Review

Nuclear functions of heterogeneous nuclear ribonucleoproteins A/B

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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-
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 RRM 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 RRM 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 RRM 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]. hnRN 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 RRM 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 X 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)(B1) tetramers and (A2), (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 hnRNPA/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)
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 stabilizes or destabilizes dsDNA is dependent on the temperature relative to the melting temperature [70]. Above this temperature A1 destabilizes 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 cooperative 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 AUUUUA-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 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]      |
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 G2 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 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.
[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,
hnRNPs 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 RRM s 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]. hnRNPA3 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 hnRNPA3 have shown an interaction with telomeric DNA repeats in rat brain extracts (S. Sara and R. Smith, unpublished observations). hnRNPA1, 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 hnRNPA2/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. hnRNPA1 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]. hnRNPA2/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]. hnRNPA3 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 hnRNPA/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 hnRNPA/B proteins in transcription. Some of these proteins, including A1 and A2, can act as either a transcriptional activator or a repressor. hnRNPA1 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]. hnRNPA2 also represses expression of the vitamin D receptor, but it is more likely to be an activator for BRCA1 transcription because suppression of this hnRNPA led to a decrease of BRCA1 at both the mRNA and protein levels [33].

How hnRNPA/B proteins contribute to transcriptional regulation is unknown. However, both direct and indirect mechanisms may be involved. Destabilization of G-quartets by hnRNPA/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 hnRNPA1, A2 and B1 are located [120]. hnRNPA/B proteins can indirectly participate in control of transcription through protein-protein interactions. hnRNPA2 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 hnRNPA2/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
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 RN1-pre-mRNA interact (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 A1b 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,
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 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.

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.
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 (Tnr1) and transportin 2 (Tnr2), 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 hnRNPA1 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
When Hmt1p is inhibited, the two hnRNP proteins Hrp1p, and another hnRNP A/B family protein, hnRNP A1 homolog, *Saccharomyces cerevisiae*ated nuclear export. The nuclear export of the Other factors might also influence hnRNP A1-mediated pathway. 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 Ser199 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 RRM. 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 nonspecific 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 cyto-
plasmic trafficking motif A2RE more tightly than
does the tandem RRM 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 tran-
scripts. 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 hnRNPs 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. An-
other 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 modif-
cations, particularly methylation and phosphoryla-
tion.
Alternatively spliced isoforms may be directed to
separate locations where they have different func-
tions. 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 concen-
trations in the nucleus and more abundantly in the
processes of oligodendrocytes and dendrites of neu-
rons (unpublished data). There are also strong tem-
poral 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 techni-
ques to knock down target genes and the generation of
conditional knock-out mice offer considerable promis-
e in exploration of the wide range of activities
associated with individual hnRNPs A/B proteins, their
alternatively spliced isoforms and breakdown prod-
ucts.
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 sub-
family 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 com-
plexity 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 ribo-
nucleoprotein particles reveals an assortment of RNA-bind-
ing 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. 258, 7877–7886.

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 Genni, 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, C., 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) hnRNP A2B1. Genomics 25, 365–371.

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., Gorlich, 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. J. Biol. Chem. 264, 8577–8590.

17 Nichols, R. C., Wang, X. W., Tang, J., Hamilton, B. J., High, F. A., Hershcomb, 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., Saconne, 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., Gharibi, B. and Schafer, K. P. (1995) Structure and expression of the gene (HNRPA2B1) encoding the human hnRNP protein A2B1. Genomics 25, 365–371.

22 Dangli, A., Plomaritoglou, A., Boutoue, E., Vassiliadou, N., Moutsopoulos, H. M. and Gualtieri, 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 splice site selection in vivo. Proc. Natl. Acad. Sci. U.S.A. 91, 6924–6928.

25 Kamma, H., Horiguchi, H., Wan, L., Matsui, M., Fujimura, 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., Sirakowa, 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) Strain radi 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 RA35/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. (1996) 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) Monovalent 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., Arentorst, 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 405 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), B1 tetramers in 40S nuclear ribonucleoprotein particles. Mol. Cell. Biol. 11, 864–871.

40 Pericpalle, P., Jonsson, A., Nachshchin, D., Karlsson, C., Bergman, T., Gualtieri, A. and Danchin, B. (2002) Nuclear actin is associated with a specific subset of hnRNP A/B-type proteins. Nucleic Acids Res. 30, 1725–1734.
hnRNP A/B nuclear functions

Ishikawa, F., Matusin, M. J., Dreyfuss, G. and Cech, T. R. (1993) Nuclear proteins that bind the pre-mRNA 3' splice site sequence (UUAG/G) and the human telomeric DNA sequence d(TTAGGG)n. Mol. Cell. Biol. 13, 4301–4310.

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.

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.

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.

Leverrier, S., Cintato, 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 spl 2 gene GAGA box. Biol. Chem. 381, 1031–1040.

Xia, H. (2005) Regulation of gamma-fibrinogen chain expression by heterogeneous nuclear ribonucleoprotein A1. J. Biol. Chem. 280, 13171–13178.

Mayeda, A. and Krainer, A. R. (1992) Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. Cell 68, 365–375.

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.

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.

Blodoe, P. S., Domsic, J. K., Mayeda, A., Krainer, A. R. and Stolzflus, 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.

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.

Pollard, V. W., Michael, W. M., Nakielny, S., Sioimi, M. C., Wang, F. and Dreyfuss, G. (1996) A novel receptor-mediated nuclear protein import pathway. Cell 86, 985–994.

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.

Brunwell, J. A., Antolik, C., Carson, J. H. and Barbarsce, E. (2002) Intracellular trafficking of the hnRNP A2 in oligodendrocytes. Exp. Cell Res. 279, 310–320.

Munro, T. P., Maggee, R. J., Kidd, G. J., Carson, J. H., Barbarsce, 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.

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.

Smith, R. (2004) Moving molecules: mRNA trafficking in mammalian oligodendrocytes and neurons. Neuroscientist 10, 495–501.

Iervolino, A., Santilli, G., Trotta, R., Guerzoni, C., Cesti, V., Bergamaschi, A., Gambaccorti-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.

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.

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.

Kwon, S., Babarrese, 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.

Herrick, G. and Alberts, B. (1976) Purification and physical characterization of nuclease acid helix-unwinding proteins from calf thymus. J. Biol. Chem. 251, 2124–2132.

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.

Chen, H., Hewison, M., Hu, B. and Adams, J. S. (2003) Heterogeneous nuclear ribonucleoprotein (hnRNPA2) binding to hormone response elements: a cause of vitamin D resistance. Proc. Natl. Acad. Sci. U.S.A. 100, 6109–6114.

Donev, R., Horton, R., Beck, S., Doneva, T., R., 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.

Cobianchi, F., Karpe, 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.

Kim, S., Merrill, B. M., Rajpurush, R., Kumar, A., Stone, K. L., Papov, V. V., Schneider, 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: Phc/Gly-Gly-Gly-Arg-Gly-Gly/Phe is a preferred recognition motif. Biochemistry 36, 5185–5192.

Rajpurush, R., Paik, W. K. and Kim, S. (1994) Effect of enzymatic methyl transfer of heterogeneous ribonucleoprotein particle A1 on its nucleic acid binding and controlled proteolysis. Biochem. J. 304, 903–909.

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 A1: nucleic acid binding properties of the COOH-terminal domain. J. Biol. Chem. 265, 17094–17100.

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.

Guillonneau, F., Guixeys, 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.

Nerzehan, 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.

Burk, C. G. and Dreyfuss, G. (1994) RNA binding specificity of hnRNP A1: significance of hnRNP A1 high-affinity binding sites in pre-mRNA. RNA 261, 1107–1124.

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 AUUA 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 AUUG-specific RNA binding protein, heterogeneous nuclear ribonucleoprotein D0. Common modular structure and binding properties of the 2XRRD-Gly family. J. Biol. Chem. 270, 22167–22175.
79 He, Y., Rothnagel J. A., Epis, M. R., Leedman, P. J. and Wilson, S. H. (1990) Studies of the strand-union and the gap region of the murine COX-2 mRNA. J. Biol. Chem. 265, 8906–8911.
80 Cok, S. J., Acton, S. J., Sexton, A. E. and Morrison, A. R. (1990) 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. 269, 8205–8210.
81 Kumar, A. and Wilson, S. H. (1990) Studies of the strand-anneling 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 Sgls helicase in the presence of NN-his[2-(1-piperidino)ethyl]-3,4,9,10-perylenetetracarboxylic dlimide, a G-quadruplex-interactive ligand. Biochemistry 39, 9316–9326.
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)n sequence with complementary strand in close vicinity under molecularly crowded condition. Nucleic Acids Res. 35, 3646–3653.
85 Fukuda, H., Katahira, M., Tsuhiya, N., Enokizono, Y., Sugimura, T., Nagaio, 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., Tsuhiya, 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. 596, 120–131.
87 Khatch, 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 homologous 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. Acad. Sci. U.S.A. 82, 2681–8887.
89 Chai, Q., Zheng, L., Zhou, M., Turci, 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 matura-
90 Vera, J., Jaumont, M., Estayol, 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 Trzcińska-Danelutti, A. M., Gorecki, A., Crubaty, A., Kowalska-Loth, B., Girstun, A., Muraw ska, M., Lesny, B. and Staron, K. (2007) RRM proteins interacting with the cap region of topoisomerase I. J. Mol. Biol. 369, 1098–1112.
94 Gupta, M., Fujimoto, A. and Pommier, Y. (1995) Eukaryotic DNA topoisomerase I from mouse myeloma. Proc. Natl. Acad. Sci. U.S.A. 92, 11296–11299.
95 Biological processes related to telomeres.
96 Slijepcevic, P. and Al-Wahiby, S. (2005) Telomere biology: integrating chromosomal and intergenic DNA damage response. Chromosoma 114, 273–286.
97 Moyer, R. K., Buckingham, J. M., Cram, L. S., Dani, M., Deaven, L. L., Jones, M. D., Meiney, J., Matlaff, R. L. and Wu, J. R. (1988) A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromo-
m 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 Griffith, J. D., Comeau, L., Rosenfeld, S., Stansel, R. M., Bianchi, A., Moss, H. and de Lange, T. (1999) Mammalian telomeres in a large double loop. Cell 97, 503–514.
102 Neumann, A. A. and Reddel, R. R. (2002) Telomere main-
tenance and cancer – look, no telomerase. Nat. Rev. Cancer 2, 879–884.
103 Reddel, R. R. (2003) Alternative lengthening of telomeres, telomerase, and cancer. Cancer Lett. 194, 155–162.
104 Weck, A. K. and De Marzo, A. M. (2004) Recent advances in telomere biology: implications for human cancer. Curr. Opin. Oncol. 16, 32–38.
105 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.
106 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.
107 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.
108 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 human hnRNP A1 (U1) complexed with single-stranded telomeric DNA. Genes Dev. 13, 1102–1115.
109 McKay, S. J. and Cooke, H. (1992) hnRNP A2/B1 binds specifically to single stranded vertebrate telomeric repeat (TTAGGG)n. Nucleic Acids Res. 20, 6461–6464.
110 Nakagama, H., Higuchi, K., Hamasaki, M. and Satoh, H. (2001) Interaction
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.

Caputi, M., Mayeda, A., Krainer, A. R. and Zalzer, A. M. (1999) hnRNP A/B proteins are required for inhibition of HIV-1 mRNA splicing. EMBO J. 18, 4060–4067.

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.

Hua, Y., Vickers, T. A., Okumola, H. L., Bennett, C. F. and Krainer, A. R. (2008) Anti-sense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. Amer. J. Hum. Genet. 82, 834–848.

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.

Zalzer, 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.

Venables, J. P., Bourgeois, C. K., Gallo, R. J., Treston, A. M. and Mulshine, J. L. (2001) Differential expression of the early lung cancer detection marker, heterogeneous nuclear ribonucleoprotein-A2/B1 in normal breast and neoplastic breast cancer. Breast Cancer Res. Treat. 66, 217–224.

Zhu, J., Miliu, S., Xu, H. and Pithor-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.

Gorlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. Annu. Rev. Cell Dev. Biol. 15, 607–660.

Lichtenstein, M., Guo, W. and Tartakoff, A. M. (2000) Control of nuclear export of hnRNP A1. Traffic 2, 261–267.

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.

Rebane, A., Aab, J. and Steitz, J. A. (2004) Transportins 1 and 2 are redundant nuclear import factors for hnRNP A1 and HuR. RNA 10, 590–599.

Bonifaci, N., Morel, J., Rudin, C. and Blobel, G. (1997) Karyopherin beta2 mediates nuclear import of a mRNA binding protein. Proc. Natl. Acad. Sci. U.S.A. 94, 5055–5060.

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.

Carson, J. H. and Barbaree, E. (2005) Systems analysis of RNA trafficking in neural cells. Biol. Cell 97, 51–62.

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.

Allemand, E., Guil, S., Myers, M., Moscat, J., Caceres, J. F. and Krainer, A. R. (2005) Regulation of heterogeneous nuclear ribonucleoprotein A1 transport by phosphorylation in cells stressed by osmotic shock. Proc. Natl. Acad. Sci. U.S.A. 102, 3605–3610.

Vautier, D., Chesne, P., Cunha, C., Calado, A., Renard, J. P. and Cattoretti, G. (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 hnRNP A/B is dependent on transcription and cell cycle stage. Biochim. Biophys. Acta 1783, 1972–1980.

Boger, H. P., Benson, R. E., Truant, R., Herold, A., Phingboodhipakkiya, M. and Cullen, B. R. (1999) Definition of a consensus transportin-specific nucleocytoplasmic transport signal. J Biol Chem 274, 9771–9777.

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.

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.

Pan, H., Lao, 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.

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.

Muncio, 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.

Cobianchi, F., Calvó, 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.

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 alpha biosynthesis and phosphorlyate 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 Ostrouski, 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)oxanthylium]-scolosinoxanthopteridine 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 Cacleres, J. F. (2007) The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. Nat. Struct. Mol. Biol. 14, 591–596.

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