Physiol. Res. 44: 193–196, 1995

Nucleoli and Argyrophil Nucleolus Organizer Regions (AgNORs) of Cells of the Megakaryocytic Line in the Rat

J. JANOUTOVÁ[1], Z. LIKOVSKÝ[1,2]

[1]*Institute of Histology and Embryology, Third Medical Faculty, Charles University and* [2]*Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

Received January 30, 1995
Accepted March 23, 1995

Summary

The main maturation stages of Norway rat megakaryocytic series, megakaryoblasts and mature megakaryocytes, stained by silver for demonstration of argyrophil nucleolus organizer regions (AgNORs) were investigated to provide basic information on the number of nucleoli and interphasic AgNORs in these cells. The results showed that megakaryoblasts as well as mature megakaryocytes possess numerous nucleoli; their number and also the number of AgNORs is significantly higher in less mature than in more mature cells. The number of AgNORs in megakaryocytes of the Norway rat and man are virtually the same, although the numbers of nucleolar organizers per haploid chromosome set differ markedly. This fact leads to the conclusion that the number of interphasic AgNORs depends on the function and metabolic state of the cell rather than on the number of nucleolar organizers.

Key words

Cell nucleolus – Megakaryoblast – Megakaryocyte – Nucleolus organizer

Introduction

Blood cells and their precursors are a very convenient model for studying changes in the nucleoli and their activity in the course of differentiation and maturation processes. The decrease of rRNA biosynthetic activity in blood cells is reflected in a change of the proportion of the main nucleolar types in maturing cells as well as by a gradual decrease in the number of interphasic AgNORs, representing the active portions of nucleolus organizing regions (NORs) of mitotic chromosomes (Busch and Smetana 1970, Grasso *et al.* 1963, Likovský and Smetana 1981, 1990, Smetana 1980, Smetana *et al.* 1975, Smetana and Likovský 1984).

In cells of the megakaryocytic series, i. e. precursors of blood platelets of mammals, the number of nucleoli and their activity in the sense of rRNA synthesis under physiological conditions has hitherto been reported in human megakaryocytes only (Matolcsy *et al.* 1992). To provide quantitative information on the number of nucleoli and their activity in rRNA synthesis in both main maturation stages of the megakaryocytic series, i.e. megakaryoblasts and mature megakaryocytes, we have studied this in white Norway rats.

Materials and Methods

Six male Wistar (130–150 g, conventional Velaz breed) were killed by cervical dislocation under ether anaesthesia. Smear preparations from the samples of bone marrow, taken from femurs (Schermer 1958) were stained for demonstration of silver stained nucleolar proteins (Likovský and Smetana 1981), and poststained by 0.01 % aqueous

solution of methylene blue. In every smear preparation, the nucleolar coefficient, i. e. the average number of nucleoli per cell (Busch and Smetana 1970) and the number of interphasic AgNORs per cell were estimated in 50 megakaryoblasts and 50 mature megakaryocytes (Williams and Levine 1982) of each animal.

From these results the sample means ± S.E.M. were calculated; the differences between means were evaluated by the paired t-test.

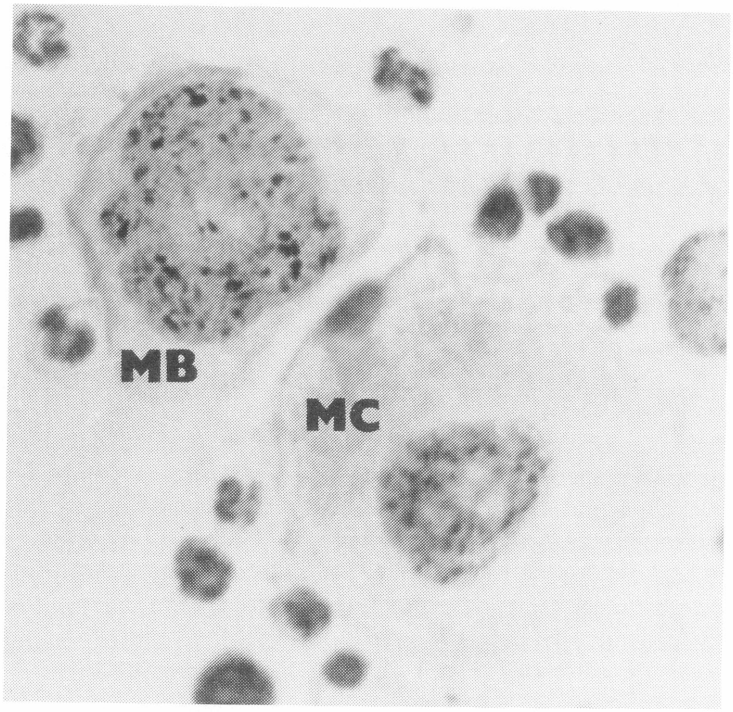

Fig. 1
Megakaryoblasts (MB) and megakaryocytes (MC) stained by silver for AgNORs, without poststaining (x740).

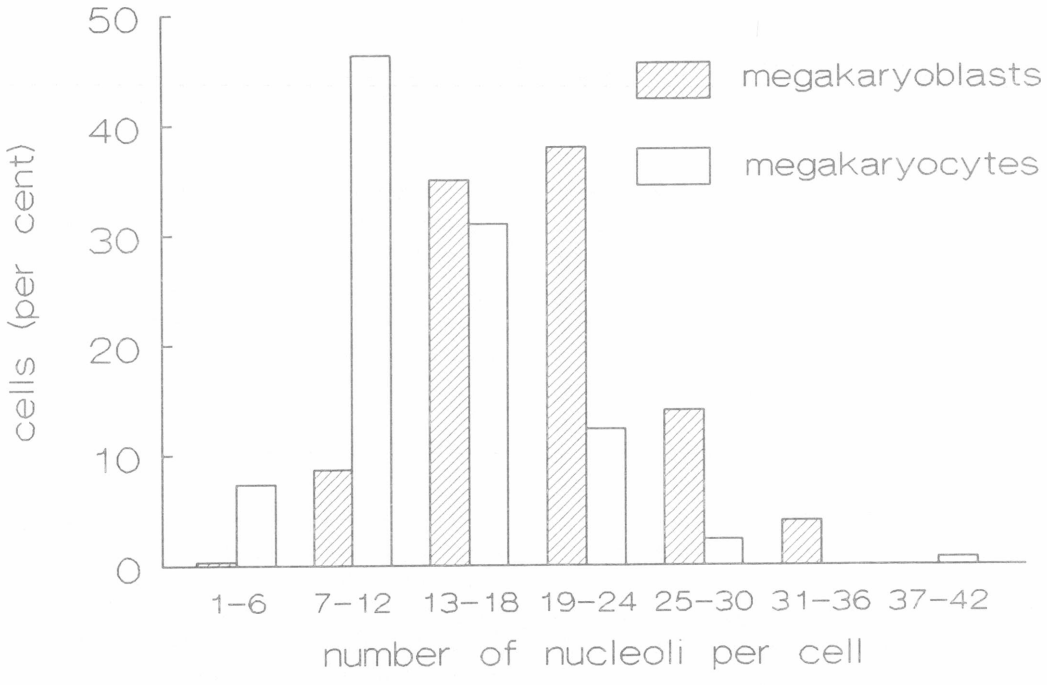

Fig. 2
Distribution of the number of nucleoli in rat megakaryoblasts and megakaryocytes.

Results

Both investigated maturation stages of megakaryocytic series of the rat were characterized by the presence of numerous nucleoli in their nuclei (Fig. 1). The number of nucleoli per cell was higher in megakaryoblasts than in mature megakaryocytes (Fig. 2); the value of the nucleolar coefficient being significantly higher in the former than in the latter (Table 1).

The number of interphasic AgNORs per cell (Fig. 3) as well as their average number per cell (Table 1) was significantly lower in the mature megakaryocytes than in the megakaryoblasts.

Table 1
The number of nucleoli per cell (nucleolar coefficient) and number of AgNORs per cell in rat megakaryoblasts and mature megakaryocytes.

	Megakaryoblasts	Megakaryocytes
Number of nucleoli	19.72±0.41	13.18±0.77*
Number of AgNORs	55.08±6.02	27.00±1.25*

*Data are means±S.E.M. * significantly different (P<0.01) from megakaryoblasts*

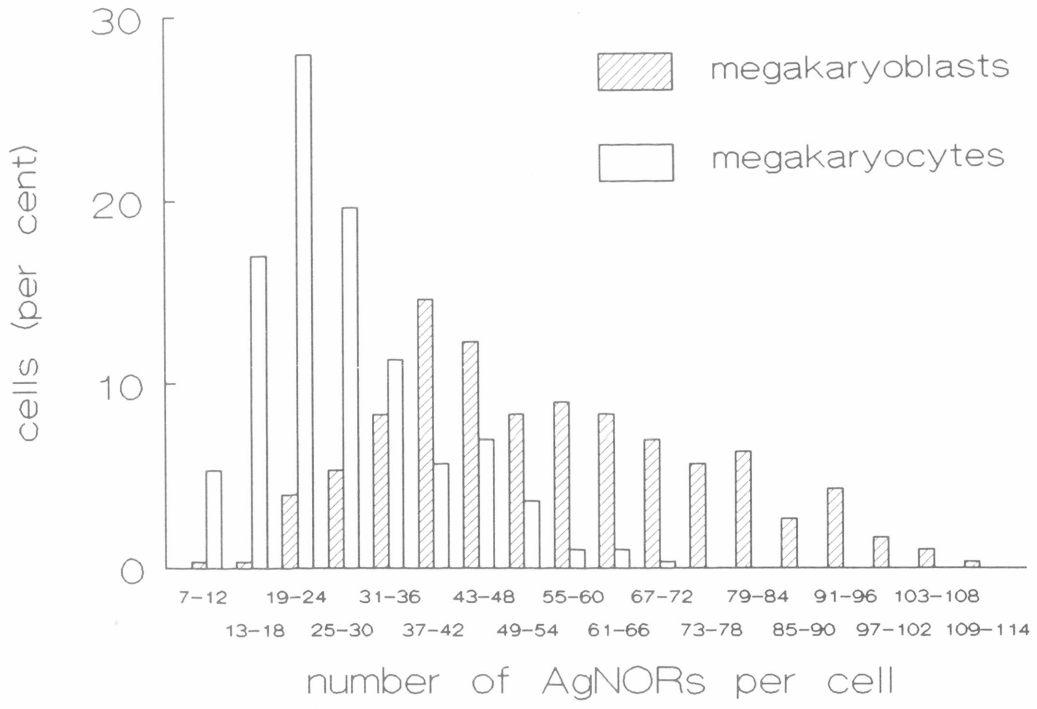

Fig. 3
Distribution of the number of AgNORs in rat megakaryoblasts and megakaryocytes.

Discussion

The observed high number of nucleoli (nucleolar coefficient) in the cells of rat megakaryocytic series and their great variation can be related to polyploidy of these cells, which can vary in rat megakaryocytes from 4 N to 64 N (Odell *et al.* 1965). The polyploidy leads to the increase of NORs, and, therefore, also to an increase in the number of nucleoli. The lower number of AgNORs in mature megakaryocytes compared to megakaryoblasts reflects the decrease of nucleolar biosynthetic activity in the sense of RNA transcription level of cell activity during maturation (Jordan and McGovern 1981, Kacerovská *et al.* 1980, Likovský and Smetana 1981, 1990, Ochs and Smetana 1989, Smetana and Likovský 1984, Trere *et al.* 1989) and is in accord with contemporary knowledge.

As the counts of AgNORs in human (Matolcsy *et al.* 1992) and rat (our results) megakaryocytes appeared to be practically identical, it may be concluded that the number of nucleoli and AgNORs depends rather on the cell metabolic activity than on the number of nucleolar organizers in the haploid chromosome set, which strikingly differ between the Norway rat and man (three and five, respectively) (Kohno *et al.* 1979, Trent *et al.* 1981, Yosida 1979).

Acknowledgement
This work was supported in part by the Ministry of Ecology and Environment of the Czech Republic (Grant GA/49/93).

References

BUSCH H., SMETANA K.: *The Nucleolus.* Academic Press, New York, 1970.

GRASSO J., WOODARD J., SWIFT H.: Cytochemical studies of nucleic acid and proteins in erythrocytic development. *Proc. Natl. Acad. Sci. USA* **50:** 134–140, 1963.

JORDAN E. G., McGOVERN J. H.: The quantitative relationship of the fibrillar centres and other nucleolar components to changes in growth conditions, serum deprivation and low doses of actinomycin D in cultured diploid human fibroblasts (strain MRC-5). *J. Cell Sci.* **52:** 373–389, 1981.

KACEROVSKÁ H., LIKOVSKÝ Z., SMETANA K.: Nucleolar silver stained granules in rat Yoshida sarcoma cells after RNA synthesis inhibition. *Neoplasma* **28:** 513–516, 1980.

KOHNO S., ABE S., MATSUI S., SANDBERG A. A.: Chromosomes and causation of human cancer and leukemia. XXXVII. Nucleolus organizers on the Ph'chromosome in chronic myelocytic leukemia. *Cancer Genet. Cytogenet.* **1:** 15–20, 1979.

LIKOVSKÝ Z., SMETANA K.: Further studies on the cytochemistry of the standardized silver staining of interphase nucleoli in smear preparations of Yoshida ascitic sarcoma cells in rats. *Histochemistry* 72: 301–313, 1981.

LIKOVSKÝ Z., SMETANA K.: Nucleoli in the cells of the erythrocyte and granulocyte series in the albino rat. *Physiol. Bohemoslov.* **39:** 505–511, 1990.

MATOLCSY A., NÁDOR R., WÉBER E., KÓNYA T.: Nucleolar organizer regions of megakaryocytes in chronic myeloproliferative disorders. *Virchows Arch. A Pathol. Anat.* **420:** 441–445, 1992.

OCHS R. L., SMETANA K.: Fibrillar center distribution in nucleoli of PHA-stimulated human lymphocytes. *Exp. Cell Res.* **184:** 552–557, 1989.

ODELL T. T. Jr., JACKSON C. W., GOSSLEE D. G.: Maturation of rat megakaryocytes studied by microspectrophotometric measurement of DNA. *Proc. Soc. Exp. Biol. Med.* **119:** 1194–1199, 1965.

SCHERMER S.: *Die Blutmorphologie der Laboratoriumstiere.* Barth, Leipzig, 1958.

SMETANA K.: Nucleoli in maturing blood cells. In: *Topical Reviews in Hematology.* S. ROATH (ed), vol. I., Wright & Sons, Bristol, 1980, pp. 115–137.

SMETANA K., LIKOVSKÝ Z.: Nucleolar silver-stained granules in maturing erythroid and granulocytic cells. *Cell Tissue Res.* **237:** 367–370, 1984.

SMETANA K., GYORKEY F., GYORKEY P., BUSCH H.: Studies on nucleoli of maturing human erythroblasts. *Exp. Cell Res.* **91:** 143–151, 1975.

TRENT J. M., CARLIN D. A., DAVIS J. R.: Expression of silver stained nucleolar organizing regions (AgNORs) in human cancer. *Cytogenet. Cell Genet.* **30:** 31–38, 1981.

TRERE D., PESSION A., DERENZINI M.: The silver-stained proteins of interphasic nucleolar organizer regions as a parameter of cell duplication rate. *Exp. Cell Res.* **184:** 131–137, 1989.

WILLIAMS N., LEVINE R. F.: The origin, development and regulation of megakaryocytes. *Br. J. Haematol.* 52: 173–180, 1982.

YOSIDA T. H.: A comparative study on nucleolus organizer regions (NORs) in 6 Rattus species with special emphasis on the organizer differentiation and species evolution. *Proc. Jpn. Acad.* **55 B:** 481–486, 1979.

Reprint Requests
Dr. J. Janoutová, Institute of Histology and Embryology, Third Medical Faculty, Charles University, Ruská 87, 100 00 Prague 10, Czech Republic.