Premeiosis and premeiotic DNA synthesis in the left ovary of the female chick embryo

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INTRODUCTION

Premeiotic DNA synthesis in the germ cells of the female mouse embryo has been studied by Peters, Levy & Crone (1962) and Crone, Levy & Peters (1965). In this species, with a close synchronization in germinal development, the process of oogenesis which is the period of multiplication by mitotic divisions of the germ cells (oogonia) seems to be followed rapidly (within 24 h) by the first step of the prophase of meiosis (see Borum, 1961).

In previous work (Callebaut & Dubois, 1965; Callebaut, 1967) we have investigated DNA synthesis in the ovarian germ cells of the chick embryo, both in vitro and in vivo, by autoradiography following the incorporation of tritiated thymidine.

In a new series of experiments with a modified technique, it has been possible to demonstrate that the nuclei of the germ cells in premeiotic S phase in the female chick embryo have a distinctive structure.

MATERIALS AND METHODS

Treatment with tritiated thymidine

Fertile eggs from White Leghorn chickens were incubated for 10 or 14 to 18 days at 39 °C. Through a hole in the shell over the air space, 100 μl of 3H-thymidine (3–6 c/mm) in 100 μl distilled water were placed on the air space membrane of the egg orientated in an upright position and left in this position for 1 h. After such a 1 h pulse the left ovary was removed from some embryos and fixed. In the remaining eggs the air space membrane was repeatedly rinsed with a Hanks balanced salt solution at 38 °C, in order to wash out the remaining tritiated thymidine.

The eggs were then incubated again and the embryos allowed to grow for a further 10 or 24 h. At that time the left ovary was dissected out and fixed.

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Histological and autoradiographic procedures

The ovaries (thirty-six in all), were fixed in acetic alcohol (1:3) at 4 °C for 18 h, embedded in paraffin and sectioned at 5 μ thickness. After removal of the paraffin wax the sections were rehydrated and stained using the Feulgen reaction, fast green being used as counterstain.

The sections were then mounted on coverslips. Photographs or drawings of the cortex were made with special attention to nuclear details. The coordinates of the photographed areas were noted. After 24 h in toluene the coverslips were removed, and following rehydration of the sections and without preliminary drying the slides were immersed in diluted nuclear emulsion (L 4, Ilford) according to Caro & Van Tubergen (1962).

The exposure time of the slides in the dark was about 3–4 weeks at 4 °C. After photographic processing the autoradiographs were stained with Croat's iron haematoxylin and fast green. The previously photographed microscopic fields were observed or photographed using an oil-immersion objective.

By comparing the observations made before and after autoradiography, it is possible to identify the nuclear details of the germ cells which have incorporated 3H-thymidine. The population of the ovarian germ cells of the chick embryo is very heterogeneous during this period. Furthermore, during a 2-day period an oocyte can undergo several stages of premeiosis or meiosis. Since each of these stages differs only slightly from the previous one, a clear picture with as many caryological details as possible is an absolute need.

Figs. 1 and 2 clearly illustrate the differences in cytological detail seen between the pre- and post-autoradiographic histology. The presence of the gelatin coat of the nuclear emulsion over the specimen and probably also the action of the developer or fixer on the histological structures interfere with the staining of the autoradiograph.

In many labelled nuclei no nuclear constituents are discernible because they are hidden by numerous silver grains. Fading of the original Feulgen stain mainly due to photographic processing makes it necessary to use iron haematoxylin as a second nuclear stain. A second counterstaining with fast green after photographic processing is necessary as the first counterstain has completely disappeared.

RESULTS

The ovarian cortex of the 10-day-old embryo contains numerous dividing oogonia and also oogonia at interphase (see Plate 1, fig. 8). The nucleus of the latter usually presents a large nucleolus and two large masses of chromatin. Granules of chromatin are studded along the inner face of the nuclear membrane and along the threads of a fine trabecular network that extends throughout the nucleus (D'Hollander, 1904). At this age about 40 % of the oogonia at interphase show nuclear labelling on the autoradiographs after a 3H-thymidine 1 h pulse in ovo.
Premeiosis in the chick

By pre-autoradiographic cytological examination of the central part of the ovary (14 days and older) we can distinguish the following types of germ cells:

**Type 1.** Cells with a reticulated (resting) nucleus. In contrast to the nucleus of oogonia at interphase, the nucleus looks like an empty vesicle except for a central nucleolus. Thin chromatin granules are studded against this nucleolus, along the inner face of the nuclear membrane and along the threads of a delicate network extending throughout the nucleus (Romanoff, 1960). The nuclear membrane is very thin but sharply defined (Plate 1, figs. 1, 6). These cells were described by D'Hollander (1904) in the 14-day embryo as more regular and fine in structure than the oogonia.

**Type 2.** Cells with less numerous but much bigger Feulgen positive chromatin masses scattered throughout the nucleus or distributed at the periphery of its surface. Usually they are connected by some thin threads (Feulgen negative) to a large central or subcentral nucleolus. There is often a paranucleolar chromatin granule visible. The nuclear membrane is absent or fragmentary (Plate 1, fig. 1).

**Type 3.** Cells with still bigger chromatin clumps and numerous very fine threads orientated as an aureole around and close to the large central nucleolus. This aureolar mass is separated from the cytoplasm by an empty halo. The nuclear membrane is absent. D'Hollander also described this type of cell in the 15-day embryo (Plate 1, fig. 1).

**Germ cells in leptotene stage of meiosis.** The very fine chromosomes form

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**Plate 1**

Fig. 1. Left ovary of a 15-day-old chicken embryo, 1 h after a tritiated thymidine pulse. Lower arrow: germ cell with reticulated nucleus, thin nuclear membrane clearly visible. Left arrows: more advanced stages of germ cells (type 2). Right arrow: germ cell of type 3 (aureolar cell). Upper arrow: germ cell in leptotene stage of meiosis. × 630.

Fig. 2. Autoradiograph of the same section as fig. 1; only the nuclei at the stage indicated by the right and left arrows (of fig. 1) incorporate ³H-thymidine. × 630.

Fig. 3. Autoradiograph of a section through the cortex of a 17-day embryo, 10 h after a ³H-thymidine pulse (nuclear details were obtained from a drawing before autoradiography). Arrows from left to right indicate cells of type 2 (not or feebly labelled). Arrows from right to left indicate cells of type 3 with labelling chiefly localized on the chromatin masses. Vertical arrow indicates a group of four leptotene oocytes heavily labelled. At the right of the picture, unlabelled cells in leptotene. × 630.

Fig. 4. Section through the cortex ovarii of a 17-day-old chicken embryo, 24 h after a tritiated thymidine pulse. Upper arrow indicates a group of three leptotene oocytes. Lower arrow indicates two zygotene oocytes. × 630.

Fig. 5. Autoradiograph of the same section as fig. 4. The leptotene and zygotene oocytes are labelled. × 630.

Fig. 6. Section through the same ovary as fig. 4. Note the two reticulated germ cells at the left. Several leptotene oocytes are visible. × 630.

Fig. 7. Autoradiograph of the same section as fig. 6. Reticulated nuclei are not labelled; the labelling over several leptotene oocytes can be seen. × 630.

Fig. 8. Oogonium at interphase (14 days incubation). × 700.
a tangled mass distributed throughout the nucleus or are partially clumped to the side neighbouring the greater part of the cytoplasm (which contains the centrosoma and mitochondrial cloud). In this region parallel parts of the leptotene threads are obvious (Plate 1, figs. 1, 4, 6).

These threads often have thickenings (probably telomeres) near their extremities. The part of the nuclear surface lying nearest to the narrow rim of cytoplasm generally looks empty. In the network of chromosomes, irregular masses are sometimes visible, probably due to visual superposition of crossing threads or to gathering by synizesis. The nucleolus is absent or very reduced in size. Germ cells in the leptotene stage generally appear in the 15-day-old embryo.

**Germ cells in zygotene stage.** At this stage, the chromosomes are thicker and weakly coiled. Large parts of them are clearly and individually visible and often show polarization (‘bouquet’ formation). The nucleolus has entirely disappeared (Plate 1, fig. 4).

In 15-day-old embryos and in older ones oogonial divisions can only be seen at the extremities of the cortex.

By combination of both caryological and autoradiographic examination we found in 15- to 18-day-old ovaries:

1. When they are treated with a 1 h pulse of tritiated thymidine *in ovo*, and fixed immediately: germ cells of type 1 are not labelled, meiocytes in leptotene or zygotene are not labelled, only germ cells of type 2 and 3 are labelled (compare Plate 1, figs. 1 and 2).

2. When they are treated *in ovo* with a \( ^3 \text{H} \)-thymidine pulse of 1 h, and fixed 10 h later: no germ cells of type 1 are labelled, germ cells of type 2 show at most a very faint labelling (0–5 grains per nucleus), germ cells of type 3 and a few oocytes in leptotene are labelled (10–30 grains or more per nucleus), some germ cells undergoing degeneration are slightly labelled (see Plate 1, fig. 3).

3. When they are treated as in (2), but fixed 24 h later: practically all labelled germ cells are in meiotic prophase—leptotene or zygotene (compare Plate 1, figs. 4 and 5 and figs. 6 and 7).

At the extremities of the cortex some labelled cells of type 1 can be found. The radioactivity is probably derived from their oogonial mother cell.

The present study clearly demonstrates that germ cells of types 2 and 3 are the direct precursors of the leptotene stage cells and also that almost all the germ cells in the central part of the cortex of 15-, 16-, 17- or 18-day embryos which incorporate \( ^3 \text{H} \)-thymidine are in their premeiotic S phase. These cells with their localized accumulations of DNA probably correspond to the pro-chromosome stage (Rhoades, 1961).
The incorporation of $^3$H-thymidine in germ cells during premeiosis was originally found (Callebaut & Dubois, 1965) by cultivating pieces of ovaries of chick embryos at different ages, on agar jelly, according to the technique of Wolff & Haffen (1952). The percentage of germ cells incorporating $^3$H-thymidine after the multiplication phase was found to be minimal in the 14-day embryo and maximal at 17 days. Intermediate values were found at 15 and 16 days. These results could be confirmed in vivo by 1 h tritiated thymidine pulses in ovo.

The importance and the extent of the whole phenomenon was demonstrated by successive tritiated thymidine pulses in ovo at 9 h intervals from 15 to 17 days (Callebaut, 1967). By this procedure it was possible to label 90–95 % of the 'stock' of germ cells in prophase of meiosis. This percentage of labelled meiocytes persists for a long period in the young pullet.

The problem of the degeneration of germ cells during this period has been examined by Hughes (1963), who described such cells as characterized by clumping of the chromatin within the nucleus and in advanced stages by wrinkling of the nuclear membrane. The less-advanced, presumed-degenerating oocytes seem to have some histological resemblance to the germ cells of type 2 or 3. By simple static histological observations it may be difficult or impossible to determine the biochemical behaviour of the development of a particular cell type (see Franchi, Mandl & Zuckerman, 1962).

**SUMMARY**

Cytological changes occurring during premeiosis in the germ cells of the ovarian cortex of the chick embryo were studied by means of Feulgen staining (specific for DNA).

The evolution of the preleptotene stages of the germ cells after $^3$H-thymidine pulses was investigated by the combination of pre- and post-autoradiographic observations and photographs.

The germ cells in premeiotic S phase present a particular structure, different from the structure found in germ cells during premitotic S phase. They are the direct precursors of the meiocytes in leptotene stage.

**RÉSUMÉ**

*La préméiose et la synthèse du DNA préméiotique dans les cellules germinales de l'ovaire gauche de l'embryon de poulet*

Les modifications cytologiques qui se déroulent pendant la préméiose des cellules germinales de l'ovaire gauche de l'embryon de poulet ont été étudiées au moyen de la réaction nucléale de Feulgen (spécifique du DNA).

L'évolution des cellules germinales au préleptotènène a été suivie après des
pulses de $^3$H-thymidine grâce à des observations et des documents photographiques réalisés avant et après le traitement autoradiographique. Les cellules germinales en phase S préméiotique présentent une structure particulière, différente de celle des cellules germinales en phase S prémítotique. Elles sont les précurseurs immédiats des cellules germinales au stade leptotène.

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REFERENCES


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