COMPERATIVE IMMUNOCYTOCHEMICAL ANALYSIS OF NUCLEOLUS-LIKE BODIES OF FULLY-GROWN OOCYTES AND NUCLEOLAR PRECURSOR BODIES OF ZYGOTIC EMBRYOS

Olga V. Zatsepina (zatsepina_olga@mail.ru), Elena A. Lavrentyeva, and Kseniya V. Shishova

Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya Street, 16/10, Moscow, 117997 Russia; Semenov Institute of Chemical Physics, Russian Academy of Sciences, ul. Kosygina 4, Moscow, 119991 Russia

Introduction

Instead of typical tripartite nucleoli, mammalian germinal vesicle (GV) oocytes contain intranuclear entities called “nucleolus-like bodies (NLBs)”, and zygotes contain the insertions named “nucleolar precursor bodies (NPBs)”. Both entities are strikingly similar in morphology, but their biochemical composition and roles in early development remain poorly understood.

Methods

Cell fixation and post-fixation treatments (see Figure legends) followed by immunocytochemistry (with Abs to UBF, fibrillarin, NPM1, nucleolin, RPL26, RPS10), FISH (with probes to different regions of 47S pre-rRNA, U3 snoRNA) and CLSM.

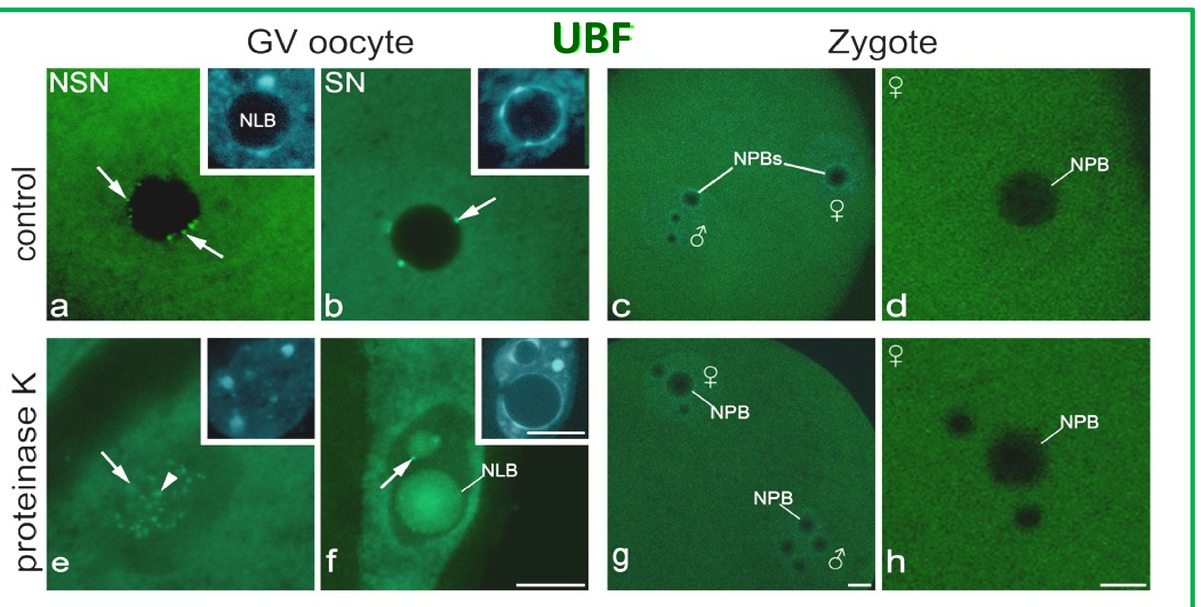

Fig. 1. Immunolabeling of NSN-type oocytes (a, e), SN-type oocytes (b, f) and zygotes (c, d, g, h) for UBF (a-h) in control (a-d) and after incubation with 1 µg/ml proteinase K for 40-45 min (e-h). The cells were fixed with 3% paraformaldehyde (PFA) in PBS; *blue* - Hoechst 33342 staining. ♂ - male pronucleus, ♀ - female pronucleus. Scale bars , 10 µm.

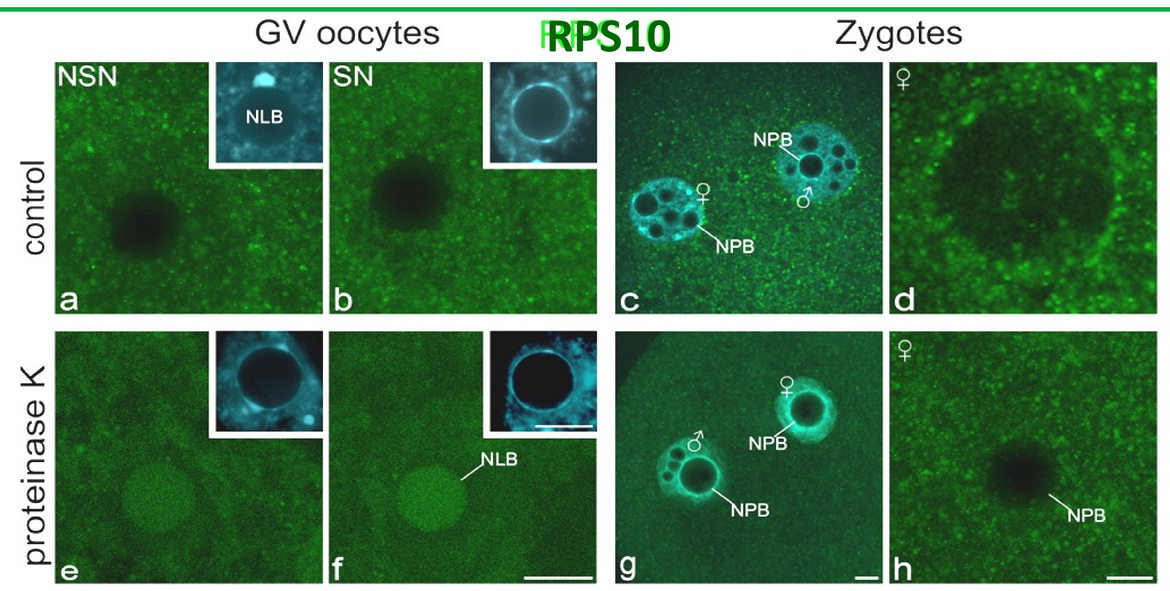

Fig. 2. Immunolabeling of NSN-type oocytes (a, e), SN-type oocytes (b, f) and zygotes (c, d, g, h) for RPS10 (a-h) in control (a-d) and after incubation with 1 µg/ml proteinase K for 40-45 min (e-h). The cells were fixed with 3% paraformaldehyde (PFA) in PBS; *blue* - staining with Hoechst 33342. ♂ - male pronucleus, ♀ - female pronucleus. Scale bars , 10 µm.

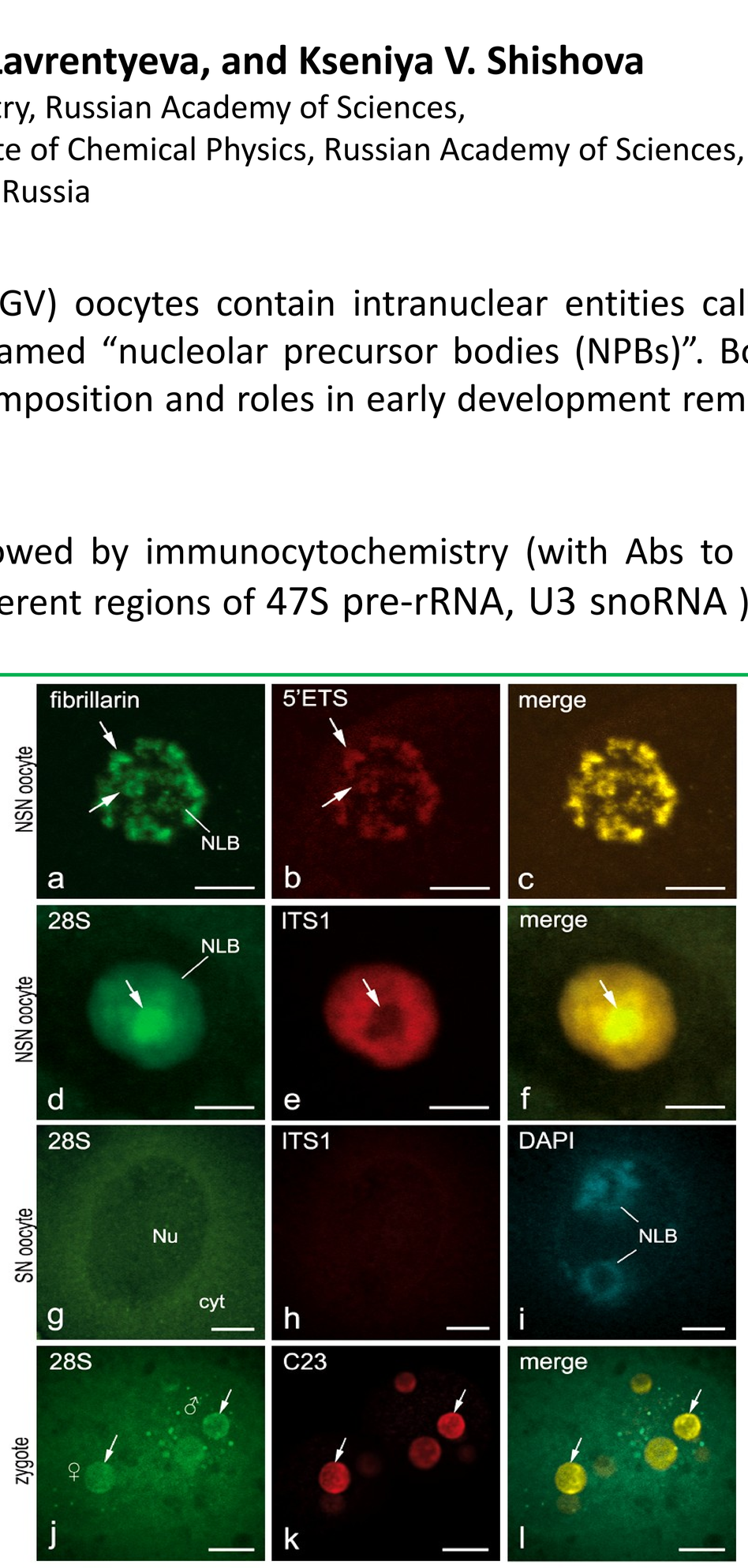

Fig. 3. Co-localization of fibrillarin (a, c) with the primary 47S pre-RNA transcripts detected with a probe to 5’ETS (b, c) and co-localization of 28S rRNA (d, f, g, j, l) with unprocessed rRNA detected with a probe to ITS1 (e, f, h) or with nucleolin (k, l) in GV oocytes (a-i) and zygotic embryos (j-l) fixed with 70% ethanol. (a-f), NSN-type oocytes; (g-i), SN-type oocytes; (i), staining with DAPI. Arrows indicate: (a-c), foci inside NLBs, (d-f), FISH-signals inside NLBs, (j-l), NPBs. Scale bars, 10 µm.

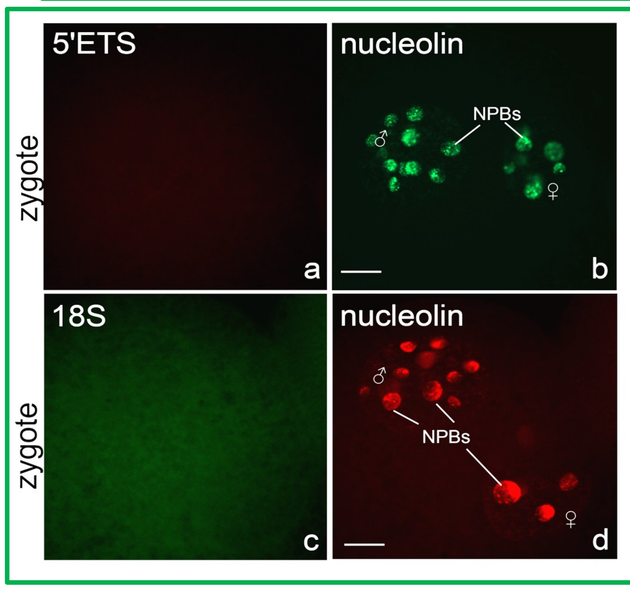

Fig. 4. Co-localization of the short-lived 5’ETS region of the primary 47S pre-RNA (a) and of 18S rRNA (c) with nucleolin (b, d). Embryos in (a-d) were fixed with 70% ethanol. ♂ - male pronucleus, ♀ - female pronucleus. Scale bars, 10 µm.

FISH signals are not observed.

Conclusions

- NLBs of NSN-type oocytes contain all factors required for rDNA transcription (UBF), early rRNA processing (fibrillarin), late rRNA processing (NPM1/B23, nucleolin/C23), and pre-ribosome assembly (ribosomal proteins RPL26 and RPS10), the primary 47S pre-rRNA transcripts, unprocessed rRNA, 18S and 28S rRNAs and U3 snoRNA.
- Transformation of NSN-oocytes to more competent SN-oocytes leads to a cessation of rDNA transcription and to a partial losing of rRNAs, RPL26 and RPS10 from the NLB interior.
- Zygotic NPBs are almost completely impoverished for RNA and completely for UBF.