sion of a cloned malaria gene in a eukaryotic vector. The sporozoite surface antigen synthesized by the infectious vaccinia virus recombinant reacted with monoclonal antibody specific for the repeating epitope of the malaria protein. Furthermore, intradermal inoculation of rabbits with infectious recombinant virus stimulated specific antibody production. Significant increases in the synthesis of the sporozoite antigen are to be expected with additional genetic engineering. On the basis of the large capacity of vaccinia virus for foreign DNA (*23*), it should be possible to express several antigens from different life stages of the malaria parasite simultaneously to create a more potent vaccine. By incorporating genes of additional infectious agents that pose health problems in areas of the world where malaria is endemic, polyvalent vaccines may be produced.

GEOFFREY L. SMITH
Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205
G. NIGEL GODSON
Department of Biochemistry, New York University Medical Center, 550 First Avenue, New York 10016
VICTOR NUSSENZWEIG
Department of Pathology, New York University Medical Center
RUTH S. NUSSENZWEIG
JOHN BARNWELL
Department of Microbiology, New York University Medical Center
BERNARD MOSS
Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases

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Repression of Rearranged μ Gene and Translocated c-*myc* in Mouse 3T3 Cells × Burkitt Lymphoma Cell Hybrids

Abstract. *The productively rearranged immunoglobulin μ chain gene and the translocated cellular oncogene c-*myc *are transcribed at high levels both in human Burkitt lymphoma cells carrying the t(8;14) chromosome translocation and in mouse plasmacytoma × Burkitt lymphoma cell hybrids. In the experiments reported here these genes were found to be repressed in mouse 3T3 fibroblast × Burkitt lymphoma cell hybrids. Such repression probably occurs at the transcriptional level since no human μ- and c-*myc *messenger RNA's are detectable in hybrid clones carrying the corresponding genes. It is therefore concluded that the ability to express these genes requires a differential B cell environment. The results suggest that the 3T3 cell assay may not be suitable to detect oncogenes directly involved in human B cell oncogenesis, since 3T3 cells apparently are incapable of transcribing an oncogene that is highly active in malignant B cells with specific chromosomal translocations.*

In Burkitt lymphoma cells with the t(8;14) chromosome translocation, the c-*myc* oncogene, normally located on band q24 of chromosome 8, translocates to the immunoglobulin heavy chain locus on chromosome 14 (*1–3*). By contrast, in Burkitt lymphoma cells with either the t(8;22) or the t(2;8) variant chromosomal translocation, the chromosomal breakpoint is distal (3′) to the c-*myc* oncogene and either the immunoglobulin λ locus or the immunoglobulin κ locus, respectively, translocates to a DNA region distal (3′) to the untranslocated and unrearranged c-*myc* oncogene (*4–5*). As a result of its close proximity to rearranged

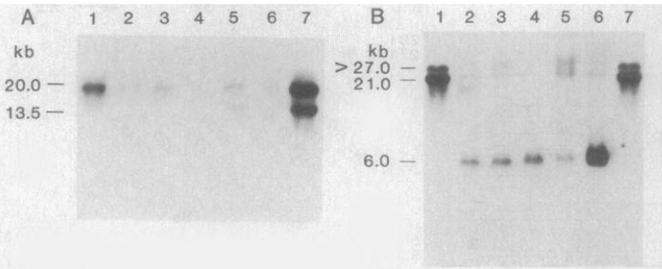

Fig. 1. Southern blotting analysis of hybrid cell DNA for (A) human μ genes and (B) c-*myc* genes. Agarose gel (0.7 or 1 percent) electrophoresis was carried out in 40 m*M* tris-HCl, 5 m*M* sodium acetate, and 2.0 m*M* EDTA, *p*H 8.0. Hind III digested phage λ DNA (0.75 μg per lane) size markers were included in every gel. Cellular DNA samples were digested with restriction enzymes and then subjected to electrophoresis in a horizontal agarose gel (10 μg of DNA per lane). Transfer of DNA from gel to nitrocellulose sheet was performed essentially as described by Southern (*14*). The DNA probes were labeled by the nick translation procedure (*21*). The DNA on nitrocellulose sheets was hybridized to ^{32}P-labeled probe DNA in a hybridization solution containing 50 percent (by volume) formamide. (A) Southern blotting analysis of hybrid cell DNA after Bam HI digestion and with the use of a 1.2-kb Eco RI genomic DNA probe of the Cμ gene (*2*). This probe includes the first, the second, and part of the third exon of the Cμ gene (*2*). Lane 1, JD38 lymphoma cell DNA; lanes 2 and 3, IT × JD-A and IT × JD-B hybrid DNA's, respectively; lane 4, IT22 mouse parental DNA; lanes 5 and 6, hybrid 272-3 and 272-5 DNA's, respectively; lane 7, ST486 Burkitt lymphoma DNA. The two IT22 × JD38 hybrid (272-3 and 272-5) DNA's (lanes 2 and 3) have the rearranged 20.0-kb band that contains the expressed μ gene. Both of the ST486 × IT22 hybrids (lanes 5 and 6) contain the rearranged but unexpressed Cμ gene (*9*) while only hybrid 272-3 contains the productively rearranged Cμ gene (13.5 kb) (*9*). (B) Southern blotting analysis of hybrid cell DNA's following Bam HI digestion and using a human c-*myc* cDNA probe (Ryc 7.4) containing sequences derived from the second and third exon of c-*myc* (*3*). Lane 1, JD38 lymphoma DNA; lanes 2 and 3, hybrid IT × JD-A and IT × JD-B DNA's, respectively; lane 4, IT22 mouse parental DNA; lanes 5 and 6, hybrid 272-5 and 272-3 DNA's, respectively; and lane 7, ST486 Burkitt lymphoma cell DNA.

immunoglobulin loci, the involved c-*myc* is activated and transcribed constitutively at high levels in Burkitt lymphoma while the normal oncogene on normal chromosome 8 is not transcribed (*3–5*). Although the involved human c-*myc* is expressed at high levels in hybrids with mouse plasmacytoma cells, the uninvolved c-*myc*, which is located on normal chromosome 8 and is derived either from Burkitt lymphomas or Epstein-Barr virus transformed lymphoblastoid cells, is repressed on a mouse plasmacytoma background (*3*). These results indicate that the involved c-*myc* escapes the normal transcriptional control to which the untranslocated and normal c-*myc* remains subjected (*3–5*). While the translocated c-*myc* on the Burkitt lymphoma $14q^+$ marker chromosome is expressed at high levels in differentiated B cells, it is repressed in mouse L cells (*3–5*). Thus mouse fibroblasts may not be the appropriate recipient cells to detect oncogenes directly involved in B cell oncogenesis.

Because they are only partially transformed in vitro, various mouse 3T3 cell lines have been used as recipients in DNA-mediated gene transfer experiments to detect and rescue human transforming genes. These cell lines, which do not form tumors in immunosuppressed mice, can be transformed into tumor cells by means of cellular DNA from tumor cell lines or malignant tissues (*6, 7*). In the present study, we investigated the expression of the translocated c-*myc* in somatic cell hybrids between Burkitt lymphoma and mouse 3T3 cells to determine whether these cells are capable of expressing the translocated c-*myc* and whether the expression of the c-*myc* and of the human μ immunoglobulin gene are regulated in a similar fashion in the hybrid cells. Previous studies have indicated that somatic cell hybrids between mouse fibroblasts and myeloma cells do not express immunoglobulins (*8*). Those hybrids, however, were not tested for the presence of the productively rearranged immunoglobulin genes and for the presence of the immunoglobulin gene transcripts.

We hybridized ST486 Burkitt lymphoma cells and JD38 lymphoma cells, which carry the t(8;14) chromosome translocation and secrete immunoglobulin M (IgM) (*9, 10*), with NIH Swiss 3T3-derived mouse IT22 fibroblasts deficient in thymidine kinase (*11*). Hybrid clones, selected in hypoxanthine aminopterin thymidine (HAT) medium (*12*) containing $10^{-4}M$ ouabain (*13*), were morphologically indistinguishable from the mouse parental fibroblasts. The clones were screened for the presence of the human rearranged μ genes and of the normal and translocated c-*myc* by Southern blotting analysis (*14*), with the use of probes specific for the human constant region μ (Cμ) gene (*2, 15*) and for the coding exons (Ryc 7.4) of the human c-*myc* oncogene (*2–3*).

Figure 1A and Table 1 show that the two hybrids between IT22 cells and JD38 cells (IT × JD-A and IT × JD-B) and one of the two hybrids between IT22 cells and ST486 cells (272-3) contain the productively rearranged human μ chain genes. Restriction enzyme analysis with Bam HI indicated that JD38 cells contain the productively rearranged μ gene within a 20-kilobase (kb) band whereas ST486 cells contain the productively rearranged Cμ gene in a 13.5-kb band (*9*). The germ line Cμ gene is 16 kb in length (*2*). In addition, hybrids 272-5, IT × JD-A, and IT × JD-B retained both the unrearranged c-*myc* on chromosome 8 (> 27-kb band) and the rearranged and translocated c-*myc* on the $14q^+$ chromosome (21-kb band), whereas hybrid 272-3 retained only the rearranged and translocated c-*myc* on the $14q^+$ chromosome (21-kb band) (Fig. 1B). The human c-*myc* complementary DNA (cDNA) probe, Ryc 7.4 (*3*), which we used for detection of human c-*myc* genes, cross hybridizes with mouse c-*myc* DNA's, giving a 6.0-kb band (Fig. 1B).

Since the mouse parental IT22 cells are near tetraploid (*11*) and the same amount of the DNA samples from parental and hybrid cells was used for Southern blotting analysis, the bands corresponding to human μ (Fig. 1A) or c-*myc* genes (Fig. 1B) appear to be weaker than those for parental JD38 or ST486 cells. We scanned the properly exposed auto-

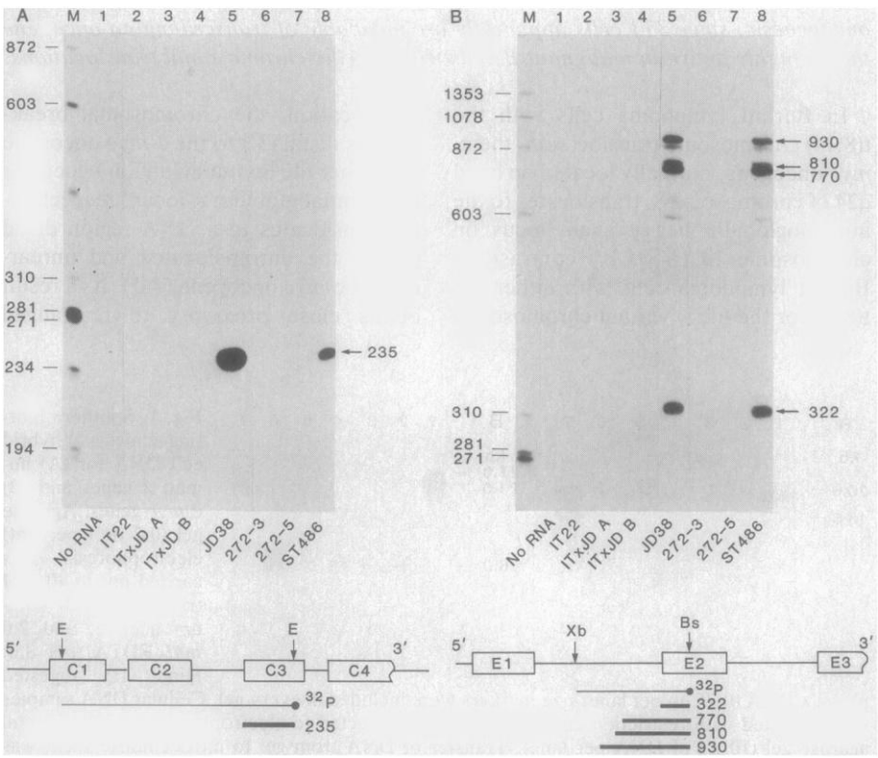

Fig. 2. (A) S_1 nuclease mapping analysis for human immunoglobulin heavy μ chain RNA's. S_1 nuclease analysis was carried out according to Sharp *et al.* (*19*) with modifications (*20*). The S_1 probe was prepared from the pHuER1.2 clone, which contained a part of human genomic μ chain gene insert at the Eco RI site of pBR322. A double-stranded Eco RI, 1.2-kb fragment which encompasses from the Cμ1, Cμ2, and Cμ3 exons, was 5′-^{32}P-labeled and hybridized in 80 percent deionized formamide to cytoplasmic RNA's (20 μg) at 55°C for 10 hours, and digested with 80 units of S_1 nuclease. Cytoplasmic RNA was extracted by the cesium chloride method as described previously (*17*). S_1 nuclease-resistant DNA products were fractionated on a 4 percent denaturing polyacrylamide gel (*18*). Lane M, size marker: ϕ × 174 digested with Hae III and 5′-^{32}P-labeled. The expected S_1 nuclease-resistant DNA product (235 nucleotides) is presented in the diagram. *E*, Eco RI restriction site. (B) S_1 nuclease mapping analysis for human c-*myc* transcripts. The S_1 probe was prepared from the human genomic c-*myc* clone pMyc 41-HE (*10*). A DNA fragment Xba I–Bst EII, encompassing a part of the first intron and the second exon, ^{32}P-labeled at the 5′ end of the Bst EII site, was used as an S_1 probe. The gel was 4 percent polyacrylamide containing 7*M* urea. The expected S_1 nuclease-resistant DNA products are presented in the diagram. *Xb*, Xba I; *Bs*, Bst EII restriction sites.

Table 1. Human c-*myc* and immunoglobulin μ gene expression in fibroblast × Burkitt lymphoma cell hybrids. The autoradiograms were scanned by densitometer to quantitate the amount of human μ genes (see Fig. 1A) or c-*myc* genes (see Fig. 1B) in the hybrid cells. The band intensity for various hybrid cells was compared to those for parental ST486 or JD38 cells. Since the parallel mouse IT22 cells were near tetraploid (*11*), we multiplied the numbers for the hybrid cells by a factor of 3 to compensate for the dilution effect of the human DNA.

Parental and hybrid cells	Origin	Productively rearranged human μ gene	Human c-*myc* gene		Human μ transcripts	c-*myc* Transcripts	
			Germ line	Rearranged		Mouse	Human
ST486	Burkitt lymphoma	1.0	1.0	1.0	+++	−	+++
272-3	ST486 × IT22 hybrid	0.45	0	0.3	−	++	−
272-5	ST486 × IT22 hybrid	0	1.0	0.45	−	+	−
IT22	Mouse 3T3 fibroblasts	0	0	0	−	+	−
JD38	B cell lymphoma	1.0	1.0	1.0	+++	−	+++
IT × JD-A	JD38 × IT22 hybrid	1.0	0.45	0.3	−	+	−
IT × JD-B	JD38 × IT22 hybrid	1.0	0.75	0.2	−	+	−

radiograms (Fig. 1, A and B) and compared the content of the human μ or c-*myc* genes (germ line and rearranged) in the various hybrid cells to those in the parental JD38 or ST486 cells (Table 1). Although hybrids between human and mouse cells tend to segregate human chromosomes, we found that at least 45 percent of the human μ genes (hybrid 272-3), 45 percent of the germ line *myc* genes (hybrid IT × JD-A), and 20 percent of the rearranged *myc* genes (hybrid IT × JD-B) were retained in the hybrid cells at the time when the DNA samples were prepared (Table 1).

Northern blotting analysis of the IT22 × JD38 and IT22 × ST486 hybrids indicates that the hybrids do not produce human immunoglobulin μ chain transcripts even if they contain the productively rearranged Cμ gene (data not shown). S_1 nuclease protection experiments with a human Cμ probe confirmed this finding (Fig. 2A). A protected 235-nucleotide human Cμ fragment is detectable only in parental JD38 and ST486 cells and not in any of the hybrids with IT22 mouse fibroblasts (Fig. 2A). These results indicate that the productively rearranged human Cμ gene, which is expressed in the parental human lymphoma cells and in hybrids between Burkitt lymphoma and mouse myeloma cells (*2, 15, 16*) is not expressed in somatic cell hybrids with mouse fibroblasts (Table 1).

We showed previously that JD38 and ST486 human lymphoma cells express aberrant human *myc* transcripts that may initiate within the first *myc* intron. Novel promoter and initiation sites appear to be activated within the first intron region (*10*). When we analyzed the c-*myc* transcripts by using the human c-*myc* cDNA probe and Northern blotting procedure, we found that, in addition to JD38 and ST486 cells, the hybrid clones expressed *myc* transcripts at levels comparable with those in IT22 mouse fibroblasts, with the exception of hybrid 272-3 which expressed higher levels (data not shown). To determine whether the *myc* transcripts detected by Northern blotting analysis in hybrids containing the normal and the rearranged human c-*myc* gene were of human or mouse origin, we carried out S_1 nuclease protection experiments using either a human or a mouse *myc* DNA probe. The RNA's of parental and hybrid cells were extracted at the same time the DNA's were extracted in order to avoid the possibility that the hybrids had lost the relevant chromosomes after subculturing. Although no expression of human *myc* transcripts was detected in any of the hybrid clones (Fig. 2B), mouse c-*myc* transcripts were present in all of them (Fig. 3). Thus we conclude that the translocated c-*myc* gene that is expressed at high levels in Burkitt lymphoma cells (*2, 3*) and in somatic cell hybrids with mouse plasmacytoma cells (*3*) is repressed in a mouse 3T3 chromosome background (Table 1). It is interesting that the *myc* oncogene on normal chromosome 8, which is silent in the parental Burkitt lymphoma cells (*10*), is also silent in the hybrid cells (Table 1). This could be due to an irreversible shut off of the normal c-*myc* oncogene on chromosome 8 in Burkitt lymphoma or, alternatively, could be due to a repression of the uninvolved c-*myc* gene on normal chromosome 8 in 3T3 cells. At present we do not know whether the c-*myc* transcripts observed in 3T3 cells are derived from all the mouse chromosomes 15 or only from one of them. Therefore it is possible that the expression of the c-*myc* oncogene in 3T3 cells is the result of a deregulation of only one of the c-*myc* alleles, while the other is repressed.

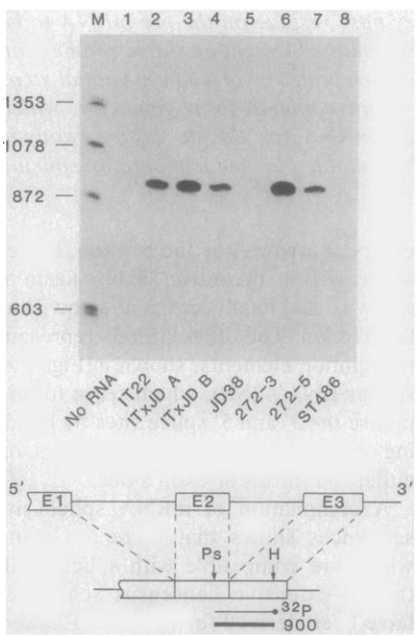

Fig. 3. S_1 nuclease mapping analysis for mouse c-*myc* transcripts. The S_1 probe was prepared from the mouse c-*myc* cDNA clone pMC-*myc* 54. The plasmid was digested by Hind III, labeled with ^{32}P at the 5′ end, and cleaved by Pst I. The resulting Pst I–Hind III 900-bp fragment, which encompasses the second and third exons, was isolated and used as an S_1 probe. Conditions for S_1 nuclease analysis are as described in Fig. 2. The gel was 4 percent polyacrylamide containing 7*M* urea. The expected S_1 nuclease-resistant DNA product (900 nucleotides) is shown in the diagram. *H*, Hind III; *Ps*, Pst I restriction sites.

These findings suggest that the B cell–specific factors that are necessary for immunoglobulin gene expression may also be necessary for the expression of the c-*myc* oncogene involved in the chromosomal translocations. Mouse 3T3 fibroblasts are evidently incapable of transcribing a productively rearranged immunoglobulin μ chain gene and an involved c-*myc* oncogene derived from malignant human B cells. If the expression of an activated oncogene depends on the differentiated state of the malignant cells harboring the involved oncogene, the 3T3 cell transformation assay

might only be able to detect genes capable of being expressed in fibroblasts. Thus the value of the 3T3 assay to detect the genes involved in most human malignancies may be questionable.

KAZUKO NISHIKURA
ABBAS AR-RUSHDI
JEAN ERIKSON
EMMA DEJESUS
DEBORAH DUGAN
CARLO M. CROCE
Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

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7 March 1984; accepted 13 March 1984

Molecular Model for Messenger RNA Splicing

Abstract. *A molecular model is presented for a messenger RNA (mRNA) "splice region." The model requires cation coordination to reduce backbone-backbone electrostatic repulsion and it allows for every base residue on the pre-mRNA to be stacked in A-form helical geometry with a recognition element on the intron or exon (or both) sides of the splice junction. The two nucleotides involved in the initial steps of the cleavage-ligation mechanism must adopt a non–A-form geometry, which ideally positions reactive groups on the pre-mRNA for the necessary catalytic chemistry. The model is also consistent with available biochemical data on splicing reactions.*

RNA splicing is now established as a major RNA processing reaction in eukaryotic cells (*1*). Proposals for the selection of the 3′ and 5′ splice sites and the removal of intron sequences have been made by Murray and Holliday (*2*), Lerner *et al.* (*3*), Rogers and Wall (*4*), as well as others (*5, 6*). These proposals are schematic and thus do not provide for the assessment of their stereochemical, thermodynamic, and mechanistic plausibility. We now describe a molecular model of a messenger RNA (mRNA) "splice region" that not only satisfactorily accounts for the stereochemical and mechanistic features of cleavage and ligation, but is thermodynamically reasonable and consistent with biochemical data on splicing reactions.

The splice region structure shown in Fig. 1 is consistent with mRNA splicing complexes discussed previously (*2–6*). Although independently derived, it is the structural counterpart of a previously proposed B-DNA crossed strand–exchange molecular model for genetic recombination (*7*). It is composed of an mRNA transcript which, reading 5′ to 3′, enters at arrow A as the 5′ exon, leaves at arrow B as the intron, enters again at arrow C, and finally leaves at arrow D as the 3′ exon. The other strands represent recognition elements, shown in Fig. 1 as splicer RNA's (SR), which serve to juxtapose the 3′ and 5′ splice sites by bonding specifically to consensus bases on either the intron or exon sides.

A compilation of mRNA splice site sequences shows that there are many which are compatible with splicing, although extensive consensus sequences have been derived for both the 5′ site,

$$(^{C}_{A})\text{-A-G//G-T-}(^{A}_{G})\text{-A-G-T}$$

and the 3′ site,

$$(^{T}_{C})_{n}\text{-N-}(^{C}_{T})\text{-A-G//G}$$

(A, adenine; C, cytosine; G, guanine; T, thymine; N, any base) (*3, 4, 8*). The important but limited role of base sequence at the 5′ site has been demonstrated by the observation that a sequence change from GT to AT (*9*) or GT to GG (*10*) abolishes splicing, but any single transition mutation at one of the other purine sites does not detectably affect splicing (*9*). It should be appreciated that the model in Fig. 1 is not dependent on the exact base sequence shown, but instead requires that there be sufficient complementarity between whatever 5′ and 3′ splice site sequences are present and the available recognition elements.

As suggested by a number of authors (*3–5*), good candidates for the recognition elements are small nuclear RNA's (snRNA). Perhaps the most likely snRNA involved in mRNA splicing is U1 RNA for a number of reasons (*11*). The 5′ terminus of the U1 RNA is highly complementary to consensus sequences on the intron side of both the 5′ and 3′ splice sites. Also, although whole HeLa nuclei were used, antibodies to Sm or ribonucleoproteins, which can precipitate U1 ribonucleoproteins, have been shown to inhibit adenovirus splicing (*12*). Finally, it has been shown that U1 RNA selectively binds to the 5′ splice site of β-globin mRNA (*13*). Also, U2 RNA is complementary with the consensus splice sequences on the exon side. Both U1 and U2 may aid in the alignment of splice sites (*5*). To demonstrate that splicer RNA's can be positioned on both the intron and exon sides (as well as ease the construction of the helical regions), two splicer RNA's have been incorporated into the molecular model seen in Fig. 1.

It is well known that helical RNA prefers the A-form (*14*) in part because of the intramolecular water bridges that form between adjacent ribose 2′-hydroxyl and 3′-phosphate groups (*15*) and the sterically unfavorable interactions of the 2′-hydroxyl group that would arise in helical forms other than A (*16*). The two double-helical regions of the molecular model shown in Fig. 1 (L and R) are in the standard A-form, although one (but probably not both) of these helical regions could be composed of the A-form mRNA transcript alone with no RNA recognition element (SR) as proposed earlier (*3–6*). Whereas amino acid residues and nucleic acid bases can undergo hydrogen-bonding with some degree of specificity (*17*), it is possible that splicing proteins can act in concert with SR's to align the splice sites. There is considerable availability of hydrogen bonding sites in the grooves of the helices (Fig. 1), and this specificity can be fostered by the ability of protein main-chain atoms and nucleic acid backbone atoms to adopt compatible structures (*18*).

Although the helical regions are in A-form, the splice region (boxed in Fig. 1) must assume a different geometry. This non–A-form region is made up of the two sugar moieties at the 3′ end of the first