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Characterization of Morphological and Cytoskeletal Changes in MCF10A Breast Epithelial Cells Plated on Laminin-5: Comparison with Breast Cancer Cell Line MCF7

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The extracellular matrix regulates functional and morphological differentiation of mammary epithelial cells both in vivo and in culture. The MCF10A human breast epithelial cell line is ideal for studying these processes because it retains many characteristics of normal breast epithelium. We describe a distinct set of morphological changes occurring in MCF10A cells plated on laminin-5, a component of the breast gland basement membrane extracellular matrix. MCF10A cells adhere and spread on laminin-5 about five times more rapidly than on fibronectin or uncoated surfaces. Within 10 minutes from plating on laminin-5, they send out microfilament-rich filopodia and by 30 minutes acquire a cobblestone appearance with microfilaments distributed around the cell periphery. At 90 minutes, with or without serum, > 75% of the MCF10A cells plated on laminin-5 remain in this stationary cobblestone phenotype, while the remainder takes on a motile appearance. Even after 18 hours, when the culture is likely entering an exponential growth phase, the majority of cells maintain a stationary cobblestone appearance, though motile cells have proportionally increased. In contrast, the fully transformed, malignant human breast epithelial cells, MCF7, never acquire a stationary cobblestone appearance, do not organize peripheral microfilaments, and throughout the early time points up to 120 min appear to be constantly motile on laminin-5. We propose that changes in morphology and microfilament organization in response to laminin-5 may represent a benchmark for distinguishing normal vs. malignant behavior of epithelial cells derived from the mammary gland. This may lead to better model systems for studying the interactions between breast epithelium and the basement membrane extracellular matrix, which appear to be deregulated in processes like carcinogenesis and metastasis.

Keywords: Breast epithelial cells, integrins, microfilaments, migration, laminin

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INTRODUCTION

Laminin-5 (Ln-5), is intimately associated with the basal surface of breast epithelial cells and likely plays an important role in regulating their behavior (Giannelli *et al.*, 1997; Koshikawa *et al.*, 2000). Ln-5 belongs to a family of extracellular matrix (ECM) molecules that consists of at least ten genetically distinct subunit chains which may form the eleven heterotrimer isoforms currently known (Tryggvason, 1993). The heterotrimers are formed by one alpha, one beta and one gamma chain that form a disulphide-bonded cross-shaped molecule (Engel, 1992). Ln-5 has been described independently by several laboratories as nicein, kalinin, epiligrin or ladsin (Carter *et al.*, 1991; Kikkawa *et al.*, 1994; Marinkovich *et al.*, 1992; Miyazaki *et al.*, 1993; Rousselle *et al.*, 1991; Verrando *et al.*, 1987). We characterized the Ln-5 heterotrimer produced by the rat cell line 804G (Falk-Marzillier *et al.*, 1998; Hormia *et al.*, 1995; Langhofer *et al.*, 1993).Genetic evidence indicates that Ln-5 plays a vital role in maintaining adhesion to the basement membrane (BM), since missense mutations in the genes for Ln-5 and or its receptor result in epithelial exfoliation to various degrees of severity (McGrath *et al.*, 1995; Uitto *et al.*, 1994; Vailly *et al.*, 1995; Videl *et al.*, 1995). Ln-5 is quite effective in promoting rapid and efficient attachment, spreading and migration of epithelial cells (Plopper *et al.*, 1998). In fact, it is one of the most adhesive ECM proteins for normal breast epithelial cells (Gorczyca *et al.*, 1993; Hewitt *et al.*, 1997; Pellegrini *et al.*, 1995; Soinin *et al.*, 1994).

Cell adhesion to laminins is mainly mediated by the ubiquitous α/β heterodimeric transmembrane glycoproteins, the integrins, signal transducing receptors involved in cell adhesion, migration, morphology, differentiation, proliferation (Clark and Brugge, 1995; Damsky and Werb, 1992; Hynes, 1987; Schaller and Parsons, 1994; Schwartz *et al.*, 1995), and generally associated with the microfilament (MF) cytoskeleton at focal or cell–cell contacts (Burridge *et al.*, 1988; Hynes,1987). As part of the BM, Ln-5 is embedded in a complex network of ECM molecules synthesized, remodeled, and maintained by the cells in contact with or anchored to it. Regulation and control of the interaction between the cell and this matrix is important for numerous biological processes, including proliferation, morphogenesis and cell migration. There is still limited understanding of function and signaling associated with integrins that bind Ln-5. In addition, there has been insufficient characterization of the morphological and cytoskeletal changes induced when cells encounter Ln-5.

The limited life span breast epithelial cell line MCF10, which was derived from the mastectomy tissue of a 36 year old parous, premenopausal woman with fibrocystic disease has, without viral or chemical intervention (Russo *et al.*, 1993), spontaneously given rise to the immortal subline MCF10A. The "A" refers to the cell line that is adherent in conventional calcium levels (1.05 mM) (Paine *et al.*, 1992; Tait *et al.*, 1990). MCF10A cells have retained many of the characteristics of normal human mammary epithelial cells (HUMEC). They have a near diploid human female karyotype (Soule *et al.*, 1990), can organize into monolayers on plastic (Russo *et al.*, 1993) and ductular structures in collagen gels (Soule *et al.*, 1990), are controlled by hormone and growth factors and are not tumorigenic in nude mice (Soule *et al.*, 1990). They are also ideal for studying mammary epithelial cell branching morphogenesis (Howlett *et al.*, 1995; Stahl *et al.*, 1997). In addition, MCF10A cells express sialomucins and keratins (Soule *et al.*, 1990; Tait *et al.*, 1990), and ultrastructurally are low cuboidal cells with numerous hemidesmosomes, desmosomes, and short microvilli, all typical attributes of normal human breast epithelium (Russo *et al.*, 1993; Stahl *et al.*, 1997). Collectively, these characteristics make this cell line closest to a normal human breast epithelial cell line, suitable for studying adhesion, spreading and migration.

The purpose of this work is to describe the morphological changes occurring in MCF10A, a normal breast epithelial cell line, and MCF7,

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a breast carcinoma cell line, when plated on matrix normally found in breast tissue BM, specifically Ln-5. Contacting Ln-5 leads to the formation of both a stationary and a migratory phenotype in MCF10A cells. The stationary phenotype dominates at early plating time points, while the migratory increases in percentage perhaps as the culture enters exponential expansion. In contrast, in MCF7 cells the migratory phenotype is predominant at any stage of plating. These results suggest that plating on Ln-5 may represent a model system for the in vitro study of the regulation of motile behavior of breast epithelial cells in contact with BM.

MATERIALS AND METHODS

Cells

MCF10A cells were passaged in DFCI medium according to Band and Sager (1989), and MCF7 cells in RPMI supplemented with 10% fetal calf serum (Gemini, Irvine CA), glutamine (2 mM), penicillin G (100 units/ml) and streptomycin sulfate (100 μg/ml) (BioWhittaker, Walkersville MD). Rat 804G cells, used for generating Ln-5 matrix, were grown in DMEM supplemented as the RPMI. All three cell types were maintained at 37 °C in a humidified incubator containing 10% carbon dioxide.

Coating of Slides

804G cells were grown on coverslips till confluent and then lysed by two incubations for 10 minutes in 20 mM sterile NH_4OH at room temperature. This procedure results in coverslips coated with cell-free deposited 804G extracellular matrix, which is composed predominantly of Ln-5 (Falk-Marzillier *et al.*, 1998; Hormia *et al.*, 1995; Langhofer *et al.*, 1993). For fibronectin coating, slides were incubated with 40 μg/ml fibronectin (Sigma) in distilled water overnight at room temperature, and blocked with bovine serum albumin for 1 hour (Hormia *et al.*, 1995; Plopper *et al.*,1998), The matrix-coated coverslips were rinsed four times for 5 minutes in phosphate buffered saline (PBS) prior to use.

Fluorescence Microscopy

Cells were plated for < 1, 10, 30, 60, 90,120 minutes, 5 and 18 hours in either growth medium or serum free medium on glass coverslips coated with either Ln-5 matrix (Langhofer *et al.*, 1993), fibronectin or uncoated. After brief rinsing in PBS, cells were fixed for 10 minutes in 3% paraformaldehyde/PBS, permeabilized for 10 minutes with 0.01% Triton X-100/PBS, and rinsed twice with PBS. Cells were incubated with a 1:10 dilution of rhodamine phalloidin (ICN Immunobiologicals, Costa Mesa, CA) for 40 minutes to stain filamentous actin. Stained coverslips were washed twice in PBS, mounted in Immunofluore mounting medium (ICN), viewed with an Axiophot I microscope equipped with epifluorescence (Zeiss, Germany) and photographed on Kodak T-max 400 film.

Video Microscopy

MCF10A or MCF7 cells were plated on coverslips coated with Ln-5 matrix and immediately placed in a sealed Dvorak Environmental Chamber equipped with a temperature controlled air curtain heated to 37 °C. Recordings of cell adhesion, spreading and migration were made using a Zeiss Axiovert 100TV equipped with a video camera (CV 730). For image analysis, images were extracted from tape using Inovision ISEE software, converted to TIFF files and then contrast stretched and sharpened using Adobe Photoshop 4.0.

Method of Categorizing Epithelial Cell Morphology

MCF10A cells were placed into 4 different categories according to morphology and MF organization (see Fig. 2). These morphologies were based on observations from at least four plating

experiments as shown in Fig. 1, and as described in the results. Morphology s10 refers to cells with many small filopodia circumferentially located around the cell that are rich in MFs. Morphology s30 and s90 refer to the polygonal, cobblestone-shaped, morphology where s90 is larger and has more peripheral and central MF bundles than s30. Morphology m90 is a fan shaped cell with no MFs or with stress fibers generally located parallel to the longitudinal axis of the cell. The "s" and "m" refer to stationary and migratory phenotypes, respectively.

Quantification and Statistical Analysis

Statistical analyses were performed using Microsoft EXCEL statistics software. The changes in morphology were quantified by using a 63 objective and counting 15 fields from $n = 4$ coverslips of cells per time point. Cells were plated at 20,000 cells per coverslip. Results were expressed as (%) mean ± standard error of the mean. A Students T-test was used to determine whether the values obtained at each time point and treatment were statistically different ($p = 0.01$). The term "preferential" is used to denote the most frequently found morphology that is statistically significant.

RESULTS

Substrate-Dependent Morphological Changes of MCF10A Cells

MCF10A cells were plated on Ln-5 matrix-coated coverslips and their morphologies and MF organization were analyzed by fluorescence microscopy at different times (Fig. 1). Immediately after plating (< 1 minute), the majority of the adherent cells were rounded and MF staining with rhodamine phalloidin was diffuse throughout the cytoplasm. The observed cells were adherent, or at least tethered to the substratum, since they resisted the mechanical shear of washing before, during and after fixation. In contrast, on uncoated glass no cells were left after fixation. At 10 minutes after plating, MF staining was still diffuse, but distinct filopodial projections encircling the cell perimete were distincly evident (Fig. 1b). By 30 minutes MCF10A cells were spread and MFs were clearly visible at the cell periphery. These MFs were aligned parallel to the circumference forming a polygonal arrangement (Fig. 1c-d). The MFs became more sharply defined between 60 and 90 minutes, as cell spreading proceeded. They eventually formed a belt-like array of MFs, evenly distributed around the entire cell. This peripheral belt thickened and widened with time, between 90–120 minutes, as the cell area approximately doubled and cells spread into a flat polygonal morphology (Fig. 1e-f). These changes in morphology and MF organization are schematically summarized in Figure 2, and referred to as the s10, s30 and s90 morphologies ["s" for "stationary"].

On fibronectin, a similar morphological progression was observed; however, the time frame was substantially longer. In fact, morphology s10 was not observed until 30 minutes, s30 was not observed until 90 minutes, and s90 was not prevalent until approximately 5 hours after plating (Fig. 3a, b, c respectively). Thus, it appears that each adhesion and spreading step took approximately 3x longer on fibronectin than Ln-5. Moreover, if cells were plated on fibronectin in the presence of a blocking antibody to human Ln-5, cells were still in the s10 morphology at 5 hours, while with a non-blocking antibody the same timing of Figure 3 was observed (not shown). Thus, at least part of the spreading observed on fibronectin may be due to endogenous Ln-5 secretion by MCF10A cells. On uncoated glass coverslips (in the presence of serum, which contains vitronectin and fibronectin), spreading was further delayed, such that s10 was only present at 5 hours (not shown).

We quantified the number of cells in each of the categorized morphologies at the 90-minute time point on these various substrates (Fig. 4). On Ln-5, >75% of MCF10A cells were in the s90

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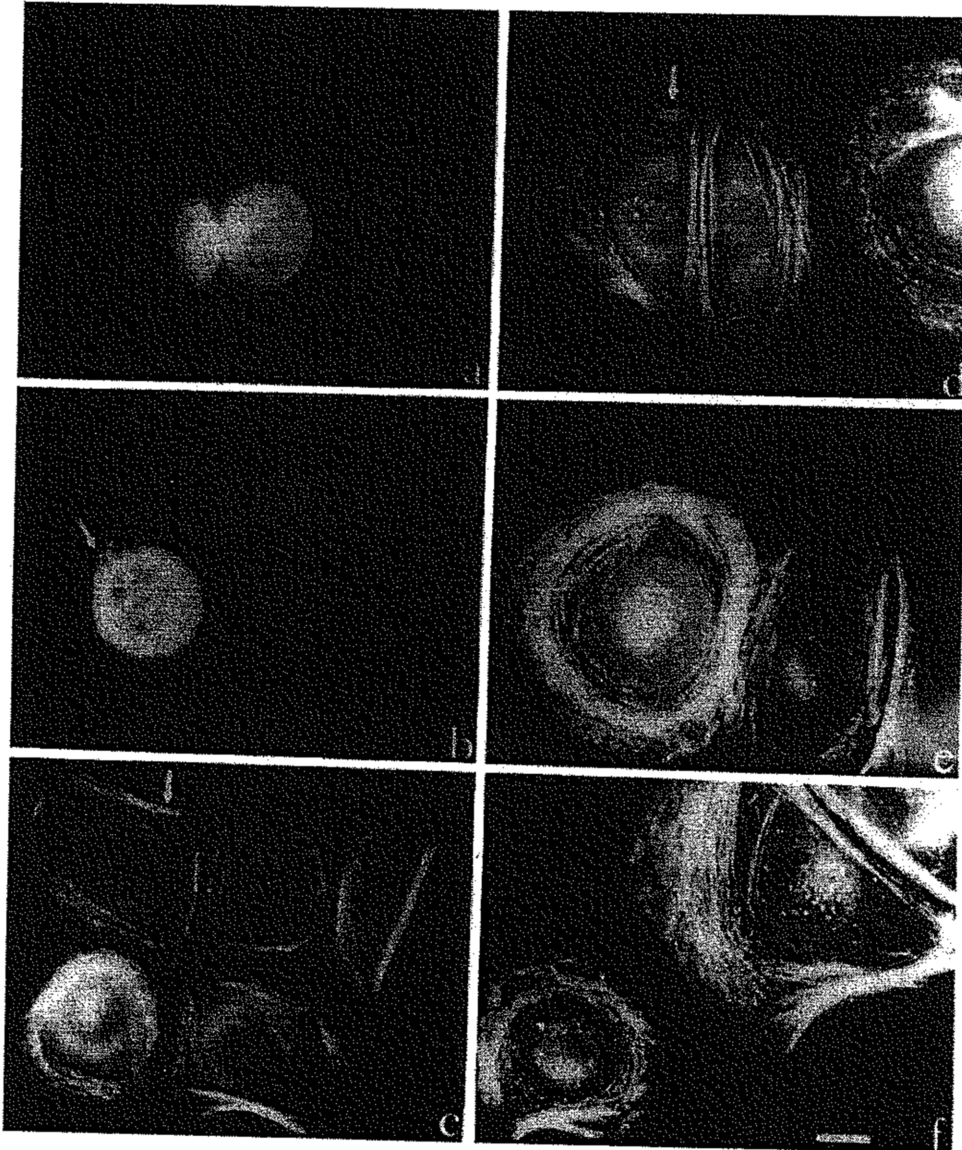

FIGURE 1 A fluorescent micrograph of MCF10A cells plated on Ln-5 and stained with phalloidin to label MFs, immediately (a), 10 minutes (b), 30 minutes (c), 60 minutes (d), 90 minutes (e), and 120 minutes (f) in the presence of growth serum (DFCI medium). Arrow in (b) indicates filopodial extensions and arrows in (c) and (d) indicate the peripheral MF bundles. Bar = 20 μm.

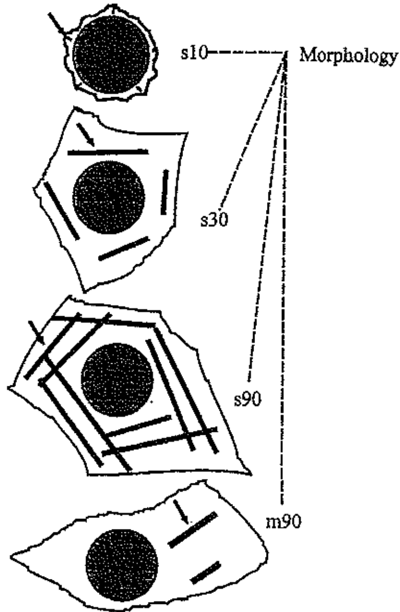

FIGURE 2 Schematic representation of the 4 distinctive morphologies of MCF10A cells plated on Ln-5. The morphologies are s10, s30, s90, and m90 and are referred to in the text where s indicates the stationary and m indicates the migratory morphology. The thick lines within each cell represent MF bundles (see arrow in each cell).

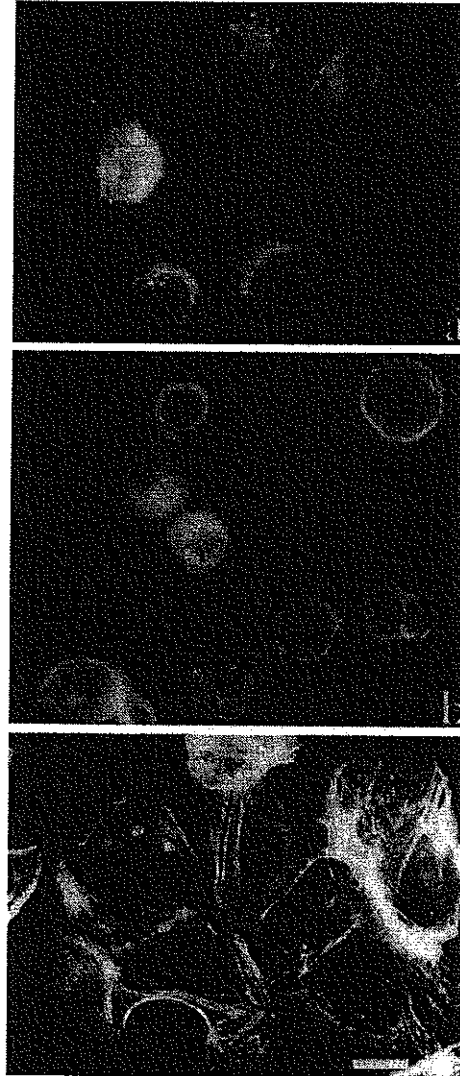

FIGURE 3 A fluorescent micrograph of MCF10a cells plated on fibronectin and stained with phalloidin to label MFs, 30 minutes (a) 90 minutes (b) and 5 hours (c) in the presence of growth serum. (DFCI medium). Bar = 20 μm.

morphology, as anticipated by our classification system. In contrast, the majority (>80%) of the cells remained in morphology s10 category after plating on fibronectin or uncoated glass (Fig. 4). Moreover, whether cells were plated in the presence of serum or serum-free, the percentage of cells in each category remained significantly the same [$p = 0.01$] (Fig. 4), suggesting that the Ln-5 matrix rather than some serum factor is responsible for initiating the morphological and MF changes observed. Collectively, these observations indicated that MCF10A cells adhered, spread and underwent a concomitant MF reorganization, rapidly and specifically on Ln-5.

We then used a different technique, time-lapse video microscopy, to confirm that the progression of shape changes on Ln-5 described above corresponded to stationary or motile phenotypes. At the 1-minute time point, >95% of the cells were

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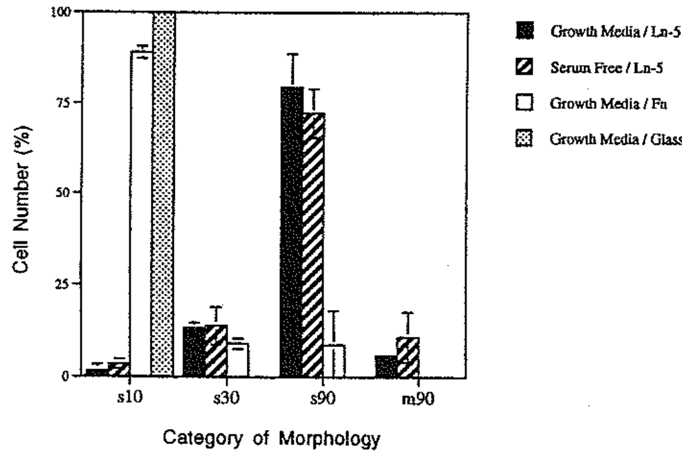

FIGURE 4 The percentage of MCF10A cells fully spread and stationary, s90, is greatest on Ln-5 (black bars) in the presence of growth media (DFCI media) and also plated on Ln-5 without growth media (hatched bars), in comparison to cells plated on fibronectin (open bars) or uncoated glass (dotted bars). Cells were classified based on criteria defined in figure 2. After 90 minutes the majority of the cells on fibronectin and glass are at s10 whereas on Ln-5 they are at s90. Values represent mean percentage of cells ± standard error of the mean.

immobile on Ln-5 coated coverslips, while on uncoated glass they floated or rolled (not shown), supporting the notion that MCF10A cells become tethered or adherent to Ln-5 substratum rapidly. By following individual cells, shape changes occurred at the expected time points. Furthermore, we observed that the cells in morphology s10, s30 and s90 were clearly stationary. However, within the 90-minute time period after plating on Ln-5 inthe presence of DFCI medium, a small population of MCF10A cells (<10%) (cell 1 in Fig. 5) were observed to be migrating rather than simply spreading. In fact, the morphology and MF distribution of these cells, noted by "m" for "migratory" and referred to as m90 (Fig. 6a, b), was clearly distinct from the "s" morphology series. In real time, we observed that the fan-shaped, migratory m90 morphology was derived only from cells that had achieved at least the s30 or s90 shape. By phalloidin staining, the m90 MCF10A cells had either no MFs present (Fig. 6a) or the MFs were presumably organized as stress fibers, aligned perpendicular tothe edge of the largest lamellipodium (Fig. 6b) and parallel to the direction of migration (Fig. 5). Up to the 90-minute time point, the relative abundance of s10–s90 and m90 morphologies was similar either in serum-free RPMI or DFCI growth medium (Fig. 4). At 18 hrs, however, in the presence of serum the percentage of MCF10A cells with stationary morphology (s90) decreased significantly, while there was a concomitant increase in the percentage of cells with migratory morphology (m90) (Fig. 7), displaying stress fibers aligned in the direction of migration (Figure 6b).

Substrate-Dependent Morphological Changes of MCF7 Cells

MCF10A are spontaneously immortalized, but non-tumorigenic cells and are considered to be a good representation of normal breast luminal epithelium. For comparison, we examined the morphological behavior on Ln-5 of a transformed, low-metastatic breast cancer cell line, MCF7 (Soule *et al.*, 1973). Interestingly, after plating on Ln-5, MCF7 cells did not take on any of the morphologies typical of MCF10A cells. By 10

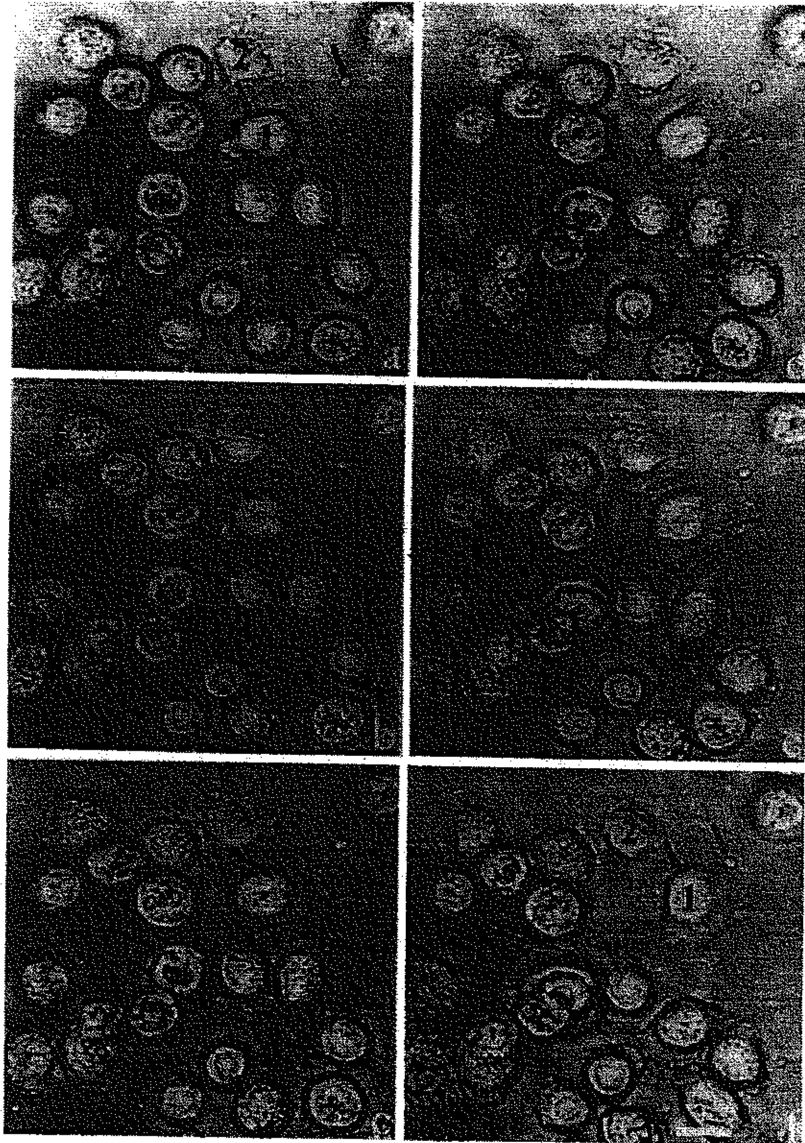

FIGURE 5 Video microscopy of MCF10A cells viewed in real time between 30 and 80 minutes after being plated on Ln-5 in the presence of growth serum (DFCI medium). Images were followed by time lapse and photographed at 30 minutes (a), 40 minutes (b), 50 minutes (c), 60 minutes (d), 70 minutes (e), and 80 minutes (f) after plating. Cells labeled 1 and 2 in (a) indicate a migrating (1) and a stationary but spreading (2) cell, that fall into the m90 and s90 category, respectively. Two arrows in (a) and (f) indicate the distance covered by the migrating cell. Bar = 10 μm.

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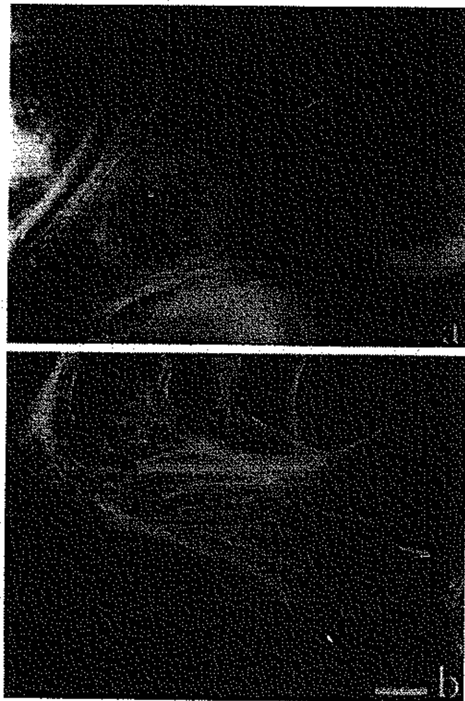

FIGURE 6 A fluorescent micrograph of a representative example of m90 MCF10A cells (a,b) plated on Ln-5 and stained with phalloidin to label MFs, 90 minutes (a) 18 hours (b) in the presence of growth serum (DFCI medium). Both Figure (a) and (b) show a cell in the fan-shaped, potentially migratory position with out stress fibers and very few peripheral MFs remaining (in a, cell labeled 1) and with stress fibers (in b, cell labeled 1) aligned parallel to the long axis of the cell. Arrows indicate the most likely direction of migration. Bar = 20 μm.

minutes on Ln-5, MCF7 cells began asymmetrically sending out long lamellipodial projections rich in MFs (Fig. 8b). By 30 minutes (Fig. 8c), 2 to 3 protrusions were substantially elongated so as to give the cell and irregular spindle shape, with filopodial extensions at their ends which were rich in MFs (Fig. 8d-f). By 90 minutes MCF7 cells had a fibroblastic appearance (Fig. 8f). By video microscopy, stationary MCF7 cells were clearly seen sending out lamellipodial projections and randomly retracting them and in most cases began migrating in the direction of the largest lamellipodium (Fig. 9, cell 3 and 1, respectively). Time lapse also showed that MCF7 cells migrated faster than MCF10A cells (Fig. 9, cell 1 and 2).

DISCUSSION

Our results show that MCF10A breast epithelial cells in contact with Ln-5 adhered, spread, and changed shape and MF organization in a sequential and distinctive manner. For clarity, we identified these stages as defined in Figure 2 (s10, s30, s90 and m90, where s = stationary and m = motile, followed by the time after plating that morphology was predominant at).

Adhesion to Ln-5 occurred rapidly, such that cells became tethered essentially upon contact with Ln-5 matrix (s10). This immediate tethering did not occur on fibronectin or Ln-1 (not shown), and may therefore be a unique property of Ln-5 and/or its receptors.

MCF10A cells then progressed through multiple stages of spreading on Ln-5, in less than 2 hours. During this period, spreading cells took on a cobblestone appearance, with MFs circumferentially distributed at the cell periphery (s30 and s90). In contrast, > 80% of MCF10A cells plated on fibronectin or uncoated glass remained in the early rounded morphology category, s10, for several hours. These results suggest that Ln-5 is a preferential substrate for adhesion and spreading of normal breast epithelial cells, at least in culture and possibly *in vivo*.

At the 10-minute time point (s10), filopodial projections were visible and, by video microscopy, some of these appeared to retract. Cells generally send out such radial projections to probe their surroundings (Albrecht-Buehler, 1976). In our system, it would appear that MCF10A cells probed their environment via filopodia, and, if they encountered other cells, they started forming cell–cell junctions that eventually resulted in cobblestone-like monolayers.

At 30 minutes, MCF10A cells were very well spread on Ln-5 (s30), touched each other at many

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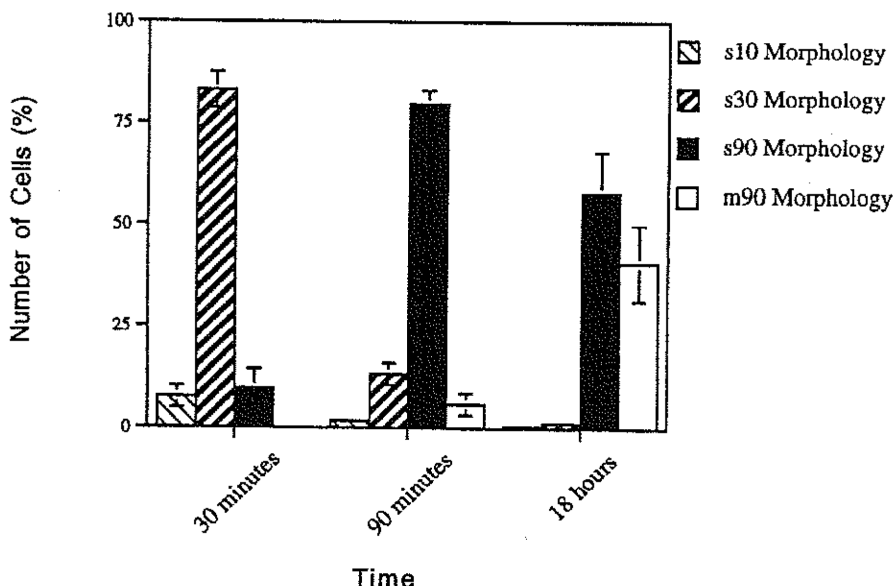

FIGURE 7 The percentage of MCF10A cells in the migratory morphology m90 increases after 18 hrs on Ln-5. MCF10A cells plated on Ln-5 in the presence of growth media for 30 minutes, 90 minutes and 18 hours are found in the various morphologies categorized as s10 (thin hatched bars), s30 (thick hatched bars), s90 (black bars) and m90 (open bars). Values represent mean percentage of cells ± standard error of the mean. ($p = 0.01$)

locations, and displayed well-formed belts of peripheral MFs, which became much more pronounced in the next 90 minutes (s90). MFs are fibers composed of two single strands of filamentous actin twisted into a helix. (Bray, 1992; Mitchison and Carmer, 1996; Preston *et al.*, 1990). Like stress fibers, the peripheral MF bundles consist of MFs aligned parallel to one another and contain also myosin, α-actinin and filamin (Byers *et al.*, 1984; Franke *et al.*, 1988; Kalnins *et al.*, 1995). Bundles of MFs in stationary epithelial cells have been described (Bray, 1992; Kalnins *et al.*, 1995) and shown to be closely associated with the intercellular junctional regions (Franke *et al.*, 1988; Schulze and Firth, 1993). Accordingly, we observed that single stationary MCF10A cells, very early after adhesion to Ln-5, began to elaborate peripheral MF bundles. As the cells reached confluency, these peripheral bundles became very prominent at regions of cell–cell apposition in monolayers of MCF10A cells (Fig. 1). Similar peripheral MF bundles are generally observed in cells belonging to epithelia with barrier functions (Bray, 1992; Kalnins *et al.*, 1995).

The majority of MCF10A cells on Ln-5 tended to assume a stationary morphology, with well-developed intercellular junction-type MFs. However, a proportion of cells, up to 40% at about 18 hours, displayed a definite motile morphology. We suspect that these cells did not enter into cell–cell contacts and were possibly moving to colonize open territory in the culture dish. Cell movement may also precede, or immediately follow mitosis. By counting the number of cells in each category as shown in Figure 2, we found the maximum number of migratory cells (m90), about 40%, at 18 hours, which corresponds to the approximate time when the cell culture would enter an exponential growth phase. Note, however, that the timing of the cell morphological adaptations were

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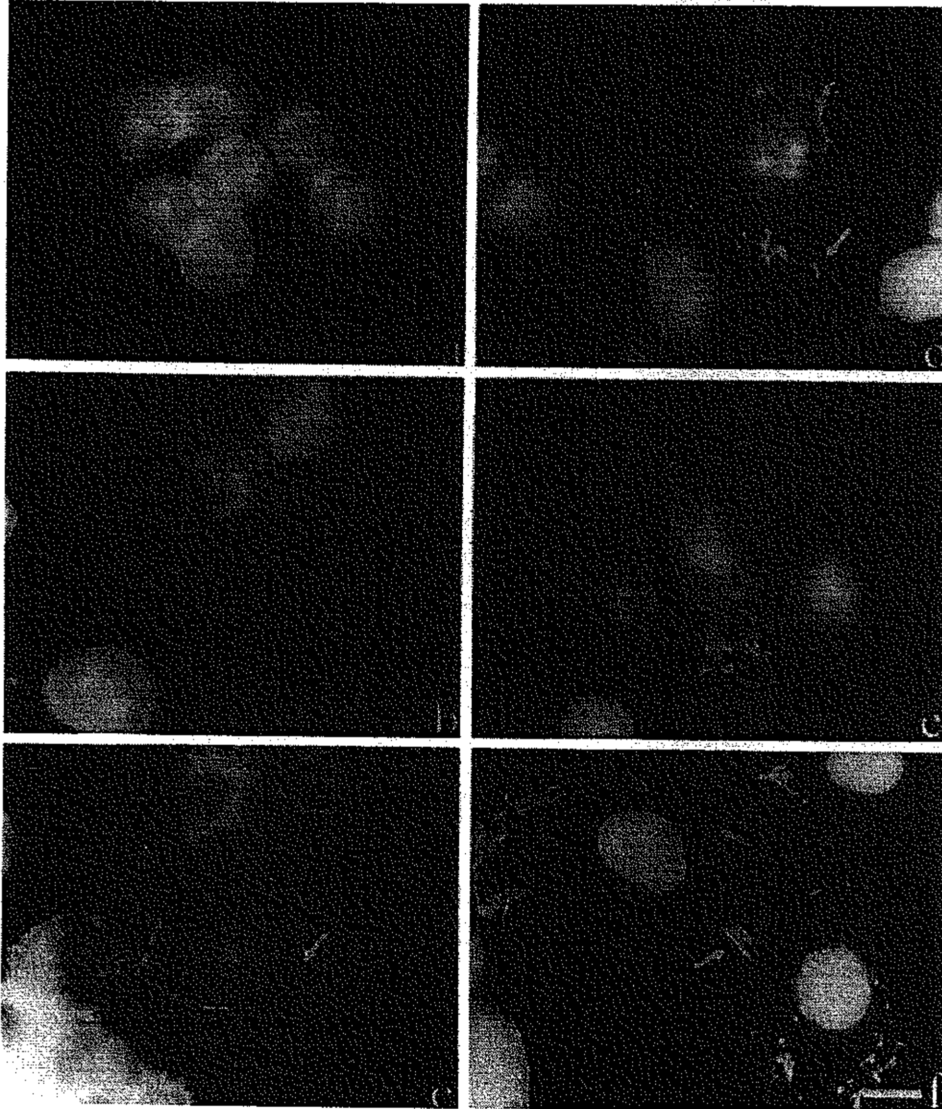

FIGURE 8 A fluorescent micrograph of MCF7 cells plated on Ln-5 and stained with phalloidin to label MFs, immediately (a), 10 minutes (b), 30 minutes (c), 60 minutes (d), 90 minutes (e), and 120 minutes (f) in the presence of growth serum (DFCI medium). Arrows in (c), (d) and, (f) indicate MF rich filopodial ends of processes that are progressively larger from (c) to (f). Bar = 20 μm.

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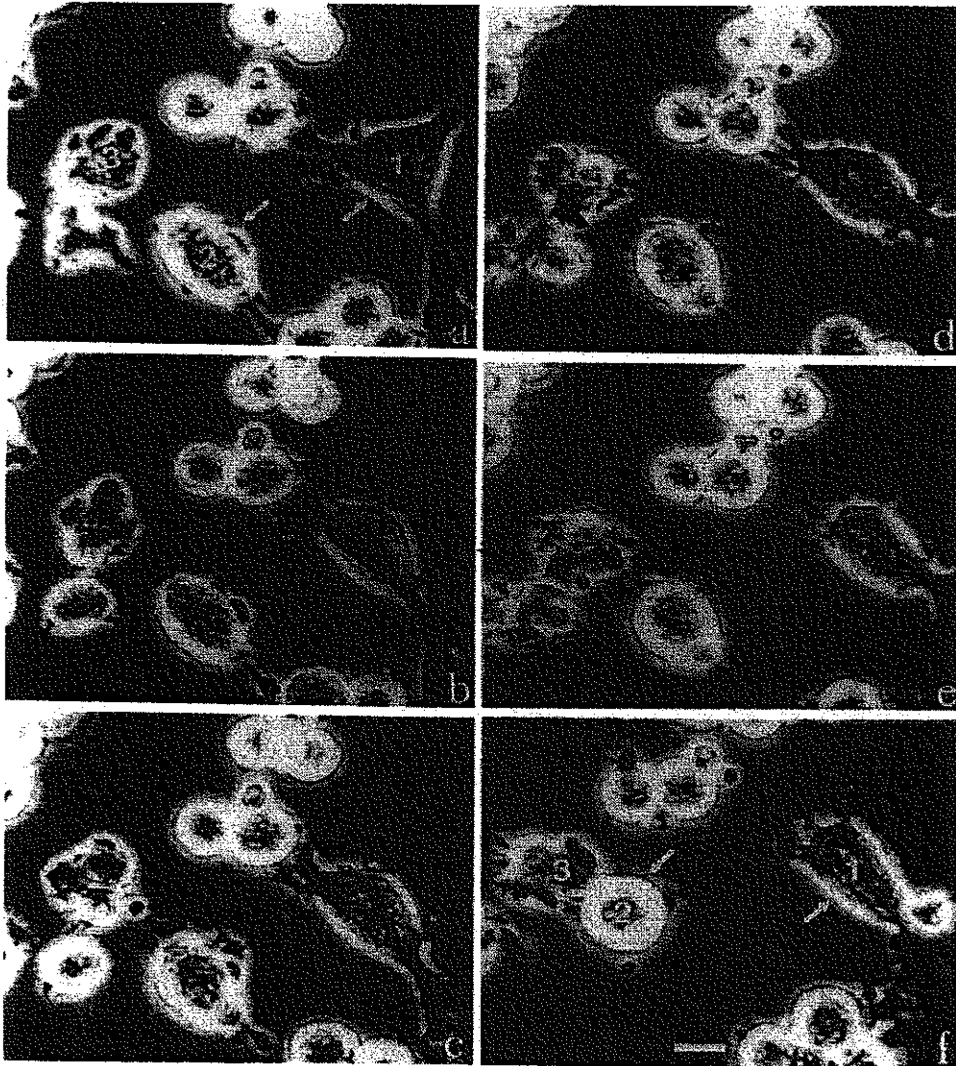

FIGURE 9 Video microscopy of MCF7 cells viewed in real time between 60 and 80 minutes after being plated on Ln-5 in the presence of growth serum (RPMI medium). Images were followed in time lapse and photographed at 60 minutes (a), 65 minutes (b), 70 minutes (c), 72.5 minutes (d), 75 minutes (e), 80 minutes (f) after plating. Cells labeled 1 and 2 are migrating past each other (see arrows for reference in (a) and (f)). The cell labeled 3 is not migrating but sending out lamellipodial projections from all directions and spreading. Bar = 20 μm.

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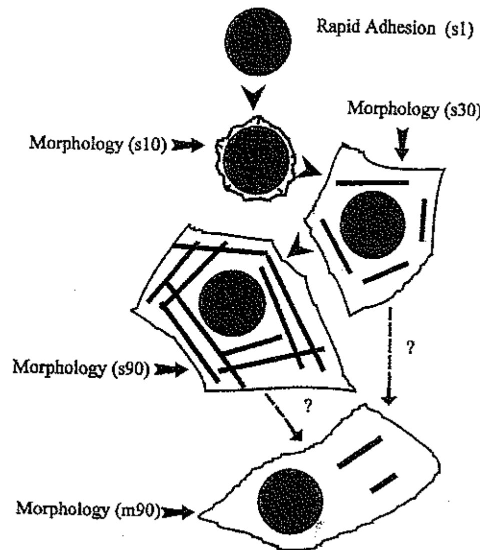

FIGURE 10 Proposed sequence of morphological changes (s10, s30, s90, m90) of MCF10A cells on Ln-5 indicating the most likely sequence of formation (see arrow heads for direction). The dotted lines, with question marks, indicate the possible transition route to the morphology designated m90 from either s30 or s90.

dependent upon the presence of Ln-5, rather than serum. The motile cells lost their elaborate peripheral MF belt and polygonal shape. Most motile cells were elongated, with a large fan shaped lamellipodium, and displayed a diffuse MF cytoskeleton, occasionally with stress fibers aligned parallel to the long axis (m90 in Fig. 2). Overall, the asymmetrical, fan-shaped morphology of migrating MCF10A cells was very reminiscent of the typical morphology and MF pattern observed in fast or slow motile fibroblasts (Abercrombie *et al.*, 1970; Abercrombie, 1980; Badley *et al.*, 1980; Lewis *et al.*, 1982). The organization of MFs in the motile MCF10A cells recalled the other type of organized actin cytoskeleton, the stress fibers, bundles of MFs found in both stationary and migrating cells that generally terminate at focal adhesions. Such adhesions are preferentially distributed behind the leading edge of slowly motile cells (Burridge *et al.*, 1988; Byers *et al.*, 1984). To migrate, a cell must acquire a spatial asymmetry which results in a polarized morphology that clearly distinguishes between the front (leading edge) and rear (trailing edge) of the cell (Lauffenburger and Horwitz, 1996). The leading edge of cells that are spreading or migrating consists of a highly compact meshworkof MFs that are arranged in membrane protrusions which are broad, flat and sheet-like,called lamellipodia (Albrecht-Buehler, 1976; Huttenlocher *et al.*, 1995; Lauffenburger and Horwitz, 1996; Small, 1981; 1995). In addition, short bundles of MFs are located in filopodia or microspikes, which are spicule-like membrane protrusions (Albrecht-Buehler, 1976; Lauffenburger and Horwitz, 1996; Nobes and Hall, 1995; Small, 1995). More detailed studies are now necessary to positively identify these type of actin-based cellular structures in MCF10A cells, and relate them to their motile (or stationary) response to Ln-5 matrices.

Previous work has characterized shape and cytoskeletal changes in both normal and malignant epithelial cells and fibroblasts on fibronectin matrices (Bannikov *et al.*, 1982). However, in keratinocytes it is clear that Ln-5 is a critical substrate both *in vitro* and *in vivo* (Giannelli *et al.*, 1996; Hormia *et al.*, 1995). Ln-5 is clearly associated with the basal membrane of normal breast epithelium (Giannelli *et al.*, 1997; Plopper *et al.*, 1998) The presence of fibronectin is less well understood (Adams and Watt, 1993; Coussens and Werb, 1996; Gorczyca *et al.*, 1993; Ronnov-Jessen *et al.*, 1996). Comparisons of the spreading and motile behavior of breast epithelial cell lines on Ln-5 versus fibronectin are therefore legitimate, in order to better understand the physiology of breast epithelium adhesion to basement membrane.

An important aspect of the breast basement membrane is that it is thought to limit, or at least initially contain, the invasivity of transformed epithelium. With this in mind, we investigated the behavior of MCF7 breast carcinoma cells on Ln-5 matrix and found that it was very distinct from that of MCF10A cells. Overall, there appeared to be no attempt on the part of MCF7 cells to form cobblestone-like monolayers, like MCF10A cells did. Rather, MCF7 cells appeared to be immediately motile right after adhesion to Ln-5 matrix, and continued to display a monotonous motile-like shape. That is, the cultures of MCF7 cells lacked the range of morphological changes and phenotypes that were observed in MCF10A cultures. We propose that perhaps transformation of MCF7 cells has reached a point where they are no longer responsive to ECM cues thought to regulate cell behavior. It is interesting to note that MCF7 are reputed to be a low invasive cell type, and are estrogen dependent. Breast carcinomas at more advanced stages of transformation may be even less responsive to ECM cues.

The structural nature of ECM cues for cell behavior, particularly for epithelial cells, is beginning to be understood. We have shown that cells become motile on Ln-5 when the latter is cleaved by the action of matrix metalloproteinases, namely MT1-MMP and MMP (Koshikawa *et al.*, 2000). MCF10A cells secrete MMP2 zymogen, but are unable to activate it because they lack the necessary cell surface amount of MT1-MMP (Koshikawa *et al.*, 2000). In contrast, MCF7 cells secrete activated MMP2. Thus, it is possible that the differences in stationary and motile behavior on Ln-5 may be at least in substantial part explained with the MMP secretion pattern, which, of course, may also be dictated by the stage of transformation. An interesting aspect here is that stromal cells may also secrete and/or activate MMPs, which may contribute to explain the role of stroma in tumor progression. Additional studies are required along these lines, and our MCF10A model may provide some useful benchmarks.

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□□	Indent two ems	⊏
⊏	Move matter to right	⊏ at left side of group to be moved
⊐	Move matter to left	⊐ at right side of group to be moved
move	Move matter to position indicated	[ ] at limits of required position
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