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Review

The emerging roles of microRNAs in the molecular responses of metabolic rate depression

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Metabolic rate depression is an important survival strategy for many animal species and a common element of hibernation, torpor, estivation, anoxia and diapause. Studies of the molecular mechanisms that regulate reversible transitions to and from hypometabolic states have identified principles of regulatory control. These control mechanisms are conserved among biologically diverse organisms and include the coordinated reduction of specific groups of key regulatory enzymes or proteins in the cell, a process likely driven by microRNA target repression/degradation. The present review focuses on a growing area of research in hypometabolism and mechanisms involving the rapid and reversible control of translation facilitated by microRNAs. The analysis draws primarily from current research on three animal models: hibernating mammals, anoxic turtles and freeze-tolerant frogs (with selected examples from multiple other sources). Here, we demonstrate a link between metabolic rate depression, a well-documented response to periods of environmental stress, and microRNA expression. Microarray-based expression profiles and PCR-driven studies have revealed that specific microRNAs are induced in response to environmental stress. Selected members of this group decrease pro-apoptotic signaling, reduce muscle wasting and reduce protein translation, whereas other members contribute to cell cycle arrest and mitogen-activated protein kinase signaling. Many of the same microRNAs are frequently deregulated in numerous disease pathologies and, hence, the hypometabolism model could provide a novel approach for the treatment of stroke and heart attack in humans.

Keywords: microRNA, metabolism, metabolic rate depression, protein translation, muscle atrophy, cell cycle, apoptosis

Introduction

The ability of an animal to lower its metabolic rate well below the standard resting rate is clearly a fascinating feat and has been the focus of numerous physiological, molecular and biochemical studies (Storey and Storey, 2007a, b). When confronted with extreme environmental stress that interferes with normal life, many animals escape by depressing their metabolic rate (often by 70%–99%) and entering a state of dormancy or torpor (Guppy and Withers, 1999). In some cases, sufficient metabolic rate depression can be achieved from behavioral or physiological responses allowing the animal to adapt to new conditions. However, when conditions are extreme (e.g. oxygen limitation, cold, freezing, dehydration) animals turn to cellular reorganization to facilitate long-term hypometabolism and survival (Storey and Storey, 2007a, b). The molecular mechanisms of metabolic rate depression include global suppression of energy-expensive cell functions (e.g. protein synthesis, gene transcription, ATP-dependent ion pumps), reprioritization of ATP use by vital cell functions and enhanced expression of multiple preservation mechanisms (e.g. antioxidants, chaperones) that protect and stabilize cellular macromolecules (Storey and Storey, 2005, 2007a, b). Therefore, the types of regulatory mechanisms that are needed for metabolic depression must be broadly applicable to all cells and all types of metabolic processes, readily coordinated as responses to a single extracellular signal, easily induced and readily reversed without wholesale reorganization of the cell (Storey and Storey, 1990). The transition into a hypometabolic state does not appear to involve an extensive array of genes; clearly, this is not the time for large-scale cellular reorganization and creation of new cellular proteins. Entrance into hypometabolism is most likely a process driven by specific wide-scale regulation of mitogen-activated protein kinase signaling and microRNA target repression or degradation.

It has been well established that in response to external stress stimuli, cellular modifications involve transcriptional, translational, post-translational and allosteric regulation (Krutzfeldt and Stoffel, 2006; Safdar et al., 2009). In recent years, however, a new level of rapid and reversible transcriptome regulation, via the action of a special class of microRNA molecules, has emerged into pathway regulation. MicroRNAs are short (18–23 nt), non-coding RNAs that are known to have central roles in regulating the post-transcriptional expression of mRNA transcripts and may play an important role in metabolic control (Humphreys et al., 2005). They are derived from RNA transcripts that fold into imperfect hairpin structures (~70 nt in length) and are processed by the endonucleases, Drosha and Dicer, to form the active microRNA. After processing and formation,

mature microRNA is incorporated into the RNA-induced silencing complex (miRISC), where the microRNA guides the associated RISC proteins to the targeted mRNA strand, annealing to the 3′ untranslated regions and promoting either mRNA degradation or reversible translational repression (Figure 1) (Bartel, 2004; Safdar et al., 2009). To date, more than 900 human microRNA species have been predicted, many of which are conserved throughout evolution (MiRBase Release 15: April 2010). Estimates indicate that microRNAs may regulate up to 20%–30% of the mammalian genome, suggesting that microRNAs may hold widespread control of gene expression (Carthew, 2006). The extent of microRNA-mediated gene regulation is extremely high due to the ability of an individual microRNA to target the transcripts of hundreds of genes, whereas individual mRNAs can be targets of multiple microRNAs, allowing for enormous regulatory potential and significant molecular crosstalk (Maziere and Enright, 2007). The characteristics of microRNA regulation dovetail with the need for metabolic depression to be broadly applicable, readily coordinated, easily induced and readily reversed, and distinct features could suggest microRNA regulation in hypometabolism.

This review begins by summarizing the most relevant biochemical information for metabolic rate depression. Following this, we draw links to microRNA regulation of the aforementioned elements with particular emphasis on key molecular pathways, including mitogen-activated protein kinase (MAPK) signaling, translational arrest, reduced muscle wasting, cell cycle arrest and anti-apoptotic signaling. The analysis draws primarily from current research on three models, mammalian hibernation, anoxia-tolerance by turtles and freeze tolerance in frogs (with selected examples from multiple other sources) and key concepts drawn from the phosphoinositide-3-kinase (PI3-K)/Akt and p53 tumor suppressor pathways. By doing this, we aim to demonstrate a link between metabolic rate depression and microRNA expression.

Stress-responsive microRNAs

Regulation of distinct microRNAs has been well established as part of the typical stress response; to date, thousands of publications have documented their role in a multitude of cell functions. An example of growth in the field can be seen in the increase in articles from 738 in 2006 to 4336 in 2010. An August 2010 search of PubMed for the phrase 'stress+microRNA' produced 343 hits and included research articles on the roles of microRNAs in ischemic injury, cardiac hypertrophy, muscle atrophy, neurodegeneration, cancer, osmotic responses, cellular proliferation and apoptosis.

Studies are now beginning to show that microRNA expression patterns differ under hypometabolic conditions. The first study of this nature examined the expression of nine microRNA species in tissues from euthermic control and hibernating 13 lined ground squirrels (*Spermophilus tridecemlineatus*) (Morin et al., 2008). The study identified several microRNAs that were differentially expressed in kidney, skeletal muscle and heart, as well as elevated amounts of the microRNA-processing enzyme, Dicer, during torpor. Similarly, a complementary study of two microRNAs (miR-16 and -21) found differential regulation in response to the freezing of body water in the wood frog (*Rana sylvatica*) (Biggar et al., 2009). Although this area of research is only beginning, it provides an indication that microRNAs may play a role in achieving a hypometabolic state among stress-tolerant animals.

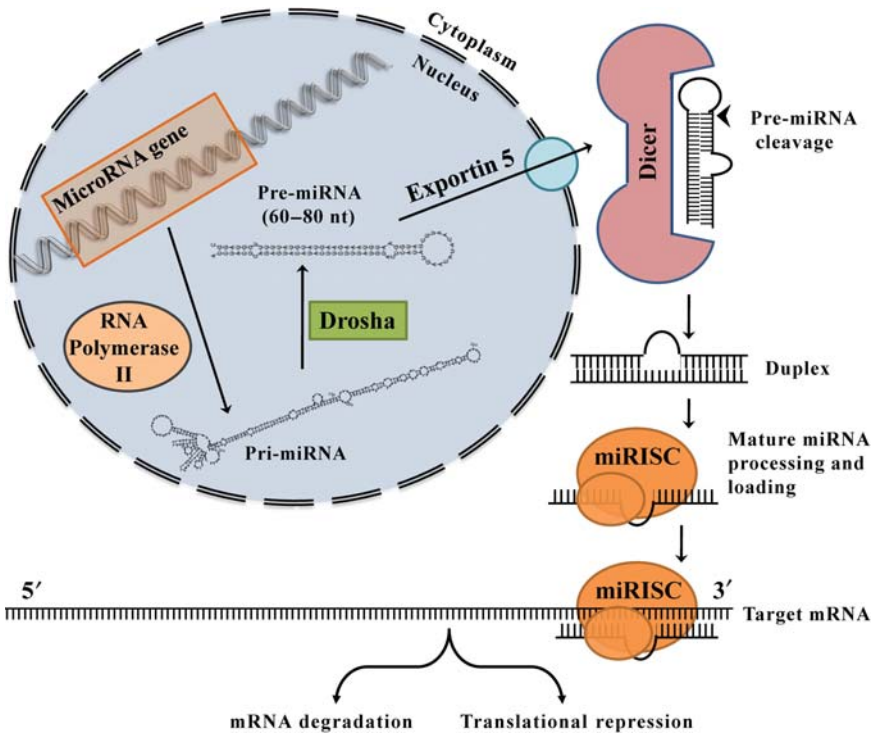

Figure 1 MicroRNA biogenesis. Primary transcripts are transcribed by RNA polymerase II and excised by a series of riboendonucleases into single-stranded mature microRNA structures.

MicroRNA influence on signaling pathways

It has been well documented that MAPK pathways are key in regulating stress responses and transducing extracellular signals to cytoplasmic and nuclear effectors (Seger and Krebs, 1995; Obata et al., 2000; Cowan and Storey, 2003). The MAPK superfamily consists of three main protein kinase families: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase and p38 (Cowan and Storey, 2003). Each of these kinases plays a major role in the regulation of gene expression and intracellular ATP metabolism (Cowan and Storey, 2003; Storey and Storey, 2004). MAPK cascades detect, amplify and integrate diverse external signals to generate responses such as changes in protein activity, gene expression and may provide a conduit for a rapid response in stress-responsive microRNA expression. Indeed, a recent study found that expression of miR-221 and miR-222 were induced by nerve growth factor stimulation and that this induction was dependent on sustained ERK1/2 activation (Terasawa et al., 2009). A similar study, further examining the role of MAPK influence on microRNA expression, showed that the ERK pathway was also capable of enhancing the stability of the microRNA-processing complex (Paroo et al., 2009). Activation of ERK signaling activated the trans-activation-responsive RNA-binding protein (TRBP), a protein responsible for recruiting the Dicer complex to Argonaute 2 for microRNA processing. Additionally, these effects were accompanied by an increase in the expression of growth-promoting microRNAs and reduced expression of let-7 tumor suppressor microRNA, resulting in a pro-growth microRNA profile (Paroo et al., 2009). These studies provide an indication that MAPK/ERK signaling could yield regulatory effects on microRNA signaling pathways, both through biogenesis as well as microRNA-influenced biological responses.

Apart from the MAPK activation of stress-responsive microRNAs, basal microRNA expression could yield significant control over MAPK pathways and their subsequent activation (Cui et al., 2006; Hagen and Lai, 2008; Inui et al., 2010). Both strength and direction of signaling networks are dictated, in part, by protein abundance (Cui et al., 2006). This characteristic makes signaling networks ideal candidates for microRNA-mediated regulation, providing the necessary means of cellular reprioritization during metabolic rate depression. It has been proposed that the regulatory potential of microRNAs may enable widespread remodeling of the signaling landscape, by targeting signal transducers either through positive or negative influences, thereby, regulating the expression of downstream effectors in a rapid manner (Inui et al., 2010). Typically, non-microRNA control over signaling pathways has been shown to involve control at the transcriptional level, through the switch between repression and activation of response elements on target genes (Inui et al., 2010). It would seem unreasonable to assume that cells would regulate important signaling pathways through such simple mechanisms. It has also been proposed that, by repressing the positive regulators of signaling cascades, microRNAs could act as crucial signal mediators by targeting key signal transducers, raising the threshold and ultimately preventing unintended activation (Inui et al., 2010). For example, in response to DNA damage, signaling networks activate the p53 tumour suppressor protein, which leads to the transcription and translation of downstream effectors capable of triggering both cell cycle arrest and apoptosis (Yu and Zhang, 2005; Inui et al., 2010). Studies have shown that miR-125b targets the 3′ UTR of p53 and is essential for complete repression of its translation (Le et al., 2009; Zhang et al., 2009). Incidentally, miR-125b is itself downregulated after DNA damage, resulting in a lower p53 activation threshold and is sufficient to allow the translation of p53 and induce p53-dependent apoptosis and cell cycle arrest, two pathways critical to the typical DNA damage response (Le et al., 2009; Zhang et al., 2009). This outlines an interesting relationship where microRNA (miR-125b) increases the activation threshold of p53 while, correspondingly decreases the activation threshold during periods of cellular stress.

Another major pathway regulating hypometabolism is the PI3-K/Akt pathway, which can initiate survival responses throughout prolonged periods of environmental stress by regulating critical cellular processes such as cell survival, cell cycle, glucose metabolism and protein translation (Eddy and Storey, 2003; Abnous et al., 2008). The phosphorylation state of Akt is the key point of regulation, determining PI3-K/Akt pathway activity or inactivity (Manning and Cantley, 2007). Protein phosphatases (primarily the phosphatase PTEN) act to suppress this signal by removing phosphate groups from members of the pathway (Manning and Cantley, 2007). Numerous microRNAs have been experimentally shown to act as positive and negative regulators of the PI3-K/Akt-signaling pathway (Yamanaka et al., 2009; Small et al., 2010). The miR-29 family negatively affects the pathway through repression of PI3-K regulatory subunits while other microRNAs, including miR-486, positively influence PI3-K/Akt signaling by targeting PTEN translation for inhibition (Small et al., 2010). PTEN mRNA is a strongly predicted target of miR-486 and recent studies have demonstrated that inhibition of miR-486 expression enhances the expression of PTEN and dampens PI3-K/Akt signaling (Small et al., 2010). Although these studies did not evaluate microRNA regulation in a hypometabolic system, microRNAs may act as a mechanism to dampen PI3-K/Akt signaling during metabolic rate depression to control downstream processes such as suppression of protein translation.

Suppression of protein synthesis

The process of protein translation accounts for up to 30%–40% of ATP turnover in some cell types and not surprisingly, the suppression of this process has been widely documented in hypometabolic systems (Storey and Storey, 2007a, b). A 65%–90% reduction in the rate of protein translation has been documented for the brine shrimp (*Artemia franciscana*), intertidal snails (*Littorina littorea*), anoxic turtles (*Trachemys scripta*) as well as hibernating mammals (Bocharova et al., 1992; Fraser et al., 2001; Larade and Storey, 2002; van Breukelen and Martin, 2002; Osborne et al., 2004; Storey and Storey, 2007a, b). In support of the global reduction in protein translation, regulatory controls on ribosomal proteins, such as eukaryotic initiation factor 2 (eIF2) occur during oxygen deprivation (anoxia) in intertidal snails (Larade and Storey, 2002). Control is also applied via differential regulation of gene expression and mRNA translation during hibernation. For example, a study of the translational state of the small mammalian hibernator,

S. tridecemlineatus, indicated that overall suppression of protein synthesis occurs during hibernation, with only a few select transcripts continuing to be translated (Hittel and Storey, 2002).

As a major signaling protein kinase, Akt is involved in numerous cell processes including cellular proliferation, cell survival and glucose metabolism, and is also involved in translation through the regulation of the eukaryotic initiation factor 4E (eIF4E) (Eddy and Storey, 2003; Zhou et al., 2005; Abnous et al., 2008). This initiation factor plays a critical role in recognizing the 7-methyl-GTP cap structure at the 5′ end of eukaryotic transcripts and acts to recruit various other initiation factors, such as eIF4G, eIF4A and eIF3 that are required for ribosome binding (Lasko, 2003). Downstream activation of mammalian target of rapamycin (mTOR) by PI3-K/Akt signaling leads to 4E-binding protein 1 (4E-BP1) phosphorylation and release from its inhibitory interaction with eIF4E, thereby releasing eIF4E for the initiation of ribosomal biogenesis and protein translation (Zhou et al., 2005). The indirect activation of eIF4E presents the critical link between Akt and the translational state. As mentioned, the control of protein synthesis is generally facilitated by changes in the phosphorylation state of the initiation factors (eIF4E) or the regulators (4E-BP) that interact with them (Lasko, 2003). A recent study examining the effect of protein synthesis for several thousand proteins (in response to microRNA transfection and microRNA knockdown) showed for the first time that changes in a single microRNA can directly decrease the production of hundreds of proteins (Selbach et al., 2008). It would therefore be expected that even moderate changes in microRNA expression would yield widespread effects similar to that seen in studies documenting translational repression during hypometabolism, facilitated by a combination of mRNA repression and degradation (Fuery et al., 1998; Smith et al., 1999; Guppy et al., 2000; Fraser et al., 2001; Larade and Storey, 2002; Guo et al., 2010). Given the nature of microRNA targeting and the translational impact of a single microRNA, small changes in microRNA expression could facilitate rapid translational repression (11%–16%) and/or degradation (84%–89%) of numerous target genes (Guo et al., 2010). The extent to which mRNA-microRNA pairing occurs may allow the expression of key genes necessary for survival. What remains to be discovered is the role of microRNA-mediated repression in regulating the global translational process facilitated through signaling pathways, such as the previously described PI3-K/Akt.

Suppression of muscle wasting

Skeletal muscle is highly organized and shows a remarkable ability to modify physiological parameters based on extrinsic demands (Hoppeler and Desplanches, 1992; Fluck, 2006). This requires a high level of integration between neuromuscular signaling, cellular morphology, contractile factors and intracellular organelles (Capetanaki et al., 2007). The myocyte enhancer factor-2 (MEF2) family of transcription factors are vital regulators of muscle plasticity and can alter the expression of a wide range of sarcomeric proteins (e.g. troponin, tropomyosin, myomesin, titin), intermediate filaments (e.g. desmin, vimentin), myofibrillar proteins (e.g. myosin heavy/light chain isoforms), structural proteins vital to signaling cascades (e.g. integrins, dystrophin, utrophin), proteins that improve aerobic capacity (e.g. glucose transporters, myoglobin, creatine kinase), ubiquitin ligases and proteases (e.g. muscle ring finger 1, muscle atrophy F-box, calpain-3) and extracellular matrix proteins (e.g. fibronectin, decorin), amongst others (Black and Olson, 1998; Bassel-Duby and Olson, 2006). Another key muscle transcription factor is myogenic differentiation (MyoD) factor that is a member of the basic helix-loop-helix (bHLH) family (Berkes and Tapscott, 2005). MEF2 and MyoD interact directly to regulate many myosin genes (Black and Olson, 1998); further, MEF2 and MyoD control the upregulation of myosin heavy chain isoforms in type I muscle fibers (Glass, 2003). Typically, this pathway is inactivated during muscle disuse, leading to loss of oxidative capacity, reduction in slow type 1 myosin heavy chain expression and, ultimately, muscle atrophy (Bassel-Duby and Olson, 2006).

Recent studies have identified a cluster of muscle-specific microRNAs that are encoded by distinct transcripts or are nestled within introns of myosin genes (van Rooij et al., 2008). In particular, miR-1, -29b, -23a, -133 and -206 have been proposed to play an important role in regulating the expression of myosin genes and key muscle signaling pathways, such as nuclear factor-kappa B and the aforementioned PI3-K/Akt, by fine-tuning gene expression patterns (Chen et al., 2005; Safdar et al., 2009; Chhabra et al., 2010). Furthermore, it has been suggested that these microRNAs may play regulatory roles in muscle atrophy (Figure 2). It has been suggested that increased miR-23a expression has also been suggested to provide protection to myocytes from atrophy. This protection is facilitated by the suppression of muscle atrophy F-box (MAFbx) translation by binding to the 3′ UTR of the mRNA (Chhabra et al., 2010). In addition to the previously mentioned microRNAs, miR-128 has been theoretically determined (using the web-based program TargetScan 4.0) to target the MyoD family inhibitor, a transcription factor that negatively regulates the transcriptional activity of other myogenic family proteins (e.g. myogenin, Mrf, MyoD) (Lewis et al., 2005). In a similar bioinformatic approach, miR-15a may reduce the atrophic effects of myostatin by reducing translation of its membrane receptor, activin receptor type-2B, thereby stopping the growth arrest effects facilitated by myostatin signaling (Lewis et al., 2005).

Not surprisingly, microRNAs are also important in regulating muscle growth and differentiation (Chen et al., 2005). For example, miR-1, -206 and -133 are expressed primarily in skeletal and cardiac muscle and are transcriptionally regulated by myogenic differentiation factors (Chen et al., 2005). miR-1 and -206 overexpression in skeletal myoblasts promotes skeletal muscle differentiation and mediates MyoD-dependent inhibition of utrophin genes in myoblasts (Chen et al., 2005; Rosenberg et al., 2006; Chhabra et al., 2010). The major myogenic transcription factors, myogenin and MyoD, are also known to control production of certain microRNAs, providing greater feed forward control under atrophy conditions (Safdar et al., 2009). These microRNAs including miR-1, -206 and -133 provide a mechanism for atrophy protection in myocytes (Safdar et al., 2009). miR-181 is also thought to play an important role as a positive regulator of skeletal muscle remodeling and maintenance through the repression of MyoD inhibitor, Hox-A11, thereby implicating a role in atrophy resistance (Safdar et al., 2009).

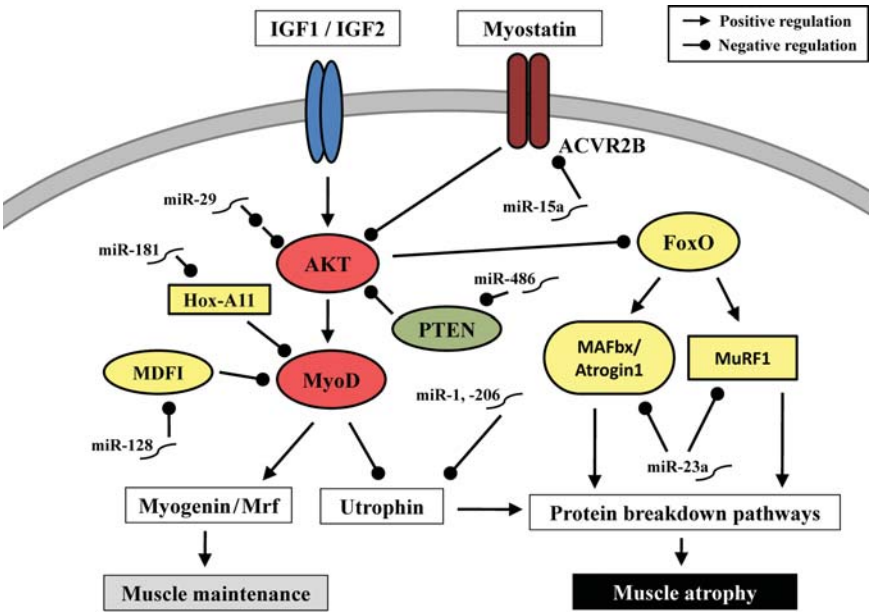

Figure 2 MicroRNA-mediated regulation of muscle remodeling and muscle atrophy. MicroRNAs may facilitate the expression of muscle remodeling proteins such as, MyoD and myogenin. Select microRNAs may also be acting to inhibit positive regulators of protein breakdown and muscle atrophy such as, MAFbx and MuRF1.

During periods of hypometabolism, stress-adapted animal models do not undergo muscle disuse atrophy (McDonagh et al., 2004; Lee et al., 2008; Tessier and Storey, 2010). These animals can spend days or weeks in a hypometabolic state without using their skeletal muscles, but show little or no sign of atrophy. For this reason, these animals prove to be valuable models in atrophy studies. Researchers have marveled at this ability and several studies have investigated aspects of atrophy resistance in hibernating rodents and anoxia tolerant turtles (McDonagh et al., 2004; Lee et al., 2008). Initial studies show that one aspect of atrophy resistance in hibernation may be differential expression of microRNA species. Targeted studies of key microRNAs known to regulate major myofibrillar proteins, signaling pathways and influence atrophy have been recently been conducted with skeletal muscle from hibernating bats (*Myotis lucifugus*) (K.K. Biggar, unpublished data). These include microRNAs involved with the PI3-K/Akt insulin pathway and the recently identified ubiquitin ligases muscle RING finger 1 (MuRF1) and MAFbx proteins. Expression levels of miR-1, -29b, -181b and -23a were elevated significantly in torpid bats. These results suggest that selected microRNAs may be acting to repress pro-atrophy genes (such as Hox-A11, PI3-K/Akt, MuRF1, MAFbx and utrophin) and favor the maintenance of muscle during hibernation (Chen et al., 2005; Safdar et al., 2009; Chhabra et al., 2010). MicroRNAs could play integral roles in torpor. With the metabolic rate depression model, future research will be able to identify the microRNA species that are linked with atrophy resistance in natural animal models.

Suppression of cellular proliferation

The extreme energy limitation imposed by metabolic rate depression should influence the energy-expensive biosynthetic process of cellular proliferation, although a few important developmental features, such as gonad development, may continue during certain hypometabolic states (Storey and Storey, 2007a, b; Biggar and Storey, 2009). The demanding energetics of mitosis provides an intriguing suggestion that the cell cycle may arrest in proliferating tissues, thereby contributing to overall energy savings. What is more, this attenuation may be implemented through conserved mechanisms of DNA damage or hypoxic response pathways, previously shown to respond to environmental stresses (Kaufmann and Paules, 1996). Indeed, studies with other systems of facultative metabolic arrest, from nematode diapause to mammalian hibernation, show induction of cell cycle arrest in hypometabolic states (Kolaeva et al., 1980; Kruman et al., 1986; Fukuyama et al., 2006). Arrest of cellular proliferation should be crucial for ATP homeostasis, since aberrant activation of the cell cycle would lead to rapid depletion of energy stores.

Fine scale regulation of the cell cycle helps maintain a timely and coordinated progression, as well as genetic stability (Pickering et al., 2009). While earlier in this review, we eluded to the microRNA regulation of p53, microRNAs regulated by p53 have been shown to increase expression levels during cell cycle arrest (Tarasov et al., 2007). The experimentally validated p53-targeted microRNAs include miR-34a and -15a/16 in addition to members of the miR-17-92 cluster (miR-20a, -17-5p, 18a and -19a) (Chang et al., 2007; Tarasov et al., 2007). Of these microRNAs, miR-34a has been shown to target key genes involved in cellular proliferation including cyclin D1, c-Myc and cyclin-dependant kinase 6 (Sun et al., 2008; Li et al., 2009). Additionally, expression of miR-15a/16 has been directly linked with cell growth and regulation of the cell cycle (Linsley et al., 2007). These two microRNA species act to repress the cell cycle by targeting a wide range of essential cell cycle-dependent targets that are found primarily within the first gap phase and

include cyclin D1, cyclin E, cdc25a, checkpoint kinase 1 and E2F1 (Kaddar et al., 2009). A recent study, focusing on microRNAs in cell cycle progression, found that cells transfected to express high levels of miR-15a/16 showed an increase in the number of cells that were quiescent with a corresponding reduction in the numbers of cells in S, G_2 and M phases (Linsley et al., 2007). Results from these experiments indicated that elevated miR-15a/16 expression alone had the ability to arrest cells and promote entry into quiescence and may play critical roles in regulating the typical stress response. Hypometabolic models appear to concur. A recent study examining the microRNA response to metabolic rate depression in freezing wood frogs (*Rana sylvatica*) showed that miR-16 levels increased in liver of frogs frozen for 24 h (Biggar et al., 2009). Elevated levels of miR-16 transcripts may act to inhibit hepatocytes from continued proliferation during freezing. This may assist cells in entering a quiescent state where energy use is reserved in order to sustain the basal needs for cellular survival. Ongoing studies in our laboratory have shown that key proteins involved in proliferation (namely cyclin D1, cyclin E, cdc25a and E2F1) decrease in wood frog tissues upon freezing stress (R. Roufayel, unpublished data). Similarly, a recent study suggested that cold stress altered miR-125b expression and may play a role in p53-mediated cell cycle regulation (Dresios et al., 2005). Although this study only presents a chill stress of 32°C (the severe temperature extremes that many other organisms endure often drop from 37°C to 4°C), it does provide another example of microRNA response to environmental stress.

Suppression of apoptosis

Normally influenced by the state of cellular proliferation, apoptosis is a process by which cells are destroyed in a regulated manner and often occurs as a response to extreme or prolonged stress in intolerant organisms (Lant and Storey, 2010). The primary mediators of apoptosis are cysteine proteases of the 'caspase' family (Rao et al., 2004). These mediators trigger activation of a host of proteins such as endoribonucleases and proteases (Rao et al., 2004). Apoptosis can be signaled by a number of different stress conditions such as DNA damage, oxygen/energy debt or starvation; most stresses initiate a conserved response in targeting mitochondrial integrity (Raha and Robinson, 2001). While we tend to focus on the well-defined characteristics of the Bcl-2 family as a means of apoptosis suppression, interactions between both pro (Bax, Bak, Bim) and anti (Bcl-2, Bcl-xl, Bcl-3) apoptotic members maintain the balance between survival and apoptosis (Lant and Storey, 2010). MicroRNAs are also likely to exert an influence on apoptosis activation (Chan et al., 2005; Lu et al., 2008; Wang and Lee, 2009).

Earlier in the review, we introduced the interaction between microRNAs and p53. The p53 signaling pathway limits cellular proliferation by inducing cell cycle arrest and is capable of stimulating apoptosis in response to cellular stresses such as DNA damage and hypoxia (Sax and El-Deiry, 2003; Hammond and Giaccia, 2006). Many apoptosis-related genes that are transcriptionally regulated by p53 have been identified and include the pro-apoptotic factors, APAF-1, PUMA, Noxa, Bax (Moroni et al., 2001; Robles et al., 2001; Sax and El-Deiry, 2003; Shibue et al., 2003; Yu and Zhang, 2005). In response to activation, p53 mediates apoptosis through a linear pathway involving Bax transactivation (Haupt et al., 2003). This is followed by the release of cytochrome c from mitochondria and caspase-9 activation and by the activation of caspase-3, -6 and -7 (Figure 3) (Robles et al., 2001; Haupt et al., 2003).

MicroRNAs have been shown to play integral roles in regulating both pro- and anti-apoptotic pathways (Lynam-Lennon et al., 2009). Studies in glioblastoma or tumor progression were amongst the first to identify the anti-apopotic role of miR-21 (Chan et al., 2005). Key mRNA transcripts targeted by miR-21 include caspase-3, APAF-1 and programmed cell death protein-4 (PDCD4), indicating that miR-21 could have a clear anti-apoptotic role under certain conditions (Chan et al., 2005; Frankel, et al., 2007; Krichevsky and Gabriely, 2009). Additionally, knockdown studies of p53 and miR-21 have suggested a fundamental link between miR-21 and the apoptotic p53 pathway, further indicating a possible role of miR-21 in inhibiting apoptosis (Lu et al., 2008). Once again, parallels can be discussed with hypometabolic models, with upregulated miR-21 identified as a key response to metabolic rate depression. Studies with wood frogs showed that miR-21 transcript levels were significantly elevated in liver and muscle from frozen frogs (Biggar et al., 2009). A similar study, looking at torpid ground squirrels showed an increase of miR-21 in kidney, suggesting that elevated miR-21 may be a conserved mechanism of stress response (Morin et al., 2008). Indeed, inhibition of apoptosis is an important component of long-term metabolic rate depression, a state in which cellular replacement processes must be minimized to facilitate long-term survival. Enhanced levels of key microRNAs (including miR-15a, -16 and -21) may act to arrest proliferation during hypometabolism without triggering the typical apoptotic response. This would

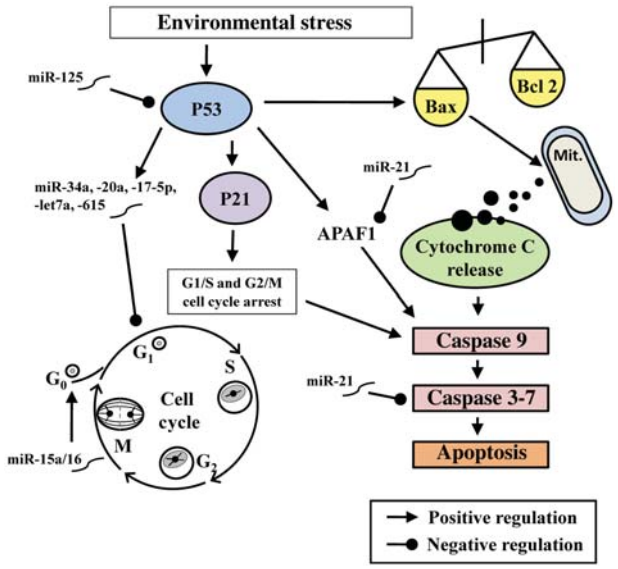

Figure 3 MicroRNA-mediated regulation of both the cell cycle arrest and apoptosis activation pathways. Focusing on p53 regulation, activation of the p53 pathway arrests the cell cycle while stimulating apoptosis, a pathway that should be reduced in an effort to reduce ATP expenditure. Key microRNAs may act to inhibit apoptosis, while allowing for p53-mediated cell cycle arrest.

confer survival while maintaining energetic homeostasis under energy limited conditions.

Concluding remarks

While this review attempts to summarize the current state of microRNA research, and draw parallels to their potential role in metabolic rate depression, the field is still expanding rapidly. The primary challenge is that the diversity and abundance of microRNA targets offer enormous combinatorial possibilities and suggest that microRNAs and their targets form a distinctly complex regulatory network in combination with signal transduction networks. Evidence gathered to date indicates microRNAs in the stress response but it is unclear how and if microRNAs impose their regulation of cellular signaling networks and whether these microRNA-regulated networks might contribute to the biological functions. Furthermore, the abundance of signal transducer proteins in a network can influence both the direction and strength of signaling (Cui et al., 2006). As microRNAs are known to play roles in altering protein amount, it has been proposed that microRNAs could hold critical roles in the regulation of cellular signaling. Important to stress survival, microRNAs offer fine-tuned regulation of gene expression, remodeling the signaling landscape to facilitate the expression of beneficial attributes and target harmful attributes for repression.

MicroRNAs may also provide a mechanism for the expression of critical genes immediately after stress removal by sequestering selected transcripts into stress granules or P-bodies known to store microRNA-targeted mRNAs during stress (Zhao and Liu, 2009). It is known that the number of P-bodies increases under a number of environmental stress conditions. These include nutrient deprivation and osmotic stress (Jud et al., 2008). This provides a useful basis for microRNAs to establish rapid biological controls, which regulate metabolic rate depression during periods of environmental stress. By temporarily storing mRNA transcripts in stress granules or P-bodies, microRNAs provide a mechanism by which hypometabolic animals can rapidly emerge from a suspended condition and reinstate normal cellular activity. However, much more remains to be determined, and many key areas are still largely unexplored in hypometabolic systems, including the expression of many key microRNAs, validation of microRNA targeting and the elucidation of microRNAs as key regulators in metabolic rate suppression.

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