臺灣二００七年國際科學展覽會

科　　別：動物學

作 品 名 稱：The unknown gene interacts with dll , abdA,Ubx

得 獎 獎 項：第一名
美國團隊正選代表：美國第 58 屆國際科技展覽會

學校 / 作者　：臺北市立建國高級中學　　賴才達
臺北市立建國高級中學　　陳泓任

About the students

Left: Lai Tsai-Ta & Right: Chen Hung-Jen

We are Lai Tsai-Ta and Chen Hung-Jen, students from Taipei Municipal Chein-Kuo Senior High School. We are both interested in molecular biology, biochemistry and zoology. These subjects are attractive to us. Since we attended senior high school, we have been concentrated on learning biology. We had a dream of participating Taiwan science fair and representing our country to the international one. So fascinating the dream was, we have participated in the Biology Program of Senior High School Students in Academia Sinica and have started this research since 2005.

As high school students in Taiwan, we have to deal with such heavy pressure from the schoolwork. Although we are now in the third year of our high school lives, which means that we are facing the entrance examination, we still make up our mind to overcome the challenge of handling schoolwork, entrance examination and science display simultaneously even we know that we will tire ourselves out.

And now, we are here participating this meaningful science fair. We will make the best of ourselves to make our dream come true.

Abstract

We operated the misexpression screen between the EP lines and the pattern lines with the genotypes of *eq1>dll*, *eq1>abdA*, *eq1>Ubx*, *eq1-GAL4*, *ey-GAL4* or *dpp-GAL4*. After the screening, we found that five of these 1,800 strains of filial generation had special phenotypes. It had shorter antennae and defects in the anterior equatorial region of eyes. We used plasmid rescue and IPCR to sequence the certain target gene, and found that it was *escargot*, abbreviated as *esg*.

To identify when, where and how the overexpression of *escargot* induces such phenotype, we operated the staining of eye-antenna disc in third-instar larval period of wild type, *eq*> *esg*×UAS-GFP and *eq*>GFP with anti-dll, anti-*caspase*3 and anti-esg. The result shows that *escargot* cannot be detected before puparium formation. But the expression of *dll*, a gene controls the eye development, was reduced in the eye disc. We except the overexpression cause the defect of distal antennae and the anterior equatorial region of eyes mainly in the 3-day-long pupal life.

中文簡介

我們用異位表現法篩選出和*eq1>dll*、*eq1>abdA*、*eq1>Ubx*、*eq1-GAL4*、*ey-GAL4*或*dpp-GAL4*這些pattern lines有交互作用的EP lines。在這1800種的果蠅子代品系中，有五種具有特殊的性狀。它們具有觸角短化以及複眼前緣中央區有缺刻的現象(形成心型眼)。我們使用質體救援法以及IPCR的方法來定序這段未知基因序列，發現這是一個叫做*escargot*的基因(簡稱*esg*)。

爲了了解過分表現此基因會造成何種分子影響，以至於產生此種性狀，因此我們使用anti-dll 、 anti-caspase3 和 anti-esg 進行野生型、 *eq*>*esg*×UAS-GFP 和 *eq*>GFP 三齡幼蟲的eye-antenna disc的螢光免疫染色。結果在幼蟲成蛹前都沒有偵測到*esg*的表現現象；不過在eye disc中，控制眼睛發育的基因*dll*的表現有被抑制的現象。因此我們推測過分表現*esg*的過程因該是發生在爲其短短三天的蛹期。也就是說，這種表型應該是在化蛹後形成。

A. Introduction

Ever since Thomas H. Morgan started to use flies for his genetic research in 1909, flies have become the main material of genetics. Using flies to understand the human development has become more and more popular among researchers. For instance, in *Drosophila melanogaster,* there is only one *Dll* gene; flies without it lack the distal portion of their appendages. Mammals, such as human, by contrast, have six Dlx genes (*Dlx1–Dlx6*), which cluster in pairs on the genome and are associated with a severe human limb defect called split-hand/foot malformation type 1 (SHFM1) (Robledo *et al*). Therefore, a variety of genetic methods have been set out to understand the interactions between different genes. Above all, a genetic screen among *Drosophila* seems to be the best option to identify genes and to link them to their physical function, because this method requires no knowledge about the identity or molecular characteristic of the genes.

However, the screening is not systematic until Dr. Rorth created a screening combining P-element insertional mutagenesis and galactose-inducible gene (*GAL4* gene) expression. *GAL4* is a yeast transcription-factor gene that can be expressed in flies under the control of cell. Along with a certain nucleotide sequence called Upstream Activation Sequence (UAS) from yeast, Gal4 protein can regulate the expression of certain gene. (Fig 1) Gal4 protein will bind to UAS and enhance the expression of the genes next to UAS. And the P-element would "jump out" from its original place with the present of transposase and insert into somewhere within the *Drosophila* genome. Mixing these two methods, Dr. Rorth created a P-element that contains UAS. Thus, the P-element can randomly insert into *Drosophila* genome while the *GAL4* gene expresses and the Gal4 protein binds to UAS on the P-element to enhance the upstream gene. Because *GAL4* usually remains silent in *Drosophila* and only expresses when it is next to a promoter, inserting a sequence which contains certain promoter and *GAL4* into a fly's egg to make transgenic flies is the best way to operate this method. Thus, by choosing the promoter which drives, or leads, *GAL4*, we can overexpress one or more genes at specific tissues.

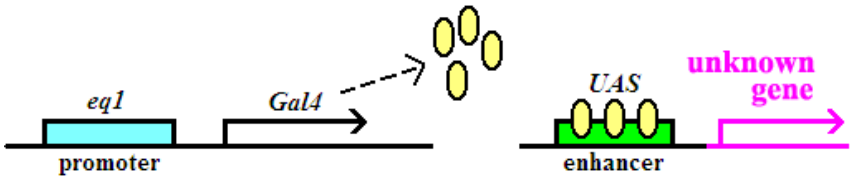

Fig 1: *GAL4*/UAS system

Thanks to Dr. Rorth's crucial breakthrough, we use his application to set out a gain-of-function screen. The screen is designed to identify the interactions between different genes. The *GAL4*/UAS system is used to set Pattern lines. A promoter called *eq1*, which is expressed in eye, antenna and notum, is chosen to drive *GAL4*. In these

operations, UAS is combined with certain gene such as *dll*, *abdA* or *Ubx.* All these genes will affect the antenna and notum development. The pattern lines with the genotypes of *eq1>dll* (causing a phenotype of groove on the nota), *eq1>abdA* (causing groove and antenna-to-leg), *eq1>Ubx* (causing groove), *eq1-GAL4*, *ey-GAL4* or *dpp-GAL4* have been set up. On the other hand, EP, which is a P-element that contains UAS, other essential sequences and several genes such as origin of replication (ori) and *kanamycin resistance*, is used to set Target lines. Thus their progeny of these two types of lines will present a gain-of-function phenotype. After the screening, we found that 5 of the 1800 filial generations had shorter antennae and defects in the anterior equatorial region of eyes.

To identify what gene caused this phenotype, we had to sequence the gene. We used plasmid rescue and inverse PCR to obtain the flanking fragments of EP insertion. After sequencing the unknown gene by suitable primers, we knew what the inserted gene really is. It is *escargot*.

According to the previous studies, the overexpression of *escargot* may be toxic to both cycling and nondividing cells by a mechanism unrelated to its role in regulating endoreduplication. *esg* RNA is expressed in wing, haltere, leg and genital imaginal discs and in abdominal histoblast nests in the embryo. Expression in imaginal tissues is also found in third-instar larvae.

Whenever an interaction is discovered, a series of methods can be used to identify how the interaction happens and the biological effect it brings about. In order to keep the *escargot*-overexpress flies, we set up a line with the genotype of *eq>escargot*/S-T.

Since overexpression of gene *esg* will cause shorten antennae and defects in the anterior equatorial region of eyes, we predicted that *esg* also has to do with cell cycle or programming cell death. Perhaps it will reduce the cell cycle or promote the programming cell death.

Using immuno-fluorescent staining, we can identify where and when the target gene will express. We have overexpressed the *escargot* and found that this operation caused such phenotype, but we didn't know how *escargot* expresses in normal. So we stained the wild type as a blank control with anti-esg (first antibody from rat) and cy5 (second antibody, combined with blue fluorescent die) to identify where, when, and how much *escargot* expressed.

Since cysteine asparate protease 3, abbreviated as caspase 3, is a functioning protein in the downstream of the programming cell death pathway, it can be used as a marker of where the apoptosis proceeds. Stained with anti-caspase3 (first antibody from rabbit) and cy3 (second antibody, combined with red fluorescence die), the region where apoptosis proceeds seriously can be observed.

On the other hand, because the overexpression of *escargot* induces the defect of the distal region of the antennae, thus cause the shorter antennae. The *escargot*'s product may have an effect on the expression of gene *distal-less*, abbreviated as *dll*, which will express

in this region and control antenna's development. Operating the staining of *dll* in eye-antenna disc with anti-dll (first antibody from rat) and cy5 (second antibody, combined with blue fluorescent die), how *escargot* affects the expression of *dll* will up to the surface.

More over, as a background of the staining, *eq*>GFP is set up. Because the green fluorescence protein is overexpressed under the leading of *eq*, as the *escargot* is, the green fluorescence represents the region where *escargot* overexpresses. In addition, according to the previous viewpoint, the wild type has been set up as a blank control.

So comparing these two strains of flies' staining (*eq*>*esg*×UAS-GFP and *eq*>GFP), we can know what mechanism induces such phenotype.

The result is that the expression of *escargot* in third-instar larvae's eye-antenna disc of wild type cannot be detected. And there is no obvious difference between the expression of caspase3 of *eq*>*esg*×UAS-GFP and *eq*>GFP. But the expression of *dll* was reduced. Since the phenotype shows that the overexpression does have an effect on the development of eye and antenna, this result may mean that *escargot* only expresses in the late period of the eye and antenna development. We except the overexpression cause the defect of distal antennae and the anterior equatorial region of eyes in the 3-day-long pupal life.

(A) What We Have Done:

1. Sieved out the fly strain containing genes which may interact with *dll*, *Ubx*, *abdA*.
2. Sieved out the unknown gene which influenced the eye development while it was leaded by *dpp-GAL4*, *ey-GAL4*, *GMR-GAL4* by using gain-of-function method.
3. Sequenced the unknown gene, K251, by using plasmid rescue method and inverse PCR. Found that this gene is *escargot*.
4. Set up and Observed the phenotype of the flies with the genotype of *eq*>*esg* /S-T.
5. Crossed *eq*>*esg*/S-T and UAS-*GFP*/S-T to induce the green fluorescence in which the promoter *eq* expressed.
6. Observed the expression of *escargot* of wild type by staining with anti-esg and cy5.
7. Observe the cell cycle in the eye-antenna disc of the *eq*>*esg* /S-T flies in larval stage by staining with anti-dll and cy5.
8. Observe the programming cell death in the eye-antenna disc of the *eq*>*esg* /S-T flies in larval stage by staining with anti-caspase3 and cy3.

(B) What We Are Going To Do Next:

1. Use UAS-*escargot* to overexpress *escargot* and do over all the experiments to avoid any possible effect caused by other genes in EP lines.

2. Observe the expression of *escargot* in eye-antenna disc after puparium formation.
3. Observe the expression of *escargot* in wing disc in different stages.
4. Observe the cell cycle in the eye-antenna disc of the *eq>esg* /S-T flies in pupal stage by staining with anti-dll and cy5.
5. Observe the programming cell death in the eye-antenna disc of the *eq>esg* /S-T flies in pupal stage by staining with anti-caspase3 and cy3.
6. Observed the expression of *escargot* of *eq>esg*×UAS-*GFP* by staining with anti-esg and cy5.
7. Observe the cell cycle in the wing disc of the *eq>esg* /S-T flies in different stages by staining with anti-dll and cy5.
8. Observe the programming cell death in the wing disc of the *eq>esg* /S-T flies in different stages by staining with anti-caspase3 and cy3.
9. Compare the previous staining with the wild-type flies'. Probe into that whether the phenotype is caused by the inhibition of cell cycle or the improvement of the programming cell death.
10. Observe the phenotype of the flies which have the genotype: $esg^{b7\text{-}2\text{-}22}$/CyO, $esg^{35Ce\text{-}1}$/SM5, esg^{k00606}/CyO, esg^{dgl}/SM1
11. Over-express *p35* and *esg* in the meantime to check whether *esg* influence the programming cell death or not.
12. Over-express *cycE* and *esg* in the meantime to check whether *esg* influence the cell cycle or not.

B. Result and Discussion

(A) Phenotype Observation:

1. *eq1>abdA*×K251

Comparing with the parental generation, the filial generation's antennae are shorter. Further more, the groove becomes smoother, and the eyes have defects in the anterior equatorial region. (Fig2)

2. *eq1>dll*×K251

The *eq1>dll*×K251 is similar to the *eq1>abdA*×K251: shorter antennae, defects in the anterior equatorial region of eyes. (Fig2)

3. *eq1>Ubx*×K251

Just like the *eq1>dll*×K251, *eq1>Ubx*×K251 has shorter antennae and defects in the anterior equatorial region of eyes, but the defects are more obvious. (Fig 2)

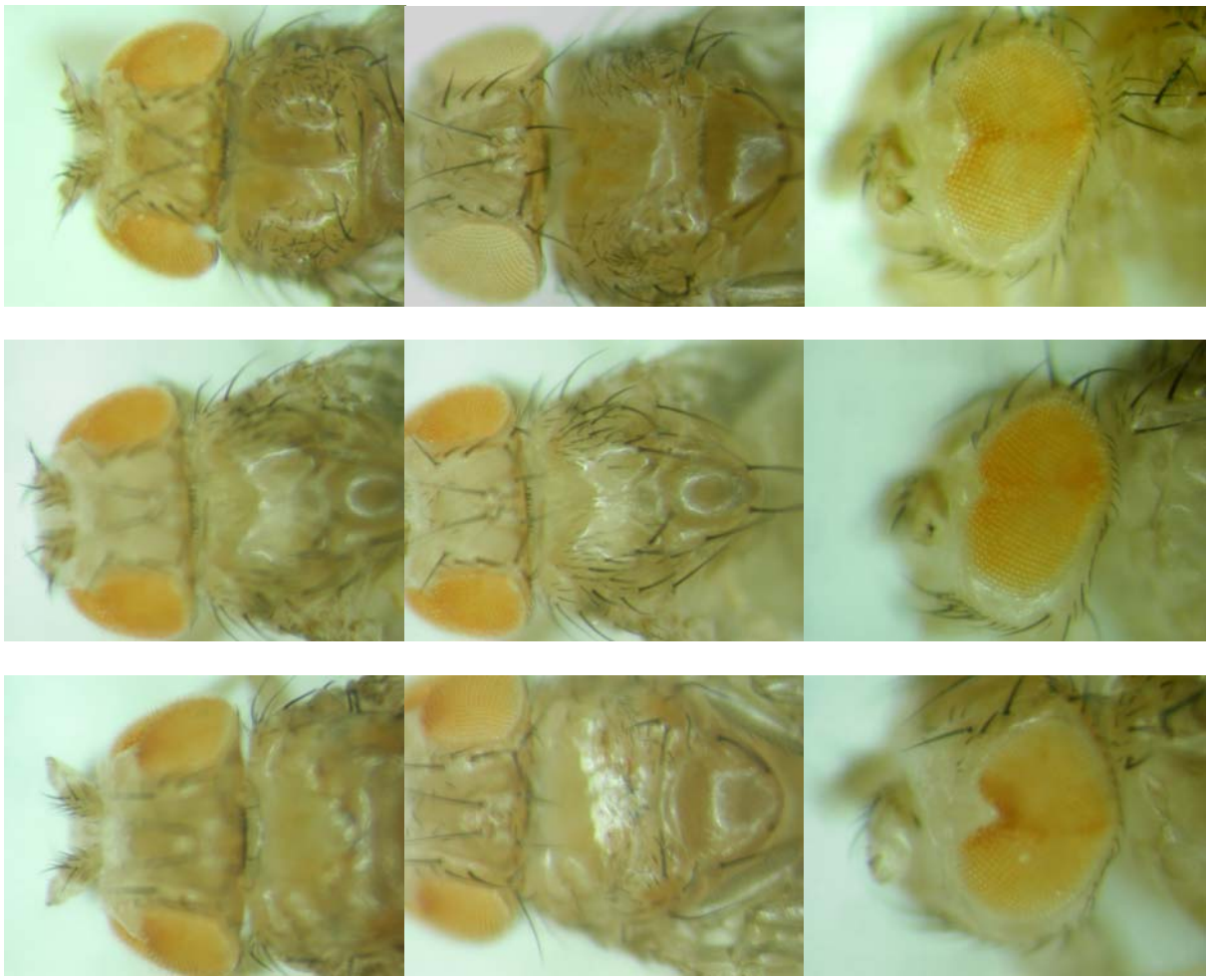

Fig 2: Results of *eq1*

eq1>abdA×K251

eq1>dll×K251

eq1>Ubx×K251

4. *eq-esg*

The *eq*-esg has shorter antennae just like the *eq1>abdA*×K251 and has groove just like the *eq1>abdA*. (Fig 3)

Fig 3: Results of *eq-esg*

5. *ey-GAL4*×K251

The *ey-GAL4*×K251 has defects in the anterior margin of eyes, causing smaller eyes. (Fig 4)

Fig 4: Results of *ey-GAL4×K251*

6. Discussion

Since overexpression of this gene will cause shorten antennae and defects in the anterior equatorial region of eyes, we predicted that it has an effect on eye-antenna disc development. Perhaps it will restrain the cell cycle or promote the programming cell death.

(B) K251(EP251) – *escargot*

Using inverse PCR, we found that EP251 was inserted in the promoter region of *escargot*. (See Appendix)

The gene *escargot*, abbreviated as *esg*, is reported here. Protein features include protein domains Rubredoxin-type Fe (Cys) 4 protein, Zn-finger, C_2H_2 type. Functions of the gene include transcriptional regulation, and its cell location is nucleus. It is located at 35D2 on the cytological map.

Mutations in the *escargot* (*esg*) locus will cause various defects in adult structures such as loss of abdominal cuticle and malformation of the wings and legs. *esg* RNA is expressed in wing, haltere, leg and genital imaginal discs and in abdominal histoblast

nests in the embryo. Expression in imaginal tissues is also found in third-instar larvae. In *esg* mutant larvae, normal diploid abdominal histoblasts replicate their DNA without cell division and become similar in appearance to the polytene larval epidermal cells.

(C) Staining:

1. Wild type

The *ESG* protein is not detected in the larval eye-antenna disc. (Pictures not shown) But it can be detected in wing disc and leg disc in this period. (Fig 5)

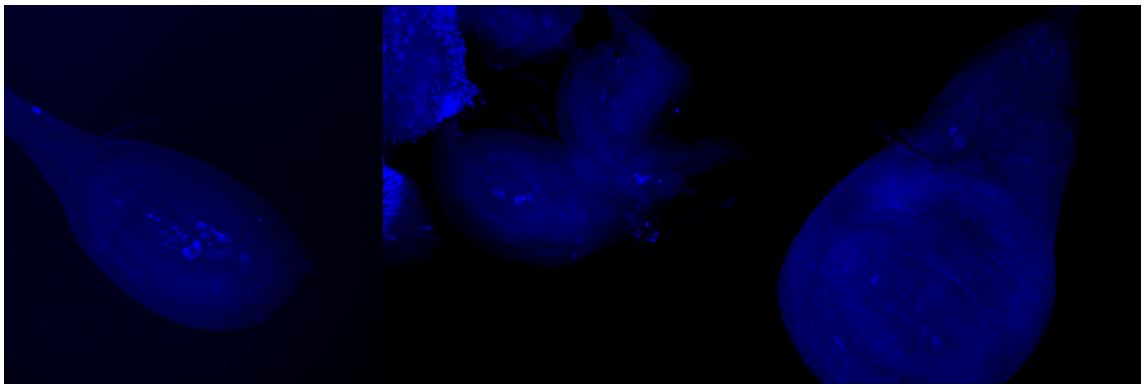

Fig 5: Staining (Blue: *esg*)
Leg disc | Leg disc | Wing disc

2. *eq*>GFP

The *dll* present in the center of the antenna disc mainly and in the peripheral regions of eye disc slightly while caspase3 expresses slightly all over the eye-antenna disc. And the GFP, leaded by the eq, is in the innermost region of antenna disc and the anterior equatorial region of the eye disc. (Fig 6)

3. *eq*>*esg*×UAS-GFP

The staining of *eq*>*esg*×UAS-GFP is similar with the *eq*>GFP, but the blue fluorescent region in the eye disc is absent. (Fig 6)

4. Discussion

Since the ESG protein is not detected in this period, the effect of *esg*-overexpression will not be very obvious. The result of staining corresponds to this prediction. There is only a difference between *eq*>*esg*×UAS-GFP and *eq*>GFP --- the overexpression restrain the expression of *dll* in the eye disc. However, we'll do over this staining to make sure that our discussion and conclusion are absolutely correct.

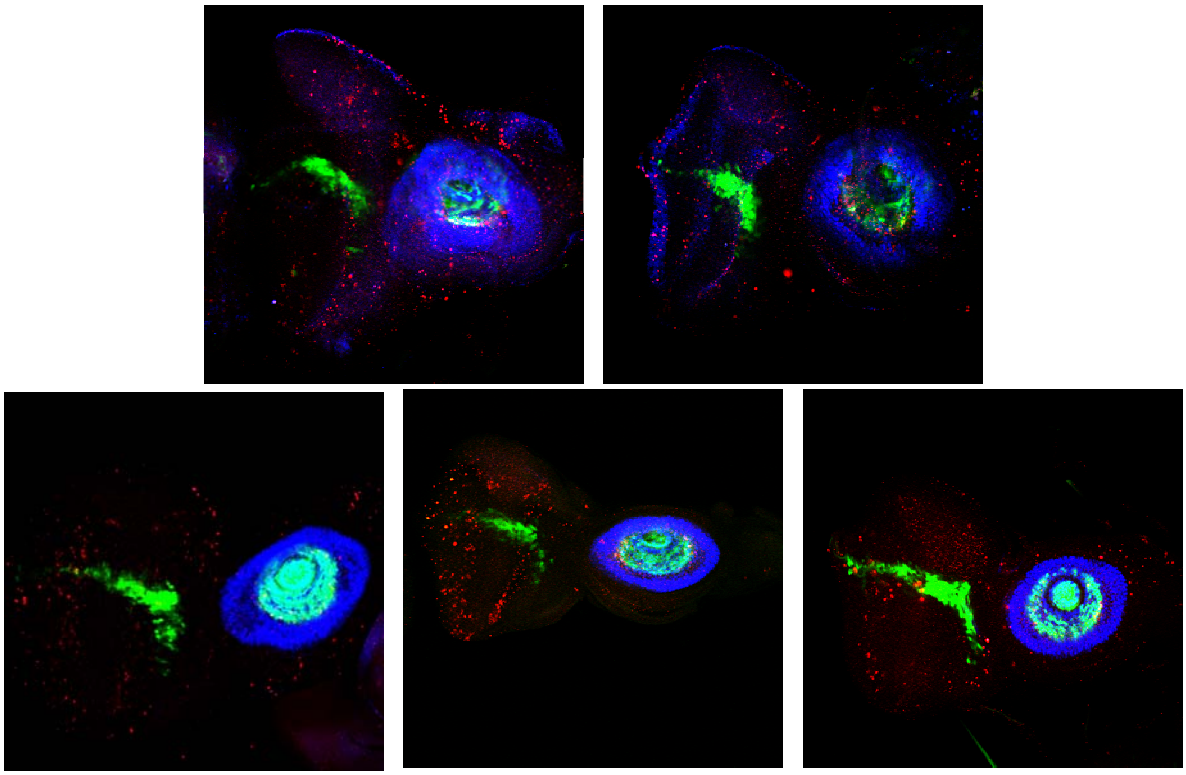

Fig 6: Staining (Green: GFP leaded by *eq*; Blue: *dll*; Red: caspase3)

eq>GFP (control)

eq>esg×UAS-GFP

(D) Prediction:

Since *ESG* protein does not express in the imaginal disc before puparium formation, its effect on other genes may present in the 3-day-long pupal life. In other words, the defect of distal antennae and the anterior equatorial region of eyes will be produced after puparium formation. In addition, because the lessening of the *dll* in larval disc, *esg* may mainly affect the cell cycle rather than programming cell death.

C. Materials and Equipment

(A) Fly Strains:

1. *eq1-GAL4*/TSS (*equational1*/TSS):

The promoter, *eq1*, mainly acts in the antenna, back and the middle of ommatidium. Chiou-Yang Tang, a senior researcher in our laboratory, discovered this promoter. Tang inserted the enhancer and the *mini-white* gene (as a marker of causing red eyes) into white-eye fly's genome randomly. Then he found that there was a red band on the middle of its white eye. This experiment showed where the gene expresses. Because of *mini-white* in the *eq1-GAL4*/UAS, there is a red band on the equator of eye. This phenotype is discovered in *eq1>dll*, *eq1>abdA*, and *eq1>Ubx* flies, too. Balancer T.S.S. induces the short bristle and notched wing. (Chiou-Yang Tang et al., 2002) (Fig 7) In addition, *eq1＞dll*, *eq1＞abdA*, *eq1＞Ubx* will inhibit the healing over of the nota. (We use the phrase "groove" to describe this phenotype in the rest of this report.)

Fig 7: *equational1*/TSS

2. *eq1＞dll*/TM6B (*equational1* drive *Distal-less*/TM6B)

The symbol "*eq1＞dll*" means activating the gene *dll* expression with the leading of promoter *eq1*. This causes the "groove" phenotype. Balancer TM6B induces the abnormal growth of pronotum bristle. (Fig 8)

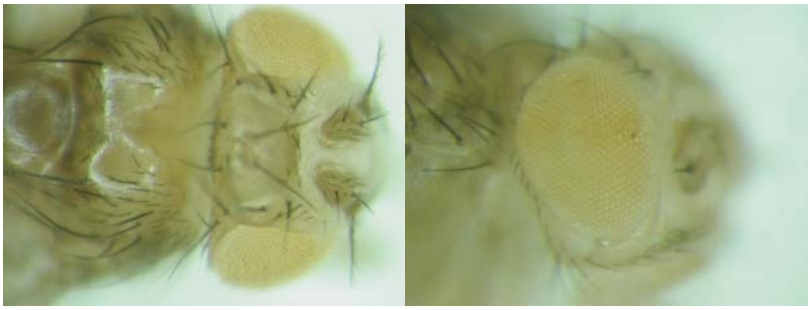

Fig 8: *equational1*/drive *Distal-less*/TM6B

3. *eq1＞abdA*/TM6B (*equational1* drive *abdominal A*/TM6B)

eq1＞abdA flies have the “groove” phenotype as well. In addition, their antennae will grow to leg-like antennae (we use the phrase “antenna to leg" to describe it). (Fig 9)

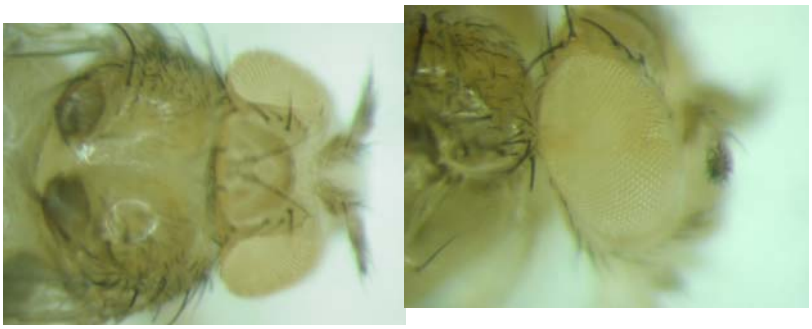

Fig 9: *eq1* drive *abdominal A*/TM6B

4. *eq1＞Ubx*/TM6B (*equational1* drive *Ultrabithorax*/TM6B)

eq1＞Ubx flies have the “groove” phenotype, but the gap is not equal to the one in *eq1＞dll* and *eq1＞abdA*. Further more, *Ubx* itself will make the flies’ halteres become larger and rounder than normal ones. (Fig 10)

Fig 10: *eq1* drive Ultrabithorax/TM6B

5. *dpp-GAL4*/TM6B (*decapentaplegic-GAL4*,[C40,1]/TM6B)

The term “decapentaplegic” means seventeen in Latin. Its homeotic gene in mammal is gene *TGF-beta/bone* (*Soluble transforming growth factor-beta*), which controls the cytokine (J Biol Chem, 2002). Gene *dpp* expresses within the compound eye and the front of the morphogenetic furrow. Because *dpp-GAL4* is inserted in the downstream site of one of the *dpp* enhancers, it only expresses at the posterior margin of the eye disc. Although *dpp* is expressed in the wings, it doesn’t induce any clear phenotype.

6. *GMR-GAL4*(*glass multimer repeat promoter-GAL4*)

GMR-GAL4 is an important promoter on compound eye development. It mainly expresses behind the morphogenetic furrow. *GMR-GAL4* produces a transcription factor, which influences the expression of gene *glass* (Hay et al., 1997). The gene *glass* encodes a DNA-binding zinc-finger protein required for the development of *Drosophila* photoreceptor cells and which appears to regulate a number of genes specifically expressed in photoreceptors (MC Ellis et al., 1993). Rather, *GMR-GAL4* itself will make

the ommatidia proliferate. Thus, the ommatidia will array irregularly. And perform a phenotype we called “irregular eye”. (Fig 11)

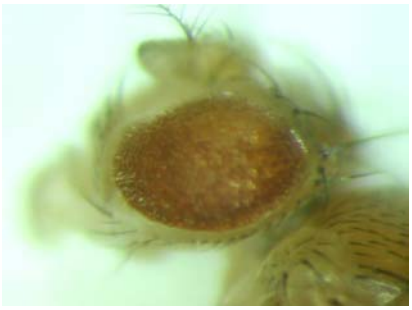

Fig 11: *glass multimer repeat promoter-GAL4*

7. *ey-GAL4* (*eyeless-GAL4*;UAS-*lacZ*/S-T)

ey-GAL4 is a master gene of eye development. Its product, PAX (*Paired box* containing protein), influences the neuron development during the embryonic period, the eye development of larvae and the development of the partial ommatidia which is in front of the morphogenetic furrow in the third-stage larvae.

Using misexpression, overexpression of *ey* can induce extra eyes on antennae, wings, etc. Further more, *PAX6*, which belongs to the *PAX* family, also exists in mice’ and humans’ genome. PAX and PAX6 are similar to each other and have the same source; therefore, they have similar effects on neurons, eyes. Some researches say that the mutation of *ey*’s familiar gene, *PAX6*, may cause defects on human being's iris. The *ey*-overexpression doesn’t induce any clear phenotype while balancer S-T induces vestigial wings. (Fig 12)

Fig 12: *eyeless-GAL4*;UAS-lacZ/S-T

8. *eq>esg* /S-T(*equational* drive *escargot*/S-T):

eq>esg /S-T flies also have a red band on the equator of eye, shorter antennae, and the defect on the anterior equatorial region of eyes while balancer S-T induces vestigial wings.

9. UAS-*GFP* (UAS-green fluorescence protein):

This strain of flies has the gene of green fluorescence protein in its genome, and there is UAS on the upstream of GFP. So with *GAL4* from another strain of fly, these flies' progeny can be an excellent reporter of where the gene of interest expresses.

10. Balancer lines

Principle: Balancer is a chromosome with continuous lethal recessive genes, so the homozygous will die. And it also avoids unwanted crossing over (recombination). When a gene of interest and which chromosome it locates are found, the operation of balancing it can keep it from any damage during crossing over (recombination). Further more, there is usually a marker of certain genes that can induce special phenotype in the balancer. This makes it easier to sieve out the flies contains balancer and the gene of interest (Michael Socolich, 2003). For example, when the homozygous fly which contains homologous chromosomes of EP/EP is found, the operation of mating them with flies of M3-12/balancer S-T will be performed. Since M3-12 is a marker of *eq1*-like phenotype and the marker of balancer S-T is vestigial wings, we can keep the EP by choosing the flies that are vestigial-winged and not with "groove". (Fig 13)

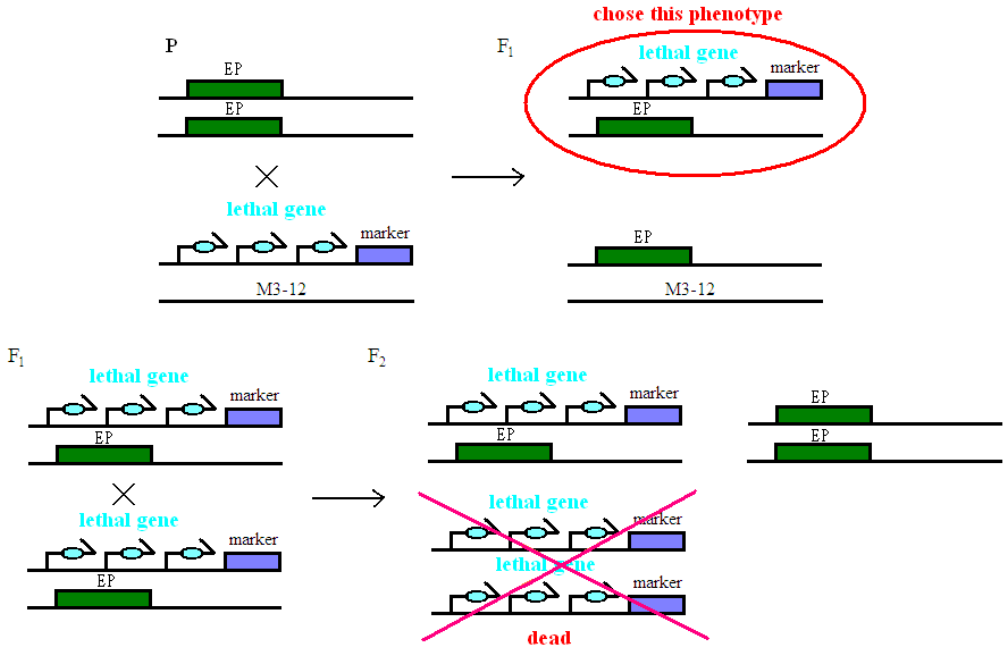

Fig 13: Balancer---using this method, genes we are interested can be reserved.

(1) Balancer TSS: "T" means the third chromosome. *Ser* (*Serrate*) also called *Beaded-Serrate*, causes wings notched, and *Sb* (*Stubble*) causes bristles short and stubby. In our laboratory, the balancer belongs to *eq1-GAL4* is TSS. (Fig 14)

Fig 14: *Ser* (*Serrate*)

(2) Balancer TM6B: It will change the two long bristles of wild type to a tuft of short bristles in pronotum. Also, "T" means the third chromosome. In our laboratory, the balancer belongs to *eq1*＞*dll*, *eq1*＞*abdA*, *eq1*＞*Ubx*, *dpp* is TM6B. (Fig 15)

Fig 15: TM6B(left) and Normal(right)

(3) Balancer S-T: It is a complex balancer. It is actually a mixture between two balancers, one is on the second chromosomes and the other is on the third ("S" means the second while "T" means the third chromosome). The phenotypes are abnormal growth of pronotum bristle and vestigial wings respectively. Because a great part of genes are on the second or the third chromosome, we can use this balancer when we are not sure which chromosomes does the gene locates.

11. EP lines:

EP vector is used as an element for seeking out unknown genes here.

With the invention of *GAL4*/UAS method (please check out the article below), Rorth combined the transposons with *GAL4*/UAS. He inserted UAS into the transposon gene vector (P-element), and the element will be randomly inserted into the genome where maybe the upstream of a gene. This unknown gene will over express with the leading of Gal4 protein (Rorth et al., 1996).

In this research, there are a *kanamycin resistance* gene, an origin of replication of *E. coli* (ori), *mini-white* (as a marker) and UAS in the P-element. Since we use a non-autonomous P element which lacks a functional transposase gene, it can only move

within the genome with the present of transposase. (Fig 16) Injecting the P-element into flies' eggs and controlling the expression of transposase make it possible to produce hundreds of different EP lines.

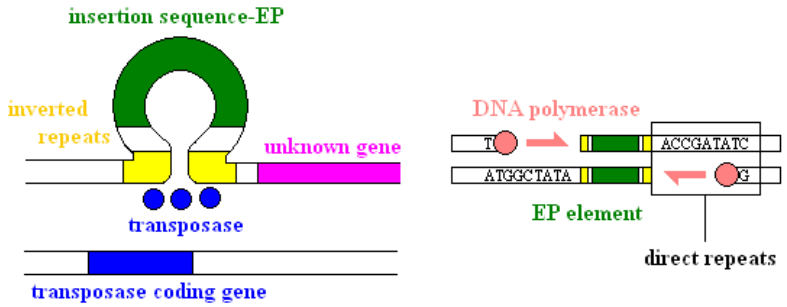

Fig 16: EP is inserted into DNA sequence by the transposase. Then the gaps in the DNA strands are filled in by DNA polymerase and sealed by ligase.

(B) Primers:

Plac4(27) - ACT GTG CGT TAG GTC CTG TTC ATT GTT
Plac1(24) - CAC CCA AGG CTC TGC TCC CAC AAT
Pry4 (23) - CAA TCA TAT CGC TGT CTC ACT CA
Pry1 (26) - CCT TAG CAT GTC CGT GGG GTT TGA AT
Pry2 (28) - CTT GCC GAC GGG ACC ACC TTA TGT TAT T
Plw3-1 (19) - TGT CGG CGT CAT CAA CTC C
Pwht1 (29) - GTA ACG CTA ATC ACT CCG AAC AGG TCA CA

(C) Enzymes:

1. Restriction enzyme:Sau3A I, HinP1 I, or Msp I (NEB)
2. T4 DNA ligase (Roche)

(D) Antibodies:

1. 1st Antibody: anti-dll (rat); 2nd antibody: cy5 (blue fluorescent)
2. 1st Antibody: anti-*caspase* 3 (rabbit); 2nd antibody: cy3 (red fluorescent)
3. 1st Antibody: anti-*escargot* (rat); 2nd antibody: cy5 (blue fluorescent)

(E) Sequencing:

We sequenced our PCR product by helping from DNA Sequencing Core Lab in IMB, Academia Sinica.

(F) Other Equipments:

1. Confocal fluorescence microscope
2. Dissecting microscope
3. Electrophoresis equipment
4. Incubator
5. Fly-operating stage
6. Tweezers and brushes
7. CO2 (to anesthetize the flies)
8. Fly stocks (100 tubes per box)

(G) Drugs:

1. TBST
2. Goat serum
3. glutaradehyde/ PBS
4. ETBr
5. Buffers
6. Inactive yeast
7. Glucose, agar and other materials composing the fly's food

D. Methods

(A) Misexpression Screen

Before Rorth invented a new way in 1996, the researches of fly genes used loss-of-function method, which cuts out certain genes, and then observed how this operation changed the fly's phenotype. Now researchers can use Roth's misexpression to operate the screening.

This method is based on the *GAL4*/UAS system. *GAL4*/UAS, which is nicknamed as "fly geneticist's Swiss army knife", had been widely used in Misexpression screen since Brand first operated it in 1993 (Brand et al., 1993). *GAL4* and UAS are additional gene and sequence that exist originally in yeast's genome. *GAL4* will be translated to a transcription factor. And the product, Gal4 protein, will bind on the UAS, which is an enhancer, causing the downstream gene to over-express.

To operate the misexpression screen, one should set a pattern line by placing the *GAL4* into the downstream of a promoter, and set the target lines by inserting enhancer UAS, which contains several Gal4 protein binding site on it, into the fly genome randomly. In flies, *GAL4* is unlikely to express actively. So it is important to set *GAL4* on the downstream site of the promoter. Thus when the promoter leads its original leaded gene express, *GAL4* will be expressed too. When the *GAL4* expresses with the leading of this promoter, the unknown gene in the downstream of UAS will be expressed as well.

In the laboratory, the operation of inserting UAS is injecting an artificial DNA sequence containing UAS into the fly eggs to make transgenic flies (Joseph Duffy, 2002). For instance, we use this method to make flies express *dll*, *abdA*, *Ubx*, in the *eq1*-expressing tissue.

In this experiment, we place *GAL4* on the downstream site of the promoter *eq1*. UAS, which will be bound by the Gal4 protein, is inserted randomly into the upstream site of an unknown gene, so this unknown gene will be over expressed in the tissue (region) where *eq1* expresses. (Fig 17)

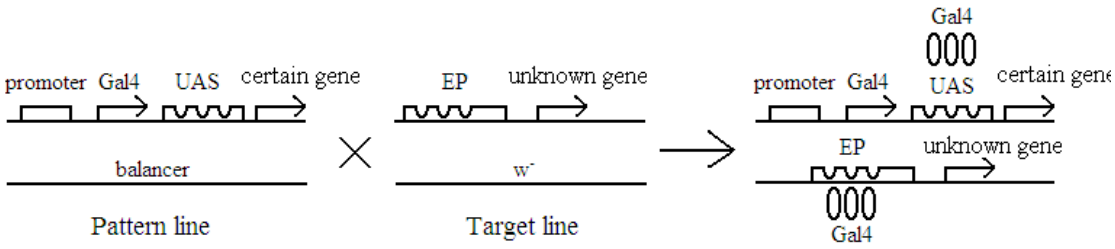

Fig 17: Probe into the Unknown Gene's Function by Using Misexpression Screen

(B) Plasmid Rescue

Electroporate the plasmid into *E. coli*, then spread the bacteria on the plates containing kanamycin. Because there is *kanamycin resistance* gene and ori (origin of replication of plasmid) on EP, only the *E. coli* with the recombinant plasmid vector where EP is located can survive on the kanamycin plate. After sequencing the plasmid, finding the EP segment and comparing the sequence with the fly genome, we can know what this unknown gene really is. (Fig 18)

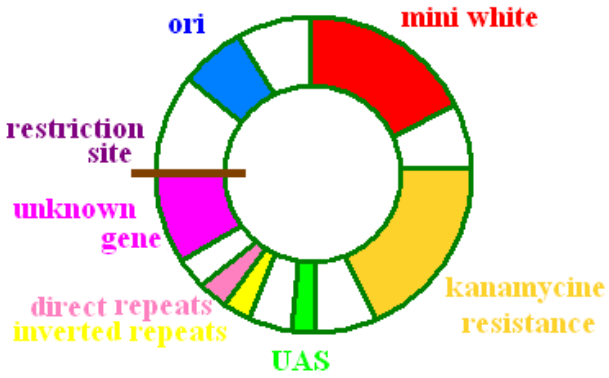

Fig 18: The Recombinant Plasmid of EP

(C) Inverse PCR (http://www.fruitfly.org/about/methods/inverse.pcr.html)

To identify the sequences with flanking EP251 insertion, we use inverse polymerase chain reaction (IPCR). IPCR, described by Ochman in 1988, is a method for the rapid in vitro amplification of DNA sequences that flank a region of known sequence. The method uses the polymerase chain reaction (PCR), but it has the primers oriented in the reverse direction of the usual orientation. The template for the reverse primers is a restriction fragment that has been ligated upon itself to form a circle.

(D) Fluorescent Staining:

1. Fluorescence (GFP):

We operate misexpression between *eq-Gal4* and UAS-GFP to overexpress GFP in specific region where *eq* leads the gene expression. We use *eq>GFP* as an experimental material. Since the imaginal disc matures in third-instar larva, the GFP will express in the eye-antenna disc. Taking out the disc from the third-instar larva and discarding the surrounding tissues make it easier to observe the green fluorescence caused by the GFP in the region where *eq* expresses under a confocal fluorescence microscope. (Fig 19 and Fig 20)

2. Immunostaining:

To identify whether a specific protein will express in certain region or not, we operate the immunostaining. Injecting the protein of interest into a mammal will make this animal produce the antibody that can bind to the target protein. Since the antibody exists in the serum, we can extract it from blood. As a tool of detecting protein, this antibody, called the first protein, will be added in the discs, so that the target protein will be bound by this antibody.

Making observing the protein-expression site possible, another antibody, the second antibody, will be used in this method. These types of antibodies will bind to the constant region of the previous antibody. In other words, they will anti specific antibodies from certain animal. More over, these antibodies have been combined with fluorescent dyes. So when they bind to the 1st antibody-target protein complex, the new complex can present fluorescence. Thus we can tell where the protein expresses through the fluorescence under a confocal fluorescence microscope.

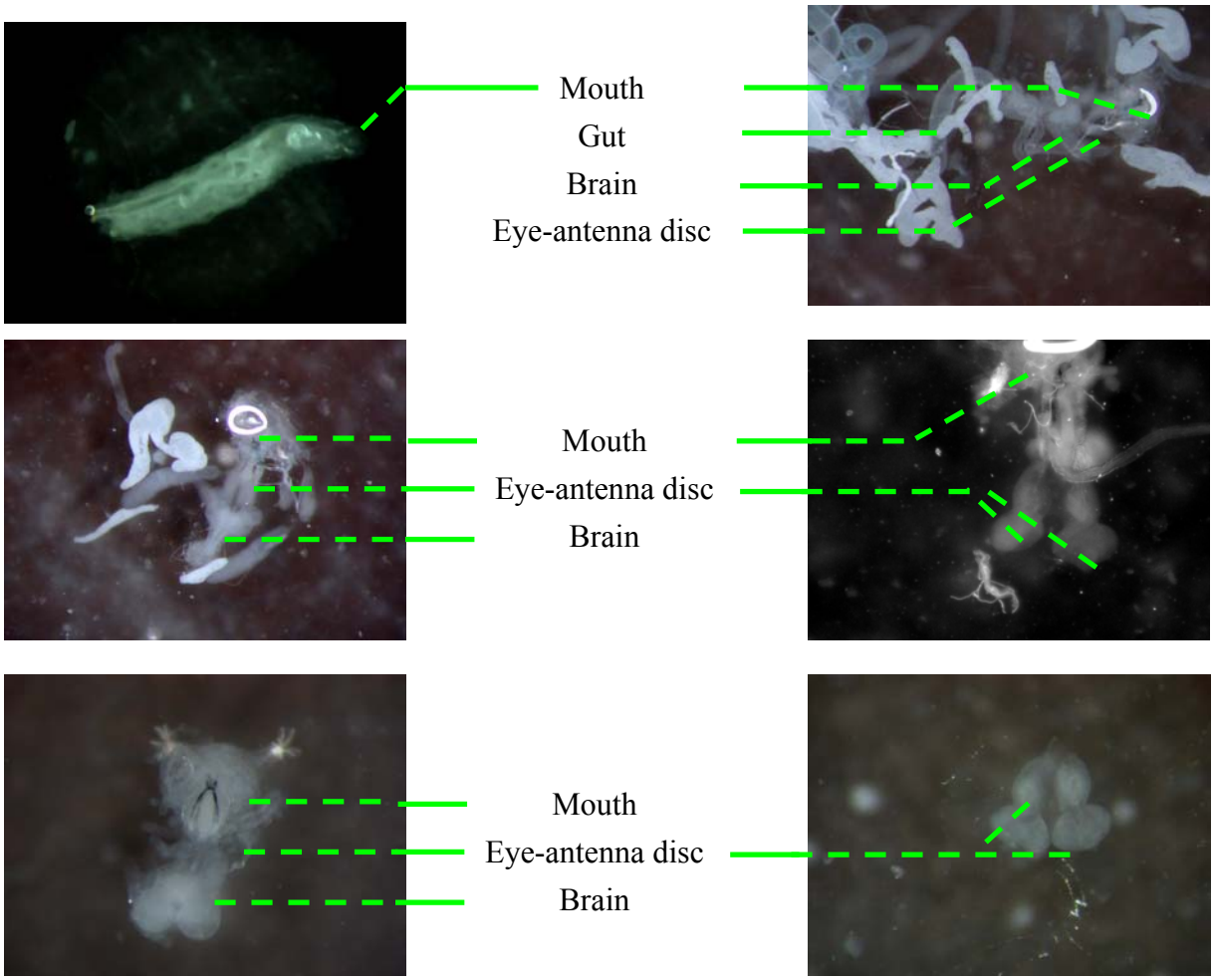

Fig 19: Taking out the Imaginal Discs (Dissecting Microscope)

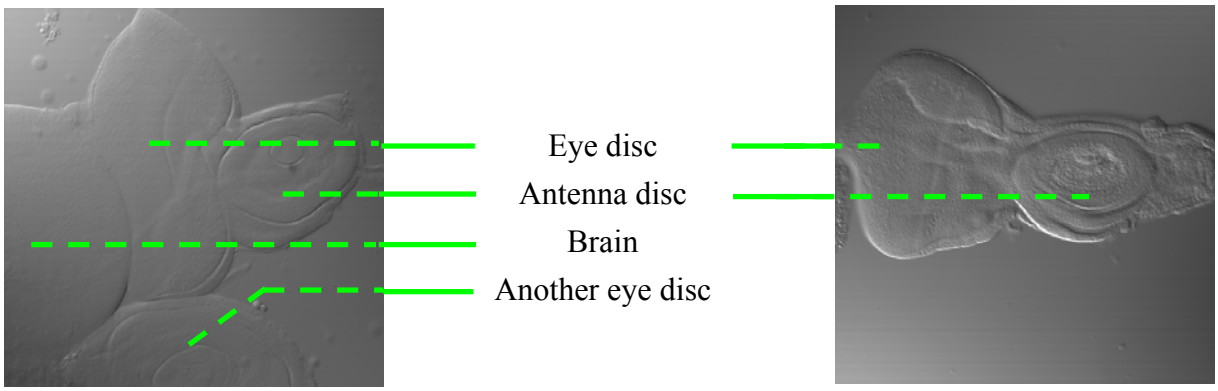

Fig 20: Eye-antenna Discs (Confocal Microscope)

(E) Other Methods:

1. PCR: We used this method to amplify the certain gene.
2. Electrophoresis: We used this method to exam whether the plasmid rescue is success or not.
3. Electroporation: We used this method to transformate the plasmid into the *E. coli*.

E. Reference

(A) Documents

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Ralph J. Greenspan (1997) Fly Pushing

(B) Literature

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Pernille Rorth (1996) A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. Proc. Natl.Acad.Sci.USA Vol93, p.12418-12422

Pernille Rorth, Kornelia Szabo (1998) Systematic gain-of-function genetics in *Drosophila*. Development Vol125, p.1049-1057

Thomas, C.M., Jones, D., English, J.J., Carroll, B.J., Bonneted, J.L., Harrison, K., Burbidge, A., Bishop, G.J. and Jones, J.D.G.（1994）Analysis of the chromosomal distribution of transposon- carrying T-DNA in tomato using inverse polymerase chain reaction. Mol. Gen. Genet.242, p.573-585

(C) Webs

Fly Base: http://flybase.bio.indiana.edu/

Japan *Drosophila* Database: http://kyotofly.kit.jp/JDD/bodyparts/

草地研究室: http://grassland.agron.ntu.edu.tw/genelab/FlyIntro.htm

F. Appendix

(A) The original sequencing data of plasmid rescue

escargot:

TTGGTGCAAGTGTACGAGTTCGGCACGTGAGGCAATTCTACATAAACAAGGCGAA
ACCTGTTTAGCCGAGTGTCGCGATTGGTCGACGCATATACCTGAATGAACATGTATA
CTCTCGCGGAACGCCCGAGAGAGCGTTC

EP251 insertion site↓

TCTTTTGGCAGCTTCGTGTAATGAGCCGAGTGCCGAGTGCAGGGGCTCTTTTGGCA
GCTTCGTGTAATGAGCCGAGTGCCGAGTGCAGGGGCAGTATAAAAAGGCCGCACC
ACCGCCAAACTCGCTCAGTTATTCCCAGACTCTAACCGATTCACATAACCATCTCTC
CCAACGCAATATACCCGAAATATAACCGATATTCAATCGCTTTGCTTAACGGAACAC
GCGTTTGGTATCTGTGCATCGATCGAGTATTTGTGCCTGTGTGAACATGACACACTG
AAAACAAAGTGACGAAAATTAAGAACATTTTACAACAATTCCGGTAAACTATCAAT
AAAAAGCCAGTAAA**ATGCATACCGTGGAAGACATGTTGGTGGAGAAAAACTAC**
AGCAAGTGCCCGCTAAAAAAGCGCCCAGTTAATTACCAGTTCGAGGCGCCTC
AAAATCACAGTAACACCCCAAATGAGCCGCAGGATTTGTGCGTAAAGAAAAT
GGAAATTCTGGAGGAAAATCCCTCCGAGGAACTGATCAACGTCAGCGATTGT
TGCGAGGACGAGGGTGTGGATGTGGATCATACAGATGATGAACACATCGAGG
AAGAGGACGAGGACGTCGATGTGGATGTGGACTCGGACCCCAATCAGACCC
AGGCTGCGGCTTTAGCTGCTGCCGCAGCTGTGGCCGCTGCTGCAGCCGCCT
CCGTGGTTGTGCCCACGCCCACATACCCGAAATATCCATGGAACAACTTCCAC
ATGTCGCCCTACACCGCCGAGTTCTACAGGACCATCAATCAGCCGGGTCATC
AGATTTTGCCGCTGCGTGGCGATCTCATTGCGCCCAGTTCGCCGAGTGACTC
GCTGGGCTCCCTGTCGCCGCCACCACATCATTACCTTCATGGTCGCGCAAGC
TCCGTTTCCCCGCCCATGAGATCGGAAATGATCCATAGACCAATCGGCGTGC
GGCAGCACCGTTTCCTGCCCTATCCCCAGATGCCCGGTTATCCGAGCTTGGG
CGGCTATACCCATACCCATCATCACCATGCGCCTATCTCGCCGGCTTATTCCG
AGAATTCCTACTACTCCATGCGATCGATGACGCCTGAATCCAGCTGCAGCTCC
TCGCTGCCCGAGGATCTATCCCTGAAACACAAGAACCTGAACTTGA

Blue: Flanking sequence of EP251 insertion

Red: *ESG* protein coding sequence

G. Appreciation

Laboratory N415 in Institute of Molecular Biology in Academia Sinica
Dr. Y. Henry Sun
Ph.D. Student Wang Cheng-Wei
Taipei Municipal Chien-Kuo Senior High School
Biology Teacher Tong Yi-Shan

Searching the Gene Essential for *Drosophila* Development: Identification of *escargot*

Students: **Hung-Jen Chen**

Tsai-Ta Lai

Professor: **Dr. Y. Henry Sun**

Instructor: **Cheng-Wei Wang**

School: **Taipei Municipal Chien-Kuo Senior High School**

Table of Contents

Abstract

Using the *Drosophila* model, we studied how overexpression of genes induced a certain phenotype of shorter antennae and defects in the anterior equatorial region of eyes. Five strains with these phenotypes were obtained from 1,800 strains by molecular screens between the EP lines (EP lines) and the pattern lines overexpressing a gene *dll*, *abdA*, or *Ubx* under the leading of promoter *eq*, or pattern lines expressing *GAL4* under the leading of one specific promoter *eq*, *ey*, or *dpp*.

In this study, we used plasmid rescue and inverse PCR to check the insertion site of the EP and get the flanking sequence of a gene responsible for the defective phenotype. Finding that EP was inserted in the upstream of gene *escargot* (*esg*), we identified the involvement of *esg* in the developmental control.

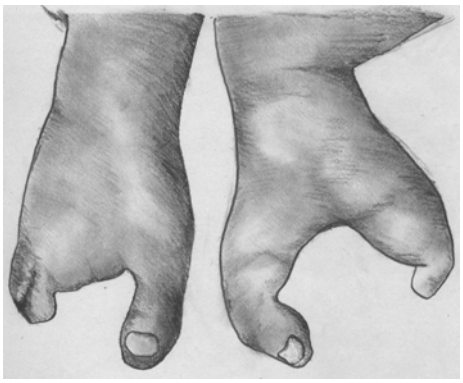
To understand the spatiotemporal control of Esg protein in *Drosophila* development, we performed the staining of imaginal discs of wild type, *eq*>*esg*×UAS-GFP, *eq*>GFP (control) and other strains with anti-Dll, anti-Caspase3, anti-Esg and other antibodies. The results showed that protein Esg cannot be detected in the eye disc of three-instar larvae in normal. Overexpression of Esg caused the defects of distal antennae and the anterior equatorial region of eyes, indicating its expression level needed to be controlled for cell status and cell destiny.

A. Introduction

(A) Motivation

Last summer, we saw an infant patient having feet of only two toes. The symptom is called lobster-claw deformity for its appearance. The disease is named split-hand/ split-foot malformation (SHFM)[1] and has been shown due to the defect of the *Dlx* family.

Dlx family contains a homologous gene in *Drosophila* called *dll.* Gene *dll* regulates embryo development. Herein, we used fly as a model to study the regulation of *dll*, and wish to understand the molecular mechanism involved in the development of the SHFM disease.



(B) Background Analysis & Theory

1. Gene *dll* controls antennae development in *drosophila*

While *Dlx* family regulates limb development in human, *dll* controls the growth of antennae in *Drosophila*. The dysfunction of *dll* will induce defect in distal antennae (Fig 1). According to previous study, *dll* regulates appendage development and expresses in embryo status[2]. Gene *dll* is important in cell proliferation in antennae.

Fig 1: Fly without *dll* displayed defect in distal antennae: compared with normal type, *dll*$^{-}$ displays shorter antennae.

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[1] Robledo, F. R., Rajan, L., Li X., and Lufkin T; **The Dlx5 and Dlx6 homeobox genes are essential for craniofacial,axial, and appendicular skeletal development**; *GENES & DEVELOPMENT*; 2002; Vol16; p.1089–1101

[2] Grace Panganiban and John L. R. Rubenstein; **Developmental functions of the Distal-less/Dlx homeobox genes**; *Development*; 2002; Vol129; p. 4371-4386

2. Overexpression of *dll* under promoter *eq* (*eq* drives *dll*, *eq>dll*)

Since the dysfunction of *dll* displays shorter antennae, the overexpression of *dll* was though to extend the length of antennae. In the experiment, we overexpressed *dll* under promoter *equational* (*eq* drives *dll*, *eq>dll*). Promoter *eq* activates in distal antennae and anterior equatorial region of eyes. These activating regions were similar to where *dll* expressed. However, overexpression of *dll* has no effect on antenna growth (Fig 2).

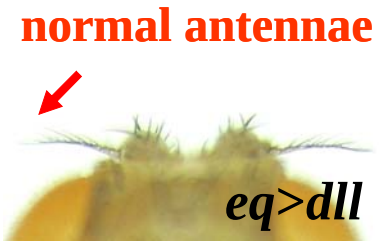


Fig 2: Overexpressing *dll* under promoter *eq* displayed normal antennae and eyes

3. *GAL4*/UAS system

The overexpression system we used is based on *GAL4*/UAS expression content. Gene *galactose-inducible* (*GAL4*) is a yeast transcription-factor gene that can be expressed in flies. Along with a certain nucleotide sequence called Upstream Activation Sequence (UAS) from yeast, Gal4 protein can regulate the expression of certain gene. When Gal4 protein is promoted by specific promoter (*eq*), it will bind to UAS and activate UAS. The activated UAS will enhance the expression of the gene next to it (*dll*)[3] (Fig 3).

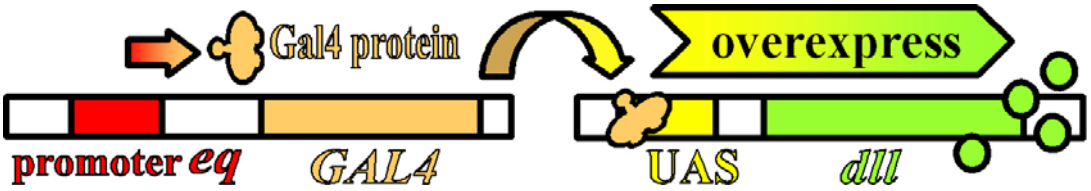


Fig 3: *GAL4*/UAS system

4. Searching gene disturbing the regulation of *dll*

The absence of *dll* presents defect in distal antennae, however, the overexpression of *dll* has no effect on antennae growth, meaning that *dll* might not be the limiting factor in the developmental process of antennae. The positive control of *dll* would be difficult to discover. We then aimed to search for gene that disturbs antennae development which requires *dll*.

[3] Joseph B. Duffy; **GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife**; 2002; *Genesis*; Vol34, p.1-15

5. EP

Here we used a transposon EP to randomly select gene from fly genome. EP is randomly inserted in *Drosophila* genome. Since EP contains UAS, by the control of *GAL4*, we can express the gene at the downstream of EP[4].

6. Modifier screen—combining *GAL4*/UAS and EP system—searching for gene disturbing *dll* regulation

To search for the gene, we performed the modifier screen. We overexpress an unknown gene X which was randomly selected by EP system. To generate the strain, we crossed the strain with *eq-GAL4* (A) with strains contained EP insertion (B). The progeny (C) contained *eq-GAL4* and EP-gene X (Fig 4). Since we were searching for gene disturbing *dll* regulation, we screened the progeny (C) with antennal defect.

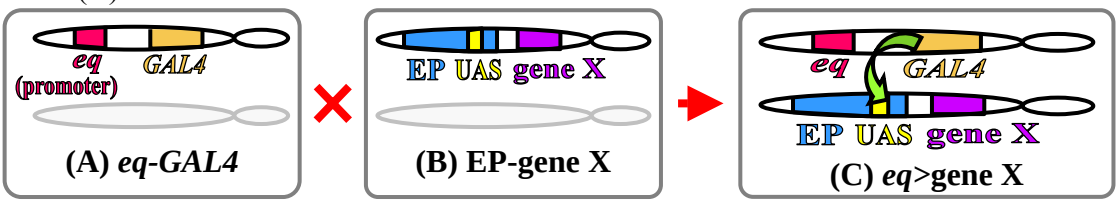


Fig 4: Modifier screen

7. Identification of gene X which is responsible for the antenna defect

After screening out the strain with defective antennae, we identify gene X which is responsible for the antennal defect. Herein, using inverse PCR, we will obtain the partial sequence of gene X and the flanking sequence of EP insertion (Fig 5). After matching with fly genome, we may identify the gene X[5].

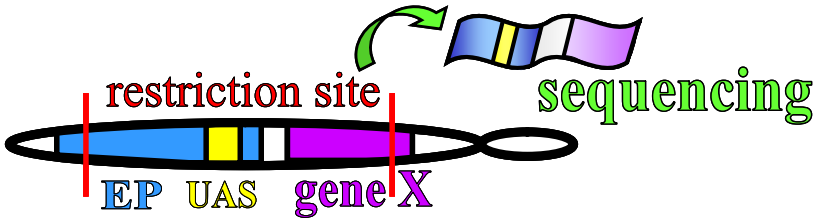


Fig 5: Inverse PCR

[4] Pernille Rorth, Kornelia Szabo; **Systematic gain-of-function genetics in *Drosophila***; 1998; *Development*; Vol125, p.1049-1057

[5] E. Jay Rehm; **Inverse PCR & cycle sequencing of P element insertions for STS generation;** 2003; Berkeley Drosophila Genome Project

8. Immuno-histo-chemical (IHC) staining—identifying gene X effect on *dll*

Gene expression in imaginal disc affects organ in adult (Fig 6). Therefore, we used IHC staining to study gene X interference with *dll* and its effect on antennae and eyes development in adult

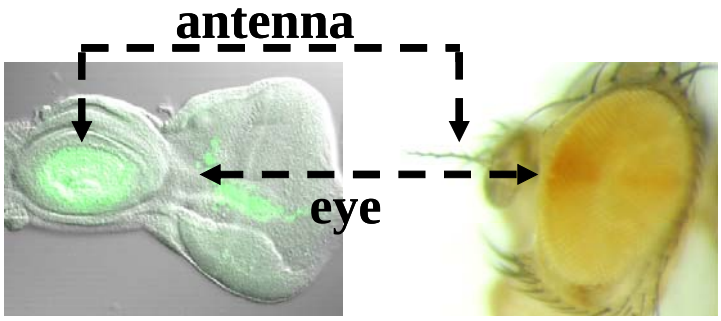


Fig 6: Gene expression in disc affects organ in adult: Green region, indicating *eq* expression, grows to the *eq*-activating site in eye and antenna in adult.

IHC staining made it possible to identify the spatiotemporal expression pattern of gene X and understand gene X effect on *dll*.

9. Identification of gene X function in apoptosis and cell cycle

In our experiment, in addition to gene X interference with *dll*, the defective phenotype was also thought to be caused by gene X function in apoptosis and cell cycle. We performed IHC staining to study gene X effect on apoptosis and cell cycle.

We then performed some analysis of gene X function.

Herein, gene *protein 35* (*p35*) was utilized. *p35* can block apoptosis process. To study gene X effect on apoptosis, we co-overexpressed gene X and *p35*. Since *p35* inhibits apoptosis process, if gene X promotes apoptosis, the overexpression of *p35* will block the process and rescue the defective phenotype (Fig 7)[6].

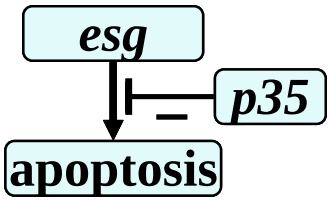


Fig 7: Overexpression of *p35* blocks the apoptosis process promoted by gene X

Likewise, Gene *cyclin E* (*cycE*) was used to analyze gene X function in cell cycle. Since *cycE* promotes cell cycle, we co-overexpressed gene X and *cycE*.

[6] Bruce A. Hay, Tanya Wolff and Gerald M. Rubin; **Expression of baculovirus *p35* prevents cell death in *Drosophila***; 1994; *Development*; Vol120; p.2121-2129

Therefore, if gene X inhibits cell cycle, overexpression of *cycE* can proceed to the cell cycle process and rescue the defective phenotype (Fig 8)[7].

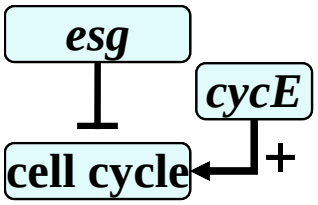
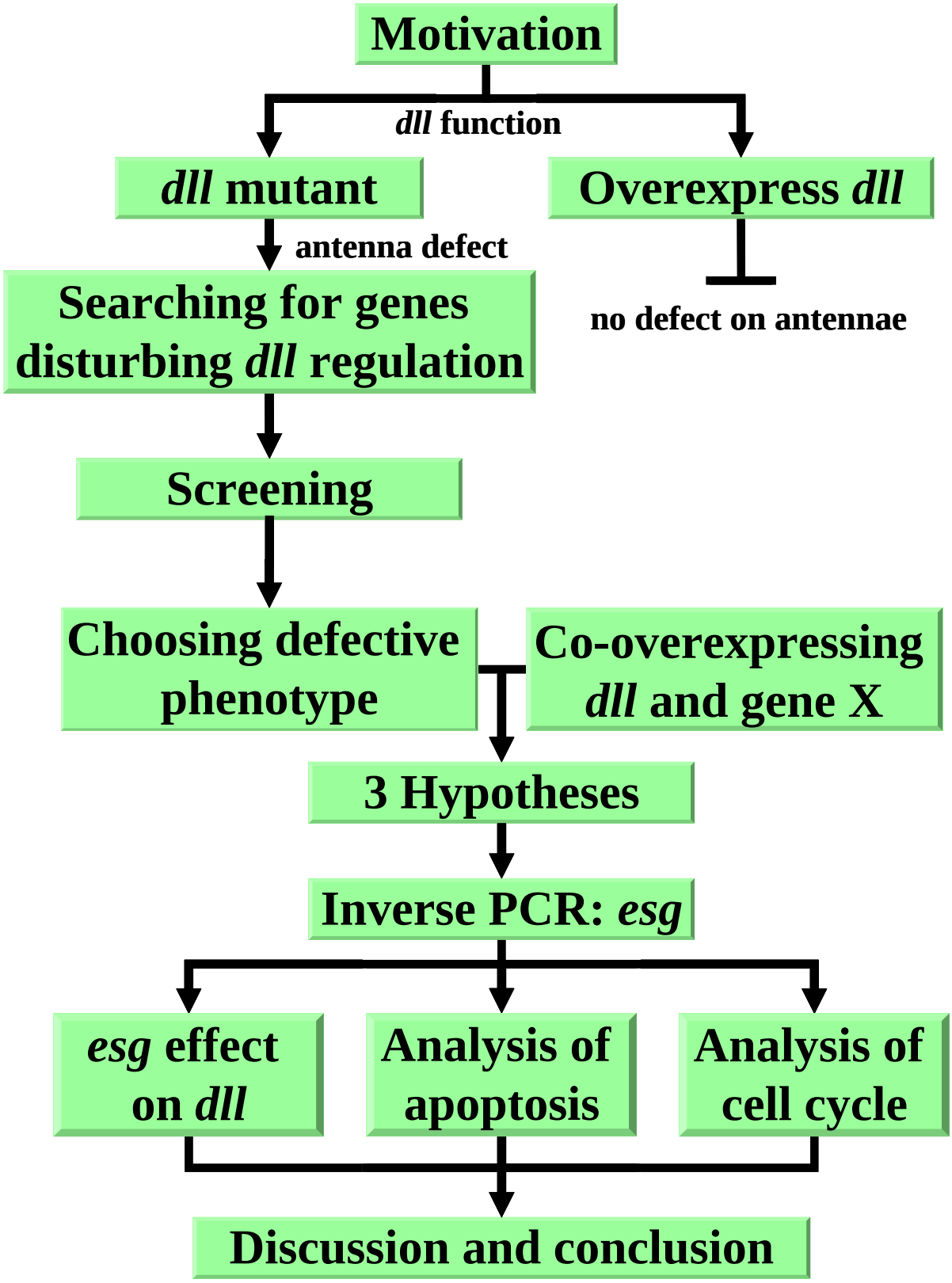


Fig 8: Overexpression of *cycE* can proceed to the cell cycle process that is blocked by gene X

[7] S. A. Trunova, T. D. Dubatolova, and L. V. Omel'yanchuk; **A study of expression of cell cycle genes in *Drosophila* using an enhancer trap and radioautography**; 2002; *Russian Journal of Developmental Biology*; Vol. 33; p.95-99

(C) Research Flow



B. Results

(A) Modifier screen－finding defective phenotype

1. Normal type (w^{1118} and EP strains)

In our study, strain w^{1118} was used as background. In addition to the white eyes, the strain displayed normal phenotype (detail information of w^{1118} are demonstrated in *C. Material and Equipments*).

The EP strains are strains with EP randomly inserted within fly genome. In these strains the UAS in EP was not activated (because the strain didn't contain *GAL4*). Therefore, EP strains displayed normal phenotype (Fig 9).

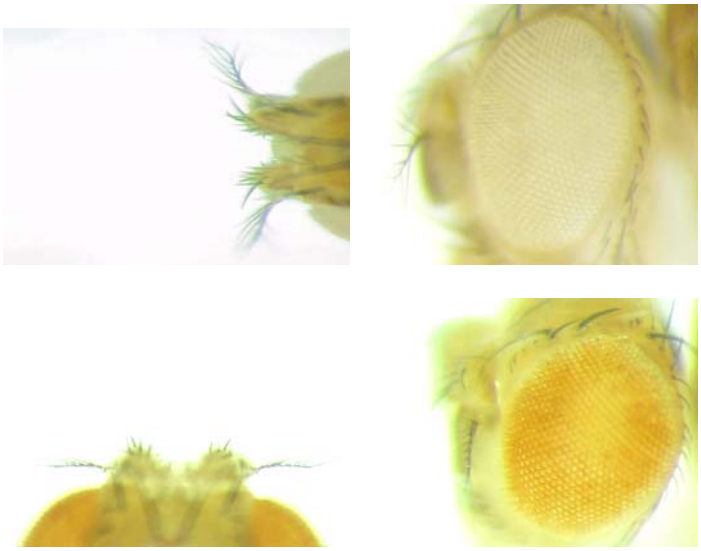


Fig 9: Phenotype of control — normal type and EP251

2. *eq-GAL4*×EP-gene X (EP251)－defective phenotype in antennae

In the modifier screen, we searched for gene disturbing *dll* regulation and inducing antennal defect. After screening 600 EP, we found only one EP induced defective antennae (EP251). However, the strain also displayed defect in the anterior equatorial region of eyes (Fig 10). The result indicates that **gene X might be inhibitory to the *dll*-mediated antenna development.**

Fig 10: Defective phenotype in antennae and eyes — *eq-GAL4* + gene X

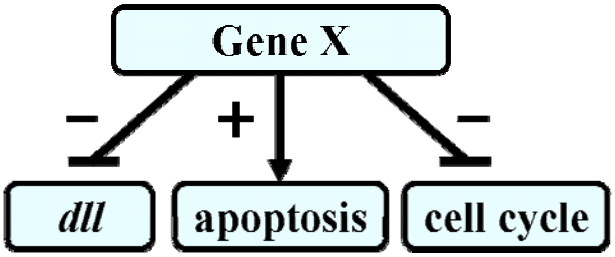
3. ***eq>dll* + gene X—indicating gene X is sufficient to interfere with *dll* regulation when *dll* is overexpressed**

We then overexpressed gene X under the condition that *dll* was also overexpressed. The strain also displayed the defective phenotype (Fig 11), indicating that gene X is sufficient to interfere with the regulation of *dll* even when *dll* is overexpressed. Defective phenotype means that the amount of cell was somehow reduced, therefore, **gene X was also considered to promote apoptosis or cell cycle.**

Fig 11: *eq*> gene X + *dll* also displayed shorter antennae and defect in eyes

(B) Hypotheses

1. **Gene X interferes with *dll* regulation, causing shorter antennae.**
2. **Gene X promotes apoptosis, causing cell death in antennae and eyes.**
3. **Gene X inhibits cell cycle, preventing cell growth in antennae and eyes.**



(C) Identification of gene X – *escargot*

Using inverse PCR, we obtain partial sequence of EP and gene X (detail data of the sequence of gene X are presented in *Appendix*). After matching with fly genome, we identified gene X as *escargot* (*esg*).

(D) IHC Staining of *escargot* in eye and antenna in larva shows that *esg* is inhibitory to *dll* in the level of post-transcription or protein function

1. normal type (*eq*>GFP)

This strain was used as a control. Protein Dll (blue fluorescent color) presented in the center of antenna disc (the region will grow to distal antennae in adult) (Fig 12).

2. *eq>dll*

This strain was another control. The bright blue color implies the overexpression of *dll* in distal antennae and the anterior equatorial region of eyes (Fig 12).

3. *eq>esg* + GFP

When overexpressing *esg* under *eq*, protein Dll (blue color) was reduced in the center of antenna disc. The reduction of Dll in the central antenna disc was thought to cause reduction of cell growth in distal antennae. Therefore, the staining result refines our first hypotheses that *esg* is inhibitory to the regulation of *dll* (Fig 12).

4. *eq>esg + dll*

When overexpressing *esg* under the condition that *dll* was promoted by *eq*, the staining result also showed reduction of Dll in the center of antenna disc.

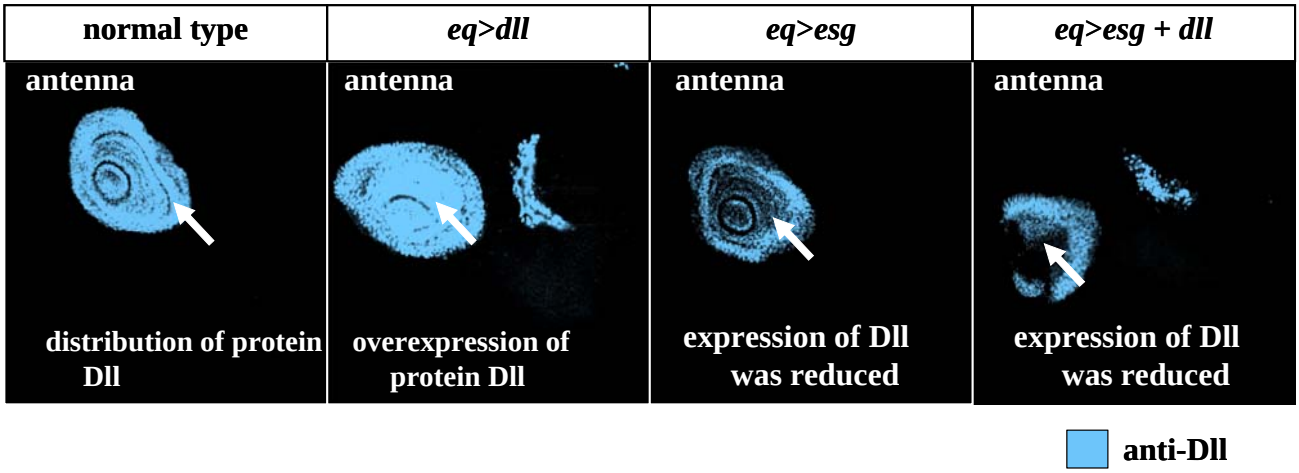


Fig 12: Staining results (Blue: Dll)

Strain *eq>esg* and *eq>esg + dll* both result in reduction of protein Dll. Since in *eq>esg + dll*, *dll* expression is controlled by *GAL4*/UAS system, it is unlikely that *dll* transcription would be affected by *esg*. Therefore, **we postulated that *esg* would control *dll* in the level of post-transcription or protein stability.**

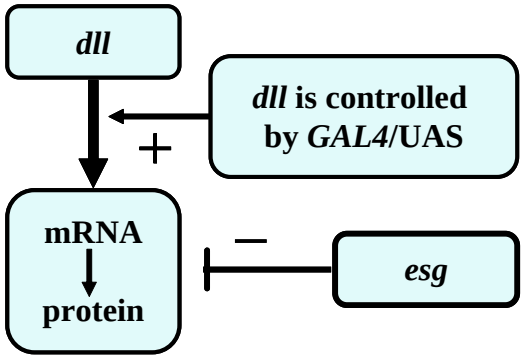


Fig 13: *esg* would control *dll* in the level of post-transcription or protein stability

However, since *dll* didn't express in eye disc endogenously, the defect in eye was thought to cause by *esg* effect on apoptosis or cell cycle.

(E) IHC staining indicating that *esg* promotes apoptosis and inhibits cell cycle

To understand the involvement of *esg* in apoptosis, we performed IHC staining upon *eq>esg*. Compared with normal type, Caspase3 (indicating apoptosis process) in *eq>esg* presented in the center of antenna disc and the anterior equatorial region of eye disc (Fig 14). The result shows that *esg* promotes apoptosis.

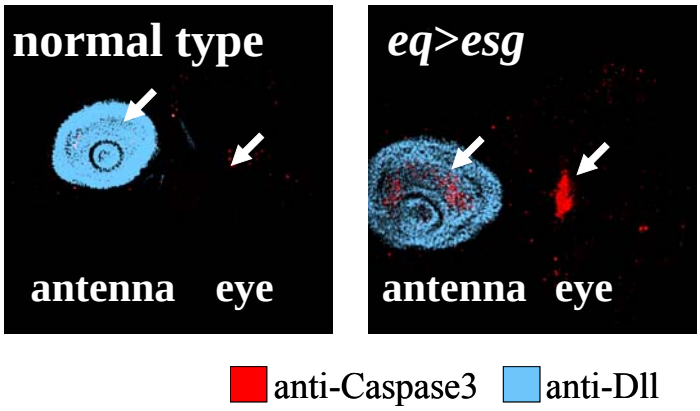


Fig 14: Staining results of *eq>esg* (Red: Caspase3; Blue: Dll)

In the analysis of *esg* function in cell cycle, Compared with normal type, protein CycE (indicating cell cycle process, pink color) was reduced in *eq>esg* (Fig). The result shows that esg would inhibit cell cycle in distal antennae and eyes.

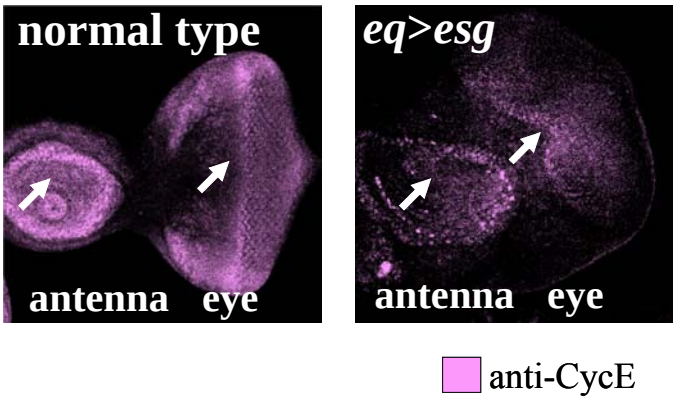


Fig 15: Staining results of *eq>esg* (Pink: CycE)

(F) Confirming *esg* function in apoptosis and cell cycle

With the used of gene *p35* and *cycE*, we confirmed *esg* function in the promotion of apoptosis and the inhibition of cell cycle.

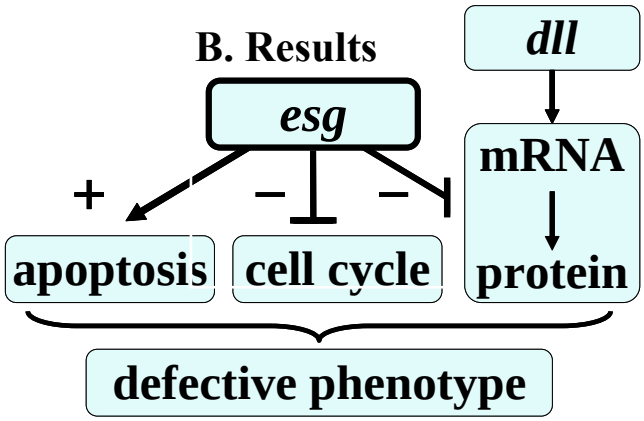
The results displayed rescued phenotype in both *eq>esg*×UAS-*p35* (Fig 16)and *eq>esg* ×UAS-*cycE* (Fig 17), meaning that *esg* would cause the promotion of apoptosis and the inhibition of cell cycle alike. These functions of *esg* were responsible for the defective phenotypes.

Fig 16: *eq>esg* + *p35* displayed rescued phenotype

Fig 17: *eq>esg* + *cycE* displayed rescued phenotype

C. Conclusions and Discussion

- The defective phenotype was caused by:
  1. *esg* inhibitory control on *dll* post-transcriptional regulation or protein function.
  2. Gene *esg* function in the promotion of apoptosis.
  3. Gene *esg* function in the inhibition of cell cycle.



- Gene *esg* could control the expression of the development-essential gene *dll*, and *esg* would also affect apoptosis and cell cycle. Therefore, *esg* was considered to be the essential gene in *Drosophila* development which we were searching for.
- *esg* is also presented in human. Since *esg* would inhibit *dll* expression in *Drosophila*, it was also thought to affect the expression of *Dlx* family (*dll* homologous gene in human). Hence, further study of *esg* might enable us to study the mechanism of SHFM disease.

D. Materials and Equipments

(A) Fly Strains:

1. w^{1118}

In our study, strain w^{1118} was used as background. All the transgenic performances were base on this background. In addition to the white eyes, the strain presents normal phenotype (Fig 18). The white eye phenotype was convenient for us to produce transgenic flies because the marker of the recombinant DNA usually displays red eye phenotype.

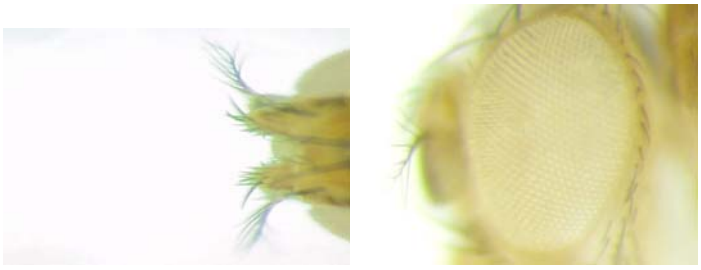


Fig 18: w^{1118}

2. *eq-GAL4*/TSS (*equational*/TSS):

The promoter, *eq*, mainly activates in antennae and the anterior equatorial region of eye. In our experiment, the marker of the strain is the red bend on white eye.

Fig 19: *equational*/TSS

3. *eq*＞*dll*/TM6B (*equational* drives *distal-less*/TM6B)

The symbol "*eq*＞*dll*" means overexpressing *dll* under promoter *eq*. This was thought to induce longer antennae. However, it displayed normal ones. (Fig 8)

Fig 20: *equational* drives *distal-less*/TM6B

4. UAS-*p35* (UAS-*protein 35*):

Gene *p35*, originally from virus, would restrain apoptosis process. The strain presented normal phenotype.

Fig 21: UAS-*protein 35*

5. UAS-*cycE* (UAS-*cyclin E*):

Gene *cycE*, controls the check point between G1 phase and S phase, therefore, the overexpression of the gene would improve cell cycle. By co-overexpression of *cycE* and a certain gene, we can identify the gene's function in cell cycle. The strain presents normal phenotype.

Fig 22: UAS--*cyclin E*

6. EP strains

EP vector is used as an element for seeking out gene X.

EP contains the UAS. While EP is randomly inserted into flies' genome, it will overexpress the downstream gene X with the presence of Gal4. Containing *mini-white* in EP, the transgenic flies will present red eyes. In our experiment, we have 600 EP lines.

Fig 23: EP lines

(B) Primers:

Plac4(27) - ACT GTG CGT TAG GTC CTG TTC ATT GTT

Plac1 (24)- CAC CCA AGG CTC TGC TCC CAC AAT

Pry4 (23) - CAA TCA TAT CGC TGT CTC ACT CA

Pry1 (26) - CCT TAG CAT GTC CGT GGG GTT TGA AT

Pry2 (28) - CTT GCC GAC GGG ACC ACC TTA TGT TAT T

Plw3-1 (19) - TGT CGG CGT CAT CAA CTC C

Pwht1 (29) - GTA ACG CTA ATC ACT CCG AAC AGG TCA CA

(C) Enzymes:

1. Restriction enzyme:**Sau3A I**, HinP1 I, or Msp I (NEB)
2. T4 DNA ligase (Roche)
3. RNAse A
4. Proteinase K
5. Taq

(D) Antibodies:

1. 1st Antibody: anti-Dll (rat)
2. 1st Antibody: anti-Caspase 3 (rabbit)
3. 1st Antibody: anti-Escargot (rat)
4. 2nd antibody: cy5 for rat (blue fluorescent)
5. 2nd antibody: cy3 for rabbit (red fluorescent)

(E) Sequencing:

We sequenced our PCR product by helping from DNA Sequencing Core Lab in IMB, Academia Sinica.

(F) Other Equipments:

1. Confocal fluorescence microscope
2. Dissecting microscope
3. Electrophoresis equipment
4. Incubator
5. Fly-operating stage
6. Tweezers and brushes
7. Fly stocks (100 tubes per box)

(G) Drugs:

1. Water
2. Carbon Dioxide (to anesthetize the flies)

3. TBST
4. NGS (normal goat serum)
5. Glutaraldehyde/ PBS (phosphate-buffer-saline)
6. ETBr
7. Buffers (HE, TE)
8. Inactive yeast
9. Glucose
10. Ethyl Ether
11. Tris (Tris-(Hydroxylmethyl)-Aminomethane, 0.1M, pH9.0)
12. EDTA (Ethylenediamine Tetraacetic Acid, 0.1M)
13. SDS (Sodium Dodecyl Sulfate, 1%)
14. Potassium Acetate
15. Ethyl Alcohol
16. Ammonium Acetate
17. Phenol/ CIA
18. Agar
19. Agarose
20. TBE (Tris/Borate/EDTA)
21. Sodium Hydroxide
22. Sodium Chloride
23. Phenol/Chloroform
24. Isopropyl Alcohol
25. dNTPs
26. Glycerol
27. PBS
28. Paraformalehyde
29. Ringer's solution
30. Sucrose
31. N-propyl gallate
32. Tryptone

E. References

(A) Documents

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(C) Webs

Fly Base: http://flybase.bio.indiana.edu/

Japan *Drosophila* Database: http://kyotofly.kit.jp/JDD/bodyparts/

F. Appendix

(A) The original sequencing data of plasmid rescue

escargot:

TTGGTGCAAGTGTACGAGTTCGGCACGTGAGGCAATTCTACATAAACAAGGCGAA
ACCTGTTTAGCCGAGTGTCGCGATTGGTCGACGCATATACCTGAATGAACATGTATA
CTCTCGCGGAACGCCCGAGAGAGCGTTC

EP251 insertion site↓

TCTTTTGGCAGCTTCGTGTAATGAGCCGAGTGCCGAGTGCAGGGGCTCTTTTGGCA
GCTTCGTGTAATGAGCCGAGTGCCGAGTGCAGGGGCAGTATAAAAAGGCCGCACC
ACCGCCAAACTCGCTCAGTTATTCCCAGACTCTAACCGATTCACATAACCATCTCTC
CCAACGCAATATACCCGAAATATAACCGATATTCAATCGCTTTGCTTAACGGAACAC
GCGTTTGGTATCTGTGCATCGATCGAGTATTTGTGCCTGTGTGAACATGACACACTG
AAAACAAAGTGACGAAAATTAAGAACATTTTACAACAATTCCGGTAAACTATCAAT
AAAAAGCCAGTAAA**ATGCATACCGTGGAAGACATGTTGGTGGAGAAAAACTAC**
AGCAAGTGCCCGCTAAAAAAGCGCCCAGTTAATTACCAGTTCGAGGCGCCTC
AAAATCACAGTAACACCCCAAATGAGCCGCAGGATTTGTGCGTAAAGAAAAT
GGAAATTCTGGAGGAAAATCCCTCCGAGGAACTGATCAACGTCAGCGATTGT
TGCGAGGACGAGGGTGTGGATGTGGATCATACAGATGATGAACACATCGAGG
AAGAGGACGAGGACGTCGATGTGGATGTGGACTCGGACCCCAATCAGACCC
AGGCTGCGGCTTTAGCTGCTGCCGCAGCTGTGGCCGCTGCTGCAGCCGCCT
CCGTGGTTGTGCCCACGCCCACATACCCGAAATATCCATGGAACAACTTCCAC
ATGTCGCCCTACACCGCCGAGTTCTACAGGACCATCAATCAGCCGGGTCATC
AGATTTTGCCGCTGCGTGGCGATCTCATTGCGCCCAGTTCGCCGAGTGACTC
GCTGGGCTCCCTGTCGCCGCCACCACATCATTACCTTCATGGTCGCGCAAGC
TCCGTTTCCCCGCCCATGAGATCGGAAATGATCCATAGACCAATCGGCGTGC
GGCAGCACCGTTTCCTGCCCTATCCCCAGATGCCCGGTTATCCGAGCTTGGG
CGGCTATACCCATACCCATCATCACCATGCGCCTATCTCGCCGGCTTATTCCG
AGAATTCCTACTACTCCATGCGATCGATGACGCCTGAATCCAGCTGCAGCTCC
TCGCTGCCCGAGGATCTATCCCTGAAACACAAGAACCTGAACTTGA

Blue: Flanking sequence of EP251 insertion
Red: *ESG* protein coding sequence

G. Appreciations

Laboratory N415 in Institute of Molecular Biology in Academia Sinica
Dr. Y. Henry Sun
Ph.D. Student Wang Cheng-Wei
Taipei Municipal Chien-Kuo Senior High School
Biology Teacher Tong Yi-Shan
Taipei First Girl Senior High School
Sylvia Lo

評語

研究工作紮實、深入，兩位作者對題目瞭解的程度在深度上和廣度上都有傑出的表現，論文完整，有創新的發現，需加強的是表達能力。