Presence, isolation and characterization of yolk DNA from chicken eggs*

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Abstract Yolk DNA was detected with immunocytochemistry in fertilized chicken eggs. Yolk spheres were purified with Ficoll-400 density gradient centrifugation, followed by extraction of DNA therein. Yolk DNA is sensitive to various restriction endonucleases, but shows no obvious bands, demonstrating its sequence heterogeneity. There is only a little, if any, methylated CpG in yolk DNA, judging from MspI and HpaII digestion. Southern blot indicated that the sequence of yolk DNA accounts for a small portion of that of genomic DNA. Its uniqueness suggests that yolk DNA may play a crucial role in early development of chickens.

Keywords: yolk DNA, immunocytochemistry, DNA methylation, Southern blot.

Extraordinary amounts of DNA were detected in the oocytes and mature eggs of amphibians and aves[1,2]. Early studies indicated that these DNA are intrinsic to yolk platelets or yolk granules[3]. Bruce[4] isolated DNA from intracellular yolk granules of chicken embryos. Our previous studies were focused on the DNA from superficial yolk granules beneath blastoderm of chicken eggs, including detection of yolk DNA with Feulgen stain and autoradiography, isolation of DNA from chromatin isolated from yolk granules and observation of yolk DNA with electron microscopy[5]. However, there has been no report on the biochemical characterization of yolk DNA up to now. Here, we provide more convincing evidence for the presence of yolk DNA with immunocytochemistry, isolated DNA with Ficoll-400 density gradient centrifugation and study the biochemical properties of this DNA.

1 Materials and methods

1.1 Materials

All experiments were carried out with fertilized, freshly laid eggs of Leghorn White chickens. Mouse anti-DNA-IgM and sheep anti-mouse-Ig-POD were purchased from Boehringer Mannheim (B. M.); all restriction endonucleases were products of Promega; DIG-DNA labeling and detection kit was purchased from B. M.

All the manipulations were carried out at 4℃, if not specifically indicated.

1.2 Immunocytochemistry

In the following experiments, every wash lasted 10 min.

Yolk was separated from album and the blastoderm was removed. 1 mL of yolk was suspended in 10 mL of cold PBS (137 mmol/L NaCl, 2.7 mmol/L KCl, 4.3 mmol/L Na₂HPO₄, 1.4 mmol/L KH₂PO₄ pH7.4). 0.1 mL of suspension was dropped on the surface of each slide. After

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staying for 10 min in a humid chamber, excess liquor was drained off and samples were fixed in 3.7% paraformaldehyde-PBS for 2 h at room temperature followed by 3 washes in cold PBS. The slides were digested in 0.04 mg/mL Proteinase K for 10 min at 37°C and washed with 0.2% Glycine-PBS twice to stop the digestion. Primary antibody (mouse anti-DNA IgM) was applied to each slide and incubated for 30 min at 37°C. After three washes with PBS the samples were incubated with secondary antibody (sheep anti-mouse-Ig-POD) at 37°C for another 30 min. Then the slide was washed 5 times with PBS and transferred to color solution (50 mmol/L Tris-Cl, pH7.5, 0.5 mg/mL DAB, 0.002% H2O2) and stayed there at room temperature overnight. On the next day, the slide was rinsed in PBS and mounted with glycerol, then observed and photographed with a Nikon Diaphot-300 microscope. For the low magnification, bright field was used; for the high magnification, differential interference contrast (DIC) was used.

1.3 Isolation of yolk DNA

Yolk was suspended in 2 volumes of cold PBS and layered on the top of a discontinuous Ficoll-400 density gradient which consisted of 4 layers of Ficoll-400 in PBS, 5%, 12%, 15%, 20%, from top to bottom. The gradient was centrifuged at 10 000 g for 30 min. The interfaces between 5% and 12% and between 12% and 15% were taken out and combined. After addition of 10 volumes of PBS, the suspension was centrifuged at 1 500 g for 10 min to pellet the yolk spheres. The pellet was washed 3 times with PBS, and resuspended in buffer K (50 mmol/L Tris-Cl pH 7.5, 50 mmol/L EDTA); Proteinase K was added to a final concentration of 0.5 mg/mL and NLS to 1%; the mixture was incubated at 55°C overnight. After extraction twice with tris-saturated phenol (pH8.0) and once with chloroform, 3 volumes of absolute ethanol was added to the supernatant; the mixture was left at −20°C overnight. The next day, DNA was pelleted, vacuum-dried and dissolved in buffer TE (10 mmol/L Tris-Cl, pH8.0, 1 mmol/L EDTA). To remove the RNA, RNaseA was added to DNA in buffer TE to a final concentration of 0.1 mg/mL and incubated at 37°C for 2 h, extracted, pelleted and dissolved in buffer TE as above.

1.4 Restriction endonuclease analysis

Each µg of yolk DNA was digested with restriction endonuclease PstI, EcoRI, BamHI, HindII and HaeIII, separately, as described in the product’s instructions. The digests were run on 0.7% agarose gel in electrophoresis buffer TAE (45 mmol/L Tris-Ac, pH8.0, 2 mmol/L EDTA), at a voltage of 2.5 V/cm, for 2 h, and stained with ethidium bromide, observed and photographed under a UV Transilluminator.

1.5 Isolation of nuclear DNA from livers, embryos and erythrocytes of chicken

Chicken livers and embryos were homogenized in PBS. The homogenate was filtered through 6 layers of cheesecloth and nuclei were pelleted by centrifugation at 1 500 g for 10 min. The blood of chicken was suspended in 10 mmol/L EDTA-PBS and stood on ice until the erythrocytes sedimented spontaneously. The supernatant was discarded, and the sediment was resuspended in EDTA-PBS-0.2% TritonX-100 and stood on ice for 10 min to dissolve the cell membrane. The nuclei were collected by centrifugation. All three types of nuclei were washed with cold PBS 3 times; DNA was extracted as described in reference [6].