Chromatin Domain Surrounding the Human Interferon-β Gene As Defined by Scaffold-Attached Regions†

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ABSTRACT: Regions attached to the nuclear scaffold have been traced after transfecting a 36-kilobase (kb) piece of DNA, surrounding the human interferon-β gene, into mouse L-cells. An extended attached region starts 1.7 kb upstream from the gene and a moderate binding site immediately downstream. These findings could be confirmed by reconstitution experiments in vitro which predict another scaffold-attached region (SAR) starting 12 kb downstream from the gene. Since no other transcripts originate from DNA between the major SARs, these elements could be involved in interferon gene regulation.

Genes introduced into host cells by transfection are expressed at levels depending on their copy number and their physical status (episomal or integrated). If a human interferon-β (IFN-β) gene containing only the immediate regulatory elements [e.g., the 1.8-kilobase (kb) *Eco*RI fragment] is introduced into a mouse cell, the induced expression level per gene copy is typically on the order of 0.5–1% of the gene in its native environment, for instance in MG63 human osteosarcoma cells. This is always true for the gene in an episomal BPV vector (Zinn et al., 1983; Klehr & Bode, 1988) and generally so for copies integrated into the mouse chromosome, although in the latter case clones with an expression 10-fold above average are eventually observed (Wirth et al., submitted for publication).

For lines obtained by transfecting longer segments of human DNA surrounding IFN-β into mouse L-cells, the expression level is consistently higher, attaining 10% (per gene copy) for a 36-kb fragment (Hauser et al., 1982; Collins, 1984). Fragments of this size also enhance the cotransfer efficiency (i.e., the percentage of cells simultaneously expressing the gene used for selection and IFN-β) to values near 100%. These findings emphasize the role of the chromosomal context for gene expression. For several of these lines, we have made the surprising observation that adjacent to the gene all integrated copies are organized into a unique nucleosome register. Upstream, this register ends at the predominant DNaseI hypersensitive site (position −1730), ahead of an extended region which is protected from the nuclease and the chemical cleaving reagent (methidiumpropylethylenediaminetetraacetato)iron(II) [cf. Bode et al. (1986)]. These properties would be expected for sequences attached to the nuclear matrix, which in the past has been a poorly defined entity. A novel low-salt extraction procedure introduced by Mirkovitch et al. (1984) avoids the rearrangements that are typical of the more classical techniques. This method leads to a nuclear substructure, now called "scaffold", which allows the precise and reproducible determination of "scaffold-attached regions" (SARs). For several *Drosophila* genes, these SARs were found to coexist with upstream regions important in regulation [reviewed by Gasser and Laemmli (1987)].

We have initiated the present work to find out if the properties of long transfected DNA could be due to the presence of SARs. Should such structures exist, it is hoped that this information can be used to construct a DNA for transfection which in the host cell will form a self-contained regulatory domain. Such a domain would possibly make gene function independent of its site of integration into the host's genome. Recent examples supporting such a hypothesis refer to P-element-mediated transformation of *Drosophila* embryos using the fushi tarazu gene [quoted by Gasser and Laemmli (1986)] and to the expression in transgenic mice of a β-globin gene contained in an engineered domain (Grosveld et al., 1987).

Materials and Methods

Cell Culture and Isolation of Nuclei. Mouse L-cells, line 2_4, were cultured and induced for IFN-β as described (Bode et al., 1986). Two plates of 150 cm^2 (5×10^7 cells) were used for each experiment. The procedure was adopted from Mirkovitch et al. (1984) and Gasser and Laemmli (1986). Cells were washed with 2 × 50 mL of isolation buffer [3.75 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, 0.05 mM spermine, 0.125 mM spermidine, 0.5 mM ethylenediaminetetraacetate potassum salt (K-EDTA), 20 mM KCl, and 1% thiodiglycol, freshly adjusted to 0.2 mM phenylmethanesulfonyl fluoride ($PhMeSO_2F$)]. They were then scraped off in 15 mL of isolation buffer containing 0.1% digitonin. Digitonin was prepared as a 1% stock solution (dissolved in H_2O by heating a few seconds in a microwave oven) and was added immediately before use. Nuclei were set free by 15–20 strokes in a tight-fitting Dounce homogenizer. After centrifugation (900*g*, 5 min, 4 °C), they were resuspended twice in the same medium and centrifuged as before.

Nuclear Halos and Nuclear Scaffolds. Nuclei were suspended in 100 μL of freshly made nuclear buffer (5 mM Tris-HCl, pH 7.4, 0.05 mM spermine, 0.125 mM spermidine, 20 mM KCl, 1% thiodiglycol, 0.1% digitonin, 0.2 mM $PhMeSO_2F$, and 1% aprotinin) and shaken gently at 37 °C for 20 min. Several controls showed that the 37 °C step could be replaced by an analogous treatment at 4 °C without affecting the general interpretation. It was kept, however, since the precise values of scaffold-bound vs free DNA proved more reproducible.

After 20 min in nuclear buffer, 4 mL of freshly prepared lithium 3,5-diiodosalicylate (LIS) buffer [25 mM LIS, 20 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes)–NaOH, pH 7.4, 0.1 M lithium acetate, 1 mM EDTA, and 0.1% digitonin] was added, and the nuclear pellet was

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carefully homogenized by two strokes in a loosely fitting Dounce homogenizer. The suspension was kept on ice for 0.5–2 min and homogenized once more. After centrifugation (2400*g*, 5 min, 4 °C), the pellet consisted of nuclear halos which were transferred to a capped centrifugation tube containing 20 mL of sterile-filtered digestion buffer (20 mM Tris-HCl, pH 7.4, 0.05 mM spermine, 0.125 mM spermidine, 20 mM KCl, 70 mM NaCl, 10 mM $MgCl_2$). The tube was gently rocked for 30 min at room temperature and centrifuged as before; this procedure was repeated at least twice. Halos were then transferred to an Eppendorf tube, filled up to 200 μL by digestion buffer, and treated with 100 units of restriction enzyme (see below).

Determination of Endogenous SARs. The above restriction mixture was shaken in a water bath at 37 °C overnight. After centrifugation (3000 rpm, 10 min), supernates were withdrawn and kept. Pellets were homogenized in 1 mL of digestion buffer and centrifuged as above. This procedure was repeated twice. Supernates were provided with 100 μL of Tris-HCl (pH 8.5), 0.1 M EDTA, and 1% sodium dodecyl sulfate (SDS), and pellets were dissolved in 300 μL of the same buffer. To each vial was added 100 μg of proteinase K. After the vials were heated to 60 °C for 2 h, 600 μL of 0.6 M LiCl in ethanol (−20 °C) was admixed. After 1 h at −70 °C, DNA pellets could be isolated by centrifugation (8000*g*, 15 min). Pellets were washed twice with 70% ethanol and dried.

Scaffold (M) and supernate (S) DNAs were diluted to an absorbance A_{260} = 8, and 30-μL aliquots were separated by electrophoresis on 0.7–1% agarose gels. DNAs were then blotted to nylon membranes (Bio-Rad Zeta Probe or NEN GeneScreen plus) by alkali transfer (Reed & Mann, 1985) and hybridized as described before (Bode et al., 1986). Blots were exposed for different times (typically 18–48 h) on Agfa Curix MK400 film with an intensifying screen. For evaluation in a laser densitometer (LKB Model 2202 Ultroscan), exposures were chosen which yielded equal integrals for the sum of DNA fragments in traces S and M.

Determination of SARs "in Vitro". For scaffold binding experiments, restriction fragments were prepared, end-labeled by Klenow polymerase, and purified either on Sephadex G50 or on an agarose gel followed by adsorption to DEAE paper (Dretzen et al., 1981). Nuclear halos, pretreated for 3 h with the respective restriction enzyme, were supplied with 200 000 cpm of labeled DNA fragments and with 20–140 μg of sonicated *Escherichia coli* competitor DNA (addition of competitor 2 h before or after the probes did not affect the results). Samples were then incubated at 37 °C overnight. The following treatments were as for the "in vivo" assay; 4000 cpm of "S" or "M" DNA was applied to an agarose gel (0.7–2.6%). After electrophoresis, the gel was dried onto a nylon membrane in a heated gel dryer and autoradiographed, typically for 18 h.

Results

Mapping Endogenous SARs 5′ to IFN-β. In 2_4 cells, 36 kb of human DNA surrounding the IFN-β gene can be screened for the existence of scaffold-attached regions (SARs). The upstream region covers 30 kb of DNA, and 5 kb are present downstream (cf. Figure 1a). Nuclei were prepared from confluent cells without induction and after activation of the gene by a 4-h treatment with poly(rI)·poly(rC) and DEAE-dextran. Loosely bound proteins and histones were extracted by a brief incubation in a diiodosalicylate–digitonin (LIS) medium, and after removal of the detergents, the resulting nuclear halos were treated with restriction enzymes. By centrifugation and washing, the final digest was separated into a residual scaffold (M) and a supernatant (S). Equal amounts of DNA from both fractions were separated on an agarose gel, blotted, and probed with a nick-translated probe from pCosIFN-β. Some results are seen in Figure 2, and a more complete summary of the data is in Figure 1a. Ten *Eco*RI fragments (A–K) are present in the digests, 8 of which (C–K) can be evaluated in fractions S or M. Among these, fragments C, E, H, and I are almost exclusively part of the M fraction; F and K have mostly been released into the supernatants (S) whereas D and G have partitioned almost equally into both fractions. The effect of induction is weak but reproducible in that the extrema in the profile become more pronounced; i.e., the transcription unit (fragments F and K) becomes somewhat less attached while the immediate flanking regions (D and C) exhibit a stronger association with the residual scaffold.

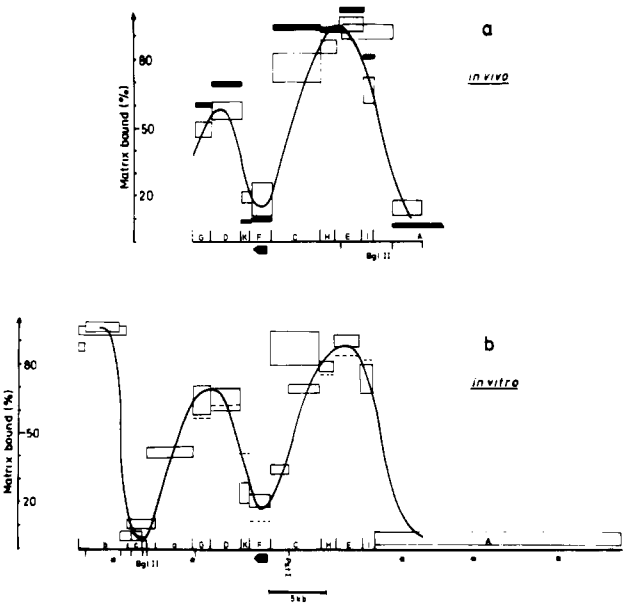

FIGURE 1: SAR potentials of DNA fragments around the human IFN-β gene (summary of data). (a) SARs for endogenous DNA ("in vivo" assays). Nuclear halos from 2_4 cells were digested extensively by *Eco*RI or *Bgl*II as indicated. The distribution of individual fragments between scaffold (M) and supernatant (S) was determined after agarose gel electrophoresis by Southern blotting and hybridization. The ratio of [M/(M + S)] × 100 has been plotted. Open boxes, data for noninduced cells from five to eight independent determinations (standard deviations); solid bars, data for induced cells from three independent determinations (average values). (b) SARs for exogenously added DNA fragments ("in vitro" assays). DNA from pCosIFN-β or C15 was digested with *Eco*RI, *Pst*I, *Bgl*II, or *Hin*dIII as indicated. Fragments were end-labeled with Klenow polymerase, and 40 ng (200 000 cpm) was reassociated with predigested nuclear halos under conditions yielding a 1:1 distribution over fractions M and S. Open boxes, data accumulated from three to five determinations using halos derived from either mouse of human cells. Data from DNA-free scaffolds (mouse) prepared according to Cockerill and Garrard (1986) have been added for comparison (dashed lines). Restrictions sites have been marked as follows: *Eco*RI, long bars above the line; *Hin*dIII, short bars above the line; *Bgl*II and *Pst*I, short bars below the line (as marked). HuIFN-β is part of fragment F; transcription is from right to left (solid arrow). Asterisks mark regions with a high content of repetitive DNA. See Figure 7 for an expanded restriction map.

The degradation of nuclear halos by *Eco*RI has been studied kinetically. During such an experiment, it was found that D, F, G, and K are released before most of the typical SAR fragments (E, H, and I). Scaffold fragment C is an exception in that it is among the first to appear in fraction M. The distribution of final fragments between S and M remained constant throughout the degradation. Therefore, it is unlikely

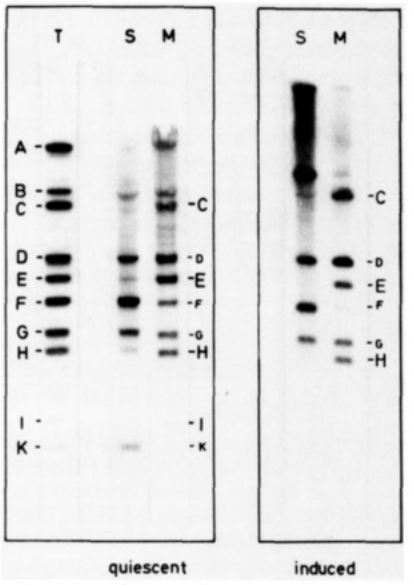

FIGURE 2: Endogenous SARs for 2_4 cells in the noninduced and induced states. Analyses are on the *Eco*RI fragments A–K as defined in Figures 1 and 7. Fragment sizes are (A) 21.2, (B) (vector) 5.6, (C) 4.2, (D) 2.7, (E) 2.25, (F) 1.9, (G) 1.45, (H) 1.25, (I) 1.0, and (K) 0.65 kb. T, control digest from pCosIFN-β; S, supernate fraction; M, scaffold-attached fraction.

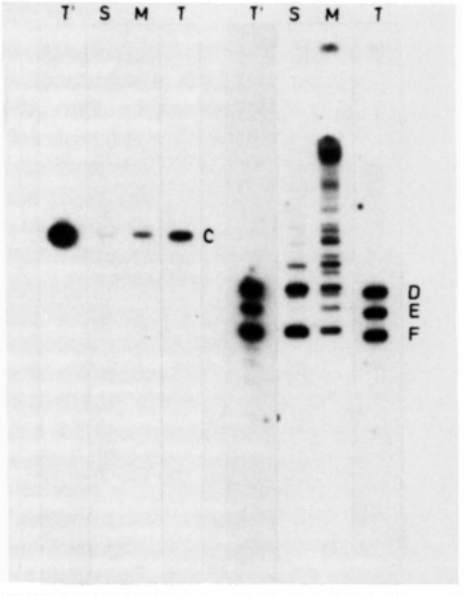

FIGURE 3: Differential degradation of fragments C–F in nuclear halos. Nuclear halos were degraded by *Eco*RI under the conditions specified under Materials and Methods but for 1 h. After electrophoresis and Southern transfer, the same blot was first probed with fragment C and (after removing the probe) for D + E + F. T', *Eco*RI digest from pCosIFN-β; S and M, supernatant and scaffold-associated fraction, respectively, after a 1-h digest of halos; T, DNA from nuclear halos that have been maximally degraded (no separation into S and M). In lanes S and M, fractions D and F have approached their final concentrations. E is only starting to appear in lane M which contains mostly partial degradation products including E.

that there is a major heterogeneity of environments for the ~100 copies of pCosIFN-β DNA in 2_4 cells, and if there were, it would not affect the interpretation of these results. Data for C and D + E + F obtained after 1 h of digestion are shown exemplarily in Figure 3.

Some efforts were undertaken in order to compare the modes of attachment for the gene transfected into a mouse host cell and the same gene in its natural environment. These studies are complicated by the fact that fragments A, G, H, I, and K (C to some extent) contain human repetitive se-

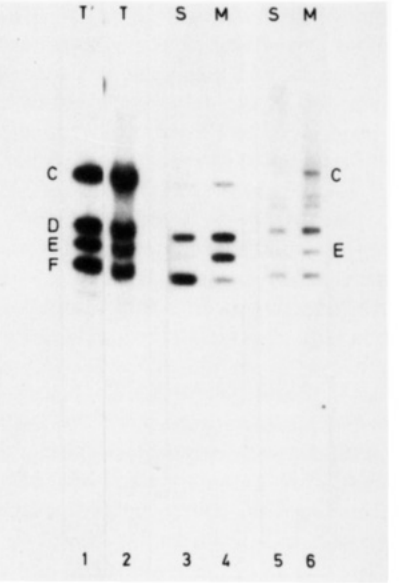

FIGURE 4: "In vivo" assays comparing the distribution of fragments C–F in transfected mouse 2_4 cells (lanes 3 and 4) and human MG63 osteosarcoma cells (lanes 5 and 6). T' *Eco*RI digest from pCosIFN-β; T, total DNA from 2_4 halos.

quences. Therefore, these fragments cannot be used to probe blots from total human DNA. In Figure 4, the composition of S and M fractions for fragments C, D, E, and F was probed for 2_4 cells (lanes 3 and 4) and MG63 human osteosarcoma cells (lanes 5 an 6). This comparison shows an analogous distribution for fragments C, D, and E. Fragment F turned out to be more variable, and in the example shown, its representation in the soluble fraction is somewhat reduced.

Mapping the 5′ SAR in Vitro. Two principal ways of performing reassociation experiments for scaffolds and radiolabeled fragments have been described. In the approach by Cockerill and Garrard (1986), pure protein scaffolds were used which were prepared by an extensive DNaseI treatment of nuclear halos followed by several washing and centrifugation steps. Gasser and Laemmli (1986), on the other hand, have observed that labeled restriction fragments will also bind specifically if added to nuclear halos during their digestion with the respective restriction enzyme. We have initially used the first procedure (Figure 5, lanes 6 and 7, and Figure 1b, dashed bars), but due to eventual problems with residual nuclease activities, we finally decided to optimize the second approach based upon the effective competition of the probes with the intrinsic SAR fragments. Figure 5 shows binding of end-labeled *Eco*RI fragments of pCosIFN-β in the absence (lanes 2 and 3) and presence (lanes 4–7) of 20 μg/200 μL sonicated *E. coli* DNA as an unspecific competitor. Only under the latter conditions is selective binding observed, which (within the investigated limits; 20–140 μg of competitor per 200 μL) is reproducible if allowed to proceed to completion (see below). If 40 ng of pCosIFN-β fragments was applied for 3×10^6 halos, equal amounts of radiolabel partitioned into the M and S fractions, allowing the evaluation both of the enrichment of SAR DNA in fraction M and of its depletion in S.

In Figure 6, the scaffold binding capacity of the 5′ SAR fragment C was investigated more closely by using an internal *Pst*I site by which subfragments of 1.2 kb (proximal to the IFN gene) and 3.0 kb (distal) are generated (Figure 6b). During the in vitro assay, the high binding potential of intact C is attained by neither subfragment, suggesting some kind of cooperativity between remote segments which is lost during

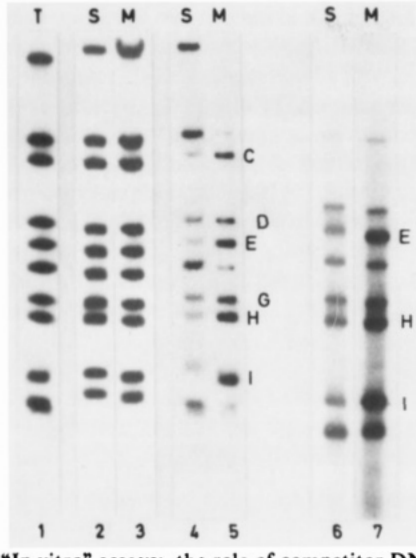

FIGURE 5: "In vitro" assays: the role of competitor DNA. Lane 1, end-labeled mixture of pCosIFN-β fragments used for the reassociation experiment; lanes 2 and 3, distribution of 40 ng of fragments over 3×10^6 nuclear halos (predigested with *Eco*RI); lanes 4 and 5, same as lanes 2 and 3 but in the presence of 20 μg of *Escherichia coli* competitor DNA; lanes 6 and 7, same as lanes 2 and 3 but using DNA-free scaffolds prepared according to Cockerill and Garrard, also in the presence of 20 μg of *E. coli* competitor DNA. Lanes 2–5 are from an experiment with scaffolds from human MG63 cells.

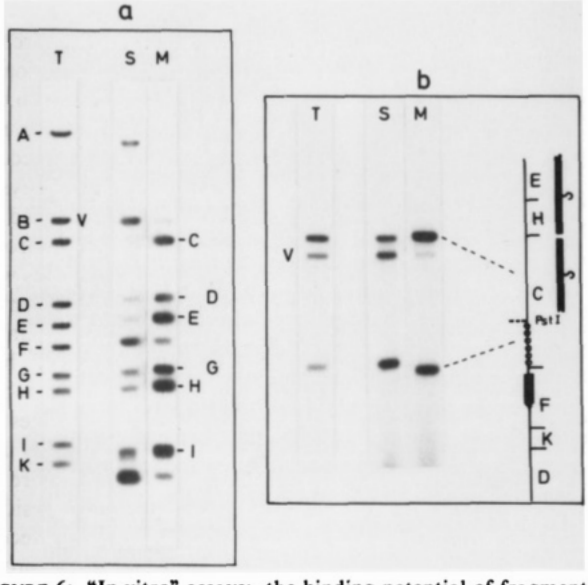

FIGURE 6: "In vitro" assays: the binding potential of fragment C. (a) Reassociation experiments as in Figure 5 (lanes 1, 4, and 5) but using halos from 2_4 cells. V, vector (pJB8) DNA. (b) Binding potential for the *Pst*I subfragments of C. C has been subcloned into the *Eco*RI site of a pTZ vector (V). For use in this experiment, the construct has been digested by *Eco*RI plus *Pst*I.

their physical separation. However, the major share clearly comes from the distal part which in vivo comprises an extended nucleosome-free region adjacent to the *Pst*I site (Bode et al., 1986).

Mapping the 3′ SAR in Vitro. At the 3′ end, the IFN-β gene is bordered by a region with 60–70% of scaffold binding potential in vitro (fragments D and G). A λ clone, C15 (Mori et al., 1981), kindly donated by M. Revel, contains a segment of human DNA extending 17 kb downstream from *Eco*RI fragment F. This opened the possibility of probing another 12.6 kb of DNA downstream from fragment D contained in a single *Eco*RI fragment. This fragment was subcloned in pBR322 and subjected to restriction analyses with *Hin*dIII and

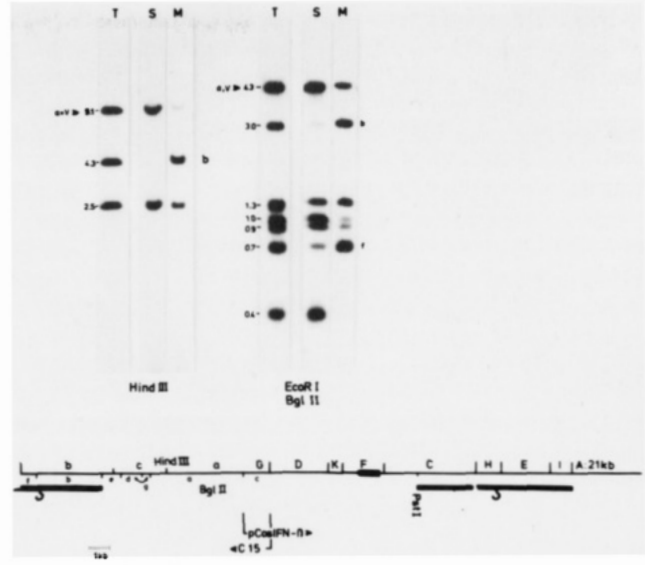

FIGURE 7: Mapping the 3′ SAR. The 11.6-kb *Eco*RI fragment of C15 starts at the D–G border of pCosIFN-β. After being subcloned into pBR322, *Hin*dIII (left part) and *Eco*RI plus *Bgl*II digests were prepared and used after end-labeling. V marks pBR322 DNA. In the *Hin*d digest, V is part of the largest fragment. In the *Eco*RI + *Bgl*II digest, it forms one of two 4.3-kb fragments which have been tested in separate control experiments, showing that V does not appear in trace M. Hence, the share of fragment a in trace M is 40% (see Figure 1).

*Bgl*II. Hereby, the approximate position of two internal *Hin*dIII sites could be confirmed [cf. Collins (1984)], and six *Bgl*II sites could be mapped in addition. Figure 7 (left part) shows matrix association of the three fragments obtained by cleaving the pBR322 derivative with *Hin*dIII. Among these, (b) contains 29 base pairs (bp) of the vector and (a) the remainder of vector DNA. A definite binding potential is only located in (b). A more precise localization became possible with a labeled *Eco*RI/*Bgl*II double digest which demonstrates that the *Hin*dIII fragment (b) binds over almost its entire length whereas a trough is located in the *Hin*dIII fragment (c). A summary of the quantitative evaluations is given in Figure 1b.

Comparative Binding of SAR and Non-SAR Fragments. One of the major SAR fragments, E, and fragment F, carrying the IFN-β structural gene including the immediate control sequences, were used in a comparative binding study. To make the comparison more rigorous, fragment E was trimmed by *Hpa*I to the precise length of F (1.9 kb).

Due to the necessary centrifugation and washing steps, individual samples could not be used repeatedly. Therefore, two series of six samples were prepared each containing 3×10^6 *Eco*RI-digested nuclear halos and 140 μg of *E. coli* competitor DNA in 200 μL. All samples were treated synchronously; i.e., their DNA concentration was raised at 2 h intervals. The scaffold-associated (M) and free (S) radioactivity was determined for one sample of the series at a time. Each sample served to construct one bar in Figure 8; the lower limit of each bar characterizes the scaffold-associated radioactivity 1 h after the last addition and the upper limit the respective value 2 h after the last addition. The kinetic information in Figure 8 suggests that under these conditions (i.e., a 9000-fold excess of *E. coli* competitor DNA on the basis of nucleotides) the association is an extremely slow process. If the incubation of all samples is extended to 18 h, the apparent sigmoidal behavior changes to linear, typical of quantitative binding (not shown). Strong and quantitative binding ends at 16 ng of fragment E, i.e., at 2500 molecules of E per individual halo.

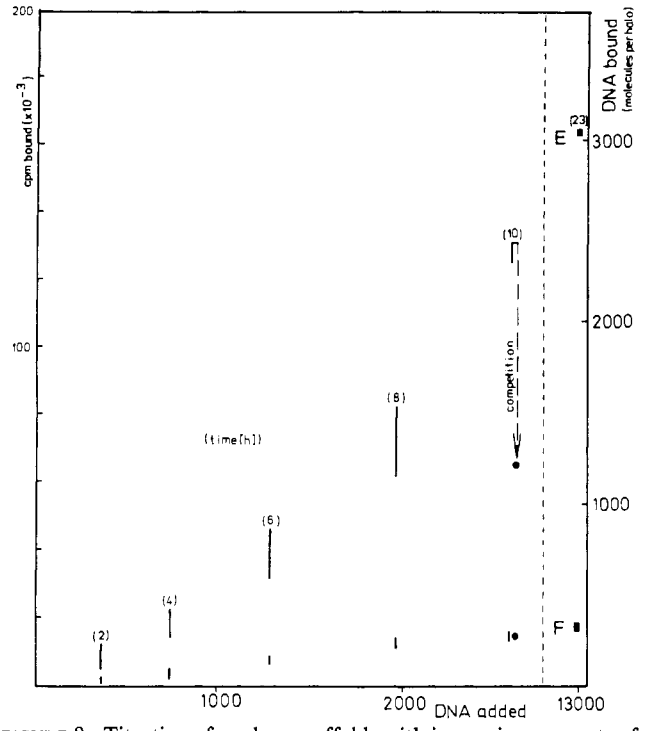

FIGURE 8: Titration of nuclear scaffolds with increasing amounts of a 1.9-kb *Hpa*I–*Eco*RI subfragment from E and of the 1.9-kb F fragment comprising IFN-β. Each bar belongs to one member of a series of parallel samples which has been measured twice at 1-h intervals. The bold bars to the right refer to samples which have been exposed to saturating amounts of E and measured after 18 and 23 h, respectively.

Addition of an equivalent amount of unlabeled fragment E to this sample displaces half of the labeled counterpart, as expected for distinct binding sites. Minor amounts of bound F were not competed off by E.

Limiting values for the binding of E and F, obtained from a sample that was treated with a 5-fold excess (83 ng) of the respective DNA, were measured after 18 and 23 h (sixth bar in each series); they are only moderately higher than the figures for stoichiometric binding.

Discussion

During the present study, we have used transfected mouse L-cells to map a 36-kb piece of human DNA including the functional IFN-β gene for the presence of endogenous SARs. A larger range of the human genome (46 kb) could be screened in an in vitro assay with cloned DNA.

Experiments in which individual segments of human DNA were transfected into mouse cells led to the conclusion that IFN-β mRNA is the only transcript from the DNA flanked by the two principal SARs, i.e., between −4.7 and +8.2 kb with respect to the IFN-β gene (H. Hauser, unpublished experiments).

We had mapped previously the chromatin structure around IFN-β using the same cell line, 2_4 (Bode et al., 1986). These studies revealed six strictly phased nucleosomes upstream from the gene, ending at the predominant DNaseI hypersensitive site (−1.7 kb) ahead of an extended protected area. This entire region is part of fragment C (−0.3 to −4.5 kb) which, as a whole, exhibits a high scaffold binding potential. After cutting with *Pst*I close to the site of hypersensitivity, a distinct (70%) SAR is left in the distal subfragment which in vivo discourages nucleosome formation. To our knowledge, this is the third example in which an SAR borders on a region of static nucleosome organization [cf. review by Gasser and Laemmli (1987)]. Moreover, coexistence of scaffold attachment sites and DNase I hypersensitivity may be a more general motiv [cf. Grosveld et al. (1987) and references cited therein].

In vitro binding studies were mostly based on scaffold preparations from mouse cells. However, several controls have shown that data obtained with scaffolds from human MG63 cells are fully accommodated by the open boxes in Figure 1b (compare also Figure 5, lanes 4 and 5, and Figure 6a, which show a comparable distribution of fragments using 2_4 and MG63 halos, respectively). For these cells, the mode of association in vivo is difficult to assess as most fragments escape detection due to their large content of repetitive human DNA. Association of the remaining fragments (with the possible exception of F) is in accord with the data from mouse cells (Figure 4). These observations demonstrate the use of transfected cell lines for a complete description of endogenous SARs surrounding a particular gene.

At first sight, there is a general agreement for the SAR profiles obtained for endogenous and exogenously added DNA fragments (panels a and b, respectively, of Figure 1). Both approaches reveal a strong extended SAR 5′ to the gene and some intermediate (50–70%) binding potential 3′ to the gene. So far, these findings agree with the notion that SARs represent constitutive points of attachment which provide genes with the potential of being transcribed but are rather unaffected by gene activity. Upon closer inspection, it appears, however, that the in vitro binding potential is not always fully utilized in vivo; see, for instance, the distribution of bands D and G in Figure 2 and 6a. During the induction process, the extrema of the in vivo profile become more pronounced, approaching the values obtained in vitro (Figure 1). This may reflect the relaxation of constraints which are typical of the inactive state [see Bode et al. (1986)].

The environment of the human IFN-β gene is peculiar in that there are two downstream areas with a scaffold binding potential which could, in principle, be utilized differentially depending on gene activity. Loop sizes of 14.5 kb (typical of moderately active genes) and 4 kb (as in highly transcribed regions) appear feasible and could alternate during induction. It is realized, however, that a different approach is needed to test these properties. The potential of chemical mapping techniques for probing intact chromatin structures has been demonstrated (Bode et al., 1986) and is presently being applied to trace induction-dependent changes for the region in question. Alternative scaffold attachment sites have been made likely for the alcohol dehydrogenase gene of *Drosophila* where choice of promoters (two) is dependent on the stage of development (Gasser & Laemmli, 1986).

The low-salt technique introduced by Mirkovitch et al. (1984) has so far been used to locate 18 SARs in nontranscribed regions near a variety of *Drosophila* genes. In these examples, the 5′ SARs cover about 0.6–1 kb which in several cases coincide with enhancer-like regulatory regions. A greater heterogeneity of length (1–5 kb) was observed for the 3′ SARs [cf. Gasser and Laemmli (1986, 1987)]. As to IFN-β, extended SAR regions flank 3.75 kb of unattached DNA including and surrounding the gene. The 5′ SAR covers about 7 kb ahead of a stretch of at least 21 kb (*Eco*RI fragment A) which proves to be totally free, even if it is subdivided into its *Bgl*II or *Pst*I fragments. In contrast, no stretches of nonbinding DNA could be demonstrated within the 5′ SAR, and this is particularly true for its most prominent component (*Eco*RI fragment E) which has recently been sequenced in our laboratory (A. Pucher, unpublished experiments). In this fragment, 31 repeats related to the topo II box [GTN(A/T)A(T/C)ATTNATNN(G/A)] (Gasser & Laemmli, 1987)

and 13 ATATTT boxes (Cockerill & Garrard, 1986) are distributed over about 2 kb of DNA; at 5 positions, these motivs overlap. None of these sequences occurs in the coding region in huIFN-β. Together with the observation that all subfragments created from E (i.e., the 1.9- and 0.35-kb *Hpa*I fragments, the 1.7- and 0.55-kb *Sph*I fragments, and the 1.1-, 0.65-, and 0.55-kb *Sau*IIIA1 fragments) bind more than 90% to the scaffold, this region appears to be part of the longest SAR that has yet been described. Brief interruptions of the binding potential may occur as between *Eco*RI fragments C and H (Figure 3) and in several short stretches within E which proved to be accessible to MPE in preliminary chemical mapping analyses (data not shown).

The most prominent upstream element, E, has also been subjected to a comparative binding study (Figure 8). The titration indicates 2000–3000, possibly composite, high-affinity binding sites per individual scaffold. Other available data have indicated about 10000 binding sites, determined by saturation binding in the absence of competitor DNA (Cockerill & Garrard, 1986). It is felt that both numbers are too small to account for organizing the entire human genome into average domains of 70 kb (which would require about 80000 sites) although they would approximate the number of active genes in a given cell. Further studies will show whether low-affinity sites contribute to SAR formation or if there is another class of high-affinity sites which is either inaccessible or lost in the preparations used here.

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