Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 7269–7273, December 1983
Genetics

DNA sequences near the site of reciprocal recombination between a c-*myc* oncogene and an immunoglobulin switch region

(switch enzyme recognition sequence/c-*myc* gene regulation)

W. DUNNICK, BRITON E. SHELL, AND CHARLOTTE DERY

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109

Communicated by Robert Perry, July 25, 1983

ABSTRACT **The chromosomal translocations found in many B-cell tumors result in the joining of a c-*myc* oncogene with an immunoglobulin heavy chain switch region. This finding is striking because the natural function of switch regions is to mediate DNA rearrangements important to the maturation of immune responses. These normal switch rearrangements are probably mediated by specific enzymes. In this paper we report the isolation of the two reciprocal products of a recombination between a c-*myc* gene on murine chromosome 15 and an immunoglobulin switch region ($S_\mu S_\gamma 2b$) on chromosome 12. We have determined the sequences of these DNA molecules near the recombination sites and show that the recombination is nearly perfectly reciprocal, with a seven-nucleotide deletion. An examination of the sequences reported in this paper, and of sequences published by other authors, shows a correlation between the points of recombination for c-*myc*–*S* segment rearrangements and for normal heavy chain switches. We suggest that this correlation implies a role for switch recombination enzymes in creating substrates for the c-*myc* recombination. The c-*myc* gene also seems to share some limited homology to sequences thought to be important in heavy chain switching. Finally, we discuss a working model that accounts for some characteristics of c-*myc*–*S* segment recombinations. The model also suggests a mechanism for increased transcriptional activity of the rearranged c-*myc* oncogene in B-cell tumors.**

A large portion of B-cell tumors (lymphomas and plasmacytomas) have chromosomal translocations (1, 2). Several workers have shown that these translocations in murine plasmacytomas result in the joining of c-*myc* gene on chromosome 15 to immunoglobulin heavy chain (chromosome 12) or light chain (chromosome 6) genes (3–8). Similar translocations are found in human B-cell tumors involving c-*myc* on chromosome 8 and immunoglobulin genes on chromosomes 14, 2, or 22 (4, 7, 9–11). c-*myc* is a ubiquitous eukaryotic gene, homologous to a viral gene (v-*myc*) in the avian retrovirus MC29 (12, 13). Because the presence of v-*myc* in the virus is strictly correlated with the ability of the virus to rapidly induce tumors in chickens, the *myc* gene is termed an oncogene. In plasmacytoma DNA, the recombination between c-*myc* and an immunoglobulin gene involves loss of the 5′ end of c-*myc* from the new complex gene and results in increased transcription of c-*myc* (7, 14, 15). The translocated c-*myc* gene in B-cell tumor DNA is often joined to sequences important in immunoglobulin heavy chain switching.

During the differentiation of B cells, a switch from IgM to IgG or IgA synthesis takes place. This protein switch reflects the shuffling of a heavy chain variable region (*V*) gene between a μ constant region (*C*) gene and a C_γ or C_α gene (Fig. 1). (In the mouse, there are four slightly different C_γ genes: $\gamma 1$, $\gamma 2a$, $\gamma 2b$, and $\gamma 3$.) This gene switch is a deletion event that uses switch sequences that lie to the 5′ end of each heavy chain *C* gene (16–19). Switch (*S*) sequences are known to consist of 50–200 copies of short sequences repeated in tandem (20–24). However, the exact signals used in the DNA deletion are just beginning to be understood (22, 25). The c-*myc* translocation nearly always recombines with S_μ or S_α. Adams, Cory, and their colleagues (7, 26) have shown, by identification and cloning of restriction fragments containing both the 5′ end of the c-*myc* gene and the 5′ end of switch sequences, that the c-*myc*–*S* segment recombination is a reciprocal event. Beyond this, the mechanism of the recombination and its relationship to normal heavy chain switching is unknown.

We have molecularly cloned both products of a c-*myc*–*S* segment reciprocal recombination from the plasmacytoma P3. We report DNA sequences around the c-*myc*–*S* segment recombination sites and show that the recombination is essentially reciprocal at the nucleotide level. Our interpretation of these sequences favors the use of immunoglobulin switch enzymes in c-*myc* rearrangements. Finally, we suggest a mechanism that accounts for the increased transcription of rearranged c-*myc* genes. This mechanism relates to the possible oncogenic role of c-*myc*.

MATERIALS AND METHODS

Molecular Cloning. Clone γM27-3 was derived from P3.X27 DNA, a cell line subcloned from the P3 line. Clone γM52 was derived from P3.26Bu4, a P3 line isolated by Margulies *et al.* (27) and given to us by M. Scharff. In both cases, plasmacytoma DNA was partially digested with *Eco*RI and ligated to Charon 4a arms (28). Phage libraries of about 400,000 plaques were screened (29, 30) with the 6.0-kilobase (kb) *Eco*RI fragment from clone γM14 (21). Positive plaques were purified twice and grown in bulk for detailed analysis. The 12-kb *Eco*RI insert of γM27-3 was subcloned into the *Eco*RI site of pBR325 and designated pX27-3.

Southern Hybridization Analysis. High molecular weight DNA was prepared from cell lines and from BALB/cJ kidneys and livers as described by Steffen and Weinberg (31). DNA samples were cut with restriction endonucleases, fractionated on 0.8% agarose gels, and blotted onto nitrocellulose paper (32). The immobilized DNA was hybridized to nick-translated DNA (33) in 6× standard saline citrate at 65°C as described (21). The final wash was in 1× standard saline citrate/0.5% sodium dodecyl sulfate at 65°C. Probes used included pγ1/IF2.E.6.0, a genomic clone in pBR325 that includes S_μ, $S_\gamma 1$, and $C_\gamma 1$ sequences and is the 6.0-kb *Eco*RI fragment from the clone γM14

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NIARD, non-immunoglobulin-associated rearranging DNA; *V*, *C*, and *S*, variable, constant, and switch regions of immunoglobulin genes; kb, kilobase(s).

(21); pSγ 2a-1, a $S_\gamma 2a$ probe (R. Lang and K. B. Marcu, personal communication); pj14, a S_μ probe (34); pα25BH3.4, a probe that includes most of the murine c-*myc* introns, exons, and 3′ flanking sequences (35); PX9-5, a probe for the 5′ exon of the murine c-*myc* gene (14); and M13/73, a probe that includes $S_\gamma 1$ and $C_\gamma 1$ sequences (21).

Restriction enzyme cleavage maps for *Eco*RI, *Bam*HI, *Xba* I, *Kpn* I, and *Hin*dIII were constructed by single and double digests and a best-fit analysis.

DNA Sequences. DNA sequences were obtained by the dideoxy method (36) in phages M13mp8 and M13mp9 (37).

RESULTS

Immunoglobulin gene expression is nearly always associated with DNA rearrangement. Using a probe that included μ and $\gamma 1$ switch sequences and $\gamma 1$ constant region sequences, we detected at least three rearranged *Eco*RI fragments in P3 plasmacytoma DNA (Fig. 1, lane 3). One fragment (8.5 kb) is probably associated with the $\gamma 1$ heavy chain gene expressed by P3 cells. We obtained molecular clones of two other fragments (12 and 14 kb). The *Eco*RI insert of the plasmid clone pX27-3 comigrates with the upper band in the 12-kb *Eco*RI doublet from P3 DNA (lanes 1–3), and the *Eco*RI insert of the Charon 4a clone γM52 comigrates with the 14-kb *Eco*RI fragment from P3 DNA (lanes 3 and 4). Both the 8.5- and 14-kb *Eco*RI fragments hybridized to a $S_\gamma 2a$ probe, as did $S_\gamma 2b$ (6.6-kb) and $S_\gamma 2a$ (4.6-kb) germ-line fragments (lanes 5 and 6).

To understand better the composition of the rearranged switch segments, we analyzed the two clones containing the 12- and 14-kb *Eco*RI fragments by Southern hybridization using S_μ, $S_\gamma 1$, and $S_\gamma 2a$ probes. We also used probes for the murine c-*myc* gene [non-immunoglobulin-associated rearranging DNA (NIARD); ref. 35] and the 5′ exon of c-*myc* (14). These analyses (Fig. 2) showed that the 14-kb *Eco*RI fragment (γM52) contained a 5.6-kb *Bam*HI fragment that included most of the c-*myc* exons and introns and a 2.8-kb *Bam*HI fragment that included some of the 5′ exon from the c-*myc* gene, $S_\gamma 2a$ or $S_\gamma 2b$ sequences, and S_μ sequences. The 12-kb *Eco*RI fragment included, in a 3.6-kb *Xba* I fragment, both S_μ sequences and c-*myc* 5′ exon sequences. However, it lacked the bulk of the c-*myc* gene. As a control for these hybridization analyses we used a Charon 4a clone called γM14 (21). This fragment hybridizes to the $S_\gamma 1$ probe but not to the $S_\gamma 2a$ probe. Furthermore, sequences in γM52 hybridize to the $S_\gamma 2a$ probe but not to the $S_\gamma 1$ probe.

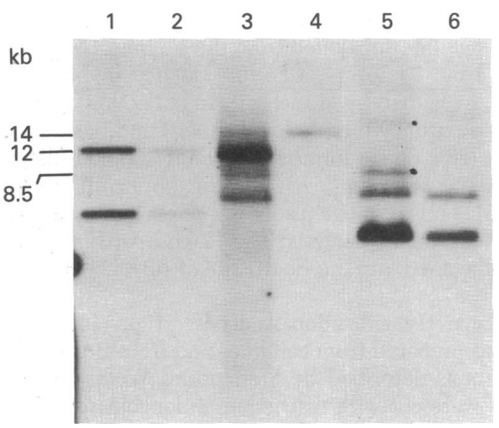

FIG. 1. Identification of cloned DNA inserts from P3 plasmacytoma DNA. DNA samples were cut with *Eco*RI, fractionated on 0.8% agarose gels, blotted onto nitrocellulose paper, and hybridized with nick-translated pγ1/IF2.E.6.0 (lanes 1–4) or pSγ2a-1 (lanes 5 and 6). DNA samples were pX27-3 (lanes 1 and 2), P3 (lanes 3 and 5), γM52 (lane 4), and BALB/cJ kidney and liver (lane 6). Because the probes used included radiolabeled plasmid DNA, plasmid vector hybridization at about 5 kb can be seen in lanes 1 and 2. Lanes 1–4 and 5 and 6 are from different experiments and thus have slightly different electrophoretic migration distances. The 14- and 8.5-kb fragments in lane 5 are shown by dots.

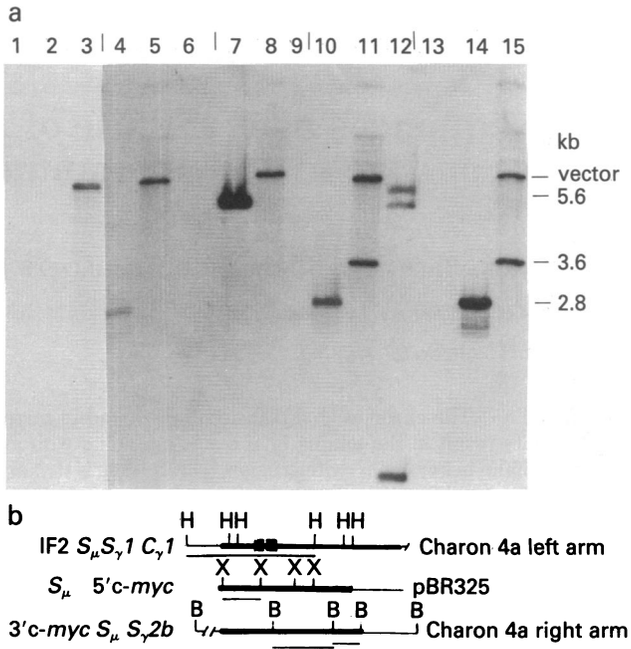

FIG. 2. (*a*) Southern hybridization analysis of c-*myc* and switch region content of recombinant DNA clones. Probes were M13/73 for $S_\gamma 1$ (lanes 1–3), pSγ2a-1 for $S_\gamma 2a$ (lanes 4–6), pα25BH3.4 for NIARD (lanes 7–9), pj14 for S_μ (lanes 10–12), and PX9-5 for the 5′ exon of c-*myc* (lanes 13–15). DNA samples were *Hin*dIII-digested γM14 (lanes 3, 6, 9, 12, and 13), *Xba* I-digested pX27-3 (lanes 2, 5, 8, 11, and 15), and *Bam*HI-digested γM52 DNA (lanes 1, 4, 7, 10, and 14). pBR325 vector DNA from the clone pX27-3 hybridizes to radiolabeled vector sequences in several of the lanes. (*b*) Restriction enzyme cleavage sites and hybridizing fragments (noted by thin lines) for γM14 (*Upper*), pX27-3 (*Middle*), and γM52 (*Lower*). H, *Hin*dIII; X, *Xba* I; B, *Bam*HI.

We constructed enzyme cleavage maps for both the 14-kb and the 12-kb *Eco*RI fragments (Fig. 3). These maps, along with the hybridization experiments presented in Fig. 2 and the restriction maps published by others (6, 7, 26, 35, 38, 39), suggest the following composition for these two rearranged fragments. The 14-kb *Eco*RI fragment includes most of the murine c-*myc* gene and 3′ flanking sequences. It includes the recombination, probably within the 5′ exon of c-*myc*, to S_μ and $S_\gamma 2b$ sequences. The clone ends with the *Eco*RI site between $S_\gamma 2b$ and $C_\gamma 2b$. The c-*myc* and switch sequences are transcriptionally in the opposite sense, in a head-to-head fashion. These results with P3 DNA segments confirm those of other workers studying similar segments derived from other plasmacytomas (4, 7, 14, 26, 35, 38, 39).

The 12-kb fragment seems to include DNA reciprocal to that in the 14-kb fragment. It includes some of the c-*myc* 5′ exon and all of the 5′ flanking sequences to the *Eco*RI site. These 5′ c-*myc* sequences are joined to S_μ sequences. Again, the DNA segments are transcriptionally in the opposite sense, being joined tail-to-tail.

These results were also confirmed by direct sequence analysis. Most of these sequences, which are in 99% agreement with published sequences (14, 20, 23, 34), are not presented, but the areas analyzed are shown in Fig. 3. Some sequences near recombination sites are presented in Fig. 4. These sequences

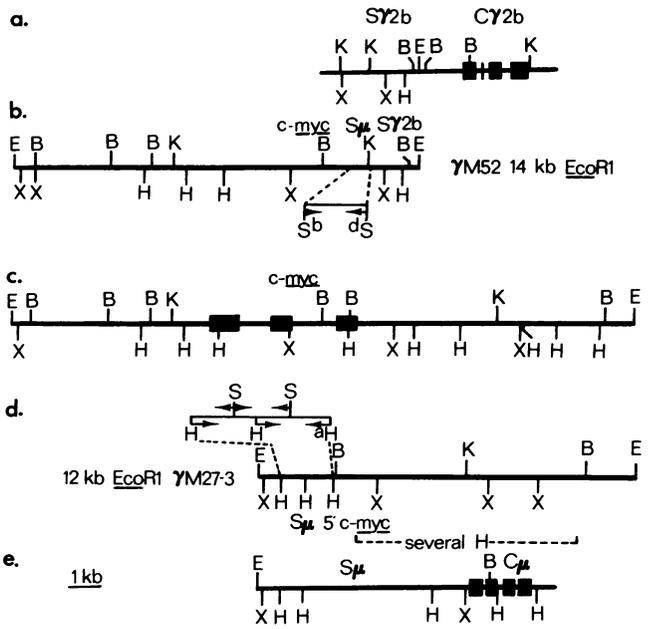

FIG. 3. Restriction enzyme cleavage maps for recombinant DNA clones and corresponding germ-line DNA. (*a*) Murine germ-line *γ2b* gene (23). (*b*) γM52 *Eco*RI insert (this paper). DNA sequence strategy for *Sau*3A fragments in phage M13 is shown by arrows in the expanded region. The letters "b" and "d" represent portions of sequence shown in Fig. 4. (*c*) Murine germ-line c-*myc* gene (6, 7, 26, 35, 38, 39). (*d*) γM27-3 *Eco*RI insert (this paper). This clone contains at least nine *Hin*dIII fragments that were not ordered. Sequence strategy of *Sau*3A fragments and *Hin*dIII fragments in phage M13 is shown by arrows in the expanded region. The letter "a" represents the portion of the sequence shown in Fig. 4. (*e*) Murine germ-line μ gene (20). Restriction enzyme cleavage sites: H, *Hin*dIII; X, *Xba* I; E, *Eco*RI; B, *Bam*HI; S, *Sau*3A; K, *Kpn* I.

demonstrate that the c-*myc*–S_μ recombination is nearly perfectly reciprocal. The recombination in the 5′ c-*myc* exon results in a deletion of only seven base pairs of c-*myc* coding sequence; the rest of the gene in either the 12- or 14-kb *Eco*RI fragment is retained. Because of the tandemly repeated nature of the S_μ region, it is impractical to determine whether the S_μ recombination is truly reciprocal. However, the position at which the recombination leaves S_μ in the 12-kb fragment and enters S_μ in the 14-kb fragment seems to be at the same position within the repeat unit. Both recombinations seem to occur after the G-G-G-G-T subunit of the S_μ repeat (Fig. 5*a*). Because of one- or two-base-pair homologies between c-*myc* and S_μ at the recombination sites, it is possible that the two recombinations took place a few nucleotides apart. The S_μ–$S_\gamma 2b$ recombination also seems to have taken place at a homologous site within the S_μ repeat unit.

DISCUSSION

The chromosomal translocations associated with many B-cell tumors result in the physical joining of a c-*myc* oncogene with an immunoglobulin gene. The c-*myc* gene is joined to the switch region of an immunoglobulin gene in a head-to-head fashion (3–7, 26). Thus, the 5′ exons and flanking sequences of both genes are absent from this new complex gene. Cory *et al.* (26) have shown that the 5′ part of the c-*myc* gene, absent from the bulk of the rearranged c-*myc* gene, is not lost from the genome. Part of the 5′ exon of the c-*myc* gene and the c-*myc* 5′ flanking sequences are found as a rearranged fragment associated with immunoglobulin switch sequences, which suggests that the c-*myc*–S region recombination is a reciprocal event. At the nucleotide level, we have shown that the recombination in P3 DNA between c-*myc* and S_μ sequences is reciprocal (with a seven-nucleotide deletion). L. Stanton, J.-Q. Yang, L. Harris, L. Eckhardt, B. Burstein, and K. Marcu (personal communication) have also identified and cloned the two reciprocal products of the c-*myc*–$S_\gamma 2a$ recombination in the MPC-11 plasmacytoma. It also appears to be reciprocal at the nucleotide level with a similar small deletion in the c-*myc* gene.

One can hypothesize that c-*myc* rearrangements are either random DNA rearrangements or are facilitated by switch recombination enzymes. In P3 plasmacytoma DNA, the productive S_μ to $S_\gamma 1$ rearrangement, the S_μ to c-*myc* rearrangement, and the S_μ to $S_\gamma 2b$ rearrangement use a homologous position in the S_μ repeat unit (Fig. 5*a*). Analysis of other published sequence data reveals a similar correlation between normal switches and c-*myc* rearrangements. The recombination sites in S_α segments for both productive S_μ–S_α rearrangements and c-*myc*–S_α rearrangements in tumors that produce α heavy chains are shown (Fig. 5*b*). The relative position of recombination for these two rearrangements is usually within a few nucleotides. In the tumor in which the c-*myc* and heavy chain switch recombination sites are the most widely separated (J558), both sites fall in the

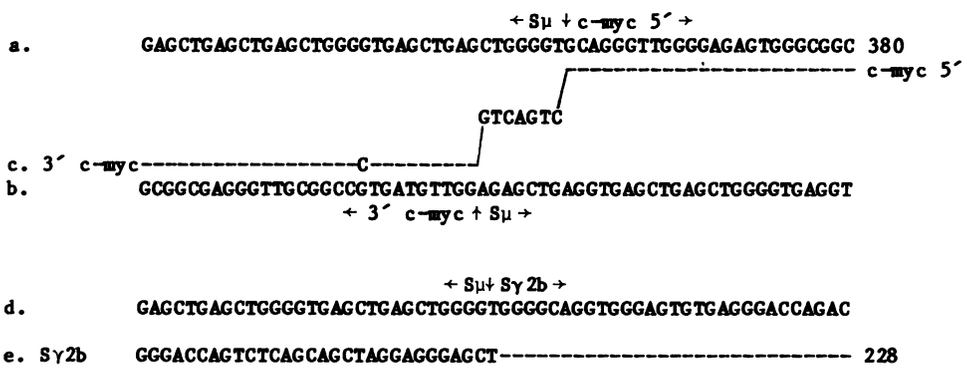

FIG. 4. Sequences near c-*myc* and switch segment recombination sites. (*a*) Sequence of the S_μ-5′ c-*myc* recombination site in pX27-3. This sequence is a portion of that derived from the M13 clone designated by an arrow and the letter "a" in Fig. 3*d*. The numbering of the c-*myc* part of this sequence corresponds to the system used by Stanton *et al.* (14). (*b*) The sequence of the 3′ c-*myc*–S_μ recombination site in γM52. This sequence is a portion of that derived from the M13 clone designated by an arrow and the letter "b" in Fig. 3*b*. (*c*) The published sequence (anti-sense strand) of murine c-*myc* (14) is shown as a dashed line when it agrees with our sequence. The seven-nucleotide deletion in c-*myc* is noted. (*d*) The sequence of the S_μ–$S_\gamma 2b$ recombination in γM52. This sequence is a portion of that derived from the M13 clone designated by an arrow and the letter "d" in Fig. 3*b*. (*e*) Identity of the sequence of the germ-line $S_\gamma 2b$ segment (23, 34) to our sequence is indicated by a dashed line. The numbering is that of Kataoka *et al.* (23).

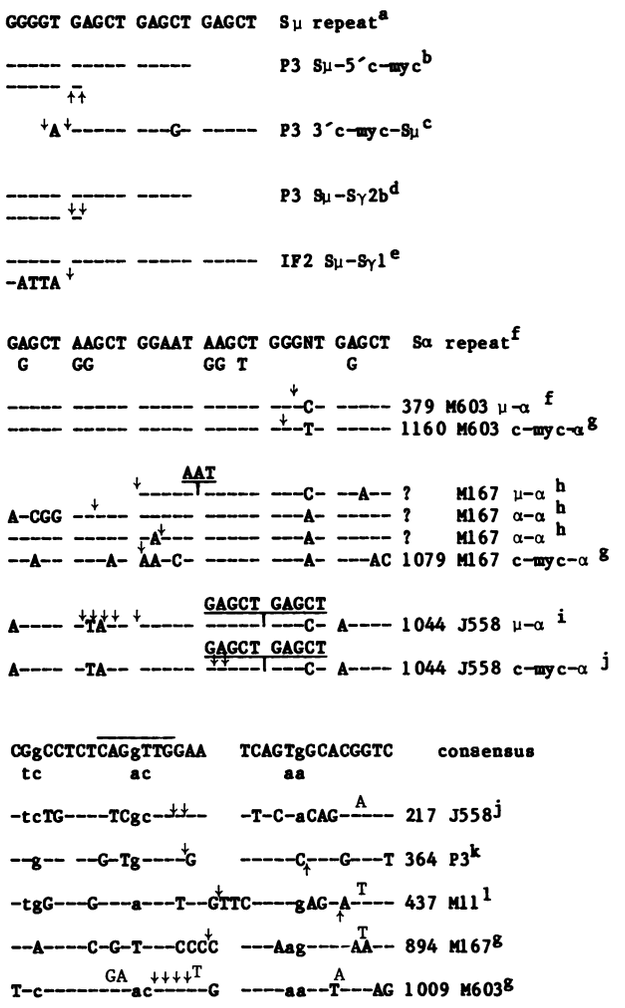

FIG. 5. Correlation between c-*myc* recombinations and normal heavy chain switch recombinations. Arrows note recombination sites. Multiple arrows indicate homology between the *S* segment and c-*myc* gene, resulting in uncertainty in the exact recombination site. Homology to the sequence at the top of each section is noted by dashes. (*a*) S_μ recombination sites in plasmacytoma P3 DNA. To emphasize their homology to the repeat element, three of these continuous S_μ sequences are presented on two lines. (*b*) S_α recombination sites in α heavy chain producing plasmacytomas. (*c*) Homology near recombination sites in the c-*myc* gene. These c-*myc* sequences, as determined by Stanton *et al.* (14) with a few corrections (personal communication), are shown for the anti-sense DNA strand. Numbering in the c-*myc* gene is according to Stanton *et al.* (14). Recombination sites for the 3′ end of the c-*myc* gene (in the transcriptional sense) are shown as downward pointing arrows; for the 5′ end of the c-*myc* gene, they are shown as upward pointing arrows. In the consensus sequence we have determined, residues having less striking homology are shown as lowercase letters in both the consensus and in the individual sequences. The sequences have been aligned with small deletions (nucleotides above the line) and insertions (blank spaces) to maximize homology. The sequence C-A-G-G-T-T-G in the c-*myc* consensus is noted by overlining. References for sequences are as follows. [a]Ref. 40. [b]This paper, Fig. 4*a*. [c]This paper, Fig. 4*b*. [d]This paper, Fig. 4*d*. [e]Ref. 21. [f]Ref. 22. [g]Ref. 38. [h]Ref. 41. [i]Ref. 25. [j]Refs. 14 and 39. [k]This paper, Fig. 4*c*. [l]L. Stanton, J.-Q. Yang, L. Harris, L. Eckhardt, B. Burstein, and K. B. Marcu, personal communication.

same repeat element (of the 20 or more available). Although these limited data can be interpreted in several ways, we suggest that, as part of the switch recombination event, recombinases create nicks in switch region sequences. Either because of recombinase specificity or because access to the substrate is limited (by repeating chromatin structure?), these nicks are at homologous sites in the switch region tandemly repeated elements. Ligation of these nicked DNA strands to similarly nicked strands in other immunoglobulin switch regions results in normal productive or nonproductive heavy chain switches. Rarely, the nicked strands may be joined to nicks in the c-*myc* gene on chromosome 15.

No correlation among recombination sites exists when different tumors are compared to one another (Fig. 5*b*). If the above interpretation is correct, different cells must have either switch recombinases of different specificity (even though they may ultimately express the same heavy chain) or different substrate accessibility patterns. On the other hand, our suggestion that switch enzymes create multiple nicks in the switch regions of both homologous chromosomes may be incorrect. In this case, the lack of correlation among tumors suggests that within a tumor some regulatory mechanism directs the rearranging c-*myc* gene to sites homologous to the recombination site of the previously switched expressed heavy chain gene.

In most B-cell tumors, c-*myc* is associated with the same *S* segment as is found in the expressed heavy chain gene (4, 6, 7, 9, 10, 14, 15, 26, 35, 38, 39). However, a few tumors do not fit this pattern: P3 expresses a γ1 protein, but its c-*myc* gene is associated with a γ2*b* gene (see above). Other exceptions are *MPC-11* gene (L. Stanton, J.-Q. Yang, L. Harris, L. Eckhardt, B. Burstein, and K. Marcu, personal communication) and several plasmacytomas that express a γ2a protein (18). We suggest that these cells attempted to switch from the expressed heavy chain genes to downstream genes, but this switch was aborted, perhaps by c-*myc* insertion into the recipient sequences. There is little experimental evidence to support the existence of such switches.

Whether or not switch enzymes recognize the c-*myc* gene in a specific fashion is a difficult question. The c-*myc* gene, and in particular its 5′ end, has no substantial homology to any heavy chain switch region (14, 39). We examined sequences in the c-*myc* gene (14) around the recombination sites in five tumors (Fig. 5*c*). We note some homology over a 32-base-pair sequence. The sequence C-A-G-G-T-T-G is part of the consensus near c-*myc* recombinations. Y-A-G-G-T-T-G has been proposed as a sequence important in normal heavy chain switching (25). The probability that we would find this particular sequence as part of our consensus by random chance is <0.001. Because c-*myc* and switch segments recombine in a head-to-head fashion, the C-A-G-G-T-T-G is in the same orientation relative to the c-*myc*–*S* segment recombination as Y-A-G-G-T-T-G is to heavy chain switch recombination sites. The distance from C-A-G-G-T-T-G to the 5′ recombination site (8–15 base pairs) is the same as the mean distance from Y-A-G-G-T-T-G sequences to normal switch recombination sites (25). It is not clear whether Y-A-G-G-T-T-G is important to heavy chain switches; more data are required to establish this proposal. Nevertheless, it is striking that analyses of normal heavy chain switches (25) and c-*myc* rearrangements reveal the same sequence (Y-A-G-G-T-T-G) as a consensus in the same relative position (Fig. 5*c*). These results suggest that switch recombinases may recognize Y-A-G-G-T-T-G, or related sequences, as part of some c-*myc* rearrangements.

Our data and the extensive data of others show that the c-*myc* gene is usually cut into two parts as a result of the c-*myc*–*S* segment recombination (4, 7, 14, 26). Cory *et al.* (26) have shown that the recombination sites lie in a restricted area; the observed recombinations occur in a region encompassing most of the first (5′) exon and intron. We suggest the following working model (Fig. 6) that accounts for this restricted region of recombination and also the increased transcription of rearranged c-*myc* genes. We assume that in normal tissue the myc protein has regulatory functions that involve DNA binding (42). We fur-

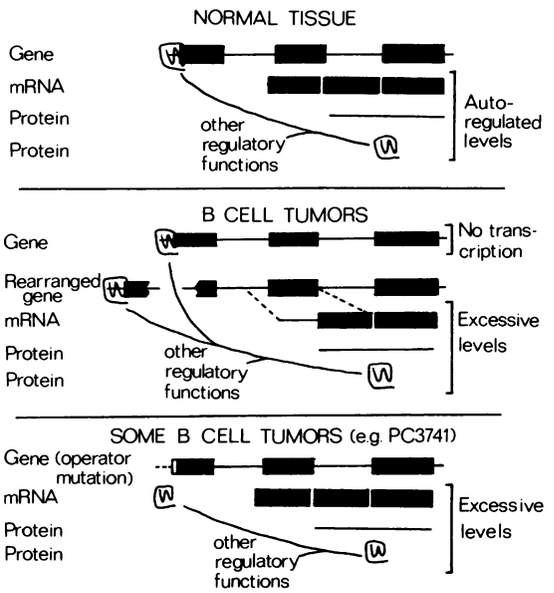

FIG. 6. Working model for c-*myc* gene regulation. We show the protein in the unfolded form to emphasize that it is encoded by the second and third exons. We also show the folded form of the protein to emphasize its function.

ther assume that the myc protein also binds to the 5′ end of the c-*myc* gene and inhibits its own transcription. The translocation in B-cell tumors separates the myc protein coding segments (in the second and third exons only; see ref. 14) from its operator in or near the 5′-most exon (7, 14, 26). Thus, transcription of the c-*myc* gene proceeds unregulated, albeit from an adventitious promoter that may or may not rely on proximity to immunoglobulin genes (7, 14). The excess of myc protein has two consequences. First, it shuts off the unrearranged gene completely, a phenomenon already noted by Stanton *et al.* (14). Second, it provides an overabundance of regulatory proteins that may result in oncogenesis. Recombinations that take place 5′ of the first exon may not separate the operator from the bulk of the c-*myc* gene; recombinations that take place 3′ of the first intron may remove transcription signals, translation signals, or protein domains important in oncogenesis. Thus, c-*myc* recombinations that lead to tumors are restricted to a small region. This model predicts the existence of operator constitutive mutations (43) that do not bind the myc protein. This may be the genotype of plasmacytomas with high levels of transcription but no c-*myc* rearrangement (e.g., PC3741) (4, 35). myc protein variants that do not bind operator are not likely to be detected because they would probably have to be homozygous to be effective. In addition to autoregulation, c-*myc* expression may be regulated by enhancing factors present only in B cells.

We are deeply indebted to Ken Marcu and his colleagues for intellectual stimulation, for generous gifts of probes, and for sending us data before publication. We thank J. L. Claflin and D. Friedman for reading the manuscript. This work was supported by grants to W.D. from the National Institute of Allergy and Infectious Diseases (AI 17778), from the Michigan Memorial Phoenix Project, and from the Horace H. Rackham Graduate School, and by Institutional Research Grant IN-40U to the University of Michigan from the American Cancer Society. B.E.S. is a Predoctoral Fellow of the National Science Foundation.

1. Klein, G. (1981) *Nature (London)* **294**, 313–318.
2. Rowley, J. D. (1982) *Science* **216**, 749–751.
3. Harris, L. J., D'Eustachio, P., Ruddle, F. H. & Marcu, K. B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6622–6626.
4. Marcu, K. B., Harris, L. J., Stanton, L. W., Watt, E. R. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 519–523.
5. Crews, S., Barth, R., Hood, L., Prehn, J. & Calame, K. (1982) *Science* **218**, 1319–1321.
6. Shen-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. (1982) *Cell* **31**, 443–452.
7. Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cory, S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1982–1986.
8. Mushinski, J. F., Bauer, S. R., Potter, M. & Reddy, E. P. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1073–1077.
9. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7837–7841.
10. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7824–7827.
11. de la Chapelle, A., Lenoir, G., Boue, J., Boue, A., Gallano, P., Huerre, C., Szajnert, M.-F., Jeanpierre, M., Lalouel, J.-M. & Kaplan, J.-C. (1983) *Nucleic Acids Res.* **11**, 1132–1142.
12. Sheiness, D. & Bishop, J. (1979) *J. Virol.* **31**, 514–521.
13. Duesberg, P. H. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 13–30.
14. Stanton, L. W., Watt, R. & Marcu, K. B. (1983) *Nature (London)* **303**, 401–406.
15. Erikson, J., Ar-Rushdi, A., Drivinga, H. L., Nowell, P. C. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 820–824.
16. Honjo, T. & Kataoka, T. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2140–2144.
17. Rabbitts, T. H., Forster, A., Dunnick, W. & Bentley, D. L. (1980) *Nature (London)* **283**, 351–356.
18. Coleclough, C., Cooper, D. & Perry, R. P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1422–1426.
19. Cory, S., Jackson, J. & Adams, J. M. (1980) *Nature (London)* **285**, 450–456.
20. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. (1980) *Nature (London)* **286**, 676–683.
21. Dunnick, W., Rabbitts, T. H. & Milstein, C. (1980) *Nature (London)* **286**, 669–675.
22. Davis, M. M., Kim, S. K. & Hood, L. E. (1980) *Science* **209**, 1360–1365.
23. Kataoka, T., Miyata, T. & Honjo, T. (1981) *Cell* **23**, 357–368.
24. Shimizu, A., Takahashi, N., Yaoita, Y. & Honjo, T. (1982) *Cell* **28**, 499–506.
25. Marcu, K. B., Lang, R. G., Stanton, L. W. & Harris, L. J. (1982) *Nature (London)* **298**, 87–89.
26. Cory, S., Gerondakis, S. & Adams, J. M. (1983) *EMBO J.* **2**, 697–704.
27. Margulies, D. H., Kuehl, W. M. & Scharff, M. D. (1976) *Cell* **8**, 405–415.
28. Blattner, F. R., Williams, B. G., Blechl, A. E., Denniston-Thompson, K., Faber, H. E., Furlong, L.-A., Grunwald, D. J., Kiefer, D. O., Moore, D. D., Sheldon, E. L. & Smithies, O. (1977) *Science* **196**, 161–164.
29. Hohn, B. (1979) *Methods Enzymol.* **68**, 299–309.
30. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
31. Steffen, D. & Weinberg, R. A. (1978) *Cell* **15**, 1003–1010.
32. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
33. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
34. Lang, R. B., Stanton, L. W. & Marcu, K. B. (1982) *Nucleic Acids Res.* **10**, 611–630.
35. Harris, L. J., Lang, R. B. & Marcu, K. B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4175–4179.
36. Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Roe, B. A. (1980) *J. Mol. Biol.* **143**, 161–178.
37. Messing, J. (1981) in *Third Cleveland Symposium on Macromolecules: Recombinant DNA*, ed. Walton, A. (Elsevier, Amsterdam), pp. 143–153.
38. Calame, K., Kim, S., Lalley, D., Hill, R., Davis, M. & Hood, L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6994–6998.
39. Adams, J. M., Gerondakis, S., Webb, E., Mitchell, J., Bernard, O. & Cory, S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6966–6970.
40. Nikaido, T., Nakai, S. & Honjo, T. (1981) *Nature (London)* **292**, 845–847.
41. Kim, S., Davis, M., Sinn, E., Patten, P. & Hood, L. (1981) *Cell* **27**, 573–581.
42. Donner, P., Greiser-Wilke, I. & Moelling, K. (1982) *Nature (London)* **296**, 262–266.
43. Jacob, F. & Monod, J. (1961) *J. Mol. Biol.* **3**, 318–356.