Divalent cations and extracellular matrix receptor function during newt epidermal cell migration

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Summary

Skin explants were placed in plastic dishes coated with fibronectin (FN), fibrinogen (FGN) or collagen. Explants were cultured for 16 h in serum-free medium containing calcium (Ca) and magnesium (Mg), or in medium containing either Ca-only, Mg-only or manganese (Mn)-only. In Ca/Mg, migrating keratinocytes on all test substrata produced a sheet of contiguous cells that formed a robust halo around each explant. When Ca was the only divalent cation added, the halos in FN- and FGN-coated dishes were approximately 70-80% as large as in Ca/Mg. On collagen, however, the halos were significantly smaller than on the other two substrata. This substratum-specific response in Ca-only suggests that migration on collagen is fundamentally different than migration on FN and FGN. Halos as large or larger than those in Ca/Mg formed on all three substrata in Mg-only. In this case, the halos were not in the form of a sheet of contiguous cells, but were composed of dissociated cells that had migrated from the explant. Individual cells likewise migrated from explants cultured in medium containing Mn-only; however, these halos were never as large as in Mg-only. Thus, while exogenous Ca appears to be an absolute requirement for maintenance of cell-cell connections, the cell-substratum interactions that lead to migration can utilize either Ca, Mg or, to a lesser extent, Mn.

Additionally we found that migration on the generally nonpermissive protein, BSA, was not improved by the presence of Mn. Hence, even though Mn can mediate to some extent migration on FN, FGN and collagen, presumably by binding to integrin-like receptors on the cell surface, Mn does not alter receptor specificity in a way that permits migration on BSA.

To analyze the possibility that individual newt keratinocytes possess receptors for more than one ECM molecule, we cultured skin explants in Mg-only on a narrow zone of FN bordered on one side by collagen and on the other by uncoated plastic. Individual keratinocytes were able to migrate across the FN strip and continue on collagen but not on uncoated plastic, indicating that at least some keratinocytes have receptors for at least two matrix molecules.

Key words: epidermal migration, extracellular matrix, divalent cations, fibrinogen, fibronectin, collagen, receptors.

Introduction

In normal skin, basal epidermal cells must remain properly attached to the basement membrane to maintain epidermal integrity. Following disruption of the epidermis by injury, basal cells and probably suprabasal cells as well, leave their existing extracellular relationships and establish new ones as they migrate to cover the wound. A full understanding of the factors regulating this transformation, from a relatively immobile population to a mobile one, requires that we know how keratinocytes respond to the individual extracellular proteins they probably encounter in these two situations.

The major proteins in basement membranes include: laminin, type IV collagen, and perhaps fibronectin (FN) (reviewed by Donaldson and Mahan, 1988). In wounds the relevant proteins include: FN and fibrinogen (Clara et al. 1982), type I collagen, and possibly vitronectin, a.k.a. serum spreading factor, epibolin, or S protein (Stenn, 1981; Barnes et al. 1983; Hayman et al. 1983; Jenne and Stanley, 1985).

Knowledge of the effects of these proteins on keratinocyte behavior has generally been obtained by seeding keratinocyte suspensions into dishes coated with various proteins and evaluating the extent of cell adhesion and/or spreading that each protein can support. Using this approach, keratinocytes have been reported to adhere and/or spread on type I collagen (O'Keefe et al. 1985), type IV collagen (Murray et al. 1979; O'Keefe et al. 1985; Wilke and Furcht, 1990), vitronectin/epibolin (Stenn et al. 1983), FN (Stenn et al. 1983; O'Keefe et al. 1985), and laminin (Wilke and Furcht, 1990). In a few studies, the movement of
individual keratinocytes seeded on various substrata has been followed. This has shown that type IV collagen and FN both support migration (O'Keefe et al. 1985; Woodley et al. 1988), but laminin apparently does not (O'Keefe et al. 1985). To study newt keratinocyte migration in more natural settings, we have performed similar experiments utilizing either skin explants in plastic dishes coated with various proteins or substratum implants, in which pieces of protein-coated plastic or glass are implanted under one edge of a fresh skin wound so that migrating epidermal cells encounter the implant. Among the proteins that support formation of a "wound epithelium" in these paradigms are: FN, fibrinogen (FGN), collagen (types I, II and IV), and vitronectin. Laminin is much less effective. Various non-matrix proteins, such as albumin, casein, fetuin and myoglobin, as well as the matrix protein tenascin, do not support newt epidermal cell migration (Donaldson and Mahan, 1983, 1984; Donaldson et al. 1982, 1987, 1989, 1991).

In addition to an adequate substratum, isolated mammalian keratinocytes require certain divalent cations, notably either calcium (Ca), magnesium (Mg), or manganese (Mn), to adhere and spread (Fritsch et al. 1979; Stenn and Core, 1986). The present study was undertaken to learn more about cation effects on newt keratinocyte interaction with extracellular matrix proteins, particularly with regard to possible differential effects on different substrata. Pieces of newt skin were placed in dishes coated with FN, FGN or type I collagen, and epidermal cell migration was evaluated in several different cation environments. We present evidence that neither Mg nor Mn can effectively substitute for Ca in maintaining cell-cell connections, though any of these three divalent cations can mediate those cell-substratum interactions that produce migration on FN, FGN and collagen. Our results also show that the cation requirement for epidermal migration on FN and FGN is significantly different from that required for migration on collagen. Finally, our data indicate that at least some newt keratinocytes exhibit Mg-mediated receptor function for migration on both FN and collagen.

Cation requirement experiments
In experiments to determine cation requirements, a central circle (250 mm²) on the bottom of 35 mm plastic dishes (Falcon no. 3001) received 300 µl of FN, FGN or collagen diluted to 100 µg/ml in either phosphate-buffered saline (FN and FGN) or 0.1 M acetic acid (collagen). The region of the dish covered by the applied solution was marked on the outside of the dish bottom and the dishes were placed at 45°C until dry. Before use all dishes were washed four times (5 min each) in distilled water and dried again at 45°C. Since there is essentially no migration on uncoated plastic, counter-coating with bovine serum albumin was not necessary.

For each animal used, a rectangular full-thickness piece of skin was removed from the dorsal surface of each hind limb between the knee and the ankle, and the pieces (skin explants) were placed on the bottoms of dishes coated as described above with FN, FGN or collagen. DMEM/F12 (Sigma) containing Zn as the only divalent cation and without L-glutamine, L-leucine, L-lysine, L-methionine, CaCl₂, MgSO₄ or MnCl₂ was diluted to 60% with distilled water to make basal medium. Skin explants (5-7 per dish) were incubated for 16 h in basal medium that, unless otherwise stated, was supplemented with CaCl₂ (final concn, 1 mM), MgSO₄ (final concn, 0.4 mM) or MnCl₂ (final concn, 0.1 mM), or both CaCl₂ and MgSO₄ (final concn, 1 mM and 0.4 mM, respectively). Usually, skin from one limb of an animal was incubated in basal medium containing both Ca and Mg (Ca/Mg) as a positive control for the contralateral piece of skin, which was cultured in basal medium supplemented with Ca-only, Mg-only or Mn-only. After overnight incubation in Mg-only, some explants on FN and Coll were changed to fresh Mg-only or Ca-only and selected fields were filmed in time-lapse on an inverted microscope for an additional 6 hrs.

Epidermal origin of migrated single cells
Having previously shown that cells that migrate as a continuous sheet from skin explants in the presence of both Ca and Mg are epidermal cells (Mahan and Donaldson, 1988), we sought to determine whether cells that migrate individually from explants in Mg-only were of dermal or epidermal origin. To address this question we treated full-thickness pieces of skin (1 piece from each hind limb of 8 newts) with dispase (Calbiochem, San Diego, CA) (0.5 units/ml) in Ca/Mg-free (CMF) medium for 1-2 h. The epidermis was then gently removed from one piece of each pair and the epidermis left in place on each contralateral piece. After washing with fresh CMF-medium (3 changes, 5 min each) to remove the enzyme, the pieces ("dermis alone" and the contralateral "dermis plus epidermis") were explanted (four per dish) in collagen-coated dishes and cultured for 16 h in basal medium containing 0.4 mM MgSO₄.

Dissociated epidermal cells were obtained as follows: full-thickness pieces of newt skin were excised and placed in dispase (2.5 units/ml of 60% CMF/F12) for 2 h at room temperature. The epidermal sheet was separated from the dermis and transferred to fresh dispase. Single epidermal cells were obtained in 2-3 h with occasional titration. The cell suspension was washed 3 times in CMF 60% DMEM/F12 and then resuspended in 60% CEM (Scott Laboratories, Fiskeville, RI). The cells were incubated for 15 min and then plated in collagen-coated dishes. After 1 h at room temperature, unbound cells were removed by gently washing with 60% CEM. Adherent cells were fixed with 10% formalin and stained with Coomassie Blue for microscopic examination and photography.

Materials and methods

General
Adult male newts (Notophthalmus viridescens) obtained from Connecticut Valley Biological (Southampton, MA) were maintained as previously described (Donaldson and Mahan, 1983).

Substratum proteins
Bovine type I collagen was kindly provided by Dr. M. Dabbous, Department of Biochemistry, University of Tennessee at Memphis. Human FGN (Kabi grade L) was purchased from Helena Laboratories (Beaumont, TX) and further purified by gelatin affinity chromatography to remove FN contamination (Donaldson et al. 1987). Human plasma CA.
**Dual receptor function experiments**

In this paradigm, experimental dishes containing a FN-coated area flanked on one side by a collagen-coated area, and on the other by uncoated plastic were produced by sequential application of the proteins (100 μg/ml) or their vehicle using nitrocellulose (nc) strips saturated with the appropriate solution. FN was applied first to the middle of the dish and its limits were marked by scoring the outside of the dish bottom. This was followed by a second coating cycle to apply an overlapping coating of collagen to one side of the FN strip and HAc (acetic acid, the collagen vehicle) to the opposite side. Each coating cycle involved incubation of the soaked nc strip in contact with the dish bottom for 2 h at room temperature, removal of the nc strip, washing the dish with a continuous stream of distilled H2O for 3 min, and drying the dish at 35°C. Control dishes were prepared exactly as described above, except that PBS (the FN vehicle) was used to treat the central strip instead of FN. For each animal, skin from one limb was explanted to the central FN-coated strip of the experimental dish while the skin from the contralateral limb was placed on the PBS-treated central region of the corresponding control dish. Basal medium containing 0.4 mM MgSO4 was added and the dishes (each containing 5 explants) were cultured for 20 h to determine whether epidermal cells would move across the FN or PBS-treated area onto the adjacent areas coated with collagen or HAc. After brief formalin fixation, standard indirect immuno-peroxidase techniques were used to verify the distribution of FN or collagen on the dishes using, as primary antibody, either rabbit anti-human FN serum that had been absorbed with human plasma proteins minus FN (Telios Pharmaceuticals, San Diego, CA) or affinity-purified goat anti-type I collagen IgG (Southern Biotechnology Assoc., Birmingham, AL) followed by the appropriate peroxidase-conjugated second antibody. The enzyme-labeled second antibody was detected using 3, 3′-diaminobenzidine as the substrate.

**Quantification of migration**

After fixation in 10% formalin, explants and migrating cells were stained with Coomassie Blue. The magnified image of each explant and the halo of migrated cells surrounding it were drawn using a dissecting microscope equipped with a drawing tube. For cells that migrated as a sheet, the leading edge of the sheet was recorded as the outer limit of the halo; when cells migrated individually, as occurred in Mg-only and Mn-only, the cells that had migrated farthest from the explant were connected by a line on the drawings to provide a measurable outer limit of the halo. A calibrated planimeter was used to measure the area containing migrated cells, i.e. the halo. All ± values accompanying a mean are standard errors. Stated P values were determined by Student’s t-test.

**Results**

When skin explants were cultured overnight on substrata coated with FN, FGN or collagen in medium containing both Ca and Mg (control conditions), a halo of contiguous epidermal cells migrating as a sheet developed around each explant (Fig. 1A). When the medium contained neither Ca, Mg nor Mn, no

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**Fig. 1.** Epidermal cell migration from newt skin explants placed in dishes coated with fibrinogen (FGN). (A) In basal medium containing Ca and Mg (Ca/Mg), a sheet of epidermal cells migrated outward to form a robust halo around the explant. A peripheral portion of the sheet is shown at higher magnification in the inset. Note how adjacent cells show so-called “intercellular bridges”, the classic picture of epidermal cells connected by desmosomes. ×19; inset, ×197. (B) In basal medium containing Mg-only considerable migration still occurred, but there were fewer cells in the migrating population and the cells did not form a sheet. ×19. Similar results were obtained in fibronectin (FN)- and collagen-coated dishes.
migration occurred on any of the three substrata (data not shown).

Migration in Ca-only
When Ca was the only divalent cation added to the basal medium, epidermal halos on FN and FGN were, respectively, only 71 and 79% as large as the halos that formed on those substrata in Ca/Mg. Under the same conditions (Ca-only) a more severe effect was seen on collagen-mediated migration, reducing it to 38% of the control (Ca/Mg) value (Fig. 2). Statistically, these differences between halo size on collagen and each of the other two substrata were significant ($P < 0.025$ for each comparison). This substratum-specific behavior, which may be more attributable to the absence of Mg than to the presence of Ca, suggests that there are important differences in the way migrating epidermal cells interact with FN and FGN versus collagen.

Migration in Mg-only
When Mg was the only divalent cation added to the basal medium, considerable epidermal migration occurred on all three substrata but the cells migrated individually, not as a sheet (compare Fig. 1A to B). Stepwise addition of Ca to Mg-containing medium in doubling increments from 0.0078 mM produced individual migrating cells at Ca concentrations up to 0.62 mM. Above this, the cells migrated as a sheet.

Although fewer cells occupied the halo in the absence of Ca than in its presence, it is clear from Fig. 2 that the epidermal halos in Mg-only were at least as large as halos surrounding their controls cultured in Ca/Mg. Apparently, at least for some keratinocytes, the divalent cation requirement for migration on FN, FGN or collagen, can be satisfied by Mg as well as Ca. The reason for fewer cells in the halo in the absence of Ca is unknown. It may be that Mg can mediate migration-promoting cell-substratum interactions in only a select subpopulation of keratinocytes (discussed further in the section dealing with the epidermal origin of cells migrating in Mg-only). Alternatively, the intercellular attachments that occur via Ca may have a positive effect on the ability of neighboring cells to migrate, resulting in a more densely populated halo.

Migration in Mn-only
Here again migration occurred on all three substrata, but since the medium was deficient in Ca the halo was composed of individual cells rather than a sheet. Two concentrations of Mn were tested, 1 mM and 0.1 mM. Since the lower concentration produced more migration than the higher on each substratum, the Mn results shown in Fig. 2 represent the lower concentration. Though halos in Mn were not as large as those in their Ca/Mg controls, Mn was clearly able to support some
Epidermal cell migration on all substrata. The differences in halo size on the three substrata in Mn-only were not statistically significant.

Since it has been previously reported that Mn, unlike Ca or Mg, can stimulate spreading of dissociated guinea pig epidermal cells on BSA (Stenn and Core, 1986), we placed some newt skin explants in BSA-coated dishes and incubated them in either Ca/Mg- or Mn (0.1 mM)-containing medium. Neither cation environment produced a significant amount of migration.

**Epidermal origin of migrating cells in Mg-only**

To verify that the cells occupying the halo in Mg-only were epidermal cells and not dermal-derived fibroblasts, pieces of skin were treated with dispase until the epidermis could be removed. Eight pieces of skin with the epidermis removed were explanted into collagen-coated dishes. Eight contralateral pieces of dispase-treated skin with the epidermis left in place were explanted to another collagen-coated dish and both sets of explants were incubated overnight in Mg-only. In no case was there any migration from epidermis-free skin (Fig. 3A). In contrast, when the epidermis was left on, individual cells migrated from all eight explants (Fig. 3B).

Moreover, the population of cells migrating from explants in Mg-only was quite heterogeneous in size (Fig. 3B), resembling the population obtained when single cell epidermal suspensions were seeded into either collagen-coated or FN-coated dishes (Fig. 3C, D). Additionally, the size heterogeneity of the cells migrating in Mg-only, shows that at least morphologically, they do not constitute a distinct subpopulation.

In a few explants that we cultured overnight in Mg-only and then switched to Ca-only, time-lapse recordings showed that approximately 6 h after the medium change, some of the migrating cells began to join in small clusters, thereby showing typical epithelial behavior (data not shown). Others joined the epidermal sheet that began issuing from the explant in response to the presence of Ca. Thus, it appears that the scattered cells migrating from explants in Mg-only are epidermally derived.

**Dual receptor function in individual keratinocytes**

Differences in the mechanism of epidermal sheet migration on FN and FGN versus collagen could involve different subpopulations of cells; one kind of epidermal cell with receptors that recognize FN and FGN, another type with receptors for collagen. Alternatively, an individual newt keratinocyte could possess receptors for both FN/FGN and collagen. To test the latter possibility the following experiment was performed: plastic dishes were coated (see Materials and methods for additional details) with a narrow strip of FN bounded on one side by a collagen-coated area and on the other side by an area treated with HAc (the collagen vehicle). Skin explants were placed on the narrow FN-coated strip and incubated in Mg-only to allow the migratory behavior of individual keratinocytes to be assessed. Control dishes, coated identically, except that the middle zone was treated with PBS instead of FN, received explants from the contralateral limbs. In this design, dual receptor function would be demonstrated by any cell that could cross the FN-coated strip and move into the collagen-coated area.
Fig. 4. Ability of individual keratinocytes to migrate on both FN and collagen. The explants shown in each panel are resting on a narrow strip of FN-coated plastic. On the right in each panel, the FN coating is partially overlapped by a coating of type I collagen that occupies the entire field on that side. To the left of each explant the plastic was treated with acetic acid (HAc), the collagen vehicle. In A the FN coating has been visualized by immunochemistry using antibodies against FN followed by peroxidase-conjugated second antibody. In B the collagen coating has been similarly visualized with anti-type I antibodies followed by the appropriate peroxidase-labelled second antibody. Note that in each example epidermal cells were able to migrate away from the explant on FN and continue over collagen. They could not, however, continue onto the area treated with HAc. Incubation conditions: Mg-only. ×19.

Two examples of the results of this experiment are shown in Fig. 4 where a large number of epidermal cells have migrated from each explant onto the FN-coated central strip. To the left of each explant there is a sharp interface where the cells were unable to continue onto the HAc-treated area of bare plastic. On the right, many cells have invaded the area coated with collagen. The results of this experiment are shown more fully in Fig. 5, which represents the various group means. Here it can be seen that when skin rectangles were explanted onto FN (on FN to start, Fig. 5) migrating cells subsequently occupied approximately four times as much area on the collagen-coated side of the dish as on the HAc-treated side. The importance of the central FN strip can be seen by noting that when skin rectangles were explanted onto an uncoated central strip (bare to start, Fig. 5), there was a great reduction in the amount of migration onto the collagen-coated area (compare the two collagen bars). The finding that the FN/collagen combination supports significantly more migration than the other combinations tested indicates that an epidermal cell can recognize and migrate on both FN and collagen.

A potential criticism of this experiment is that the long incubation required (20 h) could have allowed the migrating cells to synthesize and secrete migration-supporting proteins onto the substratum, thereby circumventing the experimental coatings. Considering that uncoated plastic has a high protein-binding capacity, it seems likely that if this had occurred, the means for all groups would have been more similar. From the differences, we conclude that the long incubation required did not compromise the design. It

Fig. 5. Importance of the FN coating in the dual receptor function experiment shown in Fig. 4. When explants were started on FN (two bars at the left) considerably more migration was found on collagen and HAc-treated plastic than was found in similar areas when the explants were started on plastic treated only with PBS, the FN vehicle (two bars at the right). This indicates that the cells migrated on FN rather than some unknown explant-derived factor to reach the adjacent areas. In the dish containing explants started on FN, significantly more migration was found on collagen than on plastic treated with HAc (P < 0.025). This experiment shows that each migrating epidermal cell has a repertoire of receptors capable of interacting with both FN and collagen. For each group, n=10.
thus appears that at least some of the keratinocytes in newt epidermis are able to interact productively with both FN and collagen. Whether this represents the involvement of one or more than one class of receptors is presently unknown.

Discussion

The present study shows that newt skin explants on FN, FGN or collagen produce an extensive “wound epithelium” when cultured in medium containing both Ca and Mg. When only Ca is present, explants produce a significantly larger “wound epithelium” on FN and FGN than they do on collagen. This suggests that Mg is more important in collagen-mediated migration than in FN- and FGN-mediated migration. Since the degree of inhibition caused by omitting Mg was so different on different substrata, the effect is probably extracellular rather than intracellular (Edwards et al. 1987). The external event most likely affected is the interaction between receptors on the keratinocyte cell surface and migration-promoting amino acid sequences in the matrix molecules we tested. On the basis of their divalent cation requirements (present paper) and their sensitivity to RGD peptides (Donaldson et al. 1987), the affected receptors appear to be members of the integrin family.

Integrins are a group of heterodimers in which α and β subunits associate noncovalently to form a complex that mediates cell-matrix and, in some cases, cell-cell interaction (reviewed by Hynes, 1987; Akiyama et al. 1990). Since the discovery of integrins, their function has been known to require divalent cations, particularly Ca and Mg. Recently, Kirchhofer et al. (1991) have suggested that integrin function is physiologically regulated by divalent cations that bind to the receptor complex. The binding of divalent cations to an integrin may cause a conformational change that either exposes a different binding site or alters the same site (Edwards et al. 1987; Yamamoto et al. 1989; Dransfeld et al. 1990). In either case this change could result in a more favorable interaction with a particular matrix molecule. Cation binding site(s) are most likely on the α subunit (see reviews by Ruoslahti and Pierschbacher, 1987; Akiyama et al. 1990; Dransfeld et al. 1990) though the β subunit on some integrins may also be involved (Gogstad et al. 1983; Kirchhofer et al. 1991).

The substratum-dependent differences that we detected in keratinocytes migrating in Ca without Mg suggests that either different receptors or different sites on a single receptor class are involved when keratinocytes interact with FN and FGN versus collagen. This conclusion is consistent with our earlier observation that FN and collagen-mediated migration are differentially affected by the peptides RGDS and RGES in competitive inhibition assays (Donaldson et al. 1988). Prior to the present study we had no definitive information about the cellular distribution of this dual receptor function. It seemed possible that formation of a “wound epithelium” on FN might involve one set of epidermal cells while its formation on collagen involves another subpopulation. In this scheme of wound healing, those cells that do not possess receptors for the available substratum would simply be carried along passively by their attachments to those that do. Alternatively, an individual keratinocyte, using one or more class of receptors, may be able to recognize both proteins. The individual migration of epidermal cells in Mg-only allowed us to differentiate between these two possibilities. We found that some cells in the epidermis were capable of migrating across FN and then continuing on collagen, indicating that certain keratinocytes have a receptor repertoire that is functionally active on more than one substratum. Though this experiment directly demonstrates dual function for receptor(s) on a relatively small percentage of epidermal cells, we have previously observed that most, if not all, cells in a migrating sheet extend lamellipodia onto a collagen-coated substratum (Mahan and Donaldson, 1986), suggesting that all the cells comprising a “wound epithelium” have migration-promoting collagen receptors. It seems likely that, had we examined epidermal sheets spreading over FN, we would have seen the same pattern.

We still do not know if the dual receptor function that we have demonstrated represents separate classes of receptors or a single receptor class with two distinct binding sites. The αβ integrin known as VLA-3 or ECMR1, a ‘promiscuous’ receptor found on many types of mammalian cells (Takada et al. 1988; Akiyama et al. 1990; Wayner et al. 1988), including keratinocytes (Wayner et al. 1988; Kaufmann et al. 1989; Carter et al. 1990; Staquet et al. 1990), is an example of an integrin with two functionally distinct ligand binding sites. Thus, the FN binding site on VLA-3 isolated from NCI-H69 cells (a small cell lung carcinoma line) can be competitively inhibited by RGD-containing peptides, but the collagen binding site cannot. Moreover, the two binding sites show different patterns of binding to their respective substrata in the presence of Ca versus Mg (Elies et al. 1991). Although adhesion is admittedly not the same as migration, our observation that newt epidermal cells migrate better on FN than on collagen in Ca-only, appears inconsistent with VLA-3 behavior on NCI-H69 cells, where in Ca, adhesion is better on collagen than on FN (Elies et al. 1991). Thus, if FN and collagen-mediated newt epidermal cell migration occur through a single receptor, it is probably not via an exact counterpart of the VLA-3 receptor on NCI-H69 cells.

The cation sensitivity of newt keratinocyte receptors involved in collagen-mediated migration resembles, to a degree, the sensitivity of VLA-2 (αβ) collagen receptors on platelets (Staatz et al. 1989) and the collagen receptors on HeLa cells (Beacham and Jacobson, 1990). In both situations, collagen receptor function is specifically dependent on Mg; Ca cannot effectively substitute. Similarly, in newt epidermal cells, Mg seems to be the significant cation for collagen-mediated migration. However, since Ca did have some effect in the present study, the newt collagen receptor...
may not precisely mimic those on platelets or HeLa cells.

Both VLA-2 and VLA-3 are known to be present on human epidermal cells (Kaufmann et al. 1989; Carter et al. 1990; Staquet et al. 1990) and both have been implicated in epidermal cell-substratum interactions (Carter et al. 1990; Levarlet et al. 1990). It therefore, seems reasonable to suppose that newt keratinocyte migration might involve amphibian counterparts of integrins implicated in epidermal cell-substratum interactions (Carter et al. 1990; Levarlet et al. 1990). It therefore, al. 1990; Staquet et al. 1990) and both have been

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also play a role in the adhesion of mammalian human epidermal cells (Kaufmann et al. 1989; Carter et al. 1990; Staquet et al. 1990) and both have been

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Likewise in our migration experiments epidermal halos were always larger in Mg than in Ca regardless of the test substratum. Some caution though must be exercised before taking these results at face value, since in Ca the halo forms a sheet and in Mg the halo was composed of individual cells. It could be that sheet behavior prevents certain cells from expressing their full migratory potential. Perhaps the halos in Ca would have been larger if sheet formation could have been circumvented. Even with this caveat, the relatively poor performance of epidermal cells in Ca-only when collagen was the substratum compared to the robust halos on this substratum in Mg-only suggests that, at least for collagen, there is a distinct preference of migrating cells for Mg.

Surprisingly, many studies have shown that Mn can stimulate adhesion and/or spreading of various cell types even on substrata where no other cation is effective. For example, mouse ascites sarcoma cells will adhere and spread in large numbers on serum-coated glass in the presence of Mn, but will adhere poorly or not at all in other cations including Ca and Mg (Rabinovitch and DeStefano, 1973). When BHK cells are seeded onto uncoated plastic dishes in the presence of Mn or a Ca/Mg mixture, they adhere in both cation environments but spread only in Mn. When the substratum is coated with albumin, BHK cells in Ca/Mg adhere very poorly and do not spread. In Mn, they not only attach, but also spread (Grinnell, 1984). Similarly, guinea pig epidermal cells in albumin-coated dishes spread in large numbers in the presence of Mn, but not in Ca or Mg (Stenn and Core, 1986). Although Mn-mediated cell spreading on non-permissive substrata may be a laboratory artifact (Stenn and Core, 1986), there is clear evidence that Mn can mediate the function of some integrins. For example, Mn effectively competes with Ca for the same cation-binding site(s) on the FN receptor and also enhances this receptor’s ligand binding activity (Gailit and Ruoslahti, 1988). In the present study we found no evidence that Mn was any better than Ca/Mg at stimulating migration over the generally non-permissive protein, BSA. However, Mn was able to mediate migration over all the permissive substrata tested (FN, FGN and collagen), although it was not as effective as Mg. Further work will be necessary to determine whether this relatively poor migratory performance in Mn is due to an excessive or insufficient level of cell-substratum adhesion.

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References


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