Incorporation of Arginine-\(^{3}\text{H}\) into Chromatin of Mouse Eggs Shortly after Sperm Penetration

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Summary. Mouse oocytes were fertilized \textit{in vitro} in a complete cultivation medium enriched by L-arginine-5-\(^{3}\text{H}\) monohydrochloride. The oocytes were isolated four hours after insemination. The incorporation of this precursor, as detected by means of autoradiography, was significantly higher in swollen sperm heads and female chromosomes at anaphase/telophase of the second meiotic division than the incorporation into ooplasm.

Introduction

At the beginning of fertilization in the mouse, both sperm and oocyte chromatin are, respectively, in a specific inactive state. The impulse(s), triggering the activation which is probably released shortly after sperm penetration, seems to be ambivalent, as the profound morphological changes in both sperm and oocyte chromatin proceed more or less \textit{pari passu} towards pronuclear formation (McGaughy and Chang, 1969). The female meiotic chromosomes, arrested originally in the oocyte in metaphase of the second meiotic division, resume further steps of meiosis shortly after sperm penetration and concomitantly with its swelling. During the swelling the sperm head loses its specific nuclear protein, which in its turn is probably replaced by another protein (Alfert, 1958; Bloch and Hew, 1960; Das \textit{et al.}, 1964; Johnson and Hnilica, 1971; Kopečný and Pavlok, 1975).

Protein synthesis was reported to increase almost immediately after fertilization in mouse and rabbit embryo (Monesi and Salfi, 1967; Tasea and Hillman, 1970; Manes and Daniel, 1969). Mintz (1964) observed by means of autoradiography newly synthesized protein in both cytoplasm and nucleus of 2-cell fertilized mouse egg. The present study was aimed at the possibility to detect autoradiographically newly formed protein distribution in earliest stages of fertilization in the mouse.

Materials and Methods

Mouse oocytes and spermatozoa were obtained from crossbred mice (from strains C57Bl/10ScSn Ph\(\times\)A/Ph). Superovulation, recovery of spermatozoa, fertilization \textit{in vitro} and removal of zona pellucida were performed as described elsewhere (Pavlok and McLaren, 1972). The complete cultivation medium for fertilization in vitro (see Pavlok and McLaren, 1972) already containing arginine was enriched by addition of L-arginine-5-\(^{3}\text{H}\) monohydrochloride (Amersham), spec. act. 500 mCi/mM up to 32 \(\mu\)Ci/ml. The oocytes were isolated four hours after insemination. The fertilization rate was normal, most oocytes were at the time of isolation in the telophase of the second meiotic division, the nuclei of spermatozoa...
were mostly swollen to the degree corresponding to the type III in classification of McGaughey and Chang (1969). The isolated oocytes were mounted in toto on slides and air dried. Some oocytes were induced to rupture by hypotony or manipulation. The slides were shortly exposed to formaldehyde vapor and afterwards fixed for 24 hours in 4 percent buffered formalin (pH 7.2) at 4°C. After fixation, the slides were washed with running tap water for 2 hours. After baths in distilled water and 70 percent alcohol the slides were dried again. The free precursor was further extracted with cold (5°C) 5 percent TCA for 5 min and the slides were washed again in running tap water for 2 hours. Slides from different experiments were coated either with L4 or K5 Ilford liquid nuclear emulsion and exposed up to 36 days. After development in D19b, the slides were stained with toluidine blue (Gurr) at pH 4.0 for 1 min.

Results

In autoradiograms of the eggs labelling in both ooplasm and chromatin was detected. The labelling in chromatin, however, was mostly significantly higher than that in the ooplasm (Figs. 1–4). In autoradiograms exposed for a shorter period the grain density above chromatin was at least twice of that found in the ooplasm. After an exposure of 38 days a heavy labelling was detected in the ooplasm and a total blackening above the chromatin. The detectability of the labelled chromatin was probably influenced by specimen geometry toward emulsion which in air dried ova was rather irregular. From three independent in vitro fertilization experiments a total of 136 suitable ova autoradiograms was obtained. In 70 ova from this number labelled male chromatin was detected, mostly as swollen sperm heads. In the same time labelled female chromosomes were detected in 65 ova in anaphase or mostly in telophase of the second meiotic division and in subsequent stages of the second polar body formation. There were further detected 48 unidentified labelled chromatin structures. In a few ova in early pronuclear stage the chromatin labelling was especially heavy. No conspicuous labelling, on the other hand, was detected in non-activated ova with chromosomes in metaphase II. Similarly, the sperm heads of unpeneetrated spermatozoa were found to be labelled only at the level of the background. Chemography was excluded, too, by checking unlabelled control slides. The staining of chromatin by toluidine blue in our material was only faint and did not interfere with grain density evaluation.

A clear intensive labelling of the prominent spheroidal Golgi complexes (Fig. 4) and of the equatorial region of the spindle of the second meiotic division ("midbody") (Figs. 1–3) was seen in some ova.

Discussion

The labelled amino acids are reasonably specific as protein precursors in autoradiography (Baserga and Malamud, 1969). It was suggested, on the other hand, that arginine-³H activity may be incorporated into DNA (Comings, 1969).