Review article

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The nexus between RNA-binding proteins and their effectors

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Abstract

RNA-binding proteins (RBPs) regulate essentially every event in the lifetime of an RNA molecule, from its production to its destruction. Whereas much has been learned about RNA sequence specificity and general functions of individual RBPs, the ways in which numerous RBPs instruct a much smaller number of effector molecules, that is, the core engines of RNA processing, as to where, when and how to act remain largely speculative. Here, we survey the known modes of communication between RBPs and their effectors with a particular focus on converging RBP–effector interactions and their roles in reducing the complexity of RNA networks. We discern the emerging unifying principles and discuss their utility in our understanding of RBP function, regulation of biological processes and contribution to human disease.

Sections

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Introduction

The regulation of gene expression at the RNA level is fundamental to essentially all biological processes. Its complexity presents one of the most formidable challenges in molecular and systems biology[1]. Human cells must ensure correct processing of each of their more than 20,000 different protein-coding RNAs, many more if one considers alternative isoforms[2,3]. These transcripts can range in level from fewer than 0.1 copies to thousands of copies per cell[4,5]. Moreover, each transcript must be processed at the right time, by the right processing molecule and at the right subcellular location, often in a manner that is specific to a particular cell type or conditions in the microenvironment[1]. There is little room for error, as RNA misprocessing can lead to disrupted cell homeostasis or cell death, as documented experimentally and by cases of human disease[3,6,7].

The scale of regulatory complexity began to emerge with the inception of next-generation DNA sequencing in the 2000s[8], which allowed RNA processing to be studied from a systems perspective. Since then, a multitude of transcriptome-wide approaches combining high-throughput sequencing with genetic perturbation, biochemistry and mass spectrometry-based approaches have transformed studies of RNA biology. In addition to allowing for the inference of RNA regulatory networks, their principal goal has been to decipher the functions of RNA-binding proteins (RBPs) as the core regulators of RNA processing[9–12]. Indeed, RNA-centric and RBP-centric systems-wide studies that entail methods based on RNA immunoprecipitation (RIP), crosslinking and immunoprecipitation (CLIP) and its many variants, RNA interactome capture, RNA antisense purification coupled with mass spectrometry (RAP–MS) and other related strategies jointly identified more than 1,500 human RBPs and characterized RNA binding preferences of several hundred of them in vitro and/or in vivo[9–12].

Together with loss-of-function analyses and other functional assays, these studies have elucidated some of the basic rules of RNA regulation for numerous RBPs. The above 'exponential technologies', however, also created a rift in the field between the rapid cataloguing of protein–RNA interactions, along with their largely correlative functional links, and a lagging understanding of the mechanisms of RBP activities. These activities commonly rely on interactions of RBPs with their effector molecules, that is, proteins or protein complexes that serve as core engines of RNA processing and thus define the activities of many RBPs.

For the purpose of this Review, we designate as 'effectors' or 'effector assemblies' those molecular entities that operate as executors of RNA processing or otherwise directly affect RNA processing but are not classified as RBPs. We define as RBPs those proteins that bind RNA in a sequence- or structure-specific manner and are not basal or auxiliary components of effector assemblies, to avoid potential confusion when referring to RBP–effector interactions. For instance, TIA1, a sequence-specific RBP, interacts with the spliceosome, an effector, to regulate alternative pre-mRNA splicing[13,14]. Furthermore, we consider RBP–effector interactions as 'converging' if there exists experimental evidence for the capacity of at least two different RBPs in a species to separately connect to the same effector of RNA processing. For example, the RBPs ZFP36 (also known as TTP) and Roquin-1 (also known as RC3H1) can both interact with the effector CCR4–NOT to regulate mRNA stability[15–18].

Like RBPs, effectors have crucial roles in essentially all stages of RNA processing; however, unlike RBPs, they and their interactions with RBPs have primarily been studied using reductionist approaches, including X-ray crystallography, in vitro studies of protein–protein interactions (PPIs) and RNA processing, RNA-tethering assays and targeted genetic experiments[17,19–22]. Studies at the nexus between RBPs and their effectors have also been outpaced by the recent resurgence of interest in the formation of biomolecular condensates, which have begun to shed light on entirely different levels of spatiotemporal control of RNA processing[23–26]. Together, these developments have propelled our understanding of RNA networks at two discrete levels of the regulatory hierarchy, while leaving behind the intervening level at which RBPs communicate with effectors of RNA processing. This disconnection has arguably hampered our understanding of RBP function as well as the overall operation of RNA networks, which recent studies are now beginning to address.

Here, we focus on the nexus between RBPs and their effectors, with a particular emphasis on its emerging roles in the control of RNA processing, regulation of biological outcomes, and its contribution to human health and disease. We first define the function of the nexus in managing the complexity of modern RNA networks, then discern the unifying features of RBP–effector interactions along with their physiological roles using illustrative examples. We then consider how cellular processes regulate and are regulated at the nexus, followed by a discussion of genetic disorders that affect, and therapeutic opportunities that emerge at, the RBP–effector interface.

The complexity of RNA networks

The evolution of progressively more complex RNA networks mandated solutions to the increasingly more challenging regulation and need for flexibility[1]. Such solutions are built into many modern RNA networks in the form of distinct molecular and systems-level features[23,27–29] (Fig. 1). These traits facilitate the regulation of RNA networks by minimizing the need for complexity, including the number of required connections, while also improving network performance and speed of adaptation to the environment.

The associations between RBPs and their effectors have a central role in the regulatory hierarchy of RNA networks by unifying the fates of multiple RBP–RNA modules and serving as a functional bridge to the formation of larger, high-concentration ribonucleoprotein (RNP) assemblies, which confer a higher level of spatiotemporal control[23,30] (Fig 1). At the RBP–effector nexus, the effectors often end up being recruited to RNA by RBPs, either directly or indirectly, and act in cis by processing the RBP-bound RNA, although there also exist other modes of RBP-dependent effector engagement with RNA that may have a substantially different impact on RNA processing (Fig. 2).

Akin to the capacity of a typical RBP to bind and regulate the fates of different RNA molecules, a typical effector of RNA processing can bind to different RBPs and subdue their bound RNAs to a common processing event. In this manner, effectors handle essentially all common RNA processing events, including the multiple steps of RNA biogenesis, modification, transport, storage, decay and, in the case of coding RNAs, translation.
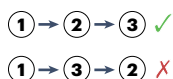
Because a large number of RBPs must converge onto a smaller number of effectors, one might expect different RBPs to use a similar strategy to contact a particular effector. Contacts between RBPs and their effectors must secure specificity to couple the right RBP–RNA modules with the right RNA processing events and be transient enough to enable a rapid reuse of both the RBP and the effector. RBP–effector interactions thus require a compromise between highly specific, high-affinity contacts, such as those that mediate the domain–domain interactions that commonly establish macromolecular complexes[31], and weaker, less-specific interactions, such as those implicated in the formation of RNP condensates[23].
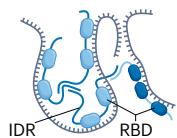
A Traits of RNA networks
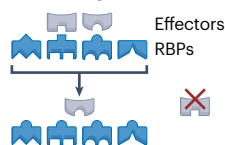
Aa RNA sequence specificity
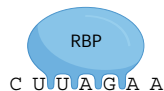
Ab Relayed RNA processing

Ac Condensate formation

IDR   RBD

Ad Convergent evolution

Effectors
RBPs

Ae Hierarchical organization

B Organization of RNA networks

Phenotype 3   Phenotype 1   Phenotype 2

Level X

Level 4   Condensate 4

Condensate 3   Condensate 2

Condensate 1

Level 3

Level 2   Effector 1

Level 1   RBP 1   RBP 2   RBP 3

RNA

Increasing valency of interactions
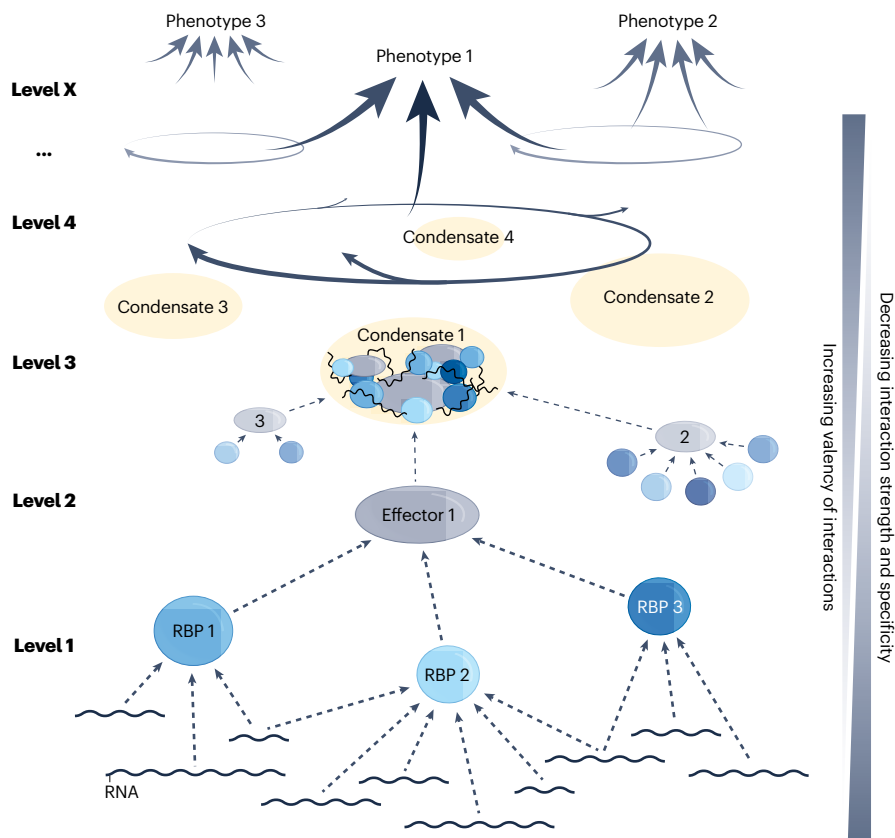
Decreasing interaction strength and specificity

Fig. 1 | Traits and organization of RNA networks associated with the management of regulatory complexity. A, Traits of RNA networks. **Aa**, The recognition of specific RNA sequences and/or structures allows *trans*-acting factors, including RNA-binding proteins (RBPs) and small RNAs, such as microRNAs (miRNAs), to act upon some but not other transcripts. This trait also allows for synchronized processing of multiple, often functionally related transcripts[184]. Recognition of specific RNA features defines the most elementary regulatory level of RNA networks that determines which transcripts will be controlled by which *trans*-acting factors. Different RNA specificities of different RBPs considerably simplify the challenge of coordinated regulation, in addition to allowing for network adaptation through rewiring of RNA targets[28,184]. **Ab**, Relayed RNA processing refers to the correct sequence of post-transcriptional processing events. For instance, a typical pipeline would ensure that RNA splicing occurs prior to RNA export and localization to a distal intracellular region, followed by localized translation and that RNA degradation occurs last[29]. Any other sequence of events could be detrimental to cell homeostasis. General molecular and cellular organization, e.g. association of the splicing machinery with RNA polymerase II (Pol II) and separation of nuclear from cytoplasmic components, respectively, as well as more specific molecular interactions contribute to the correct relay of RNA processing events[29,185]. Correct and rapid relay of RNA processing events secures directness and energy-efficient regulation of RNA processing. **Ac**, The formation of ribonucleoprotein (RNP) condensates increases local concentration of RBP–RNA modules along with their effector complexes. RNP condensation is driven primarily by intrinsically disordered regions (IDRs) of different RBP molecules, which multimerize through numerous weak, non-specific interactions, and is aided by transient secondary structures formed within IDRs as well as contributions from the associated RNA[23]. RNP condensation can stabilize the association of individual RBPs with their recognition motifs on RNA and contribute to correct RNA folding that may be required for processing[40,186]. In addition, increased local concentration of effector proteins and RNA can increase the rate of biochemical reactions, or assist in storage or transport of these molecules[23]. Upon extensive RNP condensation, a physicochemical phenomenon of liquid–liquid phase separation occurs through which various types of RNA granules, including stress granules, P-bodies, splicing speckles, neuronal granules, and others, are generated. Such granules, also referred to as 'membraneless organelles', exist in liquid-like and occasionally solid-like physical states that exhibit distinct physiological roles[23,30]. Mechanisms that govern the formation and dissolution of RNP condensates include membrane surfaces, molecular chaperones, including nuclear import receptors, RNA helicases, and post-translational modifications of condensate components[54,187]. **Ad**, Convergent molecular evolution has an important role at different levels of RNA processing and contributes to the hierarchical structure of RNA networks. At the RBP–RNA level, convergence has been observed in RNA-targeting specificities of RBPs and in the evolutionary adaptation of RNA molecules to a particular mode of post-transcriptional processing, including alternative splicing and mRNA decay[28,188,189]. Convergent evolution also occurs at the level of RBPs interacting with their effector molecules. Short linear motifs (SLiMs), which are typically located in IDRs of RBPs or adaptor proteins, are specifically recognized by cognate domains of effectors and frequently evolve in a convergent manner[93,190]. These examples point to a broad potential of convergent evolution to unify the fates of different transcripts by a common regulatory step. **Ae**, Hierarchical organization is commonly observed in biological networks and is thought to evolve due to the high cost associated with creation and maintenance of network connections[27]. **B**, Hierarchically wired networks, including RNA networks, not only exhibit fewer connections, but also adapt faster to the environment and show higher overall performance compared to non-hierarchical networks[27]. RNA networks show several hierarchical regulatory levels, with control at higher levels having broader effects on RNA processing. RBD, RNA-binding domain.

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There is currently little consensus on the nature of interactions between RBPs and their effectors. Consequently, there is limited knowledge about how these interactions enable RBPs to instruct different types of RNA processing, how they may be regulated or how they lead to human disease when misregulated. Below we draw together key aspects of how RBPs directly or indirectly interact with effector assemblies and discuss the importance of these interactions for RNA processing (Figs. 3, 4 and Supplementary Table 1). We review specific examples of RBP–effector interactions that have been characterized at both the molecular and functional levels with an aim to highlight effectors as points of convergence for diverse RBPs and their bound RNA. We gather these findings in a series of vignettes, each specific to a particular effector and its physiological role.

The nexus in and around the nucleus

RBPs and positive transcription elongation factor b. Research of RBPs has traditionally focused on their post-transcriptional activities; however, it is becoming increasingly clear that RBPs also have a role in regulating gene transcription itself[32]. One notable example involving converging RBP–effector interactions is transcriptional control of paused genes. Promoter-proximal RNA polymerase II (Pol II) pausing has emerged as a widespread mechanism of transcriptional regulation, affecting approximately 30% of metazoan genes and enabling rapid transcriptional responses to activation signals[33]. Release of paused Pol II into productive transcriptional elongation can be triggered by the kinase activity of the multiprotein complex positive transcription elongation factor b (P-TEFb) upon its delivery to paused genes[34] (Fig. 3a). A viral RBP, called Tat, and a few cellular RBPs have been found to stimulate transcription of specific genes by recruiting P-TEFb to paused Pol II while bound to nascent RNA in a sequence- and/or structure-specific manner[34–38]. It is presently unclear whether the cellular RBPs recruit P-TEFb via direct PPIs, akin to the viral Tat protein, or indirectly increase the pool of locally available P-TEFb by liberating it from the promoter-proximal 7SK RNP complex (Fig. 3a).

RBPs and the spliceosome. A large proportion of multi-exon genes in higher eukaryotes (more than 95% in humans) undergoes alternative splicing, a process that is crucial for generating proteomic diversity and is thought to sustain speciation and phenotypic complexity[39]. Sequence-specific RBPs regulate alternative splicing by modulating the activity of the spliceosome in the vicinity of their targeted loci on pre-mRNA[2]. A notable type of RBP is SR proteins, which stimulate splice site selection by locally stabilizing the 'early' components of the spliceosome through PPIs between arginine- and serine-rich intrinsically disordered regions (IDRs), also called RS domains, present in both the SR proteins and the spliceosome[21,40] (Figs. 2b,3b, Table 1 and Supplementary Table 2). Such bonding is strengthened by the observed phosphorylation of the RS domains[41], which sensitizes alternative splicing to signal transduction pathways and presents an important therapeutic opportunity, as discussed below. Regulatory strategies that rely on spliceosome stabilization are also employed by other RBPs with characterized interactions that use short stretches of IDRs to contact various protein subunits of the early spliceosomal components (Fig. 3b and Table 1). FUS, the splicing activity of which relies on an interaction with spliceosomal RNA rather than a protein subunit, represents an apparent exception to this rule[42].

RBPs and survival motor neuron protein. Survival motor neuron protein (SMN) is an essential effector protein the canonical role of which

is in the assembly of nuclear RNPs. This protein is notorious for its reduced function causing spinal muscular atrophy (SMA), a fatal autosomal recessive disorder characterized by degeneration of lower motor neurons[43,44]. Although the reason for the specific manifestation of
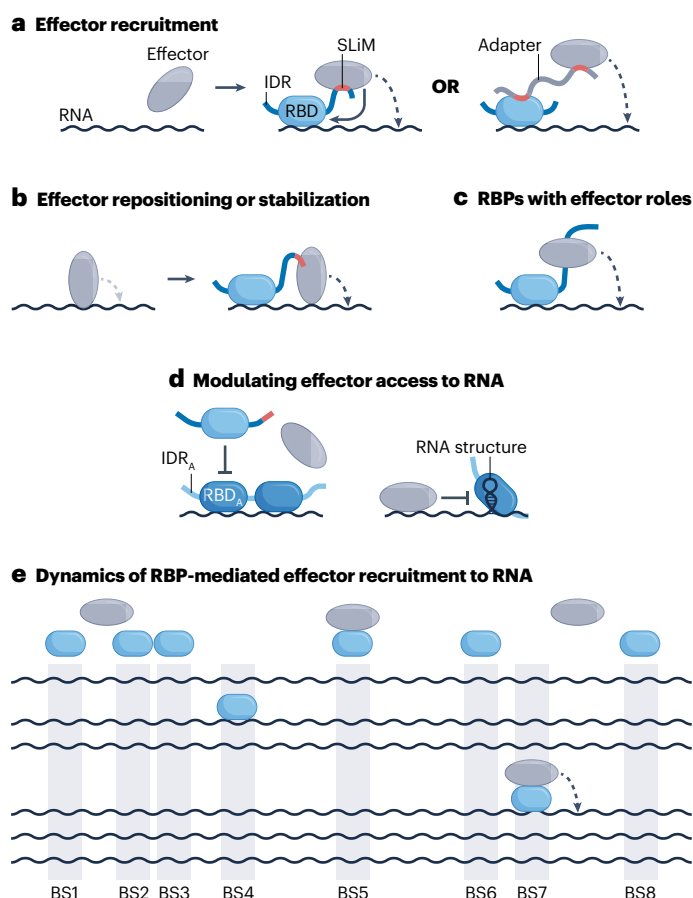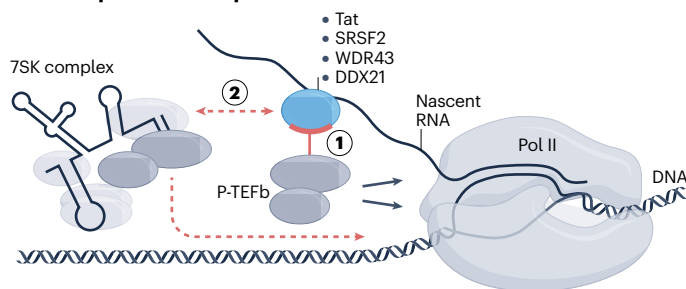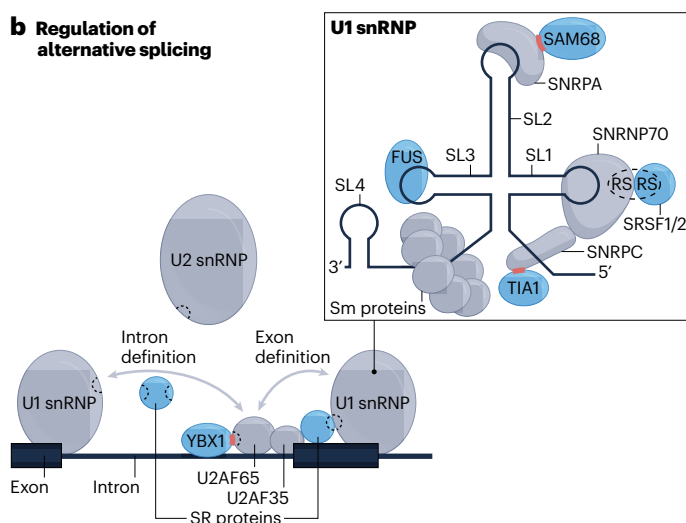

Fig. 2 | Modes and dynamics of RBP-dependent effector engagement with RNA. a, RNA-binding proteins (RBPs) (blue shapes) can recruit effectors to target RNA via direct or indirect protein–protein interactions (PPIs) that typically entail a short linear motif (SLiM; red), which typically resides in an intrinsically disordered region (IDR) of the RBP or an adaptor protein, and a structured domain located in the effector. Indirect interactions can involve additional proteins or can be mediated by non-coding RNAs (not shown). Upon recruitment, the effector can exert activity in *cis*, that is, on the RBP-bound RNA (dashed curved arrow) and occasionally also on the recruiting RBP (solid curved arrow), or in *trans*, that is, on other molecules (not shown). **b,** Instead of serving a recruiting role, some RBP–effector interactions may facilitate repositioning or stabilization of a pre-bound effector to modulate its activity[137]. **c,d,** Certain activities of RBPs do not entail contacts with effector molecules, either because RBPs themselves operate as effectors, as is the case for RBPs with enzymatic activities[11,191–194] (panel **c**), or because they operate by modulating effector access to RNA, such as heterogeneous nuclear ribonucleoproteins (hnRNPs) in regulation of splicing[40] (panel **d**). **e,** RBP-mediated recruitment of an effector to RNA is transient and occurs infrequently. Shown is a hypothetical steady-state scenario in which copies of an RBP (blue ovals), the number of which matches the number of RBP-binding sites (BS1–BS8) on RNA (black wavy lines), compete for a limiting number of available effector molecules (grey ovals). Only an RNA–RBP–effector assembly can process RNA in *cis* (dashed arrow). IDR$_A$, IDR of antagonizing RBP; RBD, RNA-binding domain; RBD$_A$, RBD of antagonizing RBP.
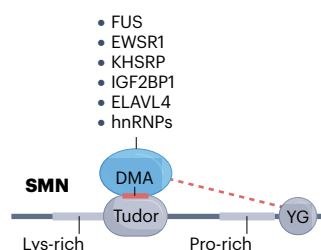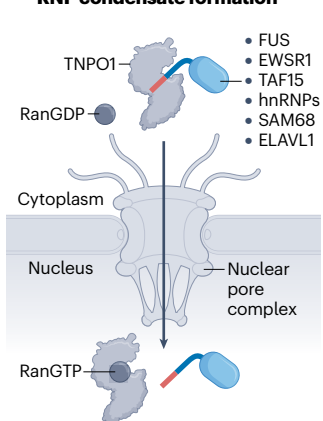
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a Gene-specific transcriptional control

b Regulation of alternative splicing

c Nuclear RNP assembly*

d Control of nuclear import and RNP condensate formation

Fig. 3 | Converging RBP–effector interactions regulating (peri-)nuclear RNA processing. a, RNA-binding protein (RBP)-dependent release of paused RNA polymerase II (Pol II) by positive transcription elongation factor b (P-TEFb). RBPs can stimulate relocation of P-TEFb from a local 7SK complex to the vicinity of Pol II either by recruiting P-TEFb via direct protein–protein interactions (PPIs) while bound to nascent RNA (step 1) or indirectly by associating with or disassociating from the 7SK complex (step 2). **b**, RBPs directly interact with spliceosomal components, including U1 small nuclear ribonucleoprotein (snRNP), U2 snRNP and U2AF subunits, to promote the early stages of spliceosome assembly. Illustrated is an overview of all interactions (left) along with a zoomed-in view of the U1 snRNP (right). Grey arrows denote intron or exon definition interactions[40], several of which are mediated by RS domains (dashed sections of shapes) of SR proteins, such as SRSF1 and SRSF2, and components of the spliceosome. RBPs other than SR proteins, including YBX1, SAM68 and TIA1, use short stretches of their intrinsically disordered regions (IDRs) (red dashes) to contact the indicated spliceosomal proteins. FUS recognizes the stem–loop (SL) region 3 (SL3) of the U1 small nuclear RNA (snRNA). Sm proteins are seven core spliceosomal proteins that make up a stable ring-like structure. **c**, Dimethylated arginines (DMAs) in the RGG/RG-rich regions of the indicated RBPs are recognized by the aromatic cage within the Tudor domain of survival motor neuron protein (SMN). Dashed red line denotes additional IDR-mediated interactions of some of the listed RBPs with the YG box domain (YG). The asterisk indicates that the same RBP–SMN interactions might also participate in processes other than RNP assembly in the nucleus[43]. For clarity, RBP-bound RNA is not drawn. **d**, RBPs that use a proline-tyrosine-rich nuclear localization sequence (PY-NLS) short linear motif (SLiM) (red dash) to interact with transportin 1 (TNPO1) for their nuclear import. The disaggregase activity of TNPO1 is not indicated (Fig. 4e). Drawings of multiple RBPs binding to the same effector molecule in individual panels solely illustrate that different RBPs can bind to a particular effector; they do not imply simultaneous interactions of multiple RBPs with different segments of the same effector or competition between different RBPs for binding to a particular region of an effector molecule.

Tudor domain of SMN[43,47,48] (Fig. 3c and Table 1). Little is known about the exact regulatory role of these contacts. However, intragenic SMA-associated mutations within the Tudor domain of SMN that disrupt RBP–SMN interactions point to their direct involvement in disease pathogenesis[45,49,50].

RBPs and nuclear import receptors. Most RBPs with a nuclear role require an active, carrier-mediated process for their import into the nucleus. This is facilitated by nuclear import receptors (NIRs) that bind RBPs exposing a nuclear localization sequence (NLS) and ferry them across a nuclear pore complex, while securing directionality of transport into the nucleus through competitive interactions with a small GTPase, Ran[51] (Fig. 3d). NIRs such as Kapβ1/Impα and transportin 1 (TNPO1) specifically recognize the classical, lysine-rich NLS (cNLS) and proline-tyrosine (PY)-NLS, respectively. Remarkably, beyond their role in nucleocytoplasmic trafficking, NIRs have recently been found to moonlight as RBP chaperones, having the capacity to prevent cytoplasmic condensation of aggregation-prone RBPs, and as 'disaggregases', denoting their ability to dissolve preformed, including aberrant, RNP condensates[52–55] (Fig 1). How do these unexpected moonlighting activities of NIRs come about? Akin to most other short linear motifs (SLiMs) of RBPs that mediate PPIs with effectors, NLSs are largely disordered, flanked by IDRs, and bind a structured surface of NIRs. However, most NLSs exhibit significantly higher effector-binding affinities than several other characterized SLiMs of RBPs (Table 1). The high-affinity interaction with NLS in turn facilitates secondary, low-affinity contacts that a NIR makes with other IDR domains, especially prion-like and RGG/RG-rich domains of the cargo RBP. These weak and dynamic

SMN deficiency in motor neurons remains unclear, several studies have pointed to additional roles of SMN in neuronal mRNA trafficking and control of local translation; these processes rely on and are regulated by direct interactions between SMN and various RBPs[43,45,46] (Table 1). Curiously, unlike most other RBP–effector contacts, these interactions rely primarily on recognition of a single residue, a mono- or dimethylated but not non-methylated arginine embedded in an arginine–glycine (RGG/RG)-rich IDR of an RBP, by the conserved

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interactions are thought to be essential in antagonizing homeostatic and pathological RNP condensate formation[53–55]. We discuss their therapeutic potential below.

Cytoplasmic activities of the nexus

RBPs and motor proteins. Subcellular localization of mRNA enables precise control over the site of protein synthesis[56]. This in turn allows a cell to configure different subcellular domains for specialized functions to support biological processes as diverse as budding in yeast, embryonic patterning in the fruitfly and synaptic activity in mammalian neurons[57]. Molecular motor-based transport of membraneless RNA granules has emerged as the prevailing mechanism of mRNA localization, and much of the mRNA transport was found to occur through a process called 'hitchhiking', whereby RNA granules dock onto membrane-bound organelles that in turn couple to motor proteins for transport[58,59]. However, certain RNA granules were found to directly connect to motor proteins via RBPs for autonomous transport of their RNA cargo, offering a plausible explanation for the observed diversity of mRNA localizations[60]. For instance, direct RBP–motor protein PPIs were found to mediate mating type-defining asymmetrical localization of *ASH1* mRNA in budding yeast[61–63], transport of *Actb* mRNA to cellular leading edge to support migration of a mouse fibroblast[20] or dendritic translocation of neurospecific mRNAs for their localized translation at the synapse[64] (Fig. 4a, Table 1 and Supplementary Table 2). A deeper understanding of this nexus should help to clarify how hundreds of different mRNAs are distinctly localized in the cell to allow for an exquisite spatial and temporal control of gene expression.

RBPs and the RNA helicase UPF1. Half-lives of cellular mRNAs are heavily dependent on stability-linked *cis*-acting RNA elements. A handful of RBPs with specificity for stem–loop structures or extended double-stranded RNA stretches are known to interact with a unique effector, the RNA helicase UPF1, to promote decay of mRNAs that harbour such elements[65]. UPF1, which is best known as a central component of a major quality control pathway known as nonsense-mediated decay[6], thus operates as a point of convergence for functionally diverse RBPs, each of which regulates the stability of its targeted mRNAs (Fig. 4b). To stimulate the RNA helicase activity of UPF1, the recruiting RBPs must bypass the intramolecular autoinhibitory interaction between the CH domain and the helicase domain of UPF1 (refs.[66,67]). How this is achieved remains unclear, although several reports point to an activating role of direct, IDR-mediated contacts between RBPs and the inhibitory domains of UPF1 (refs.[22,65,68,69]) (Table 1 and Supplementary Table 2). Upon activation, UPF1 is believed to unwind the RBP-bound RNA structure to facilitate local endonucleolytic cleavage, which, along with a decapping complex and exonucleases, ensures rapid destruction of the targeted transcript[70]. Additional RBPs are speculated to engage this pathway of mRNA decay[65].
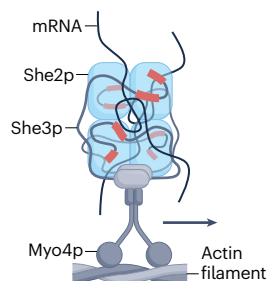
RBPs and the CCR4–NOT complex. Poly(A) tails are required for stability and translational efficiency of mRNAs, but are also subject to enzyme-mediated shortening, or deadenylation, a process that is key for initiating mRNA decay[71]. The CCR4–NOT deadenylase trims adenosines proximally to the 3′ untranslated region (UTR) with a major effect on mRNA stability. Notably, in addition to its role in 'baseline' deadenylation of bulk mRNA, CCR4–NOT is widely thought of as a hub of regulated, mRNA-specific deadenylation, much of which is driven by RBPs, microRNAs (miRNAs) and codon optimality[71,72]. In the multi-subunit CCR4–NOT complex, the CNOT9 subunit and the CNOT2–CNOT3 heterodimer constitute the major regulatory hotspots through which several RBPs are thought to recruit CCR4–NOT to select mRNAs for deadenylation[15,73–77] (Fig. 4c and Table 1). Interestingly, the concave surface of CNOT9 non-specifically binds RNA, albeit in a mutually exclusive manner with RBPs (Fig. 4d). This has led to an intriguing hypothesis that, through competition for CNOT9 binding with mRNA, RBPs might divert the deadenylating activity of CCR4–NOT from bulk mRNA to specific mRNA targets[72]. Notably, most, if not all, studied PPIs seem to conform to the general trend whereby an IDR of an RBP, often a defined SLiM, interacts with a structured domain of a CCR4–NOT subunit (Fig. 4c and Table 1). A particular case is the convex surface of CNOT9, which recognizes individual tryptophan residues present in the IDR of ZFP36 or the TNRC6 (also known as GW182) adaptor proteins; the latter recruit CCR4–NOT to Argonaute (AGO)–miRNA-targeted mRNAs and have a crucial role in miRNA-mediated gene silencing[75,78–81] (Figs. 4c,4f).
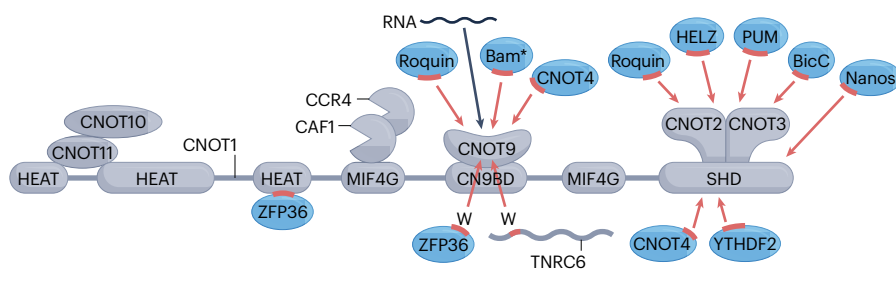
RBPs and the cytoplasmic poly(A)-binding protein. Although neither a classic effector nor a transcript-specific RBP, the abundant cytoplasmic poly(A)-binding protein (PABPC) is central to promoting efficient translation and surveillance of translation and decay rates of nearly every mRNA[71]. Canonical functions of PABPC encompass controlling the length of the poly(A) tail, physical communication between the two ends of the mRNA, and recruitment of various effectors and regulators of mRNA processing[71,82] (Fig. 4d). The latter function of PABPC is mediated via its main protein-binding platform, the MLLE domain, which specifically recognizes a SLiM, known as PAM2, found in IDRs of diverse proteins, including a handful of RBPs[82] (Fig. 4d and Table 1). Curiously, in contrast to the expected roles in regulation of mRNA stability and translation[82–84], a couple of rather unexpected roles have recently been found for RBP–PABPC interactions (Table 1). In particular, PABPC has been found to moonlight as an RNA-dependent chaperone that prevents spontaneous condensation of the intrinsically disordered RBP ataxin 2 (ATXN2)[85]. The chaperoning role of the ATXN2–PABPC interaction is especially notable because aberrant condensation of ATXN2 has been associated with two neurodegenerative disorders, spinocerebellar ataxia-2 and amyotrophic lateral sclerosis (ALS)[86]. Through a separate PAM2-mediated interaction, PABPC helps to position makorin 1 (MKRN1) upstream of premature poly(A) tails to block mRNA translation and facilitate ribosome-associated quality control[87]. Interestingly, an orthologous Mkrn1–pAbp PPI in the fruitfly is required for derepression of *oskar* mRNA translation during embryonic patterning[84].

Given the precedent of ATXN2 and MKRN1, it is tempting to speculate about other non-canonical functions of RBP–PABPC interactions. These would be important to understand especially when one considers the large number of RBPs that might potentially be in close contact with PABPC via PAM2 motif–MLLE domain interactions. Specifically, our reanalysis of published data indicates that 77 human PABPC1-bound proteins detected by in vivo proximity-dependent biotinylation (BioID) analysis (77/120, 64%)[88] are RBPs that harbour either a consensus PAM2 motif or a PAM2-like peptide sequence that is no more divergent from the consensus than the PAM2 motif of MKRN1, which differs from the consensus by three mismatched residues[11,87,88] (Fig. 4d and Supplementary Table 3). Much larger numbers of candidate MLLE domain-binding RBPs with or without mismatches to the consensus PAM2 motif are obtained if all annotated human RBPs are considered[11] (Fig. 4d and Supplementary Table 3). This is notable given that the MLLE domain is unique to PABPC and just one other, much less abundant
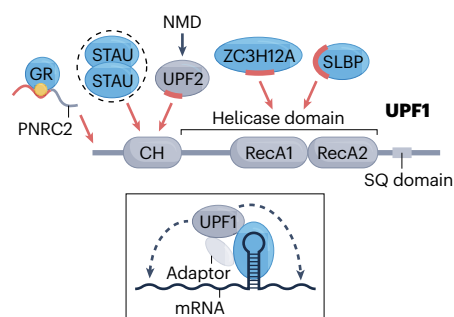
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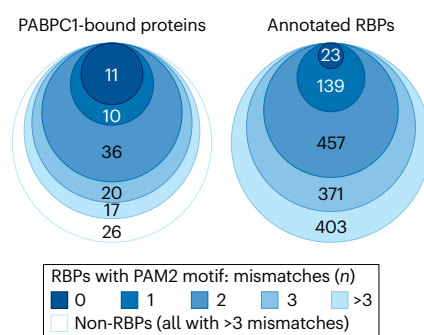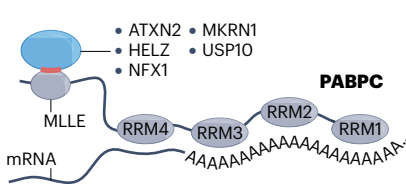## a mRNA localization

b Regulation of UPF1-mediated mRNA decay

c Control of mRNA deadenylation

d Poly(A)-dependent control of RNA metabolism

- ATXN2
- HELZ
- NFX1
- MKRN1
- USP10

PABPC

PABPC1-bound proteins

Annotated RBPs

RBPs with PAM2 motif: mismatches (n)
0 1 2 3 >3
Non-RBPs (all with >3 mismatches)

e Gene-specific control of mRNA translation

- Bicoid
- Prep1

- Scd6
- Npl3
- Sbp1
- Khd1
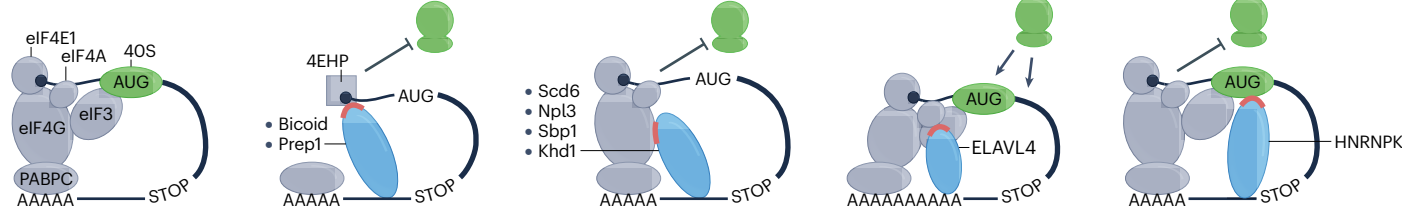
f Conductance of miRNA-mediated gene silencing
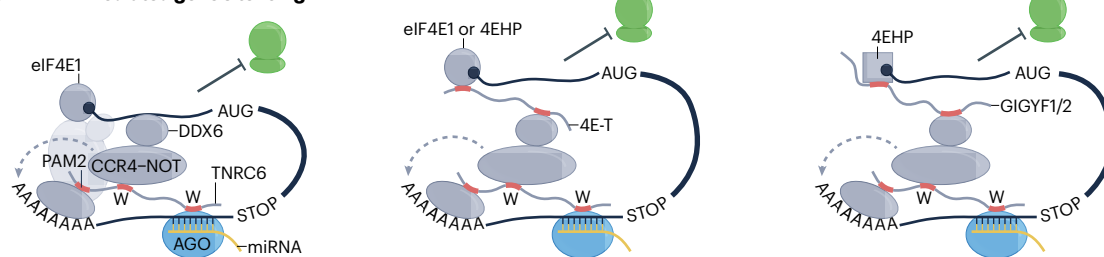
human protein, the E3 ubiquitin ligase UBR5 (ref.[82]). It would thus be of interest to probe potential interactions of these RBPs with PABPC and determine their functions.

RBPs and translation initiation factors. Rare examples of direct RBP–effector interactions with primary roles in controlling mRNA translation indicate various possible regulatory modes, most of which entail 3′ UTR-bound RBPs and components of the translation initiation machinery (Fig. 4e). For instance, in yeast, a set of RBPs directly associate with

eIF4G to block recruitment of the preinitiation complex[89], whereas in higher organisms, RBP-dependent recruitment of the translational repressor 4EHP (also known as eIF4E2) prevents eIF4E from binding to the 5′ cap on targeted transcripts (Fig. 4e and Table 1), a mechanism that sustains normal oogenesis in the fruitfly and in mammals[19,90,91]. Several other translationally linked RBP–effector interactions lack evidence of convergence; however, they largely seem to conform to the general SLiM- or IDR-structure type of interaction (Table 1). We also note that gene-specific translational control by RBPs is often coupled with

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regulation of mRNA stability and involves indirect, adaptor-mediated interactions with effectors, as is the case for AGO–miRNAs and several other RBPs[79,90,92] (Fig. 4f and Supplementary Table 2).

Plasticity and dynamics of connections

An overview of the converging RBP–effector contacts shows that the large majority of them form between a short stretch of an IDR of an RBP, or a defined SLiM residing in it, and a structured part of an effector protein (Table 1). Such interactions not only confer high specificity and span a range of affinities to satisfy the required dynamics of PPIs, but also can be regulated and exhibit significant evolutionary plasticity[93]. Notably, because SLiMs are short, harbour a small number of essential residues and often reside in IDRs, they can rapidly evolve de novo from random peptide sequences and rewire protein interaction networks[93–95]. These properties allow novel cases of a particular SLiM to evolve independently in different RBPs for binding to the same structured binding site in a given effector, thus contributing to the convergent evolution of the nexus (Fig 1). A classic example is provided by independently evolved, dissimilar-in-sequence SLiMs of RBPs and competing non-RBPs that bind to the same contact site in the concave surface of CNOT9 (refs.[15,74,96]) (Fig. 4c).

Dynamics of RBP–effector interactions should be considered together with the events that occur at the level of RBPs binding to RNA, as RNA processing by many RBP-regulated effectors eventually depends on their recruitment to RNA (Fig. 2e). Notably, a recent kinetic analysis suggested that, in cells, RBPs bind to their cognate RNA sites transiently and infrequently, that individual binding events may last only seconds or shorter, and that few if any regulatory RBPs might be bound to a given mRNA at any given time[97]. If true, then how might adding another layer of transient and possibly infrequent interactions, in this case between RBPs and their effectors, still allow for assembly of functional RNA–RBP–effector modules to secure timely processing of RNA?

The answer might depend on the RNA process in question. For instance, several forms of RBP-regulated RNA processing might require only infrequent formation of relatively short-lived RNA–RBP–effector assemblies to initiate a process that can be propagated by effector-independent means. This may apply to processes such as decapping-triggered mRNA decay, stimulation of alternative splicing and, conceivably, mRNA clamping-dependent translational repression[98]. By contrast, processes such as TNPO1-mediated RBP extraction and nuclear import, or motor protein-driven mRNP transport may require more stable associations. Indeed, the reported affinities of PY-NLS motifs for TNPO1 are generally one to two orders of magnitude higher than most other measured SLiM/IDR–effector interactions (Table 1). Alternatively, in mRNA transport granules, high concentration of RBPs could fortify their sometimes already superstoichiometric binding to motor proteins via avidity effects[62,99] (Fig. 4a).

Regulation of RBP–effector interactions

The formation and break-up of RBP–effector interactions do not rely solely on their strength and the local availability of interacting molecules in the cell but can additionally be regulated by cellular cues. The amenability to regulation can serve a host of biological processes that may require anything from fine-tuning RNA processing, enabling rapid, switch-like responses to intracellular or extracellular stimuli, to permanent rewiring of RNA networks. To accommodate such broad regulatory flexibility, RBP–effector interactions can be modulated either pre-translationally through alternative splicing (Fig. 5a), or post-translationally, by post-translational modifications (PTMs) or competitive interactions (Fig. 5b–d). Below, we discuss the principles of these regulatory strategies and list examples linked to regulation of diverse biological processes.

Rewiring of networks through pre-translational control

Alternative splicing, which affects the expression of nearly 95% of human multi-exon genes[100], has emerged as a central mechanism for functional diversification of eukaryotic proteomes[101]. When alternative splicing affects an RBP or an effector of RNA processing, an

Table 1 | Interface between RBPs and their effectors

Effector	Effector protein[a]	Effector contact region[b]	RBP[a]	RBP contact region[b]	K_d (µM)[c]	Process regulated by the RBP–effector interaction
Spliceosome	SNRNP70 (U1-70K)[d]	RS domain (IDR)	SRSF1/2 (SC35)	RS domain (IDR)		Pre-mRNA splicing
	SNRPA (U1A)[d]	RRM1 domain (SD)	SAM68 (KHDRBS1)	Y-rich (YY) region (IDR)		
	SNRPC (U1C)[d]	N terminus (SD)	TIA1	Q-rich domain (IDR)		
	U1 snRNA[d]	SL3 of U1 snRNA	FUS	RRM domain (SD)		
	U2AF65	RS domain (IDR)	YBX1	Basic/acidic repeats (IDR)		
SMN	SMN	Tudor domain (SD)	ELAVL4 (HuD)	NR		mRNA transport, translation
			IGF2BP1 (ZBP1)	KH domain region		
			KHSRP (FUBP2)	NR (methylated Arg) (IDR)		mRNA stability
			FUS	RGG box (IDR) and RRM (SD)		Pre-mRNA splicing
		Tudor domain (SD) and YG box (SD)	HNRNPR	RGG box (IDR)		mRNA transport, pre-mRNA splicing
			SYNCRIP	RGG box (IDR)		
			EWSR1	RG domain (IDR)		NR
		YG box (SD)	FMRP	Internal segment (IDR)		mRNA transport, translation
		NR	HNRNPU	RGG box (IDR)		NR
NIRs	TNPO1	HEAT repeats (SD)	HNRNPA1	PY-NLS SLiM (IDR)[e]	0.042	Nuclear import, RBP chaperoning/ disaggregation
			HNRNPA2B1			
			EWSR1			
			TAF15			
			FUS		0.0095	
			HNRNPF			Nuclear import
			HNRNPM		0.01	
			HNRNPD		0.0032	
			HNRNPDL		1	
			SAM68 (KHDRBS1)			
			ELAVL1 (HUR)			
	KAP104[(yeast)]		Nab2p[(yeast)]		0.037	
			Hrp1p[(yeast)]		0.032	
	Kapβ1/Impα	HEAT/Arm repeats (SD)	TDP43 (TARDBP)	cNLS SLiM (IDR)[e]	0.066	Nuclear import, RBP chaperoning/ disaggregation
Motor proteins	Myo4p[(yeast)]	C terminus (SD)	She3p[(yeast)]	Pseudocoiled-coil (SD)	0.058	mRNA transport
			She3p–She2p[(yeast),f]	P/R sites (IDR)–structure (SD)	1.6	
	KIF11	Tail domain (IDR/SD)	IGF2BP1 (ZBP1)	RRM domains (SD)		
	KIF3C	C terminus (IDR)	FMRP	NR		
UPF1	UPF1	Helicase domain (SD)	SLBP	N-terminal half (IDR)		mRNA stability
		RecA domain (SD) and Thr28 (IDR)	ZC3H12A (regnase 1)	Internal segment (IDR) and RNase domain (SD)		
		N terminus	STAU1/2	Tubulin-binding domain (IDR)		

Table 1 (continued) | Interface between RBPs and their effectors
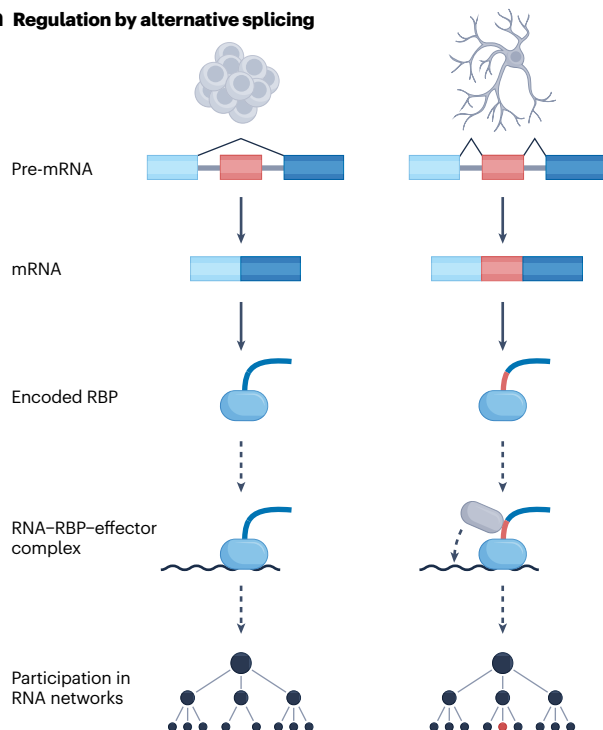
Effector	Effector protein[a]	Effector contact region[b]	RBP[a]	RBP contact region[b]	K_d (μM)[c]	Process regulated by the RBP–effector interaction
CCR4–NOT	CNOT1	HEAT domain (SD)	ZFP36 (TTP)	SLiM (IDR)	2	mRNA stability, translation
			ZFP36L1 (BRF1)			
		NR	TNRC6C (GW182)[g]	W-containing motifs (IDR)		
	CNOT9	Concave surface (SD)	Bam	CBM SLiM (IDR)	0.183	
			RC3H1/2 (Roquin 1/2)			mRNA stability
			CNOT4			mRNA stability
		Convex surface (W pockets) (SD)	ZFP36 (TTP)	Isolated W residues (IDR)	5.5	mRNA stability
			TNRC6A/C (GW182)[g]			mRNA stability, translation
	NOT module	NR (SD)	RC3H1/2 (Roquin 1/2)	C terminus (IDR)		mRNA stability
			HELZ			mRNA stability, translation
			PUM1/2	N-terminal domain (IDR)		
			Bicaudal C	KH domains (SD)		
		SHD (SD)	NANOS1–3	SLiM (IDR)		Translation
			YTHDF2	P/Q/N-rich region (IDR)		RNA stability
			CNOT4	C terminus (IDR)		mRNA stability
PABPC	PABPC1	MLLE domain (SD)	ATXN2	PAM2 SLiM (IDR)	0.7	RBP chaperoning
			HELZ			NR
		NR	USP10		26	NR
		Multiple domains	MKRN1			RQC, translation
	PABPC1/4	NR	NFX1			mRNA stability
4EHP	4EHP	Dorsal surface (SD)	Bicoid (Bcd)	SLiM (IDR)		Translation
		NR	PKNOX1 (Prep1)			
eIF4G	TIF4631/2[(yeast)]	C terminus	Scd6[(yeast)]	RGG box (IDR)		
			Khd1[(yeast)]	NR		
		Internal region	Sbp1[(yeast)]	RGG box (IDR)		
		Internal region and C terminus	Npl3[(yeast)]			

CBM, CAF40-binding motif; IDR, intrinsically disordered region; K_d, dissociation constant; NIR, nuclear import receptor; NLS, nuclear localization sequence; NR, not reported; PABPC, cytoplasmic poly(A)-binding protein; PY-NLS, proline-tyrosine-rich NLS; RBP, RNA-binding protein; RQC, ribosome quality control; RRM, RNA recognition motif; SD, structured domain; SLiM, short linear motif; SMN, survival motor neuron protein; snRNP, small nuclear ribonucleoprotein; STAU, Staufen; TNPO1, transportin 1. [a]Only those direct RBP–effector interactions are listed where a contact region has been mapped in at least one of the interacting proteins. For a fully referenced list of direct and indirect interactions, see Supplementary Table 2. Effectors and RBPs are named by their official gene symbol with text in brackets indicating popular aliases. All interacting proteins are metazoan except for the indicated yeast proteins. [b]IDR (or its part) or SD (or its part) in brackets indicates peptide organization of the contact region in its non-bound state. Where not reported, this information was obtained through modelling of the protein structure using the structure prediction algorithm AlphaFold[173]. [c]The K_d in most cases pertains to an in vitro interaction between SLiM of an RBP and the interacting effector domain or the full-length effector protein. [d]Part of U1 snRNP. [e]NIRs can also form secondary, low-affinity contacts with other domains of their cargo RBPs[53–55]. [f]Both She2p and She3p are RBPs in contact with the transported mRNA[62]. [g]TNRC6A–C are paralogous adaptor proteins that recruit CCR4–NOT to microRNA-targeted messages largely via capture of W residues by tandem W pockets present in CNOT9 and AGO (Argonaute) proteins[79,174].
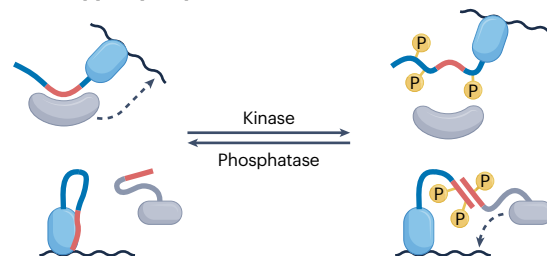
opportunity arises for the establishment of new or alteration of existing RBP–effector interactions. The observation that IDRs and their embedded SLiMs, but not structured domains of proteins, commonly reside in non-constitutive exons suggests that alternative splicing of IDR-rich RBPs, rather than their SLiM-binding effectors, more commonly impinges on RBP–effector interactions[95,102]. Pre-translational modulation of RBP–effector interactions via alternative splicing offers additional regulatory opportunities, including stable rewiring of RNA networks (Fig. 5a). Notably, rewiring of PPIs often occurs in a cell- or tissue-specific manner and can crucially contribute to development and tissue identity[103].

Several alternative RBP isoforms have been identified that harbour identical RNA-binding domains and differ only in short stretches of IDR, yet exhibit distinct effector roles and biological functions. For instance, two alternative isoforms of the *Drosophila* How RBP, one restricted to the nuclei of precursor cells and the other found in both the nuclei and the cytoplasm of mature cells, regulate a switch in tendon cell differentiation by exerting opposing effects on mRNA stability[104]. Interestingly, a similar switch mechanism enforced through differential subcellular localization but applied to the control of splicing and translation is used by alternative isoforms of the orthologous Quaking (QKI) RBP in regulating myelination in the mammalian nervous system[104–107].
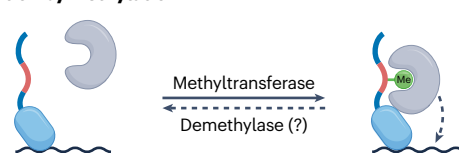
a Regulation by alternative splicing

Pre-mRNA

mRNA

Encoded RBP

RNA–RBP–effector
complex

Participation in
RNA networks

b Regulation by phosphorylation

Kinase

Phosphatase

c Regulation by methylation

Methyltransferase

Demethylase (?)

d Regulation by competitive interactions

Other RBP or non-RBP
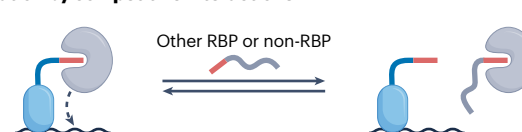
e Signal sensing and regulation of cellular responses at the RBP–effector interface

① • Hormonal stimulation
• Neurotransmission

② Growth signals

③ Immune signalling

④ • Stress
• Ageing
• Mutations

ON

OFF

PR
NMDAR

EGFR

TLR4

TLR4

CaMKII
Aurora A

SRPK

p38
MK2

4E
Maskin
NGD
CPEB PARN
AAAA

ZFP36   P   PP2A   CCR4–NOT
ZFP36

PABPC
ATXN2   NIR

4E
4G   PABPC
CPEB GLD2 ePAB
AAAAAAAAAA

SR → SR   P
CLK

PRMT

FUS, TDP43,
HNRNPA1/2,
TAF15, EWSR1

RanGTP   NIR

NIR

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Differential localization also underlies the function of ZAP (also known as ZC3HAV1), whose long isoform targets viral RNA for degradation, with the short isoform assisting by inhibiting programmed ribosomal frameshifting and resolving the antiviral response[108,109]. By contrast, with no overt difference in localization, one isoform of Musashi 2 (Msi2) RBP sustains a translation-repressive activity to promote anchorage-independent cell growth, whereas the other uniquely responds to differentiation-inducing phosphorylation[110]. Nonetheless, despite the marked biological impact of rewiring RBP–effector interactions via alternative splicing, our understanding of how such rewiring might lead to system-wide changes that underlie observed phenotypes remains minimal.

Responding to signal transduction via post-translational modulation

Reversible regulation of RBP–effector contacts is typically achieved by PTMs, of which phosphorylation has arguably garnered the most attention, particularly in the context of signal-regulated RNA processing events. The fast turnover of phosphorylation in fact renders this PTM particularly well suited to mediating rapid responses of several types of RNA processing to various signals[111,112] (Fig. 5a). For instance, phosphorylation by SR protein kinases (SRPKs) and CDC-like kinases (CLKs) affects pre-mRNA splicing by regulating interactions between SR proteins and components of the spliceosome[113]. In this manner, SRPKs operate as major transducers of growth signals; upon activation by growth factors, SRPKs relocate to the nucleus and cause widespread changes in alternative splicing[114] (Fig. 5e). By contrast, CLKs reside in the nucleus and act, for instance, in restoring the phosphorylation state of SR proteins during recovery from cellular stress[115].

The importance in regulated RBP–effector interactions has also been observed in the control of polyadenylation-induced translation, which has essential roles in diverse biological processes, such as germ-cell development, cell cycle progression and synaptic function[116]. In these settings, a sequence-specific RBP cytoplasmic polyadenylation element binding protein (CPEB) initially keeps dormant a set of mRNAs with relatively short poly(A) tails. In oocytes stimulated to mature by progesterone, a signalling cascade is initiated that activates the Aurora A kinase, which in turn phosphorylates CPEB and converts it from a repressor into an activator of translation[117] (Fig. 5e). This switch in CPEB activity results from molecular rearrangements that affect interactions of CPEB with several effectors involved in the control of poly(A) tail length and mRNA translation[118]. Remarkably, a highly similar signalling cascade leading to CPEB activation and stimulation of local translation is triggered upon synaptic stimulation of neurons[119] (Fig. 5e). In fact, loss-of-function genetic experiments in mice have demonstrated that CPEB-dependent translational control by cytoplasmic polyadenylation has an important role in synaptic plasticity, a process that is believed to form the underlying basis of learning and memory[120].

Akin to pre-mRNA splicing and protein synthesis, signalling-mediated regulation of contacts between RBPs and their effectors

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also has a central role in the control of mRNA stability, the biological significance of which is well illustrated by the regulation of innate immune responses by the AU-rich element (ARE) binding to RBPs such as ZFP36[121]. In cells that receive an inflammatory stimulus – for instance, lipopolysaccharide (LPS)-activated macrophages – a signalling cascade is triggered that revamps the transcriptional landscape to instate a pathogen-induced gene expression programme typified by induction of short-lived, ARE-containing mRNAs that encode pro-inflammatory cytokines as well as *ZFP36* mRNA[122]. The same stimulus activates a kinase cascade that phosphorylates the ZFP36 protein, preventing it from recruiting the CCR4–NOT deadenylase and allowing for accumulation of ZFP36-bound pro-inflammatory mRNAs and their encoded proteins[123] (Figs. 4c,5e). When signalling ceases, PP2A dephosphorylates ZFP36, prompting it to recruit CCR4–NOT to the bound mRNAs, which then undergo rapid deadenylation followed by degradation[75,123]. Thus, regulation of the interactions between ZFP36 and CCR4–NOT is instrumental in controlling the magnitude and duration of the inflammatory response.

Swift post-transcriptional responses to neurotransmitter release or inflammatory stimuli exemplify biological processes that require quick-acting changes in RBP–effector connectivity; such modulation can be rapidly enacted by phosphorylation. However, in other physiological settings, a longer-lasting action may be required that is less sensitive to speed of onset or termination. Although controlled phosphorylation might again serve such needs, other, generally less-studied PTMs of RBPs can have just as potent regulatory roles at the nexus of RBPs and their effectors[111]. For instance, in yeast, arginine methylation was found to fortify the interactions between Scd6 or Sbp1 and eIF4G to facilitate translational repression of targeted mRNAs in response to glucose deprivation[124,125]. Arginine methylation also strengthened interactions between KHSRP and SMN to secure correct localization and mRNA stability in neurites of differentiating mammalian neurons[126], but weakened the contact between FUS and TNPO1 to modulate the dynamics of nuclear import in HeLa cells[53,127] (Fig. 5e). These findings suggest that methylation, which displays an inherently slow turnover[128], can be adept at regulating a wide variety of post-transcriptional events at the RBP–effector interface[129]. On that note, very little is known about the spatiotemporal control of RBP methylation, including the activities of protein arginine methyltransferases (PRMTs), which catalyse the deposition of this PTM, and it remains unclear whether RBPs are ever demethylated in vivo.

System-wide studies have implicated acetylation as another common PTM of RBPs, and recent evidence suggests that acetylation, akin to phosphorylation and methylation, may also have a regulatory role at the RBP–effector juncture[111,130]. For instance, similar to arginine methylation, lysine acetylation was found to disrupt the interaction of FUS with TNPO1, reducing nuclear import and stimulating the localization of FUS in cytoplasmic stress granule-like inclusion bodies[131]. Lysine acetylation was also reported to weaken the interaction of EWSR1 with the U1 snRNP component SNRPC to regulate alternative splicing in response to DNA damage[132]. Notably, the identification of specific enzymes that catalyse acetylation and deacetylation of these RBPs indicates that the above PTM events might indeed be regulatory[131,132].

At the level of RBP–RNA interactions, competition for the same RNA target site between RBPs with different, potentially opposing effects on a given post-transcriptional process, for example, splicing or mRNA stability, leads to distinct regulatory outcomes[40,133–137]. Similar competition can occur at the level of RBP–effector interactions

(Fig. 5d). To date, few if any studies have documented such competition between different RBPs; however, experimental evidence has been gathered for competition between RBPs with non-RBPs for a common binding site on an effector. Examples supported by functionally relevant outcomes include the aforementioned competition between Staufen (STAU) RBPs and UPF2 for binding to UPF1 (ref.[138]), competition between TOB, PAN3 and eRF3 for binding to PABPC1 (ref.[139]), or the recently proposed competition between RBPs and RNF219 for binding to CCR4–NOT[96]. Given the regulatory impact of competitive effector binding on RNA processing, as well as the abundance of converging RBP–effector interactions (Supplementary Table 2), future studies are warranted to shed light on the prevalence and significance of such interactions in various biological processes.

Genetic disorders and potential therapies

Given their central roles in regulating all aspects of gene expression at the RNA level, it is not surprising that compromised function of RBPs underlies the origin of many diseases. According to a recently updated tally, a staggering 30% of all annotated human RBPs (1,054 of 3,470 RBPs) are mutated in Mendelian or somatic genetic diseases, with neurological disorders, metabolic diseases and cancer among the most common associated disease categories[10]. Similar to our minimal understanding of RBP–effector roles compared with RBP–RNA interactions, we still know very little about the pathomechanisms of RBPs in disease.

The wealth of data garnered through disease association studies suggests that disease-linked mutations occur more frequently in IDRs and RBP domains other than RNA-binding domains[10]. Furthermore, proteome-wide analyses reveal an enrichment of disease-related mutations in SLiMs within IDRs, especially at functionally important residues of SLiMs[140]. Given the central role that SLiMs have in establishing RBP–effector interactions (Table 1), it seems plausible that interference with these interactions in disease might contribute to the pathobiology of mutations in RBPs. This consideration becomes particularly worthwhile in light of the observation that the ELM database, the largest repository of experimentally validated SLiMs[141], currently lists only around 4,000 out of more than 100,000 SLiMs that a eukaryotic proteome is predicted to contain[93]. Limited general knowledge of SLiMs and RBP action thus restricts our understanding of the involvement of the RBP–effector nexus in disease to only a handful of documented examples. However, several recent studies demonstrate the therapeutic potential of targeting RBP–effector interactions genetically or pharmacologically, highlighting the regulatory and biological potency of the nexus from a novel perspective.

Mutations that disrupt the nexus

Arguably the best-characterized disease-linked mutations that interfere with RBP–effector interactions cause pathological aggregation of misfolded RBPs, a signature trait of neurodegenerative disorders[10]. For instance, mutations in FUS can cause familial ALS as well as rare cases of frontotemporal lobar degeneration (FTLD) (Table 2). Remarkably, approximately half of these mutations occur within the PY-NLS and disrupt the high-affinity interaction of FUS with TNPO1 (ref.[142]) (Table 1). This disruption compromises nuclear import and leads to aberrant cytoplasmic accumulation of the otherwise largely nuclear FUS, which eventually becomes deposited in pathological protein aggregates, as seen in brains of affected individuals[143]. Notably, the severity of the defect in nuclear import of FUS faithfully tracks with disease onset and progression, supporting a causal relationship between the mutations in PY-NLS and disease pathogenesis[143–145].

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Table 2 | RBP–effector interactions in human disease

RBP–effector interaction	Genetic disorder or developmental abnormality	Causative or associated mutation	Therapeutic approach
Disease-linked mutations that affect RBP–effector interactions			
FUS–TNPO1 HNRNPA1–TNPO1 HNRNPH2–TNPO1 TDP43–Kapβ1/Impα	ALS, rare cases of FTLD[143] ALS[175] Neurodevelopmental delay, autism[148] ALS[176,177]	Mutations in PY-NLS or cNLS of RBPs	NR
MATR3–TREX complex	ALS, vocal cord and pharyngeal weakness with distal myopathy[149,178]	Mutations in IDRs of MATR3	NR
RBP–SMN	SMA[48,179]	Mutations in the Tudor domain of SMN	NR
Therapeutically targeted RBP–effector interactions			
SRSF6–U1 snRNP	Familial dysautonomia (Riley–Day syndrome)	Intronic mutation in *ELP1*	Small-molecule CLK1 agonist-enhanced SRSF6 phosphorylation to rescue abnormal skipping of the *IKBKAP* exon 20 (ref.[156])
SRSFs–U1/U2 snRNPs	Duchenne muscular dystrophy, cystic fibrosis, anhidrotic ectodermal dysplasia with immunodeficiency	Exonic mutation in *DMD*, intronic mutations in *CFTR* and *IKBKG*	Pharmacological CLK inhibition to suppress SRSF phosphorylation and pathogenic exon inclusion[158,180,181]
SRSFs–U1 snRNP	Leukaemia, prostate cancer, colon cancer, breast cancer, lung cancer, neovascular eye disease	Cancer-associated mutations in tumour suppressor genes and oncogenes	Inhibition of SRPK activity to normalize isoform levels of genes linked to cancer progression, apoptosis and angiogenesis[159–162]
FUS–TNPO1 EWSR1–TNPO1 TAF15–TNPO1 HNRNPA1–TNPO1 HNRNPA2–TNPO1 TDP43–Kapβ1/Impα	ALS, FTLD, Alzheimer disease, multisystem proteinopathy	Aggregation-promoting mutations in RBPs or disrupted arginine methylation	Delivery of NIRs to afflicted neurons or modulation of arginine methyltransferase activity[52–55,127,164]
ATXN2–PABPC	Spinocerebellar ataxia 2, ALS	Expansion of the polyglutamine domain in ATXN2	Designer PABPC-like chaperones to counter pathogenic RNP condensate formation[85]
ZFP36–CCR4–NOT	Rheumatoid arthritis, psoriasis, multiple sclerosis, juvenile idiopathic arthritis, inflammatory diseases	Mutations in and/or reduced expression of ZFP36, aberrant signalling	PP2A agonists to activate ZFP36 or forced expression of ZFP36 in peripheral tissues or immune cells[165,166,182,183]
RBM38–eIF4E	Cancers	NR	SLiM-mimicking synthetic peptide Pep8 increases expression of p53 by blocking the inhibitory RBM38–eIF4E interaction[169]

ALS, amyotrophic lateral sclerosis; ATXN2, ataxin 2; cNLS, lysine-rich NLS; FTLD, frontotemporal lobar degeneration; MATR3, matrin 3; NIR, nuclear import receptor; NLS, nuclear localization sequence; NR, not reported; PABPC, cytoplasmic poly(A)-binding protein; PP2A, protein phosphatase 2A; PY-NLS, proline-tyrosine-rich NLS; RBP, RNA-binding protein; SLiM, short linear motif; SMA, spinal muscular atrophy; SMN, survival motor neuron protein; snRNP, small nuclear ribonucleoprotein; TNPO1, transportin 1; TREX, transport and export.

Several other RBPs associated with neurodegenerative disorders, including HNRNPA1, HNRNPH2 and TDP43, can set off pathogenic cascades remarkably similar to that of FUS, with mutations in NLS leading to defective nuclear import, aberrant cytoplasmic accumulation, eventual aggregation and neurological disease[146] (Table 2). Aside from their functional similarities, a key property common to these RBPs is their proneness to aggregation, which stems largely from their prion-like domains. Thus, the time, made longer by stress and ageing, that the aberrantly cytoplasmic RBPs spend in a condensed state is believed to be a key parameter in irreversible RBP aggregation and the resulting neuronal degeneration[54,147] (Fig. 5e). Interestingly, whereas mutations of NLS in HNRNPA1 or TDP43 can lead to neurodegeneration akin to that caused by mutated FUS, a similar mutation in HNRNPH2 was found to compromise neurodevelopment and manifest in autism[148].

Circumstantial evidence also exists for disruption of other types of RBP–effector interaction in human disease. For instance, several mutations in the IDRs of the nuclear matrix RBP matrin 3 (MATR3), which are associated with neuromuscular disorders, were shown to affect interactions of MATR3 with components of the transcription and export (TREX) effector complex and with defects in the nuclear export of mRNAs[149]. However, the directness of these interactions and their contribution to disease remain unclear, especially given that these mutations also lead to potentially pathogenic associations of MATR3 with other proteins[150]. Curiously, disease-linked mutations that compromise RBP–effector interactions might also occur in the effector rather than its bound RBPs. Approximately 5% of patients with SMA carry intragenic mutations in the *SMN1* gene; a few of these mutations, such as Glu134Lys, locate to the Tudor domain of SMN and weaken its affinity for a series of RBPs[43,48] (Fig. 3c and Table 1). Such effector mutations would be expected to broadly compromise RNA metabolism and cellular function; however, their manifestation in a specific phenotype points to the cellular context as an important determinant of disease phenotype[151–153]. It remains to be seen whether disease-linked alterations identified in other effectors might cause pathology by compromising interactions with RBPs[10,154,155]. Improved understanding of the RBP–effector nexus is sure to shed light on the underpinnings of various human disorders.

Review article

Drugging the nexus

Some recent discoveries have electrified the field by demonstrating a striking potential of manipulating RBP–effector interactions in treatment of some of the most devastating human diseases, including neuromuscular disorders and cancer. Below, we summarize opportunities for therapeutic intervention at the nexus that arise from modulating the strength of existing interactions or through establishment of novel contacts between RBPs and their effectors.

One of the most promising therapeutic modalities harnesses the regulatory role of phosphorylation and the relative ease with which one can pharmacologically manipulate kinase and phosphatase activities. Notably, phosphorylation of SR proteins, which regulates the contacts of RBPs with the spliceosome and thus their activities as splicing factors (Fig. 3b), has been successfully modulated by small molecules to affect therapeutically relevant splicing decisions (Fig. 5e). For instance, the RECTAS compound, an agonist of CLK1, has shown promise for treatment of familial dysautonomia (FD), a fatal recessive neurodegenerative disease caused by a mutation in intron 20 of the *ELP1* gene, which leads to abnormal skipping of the *ELP1* exon 20 (refs. [156,157]). RECTAS was found to directly interact with and activate CLK1, leading to enhanced phosphorylation of the SR protein SRSF6 and restorative inclusion of exon 20 in multiple FD

Fig. 6 | A network view of the nexus. Shown is a compilation of converging RNA-binding protein (RBP)–effector interactions that have been characterized at both the molecular and functional levels. Note that several RBPs (blue shapes) connect (black lines) to more than one effector (grey shapes) and that there also exist RBP-independent interactions between different effectors (grey lines). Solid and dashed lines denote direct and indirect (adaptor-mediated) protein–protein interactions (PPIs), respectively. The green line that connects FUS and the spliceosome represents an RBP–RNA interaction. Purple lines represent disease-associated or therapeutically targeted interactions (Table 2). The purple circle around survival motor neuron protein (SMN) indicates spinal muscular atrophy (SMA)-linked mutations of SMN that weaken its affinity for multiple RBPs. Only mammalian RBPs are shown. Motor proteins (motors; KIF11, KIF3C, KIF5A, KIF3AB) and translation initiation factors (eIFs; eIF4E, eIF4E2 (4EHP), eIF4G) have been grouped together and are shown as a single effector. Non-converging PPIs discussed in the text, TDP43–Kapβ2/a1 and MATR3–TREX, are omitted. CPEB, cytoplasmic polyadenylation element binding protein; MKRN1, makorin 1; PABPC, cytoplasmic poly(A)-binding protein; STAU, Staufen.

disease models[156]. By contrast, CLK inhibitors have proved effective in reducing phosphorylation of SRSFs and promoting therapeutic skipping of mutation-affected exons. One such example is TG003 inhibitor-promoted skipping of a mutated exon in the dystrophin (*DMD*) gene, which increases production of the DMD protein in cells of patients with Duchenne muscular dystrophy[158] (Table 2). Moreover, synthetic SRPK inhibitors, which likewise suppress phosphorylation of SRSFs and their interactions with the spliceosome, have shown potent tumour-suppressive activities in diverse cancer types that present with pathological exon inclusions in genes such as *VEGF*, *MYB*, *BRD4* and *MED24* (refs. [159–162]). Together, these studies exemplify how chemical control of RBP–effector interactions might provide a new mechanism-oriented therapeutic opportunity.

Neurodegenerative disorders have long been considered incurable and with few treatment options. Disorders such as ALS, FTLD and Alzheimer disease are characterized by disrupted homeostasis of aggregation-prone RBPs, which is thought to underlie the formation of abnormal inclusion bodies[146]. The pathogenesis associated with this process may result from toxic properties of the inclusion bodies themselves and/or loss of function due to RBP mislocalization[163]. The recent discovery that NIRs, including TNPO1 and Kapβ1/Impα, act as chaperones and disaggregases beyond their canonical roles in the nuclear import of RBPs has generated considerable excitement about the prospect of reversing RBP aggregation to mitigate neuronal degeneration[52,55,164] (Fig. 5e). These studies provide a rationale for therapeutic strategies that aim to increase the level of NIRs via gene therapy approaches or, conceivably, pharmacological strengthening of the interactions between mislocalized RGG/RG-rich RBPs and TNPO1 (refs. [53,127]) (Table 2). Although implementation of these strategies or the ATXN2-chaperoning PABPC in human therapy may prove challenging[85], the unique potential to combat some of the most devastating genetic diseases warrants further investigation.

Manipulating RBP–effector interactions has also shown promise in treating autoimmune and inflammatory disorders. For instance, targeting the anti-inflammatory RBP ZFP36 with agonists of PP2A, which results in dephosphorylation of ZFP36 and recruitment of the CCR4–NOT deadenylase, can confer significant protection against inflammatory arthritis and bone erosion in mice[165] (Fig. 5e). Consistently, adenoviral delivery of ZFP36 protected against bone loss and led to reduced inflammatory cell infiltration in rats[166], whereas mice with genetically stabilized ZFP36 exhibited protection against induced forms of arthritis and other immune disorders[167] (Table 2).

Aside from modulating the nexus-regulated RNA splicing, stability and RNP condensation discussed above, a case has also been made for the control of mRNA translation. The activity of the cancer-associated RBP RBM38, which suppresses p53 translation by directly interacting with eIF4E on *Trp53* mRNA[168], could be inhibited by a synthetic, RBM38 SLiM-mimicking peptide, called Pep8, that interferes with the RBM38–eIF4E interaction, derepresses p53 translation and attenuates tumour sphere formation and growth of xenograft tumours[169]. Incidentally, several small molecules and designer peptides that mimic SLiMs have shown promise as drugs that act by inhibiting various types of PPI, and some have already entered clinical trials[93].

Taken together, the above examples illustrate the breadth of therapeutic opportunities arising from manipulation of RBP–effector interactions, which is all the more significant in light of our still rudimentary knowledge of this nexus. Whereas pharmacological targeting approaches seem readily translatable to human therapy, implementation of strategies entailing gene therapy will necessitate further
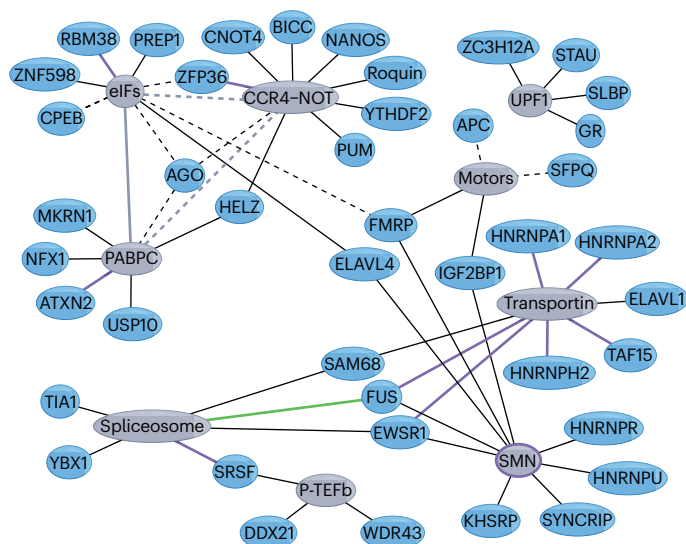
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feasibility studies. We note that with expanding understanding of the nexus, additional targeting modalities may emerge; antisense oligonucleotides, several of which are currently being used in the clinic[170], might, for instance, find therapeutic utility in the pre-translational manipulation of SLiMs (Fig. 5a).

Conclusions and perspectives

The regulation of RNA networks has largely been understood at the level of RNA recognition by RBPs and more recently at the level of RNP condensate formation. Progress in these and other areas of RNA research has revealed much about the organizational principles of RNA networks, the complexity of which is managed by converging molecular interactions that are apparent at every level of network organization. Despite these insights, relatively little is known about the mechanistic bases of RBP activities or the ways in which effectors, the chief executors of RNA processing, contribute to the overall structure and operation of RNA networks. Interestingly, an integrated view of the binary interactions considered in this Review alone suggests that the regulatory level at which RBPs communicate with their effectors might consist of a network that is perhaps more interconnected than anticipated (Fig. 6). Technological breakthroughs along with accumulating knowledge about the functions and structures of RBPs and their effectors are certain to enable a more expansive view and better understanding of this nexus.

Several pertinent questions remain. For instance, oftentimes, a mode of post-transcriptional control by an RBP becomes apparent from meta-analysis of transcriptome-wide data sets, but many, sometimes most, individual transcripts are not regulated as expected, despite being bound. Could this be explained by transcript-dependent efficiency of effector recruitment (or blockage of effector recruitment) by the RBP, alternative RBP isoforms or PTM of effectors? Is regulation of RNA binding by RBPs, as seen in budding yeast, embryonic patterning in the fruitfly or polarization of mammalian neurons, coordinated with the regulation of RBP−effector interactions[57]? Could functions of RBPs be accurately predicted based on knowledge of their interactions with effectors − or presence of SLiMs alone − in addition to RNA-binding information? Does the condensation propensity of many RBPs affect their ability to recruit effectors? How is the availability of effectors affected when condensate formation becomes pathological? Could we avert neurodegeneration by manipulating RBP−NIR interactions?

Unlike the large arsenal of high-throughput methods tailored to studies of RNA binding by RBPs, investigations of RBP−effector interactions have traditionally been limited to reductionist approaches. Although invaluable to the current understanding of communication between RBPs and effectors, such studies often yield limited and possibly inaccurate information about the dynamic nature, complexity and effects of these interactions in vivo. To tackle this problem, additional approaches that more directly capture the in vivo context of RBP−effector interactions are required. For instance, cryo-electron microscopy (cryo-EM) studies of purified RBP−effector complexes could provide views of concurrently interacting molecules and their conformations to help address the many unanswered questions about the workings of RBP−adaptor−effector conduits. These efforts could be aided by single-molecule biophysical experiments as well as kinetic assays that entail ultra-fast crosslinking strategies to investigate the dynamics of the interactions[97]. It may also be possible to devise pull-down protocols that combine, for example, enzyme-mediated proximity tagging with CLIP or RIP to study RBP−effector interactions in vivo transcriptome-wide. Such studies could centre on either the

effector, adaptor or RBP of interest and explore protein complexes that simultaneously bind to RNA in a position-specific manner.

With the large majority of the annotated RBPs still unstudied, novel adaptor and effector proteins continuing to be discovered[171,172] and with fewer than 5% of the expected number of SLiMs being functionally annotated[93,141], it is clear that we have only just begun to delve into the converging RBP−effector interface. Expanding this knowledge will be important to better understand the mechanisms of RNA processing as well as the aetiology of human disease.

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Author contributions

S.H. performed the bioinformatic analyses. All authors contributed to all other aspects of the article.

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