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Cell Population Changes in Transplanted Olfactory Placodes of Chimerae of *Xenopus laevis* and *Xenopus borealis*

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When olfactory placodes are transplanted at stages 23/24 from *Xenopus laevis* to *Xenopus borealis* hosts of the same age, it is possible to distinguish the cell populations of the host and donor due to the peculiar nuclear Q bands specific to *X. borealis*. I have replaced the eye anlage in each of a number of *X. borealis* with the transplanted olfactory placode of an individual *X. laevis*, or vice versa. In most instances, the placode of the donor fusea with that of the host. When fusion occurs, but not when the host and donor organs grow separately, the cells of the donor were replaced gradually and according to a characteristic pattern by cells of the host. The basal cells of the donor were the first to be replaced, followed by the more matured cells of the sensory epithelium. This cellular substitution, proceeding in an orderly fashion from bottom to upper layers of the epithelium, depends on the fusion of the two organs. This observation suggests intercellular contacts in the mitotic zone of the two organs favor the host's cells over those of the donor.

The influence of the environment on the differentiation of neural precursors has been demonstrated by neural crest cells (Cohen, 1972; Perris et al., 1988) and neuroblasts (McConnell and Kaznowski, 1991; Kilpatrick et al., 1994; Fishell, 1995; Magrassi and Graziadei, 1996). However, it is still unclear if neural precursors will differentiate only according to the new environment.

The neuronal renewal is a well established characteristic of the olfactory system even in adult mammals. The olfactory system acts as neurogenetic matrix which produces olfactory neurons for the replacement of those undergoing turnover (Moulton et al., 1970; Graziadei and Metcalf, 1971; Graziadei and Monti Graziadei, 1978a; Graziadei and Monti Graziadei, 1978b) and those destroyed by experimental manipulation (Monti Graziadei and Graziadei, 1979a; Monti Graziadei and Graziadei, 1979b; Monti Graziadei, 1992). Moreover, the ectopically transplanted olfactory epithelium consistently differentiates into an olfactory organ, regardless of their appropriate target (Stout and Graziadei, 1980; Heckroth et al., 1983; Morrison and Graziadei, 1983; Graziadei and Monti Graziadei, 1985; Magrassi and Graziadei, 1985; Magrassi and Graziadei, 1996). Cells that separated from the transplants migrate along the olfactory nerves into the CNS (Morrison and Graziadei, 1983; Monti-Graziadei and Graziadei, 1989), although the fate of these cells is unclear.

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In amphibians, replacement of a removed eye vesicle with the transplanted olfactory placode has been previously reported to result frequently in the fusion of the transplanted anlage with the host's placode (Magrassi and Graziadei, 1985). In spite of the fusion of the two organs, however, two separate olfactory nerves reach the brain; the host's olfactory nerve reaches its proper target, i.e. the olfactory bulb, whereas the transplanted anlage develops as a supernumerary olfactory organ and sends its nerve to the diencephalon. The removed eye fails to regenerate, presumably because of an inhibitory influence of the grafted placode. From both the host's and the donor's olfactory epithelia, a stream of cells migrate and reach, respectively, the olfactory bulb and the diencephalon, where often a conspicuous hyperplasia develops (Koo and Graziadei, 1995b).

The results of the above experiments all suggest that (1) the olfactory neurons are able to differentiate into an olfactory epithelium even in the ectopic environment without any information from their target, and (2) the olfactory epithelium is a cell supplying structure for various parts of the brain, by migration, as well as the olfactory organ. However, the influence of the local environmental conditions on the differentiation ability of olfactory neuron precursor is still unclear. *In vivo* experiments, cells dissociated from the olfactory organ have changed their fate and differentiated according to their final position (Magrassi and Graziadei, 1996).

In the present experiments I intended to study whether the olfactory placodes from donor and host show any reciprocal influence on each other in differentiative program after transplantation. I was particularly interested in examining the change in cell populations in both host and donor olfactory placodes. It was detectable because the donor cells, identified by the characteristic fluorescent Q bands in their nuclei, could be easily distinguished from those of the donor. The present work shows the changes in the cell populations of the grafted olfactory organ, when the donor placode from a *Xenopus laevis* was transplanted to a *Xenopus borealis* host and the two organs fused to form a unique long neuroepithelial groove.

Materials and Methods

Xenopus laevis and *Xenopus borealis* were used in the present experiments. The olfactory placode of a *X. laevis* was transplanted to replace a removed optic vesicle in a *X. borealis* host at stages 23/24. Animals were operated in a small Petri dish with 2% agar stained with activated charcoal. The embryos were immersed in full strength Holtfreter solution containing 1% gentamicin in the Petri dish. Sharpened minute insect pins and fine forceps were used for surgery. The grafts were gently pressed into the host by a small piece of glass coverslip for 1 h to facilitate incorporation of the graft into the host. After the graft had healed, embryos were transferred to Holtfreter solution and moved to pure well water and raise to the desired stages. Animals were sacrificed from stage 32 up to stage 60. Fixation was performed, following anethesia with MS-222 (ethyl-amino benzonate methanesulfate, 1 : 4000), in Bouin's fluid. The material was subsequently embedded in paraplast and serially sectioned. In control material the sections were stained with Gill's hematoxylin. For fluorescence-microscope observations, the animals were fixed in Carnoy's fixative and embedded in paraplast. Tissues were serially sectioned at 5 μm thickness; slides were preincubated in McIlavaine's buffer and stained in quinacrine dihydrochloride. Detailed procedures were previously reported (Koo and Graziadei, 1995b). Only 105 animals out of 201 were processed, because of premature death or unsuccessful incorporation of the transplanted tissue. Of the animals examined, 80 were processed for fluorescene microscopy and the remainder were studied by common histological methods.

Results

In all the animals, the donor placode initially developed separately from the host's and two nerves were observed to develop form each organ. The host's organ sent its nerve to the olfactory bulb (its conventional target). The donor's placode sent its nerve

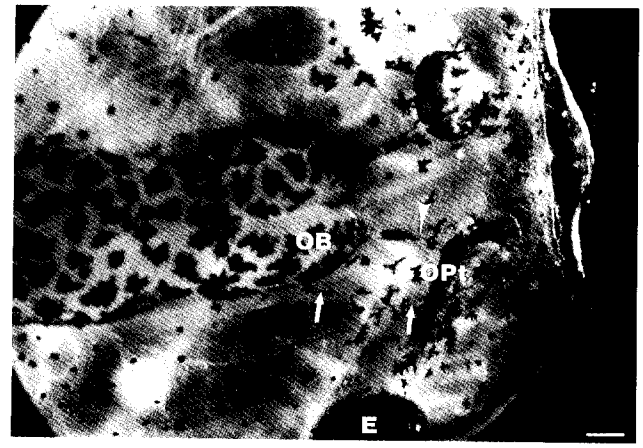

Fig. 1. Dorsal view of stage 50 *Xenopus borealis*. The transplanted olfactory organ usually fuses with the host's ipsilateral olfactory placode, forming a large strip-shaped placode (OPt). The olfactory nerve from the transplanted olfactory placode(arrows) penetrates the host brain. OB, olfactory bulb; E, regenerated host's eye; olfactory nerve from the host's olfactory organ(arrowhead). Scale bar=200 μm.

consistently to the diencephalon, where a hyperplasia developed at the point of entry (Magrassi and Graziadei, 1985; Koo and Graziadei, 1995b).

From both placodes cells were seen migrating to the central nervous system (CNS). The cells of the donor organs were followed more easily, due to their nuclear characteristics, and were seen to be incorporated into the host's diencephalon. These cells, recognizable by the absence of fluorescent nuclear Q bands, remained in the diencephalon, where they mingled with the host's neurons, forming the diencephalic nuclei. Morphologically, the nuclear characteristics of most of these cells suggested a neuronal nature. On the basis of their irregular, elongated nuclei and their sparse arrangement, a few were tentatively interpreted as glial in nature. Both types of cells form the donor organ remained in the CNS location for the duration of the experiment (until stage 60).

In the majority of cases (90%), the two placodes eventually fused, forming a unique elongated olfactory organ (Fig. 1). This fused olfactory organ distinctly showed cells with both the host's and the donor's nuclear characteristics (Fig. 2). The fusion of the two placodes followed a variable course, possibly depending on the position of the transplant or other variables that were difficult to assess. Gradually, however, cells with the host's characteristics began to replace the cells in the donor neuroepithelium. The process followed a characteristic pattern. Host cells began to invade the base of the donor epithelium, replacing the cells of the latter (Fig. 3). At this stage only the basal cells along the entire donor epithelium were from the host. In a subsequent phase, cells with host characteristics occupied the middle and upper epithelial compartments (Fig. 4), and approximately by stage 50-52 all the cells of the entire organ had the host's characteristics (Fig. 5).

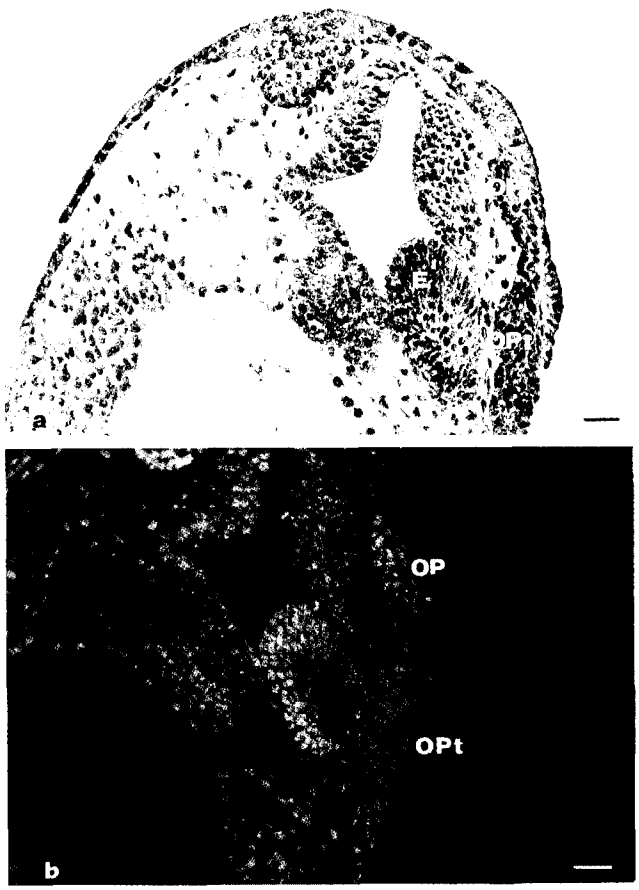

Fig. 2. Horizontal sections of stage 37/38 *X. borealis* embryo. (a) The transplanted olfactory placode is fused with the ipsilateral placode of the host, forming a large olfactory organ. The regenerating host eye(E) is temporarily fused with the brain because the overlying graft has displaced it. The regenerating eye will, however, eventually move to the right position. OP, olfactory placode of the host; OPt, transplanted olfactory placode. bar=40 microns. (b) Fluorescence micrograph of the same section in Fig. 2a. Cells of *X. laevis*(donor) show homogeneous nuclear staining, whereas cells of *X. borealis*(host) show characteristic bright spots in their nuclei. The regenerating eye and the olfactory placode(OP) of the host have also been observed. OPt, transplanted olfactory placode. Scale bar=30 μm.

The same result was observed when a *X. laevis* was the host and a *X. borealis* was the donor (Fig. 6). In the early phase of transplantation and prior to complete cell substitution, the nerve that developed from the donor placode reached the host's diencephalon, and along this nerve migration of cells with both neuronal and glial characteristics was observed. As previously reported (Koo and Granziadei, 1995b), these cells were incorporated into the diencephalic nuclei maintaining the typical donor nuclear pattern. These cells were never replaced by cells with the host's nuclear characteristics during observations, i.e. before stage 60. Thus, when the placodes fused, only the cells migrating to the brain maintained the nuclear characteristics of the *X. laevis* (donor) type; cells of the olfactory organ were gradually replaced by cells of the host (*X. borealis*). In contrast, when the two placodes developed independently and no fusion occured, the

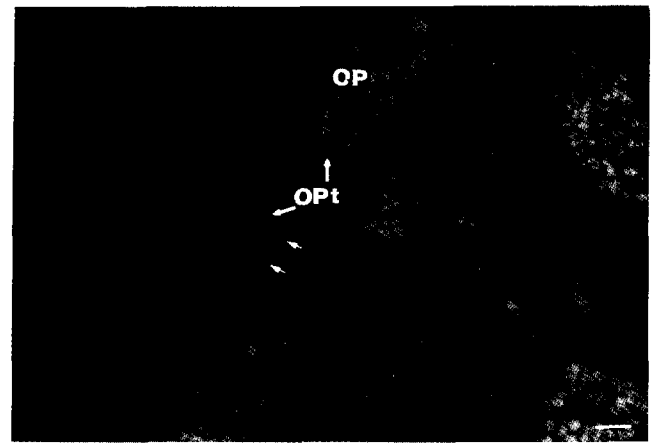

Fig. 3. Fluorescence micrograph of stage 33/34 *X. borealis*. The ipsilateral olfactory placode(OPt) has already fused. In the fused olfactory organ, boundaries between the host cells and donor cells are quite clear. Approximately half of the fused organ consists of donor cells and the other half of host cells. However, a few basal cells (small arrows) along the entire donor placode have been replaced by cells with host characteristics. Scale bar=20 μm.

two placodes retained the original nuclear characteristics of donor and host (Figs. 7 and 8)

Discussion

When the olfactory placode replaces the removed optic vesicle, the transplanted organ usually fuses with the host's ipsilateral olfactory placode. After a certain stage, the two fused placodes consist solely of cells showing the nuclear characteristic of the host. This change from the donor's to the host's characteristics occurs gradually, beginning at the level of the epithelial basal cells (the stem cells of the olfactory neurons),

Fig. 4. Fluorescence micrograph of the olfactory epithelium of stage 50 *Xenopus borealis*. Donor cells (*X. laevis*) cells in the region of the transplanted olfactory placode are gradually replaced by host (*X. borealis*) cells. Host cells show bright spots. The replacement of cells occurs in horizontal bands; the lower part of the epithelium changes first. The middle and upper parts of the olfactory epithelium undergo later cell substitution. Arrows indicate remaining donor cells (*X. laevis*). Scale bar=20 μm.

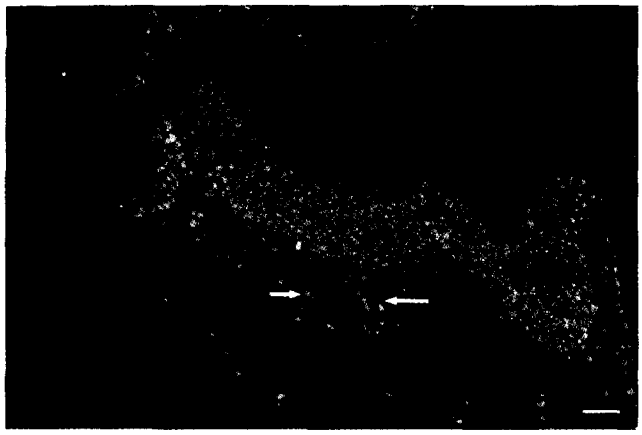

Fig. 5. Fluorescence micrograph of stage 51 *X. borealis*. All cells of the entire transplanted olfactory placode demonstrate a fluorescence that is characteristic of the host (*X. borealis*) cells. The two arrows indicate the olfactory nerves from the host (left) and from the transplanted (right) olfactory placodes. Scale bar=30 μm.

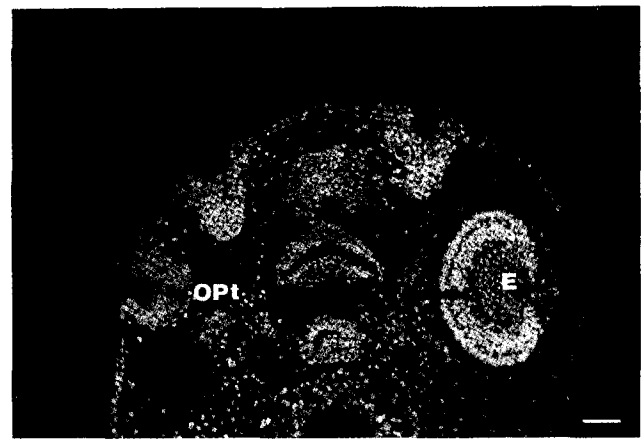

Fig. 7. Stage 41 *X. borealis* embryo. In this animal, the donor placode grows independently and has not fused with the host's placode. The transplanted placode (OPt), from *X. laevis*, does not show any changes in cell characteristics. E, host eye. Scale bar=50 μm.

is usually completed when the larva reaches stage 50-52 and persists for the duration of observations (up to stage 60). One possibility for these phenomena is that there is a change in the phenotype of the transplanted cells. This may be due to the fact that intercellular interactions are critical determinants of neuronal differentiation during development. Neuroblasts can express a number of phenotypic characteristics after migrating to their final location. It appears that contact with cells in the final location may provide a stimulus for selective expression of phenotypes and this is documented on expression of neurotransmitters (Adler and Black, 1985, 1986; Perris et. al 1989). Cortical transplant studies also showed that transplanted brain tissue is able to, at least partially, adopt the phenotype dictated by its new position in the cortex (Stanfield and O'Leary, 1985; Fischell, 1995; Magrassi and Graziadei, 1996). Since the Q bands of *X. borealis*, which appear as bright spots after staining with quinacrine, are a phenotypic character of the chromosomes, donor stem cells of the transplants might change their phenotypic traits because of the interaction with the new environment.

The other possibility is cell substitution by host placodal cells. The new population of the transplanted olfactory epithelium is continuously turning over, all the donor cells lost from the developing placode may be the same cells that were accumulated in the brain (Koo and Graziadei, 1995b). In that case, the donor cells (putative neurons and glia) that reached the host brain did not change their nuclear staining characteristics. Interestingly, it was also reported that, when an eye vesicle is transplanted in these same animals and donor retina fuses with the host's giving origin to

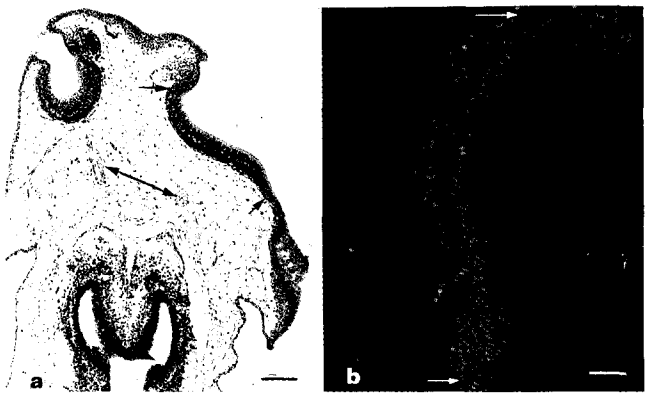

Fig. 6. Horizontal sections of stage 52 *X. laevis*. (a) hematoxylin stained section of gill shows the fused olfactory placode(between arrows). The fused olfactory placode is also shown in Fig. 6b at higher magnification. The double arrow shows the normal nerve (left) and the nerve from the fused placode (right). bar=120 microns. (b) Fluorescence photomicrograph of the animal shown in Fig. 6a. In this case, donor and host strains have been switched. Therefore, this animal had received an olfactory placode transplant from a *Xenopus borealis* instead of *Xenopus laevis*. It reiterates the result in Fig. 4 that the fused olfactory placodes (between arrows) contain only cells that possess host characteristics. Seen in this picture is the homogeneous staining that is characteristic of *Xenopus laevis* (host). Scale bar=40 μm.

Fig. 8. Fluorescence micrograph of transplanted olfactory placode at stage 51. The transplant did not fuse with the host's placode and developed independently. The cells in the transplant (between arrows) retain their donor (*X. laevis*) characteristics. Scale bar=20 μm.

a continuous chimeric retinal layer, the donor elements retain their nuclear chracteristics and a sharp boundary between areas with different nuclear characteristics can easily be detected (Koo and Graziadei, 1995a).

Because the cells only in the donor olfactory placode, but not in the retina or those of placodal origin relocated in the CNS, undergo this replacement, the change can hardly be mediated by the environment of the host. In fact, the cells of placodes that do not fuse with the host's and grow independent from it, have been observed to retain the donor's characteristics. These observations have been repeated over up to 100 specimens, and the fortuitous chance of a mistaken transplant is at best unlikely. Moreover and even more pertinently, the sequence of cell substitution from the base of the neuroepithelum, where the stem cells are located, to its upper compartment, where the matured neurons lie, indicates that the change originates from the new stem elements, which gradually replace the old ones in the course of normal turnover. Discussion of the causes of this phenomenon can only be speculative. Several laboratories have domonstrated that the vertebrate olfactory neurons are replaced during the entire lifetime of the vertebrate (Graziadei, 1973; Moulton, 1974; Graziadei and Monti Graziadei, 1978b; Graziadei, 1990). The substitution of the host's stem cells for donor stem cells, which proceeds in orderly fashion from the base of the neuroepithelium to the more mature elements, indicates that the stem cells of the neurons are the first to be affected by the change. Because the host environment alone cannot account for the change, I must presume that intercellular contacts in the mitotic zone of the two adjacent organs favors the host's cells over those of the donor. Host cells would migrate to the new sensory area of the donor organ and there divide and mature, gradually replacing the existing population. My sequential morphological observations indicate this possibility. Little is known so far that the mechanisms governing the mitotic activity of the stem cells of the normal olfactory epithelium, and that in spite of the many contributions on the subject, how the phenomenon of *in vivo* cell replacement in the olfactory organ may be regulated. In the present case, it is possible to speculate that, during the course of a programmed cell replacement, the stem cells of the host have an advantage in their reproduction over those of the donor as a result of direct contact with other host cells. The phenomena that regulate the death and replacement of the olfactory neurons, still largely unknown, may be biased in favor of the host's stem cells by this direct contact. If this should prove to be the case, these results would be a unique example of specific cell-cell interaction in which the environment facilitates but does not determine the change.

Acknowledgements

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