FRACTIONATION OF CHROMOSOMAL DEOXYRIBONUCLEOPROTEINS BY PARTITION IN TWO-PHASE AQUEOUS POLYMER SYSTEMS

Gail Turner and Ronald Hancock

Department of Cell Biology, Swiss Institute for Experimental Cancer Research, 1011 Lausanne, Switzerland

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SUMMARY: Deoxyribonucleoprotein (DNP)* prepared by shearing chromatin of mouse cells may be fractionated in 2-phase aqueous Dextran-polyethyleneglycol mixtures. A partial separation of DNPs with different non-histone protein/DNA ratios may be obtained in a single-step partition. Separation of a spectrum of fractions of DNP has been obtained by countercurrent distribution using the same 2-phase polymer system. DNP fractions which bear nascent RNA (representing approximately 1/3 of the total DNA) may be separated from the major fraction of DNP; they are found in the same region of the distribution pattern as DNP fractions with the highest non-histone protein/DNA ratio.

The chromatin of eukaryotic cells can be cut by shearing forces to give a population of molecules of DNP. This population is heterogeneous both in composition (for example, in the ratio protein/DNA) (1,6), and also in function (for example, only DNP derived from template-active regions of the chromatin bears nascent RNA). Techniques have been developed for separating sheared chromatin into two major fractions representing the template-active and the inactive regions (2-5). Fractionation procedures with higher resolution would be valuable in order to study individual regions of the chromatin in detail. One such procedure, chromatographic fractionation of DNPs on columns of

*Abbreviations: DNP, deoxyribonucleoprotein; PEG, polyethyleneglycol.

ECTHAM-cellulose, has been described (6). We are studying the use of partition in 2-phase aqueous polymer systems (7) for fractionation of DNPs, and present here some of our observations.

Materials and Methods: Chromatin was prepared by either of two procedures from growing cells of mouse line P815. In the first procedure (8), soluble chromatin was prepared by shearing nuclei after extraction with 80 mM NaCl - 20 mM EDTA. The second procedure employed lysis of cells with a non-ionic detergent followed by centrifugation of the chromatin through sucrose (9). In both cases the chromatin was sheared using a Dounce homogenizer and centrifuged (Spinco rotor SW65, 15 krpm, 30 min) to remove nucleoli and nuclear envelope fragments. The soluble DNP was then further purified by sedimenting through 60% sucrose in 0.2 mM EDTA (Spinco rotor SW65, 35 krpm, 3 hrs) to ensure complete removal of free RNP-containing particles and non-DNP proteins. The DNP was then further sheared in a French pressure cell so that the DNA molecular weight was approximately 2×10^6 (measured in alkaline sucrose gradients by comparison with a marker of polyoma form II DNA). To study the distribution of DNPs in the 2-phase polymer system, radioactive DNP was used, containing DNA and proteins labelled by growth of cells during at least two generations with radioactive precursors. DNA was labelled by growth with ^{14}C-thymidine (0.5-10 µC/litre) and unlabelled thymidine (0.2 µg/ml) (9), and total chromatin proteins with a complete ^{3}H-amino acid mixture (2.5 mC/litre); non-histone proteins were labelled specifically (9) with ^{3}H-tryptophan (2.5 mC/litre). Nascent RNA was labelled by a 7 minute pulse of ^{3}H-uridine (5 mC/litre). Radioactive compounds were obtained from NEN, Dreieichenhain, Germany.

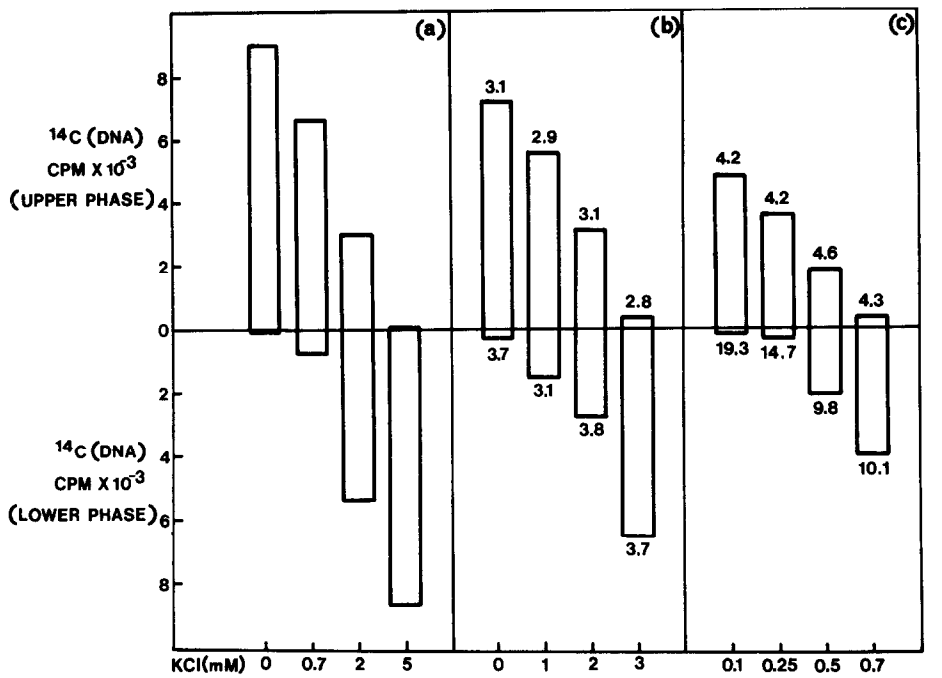

Fig. 1 Distribution of DNP containing ^{14}C-labelled DNA in a single-step partition in the 2-phase system Dextran 5%, PEG 4% in 0.1 mM tris-HCl pH 7.4 + 0.2 mM EDTA. In addition, KCl was added to the indicated concentration.

In (b) and (c) the DNP also contained ^{3}H-labelled total proteins (b) or ^{3}H-labelled non-histone proteins (c); the values above and below the columns represent the ratio ^{3}H protein/^{14}C DNA of the DNP in each phase.

Each sample contained DNP equivalent to 50 µg DNA in a volume of 2 ml.

The aqueous polymer solutions were prepared using Dextran T 500 (lot 17, Pharmacia, Sweden) and polyethyleneglycol 6000 (Carbowax 6000, Fluka, Switzerland). The volume ratio of the 2 phases was always 1.0. Single-step partitions were carried out in 10 ml nitrocellulose tubes which were inverted mechanically at about 30 cycles/min for 5 min; the tubes were then centrifuged for 20 min at 1000 g to facilitate separation of the two phases. After complete separation the upper phase was pipetted off, and the lower phase was collected by puncturing the tube bottom. For

multistep distribution, a countercurrent apparatus of the type described by Albertsson (7) was used. All operations were carried out in a cold room. To determine radioactivity in experimental samples the DNP was precipitated in 5% CCl_3COOH after addition of 100 µg of salmon sperm DNA. The precipitates were collected on Whatman GFB glass-fiber filters, which were washed in 5% CCl_3COOH and water and dried; their radioactivity was determined in a liquid scintillation counter.

Results and Discussion: The partition of DNP containing ^{14}C-labelled DNA was studied using a medium of low ionic strength (0.1 mM tris, 0.2 mM EDTA, pH 7.4) in order to reduce association between DNP molecules to a minimum (10). In the absence of other ions, DNP is concentrated in the PEG phase of the standard polymer system in this medium. The partition of DNP between the 2 phases is extremely sensitive to the concentration of other ions; increase in the concentration of KCl up to 3 mM results in progressive transfer of the DNP into the Dextran phase (Fig. 1a). (It may be noted that the sensitivity of the partition properties of DNP in this system to small changes in salt concentration may be relevant to properties of chromatin within the nucleus). The concentration of KCl at which the partition ratio K (concentration in upper phase relative to concentration in lower phase) is 1.0 was found to vary between 0.5 mM and 2 mM for different preparations of DNP; this concentration of KCl was determined for each DNP preparation before carrying out multistep fractionations. At all KCl concentrations the fraction of DNP in the lower (Dextran)phase is enriched in molecules having a higher ratio of total protein/DNA (Fig. 1b). This high ratio

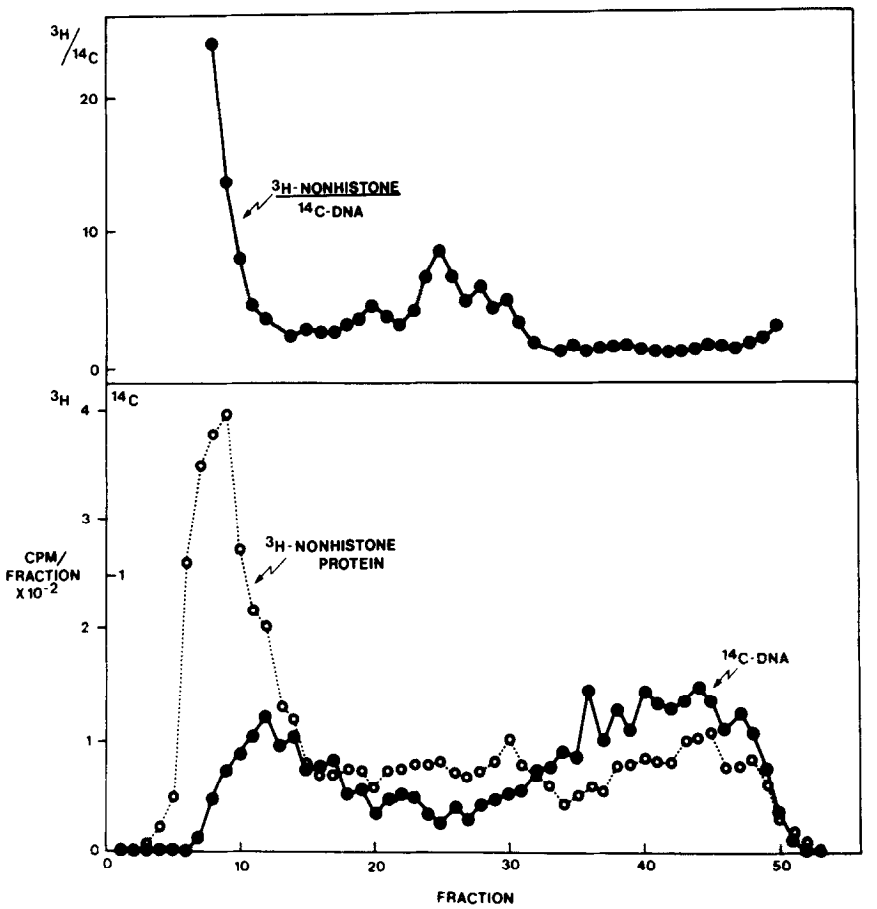

Fig. 2 Countercurrent fractionation of DNP containing ^{14}C-labelled DNA and ^3H-labelled non-histone proteins. The phase system was that described in Fig. 1, with 0.25 mM KCl. The DNP sample (containing approx. 500 µg DNA) was loaded in tube 1.

is at least partly due to a marked enrichment in molecules of high non-histone protein/DNA ratio (Fig. 1c).

The partial fractionation of species of DNP with different ratios of total protein or non-histone protein to DNA, obtained in a single step, led us to carry out multistep fractionations of DNP in the same polymer system, using a countercurrent procedure (7). In Figure 2 and 3 are shown typical multistep fractionations of DNP containing ^{14}C-labelled DNA, and bearing

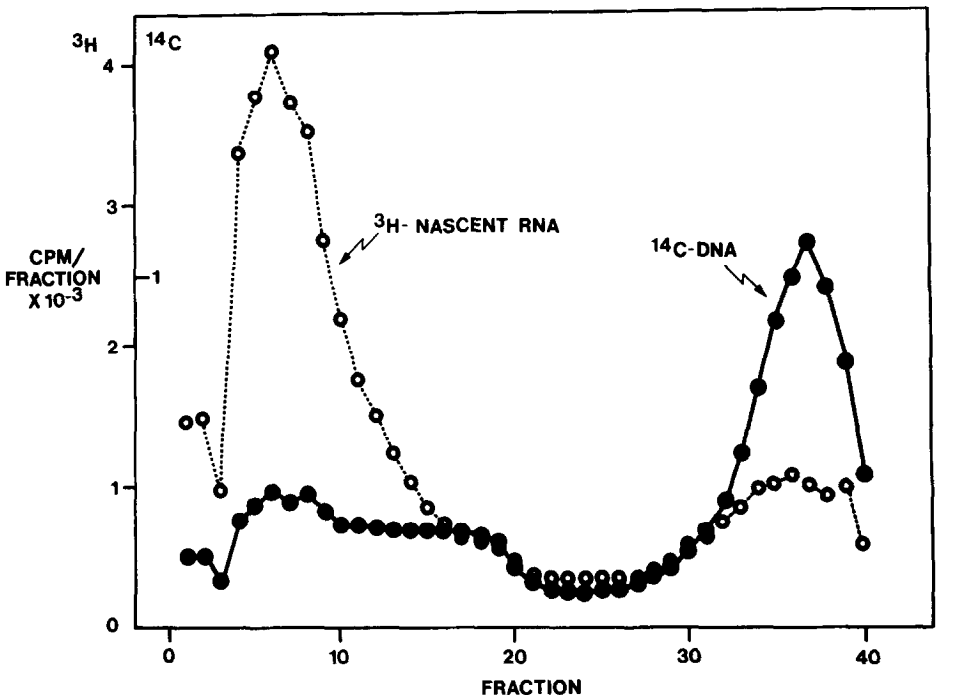

Fig. 3 Countercurrent fractionation of DNP containing ^{14}C labelled DNA and nascent RNA labelled *in vivo* for 7 minutes with ^{3}H-uridine. The phase system was that described in Fig. 1, with 0.25 mM KCl. The DNP sample (containing approx. 400 µg DNA) was loaded in tubes 1-3.

either non-histone proteins labelled with ^{3}H-tryptophan (Fig. 2) or nascent RNA pulse-labelled with ^{3}H-uridine (Fig. 3). (Individual non-histone proteins may be expected to have different contents of tryptophan, but we feel it is unlikely that systematic errors are introduced, at the present level of resolution, by regarding the tryptophan content of individual DNP fractions as proportional to their content of non-histone proteins). Under the experimental conditions used the DNP is separated, on the basis of DNA content, into two major regions of the distribution pattern; these two regions contain, respectively, about 30% and 70% of the total DNA (Figs 2 and 3).

Three major regions may be distinguished possessing high, intermediate, and low ratios of non-histone protein/DNA. (Fig. 2). The nature of the non-histone proteins in each of these major regions is at present being investigated. DNP molecules bearing nascent RNA are essentially restricted to the region of the distribution pattern containing the minor fraction of the total DNA (Fig. 3). This corresponds with the region of the distribution pattern where DNP molecules of highest non-histone protein/DNA ratio are located (Fig. 2).

DNP may be recovered from the separated fractions by carrier-free electrophoresis, using a procedure similar to that described by Albertsson (7). The buoyant density in equilibrium gradients of CsCl (11) of DNP recovered in this way from selected fractions corresponds with the relative protein/DNA ratio in the fractions as measured by radioactivity, confirming that no dissociation of proteins occurs during fractionation. Alternatively, the proteins and DNA in each fraction may be recovered separately; after adjusting the salt concentration so that the DNP is transferred to the upper (PEG) phase of each fraction (7), solid CsCl is added to this phase to give a density of 1.7. Centrifugation to equilibrium then allows banding of the DNA, while the proteins are dissociated and may be recovered from the surface of the gradient where they are concentrated together with the PEG (7).

This multistep procedure thus allows fractionation of DNPs derived from sheared chromatin into a spectrum of fractions; within this spectrum a number of major regions may be distinguished. In one region, containing about 30% of the total DNA,

are found DNP molecules which bear nascent RNA, probably derived from template-active regions of the chromatin, and which possess a relatively high ratio of non-histone protein to DNA. The second major region, representing about 70% of the total DNA, contains little nascent RNA and has a low ratio of non-histone proteins to DNA. These two major regions thus resemble the two fractions which have been separated from chromatin of other cells and tissues by other techniques (2-5).

This procedure for fractionation of DNP has a number of advantages which are inherent to this technique (7). The fractionation may be carried out under very mild conditions (low ionic strength and neutral pH), which may be varied within certain limits to give optimal fractionation. The procedure is theoretically capable of very high resolution (7). The partition of DNPs probably depends on factors (for example conformation) different from those which determine their behaviour on ECTHAM-cellulose columns (6), so that the information obtained from these two procedures may be complementary.

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