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Characterization of mouse oocytes and oocyte-cumulus complexes extracted for nuclear matrix and intermediate filaments (NM-IF)

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The extraction for nuclear matrix and intermediate filaments (NM-IF) is a three-step procedure removing most proteins while preserving essential cell architecture. We, for the first time, studied systematically mouse oocytes and oocyte-cumulus complexes after NM-IF extraction. Subjected to electrophoresis, they displayed a distinct pattern of bands. Light microscopy of extracted oocyte-cumulus complexes at germinal vesicle (GV) stage showed that much of their content was lost, leaving mainly skeletal structures. However, the general appearance, position of oocyte nucleus, zona pellucida, distribution of cumulus cells and their projections were preserved. In mature oocytes, extraction destroyed the structural organization completely. Our study shows that GV oocyte-cumulus complexes possess interconnected skeletal structures preserving the morphology even after extraction. This stability is lost in maturing oocytes, presumably due to cytoskeletal reorganization and disconnection from cumulus cells after GV breakdown.

Key words: oocyte, nuclear matrix, intermediate filaments, microscopy

Introduction

The extraction for nuclear matrix and intermediate filaments (NM-IF) is designed to preserve only these highly resistant skeletal structures, dissolving most proteins while retaining essential architectural features. It removes membranes and soluble content by non-ionic detergent, microtubules and microfilaments by high salt extraction, and chromatin by nuclease digestion [2]. The NM-IF extraction has been performed on many somatic cell types as well as spermatozoa [5]. However, no systematic study of NM-IF structures in oocytes has been published. An attempt to apply the procedure to hamster oocytes [7] produced only preliminary results due to methodological problems (cells failed to adhere on slides). Kadam et al. [3] found by immunoblotting a particular protein (MYH9) in mouse oocytes extracted for NM-IF, but did not focus on the total

protein profile. We detected by immunofluorescence keratins and vimentin in partially extracted mouse oocytes [6]. However, while immunocytochemistry is informative about selected components, it does not provide comprehensive morphological data. In the present work, we performed NM-IF extraction on immature and mature mouse oocytes and oocyte-cumulus complexes in order to study their light microscopic morphology and electrophoretic protein profile.

Materials and methods

Prepubertal virgin BALB/c females were hormonally stimulated as described in [6]. Cells were obtained by puncturing oviducts and ovaries, then washed in phosphate-buffered saline (PBS), pH 7.2. Those processed for microscopy were briefly treated with 0.5 mg/ml hyaluronidase to remove outer layers of cumulus.

Extraction for NM-IF was performed as described earlier [5, 6]. Oocytes and oocyte-cumulus complexes were placed in cytoskeletal buffer with 0.5% Triton X-100 and then in high-salt extraction buffer containing 250 mM $(NH_4)_2SO_4$ (both steps for 10 min at 4°C). Then cells were treated with 0.2 mg/ml DNase I and 0.1 mg/ml RNase A at room temperature for 20 min. $(NH_4)_2SO_4$ was added to 250 mM for additional 5 min. Some oocytes were left unextracted as controls.

For light microscopy, cells were fixed on slides with methanol and stained with 5% Giemsa in PBS (pH 7.0) for 40 min. Sodium dodecyl sulphate (SDS) electrophoresis was carried out according to [4] on 12% gels under reducing conditions, followed by silver staining as described in [8].

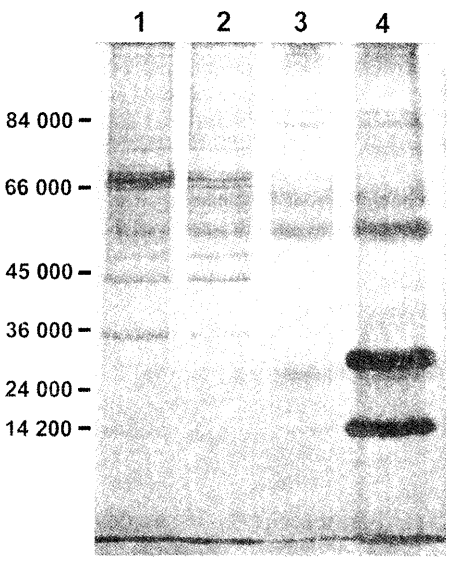

Fig. 1. Proteins from oocyte-cumulus complexes: unextracted controls (1), after cytoskeletal buffer (2), high salt (3) and nucleases (4) on silver-stained electrophoretic gel. At left, positions of molecular weight standards are indicated. The two most prominent bands on lane 4 are DNase I (Mr = 31 300) and RNase A (Mr = 13 700) used for chromatin digestion.

Results

Electrophoresis showed that after NM-IF extraction, a small fraction of the original proteins were preserved, forming a distinct pattern of bands (Fig. 1). Only the most prominent of them (Mr = 57 000) was visible also in unextracted controls.

Light microscopy showed very different effects of extraction on immature and mature oocytes (Fig. 2). Immature oocytes at germinal vesicle (GV) stage were surrounded by cumulus cells with well visible projections through zona pellucida (Fig. 2A). Extracted GV oocyte-cumulus complexes had lost much of their content, retaining mainly skeletal structures. However, the general appearance of the complex, position of oocyte nucleus, zona pellucida, distribution of cumulus cells and their projections were preserved (Fig. 2B).

Oocytes at metaphase I and metaphase II had lost their surrounding cumulus cells while being processed for microscopy. In

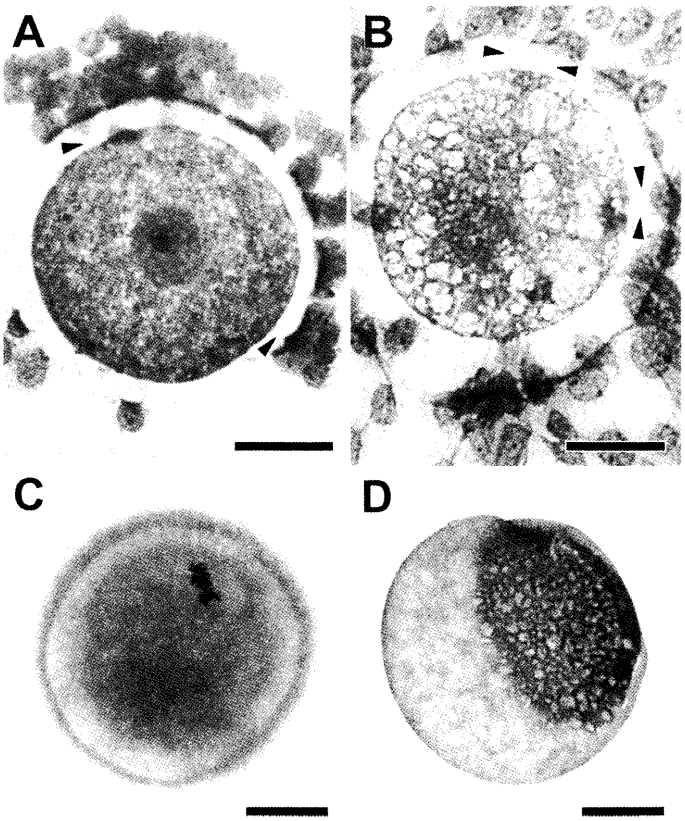

Fig. 2. Micrographs of Giemsa-stained oocytes, bars = 20 μm. **A**. Unextracted control oocyte-cumulus complex at GV stage. Arrowheads indicate projections of cumulus cells. **B**. Extracted oocyte-cumulus complex at GV stage. **C**. Unextracted control mature oocyte. The metaphase II plate is well visible. **D**. Extracted metaphase oocyte. The remaining cytoplasm has formed a sphere without any visible structure.

unextracted controls, cytoplasm had homogenous appearance, with the condensed chromosomes located at the cell periphery (Fig. 2C). Extraction of metaphase oocytes resulted in complete loss of structural organization and collapse of the remaining cytoplasmic content (Fig. 2D).

Discussion

Comparison of protein profiles after SDS electrophoresis showed that in oocyte-cumulus complexes, as in other tissues, proteins resistant to NM-IF extraction are few in number and make up only a small percentage of total protein content. The band with Mr 57 000 probably corresponded to intermediate filament proteins, known to be present in both oocytes and cumulus cells [6].

In our microscopic study, in contrast to the early report in [7], extracted GV oocyte-cumulus complexes retained their general morphology. At this stage, they appar-

ently possess well developed and interconnected skeletal structures allowing preservation of structural features even after disruptive chemical dissection. The importance of intercellular interactions in cell complexes, described for other tissues by different researchers [1, 9], in our case is best illustrated by the projections connecting cumulus cells to the GV oocyte. In later stages of oogenesis, the stability and resistance of supporting structures are lost, presumably due to reorganization of oocyte cytoskeleton and disconnection from cumulus cells after GV breakdown.

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