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CHANGES IN THE CONFORMATION OF THE INTERFERON β GENE DURING DIFFERENTIATION AND INDUCTION

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SUMMARY: DNase I sensitivity was used to investigate the chromatin conformation of the interferon β gene during differentiation of the mouse teratocarcinoma cell line PC13. These cells do not produce interferon upon viral induction in their undifferentiated state, but do so on differentiation from stem cells to endoderm. Only in induced differentiated cells were the interferon β genes digested by DNase I. A similar effect was seen in a line of human cells (MG63) upon induction. We conclude that it is induction of interferon production that brings about the change in the DNase I sensitivity of these genes, rather than differentiation.

In the last few years the ability of DNase I to distinguish between the chromatin conformation of "active" and "inactive" genes has been well documented (1,2,3,4). Most of the research has centred upon the comparison of a particular gene in different tissues and has shown that for most genes only those being transcribed in that tissue are DNase I sensitive. Studies using erythroleukemic cell lines (5) have shown that the globin genes that are capable of being transcribed upon induction, are also DNase I sensitive, suggesting that the change in chromatin configuration of a gene detected with DNase I is related to the commitment of the cell to a particular developmental pathway, and the use of that gene, rather than its actual transcriptional state. However, this does not hold true in all systems studied, for example certain muscle specific genes alter their DNase I sensitivity during myogenesis (6) as do some of the Drosophila heat shock protein genes upon a

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temperature shock (7,8), and under these conditions, transcription of the DNase I sensitive genes occurs.

The interferon (IFN) genes provide a convenient mammalian system for study of an inducible gene. Prior to induction of these cells with virus, no IFN protein or IFN mRNA is produced, whilst a few hours after the addition of virus both IFN mRNA and protein can be detected. Further, teratocarcinoma cells provide a suitable system to investigate the control of the interferon system during differentiation, since only differentiated cells are capable of induction (9). We therefore studied DNase I sensitivity of the IFN β gene in these cells before and after differentiation, and before and after induction. We also examined a differentiated human cell line (MG63) before and after induction.

MATERIALS AND METHODS

Cell culture. Non-differentiated PC13 cells were grown in modified Eagles medium plus 7.5% fetal calf serum, 2.5% newborn calf serum and antibiotics (10) on gelatinized plastic flasks. The cells were differentiated by the addition of 3 μg/ml retinoic acid and grown in non-gelatinized flasks. After 10 days the cells are capable of producing IFN upon induction (9). Induction by NDV (Strain F: 2500 HAU/ml or Sendai virus (12 HAU/ml) was for 1 hour in 2% serum media, followed by a further virus-free incubation of 3 hours. This was found to be the time when IFN could first be detected in the supernatant of differentiated cells (data not shown). MC63 cells were grown in Glasgow modified Eagles media plus 10% newborn calf serum and antibiotics in roller bottles. Induction by NDV or Sendai virus was for 1 hour followed by a further 2 hours when the first IFN activity could be measured (data not shown).

Immunofluorescence: Differentiated or non-differentiated PC13 cells were treated with NDV (75 HAU/ml) for 1 hour. Immediately after infection, or 16-22 hours later, cells were treated with rabbit anti-NDV serum (a gift from Dr. D.P. Barlow) followed by fluorescein-labelled sheep anti-rabbit IgG (Wellcome Diagnostics).

Nuclei preparation: The method was that of Schribler and Weber (11). Nuclei were used fresh or stored at -70°C in RB (25% glycerol, 5mM Mg acetate, 50 mM Tris-HCl pH 8.0, 5 mM DTT, 0.1 mM EDTA).

DNase I digestion: Nuclei were taken up at 1 mg/ml DNA in RSB (1) and digested with 1 μg/ml DNase I (BCL, EC 31.21.1) for increasing lengths of time. The reactions were stopped by the addition of SDS and EDTA to a final concentration of 0.5% SDS and 12.5 mM EDTA (12), and digested overnight with 100 μg/ml proteinase K (BCL, EC 3.4.21.14), phenol-chloroform extracted and RNase A (BCL, EC 3.1.27.5) treated. 10 μg/ml aliquots of the various DNA samples, digested with Eco RI (BRL) to completion, were loaded onto 1% agarose gels electrophoresed prior to blotting onto nitrocellulose paper (Schleicher and Schull) (13). Filters were hybridized to [^{32}P]-labelled nicktranslated probes (14). The probes used were mouse β interferon cDNA (pMIF 3/10, Pst I insert) (15) and human β interferon cDNA (D4P165, Msp I fragment) (16).

RESULTS AND DISCUSSION

DNase I Sensitivity of the Interferon β Gene In Undifferentiated PC13 Cell Nuclei.

Undifferentiated mouse teratocarcinoma cells fail to produce IFN upon viral induction. This could be because the virus fails to infect the cells and does not therefore induce the IFN system, but Figure 1 shows that undifferentiated PC13 cells, although not producing any IFN protein, do become infected by NDV and express virus proteins on the cellular outer membrane as judged by immunofluorescence. Fluorescence was seen in both undifferentiated (Figure 1a,b) and differentiated (Figure 1e,f) PC13 cells when expression of the virus proteins was allowed to occur. When virus was added only 1 hour prior to antibody treatment, very little immunofluorescence was detectable (Figure 1c,d,g,h). No immunofluorescence was observed if chick allantoic fluid and non-immune rabbit serum were substituted for virus and anti-NDV serum respectively (data not shown). Therefore, the fluorescence seen is due to newly synthesized virus proteins rather than the infecting virus particles. Thus, the failure to react to virus induction is not due to a failure in the infection by, or growth of the virus, but rather to some other process within the cell.

Nuclei were prepared as described in Materials and Methods both from non-induced and induced undifferentiated PC13 cells. These were digested with 1 μg/ml DNase for up to 5 minutes. Under these conditions about 5% of the DNA is lost. The remaining DNA was extracted and digested with an excess of Eco RI to completion. The restricted DNA was run on an agarose gel, transferred to nitrocellulose paper and probed for mouse IFN β gene sequences. This revealed a single 4000 bp fragment in DNA from non-DNase I treated nuclei (Figure 2a, 0 minutes). As can be seen from Figure 2(a,b), this fragment is not degraded by DNase I in either non-induced or induced undifferentiated PC13 cells.

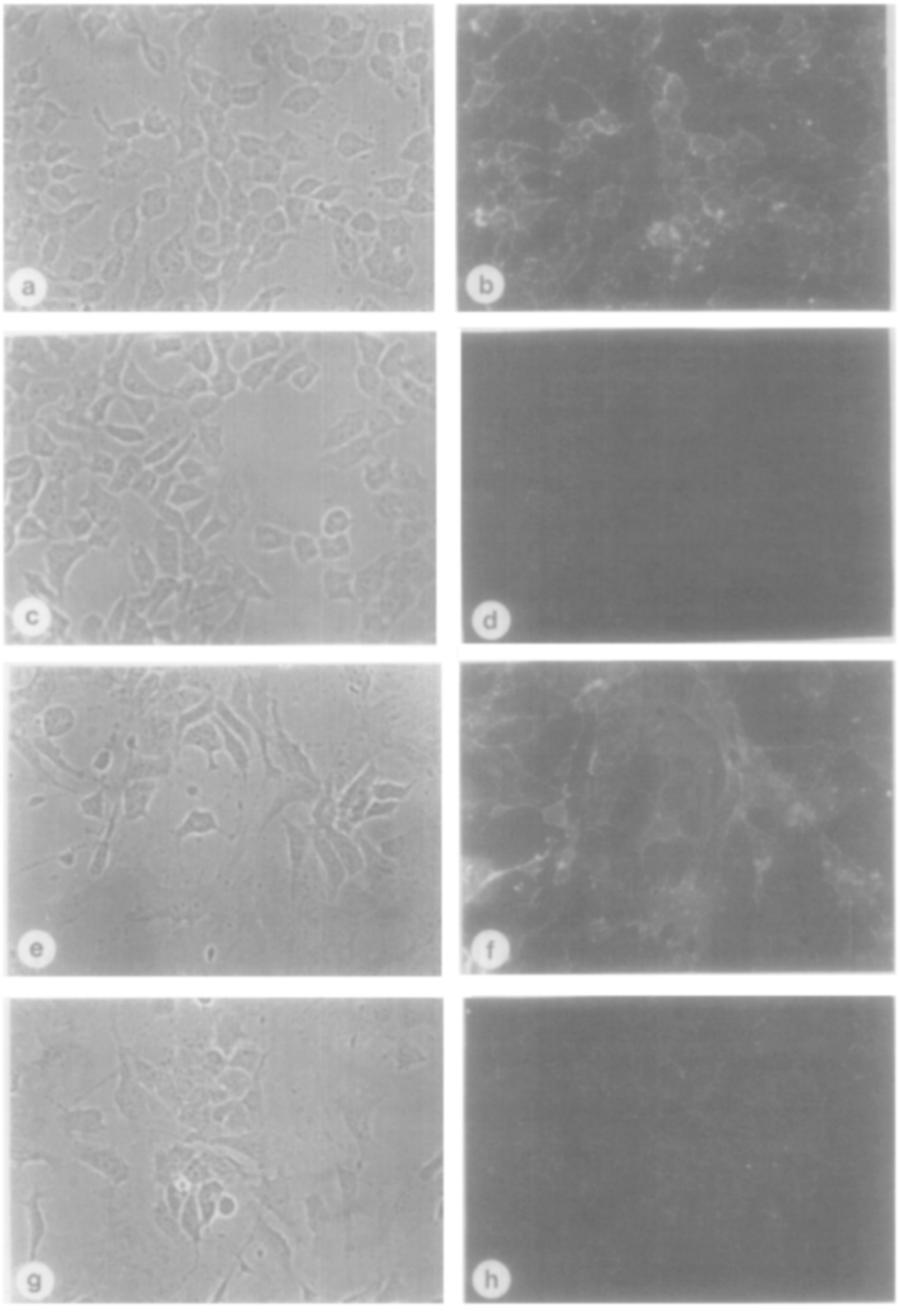

Figure 1: NDV-F Specific Immunofluorescence in PC13 Cells. Undifferentiated and differentiated PC13 cells were infected with NDV-F as described in the Materials and Methods. Cells were viewed under phase contrast (a,c,e,g) or UV illumination (b,d,f,h). 1a,b; undifferentiated cells, 16 hour incubation. 1c,d; undifferentiated cells, 1 hour incubation. 1e,f; differentiated cells, 20 hour incubation. 1g,h; differentiated cells, 1 hour incubation.

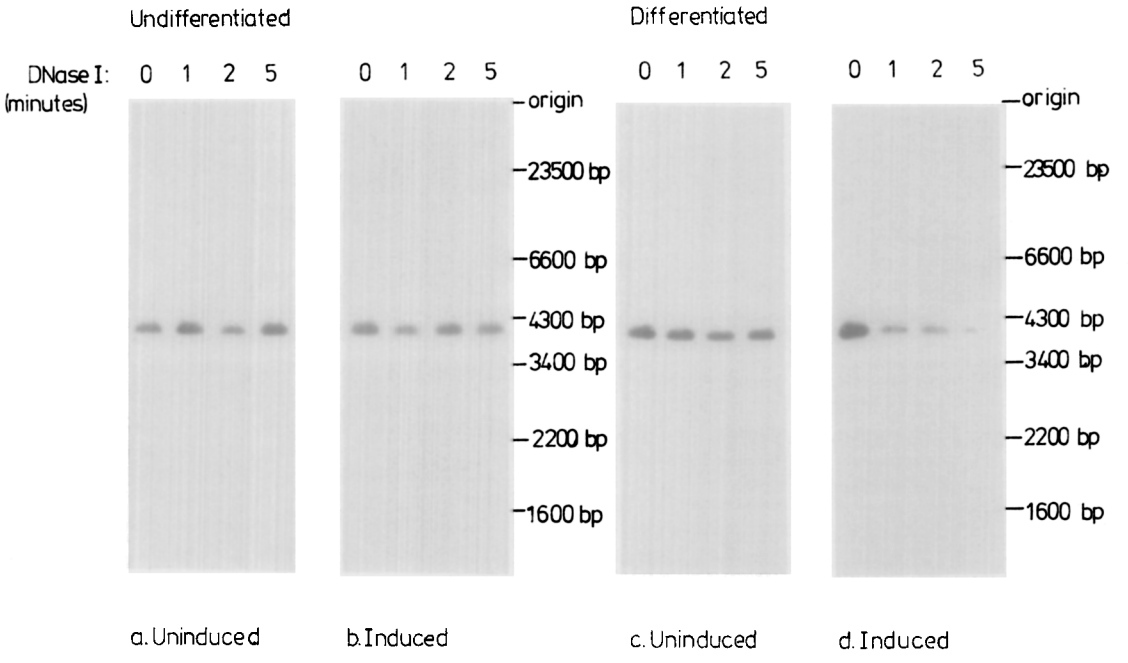

Figure 2: DNase I Sensitivity of the Interferon β Gene in PC13 Cells. Nuclei from the following cell types were digested with DNase I for 0,1,2 or 5 minutes prior to extraction, Eco RI digestion and analysis being carried out as described in Materials and Methods. 2a; non-induced, undifferentiated. 2b; induced, undifferentiated. 2c; non-induced, differentiated. 2d; induced, differentiated. In each case, the tracks are labelled with the time of DNase I digestion.

DNase I Sensitivity of the Interferon β Gene in Differentiated PC13 Cell Nuclei.

The experiment described above was repeated, but this time using non-induced and induced differentiated PC13 cells. These cells, unlike those prior to differentiation, are able to synthesize IFN after virus induction. When the DNA from DNase I-digested nuclei from non-induced cells was analyzed, no degradation of the 4000 bp fragment was seen (Figure 2c). However, upon induction of the cells, the IFN β gene does become DNase I sensitive as shown by the gradual loss of the 4000 bp fragment (Figure 2d).

Therefore, in the case of the mouse β IFN gene, the change in chromatin configuration that is detected by DNase I is a result of induction of an inducible cell. Neither the viral induction nor the differentiation alone causes the switch in chromatin structure to a more "open" and DNase I sensitive state. This is in contrast to what might be

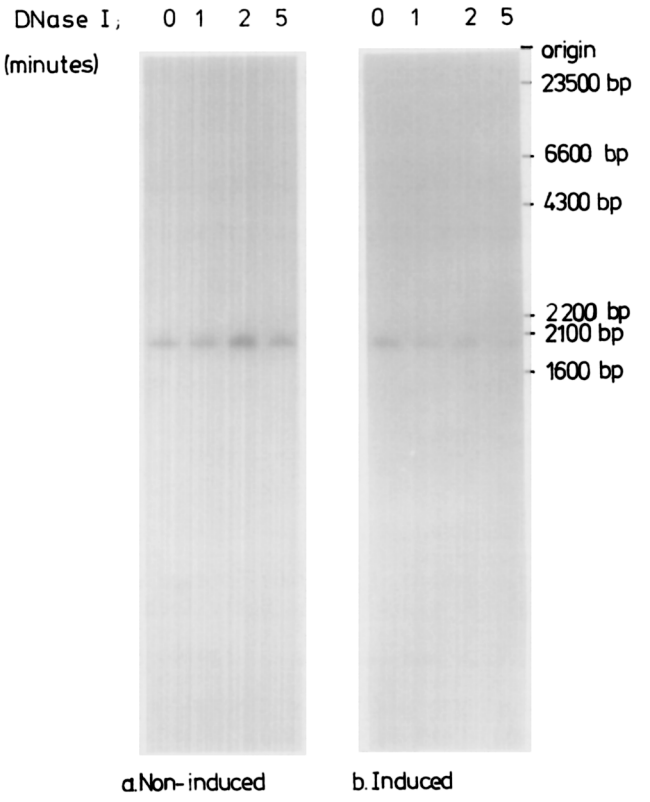

Figure 3: DNase I Sensitivity of the Interferon β Gene in MG63 Cells. Nuclei from the uninduced (3a) or induced (3b) MG63 cells were digested with DNase I for 0,1,2 or 5 minutes prior to extraction, Eco RI digestion and analysis being carried out as described previously. Tracks are labelled with the time of DNase I digestion.

expected if the change from DNase I resistance to sensitivity required DNA replication to occur.

DNase I Sensitivity of the Interferon β Gene in Human MG63 Cell Nuclei.

To investigate whether the change in DNase I sensitivity associated in PC13 cells only with induction of differentiated cells was confined to this cell line, a similar experiment was carried out using human MG63 cells. This differentiated cell line, derived from a human osteogenic carcinoma, synthesizes mostly IFN β upon virus induction. As shown in Figure 3, the single fragment of 1920 bp seen with the cDNA probe used, is not degraded in non-induced cells. In DNA isolated from DNase I treated induced MG63 cells, the IFN β gene is in a different chromatin conformation after induction, and this change is distinguished by DNase I sensitivity.

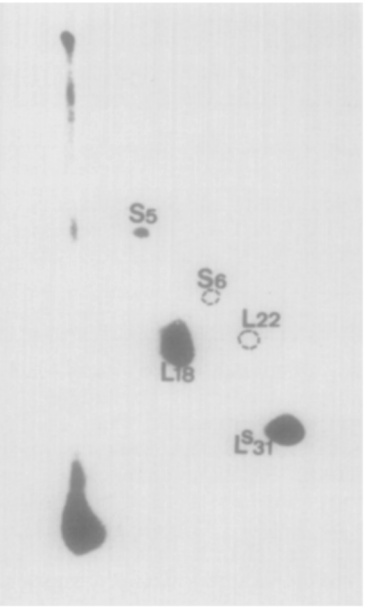

Figure 5. Autoradiogram of 70S ribosomal proteins separated by 2D-electrophoresis after in vitro incubation with [γ^{32}P]-ATP, at 30°C, for 30 min in the presence of cyclic nucleotide independent casein kinase isolated from adrenal cortex. Exposure time: 10 hours.

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