The lampbrush chromosomes and nuclear bodies of avian and amphibian oocytes studied by low-voltage scanning electron microscopy

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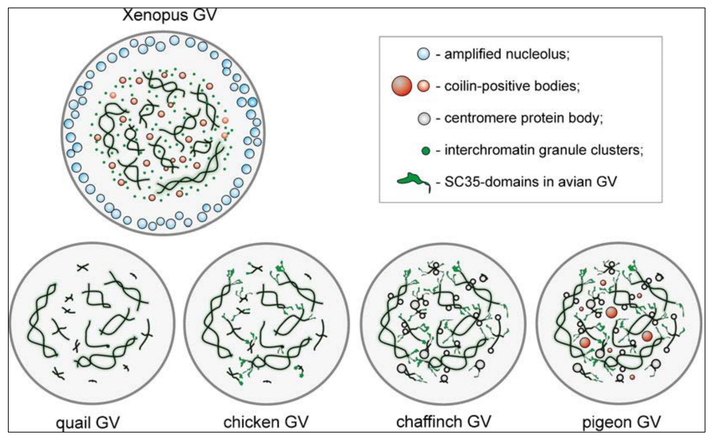
Introduction

Nucleus is a highly compartmentalized and ordered part of the cell where key processes of the genome functioning are realized through the formation of non-membranous nuclear domains. Physically nuclear domains represent liquid droplets with different viscosity stably maintained throughout interphase or during long diplotene stage of meiosis. From this viewpoint, the ultrastructural surface topography of nuclear domains is of outstanding interest since the nuclear bodies surface represents boundary of two liquid phases. The aim of our study was to examine the ultrastructural surface topography of amphibian and avian oocyte nuclear structures and to analyze the relations between the surface topography and the distribution of particular surface antigens using low-voltage scanning electron microscopy. Our results demonstrated that nuclear bodies with similar molecular composition may dramatically differ by the ultrastructural surface topography; vice versa nuclear bodies that do not have common molecular components may have similar topographical characteristics. First case was demonstrated for two coilin containing nuclear bodies: extrachromosomal histone locus bodies from amphibian oocytes and non-canonical Cajal bodies from avian oocytes; second case, for extrachromosomal histone locus bodies from amphibian oocytes and centromere protein bodies from avian oocytes. Using indirect immunogold labeling we characterized the distribution pattern of spliceosomal small nuclear RNAs (snRNAs) and coilin on the surface of heterogeneous group of coilin-containing bodies as well as the distribution pattern of double stranded DNA on the surface of lampbrush chromosomes. We conclude that low-voltage scanning electron microscopy without conductive coating allows to preserve intact ultrastructure of oocyte nuclear content and to evaluate the distribution of surface antigens using standard immunogold labeling technique.

A scheme of organisation of growing oocyte nuclei at the lampbrush stage in Xenopus and four species of birds



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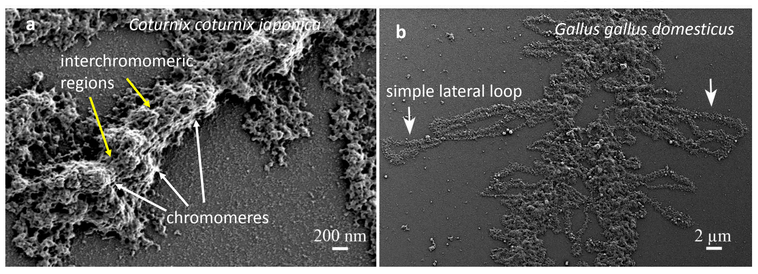
I. The investigation of ultrastructural surface topography of amphibian and avian oocyte nuclear structures

The scheme of the experiment

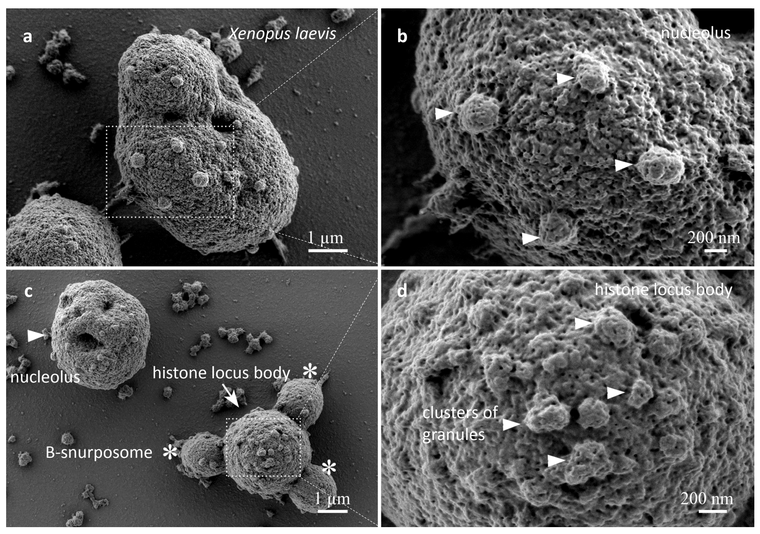
- Fixation: the mixture of 2.5% glutaraldehyde and 2% formaldehyde in 1xPBS
- Dehydratation: series of ethanol-water solutions of increasing concentration and air drying
- Without any conductive coating
- Secondary electrons detection

Avian lampbrush chromosomes morphology

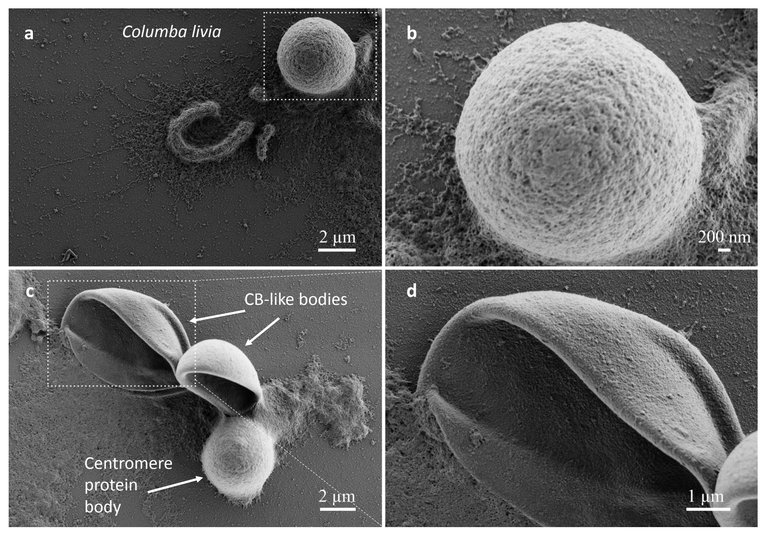


The morphology of avian lampbrush chromosomes, chromomere domains and loops. a – the surface morphology of chromomeres on the W-lampbrush bivalent. b – the fragment of macrobivalent in the lampbrush form demonstrate simple lateral loop morphology. The surface of both chromomeres and interchromomeric chromosome axes is presented by more or less loose fibrils 30 nm in diameter. Chromomere surface looks like a tangle of these fibrils and generally corresponds to chromomere morphology described earlier (Mott and Callan, 1975). b – simple lateral loops bear one or several transcription units, the direction of transcription of which is clearly visible because of thickening of RNP-matrix consisting of nascent RNPs.

Nuclear bodies morphology



The surface morphology of *Xenopus laevis* nuclear bodies in growing oocytes. a, b – the nucleoli bear dozens of large clusters of granules, with the main part of surface consisting of particles of 40-60 nm in diameter. b – 40-60 nm particles associate into irregular shaped grooves on the surface of nucleolus. The surface of patches is less textured comparing to the main part of nucleolus surface. c, d – the surface of amphibian HLBs consisting of thick and loosely packed fibrils (30-40 nm) with free spaces between individual fibrils forming cavities or depressions. The surface of HLBs also bears patches. The patches formed on nucleoli resemble patches on HLBs. However, as distinguished from patches on the surface of nucleoli the HLBs patches seem to bud off from the surface. HLB carry from zero to dozens of spherical structures called B-snurposomes (asterisks), which are attached to HLB surface by fibrillar material (12-25 µm fibrils). The surface of B-snurposomes is porous and tuberous, that can indicate that it consists of granules (30-40 nm).



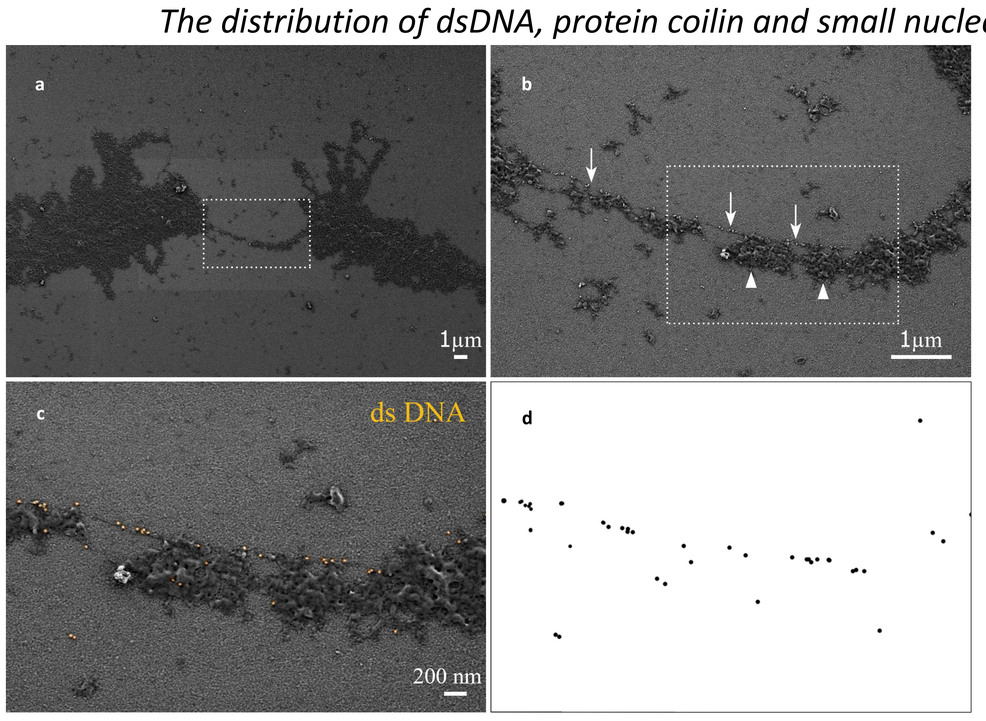
The surface morphology of *Columba livia* nuclear bodies in growing oocytes. a, b – centromere protein body and its enlarged image with tuberous and tortuous surface with pores of different size; c – CB-like bodies and centromere protein body; d – individual CB-like body; solid surface of CB-like body is uniformly covered by flat, non-regular shaped tubercles and widely spaced pores or clusters of pores. The smooth surface structure indicates compact and fibrillar nature of sphere matrix.

II. The immunogold labeling technique and the distribution of components typical for nuclear content

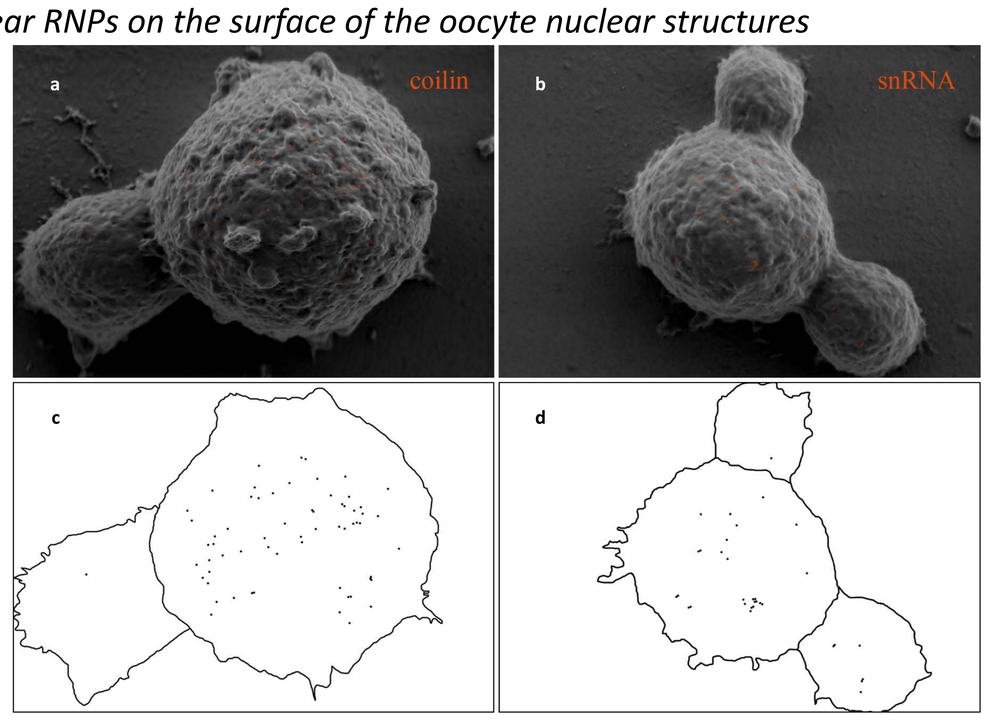
The scheme of the experiment

- Fixation: 2% formaldehyde in 1xPBS
- The immunogold labeling with secondary antibodies conjugated with 18 nm or 10 nm colloidal gold nanoparticles
- The diameter of the entire labeling complex was about 26 nm and 34 nm for 10 nm and 18 nm colloidal gold conjugates correspondingly
- Dehydratation: series of ethanol-water solutions of increasing concentration and air drying
- Without any conductive coating
- Secondary electrons detection

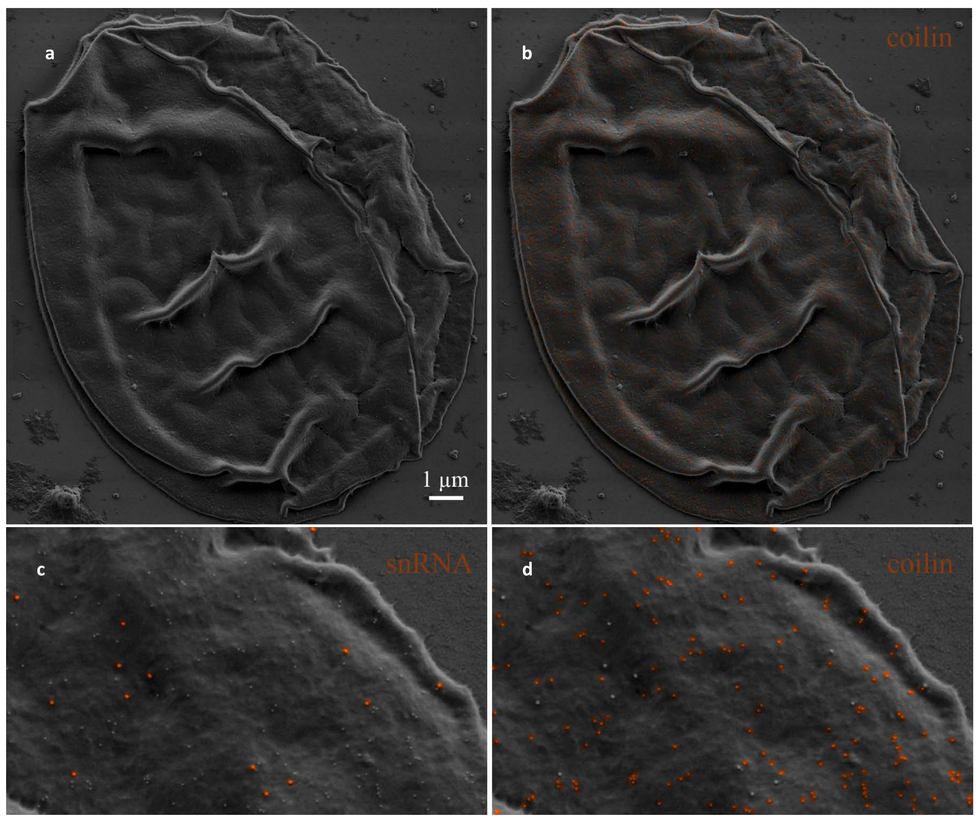
The distribution of dsDNA, protein coilin and small nuclear RNPs on the surface of the oocyte nuclear structures



The distribution of DNA along lateral loop axis of *Coturnix coturnix japonica* lampbrush chromosome. a, b, c – immunogold labelling of lateral loops with antibodies against dsDNA, b – dsDNA revealed by 18 nm gold nanoparticles (electron dense granules) along the DNP-axis (arrows) of individual lateral loop with growing nascent RNP fibrils (arrowheads); c – gold nanoparicles pseudocolured with yellow; d – the distribution pattern of gold nanoparticles.



The distribution of coilin or snRNA on the surface of histone locus body and attached B-snurposomes of *Xenopus laevis* growing oocytes. Immunogold labeling with antibodies against coilin (a, c) and snRNAs (b, d). a, b – distribution of coilin or snRNA on the surface of HLBs revealed by 10 nm and 18 nm gold nanoparticles (electron dense granules) correspondingly; c, d –distribution patterns of gold nanoparticles.



The distribution of coilin and snRNA on the surface of CB-like bodies of *Columba livia* growing oocyte. Double immunogold labeling with antibodies against coilin and snRNAs (a, b). Distribution of coilin and snRNA on the surface of HLBs revealed by 10 nm and 18 nm gold nanoparticles (electron dense granules) correspondingly; gold nanoparicles pseudocolured with yellow. c, d - enlarged images of CB-like bodies shown on panels a and b.

Conclusions

The results obtained demonstrated that LV–SEM allows to identify nucleoli, histone locus bodies (HLBs) and interchromatin granule clusters in amphibian oocyte nuclear content preparations as well as extrachromosomal and chromosome associated nuclear bodies from avian oocytes. Moreover, the individual lampbrush chromosome regions: chromomeres, transcribed and untranscribed looped out DNA regions, lateral loops with different morphology of RNP-matrix are easily recognizable.

Standard indirect immunogold labelling technique allows to detect certain nuclear antigens including coilin, small nuclear RNAs (snRNAs) and double stranded DNA (dsDNA) on the surface of oocyte nuclear structures by LV-SEM.

Nuclear bodies with the similar molecular composition may differ significantly by the surface ultrastructure while similar surface topological characteristics can be exhibited by nuclear bodies with different molecular composition.

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