

Post-transcriptional control of miRNA biogenesis

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ABSTRACT

MicroRNAs (miRNAs) are important regulators of gene expression that bind complementary target mRNAs and repress their expression. Precursor miRNA molecules undergo nuclear and cytoplasmic processing events, carried out by the endoribonucleases DROSHA and DICER, respectively, to produce mature miRNAs that are loaded onto the RISC (RNA-induced silencing complex) to exert their biological function. Regulation of mature miRNA levels is critical in development, differentiation, and disease, as demonstrated by multiple levels of control during their biogenesis cascade. Here, we will focus on post-transcriptional mechanisms and will discuss the impact of *cis*-acting sequences in precursor miRNAs, as well as *trans*-acting factors that bind to these precursors and influence their processing. In particular, we will highlight the role of general RNA-binding proteins (RBPs) as factors that control the processing of specific miRNAs, revealing a complex layer of regulation in miRNA production and function.

Keywords: microRNAs (miRNAs); terminal loop; Microprocessor; DGCR8; DROSHA; DICER; RNA-binding proteins (RBPs)

INTRODUCTION

MicroRNAs (miRNAs) are small noncoding RNAs that negatively regulate the expression of a large proportion of cellular mRNAs. They have unique, diverse expression patterns (Landgraf et al. 2007) and affect many cellular processes and developmental pathways (Ebert and Sharp 2012; Bartel 2018). Most miRNA genes are transcribed by RNA polymerase II (Pol II), with the long primary transcript, termed pri-miRNA, harboring a hairpin structure, which comprises the miRNA sequence. Whereas many of these genes are transcribed as intronic clusters within protein-coding pre-mRNAs, others can be transcribed as independent gene units, or be encoded within long non-coding RNAs (lncRNAs) (Rodríguez et al. 2004; Kim and Kim 2007).

The biogenesis of miRNAs is carried out by two RNase III enzymes, DROSHA and DICER, which catalyze two subsequent processing events, in the nucleus and in the cytoplasm, respectively (Hutvagner et al. 2001; Ketting et al. 2001; Lee et al. 2003). The nuclear event is catalyzed by the Microprocessor complex, which comprises the RNase III type enzyme DROSHA, the double-stranded RNA-binding protein (RBP) DGCR8 (DiGeorge syndrome critical re-

gion 8 gene) and associated proteins (Denli et al. 2004; Gregory et al. 2004; Han et al. 2004; Landthaler et al. 2004). This nuclear processing event results in the production of ~70 nucleotide (nt) stem-loop precursor miRNAs, termed pre-miRNAs (Han et al. 2004; Zeng et al. 2005), which are subsequently exported to the cytoplasm using the export receptor, Exportin-5 (Yi et al. 2003; Bohnsack et al. 2004; Lund et al. 2004). Once in the cytoplasm, pre-miRNAs undergo a final processing event, by another RNase type III enzyme, DICER, to give rise to miRNA duplexes (Hutvagner et al. 2001; Ketting et al. 2001). These are then incorporated into the RISC (RNA-induced silencing complex) together with an Argonaute (AGO) protein, where one strand is selected to become the mature miRNA (Kobayashi and Tomari 2016). In addition, there are also noncanonical miRNA biogenesis pathways that lead to the production of functional miRNAs. These include mirtrons that are generated via pre-mRNA splicing and miRNAs generated from small nucleolar RNA (snoRNAs) precursors (for review, see Ha and Kim 2014). Regulation of gene expression by miRNAs is also prevalent in plants; however, several aspects of their biogenesis and function differ (for comprehensive reviews, see Axtell et al. 2011; Naqvi et al. 2012; Bologna et al. 2013). Several excellent recent reviews have focused on the function of

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animal miRNAs (Bracken et al. 2016; Bartel 2018; Gebert and MacRae 2018). Here, we will focus on post-transcriptional mechanisms that regulate miRNA production in animals, with a particular focus on the role of RBPs in the post-transcriptional regulation of their biogenesis.

Nuclear step of miRNA processing: the Microprocessor

The nuclear phase of miRNA processing occurs cotranscriptionally acting on both independently transcribed and intron-encoded miRNA (Morlando et al. 2008). This cotranscriptional processing can be facilitated by HP1BP3, a histone H1-like chromatin protein, which interacts with both the Microprocessor and endogenous pri-miRNAs to promote cotranscriptional miRNA biogenesis in human cells (Liu et al. 2016). MiRNA precursors form RNA hairpins that need to be recognized by the Microprocessor. To distinguish primary miRNAs (pri-miRNAs) from other hairpin-containing transcripts, additional identifiers are required. These determinants comprise a ~35 bp stem harboring a mismatched GHG motif and also include three primary-sequence elements, a basal UG motif, an apical UGUG motif and a CNNC motif, which binds the SR protein, SRSF3, and is found downstream from approximately 60% of all pri-miRNA hairpins (Fig. 1A; Auyeung et al. 2013; Fang and Bartel 2015). Another proposed identifier of what constitutes a bona fide pri-miRNA stem-loop is the presence of an N6-methyladenosine (m⁶A) mark in the vicinity of the pri-miRNA stem-loop. This mark is bound by a reader, the hnRNP protein, A2/B1, which interacts with DGCR8 and stimulates miRNA processing (Alarcón et al. 2015a, 2015b; Knuckles et al. 2017).

The precise mechanism by which the Microprocessor recognizes pri-miRNAs and catalyzes their processing is beginning to be fully understood. In brief, the Microprocessor is a heterotrimeric complex, comprising one DROSHA and two DGCR8 molecules. The DGCR8 dimer interacts with the stem and apical elements of the pri-miRNAs through its double-stranded RNA-binding domains (dsRNA) and RNA-binding heme domain, respectively, leading to accurate and efficient processing. In contrast, DROSHA serves as a ruler to measure an 11 base pair (bp) distance from the basal single-stranded RNA-double

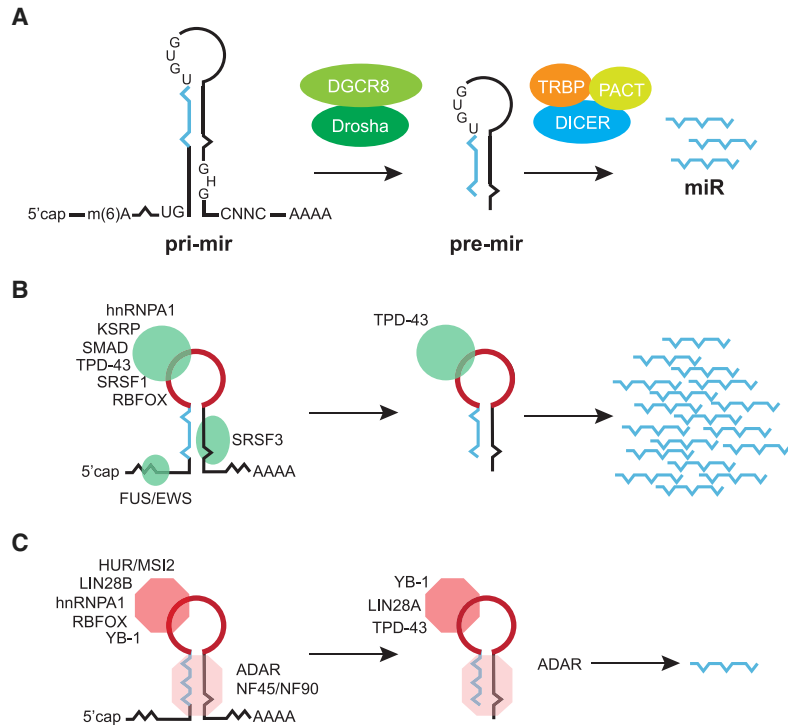


FIGURE 1. (A) The canonical pathway of miRNA biogenesis, including the Microprocessor-mediated (DROSHA/DGCR8) step in the nucleus followed by DICER processing in the cytoplasm. Structural and sequence features important for miRNA processing are highlighted in both the pri-miR and pre-miR molecules. (B) Positive regulators of miRNA biogenesis bind to the terminal loop (TL) or other elements within miRNA precursors (pri-miR and pre-miR) and stimulate Drosha and/or Dicer processing, leading to increased levels of mature miRNAs (miR). The TL, also known as apical loop, is depicted in red. (C) Negative regulators of miRNA biogenesis bind to TL or other elements within miRNA precursors (pri-miR and pre-miR) and abrogate Drosha and/or Dicer processing, leading to decreased levels of mature miRNAs (miRs). The TL, also known as apical loop, is depicted in red.

stranded RNA (ssRNA-dsRNA) junction and cleaves the stem-loop of primary miRNAs (Nguyen et al. 2015). Crucially, the orientation of this complex on the substrate is maintained by DROSHA and DGCR8 recognizing the basal UG and apical UGUG motifs, respectively (Nguyen et al. 2015; Kwon et al. 2016). It has been recently shown that SRSF3, a member of the SR protein family of splicing factors promotes miRNA processing by recruiting DROSHA to the basal junction in a CNNC-dependent manner (Kim et al. 2018). The activity of the Microprocessor can be enhanced by the binding of heme, a ferric ion-containing porphyrin, which promotes the interaction between the DGCR8 dimer and the apical UGUG motif, promoting Microprocessor activity (Quick-Cleveland et al. 2014; Weitz et al. 2014; Partin et al. 2017; Nguyen et al. 2018).

Noncanonical functions of the Microprocessor

Besides its established role in miRNA biogenesis, noncanonical functions for the Microprocessor have also been suggested (for reviews, see Macias et al. 2013; Pong and Gullerova 2018). The first hint of more extended roles for

the Microprocessor came from the observation that DROSHA cleaves pri-miRNA-like hairpins harbored within the 5'-UTR of the mRNA encoding the DGCR8 protein itself, providing a feedback loop to control DGCR8 levels (Han et al. 2009; Kadener et al. 2009; Triboulet et al. 2009). Furthermore, the phenotypic differences observed during early T-cell development in the mouse, following *Dgcr8/Drosha* and *Dicer* inactivation, were correlated to transcriptomic changes that were unique to *Drosha* but not *Dicer* highlighting the existence of DROSHA-dependent, DICER-independent processing of RNAs (Chong et al. 2010). Thus, these noncanonical activities of the Microprocessor could affect cellular RNAs, beyond the described autoregulatory feedback that controls levels of DGCR8 pre-mRNA. Identification of endogenous targets for DGCR8, revealed that the Microprocessor complex binds and regulates a large variety of cellular RNAs, other than miRNAs, including mRNAs, noncoding RNAs and transcripts derived from several human active retrotransposons (LINE-1, Alu) (Macias et al. 2012; Heras et al. 2013). Accumulating evidence suggests that these noncanonical activities of the Microprocessor do indeed have physiological relevance in the turnover of cellular RNAs. For instance, DROSHA has been shown to negatively regulate the expression of the transcription factor Neurogenin by cleaving evolutionarily conserved hairpins present in the *Neurogenin* mRNA that are similar to pri-miRNAs (Knuckles et al. 2012). Furthermore, miRNA-independent functions of DGCR8 were also shown to be essential for neocortical development in the mouse. This was attributed to the action of the Microprocessor directly regulating the cortical transcription factor, *Tbr1*, which also contains evolutionarily conserved hairpins that resemble miRNA precursors (Marinero et al. 2017). Finally, DGCR8 can also associate with other nucleases, suggesting the existence of alternative DGCR8 complexes that may regulate the fate of a subset of cellular RNAs, as shown by the DGCR8-mediated cleavage of small nucleolar RNAs (snoRNAs), which functions independently of DROSHA (Macias et al. 2015).

Cytoplasmic step of miRNA processing: DICER

In the canonical pathway, pre-miRNAs are exported to the cytoplasm and assembled into a complex containing DICER (Hutvágner et al. 2001) and the Hsp90 chaperone (Miyoshi et al. 2010). Subsequently, the pre-miRNA is cleaved by DICER, in tandem with TRBP (HIV-1 TAR RNA RBP) and PACT (protein activator of PKR) (Fig. 1A; Fukunaga et al. 2012). In this reaction, DICER serves as a molecular ruler that measures the distance from the pre-miRNA basal end to the cleavage site adjacent to the TL (also known as apical loop) (Macrae et al. 2006). This cleavage liberates the pre-miRNA TL element and creates an RNA duplex that interacts with the Argonaute 2 protein

(AGO2). Of note, due to variable structural features of pre-miRNAs, the DICER-dependent cleavage is often imprecise, generating two or more miRNA duplex variants that will give rise to distinct mature miRNAs (Starega-Roslan et al. 2015). The miRNA duplex is incorporated into an AGO2 protein to form the RNA-induced silencing complex (RISC), in an ATP-dependent manner with the assistance of HSC70/HSP90 chaperones (Iwasaki et al. 2010). Subsequently, AGO2 unwinds the RNA duplex and evicts the passenger strand forming the mature RISC complex (Kobayashi and Tomari 2016). The activated RISC then recognizes a specific mRNA sequence by complementary base-pairing resulting in translation inhibition and/or RNA degradation (for reviews, see Fabian et al. 2010; Iwakawa and Tomari 2015).

Role of RBPs in the regulation of miRNA biogenesis

Due to the important role of miRNAs in the control of gene expression and organism development, the production of mature miRNAs is tightly regulated at multiple levels, including transcriptional and post-transcriptional steps. A variety of post-transcriptional mechanisms, which affect DROSHA and DICER processing, as well as miRNA modification and turnover have been previously described (Krol et al. 2010; Siomi and Siomi 2010; Finnegan and Pasquinelli 2013; Creugny et al. 2018; Treiber et al. 2018b). Dysregulation of miRNA production can result in global defects in gene expression and lead to human disease (Mendell and Olson 2012). As an example, impaired miRNA processing promotes cellular transformation and tumorigenesis (Kumar et al. 2007), and a global miRNA depletion is frequently observed in human cancers (Hata and Lieberman 2015; Lin and Gregory 2015).

Increasing evidence suggests that general RBPs, including splicing factors and other diverse RNA processing factors, act as post-transcriptional regulators of miRNA processing (for review, see Ratnadiwakara et al. 2018). In such a scenario, the binding of an RBP to the TL or a stem of a miRNA progenitor can positively or negatively affect the Microprocessor-mediated processing of pri-miRNA in the nucleus, and/or the DICER-mediated processing of a pre-miRNA in the cytoplasm (Fig. 1B,C). Below, we will discuss the role of *trans*-acting factors that bind to precursor miRNAs and influence their nuclear and cytoplasmic processing by the Microprocessor and DICER, respectively. We will also focus on the contribution of sequence variation, exemplified by single nucleotide polymorphisms (SNPs) present in the human genome that can have a role in the biogenesis of miRNAs. Finally, we will present attempts to target miRNA regulatory events using biological and synthetic compounds that could eventually lead toward the development of therapies that correct unbalanced miRNA production in disease.

Role of LIN28 in the regulation of let-7 processing: nuclear and cytoplasmic activities

The first described example of an RBP regulating miRNA biogenesis at the post-transcriptional level involved the role of the pluripotency promoting proteins LIN28A and LIN28B in the regulation of the let-7 family of miRNAs in pluripotent embryonic stem cells (ESCs). These proteins are enriched in undifferentiated cells and their expression is gradually switched off during differentiation. LIN28 proteins harbor two RNA-binding domains, a cold shock domain (CSD) and two zinc knuckle domains that mediate recognition of the TL of let-7 in a sequence-specific manner. Binding of LIN28 proteins to let-7 precursors blocks their processing by different mechanisms at either the DROSHA and/or DICER level. Structural studies of LIN28 proteins in complex with sequences from several let-7 precursors revealed a bipartite recognition signal within the TL of let-7 precursors. The LIN28 CSD domain, which has a limited sequence specificity, binds to a let-7 closed loop to induce a conformational change of this precursor that facilitates binding of the CCHC zinc knuckles to a GGAG motif (Nam et al. 2011; Loughlin et al. 2012; Mayr et al. 2012). LIN28B binds to the TL of let-7 precursors and affects their processing by blocking the activity of the Microprocessor in the nucleus (Newman et al. 2008; Piskounova et al. 2008; Viswanathan et al. 2008). In contrast, LIN28A functions in the cytoplasm, where it recruits a TUTase (either TUT4 or TUT7) that adds a short oligo (U) stretch to the 3'-end of precursor miRNAs and blocks their processing by DICER (Heo et al. 2009). The E3 ligase TRIM25 acts as an auxiliary factor for LIN28A by binding to the let-7a precursor and stimulating TUT-mediated uridylation (Choudhury et al. 2014). Subsequently, recruitment of the 3'-5' exoribonuclease DIS3L2 causes the degradation of the uridylated pre-let-7 (Chang et al. 2013; Ustianenko et al. 2013). This inhibitory effect of let-7 production is important to block miRNA-mediated differentiation in stem cells. LIN28A also inhibits the biogenesis of the neuro-specific miRNA-9 during neuronal differentiation of mouse cells (Nowak et al. 2014, 2017), albeit using an uridylation-independent mechanism.

HnRNP A1 as a paradigm of an RBP regulating miRNA biogenesis in the nucleus

Use of an unbiased in vivo cross-linking and immunoprecipitation pro-

tol (CLIP) searching for RNA targets of the hnRNP protein, hnRNP A1, identified the miRNA precursor, pre-mir-18a (Guil and Cáceres 2007). This miRNA is expressed as part of the miR-17-92 cluster encoded as an intronic polycistron, that includes six individual miRNAs (miR-17, 18a, 19a, 20a, 19b-1, and 92a-1) and is frequently amplified and/or overexpressed in human cancers, being also termed oncomiR-1 (He et al. 2005; Concepcion et al. 2012). Of interest, hnRNP A1 has been functionally characterized as a general RBP, with a role in many aspects of RNA processing, including alternative splicing regulation, IRES (internal ribosome entry site)-mediated translation and even telomere maintenance (Mayeda and Krainer 1992; LaBranche et al. 1998; Bonnal et al. 2005; for review, see Jean-Philippe et al. 2013). HnRNP A1 has two RNA recognition motif (RRM) domains, each harboring conserved RNP-1 and RNP-2 submotifs that represent the RNA-binding region and a C-terminal glycine-rich domain (Mayeda et al. 1994). Mechanistically, we showed that hnRNP A1 binds to the TL of pri-mir-18a and induces a relaxation at the stem-loop structure near the DROSHA cleavage site, resulting in increased Microprocessor-mediated processing (Fig. 2A,B; Michlewski et al. 2008). The processing of the other miRNAs in this cluster is not affected, indicating that hnRNP A1 acts locally to influence the processing of its target pri-miRNA. Importantly, we also observed phylogenetic sequence conservation of TL sequences of precursor miRNAs, suggesting that these sequences act as a landing pad for regulatory factors that could have a positive or negative role in miRNA production, either at the level of DROSHA and/or DICER (Michlewski et al. 2008, 2010). More recently, using an integrative structural biology

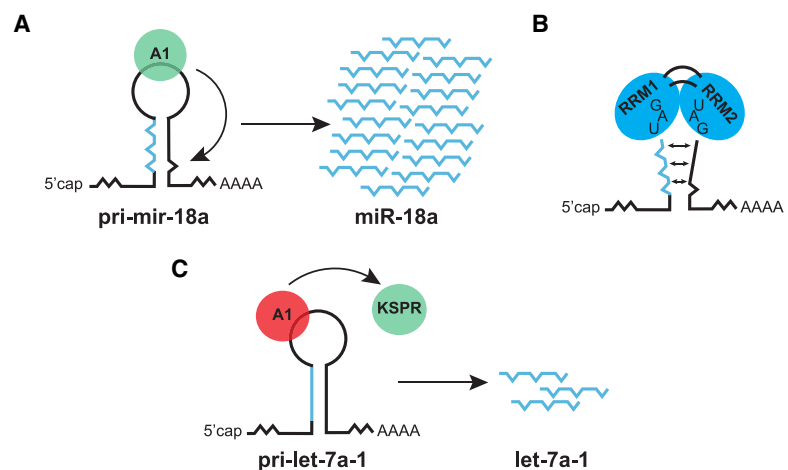


FIGURE 2. HnRNP A1 as a paradigm for RBP-mediated regulation of miRNA biogenesis in the nucleus. (A) Binding of hnRNP A1 to the TL of pri-mir-18a induces a structural rearrangement that results in enhanced Drosha processing. (B) Each RRM of hnRNP A1 recognizes an UAG motif in the TL of pri-mir-18a. (C) Binding of hnRNP A1 to the TL of pri-let-7 in differentiated cells outcompetes binding of the stimulatory factor, KSRP, resulting in decreased Drosha processing.

approach combined with biochemical and functional assays, we were able to demonstrate that hnRNP A1 forms a 1:1 complex with pri-mir-18a, in which the tandem RRM domains of hnRNP A1 recognize two UAG motifs in the pri-mir-18a TL and the proximal stem region (Fig. 2B). This structural approach also confirmed that binding of hnRNP A1 to the TL induces an allosteric destabilization of base-pairing in the pri-mir-18a stem that promotes its processing (Kooshapur et al. 2018). Of interest, binding of hnRNP A1 to the conserved TL of a precursor miRNA does not always result in enhanced miRNA processing. We showed that hnRNP A1 binding to the TL of pri-let-7 has an inhibitory role in let-7 production in differentiated cells. This is due to a different mechanism from that shown for pri-mir-18a, and involves antagonistic roles for hnRNP A1 and another hnRNP protein, the KH-type splicing regulatory protein, KSRP, which was shown to promote let-7 biogenesis in differentiated cells (Fig. 2C; Trabucchi et al. 2009; Michlewski and Cáceres 2010). Interestingly, KSRP not only regulates the processing of let-7, but also binds to the TL of a subset of pri- and pre-miRNAs that includes miR-20, miR-26b, miR-106a, miR-21, miR-16, and promotes both DROSHA- and DICER-mediated steps (Table 1; Trabucchi et al. 2009, 2010). Thus, these findings with LIN28 and hnRNP A1 suggested a previously uncharacterized role for general RBPs as auxiliary factors that influence the processing of specific miRNAs and prompted the search for novel regulators.

The terminal loop of precursor miRNAs as a hub for regulation of DROSHA and DICER activities

A flexible and long TL of approximately 10 nt was proposed to be required for efficient DROSHA processing (Zhang and Zeng 2010); however, specific sequences at the TL region of some pri- and pre-miRNAs were shown to only have a minor effect on miRNA production (Zeng and Cullen 2003; Han et al. 2006). In agreement, most precursor miRNAs display a poor phylogenetic conservation in the TL region, when compared with the high level of conservation observed in mature miRNA sequences (Berezikov et al. 2005; Akhtar et al. 2016). However, a phylogenetic analysis of human pri-miRNAs sequences revealed that approximately ~14% (74 out of 533) of all miRNAs displayed high conservation of the TL sequence (Michlewski et al. 2008), indicating that these sequences could act as a landing platform for the binding of auxiliary factors, such as hnRNP A1, that influence the post-transcriptional regulation of miRNA production. This was further validated by the use of 2'-O-methyl oligonucleotides complementary to conserved TLs, which we termed LooptomiRs (for loop-targeting oligonucleotide anti miRNAs) that block the *in vitro* processing of precursor miRNAs (Michlewski et al. 2008). We attributed this to a block exerted by looptomiRs on conserved sequences within the TL that are recognized by aux-

iliary factors required for the efficient processing of these targeted miRNAs *in vitro*. Conversely, it is also likely that looptomiRs could block the access of factors that negatively regulate processing of target precursor miRNAs.

In several cancers, the tumor suppressive role of let-7 is abrogated by the increased expression of its negative regulator, LIN28. Building on this concept, short, loop-targeting oligoribonucleotides were used to block binding of the negative regulator LIN28 to the precursor of let-7. These looptomiRs selectively antagonized the docking of LIN28, but still allowed the processing of pre-let-7a-2 by DICER, leading to suppression of growth in cancer cells (Roos et al. 2014). An RNA aptamer that specifically targets the pri-mir-17-92 cluster was identified through an *in vitro* selection process. This aptamer binds to the TL of pri-mir-18a and inhibits the biogenesis not only of miR-18a, but also of all other five miRNAs within this cluster (Lünse et al. 2010). In contrast, looptomiR-targeting miR-18a only affects the processing of this miRNA, reflecting mechanistic differences on how these two reagents influence miRNA biogenesis (Michlewski et al. 2008; Lünse et al. 2010). These evidences strongly suggest that TL recognition by RBPs could constitute a general mechanism to regulate miRNA biogenesis that operates via different mechanisms, such as altering the RNA structure of the precursor itself, recruiting additional RNA enzymes and/or affecting the recruitment and/or activity of core processing complexes associated with the Microprocessor and/or DICER. Indeed, a growing number of canonical and newly characterized RBPs have been shown to bind to TLs and regulate miRNA biogenesis (Table 1; Fig. 1B, C; Choudhury and Michlewski 2012; Castilla-Llorente et al. 2013).

Genetic variation

An increasing number of SNPs and rare mutations within precursor and/or mature miRNA sequences linked to human disease have been reported (Hogg and Harries 2014; Króliczewski et al. 2018). Despite correlations between the presence of polymorphisms in pri- and pre-miRNAs and the corresponding levels of mature miRNAs, the mechanism by which sequence variation and RNA structure control miRNA biogenesis remains mostly enigmatic. A rare genetic variation in the TL of pri-miR-30c-1 (G₂₇ to A) that was found in breast and gastric cancer patients results in increased levels of mature miR-30c (Shen et al. 2009; Fernandez et al. 2017). This genetic variant directly affects the Microprocessor-mediated processing of pri-mir-30c-1 by inducing a secondary RNA structure rearrangement that opens up the pri-miRNA stem and facilitates binding of the *trans*-acting factor SRSF3 (Fernandez et al. 2017), a factor which was described to promote Microprocessor activity on a subset of miRNAs (Fig. 3; Auyeung et al. 2013). This finding raises the interesting

TABLE 1. RNA-binding proteins that regulate miRNA biogenesis at the post-transcriptional level

RBP	miRNA biogenesis Step effect	Mechanism	Target miRNAs	References
LIN28A	Dicer –	Binds let-7 TL and blocks Dicer processing. Recruits TUTases (TUT4 or TUT7) and induces oligouridylation	let-7, miR-9, miR-107, miR-143, miR-200c	(Heo et al. 2009; Nowak et al. 2014)
LIN28B	Drosha –	Binds let-7 TL and blocks Drosha processing	let-7	(Viswanathan et al. 2008)
hnRNP A1	Drosha +	Promotes Drosha cleavage by restructuring pri-mir-18a	miR-18a	(Guil and Cáceres 2007; Michlewski et al. 2008; Kooshapur et al. 2018)
	Drosha –	Inhibits Drosha processing by competing out KSRP binding	let-7	(Michlewski and Cáceres 2010)
KSRP	Drosha and Dicer +	Unknown	let-7, miR-16, miR-21, miR-26b, miR-106a	(Trabucchi et al. 2009, 2010)
TDP-43	Drosha and Dicer +	Unknown	miR-132, miR-143, miR-558-3p, miR-574-3p	(Kawahara and Mieda-Sato 2012)
SRSF1	Drosha +	Unknown	miR-7, miR-29b, miR-221, miR-222	(Wu et al. 2010)
FUS/TLS	Drosha +	Recruits Drosha cotranscriptionally	miR-9, miR-125b, miR-132	(Morlando et al. 2012)
EWS	Drosha +	Recruits Drosha cotranscriptionally	Subset of miRNAs	(Ouyang et al. 2017)
RBFOX3	Drosha +	Binds to TL and facilitates Microprocessor recruitment	miR-15	(Kim et al. 2014a)
	Drosha –	Binds to the stem and blocks Microprocessor recruitment	miR-485	
RBFOX2	Drosha –	Binds to TL and leads to structural changes in the TL blocking Microprocessor binding and/or function	miR-20b, miR-107	(Chen et al. 2016)
HuR/MSI2	Drosha –	HuR recruits MSI2, this complex stabilizes the stem and blocks Microprocessor cleavage	miR-7	(Choudhury et al. 2013)
ADAR1,2	Drosha –	A to I editing in the stem leads to inhibition of Drosha cleavage	miR-142	(Yang et al. 2006)
ADAR1	Dicer –	A to I editing in the stem leads to inhibition of Dicer cleavage	miR-151	(Kawahara et al. 2007)
ADAR2	Drosha –	Binds to stem and blocks Drosha processing independently of catalytic activity	miR-376a	(Heale et al. 2009)
NF45/90	Drosha –	Unknown	miR-7	(Higuchi et al. 2016)
YB-1	Drosha – Dicer –	Unknown	miR-29b-2	(Wu et al. 2015)
MBNL1	Dicer +	Binds to TL and competes out binding of the negative regulator LIN28	miR-1	(Rau et al. 2011)
BCDIN3D	Dicer –	Methylates monophosphate ends of pre-miRNAs leading to inhibition of Dicer processing	miR-145	(Xhemalce et al. 2012)
SMADs	Drosha +	Binds to p68 and promotes Drosha cleavage	miR-21, miR-199a	(Davis et al. 2008, 2010)
p53	Drosha +	Binds to p68 and promotes Drosha cleavage	miR-16-1, miR-143, miR-145	(Suzuki et al. 2009)
BRCA1	Drosha +	Unknown	let-7a-1, miR-16-1, miR-145, miR-34a	(Kawai and Amano 2012)

LIN28A/B, protein lin-28 homolog A/B; hnRNP A1, heterogeneous nuclear ribonucleoprotein A1; KSRP, KH-type splicing regulatory protein; TDP-43, TAR DNA-binding protein 43; SRSF1, serine- and arginine-rich splicing factor 1; FUS/TLS, FUS RNA-binding protein/translocated in liposarcoma; EWS, EWS RNA-binding protein 1; RBFOX2,3, RNA-binding protein fox-1 homolog 2/3; HuR, Hu antigen R, also known as ELAV-like RNA-binding protein 1; MSI2, Musashi RNA-binding protein 2; ADAR1,2, adenosine deaminase acting on RNA enzymes; NF45/90, interleukin enhancer binding factor 2/3; YB-1, Y box binding protein 1; MBNL1, Muscleblind-like protein 1; BCDIN3D, BCDIN3 domain containing RNA methyltransferase; SMADs, mothers against decapentaplegic homolog; p53, tumor protein P53; BRCA1, breast cancer type 1 susceptibility protein.

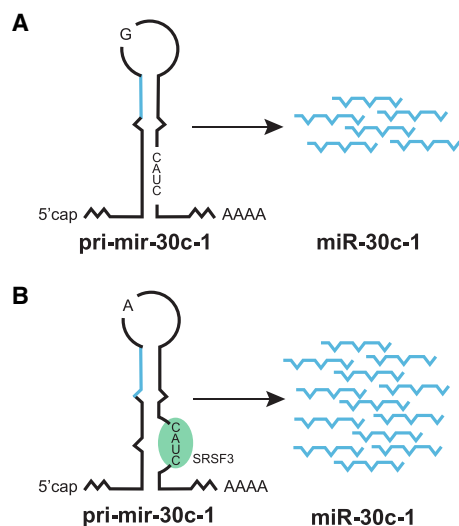


FIGURE 3. Influence of genetic variation on miRNA biogenesis. (A) Schematic structure of pri-mir-30c-1 with the CNNC motif (CAUC) occluded by the RNA secondary structure in the G₂₇ (wild-type) variant (indicated by a G in the TL). (B) A single G to A substitution (A₂₇) present in the TL of pri-mir-30c-1 in breast and gastric cancer patients leads to a secondary RNA structure rearrangement that facilitates binding of SRSF3 to the CAUC sequence determinant, causing increased Microprocessor-mediated processing and elevated miR-30c levels.

hypothesis that primary sequence determinants in conjunction with RNA structure can act as regulators of miRNA biogenesis. Although this constitutes a largely unexplored area, the emerging picture is that human genetic variation could indeed not only affect miRNA function by targeting either miRNA-binding sites in the 3'-UTRs of target genes and/or miRNA seed sequences, but it could also have an essential role in the modulation of miRNA biogenesis (Hogg and Harries 2014). Pri-miRNA secondary structure can also influence miRNA biogenesis, as observed under limiting levels of DROSHA, when miRNAs without mismatches are processed more efficiently than mismatched miRNAs (Sperber et al. 2014). Another systematic study identified additional structural elements and sequence distribution for optimal DROSHA processing (Roden et al. 2017). This study also predicts that a small but significant fraction of human SNPs could alter pri-miRNA processing, which ultimately could influence the levels of mature miRNA and their biological function. These findings highlight the interplay between genetics, RNA structure, and post-transcriptional regulation of miRNA biogenesis.

Auxiliary factors in post-transcriptional control of miRNA biogenesis

In addition to LIN28 proteins and hnRNP A1, several other RBPs recognize the TL of miRNA precursors and influence, either positively or negatively, their processing (Table 1;

Fig. 1B,C). In the following section, we will illustrate the role of a number of RBPs in the regulation of miRNA production.

TPD-43

The hnRNP protein, TDP-43 (TAR DNA-binding protein-43), also promotes the processing of a subset of precursor miRNAs acting at both the level of DROSHA and DICER processing and this activity is required for neuronal outgrowth (Kawahara and Mieda-Sato 2012; Di Carlo et al. 2013). It was shown that TDP-43 is indeed a component of the Microprocessor complex (Gregory et al. 2004) and its Microprocessor-related role affects the biogenesis of a subset of at least six miRNAs, including miR-558-3p, miR-574-3p, and both strands of miR-132 and miR-143 (Kawahara and Mieda-Sato 2012). In contrast, cytoplasmic TDP-43 was shown to bind to the TL of pre-miRNAs and interact with the DICER complex (Kawahara and Mieda-Sato 2012).

SRSF1

The splicing factor SRSF1 (also known as SF2/ASF), which is the prototype of the SR family of splicing regulators, can also promote the maturation of a subset of miRNAs, including miR-7, miR-29b, miR-221, and miR-222. In the case of pri-mir-7, this was shown to be independent of splicing and involved SRSF1 binding to a consensus motif in the stem-loop of pri-mir-7 leading to an increase in DROSHA activity, yet the exact mechanism by which this occurs remains enigmatic (Wu et al. 2010).

FUS and EWS

Two members of the TET family of proteins, FUS (also known as TLS, translocated in liposarcoma) and Ewing's sarcoma (EWS), have been shown to affect miRNA biogenesis. FUS/TLS, which is associated with familial forms of amyotrophic lateral sclerosis (ALS), promotes the biogenesis of a subset of miRNAs in neuronal cells. It is recruited to chromatin, associates with DROSHA and facilitates the recruitment of the Microprocessor complex to substrate pri-miRNAs, promoting the biogenesis of neuronal miR-9, miR-125b, and miR-132 (Morlando et al. 2012). Interestingly, mutations in both TDP-43 and FUS have been linked with the etiology of ALS, suggesting a possible link between mutations in these miRNA regulators and altered miRNA biogenesis in ALS (Goodall et al. 2013; Paez-Colasante et al. 2015).

The EWS protein has a dual and opposing role in miRNA production. On the one hand, EWS has been shown to down-regulate DROSHA at the transcriptional level (Kim et al. 2014b), but it also has a positive role in miRNA production by binding to flanking sequences in the stem-loop region of pri-miRNAs and promoting the

cotranscriptional recruitment of the Microprocessor to chromatin (Ouyang et al. 2017).

RBFOX proteins

RBFOX3 binds to a subset of pri-miRNAs and directly regulates the Microprocessor-mediated processing of selected pri-miRNAs in neuronally differentiated P19 cells and mouse brain, with stimulatory or blocking effects, depending on the miRNA (Kim et al. 2014a). Binding of RBFOX3 to the TL or to the stem of individual pri-miRNAs results in recruitment or exclusion of the Microprocessor differentially affecting the processing of the respective pri-miRNAs. Surprisingly, this role in miRNA biogenesis was independent of the cognate binding site for RBFOX3, the UGCAUG motif (Kim et al. 2014a). In contrast, a more recent study also found a role for RBFOX proteins, in the regulation of miRNA biogenesis, but in this case involved sequence-specific binding of the conserved RBFOX2 RRM to pri-mir-20b and pri-mir-107 containing the cognate motif in their TLs. This binding alters the conformation of these precursors leading to inhibition of DROSHA processing (Chen et al. 2016).

HuR and MSI2

Several miRNAs are expressed in a tissue- or cell type-specific manner, thereby contributing to tissue and cell identity and function (Landgraf et al. 2007). An example of an RBP determining the tissue-specific expression of a target miRNA is illustrated in the case of the brain-enriched expression of miR-7, which is processed from the ubiquitous hnRNP K pre-mRNA transcript. This brain specificity is achieved by the inhibition of pri-mir-7 processing in non-neural cells by the combined action of two RBPs, Musashi RNA-binding protein 2 (MSI2) and the Hu antigen R (HuR), which bind to the TL of pri-mir-7 (Choudhury et al. 2013). Mechanistically, HuR binds to the TL of pri-mir-7 and recruits MSI2. Both proteins act then synergistically to stabilize the pri-mir-7 stem-loop structure and inhibit Microprocessor cleavage. This is in agreement with a study showing that HuR depletion results in a significant increase of miR-7 without a noticeable change in the pri-mir-7 levels (Lebedeva et al. 2011). Notably, mature miR-7 is sequestered by a circular RNA, ciRS-7, which is primarily expressed in the cerebellum, indicating a sophisticated mechanism of miR-7 regulation (Hansen et al. 2013; Memczak et al. 2013). Other miRNAs, such as miR-505, miR-92a-1, or miR-224 are also sensitive to MSI2 and HuR depletion but the precise mechanism of action awaits further characterization (Choudhury et al. 2013). The biogenesis of miR-675 is inhibited by HuR in intestinal tissue by blocking processing of lncRNA *H19* (Zou et al. 2016). Moreover, maturation of miR-199a is blocked by HuR in hepatocellular carcinoma (HCC) in hypoxic conditions

that promote glycolytic metabolism and cancer proliferation (Zhang et al. 2015). Finally, HuR inhibits processing of miR-133b from linc-MD1 noncoding RNA contributing to early stages of the muscle differentiation program (Legnini et al. 2014). It is yet to be established whether HuR controls these processes alone or in a complex with MSI2.

ADARs

RNA editing and RNA editing enzymes can also act to regulate miRNA biogenesis. Adenosine deaminases acting on RNA (ADARs) are responsible for the editing of adenosine residues to inosine in dsRNA. They also affect RNA interference (RNAi) and miRNA processing by deamination of specific adenosines to inosine. RNA editing of pri-mir-142, which is expressed in hematopoietic tissues, blocks its DROSHA-mediated processing. The resulting edited pri-mir-142 is subsequently degraded by Tudor-SN, a component of RISC and also a ribonuclease specific to inosine-containing dsRNAs (Yang et al. 2006). In contrast, RNA editing of pri-mir-151 blocks its processing by DICER in the cytoplasm (Kawahara et al. 2007). It was also demonstrated that ADAR proteins can influence miRNA biogenesis independently of their enzymatic activity, as evidenced by the role of ADAR2 in blocking the Drosha-mediated processing of miR-376a-2, independently of its catalytic RNA editing activity (Heale et al. 2009).

NF45/NF-90

The heterodimer NF45-NF90 is an RBP complex that regulates the post-transcriptional expression of a large number of cellular RNAs. It also has a negative role in the processing of pri-mir-7 in HCC. The expression of this heterodimer is elevated in primary HCC tissues compared with adjacent nontumor tissues. The NF90-NF45 heterodimer binds to pri-mir-7-1 and blocks its processing. The biological effect of this repression is the elevation of EGF receptor levels that results in the promotion of cell proliferation in HCC (Higuchi et al. 2016).

YB-1

The Y box-binding protein (YB-1), a member of the DNA/RNA-binding family of proteins with an evolutionarily conserved CSD, is a modulator of miRNA processing in glioblastoma multiforme (GBM). A CLIP approach revealed that YB-1 binds to the TL of pri-/pre-mir-29b-2 and regulates its processing by blocking the recruitment of the Microprocessor and DICER to its precursors. Down-regulation of miR-29b by YB-1, which is up-regulated in GBM, is crucial for cell proliferation (Wu et al. 2015).

MBNL1

MBNL1 stimulates the production of miR-1 by binding to a UGC motif located within the TL of pre-miR-1 and competing for the binding of the negative regulator LIN28A in the cytoplasm. In myotonic dystrophy, which is an RNA gain-of-function disease caused by expansions of CUG or CCUG repeats, MBNL1 is sequestered by these expansions. This results in a decreased miR-1 processing in heart samples from patients with myotonic dystrophy (Rau et al. 2011), contributing to pathophysiology.

BCDIN3D

The RNA-methyltransferase, BCDIN3D, regulates miRNA biogenesis by methylating the 5'-monophosphate end of precursor miRNAs, thus blocking the recognition of 5'-monophosphate by Dicer and inhibiting miRNA processing. In particular, it was shown that BCDIN3D phospho-dimethylates pre-mir-145 both in vitro and in vivo leading to a reduced processing by Dicer in vitro (Xhemalce et al. 2012).

Signaling and miRNA biogenesis

Post-translational modifications of miRNA processing factors have been identified, including phosphorylation as well as ubiquitination and sumoylation that can affect DGCR8, DROSHA and/or DICER complex components (for review, see Ha and Kim 2014). It has been shown that miRNA biogenesis can also be regulated in a cell density-dependent manner (Hwang et al. 2009), and this is mediated by the tumor-suppressive Hippo pathway. At low cell density, when the Hippo signaling is suppressed, its component YAP relocalizes to the nucleus where it binds and sequesters a Microprocessor-associated component, the RNA helicase DDX17 (also known as p72), thus, down-regulating Microprocessor activity. In contrast, at high cell density the Hippo-induced cytoplasmic retention of YAP restores the association of DDX17/p72 with the Microprocessor stimulating its activity. Thus, the frequent inactivation of the Hippo pathway or expression of constitutively active YAP observed in many cancers results in a widespread miRNA suppression in cells and tumors, which explains the global down-regulation of miRNAs during cancer (Harvey et al. 2013; Mori et al. 2014).

SMADs

In particular, the Microprocessor-mediated step of miRNA biogenesis can be regulated by multiple signaling pathways, such as the transforming growth factor beta (TGF- β) and bone morphogenetic protein (BMP) pathways. Mechanistically, TGF- β and BMP-specific SMAD signal transducers are recruited to pri-mir-21 in a complex with the RNA helicase p68 and facilitate its DROSHA-mediated

processing (Davis et al. 2008, 2010). The induction of miR-21 promotes the contractile phenotype in human vascular smooth muscle cells (VSMCs); thus, regulation of miRNA biogenesis by ligand-specific SMAD proteins acts to control the VSMC phenotype.

Tumor suppressors: p53 and BRCA1

As noted above, a global down-regulation of miRNAs is commonly observed in human cancers (Lin and Gregory 2015). The tumor suppressor, p53, has been shown to act as an enhancer of miRNA biogenesis in response to DNA damage, promoting the post-transcriptional maturation of a subset of miRNAs with growth-suppressive function, which includes miR-16-1, miR-143, and miR-145. Mechanistically, p53 interacts with the Microprocessor complex by binding to the DEAD-box RNA helicase p68 (also known as DDX5) in HCT116 cells and human diploid fibroblasts and promotes the processing of pri-miRNAs (Suzuki et al. 2009). The tumor suppressor breast cancer 1 protein (BRCA1) has also been shown to promote the processing of a subset of pri-miRNAs, which include let-7a-1, miR-16-1, miR-145, and miR-34a. BRCA1 interacts with components of the Microprocessor complex, namely DROSHA and the RNA helicase DDX5, as well as with SMAD3, p53, and the DHX9 RNA helicase. This novel function of BRCA1 in miRNA biogenesis could be linked to its well-established roles in tumor suppression and maintenance of genomic stability (Kawai and Amano 2012).

Genome-wide identification of RBPs that regulate miRNA production

The initial findings that RBPs bind to TL regions of miRNAs and influence their processing, such as the examples described above (Table 1), prompted a global search for additional factors that control miRNA processing. Several strategies have been developed with the aim of identifying precursor miRNAs (both pri- and pre-miRNAs) whose biogenesis is affected by the binding of RBPs. These include the identification of additional miRNA precursors bound by cognate RBPs (Towbin et al. 2013), and identification of novel RBPs for a particular precursor miRNA sequence/s (Treiber et al. 2017, 2018a; Choudhury and Michlewski 2018). A biochemical method involving an RNA pull-down combined with SILAC mass spectrometry (RP-SMS) led to the identification of *trans*-acting factors that regulate the processing of miR-7, miR-9, and let-7 (Choudhury and Michlewski 2018). In the case of miR-9, which is specifically expressed in the brain, this approach led to the identification of LIN28A, which binds to pri-mir-9 in differentiating cells and induces the degradation of its precursor through a uridylation-independent mechanism (Nowak et al. 2014).

A recent proteomics-based pull-down approach focused on the identification of RBPs that recognize 72

different pre-miRNA hairpins used as baits in 11 different cell lines. This identified approximately 180 RBPs that interact specifically with this subset of precursor miRNAs, including known RBPs, splicing factors, as well as other mRNA processing factors (Treiber et al. 2017, 2018a). Interestingly, this approach revealed that both TLs, but also stem regions of miRNA precursors, could be specifically recognized by regulatory RBPs. In several cases, loss-of-function experiments validated the impact of these RBPs in the regulation of mRNA biogenesis, although their mechanism of action remains enigmatic. A recent study relied on a high-throughput computational screen to assess a role for 126 RBPs in miRNA biogenesis, using available eCLIP data sets from ENCODE. Those RBPs with enriched binding in the vicinity or within miR-encoding genomic loci represent candidate *trans*-acting factors for miRNA processing. This exercise resulted in the identification of 116 putative regulators that bind at 1871 annotated human precursor miRNA loci. These candidate RBPs have a potential role either positive or negative at different steps of the miRNA biogenesis cascade. Of interest, some of the interactions of individual RBPs with subsets of precursor miRNAs seem to be cell-type specific. Importantly, this difference was noted even when the corresponding pri-miRNA is expressed in both cell lines. The authors further showed that most RBPs bind fewer than 25 unique miRNA loci and in most cases, depletion of individual RBPs affects the corresponding mature miRNA levels (Nussbacher and Yeo 2018). The precise mechanisms of post-transcriptional regulation that these proteins use to control miRNA biogenesis are currently unknown.

Role of long noncoding RNAs in the control of miRNA biogenesis

A role for lncRNAs in the post-transcriptional regulation of miRNA processing has also been recently described (Table 2). Uc.283+A, a lncRNA transcribed from an ultraconserved region, inhibits the Drosha-mediated processing of pri-mir-195. This regulatory event requires complemen-

tarity between the lower stem region of the pri-mir-195 transcript and an ultraconserved sequence in Uc.283+A. The proposed mechanism involves lower-stem strand invasion by Uc.283+A, which impairs Microprocessor recognition and blocks pri-miRNA processing (Liz et al. 2014). RNA 4 (RNCR4), a retina-specific lncRNA, stimulates the timed processing of the pri-mir-183-96-182 cluster, which is repressed at an earlier developmental stage by the RNA helicase Ddx3x, during mouse retina development (Krol et al. 2015). Finally, the heterodimer of splicing factors, NONO/PSF, which are components of the paraspeckles, bind a large number of pri-miRNAs and promote the Microprocessor-mediated processing of these precursors in HeLa cells. The lncRNA, NEAT1, interacts with NONO/PSF and scaffolds RBPs and the Microprocessor to globally promote miRNA processing (Jiang et al. 2017).

Physiological relevance of miRNA regulation and human disease

Given the central role that miRNAs have in controlling the expression of target mRNAs, it is unsurprising that dysregulation of miRNA production leads to aberrant gene expression due to the misregulated expression of target mRNAs. This can affect cellular homeostasis, as well as many developmental pathways and have an impact on the development of human disease. One of the most studied examples is the altered expression of a variety of miRNAs in many different types of cancer, which arises as a consequence of mis-regulation of miRNA production, but also through the presence of mutations in miRNA processing components (for review, see Lekka and Hall 2018). Mutations in Microprocessor components, DROSHA and DGCR8 have been identified in Wilms tumors, a pediatric kidney tumor (Rakheja et al. 2014; Walz et al. 2015; Wegert et al. 2015). Conversely, DICER mutations have also been linked to several human conditions, including early childhood tumors (Heravi-Moussavi et al. 2012; Wu et al. 2013; for reviews, see Foulkes et al. 2014; Hata and Kashima 2016).

TABLE 2. Long noncoding RNAs that regulate miRNA biogenesis at the post-transcriptional level

lncRNA	miRNA biogenesis Step effect	Mechanism	Target miRNAs	References
Uc.283+A	Drosha -	Lower-stem strand invasion by Uc.283+A blocks Microprocessor recognition and efficient pri-miRNA processing	miR-195	(Liz et al. 2014)
RNCR4	Drosha +	Stimulates the timed processing of this cluster. Antagonizes repression by the RNA helicase Ddx3x	miR-183-96-182 cluster	(Krol et al. 2015)
NEAT1	Drosha +	NEAT1 interacts with the splicing factors NONO-PSF and recruits the Microprocessor	Global stimulation of miRNA production	(Jiang et al. 2017)

lncRNAs, long noncoding RNAs; Uc.283+A, transcribed ultraconserved region 283+A; RNCR4, retinal noncoding RNA 4; NEAT1, nuclear enriched abundant transcript 1.

The regulation of let-7 miRNA precursors by the LIN28 proteins is a clear example of how alterations to post-transcriptional regulation of miRNA precursors can indeed lead to cancer (Viswanathan et al. 2008, 2009; Lightfoot et al. 2011). In the section below, we will discuss novel approaches used in an attempt to influence miRNA biogenesis by targeting regulatory RBPs that regulate the production of miRNAs.

Synthetic and natural inhibitors of miRNA biogenesis

Several strategies have been developed to affect the production of miRNAs, targeting their nuclear and cytoplasmic processing machineries and/or factors that regulate their biogenesis. We have described above the use of looptomiRs and Aptamers to target the recognition of TLs by RBPs and regulate miRNA processing. Several alternative approaches have been developed to identify small molecules that bind to miRNA precursors or to RBPs that regulate miRNA biogenesis. These include the identification of a peptoid ligand that interacts with the apical loop of pri-mir-21 and inhibits cleavage by DROSHA (Diaz et al. 2014), of a benzimidazole that inhibits the biogenesis of miR-96 (Velagapudi et al. 2014) and of polyamine derivatives that block the DICER-mediated processing of pre-mir-372 processing (Staedel et al. 2018). A different strategy was developed to inhibit miRNAs that are overexpressed in human cancers, such as miR-21, in which a cyclic β -hairpin peptidomimetic binds to RNA stem-loop structures of miRNA precursors, with potent affinity and specificity. This peptide was shown to recognize the DICER cleavage site and inhibit miR-21 processing (Shortridge et al. 2017). Another study focusing on miR-21, used small molecule screening and 3D structure modeling and identified AC1MMYR2 (2,4-diamino-1, 3-diazinane-5-carbonitrile) as a potent inhibitor of pre-mir-21 cleavage by DICER (Shi et al. 2013). This inhibitor reversed epithelial-to-mesenchymal transition and suppressed tumor growth. Oleic acid (a natural monounsaturated fatty acid produced by plants and animal cells) inhibits the RNA-binding activity of Musashi RNA-binding protein 2 (MSI2), a negative regulator of pri-mir-7 processing, by binding to its N-terminal RRM (Choudhury et al. 2013; Clingman et al. 2014). Thus, the action of oleic acid could be used to disrupt the formation of a negative regulatory complex and lead to stimulation of miR-7 biogenesis (Kumar et al. 2017). Such strategies could reduce the levels of miR-7 target genes such as the EGFR oncogene, to potentially alleviate its deleterious effects in high-grade glioblastomas, where miR-7 is post-transcriptionally down-regulated (Kefas et al. 2008). Recent small-molecule screenings have identified compounds that inhibit Lin28 binding to RNA (Roos et al. 2016; Wang et al. 2018). One such compound, LI17, potently inhibited Lin28-mediated oligouridylation of pre-let-7 in vitro and

in cells, causing a concomitant increase in mature let-7 levels (Wang et al. 2018). Likewise, another compound de-repressed let-7 and inhibited proliferation and stem-like properties in human cancer cells (Roos et al. 2016). These examples demonstrate that selective pharmacologic inhibition of RBPs involved in post-transcriptional regulation of miRNA biogenesis could provide a foundation for therapeutic intervention in diseases underpinned by deregulated miRNA levels.

CONCLUSION AND PERSPECTIVES

Several layers of tightly controlled regulation have evolved to maintain the levels of mature miRNAs in order to fine-tune gene expression during development and differentiation. Such multifaceted regulation ultimately prevents gross changes in gene expression that can contribute to numerous diseases. Among these mechanisms of control, post-transcriptional steps are predominant, and increasing evidence shows the central role of general RBPs in the control of miRNA production. The binding of RBPs to TL sequences within miRNA precursors (pri- and pre-miRNAs) has emerged as a general mechanism to regulate the activity of DROSHA and/or DICER. This can encompass different mechanisms, such as conformational changes and dynamic destabilization induced by the binding of these auxiliary factors. For example, the binding of hnRNP A1 or Rbfox proteins to pri-miRNAs leads to structural changes that affect Microprocessor binding and/or activity (Table 1; Chen et al. 2016; Kooshapur et al. 2018). Another common mechanism is antagonistic binding of the regulatory RBP to either a positive or negative regulator, as seen with the competitive binding of hnRNP A1 and KSRP to let-7 precursors in differentiated cells (Michlewski and Cáceres 2010), or MBNL-1 antagonizing LIN28 binding to pri-mir-1 (Rau et al. 2011). Thus, increased knowledge of binding sites and regulatory mechanisms could facilitate the manipulation of individual miRNA expression. Similarly, recent efforts have relied on the use of oligonucleotide approaches and/or chemical or biological compounds to target the LIN28/let-7 interaction. In principle, these approaches could be expanded to compete out the binding of positive or negative regulators to individual precursor miRNAs and modify the outcome of the biogenesis pathway to correct the unbalanced miRNA levels.

Extensive genetic variation leading to altered miRNA biogenesis represents another largely unexplored mechanism of regulation. We and others have established that primary sequence determinants and RNA structure are important regulators of miRNA biogenesis. Of interest, polymorphisms and mutations within or proximal to miRNAs are frequently overlooked in disease and trait studies searching for functionally important variants, but could have an important role in determining levels of miRNA expression. In the near future, efforts will focus on the

identification and functional characterization of additional RBPs or other regulators that affect miRNA biogenesis, as illustrated by recent genome-wide efforts (Treiber et al. 2017; Nussbacher and Yeo 2018). Our expanding knowledge about the mechanisms that regulate miRNA production will be essential to understand and treat human diseases that arise from deregulated gene expression.

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REFERENCES

- Akhtar MM, Micolucci L, Islam MS, Olivieri F, Procopio AD. 2016. Bioinformatic tools for microRNA dissection. *Nucleic Acids Res* **44**: 24–44. doi:10.1093/nar/gkv1221
- Alarcón CR, Goodarzi H, Lee H, Liu X, Tavazoie S, Tavazoie SF. 2015a. HNRNPA2B1 is a mediator of m⁶A-dependent nuclear RNA processing events. *Cell* **162**: 1299–1308. doi:10.1016/j.cell.2015.08.011
- Alarcón CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF. 2015b. N⁶-methyladenosine marks primary microRNAs for processing. *Nature* **519**: 482–485. doi:10.1038/nature14281
- Auyeung VC, Ulitsky I, McGeary SE, Bartel DP. 2013. Beyond secondary structure: primary-sequence determinants license pri-miRNA hairpins for processing. *Cell* **152**: 844–858. doi:10.1016/j.cell.2013.01.031
- Axtell MJ, Westholm JO, Lai EC. 2011. Vive la différence: biogenesis and evolution of microRNAs in plants and animals. *Genome Biol* **12**: 221. doi:10.1186/gb-2011-12-4-221
- Bartel DP. 2018. Metazoan microRNAs. *Cell* **173**: 20–51. doi:10.1016/j.cell.2018.03.006
- Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk RHA, Cuppen E. 2005. Phylogenetic shadowing and computational identification of human microRNA genes. *Cell* **120**: 21–24. doi:10.1016/j.cell.2004.12.031
- Bohnsack MT, Czaplinski K, Gorlich D. 2004. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* **10**: 185–191. doi:10.1261/ma.5167604
- Bologna NG, Schapire AL, Palatnik JF. 2013. Processing of plant microRNA precursors. *Brief Funct Genomics* **12**: 37–45. doi:10.1093/bfpg/els050
- Bonnal S, Pileur F, Orsini C, Parker F, Pujol F, Prats AC, Vagner S. 2005. Heterogeneous nuclear ribonucleoprotein A1 is a novel internal ribosome entry site *trans*-acting factor that modulates alternative initiation of translation of the fibroblast growth factor 2 mRNA. *J Biol Chem* **280**: 4144–4153. doi:10.1074/jbc.M411492200
- Bracken CP, Scott HS, Goodall GJ. 2016. A network-biology perspective of microRNA function and dysfunction in cancer. *Nat Rev Genet* **17**: 719–732. doi:10.1038/nrg.2016.134
- Castilla-Llorente V, Nicastro G, Ramos A. 2013. Terminal loop-mediated regulation of miRNA biogenesis: selectivity and mechanisms. *Biochem Soc Trans* **41**: 861–865. doi:10.1042/BST20130058
- Chang HM, Triboulet R, Thornton JE, Gregory RI. 2013. A role for the Perlman syndrome exonuclease Dis3l2 in the Lin28-let-7 pathway. *Nature* **497**: 244–248. doi:10.1038/nature12119
- Chen Y, Zubovic L, Yang F, Godin K, Pavelitz T, Castellanos J, Macchi P, Varani G. 2016. Rbfox proteins regulate microRNA biogenesis by sequence-specific binding to their precursors and target downstream Dicer. *Nucleic Acids Res* **44**: 4381–4395. doi:10.1093/nar/gkw177
- Chong MM, Zhang G, Cheloufi S, Neubert TA, Hannon GJ, Littman DR. 2010. Canonical and alternate functions of the microRNA biogenesis machinery. *Genes Dev* **24**: 1951–1960. doi:10.1101/gad.1953310
- Choudhury NR, Michlewski G. 2012. Terminal loop-mediated control of microRNA biogenesis. *Biochem Soc Trans* **40**: 789–793. doi:10.1042/BST20120053
- Choudhury NR, Michlewski G. 2018. Quantitative identification of proteins that influence miRNA biogenesis by RNA pull-down–SILAC mass spectrometry (RP–SMS). *Methods pii*: S1046.
- Choudhury NR, de Lima Alves F, de Andrés-Aguayo L, Graf T, Cáceres JF, Rappsilber J, Michlewski G. 2013. Tissue-specific control of brain-enriched miR-7 biogenesis. *Genes Dev* **27**: 24–38. doi:10.1101/gad.199190.112
- Choudhury NR, Nowak JS, Zuo J, Rappsilber J, Spoel SH, Michlewski G. 2014. Trim25 is an RNA-specific activator of Lin28a/TuT4-mediated uridylation. *Cell Rep* **9**: 1265–1272. doi:10.1016/j.celrep.2014.10.017
- Clingman CC, Deveau LM, Hay SA, Genga RM, Shandilya SM, Massi F, Ryder SP. 2014. Allosteric inhibition of a stem cell RNA-binding protein by an intermediary metabolite. *Elife* **3**: e02848. doi:10.7554/eLife.02848
- Concepcion CP, Bonetti C, Ventura A. 2012. The microRNA-17-92 family of microRNA clusters in development and disease. *Cancer J* **18**: 262–267. doi:10.1097/PPO.0b013e318258b60a
- Creugny A, Fender A, Pfeffer S. 2018. Regulation of primary microRNA processing. *FEBS Lett* **592**: 1980–1996. doi:10.1002/1873-3468.13067
- Davis BN, Hilyard AC, Lagna G, Hata A. 2008. SMAD proteins control DROSHA-mediated microRNA maturation. *Nature* **454**: 56–61. doi:10.1038/nature07086
- Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A. 2010. Smad proteins bind a conserved RNA sequence to promote microRNA maturation by Drosha. *Mol Cell* **39**: 373–384. doi:10.1016/j.molcel.2010.07.011
- Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. 2004. Processing of primary microRNAs by the Microprocessor complex. *Nature* **432**: 231–235. doi:10.1038/nature03049
- Di Carlo V, Grossi E, Laneve P, Morlando M, Dini Modigliani S, Ballarino M, Bozzoni I, Caffarelli E. 2013. TDP-43 regulates the microprocessor complex activity during in vitro neuronal differentiation. *Mol Neurobiol* **48**: 952–963. doi:10.1007/s12035-013-8564-x
- Diaz JP, Chirayil R, Chirayil S, Tom M, Head KJ, Luebke KJ. 2014. Association of a peptoid ligand with the apical loop of pri-miR-21 inhibits cleavage by Drosha. *RNA* **20**: 528–539. doi:10.1261/ma.042911.113
- Ebert MS, Sharp PA. 2012. Roles for microRNAs in conferring robustness to biological processes. *Cell* **149**: 515–524. doi:10.1016/j.cell.2012.04.005
- Fabian MR, Sonenberg N, Filipowicz W. 2010. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* **79**: 351–379. doi:10.1146/annurev-biochem-060308-103103

- Fang W, Bartel DP. 2015. The menu of features that define primary microRNAs and enable de novo design of microRNA genes. *Mol Cell* **60**: 131–145. doi:10.1016/j.molcel.2015.08.015
- Fernandez N, Cordiner RA, Young RS, Hug N, Macias S, Cáceres JF. 2017. Genetic variation and RNA structure regulate microRNA biogenesis. *Nat Commun* **8**: 15114. doi:10.1038/ncomms15114
- Finnegan EF, Pasquinelli AE. 2013. MicroRNA biogenesis: regulating the regulators. *Crit Rev Biochem Mol Biol* **48**: 51–68. doi:10.3109/10409238.2012.738643
- Foulkes WD, Priest JR, Duchaine TF. 2014. DICER1: mutations, microRNAs and mechanisms. *Nat Rev Cancer* **14**: 662–672. doi:10.1038/nrc3802
- Fukunaga R, Han BW, Hung JH, Xu J, Weng Z, Zamore PD. 2012. Dicer partner proteins tune the length of mature miRNAs in flies and mammals. *Cell* **151**: 533–546. doi:10.1016/j.cell.2012.09.027
- Gebert LFR, MacRae IJ. 2018. Regulation of microRNA function in animals. *Nat Rev Mol Cell Biol*. doi:10.1038/s41580-018-0045-7
- Goodall EF, Heath PR, Bandmann O, Kirby J, Shaw PJ. 2013. Neuronal dark matter: the emerging role of microRNAs in neurodegeneration. *Front Cell Neurosci* **7**: 178. doi:10.3389/fncel.2013.00178
- Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. 2004. The Microprocessor complex mediates the genesis of microRNAs. *Nature* **432**: 235–240. doi:10.1038/nature03120
- Guil S, Cáceres JF. 2007. The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. *Nat Struct Mol Biol* **14**: 591–596. doi:10.1038/nsm1250
- Ha M, Kim VN. 2014. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* **15**: 509–524. doi:10.1038/nrm3838
- Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. 2004. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev* **18**: 3016–3027. doi:10.1101/gad.1262504
- Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, Sohn SY, Cho Y, Zhang BT, Kim VN. 2006. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell* **125**: 887–901. doi:10.1016/j.cell.2006.03.043
- Han J, Pedersen JS, Kwon SC, Belair CD, Kim YK, Yeom KH, Yang WY, Haussler D, Blelloch R, Kim VN. 2009. Posttranscriptional cross-regulation between Drosha and DGCR8. *Cell* **136**: 75–84. doi:10.1016/j.cell.2008.10.053
- Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. 2013. Natural RNA circles function as efficient microRNA sponges. *Nature* **495**: 384–388. doi:10.1038/nature11993
- Harvey KF, Zhang X, Thomas DM. 2013. The Hippo pathway and human cancer. *Nat Rev Cancer* **13**: 246–257. doi:10.1038/nrc3458
- Hata A, Kashima R. 2016. Dysregulation of microRNA biogenesis machinery in cancer. *Crit Rev Biochem Mol Biol* **51**: 121–134. doi:10.3109/10409238.2015.1117054
- Hata A, Lieberman J. 2015. Dysregulation of microRNA biogenesis and gene silencing in cancer. *Sci Signal* **8**: re3. doi:10.1126/scisignal.2005825
- He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, et al. 2005. A microRNA polycistron as a potential human oncogene. *Nature* **435**: 828–833. doi:10.1038/nature03552
- Heale BS, Keegan LP, McGurk L, Michlewski G, Brindle J, Stanton CM, Cáceres JF, O'Connell MA. 2009. Editing independent effects of ADARs on the miRNA/siRNA pathways. *EMBO J* **28**: 3145–3156. doi:10.1038/emboj.2009.244
- Heo I, Joo C, Kim YK, Ha M, Yoon MJ, Cho J, Yeom KH, Han J, Kim VN. 2009. TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. *Cell* **138**: 696–708. doi:10.1016/j.cell.2009.08.002
- Heras SR, Macias S, Plass M, Fernandez N, Cano D, Eyras E, Garcia-Perez JL, Cáceres JF. 2013. The Microprocessor controls the activity of mammalian retrotransposons. *Nat Struct Mol Biol* **20**: 1173–1181. doi:10.1038/nsmb.2658
- Heravi-Moussavi A, Anglesio MS, Cheng SW, Senz J, Yang W, Prentice L, Fejes AP, Chow C, Tone A, Kalloger SE, et al. 2012. Recurrent somatic DICER1 mutations in nonepithelial ovarian cancers. *N Engl J Med* **366**: 234–242. doi:10.1056/NEJMoa1102903
- Higuchi T, Todaka H, Sugiyama Y, Ono M, Tamaki N, Hatano E, Takezaki Y, Hanazaki K, Miwa T, Lai S, et al. 2016. Suppression of microRNA-7 (miR-7) biogenesis by nuclear factor 90-nuclear factor 45 complex (NF90-NF45) controls cell proliferation in hepatocellular carcinoma. *J Biol Chem* **291**: 21074–21084. doi:10.1074/jbc.M116.748210
- Hogg DR, Harries LW. 2014. Human genetic variation and its effect on miRNA biogenesis, activity and function. *Biochem Soc Trans* **42**: 1184–1189. doi:10.1042/BST20140055
- Hutvagner G, McLachlan J, Pasquinelli AE, Bálint E, Tuschl T, Zamore PD. 2001. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* **293**: 834–838. doi:10.1126/science.1062961
- Hwang HW, Wentzel EA, Mendell JT. 2009. Cell-cell contact globally activates microRNA biogenesis. *Proc Natl Acad Sci* **106**: 7016–7021. doi:10.1073/pnas.0811523106
- Iwakawa H, Tomari Y. 2015. The functions of microRNAs: mRNA decay and translational repression. *Trends Cell Biol* **25**: 651–665. doi:10.1016/j.tcb.2015.07.011
- Iwasaki S, Kobayashi M, Yoda M, Sakaguchi Y, Katsuma S, Suzuki T, Tomari Y. 2010. Hsc70/Hsp90 chaperone machinery mediates ATP-dependent RISC loading of small RNA duplexes. *Mol Cell* **39**: 292–299. doi:10.1016/j.molcel.2010.05.015
- Jean-Philippe J, Paz S, Caputi M. 2013. hnRNP A1: the Swiss army knife of gene expression. *Int J Mol Sci* **14**: 18999–19024. doi:10.3390/ijms140918999
- Jiang L, Shao C, Wu QJ, Chen G, Zhou J, Yang B, Li H, Gou LT, Zhang Y, Wang Y, et al. 2017. NEAT1 scaffolds RNA-binding proteins and the microprocessor to globally enhance pri-miRNA processing. *Nat Struct Mol Biol* **24**: 816–824. doi:10.1038/nsmb.3455
- Kadener S, Rodriguez J, Abruzzi KC, Khodor YL, Sugino K, Marr MT, Nelson S, Rosbash M. 2009. Genome-wide identification of targets of the *drosha-pasha/DGCR8* complex. *RNA* **15**: 537–545. doi:10.1261/ma.1319309
- Kawahara Y, Mieda-Sato A. 2012. TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. *Proc Natl Acad Sci* **109**: 3347–3352. doi:10.1073/pnas.1112427109
- Kawahara Y, Zinshteyn B, Chendrimada TP, Shiekhattar R, Nishikura K. 2007. RNA editing of the microRNA-151 precursor blocks cleavage by the Dicer-TRBP complex. *EMBO Rep* **8**: 763–769. doi:10.1038/sj.embor.7401011
- Kawai S, Amano A. 2012. BRCA1 regulates microRNA biogenesis via the DROSHA microprocessor complex. *J Cell Biol* **197**: 201–208. doi:10.1083/jcb.201110008
- Kefas B, Godlewski J, Comeau L, Li Y, Abounader R, Hawkinson M, Lee J, Fine H, Chiocca EA, Lawler S, et al. 2008. microRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma. *Cancer Res* **68**: 3566–3572. doi:10.1158/0008-5472.CAN-07-6639
- Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH. 2001. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. *Genes Dev* **15**: 2654–2659. doi:10.1101/gad.927801
- Kim YK, Kim VN. 2007. Processing of intronic microRNAs. *EMBO J* **26**: 775–783. doi:10.1038/sj.emboj.7601512

- Kim KK, Yang Y, Zhu J, Adelstein RS, Kawamoto S. 2014a. Rbfox3 controls the biogenesis of a subset of microRNAs. *Nat Struct Mol Biol* **21**: 901–910. doi:10.1038/nsmb.2892
- Kim KY, Hwang YJ, Jung MK, Choe J, Kim YK, Kim S, Lee CJ, Ahn H, Lee J, Kowall NW, et al. 2014b. A multifunctional protein EWS regulates the expression of Drosha and microRNAs. *Cell Death Differ* **21**: 136–145. doi:10.1038/cdd.2013.144
- Kim K, Nguyen TD, Li S, Nguyen TA. 2018. SRSF3 recruits DROSHA to the basal junction of primary microRNAs. *RNA* **24**: 892–898. doi:10.1261/ma.065862.118
- Knuckles P, Vogt MA, Lugert S, Milo M, Chong MM, Hautbergue GM, Wilson SA, Littman DR, Taylor V. 2012. Drosha regulates neurogenesis by controlling neurogenin 2 expression independent of microRNAs. *Nat Neurosci* **15**: 962–969. doi:10.1038/nn.3139
- Knuckles P, Carl SH, Musheev M, Niehrs C, Wenger A, Bühler M. 2017. RNA fate determination through cotranscriptional adenosine methylation and microprocessor binding. *Nat Struct Mol Biol* **24**: 561–569. doi:10.1038/nsmb.3419
- Kobayashi H, Tomari Y. 2016. RISC assembly: coordination between small RNAs and Argonaute proteins. *Biochim Biophys Acta* **1859**: 71–81. doi:10.1016/j.bbtagm.2015.08.007
- Kooshapur H, Choudhury NR, Simon B, Mühlbauer M, Jussupow A, Fernandez N, Jones AN, Dallmann A, Gabel F, Camilloni C, et al. 2018. Structural basis for terminal loop recognition and stimulation of pri-miRNA-18a processing by hnRNP A1. *Nat Commun* **9**: 2479. doi:10.1038/s41467-018-04871-9
- Krol J, Loedige I, Filipowicz W. 2010. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* **11**: 597–610. doi:10.1038/nrg2843
- Krol J, Krol I, Alvarez CPP, Fiscella M, Hierlemann A, Roska B, Filipowicz W. 2015. A network comprising short and long noncoding RNAs and RNA helicase controls mouse retina architecture. *Nat Commun* **6**: 7305. doi:10.1038/ncomms8305
- Królciewicz J, Sobolewska A, Lejnowski D, Collawn JF, Bartoszewski R. 2018. MicroRNA single polynucleotide polymorphism influences on microRNA biogenesis and mRNA target specificity. *Gene* **640**: 66–72. doi:10.1016/j.gene.2017.10.021
- Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. 2007. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet* **39**: 673–677. doi:10.1038/ng2003
- Kumar S, Downie Ruiz Velasco A, Michlewski G. 2017. Oleic acid induces miR-7 processing through remodeling of pri-miR-7/protein complex. *J Mol Biol* **429**: 1638–1649. doi:10.1016/j.jmb.2017.05.001
- Kwon SC, Nguyen TA, Choi YG, Jo MH, Hohng S, Kim VN, Woo JS. 2016. Structure of human DROSHA. *Cell* **164**: 81–90. doi:10.1016/j.cell.2015.12.019
- LaBranche H, Dupuis S, Ben-David Y, Bani MR, Wellinger RJ, Chabot B. 1998. Telomere elongation by hnRNP A1 and a derivative that interacts with telomeric repeats and telomerase. *Nat Genet* **19**: 199–202. doi:10.1038/575
- Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, et al. 2007. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* **129**: 1401–1414. doi:10.1016/j.cell.2007.04.040
- Landthaler M, Yalcin A, Tuschl T. 2004. The human DiGeorge syndrome critical region gene 8 and its *D. melanogaster* homolog are required for miRNA biogenesis. *Curr Biol* **14**: 2162–2167. doi:10.1016/j.cub.2004.11.001
- Lebedeva S, Jens M, Theil K, Schwanhäusser B, Selbach M, Landthaler M, Rajewsky N. 2011. Transcriptome-wide analysis of regulatory interactions of the RNA-binding protein HuR. *Mol Cell* **43**: 340–352. doi:10.1016/j.molcel.2011.06.008
- Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, et al. 2003. The nuclear RNase III Drosha initiates microRNA processing. *Nature* **425**: 415–419. doi:10.1038/nature01957
- Legnini I, Morlando M, Mangiacavalli A, Fatica A, Bozzoni I. 2014. A feedforward regulatory loop between HuR and the long noncoding RNA linc-MD1 controls early phases of myogenesis. *Mol Cell* **53**: 506–514. doi:10.1016/j.molcel.2013.12.012
- Lekka E, Hall J. 2018. Noncoding RNAs in disease. *FEBS Lett* **592**: 2884–2900. doi:10.1002/1873-3468.13182
- Lightfoot HL, Bugaut A, Armisen J, Lehrbach NJ, Miska EA, Balasubramanian S. 2011. A LIN28-dependent structural change in pre-let-7g directly inhibits dicer processing. *Biochemistry* **50**: 7514–7521. doi:10.1021/bi200851d
- Lin S, Gregory RI. 2015. MicroRNA biogenesis pathways in cancer. *Nat Rev Cancer* **15**: 321–333. doi:10.1038/nrc3932
- Liu H, Liang C, Kollipara RK, Matsui M, Ke X, Jeong BC, Wang Z, Yoo KS, Yadav GP, Kinch LN, et al. 2016. HP1BP3, a chromatin retention factor for co-transcriptional microRNA processing. *Mol Cell* **63**: 420–432. doi:10.1016/j.molcel.2016.06.014
- Liz J, Portela A, Soler M, Gómez A, Ling H, Michlewski G, Calin GA, Guil S, Esteller M. 2014. Regulation of pri-miRNA processing by a long noncoding RNA transcribed from an ultraconserved region. *Mol Cell* **55**: 138–147. doi:10.1016/j.molcel.2014.05.005
- Loughlin FE, Gebert LF, Towbin H, Brunschweiler A, Hall J, Allain FH. 2012. Structural basis of pre-let-7 miRNA recognition by the zinc knuckles of pluripotency factor Lin28. *Nat Struct Mol Biol* **19**: 84–89. doi:10.1038/nsmb.2202
- Lund E, Güttinger S, Calado A, Dahlberg JE, Kutay U. 2004. Nuclear export of microRNA precursors. *Science* **303**: 95–98. doi:10.1126/science.1090599
- Lünse CE, Michlewski G, Hopp CS, Rentmeister A, Cáceres JF, Famulok M, Mayer G. 2010. An aptamer targeting the apical-loop domain modulates pri-miRNA processing. *Angew Chem Int Ed Engl* **49**: 4674–4677. doi:10.1002/anie.200906919
- Macias S, Plass M, Stajuda A, Michlewski G, Eyras E, Cáceres JF. 2012. DGCR8 HITS-CLIP reveals novel functions for the Microprocessor. *Nat Struct Mol Biol* **19**: 760–766. doi:10.1038/nsmb.2344
- Macias S, Cordiner RA, Cáceres JF. 2013. Cellular functions of the microprocessor. *Biochem Soc Trans* **41**: 838–843. doi:10.1042/BST20130011
- Macias S, Cordiner RA, Gautier P, Plass M, Cáceres JF. 2015. DGCR8 acts as an adaptor for the exosome complex to degrade double-stranded structured RNAs. *Mol Cell* **60**: 873–885. doi:10.1016/j.molcel.2015.11.011
- Macrae IJ, Zhou K, Li F, Repic A, Brooks AN, Cande WZ, Adams PD, Doudna JA. 2006. Structural basis for double-stranded RNA processing by Dicer. *Science* **311**: 195–198. doi:10.1126/science.1121638
- Marinero F, Marzi MJ, Hoffmann N, Amin H, Pelizzoli R, Niola F, Nicassio F, De Pietri Tonelli D. 2017. MicroRNA-independent functions of DGCR8 are essential for neocortical development and TBR1 expression. *EMBO Rep* **18**: 603–618. doi:10.15252/embr.201642800
- Mayeda A, Krainer AR. 1992. Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. *Cell* **68**: 365–375. doi:10.1016/0092-8674(92)90477-T
- Mayeda A, Munroe SH, Cáceres JF, Krainer AR. 1994. Function of conserved domains of hnRNP A1 and other hnRNP A/B proteins. *EMBO J* **13**: 5483–5495. doi:10.1002/j.1460-2075.1994.tb06883.x
- Mayr F, Schütz A, Döge N, Heinemann U. 2012. The Lin28 cold-shock domain remodels pre-let-7 microRNA. *Nucleic Acids Res* **40**: 7492–7506. doi:10.1093/nar/gks355
- Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, et al. 2013. Circular

- RNAs are a large class of animal RNAs with regulatory potency. *Nature* **495**: 333–338. doi:10.1038/nature11928
- Mendell JT, Olson EN. 2012. MicroRNAs in stress signaling and human disease. *Cell* **148**: 1172–1187. doi:10.1016/j.cell.2012.02.005
- Michlewski G, Cáceres JF. 2010. Antagonistic role of hnRNP A1 and KSRP in the regulation of let-7a biogenesis. *Nat Struct Mol Biol* **17**: 1011–1018. doi:10.1038/nsmb.1874
- Michlewski G, Guil S, Semple CA, Cáceres JF. 2008. Posttranscriptional regulation of miRNAs harboring conserved terminal loops. *Mol Cell* **32**: 383–393. doi:10.1016/j.molcel.2008.10.013
- Michlewski G, Guil S, Cáceres JF. 2010. Stimulation of pri-miR-18a processing by hnRNP A1. *Adv Exp Med Biol* **700**: 28–35. doi:10.1007/978-1-4419-7823-3_3
- Miyoshi T, Takeuchi A, Siomi H, Siomi MC. 2010. A direct role for Hsp90 in pre-RISC formation in *Drosophila*. *Nat Struct Mol Biol* **17**: 1024–1026. doi:10.1038/nsmb.1875
- Mori M, Triboulet R, Mohseni M, Schlegelmilch K, Shrestha K, Camargo FD, Gregory RI. 2014. Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. *Cell* **156**: 893–906. doi:10.1016/j.cell.2013.12.043
- Morlando M, Ballarino M, Gromak N, Pagano F, Bozzoni I, Proudfoot NJ. 2008. Primary microRNA transcripts are processed co-transcriptionally. *Nat Struct Mol Biol* **15**: 902–909. doi:10.1038/nsmb.1475
- Morlando M, Dini Modigliani S, Torrelli G, Rosa A, Di Carlo V, Caffarelli E, Bozzoni I. 2012. FUS stimulates microRNA biogenesis by facilitating co-transcriptional Drosha recruitment. *EMBO J* **31**: 4502–4510. doi:10.1038/emboj.2012.319
- Nam Y, Chen C, Gregory RI, Chou JJ, Sliz P. 2011. Molecular basis for interaction of let-7 microRNAs with Lin28. *Cell* **147**: 1080–1091. doi:10.1016/j.cell.2011.10.020
- Naqvi AR, Sarwat M, Hasan S, Roychodhury N. 2012. Biogenesis, functions and fate of plant microRNAs. *J Cell Physiol* **227**: 3163–3168. doi:10.1002/jcp.24052
- Newman MA, Thomson JM, Hammond SM. 2008. Lin-28 interaction with the Let-7 precursor loop mediates regulated microRNA processing. *RNA* **14**: 1539–1549. doi:10.1261/rna.1155108
- Nguyen TA, Jo MH, Choi YG, Park J, Kwon SC, Hohng S, Kim VN, Woo JS. 2015. Functional anatomy of the human microprocessor. *Cell* **161**: 1374–1387. doi:10.1016/j.cell.2015.05.010
- Nguyen TA, Park J, Dang TL, Choi YG, Kim VN. 2018. Microprocessor depends on hemin to recognize the apical loop of primary microRNA. *Nucleic Acids Res* **46**: 5726–5736. doi:10.1093/nar/gky248
- Nowak JS, Choudhury NR, de Lima Alves F, Rappsilber J, Michlewski G. 2014. Lin28a regulates neuronal differentiation and controls miR-9 production. *Nat Commun* **5**: 3687. doi:10.1038/ncomms4687
- Nowak JS, Hobor F, Downie Ruiz Velasco A, Choudhury NR, Heikel G, Kerr A, Ramos A, Michlewski G. 2017. Lin28a uses distinct mechanisms of binding to RNA and affects miRNA levels positively and negatively. *RNA* **23**: 317–332. doi:10.1261/rna.059196.116
- Nussbacher JK, Yeo GW. 2018. Systematic discovery of RNA binding proteins that regulate microRNA levels. *Mol Cell* **69**: 1005–1016. e7. doi:10.1016/j.molcel.2018.02.012
- Ouyang H, Zhang K, Fox-Walsh K, Yang Y, Zhang C, Huang J, Li H, Zhou Y, Fu XD. 2017. The RNA binding protein EWS is broadly involved in the regulation of pri-miRNA processing in mammalian cells. *Nucleic Acids Res* **45**: 12481–12495. doi:10.1093/nar/gkx912
- Paez-Colasante X, Figueroa-Romero C, Sakowski SA, Goutman SA, Feldman EL. 2015. Amyotrophic lateral sclerosis: mechanisms and therapeutics in the epigenomic era. *Nat Rev Neurol* **11**: 266–279. doi:10.1038/nrneurol.2015.57
- Partin AC, Ngo TD, Herrell E, Jeong BC, Hon G, Nam Y. 2017. Heme enables proper positioning of Drosha and DGCR8 on primary microRNAs. *Nat Commun* **8**: 1737. doi:10.1038/s41467-017-01713-y
- Piskounova E, Viswanathan SR, Janas M, LaPierre RJ, Daley GQ, Sliz P, Gregory RI. 2008. Determinants of microRNA processing inhibition by the developmentally regulated RNA-binding protein Lin28. *J Biol Chem* **283**: 21310–21314. doi:10.1074/jbc.C800108200
- Pong SK, Gullerova M. 2018. Noncanonical functions of microRNA pathway enzymes—Drosha, DGCR8, Dicer and Ago proteins. *FEBS Lett* **592**: 2973–2986. doi:10.1002/1873-3468.13196
- Quick-Cleveland J, Jacob JP, Weitz SH, Shoffner G, Senturia R, Guo F. 2014. The DGCR8 RNA-binding heme domain recognizes primary microRNAs by clamping the hairpin. *Cell Rep* **7**: 1994–2005. doi:10.1016/j.celrep.2014.05.013
- Rakheja D, Chen KS, Liu Y, Shukla AA, Schmid V, Chang TC, Khokhar S, Wickiser JE, Karandikar NJ, Malter JS, et al. 2014. Somatic mutations in DROSHA and DICER1 impair microRNA biogenesis through distinct mechanisms in Wilms tumours. *Nat Commun* **2**: 4802. doi:10.1038/ncomms5802
- Ratnadiwakara M, Mohenska M, Änkö ML. 2018. Splicing factors as regulators of miRNA biogenesis—links to human disease. *Semin Cell Dev Biol* **79**: 113–122. doi:10.1016/j.semcdb.2017.10.008
- Rau F, Freyermuth F, Fugier C, Villemain JP, Fischer MC, Jost B, Dembele D, Gourdon G, Nicole A, Duboc D, et al. 2011. Misregulation of miR-1 processing is associated with heart defects in myotonic dystrophy. *Nat Struct Mol Biol* **18**: 840–845. doi:10.1038/nsmb.2067
- Roden C, Gaillard J, Kanoria S, Rennie W, Barish S, Cheng J, Pan W, Liu J, Cotsapas C, Ding Y, et al. 2017. Novel determinants of mammalian primary microRNA processing revealed by systematic evaluation of hairpin-containing transcripts and human genetic variation. *Genome Res* **27**: 374–384. doi:10.1101/gr.208900.116
- Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. 2004. Identification of mammalian microRNA host genes and transcription units. *Genome Res* **14**: 1902–1910. doi:10.1101/gr.2722704
- Roos M, Rebhan MAE, Lucic M, Pavlicek D, Pradere U, Towbin H, Civenni G, Catapano CV, Hall J. 2014. Short loop-targeting oligoribonucleotides antagonize Lin28 and enable pre-let-7 processing and suppression of cell growth in let-7-deficient cancer cells. *Nucleic Acids Res* **43**: e9. doi:10.1093/nar/gku1090
- Roos M, Pradère U, Ngondo RP, Behera A, Allegrini S, Civenni G, Zagalak JA, Marchand JR, Menzi M, Towbin H, et al. 2016. A small-molecule inhibitor of Lin28. *ACS Chem Biol* **11**: 2773–2781. doi:10.1021/acscchembio.6b00232
- Shen J, Ambrosone CB, Zhao H. 2009. Novel genetic variants in microRNA genes and familial breast cancer. *Int J Cancer* **124**: 1178–1182. doi:10.1002/ijc.24008
- Shi Z, Zhang J, Qian X, Han L, Zhang K, Chen L, Liu J, Ren Y, Yang M, Zhang A, et al. 2013. AC1MMYR2, an inhibitor of dicer-mediated biogenesis of Oncomir miR-21, reverses epithelial-mesenchymal transition and suppresses tumor growth and progression. *Cancer Res* **73**: 5519–5531. doi:10.1158/0008-5472.CAN-13-0280
- Shorridge MD, Walker MJ, Pavelitz T, Chen Y, Yang W, Varani G. 2017. A macrocyclic peptide ligand binds the oncogenic microRNA-21 precursor and suppresses dicer processing. *ACS Chem Biol* **12**: 1611–1620. doi:10.1021/acscchembio.7b00180
- Siomi H, Siomi MC. 2010. Posttranscriptional regulation of microRNA biogenesis in animals. *Mol Cell* **38**: 323–332. doi:10.1016/j.molcel.2010.03.013
- Sperber H, Beem A, Shannon S, Jones R, Banik P, Chen Y, Ku S, Varani G, Yao S, Ruohola-Baker H. 2014. miRNA sensitivity to Drosha levels correlates with pre-miRNA secondary structure. *RNA* **20**: 621–631. doi:10.1261/ma.043943.113

- Staedel C, Tran TPA, Giraud J, Darfeuille F, Di Giorgio A, Tourasse NJ, Salin F, Uriac P, Duca M. 2018. Modulation of oncogenic miRNA biogenesis using functionalized polyamines. *Sci Rep* **8**: 1667. doi:10.1038/s41598-018-20053-5
- Starega-Roslan J, Galka-Marciniak P, Krzyzosiak WJ. 2015. Nucleotide sequence of miRNA precursor contributes to cleavage site selection by Dicer. *Nucleic Acids Res* **43**: 10939–10951. doi:10.1093/nar/gkv968
- Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S, Miyazono K. 2009. Modulation of microRNA processing by p53. *Nature* **460**: 529–533. doi:10.1038/nature08199
- Towbin H, Wenter P, Guenewig B, Imig J, Zagalak JA, Gerber AP, Hall J. 2013. Systematic screens of proteins binding to synthetic microRNA precursors. *Nucleic Acids Res* **41**: e47. doi:10.1093/nar/gks1197
- Trabucchi M, Briata P, Garcia-Mayoral M, Haase AD, Filipowicz W, Ramos A, Gherzi R, Rosenfeld MG. 2009. The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. *Nature* **459**: 1010–1014. doi:10.1038/nature08025
- Trabucchi M, Briata P, Filipowicz W, Ramos A, Gherzi R, Rosenfeld MG. 2010. KSRP promotes the maturation of a group of miRNA precursors. *Adv Exp Med Biol* **700**: 36–42. doi:10.1007/978-1-4419-7823-3_4
- Treiber T, Treiber N, Plessmann U, Harlander S, Daiß JL, Eichner N, Lehmann G, Schall K, Urlaub H, Meister G. 2017. A compendium of RNA-binding proteins that regulate microRNA biogenesis. *Mol Cell* **66**: 270–284.e13. doi:10.1016/j.molcel.2017.03.014
- Treiber T, Treiber N, Meister G. 2018a. Identification of microRNA precursor-associated proteins. *Methods Mol Biol* **1823**: 103–114. doi:10.1007/978-1-4939-8624-8_9
- Treiber T, Treiber N, Meister G. 2018b. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. *Nat Rev Mol Cell Biol*. doi:10.1038/s41580-018-0059-1
- Triboulet R, Chang HM, Lapierre RJ, Gregory RI. 2009. Post-transcriptional control of DGCR8 expression by the Microprocessor. *RNA* **15**: 1005–1011. doi:10.1261/rna.1591709
- Ustianenko D, Hrossova D, Potesil D, Chalupnikova K, Hrazdilova K, Pachemik J, Cetkowska K, Uldrijan S, Zdrahal Z, Vanacova S. 2013. Mammalian DIS3L2 exoribonuclease targets the uridylylated precursors of let-7 miRNAs. *RNA* **19**: 1632–1638. doi:10.1261/rna.040055.113
- Velagapudi SP, Gallo SM, Disney MD. 2014. Sequence-based design of bioactive small molecules that target precursor microRNAs. *Nat Chem Biol* **10**: 291–297. doi:10.1038/nchembio.1452
- Viswanathan SR, Daley GQ, Gregory RI. 2008. Selective blockade of microRNA processing by Lin28. *Science* **320**: 97–100. doi:10.1126/science.1154040
- Viswanathan SR, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, O’Sullivan M, Lu J, Phillips LA, Lockhart VL, et al. 2009. Lin28 promotes transformation and is associated with advanced human malignancies. *Nat Genet* **41**: 843–848. doi:10.1038/ng.392
- Walz AL, Ooms A, Gadd S, Gerhard DS, Smith MA, Guidry Auvil JM, Guidry Auvil JM, Meerzaman D, Chen QR, Hsu CH, et al. 2015. Recurrent DGCR8, DROSHA, and SIX homeodomain mutations in favorable histology Wilms tumors. *Cancer Cell* **27**: 286–297. doi:10.1016/j.ccell.2015.01.003
- Wang L, Rowe RG, Jaimes A, Yu C, Nam Y, Pearson DS, Zhang J, Xie X, Marion W, Heffron GJ, et al. 2018. Small-molecule inhibitors disrupt let-7 oligouridylation and release the selective blockade of let-7 processing by LIN28. *Cell Rep* **23**: 3091–3101. doi:10.1016/j.celrep.2018.04.116
- Wegert J, Ishaque N, Vardapour R, Geörg C, Gu Z, Bieg M, Ziegler B, Bausenwein S, Nourkami N, Ludwig N, et al. 2015. Mutations in the SIX1/2 pathway and the DROSHA/DGCR8 miRNA microprocessor complex underlie high-risk blastemal type Wilms tumors. *Cancer Cell* **27**: 298–311. doi:10.1016/j.ccell.2015.01.002
- Weitz SH, Gong M, Barr I, Weiss S, Guo F. 2014. Processing of microRNA primary transcripts requires heme in mammalian cells. *Proc Natl Acad Sci* **111**: 1861–1866. doi:10.1073/pnas.1309915111
- Wu H, Sun S, Tu K, Gao Y, Xie B, Krainer AR, Zhu J. 2010. A splicing-independent function of SF2/ASF in microRNA processing. *Mol Cell* **38**: 67–77. doi:10.1016/j.molcel.2010.02.021
- Wu MK, Sabbaghian N, Xu B, Addidou-Kalucki S, Bernard C, Zou D, Reeve AE, Eccles MR, Cole C, Choong CS, et al. 2013. Biallelic DICER1 mutations occur in Wilms tumours. *J Pathol* **230**: 154–164. doi:10.1002/path.4196
- Wu SL, Fu X, Huang J, Jia TT, Zong FY, Mu SR, Zhu H, Yan Y, Qiu S, Wu Q, et al. 2015. Genome-wide analysis of YB-1-RNA interactions reveals a novel role of YB-1 in miRNA processing in glioblastoma multiforme. *Nucleic Acids Res* **43**: 8516–8528. doi:10.1093/nar/gkv779
- Xhemalce B, Robson SC, Kouzarides T. 2012. Human RNA methyltransferase BCDIN3D regulates microRNA processing. *Cell* **151**: 278–288. doi:10.1016/j.cell.2012.08.041
- Yang W, Chendrimada TP, Wang Q, Higuchi M, Seeburg PH, Shiekhattar R, Nishikura K. 2006. Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. *Nat Struct Mol Biol* **13**: 13–21. doi:10.1038/nsmb1041
- Yi R, Qin Y, Macara IG, Cullen BR. 2003. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* **17**: 3011–3016. doi:10.1101/gad.1158803
- Zeng Y, Cullen BR. 2003. Sequence requirements for micro RNA processing and function in human cells. *RNA* **9**: 112–123. doi:10.1261/rna.2780503
- Zeng Y, Yi R, Cullen BR. 2005. Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. *EMBO J* **24**: 138–148. doi:10.1038/sj.emboj.7600491
- Zhang X, Zeng Y. 2010. The terminal loop region controls microRNA processing by Drosha and Dicer. *Nucleic Acids Res* **38**: 7689–7697. doi:10.1093/nar/gkq645
- Zhang LF, Lou JT, Lu MH, Gao C, Zhao S, Li B, Liang S, Li Y, Li D, Liu MF. 2015. Suppression of miR-199a maturation by HuR is crucial for hypoxia-induced glycolytic switch in hepatocellular carcinoma. *EMBO J* **34**: 2671–2685. doi:10.15252/embj.201591803
- Zou T, Jaladanki SK, Liu L, Xiao L, Chung HK, Wang JY, Xu Y, Gorospe M, Wang JY. 2016. H19 long noncoding RNA regulates intestinal epithelial barrier function via microRNA 675 by interacting with RNA-binding protein HuR. *Mol Cell Biol* **36**: 1332–1341. doi:10.1128/MCB.01030-15