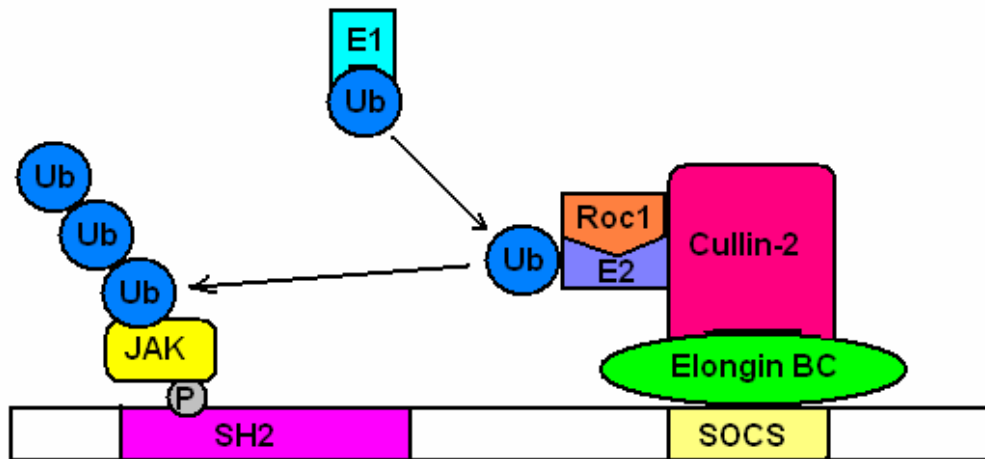
make it functionally more similar to the canonical *Socs*. The expression of *Socs44A* is not controlled by the JAK pathway and misexpression causes *JAK* mutant phenotypes in wing but not in ovary (Rawlings et al., 2004). In addition, *Socs36E* and *Socs44A* show genetic interactions with the EGFR pathway (Karsten et al., 2002; Rawlings et al., 2004). The misexpression of *Socs* implies both *Socs36E* and *Socs44A* could negatively regulate the JAK/STAT pathway; *Socs36E* can negatively regulate the EGFR pathway (Karsten et al., 2002; Rawlings et al., 2004), while *Socs44A* can upregulate the EGFR pathway (Rawlings et al., 2004). Therefore, *Socs36E* and *Socs44A* play roles in similar pathways, but they might function in different ways. The function of *Socs16D* has not been studied yet.

In this thesis, I try to understand the function of the *Drosophila Socs* genes. Since *Drosophila Socs* genes are most similar to the non-canonical *Socs* in mammals, the study on *Drosophila Socs* genes would give us valuable information on the function of the non-canonical *Socs* members in vertebrates. We used standard P element excision screens and generated mutants of the three *Drosophila Socs* genes from transposon insertion lines. We also made double mutants of *Socs36E* and *Socs16D*, *Socs44A* and *Socs16D*. I examined the eyes, wings, and the formation of melanotic tumors, where the most common phenotypes are shown in the JAK/STAT mutant flies. However, no obvious morphological phenotype is seen in those mutants. We examined the border cell number in the *Socs* mutants and the effect of *Socs* mutation in a dominant gain-of-function mutant-*hop<sup>Tum-1</sup>*. The *Socs* single mutants significantly enhance the tumor formation in *hop<sup>Tum-1</sup>*, but no significant effect on the border cell number was detected.



*Fig.1--1 Inhibition of JAK/STAT pathway by canonical SOCS: SOCS1 could directly bind to the phosphorylated JAK kinase domain and inhibit its activation; CIS and SOCS3 could inhibit the signaling by interacting with activated cytokine receptors.*





*Fig.1-2 Structure of SOCS proteins: The SH2 domain could interact with the phosphorylated tyrosine residue of other proteins; the carboxy-terminal SOCS box has been shown to interact with the elongin BC complex and targets the bound proteins for proteosomal degradation.*

## Chapter II

### Generation of *Drosophila Socs* mutants

#### INTRODUCTION

Previous studies on *Drosophila Socs* genes are all based on the mis-expression phenotypes of *Socs36E* and *Socs44A*. Mis-expression of *Socs36E* in *Drosophila* wings causes the ectopic wing vein phenotype similar to the phenotype seen in mutants of *hop* or *Stat92E* (Rawlings, 2004; Callus and Mathey-Prevot, 2002); mis-expression of *Socs36E* in *Drosophila* egg chamber causes the loss of the JAK activity marker, *pnt-LacZ*. The mis-expression phenotypes of *Socs36E* suggest a negative regulatory role of *Socs36E* in the JAK/STAT pathway. Mis-expression of *Socs44A* in *Drosophila* wing causes ectopic wing vein phenotype that is sensitive to the change of JAK activity; but the mis-expression of *Socs44A* does not cause any change of the JAK activity marker (Rawlings, 2004). The mis-expression studies of *Socs44A* indicate *Socs44A* can also regulate JAK/STAT pathway but in a mechanism different from that of *Socs36E*.

In order to clarify the function of *Drosophila Socs* genes, it is necessary to get mutants of *Socs36E*, *Socs44A* and *Socs16D*. Based on the previous studies on the mis-expression of *Socs36E* and *Socs44A*, we hypothesize the mutants of the three *Drosophila Socs* genes cause ectopic activity of the JAK/STAT pathway. Since there are no *Socs* mutants reported, we did standard P element excision screens and generated mutants of the three *Drosophila Socs* genes from transposon insertion lines. We used standard transposase-mediated mobilization to genetically remove the P element insertion and generated imprecise excision mutations of the three *Drosophila Socs* genes. We also generated double mutants *Socs16D*<sup>26A</sup>; *Socs36E*<sup>189A</sup> and *Socs16D*<sup>26A</sup>; *Socs44A*<sup>Δ291A</sup>. However, no obvious mutant phenotype was found.

## RESULTS

### Generation of *Socs36E* mutants

The reading frames and the transcription start sites of the *Drosophila Socs* genes were predicted and mapped by Jason Rawlings and Dr. Douglas Harrison in our lab. EY06665 (Fig. 2-1A) is an insertion in the second exon of *Socs36E* coding region (Fig. 2-1B). Although the EY construct is inserted in the exon of *Socs36E*, it has GAL4 enhancers and a basal promoter at the 3' end. Because of the insertion orientation, transcription may be initiated from the P element into the 3' portion of the *Socs36E* transcription unit. In addition, *SOCS36E<sup>EY06665</sup>* shows semi-dominant (can be partially complemented by wild type) ectopic wing vein (Fig. 2-2A) that is similar to the expression of *UAS-Socs36E* under *en-GAL4* control (Fig. 2-2C). Therefore, EY06665 might still have functional *Socs36E*.

I used the standard transposase-mediated mobilization to genetically remove the EY construct. The resulting excision lines were identified by loss of the *white+* and *yellow+* markers in the P element. During the mobilization of the P element, both precise and imprecise excisions can be produced. In the precise excisions, the resulting lines have the wild type sequence; in the imprecise excisions, the resulting lines may either carry a deletion of the genomic DNA flanking the P element, or have part of the P element sequence left (incomplete excision). Both kinds of imprecise excisions may cause loss-of-function mutation of the *Socs* genes. I analyzed the excision lines by PCR using primers flanking the P element insertion site and compared the PCR product size with the wild type fragment. Precise excision will give a fragment with the size identical to the wild type; imprecise excision will give a fragment with the size either larger or smaller than the wild type.

During the screen, I got about seventy viable lines and one hundred and fifty lethal lines. The lethality of those lethal lines is complemented by the deficiency line *Df(2)II31*, a deficiency that removes *Socs36E* (data not shown). Therefore, I conclude the lethality is not caused by the *Socs36E* mutation. I found the homozygotes of the original insertion line EY06665 grow much slower than wild type flies. Therefore, it is possible that the original insertion line carries another unreported P element insertion in some essential genomic region, which slows down the growth rate of those “lethal” lines and the original insertion line, making them seem to be “lethal”. A summary of the screen of the seventy *Socs36E* viable excision lines is shown in Table 1. Based on the PCR result, there were nineteen incomplete excision lines. Thirty-eight of the viable lines have the ectopic wing vein (Fig. 2-2B); nine other viable lines do not show any phenotype. I randomly picked fifteen viable lines, and sequenced their fragments from PCR using the primers flanking the P element insertion. As a result, I totally identified six incomplete excision mutants of *Socs36E* (three of them are shown in Fig. 2-1C) with part of the P element sequence left.

All the sequenced incomplete excision mutants show the semi-dominant ectopic wing vein phenotype (Fig.2-2B). I picked *Socs36E*<sup>190A</sup> as our *Socs36E* mutant, because *Socs36E*<sup>190A</sup> showed the highest penetrance of ectopic wing vein when I initially identified the first three incomplete excision mutants (189A, 190A, 313B). The penetrance of the ectopic wing vein in the homozygous incomplete excision mutants, and in the complementation test with the deficiency line *Df(2)I131* or the wild type fly is shown in Table 2. The penetrance of the wing vein phenotype in *Socs36E* mutants is increased by *Df(2)I131*, and is partially complemented by one wild type allele. The line *Df(2)I131* also has the semi-dominant wing vein phenotype by itself. These data indicate the wing vein phenotype is probably connected to the loss-of-function *Socs36E*. However, from the sequence result, I also found four lines with wild type sequence in the genomic region flanking the P element insertion site but they still show the wing vein phenotype. In addition, there are twenty lines with PCR fragment size similar to wild type showing the wing vein phenotype.

To further confirm the association of the wing vein phenotype with *Socs36E*, I used two additional molecularly defined deficiency lines, *Df(2L)Excel8038* and *Df(2L)Excel7070*, both of which remove *Socs36E* and have an overlapping deletion region (Fig. 2-3). Like the deficiency line *Df(2)I131*, *Df(2L)Excel8038* also shows the semi-dominant wing vein phenotype and increases the penetrance of the wing vein phenotype in the *Socs36E* mutants. However, *Df(2L)Excel7070* does not have the wing vein phenotype and complements the wing vein phenotype in the *Socs36E* mutants. I also used PCR to confirm the deletion of *Socs36E* in *Df(2L)Excel7070*. The wing vein phenotype might not be caused by the mutation of *Socs36E*, but something else carried by the original insertion line EY06665 that is closely connected to *Socs36E*.

#### **Generation of *Socs44A* and *Socs16D* mutants**

Using the same standard transposase-mediated mobilization, we also generated excision mutants of *Socs44A* and *Socs16D*. G2615 is a P element inserted within 50bp 5' of the *Socs44A* transcription start (Fig. 2-4). Excision lines with deletions of genomic DNA flanking P element were identified by PCR and sequencing. Among the excision lines, the line 291A removes all of *Socs44A*, including the SH2 domain and SOCS box (done by Susan W. Harrison and KBRIN students, Fig. 2-4). Therefore, *Socs44A*<sup>291A</sup> was picked as *Socs44A* mutant. The excision mutant lines do not have any visible phenotype.

NP7149 is a P insertion located 1.2kb from the predicted 5' end of *Socs16D* (Fig. 2-5). Excision mutant lines (done by Susan W. Harrison and KBRIN students, Fig. 2-5) with deletion of the genomic sequence flanking the P element insertion were identified by PCR and sequencing. The excision mutant line 14A has a 1.3 kb deletion from the P element insertion site to a small part of 5' end of the second exon; 70B has a 1.0 kb deletion without disrupting exons; 26A has a large deletion taking out all the *Socs16D*

and some part of the neighboring gene *CG6398* (the 3' end of the deletion is mapped to some region between *Socs16D* and the neighboring *CG12986*, the 5' end of the deletion takes 454bp from the 5' of the neighboring *CG6398*-mapped by Susan Harrison). 14A and 26A were picked for the mutational analysis. None of the excision mutants of *Socs16D* show any visible phenotype.

#### **Generation of double mutants of *Socs16D* with *Socs44A*, and *Socs16D* and *Socs36E***

Since there is no visible phenotype in the mutants of *Socs44A* or *Socs16D*, I think the SOCS genes might have some functional redundancy. Therefore, I made the double mutants *Socs16D*<sup>26A</sup>; *Socs36E*<sup>190A</sup> and *Socs16D*<sup>26A</sup>; *Socs44A*<sup>Δ291A</sup>. I examined the double mutants *Socs36E*<sup>190A</sup>; *Socs16D*<sup>26A</sup> and *Socs44A*<sup>Δ291A</sup>; *Socs16D*<sup>26A</sup> morphologically, especially the eye size, the wing vein and the presence of tumors, because JAK/STAT pathway plays an important role in the development of the *Drosophila* eye, wing and blood cells. However, no obvious mutant phenotype was detected yet. I have not successfully generated the double mutant of *Socs36E* and *Socs44A*, because those two genes are on the same chromosome with recombinant frequency about 0.04 and the *Socs36E* and *Socs44A* mutants have no visible phenotype.

## **DISCUSSION**

Using standard P element excision screens, we generated mutants of the three *Drosophila* *Socs* genes from transposon insertion lines. The imprecise excision mutations of *Socs36E* have part of the P element left, which cause a frameshift mutation of *Socs36E*. Both of the original insertion line EY06665 and the incomplete excision mutations show the semi-dominant ectopic wing vein. This wing vein phenotype can not be complemented by deficiency lines *Df(2) 1131* or *Df(2L)Excel8038* with *Socs36E* removed, which indicates the wing vein phenotype might be connected to *Socs36E*. However, there are twenty lines with PCR fragment size identical to the wild type showing the wing vein phenotype. Among those twenty lines, I had found four lines sequenced and found them have the wild type sequence left in the genomic region flanking the P element insertion site. In addition, the deficiency line *Df(2L) Excel17070* which also removes *Socs36E* complemented the ectopic wing vein of the incomplete excision mutation of *Socs36E*. Therefore, the ectopic wing vein seen in the original insertion line and excision lines must be caused by something else other than the mutation of *Socs36E* carried by the original insertion line. Moreover, I found the homozygotes of the original insertion line EY06665 grow much slower than wild type flies. It is possible that the original insertion line carries another unreported P insertion in some essential genomic region which slows down the growth rate.

The excision mutations of *Socs44A* and *Socs16D* do not show any visible phenotype. Double mutants of *Socs16D* and *Socs44A*, *Socs16D* and *Socs36E* do not show obvious phenotype either. It is possible that the three *Drosophila* *Socs* genes have some functional

redundancy. Further analysis on the double mutants and the making of a triple mutant might be helpful to identify the function of *Drosophila Socs* genes.

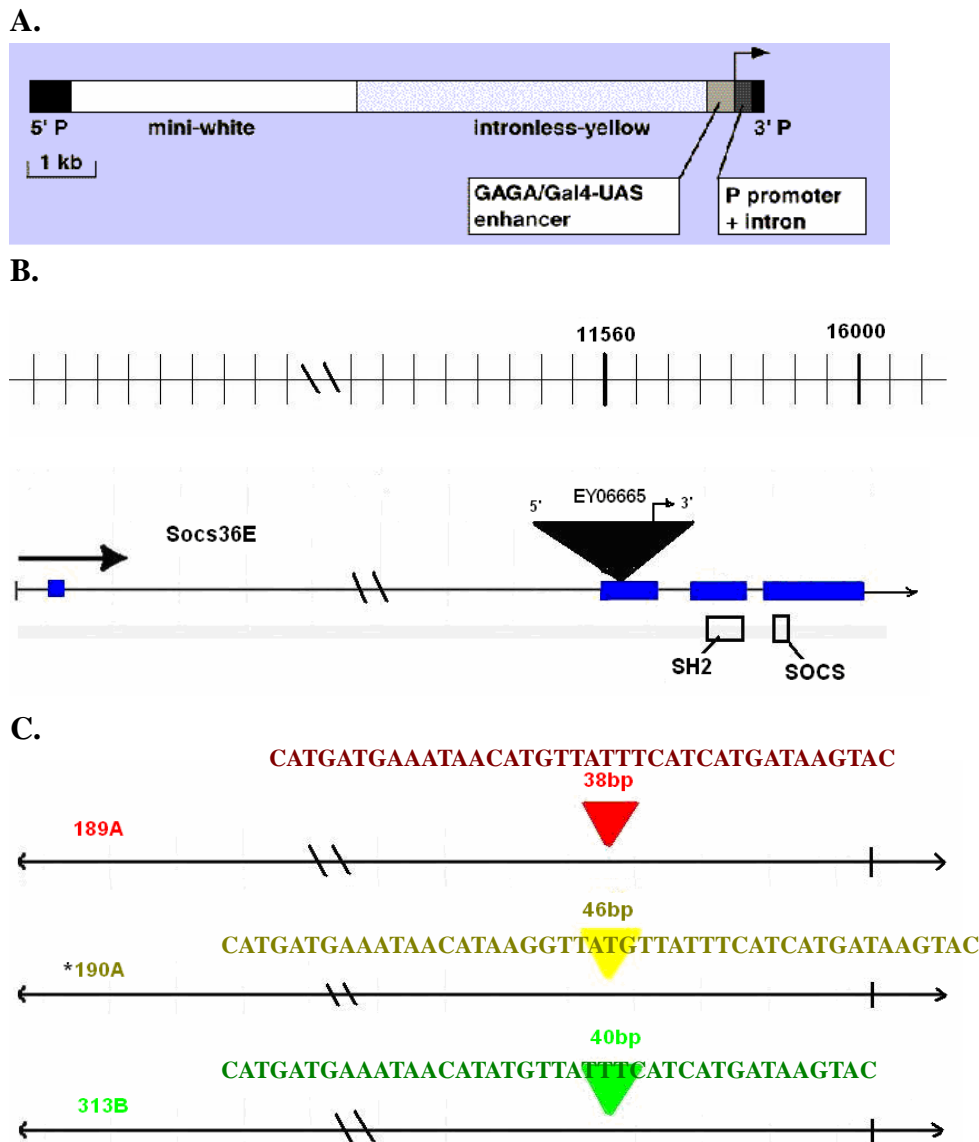


Fig. 2-1 (A) Structure of EY construct-The EY construct is inserted in the second exon of SOCS36E with GAL4 enhancers and a basal promoter at the 3' end. Transcription direction is indicated by arrow.

(B) The genomic organization of SOCS36E with direction of transcription is indicated by arrows; the P element insertion site is also indicated.

(C) Three incomplete excision mutants of SOCS36E generated from insertion line SOCS36E<sup>EY06665</sup> with part of the P element sequence left. (Asterisk indicates allele chosen for mutational analysis.). The left P element sequences are also indicated.



Fig. 2-2 Semi-dominant ectopic wing vein is seen for the insertion line  $SOCS36E^{EY06665}$  (A) and incomplete excision mutants of  $SOCS36E$  (B). Similar ectopic wing vein is also seen for expression of  $UAS-SOCS36E$  under  $en-GAL4$  control (C).

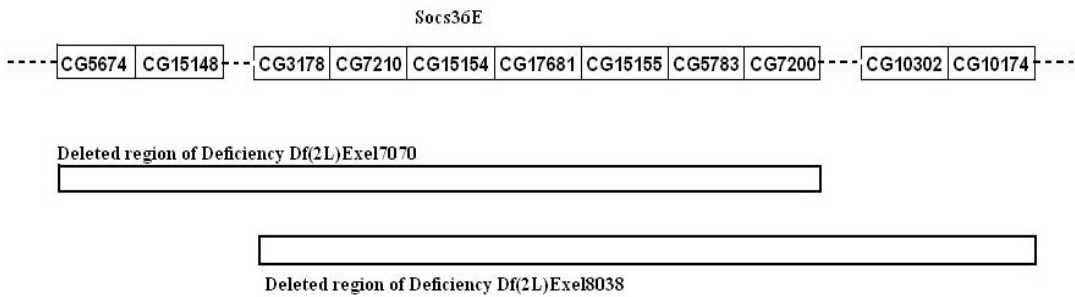


Fig. 2-3 Deletion region of deficiency lines *Excel7070* and *Excel8038*: The deficiency lines *Excel7070* and *Excel8038* have overlapping deletion region with  $SOCS36E$  ( $CG15154$ ) removed.

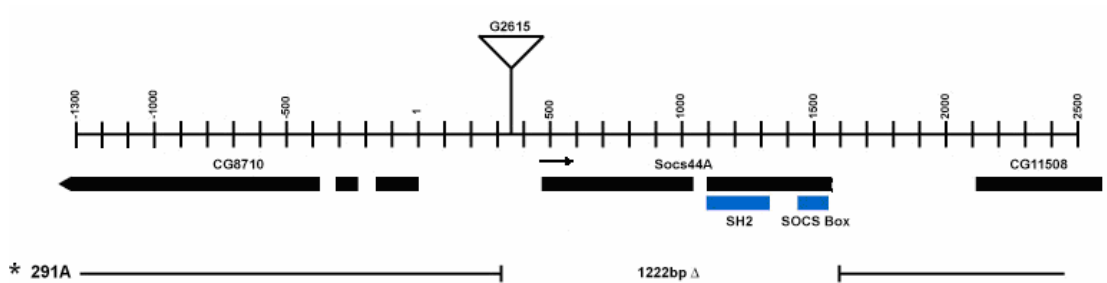


Fig. 2-4 Excision mutants of *SOCS44A* generated from insertion line *SOCS44A*<sup>G2615</sup> (by Susan Harrison) - The genomic organization of *SOCS44A* with direction of transcription and the position of *P* element insertion site are indicated. The deleted regions of the excision mutant lines are shown by gaps with sizes indicated.



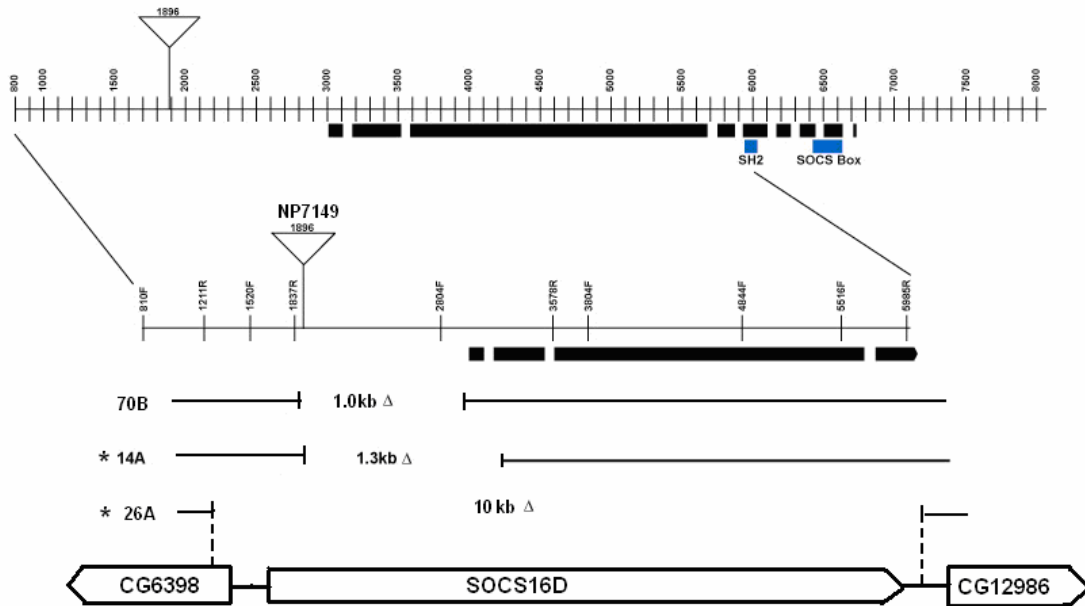
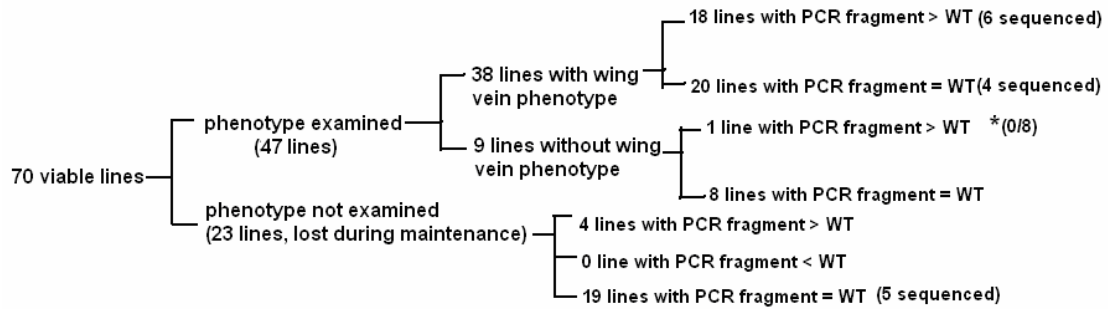


Fig. 2-5 Excision mutants of *SOCS16D* generated from insertion line *SOCS16D*<sup>NP7149</sup> (by Susan Harrison) - The genomic organization of *SOCS44A* with direction of transcription, and the position of P element insertion site are indicated; Endpoints of the deletions in 14A and 70B are shown. The deleted regions of the excision mutant lines are shown by gaps with sizes indicated. The asterisks mark the alleles chosen for mutational analysis.



*Table 2-1 PCR screen result from the SOCS36E viable excision lines. The 70 viable lines are grouped based whether they have the wing vein phenotype and the PCR fragment size compared with wild type. (Asterisk- only 8 homozygotes are available and examined, none of them has ectopic wing vein)*

	SOCS36E <sup>189A</sup>	SOCS36E <sup>190A</sup>	SOCS36E <sup>313B</sup>	SOCS36E <sup>EY06665</sup>	Df I 131
Homozygous	15/68 (22.1%)	40/122 (32.8%)	8/53 (15.1%)	17/30 (56.7%)	Lethal
/ Df I 131	17/30 (56.7%)	71/161 (44.1%)	44/75 (58.7%)	91/133 (68.4%)	Lethal
/ WT	5/45 (11%)	9/71 (12.7%)	10/108 (9.3%)	72/129 (55.8%)	20/68 (29%)

Table2-2 Frequency of ectopic wing vein phenotype: The penetrances of ectopic wing vein in homozygous incomplete excision mutants and in complementation test with deficiency line Df I 131 (a deficiency line that has SOCS36E removed) or wild type fly are shown.

	wt	SOCS36E190A	Excel7070
<b>Excel8038/</b>	<b>4/48</b> <b>(8.33%)</b>	<b>33/67</b> <b>(49.3%)</b>	<b>Lethal</b>
<b>Excel7070/</b>	<b>0/39</b>	<b>0/39</b>	<b>/</b>

Table 2-3 Frequency of ectopic wing vein phenotype: The penetrance of ectopic wing in homozygous complementation test of SOCS36E mutant and deficiency lines Excel8038 and Excel7070 with SOCS36E removed.

## Chapter III

### Interaction between *Socs* and JAK/STAT Pathway

#### The effect of *Socs* mutation on border cell number

#### INTRODUCTION

JAK/STAT pathway plays important role in *Drosophila* oogenesis. Each *Drosophila* egg chamber is covered by a monolayer of somatic cells, called epithelial follicle cells. Previous study in *Drosophila* shows the JAK activity is graded with highest concentration at the both poles of the egg, because of the secretion of the ligand *Upd* from the polar cells at the poles (Xi, McGregor et al. 2003). The gradient of JAK activity at poles plus the high level of EGFR activity at the posterior end specify the epithelial cell fates. (Fig.6, Xi, McGregor et al. 2003). Affected by the JAK and EGFR activity, at Stage 7, the epithelial cells will differentiate into five epithelial fates: border, stretched, centripetal, posterior, and main body cells. The JAK activity is shown to affect the anterior terminal fates and the identity of posterior cells.

The border cells are a cluster of 6-8 cells that migrate from the anterior to the oocyte at Stage 9. Previous studies show, at the anterior domain of an egg chamber, high level of JAK activity is shown to be necessary and sufficient to determine border cell fate. Ectopic expression of *upd* or *hop* in the anterior domain increases the number of border cells (Xi, McGregor et al. 2003). The loss of JAK activity in presumptive border cells leads to the failure of those cells to become border cells (Xi, McGregor et al. 2003). I examined the influence of *Socs* mutations on the border cell number to detect the function of *Drosophila Socs* genes.

The enhancer trap line 5A7 produces  $\beta$ -galactosidase in the border cells (Roth et al. 1995). Using the 5A7 enhancer trap line, I examined the border cell number in the *Socs36E* and *Socs44A* mutants. The change of border cell number is not statistically significant. I also used DAPI staining to preliminarily examine the border cell number in double mutants, *Socs16D*<sup>26A</sup>; *Socs36E*<sup>190A</sup> and *Socs16D*<sup>26A</sup>; *Socs44A*<sup>291A</sup>. However, since the DAPI staining is not specific for border cells, quantitative analysis can not be done by this assay.

## RESULTS

Previous study shows the level of JAK activity can effect the differentiation of the epithelial follicle cells. The JAK activity is required for the recruitment of the border cells, the identity of posterior cells, the anterior terminal fates, and the repression of main body fate. Ectopic JAK activity in the anterior domain increases the number of border cells (Xi, McGregor et al. 2003). Reduction of JAK activity reduces the number of border cells (Silver and Montell 2001; Xi, McGregor et al. 2003). If *Drosophila Socs* genes can negatively regulate JAK/STAT pathway, I would expect that the *Socs* mutants have increased JAK activity and therefore have increased border cell number.

I used the 5A7 enhancer trap line to mark the border cells in *Socs* mutants and *cn bw sp* (wild type control) flies. I grew the flies at room temperature and compared the border cell number in *Socs* mutants with that in *cn bw sp* flies using  $\beta$ -gal staining technique. The average border cell numbers in *Socs36E<sup>190A</sup>* and *Socs44A<sup>4291A</sup>* are slightly increased compared with the wild type control. The average border cell number is 5.36 in *Socs36E<sup>190A</sup>*, while the average is 4.87 in wild type control; the average border cell number is 5.25 in *Socs44A<sup>4291A</sup>* while the 4.91 in wild type control. I also record the number of flies with certain amount of border cells and looked for the shift of the border cell number distribution (Fig. 3-1; Fig. 3-2). However, when we used Wilcoxon rank sum test to compare the distribution of the border cell number in the mutants and the wild type (done by Rui Li), no significant change was detected (p-value>0.05).

## DISCUSSION

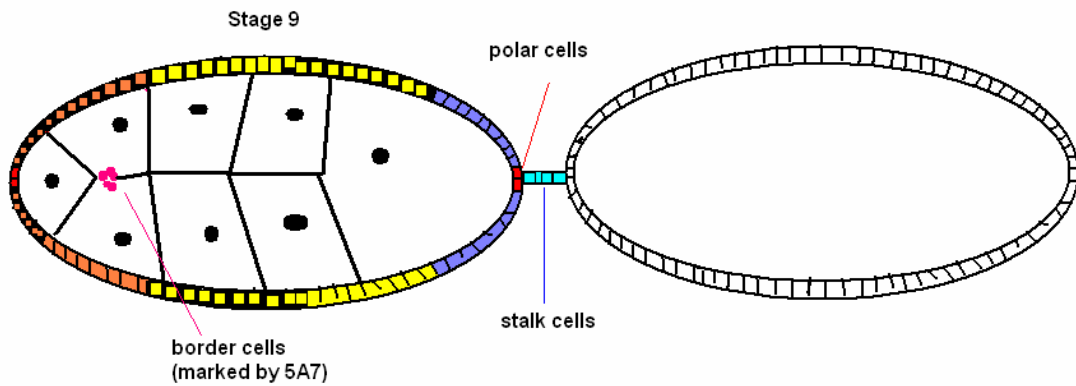
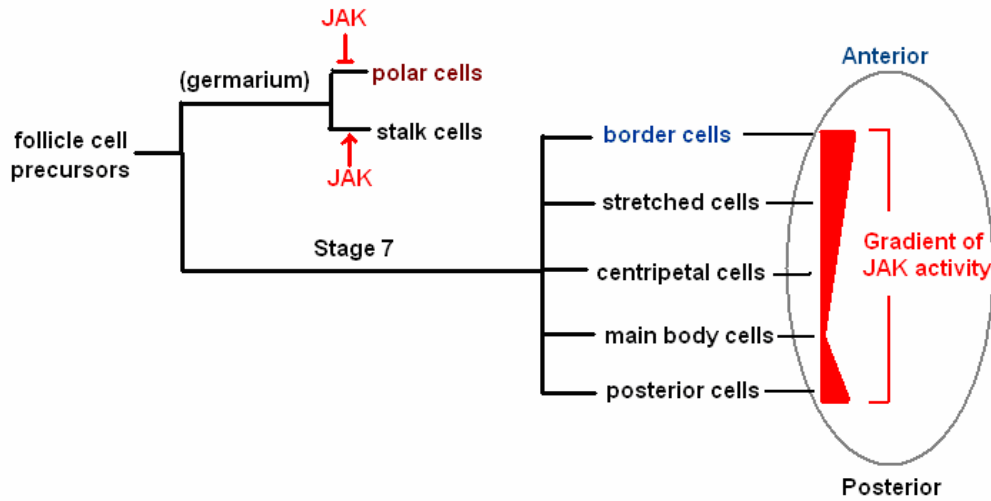
JAK/STAT pathway is required in *Drosophila* oogenesis, and its gradient affects the differentiation of follicular epithelial cells including the recruitment of border cells. Therefore, I expected to see an increase of border cell number caused by the excessive JAK activity in the *Socs* mutants. However, when I used the enhancer trap line 5A7 to mark the border cell in the *Socs36E* and *Socs44A* mutants, no significant change in the border cell number was detected. This might be caused by gene redundancy, since there are three *Drosophila Socs* genes. In addition, the line *cn bw sp* may not be a good wild type control. I should use precise excision lines from the screen as control, because the precise excision lines have the same genetic background as that of our *Socs* mutants.

When I used DAPI staining to check the border cell in the double mutants, *Socs16D*<sup>26A</sup>; *Socs36E*<sup>190A</sup> and *Socs16D*<sup>26A</sup>; *Socs44A*<sup>291A</sup>, no obvious change was detected (Fig. 3-3). However, since DAPI staining is not specific for border cell, I could not do quantitative analysis on the border cell number in the double mutant. More sensitive method, like enhancer trap lines, should be used to analyze the number of border cells in the double mutants. Because of the gene redundancy, it is possible that I did not knock out enough *Socs* genes in the double mutants. Therefore, the double mutant of *Socs36E* and *Socs44A*, and the triple mutant of the three *Socs* genes should be made to further analyze the role of *Drosophila Socs* genes in the JAK/STAT pathway and oogenesis.

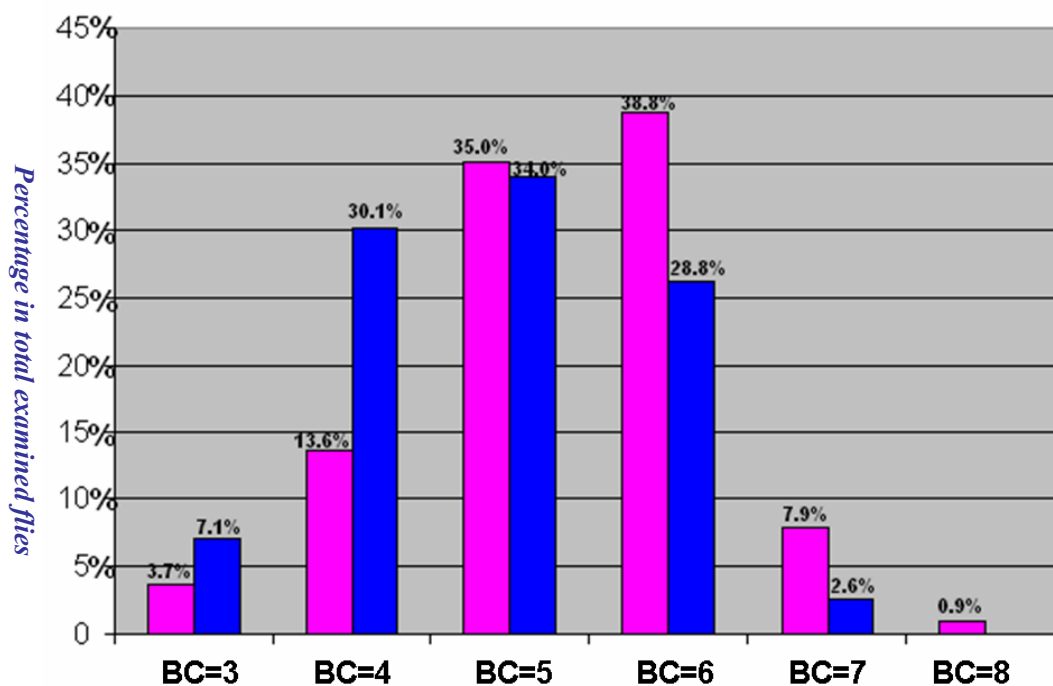
The border cells in the double mutants, *Socs16D*<sup>26A</sup>; *Socs36E*<sup>190A</sup> and *Socs16D*<sup>26A</sup>; *Socs44A*<sup>291A</sup>, were preliminarily examined by DAPI staining. We used precise excision lines *Socs36E*<sup>330B</sup> and *Socs44A*<sup>rev-129A</sup> as wild type control, which have the P element precisely excised and have the wild type sequence left. Because DAPI staining is not specific for border cells, it is very difficult to tell and count the border cells from the DAPI staining results (Fig. 3-3). More specific assay should be used to mark the border cells in the *Socs* double mutant flies.

It may be useful to check the effect of *Socs* mutations at some extreme condition, like in the mutants with ectopic JAK activity, because the compensation effect by the gene redundancy would be decreased or eliminated when the negative regulation is in great demand. I can also check the effect of *Socs* mutations on other epithelial cells, like the polar cells and the stalk cells. The differentiation of the stalk cells and the polar cells is affected by the JAK activity. Previous study shows the reduction or removal of JAK/STAT components in the ovarian somatic cells results in additional polar cells at the expense of the stalk cells (McGregor, Xi et al. 2002). Therefore, the JAK activity may either allow the adoption of the stalk cell fate or prevention of the polar cell fate (McGregor, Xi et al. 2002). If the three *Socs* genes are negative regulators of the JAK/STAT pathway as I expected, the increase of JAK activity in the *Socs* mutants

should increase the number of the stalk cells.



*Fig.3-1 The effect of JAK activity on the differentiation of follicle cells: The differentiation of stalk cells and polar cells in the germarium is affected by JAK activity. JAK activity may either allow the adoption of stalk cell fate or prevention of polar cell fate. Gradient of JAK activity effects the epithelial cell differentiation. JAK activity is required for the recruitment of border cells, the identity of posterior cells, anterior terminal fates, and repression of main body fate. The process of oogenesis is also shown with different follicular cell fates indicated.*



Number of border cell

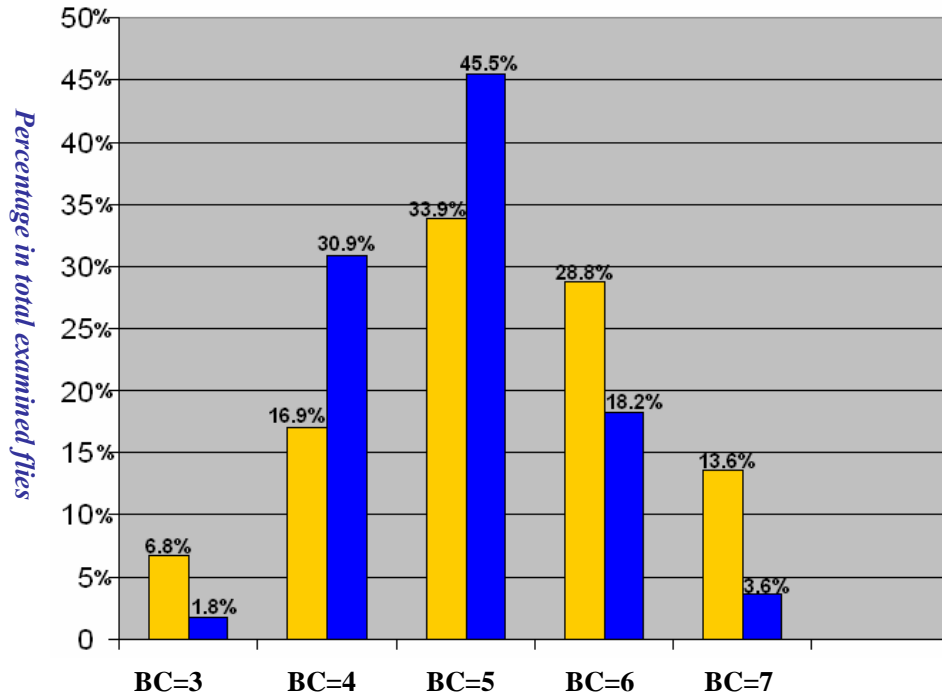
■ SOCS36<sup>190A</sup>/SOCS36<sup>190A</sup>; 5A7/+

■ +/+; 5A7/+

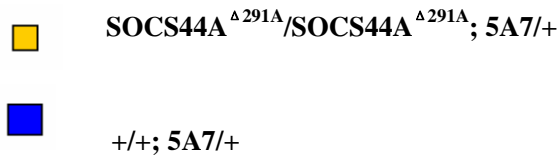
	3 BC	4BC	5BC	6BC	7BC	8BC	Ave BC
<b>190A/190A; 5A7/+</b>	<b>8</b>	<b>29</b>	<b>75</b>	<b>83</b>	<b>17</b>	<b>2</b>	<b>5.36</b>
<b>+/+; 5A7/+</b>	<b>11</b>	<b>47</b>	<b>53</b>	<b>41</b>	<b>4</b>	<b>0</b>	<b>4.87</b>

Fig. 3-2 The distributions of border cell number in SOCS36 mutant (SOCS36<sup>190A</sup>/SOCS36<sup>190A</sup>; 5A7/+) and wild type (+/+; 5A7/+) flies. Y-axis is the percentage that flies with certain amount of border cells take in total examined flies; X-axis is the number of border cell. The number of flies with different border cell number is indicated in the form below the figure. The distribution of border cell number in SOCS36E mutant is shifted a little to the right, compared with the wild type. By Wilcoxon rank sum test to analyze the location shift, the P-value is 0.6991 (calculated by Rui Li), which indicates the shift is not significant. "BC"=Border Cell



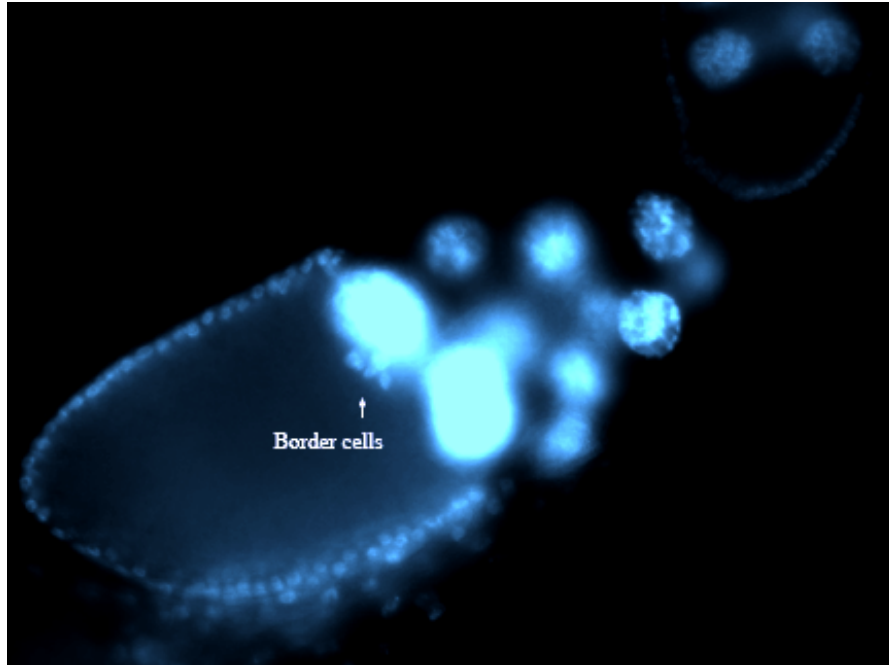


Number of border cell



	3BC	4BC	5BC	6BC	7BC	8BC	Ave BC
Δ291A/Δ291A; 5A7/+	4	10	20	17	8	0	5.25
+/+; 5A7/+	1	17	25	10	2	0	4.91

Fig. 3-3 The distributions of border cell number in SOCS44A mutant (SOCS44A<sup>Δ291A</sup>/SOCS44A<sup>Δ291A</sup>; 5A7/+) and wild type (+/+; 5A7/+) flies. Y-axis is the percentage that flies with certain amount of border cells take in total examined flies; X-axis is the number of border cell. The number of flies with different border cell number is indicated in the form below the figure. The distribution of border cell number in SOCS44A mutant is shifted a little to the right, compared with the wild type. By Wilcoxon rank sum test to analyze the location shift, the P-value >0.05 (calculated by Rui Li), which indicates the shift is not significant. "BC"=Border Cell



*Fig. 3-4 DAPI staining result of the Socs double mutant ovaries. Because DAPI staining is not specific for border cells, it is very difficult to tell and count the border cells. The position of border cells is marked by arrow.*

## The effect of Socs mutation on tumor formation of *hop*<sup>Tum-1</sup>

### INTRODUCTION

Many cytokines in mammals can stimulate JAK/STAT pathway and induce the proliferation and differentiation of many cell types, including blood cells. Excessive JAK activity can lead to leukemia. The constitutive kinase activity in the TEL-JAK2 fusion protein causes a specific form of acute lymphocytic leukemia (ALL) (Lacronique et al., 1997).

The *Drosophila* JAK activity is also involved in hematopoiesis (Harrison et al., 1995; Luo et al., 1995). *Drosophila* has two lineages of hemocytes, including plasmatocytes that can terminally differentiate into lamellocytes in hemolymph, and crystal cells (Dearolf, 1998). *hop*<sup>Tum-1</sup> is a dominant gain-of-function mutant with a single amino acid changed in *hop*, the JAK kinase in the *Drosophila* JAK/STAT pathway. *hop*<sup>Tum-1</sup> mutation causes excessive concentration of circulating plasmatocytes and premature differentiation of lamellocytes, which leads to the formation of melanotic tumors and lethality (Harrison, Binari et al. 1995; Dearolf, 1998). The formation of melanotic tumors, which are black spots on the body of *hop*<sup>Tum-1</sup> larvae or adult flies (Fig. 4-1), is dominant and temperature sensitive, which is enhanced at a temperature higher than 25°C. *hop*<sup>Tum-1</sup> is recessive lethal at restrictive temperature (Harrison, Binari et al. 1995; Dearolf, 1998). Previous studies show that the decrease in JAK/STAT activity will suppress the formation of melanotic tumors (Hou et al., 1996; Dearolf, 1998), while the over activity of *hop* will cause the melanotic tumor formation (Harrison et al., 1995).

I compared the percentage of females with tumors between the *hop*<sup>Tum-1</sup>/+ adult flies and the *hop*<sup>Tum-1</sup>/+; *Socs/Socs* adult flies at 25°C. I saw a significantly higher percentage of females with tumors in the *hop*<sup>Tum-1</sup>/+; *Socs/Socs* adult flies (Fig. 4-2; Fig 4-3), when using the C/S flies as control. Similar increase of tumor formation was seen when I used the precise excision lines from the screen as a control, which have the P element precisely excised and have wild type sequence restored (Fig. 4-4; Table 4-5).

## RESULTS

Since the melanotic tumor in *hop<sup>Tum-l</sup>* flies is caused by excessive JAK activity, if *Drosophila Socs* genes negatively regulate the JAK/STAT pathway, we would expect to see an enhancement of melanotic tumor formation in *hop<sup>Tum-l</sup>/+; Socs/Socs* flies, compared with *hop<sup>Tum-l</sup>/+* adult flies. I grew the mutant and control flies in the same 25°C incubator and calculated the percentage of adult female flies with black spots on the body. I only counted the female adult flies (*hop<sup>Tum-l</sup>/+*) with tumors, because almost all the male flies (*hop<sup>Tum-l</sup>/Y*), including both the mutant and the control, have melanotic tumors formed on the body. When I tried to compare the tumor formation at the restrictive temperature (29°C), high lethality was observed in *hop<sup>Tum-l</sup>* flies, which made it very difficult to get enough *hop<sup>Tum-l</sup>* animals.

Adult female flies were scored under a dissecting microscope as positive if they had at least one melanotic tumor on the body. When I used the C/S flies as control at first, I saw significant increase of the number of female flies with tumor formation in both the *Socs36E* and *Socs44A* mutants (*hop<sup>Tum-l</sup>/+; Socs/Socs*) at 25°C (Fig. 4-2; Fig. 4-3), compared with the *hop<sup>Tum-l</sup>/+* flies. Similar increase was also seen in the *Socs16D* mutant (done by Sudakshina Paul, data not shown). When the precise excision lines, *Socs36E<sup>rev-330B</sup>* and *Socs44A<sup>rev-129A</sup>*, were used as controls, I saw similar significant increase of tumor formation at 25°C (Fig. 4-4; Fig. 4-5). *Socs36E<sup>rev-330B</sup>* is a sequenced precise excision line, which has the wild type sequence and ectopic wing vein phenotype with penetrance of about 50%. Because *Socs36E<sup>rev-330B</sup>* and the *Socs36E* mutants are from the same screen, and *Socs36E<sup>rev-330B</sup>* also has the genetic background that causes the wing vein phenotype, the only difference between *Socs36E<sup>rev-330B</sup>* and the *Socs36E* mutants is the genomic sequence at the original P element insertion site, which makes the precise excision line a very good control. *Socs44A<sup>rev-129A</sup>* is a precise excision line with the wild type genomic sequence. Therefore, the increase of tumor formation I saw in the *Socs* mutants could not be caused by the genetic background of the original insertion stocks used in our screen, but by the *Socs* mutations.

## DISCUSSION

The JAK/STAT pathway plays an important role in immunity and hematopoiesis. Excessive JAK activity in gain-of function mutant *hop<sup>Tum-l</sup>* causes over-proliferation of blood cell which leads to the formation of melanotic tumor and lethality. If the *Drosophila Socs* genes negatively regulate the JAK/STAT pathway, the loss-of-function mutation of *Socs* should have excessive JAK activity which might enhance the tumor formation in *hop<sup>Tum-l</sup>* flies. I saw significant increase of tumor formation in all the three *Socs* mutants when I used C/S flies as the control (Fig. 4-2; Fig. 4-3). I further confirmed the result using precise excision lines from the same screen as control (Fig. 4-4; Fig. 4-5). Since the precise excision lines and imprecise lines are from the same screen, they have the same genetic background. In addition, the precise excision line *Socs36E<sup>rev-330B</sup>* also has the semi-dominant wing vein phenotype but have wild type sequence at the P element insertion site, therefore I can rule out the high frequency of tumor formation in *Socs36E* mutant is caused by something carried in the genetic background which leads to the ectopic wing vein. Because the only difference between the precise excision lines and the imprecise mutant lines is the sequence of the *Socs* genes, the higher percentage of tumor formation I saw in the *Socs* mutants must be caused by the *Socs* mutations.

The *Socs* mutations can significantly increase the tumor formation in the *hop<sup>Tum-l</sup>* flies at room temperature, while have no obvious phenotypes themselves. It is very interesting to find that the effect of *Socs* mutation is moderate in a normal condition without stress (the border cell number change is slight in the *Socs* mutants); but in some extreme condition (e.g. with too much JAK activity in the *hop<sup>Tum-l</sup>* flies), the regulatory effect of *Socs* is very obvious (the enhancement of tumor formation by the *Socs* mutants is significant). Therefore, the *Socs* genes of *Drosophila* seem to have conserved regulatory function to buffer against perturbations in the JAK activity.

We would confirm the regulatory function of *Drosophila Socs* genes under other extreme conditions. For example, we would examine the phenotype of the *Socs* mutants under some stress (high temperature/starvation); we would examine the effect of the *Socs* mutations on the border cell number of flies with ectopic JAK activity (*hop<sup>Tum-l</sup>* flies); we would also examine the effect of *Socs* double or triple mutants on the tumor formation of *hop<sup>Tum-l</sup>* flies. Other JAK/STAT mutants with ectopic JAK activity can also be used to detect the function of *Drosophila Socs*. For example, we can use *GMR-upd* flies, which have a large eye phenotype due to the increase of cell number caused by ectopic JAK/STAT pathway. We would test whether the three *Drosophila Socs* mutants could modify the large eye phenotype of *GMR-upd*. We expect to see an enhanced large eye phenotype when combining the *Socs* mutation with *GMR-upd*.



*Fig. 4-1 The melanotic tumor on  $hop^{Tum-1}$  adult flies: the melanotic tumor appears as black spots on the body of  $hop^{Tum-1}$  larvae or adult flies.*

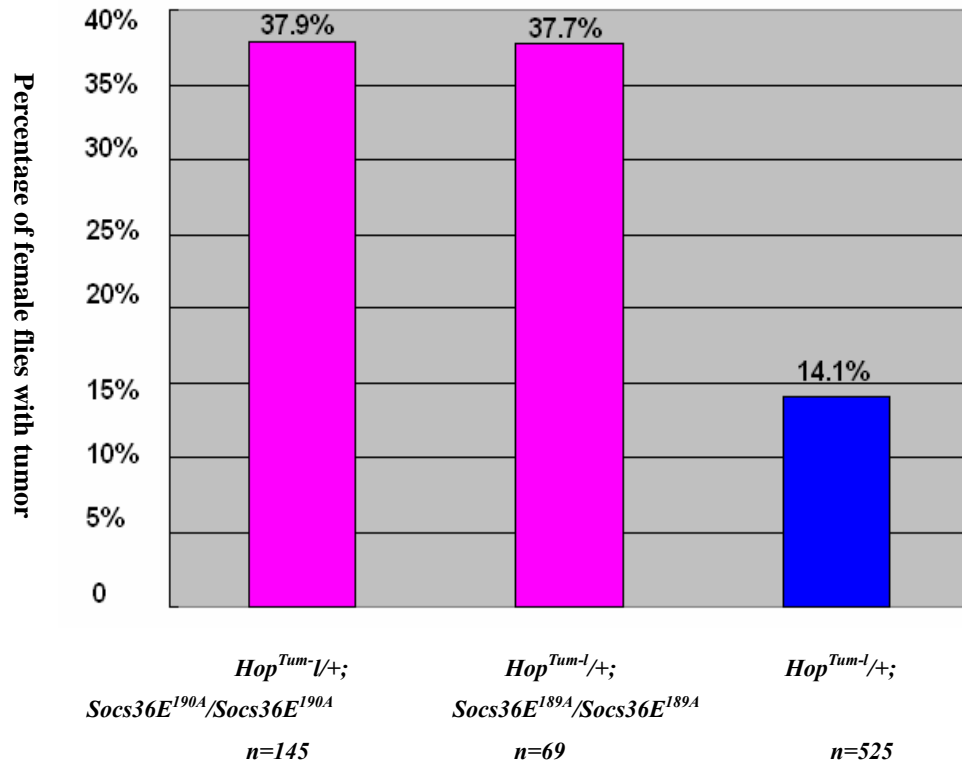


Fig. 4-2 The effect of *Socs36E* mutation on tumor formation of *Hop<sup>Tum-1</sup>* flies: *Socs36E* incomplete excision mutants (*Socs36E<sup>190A</sup>* and *Socs36E<sup>189A</sup>*) significantly enhance the tumor formation in *Hop<sup>Tum-1</sup>* flies ( $p$ -value $<0.0001$ ) compared with wild type control (C/S) at 25°C. X-axis shows the genotypes; y-axis shows the percentage of flies with tumor. The two pink bars indicate the percentage of flies with tumor in the two *Socs36E* mutants; the blue bar indicates the percentage in the wild type control. The percentage number of flies with tumor in each genotype is indicated above each bar. “N”=the total number of examined flies.

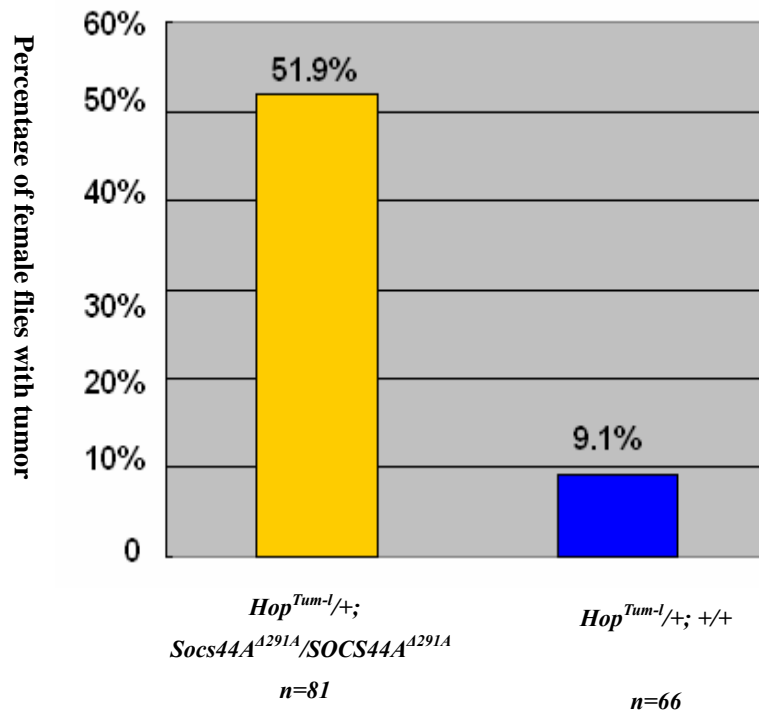


Fig. 4-3 The effect of *Socs44A* mutation on tumor formation of *Hop<sup>Tum-1</sup>* flies: *Socs44A* deletion mutants (*Socs44A<sup>A291A</sup>*) significantly enhance the tumor formation in *Hop<sup>Tum-1</sup>* flies ( $p$ -value<0.0001) compared with wild type control (C/S) at 25°C. X-axis shows the genotypes; y-axis shows the percentage of flies with tumor. The yellow bar indicates the percentage of flies with tumor in *SOCS44A* mutant; the blue bar indicates the percentage in the wild type control. The percentage number of flies with tumor in each genotype is indicated above each bar. “N”=the total number of examined flies.



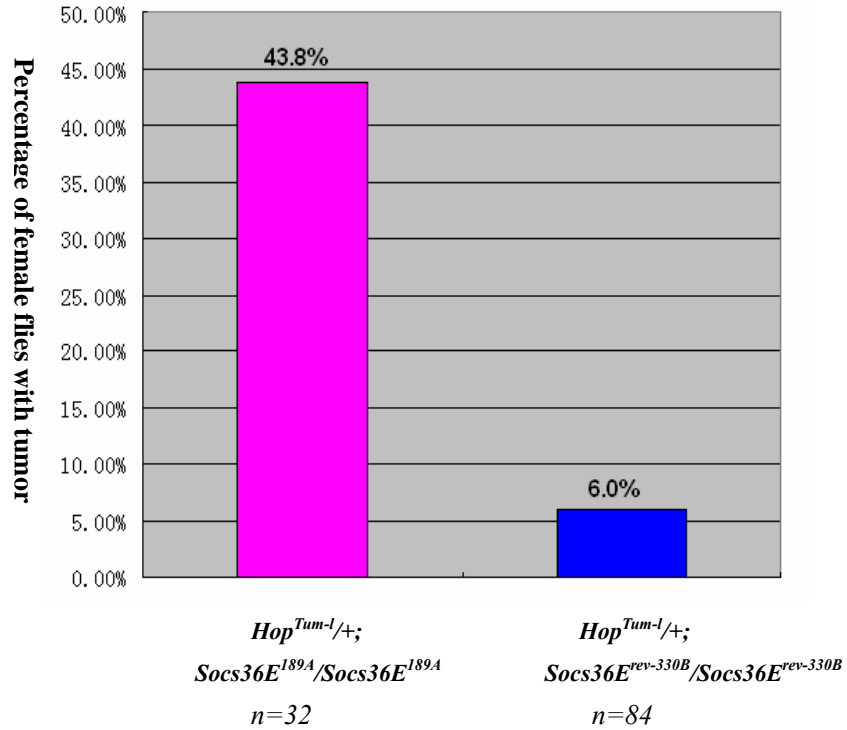


Fig. 4-4 The effect of *Socs36E* mutation on tumor formation of *Hop<sup>Tum-1</sup>* flies: *Socs36E* incomplete excision mutants (*Socs36E<sup>189A</sup>*) significantly enhance the tumor formation in *Hop<sup>Tum-1</sup>* flies ( $p\text{-value} < 0.0001$ ) compared with precise excision control (*Socs36E<sup>rev-330B</sup>*) at 25°C. X-axis shows the genotypes; y-axis shows the percentage of flies with tumor. The pink bar indicates the percentage of flies with tumor in *Socs36E* mutants; the blue bar indicates the percentage in the control. The percentage number of flies with tumor in each genotype is indicated above each bar. “N”=the total number of examined flies.

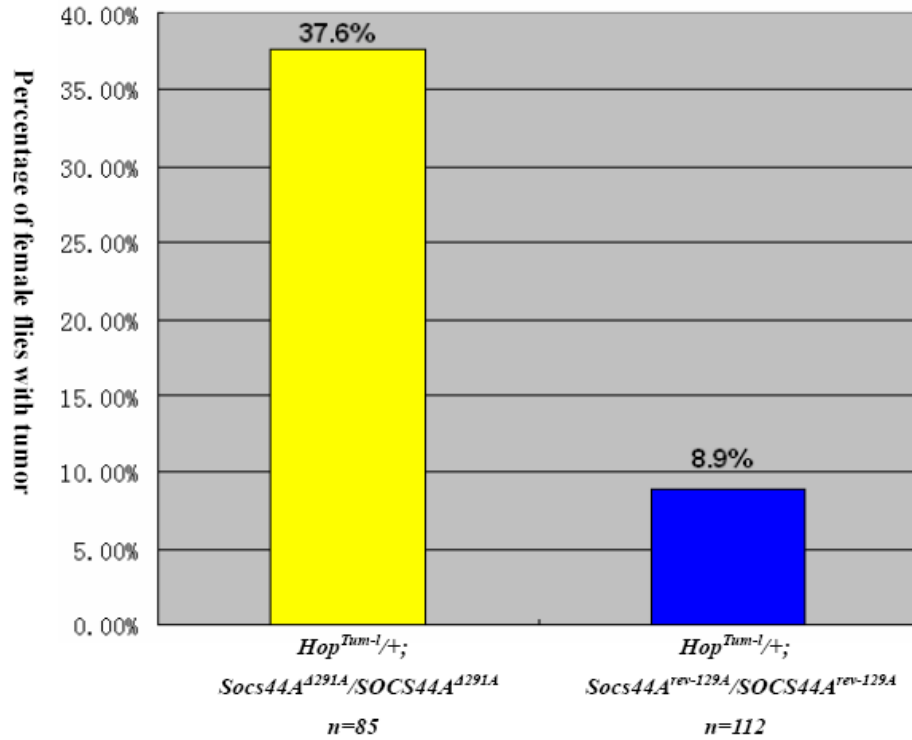


Fig. 4-5 The effect of *Socs44A* mutation on tumor formation of *Hop<sup>Tum-1</sup>* flies: *Socs44A* deletion mutants (*Socs44A<sup>291A</sup>*) significantly enhance the tumor formation in *Hop<sup>Tum-1</sup>* flies ( $p$ -value<0.0001) compared with precise excision control (*Socs44A<sup>rev-129A</sup>*) at 25°C. X-axis shows the genotypes; y-axis shows the percentage of flies with tumor. The yellow bar indicates the percentage of flies with tumor in *Socs44A* mutants; the blue bar indicates the percentage in the control. The percentage number of flies with tumor in each genotype is indicated above each bar. “N”=the total number of examined flies.

## Chapter IV

### Conclusion and Discussion

The JAK/STAT signaling pathway has biological conserved functions in the development of species. In mammals, the *Socs* are the largest class of JAK/STAT negative regulators. Three *Socs* homologues are found in *Drosophila* genome based on sequence similarity, including *Socs36E*, *Socs44A* and *Socs16D*. In this thesis, we used standard P element excision screens to generate loss-of-function mutants of the three *Drosophila Socs*. The double mutants of *Socs16D* and *Socs36E*, *Socs16D* and *Socs44A* were also made. I used these mutants to examine the function of *Drosophila Socs* and found them have potential regulatory function to buffer against perturbations in the JAK/STAT pathway.

#### *Socs mutations*

To study the function of *Drosophila Socs*, we used standard P element excision screens and generated mutants of the three *Drosophila Socs* genes from transposon insertion lines. Using PCR and sequencing, we confirmed imprecise excision mutants of *Socs36E*, *Socs44A* and *Socs16D*. *Socs36E* incomplete excision mutants have the semi-dominant ectopic wing vein. However, the wing vein phenotype can be complemented by deficiency line *Df(2L)Excel7070* which has *Socs36E* deleted. Therefore, the ectopic wing vein might be caused by something in the genetic background of the original insertion line, other than the loss of *Socs36E*. No obvious morphological phenotype is observed in the *Socs44A* and *Socs16D* mutants. I also made double mutants, *Socs16D*<sup>26A</sup>; *Socs36E*<sup>189A</sup> and *Socs16D*<sup>26A</sup>; *Socs44A*<sup>Δ291A</sup>. However, no obvious morphological mutant phenotype was found either. It is possible that the three *Drosophila Socs* genes have some functional redundancy, so even if one or two of the *Socs* are knocked-out, the effect can be compensated by the remaining *Socs*. The similar redundancy is seen in the mammalian *Socs*. SOCS4 and SOCS5 have similar structures and both negatively regulate the EGFR pathway (Kario et. al, 2005). However, the *Socs5* knockout mice develop normally and have no mutant phenotype (Brender et al, 2004), which indicates *Socs4* and *Socs5* have functional redundancy. *CIS* is most similar to *Socs2*, and both of them interact with the cytokine receptors. But the knockout mice of *CIS* have no phenotype, while the *Socs2* deficient mice develop gigantism (Cooney, 2002; Alexander, 2002), which also suggests functional redundancy between *CIS* and *Socs2*. *Socs6* and *Socs7* show high protein identities and only *Socs7* mice exhibit hydrocephalus phenotype while *Socs6* mice have very mild phenotype (Krebs et al., 2002&2004). It is possible that the functional similarity observed in mammals also exist in *Drosophila*. To answer this question, it is necessary to analyze the double and triple mutants of *Drosophila Socs*.

We would try to make the double mutant *Socs36E*<sup>189A</sup> *Socs44A*<sup>Δ291A</sup>. However, *Socs36E* and *Socs44A* are on the same chromosome (the second chromosome) and very near to each other, the recombinant frequency is about 0.04, I have not successfully generate the double mutant *Socs36E*<sup>189A</sup> *Socs44A*<sup>Δ291A</sup>. Further analysis on the double mutants and the making of triple mutant might be helpful to identify the function and explain the redundancy of *Drosophila Socs* genes.

### **The effect of *Socs* mutations on *Drosophila* oogenesis**

The JAK/STAT pathway plays an important role in *Drosophila* oogenesis. Previous studies in our lab show that ectopic expression of *upd* or *hop* in the anterior domain increases the number of border cells (Xi, McGregor et al. 2003); The loss of JAK activity in presumptive border cells leads to the failure of those cells to become border cells (Xi, McGregor et al. 2003). I used enhancer trap line *5A7* to examine the border cell number in the *Socs36E* and *Socs44A* mutants. However, no significant change in the border cell number is found. It is possible that under normal conditions the knock out of some but not all the *Socs* can be compensated by remaining *Socs*. However, it is reasonable to assume that under extreme conditions, for instance when there is too much JAK activity but not enough negative regulators to control the ectopic pathway, the compensation effect of remaining *Socs* may be weaken or get lost. Therefore, it may be useful to check the effect of *Socs* mutations at some extreme condition, like in the mutants with ectopic JAK activity. We can also check the effect of *Socs* mutations on other epithelial cells, like polar cells and stalk cells. The differentiation of stalk cells and polar cells is affected by the JAK activity. Previous study shows reduction or removal of JAK/STAT components in the ovarian somatic cells results in additional polar cells at the expense of stalk cells (McGregor, Xi et al. 2002). Therefore, the JAK activity may either allow the adoption of the stalk cell fate or prevention of the polar cell fate (McGregor, Xi et al. 2002). If the three *Socs* genes are negative regulators of the JAK/STAT pathway as we expected, the increase of JAK activity in the *Socs* mutants should increase the number of stalk cells. We can use antibody to enhancer trap marker *PZ80* to mark polar cell fate, and identify stalk cells by enhancer trap marker *93F*. If *Drosophila Socs* negatively regulate the JAK/STAT pathway, we would expect an increase of stalk cells and a decrease of polar cells in the three *Drosophila Socs* mutants. Using enhancer trap lines to examine the differentiation of follicular epithelial cells in the double or triple mutants is also helpful to understand the role of *Drosophila Socs* in oogenesis.

### **Genetic interaction between *Socs* and other JAK/STAT components**

The *Drosophila* JAK activity is involved in hematopoiesis (Harrison et al., 1995; Luo et al., 1995). *hop*<sup>Tum-1</sup>, a dominant gain-of-function mutant, causes excessive concentration of circulating plasmatocytes and premature differentiation of lamellocytes, and leads to the formation of melanotic tumors (Harrison, Binari et al. 1995; Dearolf, 1998) and

lethality. I saw significant increase of tumor formation in all the three *Socs* mutants when I used C/S flies as the control (Fig. 4-2; Fig. 4-3). I further confirmed the result using precise excision lines from the same screen as the control (Fig. 4-4; Fig. 4-5). Since the precise excision lines and imprecise lines are from the same screen, they have the same genetic background as that of the imprecise excision mutant lines. Therefore, the higher percentage I saw in the *Socs* mutants should be caused by the *Socs* mutations.

It is very interesting to find that the effect of *Socs* is moderate in a normal condition without stress (the border cell number change is slight in the *Socs* mutants); but in some extreme condition (e.g. with too much JAK activity in *hop<sup>Tum-l</sup>* flies), the regulatory effect of the *Socs* is very obvious (the enhancement of the tumor formation by the *Socs* mutants is significant). Therefore, the *Socs* genes of *Drosophila* seem to have conserved regulatory function to buffer against perturbations in the JAK activity. The function of the *Drosophila Socs* genes may be to negatively regulate the JAK/STAT pathway at extreme conditions.

To further confirm the buffering regulatory function of the *Drosophila Socs* genes, we would examine the phenotype of *Socs* mutants under other extreme conditions (high temperature/starvation); we would examine the effect of *Socs* mutations on the border cell number of flies with ectopic JAK activity (*hop<sup>Tum-l</sup>* flies). We would also try to examine the effect of *Socs* double or triple mutants on the tumor formation of *hop<sup>Tum-l</sup>*. Besides *hop<sup>Tum-l</sup>*, there are other tools we can use to examine the genetic interactions between the *Drosophila Socs* genes and the components in the JAK/STAT pathway, for example, the *GMR-upd* flies. The *GMR-upd* flies have a large eye phenotype due to the increase of cell number; the severity of the eye phenotype is very sensitive to the change of the JAK/STAT activity (Bach, Vincent et al. 2003). Since the large eye is caused by over-expression of *upd* - the ligand of the JAK/STAT, decrease in the JAK/STAT activity will reduce the large eye phenotype, while increase in the JAK/STAT activity will enhance the large eye phenotype. We would test whether the three *Drosophila Socs* mutants could modify the large eye phenotype of *GMR-upd*. We expect to see an enhanced large eye phenotype when combining the *Socs* mutation with *GMR-upd*.

### **Future work**

In the future, it would be very necessary to make the double mutant of *Socs36E* with *Socs44A*, and the triple mutant. These mutants would be helpful to clarify the function and gene redundancy of the *Drosophila Socs* family.

Because of functional redundancy, we have not detected any visible phenotype of *Socs* mutations. The use of biochemical method can help us understand the mechanism of *Socs* function. Biochemical study on mammalian SOCS suggests that SOCS proteins inhibit signal cascades by binding phosphorylated receptor or JAK. SOCS could even binds to

STAT and attenuate their nuclear translocation (Martens, Uzan et al. 2005). Identifying the interacting proteins will help us to understand how *Drosophila Socs* genes regulate the signaling pathways. We plan to use Tandem Affinity Purification (TAP) method to identify the proteins interacting with *Drosophila* SOCS. TAP system allows rapid purification of protein complex under native condition. By fusing target protein with the double-affinity TAP tag, the protein complex will be purified by two consecutive steps. The individual purified proteins will be sent to subsequent mass spectrometry identification. We hypothesize the three *Drosophila* SOCS proteins interact with ubiquitin-transferase complex, components in the JAK like DOME, JAK or STAT, components in the EGFR pathway like the EGFR receptor, RasGAP, *argos*, *Rhomboid*, and perhaps some components in other pathways.

## Chapter V

### Materials and Methods

#### ***Fly strains***

The fly strains used in the experiments were raised at 25° C, unless otherwise stated. *Socs36E<sup>EY6665</sup>*, *Df(2) I131*, *Df(2L)Exel8038*, *Df(2L)Exel7070*, *cn bw sp* can be found in Flybase. *Socs44A<sup>G2615</sup>* can be found in GenExel. *Socs16D<sup>NP7149</sup>* was purchased from Kyoto. *y v hop<sup>Tum-1</sup> m/ Basc* was obtained from Dearolf, C. R (Dearolf, 1998). Enhancer marker line *5A7* was obtained from St. Johnston (González-Reyes and St Johnston, 1994).

#### ***P-element excision mutagenesis***

The *EY6665* viable P-element was excised in the germline of males of genotype *EY6665{y+ w+}/CyO; Δ2-3, Sb/+*. These male were crossed individually with *y w; Sco/CyO* females and the progeny were examined for excision based on the loss of *y+* and *w+* markers associated with the P-element. Progeny of genotype *yw; EY6665{y-w-}/CyO* were crossed to *yw; Sco/CyO* to established balanced stocks, each representing an independent excision event. For each viable line, genomic DNA was isolated from homozygous animals. For all the viable balanced stocks, a 600bp fragment of the *Ric* locus (positive control) and a 300bp fragment of the region flanking the insertion were simultaneously amplified by PCR. In parallel, a reaction containing all the PCR reagents except the template DNA was used as a negative control. Once an imprecise excision line was found, more primer pairs and sequencing would be used to confirm the sequence of the excision region.

Similar P-element excision screens and PCR method were used for *SOCS44A* and *Socs16D* mutants (done by Susan Harrison). In the PCR screen of *SOCS44A* and *Socs16D*, deletion mutants were picked. The deletion mutants were found by the negative PCR results using primers flanking the original P element insertion site. Once a deletion mutant line was detected, more primer pairs and sequencing would be used to confirm the sequence of the deletion region.

#### ***Immunological staining***

Antibody staining was performed as previously described (McGregor et al., 2002). Primary antibody used was rabbit  $\alpha$  -  $\beta$  -galactosidase (5'-3') at 1: 500. Secondary antibody was Rhodamine Red-X-  $\alpha$  -mouse at 1: 500 (Jackson Immunolabs).

The staining results were captured by a Nikon E800 microscope.

### ***Tumor Suppression Experiments***

Melanotic tumors were identified under a dissecting microscope (ZEISS) and adult female flies were scored as positive if they had at least one melanotic tumor on the body. Flies were grown at 25° C incubator under non-crowded conditions.



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