Check for updates

REVIEW

RNA and Proteins: Mutual Respect [version 1; peer review: 3 approved]

Kathleen B. Hall

Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St Louis, MO, 63110, USA

V1 First published: 27 Mar 2017, 6(F1000 Faculty Rev):345
<https://doi.org/10.12688/f1000research.10572.1>Latest published: 27 Mar 2017, 6(F1000 Faculty Rev):345
<https://doi.org/10.12688/f1000research.10572.1>

Abstract

Proteins and RNA are often found in ribonucleoprotein particles (RNPs), where they function in cellular processes to synthesize proteins (the ribosome), chemically modify RNAs (small nucleolar RNPs), splice pre-mRNAs (the spliceosome), and, on a larger scale, sequester RNAs, degrade them, or process them (P bodies, Cajal bodies, and nucleoli). Each RNA-protein interaction is a story in itself, as both molecules can change conformation, compete for binding sites, and regulate cellular functions. Recent studies of Xist long non-coding RNP, the U4/5/6 tri-small nuclear RNP complex, and an activated state of a spliceosome reveal new features of RNA interactions with proteins, and, although their stories are incomplete, they are already fascinating.

Keywords

Ribonucleoprotein Particles, RNPs, RNA-protein interaction, Xist, lncRNA, U4/5/6 tri-snRNP complex

Open Peer Review

Approval Status

	1	2	3
version 1 27 Mar 2017

Faculty Reviews are review articles written by the prestigious Members of **Faculty Opinions**. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

1. **Xiang-Dong Fu**, Department of Cellular & Molecular Medicine, University of California San Diego, San Diego, USA
2. **Markus Wahl**, Department of Biology, Chemistry, Pharmacy, Freie Universität Berlin, Berlin, Germany
3. **Shinichi Nakagawa**, RNA Biology Laboratory, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

Any comments on the article can be found at the end of the article.

Corresponding author: Kathleen B. Hall (kathleenhal@gmail.com)

Competing interests: The author declares that she has no competing interests.

Grant information: The author(s) declared that no grants were involved in supporting this work.

Copyright: © 2017 Hall KB. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Hall KB. **RNA and Proteins: Mutual Respect [version 1; peer review: 3 approved]** F1000Research 2017, **6** (F1000 Faculty Rev):345 <https://doi.org/10.12688/f1000research.10572.1>

First published: 27 Mar 2017, **6**(F1000 Faculty Rev):345 <https://doi.org/10.12688/f1000research.10572.1>

Introduction

RNA molecules in the cell are rarely naked. Rather, proteins are bound to them in some arrangement consistent with their regulation, protection from nucleases, transport, or formation of ribonucleoprotein particles (RNPs). A 2014 compendium of RNA-binding proteins in humans¹ concluded that 7.5% of 20,500 known protein-coding genes are found in RNPs or bound to mRNAs, where they regulate RNA metabolism. This is likely to be an underestimate, since their structural heterogeneity makes them difficult to identify *de novo*.

The recent discovery of a plethora of non-coding RNAs² in cells has invigorated investigation of proteins that bind to RNA. New methods of probing the proteins in a transcriptome have allowed simultaneous identification of a protein and its RNA-binding site. Typically, these are crosslinking-immunoprecipitation (CLIP) experiments³⁻⁹. Intact cells can be irradiated with ultraviolet (UV) light or treated with formaldehyde to crosslink proteins to RNA, then the complexes are purified from the milieu by immunoprecipitation. To identify proteins bound to mRNAs, cellular UV RNA-protein crosslinking is followed by isolation of all poly(A)-RNA⁷. Alternatively, proteins bound to a specific RNA could be recovered by annealing biotin-oligonucleotides complementary to the RNA and selective purification by streptavidin⁹. Proteins bound to RNAs could then be identified by mass spectrometry. Several groups applied this method to identify mRNA-binding proteins in human cell lines, mouse embryonic stem cells (ESCs), and *Saccharomyces cerevisiae* yeast cells (reviewed in Gerstberger *et al.*¹).

Assuming that there are indeed more than 1,500 RNA-binding proteins in human cells, books will be written about them and their roles in RNA biology. Here, I focus on recent advances that reveal the variety and mystery of RNPs.

Xist, the RNA that inactivates an X chromosome

Xist is a long non-coding RNA (lncRNA) that is responsible for transcriptional silencing of one of two X chromosomes in female cells¹⁰⁻¹³. There are approximately 200 Xist molecules bound to a single X chromosome, and each 18 kb of Xist is bound by proteins (Figure 1). Proteins could participate in any aspect of its biology: Xist has to associate with the X chromosome, then spread along it, and finally inhibit RNA polymerase II (Pol II) transcription. After more than twenty years of efforts to identify those proteins, the power of mass spectroscopy has been applied to proteins crosslinked *in cellulo* to Xist.

Two research groups have recently published compendia of Xist-bound proteins. Each group first crosslinked RNA to protein *in cellulo*, selected Xist through oligonucleotide-directed annealing, then used quantitative mass spectrometry to identify bound proteins. An overall comparison of their results shows great similarity but also some curious and intriguing differences. Table 1 and Table 2 list the most abundant proteins recovered from each study.

The groups of Heard and Chang¹⁴ identified 81 proteins *in toto* bound to Xist. Using formaldehyde, they crosslinked proteins to Xist in three different mouse cell types: a male ESC line containing

Figure 1. Xist wraps around nucleosomes in the X chromosome. Approximately 200 Xist molecules bind to an X chromosome, spread along it, and inhibit RNA polymerase II from transcribing the DNA. Xist is bound by many proteins at unknown sites and with unknown stoichiometry, which subsequently interact with each other through disordered regions or structured domains. RNA is shown as a yellow/orange strand and protein linkers as blue strands. RRM, RNA recognition motif.

Table 1. Top 15 Xist-binding proteins from ChI-MS recovery in mouse cells¹⁴.

Crosslinked proteins	In order of abundance	Protein structural motifs	Length* (number of amino acids)
hnRNP M	1	3 RRM	728
hnRNP U (Saf-A)	2	RGG, KH, acidic region, DNA binding	793
hnRNP K	3	3 KH, proline-rich	463
hnRNP A2/B1	4	2 RRM, RGG, glycine-rich	353
MYEF2	5	2 non-canonical RRM, homology to hnRNP M4	591
hnRNP A1	6	2 RRM, glycine-rich, RGG	320
DDX5	7	DEAD box protein	
Spen (SHARP)	8	3 RRM, SPOC	3,640
RBM XL1	9	RRM	
hnRNP AB	10	2 RRM	
hnRNP D (AUF1)	11	2 RRM	355
hnRNP L	12	4 RRM, glycine-rich	589
hnRNP A3	13	2 RRM, glycine-rich	379
hnRNP C	14	1 RRM, acid rich	293
TARDBP (TDP-43)	15	2 RRM, glycine-rich, DNA-binding protein	414

*Many proteins have isoforms with varying lengths; the longest variant in *Homo sapiens* is listed.

Table 2. Mouse embryonic stem cells: top 10 Xist-binding proteins from RAP-MS²³.

Crosslinked proteins	In order of abundance	Protein structural motifs	Length (number of amino acids)
SHARP (SPEN)	1	3 RRM, SPOC	3,640
RBM15	2	3 RRM, SPOC	969
MYEF2 (hnRNP M)	3	3 RRMs, homology to hnRNP M4	591
CELF1	4	3 RRMs	486
hnRNP C	5	1 RRM	313
LBR	6	Chromatin-interaction domain, transmembrane region, lamin-interacting domain	626
SAF-A (hnRNP U)	7	RGG, SPRY domain, ATPase domain	793
RALY (hnRNP C)	8	1 RRM	312
hnRNP M	9	3 RRM	729
PTBP1 (hnRNP I)	10	4 RRM	555

hnRNP, heterogeneous nuclear ribonucleoprotein particle; RAP-MS, RNA antisense purification-mass spectrometry; RRM, RNA recognition motif; SILAC, stable isotope labeling by amino acids in culture; SPOC, Spen paralog and ortholog C-terminal domain.

an inducible Xist gene, an epiblast stem cell line, and trophoblast stem cells. Each cell type represents one stage of Xist expression. Combining all datasets, three proteins were identified as being most abundant: heterogeneous nuclear RNP (hnRNP) K, hnRNP U, and hnRNP M. In addition, a detailed examination of Xist 5' 0.9 kb sequence revealed several localized proteins. In particular, SPEN (aka SHARP) was found to be necessary for transcriptional silencing.

There is a preponderance of hnRNP proteins. These heterogeneous nuclear ribonucleoproteins are abundant in metazoan cells, where they are mostly found in the nucleus^{15,16}. A recent review of them traced their ancestry¹⁷, concluding that there are 13 families, each with isoforms or variants. For example, hnRNP A has four homologues in humans (A0, A1, A2, and A3), while hnRNP M has two (MYEF2 and hnRNP M). These proteins typically use RNA recognition motifs (RRMs) to bind RNA, while their other domains engage in protein-protein interactions. Several are involved in pre-mRNA splicing, where they repress splice site selection (hnRNP A¹⁸) or regulate exon inclusion (hnRNP I¹⁹). hnRNP I (aka polypyrimidine tract binding protein 1 [PTB1]) also facilitates translation from internal ribosome entry sites (IRES)^{20,21}. hnRNP functions in Xist are unknown, with the exception of hnRNP U (aka Saf-A), which facilitates Xist localization on chromatin²².

In contrast, a group of investigators headed by Guttman²³ took a different approach to finding Xist proteins during transcriptional silencing. After Xist induction in mouse ESCs, cells were UV-crosslinked, Xist RNP was recovered with long antisense oligonucleotides, and Xist proteins were identified by mass spectrometry. Two batches of mouse ESCs were cultured, one in ¹⁵N- and one in ¹⁴N-media to allow quantification by mass spectrometry (SILAC). Among their ten most abundant proteins, they found SHARP (SPEN) and RMD15, two proteins related in their architecture (they are SPEN family proteins). They also recovered six hnRNP proteins (Table 2). These are exciting findings. In a curious coincidence, SHARP has another life in a nuclear RNP with the steroid receptor RNA activator (SRA)²⁴. SRA is a lncRNA that co-regulates the transcription of nuclear receptors²⁴. Bound to SRA, SHARP represses SRA transactivation when it recruits histone deacetylase²⁵. Does it carry out a similar task in Xist^{2,10}?

In fact, McHugh *et al.* found that SHARP was required for the inhibition of Pol II transcription at sites where Xist was bound²³. The mechanism of inhibition could lie in the recruitment of SMRT and/or HDAC3²⁵. HDAC3 is a histone deacetylase²⁶ that is thought to be responsible for transcriptional repression by changing chromatin structure²⁷. Loss of SHARP, LBR, or hnRNP U in knockdown experiments was sufficient to eliminate silencing²³, but each protein appears to have unique contributions. The role of the other seven proteins was not tested directly, but since each binds directly to Xist, they could have functions in localization, recruitment of other enzymes, stabilization, etc. (for example, binding to Polycomb repressive complex 2 [PRC2]).

The identification of LBR bound to Xist explains localization of the Xist-X chromosome to the nuclear lamina¹². Transmembrane helices anchor LBR to the lamina, while its tail contacts Xist. Positioning of Xist-X on the lamina changes the structure of the DNA and facilitates protein-mediated spreading of the Xist molecules along the length of the chromatin.

Rather than discovering unknown proteins, these investigations have re-discovered known proteins. They present a new challenge: to understand why they are particularly useful in the Xist context and how their use, and corresponding abundance, is modulated according to developmental stage or cell lineage. The general challenge is not only to understand how proteins use their RNA-binding domains and intervening sequences and disordered tails to control formation of RNPs but must also account for their temporal exchange.

RNA recognition motifs

A striking feature of proteins bound to Xist is the recurring use of tandem RRM domains. There are certainly advantages to this scheme, since affinity and specificity can be modulated by increasing the number of contacts between RNA and protein. However, neither Xist-binding sites for its associated proteins nor their binding stoichiometry are known. These biochemical characterizations are important to understand how they select their target sites on

the RNA, how they bind to Xist in the milieu of other RNAs in the cell, and how they hang onto the RNA while they also bind to other cellular compartments or recruit other proteins.

RRMs¹ are the most common structural motif used in eukaryotes to bind RNA (Figure 2) and are estimated to be found in 225 human genes. When RRM s are present in multiples, deciphering the contributions of each RRM to the whole can be quite difficult^{28–31}. A recent biophysical study of two tandem RRM s revealed how they partition function.

U2 auxiliary factor (U2AF) is a heterodimer of U2AF65 and U2AF35^{32,33}, which in pre-mRNA splicing aids in the recognition of a 3' splice site^{34–38}. U2AF65 has two RRM s (RRM1 and RRM2) that bind polypyrimidine tracts, but U2AF35 has a single UHM, a “U2AF homology motif”, that is structurally homologous to an RRM^{39,40}. RRM1 and RRM2 are tethered by a short linker (~20 amino acids) that allows them to undergo relative motion and orientation³⁶. Since they bind to polypyrimidine tracts of variable length and sequence, they must be able to expand or contract to span the site⁴¹.

The Sattler and Lamb laboratories collaborated on a comprehensive study of the spatiotemporal disposition of U2AF65 RRM1 and RRM2 and their role in RNA binding. von Voithenberg *et al.*³⁵ showed that RRM1 and RRM2 undergo dynamic exchange between a closed or open orientation at equilibrium (Figure 2). In the closed state, RRM1 and RRM2 do not bind RNA, but when the conformation is open, a polypyrimidine tract can bind. If binding is weak (i.e. the polypyrimidine tract is too short or contains multiple purine nucleotides), the exchange between open and closed states is relatively unperturbed. If RNA binding is tight,

RRM1 and RRM2 will be trapped in an open state. Thus, the RNA shifts the equilibrium of U2AF RRM1 and RRM2 between open and closed states in an example of conformational selection.

These experiments were conducted using single pair Förster resonance energy transfer (spFRET) that observed single molecules, each containing a donor and acceptor fluorophore. One fluorophore was attached to either RRM, such that the open and closed orientations were distinguished by the FRET efficiency. Combining measurements of fluorophore lifetimes with spFRET facilitated temporal characterization of exchange between open and closed states. In experimental conditions, free RRM1 and RRM2 occupied an open state ~67% of the time. Addition of RNA trapped RRM1 and RRM2 in the open conformation 90% of the time.

U2AF65 and U2AF35 have been the subject of many biochemical and structural investigations, since they are essential proteins for pre-mRNA splicing. In particular, experimental studies of protein–protein interactions between U2AF and other proteins have identified sites where interactions occur^{42–45}. These latest experiments revealed a mechanism of protein–protein interaction involving the UHM of U2AF35 and U2AF65 RRM1 and RRM2. A combination of nuclear magnetic resonance (NMR) structure and dynamics experiments identified the binding site of U2AF35 UHM to be a surface of U2AF65 RRM1. Binding of the UHM to RRM1 shifts the RRM1 and RRM2 conformational equilibrium to the open state, thereby favoring RNA binding. The authors suggest that allostery drives the RRM1 and RRM2 conformational switch. Allosteric modulation of binding is a powerful mechanism to provide discrimination and affinity^{46–49}, but, by its nature, it is almost impossible to anticipate and cannot be gleaned from static structures.

Figure 2. Regulation of RNA recognition motif (RRM) binding to RNA. **A.** An RRM has a four-stranded anti-parallel β -sheet, with two α -helices on one side^{92–95}. RNA often sits on the surface of the β -sheet. **B.** The two RRM s of U2 auxiliary factor (U2AF) exhibit closed/open transitions at equilibrium, but only in the open state can RNA bind. Binding of the U2AF homology motif (UHM) from U2AF35 to RRM1 shifts the equilibrium to favor the open state, which facilitates RNA binding. The C-terminal tail of U2AF65 contacts the U2AF35 UHM. ESE, exonic splicing enhancer.

Many RNA-binding proteins are modular, with an RNA-binding domain, intervening sequences, and disordered tails. Here, U2AF uses two proteins to regulate splicing; other examples include the Sxl-Unr heterodimer that regulates translation via interactions between Sxl RRM and a Unr cold-shock domain⁵⁰, while the SR protein (serine-arginine) SRSF1 is regulated by phosphorylation of its RS tail that blocks intramolecular interaction with its RRMs^{51,52}. Regulation by intermolecular and intramolecular interactions adds another level of complexity to RNA-binding proteins.

The spliceosome and its small nuclear ribonucleoprotein particles

It is estimated that 94% of all human genes contain introns^{53–55}, thereby providing protein isoform diversity. The process of removing introns and joining exons is carried out by the spliceosome, a multi-component and dynamic assembly of RNPs⁵⁶. A great challenge in the field of pre-mRNA splicing has been to understand how the spliceosome is physically able to carry out the concerted transesterification reactions of the splicing chemistry to yield mRNAs.

The spliceosome consists of five small nuclear RNPs (snRNPs) that dynamically associate with each other and with pre-mRNA. The major spliceosome uses U1, U2, U4, U5, and U6 snRNPs in the process of splicing⁵⁷. Each snRNP contains a single RNA (snRNA) and multiple proteins, but while U1 and U2 snRNPs are independent, U4 and U6 form a di-snRNP that goes on to become a U4/U5/U6 tri-snRNP⁵⁸. The tri-snRNP is recruited to a bona-fide intron and is then remodeled, losing U4 snRNP and leaving U5 and U6 snRNPs to form the active spliceosome.

The goal of snRNP rearrangement is to allow and facilitate snRNA conformational rearrangements in the spliceosome to produce the active site for catalysis^{59–61}. Rearrangements of pre-mRNA and snRNAs to prepare and position them for catalysis are mainly accomplished by protein helicases⁶². There are eight such type SF2 helicases that associate with the spliceosome along the reaction pathway^{63,64}. These ATP-dependent RNA helicases are not sequence specific; they can unwind any RNA duplex. Rather, their specific targets appear to be defined by where and when they associate with the spliceosome. The Brr2 helicase is particularly critical in the transformation of pre-spliceosome intermediates^{64–67}. Brr2 is unusual: it has two helicase domains (only one is active) and a long (450-amino-acid) N-terminal domain^{64,65,68,69}.

Brr2, a unique RNA helicase

Brr2 enters the nucleus independently and associates with the U5 snRNP. U5 snRNP then joins the U4/U6 di-snRNP to become the U4/U5/U6 tri-snRNP⁶⁸. The tri-snRNP is recruited by U1 and U2 snRNPs to form a pre-spliceosome.

To form the active spliceosome, two snRNPs must be displaced. U1 snRNP is released from the 5' splice site, and U4 snRNP is removed from the tri-snRNP. It is the latter remodeling that requires Brr2, as U4 and U6 snRNAs are joined by 22 perfect base pairs and Brr2 is the helicase that separates them. Only when U6 snRNA is free of U4 snRNA can it rearrange to base pair with U2 snRNA and pre-mRNA and so form the catalytic center of the

spliceosome. Clearly, Brr2 activity must be regulated such that it is inactive in the tri-snRNP but active in the pre-spliceosome. How is it regulated?

Several recent studies have delved into the details of Brr2 regulation. In a series of papers from the Wahl lab^{70–74}, Brr2 structure and function were addressed by crystallography and biochemistry. The goal of Brr2 in the tri-snRNP is to maintain stasis. As biochemistry experiments of Brr2 show⁶⁴, there is a plug domain at the N-terminus of Brr2's long N-terminal region (NTR). This plug folds back over the entrance of the helicase to block access of the U4/U6 snRNA duplex to the active site of Brr2. This is a unique intramolecular regulatory device, and more experiments are required to understand how it is directed to this position (and how it is displaced).

The tri-snRNP is an intermediate in the pathway to spliceosome formation. Years of enormous efforts to map intermediates^{42,63,75–77} have now been coupled with technological advances in cryo-electron microscopy (cryo-EM) to visualize select transitional complexes^{70–72,78,79}. Those efforts have produced a cryo-EM structure of human tri-snRNP that captures Brr2 in its plugged conformation⁷² (PDB ID 3jcr). This state of the tri-snRNP, illustrated in [Figure 3](#), might represent its structure as an autonomous particle before it joins the pre-spliceosome, where U4 and U6 snRNAs are still base-paired to each other. If so, then proteins and RNAs in the tri-snRNP must rearrange to present U4 and/or U6 tails to the helicase active site.

In the tri-snRNP, Brr2 sits on the Jab1 domain of Prp8, but its orientation and contacts change during activation of the particle. In contrast to the structure of the human tri-snRNP, in a structure of yeast tri-snRNP, a single-stranded region of U4 snRNA occupies the RNA-binding tunnel of Brr2^{73,80,81} (illustrated in [Figure 3](#)). Is Brr2 now poised to completely separate U4 snRNA from U6 snRNA? Does this separation occur before the tri-snRNP is recruited to the pre-spliceosome, or is this a paused state that requires further activation?

There is another competitive inhibitor of Brr2. Prp8's Jab1 domain has a C-terminal disordered tail that sneaks into the RNA tunnel of Brr2 to compete with U4⁸². The intramolecular plug interaction and Prp8 Jab1 cooperate to inhibit unwinding. Removing the Jab1 tail activates Brr2 helicase activity; Brr2 without its intramolecular plug also has enhanced activity⁷⁵. Do both inhibitors operate in the isolated tri-snRNP?

Brr2 remains in the spliceosome after U4 snRNP has been expelled from the spliceosome. It is seen in a structure of yeast-activated spliceosome, which is defined by the loss of U1 and U4 snRNP and rearrangements of the remaining snRNAs to interact with each other and pre-mRNA. A cryo-EM structure of activated yeast spliceosomes (B^{act}) shows Brr2 perched on Prp8's Jab1 domain⁷⁹, with its helicase activity blocked by both inhibitor interactions (PDB ID 5lqw). In an illustration from this structure, U2, U5, and U6 snRNAs are remote from Brr2 ([Figure 4](#)). Although not clear from the perspective of [Figure 4](#), Prp8 is entwined with other proteins and the snRNAs in this complex, even as it binds Brr2.

Figure 3. Two tri-small nuclear ribonucleoprotein particle (snRNP) structures trap different states of Brr2. **A.** Human tri-snRNP cryo-electron microscopy (cryo-EM) at 7 Å resolution⁷² shows Brr2 sitting on Prp8 (PDB ID 3jcr). A U4/U6 snRNA duplex is visible. Sm and Lsm rings are pink; other proteins are white. **B.** In a yeast tri-snRNP complex⁷⁰, (PDB ID 5GAN), U4 snRNA is threaded through Brr2 in the RNA-binding tunnel. These structures might correspond to the tri-snRNP in the nucleus (**A**) and the tri-snRNP poised for activation by Brr2 as it joins the pre-spliceosome (**B**). Visualized with visual molecular dynamics (VMD).

Figure 4. Yeast-activated (B^{act}) spliceosome⁷⁹ (PDB ID 5LQW; cryo-electron microscopy [cryo-EM] 5.8Å). Brr2 has separated U4 and U6 small nuclear RNAs (snRNAs), and U4 small nuclear ribonucleoprotein particle (snRNP) has been expelled from the spliceosome. Brr2 is bound to the Jab1 domain of Prp8. All 27 proteins are shown in surface representation; most are colored white. Visualized with visual molecular dynamics (VMD).

As the spliceosome progresses through its cycle, there are many short RNA duplexes that need to be unwound. The other seven SF2 RNA helicases are recruited to the spliceosome when they are needed, and then they dissociate. Brr2 remains with the spliceosome until it has completed a splicing cycle, but there are no data suggesting that it is active at any time other than in the conversion from pre-spliceosome to B^{act}. If it is not required for its helicase activity, perhaps its long NTR contributes something to splicing. Brr2 is reported to contribute to catalysis^{74,83}, to stabilize U5 and U6 in the spliceosome⁶⁸, and to assist in the final disruption of the spliceosome and release of ligated exons⁸⁴. If these states of the spliceosome could be trapped for structural studies, Brr2 might be captured in action.

The spliceosome is composed of hundreds of proteins⁵⁶, many of which simply bind RNA, but others actively remodel it. In the past year, spliceosome structures have revealed connections between RNA and proteins that explain previous observations but also raise new questions. This year, structures of the spliceosome C/C* complex show another helicase, prp16, at work on remodelling^{85–87}. Slowly, this RNA enzyme is giving up its secrets.

Conclusions

There is a need to not only understand specific RNPs but also define general rules of engagement, since RNA–protein

interactions dominate RNA biology. Indeed, the most mysterious are the membrane-less organelles that contain RNAs and proteins^{88,89}. These conglomerates of RNAs bound by RNA-binding proteins are variously thought to be centers of RNA processing, degradation, transcription, and exchange: P bodies and stress granules in the cytoplasm and nucleoli, Cajal bodies, speckles, and PML bodies in the nucleus. A current model is that disordered domains of the proteins form a fluid matrix that allows a flux of molecules through these liquid droplets^{90,91}. It is a sure bet that these droplets will be objects of intense scrutiny for years to come.

Competing interests

The author declares that she has no competing interests.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgements

I thank my reviewers for their thoughtful reading and perceptive comments.

References

- Gerstberger S, Hafner M, Tuschl T: **A census of human RNA-binding proteins.** *Nat Rev Genet.* 2014; **15**(12): 829–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Melé M, Mattioli K, Mallard W, *et al.*: **Chromatin environment, transcriptional regulation, and splicing distinguish lincRNAs and mRNAs.** *Genome Res.* 2017; **27**(1): 27–37.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Jensen KB, Darnell RB: **CLIP: crosslinking and immunoprecipitation of *in vivo* RNA targets of RNA-binding proteins.** *Methods Mol Biol.* 2008; **488**: 85–98.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ascano M, Hafner M, Cekan P, *et al.*: **Identification of RNA-protein interaction networks using PAR-CLIP.** *Wiley Interdiscip Rev RNA.* 2012; **3**(2): 159–77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Scheibe M, Butter F, Hafner M, *et al.*: **Quantitative mass spectrometry and PAR-CLIP to identify RNA-protein interactions.** *Nucleic Acids Res.* 2012; **40**(19): 9897–902.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Garzia A, Meyer C, Morozov P, *et al.*: **Optimization of PAR-CLIP for transcriptome-wide identification of binding sites of RNA-binding proteins.** *Methods.* 2016. pii: S1046-2023(16)30384-X.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Munschauer M, Schueler M, Dieterich C, *et al.*: **High-resolution profiling of protein occupancy on polyadenylated RNA transcripts.** *Methods.* 2014; **65**(3): 302–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Huppertz I, Attig J, D'Ambrogio A, *et al.*: **ICLIP: protein-RNA interactions at nucleotide resolution.** *Methods.* 2014; **65**(3): 274–87.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- McHugh CA, Russell P, Guttman M: **Methods for comprehensive experimental identification of RNA-protein interactions.** *Genome Biol.* 2014; **15**(1): 203.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Minajigi A, Froberg JE, Wei C, *et al.*: **Chromosomes. A comprehensive Xist interactome reveals cohesin repulsion and an RNA-directed chromosome conformation.** *Science.* 2015; **349**(6245): pii: aab2276.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Galupa R, Heard E: **X-chromosome inactivation: new insights into *cis* and *trans* regulation.** *Curr Opin Genet Dev.* 2015; **31**: 57–66.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Chen CK, Blanco M, Jackson C, *et al.*: **Xist recruits the X chromosome to the nuclear lamina to enable chromosome-wide silencing.** *Science.* 2016; **354**(6311): 468–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
- Engreitz JM, Pandya-Jones A, McDonel P, *et al.*: **The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome.** *Science.* 2013; **341**(6147): 1237973.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Chu C, Zhang QC, da Rocha ST, *et al.*: **Systematic discovery of Xist RNA binding proteins.** *Cell.* 2015; **161**(2): 404–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Görlach M, Burd CG, Portman DS, *et al.*: **The hnRNP proteins.** *Mol Biol Rep.* 1993; **18**(2): 73–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Geuens T, Bouhy D, Timmerman V: **The hnRNP family: insights into their role in health and disease.** *Hum Genet.* 2016; **135**(8): 851–67.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Busch A, Hertel KJ: **Evolution of SR protein and hnRNP splicing regulatory factors.** *Wiley Interdiscip Rev RNA.* 2012; **3**(1): 1–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Jean-Philippe J, Paz S, Caputi M: **hnRNP A1: the Swiss army knife of gene expression.** *Int J Mol Sci.* 2013; **14**(9): 18999–9024.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Keppetipola N, Sharma S, Li Q, *et al.*: **Neuronal regulation of pre-mRNA splicing by polypyrimidine tract binding proteins, PTBP1 and PTBP2.** *Crit Rev Biochem Mol Biol.* 2012; **47**(4): 360–78.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kafasla P, Morgner N, Robinson CV, *et al.*: **Polypyrimidine tract-binding protein stimulates the poliovirus IRES by modulating eIF4G binding.** *EMBO J.* 2010; **29**(21): 3710–22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sawicka K, Bushell M, Spriggs KA, *et al.*: **Polypyrimidine-tract-binding protein: a**

- multifunctional RNA-binding protein.** *Biochem Soc Trans.* 2008; **36**(Pt 4): 641–7. [PubMed Abstract](#) | [Publisher Full Text](#)
22. Sakaguchi T, Hasegawa Y, Brockdorff N, *et al.*: **Control of Chromosomal Localization of Xist by hnRNP U Family Molecules.** *Dev Cell.* 2016; **39**(1): 11–2. [PubMed Abstract](#) | [Publisher Full Text](#)
 23. **F** McHugh CA, Chen CK, Chow A, *et al.*: **The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3.** *Nature.* 2015; **521**(7551): 232–6. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 24. Colley SM, Leedman PJ: **SRA and its binding partners: an expanding role for RNA-binding coregulators in nuclear receptor-mediated gene regulation.** *Crit Rev Biochem Mol Biol.* 2009; **44**(1): 25–33. [PubMed Abstract](#) | [Publisher Full Text](#)
 25. Shi Y, Downes M, Xie W, *et al.*: **Sharp, an inducible cofactor that integrates nuclear receptor repression and activation.** *Genes Dev.* 2001; **15**(9): 1140–51. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 26. Gregoret IV, Lee YM, Goodson HV: **Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis.** *J Mol Biol.* 2004; **338**(1): 17–31. [PubMed Abstract](#) | [Publisher Full Text](#)
 27. Tessarz P, Kouzarides T: **Histone core modifications regulating nucleosome structure and dynamics.** *Nat Rev Mol Cell Biol.* 2014; **15**(11): 703–8. [PubMed Abstract](#) | [Publisher Full Text](#)
 28. Mackereth CD, Sattler M: **Dynamics in multi-domain protein recognition of RNA.** *Curr Opin Struct Biol.* 2012; **22**(3): 287–96. [PubMed Abstract](#) | [Publisher Full Text](#)
 29. Safaei N, Kozlov G, Noronha AM, *et al.*: **Interdomain allostery promotes assembly of the poly(A) mRNA complex with PABP and eIF4G.** *Mol Cell.* 2012; **48**(3): 375–86. [PubMed Abstract](#) | [Publisher Full Text](#)
 30. Maynard CM, Hall KB: **Interactions between PTB RRM domains induce slow motions and increase RNA binding affinity.** *J Mol Biol.* 2010; **397**(1): 260–77. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 31. Clerte C, Hall KB: **The domains of polypyrimidine tract binding protein have distinct RNA structural preferences.** *Biochemistry.* 2009; **48**(10): 2063–74. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 32. Ruskin B, Zamore PD, Green MR: **A factor, U2AF, is required for U2 snRNP binding and splicing complex assembly.** *Cell.* 1988; **52**(2): 207–19. [PubMed Abstract](#) | [Publisher Full Text](#)
 33. Zamore PD, Green MR: **Identification, purification, and biochemical characterization of U2 small nuclear ribonucleoprotein auxiliary factor.** *Proc Natl Acad Sci U S A.* 1989; **86**(23): 9243–7. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 34. Shao C, Yang B, Wu T, *et al.*: **Mechanisms for U2AF to define 3' splice sites and regulate alternative splicing in the human genome.** *Nat Struct Mol Biol.* 2014; **21**(11): 997–1005. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 35. **F** Voith von Voithenberg L, Sánchez-Rico C, Kang HS, *et al.*: **Recognition of the 3' splice site RNA by the U2AF heterodimer involves a dynamic population shift.** *Proc Natl Acad Sci U S A.* 2016; **113**(46): E7169–E7175. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 36. **F** Agrawal AA, Salsi E, Chatrikhi R, *et al.*: **An extended U2AF(65)-RNA-binding domain recognizes the 3' splice site signal.** *Nat Commun.* 2016; **7**: 10950. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 37. Zorio DA, Blumenthal T: **Both subunits of U2AF recognize the 3' splice site in *Caenorhabditis elegans*.** *Nature.* 1999; **402**(6763): 835–8. [PubMed Abstract](#) | [Publisher Full Text](#)
 38. Wu S, Romfo CM, Nilsen TW, *et al.*: **Functional recognition of the 3' splice site AG by the splicing factor U2AF35.** *Nature.* 1999; **402**(6763): 832–5. [PubMed Abstract](#) | [Publisher Full Text](#)
 39. **F** Loerch S, Kielkopf CL: **Unmasking the U2AF homology motif family: a bona fide protein-protein interaction motif in disguise.** *RNA.* 2016; **22**(12): 1795–807. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 40. Kielkopf CL, Lucke S, Green MR: **U2AF homology motifs: protein recognition in the RRM world.** *Genes Dev.* 2004; **18**(13): 1513–26. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 41. Jenkins JL, Laird KM, Kielkopf CL: **A Broad range of conformations contribute to the solution ensemble of the essential splicing factor U2AF(65).** *Biochemistry.* 2012; **51**(26): 5223–5. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 42. Chen L, Weinmeister R, Kralovicova J, *et al.*: **Stoichiometries of U2AF35, U2AF65 and U2 snRNP reveal new early spliceosome assembly pathways.** *Nucleic Acids Res.* 2017; **45**(4): 2051–2067. [PubMed Abstract](#) | [Publisher Full Text](#)
 43. **F** Selenko P, Gregorovic G, Sprangers R, *et al.*: **Structural basis for the molecular recognition between human splicing factors U2AF65 and SF1/mBBP.** *Mol Cell.* 2003; **11**(4): 965–76. [PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 44. Zhang Y, Madl T, Bagdiul I, *et al.*: **Structure, phosphorylation and U2AF65 binding of the N-terminal domain of splicing factor 1 during 3'-splice site recognition.** *Nucleic Acids Res.* 2013; **41**(2): 1343–54. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 45. Corsini L, Hothorn M, Stier G, *et al.*: **Dimerization and protein binding specificity of the U2AF homology motif of the splicing factor Puf60.** *J Biol Chem.* 2009; **284**(1): 630–9. [PubMed Abstract](#) | [Publisher Full Text](#)
 46. Koshland DE Jr, Nemethy G, Filmer D: **Comparison of experimental binding data and theoretical models in proteins containing subunits.** *Biochemistry.* 1966; **5**(1): 365–85. [PubMed Abstract](#) | [Publisher Full Text](#)
 47. Cooper A, Dryden DT: **Allostery without conformational change. A plausible model.** *Eur Biophys J.* 1984; **11**(2): 103–9. [PubMed Abstract](#) | [Publisher Full Text](#)
 48. Motlagh HN, Wrabl JO, Li J, *et al.*: **The ensemble nature of allostery.** *Nature.* 2014; **508**(7496): 331–9. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 49. Williams SG, Hall KB: **Linkage and allostery in snRNP protein/RNA complexes.** *Biochemistry.* 2014; **53**(22): 3529–39. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 50. Hennig J, Militti C, Popowicz GM, *et al.*: **Structural basis for the assembly of the Sxl-Unr translation regulatory complex.** *Nature.* 2014; **515**(7526): 287–90. [PubMed Abstract](#) | [Publisher Full Text](#)
 51. **F** Serrano P, Aubol BE, Keshwani MM, *et al.*: **Directional Phosphorylation and Nuclear Transport of the Splicing Factor SRSF1 Is Regulated by an RNA Recognition Motif.** *J Mol Biol.* 2016; **428**(11): 2430–45. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 52. Cléry A, Sinha R, Anczuków O, *et al.*: **Isolated pseudo-RNA-recognition motifs of SR proteins can regulate splicing using a noncanonical mode of RNA recognition.** *Proc Natl Acad Sci U S A.* 2013; **110**(30): E2802–11. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 53. Pan Q, Shai O, Lee LJ, *et al.*: **Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing.** *Nat Genet.* 2008; **40**(12): 1413–5. [PubMed Abstract](#) | [Publisher Full Text](#)
 54. **F** Wang ET, Sandberg R, Luo S, *et al.*: **Alternative isoform regulation in human tissue transcriptomes.** *Nature.* 2008; **456**(7221): 470–6. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 55. Nilsen TW, Graveley BR: **Expansion of the eukaryotic proteome by alternative splicing.** *Nature.* 2010; **463**(7280): 457–63. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 56. Will CL, Lührmann R: **Spliceosome structure and function.** *Cold Spring Harb Perspect Biol.* 2011; **3**(7): pii: a003707. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 57. Wahl MC, Lührmann R: **SnapShot: Spliceosome Dynamics III.** *Cell.* 2015; **162**(3): 690–690.e1. [PubMed Abstract](#) | [Publisher Full Text](#)
 58. Wahl MC, Lührmann R: **SnapShot: Spliceosome Dynamics I.** *Cell.* 2015; **161**(6): 1474–e1. [PubMed Abstract](#) | [Publisher Full Text](#)
 59. Nguyen TH, Galej WP, Fica SM, *et al.*: **CryoEM structures of two spliceosomal complexes: starter and dessert at the spliceosome feast.** *Curr Opin Struct Biol.* 2016; **36**: 48–57. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 60. Wahl MC, Will CL, Lührmann R: **The spliceosome: design principles of a dynamic RNP machine.** *Cell.* 2009; **136**(4): 701–18. [PubMed Abstract](#) | [Publisher Full Text](#)
 61. Raghunathan PL, Guthrie C: **RNA unwinding in U4/U6 snRNPs requires ATP hydrolysis and the DEIH-box splicing factor Brr2.** *Curr Biol.* 1998; **8**(15): 847–55. [PubMed Abstract](#) | [Publisher Full Text](#)
 62. **F** De I, Schmitzova J, Pena V: **The organization and contribution of helicases to RNA splicing.** *Wiley Interdiscip Rev RNA.* 2016; **7**(2): 259–74. [PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
 63. **F** Semlow DR, Blanco MR, Walter NG, *et al.*: **Spliceosomal DEAH-Box ATPases Remodel Pre-mRNA to Activate Alternative Splice Sites.** *Cell.* 2016; **164**(5): 985–98. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 64. **F** Absmeier E, Santos KF, Wahl MC: **Functions and regulation of the Brr2 RNA helicase during splicing.** *Cell Cycle.* 2016; **15**(24): 3362–77. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 65. **F** Absmeier E, Wollenhaupt J, Mozaffari-Jovin S, *et al.*: **The large N-terminal region of the Brr2 RNA helicase guides productive spliceosome activation.** *Genes Dev.* 2015; **29**(24): 2576–87. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 66. **F** Zhang L, Li X, Hill RC, *et al.*: **Brr2 plays a role in spliceosomal activation in addition to U4/U6 unwinding.** *Nucleic Acids Res.* 2015; **43**(6): 3286–97. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
 67. **F** Theuser M, Hobartner C, Wahl MC, *et al.*: **Substrate-assisted mechanism of RNP disruption by the spliceosomal Brr2 RNA helicase.** *Proc Natl Acad Sci U S A.*

- 2016; **113**(28): 7798–803.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
68. Nguyen TH, Li J, Galej WP, *et al.*: **Structural basis of Brr2-Prp8 interactions and implications for U5 snRNP biogenesis and the spliceosome active site.** *Structure*. 2013; **21**(6): 910–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 69. Santos KF, Jovin SM, Weber G, *et al.*: **Structural basis for functional cooperation between tandem helicase cassettes in Brr2-mediated remodeling of the spliceosome.** *Proc Natl Acad Sci U S A*. 2012; **109**(43): 17418–23.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 70. Nguyen TH, Galej WP, Bai XC, *et al.*: **Cryo-EM structure of the yeast U4/U6.U5 tri-snRNP at 3.7 Å resolution.** *Nature*. 2016; **530**(7590): 298–302.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 71. Nguyen TH, Galej WP, Bai XC, *et al.*: **The architecture of the spliceosomal U4/U6.U5 tri-snRNP.** *Nature*. 2015; **523**(7558): 47–52.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 72. Agafonov DE, Kastner B, Dybkov O, *et al.*: **Molecular architecture of the human U4/U6.U5 tri-snRNP.** *Science*. 2016; **351**(6280): 1416–20.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 73. Cornilescu G, Didychuk AL, Rodgers ML, *et al.*: **Structural Analysis of Multi-Helical RNAs by NMR-SAXS/WAXS: Application to the U4/U6 di-snRNA.** *J Mol Biol*. 2016; **428**(5 Pt A): 777–89.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 74. Hahn D, Kudla G, Tollervey D, *et al.*: **Brr2p-mediated conformational rearrangements in the spliceosome during activation and substrate repositioning.** *Genes Dev*. 2012; **26**(21): 2408–21.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 75. Absmeier E, Becke C, Wollenhaupt J, *et al.*: **Interplay of *cis*- and *trans*-regulatory mechanisms in the spliceosomal RNA helicase Brr2.** *Cell Cycle*. 2017; **16**(1): 100–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 76. Weber G, Trowitzsch S, Kastner B, *et al.*: **Functional organization of the Sm core in the crystal structure of human U1 snRNP.** *EMBO J*. 2010; **29**(24): 4172–84.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 77. Pomeranz Krummel DA, Oubridge C, Leung AK, *et al.*: **Crystal structure of human spliceosomal U1 snRNP at 5.5 Å resolution.** *Nature*. 2009; **458**(7237): 475–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 78. Galej WP, Wilkinson ME, Fica SM, *et al.*: **Cryo-EM structure of the spliceosome immediately after branching.** *Nature*. 2016; **537**(7619): 197–201.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 79. Rauhut R, Fabrizio P, Dybkov O, *et al.*: **Molecular architecture of the *Saccharomyces cerevisiae* activated spliceosome.** *Science*. 2016; **353**(6306): 1399–405.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 80. Mozaffari-Jovin S, Santos KF, Hsiao HH, *et al.*: **The Prp8 RNase H-like domain inhibits Brr2-mediated U4/U6 snRNA unwinding by blocking Brr2 loading onto the U4 snRNA.** *Genes Dev*. 2012; **26**(21): 2422–34.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 81. Boesler C, Rigo N, Agafonov DE, *et al.*: **Stable tri-snRNP integration is accompanied by a major structural rearrangement of the spliceosome that is dependent on Prp8 interaction with the 5' splice site.** *RNA*. 2015; **21**(11): 1993–2005.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 82. Mozaffari-Jovin S, Wandersleben T, Santos KF, *et al.*: **Inhibition of RNA helicase Brr2 by the C-terminal tail of the spliceosomal protein Prp8.** *Science*. 2013; **341**(6141): 80–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
 83. Cordin O, Hahn D, Alexander R, *et al.*: **Brr2p carboxy-terminal Sec63 domain regulates spliceosome dynamics mediated by Brr2p, a DExD/H box ATPase.** *Mol Cell*. 2006; **23**(3): 389–99.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 84. Small EC, Leggett SR, Winans AA, *et al.*: **The EF-G-like GTPase Snu114p regulates spliceosome dynamics mediated by Brr2p, a DExD/H box ATPase.** *Mol Cell*. 2006; **23**(3): 389–99.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 85. Yan C, Wan R, Bai R, *et al.*: **Structure of a yeast step II catalytically activated spliceosome.** *Science*. 2017; **355**(6321): 149–155.
[PubMed Abstract](#) | [Publisher Full Text](#)
 86. Fica SM, Oubridge C, Galej WP, *et al.*: **Structure of a spliceosome remodelled for exon ligation.** *Nature*. 2017; **542**(7641): 377–380.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 87. Bertram K, Agafonov DE, Liu WT, *et al.*: **Cryo-EM structure of a human spliceosome activated for step 2 of splicing.** *Nature*. 2017; **542**(7641): 318–323.
[PubMed Abstract](#) | [Publisher Full Text](#)
 88. Banani SF, Rice AM, Peeples WB, *et al.*: **Compositional Control of Phase-Separated Cellular Bodies.** *Cell*. 2016; **166**(3): 651–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 89. Zhu L, Brangwynne CP: **Nuclear bodies: the emerging biophysics of nucleoplasmic phases.** *Curr Opin Cell Biol*. 2015; **34**: 23–30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 90. Nott TJ, Petsalaki E, Farber P, *et al.*: **Phase transition of a disordered nuage protein generates environmentally responsive membraneless organelles.** *Mol Cell*. 2015; **57**(5): 936–47.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 91. Zhang H, Elbaum-Garfinkle S, Langdon EM, *et al.*: **RNA Controls PolyQ Protein Phase Transitions.** *Mol Cell*. 2015; **60**(2): 220–30.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 92. Nagai K, Oubridge C, Jessen TH, *et al.*: **Crystal structure of the RNA-binding domain of the U1 small nuclear ribonucleoprotein A.** *Nature*. 1990; **348**(6301): 515–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
 93. Oubridge C, Ito N, Evans PR, *et al.*: **Crystal structure at 1.92 Å resolution of the RNA-binding domain of the U1A spliceosomal protein complexed with an RNA hairpin.** *Nature*. 1994; **372**(6505): 432–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 94. Hoffman DW, Query CC, Golden BL, *et al.*: **RNA-binding domain of the A protein component of the U1 small nuclear ribonucleoprotein analyzed by NMR spectroscopy is structurally similar to ribosomal proteins.** *Proc Natl Acad Sci U S A*. 1991; **88**(6): 2495–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 95. Birney E, Kumar S, Krainer AR: **Analysis of the RNA-recognition motif and RS and RGG domains: conservation in metazoan pre-mRNA splicing factors.** *Nucleic Acids Res*. 1993; **21**(25): 5803–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:

Editorial Note on the Review Process

Faculty Reviews are review articles written by the prestigious Members of **Faculty Opinions**. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

Version 1

- Shinichi Nakagawa**
RNA Biology Laboratory, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan
Competing Interests: No competing interests were disclosed.
- Markus Wahl**
Department of Biology, Chemistry, Pharmacy, Freie Universität Berlin, Berlin, Germany
Competing Interests: No competing interests were disclosed.
- Xiang-Dong Fu**
Department of Cellular & Molecular Medicine, University of California San Diego, San Diego, CA, USA
Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research