Liau et al.[7] have demonstrated that retinol is transferred from its soluble receptor, cellular retinol-binding protein (cRBP) to an undescribed chromatin acceptor(s). This results in the release of apo-cRBP and retention of retinol by a nuclear acceptor(s). Our data here suggest that such a phenomenon also occurs with retinoic acid. In support of this, figure B shows that the pattern of Coomassie blue-binding components of nuclei not preincubated with cRABP exhibited only marginal shifts in the nA a–c Rf values. No 15.0 kd shift, which might be expected if [^{3}H]RA-cRABP were a nuclear complex component, was observed.

In another aspect of this experiment we questioned whether the preferential binding of the 3 nA bands resulted from cRABP aggregates. If this were the case, first, we would not expect to see correlative bands in figures A and B in the area of the nA a–c peptides. Second, the loss of radiofluorescence demonstrated in figure E would not have been apparent even in the presence of excess unlabeled RA-cRABP. Thus, we concluded that the nA a–c components were not cRABP aggregates.

Finally, we suggest that the nA peptides are unique to the nucleus. Figure C indicates that the Coomassie blue-binding in the nA a–c peptide area associated with the microsomal fraction is significantly less than that of the nuclear fraction suggesting that the nuclear peptides were not derived from contaminating endoplasmic reticulum (ER). Further, during isolation of the cellular fractions we did add a protease inhibitor and therefore it is our contention that nA a–c peptides are distinct and did not arise as fragments of high molecular weight ER or nuclear peptides. However, given the closeness of the Mr of the nA peptides it is possible that they are, in fact, the same protein which has been subjected to limited post-translational proteolysis or glycosylation, resulting in altered or multiple ligand specificity for RA.

Thus, we conclude that rat testes interstitial cell nuclei contain at least 3 forms of RA acceptor distinct from cRABP. These are apparently native constituents of the nuclei. However, it is not possible to draw any specific conclusions regarding the role of these peptides in mediating the nuclear activity of retinoic acid.

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3 Lotan, R., Biochim. biophys. Acta *605* (1980) 33.
4 De Luca, L., in: Vitamins and Hormones, vol. 35, p. 1. Academic Press, New York 1977.
5 Shidoji, Y., and De Luca, L. M., Biochem. J. *206* (1981) 529.
6 Takase, S., Ong, D. E., and Chytil, F., Proc. natl Acad. Sci. USA *76* (1979) 2204.
7 Liau, G., Ong, D. E., and Chytil, F., J. Cell Biol. *91* (1981) 63.
8 Mancino, V., Nasca, M., Bono, A., Leutskaya, Z. K., and Fais, D., IRCS Med. Sci. *8* (1980) 115.
9 Steinberger, A., in: Methods in Enzymology, vol. 39, p. 283. Academic Press, New York 1975.
10 Salhanick, A. L., and Terner, C., Biol. Reprod. *21* (1979) 293.
11 Fleisher, S., and Kervina, M., in: Methods in Enzymology, vol. 31, p. 6. Academic Press, New York 1974.
12 Bendetto, J. P., Martel, M. B., and Got, R., Biochim. biophys. Acta *587* (1979) 1.
13 Berthillier, G., Bendetto, J. P., and Got, R., Biochim. biophys. Acta *603* (1980) 245.
14 Dewald, B., Dulaney, J. T., and Touster, O., in: Methods in Enzymology, vol. 32, p. 82. Academic Press, New York 1974.

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Egg pigment is accumulated in the tadpole's brain

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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 tadpoles[3]. Nevertheless, up to the present a reasonable explanation of their origin and functions has not yet been provided[4]. The supposition has been made that they are melanophores circulating in the cerebro-spinal fluid[3-5] since they appear as early as the other melanophores in the developing tadpole, about stage 33/34[6]. 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 melanophores[7-10]. 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 granules[9]. Spherical melanosomes are characteristic in the cytoplasm of the egg[11]. It has been previously supposed[7] 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 melanosomes[9].

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 (♂ +/+ × ♀ +/+). 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 (♂ a^p/a^p × ♀ 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 Dumont[16] 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 (♂ +/+ × ♀ 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 (♂ a^p/a^p × ♀ $a^p/+$) resulted in obtaining all darkly pigmented eggs, half of

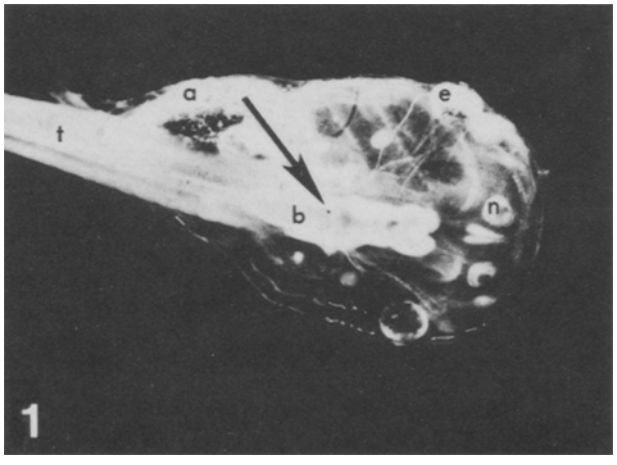

Figure 1. Albino tadpole at stage 51, displaying the presence of pigmented clusters in the brain ventricle (arrow). t, tail; a, abdomen; b, brain; e, eye; n, nostril.

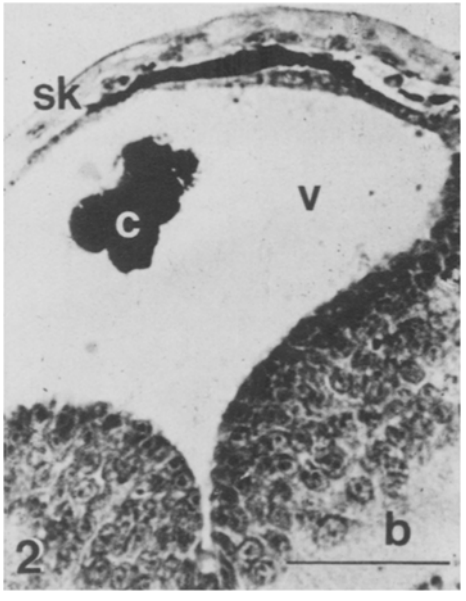

Figure 2. Cross-section of the brain of *Xenopus* tadpole at stage 42. Paraffin-wax slice stained with hematoxylin-eosin. Pigmented cluster (c) is visible within the lumen of the ventricle (v) of the brain (b). sk, skin of the head. Bar 50 μm.

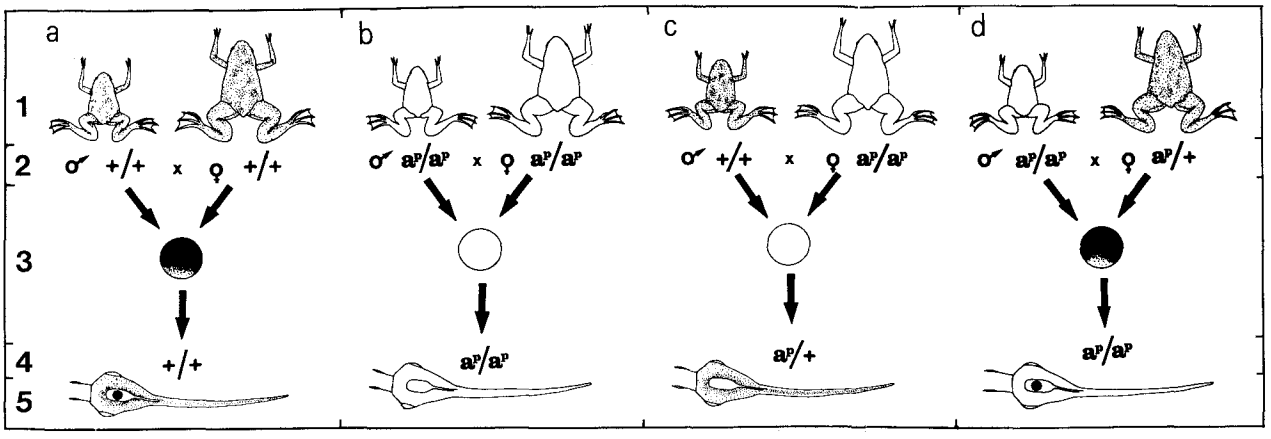

Figure 3. Schematic presentation of 4 groups of tadpoles used and the ways they were produced (a, b, c, d). *1* Parents' phenotypes, *2* parents' genotypes; *3* eggs; *4* tadpoles' genotypes; *5* tadpoles' phenotypes. Dark eggs were produced only by pigmented females (a and d), while pigmentless eggs 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.

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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.

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Electrophoretic heterogeneity exhibited by the S-allele specific glycoproteins of *Brassica*

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Summary. A number of self-incompatibility genotypes of *B. oleracea* were analyzed by sensitive electrophoretic procedures. Members of the class of S-allele specific glycoproteins that increase in correlation with the onset of the incompatibility response were resolved into several components on SDS gels. The implications of this molecular heterogeneity are discussed in relation to S locus function.

In Cruciferae, the pollen-stigma interaction of self-incompatibility is believed to be influenced, at least in stigma cells, by glycoproteins produced under the control of the S locus[2-4]. Elucidation of the structure of the S-specific glycoproteins is therefore crucial to the understanding of their role in cell-cell interactions at the stigma surface[5-7] and to a detailed molecular genetic analysis of the S locus. Our analysis of the aforementioned glycoproteins by iso-electric focusing (IEF) and by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) utilizing highly sensitive stains is reported here.
The homozygous self-incompatibility genotypes S_2, S_5, S_6, S_7, S_8, S_9, S_{11}, S_{13} and S_{14} of *Brassica oleracea* var. *acephala*, were obtained from the National Vegetable Research Station (NVRS) in Wellesbourne, U.K. (courtesy of Dr David Ockendon), and bear the station's allele designation. Genotypes S_2^c and S_{14}^c were isolated[8] at Cornell University by repeated inbreeding of homozygotes of *B. oleracea* var. *capitata*, and were shown by genetic analysis to correspond to the S_2 and S_{14} alleles of NVRS[9].
For analytical IEF, 25 flower buds were collected, often from a single plant, and their stigmas harvested. Pollen contamination was avoided by harvesting stigmas from buds at 1 day prior to anthesis; such stigmas are pollen-free and equivalent to flower stigmas in their incompatibility response. The harvested stigmas were frozen in liquid nitrogen, pulverized, dissolved in 50 µl of deionized water, and the homogenate centrifuged at 2 °C for 30 min at 10,000 × g. The resulting supernatant was assayed for total protein according to Bradford[10] with bovine gamma globulin as a standard (Bio-Rad Protein Assay, System I). Samples containing 200 µg of protein were loaded onto strips and focused for 2 h at 2 °C on pH 3.5–9.5 PAG plates (LKB). The plates were fixed and stained with fluorescein-labeled concanavalin A (Con A-FITC) according to Burridge[11]. Figure 1A shows a stained gel on which many Con A-binding bands are revealed, particularly in its acidic portion (pH 4–7). The bands that focus above pH 7.0 are well resolved at the concentration of stigmas used. Among these basic bands, some appear to be common to all genotypes albeit in varying concentrations. These common bands aside, the different S genotypes shown in figure 1A each exhibit a unique basic glycoprotein pattern, with one or more differential bands (white arrows). The S_{14}^c and S_{14} genotypes on the one hand, and the S_2^c and S_2 genotypes on the other, have very similar if not identical IEF patterns. This identity is explained by the fact that in each set, the 2 lines, which have different genetic backgrounds, nevertheless carry identical S alleles as explained above. This correlation of IEF pattern with S allele identity irrespective of genetic background, suggests the involvement of the S locus in the determination of these unique basic protein patterns. Moreover, the seemingly S-specific bands which can only be detected in the stigmatic and not in the stylar or anther tissues of the flower were functionally correlated to the self-incompatibility reaction in our analysis of stigmas at different stages of development. Immature buds at about 5 days prior to anthesis are self-compatible, and only