Cell. Mol. Life Sci. 66 (2009) 1239 – 1256
1420-682X/09/071239-18
DOI 10.1007/s00018-008-8532-1

Cellular and Molecular Life Sciences

Review

Nuclear functions of heterogeneous nuclear ribonucleoproteins A/B

Yaowu He[a,*] and Ross Smith[b,*]

[a] Institute of Health and Biomedical Innovation, Queensland University of Technology, 60 Musk Avenue, Kelvin Grove, Queensland 4059 (Australia), Fax: +61 7 3138 6030, e-mail: y.he@qut.edu.au
[b] School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, Queensland 4072 (Australia), Fax: +61 7 3365 4699, e-mail: ross.s@uq.edu.au

Received 30 August 2008; received after revision 21 October 2008; accepted 05 November 2008
Online First 16 December 2008

Abstract. The hnRNP A/B proteins are among the most abundant RNA-binding proteins, forming the core of the ribonucleoprotein complex that associates with nascent transcripts in eukaryotic cells. There are several paralogs in this subfamily, each of which is subject to alternative transcript splicing and post-translational modifications. The structural diversity of these proteins generates a multitude of functions that involve interactions with DNA or, more commonly, RNA. They also recruit regulatory proteins associated with pathways related to DNA and RNA metabolism, and appear to accompany transcripts throughout the life of the mRNA. We have highlighted here recent progress in elucidation of molecular mechanisms underlying the roles of these hnRNPs in a wide range of nuclear processes, including DNA replication and repair, telomere maintenance, transcription, pre-mRNA splicing, and mRNA nucleo-cytoplasmic export.

Keywords. hnRNP A/B, RNA, RNA-protein interactions, telomere, DNA repair, pre-mRNA splicing, nucleo-cytoplasmic RNA export.

Introduction

Heterogeneous nuclear ribonucleoproteins (hnRNPs) constitute a large family of proteins that associate with nascent pre-mRNAs, packaging them into hnRNP particles [1–3]. This family includes about 20 major polypeptides, hnRNPs A1 to U, which range in size from 34 to 120 kDa [2]. Many putative hnRNP genes that encode minor hnRNP proteins remain to be characterized [4].

Each hnRNP protein contains at least one RNA-binding motif such as an RNA recognition motif (RRM), hnRNP K homology domain (KH) or arginine/glycine-rich (RGG) box [1, 5]. Many manifest a high affinity for specific nucleic acid motifs [6, 7]. Some hnRNPs contain auxiliary domains with unusual amino acid compositions [1, 5], which mediate protein-protein interactions [5, 8]. Correlated with these diverse structural features, a multitude of cellular functions has been ascribed to hnRNP proteins, including roles in DNA maintenance and recombination, transcription and processing of primary transcripts, and nuclear export, subcellular localization, translation and stability of mature mRNA [5, 9, 10].

The A/B subfamily of hnRNPs (hnRNPs A/B) [11] were originally described as two low-molecular-

* Corresponding authors.

weight groups of hnRNP proteins isolated from the 40S "core" hnRNP particles of HeLa cells [12]. hnRNPs A0 [13] and A3 [14] were later included, as these proteins have modular structures that parallel their A1 and A2 paralogs (Fig. 1), with two tandem RRMs near the amino-terminus and a glycine-rich domain (GRD) near the carboxyl-terminus [15]. These hnRNP A/Bs share a high level of amino acid sequence identity, especially in their structural motifs [13, 14, 16]. Human hnRNPs A1 and A2 exhibit ~80% and 58% identity in the RRM and glycine-rich regions, respectively [16, 17]. The amino acid sequence of the hnRNP A3 tandem RRMs has high sequence identity with A1, though its GRD domain is more like that of A2 than A1 [14]. hnRNP A0 differs more; it has about 56% identity with human hnRNP A2 over the two RRM domains and GRD. The unrooted consensus neighbour-joining tree of tandem RRMs encoded by 10 human genes obtained from a bootstrap analysis [18] supports the view that these hnRNP A/B proteins are evolutionary products that have arisen from a single, archetypal RNA-binding protein by gene duplication [14, 19–21]. The insertion of small peptides, resulting from alternative pre-mRNA splicing, generates some of the diversity among them [16].

Another two more distantly related hnRNP proteins, B2 and AB, have also been included in this subfamily [22, 23]. hnRNP B2 may be an alternatively spliced isoform of hnRNP A1 [24] or A2 [25]. hnRNP AB was previously classified as a type C hnRNP [26], but was later found to have two RRMs and a GRD domain like the A/B type proteins [27]. This protein, however, shares limited sequence identity with hnRNP A/B subfamily proteins: it is more closely related to hnRNP D [18]. In this review we have focussed on hnRNPs A1, A2, A3, and A0, their alternatively spliced isoforms and UP1, which is a proteolytic product of hnRNP A1 generated by an unidentified trypsin-like protease [28].

hnRNPs A/B are among the smallest but most abundant hnRNP proteins [29], except for hnRNP A0, which is a minor hnRNP transcribed from a processed pseudogene [13] that has rarely been studied. hnRNPs A1 and A2 constitute 60% of the total protein mass of hnRNP particles, representing the most abundant nuclear proteins [12]. hnRNP A1 is present in $7-10 \times 10^7$ copies per HeLa cell [30].

hnRNPs A/B localize predominantly in the nucleus but are excluded from nucleoli [31–33]. Most of these proteins also shuttle between the nucleus and cytoplasm [5, 9, 34–36]. hnRNPs A1, A2, B1, and B2, together with C1 and C2, form the 40S particle obtained by sucrose gradient sedimentation of sonicated nuclei digested with RNase A [1, 37]. hnRNPs A2, B1 and B2 form $(A2)_3(B1)$ tetramers and $(A2)_3(B1)(B2)$ pentamers at the centre of core particles [38, 39], with hnRNPs A1, C1, and C2 positioned peripherally [37]. hnRNP A3 was not initially described as a component of the 40S particle, but recent mass fingerprinting has shown some of its minor isoforms to be present [40].

The tandem RRM-Gly structures of hnRNP A/B proteins enable them to bind other proteins and nucleic acids, hence their pivotal roles in packaging of nascent RNA and in many other aspects of nuclear and extra-nuclear mRNA processing. The major functions of these proteins include telomere biogenesis/maintenance [41–44], transcription [45, 46], alternative pre-mRNA splicing [24, 47–51], nuclear import [52] and export [53, 54], cytoplasmic trafficking of mRNA [14, 55–58], mRNA stability and turnover [59], and translation [60, 61]. This review focuses on the nuclear functions of these proteins. The interaction between hnRNP A/B proteins and polynucleotides or nucleic acids is reviewed first as it bears on the full repertoire of hnRNP A/B protein functions.

Association of hnRNP A/B proteins with nucleic acids

The interaction of hnRNP A/B proteins with polynucleotides was first observed for UP1, which passed through a column loaded with native dsDNA but was retained on a ssDNA-cellulose column in early attempts to identify eukaryotic DNA-binding proteins [62]. Subsequent studies have shown that hnRNP A1 and UP1 do associate with dsDNA [15, 63], suggesting that they may regulate gene expression. hnRNPs A1 and A2 interact *in vivo* with a number of elements in dsDNA, including hormone response elements [64] and other regulatory elements [46, 65]. These proteins also bind single-stranded DNA-agarose *in vitro* with low sequence specificity (Table 1, and references therein) [2].

Both the RRM and Gly-rich domains of these proteins are involved in binding DNA [66]. The hnRNP A1 tandem RRM domains are sufficient for the interaction with ssDNA, but they bind less tightly than the full-length protein. The C-terminal domain interacts with nucleic acids directly or indirectly through cooperative protein-protein interactions [66]. Post-translational *in vitro* methylation of HeLa hnRNP A1 arginine residues 193, 205, 217, and 224 [67] also affects its binding to ssDNA; compared with the unmethylated protein, the methylated A1 requires a lower concentration of NaCl to be released from a ssDNA-cellulose column [68]. As noted above, early *in vitro* data showed a preferential binding of UP1 and hnRNP A1 (cooperative for the latter) to ssDNA

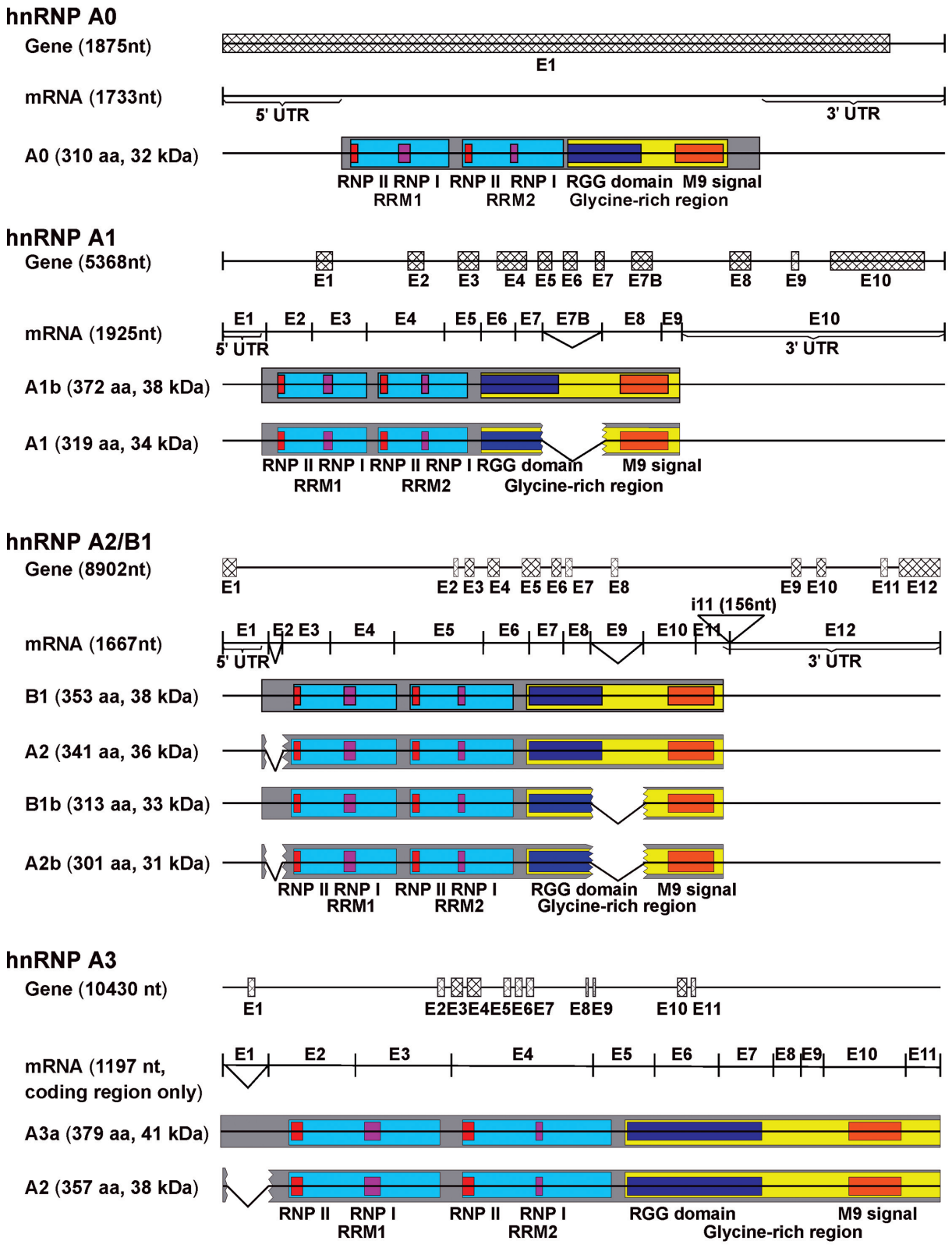

Figure 1. Structure and characteristics of main hnRNP A/B proteins (E: exon; i: intron; nt: nucleotide; UTR: untranslated region)

Table 1. DNA-binding sequences/structures for hnRNP A/B proteins

Binding sequence or structure	hnRNP	Reference
Native DNA-cellulose	UP1	[62]
$P(dT)_8$	A1, UP1	[196]
Poly(dA-dT)	A1	[63]
ssDNA	A1	[15, 63, 66]
TGCTCTC	A1	[46]
Telomeric DNA repeats	UP1, A1, A2, A3	[44, 116, 117]
dsDNA	A1	[15, 63]
DNA triplexes	A2/B1	[71]
G-quartets	A1, A3	[86]

rather than dsDNA [15, 63]. In accord with this, DNA duplex-destabilizing activity has been reported for A1 and UP1 [63], but under other conditions *in vitro* they can promote rapid renaturation of complementary strands of DNA and RNA [69]. The response of DNA to the presence of A1 is a complex function of temperature and A1 concentration. Whether A1 stabilises or destabilises dsDNA is dependent on the temperature relative to the melting temperature [70]. Above this temperature A1 destabilises dsDNA and the fraction of ssDNA is a function of the A1 concentration [70]. The interaction of A1 with ssRNA is also stronger than with ssDNA [15] and is attributed approximately equally to the tandem RRM domains of hnRNP A1 (or UP1) and the GRD [63]. Other data suggests that the GRD is needed for co-operative binding to nucleic acids [63, 69].

hnRNP A/B proteins bind structural motifs in DNA: hnRNP A2/B1 was pulled down by a DNA triplex probe together with hnRNPs K, L, E1, and I [71], and hnRNP A1 has been shown to interact with, and destabilize, G-quartets, the quadruplex structure of G-rich sequences [72]. This is believed to constitute one of the mechanisms by which these proteins trigger and coordinate their molecular functions [71].

Interaction of hnRNP A/B proteins with RNA has been well established, particularly for A1 and A2. hnRNP A1 preferentially associates with a so-called "winner" RNA sequence: UAUGAUAGGGA-CUUAGGGUG, in which the two closely-arranged UAGGGA(U) short sequences are critical [73]. Recombinant hnRNP A1 also binds to RNAs containing AUUUA-rich sequences *in vitro* [74]. For example, the granulocyte-macrophage colony-stimulating factor mRNA, which has an AUUUA-rich region in its 3′-UTR, can be immunoprecipitated using an antibody against hnRNP A1 [74]. With more extensive research on hnRNP A1 in the past two decades, additional binding sequences have been identified, as listed in Table 2.

One of the better-characterized hnRNP A2 binding sequences is the 21 nt hnRNP A2 response element (A2RE), or the derivative 11 nt oligonucleotide (A2RE11), which is essential for the cytoplasmic transport of several mRNAs in oligodendrocytes and neurons [55, 75, 76]. The A2RE sequence is evolutionarily conserved, and has been found in a number of transcripts, including *PRM2*, *MOBP81A*, *GABARα*, *GFAP*, *α-CaMKII* and *ARC* [44, 75]. The RRMs of hnRNP A2 are required to act in concert to ensure sequence-specific binding: single RRMs appear to be only capable of non-specific binding [56]. hnRNP A2 may also interact with the A2RE-like sequences, such as the A2RE-1 and A2RE-2 sequences found in a region of overlap between the *vpr* and *tat* genes of the HIV-1 virus *in vitro* [77].

The A2RE and A2RE-like sequences are not the only RNA structures that bind hnRNP A2. Early *in vitro* data suggested that hnRNP A2/B1 binds the UUAGGG sequence in addition to A1 [78]. Recently, in a microarray study to identify the downstream targets of hnRNP A2/B1 proteins, a group of transcripts was found which formed complexes with hnRNP A2/B1, but contained no A2RE or AU-rich elements (AuRE) [79], suggesting that hnRNP A2 may either associate directly with other unidentified RNA binding sequences or bind indirectly. The RNA-binding of hnRNPs A0 and A3 has been less studied, but *in vitro* evidence indicates that hnRNP A3 associates with A2RE and AuREs in the 3′-UTR of COX-2 mRNA [80].

In summary, the hnRNP A/B proteins are capable of binding a range of DNA and RNA sequences. Each of these proteins has high- and low-affinity nucleic acid binding sites [1, 56]. The eclectic binding of the hnRNP A/B proteins to DNA and RNA, specifically and non-specifically, and to consensus sequences and secondary or tertiary nucleic acid structures, can generate diverse regulatory roles.

Table 2. RNA-binding sequences for hnRNP A/B proteins

Binding sequences	hnRNP	Reference
hnRNA	A1	[15]
Coliphage MS2 RNA	A1	[15]
Poly(rU), Poly(rC), Poly(rA), or Poly[r(A+U)]	A1, UP1	[15, 63]
Py-rich 3′ splice site of introns	A1	[160]
UUAGGG	A1, A2/B1	[44, 78]
UAGGGACUUAGGGU	A1	[73]
AUUUA	A1	[74]
d(GGCAG)n	A1	[197]
UAGACUAGA	A1	[149]
UAGAGUAGG	A1	[149]
UAGAUUAGA	A1	[149]
UAG binding site	A1	[198]
nYAGGn	A1	[199]
UACCUUUAGAGUAGG	A1	[157]
AUAGAAGAAGAA	A1	[144]
UUAGAUUAGA	A1	[200]
UAGGGCAGGC	A1	[147]
UAUGAUAGGGACUUAGGGUG	A1	[201]
UUAG	A1	[78]
A2RE, A2RE-1, A2RE-2, A2RE11	A2, A3	[55]
pri-miR-18a	A1	[202]

hnRNP A/B proteins in chromosome maintenance, DNA replication and repair

As discussed in the previous section, studies of helix-destabilizing activity for the full-length hnRNP A1 protein have not yielded consistent results [15, 26, 63]. It can be a potent regulator of DNA annealing within a single strand [81] and between two complementary strands [81, 82], but it can also destabilise dsDNA.

Targets for hnRNP A1/UP1-mediated destabilization include G-quartets, which are believed to be crucial for the regulation of DNA replication, transcription, and telomere maintenance [83–85]. hnRNPs A2/B1 and A3 also associate with G-quartets [86], but no studies of the destabilization of these structures have been reported. However, hnRNP A2/B1 is capable of destabilizing G′2 d(CGG)n, a tetraplex structure similar to the G-quartet but formed by two G-rich molecules. The conserved RNP1 and RNP2 motifs of the A/B hnRNPs mediate destabilization and stabilization, respectively, of the tetraplex structure [87].

The hnRNP A/B proteins play many roles in DNA replication. UP1 stimulates the activity of DNA polymerase α [15, 88], an enzyme that synthesizes an RNA-DNA primer (the α-segment) and initiates the formation of the Okazaki fragments during lagging strand DNA synthesis [89]. hnRNP A1 then stimulates the activity of human flap endonuclease 1 (FEN-1), an enzyme that mediates processing of the α-segment, and possibly the removal of the RNA primer, during the maturation of the Okazaki fragments [89]. hnRNP A2 binds the SET oncoprotein, a key regulator of DNA replication, chromatin remodelling, and gene transcription. Both proteins act as inhibitors of protein phosphatase 2A [90], an enzyme that regulates cell proliferation and differentiation. The unfolding of tetraplex structures, which appears to be widespread across the human genome [91], by UP1, hnRNPs A1 and A2/B1 may facilitate DNA replication [92]. Finally, hnRNP A1 interacts with nuclear DNA topoisomerase I (Top1) [93], which reversibly cleaves one strand of duplex DNA, relaxing DNA supercoiling, and thereby regulating DNA topology during replication, chromosome condensation, and transcription [94]. Top1 activity is inhibited by binding to G-quartets [95].

The roles of hnRNP A/B proteins in DNA metabolism also include the maintenance of telomeres, the protein-DNA complexes that cap the chromosome ends in some cells, preventing them from being illegitimately fused by the repair machinery for DNA double-stranded breaks [96]. The telomeres for vertebrates are comprised of a TTAGGG repeat [97, 98], with a G-rich, single-stranded 3′ overhang

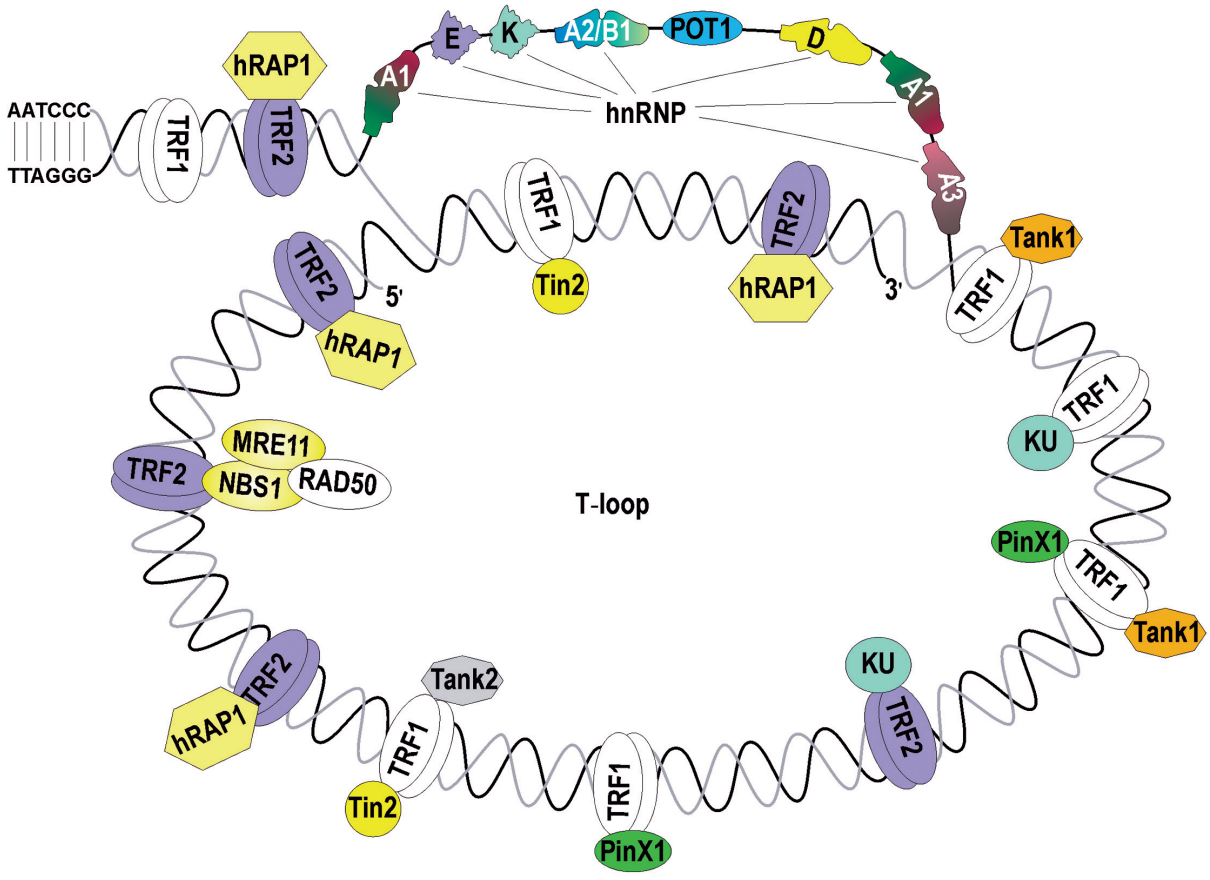

Figure 2. The putative telomere T-loop structure. The G-rich 3′ overhang of the telomere loops back, invading the duplex repeat region of the same telomere and forming a T-loop structure by annealing with the C-rich strand. The displaced strand is associated with a number of proteins including POT1 and hnRNPs A1, A2/B1, A3, D, E, and K. Binding of telomeric repeat binding factors and their interacting proteins is also indicated (Adapted from a review by Neumann and Reddel [103]).

[99–101], which invades the double-stranded region of the telomeric DNA, forming a T-loop structure (Fig. 2) [102]. To stabilize their telomeres, cells synthesize new telomeric repeat DNA using telomerase [103], or, less frequently, lengthen the telomeres using a mechanism possibly involving recombination [104]. The replication capacity of cells that lack any means of maintaining their telomeres is limited by induction of cell cycle arrest, senescence and, in a subset of cells, apoptosis [105]. Failure to detect telomeres shortened beyond a critical length leads to chromosome instability and triggers malignant transformation [106, 107].

All of the A/B hnRNP paralogs, except A0, have been demonstrated to associate with the 3′ single-stranded telomeric extension and protect it from nuclease attack. *In vivo* and *in vitro* studies have shown that hnRNP A1 and UP1 bind telomeres or single-stranded telomeric repeats [43, 108]. The crystal structure of UP1 complexed with a 12-nucleotide single-stranded telomeric DNA repeat revealed that a UP1 dimer binds to two strands of DNA, each strand interacting with the RRM1 of one monomer and RRM2 of the other [109]. Murine hnRNP A2 associates with the single-stranded telomeric repeat $(TTAGGG)_n$, as well as its RNA equivalent, UUAGGG [110]. hnRNP A2 protects the telomeric repeat sequence but not the complementary sequence [44]. A similar protective role has recently been reported for the binding of hnRNP A3 to the single-stranded telomeric repeat [42, 86] (S. Sara and R. Smith, unpublished observations). Telomeres are shorter in mouse erythroleukemic cells that do not express hnRNP A1, and are lengthened by the restoration of UP1 or hnRNP A1 expression [108], suggesting a positive role of this hnRNP in telomere elongation. Supporting this, a telomere repeat amplification protocol (TRAP) assay performed with a cell extract from HEK293, a human embryonic kidney cell line, showed that hnRNPs A1 [43] and the two isoforms of A2 that have the 12-residue N-terminal exon inclusion (B1 and B1b; Fig. 1) [111] stimulate telomerase activity. A *Caenorhabditis elegans* hnRNP A/B protein ortholog, HRP-1, also promotes telomere elongation *in vivo* [112]. At the molecular level,

hnRNP A/B proteins may serve as a bridge between the telomeric DNA template and the RNA component of telomerase. In a chromatography assay, the tandem RRMs of hnRNP A1 were found to simultaneously bind a telomeric repeat DNA oligonucleotide and the RNA component of human telomerase, suggesting that hnRNP A1 may help recruit telomerase to the ends of chromosomes [113]. hnRNP A2 also binds the RNA template of telomerase (hTERT) [44]. However it is not known if it can bind to telomeric DNA and hTERT simultaneously. The unfolding of G-quartets by these proteins also suggests a positive role for hnRNPs A/B in telomere elongation. The telomeric repeat sequence is capable of forming a quadruplex structure [114] which inhibits telomerase activity [115]. Unwinding of the G-quartets may facilitate telomerase translocation and promote telomere extension [43].

The function of hnRNP A/B proteins in telomere elongation has been controversial since inhibitory effects have also been reported. Telomerase assays using a HeLa cell extract indicated that binding of hnRNP A1 to single-stranded telomeric repeat prevented extension by telomerase [116]. hnRNP A3 appears to have a similar inhibitory effect on telomerase activity [42, 117]. Most of these studies were performed *in vitro* and additional *in vivo* studies in mammalian models are needed to fully define the effects of hnRNP A/B proteins on telomerase activity. Recently, chromatin precipitation assays with antibodies to hnRNP A3 have shown an interaction with telomeric DNA repeats in rat brain extracts (S. Sara and R. Smith, unpublished observations).

hnRNP B1, which is over-expressed in the early stages of lung cancers, may play a role in DNA repair [118]. This protein associates with the DNA-dependent protein kinase (DNA-PK) complex, which mediates the repair of DNA double-strand breaks [119] and inhibits its activity, whereas hnRNPs A1 and A2 have no effect [118]. When the expression of hnRNP A2/B1 was suppressed by siRNA, DNA repair was faster in normal human bronchial epithelial (HBE) cells. It has been suggested that this causes inappropriate rejoining of double-strand breaks, triggering cell transformation.

Functions of hnRNPs A/B in gene transcription

Although hnRNP A/B proteins preferentially bind RNA, rather than DNA [63], some have been shown to associate specifically with multiple promoter sequences and thus participate in regulation of transcription. hnRNP A1 binds the promoter regions of *c-myc* [120], *APOE* [121], thymidine kinase (*TK)* [122], and the genes encoding γ-fibrinogen [46] and the vitamin D receptor [64]. It is a component of the transcription complex of an interferon-regulated gene, protein kinase regulated by RNA (PKR), which regulates virus multiplication and cell growth, differentiation, and apoptosis [123]. hnRNP A2/B1 shares some targets with A1, such as *c-myc* [120], *APOE* [121], and the vitamin D receptor gene [64], and additionally interacts with the promoter sequences of breast cancer 1 (BRCA1) [124] and gonadotropin-releasing-hormone 1 (GnRH1) [125]. hnRNP A3 also acts as a transcription factor, binding to the regulatory region of the *Hoxc8* gene [126].

Several different oligonucleotide motifs have been reported to mediate hnRNP A/B binding to transcriptional regulatory regions. They include the ATTT motif within the cell cycle regulatory unit of the human TK promoter [122], the TGCTCTC box in the γ-fibrinogen promoter [46], and the hormone-response elements of the vitamin D receptor [64]. It is not clear if the differences in these binding motifs determine the regulatory role of hnRNP A/B proteins in transcription. Some of these proteins, including A1 and A2, can act as either a transcriptional activator or a repressor. hnRNP A1 suppresses transcription from the TK [122] and γ-fibrinogen promoters [46], as well as both basal and induced expression from vitamin D-responsive promoters [64], but it activates the apolipoprotein E (*APOE)* promoter [121]. hnRNP A2 also represses expression of the vitamin D receptor, but it is more likely to be an activator for BRCA1 transcription because suppression of this hnRNP led to a decrease of BRCA1 at both the mRNA and protein levels [33].

How hnRNP A/B proteins contribute to transcriptional regulation is unknown. However, both direct and indirect mechanisms may be involved. Destabilization of G-quartets by hnRNP A/B proteins is likely to be a factor, considering the enrichment of putative G-quadruplex formation sites in the promoter regions [91, 127]. Transcription of *c-myc* is a good example: its regulation is associated with the formation of a G-quadruplex in the promoter region [127], where the interacting sites for hnRNPs A1, A2 and B1 are located [120]. hnRNP A/B proteins can indirectly participate in control of transcription through protein-protein interactions. hnRNP A2 interacts with the SET oncoprotein, which stimulates transcription by altering histone-DNA interactions [90]. Recent pull-down assays using a glutathione S-transferase (GST)-fused p53 transcriptional activation domain (residues 1–73) detected an hnRNP A2/B1 peptide [128], suggesting the possibility of A2/B1 forming a complex with p53, which is a multi-targeting transcription factor [129]. In addition, the association of hnRNPs

A1 and A2 with 7SK RNA (an snRNA) is critical for the release of P-TEFb, a transcription elongation factor required for transcription by RNA polymerase II, from the P-TEFb–HEXIM1–7SK RNA complex [130]. Simultaneous inhibition of hnRNP A1 and A2 expression reduced the transcription-dependent dissociation of P-TEFb–HEXIM1–7SK complexes.

Apart from their role in the initiation of transcription, hnRNP A/B proteins may contribute to termination of transcription, as evidenced by studies on two yeast hnRNP A/B proteins, Npl3 and Hrp1 [131]. Hrp1 forms the B component of the CFI polyadenylation factor and, when overexpressed, increases recognition of a weakened polyadenylation site and suppresses the defective transcription termination. Npl3 also functions in polyadenylation site recognition, by competing with the CFI polyadenylation factor for an RNA binding site. It will be of interest to see if mammalian hnRNP A/B proteins share a similar biological role in transcription termination.

hnRNP A/B proteins as splicing repressors

Constitutive co-transcriptional splicing of nascent pre-mRNA results in intron removal and the fusion of exons to generate functional mRNA, a prerequisite for most eukaryotic genes [132]. Intron excision and exon ligation are directed by special sequences at the intron/exon junctions (splice sites) and catalyzed by the spliceosome, a large macromolecular complex assembled on the splice sites [133]. Alternative splicing results in excision of not only introns but also of specific exons. The hnRNP A/B proteins are essential components of the spliceosome and participate in both constitutive and alternative splicing (recently reviewed in [134]). *In vitro* dissociation of the spliceosome releases almost all hnRNP proteins, except A0 and AB, although some are only recruited to spliceosomes during certain stages of the splicing reaction. The major hnRNP A/B proteins, A1, A2, and A3 are among the few hnRNP proteins that are assembled into spliceosomes at all major splicing stages [135–142].

hnRNP A1 regulates pre-mRNA splicing by association with exonic splicing silencers (ESSs) and intronic splicing silencers (ISS), inhibiting the use of 3′ splice sites or promoting the use of more distal 5′ splice sites. This is supported by the established roles of hnRNP A1 and A2/B1 proteins as regulators of alternative splicing, which allows the cells to produce varied mRNA and protein isoforms from an identical gene by altering splice site choice, thus differentially including exons and introns, or portions of them [133]. These hnRNPs antagonise the action of serine/arginine-rich (SR) proteins, which bind to exonic and intronic splicing enhancers (ESE and ISE) and promote splicing [134],

As in most nuclear aspects of these proteins, the molecular mechanism of action of hnRNP A1 has been most intensively studied. However, hnRNP A2 appears to act similarly and it is more effective than A1 in splice-site switching. At this stage there is no direct evidence that hnRNP A3 mediates the splicing of any transcript, but it has been detected as a spliceosomal component by mass spectrometry and it is anticipated that it will function in a similar manner to A1 and A2.

The level of hnRNP A1 relative to the alternative splicing factor/splicing factor 2 (ASF/SF2) was first identified as a switch for splicing site selection using model and adenovirus E1A pre-mRNAs [47]. Later observations on bovine growth hormone (bGH) [143], HIV-1 tat and rev [144], c-Src [145], and INK4a pre-mRNA [146] correlated with this, suggesting hnRNP A1 as a *trans*-acting alternative splicing regulator *in vivo*. The two RRMs, particularly the Phe residue in the RNP-1 submotif (Fig. 1), are essential for the specific hnRNP A1-pre-mRNA interaction and for modulating alternative splicing [48]. For some transcripts, such as human fibroblast growth factor receptor 2 (FGFR2), the repression of alternative splicing may be mediated by the GRD alone [147]. These domains are conserved across the hnRNP A/B subfamily, consistent with the observation that hnRNPs A2/B1 and $A1^B$ also favour distal splice-site selection [48].

Binding of hnRNP A/B proteins to ESS elements blocks proximal exon recognition [148]. Several ESS elements in different transcripts have been identified for hnRNP A1 [50, 147, 149, 150] and hnRNP A2/B1 [50, 149, 151]. Some alternatively spliced exons, such as bGH exon 5 [152] and HIV-1 tat exon 2 [153], have ESSs that overlap with an ESE element that specifically binds SR proteins. Thus, the outcome of competition between hnRNP A/B and SR proteins for common binding sites determines the splice site selection (Fig. 3A) [145, 150, 154]. When ESE and ESS elements are overlapping, the binding of one hnRNP A1 molecule may suffice to eliminate SR association with the ESE. When these sites do not overlap, the hnRNP A/B proteins may bind cooperatively along the exon, favouring splicing repression (Fig. 3A) [47, 134]. This co-operative binding results in exclusion of the proximal exon. However, a specific binding site is not essential for hnRNP A1 to antagonize SF2 in splicing control. Cooperative, indiscriminate, and low-affinity binding of A1 to the 5′ splice site (5′SS) of β-globin mRNA inhibits U1 snRNP (small nuclear ribonucleoprotein) binding,

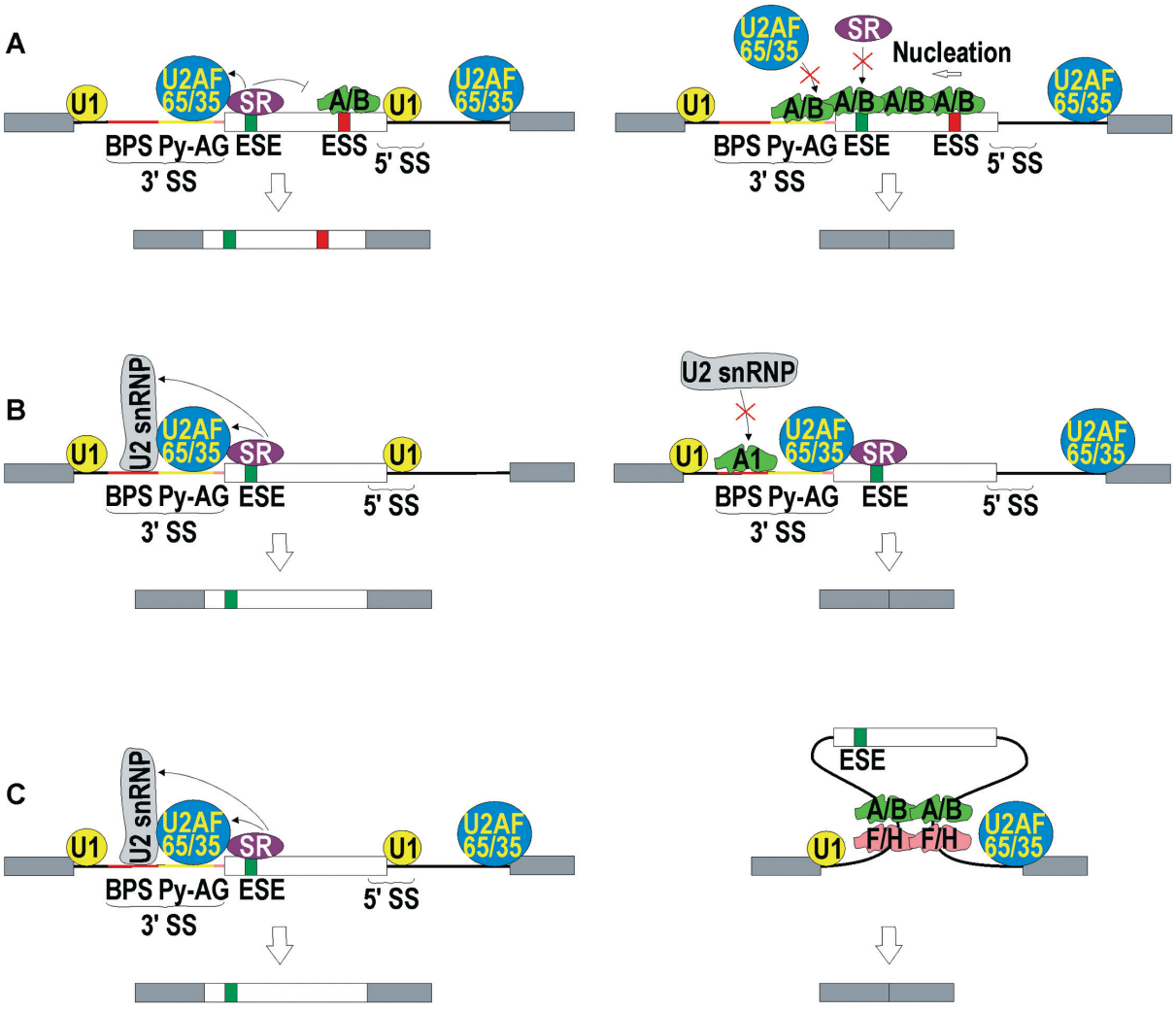

Figure 3. hnRNP A/B proteins in splicing. The intron-exon boundaries are defined by the U2 ribonucleoprotein particle (U2 snRNP), which binds a branchpoint sequence (BPS), and splicing factor U2AF, which recognizes a polypyrimidine tract (Py) at the 3′ splicing site (SS), and U1 snRNP at the 5′ SS. Binding of splicing factors, such as SR proteins, to an exonic splicer enhancer (ESE) promotes the exon definition process and suppresses the usage of exonic splicer silencers (ESS). (*A*) Binding of hnRNP A/B proteins to an ESS leads to assembly of additional hnRNP molecules along the intron-exon junction, and limits ESS accessibility to the spliceosome. (*B*) Splicing is repressed by hnRNP A/B proteins, which associate with the BPS and block entry of U2 snRNP. *(C)* The A/B- or F/H-type hnRNP proteins binding to the intronic splicing silencing sequences, which flank an exon, interact with each other and loop out the exon.

which is crucial for 5′SS recognition during spliceosome assembly, while ASF/SF2 enhances U1 snRNP binding at all 5′SSs [155].

Specific binding sites for hnRNP A/B proteins also exist in introns. For HIV-1 tat exon 3, an ISS for hnRNP A1 was found to overlap one of the branch points, a specific binding site for U2 snRNP that is required for efficient cleavage at the 3′ splice site (3′SS). Binding of hnRNP A1 physically blocks the entry of U2 snRNP and inhibits spliceosome assembly (Figure 3B) [156]. hnRNP A1 pre-mRNA exon 7b is flanked by multiple ISSs for hnRNP A/B and F/H proteins [49, 51, 157–159]. The interaction between hnRNP molecules bound to the ISSs helps to loop out exon 7b (Fig. 3C) [49].

There are other mechanisms that elicit the participation of hnRNP A/B proteins in alternative splicing control. Association of any of these proteins with pre-mRNA may represent an early step in spliceosome formation. hnRNP A1 preferentially binds the 3′ splice sites of introns in the presence of U1 and U2 snRNPs, two spliceosomal complexes, mediating the splicing of 5′ and 3′-ends of introns, respectively [160–162]. hnRNP A1 also interacts with U2 and U4 snRNPs, and RNase H excision of U2 nucleotides 28–42 impacts on the U2 snRNP-pre-mRNA interaction by abolishing the A1-U2 snRNP interaction [163]. The RNA annealing capacity regulated by the GRD domain of hnRNP A1 [48] may be involved in the annealing of the RNA components of the snRNP

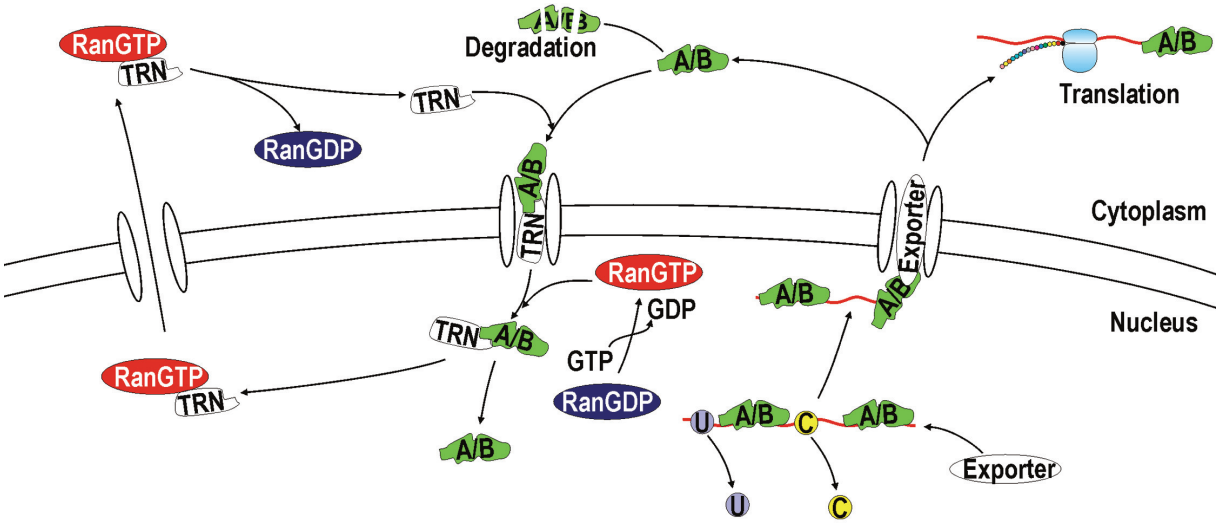

Figure 4. hnRNP A/B proteins participate in the nuclear export of mature mRNA. The exclusively nuclear-localized hnRNPs, such as hnRNPs C and U, dissociate from the mature transcripts, while the A/B type hnRNPs accompany the RNA through the nuclear pores. This may be mediated by an unidentified exporter. After directing the mRNA to its translation site, the hnRNP A/B proteins are released from the transcript, and destined for degradation or returned to the nucleus with the help of transportins (TRN). The hnRNP A/B proteins are then dissociated from transportins by the GTPase Ran (RanGTP) in the nucleus where they participate in their multiple nuclear roles.

particles and pre-mRNA [109]. Taken together, these studies support the concept that hnRNP A1 participates in the early stages of spliceosome assembly.

Disruption of alternative splicing is associated with cancer, growth hormone deficiency, Frasier syndrome, Parkinson's disease, cystic fibrosis, retinitis pigmentosa, spinal muscular atrophy, and myotonic dystrophy [164]. The targets of hnRNP A/B-mediated splicing include two transcripts essential for the replication of HIV-1 virus [165]. In addition, these hnRNPs modulate the inclusion of alternatively spliced exons of several oncogenes, such as the *K-SAM* exons of human *FGFR2* [166], exon N1 of the *c-src* gene [145], and exon 1α or 1β of *INK4a* [146]. The functions of hnRNP A/B proteins in the alternative splicing of these oncogenes or tumour-related genes may underlie the observation that hnRNP A/B proteins are frequently dysregulated in different types of cancer [167–171]. It is not known how closely the effects of hnRNP A/B proteins in alternative splicing are related to pathological conditions.

Shuttling of hnRNP A/B proteins and the nuclear export of mRNA

Mature transcripts are exported from the nucleus accompanied by an hnRNP complex [172]. The exclusively nuclear-localized hnRNPs, such as C and U dissociate from the complex and are retained in the nucleus whilst shuttling proteins, such as A1, E, and K, migrate into the cytoplasm together with mRNAs and later return to the nucleus [172, 173]. hnRNP A1 is bound to poly(A)$^+$ RNA in both the nucleus and cytoplasm, suggesting it is exported together with the mRNA [34]. More convincingly, the hnRNP A1 ortholog in *Chironomus tentans,* hrp36, has been observed under the electron microscope to accompany mRNA through the nuclear pores to polysomes [174], suggesting association between hnRNP A1 shuttling and mRNA export.

The nucleocytoplasmic transport of hnRNP proteins requires import and export factors that target them to nucleoporin [175]. Two transport receptors of the karyopherin-β family, transportin 1 (Trn1) and transportin 2 (Trn2), have been identified as regulators for the nuclear import of hnRNP A/B proteins [52, 176, 177]. Transportin is capable of binding nucleoporin and docks the hnRNP A1 at the nuclear pore complex during nuclear import (Fig. 4) [178]. Once in the nucleus, the transportin-hnRNP A/B complex is dissociated by the binding of transportin to the GTPase Ran (RanGTP) [177, 179]. The released hnRNP A/B proteins are available for their multiple nuclear functions, and the transportin returns to the cytoplasm where it is dissociated from RanGTP by the binding of the latter to the Ran binding protein (RanBP) and the GTPase activating protein (RanGAP) [173, 180].

In the GRD of hnRNPs A1 and A2/B1, a 38-residue M9 motif which bears no sequence similarity to the classical nuclear localization signal mediates their interaction with transportins [52, 176, 177]. Residues 3–21 of the M9 motif form the core signal peptide, of which residues 3–8 and 20–21 are particularly

important: a single mutation in one of the two sequences abolishes the binding of transportin and hence nuclear uptake [181]. However, the GST-tagged M9 signal exhibits different affinity and specificity for transportin compared to the full-length hnRNP A1, possibly because the M9 sequence is presented in different ways [177]. The potential conformational difference may affect the interaction of the M9 sequence with transportin, which is believed to recognize its cargo protein through secondary/tertiary structural features rather than primary sequence [179]. A 19-amino acid "F-peptide" adjacent to the M9 signal regulates the bidirectional transport of hnRNP A1. Its phosphorylation weakens the hnRNP A1-transportin interaction and decreases the nuclear import of hnRNP A1. Phosphorylation of the F-peptide may mediate the accessibility of the M9 signal by changing its tertiary structure [182].

Newly synthesized mRNA acts as an inducer for the nuclear import of hnRNP A/B proteins, although the signalling cascade is not understood. Inhibition of RNA polymerase II in HeLa cells by actinomycin D leads to hnRNP A1 retention in the cytoplasm [34, 183]. Similarly, in transcriptionally inactive mouse embryos, hnRNP A1 diffuses passively through the nuclear pores. When the actinomycin D is removed, hnRNP A1 starts to accumulate in the nucleus. Production of the new transcripts in the nucleus is necessary and sufficient to induce the nuclear accumulation of hnRNP A1, while the presence of newly synthesized RNAs in the cytoplasm has no such effect [183]. Blocking of transcription with actinomycin D or α-amanitin also disrupts the localisation of A2/B1 hnRNPs within the nucleus [184].

The molecular mechanism by which hnRNP A/B proteins regulate nuclear export of mRNA is not well understood. Although the M9 sequence is believed to be a signal for nuclear import and export of these hnRNPs [53], it is uncertain whether transportins function as bidirectional factors [177, 185, 186]. hnRNP A1 associates with the mRNA export factor [172] which represents a family of proteins involved in TAP/Mex67p-mediated mRNA export [187]. Nuclear export of hnRNP A2/B1 can be induced by the activation of the chemokine receptor CXCR4, in the presence of cyclophilin A, which forms a complex with hnRNP A2/B1 [188]. It is uncertain whether CXCR4 functions as a nuclear export factor in the M9-mediated pathway.

Other factors might also influence hnRNP A1-mediated nuclear export. The nuclear export of the *Saccharomyces cerevisiae* hnRNP A1 homolog, Hrp1p, and another hnRNP A/B family protein, Npl3p, requires arginine methylation by Hmt1p. When Hmt1p is inhibited, the two hnRNP proteins are retained in the nucleus [189]. Although methylation of human hnRNP A1 is not known to regulate the nucleocytoplasmic transport of the protein, methylation at four arginine residues (R193, R205, R217, and R224) within the RGG box affects its RNA-binding properties [67, 88].

Involvement of phosphorylation in the regulation of nuclear export and import of hnRNP A/B protein is suggested by the accumulation of hnRNP A1 in the cytoplasm when either protein kinase C ζ (PKC ζ) or protein kinase A (PKA) is over-expressed [190, 191]. An hnRNP A1 peptide including Ser^{199} has been identified as the substrate for the two kinases. In addition, phosphorylation may also affect the interaction between hnRNP A/B proteins and their cargo mRNA. The MAP kinase signal-integrating kinase (Mnk)-mediated phosphorylation of hnRNP A1 inhibits its binding to tumour necrosis factor α (TNFα) mRNA *in vivo* [192].

hnRNP A2 is a *trans*-acting factor involved in the trafficking of mRNAs possessing an A2RE11-like *cis*-acting element. It has been proposed that tetramers of A2 bind A2RE11-containing mRNAs in the nucleus and orchestrate their export from the cell nucleus [180, 193]. After export, the complex binds hnRNP E1, which represses translation until the trafficking granule has moved along the microtubules and reached its destination in the periphery of the cell.

The future and concluding remarks

The hnRNP A/B subfamily exhibits affinity for a spectrum of nucleic acid motifs, including the ssDNA telomeric repeat, the U-rich motif and A2RE. These proteins appear to possess two classes of nucleic acid binding sites, both of which largely map to the tandem RRMs. One class associates with single-stranded nucleic acid without a strong preference for a particular nucleotide sequence: these sites bind less tightly and are disrupted by addition of polyanions, such as heparin, which are commonly added to suppress non-specific protein/nucleic acid interactions [56]. These interactions are typified by the use of native ssDNA in the purification of hnRNP A/B proteins.

The second class of sites binds more tightly, with dissociation constants typically in the 10–50 nM range, and shows preference for particular motifs, but not for a strictly defined consensus sequence. These motifs may include a few highly conserved nucleotides within a matrix of less conserved or non-conserved nucleotides (e.g. the A/B hnRNPs bind the telomeric repeat sequence, but this interaction is largely unaffected by the substitution of nucleotides in many positions [44]). A second example is the binding

to exonic and intronic splicing silencer motifs and the competitive interactions that lead to the antagonistic binding directed against ASF/SF2 during splice site selection.

The hnRNP A/B glycine-rich domain binds other proteins but it may also contribute to interactions between the hnRNPs A/B and nucleic acids. For example, the full-length hnRNP A2 binds the cytoplasmic trafficking motif A2RE more tightly than does the tandem RRMs of this protein [56]. The possession of two or more binding sites on these hnRNPs may enable them to act as adaptors between DNA or RNA and other functionally specific factors. For example, hnRNP A1, A2 and A3 may help to recruit the telomerase to the telomeric RNA template [44, 113].

It will be of great interest to determine whether the individual mammalian hnRNP A/B proteins also associate with groups of functionally related transcripts. For example, the yeast homologs of two hnRNP A/B proteins, Npl3 and Nab4/Hrp1, manifest RNA binding profiles of functional significance [194]. Npl3 favours binding to mRNAs encoding ribosomal proteins and other highly expressed transcripts, whereas the transcripts for proteins involved in amino acid metabolism are enriched among the Hrp1-binding molecules [194].

Many, if not all, of the hnRNP proteins appear to be multifunctional, as noted above for the A/B hnRNPs. Some of these functions overlap for different proteins. For example, both hnRNPs A/B and K [195] have been implicated in transcription, RNA processing, translation and signal transduction processes and pathways. hnRNPs A1, A2 and A3 may manifest some similar functions, but our recent studies [184] show that all three are not expressed in the same location. In HeLa cells, A1 is concentrated close to the nuclear envelope whereas A2 and A3 are instead prominent in the perinucleolar region, suggesting that they have different intra-nuclear roles.

Further functional diversity is generated by post-transcriptional (including alternative splicing) and post-translational regulation of gene expression. Another field that has only been superficially addressed is the extent, species variation, and spatial and temporal distribution within tissues of protein molecules which have undergone different post-translational modifications, particularly methylation and phosphorylation.

Alternatively spliced isoforms may be directed to separate locations where they have different functions. One might expect, for example, to find different hnRNP A/B isoforms associated with components of the telomeres compared with the spliceosome. The clearest evidence we have to date for the hnRNPs is the differences in localisation of hnRNP A2 isoforms. The exon 9-expressing hnRNP A2 and B1 isoforms are confined to the nucleus, whereas the A2b (and probably B1b) isoform is present at far lower concentrations in the nucleus and more abundantly in the processes of oligodendrocytes and dendrites of neurons (unpublished data). There are also strong temporal variations: in rodents, the levels of A2b and B1b are low at birth, rise sharply for a few days and then decline to being barely detectable in mature adult animals. Does this molecular distribution point to differences in function for these isoforms? It is tempting to speculate that the A2b and B1b isoforms are positioned to participate in two of the major processes with which hnRNP A2 has been associated: cytoplasmic mRNA trafficking and local regulation of translation.

Some of the roles of the hnRNPs A/B can be inferred from the molecules with which they interact and the location of these proteins. It is currently difficult to predict the binding partners for these hnRNPs, but the evolving genomic or proteomic technologies present powerful new tools for high-throughput identification of the interacting molecules. In recent experiments, shRNA-induced knockdown of hnRNP A2 was used with DNA microarrays to identify downstream targets of A2 [79]. A substantial number of transcripts with no known hnRNP A2-specific binding sequence were found to form complexes with this protein, possibly by indirect binding. The increasing use of siRNA techniques to knock down target genes and the generation of conditional knock-out mice offer considerable promise in exploration of the wide range of activities associated with individual hnRNP A/B proteins, their alternatively spliced isoforms and breakdown products.

Finally, complexes within cells may exist transiently or change their composition with time, but even transient interactions may fulfil important biological functions. Thus, while there is substantial evidence to support the involvement of the hnRNPs in alternative splicing of RNA, at some stages spliceosomes can be isolated that do not include the A/B hnRNPs. Yet this protein subfamily is one of the few classes of protein that is present at most stages of splicing. The major challenge is deciphering the reasons, at the molecular and cellular levels, for the genomic and proteomic complexity of the hnRNPs.

1 Dreyfuss, G., Matunis, M. J., Piñol-Roma, S. and Burd, C. G. (1993) hnRNP proteins and the biogenesis of mRNA. Annu. Rev. Biochem. 62, 289–321.

2 Piñol-Roma, S., Choi, Y. D., Matunis, M. J. and Dreyfuss, G. (1988) Immunopurification of heterogeneous nuclear ribonucleoprotein particles reveals an assortment of RNA-binding proteins. Genes Dev. 2, 215–227.

3 Pullman, J. M. and Martin, T. E. (1983) Reconstitution of nucleoprotein complexes with mammalian heterogeneous nuclear ribonucleoprotein (hnRNP) core proteins. J. Biol. Chem. 97, 99–111.

4 Carpenter, B., MacKay, C., Alnabulsi, A., MacKay, M., Telfer, C., Melvin, W. T. and Murray, G. I. (2006) The roles of heterogeneous nuclear ribonucleoproteins in tumour development and progression. Biochim. Biophys. Acta 1765, 85–100.

5 Krecic, A. M. and Swanson, M. S. (1999) hnRNP complexes: composition, structure, and function. Curr. Opin. Cell. Biol. 11, 363–371.

6 Bennett, M., Piñol-Roma, S., Staknis, D., Dreyfuss, G. and Reed, R. (1992) Differential binding of heterogeneous nuclear ribonucleoproteins to mRNA precursors prior to spliceosome assembly in vitro. Mol. Cell. Biol. 12, 3165–3175.

7 Matunis, E. L., Matunis, M. J. and Dreyfuss, G. (1993) Association of individual hnRNP proteins and snRNPs with nascent transcripts. J. Cell Biol. 121, 219–228.

8 Cartegni, L., Maconi, M., Morandi, E., Cobianchi, F., Riva, S. and Biamonti, G. (1996) hnRNP A1 selectively interacts through its Gly-rich domain with different RNA-binding proteins. J. Mol. Biol. 259, 337–348.

9 Dreyfuss, G., Kim, V. N. and Kataoka, N. (2002) Messenger-RNA-binding proteins and the messages they carry. Nat. Rev. Mol. Cell. Biol. 3, 195–205.

10 Glisovic, T., Bachorik, J. L., Yong, J. and Dreyfuss, G. (2008) RNA-binding proteins and post-transcriptional gene regulation. FEBS Lett. 582, 1977–1986.

11 Minoo, P., Martin, T. E. and Riehl, R. M. (1991) Nucleic acid binding characteristics of group A/B hnRNP proteins. Biochem. Biophys. Res. Commun. 176, 747–755.

12 Beyer, A. L., Christensen, M. E., Walker, B. W. and LeStourgeon, W. M. (1977) Identification and characterization of the packaging proteins of core 40S hnRNP particles. Cell 11, 127–138.

13 Myer, V. E. and Steitz, J. A. (1995) Isolation and characterization of a novel, low abundance hnRNP protein: A0. RNA 1, 171–182.

14 Ma, A. S., Moran-Jones, K., Shan, J., Munro, T. P., Snee, M. J., Hoek, K. S. and Smith, R. (2002) Heterogeneous nuclear ribonucleoprotein A3, a novel RNA trafficking response element-binding protein. J. Biol. Chem. 277, 18010–18020.

15 Kumar, A., Williams, K. R. and Szer, W. (1986) Purification and domain structure of core hnRNP proteins A1 and A2 and their relationship to single-stranded DNA-binding proteins. J. Biol. Chem. 261, 11266–11273.

16 Burd, C. G., Swanson, M. S., Gorlach, M. and Dreyfuss, G. (1989) Primary structures of the heterogeneous nuclear ribonucleoprotein A2, B1, and C2 proteins: a diversity of RNA binding proteins is generated by small peptide inserts. Proc. Natl. Acad. Sci. U.S.A. 86, 9788–9792.

17 Nichols, R. C., Wang, X. W., Tang, J., Hamilton, B. J., High, F. A., Herschman, H. R. and Rigby, W. F. (2000) The RGG domain in hnRNP A2 affects subcellular localization. Exp. Cell Res. 256, 522–532.

18 Akindahunsi, A. A., Bandiera, A. and Manzini, G. (2005) Vertebrate 2xRBD hnRNP proteins: a comparative analysis of genome, mRNA and protein sequences. Comput. Biol. Chem. 29, 13–23.

19 Kim, Y. J. and Baker, B. S. (1993) Isolation of RRM-type RNA-binding protein genes and the analysis of their relatedness by using a numerical approach. Mol. Cell. Biol. 13, 174–183.

20 Biamonti, G., Ruggiu, M., Saccone, S., Della Valle, G. and Riva, S. (1994) Two homologous genes, originated by duplication, encode the human hnRNP proteins A2 and A1. Nucleic Acids Res. 22, 1996–2002.

21 Kozu, T., Henrich, B. and Schafer, K. P. (1995) Structure and expression of the gene (HNRPA2B1) encoding the human hnRNP protein A2/B1. Genomics 25, 365–371.

22 Dangli, A., Plomaritoglou, A., Boutou, E., Vassiliadou, N., Moutsopoulos, H. M. and Guialis, A. (1996) Recognition of subsets of the mammalian A/B-type core heterogeneous nuclear ribonucleoprotein polypeptides by novel autoantibodies. Biochem. J. 320 (Pt 3), 761–767.

23 Shi, S. T., Yu, G. Y. and Lai, M. M. (2003) Multiple type A/B heterogeneous nuclear ribonucleoproteins (hnRNPs) can replace hnRNP A1 in mouse hepatitis virus RNA synthesis. J. Virol. 77, 10584–10593.

24 Yang, X., Bani, M. R., Lu, S. J., Rowan, S., Ben-David, Y. and Chabot, B. (1994) The A1 and A1B proteins of heterogeneous nuclear ribonucleoparticles modulate 5′ splice site selection in vivo. Proc. Natl. Acad. Sci. U.S.A. 91, 6924–6928.

25 Kamma, H., Horiguchi, H., Wan, L., Matsui, M., Fujiwara, M., Fujimoto, M., Yazawa, T. and Dreyfuss, G. (1999) Molecular characterization of the hnRNP A2/B1 proteins: tissue-specific expression and novel isoforms. Exp. Cell. Res. 246, 399–411.

26 Kumar, A., Sierakowska, H. and Szer, W. (1987) Purification and RNA binding properties of a C-type hnRNP protein from HeLa cells. J. Biol. Chem. 262, 17126–17137.

27 Khan, F. A., Jaiswal, A. K. and Szer, W. (1991) Cloning and sequence analysis of a human type A/B hnRNP protein. FEBS Lett. 290, 159–161.

28 Pandolfo, M., Valentini, O., Biamonti, G., Morandi, C. and Riva, S. (1985) Single stranded DNA binding proteins derive from hnRNP proteins by proteolysis in mammalian cells. Nucleic Acids Res. 13, 6577–6590.

29 Steiner, G., Skriner, K., Hassfeld, W. and Smolen, J. S. (1996) Clinical and immunological aspects of autoantibodies to RA33/hnRNP-A/B proteins–a link between RA, SLE and MCTD. Mol. Biol. Rep. 23, 167–171.

30 Kiledjian, M., Burd, C. G., Portman, D. S. and Dreyfuss, G. (1994) in: RNA-Protein Interactions: Frontiers in Molecular Biology, pp. 127–149 (Nagai, K. and Mattaj, I.W., Eds.) IRL Press, Oxford.

31 Piñol-Roma, S., Swanson, M. S., Gall, J. G. and Dreyfuss, G. (1989) A novel heterogeneous nuclear RNP protein with a unique distribution on nascent transcripts. J. Cell Biol. 109, 2575–2587.

32 Leser, G. P., Escara-Wilke, J. and Martin, T. E. (1984) Monoclonal antibodies to heterogeneous nuclear RNA-protein complexes. The core proteins comprise a conserved group of related polypeptides. J. Biol. Chem. 259, 1827–1833.

33 He, Y., Brown, M. A., Rothnagel, J. A., Saunders, N. A. and Smith, R. (2005) Roles of heterogeneous nuclear ribonucleoproteins A and B in cell proliferation. J. Cell Sci. 118, 3173–3183.

34 Piñol-Roma, S. and Dreyfuss, G. (1992) Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature 355, 730–732.

35 Weighardt, F., Biamonti, G. and Riva, S. (1996) The roles of heterogeneous nuclear ribonucleoproteins (hnRNP) in RNA metabolism. BioEssays 18, 747–756.

36 Shyu, A.-B. and Wilkinson, M. F. (2000) The double lives of shuttling mRNA binding proteins. Cell 102, 135–138.

37 Lothstein, L., Arenstorf, H. P., Chung, S. Y., Walker, B. W., Wooley, J. C. and LeStourgeon, W. M. (1985) General organization of protein in HeLa 40S nuclear ribonucleoprotein particles. J. Cell Biol. 100, 1570–1581.

38 Walker, B. W., Lothstein, L., Baker, C. L. and LeStourgeon, W. M. (1980) The release of 40S hnRNP particles by brief digestion of HeLa nuclei with micrococcal nuclease. Nucleic Acids Res. 8, 3639–3657.

39 Barnett, S. F., Theiry, T. A. and LeStourgeon, W. M. (1991) The core proteins A2 and B1 exist as $(A2)_3B1$ tetramers in 40S nuclear ribonucleoprotein particles. Mol. Cell. Biol. 11, 864–871.

40 Percipalle, P., Jonsson, A., Nashchekin, D., Karlsson, C., Bergman, T., Guialis, A. and Daneholt, B. (2002) Nuclear actin is associated with a specific subset of hnRNP A/B-type proteins. Nucleic Acids Res. 30, 1725–1734.

41 Ishikawa, F., Matunis, M. J., Dreyfuss, G. and Cech, T. R. (1993) Nuclear proteins that bind the pre-mRNA 3′ splice site sequence r(UUAG/G) and the human telomeric DNA sequence d(TTAGGG)n. Mol. Cell. Biol. 13, 4301–4310.
42 Tanaka, E., Fukuda, H., Nakashima, K., Tsuchiya, N., Seimiya, H. and Nakagama, H. (2007) HnRNP A3 binds to and protects mammalian telomeric repeats in vitro. Biochem. Biophys. Res. Commun. 358, 608–614.
43 Zhang, Q. S., Manche, L., Xu, R. M. and Krainer, A. R. (2006) hnRNP A1 associates with telomere ends and stimulates telomerase activity. RNA 12, 1116–1128.
44 Moran-Jones, K., Wayman, L., Kennedy, D. D., Reddel, R. R., Sara, S., Snee, M. J. and Smith, R. (2005) hnRNP A2, a potential ssDNA/RNA molecular adapter at the telomere. Nucleic Acids Res. 33, 486–496.
45 Leverrier, S., Cinato, E., Paul, C., Derancourt, J., Bemark, M., Leanderson, T. and Legraverend, C. (2000) Purification and cloning of type A/B hnRNP proteins involved in transcriptional activation from the Rat spi 2 gene GAGA box. Biol. Chem. 381, 1031–1040.
46 Xia, H. (2005) Regulation of gamma-fibrinogen chain expression by heterogeneous nuclear ribonucleoprotein A1. J. Biol. Chem. 280, 13171–13178.
47 Mayeda, A. and Krainer, A. R. (1992) Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. Cell 68, 365–375.
48 Mayeda, A., Munroe, S. H., Caceres, J. F. and Krainer, A. R. (1994) Function of conserved domains of hnRNP A1 and other hnRNP A/B proteins. EMBO J. 13, 5483–5495.
49 Nasim, F. U., Hutchison, S., Cordeau, M. and Chabot, B. (2002) High-affinity hnRNP A1 binding sites and duplex-forming inverted repeats have similar effects on 5′ splice site selection in support of a common looping out and repression mechanism. RNA 8, 1078–1089.
50 Bilodeau, P. S., Domsic, J. K., Mayeda, A., Krainer, A. R. and Stoltzfus, C. M. (2001) RNA splicing at human immunodeficiency virus type 1 3′ splice site A2 is regulated by binding of hnRNP A/B proteins to an exonic splicing silencer element. J. Virol. 75, 8487–8497.
51 Hutchison, S., LeBel, C., Blanchette, M. and Chabot, B. (2002) Distinct sets of adjacent heterogeneous nuclear ribonucleoprotein (hnRNP) A1/A2 binding sites control 5′ splice site selection in the hnRNP A1 mRNA precursor. J. Biol. Chem. 277, 29745–29752.
52 Pollard, V. W., Michael, W. M., Nakielny, S., Siomi, M. C., Wang, F. and Dreyfuss, G. (1996) A novel receptor-mediated nuclear protein import pathway. Cell 86, 985–994.
53 Michael, W. M., Choi, M. and Dreyfuss, G. (1995) A nuclear export signal in hnRNP A1: a signal-mediated, temperature-dependent nuclear protein export pathway. Cell 83, 415–422.
54 Brumwell, C., Antolik, C., Carson, J. H. and Barbarese, E. (2002) Intracellular trafficking of hnRNP A2 in oligodendrocytes. Exp. Cell Res. 279, 310–320.
55 Munro, T. P., Magee, R. J., Kidd, G. J., Carson, J. H., Barbarese, E., Smith, L. M. and Smith, R. (1999) Mutational analysis of a heterogeneous nuclear ribonucleoprotein A2 response element for RNA trafficking. J. Biol. Chem. 274, 34389–34395.
56 Shan, J., Moran-Jones, K., Munro, T. P., Kidd, G. J., Winzor, D. J., Hoek, K. S. and Smith, R. (2000) Binding of an RNA trafficking response element to heterogeneous nuclear ribonucleoproteins A1 and A2. J. Biol. Chem. 275, 38286–38295.
57 Smith, R. (2004) Moving molecules: mRNA trafficking in mammalian oligodendrocytes and neurons. Neuroscientist 10, 495–500.
58 Iervolino, A., Santilli, G., Trotta, R., Guerzoni, C., Cesi, V., Bergamaschi, A., Gambacorti-Passerini, C., Calabretta, B. and Perrotti, D. (2002) hnRNP A1 nucleocytoplasmic shuttling activity is required for normal myelopoiesis and BCR/ABL leukemogenesis. Mol. Cell. Biol. 22, 2255–2266.
59 Hamilton, B. J., Nichols, R. C., Tsukamoto, H., Boado, R. J., Pardridge, W. M. and Rigby, W. F. (1999) hnRNP A2 and hnRNP L bind the 3′UTR of glucose transporter 1 mRNA and exist as a complex in vivo. Biochem. Biophys. Res. Commun. 261, 646–651.
60 Bonnal, S., Pileur, F., Orsini, C., Parker, F., Pujol, F., Prats, A. C. and Vagner, S. (2005) Heterogeneous nuclear ribonucleoprotein A1 Is a novel internal ribosome entry site trans-acting factor that modulates alternative initiation of translation of the fibroblast growth factor 2 mRNA. J. Biol. Chem. 280, 4144–4153.
61 Kwon, S., Barbarese, E. and Carson, J. H. (1999) The cis-acting RNA trafficking signal from myelin basic protein mRNA and its cognate trans-acting ligand hnRNP A2 enhance cap-dependent translation. J. Cell Biol. 147, 247–256.
62 Herrick, G. and Alberts, B. (1976) Purification and physical characterization of nucleic acid helix-unwinding proteins from calf thymus. J. Biol. Chem. 251, 2124–2132.
63 Nadler, S. G., Merrill, B. M., Roberts, W. J., Keating, K. M., Lisbin, M. J., Barnett, S. F., Wilson, S. H. and Williams, K. R. (1991) Interactions of the A1 heterogeneous nuclear ribonucleoprotein and its proteolytic derivative, UP1, with RNA and DNA: evidence for multiple RNA binding domains and salt-dependent binding mode transitions. Biochemistry 30, 2968–2976.
64 Chen, H., Hewison, M., Hu, B. and Adams, J. S. (2003) Heterogeneous nuclear ribonucleoprotein (hnRNP) binding to hormone response elements: a cause of vitamin D resistance. Proc. Natl. Acad. Sci. U.S.A. 100, 6109–6114.
65 Donev, R., Horton, R., Beck, S., Doneva, T., R., V., Bowen, W. R. and Sheer, D. (2003) Recruitment of heterogeneous nuclear ribonucleoprotein A1 in vivo to the LMP/TAP region of the major histocompatibility complex. J. Biol. Chem. 278, 5214–5226.
66 Cobianchi, F., Karpel, R. L., Williams, K. R., Notario, V. and Wilson, S. H. (1988) Mammalian heterogeneous nuclear ribonucleoprotein complex protein A1. Large-scale overproduction in Escherichia coli and cooperative binding to single-stranded nucleic acids. J. Biol. Chem. 263, 1063–1071.
67 Kim, S., Merrill, B. M., Rajpurohit, R., Kumar, A., Stone, K. L., Papov, V. V., Schneiders, J. M., Szer, W., Wilson, S. H., Paik, W. K. and Williams, K. R. (1997) Identification of N(G)-methylarginine residues in human heterogeneous RNP protein A1: Phe/Gly-Gly-Gly-Arg-Gly-Gly-Gly/Phe is a preferred recognition motif. Biochemistry 36, 5185–5192.
68 Rajpurohit, R., Paik, W. K. and Kim, S. (1994) Effect of enzymic methylation of heterogeneous ribonucleoprotein particle A1 on its nucleic-acid binding and controlled proteolysis. Biochem. J. 304, 903–909.
69 Kumar, A., Casas-Finet, J. R., Luneau, C. J., Karpel, R. L., Merrill, B. M., Williams, K. R. and Wilson, S. H. (1990) Mammalian heterogeneous nuclear ribonucleoprotein Al: nucleic acid binding properties of the COOH-terminal domain. J. Biol. Chem. 265, 17094–17100.
70 Pontius, B. W. and Berg, P. (1992) Rapid assembly and disassembly of complementary DNA strands through an equilibrium intermediate state mediated by A1 hnRNP protein. J. Biol. Chem. 267, 13815–13818.
71 Guillonneau, F., Guieysse, A. L., Le Caer, J. P., Rossier, J. and Praseuth, D. (2001) Selection and identification of proteins bound to DNA triple-helical structures by combination of 2D-electrophoresis and MALDI-TOF mass spectrometry. Nucleic Acids Res. 29, 2427–2436.
72 Norzehan, A.-M. and Williams, K. R. (1996) hnRNP A1 binds promiscuously to oligoribonucleotides: utilization of random and homo-oligonucleotides to discriminate sequence from base-specific binding Nucleic Acids Res. 24, 4063–4070.
73 Burd, C. G. and Dreyfuss, G. (1994) RNA binding specificity of hnRNP A1: significance of hnRNP A1 high-affinity binding sites in pre-mRNA splicing. EMBO J. 13, 1197–1204.
74 Hamilton, B. J., Nagy, E., Malter, J. S., Arrick, B. A. and Rigby, W. F. (1993) Association of heterogeneous nuclear

ribonucleoprotein A1 and C proteins with reiterated AUUUA sequences. J. Biol. Chem. 268, 8881–8887.
75 Ainger, K., Avossa, D., Diana, A. S., Barry, C., Barbarese, E. and Carson, J. H. (1997) Transport and localization elements in myelin basic protein mRNA. J. Cell Biol. 138, 1077–1087.
76 Hoek, K. S., Kidd, G. J., Carson, J. H. and Smith, R. (1998) hnRNP A2 selectively binds the cytoplasmic transport sequence of myelin basic protein mRNA. Biochemistry 37, 7021–7029.
77 Mouland, A. J., Xu, H., Cui, H., Krueger, W., Munro, T. P., Prasol, M., Mercier, J., Rekosh, D., Smith, R., Barbarese, E., Cohen, E. A. and Carson, J. H. (2001) RNA trafficking signals in human immunodeficiency virus type 1. Mol. Cell. Biol. 21, 2133–2143.
78 Kajita, Y., Nakayama, J., Aizawa, M. and Ishikawa, F. (1995) The UUAG-specific RNA binding protein, heterogeneous nuclear ribonucleoprotein D0. Common modular structure and binding properties of the 2xRBD-Gly family. J. Biol. Chem. 270, 22167–22175.
79 He, Y., Rothnage,l J. A., Epis, M. R., Leedman, P. J. and Smith, R. (2008) Downstream targets of heterogeneous nuclear ribonucleoprotein A2 mediate cell proliferation. Mol. Carcinog. Aug 4. [Epub ahead of print]
80 Cok, S. J., Acton, S. J., Sexton, A. E. and Morrison, A. R. (2004) Identification of RNA binding proteins in RAW 264.7 cells that recognize an LPS responsive element in the 3-UTR of the murine COX-2 mRNA. J. Biol. Chem. 279, 8196–8205..
81 Kumar, A. and Wilson, S. H. (1990) Studies of the strand-annealing activity of mammalian hnRNP complex protein A1. Biochemistry 29, 10717–10722.
82 Pontius, B. W. and Berg, P. (1990) Renaturation of complementary DNA strands mediated by purified mammalian heterogeneous nuclear ribonucleoprotein A1 protein: implications for a mechanism for rapid molecular assembly. Proc. Natl. Acad. Sci. U.S.A. 87, 8403–8407.
83 Han, H., Bennett, R. J. and Hurley, L. H. (2000) Inhibition of unwinding of G-quadruplex structures by Sgs1 helicase in the presence of N,N′-bis[2-(1-piperidino)ethyl]-3,4,9,10-perylenetetracarboxylic diimide, a G-quadruplex-interactive ligand. Biochemistry 39, 9311–9316.
84 Kan, Z. Y., Lin, Y., Wang, F., Zhuang, X. Y., Zhao, Y., Pang, D. W., Hao, Y. H. and Tan, Z. (2007) G-quadruplex formation in human telomeric (TTAGGG)4 sequence with complementary strand in close vicinity under molecularly crowded condition. Nucleic Acids Res. 35, 3646–3653.
85 Fukuda, H., Katahira, M., Tsuchiya, N., Enokizono, Y., Sugimura, T., Nagao, M. and Nakagama, H. (2002) Unfolding of quadruplex structure in the G-rich strand of the minisatellite repeat by the binding protein UP1. Proc. Natl. Acad. Sci. U.S.A 99, 12685–12690.
86 Nakagama, H., Higuchi, K., Tanaka, E., Tsuchiya, N., Nakashima, K., Katahira, M. and Fukuda, H. (2006) Molecular mechanisms for maintenance of G-rich short tandem repeats capable of adopting G4 DNA structures. Mutat. Res. 598, 120–131.
87 Khateb, S., Weisman-Shomer, P., Hershco, I., Loeb, L. A. and Fry, M. (2004) Destabilization of tetraplex structures of the fragile X repeat sequence (CGG)n is mediated by homolog-conserved domains in three members of the hnRNP family. Nucleic Acids Res. 32, 4145–4154.
88 Williams, K. R., Stone, K. L., LoPresti, M. B., Merrill, B. M. and Planck, S. R. (1985) Amino acid sequence of the UP1 calf thymus helix-destabilizing protein and its homology to an analogous protein from mouse myeloma. Proc. Natl. Aca. Sc. U.S.A. 82, 5666–5670.
89 Chai, Q., Zheng, L., Zhou, M., Turchi, J. J. and Shen, B. (2003) Interaction and stimulation of human FEN-1 nuclease activities by heterogeneous nuclear ribonucleoprotein A1 in alpha-segment processing during Okazaki fragment maturation. Biochemistry 42, 15045–15052.
90 Vera, J., Jaumot, M., Estanyol, J. M., Brun, S., Agell, N. and Bachs, O. (2006) Heterogeneous nuclear ribonucleoprotein A2 is a SET-binding protein and a PP2A inhibitor. Oncogene 25, 260–270.
91 Eddy, J. and Maizels, N. (2008) Conserved elements with potential to form polymorphic G-quadruplex structures in the first intron of human genes. Nucleic Acids Res. 36, 1321–1333.
92 Xu, Y. and Sugiyama, H. (2006) Formation of the G-quadruplex and i-motif structures in retinoblastoma susceptibility genes (Rb). Nucleic Acids Res. 34, 949–954.
93 Trzcinska-Daneluti, A. M., Gorecki, A., Czubaty, A., Kowalska-Loth, B., Girstun, A., Murawska, M., Lesyng, B. and Staron, K. (2007) RRM proteins interacting with the cap region of topoisomerase I. J. Mol. Biol. 369, 1098–1112.
94 Gupta, M., Fujimori, A. and Pommier, Y. (1995) Eukaryotic DNA topoisomerases I. Biochim. Biophys. Acta 1262, 1–14.
95 Marchand, C., Pourquier, P., Laco, G. S., Jing, N. and Pommier, Y. (2002) Interaction of human nuclear topoisomerase I with guanosine quartet-forming and guanosine-rich single-stranded DNA and RNA oligonucleotides. J. Biol. Chem. 277, 8906–8911.
96 Slijepcevic, P. and Al-Wahiby, S. (2005) Telomere biology: integrating chromosomal end protection with DNA damage response. Chromosoma 114, 275–285.
97 Moyzis, R. K., Buckingham, J. M., Cram, L. S., Dani, M., Deaven, L. L., Jones, M. D., Meyne, J., Ratliff, R. L. and Wu, J. R. (1988) A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc. Natl. Aca. Sci. U.S.A. 85, 6622–6626.
98 Blackburn, E. H. (1991) Structure and function of telomeres. Nature 350, 569–573.
99 Wellinger, R. J., Ethier, K., Labrecque, P. and Zakian, V. A. (1996) Evidence for a new step in telomere maintenance. Cell 85, 423–433.
100 Makarov, V. L., Hirose, Y. and Langmore, J. P. (1997) Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. Cell 88, 657–666.
101 Wright, W. E., Tesmer, V. M., Huffman, K. E., Levene, S. D. and Shay, J. W. (1997) Normal human chromosomes have long G-rich telomeric overhangs at one end. Genes Dev. 11, 2801–2809.
102 Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H. and de Lange, T. (1999) Mammalian telomeres end in a large duplex loop. Cell 97, 503–514.
103 Neumann, A. A. and Reddel, R. R. (2002) Telomere maintenance and cancer – look, no telomerase. Nat. Rev. Cancer 2, 879–884.
104 Reddel, R. R. (2003) Alternative lengthening of telomeres, telomerase, and cancer. Cancer Lett. 194, 155–162.
105 Meeker, A. K. and De Marzo, A. M. (2004) Recent advances in telomere biology: implications for human cancer. Curr. Opin. Oncol. 16, 32–38.
106 Artandi, S. E. and DePinho, R. A. (2000) A critical role for telomeres in suppressing and facilitating carcinogenesis. Curr. Opin. Genet. Dev. 10, 39–46.
107 Feldser, D. M., Hackett, J. A. and Greider, C. W. (2003) Telomere dysfunction and the initiation of genome instability. Nat. Rev. Cancer 3, 623–627.
108 LaBranche, H., Dupuis, S., Ben-David, Y., Bani, M. R., Wellinger, R. J. and Chabot, B. (1998) Telomere elongation by hnRNP A1 and a derivative that interacts with telomeric repeats and telomerase. Nat. Genet. 19, 199–202.
109 Ding, J., Hayashi, M. K., Zhang, Y., Manche, L., Krainer, A. R. and Xu, R. M. (1999) Crystal structure of the two-RRM domain of hnRNP A1 (UP1) complexed with single-stranded telomeric DNA. Genes Dev. 13, 1102–1115.
110 McKay, S. J. and Cooke, H. (1992) hnRNP A2/B1 binds specifically to single stranded vertebrate telomeric repeat TTAGGGn. Nucleic Acids Res. 20, 6461–6464.
111 Kamma, H., Fujimoto, M., Fujiwara, M., Matsui, M., Horiguchi, H., Hamasaki, M. and Satoh, H. (2001) Interaction

of hnRNP A2/B1 isoforms with telomeric ssDNA and the in vitro function. Biochem Biophys Res Commun 280, 625–630.
112 Joeng, K. S., Song, E. J., Lee, K. J. and Lee, J. (2004) Long lifespan in worms with long telomeric DNA. Nat. Genet. 36, 607–611.
113 Fiset, S. and Chabot, B. (2001) hnRNP A1 may interact simultaneously with telomeric DNA and the human telomerase RNA in vitro. Nucleic Acids Res. 29, 2268–2275.
114 Williamson, J. R., Raghuraman, M. K. and Cech, T. R. (1989) Monovalent cation-induced structure of telomeric DNA: the G-quartet model. Cell 59, 871–880.
115 Zahler, A. M., Williamson, J. R., Cech, T. R. and Prescott, D. M. (1991) Inhibition of telomerase by G-quartet DNA structures. Nature 350, 718–720.
116 Dallaire, F., Dupuis, S., Fiset, S. and Chabot, B. (2000) Heterogeneous nuclear ribonucleoprotein A1 and UP1 protect mammalian telomeric repeats and modulate telomere replication in vitro. J. Biol. Chem. 275, 14509–14516.
117 Huang, P. R., Tsai, S. T., Hsieh, K. H. and Wang, T. C. (2008) Heterogeneous nuclear ribonucleoprotein A3 binds single-stranded telomeric DNA and inhibits telomerase extension in vitro. Biochim. Biophys. Acta 1783, 193–202.
118 Iwanaga, K., Sueoka, N., Sato, A., Hayashi, S. and Sueoka, E. (2005) Heterogeneous nuclear ribonucleoprotein B1 protein impairs DNA repair mediated through the inhibition of DNA-dependent protein kinase activity. Biochem. Biophys. Res. Commun. 333, 888–895.
119 Smith, G. C. and Jackson, S. P. (1999) The DNA-dependent protein kinase. Genes Dev. 13, 916–934.
120 Takimoto, M., Tomonaga, T., Matunis, M., Avigan, M., Krutzsch, H., Dreyfuss, G. and Levens, D. (1993) Specific binding of heterogeneous ribonucleoprotein particle protein K to the human c-myc promoter, in vitro. J. Biol. Chem. 268, 18249–18258.
121 Campillos, M., Lamas, J. R., Garcia, M. A., Bullido, M. J., Valdivieso, F. and Vazquez, J. (2003) Specific interaction of heterogeneous nuclear ribonucleoprotein A1 with the -219T allelic form modulates APOE promoter activity. Nucleic Acids Res. 31, 3063–3070.
122 Lau, J. S., Baumeister, P., Kim, E., Roy, B., Hsieh, T. Y., Lai, M. and Lee, A. S. (2000) Heterogeneous nuclear ribonucleoproteins as regulators of gene expression through interactions with the human thymidine kinase promoter. J. Cell. Biochem. 79, 395–406.
123 Das, S., Ward, S. V., Markle, D. and Samuel, C. E. (2004) DNA damage binding proteins and hnRNP A1 function as constitutive KCS element components of the interferon-inducible RNA-dependent PKR kinase promoter. J. Biol. Chem. 279, 7313–7321.
124 Thakur, S., Nakamura, T., Calin, G., Russo, A., Tamburrino, J. F., Shimizu, M., Baldassarre, G., Battista, S., Fusco, A., Wassell, R. P., Dubois, G., Alder, H. and Croce, C. M. (2003) Regulation of BRCA1 transcription by specific single-stranded DNA binding factors. Mol. Cell. Biol. 23, 3774–3787.
125 Zhao, S., Korzan, W. J., Chen, C. C. and Fernald, R. D. (2008) Heterogeneous nuclear ribonucleoprotein A/B and G inhibits the transcription of gonadotropin-releasing-hormone. Mol. Cell. Neurosci. 37, 69–84.
126 Makeyev, A. V., Kim, C. B., Ruddle, F. H., Enkhmandakh, B., Erdenechimeg, L. and Bayarsaihan, D. (2005) HnRNP A3 genes and pseudogenes in the vertebrate genomes. J. Exp. Zoolog. A Comp. Exp. Biol. 303, 259–271.
127 Kostadinov, R., Malhotra, N., Viotti, M., Shine, R., D'Antonio, L. and Bagga, P. (2006) GRSDB: a database of quadruplex forming G-rich sequences in alternatively processed mammalian pre-mRNA sequences. Nucleic Acids Res. 34, D119-D124.
128 Rosonina, E., Ip, J. Y., Calarco, J. A., Bakowski, M. A., Emili, A., McCracken, S., Tucker, P., Ingles, C. J. and Blencowe, B. J. (2005) Role for PSF in mediating transcriptional activator-dependent stimulation of pre-mRNA processing in vivo. Mol. Cell. Biol. 25, 6734–6746.
129 Kastan, M. B., Canman, C. E. and Leonard, C. J. (1995) P53, cell cycle control and apoptosis: implications for cancer. Cancer Metastasis Rev. 14, 3–15.
130 Barrandon, C., Bonnet, F., Nguyen, V. T., Labas, V. and Bensaude, O. (2007) The transcription-dependent dissociation of P-TEFb-HEXIM1–7SK RNA relies upon formation of hnRNP-7SK RNA complexes. Mol. Cell. Biol. 27, 6996–7006.
131 Henry, M., Borland, C. Z., Bossie, M. and Silver, P. A. (1996) Potential RNA binding proteins in Saccharomyces cerevisiae identified as suppressors of temperature-sensitive mutations in NPL3. Genetics 142, 103–115.
132 Bentley, D. L. (2005) Rules of engagement: co-transcriptional recruitment of pre-mRNA processing factors. Curr. Opin. Cell Biol. 17, 251–256.
133 Black, D. L. (2003) Mechanisms of alternative pre-messenger RNA splicing. Annu. Rev. Biochem. 72, 291–336.
134 Martinez-Contreras, R., Cloutier, P., Shkreta, L., Fisette, J. F., Revil, T. and Chabot, B. (2007) hnRNP proteins and splicing control. Adv. Exp. Med. Biol. 623, 123–147.
135 Hartmuth, K., Urlaub, H., Vornlocher, H. P., Will, C. L., Gentzel, M., Wilm, M. and Luhrmann, R. (2002) Protein composition of human prespliceosomes isolated by a tobramycin affinity-selection method. Proc. Natl. Acad. Sci. U.S.A. 99, 16719–16724.
136 Jurica, M. S., Licklider, L. J., Gygi, S. R., Grigorieff, N. and Moore, M. J. (2002) Purification and characterization of native spliceosomes suitable for three-dimensional structural analysis. RNA 8, 426–439.
137 Makarov, E. M., Makarova, O. V., Urlaub, H., Gentzel, M., Will, C. L., Wilm, M. and Luhrmann, R. (2002) Small nuclear ribonucleoprotein remodeling during catalytic activation of the spliceosome. Science 298, 2205–2208.
138 Zhou, Z., Licklider, L. J., Gygi, S. P. and Reed, R. (2002) Comprehensive proteomic analysis of the human spliceosome. Nature 419, 182–185.
139 Rappsilber, J., Ryder, U., Lamond, A. I. and Mann, M. (2002) Large-scale proteomic analysis of the human spliceosome. Genome Res. 12, 1231–1245.
140 Jurica, M. S. and Moore, M. J. (2003) Pre-mRNA splicing: awash in a sea of proteins. Mol. Cell 12, 5–14.
141 Makarova, O. V., Makarov, E. M., Urlaub, H., Will, C. L., Gentzel, M., Wilm, M. and Luhrmann, R. (2004) A subset of human 35S U5 proteins, including Prp19, function prior to catalytic step 1 of splicing. EMBO J. 23, 2381–2391.
142 Deckert, J., Hartmuth, K., Boehringer, D., Behzadnia, N., Will, C. L., Kastner, B., Stark, H., Urlaub, H. and Luhrmann, R. (2006) Protein composition and electron microscopy structure of affinity-purified human spliceosomal B complexes isolated under physiological conditions. Mol. Cell. Biol. 26, 5528–5543.
143 Sun, Q., Mayeda, A., Hampson, R. K., Krainer, A. R. and Rottman, F. M. (1993) General splicing factor SF2/ASF promotes alternative splicing by binding to an exonic splicing enhancer. Genes Dev. 7, 2598–2608.
144 Marchand, V., Mereau, A., Jacquenet, S., Thomas, D., Mougin, A., Gattoni, R., Stevenin, J. and Branlant, C. (2002) A Janus splicing regulatory element modulates HIV-1 tat and rev mRNA production by coordination of hnRNP A1 cooperative binding. J. Mol. Biol. 323, 629–652.
145 Rooke, N., Markovtsov, V., Cagavi, E. and Black, D. L. (2003) Roles for SR proteins and hnRNP A1 in the regulation of c-src exon N1. Mol. Cell. Biol. 23, 1874–1884.
146 Zhu, D., Xu, G., Ghandhi, S. and Hubbard, K. (2002) Modulation of the expression of p16INK4a and p14ARF by hnRNP A1 and A2 RNA binding proteins: implications for cellular senescence. J. Cell Physiol. 193, 19–25.
147 Del Gatto, F. and Breathnach, R. (1995) Exon and intron sequences, respectively, repress and activate splicing of a fibroblast growth factor receptor 2 alternative exon. Mol. Cell. Biol. 15, 4825–4834.

148 Zhu, J., Mayeda, A. and Krainer, A. R. (2001) Exon identity established through differential antagonism between exonic splicing silencer-bound hnRNP A1 and enhancer-bound SR proteins. Mol. Cell 8, 1351–1361.
149 Caputi, M., Mayeda, A., Krainer, A. R. and Zahler, A. M. (1999) hnRNP A/B proteins are required for inhibition of HIV-1 pre-mRNA splicing. EMBO J. 18, 4060–4067.
150 Kashima, T. and Manley, J. L. (2003) A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. Nat. Genet. 34, 460–463.
151 Hua, Y., Vickers, T. A., Okunola, H. L., Bennett, C. F. and Krainer, A. R. (2008) Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. Amer. J. Hum. Genet. 82, 834–848.
152 Dirksen, W. P., Li, X., Mayeda, A., Krainer, A. R. and Rottman, F. M. (2000) Mapping the SF2/ASF binding sites in the bovine growth hormone exonic splicing enhancer. J. Biol. Chem. 275, 29170–29177.
153 Zahler, A. M., Damgaard, C. K., Kjems, J. and Caputi, M. (2004) SC35 and heterogeneous nuclear ribonucleoprotein A/B proteins bind to a juxtaposed exonic splicing enhancer/exonic splicing silencer element to regulate HIV-1 tat exon 2 splicing. J. Biol. Chem. 279, 10077–10084.
154 Venables, J. P., Bourgeois, C. F., Dalgliesh, C., Kister, L., Stevenin, J. and Elliott, D. J. (2005) Up-regulation of the ubiquitous alternative splicing factor Tra2beta causes inclusion of a germ cell-specific exon. Hum. Mol. Genet. 14, 2289–2303.
155 Eperon, I. C., Makarova, O. V., Mayeda, A., Munroe, S. H., Caceres, J. F., Hayward, D. G. and Krainer, A. R. (2000) Selection of alternative 5′ splice sites: role of U1 snRNP and models for the antagonistic effects of SF2/ASF and hnRNP A1. Mol. Cell. Biol. 20, 8303–8318.
156 Tange, T. O., Damgaard, C. K., Guth, S., Valcarcel, J. and Kjems, J. (2001) The hnRNP A1 protein regulates HIV-1 tat splicing via a novel intron silencer element. EMBO J. 20, 5748–5758.
157 Chabot, B., Blanchette, M., Lapierre, I. and La Branche, H. (1997) An intron element modulating 5′ splice site selection in the hnRNP A1 pre-mRNA interacts with hnRNP A1. Mol. Cell. Biol. 17, 1776–1786.
158 Blanchette, M. and Chabot, B. (1999) Modulation of exon skipping by high-affinity hnRNP A1-binding sites and by intron elements that repress splice site utilization. EMBO J. 18, 1939–1952.
159 Simard, M. J. and Chabot, B. (2000) Control of hnRNP A1 alternative splicing: an intron element represses use of the common 3′ splice site. Mol. Cell. Biol. 20, 7353–7362.
160 Swanson, M. S. and Dreyfuss, G. (1988) RNA binding specificity of hnRNP proteins: a subset bind to the 3′ end of introns. EMBO J. 7, 3519–3529.
161 Mayrand, S. H. and Pederson, T. (1990) Crosslinking of hnRNP proteins to pre-mRNA requires U1 and U2 snRNPs. Nucleic Acids Res. 18, 3307–3318.
162 Buvoli, M., Cobianchi, F., Biamonti, G. and Riva, S. (1990) Recombinant hnRNP protein A1 and its N-terminal domain show preferential affinity for oligodeoxynucleotides homologous to intron/exon acceptor sites. Nucleic Acids Res. 18, 6595–6600.
163 Buvoli, M., Cobianchi, F. and Riva, S. (1992) Interaction of hnRNP A1 with snRNPs and pre-mRNAs: evidence for a possible role of A1 RNA annealing activity in the first steps of spliceosome assembly. Nucleic Acids Res. 20, 5017–5025.
164 Venables, J. P. (2004) Aberrant and alternative splicing in cancer. Cancer Res. 64, 7647–7654.
165 Robert-Guroff, M., Popovic, M., Gartner, S., Markham, P., Gallo, R. C. and Reitz, M. S. (1990) Structure and expression of tat-, rev-, and nef-specific transcripts of human immunodeficiency virus type 1 in infected lymphocytes and macrophages. J. Virol. 64, 3391–3398.
166 Del Gatto-Konczak, F., Olive, M., Gesnel, M. C. and Breathnach, R. (1999) hnRNP A1 recruited to an exon in vivo can function as an exon splicing silencer. Mol. Cell. Biol. 19, 251–260.
167 Zhou, J., Mulshine, J. L., Unsworth, E. J., Scott, F. M., Avis, I. M., Vos, M. D. and Treston, A. M. (1996) Purification and characterization of a protein that permits early detection of lung cancer. Identification of heterogeneous nuclear ribonucleoprotein-A2/B1 as the antigen for monoclonal antibody 703D4. J. Biol. Chem. 271, 10760–10766.
168 Fielding, P., Turnbull, L., Prime, W., Walshaw, M. and Field, J. K. (1999) Heterogeneous nuclear ribonucleoprotein A2/B1 up-regulation in bronchial lavage specimens: a clinical marker of early lung cancer detection. Clin. Cancer Res. 5, 4048–4052.
169 Sueoka, E., Goto, Y., Sueoka, N., Kai, Y., Kozu, T. and Fujiki, H. (1999) Heterogeneous nuclear ribonucleoprotein B1 as a new marker of early detection for human lung cancers. Cancer Res. 59, 1404–1407.
170 Zhou, J., Allred, D. C., Avis, I., Martinez, A., Vos, M. D., Smith, L., Treston, A. M. and Mulshine, J. L. (2001) Differential expression of the early lung cancer detection marker, heterogeneous nuclear ribonucleoprotein-A2/B1 (hnRNP-A2/B1) in normal breast and neoplastic breast cancer. Breast Cancer Res. Treat. 66, 217–224.
171 Xu, X., Joh, H. D., Pin, S., Schiller, N. I., Prange, C., Burger, P. C. and Schiller, M. R. (2001) Expression of multiple larger-sized transcripts for several genes in oligodendrogliomas: potential markers for glioma subtype. Cancer Lett. 171, 67–77.
172 Mili, S., Shu, H. J., Zhao, Y. and Piñol-Roma, S. (2001) Distinct RNP complexes of shuttling hnRNP proteins with pre-mRNA and mRNA: candidate intermediates in formation and export of mRNA. Mol. Cell. Biol. 21, 7307–7319.
173 Gorlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. Annu. Rev. Cell Dev. Biol. 15, 607–660.
174 Visa, N., Alzhanova-Ericsson, A. T., Sun, X., Kiseleva, E., Bjorkroth, B., Wurtz, T. and Daneholt, B. (1996) A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. Cell 84, 253–264.
175 Lichtenstein, M., Guo, W. and Tartakoff, A. M. (2001) Control of nuclear export of hnRNP A1. Traffic 2, 261–267.
176 Fridell, R. A., Truant, R., Thorne, L., Benson, R. E. and Cullen, B. R. (1997) Nuclear import of hnRNP A1 is mediated by a novel cellular cofactor related to karyopherin-beta. J. Cell Sci. 110, 1325–1331.
177 Rebane, A., Aab, A. and Steitz, J. A. (2004) Transportins 1 and 2 are redundant nuclear import factors for hnRNP A1 and HuR. RNA 10, 590–599.
178 Bonifaci, N., Moroianu, J., Radu, A. and Blobel, G. (1997) Karyopherin beta2 mediates nuclear import of a mRNA binding protein. Proc. Natl. Acad. Sci. U.S.A. 94, 5055–5060.
179 Siomi, M. C., Eder, P. S., Kataoka, N., Wan, L., Liu, Q. and Dreyfuss, G. (1997) Transportin-mediated nuclear import of heterogeneous nuclear RNP proteins. J. Cell Biol. 138, 1181–1192.
180 Carson, J. H. and Barbarese, E. (2005) Systems analysis of RNA trafficking in neural cells. Biol. Cell 97, 51–62.
181 Iijima, M., Suzuki, M., Tanabe, A., Nishimura, A. and Yamada, M. (2006) Two motifs essential for nuclear import of the hnRNP A1 nucleocytoplasmic shuttling sequence M9 core. FEBS Lett. 580, 1365–1370.
182 Allemand, E., Guil, S., Myers, M., Moscat, J., Caceres, J. F. and Krainer, A. R. (2005) Regulation of heterogenous nuclear ribonucleoprotein A1 transport by phosphorylation in cells stressed by osmotic shock. Proc. Natl. Acad. Sci. U.S.A. 102, 3605–3610.
183 Vautier, D., Chesne, P., Cunha, C., Calado, A., Renard, J. P. and Carmo-Fonseca, M. (2001) Transcription-dependent nucleocytoplasmic distribution of hnRNP A1 protein in early mouse embryos. J. Cell Sci. 114, 1521–1531.

184 Friend, L. R., Han, S. P., Rothnagel, J. A. and Smith, R. (2008) Differential subnuclear localisation of hnRNPs A/B is dependent on transcription and cell cycle stage. Biochim. Biophys. Acta 1783, 1972–1980.
185 Bogerd, H. P., Benson, R. E., Truant, R., Herold, A., Phingbodhipakkiya, M. and Cullen, B. R. (1999) Definition of a consensus transportin-specific nucleocytoplasmic transport signal. J Biol Chem 274, 9771–9777.
186 Yi, R., Bogerd, H. P., Wiegand, H. L. and Cullen, B. R. (2002) Both ran and importins have the ability to function as nuclear mRNA export factors. Rna 8, 180–187.
187 Stutz, F., Bachi, A., Doerks, T., Braun, I. C., Seraphin, B., Wilm, M., Bork, P. and Izaurralde, E. (2000) REF, an evolutionary conserved family of hnRNP-like proteins, interacts with TAP/Mex67p and participates in mRNA nuclear export. RNA 6, 638–650.
188 Pan, H., Luo, C., Li, R., Qiao, A., Zhang, L., Mines, M., Nyanda, A. M., Zhang, J. and Fan, G. H. (2008) Cyclophilin A is required for CXCR4-mediated nuclear export of heterogeneous nuclear ribonucleoprotein A2, activation and nuclear translocation of ERK1/2, and chemotactic cell migration. J. Biol. Chem. 283, 623–637.
189 Shen, E. C., Henry, M. F., Weiss, V. H., Valentini, S. R., Silver, P. A. and Lee, M. S. (1998) Arginine methylation facilitates the nuclear export of hnRNP proteins. Genes Dev. 12, 679–691.
190 Municio, M. M., Lozano, J., Sanchez, P., Moscat, J. and Diaz-Meco, M. T. (1995) Identification of heterogeneous ribonucleoprotein A1 as a novel substrate for protein kinase C zeta. J. Biol. Chem. 270, 15884–15891.
191 Cobianchi, F., Calvio, C., Stoppini, M., Buvoli, M. and Riva, S. (1993) Phosphorylation of human hnRNP protein A1 abrogates in vitro strand annealing activity. Nucleic Acids Res. 21, 949–955.
192 Buxade, M., Parra, J. L., Rousseau, S., Shpiro, N., Marquez, R., Morrice, N., Bain, J., Espel, E. and Proud, C. G. (2005) The Mnks are novel components in the control of TNF alpha biosynthesis and phosphorylate and regulate hnRNP A1. Immunity 23, 177–189.
193 Carson, J. H., Blondin, N. and Korza, G. (2006) Rules of engagement promote polarity in RNA trafficking. BMC Neuroscience 7 (suppl.1), S3.
194 Kim Guisbert, K., Duncan, K., Li, H. and Guthrie, C. (2005) Functional specificity of shuttling hnRNPs revealed by genome-wide analysis of their RNA binding profiles. RNA 11, 383–393.
195 Bomsztyk, K., Denisenko, O. and Ostrowski, J. (2004) hnRNP K: one protein multiple processes. Bioessays 26, 629–638.
196 Merrill, B. M., Stone, K. L., Cobianchi, F., Wilson, S. H. and Williams, K. R. (1988) Phenylalanines that are conserved among several RNA-binding proteins form part of a nucleic acid-binding pocket in the A1 heterogeneous nuclear ribonucleoprotein. J. Biol. Chem. 263, 3307–3313.
197 Myers, J. C. and Shamoo, Y. (2004) Human UP1 as a model for understanding purine recognition in the family of proteins containing the RNA recognition motif (RRM). J. Mol. Biol. 342, 743–756.
198 Del Gatto, F., Gesnel, M. C. and Breathnach, R. (1996) The exon sequence TAGG can inhibit splicing. Nucleic Acids Res. 24, 2017–2021.
199 Myers, J. C., Moore, S. A. and Shamoo, Y. (2003) Structure-based incorporation of 6-methyl-8-(2-deoxy-beta-ribofuranosyl)isoxanthopteridine into the human telomeric repeat DNA as a probe for UP1 binding and destabilization of G-tetrad structures. J. Biol. Chem. 278, 42300–42306.
200 Li, H. P., Zhang, X., Duncan, R., Comai, L. and Lai, M. M. (1997) Heterogeneous nuclear ribonucleoprotein A1 binds to the transcription-regulatory region of mouse hepatitis virus RNA. Proc. Natl. Acad. Sci. U.S.A. 94, 9544–9549.
201 Shen, X. and Masters, P. S. (2001) Evaluation of the role of heterogeneous nuclear ribonucleoprotein A1 as a host factor in murine coronavirus discontinuous transcription and genome replication. Proc. Natl. Acad. Sci. U.S.A. 98, 2717–2722.
202 Guil, S. and Caceres, J. F. (2007) The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. Nat. Struct. Mol. Biol. 14, 591–596.