Recognizing the 35th anniversary of the proposal that snRNPs are involved in splicing

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ABSTRACT **Thirty-five years ago, as young graduate students, we had the pleasure and privilege of being in Joan Steitz's laboratory at a pivotal point in the history of RNA molecular biology. Introns had recently been discovered in the laboratories of Philip Sharp and Richard Roberts, but the machinery for removing them from mRNA precursors was entirely unknown. This Retrospective describes our hypothesis that recently discovered snRNPs functioned in pre-mRNA splicing. The proposal was proven correct, as has Joan's intuition that small RNAs provide specificity to RNA processing reactions through base pairing in diverse settings. However, research over the intervening years has revealed that both splice site selection and splicing itself are much more complex and dynamic than we imagined.**

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In the summer of 1979, we both began work in Joan Steitz's laboratory in the Department of Molecular Biophysics and Biochemistry at Yale University (Figure 1). Steve had chosen the Steitz laboratory for his thesis work after a rotation during his first year. Sandy had completed her first year of Yale's MD-PhD program and was trying out the Steitz laboratory as a rotation student. It was an exciting time to be a student of molecular biology. Only 2 years earlier, the first intervening sequences had been discovered in mRNA (Berget *et al.*, 1977; Chow *et al.*, 1977). Now attention was focused on finding a mechanism for their removal. What still entirely mysterious machinery could identify and remove intervening sequences?

Experiments in the Steitz laboratory had very recently identified a new class of ribonucleoproteins (RNPs) in mammalian cells: small nuclear ribonucleoproteins (snRNPs), known in the lab as "snurps." An older MD-PhD student, Michael Lerner, had just shown that "anti-RNP" and "anti-Sm" autoantibodies from patients with the rheumatic disease systemic lupus erythematosus recognize snRNPs containing a specific set of small RNAs complexed with proteins (Lerner and Steitz, 1979). These RNAs included U1 and U2, small nuclear RNAs (snRNAs) previously identified in the laboratories of Harris Busch and Sheldon Penman (Hodnett and Busch, 1968; Weinberg and Penman, 1968), as well as three new RNAs that Lerner and Steitz (1979) named U4, U5, and U6 snRNA.

Our 1980 article, Are snRNPs Involved in Splicing? (Lerner *et al.*, 1980), presented a few simple yet powerful arguments implicating these newly discovered Sm snRNPs in intron removal. These included the finding that RNAs similar in size to the mammalian U1, U2, U4, U5, and U6 RNAs were present in anti-Sm immunoprecipitates from insect cells, as would be expected for components of a highly conserved process, and that both "30S hnRNPs" (likely equivalent to pre-mRNAs complexed with proteins) and snRNAs were more abundant in the nuclei of metabolically active cell types (Lerner *et al.*, 1980). Supporting these relatively nonspecific pieces of data were two highly intriguing observations: 1) the 5′ end of the U1 snRNA was capable of base pairing with conserved sequences at the intron–exon junctions, and 2) a degraded form of U1 RNA lacking these sequences differs from the full-length RNA in that it no longer sediments with 30S hnRNPs. These observations were woven into a model in which RNA:RNA base pairing between the 5′ end of U1 snRNA and splice junction sequences contributes to the recognition of splice junctions (Lerner *et al.*, 1980).

This paper was not so much the report of a discovery as the presentation of a hypothesis accompanied by preliminary, tantalizing clues. In fact, the article contains five specific speculations, and it is interesting to consider them one at a time.

First and foremost is the hypothesis addressed by the title of the paper: are snRNPs involved in splicing? This is the simplest and most general of the hypotheses, and it has proven correct. All of the Sm snRNPs mentioned in the article turned out to be components

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Abbreviations used: hnRNA, heterogeneous nuclear RNA; hnRNP(s), heterogeneous nuclear ribonucleoprotein(s); RNase, ribonuclease; RNP(s), ribonucleoprotein(s); snRNA, small nuclear RNA; snRNP(s), small nuclear ribonucleoprotein(s).

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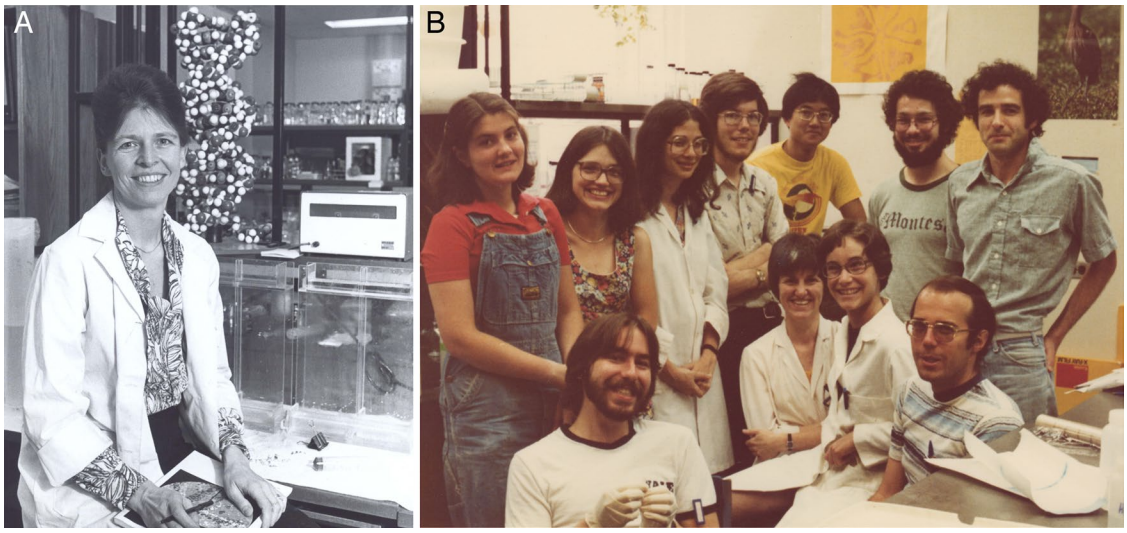

FIGURE 1: The Steitz laboratory. (A) Joan Steitz (photograph taken in January 1983 by T. Charles Erickson; courtesy of John Curtis, Yale School of Medicine Institutional Planning and Communication). (B) The Steitz laboratory in the summer of 1979. Standing, left to right: Nancy Andrews, Martha Krikeles, Sandra Wolin, Stephen Mount, Bernard Shen, James Spilsbury, Michael Lerner. Seated: Richard Bram, Margaret Rosa, Joan Weliky (now Conaway), John Boyle (photograph by Randall Reed, who is missing from the picture).

of the spliceosome, a large dynamic structure that forms anew on each intron in an ordered way; RNPs containing the five snRNAs work together in a coordinated way to remove most introns found in protein-coding genes (Brody and Abelson, 1985; Grabowski *et al.*, 1985; Will and Luhrmann, 2011).

A more specific hypothesis, supported by the complementarity just noted, suggested that U1 snRNA contributed to the recognition of splice sites through base pairing. This proposal, together with a similar one from Rogers and Wall (1980), immediately attracted much attention. It was quickly shown that U1 snRNPs in a crude extract could specifically bind and protect 5′ splice-site sequences (Mount *et al.*, 1983) and that anti-Sm and related patient autoantibodies against only the U1 snRNP inhibited splicing in extracts (Padgett *et al.*, 1983). Formal genetic proof of this hypothesis was obtained by Alan Weiner's group (Zhuang and Weiner, 1986), which, not coincidentally, resided down the hall from the Steitz laboratory. Very recently, a crystal structure of the U1 snRNP complexed with the 5′ splice site (Kondo *et al.*, 2015) indeed shows the proposed base pairing.

The original proposal involved extended base pairing between the 5′ end of U1 RNA and both the 5′ and 3′ splice sites in the form of a crossover structure resembling half of a Holliday junction, an intermediate in homologous recombination of DNA (Holliday, 1964). So, a third hypothesis was that base pairing might join splice sites via a crossover structure resembling the Holliday junction. One aspect of this proposal was nicely confirmed with the discovery that U5 snRNA interacts with exon nucleotides at both ends of the intron (Newman and Norman, 1991; Sontheimer and Steitz, 1993). However, the role of U1 was quickly modified to involve only the 5′ splice site, based on the finding that only the 5′ site was bound in vitro (Mount *et al.*, 1983) and only nucleotides 1–11 were conserved in *Drosophila melanogaster* U1 RNA (Mount and Steitz, 1981). Subsequent analysis showed that the 3′ splice site is recognized in relation to (and after) the branch site, and that U1 leaves the spliceosome before the first step of splicing (Konforti *et al.*, 1993). The branch site is recognized by U2 via base pairing, and each of the other snRNAs contributes to spliceosome formation or function through base pairing of some sort (Will and Luhrmann, 2011). Thus, although the specific crossover model was wrong, the idea that multiple base-pairing interactions play a critical role in bringing things together has proven correct.

More generally, we hypothesized that base pairing to small RNAs might provide specificity to splicing, much as base pairing between the 16S rRNA and the Shine–Dalgarno sequence had been shown to increase specificity at translation initiation sites (Shine and Dalgarno, 1975; Steitz and Jakes, 1975). In fact, we envisioned a family of RNAs that might recognize specific subsets of splice sites: "different RNA sequences [in the other snRNAs] could facilitate more precise recognition of variant splice junction sequences in hnRNA or in other RNA molecules" (Lerner *et al.*, 1980). Although the snRNAs we knew about (U2, U4, U5, and U6) act together to remove the same introns, this prediction was borne out in a surprising way by the discovery of the minor spliceosome, in which case, variant splice sites are indeed recognized by a variant spliceosome (containing U11, U12, U4atac, and U6atac in place of U1, U2, U4, and U6 but the same U5) (Hall and Padgett, 1994, 1996; Tarn and Steitz, 1996a,b).

Moreover, a role for specificity provided by base pairing to small RNAs within a class later emerged as a theme in other large classes of small RNAs. The first large class to be described was small nucleolar RNAs, which direct specific base modification (pseudouridylation and 2′ O-methylation) of their targets (Cavaille *et al.*, 1996; Kiss-Laszlo *et al.*, 1996; Ganot *et al.*, 1997; Ni *et al.*, 1997). The second, even more spectacular story was, of course, microRNAs (Lee *et al.*, 1993; Wightman *et al.*, 1993). Thus base pairing by small RNAs to their RNA targets has proven a key means to provide specificity in several biological processes.

The last hypothesis of note in our original article was that the snRNAs themselves might be catalytic. This speculation ("U1-containing snRNPs…could either be the splicing enzyme itself [note the example of *E. coli* RNase P, which contains a small RNA complexed with several small proteins]") had little basis in any data shown in the paper. It was pure Joan Steitz and showed her deep insight into RNA function. The work on RNase P was being carried out elsewhere at Yale by Sidney Altman and would later earn him

the Nobel prize (shared with Tom Cech) for the discovery of catalytic RNA (Stark *et al.*, 1978; Guerrier-Takada *et al.*, 1983). Indeed, spliceosomal splicing is probably catalyzed, at least primarily, by snRNAs, but by U6 and U2 rather than U1 (Madhani and Guthrie, 1992; Valadkhan, 2005), and fully self-splicing group II intron RNAs are homologous (Jacquier, 1990; Sashital *et al.*, 2004). Remarkably, the recent 3.6 Å cryo-electron microscopy structure of the *Schizosaccharomyces pombe* spliceosome has now allowed visualization of both the catalytic center and the resemblance to group II introns in three dimensions (Hang *et al.*, 2015; Yan *et al.*, 2015).

Overall the hypotheses detailed in our original article proved remarkably prescient and influential, as evidenced by the >1000 citations it has received. Much of the focus in the splicing field is now on obtaining high-resolution structures of splicing complexes, integrating splicing with other processes, such as transcription, mRNA stability and translation, and in deciphering the myriad ways in which splicing and splice-site choice can be regulated. In addition, in 1980, we did not foresee the plethora of disease-causing mutations in splice sites, auxiliary splicing signals and in components of the splicing machinery or the ways in which genetic variation can affect splicing (Sterne-Weiler and Sanford, 2014; Xiong *et al.*, 2015). Yet, the idea of using base pairing between short RNAs and their targets to influence splice-site selection underlies several current efforts to use antisense oligonucleotides as therapeutics to sequester inhibitory sequences and/or correct splicing defects (Hua *et al.*, 2010; Osorio *et al.*, 2011; Lentz *et al.*, 2013).

Thirty-five years is a long time, giving us an opportunity to also reflect on how the practice of science has changed. Several of the experiments in our original article involved metabolically labeling tissue culture cells with 10 mCi/l [^{32}P]orthophosphate, something that would be very difficult to convince a student or a postdoc to do today. The experiment that showed snRNPs were present only in metabolically active cells involved comparing nuclei from chicken liver and erythrocytes, for which purpose a chicken was kept in the laboratory, a housing choice that would be unlikely to be allowed now. The compilation of consensus splice sites was done by hand, as was the observation of complementarity between these sequences and the 5′ end of the U1 snRNA. Moreover, since *Molecular Cloning* (Maniatis *et al.*, 1982) had not yet been written and there was no Internet to consult for protocols and certainly no kits to purchase, we were forced to rely on our colleagues, both at Yale and elsewhere, to learn new techniques. We formed deep friendships within the Steitz laboratory, with students and postdocs in nearby laboratories, and with colleagues elsewhere, which have endured to this day. Despite changes in the practice of science, we are confident that graduate students today continue to find the same excitement in discovery and in a collegial atmosphere like the one that characterized our time in the Steitz laboratory.

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