Changes of chromatin conformation around mouse interferon- β gene associated with induction of interferon synthesis

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ABSTRACT

Changes of chromatin structure around the mouse interferon- β gene associated with induction were examined by the use of sensitivity to digestion with DNaseI. Upon induction, the DNaseI-sensitivity of mouse interferon- β gene was increased and seven DNaseI-hypersensitive sites were mapped. One of them, which was located at approximately 100bp upstream and was insensitive in the uninduced state, showed a marked sensitivity upon induction. Four others, located at 580bp and 430bp upstream, and 1.9kb and 2.2kb downstream, whose levels of DNaseI-sensitivity were very low in the uninduced state, showed significant sensitivity in the induced state. Two remaining sites, located at 7.5kb upstream and 7.6kb downstream, had relatively high DNaseI-sensitivities even in the uninduced state and slightly increased their sensitivities upon induction.

INTRODUCTION

It has been shown that transcriptionally active genes have an altered chromatin structure which is reflected in their increased sensitivity to DNase-digestion (1,2,3). In these DNase-sensitive regions, discrete short stretches of DNA which are hypersensitive to DNase have also been detected. Frequently, but not always, these hypersensitive sites were observed at or near the 5'-ends of actively transcribed genes (4,5,6), although more complex pattern of location of DNase-hypersensitive sites could be seen in some developmentally or hormonally regulated genes (7,8).

Interferon (IFN) is one of the most useful genes for studying the regulatory mechanism of eukaryotic gene expression because it is highly inducible by specific inducers such as viruses or synthetic double stranded RNA (9). cDNA and genomic DNA for human and mouse IFN- α (10,11) and β (12,13) have been cloned. The analyses of 5'-flanking sequences necessary for

regulated expression of IFN genes were performed and the regions of 100-200bp length, which seem to be sufficient for induction of IFN genes, were defined (14,15).

Recently, Coveney et al reported that the DNaseI-sensitivities of human and mouse IFN- β genes were increased upon induction in differentiated cells, but not in undifferentiated cells which cannot produce IFN (16). No further information about the chromatin structure around IFN- β gene, such as the specific DNaseI-hypersensitive sites and changes associated with IFN induction, has yet been obtained. We report here the difference of DNaseI-sensitivity of mouse IFN- β gene between induced and uninduced state. The IFN- β gene was more susceptible to DNaseI in the induced state than in the uninduced state, and several DNaseI-hypersensitive sites around this gene were mapped. One of these DNaseI-hypersensitive sites, which is located near the 5'-end of mouse IFN- β gene, was shown to appear in an induction specific manner.

MATERIALS AND METHODS

Materials

Mouse L cells, which have been recloned in our laboratory for high production of IFN by Newcastle disease virus (NDV), are maintained in Eagle's minimal essential medium (MEM) containing 5% calf serum. NDV used for IFN induction is Miyadera strain, a wild type strain (17).

DNaseI and restriction enzymes were purchased from Takara Shuzo Co. Ltd. and New England Biolabs. Other chemical reagents and biochemicals were from Wako Pure Chemicals and Nakarai Chemicals Co. Ltd.

Mouse IFN- β cDNA pM β -3 was reported previously (13). The 3kb-EcoRI fragment of mouse IFN- β genomic DNA was provided by T.Taniguchi (T.Kuga and T.Taniguchi, unpublished result). Mouse IFN- α cDNA (11) was from H.Taira and mouse IFN- γ cDNA was from H.Teraoka (unpublished results). Mouse β -globin cDNA (5'-pCRI/MG β -1) (18) was obtained from C.Weissmann through S.Nagata.

Methods

IFN induction For induction of IFN, mouse L cells were grown

to confluence in Eagle's MEM containing 5% calf serum, and infected with NDV at m.o.i.10. After 1 hr of adsorption, cells were washed and further incubated at 37°C in Eagle's MEM containing 0.5% calf serum.

Isolation of nuclei and DNaseI-digestion Isolation of nuclei and DNaseI-digestion were performed according to the method described by Wu et al (19). About 3×10^8 cells suspended in 20ml of 0.25M sucrose-nuclear buffer (0.25M sucrose, 60mM KCl, 15mM NaCl, 3mM MgCl₂, 15mM Tris-HCl pH 7.5) were lysed by adding 0.5ml of 10% NP40, mixed once with vortex, and placed on ice for 5 min for complete lysis. The lysate was centrifuged through 0.9M sucrose-nuclear buffer containing 0.5mM DTT at 1000xg for 15 min. The nuclear precipitate was washed in 0.25M sucrose-nuclear buffer containing 0.5mM DTT and recollected by centrifugation at 400xg for 10 min. The isolated nuclei were resuspended in DNaseI-digestion buffer (0.05mM CaCl₂, 3mM MgCl₂, 60mM KCl, 15mM NaCl, 0.5mM DTT, 0.25M sucrose, 15mM Tris-HCl pH 7.5) at 1×10^8 /ml. Aliquots (10^7 nuclei) were dispensed into 1.5ml-Eppendorf tubes and increasing amounts of DNaseI were added to a series of tubes. After incubation at 25°C for 10 min, reactions were terminated by addition of EDTA and SDS to a final concentration of 10mM and 0.5%, respectively.

Isolation of total DNA Lysed nuclei were treated with Proteinase K (200µg/ml) for overnight at 37°C and extracted twice with phenol and once with chloroform. DNA was purified by ethanol precipitation.

Blot-hybridization analysis Southern-blotting and hybridization were carried out as described previously (13). For Northern-blotting, formaldehyde-agarose gel electrophoresis was used and hybridization was carried out as described previously (20). Total RNA was isolated according to the procedure using guanidium thiocyanate (21) and poly(A)⁺RNA was obtained by oligo(dT)-cellulose chromatography.

RESULTS

Induction kinetics of mouse IFN-β mRNA in L cells

Confluent monolayers of mouse L cells were challenged with

Figure 1. Time course of induction of mouse IFN- β mRNA synthesis. a, total RNA was isolated from L cells at the time indicated after induction with NDV. RNA (40 μ g/lane) was electrophoresed in 1.3% agarose gel containing 2.2M formaldehyde and analyzed by Northern-blot-hybridization using mouse IFN- β genomic 3kb-EcoRI fragment as a probe. b, Poly(A)⁺RNA prepared from total RNA preparation at 12 hr.

NDV and at the times indicated, total RNA or poly(A)⁺RNA was prepared. As shown in Fig 1, mouse IFN- β mRNA which was analyzed by Northern-blot-hybridization could be detected at 4 hr post induction and was maximal at 12 hr. The level of the mRNA soon declined and it completely disappeared during the following 12 hr incubation. IFN activity in the culture fluids (a mixture of IFN- α and β) also increased in parallel with the transcriptional level of IFN- β (data not shown). Because active transcription of IFN- β seemed to start at about 6 hr, the nuclei were prepared from L cells harvested at 6.5 hr after induction with NDV in order to examine the chromatin structure around active IFN- β gene. The nuclei from mock-induced L cells were also prepared as control.

DNaseI-sensitivity of mouse IFN- β gene in induced and uninduced state

For the investigation of the chromatin structure around a IFN gene, we have chosen mouse IFN- β gene for the following reasons : 1) Relatively high titer of IFN can be produced when

mouse L cells are induced with NDV in comparison to human cells (22). 2) While the α -type IFN genes comprise a multi-gene family (23), the β -type IFN gene corresponding to pM β -3, a cDNA for mouse IFN- β , is unique (13), allowing us to expect simpler results.

Nuclei from NDV-induced or mock-induced mouse L cells were incubated with increasing amounts of DNaseI, and total DNA was isolated and electrophoresed in 0.6% agarose gel. As shown in Fig 2-a, DNA fragments of smaller size were gradually discernible according to the increasing extent of DNaseI digestion. Little difference between the DNA preparations from induced and uninduced nuclei could be detected in the digestion pattern, except in the DNA sample treated with 60 units/ml of DNaseI, where the DNA from uninduced nuclei was somewhat more digested than that from induced nuclei.

After blotting the DNA in the gel on to a nitrocellulose filter, hybridization was performed with ^{32}P -labelled IFN- β gene as a probe (Fig 2-b). With increasing DNaseI-digestion, the size of the DNA fragments containing IFN- β sequences prepared from the induced nuclei was more rapidly reduced and converted to smaller size in comparison to the DNA from uninduced nuclei. Thus, the DNA containing the IFN- β gene in the induced state was more sensitive to DNaseI than that in the uninduced state.

In the case of IFN- α genes, preferential DNaseI-sensitivity was also observed in the induced DNA preparation (Fig 2-c): The smeared DNA of smaller size was more evident in the induced DNA sample than in the uninduced. However, the difference between DNA preparations from induced nuclei and that from uninduced nuclei was much less distinct compared to IFN- β gene, especially in DNA of larger size. This is probably because of the presence of several inactive IFN- α genes, which are resistant to DNaseI, but can cross-hybridize with the probe under the conditions used.

The same DNA preparations were hybridized with IFN- γ and β -globin sequences respectively. The IFN- γ gene is unique and it is dormant, like the β -globin gene, in L cells (24). Little difference between the induced and the uninduced sample was

observed in the DNaseI-digestion pattern of these genes (Fig 2-d,e).

These results suggest that the preferential DNaseI-sensitivity of mouse IFN- β and α genes results from a specific change in chromatin conformation associated with active transcription. It should be noted that two discrete bands, corresponding to approximately 20kb and 9kb, respectively, were observed with the IFN- β probe (indicated by arrows), suggesting that specific sites which are susceptible to DNaseI-digestion exist near the mouse IFN- β gene. Although they were detected in both the induced and the uninduced state, they became more prominent in the induced state with increasing extent of DNaseI-digestion.

Mapping of the DNaseI-hypersensitive sites

To examine DNaseI-hypersensitive sites around the mouse IFN- β gene, the partial restriction map for the mouse IFN- β gene with its flanking region was determined by Southern-blot-hybridization analysis of total genomic DNA using mouse IFN- β cDNA probe, and by restriction mapping analysis of the cloned 3kb-EcoRI fragment containing the mouse IFN- β gene (T.Kuga and T.Taniguchi, unpublished result). From this restriction map, EcoRI, BglII and BamHI were selected for the restriction digestion of DNaseI-treated total DNA to search for DNaseI-hypersensitive sites around the mouse IFN- β gene.

The same DNA preparations used in the experiments of Fig 2, were digested with EcoRI, blotted, and hybridized with the 32 P-labelled EcoRI-PvuII 300bp fragment (probe 2, Fig 3). Digestion with EcoRI produced a single band of 3kb length in Southern-blotting of total DNA. The additional degradation subfragments, which were smaller than the 3kb-EcoRI fragment,

Figure 2. The preferential DNaseI-sensitivities of mouse IFN- β and α genes. Isolated nuclei from NDV-induced or mock-induced L cells were treated with increasing amounts of DNaseI (0 - 60 unit/ml). Total DNA was isolated and electrophoresed in 0.6% agarose gels. -, the lanes of mock-induced cell DNA samples ; +, the lanes of NDV-induced samples. The right-most lanes shows EcoRI-digested total L cell DNA. a, agarose gel of total DNA. The four parallel gels were blotted and hybridized with 32 P-labelled probes as follows: b, IFN- β genomic 3kb-EcoRI fragment. c, IFN- α cDNA. d, IFN- γ cDNA and e, β -globin cDNA.

Figure 3. Restriction map of mouse IFN- β gene and its flanking region. Restriction map of mouse IFN- β gene and its flanking sequence was determined by the total DNA Southern-blot-hybridization using mouse IFN- β cDNA probe, and by restriction mapping analysis of the cloned 3kb-EcoRI fragment containing mouse IFN- β gene (T.Kuga and T.Taniguchi, unpublished result). The horizontal lines above the map indicate the probes used in this study.

1.1kb, 0.73kb and 0.62kb were observed in the induced cell DNA preparation (Fig 4-b), whereas the only one subfragment, which was rather faint but seemed corresponding to the two closely associated 0.73kb and 0.62kb subfragments in the induced cell DNA sample, was detected at higher concentration of DNaseI in the uninduced cell DNA sample (Fig 4-a). These results clearly show the appearance of DNaseI-hypersensitive sites upon induction within the 3kb region including the IFN- β gene. One of them, which could be seen specifically in the induced state as the 1.1kb subfragment, was mapped at approximately 100bp upstream from the transcription initiation site. Two others, whose levels of DNaseI-sensitivity seemed to be enhanced upon induction, were mapped at 580bp and 430bp upstream, respectively (Fig 4-b).

Next we determined the DNaseI-hypersensitive sites in the 15kb-BglII fragment encompassing the upstream flanking sequence of the mouse IFN- β gene. BglII digestion produced two fragments (15kb and 1.3kb) which were hybridized with the 3kb-EcoRI fragment probe (Fig 5). An additional degradation

Figure 4. Mapping of the DNaseI-hypersensitive sites in the 3kb-EcoRI fragment. The same DNA preparations as in Fig 2 were digested with EcoRI, electrophoresed in 0.9% agarose gel, blotted and hybridized with the 32 P-labelled EcoRI-PvuII genomic DNA fragment. a, Mock-induced cell DNA sample. b, NDV-induced cell DNA sample. The 1.1kb subfragment is indicated by closed arrow. The 0.73kb and 0.62kb subfragments are indicated by hatched arrows. On the right side, approximate map positions of the DNaseI-hypersensitive sites and the hybridization probe are shown.

subfragment of approximately 8.3kb length was clearly identified, suggesting that the DNaseI-hypersensitive site was located at about 7.5kb upstream from the mouse IFN- β gene (Fig 5). In contrast to the sites found in the 3kb-EcoRI fragment (

Figure 5. Mapping of the DNaseI-hypersensitive sites in the 15kb-BglIII fragment. The same DNA preparations as in Fig 2 were digested with BglII, electrophoresed in 0.7% agarose gel, blotted and hybridized with the ^{32}P -labelled mouse IFN- β genomic 3kb-EcoRI fragment. a, Mock-induced cell DNA sample. b, NDV-induced cell DNA sample. The rightmost lanes show BglII-digested L cell DNA. The 8.3kb subfragment is indicated by open arrow. On the right side, approximate map positions of the DNaseI-hypersensitive sites and the hybridization probe are shown.

Figure 6. Mapping of the DNaseI-hypersensitive sites in the 10kb-BamHI fragment. The same DNA preparations as in Fig 2 were digested with BamHI, electrophoresed in 0.7% agarose gel, blotted and hybridized with the ^{32}P -labelled KpnI-EcoRI genomic DNA fragment. a, Mock-induced cell DNA sample. b, NDV-induced cell DNA sample. The rightmost lanes show BamHI-digested L cell DNA. The 7.5kb subfragment is indicated by open arrow. The 2.1kb and 1.8kb subfragments are indicated by hatched arrows. On the right side, approximate map positions of the DNaseI-hypersensitive sites and the hybridization probe are shown.

Fig 4), this DNaseI-hypersensitive site seemed to be more susceptible to DNaseI-digestion, not only because it was discernible even at the lower DNaseI concentration, but also because all the 15kb fragments were degraded into the 8.3kb subfragment at the higher DNaseI concentration. Since the 8.3kb subfragment was clearly seen in both the induced and the uninduced state, this site seemed independent of induction. However, the induced state made this hypersensitive site more susceptible to DNaseI than in the uninduced state : at lower concentration of DNaseI, the 8.3kb subfragment was detected more distinctly in the induced state than in the uninduced state.

Finally, we mapped the DNaseI-hypersensitive sites in the 10kb-BamHI fragment spanning the downstream flanking sequence, using BamHI for the restriction digestion and the KpnI-EcoRI 600bp genomic fragment for the hybridization probe (probe 3, Fig 3). In this region, three DNaseI-hypersensitive sites were observed in the induced state (Fig 6-b). One of them was detected as the major degradation subfragment with the size of approximately 7.5kb just below the 10kb-BamHI fragment (indicated by the open arrow; overexposure made the band appear broad, overlapping partially with the 10kb-BamHI fragment). The 7.5kb subfragment was seen in both the induced and the uninduced state (Fig 6-a,b). Two further bands were relatively weak, but distinctly identified as 1.8kb and 2.1kb subfragments. The subfragment detected faintly at approximately 1.9kb length in the uninduced cell DNA sample seemed to correspond to the 1.8kb and 2.1kb subfragments in the induced cell DNA sample (Fig 6-a,b, indicated by hatched arrows). These two sites were mapped at 1.9kb and 2.2kb downstream, respectively. They showed very weak sensitivities in the uninduced state, but the sensitivities were increased in the induced state.

DISCUSSION

The increase of DNaseI-sensitivity of the mouse IFN- β gene upon induction indicates a specific change of chromatin conformation surrounding the mouse IFN- β gene to a relaxed, or

Figure 7. DNaseI-hypersensitive sites around mouse IFN- β gene. The central box including the hatched region indicates the mouse IFN- β transcription unit. The hatched portion is the coding region. The small vertical line indicates the TATA box. The filled box corresponds to the conserved region. See details in DISCUSSION

open structure which is more susceptible to DNaseI. The increased DNaseI-sensitivity was clearly detected at 6.5 hr post induction, but not at 1.5 hr post induction when no mRNA for mouse IFN- β was observed by Northern-blot analysis (data not shown). This suggests that the formation of "active chromatin" is closely coincident with active transcription.

Recent mapping studies of DNaseI-hypersensitive sites in several different genes shows that they are frequently, but not always located at the 5'-end of active genes (6). As for mouse IFN- β gene, seven DNaseI-hypersensitive sites were mapped in the flanking sequences, approximately from 8kb of upstream to 8kb downstream (Fig 7). These hypersensitive sites were classified into three groups according to the degree of their dependency on induction.

The first group of hypersensitive site (Fig 7, site 1) was located at approximately 100bp upstream from the transcription initiation site of mouse IFN- β gene . While this site had no sensitivity in uninduced state, it showed the marked sensitivity to DNaseI upon induction (Fig 4). Interestingly, this site corresponds to the region that is well conserved among several types of IFN genes and considered to be a regulatory element for IFN induction (14,15,25). Similar results have

been reported in the heat shock gene system : The 5'-flanking region containing the sequences conserved in the heat shock gene family became hypersensitive to DNaseI upon heat shock induction (2). Moreover, the presence of an induction-specific factor which binds to this site has been reported (26,27). Taking account of these data, the hypersensitivity at 100bp upstream may reflect some functional role of this region in the induction of the mouse IFN- β gene.

The second group including site 2, 3, 5 and 6 (Fig 7, indicated by hatched arrows), which had very low levels of DNaseI-sensitivity in the uninduced state, became significantly sensitive upon induction. Because the enhancement of the DNaseI-sensitivity of this group depends on induction and these sites locate relatively near the IFN- β gene, they could be potential sites for regulation of IFN induction. Recently, enhancer elements have been identified in some viral and cellular DNA, such as SV40 and immunoglobulin genes. The DNaseI-hypersensitive sites which have been mapped to the enhancer regions (28,29) are closely associated with the active transcription (30). Although the regulatory mechanism of enhancer elements is so far poorly understood, it would be interesting to know whether the DNaseI-hypersensitive sites observed here have enhancer-like sequences or not.

The last group consisting of site 4 and site 7 (Fig 7, indicated by open arrow) had significant DNaseI-sensitivities even in the uninduced state, though the sensitivities were slightly increased upon induction. Moreover, these sites are located at relatively long distance from the mouse IFN- β gene. It seems less probable that they have something to do with induction mechanism of IFN- β gene in comparison with the other sites.. They may rather represent the structural requirements of chromatin surrounding the mouse IFN- β gene. The discrete bands observed in the Southern-blot-hybridization of total DNA which was not digested with restriction enzymes might be generated from this kind of DNaseI-hypersensitive sites (Fig 2-b). Recently, Weintraub proposed a chromosomal unit which changes its supranucleosomal structure in association with active transcription; The unit spans about 20 - 40kb around the chicken β -

globin gene (31). In the case of the mouse IFN- β gene, the two sites, site 4 and site 7, may be included in such a chromosomal unit which changes its supranucleosomal structures associated with the activation of the mouse IFN- β gene.

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