RNA SYNTHESIS IN PREOVULATORY MOUSE OOCYTES

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Previous biochemical studies have demonstrated that at least part of the RNA stored in ovulated mouse oocytes is synthesized during their growth phase (1). Those studies, however, failed to detect incorporation of RNA precursors during the 4-day preovulatory period, which includes part of the 5-day growth-quiescent phase (12), germinal vesicle breakdown (GVBD), chromosomal condensation, and the first meiotic division. As previously suggested (16), RNA synthesized just before GVBD could include messenger RNAs coding for proteins involved in those events of maturation, as distinct from ribosomal and messenger RNAs to be stored for use in early embryogenesis. The studies reported here, therefore, were designed to determine whether RNA synthesis in the 4-day preovulatory period could be detected by the use of more sensitive techniques and, further, whether the time of cessation of RNA synthesis in relation to GVBD could be precisely defined. The possibility that RNA, synthesized by either growing or growth-quiescent oocytes, is retained on the condensed post-GVBD chromosomes as an alternative to cytoplasmic storage was also examined.

MATERIALS AND METHODS

Maturation and ovulation of oocytes were induced by intraperitoneal injection of 7.5 IU of a follicle-stimulating hormone, pregnant mare’s serum gonadotrophin (PMS), followed 44–48 h later by 7.5 IU of a luteinizing hormone, human chorionic gonadotrophin (HCG). [5,6-3H]Uridine (sp act 40–49 Ci/mmol) was administered to 24–26-day old ICR mice as a single injection into each ovarian bursa, as previously described (1).

For Series I, the dose of tritiated uridine was 40 μCi per ovary, and was administered 1–7 days before collection of oocytes from both ovaries and oviducts of 20 mice. All collections were made 18–22 h after HCG. All ovulated oocytes and those ovarian oocytes without germinal vesicles (ascertained by observation of the intact oocyte at × 50) were treated by a hypotonic air-drying method for meiotic chromosome spreads in which the ooplasm is dispersed (13).

In Series II, ovarian oocytes were collected from one group of six mice at 30 min after HCG and from another group of seven mice at 4 h after HCG, to obtain, for each labeling interval, a spectrum of oocyte stages, including late dictyate as well as the various phases of meiosis from diakinesis to metaphase II. In each of the two groups, the [3H]uridine was administered at a series of intervals before collection (Table II). The dose at the 24–29-h
interval was, as in Series I, 40 μCi per ovary. For the intervals of 0.5–8 h, the dose was increased to 100 μCi to compensate for the shorter periods of availability of precursor. Oocytes without germinal vesicles (diakinesis-metaphase II) were treated like those of Series I. Oocytes with germinal vesicles (dictyate) were fixed on slides without being subjected to hypotonic treatment or dispersion of the ooplasm.

All preparations were flooded with fixative (ethanol-acetic acid, 3:1), stained for 0.5 h with aceto-orcein (2% orcein in 40% acetic acid), and photographed. Autoradiography was carried out by standard techniques (15), using Kodak NTB-2 emulsion, and the preparations were again photographed. The fixation and subsequent processing steps were adequate to remove unincorporated acid-soluble precursor (8).

RESULTS
Series I

These experiments were designed to determine whether, during the last 7 days before ovulation, large oocytes incorporate [5,6-3H]uridine into RNA which is retained either on the chromosomes or in the cytoplasm until ovulation. Both preovulatory and ovulated oocytes were collected at various times after intrabursal injection of tritiated uridine, and autoradiographs were prepared. In no instance was significant label above that of background found concentrated on the chromosomes from the time they were recognizable as diakinesis figures in either this or the subsequent series described below (Figs. 1, 2, 4, and 5).

Whereas the oocyte population of the ovary includes all stages from early dictyate to metaphase II, those of the oviduct are a homogeneous and synchronized population at metaphase II. Therefore, the 174 ovulated oocytes of this series were analyzed with respect to the relationship between the interval from administration of [3H]uridine to collection and the incidence and density of ooplasmic label. Table I shows that the fraction of those oocytes in which the ooplasm was significantly labeled and the average grain density of ooplasmic label (Figs. 1, 2, 4, and 5). The metaphase II ovarian oocytes of the 24-29-h interval (Table II) are similar, with respect to their stage at the time of incorporation of label, to the ovulated oocytes of the 2-day interval (Table I).

Series II: Post-GVBD

These experiments were designed to define more narrowly the time within the last 2 days before ovulation at which incorporation of [3H]uridine could be detected. The terminal intraovarian period is that in which GVBD, chromosomal condensation, and the meiotic events from diakinesis to metaphase II take place (7). Therefore, ovarian oocytes representative of that period (no germinal vesicle) and those representative of late dictyate were selected for each labeling interval (Table II). Autoradiographs of ovarian oocytes at various stages of meiosis demonstrated, again, that the condensed meiotic chromosomes are not significantly labeled above background (Figs. 4, 5), although ooplasmic label was detected at short labeling times (Figs. 4, 5). The fraction of oocytes with labeled ooplasm at each stage of meiosis is shown in Table II. Oocytes at diakinesis that were collected 30 min after injection of [3H]uridine were not labeled, but those collected after 2 h or more were significantly labeled (Fig. 4). Ooplasms of metaphase II preparations were labeled after 8 h or more (Fig. 5). Those observations on post GVBD oocytes demonstrate, by extrapolation from the timetable of intraovarian post-GVBD events (7), that RNA synthesis continues up to within the last 2 h of the dictyate stage.

Series II: Pre-GVBD

The dictyate oocytes collected from these ovaries were considered to be close to the time of GVBD since they were obtained from mature follicles, the follicular cells were dispersed, and the zonae pellucidae were large and clearly visible (Fig. 6). Labeled oocytes were seen at all time intervals (Table II). At 0.5 h, only the germinal vesicle (nucleolus and nucleoplasm) was significantly labeled in 75% of the oocytes (e.g., Fig. 6). The metaphase II ovarian oocytes of the 24-29-h interval (Table II) are similar, with respect to their stage at the time of incorporation of label, to the ovulated oocytes of the 2-day interval (Table I).
Figure 1. Ovarian oocyte at diakinesis collected 7 days after intrabursal injection of [3H]uridine. Ooplasm is heavily labeled and dispersed beyond the limits of the micrographic field. The grain count over a unit area containing the chromosomes is equal to or less than the average of six grain counts measured over unit areas of ooplasm alone. × 1,000.

Figure 2. (a) Ovulated oocyte collected 4 days after intrabursal injection of [3H]uridine. Ooplasm is unevenly dispersed and labeled (arrows at limits of ooplasm). The grain count over the metaphase II chromosomes of either (b) oocyte or (c) polar body is below that of the surrounding region of ooplasm. (a) × 200; (b, c) × 1,000.
### Table I

| Days between injection of precursor and collection of oocytes | Total number | - | ± | + | ++ |
|-------------------------------------------------------------|--------------|---|---|---|----|
| 7                                                           | 33           | 5 | 0 | 4 | 24 |
| 4                                                           | 51           | 23| 4 | 8 | 16 |
| 2                                                           | 34           | 17| 7 | 8 | 2  |
| 1                                                           | 56           | 56| 0 | 0 | 0  |

All mice received 40 μCi of precursor per ovary.

± Represents estimated grain count of three to four times over background.

+ Represents estimated grain count of five to seven times over background.

++ Represents label too dense for grains to be counted.

At progressively longer intervals the proportion of oocytes with label over both nucleus and cytoplasm and the density of cytoplasmic label increased (Table I, Fig. 7). The nucleus remained significantly labeled even at 1 day after exposure to [3H]uridine. These observations on dictyate oocytes demonstrate directly that nuclear synthesis of RNA takes place in the terminal period of the dictyate stage.

**Discussion**

Although RNA synthesis in growing oocytes has been amply demonstrated (2, 10, 11), these experiments show that, in addition, nuclear RNA synthesis in mouse oocytes in vivo continues throughout the period between cessation of growth and GVBD. This has been demonstrated by two lines of evidence: first, the relationship between the time of precursor injection and the incorporation observed in ovulated and ovarian oocytes at various stages of meiosis after GVBD; and second, the direct observation of the incorporation of [5,6-3H]uridine by large dictyate oocytes from large follicles.

The analysis of the number of significantly labeled ovulated oocytes collected at various times after [3H]uridine injection (Table I) indicates that incorporation takes place at least until 2 days before ovulation, a period that is well within the growth-quiescent stage (12), and suggests that the level of incorporation into stable RNA decreases as ovulation approaches. Those data are consonant with the reported decline in incorporation into 1-h pulse-labeled RNA by growth-quiescent oocytes in progressively more mature follicles (10).

To more precisely define the time of cessation of detectable incorporation of precursor into RNA, oocytes were collected directly from hormone-stimulated ovaries at short times after exposure to a higher dose of [3H]uridine. The results recorded in Table II show a progressive appearance of labeled ooplasm at successive stages of meiosis as the interval between injection of [3H]uridine and collection was increased. The stage of the oocyte at the time of precursor injection could be extrapolated from the observed stage at collection. The time from GVBD to the appearance of diakinesis figures in mouse oocytes has been estimated as 0.5-2 h, and to the appearance of metaphase II complements as approximately 8 h (7). Therefore, the observation of ooplasmic label in oocytes at diakinesis at 2 h after injection (Fig. 4) and in oocytes at metaphase II at 8 h (Fig. 5) permits the conclusion that those oocytes were close to GVBD at the time of injection and that incorporation took place within the final 2-h period of the dictyate stage.

In no instance was there a concentration of grains over meiotic chromosomes that could be considered specific chromosomal label. Thus, no detectable amounts of RNA are retained, nor is there any detectable RNA synthesis by condensed meiotic chromosomes. These findings are consistent with observations on mitotic metaphase chromosomes (6) and with those on amphibian oocytes indicating that post-GVBD synthesis (5) is mitochondrial rather than chromosomal (18).

Observations on the labeling patterns of large pre-GVBD dictyate oocytes permit some conclusions concerning the gene activity which results in labeled RNA observed in the ooplasm after GVBD. The time course of appearance of label first in the nucleus and then in the cytoplasm (Figs. 6, 7) indicates that [3H]uridine is incorporated mainly into nuclear RNA rather than into mitochondrial RNA or DNA. It may be assumed that the observed incorporation was not that of nuclear DNA since no label was retained on meiotic chromosomes, and further, it is well established that bulk nuclear DNA replication ceases in mouse oocytes before birth (4). In addition, the autoradiographic demonstration of incorporation into both nucleolus and nucleoplasm of large dictyate oocytes at short labeling times suggests that heterogeneous RNA as well as ribosomal RNA is synthesized in the growth-quiescent period.

Incorporation of tritiated uridine into oocytes undergoing maturation in vitro has been observed and interpreted to have occurred within the last 6 h before GVBD (3). The data reported here es-
Figure 3 (a) Follicle cells from the same ovary as that represented in Fig. 1 collected 7 days after intrabursal injection of [3H]uridine. (b) Follicle cells from the same ovary as that represented in Fig. 6 collected 30 min after injection. × 1,000.

Figure 4 (a) Ovarian oocyte at diakinesis collected 2 h after administration of label. Arrows at limits of ooplasm. (b) Ooplasm is significantly labeled. No label attributable to chromosomes. (a) × 200; (b) × 700.

Figure 5 (a) Ovarian oocyte at metaphase II collected 8 h after administration of label. (b) Ooplasm is significantly labeled, but no significant label above background was retained on the chromosomes. (a) × 200; (b) × 1,000.
**TABLE II**

Labeling of Ovarian Oocytes at Successive Stages of Meiosis at Short Times after Bursal Injection of [5,6-3H]Uridine

| Interval between [3H]Uridine and sacrifice | [3H]Uridine per ovary | Dictyate | Diakinesis cytoplasm | Metaphase II cytoplasm |
|-------------------------------------------|-----------------------|----------|---------------------|----------------------|
|                                           | µCi                   | Nucleus  | Cytoplasm           |                      |
| 0.5                                       | 100                   | 12/12    | 3/12                | 0/2                  | 0/7                  |
| 2                                         | 100                   | 8/8      | 4/8                 | 6/7                  | 0/2                  |
| 3.5 and 5.5                               | 100                   | 5/5      | 5/5                 | 3/8                  | 0/3                  |
| 8                                         | 100                   | –        | –                   | 1/1                  | 4/6                  |
| 24–29                                     | 40                    | 16/20    | 16/20               | 4/6                  | 5/26                 |

**Figure 6** Dictyate oocyte collected 30 min after injection of label. Nucleolus and nucleoplasm are heavily labeled; ooplasmic label is slightly over background. The size of the zona pellucida (arrow) indicates that this oocyte is fully grown. × 650.

**Figure 7** Dictyate oocyte collected 5.5 h after injection of label. Ooplasm as well as nucleus is heavily labeled. × 650.
establish that the late RNA synthesis in vitro does not represent a temporally isolated burst but, instead, represents a continuation of the RNA synthesis that takes place throughout the dictyate stage. This is consonant with recent evidence obtained from an amphibian system in which it was demonstrated that, contrary to previous concepts, RNA synthesis takes place in fully grown preovulatory oocytes as well as in oocytes of the growing lambrush stage (9).

The progression of meiosis in mouse oocytes undergoing maturation in vitro is arrested by puromycin (17) and by actinomycin D (3). Cytological studies suggest that changes in chromosome configuration from diakinesis to metaphase II (14) involve changes in chromosomal compaction that may be related to a turnover of chromosomal basic proteins. It is possible that a fraction of the RNA synthesized in the late dictyate pre-GVBD oocyte is messenger RNA that codes for proteins essential for those chromosome dynamics and/or other events associated with GVBD.

SUMMARY
RNA synthesis, previously shown to take place during oocyte growth, has been demonstrated throughout the growth-quiescent period preceding ovulation of the mouse oocyte. In the final 7-day preovulatory period, the level of incorporation of [5,6-3H]uridine into ovulated oocytes decreased as the interval between exposure to precursor and ovulation decreased; significant incorporation was detectable within 2 days before ovulation. Analysis of the frequency and density of label in ovarian oocytes at successive stages of meiosis in relation to the interval between administration of labeled precursor and collection of oocytes revealed that RNA synthesis continues up to within 2 h before GVBD.

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