THE AXOLOTL EMBRYO AS A MODEL FOR STUDIES OF NEURAL CREST CELL MIGRATION

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Directional cell migration is a fundamental process during the morphogenesis of the vertebrate embryo (Trinkaus, 1984). To elucidate the mechanisms that regulate such processes, we are currently using neural crest cell migration in the axolotl embryo as a model system. For several reasons the axolotl embryo is a convenient material for our experiments: Its large size permits detailed microsurgery; during the initial stages of migration, the neural crest cells are the only migrating cells in the dorsal region of the embryo and they are readily available for experiments; graft rejection mechanisms are not yet developed and tissue transplantations between individuals can be performed freely; the embryo has a marvellous wound healing capacity; neural crest cells can be cultured for up to 5 days in simple salt solutions without the addition of uncharacterized serum factors, which might interfere with cell behavior; a white mutant is available with a defective migration pattern of neural crest-derived chromatophores.

Before we started our experimental series, a basic study of the early phases of neural crest cell migration in the embryonic axolotl trunk was made with light microscopy (LM), TEM and SEM (Löfberg and Ahlfors, 1978; Löfberg *et al.*, 1980). A special method was developed to enhance the contrast of extracellular matrix (ECM) components in LM sections (Löfberg and Ahlfors, 1978). The method is based on staining of glucosaminoglycans in the ECM with ruthenium red in combination with OsO_4 (Luft, 1971 a, b). Epon sections of these specimens are then stained with silver and gold salts (Fig. 4). RR staining also made it possible to study details of cell-ECM interactions with TEM. For SEM studies, the epidermis was removed to expose the dorsal region of the embryos, and the early phases of neural crest cell migration could be followed stage by stage

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(Bordzilovskaya and Dettlaff, 1979). Embryos cryofractured in frozen ethanol (Humphreys *et al.*, 1974) provided additional information.

These studies demonstrated a marked craniocaudal gradient of initiated neural crest cell migration along the body axis (Fig. 3). Evidence was obtained that the neural crest cells largely use ECM fibrils as substrate for migration (Fig. 4). The fibrils were indicated to contain collagen and glycosaminoglycans (GAG). An increase of ECM fibrils in the extracellular spaces of migration was temporally and regionally correlated with expansion of these spaces and with progressing neural crest cell migration into them. These results suggested that the ECM which is associated with the dorsal epidermis and the neural tube might influence the initial migration (Löfberg *et al.*, 1980).

With these basic data in our hands, we turned our interest to mechanisms regulating the onset of the migration. A principal question is whether the coordinated onset of the neural crest cell migration is regulated by mechanisms intrinsic to the cells themselves, regardless environmental influences, or whether the cells in the original position possess migratory capability but need some stimuli from the surrounding tissues to start migration. We have tested the latter alternative by taking tissue grafts from regions where neural crest cell migration had started, or was soon to begin, and grafting these onto host embryos in regions where the migration had not yet started. The trunk region was chosen for the tests and the tissues grafted comprised the dorsal and ventral epidermis and the somites. The epidermal grafts were placed as close as possible to the premigratory neural crest cell cord on the neural tube (Fig. 1). Control grafts consisted of tissues transplanted between embryos of the same stage and between the same regions.

The results of the graftings (Löfberg *et al.*, 1985) indicate that the dorsal epidermis is particularly effective in locally stimulating neural crest cell migration. In the region under the epidermal grafts, a tongue of crest cells protruded out from the original position ahead of adjacent cells in the premigratory cord, indicating a precocious onset of migration (Figs. 5 and 6).

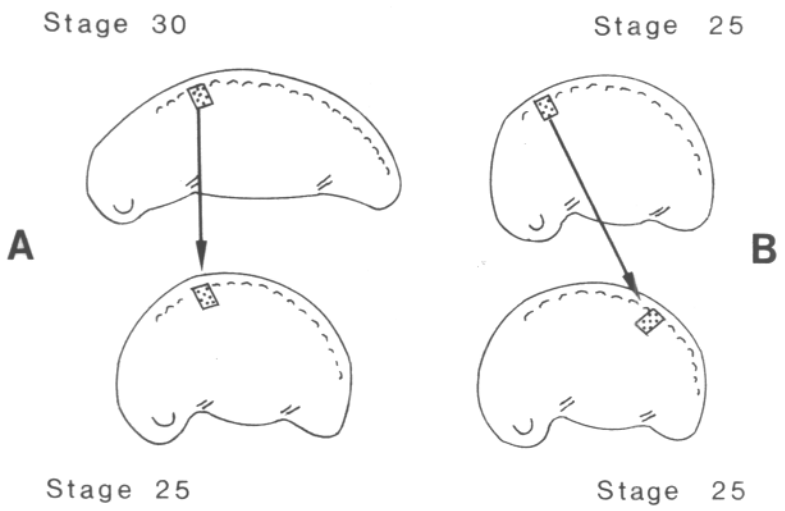

Fig. 1. Diagram showing two types of the epidermal graftings performed. In A, dorsal epidermis from older embryos was grafted to the same trunk segment of younger hosts. In 13 individual cases out of a total of 16, the grafts triggered precocious, local onset of neural crest cell migration (See Fig. 5). In B, dorsal epidermis was transferred from cranial trunk segments to posterior segments between embryos of the same age. In this series too, in 6 cases out of 7, precocious onset of migration was indicated under the grafts (See Fig. 6).

All controls showed normal migration. These results suggest that the neural crest cells in the original position along the neural tube have latent migratory capability but that they need a stimulus from the surroundings to start moving. Apparently, a stimulus triggering migration emanates from the dorsal epidermis and is expressed in a craniocaudal direction.

SEM studies indicated that those epidermal grafts which were most effective in stimulating migration had a large amount of ECM associated with its ventral side. Therefore, we wanted to test the possible effects of just the extracellular materials on the migration. For this purpose, we developed a "microcarrier" system which allows transplantation of adsorbed extracellular materials from one embryo to another (Fig. 2). A carrier consisted of a

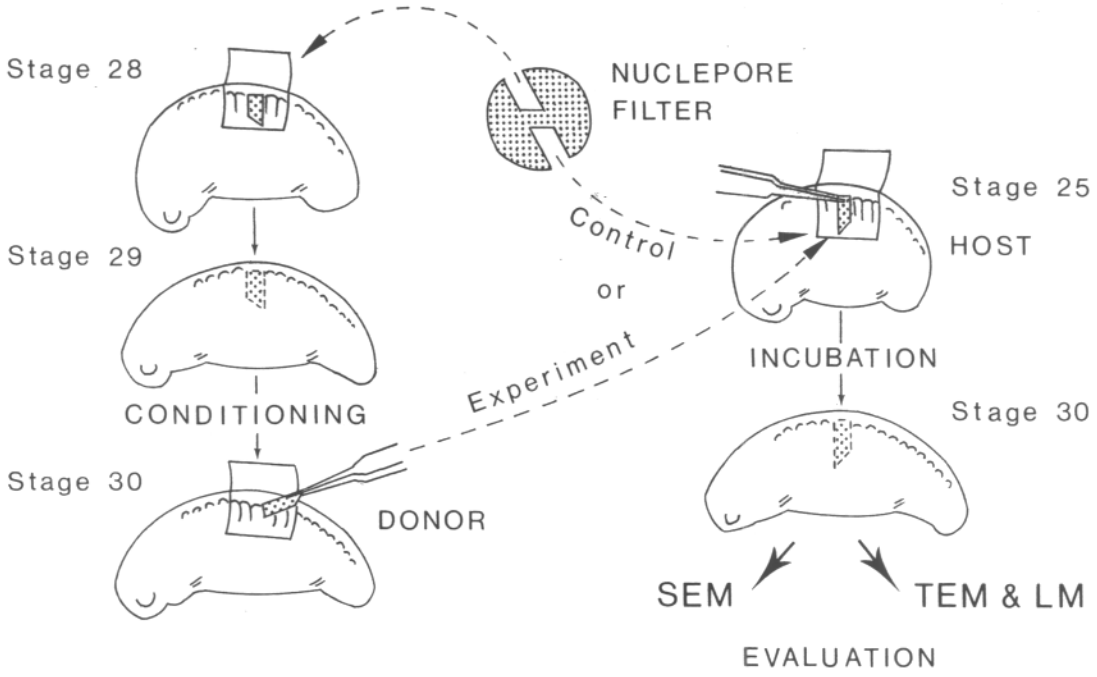

Fig. 2. Diagram of the microcarrier system developed to test the effects of extracellular materials on initial neural crest cell migration in the axolotl embryo. A SEM micrograph of a carrier is shown in Fig. 7, and a carrier with extracellular matrix materials adsorbed during conditioning is shown in Fig. 8. Results of the experiments: see Figs. 9 and 10.

small piece of Nuclepore filter material (Fig. 7) which was cut out and inserted under the epidermis of a donor embryo. After conditioning over night, extracellular materials were adsorbed to the carrier and remained attached, as confirmed with SEM (Fig. 8). The materials attached had a similar organization as that of the subepidermal ECM of the embryo. Consequently, we assumed that the material mainly consisted of ECM. The conditioned carriers were inserted under the epidermis of host embryos close to the neural crest cell cord. The embryos were then incubated till they had reached a specific stage, and finally fixed and prepared for LM, TEM and SEM. Controls consisted of embryos which received untreated Nuclepore carriers (Fig. 2).

It was found that the conditioned carriers triggered precocious neural crest cell migration, whereas normal migration was indicated in the controls (Figs. 9 and 10). In the experiment embryos, the migration was localized to the region of the carrier, and TEM studies revealed that the neural crest cells contacted a ruthenium red positive material, probably ECM, adsorbed to the carrier. These results indicate that the extracellular materials adsorbed to the carriers elicited migration, suggesting that a similar mechanism might be responsible for the onset of the migration in the embryo. Our working hypothesis is therefore that the dorsal, subepidermal ECM triggers onset of neural crest cell migration in the embryo, probably as substrate for cell locomotion. It remains to be studied whether the stimulating factor(s) which is adsorbed to the carrier consists of ECM components, or if some other extracellular constituents are the effectors.

In embryos of the white mutant axolotl (Frost and Malacinski, 1980; Frost *et al.*, 1984), the subepidermal migration of neural crest-derived chromatophores is inhibited, apparently due to an environmental defect along the migratory pathway (Keller *et al.*, 1982; Spieth and Keller, 1984; Keller and Spieth, 1984). This defect could be compensated for, allowing migration, by grafts of dorsal epidermis from wildtype embryos (Keller *et al.*, 1982). Using our microcarrier system, we have now been able to transplant extracellular materials adsorbed to microcarriers from wildtype embryos to white mutant embryos (unpublished). A dramatic stimulation of neural crest cell migration was obtained in the region of the carrier. This indicates that the defect in the tissue environment of the mutant is localized to the extracellular materials which becomes adsorbed to the carrier, probably extracellular matrix. Studies are now in progress to analyze the composition of the ECM in the white mutant in comparison with the wildtype. These studies are motivated by the possibility this mutant offers to correlate an extracellular, cell migration regulating principle with gene expression.

In the axolotl larva, chromatophores migrate out from the neural crest and form a characteristic barred pigment pattern, consisting of alternating transverse bands of black melanophores and yellow xanthophores along the dorsal side of the trunk. The

initiation of this pattern was studied in collaboration with Dr. Hans Epperlein, Freiburg University, FRG (Epperlein and Löfberg, 1984). Specific markers for chromatophore differentiation before this was externally visible were used. It was found that melanophores appeared in the premigratory neural crest already at stages 30-31, and xanthophores between stages 35-36. The xanthophores were arranged in a "prepattern" of distinct chromatophore groups along the trunk. In SEM, these groups were correlated with humps on the premigratory neural crest cord. Xanthophores mainly occurred in the groups, whereas melanophores were ubiquitous along the dorsal trunk, including the chromatophore groups, and over the flank. The barred pigment pattern emerged as a kind of "laterad projection" and was established by cell migration, by appearance of visual pigments and by fading of melanophores in xanthophore regions. We are currently using our grafting technique and the microcarrier system in further studies of the mechanisms that control pigment pattern formation in wildtype and mutant axolotl embryos (Frost *et al.*, 1984).

Thus, the axolotl embryo has proven to be a most useful material for our grafting experiments which are performed with classical methods but evaluated with modern electron microscopical techniques. Our microcarrier system, as far as we know, is a new approach in experimental embryology. With the carrier as a probe, it is possible to take samples of extracellular materials from different regions of the axolotl embryo and test the effects of the materials adsorbed to the carrier on neural crest cell migration (Löfberg *et al.*, 1985) and differentiation (Perris and Löfberg, 1984) both *in vivo* and *in vitro*. Such microcarrier systems may thus be useful in a wide range of studies on how extracellular materials influence migrating cells during embryonic development.

Acknowledgements

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Text to Figures

Abbreviations used: ECM = extracellular matrix; EP = epidermis; NC = neural crest cells; NT = neural tube; M = microcarrier; S = somite. Arrows indicate the apparent main direction of migration.

Fig. 3. SEM micrograph showing the cranial trunk region of a stage-30 axolotl embryo; the head is on left. Neural crest cells have started spreading laterally. The craniocaudal gradient of initiated migration is evident. X 100.

Fig. 4. Cross section through the cranial trunk of a stage-32 embryo. NC cells apparently using a subepidermal network of ECM fibrils as substrate for locomotion. The contrast of the fibrils is enhanced by block staining with ruthenium red-OsO_4 followed by section staining with silver and gold salts. X 950.

Fig. 5. The result of a grafting experiment from series A (see Fig. 1). A tongue of neural crest cells extend out over the somite where an older epidermal graft was placed, indicating precocious onset of migration triggered by the graft. SEM, X 100.

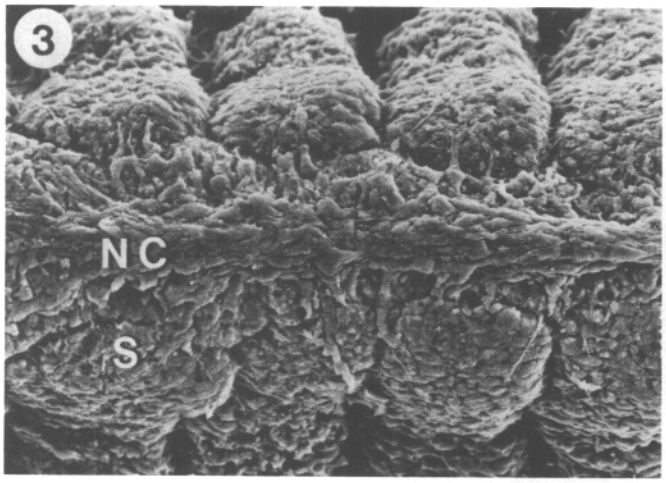
Fig. 6. An experimental embryo from series B (see Fig. 1). An epidermal graft from the 3rd trunk segment of a stage-25 donor was transferred to the 8th segment of a stage-25 host. Here, at stage 30, a local stimulation of neural crest cell migration is demonstrated where the graft was placed. The contralateral, control side shows the normal situation for this stage and region. SEM, X 100.

Fig. 7. SEM micrograph of a microcarrier (see Fig. 2). X 100.

Fig. 8. SEM micrograph showing the appearance of subepidermal, extracellular matrix materials which was adsorbed onto the microcarrier during conditioning (see Fig. 2). X 2,000.

Fig. 9. Cross section of a stage-30 embryo which had a control microcarrier inserted under the epidermis. Most neural crest cells apparently remain in the premigratory position which is the normal situation for this region. X 200.

Fig. 10. Cross section of a stage-30 experimental embryo which had a conditioned microcarrier, with ECM materials adsorbed, inserted under the epidermis. The neural crest cells have been stimulated to start migration in the region of the carrier. On the neural tube where most of the cells should have remained in this region (see Fig. 9), only sporadic cells occur. Thus, extracellular materials adsorbed to the carrier has triggered a local, precocious onset of neural crest cell migration. X 200.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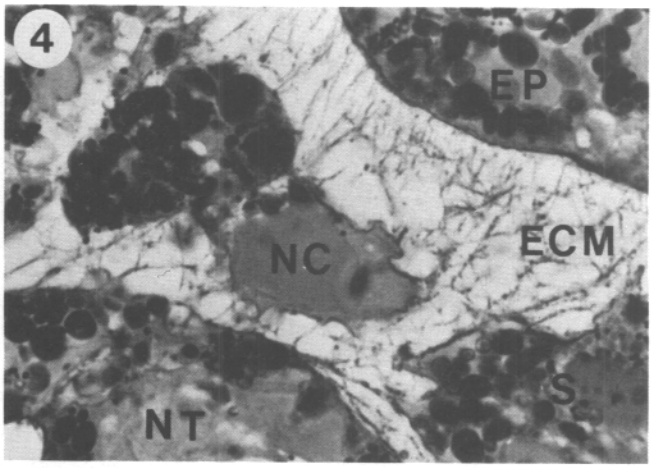
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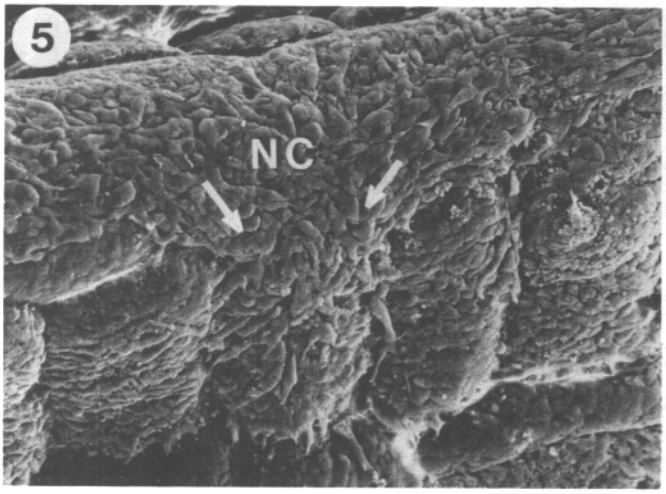
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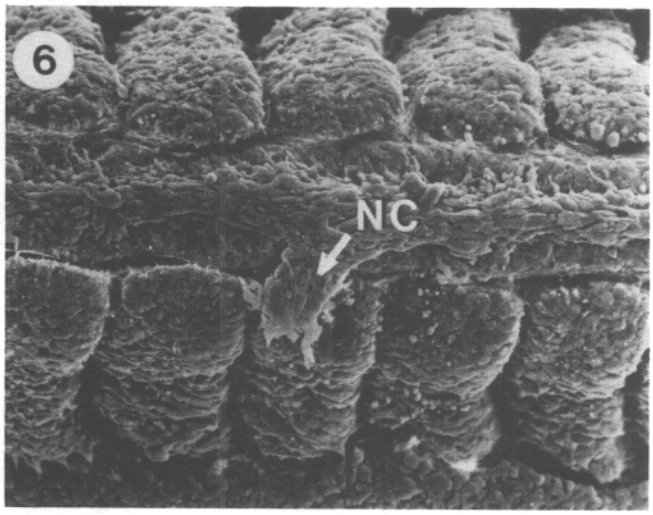
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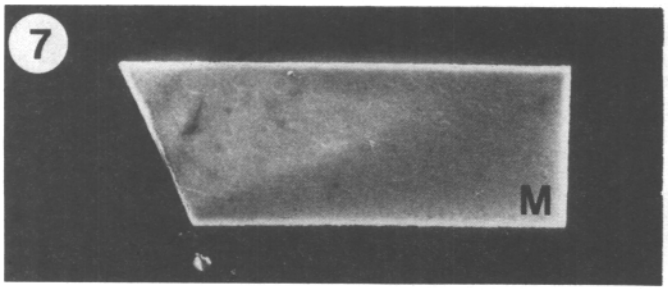
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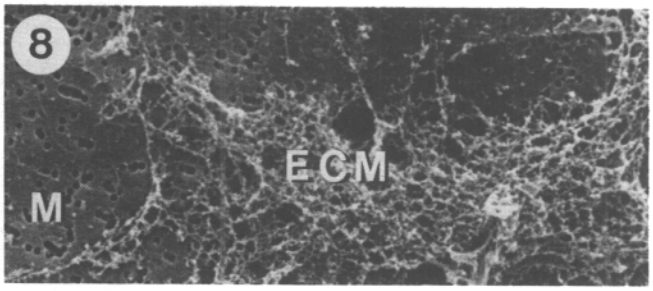
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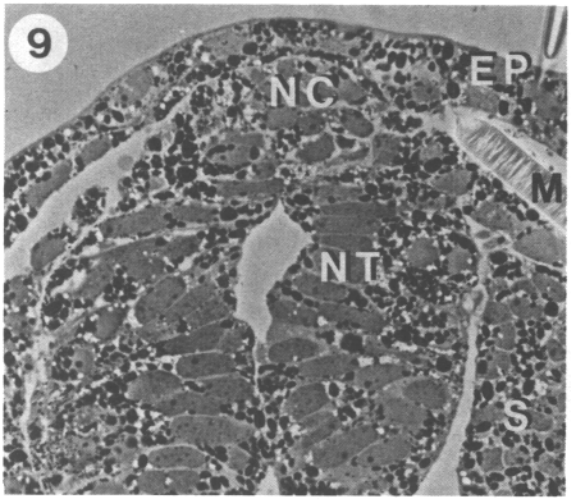
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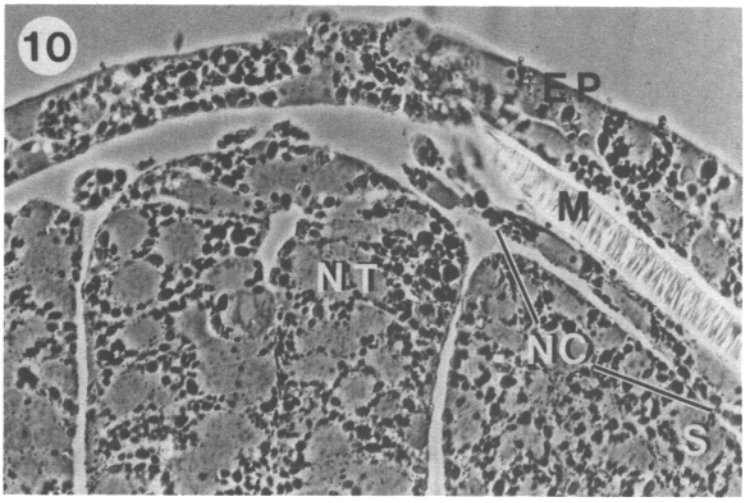
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