Incorporation of Exogenous (Phage) DNA into Amphibian Oocytes

DAWID[1,2] has identified cytoplasmic DNA in amphibian eggs as mitochondrial DNA. This, however, does not comprise all the cytoplasmic DNA one can find in amphibian eggs. There is an important acid-soluble fraction with unknown biological function and cytological origin[3,4]. Leaving aside the biosynthesis of mitochondrial DNA, one wonders whether amphibian egg cytoplasmic DNA is synthesized *de novo*. In insect eggs, the DNA found in the ooplasm comes partly from the nurse cells which are disintegrated and incorporated into the ooplasm during oogenesis[5-7]. A similar phenomenon has not been observed in amphibian eggs, where the follicle cells only form a thin layer of flat cells around the oocyte. On the other hand, the amphibian oocytes are equipped with microvilli connecting the egg cortex with the surrounding follicle cells[8,9]. This suggests that the ingestion of foreign macromolecules occurs by pinocytosis.

In order to investigate this possibility, we have treated *Triton* oocytes with ^{14}C-DNA (phage) *in vivo* and followed its incorporation by autoradiography. Phage DNA, randomly labelled with carbon-14, was given by Schwarz. The radioactive DNA had a specific activity of 1·34 μc./mg and appeared as slightly yellowish fibres. It was first dissolved in N/5 saline-citrate (0·15 molar sodium chloride, 0·015 molar sodium citrate, *p*H 7·4) by continuous stirring in the cold room. When the DNA was completely dissolved an excess of cold absolute alcohol was added to the solution which was left overnight in the cold room. The precipitated radioactive DNA was recovered by centrifugation and redissolved in physiological saline. A quantity equal to 5 μc. of labelled DNA was injected intraperitoneally into a female *Triton*. The animal was killed 24 h later. The ovaries were removed and prepared for autoradiography according to the procedure of Ficq[10].

Young oocytes, particularly before the onset of vitellogenesis, show considerable radioactivity. The site of incorporation is mainly limited to their germinal vesicles. Follicle cells are labelled randomly, whether they are associated with large or small oocytes. Radioactivity in the germinal vesicle can still be observed in mature oocytes reaching the end of vitellogenesis, but they are much less radioactive than the young oocytes.

A wash with 0·5 normal perchloric acid at 0° C removes a great part of the nuclear radioactivity. Treatment by DNase or RNase removes about half of the total radioactivity; but none of these treatments can remove it completely.

The incorporation of exogenous DNA by living cells has been reviewed by Ledoux[11]. Of particular interest are the results of Kay[12] and Meizel and Kay[13], who showed that ascites tumour cells in suspension can ingest homologous DNA without previous degradation. The ingested DNA becomes part of the nuclear chromatin. The incorporation that we observed could be more comprehensible if the phage DNA was subjected to degradation before it was incorporated into the oocytes. Denatured DNA is more extensively degraded into an acid-soluble state by the ascites tumour cell homogenates[14], or after it has been ingested into the host cell[15]. We do not know to what degree the phase DNA we used was denatured. We also ignored the activity of DNase in the lymphatic fluid or blood of amphibians. According to Bendich[16] tumour-inducing polyoma virus, after being injected intraperitoneally into mice, can be recovered in the blood in a biologically active form.

A similar experiment was performed by one of us (A. F.) some years ago. It was observed that phage DNA labelled with carbon-14 was incorporated into the germinal vesicle of amphibian oocytes and follicle cells. It was further noticed that after centrifugation of the whole oocyte, the nuclear radioactivity sedimented inside the germinal vesicle, indicating that the exogenous DNA was bound to the lampbrush chromosomes. Other work in this laboratory has shown that ^{3}H-DNA from *E. coli*, when micro-injected into one of the two first blastomeres of *Pleurodeles* embryo, can be incorporated into the nucleus and later distributed between the daughter nuclei (Sempinska, unpublished results). There is some biophysical evidence to indicate that part of the incorporated DNA remains in a high molecular form.

This work was supported by Euratom.

Y. C. KONG
A. FICQ

Laboratoire de Morphologie animale,
Université libre de Bruxelles.

[1] Dawid, I. B., *J. Mol. Biol.*, **12**, 581 (1965).
[2] Dawid, I. B., *Proc. U.S. Nat. Acad. Sci.*, **56**, 269 (1966).
[3] Haggis, A. J., *Dev. Biol.*, **10**, 558 (1964).
[4] Løvtrup, S., *Exp. Cell Res.*, **43**, 65 (1966).
[5] Durand, M., *Exp. Cell Res.*, **15**, 257 (1958).
[6] Anderson, E., *J. Cell Biol.*, **20**, 131 (1964).
[7] Eschenberg, K. M., and Dunlap, H. L., *J. Morphol.*, **118**, 297 (1966).
[8] Balinski, B. I., and Davies, R. J., *Acta Embryol. Morphol. Exp.*, **6**, 55 (1963).
[9] Van Gansen, P., *J. Embryol. Exp. Zool.*, **15**, 255 (1966).
[10] Ficq, A., *Arch. Biol.*, **66**, 509 (1955).
[11] Ledoux, L., in *Progress in Nucleic Acids Research*, **4** (Academic Press, 1965).
[12] Kay, E. R. M., *Nature*, **191**, 387 (1961).
[13] Meizel, S., and Kay, E. R. M., *Biochim. Biophys. Acta*, **103**, 399 (1965).
[14] Sung, S.-C., and Quastel, J. H., *Nature*, **200**, 781 (1963).
[15] Meizel, S., and Kay, E. R. M., *Biochim. Biophys. Acta*, **123**, 34 (1966).
[16] Bendich, A., *Science*, **148**, 374 (1965).

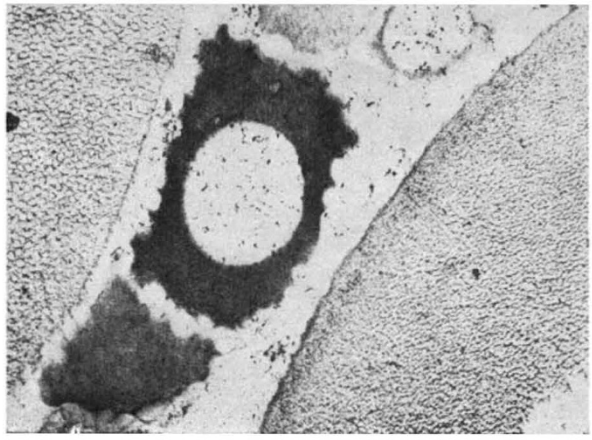

Fig. 1. Incorporation of ^{14}C-DNA (phage) into *Triton* oocytes.

Iodine Contents of Normal Human Eye Tissues

THE iodine content in normal human eye tissues is of interest not only because it gives knowledge of chemical parameters in the living organism but also because thyrotropic oculopathy is related to iodine metabolism. There have been no data available on the iodine content in the eye tissues, and we have therefore undertaken the present investigation.

Five post-mortem enucleated adult normal eyes were used as donors for corneal grafts for iodine analysis. The eye was dissected by a technique similar to that of Azevedo and De Jorge[1]. After determination of the fresh weight, the tissues were dried overnight in an electric oven at 95° C, and thus the dry weight was determined. The total iodine content of the tissues was determined by the method of Bird and Jackson[2]. The results for fresh and dry tissues (μg of iodine/100 g of tissues) are summarized in Table 1.

The iodine content of the scleral tissues is very small and that of the uvea is the greatest of all the ocular tissues. The value found in the aqueous humour corresponds to