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Gamma Interferon Regulates Long Terminal Repeat-Controlled Oncogene Expression in Transformed Mouse Fibroblasts at the Level of mRNA Transcription

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In transformed NIH 3T3 cells, murine gamma interferon reduces the expression of the long terminal repeat-controlled oncogenes v-*mos*, c-*myc*, and v-Ha-*ras* by a direct effect on the activity of retroviral promoters, as revealed by analyses of RNA half-life and transcriptional activity of retroviral genes as well as by analyses of chloramphenicol acetyltransferase activity in cells transformed with the *cat* gene under the control of long terminal repeats.

Interferons (IFNs) are a group of related cytokines with multiple biological activities, some of which, e.g., cytostatic action or induction of differentiation of tumor cells, may contribute to the control of malignant disease (5, 8, 10, 18, 23). From models of oncogenic transformation of fibroblasts, it has been implicated that modulation of oncogene expression is one of the crucial mechanisms of antitumoral activities of IFNs (5, 7, 12, 13, 19, 20, 21), although no general rules have emerged as to the mechanisms by which IFNs exert control of oncogene expression. Using NIH 3T3 cells transformed by oncogenes of different transforming capacity, we studied the mechanisms of gamma IFN-mediated inhibition of long terminal repeat (LTR)-controlled oncogene expression. The data show that murine recombinant gamma IFN (mu-rIFN-gamma) downregulates the expression of v-Ha-*ras*, v-*mos*, and c-*myc* oncogenes at the transcriptional level and causes phenotypical reversion of v-*mos* and c-*myc* but not of v-Ha-*ras* transformants.

Oncogene-mediated transformation was obtained after transfection of NIH 3T3 cells with retroviral vectors Neo^r MPSV (v-*mos*) (17), ZIP-myc (c-*myc*), and ZIP-Ha-*ras* (v-Ha-*ras*) (Fig. 1). The respective oncogene-negative control vectors Neo^r mos$^-$ (17) and ZIP-SVX (3) served as a control (Fig. 1). Stable retrovirally transformed cell lines were established by using the distinct vectors and were analyzed for responsiveness to mu-rIFN-gamma (specific activity, 2×10^7 U/ml; produced by Genentech and kindly provided by Boehringer Ingelheim, Vienna, Austria). Total cellular RNA was extracted from untreated and gamma IFN-treated cells and analyzed by Northern (RNA) blotting for the expression of specific oncogenes as described previously (21) with cDNA probes labeled with ^{32}P by random priming (9). In accordance with earlier findings (21), 24-h treatment with 400 U of gamma IFN per ml selectively reduced the retroviral steady-state RNA levels in four v-*mos*-, two v-Ha-*ras*-, and two c-*myc*-transformed cell lines (data not shown). Inhibition of protein synthesis by a 1-h pretreatment of these cell lines with cycloheximide (10 μg/ml) did not abrogate downregulation of oncogene expression during the subsequent 4-h gamma IFN treatment (Fig. 2A). As integration of the provirus into the cellular genome is random, the integration site appears not to be important for this gamma IFN action, and this suggests that inhibition of oncogene expression is due to a direct effect on the retroviral genes.

To define the level(s) at which gamma IFN downregulates the expression of the respective oncogene, three distinct cell lines (3T3mos4, 3T3myc2, and 3T3ras11) were investigated for both the stability of retroviral RNA and transcriptional activity of retroviral genes in the absence or presence of gamma IFN. To determine the influence of gamma IFN on RNA half-life, cells were preincubated for 1 h with gamma IFN (400 U/ml) or left untreated before actinomycin D (5 μg/ml) was added to shut off transcriptional activity. Total cellular RNA was extracted (2×10^7 cells for each group and time point), and oncogene-specific RNA levels were analyzed by Northern blotting. In the three distinct transformants, gamma IFN treatment did not change the RNA half-life, which was approximately 1 h for v-*mos* and 30 min for v-Ha-*ras* and c-*myc* RNA (Fig. 2B).

To determine whether gamma IFN affected the transcriptional activity of LTR-controlled oncogenes, two cell lines, transformed by distinct vectors, were investigated by nuclear run-on assays. Nuclei from both untreated and gamma IFN-treated 3T3mos4 and 3T3ras11 cells (5×10^7 cells per group) were isolated, and in vitro transcripts were generated by elongation of previously initiated RNA chains in the

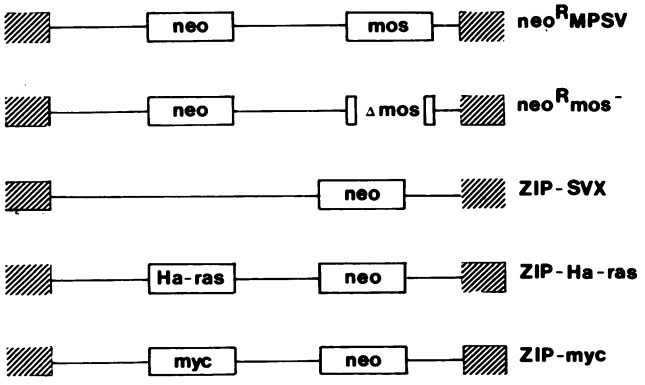

FIG. 1. Schematic diagram of the retroviral vectors used. Hatched boxes indicate the positions of the LTRs.

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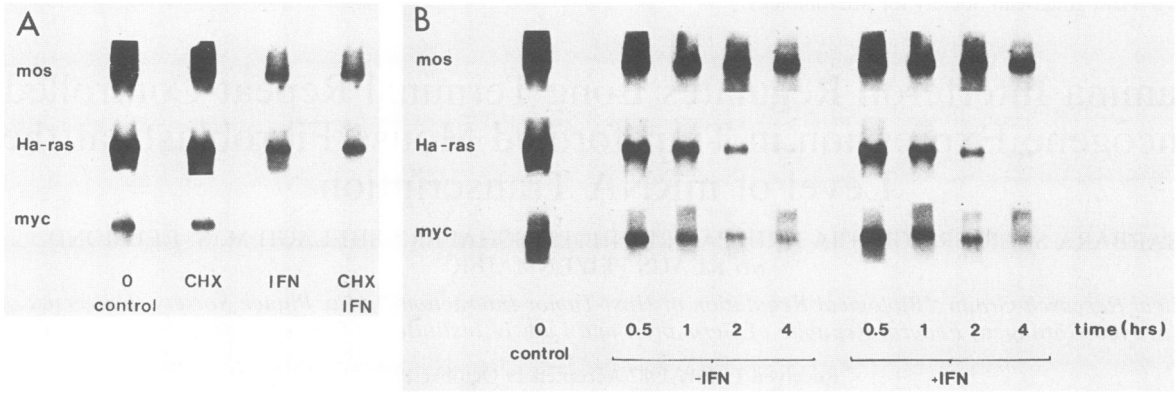

FIG. 2. Northern blot analysis of gamma IFN influence on steady-state levels (A) and half-life (B) of oncogene-specific RNAs in distinct retrovirally transformed cell lines. CHX, Cycloheximide. Probes are shown to the left.

presence of [α-^{32}P]UTP (14, 16). The transcripts (2×10^6 cpm of deproteinized RNA per group) were then hybridized to nitrocellulose filters loaded with 5 μg of alkali-denatured cDNA probes specific for the LTR and the respective genes. A significant inhibition of transcriptional activity of retroviral genes was observed in gamma IFN-treated 3T3mos4 and 3T3ras11 cells, whereas no modulation of the β-actin gene was noted (Fig. 3). A similar mechanism of gamma IFN action can be assumed for regulation of c-*myc* expression in 3T3myc2 cells, as the oncogene is under control of the same LTR as v-Ha-*ras* in 3T3ras11 cells, and no evidence for reduced mRNA half-life was found (Fig. 2B). Therefore, for all three types of transformants, the gamma IFN-mediated decrease of steady-state retroviral RNA (Fig. 2A) was largely due to reduction in gene transcription (Fig. 3) rather than to an immediate effect on the stability of v-*mos*, c-*myc*, and v-Ha-*ras* RNA, suggesting a direct effect of gamma IFN on LTR activity. Support for this reasoning was obtained from experiments in which the influence of gamma IFN on the Moloney virus-derived promoter was investigated by using *cat* gene expression as an indicator system (11). Gamma IFN treatment (1,000 U/ml, 24 h) of stable LTR-CAT transformants of 3T3 cells indeed caused a significant reduction in CAT activity (Fig. 4). Together, the data suggest that gamma IFN downregulates the expression of LTR-controlled oncogenes *mos*, *myc*, and Ha-*ras* primarily at the level of transcription by affecting the activity of the retroviral promoters.

The detailed molecular mechanism(s) and specificity of gamma IFN action on LTR activity largely remains to be defined. Although three distinct constructs were used in this study, the vectors were all based on Moloney murine leukemia virus- and Moloney murine sarcoma virus-derived sequences, which show close homology in their LTRs (3). Therefore, it needs to be established whether IFN-mediated inhibition of retroviral gene transcription is selective for LTRs related to Moloney virus or whether these mechanisms of gamma IFN action are effective on a broader range of nonrelated retroviruses. In any case, regulation of LTR activity by gamma IFN implies a common mechanism of gamma IFN action, by which both antineoplastic and antiviral effects can be exerted.

We have previously shown that downregulation of v-*mos* expression in 3T3mos4 cells was associated with a nearly complete but fully reversible inhibition of the transformed phenotype (21). Similar results were obtained with three other independently derived v-*mos*-transformed cell lines with an average reduction of soft agar colonies of greater than 95% (Table 1). Likewise, colony formation of two c-*myc* transformants was completely inhibited when gamma IFN (400 U/ml) was added at the beginning of soft agar culture (Table 1). In contrast, only weak inhibition of anchorage-independent growth was observed for the two v-Ha-

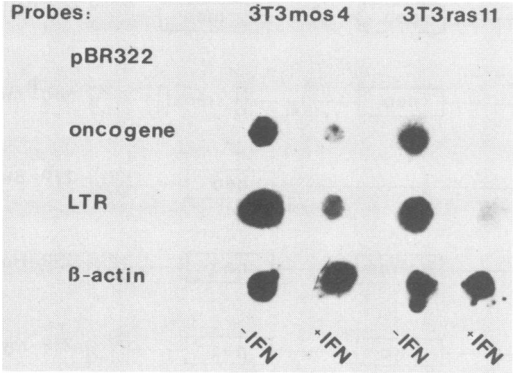

FIG. 3. Nuclear transcription analysis of retroviral and cellular genes after gamma IFN treatment of 3T3mos4 and 3T3ras11 cells.

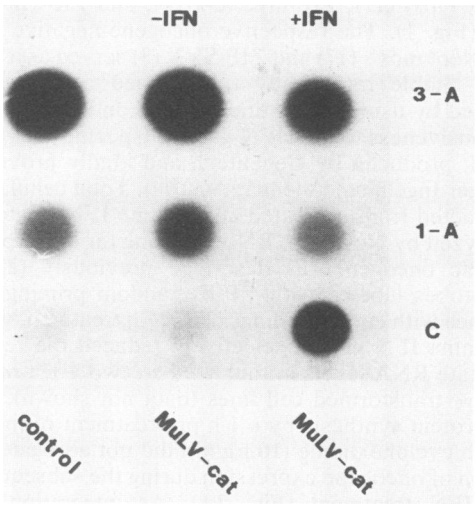

FIG. 4. Gamma IFN-mediated reduction of CAT activity in 3T3 cells stably transformed by an LTR-CAT vector. MuLV, Murine leukemia virus. C, Unacetylated chloramphenicol; 1-A and 3-A, 1- and 3-monoacetylated chloramphenicol.

TABLE 1. Influence of mu-rIFN-gamma on soft-agar colony formation of fibroblasts transformed by oncogenic and nononcogenic retroviral vectors

Cell line	No. of cell clones analyzed	Mean no. of soft-agar colonies per 100 cells plated[a] ± SD	
		Untreated	Gamma IFN (400 IU/ml)
NIH3T3	1	0	0
3T3mos$^-$	3	0	0
3T3mos$^+$	4	83.4 ± 5.7	3.0 ± 2.5
3T3 SVX	2	0	0
3T3myc	2	9.0 ± 3.1	0
3T3ras	2	91.5 ± 8.1	75.7 ± 5.3

[a] Obtained from two to four independently derived clones in at least three experiments for each clone.

ras transformants (Table 1). This differential gamma IFN response was confirmed by analysis of the proliferative capacity of the various transformed cells in monolayer cultures (data not shown). Although the number of cell lines investigated was limited, and thus cell-specific factors influencing responsiveness cannot be excluded, we observed within a given group of oncogene-transformed cells a homogeneous gamma IFN response pattern. However, despite a similar mechanism and efficacy in the reduction of steady-state RNA levels of v-*mos*, c-*myc*, and v-Ha-*ras* (Fig. 2), a differential cellular response of the various oncogene-transformed cell lines to gamma IFN treatment was observed, which may reflect the differential function and tumorigenic potential of these oncogenes (1, 2, 15). In this context, the observation of dose-response relationships between oncogene product and transformed phenotype (22, 24) may be of relevance. It is conceivable that the oncogenes analyzed here have different threshold levels of transformation due to completely different targets in the cellular metabolism. Accordingly, our data suggest that v-*mos* and c-*myc* but not v-Ha-*ras* mRNA levels and oncogene products dropped below the threshold required for maintenance of the transformed phenotype. In support of this reasoning is the recent finding of retransformation of reverted cells concomitant with an increase in v-*mos* mRNA levels upon withdrawal of gamma IFN (21) as well as earlier studies showing a differential effect of type I interferons on v-Ha-*ras*- versus EJ/T24-*ras*-induced transformation of NIH 3T3 cells (19, 20).

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