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Organization and Functional Analysis of the Mouse Transporter Associated with Antigen Processing 2 Promoter

Evgeny Arons, Victor Kunin, Chana Schechter, and Rachel Ehrlich

In accordance with the key role of MHC class I molecules in the adaptive immune response against viruses, they are expressed by most cells, and their expression can be enhanced by cytokines. The assembly and cell surface expression of class I complexes depend on a continuous peptide supply. The peptides are generated mainly by the proteasome and are transported to the endoplasmic reticulum by a peptide transport pump consisting of two subunits, TAP1 and TAP2. The proteasome low molecular weight polypeptide (2 and 7), as well as TAP (1 and 2) genes, are coordinately regulated and are induced by IFNs. Despite this coordinate regulation, examination of tumors shows that these genes can be discordantly down-regulated. In pursuing a molecular explanation for these observations, we have characterized the mouse TAP2 promoter region and 5′-flanking sequence. We show that the 5′ untranslated regions of TAP2 genes have a characteristic genomic organization that is conserved in both the mouse and the human. The mouse TAP2 promoter belongs to a class of promoters that lack TATA boxes but contain a MED1 (multiple start site element downstream) sequence. Accordingly, transcription is initiated from multiple sites within a 100-nucleotide window. An IFN regulatory factor 1 (IRF1)/IRF2 binding site is located in this region and is involved in both basal and IRF1-induced TAP2 promoter activity. The implication of the extensive differences found among the promoters of class I heavy chain, low molecular weight polypeptide, and TAP genes, all encoding proteins involved in Ag presentation, is discussed. The Journal of Immunology, 2001, 166: 3942–3951.

The MHC in both the mouse and in humans spans about four megabases, and its gene products are predominantly associated with the immune system. MHC class I and II molecules are membrane-bound glycoproteins that fulfill a key role in the immune response by virtue of their ability to present antigenic peptides to T lymphocytes (1–3). MHC class I molecules are ubiquitously expressed (4) in accordance with their essential function in mediating immune responses against viruses and tumor cells. The class I binding peptides are generally produced in the cytosol by the multicatalytic protease complex (proteasome), whose cleavage specificity can be altered by the IFN-inducible subunits low molecular weight polypeptide (LMP)2,3 LMP7, multicatalytic endopeptidase complex-like-1, and the 11S regulator proteasome activator 28 (5). The peptides are transported by specialized peptide transporters (TAP1 and TAP2) into the endoplasmic reticulum, where assembly with class I heavy chain and β2-microglobulin (β2m) takes place (1–3). TAP1 and TAP2 as well as LMP2 and LMP7 genes are embedded in the class II region of the MHC locus. It appears that the class I chains, β2m, TAP1, and TAP2, as well as LMP2 and LMP7 genes are coordinately regulated on the transcriptional level to enable efficient class I-mediated Ag presentation.

The transcriptional regulation of MHC class I genes and the human bidirectional TAP1/LMP2 promoter have been analyzed in detail (6–8). In addition to the TATA and CCAAT boxes, a number of conserved cis-acting, positive regulatory elements were characteristic in the 5′ region of class I heavy chain genes: enhancer A, the IFN response element (ISRE), site α (a regulatory element that is homologous to the cAMP response element), and enhancer B. The TAP1/LMP2 promoter contains a single NF-κB site that depends on the flanking Sp1 site for TNF-α-induced transcription by NF-κB. The most potent regulators of antiviral activities are IFN-α, -β, and -γ (9, 10). IFN-γ exerts its effect by inducing phosphorylation of Stat1, which then homodimerizes, allowing it to interact with the IFN-γ activation site (GAS) (11) or, in combination with p48 (IFN-stimulated gene factor), with the ISRE, thereby trans-activating genes bearing either of these sites in their promoter (12). Transcription factors of the IFN regulatory factor (IRF) family, such as IRF1, IRF2, and IFN consensus sequence binding proteins (ICSBP), are induced by this route. The ISRE can bind factors of the IRF family, thereby mediating both constitutive expression and cytokine induction of MHC class I expression (4, 13–15). Although regulatory elements are generally conserved among MHC class I genes, nucleotide variation exists, particularly in the ISRE and enhancer A. These nucleotide differences determine the binding affinities of the relevant factors and, consequently, the contribution of these elements to promoter trans-activation (16, 17). The mouse and human LMP7 promoters as well as the mouse TAP1/LMP2 bidirectional promoter have not been fully functionally analyzed (18, 19). Despite the coordinate regulation of human TAP1 and LMP2 genes observed in several cell lines (20–22), it appears that binding of IRF1 or Stat1 to ICS2/GAS is sufficient to regulate transcription of the TAP1 gene, but binding of both factors is required for transcription of the LMP2 gene (23).
Transcriptional down-regulation of genes associated with peptide production and transport as well as inefficient assembly and cell surface expression of class I complexes are common in mouse and human tumors (24–27). Despite evidence that TAP2 transcription is completely suppressed in some tumors (28, 29), to the best of our knowledge the promoter and the regulatory elements associated with this gene have not been characterized or analyzed. We now present the initial characterization of the mouse TAP2 promoter and 5′ regulatory elements. We also demonstrate that the 5′ untranslated region (UTR) of the mouse TAP2 gene shows unique organization compared with that of other genes in the MHC locus, and that both the constitutive activity and the IFN-induced activity of the TAP2 promoter depend on an I RF-1 binding element. The molecular basis for coordinate or discordant expression of TAP (1 and 2), LMP (2 and 7), and class I heavy chain genes are discussed.

Materials and Methods

Plasmids

The PBr-based plasmid 5.9AII (30, 31) containing the mouse LMP7 gene, the intergenic region, and the first four exons and introns of the TAP2 gene was a gift from Dr. J. Monaco (Cincinnati, OH). The IRF1, IRF2, and ICSBP expression vectors as well as pRD1X were gifts from Dr. B. Z. Levi (Technion, Haifa, Israel) (32, 33). The pGEL expression system (Promega, Madison, WI) was used for analysis of the TAP2 promoter. PUC19 was used to clone and sequence PCR products.

Cell cultures

The E1Ad5-transformed fibroblast cell lines (A505 and A501), the Ad12-transformed fibroblast cell lines (VAD12.79), and the T cell leukemia PD1.2 line were cultured in the same medium supplemented with 5% FCS, penicillin, streptomycin, gentamicin, and amphotericin B at the recommended concentrations (37). The T cell leukemia PD1.2 line was cultured in the same medium supplemented with 5 × 10−3 M β-ME and 0.1 mM nonessential amino acids. Media and supplements were purchased from Biological Industries (Bet Ha’emek, Israel). Cell lines were treated with 100 U/ml IFN-γ (Roche, Mannheim, Germany) for 48–72 h before harvesting.

RNA analysis

Tri-Reagent (Molecular Research Center, Cincinnati, OH) was used for RNA preparation.

DNA sequencing

The LMP7/TAP2 intergenic region in 5.9AII was sequenced by the dye-deoxy chain termination method (38), using a Big Dye RMX (PE Applied Biosystems, Foster City, CA) and an ABI Prism 377 DNA Sequencer (Applied Biosystems). The two DNA strands were sequenced. All the amplified fragments were fractionated on agarose gels, and the specific bands were excised and cloned into the HindIII/Xhol site of the pUC19 vector. Plasmids purified from individual clones were sequenced using the M13pUC reverse primer (New England Biolabs, Beverly, MA).

RNase protection assay

TRP5 and TRP6 (illustrated in Fig. 3A) were generated by PCR, using the 5.9AII plasmid as template. The TRP5 fragment (94 nucleotides (nt)) was synthesized using primers T2B and T2H2; it contains 40 nt of intron 1 and 54 nt of exon 2. The TRP6 fragment (266 nt) was synthesized using primers T22 and T54H; it contains the 5′ UTR and the flanking sequences of the TAP2 gene. Following digestion with HindIII and BglII restriction enzymes (New England Biolabs), the fragments were cloned into the HindIII/BamHII sites of pGEM 3zf− (Promega, Madison, WI). The sequence of the cloned fragments was verified, using the PGL primer (5′-GGTACCGAGGCCCTTATCGCTGC-3′). DNA templates were prepared for in vitro run-off transcription by digestion with RsalI (New England Biolabs). Antisense Sp6 riboprobes were synthesized as specified by the Riboprobe In Vitro Transcription System (Promega) with [α-32P]UTP (Amersham, Aylesbury, U.K.). Including vector sequences, the RNA transcription products resulted in 525 nt (TRP5) and 697 nt (TRP6). RNase protection was performed using an RNase protection kit (Roche). A quantity of 100 μg of total RNA was co precipitated with the radio probe (3 × 106 cpm) at the presence of 0.3 M sodium acetate. The pellets were dissolved in 30 μl of hybridization solution, denatured at 90°C for 5 min, and hybridized at 45°C for 16 h. The hybridized probe and sample RNAs were digested for 30 min at 37°C with an RNase A/T1 mix (Roche). The protected fragments were fractionated on 6% sequencing gels against m.w. markers.

 Luciferase reporter gene constructs

To map the TAP2 promoter, fragments including the 5′UTR and flanking sequences (Figs. 4A and 5A) were cloned into the BglII/Hind sites of the PGL3/Enhancer vector (Promega). The BglII/HindIII fragments were generated by PCR using specific primers (illustrated in Figs. 4A and 5A) and the 5.9AII plasmid as template. The sequence was verified using sequencing PCR for specific primers.

Mutation analysis of the IRF1/IRF2 element

The generation of constructs containing a mutated IRF1/IRF2 binding sequence (PT5S and PT5P) is illustrated in Fig. 7. The 5.9AII plasmid was used as a template for the generation of both fragments. For the generation of PT5S (Fig. 7A), mutated primers (T6bS and T62S) were used. PCR-generated fragments (T52B-T61S and T62S-T54H) were digested with XbaI and ligated. The resulting construct (3 × 106 cpm) was subjected to the run-off transcription assay. Following digestion with HindIII and BglII, the purified fragment was cloned into the HindIII/BglII site of the pGEL3/Enhancer vector. PT5S was generated by sequential PCR amplification using the mutated primers T63 and T64 and matching primers T52B and T54H. The first set of fragments (T52B-T64 and T63-T54H) was purified, mixed, and used as a template for a second PCR with T52B and T54H. The purified fragments were digested with HindIII and BglIII and then cloned into the HindIII/BglII site of the pGEL3/Enhancer vector.

Transient transfections

Transient transfections were performed using the (N-[2,3-dioleoyloxy]-propyl)-N,N,N,N-tetramethy lammonium methyl-sulfate (DOTAP) liposomal transfection reagent (Roche). A quantity of 106 cells was plated on 60-mm plates 24 h before transfection. The complete medium was then removed from the plates, the cells were washed twice with serum-free medium, and 2 ml of serum-free medium with 5 μl of DOTAP, 40 μl of 20 mM HEPES, and 5 μg of the test plasmid were added. After 4 h, the mixture was replaced with complete medium. The transfected cells were incubated at 37°C for an additional 24 h. To check transfection efficiency, the SVS2CAT (CAT, chloramphenicol acetyltransferase) was cotransfected with the test plasmid or a pRSV LUC construct was transfected in parallel to the test construct. The latter was performed regularly, since cotransfection with pSV2CAT resulted in a general 10-fold decrease in LUC expression. To maximize accuracy, the protein concentration in the cell extracts was determined using Bradford’s reagent (Sigma, St. Louis, MO), and equivalent amounts of protein were used in the luciferase assay. Transfections were performed in triplicate, and each experiment was repeated at least three times.

Measurement of LUC activity

LUC activity was measured using a luciferase assay system according to the manufacturer’s instructions (Promega).
Characterization of the mouse TAP2 transcriptional start sites

The transcription start sites of the TAP2 gene were determined by the 5′RACE method. Total RNA from a leukemic cell line (PD1.2) or a transformed fibroblast cell line (A505) and two sets of TAP2-specific primers (exon 2- and exon 3-specific primers) were used to ensure the cloning of rare transcripts that might initiate in exon 1 or intron 1 (Fig. 2A). DNA inserts from 35 colonies were sequenced. A total of 23 DNA inserts corresponding to mouse TAP2 cDNA revealed the existence of at least nine transcriptional start sites (Fig. 2, B and C). All the start sites were located within a region spanning from −63 to −147 nt (the translation initiation site was designated nucleotide 1). Sequence analysis of the cDNAs confirmed the existence and length of the intron separating the first two exons. Multiple transcription start sites are typical of genes with TATA-less promoters. Among these are the TAP1 and LMP2 genes, which, like the TAP1 gene, are located in the class II MHC region. Analysis of the TAP2 5′UTR and flanking sequence with TFSSEARCH and TRANSFACT computer software revealed that the TAP2 5′UTR indeed lacks TATA and CCAAT boxes, but contains two MEDI (multiple start site element downstream) consensus sequences (gtcctcgcg) at positions −98 and −18 nt (Fig. 2C). The MEDI sequence appears to define a new subclass of promoters, since it is frequently present in the 5′-flanking region of genes with TATA-less promoters and multiple transcriptional start sites (39). Two regulatory elements commonly associated with genes of the immune system were identified: an IRFI/IRF2-binding site (tgaagtaagca) at −126 nt and a CREB site (tgcacgctg) at −593 nt.

To determine the extent of in vivo utilization of the TAP2 transcriptional start sites, an RNAse protection assay was performed with total RNA from the leukemic cell line (PD1.2) and an Ad12-transformed cell line (VAD12.79). The latter does not express detectable levels of TAP2 mRNA unless treated with IFNs (28). Two antisense probes were generated (Fig. 3A). TRP5 spans 54 nt of exon 2 and 40 nt of intron 1 and was designed to detect possible transcripts initiated in intron 1. TRP6 spans exon 1 and 5′-flanking sequences of the TAP2 gene. Specific bands appeared following hybridization with RNA from PD1.2 cells, but not with RNA from untreated VAD12.79 cells (the bands are marked by arrows in Fig. 3B). The strong band observed following hybridization with TRP5 at position −60 nt (Fig. 3B) corresponds to the expected size of the protected exon 2 plus the 6 nt repeat (CCGCAG) present at the end of intron 1 (and, therefore, in the probe) and at the 3′ end of exon 2 (Fig. 1B, underlined). Since this is the only band that appears following hybridization with TRP5, we conclude that there are no major transcriptional start sites in intron 1. As evident from the hybridization with TRP6, the major protected bands are induced by IFN-γ and result from transcriptional initiation sites located within 45 nt, spanning from −92 to −47 nt. The hybridization with RNA from IFN-γ-treated PD1.2 cells revealed the existence of transcription start sites at −47, −63, −80, and −92 nt. The transcription start sites at −63 and −80 nt were also detected by the 5′RACE method. The start sites at −47 and −63 nt were detected in untreated PD1.2 cells, indicating that these are major transcriptional initiation sites in the leukemic cells. Only one transcription start
site, at −63 nt (detected using both methodologies), was clearly observed in IFN-γ-treated VAD12.79 cells, raising the possibility that it is a predominant TAP2 transcription start site for both lymphoid and fibroblast cell lines. Verification of this will require analyses of additional cell lines. The upstream transcription start sites (between 2113 and 2147 nt) identified by the 5'RACE method were not detected by the RNase protection assay, either because of the lack of sensitivity of this assay or because these initiation sites are rarely used. The data obtained by both methodologies localize the mouse TAP2 promoter region to a sequence spanning from −46 to −147 nt (Fig. 2C).

**Functional characterization of the TAP2 promoter**

To map the TAP2 promoter region, fragments spanning the 5’UTR and 5’-flanking sequences (Fig. 4A) were inserted into the LUC/Enhancer expression vector (pGL3/Enhancer) and used in transient transfections of the fibroblast cell line A5O5. The expression constructs were divided into three groups, each including different
parts of the 5'-UTR. Group 1 (PT1–PT4) contains TAP2 5'-flanking sequences, but none of the identified transcription start sites, neither the IRF1/IRF2 binding site nor the MED1 elements. Group 2 (PT5–PT8) contains the IRF1/IRF2 binding site, the MED1 elements, and all the identified transcription start sites. Group 3 (PT9–PT12) lacks one MED1 element and is devoid of two transcription start sites: the first (−47 nt) was detected by the RNase protection assay in PD1.2 cells, and the second (−63 nt) was identified by both methods and appears to be a major start site in both cell lines. However, these constructs contain the IRF1/IRF2 binding element. A compilation of the data from three experiments is presented in Fig. 4B. Maximal promoter activity is mediated by the second group constructs (PT5–PT8), which contain all the identified transcription start sites as well as the IRF1/IRF2 binding site and the MED1 elements. Constructs of the third group, which lack the two transcription start sites and one MED1 element, display reduced (~50% reduction) promoter activity. Constructs of the first group, devoid of all transcription start sites, did not show any LUC activity, as anticipated. The TAP2 5'-flanking sequences (~419 to −149 nt) did not affect LUC expression, implying that either this region does not contain regulatory elements or that if there is any regulatory element in this region, it does not function in the presence of the SV40 enhancer.

To explore the relative contributions of the transcription start sites, the MED1, and the IRF1/IRF2 binding elements to TAP2 promoter activity, the PT5 fragment was divided into a proximal...
**FIGURE 3.** In vivo utilization of TAP2 transcriptional start sites. A, Two riboprobes (TRP6 and TRP5) were used for synthesis of antisense RNA and mapping of possible transcriptional start sites in exon 1 and intron 1, respectively. In the absence of transcription start sites in intron 1, TRP5 is expected to protect a 60-nt band (54 nt of exon 2 plus a 6-nt repeat that appears at the 3’ end of exon 1 as well as in the labeled probe; see Fig. 1B, underlined sequence). TRP6 is expected to protect TAP2 transcripts that initiate in exon 1. Total RNA was prepared from untreated and IFN-γ-treated PD1.2 and VAD12.79 cells. B, Major specific bands (bands corresponding to protection by PD1.2, but not by VAD12.79 RNA) are marked by an arrow.

**FIGURE 4.** Functional characterization of the TAP2 promoter. TAP2 expression constructs based on the pGL3/Enhancer vector were generated as illustrated in A. The lengths of the TAP2 inserts (brackets) and their positions within the 5’UTR are indicated. The results of transient transfection of A505 cells are presented as luciferase activity (activity, arbitrary units) in B as the mean ± SE of three individual experiments. The activities of the parental vector (pGL3/Enhancer) and the positive control (pRSV luc) are shown.
part (PT13) containing the MED1 elements and major transcription start sites, and a distal part (PT14) containing the IRF1/IRF2 binding element and five transcription start sites that had been detected by 5' RACE (Fig. 5A). The data in Fig. 5B show that each of the constructs can mediate partial promoter activity, suggesting the existence of multiple promoter elements in this region. However, the full-length PT5 insert is required for maximal promoter activity, implying that more than one promoter or all the regulatory elements contribute to LUC expression.

Functional characterization of the TAP2 IFN response element

Two regulatory elements were identified in the promoter region of the TAP2 gene: an IRF1/IRF2 binding sequence and MED1. Since TAP2 expression is induced by IFNs, and as IFN response elements are known to be involved in both constitutive and IFN-

induced activity of class I heavy chain and TAP1 genes, we further studied the function of this element. To determine directly the responsiveness of the TAP2 IRF1/IRF2 binding sequence to relevant binding factors, a representative from each group of expression constructs (PT1, PT5, and PT9) as well as a control expression plasmid (pSV2CAT) were cotransfected with vectors expressing IRF1, IRF2, or ICSBP. Control cotransfections were performed with pSV2CAT or pSV2CAT or vectors expressing IRF1 and IRF2. Luciferase activity (activity, arbitrary units) is presented as the mean ± SE of three individual experiments.

FIGURE 6. Functional characterization of the TAP2 IFN response element. A501 and A505 cells were cotransfected with PT5 and PT9 (containing the IRF1/IRF2 binding site) as well as with PT1 (lacking the IRF1/IRF2 binding site) and a control plasmid (pSV2CAT) or plasmids expressing IRF1, IRF2, and ICSBP. Control cotransfections were performed with pRD1×8 and pSV2CAT or the IRF1 expression vector. Luciferase activity (relative activity) was normalized to that of the control cotransfection (LUC expression vectors cotransfected with pSV2CAT) and is presented as the mean ± SE of three individual experiments.

FIGURE 7. The TAP2 IFN response element is essential to both basal and IFN-γ-induced promoter activity. The IRF1/IRF2 binding site in PT5 was mutated to generate PT5P and PT5S, as illustrated in A and B. A505 cells were cotransfected with PT1, PT5, PT5P, PT5S, and a control plasmid (pSV2CAT) or vectors expressing IRF1 and IRF2. Luciferase activity (activity, arbitrary units) is presented in C as the mean ± SE of two individual experiments.

FIGURE 5. The entire promoter region is required for maximal TAP2 promoter activity. Expression constructs containing the full-length promoter region (PT5) and constructs containing the 3' (PT13) and 5' (PT14) promoter regions (A) were analyzed in transient transfections of A505 cells. Luciferase activity (activity, arbitrary units) is presented in B as the mean ± SE of three individual experiments.
7C, cotransfection with pSV2CAT). The fact that only nonmutated PT5 was activated by IRF1 (compare the second set of columns in Fig. 7C) substantiates the importance of the IRF1/IRF2 binding site for induced promoter activity. Hence, the TAP2 IRF1 binding element is essential to IFN-γ induction and contributes to the basal activity of the TAP2 promoter(s).

Discussion

Ag presentation to CTL depends upon efficient transport of class I MHC complexes (class I heavy chains associated with β2m and a peptide) to the cell surface (1, 2). The regulation of expression of MHC class I genes is important during development (40, 41), differentiation (42), and infection (1, 2), because of the requirement for class I complexes in T cell development and immune recognition of pathogen-infected cells (43). Thus, it might be expected that the genes encoding proteins essential for peptide production and transport as well as for class I complex assembly are coordinately regulated. Indeed, class I heavy chain genes as well as β2m, TAP, and LMP genes are expressed at relatively high levels in mature lymphocytes; the expression of these genes is enhanced by IFNs and TNF-α, and in some tumors all of these genes are down-regulated, resulting in a loss of recognition by CTL (26). Moreover, mutations in the IRF1 binding site core sequence resulted in reduced constitutive activity of the TAP2 promoter (Figs. 6 and 7C). Since all these studies were performed with the pGL3/Enhancer construct, it is conceivable that the SV40 enhancer present in this construct increases TAP2 promoter activity. It is not clear whether an enhancer element is essential to IRF1-mediated constitutive and induced TAP2 promoter functions.

![Diagram](http://www.jimmunol.org/)

**FIGURE 8.** Different regulatory elements are associated with TAP (1 and 2) and LMP (2 and 7) genes in mice and humans. A, One imperfect and two perfect MED1 elements are associated with the mouse TAP2 gene. The TAP2 gene belongs to a novel group of genes containing a promoter that lacks a TATA box, has multiple transcription start sites within a 100-nt window, and a MED1 element. The major transcriptional start sites (detected by the RNase protection assay) are marked by an asterisk. The schematic map of the TK and HPRT promoters was adapted from the report by Scotto et al. (51). B, The mouse and human TAP2 5'UTRs have only one common regulatory element (CREB). C, Mouse TAP1, TAP2, LMP2, and LMP7 5'UTRs have only one common regulatory element (CREB).
The finding that the IRF1 binding element contributes to the constitutive promoter activity of the TAP2 gene agrees with other studies demonstrating the involvement of ICS in the regulation of basal promoter activity and supports the observation that some NFs, which are associated with IFN responsiveness, are expressed constitutively. Among the latter are components of the ISGF-3 (Stat1α/Stat1β/Stat2/ISGF-3γ) that binds to the ISRE (48), Stat1 (49), and also minute amounts of IRF1 (50). Min and Johnson (8) demonstrated that the IFN response elements of the human TAP1 gene are required for both constitutive activity and cytokine-induced stimulation of the human TAP1 promoter in HeLa cells. However, while the ICS (AAANNGAAA C/G T/C) was demonstrated that the IFN response elements of the human TAP1 gene are required for both constitutive activity and cytokine-induced stimulation of the TAP1 promoter, it was not necessary for IFN-γ-mediated induction. On the other hand, the GAS element (TTCNNNGAAA) was required for IFN-γ-mediated induction, but not for the constitutive activity or the TNF-α-mediated induction of the TAP1 promoter. In another cell system (normal lymphocytes), Lee et al. (15) demonstrated that Stat1 is required for basal expression of MHC class I genes. IRF1, which is a Stat1 target whose expression is substantially reduced in Stat1−/− mice, is likely to be the main downstream mediator of Stat1 in lymphocytes. It appears that in the mouse, the TAP2-associated IRF1 binding element mediates both the constitutive and the induced expression of TAP2 via its binding to IRF1. However, while the activity of the human TAP1 (as well as of the human LMP2 gene) promoter is assisted by factors binding to the NF-κB and Sp1 elements (7, 8), these elements are not obvious in the 5′UTR and flanking region of the mouse TAP2 gene (Fig. 2C). It is, therefore, likely, that the IRF1 binding site is the major regulatory element of the mouse TAP2 gene.

Most genes encoding proteins that participate in immune responses are regulated by members of the NF-κB/Rel family. Since the organization of the TAP2 gene is unique for MHC genes, characterized by the presence of an untranslated first exon, and as it is well known that first introns may contain regulatory elements, especially enhancers (51, 52), we analyzed the sequence of the first intron for the presence of NF-κB-like binding sites. Indeed, sequences homologous to both NF-κB-like and Sp1 sites are present in the TAP2 first intron. However, a detailed functional analysis of this intron, following its insertion into an SV40 promoter/LUC reporter system and transient transfection of E1A5-transformed cell lines, did not reveal the existence of an enhancer element (data not shown). Whether these sequences are functional when located in the natural context of the TAP2 gene is under investigation.

Similarly to the TAP1 and LMP2 genes (7, 19), the TAP2 promoter lacks a TATA box. Accordingly, transcription start sites were detected by the 5′RACE method (Fig. 2, B and C). Two additional start sites were detected by the RNase protection assay (Figs. 2C and 3). At least one transcription start site (−63 nt) was identified by both methods and is expressed in both leukemic (PD1.2 and PD1.1; data not shown) and fibroblast cell lines (IFN-γ-treated VAD12.79 and ASO5; data not shown), suggesting that this is a major TAP2 transcription start site. The four initiation sites observed in the leukemic cell line PD1.2 were induced by IFN-γ. The combined data localize the TAP2 promoter region to a sequence that spans −147 to −47 nt. As shown in Figs. 4 and 5, these 100 nt are required for maximal promoter activity, suggesting that all the transcription start sites and/or the DNA regulatory elements within this region contribute to promoter activity. Deletion of fragments containing 3′ transcription start sites (Figs. 4 and 5) or fragments containing 5′ transcription start sites and the IRF1/IRF2 binding site (Fig. 5) or the MED1 elements (Fig. 5) resulted in an approximately 50% reduction of LUC expression. Thus, each of the deleted fragments could mediate partial promoter activity, suggesting the existence of multiple promoter elements or alternative, independent promoters in this region able to mediate LUC expression. This promoter(s) can also function in the absence of the MED1 element. The deletion of the 5′-flanking sequences (from −199 to −419 nt; Fig. 4), did not affect TAP2 promoter activity, implying that at least in the presence of heterologous enhancer, the latter region is not essential for TAP2 promoter activity.

The MED1 element is associated with TATA-less promoters that have multiple initiation sites (39) (two examples shown in Fig. 8A). This downstream element appears to define a new class of RNA polymerase II promoters containing multiple start sites that span less than 100 nt. Mutations in the MED1 consensus sequence of the pgp 1 promoter reduced promoter activity. As illustrated in Fig. 8A, the TAP2 exon 1 harbors two perfect MED1 sequences and an imperfect one. Interestingly, the TAP2 transcription start sites detected by the RNase protection assay are located between the MED1 elements (Fig. 8A). Functional analysis of these elements and their effect(s) on transcription efficiency from the various transcriptional start sites await further investigation.

Sequence comparison of the mouse and human TAP2 gene 5′UTRs as well as computer analysis of the associated regulatory elements highlighted two interesting points. First, the organizations of the 5′UTRs of mouse and human TAP2 genes are identical (Fig. 1, A and C); both have an untranslated first exon and translation starts in exon 2. The sequence of this region is highly conserved among rodents (Fig. 1B) and probably among higher mammals (Fig. 1D), but there is only 54% homology between mouse and human sequences (Fig. 1D). Second, a computer search for known regulatory elements revealed that the 5′UTRs of the mouse and human TAP2 genes share only one common regulatory element, the CREB site, located at −597 and −552 nt in the mouse and human, respectively (Fig. 8B). These comparisons show that the 5′-flanking sequences of the mouse and human TAP2 genes have diverged significantly. Since these genes encode proteins of identical function, we assume that the transcriptional regulation of mouse and human TAP2 genes is dictated by the basal promoter elements and by unidentified elements that acquired similar regulatory functions. Of special interest is the lack of any sequence homology to IFN response elements in the 5′-flanking sequence of the human TAP2 gene.

A comparison between the human and mouse TAP1/LMP2 and mouse LMP7 and TAP2 genes (Fig. 8C) shows that the only common element shared by mouse TAP2, LMP2, LMP7, as well as by mouse and human LMP genes, is the CREB site (18). Cumulatively, these observations suggest that this element might have an important function in the regulation of genes associated with peptide production and transport. The TAP1/LMP2 intergenic mouse and human region contains GC-rich regions and an NF-κB site, but the human sequence contains also Sp1, ICS, and GAS functional elements (7, 8, 19). Thus, which element mediates IFN induction of the human TAP2 and the mouse TAP1, LMP2, and LMP7 genes in the absence of known IFN response elements remains an enigma.

While class I heavy chain genes are regulated by multiple elements and some of these are also shared with class II genes (53), the only regulatory element common to mouse TAP2 and class I heavy chain genes is the IRF1 binding element. This observation supports the idea that the IRF1 binding element, which is considered a secondary regulatory module for genes other than TAP2 and is acquired late in evolution (54) is a major regulatory element of the mouse TAP2 gene. The lack of conservation of common regulatory elements among class I, TAP, and LMP genes raises the possibility that the genetic linkage of these genes provides them with another mechanism to support their coordinate regulation.
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