Interchromatin Granules in the Dividing Embryonic Ectoderm Cells of Postimplantation Rat Embryos: An Electron Microscopic Silver-Staining Study

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The behavior of interchromatin granules (IG) during cell division of rat embryonic ectoderm cells was studied by the electron microscopic silver nitrate-ammoniacal silver nitrate (Ag-AS) staining method. In the interphase cells, the IG formed various-sized clusters which were localized in the nuclei with small-sized accumulations of silver grains after the Ag-AS staining. These IG clusters persisted throughout the subsequent cell division of embryonic ectoderm cells, and were left behind in the cytoplasm of telopbase cells. In the cytoplasm of interpbase cells, however, no IG clusters were present. It appeared that the cytoplasmic IG clusters might break up and that the IG might either disperse into the cytoplasm or disintegrate during the transition from the telophase stage to the interphase stage.

Key words = interchromatin granule: cell division: silver-staining: embryonic ectoderm: rat embryo

INTRODUCTION

Interchromatin granules (IG), measuring about 20-25 nm in diameter, are commonly found in the interchromatin area of the interphase nuclei of normal and abnormal cells. They usually appear as clusters interconnected by fine fibrils. Although some controversy remains concerning their chemical nature, it is widely considered that IG may consist of proteins and a limited amount of RNA. The exact function of IG remains unclear.1,4

There have been only a few brief descriptions made in the literature on the behavior of IG during cell division. Swift3 reported that the IG in ascites tumor cell were moved from the nuclei to the cytoplasm during mitosis. Fakan and Nobis4 indicated that the IG persisted during mitosis of cultured CHO cells, but did not ascertain the fate of IG in the daughter cells. Clevenger and Epstein5 showed that the IG clusters in the transformed Raji and HeLa cells persisted in the mitotic cytoplasm throughout anaphase up until late telophase.

Recently, Hernandez-Verdun et al.6 adapted the light microscopical silver nitrate-ammoniacal silver nitrate (Ag-AS) staining method to the electron microscopic examination of the interphase cell nucleus. After staining with this method, silver grains were shown to be deposited not only on the fibrillar centers and the RNP fibrillar components of the nucleolus, but also on some other nuclear structures including IG.7,8

In the present study, the Ag-AS staining method was used to investigate the behavior of IG during the cell division of rat embryonic ectoderm cells.

MATERIALS AND METHODS

Female Wistar rats were housed with males overnight, and those with sperm in vaginal smears following mating were considered in gestational day 0. On the morning of gesta-
tional day 9, the neurulation-stage embryos were removed from the uteri of pregnant rats. For the standard electron microscopy, they were pre-fixed with Karnovsky's paraformaldehyde-glutaraldehyde fixative (cacodylate-buffered at pH 7.4) for 1 hr at room temperature, post-fixed with Dalton's chrome-osmium fixative (pH 7.2) for 1 hr at 4°C, and embedded in Quetol 812.

For the Ag-AS staining, the embryos were quickly bisected into mesometrial halves and anti-mesometrial halves in Karnovsky's fixative, and the anti-mesometrial halves containing the embryonic ectoderm were pre-fixed with the same fixative for 10 min at room temperature. These materials were post-fixed with Carnoy's solution for 5 min at room temperature, treated with the Ag-AS method described by Hernandez-Verdun et al., and embedded in Quetol 812.

All the ultrathin sections were observed using the JOEL JEM 100B electron microscope after routine double-staining with uranyl acetate and lead nitrate.

RESULTS

Interphase cells

As already reported elsewhere, the nuclei of interphase embryonic ectoderm cells contain highly enlarged and irregular nucleoli. The nucleolonas are reticulated and consist of RNP granular components and RNP fibrillar components. The discrete regions corresponding to the proteinaceous fibrillar center are almost never found in these nucleoli. Loosely aggregated chromatin materials are observed to make contact with the nuclear envelope and the nucleolonema. In the present study, interchromatin granules, measuring about 20–25 nm in diameter, formed loosely-aggregating clusters of various sizes in the interchromatin area (Figs. 1 and 2). Fine fibrillar materials appeared to interconnect these granules (Fig. 2).

After the Ag-AS staining, dense accumulations of silver grains were formed on the fibrillar components of nucleolonema, whereas only a few were observed on the granular components (Fig. 3). The IG clusters were also stained with silver grains, but their grain density appeared to be less than that of the fibrillar components of nucleolonema (Fig. 3). Some spot-like accumulations of silver grains were dispersed on other unidentified nuclear structures, and a few were also noted in the cytoplasm (Fig. 3).

Prophase cells

Chromatin materials were condensed near the nuclear envelope. The nucleolonemas were aggregated into the round form, and their fibrillar components had decreased in amount. After the Ag-AS staining, the density of silver grains on the fibrillar components appeared to be somewhat more reduced than that of the interphase cells, whereas that of the IG clusters appeared to be unchanged (Fig. 4).

Metaphase cells

Both the nuclear envelope and the nucleolus had disappeared, and the condensed chromatin materials were arranged near the central plane of the cell. The various-sized IG clusters were scattered throughout the cytoplasm.

After the Ag-AS staining, numerous spot-like accumulations of silver grains were homogeneously dispersed not only on the condensed chromatin materials but also on the unidentified cytoplasmic structures (Fig. 5). The IG clusters were localized with smaller-sized accumulations of silver grains (Fig. 5).

Telophase cells

The nuclear envelope had been reconstructed, and the chromatin materials reverted from the condensed form into the loosely aggregated state (Fig. 6). The IG clusters were left behind in the cytoplasm, and never incorporated into the daughter cell nuclei (Fig. 6). The size of clusters varied greatly, but rarely exceeded 2 μm in diameter. The
degrees of IG aggregation differed; in some clusters the IG appeared to disperse into the cytoplasm (Fig. 7). Most of the telophase cells contained from one to three cytoplasmic IG clusters in one ultrathin section, and those containing four or five IG clusters were occasionally found (Fig. 6), but no cells having more than five IG clusters were encountered in the present study.

After the Ag-AS staining, dense accumulations of silver grains were localized on the reappearing fibrillar components of the nucleolonema, and the spot-like accumulations were observed on the unidentified nuclear structures (Fig. 8). Smaller-sized accumulations of silver grains were localized on the cytoplasmic IG clusters (Fig. 8).

DISCUSSION

The Ag-AS staining was originally developed to visualize light-microscopically the nucleolus-organizer regions in metaphase chromosomes. Recently, this method has been adapted to the ultrastructural study of interphase cell nuclei, and it has been shown that the RNP fibrillar components of nucleolonema and the proteinaceous fibrillar centers are mainly stained with this method. From many histochemical studies, the materials reactive to the Ag-AS method have been considered to be the non-histone, acidic proteins which are numerous at the sites where ribosomal RNA genes are transcribed actively. From biochemical studies, Busch and his colleagues reported that the major proteins stained with the Ag-AS method are C23 and B23 acidic proteins which are characterized by their high contents of phosphate groups and acidic amino acids. On the other hand, Locke and his colleagues, and also Wassef, showed histochemically that the IG contains large amounts of phosphoproteins. Although there is no evidence that these phosphoproteins in IG are similar to the C23 and B23 proteins, it is plausible that the stainability of IG by the Ag-AS method is due to the presence of these phosphoproteins.

The present study showed that the IG are released into the cytoplasm in cluster form during cell division of the embryonic ectoderm cells. This finding accords well with the recent work of Clevenger and Epstein, indicating that the IG clusters in the transformed Raji and HeLa cells persist in the cytoplasm during cell division. These clusters of granular materials in the cytoplasm of dividing cells have already been reported in certain kinds of normal and pathological cells. Many authors have called these structures the persistent nucleoli, since they consider that these structures may be the remnant of nucleoli dissolving during cell division. In the present study, the Ag-AS method densely stained the fibrillar components of the nucleolonema, but the granular components remained almost unstained. This result accords well with the previous studies using the electron microscopic Ag-AS staining method and indicates that the granular components of the nucleolonema have no direct relationship to the nuclear IG clusters, nor to the cytoplasmic IG clusters.

The function of IG is still unclear. Many investigators have speculated that IG may have a role in the synthesis or transport of ribosomes in the nucleus. The present study clearly showed that IG are completely different from the granular components of the nucleolonema, which are believed to be the precursor of cytoplasmic ribosomes, in cytochemical nature and also in their behavior during cell division. From the present observations that the telophase cells contained not only compactly aggregating IG clusters but also those aggregating loosely, and also that there were no IG clusters in the cytoplasm of interphase cells, it appears that the IG become dispersed throughout the cytoplasm during the transition from the telophase stage to the interphase stage. However, it is uncertain in the present study whether the IG remain intact or disintegrate in the cytoplasm.
of interphase cells. In the interphase cells, the spot-like accumulations of silver grains were sometimes formed after the Ag-AS staining. However, it was difficult to identify whether these grains represent the presence of dispersed IG or the staining artifact. The present study did not clarify the function of IG, but may offer some new clues for its investigation.

REFERENCES

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EXPLANATION OF FIGURES

Fig. 1. Nucleus of the interphase embryonic ectoderm cell of 9-day rat embryo. A large irregular nucleolus consisting of twisted nucleolonema made up of granular components (GC) and fibrillar components (FC), loosely aggregated chromatin materials (CM) and various-sized clusters of interchromatin granules (IG) is observed. NE, nuclear envelope. ×17,700.

Fig. 2. Enlarged view of IG cluster. Fine fibrillar materials appear to interconnect IG (arrow). ×90,600.

Fig. 3. Ag-AS-stained interphase embryonic ectoderm cells. Dense accumulations of silver grains are formed on the fibrillar components of the nucleolonema (FC), whereas only a few are found on the granular components (GC). Smaller-sized accumulations of silver grains are noted on the IG clusters (IG). Numerous spot-like accumulations of silver grains are dispersed on the unidentified nuclear structures (arrows); fewer are seen in the cytoplasm (crossed arrow). ×14,800.

Fig. 4. An Ag-AS-stained prophase embryonic ectoderm cell. Chromatin materials (CM) are condensed near the nuclear envelope (NE), and the nucleolonema takes on a round shape in the central region of the nucleus. The amount of fibrillar components of nucleolonema has decreased, and the silver grains on them appear to be somewhat less dense than in the interphase cell (FC). The accumulations of silver grains on the IG cluster are unchanged (IG). GC, granular component of nucleolonema. ×15,900.

Fig. 5. An Ag-AS-stained metaphase embryonic ectoderm cell. The nuclear envelope and the nucleolus have disappeared, but the IG cluster remains unchanged (IG). Numerous spot-like accumulations of silver grains are dispersed on the condensed chromatin materials (CM) and on the unidentified cytoplasmic structures (arrows). ×13,900.

Fig. 6. A telophase embryonic ectoderm cell with five IG clusters in its cytoplasm (arrows). The nuclear envelope has been reconstructed (NE), and the chromatin materials have reverted to the loosely aggregated state (CM). ×10,500.

Fig. 7. Two IG clusters in the cytoplasm of telophase embryonic ectoderm cell. The IG in the cluster marked “A” are compactly aggregated, whereas in the cluster marked “B” the dispersion of IG into the cytoplasm appears to occur. ×20,300.

Fig. 8. An Ag-AS-stained telophase embryonic ectoderm cell. Dense accumulations of silver grains are obvious on the re-appearing fibrillar components of the nucleolonema (FC), and numerous spot-like accumulations are found on the unidentified nuclear structures (arrows). The IG clusters deposited with smaller-sized accumulations of silver grains are still found in the cytoplasm (IG). ×18,800.
Interchromatin Granules in Dividing Cells