tion with a full-length cDNA (*13*) to the human ER revealed a single species of approximately 6.4 kb, identical in size to that found in MCF-7 cells (*13, 14*), an ER-positive human mammary carcinoma cell line (Fig. 4). Expression of ER mRNA was similar in all three strains tested.

In summary, cultured human osteoblast-like cells possess the properties of target cells for estrogen, in that they display a steroid-specific, saturable, temperature-dependent nuclear binding, and an induction of progesterone receptor in response to estrogen treatment. Lastly, they contain mRNA for the ER. Since estrogen is the major hormone responsible for the maintenance of bone mass, the presence of specific ERs in osteoblasts could indicate a direct effect of estrogen on these cells.

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Local Embryonic Matrices Determine Region-Specific Phenotypes in Neural Crest Cells

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Membrane microcarriers were used to determine the ability of regional extracellular matrices to direct neural crest cell differentiation in culture. Neural crest cells from the axolotl embryo responded to extracellular matrix material explanted from the subepidermal migratory pathway by dispersing and by differentiating into pigment cells. In contrast, matrix material from the presumptive site of dorsal root ganglia stimulated pronounced cell-cell association and neurotypic expression. Cell line segregation during ontogeny of the neural crest that leads to diversification into pigment cells of the skin or into elements of the peripheral nervous system appears to be controlled in part by local cell-matrix interactions.

DURING EMBRYONIC DEVELOPMENT, neural crest (NC) cells of the trunk region migrate from their original position along the dorsal neural tube in two principal directions. Cells following the subepidermal migratory pathway give rise to pigment cells of the skin, whereas NC cells proceeding along the medioventral route will form various structures of the peripheral nervous system (*1–3*). Although the initial state of phenotypic commitment is probably heterogeneous within the premigratory NC cell population (*1, 4, 5*), there is evidence that expression of various phenotypes is affected by environmental cues encountered during migration (*1, 4–9*). The extracellular matrix contacted by the moving NC cells has been proposed to constitute a site from which instructive stimuli for both cell differentiation and morphogenetic processes occurring during NC development may emanate (*6, 9–13*).

We showed earlier that by implanting Nuclepore membranes into living axolotl embryos (*Ambystoma mexicanum*) it is possible to explant regional matrix materials that are synthesized and deposited onto the surface of the membranes in situ (*3, 6, 12, 13*). When tested in a defined culture system, matrix material explanted on such membrane microcarriers was found to promote NC cell differentiation into pigment cells (*6*). We have now examined the function of the extracellular matrix in cell line segregation during NC development by use of a novel nitrocellulose-based membrane microcarrier with high adsorbance capacity.

Microcarriers were introduced into the subepidermal space (subepidermal matrix material) or in the presumptive region of dorsal root ganglia (preganglionic matrix material) of living axolotl embryos (Fig. 1). After 10 to 12 hours of conditioning, the microcarriers were removed from the embryo and covered with isolated NC cells or processed for analysis of the explanted matrix materials. Scanning electron microscopy revealed that the material adsorbed onto the surfaces of the microcarriers was organized as sparse patches of short fibrils densely decorated with ruthenium red–precipitated granules, which might represent proteoglycan-based complexes as well as matrix-affiliated growth factors. Although the amounts of material isolated from the two embryonic regions were not quantitated, they appeared comparable. Both matrix materials became adsorbed onto the microcarrier surfaces as fibrillar tufts and smaller fragments, distributed in different proportions (Fig. 2, A and B). Incubation with selected antibodies demonstrated the presence of characteristic matrix components, including fibronectin (*14*), laminin (*15*), collagen types I and III (*16*), and sulfated proteoglycans as revealed by identification of their chondroitin-6-sulfate moieties (*17*). No differences between the two regional matrices were detected after immunohistochemical labeling of matrix-covered microcarriers. The nanogram quantities of subepidermal and preganglionic matrices isolated on microcarriers were also analyzed by polyacrylamide gel electrophoresis with a newly developed technique that permits detection of protein amounts below 10^{-12} g (*18*). Primarily, two-dimensional separation according to this procedure (*19*) revealed differences in the protein contents of the two matrices that were predominantly localized in regions corresponding to molecular sizes of 45 to 50 kD and 70 to 95 kD (Fig. 3).

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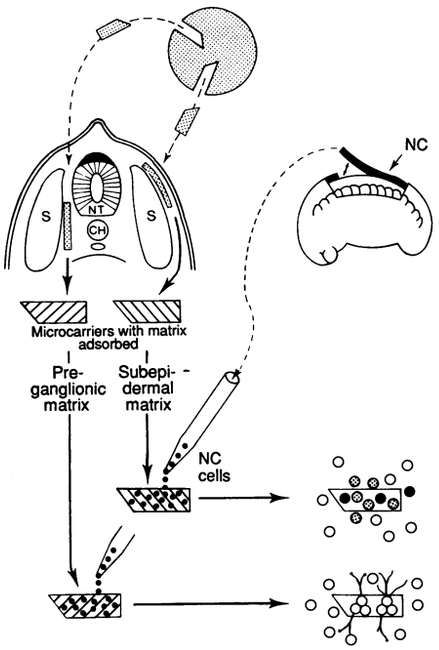

Fig. 1. Diagram illustrating the experimental design and summarizing the results obtained in the present study. Membrane microcarriers were manufactured from nitrocellulose sheets (Trans-Blot, Bio-Rad) by dissolving a piece of nitrocellulose paper (0.45 mm), about 2 cm^2 in size, in 1 ml of pure acetone with vigorous stirring. A volume of 7 to 10 μl of this solution was evenly smeared on a sterile hydrophobic metal surface. This surface was made hydrophobic by being dipped into a solution of dimethyldichlorosilane (Merck) in trichloroethane and then sterilized with ultraviolet light. The metal surface covered with the acetone-nitrocellulose solution was then exposed to sterilizing ultraviolet light, and the acetone was allowed to evaporate under constant irradiation for up to 5 minutes. A 3- to 5-μm-thick transparent membrane was formed. The membrane was gently detached from the metal surface, extensively washed in sterile PBS (phosphate-buffered saline), and cut under liquid asymmetric "microcarriers" (*6, 12, 13*). The protein-binding capacity of these membrane microcarriers was estimated fluorimetrically by an enzyme-linked protein-binding assay (*29*). Microcarriers, approximately 0.15 by 0.4 mm, were implanted into the trunk region of stage 25 Mexican axolotl embryos (*Ambystoma mexicanum*) either subepidermally (*6, 12, 13*) or in the presumptive region of the dorsal root ganglia. Implanted microcarriers were left in the embryo for adsorbance of matrix material for 10 to 12 hours. Just before the onset of local NC cell migration, they were removed from the embryo and transferred to plastic dishes for cell culture, or further processed for characterization of the adsorbed matrix material. Pure populations of premigratory NC cells were obtained from the dorsal NC cord according to a microsurgical procedure previously described (*6*). By our culture technique (*6*), explanted NC cells can be deposited site specifically onto the matrix-covered microcarriers, or within the same culture dish, onto the plastic surface beside the carriers and incubated for up to 5 days at 20° to 22°C in a serum-free PL-85 medium (*29*). Empty circles, undifferentiated cells; filled circles, melanocytes; dotted circles, xanthophores; and circles with projections, neurons.

When pure populations of premigratory NC cells, isolated from the axolotl embryo according to a microsurgical technique previously described (*6*), were cultured on microcarriers bearing subepidermal matrix material, the NC cells distributed themselves uniformly over the surface of the carriers and subsequently differentiated into pigment cells (Fig. 2, C and H). This was revealed by detectable activity of the melanogenic enzyme tyrosinase in melanocytes and by fluorescence of pteridine pigments in xanthophores (*20, 21*). In 46 of 49 cultures examined, we found five or more individual cells expressing these phenotypic traits. In a total of 28 cultures examined, NC cells grown on subepidermal matrix material showed no tendency to aggregate and did not develop any morphological characteristics of neurons. Moreover, in a total of 19 of this type of cultures examined, NC cells did not express immunohistochemically detectable neural adhesion molecules (N-CAMs) (*22*) or neural intermediate filaments (*23*). The presence of pigment cells among NC cells that had migrated away from their initial position on the microcarriers (Figs. 1 and 2C) indicates that an inceptive contact with the underlying subepidermal matrix was sufficient to direct pigment cell differen-

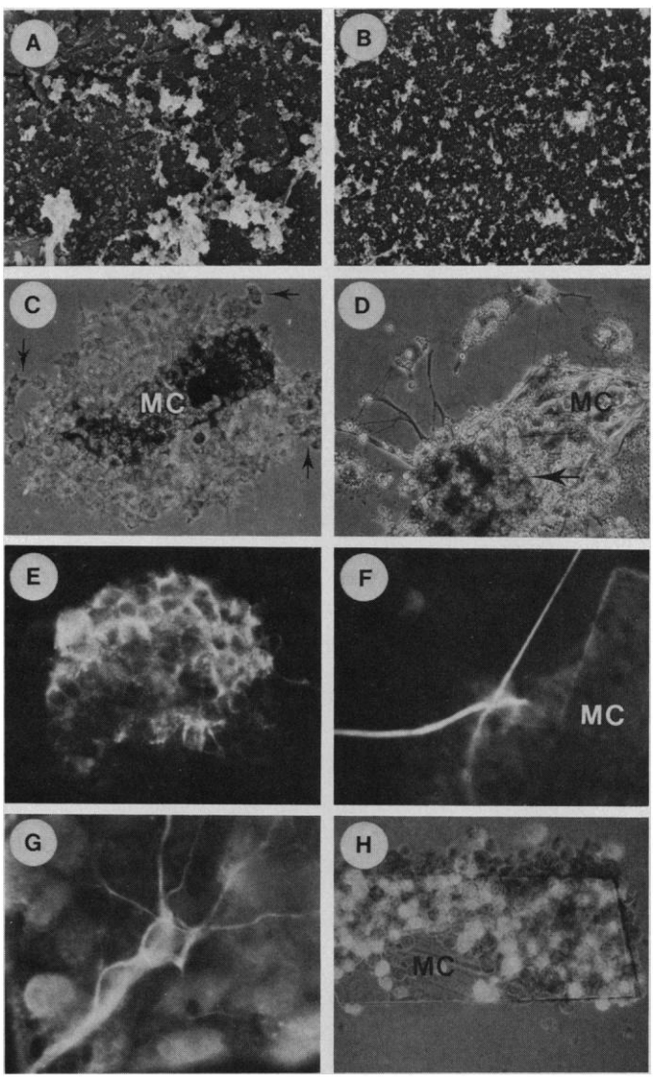

Fig. 2. Scanning electron micrographs showing matrices explanted on nitrocellulose microcarriers from the subepidermal (**A**) and preganglionic (**B**) regions of the embryo (×1400). Subepidermal and preganglionic matrix materials were observed on the microcarrier surfaces as granulofibrillar tufts of various sizes and smaller fragments interspaced by empty areas. For scanning electron microscopy the matrix-covered microcarriers were fixed in ruthenium red–containing fixatives following routine procedures (*6, 12, 13*). (**C**) NC cells induced by subepidermal matrix to express tyrosinase activity appear black after incubation with DL-dopa (*6, 20, 21*). A modification of the procedure previously used consisted in a postfixation with 1% OsO_4 in 0.1*M* cacodylate buffer (*p*H 7.4) for 45 minutes at 20°C. Positively stained NC cells (arrows) were also visible in the outgrowths formed by cells that emigrated from their initial position on the matrix-covered carriers (×80; see also Fig. 1). (**D**) Phase contrast micrograph showing aggregation of NC cells (arrow) and neurite extension on a microcarrier bearing preganglionic matrix (×150). Only cells forming aggregates extended neurofilament-containing neurites. (**E**) N-CAM immunoreactivity (*22–24*) detected on a 4-day-old cell aggregate on preganglionic matrix. Immunolabeling with antibodies to N-CAM was especially strong in regions of cell-cell contact (×250). (**F**) Neurofilament-immunoreactive neurites extended from NC cells grown for 4 days on preganglionic matrix (×400). (**G**) A multipolar neuron located deeply inside a similar cell aggregate as in (D) and (E) and stained by the 31EB antibody (*23, 25*). The diffuse background fluorescence in some of the surrounding cells is due to autofluorescence of yolk proteins (×350). (**H**) Pteridine-fluorescent xanthophores that differentiated after 3 days of culture on a microcarrier covered with subepidermal matrix and placed on a polylysine-coated substrate (×120). Although the majority of the NC cells remained constrained to the microcarrier surface or assembled along its edges, cells retained the ability to differentiate into pigment cells in response to the underlying matrix. MC, microcarrier.

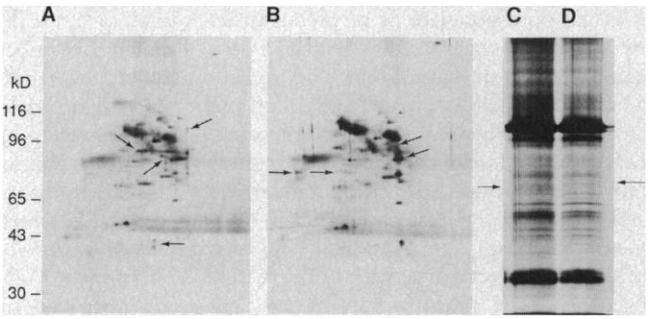

Fig. 3. One- and two-dimensional polyacrylamide gel electrophoretic separation of subepidermal (**A** and **C**) and preganglionic (**B** and **D**) matrix materials explanted on microcarriers under reducing conditions (*19*). After one-dimensional separation, protein banding was virtually identical for the two types of matrices, with the exception of a doublet (subepidermal matrix) versus single band (preganglionic matrix), which appeared at approximately 70 kD (arrows). Two-dimensional separation revealed several discrepancies in the protein patterns (major differences indicated by arrows). The prominent plaques in the 100-kD region (particularly evident on the one-dimensional gels) probably represent procollagen-proteoglycan complexes or similar aggregations of matrix components. Molecular weight standards are indicated at the left.

tiation. This observation is consistent with our previous findings (*6*).

Premigratory NC cells cultured on microcarriers covered with preganglionic matrix initially dispersed over the surface and later beyond the edges of the carriers in a manner similar to that of NC cells on subepidermal matrix. On preganglionic matrix, however, a portion of the NC cell population remained condensed in clusters and formed one or, occasionally, two distinct cell aggregates on each microcarrier (Fig. 2D). Tyrosinase activity and pteridine-fluorescence was not detected in any of the 25 cultures analyzed of this type. In contrast, incubation with antibodies to N-CAM (*24*) showed that NC cells forming aggregates expressed N-CAM immunoreactivity (Fig. 2E) (17 of 21 antibody-incubated cultures). Many of the aggregated cells assumed a spherical shape, became birefringent, and extended neurite-like processes that terminated in growth cones (Fig. 2D). Neurites always extended from cells localized within the aggregates (Fig. 2D).

Thirty-four cultures of NC cells grown on preganglionic matrix were incubated with antibodies directed against neurofilament peptides and in 29 of these cultures neurites extended from aggregated NC cells were immunoreactive (Fig. 2F). Similar cultures were also incubated with the monoclonal antibody 31EB, which recognizes neurons of the peripheral nervous system of the axolotl (*25*) and several of the NC cells in the aggregates appeared intensely stained (20 of 22 cells) (Fig. 2G). Double-labeling with antibodies to neurofilament peptides and the antibody 31EB revealed simultaneous expression of both neuronal traits in individual cells (seven cultures examined). Similar double-labeling with antibodies to the N-CAM and with the 31EB antibody indicated co-expression of cell adhesion molecules and the neuronal marker in several of the aggregated cells (nine cultures). Those NC cells that displayed neuronal traits were invariably located on the matrix-covered microcarriers, an indication that prolonged cell-matrix contact is required for the establishment of this differentiation pathway. This finding implies that expression of neurotypic properties in NC cells may involve a regulative mechanism different from that underlying differentiation into pigment cells.

Control NC cell populations were grown directly on the surface of the culture dishes but at distant locations from the matrix-covered microcarriers. This prevented dispersing control cells from establishing contact with the matrix material or with NC cells migrating away from matrix-covered microcarriers. In none of these control populations (58 total) did the NC cells form aggregates or express any of the phenotypic characteristics of pigment or neuronal cells. The extent of mitotic activity in NC cells grown on the local matrix materials was not quantitated, but proliferating cells were rarely seen during the 4 days of culture. Although we cannot preclude the possibility that some of the aggregated cells on preganglionic matrix material were in the process of dying, Trypan blue exclusion did not reveal any dead cells during the culture period.

Two alternative mechanisms could account for the matrix-induced expression of different phenotypes. The state of cellular aggregation or dispersion and lack of stable cell-cell association could be the prerequisites for the effect of the extracellular matrix on cytodifferentiation. Alternatively, the matrix could differentially induce the expression of phenotypes, which in turn would result in aggregation or dispersion of the phenotypically specified cells. In an attempt to distinguish between these alternatives, we perturbed the dispersive behavior of premigratory NC cell populations grown on subepidermal matrix. This was accomplished by culturing NC cells on matrix-covered microcarriers that were placed on polylysine-coated substrates. Under this experimental condition, NC cells were initially able to spread on the matrix-covered surface of the microcarriers but were strongly restrained in their subsequent dispersion onto the polylysine-coated plastic beyond the edge of the microcarrier (Fig. 2H). NC cells restricted in their migration by the highly adhesive polylysine substrates tended to assemble at the edges of the microcarrier but did not form delimited aggregates analogous to those observed on preganglionic matrix. The cells also failed to express N-CAM immunoreactivity and to project neurofilament-containing neurites (22 cultures). Differentiation into pigment cells, however, did occur in 33 of 35 cultures examined, as revealed by detectable tyrosinase activity and pteridine-fluorescence (Fig. 2H). Control NC cells deposited beside the matrix-covered microcarriers—that is, directly onto the polylysine substrate—remained assembled but expressed neither neurotypic nor pigment cell traits (17 cultures). These results suggest that the disparate phenotypic expression may not be primarily governed by a differential degree of cell dispersion (*9*) but may be regulated by specific information imparted by the local matrices. Pigment cell expression seemed to occur even after a short response to the underlying subepidermal matrix, whereas a prolonged contact of the NC cells with preganglionic matrix material appeared essential to induce neurotypic expression. In conclusion, our findings indicate that local matrices explanted on membrane microcarriers determine region-specific NC cell phenotypes according to their original location in the embryo.

The dissimilar cell behavior—that is, aggregation versus dispersion—and the divergent differentiation pathways embarked on by NC cells grown on the two regional matrices seem to correlate with the situation observed in vivo. In the embryo, externally visible pigment cell differentiation coincides with dispersion of the pigment cell precursors, which progressively colonize the subepidermal space (*3, 21, 26*). In contrast, NC cells translocating along the medioventral migratory route proceed to specific sites where they stop and coalesce into ganglia or contribute to the formation of other peripheral nervous structures (*1, 2*). It has been proposed that expression of N-CAM is concomitant with neurogenesis and that this class of molecules may have a role in multivalent cell-cell and cell-substrate interactions (*22*). Our finding that the expression of N-CAM on NC cell surfaces is promoted by preganglionic matrix material suggests that expression of N-CAMs might be important

during ontogeny of the peripheral nervous system.

Although the developmental potential of premigratory NC cells may be restricted, our results suggest that regional differences in the extracellular matrix encountered during migration in the embryo provide environmental cues responsible for cell line segregation during development of the neural crest. Further investigation is required to elucidate whether the local extracellular matrix instructs developmentally labile NC cells to express a specific phenotype or whether the matrix promotes selective survival and proliferation of phenotypically committed subpopulations. The experimental technique developed in this study offers opportunities for addressing these questions at the molecular level and for characterizing the factors in the extracellular matrix that govern various developmental processes.

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Cellular Transcription Factors and Regulation of IL-2 Receptor Gene Expression by HTLV-I *tax* Gene Product

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Expression of the interleukin-2 receptor (IL-2Rα) gene is activated by the transcriptional activator protein, Tax (previously referred to as the *tat* gene product), encoded by the human T-cell leukemia virus (HTLV-I). Multiple protein binding sites for specific DNA-protein interactions were identified over the upstream IL-2Rα transcriptional regulatory sequences. However, only one region, which includes the sequence motif GGGGAATCTCCC, was required for activation by both the *tax* gene product and mitogenic stimulation. Remarkably, this sequence also bound the nuclear factor NFκB, which is important for induction of κ-immunoglobulin gene expression. A model is presented whereby regulation of cellular gene expression by the HTLV-I *tax* gene product occurs via an indirect mechanism that may involve a post-translational modification of preexistent cellular transcription factors.

THE HUMAN T-CELL LEUKEMIA VIrus (HTLV-I) is the etiological agent of adult T-cell leukemia/lymphoma (ATL) (*1*). The genome of this retrovirus encodes a nuclear transcriptional activator protein, Tax (previously referred to as the *tat* protein), (*2*) that activates gene expression directed by the viral long terminal repeat (LTR) sequences (*3*). Likewise, expression of the *tax* gene activates expression of several cellular genes including the interleukin-2 receptor (IL-2Rα) (*4, 5*). HTLV-I sequences responsive to the *tax* gene product are present on three 21-bp repeats within the LTR (*6, 7*). Recent stud-

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