ZOFIA KILIAŃSKA and LEOKADIA KŁYSZEJKO-STEFANOWICZ

COMPARISON OF NON-HISTONE PROTEINS FROM HAMSTER KIRKMAN-ROBBINS HEPATOMA AND LIVER*

Department of Cell Structures Biochemistry
University of Łódź, 90-237 Łódź, Banacha 12/16

1. Three classes of non-histone proteins were obtained from hamster Kirkman-Robbins hepatoma and liver nuclei following separation of nucleic acids with the polyethylene glycol-dextran mixture and fractionation of nuclear proteins on hydroxyapatite in a salt-glycerol-phenylmethylsulphonyl fluoride system at increasing concentrations of Na⁺ and K⁺ phosphate buffer, pH 6.8.

2. Two-dimensional polyacrylamide gel electrophoresis of these proteins documented their high heterogeneity; many spots were common but some spots specific only for neoplastic or normal tissue were also observed.

Various lines of evidence indicate that non-histone proteins may be involved in gene regulation (Paul & Gilmour, 1968; Elgin & Weintraub, 1975; Kleinsmith, 1978). The postulated regulatory role of non-histone proteins at the level of gene transcription is of particular interest with regard to cell differentiation. Since neoplastic transformation is likely to involve alterations in the differentiated state of the cell, the behaviour of non-histone proteins in the process of neoplasia requires special attention.

Recent improvements in the techniques of analyses of complex mixtures of proteins have provided tools for exploring the complexity and diversity of non-histone proteins of tumours and other tissues. Two-dimensional polyacrylamide gel electrophoresis developed by O'Farrell (1975) allows for rapid advancement of the identification and characterization of non-histone proteins originating from neoplastic and normal tissue.

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MATERIALS AND METHODS

Tissues. A Kirkman-Robbins hamster hepatoma was obtained from Chester Beatty Research Institute in London via the Department of Oncology, School of Medicine in Łódź.

Samples (0.2 ml) of a mechanically dispersed neoplastic cell suspension taken from an 8-day old cancer were inoculated subcutaneously above the axilla of male Syrian hamsters. Livers of control Syrian hamsters were used as the reference tissue. Groups of 20 hamsters were used for each experiment.

Preparation of nuclei. All solutions used were at 0 - 4°C and contained phenylmethylsulphonyl fluoride (PMSF). Freshly removed hepatoma tissue was kept on ice and washed in isotonic sucrose containing 3 mM-CaCl₂ 0.8-mM-KH₂PO₄, pH 6.7, and 1 mM-PMSF. The nuclei of hamster Kirkman-Robbins hepatoma and liver were isolated by the method of Chauveau et al. (1956) with additional treatment of nuclei with 0.4% Triton X-100.

Preparation and fractionation of non-histone proteins. Non-histone proteins were isolated from nuclei and fractionated as described previously (Kiliańska et al., 1980). The procedure is based on separation of chromatin proteins from DNA with a polyethylene glycol-dextran mixture and their fractionation by hydroxylapatite chromatography. Non-histone protein fractions called: NHCP1, NHCP2, and NHCP3, were eluted from hydroxylapatite with a stepwise gradient of 50 mM (Na⁺ salt), 100 and 200 mM (K⁺ salt) phosphate buffer, pH 6.8, in 2 M-NaCl, 8% glycerol and 0.1 mM-PMSF.

Two-dimensional gel electrophoresis. The method of O'Farrell (1975), slightly modified, was used throughout. Protein samples were dissolved in 8 M deionized urea. The gels composed of 3.77% acrylamide, 0.22% bis-acrylamide, 9 M-urea, 2% Triton X-100, 2% ampholine (made up of 1.6% ampholine pH range 5.0 to 8.0, and 0.4% ampholine pH range 3.5 to 10.0) were cast in glass tubes (10×0.2 cm). The gels were electrophoresed for 14 h at 300 V, and 2 h at 400 V; the electrolyte solutions were 0.02 M-NaOH (cathode) and 0.01 M-H₃PO₄ (anode). The pH gradient formed in the gel was measured by means of a pH-meter in 0.01 M-KCl extracts of 0.5 cm sections of a control gel. After isoelectric focusing, the gels were equilibrated for 1.5 h in the buffer containing 10% glycerol, 5% 2-mercaptoethanol, 2.3% sodium dodecyl sulphate and 0.0625 M-Tris/HCl (pH 6.8). Electrophoresis in the second dimension was carried out on 11.2% polyacrylamide according to Laemmli (1970). The slab gels were stained according to Fairbanks et al. (1971). The proteins used as standards were: phosphorylase b (M, 94 000), bovine serum albumin (M, 67 000), ovalbumin (M, 43 000), carbonic anhydrase (M, 30 000) and soybean trypsin inhibitor (M, 20 100).

Other methods. Protein was determined as described by Lowry et al. (1951) and DNA was measured by the Burton method (1956).
Reagents. Acrylamide was a product of Koch-Light Lab. (Colnbrook, Bucks., England). N,N’-Bisacrylamide, ammonium persulphate and hydroxylapatite were from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Ampholine was from LKB-Producent AB (Bromma, Sweden), and Coomassie Brilliant Blue R-250 from Sigma Chem. Co. (St. Louis, MO, U.S.A.). Dextran T-500 (M, 500,000) and molecular weight standard proteins kit were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). PMSF was from Calbiochem (San Diego, CA, U.S.A.). Polyethylene glycol, type 6000 (M, 6000 - 7500) was a product of Serva Feinbiochemica (Heidelberg, F.R.G.). SDS, specially pure, was supplied by B.D.H. Chemicals (Poole, Dorset, England). Other reagents were analytical grade products supplied by POCh (Gliwice, Poland).

RESULTS AND DISCUSSION

The extremely high heterogeneity of non-histone chromatin proteins requires their preliminary fractionation prior to the analysis (Klyszejko-Stefanowicz & Hnilica, 1983). The chromatin proteins were initially dissociated from DNA with a mixture of polyethylene glycol-dextran and subsequently fractionated on hydroxylapatite (Kiliańska et al., 1980). Elution with a step-wise gradient of phosphate buffer in the salt-glycerol-PMSF system yielded three classes of non-histones, i.e., NHCP1, NHCP2 and NHCP3. Taking advantage of the high resolving power of two-dimensional gel electrophoresis (O’Farrell, 1975) we compared the non-histone polypeptide patterns of hamster Kirkman-Robbins hepatoma and liver. Two-dimensional gel electrophoretic patterns of the foregoing three classes of non-histone proteins from these tissues revealed higher heterogeneity than that observed using one-dimensional SDS-polyacrylamide gel electrophoresis (Krajewska et al., 1985). The two-dimensional electropherograms show many common spots and some spots specific only for the neoplastic or normal tissue (Plates 1 - 3).

Non-histone chromatin protein fraction 1. Two-dimensional gel electrophoretic patterns of the main class of non-histone proteins from both tissues, i.e., NHCP1, eluted from hydroxylapatite with 50 mM-phosphate buffer are presented in Plate 1. Most of the components of NHCP1 are distributed within the M, range from about 25000 to 80000. Intensely stained spots with M, × 10^-3/pI values of 56/6.2, 74/5.9 and 85/6.3-6.6 were present in similar amounts in Kirkman-Robbins hepatoma and liver (Plate 1H, L). The predominant spots in hepatoma were 130/6.5-6.7 while those in liver were 28-31/7.3-8.4. The electrophoretic pattern of low molecular weight components in the region from about 14000 to 23000 with pI from 6.8 to 7.8 is different in liver NHCP1 than in hepatoma. Hepatoma NHCP1 is characterized by specific components: 110/6.9, 110/7.0, 110/7.1, 110/7.2, 95/5.8, 81/6.1, 81/6.2, 73/5.5, 66/6.9,
43/6.0, 42/5.7, 36/5.7, 31/7.2 and 25/7.2, whereas the 102/5.2, 100/5.0, 76/6.3, 76/6.4, 35/6.8, 35/6.9, 31/6.6, 30/6.8, 28/6.8, 28/6.6, 27/6.5, 26/6.8, 23/6.9 and 21/7.0 components are present only in normal liver. It is interesting to note that the polypeptide of $M_r$ 110 000 but more basic, with pI of 8.4 was described by Durban et al. (1981) in four tumour tissues, fast-growing Novikoff hepatoma, Morris hepatoma 3924, HeLa and Namalwa cells, as well as in fetal rat liver. The 110 000 non-histone component was not detected in normal or regenerating liver and, according to these authors may represent an “oncofetal marker”. It is possible that some of 110 000 polypeptides (pI from 6.9 to 7.2) detected in Kirkman- Robbins hepatoma may exhibit the properties suggested by Durban et al. (1981).

Non-histone chromatin protein fraction 2. Most components of the non-histone class eluted from hydroxylapatite with 100 mm-phosphate buffer range in $M_r$ from 50 000 to 100 000 (Plate 2H, L). The main spots of neoplastic NHCP2, i.e., 100/5.6, 75/5.7 and 61/6.1, occurred in hamster liver in small amount, whereas the predominant 75/7.1 polypeptide of liver NHCP2 was significantly reduced in the case of hepatoma. The polypeptides of 102/5.1, 102/5.2, 90/5.2, 81/5.0, 65/4.9, 40/5.2, 24/5.2, and 20/8.4 seem to be specific for hepatoma NHCP2; 140/5.5, 73/4.9, 41/5.0, 26/6.9 and 23/7.1 are characteristic for hamster liver.

Non-histone chromatin protein fraction 3. These non-histones belong to the class of proteins tightly bound to DNA. A small amount of NHCP3 is present in the nuclei, i.e., about 1 mg per 100 mg of DNA. Most of NHCP3 components are distributed on two-dimensional gels within the $M_r$ range from 30 000 to 100 000. In this area of the gel many spots specific for either tissue were detected (Plate 3H, L). Neoplastic tissue is characterized by the presence of polypeptides 100/6.2, 88/6.4-6.6, 80/5.8, 80/5.9, 75/6.8, 38/7.0 and 32/7.0 while the polypeptides 98/5.9, 77/7.1, 58/5.2, 49/5.2, 42/5.2, 30-31/6.5-6.9 and 15/5.1 are present only in normal liver tissue.

It is worth noting that NHCP1, NHCP2 and NHCP3 of both tissues contain also some slightly stained spots which are clearly visible on the original gels and indistinguishable on the photographs.

Two-dimensional gel electrophoresis of nuclear proteins (sometimes radioactively labelled) was recently used by other laboratories for detection of differences between neoplastic and normal tissues (Takami & Busch, 1979; Ruoslahti et al., 1980; Unteregger et al., 1983; Ochs & Busch, 1983; MatheWS et al., 1984). Non-histone proteins characteristic for malignant tissues may provide some clues towards the understanding of gene regulation in neoplastic cells and some of them may become tumour markers (Durban et al., 1981; Celis et al., 1984). It seems that an answer to the question of whether differences in non-histone proteins of neoplastic and normal
Plate 1. Analysis of NHC\(\Pi\) (250 \(\mu\)g protein) from hamster Kirkman-Robbins hepatoma (H) and liver (L). The isoelectric focusing in polyacrylamide gel (IFPA) was in the horizontal dimension and electrophoresis in polyacrylamide gel containing SDS (SDS-PAGE) was in the vertical dimension. The positions of molecular weight markers \((\times 10^{-3})\) and distribution of pI values are shown. The arrows indicate specific spots.

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Plate 3. Analysis of NHCP3 (150 μg protein) from hamster Kirkman-Robbins hepatoma (H) and liver (L). For explanation see Plate 1.
Plate 2. Analysis of NHCP2 (250 μg protein) from hamster Kirkman-Robbins hepatoma (H) and liver (L). For explanation see Plate 1.
tissues are really important in malignancy must await further characterization of the individual proteins. The problem of how the presence of non-histone proteins specific for neoplastic tissue reflects their biological function requires further investigation.

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