1 The studies described in this report were performed on dead aborted human fetuses. The abortion did not have any connection with the protocol of the study[21].
2 The authors wish to thank Prof. M. Zydowo for his interest in this work and reading of the manuscript, and Prof. E. W. Holmes and Dr R. Sabina (Duke University Medical Center, Durham, USA) for discussion. This work was supported by the Polish Academy of Science within the project 04.01.2.05.
3 Purzycka, J., Acta biochim. polon. *9* (1962) 83.
4 Ashby, B., and Frieden, C., J. biol. Chem. *252* (1977) 1869.
5 Lowenstein, J. M., Physiol. Rev. *52* (1972) 382.
6 Parnas, J. K., Mozolowski, W., and Lewinski, W., Biochem. Z. *184* (1927) 399.
7 Kendrick-Jones, J., and Perry, S. V., Biochem. J. *103* (1967) 207.
8 Kaletha, K., and Zydowo, M., Int. J. Biochem. *2* (1971) 20.
9 Sammons, D. W., and Chilson, O. P., Archs Biochem. Biophys. *191* (1978) 561.
10 Kaletha, K., Biochim. biophys. Acta *759* (1983) 99.
11 Kaletha, K., Biochim. biophys. Acta *784* (1984) 90.
12 Fishbein, W. N., Armbrustmacher, V. W., and Griffin, J. L., Science *200* (1978) 545.
13 Fishbein, W. N., Griffin, J. L., and Armbrustmacher, V. W., Archs Path. Lab. Med. *104* (1980) 462.
14 Sabina, R. L., Swain, J. L., Olanow, C. W., Bradley, W. G., Fishbein, W. N., DiMauro, S., and Holmes, E. W., J. clin. Invest. *73* (1984) 720.
15 Fishbein, W. N., Biochem. Med. *33* (1985) 158.
16 Smiley, K. L., Berry, A. J., and Suelter, C. H., J. biol. Chem. *242* (1967) 2502.
17 Chaney, A. L., and Marbach, E. P., Clin. Chem. *8* (1962) 130.
18 Bradford, M. M., Analyt. Biochem. *72* (1976) 248.
19 Endrenyi, L., Fajszi, C., and Kwong, F. H. F., Eur. J. Biochem. *51* (1975) 317.
20 Rahim, Z. H. A., Perret, D., and Griffith, J. R., FEBS Lett. *69* (1976) 203.
21 Zetterstrom, R., Clin. Biochem. *17* (1984) 57.
22 Kaletha, K., Stankiewicz, A., Makarewicz, W., and Zydowo, M., Int. J. Biochem. *7* (1976) 67.
23 Ogasawara, N., Goto, H., Yamada, Y., Watanabe, T., and Asano, T., Biochim. biophys. Acta *714* (1982) 298.

0014-4754/87/040440-04$1.50 + 0.20/0

Transdifferentiation of larval *Xenopus laevis* iris implanted into the amputated hindlimb[1]

C. Cioni, S. Filoni[2], L. Bosco, C. Aquila and S. Bernardini

Dipartimento di Biologia animale e dell'uomo, Università di Roma 'La Sapienza', via A. Borelli 50, I-00161 Roma (Italy), and Dipartimento di Biologia, Università di Roma 'Tor Vergata', Via Orazio Raimondo, I-00173 Roma (Italy), 9 July 1986

Summary. Fragments of larval *Xenopus laevis* iris, autoplastically implanted into the stump of the amputated hindlimb, transdifferentiated into neural retina. However, when such iris fragments were implanted into the caudal fin, no transdifferentiative process was observed.
Key words. Transdifferentiation; dorsal iris; larval *Xenopus*; amputated limb.

Lens regeneration from the outer cornea in larval *Xenopus laevis* depends on the presence of ocular factor(s), probably proteic in nature, coming from the neural retina[3-6]. However, it was demonstrated that the neural retina is not the only source of stimulus for transdifferentiation of corneal cells into lens, since promoting factors of lens-forming transformations of the outer cornea are also produced by several larval tissues. In particular, the outer cornea also responds to stimuli coming from regenerating limb, limb bud, limb blastema, tentacle blastema and spinal ganglia[5,7-9]. These data have been explained by assuming that a neurotrophic factor, produced by the ganglion cells and also by dedifferentiated cells of buds and blastema, is responsible for promoting the lens-forming transformations of the outer cornea[8,9].

In contrast to the outer cornea, iris epithelial cells of lentectomized *Xenopus laevis* larvae do not show any lens-forming transformation capacity even when this tissue has been injured in order to stimulate latent potentialities[10]. However, we recently demonstrated that iris fragments from *Xenopus laevis* larvae implanted in the vitreous chamber of lentectomized eyes can transdifferentiate into neural retina under the influence of promoting factors present in this eye territory[11]. Other investigations indicated that dorsal iris epithelial cells from adult frogs (*Rana temporaria*) show different transdifferentiative capacities under different experimental conditions[12]. The aim of the present work was to test the influence of the amputated hindlimb environment on transdifferentiative capacities of the dorsal iris from larval *Xenopus laevis*.

Materials and methods. A total of 55 *Xenopus laevis* larvae at stage 54-55 (according to Nieuwkoop and Faber[13]), anesthetized with MS 222 Sandoz at a concentration of 1:5000 in full strength Holtfreter's solution, were operated on. The animals were then gradually transferred into tap water and reared until the 20th day after the operation. The sacrificed animals were processed for O. M. (fixed in Bouin's solution, embedded in paraffin, cross-sectioned at 7 µm and stained with hematoxylin-eosin) or E. M. (fixed in 2% paraformaldehyde, 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4, postfixed in OsO_4, dehydrated through an ethanol series, stained 'en bloc' with uranyl acetate and embedded in Spurr's resin). Thin sections were stained with lead citrate and observed on AEI 801. Two types of experiments were carried out: Experiment 1: Autoplastic implant of dorsal iris into the amputated hindlimb (fig. 1a). Experiment 2: Autoplastic implant of dorsal iris into the caudal fin (fig. 1b).

Results and discussion. The results are shown in the table. The data obtained in experiment 1 show that dorsal iris fragments implanted into the limb stump transdifferentiate into neural retina in 52% of cases examined, while 19% of cases undergo only a partial depigmentation of iris epithelial cells. The newly-formed neural retina has a typical stratification, with clearly identifiable photoreceptors (figs 2, 3, 4).

Summary of the results of experiments 1 (autoplastic implant of dorsal iris into the amputated hindlimb) and 2 (autoplastic implant of dorsal iris into the caudal fin)

Experiment	N° of cases operated*	N° of cases dead/ discarded	N° of cases examined	N° of cases with partial transformation	N° of cases transdifferentiated into retina
1	30	9	21	4	11
2	25	3	22	–	–

* Moreover, in both experiments five additional larvae were operated on and fixed immediately after the operation to serve as controls of the operative procedure.

The data obtained in experiment 2 show that the dorsal iris fragments implanted into the caudal fin undergo various degrees of regression and a partial depigmentation.

Experientia 43 (1987), Birkhäuser Verlag, CH-4010 Basel/Switzerland

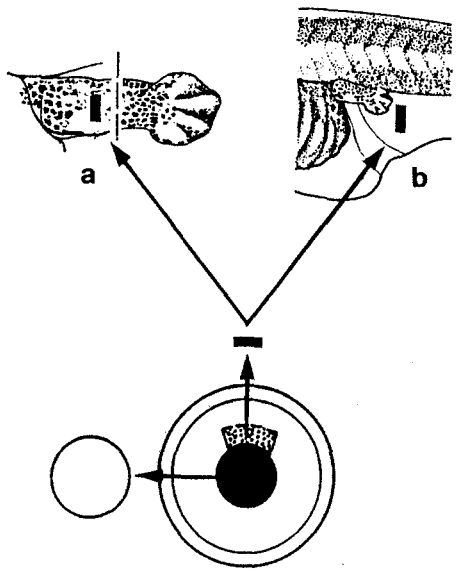

Figure 1. Diagram of operating technique. A fragment of the marginal ring of the dorsal iris was autoplastically implanted either into the surface amputation of a hindlimb amputated at knee level (a, experiment 1), or into the caudal fin (b, experiment 2). In order to avoid contamination of the iris by fragments of lens epithelium, the isolation of the iris was accomplished after lentectomy.

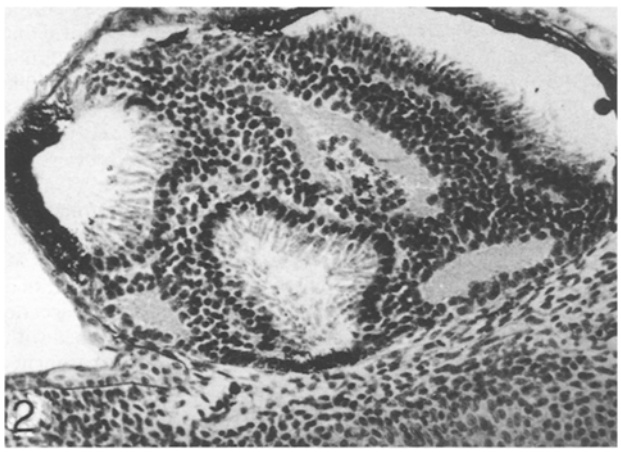

Figure 2. Transdifferentiation of the iris into neural retina 20 days after implantation into the amputated hindlimb. × 240.

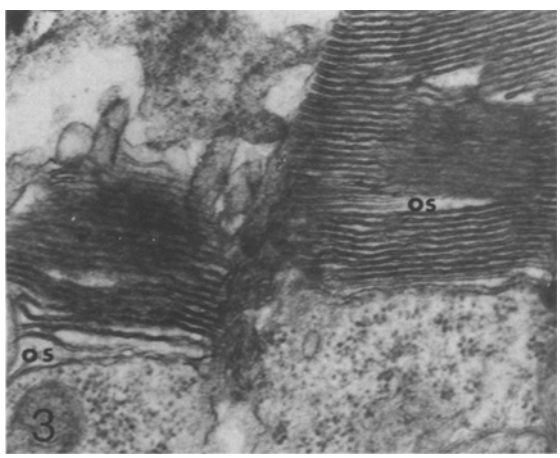

Figure 3. Outer segments of two retinal receptors surrounded by narrow processes, coming from the inner segments, are clearly seen. o.s.: outer segment. × 400,000.

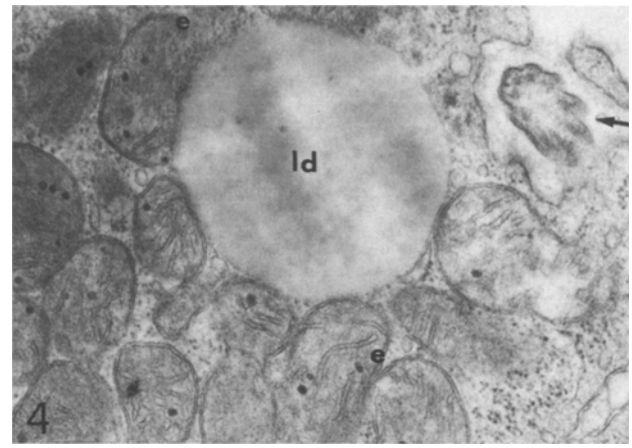

Figure 4. Cross section of a cone inner segment. The picture shows the lipid droplet in the ellipsoid and the eccentrically located connecting structure (arrow). l.d.: lipid droplet; e: ellipsoid. × 32,000.

The results of the present research suggest that the regenerating limb environment possesses some factor(s) capable of promoting the retinal transformation of the iris implants, thus replacing the action of the vitreous chamber. It is known that the regenerating limb environment can also replace that of the vitreous chamber in the process of lens-forming transformation of the outer cornea in larval *Xenopus laevis*[7].

These data may be explained by assuming that the two environments contain some factor(s) which are permissive in nature; that is, factor(s) capable of promoting successive events permitting the phenotypic expression of latent transdifferentiative capacities residing inside the reacting tissue. It seems probable that in the regenerating limb environment the factor(s) involved is the neurotrophic factor that plays a fundamental role in amphibian limb regeneration[14].

On the other hand, it could also be possible that the retina-forming transformation of the iris and the lens-forming transformation of the outer cornea are due to inductive action of two different factors instructive in nature; that is, factors capable of modifying the determination of the reacting tissue, probably operating changes in the genome. If this is so, we have to admit that both specific ocular factors present in the vitreous chamber are also present in the regenerating limb environment.

1 Supported by grant from the P.I. ministry.
2 Reprint requests to S.F., Dipartimento di Biologia, II Università di Roma 'Tor Vergata', Via Orazio Raimondo, I-00173 Rome (Italy).

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 Reeve, J.G., and Wild, A.E., J. Embryol. exp. Morph. *63* (1981) 121.
6 Filoni, S., Bosco, L. Cioni, C., and Venturini, G., Experientia *39* (1983) 315.
7 Waggoner, P.R., J. exp. Zool. *186* (1973) 97.
8 Filoni, S., Bosco, L., Cioni, C., and Burani, P., J. exp. Zool. *230* (1984) 409.
9 Bosco, L., Filoni, S., and Cioni, C., J. exp. Zool. *233* (1985) 221.
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 Hoperskaya, O.A., and Zviadadze, K.G., Devl. Growth Diff. *23* (1981) 201.
13 Nieuwkoop, P.D., and Faber, J., Normal table of *Xenopus laevis*. Daudin – North-Holland Publ., Amsterdam 1956.
14 Singer, M., in: Regeneration in Animals and Related Problems, p. 20. Eds W. Kiortsis and H.A.L. Trampush. North-Holland, Amsterdam 1965.

0014-4754/87/040443-02$1.50 + 0.20/0