ACID SOLUBLE NUCLEAR PROTEINS OF RAT LIVER: DIFFERENTIAL ABSORBANCE OF BOUND DYES AND CHANGES IN NEOPLASIA

by

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Summary

Evidence was obtained for the presence in several hepatomas of a minor histone previously reported to be absent in tumors. The concentration of this protein was inversely related to hepatoma growth rate. Comparison of light absorbance by bound dyes showed that the protein appeared to be a minor component of acid soluble nuclear proteins when stained with Amido Black or Fast Green but a major component when stained with Coomassie blue.

Introduction

Despite the potential significance of histones in the regulation of genetic expression, studies of these molecules have generally indicated their similarity in normal and neoplastic cells. Differences have been observed in the rates of synthesis and the modification of side chains (1-4). A report by Panyim and Chalkley noted the presence of a new and minor histone fraction which was absent in rapidly dividing cells (5). This fraction has received little attention although it may be observed in a number of published photographs or densitometric tracings of histones separated by polyacrylamide gel electrophoresis e.g fraction 5 of Chanda et al. (6). Changes in the concentration of this histone, termed Fla by Fitzgerald and coworkers, have been reported in regenerating rat pancreas (7). In this report we present evidence for the correlation of the level of the fla histone with hepatoma growth rate and the influence of different dyes on the apparent relative concentration of acid soluble nuclear proteins.

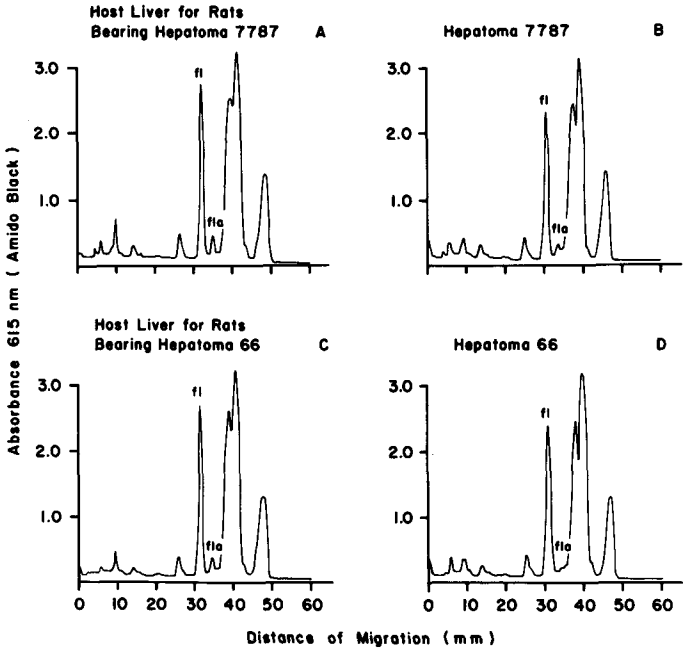

Fig. 1. Polyacrylamide gel electrophoresis of acid soluble nuclear proteins stained with Amido Black: (A) host liver, (B) hepatoma 7787, (C) host liver and (D) hepatoma 66.

Material and Methods

Hepatomas were maintained by subcutaneous transplants in Buffalo strain rats (8). Nuclei were isolated in the presence of 3 mM $MgCl_2$ and 5 mM $NaHSO_3$ essentially as described previously (9) and were washed with 0.32 M Sucrose, 1% Triton x-100 and 0.14 M NaCl. Acid-soluble proteins were extracted with 0.24 N HCl and precipitated with ten volumes of acetone. Polyacrylamide gel electrophoresis with 50 µg proteins was performed by the method of Panyim and Chalkley (10). Gels were stained and destained with methanol: acetic acid: water (3:1:6) using 0.1% Amido Black and Coomassie Blue or 0.2% Fast Green as dyes. Densitometric tracings were obtained on a Gilford model 2000 kindly made available by Dr. Vincent Allfrey, Rockefeller University, New York, N. Y.

Results and Discussion

In Fig. 1 are shown densitometric tracings of polyacrylamide

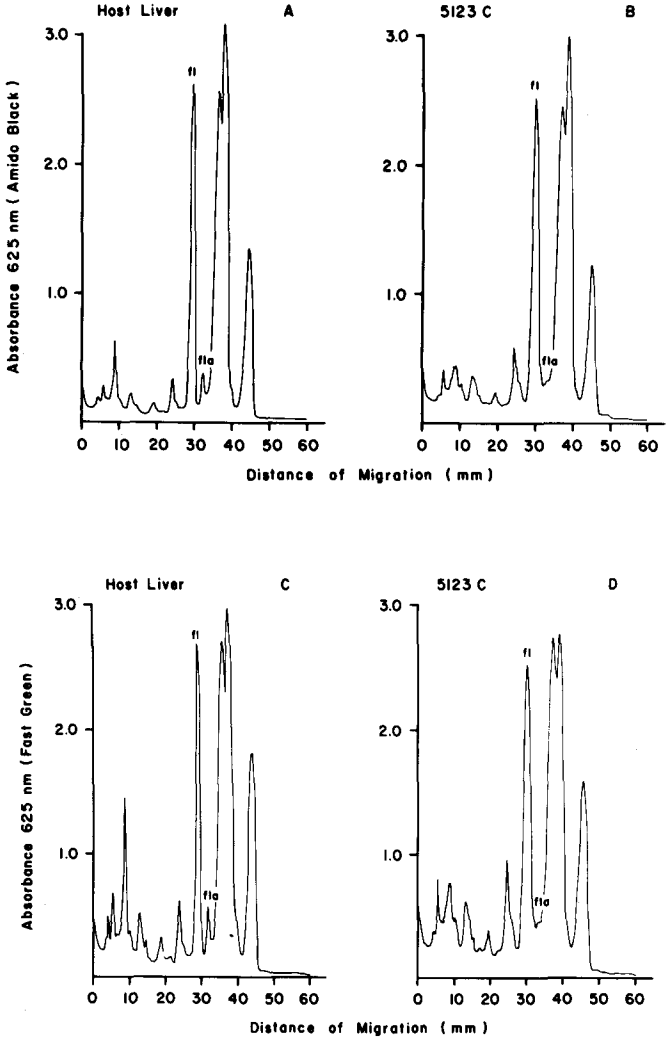

Fig. 2. Polyacrylamide gel electrophoresis of acid soluble nuclear proteins. Absorbance measured at 625 nm after staining with either Amido Black (A and B) or Fast Green (C and D) using proteins from either host liver (A and C) or hepatoma 5123C (B and D).

gel electrophoretic patterns of acid-soluble nuclear proteins from two slowly growing hepatomas and host livers. Staining with Amido Black indicates the presence of the fla histone in the very slowly growing hepatoma 7787 (approximately one year after transplantation)with a lesser amount in hepatoma 66 (approximately 3 months after transplantation).

Fast Green and Amido Black give a similar staining pattern with acid-soluble nuclear proteins (Fig. 2) and both indicate an absence or near

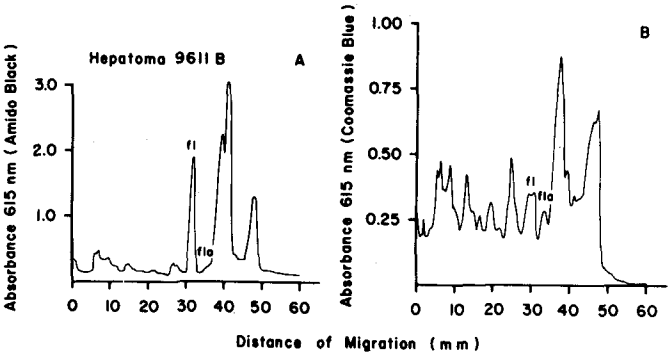

Fig. 3. Polyacrylamide gel electrophoresis of acid soluble nuclear proteins from hepatoma 9611B stained with Amido Black (A) or Coomassie Blue (B).

absence of the f1a histone in hepatoma 5123C, a tumor of intermediate growth rate. This was also the case for another tumor of similar growth rate, hepatoma 9611B (Fig. 3) when Amido Black was used as a dye. Coomassie Blue gave a greater relative absorption with minor constituents revealed by Amido Black such that the f1a histone could be detected in hepatoma 9611B using Coomassie Blue. This may in part reflect a more marked deviation from Beers Law by Coomassie Blue stained proteins (11). However, it is shown in Fig. 4 that the wavelength selected for absorption measurement influences the relative intensity of the different proteins so that the histones such as f1 appear more significant at 615 nm than at 550 nm. Using Coomassie Blue the amount of f1a histone in some normal livers may appear as great or greater than the f1 histone. After addition of a variety of proteins to solutions of Coomassie Blue we have noted red shifts in peak absorbance which differ in degree according to the individual protein.

We have observed that the f1a histone is not extracted by the medium of Gronow and Griffiths (12) containing 8 M urea, 50 mM sodium phosphate pH 7.6 which removes some of the arginine rich histone and most of the non-histone proteins extracted from nuclei by 0.24 N HCl. The amount of f1a histone was very similar in male and female control rats. The level was decreased in regenerating liver 24 hours after

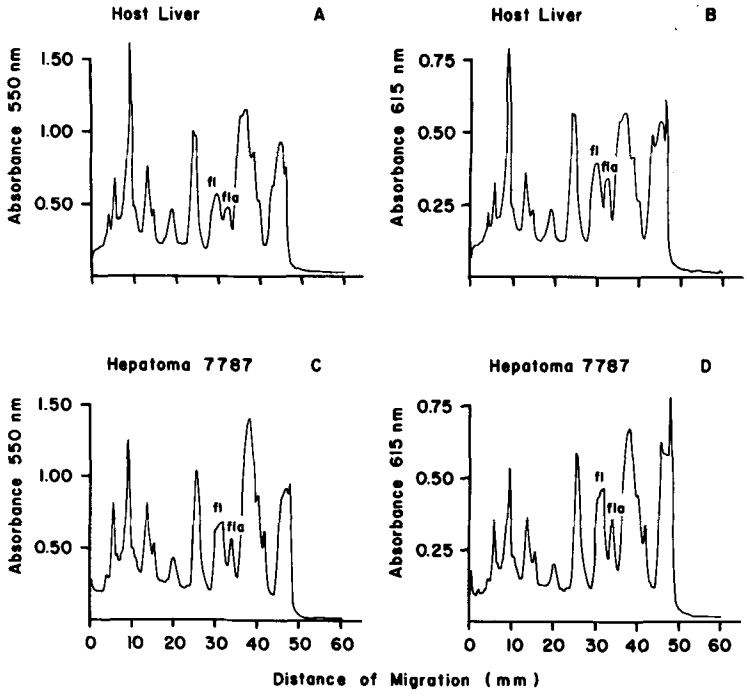

Fig. 4. Polyacrylamide gel electrophoresis of acid soluble nuclear proteins stained with Coomassie Blue. Absorbance measured at 550 nm (A and C) or 615 nm (B and D) using proteins from either host liver (A and B) or hepatoma 7787 (C and D).

partial hepatectomy but not to the extent seen in rapidly growing hepatomas (7777 and 9618A2) of similar growth rate. These observations suggest that the synthesis of fla histone may be more closely related to the degree of differentiation of tissues than the growth rate per se. The data presented here indicate that until the light absorbance characteristics of individual protein complexes have been established for purified histones including the fla histone, the published values for the amount of fla histone as a percentage of the total histones (5,7) must be viewed with caution.

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References

1. Arnold, E. A., Buksas, M. M., and Young, K. E. (1973) Cancer Res. 33, 1169-1176.

2. Balhorn, R., Balhorn, M., Morris, H. P., Chalkley, R. (1972) Cancer Res. 32, 1775-1784.
3. Hnilica, L. S. (1972) The Structure and Biological Functions of Histones, CRC Press, Cleveland, Ohio.
4. Stein, G. S., Criss, W. E., and Morris, H. P. (1974) Life Sciences 14, 95,-105.
5. Panyim S., and Chalkley, R. (1969) Biochem. Biophys. Res. Commun. 37, 1042-1049.
6. Chanda, S. K., Ickowicz, R., and Dounce, A. L. (1973) Biochem. J. 135, 115-123.
7. Marsh, W. H., and Fitzgerald, P. J. (1973) Fed. Proc. 32, 2119-2125.
8. Morris, H. P., and Meranze, D. R. (1974) Recent Results in Cancer Research 44, 103-114.
9. Lea, M. A., Khalil, F. L., Rey, M. I., and Morris, H. P. (1973) Chem.-Biol. Interactions 6, 339-349.
10. Panyim, S., and Chalkley, R. (1959) Arch. Biochem. Biophys. 130, 337-346.
11. Gorovsky, M. A., Carlson, K., and Rosenbaum, J. L. (1970) Anal. Biochem. 35, 359-370.
12. Gronow, M., and Griffiths, G. (1971) FEBS Letters 15, 340-344.