Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 26 (1-2)
Sofia ● 2019

Germ Cell Marker DDX4 (Vasa) Transiently Accumulates in Balbiani Body of Early Mouse Oocytes

Valentina Hadzhinesheva, Maya Markova, Stefka Delimitreva, Irina Chakarova, Venera Nikolova, Ralitsa Zhivkova*

Department of Biology, Medical Faculty, Medical University of Sofia, 2 Zdrave Street, 1431 Sofia, Bulgaria

* Corresponding author e-mail: mayamarkov@gmail.com

Abstract

RNA helicase DDX4 (Vasa) is a marker of germ cells. There is controversy about its intracellular distribution in early oocytes, reported by different authors to be uniform throughout the cytoplasm in mice or transiently associated with the oocyte-specific complex of organelles known as Balbiani body in humans. We performed immunohistochemical localization of DDX4 in sections from neonatal mouse ovaria. Prophase I oocytes from 1-day old mice showed reaction in a perinuclear aggregate, while in dictyate oocytes from 2-day old mice the reaction had diffuse staining throughout the cytoplasm. These results indicate that DDX4 is associated with the Balbiani body in early oogenesis of both mice and humans, and suggest that the Balbiani body in mammals may be a site of storage and regulation of mRNA as in other vertebrates.

Key words: DDX4, Vasa, oocytes, Balbiani body, meiosis

Introduction

RNA helicases are enzymes participating in splicing of primary transcripts, biogenesis of ribosomes, initiation of translation and other processes requiring changes in RNA secondary structure. The cytoplasm of mammalian germ cells at all stages of differentiation contains an RNA helicase known as DDX4 (Dead-box helicase 4) or Vasa after its homolog in *Drosophila*. It is not present in somatic cells and is regarded as a germ cell marker [7, 8]. There are reports that DDX4 keeps the germ cell genome stable by inhibiting the expression and propagation of retrotransposons [5]. Despite the apparent functional importance of this protein for germ cells, there is still controversy about its intracellular localization in immature oocytes. Uniform distribution of DDX4 has been described in early mouse oocytes [6], while in human oocytes, it has been found concentrated in the Balbiani body in primordial follicles and spread over wider areas of cytoplasm in primary follicles [1]. The Balbiani body is a perinuclear aggregate of membrane organelles (endoplasmic reticulum, Golgi cisternae and mitochondria) observed in early oocytes and in non-mam-

malian vertebrates related to polarity determination during oogenesis [3]. To examine the intracellular distribution of DDX4 protein in early mammalian oocytes, we localized it immunohistochemically in ovarian sections from newborn mice.

Materials and Methods

Experiments were performed in compliance with the European Union and Bulgarian legislature concerning use of animals as research objects. Tissue processing and immunocytochemistry was carried out as described before [4]. Briefly, ovaria of newborn female BALB/c mice were isolated, fixed and paraffin embedded at the first and second day after birth. Sections were prepared, mounted on slides, deparaffinized and rehydrated. Then immunofluorescent detection of DDX4 was performed on them using rabbit DDX4-specific primary antibody diluted 1:40 (Quartett, Germany) and FITC-labeled secondary antibody diluted 1:80 (Sigma – Aldrich, Germany). Chromatin was counterstained by Hoechst 33258. The result was observed and documented using Leica TSC SPE laser scanning confocal microscope.

Results

In ovaria of one-day old mice, oocytes were surrounded by few, irregularly positioned somatic cells. Oocyte nuclei were characterized by presence of condensed meiotic chromosomes. Antibodies against DDX4 produced a bright localized reaction in oocytes, staining a perinuclear aggregation (**Fig. 1**).

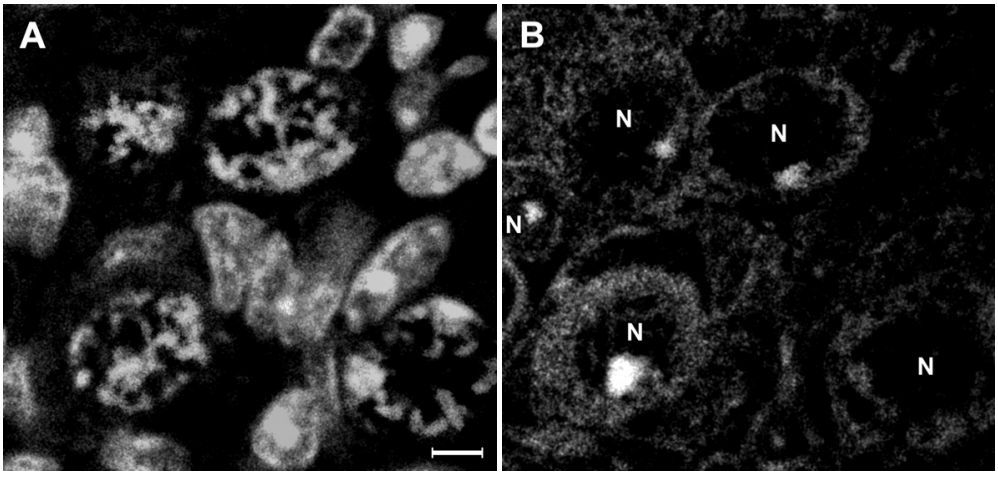

Fig. 1. Staining of a section from 1-day old mouse ovary for chromatin (**A**) and DDX4 (**B**). N, oocyte nuclei. Bar = 5 μm.

In ovaria of two-day old mice, well-formed primordial follicles were observed, with elongated somatic cells surrounding the oocytes. Oocyte nuclei had homogeneous appearance of chromatin corresponding to the first meiotic arrest in dictyate. The positive reaction for DDX4 protein was finely distributed in the oocyte cytoplasm (**Fig. 2**).

Somatic cells were negative for DDX4 in all ovarian samples.

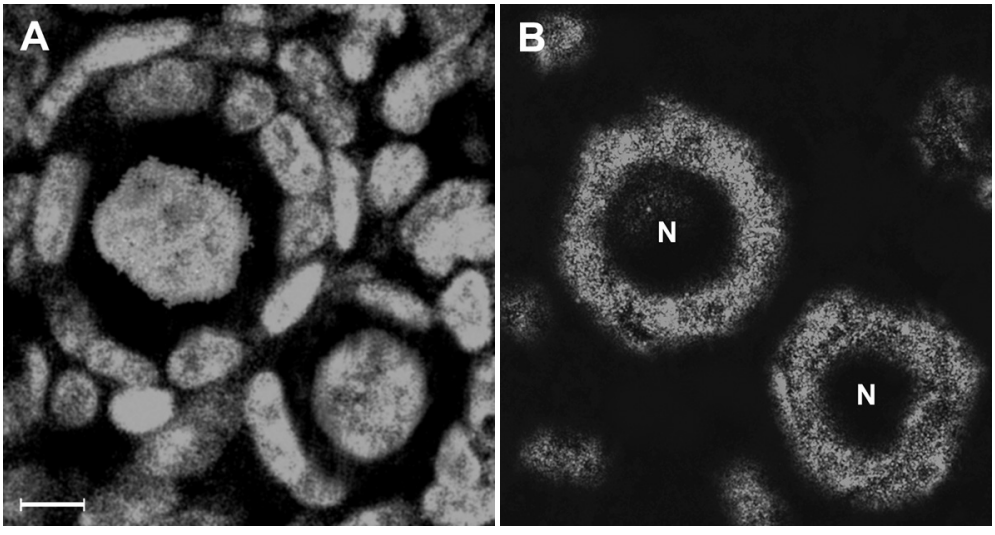

Fig. 2. Staining of a section from 2-day old mouse ovary for chromatin (**A**) and DDX4 (**B**). N, oocyte nuclei. Bar = 5 μm.

Discussion

This study was carried out on newborn mouse ovaria because in mice, prophase I of meiosis is still ongoing by the time of birth, and oocytes are arrested in dictyate only on the 2nd or 3rd day of postnatal life [2]. Comparing the reaction for DDX4 in ovaria of one-day versus two-day old newborn mice allowed us to trace the dynamics of DDX4 during late meiotic prophase I and transition to dictyate. The different appearance of chromatin (condensed in meiotic chromosomes in one-day old ovaria vs. homogenous in two-day old ovaria) and the cellular organization of ovaria (recognizable primordial follicles only in the two-day old group) confirmed that oocyte differentiation stage differed between the two sets of samples: ongoing prophase I in 1-day-old mice versus dictyate in 2-day-old ones.

There is controversy about the distribution of DDX4 in early mammalian oogenesis: uniform throughout the cytoplasm in fetal and neonatal mouse oocytes [6] or associated with the Balbiani body in human oocytes from primordial follicles and dispersing in oocytes from primary follicles [1]. More data are needed to clarify this discrepancy. Our finding of DDX4 in neonatal mouse oocytes concentrated in a large perinuclear cluster before dispersing in the cytoplasm suggests that in both mice and humans it is a component of the Balbiani body during prophase I. Messenger RNAs have been detected in the Balbiani body of fishes and amphibians but so far not in mammals [3]. The transient perinuclear concentration of the RNA-associated enzyme DDX4 revealed in this study makes it likely that the Balbiani body in mammals may be a site of storage and regulation of mRNA as in other vertebrates.

Conclusions

The germ-cell specific RNA helicase DDX4 colocalizes with Balbiani body in prophase I mouse oocytes before dispersing in the cytoplasm at dictyate. This result suggests that mammalian Balbiani body may have a function to accumulate and regulate mRNA.

Acknowledgements: We thank Assoc. Prof. Pavel Rashev and Assoc. Prof. Milena Mourjeva from the Institute of Biology and Immunology of Reproduction at the Bulgarian Academy of Sciences for valuable help in providing the specimens and documenting the results. This work was supported by the Bulgarian Ministry of Education and Science uder the National Program for Research "Young Scientists and Postdoctoral Students".

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