Zygotic Drosophila E-cadherin expression is required for processes of dynamic epithelial cell rearrangement in the Drosophila embryo

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Dynamic epithelial reorganization is essential for morphogenesis of various organs. In Drosophila embryos, for example, the Malpighian tubule is generated by cellular rearrangement of a preexisting epithelium and the tracheal network is formed by outgrowth, branching, and fusion of epithelial vesicles. Here we report that the previously identified locus shotgun (shg) encodes DE-cadherin, an epithelial cell–cell adhesion molecule of the classic cadherin type and that zygotic shg mutations rather specifically impair processes of the dynamic epithelial morphogenesis. In the mutants, the Malpighian tubule disintegrated into small spherical structures, and the tracheal network formation was blocked in selected steps. The malformation of these organs could be rescued by overexpression of DE-cadherin cDNA under a heat shock promoter. Unexpectedly, the zygotic null condition did not severely affect general epithelial organization; most epithelial tissues maintained not only their cell–cell associations but also their apicobasal polarity in the mutants. The zygotic null mutant retained a certain level of maternally derived DE-cadherin molecules until the end of embryogenesis. These results suggest that zygotic DE-cadherin expression is critical for the rearrangement processes of epithelial cells, whereas the maternally derived DE-cadherin may serve only for the maintenance of the static architecture of the epithelia.

[Key Words: DE-cadherin; tubulogenesis; cell rearrangement; Drosophila]

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Epithelial cells can reposition themselves without breaking the cell layer. This type of cellular rearrangement plays an important role in the production of tissues or organs of diverse morphology (for review, see Gumbiner 1992). A well-known example is amphibian gastrulation, in which a process of intercellular movement, called convergent extension, causes a dramatic elongation of the mesodermal tissue (Keller et al. 1992). In Drosophila embryos, a similar mechanism operates for morphogenesis of tubular organs. These include Malpighian tubules (MTs) and probably tracheal trees as well, both of which are of ectodermal origin and simple epithelial monolayers. MTs arise from the posterior region of the hindgut (for review, see Skaer 1992, 1993). Primordia of the tubules first grow out by cell divisions, and when the proliferation is complete, 12–14 cells encircle the lumen. Subsequently the cells start shifting their relative positions, generating elongated tracts with only two to three cells surrounding the lumen. Embryonic tracheal development is initiated by invagination of a cluster of cells in each of hemisegments T2 through A8 (for review, see Manning and Krasnow 1993). Each cluster gives rise to one metameric unit, which undergoes branching and outgrowth. Then a subset of branches fuses to one another to establish the basic framework. Once the invagination takes place, no further mitosis is observed, so this tracheal patterning most likely depends on cell rearrangement and/or cell shape change, although cellular mechanisms for individual branching processes have not been studied in detail.

In expectation of its possible functions in epithelial morphogenesis, we identified previously a Drosophila counterpart of the major vertebrate epithelial cadherin [E-cadherin] and designated it as DE-cadherin (Oda et al. 1994). The vertebrate E-cadherin belongs to a subgroup of the cadherin superfamily (for review, see Takeichi 1995). Members of this subfamily are named classic cadherins and they form multigene complexes with at

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least two classes of cytoplasmic molecules, α-catenin and β-catenin. This assembly is essential for the cell adhesion activities of cadherins (Hirano et al. 1992; Kawanishi et al. 1994; Oyama et al. 1995). Besides having structural similarities between itself and the vertebrate classic cadherins, DE-cadherin was shown to mediate cell adhesion in vitro and bind catenins, α-catenin (Oda et al. 1993) and Armadillo (Arm), a β-catenin homolog (Peifer and Wieschaus 1990; McCrea et al. 1991). Arm was originally discovered as the product of a segment polarity gene belonging to the wingless (wg) class and is considered to have dual roles as an element of the adhesion apparatus and a transducer of the Wg signal reception (for review, see Hinck et al. 1994; Peifer 1995). DE-cadherin is expressed in all embryonic ectodermal epithelia, subsets of neural and mesodermal cells, and endodermal cells (Oda et al. 1994; H. Oda, and T. Uemura, unpubl.). At boundaries between epithelial cells, DE-cadherin is localized at the position of the actin-based zonula adherens (ZA); this suggests that DE-cadherin is a component of ZA, as is the case for the vertebrate E-cadherin (Tsukita et al. 1992; Tepass and Hartenstein 1994a). In invertebrates, three other molecules of the cadherin superfamily were reported: Fat [Mahoney et al. 1991], Dachsous [Clark et al. 1995], and the toxin receptor BT-R1 [Vadlamudi et al. 1995]. However, their roles in cell adhesion remain to be investigated.

Vertebrate classic cadherins have been shown to be crucial for the integrity of polarized epithelial architecture (for review, see Eaton and Simons 1995). This was demonstrated by a number of methods, including cDNA transfection of cell lines (McNeill et al. 1990; Watabe et al. 1994), expression of dominant-negative constructs [Kintner 1992, Levine et al. 1994, Hermiston and Gordon 1995; Lee and Gumbiner 1995], injection of antisense oligonucleotides [Heasman et al. 1994], and targeting of the mouse gene [Larue et al. 1994; Riethmacher et al. 1995]. However, functions of cadherins in dynamic organogenesis have not been critically investigated in vivo by means of loss-of-function mutations. In this study we have isolated and characterized complete or partial loss-of-function mutants of the DE-cadherin gene. Phenotypes of these mutants indicated that zygotic expression is crucial for processes of cell rearrangement, including the extension of MTs and tracheal tree patterning. Unexpectedly, these epithelial tissues did not dissociate into single cells in the zygotic null mutant, nor were most of the other epithelial organs so severely affected by the mutation.

Results

DE-cadherin is encoded by shotgun

We cytologically located the DE-cadherin gene (cadE) very close to or in the chromosomal band 57B13-14. We found that two partially overlapping chromosomal deletions, Df(2R)D17 (O’Donnell et al. 1989) and Df(2R)E2 [Kraut and Campos-Ortega 1996], uncovered this gene [Fig. 1A]. The transheterozygote of these two deficiencies (E2/D17) thus represented a complete loss-of-function mutant for cadE, and it was designated as the zygotic null mutant or simply null mutant m this study. Within that overlap interval, we mapped an embryonic lethal mutation, shotgun (shg), which had been isolated by mutant searches for aberrant cuticle patterns (Nüsslein-Volhard et al. 1984). As described below, we isolated a P-element allele of shg in which the transposon was inserted in the DE-cadherin gene. Furthermore, we could rescue shg phenotypes by DE-cadherin cDNA expression. Therefore, shg most likely encodes DE-cadherin.

Besides the null mutant, we studied three embryonic lethal shg alleles [shg<sup>2</sup>, shg<sup>217b</sup>, and shg<sup>1H1N-1</sup>], and one semilethal allele [shg<sup>2P10460</sup>; see Materials and methods]. The embryonic lethal alleles were partial loss-of-function mutants; shg<sup>2</sup> showed intermediate defects and shg<sup>217b</sup> and shg<sup>1H1N-1</sup> gave weaker phenotypes. The shg<sup>2P10460</sup> had an insertion of a PZ element [Moldzik and Hiromi 1992] behind nucleotide 265 in the 5′-untranslated region of the DE-cadherin cDNA [Fig. 1B]. Revertant analyses confirmed that this insertion did cause a
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shg mutation: Of 32 independent revertant lines, 10 were completely viable both in a homozygous condition and in a trans relation to shg^p10469. Genomic Southern analyses indicated that the excision appeared to be precise in eight strains and near precise in 2 of the 10 strains (data not shown). The remaining 22 lines were homozygous lethal, and most of them produced embryos of weak shg phenotypes.

We immunostained mutant embryos for DE-cadherin. In ectodermal epithelia of the wild-type embryo, DE-cadherin was restricted to an apical position of cell–cell contacts, and its distribution was segregated from the territory of fasciclin III, a marker for the septate junction, which is located in the subapico-lateral region (Patel et al. 1987; Woods and Bryant 1993; see Fig. 2A). The shg^E17B [Fig. 2B], shg^SHN-1, and shg^p10469 embryos exhibited normal apical distribution of DE-cadherin; but the signal intensity in the shg^p10469 homozygotes was slightly reduced compared to that in heterozygotes. Embryos of E2/D17 showed no intense immunostaining for DE-cadherin (Fig. 2C), although maternally derived molecules could be detected by Western analysis as described in the last section of this paper. In this null mutant, fasciclin III patterns were normal [Figs. 2E,F], suggesting little effect of this mutation on septate junction formation. shg^E showed an abnormal staining pattern for DE-cadherin; most signals were diffusely scattered in the cytoplasm although its apical concentration was also observed to some degree (Fig. 2D). Western analyses indicated that the shg^E mutant cadherin molecule was partly degraded in vivo (Fig. 2C, below). The shg^E product is probably mutated in such a way as to become more susceptible to proteolysis.

DE-cadherin is present in all epithelia in the normal embryo; therefore, extensive epithelial defects had been expected in the mutants. However, gross disorganization of epithelial tissues was limited to the head and ventral epidermis, Malpighian tubules, and tracheal ducts. In the null mutant embryo, ventral and head regions failed to form contiguous epidermis; its disruption became obvious at stage 11, and most cuticles were missing in those regions at the end of embryogenesis [Fig. 3B]. In the remaining ventral cuticle, some denticles were formed and they were flanked by naked regions. This pattern was not similar to that of a strong loss-of-function arm mutant that produced ectopic denticles at the expense of naked regions (Klingensmith et al. 1989). The cuticles of the shg^E mutant were similarly destroyed and tended to wrinkle. In 50% of the weak shg^E17B mutants, one or two small holes per embryo were seen in the cuticle [Fig. 3C]. Mutant embryos of all the alleles showed defects in head involution and did not hatch, whereas no rupture was observed in their dorsal epidermis, as confirmed by staining for fasciclin III [Fig. 2F].

Figure 2. Subcellular localization of DE-cadherin and fasciclin III in the deficiency and shg mutant embryos [stage 15]. [A] A wild-type hindgut was double-labeled for DE-cadherin [green] and a marker for the septate junction, fasciclin III [red]. [B–D] DE-cadherin staining of hindguts in shg^E17B [B], E2/D17 [C], and shg^E [D]. Confocal images were adjusted to increase brightness for C and D relative to B. [E,F] Staining for fasciclin III in the hindgut [E] and dorsal epidermis [F] of the deficiency embryo. Note apparently normal distribution of fasciclin III in these tissues. Arrows in F demarcate the dorsal midline. Scale bar, 30 μm in A and 50 μm in B–F.
To study whether the mutant phenotypes could be rescued by DE-cadherin expression, we attempted expression of DE-cadherin cDNA under a heat shock promoter (hsp-cadE). In both the null and shg<sup>2</sup> backgrounds, the cDNA expression restored the destroyed ventral cuticles, although the head defect was not recovered perfectly (Fig. 3D). In the following three sections, we focus on the phenotypes of MTs and tracheal ducts.

**Disruption of MTs in shg mutants**

The development of the MT is initiated by local evagination from the hindgut primordium (Skaer 1993). Following cell proliferation, cell rearrangement drives tubular elongation. Both mechanisms are regulated by specialized cell types known as tip cells (Skaer 1989). As mentioned above, the MT structure was destroyed in the shg null mutant [Fig. 4B]. However, the tip cells were present and differentiated into neurons in this mutant, as in the wild type (data not shown). We studied malformation of this organ by use of an antibody to a transmembrane protein, Crumbs (Crb), located at the outermost apical region of cell–cell boundaries as well as on the luminal surface (Tepass and Knust 1993; Wodarz et al. 1995). A structural abnormality of the MT was first detected at late stage 12 or early stage 13, when the proliferation is still continuing in the wild type. At these stages, instead of having the slender MT morphology of the normal embryo [Fig. 4C], the MT of the null mutant was rounded [Fig. 4D] and the single-layered epithelium was locally kinked [Fig. 4E]. When the mutant entered the initial stage of cell rearrangement (late stage 13), the tissues started falling apart and eventually disintegrated into small cell clumps [Fig. 4G], although they did not disperse into single cells even at stage 17. Each spherical structure was composed of several cells, and cells in the aggregate adhered to each other. Interestingly, the individual aggregate was a closed vesicle with Crb concentrated on its inner surface. Therefore, the MT cells appeared to preserve apical polarity.

Further evidence for the conservation of cell polarity was provided by monitoring intracellular distribution of a membrane cytoskeletal element, β<sub>heavy</sub>-spectrin (Thomas and Kiehart 1994). When embryos were fixed by heat methanol treatment [Miller et al. 1989], the cytoplasmic pool of β<sub>heavy</sub>-spectrin was washed away, resulting in an exclusive localization of the remaining molecules at an apical site of cell–cell contacts (Thomas and Kiehart 1994; as shown in Fig. 4H). In the mutant MT cell cysts, the β<sub>heavy</sub>-spectrin-enriched domain was located inside the aggregates, as seen in the case of Crb staining [Fig. 4I, arrows].

At the end of embryogenesis, the MT cells secrete uric acid, which accumulates in the lumen and thus makes this organ visible under polarized light. The null mutant produced no trace of the tubular structure [Fig. 5B]; as opposed to the wild-type, elongated tracts filled with the crystal [Fig. 5A]. A small fraction (~10%) of shg<sup>2</sup> embryos was able to generate short and discontinuous tubes [Fig. 5C]. In the mutant embryos with the null or shg<sup>2</sup> genetic background, the induction of hsp–cadE rescued the organism from the MT malformation; an example of a rescued shg<sup>2</sup> is shown in Figure 5D.

**Defective outgrowth and fusion of tracheal branches in shg**

The shg mutation blocked specific steps of another tubulogenesis, that is, tracheal formation. To study fine structures of the tracheal network pattern, we used an antibody to an anonymous component that is deposited at late stage 13Heavy-Spectrin was washed away, resulting in an exclusive localization of the remaining molecules at an apical site of cell–cell contacts (Thomas and Kiehart 1994; as shown in Fig. 4H). In the mutant MT cell cysts, the β<sub>heavy</sub>-spectrin-enriched domain was located inside the aggregates, as seen in the case of Crb staining [Fig. 4I, arrows].

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Figure 4. Disintegrated malpighian tubules (MTs) in the deficiency embryo. (A,B) Dorsal views of the wild-type (A) and the E2/D17 (B) embryos at stage 15. Nuclei of MTs were visualized with an enhancer trap marker A154 [Bellen et al. 1989]. In the mutant, the tract was converted into discontinuous cell aggregates [arrow]. (HG) Hindgut. (C–G) Immunostaining of wild-type (C,F) and deficiency (D,E,G) embryos with anti-Crb antibody. (C,D) Dorsal views of stage 13 embryos. (E) A dorsolateral view of a deficiency embryo slightly older than that in D. [E,G] Dorsolateral views of late stage 15 embryos. In the mutant, cells of the MT formed spherical structures with a polarized Crb distribution [arrow]. Elements of the dorsal longitudinal trunk (DT) of the tracheal system contacted each other, but they were not interconnected [arrowheads]. (H,I) Distribution of β-heavy-spectrin in the wild-type (H) and the deficiency embryos (I) fixed by the heat methanol method. Dorsal views of stage 15 embryos. β-heavy-spectrin is located at the apical pole of cell–cell boundaries. In the deficiency embryo, β-heavy-spectrin was concentrated on inner faces of cysts derived from disrupted MTs [arrows]. The staining pattern in the hindgut was not altered. [A,B,F,G,H,I] Composite pictures of different focal planes. Anterior is to the left. Scale bar, 135 μm in A and B, 35 μm in C–G, and 50 μm in H and I.

DE-cadherin in epithelial rearrangement

As cadherin function requires β-catenin [Kawanishi et al. 1994; Oyama et al. 1995], we examined tracheal patterning phenotypes in mutants of arm, which encodes a Drosophila β-catenin [Peifer and Wieschaus 1990; McCrea et al. 1991]. Tracheal phenotypes of arm were similar, though not identical, to those of shg [Figs. 7A–C]. In one arm null mutant, armΔDS5, DTs were completely missing and LTs did not link to each other at all [Fig. 7A,B]. In an arm hypomorph, armΔVR5, DTs exhibited irregular width, and the connections between LTs were incomplete [Fig. 7C]. We also attempted to determine whether the role of Arm in tracheal development is dependent on the signaling molecule Wg, as Arm is known to function downstream of Wg in the pattern formation of segment polarity [Noordermeer et al. 1994; Peifer et
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Figure 5. Structures of MTs in shg mutants and a phenotypic rescue by DE-cadherin cDNA expression. MTs at the end of embryogenesis or in first-instar larvae were observed under polarized light. (A) Wild type; (B) E2/D17 did not display any tubular organization; (C) generation of short tracts in a shg<sup>+</sup> homozygote; (D) heat shock-treated shg<sup>+</sup> embryo that had the hsp-cadE transgene. The induction of DE-cadherin rescued both MTs and a tracheal network (arrowhead). The recovered tracheal branches at this stage are visible if filled with gas. In A, tracheal trees were out of focus. D is a composite panel of two different focal planes. All embryos are oriented with anterior to left and dorsal down. Scale bar, 50 μm.

al. 1994; Siegfried et al. 1994). In contrast to the indistinguishable ventral cuticle patterns in the arm and wg mutants, tracheal defects were quite different between these two mutant embryos. In a wg null mutant, wg<sup>CX4</sup>, the entire dorsolateral ectoderm shows a transformation toward tracheal cells, but most of them do not invaginate and remain at the surface [Fig. 7D star; P. Green and V. Hartenstein, pers. comm.]. DT-like branches were, however, formed partially on each side of the body [Fig. 7D, arrow], indicating that DT formation does not depend on wg activity completely. Therefore, the role of Arm in tracheal morphogenesis is at least partly separate from that of Wg. Although the tracheal system was defective in both shg and arm mutants, MTs extended normally without any breakage in arm<sup>Y235</sup>.

Maternally derived DE-cadherin and catenins in the shg zygotic null mutant

In shg mutants, severe destruction was not seen in epithelial organs of the intestinal tract other than the MT. Invagination of the salivary gland, foregut, and hindgut primordia proceeded normally, and their epithelial monolayers were not torn, at least at a gross level. The null mutants developed an esophagus and proventriculus from the foregut primordia as in the wild type, although their shape was slightly distorted. All of these ectodermal epithelia showed typical apical distributions of both Crb and β<sub>Heavy</sub>-spectrin [for β<sub>Heavy</sub>-spectrin, see Fig. 4I].

In an attempt to understand why severe phenotypes were limited to particular organs, we studied whether the zygotic null mutant produced DE-cadherin proteins from maternal transcripts. Unhatched embryos [22–26 hr at 25°C after egg laying] of E2/D17 were selected on the basis of their morphological features, and levels of DE-cadherin and catenins were compared to those of the wild type, which had two copies of shg<sup>+</sup> [Fig. 8A]. The E2/D17 embryos of this age contained nearly 20% of the wild-type level of DE-cadherin protein expression. This abundance was confirmed in embryos homozygous for Df(2R)D17 [Fig. 8B]. These embryos were selectable at earlier stages [13–16 hr] because their whole morphology was in disarray. In immunostaining for DE-cadherin, the mutant embryos stained less brightly than wild-type siblings at stage 8, and they did not give signals at cell–cell junctions except for weak ones in the amnioserosa at stage 13. At later stages, no signals were obtained at cellular boundaries throughout the embryos [Fig. 2C]. Thus, despite the presence of a certain level of maternally derived DE-cadherin, we could not clearly detect them at cell–cell contact sites within the range of the sensitivity of immunohistology.

The zygotic removal of DE-cadherin expression also reduced the expression of β-catenin. Two forms of Arm/β-catenin are generated by alternative splicing [J. Loureiro and M. Peifer, pers. comm.]. The larger isoform, which is thought to be involved in Wg signaling, is predominantly associated with DE-cadherin [Oda et al. 1994]. Western blot analysis showed that the shg null mutation dramatically decreased the level of this large form [Fig. 8A,B, arrow]. In sharp contrast, the amount of the smaller neural-specific isoform was not altered [Fig. 8A,B, arrowhead]. As for Da-catenin, only a slight decrease in its total level was observed [Fig. 8A,B]. This would be at least partly attributable to the strong expression of Da-catenin in axon tracks that were not affected by shg mutations [see below].

Images of embryos immunostained for Arm were consistent with the results of the Western analyses [Fig. 9A–D]. In the wild-type embryo, in addition to the ubiquitous expression of membrane-associated Arm molecules, cells that received the Wg signal also accumulated Arm in the cytoplasm [Peifer et al. 1994], generating the well-known Arm transversal stripes [Fig. 9A]. The shg null embryo displayed the normal Arm stripes until stage 9, but signals at both the stripes and interstripe regions started to decay at stage 10 [Fig. 9B]. This alteration of the Arm patterns did not appear to affect epidermal cell fates, as shown by denticle patterns [Fig. 3B]. In epithelial tissues of the wild-type embryo at later developmental stages, the apical zone of cell–cell boundaries [Fig. 9C], where ZA develops, was enriched with Arm [Peifer 1993]. However, in every shg mutant embryo, these signals of Arm were greatly weakened not only in MTs and trachea but also in all the other epithelia [Fig. 9D]. We could not detect any organized arrays of staining signals along intercellular junctions for Da-catenin either [Fig. 9E,F]. Finally, the central nervous system [CNS] in the mutant embryo exhibited intense immunostaining signals for both Da-catenin and Arm, as in the wild type. Images for Da-catenin staining are shown in Figure 9, G and H.
Discussion

We presented evidence that the processes of dynamic epithelial reorganization require the zygotic expression of DE-cadherin, encoded by shotgun (shg). Elimination of the zygotic expression destroyed the MT and the ventral epidermis, and blocked distinct aspects of tracheal tree formation. A strong shg mutation was reported previously to affect the cell morphology of midgut epithelia, which are of endodermal origin (Tepass and Hartenstein 1994b). Assembled midgut epithelial cells remained round or cuboidal instead of becoming columnar. This phenotype is consistent with our finding of the role of DE-cadherin in epithelial reorganization processes.

The role of maternal DE-cadherin expression

Unexpectedly, most of the epithelial tissues were not severely disorganized by shg mutations. They maintained not only their mutual associations but also apico-basal polarity. Even after fragmentation of the MT, the mutant cells still preserved these two aspects of cellular characters. The zygotic null mutant contained a significant amount of maternally derived DE-cadherin, suggesting that this maternal pool may contribute to the maintenance of the general epithelial architecture. To test this possibility, we attempted to generate germ-line clones, but the obtained clones displayed blocked oogenesis (H. Oda, unpubl.). For this reason, we were unable to

Figure 6. Outgrowth and fusion of tracheal branches were blocked in shg mutants. All photographs are lateral views, and oriented with anterior to the left and ventral down. (A–H) Images of tracheal lumina, stained with no. 84, which were constructed by overlaying multiple optical sections. (A,B) Mid-stage 13 embryos of the wild type and E2/D17, respectively. In the wild type, DTs were about to fuse together. (C,D) Stage 14 embryos of the wild type and the deficiency, respectively. The DT fusion has already taken place at late stage 13 in the wild type. Arrows represent visceral branches, and arrowheads point to LTs. (E) Stage 14 deficiency embryo that was partially rescued by the expression of the hsp-cadE transgene. (F) Stage 14 shg2 mutant. Arrows indicate examples of constrictions at the fusion points of DTs. Interruptions of tracts are labeled by asterisks (*). (G,H) Stage 16 wild-type and shgE17B embryos, respectively. This shgE17B mutant (H) is slightly younger than the normal embryo of G. In the mutant, every DT joint was pinched (arrows) and some LTs were not connected (asterisks). (I,J) Early stage 13 embryos of the wild type and the deficiency, respectively, that were double-stained for β-gal expression of a breathless-enhancer trap line (red) and esg (yellow or green). Tracheal tip cells expressed esg (arrowheads in I and J). Scale bar, 50 μm.
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**Figure 7.** Tracheal development in arm and wg mutants. Lateral views oriented with anterior to the left and ventral down. Staining and image reconstruction were done as explained in Fig. 6. (A,B) arm null mutants (armYD3s) of late stage 13 and early stage 16, respectively. Dorsal longitudinal trunks were totally missing (cf. A with Fig. 6C and B with Fig. 6G). Lateral trunks (arrowhead) did not fuse with each other in B. Arrows represent visceral branches. (C) Stage 16 arm hypomorph (armHS6). Connections of dorsal and lateral trunks were incomplete (arrow and asterisk, respectively). (D) Stage 14 wg null mutant (wgCX4). Many of the tracheal cells remained on the surface ectoderm (star), but a dorsal trunk was generated (arrow). In armYD3s, no immunoreactivity of no. 84 could be detected on the embryonic surface. Scale bar, 50 μm.

study the effect of the removal of maternal DE-cadherin on embryogenesis. The same type of experiment was done successfully by another group who employed weak alleles [Tepass et al., this issue]. Those embryos produced only small patches of the cuticle over their whole body. This cuticle pattern strongly suggests that all of the cuticle-secreting epithelia, including the entire epidermis, were totally destroyed. This observation supports the idea that the maternal DE-cadherin plays an essential role in the initial formation and maintenance of epithelial tissues.

The above argument about the maternal contribution of DE-cadherin, however, appears inconsistent with our immunohistological observations of the zygotic shg mutants; that is, neither DE-cadherin nor its associated proteins, Da-catenin and Arm, were highly concentrated at cell–cell boundaries in embryos at late stages, for example, after stage 13. Nevertheless, the results of the shg germ-line clones, together with those of the zygotic mutants, indicate that the drastically reduced levels of the DE-cadherin/catenin complex can still manage to maintain the epithelial architecture once it has been established. Perhaps, our immunohistological method was not sensitive enough for fully detecting the maternal molecules.

Previously we showed similar features in the expression patterns and in vitro activities of DE-cadherin and vertebrate E-cadherin, and considered DE-cadherin to be a Drosophila homolog of the vertebrate E-cadherin (Oda et al. 1994). The shg studies reinforce the notion that these two proteins appear to be equivalent in the sense of in vivo functions as well.

The role of DE-cadherin in dynamic epithelial rearrangements

How does the zygotic DE-cadherin control epithelial reorganization? During cell rearrangement within a tissue like the MT, cells must cycle a rapid breakdown and a re-establishment of their mutual adhesion without creating intercellular breakages (Gumbiner 1992). Another type of dynamic cell adhesion is found in the ventral neurogenic region of the ectoderm, where epidermal precursors have to remake new contacts as soon as neuroblasts delaminate (Hartenstein and Campos-Ortega 1984). In the shg zygotic mutants, epithelial structures were broken down in both the MT and the neuroectoderm (this study, Tepass et al., this issue). These severe damages present a sharp contrast to the undisrupted tissues including the dorsal epidermis and the hindgut. The dorsal epidermis is morphogenetically less active than the ventral epidermis at the stage of neuroblast segregation, and so is the hindgut than the MT during the elongation of the latter. Maternal DE-cadherin molecules, detected in the ventral epidermis and also presumably present in the MTs, could not support the reorganization of these tissues. We therefore speculate that the zygotic DE-cadherin expression is critically required for the establishment of new cell–cell associations and that the maternal molecules cannot perform this task. It is conceivable that the pool of the maternal DE-cadherin is not sufficient in amount, and/or it cannot be recycled, to establish new cell–cell adhesions. That is, the maternal molecules may serve only for the maintenance of the already established cell–cell junctions, whereas the ones supplied by the zygotic expression are utilized for active cell repositioning.

In the absence of well-controlled intercellular adhesion systems, the activated cell motility would cause a disruption of epithelial monolayers. Adhesion molecules need to be delivered rapidly to appropriate positions to form new cell–cell contacts in a manner coordinated with the cell movement. Interaction of DE-cadherin with the actin-based cytoskeleton via catenins likely supports an efficient redistribution of the molecules on the plasma membrane. Also, detachment of cells must
junctions persist (Tepass and Hartenstein 1994a), and epithelial cell contacts (Woods and Bryant 1993; Hartenstein 1994). DF-cadherin and catenins stay at the apical positions of MT cells during cell proliferation. Therefore, cadherins seem to be ideal regulators of dynamic cell-cell adhesion. Recently, one of the cadherins was implicated in gastrulation movements (Lee and Gumbiner 1995); this finding appears to be consistent with ours on the function. However, the situation in the zygotic mutants, the shg phenotypes are unique in that the defects were rather restricted to a subset of branches that perform fusion, that is, fusion branches (Manning and Krasnow 1993). Although cellular mechanisms for elaboration of individual branch extensions are not fully understood, such restricted effects of the shg mutations suggest that different mechanisms for tubular outgrowth are operative between the fusion branches and the other branches. For the elongation of the fusion branches, it is likely that cell rearrangement may be a major force, as is the case in MT development. In contrast, for the other branches, for example, visceral branches, mechanisms such as simple cell shape changes, which would not accompany cellular repositioning, might be essential. The tracheal epithelial structure was damaged much less seriously than that of the MTs. This could be attributable to possible involvement of multiple mechanisms, as described above, operating in the elongation processes.

Analysis of partial loss-of-function shg mutants revealed another DE-cadherin-dependent process in the tracheal fusion. In the weak allele shgD17B, DTs were able to extend far enough so that their tracheal tip cells established physical contact with each other, and thus lumina were generated to connect flanking branches. However, widening of the tract diameter was blocked at every fusion point. To understand more definitively the roles of DE-cadherin in the fusion processes, we must clarify the cellular basis of the fusion, in particular, the behavior of the tip cells.

Besides the shg mutants, arm mutants also showed malformation of the fusion branches. This is not surprising superficially, because Arm and DE-cadherin are supposed to interact physically with each other in their function. However, the situation in the arm mutant is somewhat complicated. In the arm zygotic null mutant, DE-cadherin proteins were detectable at the apical side of cell boundaries in all epithelia, although the signal was weaker than that in wild-type siblings (data not shown). Those cadherin molecules probably form complexes with maternally derived Arm (Riggleman et al.

**Figure 8.** Western blotting analyses of deficiency and shg2 mutant embryos. (A,B) DE-cadherin (DE-cad), β-catenin/Arm, Δcat-catenin (Δcat)-cat in deficiency embryos (DF) were compared with those in wild-type Oregon R (WT). E2/D17 embryos (22–26 hr (A) or 13–16 hr Df(2R)D17 homozygotes (B) were used. In B, besides the mature form of DE-cadherin (150 kD, short arrow), a larger precursor is seen. Two Arm isoforms are generated, an epithelial type (long arrow, 105–110 kD) and a smaller neural type (arrowhead, 82 kD) (J. Loureiro and M. Peifer, pers. comm.). Δcat is 110 kD in its full size, and smaller bands seen are most likely degradation products. The 65-kD subunit (A subunit) of protein phosphatase 2A (PP2A) was used as a standard to compare the amounts of proteins loaded. In each lane, a whole lysate equivalent to ~15 embryos was loaded. Proteins in the deficiency embryos were more extractable, probably because of the elimination of most ventral cuticle. (C) Patterns of DE-cadherin in Oregon R (WT) and shg2 homozygote (shg2). Degraded DE-cadherin molecules were detected in the shg2 extract (arrowheads). A position of β-galactosidase (116 kD) is indicated by a bar in C.
1990), suggesting that they are functioning as adhesion machinery to a certain extent. Consistently, the arm null mutant exhibited normal extension of MTs as opposed to the shg mutant. Therefore, the tracheal malformation in the arm embryo cannot simply be explained by a reduction in the activity of the cadherin–catenin adhesion system. Arm is considered to work as a signal transducer downstream of Wg in patterning of the embryonic epidermis [for review, see Peifer 1995]. However, tracheal phenotypes in arm and wg mutants indicate that these two genes function, at least in part, separately in this tubulogenic morphogenesis.

Finally, it would be intriguing to ask whether DE-cadherin is involved in the Wg signaling cascade for segment polarity determination. The denticle patterns in the shg null mutant did not provide positive evidence for this possible linkage, although the loss of most ventral cuticles hindered careful analyses of the patterning. Weakening of the overall Arm stripe pattern was observed in the shg null mutant. However, this reduction of the Arm level became apparent during stage 10, when most cells no longer require active Wg for stable engrailed (en) expression [Bejsovec and Martinez Arias 1991; Heemskerk et al. 1991]. The maternally derived DE-cadherin perhaps sustained the Arm level at the critical period for Wg signaling, and thus the effect of loss of DE-cadherin on segment polarity could not be examined. Further studies are necessary to disclose whether DE-cadherin is actively involved in the Wg pathway.

Materials and methods

Drosophila strains

Isolation of deficiency chromosomes in the 57B region is described elsewhere [Kraut and Campos-Ortega 1996]. Embryos from each line were stained with an antibody to DE-cadherin,
and we found that signals were undetectable in late homozygous Df(2R)E2 embryos. Df(2R)E2/+ and Df(2R)D17/+ polytene chromosomes were then hybridized with DE-cadherin cDNA probes, and we confirmed that both deficiencies completely lacked shg. Df(2R)D17, Df(2R)P13, shg
 and wg
 were provided from the Bloomington Stock Center. Df(2R)F36 and Df(2R)PL3 were reported previously (O’Donnell et al. 1989).

Before isolation of Df(2R)E2 and finding that shg encodes DE-cadherin, we tried to find candidates of cadE mutants among a collection of lethal strains that mapped within Df(2R)D17. For this purpose, we used a subset of the lethal lines that positioned within Df(2R)D17 but outside all internal deficiencies like Df(2R)F36 and Df(2R)PL3. These lethal strains were provided from Dr. Janis O’Donnell (University of Alabama, Tuscaloosa). Embryos of those individual lines were stained with the anti-DE-cadherin antibody; two lines, l(2)E17B and l(2)SHN-1, showed ectopic signals in axon tracks in the CNS. However, cadE mRNA was detected only in midline glial cells in these lines as in the wild type [T. Usui and T. Uemura, unpubl.]. We did not study further the molecular basis of the ectopic protein distribution in these lines. By complementation tests with shg
, we found that those two were allelic to shg, so they were renamed shg
 and shg
, respectively.

In FlyBase, we found that the Berkeley Drosophila Genome Project identified three strains with PZ-element insertions into 57B1-13: l(2)E03050, l(2)E07206, and l(2)E10469 [FlyBase 1994, Spradling et al. 1995, Berkeley Drosophila Genome Project, pers. comm.]. These three lines were provided from the Bloomington Stock Center. l(2)E10469 did not complement the three shg alleles that we described above, and we renamed this line shg
. The PZ-element was mobilized by ∆2-3 (Robertson et al. 1988). Adult escapers homozygous for shg
 showed a patterning defect along the anterior wing margin [Y. Kataoka and T. Uemura, unpubl.]. arm
 and arm
 were provided by Dr. Mark Peifer [University of North Carolina, Chapel Hill], arm
 is a temperature-sensitive allele (Klingensmith et al. 1989), and embryos homozygous for it were incubated at 25°C for tracheal analyses.

The following enhancer trap lines were used: A154.2M3 (Bellen et al. 1989) to study MTs and 6-81a (Klämbt et al. 1992) for tracheal cells.

Isolation of genomic DNA clones of the DE-cadherin gene

We isolated phage clones from an Oregon-R genomic library [L. Jan and Y.N. Ian, University of California, San Francisco] using DE-cadherin cDNA clones [Oda et al. 1994]. About 20 kb of genomic DNA in total was isolated, and we roughly determined positions of exons by hybridizing various cDNA fragments to the genomic clones, comparison of restrictions sites, and partial genomic sequencing. The position of the shg
 insertion was determined by sequencing the plasmid rescue fragment.

Antibodies and Western blotting

Rat antibodies to DE cadherin [DCAD1 and DCAD2], Δa-catenin [DCAT1], Eσg, and protein phosphatase 2Aα were generated previously [Oda et al. 1993, 1994, Fuse et al. 1994, Shiomura et al. 1994]. DCAD1 was used for Western analyses and DCAD2 for immunostaining embryos. Other antibodies used were as follows: 7A1 [mouse anti-Arm; Peifer 1993], C4 [mouse anti-CRB; Tepass and Knust 1993], no. 84 [guinea pig tracheal-specific antibody; Klämbt et al. 1992], DA1B6 [mouse anti-fasciclin III; Gauger et al. 1987], no. 243 [rabbit anti-β
 spectrin antibody; Thomas and Kiehart 1994], and rabbit anti-β-galactosidase [Cappel]. To prepare samples for Western blots, we homogenized dechorionated embryos in a 1× Laemmli sample buffer containing 1 mm phenylmethylsulfonyl fluoride, sonicated the homogenate, and immediately boiled it for 3–4 min. To detect signals in Western blots, we employed an ECL kit (Amersham).

Observation of embryos

For immunostaining, embryos were usually fixed in 3.5% formaldehyde in 0.1 M phosphate buffer [pH 7.4], or a protocol of heat methanol fixation was followed [U. Tepass, pers. comm.]. The heat methanol fix was employed for pHev-spectrin [Fig. 4H,I] and Δα-catenin [Fig. 9E,F] stainings. Δα-catenin is concentrated at the ZA in heat-fixed embryos [U. Tepass, pers. comm.]. We did not employ any fix solutions containing EDTA or EGTA, because cadherins are degraded easily under Ca
-free conditions. Signals were detected by use of an ABC Elite kit [ Vectastain] or by fluorescence labeling [Oda et al. 1994]. A combination of FITC and Texas Red was employed for double labeling, and embryos were viewed with a Zeiss laser scanning confocal microscope. MTs in live embryos were observed with Normarski optics except that a condenser for dark field microscopy was used.

Heat shock experiments

A 5-kb fragment of the DE-cadherin cDNA was isolated from pRMHa3–DECH [Oda et al. 1994] and inserted into pCaSpeR–hs [Thummel et al. 1988]. The resultant plasmid, phs–DECH, was microinjected into w
 – Δα-3 TM3 Sb/Dr [Robertson et al. 1988] to produce transgenic flies. Two established lines were used for rescue experiments: DECH 1-1-2, which has phs–DECH inserted into the X chromosome, and DECH 2-3 (with insertion into the third chromosome). For the rescue experiments we collected males and virgin females, each of which was heterozygous for Df or for shg
 and carried one copy of the DECH chromosome. Those males and females were crossed to each other. Embryos were collected, aged for 3.5–5 hr at 25°C, and heat-shocked for 30 min in a 37°C water bath. They were then aged to stage 13–14, to stage 16 for observation of tracheal development, or to the end of embryogenesis for examination of cuticles and MTs. Embryos of the deficiency or shg
 strains without phs–DECH were heat-shocked in parallel for the control. As for DECH 1-1-2 and DECH 2-3, the overexpression was confirmed by RNA in situ hybridization, Western blot analysis, and immunostaining. Both chromosomes exhibited similar levels of the DE-cadherin induction and equally rescued the embryos from the mutant phenotypes.

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