between different currents that leave the same quantum conductor. One result is that current-current correlations can show two-electron interference effects that are periodic in magnetic flux. The first researcher to perform such an experiment will definitely not be annoyed when his apparatus picks up noise that is periodic with a magnetic flux quantum.

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AT-AC Introns: An ATtACk on Dogma

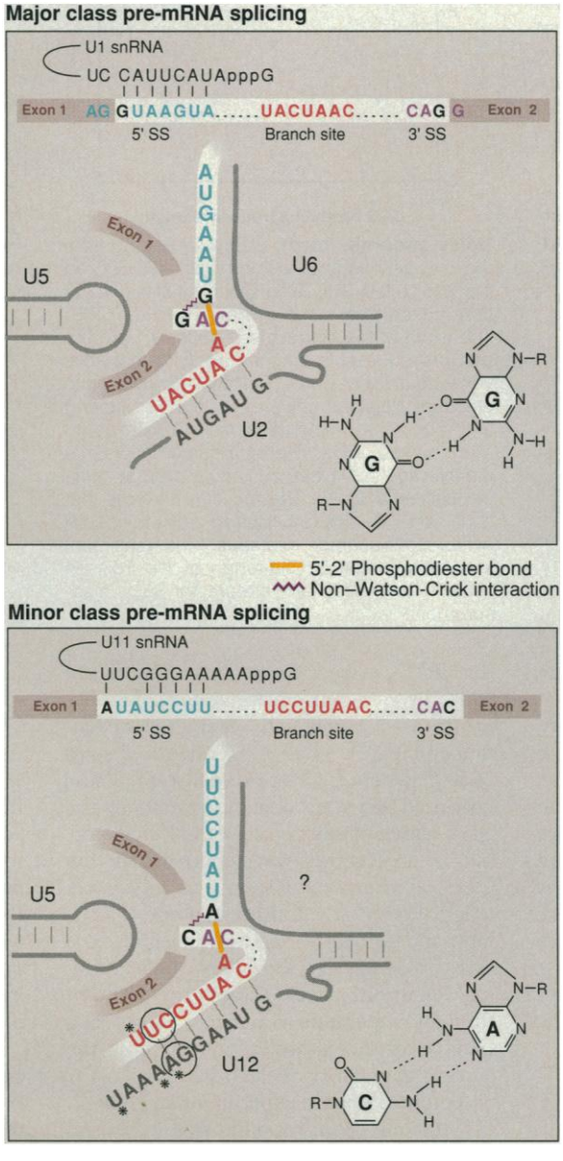
Stephen M. Mount

In the years immediately after the discovery that messenger RNAs (mRNAs) were spliced together from larger precursors, a common sequence feature was described in these precursors—invariant GT and AG dinucleotides at the ends of the intron (corresponding to GU and AG in the RNA). These short, conserved sequences (see figure) reflect the mechanism by which introns are removed from the precursor, and are not found in self-splicing, organelle, or transfer RNA introns (which are removed by other mechanisms). Among pre-mRNA introns, exceptions to this consensus are rare, and greater than 99% of pre-mRNA splice sites conform to the consensus sequences in the figure (*1*). The extent of agreement varies, but the "GT-AG" rule is followed particularly well. Apparent exceptions that prove the rule include 5′ splice sites with C at the second position and an otherwise excellent match to consensus. Most exceptions in the databases can be attributed to sequencing errors, genetic polymorphisms, somatic mutation, or errors in database annotation (*1*).

Pre-mRNA introns are spliced by the spliceosome, a large complex consisting of numerous small nuclear ribonucleoprotein (snRNP) particles and other factors. During the orderly assembly of this complex, the splice site consensus sequences are recognized by small nuclear RNAs (snRNAs). The evidence that all pre-mRNA introns are removed by a single conserved mechanism had appeared to be compelling (*2*): There are few counter-examples; the splicing machinery is complex and splicing factors are strongly conserved among all eukaryotes. Certainly, there is variation among species in consensus sequences and in mechanisms of splice site selection (*2*), but until now the evidence has suggested that all pre-mRNA introns are spliced similarly—in two steps via a branched intermediate by machinery with an active site that includes the conserved snRNAs U2 and U6 (*2*) (see figure).

Results in this issue of *Science* (*3*) and in a recent issue of *Cell* (*4*) are therefore quite surprising. These papers present data showing that the splicing of a specific minor class of intron (representing something less than 0.1% of all known pre-mRNA introns) is accomplished by a mechanism involving a distinct and correspondingly rare class of snRNAs, U11 and U12. The existence of such a class of intron with bona fide nonconsensus splice sites was first proposed by Jackson (*1*). Introns in this group have AT rather than GT at the 5′ end of the intron, and AC rather than AG at the 3′ splice site. Five such AT-AC introns are known (*5*–*7*), and in three of these instances the intron is conserved in distinct vertebrate species. Strikingly, all of these introns, including one in the *Drosophila* homeodomain protein gene *prospero*, share not only the AT and AC dinucleotides, but a much longer consensus at both splice sites and a nearly invariant sequence (TCCTTAAC) at a consistent distance (8 to 11 nucleotides) upstream of the 3′ splice site (see figure). On the basis of potential base pairing between these consensus sequences and the minor snRNAs U11 and U12, it was proposed (*5*, *8*) that AT-AC introns are recognized by a distinct class of factors that includes U11 and U12.

This proposal has now been subjected to experimental test. Hall and Padgett (*3*) show that mutation of the putative branch point consensus (UCCUUAAC in the RNA) interferes with splicing in vivo; alteration of two nucleotides that do not occur in the branch point consensus for the major class of intron (UC to AG at the position circled in the figure) prevented splicing in transfected cells. Furthermore, splicing could be restored by providing U12 with an alteration of the complementary nucleotides (GA to CU



The major (GT-AG) and minor (AT-AC) classes of pre-mRNA introns. (**Top**) GT-AG introns base pair with snRNPs U1, U2, and U6. (**Bottom**) The corresponding interaction between AT-AC introns and U11 remains unproven. Nucleotides mediating the AT-AC intron branch point–U12 pairing established by genetic suppression (*3*) are circled. Asterisk (*), intron and U12 nucleotides shown to be in proximity. Potential non–Watson-Crick base pairs between nucleotides at the two termini of the intron are indicated at the lower right of each panel.

The author is in the Department of Zoology, Room 3202, University of Maryland, College Park, MD 20742-4415, USA. E-mail: mount@zool.umd.edu

UPDATE

Releasing the Brakes on Antitumor Immune Response

Drew Pardoll

As is the case with firearms, the destructive capacity of the immune system must be carefully aimed and just as carefully regulated. A report by Leach *et al.* (*1*) in this issue is an encouraging example of how the receptors that regulate immune responses—in this case, CTLA-4, which was discussed in a recent Perspective (*2*)—can be directly targeted for immunotherapy.

T cell activation is normally self-limited so that the immune system returns to a baseline state once the offending antigen has been cleared. Initiation of T cell activation requires two signals, a major histocompatibility complex (MHC) antigen-specific signal delivered through the T cell receptor (*3*) and a "constimulatory" signal, the best characterized of which is delivered through the CD28 receptor (*4*). Each of these receptors has a counterregulatory cousin that recognizes the same ligand but delivers an opposing negative signal. The counterregulatory receptor for the T cell receptor is from the natural killer (NK) receptor gene family [first discovered on NK cells but also expressed on T cells (*5*)] CTLA-4 is the counterregulatory receptor for CD28; it binds B7 with roughly 10-fold higher affinity than does CD28. Occupancy of CTLA-4 directly counters the effects of CD28 on T cell activation (*6*): CD28 cross-linking increases lymphokine secretion by activated T cells, whereas CTLA-4 cross-linking decreases lymphokine production.

Full text of this and a previous Perspective (*2*) is available at http://science.mag.aaas.org/science/

Neither the NK receptor nor CTLA-4 is expressed on resting T cells; rather, they become induced after T cell activation. These delayed kinetics of expression are responsible for the characteristic self-limited nature of T cell activation. The importance of CTLA-4 in T cell homeostasis in vivo is most dramatically exemplified in CTLA-4 knockout mice. These mice develop a severe lymphoproliferative disease with immunologically mediated organ destruction (*7*).

Manipulation of these counterregulatory receptors in vivo has now entered the realm of cancer immunotherapy. Leach *et al.* have registered a success in this endeavor by injecting antibodies to CTLA-4 (anti–CTLA-4) into mice with tumors, thereby releasing the brake on T cell activation and favoring the accelerator. In two murine cancer models, infusions of antibodies to CTLA-4 produced an enhanced antitumor effect without overt toxicity. The success of this approach depends on the presence of a preexisting T cell response against the tumor antigens, which, under normal circumstances, fails to develop enough amplitude to win the battle against the tumor. This appears to be the case for at least one human tumor, melanoma, in which tumor-specific T cell responses can be readily identified in tumor-infiltrating lymphocytes (*8*).

CTLA treatment itself is not antigen-specific, and the window between development of an antitumor response and hyperimmunity or autoimmunity may be narrow. However, in contrast to genetic knockout, CTLA-4 blockade with antibodies allows a limited duration of therapy to take maximal advantage of even a small therapeutic window. Another intriguing possibility is the combination of cancer vaccines with short-term anti–CTLA-4 treatment. Cancer vaccines seek to present tumor antigens to the immune system in a fashion that favors T cell activation rather than tolerance. For a cancer vaccine to be successful, T cells specific for the vaccinating antigen must be activated above a threshold that provides them the edge over the tumor's growth. CTLA-4 blockade at the time of vaccination might allow selective amplification of immune responses against the vaccinating antigen while limiting induction of undesirable immune responses.

The author is in the Department of Oncology, Johns Hopkins University Medical School, Baltimore, MD 21205, USA.

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at the circled positions). Such a suppression result is very likely to mean that the splicing mechanism occurs by base pairing between U12 and the branch point region.

By inactivating U2, Tarn and Steitz (*4*) were able to obtain splicing of the AT-AC intron F from the human *P120* gene in nuclear splicing extracts derived from HeLa cells. With this in vitro system, they were able to map the branch point and confirm that it lies at the expected location within the TCCTTAAC consensus. Interaction between these nucleotides and U12 was demonstrated by psoralen cross-linking. These authors could also detect incorporation into spliceosomes not only of U12, but also U11 and U5. However, they were unable to detect U6, and inactivation of U6 did not interfere with P120 intron F splicing. This result is surprising in light of potential base pairing between U6 and U12 resembling that between U6 and U2 (*8*) and points to the existence of a minor snRNA related to U6. This unknown RNA should be capable of intimately associating with U12 and would also be expected to pair to the extended 5′ splice site consensus of AT-AC introns.

Like all results describing new phenomena, the two new reports raise more questions than they answer. Foremost among these is the extent to which the two mechanisms differ. We already know that U5 is present in both types of spliceosome and that both involve branch formation at a bulged A residue. Deeper kinship is suggested by an earlier result (*9*) that the yeast pre-mRNA splicing machinery (which is probably limited to the major class) shows some ability to recognize standard introns carrying mutations that alter the termini to AU and AC. Specifically, it had been known for some time that mutation of G at the first position of the intron blocked splicing after the first step. AC (and, to a lesser extent, A) at the last position of the intron can suppress this block (*9*). These and subsequent data [reviewed in (*10*)] argue persuasively for a non–Watson-Crick base pair between the nucleotides at the extreme ends of the intron that is important in the second step of splicing (see figure). These results imply that

standard U2-containing splicing machinery can remove introns with AU and AC at their termini in the context of splice sites that otherwise fit the standard consensus. A similar observation has been made in mammalian cells, where a G to A mutation in the last position of the fourth intron of the CHO cell *dhfr* gene was isolated as a suppressor of a G to A mutation in the first position (*11*). (The reader may want to verify that non–Watson-Crick A-A pairs with the geometry shown in the figure for G-G and A-C pairs are possible.) Without a good match to the minor class branch point consensus, it is likely that the doubly mutant AT-AA *dhfr* intron is removed by splicing machinery containing U2, but it will be interesting to verify this supposition and related questions. For example, is mutation of the terminal AT-AC nucleotides alone sufficient to allow splicing of introns carrying GT-AG termini by a U2-mediated process (with its less stringent branch point requirement)? There are already results indicating an in vivo preference for splicing between splice sites of the same class (recognized as skipping of both exons flanking a crippled AT-AC intron), as in P120 (*3*) and mutant alleles of the mouse sodium channel Scn8a (*7*).

Finally, there is the question, Why? The possibility that AT-AC introns could provide a mechanism for alternative splicing springs immediately to mind, but there is so far no evidence for regulated use of AT-AC introns. I favor the idea that AT-AC introns are yet another molecular fossil, a way of getting the job done that, like diesel cars or Beta videotapes, is clearly less popular, but whose adherents (people who own diesel cars or Betamax VCRs; genes that contain AT-AC introns) create a continuing demand for the machinery. In any case, AT-AC introns will surely provide a counterpoint to GT-AG introns for testing ideas about the mechanism of pre-mRNA splicing.

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Hydrogen: The First Metallic Element

Friedrich Hensel and Peter P. Edwards

Most elements are metals, which remain metallic when they are heated through their melting points at normal (1 atm) pressure. However, the lightest element, hydrogen, is unquestionably a molecular nonmetal in its normal solid and liquid states. Recent experiments by Weir, Mitchell, and Nellis (*1*) on shock-compressed fluid hydrogen give the first direct experimental evidence for metallic hydrogen. In a news story on page 1667 of this issue, Kerr discusses the implication of this work for jovian interiors (*2*).

The key to the Livermore experiments is the shock compression of a thin (0.5 mm) layer of liquid hydrogen contained between two single-crystal Al_2O_3 anvils. The high shock pressures, up to 2 million atm (200 GPa), are generated by high-velocity impact (7 kms^{-1}) of a metal plate onto the surface of a ductile aluminum sample cell. A reverberating shock in the liquid generates a temperature of a few tenths of an electron volt (≈3000 K), low compared to both the initial electronic energy gap of 15 eV and the molecular dissociation energy of ≈2 eV. Importantly, the Livermore group also measured the electrical resistance of the sample at high pressure using electrodes inserted through the rear walls of the cell, flush with the liquid hydrogen–Al_2O_3 interface. The conductivity rises continuously, increasing by over five orders of magnitude, from 50 to 140 GPa and is then essentially constant at 2000 $(\Omega \cdot cm)^{-1}$ from 84 to 180 GPa.

These remarkable experiments also highlight a fascinating intellectual problem; namely, how do we know that the measured conductivity at high pressure is indeed indicative of a "metallic" form of hydrogen? The only rigorous criterion for differentiating between a metal and a nonmetal is the value of the electrical conductivity at the absolute zero of temperature; there, metals have a finite electrical conductivity (or infinite in the case of a superconductor), whereas nonmetals have zero conductivity. At finite temperatures, as in the situation at hand, the thermal excitation of conduction electrons will blur the distinction between metals and nonmetals. This dilemma has led to numerous attempts at predicting the actual value of electrical conductivity at the nonmetal-to-metal borderline. A favored view is that high-temperature fluids metallize when the characteristic mean free path for the valence electrons becomes comparable to, or exceeds, the average distance between particles providing the electrons. This simple but powerful argument leads to an estimate of ≈2000 $(\Omega \cdot cm)^{-1}$ for the conductivity of fluid hydrogen at the metallization threshold (*3*, *4*). Interestingly, not only does the measured electrical conductivity of compressed fluid hydrogen attain this value, but it is essentially the same as that of the alkali metals Cs and Rb in their expanded fluids at 2000 K. This is a testament to the inherent similarities of hydrogen and the other alkali metals.

Although the metallization of the lightest and most abundant element in our universe requires unearthly pressures and temperatures, such conditions are normal in the interior of Jupiter (*2*). That planet, itself a fluid, is composed of an outer layer of nonmetallic, molecular hydrogen that continuously transforms to metallic fluid hydrogen within the core. The outstanding ingenuity and technical skills—and perseverance—of the earthly Livermore researchers will undoubtedly lead to major new insights into the very nature of the jovian planet. Finally, the metallization of hydrogen casts a new perspective on the periodic table itself, devised over the centuries from the observed periodicity in properties of the chemical elements at a pressure of 1 atm. Now that the first element has finally succumbed to metallization, we must add to the age-old question, "What is a metal?" an equally important inquiry, "When is a metal?"

F. Hensel is at the Institute of Physical Chemistry, University of Marburg, 35032 Marburg, Germany. P. P. Edwards is in the School of Chemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

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