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Short Communication

# Constitutive interferon expression from retroviral vector

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**Summary**

A genomic fragment with the human β-interferon gene was cloned into a pL3-4, a defective Moloney murine leukemia virus (M-MuLV) vector. Here we show that clones selected after viral infection of mouse NIH 3T3 cells constitutively produced 128 IU/ml of human β-interferon. Constitutive synthesis of retroviral RNA was confirmed by dot blot hybridization of RNA isolated from two of the selected clones. Poly(I)·poly(C) and cycloheximide induction resulted in an increased RNA level, but this was not reflected in an increased production of biologically active interferon.

Retrovirus; Interferon; Constitutive expression

Many cell lines respond to viral infection or treatment with double-stranded RNA (ds RNA) by increasing the expression of a number of genes (Pestka and Baron, 1981). The best characterized genes of this type are the interferons (IFNs), which curtail further replication of viruses and which play a role in regulating the cell growth (Stewart, 1979; DeMaeyer and DeMaeyer-Guignard, 1988).

Transcriptional activation is a major component of interferon gene induction, and *cis*-acting sequences that mediate this process were identified (Zinn et al., 1983). The sequence required for efficient induction of the human

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β-interferon gene, interferon regulatory element (IRE), is both necessary and sufficient for induction in mouse cells (Goodbourn et al., 1985).

In this study, we investigated the regulation of the expression of the human β-interferon gene inserted in a M-MuLV based retroviral vector (Keller et al., 1985).

The inducibility of the human β-interferon gene was detected at the mRNA level, and the synthesis of biologically active human interferon proceeded constitutively, regardless of the synthesized amounts of specific transcripts.

Plasmid DNA (without carrier DNA) was transfected into the Psi-2 cells (Mann et al., 1983), using the calcium phosphate precipitation procedure (Graham and Van der Eb, 1973). NIH 3T3 cells were infected by recombinant viruses in medium containing 8 μg/ml of polybrene. 2 days later the medium was replaced by the selective medium containing 800 μg/ml of G418. Drug-resistant colonies were counted 14 days later.

RNA was isolated from $2 \times 10^7$ cultured cells by CsCl density gradient centrifugation method (Glisin et al., 1974) modified by Chirgwin (Chirgwin et al., 1979). Purification of poly(A)-rich RNA directly from cell culture has been performed with Dynabeads® $Oligo(dT)_{25}$ (Dynal, Oslo, Norway) (Hornes and Korsnes, 1990). The amount of RNA on blots was monitored by RNA electrophoresis and EtBr vizualisation of 28S and 18S RNA (data not shown).

Interferon and *neo* DNA probe were radioactively labeled (Feinberg and Vogelstein, 1983) with the oligolabelling kit (Pharmacia). Northern and dot blots were hybridized, washed and autoradiographed by standard procedures (Church and Gilbert, 1984).

The 550 bp double-stranded DNA interferon probe was obtained by *Eco*RI/*Pvu*II digestion from pIF plasmid (Crkvenjakov et al., 1984). The 920 bp double-stranded DNA *neo* probe was prepared by *Pst*I digestion of pL3-4 plasmid (Keller et al., 1985).

Interferon was titrated on WISH cells by a cytopathogenic effect-inhibition test using vesicular stomatitis virus as a challenge (Sanceau et al., 1987). Interferon titers were expressed in international units, using NIH human β-interferon (G-023-902-527) as a standard.

In order to investigate the human β-interferon gene expression from murine retroviral vector, we inserted the *Eco*RI-*Hpa*II DNA fragment from pIF into the unique *Xho*I site of pL3-4 vector downstream from the *neo* gene, in the same transcriptional orientation as 5′ LTR (Fig. 1).

The 922 bp *Eco*RI-*Hpa*II fragment of human β-interferon gene contains the 350 bp of the 5′ regulatory region, and the coding sequence terminating with a UGA stop codon (Fig. 1.) (Crkvenjakov et al., 1984).

Four NIH 3T3 clones (A, E, C and G) and pool of clones (1NIH 3T3), obtained after infection with L3-4-IF virus, were used for further analysis. Southern blot demonstrated integration of the complete L3-4-IF virus sequence into these clones (data not shown).

In order to determine the level of human interferon mRNA, two individual clones, A and E were superinduced. After 4 h, total cellular RNA was isolated

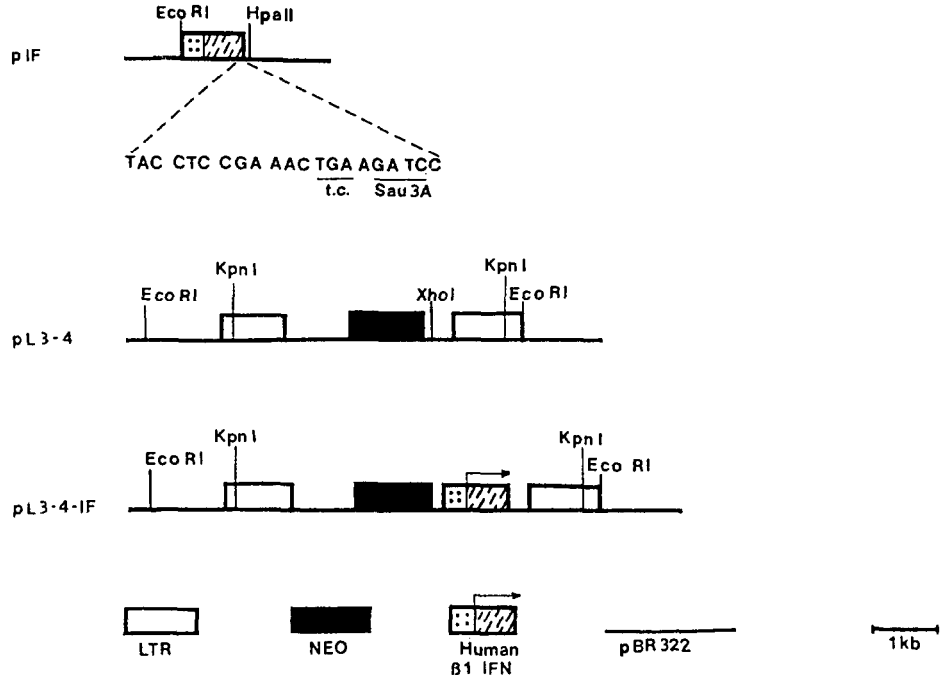


Fig. 1. Partial restriction maps of used vectors. pIF (Crkvenjakov et al., 1984) and pL3-4 (Keller et al., 1985) have been previously described. pL-3-4-IF was constructed by inserting an *Eco*RI/*Hpa*II 934 bp blunt end fragment from pIF, into the *Xho*I site of pL3-4. Transcriptional orientation of the human β-interferon genomic fragment is indicated by the arrow. The following regions are indicated: LTR, viral long terminal repeat; NEO, neomycin resistance gene; IFN, human β-interferon genomic fragment; pBR322, bacterial plasmid sequences; t.c., termination codon.

from induced and uninduced cells. Total and poly(A)-RNA were analyzed by dot blot hybridization with human β-interferon probe. RNAs from induced and uninduced human WISH cells were used as controls.

Uninduced clones produced significant amounts of interferon mRNA (Fig. 2b lines 4 and 6). This result indicates a constitutive transcription from the integrated human interferon gene. Similarly, some other cellular and RNA tumor virus genes are also expressed constitutively following the transfection, although at the same time their resident counterparts remain silent (Pitha et al., 1982., Zinn et al., 1983). However, after induction the amount of interferon mRNA was doubled (Fig. 2b lanes 3 and 5).

It could be argued that the increase in interferon mRNA level after poly(I) · poly(C) induction may be the consequence either of the overall increase from LTR promoter, or of specific transcription from the interferon promoter. If the transcription is controlled by the LTR promoter, *neo* transcription should be increased as well. Hybridization of E clone RNA with *neo* probe, however, did not show differences in *neo* mRNA level in induced and uninduced E clone (Fig. 2a lanes 3 and 4). Indirectly, this dot blot hybridization

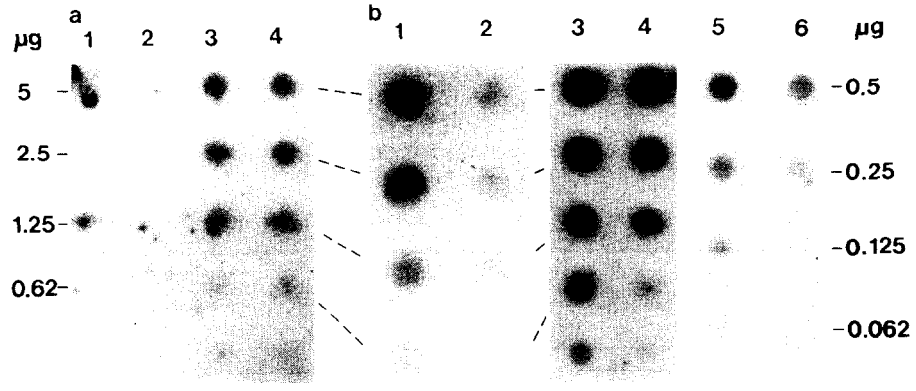


Fig. 2. Quantification of the level of *neo* (a) or human β-interferon mRNA (b) produced in induced and uninduced clones. Total RNA from human WISH cells used as negative and positive control. Dot-blot analysis was performed as described in Materials and Methods. 5 μg of total RNA (from clone E) and 0.5 μg of poly(A)-RNA (from clone A) was applied in the first row, followed by two-fold dilutions. The lanes contain the following: (a) total RNA from induced and uninduced WISH cells (lanes 1 and 2); total RNA from induced and uninduced clone E (lanes 3 and 4), (b) total RNA from induced and uninduced WISH cells (lanes 1 and 2); total RNA from induced and uninduced clone E (lanes 3 and 4); poly(A)-RNA from induced and uninduced clone A (lanes 5 and 6). The amounts of used RNA are outlined on the left and right. The panel represents results from three independent experiments. Each experiment was done in triplicate. Dots intensity differ depending of probe labelling and time of film exposure.

with the *neo* probe suggests that the increase in mRNA transcription is from the interferon promoter only.

The human β-interferon probe was used in Northern blot analysis of total RNA isolated from uninduced and induced A cells. The results presented in Fig. 3, lanes A (+) and (−), showed three different sizes of human β-interferon transcripts detected in both induced and uninduced cells. These transcripts may represent the following mRNAs:

- the large transcript (4.6 kb) – mRNA initiated in the M-MuLV 5′ LTR and terminated in the viral 3′ LTR polyadenylation site,
- the transcript of 3.6 kb – spliced viral mRNA containing both neo and interferon transcripts,
- the smallest transcript of 1.9 kb – β-interferon mRNA properly initiated at the interferon mRNA cap site and terminated in the MuLV 3′ LTR polyadenylation site.

Verification of the start sites of these transcripts would need more precise analysis. Yet, undoubtedly, Northern blot analysis detects all three transcripts in uninduced and induced cells. Therefore, the induction of interferon promoter in retroviral background does not result in new transcript, although we can not exclude quantitative changes.

In order to determine synthesis of biologically active human interferon, we analyzed the antiviral interferon activity produced from uninduced and induced INIH 3T3, A and E clones. Uninduced and induced human WISH amnion cells served as the induction control. Mouse interferon cannot be

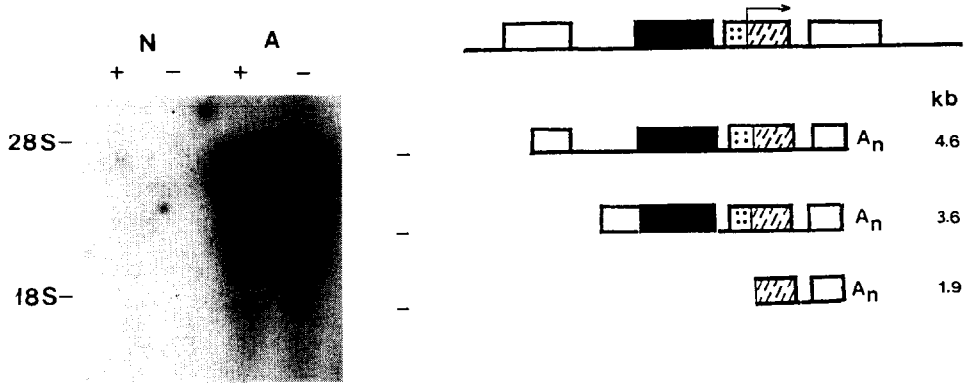


Fig. 3. Identification of human β-interferon transcripts in mouse NIH 3T3 fibroblasts containing the integrated human β-interferon gene. Total RNA (15 μg) isolated from induced and uninduced control NIH 3T3 cells (N), and L3-4-IF virus infected NIH 3T3 cells (clone A), was analyzed by Northern hybridization with the human interferon probe. The hybridization was done as described in Materials and Methods. (+) and (−) indicate the poly(I)·poly(C) induced and uninduced cells. The possible structure of the detected transcripts is indicated.

detected in these assay conditions (Table 1, NIH 3T3 cells).

The measurement of relative levels of human β-interferon synthesized in uninduced A and E clones showed the synthesis of 128 IU interferon/ml (Table 1). However, the superinduction of interferon synthesis of same clones did not lead to adequate increase in the amount of the synthesized human interferon (Table 1). The same result has been obtained with a pool of infected cells (1NIH 3T3). The nonparallel increase in interferon mRNA synthesis and interferon biological activity was also observed in the induced human lymphoblastoid cells (Raj et al., 1985; Shuttleworth et al., 1983). Interferon synthesis is controlled at post-transcriptional level through translational

TABLE 1

Production of human β-interferon in analyzed cells

| Cells | Induction | Hu IFN (IU/ml) |
|---|---|---|
| NIH 3T3 | + | 2 ± 2 |
| NIH 3T3 | − | 0 ± 0 |
| WISH | + | 256 ± 0 |
| WISH | − | 0 ± 0 |
| 1 NIH 3T3 | + | 128 ± 0 |
| 1 NIH 3T3 | − | 128 ± 0 |
| A | + | 128 ± 0 |
| A | − | 128 ± 0 |
| E | + | 128 ± 0 |
| E | − | 128 ± 0 |

Interferon levels were measured from the supernatants of uninduced and poly(I)-poly(C) induced cell lines as described in Materials and Methods. Measurements represent the average of three separate inductions; each interferon assay was done in triplicate. Interferon standard was included in each set of assay.

efficiency or mRNA stability (Ray and Pitha, 1983; Mosca and Pitha, 1986). However, a sequence in the 3′ end of the β-interferon mRNA which inhibits translational efficiency (Kruys et al., 1987) is not present in our interferon transcription unit.

In summary, in both vectors (Engelhardt et al., 1990 and this paper), which differ significantly in their interferon 3′ untranslated regions, the transcription is inducible but not followed by an adequate increase of protein synthesis.

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