Ectopic Expression of dWarts Containing Multiple Mutations in the Insert Segment Caused Enhanced Phenotype in Transgenic *Drosophila*

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Abstract

dWarts is a NDR kinase that plays a key role in the regulation of cell proliferation and apoptosis. Loss of function in dwarts causes cell over-proliferation and cancer. In this study, one of regulatory mechanisms of dwarts was reported. Similar to human Lat1, the insertion segment of dwarts in the catalytic domain was identified as negative regulatory domain, as multiple mutations in the insertion segment by substituting the conserved alkaline amino acids resulted in the enhanced small compound eye phenotypes when expressed in the eye discs of *Drosophila*. Moreover, our results demonstrated that regardless of identical genetic background, both wild type dwarts and mutant *dwarts* could confer incomplete penetrance phenotype when ectopically expressed in the eye discs of transgenic *Drosophila*, suggesting multiple regulatory mechanisms may be associated with dwarts.

Keywords: dWarts, Insertion segment, Cell proliferation, Incomplete penetrance

1. Introduction

During the development of *Drosophila*, both cell division and programmed cell death (apoptosis) contribute the formation of any organs with patterned tissues (Saucedo *et al*. 2007; Harvey *et al*. 2007). The Hippo pathway, first identified in *Drosophila*, coordinates cell proliferation and apoptosis and opens a new field to decipher the mechanisms of organ size control and tissue homeostasis (Saucedo *et al*, 2007; Harvey *et al*. 2007; Hariharan *et al*. 2006).

The loss-of-function mutants of Hippo pathway components exhibited overgrowth phenotypes and inhibited apoptosis, suggesting this pathway plays a key role in inhibiting cell proliferation and promoting apoptosis during *Drosophila* development (Saucedo *et al*. 2007; Harvey *et al*. 2007; Hariharan *et al*. 2006; Lai *et al*. 2005; Pan 2007; Cho *et al*. 2006; Goulev *et al*. 2008; Wu *et al.* 2008; Zhang *et al*. 2008; Shimizu *et al*. 2008; Thompson *et al*. 2006; Feng *et al*. 2007). The dwarts, a key components of Hippo pathway, encodes a 121kD Ser/Thr protein kinase that belongs to NDR kinase (nuclear Dbf2-related), a subfamily of the AGC kinase (protein A, protein G, protein C kinase). The NDR kinases are highly conserved from mammals to yeast and regulate important cell processes such as mitotic exit, morphological change, cell proliferation and apoptosis. The primary structures of NDR kinases share some common features (Hergovich *et al*. 2007). For example, their catalytic domains consist of 12 subdomains; their activation segments are located in subdomain VIII and the hydrophobic motif is in the C-terminus; they contain an N-terminal regulatory domain (NTR domain) and an

insert segment of 30-60 amino acids between subdomain VII and VIII (Hergovich *et al*. 2007). The dwarts shares 74% sequence identity with human Lats1 and 50% identity with hNDRs in the C-terminal kinase domain.

The *hLATS1* can rescue the lethality of *dwarts* mutants in fly (Tao *et al*, 1999). In the homozygous *dwarts-/-* mutant larvae, size of the body, eye disc and brain are larger than wild type siblings, but the cell differentiation is not affected (Xu *et al*. 1995). Different *dwarts* mutant alleles exhibited phenotypes from viable to 1st instar larval lethality (Xu *et al*. 1995; Justice *et al*. 1995). The dwarts has been found to regulate CDC2, cyclin A and cyclin B activity, which suggests Hippo/ Warts also function in mitotic regulation (Shimizu *et al.* 2008; Tao *et al*. 1999; St John *et al*. 1999).

The molecular regulatory mechanisms of NDR kinase are better known in the mammalian model systems than other systems. Consequently, the regulatory mechanisms of NDR kinase in Drosophila (dwarts) have not been clarified yet. In this study, we report new regulatory mechanisms for dwarts: dwarts can be activated by mutations of its insert segment and ectopic expression of either wild type dwarts or mutant dwarts resulted in incomplete penetrance phenotype in eye discs.

2. Material and Methods

2.1 Strains used in this study

Drosophila strains used in this study were listed in the Table 1.

2.2 Drosophila strains culture

All strain stocks were grown in 10 ml disposable vials in an incubator at 25 ℃ unless the temperature was indicated. Hard agar fly food was made according to the recipe described previously (Wirtz *et al*. 1982).

2.3 Tissue culture of HEK 293T cell and transfection

HEK 293T cells (a gift from Dr Yanming Wang, PSU) were grown in the 37 ℃ incubator with 5% CO_2 supply. Medium used was DMEM (Invitrogen, CA) with addition of 10% fetal bovine serum, 100 U/ mL ampicillin and 100 U/mL streptomycin. Cells were passaged according to the ATCC protocol. In brief, medium was aspirated and cells were rinsed with 1x PBS. Cells were digested by 0.25% Trypsin-EDTA (Invitrogen, CA) for 5-15 min and complete medium was added to stop the digestion. Cells were spun down, resuspended in the complete medium and seeded to new plate. DNA was isolated with mini-prep kit (Promega, WI). Cells were transiently transfected with PolyFect Transfection Reagent (Qiagen, CA) according to the manufacturer's protocol and were harvested 36 hrs after transfection.

2.4 Site- directed mutagenesis and sub-cloning

By BLASTing dwarts with other NDR kinases, we identified the conserved insert segment sequence between subdomain VII and VIII (supplement 1A). The four point-mutations were introduced into wild type myc-dWarts on the vector pcDNA3 by site-directed mutagenesis according to the manufacture protocol (Stratagene, CA). The Arg of 907, 908, 909, and 915 were substituted with Ala by site-directed mutagenesis. Wild type dwarts fused with myc tag at its C-terminal was used as the template. The following mutagenesis primers were used: dwarts RRRR907 AAAA (F 5'- gcccaccgtgctggaggcggca gcgatgcgcgatcacc; R 5'-ggtgatcgcgcatcgctgccgcctccagcacggtgggc). The PCR cycle was 95 ℃ 30 sec, and 16-18 cycles of 95 ℃ 30 sec, 55 ℃ 1min, and 68 ℃ 10min. 1-10 ul DNA was transformed into chemical competent cells by 1 min heat shock at 42 ℃. Plasmids of positive clones were isolated with mini-prep kit (Promega, CA). The mutations were confirmed by DNA sequencing of full –length inserts. The mutant *dwarts* DNA was cut from pcDNA3 by digestion with EcoR I and Not I. Then the insertion was ligated with pUAST digested with EcoR I and Not I by T4 ligase (New England Labs, MA) according to the manufacturer's protocols. The ligation was transformed into chemical competent cells and positive clones were confirmed by restriction analysis.

2.5 Western Blotting

HEK293T cells transfected with pcDNA-myc-dwarts or pcDNA-myc- AAAA *dwarts* were harvested with RIPA lysis buffer (150 mM sodium chloride, 1.0% NP-40 or Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 50 mM Tris, pH 8.0, 1 mM PMSF) and then cell lysates were treated with sonicator 30sec 3 times at 4 ℃. Fly samples from USA- myc- AAAA dwarts transgenic fly crossed with *ey-Gal4/S.T*. The eye discs were isolated from the progenies' third instar larval stage. Eye discs were homogenized in lysis buffer (1% Triton X-100, 0.5% NP-40, 50mM NaCl, 10mM Tris, pH7.4, 1mM EDTA, 1mM EGTA, 0.2 mM PMSF).15-20 ug total proteins were loaded per lane. Western blotting was performed according to the Amersham ECL protocol (Amersham, USA). Primary antibody was rabbit anti-Myc (9E10, 1:5000) (Santa Cruz

Biotechnology, CA), mouse anti-α-tubulin (1:5000). The second antibody was anti-mouse HRP, and anti-rabbit HRP.

2.6 Drosophila microinjection and transgenic fly balancing

Drosophila strain (*w, Dr/TMS Sb Δ 2-3*) was cultured in cage covered with Molasses agar plate (1112 ml H_2O, 44g agar, 180 ml molasses) to collect fly eggs. Transgenic DNA (500-1000 ug/ml) 1-2 ul mixed with green food color was loaded into glass needle with syringe. Fly eggs received microinjection were cultured in moisture chambers at 18 ℃ for 3 days. Then the hatched embryos were picked up and cultured in the vials at 25 ℃. The transgenic flies were crossed with wild type strain (w^{1118}); the progenies with red eyes were identified as transgenic positive. The male transgenic flies were balanced with *w; Adv/SM1; Sb/TM6*. To examine the phenotype, the transgenic flies were crossed with *w, GMR-Gal4; or w, ey-Gal4/ S.T.* to examine the eye morphology.

2.7 Scanning electronic microscope (SEM)

The selected flies were treated with different concentrations of ethanol for dehydration at room temperature. Flies were in 5 ml of 25% ethanol for 12-24 hrs, in 50%, 75% ethanol for 12-24 hrs, and 2 times in 100% ethanol for at least 12-24 hrs. The following steps were performed at Electron Microscopy Facility, PSU: samples were subjected to critical point drying, and then were put in cages and the ethanol was exchanged in the sample by liquid CO_2 under high pressure. The dried samples were mounted on the T.V. coated S.E.M. stubs, mounted samples were coated with a layer of 25nm platinum. Samples were visualized and were taken photos by the SEM microscope JEOL JSM 5400.

3. Results and Discussion

3.1 Generation of insertion segment mutations in the dwarts

It was reported that *dwarts* deficiency in flies showed diverse phenotypes from larvae to adults in the process of fly development such as giant larvae, enlarged eye discs, enlarged central nerve system, and larvae lethality or giant adults. These phenotypes suggested that dwarts played important roles in inhibiting cell proliferation and promoting apoptosis (Xu *et al*. 1995; Tao *et al*. 1999; Dong *et al.* 2007; Udan *et al.* 2003). In order to understand the regulatory mechanisms underlying dwarts activity, we designed the mutations in the insertion segment between subdomain VII and VIII, which was reported to inhibit human Lats1 kinase activity and other NDR kinases (Hergovich *et al*. 2007; Bichsel *et al*. 2004). The conserved alkaline amino acids in dwarts insert segment were identified by aligning dwarts with other NDR kinases (supplement 1A). The mutant *dwarts*, containing four substitution mutations (AAAA-*dwarts*, R907AR908AR909AR915A) in the insertion segment, was confirmed by DNA sequencing. The mutated protein was stable expressed in HEK293T cells, indicating the mutations we introduced didn't change the stability of dwarts (supplement 1C).

3.2 The AAAA-*dwarts transgenic fly showed diverse eye phenotypes*

The mutated *dwarts* (AAAA-*dwarts*) was introduced into fruit fly by microinjection and the phenotypes of the positive transgenic strains were examined in their eye discs. The mutant *dwarts* transgenic fly (w, UAS-AAAA-*dwarts)* crossed with *w; ey-Gal4/S.T.* to generate progenies (*w; ey-Gal4, UAS-AAAA-dwarts*). The progenies showed smaller compound eye phenotypes (Figure 1, Figure 3, Supplement 2). The diameters of compound eyes of AAAA-*dwarts* transgenic fly reduced comparing to those of wild type (Caton S) and the dwarts transgenic fly (*w; ey-Gal4, UAS-dwarts*) (Figure 3, Supplement 2). With much smaller size of compound eye in the AAAA-*dwarts* transgenic fly, some compound eyes showed small diameters; while others showed protruded surface; and it was common that the surface of compound eye formed very narrow cones (Supplement 2). The surface morphology of compound eye was changed too and the pattern of ommatidia and mechanosensory bristles were disrupted in the AAAA-*dwarts* transgenic fly (Figure 1). For example, some ommatidia fused together (Figure 1B, 1C, 1D); some of them disappeared with only trace left (Figure 1C); some of the mechanosensory bristles disappeared due to the merged ommatidia (Figure 1B, 1C, 1D); some mechanosensory bristles clustered due to merged/missed ommatidia (Figure 1D). The dwarts is known that it enhances cell apoptosis and inhibits cell proliferation (Xu *et al*. 1995). Comparing to the dwarts transgenic flies, the AAAA- *dwarts* transgenic flies showed reduced size of compound eye suggested the reduced number of ommatidium. The morphology of some ommatidia was disrupted, such as missed mechanosensory bristles, the edges of corneal lens became unclear and fused together (Figure 1B, 1C, 1D). Moreover, the impaired morphology of ommatidia might be due to the decreased cell number during eye disc development. Besides, the compound eyes of transgenic fly exhibited more ommatidia fusion and missed mechanosensory bristles at the posterior side (Figure 1). In fruit fly, compound eye is developed from eye-antennal disc, which is derived from

around 20 cells of the optic primordium in the embryonic blastoderm (Krafka *et al*. 1924). In the middle of third instar larva stage, a morphorgenic furrow progresses in the eye-antennal disc from the posterior to anterior driven by hedgehog (Thomas *et al*. 2003). Cells in eye discs precede cell proliferation and apoptosis, the phenotypes in compound eye of AAAA-*warts* transgenic flies suggested reduced cell proliferation and enhanced apoptosis (Wolff *et al*. 1991).

3.3 Similar to the wild type dwarts transgenic line (6R), the AAAA-dwarts transgenic flies showed incomplete penetrance phenotype in the compound eyes

In this study, we surprisingly found a new phenomenon associated with dwarts ectopic expression in addition to its smaller eye phenotype (Lai *et al*. 2005). Diverse small eye phenotypes were observed in compound eyes of dwarts transgenic flies (Figure 2, Figure 4), such as small compound eye, single cone eye and double cone eye. Similarly, we also observed varied compound eye phenotypes in multiple independent transgenic lines of AAAA-dwarts (Figure 2, Figure 4, Supplement 2). While wild type fly (Caton S) showed complete penetrance with 100% consistent eye size (Pei *et al.* 2007); transgenic flies expressing dwarts (6R) showed 86% small compound eyes, 8.6% single cone eye and only 1% double cone eye; similarly, eleven lines of *AAAA-dwarts* transgenic flies all demonstrated varied eye phenotypes though the percentage varied (Figure 2A). Therefore, it is clear that the genetically-identical fly individuals ectopically expressing dwarts (6R) or AAAA-*dwarts* exhibited varied eye phenotypes, which is so called incomplete penetrance (Griffiths *et al*. 1993).

It has reported that genetic modifiers and environments factors all contribute to the variation of phenotype during development. In zebrafish, the cyclopia penetrance phenotype was identified in sqt deficient mutant; the phenotype was affected by the heritable factors and the temperature could also play roles in the degrees of the sqt penetrance phenotype (Pei *et al*. 2007). The cyclopia penetrance phenotype was associated with the degree of the downstream Activin-like signaling activity. The sqt worked together with the other molecules such as Cyc, Spaw, Dvr1, and several Activin subunits to form active Smad/FoxH1 complex; it is found that the penetrance phenotype was inversely related to the Activin-like signaling activity. Besides, the temperature could also play roles in the degrees of the sqt penetrance phenotype (Pei *et al*. 2007). However, the phenotypes varied even in the genetically identical organisms in homogeneous environments, indicating that randomness in developmental processes such as gene expression may also generate diversity. In the C. elegans, the intestinal cell differentiation process was controlled by a transcription network. The *skn1* mutant showed varied phenotype of intestinal cell fate determination, which was affected by chromatin remodeling, and the redundant genes to regulate elt2, such as med-1/2, end-1 and end-3. The function of upstream genes of elt2 affected the degree of the *skn1* penetrance phenotype differently (Raj *et al*. 2010).

Similarly, the incomplete penetrance phenotype of dwarts transgenic lines indicated multiple mechanisms in the dwarts regulation *in vivo* since the genetics modifiers such as gene expression or other molecular regulators could cause the penetrance during development (Pei *et al*. 2007; Raj *et al*. 2010). For example, the upstream regulators Hippo and Salvador activate dwarts kinase activity via phosphorylation (Udan *et al.* 2003); the other upstream molecules such as Fat, Dchs and disks overgrown were involved in the dwarts regulation as well (Cho *et al*. 2006). The eye phenotype in the dmats transgenic fly is greatly different from that of dmats /dwarts double transgenic line indicated the dmats regulated dwarts kinase activity (Lai *et al.* 2005; Wei *et al*. 2007). Moreover, the membrane localization of dmats could also play a part in the dwarts kinase activity regulation (Ho *et al*. 2010). As an important kinase for cell number control in the development, warts are conserved from yeast to mammals. Thus, it is not surprising to identify multiple regulatory controls of dwarts kinase activity. In addition to these, we can't rule out other new regulatory mechanisms.

3.4 Mutant AAAA-dwarts transgenic flies showed stronger phenotype than the wild-type transgenic fly

We have identified 16 independent positive lines of mutant *dwarts* transgenic flies. After balancing, the individual line was examined for the phenotypes in compound eyes. According to the phenotypes in compound eye, seven lines showed strong phenotypes with more percentage of double cone eyes (from 38/51 to 12/37); four lines showed medium phenotype with big portion of single cone eye (from 13/46 to 9/36); one line of weak phenotype showed more flies with small eyes (22/23). The other lines showed wild type looking in the most of the population. The eleven AAAA-*dwarts* transgenic lines with stronger compound eye phenotypes were analyzed and showed in Figure 2A. However, the wild type dwarts transgenic line 6R showed small eye phenotype in most of its population (80/93), which is the strong line among the dwarts transgenic strains. The eye phenotypes of eleven AAAA- *dwarts* transgenic lines was stronger than the wild type dwarts transgenic line 6R, which only showed big portion of small eye phenotype in the population (Figure 3, supplement 2). The phenotype implied that AAAA-*dwarts* had more activity than wild type dwarts in reducing cell numbers. In

some cases, the transgenic fly strains may express the protein in different levels according to the genome context where it located. Western blotting was performed to examine the dwarts expression in transgenic flies. The results showed that there were no much differences in the dwarts levels between the transgenic flies expressing wild type or mutant dwarts although the mutant dwarts transgenic lines (#17, #27, #42) were slightly lower in the dwarts level compared with 6R considering the internal loading control (Figure 2B). Thus, the reason that AAAA-dwarts transgenic lines showed stronger phenotype than wildtype dwarts transgenic line may be due to high kinase activity of AAAA-dwarts that inhibited cell proliferation as indicated by previous studies with the warts mutants (Bichsel *et al.* 2004). Different *AAAA-dwarts* lines showed different percentages of cone eyes and small eyes, but all the eleven lines showed higher percentages of single cone eye (21%-35%) and double cone eye (range from 4% to 75%) than the wild type dwarts transgenic line(6R). The seven strong lines showed much higher percentage of double cone phenotype (Figure 2A). Besides, considering the similarity of protein expression in the transgenic flies between the 6R transgenic strain and line 42, the phenotypes difference between wild type dwarts and mutant *dwarts* could be due to the difference in their activities of the two versions of dwarts. Insertion segment in the catalytic domain of NDR family kinase such as human NDR and mammalian Lats1 played an auto-inhibitory role in their kinase activities. The sequence of insertion segment is featured with high alkaline amino acids content where the hMob1 binds. The mutations introduced in the conserved alkaline amino acids in the insertion segment of NDR greatly increased the kinase activity (Hergovich *et al*. 2006; Bichsel *et al*. 2004). The phenotypes of reduced compound eye of mutant *dwarts* transgenic fly than wild type dwarts transgenic flies suggested that the mutations we introduced to dwarts in the insertion segment most likely increased the kinase activity of mutant *dwarts*.

In summary, the phenotypes of transgenic *dwarts* mutant in fly showed reduced compound eye size, reduced mechanosensory bristles, and fused the corneal lens. These phenotypes revealed that reduced number of ommatidia in compound eye were possibly due to an enhanced cell apoptosis and reduced cell proliferation in the compound eye development. The stronger eye phenotype conferred by mutant *dwarts* than the wild type dwarts implied the mutations in the insertion segment of the catalytic kinase domain played a negative regulatory role in regulating the kinase activity of dwarts. The incomplete penetrance phenotypes of compound eye demonstrated by each transgenic line of dwarts were firstly reported, which may manifest a novel regulatory mechanism of dwarts regulation and requests further study.

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Table 1. *Drosophila* strains used in this study

	Genotype	
1	*w* ^{1118}	(Lai *et al*. 2005)
2	*w, ey-Gal4/S.T.*	(Lai *et al*. 2005)
3	*w, GMR-Gal4*	(Lai *et al*. 2005)
4	*w, C5-Gal4*	(Lai *et al*. 2005)
6	*w; Adv/SM1; Sb/TM6*	(Lai *et al*. 2005)
7	*w; UAS-Lats(6R); Sb/TM6*	(Lai *et al*. 2005)
8	*w; Dr-/TMS SbΔ2-3*	(Lai *et al*. 2005)
9	*Caton S*	(Lai *et al*. 2005)

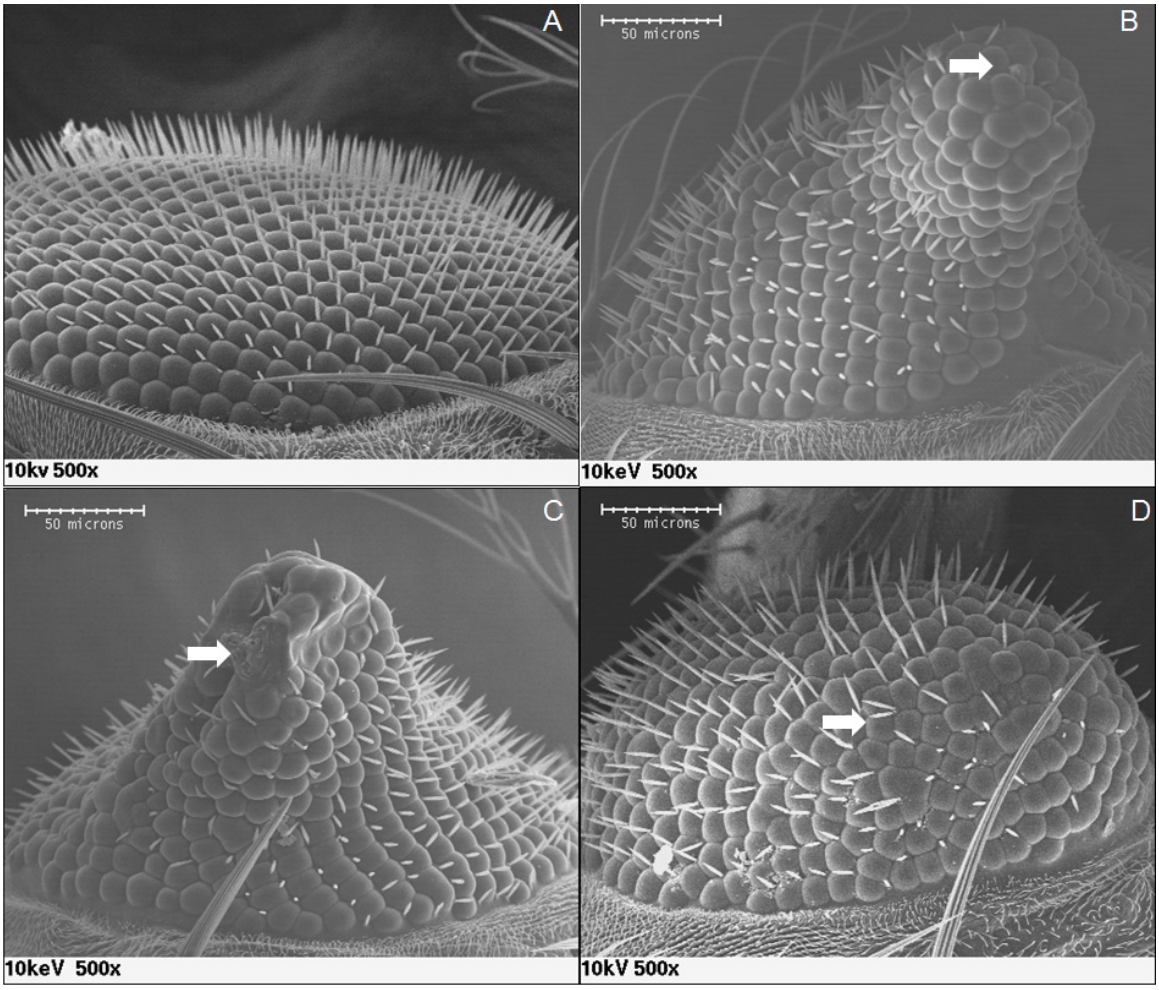

Figure 1. The transgenenic flies expressing mutant AAAA-dwarts exhibited ommatidia and mechanosensory bristles change

The transgenic mutant AAAA-dwarts flies (w, UAS-myc-AAAA-dwarts) were crossed with eyeless diver (w, ey-Gal4/S.T.), and then the samples were dehydrated and examined by SEM. A, wild type compound eye showed the pattern of ommatidia and mechanosensory bristles. B-D, the mutant dwarts transgenic fly showed the patternchange of ommatidia and mechanosensory bristles. B, the fused ommatidia and missed mechanosensory bristles. C, the dead ommatidia and curved mechanosensory bristles. D, fused ommatidia and clustered mechanosensory bristles.

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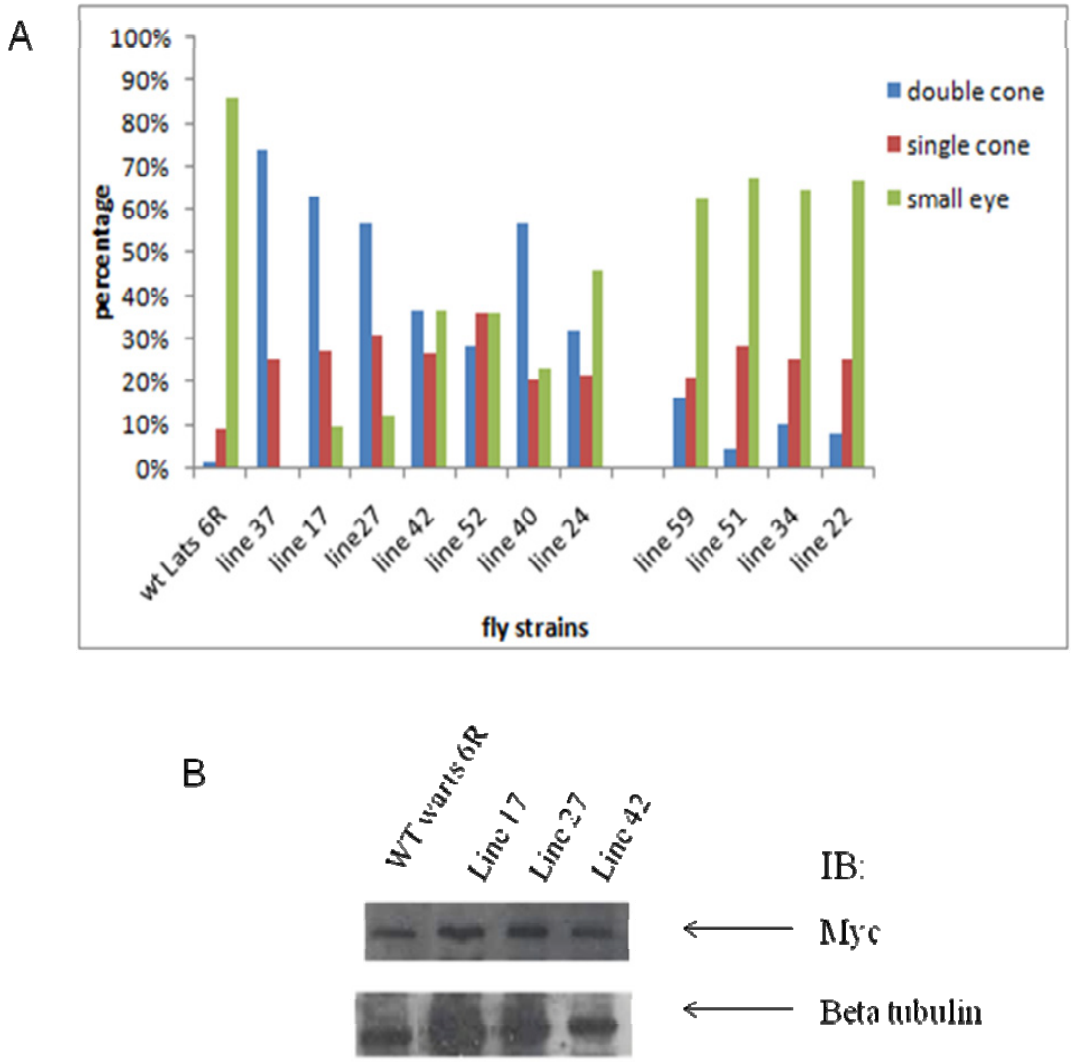

Figure 2. The diverse compound eye phenotype and their percentages in the multiple lines of transgenic strain expressing wildtype or mutant dwarts

The 11 transgenic mutant dwarts strains were observed and compound eye phenotypes were analyzed. A, the distribution of compound eye phenotype in the 11 mutant dwars transgenic strains (w, ey-Gal4, UAS-*AAAA-warts*) and wild type dwarts transgenic fly (w, ey-Gal4, UAS-warts(6R)). B, dwarts protein levels in the transgenic strains were detected by Western Blotting with myc antibody.

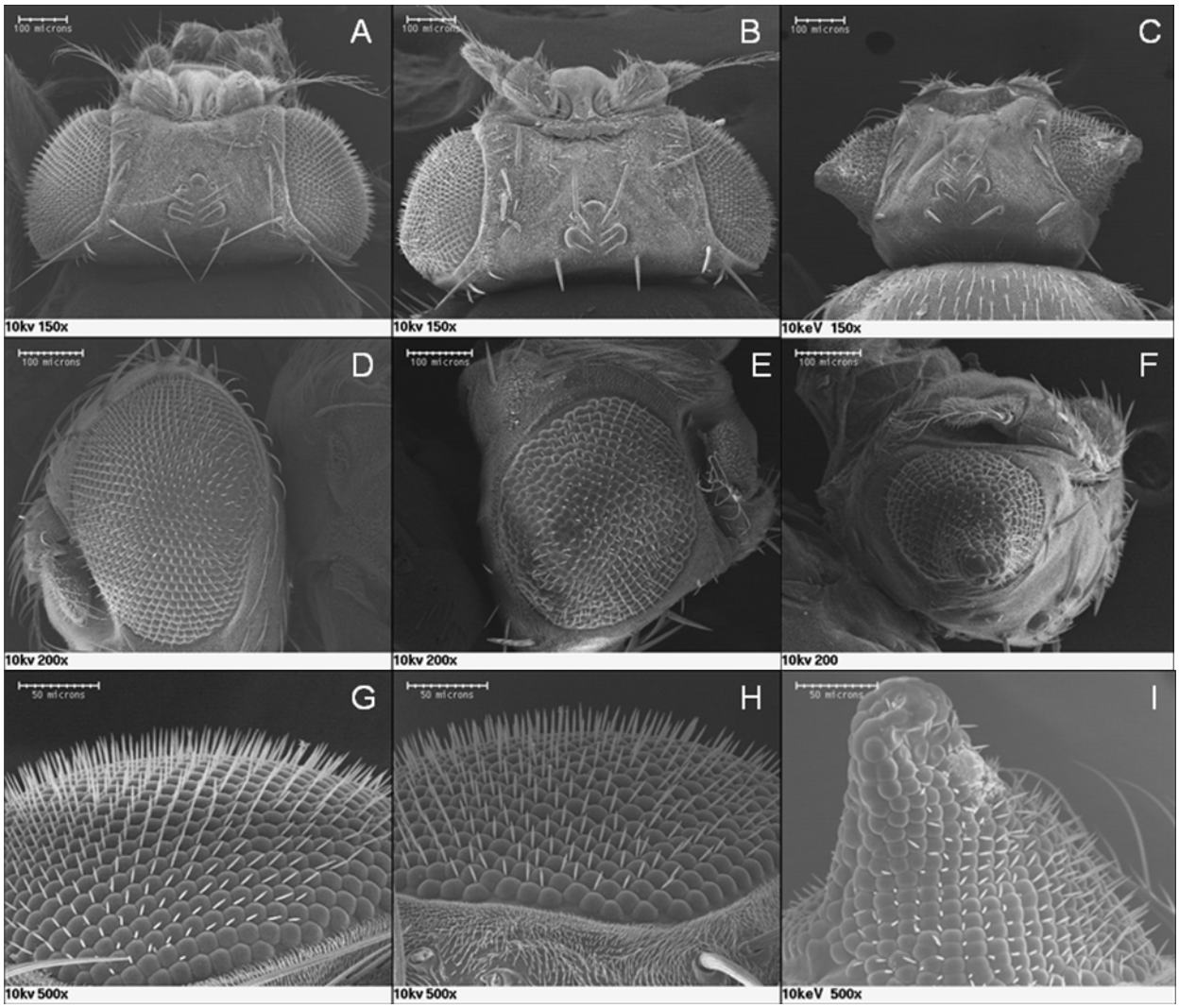

Figure 3. The compound eye phenotypes of transgenic fly strains and wild type strain

The transgenic fly strains (w, ey-Gal4, UAS-warts or w, ey-Gal4, UAS-*AAAA-warts*) were observed by SEM. Comparing with wild type strain, the two transgenic strains had smaller eyes than wild type strain. The figure also showed the predominant eye phenotypes in wild type dwarts transgenic fly and mutant dwarts transgenic fly strains. A-C, dorsal view. D-F, side view. G-I, ventral view. A, D, G: WT (Caton S); B, E, H: wild type dwarts transgenic strain (6R); C, F, I: mutant AAAA-d*warts* transgenic strain (#17).

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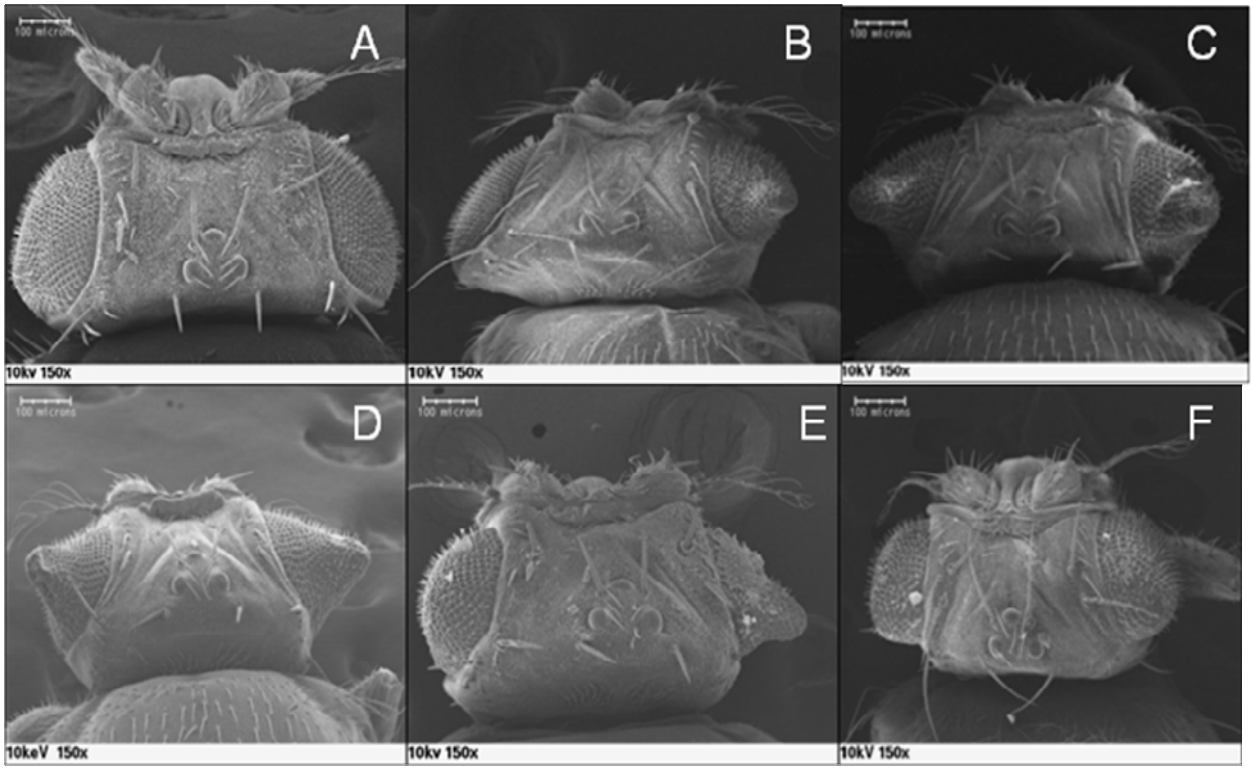
86% **8.6%** **1%**

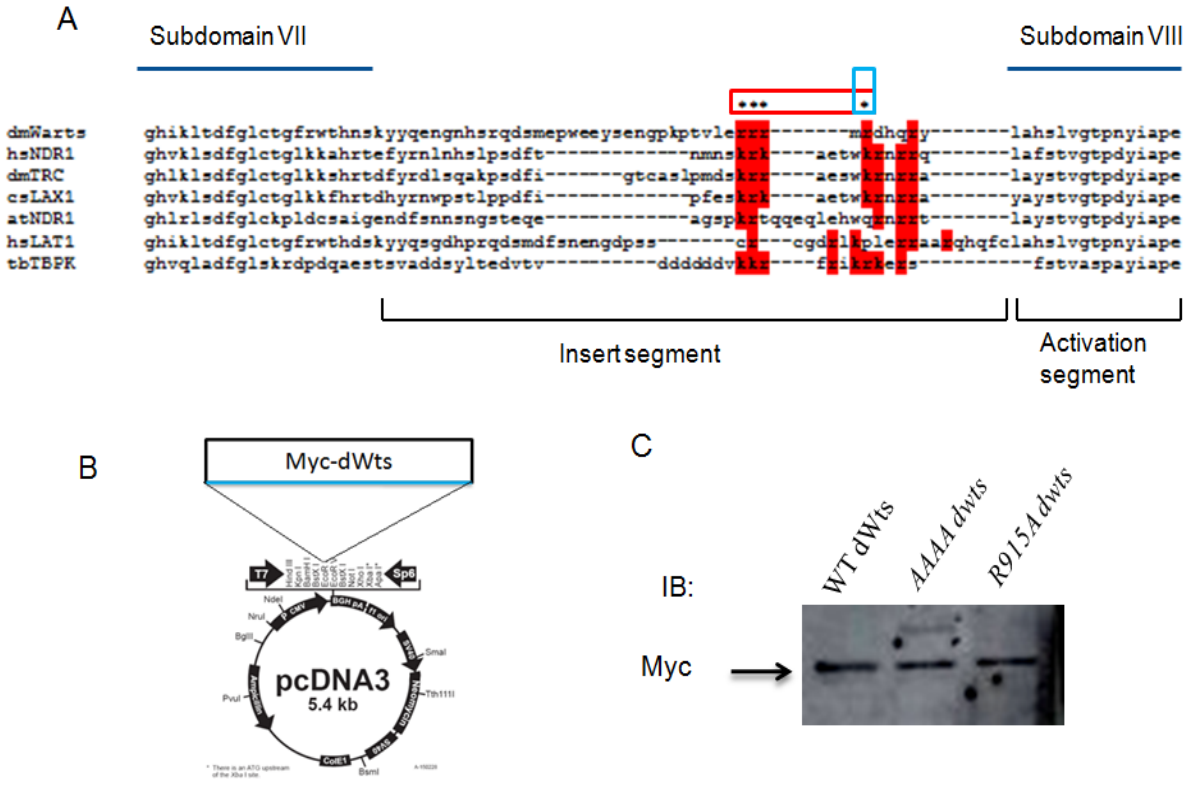


63.5% **26.9%** **9.6%**

Figure 4. The eye phenotype in the dwarts transgenic fly strains demonstrated incomplete penetrance

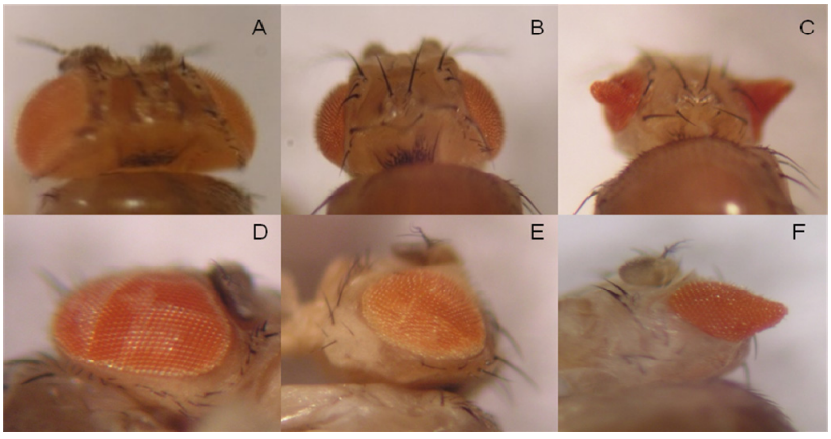
The transgenic strains (w, ey-Gal4, UAS-warts or w, ey-Gal4, UAS-*AAAA-warts*) were observed under SEM. The phenotypes of compound eyes were analyzed according to their morphology and percentage in the total samples.

A-C, the eye phenotypes of wild type dwarts transgenic strain (w, ey-Gal4, UAS-warts, (6R)) were shown from the high percentage to the low percentage. A, small compound eye, 86%; B, single cone compound eye, 8.6%; C, double cone compound eye, 1%. D-F, the eye phenotypes of mutant *AAAA-dwarts* transgenic fly strain (w, ey-Gal4, UAS-*AAAA-warts,* #17) were shown from the high percentage to the low percentage. D, double cone compound eye, 63.5%; E, single cone compound eye, 26.9%; F, small compound eye, 9.6%.



Supplement 1. Mutations were introduced into insert segment of pcDNA3 -dWarts

A, The conserved alkaline amino acids were identified in the insertion segment of dWarts between subdomain VII and subdomain VIII of its catalytic domain by alignment dWarts with other NDR kinases. The red amino acids indicated the conserved alkaline amino acids. *, indicated the mutated amino acids in the dWarts. The red frame showed the 4 mutations in AAAA-dwarts, the blue frame indicated the mutation in the R915A-dwarts. B, the construction was used as template for site directed mutagenesis. By site-directed mutagenesis, 1 mutation and 4 mutations were introduced into dWarts. C, Western Blotting indicated that dWarts and mutant dwarts protein expressed in the HEK 293T cell.



Supplement 2. Mutant AAAA-dwarts transgenic fly exhibited smaller compound eye

The transgenic dwarts flies (w, UAS-myc-dwarts or w, UAS-myc-AAAA-dwarts) were crossed with eyeless diver (w, ey-Gal4/S.T.) and the eye phenotypes were observed. A and D, the wild type control fly (Caton S). B and E, wild type dWarts transgenic fly. C and F, the mutant AAAA-dwarts transgenic fly. A-C, dorsal view. D-F, side view.

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