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RELATIVE AMOUNT OF HMG 1 AND HMG 2 PROTEINS  
IN DIFFERENT SYRIAN HAMSTER TISSUES

Relative amount of HMG 1 and HMG 2 fractions have been established in different Syrian hamster tissues. It has been detected that in cerebral hemispheres, the liver of healthy animals and in Kirkman-Robbins hepatoma as well as in the liver of tumor bearing animals, the amounts of HMG 1 and HMG 2 have been basically the same. A significant increase of HMG 2 fraction have been found in Syrian hamster testes.

INTRODUCTION

Among the more abundant non-histone proteins there is the high mobility group (HMG). In mammals, the four major HMG can be divided into two categories: the proteins HMG 1 and HMG 2 as well as HMG 14 and HMG 17. HMG 14 and HMG 17 have been involved in the maintenance of the chromatin configuration necessary for transcription [8]. HMG 1 and HMG 2 are of the same size and show homology in sequence. Functions of HMG 1 and HMG 2 have not been demonstrated yet. Studies carried out indicate that HMG 1 and HMG 2 replace histone H 1 in nucleosomes [5]. Seyedin and Kistler [12, 13] assumed that HMG 2 and H 1<sup>o</sup> histone perform alternative functions in nucleosome linkers. H 1<sup>o</sup> is characteristic of non-replicative tissues, whereas HMG 2 appears in the proliferating tissues. Levels of chromosomal protein HMG 2 are parallel with the proliferative activity of testis cells, skeletal muscle and other organs. In our tissue model an interesting fact been found, i.e. in Kirkman-Robbins hepatoma an extra subfraction of H 1 histone appeared, called H 1 "slow" [4].

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The purpose of this work was the search for correlation between the tissue proliferation activity, H 1 subfraction subset and on the other hand, the amount of HMG 1 and HMG 2 proteins.

#### MATERIAL AND METHODS

The transplantable hamster hepatoma derived from the line originally induced by Kirkman and Robbins [6]. The neoplastic material was collected on the eight day after the transplantation of the tumor. The HMG 1 and HMG 2 proteins were isolated from the tumor and other tissues according to Seydin and Kistler [12] through extraction by means of 0,2 M  $H_2SO_4$ , 10 mM PMSF, 10 mM  $\beta$ -merkaptoethanol. HMG 1 and HMG 2 were isolated from total fraction by fractionated precipitation with trichloroacetic acid. HMG proteins were separated by polyacrylamide gel electrophoresis according to Panyim and Chalkley [10]. In 25% acrylamide gel slabs containing 2.5 M urea, 30 cm long were run for 72 h at 4°C and 210 V. The gels were stained with Amido-Black 10 B and after destaining they were stained once again with Comassie blue R-250. HMG 1 and HMG 2 isolated from calf thymus were used as standards [2]. The densitometric patterns of the gel fragments containing HMG proteins were obtained with the use of a densitometr. The amounts of HMG 1 and HMG 2 were estimated by calculating the ratios of the areas under the proper peaks. The preparates chosen for the studies were those whose band absorption was in the linear dependence to the protein concentration, which had previously been stated with standard proteins.

#### RESULTS AND DISCUSION

HMG proteins were extracted from the normal and pathologic tissues of the hamster. The densitometric patterns of polyacrylamide gel fragments containing HMG 1 and HMG 2 fractions have been shown on Fig. 1. The densitograms obtained from the protein extracts taken from cerebral hemispheres, healthy liver, Kirkman-Robbins hepatoma as well as the liver of tumor bearing animal were basically similar.

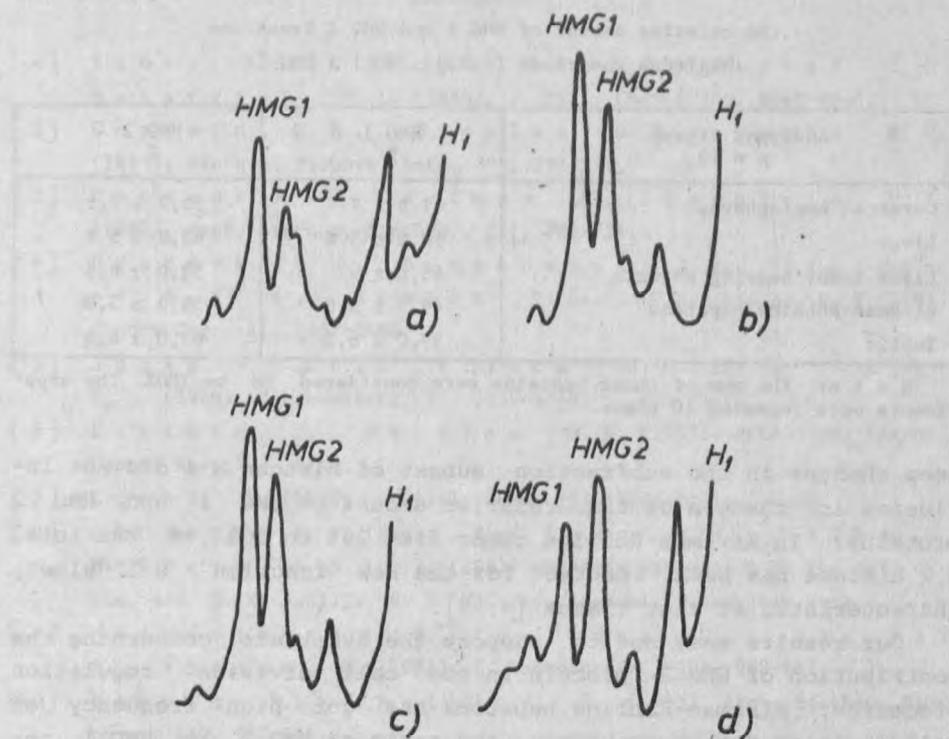


Fig. 1. Densitometric patterns of the HMG proteins extracts obtained from different cells of the normal and pathological tissues

a) from the cerebral hemispheres, b) from the normal liver, c) from the Kirkman-Robbins hepatoma, d) from the Syrian hamster testis

Rys. 1. Densytogram ekstraktu białek HMG otrzymanego z różnych komórek tkanek normalnych i patologicznych

a) z półkul mózgowych, b) z normalnej wątroby, c) z mięsaka Kirkmana-Robbinsa d) z jąder chomika syryjskiego

The relative amount of HMG 1 and HMG 2 proteins as revealed by electrophoresis and densitometric tracing of the gels were almost equal in various tissues (Tab. 1). A quite different densitometric pattern was obtained for the extracts from the Syrian hamster testes. In this case the amount of HMG 2 fraction exceeded that of HMG 1 fraction. (Fig. 1d and Tab. 1).

The fact that no increase of the relative amount of HMG 2 protein was found in Kirkman-Robbins hepatoma suggested that even

Table 1

The relative amount of HMG 1 and HMG 2 fractions  
Względna zawartość frakcji HMG 1 i HMG 2

Analysed tissue n = 10	HMG 1 %	HMG 2 %
Cerebral hemispheres	47,8 ± 7	52,2 ± 7,5
Liver	56,0 ± 5,8	43,8 ± 5,8
Liver tumor bearing animals	48,0 ± 9	52,0 ± 8,5
Kirkman-Robbins hepatoma	49,7 ± 5,9	51,3 ± 3,9
Testis	39,0 ± 8,5	61,0 ± 8,5

Note: The sum of these proteins were considered to be 100%. The experiments were repeated 10 times.

deep changes in the subfraction subset of histone H 1 did not influence in changes of the relative amount of HMG 1 and HMG 2 proteins. In Kirkman-Robbins tumor from 20% to 30% of the total H 1 histone has been reserved for the new fraction - H 1 "slow", characteristic of that tissue [4, 9].

Our results seem not to support the hypothesis concerning the contribution of HMG 2 protein in the cell division regulation processes. Kirkman-Robbins hepatoma has got high frequency of cell division but nevertheless the ratio of HMG 2 to HMG 1 remains constant, even in comparison with the cerebral hemispheres which lost their ability to cell division.

There have been several reports suggesting that the ratio of HMG 2 to HMG 1 increases parallelly with proliferative activity in muscle [12, 3], testis [12] and salivary gland tissues [11]. However other authors reports on regenerating rat liver [7] and mouse neuroblastoma cells [13] do not note an increased HMG 2/HMG 1 ratio during proliferation.

The increase of the amount of HMG 2 in Syrian hamster testis seems to be of particular interest. An additional fraction of H 1 histone appears in this organ as well. However, the increase of HMG 2 cannot be directly associated with the increase of the amount of H 1 "slow" as there has been no correlation stated between these two facts in case Kirkman-Robbins hepatoma. Recently B u c c i et al [1] fractionated various cells from rat testis and on the basis of the results framed a new hypothesis saying that this enormous increase of HMG 2 is associated with meiosis but not proliferation.

## REFERENCES

- [ 1 ] Bucci L. R., Brock W. A.; Goldknopf I. L. Meistrich M. L. (1984), *J. Biol. Chem.*, 259, 8840-8846.
- [ 2 ] Goodwin G. H., Nicolas R. H., Johns E. W. (1975), *Biochim. Biophys. Acta*, 405, 280-291.
- [ 3 ] Gordon J. S., Kaufman R., Rosenfeld V. I. (1981), *Arch. Biochem. Biophys.*, 211, 709-721.
- [ 4 ] Graczyk G. M., Bartkowiak J. K., Płucieniczak A., Hrabec E. L., Panusz H. T. 1981, *Cancer Res.*, 41, 2457-2464.
- [ 5 ] Jacks J. B., Pollock J. M. (Jr.), Rill R. L. (1979), *Biochemistry*, 18, 3739-3748.
- [ 6 ] Kirkman H., Robbins M. A. (1955), *Proc. Am. Assoc. Cancer Res.*, 2, 38-45.
- [ 7 ] Kuehl L. (1979), *J. Biol. Chem.* 254, 7276-7281.
- [ 8 ] McCarty K. S. (Sr.), Kellner D. N., Wilke K., McCarty K. S. (Jr.), (1982), *Genetic expression in the cell cycle*, eds G. M. Padilla, K. S. McCarty, Academic Press, New York.
- [ 9 ] Modrzejewska H., Gałązka G., Szemraj J., Panusz H. (1984), *Z. Naturforsch.*, 39c, 958-961.
- [ 10 ] Panyim S., Chalkley R. (1969), *Arch. Biochem. Biophys.*, 130, 337-346.
- [ 11 ] Pipkin J. L., Hinson W. G., Hudson J. L., Anson J., Pack L. D. (1981), *Biochim. Biophys. Acta*, 655, 421-431.
- [ 12 ] Seyedin S. M., Kistler W. S. (1979), *J. Biol. Chem.*, 254, 11204-11271.
- [ 13 ] Seyedin S. M., Pehrson J. R., Cole R. D. (1981), *Proc. Natl. Acad. Sci. USA*, 78, 5988-5992.

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WZGLĘDNA ILOŚĆ BIAŁEK HMG 1 I HMG 2  
W RÓŻNYCH TKANKACH CHOMIKA SYRYJSKIEGO

Przebadano względną zawartość ilościową białek HMG 1 i HMG 2 w różnych tkankach chomika syryjskiego. Stwierdzono, że zawartość tych białek w półku-

lach mózgowych, wątrobie prawidłowej, wątrobie nosiciela guza i w wątrobiaku Kirkmana-Robbins jest bardzo podobna i niezależna od występowania dodatkowej subfrakcji H 1 - H 1 "slow".

Względna zawartość białek HMG 1 i HMG 2 różni się w jądrach samców chomika syryjskiego. Ilość HMG 2 przewyższa znacznie zawartość HMG 1.