Evidence for a Novel Enzymatic Mechanism of Neural Crest Cell Migration on Extracellular Glycoconjugate Matrices

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Abstract. Migrating embryonic cells have high levels of cell surface galactosyltransferase (GalTase) activity. It has been proposed that GalTase participates during migration by recognizing and binding to terminal *N*-acetylglucosamine (GlcNAc) residues on glycoconjugates within the extracellular matrix (Shur, B. D., 1982, *Dev. Biol.* 91:149–162). We tested this hypothesis using migrating neural crest cells as an in vitro model system. Cell surface GalTase activity was perturbed using three independent sets of reagents, and the effects on cell migration were analyzed by time-lapse microphotography.

The GalTase modifier protein, alpha-lactalbumin (α-LA), was used to inhibit surface GalTase binding to terminal GlcNAc residues in the underlying substrate. α-LA inhibited neural crest cell migration on basal lamina–like matrices in a dose-dependent manner, while under identical conditions, α-LA had no effect on cell migration on fibronectin. Control proteins, such as lysozyme (structurally homologous to α-LA) and bovine serum albumin, did not effect migration on either matrix. Second, the addition of competitive GalTase substrates significantly inhibited neural crest cell migration on basal lamina–like matrices, but as above, had no effect on migration on fibronectin. Comparable concentrations of inappropriate sugars also had no effect on cell migration. Third, addition of the GalTase catalytic substrate, UDPgalactose, produced a dose-dependent increase in the rate of cell migration. Under identical conditions, the inappropriate sugar nucleotide, UDPglucose, had no effect. Quantitative enzyme assays confirmed the presence of GalTase substrates in basal lamina matrices, their absence in fibronectin matrices, and the ability of α-LA to inhibit GalTase activity towards basal lamina substrates. Laminin was found to be a principle GalTase substrate in the basal lamina, and when tested in vitro, α-LA inhibited cell migration on laminin. Together, these experiments show that neural crest cells have at least two distinct mechanisms for interacting with the substrate during migration, one that is fibronectin-dependent and one that uses GalTase recognition of basal lamina glycoconjugates.

NEURAL crest cells are the embryonic progenitors of several adult cell types, including neurons and Schwann cells of the peripheral nervous system, melanocytes, cells of the adrenal medulla, and skeletal and connective tissue cells of the head and face (16, 26, 45). During development, neural crest cells migrate along the epithelial basal lamina, invade extracellular spaces, and interact with other embryonic cell types (4, 41). Neural crest cells encounter various glycoconjugates during migration in vivo, and their migration in vitro has been analyzed on many of these glycoconjugates (2, 3, 7, 9, 20, 24, 25, 27, 31, 43). Two extracellular matrix glycoproteins in particular, fibronectin and laminin, have been shown to support neural crest cell migration in culture (24, 31). Neural crest cells appear to interact with the peptide portion of the fibronectin molecule rather than its oligosaccharide portion, since the peptide fragment of the fibronectin cell-binding domain inhibits cell migration (3). In contrast, there is evidence that cells interact with the oligosaccharide rather than the peptide portion of the laminin molecule (8, 10, 14). In either case, it is likely that multiple mechanisms of cell–matrix interaction are required as the cells migrate through different areas of the embryo, including the basal lamina and the extracellular matrix.

In this paper, we identify a novel mechanism for neural crest cell migration based upon the binding of a cell surface enzyme, galactosyltransferase (GalTase),[1] to its glycoconjugate substrates in the extracellular matrix. Since cell surface glycosyltransferases were first suggested to function as recognition molecules in cellular interactions (29, 30), UDPgalactose:*N*-acetylglucosamine (GlcNAc) GalTase has been shown to function as a surface receptor in a number of systems. For

[1] *Abbreviation used in this paper:* DME, Dulbecco's modified Eagle's medium; EHS, Englebreth Holm Swarm; GalTase, galactosyltransferase; GlcNAc, *N*-acetylglucosamine; α-LA, alpha-lactalbumin.

example, mouse sperm surface GalTase is the principal receptor for sperm binding to the zona pellucida during fertilization (18, 37, 38). In addition, surface GalTase participates during embryonal carcinoma and preimplantation mouse embryo adhesions (1, 34, 36). Several other studies also suggest that cell surface GalTase is involved in mesenchymal cell migration in the early embryo. For example, the distribution of cell surface GalTase in avian embryos is heaviest on migrating mesenchymal cells (32, 33, 39). Similarly, in mouse embryos, surface GalTase is preferentially localized to migrating mesenchymal cells, and in *T/T* mutant embryos, defective mesenchymal cell migration is associated with abnormal levels of surface GalTase (35). Finally, cultured fibroblasts spontaneously glycosylate extracellular glycoconjugate substrates during migration in vitro (44).

Based on these data, it was proposed that surface GalTase serves as a matrix receptor during migration by recognizing and binding to terminal GlcNAc residues in the extracellular matrix (32, 35). Continued cell migration would result from catalytically dissociating the GalTase/GlcNAc complex with the appropriate galactose donor (e.g., UDPGal), thus releasing the enzyme and enabling it to bind unoccupied GlcNAc residues.

Using neural crest cells as an in vitro model system, the present study directly examines the function of surface GalTase during cell migration on a variety of glycoconjugate substrates. Neural crest cell migration was analyzed by time-lapse microphotography in the presence of three sets of reagents that perturb surface GalTase activity. First, the addition of the GalTase modifier protein, alpha-lactalbumin (α-LA), which reduces the enzyme's affinity for GlcNAc residues (22, 23), produced a dose-dependent inhibition of neural crest migration on basal lamina–like matrices. In contrast, α-LA had no effect on cell migration on fibronectin. As control, identical levels of the structurally homologous protein, lysozyme, had no effect on cell migration on either substrate. Second, the presence of competitive GalTase substrates (i.e., GlcNAc or chitotriose) inhibited cell migration on basal lamina–like matrices, but not on fibronectin. Identical levels of inappropriate sugars did not inhibit cell migration. Third, addition of the GalTase catalytic substrate (i.e., UDPGal) increased the rate of cell migration on basal lamina–like matrices, whereas an inappropriate sugar nucleotide (UDP-glucose) had no effect on cell migration. Finally, quantitative enzymatic assays confirmed the presence of GalTase substrates in the basal lamina matrix, their absence in fibronectin matrices, and the ability of α-LA to inhibit GalTase activity towards basal lamina substrates. Laminin was identified as a principal GalTase substrate in the basal lamina matrix.

Materials and Methods

Materials

α-LA (type I) and lysozyme (grade I) (Sigma Chemical Co., St. Louis, MO) were suspended in water (1 g/10 ml) and dialyzed twice (24 h each) against 6 liters of deionized water to remove buffer salts. The protein solutions were then lyophilized and stored at −20°C until used. UDPGal and UDPglucose were also obtained from Sigma Chemical Co. Bovine plasma fibronectin was obtained from Bethesda Research Laboratories (Gaithersburg, MD). The Engelbreth Holm Swarm (EHS) matrix (described below) and purified laminin were kind gifts of Drs. H. K. Kleinman and G. T. Kitten at the National Institute of Dental Research.

Preparation of Substrates

Two different basal lamina–like matrices were used. One was a basal lamina–like matrix secreted by mouse endodermal PYS-2 cells onto the tissue culture dish. PYS cells were grown in 15-mm (diam) tissue culture wells for 6 d past confluency in Dulbecco's modified Eagle's medium (DME, Gibco, Grand Island, NY) containing 15% heat-inactivated (56°C, 30 min) fetal calf serum (HyClone Laboratories, Logan, UT). The PYS cells were removed with 2.2 mM EDTA in calcium- and magnesium-free Earle's balanced salt solution, and the underlying basal lamina–like matrix (PYS matrix) was rinsed twice with salt solution and frozen at −20°C until needed. The dishes were rinsed twice with fresh medium immediately before use. The second basal lamina–like matrix (EHS matrix) was obtained in soluble form by extracting the EHS with 1.0 M NaCl, 50 mM urea, and subsequently dialyzing it against DME at 4°C. When desired, EHS matrix (0.3 ml/15-mm well) was polymerized into a solid substrate by warming to room temperature and was rinsed twice before use. Fibronectin and laminin substrates were made by adding 25–33 μg of bovine plasma fibronectin, or 20 μg of laminin, in 0.5 ml of medium to a 15-mm tissue culture well. After 1 h at 37°C, the medium was decanted and replaced with fresh medium for cell culture.

Neural Crest Cell Culture

Quail (*Coturnix coturnix*) blastoderms were excised in either Earle's or Hank's salt solutions (Gibco) containing 25 mM Hepes and staged according to the equivalent chick embryo stages of Hamburger and Hamilton (11). Stage 11–12 embryos were used for the collection of cranial neural crest, and stage 15–16 embryos were used for the isolation of trunk neural crest. The neural tubes were dissected free with tungsten needles and digested in 0.5% pancreatin (Gibco) for 10–15 min at room temperature to remove adherent tissue. After three rinses in Earle's salt solution, the neural tubes were placed in 15-mm tissue culture wells with an underlying substrate and 0.5 ml of serum-free medium. The medium used was a mixture of 50% Ham's F12 and 50% DME (both Gibco) containing 5 μg/ml transferrin, 5 μg/ml insulin, 5 ng/ml selenium, 100 μM putrescine, and 20 nM progesterone (all Collaborative Research Inc., Lexington, MA). After an overnight incubation at 37°C in 5% CO_2, the neural crest cells had migrated from the explant sufficiently for experimentation.

Experimental Protocol

The tissue culture dishes containing neural tube explants were placed on an inverted microscope stage that was surrounded by a plexiglass incubator box. The box was warmed with a Sage Air Curtain (Sage Instruments Div., MA) set at 37°C and enriched with CO_2 by infusion with 100% CO_2 at a rate sufficient to keep the media at pH 7.3. Initial experiments were done in closed 25-cm^2 tissue culture flasks warmed directly with the Air Curtain. The rates of migration were similar under both conditions, but the initial method required greater quantities of reagents. Neural crest cells were examined by time-lapse microphotography with an Olympus OM-2 camera and Olympus M.AC. (Olympus Corporation of America, New Hyde Park, NY) control box modified to take pictures at 4.3-min intervals. Owing to variation in mean cell migration rate between explants, each neural crest cell population was photographed for 70–140 min to establish a control migration rate. Immediately afterward, the culture was treated with the experimental reagent, and the identical cell population was photographed for a similar period of time to determine effects on the rate of cell migration.

Analysis of Data

The 35-mm time-lapse films were projected in a photographic enlarger (~7× magnification). Peripheral cells from each explant (i.e., cells with minimal or no cell-to-cell contact) were traced between frames by monitoring movement of the nucleus. Analysis of successive frames resulted in a tracing of cell migrations by the population of cells during each treatment. Each cell path was traced on an X-Y digitizing board (Kurta Corp., Phoenix, AZ), and a path-length was calculated (VIAS software, Pella Inc., Tustin, CA). An average of 31 cells (range 10–73) were traced for each treatment, and population parameters between control and experimental time periods were compared. The experimental data are expressed as a percentage of the rate of migration observed during the preceding control period by the same population of cells.

GalTase Assay

Chicken serum (Gibco) and/or quail neural crest cells were used as sources of avian GalTase to confirm both the mode of action of α-LA and the appropriate substrate specificity of the extracellular matrices. GalTase was assayed as

described (33, 34) in a final volume of 50 μl. Each assay contained 1 μCi of UDP-[^{3}H]galactose (New England Nuclear, Boston, MA) (specific activity 12.0 Ci/mmol), 5 μmol of unlabeled UDPGal, 0.5 μmol $MnCl_2$, and either chicken serum or neural crest cells (see below). After 30 min of incubation at 37°C, the reactions were stopped with the addition of 10 μl of 0.2 M EDTA in 50 mM Tris buffer, pH 7.2. 50 μl of each incubation mixture was applied to the origins of Whatman 3MM paper (Whatman Inc., Clifton, NJ) and subjected to high voltage borate electrophoresis (300 mA, 45 min) to remove noncovalently bound sugars and sugar nucleotides from the galactosylated product. The origin of the electrophoretogram was dried, and the amount of radioactivity present was determined by liquid scintillation counting. Control incubations (i.e., kept on ice) averaged 179 cpm and were subtracted from all assays incubated at 37°C.

To determine the GalTase substrate activity of the various glycoconjugate matrices, the incubations were supplemented with 20 μl of chicken serum, and varying amounts of fibronectin (1 μg/μl), EHS matrix (5.7 μg/μl protein, 10.7 μg/μl dry wt), or laminin (3 μg/μl). None of the glycoconjugate matrices assayed contained any detectable GalTase activity. Neural crest cell surface GalTase activity was assayed as described for other cultured cells (34). 54 neural tube explants were cultured in complete medium (21). After 3 d, the neural tubes were removed, and the neural crest cells were harvested with 2.2 mM EDTA in calcium- and magnesium-free Earle's balanced salt solution. The cells were

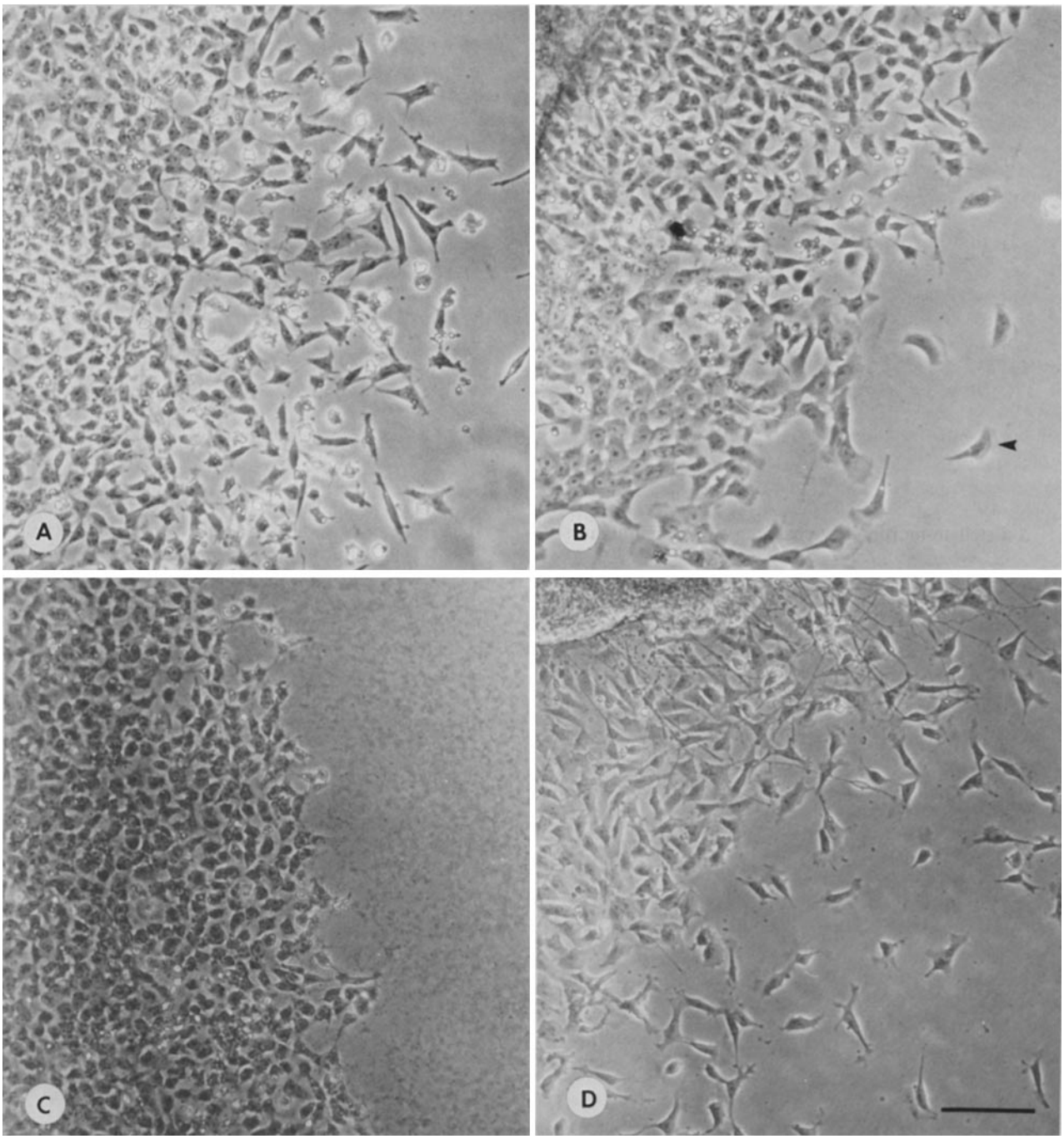

Figure 1. Appearance of neural crest outgrowths on various glycoconjugate substrates. PYS matrix (*A*), fibronectin (*B*), EHS matrix (*C*), and laminin (*D*) matrices were prepared as described in the Materials and Methods. On PYS matrix (*A*), the cells assume a typical fibroblastic morphology and show some cell–cell separation at the periphery of the outgrowth. The appearance of the cells on fibronectin (*B*) is similar, but many of the cells demonstrate a flattened morphology with large lamellipodia (arrow). Cells on EHS matrix (*C*) form a migrating sheet of small cells with less cell–cell separation than that seen on other substrates. On laminin (*D*), the cells appear similar to those on PYS matrix, but are sparser than on other substrates. Bar, 100 μm.

rinsed extensively by centrifugation in medium B (NaCl, 7.5 g/liter; KCl, 0.4 g/liter; Hepes buffer, 4.76 g/liter; pH 7.2). Aliquots of 50,000 cells (in medium B) were added to each incubation, and activity towards saturating levels of GlcNAc substrate (1.5 μmol/assay) was assayed as above. When desired, GalTase activity was modified away from GlcNAc by the addition of 5 μg α-LA to the incubation, and the GlcNAc was replaced by 1.5 μmol glucose.

Results

Neural Crest Cell Migration on Complex Glycoconjugate Matrices

Neural crest cell migration was analyzed on four different glycoconjugate matrices. Two of these matrices were derived from basal lamina. The first, PYS matrix, was produced by cultured endodermal cells, which when removed with EDTA (see Materials and Methods) leave behind a native basal lamina on the dish. Alternatively, a soluble basal lamina–like matrix was prepared from EHS. EHS matrix could be polymerized into a solid substrate and subsequently used for migration, or used in soluble form for biochemical assays. Both matrices are known to contain the variety of components normally identified in the basal lamina including type IV collagen, laminin, and heparan sulfate (12, 13, 15). By having both native (PYS) and repolymerized (EHS) basal lamina–like matrices available, we could control for any differences due to substrate organization and/or polarity, while still being able to use the soluble EHS matrices for biochemical analysis. Finally, for comparative purposes, neural crest cell migration was also monitored on substrates of purified fibronectin and laminin.

The appearance of the neural tube cell outgrowths on each of the substrates is shown in Fig. 1. The cells in the outgrowths assumed a stellate morphology characteristic of neural crest cells on all substrates. However, on EHS matrix the cells appeared closer together than on other substrates. All of the cells that had left the neural tubes cultured on EHS matrices were shown to be neural crest cells, since they reacted with anti-HNK-1/NCl antibody diagnostic for early neural crest cells (data not shown) (42). Additional confirmation of the neural crest origin of the outgrowths was obtained by shifting representative cultures into complete medium permissive for melanocyte differentiation (21). Within 7 d, a large fraction of the cells had differentiated into neural crest–derived melanocytes (data not shown).

The mean rate of migration for cells cultured on each of the four glycoconjugate substrates varied between individual explants. Nevertheless, the average rate of migration reflected characteristic differences on each substrate. The average rate of cell migration was: 41.0 ± 18.6, 54.9 ± 29.7, 56.5 ± 17.7, and 125.0 ± 27.4 μm/h (± SD) for EHS matrix, fibronectin, PYS matrix, and laminin, respectively. Neural tube explants were unable to attach, and neural crest cells were unable to migrate, when cultured directly on tissue culture plastic in serum-free medium without an underlying glycoconjugate substrate.

Effect of a GalTase Substrate Modifier on Neural Crest Cell Migration

To examine the function of surface GalTase during neural crest migration, we examined the effect of α-LA on the rate of cell migration. α-LA binds to and modifies GalTase, reducing its affinity for its conventional GlcNAc substrate. We reasoned that by modifying the surface GalTase away from its GlcNAc substrate, α-LA would inhibit GalTase binding to the matrix and thereby inhibit neural crest cell migration. Fig. 2 shows that α-LA inhibited neural crest cell migration in a dose-dependent manner on native basal lamina–like (PYS) substrates. α-LA produced similar inhibition on the repolymerized soluble basal lamina–like substrate (EHS matrix). α-LA dramatically inhibited the length of the individual cell migration pathways relative to the preceding control period (Fig. 3). The appearance of neural crest cell cultures at the end of the control migration period and after subsequent treatment with α-LA is shown in Fig. 4. At low concentrations (1 mg/ml), α-LA had no effect on cell morphology, even though migration was inhibited. However, at higher concentrations (i.e., 5 mg/ml), α-LA inhibited cell–substrate adhesion to the point where cells began to round up. When neural crest cultures that had been incubated for 2 h in 5 mg/ml α-LA were returned to control medium, the cell morphology and migration rate returned to normal (data not shown).

Two controls were used to test for nonspecific effects of α-LA on neural crest cell migration. Neural crest cultures were incubated with varying concentrations of lysozyme, a protein of similar molecular weight and homologous protein sequence to α-LA, but without GalTase modifying ability. Figs. 3 and 4 show the migration paths and morphology of the cells incubated in control medium and lysozyme. Lysozyme had no effect on cell morphology, migration path, or rate of cell migration, even at the highest dose assayed, (i.e., 5 mg/ml) (Figs. 2, 3, and 4). Similarly, the presence of 5 mg/ml bovine serum albumin (BSA) had no effect on the rate of cell migration (Fig. 2).

Cranial neural crest cells were more sensitive to α-LA than were trunk cells (36% inhibition for trunk cells compared with 80% inhibition for cranial cells at 1.0 mg/ml α-LA). In addition, there appeared to be two distinct subpopulations of cranial neural crest cells as revealed by their sensitivity to α-LA (Fig. 5). The migration rate of cells farthest from the explant was inhibited 80%, relative to control rates, whereas cells closer to the explant retained a normal stellate morphology, and their migration rate was inhibited by only 20%.

Neural Crest Cell Migration on Laminin

Laminin is a principal component of the basal lamina, and neural crest cell migration on laminin has been reported by others (24). Therefore, we wanted to determine whether α-LA would inhibit trunk neural crest cell migration on laminin. The presence of α-LA (5 mg/ml) inhibited cell migration on laminin by 51% during the first 78 min of treatment, during

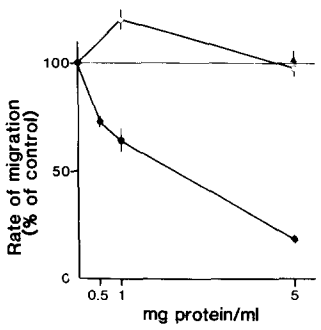

Figure 2. Migration on PYS matrix is inhibited by α-LA. Compared with control rates for each explant, α-LA (●) produced a dose-dependent inhibition in the rate of cell migration. Identical concentrations of the structurally homologous protein lysozyme (○) or bovine serum albumin (▲) did not inhibit cell migration. The control (i.e., 100%) rate of migration is shown by the horizontal line.

Figure 3. Migratory pathways of neural crest cells are reduced in the presence of α-LA but not lysozyme. (*A*) Tracings of the pathways produced by peripheral neural crest cells on PYS matrix after 70 min. (*B*) Pathways of cells from the same explant during a sequential 70-min period in the presence of 5 mg/ml α-LA are visibly shorter. (*C*) Pathways produced by peripheral neural crest cells on PYS matrix over a period of 73 min. (*D*) Pathways of cells from the same explant shown in (*C*) during a sequential 73-min interval in the presence of 5 mg/ml lysozyme show no inhibition of migration.

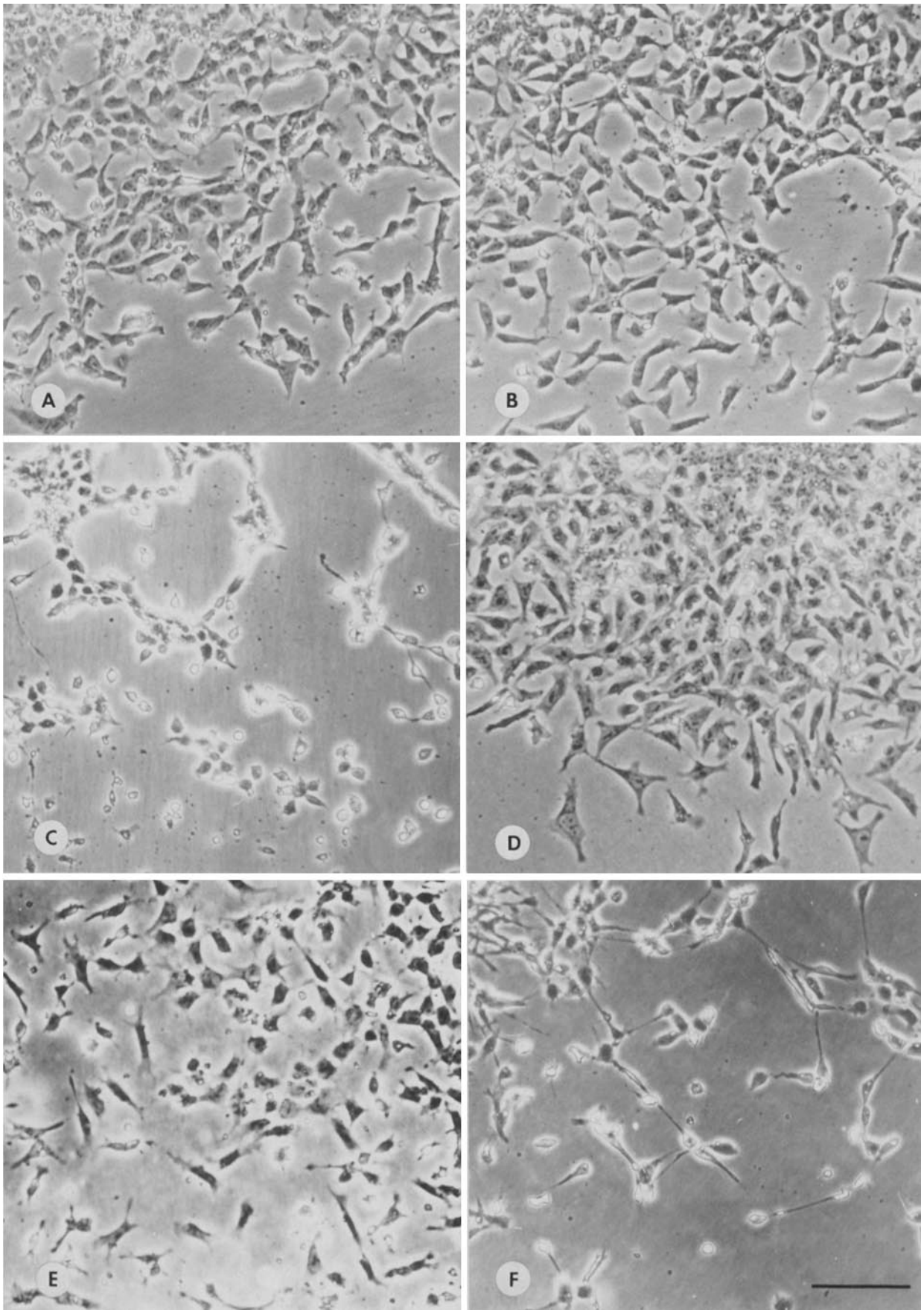

Figure 4. Neural crest cells show both dose-dependent and substrate specific sensitivity of α-LA. Cells on PYS matrix after 120 min of incubation in the absence (*A*) or presence (*B*) of 1 mg/ml α-LA. This level of α-LA inhibited migration by 36%, but did not visibly change the cellular morphology. (*C*) Cells on PYS matrix after 120 min with 5 mg/ml α-LA show a rounded appearance concomitant with a near total inhibition of migration. (*D*) The addition of the homologous protein, lysozyme, at 5 mg/ml to cells on PYS matrix did not alter cellular morphology or migration rate after 120 min of incubation. (*E*) Treatment of cells on fibronectin for 180 min with 5 mg/ml α-LA did not effect cell morphology or migration rate. (*F*) Cells on laminin after 112 min with 5 mg/ml α-LA maintain extended cell processes, while the cell bodies have assumed a rounded shape. Within 25 min after this picture was taken all cells were completely rounded and had ceased migrating. Bar, 100 μm.

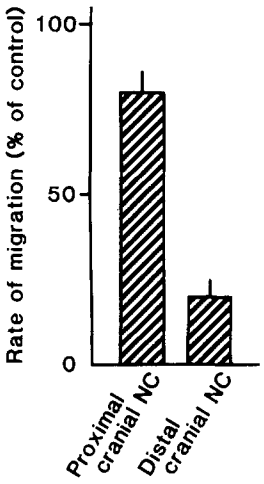

Figure 5. A subpopulation of cranial neural crest cells is more sensitive to α-LA. Examination of cranial neural crest cells in the presence of 1 mg/ml α-LA revealed that the most distal cells of the explant had become rounded. The distal and more proximal cell populations of the explant were examined separately for changes in the rate of cell migration. The distal cells are more sensitive to α-LA than are the proximal cells at this concentration.

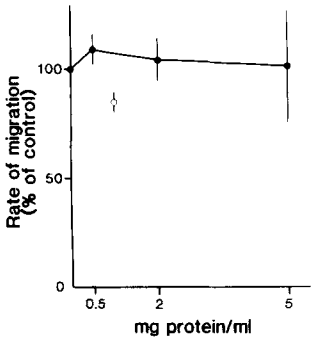

Figure 6. Migration on fibronectin is not inhibited by α-LA. Cell migration rates in the presence of α-LA (●) or lysozyme (○) were not significantly different from control rates at any concentration tested.

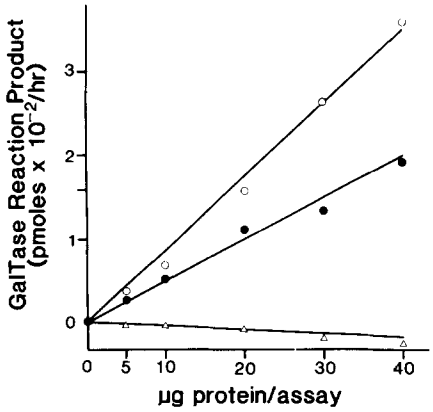

Figure 7. GalTase substrate activity of extracellular matrices. The substrate activities of EHS matrix (○), laminin (●), and fibronectin (△) were assayed from 0–40 µg of protein per assay. GalTase is able to glycosylate the basal lamina-derived EHS matrix and one of its components (laminin). Fibronectin is not a substrate for GalTase and produces a slight inhibition of the GalTase activity endogenous to the chicken serum.

which time neural crest cell morphology remained normal. During continued incubation, α-LA inhibited the migration rate by 86% and cell–substrate adhesion as well, resulting in a rounded morphology. The appearance of neural crest cells on laminin in the presence of 5 mg/ml α-LA is shown in Fig. 4.

Neural Crest Cell Migration of Fibronectin

Peptide fragments of the cell-binding domain of fibronectin were shown previously to inhibit neural crest cell migration

Table I. GalTase Substrate Specificity and Modification with α-LA

Enzyme source	Acceptor substrate	α-LA	pmol GalTase product/h
Neural crest cells	GlcNAc	–	620*
Neural crest cells	GlcNAc	+	92.3 (85% inhibition)
Neural crest cells	Fibronectin	–	48.7
Neural crest cells	Laminin	–	157
Chicken serum	GlcNAc	–	2,697 ± 590‡
Chicken serum	GlcNAc	+	627 ± 75 (77% inhibition)
Chicken serum	Glucose	–	113 ± 12
Chicken serum	Glucose	+	1,589 ± 300 (1,412% stimulation)

GalTase activity was assayed under optimal enzymatic conditions, and neural crest cells were prepared as described in the Materials and Methods. α-LA was added at a final concentration of 1 mg/ml (neural crest cells) and 0.1 mg/ml (chicken serum). Fibronectin and laminin were added to the assays at a final concentration of 0.143 mg/ml.

* Neural crest cell surface GalTase activity is normalized per 10^6 cells, and the data shown are the average of duplicate determinations.

‡ ± SD.

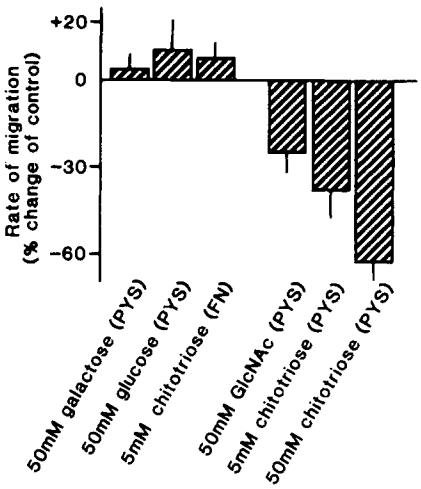

Figure 8. Competitive GalTase substrates inhibit migration on basal lamina-like matrix. The competitive GalTase substrates, GlcNAc and chitotriose (tri-*N*-acetylglucosamine), inhibit cell migration on PYS matrix, but equal concentrations of inappropriate sugars (glucose, galactose) do not. Similarly, chitotriose has no effect on cell migration on fibronectin matrix, at the concentration tested.

on this substrate, suggesting that cells recognize the peptide rather than oligosaccharide portion of the fibronectin molecule (3). To test the biological specificity of α-LA inhibition of neural crest cell migration, and to rule out nonspecific metabolic or cytotoxic effects of the reagent, we examined the effect of α-LA on neural crest cell migration on fibronectin. α-LA did not significantly affect the rate of cell migration at any concentration tested (Figs. 4 and 6); and as before, lysozyme had no effect on cell migration.

GalTase Activity Towards Extracellular Matrices

Past studies have documented GalTase activity on neural crest cell surfaces (32, 33, 36, 39). Nevertheless, we thought it necessary to determine the substrate specificity of surface GalTase on cultured neural crest cells as well as its sensitivity to α-LA. Results in Table I show the presence of GalTase activity on neural crest cells using incubation conditions

designed to assay specifically cell surface GalTase activity (33, 34). Furthermore, results confirmed the ability of α-LA to modify the substrate specificity of the neural crest cell surface GalTase activity, as well as the ability of surface GalTase to recognize laminin with higher affinity than fibronectin.

Due to the limited number of neural crest cells obtainable, we also used a soluble form of avian GalTase (i.e., chicken serum) to better define this substrate specificity. Fig. 7 shows the presence of GalTase substrates in basal lamina-like (EHS) matrix, but not in fibronectin matrix. (In fact, fibronectin displayed a slight inhibition of the endogenous GalTase activity found in chicken serum. The mechanism of this inhibition is unknown.) Purified laminin could account for a significant portion of the GalTase substrate activity found in the basal lamina-like matrix. As shown in Table I, α-LA produced the appropriate modification of soluble GalTase substrate specificity (i.e., activity towards GlcNAc substrates was inhibited while simultaneously stimulating activity towards glucose).

Cell Migration in the Presence of Competitive Exogenous Substrates

If surface GalTase participates in neural crest migration, then we reasoned that the addition of competitive GalTase substrates should inhibit cell migration by preventing the enzyme from binding to GlcNAc residues in the underlying matrix. The presence of 50 mM GlcNAc significantly inhibited cell migration relative to control levels, whereas an equal concentration of either glucose or galactose had no effect on migration (Fig. 8). The addition of the repeating GlcNAc polymer, tri-*N*-acetylglucosamine (chitotriose), also significantly inhibited cell migration on basal lamina-like substrates, but had no effect on cell migration on fibronectin, again demonstrating the appropriate substrate specificity for GalTase.

Cell Migration in the Presence of Exogenous UDPGal

Finally, we attempted to increase the rate of cell migration by forcing premature catalysis of the GalTase reaction, thus releasing the GalTase from its extracellullar matrix substrate and enabling it to recycle. The addition of UDPGal to neural crest cultures on basal lamina-like matrices produced a dose-dependent increase in the mean migration rate relative to controls (Fig. 9). Under identical conditions, the inappropriate sugar nucleotide, UDPglucose, had no effect on cell migration, thus demonstrating the appropriate substrate specificity for GalTase. UDPGal produced a greater relative increase in cell migration on EHS matrix than on PYS matrix (Fig. 9).

Discussion

The results presented here show that cell surface GalTase participates during neural crest cell migration in vitro. Furthermore, the data suggest that GalTase functions only during cell migration on particular matrices, since perturbation of surface GalTase activity only affects cell migration on matrices that contain GalTase substrates. Cell migration is unaffected on fibronectin matrices, which do not contain GalTase substrates. The substrate specificity of these results clearly eliminates any consideration of nonspecific or cytotoxic effects of these reagents, as does the ability of treated cells to return to a normal rate of migration after being transferred back to control medium.

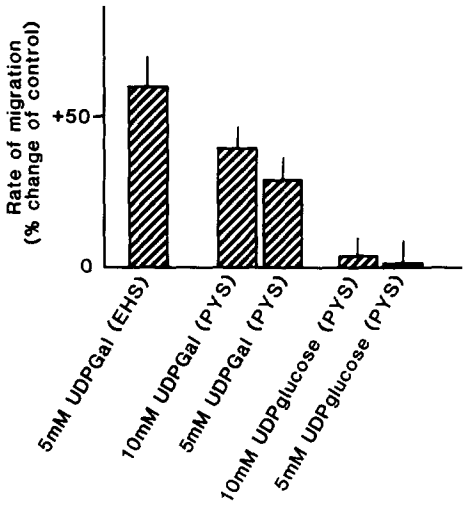

Figure 9. Catalytic GalTase substrates increase the rate of neural crest cell migration. UDPGal stimulates the rate of cell migration on both EHS and PYS basal lamina matrices relative to the preceding control period. The inappropriate sugar nucleotide, UDPglucose, has no effect on the rate of migration.

GalTase was shown to serve as an extracellular matrix receptor by using three different sets of reagents that perturb GalTase. First, α-LA was added to cultures to modify the substrate recognition of cell surface GalTase and inhibit cell attachment to terminal GlcNAc residues within the matrix. α-LA produced a dose-dependent inhibition of cell migration. The concentrations of α-LA used, while relatively high, are similar to, or less than, those used in other systems to inhibit surface GalTase activity toward GlcNAc substrates (1, 38). More significantly, controls using either a matrix devoid of GalTase substrates (i.e., fibronectin), BSA, or the structurally homologous protein, lysozyme, show the appropriate biological specificity and mode of action of α-LA in this system. Furthermore, quantitative enzyme assays demonstrated the ability of α-LA to inhibit GalTase activity towards basal lamina substrates. Laminin was shown to be one of the principle GalTase substrates in the basal lamina, and when retested in vitro, α-LA inhibited cell migration on laminin matrices. These results show that shifting the substrate specificity of the surface GalTase produces a coincident shift in the migratory behavior of the neural crest cells.

Second, the addition of competitive GalTase substrates (i.e., GlcNAc, chitotriose) to the culture medium inhibited cell migration in a dose-dependent manner, whereas identical levels of inappropriate sugars (i.e., glucose, galactose) did not. Presumably, exogenous substrates compete with terminal GlcNAc residues within the extracellular matrix for the GalTase active site, thus preventing the enzyme from binding to its appropriate substrate in the matrix and inhibiting cell migration. The GlcNAc polymer, chitotriose, proved to be a more potent inhibitor of cell migration than GlcNAc, suggesting that the GalTase substrate in the basal lamina has lactosaminoglycan core structures (34, 36). As above, these reagents had no effect on cell migration on fibronectin matrices, clearly eliminating any considerations of cytotoxicity.

Third, the addition of the catalytic GalTase substrate (UDPGal) stimulated cell migration in a dose-dependent manner. In controls, cell migration was unaffected by identi-

cal levels of the inappropriate sugar nucleotide, UDPglucose. Presumably, the exogenous UDPGal forces premature catalysis of the GalTase reaction, thus releasing the GalTase from its galactosylated substrate. The GalTase would then be available for binding to unoccupied substrates within the extracellular matrix. The ability of UDPGal to increase the rate of cell migration suggests that dissociation of the cell–matrix interaction is a rate-limiting step during migration. Premature release of the cell–substrate linkage thus enables the cell to move at a faster rate. Consistent with this suggestion is the finding that UDPGal stimulated migration on EHS matrices (where migration is normally slow), more than it stimulated migration on PYS matrices (where migration is more rapid).

Whether the enzyme functions catalytically during normal cell migration or rather functions as a lectin-like recognition molecule is not clear at present. Catalytic release would require the presence of the appropriate galactose donor at the binding site, but UDPGal is not normally found in the extracellular environment. However, Turley and Roth (44) observed spontaneous glycosylation of extracellular glycoconjugates by migrating fibroblasts prelabeled with sugar nucleotide precursors. This observation suggests that a localized mechanism of sugar nucleotide transport or a lipid-sugar intermediate may exist. Alternatively, if GalTase functions like a lectin in glycoconjugate recognition and binding, it might be either degraded or released from the cell membrane to permit continued cell movement.

These experiments do not prove that neural crest cells use GalTase as a mechanism for substrate recognition during migration in vivo. However, there are a number of observations that support this view. For example, autoradiographic studies show that surface GalTase is localized preferentially on a variety of migrating cells within the embryo (32, 33, 39). In developing chick embryos, UDPGal proves to be teratogenic by preferentially disturbing the development of mesenchyme-derived structures (32). In addition, defective mesenchymal cell migration in mutant *T/T* mouse embryos is associated with abnormal levels of surface GalTase activity on these cells (35).

Interestingly, neural crest migration in vivo occurs at the interface between the epithelial basal lamina and the underlying extracellular matrix rich in fibronectin (4). Thus, neural crest cells are exposed to multiple types of extracellular matrices during migration. The results presented here indicate that GalTase functions as a surface receptor only during migration on those matrices that contain GalTase substrates. This suggests that different molecular mechanisms of neural crest cell/substrate interaction are involved in different regions of the extracellular matrix. Which cell surface receptors are used in any given circumstance may depend on which molecules the cell encounters within the extracellular matrix. Alternatively, subpopulations of cells may exist within the neural crest that possess different cell surface receptors for matrix components, thus specifying the migratory pathway of the various cell populations. The ability of a cell surface receptor to interact with its complementary ligand in the extracellular matrix may in turn activate, or organize, cytoskeleton components involved in cell motility. Although they did not examine motile cells, Sugrue and Hay (40) demonstrated that a number of different extracellular matrix molecules are able to organize elements of the cytoskeleton.

The finding that laminin is a substrate for GalTase and that the migration of neural crest cells on laminin is inhibited by the GalTase substrate modifier protein α-LA is of interest. It appears that GalTase functions as a laminin "receptor" in this system, though the interaction of GalTase with other glycoconjugates within the basal lamina is not ruled out. It is unclear what the relationship is, if any, between GalTase and the laminin receptors recently identified in other laboratories (5, 6, 17, 19, 28). There is evidence that at least some cells recognize, and bind to, the oligosaccharide portion of the laminin molecule (8, 10, 14), although there are probably multiple mechanisms of cell attachment to laminin (5).

In conclusion, these experiments show that cell surface GalTase participates in neural crest cell migration by recognizing and binding to its appropriate substrates in the underlying matrix. GalTase functions in a substrate-dependent manner, since reagents that perturb GalTase activity coincidently perturb migration only on basal lamina matrices and not on fibronectin. These data, together with studies of neural crest migration on fibronectin (3, 9, 24, 25, 31), suggest that neural crest cells employ at least two distinct mechanisms for interacting with the extracellular matrix during migration. Which mechanism is used may be governed by the immediate embryonic environment encountered by the migrating neural crest cell.

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