Repetitive DNA Associated with Rodent Liver Nuclear Envelopes

Electron microscopy of ultra-centrifuged, interphase nuclei[1] and of spread metaphase chromosomes[2] reveals an intimate association of chromatin and the nuclear envelope. Such an association is also indicated by the presence of residual amounts of DNA in nuclear envelopes isolated by a variety of procedures[3]. These, and other results, strongly suggest the in vivo attachment of interphase chromatin to specific envelope sites. Possibly this attachment has a role in ordering interphase chromatin, though not in DNA replication[3]. We have previously studied the DNA associated with isolated rat liver nuclear envelopes (n.e.-DNA)[4,5] and we report here further experiments to characterise this DNA and the nature of its attachment to the envelope.

Materials and methods. Nuclear envelopes were isolated from albino rat or mouse liver nuclei as previously[5] and further purified on sucrose density gradients[4]. DNA was purified from nuclei and nuclear envelopes[6], dissolved in 0.5 × SSC (1 × SSC is 15 m*M* tri-sodium citrate, 150 m*M* NaCl, pH 7.0) and in some cases sonicated for 3 min. The melting temperature[7] and reassociation kinetics of DNA samples were determined optically using a Cary 14 recording spectrophotometer[8,9]. Analytical CsCl density gradient centrifugation[10] was performed using a Spinco Model E analytical ultra-centrifuge. DNA and protein were estimated as before[5].

Results and discussion. Isolated nuclear envelopes from rat or mouse liver nuclei contained 1–3% of the total nuclear DNA, the recoveries being very similar for male (2.0 ± 0.3%) or female (1.6 ± 0.3%) animals. When the envelopes were pelleted (60 min × 100,000 *g*) from suspensions in 10 m*M* *Tris*-HCl pH 7.4, containing increasing salt concentrations, there was a progressive loss of DNA from the envelope pellet, as shown in Figure 1. 0.2 *M* NaCl was sufficient to remove 50% of the DNA from the envelope pellet, suggesting as the simplest explanation that most of the n.e.-DNA is bound to the envelope by ionic forces.

The content of highly repetitive DNA in the purified DNA samples was estimated at various reassociation temperatures, as described by HENNIG and WALKER[8]. Figure 2 shows second order rate plots of the reassociation of rat bulk and n.e.-DNA's used for such an estimation. Since some of the DNA samples had different Tm's (due to the use of DNAase 1 in the envelope isolation procedure, as shown by control experiments) the contents of repetitive DNA in the various samples was estimated at 25 °C below the Tm of the particular sample, allowing valid comparison between them[11,9]. The results are shown in the Table, each value being based on at least 3 estimates and these show that n.e.-DNA is moderately enriched in repetitive sequences relative to bulk DNA.

Content of highly repetitive DNA in nuclear envelope – associated and bulk DNA from rat and mouse liver nuclei

Source of DNA	Repetitive DNA (%)	Enrichment
Rat nuclei	16	1.00
Rat nuclear envelopes	25	1.59
Mouse nuclei	22	1.00
Mouse nuclear envelopes	30	1.37

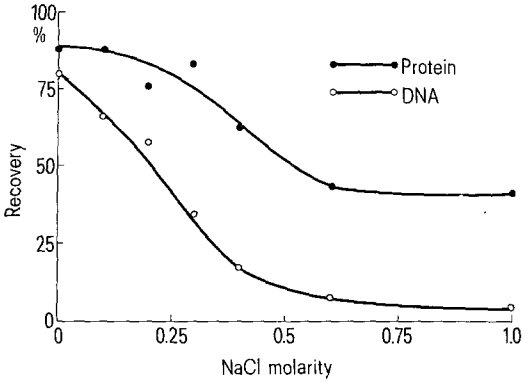

Fig. 1. Recovery of DNA and protein in nuclear envelopes pelleted from solutions containing increasing NaCl concentrations.

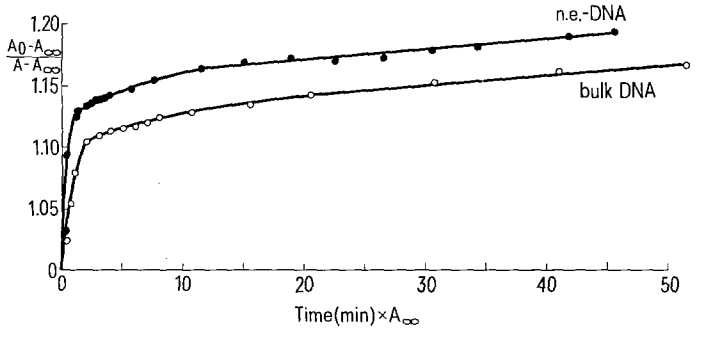

Fig. 2. Second order rate plots of the reassociation of rat liver bulk nuclear and nuclear envelope-associated DNA's, at 60 °C in 0.5 × SSC. A_0, absorbance at 260 nm of denatured DNA; A_∞, absorbance of native DNA; A, absorbance of renaturing DNA at a particular time.

[1] H. W. BEAMS and S. MÜLLER, Z. Zellforsch. *108*, 297 (1970).
[2] D. E. COMINGS and T. A. OKADA, Expl. Cell Res. *63*, 62 (1970).
[3] R. R. KAY and I. R. JOHNSTON, Subcell. Biochem. *2*, 127 (1973).
[4] R. R. KAY, M. E. HAINES and I. R. JOHNSTON, FEBS Lett. *16*, 233 (1971).
[5] R. R. KAY, D. FRASER and I. R. JOHNSTON, Eur. J. Biochem. *30*, 145 (1972).
[6] C. L. SCHILDKRAUT and J. J. MAIO, Biochim. biophys. Acta *161*, 76 (1968).
[7] M. MANDEL and J. MARMUR, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1968), vol. 12B, p. 195.
[8] W. HENNIG and P. M. B. WALKER, Nature, Lond. *225*, 915 (1970).
[9] R. R. KAY, Ph. D. Thesis, University of London (1973).
[10] M. MANDEL, C. L. SCHILDKRAUT and J. MARMUR, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1968), vol. 12B, p. 184.
[11] C. D. LAIRD, B. L. MCCONAUGHY, and B. J. MCCARTHY, Nature, Lond. *224*, 149 (1969).

Mouse bulk and n.e.-DNA's were also compared by analytical CsCl density gradient centrifugation. Bulk DNA banded as a sharp zone of peak density 1.701 g/cm^3 with satellite at 1.690 g/cm^3. 4 samples of n.e.-DNA banded as broader zones with peak density at 1.705 g/cm^3 and without a separate satellite. Low M. W. bulk DNA also banded in this way. In these cases it was not possible to accurately estimate the satellite content of mouse n.e.-DNA, but it was obviously not greatly enriched in this DNA, relative to bulk.

The present results reinforce the view that the DNA associated with isolated nuclear envelopes is to some extent a distinct sub-fraction of the total DNA. It is characterized by its close association with the envelope and by moderate enrichments in late-replicating[4,5] and repetitive DNA sequences and presumably also by an enrichment in sequences most intimately associated with the envelope in vivo. These results are consistent with the n.e.-DNA being derived from peripheral heterochromatin, a conclusion also reached by FRANKE et al[12]. Finally it seems that the association of this peripheral heterochromatin with the envelope is mainly mediated by ionic forces.

Zusammenfassung. Nachweis, dass aus Ratten- und Mausleberzellen isolierte, an Zellkernmembrane gebundene DNS, im Vergleich zum totalen Zellkern-DNS besonders reich an wiederholenden Sequenzen waren. Diese DNS stammt vermutlich aus peripherem Heterochromatin und ist an die Membran hauptsächlich durch Ionen gebunden.

R. R. KAY and I. R. JOHNSTON[13]

Department of Biochemistry,
University College, Gower Street,
London WC 1E 6BT (England), 9 November 1973.

[12] W. W. FRANKE, B. DEUMLING, H. ZENTGRAF, H. FALK and P. M. M. RAE, Expl. Cell Res., *81*, 365 (1973).

[13] We wish to thank the Medical Research Council for Financial support.

The Localization of Adenosine Triphosphatase Activity in the Alveolar Macrophages of Hamster Lung

The alveolar macrophages of mammalian lung are known to contain a variety of hydrolytic enzymes[1–4] although the precise intracellular location of many of these enzymes has yet to be determined. In this study the adenosine triphosphatase (ATPase) activity in the alveolar macrophages of hamster lung was localized using an electron cytochemical technique.

Materials and method. Young adult hamsters were killed by cervical dislocation and small blocks of lung tissue were excised and fixed for 90 min in ice-cold 2.5% glutaraldehyde in cacodylate buffer, pH 7.3. The blocks were washed for 30 min in cacodylate buffer containing 0.15 *M* sucrose and then cut into sections (about 0.5 mm thick) with a razor blade. The sections were incubated for 20–30 min in a lead capture medium at 37 °C. The medium consisted of 11 ml of 0.2 *M Tris*-maleate buffer (pH 7.4), 11 ml of 2.5 m*M* ATP (Sigma London), 2 ml of 0.1 *M* magnesium sulphate, and 1 ml of 10 m*M* lead nitrate. Control sections were incubated in a substrate-free medium or, alternatively, in a medium in which ATP had been replaced by an equimolar quantity of Na β-glycero-phosphate. After incubation all sections were post-fixed for 45 min in buffered 1% osmium tetroxide, rapidly dehydrated in absolute ethanol, and embedded in Araldite[5]. Ultrathin sections were cut on a Reichert ultramicrotome and viewed without further staining in an AEI 801 electron microscope at an accelerating voltage of 60 kV.

Observations. Enzymic hydrolysis of ATP in the presence of free lead ions results in an insoluble electron-opaque deposit of lead phosphate. In tissues incubated for 30 min or longer a fine particulate deposit of lead phosphate was present in the plasma membranes of the alveolar macrophages. Although membranous structures are generally poorly delineated in sections of unstained material, sufficient contrast was present in the electron micrographs to confirm that the reaction product was associated with the outer leaflet of the plasma membrane. The intensity of the reaction was greatest over the surface of pseudopodia (Figure 1). A moderately intense reaction was present in the membranes of phagocytic and micropinocytotic pits. Many of the digestive vacuoles and dense bodies were labelled (Figure 2) but the mitochondria and Golgi elements were unreactive even after prolonged incubation (120 min). Non-specific deposition of lead salts was commonly seen within the nuclei of macrophages. Control sections incubated in a medium lacking the substrate (ATP) or in a medium in which ATP had been replaced by Na β-glycerophosphate showed no reaction.

Discussion. In this study vigorous hydrolysis of ATP was observed on the plasma membranes of alveolar macrophages, especially in areas where pseudopodia or phagocytic and micropinocytotic pits are present. The absence of reactivity in control incubations where Na β-glycerophosphate was substituted for ATP in the medium suggests that the hydrolysis of ATP was due in normal circumstances to the action of a specific ATPase rather than a non-specific phosphomonoesterase.

In previous cytochemical studies ATPase has been observed on the microvilli of intestinal epithelium[6], the endothelium of capillaries[7], the basal infoldings of cell membranes in kidney tubules[8], and the microvilli of bile canaliculi[9]. In each of these sites extensive transfer of fluid is known to take place. Furthermore, strong ATPase activity has been detected within motile cell processes[10,11]. It is probable therefore that much of the ATPase contain-

[1] A. M. DANNENBERG, M. S. BURSTONE, P. C. WALTER and J. W. KINSLEY, J. Cell Biol. *17*, 465 (1963).
[2] Z. A. COHN and E. WEINER, J. exp. Med. *118*, 991 (1963).
[3] E. S. LEAKE and Q. N. MYRVIK, Br. J. exp. Path. *45*, 384 (1964).
[4] S. P. SOROKIN, J. Histochem. Cytochem. *14*, 884 (1967).
[5] J. H. LUFT, J. biophys. biochem. Cytol. *9*, 409 (1961).
[6] C. T. ASHWORTH, F. J. LUIBEL and S. C. STEWART, J. Cell Biol. *17*, 1 (1963).
[7] V. T. MARCHESI and R. T. BARRNETT, Anat. Rec. *142*, 317 (1962).
[8] H. W. SPATER, A. B. NOVIKOFF and B. MASEK, J. biophys. biochem. Cytol. *4*, 765 (1958).
[9] E. ESSNER, A. B. NOVIKOFF and B. MASEK, J. biophys. biochem. Cytol. *4*, 711 (1958).
[10] I. R. GIBBONS and A. J. ROWE, Science *149*, 424 (1965).
[11] F. L. RENAUD, A. J. ROWE and I. R. GIBBONS, J. Cell Biol. *36*, 79 (1968).