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Increased rate of degradation of c-myc mRNA in interferon-treated Daudi cells

(oncogene/HLA histocompatibility complex/post-transcriptional regulation)

CH. DANI, N. MECHTI, M. PIECHACZYK, B. LEBLEU*, PH. JEANTEUR, AND J. M. BLANCHARD

Equipe de Recherche Associée au Centre National de la Recherche Scientifique No. 482, Université des Sciences et Techniques du Languedoc, 34060 Montpellier, France; and Centre Paul Lamarque BP5054, 34033 Montpellier, France

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ABSTRACT **The recent observation made in our laboratory that cellular myc (c-myc) mRNA has a very short half-life in a variety of normal and transformed human cells emphasized the potential importance of post-transcriptional regulation of *c-myc* gene expression. Jonak and Knight [Jonak, G. J. & Knight, E., Jr. (1984) *Proc. Natl. Acad. Sci. USA* 81, 1747–1750] have reported a selective reduction of c-myc mRNA accumulation in lymphoblastoid Daudi cells treated with human β interferon. This provided a suitable situation in which to examine a possible action of negative modulators of *c-myc* expression at the level of mRNA stability. Our results confirm the observation by Jonak and Knight that c-myc mRNA level is depressed in cells treated with β interferon and extend it to $\alpha 2$ interferon. Furthermore, we now demonstrate that interferon has no effect on *c-myc* transcription rate in isolated nuclei but rather reduces the half-life of its mRNA. Conversely, we show that it increases the level of HLA-A2 mRNA by stimulating its transcription.**

The pathway that leads from a normal cell to a fully transformed one is controlled by at least two distinct classes of oncogenes acting in concert (for a review, see ref. 1). *c-myc*, the cellular homologue of the avian myelocytomatosis virus (MC29) oncogene, belongs to the establishment-immortalization class along with adenovirus *E1a* and the polyoma gene encoding the large tumor (T) antigen. To the second class, referred to as transforming genes, belong the *ras* family of oncogenes and the polyoma gene encoding middle T antigen.

The expression of the *c-myc* gene can be modulated in opposite directions. Enhanced expression of c-myc mRNA has been observed in a wide spectrum of neoplasms. It also can be induced experimentally early after mitogenic stimulation of lymphocytes by lipopolysaccharides or concanavalin A and of fibroblasts by platelet-derived-growth factor (2). Conversely, HL60, a human promyelocytic leukemia cell line, has been shown to respond by an early decrease in *myc* expression to agents that induce its granulocytic differentiation (3, 4). Along the same vein, Jonak and Knight (5) have reported a selective reduction of c-myc mRNA in Daudi cells treated with human β interferon (Hu-IFN-β).

Several models of *c-myc* regulation by *trans*-acting elements, possibly repressors, have been proposed (6–8). On the other hand, we have shown that c-myc mRNA is extremely unstable in both normal and transformed cells (9), suggesting that it might, at least for a large part, be post-transcriptionally regulated at the level of its degradation. Thus, it was tempting to assess the contribution of transcriptional and post-transcriptional events to the effects of positive and negative modulators of *c-myc* expression. We selected IFNs as representatives of this latter type of modulators. The present results show that the reduced level of steady-state c-myc mRNA in IFN-treated cells can be accounted for by an increased rate of its degradation. A preliminary account of this work has recently been published in abstract form (10).

MATERIALS AND METHODS

Daudi lymphoblastoid cells were grown in suspension in RPMI 1640 medium containing 10% fetal calf serum. Human recombinant IFNs, Hu-IFN-β at a specific activity of 5×10^7 international units/mg of protein and Hu-IFN-$\alpha 2$ at a specific activity of $>1.3 \times 10^8$ international units/mg of protein, were obtained from W. Fiers and L. Gauci, respectively. Cell growth rates were measured with a Coulter Counter.

Human c-myc cDNA clones were obtained from K. Marcu and D. Stehelin, and an HLA-A2 cDNA clone was from B. Jordan.

All techniques were as described previously (9).

RESULTS

Hu-IFN-$\alpha 2$ (11) and Hu-IFN-β (5, 11) are known to have a dramatic inhibitory effect on the growth of Daudi cells. Jonak and Knight (5) have shown that treatment with Hu-IFN results in a rapid decrease in c-myc mRNA accumulation. In order to confirm this observation and extend it to Hu-IFN-$\alpha 2$, poly(A)$^+$ RNAs were prepared from Daudi cells grown for various times in the absence or presence of different concentrations of Hu-IFN-$\alpha 2$ or Hu-IFN-β and analyzed by RNA blotting. Results obtained with cells treated for 16 hr with either interferon demonstrate a dose-dependent decrease (Fig. 1) in c-myc mRNA accumulation which occurs before any effect on cell growth can be detected (data not shown). It seems to be specific for c-myc mRNA since rehybridization of the same blots with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe did not show any variation in the levels of this mRNA. A 75–80% reduction in myc mRNA levels was reproducibly estimated by densitometry scanning of blot hybridizations from several experiments.

In order to determine the level(s) at which IFNs inhibit *c-myc* gene expression, we determined both its transcription rate in isolated nuclei and the stability of its messenger. *In vitro* transcripts were generated by elongation of previously initiated RNA chains during incubation of nuclei isolated from either normal or IFN-treated cells in the presence of [α-^{32}P]UTP as described by Schibler *et al.* (19). As pointed out by these authors, this assay provides a reasonable

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Abbreviations: *c-myc*, cellular *myc* gene; T antigen, tumor antigen; Hu-IFN-α and -β, human α and β interferons; GADPH, glyceraldehyde-3-phosphate dehydrogenase.

*To whom reprint requests should be addressed.

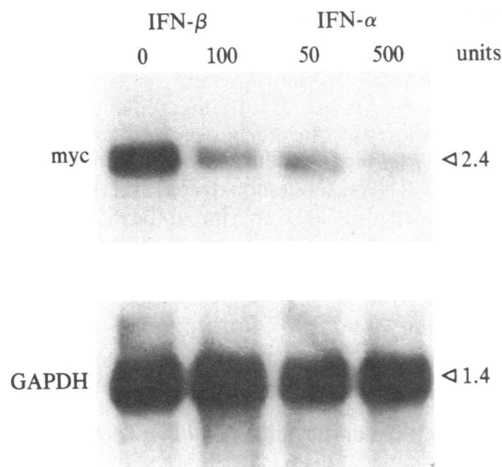

FIG. 1. RNA blot analysis of c-myc and GAPDH mRNAs in Daudi cells treated with Hu-IFN-α2 or Hu-IFN-β. Daudi cells were seeded at a starting density of 2×10^5 cells per ml and were grown for 16 hr in the presence of the indicated concentrations of Hu-IFN-α2 or -β. RNA was extracted from 7×10^7 cells as described by Auffray and Rougeon (12), and poly(A)$^+$ RNA was selected on oligo(dT)-cellulose (13), fractionated on formaldehyde-agarose gels, and transferred to nitrocellulose membranes (Schleicher & Schüll, BA85) (14). Filters were successively probed with nick-translated (15) human c-myc (pRYC7.4) (16) and GAPDH (pRGAPDH13) cDNAs (17). Hybridizations were carried out at 42°C for 24 hr in the presence of dextran sulfate (18). Sizes in kilobases were estimated with reference to ribosomal RNAs.

assessment of the number of polymerase molecules actively engaged in transcription at the time nuclei were prepared. This assumption is valid provided that *in vitro* elongation rates were comparable in all cases, which we have actually verified (not shown). These transcripts were then hybridized to dot-spots of plasmid DNA containing c-myc or GAPDH cDNA. Interference from possible transcripts of repetitive sequences, frequently found in introns, was prevented by the use of cDNA rather than genomic probes and by the presence of homologous genomic DNA in hybridization reactions. Under these conditions, the intensity of hybridization signals reflects the transcriptional activity of a given gene. A typical result, as shown in Fig. 2*a*, did not reveal any significant difference between c-*myc* transcription rates in control cells or cells treated for 16 hr with Hu-IFN-α2. The same result was obtained with Hu-IFN-β (not shown). In both cases, several experiments gave reproducible results. We excluded the possibility that the nuclear transcripts observed could originate from the noncoding strand, as established by the use of single-stranded probes. On the other hand, examination of the stoichiometry of hybridization signals obtained with probes covering different regions of the gene failed to reveal any premature termination related to IFN treatment (Fig. 2 *a* and *b*).

Since IFN did not affect c-*myc* or *GAPDH* in this system, it was important to assess the ability of our nuclear run-off assay to detect any modulation of gene transcriptional activity. Among the genes whose expression is enhanced by IFNs at both the protein and mRNA level are members of the major histocompatibility complex (20–25). As described in Fig. 2*c*, IFN increases HLA-A2 mRNA concentration in agreement with published data (23–25). This accumulation of HLA-A2 mRNA in IFN-treated Daudi cells results, at least in part, from an increase in its transcription rate (Fig. 2*d*). It is worth noticing, however, that this enhanced rate of transcription is transient and returned to pretreatment level by 24 hr.

The above data suggest that the observed reduction in c-myc mRNA levels upon IFN treatment cannot be accounted for by differences in transcription rates and, therefore,

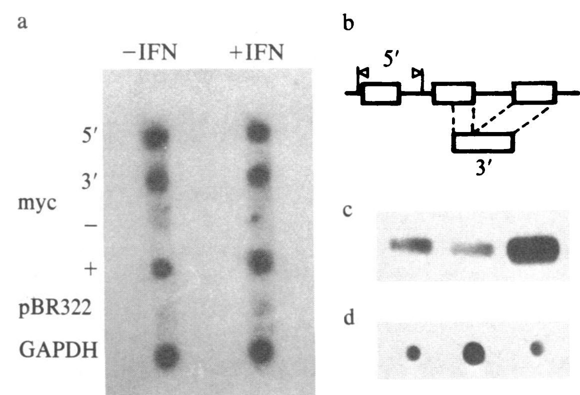

FIG. 2. Effects of Hu-IFN-α2 on the transcription of *GAPDH*, *HLA-A2* and c-*myc* genes in isolated nuclei. (*a*) Nuclei from 5×10^7 Daudi cells either untreated or treated for 16 hr with 100 units of Hu-IFN-α2 per ml were prepared and incubated for 10 min with 200 μCi (1 Ci = 37 GBq) of [α-^{32}P]UTP (Amersham, 400 Ci/mM) under the conditions described by Schibler *et al.* (19) for elongation of nascent RNA chains. Then 4×10^6 cpm of deproteinized RNA was hybridized to nitrocellulose filters carrying five dot-spots of 5 μg of alkali-denatured DNA from pBR322, pRGAPDH13 (Ph. Fort, personal communication), c-*myc* genomic DNA fragment (5′), and c-myc cDNA (pRYC7.4) (16) in either double (3′)- or single-stranded form [in the latter case, both coding (−) and noncoding (+) strands cloned in phage M13 were spotted]. Hybridization and washing conditions were as described (19). Autoradiographs were exposed for 72 hr. (*b*) Diagrammatic representation of the 5′ c-*myc* and 3′ c-*myc* probes used in *a*. The 5′-end probe is a 1.3-kilobase *Sac* I–*Sac* I genomic subfragment containing the first exon and part of the first intron. The 3′-end probe is a 1.2-kilobase cDNA clone containing half of the second exon and the entire third exon. (*c* and *d*) Control experiments showing the effect of Hu-IFN-α2 on *HLA-A2* expression. mRNA accumulation was analyzed by RNA blot as described in Fig. 1 with an HLA-A2 cDNA probe (*c*). Nuclear transcription was assayed as in *a* (*d*). For both *c* and *d* and from left to right, samples correspond respectively to 0, 2, and 20 hr of exposure to Hu-IFN-α2.

must reflect a post-transcriptional regulation. This led us to search for a possible reduction in c-myc mRNA half-life as a result of IFN treatment. We have shown previously that actinomycin D could be used to determine c-myc mRNA turnover without grossly affecting post-transcriptional steps leading to its degradation (9). Control cells or cells treated for 16 hr with Hu-IFN-α2 were exposed to high doses of actinomycin D to shut off all transcriptional activity. The survival of c-myc and GAPDH mRNAs in poly(A)$^+$ RNA from cells taken at various times during the actinomycin D chase was determined on RNA blots. A typical experiment is shown in Fig. 3 *Upper*. Densitometric scanning of several autoradiographs from the same blots after different exposure times were combined to generate the kinetic curve of Fig. 3 *Lower*. Hybridization signals obtained with a GAPDH cDNA probe were used as an internal standard not affected by IFN (see Fig. 1). Under these conditions, we observed a 67–80% reduction in c-myc mRNA half-life in IFN-treated cells as compared to control cells (Fig. 3 *Lower*).

DISCUSSION

The accelerated degradation of c-myc mRNA and the unaltered rate of transcription of c-*myc* gene that we report here are consistent with the observed 75–80% reduction in steady state c-myc mRNA levels after IFN treatment (5, 10). Similar data concerning the absence of transcriptional inhibition of c-*myc* in IFN-treated Daudi cells have been reported by G. Jonak and E. Knight at the last Netherland Organization for Applied Scientific Research (TNO) Meeting in Interferon Research.

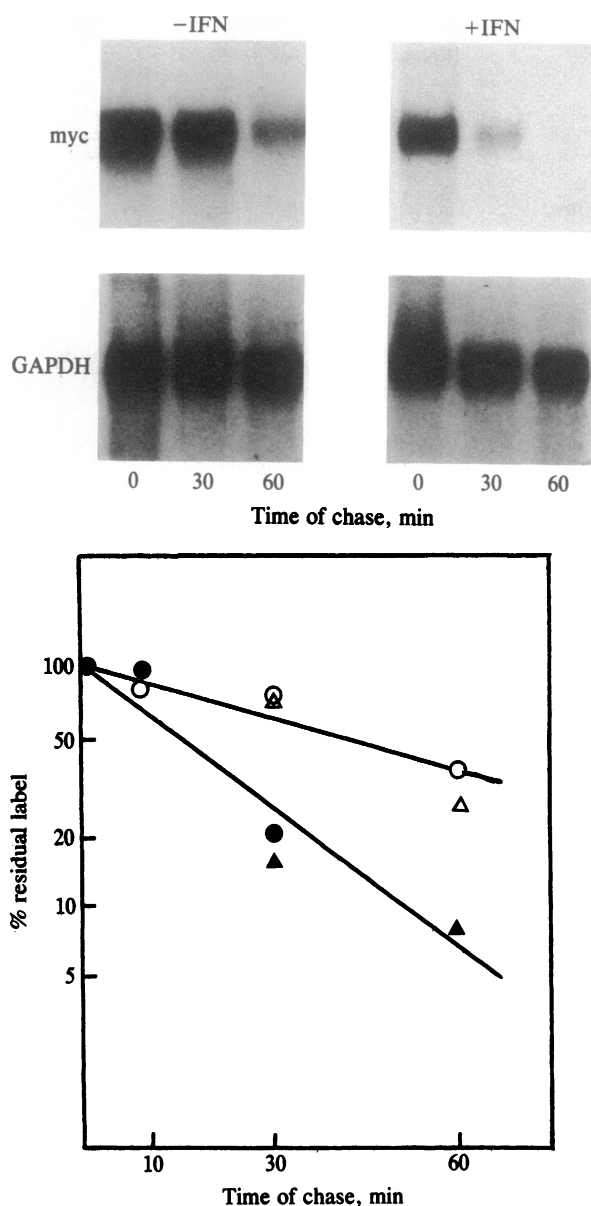

FIG. 3. Actinomycin D chase of c-myc and GAPDH mRNAs from Daudi cells grown in the absence or presence of Hu-IFN-α2. (*Upper*) RNA blot analysis. Cells were treated (+IFN) or not (−IFN) for 16 hr with 100 units of Hu-IFN-α2 per ml as described in Fig. 1 and were incubated further with 5 μg of actinomycin D per ml for the indicated times. For each time point, 10 μg of poly(A)$^+$ RNA was prepared and analyzed as in Fig. 1. (*Lower*) Half-life determination of c-myc mRNA. Autoradiographs of two independent RNA blots (triangles and circles) were quantitated by densitometric scanning and normalized with respect to GAPDH mRNA to correct for small variations in the amounts of input RNA. ○ and △, Control cells; ● and ▲, IFN-treated cells.

As illustrated here for *HLA-A2*, IFNs have until now been mainly recognized as inducers of gene activity. The level of mRNAs for several cellular genes is indeed increased by IFNs among which are (2′-5′)adenylyladenosine oligonucleotide synthetases [(2′-5′)A synthetases, called the 2-5A system] (26, 27), histocompatibility antigens (23–25), and a number of proteins of unknown functions (28–33). Although this was not the main purpose of these studies, it is worth noting that IFN increases rapidly but transiently the transcriptional activity of *HLA-A2*, whereas its mRNA accumulates over longer periods of time, in agreement with published data (23–25). This can only be explained by postulating a contribution of post-transcriptional events to the regulation of HLA-A2 mRNA accumulation. Similar observations have been made and similar conclusions have been reached recently by several groups working on the regulation of IFN-induced proteins (32, 33). In infected cells, on the other hand, IFNs increase the turnover rate of several viral mRNAs, while leaving unchanged the stability of bulk cellular poly(A)$^+$ RNAs, as demonstrated in reovirus-infected cells, for instance (34, 35). Experimental evidence is consistent in these cases with an involvement of the (2′-5′)A synthetase–RNase L pathway (see ref. 36 for a review) after its localized activation by double-stranded intermediates of virus replication (see ref. 37 for a review). Would double-stranded RNA structures, which could possibly exist in c-myc mRNA (38), be capable of activating this pathway in IFN-treated cells, thus leading to a preferential degradation of c-myc mRNAs over the bulk of cellular mRNAs? Although this degradation mechanism is certainly worth considering in the light of the above data, more specific pathways involving Myc protein, for example, should be considered as well.

In any case, a more general understanding of c-*myc* regulation calls for further knowledge along two lines. (*i*) Is alteration of c-myc mRNA stability a general way of turning off and on *myc* expression? Along this line, it is important to ask whether positive modulators (for example, growth factors) would induce a stabilization of c-myc mRNA. (*ii*) Is it possible to identify target sequences in c-myc mRNA that specifically would designate it to the degradation machinery?

Similar data reporting the absence of any significant modulation of the transcription rate of c-*myc* gene by IFN have been obtained recently (39).

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