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A cassette vector for high-level reporter expression driven by a hybrid invariant chain promoter in transgenic mice

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Abstract

A plasmid cassette vector was designed to generate transgenic mice expressing reporter cDNAs at high levels in antigen-presenting cells under the control of the murine invariant chain (Ii) promoter. Analysis of several transgenic mice harboring a chimeric Ii cDNA placed in this vector showed that it can drive expression of the reporter protein to levels comparable to those of endogenous Ii. Furthermore, its expression pattern overlaps quite well with that of endogenous Ii. This vector should therefore be a convenient and versatile tool for the generation of transgenic mouse lines in which a desired protein may be expressed in Ii-positive cells at levels similar to those of endogenous Ii. Such a vector would be ideal for complementation studies of Ii-deficiency by specific Ii variants.

Keywords: Transgenic mouse; Vector; Invariant chain; Major histocompatibility complex

1. Introduction

The invariant chain (Ii) is an ancillary molecule endowed with chaperone and intracellular targeting functions which plays an essential role in the transport and maturation of class II molecules of the major histocompatibility complex (MHC-II) (Castellino et al., 1997). It helps in their heterodimeric assembly in the endoplasmic reticulum, guides their transport towards compartments of the endocytic pathway, and shields the antigen-binding groove until proper peptide loading can be effected. Fittingly, the tissue and cell-type distribution of Ii gene expression closely matches that of MHC-II (Long, 1985), but the Ii gene is expressed at significantly higher levels than those of αβ class II genes.

Analyzing the function of Ii isoforms and variants has previously relied on transgenesis to complement knockout mutations (e.g., Naujakos et al., 1995; Shachar et al., 1995; Serwe et al., 1997; Barton and Rudensky, 1998). Several of these studies (Naujakos et al., 1995; Shachar et al., 1995) have used the pDOI-5 cassette vector to drive transgene expression. Since this vector is based on promoter and enhancer elements of MHC-II genes (Kouskoff et al., 1993), the levels of Ii expression observed in the transgenic mice have often been considerably less than those of

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normal endogenous Ii gene products, severely complicating the interpretation of these experiments. Only when the transgene contained large segments of Ii genomic sequences was respectable expression achieved (Serwe et al., 1997; Barton and Rudensky, 1998). Yet manipulation of whole genomic fragments can be cumbersome, particularly if one wishes to target the expression of genetic elements other than Ii itself. We have thus created a cassette vector that directs reliable high-level expression of cDNA sequences in Ii-positive antigen-presenting cells.

2. Results and discussion

2.1. The vector

To boost expression levels while maintaining tissue specificity, we replaced several elements from the existing pDOI-5 vector: the promoter region (from the MHC-II Eα gene) and the splice element (from rabbit β-globin). In their place was inserted a segment of the Ii gene, bringing together the promoter region with the enhancer elements from the first intron. The promoter region of the resulting plasmid (pDOI-6, Fig. 1) is comprised of residues −824 to +1 of the Ii gene (numbering starts from the transcription initiation site, according to Zhu and Jones, 1989; residues 991–1822 in Fig. 1). It seemed preferable that translation of reporter cDNAs be initiated from their own initiation codons which necessitated the elimination of ATG motifs from the vector upstream of the cDNA cloning sites. To this end the normal Ii translation initiation codon at +90 was mutated along with two other adjacent ATGs, and replaced with a unique *Bam*HI site. This *Bam*HI site was followed by the first intron of the Ii gene which contains the enhancer element (Zhu and Jones, 1990) as well as splice donor and acceptor sites. A unique *Cla*I site was introduced directly downstream of the splice acceptor site. The remainder of the plasmid segment from pDOI-5 provides the 3′ untranslated region and poly-adenylation signals from rabbit β-globin as well as the plasmidic replication origin and ampicillin resistance. We preserved the −2172 to −1180 region of the Eα gene upstream of the Ii promoter, which provides enhancer activity in B lymphocytes (Koch et al., 1989). Either the

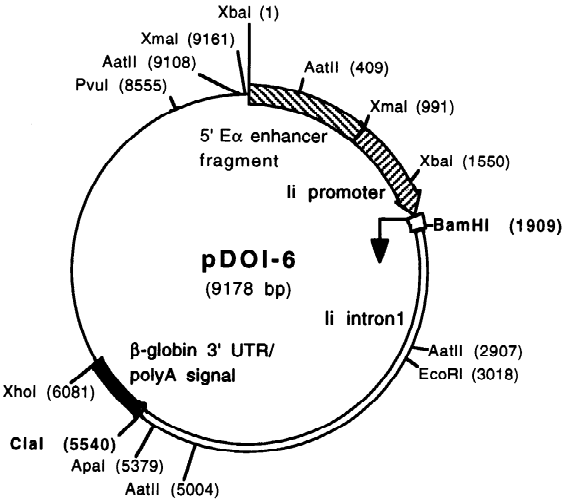

Fig. 1. Schematic representation of the pDOI-6 expression vector. The Ii-promoter and the upstream Eα enhancer fragment are indicated by a thick, striped arrow. The small black arrow indicates the position of initiation and the direction of transcription. Open boxes represent sequences derived from the first exon, first intron and second exon of Ii. The intron is indicated as a narrower box. The β-globin-derived sequences are depicted as a shaded box and the pBR322-derived sequences as a thin line. The unique *Bam*HI and *Cla*I sites that can be used for inserting cDNAs are indicated in bold. The Ii-promoter fragment was generated by PCR using Pfu-polymerase with OLA129/Sv ES cell DNA as template. The exon1–intron1–exon2 fragment was generated by using a Boehringer long-range PCR kit with a Ii genomic clone (Stockinger et al., 1989) as template.

*Bam*HI and *Cla*I sites can in principle be used to insert cDNAs containing their own translation initiation codon, although we have only tested the *Cla*I site so far.

2.2. Generation of test transgenic mice

To test the activity of the pDOI-6 vector in mice, we inserted a chimeric Ii cDNA as a reporter into the *Cla*I site. The CLIP region of this Ii variant was replaced by a segment of moth cytochrome c (MCC) corresponding to the dominant E^k-restricted T cell epitope. The C-terminus of this protein was also enlarged with a 42-amino acid tail (Nakano, van Santen, Benoist and Mathis, unpublished data). This chimeric protein, Ii-MCC, does not associate with MHC class II molecules and is therefore unable to fulfil its role as a chaperone of these MHC proteins

(data not shown). Its effects on T cell selection and antigen recognition are currently being studied and will be reported elsewhere. To generate transgenic lines, most of the bacterial backbone of pDOI-6 was removed by digestion with *Xho*I and *Pvu*I (see Fig. 1), and the purified fragment was injected into fertilized mouse eggs (C57Bl/6xSJL F2). The resulting mice were tested for integration of the transgene by PCR analysis of tail DNA. Several founders were thus obtained (hereafter referred to as Tg^+ mice). Mice were kept in the conventional animal facility of the institute in accordance with institutional guidelines.

2.3. Expression of pDOI-6-driven reporter transgenes

Transgenic lines derived from four independent founders were tested for expression of reporter RNA in thymus and spleen via quantitative S1-nuclease mapping experiments. Representative results for three of these lines are illustrated in Fig. 2. The end-labeled probe used for these experiments yields distinct fragments when protected by transgenic or endogenous Ii transcripts (Fig. 2a; Ii-Tg and Ii-end., respectively), allowing for a direct comparison of the relative expression level of the two RNA species. Expression of Ii-Tg RNA in the different transgenic lines ranged from about 6% (line 17) to 150–200% (line 34), relative to the expression of endogenous Ii (Fig. 2b). Based on the size of the probe fragment protected by the transgenic RNA, the splicing of the intronic sequence of pDOI-6 also appears to occur correctly. The pDOI-6 vector thus drives reporter RNA expression at levels that readily reach those of the endogenous Ii gene.

RNA was isolated from different organs of mice from lines 34 and 50 to assess whether reporters expressed from pDOI-6 display the same expression pattern as endogenous Ii (Fig. 2c). This was the case in most of the organs tested (thymus, spleen, lymph node, kidney and lung) for both lines. Importantly, the relative expression of the Ii-MCC transgenes versus endogenous Ii did not change between the different organs, indicating that at least in these organs transgene expression is controlled similarly to that of endogenous Ii. There was one clear difference — abundant Ii-Tg mRNA was detected in the livers

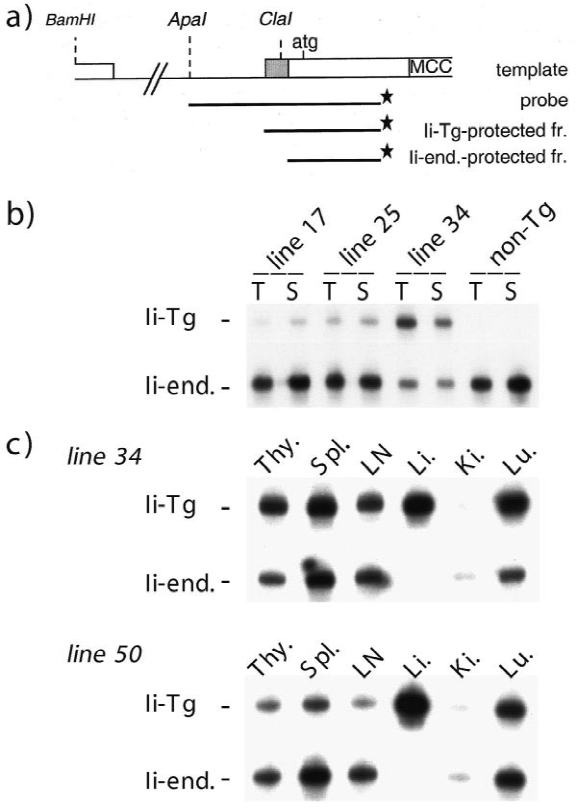

Fig. 2. Analysis of expression of Ii-Tg RNA in transgenic mice. (a) Schematic representation of the S1 probe and fragments protected by mRNA derived from the Ii-MCC transgene (Ii-Tg) and the endogenous Ii gene (Ii-end.). A reverse primer complementary to bases 142–159 of the Ii cDNA was end-labeled using [γ-^{32}P]dATP and T4 polynucleotide kinase (indicated by an asterisk), elongated by Klenow fragment with the Ii-MCC construct cloned into pDOI-6 as template, digested with *Apa*I and size-purified on a polyacrylamide gel. The purified probe is complementary to bases 1–159 and 15 upstream untranslated bases of Ii-end. mRNA and complementary to the same translated and untranslated bases of Ii-Tg RNA plus 28 extra upstream untranslated bases up to the splice acceptor site of pDOI-6 (indicated by the grey box). (b) Quantitative S1-nuclease mapping of RNA purified from thymus (T) and spleen (S) from mice from the indicated lines. The purified probe was incubated o/n with the indicated RNA samples and subsequently digested with S1 nuclease. Protected fragments were run out on a polyacrylamide gel together with a size marker, and quantification was performed by densitometric scanning of exposed X-ray films and by exposing the gel to phospho-imaging plates. (c) Tissue distribution of Ii-Tg RNA expression. RNA was prepared from thymus (Thy.), spleen (Spl.), lymph node (LN), liver (Li.), kidney (Ki.), and lung (Lu.) and quantitative S1-nuclease mapping was performed as described above.

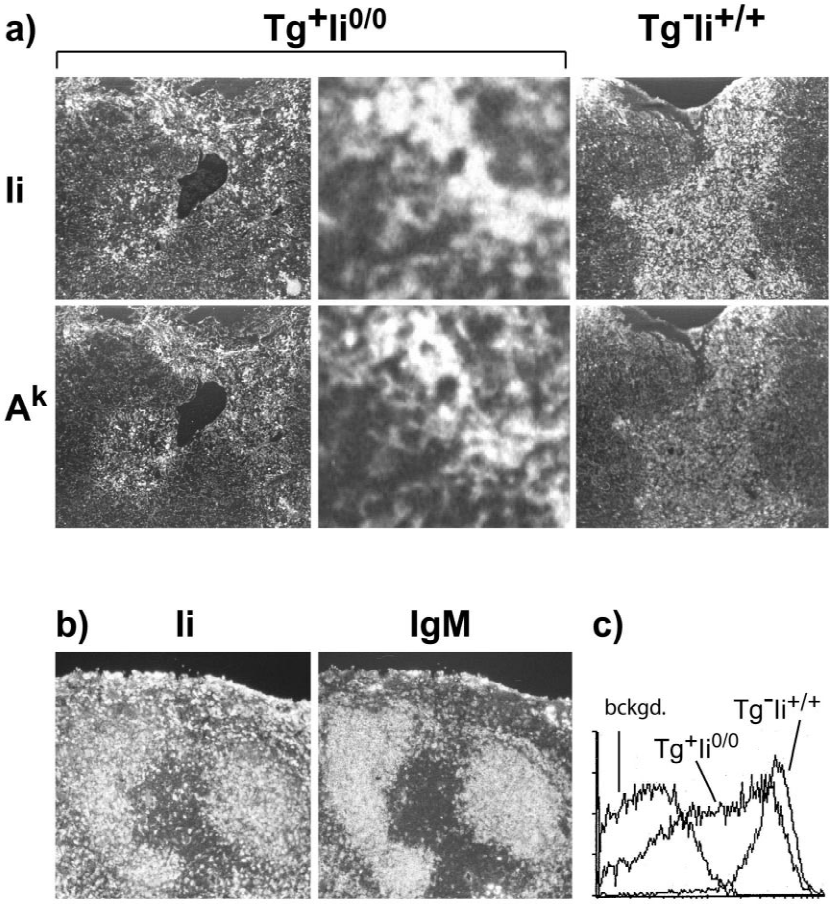

Fig. 3. Immunohistochemistry on thymus and spleen of Ii-MCC-expressing mice. (a) Thymus cryostat sections from a $Tg^{+}Ii^{0/0}$ mouse and a B10.BR ($Tg^{-}Ii^{+/+}$) mouse were double-stained with the rat anti-murine Ii mAb In1 (upper panels) and the murine, A^{k}-specific mAb 10.2.16 (lower panels), followed by Alexa594-conjugated goat anti-rat IgG(H+L) and FITC-coupled goat anti-mouse IgG ($F(ab')_2$ fragment (Fcγ-specific). A higher magnification of the In1 and 10.2.16 staining at the cortico-medullary boundary of the thymus of the $Tg^{+}Ii^{0/0}$ mouse is shown in the central panels. (b) Adjacent frozen spleen sections from a $Tg^{+}Ii^{0/0}$ mouse were stained with mAb In1 followed by Alexa594-conjugated goat anti-rat IgG(H+L) (left panel), and a FITC-coupled goat anti-mouse IgM antibody (right panel). (c) Spleen cells from a $Tg^{+}Ii^{0/0}$ mouse and a B10.BR ($Tg^{-}Ii^{+/+}$) mouse were permeabilized with saponin and stained with mAb In1, followed by a FITC-coupled goat anti-rat IgG(H+L) antibody and a PE-coupled goat anti-mouse IgM antibody. A sample stained with the the secondary reagents only is included as a background control (bckgd). Gated on IgM-positive cells.

of transgenic mice from both lines. There is no clear explanation for this mis-expression, although there is certainly precedent for unexpected expression stemming from the combination of enhancer/promoter elements from different origins. On the whole, however, the pDOI-6 cassette is active in all MHC-II positive tissues.

To verify that MHC-II positive cells expressed the reporter carried in pDOI-6, immunohistology was performed on thymi and spleens of transgenic mice. Because of the lack of reagents which discriminate endogenous Ii from Ii-MCC proteins, mice of line 34 were crossed with Ii-deficient mice ($Ii^{0/0}$ mice; Viville et al., 1993). Thymus sections from a $Tg^{+}Ii^{0/0}$ mouse were doubly stained with the Ii-specific mAb In1 (Koch et al., 1982) and the $A\beta^{k}$-specific mAb 10.2.16 (Oi et al., 1978). Thymus sections from an inbred B10.BR mouse were included as controls. From the representative sections in Fig. 3a, it is clear that the expression pattern of the Ii-MCC protein overlaps quite well with that of the endogenous $A\beta^{k}$ protein: strong and heterogeneous

in the epithelial and dendritic cells of the medulla, less intense in the fine network of epithelial cells in the cortex. Indeed, essentially all class II-positive cells also express the reporter (and vice versa), as evidenced on higher magnification (Fig. 3a, middle panels).

Staining of spleen sections from a $Tg^{+}Ii^{0/0}$ mouse with the In1 mAb (Fig. 3b, left panel) also demonstrated the synthesis of the Ii-MCC protein by B cells in the follicles (identified by staining of serial sections with anti-IgM, right panel), and by non-B cells: red pulp macrophages, dendritic cells in the T areas (Fig. 3b). To confirm the presence of the Ii-MCC protein in B cells, we performed flow cytometry analysis of permeabilized splenocytes from line 34 with the In1 mAb, surface counterstained with anti-IgM. The profiles in Fig. 3c show that there is strong Ii-MCC expression in many of the B cells, at levels similar to that of normal Ii.

3. Concluding remarks

It thus appears that pDOI-6 fulfills the goal of a readily manipulable cassette for expression of reporter cDNAs at high levels in MHC class II-positive cells of transgenic mice. The inclusion of promoter and enhancer elements from the Ii gene, together with an enhancer specifically active in B cells from an MHC-II gene, promotes its expression in all class II-positive cells of the thymus and peripheral lymphoid organs, with an efficiency that mirrors that of the Ii gene itself. The only unexpected finding was the detection of reporter RNA in the liver of two independent lines. This vector should therefore be useful for expression of Ii variants, or of other desired proteins in cells involved in the selection and/or activation of T lymphocytes.

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References

Barton, G.M., Rudensky, A.Y., 1998. An altered invariant chain with an antigenic peptide in place of CLIP forms SDS-stable complexes with class II αβ dimers and facilitates highly efficient peptide loading. Int. Immunol. 10, 1159.

Castellino, F., Zhong, G., Germain, R.N., 1997. Antigen presentation by MHC class II molecules: invariant chain function, protein trafficking, and the molecular basis of diverse determinant capture. Hum. Immunol. 54, 159.

Koch, N., Koch, S., Hämmerling, G., 1982. Ia invariant chain detected on lymphocyte surfaces by monoclonal antibody. Nature 299, 644.

Koch, W., Benoist, C., Mathis, D., 1989. Anatomy of a new B-cell-specific enhancer. Mol. Cell. Biol. 9, 303.

Kouskoff, V., Fehling, H.-J., Lemeur, M., Benoist, C., Mathis, D., 1993. A vector driving the expression of foreign cDNAs in the MHC class II-positive cells of transgenic mice. J. Immunol. Methods 166, 287.

Long, E.O., 1985. In search of a function for the invariant chain associated with Ia antigens. Surv. Immunol. Res. 4, 27.

Naujakos, M.F., Arneson, L.S., Fineschi, B., Peterson, M.E., Sitterding, S., Hammond, A.T., Reilly, C., Lo, D., Miller, J., 1995. Potent effects of low levels of MHC class II-associated invariant chain on $CD4^{+}$ T cell development. Immunity 3, 359.

Oi, V.T., Jones, P.P., Goding, J.W., Herzenberg, L.A., Herzenberg, L.A., 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. Curr. Top. Microbiol. Immunol. 81, 115.

Serwe, M., Reuter, G., Sponaas, A., Koch, S., Koch, N., 1997. Both invariant chain isoforms Ii31 and Ii41 promote class II antigen presentation. Int. Immunol. 9 (7), 983.

Shachar, I., Elliott, E.A., Chasnoff, B., Grewal, I., Flavell, R.A., 1995. Reconstitution of invariant chain function in transgenic mice in vivo by individual p31 and p41 isoforms. Immunity 3, 373.

Stockinger, B., Pessara, U., Lin, R.H., Habicht, J., Grez, M., Koch, N., 1989. A role of Ia-associated invariant chains in antigen processing and presentation. Cell 56, 683.

Viville, S., Neefjes, J., Lotteau, V., Dierich, A., Lemeur, M., Ploegh, H., Benoist, C., Mathis, D., 1993. Mice lacking the MHC class II-associated invariant chain. Cell 72, 635.

Zhu, L., Jones, P.P., 1989. Complete sequence of the murine invariant chain (Ii) gene. Nucleic Acid Res. 17 (1), 447.

Zhu, L., Jones, P.P., 1990. Transcriptional control of the invariant chain gene involves promoter and enhancer elements common to and distinct from Major Histocompatibility Complex class II genes. Mol. Cell. Biol. 10 (8), 3906.