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Alternative splicing and alternative initiation of translation explain the four forms of the Ia antigen-associated invariant chain

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The Ia antigen-associated invariant chain (In) exists in humans as four related polypeptides, p33, p35, p41 and p43, all associated with HLA-class II antigens. As described previously, two of these forms of In chain, p33 and p35, result from the use of two in-phase initiation AUG codons on the unique In p33 mRNA. In addition to cDNA clones derived from In p33 mRNA, we have isolated a new cDNA clone, called p41-1, which differs from p33-1 by an additional segment in the coding region. The DNA sequence encoding the segment unique to p41-1 was identified in the genomic sequence in the intron between exon 6 and 7, and we refer to it as exon 6b. Cells transfected with a full length p41 cDNA clone in an expression vector synthesize the two larger forms of the In chain, p41 and p43. We propose that the larger mRNA, encoding p41, results from alternative splicing of exon 6b, and that p41 and p43 result from the use of the two functional initiation AUG codons identified in p33 mRNA. Alternative splicing, together with alternative initiation of translation, allows therefore the synthesis of four related In chain polypeptides from a single gene.

Key words: Ia antigen/invariant chain/alternative splicing/translation initiation

Introduction

Since the discovery that eukaryotic genes are split into several exons, the synthesis of related forms of a given protein from a unique gene as the result of differential splicing patterns has been firmly established. Alternative RNA splicing with mRNAs differing in their protein coding region can be related to different steps in cellular differentiation, as reported for instance in the case of membrane and secreted forms of immunoglobulin heavy chains (Blattner and Tucker, 1984). RNA splicing can also be regulated in a tissue-specific way, with different pathways operating in different cell types (Breitbart *et al.*, 1985). As reported in this paper, the Ia antigen-associated invariant chain (In) represents a case where two related forms of a protein result from alternative splicing events within the same cell type.

The invariant chain is transiently associated with class II antigens of the major histocompatibility complex (MHC) during intracellular transport (Jones *et al.*, 1979; Charron and McDevitt, 1979). Class II (Ia) antigens are products of the highly polymorphic immune response genes located in man in the HLA-D region of the MHC. They function as restriction elements in the recognition of foreign antigens by regulatory T lymphocytes. Expressed primarily on macrophages, B lymphocytes and activated T lymphocytes, Ia antigens consist of two non-covalently associated glycoproteins, the α and the β chains (for a review, see Kaufman *et al.*, 1984). The HLA-D-associated In chain (also referred to as the Ii or the γ chain) is not polymorphic. We have previously cloned the cDNA and genomic genes for the In chain and reported the nucleotide sequence of the mRNA (Long *et al.*, 1983; Strubin *et al.*, 1984). From the sequence analysis, we concluded that the In chain does not have a signal sequence, and that it is inserted in the microsomal membrane with an inverted polarity compared with most transmembrane proteins, thus exhibiting an N-terminal cytoplasmic tail. Even though the In chain gene is located outside the MHC (Long *et al.*, 1983), it is usually regulated in a coordinate fashion with HLA class II genes (Collins *et al.*, 1984).

At the biochemical level, the In chain displays an interesting heterogoneity. In man, in addition to the well-characterized 33-kd In chain form (p33), no less than five other polypeptides are associated with class II antigens. Serological and biochemical analysis have indicated that most of these polypeptides are antigenically and structurally related to the major p33 form (Charron, 1983; Zecher *et al.*, 1984). We have previously reported that the use of two in-phase initiation AUG codons on a unique mRNA is responsible for the synthesis of two of these different forms of the In chain, p33 and p35 (Strubin *et al.*, 1986). In this communication we present evidence for the existence of an alternative splicing event as an additional mechanism responsible for the heterogeneity of the In chain in the same cell type. This, together with the alternative use of the two in-phase AUG codons, accounts for the synthesis of four distinct forms of the Ia antigen-associated In chain from a single gene.

Results

Multiple forms of the In chain are associated with Ia antigens

Figure 1 shows a typical example of two-dimensional gel electrophoresis of HLA-DR molecules, the prominent Ia antigens in

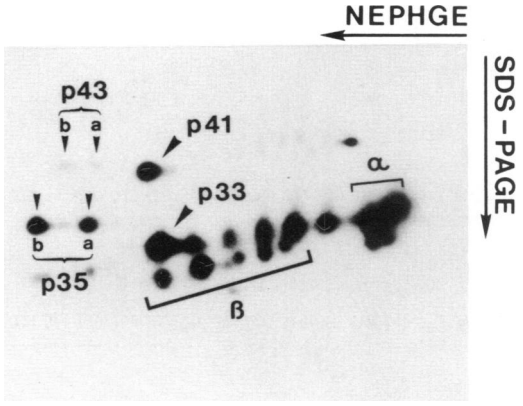


Fig. 1. Electrophoretic analysis of In chain heterogeneity. HLA-DR antigens were analyzed by two-dimensional gel electrophoresis following immunoprecipitation by monoclonal antibody D1-12 (Carrel *et al.*, 1981) from [^{35}S]-methionine-labeled HHK cells. First dimension is NEPHGE, with the basic side on the left. The position of α spots and of β spots is indicated by brackets. The different forms of the In chain are indicated by arrows. The additional spots extending from p33 up to the cluster of α-chain spots have been characterized as intermediates in p33 maturation (Machamer and Cresswell, 1984).

man, immunoprecipitated from the B cell line HHK with an anti-DR monoclonal antibody (mAb). The major form of the In chain associated with HLA-DR has an isoelectric point of 7.5 and an apparent mol. wt of 33 kd (p33). A larger and more basic polypeptide of 35 kd, usually appearing as a doublet, is also found (p35a and p35b). In addition to p33 and p35, one observes another group of DR-associated polypeptides of higher mol. wt. It consists of a major spot of 41 kd (p41) and two minor and more basic spots at 43 kd (p43a and p43b).

The origin of In chains p33 and p35 (a and b) is now well understood. The two forms result from the use of two in-phase translational initiation sites on the unique p33 mRNA (Strubin *et al.*, 1986). From the DNA sequence, p35 differs from p33 in having an additional 16-amino acid NH_2-terminal segment. Very little is known concerning the human p41 protein, except that it is also observed in cell-free translation products of In mRNA purified by hybridization selection (Claesson *et al.*, 1983; and unpublished). In mouse, it has been shown that p41 is structurally related to In p33 (Zecher *et al.*, 1984). Transfection experiments with a murine genomic Invariant chain clone revealed that the two polypeptides are encoded by a single gene (Yamamoto *et al.*, 1985). The two minor human p43 spots have not been detected previously.

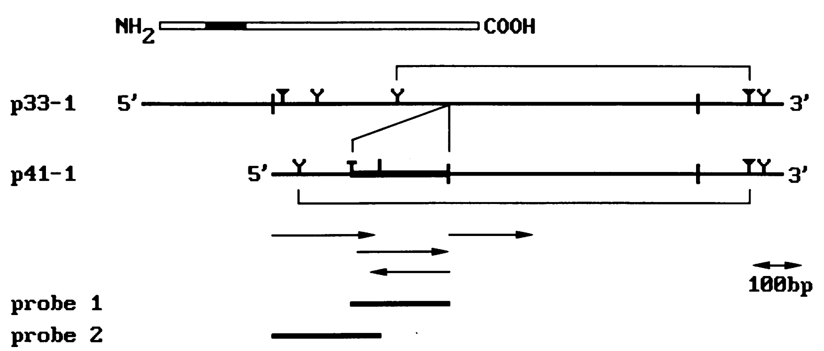
Evidence for two different In chain mRNAs species

A large number of cDNA clones have been isolated from a size-selected cDNA library derived from mRNA of the B cell line Raji (Wake *et al.*, 1982). The isolation of the nearly full-length cDNA clone p33-1 encoding p33 and p35 In chains (Figure 2A) and the complete sequence of the p33 mRNA have been previously reported (Strubin *et al.*, 1984). Based on restriction analysis, another type of In chain cDNA clone, called p41-1 (Figure 2A), was identified. Partial nucleic acid sequencing analysis revealed that clone p41-1 is identical to p33-1 except for an additional 192 bp long segment located in the coding region at position 632 from the cap site. The reading frame which codes for p33 and p35 remains open throughout this segment (Figure 2B). The mRNA from which clone p41-1 is derived has therefore the potential to direct the synthesis of a protein which would be identical to p33 except for a 64-amino acid additional segment located near the carboxy terminus.

cDNA clone p41-1 corresponds to a mature form of In mRNA

To exclude the possibility that clone p41-1 was derived from a splicing intermediate, poly(A)+ RNAs from various B cell lines were analyzed by Northern blot hybridization for the presence of a mRNA specific for clone p41-1. The same blot was successively hybridized with the segment unique to cDNA clone p41-1 (probe 1 in Figure 2A) and with the first exon (134 bp) of the In chain gene isolated from a genomic clone (kindly provided by J.Gorsky). As shown in Figure 3, an mRNA specific for probe 1 of clone p41-1 is easily detected in these preparations. Its migration corresponds to an In chain mRNA containing the additional segment observed in clone p41-1. With the

A



B

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         192                     200                                210                                  220
 P   P   K   V   L   T   K   C   Q   E   E   V   S   H   I   P   A   V   H   P   G   S   F   R   P   K   C   D   E   N   G   N   Y   L   P

CCA CCG AAA gTA CTG ACC AAG TGC CAG GAA GAG GTC AGC CAC ATC CCT GCT GTC CAC CCG GGT TCA TTC AGG CCC AAG TGC GAC GAG AAC GGC AAC TAT CTG CCA   727
cctgtcgcttcag-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---T--- --- --- --- --- ---T--- --- --- --- ---   9654
              ▲
                     230                                240                                  250                                  257
 L   Q   C   Y   G   S   I   G   Y   C   W   C   V   F   P   N   G   T   E   V   P   N   T   R   S   R   G   H   H   N   C   S   E   S   L

CTC CAG TGC TAT GGG AGC ATC GGC TAC TGC TGG TGT GTC TTC CCC AAC GGC ACG GAG GTC CCC AAC ACC AGA AGC CGC GGG CAC CAT AAC TGC AGT GAG TCA CTG   832
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---G--- --- --- --- --- --- --- -   --- --- --- --- --- --- --- -gtaagcagtg   9759
                                                                                                                                  ▲
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Fig. 2. Representation of p41-1 cDNA and sequence of the insert segment. (**A**) cDNA clones p33-1 and p41-1 were isolated from a size-selected cDNA library as described (Strubin *et al.*, 1984). The upper line shows the coding region with the transmembrane region boxed. The segment unique to p41-1 cDNA is represented by a thick line. Sites for restriction endonucleases are indicated with the following symbols: *Pst*I (|); *Hin*dIII (▼); *Nco*I (Y); *Rsa*I (T); *Sma*I (⊥). The *Rsa*I site represented on the p41-1 map is not unique. The *NcoI*/*Hin*dIII fragments used in the construction of full-length p41 cDNA are designated by brackets. The sequencing strategy is shown below the cDNAs representation. Arrows indicate the direction and extent of sequence analysis performed according to Maxam and Gilbert (1980). The two boxes at the bottom represent the restriction fragments used as probes in Northern and RNase protection experiments presented in Figures 3 and 6, respectively. (**B**) Nucleotide sequence of the region of difference between the two In chain cDNAs. The borders of the segment unique to p41-1 cDNA are indicated by arrows. Numbering starts with the first nucleotide of the p33 mRNA (Strubin *et al.*, 1984). The deduced amino acids sequence is given in the single-letter code. The genomic sequence identified by homology with this segment is shown below. Hyphens indicate identity with p41-1 cDNA sequence. Nucleotide differences, which are most likely due to sequencing errors, are indicated.

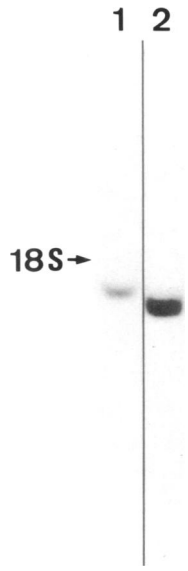

Fig. 3. Detection of a mature mRNA specific for p41-1 cDNA clone. 10 μg of poly(A)$^+$ RNAs from B cell lines Raji, HHK, DR4/6, QBL and B cells from a patient with chronic lymphocytic leukemia were separated by electrophoresis on a 1.4% agarose gel and transferred to Biodyne filter as described in Materials and methods. The results with RNAs from all sources were the same as with HHK poly(A)$^+$ RNA presented here. The same filter was hybridized with ^{32}P-labeled SP6 polymerase transcript of: **lane 1**, probe 1 depicted in Figure 2 and containing the p41-1 insert coding sequence, and **lane 2**, the entire first exon isolated from a genomic clone. To optimize discrimination between the two mRNAs, filters were exposed on film for different periods. Consequently, the relative abundance of the two mRNAs cannot be determined from this experiment.

first exon as probe, in addition to p33 mRNA, the mRNA specific for clone p41-1 is also visible, but only after a long exposure. It indicates that this region is common to both mRNAs, and that p41 mRNA is present in lower amount than the p33 mRNA. The poor resolution of these gels does not allow a complete separation of the two different mRNAs. From this experiment, we concluded that clone p41-1 was derived from a mature mRNA, called p41 mRNA, which has the capacity to direct the synthesis of a protein related to the p33 In chain.

p41 mRNA is the result of a differential splicing event

Southern blot data and analysis of genomic clones have supported the existence of a single In chain gene (Kudo *et al.*, 1985; and unpublished). From a comparison of the sequence of p41-1 cDNA with the In chain gene sequence published by Kudo *et al.* (1985), we observed that the position corresponding to the boundary of the additional segment of clone p41-1 is precisely at the splice junctions between exon 6 and exon 7. Moreover, the complete sequence of the additional intervening segment of clone p41-1 was found in the sequence of the genomic clone within the intron separating exons 6 and 7, 1353 bp from the 3′ end of exon 6 (Kudo *et al.*, 1985; Figure 2B). p41 mRNA contains therefore an additional coding region, encoded in an additional exon denoted 6b (Figure 4A). This segment is absent in p33 mRNA. Since the In chain gene is unique, the two mRNAs must be derived from a common precursor according to the different splicing patterns indicated in Figure 4A.

p41 mRNA codes for both the p41 and the p43 forms of the In chain

A transient expression assay with cDNA clones transfected into simian COS cells was used to determine which product is en-

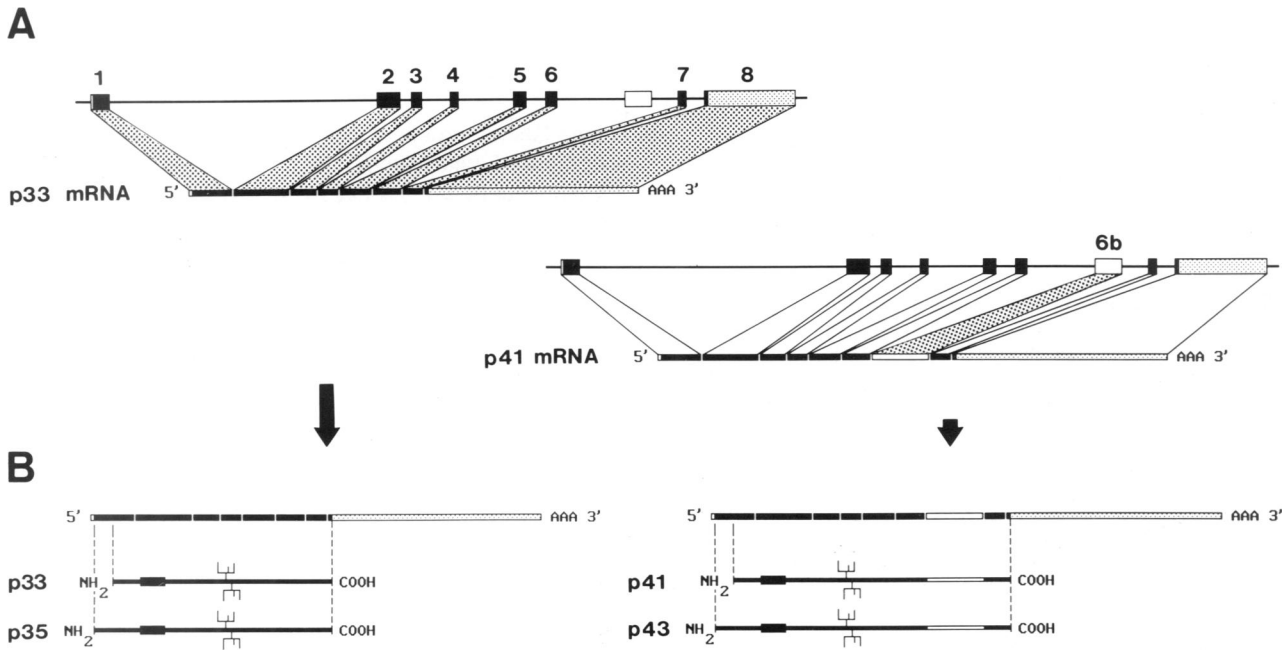

Fig. 4. Different patterns of splicing that yield In chain p33 and p41 mRNA. (**A**) The structure of the In chain gene is according to Kudo *et al.* (1985). Solid boxes represent exons, with black areas for protein coding and dotted areas for untranslated regions. Exons are represented on a larger scale than introns. The newly identified 6b exon which is only found in mature p41 mRNA is represented by a white box. The structure of the resulting mRNAs is shown in the same way but on a different scale. (**B**) Schematic representation of the four polypeptides resulting from the use of two in-phase initiation codons on both p33 and p41 mRNAs. The structure of the mRNAs is diagrammed on the top as in **A**. The last two lines represent the different forms existing for the human In chain: p35 and p33, initiation of translation at the first and the second AUG on p33 mRNA, respectively; p43 and p41, initiation at the first and the second AUG on p41 mRNA. The transmembrane region is boxed.

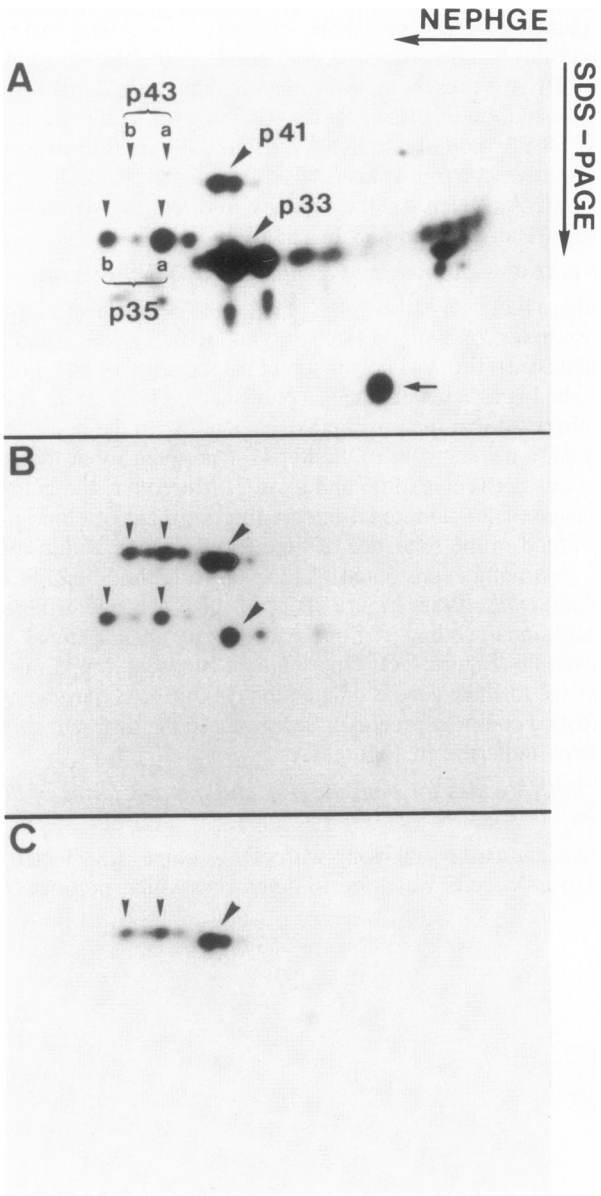

Fig. 5. Two-dimensional analysis of In chain molecules immunoprecipitated from COS cells transfected with full-length p41 cDNA. Cells were labeled with [^{35}S]methionine for 2 h, and In chain molecules were immunoprecipitated from cell lysates with the anti-In monoclonal antibody VIC-γ1 (Quaranta *et al.*, 1984). (**A**) Control B cell line HHK. The different forms of the In chain are indicated by arrows. The horizontal arrow indicates the additional In chain form which is absent in anti-DRβ immunoprecipitates (Figure 1). The other spots have been defined in Figure 1. (**B**) COS cells labeled with [^{35}S]methionine 48 h after transfection with full-length clones p33 and p41. The arrows indicate the same forms of the In chain seen in **A**. Co-transfection with full-length p33 cDNA encoding p33 and p35 (a and b) was performed to facilitate correct superimposition of the autoradiographs. (**C**) As in **B**, but COS cells were transfected with only p41 cDNA clone.

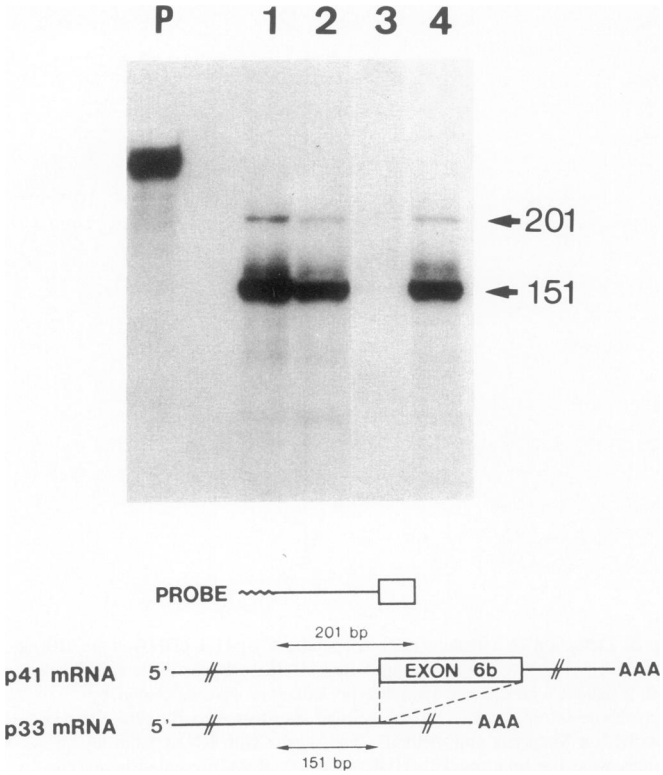

Fig. 6. Determination of the relative abundance of the two In chain mRNA species. The diagram of the cRNA probe and the expected fragments after RNase digestion are shown at the bottom. The probe is a ^{32}P-labeled SP6 polymerase transcript from the *PstI*/*SmaI* restriction fragment of p41-1 insert shown in Figure 2 (probe 2). The wavy line represents SP6 vector sequences. The lengths of the fragments protected by p41 mRNA and p33 mRNA are 201 and 151 bp long, respectively. The labeled probe was hybridized with 10 μg of total RNA from : **lane 1**, Raji; **lane 2**, HHK; **lane 3**, 143B cell line and **lane 4**, IFN-γ-treated 143B cells. P: the probe alone. The RNase digestion products were analyzed on a 20% acrylamide–urea sequencing gel.

coded by p41 mRNA. Since p41-1 cDNA was incomplete at its 5′ end, a full length p41 cDNA was constructed in an SV40-derived expression vector as outlined in Materials and methods. This clone was then used to transfect In chain-negative monkey cells (COS) in the presence or absence of the full-length p33 cDNA clone which is known to code for the p33 and p35 (a and b) forms of the In chain (Strubin *et al.*, 1986). [^{35}S] Methionine-labeled products were immunoprecipitated with a mAb specific for In and analyzed by two-dimensional electrophoresis. As shown in Figure 5, three major spots could be resolved when transfection was done with clone p41 alone. The major spot co-migrates precisely with the p41 protein present in the control B cell lysate immunoprecipitated with the same mAb, whereas the two additional more basic spots correspond to p43 a and b. Thus, the data demonstrate that the p41 cDNA clone codes for the p41 protein previously characterized *in vivo* as well as for the p43 form.

Determination of the quantitative ratio between the p33 and p41 In chain mRNAs

The Northern experiment presented in Figure 3 does not allow precise estimates of the relative amount of the p33 and p41 mRNAs. To address this question, an RNase protection experiment was performed with the use of a probe isolated from clone p41-1 (probe 2 in Figure 2A) and subcloned in an SP6 vector. It consists of a restriction fragment containing the 5′ end of p41-1 cDNA common to both mRNAs and extending 50 bp into the sequence unique to p41 mRNA. The ^{32}P-labeled transcript was hybridized to total RNA, digested with RNase and the protected fragments were fractionated on a denaturing polyacrylamide gel (Figure 6). The 201 bp and 151 bp bands result from protection with p41 and p33 mRNA, respectively. The intensity of the bands was quantitated by densitometry, and the values were adjusted

to reflect labeled nucleotide content. It can be concluded from this experiment that p41 mRNA represents ~10% and p33 mRNA 90% of In chain mRNA. This is true for several different B cell lines as shown in Figure 6 in the case of Raji and HHK lines.

We have also investigated the expression of the p41 mRNA under conditions of induction of In chain gene expression by immune interferon (IFN-γ). Both HLA class II mRNAs and In chain mRNA are known to be induced by this lymphokine in a variety of cell types (Collins *et al.*, 1984). As seen in Figure 6, IFN-γ induces both p33 and p41 In chain mRNAs in cell line 143B, and the ratio of the two forms is similar to that observed in B cells.

Table I. Splicing signals involved in the alternative splicing patterns

	Donor	Acceptor	
Exon 6	GAAAG GTACAG	TCCTTTGCAG AGTCA	Exon 7
Exon 6_b	CAGTG GTAAGC	GTCGCTTCAG TACTG	Exon 6_b
	5′ $\begin{matrix}C\\A\end{matrix}$AG $GT\begin{matrix}A\\G\end{matrix}$AGT ... Intron ... $Y_6N\begin{matrix}C\\T\end{matrix}$AG G 3′		

Asterisks indicate identity with the consensus sequence for splice sites shown at the bottom (Breathnach and Chambon, 1981). N: unspecific nucleoside; Y: unspecific pyrimidine nucleoside.

Discussion

The biosynthesis of two similar forms of a given protein as the result of different splicing events, producing different mRNA species, has been described for a number of genes, both in viral and non-viral systems. The resulting mRNAs produced can differ either in their leader sequence (Nabeshima *et al.*, 1984), in a region coding for an internal exon (King and Piatigorsky, 1983; De Ferra *et al.*, 1985) or in their 3′ coding exon (Kress *et al.*, 1983). We have shown here that the In chain gene represents such a case where an additional splicing event results in an mRNA containing an additional exon in the coding region and directing the synthesis of a protein variant of the main gene product.

A new In chain cDNA clone, called p41-1, derived from a mature mRNA and differing from the previously identified In chain p33 cDNA (Strubin *et al.*, 1984) by an additional 192-bp segment in the coding region, has been isolated and sequenced. Comparison of this clone with the sequence of the In chain gene deduced from genomic clones (Kudo *et al.*, 1985) revealed that the sequence corresponding to the additional segment in the p41 cDNA is located within the intron between exon 6 and exon 7 of the genomic sequence published by Kudo *et al.* (1985). One can therefore refer to that segment as exon 6b. The more abundant In chain p33 mRNA is produced when exon 6 is linked directly to exon 7, as indicated in Figure 4A. The alternative splicing product is generated by the insertion of the transcript of the new exon (6b) in the mature p41 mRNA. Transfection experiments revealed that this longer mRNA directs the synthesis of the larger forms of the HLA-DR-associated In chain, p41 and p43.

The availability of probes specific for the two alternative splicing forms of mRNA allowed us to estimate the relative amount of p33 and p41 mRNA. In B cells, which express HLA-class II and the In chain genes constitutively, there is a 10-fold excess of p33 mRNA over p41, indicating preferential splicing between exon 6 and 7, without inclusion of exon 6b. This was observed in several different B cell lines. Interestingly, the induction of an unexpressed In gene by IFN-γ in another cell type resulted in the same pattern of expression of p33 and p41 mRNA, indicating in this case also, a much less efficient splicing of exon 6b. Therefore, under both constitutive and induced conditions of gene expression, and in two different cell types, the two specific splicing events responsible for the two forms of the In chain occur with the same relative efficiency. Using the flanking sequences of exon 6b, as well as the flanking sequences of exon 6 and 7 (Kudo *et al.*, 1985; O'Sullivan *et al.*, 1986), we have identified classical consensus donor and acceptor splicing sites for the two alternative splicing events generating p33 or p41 mRNA (see Table I). The low frequency of insertion of exon 6b in the mature In mRNA is therefore not due to a deviation from the highly conserved GT/AG dinucleotides bordering mammalian introns as has been described in the case of the αA-crystallin gene (King and Piatigorsky, 1983).

From the DNA sequence, the In chain polypeptide p41 contains 64 additional amino acids compared with p33, and these are located 24 residues from the carboxy terminus. Since the polarity of membrane insertion of the In chain is inverted relative to most transmembrane proteins (Strubin *et al.*, 1984), the structural difference between p33 and p41 concerns their extracytoplasmic region. This difference does not prevent the association of p41 with Ia antigens since p41 is immunoprecipitated with an anti-DR mAb, together with HLA-DR α and β chains. Two additional potential N-linked glycosylation sites are found in p41 form at positions 240 and 254 (Figure 2A). They are probably not used, since the difference in size between p33 and p41 made *in vivo* is the same as that observed under cell-free conditions where glycosylation does not take place (data not shown). The most striking feature of the additional polypeptide segment specific of p41 is that it contains six cysteine residues (Figure 2B), whereas there is only one in the rest of the molecule. In chain p33 and p41 must therefore display a very different three-dimensional structure.

When HLA-DR molecules are immunoprecipitated with an anti-DR mAb, the major form of the In chain observed is p33. Most DR α/β dimers are therefore associated with p33 alone. From similar immunoprecipitations it is clear that p41 is also associated with DR α/β complexes. In these cases, p41 could either take the place of p33 in the association with DR, or it could be linked to DR in addition to p33. The use of an antiserum directed against the peptide segment unique to p41 could clarify this issue. The exact role of the In chain is still unknown. It was believed to be involved in the transfer of Ia antigens to the plasma membrane (Owen *et al.*, 1981; Kvist *et al.*, 1982). However, from recent transfection experiments, we have concluded that cell surface expression of HLA-DR occurs in the absence of the In chain (Sekaly *et al.*, 1986). We therefore consider it more likely that the In chain is implicated in interactions of Ia molecules with foreign antigens following internalization and cleavage within antigen presenting cells, and that it is thus involved in the complex process of antigen processing. A role for each of the distinct forms of the In chain in such a process could be envisaged.

A unique feature of the expression of In p33 mRNA is the use of two in-phase AUG codons for alternative initiation (Strubin *et al.*, 1986). This generates, from the same mRNA, two distinct polypeptide chains differing in their NH_2 end, p33 and p35 (a and b). This translational mechanism accounts therefore for the p33 and p35 heterogeneity of the In chain. The larger form of In mRNA which results from splicing of exon 6b (p41 mRNA)

could also display alternative initiation at the same two functional initiation codons. Interestingly, monkey COS cells transfected with cDNA clone p41 synthesize not only protein p41 but also p43 a and b, these three polypeptides corresponding exactly to the p41, p43a and p43b In chains associated with Ia molecules in B cell lines. We conclude therefore that, in the case of p41 mRNA, the p41 and p43 protein products result from an alternative use of the two functional in-phase initiation AUGs triplets, as has been demonstrated for proteins p33 and p35 (Figure 4B).

As shown in Figure 4, the primary transcript derived from the unique In chain gene is spliced differently to yield two distinct mature mRNAs. The two transcripts differ in that exon 6b is found only in the less abundant p41 mRNA. In addition, the existence of two functional in-phase AUG initiation codons at the 5′ end of the In chain mRNAs allows an alternative initiation of translation. Two polypeptides with a different NH_2-terminal segment are then produced from each of these two In chain mRNAs. The In chain gene represents therefore an exceptional case where a single gene encodes four variant forms of a given protein that are generated by two distinct mechanisms operating at the splicing and at the translational level, respectively.

Materials and methods

Plasmids

The full length In chain p33 cDNA clone with both ATG initiation codons has been constructed in an SV40-derived expression vector as described elsewhere (Strubin *et al.*, 1986). The full length In chain p41 cDNA was constructed as follows. The *NcoI*/*Hin*dIII fragment of clone p41-1 (bracket in Figure 2A), which contains the segment unique to p41 mRNA, was inserted in the full length p33 cDNA in place of the corresponding *NcoI*/*Hin*dIII fragment of clone p33-1. The resulting construct was then used for transient expression experiments.

Cell lines

Human B-lymphoblastoid cell lines Raji (human Burkitt's lymphoma) and HHK (EBV-transformed cell line) were used as the source of mRNA. The simian COS-7 cells (Gluzman, 1981) have been described. 143B is a human osteosarcoma cell line (ATCC CRL8303).

Preparation of RNA and screening of the cDNA library

Total RNA (de Préval and Mach, 1983) and poly(A)$^+$ RNA (Long *et al.*, 1982) were prepared as described. The cDNA clones were constructed from an enriched mRNA fraction as reported by Wake *et al.*, (1982). Screening of the cDNA library and preparation of plasmid DNA have already been described (Long *et al.*, 1983). The 5′ end labeling of DNA and nucleotide sequence determination were according to Maxam and Gilbert (1980).

Northern blot analysis and RNase protection experiment

RNA was denatured in glyoxal, electrophoresed in a 1.4% agarose gel before being transferred to Biodyne filter as described (de Préval and Mach, 1983). The hybridization with the SP6 polymerase [α-^{32}P]UTP-labeled transcript (probe 1 in Figure 2A) was in 50% formamide, 50 mM Pipes pH 6.0, 0.8 M NaCl, 2 mM EDTA 0.1% SDS, 2 × Denhart and 1 mg/ml yeast RNA at 58°C for 15 h. Filters were washed according to Melton *et al.* (1984).

The probe used in the RNase protection experiment shown in Figure 6 was a SP6 polymerase [α-^{32}P] UTP-labeled transcript from a *PstI*/*Sma*I restriction fragment derived from cDNA clone p41-1 (probe 2 in Figure 2A). Transcription reaction and RNase protection were carried out exactly as described (Melton *et al.*, 1984), except that RNase digestion was performed at 25°C. After ethanol precipitation, samples were denatured and loaded on a 20% acrylamide–8 M urea sequencing gel.

Transfection, radiolabeling of cells and immunoprecipitation

Transfection of the COS cells by the calcium phosphate co-precipitation technique, and radiolabeling were performed as described (Graham and Van der Eb, 1973; Strubin *et al.*, 1986), except that cells were labeled for 2 h instead of 6 h. The reactivity of the monoclonal antibodies used in this study has been described in previous publications (D1-12 in Carrell *et al.*, 1981; VIC-γ1 in Quaranta *et al.*, 1984). Immunoprecipitation of translation products and two-dimensional gel electrophoresis have already been described (Long *et al.*, 1982).

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