Oriented cell divisions in the extending germband of *Drosophila*

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Tissue elongation is a general feature of morphogenesis. One example is the extension of the germband, which occurs during early embryogenesis in *Drosophila*. In the anterior part of the embryo, elongation follows from a process of cell intercalation. In this study, we follow cell behaviour at the posterior of the extending germband. We find that, in this region, cell divisions are mostly oriented longitudinally during the fast phase of elongation. Inhibiting cell divisions prevents longitudinal deformation of the posterior region and leads to an overall reduction in the rate and extent of elongation. Thus, as in zebrafish embryos, cell intercalation and oriented cell division together contribute to tissue elongation. We also show that the proportion of longitudinal divisions is reduced when segmental patterning is compromised, as, for example, in *even skipped* (*eve*) mutants. Because polarised cell intercalation at the anterior germband also requires segmental patterning, a common polarising cue might be used for both processes. Even though, in fish embryos, both mechanisms require the classical planar cell polarity (PCP) pathway, germband extension and oriented cell divisions proceed normally in embryos lacking *dsh* (*dsh*), a key component of the PCP pathway. An alternative means of planar polarisation must therefore be at work in the embryonic epidermis.

**KEY WORDS:** Tissue elongation, Planar cell polarity, Pair rule, *string*, Mitosis, *Drosophila*

**INTRODUCTION**

The best-known example of tissue elongation is the extension of the vertebrate axis following gastrulation. In *Xenopus*, elongation of the prospective notochord is driven by convergence and extension, a process that requires cell intercalation. In fish embryos, cell intercalation also contributes to axis elongation during gastrulation (Wallingford et al., 2002). In these embryos, oriented cell division is a key additional contributory factor. Both processes appear to be controlled by the planar cell polarity (PCP) pathway (Gong et al., 2004; Wallingford et al., 2000). *Drosophila* embryos also undergo dramatic tissue elongation: during early embryogenesis, the germband, which gives rise to the segmented trunk of the larva, doubles in length while thinning commensurately. In this case, the elongating tissue is constrained by external membranes and the germband folds over itself as it elongates. At the end of elongation, the posterior half of the germband (segments A3-A9) ends up on the dorsal side of the egg, while the anterior half (segments T1-A2) remains on the ventral side throughout (Sonnenblick, 1950) (Fig. 1A). Upon completion of germband extension (GBE), the posterior tip of the germband has travelled over 70% of the egg length towards the head region. Therefore, the displacement of the posterior tip provides a quantitative measure of the progression of GBE. Using this simple assay, two phases can be distinguished during GBE (Hartenstein and Campos-Ortega, 1985). Most of the elongation takes place during the fast phase, which lasts approximately 25 minutes. Extension is completed during the following 70 minutes, which make up the slow phase.

What are the cellular behaviours that drive GBE? Early morphological studies suggested that the contraction of an actin network underlying the forming epidermis could be responsible (Rice and Garen, 1975; Rickoll and Counce, 1980). Subsequent work assessed the role of cell rearrangements (Bertet et al., 2004; Irvine and Wieschaus, 1994; Zallen and Wieschaus, 2004). These observations were focused on the anterior of the germband because it remains within the same field of view throughout extension (remaining at the anterior of the fold). In this region, no cell division occurs during the first 15 minutes of GBE (Foe, 1989; Hartenstein and Campos-Ortega, 1985), excluding the possibility that oriented cell divisions could contribute to elongation, at least at these early times and in this part of the germband. Instead, early extension of the anterior region of the germband is powered by an orderly process of cell intercalation driven by junctional remodelling (Bertet et al., 2004; Blankenship et al., 2006; Irvine and Wieschaus, 1994). Polarisation of this intercalary behaviour requires cues from the genetic cascade that pattern the anteroposterior axis. Indeed, no extension is seen in embryos laid by triple-mutant *bicoid nanos torso-like (bcd nos tsl)* mothers, hence lacking all anteroposterior information (Irvine and Wieschaus, 1994). GBE is reduced in embryos lacking the pair-rule transcription factor encoded by *even skipped* (*eve*). These embryos are partly segmented, and this is correlated with a strong decrease in intercalary behaviour (Blankenship et al., 2006; Irvine and Wieschaus, 1994). Little attention has been given to the posterior of the germband (the region that ends up posterior to the fold during extension; see diagram in Fig. 1). This region is known to undergo mitoses shortly after the beginning of GBE. Indeed, so-called mitotic domain 4 is recognisable at the posterior tip of the extending germband shortly after the onset of extension (Foe, 1989). Are these divisions oriented and do they play a role in the extension of the posterior region of the germband? We found that, in the posterior region of the germband, mitoses are oriented along the axis of elongation. Moreover, in the absence of mitosis, germband elongation is reduced and no tissue...
Fig. 1. Cell divisions during the fast phase of GBE are predominantly oriented along the anteroposterior axis. (A) Side-view diagram illustrating the process of germ band elongation (GBE). Images (C-F) were taken from the dorsal side of the embryo (eye icon). Notice how the posterior tip of the germ band (red) moves towards the anterior of the embryo (left side). (B) Cell-division angles were measured relative to the ventral midline. (C-F) Cell divisions during the fast and slow phases of GBE. Examples of snapshots taken after 5 minutes (C) and 10 minutes (D) (during the fast phase), and at 45 minutes (E) and 50 minutes (F) (during the slow phase) are shown. The time when the pole cells become visible on the dorsal side was arbitrarily taken as t=0. Note how cell divisions tend to be longitudinally oriented during the fast phase (white lines). (G, H) Quantification of cell division angles during the two phases of GBE. The fast phase occurs during the first 25 minutes, whereas the slow phase takes place during the subsequent 70 minutes. A total of 50 and 100 cell divisions were counted (per embryo) for the fast and slow phases, respectively. Each bar represents the average obtained from five embryos. The standard error is also shown. Longitudinal divisions are predominant during the fast phase of GBE (white lines in D). A, anterior; P, posterior.

deformation is seen in the posterior region. We also present evidence that segmental patterning provides a cue for the orientation of cell division.

MATERIALS AND METHODS

Fly strains

Df(2R)eve113 was obtained from the Tubingen Centre. The string alleles used were stgG26, which causes a deletion of the coding region (Edgar et al., 1994) and stgW2, an amorphic point mutation. Flies carrying His2AvDGF were kindly provided by Rob Saint (Australian National University, Canberra, Australia). Embryos lacking all segmental patterning were obtained from bicoid nanos torso-like triple-mutant females (Nusslein-Volhard et al., 1987) that also carried His2AvDmRFP (Schuh et al., 2007).

Embryos deficient in PCP-specific dsh activity were obtained from dsh1/dshV26 females crossed to dsh1 males. The dshV26 allele results from a frame shift after amino acid residue 94 and is presumed to be a null. The dsh1 mutation results from a single amino acid replacement that specifically affects PCP (Penton et al., 2002). Note that the zygotes could be either dsh1/dshV26 or dsh1/dsh1. In both cases, no PCP-specific dsh activity is expected.

Image capture

All images were acquired at 25°C. Five embryos were analysed for each genotype (except for the embryos from bicoid nanos torso-like triple mutants). Embryos were dechorionated in 10% bleach for 3 minutes and mounted in Voltalef oil on a coverslip. The images were acquired with a Perkin-Elmer UltraView spinning disc confocal scanner mounted on an Olympus IX70 inverted microscope with a 20× (0.5 NA) or a 40× (0.8 NA) objective lens. Ten z-sections (covering 10 μm) were collected every 30 seconds. The time-lapse series was assembled and analysed with Velocity (Improvision) and ImageJ [National Institutes of Health (NIH)]. Angles and lengths were measured with the angle and measure tools on ImageJ. Polynomial regression curves (Fig. 2Q) were determined with Excel (Microsoft) as: y=(−3×10−8)x+(4×10−4)x2−0.0003x4+0.0085x3−0.1249x2+0.5932x+2.6912 (R2=0.9558) for wild-type embryos; y=(4×10−8)x−(6×10−5)x2+0.0003x3−0.0077x2+0.0738x−0.3718x+2.9431 (R2=0.9483) for stg embryos; and y=(4×10−8)x−(6×10−5)x2+0.0003x3−0.0062x2+0.0453x−0.0232x+2.1822 (R2=0.9905) for eve embryos.

RESULTS AND DISCUSSION

Cell divisions are oriented during the fast phase of germ band extension

We monitored the timing and orientation of cell divisions in the posterior of the Drosophila germband as it comes into view when the egg is observed from its dorsal side (posterior to the fold, Fig. 1A). Embryos uniformly expressing a histone-green fluorescence protein (His-GFP) fusion protein (Clarkson and Saint, 1999) were imaged by 4D confocal microscopy. By virtue of marking chromatin, His-GFP reports on the various stages of the cell cycle in live embryos. We confirmed that mitoses take place as soon as the posterior tip of the germ band comes into view, early during germ band elongation. Although easily monitored, the orientation of the metaphase plate did not provide a reliable measure of the angle of division because it often rotates before anaphase (data not shown). Orientation of division was therefore assessed at telophase as the angle between the spindle and the ventral midline. This was measured for dividing cells within view (up to 10-cell diameter from either side of the midline) (Fig. 1B). The data was compiled in two
Fig. 2. Quantification of GBE in wild-type and mutant backgrounds. Progression of the posterior tip of the germband in wild type (wt, A–D), and in string (E–H) and eve (I–L) mutants. Representative frames are shown at 5 (A,E,I), 15 (B,F,J), 25 (C,G,K) and 180 (D,H,L) minutes. (M–P) Quantification of germband elongation (GBE) progression. This was plotted as a percentage of egg length progressed over time. The fast phase occurs during the first 25 minutes, whereas the slow phase takes place during the subsequent 70 minutes. The average from five embryos is shown (with standard error). Notice that, in string mutants (N) (cell divisions are absent), the germband elongates more slowly than in wild-type embryos (M). Total elongation is less in eve embryos than in string mutants or in wild-type embryos (P). (P) Composite of M, N and O, with the error bars removed for clarity. (Q) Average velocity of the tip of the germband in the three genetic backgrounds during the first 45 minutes of extension. Fitting curves were drawn for a better view of the data.
length in *string* mutants. Therefore, lack of cell division leads to a reduction in the elongation rate. The absence of cell division also caused increased variability in the rate of elongation. Perhaps mitoses help synchronise the mechanical behaviour of the cell. Beyond the initial phase, the germband continued to extend at a relatively slow rate in *string* mutants and never extended as much as in wild-type embryos. GBE in *string* mutants reached a maximum of approximately 55% egg length (compared with 70% for wild-type embryos). Velocity measurements (Fig. 2Q) show that *string* embryos (and to a lesser extent *eve* embryos) were specifically defective during the fast phase of elongation, whereas they exhibited a near wild-type elongation rate during the slow phase. It is unlikely that cell intercalation (or junctional remodelling) is affected on the ventral side of *string* mutants (Bertet et al., 2004; Irvine and Wieschaus, 1994; Zallen and Wieschaus, 2004). We therefore conclude that the reduction of extension seen in *string* mutants is a consequence of the lack of cell divisions. One interpretation is that mitoses could provide a driving force for elongation. Alternatively, as cells turn around the fold, they might encounter reduced resistance, which would allow longitudinal mitoses and tissue expansion.

**Local tissue deformation at the posterior of *eve*, *string* and wild-type embryos**

To assess the roles of *string* and *eve* specifically at the posterior of the germband, we studied local tissue deformation in this region. This was done by outlining 25 nuclei at the onset of GBE and assessing the deformation of this outline during a 6-minute window. Examples for wild-type, *eve* and *string* embryos are shown in Fig. 3. To help in the assessment of deformation, the aspect ratio (AR) of the outlines (before and after elongation) were crudely measured as indicated Fig. 3I. As expected, in wild-type embryos, a roughly isotropic outline becomes elongated (Fig. 3C,D) and this occurs without apparent cell intercalation (Fig. 3A,B). In *eve* mutants, elongation takes place but to a reduced extent (Fig. 3C,D). Most relevant to this paper, little deformation is seen in *string* mutants (Fig. 3E,F), confirming the key role of cell divisions in the elongation of this region of the germband. We favour the interpretation that it is the longitudinal aspect of divisions that supports elongation. However, we cannot exclude the possibility that the act of division itself (irrespective of orientation) contributes, although we note that little growth is thought to take place in *Drosophila* embryos. In any case, our results show that cell divisions (not cell intercalations) are essential for the posterior part of the germband (posterior to the fold) to elongate. As shown previously by others, cell intercalations drive elongation at the anterior of the fold. Therefore, two distinct mechanisms acting in two distinct regions ensure elongation.

**Segmental patterning polarises the orientation of cell divisions**

Embryos mutant for *eve* are defective in GBE and this is correlated with a strong reduction in cell intercalation (Irvine and Wieschaus, 1994). It is thought that the segmentation cascade could provide cells with a polarising signal that orients junctional remodelling and intercalatory behaviour (Bertet et al., 2004; Zallen and Wieschaus, 2004). Weakening of this signal in *eve* mutants would deprive cells of the cue that polarises intercalary behaviour and thus would reduce GBE. In order to find out whether *eve* activity also affects the orientation of cell divisions, we imaged mitoses at the posterior germband of *eve* mutants and, as before, compiled separate data for the first 25 minutes and the subsequent 70 minutes (Fig. 4 and see Movie 3 in the supplementary material). During the first period, the longitudinal bias of cell divisions is much reduced compared with the situation in wild-type embryos. Out of 260 mitoses, 38% occurred between 0° and 30°, 41% between 30° and 60°, and 21% between 60° and 90° (Fig. 4E). In the subsequent period, no longitudinal bias could be seen. Out of 150 mitoses, 32% were oriented along the...
DEVELOPMENT

The elongation axis (0°-30°), 27% were in the 30°-60° bracket, and 41% occurred between 60° and 90° (Fig. 4G). While performing this analysis, we noticed that the longitudinal divisions tended to take place near the midline. To verify this apparent bias, we monitored separately the orientation of cell divisions in the medial half of the germband (Fig. 4F, diagram) and in the more lateral half. Out of 130 mitoses near the midline, 52% occurred between 0° and 30°, 36% between 30° and 60°, and 12% between 60° and 90°. Out of 130 mitoses counted in the more lateral region, 23% were oriented along the elongation axis (0°-30°), 45% between 30° and 60°, and 32% between 60° and 90° (Fig. 4F). Therefore, in eve mutants, longitudinal orientation of cell divisions is preferentially lost in more-lateral cells. The residual longitudinal bias near the midline of eve embryos suggests that an eve-independent orientation cue might be present near the midline. Residual polarisation in eve mutants must come from residual segmental patterning in these embryos, because, in embryos lacking all segmental information (obtained from bicoid nanos torso-like triple-mutant females), cell divisions appeared to be completely randomised (Fig. 4H-K; see Movie 4 in the supplementary material). Anterior to the fold, lack of segmental information caused a loss in cell intercalation, whereas, at the posterior, it led to a reduction in the orientation of cell divisions.

dsh-dependent PCP is not required for oriented cell divisions or for germband elongation in Drosophila embryos

In zebrafish embryos, axis elongation involves cell intercalation and oriented cell divisions, and both processes are under the control of the PCP pathway. Indeed, no elongation takes place in fish embryos with a disrupted PCP pathway (Gong et al., 2004). As we have shown, GBE in Drosophila also follows from a combination of oriented cell divisions (posterior to the fold) and cell intercalations (anterior to the fold). However, in Drosophila, mutations in frizzled or dishevelled, two genes involved in the classical PCP pathway, have no noticeable effect on extension (Perrimon and Mahowald, 1987; Zallen and Wieschaus, 2004) (Fig. 5A-D). We assessed the orientation of cell divisions in embryos carrying a combination of dsh alleles known to cause PCP defects in the adult wing (Penton et al., 2002) (Fig. 5A). From a total of 250 fast-phase divisions (five embryos), 58% occurred at
Fig. 5. A PCP-specific mutation in dsh does not affect the orientation of cell divisions. In the same genetic background, wing hairs appear dishevelled, indicating a PCP defect (A). Nevertheless, denticle orientation appears normal in first instar larvae (B). (C,D) Orientation of cell divisions in dsh-deficient embryos during germ band extension (GBE). Data for five embryos are shown with average and standard error. For each embryo, 50 and 100 (randomly chosen) divisions were counted for the fast (C) and slow (D) phases, respectively. Longitudinal divisions are predominant during the fast phase of GBE. As in wild-type embryos, a majority of fast-phase mitoses are oriented longitudinally in dsh embryos. For each embryo, we calculated an index of longitudinal bias as the absolute slope of the line relating the angle of division to the proportion of cells dividing along that angle (obtained from linear regression). We then compared the value of this index for five dsh and five wild-type embryos and found no statistically significant difference between the two groups (t-test, P>0.05). We conclude, therefore, that the orientation of cell divisions is unaffected in dsh mutant embryos.

an angle of less than 30°, while 36% were between 30° and 60° from the midline. Only 6% of fast-phase mitoses took place at an angle between 60° and 90°. This suggests that, during the fast phase, divisions in dsh mutants are polarised, as they are in wild type (Fig. 5C,D; Fig. 1G,H; and see Movie 5 in the supplementary material). In order to compare the extent of polarisation in the two genetic backgrounds, we devised a simple index of longitudinal bias as the absolute slope of the line relating the angle of division to the proportion of cells dividing along that angle (obtained from linear regression). We then compared the value of this index for five dsh and five wild-type embryos and found no statistically significant difference between the two groups (t-test, P>0.05). We conclude, therefore, that the orientation of cell divisions is unaffected in dsh mutant embryos.

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References


