DNA-Binding Specificity of a Chromatin Non-Histone Protein Fraction of HeLa Cells

James H. J. DUNN,* Raymond M. LYALL,* Robert C. BRIGGS,† Ailsa M. CAMPBELL* and
Lubomir S. HNILICA†

*Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.,
and †Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232, U.S.A

(Received 15 October 1979)

The DNA-binding site of a previously characterized non-histone chromosomal protein antigen(s) from HeLa cells was investigated for its species specificity. Treatment with large amounts of micrococcal nuclease abolishes immunoactivity, which can then be recovered by the subsequent addition of human or HeLa DNA to reconstitute the immune complex. Neither rat nor calf DNA exhibits this property, but DNA from monkey cells gives considerable activity. The antigen is not, however, detectable in monkey chromatin.

Among the diverse groups of non-histone chromosomal proteins which are amenable to immunological analysis, the tight-binding group is the most difficult to subject to a detailed study. Many of the proteins that show cell specificity, and consequently have potential for analysis as determinants of phenotypic expression, have such altered physicochemical characteristics in the absence of DNA that they precipitate under normal solution conditions (Campbell *et al.*, 1978, 1979; Briggs *et al.*, 1979). One such protein, or group of proteins, previously shown to be absolutely specific to HeLa cells falls into this category. Treatment with the small amounts of micrococcal nuclease sufficient to fragment chromosomal DNA into nucleosome-sized pieces does not dislodge the antigen(s), and even doses up to 500 units/ml per mg of chromatin have no effect on immunoactivity as judged by complement-fixation and immunocytochemical techniques. However, treatment with 2000 units of nuclease/ml per mg eventually abolishes immunoactivity. The antigen has previously been shown to be physically located on the scaffold region of metaphase chromosomes, where certain DNA sequences are suggested to occur with high frequency (Campbell *et al.*, 1979; Adolph *et al.*, 1977). The very small amounts of cell-specific HeLa antigen present in each cell make it difficult to attempt isolation of the DNA fragments to which it is bound. However, it is possible to attempt simple reconstitution experiments on nuclease-digested material to examine the nature of the DNA required for resolubilization of the precipitated antigen released by the extensive micrococcal nuclease treatment. The experiments described in the

present paper were undertaken to ascertain firstly whether reconstitution was possible or whether the abolition of reactivity by massive amounts of nuclease represented some irreversible phenomenon. Secondly they were undertaken to ascertain whether the resolubilization of the antigen by DNA reflected a physicochemical requirement for DNA or some similar polyanion, or a genuine sequence-specific binding phenomenon. Thirdly the data were required to investigate the evolutionary pattern of both the protein and its binding site. Such experiments provide the necessary preliminary information for the determination of the suitability of cross-species cell-fusion procedures as analytical probes for the location of the antigen and its binding site on the appropriate HeLa chromosomes.

Methods

HeLa cells and green-African-monkey kidney cells (BSC 1) were cultured in Glasgow-modified Eagle's medium (Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.) supplemented with 5% (v/v) calf serum. Chromatin was prepared as described previously (Campbell *et al.*, 1979). Human placental DNA, rat liver DNA, green-African-monkey kidney DNA and HeLa DNA were prepared by phenol extraction in the presence of 0.5% sodium dodecyl sulphate and 10mM-EDTA (Thomas *et al.*, 1966). The DNA was extracted twice, ethanol precipitated and treated with ribonuclease before a further phenol extraction. Calf thymus DNA was purchased from Sigma Chemical Co.

Complement-fixation reactions were undertaken

by the method of Wasserman & Levine (1961). The antisera were from the same group of animals (Campbell *et al.*, 1979).

Staphylococcal-nuclease digestion was carried out in 10 mM-Tris/HCl/40 mM-NaCl/1 mM-phenylmethanesulphonyl fluoride/1 mM-CaCl₂, pH 7.5. The nuclease (Worthington) was used at 2250 units/100 µg of chromatin DNA and incubated at 37°C for 90 min. Some batches of nuclease did not have the stated specific activity, so activity was monitored by the manufacturers' assay before use. The reaction was stopped by adding EDTA to a final concentration of 2.5 mM. The DNA was then added back to the final concentration shown, and the complement-fixation assay was carried out as for normal chromatin without any additional incubation periods.

Results

Fig. 1 demonstrates two main features of the reconstitution experiment. Firstly it shows that almost total recovery of activity is possible on the re-addition of DNA, thus indicating that the loss of activity on digestion with massive amounts of enzyme reflects genuine dislodgement of the protein from the DNA and not the presence of endogenous proteinase or some other inhibitory factor in the commercial nuclease preparation. Secondly it shows that human and HeLa-cell DNA are virtually interchangeable for this purpose. This second factor of

information was not necessarily to be expected, since the HeLa cell line is known to have unusual ploidy and to have been in culture for more than 20 years, so that in fact some sequences might well be expected to be present in different ratios from those in human placental DNA. The data show either that this is not the case, or that there are ample sites on the DNA for the numbers of antigen molecules present in the chromatin, so that the availability of a DNA-binding site for the reconstitution process is not a limiting factor. Fig. 1 also confirms that the DNA itself has no immunological reactivity.

In Fig. 2, the question of the nature of the DNA required for reconstitution is investigated. It is immediately clear that not all types of DNA are effective and that specific DNA sequences must be involved in binding. Thus neither calf nor rat DNA can re-solubilize the antigens, so that complement-fixation activity is regained even at high concentrations. On the other hand, the DNA from green-African-monkey kidney cells leads to a reconstitution almost as good as with human or HeLa DNA. No trace of the antigen can be found in the chromatin proteins of green-African-monkey kidney cells, as would be expected, since the antigen seems to be totally confined to HeLa cells in its location (Campbell *et al.*, 1979; Briggs *et al.*, 1979).

Fig. 1. Digestion and reconstitution of HeLa chromatin with micrococcal nuclease, studied by complement-fixation assay

For details see the text. Key: ○, HeLa chromatin, undigested; △, HeLa chromatin digested with micrococcal nuclease; □, HeLa chromatin digested with nuclease and reconstituted with human DNA; ●, HeLa chromatin digested with nuclease and reconstituted with HeLa DNA; ■, human or HeLa DNA.

Fig. 2. Digestion and reconstitution of HeLa chromatin with micrococcal nuclease and reconstitution with heterologous DNA types

For details see the text. Key: ▽, HeLa alone; ○, HeLa chromatin, undigested; △, HeLa chromatin digested and reconstituted with human DNA; □, HeLa chromatin digested and reconstituted with HeLa DNA; ●, HeLa chromatin digested and reconstituted with calf thymus DNA; ▲, HeLa chromatin digested and reconstituted with rat liver DNA; ■, HeLa chromatin digested and reconstituted with green-African-monkey kidney-cell DNA.

Discussion

Any chromosomal complex that can only be assayed in the presence of two discrete components is difficult to analyse in terms of the nature and extent of the role played by each of the two individual parts of the system. This is particularly true in the case of the DNA-binding group under analysis, and yet is a desirable aim.

One of the most reliable methods of allocation of specific gene functions to certain chromosome locations is by interspecies somatic-cell fusion and subsequent isoenzyme analysis (Ringertz & Savage, 1976). Experiments with HeLa cells and Chinese-hamster kidney cells have shown that this antigen can be expressed in some interspecies hybrids and not in others (J. Dunn, B. Carrit & A. M. Campbell, unpublished work), and it has therefore become necessary to ask whether in such hybrids one is looking for two genes which are not necessarily on the same chromosome(s). The presence of the gene coding for the HeLa-cell antigen(s) itself is not sufficient for the detection of immunoactivity if the DNA-binding site is not present as well.

The experiments described in this paper show that total reconstitution of DNA-protein complexes to their original immunoactivity is possible in this system. Many of the recent experiments involving reconstitution of chromatin proteins have been held in some doubt with respect to their transcriptional properties, but this dissociation would appear to be entirely reversible. The nature of the binding of the two components is not clear and has been discussed before (Campbell *et al.*, 1979), but it would appear that the DNA sequence is essential to solubilize the antigen and prevent its aggregation. It could also be argued that there is a different massive conformational change in the antigen in the absence of the DNA, such that the antigenic sites are no longer recognized but aggregation is not involved. It may be interesting to note in this context that the antibody appears to give little protection against digestion of the DNA-binding site by micrococcal nuclease (A. M. Campbell, unpublished work).

The evolutionary conclusion that can be drawn from the experiments described is that the sequence is largely conserved among primates and is not undergoing the rapid evolution characteristic of satellite-DNA molecules. In this context it will be of interest to attempt an analysis of the frequency class of the DNA molecules required for reconstitution, since it seems unlikely that a complex that occurs only once per haploid genome would be readily detectable in the small amounts of material used for assay, however sensitive the assay. If the sequence is

primate-specific, then it can be argued that some evolutionary advantage has been conferred by the possession of the sequence, but that the only cell line yet shown to use this advantage is the HeLa one. However, it may also be that a sequence that performs a similar task is present in other mammalian genomes such as calf, but that it does not recognize the HeLa-cell protein. It is perhaps worth noting that the decrease in affinity is not a minor gradation, since much larger amounts of DNA in reconstitution experiments do not increase the complement-fixation activity of the complex.

The species variation of the non-histone chromosomal proteins has been the subject of detailed analysis in several laboratories (Zardi, 1975; Sevall *et al.*, 1975), and in at least one case species specificity of the tight-binding proteins in a cross-species cell hybrid has been reported (Tsusui *et al.*, 1977). The present paper reports the analysis of the species variation of the binding site of a partially characterized cell-specific protein whose chromosomal location on the scaffold has been established. Actual isolation and sequencing of the DNA-binding site will depend on progress in purification of the adequate amounts of the cell-specific HeLa antigen.

We acknowledge the support of the Science Research Council of the U.K. and the National Cancer Institute and the U.S. Public Health Service grant CA18389.

References

- Adolph, K. W., Chang, S. M., Poulson, J. R. & Laemmli, U. K. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4937–4941
- Briggs, R. C., Campbell, A. M., Chiu, J. F., Hnilica, L. S., Lincoln, G., Stein, J. & Stein, G. (1979) *Cancer Res.* **39**, 3683–3688
- Campbell, A. M., Briggs, R. C., Zimmer, M. S., Krajewska, W. M., Chiu, J. F. & Hnilica, L. S. (1978) in *Biological Markers of Neoplasia* (Ruddon, R., ed.), pp. 369–384, Elsevier/North-Holland, Amsterdam
- Campbell, A. M., Briggs, R. C., Bird, R. E. & Hnilica, L. S. (1979) *Nucleic Acids Res.* **6**, 205–218
- Ringertz, N. R. & Savage, R. E. (1976) *Cell Hybrids*, chapter 13, Academic Press, New York
- Sevall, J. S., Cockburn, A., Savage, M. & Bonner, J. (1975) *Biochemistry* **14**, 782–789
- Thomas, C. A., Berns, K. I. & Kelly, T. J. (1966) in *Procedures in Nucleic Acid Research* (Cantoni, G. L. & Davies, D. R., eds.), pp. 539–540, Harper and Row, London
- Tsusui, Y., Chang, H. L. & Baserga, R. (1977) *Cell Biol. Int. Rep.* **1**, 301–308
- Wasserman, E. & Levine, L. (1961) *J. Immunol.* **87**, 290–295
- Zardi, L. (1975) *Eur. J. Biochem.* **55**, 231–238