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Interferon-Induced Gene Expression in Wild-Type and Interferon-Resistant Human Lymphoblastoid (Daudi) Cells

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Interferon-induced gene expression was analyzed in wild-type and interferon-resistant Daudi cells. Two classes of α-interferon-induced mRNAs and proteins were observed: those that were similarly induced in both types of cell and those that were induced only in the wild-type cells. Furthermore, the level of c-*myc* mRNA decreased in the wild-type but not in the resistant cells. This differential control in the wild-type and resistant cells indicates that there must be either functionally distinct α-interferon receptors or more than one pathway leading to altered gene expression triggered by a single receptor.

Binding of interferon to specific high-affinity cell surface receptors leads to increased expression of a set of genes (6), the development of an antiviral state, and, in some cases at least, the inhibition of cell growth. The Daudi line of human lymphoblastoid cells is extremely sensitive to the antigrowth activity of α-interferons, whereas the growth of a mutant line which retains α-interferon receptors is resistant to up to 100,000 reference units/ml (20). Furthermore, although most viruses grow poorly in Daudi cells, the mutant line shows partial resistance to the antiviral effects of the α-interferons when assayed with vesicular stomatitis or Semliki Forest virus (E. Meurs, F. Balkwill, and I. M. Kerr, unpublished data). Similar results with independently isolated clones of interferon-resistant Daudi cells have been reported by Dron et al. (7).

We and others have recently isolated a number of cDNAs corresponding to α-interferon-inducible mRNAs (3, 9, 14, 16, 19). The transcription of these RNAs increases rapidly in response to interferon, but they also appear to be subject to posttranscriptional control (9, 10). Here the cDNAs were used to examine the response of the wild-type and resistant Daudi cells to α-interferons. In addition, the treatment of Daudi cells with type 1 interferons leads to a decrease in the level of c-*myc* mRNA, and it has been suggested that this decrease may play a part in the growth-inhibitory response (8, 11, 12). Accordingly, the behavior of c-*myc* mRNA in response to α-interferons was also investigated in the wild-type and resistant cells.

Cells were grown in RPMI 1640 medium with 10% heat-inactivated fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) and were treated with α-interferons from human lymphoblastoid (Namalwa) cells ($\geq 10^8$ units/mg of protein; from K. Fantes, Wellcome Research Laboratories [concentrations are given in reference units throughout]). The cDNAs used were those to the interferon-inducible mRNAs 1-8, 9-27, 6-16, 6-26, MTII (metallothionein II), and 2A (a class I histocompatibility antigen [HLA]) described previously (9) and to mRNAs for c-*myc* and a ppp(A2′p)$_n$A; $n \geq 2$ (2-5A) synthetase (from T. H. Rabbits and B. R. G. Williams, respectively). Further sequence analysis has indicated that 9-27 corresponds to a 3′ end of a message(s) of the 1-8 family and that 6-26 is highly homologous to a cDNA corresponding to rat spleen thymosin β4 (22). In experiments with a number of cell lines the 2-5A synthetase cDNA probe hybridizes to several RNAs of between 1.6 and 4 kilobases (1; J. Kelly, personal communication). With Daudi cells, however, only a 1.8-kilobase mRNA is detected (1) (Fig. 1A).

Four of the mRNAs (6-26, 2A, MTII, and 2-5A synthetase) were induced similarly in the wild-type and resistant cells upon treatment for 9 h with 200 units of α-interferons per ml (Fig. 1A). The extent of induction varied from 3-fold for 2A to 10- to 20-fold for MTII. The mRNA for β_2-microglobulin responded in the same manner as 2A (data not shown). In contrast, mRNAs 1-8, 9-27, and 6-16 were induced strongly (10- to 50-fold) in the wild-type but not the resistant cells (Fig. 1B). This was true at time points from 2 to 60 h after the addition of the interferons. It was also true for treatment for 16 h with 100,000 units/ml for 1-8 and at least 3,000 units/ml for the others. Higher concentrations and additional times were not tested.

In wild-type cells the level of c-*myc* mRNA decreased substantially after exposure to 200 units of α-interferons per ml for 24 h. No such decrease was observed in the resistant cells at any time up to 92 h after addition of the α-interferons (Fig. 2). The levels of mRNAs for β-actin and class II HLA were not affected by α-interferons in either the wild-type or resistant cells (data not shown) and provided a convenient internal standard.

An electrophoretic analysis of the proteins synthesized after treatment of wild-type and resistant cells for 16 h with 200 units of α-interferons per ml is shown in Fig. 3A. In wild-type cells proteins of apparent M_r 15,000, 16,000, 20,000, 53,000, 79,000, 87,000, and 105,000 were induced (Fig. 3A, a to c and e to h) and one of M_r 23,000 decreased (Fig. 3A, d, broken arrow). The resistant cells showed some induction of the 79,000-, 87,000-, and 105,000-M_r proteins and possibly a very low-level induction of those of 16,000, 20,000, and 53,000 M_r. The 15,000-M_r protein, however, was clearly not induced in the resistant cells. When cell surface proteins were labeled with ^{125}I, a major protein of apparent M_r 16,000 was induced by the α-interferons in the wild-type cells only (Fig. 3B, A). This ^{125}I-labeled protein did not comigrate with the 15,000-M_r ^{35}S-labeled protein (Fig. 3A, a) when analyzed in the same gel.

A number of mRNAs and proteins were induced in both the wild-type and resistant cells (Fig. 1A and 3A). These are unlikely to be involved in mediating the antigrowth effects of the α-interferons unless they do so by subtle differences in

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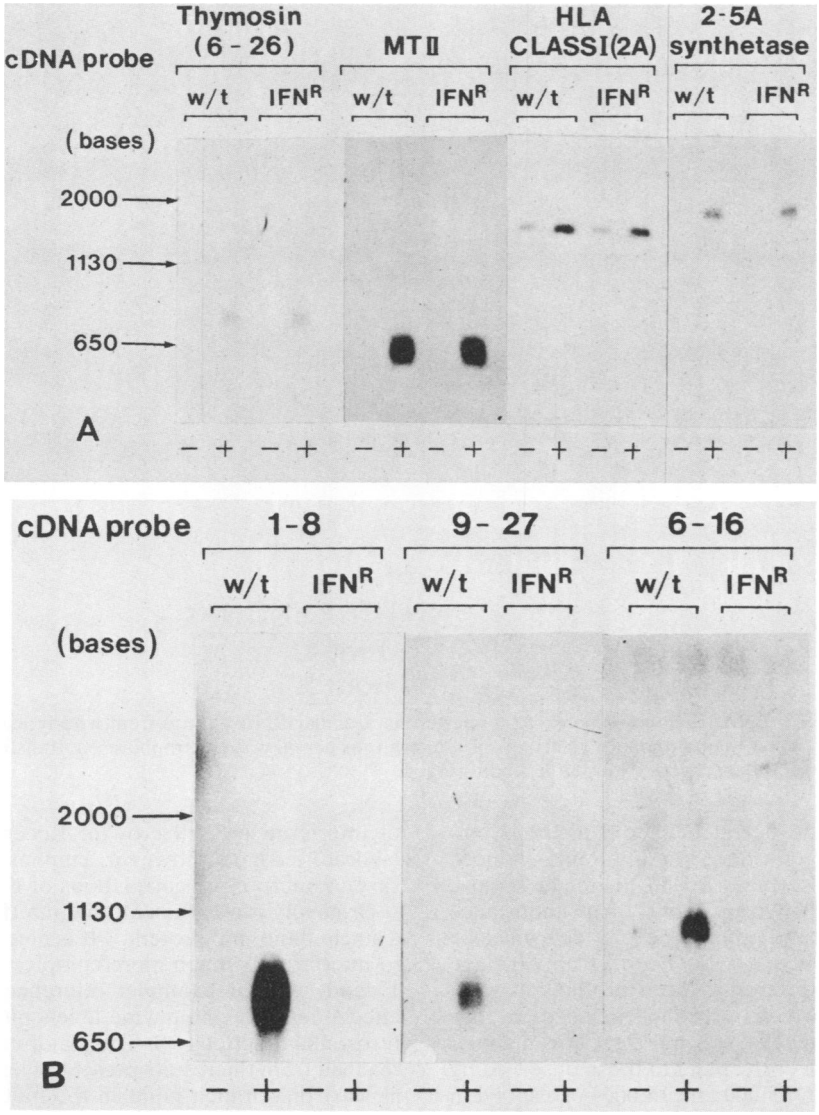

FIG. 1. Induction of mRNAs by α-interferons. Daudi wild-type (w/t) and resistant (IFN^R) cells were incubated for 9 h with (+) or without (−) 200 units of α-interferons per ml. RNA was isolated with guanidinium thiocyanate (5). Samples (25 μg) were glyoxalated, separated by electrophoresis in agarose gels, and transferred to NNG (Pall Corp.) nylon membranes (21). Hybridization was with 5×10^6 cpm of nick-translated (18) cDNA probes per ml ($\geq 10^8$ cpm/μg) according to the manufacturer's protocol. After hybridization the membranes were washed twice for 15 min each in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.2% sodium dodecyl sulfate at room temperature and four times for 15 min each in 0.2× SSC–0.2% sodium dodecyl sulfate at 50°C. The dried membranes were exposed to Kodak XAR5 X-ray film at −80°C, using intensifying screens (Du Pont). The cDNA probes were to mRNAs 6-26, MTII, 2A, and 2-5A synthetase in panel A and 1-8, 9-27, and 6-16 in panel B. Size markers (in bases) indicate the mobility of single-stranded DNA molecules in the same gel.

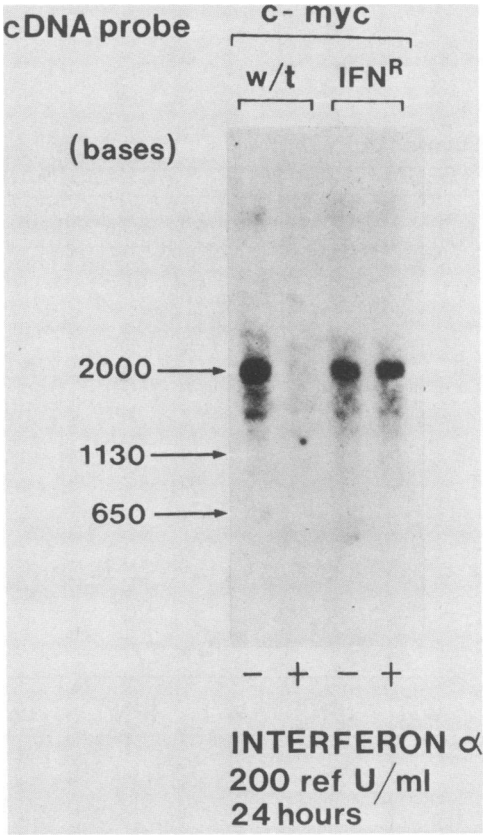

FIG. 2. Reduction in c-*myc* mRNA levels in response to α-interferons. Cellular RNA (25 μg) from wild-type (w/t) and resistant (IFN^R) Daudi cells incubated for 24 h with (+) or without (−) 200 units of α-interferons per ml was electrophoresed, transferred, and hybridized with a cDNA probe to the c-*myc* mRNA as described in the legend to Fig. 1.

expression too small to be detected by the methods employed. The second group of mRNAs and proteins, induced only in the wild-type cells (Fig. 1B and 3), might seem a priori more likely to be involved, but their importance remains to be established as other gene products may be crucial in this regard. For example, Dron et al. (7) have recently detected 15 α-interferon-induced proteins in wild-type Daudi cells, 7 of which were not induced in four different clones of resistant cells. It is not clear how many of the proteins described here correspond to those observed by Dron et al., but those at M_r 15,000 and 16,000 correspond in electrophoretic mobility at least. Similarly, the relationship of the α-interferon-induced cell surface protein (Fig. 3B) to that reported by Burrone and Milstein (2) remains to be established.

The absence of an effect of α-interferons on the level of c-*myc* mRNA in the resistant cells (Fig. 2) (11; E. Knight and M. Tovey, personal communication) is consistent with a possible role for the reduction in this message in the effects of interferon on cell growth. Recent results from several laboratories have, however, emphasized the caution necessary in interpreting correlations of this type.

Originally it was thought that interferon might act through a single "antiviral protein." It is now clear that the response to interferon is much more complex (reviewed in reference 15 and see, for example, reference 17) and may involve effects not only on enzyme levels but also, for example, on lysosomal functions (4). The major conclusion which can be reached from the results presented here is that there must be at least one branch point in the induction pathway leading from occupied α-interferon receptors on the cell surface to the accumulation of interferon-inducible mRNAs. Such a branch could reflect functional heterogeneity among receptors which have similar high affinity for α-interferons. Alternatively, a single intermediate could trigger the formation of two or more discrete signals which activate different sets of genes. Differential effects on the processing or stability of the α-interferon-induced mRNAs may also be involved.

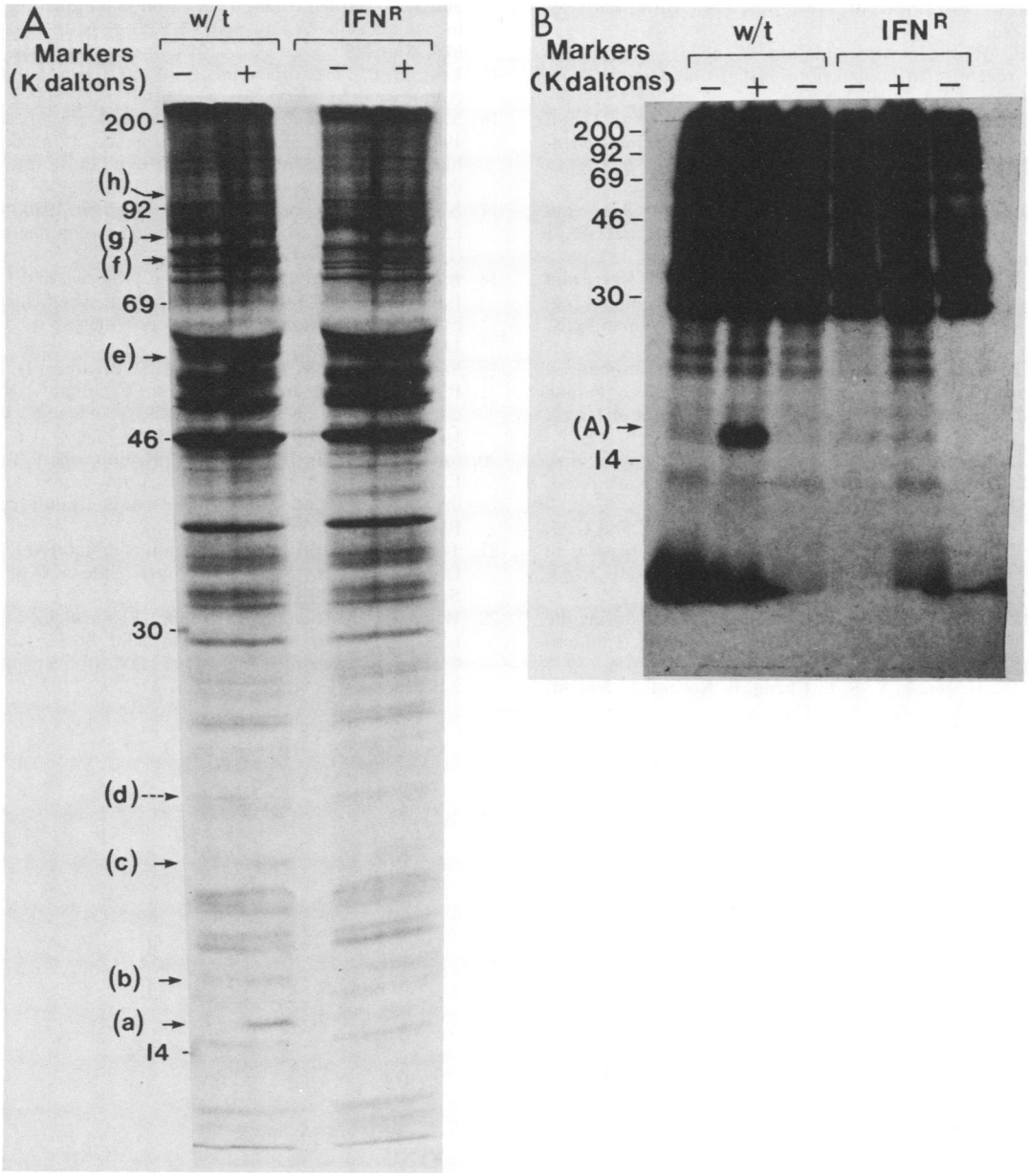

FIG. 3. Induction and repression of proteins by α-interferons. Cell extracts from wild-type (w/t) and resistant (IFN^R) Daudi cells which had been incubated with (+) or without (−) 200 units of α-interferons per ml for 9 h were analyzed by electrophoresis in sodium dodecyl sulfate–12.5% polyacrylamide gels (13). (A) Cells (5×10^6) were labeled in 0.5 ml of methionine-free medium with 100 μCi of [^{35}S]methionine (>1,000 Ci/mmol; Amersham International) for 90 min at 37°C. (B) Cells (2×10^7) were labeled with 500 μCi of sodium [^{125}I]iodide (100 mCi/ml; Amersham International) in 1.0 ml of phosphate-buffered saline with 20 μg of lactoperoxidase (Calbiochem-Behring, La Jolla, Calif.), 0.18% (wt/vol) glucose, and 150 U of glucose oxidase (Sigma Chemical Co., St. Louis, Mo.) per ml at room temperature for 10 min. Labeled cells were lysed in 0.5% Nonidet P-40, and a 13,000 × *g* supernatant solution was added to an equal volume of loading buffer, heated to 95°C for 5 min, and analyzed. Autoradiographs of the dried gels are shown. The molecular sizes (in kilodaltons) of marker polypeptides are indicated to the left of each gel. Proteins induced by α-interferons are labeled with solid arrows. The repressed protein is labeled with a broken arrow.

LITERATURE CITED

1. **Benech, P., C. Merlin, M. Revel, and J. Chebath.** 1985. 3′ end structure of the human (2′-5′)oligoA synthetase gene: prediction of two distinct proteins with cell type-specific expression. Nucleic Acids Res. **13**:1267–1281.
2. **Burrone, O. R., and C. Milstein.** 1982. Control of HLA-A, B, C synthesis and expression in interferon treated cells. EMBO J. **1**:345–349.
3. **Chebath, J., G. Merlin, R. Metz, P. Benech, and M. Revel.** 1983. Interferon-induced 56,000 Mr protein and its mRNA in human cells: molecular cloning and partial sequence of the cDNA. Nucleic Acids Res. **11**:1213–1226.
4. **Chelbi-Alix, M. K., and M. N. Thang.** 1985. Chloroquine impairs the interferon-induced antiviral state without affecting the 2′,5′-oligoadenylate synthetase. J. Biol. Chem. **260**:7960–7964.
5. **Chirgwin, J. M., A. E. Przbyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid

from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
6. **Colonno, R. J.** 1981. Accumulation of newly synthesized mRNAs in response to human fibroblast (β) interferon. Proc. Natl. Acad. Sci. USA **78**:4763–4766.
7. **Dron, M., M. G. Tovey, and P. Eid.** 1985. Isolation of Daudi cells with reduced sensitivity to interferon. III. Interferon-induced proteins in relation to the phenotype of interferon resistance. J. Gen. Virol. **66**:787–795.
8. **Einat, M., D. Resnitzky, and A. Kimchi.** 1985. Close link between reduction of c-*myc* expression by interferon and G0/G1 arrest. Nature (London) **313**:597–600.
9. **Friedman, R. L., S. P. Manly, M. McMahon, I. M. Kerr, and G. R. Stark.** 1984. Transcriptional and post-transcriptional regulation of interferon-induced gene expression in human cells. Cell **38**:745–755.
10. **Friedman, R. L., and G. R. Stark.** 1985. α-Interferon-induced transcription of HLA and metallothionein genes containing homologous upstream sequences. Nature (London) **314**: 637–639.
11. **Jonak, G. J., and E. Knight.** 1984. Selective reduction of c-*myc* mRNA in Daudi cells by human β interferon. Proc. Natl. Acad. Sci. USA **81**:1747–1750.
12. **Knight, E., E. D. Anton, D. Fahey, B. K. Friedland, and G. J. Jonak.** 1985. Interferon regulates c-*myc* gene expression in Daudi cells at the post-transcriptional level. Proc. Natl. Acad. Sci. USA **82**:1151–1154.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Larner, A. C., G. Jonak, Y.-S. E. Cheng, B. Korant, E. Knight, and J. E. Darnell.** 1984. Transcriptional induction of two genes in human cells by interferon-β. Proc. Natl. Acad. Sci. USA **81**:6733–6737.
15. **Lengyel, P.** 1982. Biochemistry of interferons and their actions. Annu. Rev. Biochem. **51**:251–282.
16. **Merlin, G., J. Chebath, P. Benech, R. Metz, M. and M. Revel.** 1983. Molecular cloning and sequence of partial cDNA for interferon-induced (2′-5′) oligo A synthetase mRNA from human cells. Proc. Natl. Acad. Sci. USA **80**:4904–4908.
17. **Nilsen, T. W., D. L. Wood, and C. Baglioni.** 1980. Virus-specific effects of interferon in embryonal carcinoma cells. Nature (London) **286**:178–180.
18. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
19. **Samanta, H., J. P. Dougherty, M. E. Brawner, H. Schmidt, and P. Lengyel.** 1982. Interferon action: cloning of cDNA segments complementary to messenger RNAs induced by interferons. UCLA Symp. Mol. Cell. Biol. **25**:59–72.
20. **Silverman, R. H., D. Watling, F. R. Balkwill, J. Trowsdale, and I. M. Kerr.** 1982. The ppp(A2′p)$_n$A and protein kinase systems in wild type and interferon-resistant Daudi cells. Eur. J. Biochem. **126**:333–341.
21. **Thomas, P. W.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocelulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
22. **Wodnar-Filipowicz, A., U. Gubler, Y. Furuichi, M. Richardson, E. F. Nowosiat, M. S. Poonian, and B. L. Horecker.** 1984. Cloning and sequence analysis of cDNA for rat spleen thymosin β4. Proc. Natl. Acad. Sci. USA **81**:2295–2297.