Oncogenic *Myc* translocations are independent of chromosomal location and orientation of the immunoglobulin heavy chain locus

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Many tumors are characterized by recurrent translocations between a tissue-specific gene and a proto-oncogene. The juxtaposition of the Ig heavy chain gene and *Myc* in Burkitt's lymphoma and in murine plasmacytoma is a classic example. Regulatory elements within the heavy chain constant region locus are required for *Myc* translocation and/or deregulation. However, many genes are regulated by cis-acting elements at distances up to 1,000 kb outside the locus. Such putative distal elements have not been examined for the heavy chain locus, particularly in the context of *Myc* translocations. We demonstrate that a transgene containing the Ig heavy chain constant region locus, inserted into five different chromosomal locations, can undergo translocations involving *Myc*. Furthermore, these translocations are able to generate plasmacytomas in each transgenic line. We conclude that the heavy chain constant region locus itself includes all of the elements necessary for both the translocation and the deregulation of the proto-oncogene.

Many tumor types, including most leukemias and lymphomas, are characterized by reciprocal translocations of the same two chromosomal loci in independent tumors. A number of B lineage lymphomas harbor recurrent translocations that involve the Ig locus, whereas the T-cell receptor locus is involved in most T lineage lymphoma translocations (1, 2). As a result of these recurrent translocations, promoters or enhancers in one translocation partner often change the regulation of the proto-oncogene in the other partner. A classic example is the translocation of the *Myc* proto-oncogene on human chromosome (Chr) 8 (mouse Chr 15) to the Ig heavy chain locus (*Igh*) on human Chr 14 (mouse Chr 12) (3–6). This translocation results in *Myc* being placed under the strong transcriptional control of *Igh*. The *Myc:Igh* translocation occurs in more than 85% of human Burkitt's lymphoma and mouse plasmacytoma, and it is one of the earliest events in tumorigenesis, indicating it to be the driving force of these tumors. Molecularly, the translocation junction occurs most frequently in the first (noncoding) exon or first intron of the *Myc* gene, joining the "tail" (second and third exons) of *Myc* to the tail (3′ or constant region) end of *Igh*. Because it is retained in the majority of these translocations, enhancer elements in the tail (3′ regulatory region) of the *Igh* locus presumably deregulate *Myc* expression, a primary event in the tumorigenesis (7). In part, the prevalence of this recurrent translocation is due to strong selection for deregulated *Myc* expression (3–6).

Chromosomal location also has a role in recurrent translocations. Although the *Myc* and *N-myc* genes are very similar, *Myc*, but not *N-myc*, is found as a translocation partner in murine pro–B-cell lymphomas that arise in mice deficient in the DNA repair factors p53 and Ligase 4. Gostissa et al. tested whether this cell-type specific use of *Myc* in translocations was due to selection for specific activities of the protein encoded by the *Myc* gene, by replacing the *Myc* coding exons with the *N-myc* coding exons (8). They found translocations in pro–B-cell lymphomas now joined heavy chain genes to the *N-myc* gene in the *Myc* location. These investigators concluded that, at least for this pair of genes in this genetic background, selection for the activities of the specific protein is less important than cis-acting elements in the *Myc* locus that target translocations with some degree of cell-type preference (8). Apparently, preferential targeting of specific loci can vary depending on the cell type and on DNA repair pathways used; the *N-myc* locus is a target for chromosomal rearrangements in other genetic backgrounds (9, 10).

Myc:Igh translocations in plasmacytoma are thought to result from aberrant heavy chain class switch recombination (1, 2). Normal switch recombination occurs through double-stranded breaks that are introduced into 2- to 8-kb switch (S) regions that precede the *Igh* constant region genes (11). S regions are characterized by multiple copies of simple sequences, some of which are preferred sites for action by the activation-induced cytidine deaminase (AID), the enzyme that initiates class switching (12, 13). Recombination joins double-stranded breaks in two S regions, bringing the exon encoding the *Igh* variable region into physical and functional association with a new heavy chain constant region with different effector functions (11). Like class switch recombination, translocations to *Myc* usually involve S regions (1, 4–6) and depend on AID (14–16).

The known regulatory elements contained within the *Igh* locus have been examined for a role in *Myc* translocation and deregulation. The *Igh* intronic enhancer (Eμ) is not physically associated with the *Myc* coding sequences after translocation and is, therefore, unlikely to be important for *Myc* deregulation (3–6). Gostissa et al. demonstrated that elements in the *Igh* 3′ regulatory region are required for *Myc* translocation and/or deregulation (17). The potential for additional cis-acting elements outside the *Igh* constant region locus has not been investigated. In this study, we addressed a fundamental question: Are DNA sequences flanking the *Igh* constant region locus in its normal chromosomal location required for tumorigenic translocations? Or, are the sequences within the constant region locus sufficient? We used an *Igh* transgene in five genomic locations and determined that all five different chromosomal locations are permissive for translocations with *Myc* that result in plasmacytoma.

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Results

***Igh* Transgene Is Able to Undergo Translocations with *Myc* at Multiple Chromosomal Locations.** To determine whether the chromosomal environment impacts the development of *Myc:Igh* translocations, we analyzed these events in transgenic mouse models expressing *Igh* from five different chromosomes. The transgene consists of a 230-kb bacterial artificial chromosome (BAC) carrying a prerearranged VDJ exon and the entire heavy chain constant domain, including the 28-kb 3′ regulatory region (7) and an additional 15 kb (Fig. 1*A*) (18). This heavy chain transgene undergoes normal class switch recombination that is qualitatively and quantitatively comparable to that of the endogenous locus, regardless of the chromosomal insertion site (18). To determine whether this construct can participate in *Myc* translocations, transgenic B cells were induced ex vivo to undergo class switching. To examine a reasonable number of translocation events, we increased the frequency of them by overexpression of AID from a retrovirus. Three days after activation, *Myc:Igh* translocations were cloned by using a PCR-based approach (19, 20) and characterized by DNA sequencing. Three independent transgenic lines were studied. Line 995 contains three complete copies of the transgene on chromosome 4. The variable region-encoding exon of at least one transgene copy in line 995 is proximal to the centromere and inverted compared with the endogenous locus (Fig. 1*B*). Line 820 contains a single copy of the *Igh* transgene inserted into chromosome 7 and inverted compared with the endogenous locus. Line 556 contains two complete and two partial copies of the transgene inserted near the telomere of chromosome 17 in the same orientation as the endogenous *Igh*. We found that the *Igh* transgenes were a frequent partner for *Myc* translocations in tissue culture; 79% of translocations were to the transgene (Fig. 1*B*). The translocations to the transgene were indistinguishable from translocations to the endogenous locus, in that they were distributed over a 2-kb region, including the 5′-most *Myc* exon and intron, and the translocations were widely distributed in S regions (Table S1). The interpretation of these experiments includes the assumption that the ratio of translocations involving the transgene to translocations involving the endogenous genes will not be changed significantly by AID overexpression. Even considering this assumption, these results established that the *Igh* transgene, in three different chromosomal locations, can undergo recombination with the *Myc* proto-oncogene. Recombination of *Myc* with the transgene is at least as efficient as recombination with the endogenous *Igh* genes.

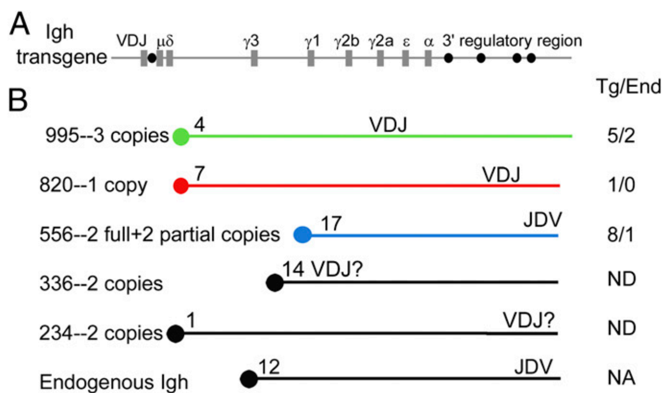

Fig. 1. *Myc:Igh* translocations. (*A*) Structure of the 230-kb *Igh* transgene. Coding exons are depicted as gray rectangles, and regulatory elements are depicted as black circles. The transgene is drawn approximately to scale. (*B*) Five transgenic lines used in this study are shown, with copy number, a schematic of chromosome location, and transgene orientation relative to the centromere depicted by the orientation of the VDJ exon. The location of the endogenous *Igh* locus on Chr 12 is shown for comparison. To the right of the chromosome schematics is shown an enumeration of *Myc:Igh* translocation sites cloned from in vitro cultures. Translocation to the transgene or to the endogenous genes (number of sequences of each type shown, separated by a slash) was determined by evaluating three to 52 polymorphisms in the various S regions (see *SI Materials and Methods* for sequence data and methods).

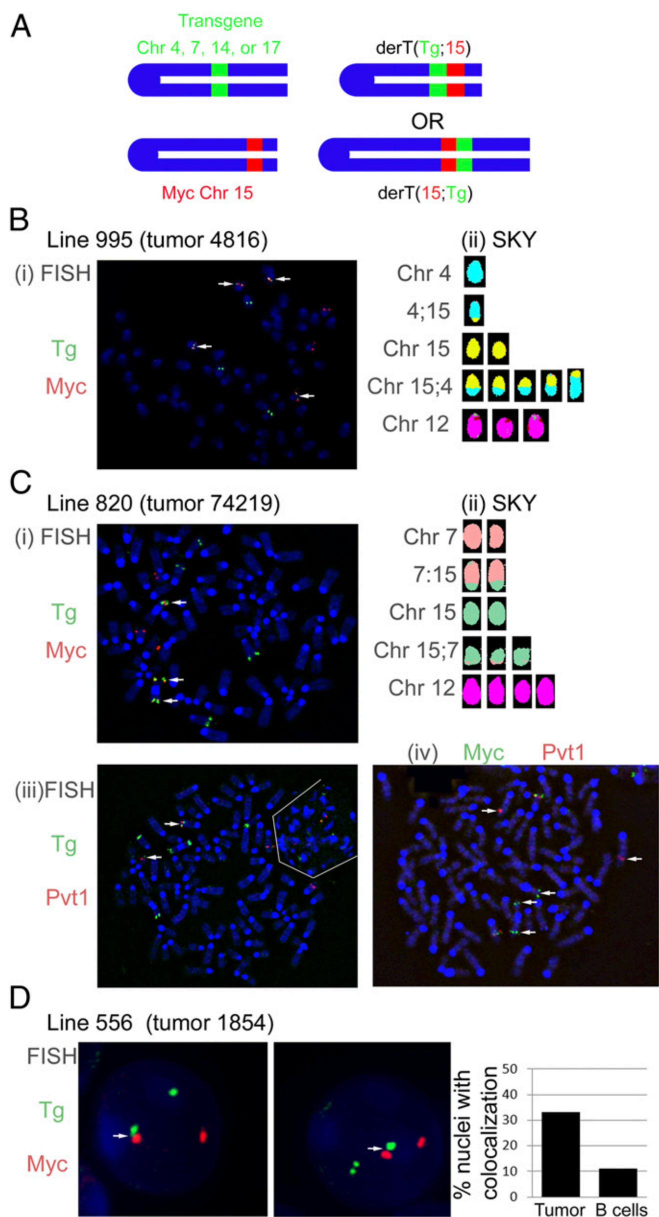

Fig. 2. Plasmacytomas harbor translocations to the *Igh* transgene. (*A*) Colocalization of the *Igh* transgene with *Myc* or *Pvt1*. Colocalization of BACs spanning the *Igh* transgene insertion site on Chr 4 (line 995), 7 (line 820), or 17 (line 556) and either *Myc* or *Pvt1* reveal translocations involving the two loci. White arrows note separation of probes (*Civ*) or colocalization (*C i–iii*). (*B*) Two-color FISH (*i*) and SKY (*ii*) on metaphase spreads from tumor no. 4816, line 995. The color coding for Chr 15 for the SKY analysis of these metaphases was changed to yellow to better contrast with the aqua color coding for Chr 4. (*C*) Two-color FISH and SKY analysis of tumor no. 74219, line 820. In *Ci*, the *Igh* transgene is shown to colocalize with *Myc*. In *Ciii*, the *Igh* transgene is shown to colocalize with *Pvt1*. The white lines delimit a second, interphase cell that lies next to the metaphase. In *Civ*, the *Myc* and *Pvt1* probes are rearranged onto different chromosomes. (*D*) Interphase two-color FISH analysis of tumor no. 1854, line 556 (two representative cells shown). *Myc* and the *Igh* transgene were colocalized in 33% of the interphases from tumor no. 1854 ascites, and in 11% of the interphases from normal B-cell controls ($P < 0.001$, Fisher's exact test).

***Myc:Igh* Translocations to the Transgene Are Found in Plasmacytoma.** To determine whether these translocations involving the *Igh*

Table 1. *Myc:Igh* translocations

Transgenic line	Primary tumor	Genetic background	Translocations	Other
995 Chr 4 at 98.3 of 154.3 Mb	4816	C57BL/6, Bcl-xL	4;15	*Myc*-Sμ (cloning), secretes IgA
VDJ centromere proximal	1022	C57BL/6, Bcl-xL	4;15	
820 Chr 7 at 122 of 145 Mb	74149	Mixed, Bcl-xL*	7;15	
VDJ centromere proximal	74150	Mixed, Bcl-xL	7;15	
	74160	Mixed, Bcl-xL	7;15 and 12;18	Secretes IgA
	74162	Mixed, Bcl-xL	12;15	
	74163	Mixed, Bcl-xL	7;15	Secretes IgA, *Pvt1*-Sα (cloning)
	74219	Mixed, Bcl-xL	7;15 and 6;13	
	74220	Mixed, Bcl-xL	12;15	
	74223	Mixed, Bcl-xL	7;15	
	74997	Mixed, Bcl-xL	12;15	
	75000	Mixed, Bcl-xL	12;15	
	75001	Mixed, Bcl-xL	7;15	Chr 4 insertion into 7;15
	75002[†]	Mixed, Bcl-xL	7;15	Secretes IgA and IgG2a
	75002[†]	Mixed, Bcl-xL	12;15	
	75004	Mixed, Bcl-xL	12;15	
336 Chr 14 near centromere	78286	Mixed, Bcl-xL*	14:15	
orientation unknown	79130	Mixed, Bcl-xL	14?;15[‡], 15;18, and 1;4	*Myc*-Sγ2a (cloning)
	79134	Mixed, Bcl-xL	14;15	
	79710	Mixed, Bcl-xL	14;15	
	79711	Mixed, Bcl-xL	14;15	
	79716	Mixed, Bcl-xL	14;15 and 9;16	
	80635	Mixed, Bcl-xL	14;15	
	80639	Mixed, Bcl-xL	12;15	
	80648	Mixed, Bcl-xL	14;15	
	82028	Mixed, Bcl-xL	12;15	
	82043	Mixed, Bcl-xL	14;15	
	82048	Mixed, Bcl-xL	12;15	
	82181	Mixed, Bcl-xL	14;15	
	83353	Mixed, Bcl-xL	12;15	
556 Chr 17 at 89.6 of 95.2 Mb	1854	BALB/cAn	15;17	Interphase, *Myc*-Sα (cloning)
VDJ centromere distal	2731	C57BL/6, Bcl-xL	not 15;17	Interphase
234 Chr 1 near telomere	78709	Mixed, bclxL*	12;15	
	79145	Mixed, bclxL	12;15	
	80616	Mixed, Bcl-xL	1;15 and 6;13	
	80621	Mixed, Bcl-xL	12;15	
	83366	Mixed, Bcl-xL	12;15	

*In addition, lines 820, 336, and 234 had a B1-8 VH recombined into one of the endogenous *Igh* loci (41).

[†]The ascites in mouse no. 75002 included two tumor clones.

[‡]The ambiguity concerning the translocation in tumor no. 79130 is detailed in the data and legend of Fig. S2.

transgene could promote tumor development, mice from each of the three transgenic lines, and two additional transgenic lines, designated line 336 (two copies integrated near the centromere of Chr 14) and 234 (two copies integrated near the telomere of Chr 1), were injected with pristane to induce plasmacytoma. Many of the mice developed abundant ascites containing cells with the characteristic cytology of malignant plasma cells. Primary tumor cells, tumor cells adapted to tissue culture, or highly homogenous tumor cells passaged in SCID or *nude* mice were characterized for chromosomal translocations by two-color fluorescent in-situ hybridization (FISH) and whole genome spectral karyotyping (SKY) (21). For FISH, metaphase or interphase chromosome spreads were hybridized with a BAC probe spanning the *Myc* gene on Chr 15. The same chromosome spreads were also hybridized with a second BAC (labeled with a different fluorochrome) that spans the insertion site of the transgene for the given line (Fig. 2*A*). This second probe lacked any *Igh* sequences, containing only sequences from Chr 1, 4, 7, 14, or 17. Translocation between the *Igh* transgene and *Myc* were defined by colocalization of the two BAC probes. Representative two-color FISH data are shown in Fig. 2 *B–D*. For example, the tumor from line 995 (Fig. 2*B*, *i*) has four Chr with colocalization of the *Myc* and *Igh* transgene probes. SKY analysis confirmed the presence of both der (4, 15) and der (15, 4) chromosomes within this tumor (Fig. 2*B*, *ii*). Notably, Chr 12 carrying the endogenous *Igh* was not rearranged. Seven tumors with translocations between *Myc* and the *Igh* transgene in line 820 had no rearrangements involving Chr 12 (Table 1). In a single tumor with a T(7;15) translocation, Chr 12 was involved in another translocation (Table 1). More than 50% of the plasmacytomas demonstrated colocalization of the *Myc* probe and the *Igh* transgene probe and, therefore, contained translocations between *Myc* and the *Igh* transgene (Table 1 and Figs. S2–S5). As expected, the plasmacytomas that lacked a translocation between *Myc* and the *Igh* transgene had translocations involving the endogenous *Igh* locus and *Myc* (Table 1). Of the five chromosomal locations we tested, all were permissive for translocations to *Myc*. Apparently, the chromosomal location for line 234 (telomeric on Chr 1) is relatively inefficient for translocations with *Myc* that lead to plasmacytoma.

A concern of the transgenic approach is that unusual transcription rates or chromosomal structure of the *Igh* transgenes may create a nonphysiologic target for *Myc* translocations. It is possible that multiple copies of large Igh transgenes, with their strong regulatory elements, result in nonphysiologic chromatin structures. Such a criticism is difficult to rule out in the absence of any experimental data for or against this possibility. To equalize the *Igh* transgenic and endogenous loci to the extent possible, we “knocked-in” an assembled V_H region segment,

with its physiologic promoter, into one of the endogenous loci in the single-copy line 820. In line 820, where the transcriptional activity of the transgene and the endogenous genes should be similar, the frequency of translocation to the transgene and endogenous *Igh* genes was virtually identical (Table 1). Analogous results were obtained with the two-copy line 336 (Table 1).

Either Orientation of the *Igh* Transgene Is Able to Undergo Translocation. In approximately 85% of mouse plasmacytomas and human Burkitt lymphomas, the *Igh* locus is translocated 5′ of *Myc* coding exons (3–6). Conversely, the remaining 15% of plasmacytomas juxtapose *Igκ* or *Igλ* sequences 3′ of *Myc*, to a region known as plasmacytoma variant translocation locus or *Pvt1* (3, 22, 23). This bias is best explained by the orientation of heavy and light chain loci relative to their respective centromeres. Light chain gene rearrangements 5′ of *Myc* are thought to be precluded because they would generate dicentric chromosomes that are incompatible with cell viability. Because the orientation of the transgene in line 820 is the same as *Igκ* and *Igλ* (Fig. 1*B*), we speculated that translocations involving the *Igh* transgene in this line would deregulate *Myc* primarily through *Pvt1* rearrangments. Indeed, two-color FISH analysis showed colocalization of *Pvt1* and the *Igh* transgene in tumors from this line (Fig. 2*C*, *iii*). Furthermore, two-color FISH revealed that *Myc* and part of the *Pvt1* probe are rearranged to separate chromosomes, as would be expected if the *Pvt1* locus was used in the translocation (Fig. 2*C*, *iv*). SKY confirmed the presence of a T(7;15) translocation (Fig. 2*C*, *ii* and Fig. S4).

Translocations of *Myc* to the Transgene and to the Endogenous *Igh* Are Similar at the Molecular Level. To characterize the translocations at the molecular level, the translocation site from at least one tumor from each of four transgenic lines was cloned and sequenced (Fig. 3). The translocation event from a line 995 tumor was typical of *Myc:Igh* translocations, joining exons 2 and 3 of *Myc* to the 3′ end of *Igh*, in the opposite transcriptional orientation (Fig. 3*B* and Table S2). The recombination event occurred in the first intron of *Myc* and in the Sμ region of the *Igh* transgene. Recombination sites in two tumors from line 336 were also typical *Myc:Igh* translocations. Recombination in the first intron of *Myc* and in *Igh* Sγ2 joined exons 2 and 3 of *Myc* to the 3′ end of the *Igh* locus (Table S2). Both products of the reciprocal translocation from a tumor from line 556 were cloned and sequenced. Both recombination products were similar to *Myc:Igh* translocations involving the endogenous *Igh* in terms of translocation sites in *Myc*, Sμ, and Sα, and in terms of orientation (Fig. 3*B*). Molecular cloning of the translocation site in a tumor from line 820 was consistent with the results of FISH and SKY analysis—sequences in the fourth intron of the *Pvt1* gene were joined to Sα from the transgene in a 3′ to 5′ orientation (Fig. 3*B* and Table S2). Molecular cloning revealed that the translocation sites in a second line 820 tumor and in a line 336 tumor were also in the fourth intron/fifth exon of *Pvt1* (Table S2). The fourth intron/fifth exon/fifth intron of the *Pvt1* gene may be unusually susceptible to dsDNA breaks in B cells, because it is a site of RNA polymerase initiation or pausing and of AID binding (24). Most *Pvt1*:kappa light chain translocations are located in this part of the *Pvt1* gene (Fig. 3*C*) (25). Frequent translocation by the *Igh* transgene in line 820 suggests that the orientation of the Igh locus is more or less irrelevant for oncogenic rearrangements. Apparently, the cell population examines all possible translocations (26, 27), and those translocations that allow cell viability and neoplastic transformation are selected.

Discussion

We sought to determine whether the recurrent *Igh:Myc* translocations in human Burkitt lymphoma and in murine plasmacytoma depend on the particular chromosomal location of *Igh* and its orientation relative to the centromere. To determine the role of chromosomal location, we used a 230-kb *Igh* transgene inserted into five different chromosomal locations and in both orientations relative to the centromere. Each of the five *Igh* transgenes is capable of class switch recombination at near physiologic levels (18, 28), a likely prerequisite for translocation (1, 4–6). We found that the *Myc* proto-oncogene can translocate to a 230-kb *Igh* locus inserted into any of five different chromosomal locations (Fig. 2 and Table 1). Not only was the transgene able to undergo translocations with *Myc*, but the frequency of translocation to the transgenic *Igh* was at least equal to the frequency of translocation to the endogenous *Igh*. At the molecular level, *Myc* translocations to the *Igh* transgene are similar, if not

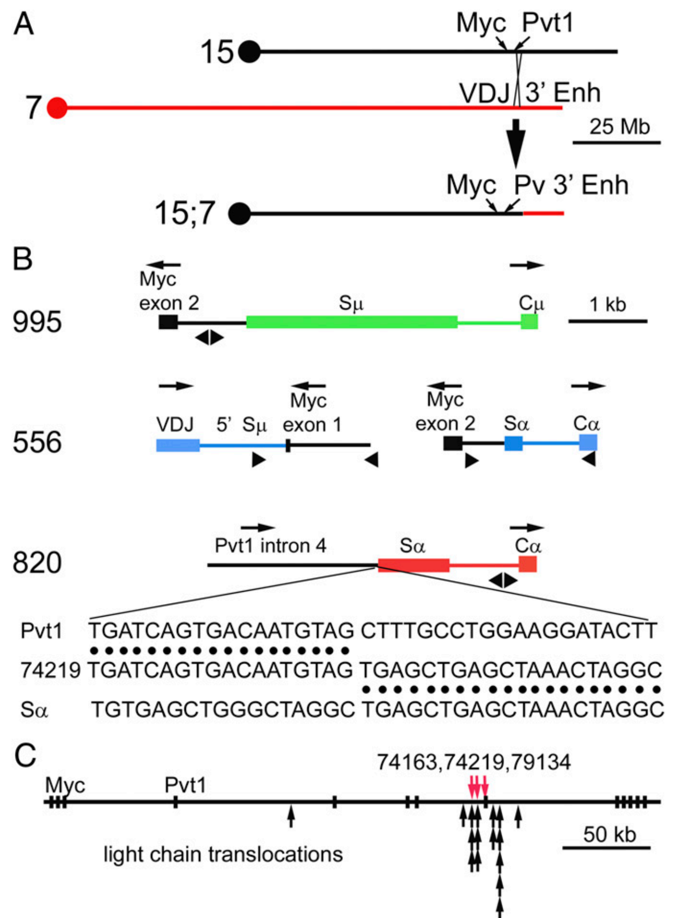

Fig. 3. Molecular characteristics of translocations between the *Igh* transgene and *Myc*. (*A*) Schematic diagrams of Chr 15 with the approximate location of the *Myc* and *Pvt1* genes, Chr 7 with the *Igh* transgene, and the resulting derivative Chr 15 after translocation. (*B*) Molecular clones of translocations to the *Igh* transgenes. These molecular clones are from primary tumor no. 4816 (line 995), from primary tumor no. 1854 [line 556, both der (15) and der (17) translocation products], and from tumor no. 74219 (line 820). *Igh* transgenic sequences at the translocation site are color coded according to the chromosome on which the transgene resides. *Myc*- or *Pvt1*-associated sequences (Chr 15) are shown in black. The transcriptional direction of each gene is shown above each schematic. Triangles below each schematic approximate the location of PCR primers used to clone these translocation sites. The sequence data corresponding to the structure shown in the line 820 schematic is presented below the schematic. Sequence data for other translocation sites in tumors are shown in Table S2. (*C*) Structure of the *Myc* and *Pvt1* loci on Chr 15. Exons are depicted as black rectangles. The *Myc* gene is encoded by the three exons on the left of the map; the *Pvt1* transcripts are encoded by the next exon designated "Pvt1" and the remaining nine exons (and additional exons not included in this figure). The translocation sites in tumors 74163, 74219, and 79134 are shown as red arrows. Translocation sites to light chain genes in plasmacytomas (25) are shown below as black arrows.

identical, to translocations to the endogenous *Igh* (Fig. 3, Table S1, and Table S2).

Lymphoid cells expressing the RAG endonuclease or AID can suffer any one of thousands of different translocations that are spread throughout the genome (26, 27, 29, 30). Presumably it is selection for the *Igh:Myc* translocation, and its resulting deregulated *Myc* expression, that leads to the recurrence of this translocation in human lymphoma and murine plasmacytoma. However, *Igh:Myc* translocations are overrepresented in the primary pool of translocations (26, 27, 29, 31). Several factors have been hypothesized to increase the frequency of *Igh:Myc* translocation, including gene proximity due to chromosome territories (32, 33), gene proximity perhaps due to shared transcription factors (34, 35), high frequency of DNA breaks after AID activity (29), gene proximity after DNA breaks perhaps due to sharing of repair machinery (32), and failure to repair after DNA breaks (16, 36). Chromosome environment is likely to play a role in some or all of these factors. The *Igh* transgene therefore represents a valuable model for future studies on the effect of different chromosome environments on translocation and each of the potential factors mentioned above.

The 230-kb transgene is sufficient not only for the recombination event with *Myc*, but it also includes all of the regulatory elements necessary for oncogenic deregulation of *Myc* (Table 1). Because the *Igh* 3′ regulatory region is required for the genesis of plasmacytomas (17, 37–39), it is likely that the 3′ regulatory region in the *Igh* transgene both enhances *Myc* recombination to the transgene and deregulates *Myc* expression, resulting in tumor development. Thus, putative regulatory elements outside of the 230-kb constant region gene region play only a minor role for oncogenic translocation and for *Myc* deregulation.

Materials and Methods

Identification of Transgene Insertion Sites into the Mouse Genome. Transgenic DNA was digested with *Sau*3A and ligated to form circles. Primers were used from the 3′ end of the *Igh* transgene that would amplify around the circle (including the adjacent chromosomal insertion sequences—see Fig. S1 and Table S3). The resulting PCR product was cloned and sequenced as described in detail in *SI Materials and Methods*.

Plasmacytoma induction and analysis. Plasmacytomas were induced with pristane in mice expressing a Bcl-xL transgene or on a BALB/cAN background (*SI Materials and Methods*). All mouse studies were approved by the institutional animal use committees at the University of Michigan or the National Cancer Institute. FISH and SKY analysis of tumor cells used standard reagents and approaches (*SI Materials and Methods* and Table S4). Molecular clones of tumor translocation sites were obtained by PCR-based approaches as described in detail in *SI Materials and Methods*.

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