MOLECULAR AND CELLULAR BIOLOGY, June 1996, p. 2814–2822 Vol. 16, No. 6
0270-7306/96/$04.00+0

2-Aminopurine Selectively Inhibits Splicing of Tumor Necrosis Factor Alpha mRNA

NAYEF JARROUS, FARHAT OSMAN, AND RAYMOND KAEMPFER*

Department of Molecular Virology, The Hebrew University-Hadassah Medical School, 91120 Jerusalem, Israel

Received 1 February 1996/Returned for modification 20 February 1996/Accepted 11 March 1996

2-Aminopurine (2-AP) inhibits specific kinases that phosphorylate the α subunit of eukaryotic translation initiation factor 2. One of these, PKR, is also involved in signal transduction. We show here that 2-AP selectively inhibits expression of tumor necrosis factor alpha (TNF-α) mRNA in primary human lymphoid cells. 2-AP does not inhibit transcription of the human TNF-α gene, nor does it affect mRNA stability. Instead, the flow of short-lived precursor transcripts into mature TNF-α mRNA is blocked. When 2-AP is present during induction, unspliced TNF-α precursor transcripts accumulate at the expense of mRNA. Using RNase protection analysis with genomic probes for different exon-intron junctions, we show that 2-AP blocks splicing of TNF-α mRNA. Neither the TNF-β nor the interleukin-1β gene shows such regulation. 2-AP also inhibits splicing of precursor RNA transcribed from an exogenous human TNF-α gene. Sequences within this gene thus confer sensitivity to 2-AP. Yet, control is not exerted at a specific splice site. Our results reveal the involvement of a 2-AP-sensitive component, expressed in functional form before induction, in the splicing of TNF-α mRNA.

2-Aminopurine (2-AP), an isomer of adenine, is an inhibitor of the ATP-dependent phosphorylation of the α subunit of eukaryotic initiation factor 2 (eIF-2α) by two specific kinases, one controlled by heme (12, 21) and the other, PKR, activated by double-stranded RNA (dsRNA) (8, 12). Phosphorylation of eIF-2α results in inhibition of the initiation of translation (26, 41). 2-AP also inhibits the autophosphorylation of PKR necessary for its activation (53). PKR is expressed constitutively at low levels in most cell types (2, 28) but is induced by viruses, dsRNA, and interferon (IFN). 2-AP prevents the establishment of the antiviral state in IFN-treated cells (56). To allow viral protein synthesis, many viruses depend critically on strategies to inhibit the function of PKR or its activation by viral dsRNA. Thus, the vaccinia virus E3L gene product interferes with the binding of PKR to dsRNA, while its K3L gene product exhibits homology to eIF-2α and acts as a decoy substrate (7).

In addition to its role in translational regulation, PKR functions in signal transduction (23, 27, 42, 44). This kinase is a transducer for the activation of transcription factor NF-κB signaling by dsRNA but not by tumor necrosis factor alpha (TNF-α) (34, 39). 2-AP inhibits transcription of murine TNF-α (24), IFN-α, and IFN-β genes (6); inhibition of murine IFN-β gene induction by 2-AP is cell type specific (6). Moreover, 2-AP inhibits induction of human TNF-α and IFN-β mRNA in monocyte and B-cell lines (18) and expression of the murine c-*myc* and c-*fos* genes (59). 2-AP prevents induction of gene expression by phorbol myristate acetate (PMA) or epidermal growth factor without affecting activation of protein kinase C (37). By blocking induction of mRNA encoding the 48-kDa DNA binding component of IFN-stimulated gene factor 3 (1), 2-AP prevents transcriptional activation of human IFN-induced genes (54, 56).

TNF-α plays an essential role in inflammatory, antitumor, and antiviral responses (3, 14). TNF-α shares all of its biological activities with TNF-β and some of them with interleukin-1β (IL-1β) (35, 49). Human TNF-α and TNF-β genes show structural and functional homology (46, 49); they are arranged in tandem within the major histocompatibility gene cluster and transcribed in the same direction (46, 50). TNF-α is expressed in activated monocytes and T cells, while TNF-β, like IL-2, which regulates the clonal expansion of T cells, is a T-cell product (13). All-*trans*-retinoic acid, lipopolysaccharide (LPS), PMA, and inhibitors of translation induce the coordinate expression of human TNF-α and IL-1β genes (29).

Recently, it was shown that cytokine gene expression is regulated when nuclear processing of precursor transcripts occurs. The induction of human IL-2 gene expression elicits a transient wave of unstable mRNA (10, 11, 15). Transcription continues unabated well beyond the time when the wave has subsided but does not yield more mRNA. Instead, IL-2 precursor transcripts accumulate. The flow of precursor transcripts into mature mRNA becomes inhibited in the course of induction. When translation is blocked by cycloheximide (CHX) or emetine, expression of IL-2 mRNA can be superinduced by 2 orders of magnitude, without a significant increase in the level of primary transcription or mRNA stability. Instead, splicing of nuclear IL-2 precursor transcripts is facilitated (15). Induction of the IL-1β gene by the physiological inducer all-*trans*-retinoic acid is similarly regulated, processing of precursor transcripts being the dominant element of control in induced cells (29).

In this report, we show that 2-AP does not inhibit transcription of the human TNF-α gene in primary human lymphoid cells. Instead, the flow of unspliced precursor transcripts into mature TNF-α mRNA is blocked. Neither the TNF-β gene nor the IL-1β gene shows such regulation. Our results reveal the involvement of a 2-AP-sensitive component in the splicing of human TNF-α mRNA.

These observations support the concept that precursor mRNA splicing constitutes, more widely than hitherto thought, an essential step for regulating cytokine mRNA expression. Only after mRNA has been generated can the control of its stability begin to serve as a secondary target for posttranscriptional regulation.

* Corresponding author. Mailing address: Department of Molecular Virology, The Hebrew University-Hadassah Medical School, 91120 Jerusalem, Israel. Phone: (972) 275-8389. Fax: (972) 278-4010. Electronic mail address: kaempfer@cc.huji.ac.il.

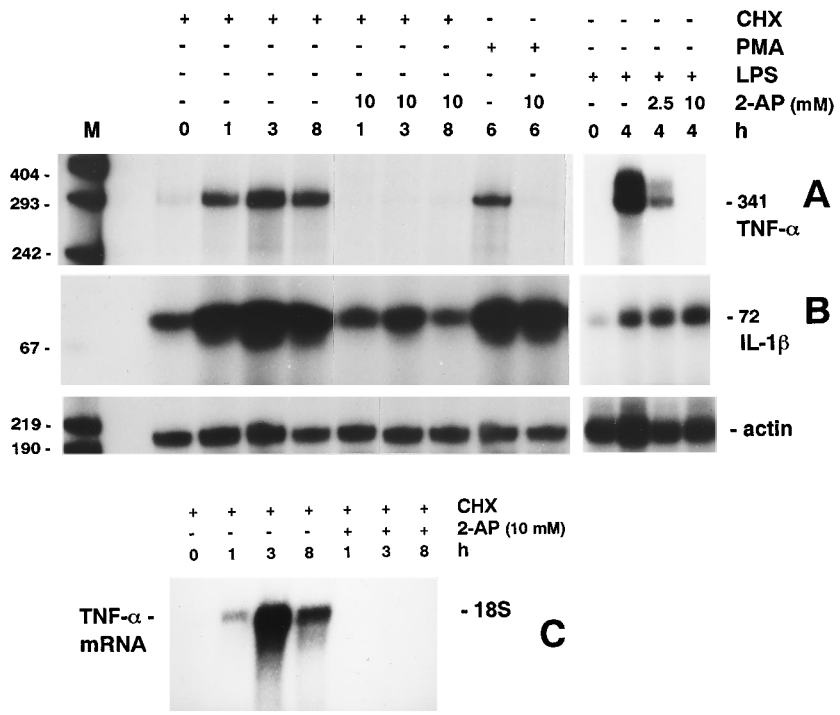

FIG. 1. Inhibition of human TNF-α mRNA induction by 2-AP. PBMC were induced with CHX, PMA, or LPS as indicated. 2-AP was included from the onset of induction at the concentrations shown. Cell viability remained constant. At the times shown, total RNA was isolated, subjected to agarose-formaldehyde gel electrophoresis, and blot hybridized with a ^{32}P-labeled TNF-α antisense RNA probe covering a portion of the 3′ untranslated region (C), or it was subjected to RNase protection analysis with antisense RNA probes complementary to a 341-nt portion of TNF-α exon 4 (A) (for a map, see Fig. 4), a 72-nt portion of IL-1β exon 1 (B) (for a map, see Fig. 2), or a 215-nt portion of α-actin mRNA. Marker (M) lanes show end-labeled pGEM-3 DNA digested with *Msp*I.

MATERIALS AND METHODS

Materials. 2-AP, PMA, LPS, CHX, cordycepin triphosphate, staurosporine (STS), H7 [1-(5-isoquinolinylsulfonyl)-2-methylpiperazine], and W7 [*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide] were from Sigma.

Cell culture and induction. Peripheral blood mononuclear cells (PBMC) were separated from buffy coats from healthy donors, washed, resuspended at 4×10^6 cells per ml, and preincubated overnight at 37°C in RPMI 1640 medium (29). BHK-21 cells were grown in Dulbecco modified Eagle medium supplemented with 2 mM glutamine, 40 mM $NaHCO_3$, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.3), 100 μg each of penicillin and streptomycin per ml, 10 μg of nystatin per ml, and 10% fetal calf serum. HL-60 cells were grown in RPMI 1640 medium containing the above supplements except $NaHCO_3$. PMA was used at 10 ng/ml, and LPS was used at 15 μg/ml. Unless otherwise indicated, 2-AP was present at 10 mM. To prepare a stock solution of 0.15 M, 2-AP was dissolved in phosphate-buffered saline by heating to 70°C for 10 min. Where indicated in Fig. 1 and 5 through 7, CHX was added to a final concentration of 20 μg/ml and actinomycin D was added to 4 μg/ml.

Plasmids for transfection. phTNF-α, which contains the entire human TNF-α gene, including the upstream regulatory sequences (17), in pUC13pML, was obtained from Arjun Singh (Genentech, South San Francisco, Calif.) and used for transfection. pSV_2CAT containing the bacterial chloramphenicol acetyltransferase (CAT) gene (20) was used to monitor transfection efficiency.

Cell transfection. Monolayers of BHK-21 cells were seeded at a density of 1×10^6 to 2×10^6 cells per 8-cm-diameter petri dish and grown overnight; 4 h before cotransfection, the culture medium was replaced. A mixture of 10 μg of phTNF-α DNA, 2 μg of pSV_2CAT DNA, and 4 μg of salmon sperm DNA carrier was allowed to permeate cells by the calcium phosphate-DNA coprecipitation technique. After 16 h, the culture medium was replaced with fresh medium prewarmed to 37°C.

Plasmids and hybridization probes. For RNase protection analysis, DNA was subcloned in pBS (Stratagene) under the control of the T3 or T7 promoter and transcribed with [α-^{32}P]UTP to generate labeled antisense RNA transcripts. A TNF-α RNA probe (see Fig. 2) was generated from a 259-bp *Ava*I-*Ava*I fragment consisting of adjoining segments of exon 3 and intron 3 (29). A second TNF-α RNA probe (see Fig. 4) was generated from a 700-bp *Sau*3AI-*Sau*3AI fragment consisting of 10 bp of intron 2, exon 3, intron 3, and part of exon 4. A third TNF-α RNA probe (see Fig. 6C) was generated from an 821-bp *Eco*RI-*Sau*3AI fragment containing the 5′ flank and 169 bp of the 5′ untranslated region. A TNF-β RNA probe (see Fig. 2) was generated from a 324-bp *Eco*RI-*Bsu*36I DNA fragment containing part of exon 1, exon 2, exon 3, and a portion of intron 3 that was derived from pSV-LT, obtained from Rao Movva (Biogen S.A., Geneva, Switzerland). A human IL-1β genomic clone in λEMBL3 phage was from Alan R. Shaw. An IL-1β RNA probe (see Fig. 1 and 2) was generated from a 516-bp *Hin*dIII-*Xba*I fragment consisting of a 72-bp segment of exon 1 adjoining part of intron 1; a second IL-1β RNA probe (see Fig. 6D) was generated from a 267-bp *Nco*I-*Pst*I fragment consisting of adjoining segments of exon 2 and intron 2 (29). A probe of 250 nucleotides (nt), generated from a *Hin*dIII-*Eco*RI fragment of the coding region of the CAT gene (20), was used to quantitate CAT mRNA in cotransfection experiments. The intact size of each RNA probe was assessed by polyacrylamide gel electrophoresis.

For RNA blot hybridization, an antisense RNA probe was generated from a human TNF-α cDNA fragment covering a portion of the 3′ untranslated region, subcloned under the phage T7 promoter of pGEM-3 (Promega Biotec) (29); the DNA was derived from p-hTNF5 obtained from Jan Tavernier (Roche Research, Ghent, Belgium).

RNase protection analysis. Total RNA was isolated with guanidinium isothiocyanate-CsCl. RNase protection analysis with RNases A and T_1 was performed with genomic riboprobes as indicated for each figure. Protected RNA fragments were separated by electrophoresis in 5% polyacrylamide–8 M urea gels. Size markers were generated by *Msp*I digestion of pGEM-3 DNA and by filling in with [α-^{32}P]dCTP (29), except for the experiment shown in Fig. 8A, for which digestion was with *Hin*fI, and filling in was with [α-^{32}P]dATP.

RESULTS

Inhibition of TNF-α mRNA expression by 2-AP. Human PBMC were stimulated with the translation inhibitor CHX, PMA, or LPS, and inductions of TNF-α and IL-1β mRNA were analyzed by RNase protection analysis. In Fig. 1A, a 341-nt fragment of the genomic antisense RNA probe is protected by exon 4 of TNF-α mRNA. CHX elicited a rapid accumulation of TNF-α mRNA that reached a maximum within 3 h and then declined. CHX-mediated induction of TNF-α mRNA was inhibited completely by 10 mM 2-AP. Likewise, 10 mM 2-AP blocked the induction of TNF-α mRNA by PMA or LPS (Fig. 1A). Even at 2.5 mM, 2-AP severely inhibited induction of TNF-α mRNA. Although the kinetics of the CHX-

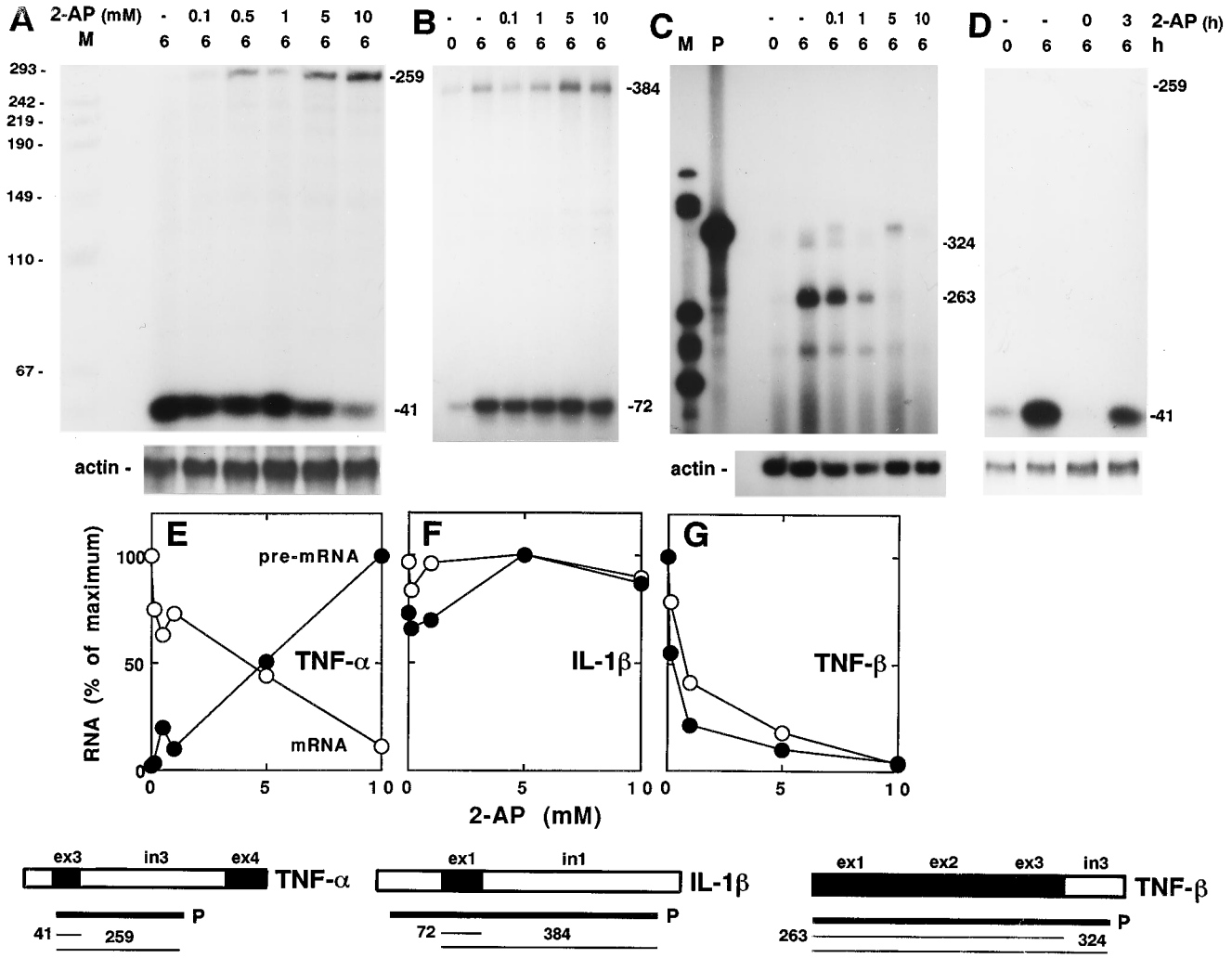

FIG. 2. 2-AP selectively elicits accumulation of unspliced TNF-α precursor RNA. PBMC were induced with PMA for 6 h in the presence of increasing concentrations of 2-AP as indicated. Cell viability remained constant. Total RNA was isolated and subjected to RNase protection analysis with ^{32}P-labeled antisense RNA probes (maps) complementary to adjacent portions of an exon and an intron of the TNF-α (A, D, and E), IL-1β (B and F), or TNF-β gene (C and G). For panels A to C, mRNA protects 41, 72, and 263 nt, respectively, while unspliced precursor transcripts protect 259, 384, and 324 nt, respectively, in these probes (P). Amounts of precursor transcripts (●) and mRNA (○) in panels A to C were quantitated by microdensitometry and are plotted in panels E to G, respectively. (D) Cytoplasmic RNA was isolated and analyzed as described for panel A. Panels A, B and C, and D represent three different experiments; α-actin RNA controls are shown for each. M, size marker.

mediated induction of IL-1β mRNA closely resembled those for TNF-α mRNA (Fig. 1A and B), 2-AP caused only a partial reduction in CHX-induced expression of IL-1β mRNA. Indeed, 2-AP failed to inhibit IL-1β mRNA expression induced by PMA or LPS (Fig. 1B). CHX-mediated induction of mature TNF-α mRNA, analyzed by Northern (RNA) blotting (Fig. 1C), was prevented equally by 2-AP. These results show that 2-AP inhibits the expression of TNF-α mRNA elicited by different inducers yet has little, if any, effect on the expression of IL-1β mRNA.

2-AP selectively elicits accumulation of TNF-α precursor transcripts. In Fig. 2A, PMA-induced expression of TNF-α precursor transcripts and spliced mRNA was monitored by RNase protection analysis, using an antisense RNA probe covering adjacent portions of exon 3 and intron 3. A 41-nt fragment is protected by mRNA, and the entire 259-nt probe is protected by unspliced precursor transcripts (29). Increasing concentrations of 2-AP caused a progressive decrease in the level of expression of TNF-α mRNA at 6 h (the time of maximal expression), which was matched by a pronounced increase in the level of precursor transcripts (Fig. 2A and E). Both effects were maximal with 10 mM 2-AP; higher concentrations had no additional effect (30). The reciprocal shifts in the expression of TNF-α mRNA and precursor transcripts observed in the presence of 2-AP, with half-maximal expression of each RNA species occurring at the same 2-AP concentration (Fig. 2E), demonstrate that the inhibition of mRNA expression does not result from a lack of precursor RNA.

The induction of IL-1β mRNA, by contrast, was insensitive to 2-AP (Fig. 2B). IL-1β precursor transcripts protect a 384-nt RNA fragment consisting of exon 1 and an adjacent segment from intron 1, while mRNA protects 72 nt of exon 1 (29). The level of IL-1β mRNA remained constant throughout the range of 2-AP concentrations tested. With 10 mM 2-AP, the level of IL-1β precursor transcripts increased 1.4-fold (Fig. 2F), an insignificant effect when compared with the 60-fold increase in

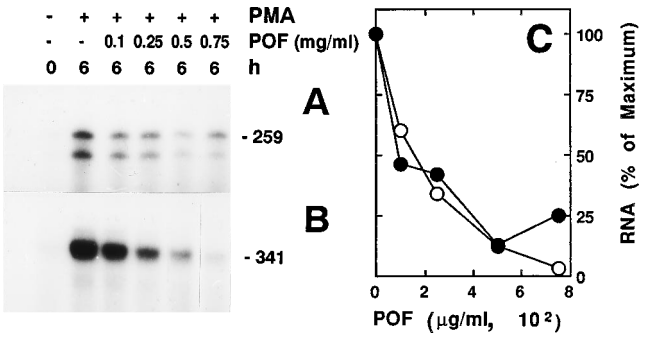

FIG. 3. Effect of pentoxifylline on human TNF-α RNA expression. PBMC were induced with PMA for 6 h in the presence of pentoxifylline (POF) at the indicated concentrations. Total RNA was isolated and subjected to RNase protection analysis with ^{32}P-labeled antisense RNA probes complementary to adjacent portions of an exon and an intron of the TNF-α gene as performed for the experiment shown in Fig. 2A to quantitate precursor transcripts (A) or as performed for the experiment shown in Fig. 4 to quantitate mRNA (B). Amounts of precursor transcripts in panel A (●) and mRNA in panel B (○) were quantitated by microdensitometry and are plotted in panel C.

the level of TNF-α precursor RNA observed in the experiment shown in Fig. 2E. Similar results were obtained with an IL-1β genomic antisense RNA probe covering adjacent portions of exon 2 and intron 2 (30).

Expression of the TNF-β gene was examined in the same experiment. In Fig. 2C, TNF-β precursor transcripts protect a 324-nt RNA fragment consisting of part of exon 1, exon 2, exon 3, and a portion of intron 3, while mRNA protects 263 nt of exons 1 to 3. In contrast to the reciprocal changes in levels of TNF-α precursor transcripts and mRNA (Fig. 2A) and the unabated expression of IL-1β precursor transcripts and mRNA (Fig. 2B), levels of TNF-β precursor transcripts and spliced mRNA declined in parallel, indicating that here, inhibition of mRNA expression by 2-AP resulted from a lack of precursor transcript synthesis (Fig. 2C and G). 2-AP similarly inhibited the induction of IL-2 precursor RNA (16). In the absence of an inducer, 2-AP had no effect on the basal level of expression of the TNF-α, IL-1β, or TNF-β gene (30).

These results were reproducible. They strongly suggest that the inhibition of TNF-α mRNA expression by 2-AP and the concomitant, sharp rise in the level of precursor transcripts result from a failure of precursors to be spliced into mRNA. The inhibition is selective for the TNF-α gene.

Inhibition of splicing by 2-AP is not narrowly localized within TNF-α precursor RNA molecules. Results similar to those of the experiments shown in Fig. 2A and E were obtained with another genomic probe (for a map, see Fig. 4) that detects splicing at the intron 2-exon 3, exon 3-intron 3, and intron 3-exon 4 junctions in TNF-α precursor transcripts (30).

As shown for murine TNF-β RNA (47, 57), RNA species that retain an intron may be transported into the cytoplasm and even translated. In Fig. 2D, abundant amounts of spliced TNF-α mRNA appeared in the cytoplasm within 6 h of induction by PMA. This accumulation was prevented completely when 10 mM 2-AP was added at the onset of induction and was reduced when 2-AP was added later, at 3 h. Even though this concentration of 2-AP induced a massive accumulation of precursor transcripts in total cellular RNA (Fig. 2A), precursors could not be detected in the cytoplasm (Fig. 2D). Thus, when 2-AP inhibits induction of TNF-α mRNA, unspliced precursor transcripts are not exported into the cytoplasm, as would be expected during a splicing block.

Pentoxifylline inhibits expression of TNF-α precursor transcripts. Pentoxifylline, a methylxanthine, was suggested to inhibit the processing of murine TNF-α precursor RNA into mRNA (24). It was thus of interest to compare the effects of pentoxifylline and 2-AP for the human TNF-α gene. However, the presence of increasing concentrations of pentoxifylline, in the range used to study murine TNF-α gene expression (24), induced a parallel decline in the levels of human TNF-α precursor transcripts (Fig. 3A) and mRNA (Fig. 3B), indicating an inhibition of synthesis of precursor RNA rather than an effect on their splicing (cf. Fig. 2E and 3C).

2-AP inhibits processing of TNF-α precursor transcripts in transfected cells. To examine if processing of precursor RNA transcribed from an exogenous TNF-α gene is sensitive to 2-AP, the human TNF-α gene was transiently expressed by transfection into BHK-21 cells (Fig. 4). Endogenous hamster TNF-α mRNA could not be detected with the probes used (30). We quantitated the transient expression of human TNF-α precursor transcripts (the 259-nt band in Fig. 4A and the 700-nt band in Fig. 4B) and spliced mRNA (the 341-nt band in Fig. 4C). In the absence of 2-AP, precursors were abundantly expressed at 18 h after transfection but declined to low levels by 30 h (Fig. 4A and B). These precursor transcripts were processed, resulting in an accumulation of mRNA that was maximal by 24 h (Fig. 4C). The addition of 2-AP at 16 h after transfection led, however, to a greatly enhanced accumulation of precursor transcripts within 2 h and to their sustained ex-

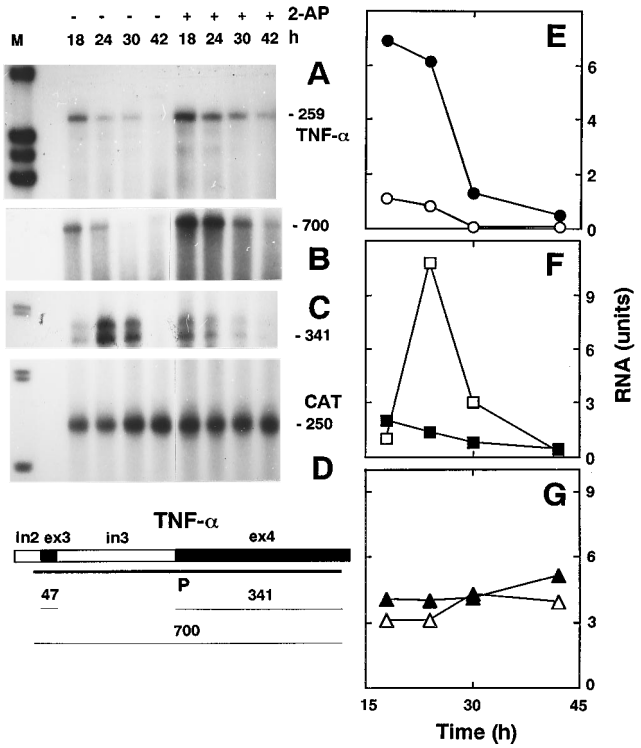

FIG. 4. Effects of 2-AP on splicing of precursor RNA transcribed from a transfected TNF-α gene. BHK-21 cells were cotransfected with phTNF-α and pSV$_2$CAT DNA. 2-AP was added at 16 h after transfection. Cell viability remained constant. Total RNA was isolated at the times shown after transfection and subjected to RNase protection analysis with a ^{32}P-labeled antisense RNA probe as performed for the experiment shown in Fig. 2A to quantitate TNF-α precursor transcripts (A) or as shown in the map to quantitate TNF-α precursor transcripts (the 700-nt protected fragment) (B and E) and mRNA (the 341-nt protected fragment) (C and F). CAT mRNA protects 250 nt complementary to the coding region (D and G). The intensities of the indicated bands in panels B to D, expressed in the absence (○, □, and △) or presence (●, ■, and ▲) of 2-AP, were quantitated by microdensitometry and plotted in panels E to G, respectively. M, size marker; in, intron; ex, exon.

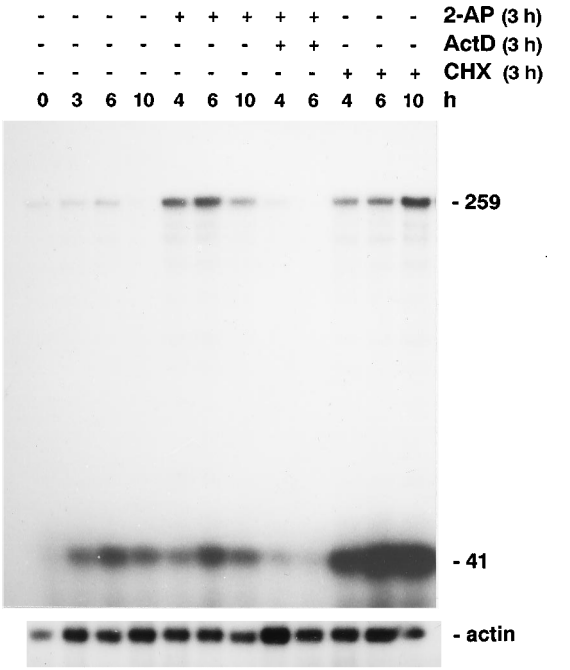

FIG. 5. Effects of 2-AP and CHX on splicing of unstable TNF-α precursor transcripts. PBMC were induced with PMA. 2-AP, CHX, or actinomycin D was added at 3 h where shown. Cell viability remained constant. Total RNA was isolated at the times indicated and subjected to RNase protection analysis to quantitate TNF-α precursor transcripts (the 259-nt band) and mRNA (the 41-nt band) as performed for the experiment shown in Fig. 2A.

pression (Fig. 4A and B). Expression of spliced TNF-α mRNA, by contrast, was strongly inhibited at 24 h and beyond (Fig. 4C). In the presence of 2-AP levels of precursor transcripts had increased by 7.6-fold at 24 h, relative to that of the untreated control (Fig. 4E), while levels of mRNA had decreased by 7.7-fold (Fig. 4F). These representative kinetic data demonstrate a significant decline in the flow of precursors into TNF-α mRNA. They show that the accumulation of precursor transcripts occurs promptly and in the same cells in which the accumulation of TNF-α mRNA is inhibited. In agreement with an earlier report (32), expression of mRNA from a cotransfected CAT gene was not noticeably affected by 2-AP (Fig. 4D and G), showing that efficiency of transfection was constant. The 2-AP-sensitive mechanism that supports splicing of TNF-α precursor RNA in PBMC thus is also present in BHK-21 cells.

2-AP elicits accumulation of unstable, functional TNF-α precursor transcripts. The accumulation of unspliced TNF-α precursor transcripts occurred when 2-AP was present from the outset of induction (Fig. 2A). In the experiment shown in Fig. 5, 2-AP was introduced later, at 3 h, a time at which precursor levels were still rising. Within 1 h, an extensive increase in the level of precursor transcripts was observed. Their level became maximal within 3 h before declining gradually, with large amounts of precursors still present 4 h later (Fig. 5). However, this increase in the level of precursor transcripts was not matched by a decrease in the level of TNF-α mRNA. Indeed, when added after 3 h of induction, 2-AP failed to affect the subsequent expression of mRNA perceptibly, including the rise between 4 and 6 h, showing that splicing was no longer blocked by that time and that the presence of 2-AP did not affect TNF-α mRNA stability. Because mRNA could still be generated, the 2-AP-induced rise in the precursor transcript level apparently involves functional TNF-α RNA molecules rather than abnormal ones that cannot be processed. In this experiment, expression of IL-1β RNA was insensitive to 2-AP (30).

The increase in the level of precursors was blocked when actinomycin D was added together with 2-AP; instead, the level of precursors declined completely within 3 h (Fig. 5). Hence, the accumulation of precursors, induced by 2-AP, results from the synthesis of short-lived precursor transcripts and not from the stabilization of preexisting ones. The concomitant decline in the level of TNF-α mRNA shows that it likewise remained unstable (29) when 2-AP was present.

An increase in the level of precursor transcripts could be observed also upon the addition of CHX at 3 h, becoming particularly pronounced by 10 h. In this case, however, the rise in the level of precursor RNA resulted in an immediate superinduction of TNF-α mRNA (Fig. 5). Upon the addition of 2-AP, by contrast, mRNA levels failed to increase, even though by 4 and 6 h, precursor transcripts exceeded the levels seen in the presence of CHX. Thus, short-lived TNF-α precursor transcripts are processed smoothly into mRNA in the presence of CHX but not when 2-AP is present.

Effects of other protein kinase inhibitors on TNF-α precursor RNA expression. Protein kinases and phosphatases regulate TNF-α gene activity. TNF-α gene expression is blocked by tyrosine kinase inhibitors (48) and stimulated by inhibitors of phosphatases 1 and 2A (51). Considering the ability of 2-AP to inhibit splicing of TNF-α mRNA from precursor transcripts, we examined the effects of H7 and STS, inhibitors of protein kinases C and A, on PMA-induced TNF-α RNA expression (Fig. 6A and B). H7 and STS each blocked PMA-induced mRNA expression by inhibiting the synthesis of precursor RNA, resembling actinomycin D in their effects. H7 and STS thus inhibit TNF-α gene transcription.

Cordycepin, an inhibitor of polyadenylation (43), affected neither formation nor splicing of precursors into TNF-α mRNA (Fig. 6A and B).

2-AP inhibits CHX-mediated superinduction of TNF-α mRNA. The results of the experiment shown in Fig. 5 raised the question of whether 2-AP is able to block the superinduction of TNF-α mRNA mediated by CHX (29). In Fig. 6C, accurately initiated precursor transcripts and mRNA (46) protect 169 nt in a 5′ RNA probe used to map and quantitate TNF-α RNA. Addition of CHX to PMA-induced cells at 6 h resulted in a marked superinduction of TNF-α RNA, most of which is mRNA (29). This superinduction was prevented almost totally by 8 mM 2-AP, added 3 h earlier (Fig. 6C). By contrast, 2-AP had no effect on superinduction of IL-1β mRNA (Fig. 6D). Thus, 2-AP selectively inhibits both induction of TNF-α mRNA and its superinduction by CHX.

H7, STS, and W7, a specific inhibitor of calcium-calmodulin-dependent protein kinase, eliminated expression of TNF-α RNA even when superinduced by CHX (Fig. 6C). This effect was not selective, because IL-1β precursor transcripts were not detected in the presence of these three agents and mRNA levels were strongly reduced (Fig. 6D). Clearly, 2-AP inhibits TNF-α gene expression through a mechanism distinct from that used by protein kinase inhibitors of transcription.

Inhibition of TNF-α gene transcription by 2-AP in HL-60 cells. Previous studies with cell lines showed that 2-AP inhibits transcription of a number of cytokine genes and proto-oncogenes (18, 56, 59). This raised the question of whether the posttranscriptional inhibition of TNF-α gene expression by 2-AP, seen in primary human lymphoid cells (Fig. 1 and 2) and transfected BHK-21 cells (Fig. 4), also operates in a human cell line capable of expressing its endogenous TNF-α gene.

Exposure of HL-60 cells, a promyelocytic human cell line, to

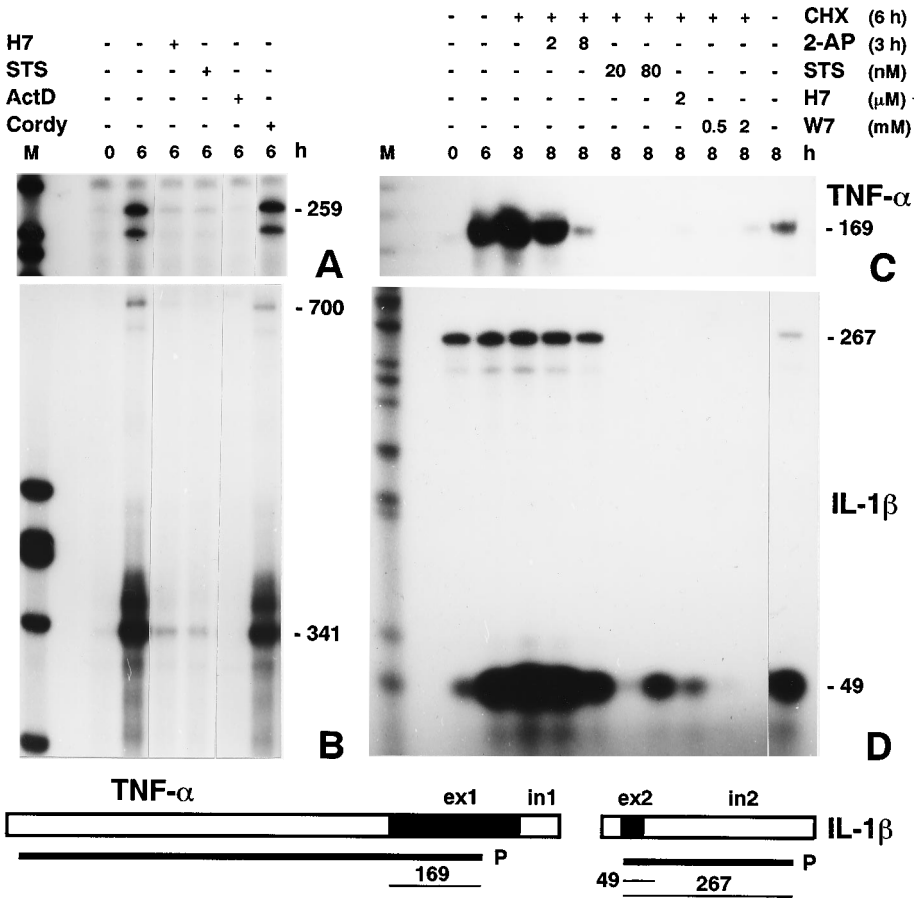

FIG. 6. Effects of 2-AP and other kinase inhibitors on TNF-α and IL-1β gene expression. PBMC were induced with PMA for the times shown. H7 (5 μM), STS (80 nM), actinomycin D, or cordycepin triphosphate (Cordy; 50 μg/ml) were added at 3 h (A and B). In a separate experiment, 2-AP, STS, H7, or W7 at the indicated concentrations was added together with CHX at 6 h (C and D). Total RNA was isolated and subjected to RNase protection analysis with ^{32}P-labeled antisense TNF-α RNA probes as performed for the experiments shown in Fig. 2A (A) (the 259-nt fragment, precursor transcripts) and Fig. 4 (B) (the 700-nt fragment, precursor transcripts; the 341-nt fragment, mRNA) or with a probe (map) complementary to untranscribed 5′-flank sequences and 169 nt of exon 1 (C). In panel D, total RNA was analyzed with a ^{32}P-labeled antisense IL-1β RNA probe (map) (the 267-nt fragment, precursor transcripts; the 49-nt fragment, mRNA) (28). M, size marker; in, intron; ex, exon.

PMA elicited a transient wave of TNF-α precursor transcripts (Fig. 7A) and mRNA (Fig. 7B) that reached a maximum at 3 h and then declined rapidly, showing that transcription had been activated. The appearance of both precursor transcripts and mRNA was blocked by 2-AP, whether it was added at the onset of induction or 3 h later. Moreover, 2-AP blocked the superinduction of precursor transcripts and mRNA by CHX. Expression of TNF-α mRNA was reduced to levels even lower

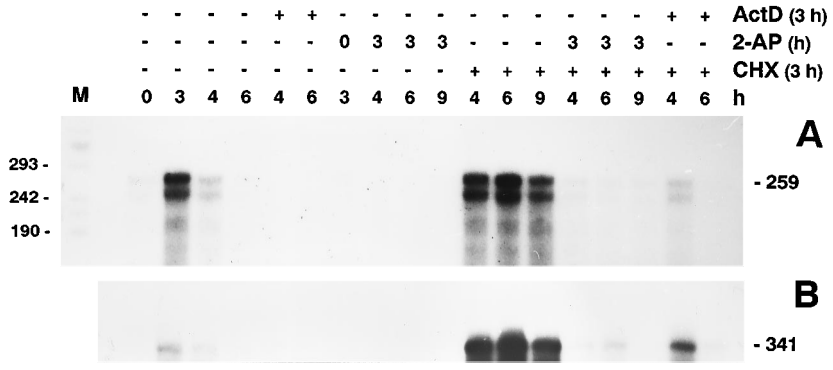

FIG. 7. Inhibition of TNF-α gene transcription by 2-AP in HL-60 cells. Aliquots of HL-60 cells were induced with PMA. Actinomycin D (ActD), 2-AP, or CHX was added as shown. Cell viability remained constant. Total RNA was isolated at the times indicated and subjected to RNase protection analysis to quantitate precursor transcripts, as performed for the experiment shown in Fig. 2A (A), or mRNA, as performed for the experiment shown in Fig. 1A (B) (for a map, see Fig. 4). M, size marker.

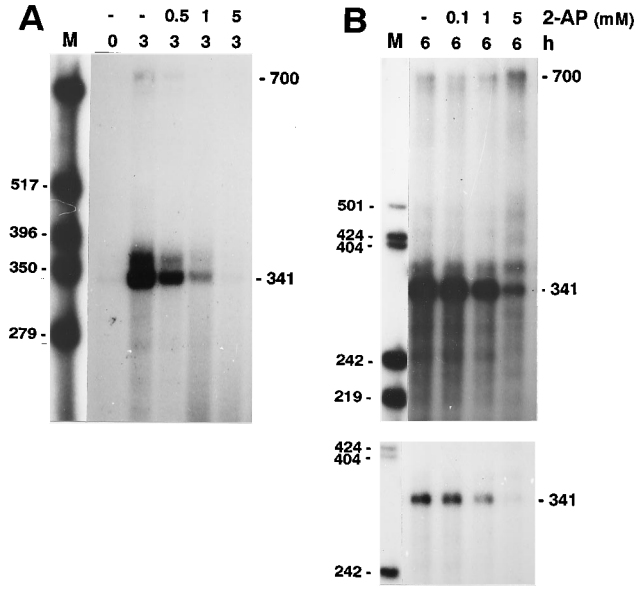

FIG. 8. Inhibition of splicing of TNF-α precursor transcripts by 2-AP at multiple sites. HL-60 cells (A) or PBMC (B) were induced with PMA. 2-AP was added at the onset of induction in the indicated concentrations. Total RNA was isolated at 3 h (A) or 6 h (B) and subjected to RNase protection analysis to quantitate precursor transcripts (the 700-nt band) and mRNA (the 341-nt band) (for a map, see Fig. 4). In panel B, a lower exposure of the 341-nt band is shown at the bottom. M, size marker.

than those seen in the control without CHX. The inhibitory effect of 2-AP on induction or superinduction was at least as effective as that of actinomycin D, indicating that in HL-60 cells, 2-AP blocks TNF-α gene transcription.

2-AP inhibits splicing of TNF-α precursor transcripts at multiple sites. In Fig. 8, the responses of the expression of TNF-α mRNA and precursor transcripts to increasing concentrations of 2-AP in HL-60 cells and PBMC were compared by using the TNF-α probe shown in Fig. 4. This probe detects splicing at different sites within human TNF-α primary transcripts: the intron 2-exon 3, exon 3-intron 3, and intron 3-exon 4 junctions. In HL-60 cells, the presence of increasing concentrations of 2-AP led to a parallel decline in levels of precursor transcripts (the 700-nt band) and spliced mRNA (the 341-nt band), confirming an inhibition at transcription (cf. Fig. 7). In PBMC, by contrast, increasing concentrations of 2-AP induced a decline in the level of spliced mRNA concomitant with a rise in the level of precursor transcripts (Fig. 8B). These reciprocal shifts extend the data of the experiment shown in Fig. 2A, for which splicing only at the exon 3-intron 3 junction was analyzed. Thus, as in transfected BHK-21 cells (Fig. 4), 2-AP inhibits the splicing of TNF-α precursor transcripts in PBMC at multiple sites, including intron 2 and intron 3 splice junctions.

DISCUSSION

We show here that 2-AP selectively inhibits expression of human TNF-α mRNA in PBMC. This inhibition is coupled with the prompt and extensive accumulation of newly transcribed, unspliced precursor transcripts. Two possible explanations may be considered for the finding that when 2-AP is present during induction, short-lived TNF-α precursor transcripts accumulate at the expense of mRNA. One explanation is that 2-AP causes extensive destabilization of TNF-α mRNA, concomitant with an increased transcription level that leads to a rise in the pool of precursor transcripts. However, although TNF-α mRNA is unstable (29), the stability of TNF-α mRNA is not detectably affected by 2-AP (Fig. 5). There is also no evidence that 2-AP can stimulate transcription of endogenous genes; by contrast, 2-AP is known to inhibit transcription of a number of cytokine genes and proto-oncogenes in cell lines, and in agreement with these observations (56, 59), we show that it inhibits transcription of the TNF-α gene in HL-60 cells (Fig. 7 and 8A). Even if 2-AP were to enhance transcription of the TNF-α gene in PBMC, this should have resulted primarily in a rise in the level of mRNA rather than the selective accumulation of precursor RNA that is observed. The inhibition of mRNA expression shows that in the presence of 2-AP, precursor transcripts are not processed. Indeed, the results reported here are consistent with the explanation that in PBMC, 2-AP inhibits the splicing of TNF-α mRNA. Neither the linked TNF-β gene nor the IL-1β gene shows such regulation.

2-AP inhibits splicing of TNF-α precursor transcripts not only in primary human lymphoid cell populations but also in BHK-21 cells transfected with the human TNF-α gene (Fig. 4). Thus, the 2-AP-sensitive component that supports splicing of TNF-α precursor RNA in PBMC is also active in a cell line that does not express its endogenous TNF-α gene.

PMA-induced expression of TNF-α and IL-1β mRNA can be superinduced coordinately by CHX (29). Although for both genes, superinduction depends on processing of precursor transcripts (29), 2-AP inhibits superinduction of TNF-α mRNA but not of IL-1β mRNA (Fig. 6). Thus, both induction and superinduction of the TNF-α gene are inhibited by 2-AP in a similarly selective manner.

2-AP does not inhibit TNF-α gene transcription in PBMC. TNF-α precursor transcripts accumulate in the presence of 2-AP (Fig. 2A, 5, and 8B), yet accumulation is prevented by actinomycin D, indicating that it depends on transcription (Fig. 5). The action of 2-AP on TNF-α gene expression in PBMC is distinct from those of the kinase inhibitors, H7, STS, and W7, which inhibit transcription of the TNF-α and IL-1β genes (Fig. 6), and also that of pentoxifylline, which induces a parallel decline in levels of TNF-α precursor transcripts and spliced mRNA (Fig. 3), indicating that this agent inhibits transcription, as reported earlier for the murine TNF-α gene (24). Finally, we show that 2-AP affects levels of TNF-α precursor transcripts and spliced mRNA in opposite ways (Fig. 2A and 8B) but induces parallel declines in levels of TNF-β precursor transcripts and mRNA (Fig. 2C), supporting the concept that expression of the TNF-β gene, but not of the TNF-α gene, is inhibited by 2-AP at transcription. Methods used here to study TNF-α gene expression in PBMC were capable of detecting an inhibitory effect of 2-AP on transcription, had it existed. Moreover, using electrophoretic mobility shift analysis of an NF-κB oligodeoxyribonucleotide, we could show that 2-AP does not interfere with NF-κB signaling (30).

The 2-AP-induced accumulation of TNF-α precursor transcripts results from synthesis of short-lived RNA molecules, not from stabilization of preexisting ones. These precursor transcripts decay rapidly in the presence of actinomycin D (Fig. 5). Although in the presence of 2-AP the rate of synthesis of short-lived precursors must exceed the rate of their degradation in order to result in a net accumulation, the instability of TNF-α precursor transcripts prevents their accumulation to the level of mRNA seen in the control without 2-AP, thus resulting in an overall reduction in the total level of TNF-α RNA expressed (Fig. 2A and 8B). TNF-α precursor transcripts accumulating in the presence of 2-AP do not enter the cytoplasm (Fig. 2D) and retain their ability to be spliced (Fig. 5).

In certain cell lines, 2-AP induces a reversible G_2 arrest associated with the induction of expression of transfected genes (5, 38). In the U937 human promonocytic cell line, increased levels of transcription and stabilization of mRNA accompanied the induction of exogenous gene expression by 2-AP; the stability of histidinol dehydrogenase mRNA and c-*myc* mRNA increased 15- and 2-fold, respectively (38). In BHK-21 cells transfected with the TNF-α gene, however, the 2-AP-induced rise in the level of TNF-α precursor RNA was not accompanied by an increase in the level of TNF-α mRNA. Instead, 2-AP inhibited TNF-α mRNA expression (Fig. 4).

The accumulation of unspliced TNF-α precursor transcripts shows that in the presence of 2-AP, splicing is not coupled to transcription. 2-AP does not affect the accuracy of initiation of TNF-α RNA synthesis (Fig. 6C). Using a genomic antisense RNA probe extending beyond the 3′ end of TNF-α precursor transcripts, we found that specific cleavage of the 3′ end also is not altered in the presence of 2-AP (30). Capping of precursor RNA is not required for splicing, although it influences accuracy and efficiency (33). Cordycepin, an inhibitor of polyadenylation (43), affected neither formation nor splicing of TNF-α precursor transcripts (Fig. 6), in agreement with evidence that polyadenylation also is not essential for splicing (43). The finding that 2-AP inhibits splicing of TNF-α mRNA, but not of IL-1β mRNA, also argues against an effect on general processing events.

Control of splicing is not restricted to a specific TNF-α splice site. Inhibition by 2-AP is observed with probes that monitor excision of intron 3, as well as splicing at different sites within human TNF-α primary transcripts: the intron 2-exon 3, exon 3-intron 3, and intron 3-exon 4 junctions. 2-AP thus blocks splicing at multiple sites. By contrast, at least 2 exons of the control IL-1β transcript are spliced normally in the presence of 2-AP. A possible explanation for this finding could be that in the presence of 2-AP, splicing of one TNF-α intron becomes rate limiting for splicing of other introns. Such cooperation was demonstrated during normal expression of the murine TNF-β gene (47). Most likely, regulation by 2-AP is mediated through a particular sequence within the TNF-α primary transcript to produce a general inhibition of the splicing of this transcript. The inhibitory action of 2-AP on splicing of precursor transcripts for the TNF-α gene but not for the IL-1β or TNF-β gene could be used to identify sequences that confer sensitivity to 2-AP and differential regulation of splicing of TNF-α mRNA in intact cells. We have indeed made progress on this issue by showing, in transfection experiments, that deletion of a particular sequence from the TNF-α gene renders splicing of the encoded precursor transcripts resistant to inhibition by 2-AP, while introduction of that sequence into the TNF-β gene shifts the inhibitory effect of 2-AP on TNF-β gene expression from transcription to splicing (30).

Expression of TNF-α mRNA is inhibited by 2-AP, whether it is induced with PMA, CHX, or LPS (Fig. 1). Precursor transcripts are observed in each case (Fig. 2A) (30). Because these inducers act through distinct pathways of signal transduction to activate TNF-α gene expression, the 2-AP-sensitive component either is activated by each or functions constitutively. The ability of 2-AP to block expression of TNF-α mRNA in cells treated with CHX (Fig. 1) shows that the 2-AP-sensitive component is expressed in functional form before induction.

What is the basis for this novel type of posttranscriptional control? The fact that splicing of IL-1β mRNA proceeds unabated in 2-AP-treated cells (Fig. 1, 2, and 6) shows that no common step is affected. Sequences within the TNF-α gene confer sensitivity of splicing to 2-AP. Our results reveal a distinct requirement for a 2-AP-sensitive component, most likely a kinase, in splicing of TNF-α mRNA.

There is evidence for the involvement of protein kinases, such as SR protein kinase I (22), in pre-mRNA splicing (22, 40, 52, 58), but these are not known to have specific roles in splicing of particular genes. However, 2-AP is a known inhibitor of the ATP-dependent phosphorylation of eIF-2α by PKR or by the heme-controlled kinase (8, 12, 21). eIF-2, an mRNA-binding protein, forms a ternary complex with Met-$tRNA_f$ and GTP during the initiation of translation and possesses a binding site for ATP (19). Conceivably, certain proteins that function in translation could also participate in splicing and vice versa. Thus, the cap-binding initiation factor, eIF-4E, localizes to the nucleus (36), although its role there remains unknown. Pyrimidine tract-binding protein, a nuclear protein originally implicated in the splicing of precursor transcripts, facilitates internal ribosome binding on poliovirus and encephalomyocarditis virus RNA (4, 25) as well as their translation (25) and binds to the 5′ untranslated region of human insulin-like growth factor 2 mRNA (9).

PKR is thought to participate in activation of the immediate-early genes c-*myc*, c-*fos*, and JE, because their induction can be blocked by 2-AP as well as by $p21^{ras}$, which is believed to cause an inhibition of PKR (44, 45). Our results indicate that a 2-AP-sensitive component is essential for nuclear splicing of TNF-α precursor transcripts, but its relationship, if any, to PKR or heme-controlled eIF-2α kinase remains to be established. PKR was found in the nucleus, but its function there is unknown (31). Accumulation of unspliced c-*fos* and metallothionein precursor transcripts, in a manner similar to that reported here for the TNF-α gene, was detected in the presence of 2-AP in serum- and cadmium-induced mouse NIH 3T3 cells, respectively (59), supporting the wider involvement of a 2-AP-sensitive mechanism in the regulation of splicing.

These findings strengthen the concept emerging from studies of IL-1β (29) and IL-2 gene expression (11, 15, 55) that the rate of splicing of precursor RNA is tightly regulated and serves as a limiting step in expression of cytokine mRNA. The sensitivity of splicing of TNF-α precursor transcripts to 2-AP can serve as a valuable tool for further study of this type of posttranscriptional control.

ACKNOWLEDGMENT

We thank Tom Maniatis for valuable comments on the manuscript.

REFERENCES

1. **Bandyopadhyay, S. K., and G. C. Sen.** 1992. Role of protein phosphorylation in activation of interferon-stimulated gene factors. J. Biol. Chem. **267:**6389–6395.
2. **Barber, G. N., S. Thompson, T. G. Lee, T. Strom, R. Jagus, A. Darveau, and M. G. Katze.** 1994. The 58-kilodalton inhibitor of the interferon-induced double-stranded RNA-activated protein kinase is a tetratricopeptide repeat protein with oncogenic properties. Proc. Natl. Acad. Sci. USA **91:**4278–4282.
3. **Beyaert, R., and W. Fiers.** 1994. Molecular mechanisms of tumor necrosis factor-induced cytotoxicity. What we do understand and what we do not. FEBS Lett. **340:**9–16.
4. **Borman, A., M. T. Howell, J. G. Patton, and R. J. Jackson.** 1993. The involvement of a spliceosome component in internal initiation of human rhinovirus RNA translation. J. Gen. Virol. **74:**1775–1788.
5. **Brown, F. L., E. Tahaoglu, G. J. Graham, and J. J. Maio.** 1993. Inducible transcriptional activation of the human immunodeficiency virus long terminal repeat by protein kinase inhibitors. Mol. Cell. Biol. **13:**5245–5254.
6. **Daigneault, L., A. Haggarty, Q. H. Meng, and D. Skup.** 1992. Two distinct pathways of interferon induction as revealed by 2-aminopurine. Nucleic Acids Res. **20:**2749–2754.
7. **Davies, M. V., H. W. Chang, B. L. Jacobs, and R. J. Kaufman.** 1993. The E3L and K3L vaccinia virus gene products stimulate translation through inhibition of the double-stranded RNA-dependent protein kinase by different mechanisms. J. Virol. **67:**1688–1692.
8. **De Benedetti, A., and C. Baglioni.** 1983. Phosphorylation of initiation factor

eIF-2α, binding of mRNA to 48 S complexes, and its reutilization in initiation of protein synthesis. J. Biol. Chem. **258:**14556–14562.
9. **De Moor, C. H., M. Jansen, E. J. Bonte, A. A. Thomas, J. S. Sussenbach, and J. L. Van den Brande.** 1995. Proteins binding to the leader of the 6.0 kb mRNA of human insulin-like growth factor 2 influence translation. Biochem. J. **307:**225–231.
10. **Efrat, S., and R. Kaempfer.** 1984. Control of biologically active interleukin 2 messenger RNA formation in induced human lymphocytes. Proc. Natl. Acad. Sci. USA **81:**2601–2605.
11. **Efrat, S., S. Pilo, and R. Kaempfer.** 1982. Kinetics of induction and molecular size of mRNAs encoding human interleukin-2 and γ-interferon. Nature (London) **297:**236–239.
12. **Farrell, P. J., K. Balkow, T. Hunt, R. J. Jackson, and H. Trachsel.** 1977. Phosphorylation of initiation factor eIF-2 and the control of reticulocyte protein synthesis. Cell **11:**187–200.
13. **Ferreri, N. R., T. Sarr, P. W. Askenase, and N. H. Ruddle.** 1992. Molecular regulation of tumor necrosis factor-α and lymphotoxin production in T cells. Inhibition by prostaglandin E_2. J. Biol. Chem. **267:**9443–9449.
14. **Fiers, W.** 1991. Tumor necrosis factor. FEBS Lett. **285:**199–212.
15. **Gerez, L., G. Arad, S. Efrat, M. Ketzinel, and R. Kaempfer.** 1995. Post-transcriptional regulation of human interleukin-2 gene expression at processing of precursor transcripts. J. Biol. Chem. **270:**19569–19575.
16. **Gerez, L., and R. Kaempfer.** Unpublished data.
17. **Goldfeld, A. E., C. Doyle, and T. Maniatis.** 1990. Human tumor necrosis factor α gene regulation by virus and lipopolysaccharide. Proc. Natl. Acad. Sci. USA **87:**9769–9773.
18. **Goldfeld, A. E., and T. Maniatis.** 1989. Coordinate viral induction of tumor necrosis factor α and interferon-β in human B cells and monocytes. Proc. Natl. Acad. Sci. USA **86:**1490–1494.
19. **Gonsky, R., M. A. Lebendiker, R. Harary, Y. Banai, and R. Kaempfer.** 1990. Binding of ATP to eukaryotic initiation factor 2. Differential modulation of mRNA-binding activity and GTP-dependent binding of methionyl-$tRNA^{Met}_f$. J. Biol. Chem. **265:**9083–9089.
20. **Gorman, C. M., L. F. Mofat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
21. **Gross, M., J. Rynning, and W. M. Knish.** 1981. Evidence that the phosphorylation of eukaryotic initiation factor 2α by the hemin-controlled translational repressor occurs at a single site. J. Biol. Chem. **256:**589–592.
22. **Gui, J. F., H. Tronchere, S. D. Chandler, and X. D. Fu.** 1994. Purification and characterization of a kinase specific for the serine- and arginine-rich pre-mRNA splicing factors. Proc. Natl. Acad. Sci. USA **91:**10824–10828.
23. **Hall, D. J., S. D. Jones, D. R. Kaplan, M. Whitman, B. J. Rollins, and C. D. Stiles.** 1989. Evidence for a novel signal transduction pathway activated by PDGF and dsRNA. Mol. Cell. Biol. **9:**1705–1713.
24. **Han, J., G. Huez, and B. Beutler.** 1991. Interactive effects of the tumor necrosis factor promoter and 3′ untranslated regions. J. Immunol. **146:**1843–1848.
25. **Hellen, C. U., T. V. Pestova, M. Litterst, and E. Wimmer.** 1993. The cellular polypeptide p57 (pyrimidine tract-binding protein) binds to multiple sites in the poliovirus 5′ nontranslated region. Proc. Natl. Acad. Sci. USA **90:**7642–7646.
26. **Hershey, J. W.** 1991. Translational control in mammalian cells. Annu. Rev. Biochem. **60:**717–755.
27. **Ito, T., R. Jagus, and S. May.** 1994. IL-3 stimulates protein synthesis by regulating dsRNA-dependent protein kinase (PKR). Proc. Natl. Acad. Sci. USA **91:**7455–7459.
28. **Jagus, R., and M. M. Gray.** 1994. Proteins that interact with PKR. Biochimie **76:**779–791.
29. **Jarrous, N., and R. Kaempfer.** 1994. Induction of human interleukin-1 gene expression by retinoic acid and its regulation at processing of precursor transcripts. J. Biol. Chem. **269:**23141–23149.
30. **Jarrous, N., F. Osman, and R. Kaempfer.** Unpublished data.
31. **Jeffrey, I. W., S. Kadereit, E. F. Meurs, T. Metzger, M. Bachmann, M. Schwemmle, A. G. Hovanessian, and M. J. Clemens.** 1995. Nuclear localization of the interferon-inducible protein kinase PKR in human cells and transfected mouse cells. Exp. Cell Res. **218:**17–27.
32. **Kalvakolanu, D. V. R., S. K. Bandyopadhyay, R. K. Tiwari, and G. C. Sen.** 1991. Enhancement of expression of exogenous genes by 2-aminopurine. J. Biol. Chem. **266:**873–879.
33. **Krainer, A. R., T. Maniatis, B. Ruskin, and M. R. Green.** 1984. Normal and mutant human β-globin pre-mRNAs are faithfully and efficiently spliced in vitro. Cell **36:**993–1005.
34. **Kumar, A., J. Haque, J. Lacoste, J. Hiscott, and B. R. Williams.** 1994. Double-stranded RNA-dependent protein kinase activates transcription factor NF-κB by phosphorylating IκB. Proc. Natl. Acad. Sci. USA **91:**6288–6292.
35. **Le, J., and J. Vilcek.** 1987. Biology of disease. Tumor necrosis factor and interleukin-1: cytokines with multiple overlapping biological activities. Lab. Invest. **56:**234–248.
36. **Lejbkowicz, F., C. Goyer, A. Darveau, S. Neron, R. Lemieux, and N. Sonenberg.** 1992. A fraction of the mRNA 5′ cap-binding, eukaryotic initiation factor 4E, localizes to the nucleus. Proc. Natl. Acad. Sci. USA **89:**9612–9616.
37. **Mahadevan, L. C., A. J. Wills, E. A. Hirst, P. D. Rathjen, and J. K. Heath.** 1990. 2-Aminopurine abolishes EGF- and TPA-stimulated pp33 phosphorylation and induction without affecting the activation of protein kinase C. Oncogene **5:**327–335.
38. **Maio, J. J., G. J. Graham, and F. L. Brown.** 1995. Induction of G2 arrest and gene expression by 2-aminopurine in human U937 promonocyte-macrophage cells. Exp. Cell Res. **219:**442–448.
39. **Maran, A., R. K. Maitra, A. Kumar, B. Dong, W. Xiao, G. Li, B. R. G. Williams, P. F. Torrence, and R. H. Silverman.** 1994. Blockage of NF-κB signaling by ablation of an mRNA target by 2-5A antisense chimeras. Science **265:**789–792.
40. **Mermoud, J. E., P. T. Cohen, and A. I. Lamond.** 1994. Regulation of mammalian spliceosome assembly by a protein phosphorylation mechanism. EMBO J. **13:**5679–5688.
41. **Merrick, W. C.** 1992. Mechanism and regulation of eukaryotic protein synthesis. Microbiol. Rev. **56:**291–315.
42. **Meurs, E. F., J. Galabru, G. N. Barber, M. G. Katze, and A. G. Hovanessian.** 1993. Tumor suppressor function of the interferon-induced double-stranded RNA-activated protein kinase. Proc. Natl. Acad. Sci. USA **90:**232–236.
43. **Moore, C. L., and P. A. Sharp.** 1985. Accurate cleavage and polyadenylation of exogenous RNA substrate. Cell **41:**845–855.
44. **Mundschau, L. J., and D. V. Faller.** 1992. Oncogenic *ras* induces inhibition of PKR. J. Biol. Chem. **267:**23092–23098.
45. **Mundschau, L. J., and D. V. Faller.** 1995. Platelet-derived growth factor signal transduction through the interferon-inducible kinase PKR. Immediate early gene induction. J. Biol. Chem. **270:**3100–3106.
46. **Nedwin, G. E., S. L. Naylor, A. Y. Sakaguchi, D. Smith, J. Jarrett-Nedwin, D. Pennica, D. V. Goeddel, and P. W. Gray.** 1985. Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. Nucleic Acids Res. **13:**6361–6373.
47. **Neel, H., D. Weil, C. Giansante, and F. Dautry.** 1993. *In vivo* cooperation between introns during pre-mRNA processing. Genes Dev. **7:**2194–2205.
48. **Novogrodsky, A., A. Vanichkin, M. Patya, A. Gazit, N. Osherov, and A. Levitzki.** 1994. Prevention of lipopolysaccharide-induced lethal toxicity by tyrosine kinase inhibitors. Science **264:**1319–1322.
49. **Ruddle, N. H.** 1992. Tumor necrosis factor (TNF-α) and lymphotoxin (TNF-β). Curr. Opin. Immunol. **4:**327–332.
50. **Spies, T., C. C. Morton, S. A. Nedospasov, W. Fiers, D. Pious, and J. L. Strominger.** 1986. Genes for the tumor necrosis factor α and β are linked to the human major histocompatibility complex. Proc. Natl. Acad. Sci. USA **83:**8699–8702.
51. **Sung, S. J., J. A. Walters, and S. M. Fu.** 1992. Stimulation of tumor necrosis factor α production in human monocytes by inhibitors of protein phosphatase 1 and 2A. J. Exp. Med. **176:**897–901.
52. **Tazi, J., U. Kornstadt, F. Rossi, P. Jeanteur, G. Cathala, C. Brunel, and R. Luhrmann.** 1993. Thiophosphorylation of U1-70K protein inhibits pre-mRNA splicing. Nature (London) **363:**283–286.
53. **Thomis, D. C., and C. E. Samuel.** 1993. Mechanism of interferon action: evidence for intermolecular autophosphorylation and autoactivation of the interferon-induced, RNA-dependent protein kinase PKR. J. Virol. **67:**695–700.
54. **Tiwari, R. K., J. Kusari, R. Kumar, and G. C. Sen.** 1988. Gene induction by interferons and double-stranded RNA: selective inhibition by 2-aminopurine. Mol. Cell. Biol. **8:**4289–4294.
55. **Umlauf, S. W., B. Beverly, O. Lantz, and R. H. Schwartz.** 1995. Regulation of interleukin-2 gene expression by CD28 costimulation in mouse T-cell clones: both nuclear and cytoplasmic RNAs are regulated with complex kinetics. Mol. Cell. Biol. **15:**3197–3205.
56. **Wathelet, M. G., I. M. Clauss, F. C. Paillard, and G. A. Huez.** 1989. 2-Aminopurine selectively blocks the transcriptional activation of cellular genes by virus, double-stranded RNA and interferons in human cells. Eur. J. Biochem. **184:**503–509.
57. **Weil, D., S. Brosset, and F. Dautry.** 1990. RNA processing is a limiting step for murine tumor necrosis factor β expression in response to interleukin-2. Mol. Cell. Biol. **10:**5865–5875.
58. **Woppmann, A., C. L. Will, U. Kornstadt, P. Zuo, J. L. Manley, and R. Luhrmann.** 1993. Identification of an snRNP-associated kinase activity that phosphorylates arginine/serine rich domains typical of splicing factors. Nucleic Acids Res. **21:**2815–2822.
59. **Zinn, K., A. Keller, L.-A. Whittemore, and T. Maniatis.** 1988. 2-Aminopurine selectively inhibits the induction of β-interferon, c-*fos*, and c-*myc* gene expression. Science **240:**210–213.