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Nearly identical members of the heterogeneous IAP gene family are expressed in thymus of different mouse strains

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ABSTRACT

Poly(A)RNAs prepared from the thymuses of C57BL/6J and DBA/2J mice were used to construct cDNA libraries in the bacterial expression vector λgt11. The libraries were scanned first for protein production with polyvalent antiserum prepared against the 73kDa gag protein of mouse intracisternal A-particles (IAP). Reactive plaques were crossed-screened by hybridization with an IAP-specific DNA probe. Two IAP-specific protein-producing plaques were obtained from the C57BL/6 library and 4 from the DBA/2 library. One C57BL/6 cDNA clone (B12) and two DBA cDNA clones (D8 and D20) were sequenced in their entirety. Clones B12 and D8 were remarkably similar, particularly when compared to the 6 other IAP elements that have been sequenced thus far. We discuss the evidence which leads us to suggest that these clones may be derived from allelic IAP elements expressed in mouse thymus.

INTRODUCTION

Intracisternal A-particles (IAP) in the mouse are defective retroviruses encoded by members of a large family of endogenous proviral elements dispersed throughout the genome (1-5). There are some 1000 homologous but individually distinctive elements per haploid genome. Cloned sequenced IAP elements have all differed significantly from one another at the level of individual nucleotides and by virtue of various deletions and insertions (6-9). Certain types of structural variants are particularly abundant and produce transcripts and proteins that differ characteristically from those derived from the full-sized elements (5,10).

IAP are abundant in many mouse tumor cells where, once established, particle expression tends to persist indefinitely. In contrast, the level of IAP expression in mouse embryos is regulated during preimplantation development both temporally and with regard to genetic background (11).

Among normal mouse somatic tissues, IAP expression is highest in the thymus of young animals (10). The proportions and amounts of IAP-related RNAs and their protein products vary characteristically in the thymus of different inbred mouse strains, and analysis of F1 progeny from two strains that differ

markedly in these respects reveals a reproducibly mixed pattern of expression. IAP expression is clearly under genetic control in the thymus, indicating that specific IAP proviral elements are activated in this tissue. Several IAP elements encoding secreted IgE-binding factors (IgE-BF) also appear to be specifically regulated in a murine T-cell hybridoma (8,12).

Tissue-specific activation of IAP proviral elements could reflect their response to differentiation-related regulatory factors, as in the case of the IgE-BF genes, or their proximity to particular loci of chromatin activation (13). As a step toward identification and ultimate genomic localization of such elements, we have examined cDNA libraries from thymuses of two different inbred mouse strains with similar patterns of IAP expression for evidence of identical or near-identical IAP transcripts. We describe here a pair of protein-encoding partial cDNA clones which have remarkable homology over their entire span of 2.2 kb.

MATERIALS AND METHODS

Isolation and Size Fractionation of mRNA

Total cellular poly(A)RNA was isolated from thymuses of 1 month old C57BL/6J andDBA/2J mice as described (10), and fractionated according to size on 15-33% w/v sucrose gradients. Fractions containing poly(A)RNA greater than 4 kb were pooled and used for constructing the cDNA libraries.

Construction and Screening of cDNA Libraries

The cDNA libraries were constructed in bacteriophage λgt11 essentially following the procedure of Huynh et al. (14), but with several modifications. Second strand synthesis was performed according to Gubler and Hoffman (15), using DNA polymerase I, RNase H and ligase. S1 nuclease was not used to blunt-end the cDNA; instead, the ends were filled in with DNA polymerase I and T4 polymerase. Synthetic adaptors containing ClaI-EcoRI-ClaI sites were used as linkers in order to enclose the inserts with both ClaI and EcoRI sites.

Phage libraries were screened for specific protein production with a polyclonal rabbit antiserum A3706.4 prepared against electrophoretically purified p73 as previously described (16), using a peroxidase-coupled goat anti-rabbit IgG to detect bound antibody. Positive plaques were picked and purified to homogeneity. Inserts were cut out of phage DNA with ClaI and subcloned into AccI-digested calf intestinal phosphatase-treated pT7/T3-18 transcription vector (BRL). Clone 8.3, which had been originally prepared in pCD vector (12,17), was cut from the plasmid and recloned into λgt11 in a similar way.

Other Genomic and cDNA Clones

The mouse MIA14 element was isolated previously from a BALB/c mouse embryo genomic library in phage Charon 4A (2,4). The full 7.1 kb nucleotide sequence and genomic structure of MIA14 will be published elsewhere (J.A. Mietz, et al., submitted). IL3 represents a transposed IAP element isolated from the WEH1-3B leukemia of BALB/c origin; the complete sequence has been published (7).

cDNA clones 8.3, 9.5 and 10.2, which all encode IgE-binding factors (8,12), were derived from the T-cell hybridoma 23B6 formed by fusion of cells from a rat mesenteric lymph node with the AKR mouse T-lymphoma BW35147 (18). The 60 kDA protein product of clone 8.3 is known to react with rabbit antisera against IAP p73 (8). The complete sequence of clone 8.3 has been published (8,12). Clone 9.5 was provided to us by K.W. Moore of the DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA. It was sequenced as part of the present study. The sequence of clone 10.2 was provided by M.L. Trounstine of DNAX.

DNA Sequencing

DNA sequencing was done by the dideoxy chain termination method of Sanger (19) on double stranded plasmid DNA (20), using a series of synthetic 18-20 nucleotide primers based on the known sequences of MIA14 and cDNA clone 8.3. All of the IAP-specific oligonucleotides, as well as the ClaI-EcoRI-ClaI adaptors, were chemically synthesized in the laboratory of Michael Brownstein, NIH.

Computer Analysis

Sequence data were analyzed using the program of Korn and Queen (21) for all sequence alignments, homology comparisons, and amino-acid translations.

RESULTS

IAP RNA in Thymus

IAP-related RNAs ranging in size from 7.2 to 3.5 kb are found in various normal and transformed mouse cells (3,5,10,22-25). The predominant species in normal thymuses are 7.2 and 5.4 kb, representing transcripts of full-sized and deleted IAP elements, respectively (10). The 7.2 kb RNA codes for the major 73 kDa gag protein, p73, while 5.4 kb transcripts code for a group of gag-pol fusion proteins with sizes between 115 and 120 kDa (10). The total amounts and relative proportions of these RNA species and their protein products vary characteristically among thymuses of different inbred mouse strains. The

5.4 kb component is strongly predominant in thymuses of both strains of mice used in this study, but some 7.2 kb RNA is present (10).

cDNA Libraries from Poly(A)RNA of Normal Thymus Cells

In order to search for protein-expressing IAP genes, we constructed cDNA libraries in λgt11 from size-fractionated poly(A)RNAs of C57BL/6J and DBA/2J thymuses. From 80,000 to 100,000 independent clones were obtained per μg of

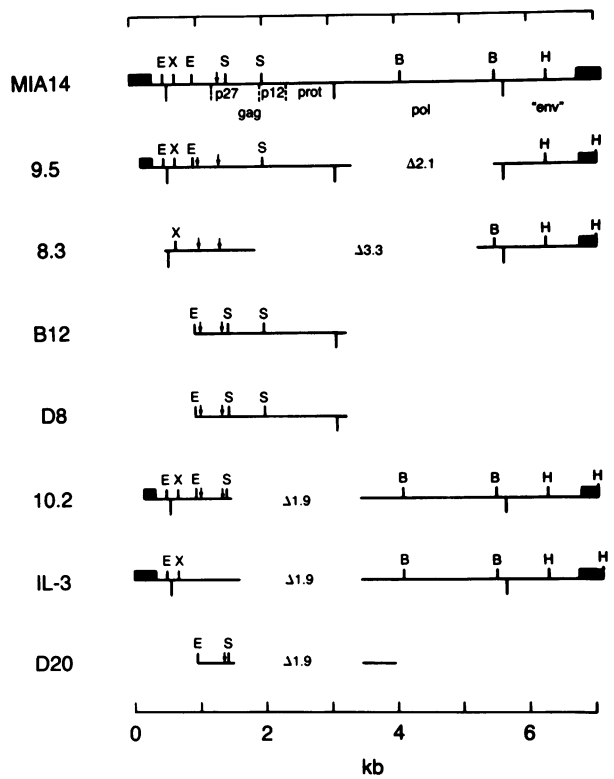

Figure 1: Structural relations between the 8 genomic and cDNA clones discussed in the paper. MIA14 is a full-size genomic clone isolated from BALB/c. IL-3 is a transposed deleted IAP element found inserted near the interleukin-3 gene of the leukemia cell line WEHI-3B (BALB/c origin). The IgE-binding factors (IgE-BF) cDNA clones 8.3, 9.5 and 10.2 were derived from the T-cell hybridoma 23B6 (AKR origin). The cDNA clones B12, D8 and D20 were isolated from thymuses of C57BL/6 and DBA/2 mouse strains. Clones 10.2 D20 and IL-3 have identical deletions of 1.9 kb (nucleotide 1576 to 3476 on the MIA14 maps. Origins of the clones are given in Materials and Methods.

Long terminal repeats (LTRs) are shown in filled boxes. Arrows indicate N-glycosylation sites. Solid and dotted lines delimit the major IAP genetic regions and the segments corresponding to processed gag proteins in related retroviruses, respectively. Restriction sites: B, BamHI; E, EcoRI; H, HindIII; S, SstI; X, XbaI.

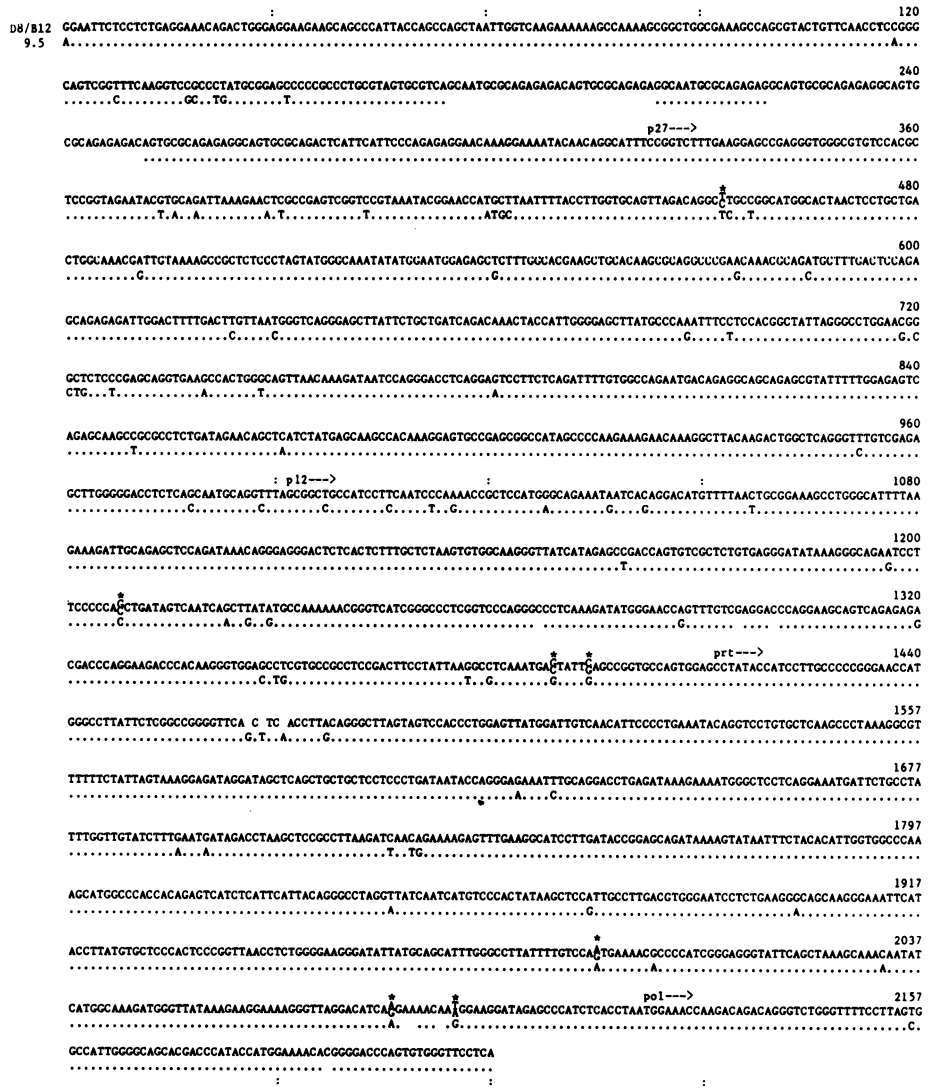

Figure 2: Nucleotide sequences of IAP-related cDNA clones D8 and B12, derived from normal thymus of DBA/2 and C57BL/6 mice, respectively, compared to the equivalent region of cDNA clone 9.5 which is derived from a rat-mouse T-cell hybridoma and encodes an IgE-binding factor (9,10). The 2217 bp sequences of D8 and B12 differ in 7 positions, indicated by asterisks (*); in these positions, the D8 nucleotide appears above the B12 nucleotide. In the squence of clone 9.5 (lower line), dots indicate nucleotides identical to those in D8/B12. Coding domains for p27, p12, protease and polymerase (J. Mietz, submitted) are indicated. Clones D8 and B12 began 322 bp upstream of p27 and end 99 bp into pol.

poly(A)RNA, and 50,000 to 80,000 clones from each library were screened with a rabbit polyclonal antiserum against p73. Thirty and sixty antigen-positve clones were picked from the two libraries, respectively. This antiserum is known to cross-react with antigenic determinants on some hnRNP components (J.E. Fewell and E.L. Kuff, in preparation) and perhaps with other cellular proteins. After the antibody-reactive clones were purified to homogeneity, only 10% of them were found to contain authentic IAP sequences when tested by hybridization with an IAP DNA probe representing the 5' portion of the IAP gag-coding region. The data presented here were obtained from 3 clones: one of them (B12) was isolated from the C57BL/6 cDNA library and the other two (D8 and D20) from the DBA/2 cDNA library.

Sequence Comparison Between cDNA and Genomic Clones

Figure 1 illustrates schematically the structure of several IAP-related clones whose complete nucleotide sequence is known. The cDNA clones are compared to two IAP genomic clones. MIA14 represents a "typical" full length element which is colinear with the particle-specific 7.2 kb genomic RNA (4). The IL-3 clone (7) is a transposed IAP element found inserted near the interleukin-3 gene of the leukemia cell line WEHI-3B (BALB/c origin); the insertion causes constitutive expression of interleukin-3 in these cells. The full nucleotide sequences of the T-cell hybridoma-derived cDNA clone 9.5 and the thymus cDNA clones were determined by us as part of this study. The region common to the three clones is shown in Fig. 2. Clone 9.5 is 4.7 Kb in size and contains open reading frames for the entire gag domain and that portion of pol upstream of the deletion. Clones B12 and D8 are 2217 nt in length and begin 322 nt upstream of p27 in the gag coding region and extended 99 nt into the pol gene.

MIA14 represents the most abundant class of IAP genomic elements, present at a copy number of about 700 per haploid genome of laboratory mice. These elements have been designated Type I (5). The IgE-BF cDNA clones (8.3, 9.5 and 10.2) and IL-3 genomic clone all represent deleted IAP elements. Clones 10.2 and IL-3 are members of a large subset of IAP genes, designated type IΔ (5), which carry deletions of similar size, 1.9 kb, and location, and produce RNA transcripts of 5.4 kb (10,24,25). The deletions in clones 10.2 and IL-3 begin and end at identical residues (nucleotides 1576 and 3476 on the MIA14 sequence). Thymus cDNA clone D20, although only 1.2 kb long, can be definitively identified as a type IΔ gene product because it has a 1.9 kb deletion in precisely the same position.

The nucleotide sequences of thymus-derived cDNA clones D8 and B12 are

shown in Fig. 2, together with a partial sequence of cDNA clone 9.5 (Fig. 1) obtained from a T-cell hybridoma (8,9,12). Clones B12 and D8 are each 2217 kb long and truncated at the same positions at both ends. The reasons for this are not clear. In each clone, the 5' end lies within an EcoRI site, perhaps because this site in the cDNAs was not fully methylated before the EcoRI digestion involved in the subsequent cloning procedure. The 3' truncations most likely represent incomplete second strand synthesis. However, the identical termination sites are more difficult to explain. It may be related to the presence of a palindromic sequence just upstream from it (..ACG GGGACCCA GTG TGGGTTCC TCA/linker). Neither clone contains any major deletion with respect to the corresponding region in MIA14 or 9.5. B12 and D8 could be derived from full-sized IAP elements or ones like clone 9.5, with deletions in a more 3' location (nucleotide 3432 to 5615 on the MIA14 map). Probability favors a full-sized element since they are far more common in the mouse genome.

TABLE 1: VARIABILITY AMONG IAP-RELATED CLONES+

	GROUP I					GROUP II (TYPE IΔ)			
clone	14	9.5	8.3	B12	D8	10.2	IL-3	D20	
size of compared region (bp)	2217	2217	970	2217	2217	654 1060	654 1060	654* 1060**	
	per cent difference				group average	per cent difference			group average
14		2.3	3.3	3.7	3.7	4.8	4.6	6.6	
9.5			1.4	3.4	3.2	5.1	5.3	6.5	
8.3				3.4	3.4	4.7	4.7	7.2	5.7
B12					0.3	5.3	4.6	7.9	
D8						5.4	4.7	7.8	
10.2							1.9	3.1	
IL-3								4.0	

2.8

3.0

+ Values derived by pairing clones as indicated.
* Comparison with Group I clones
**Comparison with Group II clones

TABLE II: VARIABILITY OF cDNA CLONES B12 AND D8 WITHIN IAP GENE REGIONS[+]

Gene Region	Base Pairs Compared	Number of Differences From One Another	Average Number of Differences from Other Clones in Group I
5' to p27	322	0	10
p27	669	1	34
p12	420	3	13
Protease	744	3	20
Polymerase	99	0	2
Total	2254*	7	79

[+]Values derived by pairing B12 and D8 with one another and with each of the other clones in Group I.

*Total compared exceeds the number of nucleotides in D8 and B12 (2216) because of overlap of 37 bp at the protease-pol junction.

Both B12 and D8 have a single open reading frame through their entire gag coding regions, and enter pol with a -1 frame shift typical of IAP elements (J. Mietz, submitted).

Nucleotide comparisons were performed for all possible pairings between the 8 clones shown in Figure 1, with the results summarized in Table I. In calculating the variability between clones, each point difference and any change (insertion, deletion or substitution) of 2 or more consecutive nucleotides were considered as single events. In Table I, the 3 type IΔ clones are designated as group II, the remaining clones - Group I.

The average diversity between different members of each group was about 3%, and between members of different groups nearly 6%. Against this background the similarity between the two thymus-derived clones B12 and D8 is striking, with only 7 point mutations among 2217 bp, corresponding to a 0.3% divergence. Clones 8.3 and 9.5, which each were derived from the same cell and code for IgE-binding and potentiating factors (12), were also more closely related to one another than to any of the other clones. However, the variability between these two clones was 4 times greater than that between clones B12 and D8. The DBA/2 thymus cDNA clone 20 was not highly related either to B12 and D8 or to the other type 1Δ elements.

The resemblance between B12 and D8 extended through all of the genetic regions represented in these clones (Table 2). The distinction between this pair and the other members of group I was particularly marked in the 838 bp

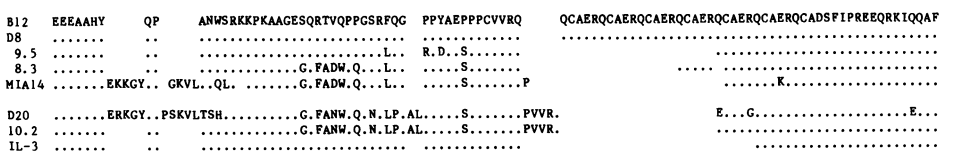

Figure 3: Partial amino acid sequence of the IAP clones. The amino acid sequences (standard one-letter designations) were derived from the nucleotide sequences in the gag regions upstream of p27 (see Fig. 1). Letters indicate differences between the amino acids of B12 and the other clones in this region, which reaches from nucleotide 987 to 1244 on the MIA14 sequence.

region upstream of the putative p12 gene segment, where B12 and D8 differ from each of the other clones by an average of 44 events but from one another by a single point mutation. There are 6 more differences between B12 and D8 in the remaining 1389 bp of these clones. The relative conservation of sequence in the 5' portion could reflect a more stringent functional requirement or a gene conversion event antecedent to the separation of the BL/6 and DBA/2 strains. Three of the seven nucleotide differences between B12 and D8 are silent with respect to amino acids, and three others are conservative changes (Thr to Ser, Gln to Glu, and Pro to Ala).

Amino-acid Sequence Comparison Between cDNA and Genomic Clones

The conservation between B12 and D8 is further illustrated in Fig. 3, which compares the deduced amino acid sequences of the various IAP-related clones in a region beginning at nucleotide 987 on the MIA14 map (corresponding to the first codons in B12 and D8). B12 and D8 are the only two clones that are identical throughout this region. In addition, they each contain a distinctive 6-fold repeat of the motif QCAER which is present in one to three copies in the other clones.

The sequences shown in Figure 3 indicate an interesting relationship between the clones, best exemplified by the three type IΔ clones D20, 10.2 and IL-3. As mentioned earlier, each of these clones contains the same 1.9 kb gag-pol deletion. In this sense, they form a highly related family. Yet in the region shown in Fig. 3, D20 and 10.2 share an extensive sequence variation (beginning G.FAN., etc.) which is missing in IL-3, while 10.2 and IL-3 lack two short sequence elements (ERKGY and KSKVL) which are present in D20. Thus, there are overlapping subsets of sequence variants within the type IΔ group, and as is evident from further inspection of the data in Fig. 3, among the other genes as well. This type of sequence variation is difficult to understand solely on the basis of the independent amplification and evolution

of a few primary IAP variants, but seems more likely to have arisen through multiple gene conversion events either at the chromosomal level or perhaps during reverse transcription in IAPs containing RNA products of two different IAP genes. Judging from the number of variations already encountered within a limited number of examples, such conversion events must be common.

DISCUSSION

This study demonstrates a remarkably close resemblance between two protein-producing partial cDNA clones isolated from thymus of different inbred mouse strains. The C57BL/6 and DBA/2 lines are not closely related: these two strains showed the largest pair-wise differences among 10 common inbred strains when compared at 97 loci for which allelic protein products are recognized (27). The two clones, B12 and D8, showed strong homology at the individual nucleotide level (7 single-base changes in 2217 bp) and a common distinctive sequence variation relative to 6 other IAP-related clones. At the protein level they differ by 4 amino acids, with only one non-conservative change. Although these cDNA clones do not repesent the complete IAP elements from which the transcripts originated, they are large enough to provide a significant basis for comparison with one another and with other IAPlements. These isolates, which are the first known examples of such closely related IAP elements, were detected within a very small number of cDNA clones analyzed.

The sequence variation between B12 and D8 (0.3%) is low compared to that observed for alleles of other mouse genes. For example, four genes in the T15 heavy chain variable region of BALB/c mice differ from their four allelic counter parts in a C57BL/6 substrain (B10.P) by 2-5% at the nucleotide level (5 to 14 single changes in 672 bp) and by 0-5 amino acids out of 224 (28). Similarly, the allelic βdmaj and βs globin chains in these same mouse strains differ by 3 out of 146 amino acids (29). Two highly conserved genes in the Qa-2,3 region of the C57BL/10 major histocompatibility complex, Q10 and Q7, differ from their alleles in SWR/J and BALB/c mice, respectively, by 0.5% and 0.4% at the nucleotide level (30,31). In contrast, the k^d and k^b alleles at the highly polymorphic H-2K locus differ by 16% (32).

Feenstra et al. (33) showed that demethylation of certain sites in the IAP 5' LTR is essential for transcriptional activity and also that the level of demethylation at these LTR sites in normal thymus is so low that only a few gene copies could be expected to be active. It seems unlikely that two such highly related elements as B12 and D8 would be randomly activated or occur by chance among the putatively small number of active genes in the thymuses of

the two mouse strains. Rather, cDNA clones B12 and D8 could be derived from transcripts of allelic IAP elements selectively activated in the thymus of C57BL/6J and DBA/2J mice, or, alternatively, close copies located at different chromosome positions on the two strains but responding in like fashion to tissue regulatory signals.

Resolution of these possibilities can be achieved by cloning the genomic elements corresponding to clones B12 and D8 and comparing their flanking sequences. Barklis et al. (13) have shown that the activation of integrated retroviral elements in embryonal carcinoma cells can be position-dependent and that flanking sequences can mediate provirus expression. They suggest that transcriptionally active cellular sequences might be isolated by selection for retroviral expression. In the present instance, identification and cloning of allelic B12 and D8 elements may provide entry to a region of specific gene activity in thymus DNA. The possibility must also be kept in mind that the active IAP elements themselves code for functional thymus-specific cellular products, in a manner analogous to the production of IgE-binding proteins by IAP genes in suitably stimulated T-cells (8,9,12).

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