Indian J. Genet., 77(2): 304-311 (2017)
DOI: 10.5958/0975-6906.2017.00041.4

Identification of functions of *DWnt4* gene in ventral epidermis of embryos and abdomen of adult *Drosophila* during development

Prachi Yadav*, Adita Joshi[1] and Akshay Talukdar

Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012; [1]Institute of Genomics and Integrative Biology, Mathura Road, New Delhi 110 020

(Received: June 2016; Revised: February 2017; Accepted: April 2017)

Abstract

Wnt protein forms a multigenic family secreting signaling molecules important for various key developmental events in animals. *Wingless* (*wg*) is the prototype member of Wnt gene family, while *DWnt4* has been identified recently from a clonal library of proteins associated in vivo with *Ubx* protein. DWnt4 has been reported to function in embryo epidermis patterning, female gonade development, motor neuron axon guidance. In this study, validation was done for some reported functions of *DWnt4* with ectopic expression and identified some novel functions using RNA interference. Knockdown of *DWnt4* caused in ventral ectoderm reduced denticles with loss of one or two rows or entire denticle belt. Loss of *DWnt4* also loss of polarity of wing hairs, loss of wing veins and notched wing phenotype. All the bristles from adult abdomen were lost when *DWnt4* was driven with ubiquitous driver *actineGal4*. Tissue specific drivers *enGal4* caused loss of polarity of dorsal tergite bristles and patches of tergites from adult abdomen. Thus, the *DWnt4* seems to function in the cuticular differentiation of adult abdomen polarity of tergites bristles in *Drosophila*.

Key words: *DWnt4*, RNAi, UAS-Gal4, *wg*, tergites, adult abdomen, polarity

Introduction

Wnt genes (and proteins) belong to a family of secreted signaling molecules that are homologues to the *wingless* prototype gene of *Drosophila and int1* gene from mammary tumor virus. The *wingless* (*wg*) gene was identified from EMS mutagen treated *Drosophila* stock that lost one or both adult wings (Sharma and Chopra 1976). Multiple Wnts has been identified afterwards across entire animal kingdom. Wnt pathway is one of the most important cell signaling pathways for all eukaryotes which plays vital role from embryogenesis to cell differentiation to adult morphogenesis (Logan and Nusse 2004). *Drosophila* has seven Wnt genes, out of which four Wnt genes *viz*., *DWnt4, wg, DWnt6* and *DWnt10* are clustered in cytogenetic interval 27E7 to 27F3 on left arm of second chromosome (Adams et al. 2000).

DWnt4 gene maps 30 kb upstream of *wg* and transcribes in opposite direction of *wg*. *DWnt4* is not an ortholog or paralog of *wg*, and shows 35% of sequence similarity (Graba et al. 1995). It contains 22 cysteins found in all Wnt genes plus extra two cystein residues, at position 90 and 103, which are not present in *wg* (Graba et al. 1995). Functionally *DWnt4* shows segment polarity like pattern in ectoderm (Graba et al. 1995) and have antagonistic and distinct activities in ventral ectoderm patterning of embryo when compared to *wingless* (Gieseler et al. 1999). During dorsal ectoderm patterning of embryo, *DWnt4* requires *hedgehog* (*Hh*) for specification of 3^{o} cells while *wg* expresses independent of *DWnt4* and *hh* and specifies 4^{o} cells (Buratovich et al. 2002). Ectopic expression of *DWnt4* in wing imaginal dics provides wg-like activity and can give rise to ectopic wing blade by notum to wing tranformation (Gieseler et al. 2001).

Semi - lethal and sterile *DWnt4* mutants (Cohen et al. 2002) demonstrated role of *DWnt4* in ovariolar sheath development and salivary gland migration (Harris and Stevens 2007) through non canonical pathway. *DWnt4* is using planar cell polarity for giving omanatidial polarity in ventral region of eye and it interacts with *four jointed*(*fj*) which is a component of

*Corresponding author's e-mail: pallu.prachi@gmail.com
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com; http://epubs.icar.org.in/journal/index.php/IJGPB

Frizzled (Fz)/PCP pathway (Lim et al. 2005), *DWnt4* functions in motor neuron axon guidance, neural retina development through non canonical Wnt signaling (Sato et al. 2006). Till date no lethal allele of *DWnt4* is reported for embryonic cuticle patterning. *DWnt4* can antagonize canonical Wnt pathway by interacting with *PTK/Otk* protein (Peradziryi et al. 2011). *DWnt4/wg* are involved in long range directional input for hair polarity of wings through *Fz/Vang (Van Gogh)* PCP pathway (Wu et al. 2013).

Ectopic expression of gene has been used to study functions of *DWnt4* (Gieseler et al.1995, 1999, 2001). In this study, UAS-Gal4 system was used to knockdown *DWnt4* gene throughout different developmental stages of *Drosophila* using different *Gal4* drivers. Ubiquitously expressed *heatshockGal4* (*hsGal4*), *actineGal4* (*actGal4*) and tissue specific driver *engrailedGal4* (*enGal4*) and *patchedGal4* (*ptcGal4*) were used to study the functions of *DWnt4* gene in the embryos of *Drosophila* during development.

Materials and methods

Drosphila stocks

Canton S was used as wild type. *UASDWnt4 (obtained from Marta Llimargass)* and *UAS DWnt4RNAi* (BDSC stock No. 29442) ordered from Bloomington *Drosophila* Stock Center (USA) and were used as *Gal4* responsive stocks with *heatshock Gal4 (hs Gal4), actineGal4 (actGal4), daughterlessGal4 (daGal4), engrailed Gal4 (enGal4) and patched Gal4 (ptcGal4)* driver lines. Gal4 driver lines were used as female and crossed with either *UASDWnt4* or *UASDWnt4RNAi* according to experiment.

Crosses set were as follows: *daGal4 X UAS DWnt4; daGal4 X UAS DWnt4RNAi; hsGal4 X UAS DWnt4*; *hsGal4 X UAS DWnt4RNAi*; *enGal4 X UAS DWnt4*; *enGal4 X UAS DWnt4RNAi*; *ptcGal4 X UAS DWnt4*; *ptcGal4 X UAS DWnt4RNAi*

In order to check possible OTEs (Off Target sites) (Hinno and Gotto 2013) of the RNAi constructs we examined the sequence of JF03378 construct, which forms double stranded RNA forming hairpin loop against *DWnt4* gene in the *DWnt4RNAi* line, (BDSC 29442) using the dsCheck software.

The crosses were set as mentioned above and embryos were collected for 4 hours and allowed to grow at 25^{o}C. Unhatched embryos were collected after 24-30 hrs. For heat shock Gal4 driver, embryos were collected overnight at 25^{o}C and heat shocked for 1 hr at 37^{o}C in waterbath. Cuticles of embryos were made according to Stern and Sucena (2000).

For mounting the wings**,** the crosses were set as above and allowed to lay eggs overnight at 25^{o}C and further shifted to 29^{o}C; the latter provides better expression of UAS constructs. The wings of the flies which emerged from these crosses were removed in ethanol and mounted in DPX mountant for observation under microscope for any deviation from wild type wing phenotype. Abdominal cuticles were made both from eclosed adult flies and pharate adults. Flies were dissected from pupae and transferred to 70% ethanol/ 30% glycerol. Prior to examination, the adults were dissected to separate abdomen, rehydrated and transferred to Hoyer's medium for clearing of soft tissues, the slides were cleared at 50^{o}C.

Results

Effect of over expression DWnt4 on ectopic denticles

Over expression of *DWnt4* resulted in ectopic denticles in ventral cuticles of embryos. When *UASDWnt4* transgene was over expressed with any of the *Gal4* i.e., *daughterless* (*da*) or *engrailed* (*en*) or *heatshock* (*hs*), the ectopic denticles were formed in the region of naked cuticle (Fig. 1c) and showed embryonic lethality of 14 to 25%, larval lethality of 4 to 7% and pupal lethality of 15 to 30%. It was observed that naked cuticles became decorated with denticles when expression of *DWnt4* was increased by shifting the developing embryos to 29^{o}C. The diversity and identity of the segment belts remained unaltered.

Effect of over expression of DWnt4 on wings and abdominal segment

The *DWnt4* was over expressed using *engrailed Gal4* (*enGal4*) and patched *Gal4* (*ptcGal4*). The *enGal4* expresses in the posterior wing compartment and the (*ptcGal4*) expresses at A/P compartment boundary of wing imaginal disc. Similarly, *heatshock Gal4* (*hsGal4*) was used to ubiquitously over express *DWnt4*. Pupal lethality was 15-30% for all the three Gal4 drivers and around 70% of the adults and pharate adults showed open wings or wings perpendicular to the body axis except for *hsGal4*. Similarly, the ectopic wing veins and ectopic bristles were observed on scutellum and notum of the flies. Loss of wing hair polarity particularly in the posterior compartment of the wing was observed when *DWnt4* was expressed with *enGal4*.

Effect of loss of DWnt4 on denticles on ventral ectoderm

Down regulation of *DWnt4* during embryo development gave opposite phenotype to the over expression of *DWnt4* (Fig. 1). Over expression of *DWnt4* caused loss

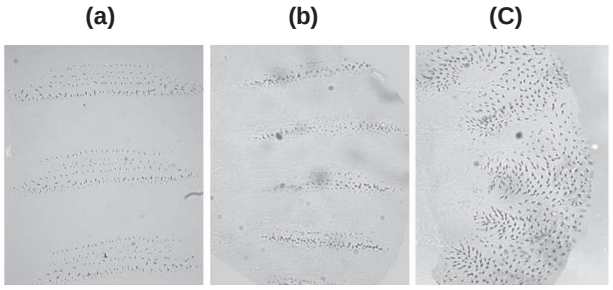

Fig. 1. Effects of *DWnt4* over expression and knockdown on ventral ectoderm of embryonic cuticle a. Wild type b. *hsGal4>UASDWnt4RNAi* c. *hsGal4>UASDWnt4*

of naked cuticle while down regulation increased naked cuticle. About 25-30% embryonic lethality was observed with different degree of denticle deformities which were classified as (A) Denticle reduced, (B) row missing + denticle reduced, and (C) segment missing+ row missing + denticle reduced (Fig. 2). The frequency of these phenotypes is summarized in Table 1.

Effect of DWnt4 knockdown on wings

To see the effect of absence of *DWnt4* expression on wing development, *UASDWnt4RNAi* line was over-expressed using *enGal4*. The *hsGal4* was used to knock down *DWnt4* from entire wing disc. Heat shock was given to third instar larva at 37^{o}C and then shifted to 29^{o}C for further development. For experiments using *enGal4* and *ptcGal4,* the egg laying was done at 25^{o}C and embryos were reared at 29^{o}C till adult stage. The *enGal4* driven down regulation of *DWnt4* resulted in flies (2-4%) with only one fully developed wing while the other wing was absent (Fig. 3a). Similarly, 12.5% of flies with *hsGal4* driven down regulation and 4.8% of flies with *enGal4 driven down regulation* showed unequal wing development (Fig. 3b and 3c). Pharate adult showed the same phenotypes.

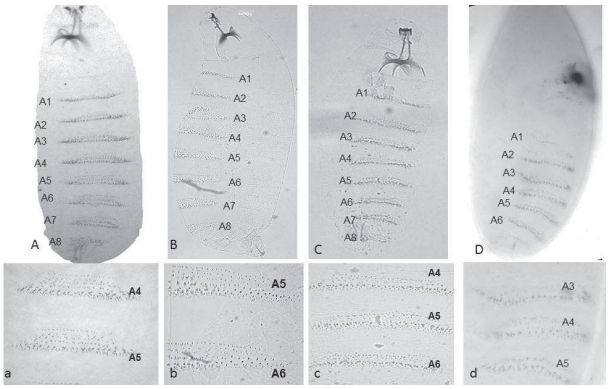

Fig. 2. Ventral ectoderm pattern of embryos after down regulating *DWnt4* using RNAi line. Different degrees of cuticular defects classified into three classes (A and a)) wild type ventral epidermis (B and b) Reduced denticle size (C and c) Reduced denticles + row missing from each belt (D and d) reduced denticles + row missing + segment missing

Loss of *DWnt4* also lead to loss of polarity of wing hairs (trichomes) and notched wing margin (Fig. 4). The notched wing manifested in 3-5% flies with *enGal4* and *ptcGal4* but not with *hsGal4 or actGal4.*

Knockdown of DWnt4 caused loss of abdominal tergites and polarity of abdominal bristles

The abdomen of adult *Drosophila* is made up of cuticular structures. The dorsal surface is made of tanned cuticles called tergites and the ventral surface is covered with cuticles called sternites. Tergites bear different types of bristles namely macrochaetae, microchaetae and trichomes. Between the tergites and sternites trichomes (pleura) are present. Spiracular opening are present at lateral edge of each tergite (Fig. 6A,a). Ectopic tergite bristles were found near spiracles upon over expression of *DWnt4* (Fig. 6B,b). When *DWnt4* was knocked down with *enGal4* and *hsGal4*, adult and pharate adults with loss of tergite bristles, patches of missing abdominal bristles and loss of polarity of bristles (Fig. 5) were seen. When RNAi for *DWnt4* was driven with *actGal4*(*actinGal4*),

Table 1. Embryonic defects due to knock down of *DWnt4*

Cross	Segment absent (%)	Denticle row missing (%)	Reduced denticles (%)	No. of dead embryos observed
daGal4>UASDWnt4RNAi	21.8 (120/455)	24.8 (137/455)	36 (198/455)	10% (455/4550)
hsGal4>UASDWnt4RNAi	27 (135/500)	20.6 (103/500)	40 (200/500)	12.4% (440/3532)
enGal4>UASDWnt4RNAi	23 (138/600)	19.8 (119/600)	31 (186/600)	19.5% (443/2,271)

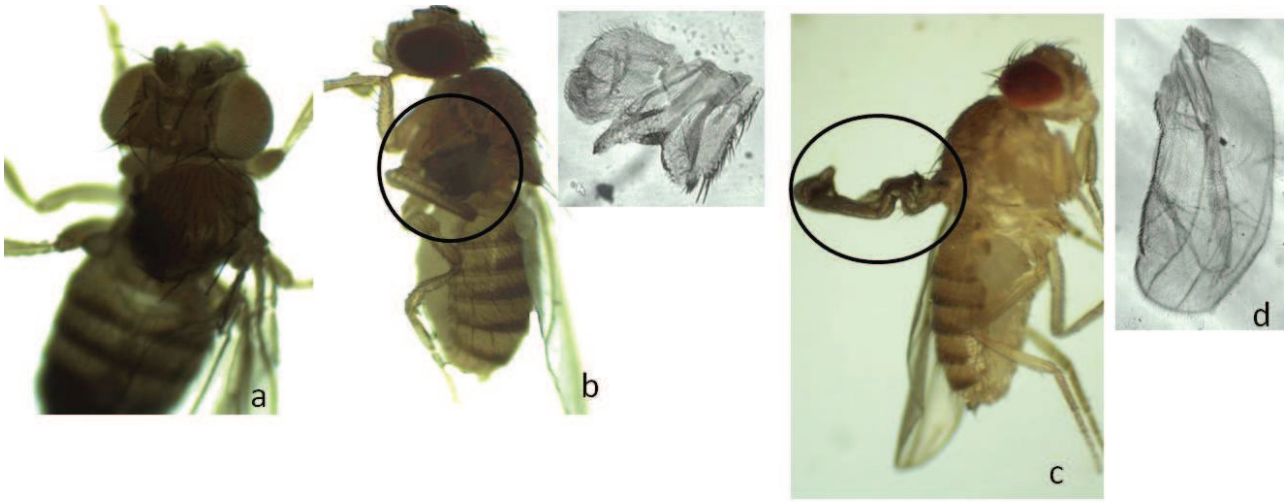

Fig. 3. Wing phenotype induced after knock down of *DWnt4* transcript, a) one wing missing, b) Wing reduced (inset wing mount from reduced wing of respective fly, c) unopened wing blade and d) wing mount from unopened wing phenotype

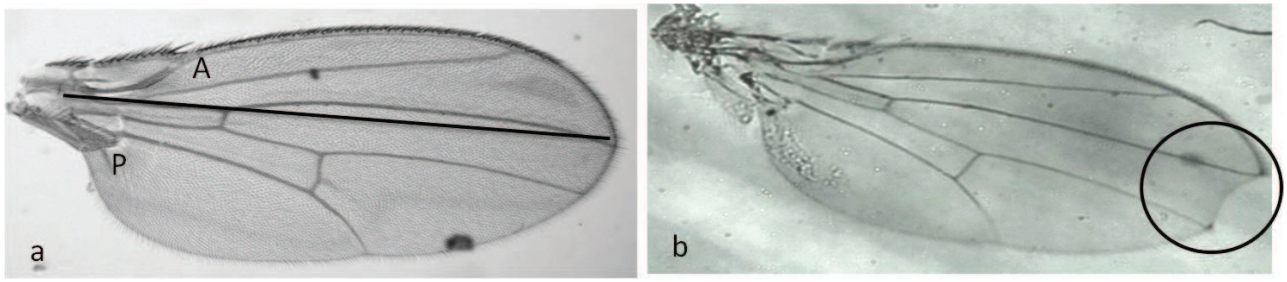

Fig. 4. Effect of loss of *DWnt4* on wing blades; a) Wild type wing blade A for anterior and P for posterior wing compartment; b) A notch in the wing blade is observed in a cross with *enGal4>UASDWnt4RNAi*

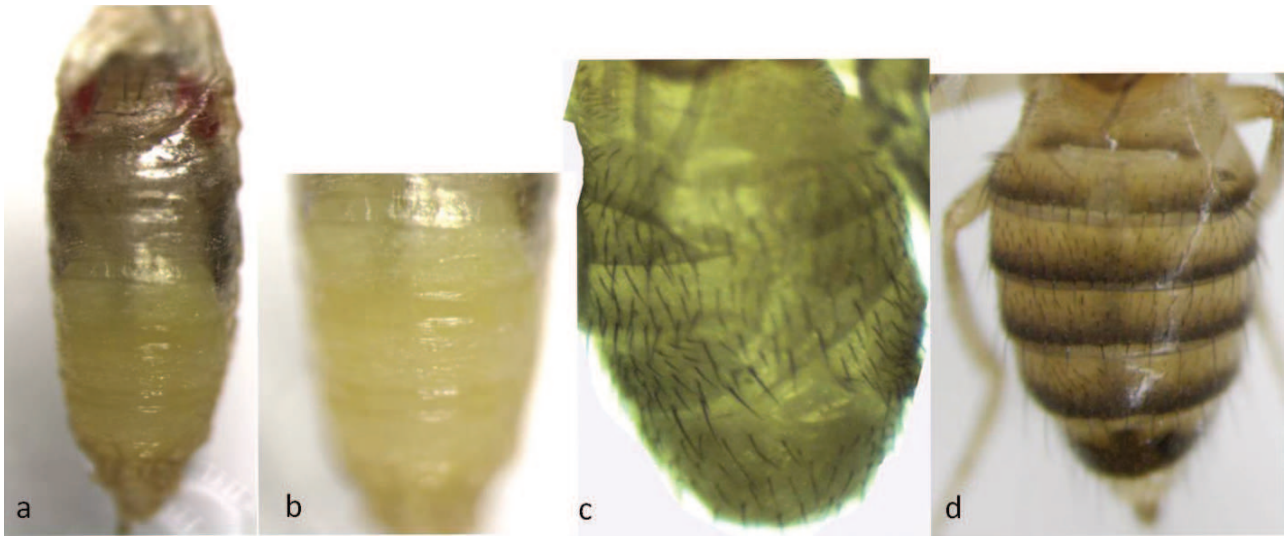

Fig. 5. Effect of loss of *DWnt4* on abdominal patterning and polarity of bristles; a) *actGal4>UASDWnt4RNAi* causes loss of abdominal segments and tergites; b) magnification of (a); c) *enGal4>UASDWnt4RNAi* results in eclosion of flies with perturbed dorsal abdomen where distinct bands of abdominal tergites are not formed, the polarity of bristles is not uniformaly posterior but instead some of bristles are oriented perpendicular, a clear patch of bristleless cuticle is visible d) wild type abdomen (female)

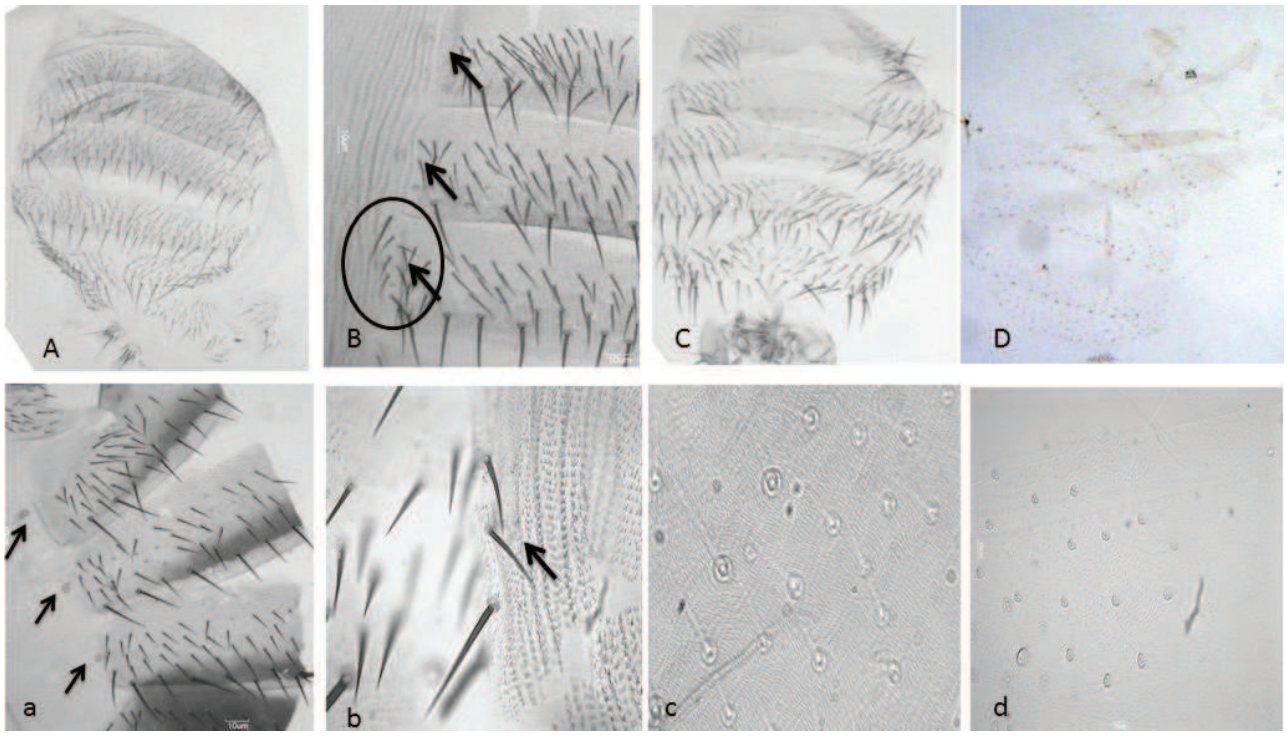

Fig. 6. Adult abdominal cuticles after over expression and knock down of *DWnt4* (A and a) wild type (B and b) over expression of *DWnt4* causes ectopic tergite bristles near spiracle (arrow) (C and c). Down regulation of *DWnt4* results in patches of missing tergites, trichomes are formed but no micro or macrochaetae (D and d) Dorsal bristles are absent but cuticular structures are trichomes are present after knock down of *DWnt4*

25-30% of pupal lethality was observed. The pharate adults had normal development of wings, thoracic bristles but were lacking of tergite bristles. However the microchaetae and bristle precursors were present on the abdomen of pharate adults (Figs. 5 and 6D,d). Patches of bristleless tergites and stermites similar to *enGal4* driven knock down were also observed. These patches had well patterned cuticle and bristle precursors but no bristles (Fig. 6 C,c),

Discussion

This study was carried out to see the effects of *DWnt4* over expression and knock down throughout the fly development starting from ventral ectoderm patterning of embryo till adult fly morphogenesis. The UAS-Gal4 system and RNAi has offered an unequivocal tool to study the functions of gene in the absence of lethal mutations. The study could identify novel functions of *DWnt4* during patterning of adult abdomen in fly development.

DWnt4 expresses in segment polarity in pattern similar to *wg* in ventral ectoderm, only difference being *DWnt4* expresses in middle of each parasegment and anterior to stripes of *wg*. *DWnt4* forms ectopic denticles on over expression (Gieseler et al. 1999). Similar observation was made in this study. Compromising *DWnt4* activity using RNAi led to different degree of loss of denticles from embryonic cuticles (Table 1). These phenotypes are quite contradictory to that of *wg* where overexpression lead to formation of naked cuticles **(**Noordermeer et al. 1992). Again, *wg* loss of function leads to formation of ectopic denticles in the region of naked cuticles (Bejsovec and Martinez Arias 1991). In early phase, *wg* maintains *en* (*engrailed*) transcription in adjacent two cell rows of parasegment, and during late phase maintains the denticle diversity of anterior half of parasegment (Bejsovec and Martinez Arias 1991). Looking at the ectopic expression phenotypes in ventral epidermis of embryos, it became clear that *DWnt4* was functioning during late phase of *wg* expression as knocking down of *DWnt4* resulted in development of embryos with only one or two rows of denticles. Majority of embryos had six rows of reduced denticles, however, the denticle diversity was lossed. Denticle diversity remained unchanged on over expression of *DWnt4*. Loss of *DWnt4* also led to missing of rows of denticle belts from each segment of ventral

ectoderm. The rows were missed at random, sometime the anterior rows (Gieseler et al. 1999) and sometimes the middle two rows were lost from each denticle belt (Fig. 2). Graba et al. (1995) reported that *DWnt4* transcript appears in segmental pattern after rapid extension of germ band elongation. This stage specifies the late phase of *wg* function in ectoderm patterning where function of *wg* and *en* become independent of each other. The *DWnt4 and wg* transcript got reduced in null mutants of *hh* and *gsb (gooseberry),* the genes which are necessary for activation of *wg* signaling. Results of the knock down experiments are supporting the hypothesis that *DWnt4* and *wg* are sharing common regulatory and signaling cascasde and *DWnt4* can antagonize *wg* by either substituting its function or interacting with other segment polarity gene like *hedghog*.

DWnt4 can induce supernumerary wing, haltere and bristles formation when over expressed strongly under the control of *ptcGal4*. The supernumerary wings are formed at the expense of notum, with ectopic D/V (Dorsal-Ventral) boundary and quite high levels of expression of *Wg, En* and *Vg* (Vestigial) while supernumerary bristles are formed at the expense of scutellum (Gieseler, et al. 2001). It is well documented that *Wg* protein has intrinsic capacity to form a new wing margin (Ng et al. 1996), formation of ectopic D/V boundary for future wing blade, (Baker, 1988), and specification of wing margin bristles (Couso et al. 1994).

Knocking down of *DWnt4* can cause loss of wing. However, in this experiment, flies were obtained with one wing absent, notched wing margin, loss of wing veins and loss of polarity of wing hair. This can be attributed to the endogenous activity of *wg* functioning to pattern the wing. Flies without wings did not show any transformation of notum, although sometimes reduced scutellum was observed.

DWnt4 transcript down regulation resulted in reduced wing blade size or unequal size of wing blades. Wing hair showed loss of polarity both on over and reduced expression of *DWnt4.* No Wnt gene has been assigned for planner cell polarity (PCP) function in Drosophila (Chen et al. 2008). Results of this study indicated that *DWnt4* is functioning in PCP for wing hair polarity and wing size variation. Size of the organ is attributed to cell's perception for proliferation and cell death which was controlled by gradient of concentration of polarizing factor. (Day and Lawrence, 2000). The PCP pathways in Drosophila are regulated globally by *Fz/PCP* pathway (Carvajal-Gonzalez and Mlodzik 2014, Shulman et al. 1997). The double mutant for *wg-DWnt4* showed loss of polarity of wing hair and this loss of polarity was caused by interaction of *wg-DWnt4* with Fz/vang PCP pathway (Wu et al. 2013). *DWnt4* interacts with *four jointed (fj)* to induce ommatidial polarity in ventral domain of eye (Lim et al. 2005). PTK7 and otk (Drosophila ortholog) are *Frizzled* co receptors and they activate non canonical Wnt pathway by forming Wnt/Fz/PTK7 or Wnt/Fz/otk comple (Peradziryi et al. 2011). These reports support the fact that *DWnt4* can be a member of Wnt gene family which is functioning in non canonical Wnt pathway and PCP pathway. Occurrence of notched wing on knockdown of *DWnt4* with *en* or *ptcGal4,* invokes the question, is *DWnt4* able to form the wing hair margin like *wg* (Couso et al. 1994) or is it interacting with *Notch* signaling pathway during wing patterning? Further experiments need to be done to find the answer for this question.

The abdomen of Drosophila consists of segments which are subdivided into anterior (A) and posterior (P) compartments. The selector gene *engrailed* (*en*) acts in P (posterior) compartment and *Hh* acts on A (anterior) compartment, The gradient of *Hh* actually gives rise to different bristle fate in each segment in A compartment of abdomen which are called tergites (Struhl et al. 1997a). *Hh* activates *wg* in A compartment and then *wg* spreads back in P compartment to pattern it (Lawrence et al. 2002). Lack of *wg* leads to lack of bristle formation from sternite and tergite, similarly ectopic expression of *wg* leads to formation of ectopic bristles on abdomen and expansion of sternite and tergite (Shirras and Couso 1996).

DWnt4 over expression with ubiquitous Gal4 drivers like *actGal4* or *hsGal4*, or with tissue specific driver like *enGal4* results in ectopic bristles near spiracles (Fig. 6). When *DWnt4* was removed using RNAi, with same drivers showed loss of tergites and polarity of abdominal bristles (Figs. 5 and 6). It may be hypothesized that *DWnt4* might be functioning in adult abdomen patterning and was involved in planer polarity of abdominal bristles. It is well established that *wg* is necessary for determination of bristle precursor 18-28 hr after puparium formation (Shirras and Couso, 1996). Working model given for patterning of Drosophila abdomen suggests that there is Factor X which determines the polarity and size of bristles and it may be induced by *Hh* (Lawrence et al. 2002).

DWnt4 interacts with *hh* in both ventral and dorsal ectoderm patterning of embryo (Gieseler et al. 1995; Buratovich et al. 2000). *Hedgehog* is the gene which patterns the dorsal abdomen in adult (Struhl et al. 1997b). It may be possible that *Hh* is patterning abdomen indirectly through *DWnt4* and giving polarity to bristles when *wg* has already decided the cell fate for bristle precursor and onset of their differntiation. Thus *DWnt4* can be candidate of Wnt family of gene as a link between patterning the adult abdomen and giving polarity to the bristles.

Authors' contribution

Conceptualization of research (PY, AJ); Designing of the experiments (PY, AJ); Contribution of experimental materials (PY, AJ); Execution of field/lab experiments and data collection (PY); Analysis of data and interpretation (PY, AJ, AT); Preparation of manuscript (PY, AJ, AT).

Declaration

The authors declare no conflict of interest.

Acknowledgement

The first author is thankful to ICAR-Indian Agricultural Research Institute, New Delhi for providing the fellowship. The authors are indebted to Prof. Vani Brahmachari for her valuable input for this work. The authors also thankful to Bloomington Drosophila Stock Center (NIH P40OD018537) for providing the Drosophila stocks used in this study.

References

Adams et al. 2000. The genome sequence of *Drosophila melanogaster*. Science, **287**: 2185-2195.

Bejsovec A. and Martinez-Arias A. 1991. Roles of wingless in patterning the larval epidermis of *Drosophila*. Development, **113**: 43-54.

Buratovich M. A., Anderson S., Gieseler K., Pradel J. and Wilder E. L. 2000. *DWnt4* and *wingless* have distinct activities in the *Drosophila* dorsal epidermis. Dev. Genes Evol., **210**(3): 111-119.1.

Carvajal-Gonzalez J. M. and Mlodzik M. 2014. Mechanisms of planar cell polarity establishment in Drosophila. F1000 Prime Reports 2014, **6**(98): 1-10.

Chen Wei-S., Antic D., Matis M., Logan, C. Y., Povelones M., Anderson G. A., Nusse R. and Axelrod J. D. 2008. Asymmetric homotypic interactions of the atypical cadherin Flamingo mediate intercellular polarity signaling. Cell, **133**: 1093-1105.

Cohen E. D., Mariol M. C., Wallace R. M. H., Weyers J., Kamberov Y. G., Pradel J. and Wilder E. L. 2002. Dwnt 4 regulates cell movement and focal adhesion kinase during *Drosophila* ovarian morphogenesis. Dev. Cell, **2**: 437-448.

Couso J. P., Bishop S. A. and Martinez-Arias A. 1994. The wingless signalling pathway and the patterning of the wing margin in *Drosophila*. Development, **120**: 621-636.

Day S. J. and Lawrence P. A. 2000. Measuring dimensions: the regulationof size and shape. Development, **127**: 2977-2987.

dsCheck. Available online: http://dscheck.rnai.jp/ (accessed on 19 November 2013).

Gieseler K., Graba Y., Mariol M. C., Wilder E. L., Martinez-Arias A., Lemaire P. and Pradel J. 1999. Antagonost activity of *DWnt4* and *wingless* in the *Drosophila* embryonic ventral ectoderm and in the heterologous Xenopus assays. Mech. Dev., **85**: 123-131.

Gieseler K., Mariol M. C., Sagnier T., Sagnier T., Graba Y. and Pradel J. 1995. *Wingless* and *DWnt4*, two Drosophila *Wnt* genes, have related expression, regulation and function during embryonic development. C.R . Acad. Sci. III, **318**: 1101- 1110.

Gieseler K., Wilder E., Mariol M. C., Buratovich M., Berenger H., Graba Y. and Pradel J. 2001. *DWnt4* and *Wingless* elicit similar cellular responses during imaginal development. Dev. Biol., **232**: 339-350.

Graba Y., Gieseler K., Aragnol D., Laurenti P., Mariol M. C., Berenger H., Sagnier T. and Pradel J. 1995. DWnt-4, a novel Drosophila Wnt gene acts downstream of homeotic genes in the visceral mesoderm. Development, **121**: 209-218.

Harris K. E. and Beckendorf S. K. 2007. Different Wnt signals act through the Frizzled and RYK receptors during *Drosophila* salivary gland migration. Development, **134**: 2017-2015.

Hino Y. M. and Goto S. 2013. *In Vivo* RNAi-Based Screens: Studies in Model Organisms. Genes, **4**: 646-665.

Lawrence P. A., Casal J. and Struhl G. 2002.Towards a model of the organization of planar polarity and pattern in the *Drosophila* abdomen. Development, **129**: 2749-2760.

Lim J., Norga K. K., Chen Z. and Choi K. W. 2005. Contril of cell polarity by interaction of *DWnt4* and four-Jointed. Genesis, **42**: 150-161.

Logan C. Y. and Nusse R. 2004. The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol., **20**: 781-810.

Ng M., Diaz-Benjumea F. J., Vincent J. P., Wu. J. and Cohen S. M. 1996. Specification of wing by localized expression of Wingless protein. Nature, **381**: 316-318.

Noordermeer J., Johnston P., Rijsewijk, Nusse R. and Lawrence P. A.1992. The consequences of ubiquitous expression of the wingless gene in the

Drosophila embryo. Development, **116**: 711-719.

Peradziryi H., Kaplan N. A., Podleschny M., Liu X., Wehner P., Borchers A. and Tolwinski N. S. 2011. PTK/Otk interacts with Wnts and inhibits canonical Wnt signaling. EMBO J., **30**: 3729-3740.

Sato M., Umetsu D., Murakami S., Yasugi T. and Tabata T. 2006. DWnt4 regulates the dorsoventral specificity of retinal projections in the *Drosphila melanogaster* visual system. Nat. Neuro., **9**: 66-75.

Sharma R. P. and Chopra V. L. 1976. Effect of the *wingless wg* mutation on wing and haltere development in *Drosophila melanogaster*. Dev. Biol., **48**: 461-465.

Shirras A. D. and Couso J. P. 1996. Cell fates in the adult abdomen of *Drosophila* are determined by *wingless* during pupal development. Dev. Biol., **175**: 24-36.

Shulman J. M., Perrimon N. and Axelrod J. D. 1997. Frizzled signaling and the developmental control of cell polarity. Trends. Genet., **14**: 452-458.

Stern D. L. and Sucena E. 2000. Preparation of larval and adult cuticles for light microscopy. *Drosophila* protocols. CSHL press. New York. Pp 601-615.

Struhl G., Barbash D. A. and Lawrence P. A. 1997a. Hedgehog organizes the pattern and polarity of epidermal cells in the *Drosophila* abdomen. Development, **124**: 2143-2154.

Struhl G., Barbash D. A. and Lawrence P. A. 1997b. Hedgehog acts by distinct gradient and signal relay mechanisms to organize cell type and cell polarity in the *Drosophila* abdomen. Development, **124**: 2155-2165.

Wu J., Roman, Angel-Carlos Carvajal-Gonzalez J. M. and Mlodzik M. 2013. Wg and Wnt4 provide long-range directional input to planar cell polarity orientation in *Drosophila.* Nat. Cell Biol., **15**(9): 1045-1055.