

Egg pigment is accumulated in the tadpole's brain

L. Kordylewski^{1,2}

Department of Comparative Anatomy, Institute of Zoology, Jagiellonian University, Karasia 6, 30-060 Kraków (Poland), 28 March 1983

Summary. Four different crosses with wild-type and albino mutants of *Xenopus laevis* show 1. that the occurrence of the pigmented cell clusters in the tadpole's brain is independent of the presence of pigmentation of the body and 2. that there is a strict correlation between a pigmentation of the egg and the appearance of pigmented cells in the brain of the developing larva. The data strongly support the idea that the egg pigment appears in the brain later in development.

In a tadpole of *Xenopus* there are conspicuous darkly pigmented clusters of cells floating in the brain cavities. They are visible in a living animal when inspected with a stereomicroscope (fig. 1) and can also be examined on cut slices (fig. 2). They occur in other anuran species and seem to be common in the tadpoles³. Nevertheless, up to the present a reasonable explanation of their origin and functions has not yet been provided⁴. The supposition has been made that they are melanophores circulating in the cerebro-spinal fluid³⁻⁵ since they appear as early as the other melanophores in the developing tadpole, about stage 33/34⁶. Moreover, as in the melanophores, their content of melanin increases with age. Morphological data indicate, however, that the cells that build the clusters cannot be

melanophores⁷⁻¹⁰. It has been shown by electron microscopy that they contain exclusively spherical melanosomes of uniform size (0.6-1.1 μm), while the melanophores of the same animal are filled in addition with smaller, usually elongated, newly-formed pigment granules⁹. Spherical melanosomes are characteristic in the cytoplasm of the egg¹¹. It has been previously supposed⁷ that the egg melanin, which is distributed among the cells of the tadpole's body, is next discharged (mainly from the ectodermal tissues) into the external space^{12,13}. Since the deposits of free melanosomes in the enclosed neural tube lumen increase with age, the clusters under observation were supposed to be aggregates of scavenger cells that keep the cerebro-spinal fluid free of the excreted melanosomes^{7,8}.

This assumption has also been supported by experimental data^{10,12,13}, which indicate that the cells under observation maintain their pigmentation in the tadpoles in which the melanization had been blocked with phenylthiourea (PTU)^{14,15}. It has also been shown by electron microscopy that the pigmented clusters increase their content of melanin granules by engulfing the free melanosomes⁹.

The breeding experiment reported in the present paper strongly supports the idea that the clusters accumulate pigment of egg origin. 4 groups of tadpoles were under observation. Each contained about 300 individuals anesthetized with MS 222 and observed by stereomicroscope between the developmental stages 39 and 46. The tadpoles were obtained by crossing parental toads of various genotypes: wild-type, periodic albino mutants and their hybrids (fig.3). The pigmented clusters occurred exclusively in those tadpoles which were reared from pigmented eggs (fig.3, a and d), e.g. in the offspring of both wild-type parents ($\delta +/+ \times \delta +/+$). On the other hand they have never been observed in the homozygote periodic albino tadpoles (a^p/a^p) obtained by crossing both homozygote

parents of the same genotype ($\delta a^p/a^p \times \delta a^p/a^p$), which produced pigmentless eggs (fig.3, b). Similarly, no pigmented cells in the cerebro-spinal fluid were observed by Eppig and Dumont¹⁶ in the tadpoles of *Xenopus* reared from pigmentless eggs produced by inhibition of melanogenesis during oocyte maturation. What is more, the pigmented clusters never appeared in the present experiments within the brains of the heterozygote tadpoles which were reared from pigmentless eggs obtained from a wild-type father and homozygote periodic albino mother ($\delta +/+ \times \delta a^p/a^p$), although normal pigmentation of the body and typical melanophores developed in such heterozygote tadpoles ($a^p/+$) (fig.3, c). Furthermore, the backcross performed with homozygote periodic albino male and heterozygote darkly pigmented female ($\delta a^p/a^p \times \delta a^p/+$) resulted in obtaining all darkly pigmented eggs, half of

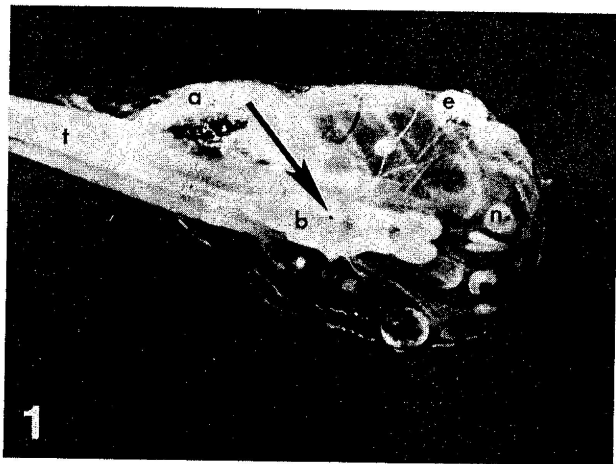


Figure 1. Albino tadpole at stage 51, displaying the presence of pigmented clusters in the brain ventricle (arrow). t, tail; a, abdomen; b, brain; e, eye; n, nostril.

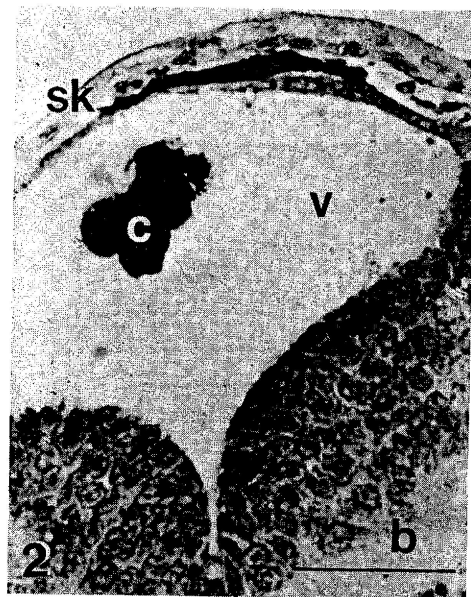


Figure 2. Cross-section of the brain of *Xenopus* tadpole at stage 42. Paraffin-wax slice stained with hematoxylin-eosin. Pigmented cluster (c) is visible within the lumen of the ventricle (v) of the brain (b). sk, skin of the head. Bar 50 μ m.

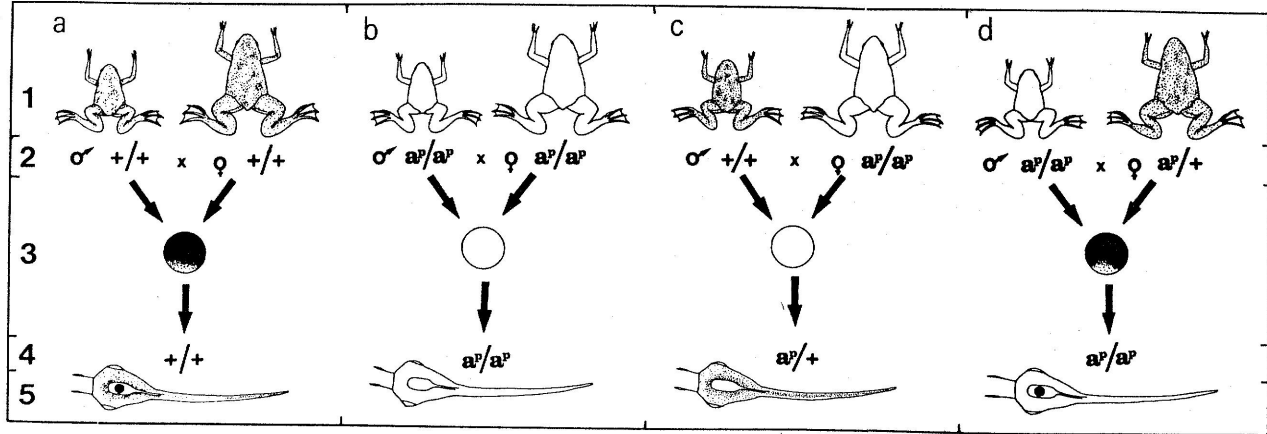


Figure 3. Schematic presentation of 4 groups of tadpoles used and the ways they were produced (a, b, c, d). 1 Parents' phenotypes, 2 parents' genotypes; 3 eggs; 4 tadpoles' genotypes; 5 tadpoles' phenotypes. Dark eggs were produced only by pigmented females (a and d), while pigmentless eggs originated exclusively from homozygote albino females (b and c). Black globes in the tadpoles' brains indicate the presence of the pigmented clusters (a and d). The occurrence of the clusters is independent of a pigmentation of the body, but there is a strict correlation between a pigmentation of the egg and the appearance of the pigmented cells in the larva's brain.

which (of a^P/a^P genotype) gave rise to non-pigmented tadpoles which contained pigmented clusters in their brains (fig. 3, d). No wild-type pigmentation occurred in those tadpoles other than that of egg origin.

Since presumably the same mechanism of egg pigment accumulation in the brain cavity occurs also in the wild-type tadpoles, it has been concluded that in all tadpoles in which the clusters occur, the clusters are composed of melanophages that accumulate the egg melanosomes.

- 1 Present address for reprint requests: Department of Medicine, University of Chicago, 950 East 59th Street, Box 137, Chicago, Illinois 60637, USA.
- 2 The author wishes to express his deep gratitude to Dr J.B. Gurdon (Cambridge) for kindly providing heterozygous females of *Xenopus*. The receipt of albino mutants from Dr K. Rzehak (Cracow) is gratefully acknowledged. The author warmly thanks Prof. H. Krzanowska (Cracow) for encouraging him to carry out the breeding experiments. The author is also grateful to Dr G. MacMillan (Aberdeen) and Dr O. Hoper-skaya (Moscow) for valuable comments and Dr D. Doyle (Chicago) for help in preparing the final draft of the manuscript. The author is also indebted to Polish Airlines LOT for

kindly transporting two living toads from London to Cracow. The paper has been partially supported by a grant-in-aid from The British Council.

- 3 Adam, H., Z. mikrosk.-anat. Forsch. 60 (1954) 6.
- 4 Deuchar, E.M., *Xenopus: The South African Clawed Frog*, p. 175. Wiley, New York 1975.
- 5 Komnick, H., Wilhelm Roux Arch. 153 (1961) 14.
- 6 Nieuwkoop, P.D., and Faber, J., Normal table of *Xenopus laevis*. North Holland Publ., Amsterdam 1967.
- 7 Kordylewski, L., Bull. Acad. Pol. Sci. 17 (1969) 347.
- 8 Kordylewski, L., J. Anat. 129 (1979) 862.
- 9 Kordylewski, L., J. Morph. 176 (1983) 315.
- 10 Kordylewski L., J. exp. Zool. 227 (1983) 93.
- 11 Noda, K., Nomaguchi, T., and Tanaka, Y., Cell Tissue Res. 185 (1977) 331.
- 12 Millott, N., and Lynn, W.G., Biol. Bull. 129 (1965) 562.
- 13 Millott, N., and Lynn, W.G., Nature 518 (1966) 99.
- 14 Sims, R.T., Q. Jl microsc. Sci. 102 (1961) 227.
- 15 Sims, R.T., Q. Jl microsc. Sci. 103 (1962) 439.
- 16 Eppig, J.J., and Dumont, J.N., J. exp. Zool. 177 (1971) 79.