ARE DNases OF AVIAN OOCYTES ABLE TO DESTROY SPERM AT POLYSPERMIC FERTILIZATION?

U. Stepinska and B. Olszanska

Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec n/Warsaw, 05-552 Wolka Kosowska, Poland

INTRODUCTION
Fertilization in birds is polyspermic, i.e. numerous sperm enter the cytoplasm of an ovum (Fovanova, 1965; Perry, 1987; Waddington et al., 1998), in contrast to the situation in mammals, where natural fertilization is monospermic (Yanagimachi, 1994). Nevertheless, in birds only one of the sperm - the one located in the centre of germinal disc - participates in formation of a zygote nucleus, whereas the supernumerary sperm disperse towards the periphery of the germinal disc and degenerate at the early cleavage stages (Perry, 1987; Waddington et al., 1998). Besides, exogenous DNA injected into germinal disc of the fertilized chick ovum initially persisted episomally, then the majority was gradually degraded during early embryogenesis, (Perry et al., 1991; Sang and Perry, 1989), what may contribute to the poor efficiency in obtaining transgenic birds (Love et al., 1994; Naito et al., 1994). The above data led us to suppose that avian oocytes should contain nuclease(s) responsible for degradation of excess DNA in the early embryos.

The purpose of the study was to establish whether quail oocytes contain DNases, to characterize them and to estimate the level of their activity during oogenesis.

MATERIAL AND METHODS
Biological material. The material consisted of Japanese quail oocytes, free from granulosa cells, at different stages of oogenesis. The entire previtellogenic (1-2 mm), small vitellogenic (3-4 mm) and the germinal discs of the largest vitellogenic (preovulatory) oocytes were used for preparation of oocyte extracts, according to Takeshita et al. (1994). Additionally, superovulated mouse oocytes were used for comparison.

Assay for DNase I and DNase II activities. The DNase activity of the oocytes was assayed in vitro by digestion of substrate DNA (naked λDNA/Hind III or quail sperm) with the oocyte extracts and visualization of the degradation products by electrophoresis on 0.7% agarose gel stained with ethidium bromide. Undigested λDNA/Hind III produced on electrophoresis six sharp bands, whereas after incubation with the oocyte extract the bands were degraded and formed smear or disappeared completely from the gel. The incubation conditions (pH, presence of Mg2+ or EDTA) were chosen to reveal the presence of DNase I or DNase II activities, separately (Ausubel et al., 1992; Takeshita et al., 1994). The incubation mixture also contained DNase-free RNase A, to remove RNA contained in the oocyte material. In the control reaction, the substrate was incubated with bovine serum albumin (BSA) instead of oocyte extract.
RESULTS

Characteristic of DNase activities in quail oocytes. The results show the existence of high DNase activity in the germinal discs of the preovulatory quail oocytes (Stepinska and Olszanska, 2001). This activity represents both DNase I-like and DNase II-like enzymes, as the substrate DNA was digested by the oocyte extract both in the conditions optimal for DNase I (neutral pH, presence of Mg²⁺) or for DNase II (acidic pH, presence of EDTA) (Fig. 1). In both cases degradation of the substrate depended on the protein concentration of the oocyte material. We did not observe the substrate degradation by the ovulated mouse oocytes under the conditions optimal for DNase I nor DNase II (Fig. 1).

![Figure 1. DNase I and II activities in the quail and mouse oocytes. Electrophoretic pattern of λDNA/Hind III incubated with oocyte extracts (50 µg protein/ml) under the conditions optimal for DNase I (a-c) or for DNase II (d-f). Lanes a,d, control (DNA + BSA); lanes b,e, DNA + germinal discs from the preovulatory quail oocytes; lanes c,f, DNA + the ovulated mouse oocytes.](image)

Estimation of DNase I and II activities in quail oocytes. Comparing the electrophoretic pattern of λDNA/Hind III degradation products after incubation with oocyte material to that obtained after commercial DNase I or DNase II treatment, we could roughly estimate the enzyme activities present in a germinal disc. The activities were calculated approximately as ~3x10⁻³ KU (Kunitz Units)/germinal disc for DNase I (~0.08 KU/mg of protein) and ~4x10⁻² KU/germinal disc for DNase II (~0.08 KU/mg of protein). Thus DNase II activity seems to be at least 10 times higher than that of DNase I.

Degradation of quail sperm DNA by oocyte extracts. Our results with quail sperm as a substrate show that the germinal disc extracts from the largest vitellogenic oocytes were able to digest not only naked DNA (λDNA/Hind III) but also DNA contained in quail spermatozoa, both in the conditions optimal for DNase I as for DNase II.

Comparison of DNase I and II activities during oogenesis. To establish the level of DNase I and II during oogenesis, we compared the degradation of λDNA/Hind III substrate by extracts...
DISCUSSION
The results of our in vitro studies showed, in the oocytes of Japanese quail, the existence of DNase I and DNase II activities able to digest naked DNA (λDNA/Hind III) and DNA contained in quail spermatozoa. Both these DNase activities accumulate during oogenesis and are the highest in the germinal disc of the largest vitellogenic oocytes. The opposite situation was observed in rat oocytes, where Boone and Tsang (1997) showed, by an immunohistochemical method, the presence of DNase I in the oocytes of preantral follicles, whereas staining of the oocytes in antral follicles was never seen. We did not also observe the substrate degradation by the extracts from the ovulated mouse oocytes, under the conditions optimal for DNase I nor for DNase II. The absence of DNase activity in healthy mature oocytes of mouse and rat, which are monospermic animals, seems to be reasonable, as the presence of the enzyme would be needless or even harmful, while in quail oocytes the enzyme may be needed for degradation of supernumerary sperm soon after fertilization. Most animals exhibit monospermic fertilization, which is ensured by the block to polyspermy operating about the time of sperm - egg fusion (Jaffe and Gould, 1985 ; Yanagimachi, 1994). The lack of such a block preventing multiple sperm penetration into the avian ovum, in the course of natural polyspermic fertilization, might be compensated by the high DNase activities degrading DNA from supernumerary sperm nuclei in the cytoplasm after fertilization had occurred. However, the question remains open why there are two DNases present in avian oocyte and which one is active in vivo after fertilization? This could possibly be regulated by pH and/or the availability of divalent cations in the sperm environment after entering the egg. Another question is why all entering sperm are not destroyed by the DNases present in the avian oocyte cytoplasm but, somehow, a single one is protected and, together with a female pronucleus, forms a zygote nucleus. The mechanism of this protection is still obscure; it might depend on localization of the sperm in the germinal disc, on the presence of some specific DNase inhibitors, or on the intrinsic property of the spermatozoon. The presence of DNases in the avian germinal discs could also explain the loss, during early embryonic development, of exogenous DNA introduced into the cytoplasm of fertilized ovum (Perry et al., 1991 ; Sang and Perry, 1989) and the low efficiency of transgenesis by the method of DNA microinjection into a germinal disc (Love et al., 1994 ; Naito et al., 1994).

CONCLUSION
The presence of high DNase I and II activities was detected in the germinal discs of the largest quail vitellogenic oocytes, what could explain degradation of DNA from accessory sperm entering an avian egg at polyspermic fertilization. Thus the enzymes could be a factor of the late block to polyspermy in the cytoplasm of the avian eggs. It is also suggested that the DNase activities might be responsible for poor efficiency in obtaining transgenic birds by microinjection of exogenous DNA into the fertilized chick ovum.
ACKNOWLEDGEMENT
This work was supported by The Committee for Scientific Researches grant PO6D 012 18.

REFERENCES