

Mutations in tumour suppressor genes, *l(2)gl* and *l(2)gd*, alter the expression of *wingless* in *Drosophila*

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MS received 3 February 1995; revised 10 April 1995

Abstract. To understand the roles of two well known tumour suppressor genes, *l(2)gl* and *l(2)gd* in normal imaginal disc development in *Drosophila*, we have initiated a study to examine effect of mutations of these genes on the expression of genes involved in the patterning of the imaginal discs. In this study we show that the expression of *wingless*, the *Drosophila* orthologue of the mammalian oncogene *Wnt*, is affected in the imaginal discs of *l(2)gl^l* and *l(2)gd^l* mutant individuals. In the tumourous wing imaginal discs from *l(2)gl* mutant larvae, the pattern of *wingless* expression was progressively disrupted with an increase in the area of expression. Tumourous wing imaginal discs from *l(2)gd* homozygous individuals exhibited progressive broadening and extension of the *wingless* expressing domains. We suggest that *l(2)gl* and *l(2)gd* might be involved in regulating post embryonic expression of *Wingless*.

Keywords. Segment polarity genes; *wingless*; tumour suppressor genes; *l(2)gl*; *l(2)gd*

1. Introduction

The *lethal(2)giant larvae [l(2)gl]* and *lethal(2)giant discs [l(2)gd]* are two of the best studied tumour suppressor genes in *Drosophila*. Recessive lethal mutations of the *l(2)gl* gene lead to malignant transformation of neuroblasts of the presumptive adult optic centres in the larval brain and of imaginal disc cells (Gateff and Schneiderman 1974) while recessive lethal mutations of the *l(2)gd* produce hyperplastic overgrowth of imaginal discs (Bryant and Schubiger 1971). Genetic and molecular analyses of these genes revealed that in addition to their tumour suppressing activity they have important functions in the regulations of specific cell fates and patterns of differentiation (Bryant 1987; Gateff and Mechler 1989). In another study (Mukherjee *et al* 1995) we have shown that absence of *l(2)gl* function completely disrupted the anterior posterior compartment boundary in late larval imaginal discs and led to ectopic expression of the compartment specific segment polarity genes *cubitus interruptus-Dominant (ci^D)* and *engrailed (en)* in anterior and posterior compartments, respectively; on the other hand, *l(2)gd* did not affect expression of either of these two genes. In view of these findings, we suggested that *l(2)gl* regulates expression of *en* as well as *ci^D* during late stages of development in *Drosophila* (Mukherjee *et al* 1995).

The *wingless (wg)* gene of *Drosophila* encodes a secreted growth factor similar to the mammalian Wnt 1 protein (Rijsewijk *et al* 1987; Gonzalez *et al* 1991). The

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function of this is required in several developmental processes such as embryonic segmentation, cell fate determination, cell proliferation and axis specification in the embryo and imaginal discs (see review by Siegfried and Perrimon 1994). In association with other segment polarity genes, this gene plays crucial roles in segment patterning in the anterior-posterior axis during embryonic development (Ingham 1988; Vincent and Lawrence 1994) and in patterning the dorso-ventral axis in post embryonic imaginal discs (Bryant 1993; Couso *et al* 1993; Struhl and Basler 1993; Williams *et al* 1993). The present study was undertaken to examine if *wg* expression in imaginal discs is also regulated by *l(2)gl* or *l(2)gd*.

We report that individuals mutant for *l(2)gl* exhibit progressive disruption of *wg* expression pattern during their tumourous growth, while in the *l(2)gd* mutant discs, the *wg* expressing stripes are considerably broadened and extended.

2. Materials and methods

2.1 Fly strains and generation of recombined stocks

The following fly stocks were used: *l(2)gl⁴ or/SM5*, *l(2)gd¹ a px or/SM5* and *wg^{lacZ}/CyO*. For details of mutations and balancer chromosomes, see Lindsley and Zimm (1992). The *wg^{lacZ}* allele allows monitoring of *wg* expression through histochemical staining for the β -galactosidase activity using the chromogenic substrate X-gal (Kassis *et al* 1992). The *l(2)gd¹ wg^{lacZ}/SM5* stock was generated from the above through appropriate crosses.

All flies and larvae were reared in uncrowded condition on standard yeast supplemented food at $22 \pm 1^\circ\text{C}$.

2.2 Expression of *wg* in imaginal discs

Expression of *wg* was monitored either by *in situ* hybridization of a digoxigenin labelled *wg* probe to detect *wg* transcripts in intact wing imaginal discs of *l(2)gl⁴/l(2)gl⁴* or *l(2)gl⁴/SM5* larvae or by X-gal staining of wing imaginal discs of *l(2)gd¹ wg^{lacZ}/l(2)gd¹ +* or *l(2)gd¹ wg^{lacZ}/SM5* larvae.

For *in situ* hybridization, the 3 kb *Bam*HI fragment of the *wg* cDNA clone (Rijsewijk *et al* 1987) was digested with *Alu*I and *Rsa*I. The resulting fragments were labelled with digoxigenin by random-primed synthesis and were hybridized to *wg* transcripts in intact wing imaginal discs from *l(2)gl⁴* homozygous or heterozygous larvae as described earlier (Mukherjee *et al* (1995). X-gal staining of wing discs of *l(2)gd¹ wg^{lacZ}/l(2)gd¹ +* or *l(2)gd¹ wg^{lacZ}/SM5* larvae to monitor the *wg* promoter driven β -galactosidase activity was performed as described earlier (Mukherjee *et al* 1995).

3. Results

3.1 Expression of *wg* in wing imaginal discs from *l(2)gl* mutant larvae

The pattern of distribution of *wg* transcripts in intact wing imaginal discs from *l(2)gl⁴* homozygous or heterozygous larvae (progeny obtained from *l(2)gl⁴ or/SM5*

parents) of different stages was examined by *in situ* hybridization of the *wg* probe to cellular RNA. The pattern of hybridization in all imaginal discs (about 100 wing imaginal discs each from early and mid third instar larvae) till the onset of tumorigenesis was comparable to that in the wild type (figure 1a). Although many of these discs would have been homozygous for *l(2)gl*, the fact that none of these discs displayed any aberrant pattern of hybridization, revealed that *wg* expression was not affected in *l(2)gl* homozygous individuals till mid third instar stage, i.e., before the onset of tumorigenesis. However, tumorous wing imaginal discs (which coalesce with the neighbouring haltere and third teg discs) from *l(2)gl^t* homozygous late third instar larvae—identified by their prolonged larval life and bloated appearance—exhibited a progressively complete disruption in *wg* expression pattern during tumour progression; the total area covered by patches of *wg* expressing cells was more extensive in the tumorous discs (figure 1b, c).

3.2 Expression of *wg* in wing imaginal discs from *l(2)gd* mutant larvae

The expression of *wg* in *l(2)gd* mutant was examined by X-gal staining in imaginal discs from different stages of larval development in the progeny derived from crosses between *l(2)gd^l wg^{lacZ}/SM5* and *l(2)gd^l a px or/SM5*. In these progeny larvae, all imaginal discs (about 50 wing imaginal discs each from early and mid third instar larvae) showed a typical wild type *wg* expression pattern till the onset of tumorigenesis (figure 2a). Since none of these discs displayed any aberrant X-gal staining pattern, it revealed that *wg* expression was not affected in *l(2)gd* homozygous individuals till mid third instar stage. Unlike the case in *l(2)gl* tumorous discs, the hyperplastic imaginal discs of *l(2)gd* homozygous late third instar larvae did not show disruption of *wg* expression pattern during their extended larval life but the *wg* expressing areas were considerably broadened with progression of tumorigenesis. The posteriorly directed end of *wg* expressing incomplete ring along the edge of the disc was extended further in the posterior compartment; the central *wg* expressing stripe in the wing pouch in the dorso-ventral axis became progressively broader and ultimately fused with the ring thereby covering the entire areas of presumptive mesopleura and pteropleura. The faint second stripe adjacent to the ring, which normally remains restricted to the anterior half of the disc in imaginal discs from wild type larvae (figure 2a), was also extended posteriorly (figure 2b, c).

4. Discussion

Our results showed that *wg* expression is affected by both the tumour suppressor genes, *l(2)gl* and *l(2)gd*. In tumorous wing discs from *l(2)gl* mutant individuals, the pattern of *wg* expression was completely disrupted and it was expressed in apparently larger areas as was also observed earlier for *en* and *ci^D* (Mukherjee *et al* 1995). The disruption of *wg* expression in tumorous discs of *l(2)gl* homozygous larvae could be related to the general loss of organization due to invasion and/or mixing of cells from one area to other. However, the apparent increase in the total area of *wg* expressing cells in *l(2)gl* tumorous tissues suggests that this may instead be due either to a higher rate of proliferation of *wg* expressing cells or to ectopic expression of *wg* in cells which do not normally express *wg*. Present data

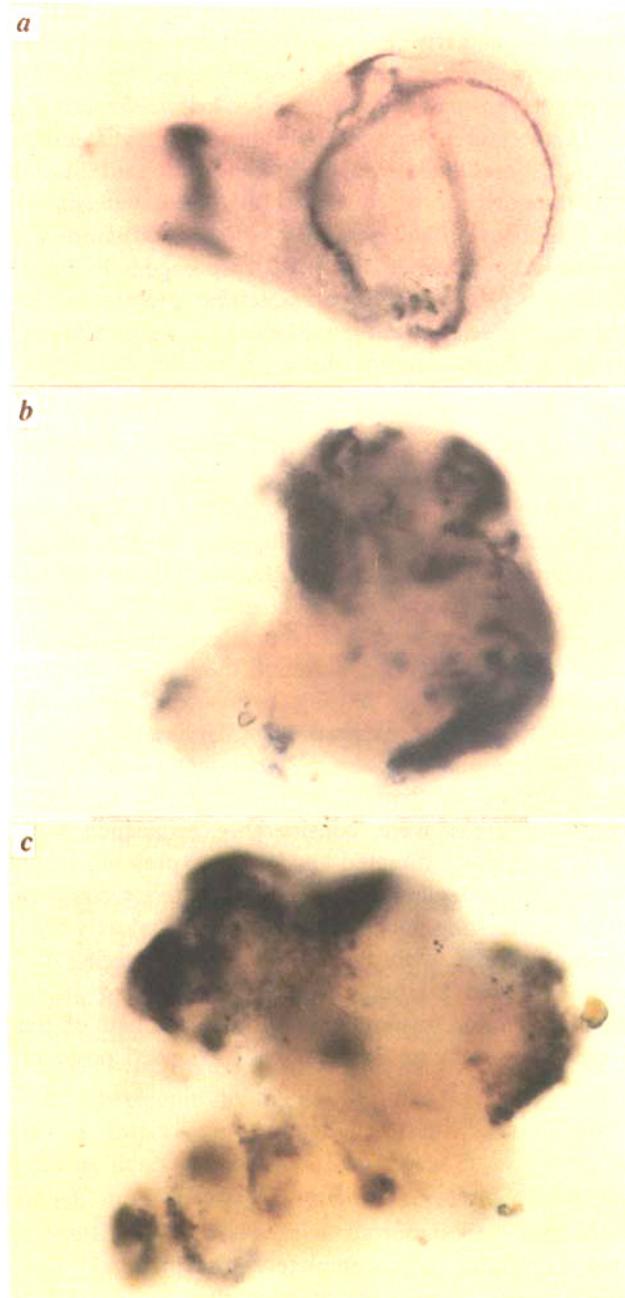


Figure 1. Expression of *wg* in wing imaginal discs from third instar larva of wild type (a) or $l(2)gt^1/l(2)gt^1$ genotype (b, c) of *D. melanogaster* as revealed by *in situ* hybridization of the DIG-labelled *wg* probe to transcripts. Note the normal pattern of *wg* expression in imaginal disc from wild type larva (a) and a progressively disrupted pattern of expression in imaginal discs from 9 day (b) and 12 day (c) old $l(2)gt^1/l(2)gt^1$ larvae.

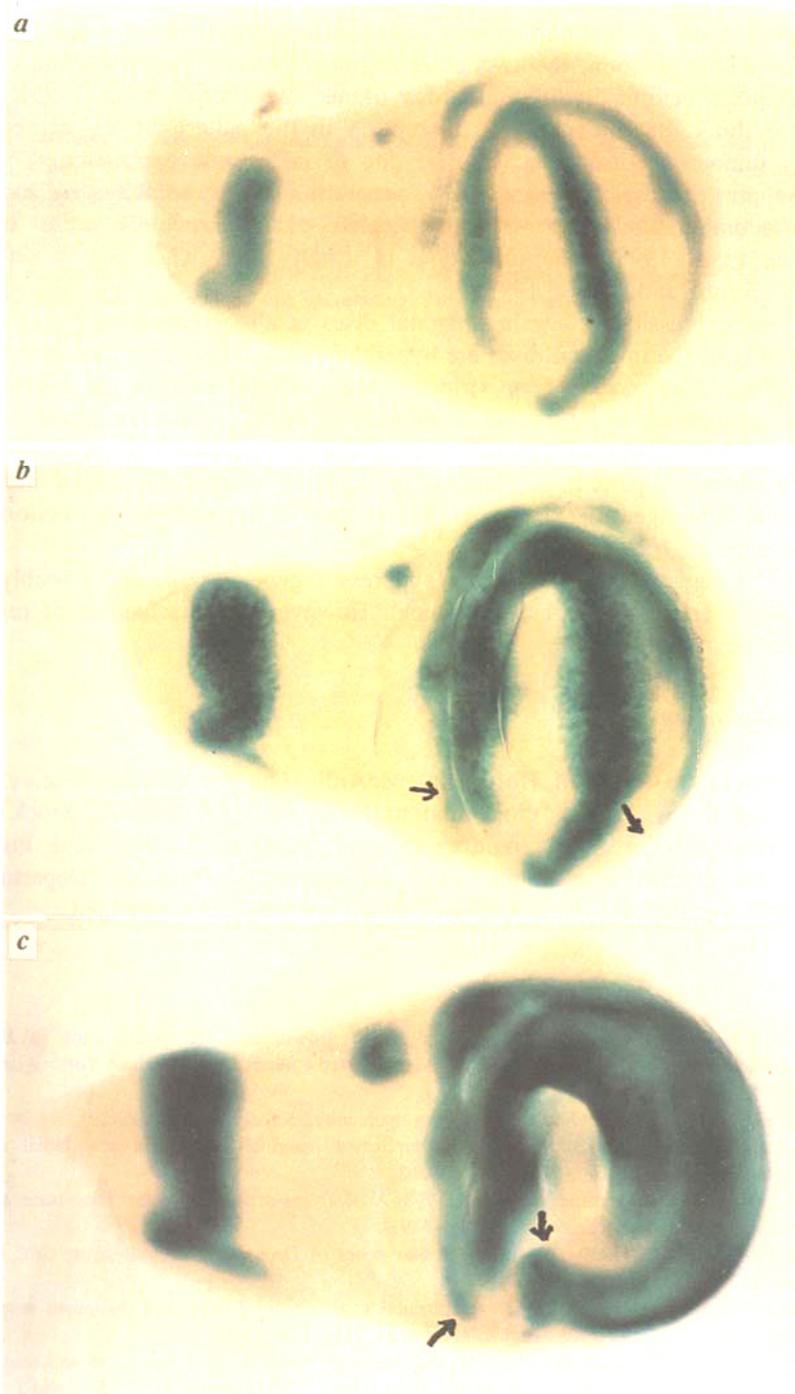


Figure 2. Expression of *wg* in wing imaginal discs from third instar larvae of *l(2)gd¹ wg^{lacZ}/l(2)gd¹ +* genotype as revealed by X-gal staining. Note the normal pattern of *wg* expression in imaginal disc from 7 days old larva prior to the onset of tumourigenesis (a) and broadening as well as extension (arrows) of *wg* expressing stripes in imaginal discs from 9 days (b) and 11 days (c) old *l(2)gd¹ g^{lacZ}/l(2)gd¹ +* larvae.

do not permit discrimination between these possibilities. However, since in the absence of *l(2)gl* function, the two other segment polarity genes, *en* and *ci^D* were seen to express ectopically in tumourous tissues (Mukherjee *et al* 1995), we are inclined to think that the observed disruption in the pattern of *wg* expression in the *l(2)gl* tumourous discs may also be due to its ectopic expression. This finds further support in the fact that while generating *l(2)gl* homozygous clones by somatic recombination, legs with duplicated distal structures were observed (Mukherjee *et al* 1995); this phenotype is usually seen when *wg* is ectopically expressed (Struhl and Basier 1993).

An altered expression of *wg* in imaginal discs is also evident in *l(2)gd* mutants. In these mutants, the imaginal discs are hyperplastic but do not lose their morphology. Thus the distinct extension of *wg* stripes to the posterior edge in the *l(2)gd* mutant wing discs is unlikely to be due to different rates of cell proliferation and migration but appears more likely to be due to ectopic expression of *wg*. Furthermore, the frequently observed pattern duplication in imaginal discs from *l(2)gd* individuals (Bryant and Schubiger 1971) also could be due to the ectopic expression of *wg* in the absence of *l(2)gd* function.

Our results suggest that the tumour suppressor gene, *l(2)gd*, and possibly *l(2)gl*, regulate *wg* at later stages of development. However, the mechanism of regulation remains to be investigated.

Acknowledgements

We are grateful to Dr F M Hoffmann, McArdle Lab for Cancer Research, USA for providing the *wg* cDNA clone and to Dr P Sinha, *Drosophila* Stock Centre, D A University, Indore, for providing the *wg^{lacZ}/CyO* stock. We thank Prof. S C Lakhotia, for critical suggestions. Financial assistance from the Department of Science and Technology, New Delhi is also gratefully acknowledged.

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Corresponding editor: VIDYANAND NANJUNDIAH