Dynamic Behavior of the Cadherin-Based Cell-Cell Adhesion System during Drosophila Gastrulation

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During Drosophila gastrulation, morphogenesis occurs as a series of cell shape changes and cell movements which probably involve adhesive interactions between cells. In the present study, we examined the dynamic aspects of cadherin-based cell–cell adhesion in the morphogenetic events to assess its contribution to morphogenesis. DE- and DN-cadherin show complementary expression patterns in the presumptive ectoderm and mesoderm at the mRNA level. We found that switching of cadherin expression from the DE- to the DN-type in the mesodermal germ layer occurred downstream of the mesoderm-determination genes twist and snail. However, examination of their protein expression patterns showed that considerable amounts of DE-cadherin remained on the surfaces of mesodermal cells during invagination, while DN-cadherin did not appear on the cell surfaces at this stage. Further immunocytochemical analysis of the localizations of DE-cadherin and its associated proteins Armadillo (β-catenin) and Dα-catenin revealed dynamic changes in their distributions which were accompanied by changes in cell morphology in the neuroectoderm and mesoderm. Simultaneously, adherens junctions (AJs), based on the cadherin–catenin system, were shown to change their location, size, and morphology. These dynamic aspects of cadherin-based cell–cell adhesion appeared to be associated with the following: (1) initial establishment of the blastoderm epithelium, (2) acquisition of cell motility in the neuroectoderm, (3) cell sheet folding, and (4) epithelial to mesenchymal conversion of the mesoderm. These observations suggest that the behavior of the DE-cadherin–catenin adhesion system may be regulated in a stepwise manner during gastrulation to perform successive cell-morphology conversions. Moreover, the processes responsible for loss of epithelial cell polarity and elimination of preexisting DE-cadherin-based epithelial junctions during early mesodermal morphogenesis are discussed. © 1998 Academic Press

Key Words: cadherin; catenin; Drosophila; gastrulation; adherens junction.

INTRODUCTION

1 Morphogenesis in the development of multicellular organisms involves a variety of cellular mechanisms for control of adhesive interactions between cells. In Drosophila gastrulation, a complex multilayered cell structure is generated from a simple monolayer of homogeneous columnar epithelial cells within an hour (Poulson, 1950; Sonnenblick, 1950; Turner and Mahowald, 1977; Fulilove et al., 1978; Campos-Ortega and Hartenstein, 1985; Wieschaus and Nüsslein-Volhard, 1986; Costa et al., 1993). During this event, the cells presumably control intercellular adhesion, resulting in gradual changes in cell shape and position relative to neighboring cells. Cellular dynamics during Drosophila gastrulation has been observed by scanning electron microscopy and three-dimensional fluorescence microscopy (Sweeton et al., 1991; Kam et al., 1991).

In Drosophila, the cellular blastoderm establishes a monolayer of epithelial cells. At the gastrula stage, prospective neuroectodermal cells inherit epithelial properties and acquire motility to perform the germ band extension by cell intercalation (Hartenstein and Campos-Ortega, 1985; Irvine and Wieschaus, 1994), whereas prospective mesodermal cells lose epithelial properties and instead acquire mesenchymal characteristics to penetrate to within the embryo. These cell-state conversions are accompanied by cell-shape changes and cell movements. Morphogenesis is considered to be the sum of such dynamic cellular events.

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In this study, we focused on the cell–cell adhesion apparatus based on the cadherins and catenins to assess how adhesion contributes to morphogenesis. This adhesion system, which is molecularly and functionally conserved between vertebrates and invertebrates, plays important roles in a variety of morphogenetic events during the animal development (for review see Takeichi, 1995; Huber et al., 1996). In this system, the classic cadherin binds to β-catenin which interacts with α-catenin (Tsukita et al., 1992; Cowin and Burke, 1996). α-Catenin is thought to mediate interactions between the cadherin–β-catenin complex and the actin cytoskeletal network (Knudsen et al., 1995; Rimm et al., 1995). Evidence from studies of early mouse embryos and human carcinoma cells indicated that each of these molecules is essential for establishment and maintenance of organized multicellular structures with epithelial polarity (Larue et al., 1994; Riethmacher et al., 1995; Høegel et al., 1995; Hirano et al., 1992; Torres et al., 1997). In addition, many cell biological studies using cultured cells have accumulated which show that the cadherin–catenin system has cross-talk with other cellular systems to control cell morphology, behavior, growth, and differentiation (for review see Barth et al., 1997). Recently, studies in Drosophila have provided valuable evidence regarding the function of the cadherin adhesion system. Two classic cadherins, DE- and DN-cadherin, Armadillo, and Dα-catenin are Drosophila counterparts of E- and N-cadherin, β-catenin, and α-catenin, respectively (Oda et al., 1994; Iwai et al., 1997; Peifer and Wieschaus, 1990; McCrea et al., 1991; Oda et al., 1993). Phenotypes of DE- and DN-cadherin mutants have revealed their critical roles in establishment of well-defined multicellular structures in epithelial tissues and the central nervous system, respectively (Uemura et al., 1996; Tepass et al., 1996; Iwai et al., 1997). The function of Armadillo is related not only to the cadherin adhesion system but also to the wingless signal transduction pathway (Peifer, 1995). Germ-line clones mutant for a moderate allele of armadillo which can overcome oogenesis fail to exhibit normal cell morphology at gastrulation (Cox et al., 1996). There have been no reports of genetic analysis of the Dα-catenin gene.

The cadherins and catenins are major constituents of the adherens junctions (AJs) which are associated with actin filaments (Tsukita et al., 1992). Many other molecules also participate in these junctions and play roles not only in cell–cell adhesion but also in interactions with the cytoskeleton and intra- and intercellular signaling (for review see Tsukita et al., 1992; Cowin and Burke, 1996). In epithelial cells, a specialized type of the AJ, the zonula adherens (ZA), continuously surrounds apical surfaces and contributes to establishing and maintenance of cell shape and polarity (McNeill and Nelson, 1992). Cooperative actions of the adhesion system and the cytoskeleton in the ZA are thought to produce morphogenetic movements in developing epithelial tissues. The AJ of Drosophila ultrastructurally resembles its vertebrate counterpart, and DE-cadherin, Armadillo, and Dα-catenin were shown to be localized to these junctions in embryonic and ovarian epithelial cells (Oda et al., 1993, 1994, 1997; Peifer et al., 1993). AJ formation in the embryonic epithelium is affected by several known mutations including crumbs, stardust, bazooka, and armadillo (Grawe et al., 1996; Müller and Wieschaus, 1996). These mutants fail to maintain epithelial integrity, and thereby, fail to undergo normal epithelial morphogenesis.

DE-cadherin, which has a major role in early embryonic cell–cell adhesion in Drosophila, is continuously present in ectodermally and endodermally derived epithelial tissues (Oda et al., 1994; Tepass et al., 1996). DN-cadherin begins to be expressed in the presumptive mesoderm during gastrulation and subsequently in the nervous system, although no mesodermal defects have been found in its mutants (Iwai et al., 1997). Armadillo and Dα-catenin are colocalized with these cadherins at cell–cell boundaries from the cellular blastoderm stage through embryogenesis (Oda et al., 1994; Iwai et al., 1997). Despite previous studies of the expression of cadherins and catenins, detailed descriptions on their behavior in early embryogenesis are still lacking.

We studied dynamic aspects of cadherin-based cell–cell adhesion in morphogenetic movements at the ventral part of gastrulating embryos. We will describe changes in the expressions and subcellular localizations of DE- and DN-cadherin, Armadillo, and Dα-catenin, which were correlated with cell morphology changes in the developing neuroectoderm and mesoderm. Our observations and results suggest important roles of DE-cadherin-based adhesion and its step-wise regulation in morphogenetic movements during gastrulation.

**MATERIALS AND METHODS**

**Drosophila Strains**

Wild-type embryos were of the Oregon R strain. The following mutants were used: twi106E, snail2G08 (Simpson, 1983), fogg23G (Zusman and Wieschaus, 1985), and shg10469 (Uemura et al., 1996).

**Whole-Mount in Situ Hybridization**

In situ hybridization to RNA in whole-mount embryos was performed as described (Lehmann and Tautz, 1994). The digoxigenin-labeled RNA probes generated with 2.2- and 4.5-kb fragments of DE- and DN-cadherin cDNA, respectively, which contained cadherin repeats in the N-terminal region (Oda et al., 1994; Iwai et al., 1997), were hybridized with embryos, and signals were detected with antidigoxigenin antibody conjugated with alkaline phosphatase (Boehringer Mannheim). Stained whole-mount embryos were embedded in polyester wax (BDH Laboratory Supplies) and sectioned at a thickness of 8 μm on a microtome (Yamato Kohki).

**Antibodies**

A mixture of DCAD1 and 2 (rat MAb) was used for DE-cadherin staining at 1:20 dilution (Oda et al., 1994), DN-EX8 (rat MAb) for DN-cadherin at 1:4 (Iwai et al., 1997), N2-7A1 (mouse MAb) for Armadillo at 1:100 (Peifer, 1993), and DCAT-1 (rat MAb) for Dα-catenin at 1:100 (Oda et al., 1993).
Fixation for Immunofluorescent Microscopy

Embryos were placed in 0.7% NaCl, 0.3% Triton X-100 and dechorionated in 50% sodium hypochlorite. The embryos were fixed for 15 min at room temperature in a mixture of 4% paraformaldehyde in Chan and Gehring's buffered saline (CBGS: 55 mM NaCl, 40 mM KCl, 15 mM MgSO4, 5 mM CaCl2, 10 mM Tricine (pH 6.9), 20 mM glucose, 50 mM sucrose) with heptane. They were devitrillized in 80% EtOH, rehydrated in a graded series of EtOH (75%, 35%), and placed in PBS containing 0.1% Tween 20 (PBST).

Cryosectioning of Embryos

Fixed embryos were embedded in 1.5% agarose gel, incubated in a graded series of sucrose solutions in PBS (12–25%) and frozen in Tissue-Tech (Miles Scientific) using liquid nitrogen. Cryostat sections (14-μm-thick) were then cut, mounted on slides coated with 1% gelatin and 0.1% chromium sulfate, and air-dried.

Immunofluorescence

Whole-mount or sectioned embryos for immunohistochemistry were incubated in PBS containing 0.3% Triton X-100 for 30 min at 4°C, blocked in 5% skim milk in PBST (blocking buffer) for at least 1 h at room temperature, and then incubated with primary antibody diluted with blocking buffer overnight at 4°C. After being washed in PBST four times (5 min each), the samples were incubated with secondary antibody for 2 h at room temperature and washed again in PBST. For double staining, the embryos were further incubated with next primary antibody and treated as described above. Texas-red-labeled goat anti-rat (Cappel) and Cy5-labeled donkey anti-mouse (Chemicon) antibodies were used as secondary antibodies. To avoid cross-reactivity of the anti-rabbit antibody to other species on double staining, staining with rat antibodies was performed at first. For F-actin staining, rhodamine- or FITC-labeled phalloidin (Molecular Probes) was used. Stained samples were mounted in a mixture of 90% glycerol containing 1 mg/ml p-phenylenediamine and examined with a Zeiss Axiophot II equipped with a Bio-Rad laser scanning confocal microscope (MRC1024).

Electron Microscopy

For fixation, embryos were incubated in a mixture of 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.0) with heptane for 15 min at room temperature, and then hand peeling further incubated in the fixative for 2 h at 4°C. After being rinsed with sodium cacodylate buffer, embryos were postfixed in 1% OsO4 in 0.1 M sodium cacodylate buffer for 2 h at 4°C and rinsed with distilled water. Embryos were subsequently dehydrated through an ethanol series and embedded in Epon 812 (Polysciences, Inc.). Ultrathin sections were cut with a diamond knife (Drukker International), double stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope (JEM-1010, JEOL).

RESULTS

Switching of Cadherin Expression from the E- to the N-Type during Gastrulation

Two cadherins, DE- and DN-cadherin, are expressed in early Drosophila embryos (Oda et al., 1994; Iwai et al., 1997). The expression of DE-cadherin is specific to ectoderm- and endoderm-derived epithelia, while DN-cadherin is specifically expressed in the mesoderm and nervous system. Before gastrulation, all cells of the blastoderm express DE-cadherin which is provided maternally and zygotically. After gastrulation, however, DN-cadherin is substituted for DE-cadherin in the mesoderm.

We investigated this process of the cadherin switching. The distributions of mRNA and protein of DE- and DN-cadherin were observed at the cellular blastoderm stage and successive stages of gastrulation. DE-cadherin mRNA was present uniformly in the embryo until late stage 5, but it began to disappear in the presumptive mesoderm shortly before the onset of ventral furrow formation (Fig. 1A). As apical constriction of the most ventral cells occurred at the beginning of ventral furrow formation, amounts of DE-cadherin mRNA rapidly decreased in these cells (Fig. 1B). No signals for the mRNA were visible in the mesoderm from stage 7 (Figs. 1C–1E). We also found that DE-cadherin mRNA tended to accumulate in the apical cytoplasm in cells of the cellular blastoderm and ectoderm at stages 5–7 (Figs. 1A–1C). However, after stage 7 the apically restricted pattern of the mRNA became obscure (Figs. 1D and 1E).

DN-cadherin mRNA was first seen in presumptive mesodermal cells of the cellular blastoderm (Fig. 1F). Initial signals with a probe for the mRNA were restricted to two dots within the nucleus, as previously reported (Iwai et al., 1997). Most signals persisted within the nucleus until the beginning of stage 8 (Fig. 1G, H).

From stage 8, cytoplasmic signals for DN-cadherin mRNA became apparent (Figs. 1I and 1J). Expression profiles of DE- and DN-cadherin proteins were consistent with those of their mRNAs, although there were considerable time lags. Detectable levels of DE-cadherin protein were present in the mesoderm until stage 8 (Figs. 1K–1M). As the mesodermal cells became round in shape and started to migrate dorsally, the protein began to disappear from the mesoderm (Fig. 1N). On the other hand, faint staining for DN-cadherin protein could be detected in the cytoplasm of mesodermal cells from early stage 9 (Figs. 1O and 1P). Although some of the DN-cadherin appeared on mesodermal cell surfaces at stage 9, its cell surface signals were less apparent. Most of the mesoderm morphogenesis during gastrulation seems to occur where DE-cadherin protein is present on the cell surface but DN-cadherin protein is not.

Behavior of the DE-Cadherin-Based Adhesion System Associated with Cellular Changes

Blastoderm and neuroectoderm. During the cellular blastoderm stage (stage 5), cell–cell contacts arise as a result of the invagination of the surface membrane. DE-cadherin, together with Armadillo and Ds-catenin, immediately localized at the newly forming contact sites (Fig. 2A) (Müller and Wieschaus, 1996). There were no detectable differences in the levels of DE-cadherin protein expression between the prospective neuroectodermal and mesodermal cells by the
end of stage 5. In cells of the late cellular blastoderm, DE-cadherin mRNA preferentially accumulated in the apical cytoplasm (Fig. 2B). In contrast, lacZ transcripts expressed in shgP10469 were diffusely distributed around nuclei (Fig. 2C), indicating that DE-cadherin mRNA is specifically transported and/or is stable in the apical cytoplasm. The apical localization of the mRNA persisted in the neuroectodermal cells until the germ band started to elongate (Fig. 1D). DE-cadherin protein was preferentially accumulated within about the apical one-third of lateral membranes, although it was present in small amounts in the other lateral domains (Fig. 2D). Additional strong signals for DE-cadherin were seen close to basal ends of lateral membranes (Fig. 2D). The localization patterns of DE-cadherin resembled those of Armadillo and Dα-catenin with respect to their membrane-associated pools (Figs. 2D–2F). F-actin, some of which may interact with the DE-cadherin adhesion system, showed a relatively uniform distribution along lateral membranes with occasional concentrations preferentially at more apical portions (Fig. 2G). Weak F-actin signals were additionally detected in cortical regions of apical cell surfaces and at high levels at contractile rings. These F-actin distribution patterns were almost the same as those reported by Warn (1990).

By electron microscopy, we frequently found type I spot AJs termed in Tepass and Hartenstein (1994) at...
lateral cell surfaces where DE-cadherin and the catenins were accumulated (Figs 2D–2F, 3A, and 3B). At stage 7, when ingression of the mesodermal cells had almost completed, DE-cadherin together with Armadillo and D\(\alpha\)-catenin was more strongly accumulated at apical contacts of neuroectodermal cells (Fig. 2H). In the same stage embryos, larger AJs were observed at the corresponding sites (Figs. 3A, 3B, 3F, and 3G), some of which consisted of several smaller AJs aligned in tandem. The AJs were closer to the apical surface than at the previous stage. As soon as the germ band started to elongate (stage 8), the DE-cadherin distribution changed markedly. The apical accumulation pattern along lateral cell surfaces became disordered or obscure, although some of the DE-cadherin remained concentrated at the apical poles (Fig. 2I). Curiously, small patches of signals for DE-cadherin were seen within cells and on apical cell surfaces at this stage. Electron microscopy revealed interdigitated or protruded foldings between cells and on apical cell surfaces, and vesicles with membranous contents in cells (Figs. 3C–3E). Thus, marked rearrangements in cell surface domains appeared to occur as germ band extension began. Large AJs as observed at the previous stage were rarely found at stage 8. Although it was difficult to find typical AJs in neuroectodermal cells of stage 8 embryos, compact types of AJs were occasionally found at the apical ends of lateral membranes (Figs. 3C and 3H). At the following stage (stage 9), DE-cadherin protein was seen continuously at sites of cell–cell contact not only in the surface ectoderm but also in delaminating neural precursors (Fig. 1N).

Mesoderm. At the early stage of gastrulation, ventralmost cells are flattened by constriction of their apices. These cells change from a columnar to a wedged shape and begin ingression to within the embryo to generate the ventral furrow. DE-cadherin was strongly concentrated at

**FIG. 2.** Distributions of DE-cadherin, Armadillo, D\(\alpha\)-catenin, and F-actin in the blastoderm and neuroectodermal cells. Sections of wild-type embryos were stained for DE-cadherin (A, D, H, I), Armadillo (E), and D\(\alpha\)-catenin (F) proteins, and for F-actin (G). Sections of wild-type and shg \(^{10469}\) embryos were stained for DE-cadherin (B) and lacZ (C) mRNAs, respectively. Dorsal is up; ventral is down. In each panel, the apical side of the epithelium is to lower right; the basal side to upper left as indicated in A. (A) Early cellular blastoderm-stage. DE-cadherin was localized at newly forming cell–cell contacts. (B–G) Late cellular blastoderm-stage. DE-cadherin mRNA was preferentially accumulated in the apical cytoplasm (B), while lacZ mRNA was diffusely distributed around nuclei (C). DE-cadherin protein, together with Armadillo and D\(\alpha\)-catenin, was preferentially accumulated within the apical one-third of lateral cell contacts (between arrowheads) (D–F). F-actin also concentrated at corresponding regions (arrowheads), although it was also seen in the entire cell cortices and contractile rings. (G). (H) Stage 7 (just before the start of germ band extension). DE-cadherin protein was highly concentrated at lateral cell contacts near apical poles (arrowheads). (I) Stage 8 (rapid phase of germ band extension). DE-cadherin protein was seen at various sites including apical cell poles (arrowheads), more basal parts of lateral cell contacts (fat arrows), and apical cell surfaces and the inside of cells (thin arrows). Asterisks indicate nuclei. Scale bar, 10 \(\mu\)m.
the apical ends of lateral surfaces of the constricted cells (Fig. 4A). As expected, Armadillo was distributed in a similar pattern (Fig. 4B) but not such prominent apical concentration of D\textalpha-catenin and F-actin was observed (Figs. 4C and 4D), although some apical concentration was observed in more lateral cells on constriction of their apices. Whole-mount embryos double stained for DE-cadherin or D\textalpha-catenin and F-actin also showed that the former was concentrated at interfaces between apical and lateral surfaces of the apically constricted cells, but no proportional concentration of D\textalpha-catenin and F-actin occurred (Figs. 4E–4H). The distribution pattern of D\textalpha-catenin was more similar to that of F-actin than to that of DE-cadherin.

By electron microscopy, AJs were observed at almost all of the apical poles of the constricted cells at the early stage of gastrulation (Figs. 5A and 5B). Although we did not detect highly concentrated signals for F-actin by phalloidin staining, the observed AJs had undercoats that appeared to contain considerable amounts of actin filaments (Fig. 5B). Coated vesicles were frequently found near the AJs in invaginating mesodermal cells (Figs. 5B and 5E). It is possible that the production of these vesicles contributes to the reduction of DE-cadherin-based cell–cell contact in the mesoderm. During mesoderm ingression, the lines of concentrated DE-cadherin along the apical–lateral interfaces became discontinuous. In cross sections of stage 7 embryos, the signals were detected as intense dots close to the transient lumen of the tube-like structure of the mesoderm (Fig. 5A). As the invagination proceeded, AJ became less frequent in the sections, although various sizes and morphologies of AJs were found at interfaces between apical and lateral membranes (Figs. 5C–5E). In some cases, AJs were flanked by cell contact-free membranes with electron-dense undercoats and outer surfaces (Fig. 5E). These seem to reflect the transient appearance of the AJs during their disruption. After completion of inward movement of the mesoderm, only a few AJs were found at plasma membranes close to the lumen corresponding to the apical side of the initial epithelium (Figs. 5F and 5G).

In lateral domains of cell surfaces of the invaginating mesoderm, DE-cadherin, Armadillo, and D\textalpha-catenin were detected at comparable levels (Figs. 4A–4D, 6A, and 6B). These molecules were distributed in relatively uniform patterns along the lateral cell surfaces. Electron microscopy revealed smooth parallel lines of contacting plasma membranes (Figs. 5A, 5D, and 5F). However, as the invaginated mesodermal cells were converted from wedge-shaped to round cells, the contacting plasma membranes became loose and separated, producing spaces between mesodermal cells (Figs. 5H and 5I). Nonetheless, contacts of membranes were still seen not only between mesodermal cells but also between mesodermal cells and the yolk. In addition, there were many small blebs and fragments probably derived from parts of cells in the intercellular spaces. DE-cadherin, Armadillo, and D\textalpha-catenin became distributed over the entire cell surface including the cell contact-free areas in rounded mesodermal cells at stage 8 (Figs. 6C–6G). After this stage, DE-cadherin was completely eliminated from the mesoderm. Simultaneously, Armadillo and D\textalpha-catenin on cell surfaces were reduced to undetectable levels.

**Effects of twi, sna, and fog Mutations on Cadherin Expression**

We examined the regulatory mechanisms which control the switching of cadherin expression from DE- to DN-cadherin during early mesoderm development. Expression patterns of these cadherin mRNAs were investigated in twist (twi), snail (sna), and folded gastrulation (fog) mutant embryos. twi and sna encode essential transcription factors, whose expressions are zygotically induced by activities of maternal components to produce the mesodermal fate (Thissel et al., 1987; Boulay et al., 1987). Thus, twi and sna mutants produce incorrect cell fates at the ventral part of the embryos corresponding to the wild-type mesodermal region. In twi and sna, we found that downregulation of DE-cadherin mRNA expression in the ventral part of the embryos did not occur even after the onset of gastrulation (Figs. 7C and 7E). Moreover, shg\textsuperscript{10469}, in which the reporter lacZ gene is transcribed under control of the enhancer for the DE-cadherin gene (Uemura et al., 1996), was used to investigate the effects of twi and sna mutations on transcriptional activities of the DE-cadherin gene. Results similar to those yielded by analysis of DE-cadherin transcripts were obtained by examining lacZ transcripts in twi, shg\textsuperscript{10469}/twi, and sna, shg\textsuperscript{10469}/sna (data not shown).

DN-cadherin expression did not occur in twi, while it was initiated in sna, although its level did not increase sufficiently (Figs. 7D and 7F). These results indicated that twi is essential for the initiation of DN-cadherin expression but sna is not. Also, sna was shown to be required for the increased expression of DN-cadherin on and after gastrulation. fog encodes a secreted protein, which is one of morphogenetic regulators downstream of twi and sna (Costa et
We found that fog mutation did not affect normal cadherin expression at the mRNA level in gastrula embryos (Figs. 7G and 7H). Taken together, these observations indicated that the switching of cadherin expression from DE- to DN-cadherin occurs downstream of twi and sna independently of fog, as summarized in Fig. 7I (see Discussion for detail).

In twi, sna, and fog mutants, morphogenetic movements at the ventral side of the embryo during gastrulation are grossly affected (Leptin and Grunewald, 1990; Sweeton et al., 1991; Costa et al., 1993, 1994). We further examined the effects of these mutations on the behavior of DE-cadherin. twi and sna mutants do not produce any mesenchymal cells even at stage 8 (Leptin and Grunewald, 1990). The cells corresponding to the wild-type mesodermal cells fail to penetrate into the inside of the embryo and remain keeping their monolayered structure. In these mutant embryos, high levels of DE-cadherin protein persisted in the ventral...
cells as well as in more lateral cells (Figs. 8A and 8B). In twi, incomplete furrow formation occurs at the most ventral region, giving rise to wedge-shaped cells (Leptin and Grunewald, 1990). DE-cadherin was highly concentrated at apical poles of the cells where Armadillo and D\\textalpha-catenin were also present at comparable levels (Fig. 8A; data not shown). In sna, the ventral region is known to give rise to cells with features similar to those of the mesectodermal cells in wild-type embryos (Rao et al., 1991; Arora and Nüsslein-Volhard, 1992). In these cells, DE-cadherin together with Armadillo and D\\textalpha-catenin was localized at apical poles of lateral cell surfaces, although these molecules were additionally distributed in irregular patterns along the lateral cell contacts and in the cells (Fig. 8B; data not shown). Thus, the ventral cells in twi and sna mutants fail to eliminate DE-cadherin-mediated adhesion and to lose the apical localization of DE-cadherin characteristic of epithelial cells.

In contrast to twi and sna mutants, fog mutants show normal replacement of DE-cadherin with DN-cadherin in cells corresponding to the mesoderm. The cells can penetrate into the inside of the embryo and exhibit mesenchymal features, although these cells fail to undergo normal concerted cell shape changes during the invagination process (Sweeton et al., 1991; Costa et al., 1994). As expected from the mRNA expression pattern, the reduction of DE-cadherin protein expression in the ventral cells took place normally in fog at stage 8 (Fig. 8C). However, we frequently found unusual concentrations of DE-cadherin between these mesenchymal cells which appeared to be adhering to one another. It seems that fog mutants fail in normal elimination of the preexisting DE-cadherin-based epithelial junctions in the invaginating mesoderm.

**DISCUSSION**

**Switching of Cadherin Expression and Its Roles**

Switching of cadherin expression from one to another type is seen in a variety of morphogenetic events during the development of higher multicellular organisms (Takeichi, 1988). However, there have been few studies in which the switching processes are precisely described. Our observations of Drosophila embryos at successive stages of gastrulation revealed a process in which the cadherin expression is turned from the DE- to the DN-type in formation of the mesoderm. We found that the control of cadherin transcription is governed by twi and sna activities as summarized in Fig. 7f. Generally, Twi is thought to function as a transcriptional activator (Ip et al., 1992a; Kosman et al., 1991; Leptin, 1991), while Sna is a repressor (Ip et al., 1992b; Gray et al., 1994). As expected, DN-cadherin transcription was activated by twi, and DE-cadherin transcription was repressed by sna. In addition, our results suggested that Twi may function as a repressor. In twi mutants, DE-cadherin expression is not repressed in the ventral region. At present, we do not know whether DE-cadherin transcription is regulated by Twi directly or indirectly. It is possible that Twi activates transcription of a gene(s) the products of which repress DE-cadherin transcription. Moreover, it is worth noting that sna was not required for initiation of DN-cadherin expression, but was required for an increase in its level.

Patterns of DE- and DN-cadherin mRNA subcellular localization are associated with cell polarity. In initial blastoderm epithelial cells, DE-cadherin mRNA is accumulated in the apical part of the cytoplasm. This apical accumulation resembles that of crumbs mRNA (Leptin, 1991). DE-cadherin mRNA may be preferentially transported to the apical cytoplasm and/or be stable in the apical cytoplasm in blastodermal cells. The loss of the apical localization and expression of DE-cadherin mRNA precedes the generation of mesenchymal mesoderm cells. The strong nuclear persistence of DN-cadherin mRNA, which might be a cause for delay in the appearance of the protein, continues until the cells become mesenchymal at stage 8. These mRNA expression patterns appear to be concerted with the cell-state changes involved in epithelial–mesenchymal conversion of the mesodermal germ layer.

Cadherin switching is a dynamic process, but its roles in morphogenetic movements seem to be restricted. In vertebrate embryos, as well as in Drosophila; cadherin switching from the E- to the N-type takes place in the initial mesoderm (Hatta and Takeichi, 1986). In the mouse, knock-out experiments indicated that N-cadherin is not essential for mesoderm morphogenesis during gastrulation (Radice et al., 1997). Even in Drosophila, DN-cadherin mutants have been analyzed, but no obvious defects on gastrulation have been found (Iwai et al., 1997). Consistent with these genetic results, our observations showed that the DN-cadherin protein appears too late to participate in the dynamic morphogenetic movements of the mesoderm as an adhesion molecule. In contrast to DN-cadherin, the preexisting cadherin, DE-cadherin, was shown to have potential key roles in adhesive interactions between cells not only in the presumptive ectoderm but also in the presumptive mesoderm before and during gastrulation.

**Cadherin Behavior and Cell Morphology Changes**

With respect to the presumptive neuroectoderm, the behavior of DE-cadherin could be divided into two phases. First, DE-cadherin was accumulated at apical parts of lateral cell contacts and gradually restricted to narrower regions close to the apical ends (Figs. 9A and 9B). The AJs concomitantly become enlarged. During this phase, the blastoderm epithelium remains static with small changes in cell shape.

In the second phase, DE-cadherin becomes distributed in irregular patterns, although apically concentrated DE-cadherin is still present (Fig. 9C). This unusual distribution of DE-cadherin may result not only from newly synthesized products but also from redistribution of the preexisting molecules. During this phase, the neuroectoderm epithe-
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Lium is dynamic mainly due to germ band extension. The cells form interdigitated or protruded foldings between neighboring cells and on apical cell surfaces. Cell intercalation in the extension of the ectoderm epithelium (Hartenstein and Campos-Ortega, 1985; Irvine and Wieschaus, 1994) may accompany marked alterations in the adhesive

**FIG. 5.** Transmission electron microscopic observation of the AJ in the mesoderm. Dorsal is up; ventral is down. (A, B) Ventralmost prospective mesodermal cells in a stage 6 embryo with constricted apices. (B) High magnification of the AJs indicated by arrowheads in A. AJs are present at almost all of apical ends of lateral cell contacts. Coated vesicles were frequently found near the AJs (arrows). (C–E) Mesodermal cells moving inward in a stage 7 embryo. (C) Light micrograph of a semithin section. (D) AJs with electron-dense undercoats were seen in the vicinity of apical cell surfaces in a region corresponding to the frame in C. (E) High magnification of the region indicated by an arrowhead in D. In some cases, AJs were flanked by contact-free plasma membranes with dense undercoats presumably containing large amounts of actin filaments (bracket). Note a small vesicle indicated by an arrow. (F, G) Mesodermal cells which have completed their inward movement. They formed a tube-like structure with a lumen (asterisks). (G) High magnification of a region close to the lumen in F. Few AJs were present around the lumen. (H, I) Mesodermal cells which have become round in shape in a stage 8 embryo. (H) Light micrograph of a semithin section. Small arrows indicate divided cells. (I) High magnification of a region corresponding to the frame in H. Regions of cell-cell contact were reduced resulting in intercellular spaces (arrows). Membrane-membrane contacts were still present not only between mesodermal cells but also between mesodermal cells and the yolk (yo). Scale bar: 2 μm for A, F, I; 320 nm for B, E, G; 26 μm for C, H; 533 nm for D.

**FIG. 6.** Distributions of DE-cadherin, Armadillo, and Dα-catenin in the invaginated mesoderm. (A, B) Sections of a stage 7 embryo stained for DE-cadherin (A) and for Dα-catenin (B). Strong concentrations of DE-cadherin were seen at apical cell poles of the invaginated mesoderm (arrowheads), but staining for Dα-catenin did not show prominent concentrations at corresponding sites. Note that the intensity of the concentrated signals for DE-cadherin was varied and not all of the apical poles showed such signals. At lateral cell surfaces, DE-cadherin and Dα-catenin were similarly distributed (arrows). (C–G) Sections of a stage 8 embryo stained for DE-cadherin (C, F), for Armadillo (D), and for Dα-catenin (E, G). (F and G) Higher magnifications of C and E, respectively. Cadherin and the catenins became distributed over the entire cell surface including cell contact-free areas (arrows). Strong cytoplasmic signals for Armadillo made it difficult to observe cell-surface signals. Scale bar: (A–E and F, G) 10 μm.
interactions and morphology of the cells. At the transition of the blastoderm epithelium from a static to a dynamic state, the distribution of DE-cadherin seems to become largely disordered, although tight cell contact at apical portions remains retained.

In the mesoderm, the DE-cadherin behavior could be divided into at least three phases. In the first phase, a subpopulation of the DE-cadherin is concentrated at apical ends of lateral cell contacts giving rise to AJs. This occurs concomitantly with cell shape changes from columnar to wedged forms, which result in formation of the ventral furrow. At this time, cytoplasmic myosin seems to generate forces to slide actin filaments leading to constriction of the cell apices (Young et al., 1991; Callaini, 1989). Coordinated actions of the cadherin-based adhesion system and the actin-based cytoskeleton are generally thought to play roles in bending of epithelial sheets. Our observations agree with this hypothesis. We speculate that the behavior of the cadherin-catenin system is largely affected by rearrangements of the actin network in the first phase.

In the second phase of DE-cadherin behavior, the AJs once established at the apical cell poles are degenerated or

**FIG. 7.** Whole-mounts of wild-type (A, B), twi (C, D), sna (E, F), and fog (G, H) mutant embryos stained for DE-cadherin (A, C, E, G) or DN-cadherin (B, D, F, H) mRNA. All embryos were at stage 7–8 and were viewed from the ventral side. Brackets indicate ventral regions which correspond to the prospective mesodermal region of the wild-type embryo. In twi and sna, there were no differences in the expression level of DE-cadherin mRNA between the ventral and other parts (C, E). DN-cadherin mRNA was undetectable in twi (D) but could be detected in sna (F), although its expression level was markedly reduced. In fog, both DE- and DN-cadherin mRNAs showed normal expression (G, H). (I) Summary of the regulatory cascade for the DE- to DN-cadherin switching at the ventral region of the embryo. Dotted line indicates a requirement for enhancement or maintenance of the expression. Scale bar, 10 μm.
disrupted. DE-cadherin-based epithelial junctions are eventually eliminated from the apical portions (Fig. 9E). During this elimination process, the amounts of DE-cadherin and Armadillo are not comparable to those of Dα-catenin and F-actin at the apical cell contacts. This suggests that DE-cadherin and Armadillo are more concentrated and/or remain longer at the apical portions than Dα-catenin and F-actin. It is likely that the cadherin–β-catenin complexes remain anchored to the membrane at apical–lateral borders, but considerable amounts of Dα-catenin and F-actin become separated from the adhesion complexes. Dα-catenin may be a key molecule to regulate the interaction between the cadherin–β-catenin adhesion complexes and the actin cytoskeleton for the control of cell morphology as suggested by previous studies on vertebrate α-catenin (Nagafuchi et al., 1994; Hirano et al., 1992; Rimm et al., 1995; Torres et al., 1997).

In the third phase, DE-cadherin is distributed over the entire cell surface, followed by complete degradation (Fig. 9F). At the same time, the mesodermal cells enter the 14th mitosis (Hartenstein and Campos-Ortega, 1985; Foe and Odell, 1989; Arora and Nüsslein-Volhard, 1992) while be-
coming round in shape. It is likely that this cell morphology change is mainly due to reorganization of the tubulin-based cytoskeletal network. However, it is independent of mitosis since mesoderm morphogenesis looks normal in string mutant gastrulae, in which the 14th and later mitosis do not take place (Leptin and Grunewald, 1990). After the apically located AJs have been degenerated, lateral cell contacts mediated by DE-cadherin may become weak and flexible. It should be stressed that the loss of epithelial junctions allows the cells to change to a round shape, disrupt their monolayer structure and begin to exhibit mesenchymal features.

**Mesoderm Formation and Cell Polarity**

Marked conversion of the presumptive mesodermal cells from an epithelial to a mesenchymal state is common during gastrulation of a wide range of metazoans. In this study, we observed successive cellular changes in the epithelial–mesenchymal transition of the initial mesoderm. Our observations suggest that the ingestion of the mesoderm occurs concomitantly with disruption of the DE-cadherin-based epithelial junctions. This junctional disruption leads to the relatively even appearance of cell surfaces, allowing the mesodermal cells to enter a nonpolarized state.

Mutations in twi and sna fail to eliminate DE-cadherin-based cell–cell junctions from ventral cells. This seems to result from a failure in the loss of epithelial cell polarity. At the ventral region of twi mutants, delayed constriction of the cell apices occurs, but the cells never penetrate to within the embryo (Leptin, 1991). In twi–sna double mutants, exogenous sna expression induces furrows, but does not to generate motile cells which can disperse (Ip et al., 1994). It is conceivable that the persisting epithelial junctions interfere with dispersal of the cells. In contrast to twi and sna, fog function seems to be independent of cell polarity control but to be associated with junctional disruption. In fog mutants, the ventral cells appear to be normally committed to the mesenchymal fate. However, unusual DE-cadherin concentrations remaining between the invaginated mesodermal cells in fog mutants suggest a defect in a process by which DE-cadherin-based junctions are lost. It is possible that this junctional disruption process is linked with reorganization of the actin cytoskeleton, which is presumably affected in fog mutants (Costa et al., 1994; Barrett et al., 1997).

In normal embryos, epithelial cell polarity of the presumptive mesoderm is disrupted downstream of twi and sna activities, which control both mesodermal morphogenesis and differentiation. Our analyses of the expression patterns of cadherin mRNAs and proteins suggested that the cadherin-based cell–cell adhesion system is closely linked to a variety of cellular functions including cell

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**FIG. 9.** Summary of the expression patterns of DE-cadherin in neuroectodermal and mesodermal cells during gastrulation. The ventral regions of the gastrula embryos at stages 6 (A, D), 7 (B, E), and 8 (C, F) are illustrated. N and M indicate neuroectodermal (A–C) and mesodermal (D–F) cells schematically drawn below, respectively. See text for detail.
movement, polarity, and differentiation. It is speculated that, even in the gastrulation of other organisms including vertebrates and sea urchin, some of these aspects of the cadherin adhesion system may be conserved (Hatta and Takeichi, 1986; Miller and McCay, 1997a,b).

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