

# miRNAs at the interface of cellular stress and disease

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## Abstract

**microRNAs (miRNAs) are a family of small, non-coding RNAs, which provides broad silencing activity of mRNA targets in a sequence-dependent fashion. This review explores the hypothesis that the miRNA machinery is intimately linked with the cellular stress pathway and apparatus. Stress signaling potentially alters the function of the miRNA-bioprocessing core components and decompensates regulation. In addition, dysregulation of miRNA activity renders the cell more prone to stress and emerges as a new pathway for age-related insults and diseases, such as neurodegeneration.**

**Keywords** cellular stress; dicer; disease; microRNAs; stress granules

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## Introduction

### microRNAs

miRNAs are genome-encoded small RNAs that mediate post-transcriptional silencing. Considering hundreds of miRNA genes, each with many dozens of targets, regulation by miRNAs is very broad and met in any cellular activity in health or in disease (Bushati & Cohen, 2008; Bartel, 2009; Carthew & Sontheimer, 2009; Fabian *et al.*, 2010). miRNA precursors are processed in two steps (Fig 1). The initial miRNA transcript (pri-miRNA) is subjected to a nuclear processing by the Drosha–DGCR8 ‘microprocessor’ complex. The resulting intermediate precursor (pre-miRNA) is exported to the cytoplasm and then further identified and cut by Dicer, yielding a 22-nt mature miRNA (Gregory *et al.*, 2005). Partners of Dicer are required for efficient pre-miRNA processing and include Argonaute (AGO), protein kinase interferon-inducible double-stranded RNA-dependent activator (Pact) and TAR RNA-binding protein (Trbp; Chendrimada *et al.*, 2005; Haase *et al.*, 2005; Diederichs & Haber, 2007; Koscianska *et al.*, 2011). Dicer and its co-factors load the mature miRNA onto AGO in the RNA-induced silencing complex (RISC), providing sequence-specific silencing activity.

Argonaute RISC catalytic component 2 (AGO2) has dual functions in the processing of miRNA precursors and in target silencing. First, AGO2 functions as a Dicer co-factor in pre-miRNA processing, as part

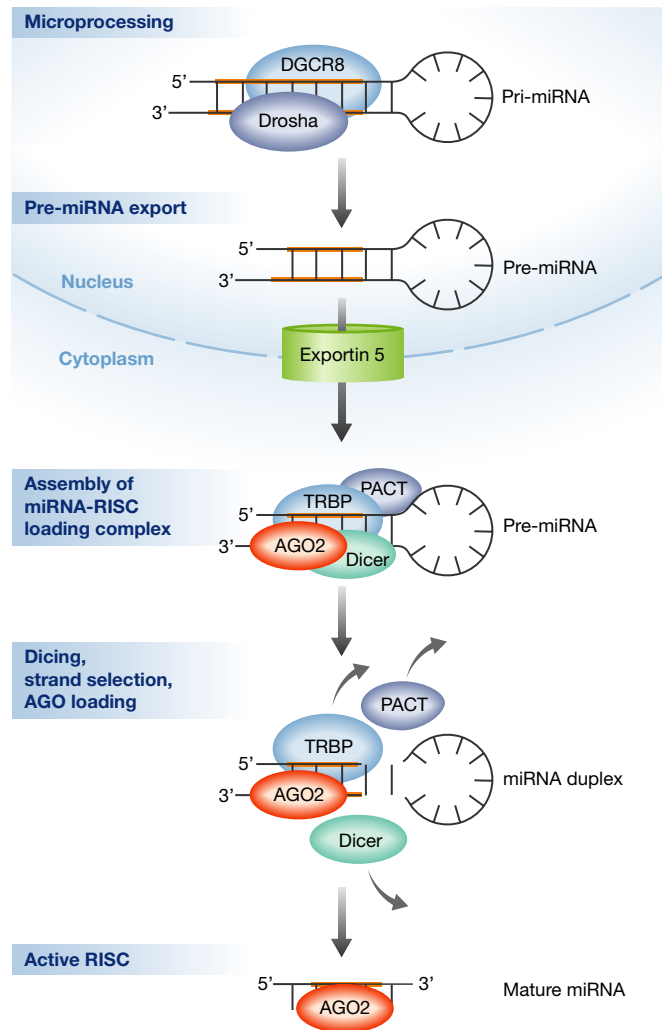
of the RISC-loading complex. Next, AGO2 is loaded with a guide miRNA strand, making an active RISC (Chendrimada *et al.*, 2005; Gregory *et al.*, 2005). Once programmed with a particular miRNA sequence, RISC acts as an effector that facilitates miRNA-dependent mRNA silencing (Fig 1). Therefore, miRNA processing and target-RNA repression are physically and functionally interlinked by sharing many common protein components.

miRNA circuitry buffers unwanted gene expression, and loss of miRNA activity enables drift from normal cellular function (Hornstein & Shomron, 2006; Li *et al.*, 2009; Herranz & Cohen, 2010; Mukherji *et al.*, 2011; Pelaez & Carthew, 2012; Cassidy *et al.*, 2013; Siciliano *et al.*, 2013). In recent years, an intimate link between miRNA activity and the cellular stress response has been uncovered and is the focus of this review. We explore the evidence that stress signaling and the miRNA biosynthesis machinery are interlinked at various levels of activity. miRNAs emerge as critical regulators of the stress response, and dysregulation of miRNA expression or activity renders the cell more prone to stress and to its insults.

### Stress and stress granule formation

Extreme environmental conditions and chemical stressors invoke a cellular cascade, which reduces the damage and conserves integrity. This adaptive response for coping with stress is executed primarily by transient blockade in translation of most cellular mRNAs and directing cellular RNA metabolism toward damage repair (Ron & Walter, 2007). The phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2a) is the canonical signal for blocking translation initiation and promoting polysome disassembly. Accordingly, the phosphorylation of eIF2a is controlled by stress-activated kinases, PKR, HRI, PERK, or GCN2 (Chen *et al.*, 1991; Dever *et al.*, 1992; Carroll *et al.*, 1993; Harding *et al.*, 1999).

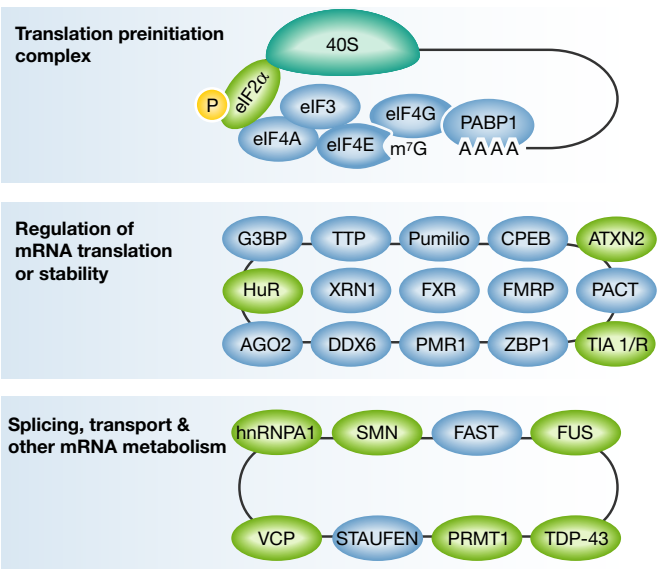
mRNA from disassembling polysomes is often found in cytoplasmic stress granules (SGs), the structural correlate of the stress response (Kedersha *et al.*, 1999; Anderson & Kedersha, 2006; Anderson & Kedersha, 2008; Buchan & Parker, 2009). SGs are composed of stalled translation preinitiation complexes: 40S ribosomal subunits, translation initiation factors (eIFs) and RNA-binding proteins that are involved in other facets of RNA metabolism (Fig 2). Recruitment of proteins into SGs is regulated and in specific conditions is facilitated by so-called ‘piggyback’



**Figure 1. Canonical pathways for miRNA maturation and loading of the RISC.**

After transcription, an initial pri-miRNA transcript is subject to microprocessing by the Drosha/DGCR8 complex. The resultant intermediate precursor, pre-miRNA, is a ~70-nt hairpin that is exported from the nucleus into the cytoplasm in a regulated manner by Exportin 5. Assembly of the Dicer complex/miRNA-RISC-loading complex is required for further processing and for AGO loading. Upon disassembly of the RISC-loading complex, AGO is the chief protein factor of an active RISC, which is programmed with a specific miRNA guide strand.

protein-protein interactions (Anderson & Kedersha, 2008). Several of the reported SG inhabitant RNA-binding proteins are: poly(A)-binding protein (PABP1), GTPase activating protein (SH3 domain) binding protein (G3BP), Tristetraprolin (TTP), Pumilio, cytoplasmic polyadenylation element binding protein (CPEB), Ataxin-2 (ATXN2), ELAV like RNA-binding protein (HuR), 5'-to-3' exonuclease (Xrn1), fragile X mental retardation protein (FMRP) and its autosomal homolog FXR, DEAD box polypeptide 6 (DDX6/RCK), polysomal ribonuclease 1 (PMR1/PXDNL), Zipcode-binding protein 1 (ZBP1), TIA-1 and its homolog TIAR, STAUFEN and Fas-activated serine/threonine kinase (FAST). PACT and AGO2, two RISC-loading complex proteins are also found in SGs (Leung *et al*, 2006; Pare *et al*, 2009; Johnston *et al*, 2010).



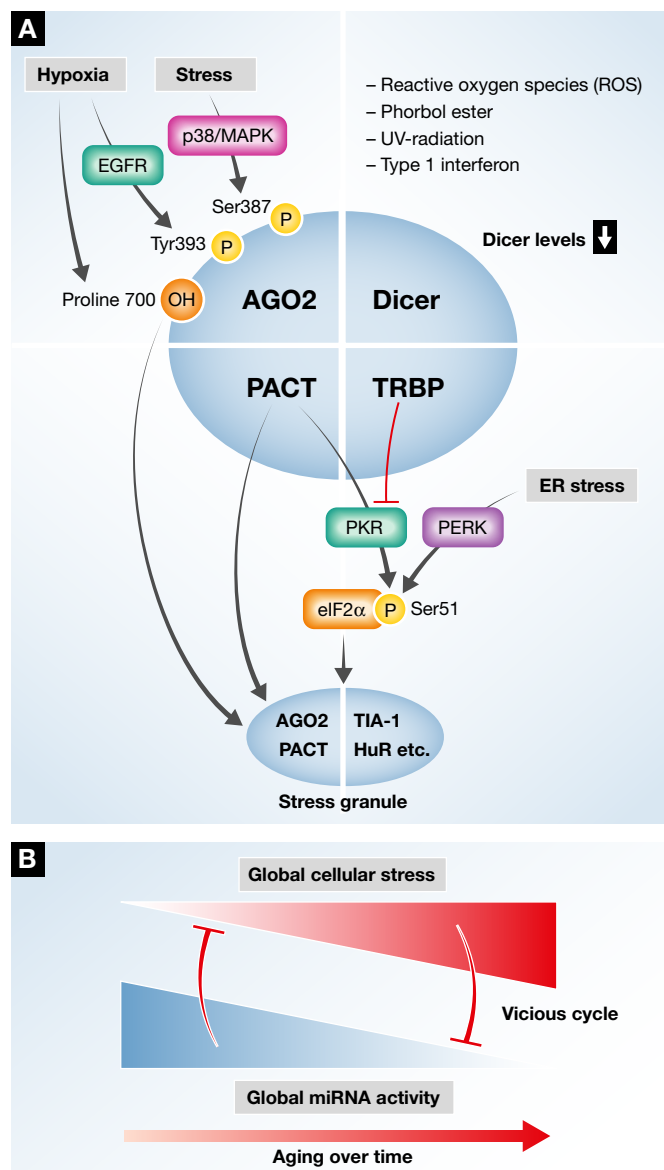
**Figure 2. A diagram of representative components present in stress granules, grouped according to the known protein function.**

The translation preinitiation complex, including the small ribosomal subunit (40S; upper panel); RNA-binding proteins with role in regulation of mRNA translation or stability (middle panel); Splicing and other mRNA metabolism activities (lower panel). For comprehensive reviews see Anderson & Kedersha (2006, 2008), Buchan & Parker (2009) and references therein. Several of the presented RNA-binding proteins, depicted in green, were suggested to be involved in neurodegeneration. Incomplete list of references, linking particular stress granule components to motor neuron diseases include: eIF2 $\alpha$  (Kim *et al*, 2014); HuR (Lu *et al*, 2009); TIA1 (Lu *et al*, 2009); SMN (Hua & Zhou, 2004); TDP-43 (Liu-Yesucevitz *et al*, 2010; McDonald *et al*, 2011); FUS (Bosco *et al*, 2010; Vance *et al*, 2013); hnRNPA1 (Kim *et al*, 2013); ATXN2 (Farg *et al*, 2013); VCP (Johnson *et al*, 2010; Buchan *et al*, 2013); PRMT1 (Tradewell *et al*, 2012; Yamaguchi & Kitajo, 2012).

Several RNA-binding proteins that are identified in SGs are encoded by genes that are mutated in different neurodegenerative states, including TAR DNA-binding protein 43 (TDP-43; Liu-Yesucevitz *et al*, 2010; McDonald *et al*, 2011), fused in sarcoma (FUS; Bosco *et al*, 2010; Vance *et al*, 2013), heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1; Kim *et al*, 2013), valosin containing protein (VCP; Johnson *et al*, 2010; Buchan *et al*, 2013) and SMN (survival motor neuron 1; Hua & Zhou, 2004). Other SG resident proteins were also linked to the molecular pathogenesis of neurodegeneration, including eukaryotic translation initiation factor 2A (eIF2 $\alpha$ ; Kim *et al*, 2014), HuR, TIA1 (Lu *et al*, 2009), ATXN2 (Farg *et al*, 2013) and protein arginine methyltransferase 1 (PRMT1; Tradewell *et al*, 2012; Yamaguchi & Kitajo, 2012). Therefore, SG proteins are intriguingly linked to human neurodegeneration.

## The interconnection between miRNAs and the cellular stress response

**Association between the Dicer complex and the cellular stress response**  
Several lines of evidence reveal links between the Dicer complex and the cellular stress response. Stress may be experimentally induced by many reagents. Stressors, including reactive oxygen species (ROS),



**Figure 3. Interrelations between the miRNA-bioprocessing machinery, stress and stress granules.**

(A) A simplified illustration of the Dicing complex with its documented components: Dicer, AGO2, TRBP and PACT. Stress was suggested to modify AGO2 at a post-translational level: Hypoxia induces Proline 700 hydroxylation, which recruits AGO2 into stress granules. Likewise, EGFR-mediated phosphorylation of Tyrosine 393 leads to impaired miRNA processing of specific pre-miRNAs. Stress-mediated p38/MAPK signaling phosphorylates AGO2 on Serine 387, but the impact of this phosphorylation event on miRNA maturation is not characterized. Several types of cellular stress down-regulate Dicer levels and inhibit miRNA processing. Kinases of eukaryotic translation initiation factor 2 alpha (eIF2a) phosphorylate it on Serine 51, which is a critical step in halting translation and inducing stress granules. eIF2a is phosphorylated by eIF2aK3/PERK as part of the unfolded protein response, in response to endoplasmic reticulum stress (ER stress), or by eIF2aK2/PKR in response to viral double-stranded RNA. For simplicity, eIF2aK1 and eIF2aK4 are not mentioned. eIF2aK2/PKR activity is modulated by PACT and TRBP in a reciprocal manner. When stress granules are formed, both PACT and AGO2 are recruited into them. The interactions between cellular stress and the Dicing complex/miRNA-RISC-loading complex core components point toward a dynamic regulation of Dicing activity. (B) Stress signaling potentially alters the function of the miRNA-bioprocessing core components and leads to decompensated regulation. The consequences may include a vicious cycle, whereby stress impairs miRNA processing which in turn renders cells even more prone to stress.

AGO2 post-translationally as well, via hydroxylation of proline 700 (Qi *et al.*, 2008), which leads to AGO2 SG localization (Wu *et al.*, 2011). Taken together, it appears that various stress signaling cascades control AGO2 activity and contribute to AGO2 translocation into SGs and to attenuation of miRNA processing. However, further mechanistic studies will be required for understanding of the full picture.

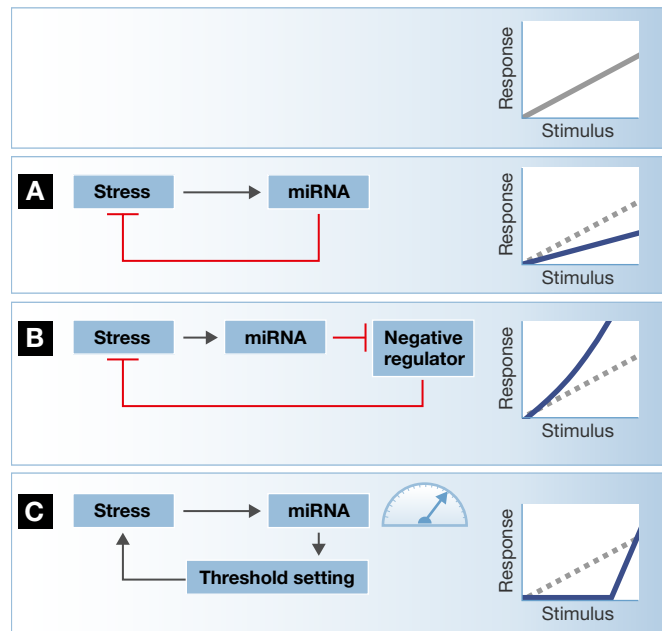
PACT and TRBP are two co-factors of Dicer, which are engaged in the cellular stress response via direct interaction with PKR. PKR is a dsRNA-dependent serine/threonine protein kinase sensor of viral dsRNA that phosphorylates eIF2a on Ser51, thereby causing global reduction of protein synthesis (Taylor *et al.*, 2005; Garcia *et al.*, 2006). PACT and TRBP both contribute to Dicer complex functions but possess distinct, non-redundant, activities (Lee *et al.*, 2013). Intriguingly, they reciprocally modulate PKR, as PACT activates PKR, whereas TRBP inhibits PKR via dsRNA sequestration and direct protein-protein interactions (Park *et al.*, 1994; Cosentino *et al.*, 1995; Patel & Sen, 1998; Lee *et al.*, 2006; Daniels & Gatignol, 2012). The interaction of both TRBP and PACT with PKR links miRNA processing to stress signaling, but it is not known whether or how TRBP and PACT provide crosstalk between these systems.

#### Stress granules and the miRNA machinery are interlinked

SGs recruit proteins, which are involved in miRNA regulation pathways. This further suggests that SGs are integrated with miRNA-induced translational silencing pathways. Indeed, fragile X mental retardation protein (Caudy *et al.*, 2002; Jin *et al.*, 2004; Li *et al.*, 2008) and Pabp1 (Moretti *et al.*, 2012) interact with the miRNA machinery. Furthermore, AGO2 and PACT are recruited into stress granules, a process that depends at least partially on HSP90 activity (Leung *et al.*, 2006; Pare *et al.*, 2009; Johnston *et al.*, 2010). In this context, Leung and Sharp suggested that AGO translocation into SGs may reflect a dynamic change in its activity (Leung *et al.*, 2006). Stress response is extended also to the regulation of RISC activity, by poly-ADP ribosylation and probably other mechanisms. Additional investigations will substantiate initial reports in this field that focus on specific subsets of

phorbol ester, UV radiation or type I interferon, cause a reduction in the expression of Dicer (Wiesen & Tomasi, 2009; Mori *et al.*, 2012). These changes in Dicer levels appear to be particularly relevant for further coping with stress, since loss of Dicer function reduces stress tolerance, whereas Dicer over-expression confers stress resistance, at least in invertebrates (Lim *et al.*, 2011; Mori *et al.*, 2012). Therefore, Dicer activity contributes to stress resistance. In addition, Dicer co-factors AGO, PACT and TRBP participate at multiple levels of stress signaling, as summarized in Fig 3, substantiating the connection between the Dicer/miRNA-RISC loading complex and the stress response.

Stress signaling alters AGO2 function by post-translational modifications, and these are suggested to impact RISC and Dicer activity. Epidermal growth factor receptor (EGFR) signaling phosphorylates tyrosine 393 under hypoxic conditions, which reduces the binding of AGO2 to Dicer and inhibits pre-miRNA processing (Shen *et al.*, 2013). In addition, stress-induced P38/MAPK signaling phosphorylates AGO2 at Serine 387 (Zeng *et al.*, 2008). Hypoxic stress regulates



**Figure 4. Potential network architecture for miRNA influence on stress signals.**

The cellular response to stressful stimuli is modulated by miRNAs. (A) A miRNA may be wired in a negative-feedback loop, inhibiting the expression of a target that is involved in stress signaling. In this case, miRNA activity dampens the cellular stress cascade activity. (B) However, a miRNA may contribute to pathway activation via the inhibition of negative regulators, as part of a positive-feedback loop. (C) The relative levels of miRNAs and their cognate target mRNAs determine how much target protein is effectively produced. Therefore, if the miRNA is in excess, there is effective silencing of the target, which activates the stress cascade with defined threshold. However, either a continuous stress stimulus or a dynamic change in the levels of the miRNA may enable adaptation or sensitization of the cellular stress response. See also reviews by Mendell & Olson (2012) and Leung & Sharp (2007, 2010).

mRNAs (Leung *et al*, 2011; Karginov & Hannon, 2013). In addition, we believe that it will be indeed important to test whether the translocation of AGO2 and/or PACT into SGs impairs the various activities of Dicer and the RISC complex, for example, pre-miRNA processing.

#### *The activity of specific miRNA species is involved in the stress response*

miRNAs contribute in different contexts to modulation of the stress response, by adjusting the levels of key unfolded protein response (UPR) components. For example, miR-214 regulates ATF4 levels (Wang *et al*, 2013), and miR-30c-2\* is upstream of Xbp1 (Byrd *et al*, 2012). In addition, maturation of a few miRNA species is downstream of IRE1, one of the three arms of the cellular stress response. IRE1, commonly known for its activity in splicing Xbp1 in the cytoplasm, terminates specific pre-miRNAs by preventing them from being further processed by Dicer into their mature forms (Upton *et al*, 2012). Therefore, miRNAs fine-tune the expression of components of the UPR signaling cascade and modulate cellular adaptation to stress, which is further reviewed in (Byrd & Brewer, 2013; Chitnis *et al*, 2013; Maurel & Chevet, 2013).

Additional influential reviews (Mendell & Olson, 2012 and Leung & Sharp, 2007, 2010) describe potential network architectures for the influence of miRNAs on stress signals: (i) miRNAs may dampen

a stress signal if wired in a negative feedback loop or (ii) miRNAs may promote pathway activation via the inhibition of negative regulators within a positive-feedback loop. (iii) Finally, miRNAs may generate a threshold in responsiveness, because the relative levels of miRNAs and mRNA targets determine how much target protein is effectively produced (See Fig 4).

This last aspect of miRNA activity warrants further discussion. Long-lived miRNAs generate threshold for activation, which contributes to filtering transient or low-amplitude signals. Dynamic increase in the expression of a miRNA would contribute to a higher threshold and hence to tolerance toward specific stressor agents or to stress pre-conditioning. Examples for buffering may be observed in regulation of MICA and MICB, two stress-induced ligands that mark cells for destruction. The miR-17~92 family chronically represses MICA/MICB mRNAs levels, thereby filtering fluctuations in expression under normal conditions. However, when MICA/MICB transcription is induced at higher levels, to an extent that overcomes the threshold of miRNAs, cell destruction ensues (Stern-Ginossar *et al*, 2008). In another intriguing example, miRNAs encoded by herpes simplex virus 1 continuously repress viral proteins and provide the molecular basis of long-term latency in neurons. In this case, stress enables breakdown of the miRNA-based inhibitory mechanism and allows for reactivation of dormant viruses (Umbach *et al*, 2008).

## miRNA activity and stress signaling in human disease

### *miRNA activity and stress signaling in animal models*

Under favorable laboratory conditions, the loss of miRNA function infrequently exhibits dramatic phenotypes, unless aggravated by stress. Horvitz and co-workers have shown that many *C. elegans* miRNAs are dispensable under normal conditions and even the elimination of whole miRNA gene families was of surprisingly limited impact. However, genetic knockout of specific miRNAs renders the mutant animals incapable of coping with stress (Miska *et al*, 2007; Alvarez-Saavedra & Horvitz, 2010). This conclusion holds true also in mammals. For example, loss of miR-208a activity does not affect cardiovascular function, yet it abrogates stress-responsive cardiac remodeling (van Rooij *et al*, 2007). Likewise, nullification of miR-375, a miRNA highly expressed in the endocrine pancreas, exhibits mild impairments in glucose homeostasis, but these are aggravated by leptin-resistance obesity (Poy *et al*, 2009). These examples suggest that miRNAs will be particularly relevant to investigations of the response of fully developed tissues to stress (Leung & Sharp, 2010). In the same train of thought, it is plausible that miRNA insufficiency will be particularly evident in disease states.

### *Stress is a component of many adult-onset diseases*

Cellular stress is often considered a vector of pathology. Aberrant protein folding and aggregation ignite UPR, which is evident in many chronic diseases including cardiovascular diseases (Minamino *et al*, 2010) and diabetes. Diabetes is a common metabolic disease, whereby insulin supply does not meet the body's demands. The endocrine pancreas is under high demand for insulin synthesis in diabetes. The typical progressive reduction in beta cell mass observed in diabetes requires that the remaining beta cells increase

insulin synthesis, which subjects them to increased UPR stress. Accordingly, modulation of the stress response is considered a potential therapeutic target in diabetes. For example, a chemical chaperone of unfolded proteins, sodium phenylbutyrate, was able to reduce UPR/ER stress and to restore glucose homeostasis in a mouse model of diabetes (Ozcan *et al*, 2006; Ozcan & Tabas, 2012).

Stress is evident also in brain diseases and particularly in neurodegeneration. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the motor system (Al-Chalabi & Hardiman, 2013). Motor neurons in ALS are thought to agonize under stress (Atkin *et al*, 2008), and ER stress components including Perk (Wang *et al*, 2011), Gadd34 (Wang *et al*, 2014), and phosphorylation of eIF2a (Kim *et al*, 2014) were shown to modulate disease progression in a mouse model of ALS. Moreover, Salubrinal, an ER stress-protective agent, attenuates ALS manifestations and delayed progression in similar disease models (Saxena *et al*, 2009). Stress is plausibly playing comparable roles in other neurodegenerative states, including Alzheimer's disease, where deposition of aggregated beta-amyloid triggers ER stress (Lee do *et al*, 2010).

#### RNA-binding proteins and stress granules in neurodegeneration

Accumulation of cytoplasmic granules is a patho-histological hallmark of several neurodegenerative diseases, including ALS. Mutations in the genes encoding for RNA-binding proteins TDP-43 and FUS were recently identified as causative in human ALS (Sreedharan *et al*, 2008; Kwiatkowski *et al*, 2009; Vance *et al*, 2009). These RNA-binding proteins are commonly found within SGs (Bosco *et al*, 2010; Parker *et al*, 2012; Daigle *et al*, 2013). TDP-43 is essential for proper SG assembly, and both TDP-43 and FUS interact with SG core components (McDonald *et al*, 2011; Aulas *et al*, 2012; Vance *et al*, 2013). Additionally, the mutated forms of TDP-43 and FUS both have the propensity to enhance aggregation (Bosco *et al*, 2010; Liu-Yesucevitz *et al*, 2010), and SGs containing TDP-43 or FUS tend to be more persistent, larger, and more abundant (Vance *et al*, 2013; Baron *et al*, 2013). Altogether, ALS-causing mutations in RNA-binding proteins contribute to the emergence of stress granules, which may aggravate other cues for SG formation in neurodegeneration. Importantly, TDP-43 and FUS are involved in miRNA processing. TDP-43 is a co-factor of both Drosha and Dicer, promoting the biogenesis of a subset of miRNAs (Buratti *et al*, 2010; Kawahara & Mieda-Sato, 2012; Li *et al*, 2013), and FUS stimulates biogenesis of a specific subset of miRNAs as well (Morlando *et al*, 2012). Therefore, these specific RNA-binding proteins provide a new intriguing link between miRNA bioprocessing, SGs and neurodegeneration.

Altered SG dynamics occur also in other neurodegenerative diseases. ATXN2 concentration impacts SG assembly by interaction with SG components PABP1 and Dead box helicase 6 (DDX6/RCK) and by regulating the translocation of FUS to the cytoplasm (Nonhoff *et al*, 2007; Farg *et al*, 2013). This may be relevant to the increased risk for ALS observed in ATXN2 intermediate-length polyglutamine expansions (Elden *et al*, 2010). SGs also play a role in Alzheimer's disease (Vanderweyde *et al*, 2012), where SG components TIA-1 and tristetraprolin (TTP) bind phospho-tau. DDX6/RCK and ribosomal protein S6 were observed in degenerating granulo-vacuolar pyramidal neurons (Castellani *et al*, 2011). Altogether, the modulation of stress and SG formation plays a role in several neurodegenerative diseases, suggesting potential molecular commonalities

for these conditions, regardless of the neuronal cell type involved or the particular clinical manifestations.

#### miRNAs in control of aging and age-related diseases

Aging is often characterized by the declined ability to cope with cellular stress. Several sources of internal cellular stress were suggested to impact longevity. Such insults include impaired mitochondrial activity, excessive production of ROS, DNA damage, and excessive accumulation of misfolded proteins (Finkel & Holbrook, 2000; Ishii *et al*, 2002; Gerstbrein *et al*, 2005; Heidler *et al*, 2009; Brown & Naidoo, 2012; Van Raamsdonk & Hekimi, 2012; Taylor & Dillin, 2013).

miRNAs ensure developmental robustness and homeostasis (Hornstein & Shomron, 2006) and reinforce cellular programs also in the adult life (Li *et al*, 2009; Pelaez & Carthew, 2012; Cassidy *et al*, 2013). Therefore, dysregulation of miRNA function may contribute to aging by allowing the activation of aberrant pathways that are repressed by normal miRNA activity in younger individuals (Ibanez-Ventoso & Driscoll, 2009).

One of the most established examples in this emerging field focuses on miR-34, a specific miRNA family with conserved functions in control of aging and age-related diseases. miR-34 is up-regulated in aging nematodes (Kato *et al*, 2011), and miR-34 over-expression extends *Drosophila* life span and reduces the propensity to acquire age-related neurodegeneration (Liu *et al*, 2012). In human neurodegenerative diseases, both up- and down-regulation of miR-34 have been observed. miR-34 is down-regulated in human Parkinson's disease brains (Minones-Moyano *et al*, 2011). In contrast, miR-34c is up-regulated in aging mice, in mouse models for Alzheimer's disease, and in patients. Furthermore, miR-34c over-expression impairs memory, whereas targeting miR-34c rescues memory function in a mouse model for AD-linked amyloid pathology (Zovolis *et al*, 2011). miR-34 is also up-regulated in plasma of patients of Huntington's disease (Gaughwin *et al*, 2011) and in the organ of Corti in a mouse model of age-related hearing loss (Zhang *et al*, 2013). Altogether, it appears that miR-34 is involved in regulation of proper aging and of age-related brain diseases, suggesting potentially complex roles, which may include compensatory mechanisms.

miRNA activity *per se* is also critical in the *C. elegans* life cycle. Dysregulation of many miRNAs was observed in aging *C. elegans* with impact on longevity (Ibanez-Ventoso *et al*, 2006; Kato *et al*, 2011). A series of exciting studies on this topic from the laboratory of Frank Slack are reviewed in Smith-Vikos & Slack (2012) and Inukai & Slack (2013). One of the earliest works in this field showed that lin-4 and its target, lin-14, regulate the insulin growth factor (IGF)/DAF-16 pathway, which is a primary cascade for control of aging in nematodes (Boehm & Slack, 2005). Intriguingly, the expression of other miRNAs is up-regulated in aging animals, which may be a protective, compensatory response. For example, miR-71, miR-238, and miR-246 prolong life span and at least miR-71 functions via the IGF/DAF-16 (de Lencastre *et al*, 2010; Pincus *et al*, 2011; Boulias & Horvitz, 2012).

In addition to the function of individual miRNA genes, a global decrease in miRNA abundance or activity has been observed in aging organisms and suggests that the whole miRNA regulatory network may play a role in aging and late-onset diseases (Ibanez-Ventoso *et al*, 2006; Drummond *et al*, 2011; ElSharawy *et al*, 2012; Inukai *et al*, 2012; Liu *et al*, 2012; Mori *et al*, 2012). In nematodes,

miRNA-binding proteins Argonaute-like 1 (alg-1), Dicer, Drosha, and DGCR8/Pasha regulate aging and control life span (Kato *et al*, 2011; Lehrbach *et al*, 2012; Mori *et al*, 2012). The activity of at least one of these miRNA-binding proteins, Dicer, decreases also in aging mammals; Dicer was found to be globally down-regulated in old mouse tissues in comparison with their younger adult counterparts (Nidadavolu *et al*, 2013).

In another study, Dicer activity was reduced in aging adipocytes of rodents and humans, noting concomitant reduction of miRNA expression (Mori *et al*, 2012). Likewise, Dicer activity is reduced in cerebro-microvascular endothelial cells of aged rats (Ungvari *et al*, 2013) and in age-related macular degeneration in humans (Kaneko *et al*, 2011; Tarallo *et al*, 2012), although the critical role of Dicer in this model was suggested to regulate ALU mobile elements.

Together, these observations suggest a link between age-related Dicer dysfunction and loss of robust cellular function. Reduction in Dicer levels lessens stress tolerance, whereas increased stress further inhibits Dicer activity (Lim *et al*, 2011; Mori *et al*, 2012). Because normal Dicer activity contributes to stress resistance, our proposed model suggests a vicious cycle, in which Dicer activity is progressively reduced as part of the aging process, whereas stress is increasingly built. Over time, cells and organisms are rendered more prone to disease (Fig 3).

## Conclusion

miRNA regulation and various aspects of the cellular stress response are tied together. The data reviewed above suggest the presence of compensatory mechanisms, whereby miRNA functionality takes part in the physiological stress response, which are required for appropriate execution of the stress response and for proper cellular adaptation. However, stress signaling potentially alters the function of the miRNA-bioprocessing core components and leads to decompensated regulation. The consequences may include a potentially vicious cycle, whereby stress in the aging organism impairs miRNA processing, which in turn renders cells even more prone to stress.

Aging is characterized by progressive decline of robustness and stress resistance. miRNAs are used to ensure cellular robustness and homeostasis (Hornstein & Shomron, 2006; Ibanez-Ventoso & Driscoll, 2009; Pelaez & Carthew, 2012; Cassidy *et al*, 2013). Alterations of miRNA expression can occur as a response to stress-related phenomena in a variety of diseases. However, currently, it is not clear whether the dysregulation of a single miRNA or a subset of miRNAs presents the cause or the consequence of an altered cellular phenotype. What pathways may modulate dysregulation of miRNAs in aging and disease? Many research efforts will be directed in coming years towards deciphering the role of miRNAs of various aspects of human disease and to uncovering the potential therapeutic activity of manipulating miRNA expression.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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