Transdifferentiation of larval *Xenopus laevis* iris under the influence of the pituitary

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Summary. Fragments of larval *Xenopus laevis* dorsal iris implanted together with the pituitary into the tail fin transdifferentiate into neural retina. On the contrary, in the control experiments the implanted tissues, dorsal iris alone, pituitary, or dorsal iris with liver fragments, do not undergo any retinal transformation.
Key words. Transdifferentiation; iris; larval *Xenopus*; pituitary.

Lensectomized *Xenopus laevis* larvae can regenerate a new lens from the inner layer cells of the outer cornea [1]. In situ, both the outer cornea and the pericorneal epidermis undergo lens-forming transformations whenever they come into direct communication with the vitreous chamber environment, which contains some inducing factor(s), probably proteic in nature, produced by the neural retina [2–7].

Several larval tissues other than neural retina can promote the lens-forming transformations of the outer cornea (see Bosco [8]).

After lensectomy, in contrast to the outer cornea, the iris does not exhibit transdifferentiative capacities, either in the lens-forming direction or towards other differentiative pathways, even when the tissue had been damaged in order to stimulate its latent competence [9, 10]. However, after having been isolated from surrounding tissues and implanted in the vitreous chamber of a lensectomized larval eye, the dorsal iris transdifferentiates into neural retina under the influence of the host neural retina [1]. As in the process of lens-forming transformation of the outer cornea, the regenerating limb environment can replace the action of the neural retina in promoting the retina-forming transformation of the iris implants [12]. The aim of our work is to establish whether the capacity to induce retina-forming transformations is also present in other larval tissues. In the present paper data concerning the inducing capacities of the pituitary are reported.

Materials and methods

A total of 71 *Xenopus laevis* larvae at stage 54–55 (according to Nieuwkoop and Faber [13]) were used. As donors for pituitary and liver 36 neo-metamorphosed individuals were used. The animals were obtained after ovulation and mating induced by gonadotropic hormones (Coriantin, Richter). During the operations described below, the animals were anesthetized with MS 222 (Sandoz) at a concentration of 1:3000 in full strength Holtfreter's solution.

The operated larvae were then gradually transferred into tap water and reared until the 20th day after the operation. After fixation in Bouin's solution and embedding in

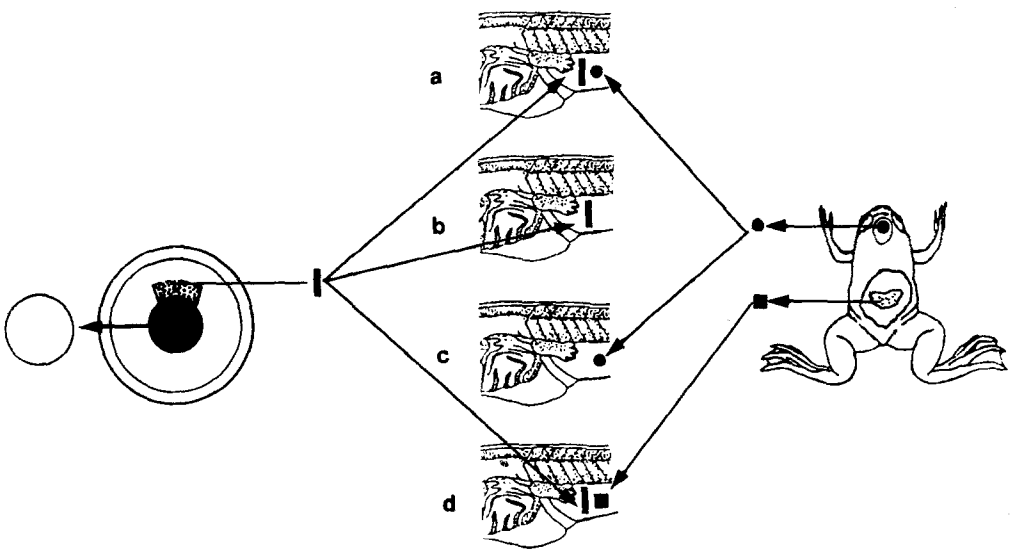

Figure 1. Diagram of operating technique. After lensectomy, a fragment of the marginal ring of the dorsal iris was autoplastically implanted into the tail fin with the pituitary of a metamorphosed individual (a, experiment I), alone (b, experiment II) or with a fragment of liver of a metamorphosed individual (d, experiment IV). In experiment III (c) the pituitary of a metamorphosed individual was omoplastically implanted into the tail fin.

paraffin, the larvae were cut into 7-μm-thick serial transverse or sagittal sections, which were then stained with hematoxylin and eosin.

Four types of implants into the tail fin were carried out (fig. 1). Experiment I: Autoplastic implant of dorsal iris with the pituitary of metamorphosed individuals. Experiment II: Autoplastic implant of dorsal iris. Experiment III: Omoplastic implant of pituitary. Experiment IV: Autoplastic implant of dorsal iris with liver of metamorphosed individuals.

Results and discussion

The results are summarized in the table. The results obtained in experiment I show that dorsal iris fragments implanted into the tail fin with the pituitary transdifferentiated into neural retina in 42% of the cases examined. The neo-formed retinal vesicles show a thick orderly layered wall and recognizable visual cells converging into the lumen (fig. 2). In a further 42% of cases the implanted iris underwent different degrees of partial transformation: depigmentation, proliferation of the depigmented iris epithelial cells and initial stratification of the newly-formed retinal cells.

In contrast, in the control experiments, the implanted tissues – dorsal iris alone (experiment II), pituitary (experiment III) and dorsal iris with liver fragments (experiment IV) – did not undergo any retinal transformation (fig. 3). In some cases in experiments II and IV the implanted dorsal iris showed various degrees of depigmentation and regression.

Previous data reported the differentiation of retinal structures in contact with the pituitary in the adult crested newt, after the insertion of a mechanical barrier at the level of the median eminence to isolate the pituitary from the brain [14]. Moreover, both retina and lens potential appear to reside in the embryo hypophysis [15]. The results of experiment III confirm that the retinal structures observed in experiment I originate from the implanted iris, as the isolated pituitary appears to maintain a high phenotypic stability. The results obtained in the present research indicate that, in addition to the neural retina and the amputated limb environment [11, 12] the pituitary can also promote and sustain the retinal transformation of the dorsal iris. On the other hand, the liver induced no significant response in the iris implants; similarly, the simple implantation of the iris into the tail fin did not promote the retinal transdifferentiation of this tissue.

Summary of the results of experiments I (autoplastic implant of dorsal iris with the pituitary of metamorphosed individuals), II (autoplastic implant of dorsal iris), III (omoplastic implant of pituitary) and IV (autoplastic implant of dorsal iris with liver of metamorphosed individuals)

Experiment	Cases operated*	Cases dead/discarded	Cases examined	Cases with partial transformation	Cases transdifferentiated into retina
I	13	1	12	5	5
II	15	2	13	–	–
III	13	3	10	–	–
IV	10	1	9	–	–

*In all experiments five additional larvae were operated and fixed immediately after the operation to serve as controls of the operative procedure.

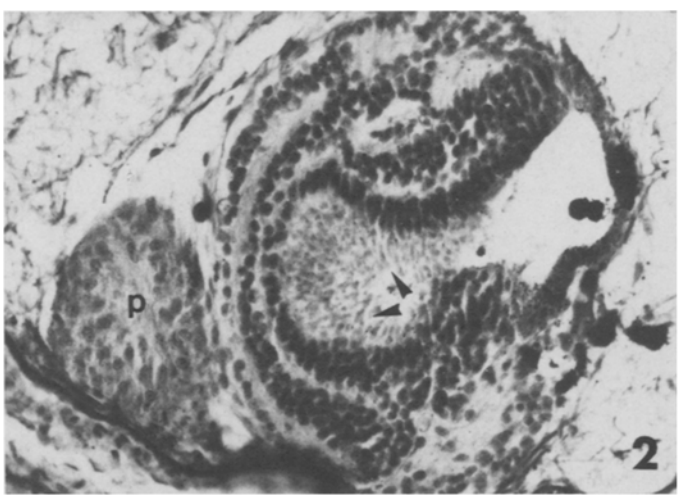

Figure 2. Experiment I, 20 days after the operation. From the implanted dorsal iris a retinal vesicle is formed. The newly-formed retina shows a typical stratification and recognizable photoreceptors converging into the lumen of the vesicle (arrowheads). p, pituitary. ×250.

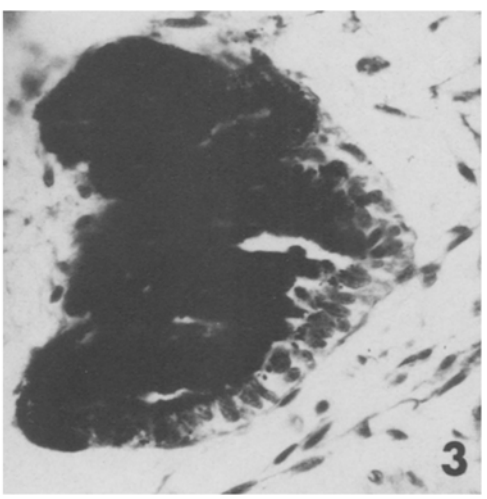

Figure 3. Experiment II (control experiment), 20 days after the operation. The dorsal iris fragment implanted alone does not show any retinal transformation. ×400.

It is known that the pituitary is also effective in stimulating the lens-forming transformation of the outer cornea, likewise the neural retina, the regenerating limb, the limb bud and its blastema, the tentacle blastema and the spinal ganglia[7,16,17]. These data have been explained by assuming that, in addition to the retinal factor, a pituitary factor and a neurotrophic factor, produced by the ganglion cells and also by dedifferentiated cells of buds and blastema, are responsible for promoting the lens-forming transformations of the outer cornea[17,18]. Similar factors could be responsible for triggering the retinal transdifferentiation of the iris.

The available data concerning the lens-forming transformation of the outer cornea and the retinal transdifferentiation of the iris could be explained by assuming that the influence exerted by the triggering factors is permissive in nature, allowing the already-committed responding tissues (outer cornea, dorsal iris) to realize their latent intrinsic developmental capacities (see also Bosco[8]). Therefore, whereas the outer cornea is always transformed into lens, the dorsal iris, under the influence of the same tissues, always transdifferentiates into neural retina. Alternatively, one could suggest that different factors, instructive in nature, are responsible for promoting the lens-forming transformations of the outer cornea and the retinal transdifferentiation of the iris. In this hypothesis it is necessary to admit that factors promoting lens- and retinal-transdifferentiation are simultaneously present in all the different inducing tissues. However, this hypothesis seems to be less likely than the first.

As far as the identity of the inducer is concerned, the pituitary could exert its inducing effect by releasing hormones. However, the available in vitro data concerning the influence of bovine pituitary hormones on lens regeneration from newt iris epithelium failed to demonstrate a link between activity stimulating lens-regeneration and purified hormonal activity[19,20].

On the other hand, it could be argued that the pituitary factor is a neural growth factor. In this connection, it is interesting to note that the mitogenic protein called glial growth factor (GGF) originally purified from bovine pituitary[21] has recently also been detected in newt brain extracts[22]. It has been demonstrated that GGF is present in the innervated blastema of the newt regenerating limb, and is lost on denervation. A role of GGF in the nerve-dependent proliferation of the blastemal cells has been suggested[22].

1 Freeman, G., J. exp. Zool. *154* (1963) 39.
2 Bosco, L., Filoni, S., and Cannata, S., J. exp. Zool. *209* (1979) 261.
3 Filoni, S., Bosco, L., and Cioni, C., Acta embryol. morph. exp. *2* (1981) XXVI.
4 Filoni, S., Bosco, L., and Cioni, C., Acta embryol. morph. exp. *3* (1982) 15.
5 Filoni, S., Bosco, L., Cioni, C., and Venturini, G., Experientia *39* (1983) 315.
6 Reeve, J. G., and Wild, A., J. Embryol. exp. Morph. *48* (1978) 205.
7 Reeve, J. G., and Wild, A., J. Embryol. exp. Morph. *64* (1981) 121.
8 Bosco, L., Differentiation *39* (1988) 4.
9 Filoni, S., Bosco, L., and Cioni, C., Acta embryol. exp. *3* (1976) 319.
10 Bosco, L., Filoni, S., and Paglioni, S., J. exp. Zool. *216* (1981) 267.
11 Cioni, C., Filoni, S., Aquila, C., Bernardini, S., and Bosco, L., Differentiation *32* (1986) 215.
12 Cioni, C., Filoni, S., Bosco, L., Aquila, C., and Bernardini, S., Experientia *43* (1987) 443.
13 Nieuwkoop, P. D., and Faber, J., Normal Table of *Xenopus laevis*. Daudin-North-Holland Publ., Amsterdam 1956.
14 Sacerdote, M., Z. Anat. Entwickl. Gesch. *134* (1971) 49.
15 Clayton, R. M., Adv. exp. Biol. Med. *158* (1982) 23.
16 Waggoner, P. R., J. exp. Zool. *186* (1973) 97.
17 Bosco, L., Filoni, S., and Cioni, C., J. exp. Zool. *233* (1985) 221.
18 Filoni, S., Bosco, L., Cioni, C., and Burani, P., J. exp. Zool. *230* (1984) 409.
19 Connelly, T. G., Differentiation *16* (1980) 85.
20 Cuny, R., and Zalik, S., Exp. Eye Res. *41* (1985) 629.
21 Lemke, G. E., and Brockes, J. P., J. Neurosci. *4* (1984) 75.
22 Brockes, J. P., and Kintner, C. R., Cell *45* (1986) 301.

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Hydrocarbons in tarsal glands of *Bombus terrestris*

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Summary. Chemical components of tarsal glands of *Bombus terrestris* workers were identified by combined gas chromatographic/mass-spectrometric analysis. The 17 components are exclusively saturated and unsaturated hydrocarbons.
Key words. Bombus; tarsal gland; forage-marking; hydrocarbons.

In honeybees, tarsal glands are situated in the pretarsae of all legs and were first described by Arnhart[1] (Arnhart's glands). Although honeybees are one of the best examined insects, a comprehensive chemical analysis of tarsal glands is still lacking. Chauvin[2] examined infrared spectra of the gland compounds and found a waxy composition with aromatic substances. In bumblebees, tarsal glands are located in the pretarsae too and are less devel-