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Specification of left-right asymmetry in the embryonic gut of *Drosophila*

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SUMMARY

Most animals exhibit stable left-right asymmetries in their body. Although significant progress has been made in elucidating the mechanisms that set up these asymmetries in vertebrates, nothing is known about them in *Drosophila*. This is usually attributed to the fact that no reversals of stable left-right asymmetries have been observed in *Drosophila*, although relevant surveys have been carried out. We have focused on the asymmetry of the proventriculus in the embryonic gut of *Drosophila*, an aspect of left-right asymmetry that is extremely stable in wild-type flies. We show that this asymmetry can be reversed by mutations in the *dicephalic* and *wunen* genes, which also cause reversals in the antero-posterior axis of the embryo relative to its mother. This is the first observation to suggest that left-right asymmetries in *Drosophila* can be reversed by genetic/developmental manipulations. It also suggests that maternal signals may initiate the specification of some left-right asymmetries in the embryo.

Key words: Left-right asymmetry, Body patterning, *Drosophila*, Gut

INTRODUCTION

The left and right sides of the body exhibit stable asymmetries in most animals; for example, it is well known that organs like the heart and spleen are normally displaced to the left side of the body in humans, while the liver is normally displaced to the right. Recently, significant progress has been made in elucidating the genetic and developmental mechanisms that set up these asymmetries in vertebrates (see Capdevila et al., 2000 for a review), but little research on this topic has been carried out in other organisms (see Freeman and Lundelius, 1982; McCain and McClay, 1994; Wood, 1991). Most surprisingly, very few studies on the specification of left-right asymmetry have been carried out in *Drosophila*, an organism that normally offers great opportunities for genetic and developmental research. The lack of such studies is often attributed to the idea that stable left-right asymmetries may not be subject to genetic control in *Drosophila*, as no reversals of such asymmetries have so far been observed in this species (see Corballis and Morgan, 1978; Klingenberg et al., 1998; Tuinstra et al., 1990). We have approached this question by considering the implications of two types of mechanisms that may be responsible for the initiation of left-right asymmetries in all animals.

The left and right sides of the body can only be defined with reference to the other two body axes, the anteroposterior (AP) and dorsoventral (DV) axes. The mechanisms that generate stable left-right asymmetries must therefore be able to 'sense' the orientation of the AP and DV axes and to co-ordinate their activities with respect to them, in order to polarise the development of left-right asymmetries consistently in a particular orientation. We can envision two general ways by which this could occur: the co-ordination could either take place within the embryo, or it could be set up by external signals.

Brown and Wolpert have proposed a model by which left-right asymmetries can be initiated entirely within the embryo, postulating that chiral molecules (or macromolecular structures) that are specifically oriented with respect to the AP and the DV axes within the embryo may provide the molecular basis for 'sensing' these axes and co-ordinating the development of left-right asymmetries (Brown and Wolpert, 1990). Thus, the polarity of left-right asymmetries in the body would depend on the intrinsic molecular handedness of those chiral elements (Fig. 1A). This is an attractive model for the specification of left-right asymmetries in organisms where co-ordinated positional information cannot be provided by the external environment, and indeed there is evidence to suggest that such a mechanism might operate in vertebrates (Capdevila et al., 2000; Nonaka et al., 1998).

Alternatively, left-right asymmetries may depend on positional information that is provided to embryos by an asymmetric external signal, for example originating from the surrounding maternal tissues (Fig. 1B). In this situation, maternal left-right asymmetries could give rise to an asymmetric signal that would directly imprint left-right asymmetry on the embryo. This type of mechanism seems plausible in embryos whose body axes are co-ordinated with those of maternal tissues, as happens in *Drosophila* (the AP axis of the *Drosophila* embryo is specified with respect to the AP axis of its mother; the DV axis is probably specified randomly, but it becomes aligned with the DV axis of the mother at later stages; see van Eeden and St Johnston, 1999).

The two models make different predictions that can be tested

experimentally in *Drosophila*. The first model predicts that reversing the AP axis of the embryo should have no effect on left-right asymmetry, as the internal mechanisms (the chiral structures) that polarise left-right asymmetries would simply re-orient with respect to the new AP axis (see Fig. 1A). According to the second model, however, reversal of the AP (or the DV) axis of the embryo would place the external signal on the opposite side of the embryo, giving rise to embryos with reversed left-right asymmetries (see Fig. 1B). To address this issue, we have used genetic means to invert the polarity of the AP axis in *Drosophila* embryos.

MATERIALS AND METHODS

Drosophila stocks

We have used a $dic^1 st^1 th^1 ru^1/TM3\ Sb^1$ stock obtained from the Bloomington stock centre and wun^{GL}/CyO and wun^{k15909}/CyO stocks that were kindly provided by Ruth McCaffrey and Acaimo Gonzalez-Reyes. A significant number of egg chambers with inverted AP polarity were observed in dissected ovaries of both dic^1/dic^1 and wun^{GL}/wun^{k15909} females, as previously reported (Gonzalez-Reyes and St Johnston, 1998; Nian Zhang and Ken Howard, personal communication). Overall, we scored 195 egg chambers with normal polarity, 16 egg chambers with reversed polarity and 14 egg chambers with mislocalised oocytes in the ovaries of dic^1/dic^1 mothers; we noticed some variation in these frequencies among different vials of flies, which may depend on age, feeding and crowding conditions.

Embryo staining

Embryos from wild-type and mutant *Drosophila* were collected and allowed to develop for 12-18 hours at 25°C on apple juice plates. The embryos were then dechorionated, fixed and immunochemically stained using standard techniques (Patel, 1994). The embryos were stained with an anti-Nubbin antibody (Averof and Cohen, 1997) to facilitate visualisation of the proventriculus.

RESULTS

Drosophila embryos exhibit a number of left-right asymmetries in the coiling of their gut, some parts being consistently displaced to the left while others are displaced to the right. We have focussed on the proventriculus, a distinctive structure that forms at the posterior part of the foregut, because it is a part of the gut that can be identified unambiguously in all embryos (by its characteristic morphology and staining, see Fig. 2) and exhibits a very stable left-right asymmetry. The proventriculus shows left-right asymmetry in its positioning and orientation, its posterior part being consistently displaced to the right side of the embryo after embryonic stage 15 (Fig. 2A-C). We have observed only 1 in 1010 wild-type embryos where this asymmetry was partly reversed; this aspect of left-right asymmetry is therefore extremely stable in wild-type flies.

In order to test the two general models for the specification of left-right asymmetries (outlined earlier), we have looked for genetic conditions where the AP axis of embryos could be reversed within the ovary of their mothers. Mutants of genes known to play an important role in the establishment of the AP axis can give rise to embryos with severe defects in AP polarity, but no perfect reversals of the AP axis; these embryos do not develop to a stage when overt left-right asymmetries can be assessed. Therefore, we have had to rely on two mutants that produce a small proportion of embryos (less than 10%) that have a fully reversed AP axis, namely mutants in the genes *dicephalic* (*dic*) and *wunen* (*wun*).

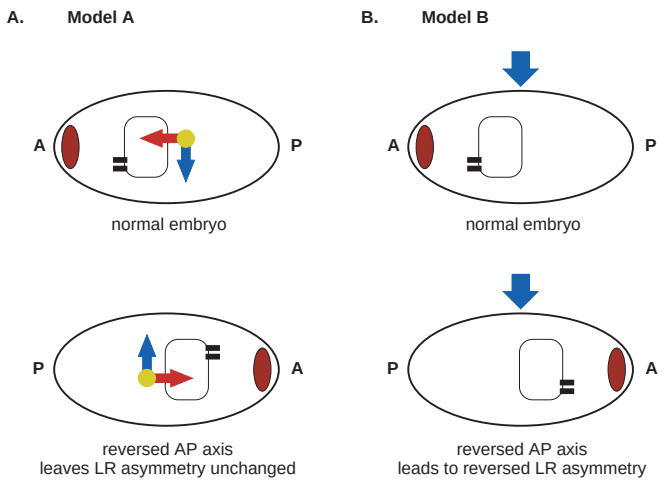

Fig. 1. Two general models for the specification of left-right asymmetry. All embryos are shown from a ventral view. (A) The first model suggests that left-right asymmetry is initiated within the embryo, by the intrinsic handedness of a chiral structure that is specifically oriented with respect to the AP and DV axes. The handed structure is illustrated by a set of red, yellow and blue arrows (yellow arrow points ventrally towards the observer). Reversal of the AP axis should have no effect of left-right asymmetry, as the chiral structures that initiate this asymmetry should simply re-orient in relation to the new AP axis. (B) The second model suggests that left-right asymmetry is specified by an external signal (illustrated by the blue arrow) that is provided asymmetrically by the surrounding maternal tissues. Reversal of the embryonic AP axis would place this signal on the opposite side of the embryo, leading to a reversed pattern of left-right asymmetry.

Mothers that are homozygous mutant for *dic* produce a small proportion of eggs that have a reversed AP orientation within their egg-chambers, in the *Drosophila* ovary (see Materials and Methods; Gonzalez-Reyes and St Johnston, 1998). These eggs are expected to give rise to embryos with a fully reversed AP axis. We examined embryos laid by dic^1/dic^1 mutant mothers at embryonic stages 15-17 and found that a significant proportion of these embryos (29/341 embryos in total) show perfect reversals in the left-right asymmetry of the proventriculus; its orientation is reversed, with its posterior part displaced to the left (Fig. 2D-F). As already mentioned, such reversals occur extremely rarely in wild-type *Drosophila* (1/1010 embryos). These reversals were also extremely rare in embryos derived from $dic^1/+$ parents (1/238 embryos in total), suggesting that they are not associated with a zygotic effect of the dic^1 mutation (see Table 1).

We have obtained similar results using mutations in the apparently unrelated gene *wun*. As with *dic*, mothers that are homozygous mutant for *wun* produce a small proportion of eggs that have a reversed AP axis (Nian Zhang and Ken Howard, personal communication). We observed that a significant proportion of embryos laid by wun^{GL}/wun^{k15909} mutant mothers (3/26 embryos) show reversals in the left-right asymmetry in their proventriculus (these embryos also show additional defects in the morphogenesis of their gut; data not

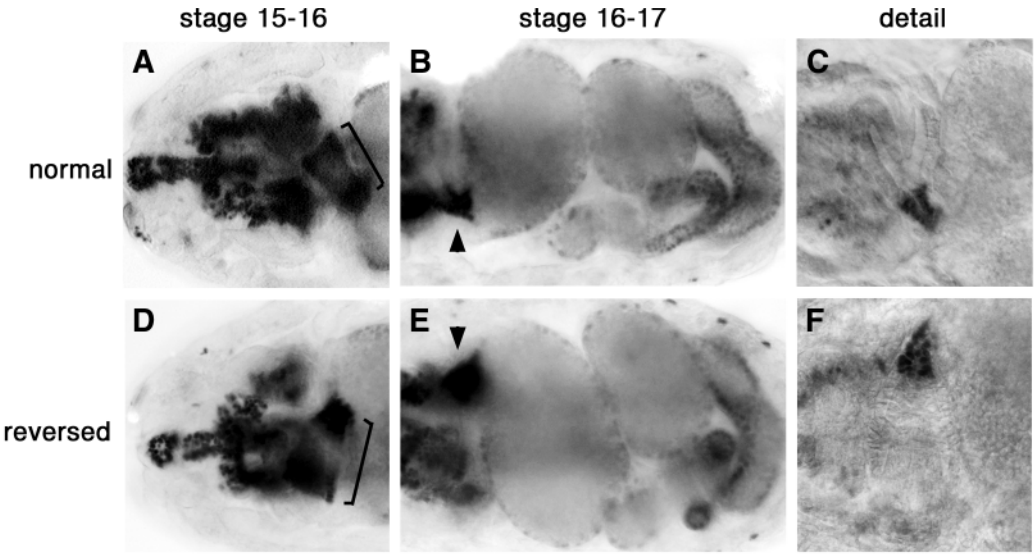

Fig. 2. *Drosophila* embryos with normal and reversed left-right asymmetry of the proventriculus at stages 15-17. (A-C) Wild-type embryos showing a normal pattern of left-right asymmetries in the gut. (D-F) Embryos laid by dic^1/dic^1 mutant mothers showing reversed left-right asymmetry in the positioning of the proventriculus. The proventriculus is indicated by a bracket at stage 15-16 (A,D), and the strongly stained posterior part of the proventriculus is indicated by an arrowhead at stage 16-17 (B,E). Higher magnifications of the proventriculus are shown at stage 16-17 (C,F). All panels show mid-ventral views, with anterior towards the left. All embryos have been stained with an antibody against Nubbin.

Table 1. Frequency of reversals of left-right asymmetry of the proventriculus

Maternal genotype	Paternal genotype	AP reversals	Embryos with LR reversal	% Embryos with LR reversal
Wild type	Wild type	–	1/1010*	0.1
$dic^1/+$	$dic^1/+$	–	0/130*	0.0
$dic^1/+$	dic^1/dic^1	–	1/108*	0.9
dic^1/dic^1	Wild type	+	3/50‡	6.0
dic^1/dic^1	$dic^1/+$	+	10/110‡	9.0
dic^1/dic^1	dic^1/dic^1	+	16/181‡	8.8
wun^{GL}/wun^{k15909}	Wild type	+	3/26§	11.0

*Not significantly different from each other.
‡Not significantly different from each other, but significantly different from the values marked with one asterisk (chi-squared test $P<0.01$).
§Significantly different from wild type (chi-squared test $P<0.01$).

shown). Thus, the observed reversals of left-right asymmetry appear to be associated with reversals of the AP axis, irrespective of the particular mutants used to generate the reversals of the AP axis.

It has not been possible to prove that the embryos showing reversals of left-right asymmetry are precisely those that developed with a reversed AP axis. This one-to-one correlation was not possible to establish, as the AP reversals caused by the *dic* and *wun* mutations appear perfect (involving also the reversal of AP polarity in the chorion of the egg), leaving no trace on the eggs once they have been laid. Our suggestion that the reversals of left-right asymmetry are associated with AP reversals is therefore an inference based on the coincident occurrence of these two phenotypes in different genetic conditions (see Table 1).

DISCUSSION

We have shown that a particular aspect of left-right asymmetry, the asymmetry of the proventriculus in the embryonic gut of *Drosophila*, can be reversed in embryos laid by *dic* or *wun* mutant mothers. These mutants were chosen for their ability to produce embryos with a fully reversed AP axis, suggesting that the reversals of left-right polarity are associated with reversals of the AP axis. Our results are therefore in agreement with the predictions of the model in Fig. 1B, suggesting that the direction of left-right asymmetry in the proventriculus is specified by a maternally provided signal.

The nature of this signal remains unknown. In egg chambers that arise from *dic* or *wun* mutant mothers, the AP polarity of the follicular epithelium appears to correlate with the polarity of the oocyte (reversed egg chambers develop dorsal appendages at the opposite end of the egg), suggesting that the co-ordination of this signal with respect to the AP axis probably occurs outside the follicular layer, in the surrounding maternal tissues. The signal itself could be a molecular signal emanating asymmetrically from one side of the maternal genital tract, or even a mechanical signal arising from anatomical left-right asymmetries in the mother's internal organs.

We would also like to point out that embryos showing a reversed left-right asymmetry in their proventriculus do not show reversals of left-right asymmetry in other parts of their gut (compare gut coiling in Fig. 2B with 2E). This observation implies that other aspects of left-right asymmetry may be specified by an intrinsic molecular mechanism, according to the model in Fig. 1A, as in vertebrates. This suggests that fundamentally different mechanisms may be involved in the specification of different aspects of left-right asymmetry, both within an organism and between species.

An alternative interpretation of our results is that *dic* and *wun* mutations have a direct effect on left-right asymmetry that is not dependent on the AP axis reversals. In that case, the maternal products of *dic* and *wun* would be more directly implicated in the specification or maintenance of left-right asymmetry in the proventriculus of *Drosophila*. We find this

possibility equally interesting, although less likely; it would imply that we fortuitously identified two genes that are involved in the specification of left-right asymmetry. In either case, our results demonstrate for the first time that stable left-right asymmetries in *Drosophila* can be reversed by genetic/developmental manipulations and are therefore amenable to further genetic studies.

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