**INTRODUCTION**

Morphogenesis studies the process by which an immature tissue acquires its final form during development. During this process, cells follow different strategies using one or a combination of mechanisms that include control of cell movements, shape, death and proliferation (Conlon and Raff, 1999; Fristrom, 1988; Vaux and Korsmeyer, 1999).

The main morphogenetic mechanisms resulting in the movement of cells in the organism are migration and rearrangement. Migratory cells usually go through a mesenchymal phase, detaching from their neighbours and moving more or less independently. In contrast, during rearrangement the cells maintain their epithelial characteristics, not abandoning the tissue but changing their relative position in it. When cell rearrangements are consistently biased in one direction, one axis shortens while the other elongates causing global change of the organ's shape (Wilson and Keller, 1991).

**Drosophila grain** encodes a GATA transcription factor required for cell rearrangement during morphogenesis

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Accepted 6 September; published on WWW 24 October 2000

**SUMMARY**

The genetic mechanisms controlling organ shape are largely unknown. We show that the *Drosophila grain* gene is required during development for shaping the adult legs and the larval posterior spiracles. Mutant legs are short and wide rather than long and thin, while the spiracles are flat instead of dome-shaped. We demonstrate that *grain* encodes the GATAc transcription factor. Analysis of loss-of-function mutations at the cellular level indicates that *grain* affects organ shape by locally controlling cell rearrangement. Ectopic *grain* expression causes major morphogenetic movements, resulting in the invagination of the posterior segments into the embryo. This is the first gene that has been shown to affect epithelial morphogenesis by controlling cell rearrangements, and suggests a novel function for GATA transcription factors.

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cell rearrangement has been observed during the extension of the germ band and in the formation of the posterior spiracles, but little is known about the genetic mechanisms that regulate it (Hu and Castelli-Gair, 1999; Irvine and Wieschaus, 1994). The posterior spiracles are composed of two structures: an internal tube that forms a refringent filter, the filzkörper; and an external protruding structure that develops by cell rearrangement, the stigmatophore (Hu and Castelli-Gair, 1999).

To identify genes controlling cell rearrangements, we have studied mutations affecting the stigmatophore. Here we report that the grain (grn) gene encodes a GATA transcription factor controlling epithelial morphogenesis in the embryo and imaginal discs of Drosophila. Loss of grn function affects the shape of the posterior spiracles and legs in a way that is consistent with grn controlling cell intercalation.

MATERIALS AND METHODS

In vivo analysis of embryos

Time-lapse images were taken in a confocal microscope every 30 seconds for about 30 minutes and the position of neighbouring cells studied in 3D. The cell membranes were visualised using a UAS-GFP-GAP construct (A. Chiba and M. D. Kim, unpublished). GFP-GAP is a fusion protein attaching the miristilation domain of GAP43 to the GFP molecule which results in GFP being attached to the membrane (Moriyoshi et al., 1996). UAS-GFP-GAP flies were crossed to the 459.2-GAL4 line that drives expression in the stigmatophore. To obtain strong expression, the offspring were crossed, generating flies with two GAL4 and two GFP-GAP inserts. GFP expression was detected from stage 14 (as 459.2GAL4 expresses from stage 11, this technique adds a further delay to the GAL4 system). Embryos dechorionated in 50% bleach were staged under the dissecting microscope and mounted individually in halocarbon oil series 27. The embryos were incubated at 25°C under a coverslip fragment (0.5 cm). By mounting the embryos individually we could confirm that the embryos analysed had completed mitotic recombination.

Generation of new grn alleles

The P[24-20c] insertion was mobilised by crossing it to the grn 7L12 starter (Robertson et al., 1988). Of 75 revertants, 51 were viable over l(3)84Fi 1. Of the viable revertants, 47 gave viable progeny suggesting that they are imperfect. The P[24-20c] insertion was mobilised by crossing it to the grn 7L12 allele. Of 75 revertants, 51 were viable over l(3)84Fi 1. Of the viable revertants, 47 gave viable progeny suggesting that they are imperfect.

In situ probes and antibodies

An antisense RNA probe was made from dGATAc cDNA4 (F.-S. Tsai, unpublished). GFP.GAP is a fusion protein attaching the miristilation domain of GAP43 to the GFP molecule which results in GFP being attached to the membrane (Moriyoshi et al., 1996). UAS-GFP-GAP flies were crossed to the 459.2-GAL4 line that drives expression in the stigmatophore. To obtain strong expression, the offspring were crossed, generating flies with two GAL4 and two GFP-GAP inserts. GFP expression was detected from stage 14 (as 459.2GAL4 expresses from stage 11, this technique adds a further delay to the GAL4 system). Embryos dechorionated in 50% bleach were staged under the dissecting microscope and mounted individually in halocarbon oil series 27. The embryos were incubated at 25°C under a coverslip fragment (0.5 cm). By mounting the embryos individually we could confirm that the embryos analysed had completed mitotic recombination and hatched as normal healthy larvae.

Sequencing of grn alleles

grn alleles were sequenced using RT-PCR made from homozygous mutant embryos. To select homozygous mutant embryos each grn allele was recombined with klu-GAL4 and UAS-NodlacZ (Clark et al., 1997; Klein and Campos-Ortega, 1997). The grn, klu-GAL4 /TM6B and grn, UAS-NodlacZ/TM6B recombinant stocks for each allele were then crossed and live embryos stained with X-gal (Rastelli et al., 1993). Embryos homozygous for the grn allele, stain, as they also carry klu-GAL4 and UAS-NodlacZ. Ten homozygous embryos at stages 10-14 were hand-picked and total RNA made using Qiagen RNA columns. First-strand cDNA was generated by reverse transcriptase (Boehringer Mannheim) primed by oligo dT, using standard methods. grn cDNA was amplified using either Pfu Turbo (Stratagene) or Pfx (Gibco) with the combination of primers 5′-CGCGGGAA TTCTCTAG- and 3′-CGCCGAAATCTCTTCTGAG-ATCACGTAGATGCAGGACC. The fragment was phosphorylated, blunt-end subcloned into Ssr1 cut PCRscript (Stratagene) and sequenced.

Constructs

grn cDNA was amplified by PCR using Pfu Turbo (Stratagene) with the above oligos and blunt-end cloned into Ssr1 cut PCRscript (Stratagene). The cDNA consisted of the 5′ UTR, the complete coding sequence and included the stop codon (Lin et al., 1995). The cDNA was sequenced and subcloned into NotI KpnI cut pUAST (Brand and Perrimon, 1993). Three transformant lines inserted on the second chromosome were generated. All three lines have similar effects when expressed but they can be ordered by decreasing strength as UAS-grn 2; UAS-grn 12 and UAS-grn 11. To test the effect of ectopic GRN, homozygous lines carrying one or two UAS-grn 2; UAS-grn 12 constructs where crossed to GAL4 driving lines at either 25°C or 29°C.

RESULTS

The cells of the stigmatophore express the sal gene before their morphogenesis starts. Using this marker in fixed embryos it has been shown that from stage 12 to stage 15, the location of the sal expressing cells changes in a manner consistent with the cells rearranging their position with their neighbours. This movement is a major morphogenetic mechanism for spiracle formation as it both contributes to close the spiracle and to elongate the stigmatophore (Fig. 1A; Hu and Castelli-Gair, 1999). The process is similar to the change of shape produced...
by folding a pearl necklace twice: the internal area decreases while the necklace width increases from one to four tiers.

**In vivo observation of cell rearrangements**

To find out the speed of the cell rearrangements we analysed living embryos. We marked the spiracle cells using a GFP.GAP fusion protein that localises to the cell membrane. The fusion protein was expressed in the stigmatophore using the 459.2GAL4 line, an enhancer trap line where GAL4 has probably inserted in the *sal* gene. The overall shape of the stigmatophore cells did not change during the period of rearrangement.

Using time-lapse recording in a confocal microscope we studied the position of individual cells from stage 14. The study was complicated by the various morphogenetic movements occurring at this time (final stages of germ band retraction, dorsal closure and spiracle formation movements) that take the cells in and out of the focal plane. In four embryos, we were capable of following groups of cells for at least 30 minutes. These observations showed that the cell rearrangement movements did not occur at a uniform speed. In all cases, some cells kept their relative positions during the period analysed, while others rearranged. Fig. 1 shows two groups of cells that could be continuously followed for half an hour. During the recording period the four cells labelled as ‘a’ in Fig. 1B maintained their relative positions, while in the group labelled ‘b’, one cell shifted position with respect to its neighbours. The shift occurred during the last 15 minutes after a static period. These results show that the spiracle cell rearrangements occur discontinuously. During the recorded period the shape of the stigmatophore changed very subtly, in agreement with the pace at which cell rearrangement is observed.

**grn is required for the cell rearrangements in the stigmatophore**

To find out the identity of the genes affecting cell rearrangement, we have studied mutants affecting the formation of the stigmatophore. *grn* has been identified in a mutagenesis designed to find zygotic lethal mutations in *Drosophila* (Jürgens et al., 1984). Two embryonic lethal alleles with similar phenotypes were isolated, *grn*7L12 and *grn*7J86. Embryos mutant for *grain* had defects in the head skeleton and in the posterior spiracles (Fig. 2). The head skeleton was mostly affected in the dorsal bridge and in the lateralgräten, structures that derive, respectively, from the acron and the mandibular segment of the embryo (Jürgens and Hartenstein, 1993). The posterior spiracles have a shorter filzkörper and do not form the protruding stigmatophore.

To determine the primary cause of the defects in *grn* mutants we first determined whether this gene is required for the specification or the morphogenesis of the posterior spiracles. *sal* and *cut* (*ct*) are the first genes required for posterior spiracle patterning. In *grn* mutants, the activation of both genes was not affected, showing that the patterning of the spiracles was normal (Fig. 3 and not shown). Analysing the position of *sal*-expressing cells at different stages is a good way to follow the cell rearrangements in the stigmatophore (Hu and Castelli-Gair, 1999). In the wild type, the number of cells directly abutting the invaginating spiracular chamber was approximately 25 at stage 12. After 5.7 hours of development (depending on the temperature) the number of stigmatophore cells abutting the spiracular chamber decreased to eight and the number of tiers increased because the cells had shifted position (Fig. 3). In *grn* mutants, the spiracle did not close and similar cell counts showed that neither the number of cells abutting the spiracular chamber nor the number of cell tiers changed during development (Fig. 3). As this result suggests that *grn* is required for cell rearrangements during morphogenesis, we decided to characterise the gene in detail.

**Genetic identification of grn**

To localise *grn*, we used 13 deficiencies that delete between the cytological regions of 84A to 85D (Fig. 4). Deficiencies deleting the 84F1-2 bands are lethal over *grn*. The 84D-F region has been mutagenised to saturation and a detailed complementation map obtained (Baker et al., 1991). Crosses of *grn* alleles to mutations in 11 complementation groups indicate that *grn* is allelic to *l(3)84FdH11* (henceforth referred to as *grn*84Fd). As *grn*84Fd was the only gene found in the region
Fig. 2. Cuticle phenotypes of wild-type and grn mutant embryos. Heads and tails of late wild-type (A,B) and grn embryos (C-F). The head skeleton (A) forms by the fusion of several segments after head involution (arrowhead points to the dorsal bridge in A-C). In the posterior spiracles (B), the filzkörper (black arrowhead in B-F) is located inside the protruding stigmatophore (white arrowhead in B-F). In the grn mutant embryos (C,D) the head skeleton is abnormal with aberrant lateralgräten and dorsal bridge (C), the stigmatophore does not protrude and the filzkörper is short (D). Other cuticular structures are normal. Embryos with the overlapping deficiencies Df(3R)p13/Df(3R)dsx10M that remove the grn gene (E,F) show the same phenotype as grn7L12.

deleted by the overlapping deficiencies Df(3R)p13 and Df(3R)dsx10M (Baker et al., 1991), we compared the phenotype of flies double heterozygous for these deficiencies with that of the original grn alleles. The phenotype of grn7L12 and grn786 in the spiracles was indistinguishable from the double deficiency (Fig. 2). Germline clones showed that grn was not required maternally (not shown).

The P-element insertion P[24-20C] was lethal over grn alleles. This P-element has been report (under the synonym P812) to be inserted 34 bp upstream of the transcription start site of the gene encoding the GATAc protein (Lin et al., 1995). GATAc RNA is expressed, among other places, in the primordia of the head and the posterior spiracles (Fig. 5A and Lin et al., 1995) suggesting that the grn alleles could be mutations in GATAc. As the P[24-20C] insertion showed no embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele. We mobilised the insertion to generate embryonic phenotypes, we reasoned that it could be a weak hypomorphic allele.

The grn alleles could be ordered according to the severity of their phenotype on head and spiracle. The overlapping deficiencies, grn786 and grn7L12, represented a complete lack of function (Fig. 2). The other alleles were hypomorphic mutations: grnSPJ9 and grnSPJ1 had intermediate phenotypes; and grn84-20C was the weakest.

Molecular characterisation of the grain alleles

We have amplified and sequenced the gene encoding GATAc in grn7L12 homozygous embryos. Using RT-PCR with oligos specific for the GATAc sequence (Lin, et al., 1995), we found that the GATAc cDNA in grn7L12 had an 11 bp deletion of bases 379-389. This deletion introduced a frameshift and produced a truncated protein that was identical to wild-type GATAc over the first 52 amino acids, but aberrant after the deletion and lacking both zinc fingers (Fig. 4B). This result proves that grn encodes GATAc.

grn expression in embryos and larval tissue

At late blastoderm stage, grn transcripts were prominently expressed in the procephalic region that forms the acron (data not shown). This region requires grn function during development as indicated by the head phenotypes observed in grn mutants. At the extended germ band, grn was expressed in the CNS, in the primordia of the anterior and posterior midgut, and in a dorsolateral stripe running anteroposteriorly at the level where the tracheal spiracular branches join to the ectoderm (Lin et al., 1995; Fig. 5). In the posterior spiracles, expression was detected in a subset of the cells that give rise to the spiracular chamber, accounting for the abnormal filzkörper in grn mutants; and in an area coinciding with the sal-expressing cells, accounting for the abnormal stigmatophore. We have analysed grn null mutant embryos using the neural marker 22C10 antibody and HNT, a protein expressed in the endoderm (Yip, et al., 1997; Zipursky, et al., 1984). Neither the CNS nor the gut showed any obvious abnormalities.

At the wandering larva stage (just before pupariation) grn transcripts were detected in the imaginal discs from which the adult ectodermal structures form. In the leg discs, high levels were seen in the region that gives rise to the proximal parts of the leg (femur and tibia), with the exception of a small patch of cells (Fig. 5E-G). Lower levels of grn were expressed in the central parts of the disc that form the tarsal segments. In the wing and haltere discs, grn was expressed in the areas that form the hinge, with little or no expression in the wing pouch. grn...
Control of shape by a GATA transcription factor

Requirement of grn in imaginal tissues

As grn mutants were embryonic lethal, we used mosaic analysis to study the function of grn in imaginal tissues. We induced clones using the minute technique (Morata and Ripoll, 1975), which generates clones occupying large areas of the adult tissue, owing to growth advantage from the surrounding cells. Clones of grn\textsuperscript{T986}, grn\textsuperscript{T112} and grn\textsuperscript{SP9} alleles gave identical results and will be considered together. We did not detect any abnormalities when the mutant clones occupied the wing or the distal leg segments. In contrast, grn clones on the proximal leg segments severely affected the shape of the femur and tibia.

Out of a total of 25 M\textsuperscript{+} forked clones generated in the leg, 13 affected the shape of the femur and tibia, which became shorter and broader than in the wild type (Fig. 6A,B). In these cases the segments were approximately one third shorter and one third broader, but leg segmentation was normal, indicating that grn does not affect leg patterning. To find out if the reason for the different shape was due to an abnormal packaging of the cells, we counted the leg trichomes. In the leg, each trichome is formed by a single cell. Therefore, by comparing the distribution of the trichomes in a region occupied by a grn clone with the distribution of trichomes in the same region of a wild type leg, we could indirectly find out if the abnormal leg shape was due to shape changes of the individual cells. We observed that the number of cells and their spatial distribution in grn clones did not differ from the wild type (Fig. 6D,E and legend), indicating that the shape change was not caused by a change in cell density.

As grn is proximal to the forked duplication used to label the clones, we expect that some clones marked with forked will not be grn. This may account for some of the forked clones that did not affect the leg shape, but it is unlikely that it will account for all. The high number of forked clones that did not affect leg shape could be explained if some grn clones were induced in areas of the leg where grn was not expressed (Fig. 5I). Alternatively, grn requirement could be non cell autonomous and some mutant clones could be rescued by the wild-type neighbouring cells.

To test if the absence of grn phenotypes was due to non autonomy, we made smaller non-minute grn clones and compared their size with control clones generated at the same stages. Wild-type control clones generated at second larval stage occupied long and thin stripes of tissue when labelled either with forked or yellow cuticle markers. Of 17 control forked or yellow clones induced on the femur or tibia, all occupied more than one leg segment, with most of them (13) occupying a long and narrow line running along femur and tibia. None of the control clones generated at this stage occupied a single leg segment. These results are in agreement with those previously published (Bryant and Schneiderman, 1969). In contrast, in a grn background out of 18 forked or yellow clones induced on the femur or tibia, more than half of them (10) were short and broad. Of the latter, eight occupied a single segment and the two that occupied two segments were localised straddling the femur and tibia junction (Fig. 6C). These results suggest that wild-type neighbouring cells cannot rescue grn mutant clones and that the reason why only half of the clones show grn phenotypes is due to the fact that not all leg cells express grn.

The grn clones marked with forked were generated in a
Fig. 4. Genetic and molecular identification of grn. (A) Deficiencies used for the chromosomal location of grn. All grn alleles are lethal over deficiencies deleting the 84F2 region. The double heterozygous combination of Df[p13]/Dfdsx10M results in the exclusive deletion of 84F1-2. (B) GRN protein compared with the predicted truncated protein formed in the GRN7L12. The mutant protein diverges from the wild type after 52 amino acids and lacks both zinc fingers.

Ectopic expression of grn

To test the effect of ectopic grn expression we have made UAS-grn constructs. First we analysed if the constructs are capable of rescuing the grn mutant phenotype. We made flies simultaneously mutant for grn and carrying the 459.2-GAL4 and UAS-grn constructs. UAS-grn expression driven by 459.2-GAL4 rescues the mutant phenotype in the stigmatophore (Fig. 7C), proving that the construct produces functional protein.

We next investigated the effects of expressing GRN in areas outside of its normal pattern. Ectopic expression of grn using several GAL4 driver lines cause lethality. Analysis of the embryos using different driver lines show some common features: the ventral denticle belts tend to disappear, head involution is distorted and in a small percentage of the embryos the tail invaginates abnormally.

We studied in detail these defects using the h-GAL4 line, a pair-rule gene that drives expression in alternate segments (Brand and Perrimon, 1993) using either one or two UAS-grn inserts. As expected, the penetrance of the transformations increased with two UAS inserts, although the phenotypes were not qualitatively different.

In most h-GAL4 UAS-grn larvae, the denticle belts of the alternating segments were reduced or missing, leaving a naked cuticle area. We did not see any indication that the denticle cells have invaginated or that this is a segmentation defect, as staining with anti-EN antibody reveals that the segments form normally (Data not shown). The head skeleton is abnormal in about 9-32% of the embryos and tail invagination is observed in 6-20%, depending on the number of inserts and the temperature of the culture (Materials and Methods). Closer examination of the embryos with invaginated tails reveals that the spiracles are now at the end of a long tubular structure formed by segments posterior to A8 that normally would lay in the outside of the embryo. This structure includes cells from A8, A9, A10 and the telson (Fig. 7D). Although due the absence of specific cell markers we cannot know if these defects are due to abnormal cell rearrangement, these results show that ectopic grain expression affects morphogenetic movements during gastrulation.

DISCUSSION

GATA zinc-finger proteins were identified as transcription factors required for the expression of tissue-specific genes (Evans and Felsenfeld, 1989; Tsai et al., 1989). Subsequent studies have shown that they play a central role during development. Six different genes encoding GATA proteins have been isolated in vertebrates, where they are required for the development of CNS, heart, endoderm and haematopoietic system (Kuo et al., 1997; Molkentin et al., 1997; Morrisey et al., 1998; Simon, 1995).

To date, three genes encoding GATA proteins have been
reported in *Drosophila*, for which mutations had been previously isolated in only two. GATAaa, encoded by the *pannier* (*pnr*) gene, is required for the development of extra-embryonic tissue and dorsal ectoderm derivatives of both embryo and adult (Heitzler et al., 1996; Ramain et al., 1993; Winick et al., 1993). GATAb, encoded by the *serpent* (*srp*) gene, is required for the development of the gut and the fat body, as well as the extra-embryonic tissue (Abel et al., 1993; Rehorn et al., 1996; Reuter, 1994; Sam et al., 1996). The lack of mutations for GATAc has prevented functional studies (Lin, et al., 1995). In this paper we prove that GATAc is encoded by the *grn* gene (Jürgens, et al., 1984). We have identified a number of alleles and show that *grn*1L12, which genetically produces a complete lack of function, has an 11 bp deletion that should encode a truncated GATAc protein lacking both zinc fingers.

*grn* is expressed in the CNS, midgut and lateral ectoderm of wild-type embryos (Fig. 5). Embryos homozygous for *grn* null alleles complete embryogenesis but the larvae are unable to hatch. At the resolution we have studied, these embryos formed an apparently normal CNS and gut. Analysis of the cuticular structures revealed defects in head development and posterior spiracles. Spiracle patterning gene expression is normal in *grn* mutant embryos but the morphogenesis is affected. Lack of *grn* in the imaginal tissues also affects adult ectodermal derivatives. Legs with large *grn* clones have all the normal segments but the femur and tibia become short and broad. The formation of legs with all segments suggests that the patterning genes required for leg development are also unaffected, and that *grn* is required for morphogenesis.

**grn is required for cell rearrangement during morphogenesis**

Small amounts of cell rearrangement may have strong effects on organ shape. In the posterior spiracle of *Drosophila*, when the cells that form the tubule connecting to the trachea invaginate, the epithelial integrity remains intact by rearrangement of the surrounding cells (Hu and Castelli-Gair, 1999). This rearrangement process in the stigmatophore results in a decrease from 25 to eight in the number of cells abutting the invaginating tissue (Fig. 3). This decrease can be obtained if cells rearrange twice between stage 12 and stage 15, a period that lasts 5 hours at 25°C (or 7.5 hours at 20°C, the temperature at which we carried out the in vivo analysis). Our in vivo observations show that cell rearrangement in the spiracle does not occur synchronously. Cells alternate static periods with others of rearrangement that can last about 15 minutes (Fig. 1B).

Scoring the spatial distribution of cells in the stigmatophore of *grn* mutant embryos, showed that their position remained almost unchanged from stage 12 to stage 16 (Fig. 3). This observation suggests that *grn* may be required for the cell rearrangements. Evidence from previous studies suggests that the leg, which requires *grn*, also develops using cell...
Fig. 6. Effect of *grn* clones on leg shape. Legs carrying a homozygous *grn*7J86 (A) or *grn*3L12 (B) clone in the femur and tibia affect the shape of the leg but not the number of segments. In A and B, the left and right legs of the same fly have been mounted for comparison. Note that the leg carrying the clone (red arrowhead) has a shorter and broader femur and tibia than the wild-type leg (black arrowhead). (D,E) Higher power photos of the legs in A. Note that the cell density inside the homozygote *grn* clone (D) is the same as that of the wild type leg (E). The base of the trichomes has been highlighted inside the circled area. The number of trichomes are 137 and 139 in the mutant clone and 134 and 130 in the wild-type area. (C) A leg with a yellow clone and a *grn*, forked twin clone (the clones have been outlined in yellow and black respectively). Although both clones were induced simultaneously the yellow clone is elongated in the proximo-distal axis whereas the forked, *grn* clone is shorter and broader. The yellow clone has grown normally beside the *grn* clone, showing that the *grn* defect is cell autonomous. The leg in C was folded but the image has been artificially straightened to save space in the figure. This leg is shorter than its wild-type control (not shown). (F) The width and length of the femur and tibia of the *grn* legs shown in A and B compared with those of their internal control (red bar, *grn* leg; black bar, wild type leg). The bars were traced over the leg segments of A and B, and then aligned side by side to allow size comparison. (G,H) *grn* and wild-type twin clones in the imaginal disc before leg eversion. The heterozygous *grn*7L12 disc is labelled with medium levels of GFP expressed from a ubiquitin-GFP construct. Recombination results in twin clones, one homozygous for *grn*7L12 cells lacking GFP, which look black, and a wild-type clone with stronger GFP signal, owing to the expression of two copies of the homozygous construct. Both elements of the twin have been encircled by a white line. Note that both clones have a similar shape and size, and that, at this stage, the wild-type clone is still not thin and long as the yellow clone in C. (G) Two twin clones in a L2 leg disc. (H) A twin clone in a L1 leg disc.

rearrangement. First, mosaic analysis shows that clones generated in the leg imaginal disc always occupy a long and thin stripe that runs from proximal to distal in the adult leg (Bryant and Schneiderman, 1969). These clones can be 100 cells in length, without exceeding three cells in width. This shape is markedly different from the shape of clones in the imaginal disc (Fig. 6G-H, GFP twin spot). Second, in many cases leg clones are split by neighbouring cells that have intercalated breaking the continuity of the clone (Bryant and Schneiderman, 1969). Third, it has been shown that bristles adjacent in the adult leg originate from cells that were not adjacent in the leg primordium (Held, 1979). Finally, direct scanning electron microscope (SEM) observations at the stage when the leg discs evert, suggest that there is cell intercalation (Fristrom, 1976).

The similarity of the effects controlled by *grn* in leg and spiracle suggest that both phenotypes are due to the control of cell rearrangement. The short and wide shape of legs formed by *grn* mutant cells can be accounted for by an alteration in cell rearrangement in the primordium, resulting in a leg with altered shape. This hypothesis is supported by the shorter and broader appearance of *grn* clones compared with wild-type clones generated at the same stage. This effect is most evident when the clones are generated simultaneously in twin analysis (Fig. 6C). The different shape of these clones was not due to cell death, as shown by the fact that *grn* clones could form most of the leg. The involvement of *grn* in the control of cell shape can also be discarded as a cause for the different shape of *grn* clones by observing the distribution of the cells in mutant clones. In *Drosophila*, each cell in the leg forms a single trichome; therefore, an altered distribution of the trichomes in the leg would indicate a change in the apical shape of the cells forming them. Close observation of the cuticle in legs with *grn* clones shows that trichome density in all axes was unaffected, indicating that the cell shape had not changed. Most of the leg cell divisions have ended by third instar larva, and we have shown that *grn* is required after this stage, discarding a function of *grn* in controlling spindle orientation. Similarly, *grn* is required in the spiracle after the divisions have finished. These results allow us to conclude that *grn* has a role in the control of cell rearrangement during morphogenesis and that abnormal convergent extension in the epidermis is the cause for the defects observed in the shape of the leg and the posterior spiracle.

*grn* can modify morphogenetic movements

During normal gastrulation the posterior 10% of the blastoderm invaginates, forming the primordium of the posterior midgut and the hindgut. The area immediately anterior to that (from 10% to 20% of the blastoderm length as measured from the posterior end) does not invaginate, forming the external cuticle of A8, A9, A10 and the unsegmented telson (Jürgens, 1987; Jürgens and Hartenstein, 1993). Ectopic *grn* expression results in the invagination of this area, doubling the amount of blastoderm tissue that invaginates at the posterior.
end of the embryo. This major morphogenetic movement occurs without a change in the specification of the segments, which form the normal cuticle structures but in internal positions. This observation and the normal patterning of legs with mutant clones indicates that the main function of grn is to control the morphogenetic movements of the cells rather than to specify the structure they will form. This contrasts with the function of serpent and the Hox genes, which affect morphogenesis indirectly by changing the fate of the cells. Thus, grn belongs to a new category of genes that controls the morphogenesis of organs without affecting their early specification during development.

Control of cell rearrangement in other organisms

The conservation of the GATA family in all animals, suggests they may control cell rearrangement in other organisms. In vertebrates, GATA factors are associated with blood formation and the morphogenesis of endoderm, CNS and heart (Kuo et al., 1997; Molkentin et al., 1997; Morrisey et al., 1998; Simon, 1995), but to date there have been no reports associating them with the control of convergent extension. However, this association cannot be ruled out as the mutants have not been studied at the cellular level. Also, the expression of GATA factors in overlapping patterns in vertebrates (Read et al., 1998), suggests that redundancy could be obscuring possible effects on cell rearrangements. In this respect, it has been shown that GATA3 is capable of partially rescuing lack of function of GATA1 in mouse erythroid cells (Tsai et al., 1998).

In zebrafish, some mutants have been reported to block convergent extension of mesodermal derivatives. The trilobite and knypek mutants form somites that are broader and shorter than the wild type (Marlow et al., 1998). As yet these genes have not been cloned. Mutations for the zebrafish spadetail gene also affect convergence of the trunk somitic mesoderm towards the midline (Kimmel et al., 1989). spadetail encodes a T-BOX transcription factor (Griffin et al., 1998). This could either be interpreted as vertebrates and invertebrates using different class transcription factors to control convergent extension; or alternatively, as different classes of transcription factors controlling convergent extension in different germ layers. We favour the second possibility, as cell rearrangement in an epithelium has to involve different mechanisms from those used by mesenchymal tissues where specialised apical adhesive junctions are not present.

It is likely that other genes besides grn regulate cell rearrangement in Drosophila. For example, the extension of the germ band is driven by cell rearrangements in the ventral ectoderm (Irvine and Wieschaus, 1994), a tissue that does not express grn. The normal development of the endoderm in mutant embryos also suggests a possible redundancy of grn. The midgut is formed by an external layer of visceral mesoderm lined by a layer of endoderm. During development, the endoderm cells spread over the visceral mesoderm until they cover it. During this process cell rearrangements must occur in the endoderm, suggesting that grn would be controlling them. Although grn is expressed during midgut formation, the gut epithelium forms normally in grn mutants. It would be interesting to test if the GATAb protein, which is expressed in the gut, can substitute for grn.

The study of downstream targets of grn should further our understanding of the mechanisms involved in the regulation of cell rearrangement during morphogenesis.

We thank A. Hidalgo, J. F. de Celis, Y. N. Jan, the Tübingen and Bloomington stock centres; P. Lawrence, N. Brown, Y. N. Jan, R. Schuh and M. Ruiz Gómez for antibodies; C. Mirth and B. Sanson for suggestions and M. J. Sánchez, N. Hu, C. Sharpe and D. St Johnston for comments on the manuscript. We are grateful to F. S. Tsai for the GATAc cDNA and for communicating unpublished results, and to Akira Chiba and Michael Dom Kim for letting us use the UAS-GFP.GAP construct. This work was supported by The Royal Society and The Wellcome Trust.
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