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Wingless can bring about a mesoderm-to-ectoderm induction in *Drosophila* embryos

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SUMMARY

By means of nuclear transplantations, we make mosaics in which largely *wingless*$^-$ embryos contain patches of *wingless*$^+$ cells. In these genetic mosaics, using a standard assay for *wingless* function (the maintenance of *engrailed* expression), we uncover an induction across germ layers: Wingless made in the mesoderm can sustain *engrailed* expression in the ectoderm. This result makes clear that Wingless is expressed in the mesoderm until at least one hour after gastrulation and may function in this germ layer in the wild type.

Key words: embryonic induction, mesoderm, *Drosophila*, *wingless*, *engrailed*

INTRODUCTION

At gastrulation, the *Drosophila* embryo becomes subdivided into an outer ectoderm and an inner mesoderm. Both these layers of cells, although they form different organs, are segmented and must be kept in registration with one another, presumably by interactions between them. Several experiments show that the pattern of the mesoderm is partly dictated by the ectoderm (Bock, 1942; Lawrence and Johnston, 1986) but it is conceivable that there is some feedback the other way, from mesoderm to ectoderm. Here we demonstrate that the mesoderm can indeed influence ectodermal patterning.

One of the essential genes for pattern formation in the ectoderm is *wingless* (a member of the *Wnt* gene family; Nusse and Varmus, 1992); in its absence the cuticle develops as a lawn of unsegmented denticles. The *wingless* gene product is needed to maintain expression of the *engrailed* selector gene in cells that are adjacent to the *wingless*-expressing cells. We ask whether *wingless* has any function within the mesoderm and, if so, whether it can act across germ layers onto the overlying ectoderm. To investigate, we transplanted wild-type *wingless*$^+$ nuclei that were genetically marked into *wingless*$^-$ hosts and made embryos that were mosaics of wild-type and mutant cells. In some of these mosaics, parts of the mesoderm are *wingless*$^+$ and the ectoderm is *wingless*$^-$. In these, we show that *wingless* function in the mesoderm can affect *engrailed* expression in the overlying ectoderm. Although *wingless* is thought to be only fleetingly transcribed in the mesoderm (Baker, 1987, 1988), our results argue that it can function there, at least until about one hour after gastrulation.

MATERIALS AND METHODS

Nuclear transplantations were carried out as before (Vincent and Lawrence, 1994), except that the recipients were oriented so that donor nuclei were placed near to the ventral midline (to increase mesoderm colonisation). The donor embryos were at the late blastoderm stage while hosts were in early cleavage. Donors were marked with *armadillo-lacZ* and hosts were a stock in which one quarter of the embryos were homozygous *wingless*$^-$. For fixation and staining of embryos see Vincent and Lawrence (1994). Some mosaics were embedded in Durcupan resin and sectioned at 10-15 μm, others were dissected with a piece of broken razor blade and mounted in Araldite resin. Heat shocks were given in a water bath at 36°C for 20 minutes. Genotypes used: *wg*^{CX4} a null allele for *wingless*, hs-*wingless* (Noordermeer et al., 1992), *wingless-lacZ* (*CyO*, P(en 11); Kassis et al., 1992).

RESULTS AND DISCUSSION

Nuclei were taken from wild-type embryos that carried a gratuitous cell marker, the *armadillo-lac Z* gene (Vincent et al., 1994) and transplanted into unmarked embryos, a quarter of which were mutant for the *wingless* gene. After development, the fixed embryos were stained for β-galactosidase to detect the patches of donor cells. Mosaics made by nuclear transplantation generally develop normally with the cells of donor and host tissue being well integrated (Santamaria, 1975; Vincent and Lawrence, 1994).

In *wingless*$^-$ embryos, although *engrailed* expression is activated normally under the control of the pair-rule genes, it fails to be maintained. This decay of *engrailed* expression is thought to be due to the absence of a signal that normally comes from the *wingless*-expressing cells (DiNardo et al., 1988; Martinez-Arias et al., 1988). In those mosaics in which patches of wild-type cells colonised the **mesoderm** of an otherwise *wingless*$^-$ embryo, we find some **epidermal** *wingless*$^-$ cells expressing *engrailed*. These *engrailed*-express-

ing cells occur within large areas of *wingless*$^-$ epidermis, so they could not be responding to an epidermal source of Wingless. (Fig. 1A,B). The numbers and arrangement of such cells vary; they usually do not form a proper stripe and often there are one or two cells per parasegment, or there is an intermittent line. Out of 17 mosaics in which patches of donor mesoderm underlie large areas of host ectoderm, 14 show *engrailed* expression in that ectoderm. These 14 embryos include *engrailed*-expressing cells in 1 to 5 segments, depending on the extent of the mesodermal patch. The positions of the expressing cells, with respect to the *engrailed* stripes that normally persist in the CNS, make it likely that they are descendants of cells which expressed *engrailed* earlier. As these *engrailed*-expressing cells invariably overlie patches of *wingless*$^+$ territory in the somatic mesoderm, some effect must have come from there.

Our results show that *wingless* must be produced in the mesoderm and still be active there at least one hour after gastrulation (see below). This was not clear before: *wingless*

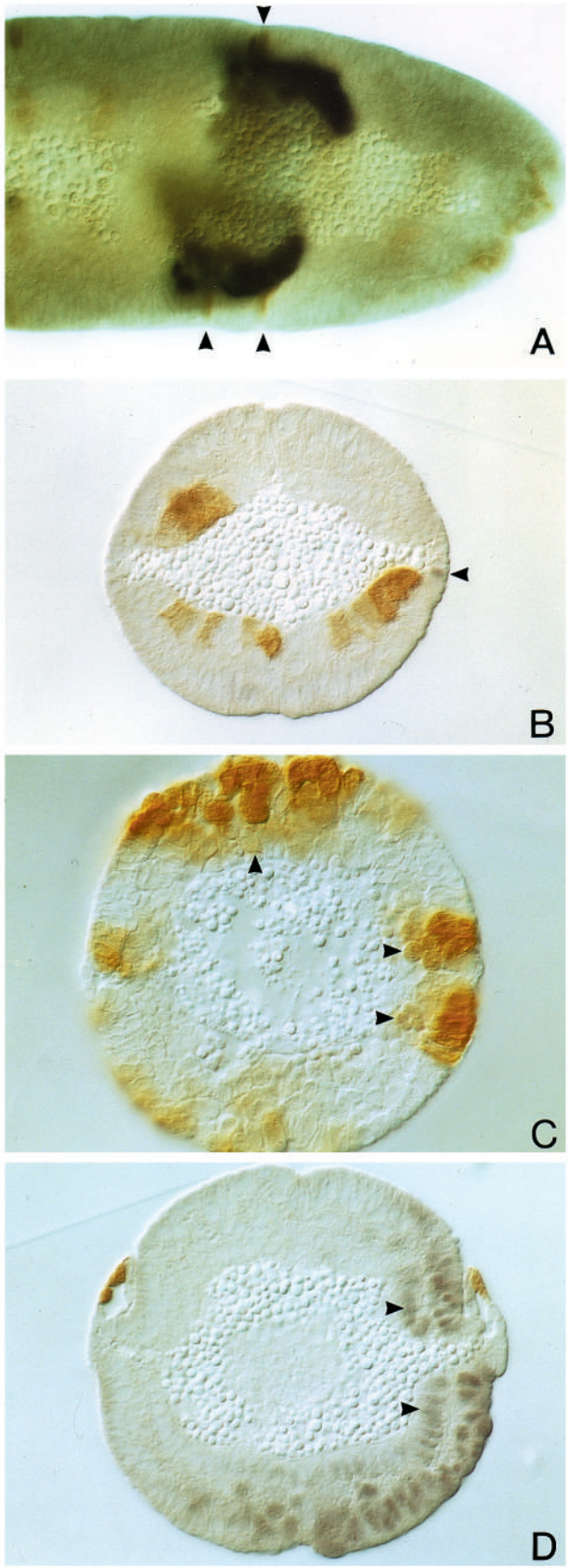

Fig. 1. (A) Dorsal view of a mosaic in which part of the mesoderm, both left and right, has been formed by *wingless*$^+$ cells, which stain black. The remainder of the mosaic does not stain black and is *wingless*$^-$ in genotype. The embryo was stained for the Engrailed protein. Within the *wingless*$^-$ epidermis on both sides, some nuclei contain Engrailed (brown streaks in the columnar epithelium of the ectoderm). All of this ectoderm consists of *wingless*$^-$ host cells. Note also that, associated with both groups of *engrailed*-expressing nuclei, there is a suspicion of grooves (arrowheads), which are appropriately spaced and positioned (just anterior to the *engrailed*-expressing nuclei) and resemble those normally seen at the parasegmental borders. We have seen this occasionally, but not invariably, in other mosaics suggesting that the parasegmental grooves in the epidermis might depend, partially or entirely, on the underlying mesoderm. (B) A transverse section of a similar mosaic, probably at stage 10; it shows the *wingless*$^+$ mesoderm stained in brown and a single dorsal nucleus that expresses *engrailed* in the epidermis (stained in black, arrowhead). One of a row of Engrailed-positive nuclei was caught in this section. Nuclei in this region of the *wingless*$^-$ epidermis never normally show Engrailed protein. (C) A section of a stage 11 embryo carrying the *wingless-lacZ* transgene; β-galactosidase is stained in brown. Note that mesoderm cells (arrowed; only some of which are caught in the section) contain β-galactosidase and are therefore presumed to express *wingless*. Note also that these cells are in register with the ectodermal *wingless* stripes. It is possible that the mesodermal β-galactosidase might have perdured from expression of *wingless* around the time of gastrulation, but we doubt this could account for all the β-galactosidase detected. *wingless* expression in the mesoderm, as visualised with a *wingless-lacZ* enhancer trap gene, does not require *wingless* function in the ectoderm; we transplanted *wingless-lacZ* nuclei into *wingless*$^-$ hosts, and showed that donor cells express β-galactosidase in the mesoderm, even when they were covered by *wingless*$^-$ epidermis (not shown). (D) An example of a control mosaic (stage 10) in which *wingless*$^+$ donor cells (limited to amnioserosa) are stained in brown and Engrailed in black. The host is also *wingless*$^+$ and the whole embryo (donor and host cells) shows Engrailed stripes. On the right, the section cuts through a stripe and we see Engrailed in the epidermis and in the underlying mesoderm (arrowheads; this is unexpected as *engrailed* was thought to turn off shortly after gastrulation (Ingham et al., 1985). On the left, the section largely misses the stripe and therefore, there is no Engrailed to be seen either in the dorsolateral ectoderm or the underlying mesoderm. There is some Engrailed in the epidermis and CNS of the ventral region.

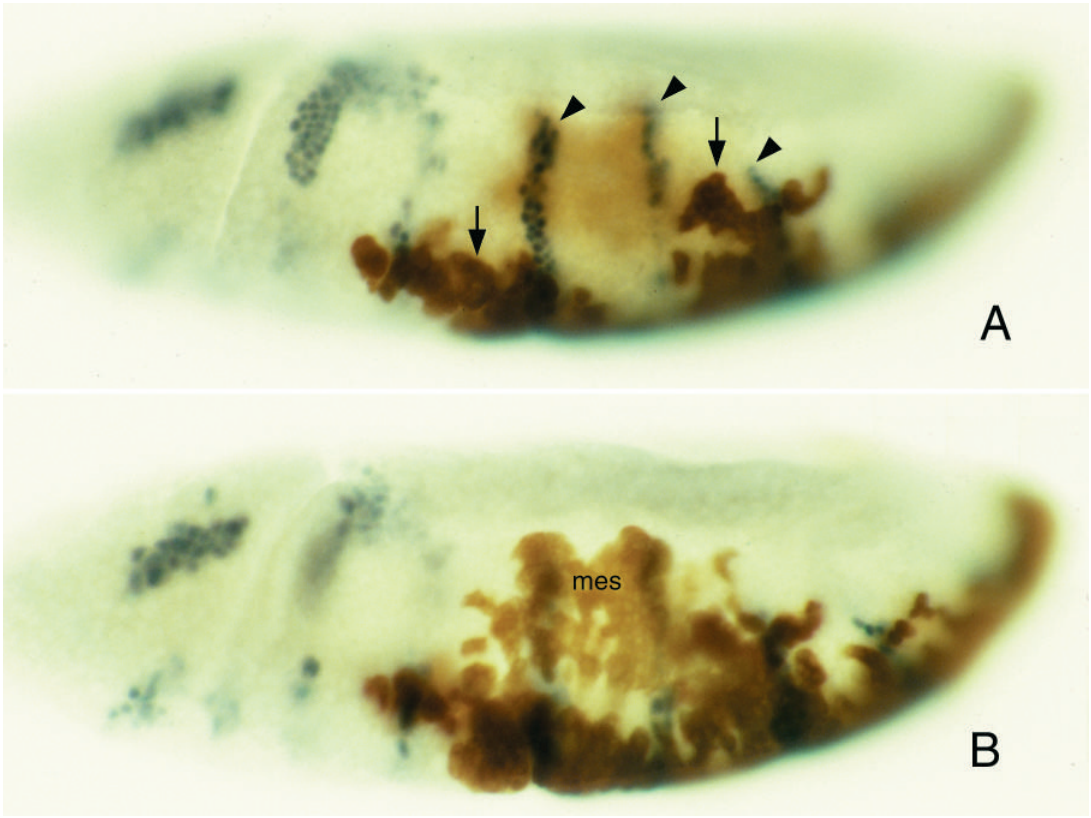

Fig. 2. Dissected mosaic embryo of early stage 11 showing *engrailed* expression in a *wingless*$^{-}$ patch overlying wild-type mesoderm. (A) Ectodermal plane of focus. The *wingless*$^{+}$ tissue is stained in brown. A large mesodermal patch (wild type, out of focus, light brown) occupies the centre of the field. Wild-type ectodermal tissue appears dark brown (in focus) and is mostly found medially (arrows). Engrailed is stained in black. Two stripes of Engrailed are strongly and substantially maintained (the two left-most arrowheads). These two stripes overlie faint brown, mesodermal, wild-type tissue. Sometimes *wingless*$^{-}$ embryos have 3-4 Engrailed-positive cells at the dorsal tip of the left-most stripe (at the position of the arrowhead). But a proper stripe such as the one seen is never maintained. Moreover, no *engrailed* expression was detectable on the contralateral side (which was completely *wingless*$^{-}$). Although overlying wild-type mesoderm, the third bit of stripe (arrowhead on the right) may or may not be maintained by an influence from the mesoderm. It could also be due to a non-autonomous effect of the neighbouring epidermis (Vincent and Lawrence, 1994). (B) The same mosaic photographed at a lower depth of focus to show the mesodermal cells (mes).

expression was detected in the mesoderm of stage 9 embryos by in situ hybridisation but some doubt remained as the preparations were of low resolution (see Fig. 3A in Baker, 1988). The protein was also seen with antibodies at stage 10 (van den Heuvel et al., 1989) but, since it is secreted (van den Heuvel et al., 1989; van Leeuwen et al., 1994), it might have been produced in the ectoderm and diffused from there to the underlying mesoderm. We also see β-galactosidase in the mesoderm of embryos from an enhancer trap line in which the *wingless* promoter directs the expression of the *lacZ* gene (Fig. 1C), but this might follow from perdurance of the β-galactosidase after the *wingless* promoter has shut off (see legend).

Qualifications weaken each of the above three lines of evidence that *wingless* is functional in the mesoderm, but we believe our assay in mosaic embryos provides an incontrovertible argument that it is, at least until stage 9. This upper time limit can be deduced from Fig. 1B, which shows the *wingless*-dependent induction of a dorsal *engrailed*-expressing cell — a cell that could not have received any influence before some wild-type mesodermal cells came close to it. Mesodermal cells appear ventrally during gastrulation and migrate dorsally. They do not reach their dorsal-most position until well into stage 9 (around 4 hours of development, Azpiazu and Frasch, 1993; our observations).

Why are the induced *engrailed* stripes discontinuous? We tried to increase the amount of Wingless protein produced by the mesoderm by transplanting nuclei, that, in addition to being wild type for *wingless*, carried a transgene in which the *wingless* cDNA is driven by a heat-shock promoter (hs-*wingless*). As expected from previous studies (Noordermeer et al., 1992), within **epidermal** patches of such cells, the *engrailed* stripes broaden following a heat shock. Amongst the mosaics containing hs-*wingless* cells in the **mesoderm** we have, in one case only, found an abnormally broad, although somewhat incomplete, *engrailed* stripe in the overlying *wingless*$^{-}$ epidermis (not shown). This suggests that incompleteness of the stripes might be partly or completely due to the low level of Wingless protein coming from the mesoderm.

Patchy *engrailed* expression could also follow from inadequate contact between the two germ layers: since the mesodermal and ectodermal stripes of cells expressing *wingless* overlie each other rather exactly, the mesodermal *wingless*-

expressing cells will be only intermittently and narrowly in contact with the overlying *engrailed*-expressing cells, which are offset by one row. We noticed that, in mosaics fixed at later stages, induced *engrailed* expression is often stronger and the epidermal stripes more continuous and broader (4 out of 5 stage 11 or 12 embryos, see Fig. 2). It is possible that, as development proceeds and cell movements occur, additional *engrailed*-expressing cells might gain contacts with the *wingless*-expressing cells underneath. This interpretation is consistent with *wingless* having a short range (Vincent and Lawrence, 1994).

It is still not known whether the *wingless*-dependent signal is the Wingless protein itself, but if it is, our results bear on how Wingless must be presented to *engrailed*-expressing cells where, normally, it is provided from within the same epithelium. They suggest that the protein simply needs to be there, its provenance and exact distribution being unimportant; a view put forward and discussed before — because uniformly distributed Wingless protein within the whole embryo, including ectoderm (Sampedro et al., 1993) or within the mesoderm only (Baylies, Bate, Bishop, Wilder and Martinez-Arias, personal communication), can restore a considerable amount of segmental pattern to *wingless*$^-$ embryos. Moreover, one possibility suggested by our result is that the Wingless protein can be presented to the basal surface of the epidermal cells and function there. Alternatively, if the target of Wingless action is located on the apical surface of epidermal cells, our result implies that the protein can diffuse between epidermal cells to reach its destination. Clearly, it is relevant to know how closely apposed the mesodermal cells are to the overlying ectoderm during the interaction. It is not known whether the two layers are separated by a basement membrane at stages 9 to 10. But laminin A and collagen, constituents of basement membranes, are not detected there before late stage 11 (Kusche-Gullberg et al., 1992) suggesting that there is no intervening basement membrane and that the cells might be in direct contact.

The simplest interpretation of our results is that, just anterior to the parasegment border, *wingless* is expressed in both the ectoderm and the mesoderm and that the secreted protein can move both within and between the germ layers, or affect the transfer of some other signal. The significance of this action of *wingless* across germ layers is unclear. In mosaics with the converse arrangement of cells (*wingless*$^+$ ectoderm overlying *wingless*$^-$ mesoderm), *engrailed* is expressed within the ectoderm in the normal pattern (Vincent and Lawrence, 1994) so the effect we have been studying is not necessary for *engrailed* expression in the ectoderm as such.

What could be the wild-type function of *wingless* in the mesoderm? The mesoderm includes at least two distinct tissues, the somatic and visceral mesoderms. In the visceral mesoderm, *wingless* and other segmentation genes, such as *even-skipped*, are needed for expression of homeotic genes in specific sets of parasegments (Tremml and Bienz, 1989), suggesting that segmentation of the visceral mesoderm occurs and is important. The somatic mesoderm is also clearly segmented: there are segmental sets of muscle, the heart is overtly segmented and the homeotic selector genes are expressed in parasegmental sets (reviewed in Lawrence, 1992). Even *engrailed* is expressed in segmental stripes in the mesoderm, at least until stage 9 or 10 (Fig. 1D), although it appears that Engrailed may not be functional there since transplanted *engrailed*$^-$ nuclei participate normally in mesodermal development (Lawrence and Johnston, 1984). It is possible that *wingless* is involved in maintaining this mesodermal *engrailed* expression. Alternatively, *wingless* might have other roles in the mesoderm which may or may not be related to segmentation. In any case, our assay shows that Wingless is being made in the mesoderm and implies that it may function there in the wild type.

This is not the only case of *wingless* being instrumental in an induction between germ layers; it has been shown that the gene is necessary in the visceral mesoderm for the pattern of expression of *labial* in the underlying endoderm (Immergluck et al., 1990; Panganiban et al., 1990). But we believe it is the first example of an induction from the mesoderm to the ectoderm.

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