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THE NATURE OF THE FOLDING OF THE INTERPHASE CHROMATIN

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It is generally accepted that the way which interphase chromatin is accomodated within the nucleus depends ultimately on its interaction with scaffolding proteins, so that an efficient packaging may be achieved either by crosslinking the fibres or by subdividing the genome into independent loops. The former view has been favoured by Bram and Ris (1), the latter is supported by hydrodynamic investigations on the protein-DNA complex (in jargon nucleoid) isolated by high-salt extraction (2). The binding of proteins to DNA, however, hardly could represent the sole factor responsible for the packaging of chromatin and we were recently able to detect within large ethidium-relaxed nets of DNA from rat liver several conformational singularities which perturb the worm-like trend of the chain. A few examples are shown in the micrographs of Fig. 1 A-C. They include strong local bending which often causes the chain to fold back and wind up on itself (A,B) and long-range DNA-DNA crosslinking (C). These singularities occur regularly along the path of the duplex and their spatial co-ordination could actually direct the collapse of nuclear chromatin. Within the different models for chromatin assembly nothing is said about the nature of the proteins that clamp the DNA, although several workers dealing with ultrastructural analysis have called attention to the possible role of lamins, the main constituents of the inner shell of the nuclear envelope and, more recently, of heterogeneous nuclear RNA - protein complexes (hnRNP). We have re-examined this matter by preparing nucleoids from hepatocytes according to a steady extraction method in the presence of a non ionic detergent (Triton X-100) and high concentration of monovalent salt (2 M NaCl). In this way spurious protein reassortment, which is invariably met whenever discontinuous (involving a pelleting step) procedures are used, can be circumvented. The progress of the isolation of nucleoids from rat liver nuclei is reported in Fig. 1D. An effective separation of nuclear proteins from cytoplasmic material is achieved (lanes a and b); lane c shows that after high-salt wash the nuclear DNA is associated only with lamins (at

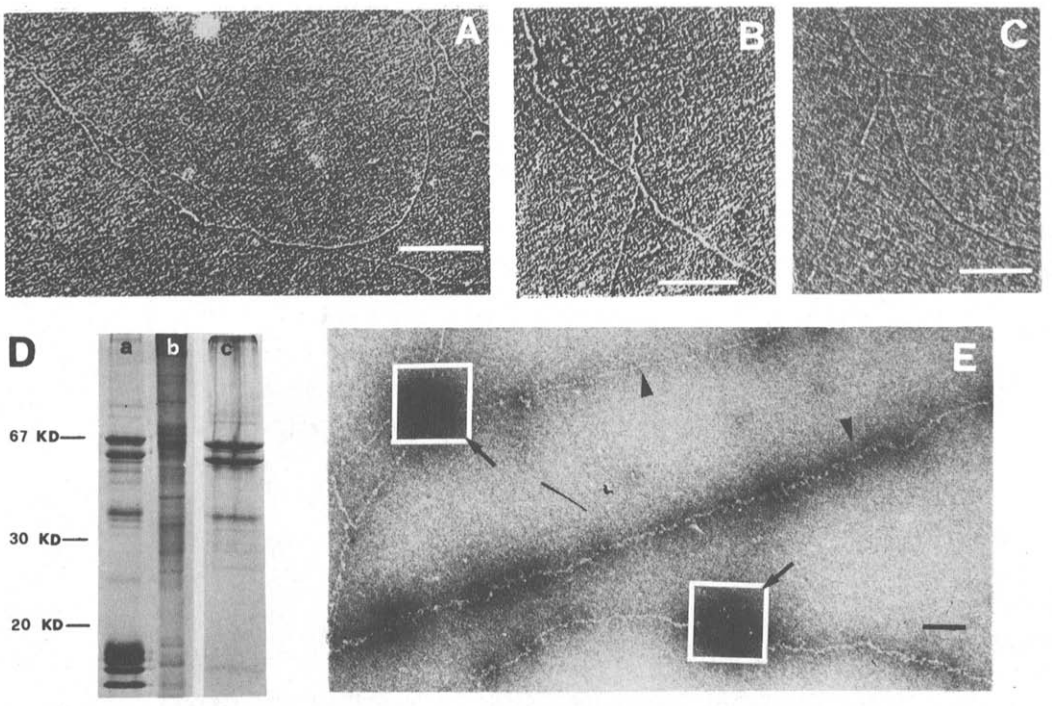

Fig. 1. A-C. Conformational singularities within a DNA net. Gently dehistonized chromatin from rat liver has been relaxed by ethidium. D. The electrophoretic patterns on 12,5% polyacrylamide gels containing 0,1% SDS of nuclear proteins (lane a), cytoplasm (lane b) and nucleoids (lane c). The molecular weight scale in KDaltons is reported on the left. E. A negatively stained peripheral region of a nucleoid. hnRNP cluster around the DNA. In A,B,C and E the bar corresponds to 200 nm.

65-60 KD) and hnRNP (at 40-30 KD). Which might be the structural connection among these components is reported in Fig. 1E, showing clumps of recognizable (arrowed) hnRNP particles bound to DNA stretches (arrow-heads). 10 nm thick laminar fragments are often associated with hnRNP (not shown). We inferred that these latter bridge between the nuclear scaffold and chromatin. In the presence of ionic detergents (sodium dodecylsulfate or deoxycholate) the nucleoid structure becomes unstable, and the protein core undergoes dissociation; laminar fragments, as viewed in the electron microscope, slide onto the DNA loops. This disintegration process culminates in the loss of any discernible radial symmetry.

Both conformational singularities and binding to large protein clusters of the DNA apparently direct the packaging of the genome. The interrelation between these factors is still unclear. Regarding this fact it is worth noting that regularly spaced kinks, in association with laminar fragments, punctuate the path of interphase chromatin (3).

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REFERENCES

1. S. Bram and H. Ris, J. molec. Biol. 55, 325 (1971).

2. P.R. Cook and I.A. Brazell, J. cell Sci. 19, 261 (1975).

3. B. Cavazza, V. Trefiletti, F. Pioli, E. Ricci and E. Patrone, J. cell Sci. 62, 81 (1983).