Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

A large scale screen for genes (3rd chromosome) related to Wingless signaling pathway*

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Received Mar. 4, 2003; revision accepted May 5, 2003

Abstract: A wing specific F1 genetic screen was carried out using the powerful *Drosophila* genetic system, combined with yeast FRT/FLP and GAL4/UAS system. Form the wing phenotypes and germline clone embryonic cuticle phenotypes observed in these mutant alleles, a number of mutant alleles of known or unknown genes were isolated. Among them, fifteen mutant alleles related to Wingless signal transduction were further isolated; the arm of these mutations located were determined, and their location in the chromosome were roughly mapped.

Key words: *Drosophila*, Genetic screen, Wingless signal transduction, Mutant allele
Document code: A **CLC number:** Q344.5

INTRODUCTION

The Wingless (Wg) signal transduction pathway is an essential pathway in animal development (Wodarz and Nusse, 1998; Siegfried and Perrimon, 1994). The Wg signaling pathway is evolutionarily conserved and involved in numerous developmental processes in both *Drosophila* and vertebrates. Homologs of both Wg and their signaling components have been identified in vertebrates. The powerful genetics of *Drosophila melanogaster* provides great opportunity for dissecting the complex developmental processes that transformed the fertilized egg cell into the spatial pattern observed in the differentiated embryo.

Analysis of the *Drosophila* genome sequence led to the identification of a number of previously uncharacterized disease gene orthologues. Powerful genetic tools and the finished genome sequence were used to design and carry out tissue (wing) specific screening. The isolation and characterization of the mutant alleles related to the Wg signaling pathway provides a means for obtaining information on different aspects of this signaling pathway during the *Drosophila* development (Wehrli *et al*., 2000; Bhanot *et al*., 1999; van de Wetering *et al*., 1997; Cadigan and Nusse, 1997).

The use of the *Drosophila* as large-scale genetic screen is a very powerful tool for analyzing complex biological processes, as had been exemplified by screens for genes affecting embryonic development in flies (Nüsslein-Volhard and Wieschaus, 1980). Conventional ways to do screen are use of chemical mutagens, ionizing radiation or transposable elements to generate mutations randomly. A more recent way is using the mis-expression approach to direct the expression level of certain

* Project (No.30230070) supported partially by the National Natural Science Foundation of China

genes to do gain-of-function screens (Spradling *et al.*, 1995; Rorth *et al.*, 1998). Genetic screens would allow us to isolate novel genes and identify their functions required for specific developmental and cellular processes. Importantly, the genome of *Drosophila* has been sequenced and annotated in detail. The available information from the *Drosophila* genome has greatly facilitated the identification and isolation of new genes.

It has been known that the yeast site-specific recombinase Flipase (FLP) can catalyze recombination at sequence motifs termed FRTs (Flp Recognition Targets). So by integrating the *cis* and *trans* FRTs into the fly genome, site specific recombination would be induced (Chou *et al*., 1993; Xu and Rubin, 1993). Using the GAL4-UAS system to target the FLP to specific tissues, the loss of the genes of interest could be directed to any tissue or biological process of interest (Brand and Perrimon, 1993; Duffy *et al.*, 1998; Duffy, 2002). According to the principles above, application of the wing specific FLP should be able to induce the FLP specifically in the wing, which means that a screen that aims specifically at the wing is feasible. The obvious advantages of this approach are that it provides an efficient way to bypass the vital requirements of lethal mutations and assay their effects specifically in the wing, not in the other tissues. By combining the mutant alleles isolated from this wing specific F1 genetic screen with the available annotated genome sequence, the *Drosophila* genetics would be a more powerful tool for studying the complex biological process.

In this study, we conducted a F1 genetic screen using the strategy described above. A number of mutant alleles of known or unknown genes were isolated from this F1 screen. From the wing phenotypes and embryonic cuticle phenotypes observed in these mutant alleles, one major group of mutant alleles required for Wg signaling was isolated.

MATERIALS AND METHODS

Drosophila melanogaster strains

Lines used for F1 screen: *w*; *FRT*2*A FRT* ^{82B} *e* / *TM*3, *w*; *vg Q*1206-*Gal*4 *UAS-flp*; FRT^{2A} *FRT* ^{82B}/ *TM*3, *w*; *Dr/TM*3, *w*; *vg Q*1206-*Gal*4 *UAS-flp*; FRT^{2A}/*TM*3, *w*; *vg Q*1206-*Gal*4 *UAS-flp*; FRT^{82B} /*TM*3.

EMS (Ethyl MethaneSulfonate, also known as methane sulfonic acid ethyl ester)

Bought from Sigma Aldrich Company (liquid).

EMS treatment

After injecting 0.24 cc of EMS solution into 100 ml of 1 percent sucrose solution, mix them completely. Aspirate 10 cc of the mixture into a bottle with kinwipes at the bottom. Put approximately 100 adult males, which are 0−3 days old and 200−300 virgin females into each bottle. Tap the flies down carefully into the bottle. Feed for 12−24 hours. Clean and move the flies to new bottles with food. Let the flies stay in the first bottle for 2 days and then transfer everyday up to total 5 bottles.

Strategy for F1 Screen (on 3rd chromosome)

A wing-specific Gal4 line, *vg Q*1206-*Gal*4 (Simmonds *et al*., 1995) was used to drive high level expression of Flipase in dorso-ventral compartment boundary of the wing imaginal disc. Males of the genotype *w*; FRT^{2A} FRT^{82B} *e/TM*3 were mutagenized with EMS (Sigma) and crossed to females of the genotype *w*; *vg Q*1206-*Gal*4 *UAS-flp*; FRT^{2A} *FRT* ^{82B} /*TM*3. F1 flies of the genotype *w*; *vg Q*1206-*Gal*4 *UAS-flp/+*; FRT^{2A} FRT^{82B} *e*/FRT^{2A} FRT^{82B} expressed Flp primarily in wing imaginal cells under *vg Q*1206-*Gal*4 control. This Flp activity mediated a high frequency of mitotic recombination, generating clones of cells homozygous for the mutagenized FRT^{2A} FRT^{82B} *e* chromosome specifically in the wing. The wings of the resulting F1 flies were screened for wing phenotypes like notches, lost or ectopic veins and ectopic margin bristles or overgrown bristles. The F1 male flies with interesting wing phenotypes were further crossed with females of the genotype *w*, *Dr/TM*3 to keep the mutation. The resulting F2 flies with genotype of FRT^{2A} FRT^{82B} *e/TM*3 were isolated based on ebony marker. We further determined the chromosome arm of interesting mutations by crossing them with the stocks of genotype *w*; *vg Q*1206-*Gal*4 *UAS-flp*; FRT^{2A}/*TM*3 or with *w*; *vg Q*1206-*Gal*4 *UAS-flp*;

FRT^{82B}*/TM*3 to double confirm the phenotypes (Fig.1).

To identify molecular lesions associated with alleles, genomic DNA was prepared from larvae homozygous for alleles and amplified by PCR using synthetic oligonuleotide primers against the gene of interest. Mutations were identified by sequencing the PCR products on both directions.

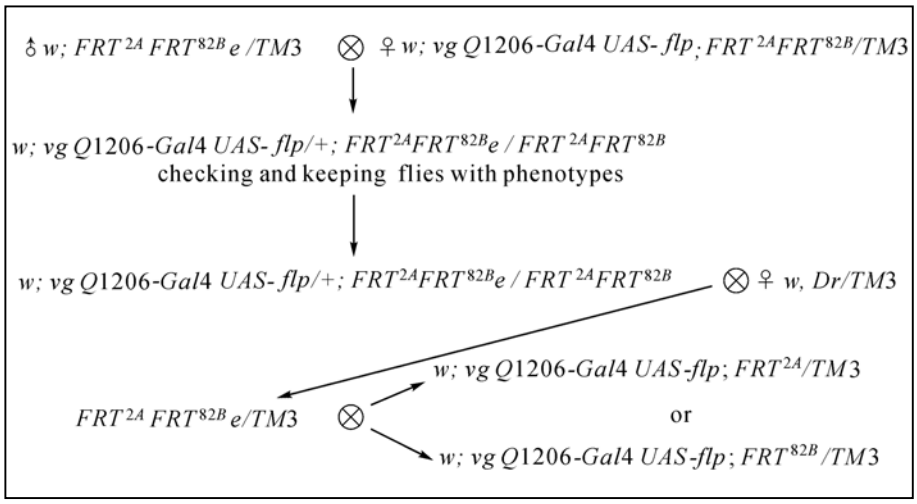

Fig.1 General scheme for Wing Specific F1 Screen on 3rd chromosome

Germline Clone (GLC)

Scheme for GLC on 3 L was done as follows: virgin females of the genotype *m FRT*^{2A}*/TM*3, *Sb* were mated with males of the genotype *y w FLP*22/+, *FRT*^{2A} *P*[*ovo*^{D1}]*/TM*3, *Sb*. The resulting progeny were heat-shocked at 37 ºC for 2 hours at the larval stages, and *y w FLP*22/+; *m FRT*^{2A}*/P* [*ovo*^{D1}]*FRT*^{2A} females carrying *mutant* homozygous germline clones were selected. Females with germline clones were generated using the autosomal 'FLP-DFS' technique (Chou and Perrimon, 1996). GLC on the 3R mutant was done in a similar way.

Cuticle preparation

First vortex and then let the vial stand; wait for embryos to precipitate. Take out upper Triton with food by aspiration. Then add a little bleach (less than 50 μl), wait for 2−3 min. Then add triton till full; let it stand to let embryos precipitate, and then get rid of the solution by aspiration. Wash with Triton for one−two or more times; then with a small tip, transfer embryos with triton into the slide. Separate embryos from food, shell and whatever with needle. Get rid of these stuff and liquid with absorbing paper. Add Hoyer's goop, when they are not too wet or too dry, cover with cover slide. Put the slide in the slide warmer (53 °C), when it is getting dried, add one small fishing sinker on cover glass. Keep the slide on the warmer for a couple of days, then save it for analysis.

Arm determination and location mapping

To determine the mutation located at which chromosome arm, the mutant fly was crossed with both the *w*; *Dr/TM*3, *w*; *vg Q*1206-*Gal*4 *UAS-flp*; *FRT*^{2A}*/TM*3 and *w*; *vg Q*1206-*Gal*4 *UAS-flp*; *FRT*^{82B} */TM*3, if the wing phenotype was observed by the previous cross, but not by the latter cross, then we could know the mutant is at the 3 L arm, and vice versa. Location mapping was done by crossing the mutant lines with the 3rd chromosome deficiency kit lines, and by crossing these mutant lines with each other.

RESULTS

Known genes identified in the F1 screen

Proteins like Vestigal, Wg, Hh, Dpp, Costal-2 etc., play key roles in the normal wing development and embryo denticle formation. It could be predicted that the wing specific F1 screen, which disrupted genes, might be used to identify the components of these signaling transduction pathways. The known

genes identified in this wing specific F1 screen are wg, arrow, hh, vgl, cos-l, sfl, axin, ptc, ttv, *et al*. These mutant alleles were firstly selected according to their wing phenotypes that exhibited, and then were further confirmed by complementation test with corresponding flies of available mutant alleles and germ-line clone. Some of these phenotypes are shown below (Figs.2 and 3).

Multiple wing hairs (*mwh*) as an internal control

Multiple wing hairs (*mwh*) is a recessive and viable mutation of spontaneous origin which had been used extensively as a genetic marker and had been mapped to the 3rd chromosome. The mutant heterozygote *mwh*/+ fly was used in the screen as an internal control to see the efficiency of the F1 screen. The ratio of the *multiple wing hairs* fly indicated the

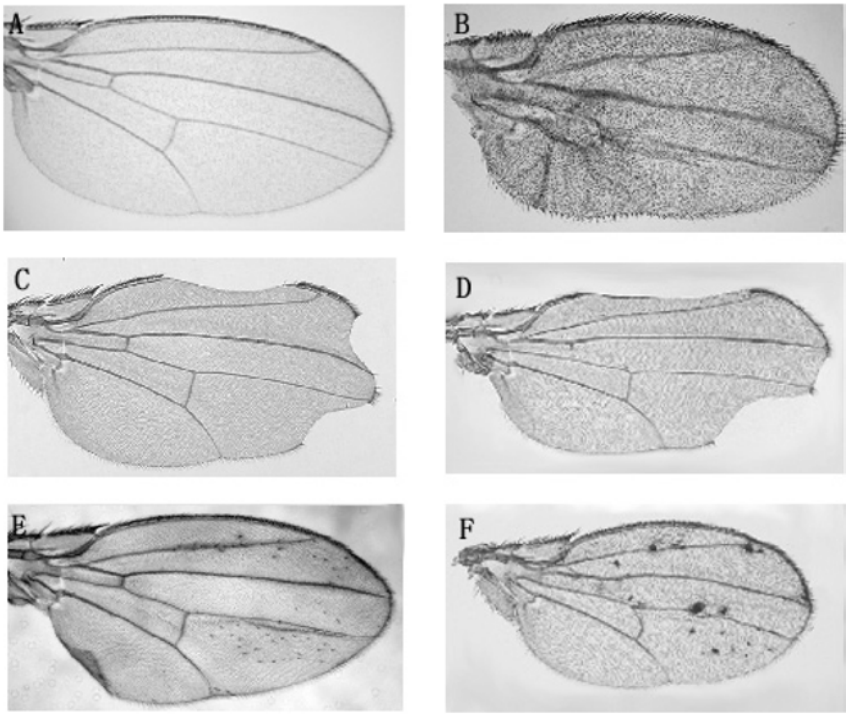

Fig.2 Wing phenotypes related to Wg signaling of adult mutant flies from F1 screen. The dorsal side is shown here. Up is anterior and down is posterior

(A) A wing from wild-type; (B) *multiple wing hairs* (*mwh*); (C) Loss-of-function of wg. Note the notch and the loss of wing margin structures; (D) Wing of mutant fly from F1 screen shows loss-of-function of *wg* like phenotype. Note the notch and the loss of wing margin structures; (E) Gain-of-function of Wg signaling. Note the ecotopic bristles grown inside the wing (B140); (F) A wing of mutant fly from F1 screen shows gain-of-function of *wg* like phenotype. Note the ecotopic bristle growth inside the wing (F66)

efficiency of the recombination of the wing. In the wings in Fig.2B and Fig.3B, the hairs and denticles are smaller, more in number and less orderly than those in wild type (Fig.2A and Fig.3A); which indicated that the recombination efficiency of the F1 screen system was very high.

Wg-related wing phenotype from F1 screen

The F1 progenies were screened firstly according to their wing phenotypes. The F1 adult flies exhibited various wing phenotypes reminiscent of many signaling pathways and biological processes. There were notched wing, small or narrow wing, enlarged wing, ecotopic wing and double-edge wing. There were also wings with big bristles, vein lost, ecotopic veins, etc. From these wing phenotypes, one group of phenotype, the notched wing and loss of wing margin structures as well as the wing with ecotopic bristles, which were reminiscent of the *wg*-related phenotypes were roughly separated from the F1 progeny. Fig.2 shows the various wing phenotypes of this group from the F1 screen. Both loss-

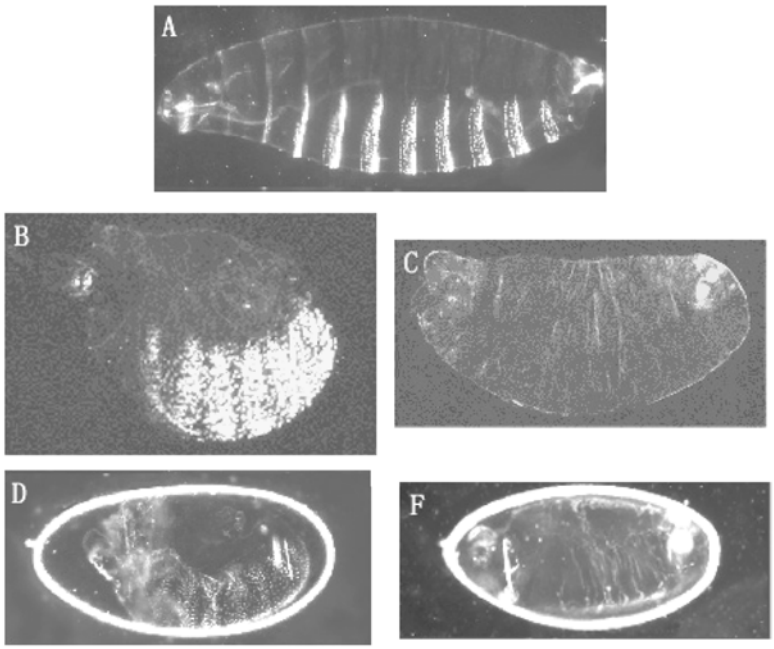

Fig.3 Cuticle patterning of embryos from Germline Clone (all the embryos with anterior to the left)
(A) Cuticle of wild-type GLC embryo; (B) Loss-of-function of Wg GLC embryo with cuticle fusion phenotype shown; (C) Gain-of-function of Wg signaling GLC embryo with naked cuticle phenotype (*axin*) shown here; (D) GLC embryo from the F1 screen. The GLC embryo with cuticle fusion phenotype is shown (B173); (F) GLC embryo from the F1 screen. Gain of function of Wg signaling GLC embryo with naked cuticle phenotype is shown

of-function of Wg signaling and gain-of-function of Wg signaling phenotypes were observed from the F1 screen. The notched wing shown in Fig.2C is the typical *wg* phenotype; complementation test with the *wg* mutant allele and germ-line clone, finally showed it was a new *wg* allele. The wing shown in Fig.2E is a phenotype related to gain-of-function of Wg signaling and later on turned out to be a new *axin* mutant allele. Fig.2D and Fig.2F are wings also from the F1 screen and showed phenotypes very much similar to those of Fig.2C and Fig.2E. Both Fig.2D and Fig.2F are potential new components or at least new mutant alleles of previously identified genes.

Wg-related cuticle phenotypes of GLC embryos made from adult flies of F1 screen (3rd chromosome)

For the interesting wing phenotypes, germ line clone (GLC) was made to further check the cuticle phenotype and double confirm which group they belonged to. There were also various phenotypes present in those GLC embryos: U shape embryo, head defect, dorsal open, denticle fusion or deletion, short or narrow denticles; from which only those with denticle fusion or lost wing margin structures that are reminiscent of the *wg*-related phenotypes were selected. Through the germline clone, whether the gene product was necessary in the germline or in the non-germline supporting tissues was determined, and also, whether the gene had maternal contribution, whether this maternal contribution was necessary for embriogenesis or the development of the subsequent embryo was also determined. Fig.3 shows the cuticle phenotype of GLC embryos of Wg signaling related mutants from this F1 screen. Obviously the maternal contribution of this gene was necessary for the embryogenesis and development of the subsequent embryo.

Interesting mutants from the F1 screen

The F1 screen is a very efficient screen system. Around 100 000 flies were screened totally. Many of the mutant alleles from of the screen had very interesting phenotypes. There were mutants phenocopy that of various of pathways and biological

processes, they probably related to the Wg signaling pathway, Hh signaling pathway or Jak/Stat signaling pathway; they could also be involved in the tumorigenesis of fly, or be involved in all kinds of fly pattern formation and metabolism, etc. Many of the alleles isolated from the F1 screen are believed to be alleles of important players in the *Drosophila* development according to the wing and GLC embryo phenotypes observed. Among the mutant alleles isolated from this F1 screen according to wing phenotype and GLC embryo cuticle phenotype, mutant alleles (Table 1) related to the Wg signal transduction pathway were isolated. The arm determination was done as described in materials and methods; the results are shown in Table 1 and Fig.4. Besides the mutant alleles of known genes, some mutant alleles like *dly* were recently identified; some others were probably new genes required for Wg signaling pathways. The relative locations of the genes corresponding to mutant alleles were done by mapping as described in materials and methods and are shown in Fig.4. It was found that some alleles were mapped in the same location, which indicated that most likely they were mutant alleles of the same gene; and that the screen was almost saturated.

Table 1 Wg signaling related mutants from the F1 screen on the 3rd chromosome

3rd left	3rd right
B138, *dly*, A61, B104, B164	B173, B140, B158, B161, B6, 22C, F66, F15-108, F126, F107

Fig.4 Chromosome mapping of Wg signaling related mutants from the F1 screen on the 3rd chromosome

DISCUSSION AND CONCLUSION

Drosophila represents an almost perfect model system for the genetic and biochemical dissection of signaling pathways and other biological processes. Although searching for different lethal mutations according to various phenotypes by screen is a powerful genetic tool for dissecting developmental processes, the conventional approach to recover such mutations requires generating lethal mutation to be maintained as individual stock before analysis. Also, this type of F2 genetic screen is not only time consuming, but also labor intensive. Fortunately, the F1 genetic screen strategy provides a more efficient choice and allows us to check the phenotypes and analyze from the first generation of the progeny. For the lethal and recessive mutations, a potential problem is that the fly would die, being a homozygous mutant; which makes it impossible to check phenotypes from the F1 progeny. The mosaic technique is used to solve this problem. Because homozygous cells only exist in certain or in some cases specifically in one tissue, the high viability of the mosaic flies were retained. The wing specific vg-Gal4 is very efficient as vg-GAL4 directs the Flipase only to the wing, not in any other tissues. Compared with other tissue specific screens, the wing specific genetic screen also has advantages, as mutant clones formed in the wing is usually viable and the mosaic formed in the wing could be observed easily.

The mutant alleles isolated from the F1 screen were either new alleles of known genes or potential alleles of new genes. The next step will be identifying genes that the mutant alleles correspond to. In this study, we determined whether the mutant alleles related to Wg signaling according to both the wing phenotype and GLC embryo phenotype observed, which were obviously not enough to determine conclusively whether this was really the case, as the wing and cuticle phenotypes observed could be generated by many other biological processes. Also,

whether the genes corresponding to these mutant alleles are the genes which are the components of the Wg signaling pathway or other genes related to Wg signaling (for example, genes required for the movement of Wg morphogen between cells) is also not clear yet. At present, for most of the mutant alleles, only wing and GLC embryo cuticle phenotypes were observed, more work like embryonic and imaginal disc clonal analysis to see whether Wg signaling is affected would be done in the future to determine their functions.

References

Bhanot, P., Fish, M., Jemison, J.A., Nusse, R., Nathans, J., Cadigan, K.M., 1999. Frizzled and DFrizzled-2 function as redundant receptors for Wingless during *Drosophila* embryonic development. *Development*, **126**: 4175-4186.

Brand, A.H., Perrimon, N., 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, **118**(2):401-415.

Cadigan, K.M., Nusse, R., 1997. Wnt signaling: a common theme in animal development. *Genes & Dev*, **11**:3286-3305.

Chou, T.B., Noll, E., Perrimon, N., 1993. Autosomal *P*[*ovo*^{D1}] dominant female sterile insertions in *Drosophila* and their use in generating germ-line chimeras. *Development*, **119**(4):1359-1369.

Chou, T.B., Perrimon, N., 1996. The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. *Genetics*, **144**(4):1673-1679.

Duffy, J.B., 2002. GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. *Genesis*, **34**(1-2):1-15.

Duffy, J.B., Harrison, D.A., Perrimon, N., 1998. Identifying loci required for follicular patterning using directed mosaics. *Development*, **125**(12):2263-2271.

Nüsselein-Volhard, C., Wieschaus, E., 1980. Mutations affectiong segment number and polarity in Drosophila. *Nature*, **287**:795-801.

Rorth, P., Sazbo, K., Balley, A., Laverty, T., Rehm, J., Rubin, G.M., Weigmann, K., Milan, M., Benes, V., Ansorge, W., Cohen, S.M., 1998. Systematic gain-of–function genetics in *Drosophila*. *Development*, **126**:1049-1057.

Siegfried, E., Perrimon, N., 1994. *Drosophila* wg: a paradigm for the function and mechanism of Wnt signaling. *Bioessays*, **16**:395-404.

Simmonds, A.J., Brook, W.J., Cohen, S.M., Bell, J.B., 1995. Distinguishable functions for engrailed and invected in anterior-posterior patterning in the *Drosophila* wing. *Nature*, **376**:424-427.

Spradling, A.C., Stern, D.M., Kisss, I., Roote, J., Laverty, T., Rubin, G.M., 1995. Gene disruptions using P transposable elements: an integral component of the *Drosophila* genome project. *Proc Natl Acad Sci USA*, **92**: 10824-10830.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., Peifer, M., Mortin, M., Clevers, H., 1997. Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell*, **88**:789-799.

Wehrli, M., Dougan, S.T., Caldwell, K. O'Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Schejter, E., Tomlinson, A., DiNardo, S., 2000. arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature*, **407**:527-530.

Wodarz, A., Nusse, R., 1998. Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol*, **14**:59-88.

Xu, T., Rubin, G.M., 1993. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development*, **117**(4):1223-1237.