Development 118, 427-438 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

wingless expression mediates determination of peripheral nervous system elements in late stages of *Drosophila* wing disc development

R. G. Phillips* and J. R. S. Whittle

School of Biological Sciences, University of Sussex, Brighton BN1 9QG, UK

*Author for correspondence

SUMMARY

We have used conditional *wingless* genotypes to dissect the role of this gene in late stages of wing disc development. One of these genotypes (wg^{IL}/*wg-lacZ*) is simultaneously a reporter of *wingless* transcription and temperature-sensitive for *wingless* function, and has allowed us to define its pattern of transcription in the absence of *wingless* activity. The primordia of a subset of the bristles of the notum, which develop in or immediately adjacent to *wingless*-expressing cells, depend upon *wingless* activity. The time-course of this contribution and the effect on proneural gene expression together suggest that *wingless* may regulate the activity of products of the *achaete-scute* complex in proneural clusters. *wingless* activity is also required at the presumptive wing margin and is a necessary precondition for the change in proliferation pattern in this region. The involvement of *wingless* in transducing or mediating positional signals for spatial patterning in imaginal disc development is discussed.

Key words: *wingless*, *Drosophila* imaginal discs, signal transduction, sensory organs

INTRODUCTION

The appearance of spatial patterns of cell differentiation in the epithelia of imaginal discs in *Drosophila* depends upon signalling mechanisms between adjacent cells (Haynie and Bryant, 1976; Wilcox and Smith, 1977; Mohler, 1988; Phillips et al., 1990). In particular, the specification and differentiation of the wing cuticular elements, including bristles in stereotyped distributions, presents a very accessible system for analysis (Campuzano and Modolell, 1992). The application of genetic methods, particularly molecular probes to gene products in situ in the disc tissue, has suggested that the patterning is a series of cell-specific gene activations, acting in a cascade to increase the precision of the pattern.

In a wing imaginal disc, each sensillum comprises four different cells, derived clonally from a single precursor cell (Hartenstein and Posakony, 1989; Huang et al., 1991). This precursor or sensory mother cell (SMC) in turn arises within a group of cells, called a proneural cluster, characterised by expression of the 'proneural' genes of the *achaete-scute* complex (Cubas et al., 1991; Skeath and Carroll, 1991; Romani et al., 1989). The *achaete-scute* genes are expressed in the proneural clusters, dependent upon *cis*-regulatory regions (Ruiz-Gómez and Modolell, 1987). Proneural expression is refined by a balance between activators and inhibitors of a positive feedback system (Cubas and Modolell, 1992; Martinez and Modolell, 1991). Inhibitory factors include gene products that influence the specification of an SMC (Cubas and Modolell, 1992; Blair, 1992) as well as components of a system of lateral inhibition, which act after SMC specification to inhibit formation of additional SMCs in adjacent cells (Heitzler and Simpson, 1991; Simpson, 1990). Subsequently, genes such as *neuralised* and *cut*, which may be specific for neural functions, maintain this state of determination (Jack et al., 1991; Jan and Jan, 1992).

We chose to examine the contribution of the *wingless* gene to these events because it appears to be a component in a cell-to-cell signal transduction pathway both in embryogenesis (Martinez-Arias et al., 1988; DiNardo et al., 1988) and during imaginal disc development. It is a member of the highly conserved *Wnt* family of genes, which code for putative intercellular signalling molecules (Nusse and Varmus, 1992). It is expressed in a complex pattern in third instar imaginal discs implying a role in local developmental events (Baker, 1988a) and mutations in it affect disc-derived structures, including the wing (Sharma and Chopra, 1976; Morata and Lawrence, 1977).

We describe the time-course and pattern of activation of *wingless* in late stages of the wing disc development using a *wingless* reporter genotype which is simultaneously a temperature-sensitive genotype for *wingless.* Its role may be to establish a link between proximodistal positional cues and specification of sense organ primordia. Our findings are consistent with its participation in post-transcriptional regulation of the *achaete-scute*-complex (AS-C), in antagonism to the action of the gene *zeste-white 3-shaggy* (*zw3-sgg*). At the presumptive wing margin, *wingless* activity affects cell proliferation.

MATERIAL AND METHODS

Culture and staging methods

Flies were reared on standard maize medium at 25°C. For the temperature shifts of conditionally expressed genotypes, collections were made at 16°C for 12 hour periods and upshifts were to 28°C. The precise stage reached at any shift point was determined (a) by X-gal staining a sample of the larvae and matching that pattern with the time course for the reporter in a wild-type genetic background at 25°C and (b) by inspecting morphological features associated with particular developmental stages. Those we have used include embryonic stages judged by differential interference optics, identification of the mouth parts, which are instar-specific, and the presence of wandering stage larvae, white prepupae, brown prepupae and pupae. We have distinguished five periods in the 3rd instar; stages $III_{(1)}$, $III_{(1/2)}$, stage $III_{(2)}$ and $III_{(3)}$ are separated by changes in their *wingless* reporter expression (see Fig. 2A-C), whilst stage $III_{(w)}$ is the wandering stage.

Adult cuticles were softened in 10% potassium hydroxide, then mounted in Berlese fluid and examined under phase-contrast optics. The frequency per hemi-notum was determined for each notal bristle, and wing margins were scored for a complete set of costal, triple row and double row bristles and hairs per wing.

Genotypes

The temperature-sensitive *wingless* mutation used was wg^{1L} (Nüsslein-Volhard et al., 1984) and the *wingless* reporter was *wg-lacZ* (Kassis et al., 1992) which is simultaneously a recessive lethal mutation in *wingless.* For the temperature-shift experiments, eggs were collected from a cross between *CyO/wg-lacZ* females and *en-lacZ/*wg^{1L} males. Positive X-gal staining in a wing disc but without the typical *engrailed* reporter pattern (Hama et al., 1990) identifies it as coming from the genotype *wg-lacZ/*wg^{1L}. The development of SMCs and proneural expression was examined in temperature-shift experiments where eggs were collected from a cross between *T(2;3) CyO/*wg^{1L}*;TM2/Ch Tb* females and *T(2;3) CyO/*wg^{CX4}*;neu-lacZ*^{B28}*/Ch Tb* males. Larvae and pupae of the wg^{1L}/wg^{CX4};*neu-lacZ*^{B28}/TM2 genotype can be distinguished from their 'Tubby' sibs. *neu-lacZ*^{B28} is an enhancer trap allele of *neu - ralised* (D. Clements and J. Merriam, unpublished observations) which shows an imaginal disc *lacZ* pattern identical to that of the A101 strain (Huang et al., 1991; Phillips et al., 1990; Clements and Phillips, unpublished observations). Except where otherwise referenced, genotypes are described in Lindsley and Zimm (1992).

In situ hybridisation and X-gal staining

In situ hybridization was performed by the digoxigenin method and used in combination with X-gal staining as described previously (Phillips et al., 1990). The template for probe synthesis was the *wingless* cDNA wg7121 (Cabrera et al., 1987).

Bromodeoxyuridine labelling of disc cells

Wandering 3rd instar larvae were selected by the appearance of mature salivary glands, characteristic of the end of the larval period, which are visible as large clear areas through the ventral body wall. They were injected with 10 mM bromodeoxyuridine (BrdU) in Ringers solution, kept for 2 hours and then assayed for β-galactosidase activity and BrdU incorporation. The anterior third of the animal was inverted in phosphate-buffered saline (PBS, pH 7.2) and fixed in PBS containing 4% paraformaldehyde for 5 minutes at 22°C. The sample was then stained in X-gal as described previously (Phillips et al., 1990), washed in PBS and postfixed in Carnoy's fixative for 15 minutes at 22°C. After rehydration, the sample was incubated for 1 hour at 22°C in mouse anti-BrdU monoclonal antibody with DNase (Amersham, RPN202). Subsequently bound antibody was detected using a biotinylated goat anti-mouse secondary antibody and avidin/biotin/horseradish peroxidase complex (Vector Laboratories). The heads were washed in PBS containing 1% bovine serum albumin (fraction V, Sigma) and 0.1% Triton X-100 (PBT), blocked in PBT plus 2% goat serum for 1 hour and then incubated in secondary antibody in PBT with serum at 4°C overnight. The samples were washed twice in PBT, twice in PBS with 0.1% Tween 20 (PBW) and incubated with avidin/biotin/horseradish peroxidase complex in PBW prepared according to the manufacturer's instructions. The samples were washed three times in PBW and then incubated 2 to 10 minutes at 22°C in PBW with 0.1% 3, 3′-diaminobenzidine and 0.05% hydrogen peroxide. The reaction was stopped by washing in PBW. Discs were dissected from the inverted heads and mounted in PBS plus 0.1% sodium azide for examination and photography using differential interference contrast microscopy.

A ratio of replication indices between the margin and the pouch of wing imaginal discs was calculated by dividing the proportion of cells that incorporate BrdU in the marginal region by the proportion in the pouch away from the margin. The margin was defined by expression of the *wg-lacZ* reporter. The sample was taken from the region of the pouch anterior to the M line (see Results) and distal to the distal ring of *wg-lacZ* expression. Significance was tested by Student's *t*-test after arc-sine transformation.

Immunohistological detection of achaete protein

Larvae and prepupae were dissected as for BrdU staining and fixed for 1 hour at 4°C in 0.1 M Pipes (pH 6.9), 1 mM EGTA, 2 mM $MgSO_4$ with 1% Triton X-100 and 1% formaldehyde (final concentration). Samples were washed and blocked for 2-4 hours at 22°C in PBT, incubated overnight at 4°C in PBT with 10% anti-Achaete mouse monoclonal supernatant (Skeath and Carroll, 1991), which was generously provided by M. Haenlin and P. Simpson. The samples were washed and primary antibody binding was detected as described for anti-BrdU immunohistology, except that the secondary antibody incubation was for 3 hours at 22°C and the staining buffer included 0.075% each of $CaCl_2$ and $NiCl_2$.

RESULTS

The transcription pattern of *wingless* in the third instar wing disc

The pattern of *wingless* expression is neither spatially uniform nor static during larval development. Our results confirm the principle elements of the transcription pattern of *wingless* in imaginal discs from wandering third instar larvae as shown by in situ hybridization and autoradiography (Baker, 1988a). A stripe of intensely labelled cells bisects the wing pouch (wp, Fig. 1A), which Baker correctly suggested might lie at the presumptive wing margin. The marginal sensory bristles form on either side of the wing margin, which is also the dorsoventral compartment border (Garcia-Bellido et al., 1973). The stripe of *wingless* expression (between arrowheads, Fig. 1A,B,D) is two cells wide. Simultaneous detection of *neu-lacZ* and *wingless* shows that the stripe lies between the SMCs of the dorsal and ventral rows of the marginal chemosensory bristles (drc, vrc in Fig. 1D). The cells in this stripe form the mechanoreceptor bristles of the anterior margin and the posterior marginal hairs (Hartenstein and Posakony, 1989). In the rest of the wing pouch, cells show an intermediate level of *wingless* expression. Parts of two rings of *wingless*-expressing cells can be resolved at the base of the wing

pouch in the presumptive hinge region (e.g. at arrows in Fig. 1A-D). The distal ring (Fig. 1D) includes SMCs of the giant sensillum of the radius (gsr) and the dorsal humeral crossvein sensillum (dhcv), and *wingless*-expressing cells extend from the ring near the anterioposterior border towards the SMCs of the vein 3 sensilla (Fig. 1D, open arrow). The SMCs of the sensilla of the dorsal radius (dr, Fig. 1D) and the ventral radius (arrowhead in Fig. 1C) are between the rings. A group of *wingless*-expressing cells surrounds the SMCs of the tegula (t, Fig. 1E). A proximal band of expression (3-5 cells wide) extends along the A/P border and anteriorly across the presumptive notum (n, Fig. 1A; see also B,C,E). The SMCs of the scutellar (sc, Fig. 1C) and posterior postalar (ppa, Fig. 1E) bristles are within this band while the dorsocentral bristles (dc) are on its proximal edge (Fig. 1B,E).

The RNA expression profile does not differ substantially at the third instar and prepupal stages, or in the embryo, from the pattern of β-galactosidase expression of the *wing-less* enhancer detector (*wg-lacZ*), stock 1-en-11 (Kassis et al., 1992; also compare Fig. 1A,D and Fig. 2C,D). We have therefore taken the latter to inform us of the *wingless* transcription pattern. The late third instar pattern (Fig. 2C) begins to emerge in the early third instar when the marginal stripe appears as a row of intensely stained cells in the otherwise uniform expression in the distal half of the wing disc ($III_{(1)}$; Fig. 2A). During the subsequent six hours of development, staining in four quadrants of the wing pouch decreases to leave a ring and cross, and at 12 hours into the third instar, the notal band of *wingless* expression has appeared in most wing discs ($III_{(2)}$; n in Fig. 2B). By 30 hours into the third instar ($III_{(3)}$) a group of cells on the presumptive tegula begin expressing, completing the final pattern.

The topological transformation that results from eversion of the wing disc can be appreciated by following the position of the principal components of the *wg-lacZ* pattern. During eversion, the dome-shaped wing pouch (Fig. 2C) is extended and flattened to form a mitten-like shape (Fig. 2D) moving the marginal stripe (arrowheads in Fig. 2C,D) from the face of the disc to the edge and bringing the dorsal (●) and ventral (★) arcs of the rings (Fig. 2C,D) into superposition. The presumptive tegula (t, Fig. 2D) lies on the edge of a deep sulcus which separates the wing from the notum. This topology and staining pattern is maintained until 80 hours after pupariation when the cells of the wing disc have secreted adult cuticle and the bristles of the notum are visible (Bainbridge and Bownes, 1981). This permits direct comparisons between the position of *wg-lacZ* expression and the sites of sensory organs, which confirm and extend the relationships shown in the wing disc. In addition to the scutellar (sc) and dorsocentral (dc) bristles, the posterior postalar (ppa) bristle forms in the notal band of *wg-lacZ* expression (Fig. 2E). The epithelium that constitutes the wing hinge is elaborately folded and the cells are densely packed, which contributes to its complexity. The proximal ring extends from the base of the costa across the proximal tip of the radius, distal to the axillary sclerites, ending at the axillary cord and the distal ring extends from the medial costa across the humeral crossvein to the distal radius and then to the base of the alula (Fig. 2F). A branch of expression along vein 3 near the A/P border reaches the anterior crossvein (acv), and the posterior crossvein also stains (pcv, Fig. 2G). The chemosensory bristles of the anterior margin, which lie on the edge of the marginal stripe, also express and can be seen against the background of weaker staining from underlying cells (arrowheads in Fig. 2G).

The late pattern of *wingless* expression in wing discs suggests that it has a local role in cell behaviour which might be separable from the earlier, more global effect on the distal half of the disc (Phillips and Whittle, unpublished observation). We therefore followed the consequences for imaginal cuticular differentiation of interrupting *wingless* activity at selected developmental stages using a temperature-sensitive genotype.

wingless function and bristle pattern

A maximum of five of the eleven bristles of the notum is sensitive to non-permissive conditions in the conditional *wingless* genotype, *wg-lacZ*/wg^{IL} called *wg-ts*. When shifted up before the third instar, *wg-ts* larvae formed pharate adults lacking specific bristles on the notum including the posterior postalar, the anterior and posterior dorsocentral and scutellar bristles (compare Fig. 3B with the wild-type notum in Fig. 3A). The anterior dorsocentral bristle has a very strong temperature dependence in this genotype, with a sharp transition between stages $III_{(3)}$ and $III_{(w)}$ (Fig. 4A). The posterior postalar bristle (ppa) is most sensitive to decrease in *wingless* activity. The ppa bristles are lost in some *wg-ts* individuals even at the permissive temperature, and up-shifts as late as the wpp stage still significantly increase the penetrance of this phenotype (Fig. 4B). However, like the dorsocentral and scutellar bristles, the ppa bristles are not rescued by down-shift after the late IIIrd instar, indicating a *wingless* requirement at this stage. The anterior scutellar bristle (asc) is deleted with high penetrance by up-shifts through the beginning of the IIIrd instar, and a significant level of sensitivity persists through the $III_{(3)}$ stage (Fig. 4C). The frequency of loss of the posterior dorsocentral (pdc) and scutellar (psc) bristles is much lower and more variable in this *wg-ts* genotype, but these bristles are completely absent in wg^{ts}/wg^{CX4} as discussed later. After up-shift in the $III_{(1/2)}$ stage, the number of psc bristles formed is 0.78 of the wild-type number, which increases to 0.94 after shifts in the $III_{(2)}$ stage, while for the pdc bristles the fraction increases from 0.94 to 1.00 over the same period. Shifts before the IIIrd instar do not increase penetrance of this phenotype. These five sensitive bristles form from SMCs that arise within or adjacent to *wingless*-expressing cells of the notal band and the decreased sensitivity of asc, psc and pdc bristles coincides with the beginning of *wingless-lacZ* expression in the notum.

wingless requirement during the formation of the wing margin

The sensory bristles of the wing margin in the *wg-ts* genotype are temperature sensitive during the late third instar and early prepupal stages. Up-shifts prior to the wpp stage delete bristles of the triple row (tr) and double row (dr), and down-shifts from the $III_{(w)}$ stage onwards fail to rescue the normal margin bristle pattern (Fig. 5). When *wg-ts* larvae are held at the non-permissive temperature through-

out the period from the middle third instar ($III_{(2)}$) to the bpp stage, they develop normal-sized wing blades with wild-type venation but are devoid of marginal structures save an occasional bristle or two of the medial tr or dr located at the distal extremity of the wing blade (arrowhead, Fig. 3D). More bristles in the proximal margin are formed following up-shifts at progressively later stages after $III_{(2)}$. The posterior row hairs and the long hairs of the alula reappear after down-shift in the $III_{(3)}$ stage but are still sensitive to up-shift during the bpp stage (data not shown).

wg-lacZ reporter gene expression is similar to the wild-type pattern until after the prepupal stages, when *wg-ts* larvae are up-shifted at the end of the second instar (compare Figs 2C and 6B). It therefore follows that, although this period includes the temperature-sensitive period for the

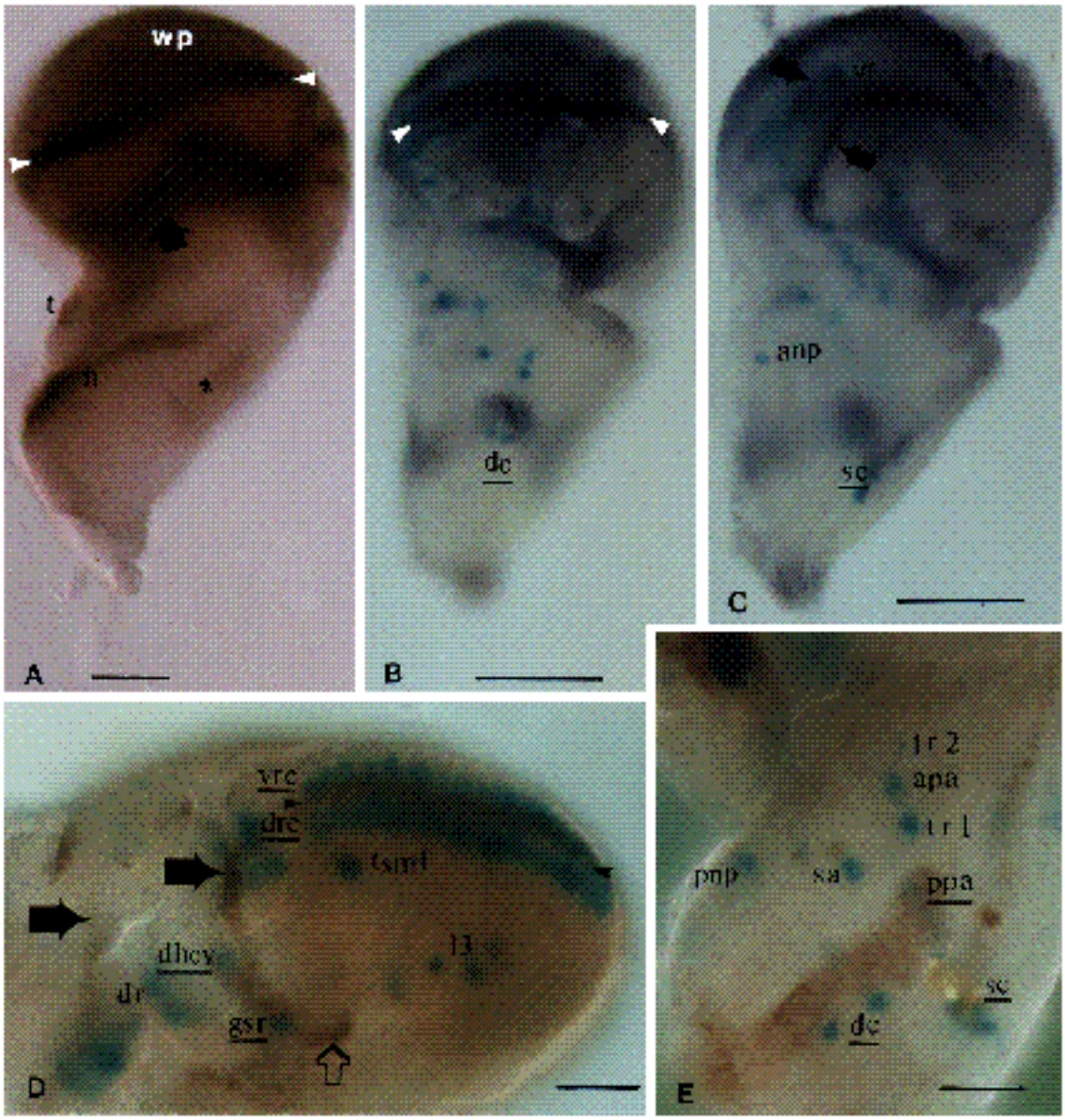

Fig. 1. Expression of *wingless* RNA in wing discs. The wild-type pattern of *wingless* expression (brown in A, D and E; purple in B, C) is shown in a wandering third instar wing disc (A) and relative to the position of *neuralised-lacZ*-stained SMCs (blue-green in B-E) in discs from wandering third instar larvae (B, C) and prepupae (D, E). SMCs of most *wingless*-sensitive sensilla (labels underlined) including the chemosensory bristles of the anterior wing margin (between arrowheads in B, D), anterior and posterior scutellar bristles (sc in C, E), anterior and posterior dorsocentral bristles (dc in B, E) and posterior postalar bristle (ppa in E) lie in or adjacent to regions of high *wingless* expression while the insensitive bristles are in regions of low expression (anterior notopleural bristle, anp, in C; posterior notopleural, pnp, anterior and posterior supraalars, sa, tricoid sensilla 1 and 2, tr1and tr2, and anterior postalar, apa in E). SMCs of the giant sensillum of the radius (gsr) and the sensillum of the dorsal humeral crossvein (dhcv in D) lie in the distal ring of *wingless* expression and are variably sensitive in the *wingless* genotypes that we have examined. The SMCs of the sensilla of the dorsal and ventral radius (vr in C; dr in D) are between the proximal and distal rings of *wingless* expression. The bar represents 100 μm in A, B and C, 50 μm in D and 20 μm in E.

effects on the wing margin and bristles, concurrent *wingless* activity is not required for cellular viability or maintenance of *wingless* transcriptional regulation in the *wingless*-expressing cells.

The relationship between cell proliferation at the presumptive DV border and *wingless* expression

The pattern of cell division in the wing disc immediately before pupariation is not uniform (O'Brochta and Bryant,

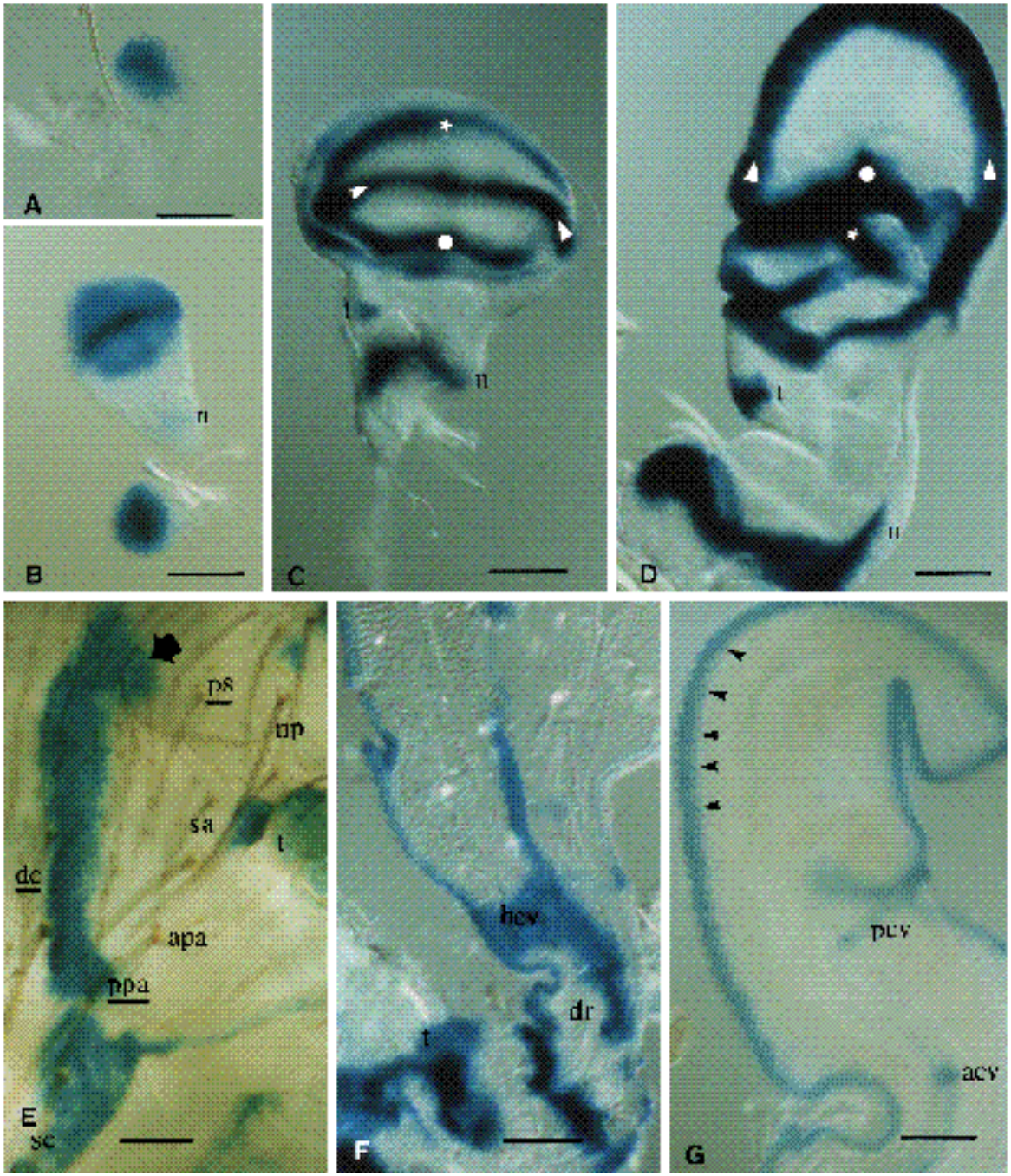

Fig. 2. *wingless-lacZ* expression (blue-green) in wild-type wing discs and its derivatives at various stages. (A) In the wing disc from an early third instar larva ($III_{(1)}$), the marginal stripe and pouch ring begin to emerge from the background of uniform staining which was characteristic of the distal half of the second instar wing. After 12 hours ($III_{(2)}$), the marginal stripe is distinct and the notal stripe (n) appears (B). During metamorphosis, the pattern of expression does not change but the topology of the wing pouch is transformed such that the marginal stripe (arrowheads) across the pouch in the wandering third instar disc (C) is moved to the edge of the everted prepupal disc (D). *wingless-lacZ* expression is still detectable in pharate individuals when the adult cuticular structures begin to tan. Thus the relative position of SOs (abbreviations as in Fig. 1) and regions of *wingless* expression can be compared directly in the notum (E), hinge (F) and proximal wing blade (G). The *wingless*-sensitive bristles (labels underlined) are located in or adjacent to regions of *wingless* expression. One exception to this correlation is the presutural bristle (ps in E) which is several cell diameters anterior to the notal *wingless* stripe but shows sensitivity under some conditions (see text). The bar represents 100 μm.

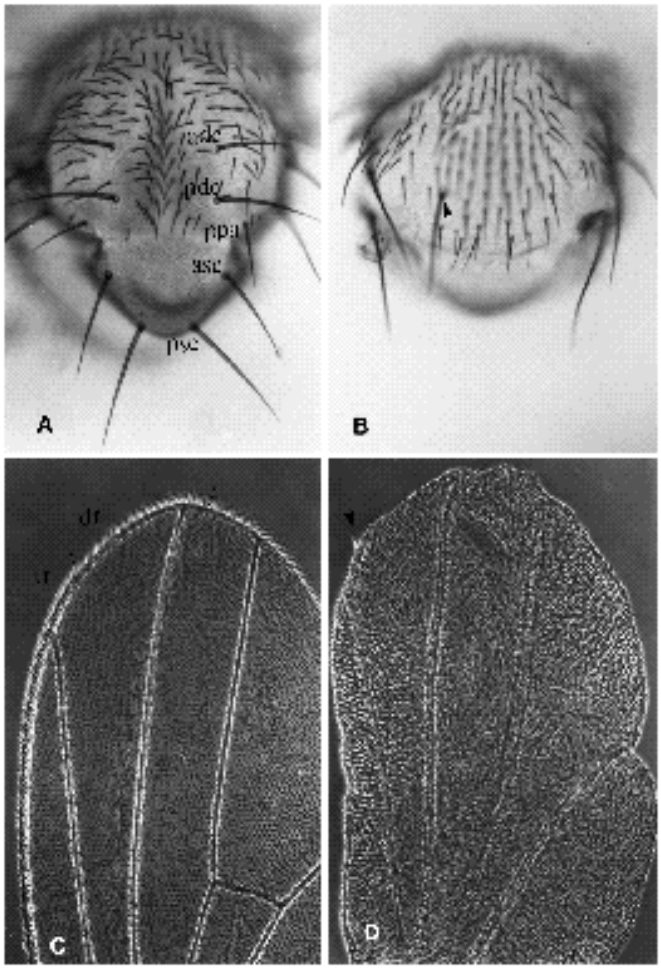

Fig. 3. Cuticular phenotypes of *wg-ts* flies reared at non-permissive temperature during the third instar and pupal stages. (A) The wild-type notum shows the disposition of the macrochatetes affected in *wg-ts* flies: anterior and posterior scutellars (asc and psc), the anterior and posterior dorsocentrals (adc and pdc) and the posterior postalars (ppa). Compare to a *wg-ts* notum (B) in which one dorsocentral remains, and the postalars and scutellars are absent. (C) A wild-type wing shows the triple row (tr) and double row (dr) marginal bristles whereas the *wg-ts* wing (D) which has been dissected from a pharate adult is entirely devoid of margin bristles save one (arrow).

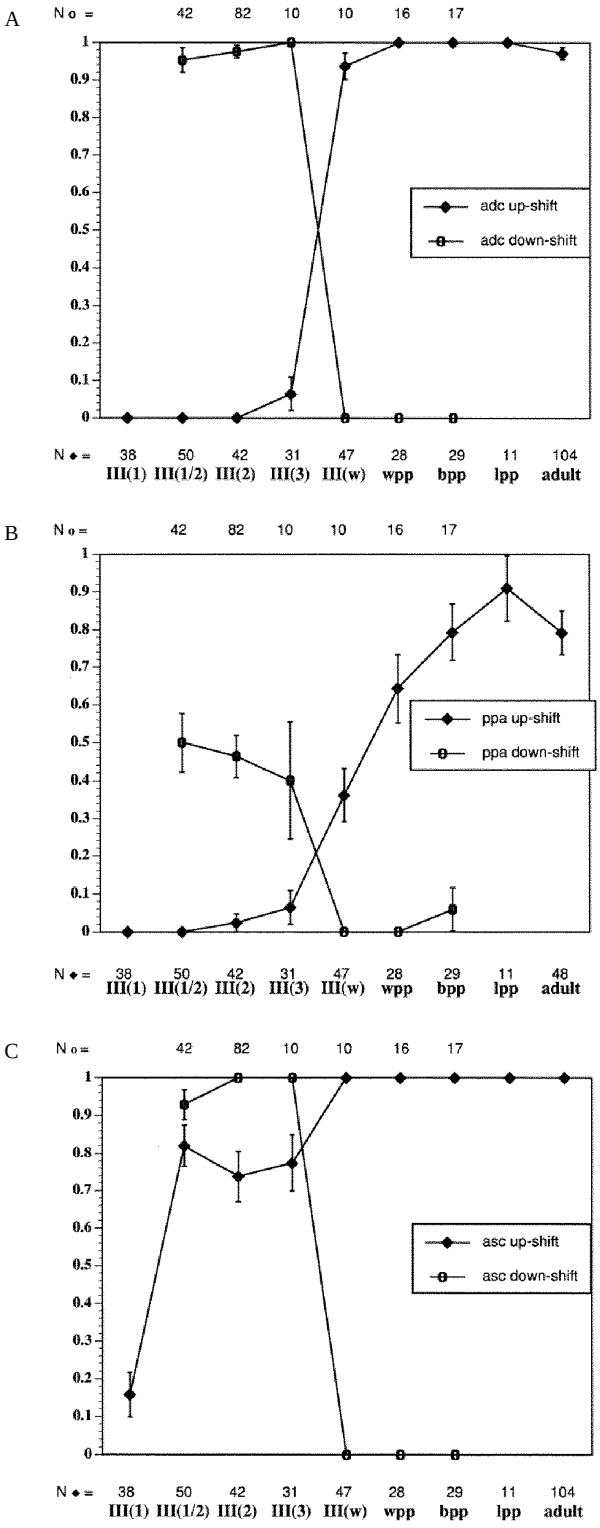

Fig. 4. Stage dependence of bristle temperature sensitivity in *wg-ts* flies. The frequency of adc (A), ppa (B) and asc (C) bristles per hemi-notum (ordinate) varies from 0 to 1, the wild-type number, depending upon stage of development at temperature shift (abscissa). Samples of each culture were killed and stained with X-gal at the time of the shift to determine the developmental stage of the population of *wg-ts* larvae. The number of hemi-nota examined for down-shift (○) and for up-shift (◆) at each stage is given above ($N_{○}$) and below ($N_{◆}$) the graph, respectively.

1985; Schubiger and Palka, 1987). Regions of mitotic quiescence are associated with the site of specification of SMCs (Hartenstein and Posakony, 1989; Usui and Kimura, 1992). As their immediate neighbours stop replication, the SMCs initiate a specialized series of mitoses to produce the sensory organ.

At the presumptive wing margin, this pattern of mitotic regulation is dependent upon *wingless* activity. In wing discs from larvae of the *wg-lacZ*/*en-lacZ* genotype (*wg*+) that are close to pupariation (late $III_{(w)}$ stage), fewer marginal cells, which express *wg-lacZ*, show BrdU incorporation than in the rest of the pouch (Fig. 6A between arrowheads). The change in labelling often extends several cells dorsally beyond the region of *wingless* expression. In discs from sibling *wg-ts* larvae reared at the non-permissive tem-

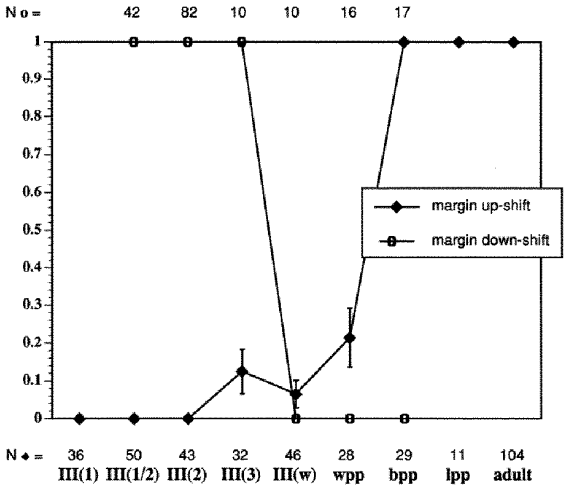

Fig. 5. Stage dependence of wing margin bristle sensitivity in *wg-ts* flies. As in Fig. 4, except that the frequency of wings with complete costa, triple row and double row per wing is plotted (ordinate).

perature during the IIIrd instar, suppression of replication is reduced (Fig. 6B). Discs from larvae reared at the restrictive temperature from the end of the second instar were staged by the presence of a region of reduced replication associated with the presumptive third vein (the M line), which forms just after division stops at the presumptive margin (Schubiger and Palka 1987; Hartenstein and Posakony, 1989). The ratio of replication indices between the margin and the pouch in *wg-ts* discs was five times greater than the ratio in *wg+* discs (Students *t*, $P<0.01$).

Birth of SMCs in *wingless*-deficient wing discs

SMCs arise at fixed locations and times during wild-type imaginal disc development as judged by activation of a *lacZ* reporter in the *neuralised* gene (Huang et al., 1991). We used the null allele wg^{CX4} (Baker, 1987) in the genotype wg^{ts}/wg^{CX4} instead of *wg-lacZ* so that the expression of the *neu-lacZ* reporter would not be obscured. This allele shows a more severe phenotype than *wg-lacZ* in combination with wg^{ts} or with other viable alleles of *wingless* (Phillips, unpublished). The SMCs of the *wingless*-sensitive SOs are not formed in discs from wg^{ts}/wg^{CX4} larvae that have been cultured at the non-permissive temperature during the III instar. The SMCs of most sense organs that are unaffected by the *wg-ts* genotype, including anp, pnp, psa, tr1 and apa, are present in the wing disc of the wg^{ts}/wg^{CX4}; *neu-lacZ* wandering third instar larvae (arrowheads on presumptive notum, Fig. 7B) or appear after pupariation (asa, data not shown). However, the SMCs of the *wingless*-sensitive SOs (dc, sc and ppa) are absent. The SMCs of the chemosensory bristles of the margin appear within a triangle formed by the SMCs of the sensilla of the anterior crossvein and ventral sensilla of vein 3 and the campaniform sensillum of the margin (tsm1) in discs from *wg+* sibs (arrowheads on wing pouch, Fig. 7A) but are absent in wg^{ts}/wg^{CX4} discs (Fig. 7B). The pdc and psc are always missing in this genotype (n=12) although in wild-type flies they are amongst the first SMCs formed (Huang et al., 1991). These SMCs could not be found at any time during the third instar or prepupal stages. In addition, the SMC of the ps bristle, which is unaffected in the *wg-ts* genotype, is sometimes absent in wg^{ts}/wg^{CX4} discs although the asa bristle, which normally forms later, is present (data not shown). The SMCs of the dhcv and gsr are also sometimes affected.

wingless function and *achaete* expression

Each sensory organ of the wing and notum is formed by the progeny of an SMC arising amongst the cells of a proneural cluster. The dorsocentral bristles require *achaete* activity while the recurved bristles of the margin and the rest of the *wingless*-dependent bristles of the notum require *scute* activity (reviewed in Ghysen and Dambly-Chaudière, 1988). However *achaete* and *scute* cross-activate (Martinez and Modolell, 1991; Skeath and Carroll, 1991; Cubas et al., 1991). The distribution of Achaete protein is therefore a measure of proneural activity during the formation of both *achaete* and *scute*-dependent sensory organs. We have followed this distribution in wg^{ts}/wg^{CX4}, *wg+* and *wg-ts* wing discs after development at the restrictive temperature using a monoclonal antibody against the Achaete protein (Skeath and Carroll, 1991). Wing discs from *wg+* wandering third instar larvae showed an age-dependent pattern as previously described (Skeath and Carroll, 1991; Cubas et al., 1991), always including two parallel rows of Achaete-expressing cells corresponding to the precursors of the chemosensory bristles of the anterior margin. In contrast, in *wg-ts* or wg^{ts}/wg^{CX4} wing discs, we found only occasional isolated clusters of Achaete-expressing cells near the presumptive margin (Fig. 8B) which probably correspond to the ventral sensillum of vein three and the acv (compare with Fig. 7B). In wg^{ts}/wg^{CX4} discs, there is no Achaete staining where the dorsocentral and scutellar clusters would normally form (open arrow in Fig. 8B, compare to 8A), proximal to the proneural clusters of the sense organs which do not depend upon *wingless* (namely the anp, pnp, psa and apa bristles and the trichoid sensillum 1, Fig. 8B, arrowheads shown in the notum). Achaete protein is also reduced in this area in *wg-ts* discs (data not shown) but SMCs of the pdc and psc appear as isolated stained cells.

DISCUSSION

The correct spatial pattern of differentiation in an epithelium like that in the wing imaginal disc will include signalling events between adjacent cells which constrain the possible alternative fates that they can assume (Greenwald and Rubin, 1992). We have correlated the precision of the pattern of *wingless* transcription with the assignment of particular cell fates in the presumptive wing margin and for peripheral sense organs on the notum, and we propose that the *wingless* protein mediates changes in the competence of cells to undergo fate restrictions including commitment to form particular sense organs.

The position of the *wingless* product in the hierarchy of events establishing single SMCs in 'proneural' clusters.

Cells of the peripheral sensory organs and of the central nervous system follow a similar developmental programme including progressive specification of precursor cells fol-

lowed by differentiative proliferation. Sensory organs of the imaginal thorax and head, excepting the eye, derive from precursor cells called sensory mother cells (SMCs) in the imaginal disc epithelia. Most thoracic sensory organs, excepting the mechanosensory bristles of the wing margin, depend either on *scute* or *achaete* (reviewed by Ghysen and Dambly-Chaudière, 1988). Transcription of one of these genes is intitiated in each proneural cluster, and then, in the presence of active product, transcription of both genes is enhanced by autoactivation and cross-regulation (Romani et al., 1989; Cubas et al., 1991; Skeath and Carroll, 1991; Martinez and Modolell, 1991). One cell in the cluster becomes the SMC concomitant with a further increase in *achaete* (*ac*)/*scute* (*sc*) expression, then the neurogenic gene *neuralised* is activated in this cell whilst *ac*/*sc* expression in adjacent cells of the cluster is repressed.

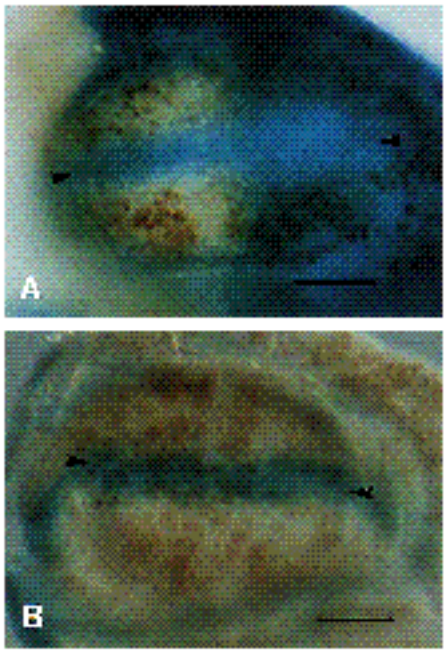

Fig. 6. DNA replication in *wg*+ and *wg-ts* wing discs reared at non-permissive temperature during the third instar. Cells near the presumptive margin stop replication as assayed by BrdU incorporation (brown) shortly before pupariation in *wg*+discs (A) but not in *wg-ts* discs (B). The zone of non-proliferating cells includes the stripe of *wingless-lacZ*-expressing cells (blue-green, between arrowheads in A). The marginal stripe is present in *wg-ts* discs (blue-green, between arrowheads in B) but many of these cells are replicating. The bar represents 50 μm.

A transcriptional regulatory mechanism is implicated in the specification of the SMCs (reviewed in Campuzano and Modolell, 1992). The distribution of multiple stimulatory and inhibitory factors including the products of *ac*, *sc* and *extramacrochaetae* (*emc*) influence the pattern of SMCs (Romani et al., 1989; Cubas et al., 1991; Skeath and Carroll, 1991; Cubas and Modolell, 1992). *achaete* and *scute* encode DNA-binding helix-loop-helix (bHLH) proteins (referred to here as AC and SC respectively) which associate with other bHLH proteins in heterodimers to promote DNA binding and transcriptional activation (Villares and Cabrera, 1987; Murre et al., 1989; Cabrera and Alonzo, 1991). The Extramacrochaetae product is an HLH protein lacking a DNA-binding domain which inhibits AC/SC DNA binding and bristle formation (Van Doren et al., 1991; Garrell and Modolell 1990; Ellis et al., 1990).

We find that *wingless* is necessary to specify a subset of the wing and notum sensory organs which arise in or adjacent to *wingless*-expressing cells. Inactivation of *wingless* suppresses the earliest manifestations of sensory organ development but this block is reversible until the stage when the SMCs would normally be established. The timing and developmental phenotype are compatible with a role in regulation of AC/SC activity. Without *wingless*, development of *wingless*-sensitive sensory organs is arrested before SMC formation, as judged by *neuralised-lacZ* expression, and AC is not detectable either in single intensely labelled cells characteristic of SMCs or in proneural clusters in the region of the affected sense organs. Either *wingless* is required upstream of the transcriptional activation of the *ac* and *sc* genes or in the process of auto/cross-regulation which amplifies *ac/sc* expression in the proneural cluster.

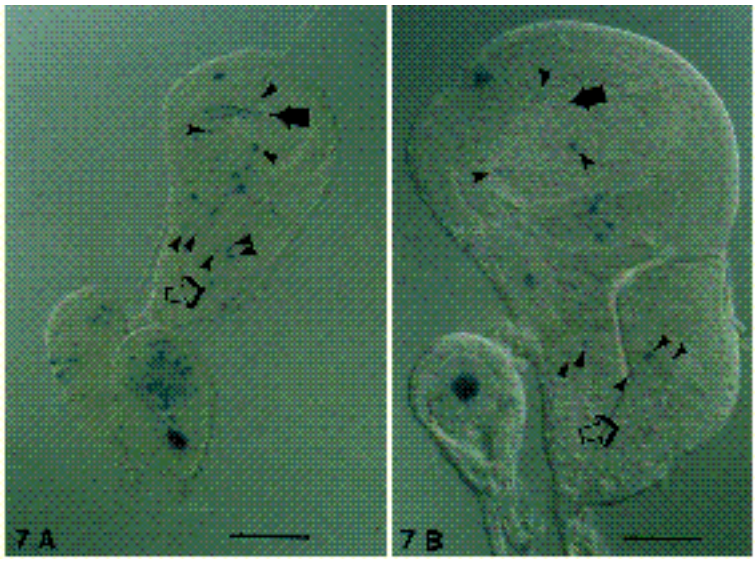

Fig. 7. *neuralised-lacZ* expression in wing discs from wg^{+};*neu-lacZ*(A) and wg^{ts}/wg^{CX4}; *neu-lacZ* (B) white prepupae reared at non-permissive temperature during the third instar. SMCs of *wingless*-insensitive bristles (arrowheads in A and B) are present in both genotypes while the SMCs of *wingless*-sensitive bristles are present only in the wg^{+} disc (compare region marked by arrows in A and B). The bar represents 100 μm in A and 50 μm in B.

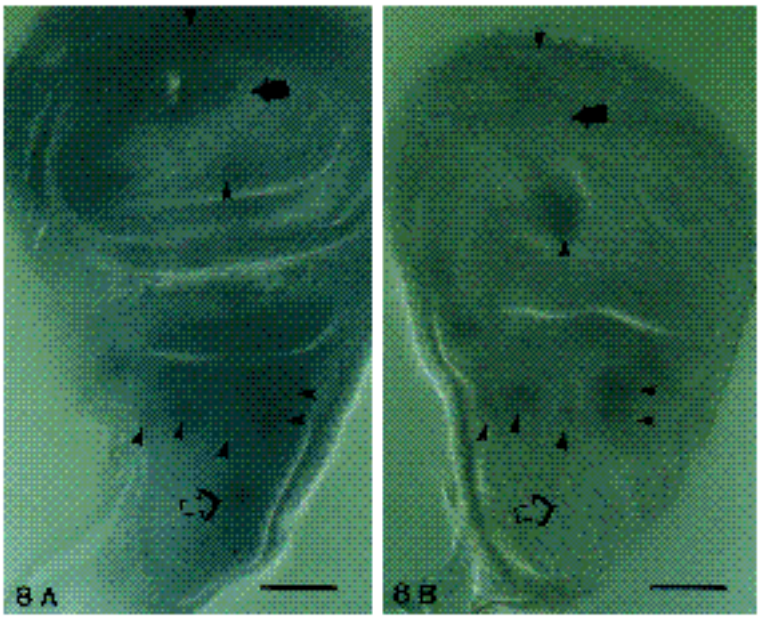

Fig. 8. Achaete protein expression in wing discs from wild-type and wg^{CX4}/wg^{ts} wandering third instar larvae reared at non-permissive temperature during the third instar. Like the wild-type disc (A), the wg^{CX4}/wg^{ts} disc (B) shows *achaete* expression in the distal half of the presumptive notum corresponding to the proneural clusters of the anp, pnp, psa and apa bristles and tr1 (arrowheads in A and B). However, more proximally, the staining corresponding to the dc and sc proneural clusters is absent in the wg^{CX4}/wg^{ts} disc, but present in wild-type (open arrow). In the presumptive wing, proneural clusters of the anterior crossvein and the l3v sensilla are present in both discs (arrowheads in A and B), while staining associated with precursors of the marginal bristles are present only in the wild-type disc (solid arrow in A). The bar represents 50 μm.

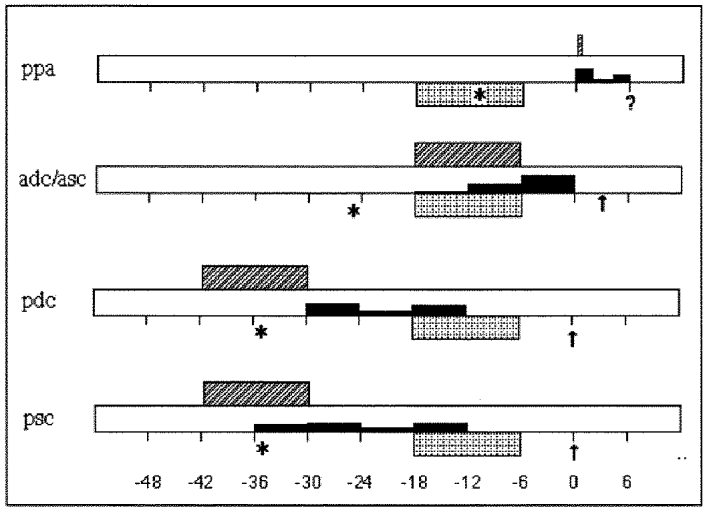

Fig. 9. Correlation between timing of bristle development and *wingless* requirement. Developmental parameters are shown for *wingless*-sensitive bristles including those that develop early (psc and pdc), at an intermediate (asc/adc combined) and late (ppa) stage. The percentage of SMCs born during each 6 hour period (Huang et al., 1991) is indicated by the step graph ( ) and the initial differential divisions by an arrow (↑; *ibid.*) or a question mark if not determined (?). The periods of the latest effective upshift and downshift of the *wg-ts* genotype are shown, respectively, above ( ) and below ( ) the step graph for each bristle. The earliest stage at which transcription of *scute* or *achaete* has been observed in proneural clusters that are associated with these SOs (Cubas et al., 1991) is indicated by an asterix (*).

At present, we cannot exclude either of these hypotheses, but we discuss later several observations that suggest that *wingless* might regulate AC and SC post-transcriptionally. We have summarised our own and other published data on the events during the progress of SMC formation in Fig. 9.

Correlation of *wingless*-temperature sensitivity with bristle development

The requirement for *wingless* activity in the development of *wingless*-dependent SOs can be satisfied by expression during or shortly before the period of SMC specification as defined by *neuralised-lacZ* expression but not by earlier activity (Fig. 9). For the asc, adc and ppa bristles, this period is substantially later than the beginning of proneural expression in the surrounding cluster of cells (indicated by * in Fig. 9; Cubas et al., 1991; Cubas and Modolell, 1992). Therefore *wingless* is required after the initiation of proneural gene transcription. The limit to its ability to rescue the formation of particular bristles by down-shift appears to anticipate the beginning of differentiation in that the last effective down-shift for each sensory organ ends 12-15 hours before the first SMC division (Fig. 9). The fact that *wingless* activity can rescue sensory organ development until the end of the normal period of psc and pdc sensory organ specification makes clear the persisting role for *wingless* in proneural expression. The *wingless* product is required continuously to enhance the activity of factors that promote proneural expression.

Possible antagonism by kinase activity in regions of *wingless* expression

When we compare the *wingless* bristle phenotype to that of other mutations that affect thoracic sensory organs, there is only one similar pattern. The 'pecking order' of loss of thoracic bristles after interrupting *wingless* is not the same as that in mutations of *achaete, scute, Enhancer of split, extra-macrochaetae, Notch, Delta* or *Hairless* (de Celis et al., 1991). The *wingless*-sensitive bristles do not depend upon a common set of proneural cluster-specific regulatory regions in the *scute* and *achaete* genes (Ruiz-Gómez and

Modolell, 1987; Leyns et al., 1989). However, the pattern closely resembles the spatial requirement for *zw3-sgg*. Clones homozygous for embryonic lethal alleles of *zw3-sgg* (*sgg*$^-$) induced during larval development produce supernumerary sensory organs throughout the wing blade and around some bristles of the notum. In these mosaics, most of the extra notal bristles arose close to the dorsocentral and scutellar bristles, others were around the posterior postalar and the presutural bristles but far fewer were close to the other notal bristles (Simpson and Carteret, 1989). *sgg*$^-$ clones in the wing blade form triple row bristles, double row bristles or posterior row hairs depending upon their position in the wing (Simpson et al., 1988). *zw3-sgg* activity suppresses ectopic development of bristles which are normally dependent upon *wingless* activity in regions where *wingless* is expressed. The spatial correspondence between *wingless* expression and the pattern of ectopic bristles formed by *sgg*$^-$ mosaics becomes more striking in the absence of the repressive effect of *extramacrochaetae* (Simpson and Carteret, 1989). Under these conditions, ectopic bristles in *sgg*$^-$ clones cover the entire area of the notal *wingless* stripe including the area anterior to the dorsocentrals and extend into the prescutum (Fig. 2E, arrow), without increasing the number of bristles outside this region. Simpson and Carteret suggest that *zw3-sgg* is acting during lateral inhibition, but Blair (1992) has shown that *sgg*$^-$ clones do induce ectopic regions of *achaete* expression in the anterior wing blade, a region where neither *achaete* nor *scute* are normally detectable. We propose that *zw3-sgg*, the *Drosphila* homologue of the mammalian serine kinase GSK3 (Bourouis et al., 1990; Siegfried et al., 1990, 1992; Woodgett, 1991), antagonises a *wingless*-dependent activation of the proneural genes. This might occur by modification of the protein products of these genes or by modification of a common activator of these proteins, for example, *daughterless* (Dambly-Chaudière et al., 1988). We are now testing the genetic and molecular interactions between *wingless* and *zw3-sgg*. Independent evidence for a kinase-mediated regulation of AS-C gene activity has been reported by Cabrera (1990, 1992). Antibodies were raised against synthetic peptides from *lethal-of-scute* and *achaete* which contain consensus phosphorylation sites for casein kinase II. These antibodies only recognize protein in the sensory mother cells although other antibodies show that the protein is present throughout the proneural cluster (Martin-Belmudo et al., 1991) suggesting that the proteins might be phosphorylated when less active.

The antagonism between *wingless* and *zw3-sgg* activity that we have identified in imaginal disc cells also exists during embryonic segmentation and may be a general characteristic of *wingless* signal transduction. The embryonic cuticular phenotypes of *zw3-sgg* and *wingless* are opposites; the former has no ventral denticle bands, retaining only the 'naked' regions (Perrimon and Smouse, 1989), whilst *wingless* mutants have a lawn of denticles (Nüsslein-Volhard et al., 1984). Heat-shock-induced ectopic expression of a *wingless* cDNA causes stable ectopic expression of *engrailed* and *wingless*, and a 'naked' phenotype (Noordermeer et al., 1992). Ectopic *wingless* activity is also responsible for this phenotype in the mutation *naked* (Dougan and DiNardo, 1992). Siegfried et al. (1992) have shown that *zw3-sgg* acts as a repressor of *engrailed* autoactivation and that *wingless* inactivates this function.

The role of *wingless* in modulation of cell proliferation at the presumptive DV margin

The proposed relationship between sensory organ development and changes in cell cycle regulation (Garcia-Bellido and Merriam, 1971; Hartenstein and Posakony, 1989; Huang et al., 1991) has been confirmed by simultaneous detection of BrdU incorporation and of the *neuralised-lacZ* reporter activity (Usui and Kimura, 1992). We find *wingless* is necessary for the normal decrease in cell proliferation at the wing margin. The murine *Wnt1* was originally identified on the basis of its oncogenic phenotype in mouse mammary tissue (Nusse and Varmus, 1982). In addition *wingless* is required for cell proliferation in the primordia of the fly Malpighian tubules (Skaer and Martinez Arias, 1992). In these cases, activity results in increased proliferation. However, over-expression of *Wnt1* does not increase proliferation in most other cell types (unpublished data of Shackleford and Varmus, reported in Nusse and Varmus, 1992). Like other growth factors (Sporn and Roberts, 1988), the response to *Wnt* signals appears to be context-dependent. Regulation of proliferation in the MQC cells is not dependent upon *scute* or *achaete* activity (Usui and Kimura, 1992). Therefore continued proliferation at the margin in the absence of *wingless* is independent of decreased *ac*/*sc* activity. The converse causal relationship, that proneural expression is dependent upon cessation of cell division, has not been tested.

Non-autonomy of *wingless* in clones and its 'local' action

We have presented evidence for a spatially localised role of *wingless* in the late stages of imaginal disc patterning. Mosaics of the wg^1 allele showed that the earlier global role of *wingless* in distalization of imaginal discs was not cell-autonomous (Morata and Lawrence, 1977) but we know that wg^1 complements *wingless* alleles that interrupt wing margin integrity (Phillips, unpublished). Mosaics homozygous for the lethal allele wg^{L2} induced during embryogenesis were associated with losses of wing margin (Baker, 1988b) but clones initiated postembryonically showed no such effect. This apparent contradiction may be resolved either if the perdurance of *wingless* after embryonic expression is long or if there is local non-autonomy at the presumptive DV border, because mosaics initiated after the DV compartmentation event (Garcia Bellido et al., 1973) would not interrupt the *wingless* stripe of expression in both compartments simultaneously.

wingless and positional cues in sense organ patterning

We interpret our experiments as evidence that *wingless* participates in the transduction of positional cues that arise in the main axes of the disc. *wingless* activity is necessary to establish the locations at which particular SMCs form. However, it is not a global signal, because it is not necessary for all SMCs or for the normal veination pattern. The *wingless*-sensitive SOs arise in very precise positions relative to *wingless* expression suggesting that their siting might

be *wingless*-concentration dependent. For instance, the dorsocentral bristles develop just outside the region of intense *wingless* transcription whilst most of that proneural cluster is distal to the SMCs (Cubas et al., 1991) and therefore within the *wingless* stripe of expression. Either *wingless* or another gene coordinately regulated with *wingless* may inhibit SMC formation within the stripe. *wingless* expression in the mature wing disc can be characterized as a series of concentric rings or arcs centred at the distal-most point of the disc. We propose that *wingless* acts as a link between proximodistal patterning processes, which determine its own expression pattern, and specification of part of the SO pattern. Because *wingless* can provide either a paracrine or an autocrine signal (Nusse and Varmus, 1992), this linkage would extend the influence of the cell-autonomous transcription factors which regulate *wingless* to nearby cells providing a gradient of information for positioning sensory organs.

We acknowledge project grant support from the Science and Engineering Research Council of Great Britain, and are grateful to M. Haenlin and P. Simpson for the gift of the *achaete* antibody, and to J. Kassis for the *wg-lacZ* reporter stock.

REFERENCES

Bainbridge, S. P. and Bownes, M. (1981). Staging the metamorphosis of *Drosophila melanogaster*. *J. Embryol. Exp. Morph.* **66**, 57-80.

Baker, N. E. (1987). Molecular cloning of *wingless*, a segment polarity gene in *Drosophila:* the spatial distribution of a transcript in embryos. *EMBO J.* **6,** 1765-1773.

Baker, N. E. (1988a). Transcription of the segment-polarity gene *wingless* in the imaginal discs of *Drosophila*, and the phenotype of a pupal-lethal *wg* mutation. *Development* **103,** 289-298.

Baker, N. E. (1988b). Embryonic and imaginal requirements for *wingless*, a segment-polarity gene in *Drosophila. Dev. Biol.* **125,** 96-108.

Blair, S. S. (1992) *shaggy (zeste-white 3)* and the formation of supernumerary bristle precursors in the developing wing blade of *Drosophila. Dev. Biol.* **152,** 263-278.

Bourouis, M., Moore, P., Ruel, L., Grau, Y., Heitzler, P. and Simpson. P. (1990). An early embryonic product of the gene *shaggy* encodes a serine/threonine protein kinase related to the CDC28/cdc2$^+$ subfamily. *EMBO J.* **9**, 2877-2884.

Cabrera, C. V. (1990). Neuroblast determination and segregation in *Drosophila:* the interactions between *scute*, *Notch* and *Delta*. *Development* **109,** 733-742. (reprinted in volume **110 (1).)**

Cabrera, C. V. (1992). The generation of cell diversity during early neurogenesis in *Drosophila. Development* **115,** 893-901.

Cabrera, C. V. and Alonso, M. C. (1991). Transcriptional activation by heterodimers of the *achaete-scute* and *daughterless* gene products of *Drosophila. EMBO J.* **10**, 2965-2973.

Cabrera, C. V., Alonso, M. C., Johnston, P., Phillips, R. G. and Lawrence, P. A. (1987). Phenocopies induced with antisense RNA identify the *wingless* gene. *Cell* **50**, 659-663.

Campuzano, S. and Modolell, J. (1992). Patterning of the *Drosophila* nervous system: the *achaete-scute* gene complex. *Trends Gen.* **8**, 202-208.

de Celis, J. F., Mari-Beffa, M. and Garcia-Bellido, A. (1991). Function of trans-acting genes of the *achaete-scute* complex in sensory organ patterning in the mesonotum of *Drosophila. Roux's Arch. Dev. Biol.* **200,** 64-76.

Cubas, P., deCelis, J-F., Campuzano, S. and Modolell, J. (1991). Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. *Genes Dev.* **5,** 996-1008.

Cubas, P. and Modolell, J. (1992). The *extramacrochaetae* gene provides information for sensory organ patterning. *EMBO J.* **11,** 3385-3393

Dambly-Chaudière, C., Ghysen, A., Jan, L. Y. and Jan, N. J. (1988). The determination of sense organs in *Drosophila*: interaction of *scute* with *daughterless. Roux's Arch. Dev. Biol.* **197,** 419-423.

DiNardo, S., Sher, E., Heemskerk-Jongens, J., Kassis J. and O'Farrell, P. H. (1988). Two-tiered regulation of spatially patterned *engrailed* gene expression during *Drosophila* embryogenesis. *Nature* **332,** 604-609**.**

Dougan, S. and DiNardo, S. (1992). *Drosophila wingless* generates cell type diversity among *engrailed* expressing cells. *Nature* **360**, 347-350.

Ellis, H. M., Soann, D. R. and Posakony, J. (1990). *extramacrochaetae*, a negative regulator of sensory organ development in *Drosophila*, defines a new class of helix-loop-helix proteins. *Cell* **61**, 27-38.

Garcia-Bellido, A. and Merriam, J. R. (1971). Parameters of the wing imaginal disc development of *Drosophila melanogaster*. *Dev. Biol.* **24**, 61-87.

Garcia Bellido, A., Ripoll, P. and Morata, G. (1973). Developmental compartmentalisation of the wing disc of *Drosophila. Nature New Biology* **245,** 251-253.

Garrell, J. and Modolell, J. (1990). The *Drosophila extramacrochaetae* locus, an antagonist of proneural genes that, like these genes, encodes a Helix-Loop-Helix protein. *Cell*, **61,** 39-48.

Ghysen, A. and Dambly-Chaudière, C. (1988). From DNA to form: the *achaete-scute* complex. *Genes Dev.* **2,** 495-501.

Greenwald, I. and Rubin, G. (1992). Making a difference: the role of cell-cell interactions in establishing separate identities for equivalent cells. *Cell* **68,** 271-281.

Hama, C., Ali, Z. and Kornberg, T. B. (1990). Region-specific recombination and expression are directed by portions of the *Drosophila engrailed* promoter. *Genes Dev.* **4**, 1079-1093.

Hartenstein, V. and Posakony, J. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. *Development* **107**, 389-405.

Haynie, J. L. and Bryant, P. J. (1976). Intercalary regeneration in imaginal wing discs of *Drosophila melanogaster. Nature* **259**, 659-662.

Heitzler, P. and Simpson, P. (1991). The choice of cell fate in the epidermis of *Drosophila. Cell* **64**, 1083-1092.

Huang, F., Dambly-Chaudière, C. and Ghysen, A. (1991). The emergence of sense organs in the wing disc of *Drosophila. Development* **111,** 1087-1095.

Jack, J., Dorsett, D., Delotto, Y. and Liu, S. (1991). Expression of the *cut* locus in the *Drosophila* wing margin is required for cell type specification and is regulated by a distant enhancer. *Development* **113,** 735-747.

Jan, Y. N. and Jan, L. Y. (1992). Neuronal specification. *Current Opinion in Genetics & Development* **2,** 608-613.

Kassis, J. A., Noll, E., Van Sickle, E. P., Odenwald, W. F. and Perrimon, N. (1992). Altering the insertional specificity of a *Drosophila* transposable element. *Proc. Natn. Acad. Sci. USA* **89,** 1919-1923.

Leyns, L., Dambly-Chaudière, C. and Ghysen, A. (1989). Two different sets of *cis* elements regulate *scute* to establish two different sensory patterns. *Roux's Arch. Dev. Biol.* **198**, 227-232.

Lindsley, D. L. and Zimm, G. (1992). *The Genome of* Drosophila melanogaster. San Diego: Academic Press.

Martin-Belmudo, M. D., Martinez, C., Rodriguiz, A. and Jimenez, F. (1991). Distribution and function of the *lethal of scute* gene product during early neurogenesis in *Drosophila*. *Development* **113,** 445-454.

Martinez, C. and Modolell, J. (1991). Cross-regulatory interactions between the proneural *achaete* and *scute* genes of *Drosophila. Science* **251,** 1485-1487.

Martinez-Arias, A., Baker, N. A. and Ingham, P. W. (1988). Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. *Development* **103**, 157-170.

Mohler, J. (1988). Requirements for *hedgehog*, a segment polarity gene, in patterning larval and adult cuticle of *Drosophila. Genetics* **120**, 1061-1072.

Morata, G. and Lawrence, P. A. (1977). The development of *wingless*, a homeotic mutation of *Drosophila. Dev. Biol* **56,** 227-240.

Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B., Weintraub, H. and Baltimore, D. (1989). Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* **58,** 537-544.

Nordemeer, J., Johnston, P., Rijsewijk, F., Nusse, R. and Lawrence, P. A. (1992). The consequences of ubiquitous expression of the *wingless* gene in the *Drosophila* embryo. *Development* **116**, 711-719.

Nusse, R. and Varmus, H. E. (1982). Many tumours induced by the mouse

mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* **31**, 99-109.

Nusse, R. and Varmus, H. E. (1992). *Wnt* genes. *Cell* **69,** 1073-1087

Nüsslein-Volhard, C., Wieschaus, E. and Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster.* I. Zygotic loci on the second chromosome. *Roux's Arch. Dev. Biol.* **193,** 267-282.

O'Brochta, D. A. and Bryant, P. J. (1985). A zone of non-proliferating cells at a lineage restriction boundary in *Drosophila. Nature* **313,** 138-141.

Perrimon, N. and Smouse, D. (1989). Multiple functions of a *Drosophila* homeotic gene, *zeste-white3,* during segmentation and neurogenesis. *Dev. Biol.* **135**, 287-305.

Phillips, R. G., Roberts, I. J. H., Ingham, P. W. and Whittle, J. R. S. (1990). The *Drosophila* segment polarity gene *patched* is involved in a position-signalling mechanism in imaginal discs. *Development* **110,** 105-114.

Romani, S., Campuzano, S., Macagno, E. R. A. and Modolell, J. (1989). Expression of *achaete* and *scute* genes in *Drosophila* imaginal discs and their function in sensory organ development. *Genes Dev.* **3,** 997-1007.

Ruiz-Gómez, M. and Modolell, J. (1987). Deletion analysis of the *achaete-scute* locus of *Drosophila melanogaster. Genes Dev.* **1**, 1238-1246.

Schubiger, M. and Palka, J. (1987). Changing spatial patterns of DNA replication in the developing wing of *Drosophila. Dev. Biol.* **123,** 145-153.

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

Siegfried, E., Chou, T-B. and Perrimon, N. (1992). *wingless* signaling acts through *zeste-white 3,* the *Drosophila* homolog of glycogen synthase kinase-3, to regulate *engrailed* and establish cell fate. *Cell* **71**, 1167-1179.

Siegfried, E., Perkins, L. A., Capaci, T. M. and Perrimon, N. (1990). Putative protein kinase product of the *Drosophila* segment-polarity gene *zeste-white3. Nature* **345**, 825-829.

Simpson, P. (1990). Lateral inhibition and the development of the sensory bristles of the adult peripheral nervous system of *Drosophila. Development* **109,** 509-519.

Simpson, P. and Carteret, C. (1989). A study of *shaggy* reveals spatial domains of expression of *achaete-scute* alleles on the thorax of *Drosophila. Development* **106,** 57-66.

Simpson, P., El Messal, M., Moscoso del Prado, J. and Ripoll, P. (1988). Stripes of positional homologies across the wing blade of *Drosophila melanogaster. Development* **103**, 391-401.

Skaer, H. and Martinez Arias, A. (1992). The *wingless* product is required for cell proliferation in the Malpighian tubule anlage of *Drosophila melanogaster. Development* **116**, 745-754.

Skeath, J. B. and Carroll, S. B. (1991). Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev.* **5,** 984-995.

Sporn, M. B. and Roberts, A. B. (1988). Peptide growth factors are multifunctional. *Nature* **332,** 217-219.

Usui, K. and Kimura, K.-I. (1992). Sensory mother cells are selected from among mitotically quiescent cluster of cells in the wing disc of *Drosophila*. *Development* **116**, 601-610.

Van Doren, M., Ellis, H. M. and Posakony, J. W. (1991). The *Drosophila extramacrochaetae* protein antagonizes sequence-specific DNA binding by *daughterless/achaete-scute* protein complexes. *Development* **113**, 245-255.

Villares, R. and Cabrera, C. V. (1987). The *achaete-scute* gene complex of *Drosophila melanogaster:* conserved domains in a subset of genes required for neurogenesis and their homology to *myc. Cell* **50**, 415-424.

Woodgett, J. R. (1991). A common denominator linking glycogen metabolism, nuclear oncogenes and development. *TIBS* **16,** 177-181.

Wilcox, M. and Smith, R. J. (1977). Regenerative interactions between *Drosophila* imaginal discs of different types. *Dev. Biol.* **60**, 287-297.

(Accepted 12 March 1993)