Original Article

Chromosome activities in cleaving mouse embryos to construct prenucleolar bodies orienting an intranuclear chromosome-chromosome configuration

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Most chromosomes of cleaving mouse embryos formed spherical structures called as prenucleolar bodies. Embryos stained with fluorescing dyes selective for AT-rich regions of DNA indicated that chromosome centromeres were organized by prenucleolar bodies and that chromosomes at pre-prophase of the cleavage division started to condense with their centromere regions aligned towards the peripheries of spheres. Embryo chromosomes as well as prenucleolar bodies were shown to bind a monoclonal antibody to spliceosomal snRNAs, which are contrasted to the nucleoli or chromosomes of somatic cells. More than 10 prenucleolar bodies were found in the pronuclei at the earliest stage after fertilization and 40 of them at most, diploid number of chromosomes of the mouse, in the nuclei of the 2-cell embryos. The number was decreased later in the cell cycles and at the 4-cell stage. Hence, every chromosome of cleaving mouse embryos is highly active in constructing prenucleolar bodies and behaves during the interphase until mitosis as a unit of the body; either as a single unit or as a member of the units fused. In other words, the prenucleolar bodies function as centers orienting chromosome-chromosome configuration in the fertilized eggs.

Key words: Fertilized eggs, chromosome orientation, prenucleolar bodies

Introduction

The nucleoli of cells are prominent intranuclear organelles organized by chromosomes, which function as factories for ribosome biosynthesis and their activities. Their structures strikingly different during development of organisms, cell proliferation stages, and pathological states of cells. Particularly, nucleoli in oocytes of various species are different in their numbers per nucleus, and in their sizes during meiotic maturation and after fertilization. They are referred to under various names such as access nucleoli, micronuclei, nuclear spheres, pseudonucleoli and sometimes viewed as non-nucleoli. In amphibian oocytes, such spheres are organized by sphere organizing regions of chromosomes and composed of spliceosomes, with a pre-mRNA editing function. Similarly, the nuclear architecture of fertilized mammalian eggs is characterized by the spherical bodies named as primary and secondary nucleoli. These bodies are composed of nucleolus-specific proteins, B23 and nucleolin. However, the cleaving embryos of mammals are inactive or less active in their ribosomal RNA synthesis. The spherical bodies are devoid of silver staining specific to nucleolar organizing regions of chromosomes except for peripheries of the bodies and morphologically they are not differentiated to “true” nucleoli. Such spherical bodies in the mouse are called as prenucleolar bodies by Biggigera et al. and so abbreviated as prenucleoli in this communica-
tion.

At meiotic prophase in oocytes and spermatocytes of invertebrate, the nucleolus is closely associated with the condensing chromosomes which take the polarized "bouquet" arrangement, that is, Rabl configuration. Recent studies on mammalian cells focus on the involvement of nucleolus in directing the orientation of interphase chromosomes as well as nuclear envelope, nuclear matrix, and pre-mRNA splicing organelles. The interphase chromosomes of a mammalian cell take a radial configuration conforming to Rabl configuration with inward facing centromeres, which was confirmed by the premature chromosome condensation of Chinese hamster ovary cells fused with metaphase HeLa cells. Such a radial configuration has been discussed for the metaphase chromosome arrangements of somatic cells. An alternative view is stated by Holowacz and De Boni that the chromosomes take intranuclear spatial domains in interphase neuronal cell nuclei without conforming to the classical Rabl configuration.

During the cleavage stage of the mouse, maternal gene products such as a transcription factor Oct-3, mos proto-oncogene product, and DNA-methylase are present and the embryonic genes become activated. The cell cycles of 1-cell and 2-cell mouse embryos are characterized by a short S period and a long G2/M period, which is contrasted to those cell cycles of amphibian cleaving embryos and mammalian somatic cells. In mammalian zygotes female and male pronuclei are never fused before the first cleavage division, and so maternal and paternal genes are transcribed in spatially-separated microenvironments. In fact, a luciferase reporter gene expression is detected in G2 of the 1-cell mouse embryo when the gene is injected in S to the male pronucleus but not to the female pronucleus. A question remains to be investigated whether or not such regulation of gene activity transitions and characteristic cell cycle changes reflects chromosomal spatial arrangements in the blastomeres of mouse zygotes, as mentioned above. This paper presents the chromosomal activities to construct pronucleoli and their functions as centers for chromosome arrangements during the cleavage stage.

Materials and Methods

**Embryos and their culture**

Eight-week old female mice of (D2B6)F1, strain were superovulated and mated with male mice of ICR strain (Charles River Japan, Japan). The embryos were collected at the pronuclear, 2-cell and 4-cell stage and handled in Whitten's culture medium of mouse embryos (WCM) from which sodium bicarbonate was replaced by 20 mM HEPES (WCM-H), with its osmotic pressure adjusted to that of WCM. The staging of embryos in the present strain of mice and their culturing method in WCM was previously described.

In the present study, embryos were cultured in WCM supplemented with 100 μM disodium EDTA (Sigma, U.S.A.).

**Embryo fixing, extraction and staining**

The embryos were fixed with 2% paraformaldehyde in WCM-H for 10 min, extracted for 60 min by a microtubule stabilizing buffer reported by Shattan et al. and stained for 10 min with 1 μg/ml Hoechst 33258 (Wako Pure Chemical, Japan) or 10 μM 4′,6-diamidino-2-phenyl-indole (DAPI) (Sigma, U.S.A.) in PBS containing 25% glycerin. The formaldehyde solution was freshly prepared. The stained embryos were transferred into a drop of 80% glycerin/PBS on microscope slide glass, over which coverglass was gently placed at later than 10 min. The coverglass was immobilized on the slide glass by sealing its two sides with Entellan New (Merck, Germany). When the stained embryos were photographed by a confocal laser scanning inverted microscope (Olympus Optical, Japan), they were transferred into PBS-containing wells made of 0.15-0.18 mm thick coverglass glued onto microscope slide glass. The objective Olympus lens had a maximal resolution when immersed in water for the ultraviolet-light-excitation of the microscopy and so glycerin was not added into the PBS solution in the wells.

**Immunostaining of embryos**

The embryos were fixed and extracted as mentioned above followed by washing twice in 25% glycerin/PBS, since the extraction buffer contained 25% glycerin. For immunostain of UsnRNAs of splicing complexes, the extracted embryos were exposed to a monoclonal antibody to 2,2,7-trimethylguanosine (m7G) (mAb K121, Oncogene Science, U.S.A.) for 18 hr. The snRNAs of pre-mRNA splicing organelles are capped with m7G. The monoclonal antibody IgG conjugated with fluorescein-isothiocyanate (FITC) was used at the concentration of 200 μg/ml in 25% glycerin/PBS. For a scanning laser microscopy which is optimal for the more intense fluorescence light, mAb
K121-treated embryos were exposed for 4 hr to goat anti-mouse IgG (E. Y. Lab., U.S.A.) conjugated with FITC. For DNA immunostaining, embryos were exposed for 18 hr to a monoclonal antibody IgM to double- and single-stranded DNA, AC-30-10 at 1:200 dilution (Progen Biotechnik, Germany)36 and then exposed to the FITC-conjugated goat anti-mouse IgG, IgM, IgA (Cappel, Organon Teknika, U.S.A.) at 1:100 dilution for 4 hr. The antibody treatments were done at 0–4°C in 25% glycerin/PBS containing 0.1% NaN₃.

Microphotography

The microphotographs of embryos under Nomarski interference contrast (NIC) were taken by Olympus microscope BHS-N using Kodak technical pan film, which was developed by the F-dilution of HC-110 Kodak developer (Kodak, U.S.A.). Epifluorescence microphotographs of embryos were taken by Olympus microscope BHS-RF-A using Kodak Tri-X pan 400 film, which was developed by the B-dilution of HC-110 Kodak developer. The fluorescing images of embryos were also photographed by confocal laser scanning microscopes, Olympus LSM-GB200 (UV type) (Olympus Optical, Japan) and Zeiss, LSM310 (Carl Zeiss, Germany).

Statistical analysis

The embryo blastomeres prepared for microscopy were scanned and the numbers of pronuclei per nucleus were counted by adjusting the focal distance under NIC microscopy. For pronuclear embryos, the data were registered by the number per individual pronucleus, since a female and a male pronucleus of the mouse are never fused. The percentage cumulative frequencies of nuclei were plotted on a probit scale versus the axis of number of pronuclei per nucleus on a log scale. The plot for pronuclear embryos were fitted to curves of second order polynomials and those of 2-cell and 4-cell embryos were fitted to lines calculated by probit analysis with χ² of less than their degrees of freedom33.

Results

At the pronuclear to 2-cell stage, a few pronucleoli per nucleus and frequently single ones per pronucleus can be visualized by microscopy of living embryos37. By fixing embryos with paraformaldehyde, the pronucleoli were stabilized allowing detailed scanning of them under microscopic observations of their structures. Both male and female pronuclei formed numerous pronucleoli of 1–2 μm at the early stage after fertilization (Fig. 1, A and C), when the pronuclei stayed at subcortical regions of blastomeres. The pronucleoli of blastomeres as well as those of polar bodies produced images of fluorescing rings when stained with a DNA selective dye, Hoechst 33258 (Fig. 1 B and D). The sperm heads in addition intensely fluoresced (data not shown). At a G₂ phase of the 1-cell stage, pronuclei migrated to the centers of blastomeres and pronucleoli were decreased in number while increased in size and in fluorescence intensity (Fig. 1, E and F). They became undetectable at mitosis of the first cleavage division under NIC microscopy as described previously by others34. At the early 2-cell stage they re-formed in larger numbers per blastomere nucleus (Fig. 1, G and H).

To see statistically the changes during development, the numbers of pronucleoli per nucleus were counted at the 1-cell to 4-cell stage. As seen in Fig. 2, the distributions of pronucleoli numbers gave different patterns between developmental stages and between cell cycle phases of cleaving embryos. Particularly, a striking contrast was noticed between the 1-cell stage and the 2-cell stage. At the early 1-cell stage, more than 10% pronuclei had 10–20 pronucleoli and at the late stage 60% pronuclei had single pronucleoli. Similarly, at the early 2-cell stage more than 50% nuclei had 19–40 pronucleoli with mean of 19.3 and at the late stage, the mean became 12.4, which were calculated by probit analysis33. The means for the early 4-cell stage was 9.6. Thus, immediately after decondensation of chromosomes after fertilization or cleavage division, the maximum number of pronucleoli appeared to reach 20 for the 1-cell stage or 40 for the 2-cell stage, which is a haploid or diploid chromosome number of the mouse, respectively35. Namely, the pronucleoli per nucleus is decreased in number at later stages of the cell cycles and at 4-cell stage. The maximum number indicates that every chromosome has the capacity to organize a pronucleolus in fertilized mouse eggs. As referred to by Hay1, Gates commented similar findings on the nucleolus formation in annelid eggs.

The spherical arrangements of chromosome centromeres were revealed by fluorescence microscopy of Hoechst-stained embryos at prophase or earlier, while not imaged by NIC microscopy as mentioned above (Fig. 3). At a late G₂ phase of the 1-cell stage, the ring-like fluorescence of pronucleoli became more intense (Fig. 1F) with radiating thin fibers of condensing chro-
Fig. 1. Prenucleoli in whole mouse embryos stained with Hoechst 33258. Figures (A, C, E, G) show Nomarski interference contrast images and figures (B, D, F, H), fluorescence images. The side-by-side pairs of figures are the images of same embryos, respectively. The first pair a, B give the images of small and numerous prenucleoli at the earliest stage after pronucleus formation. The lower polar body shows prenucleoli and the upper one is still at the condensed chromosome stage. The second pair C, D are the images of prenucleoli at a G1 phase of the 1-cell stage; the third pair E, F are the images of two pronuclei with single prenucleoli at a G2 phase of the 1-cell stage and the fourth pair G, H are the images of 20–30 prenucleoli per nucleus at an early G1 phase of the 2-cell stage. The bar indicates 30 µm.
mosomes (Fig. 3A). As embryos progressed into prophase, the chromosomes were more condensed maintaining their radial configurations and centering on a few spheres (Fig. 3, B). Up to late prophase, male chromosomes (upper group in Fig. 3, B) were less condensed compared with female chromosomes (lower group in Fig. 3, B). The disappeared prenucleolus NIC images with more intense spherical fluorescence indicate that the prenucleoli decay and their remnants still function as centers of condensing chromosomes.

To see the spherical arrangements of condensing chromosomes, the DAPI-stained embryos were photographed by Olympus confocal laser scanning microscope. At late G2 phase the spherical prenucleolar bodies were attracting chromosomal DNA on their peripheries (Fig. 4). At prophase, the condensing chromosomes surrounding two spheres devoid of fluorescence, taking a Rabl configuration (Fig. 5). Namely, the spheres, which may be constructed by prenucleolar remnants, take central positions of the condensing chromosomes.

Small nuclear RNAs (snRNAs) associated with splicosomes of pre-mRNA editing organelles were more concentrated on the peripheries of prenucleoli as indicated by interphase embryos immunostained with antibody to the snRNAs, mAb K1211 (Fig. 6, A). It should be emphasized that the embryos were not exposed to any DNA-staining chemicals for this experiment and so the fluorescence images were produced by mAb K121-binding structures. At late G2 phase, the peripheries of spheres were of more intense fluorescence (Fig. 6, B). At prophase to metaphase, the immunostained chromosomes at the second cleavage division showed images with larger intensities on their centromere regions (Fig. 6, C).

**Fig. 2.** Changes in numbers of prenucleoli under NIC microscopy of the embryos at the 1-cell, 2-cell, and 4-cell stage. The number of prenucleoli per nucleus decreases from the early stage (G1 phase) to the late stage (G2 phase) of 1-cell embryos or 2-cell embryos. Two hundred and four (Early 1-cell stage), 116 (late 1-cell stage), 178 (early 2-cell stage) and 159 (late 2-cell stage), and 78 (early 4-cell stage) are the numbers of nuclei counted.

**Fig. 3.** Hoechst-stained mouse embryos. The figure A shows a single prenucleolus per pronucleus of the 1-cell embryo at late G2 phase. The figure B gives the images of a condensing male chromosome set (upper) and a female chromosome set (lower) at early prophase. The bar in (A) indicates 10 μm and the bar in (B), 20 μm.
Fig. 4. Consecutive slices of 1 μm-thick images from a DAPI-stained pronucleus mouse embryo printed by the Olympus confocal laser scanning microscope. The photographs show two pronuclei, female (lower) and male pronucleus (upper), with single pronuclei. The AT-rich regions of chromosomal DNA are assembled surrounding the two spheres. The bar indicates 30 μm.

Chromosomal localization of mAb K121-binding snRNAs is in a sharp contrast to their distribution in somatic cells, in which such snRNAs localize in non-nucleolar regions\(^{12}\) or in the regions independent of the presence of DNA\(^{13}\). Xenopus oocytes and early embryos store larger amounts of spliceosomal snRNAs than somatic cells\(^{15,36}\). And the nuclear spherical bodies of newt oocytes contain more mRNA-splicing snRNAs as well as the lampbrush chromosomes, while the nucleolus, which is composed of \(U_1\) snRNA, are devoid of splicing organelles\(^5\). Hence, chromosomes and pronucleoli of mouse embryos appeared to have larger affinities to spliceosomal snRNAs during early development as those of amphibian oocytes.

To show the staining images by Zeiss confocal
laser scanning microscope, embryos immunostained with mAb K-121 were secondly treated with FITC-conjugated goat anti-mouse IgG. The confocal images of the embryos are shown in Fig. 7, A. The peripheries of prenucleoli more intensely fluoresced for the embryos stained with mAb K121 than that for the embryos stained with mAb of anti-DNA (Fig. 7, B). Hence, the confocal images are consistent with the image in Fig. 6, A revealing that splicosomal snRNAs are associated with prenucleoli, though not selectively.

The satellite DNAs of chromosomes are associated with nucleolus in somatic cells which are methylated at high levels during the cleavage stages of mice due to their larger activities of DNA methylase. A cytosine analog, 5-azacytidine inhibits the DNA methylation when incorporated into DNA. Therefore, an experiment was designed to see whether the analog interfered with prenucleolus formation when embryos were cultured from 1-cell to 2-cell stage. As shown in Fig. 8, 2-cell embryos treated with 40 μM 5-azacytidine formed 7.1 prenucleoli per nucleus of the mean compared with 8.8 prenucleoli for control embryos. Since the mean number of 12.4 for control in vivo embryos, the cultivating itself affected the prenucleolus formation and so 5-azacytidine effects appeared to be non-significant. Next, the chemicals to inhibit cytoskeleton...
Fig. 6. Fluorescence images of embryos immunostained with mAb K121. The stains of peripheries of prernucleoli and centromeres of chromosomes are more intense. The figures A, and B show the prernucleoli of 1-cell embryos. The figure C shows the metaphase chromosomes in two blastomeres of a 2-cell embryo. The bar indicates 30 μm.

Fig. 7. Images of 1 μm-thick slices printed by the Zeiss confocal laser scanning microscopy of 1-cell embryos immunostained with mAb K121 (A) and with monoclonal DNA antibody, mAb AC-30-10 (B), respectively. Both photos show two pronuclei with single prernucleoli (A) or single or a few ones (B). The bar indicates 30 μm.

function were examined for their effects on the prernucleolus formation. The embryos treated with 10 μM colchicine (Merck) from the early 1-cell stage formed single prernucleoli per pronucleus by 74% (53/72) at the late 1-cell stage, which are at a level similar to that for untreated embryos (Fig. 2). Colchicine treated-embryos progressed to late prophase of the first cleavage division and their chromosomes were condensed in a female and a male pronucleus separately. The first cleavage division was inhibited and the double sets of chromosomes in the blastomeres persisted for the additional culture period of 20 hr when control embryos reached the late 2-cell stage (data not shown). One-cell embryos treated with 40 μM cytochalasin D (Sigma), an inhibitor of actin polymerization were blocked for their cytokinesis of the first cleavage division and stayed at the single binucleated blastomeres. The number of prernucleoli per nucleus was similar to that of normal 2-cell embryos (data analysis not shown). Thus, the prernucleolus formation
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The DNA binding chemicals used, Hoechst 33258 and DAPI, are selective for AT-rich DNA grooves\(^{38,39}\), and the monoclonal antibody AC-30-10 is not selective for DNA bases\(^5\). Such binding characteristic of Hoechst and DAPI may be reflected by the prenucleolar-associated fluorescing patterns of embryos, compared with the patterns by the DNA antibody (Fig. 3, A and 6, A). By a nucleic acid-binding dye, propidium iodide, the stained embryos revealed less distinct prenucleolar fluorescing patterns, while nuclear staining was evident (data not shown). By Feulgen staining used for measuring DNA contents of the cell, the ring-like patterns of prenucleoli were barely discernible at best preparations of stained embryos (data not shown). Hence, the prenucleolar fluorescing patterns (Figs. 3 and 4) by Hoechst and DAPI do not simply indicate the non-uniform presence of DNA and rather reveal polarized distributions of AT-rich DNA regions of chromosomes in embryos. At mitosis of cleavage divisions of the mouse, centromeric regions of acrocentric chromosomes most intensely bound the DNA probes and dot-like fluorescence images were taken for embryos stained with human autoimmune polyclonal antibody to centromere/kinetochore prepared from CREST-type scleroderma patients\(^{19,40}\) (data not shown). Simler et al.\(^{40}\) report that scattered prenucleoli were induced in early 2-cell mouse embryos injected with anti-centromere/kinetochore human antibody. These results support the view that the prenucleoli are organized by centromeric regions of chromosomes in cleaving mouse embryos. Next, every chromosome of cleaving mouse embryos may have the capacity to form the prenucleolus, since the estimated maximum number of prenucleoli is fitted to the number of chromosomes of the mouse (Fig. 2). At later phases in the cell cycles, the prenucleolus number was decreased with enlarged sizes and so the prenucleoli were fused into larger ones with bundling several chromosomes (Fig. 5). Namely, every chromosomes are arranged in the nucleus as a unit of prenucleolus and organized into larger groups by fusion of prenucleoli.

The intranuclear spheres composed of pre-mRNA-editing spliceosomes are viewed as organelles regulating biosynthetic activities during amphibian oocyte maturation\(^3\). As mentioned in Introduction, the activity to synthesize RNA is at a lowest level in the pronuclear stage of the mouse and so the prenucleoli cannot be viewed as organelles involved in the active biosynthesis of ribosomes, though the snRNAs of spliceosomes are detected in blastomere nuclei. The present study leads to an alternative view on their function as organelles to orient interphase embryo chromosomes. This viewpoint may be fit to studies on the nucleolus-chromosome arrangement at prophase of meiosis in invertebrate species, which precedes to polarized chromosome configuration, e.g. Rabl configuration\(^1\). The prenucleoli are expected to play roles in regulating chromosome arrangements by binding the centromere/kinetochore of chromosomes during interphase and chromosome condensation stage of mouse embryos. The involvement of kinetochores in the chromosome movement at mitosis is previously confirmed for the cleavage division of the 1-cell to 2-cell stage\(^{42}\). The kinetochores associated with nucleoli in fibroblasts correspond to the number of chromo-

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**Fig. 8.** Number of prenucleoli per blastomere nucleus of late 2-cell embryos when cultured from the early 1-cell stage. The treated embryos were cultured in the presence of 40 µM 5-azacytidine. The cultured control embryos were reduced in their number of prenucleoli per nucleus at late 2-cell stage, with mean of 8.8, compared with embryos in vivo with mean of 12.4 (see Fig. 2).

appeared to be more dependent on their developmental stages and positions in the cell cycle.

**Discussion**

The DNA binding chemicals used, Hoechst 33258 and DAPI, are selective for AT-rich DNA grooves\(^{38,39}\), and the monoclonal antibody AC-30-10 is not selective for DNA bases\(^5\). Such binding characteristic of Hoechst and DAPI may be reflected by the prenucleolus-associated fluorescing patterns of embryos, compared with the patterns by the DNA antibody (Fig. 3, A and 6, A). By a nucleic acid-binding dye, propidium iodide, the stained embryos revealed less distinct prenucleolar fluorescing patterns, while nuclear staining was evident (data not shown). By Feulgen staining used for measuring DNA contents of the cell, the ring-like patterns of prenucleoli were barely discernible at best preparations of stained embryos (data not shown). Hence, the prenucleolar fluorescing patterns (Figs. 3 and 4) by Hoechst and DAPI do not simply indicate the non-uniform presence of DNA and rather reveal polarized distributions of AT-rich DNA regions of chromosomes in embryos. At mitosis of cleavage divisions of the mouse, centromeric regions of acrocentric chromosomes most intensely bound the DNA probes and dot-like fluorescence images were taken for embryos stained with human autoimmune polyclonal antibody to centromere/kinetochore prepared from CREST-type scleroderma patients\(^{19,40}\) (data not shown). Simler et al.\(^{40}\) report that scattered prenucleoli were induced in early 2-cell mouse embryos injected with anti-centromere/kinetochore human antibody. These results support the view that the prenucleoli are organized by centromeric regions of chromosomes in cleaving mouse embryos. Next, every chromosome of cleaving mouse embryos may have the capacity to form the prenucleolus, since the estimated maximum number of prenucleoli is fitted to the number of chromosomes of the mouse (Fig. 2). At later phases in the cell cycles, the prenucleolus number was decreased with enlarged sizes and so the prenucleoli were fused into larger ones with bundling several chromosomes (Fig. 5). Namely, every chromosomes are arranged in the nucleus as a unit of prenucleolus and organized into larger groups by fusion of prenucleoli.

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omes per nucleus and those in neuronal cells are fused into prominent nucleoli\textsuperscript{19}. In terms of organizing chromosomes, the prenucleoli may have a similar capacity as nucleoli of such somatic cells.

The present study leads to the conclusion that every chromosome of the embryo is highly active in constructing the prenucleolus by pericentromeric AT-rich regions of DNA. The question remains what is the factor to give rise to such a chromosome activity in embryos. Previous studies by others converge to a high level of DNA methylation of embryo chromosomes\textsuperscript{22,23} as a candidate. It is demonstrated that DNA methylation is related to altered chromatin structure in the nuclei of mouse cell lines\textsuperscript{41} and that a rat chromosomal protein with methyl-CpG-binding activity is localized in pericentromeric regions of chromosomes and binds to narrow groove of AT-rich duplex DNA as Hoechst 33258 does\textsuperscript{42}. Lewis et al.\textsuperscript{32} suggest a possibility that MeCP\textsubscript{2} is involved in compaction of methylated DNA in chromatin, which may result in lowered transcriptional activity of DNA. Hence, further attempts may be worthwhile to identify such proteins as MeCP\textsubscript{2} in embryos and to examine their roles in constructing prenucleoli, though the 5-aza-cytidine experiment has not confirmed the roles of DNA methylation (Fig. 8).

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References


