Changes in the number and volume of fibrillar centres with the inactivation of nucleoli at erythropoiesis

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Summary

The number and volume of fibrillar centres, the structural components of interphase cell nucleoli on the surface of which rRNA is synthesized, have been studied in differentiating erythroblasts of mouse embryo liver. Complete series of ultrathin sections of erythroblast nuclei have been used at the main stages of differentiation: proerythroblast, basophilic erythroblast, polychromatophilic erythroblast and normoblast. It has been shown that in the active nucleoli of proerythroblasts the number of fibrillar centres is correlated with cell ploidy and exceeds by several-fold the number of nucleolus-organizing regions of chromosomes. The total volumes of fibrillar centres in 1C (0-369 nm3) and 4C (0-749 nm3) proerythroblasts are proportional to number of nucleolus-organizing regions. With the maturation of erythroblasts the total number of fibrillar centres declines and in normoblasts it is 3- to 10-fold less than that of the nucleolus-organizing regions. The total volume of fibrillar centres in normoblasts (0-102 nm3) is threefold smaller than that in proerythroblasts (0-369 nm3), even though the mean volumes of individual fibrillar centres are significantly higher (0-0042 nm3 in proerythroblasts and 0-039 in normoblasts). The optical density of fibrillar centres in normoblasts can be higher compared with that of proerythroblasts. It has been suggested that the inactivation of nucleoli at erythropoiesis is accompanied by the fusion of individual fibrillar centres and, possibly, by the compaction of their material.

Key words: erythropoiesis, fibrillar centres, nucleolus-organizing regions, morphometric analysis.

Introduction

It is known that clusters of ribosomal genes (r-genes) are located in definite regions of chromosomes, the so-called 'nucleolus-organizing regions' (NORs) (Busch & Smetana, 1970; Smetana & Busch, 1974; Goessens, 1984; Hadjiolov, 1985). A caryologic analysis of metaphase chromosomes shows a strictly definite and stable number of NORs, specific for each species (Long & Dawid, 1980), although the number of active NORs determined by silver-staining may show considerable variation even among cells of the same organism (Goessens, 1984). In interphase nucleoli the r-genes are concentrated in fibrillar centres (FCs) and in the dense fibrillar component, which may correspond to the regions of newly synthesized rRNA (Goessens, 1984; Hadjiolov, 1985). A comparison of the ultrastructure of FCs in many cells indicates their significant morphological similarity. But far less constant are the quantitative characteristics of FCs, i.e. the number and the volume. The causes of such variety are still obscure. According to some authors, the number of FCs indicates, first and foremost, the nucleolar functional activity level (Jordan & McGovern, 1981; Mirre & Kniebiehler, 1982, 1984; Kniebiehler et al. 1983; Vagner-Capodano et al. 1984; Hozák et al. 1986). This supposition is based on investigations of cells (e.g. mouse oocytes, human fibroblasts, lymphocytes of peripheral human blood, pig kidney cells, neurones of rat spinal ganglia) in which the number of FCs increases with the activation of r-genes and decreases with their inactivation (Jordan & McGovern, 1981; Chelidze, 1982; Raška et al. 1983; Mirre & Kniebiehler, 1984; Vagner-Capodano et al. 1984; Pèbusque et al. 1985; Hozák et al. 1986). Much less is known about the dependence of the number of FCs on the amount of r-gene copies. It is only in the work of Mirre & Kniebiehler (1984) that data have been cited showing that the maximal number of FCs may be correlated with the number of ribosomal cistrons; in mouse Sertoli diploid cells as many as 50 FCs, and in tetraploid oocytes about 100 FCs, were detected. The dependence of changes in FC volume with the degree of nucleolar functional activity has not been explored either. According to preliminary data, the linear dimensions of individual FCs decrease with the growth and activation of
nucleoli, whereas the total volume of FCs is progressively on the increase (Mirre & Kniebiehler, 1984; Hozák et al. 1986). There is to date contradictory evidence on the correlation between the number of FCs and that of NORs. There are cells in which the number of FCs significantly exceeds that of NORs (Mirre & Kniebiehler, 1982, 1984; Seite & Pêbusque, 1985; Pêbusque et al. 1985; Hozák et al. 1986), but there are also others in which the reverse has been observed (Raška et al. 1983; Devictor et al. 1984).

The present work deals with the structural and quantitative parameters of FCs in the process of natural inactivation of nucleoli. As test models we used differentiating mouse erythroblasts: proerythroblasts (PEB), basophilic erythroblasts (BEB), polychromatophilic erythroblasts (PCB) and normoblasts (NB) in which rRNA synthesis is arrested by the maturation process (Busch & Smetana, 1970; Smetana & Busch, 1974). We also attempted to determine the dependence of changes in the number and volume of FCs on the number of NORs in the cell, by analysing active nucleoli in 2C and 4C PEBs. Cell ploidy was determined according to the data on the number and structure of centrioles in mouse cells (Onishchenko, 1978; Vorobjev & Chentsov, 1987).

Materials and methods

The liver of 12- to 14-day mouse embryos is a convenient organ in which to observe maturing erythroid cells (Rhodin, 1974). Liver pieces from a 12-day embryo were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4) for 2h at 4°C, washed with the same buffer and postfixed with a buffered 1% solution of OsO4 for 2h at 4°C. Then the pieces were dehydrated in a series of ethanol at increasing concentrations (70% ethanol contained 2% uranyl acetate), washed in absolute acetone and embedded in Epon. Ultrathin serial sections (0.1 μm) were prepared on a LKB-II ultramicrotome. After the ultrathin sections had been contrasted with lead citrate according to the Reynolds procedure, they were examined under HU-11B and HU-12 electron microscopes. The nucleoli were photographed at a magnification of ×10000. Then under a photographic enlarger (final magnification was ×50 000), the contours of the nucleoli and FCs were drawn and, with a Hewlett-Packard computer, their surface areas were determined. The volumes of the nucleoli and FCs were calculated according to the formula:

$V = \sum \frac{s \cdot d}{T}$

where $S$ is the cross-section area, $D$ is the thickness of the section, equal to 0.1 μm, and $n$ is the number of sections. To obtain planar images of the nucleoli and FCs, the contours of the structures were drawn on transparent films, which then were collated and photographed. The compaction of the dense fibrillar material within FCs was determined with an MF-4 densitometer. Optical density measurements were performed on the negative cell images, photographed on the same section and developed under identical conditions. In all these techniques we also took proper account of background optical densities (i.e. in intercellular spaces devoid of any structures). We made 20–30 measurements of the optical density of each FC. The statistical processing of the results was performed according to Student’s criterion.

We know that proerythroblasts and basophilic erythroblasts are proliferation-capable cells (Rhodin, 1974; Smetana & Busch, 1974). For this reason they may be at any stage of the cell cycle: G1, S or G2 period, and thus have different ploidy. In order to determine cell ploidy on ultrathin sections, we used the structural data of the centriolar apparatus in interphase mouse cells (Onishchenko, 1978; Vorobjev & Chentsov, 1987). It was shown that mouse cells containing two adjacent centrioles are in the G1 period, while the two pairs of centrioles are at the end of the S and in the G2 period. It may be inferred on the basis of these data that of the five PEBs studied by us, three persisted in the G1 (or early S) period, i.e. were 2C, and the other two were in the late S and in the G2 period, i.e. were 4C. All the basophilic erythroblasts were 2C.

Overall, we studied five PEBs, five BEBs, three PCBs and six NBs.

Results

Fig. 1A represents a region of mouse embryo liver section photographed at low magnification. It shows distinctly dividing and non-dividing erythroid cells at different maturation stages. Proerythroblasts, basophilic erythroblasts, polychromatophilic erythroblasts and normoblasts are easily identifiable by size, the structure of the nucleus and the presence of haemoglobin in the cytoplasm.

Proerythroblasts (Figs 1, 2A,B)

These are large cells, measuring 10–12 μm in diameter, containing round or ovoid nuclei with a small amount of compact chromatin. Usually a single large nucleolus of the nucleolomeral type is detectable in the nuclei (Fig. 1A,B). Distinctly visible in the nucleoli are RNP granules, FCs surrounded by a layer of the RNP fibrillar component and numerous small vacuoles (interstices) (Figs 1D, 2A). The peri- and intranucleolar chromatin is not significantly present.

An analysis of ultrathin sections shows that in PEBs FCs have predominantly spherical or ellipsoid forms and are evenly distributed in the nucleolar volume (Fig. 3). The results of the morphometric analysis of FCs in 2C and 4C PEBs are shown in Table 1.

Basophilic erythroblasts (Fig. 2C-E)

The nuclei of this group of erythroblasts have an irregular, polygonal form. They are smaller in size then those of PEBs and are distinguished by the greater compaction of their chromatin (Fig. 2C,E). Attached to the nucleoli are large blocks of perinucleolar chromatin, thus blurring the nucleolar boundaries. Compared with PEBs nucleoli, the BEBs nucleoli have smaller dimensions and contain less of the granular component (Fig. 2E). FCs are distinct (Fig. 2C,E).

As shown by the ultrathin section analysis data, FCs in BEBs are fairly diverse in form: as well as the spherical FCs, one often comes across FCs with a complex and ramified configuration (Fig. 4). Most of the FCs are located in peripheral nucleolar regions, at the border of the perinucleolar chromatin (Fig. 2C,E).

The morphometric analysis data on FCs in five 2C BEBs are given in Table 2.

Polychromatophilic erythroblasts (Fig. 5A,B)

In volume, the nuclei of PCBs are no larger than 4 or
Fig. 1. Ultrathin section of 12-day-old mouse embryo (A), general view of 2C proerythroblast (B), its centriolar apparatus (C) and fragment of the nucleolus in proerythroblast (D). D. Arrows indicate FCs. PEB, proerythroblast; BEB, basophilic erythroblast; PCB, polychromatophilic erythroblast; NB, normoblast. dc. Dividing cell; n, nucleus; nu, nucleolus; c, cytoplasm; f, fibrillar component; g, granular component. Bars: A, 3 μm; B,C, 1 μm; D, 0.5 μm.

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Fig. 2. Fragment of proerythroblast nucleolus (A) containing four centrioles (B), general view of the basophilic erythroblast nucleus (C), containing two centrioles (D) and a region of its nucleolus (E). A, E. Arrows indicate FCs. n, nucleus; nu, nucleolus; c, cytoplasm; cch, condensed chromatin; g, granular component; f, fibrillar component. Bars: E, 0.5 μm; A–D, F, G, 1 μm.
Nonnoblats (Fig. 5C,D) tana & Busch, 1974). As well as ring-shaped nucleoli, the nuclei of some PCBs may contain zones of residual fibrils (Fig. 5B). In structure, they are similar to the so-called devoid of the granular component and are represented by FCs surrounded by a layer of densely packed fibrils (Fig. 5C). In form, they are similar to the so-called ring-shaped nucleoli characteristic of many specialized cells, including myelocytes and lymphocytes (Smetana & Busch, 1974). As well as ring-shaped nucleoli, the nuclei of some PCBs may contain zones of residual fibrillar material corresponding to ‘free’ FCs. Their number per nucleus does not exceed two or three.

The BEB nuclei have (Fig. 5A). The nucleoli are represented by FCs surrounded by a layer of densely packed fibrils. Their volume per cell is as good as devoid of organelles and filled with haemoglobin granules (Fig. 5C). Their cytoplasm is as good as devoid of organelles and filled with condensed chromatin blocks but are separated from them by a narrow electron-transparent rim (Fig. 5C,D). In form, the FCs are rather spherical or ellipsoid (Fig. 7).

Normoblasts, as well as PCBs, are incapable of division; accordingly, we did not count the number of centrioles. Normoblast ploidy is equal to 2C (Table 4). The data of nucleolar volume, and FC number and dimensions in PEBs, BEBs, PCBs and NBs are summarized in Table 5.

Results of densitometric analysis of the compaction of the FC material in PEBs are summarized in Table 6. They show that the optical density of FCs in PEBs is nearly 1.5 times lower than that of FCs in normoblasts.

5 μm, and have much more condensed chromatin than the BEB nuclei have (Fig. 5A). The nucleoli are practically devoid of the granular component and are represented by FCs surrounded by a layer of densely packed fibrils (Fig. 5B). In structure, they are similar to the so-called ring-shaped nucleoli characteristic of many specialized cells, including myelocytes and lymphocytes (Smetana & Busch, 1974). As well as ring-shaped nucleoli, the nuclei of some PCBs may contain zones of residual fibrillar material corresponding to ‘free’ FCs. Their number per nucleus does not exceed two or three.

Reconstruction of serial ultrathin sections shows that in PCBs FCs have as many forms as in BEBs (Fig. 6). It is known that at the polychromatophilic erythroblast stage the cells lose their capacity for division, with their ploidy being equal to 2C. For this reason we did not determine the number of centrioles in polychromatophiles. The results of the respective morphometric analysis of FCs are shown in Table 3.

Table 1. Quantitative characteristics of nucleoli (Nu) and fibrillar centres (FCs) in proerythroblasts

<table>
<thead>
<tr>
<th>No. of cell</th>
<th>Ploidy (C)</th>
<th>Nu number</th>
<th>Nu volume (μm³)</th>
<th>FC number</th>
<th>FC mean diameter (μm)</th>
<th>FC mean volume (μm³)</th>
<th>FC total volume per cell (μm³)</th>
</tr>
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<tr>
<td>1</td>
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<td>1</td>
<td>17-727</td>
<td>88</td>
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<td>0-0041</td>
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<td>1</td>
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<td>0-20</td>
<td>0-0042 ± 0-0007</td>
<td>0-369 ± 0-008</td>
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<tr>
<td>4</td>
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<td>1</td>
<td>28-336</td>
<td>114</td>
<td>0-23</td>
<td>0-0062</td>
<td>0-707</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1</td>
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<td>121</td>
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<td>0-0066</td>
<td>0-790</td>
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<td>29-443</td>
<td>118</td>
<td>0-23</td>
<td>0-0064 ± 0-0004</td>
<td>0-749 ± 0-042</td>
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</table>

M ± m, mean and standard error of the mean.

Table 2. Quantitative characteristics of nucleoli (Nu) and fibrillar centres (FCs) in basophilic erythroblasts

<table>
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<th>No. of cell</th>
<th>Ploidy (C)</th>
<th>Nu number</th>
<th>Nu volume (μm³)</th>
<th>FC number</th>
<th>FC mean diameter (μm)</th>
<th>FC mean volume (μm³)</th>
<th>FC total volume per cell (μm³)</th>
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<td>2</td>
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<td>12</td>
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<td>0-0120</td>
<td>0-1440</td>
</tr>
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<td>2</td>
<td>2</td>
<td>2-388</td>
<td>8</td>
<td>0-338</td>
<td>0-0201</td>
<td>0-1608</td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>5-766</td>
<td>4</td>
<td>0-410</td>
<td>0-0355</td>
<td>0-1420</td>
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<td>2</td>
<td>4-026</td>
<td>6</td>
<td>0-411</td>
<td>0-0360</td>
<td>0-2160</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>4-336</td>
<td>8</td>
<td>0-340</td>
<td>0-0203</td>
<td>0-1624</td>
</tr>
<tr>
<td>Mean</td>
<td>2</td>
<td>2</td>
<td>4-568</td>
<td>7-6</td>
<td>0-360</td>
<td>0-0248 ± 0-005</td>
<td>0-1700 ± 0-013</td>
</tr>
</tbody>
</table>

M ± m, mean and standard error of the mean.

Discussion

The evidence obtained in this work shows that mouse differentiating erythroblasts are a very suitable object for solving many problems related to nucleolar restructuring and quantitative analysis of FCs. Comparing different types of cells, we may trace the dependence of FC number and volume on the degree of functional activity of the nucleoli and cell ploidy, i.e. the number of NORs and the amount of r-genes. It will be noted, however, that there is practically no evidence of a correlation between the morphometric parameters of FCs and the number of r-genes and NORs.

Cells with equal functional activity but different ploidy may well be exemplified by 2C and 4C PEBs, which are in the G₁ and G₂ periods of the cell cycle, respectively. From Table 5 we can see that the volume of nucleoli and the total volume of FCs in these PEBs differ by twofold and are explicitly correlated with the amount of NORs.
Fig. 3. Three-dimensional structure and distribution of FCs in proerythroblast nucleolus. A. Sections N13, 14, 15. B. Sections N15, 16, 17. FCs have predominantly spherical or ellipsoid forms and are evenly distributed in the nucleolar volume. Bar, 0.5 μm.

Fig. 4. Three-dimensional structure and distribution of FCs in basophilic erythroblast nucleolus. Besides the spherical FCs (A), one often comes across FCs with a complex and ramified configuration (B). Bars, 0.5 μm.

The evidence for duplication of nucleolar volume with the transition of cells from the G1 to the G2 period is well known (Lepoint & Goessens, 1982; Goessens, 1984). It was also shown with Ehrlich cells that an increase in nucleolar size in the cell cycle does not result in a change in the ratio between areas of nucleoli and those of FCs (Goessens, 1984). This may be possible only in the case when, concurrently with the duplication of the area (and volume) of the nucleoli, the area (and volume) of FCs are also doubled. Thus, there is good reason to believe that the total volume of FCs is proportional to the number of NOR sets contained in the cell.

Unlike the total volume, the number of FCs is not strictly correlated with cell ploidy. Thus, PEBs in the G2 period (4C) contain only 1.4-fold more FCs than PEBs in the G1 period (2C). This must indicate that rDNA replication, which terminates by the G2 period, is not always accompanied by separation of the 'daughter' and 'mother' FCs. This conclusion is likewise supported by the fact that the mean volumes of individual FCs in the G2 period are enhanced compared with those in the G1 period (Table 5). Unfortunately, there is scant direct evidence in the literature on the dependence of the FC number on cell ploidy. According to light-microscopic data, the number of Ag granules identifying FCs and the dense RNP fibrillar component is not precisely doubled with the transition of cells from the G1 to the G2 period (Hubbell et al. 1980; Vagner-Capodano et al. 1984).

So, the results of the present work enable us to conclude that in 2C and 4C PEBs, the nucleoli of which actively synthesize rRNA, the number of FCs and the total volume of FCs are correlated with cell ploidy and the number of r-genes.

To find out how a change in ribosomal gene activity affects the number of FCs and their dimensions, we compared 2C erythroblasts at the main differentiation stages: proerythroblast, basophilic erythroblast, polychromatophilic erythroblast and normoblast. We know that with maturation of erythroblasts, the level of rRNA synthesis in them declines. This is indicated by copious published evidence (Busch & Smetana, 1970; Smetana & Busch, 1974; Smetana, 1980; Goessens, 1984; Smetana & Likovsky, 1984), as well as by our own observation of nuclear and nucleolar morphology at erythropoiesis (Chelidze et al. 1984). In maturing erythroblasts, the chromatin condensation rises gradually, the volumes of nucleoli decrease and the RNP fibrillar and granular material is lost. As a result, of all the nucleolar components, only free FCs, devoid of dense fibrillar component and not capable of rRNA synthesis, persist in NBs. Similar changes in the nuclear apparatus have also been described in the columnar cells of rat jejunum (Altmann & Leblond, 1982).

An analysis of the total number of FCs in differentiating erythroblasts shows that they are progressively on the decrease, with the transition of the nucleoli from the active to the inactive state: the number of FCs in NBs is 12-fold smaller than in PEBs. The decrease in the number of FCs proceeds along with an increase in the volume of individual centres, and so in PCBs (0.026 μm³) and NBs (0.039 μm³) they were 6- to 10-fold larger than
Fig. 5. General view of nuclei (A,C) and nucleoli (B,D) in polychromatic erythroblast (A,B) and normoblast (C,D). A,B. The nucleolus consists of one FC (arrows) and a small amount of the dense fibrillar component (broken lines). C,D. Of all the nucleolar components only free FCs (arrows) persist, within an FC one can see electron-dense material corresponding to perinucleolar condensed chromatin. n, nucleus; c, cytoplasm; cch, condensed chromatin; f, fibrillar component. Bars: A,C, 1 μm; B,D 0.5 μm.
Table 3. Quantitative characteristics of nucleoli (Nu) and fibrillar centres (FCs) in polychromatophilic erythroblasts

<table>
<thead>
<tr>
<th>No. of cell</th>
<th>Nu number</th>
<th>Nu volume (µm$^3$)</th>
<th>FC number</th>
<th>FC mean diameter (µm)</th>
<th>FC mean volume (µm$^3$)</th>
<th>FC total volume per cell (µm$^3$)</th>
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<td>1</td>
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<td>Mean</td>
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<td>4-3</td>
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<td>0.0239 ± 0.0013</td>
<td>0.1110 ± 0.0022</td>
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</tbody>
</table>

M ± m, mean and standard error of the mean. Cell ploidy, equal to 2C.

Table 4. Quantitative characteristics of residual nucleoli (Nu) and free fibrillar centres (FCs) in normoblasts

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<thead>
<tr>
<th>No. of cell</th>
<th>Nu number</th>
<th>Nu volume (µm$^3$)</th>
<th>FC number</th>
<th>FC mean diameter (µm)</th>
<th>FC mean volume (µm$^3$)</th>
<th>FC total volume per cell (µm$^3$)</th>
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<td>6</td>
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<td>0.039 ± 0.002</td>
<td>0.1020 ± 0.0117</td>
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M ± m, mean and standard error of the mean.
3FCs: one is within the residual nucleolus, the two others are free.

Table 5. Morphometric analysis data on nucleoli (Nu) and fibrillar centres (FCs) at erythropoiesis in mouse embryo cells

<table>
<thead>
<tr>
<th>Stage</th>
<th>Nu volume per cell (µm$^3$)</th>
<th>FC number</th>
<th>FC mean diameter (µm)</th>
<th>FC mean volume (µm$^3$)</th>
<th>FC total volume per cell (µm$^3$)</th>
<th>Correlation of total FC volumes*</th>
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<tr>
<td>Proerythroblast, 2C</td>
<td>17 700</td>
<td>88</td>
<td>0.20</td>
<td>0.0042 ± 0.0001</td>
<td>0.369 ± 0.008</td>
<td>3.6</td>
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<td>Proerythroblast, 4C</td>
<td>29 443</td>
<td>118</td>
<td>0.23</td>
<td>0.0064 ± 0.0004</td>
<td>0.749 ± 0.042</td>
<td>7.3</td>
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<td>Basophilic erythroblast, 2C</td>
<td>4 568</td>
<td>7.6</td>
<td>0.36</td>
<td>0.0248 ± 0.005</td>
<td>0.170 ± 0.013</td>
<td>1.7</td>
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<tr>
<td>Polychromatophilic erythroblast, 2C</td>
<td>0.547</td>
<td>4.3</td>
<td>0.37</td>
<td>0.0259 ± 0.001</td>
<td>0.111 ± 0.002</td>
<td>1.1</td>
</tr>
<tr>
<td>Normoblast, 2C</td>
<td>0.102</td>
<td>2.7</td>
<td>0.42</td>
<td>0.0390 ± 0.002</td>
<td>0.102 ± 0.012</td>
<td>1.0</td>
</tr>
</tbody>
</table>

M ± m, mean and standard error of the mean.
*Total volume of FCs in normoblasts is assumed to 1.

Table 6. Optical density of the electron-microscopic negatives of fibrillar centres (FCs) in proerythroblasts (PEB) and normoblasts (NB)

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of cell</th>
<th>FC optical density* per cell</th>
<th>FC mean optical density* per stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEB</td>
<td>1</td>
<td>73 ± 1.3</td>
<td>76 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>69 ± 0.8</td>
<td>87 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>87 ± 0.7</td>
<td>94 ± 0.9</td>
</tr>
<tr>
<td>NB</td>
<td>1</td>
<td>111 ± 1.1</td>
<td>103 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>111 ± 1.1</td>
<td>103 ± 0.21</td>
</tr>
</tbody>
</table>

M ± m, mean and standard error of the mean.
*Optical density is indicated in arbitrary units.
FCs broken lines). in polychromatophilic erythroblasts are by FCs surrounded by a layer of densely packed fibrils (/, broken lines). B. The nucleolus is represented and a small amount of the dense fibrillar component (/, broken lines). B. The nucleolus is represented by FCs surrounded by a layer of densely packed fibrils (/, broken lines). FCs in polychromatophilic erythroblasts are multiform as in basophilic erythroblasts. Bar, 0-5 μm.

Fig. 6. Three-dimensional structure of residual nucleoli in polychromatophilic erythroblasts. A. The nucleolus consists of one FC and a small amount of the dense fibrillar component (/, broken lines). B. The nucleolus is represented by FCs surrounded by a layer of densely packed fibrils (/, broken lines). FCs in polychromatophilic erythroblasts are multiform as in basophilic erythroblasts. Bar, 0-5 μm.

Fig. 7. Three-dimensional structure and distribution of free FCs in a normoblast nucleus (n). Bar, 0-5 μm.

in PEBs (0.0042 μm³). On the whole, these observations are very consistent with the data of other authors, who have shown that inactivation of nucleoli in some cells leads to a decrease in the number of FCs on ultrathin sections and to an increase in their linear dimensions. By way of an example, we may cite the results of investigations with pig kidney (PK) cells in the G₀ and G₂ periods (Hozák et al. 1986), epithelial cells of jejunum (Altmann & Leblond, 1982), human fibroblasts (Jordan & McGovern, 1981) and a study of PK cells incubated in the presence of actinomycin D (Chelidze, 1982).

The simplest explanation for the decrease in the number of FCs per cell and the increase in their individual dimensions would be that, with the inactivation of nucleoli, individual FCs associate with one another (Hernandez-Verdun, 1986). Yet, a comparison of total FC volume argues against this conclusion: a decrease in the number of FCs is accompanied by a decrease in their total volume (Table 5). This may be partially explained by a tighter compaction of material within FCs, as testified by a comparison of the optical density of some of the FCs in PEBs and in NBs (Table 6). Another reason may be the loss of part of the protein component, e.g. RNA polymerase I (Goessens, 1984), which is always present in active nucleoli (Hadjiolov, 1985). Both processes in differentiating erythroblasts must be taking place simultaneously.

The correlation between the number of NORs and FCs has been actively debated in the literature. The key to this problem is to find out how many FCs are formed by one NOR. According to the data from the present work, in active PEB nucleoli the number of FCs exceeds that of NORs 5- to 10-fold. Thus, no less than 100 FCs are detected in tetraploid PEBs, whereas the number of NORs in them should not exceed 20 (we know that in mouse cells there are five NO chromosomes per haploid set and only three or four are the ‘working ones’ (Dev et al. 1977). This means that in active nucleoli one NOR forms more than one FC. Apparently this regularity applies to the majority of animal cells (Jordan & McGovern, 1981; Mirre & Kniebiehler, 1982, 1984; Kniebiehler et al. 1983; Seite & Pébusque, 1985; Hozák et al. 1986).

With the inactivation of nucleoli the number of FCs formed by one NOR progressively declines. The persisting FCs tend to aggregate and, as a consequence, the overall number of FCs proves to be smaller than that of NORs.

References


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