spreading genotypes. Thus, extensive areas now occupied by single genotypes may circumscribe centers of allopolyploid origin. This may well apply to *A. bradleyi* in the Ozark-Ouachita region. Three widely separated populations from this region (Garland and Yell counties, Arkansas and Callaway County, Missouri) shared essentially identical genotypes; the only exception is that *A. bradleyi* plants from Yell County are variable for *IDH*, being either heterozygous (100^2, 63^2), homozygous (63^4), or asymmetrically heterozygous (100^1, 63^3). The latter pattern probably resulted from crosses between the former two genotypes.

How general is the phenomenon of recurrent origins of allopolyploid species? Studies of allozyme variation in polyploid complexes are few, and most consider crops or anthropogenic weeds (*20*). There are indications from these and from morphological and cytological data on other taxa that the phenomenon of multiple allopolyploidizations may be much more general than has been thought. Studies of wheat (*21*), *Phlox* (*22*), and European *Asplenium lepidum* (*23*) have shown that differently named species may represent analogous combinations of different morphotypes of the same diploid progenitor species. Variation in esterase genotypes indicates that *Tragopogon mirus*, a recent allotetraploid derivative of two species introduced into North America, originated independently at widely separated localities (*4*). Allozyme data also suggest multiple origins for the fern allotetraploid *Pellaea wrightiana* (*24*). These studies suggest an important aspect of the dynamics of reticulate evolution: species genetically isolated at the diploid level may repeatedly contribute to a common gene pool at the polyploid level. The continued acquisition of genetic diversity by allopolyploids, such as those in *Asplenium*, may substantially augment their potential to evolve and speciate.

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11. Putative heterozygotes have been found to segregate electromorphs into gametophyte progeny, supporting the interpretation of the electromorphs as alleles at genetic loci (C. R. Werth, unpublished observations).
12. The *PGM-2* homozygotes in the other two diploid species have a single band of corresponding intensity and general position.
13. The heterozygosity referred to here is for equivalent genes coded in the nonhomologous genomes of the two parental species. Genotypes are only inferred since segregation does not ordinarily occur in allotetraploids.
14. Compare loss of parental gene expression in *Chenopodium* [H. Wilson, S. C. Barber, T. Walters, *Biochem. Syst. Ecol.* **11**, 7 (1983)] attributed to nonfunctional (null) alleles.
15. Recombination could be accomplished through chromosome breakages or through rare pairing of nonhomologous chromosomes, either in multivalents as suggested by Roose and Gottlieb (*4*) or in bivalents, as suggested by E. J. Klekowski, [*Am. J. Bot.* **60**, 535 (1973)].
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17. An exception is the previously mentioned instances of nonexpression of parental alleles.
18. *Asplenium montanum* occurs in small isolated populations that show considerable interpopulational differentiation resulting from founder effect [C. R. Werth, thesis, Miami University, Oxford, Ohio (1983); T. Reeves, *Am. Fern J.* **64**, 105 (1974)]. A more extensive survey of populations in this species may well turn up these "orphan" alleles [see B. J. Turner, B-L. H. Brett, E. M. Rasch, J. S. Balsano, *Evolution* **34**, 246 (1980)].
19. Although alleles may, of course, arise through mutation, it is unlikely that most or all the variation in the heterozygous genotypes of the allotetraploid genotypes resulted from mutations following a special hybridization. Most mutations would give rise to nonfunctional alleles that would be sheltered from selection by the fixed heterozygosity of the allopolyploids. However, for those loci that are variable in the diploids, most of the variation in the allotetraploids involves functional alleles present in the diploids. In contrast, for loci that are invariant in the diploids, the only type of variation in the allotetraploids involves nonexpression of parental alleles. Moreover, the occurrence of a single *A. bradleyi* genotype (with the exception of *IDH*) occupying the entire range of this species west of the Mississippi is not consistent with a supposed origin of all or most variability in the allotetraploids from posthybridization mutations.
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Human Immunoglobulin D: Genomic Sequence of the Delta Heavy Chain

Abstract. *The DNA coding for the human immunoglobulin D (IgD) heavy chain (δ, delta) has been sequenced including the membrane and secreted termini. Human δ, like that of the mouse, has a separate exon for the carboxyl terminus of the secreted form. This feature of human and mouse IgD distinguishes it from all other immunoglobulins regardless of species or class. The human gene is different from that of the mouse; it has three, rather than two, constant region domains; and its lengthy hinge is encoded by two exons rather than one. Except for the third constant region, the human and mouse genes are only distantly related.*

Immunoglobulin D (IgD) was discovered in human serum in 1965 (*1*), and the corresponding mouse Ig was identified subsequently (*2, 3*). In both the mouse and the human, IgD is a predominant surface component of B cells and is only a minor component of serum Ig (*1, 4*). The function of IgD has been a subject of considerable speculation, but despite a wealth of structural information, the function is basically unknown (*5, 6*).

IgD has been studied in great detail at physiological and molecular levels in the mouse. The majority of antigen-reactive splenic B cells co-express surface IgM and IgD of shared light chain type, idiotype, and antigen specificity (*7, 8*). The ratio of IgM to IgD varies with the state of B-cell differentiation (*9*), with early B cells expressing IgM only. As cell differentiation progresses, IgD is turned on; this leads to "double-producers" expressing both IgM and IgD on the cell surface with identical variable regions. Most mature B cells have ten times as much IgD as IgM on the surface (*10*). As the B cell is activated, IgD is shut off rather than being secreted copiously as with IgM and other immunoglobulin classes.

The murine δ chain has several properties that set it apart from all other immunoglobulin heavy (H) chains: (i) Murine IgD has only two H-chain constant re-

gion domains (Cδ1 and Cδ3, by homology considerations) separated by a hinge, which makes it the smallest known immunoglobulin H chain (*11*). All other immunoglobulins have either three or four constant region domains. (ii) The hinge is unusually long, but it lacks cysteine residues that normally provide disulfide bridges between H chains of the tetrameric antibody. As a result, mouse IgD is observed in dimeric as well as tetrameric forms (*12, 13*). (iii) The secreted terminus is encoded on a separate exon, which is distal to the body of the gene rather than as an extension of the carboxyl terminal domain as is the case in all other immunoglobulins (*14*).

The genomic arrangement of μ and δ exons of mouse Ig genome suggested a rationale for regulation of expression of IgD (*6*). Since the μ and δ genes are very close together (2.5 kbp in mouse), messenger RNA (mRNA) precursors of varying lengths can be produced that contain V, μ, and δ sequences. Hence, the choice of 3′ end of the mRNA precursor would determine how the precursor RNA is spliced and whether IgM or IgD in membrane or secreted form is expressed.

In view of such hypotheses, it is of interest to compare the organization of the μ-δ locus in different species to ascertain whether the spatial arrangements or any other peculiarities have been preserved over evolutionary time. A large number of species have been reported to possess an IgD system (*15*). We now report the detailed sequence analysis of the coding regions of the human δ gene. Previous restriction mapping and partial sequence studies of the human δ gene show that the human δ gene is located 3 kbp (*16*) to 6 kbp (*17, 18*) downstream of human μm2; the complete sequence of the region from μ to δ has been reported (*18*). The amino acid sequence of the secreted form of human δ protein was determined from human myeloma sources (*19, 20*), but there has been no information concerning the detailed ar-

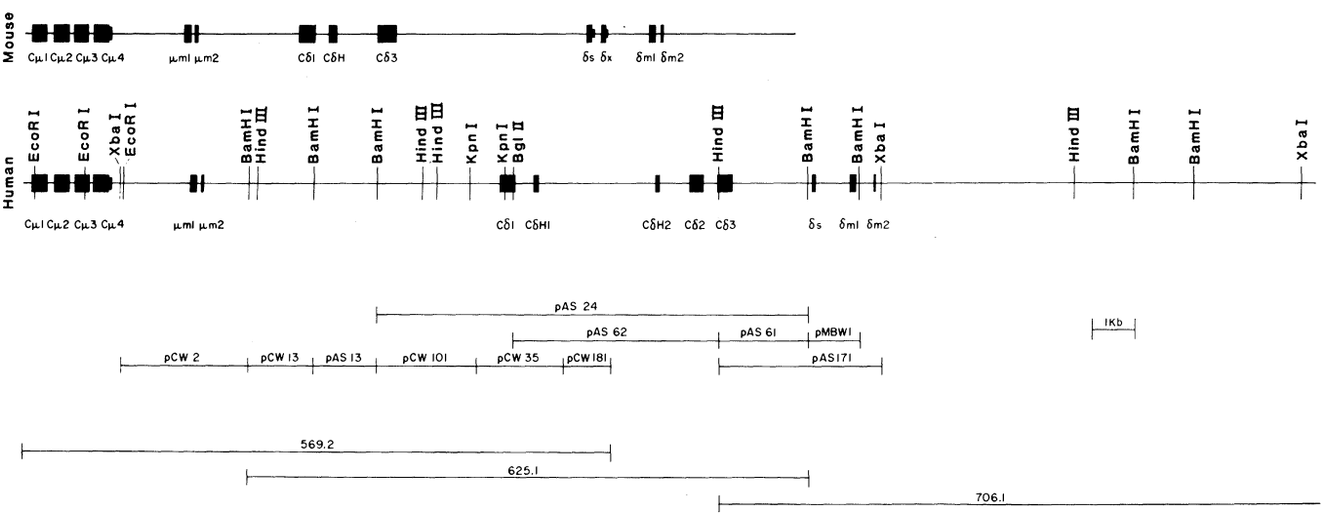

Fig. 1. Restriction map of the human μ–δ region and comparison with mouse μ–δ exons. Coding regions are indicated by black rectangles. Phage and plasmid clones are shown below. The mouse μ–δ region is shown above (*11*). Phage libraries were constructed with the use of DNA isolated from lymphocytes from patients with chronic lymphocytic leukemia. The phage clone 569.2 was isolated from a partial Sau 3A library in Charon 28 (*31*); the probe consisted of the 1.2- and 0.9-kb Eco RI fragments carrying Cμ1 to Cμ4 (*32*). The plasmid pCW35 was used to isolate the phage 625.1 from a complete Bam HI library of the same patient in Charon 30. A complete H3 library in Charon 35 (*33*) was constructed from DNA of another patient and screened with the plasmid pAS61 to isolate the phage 706.1. The plasmid pAS24 was used to screen a cDNA library prepared from the human plasmacytoma ODA. Sequencing of the cDNA clones identified clones carrying the δ secreted and membrane termini (*21*). The positions of the Cδ1, CδH1, CδH2, Cδ2, and Cδ3 exons were determined by DNA sequencing. The locations of the exons coding for the membrane (δm) and secreted (δs) termini were determined by hybridization to cDNA clones of the secreted or membrane forms and confirmed by DNA sequencing.

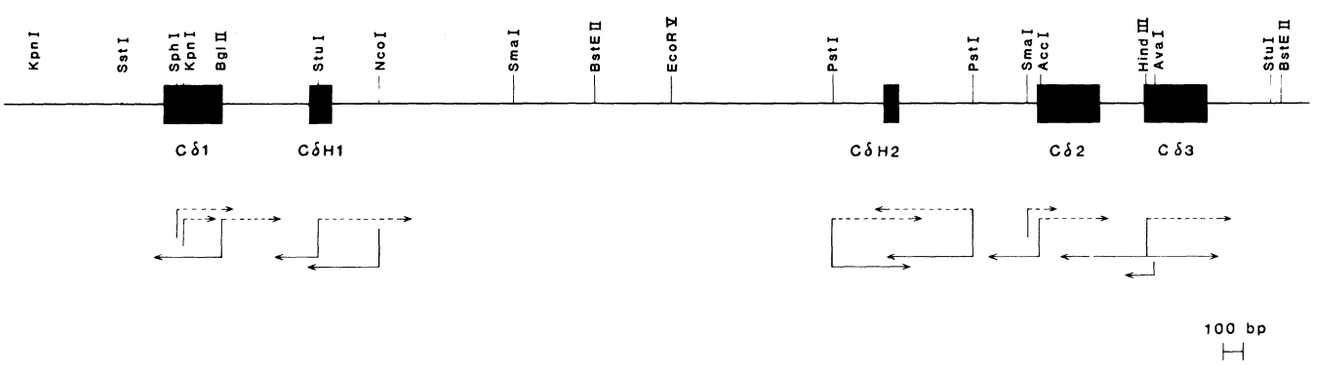

Fig. 2. Organization and sequence strategy of the human δ heavy chain constant region. Exons are indicated by black rectangles. All sequencing was performed by the chemical degradation method of Maxam and Gilbert (*34*). Dashed horizontal lines represent sequencing reactions for the coding strand and solid horizontal lines represent those for the noncoding strand. 5′ and 3′ end labelings are indicated by the direction sequenced (indicated by arrowheads). All restriction sites used for end labelings are presented on the map. For the sequencing of CδH2, the 750-bp Pst I fragment containing this exon was cloned into pUC13 (*35*) and labeled at the Bam HI and Hind III sites of the vector polylinker.

rangement or DNA sequence of the human δ exons and no information on the membrane terminus of human IgD.

Lambda phage libraries of lymphocyte DNA from a patient with chronic lymphocytic leukemia (CLL) were screened with a human Cμ probe to obtain clones carrying Cμ and linked Cδ1 and CδH1 genes. Plasmid subclones lacking repetitive DNA were used in chromosome "walking" to obtain the rest of the δ gene. A large fragment carrying Cδ3 and its 3′ flanking region was used to probe a human plasmacytoma complementary DNA (cDNA) library in order to isolate representatives of membrane and secreted forms of human mRNA. These were sequenced (*21*) and in turn used as probes to locate the coding regions of alternate δ carboxyl termini in genomic DNA (legend to Fig. 1).

Comparison of the coding regions of the human μ and δ genes (Fig. 1) with Southern blots of human liver DNA from six individuals of various races as well as lymphocyte DNA from four patients with chronic lymphocytic leukemia confirmed that the human δ gene was 6 kbp 3′ to μm2 and that no major rearrangements had occurred during cloning in *Escherichia coli*. The Cδ and δs exon boundaries were located by comparing the translated nucleotide sequences with the known amino acid sequences (*19, 20*). The δs and δm1 and δm2 coding region boundaries were located by reference to the amino acid sequence (for δs) and the sequenced cDNA clones (for the membrane exons) (*21*).

Organization of the human δ locus. In comparing the map of human δ with that of the mouse (Fig. 1), we found that, as with all other immunoglobulins, each domain of the protein chain is encoded by a separate exon. As we anticipated from the protein sequence, the human form of δ has an additional (Cδ2) domain not present in mouse. Both mouse and human δ genes span approximately 10 kbp, but the sizes of the intervening sequences vary. The distance from μ to δ (that is, from μm2 to Cδ1) of human is approximately three times greater than that of mouse (6.6 compared to 2.5 kbp). The human δ hinge is coded on two exons, CδH1 and CδH2. The secreted terminus comprising the last seven residues of the sequenced human myeloma protein is coded on a separate exon located 1.8 kb downstream of the third constant region domain, and the membrane terminal exons (δm1 and δm2) are located 0.8 and 1.3 kbp further downstream, respectively. Thus, the general arrangement of the membrane and secreted exons of the human δ gene is the same as the murine form and differs from the other immunoglobulins.

Sequences of the human δ coding regions. The nucleotide sequence of each of the coding regions of δ is presented in Fig. 3 with flanking intron sequences on each side. The subclones and strategy used for sequence determination are presented in Fig. 2. The translated amino acid sequence is presented above the DNA sequence. The biased codon usage is typical of other eukaryotic genes (*22*). The predicted amino acid sequence agrees with the published protein sequence except for two residues. (i) An arginine (AGG) in place of glycine (GGG) in the third domain [position C330 according to Putnam *et al.* (*19*) and Shinoda *et al.* (*20*) and indicated in Fig. 3 by parentheses]; and (ii) an extra lysine at the secreted carboxyl terminus. An extra carboxyl-terminal lysine that is apparently removed posttranslationally is a common feature of mouse immunoglobulins (*23*), and it is probable that this is also the case with human IgD. The sequence GGG, however, is found in the human δ cDNA clone (*21*) and in a genomic clone from another CLL patient. We have reconfirmed this genomic DNA sequence by restriction enzyme digestion; the sequence GGG is part of a

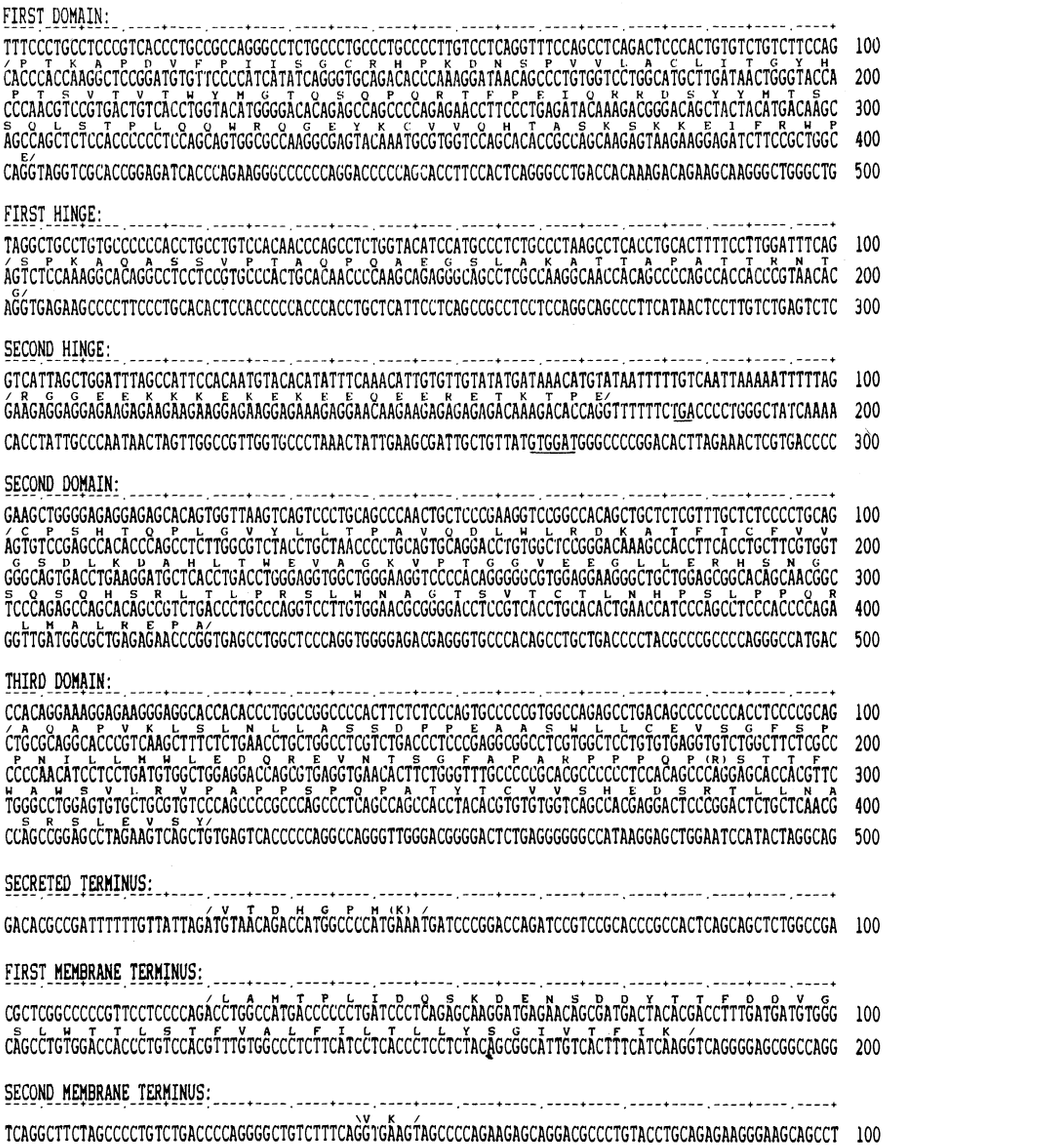

Fig. 3. DNA sequence of the human delta heavy chain exons. DNA sequences of the exons are presented along with adjacent intron segments in both the 5′ and 3′ directions. The predicted amino acids are shown directly above the first base of the corresponding codon. Single letter abbreviations for amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Parentheses indicate the only amino acids that are generated by our sequences and are in disagreement with the amino acid sequence data (*19, 20*). The alternative donor splice site for CδH2 is underlined as well as the in-phase stop codon for this exon. RNA splicing positions are represented by (/).

Table 1. DNA sequence comparison of human and mouse IgD heavy chain exons showing the similarities between the DNA sequences of the compared exons. The "similarity index" (*36*) is defined as the total number of matched bases divided by the sum of the number of mismatched bases plus the number of gaps in alignment. This program would generate a similarity index of 25 percent if two completely random sequences were compared. The program employed for this comparison is ALIGN (*37*) which produces a locally optimal alignment of two partially homologous DNA sequences using the method of Wilbur and Lipman (*38*). The number of gaps used to generate the corresponding similarity is also presented.

Human	Cδ1	CδH1	Cδ2	Cδ3	δs	δm1	δm2
Mouse	Cδ1	CδH	Pseudogene	Cδ3	δs	δm1	δm2
Similarity index	37.5	51.9	37.8	66.0	73.7*	70.0	100.0
Number of gaps	1	0	1	1	1	0	0

*This value was obtained with the program GAP (*37*), which produces an alignment between two DNA sequences by introducing gaps in either strand, thereby maximizing the number of matching bases. The number generated with this program represents the homology existing between the human δs and the carboxyl half of the mouse δs:

```
                                        V  T  D  H     G  P  M  K
HUMAN                                   ATGTAACAGACC--ATGGCCCCATGAAA

MOUSE   GATGCTACCACCTCCTGCCTGAGTCAGACGGTCCTTCTAGGAGACCTGATGGTCCTGCCCTTGCC
          C  Y  H  L  L  P  E  S  D  G  P  S  R  R  P  D  G  P  A  L  A
```

Table 2. A list of acceptor and donor splice sites for the exons of the human δ gene. Boundaries between introns and exons are indicated by (/).

	ACCEPTOR SITE	DONOR SITE
EXON	INTRON/EXON	EXON/INTRON
Cδ1	ACTCCCACTGTGTCTGTCTTCCAG/CACCC	GCCAG/GTAGGTCGCACC
CδH1	CCTGCACTTTTCCTTGGATTTCAG/AGTCT	CACAG/GTGAGAAGCCCC
CδH2	TTTTTGTCAATTAAAAATTTTTAG/GAAGA	ACCAG/GTTTTTTCTGAC
Cδ2	GCTCTCGTTTGCTCTCCCCTGCAG/AGTGT	ACCCG/GTGAGCCTGGCT
Cδ3	GACAGCCCCCCCACCTCCCCGCAG/CTGCG	CAGCT/GTGAGTCACCCC
δS	GACACGCCGATTTTTTGTTATTAG/ATGTA	----------
δM1	CGCTCGGCCCCCGTTCCTCCCCAG/ACCTG	TCAAG/GTCAGGGGAGCG
δM2	CTGACCCCAGGGGCTGTCTTTCAG/GTGAA	----------

Sma I restriction site that is not found in this clone. Thus, this amino acid change is probably due to a polymorphism or to a fortuitous mutation in the particular clone we sequenced.

All of the RNA splicing signals at δ exon boundaries follow the GT/AG rule (*24*), but the sequences flanking the CδH2 exon (Table 2) are quite unusual. The CδH2 donor site, /GTTTTT is most unusual in having three pyrimidine residues after the GT. Of 282 donor sites in the Genbank (Tm®) database (*25*), the vast majority have three purines (75 percent) or two purines (23 percent) in that position. Only one other sequence (/GTCTT in exon 4 of adenovirus) is devoid of purines. A much better consensus donor splice site occurs approximately 90 bp downstream (underlined in Fig. 3) but apparently is not used. (Such an RNA would contain an in-frame termination codon and could not be productively expressed.) The CδH2 acceptor splice sequence AAAAATTTTTAG/ is also unusual in having a run of purines in an area that is normally very pyrimidine rich. Only three of 271 acceptor sites in the database appeared to be purine rich.

We noted previously that immunoglobulin genes have a consistent relationship between the phase of translation and the position of RNA splicing (*26*). Splices that join m1 to m2 exons are made in exact register with the translation phase, whereas all other splices occur after the first base of the codon register. The human δ gene follows this pattern.

The human δ hinge. The domain boundary assignments agree very well (±4 amino acids) with those based on papain cleavage of the protein (*19*), although the genomically defined length of the hinge is 58 amino acids rather than 64. This is still considerably longer than hinges of other isotypes including the 35-amino-acid-long mouse δ hinge. Interestingly, the interchain disulfide bridge assigned to the hinge by papain cleavage (*19*) is actually coded by the Cδ1 exon. Thus the human δ hinge, like that of the mouse, is free of cysteine residues.

The splitting of the human δ hinge into two exons is unusual. Other immunoglobulin hinges are coded as single exons except for two: the murine α chain, which has a hinge that is coded as part of the second domain exon (*27*) and the human γ3 gene in which the hinge is quadruplicated into four homologous exons (*28, 29*). Putnam *et al.* (*19*) and Tucker *et al.* (*14*) observed that the human δ hinge has a strong sequence homology to the mouse only in the amino terminal portion, while the remainder of the hinge consists of a bizarre cluster of charged amino acids. We find that this corresponds exactly to the division of the gene into two exons. Thus, the two parts of the human δ hinge are unlikely to have arisen by duplication. The charged segment is encoded by a purine-rich sequence containing a 44-bp and a 15-bp uninterrupted polypurine tract. No purine-rich remnant of this hinge segment is found downstream of the mouse hinge (*11*).

Comparisons between corresponding exons of human and murine δ are shown in Table 2. As was evident from comparisons of the protein sequences, the first domains are quite distantly related, whereas the third domains are quite similar. The 37.5 percent similarity between the Cδ1 exons is in fact no higher than is found between noncorresponding immunoglobulin exons. The amino terminal exon of the hinge shares 51.9 percent similarity with the complete mouse hinge, but no part of the mouse gene has a significant similarity to the purine-rich second portion of the hinge. The Cδ2 exon is missing in mouse. However, a comparison of the mouse pseudodomain identified by Richards *et al.* (*30*) with human Cδ2 results in a similarity index of 37.8 percent (one gap) which is the same as that between the corresponding Cδ1 exons and suggests that the pseudodomain in mouse may be a remnant of the missing Cδ2. The human secreted terminus (δs) exon codes for a peptide that is completely different from that of mouse, which is almost three times as long (21 compared to 8 amino acids). In this case though, there is a surprising stretch of similarity in the 3′ half of the DNA sequence at the DNA level (legend to Table 1). The membrane exons, δm1 and δm2, of human and mouse IgD are similar. The length of the spacer peptide, the region between Cδ3 and the membrane, as well as the transmembranal sequence are conserved (Table 1). The cytoplasmic domain (Lys-Val-Lys) coded by the last codon of δm1 and by δm2 are identical in both the μ and δ genes of the two species, a remarkable coincidence.

IgD is widely distributed, having been found in each of the vertebrates studied (*15*), yet its function is unknown. The extensive differences we have observed between human and murine forms raise a

question as to how a molecule that shows such evolutionary variation can serve an important function. The three aspects of IgD gene structure that are very unusual and are conserved between mouse and human are the proximity to the μ gene, the distal coding of the secreted terminus, and the identical cytoplasmic domain of the membrane terminus. All of these are connected with the unusual way in which the expression of the molecule is regulated by mRNA processing. This suggests perhaps that the maintenance of a system of dual antigen receptors on the B-cell surface is more important in evolution than the specific structure of the receptor itself.

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Vaccinia Virus Recombinant Expressing Herpes Simplex Virus Type 1 Glycoprotein D Prevents Latent Herpes in Mice

Abstract. *In humans, herpes simplex virus causes a primary infection and then often a latent ganglionic infection that persists for life. Because these latent infections can recur periodically, vaccines are needed that can protect against both primary and latent herpes simplex infections. Infectious vaccinia virus recombinants that contain the herpes simplex virus type 1 (HSV-1) glycoprotein D gene under control of defined early or late vaccinia virus promoters were constructed. Tissue culture cells infected with these recombinant viruses synthesized a glycosylated protein that had the same mass (60,000 daltons) as the glycoprotein D produced by HSV-1. Immunization of mice with one of these recombinant viruses by intradermal, subcutaneous, or intraperitoneal routes resulted in the production of antibodies that neutralized HSV-1 and protected the mice against subsequent lethal challenge with HSV-1 or HSV-2. Immunization with the recombinant virus also protected the majority of the mice against the development of a latent HSV-1 infection of the trigeminal ganglia. This is the first demonstration that a genetically engineered vaccine can prevent the development of latency.*

In humans, a primary infection of the lips, cornea, or genitalia with herpes simplex virus (HSV) type 1 or type 2 is often followed by the establishment of a latent ganglionic infection that persists for the life of the individual (*1*). These latent infections can reactivate intermittently and give rise to recurrent herpetic lesions even in the presence of high titers of neutralizing antibody (*2*). Our understanding of this process in man is based largely on research carried out on experi-

Table 1. Effect of immunization with vaccinia HSV-1 gD recombinant virus on the lethality of mice challenged with HSV-1 or HSV-2. BALB/c mice, 6 to 8 weeks old, were vaccinated with either 1×10^8 plaque-forming units (pfu) of vaccinia HSV-1 gD (vgD52) or vaccinia HBsAg (vHBs4) or with 1×10^8 pfu of wild-type vaccinia. Sera for antibody determinations were collected from the retro-orbital plexus 4 weeks after immunization. HSV neutralizing antibody titers were determined in a complement-dependent microneutralization assay (*21*). End points were expressed as the reciprocal of the highest twofold serum dilution that prevented a cytopathic effect by 100 tissue culture infectious doses of HSV. The geometric means of the antibody titers were based on data from groups of 14 to 19 mice. In lethality experiments, mice were challenged intraperitoneally with 1×10^8 pfu of HSV-1 (strain F) or 2×10^6 pfu of HSV-2 (strain G).

Immunizing agent	Route of immunization	Antibody titer (geometric mean)	Challenge virus: HSV-1 Dead/inoculated	HSV-1 Mortality (%)	HSV-2 Dead/inoculated	HSV-2 Mortality (%)
None		<4	58/72	81	39/40	98
Vaccinia (wild type)	Footpad	<4	9/14	64	20/21	96
	Tail	<4			19/21	90
Vac HBsAG	Intraperitoneal	<4			18/19	95
	Footpad	<4	18/21	86		
	Tail	<4			20/21	96
Vac HSV-1 gD	Intraperitoneal	54	1/19	5	1/19	5
	Footpad	75	0/28	0	0/20	0
	Tail	60	1/45	2	1/20	5
	Total	62	2/92	2	2/59	3