11. The W-100 line carries ten homozygous recessive mutations with visible phenotypes, two on each chromosome. The *ap-1* (apetala) locus has been mapped to position 103.5 on chromosome 1 [M. Koornneef *et al.*, *J. Hered.* **74**, 265 (1983)]. Seeds of W-100 were kindly supplied by M. Koornneef, Department of Genetics, Agricultural University, Wageningen, The Netherlands.
12. Values given for linkage analysis are percent recombination ± the standard error as calculated with maximum likelihood formulas. Calculations were performed with the Linkage-1 computer program [K. A. Suiter, J. F. Wendel, J. S. Case, *J. Hered.* **74**, 203 (1983)].
13. Freshly harvested seeds of the wild type showed >95% germination if imbibed seeds were preincubated at 4°C for 3 days. The *etr* mutant seeds did not germinate under these conditions. The mutant line showed >90% germination if seeds were treated with 5 μM gibberellic acid (GA_3) during the 3-day preincubation.
14. Ethylene-induced increases in peroxidase activity have been reported for a number of plant species [F. B. Abeles, *Ethylene in Plant Biology* (Academic Press, New York, 1973)].
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Isotypic Exclusion of γδ T Cell Receptors in Transgenic Mice Bearing a Rearranged β-Chain Gene

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The rearrangement of T cell antigen receptor β- and γ-chain gene segments was studied in transgenic mice that bear a functional β-chain gene. Virtually all CD3-positive T cells derived from transgenic mice express β chains containing the transgene-encoded $V_\beta 8.2$ variable region on their surfaces and do not express endogeneous β-chain variable regions. Expression of endogenous V_β genes is inhibited at the level of somatic recombination during thymic ontogeny. Furthermore, rearrangements of the TCR γ-chain genes are also markedly inhibited in these transgenic animals. Hence expression of the TCR β transgene has led to allelic exclusion of αβ receptors and isotypic exclusion of γδ T cell receptors.

THE DIVERSITY OF THE T CELL receptor (TCR) for antigen is generated during intrathymic development by a regulated process involving somatic recombination of variable (V), diversity (D), and joining (J) gene segments. Findings that individual T cells can express on their surface either a γδ or an αβ receptor (*1, 2*) and that the γ and δ genes rearrange early in ontogeny, followed by β and then α (*3*), have led to models in which products of TCR rearrangement determine not only the antigenic specificity of the T cell but also the lineage to which it will belong. Although recombination of TCR gene segments involves a mechanism whose features appear similar to those of the immunoglobulin (Ig) system (*4*), the signals that sequentially activate each locus to begin recombination and those that terminate these events are unknown.

Each T cell expresses only one functional idiotypic receptor (*2*), and therefore mechanisms must exist to exclude further TCR gene rearrangements once a functional receptor is produced, a phenomenon referred to as allelic exclusion. Allelic exclusion in B cells has been studied by introducing functional heavy or light chain Ig genes into the germline of mice and defining the effects of the transgene on endogenous Ig gene rearrangements. Expression of injected μ heavy chain genes significantly decreases the expression of endogenous heavy chain loci by inhibiting V-to-DJ and D-to-J recombination events (*5*). Several lines of evidence, including the characterization of transgenic mice bearing a productive κ-chain gene, indicate that allelic exclusion of light chain loci is mediated by intact Ig (*6*).

To study the mechanisms by which productively rearranged TCR genes regulate developmental events during T cell ontoge-

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Table 1. Phenotype of peripheral T cells and thymocytes by FACS analysis. Screening of purified peripheral blood T cells showed that all animals were heterozygous H-2^b × H-2^s and homozygous for the SJL endogenous TCR β locus. Lymph node T cells were purified by passage over nylon wool. Primary antibodies were KJ23a (*20*), 2C11 (*21*), F23.1 (*9*), GK1.5 (*22*), and 53.6.72 (*23*). RR-4.7 is a rat monoclonal antibody specific for $V_\beta 6$ (*24*). T cells and thymocytes were stained and analyzed as previously described (*20*). FACS analysis performed on other mice from the TCRE-1 and TCRE-2 lines confirms the data shown here.

Determinant	Antibody	Peripheral T cells (%)		Thymocytes (%)	
		Control	TCRE-1	Control	TCRE-1
CD3	2C11	99	94		
$V_\beta 8$	F23.1	0	97	0.06	76
$V_\beta 17a$	KJ23a	5.45	0.05	3.28	0.2
$V_\beta 6$	RR-4.7	12.12	0.34		
CD4	GK1.5	58.78	41.7		
CD8	53.6.72	36.93	54.78		

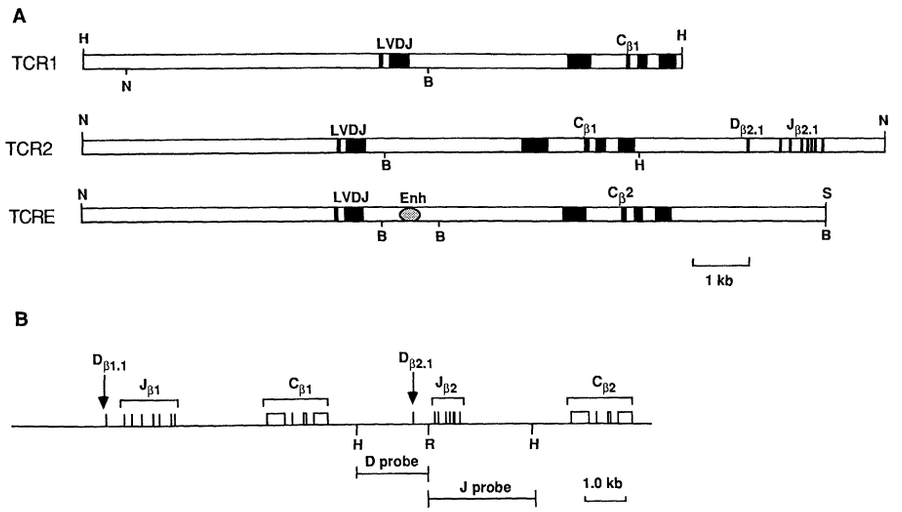

Fig. 1. (**A**) Schematic representation of the three constructs injected into β-chain transgenic mice (*14–18*). Exons are indicated as closed boxes; introns and intergenic regions are open. LVDJ refers to the leader, variable, diversity, and joining DNA segments of the productive DO-11.10 β-chain allele. The approximate position of the Ig heavy chain enhancer (Enh) is shown within the 1-kb Bam HI restriction fragment. Restriction enzyme sites indicated above each construct were used to cleave cloned sequences from the vector for injection into fertilized eggs; other relevent restriction sites are shown below each construct. Nru I, N; Bam HI, B; Hind III, H; and Sma I, S. (**B**) Diagrammatic representation of the murine germline β-chain locus (*2*). The map positions of the $D_\beta2.1$ and $J_\beta2$ probes are shown. Exons are indicated as vertical bars or boxes above the horizontal axis.

ny, we introduced a productively rearranged TCR β-chain gene from the T cell hybridoma DO-11.10 into transgenic mice. DO-11.10 has reactivities for chicken ovalbumin in association with IA^d, and alloreactivity for ovalbumin associated with IA^b as well as for IA^b alone (*7*). The productive DO-11.10 β-chain allele utilizes the $V_\beta8.2$ variable gene (*8*) and therefore reacts with the allotypic monoclonal antibody F23.1 [which recognizes all three members of the $V_\beta8$ subfamily (*9*)]. This productively rearranged allele was cloned into lambda phage 2001 as a 13-kb Hind III fragment (TCR1) (Fig. 1A) and was introduced into transgenic mice. Four independent transgenic lines, each of which contained multiple tandem repeats of the transgene, failed to express significant levels of transgenic RNA or protein product in the thymus or peripheral T cells [as assayed by fluorescence-activated cell sorter (FACS) and RNA blot analysis]. Transgenic mice containing a second construct, TCR2 (which contains an additional 4 kb on the 3′ side of $C_\beta1$) (Fig. 1A), also failed to express significant levels of the transgene in lymphoid organs. Recently, a TCR enchancer (TCRE) was mapped 5 kb to the 3′ side of the $C_\beta2$ coding region (*10*); our data suggest that this enhancer drives transcription of productive rearrangements to the $C_\beta1$ locus as well, at a distance of more than 25 kb.

The murine Ig heavy chain enhancer functions in T cells of transgenic mice (*11*). We therefore introduced this enhancer element into a Bam HI site between the variable and constant regions of the cloned rearranged DO-11.10 β-chain gene (TCRE in Fig. 1A). Five transgenic mouse lines were established, and two of these, TCRE-1 and TCRE-2, contained tandem insertions of the transgene (see below) and were studied further.

To test for cell surface expression of the transgene in the TCRE lines, we used a panel of monoclonal antibodies to stain nylon wool–purified T cells from lymph nodes of an F_2 animal of the TCRE-1 line and a transgene-negative littermate; the cells were then subjected to FACS analysis. Nearly all lymph node T cells from the control and the TCRE-1 animal were $CD3^+$ (Table 1). Transgene-negative siblings did not stain with F23.1, as expected, since both TCRE transgenic lines had been bred from strains that lack endogenous $V_\beta8$ genes (SJL or C57L). In contrast, 97% of purified TCRE-1 T cells reacted with F23.1. Since 94% of this T cell population was $CD3^+$ and 97% expressed $V_\beta8$ determinants, these data indicate that most peripheral T cells expressed the transgene on their surfaces. Antibodies specific for two other variable region genes of the endogenous β-chain locus ($V_\beta17a$ or $V_\beta6$) stained T cells from control animals (5.45% and 12.12%, respectively), whereas TCRE-1 T cells showed negligible staining. When thymocytes from TCRE-1 animals were stained with F23.1 (Table 1), 76% were reactive, with the expected broad distribution between dull (cortical) and bright (medullary) staining; thymocytes from transgene-negative siblings were not reactive with F23.1, as expected. In contrast, there were virtually no $V_\beta17a$-positive thymocytes in TCRE-1 animals, whereas 3.28% of thymocytes from transgene-negative siblings reacted with $V_\beta17a$. These data demonstrate that transgene expression has led to a nearly complete absence of expression of the endogenous V_β genes, and indicate that the inhibitory effect occurs during T cell development in the thymus. In support of these data, we observed very high levels of $V_\beta8$-specific RNA in the thymus of TCRE-1 and TCRE-2 mice, whereas little RNA was detected with probes specific for $V_\beta6$ or $V_\beta16$, two endogenous V_β genes that were expressed at easily detectable levels in control animals. Therefore, the murine Ig heavy chain enhancer can direct high levels of transcription from the TCR β-chain promotor in thymocytes and T cells. In addition, high levels of transgene-specific RNA were observed in splenic B cells, whereas low but detectable levels were seen in other nonlymphoid tissues, including skeletal muscle, brain, and intestine.

The ratio of peripheral T cells expressing CD4 or CD8 (normal 60:40) is reversed in the TCRE-1 line (Table 1). Models of either positive or negative selection during thymic ontogeny can be invoked to explain these results (presumably involving interactions between the thymocyte TCR and thymic major histocompatibility determinants); further studies will be required to resolve this issue.

Previous studies with transgenic mice bearing functional Ig transgenes (*5*) indicated that a likely mechanism for the inhibition of endogenous β-chain expression observed in the TCRE lines would be decreased gene rearrangements at the β-chain locus. We tested this model by subjecting genomic DNA from the liver and thymus of transgenic animals and their transgene-negative siblings to DNA blot analysis and hybridizing with either $J_\beta2$- or $D_\beta2.1$-specific probes (Fig. 2, A and B, respectively). Thymus DNA from nontransgenic control animals yielded many bands (labeled VDJ) that represent complete V-to-DJ recombinations, whereas the restriction pattern of thymus DNA from the TCRE mice was devoid of these rearrangements. Incomplete DJ recombinations were observed in the transgenic animals, but to a lesser degree than in control animals. These data indicate that allelic exclusion of the murine β-chain loci in the TCRE lines occurs in the thymus during T cell ontogeny, and that the mechanism is inhibition of V-to-DJ recombination events and a reduction of D-to-J recombination. These data are in general agreement with studies of transgenic mice bearing a different TCR β-chain gene (*12*). However, in con-

trast to our results, only a low level of D-to-$J_\beta 2$ recombination was reported, and a normal CD4/CD8 ratio was observed (*12*).

The genes encoding the γ chain of the murine TCR begin to rearrange early in T cell development, prior to the expression of the TCR β-chain genes (*3*) and continue to rearrange in the adult thymus. We examined the possibility that expression of the transgene alters the pattern of γ gene rearrangements in the thymus of TCRE animals. For this purpose, genomic DNA from the same animals as studied in Fig. 2A was digested with Pvu II and DNA blots were hybridized to a Cγ probe. Five germline bands were observed in the liver of transgene-negative siblings and in the TCRE-1 and TCRE-2 animals (Fig. 2C). A single band (arrow) representing γ gene rearrangements was observed in the thymus DNA of control animals but was barely detectable in thymus DNA of TCRE-1 and TCRE-2 animals.

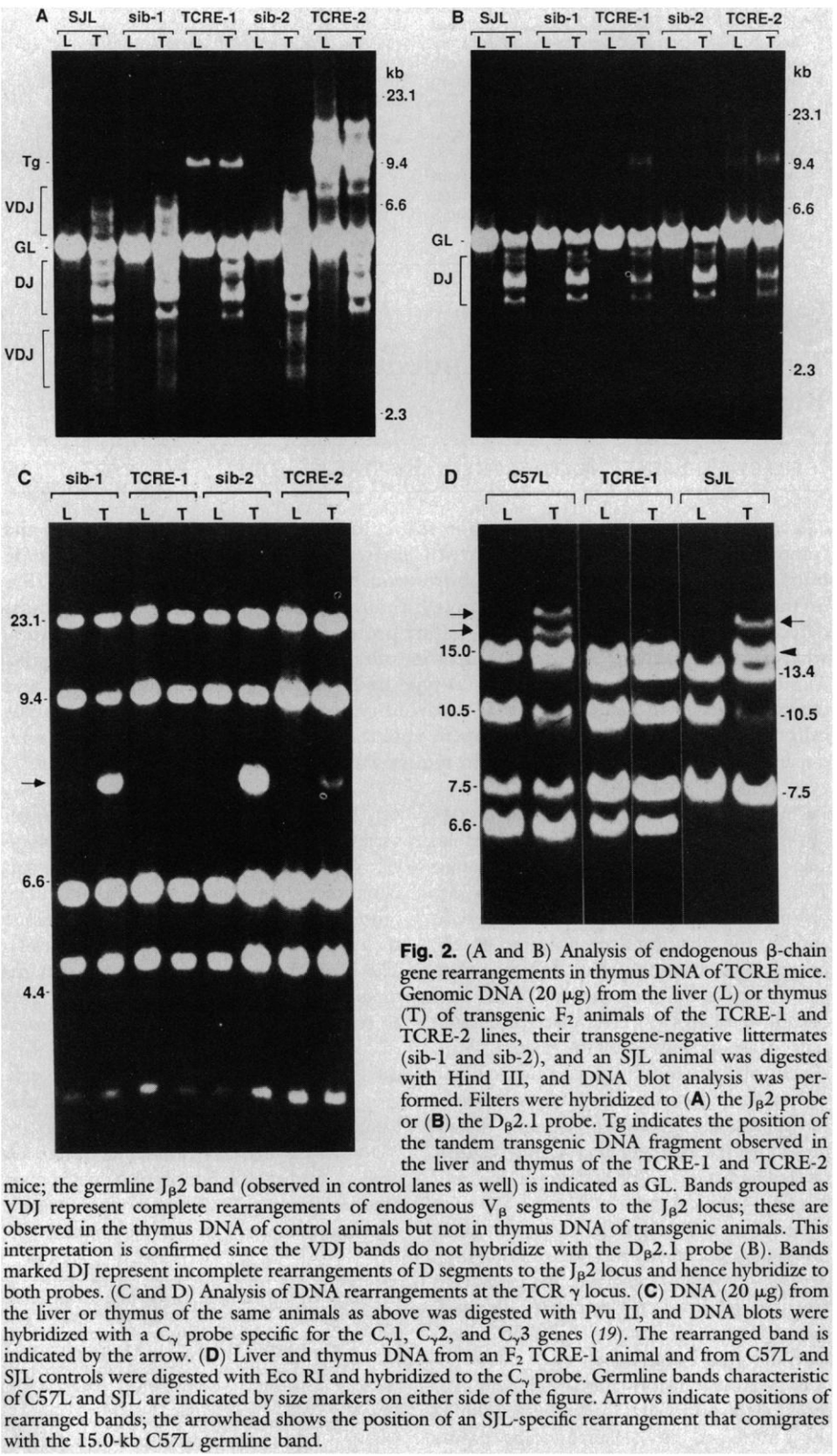

Fig. 2. (A and B) Analysis of endogenous β-chain gene rearrangements in thymus DNA of TCRE mice. Genomic DNA (20 μg) from the liver (L) or thymus (T) of transgenic F_2 animals of the TCRE-1 and TCRE-2 lines, their transgene-negative littermates (sib-1 and sib-2), and an SJL animal was digested with Hind III, and DNA blot analysis was performed. Filters were hybridized to (**A**) the $J_\beta 2$ probe or (**B**) the $D_\beta 2.1$ probe. Tg indicates the position of the tandem transgenic DNA fragment observed in the liver and thymus of the TCRE-1 and TCRE-2 mice; the germline $J_\beta 2$ band (observed in control lanes as well) is indicated as GL. Bands grouped as VDJ represent complete rearrangements of endogenous V_β segments to the $J_\beta 2$ locus; these are observed in the thymus DNA of control animals but not in thymus DNA of transgenic animals. This interpretation is confirmed since the VDJ bands do not hybridize with the $D_\beta 2.1$ probe (B). Bands marked DJ represent incomplete rearrangements of D segments to the $J_\beta 2$ locus and hence hybridize to both probes. (C and D) Analysis of DNA rearrangements at the TCR γ locus. (**C**) DNA (20 μg) from the liver or thymus of the same animals as above was digested with Pvu II, and DNA blots were hybridized with a C_γ probe specific for the $C_\gamma 1$, $C_\gamma 2$, and $C_\gamma 3$ genes (*19*). The rearranged band is indicated by the arrow. (**D**) Liver and thymus DNA from an F_2 TCRE-1 animal and from C57L and SJL controls were digested with Eco RI and hybridized to the C_γ probe. Germline bands characteristic of C57L and SJL are indicated by size markers on either side of the figure. Arrows indicate positions of rearranged bands; the arrowhead shows the position of an SJL-specific rearrangement that comigrates with the 15.0-kb C57L germline band.

Since the γ locus contains three functional constant region genes and seven variable region genes (*13*), we examined the possibility that γ rearrangements could be detected in the TCRE lines with enzymes that show rearrangements of other γ-chain variable and joining segments. For this purpose, DNA blot analysis was performed on Eco RI–cleaved genomic DNA. This analysis is complicated by extensive polymorphism in the γ locus. Since the TCRE mice used in this experiment were heterozygous for γ alleles from C57L and SJL mouse strains, DNA from these mouse strains were included as controls. TCRE-1 liver DNA contained five germline bands as expected from a merging of the SJL and C57L germline patterns (Fig. 2D). C57L thymus DNA contained at least two rearranged bands (arrows), as did SJL thymus DNA, one of which comigrates with the 15.0-kb germline band of C57L (arrowhead). In contrast, the Eco RI digestion pattern of TCRE-1 thymus DNA was identical to the germline pattern in liver. None of the rearrangements observed in C57L or SJL thymus were observed in TCRE thymus DNA, although a small amount of an SJL-type rearrangement could be hidden by the 15.0-kb germline band. Thymus DNA from transgene-negative siblings showed both the C57L and SJL rearrangement patterns. These data were confirmed by hybridization of DNA blots to probes specific for the $V_\gamma 1.2$ or $V_\gamma 2$ variable region genes; thymus DNA from TCRE mice showed a marked decrease in rearranged V_γ genes as compared to DNA from control animals.

These results demonstrate that expression of a productively rearranged β-chain gene can lead to a decrease in rearrangements at the TCR γ locus in the thymus of adult transgenic animals. This observation correlates with the apparent deficit of $CD3^+CD4^-CD8^-$ lymph node T cells, which usually express γδ receptors (Table 1). During normal T cell ontogeny, γ genes rearrange before β chains are expressed. Presumably in the TCRE animals, the Ig enhancer directs expression of the transgene in developing thymocytes before rearrangement of γ-chain and endogenous β-chain genes. The transgene product, either alone or in a membrane complex with CD3 and

perhaps the TCR α chain, would then elicit an intracellular signal to block further rearrangements of the γ locus. We believe that the transgene-induced inhibition of rearrangements at the endogenous β-chain locus and the γ locus are mediated by the same molecular signal. This could act at the level of the recombinase (or associated factors), or by rendering germline V_γ and V_β segments inaccessible to the recombinase, perhaps by alterations in chromatin configuration or inhibition of transcription at these loci. Whether transgene expression in the TCRE lines causes accelerated developmental activation of the α-chain locus (and hence deletion of δ-chain alleles), and how this might play a role in excluding further TCR gene rearrangements, is unknown. A recent report (*12*) indicates that a TCR β-chain transgene driven by its own promotor and enhancer elements has no discernible effects on γ gene rearrangements. This difference from our observations may reflect the earlier developmental activation of the Ig enhancer in the TCRE mice.

The inherent ability of a functional β-chain complex to block further γ rearrangements would help ensure that each T cell expresses a single idiotypic antigen receptor on its surface. This inhibition of γ rearrangements leads to the prediction that in clonal T cell lines derived from TCRE mice, the γ TCR genes will be in germline configuration and suggests that αβ-bearing T cells can arise without an obligatory developmental stage in which γ genes rearrange. It will be of interest to ascertain whether allelic exclusion also occurs at the TCR δ-chain locus, and to determine directly whether γδ cells can be detected in the skin, thymus, or peripheral lymphoid organs of TCRE animals.

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14. Hind III–digested genomic DNA from DO-11.10 were size-fractionated on 10% to 40% sucrose gradients, and appropriate fractions were ligated to λ2001 arms (Stratagene) and packaged in vitro. Plaques hybridizing to the $V_\beta 8$ probe were purified and the 13-kb insert (TCR1) was subcloned. Construct TCRE was produced by cleaving TCR1 with Bam HI and replacing the $C_\beta 1$ Bam HI fragment with the germline $C_\beta 2$ Bam HI fragment. This construct was partially cleaved with Bam HI and a 1-kb fragment containing the murine Ig heavy chain enhancer (*15*) was inserted as shown. V_β probes were kindly provided by D. Y. Loh (*16*). Transgenic mice were produced according to published procedures (*17*). DNA blot analysis and hybridizations were performed as described earlier (*18*).
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Tumor Formation Dependent on Proteoglycan Biosynthesis

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The role proteoglycans play in tumor formation was examined by measuring the tumorigenicity of proteoglycan-deficient Chinese hamster ovary cell mutants in nude mice. When 10^7 cells were injected subcutaneously, mutants with less than about 15% of the wild-type level of proteoglycan synthesis did not produce tumors. Mutants defective in the synthesis of heparan sulfate proteoglycans also did not form tumors, whereas mutants with altered chondroitin sulfate proteoglycans were tumorigenic. Tumors arose from mixtures of wild-type and nontumorigenic mutant cells and contained both cell types, suggesting that wild-type cell proteoglycans enabled mutant cells to survive. The failure of heparan sulfate–deficient mutants to form tumors depended on the ability of the host to mount a B cell–mediated immune reaction.

PROTEOGLYCANS OCCUR ON THE plasma membranes of mammalian cells and in the extracellular matrix. The various classes of proteoglycans are distinguished by differences in core proteins and in the chemical composition of the linear polysaccharide chains (glycosaminoglycans) covalently attached to serine residues in the cores (*1*). The glycosaminoglycans of chondroitin sulfate proteoglycans consist of alternating D-glucuronic acid and *N*-acetyl-D-galactosamine residues, whereas the glycosaminoglycans of heparan sulfate proteoglycans contain alternating residues of D-glucuronic acid or L-iduronic acid and *N*-acetyl-D-glucosamine. Because of the presence of sulfate groups at various positions along the chains and the resulting high density of negative charges, proteoglycans are able to interact electrostatically with numerous soluble ligands and matrix proteins involved in cell adhesion (*2, 3*).

Neoplastic transformation of cells dramatically alters proteoglycan synthesis both in the tumor and in the surrounding tissues (*4*). Although the pattern of these changes varies among tumors of different histological types, in general more chondroitin sulfate proteoglycans and fewer heparan sulfate proteoglycans are present in tumorous tissue than in the corresponding normal tissue (*4*). The heparan sulfate proteoglycans that tumors produce also tend to be poorly sulfated as compared to their normal counterparts (*5*). These alterations in proteoglycan synthesis are thought to stimulate tumorigenic growth by decreasing the adhesion of transformed cells to the extracellular matrix (*6*).

Numerous animal cell mutants have been used to study the function of glycoproteins in tumorigenesis (*7*). A similar approach for studying proteoglycans has not been possi-

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