EPIGENETICS AND GENETICS

MicroRNA regulation by RNA-binding proteins and its implications for cancer

Marieke van Kouwenhove, Martijn Kedde* and Reuven Agami*‡*

Abstract | Non-protein-coding transcripts have been conserved throughout evolution, indicating that crucial functions exist for these RNAs. For example, microRNAs (miRNAs) have been found to modulate most cellular processes. The protein classes of RNA-binding proteins include essential regulators of miRNA biogenesis, turnover and activity. RNA–RNA and protein–RNA interactions are essential for post-transcriptional regulation in normal development and may be deregulated in disease. In reviewing emerging concepts of the interplay between miRNAs and RNA-binding proteins, we highlight the implications of these complex layers of regulation in cancer initiation and progression.

MicroRNAs (miRNAs) are small, non-coding RNAs that repress gene expression through interaction with 3′ untranslated regions (3′ UTRs) of mRNAs (reviewed in REF. 1). miRNAs are predicted to target over 50% of all human protein-coding genes, enabling them to have numerous regulatory roles in many physiological and developmental processes[2]. Global downregulation of miRNA expression is an emerging feature in cancer, and the specific deregulation of certain miRNAs is seen in specific tumour types[3,4] (BOX 1). RNA-binding proteins (RBPs) are key components in the determination of miRNA function, as they control different stages of miRNA biogenesis and their localization, degradation and activity. Indeed, alteration of RBP function can lead to impairment in any of the crucial steps of the miRNA pathway. Deregulation of RBP expression or activity has been reported in several malignancies. Recently, several groups obtained evidence for more specific miRNA–RBP interplay under various physiological conditions or in response to external stimuli. Such regulatory mechanisms rely on miRNA and RBP binding activity to common target RNAs and are probably under tight spatio-temporal control. These insights uncover a wide variety of new mechanisms in RNA regulation, which could have relevance for cancer development and progression.

RBPs modulate post-transcriptional regulation

RBPs are essential players in RNA metabolism, regulating RNA splicing, transport, localization, stability, translation and degradation. Some RBPs recognize common mRNA features such as the 5′ cap or the 3′ poly(A) tail, but most RBPs contain RNA-binding domains for recognition of specific sequence motifs or secondary structures in mRNA (reviewed in REF. 5) (TABLE 1). In the past decade, numerous roles of RBPs in miRNA processing and function have emerged.

More than 500 human RBPs are known, but only a few have been assigned an oncogenic or tumour-suppressive function (reviewed in REF. 6). Examples of RBPs implicated in tumorigenesis are the TET family of RBPs[7], the STAR family of RBPs (such as Src-associated in mitosis 68 kDa protein (SAM68; also known as KHDRBS1)[8], β-catenin[9] and multiple RBPs involved in alternative splicing (see REF. 10 for a review of alternative splicing in cancer). In addition, many RBPs are involved in RNA stability, in which simultaneous assembly of these RBPs on target RNA has either a synergistic or antagonistic effect. RNA-binding activity can be rapidly modulated in response to external stimuli, for example through RBP expression levels, nucleocytoplasmic translocation, post-translational modifications or changes in secondary structure. The specifics of RNA binding remain largely undiscovered, but the function of various classes of RBPs in miRNA-mediated post-transcriptional regulation has started to receive more attention.

The biogenesis of miRNAs

The biogenesis of miRNAs starts with RNA polymerase II-dependent transcription of a miRNA gene locus, generating a long primary transcript (pri-miRNA) that folds into a hairpin structure. These pri-miRNAs are first 5′ 7-methyl-guanosine (m^7G) capped and 3′ polyadenylated before further processing occurs (FIG. 1). In the nucleus, recognition by the microprocessor complex results in cleavage of the pri-miRNA, which is attained through catalytic cleavage of the double-stranded stem

**Division of Gene Regulation, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, The Netherlands.*
‡The Centre for Biomedical Genetics, UMCU, Stratenum 3.217, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands.
Correspondence to R.A.
e-mail: r.agami@nki.nl
doi:10.1038/nrc3107
Published online 5 August 2011

At a glance

- Global downregulation of microRNA (miRNA) expression is an apparent feature of many tumours. Oncogenic or tumour-suppressive functions have been assigned to numerous miRNAs.
- Alterations in key players of miRNA biogenesis affect mature miRNA levels in a global manner, whereas RNA-binding proteins (RBPs) regulating specific miRNAs can contribute to differences in the production of specific (subsets of) miRNAs.
- The observation that miRNA binding to target mRNAs can repress gene expression through distinct mechanisms suggests the involvement of accessory proteins, some of which are linked to cancer.
- Interplay between miRNAs and RBPs on target 3′ untranslated regions can rapidly modulate target expression under specific conditions. Binding of RBPs near miRNA target sites can potentially regulate miRNA function either directly by affecting miRNA binding or indirectly through a switch in RNA secondary structure.
- The activity of RBPs is temporally and spatially regulated through changes in transcription rate, post-translational modifications and subcellular localization, and is sometimes deregulated in cancer and other diseases.
- The data discussed in this Review illustrate several examples of mechanisms for miRNA–RBP interplay that could hold true for other miRNAs and RBPs. As some of these mechanisms are linked to oncogenesis, the challenge now is to connect the mechanisms of action to disease by applying state-of-the-art genome-wide approaches.

by the RNase III endonuclease Drosha, while the hairpin is correctly positioned by DiGeorge syndrome critical region 8 (DGCR8; also known as Pasha)[11,12]. After cleavage, the secondary structure of the resulting ~70 nucleotide (nt) precursor miRNA (pre-miRNA) is recognized by a complex of exportin 5 (XPO5) and RAN-GTP[13,14]. The stabilized pre-miRNAs are then shuttled to the cytoplasm and released on GTP hydrolysis.

In the cytoplasm, the pre-miRNA terminal loop is cleaved by another double-stranded RNA (dsRNA)-specific RNase III, Dicer, in collaboration with the human immunodeficiency virus transactivation responsive RNA-binding protein 2 (TARBP2)[15–18]. A PAZ domain within Dicer binds the pre-miRNA 2-nt 3′ overhang, while the RNA-binding domain of Dicer binds the double-stranded stem and defines the site of cleavage by measuring 22 nt from the 3′ overhang[19,20]. The cofactor TARBP2 uses two dsRNA-binding domains (dsRBDs) to interact with the pre-miRNA, stimulating Dicer-mediated cleavage, and a third dsRBD to increase the stability of the Dicer–RNA complex[21,22]. Another cofactor, the protein activator PACT (also known as PRKRA), has a similar function to TARBP2 in that it recognizes the same binding domain of Dicer[23]. TARBP2 and PACT therefore selectively promote miRNA processing but are not essential for Dicer-mediated cleavage. After cleavage of the ~22-nt RNA duplex, now consisting of two 5′ phosphorylated sequence strands with 3′ overhangs, the functional strand, referred to as the guide strand, is loaded into an Argonaute (AGO) protein[24]. TARBP2 secures the recruitment of the AGO protein and the formation of a ternary complex together with Dicer[18]. All AGO proteins are characterized by evolutionarily conserved MID and PAZ domains involved in RNA binding and an RNase H-like PIWI domain for endonuclease activity[25,26]. The 5′ phosphate group of the miRNA guide strand is stably bound by the MID domain while the PAZ domain recognizes the dinucleotide 3′ overhang that is characteristic of Dicer-mediated cleavage[27]. Occasionally, a processing intermediate is generated by AGO2-mediated cleavage of a pre-miRNA that probably facilitates removal of the passenger strand[28]. Rarely, an miRNA with a short stem region that cannot be recognized by Dicer, as occurs for pre-miR-451 in mice and zebrafish, is trimmed by the endonuclease activity of AGO2 (REFS 29,30). Although this provides evidence for an miRNA biogenesis pathway that does not require Dicer cleavage, the extent to which such phenomena occur is unclear. In general, pre-miRNA characteristics determine which strand is retained, whereby the suffixes 5p and 3p (or *) designate the 5′ and 3′ duplex arms, respectively[31,32].

Passenger strand
The strand of the microRNA duplex that is complementary to the guide strand and is destined for degradation upon loading of the guide strand into the microRNA-induced silencing complex (miRISC).

Global control of miRNA biogenesis in cancer

In human tumours, global downregulation of miRNA expression is an apparent feature[3,4]. Another striking observation in primary tumours is the accumulation of pri-miRNAs compared with normal tissue[33]. Hence, impairment of crucial steps in miRNA production, either in the nucleus or in the cytoplasm, could be the underlying cause. In recent years, an increasing amount of evidence has been obtained for cancer-related alterations in RBPs that are intimately involved in miRNA biogenesis.

At the genetic level, copy number abnormalities of *DICER1*, *AGO2*, *XPO5* and other genes that are essential for miRNA biogenesis occur often in breast and ovarian cancer, as well as in melanoma[34,35]. In various human cancer cell lines, mature miRNA expression levels are inconsistent with pre-miRNA expression levels owing to nuclear retention of pre-miRNAs[36]. Mutations that inactivate *XPO5* in human tumours lead to precursor accumulation in the nucleus and lower levels of mature miRNAs[37]. Moreover, *XPO5* knockdown enhances the tumorigenicity of cells injected into mice, and the reverse effect is seen on overexpression of wild-type *XPO5* in colorectal cancer cells expressing mutant *XPO5* (REF. 37).

Intriguingly, disruption of miRNA production by depletion of any of the miRNA processing factors Drosha, DCGR8 or Dicer has been shown to promote oncogenesis[38]. In a mouse model of retinoblastoma, the upregulation of an miRNA subset in response to *Rb1* inactivation is abolished on monoallelic loss of *Dicer1*, resulting in accelerated tumour formation[39]. Indeed, the frequent occurrence of heterozygous, but not homozygous, genetic deletions in various human tumours implicate *DICER1* as a haploinsufficient tumour suppressor[40,41]. Piccolo and colleagues[42] have also recently shown that the miRNA family miR-103/107 abrogates miRNA maturation by targeting the 3′ UTR of *DICER1*. Low Dicer protein levels result in stimulation of migration and metastasis that is at least partially due to blocked processing of miR-200.

Several reports suggest that TARBP2 function is also impaired in cancer. The occurrence of frame-shift mutations in *TARBP2* in colon tumours with microsatellite instability correlates with lower levels of Dicer and mature miRNAs[43,44]. Indeed, the downregulation of TARBP2 expression by RNA interference (RNAi)

Seed
Six to eight nucleotides at the 5′ end of the mature microRNA that are involved in the recognition of target mRNAs.

destabilizes the Dicer protein, resulting in concurrent impairment of miRNA biogenesis[18]. As expected, restoration of wild-type TARBP2 levels in cell lines expressing truncated TARBP2 reconstitutes normal levels of Dicer and mature miRNAs[43]. Importantly, rescue of miRNA production by TARBP2 restoration is accompanied by a decline in tumour growth *in vivo*, showing that the normal production of miRNAs is tumour suppressive in this setting. Interestingly, under normal growth conditions TARBP2 is phosphorylated, which increases the stability of TARBP2 and Dicer[45]. On growth factor stimulation, the MAPK–ERK pathway increases TARBP2 phosphorylation, resulting in increased levels of a subset of miRNAs but lower levels of the let-7 miRNA family[45]. This illustrates how mitogenic signalling can be translated into changes in cell viability and proliferation through the miRNA biogenesis pathway. Thus, the role of TARBP2 in miRNA processing is important for preservation of a normal, untransformed cell state.

Specific control of miRNA biogenesis in cancer

In addition to global changes in miRNA levels, differential expression of specific miRNAs is also apparent in tumours, and this could result from changes in various RBPs.

DDX5 and DDX17. The stability of the microprocessor complex is controlled by post-transcriptional cross-regulation between Drosha and DGCR8 (REF. 46). Furthermore, Drosha-mediated cleavage of specific pri-miRNAs is modulated by accessory RBPs such as DEAD-box 5 (DDX5; also known as p68) and DDX17 (also known as p72), a number of heterogeneous nuclear ribonucleoproteins (hnRNPs) and other factors[47]. Interestingly, both DDX5 and DDX17 are often highly expressed in human breast, prostate and colon tumours (reviewed in REF. 48). In mice, both DDX5 and DDX17 are required for efficient processing of a subset of pri-miRNAs, as depletion of either protein results in lower levels of mature forms[49]. This has consequences for cell proliferation and survival, as *Ddx5*-deficient or *Ddx17*-deficient mouse embryonic fibroblasts (MEFs) are characterized by slower cell growth and increased apoptosis[49]. Moreover, joint knockdown of *DDX5* and *DDX17* in human cervical carcinoma cells suppressed cell proliferation, whereas overexpression of wild-type DDX5 stimulated keratinocyte proliferation, and overexpression of a constitutively phosphorylated DDX5 mediated cell proliferation and epithelial-to-mesenchymal transition (EMT) on growth factor stimulation[48]. These functions provide a causal explanation for the overexpression of DDX5 and DDX17 in human cancer, but evidence for a specific role of the miRNAs regulated by DDX5 and DDX17 in tumorigenesis is still lacking.

Intriguingly, DDX5 and DDX17 can act as a bridge between Drosha activity and other regulators through protein–protein interactions. The tumour suppressor p53, for example, interacts with DDX5 to enhance processing of miRNAs that function in growth suppression[50]. Cancer-related mutations in p53 result in loss of DDX5 interaction with Drosha and inefficient miRNA maturation[50]. As a transcription factor, p53 is known to activate transcription of a subset of miRNA genes that partially overlaps with the group of miRNAs that is targeted by p53 during processing (reviewed in REF. 51).

Another case is illustrated by oestrogen receptor-α (ERα), a transcription factor that is overexpressed in the largest subgroup of breast tumours. Yamagata *et al.*[52] reported that the processing of a set of DDX5- and DDX17-dependent pre-miRNAs is blocked by the binding of activated ERα to DDX5 and DDX17. Thus, steroid hormones can affect miRNA maturation through their cognate nuclear receptors, resulting in the stabilization and efficient expression of ERα-target mRNAs.

SMAD signal transducers also facilitate processing of a subset of miRNAs following transforming growth factor-β (TGFβ) and bone morphogenetic protein (BMP) growth factor activation[53]. The SMADs (in a complex with the microprocessor component DDX5) promote miRNA maturation by binding a consensus sequence in the pri-miRNA stem region, which is similar to the SMAD-binding sequences in gene promoters[54]. SMAD nuclear interacting protein 1 (SNIP1) induces processing of some miRNAs as well, either by pri-miRNA binding or by direct interaction with Drosha[55]. Whether enhanced processing of these specific pri-miRNAs contributes to TGFβ-induced tumour progression remains to be established (reviewed in REF. 56).

The serine/arginine-rich splicing factor 1 (*SRSF1*) gene encodes the mRNA splicing factor SRSF1 (also known as SF2 and ASF), which recognizes the stem region of specific pri-miRNAs, resulting in enhanced cleavage by Drosha[57]. Amplification of the *SRSF1* proto-oncogene is found in various tumours, along with increased levels of miR-221 and miR-222, which are targets of SRSF1. However, the contribution of miRNAs to the tumorigenic capacity of SRSF1 is uncertain[58,59]. Some

Box 1 | **MicroRNAs as regulators of gene expression**

At present, almost 1,500 unique microRNAs (miRNAs) are produced from mostly evolutionarily conserved regions in the human genome (miRBase, release April 2011)[161–163]. miRNAs regulate gene expression by seed base-pairing to one or more partially complementary sites in target mRNAs (reviewed in REF. 1). Gene expression is mainly and most efficiently controlled via target sites located in the mRNA 3′ untranslated region (UTR), although a small number of target sites in open reading frames (ORFs) or 5′ UTRs have also been reported[164]. During animal development, expression of miRNAs is pivotal for the timing and regulation of many processes[165]. Subsequent differentiation is associated with a global increase in expression of miRNAs that define tissue-specific gene expression patterns[3,166]. Moreover, miRNA expression profiles can discriminate tumour tissue from normal tissue, associating low miRNA expression levels with a loss of cellular differentiation in tumours (reviewed in REF. 167). In almost all cancer types, alterations in miRNA-mediated regulation are implicated in key processes of tumorigenesis, such as apoptosis[168], proliferation[169], angiogenesis[170], migration[171] and invasion[172,173]. Whether miRNAs act as oncogenes or as tumour suppressor genes is dependent on the presence of their targets and the cellular context[174] (reviewed in REFS 175,176). In addition, expression profiling of human tumours has identified miRNA signatures associated with diagnosis, progression, prognosis and treatment response (reviewed in REF. 177). In cancer, miRNA-mediated repression can be altered owing to genetic variation and small- or large-scale mutations in miRNA genes and mRNA target genes (reviewed in REFS 178,179). Also, the production of miRNAs can be induced or disturbed by changes in miRNA transcription or further processing (reviewed in REFS 178,180,181).

Table 1 | **Classes of proteins with RNA-binding capacity and their motifs**

Classes of RBPs	Structure elements	RNA recognition	Examples
RNA recognition motif (RRM)	β-α-β-β-α-β (canonical)	~4-bp ssRNA nucleotide	DAZ, DAZL, DND1, ELAV, GW182, hnRNPs, IGF2BP1, PABP1 and SRSF1
dsRNA-binding domain (dsRBD)	α-β-β-β-α (canonical)	dsRNA	DGCR8, Dicer, Drosha and TARBP2
HNRNPK homology (KH) domain	Variable loop of α/β and Gly-X-X-Gly	~4-bp ssRNA nucleotide	FMRP, FXR1, FXR2, IGF2BP1, KHSRP and STAR
PIWI, AGO and Zwille (PAZ) domain	OB-like β-barrel fold	3′ single-stranded overhangs	AGO and Dicer
PIWI domain	RNase H-like fold	5′ phosphate group*	AGO
MID domain	Rossman-like fold	5′ phosphate group*	AGO
GW domain	Gly-Trp repeats	–	GW182
Zinc-binding motif	Cys_n/His_n in a β-β-α structure	–	LIN28, TRIM proteins with NHL domain and TTP
PUF repeat	Eight base-specific repeats of three α-helices	UGUAHAUA	PUM1 and PUM2
DEAD-box motif	Asp-Glu-Ala-Asp	–	DDX5, DDX6, DDX17, DDX20 and DDX42
DExD/H-box motif	Asp-Glu-X-Asp/His	–	MOV10

α, alpha helix in secondary protein structure; β, beta sheet in secondary protein structure; AGO, Argonaute; DAZ, deleted in azoospermia; DAZL, DAZ-like; DGCR8, DiGeorge syndrome critical region 8; DDX, DEAD-box; DND1, dead end 1; dsRNA: double-stranded RNA; ELAV, embryonic lethal abnormal vision; FMRP, fragile X mental retardation protein; FXR, fragile X mental retardation syndrome-related protein; hnRNP, heterogenous nuclear ribonucleoprotein; IGF2BP1, insulin-like growth factor 2 binding protein 1; KHSRP, KH-type splicing regulatory protein; MOV10, Moloney leukaemia virus 10; PABP1, poly(A) binding protein 1; PUM1, pumilio 1; SRSF1, serine/arginine-rich splicing factor 1; ssRNA, single-stranded RNA; STAR, steroidogenic acute regulatory protein; TARBP2, transactivation responsive RNA-binding protein 2; TRIM, tripartite motif; TTP, tristetraprolin.
*The 5′-phosphate binding pocket lies at the interface between the MID and PIWI domains; the MID domain alone binds nucleotides with low affinity.

common ALL1 (also known as MLL) leukaemogenic fusion proteins interact with Drosha and have a role in the biogenesis of specific miRNAs, which could contribute to the abundant levels of some miRNAs observed in acute myeloid leukaemias[60].

The let-7 miRNA family. Another regulatory circuit is provided by the let-7 miRNA family and LIN28, an RBP exclusively expressed in undifferentiated cells[61]. The role of LIN28 in development is well studied and involves suppression of let-7 miRNA levels by binding to the terminal loop of pri-let-7, thereby blocking Drosha cleavage (reviewed in REF. 62). In the cytoplasm, LIN28 recruits terminal uridyltransferase (TUT4; also known as ZCCHC11) to a 4-nt sequence motif in the terminal loop of pre-let-7, and of other pre-miRNAs, resulting in oligo-uridylation. This causes resistance to Dicer cleavage and the subsequent precursor degradation decreases let-7 abundance (see REF. 62). In response to inflammation, upregulation of LIN28 blocks let-7-mediated repression of interleukin-6 (*IL6*). This activates STAT3 and reinforces LIN28 expression, both of which can lead to a transformed state in the absence of external signals[63].

Interestingly, in differentiated cells lacking LIN28 expression, mature let-7 levels differ substantially, pointing towards a more complex regulation of miRNA processing by distinct RBPs. KH-type splicing regulatory protein (KHSRP; also known as KSRP) recognizes a conserved sequence in the terminal loop of let-7 and promotes its maturation and that of other miRNAs by facilitating an association with Drosha in the nucleus or with Dicer in the cytoplasm[64]. By contrast, the binding of the splicing regulator HNRNPA1 to the same sequence in the pri-let-7 terminal loop represses Drosha-mediated processing[65]. In differentiated cells that lack LIN28 expression, KHSRP and HNRNPA1 compete for pri-let-7 binding to regulate the extent of Drosha cleavage. In addition, the pre-miR-18a hairpin is recognized by HNRNPA1, and this promotes microprocessor interaction and maturation of pre-miR-18a[66]. Other members of the *mir-17-92* polycistron are not subject to regulation by HNRNPA1.

Altogether, the findings described above illustrate that RBP binding can have a dual effect on miRNA processing, and thus on development and cancer. Whether the eventual effect is determined by the relative abundance of each RBP, by differences in RNA binding affinity or by both generally requires additional clarification. Interestingly, both the pri-miRNA stem region and the terminal loop harbour specific RNA-binding motifs serving as control regions[67,68]. It remains a challenge to unravel sequence and structural characteristics that confer RBP binding specificity and dictate further processing.

RBPs, miRNA-mediated repression and cancer

The RNA silencing process involves assembly of the miRNA-induced silencing complex (miRISC), also called the miRNA ribonucleoprotein complex (miRNP) (reviewed in REF. 69). This complex minimally consists of three components: an active miRNA strand associated with an AGO protein and a GW182 protein[70]. The incorporated miRNA strand guides the RISC machinery

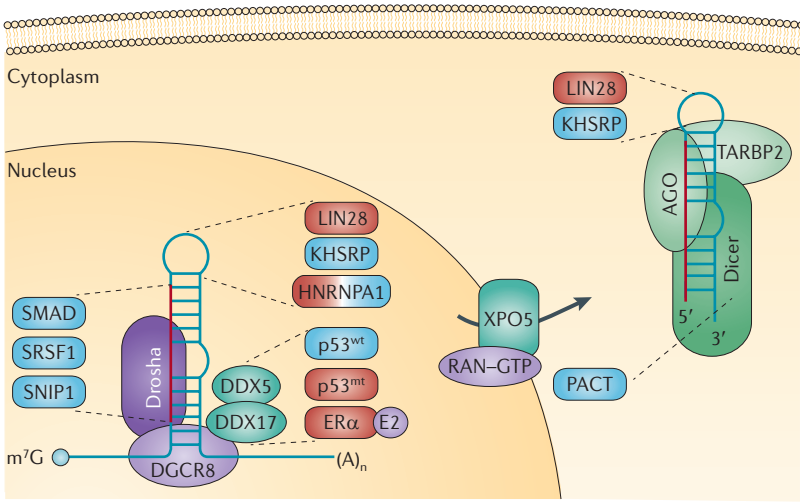


Figure 1 | **Regulation of the microRNA biogenesis pathway by processing factors.** Several RNA-binding proteins (RBPs) influence the processing of (a subset of) microRNAs (miRNAs) by recognizing specific RNA features or by associating with key components of the miRNA biogenesis pathway. Some of these factors are overexpressed or lost in tumours. RBPs can influence miRNA processes both in the nucleus and in the cytoplasm, depending on which steps in the biogenesis of miRNAs they affect. Blue and red boxes indicate a stimulative or inhibitive effect, respectively, of RBPs on miRNA processing. AGO, Argonaute; DGCR8, syndrome critical region 8; E2, 17β-oestradiol; ERα, oestrogen receptor-α; HNRNPA1, heterogeneous nuclear ribonucleoprotein A1; KHSRP, KH-type splicing regulatory protein; m^7G, 7-methyl-guanosine; $p53^{mt}$, mutant p53; $p53^{wt}$, wild-type p53; SNIP1, SMAD nuclear interacting protein 1; SRSF1, serine/arginine-rich splicing factor 1; TARBP2, transactivation responsive RNA-binding protein 2; XPO5, exportin 5.

to a matching mRNA, resulting in specific target repression[71,72]. The activity of miRISC is regulated by interacting proteins, which either directly bind AGO or GW182 or are integrated through binding to RNA molecules[74].

The AGO protein family is active in embryogenesis, germ cell maintenance and cell differentiation[74]. The four ubiquitously expressed human AGO proteins (AGO1–4), with AGO2 as the best-characterized member[24], seem to have overlapping functions in miRNA-mediated repression[75,76]. The second essential RISC component, GW182, is probably responsible for translational repression, as GW182-deficient cells have impaired miRNA function (reviewed in REF. 77). In humans, the PIWI domain of AGO proteins interacts with trinucleotide repeat-containing gene 6A (TNRC6A), TNRC6B and TNRC6C (homologues of *Drosophila melanogaster* GW182). All three homologues contain a bipartite carboxy-terminal silencing domain that is required for translational repression (see REF. 77).

Modulation of miRISC function by RBPs in cancer. According to the current view, miRNAs accomplish gene repression by inhibiting translation or by reducing mRNA stability, which generally results in target degradation (reviewed in REFS 78,79) (FIG. 2a,b). It is unknown what determines the decision between mRNA storage in a translationally repressed state or destabilization. However, although inhibition of translation results in decreased protein levels, the changes in target protein levels following miRNA binding are found to mainly coincide with actual destabilization of the target mRNA[80]. Remarkably, in mammalian cells, observed changes in ribosome occupancy on miRNA targeting largely reflect concurrent mRNA destabilization, rather than persistence of repressed mRNAs[81].

Human miRISC is found associated with several proteins characterized by RNA helicase domains (FIG. 2c). By RNA unwinding, RNA helicase activity could facilitate either the incorporation of the active miRNA in AGO or miRISC target binding. The DEAD-box RNA helicase DDX6 (also known as RCK and p54) interacts with AGO2 and has been assigned a function both in translational repression and decapping[82,83]. Overexpression of DDX6 is found in colon cancer and RNAi-mediated downregulation results in antitumour effects, suggesting an oncogenic function[84]. Another DEAD-box member, DDX5, has also been shown to have helicase activity when in the cytoplasm and facilitates miRISC loading by unwinding the let-7 precursor duplex[85]. The proteins described next are also reported to co-purify with miRISC, but their exact role in miRNA-mediated repression has not been validated yet. The DExD-box RNA helicase Moloney leukaemia virus 10 (MOV10) interacts with either of the core miRISC components in the cytoplasm, whereas when in the nucleus it is thought to bind chromatin[86,87]. Furthermore, the co-purification of the DEAD-box helicases DDX20 (also known as gemin 3) and DDX42 (also known as gemin 4) from HeLa cells suggests that they reside in a complex with AGO2 and miRNAs[71,88]. Whereas knockdown of MOV10, DDX20 or DDX42 does not disrupt miRISC localization in P-bodies, an intact helicase domain of DDX6 is required for P-body assembly and for miRNA-mediated repression[82]. Interestingly, HeLa cells in which P-bodies have been disrupted by depletion of the P-body component LSM1 retain functional miRNA-mediated repression[89]. Thus, the function of DDX6 in miRNA-mediated targeting is probably not dependent on P-body localization. Moreover, these data indicate that the aggregation of stalled translation complexes in cytoplasmic foci is merely a consequence of translational repression, but leave the exact role of RNA helicase activity in facilitating miRNA function unexplained.

A number of heat shock proteins, a class of proteins that is frequently overexpressed in cancer, were found to reside in complex with AGO proteins[73,90] (FIG. 2c). For example, heat shock protein 90 (HSP90), an essential member of an ATP-dependent chaperone complex, stabilizes unloaded AGO2 but also influences miRISC, eukaryotic translation initiation factor 4E (eIF4E) and eIF4E transporter (eIF4E-T) localization[91–93]. eIF4E-T is thought to compete with eIF4G for binding to eIF4E, thereby preventing mRNA circularization[94]. Inhibition of HSP90 prevents P-body formation and, through AGO protein destabilization, affects miRNA function indirectly[91,95].

Several tripartite motif (TRIM) domain proteins, which are known for ubiquitin ligase activity, influence the function of specific miRNAs. *TRIM71* is a mammalian homologue of *Caenorhabditis elegans lin-41* and a target of let-7. TRIM71 drives AGO degradation through ubiquitylation, thereby interfering with miRNA

P-bodies
Cytoplasmic foci containing proteins involved in diverse post-transcriptional processes, such as mRNA degradation.

function[96] (FIG. 2c). Together with LIN28, TRIM71 forms a feedback circuit for let-7 regulation. As a positive modulator, TRIM32 stimulates the function of a specific set of miRNAs in mice, including let-7a, independently of its E3 ubiquitin ligase domain[97] (FIG. 2c). However, the mechanism by which TRIM32 operates, or whether TRIM32 binds RNA, is unknown. Whereas TRIM32 and TRIM71 do not change miRNA levels, interaction of a TRIM protein that contains an NCL1, HT2A and LIN41 (NHL) domain and AGO1 interferes with miRNA biogenesis in *D. melanogaster*[98]. In head and neck carcinoma, TRIM32 is overexpressed and promotes tumour growth in part through its ubiquitin activity[99].

Importin 8 (IPO8) binds to AGO proteins and influences AGO nuclear import. It also functions as a chaperone and targets AGO2-associated mRNAs, possibly through RNA-independent interactions with miRISC that may alter the structure of the complex[100] (FIG. 2c). In addition, 5′–3′ exoribonuclease 1 (XRN1), a cellular exoribonuclease that is essential for efficient decay of uncapped mRNA[101], is often lost in osteogenic sarcomas and may act as a tumour suppressor protein in these types of tumours[102].

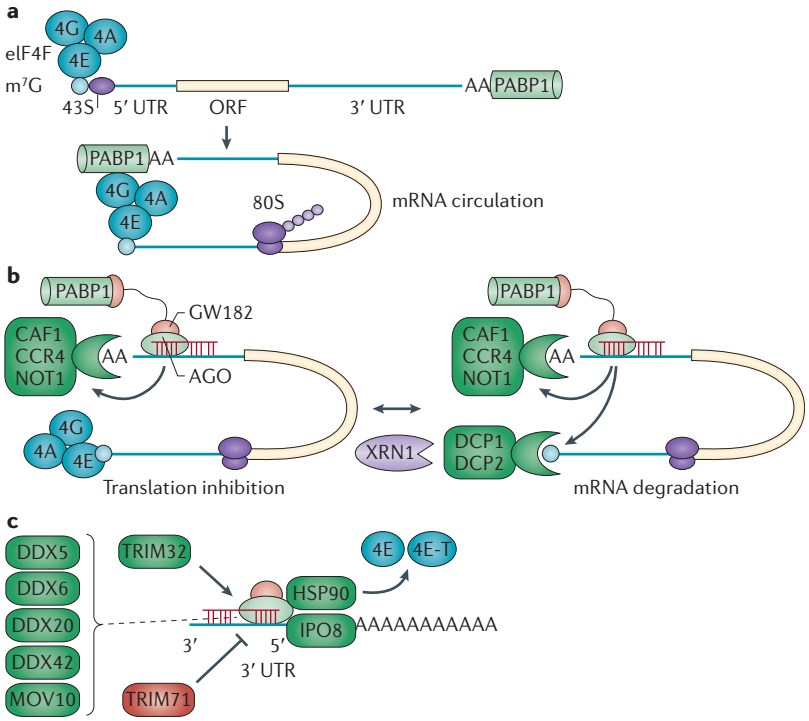


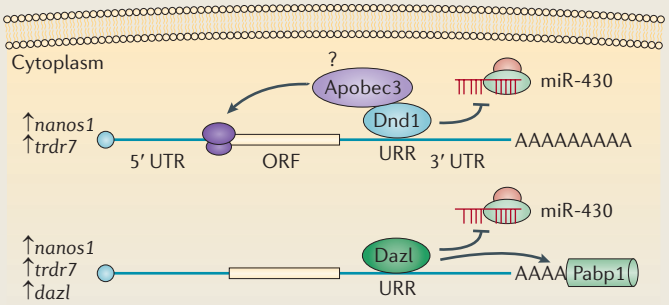
Figure 2 | **RISC-associated factors regulate efficient microRNA-mediated repression. a** | The common process of cap-dependent mRNA translation begins with modifications at the mRNA 5′ methylated guanosine cap structure and 3′ poly(A) tail. The cap-initiation complex eukaryotic translation initiation factor 4F (eIF4F, which consists of the RNA helicase eIF4A, the cap-binding protein eIF4E and the scaffolding protein eIF4G) associates with the 5′ cap and recruits the 43S pre-initiation complex (which contains the 40S ribosomal subunit) to the mRNA 5′ cap. The cytoplasmic poly(A) binding protein 1 (PABP1) is bound to the 3′ poly(A) tail and interacts with the cap-initiation complex through eIF4G. This interaction effectively establishes mRNA circularization, which stimulates translation. The 43S pre-initiation complex starts scanning in a 3′ direction and then, on recognition of an initiation codon, the 60S large ribosomal subunit is recruited to form a ribosome (80S) that enables translation elongation. **b** | When human miRNA-induced silencing complex (miRISC) recognizes the 3′ untranslated region (UTR) of a target mRNA, interference with the process of translation can occur either at the initiation step or during mRNA translation processes following initiation (reviewed in REF. 189). According to the conventional model, the mechanism of gene repression by miRISC is through translational inhibition at initiation and removal of the target poly(A) tail, which is facilitated by the cytoplasmic deadenylase complex made up of CC chemokine receptor type 4 (CCR4), CCR4-associated factor 1 (CAF1; also known as CNOT7) and negative regulator of transcription subunit 1 (NOT1; also known as CNOT1). It is plausible that this deadenylation is enabled by direct interaction between the carboxy-terminal silencing domain of trinucleotide repeat-containing gene 6 (TNRC6) and PABP1, which interferes with the function of eIF4F and PABP1 and impedes mRNA circularization (reviewed in REFS 78,190). Actual target degradation can follow recruitment of the DCP1–DCP2 decapping complex and several decapping activators to the 5′ cap, allowing mRNA degradation by the major cytoplasmic 5′-to-3′ exonuclease XRN1. **c** | Some miRISC-associated RNA helicases, such as specific DEAD-box (DDX) proteins and Moloney leukaemia virus 10 (MOV10), may facilitate miRISC loading or target binding by RNA unwinding. The RNA-binding tripartite motif (TRIM) proteins, heat shock proteins (HSPs) and importin 8 (IPO8) co-regulate mRNA translation at various points, and this function can be deregulated in cancer. Green boxes indicate a stimulatory effect and red boxes an inhibitory effect on miRNA function. 4E-T, eIF4E transporter; AGO, Argonaute; m^{7}G, 7-methyl-guanosine.

miRNA–RBP interplay on the target mRNA

Although several proteins can modulate the efficacy of miRNA biogenesis, two observations support the existence of an alternative regulatory mechanism that influences miRNA activity. First, certain genes are exclusively subject to miRNA regulation in particular conditions, but no significant change is observed in the level of the targeting miRNA. Second, of all potential high affinity targets of a specific miRNA, only a subset is subjected to miRNA regulation. An expanding number of reports now provide mechanistic explanations, often demonstrating interplay between miRNAs and RBPs on target 3′ UTRs under specific conditions, some of which are linked to differentiation (BOX 2) or oncogenesis (FIG. 3).

The ELAV RBP family. Positive modulators of RNA stability include the Hu proteins. They share homology with the *D. melanogaster* embryonic lethal abnormal vision (ELAV) protein and have a broad function in stabilizing AU-rich element (ARE)-containing mRNAs in the cytoplasm (reviewed in REF. 103). Whereas HuB, HuC, and HuD are neuronal or gonadal proteins, HuR is ubiquitously expressed and mediates cellular responses to different types of stress (FIG. 3a). Bhattacharyya and colleagues[104] found that HuR relieves cationic amino acid transporter 1 (*CAT1*) mRNA from miR-122-mediated repression under stress conditions in human liver cells. On stress induction, nuclear HuR is translocated to the cytoplasm and specifically recruits target mRNA to polysomes to secure translation[104,105]. In rare cases, HuR and/or HuD inhibit target expression[106–108]. HuR can bind to untranslated mRNA regions or can modulate miRNA binding to a nearby site, as illustrated by the requirement of HuR binding for let-7-mediated repression of *MYC*[109]. Interestingly, the activity of HuR is controlled by phosphorylation and other post-translational modifications that affect HuR subcellular localization and RNA binding activity[110]. Furthermore, miR-125a and miR-519 levels in cells generally inversely correlate with HuR target levels in various tumours. In fact, re-expression of these miRNAs decreased HuR protein levels and tumorigenicity *in vitro* and in a nude mouse xenograft model[111–113].

Box 2 | **RNA–RNA-binding protein interplay during differentiation**



Specifically expressed in germ cells, dead end 1 (DND1) is a negative modulator of microRNA-induced silencing complex (miRISC) activity. In zebrafish and human germ cells, DND1 binds uridine-rich regions (URRs) in the 3′ untranslated regions (UTRs) of germline-specific genes, which either sequesters mRNAs or physically displaces miRISC to alleviate microRNA (miRNA)-mediated suppression[182]. In zebrafish, the miR-430 family represses translation of the germ cell factors *nanos1* (also known as *nanos3*), *tdrd7* and *deleted in azoospermia-like* (*dazl*). The human orthologues of the miR-430 family, which comprises miR-371–373 and miR-518–520, are oncogenic in germ cells and associated with increased proliferation, migration, invasion and metastasis[183,184]. DND1 alleviates miR-372-mediated repression of large tumour suppressor 2 (*LATS2*) and also miR-221- and miR-222-mediated repression of cyclin-dependent kinase inhibitor 1B (*CDKN1B*)[182]. In mouse germ cells, DND1 directly interacts with apolipoprotein B mRNA-editing complex 3 (APOBEC3), but whether these RNA-binding proteins (RBPs) cooperatively de-repress germ-cell-specific genes is not known[185]. DAZL also antagonizes miRNA function in human germ cells. During embryogenesis, DAZL binds URRs and drives polyadenylation through interaction with poly(A)-binding protein 1 (PABP1), thereby antagonizing the miRNA effect[186]. DAZL in humans is found associated with pumilio 2 (PUM2), either as an RNA-binding complex or bound to separate motifs[187,188]. Although PUM proteins generally function in gene silencing, the fate of mRNAs jointly targeted by DAZL and PUM2 in the context of human germ cells is unclear. The RBPs DND1, DAZL and PUM2 are essential for germline development and form part of an extended network of post-transcriptional regulation for the maintenance of stemness. Although these RBPs are mainly expressed in germ cells, their regulatory mechanisms could be relevant in tumours with acquired multipotency or pluripotency. This hypothesis needs to be examined in the future. ORF, open reading frame.

The relevance of HuR to cancer is further shown by the high levels of HuR protein in various tumours and direct or miRNA-mediated regulation of many mRNA targets involved in cell proliferation, survival, evasion of immune recognition, metastasis, invasion and local angiogenesis (reviewed in REF. 110).

Occasionally, more complex interactions between HuR and other RBPs in post-transcriptional regulation are observed[114]. For example, HuR and the cap-binding protein eIF4E cooperatively stimulate translation of proteins involved in growth, survival and malignancy, whereas *eIF4E* mRNA itself is concurrently stabilized by HuR binding[115]. As eIF4E is overexpressed in many cancers and closely correlates with HuR expression levels and poor prognosis, these mechanisms are likely to influence tumour progression[115–117].

hnRNPs. Another important RBP family is the hnRNPs. These are sequence-specific repressors of mRNA splicing but are being increasingly associated with a broader range of functions. Whereas some hnRNPs strictly localize to the nucleus, others constantly shuttle between the nucleus and the cytoplasm, indicating a putative role in translational control[118]. Nuclear AU-rich element RNA-binding protein 1 (AUF1; also known as HNRNPD) and HuR simultaneously bind separate regions in the 3′ UTR of common targets but competitively bind shared targets in the cytoplasm in a manner dependent on the abundance of either RBP[115,119]. HuR-bound mRNAs are localized to polysomes for protein synthesis, whereas mRNAs bound by AUF1 are destined for degradation[120]. Several environmental cues can cause cytoplasmic enrichment of these, and other, RBPs[105]. However, the contrasting fate of the common targets cyclin-dependent kinase inhibitor 1A (*CDKN1A*) and cyclin D1 (*CCND1*) after ultraviolet C (UVC) irradiation argues for target-specific regulatory mechanisms in addition to RBP enrichment[119]. Indeed, access of AUF1, but not of HuR, to AREs in target 3′ UTRs can be inhibited by local changes in secondary ARE structure[121]. However, RBPs tend to induce a local change in secondary RNA structure on binding, possibly modulating access of other *trans*-acting factors[122,123].

Hypoxia, a hallmark of the tumour microenvironment, coincides with translocation of nuclear HNRNPL and stabilization of vascular endothelial growth factor A (VEGFA)[124] (FIG. 3b). During hypoxia, translocated HNRNPL competes with several miRNAs that repress *VEGFA* under normoxic conditions through a CA-rich element in the 3′ UTR[125]. In addition to relieving miRNA-mediated repression, translocation of HNRNPL alleviates the repression of VEGFA in response to an inflammatory cytokine, interferon-γ (IFNγ)[126]. In this case, HNRNPL induces a change in RNA secondary structure, whereby access of the IFNγ-activated inhibitor of translation (GAIT) complex to the *VEGFA* 3′ UTR is prevented[126]. Furthermore, *VEGFA* mRNA can be stabilized by binding of HuR to AREs downstream in the 3′ UTR in a hypoxia-responsive manner[127].

An interesting interplay has been observed between HNRNPE2 (also known as PCBP2) and miR-328 (REF. 128) (FIG. 3c). The binding of HNRNPE2 to C-rich regions in the 5′ UTR of CCAAT/enhancer binding

AU-rich element
(ARE). An element that is present in certain 3′ untranslated regions. It often contains a repeat of the AUUUA motif, which has a destabilizing effect on the mRNA in which it resides.

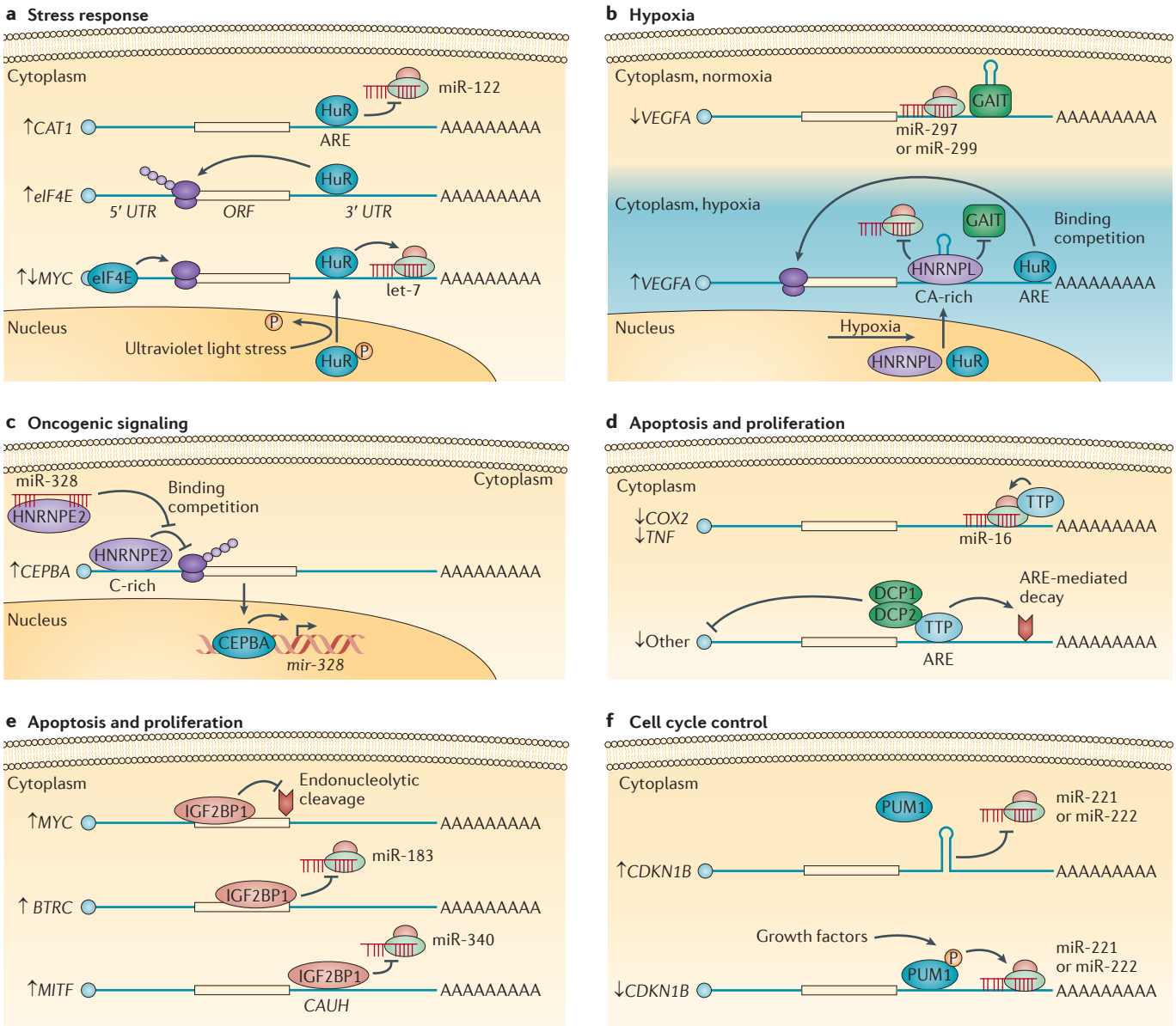


Figure 3 | **Mechanisms of microRNA–RNA-binding protein interplay in various cellular processes.** Schematics of the versatile roles of RNA-binding proteins (RBPs) in regulation of microRNA (miRNA)-mediated repression under various conditions. Deregulation of miRNAs or RBPs in cancer can be due to altered expression, localization, activity or stability of these regulators. Also, both RBPs and miRNAs are dependent on the secondary structure of their target RNA for accessibility. The mRNA is presented linearly for simplicity. **a** | In response to cellular stress, HuR is dephosphorylated and translocates, which relieves cationic amino acid transporter 1 (*CAT1*) mRNA from miR-122-mediated repression. HuR is also required for let-7-mediated repression of *MYC* mRNA. Binding of HuR to AU-rich elements (AREs) in the 3′ untranslated region (UTR) of eukaryotic translation initiation factor 4E (*eIF4E*) mRNA promotes production of eIF4E protein, which is a positive modulator of *MYC* mRNA stability. **b** | Under hypoxic conditions, heterogeneous nuclear ribonucleoprotein L (HNRNPL) and HuR translocate and target the 3′ UTR of vascular endothelial growth factor (*VEGF*) mRNA. Through competitive binding of CA-rich sites, HNRNPL relieves miR-297- or miR-299-mediated repression of *VEGF* mRNA. Also, HNRNPL binding causes a change in secondary structure of *VEGF* mRNA that impedes translational repression by interferon-γ-activated inhibitor of translation (GAIT). *VEGF* mRNA can be stabilized by HuR binding to AREs downstream of the 3′ UTR. **c** | The binding of HNRNPE2 to C-rich regions in the 5′ UTR of CCAAT/enhancer binding protein-α (*CEPBA*) mRNA represses protein translation. The C-rich mature form of miR-328 competes with *CEPBA* for binding of HNRNPE2, which is induced by CEPBA-stimulated transcription of miR-328. **d** | Tristetraprolin (TTP) interaction with an Argonaute (AGO) protein enables miR-16-mediated repression of tumour necrosis factor (*TNF*) and cyclooxygenase 2 (*COX2*). TTP is also known to bind AREs and promote ARE-mediated mRNA decay by recruiting components of the degradation machinery, as is demonstrated for *VEGFA*, hypoxia inducible factor 1α (*HIF1A*), large tumour suppressor 2 (*LATS2*) and *MYC*. **e** | On binding of insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) to the coding region of *MYC* mRNA, the mRNA is sequestered and protected from cleavage and subsequent decay by an endoribonuclease within a region termed the coding region determinant (CRD). IGF2BP1 also relieves miR-183-mediated targeting of the coding region of *BTRC* mRNA and miR-340-mediated targeting of the 3′ UTR of microphthalmia-induced transcription factor (*MITF*) mRNA, where IGF2BP1 is thought to bind on recognition of a CAUH motif. **f** | Phosphorylation of pumilio 1 (PUM1) activates binding of this RBP to the UGUANAUA pumilio recognition element (PRE). The change in secondary structure of cyclin-dependent kinase inhibitor 1B (*CDKN1B*) mRNA enables miR-221 or -222 to access its binding site in the 3′ UTR.

protein-α (*CEPBA*) mRNA, a key regulator of differentiation, represses protein translation and myeloid differentiation[129]. The C-rich mature form of miR-328 competes with *CEPBA* for binding of HNRNPE2 in a RISC-independent manner[128]. Intriguingly, this competition is induced by CEPBA-stimulated transcription, mainly of miR-328 and other miRNA genes[128,130]. It is conceivable that miR-328 sequesters other C-rich region-binding proteins, such as PCBP4 and HNRNPK, in a similar way or that other miRNAs containing an RBP recognition motif function likewise. The importance of this interaction in the context of cancer is stressed by the loss of miR-328 in chronic myelogenous leukaemia. Here, HNRNPE2 activity is upregulated by oncogenic signalling and results in lower miR-328 levels. Subsequent deregulation of direct HNRNPE2 targets, as well as miR-328 targets (such as *MYC* and *PIM1*), contributes to cancer progression[128,129,131].

Tristetraprolin. Another ARE-targeting RBP is tristetraprolin (TTP; also known as ZFP36), an mRNA decay factor that recruits components of the mRNA degradation machinery to the bound target and directs ARE-mediated decay[120,132]. Tumour necrosis factor (*TNF*), *VEGFA*, hypoxia inducible factor 1α (*HIF1A*), cyclooxygenase 2 (*COX2*; also known as *PTGS2*), large tumour suppressor 2 (*LATS2*) and *MYC* are among numerous TTP targets, establishing links with apoptosis and proliferation[133–138] (FIG. 3d). Interestingly, human tumours frequently lack TTP expression, and low *TTP* mRNA levels correlate with a poor prognosis[139]. Several studies have also reported interactions between TTP and components of the RISC machinery, as well as overlap between the associated mRNA degradation machinery (reviewed in REF. 140). For example, TTP can induce mRNA decay by decapping in a manner that requires both binding to an ARE in the 3′ UTR and interaction of the RBP with the decapping complex. However, it has been reported that TTP interaction with AGO2 instead of binding to AREs enables miR-16-mediated repression of *TNF* and *COX2* in human cells[141]. These data suggest that there may be multiple mechanisms whereby TTP and miRNAs can synergistically promote mRNA degradation.

Cell cycle control by RBP–miRNA interplay. On activation of β-catenin signalling, the insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1; also known as CRDBP or IMP1) increases levels of MYC and an F-box protein, βTrCP1 (also known as FBW1A), by targeting a recognition motif in the protein coding region[142,143] (FIG. 3e). IGF2BP1 captures the mRNAs in cytoplasmic particles, thereby providing protection from decay, possibly with assistance from IGF2BP1-associated RBPs[144]. Moreover, IGF2BP1 was found to control degradation of *BTRC* (which encodes βTrCP1) by disrupting the association of miR-183 and AGO2 with a target site in the *BTRC* coding region[145]. Additionally, IGF2BP1 targets the 3′ UTR of a microphthalmia-induced transcription factor (*MITF*) isoform that is predominantly expressed in melanoma and prevents miR-340-mediated repression of *MITF*[146]. Whereas IGF2BP1 proteins are barely detectable in normal cells, overexpression is seen in human tumour cells and correlates with high *MYC* mRNA levels and poor prognosis[145,147]. Moreover, knockdown of IGF2BP1 in colorectal cancer cells reduces colony formation and stimulates apoptosis[142]. Thus, part of IGF2BP1's multifunctional activity is to bind mRNAs, prevent miRNA-mediated repression and regulate tumour progression.

The mRNA 3′ UTR of *CDKN1B* (also known as p27), a crucial inhibitor of cell cycle progression, harbours an 8-nt recognition motif for pumilio 1 (PUM1) near a miR-221 and miR-222 target region[59,148] (FIG. 3f). Under starved conditions, these reverse complementary regions form a stem–loop RNA structure, prohibiting miRNA binding[149]. However, on growth factor stimulation, both phosphorylation and upregulation of PUM1 promote RNA binding activity, exposing the miR-221 and miR-222 site and allowing repression of *CDKN1B*. In fact, several other cell cycle regulators have been identified as human PUM1 targets and are repressed through their mRNA 3′ UTR[148,150]. Furthermore, enrichment of PUM motifs in low-accessibility target 3′ UTRs of miR-410, together with consistent target expression data in various cancer cell lines, support the idea that PUM1 cooperates with miRNAs by inducing conformational changes[151].

As reported by Steitz and colleagues[152], the repression of ARE-containing mRNAs switches to translational activation on growth factor deprivation and subsequent cell cycle exit. Whereas miRNAs act as repressors under proliferative conditions, the binding of miRNAs to AREs now stabilizes target mRNAs by an unspecified mechanism[153]. Strikingly, besides complementary miRNA binding, this process requires recruitment of the RBPs AGO2 and fragile X mental retardation syndrome-related protein 1 (FXR1) to the ARE. FXR1 may function as a translational repressor by binding to the ARE in proliferating cells[154]. However, despite these and other data, the mechanism for activation has not yet been elucidated.

Alternative splicing and polyadenylation. Genome-wide analysis of mature mRNAs reveals alternative transcripts of individual genes with diverse composition and length. About half of the mammalian genes express mRNA isoforms varying in 3′ UTR length or sequence as a result of alternative polyadenylation (APA)[155]. APA can occur in two modes: 3′ exon switching, which requires splicing-dependent terminal exon selection, and tandem UTRs, where different polyadenylation sites (PASs) occur in the same terminal exon. Recognition of a PAS by the polyadenylation machinery is directed by RNA cleavage factors, which select the site of cleavage and determine 3′ UTR length. Interestingly, switching to shorter 3′ UTR forms may circumvent 3′ UTR-mediated regulation by miRNAs and RBPs, conveying changes in mRNA and protein abundance[156,157].

Whereas in the majority of 3′ UTRs with APA the canonical poly(A) signal is distally located, substantial expression of mRNAs with shorter 3′ UTRs is observed in proliferating and less differentiated cells[146,156,158]. For several genes, mRNA isoforms with shorter 3′ UTRs are expressed at higher levels in transformed cells than in

Table 2 | **Current methods for isolating RNPs to identify interactions between RNA and RBPs or miRISC**

Method	Procedure	Refs
RIP-chip	Microarray profiling of endogenous mRNAs associated with immunoprecipitated RBPs and identification of sequence motifs among the bound targets	191
SELEX	Immunoprecipitation of RBPs bound to artificial 52-nt RNAs *in vitro*, followed by cDNA sequencing to identify sequence motifs	192
Genomic SELEX	Analogous to SELEX, but using a genome-based RNA pool, generated by random priming and *in vitro* transcription to reduce complexity and increase sensitivity	193
HITS-CLIP	RBP immunoprecipitation with prior *in vivo* ultraviolet-light-mediated crosslinking of RNA–protein interactions, followed by deep sequencing of linked RNA fragments	194
Ribotrap	Expression of a reporter mRNA containing a 3′ untranslated region recognition site for a known RBP, followed by RBP immunoprecipitation and analysis of associated RNP components by mass spectrometry	195
RNA competition	Definition of RBP binding preferences by microarray analysis of RBP-bound sequences *in vitro* from an abundant pool of 29–38-nt small RNAs. This approach yields binding preferences for either structured or unstructured RNA	196
PAR-CLIP	Analogous to HITS-CLIP, but using incorporation of the nucleotide analogue 4SU into the RNA, which allows efficient crosslinking and direct identification of the RBP binding site by deep sequencing	143
RaPID	Identification of RNP components associated to RNA-aptamer tagged mRNA *in vivo* by mass spectrometry, which allows detection of different RNA species captured in the same RNP by quantitative real-time PCR	197

4SU, 4-thiouridine; HITS-CLIP, high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation; miRISC, microRNA-induced silencing complex; nt, nucleotide; PAR-CLIP, photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation; RaPID, RBP purification and identification; RBP, RNA-binding protein; RIP-chip, RBP immunoprecipitation on cDNA array chip; RNP, ribonucleoprotein; SELEX, systemic evolution of ligands by exponential enrichment.

non-transformed cells, possibly owing to changes in RBP activity[158]. In human colorectal cancer cell lines, binding of TTP to the *COX2* 3′ UTR promotes the use of the proximal PAS[134]. Overexpression of a shorter, but not full length mRNA isoform of *IGF2BP1* leads to oncogenic transformation[158]. However, the exact changes in APA that correlate with tumour development, as well as the mechanisms that operate to control them, are largely unknown.

Conclusions and perspectives

miRNAs are often deregulated in cancer. However, miRNA deregulation is rarely caused by gene amplification or disruption, most probably owing to redundant functions in different genomic loci. Frequently, changes in transcription rate and processing, as well as miRNA activity, are observed. Although transcription factors and chromatin modulators account for alterations in miRNA production, which explain several cases of miRNA overexpression in cancer, RBPs and their interacting partners mostly cause the changes observed in miRNA processing and activity. In cancer these range from disruption of miRNA biogenesis core components to changes in the secondary structure of miRNA target sites, and from a global effect on miRNA processing to specific regulation of select 3′ UTRs. Interestingly, in response to external or internal stimuli, RBPs can dynamically shape the extent of miRNA-mediated repression to maintain robust gene expression. In this way, the 3′ UTR can be considered a multi-faceted docking platform for post-transcriptional regulators that either synergistically or antagonistically fine-tune gene expression in time and space. The fact that 3′ UTRs frequently contain multiple evolutionary conserved binding sites for both miRNAs and RBPs suggests that the interplay between RBPs and miRNAs is a crucial component of gene regulation. Novel high-throughput techniques to measure RNA–RNA and RNA–protein interactions (reviewed in REF. 159) (TABLE 2), as well as to monitor mRNA secondary structure[160], should enable us to connect networks of post-transcriptional regulation and decipher their relevance for cancer initiation and progression. This exciting playground of RBPs and miRNAs still holds secrets that, when uncovered, hopefully will reveal networks with potential therapeutic benefits.

1. Bartel, D. P. MicroRNAs: target recognition and regulatory functions. *Cell* **136**, 215–233 (2009).
2. Friedman, R. C., Farh, K. K., Burge, C. B. & Bartel, D. P. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* **19**, 92–105 (2009).
3. Lu, J. *et al.* MicroRNA expression profiles classify human cancers. *Nature* **435**, 834–838 (2005). **The results presented in this paper had a big impact. It was found that miRNA expression profiles could be used to discriminate between normal and tumour tissue and, in addition, they reflected the developmental origin and differentiation state of the tumour. This led to discussion of the possible application of this finding in diagnosis and raised queries about the cause of global miRNA deregulation in tumours.**
4. Volinia, S. *et al.* A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc. Natl Acad. Sci. USA* **103**, 2257–2261 (2006). **Based on a panel of 540 solid tumour samples, an miRNA expression signature was presented that contains miRNAs that are differentially expressed in tumours. Many of their predicted targets were tumour suppressors or oncogenes, turning the miRNA genes into novel cancer genes.**
5. Lunde, B. M., Moore, C. & Varani, G. RNA-binding proteins: modular design for efficient function. *Nature Rev. Mol. Cell Biol.* **8**, 479–490 (2007).
6. Lukong, K. E., Chang, K. W., Khandjian, E. W. & Richard, S. RNA-binding proteins in human genetic disease. *Trends Genet.* **24**, 416–425 (2008).
7. Riggi, N., Cironi, L., Suvà, M. L. & Stamenkovic, I. Sarcomas: genetics, signalling, and cellular origins. Part 1: the fellowship of TET. *J. Pathol.* **213**, 4–20 (2007).
8. Rajan, P. *et al.* Regulation of gene expression by the RNA-binding protein Sam68 in cancer. *Biochem. Soc. Trans.* **36**, 505–507 (2008).

9. Lee, H. K. *et al.* β-catenin regulates multiple steps of RNA metabolism as revealed by the RNA aptamer in colon cancer cells. *Cancer Res.* **67**, 9315–9321 (2007).
10. David, C. J. & Manley, J. L. Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. *Genes Dev.* **24**, 2343–2364 (2010).
11. Denli, A. M., Tops, B. B., Plasterk, R. H., Ketting, R. F. & Hannon, G. J. Processing of primary microRNAs by the Microprocessor complex. *Nature* **432**, 231–235 (2004).
12. Gregory, R. I. The Microprocessor complex mediates the genesis of microRNAs. *Nature* **432**, 235–240 (2004).
13. Yi, R., Qin, Y., Macara, I. G. & Cullen, B. R. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.* **17**, 3011–3016 (2003).
14. Lund, E., Güttinger, S., Calado, A., Dahlberg, J. E. & Kutay, U. Nuclear export of microRNA precursors. *Science* **303**, 95–98 (2004).
15. Bernstein, E., Caudy, A. A., Hammond, S. M. & Hannon, G. J. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **409**, 363–366 (2001).
16. Grishok, A. *et al.* Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. *Cell* **106**, 23–34 (2001).
17. Hutvágner, G. *et al.* A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* **293**, 834–838 (2001).
18. Chendrimada, T. P. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* **436**, 740–744 (2005).
19. Zhang, H., Kolb, F. A., Jaskiewicz, L., Westhof, E. & Filipowicz, W. Single processing center models for human Dicer and bacterial RNase III. *Cell* **118**, 57–68 (2004).
20. MacRae, I. J., Zhou, K. & Doudna, J. A. Structural determinants of RNA recognition and cleavage by Dicer. *Nature Struct. Mol. Biol.* **14**, 934–940 (2007).
21. Haase, A. D. *et al.* TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. *EMBO Rep.* **6**, 961–967 (2005).
22. Chakravarthy, S., Sternberg, S. H., Kellenberger, C. A. & Doudna, J. A. Substrate-specific kinetics of dicer-catalyzed RNA processing. *J. Mol. Biol.* **404**, 392–402 (2010).
23. Lee, Y. *et al.* The role of PACT in the RNA silencing pathway. *EMBO J.* **25**, 522–532 (2006).
24. Meister, G. *et al.* Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol. Cell* **15**, 185–197 (2004).
25. Cerutti, L., Mian, N. & Bateman, A. Domains in gene silencing and cell differentiation proteins: the novel PAZ domain and redefinition of the Piwi domain. *Trends Biochem. Sci.* **25**, 481–482 (2000).
26. Song, J. J., Smith, S. K., Hannon, G. J. & Joshua-Tor, L. Crystal structure of Argonaute and its implications for RISC slicer activity. *Science* **305**, 1434–1437 (2004).
27. Ma, J. B., Ye, K. & Patel, D. J. Structural basis for overhang-specific small interfering RNA recognition by the PAZ domain. *Nature* **429**, 318–322 (2004).
28. Diederichs, S. & Haber, D. A. Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. *Cell* **131**, 1097–1108 (2007).
29. Cheloufi, S., Dos Santos, C. O., Chong, M. M. & Hannon, G. J. A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. *Nature* **465**, 584–589 (2010).
30. Cifuentes, D. *et al.* A novel miRNA processing pathway independent of Dicer requires Argonaute2 catalytic activity. *Science* **328**, 1694–1698 (2010).
31. Ambros, V. *et al.* A uniform system for microRNA annotation. *RNA* **9**, 277–279 (2003).
32. Hutvágner, G. Small RNA asymmetry in RNAi: function in RISC assembly and gene regulation. *FEBS Lett.* **579**, 5850–5857 (2005).
33. Thomson, J. M. *et al.* Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev.* **20**, 2202–2207 (2006).
34. Zhang, L. *et al.* microRNAs exhibit high frequency genomic alterations in human cancer. *Proc. Natl Acad. Sci. USA* **103**, 9136–9141 (2006).
35. Kim, M. S. *et al.* Somatic mutations and losses of expression of microRNA regulation-related genes AGO2 and TNRC6A in gastric and colorectal cancers. *J. Pathol.* **221**, 139–146 (2010).
36. Lee, E. J. *et al.* Systematic evaluation of microRNA processing patterns in tissues, cell lines, and tumors. *RNA* **14**, 35–42 (2008).
37. Melo, S. A. *et al.* A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. *Cancer Cell* **18**, 303–315 (2010).
38. Kumar, M. S., Lu, J., Mercer, K. L., Golub, T. R. & Jacks, T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nature Genet.* **39**, 673–677 (2007).
This report provided evidence that impairment of miRNA biogenesis contributes to oncogenic transformation and is not merely a result of it. Independent knockdown of miRNA pathway components caused a global loss of miRNA (as observed in reference 3) and promoted tumour formation.
39. Lambertz, I. *et al.* Monoallelic but not biallelic loss of *Dicer1* promotes tumorigenesis *in vivo*. *Cell Death Differ.* **17**, 633–641 (2010).
40. Hill, D. A. *et al. DICER1* mutations in familial pleuropulmonary blastoma. *Science* **325**, 965 (2009).
41. Kumar, M. S. *et al. Dicer1* functions as a haploinsufficient tumor suppressor. *Genes Dev.* **23**, 2700–2704 (2009).
42. Martello, G. *et al.* A microRNA targeting Dicer for metastasis control. *Cell* **141**, 1195–1207 (2010).
43. Melo, S. A. *et al.* A *TARBP2* mutation in human cancer impairs microRNA processing and DICER1 function. *Nature Genet.* **41**, 365–370 (2009).
44. Garre, P., Pérez-Segura, P., Díaz-Rubio, E., Caldés, T. & de la Hoya, M. Reassessing the *TARBP2* mutation rate in hereditary nonpolyposis colorectal cancer. *Nature Genet.* **42**, 817–818 (2010).
45. Paroo, Z., Ye, X., Chen, S. & Liu, Q. Phosphorylation of the human microRNA-generating complex mediates MAPK/Erk signaling. *Cell* **139**, 112–122 (2009).
46. Han, J. Posttranscriptional crossregulation between Drosha and DGCR8. *Cell* **136**, 75–84 (2009).
47. Obernosterer, G., Leuschner, P. J., Alenius, M. & Martinez, J. Post-transcriptional regulation of microRNA expression. *RNA* **12**, 1161–1167 (2006).
48. Fuller-Pace, F. V. & Moore, H. C. RNA helicases p68 and p72: multifunctional proteins with important implications for cancer development. *Future Oncol.* **7**, 239–251 (2011).
49. Fukuda, T. *et al.* DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nature Cell Biol.* **9**, 604–611 (2007).
50. Suzuki, H. I. *et al.* Modulation of microRNA processing by p53. *Nature* **460**, 529–533 (2009).
51. Feng, Z., Zhang, C., Wu, R. & Hu, W. Tumor suppressor p53 meets microRNAs. *J. Mol. Cell Biol.* **3**, 44–50 (2011).
52. Yamagata, K. Maturation of microRNA is hormonally regulated by a nuclear receptor. *Mol. Cell* **36**, 340–347 (2009).
53. Davis, B. N., Hilyard, A. C., Lagna, G. & Hata, A. SMAD proteins control DROSHA-mediated microRNA maturation. *Nature* **454**, 56–61 (2008).
54. Davis, B. N., Hilyard, A. C., Nguyen, P. H., Lagna, G. & Hata, A. Smad proteins bind a conserved RNA sequence to promote microRNA maturation by Drosha. *Mol. Cell* **39**, 373–384 (2010).
55. Yu, B. The FHA domain proteins DAWDLE in *Arabidopsis* and SNIP1 in humans act in small RNA biogenesis. *Proc. Natl Acad. Sci. USA* **105**, 10073–10078 (2008).
56. Wendt, M. K., Allington, T. M. & Schiemann, W. P. Mechanisms of the epithelial-mesenchymal transition by TGF-β. *Future Oncol.* **5**, 1145–1168 (2009).
57. Wu, H. A splicing-independent function of SF2/ASF in microRNA processing. *Mol. Cell* **38**, 67–77 (2010).
58. Karni, R. *et al.* The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nature Struct. Mol. Biol.* **14**, 185–193 (2007).
59. le Sage, C. *et al.* Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *EMBO J.* **26**, 3699–3708 (2007).
60. Nakamura, T., Canaani, E. & Croce, C. M. Oncogenic All1 fusion proteins target Drosha-mediated microRNA processing. *Proc. Natl Acad. Sci. USA* **104**, 10980–10985 (2007).
61. Rybak, A. A feedback loop comprising lin-28 and let-7 controls pre-let-7 maturation during neural stem-cell commitment. *Nature Cell Biol.* **10**, 987–993 (2008).
62. Viswanathan, S. R. & Daley, G. Q. Lin28: a microRNA regulator with a macro role. *Cell* **140**, 445–449 (2010).
63. Iliopoulos, D., Jaeger, S. A., Hirsch, H. A., Bulyk, M. L. & Struhl, K. STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. *Mol. Cell* **39**, 493–506 (2010).
64. Trabucchi, M. The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. *Nature* **459**, 1010–1014 (2009).
65. Michlewski, G. & Cáceres, J. F. Antagonistic role of hnRNP A1 and KSRP in the regulation of let-7a biogenesis. *Nature Struct. Mol. Biol.* **17**, 1011–1018 (2010).
66. Guil, S. & Cáceres, J. F. The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. *Nature Struct. Mol. Biol.* **14**, 591–596 (2007).
67. Michlewski, G., Guil, S., Semple, C. A. & Caceres, J. F. Posttranscriptional regulation of miRNAs harboring conserved terminal loops. *Mol. Cell* **32**, 383–393 (2008).
68. Zhang, X. & Zeng, Y. The terminal loop region controls microRNA processing by Drosha and Dicer. *Nucleic Acids Res.* **38**, 7689–7697 (2010).
69. Filipowicz, W., Bhattacharyya, S. N. & Sonenberg, N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nature Rev. Genet.* **9**, 102–114 (2008).
70. Eulalio, A., Huntzinger, E. & Izaurralde, E. Getting to the root of miRNA-mediated gene silencing. *Cell* **132**, 9–14 (2008).
71. Hutvágner, G. & Zamore, P. D. A microRNA in a multiple-turnover RNAi enzyme complex. *Science* **297**, 2056–2060 (2002).
72. Martinez, J., Patkaniowska, A., Urlaub, H., Lührmann, R. & Tuschl, T. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. *Cell* **110**, 563–574 (2002).
73. Höck, J. *et al.* Proteomic and functional analysis of Argonaute-containing mRNA-protein complexes in human cells. *EMBO Rep.* **8**, 1052–1060 (2007).
74. Carmell, M. A., Xuan, Z., Zhang, M. Q. & Hannon, G. J. The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance, and tumorigenesis. *Genes Dev.* **16**, 2733–2742 (2002).
75. Azuma-Mukai, A. Characterization of endogenous human Argonautes and their miRNA partners in RNA silencing. *Proc. Natl Acad. Sci. USA* **105**, 7964–7969 (2008).
76. Su, H., Trombly, M. I., Chen, J. & Wang, X. Essential and overlapping functions for mammalian Argonautes in microRNA silencing. *Genes Dev.* **23**, 304–317 (2009).
77. Eulalio, A., Tritschler, F. & Izaurralde, E. The GW182 protein family in animal cells: new insights into domains required for miRNA-mediated gene silencing. *RNA* **15**, 1433–1442 (2009).
78. Huntzinger, E. & Izaurralde, E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nature Rev. Genet.* **12**, 99–110 (2011).
79. Djuranovic, S., Nahvi, A. & Green, R. A parsimonious model for gene regulation by miRNAs. *Science* **331**, 550–553 (2011).
80. Baek, D. *et al.* The impact of microRNAs on protein output. *Nature* **455**, 64–71 (2008).
81. Guo, H., Ingolia, N. T., Weissman, J. S. & Bartel, D. P. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* **466**, 835–840 (2010).
82. Chu, C. Y. & Rana, T. M. Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54. *PLoS Biol.* **4**, e210 (2006).
83. Eulalio, A. *et al.* Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. *Genes Dev.* **21**, 2558–2570 (2007).
84. Lin, F. *et al.* Knockdown of RCK/p54 expression by RNAi inhibits proliferation of human colorectal cancer cells *in vitro* and *in vivo*. *Cancer Biol. Ther.* **7**, 1669–1676 (2008).
85. Salzman, D. W., Shubert-Coleman, J. & Furneaux, H. P68 RNA helicase unwinds the human let-7 microRNA precursor duplex and is required for let-7-directed silencing of gene expression. *J. Biol. Chem.* **282**, 32773–32779 (2007).
86. Meister, G. *et al.* Identification of novel argonaute-associated proteins. *Curr. Biol.* **15**, 2149–2155 (2005).
87. Chendrimada, T. P. *et al.* MicroRNA silencing through RISC recruitment of eIF6. *Nature* **447**, 823–828 (2007).

88. Mourelatos, Z. *et al.* miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. *Genes Dev.* **16**, 720–728 (2002).
89. Eulalio, A., Behm-Ansmant, I., Schweizer, D. & Izaurralde, E. P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. *Mol. Cell. Biol.* **27**, 3970–3981 (2007).
90. Landthaler, M. *et al.* Molecular characterization of human Argonaute-containing ribonucleoprotein complexes and their bound target mRNAs. *RNA* **14**, 2580–2596 (2008).
91. Pare, J. M. Hsp90 regulates the function of argonaute 2 and its recruitment to stress granules and P-bodies. *Mol. Biol. Cell* **20**, 3273–3284 (2009).
92. Suzuki, Y. *et al.* The Hsp90 inhibitor geldanamycin abrogates colocalization of eIF4E and eIF4E-transporter into stress granules and association of eIF4E with eIF4G. *J. Biol. Chem.* **284**, 35597–35604 (2009).
93. Iwasaki, S. *et al.* Hsc70/Hsp90 chaperone machinery mediates ATP-dependent RISC loading of small RNA duplexes. *Mol. Cell* **39**, 292–299 (2010).
94. Andrei, M. A. *et al.* A role for eIF4E and eIF4E-transporter in targeting mRNPs to mammalian processing bodies. *RNA* **11**, 717–727 (2005).
95. Johnston, M., Geoffroy, M. C., Sobala, A., Hay, R. & Hutvágner, G. HSP90 protein stabilizes unloaded argonaute complexes and microscopic P-bodies in human cells. *Mol. Biol. Cell* **21**, 1462–1469 (2010).
96. Rybak, A. The let-7 target gene mouse *lin-41* is a stem cell specific E3 ubiquitin ligase for the miRNA pathway protein Ago2. *Nature Cell Biol.* **11**, 1411–1420 (2009).
97. Schwamborn, J. C., Berezikov, E. & Knoblich, J. A. The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors. *Cell* **136**, 913–925 (2009).
98. Neumuller, R. A. Mei-P26 regulates microRNAs and cell growth in the *Drosophila* ovarian stem cell lineage. *Nature* **454**, 241–245 (2008).
99. Kano, S., Miyajima, N., Fukuda, S. & Hatakeyama, S. Tripartite motif protein 32 facilitates cell growth and migration via degradation of Abl-interactor 2. *Cancer Res.* **68**, 5572–5580 (2008).
100. Weinmann, L. Importin 8 is a gene silencing factor that targets argonaute proteins to distinct mRNAs. *Cell* **136**, 496–507 (2009).
101. Parker, R. & Song, H. The enzymes and control of eukaryotic mRNA turnover. *Nature Struct. Mol. Biol.* **11**, 121–127 (2004).
102. Zhang, K. *et al.* The human homolog of yeast *SEP1* is a novel candidate tumor suppressor gene in osteogenic sarcoma. *Gene* **298**, 121–127 (2002).
103. Hinman, M. N. & Lou, H. Diverse molecular functions of Hu proteins. *Cell. Mol. Life Sci.* **65**, 3168–3181 (2008).
104. Bhattacharyya, S. N., Habermacher, R., Martine, U., Closs, E. I. & Filipowicz, W. Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell* **125**, 1111–1124 (2006).
In this paper, the first example of stress-induced relief of miRNA-mediated repression through binding of an RBP to the 3′ UTR was described.
105. Wang, W. *et al.* HuR regulates p21 mRNA stabilization by UV light. *Mol. Cell. Biol.* **20**, 760–769 (2000).
106. Kullmann, M., Göpfert, U., Siewe, B. & Hengst, L. ELAV/Hu proteins inhibit p27 translation via an IRES element in the p27 5′UTR. *Genes Dev.* **16**, 3087–3099 (2002).
107. Leandersson, K., Riesbeck, K. & Andersson, T. Wnt-5a mRNA translation is suppressed by the Elav-like protein HuR in human breast epithelial cells. *Nucleic Acids Res.* **34**, 3988–3999 (2006).
108. Saunus, J. M. *et al.* Posttranscriptional regulation of the breast cancer susceptibility gene *BRCA1* by the RNA binding protein HuR. *Cancer Res.* **68**, 9469–9478 (2008).
109. Kim, H. H. *et al.* HuR recruits let-7/RISC to repress c-Myc expression. *Genes Dev.* **23**, 1743–1748 (2009).
In contrast to the stabilizing function of HuR described in reference 104, this RBP was found to be required for let-7-mediated target repression.
110. Abdelmohsen, K. & Gorospe, M. Posttranscriptional regulation of cancer traits by HuR. *RNA* **1**, 214–229 (2010).
111. Abdelmohsen, K., Srikantan, S., Kuwano, Y. & Gorospe, M. miR-519 reduces cell proliferation by lowering RNA-binding protein HuR levels. *Proc. Natl Acad. Sci. USA* **105**, 20297–20302 (2008).
112. Guo, X., Wu, Y. & Hartley, R. S. MicroRNA-125a represses cell growth by targeting HuR in breast cancer. *RNA Biol.* **6**, 575–583 (2009).
113. Abdelmohsen, K. *et al.* miR-519 suppresses tumor growth by reducing HuR levels. *Cell Cycle* **9**, 1354–1359 (2010).
114. Pullmann, R. *et al.* Analysis of turnover and translation regulatory RNA-binding protein expression through binding to cognate mRNAs. *Mol. Cell. Biol.* **27**, 6265–6278 (2007).
115. Topisirovic, I. *et al.* Stability of eukaryotic translation initiation factor 4E mRNA is regulated by HuR, and this activity is dysregulated in cancer. *Mol. Cell. Biol.* **29**, 1152–1162 (2009).
This paper reports that HuR and eIF4E cooperatively stabilize mRNA targets, while eIF4E mRNA is stabilized by HuR. Such regulative loops add a layer of complexity onto the interplay of RBPs.
116. Wendel, H. G. *et al.* Dissecting eIF4E action in tumorigenesis. *Genes Dev.* **21**, 3232–3237 (2007).
117. Furic, L. *et al.* eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. *Proc. Natl Acad. Sci. USA* **107**, 14134–14139 (2010).
118. Mili, S., Shu, H. J., Zhao, Y. & Piñol-Roma, S. 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 (2001).
119. Lal, A. *et al.* Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. *EMBO J.* **23**, 3092–3102 (2004).
120. Chen, C. Y. *et al.* AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell* **107**, 451–464 (2001).
121. Fialcowitz, E. J., Brewer, B. Y., Keenan, B. P. & Wilson, G. M. A hairpin-like structure within an AU-rich mRNA-destabilizing element regulates trans-factor binding selectivity and mRNA decay kinetics. *J. Biol. Chem.* **280**, 22406–22417 (2005).
122. Wilson, G. M., Sutphen, K., Chuang, K. & Brewer, G. Folding of A + U-rich RNA elements modulates AUF1 binding. Potential roles in regulation of mRNA turnover. *J. Biol. Chem.* **276**, 8695–8704 (2001).
123. Wilson, G. M., Sutphen, K., Moutafis, M., Sinha, S. & Brewer, G. Structural remodeling of an A + U-rich RNA element by cation or AUF1 binding. *J. Biol. Chem.* **276**, 38400–38409 (2001).
124. Shih, S. C. & Claffey, K. P. Regulation of human vascular endothelial growth factor mRNA stability in hypoxia by heterogeneous nuclear ribonucleoprotein L. *J. Biol. Chem.* **274**, 1359–1365 (1999).
125. Jafarifar, F., Yao, P., Eswarappa, S. M. & Fox, P. L. Repression of VEGFA by CA-rich element-binding microRNAs is modulated by hnRNP L. *EMBO J.* **30**, 1324–1334 (2011).
The binding of HNRNPL to the *VEGFA* 3′ UTR competes with miRNA binding and changes the RNA conformation.
126. Ray, P. S. *et al.* A stress-responsive RNA switch regulates VEGFA expression. *Nature* **457**, 915–919 (2009).
Under hypoxic conditions, the binding of HNRNPL to the *VEGFA* 3′ UTR changes the RNA secondary structure and relieves IFNγ-induced repression of *VEFGA* by GAIT, showing how the response to combined stimuli is orchestrated. Together with reference 125, these data demonstrate how HNRNPL can relieve two repressive mechanisms simultaneously.
127. Goldberg-Cohen, I., Furneauxb, H. & Levy, A. P. A 40-bp RNA element that mediates stabilization of vascular endothelial growth factor mRNA by HuR. *J. Biol. Chem.* **277**, 13635–13640 (2002).
128. Eiring, A. M. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. *Cell* **140**, 652–665 (2010).
This paper uniquely describes a decoy function of an miRNA. miR-328 competes with *CEPBA* mRNA for binding of HNRNPE2. CEPBA is a transcriptional activator of the miR-328 gene, thus forming a positive feedback loop.
129. Chang, J. S. *et al.* High levels of the BCR/ABL oncoprotein are required for the MAPK-hnRNP-E2 dependent suppression of C/EBPα-driven myeloid differentiation. *Blood* **110**, 994–1003 (2007).
130. Fazi, F. *et al.* A minicircuitry comprised of microRNA-223 and transcription factors NFI-A and C/EBPα regulates human granulopoiesis. *Cell* **123**, 819–831 (2005).
131. Evans, J. R. *et al.* Members of the poly (rC) binding protein family stimulate the activity of the c-*myc* internal ribosome entry segment *in vitro* and *in vivo*. *Oncogene* **22**, 8012–8020 (2003).
132. Fenger-Grøn, M., Fillman, C., Norrild, B. & Lykke-Andersen, J. Multiple processing body factors and the ARE binding protein TTP activate mRNA decapping. *Mol. Cell* **20**, 905–915 (2005).
133. Carballo, E., Lai, W. S. & Blackshear, P. J. Feedback inhibition of macrophage tumor necrosis factor-α production by tristetraprolin. *Science* **281**, 1001–1005 (1998).
134. Sawaoka, H., Dixon, D. A., Oates, J. A. & Boutaud, O. Tristetraprolin binds to the 3′-untranslated region of cyclooxygenase-2 mRNA. A polyadenylation variant in a cancer cell line lacks the binding site. *J. Biol. Chem.* **278**, 13928–13935 (2003).
135. Lai, W. S., Parker, J. S., Grissom, S. F., Stumpo, D. J. & Blackshear, P. J. Novel mRNA targets for tristetraprolin (TTP) identified by global analysis of stabilized transcripts in TTP-deficient fibroblasts. *Mol. Cell. Biol.* **26**, 9196–9208 (2006).
136. Marderosian, M. *et al.* Tristetraprolin regulates Cyclin D1 and c-Myc mRNA stability in response to rapamycin in an Akt-dependent manner via p38 MAPK signaling. *Oncogene* **25**, 6277–6290 (2006).
137. Lee, H. H. *et al.* Stability of the *LATS2* tumor suppressor gene is regulated by tristetraprolin. *J. Biol. Chem.* **285**, 17329–17337 (2010).
138. Stoecklin, G. *et al.* Genome-wide analysis identifies interleukin-10 mRNA as target of tristetraprolin. *J. Biol. Chem.* **283**, 11689–11699 (2008).
139. Brennan, S. E. *et al.* The mRNA-destabilizing protein tristetraprolin is suppressed in many cancers, altering tumorigenic phenotypes and patient prognosis. *Cancer Res.* **69**, 5168–5176 (2009).
140. von Roretz, C. & Gallouzi, I. E. Decoding ARE-mediated decay: is microRNA part of the equation? *J. Cell Biol.* **181**, 189–194 (2008).
141. Jing, Q. *et al.* Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell* **120**, 623–634 (2005).
These data indicate that the RBP TTP, an mRNA decay factor that binds AREs and generally operates by uncapping, assists miR-16 in targeting AREs by interacting with AGO2 or other RISC factors, showing the versatility of RBPs.
142. Noubissi, F. K. *et al.* CRD-BP mediates stabilization of *βTrCP1* and *c-myc* mRNA in response to β-catenin signalling. *Nature* **441**, 898–901 (2006).
143. Hafner, M. *et al.* Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell* **141**, 129–141 (2010).
144. Weidensdorfer, D. *et al.* Control of c-myc mRNA stability by IGF2BP1-associated cytoplasmic RNPs. *RNA* **15**, 104–115 (2009).
145. Elcheva, I., Goswami, S., Noubissi, F. K. & Spiegelman, V. S. CRD-BP protects the coding region of β*TrCP1* mRNA from miR-183-mediated degradation. *Mol. Cell* **35**, 240–246 (2009).
This paper shows that the RBP IFG2BP1 can bind a coding region in a specific target and can alleviate the interaction between miR-183 in complex with RISC and its target site, which is also located in the coding region.
146. Goswami, S. *et al.* MicroRNA-340-mediated degradation of microphthalmia-associated transcription factor mRNA is inhibited by the coding region determinant-binding protein. *J. Biol. Chem* **285**, 20532–20540 (2010).
The RBP IGF2BP1 can also antagonize miRNA-mediated repression through the 3′ UTR, illustrating the multifunctional character of this protein.
147. Köbel, M. *et al.* Expression of the RNA-binding protein IMP1 correlates with poor prognosis in ovarian carcinoma. *Oncogene* **26**, 7584–7589 (2007).
148. Galgano, A. *et al.* Comparative analysis of mRNA targets for human PUF-family proteins suggests extensive interaction with the miRNA regulatory system. *PLoS ONE* **3**, e3164 (2008).
149. Kedde, M. *et al.* A Pumilio-induced RNA structure switch in p27-3′ UTR controls miR-221 and miR-222 accessibility. *Nature Cell Biol.* **12**, 1014–1020 (2010).
This is the first report of a reverse complementary RBP recognition site and miRNA site that form a stem–loop structure in the absence of the RBP PUM1. This emphasizes the importance of RNA secondary structure for miRNA accessibility.

150. Morris, A. R., Mukherjee, N. & Keene, J. D. Ribonomic analysis of human Pum1 reveals *cis-trans* conservation across species despite evolution of diverse mRNA target sets. *Mol. Cell. Biol.* **28**, 4093–4103 (2008).
151. Leibovich, L., Mandel-Gutfreund, Y. & Yakhini, Z. A structural-based statistical approach suggests a cooperative activity of PUM1 and miR-410 in human 3′-untranslated regions. *Silence* **1**, 17 (2010).
152. Vasudevan, S. & Steitz, J. A. AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. *Cell* **128**, 1105–1118 (2007).
153. Vasudevan, S., Tong, Y. & Steitz, J. A. Switching from repression to activation: microRNAs can up-regulate translation. *Science* **318**, 1931–1934 (2007).
154. Garnon, J. *et al.* Fragile X-related protein FXR1P regulates proinflammatory cytokine tumor necrosis factor expression at the post-transcriptional level. *J. Biol. Chem.* **280**, 5750–5763 (2005).
155. Zhang, H., Lee, J. Y. & Tian, B. Biased alternative polyadenylation in human tissues. *Genome Biol.* **6**, R100 (2005).
156. Sandberg, R., Neilson, J. R., Sarma, A., Sharp, P. A. & Burge, C. B. Proliferating cells express mRNAs with shortened 3′ untranslated regions and fewer microRNA target sites. *Science* **320**, 1643–1647 (2008).
This paper shows that shorter 3′ UTRs, as expressed in proliferating and undifferentiated cells, confer post-transcriptional mRNA stability and higher protein levels. This implies that the function of the 3′ UTR is mainly repressive and that regulation by repressors acting on the 3′ UTR seems to be essential for maintaining correct gene expression.
157. Legendre, M., Ritchie, W., Lopez, F. & Gautheret, D. Differential repression of alternative transcripts: a screen for miRNA targets. *PLoS Comput. Biol.* **2**, e43 (2006).
158. Mayr, C. & Bartel, D. P. Widespread shortening of 3′UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell* **138**, 673–684 (2009).
In this paper, the consequences of alternative PAS usage for oncogenic transformation are shown by isoforms of IGF2BP1, in agreement with the data presented in reference 156.
159. Licatalosi, D. D. & Darnell, R. B. RNA processing and its regulation: global insights into biological networks. *Nature Rev. Genet.* **11**, 75–87 (2010).
160. Kertesz, M. *et al.* Genome-wide measurement of RNA secondary structure in yeast. *Nature* **467**, 103–107 (2010).
161. Griffiths-Jones, S., Grocock, R. J., van Dongen, S., Bateman, A. & Enright, A. J. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res.* **34**, D140–D144 (2006).
162. Berezikov, E., Cuppen, E. & Plasterk, R. H. Approaches to microRNA discovery. *Nature Genet.* **38**, S2–S7 (2006).
163. Landgraf, P. *et al.* A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* **129**, 1401–1414 (2007).
164. Forman, J. J. & Coller, H. A. The code within the code: microRNAs target coding regions. *Cell Cycle* **9**, 1533–1541 (2010).
165. Stefani, G. & Slack, F. J. Small non-coding RNAs in animal development. *Nature Rev. Mol. Cell Biol.* **9**, 219–230 (2008).
166. Farh, K. K. *et al.* The widespread impact of mammalian microRNAs on mRNA repression and evolution. *Science* **310**, 1817–1821 (2005).
167. Kloosterman, W. P. & Plasterk, R. H. The diverse functions of microRNAs in animal development and disease. *Dev. Cell* **11**, 441–450 (2006).
168. Lima, R. T. *et al.* MicroRNA regulation of core apoptosis pathways in cancer. *Eur. J. Cancer* **47**, 163–174 (2011).
169. Negrini, M., Nicoloso, M. S. & Calin, G. A. MicroRNAs and cancer — new paradigms in molecular oncology. *Curr. Opin. Cell Biol.* **21**, 470–479 (2009).
170. Urbich, C., Kuehbacher, A. & Dimmeler, S. Role of microRNAs in vascular diseases, inflammation, and angiogenesis. *Cardiovasc. Res.* **79**, 581–588 (2008).
171. Gregory, P. A., Bracken, C. P., Bert, A. G. & Goodall, G. J. MicroRNAs as regulators of epithelial-mesenchymal transition. *Cell Cycle* **7**, 3112–3118 (2008).
172. Nicoloso, M. S., Spizzo, R., Shimizu, M., Rossi, S. & Calin, G. A. MicroRNAs — the micro steering wheel of tumour metastases. *Nature Rev. Cancer* **9**, 293–302 (2009).
173. Olson, P. *et al.* MicroRNA dynamics in the stages of tumorigenesis correlate with hallmark capabilities of cancer. *Genes Dev.* **23**, 2152–2165 (2009).
174. Volinia, S. *et al.* Reprogramming of miRNA networks in cancer and leukemia. *Genome Res.* **20**, 589–599 (2010).
175. Voorhoeve, P. M. MicroRNAs: oncogenes, tumor suppressors or master regulators of cancer heterogeneity? *Biochim. Biophys. Acta* **1805**, 72–86 (2010).
176. Farazi, T. A., Spitzer, J. I., Morozov, P. & Tuschl, T. miRNAs in human cancer. *J. Pathol.* **223**, 102–115 (2011).
177. Calin, G. A. & Croce, C. M. MicroRNA signatures in human cancers. *Nature Rev. Cancer* **6**, 857–866 (2006).
178. Ventura, A. & Jacks, T. MicroRNAs and cancer: short RNAs go a long way. *Cell* **136**, 586–591 (2009).
179. Ryan, B. M., Robles, A. I. & Harris, C. C. Genetic variation in microRNA networks: the implications for cancer research. *Nature Rev. Cancer* **10**, 389–402 (2010).
180. Kim, V. N., Han, J. & Siomi, M. C. Biogenesis of small RNAs in animals. *Nature Rev. Mol. Cell Biol.* **10**, 126–139 (2009).
181. Krol, J., Loedige, I. & Filipowicz, W. The widespread regulation of microRNA biogenesis, function and decay. *Nature Rev. Genet.* **11**, 597–610 (2010).
182. Kedde, M. *et al.* RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. *Cell* **131**, 1273–1286 (2007).
This paper describes an RBP, which interacts with the 3′ UTR of some germ cell genes and allows their expression by preventing miRNA-mediated repression during germ cell development in zebrafish.
183. Voorhoeve, P. M. *et al.* A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell* **124**, 1169–1181 (2006).
184. Huang, Q. *et al.* The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. *Nature Cell Biol.* **10**, 202–210 (2008).
185. Bhattacharya, C., Aggarwal, S., Kumar, M., Ali, A. & Matin, A. Mouse apolipoprotein B editing complex 3 (APOBEC3) is expressed in germ cells and interacts with dead-end (DND1). *PLoS ONE* **3**, e2315 (2008).
186. Takeda, Y., Mishima, Y., Fujiwara, T., Sakamoto, H. & Inoue, K. DAZL relieves miRNA-mediated repression of germline mRNAs by controlling poly(A) tail length in zebrafish. *PLoS ONE* **4**, e7513 (2009).
This paper states that miRNA-mediated repression can be counteracted through poly(A)-tail elongation controlled by an RBP during germ cell development in zebrafish.
187. Moore, F. L. *et al.* Human Pumilio-2 is expressed in embryonic stem cells and germ cells and interacts with DAZ (Deleted in AZoospermia) and DAZ-like proteins. *Proc. Natl Acad. Sci. USA* **100**, 538–543 (2003).
188. Fox, M., Urano, J. & Reijo Pera, R. A. Identification and characterization of RNA sequences to which human PUMILIO-2 (PUM2) and deleted in Azoospermia-like (DAZL) bind. *Genomics* **85**, 92–105 (2005).
189. Fabian, M. R., Sonenberg, N. & Filipowicz, W. Regulation of mRNA translation and stability by microRNAs. *Annu. Rev. Biochem.* **79**, 351–379 (2010).
190. Tritschler, F., Huntzinger, E. & Izaurralde, E. Role of GW182 proteins and PABPC1 in the miRNA pathway: a sense of déjà vu. *Nature Rev. Mol. Cell Biol.* **11**, 379–384 (2010).
191. Tenenbaum, S. A., Carson, C. C., Lager, P. J. & Keene, J. D. Identifying mRNA subsets in messenger ribonucleoprotein complexes by using cDNA arrays. *Proc. Natl Acad. Sci. USA* **97**, 14085–14090 (2000).
192. Galarneau, A. & Richard, S. Target RNA motif and target mRNAs of the Quaking STAR protein. *Nature Struct. Mol. Biol.* **12**, 691–698 (2005).
193. Lorenz, C., von Pelchrzim, F. & Schroeder, R. Genomic systematic evolution of ligands by exponential enrichment (Genomic SELEX) for the identification of protein-binding RNAs independent of their expression levels. *Nature Protoc.* **1**, 2204–2212 (2006).
194. Licatalosi, D. D. *et al.* HITS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* **456**, 464–469 (2008).
195. Beach, D. L. & Keene, J. D. Ribotrap: targeted purification of RNA-specific RNPs from cell lysates through immunoaffinity precipitation to identify regulatory proteins and RNAs. *Methods Mol. Biol.* **419**, 69–91 (2008).
196. Ray, D. *et al.* Rapid and systematic analysis of the RNA recognition specificities of RNA-binding proteins. *Nature Biotech.* **27**, 667–670 (2009).
197. Slobodin, B. & Gerst, J. E. A novel mRNA affinity purification technique for the identification of interacting proteins and transcripts in ribonucleoprotein complexes. *RNA* **16**, 2277–2290 (2010).

Acknowledgements
We thank A. Morris for critical reading of the manuscript and the members of the R.A. group for their suggestions. This work was supported by the EURYI (European Research Young Investigator Award), ERC (European Research Council), KWF (Koningin Wilhelmina Fonds; Dutch Cancer Foundation) and Horizon-NWO (Nederlandse Organizatie voor Wetenschappelijk Onderzoek; R.A.). We apologize to colleagues whose work was not cited because of space limitations.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Reuven Agami's homepage: http://research.nki.nl/agamilab
CLIPZ (open access database and analysis environment): http://www.clipz.unibas.ch
***Homo sapiens* in the UCSC Genome Browser:** http://genome.ucsc.edu/cgi-bin/hgGateway
miRBase: http://www.mirbase.org
RBPDB (RNA Binding Protein Database): http://rbpdb.ccbr.utoronto.ca
RNAfold WebServer: http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi
starBase: http://starbase.sysu.edu.cn

ALL LINKS ARE ACTIVE IN THE ONLINE PDF