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Regulation of Interferon Gene Expression: Mechanism of Action of the *If-1* Locus

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We have examined the mechanism of action of the *If-1* interferon (IFN) regulatory locus. This locus controls the level of circulating IFN produced in inbred mice in response to intravenous injection of Newcastle disease virus. Mice carrying the *If-1*^{h} (high) allele show circulating IFN levels 10- to 15-fold higher than those carrying the *If-1*^{l} (low) allele. In this report we show that induced splenocytes from *If-1*^{h} and *If-1*^{l} mice produce IFN at levels which are in the same proportions as those found in the circulation. Higher levels of IFN-specific mRNA were observed in splenocyte populations from *If-1*^{h} animals. This was due to increased transcription of IFN genes. At the same time, the high- and low-producing populations showed no significant difference in the number of IFN mRNA-containing cells. We conclude that the effect of *If-1* in the spleen is to control the levels of transcription of the IFN genes in individual induced splenocytes.

The interferons (IFNs) are the products of a family of inducible genes which are ubiquitous to all vertebrates and which possess varied and potent biological effects on cultured cells or animals (40). In general, the IFNs are subdivided into the type I or viral IFNs and the type II, immune (or γ) IFNs, according to the nature of induction. The type I IFNs are further subdivided into α and β. In humans this subdivision into antigenically distinct subtypes is reinforced by the fact that the production of IFN-α and IFN-β is segregated, with different cell types producing predominantly one or the other (40). In contrast, cultures of murine cells as a rule produce both IFN-α and IFN-β (9, 15). The use of recombinant DNA technology has shown that the IFN-α genes constitute a family made up of a large number of highly homologous genes both in humans (3) and in mice (25, 28, 38). One human IFN-β gene has been well defined (17, 41), but evidence for other human IFN-β genes exists (26, 36, 37, 42). In the mouse, cDNAs corresponding to the IFN-β_1 have also been cloned and have been shown to represent a single copy gene (24).

Generally speaking, uninduced cells or organisms do not secrete detectable levels of IFN (40). After exposure to an inducer, typically a virus or a double-stranded RNA, IFN production can occur both in tissue culture and in the animal (40). The onset of IFN production is due to transcriptional activation of the IFN genes (34). Several groups have characterized induction-regulatory elements in the 5′ flanking regions of the human IFN genes (18–20, 33, 43, 44). Both indirect, and recently direct, evidence for a role for *trans*-acting transcription factors in the induction mechanism has been obtained (19, 44).

Regulation of IFN production occurs at another level besides the on-off regulation of induction/repression. Indeed, the levels of production of IFN in the mouse are regulated by a series of factors. These include age (7), hormones (30, 35), and the genotype (7–14). This last is of particular interest. It has been found that different inbred mouse strains produce high or low levels of circulating IFN in response to various inducers (7–14). The loci involved in regulating this response have been called the *If* loci and have been studied extensively from the viewpoint of classical genetics (7–14). The response to different inducers is regulated by independently segregating loci (12). The level of IFN in the circulation of mice in response to Newcastle disease virus (NDV) is regulated by the locus *If-1* (7–14). Two alleles have been described: *If-1*^{h} and *If-1*^{l}, corresponding to high and low levels of circulating interferon, respectively. In mice carrying the *If-1*^{h} allele (e.g., the C57BL/6 strain), circulating IFN levels after intravenous injection of NDV are 10- to 15-fold higher than in strains which carry the *If-1*^{l} allele (e.g., the BALB/c strain) (7–14). Recombinant inbred and congenic strains for *If-1* have been obtained (11), and these mice have been used to assign *If-1* to murine chromosome 3 (31).

As is often the case for regulatory loci, the lack of information on the site of action of *If-1* has hampered the development of further studies. The cells of the lymphatic system are largely responsible for IFN production after intravenous injection of viruses (13) and can be carriers of the *If-1* phenotype (13). For this reason we decided to examine the action of *If-1* in the spleen.

The mechanism of action of a regulatory locus such as *If-1* could of course be due to any of many different possibilities. Higher IFN levels in *If-1*^{h} mice could be due to greater specific activity of IFNs translated from equivalent amounts of IFN-specific mRNA. On the other hand, higher levels of such mRNA could be present in the high-producing animals. These levels could in turn be controlled transcriptionally or posttranscriptionally. Finally, the effect could be due to differences in the number of IFN-producing cells (which we call a cellular mechanism) or to higher levels of production per cell (which we call a molecular mechanism).

In the present report we have addressed the problem of the mechanism of *If-1* action in one target organ, the spleen. We conclude that splenocytes from *If-1*^{h} mice show higher levels of transcription of IFN mRNA and that the numbers of IFN-producing cells in *If-1*^{h} and *If-1*^{l} spleens are equivalent.

MATERIALS AND METHODS

Animals, cell lines, and virus stocks. Male mice of the C57BL/6, BALB/c, and HW81 (B6.C *H-28*^{c}; *If-1*^{l}) (7, 11)

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lines, aged from 5 to 6 weeks, were obtained from the Jackson Laboratory, Bar Harbor, Maine.

L cells were grown in Eagle minimal essential medium supplemented with 10% fetal bovine serum. Vesicular stomatitis virus, Indiana strain, was propagated on L cells, and titers were determined by plaque assay on the same cells. The Kumarov strain of NDV was propagated on primary or secondary cultures of chicken embryo fibroblasts after the virus was seeded at approximately 1 PFU/cell. Titers of NDV were determined by plaque assay on secondary cultures of chicken embryo fibroblasts.

IFN inductions and titrations. For in vivo inductions, 200-μl volumes of Eagle minimal essential medium, containing approximately 10^8 PFU of NDV, were injected into the extraorbital sinus of mice. Sera and spleens were taken 6 h after infection.

For the preparation of splenocyte suspensions, spleens were removed and dissected onto a Millipore filter support (AP 32; 75-mm diameter). The filter support was discarded, and the suspension was centrifuged and washed twice in Eagle minimal essential medium without fetal bovine serum.

Isolated single-cell suspensions of splenocytes were infected at a multiplicity of infection of 100 PFU/cell. Virus was left in contact with the cells for 2 h, removed, and replaced with Eagle minimal essential medium.

IFN titrations were carried out by the method of cytopathic effect reduction in L-cells infected with vesicular stomatitis virus as previously described (4).

Isolation and analysis of RNA. RNA from splenocytes was isolated either by the guanidine hydrochloride method described previously (39) or by the method of Murphy et al. (32). For the preparation of polyadenylated RNA, total RNA preparations were passaged twice through oligo(dT)-cellulose (type 7, Pharmacia).

For Northern (RNA) blot analysis, RNA samples were fractionated on 1.5% agarose gels containing 4 mM methylmercury hydroxide (1). RNA was transferred by capillarity to Pall Biodyne membranes. Hybridizations and washes were done by the methods of Coulombe and Skup (4).

Probes used were $pM\beta_3$, which contains a cDNA for the mouse IFN-β_1 (MuIFN-β_1) gene (24), kindly provided by Y. Higashi and Y. Kawade, and pMIF1204, which contains a cDNA of the MuIFN-α_2 gene (25). These plasmids were labeled with [^{32}P]dCTP by nick translation (4).

RNase protection mapping of mRNA was essentially as described by Zinn et al. (43). The probe was a ^{32}P-labeled cRNA complementary to the MuIFN-β mRNA. This probe was synthesized using the MuIFN-β_1 (24) cDNA recloned into plasmid pGEM-1 (Promega Biotec) linearized with *Hin*dIII and transcribed with the T7 RNA polymerase (27, 29). Hybridizations (to 2 μg of polyadenylated RNA) were carried out at 45°C for 17 h, and RNase digestion was for 1 h at 30°C.

Transcription in isolated nuclei. Isolated single-cell suspensions of splenocytes were induced for 2 and 4 h, nuclei were isolated, and nascent transcripts were elongated essentially as described by Greenberg and Ziff (21), except that 0.5 mM $MnCl_2$, 2.5 mM dithiothreitol, and 2 U of RNasin per ml were present in the reactions. The labeled nascent transcripts were isolated by the method of Groudine et al. (22) except that the unincorporated nucleotides were removed by centrifugation through a Sephadex G-50 column.

Labeled transcripts were suspended in the hybridization buffer at 42°C and hybridized to Pall Biodyne filters carrying 5 μg of plasmids for 72 h at 42°C. Hybridization and washes were done by the method of Hannigan and Williams (23). Filters were exposed to Kodak XAR-5 film with Cronex Lightning-Plus intensifying screens (Du Pont) for autoradiography. Multiple exposures of the filters were taken and were quantified by densitometry (GS 300 densitometer, Hoefer Scientific Instruments). All signals observed corresponded to transcription which was largely sensitive to α-amanitin.

In situ hybridization. Probes for in situ hybridizations were ^{3}H-labeled cRNAs synthesized using the MuIFN-β_1 (24) and MuIFN-α_2 (25) cDNAs recloned into plasmid pGEM-1 (Promega Biotec), linearized with *Bgl*II, and transcribed with the RNA polymerases of bacteriophages SP6 or T7 (27, 29).

Samples of 50 μl of suspension at a concentration of 10^8 cells per ml were put onto slides, rapidly air dried, fixed with Carnoy fixative (methanol-acetic acid, 3:1), and air dried for at least a further 2 h after dehydration.

Hybridizations were by the method of Cox et al. (5). Hybridization buffer was 50% formamide–0.3 M NaCl–10 mM Tris hydrochloride (pH 8)–1 mM EDTA–1× Denhardt solution (16)–500 μg of yeast tRNA per ml–10% dextran sulfate. Probe concentration was 0.2 to 0.3 μg/ml per kilobase of probe complexity.

Hybridizations were carried out in moist chambers for 36 to 48 h at 42°C.

Slides were washed twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature (30 min per wash), four times with 2× SSC at 65°C (15 min per wash), and twice with 0.1× SSC at 65°C (15 min per wash).

After washes, slides were dehydrated with two treatments of 10 min each with 70% ethanol and two treatments with 95% ethanol, all at room temperature. Dehydrated slides were dipped in Kodak Nuclear Track NTB emulsion type 2 and stored at 4°C in the dark.

After development, slides were stained with Giemsa and mounted.

RESULTS

***If-1* action in splenocytes.** The first question that we addressed was whether or not IFN production in the spleens of inbred mice was regulated by the *If-1* locus. For this purpose, mice of the C57BL/6, BALB/c, and HW81 lines (the latter a congenic line which carries the *If-1*^{l} allele of BALB/c mice on a C57BL/6 genomic background; 11) were injected intravenously with NDV. IFN levels were determined in the sera and in the culture fluids of isolated splenocytes from the infected mice. The expected difference in IFN levels between the *If-1*^{h} and the *If-1*^{l} mice was obtained (Table 1). Splenocytes removed from the infected mice continued to produce IFN for some time, and those of high-producer mice made more, in roughly the same proportions as found for circulating IFN. The HW81 congenic mice behaved in exactly the same way as their BALB/c counterparts, indicating that the difference in levels of IFN produced by spleens is indeed due to *If-1*.

Similar results were obtained when the splenocytes of uninfected mice were induced in vitro with NDV (Table 1). Although higher titers were obtained for both strains, the cells from C57BL/6 mice produced roughly 10-fold more IFN than did those from BALB/c mice.

The differences in the levels of IFN produced by the animals which carry the different *If-1* alleles are not due to a shift in the kinetics of the induction. IFN accumulated in the sera of the high- and low-producing animals with an identical time course (Fig. 1A). This corresponds to the results of the group of De Maeyer (10). A time course of IFN production

TABLE 1. IFN titers in sera and in culture media of spleen cell suspensions from mice inoculated intravenously with NDV (in vivo) or from spleen cell suspensions induced with NDV (in vitro)

Source of IFN	Titers[a]
BALB/c serum	240, 768, 480
C57BL/6 serum	4,800, 7,680, 9,600
HW81 serum	240, 720, 720
BALB/c spleen (in vivo)	24, 36, 18[b]
C57BL/6 spleen (in vivo)	192, 384, 576[b]
HW81 spleen (in vivo)	12, 18, 24[b]
BALB/c spleen (in vitro)	4,800, 9,800, 2,400[c]
C57BL/6 spleen (in vitro)	48,000 36,000, 24,000[c]

[a] All titers are in laboratory units. In this system 1 U corresponds to 1 IU as measured by reference MuIFN preparation no. G-002-904-511. Each figure is the average from three mice of each strain.

[b] Titers were determined after 16 h in culture. In all cases, the titer at the time incubation was begun was <6.

[c] Titers were determined after 6 h of induction. NDV stock used was a new one which yields higher responses.

by splenocytes induced in vitro (Fig. 1B) also shows that the induction occurred with similar kinetics, although the C57BL/6 IFN seemed to be degraded more rapidly, leading to a less significant difference at late times.

Levels of IFN-specific mRNA in the spleen. Figure 2 shows

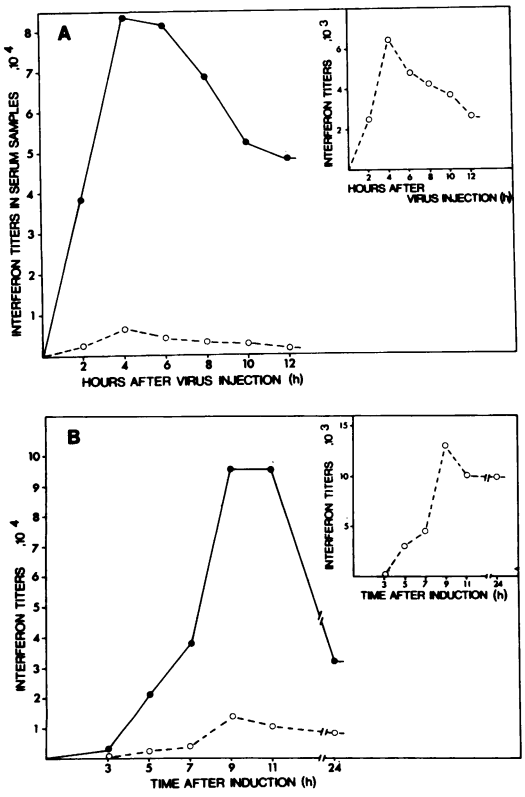

FIG. 1. Kinetics of accumulation of MuIFN. (A) Six mice were injected with 200 μl each of NDV for each point. (B) Cell suspensions were prepared from spleens of 15 mice. The suspensions were infected with NDV, and supernatants were collected at the times indicated. Symbols: ●, C57BL/6 mice; ○, BALB/c mice. Insets show the kinetics of accumulation of IFN for BALB/c mice on a different scale.

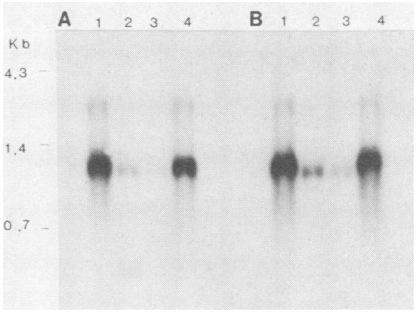

FIG. 2. Northern blot analysis of poly(A)$^+$ RNA from the spleens of NDV-induced mice. Lanes: 1, 5 μg of RNA from C57BL/6; 2, 5 μg of RNA from BALB/c; 3, 5 μg of RNA from HW 81; 4, 0.5 μg of RNA from NDV-induced c243 cells. (A) Probe was nick-translated pMβ_3; (B) probe was nick-translated pMIF1204. The markers were *Pst*I-digested pBR322 (4.3 kilobases) and *Taq*I-digested pUC8 (1.4, 0.7, and 0.5 kilobases).

Northern blots of poly(A)$^+$ RNA from spleens of the same animals used to obtain the data in the first part of Table 1. In no case was any signal obtained when RNA from the spleens of uninduced animals was used (data not shown). The signal obtained with both MuIFN-β_1 (Fig. 2A) and MuIFN-α_2 (Fig. 2B) specific probes was much stronger for the high-producing C57BL/6 mice than for either the BALB/c or the HW81 strain. This indicates that the effect of *If-1* in the spleen is to cause higher levels of IFN-specific RNA in *If-1*^{h} than in *If-1*^{l} mice. The identity of the results obtained from HW81 or BALB/c mice confirms that we are indeed dealing with *If-1* and not some other IFN regulatory locus.

In the splenocytes which were induced in vitro, the levels of IFN-specific mRNA were also higher in the cells from the high-producing *If-1*^{h} mice throughout the length of the induction (Fig. 3). Kinetics of accumulation were again similar, and the maximal accumulation occurred at 3 h after induction. With longer exposures, signals became detectable at 6 and 9 h of induction (data not shown), and the difference between the high-producing C57BL/6 mice and the low-producing BALB/c mice was maintained.

Analysis of IFN-specific gene transcription. To determine whether the difference in the accumulation of IFN-specific mRNA between C57BL/6 mice and BALB/c mice is due to a higher transcriptional level of the IFN genes in the former, we performed nuclear run-on transcription experiments with nuclei isolated from induced splenocytes.

The relative transcription rate of the MuIFN genes (Fig. 4) was three to five times higher in the nuclei of induced splenocytes of C57BL/6 mice than in those of BALB/c mice.

Determination of numbers of IFN-producing cells in populations of induced splenocytes. Higher levels of transcription of IFN genes in the splenocyte populations obtained from C57BL/6 mice could be due to a higher number of IFN-producing cells in this population or to higher levels of transcription per IFN-producing cell. We carried out in situ hybridization experiments with cRNA probes corresponding to the MuIFN-β gene to compare the number of splenocytes producing IFN mRNA. The first experiments were carried out on tissue slices. Positive cells were found only in spleens of induced animals with the appropriate probes (data not shown), but quantitation of the number of positive cells was difficult.

To get a better quantitation of the results obtained by in situ hybridization, hybridizations were carried out on splenocytes isolated as a cell suspension from spleens of normal

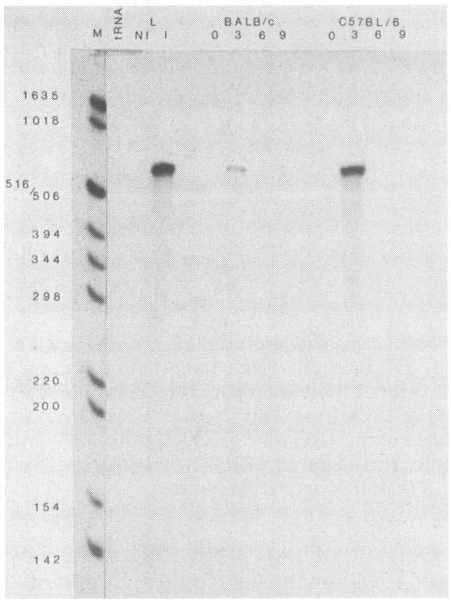

FIG. 3. Kinetics of accumulation of MuIFN-β_1 mRNA in splenocytes induced in vitro. RNase mapping with 2 μg of polyadenylated RNA from splenocytes at the times indicated postinfection was carried out as described by Zinn et al. (44). The probe was a ^{32}P-labeled cRNA complementary to MuIFN-β mRNA. Numbers at the top indicate time in hours. The signal obtained corresponds to the predicted 680-nucleotide band. The negative controls were tRNA (25 μg) and 0.5 μg of poly(A)$^+$ RNA from uninduced L cells (NI) mixed with the probe. The positive control was 0.5 μg of poly(A)$^+$ RNA from L cells induced with NDV (I) (8 h of induction). Markers are the 1-kilobase DNA ladder from Bethesda Research Laboratories.

and NDV-infected mice and on splenocytes isolated from uninfected mice and induced in vitro with NDV (Fig. 5). In no case were positive cells obtained when the splenocytes were isolated from uninduced animals. However, significant numbers of positive cells were found when splenocytes were induced, either in vivo or in vitro. Hybridization with labeled RNA of the same polarity as the mRNA or with RNA containing nonspecific (λ-phage) sequences never yielded signals (data not shown). The results of analysis of a large number of cells of both the BALB/c (*If-1^l*) and C57BL/6 (*If-1^h*) strains are shown in Fig. 6. The insets confirm that no cells that could be classified as positive were obtained in the uninduced populations. In induced populations the total number of IFN-positive cells from C57BL/6 and BALB/c mice was not significantly different, and the number of highly positive cells was obviously greater in the population from *If-1^h* mice. Comparison of Fig. 6A and 6B shows that *If-1* action was equivalent both in vivo and in vitro.

DISCUSSION

In this report, we have investigated the site and mode of action of the *If-1* IFN regulatory locus. We have identified the spleen as a site of action and shown that in this organ the effect of the *If-1* locus is to cause greater or lesser levels of IFN mRNA in splenocytes for the high- and low-producing alleles, respectively. These differences in mRNA levels are at least in part due to transcriptional regulation.

It had been shown previously (13) that transplantation of bone marrow cells from *If-1^h* or *If-1^l* mice into irradiated rats restored the ability of the rats to produce IFN. The IFN levels obtained corresponded to those expected for the genotype of the donor (13). *If-1* is also expressed in peritoneal macrophages (10). Here we have shown that IFN production in splenocytes is also controlled by this locus (Table 1) after both in vivo and in vitro induction. The differences observed are not due to shifts in kinetics (Fig. 1) in either case, and the results with the HW81 congenic line (11) confirm that the effect is indeed due to *If-1*. Thus *If-1* action in cells of the lymphatic system seems to be a general phenomenon. The group of De Maeyer has reported that mouse embryo fibroblasts from C57BL/6 and BALB/c mice produce identical amounts of IFN in response to NDV (10), and we have obtained the same result (data not shown). This indicates that the *If-1* phenotype is not expressed in all cell types.

Examination of the levels of IFN mRNA in the spleen cells of the induced animals showed that they correlated well with the levels of IFN activity (Fig. 2). The same result was found for RNA from splenocytes induced in vitro (Fig. 3). Thus the differences in levels of IFN activity are in all probability due to a real physical difference in the amount of IFN produced from an increased amount of translatable message. The difference in the levels of IFN-specific mRNA in splenocytes of induced C57BL/6 and BALB/c mice is due to increased transcription in the former, as shown by run-on transcription (Fig. 4).

The question of whether or not a larger number of IFN-producing cells in *If-1^h* mice could account for the higher levels of IFN gene transcription observed was answered by in situ hybridization on isolated splenocytes. In all cases in the induced population, no significant difference in the number of cells producing IFN-specific mRNA was found when BALB/c and C57BL/6 mice were compared. Cells obtained from C57BL/6 mice showed higher signals than did those obtained from the BALB/c mice. In these experiments, we have concentrated on obtaining, as far as possible, the maximum number of positive cells to compare their numbers. Thus exposure times were such that the overall tendency was for the C57BL/6 cells to show a distribution

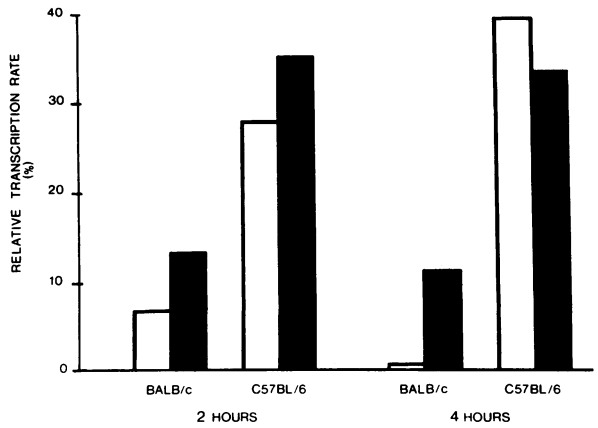

FIG. 4. Relative rate of transcription of the IFN genes in isolated nuclei from induced splenocytes. Nuclei from approximately 2×10^7 cells were prepared from isolated splenocytes from C57BL/6 and BALB/c mice at 2 and 4 h after induction with NDV. Results were quantitated by densitometry, and all results were standardized to the level of transcription of RNA corresponding to ribosomal protein L27′ (2), which can be considered as a housekeeping gene. The relative transcription rates of IFN genes in the induced nuclei were evaluated by the subtraction of the basal level observed in the uninduced nuclei (30 to 40% of the ribosomal protein L27′).

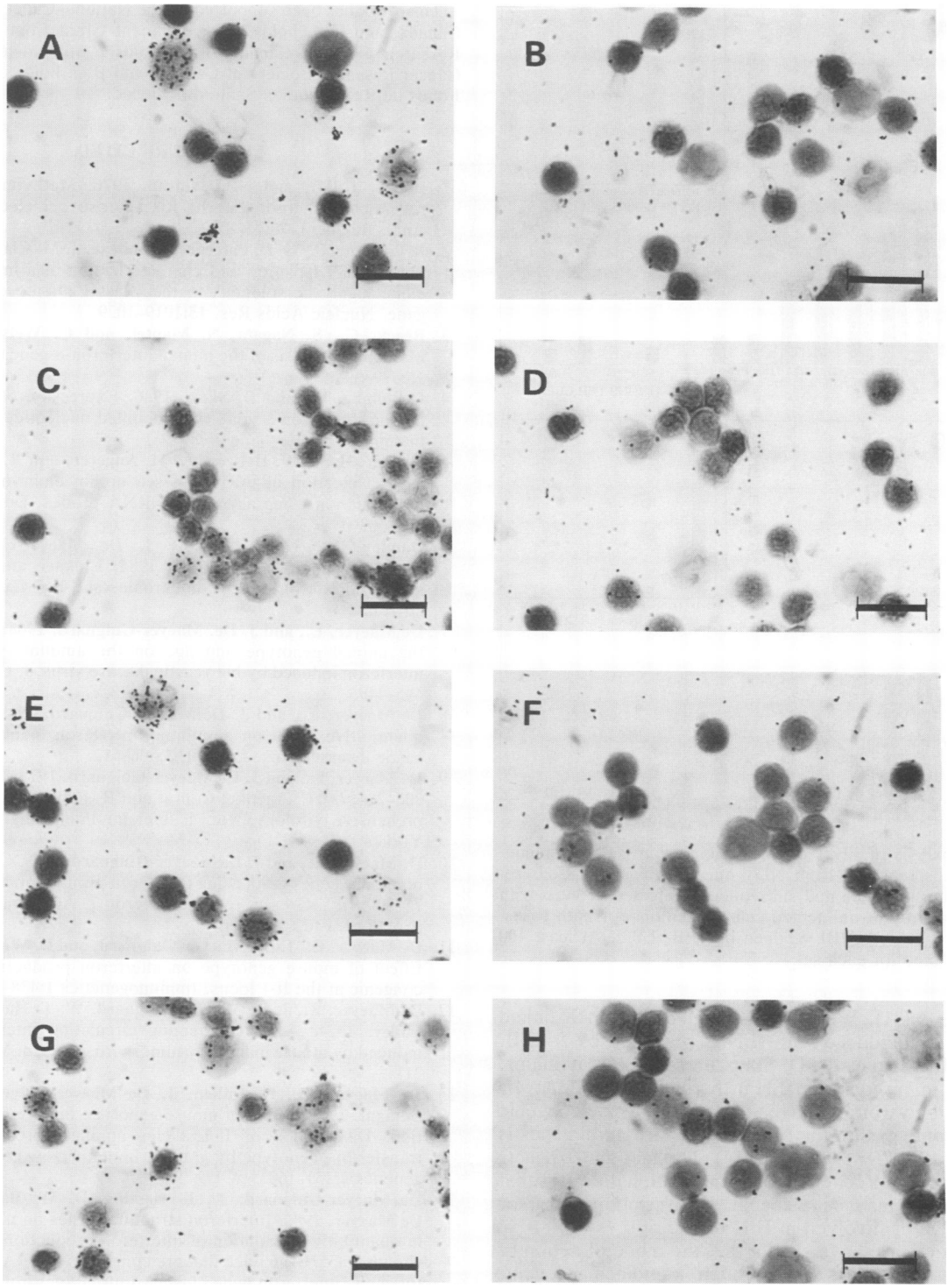

FIG. 5. In situ hybridizations on isolated splenocytes. (A) NDV-infected and (B) uninfected C57BL/6 mice; (C) NDV-infected and (D) uninfected BALB/c mice; (E) in vitro NDV-infected and (F) uninfected C57BL/6 mice; (G) in vitro NDV-infected and (H) uninfected BALB/c mice. Hybridizations were for 48 h at 42°C with 10 ng of ^{3}H-labeled MuIFN-β_1 cRNA (specific activity, 6.5×10^7 cpm/μg). Autoradiographic exposure was for 25 days. Bars, 25 μm.

which was shifted towards cells with more silver grains, but this was not necessarily in proportion to the ratio in mRNA levels. In sum, we interpret our results as meaning that in the spleens of *If-1^l* and *If-1^h* mice roughly equal numbers of cells respond to NDV by producing IFN; thus the cells from the high-producing animals transcribe higher levels of IFN mRNA.

If-1 acts in *trans*. This is evident as the structural genes for both IFN-α and IFN-β are on chromosome 4 (6, 25, 28), whereas the *If-1* locus has been mapped genetically to

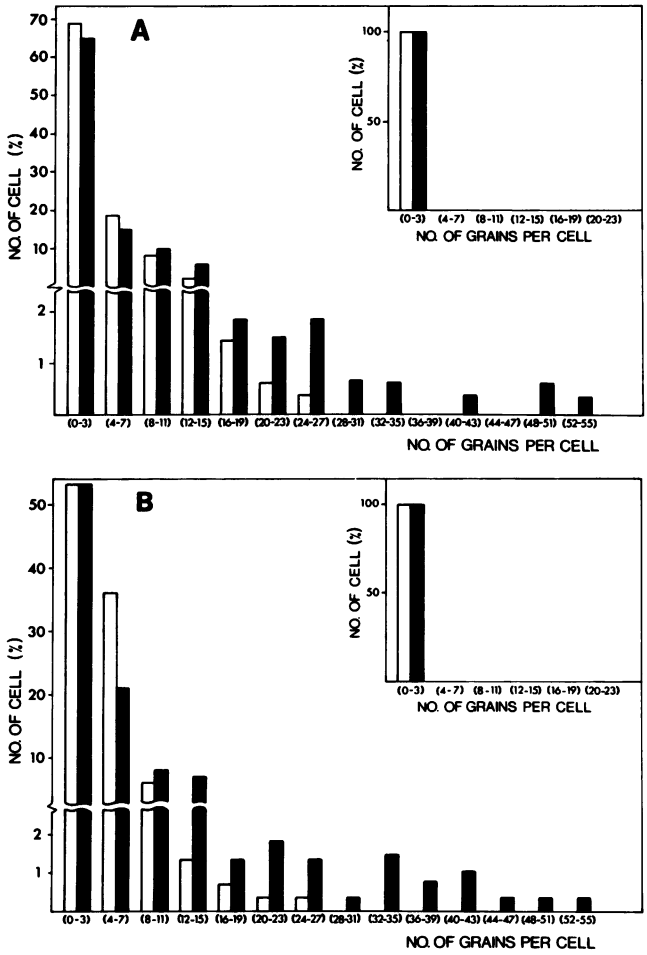

FIG. 6. Analysis of silver grain distribution in isolated splenocytes. (A) Cells removed from NDV-infected mice. (B) Cells removed from normal mice and subsequently infected in vitro with NDV. Insets represent uninfected cell populations for both cases. Symbols: Solid bars, C57BL/6; open bars, BALB/c. At least 400 cells were counted for each set.

chromosome 3 (31). We have also found that an X-linked gene which is coinduced with IFN is regulated by *If-1* (B. Coulombe, L. Daigneault, D. Skup, and B. R. G. Williams, manuscript in preparation). Furthermore, De Maeyer-Guignard and co-workers have shown, using mice congenic for chromosome 4, that the origin of the structural genes is not important for *If-1* regulation (14). The fact that *If-1* acts in *trans* to exert a direct effect on transcription indicates that the *If-1* product may be a specific transcription factor or some other factor involved in IFN induction.

In conclusion, we have shown that the mode of action of the *If-1* regulatory locus is through a molecular event. Individual IFN-producing cells in spleen transcribe higher levels of IFN-specific mRNA in *If-1*^{h} mice. This fact, in conjunction with the findings that splenocytes induced in vitro produce high levels of IFN and that this production is regulated by *If-1*, will now permit study of the molecular mechanisms of *If-1* action.

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LITERATURE CITED

1. **Bailey, J. M., and N. Davidson.** 1976. Methylmercury as a reversible denaturing agent for agarose gel electrophoresis. Anal. Biochem. **70**:75–85.
2. **Belhumeur, P., G. D. Paterno, G. Boileau, J. M. Claverie, and D. Skup.** 1987. Isolation and characterization of a murine cDNA clone highly homologous to the yeast L29 ribosomal protein gene. Nucleic Acids Res. **15**:1019–1029.
3. **Brack, C., S. Nagata, N. Mantei, and C. Weissman.** 1981. Molecular analysis of the human interferon-α gene family. Gene **15**:379–394.
4. **Coulombe, B., and D. Skup.** 1986. Expression of a synthetic human interferon-α gene with modified nucleotide sequence in mammalian cells. Gene **46**:89–95.
5. **Cox, K. H., D. V. DeLeon, L. M. Angerer, and R. C. Angerer.** 1984. Detection of mRNA in sea urchin embryos by *in situ* hybridization using asymmetric RNA probes. Dev. Biol. **101**: 485–502.
6. **Dandoy, F., K. A. Kelley, J. De Maeyer-Guignard, E. De Maeyer, and P. M. Pitha.** 1984. Linkage analysis of the murine interferon-α locus on chromosome 4. J. Exp. Med. **160**: 294–302.
7. **De Maeyer, E., and J. De. Maeyer-Guignard.** 1968. Influence of the animal genotype and age on the amount of circulating interferon induced by Newcastle disease virus. J. Gen. Virol. **2**: 445–449.
8. **De Maeyer, E., and J. De Maeyer-Guignard.** 1969. Gene with quantitative effect on circulating interferon induced by Newcastle disease virus. J. Virol. **3**:506–512.
9. **De Maeyer, E., and J. De Maeyer-Guignard.** 1979. Interferon, p. 205–284. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 1. Plenum Publishing Corp., New York.
10. **De Maeyer, E., and J. De Maeyer-Guignard.** 1979. Consideration on mouse genes influencing interferon production and action, p. 75–100. *In* I. Gresser (ed.), Interferon 1. Academic Press, Inc. (London), Ltd., London.
11. **De Maeyer, E., J. De. Maeyer-Guignard, and D. W. Bailey.** 1975. Effect of mouse genotype on interferon production. I. Lines congenic at the If-1 locus. Immunogenetics **1**:438–443.
12. **De Maeyer, E., J. De Maeyer-Guignard, W. T. Hall, and D. W. Bailey.** 1974. A locus affecting circulating interferon levels induced by mouse mammary tumor virus. J. Gen. Virol. **23**:209–211.
13. **De Maeyer, E., P. Jullien, J. De Maeyer-Guignard, and P. Démant.** 1975. Effect of mouse genotype on interferon production. Distribution of If-1 alleles among inbred strains and transfer of phenotype by grafting bone marrow cells. Immunogenetics **2**:151–160.
14. **De Maeyer-Guignard, J., F. Dandoy, D. W. Bailey, and E. De Maeyer.** 1986. Interferon structural genes do not participate in quantitative regulation of interferon production by *If* loci as shown in C57BL/6 mice that are congenic with BALB/c mice at the alpha interferon gene cluster. Journal of Virol. **58**:743–747.
15. **De Maeyer-Guignard, J., M. G. Tovey, I. Gresser, and E. De Maeyer.** 1978. Purification of mouse interferon by sequential affinity chromatography on poly(U)- and antibody-agarose columns. Nature (London) **271**:622–625.
16. **Denhardt, D. T.** 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
17. **Derynck, R., S. Content, E. De Clerq, G. Volckaert, J. Tavernier, R. Devos, and W. Fiers.** 1980. Isolation and structure of a human fibroblast interferon gene. Nature (London) **285**:542–547.
18. **Fujita, T., S. Ohno, H. Yasumitsu, and T. Taniguchi.** 1985.

Delimitation and properties of DNA sequences required for the regulated expression of human interferon-β gene. Cell **41**:489–496.

19. **Goodbourn, S., H. Burstein, and T. Maniatis.** 1986. The human β-interferon gene enhancer is under negative control. Cell **45**: 601–610.
20. **Goodbourn, S., K. Zinn, and T. Maniatis.** 1985. Human β-interferon gene expression is regulated by an inducible enhancer element. Cell **41**:509–520.
21. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation on 3T3 cells induces transcription of the c-fos proto-oncogene. Nature (London) **311**:433–438.
22. **Groudine, M., M. Peretz, and H. Weintraub.** 1981. Transcriptional regulation of hemoglobin switching in chicken embryos. Mol. Cell. Biol. **1**:281–288.
23. **Hannigan, G., and B. R. G. Williams.** 1986. Transcriptional regulation of interferon-responsive genes is closely linked to interferon receptor occupancy. EMBO J. **5**:1607–1613.
24. **Higashi, Y., Y. Sokawa, Y. Watanabe, Y. Kawade, S. Ohno, C. Takaoka, and T. Taniguchi.** 1983. Structure and expression of a cloned cDNA for mouse interferon-β. J. Biol. Chem. **258**:9522–9529.
25. **Kelley, K. A., C. A. Kozak, F. Dandoy, F. Sor, D. Skup, J. D. Windass, J. De Maeyer-Guignard, P. M. Pitha, and E. De Maeyer.** 1983. Mapping of murine interferon-α genes to chromosome 4. Gene **26**:181–188.
26. **Kohase, M., D. Henrikson-Destefano, L. T. May, J. Vilcek, and P. B. Sehgal.** 1986. Induction of β_2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. Cell **45**:659–666.
27. **Krieg, P. A., and D. A. Melton.** 1984. Functional messenger RNAs are produced by SP6 in vitro transcription of cloned cDNAs. Nucleic Acids Res. **12**:7057–7069.
28. **Lovett, M., D. R. Cox, D. Yee, W. Boll, C. Weissmann, C. J. Epstein, and L. B. Epstein.** 1984. The chromosomal location of mouse interferon-α genes. EMBO J. **3**:1643–1646.
29. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12**:7035–7056.
30. **Mendelson, J., and L. A. Glasgow.** 1966. The *in vitro* and *in vivo* effects of cortisol on interferon production and action. J. Immunol. **96**:345–352.
31. **Mobraaten, L. E., H. P. Bunker, J. De Maeyer-Guignard, E. De Maeyer, and D. W. Bailey.** 1984. Location of histocompatibility and interferon loci on chromosome 3 of the mouse. J. Hered. **75**:233–234.
32. **Murphy, D., P. M. Brickell, D. S. Latchman, K. Willison, and P. W. J. Rigby.** 1983. Transcripts regulated during normal embryonic development and oncogenic transformation share a repetitive element. Cell **35**:865–871.
33. **Ohno, S., and T. Taniguchi.** 1983. The 5′ flanking sequence of human interferon-β gene is responsible for viral induction of transcription. Nucleic Acids Res. **11**:5403–5412.
34. **Raj, N. B. K., and P. M. Pitha.** 1983. Two levels of regulation of β-interferon gene expression in human cells. Proc. Natl. Acad. Sci. USA **80**:3923–3927.
35. **Rytel, M. W., and E. D. Kilbourne.** 1966. The influence of cortisone on experimental viral infection. VIII. Suppression by cortisone of interferon formation in mice injected with Newcastle disease virus. J. Exp. Med. **123**:767–776.
36. **Sagar, A. D., P. B. Sehgal, L. T. May, M. Inouye, D. L. Slate, L. Shulman, and F. H. Ruddle.** 1984. Interferon-β-related DNA is dispersed in the human genome. Science **223**:1312–1315.
37. **Sehgal, P. B., and A. D. Sagar.** 1980. Heterogeneity of poly(I). Poly(C)-induced human fibroblast interferon mRNA species. Nature (London) **288**:95–97.
38. **Shaw, G. D., W. Boll, M. Taira, N. Mantei, P. Lengyel, and C. Weissmann.** 1983. Structure and expression of cloned murine IFN-α genes. Nucleic Acids Res. **11**:555–573.
39. **Skup, D., H. Zarbl, and S. Millward.** 1981. Regulation of translation in L-cells infected with reovirus. J. Mol. Biol. **151**: 35–55.
40. **Stewart, W. E., II.** 1979. The interferon system. Springer-Verlag, Vienna.
41. **Taniguchi, T., S. Ohno, Y. Fujii-Kuriyama, and M. Muramatsu.** 1980. The nucleotide sequence of human fibroblast interferon cDNA. Gene **10**:11–15.
42. **Weissenbach, J., J. Chernajovsky, M. Zeeni, L. Shulman, H. Soreq, U. Nir, D. Wallach, M. Perricaudet, P. Tiollais, and M. Revel.** 1981. Two interferon mRNAs in human fibroblasts: in vitro translation and *Escherichia coli* cloning studies. Proc. Natl. Acad. Sci. USA **77**:7152–7156.
43. **Zinn, K., D. Di Maio, and T. Maniatis.** 1983. Identification of two distinct regulatory regions adjacent to human β-interferon gene. Cell **34**:865–879.
44. **Zinn, K., and T. Maniatis.** 1986. Detection of factors that interact with human β-interferon regulatory region *in vivo* by DNAase I footprinting. Cell **45**:611–618.