Data Article

Data on morphology, large-scale chromatin configuration and the occurrence of proteins and rRNA in nucleolus-like bodies of fully-grown mouse oocytes in different fixatives

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Here we provide data on accessibility of nucleolus-like bodies (NLBs) of fully-grown (GV) mouse oocytes to fluorescence in situ hybridization (FISH) probes and anti-nucleolar antibodies as well as on oocyte general morphology and large scale chromatin configuration, which relate to the research article “High-resolution microscopy of active ribosomal genes and key members of the rRNA processing machinery inside nucleolus-like bodies of fully-grown mouse oocytes” (Shishova et al., 2015 [1]). Experimental factors include: a cross-linking reagent formaldehyde and two denaturing fixatives, such as 70\% ethanol and a mixture of absolute methanol and glacial acetic acid (3:1, v/v).

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Value of the data

- These data demonstrate that the occurrence of nucleolar proteins and RNAs in NLBs should be examined not only after oocyte fixation with paraformaldehyde but also after their fixation with 70% ethanol.
- These data are valuable to researchers interested in investigating the molecular composition of NLBs in mammalian oocytes.

1. Data

Cross-linking (formaldehyde) and denaturing (70% ethanol and methanol/glacial acidic acid, 3:1, v/v) fixatives exert different effects on oocyte and NLB morphology (Fig. 1a, a', a''), large-scale chromatin configuration (b, b', b'') and on accessibility of the NLB material to immunofluorescence (c, c', c'') and fluorescence in situ hybridization (FISH) probes (Fig. 2). Fixation with paraformaldehyde (PFA) best preserves the oocyte phenotype (Fig. 1a) and chromatin configuration (b), but it does not permit to label the nucleolar protein NPM1 (c) and rRNAs (Fig. 2a–c) inside NLBs. In the NLB mass, rRNAs became accessible to different FISH probes [1] only after oocyte fixation with 70% ethanol (Fig. 2a'–c') or with a mixture of methanol and acidic acid (2a''–c'') despite the mixture can deteriorate resolution labeling as compared with that in the ethanol-fixed oocytes (a', a'') (Table 1).

2. Experimental design, materials and methods

2.1. Oocyte collection and fixation

Fully-grown oocytes were collected from C57Bl/6 mice aged 4–8 weeks following the standard hormone administration with PMSG as described in [1]. Oocytes were fixed either with freshly made 3% PFA in PBS (140 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, and 8.1 mM Na2HPO4, pH 7.2) or with 70% ethanol in bidistilled water or with a mixture of absolute methanol and glacial acetic acid (3:1, v/v). In all cases, the fixation procedure continued for 20–25 min at room temperature. PFA-fixed oocytes were then treated with 0.5% Triton X-100 in PBS for 10 min, and the other oocytes were exposed to
0.2% Triton X-100 for 5 min to increase accessibility of the used probes and antibodies to target biomolecules.

2.2. Phase contrast

Oocytes were fixed with either of three fixatives, placed in PBS and examined as described in Section 2.6.
2.3. DNA staining

Large-scale chromatin configuration was examined in oocytes stained with a DNA binding dye Hoechst 33342 (1 μg/ml in PBS) for 10–15 min at room temperature.

2.4. Immunofluorescence

Fixed oocytes were washed in PBS (3 × 10 min), incubated with the mouse monoclonal anti-NPM1 (B23/nucleophosmin) antibody (Sigma-Aldrich, USA, cat. B0556) diluted 1:200 in PBS for 1 h at room temperature.
temperature, washed in PBS (3 × 10 min) and placed in Alexa Fluor® 488 goat anti-mouse IgGs (H+L) (Molecular probes Inc., cat. A11029) for 45 min at room temperature.

2.5. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed with antisense oligonucleotide probes recognizing the mouse 47S pre-rRNA. The probe “5’ETS” (5’atc ggg aga aag cga gat agg aat gtc tta) hybridizes with the short-lived 5’-external transcribed spacer (5’ETS) segment. The probe “ITS1” (5’aaa cct ccg cgc cgg aac gcg aca gct agg) hybridizes with the internal transcribed spacer 1. The probe “28S” (5’gag gga acc agc tac tag atg gtt cga tta) hybridizes with the 28S rRNA sequence (see [1] for the position of the probes along the 47S pre-rRNA). The probes were synthesized by DNA-synthesis Ltd. (Russia), conjugated with Cy3 at the 5’-terminal end and had the stock concentration about 2 μg/μl.

Oocytes fixed with either of three fixatives were washed in PBS (3 × 10 min), then in saline–sodium citrate buffer (2 × SSC, 0.3 M NaCl, 0.03 M Na2Cit, pH 7.0; 2 × 10 min), and placed into the hybridization mix (50% deionized formamide (Sigma-Aldrich), 10% dextran sulfate (Loba Feinchemie GMBH, Austria), 5% 20 × SSC (3 M NaCl, 0.3 M Na2Cit, pH 7.0), and 8 ng/μl probes) for 18 h at 42 °C in a wet chamber. Oocytes were sequentially washed with 50% formamide (Panreac, Spain) in 2 × SSC (3 × 10 min) at 42 °C, 2 × SSC at 42 °C (10 min), and 2 × SSC (10 min) at room temperature.

After DNA-staining, IF and FISH oocytes were mounted in Vectashield® (Vector Laboratories, USA), and examined under a confocal microscope within the next one–two days.

2.6. Image acquisition

Eight-bit digital images of oocytes were acquired with a DuoScanMeta LSM510 confocal laser scanning microscope (Carl Zeiss, Germany) equipped with a Plan-Apochromat 63 ×/1.40 (numerical aperture) oil Ph3 objective.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.03.085.
Reference

[1] K.V. Shishova, Yu. M. Khodarovich, E.A. Lavrentyeva, O.V. Zatsepina, High-resolution microscopy of active ribosomal genes and key members of the rRNA processing machinery inside nucleolus-like bodies of fully-grown mouse oocytes, Exp. Cell Res. 337 (2015) 208–218.