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NOTES

Alternate Splicing of Rabbit Polymeric Immunoglobulin Receptor

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Rabbits have a minimum of two polymeric immunoglobulin receptor primary translation products. A cDNA clone of the smaller product lacked two of the five receptor domains. These two domains were on a single exon. As there was one receptor gene, we suggest that this exon can be spliced in or out.

The polymeric immunoglobulin receptor (poly-Ig-R) transports the polymeric immunoglobulins, A and M across certain epithelial cells. During this transport, the receptor, an integral membrane protein, is cleaved so that a large portion is released into external secretions with the immunoglobulins. This cleaved fragment is known as secretory component (SC) (9). We have recently sequenced a cDNA clone containing the complete coding region of the rabbit poly-Ig-R (10). The deduced amino acid sequence shows that the portion that becomes SC contains five repeating units of 100 to 110 residues each that are homologous to members of the immunoglobulin superfamily. These domains are numbered I to V.

The rabbit poly-Ig-R is heterogeneous. Four translation products of poly-Ig-R mRNA can be immunoprecipitated: an upper doublet of 87 to 90 kilodaltons (kDa) and a lower doublet of 64 to 67 kDa (11). All four can bind to immunoglobulin A and give rise to functional receptors. The poly-Ig-R isolated from rabbit liver plasma membranes is even more heterogeneous, forming two groups of poorly resolved bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 115 to 120 kDa and 90 to 95 kDa (6). The cleaved SC isolated from milk is similarly heterogeneous, containing two groups of bands of 77 to 84 kDa and 51 to 59 kDa (6). This heterogeneity has several origins. First, at least two alleles of rabbit poly-Ig-R, designated t^{61} and t^{62}, have been identified serologically (5), and the outbred rabbits used in most previous studies may have been heterozygous. Second, amino- and carboxy-terminal protein sequencing as well as peptide mapping of SC strongly suggest that the lower group of bands of SC has an internal deletion relative to the upper group of bands (6). Finally, part of the heterogeneity results from variability in posttranslational processing (2). In this report, we consider only the factors leading to the four primary translation products.

We have previously shown that mammary and liver mRNAs from an outbred rabbit of unknown (presumably heterogeneous) allotype produced four primary translation products of poly-Ig-R (11). We have now translated liver mRNA from a rabbit that was homozygous for the poly-Ig-R allotype t^{62} and was kindly provided by K. Knight and C. Hanly, University of Illinois Medical Center. Translation and immunoprecipitation were performed as described previously (11). The immunoprecipitated poly-Ig-R primary translation products from the outbred rabbit and the t^{62} homozygous rabbit are shown in Fig. 1, lanes 1 and 2, respectively. The t^{62} homozygous rabbit had only one band of 67 kDa and one band of 90 kDa, whereas the outbred rabbit had two bands of 64 to 67 kDa and two bands of 87 to 90 kDa. Unfortunately, we were unable to obtain mRNA from a t^{61} rabbit to confirm that it produced 64- and 87-kDa forms. Nevertheless, we can conclude that even a homozygous rabbit has two translation products.

We sought to determine if these two translation products are the result of two genes or alternative splicing of one gene. We previously observed that the complete cDNA clone of poly-Ig-R hybridized to two broad bands on Northern blots of outbred rabbit mammary and liver mRNAs (10). Presumably, these corresponded to the mRNAs coding for

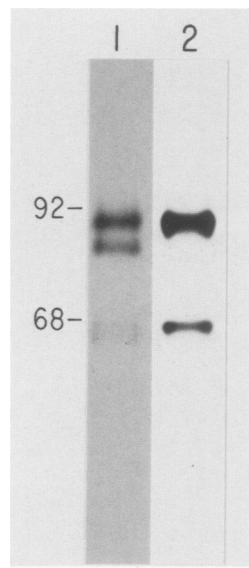

FIG. 1. Translation products of poly-Ig-R from heterozygous and homozygous rabbits. Liver mRNAs were prepared from the presumed heterozygous rabbit used previously (rabbit A, reference 11) and from a rabbit homozygous for the poly-Ig-R allotype t^{62}. The mRNAs were translated in the wheat germ system. Products were immunoprecipitated with anti-SC antiserum, analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7% polyacrylamide), and visualized by fluorography. Lanes: 1, heterozygous rabbit; 2, homozygous rabbit. The positions of the molecular mass standards are indicated at the left (phosphorylase *a*, 92 kDa; bovine serum albumin, 68 kDa).

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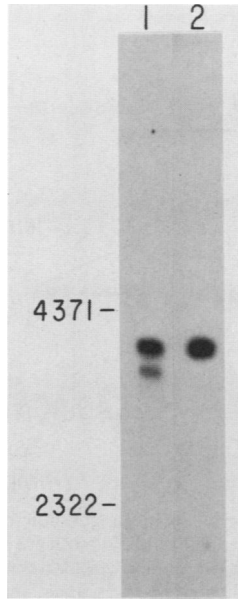

FIG. 2. Northern blot analysis. Liver mRNA (1 μg) from the presumed heterozygous rabbit was run in each of two lanes on a 1% agarose gel in formaldehyde. The mRNA was blotted onto nitrocellulose and probed with either the domain I-specific probe (lane 1) or the domain III-specific probe (lane 2). The positions of the end-labeled *Hin*dIII fragments of lambda DNA used as markers are indicated at the left.

the 64- to 67-kDa and 87- to 90-kDa primary translation products. We now probed the Northern blots with isolated restriction fragments of the cDNA clone that each code for a portion of one of the five immunoglobulin-like domains (8). The domain III-specific probe was a *Sau*3AI fragment from positions 1011 to 1053, whereas the domain I-specific fragment was a *Hin*cII fragment from positions 280 to 427. (Numbering of the nucleotides is described in reference 10.) We found that the probe coding for part of domain III hybridized only to the upper band on the Northern blot (Fig. 2, lane 2), whereas the probe coding for a portion of domain I hybridized to both bands (Fig. 2, lane 1). This result suggested that at least part of domain III is missing in the smaller form of the receptor.

To characterize this deletion, we screened our previously described rabbit liver cDNA library for clones hybridizing to the domain I probe but not to the domain III probe (10). Three such clones were identified (Fig. 3). One was sequenced entirely (10). It was identical to our original poly-Ig-R sequence, except for a deletion of bases 527 to 1161. It also lacked parts of the 5′ and 3′ untranslated regions; this absence may have been due to the use of S1 nuclease in constructing the library. When translated, the deletion in the clone yielded a precise in-frame deletion of domains II and III of the protein. To rule out a cloning artifact, we sequenced the relevant regions of two other independent cDNA clones and found precisely the same deletion. Note that the library was constructed from mRNA from the presumed heterozygous rabbit, and we do not know whether the cDNA clones were for the t^{61} or t^{62} allotype.

To determine whether this deletion could result from alternative splicing, we screened a genomic library (gift of K. Knight) that had been constructed by partially digesting rabbit germ line DNA with *Mbo*II and cloning this DNA into the lambda phage EMBL 4 (4). The poly-Ig-R allotype of the rabbit used is unknown. We probed the genomic library with an *Sma*I fragment of the cDNA clone (nucleotides 214 to 2261) that contained most of the coding region. A total of 5×10^5 clones were screened to yield two positive clones (8).

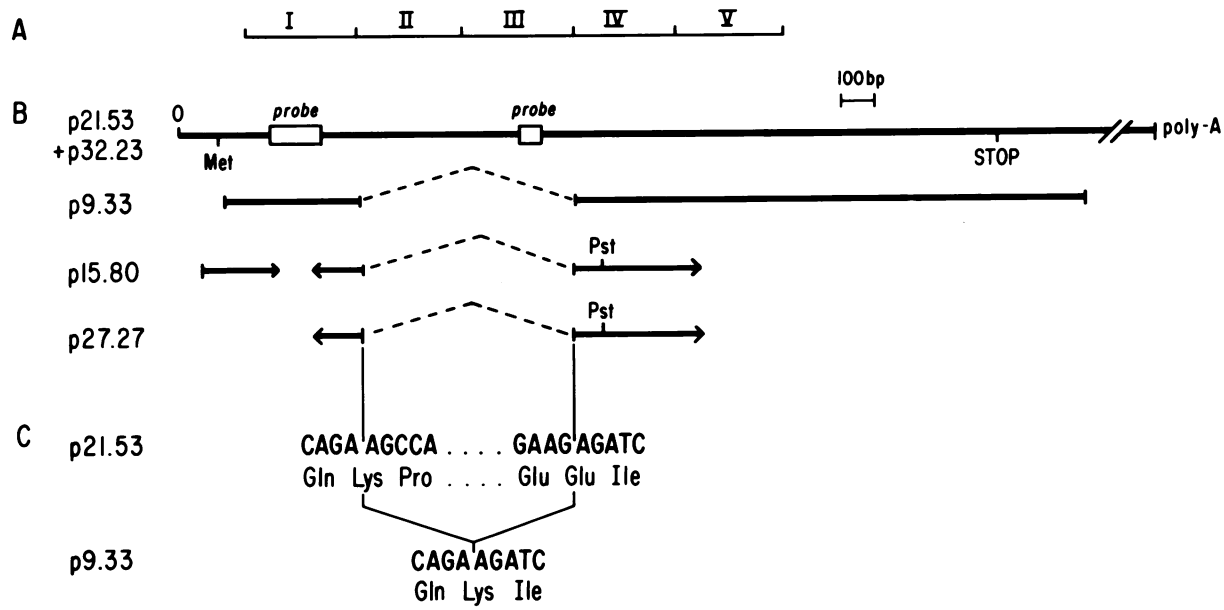

FIG. 3. Comparison of cDNA sequences. (A) Relative positions of the five homologous domains in the protein structure. (B) Maps of sequenced portions of cDNA clones. The composite of clones p21.53 and p.32.23, which were previously sequenced, is shown on the first line. The positions of the initiator methionine (Met), the stop codon, the poly(A) tail, and the domain I- and domain III-specific probes are shown. Clones p9.33, p15.80, and p27.27 contained an internal deletion relative to clone p21.53 (indicated by the dashed line). Clone p9.33 was sequenced entirely, whereas clones p15.80 and p27.27 were only sequenced partially. The *Pst*I site used for sequencing clones p15.80 and p27.27 is shown. The 5′ end of p15.80 was also sequenced. The direction and extent of sequencing are shown by the arrows. bp, Base pairs. (C) Nucleotide and corresponding protein sequences at the boundaries of the deletion for clones p21.53 and p9.33.

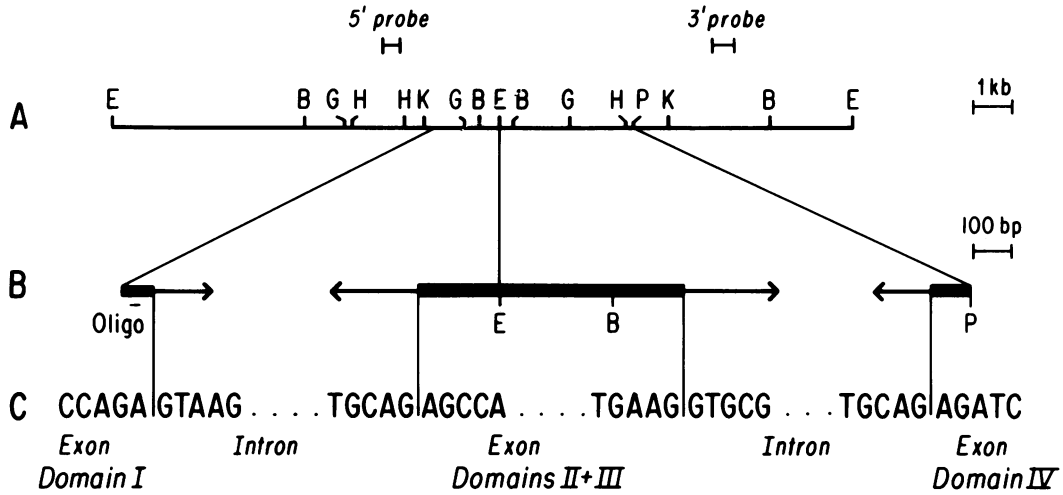

FIG. 4. Organization and sequencing of the genomic clone. (A) Partial restriction map of the insert in the genomic clone. Only restriction sites that were used in sequence determinations, or that are relevant to interpretation of the genomic Southern blots are shown above the map. Abbreviations: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; and K, *Kpn*I. The EcoRI sites on the ends were contributed by the polylinker of the vector. kb, Kilobases. (B) Enlarged map showing intron-exon organization. Exons are indicated by thick lines, and introns are indicated by thin lines. The *Eco*RI (E), *Bam*HI, (B), and *Pst*I (P) sites used for sequencing are indicated. In one case, sequencing was done with an oligonucleotide primer. The oligonucleotide was identical to nucleotides 473 to 492 of the cDNA sequence. Arrows indicate the direction and extent of sequencing. bp, Base pairs. (C) Nucleotide sequences at the intron-exon boundaries.

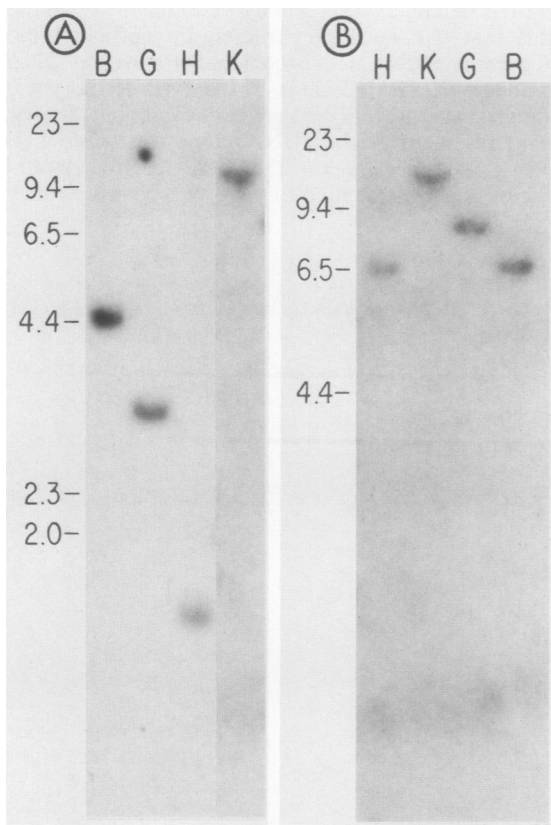

FIG. 5. Genomic Southern blot analysis. Liver DNA was prepared from the presumed heterozygous rabbit and digested with the indicated enzymes. Digests were separated on 0.8% agarose gels and blotted onto Biodyne-Pall nylon membranes. Blots were probed with either the 5′ (A) or 3′ (B) cDNA probe. Abbreviations for restriction enzymes are as described in the legend to Fig. 4. The molecular mass markers are the *Hin*dIII fragments of lambda DNA (in kilodaltons).

By restriction mapping and probing with various short probes, we found that one clone apparently contained the entire reading frame of poly-Ig-R on a single 19-kDa insert (Fig. 4A). We then sequenced the portions of the genomic clone coding for domains II and III and part of the surrounding region (Fig. 4B and C). Domains II and III were encoded by a single exon. Introns were found precisely at the breakpoints of the deletion that yielded the smaller cDNA clones. All splice junctions fit the consensus sequence (12).

To determine the number of genes for the receptor, we digested rabbit liver DNA with four different restriction enzymes (*Bam*HI, *Bgl*II, *Hin*dIII, and *Kpn*I) and probed genomic Southern blots with cDNA probes from the 5′ and 3′ portions of the reading frame (Fig. 5). The 5′ probe was an *Msp*I fragment from positions 42 to 215, whereas the 3′ probe was an *Rsa*I fragment from positions 1590 to 1828. (We found that probes from the 3′ untranslated region hybridized to many bands, suggesting that it contained a repetitive element.) We found a single band in each case. Considering the restriction map of the genomic clone (Fig. 4), the results strongly suggest that there is only one gene for poly-Ig-R.

Taking together the data from the Northern blots, cDNA and genomic sequencing, and genomic blots, we suggest that the transcript of the poly-Ig-R gene can be spliced in two ways. The exon coding for domains II and III can be left in to produce the 90-kDa translation product or spliced out to produce the 67-kDa product. (We have not examined whether the transcripts leading to these two mRNAs have the same 5′ or 3′ ends.) Our conclusion is consistent with the protein sequencing and peptide mapping data that suggest that the smaller forms of SC have an internal deletion relative to the larger forms (6). Our analysis of the gene structure did not reveal the basis of the difference between the two allotypes of the receptor.

Alternative splicing has been described for a wide variety of genes. Splicing in or out of an entire exon occurs in lens alpha crystallin (3) and myelin basic protein (13). Of particular interest in poly-Ig-R is that the alternative splicing

involves immunoglobulin-like domains. Differential processing of mRNAs occurs in immunoglobulin heavy-chain switching but involves a different mechanism (1). We examined the relative levels of the mRNAs for the 67- and 90-kDa translation products in rabbit liver, lactating mammary gland, and small intestines. No statistically significant differences were observed (data not shown), suggesting that the alternative splicing is not tissue specific. However, it is possible that tissue-specific regulation exists in other tissues, such as the uterus, where the production of poly-Ig-R is hormonally controlled (14). We do not know if there are functional differences between the alternatively spliced receptors (7).

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