attaches by oxygen or hydrogen bonding, slightly hindered internal rotation about the weak bond will occur. In the event that the water hydrogen-bonds, an unusual molecular motion, that of hydrogen-bond interchange, will also occur. Because all of the examples are at very low effective temperatures, these motions occur by tunneling through the barrier rather than through classical, over-the-barrier motion (*7*).

The structural characterization of binary gas-phase complexes of common, important species is by no means well balanced. There are numerous interesting complexes of monomeric water and a few containing the water dimer. Of the common atmospheric species, complexes of molecular oxygen appear conspicuously absent. Whether this insufficiency is of material significance, in view of the large number of condensed-phase magnetic resonance spectroscopic studies, is unclear.

The nature of intermolecular interactions is being explored empirically. Questions such as the uniqueness of hydrogen bonding as the directional weak interaction will in all likelihood be answered as relatively nonunique. It is likely that stereospecificity will be the rule in intermolecular interactions.

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Splicing Takes a Holliday

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A central problem in pre–messenger RNA (pre-mRNA) splicing is to determine how the ends of an intron are juxtaposed for cutting and subsequent exon ligation. Small nuclear ribonucleoprotein particles (snRNP's) containing U1, U2, U4, U5, and U6 RNA's, as well as many protein factors, are essential to the splicing process (*1*). Also, a two-step mechanism has been described in which the 2′-hydroxyl of an adenosine residue located upstream of the 3′ splice site first attacks the 5′ splice site to form a lariat intermediate, and then the two exons are joined with release of the lariat intron. Consensus sequences at the 5′ splice site and at the branch point are recognized by base pairing with the U1 and U2 snRNP's, respectively (Fig. 1A). But the contributions of the U4, U5, and U6 RNA's, which assemble later as a tri-snRNP complex to form a splicing-competent body called the spliceosome, have remained obscure.

Three recent elegant studies that utilized yeast genetics suggest that the U5 RNA collaborates with U1 to bring the splice sites together in the newly assembled spliceosome. (i) Newman and Norman (*2*) discovered that point mutations within an evolutionarily invariant nine-nucleotide loop sequence in U5 RNA allowed use of novel 5′ splice sites when the normal 5′ splice site in a *Saccharomyces cerevisiae* pre-mRNA was mutated. (ii) Equally unanticipated were results of subsequent suppression analyses, implicating the same conserved U5 loop sequence in 3′ splice site activation (*3*). Specifically, splicing of defective introns was restored when positions 5 or 6 of the invariant U5 loop (see Fig. 1B) were mutated so that they were complementary to the nucleotides at positions 2 and 3 upstream of the novel 5′ splice site, or when positions 3 or 4 of the U5 loop sequence were mutated to allow pairing with the first two nucleotides of the 3′ exon. (iii) Reich, VanHoy, Porter, and Wise (*4*) found that appropriate substitutions in the invariant nucleotides 9 and 10 of U1 RNA could suppress splicing-defective changes in the **ag** at the 3′ splice site of a *Schizosaccharomyces pombe* pre-mRNA—but just for the first step of the reaction. In summary, these new observations suggest first that U1 base pairs with intron nucleotides at the 3′ as well as the 5′ splice site, as in an earlier crossover model (*5*), and second that U5 can pair with nucleotides in both exons to specify the exact points of cleavage at the 5′ and 3′ splice sites.

Evidence that all these base pairs between the pre-mRNA, U1, and U5 form simultaneously is currently lacking; but the interactions are not incompatible with one another. I therefore propose that a structure mimicking a Holliday junction (*6*)—a well-characterized intermediate in homologous recombination of DNA molecules—may exist in a newly assembled spliceosome and serve to juxtapose the 5′ and 3′ splice sites. Clearly, formation of every one of the base pairs shown in Fig. 1, B and C, is not essential since only the U1 and U5 sequences shown are absolutely conserved, whereas pre-mRNA 5′ and 3′ splice sites each conform to a consensus. Moreover, the first step of splicing can occur on some pre-mRNA's that lack the **ag** dinucleotide at the 3′ splice site (*7*) and therefore would lack branch **2** of the structure. Evidence that the U5 loop shifts between the first and second steps of splicing (*3*) argues that the structure is poised to undergo conformational change and that all four arms of the crossover may not simultaneously be comprised of duplex RNA. Nonetheless, the model suggests how the U1, U5, and pre-RNA molecules may be positioned relative to one another immediately after spliceosome assembly.

To form the proposed Holliday structure, U1 nucleotides 9 and 10 would exchange their initial pairing with 5′ exon nucleotides −1 and −2 (Fig. 1A) for pairing with the **ag** at the 3′ end of the intron (*4*) (forming branch **2** in Fig. 1, B and C). Simultaneously, the U5 invariant loop would contact the 5′ exon (forming branch **3**). Exon sequences are not highly conserved. Therefore, the multiple U residues in the U5 loop may have been selected during evolution for their ability to pair nondisruptively with all other nucleotides, as in mitochondrial decoding (*8*). Holliday structures are characterized by their potential for isomerization and strand exchange (branch migration). The release of U1 RNA sometime prior to the first step of splicing would destroy branches **1** and **2** and allow U5 to establish closer contacts with the 3′ exon (branch **4**) by branch migration of the U at the top of the conserved loop, as suggested by Newman and Norman (*3*). Additional conformational changes seem likely to occur at this point. Biochemical data support re-recognition of the **ag** dinucleotide at the 3′ end of the intron (*9*) and a change in the environment of the 3′ splice site (*10*) as the spliceosome proceeds from the first to the second step of the reaction.

If a Holliday-like structure is critical for defining splice sites, it should be formed by all types of spliceosomes. Trans splicing is a special circumstance in which a 5′ exon carried on an snRNP-like RNA (called an SL RNA) is joined to a 3′ exon on a separate cellular transcript. Organisms such as trypanosomes, which engage exclusively in trans splicing, curiously have only U2, U4, and U6 RNA's, whereas organisms like nematodes that carry out both normal and

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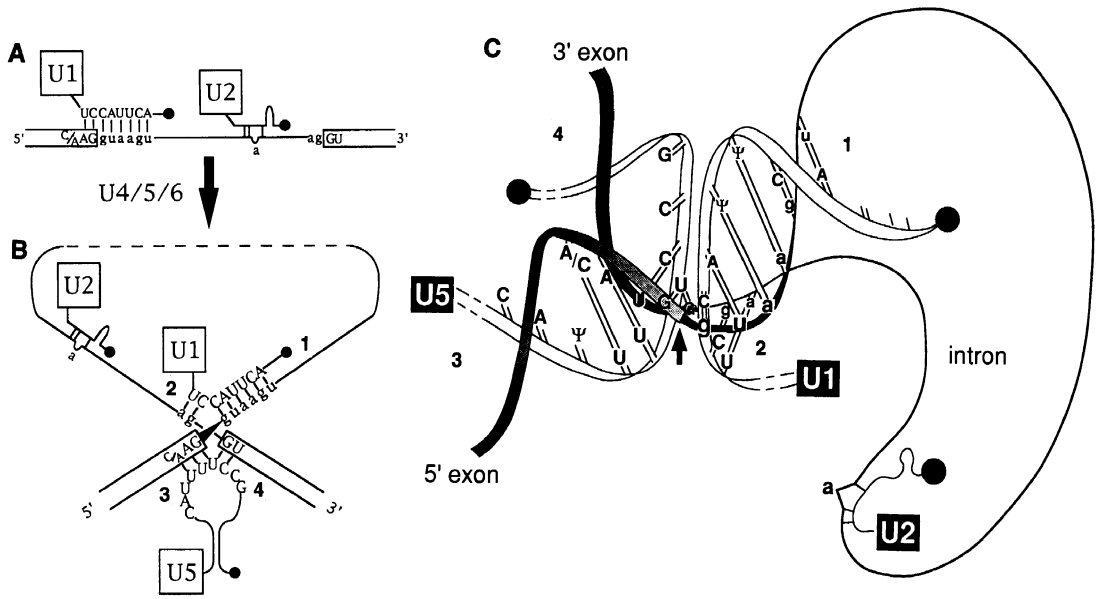

Fig. 1. Formation of Holliday-like structure. During assembly of the mammalian spliceosome, the early complex (**A**) (*1*) is joined by the U4/5/6 tri-snRNP to form the postulated Holliday-like structure (**B**). Exon sequences (uppercase) and intron sequences (lowercase) are consensus nucleotides for vertebrate 5′ and 3′ splice sites (*15*). ● indicates the 5′ cap of the snRNA's. (**C**) A ribbon-form view of the Holliday-like structure, arbitrarily stacked, after the DNA structure (*17*). The 5′ splice site is indicated by an arrow.

trans splicing contain a full complement of spliceosomal RNA's (*11*). We previously proposed that SL RNA sequences functionally substitute for U1 during trans splicing in both trypanosomes and nematodes (*12*). But if presentation of the 5′ splice site also requires U5, how can trypanosomes survive without it? In fact, the SL RNA's of trypanosomes have distinctively longer 5′ exons (about 40 nucleotides) than those of nematodes (22 nucleotides) and contain three stretches of conserved sequence (Fig. 2A). I propose that these associate with the pre-mRNA to form a version of the Holliday structure (Fig. 2B) that lacks the fourth branch because trypanosome 3′ exon sequences are not conserved. The invariant sequence at the extreme 5′ end of trypanosome SL RNA's would substitute for U5 by pairing with nucleotides upstream of the 5′

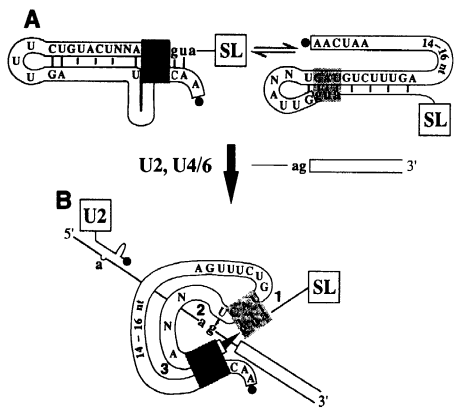

Fig. 2. Three-branch Holliday-like structure. Alternative secondary structures (*13*) assumed by the invariant sequences (those written) in trypanosomatid SL RNA's (**A**) contribute to a modified Holliday-like structure proposed for the trans spliceosome (**B**). Discarded (intron-like) sequences are indicated by lines. Shading shows corresponding regions in (A) and (B).

splice site (branch **3**), and at the same time the middle conserved sequence would allow the SL to fulfill the role of U1 at both the 5′ and 3′ splice sites (branches **1** and **2**). Le Cuyer and Crothers (*13*) have found the intriguing result that the 5′ portion of *Leptomonas collosoma* SL RNA forms two alternative secondary structures in vitro (Fig. 2A). The structure on the right is that proposed earlier (*12*); the one on the left (*13*) pairs the 5′ splice site with the extreme 5′ end of the SL. Fig. 2B incorporates elements of both alternative structures (branches **1** and **3**, respectively). Re-recognition of the **ag** at the 3′ splice site before the second step of trans splicing would be required as for normal splicing. In the shorter SL RNA's of nematodes, the sequence upstream of the 5′ splice site is not UUG but PuAG, which could pair as in Fig. 1B with the invariant loop sequence in U5 RNA. I predict that U5 does participate in trans splicing in nematodes.

As discussed by Newman and Norman (*3*), the idea that U1 and U5 collaborate to identify the splice sites prior to the first step of pre-mRNA splicing nicely dovetails with our growing understanding of protein-free group II self-splicing (*14*). Group II splicing proceeds by way of the same lariat intermediate as does the spliceosome-mediated reaction. A six-nucleotide intron sequence called EBS1 exhibits complementarity to six nucleotides of the exon at the 5′ splice site, and a nucleotide adjacent to EBS1 often covaries with the first nucleotide of the 3′ exon—comparable to the postulated role of the U5 loop in the early spliceosome (*3*). A distinct intron sequence (called ϵ), which base pairs with two nucleotides just downstream of the 5′ splice site (called ϵ') (*14*), perhaps mimics one of the roles of U1. These interactions could comprise three branches (**3**, **4**, and **1**, respectively) of a Holliday structure, and, as in the spliceosome, branch **4** is required only for the second step of splicing (*14*). Although all the parallels are not yet clear, the newly identified U5 and U1 interactions bring us significantly closer to understanding the roles of snRNA's in the spliceosome.

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