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RNA Regulation of Lipotoxicity and Metabolic Stress

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Noncoding RNAs are an emerging class of nonpeptide regulators of metabolism. Metabolic diseases and the altered metabolic environment induce marked changes in levels of microRNAs and long noncoding RNAs. Furthermore, recent studies indicate that a growing number of microRNAs and long noncoding RNAs serve as critical mediators of adaptive and maladaptive responses through their effects on gene expression. The metabolic environment also has a profound impact on the functions of classes of noncoding RNAs that have been thought primarily to subserve housekeeping functions in cells—ribosomal RNAs, transfer RNAs, and small nucleolar RNAs. Evidence is accumulating that these RNAs are also components of an integrated cellular response to the metabolic milieu. This Perspective discusses the different classes of noncoding RNAs and their contributions to the pathogenesis of metabolic stress.

Much of the morbidity and mortality in diabetes relates to complications that result from the underlying metabolic alterations in this disease. In type 2 diabetes, hyperlipidemia, as well as hyperglycemia, have been implicated as triggers for complications that impact the heart, liver, kidney, and the endothelium of blood vessels of many tissues including the eye (1–4). Furthermore, these metabolic stressors have been implicated in the decline in β -cell function that contributes to progressive insulin insufficiency and eventual requirement for insulin therapy in type 2 diabetes (5).

High levels of circulating glucose, fatty acids, and triglycerides result in delivery of quantities of substrates that exceed the ability of tissues to safely metabolize or store these molecules. Glucotoxicity, lipotoxicity, and glucolipotoxicity engage endoplasmic reticulum stress and oxidative stress pathways that cause organ dysfunction and, in some cases, cell death (6). Protein-mediated signaling

clearly plays important roles in these metabolic stress responses, and metabolic stress-induced changes in gene expression have been described in many cell types and physiological contexts. With the advent of high-throughput RNA sequencing technologies over the past 15 years, there is a growing appreciation of the functional role of noncoding RNAs in physiological and pathological processes. This review will focus on noncoding RNAs that play key roles in directing cell and tissue responses to lipotoxicity and glucotoxicity.

microRNA

Since their initial discovery in the mid-1990s, microRNAs (miRNAs) have come to be recognized as a ubiquitous class of noncoding RNA modulators of mammalian physiological responses that act through posttranscriptional regulation of gene expression. Primary miRNA molecules are generated by RNA polymerase II from independent transcriptional units or from mirtrons embedded within the introns of protein coding genes, and they are processed by the enzymes Drosha and Dicer to generate miRNAs of 19–23 nucleotides in length (7). These mature miRNAs are loaded onto Argonaute proteins to form the functional RNA-induced silencing complex that targets complementary sites within mRNAs, leading to degradation of the mRNA in most cases or inhibition of translation in rare instances (8).

Observations that levels of some miRNAs are regulated by lipotoxic and glucotoxic conditions have implicated these noncoding RNAs in the pathogenesis of diabetes complications. Microarray analyses have revealed that in the MIN6 pancreatic β -cell line, more than half of the 108 detectable miRNAs are glucose regulated (9), whereas relatively fewer miRNAs are regulated by prolonged exposure to lipids (10). However, only a subset of these glucose- and lipid-regulated miRNAs have been demonstrated to function in the pathophysiological response to metabolic stress (Fig. 1

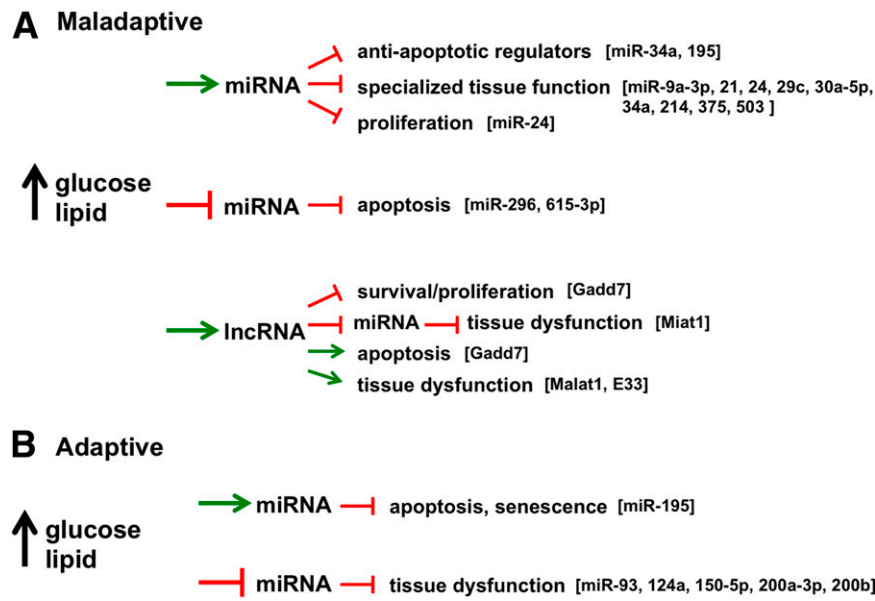


Figure 1—Noncoding RNA mediators of metabolic stress that impact gene expression. Hyperglycemic and hyperlipidemic conditions induce changes in miRNAs and lncRNAs that serve maladaptive (A) and adaptive (B) cellular roles in the response to metabolic stress. Broad classes of downstream effectors are highlighted. Specific noncoding RNAs described in the text are shown in brackets.

and Table 1). Abundance of miRNAs can be regulated at the level of transcription, processing, and/or degradation. While the molecular details of transcriptional and processing steps of many miRNAs are well understood, relatively less is

known about how these small RNAs are degraded. For most metabolic stress-regulated miRNAs, future studies will be required to determine the mechanisms that lead to altered abundance of the miRNA.

Table 1—miRNAs that function in metabolic stress

miRNA	Context	Target	Function	Change in metabolic stress
Maladaptive				
miR-34a	MIN6 β-cells	Bcl2, VAMP2	Promotes lipotoxic cell death	Induced by palm
miR-146	MIN6 β-cells		Promotes lipotoxic cell death	Induced by palm
miR-195	Cardiomyocytes	Bcl2, sirtuin1	Promotes lipotoxic cell death	Induced by palm
miR-296	Hepatocytes	PUMA	Inhibits apoptosis	Decreased by palm
miR-615-3p	Hepatocytes	CHOP	Inhibits apoptosis	Decreased by palm
miR-24	Islets, MIN6 β-cells	Hnf1a, Neurod1	Inhibits proliferation, insulin secretion	Induced by palm and HFD
miR-375	Islets, β-cells	Pdpk1	Dampens phosphatidylinositol 3-kinase signaling	Induced by high glucose
miR-30a-5p	Islets, β-cells	Beta2/NeuroD	Decreases transcription of Ins, Sur1	Induced by high glucose
miR-214	Monocytes	Pten	Prolongs inflammation	Induced by AGEs
miR-21	Renal mesangial cells	Pten	Increases AKT and TORC1 activation	Induced by high glucose
miR-9a-3p	Vascular SMCs	Sur2b	Compromises K _{ATP} channel function	Induced by methylglyoxal
miR-29c	Renal ECs, podocytes	Spry1	Increases extracellular matrix, apoptosis	Induced by hyperglycemia, DM
miR-503	ECs	Cdc25A	Inhibits proliferation, migration	Induced by glucose, DM
Adaptive				
miR-195	Retinal ECs	Sirt1	Limits senescence	Induced by high glucose
miR-93	Podocytes	VEGF	Limits microvascular complications	Decreased by hyperglycemia
miR-124a	INS-1 β-cells	FOXA2	Decreases islet amyloid polypeptide	Decreased by hyperglycemia
miR-200a-3p	Renal mesangial cells	TGFβ	Decreases fibrosis	Decreased by high glucose
miR-200b	Retinal ECs	VEGF	Decreases vascular permeability	Decreased in DM

DM, diabetes; ECs, endothelial cells; HFD, high-fat diet; palm, palmitate; SMCs, smooth muscle cells.

Several miRNAs contribute to cell death after prolonged exposure of cells in culture to media containing pathophysiologically high concentrations of the saturated fatty acid palmitate. In MIN6 cells, palmitate induces expression of miR-34a and miR-146 (10). Overexpression of either miRNA enhances, whereas knockdown inhibits, palmitate-induced apoptosis of MIN6 cells. Targets of miR-34a in these cells include Bcl2, an important antiapoptotic regulator, as well as VAMP2, which plays a critical role in exocytosis of insulin granules, providing a mechanistic understanding of the contribution of miR-34a to cell dysfunction and cell death. In cardiomyocytes, Bcl2 and sirtuin 1 are targeted by palmitate-induced miR-195, resulting in changes in gene expression that promote apoptosis (11). On the other hand, lipotoxic conditions also decrease expression of miRNAs that target proapoptotic proteins and can thus disrupt constitutive prosurvival functions. Incubation of hepatocytes with palmitate decreases generation of miR-296, which leads to increased expression of its proapoptotic target p53 upregulated modulator of apoptosis (PUMA), whereas forced overexpression of this miR-296 decreases PUMA expression and protects against lipoapoptosis (12). Similarly, decreases in miR-615-3p under lipotoxic conditions have been linked to increases in expression of CHOP, a proapoptotic transcription factor that is induced during lipoapoptosis (13,14). Beyond the inverse relationship between miRNA abundance and putative target expression for each of these palmitate-regulated miRNAs, experiments using reporter constructs have identified functional binding sites in the 3'-untranslated region of the putative targets. Nonetheless, no studies to date have captured these miRNAs with their targets within the RNA-induced silencing complex.

Lipid-induced miRNAs also drive dysfunction of some cell types under lipotoxic conditions. miR-24 is highly upregulated in islets isolated from *db/db* mice and from wild-type mice fed a high-fat diet (15). Furthermore, in MIN6 cells, palmitate induces miR-24, and overexpression of miR-24 inhibits MIN6 cell proliferation, an effect that is mediated through miR-24 downregulation of *Hnf1a* and *Neurod1*. Overexpression of miR-24 also decreases glucose- and potassium-stimulated insulin secretion. By contrast, knockdown of miR-24 in islets from HFD mice restores normal glucose-stimulated insulin secretion. Thus, a single miRNA induced during lipid stress can have broad impact on normal tissue function, presumably through its effects on multiple RNA targets.

High glucose and its resulting metabolites, such as advanced glycation end products (AGEs) and methylglyoxal, also induce expression of miRNAs that contribute to cellular dysfunction. In primary islets and β -cell lines, high glucose increases expression of miR-375 and miR-30a-5p, which contribute to impairment of glucose-stimulated insulin secretion by targeting 3' phosphoinositide-dependent protein kinase-1 (*Pdpk1*) and *Beta2/NeuroD*, respectively (16,17). Decreases in PDPK1 expression dampen phosphatidylinositol 3-kinase signaling, whereas BETA2/NEUROD is a transcription

factor that regulates transcription of the insulin gene and the SUR1 subunit of the K_{ATP} channel. Interestingly, high-level expression of miR-375 has been found in postmortem studies of pancreases from humans with type 2 diabetes (18). Several other miRNAs upregulated by glucose target the phosphatase and tensin homolog (*Pten*) mRNA—in monocytes treated with AGE, miR-214 downregulates PTEN expression and prolongs inflammation (19), and in glucose-treated renal mesangial cells, miR-21-mediated decreases in PTEN expression lead to increased AKT and TORC1 activation (20). Treatment of vascular smooth muscle cells with the reactive carbonyl species methylglyoxal, a by-product of persistent hyperglycemia, induces miR-9a-3p, which targets the mRNA encoding the sulfonylurea receptor 2B (SUR2B) subunit of the vascular K_{ATP} channel, thereby compromising K_{ATP} channel function (21). Overall, these glucose-induced miRNAs are expressed in a context that is consistent with a potential role in progressive cellular dysfunction.

On the other hand, many miRNAs serve an adaptive role in the face of metabolic stress. Some, like miR-195, are induced by treatment of cultured endothelial cells with high glucose and are also found to be upregulated in the diabetic retina (22). Experiments using an miR-195 antagomir demonstrate that this miRNA functions to limit hyperglycemia-induced senescence of cultured endothelial cells through downregulation of *Sirt1*. By interfering with the expression of this key mediator of glucose-induced damage to microvessels, miR-195 may protect against retinal complications of hyperglycemia. There are also miRNAs that serve a protective role under homeostatic conditions but whose expression is diminished by hyperglycemia. miR-93 targets vascular endothelial growth factor (VEGF), high levels of which have been implicated in diabetes microvascular complications (23). Hyperglycemia downregulates miR-93 in both the diabetic kidney and in cultured podocytes, and in podocytes transcriptional downregulation of miRNA-93 is associated with excessive secretion of VEGF. miR-124a is another example of an miRNA that physiologically serves to maintain homeostasis in cultured INS-1 β -cells by targeting the transcription factor FOXA2, the net effect of which decreases expression of islet amyloid polypeptide (24). Levels of miR-124a are downregulated by glucose-induced thioredoxin-interacting protein (TXNIP). As deposits of islet amyloid polypeptide are associated with islet degeneration in type 2 diabetes, this observation implicates glucose-induced downregulation of miR-124a in progressive loss of functional β -cells.

Although levels of many miRNAs are altered in the serum and in tissues affected by complications in human subjects with type 1 or type 2 diabetes (25,26), causal relationships among altered miRNA expression, metabolic substrate excess, and pathogenesis of complications are difficult to establish in humans. In contrast, studies in rodent models, in which miRNAs are targeted biochemically or genetically, have provided key insights into their contributions to

the response to metabolic stress. Nonetheless, while there are many examples in which manipulation of miRNA expression leads to anticipated changes in target mRNA expression in animal models (27,28), only in a few instances have such changes been functionally linked to the pathogenesis of complications.

Two glucose-regulated miRNAs play a role in the development of renal abnormalities in diabetes mouse models. High glucose induces expression of miR-29c, which directly targets sprouty homolog 1 (*Spry1*), a negative regulator of Rho kinase and Wnt signaling that is known to promote diabetic nephropathy (29). In *db/db* mice, antisense oligo knockdown of miR-29c decreases albuminuria and mesangial matrix accumulation, hallmarks of diabetic nephropathy. By contrast, high glucose downregulates miR-200a-3p, a negative regulator of profibrotic genes such as TGF- β 2 (30). Treatment of mice with a lentiviral short hairpin RNA targeting miR-200a-3p exacerbates urinary albumin excretion and renal fibrogenesis in streptozotocin-treated mice.

Furthermore, a number of miRNAs, which are regulated by glucose in cell culture and dysregulated in tissues of diabetic mice, have been shown to contribute to vascular abnormalities in diabetes models relevant to retinopathy. In retinal capillary endothelial cells of streptozotocin-treated rats, decreased miR-200b is associated with upregulation of both mRNA and protein for its target VEGF, similar to effects observed in glucose-treated endothelial cells (31). Injection of miR-200b mimic or antagomir into the vitreous cavity causes anticipated changes in the abundance of miR-200b and corresponding changes in its VEGF target. Furthermore, treatment with miR-200b mimic mitigates against increases in albumin permeability in the streptozotocin-induced diabetes model. Another aspect of vascular function that is important in the pathogenesis of diabetes complications is reparative angiogenesis that occurs after ischemic insult. Glucose-induced miR-503 inhibits endothelial proliferation, migration, and network formation in vitro by targeting mRNAs such as *Cdc25A* (32). Levels of miR-503 are higher in ischemic limb muscles of diabetic compared with control mice, and transduction with an adenoviral decoy for miR-503 upregulates CDC25A expression and increases capillary and arteriolar densities in ischemic diabetic limb muscle.

LONG NONCODING RNA

Long noncoding RNAs (lncRNAs) are distinct from miRNAs in both their structure and biogenesis. By definition, these RNAs have limited protein-coding potential, even though they are typically >200 nucleotides in length, transcribed by RNA polymerase II, spliced, and maintain a 5'-cap and polyA tail (33). Initially, lncRNAs were considered products of aberrant transcription, given their low expression, lack of sizable open reading frames or identified translation products, and poor sequence conservation across species. However, functional analyses and genetic models of lncRNAs have revealed that lncRNAs are a diverse class of noncoding RNAs that regulate transcriptional activity through roles as

scaffolds, guides, and decoys for transcription factors and epigenetic modifiers. lncRNAs can also alter RNA function by serving as decoys for miRNAs and splicing factors. In these varied roles, lncRNAs complex not only with RNAs but also with proteins to form bioactive regulatory complexes that impact cellular homeostasis. Many of the ~30,000 lncRNAs encoded within the mammalian genome have cell-, tissue-, and developmental stage-specific patterns of expression or respond to environmental stimuli, providing an additional dimension to gene regulation in physiological and pathological settings.

A genetic screen identified lncRNA *Gadd7* as a critical mediator of lipotoxic cell death in Chinese hamster ovary cells (6). *Gadd7* is upregulated by lipid-induced reactive oxygen species (ROS), and its induction is critical for propagation of ROS, palmitate-induced endoplasmic reticulum stress, and palmitate-induced cell death. Oxidative stress is a key downstream response pathway, not only after lipotoxicity, but also in the setting of genotoxic stress, and *Gadd7* has been shown to be upregulated in response to DNA damaging agents and ultraviolet radiation (34). While the mechanism through which *Gadd7* functions in lipotoxic stress is unknown, during DNA damage-induced cell cycle arrest, *Gadd7* binds to TAR DNA-binding protein (TDP-43), causing destabilization of cyclin-dependent kinase 6 (*Cdk6*) mRNA (35). Knockdown of *Gadd7* preserves the levels of *Cdk6* mRNA and protein and prevents G1/S-phase arrest after genotoxic stress. Whether *Gadd7* similarly regulates cell cycle changes during metabolic stress responses remains to be explored.

The most compelling evidence for a role of lncRNAs in the response to the altered metabolic environment and the pathophysiology of diabetes complications comes from studies of glucose-regulated lncRNAs in diabetic retinopathy. MALAT1 is upregulated in retinal endothelial cells cultured in high glucose, in the retinas of streptozotocin-treated mice and rats and mice that demonstrate impaired electroretinograms, and in the fibrovascular membranes and aqueous humor from the eyes of human subjects with diabetic retinopathy (36,37). Knockdown of MALAT1 in rodents using intraocular injection of short hairpin RNA improves retinal function as documented by electroretinograms, decreases apoptotic retinal cell death, and improves survival of retinal pericytes and decreases retinal vascular leakage. In cultured endothelial cells, knockdown of MALAT1 significantly decreases endothelial cell migration and tube formation, both of which may contribute to progression of retinopathy. The precise mechanism of these MALAT1 effects are not known, but experiments using chemical inhibitors of signaling molecules suggest that p38 MAPK signaling pathways are likely to be important downstream effectors (37). Another lncRNA that is induced by culture of cells in high glucose, myocardial infarction-associated transcript 1 (MIAT1), is also upregulated in the retinas of diabetic rodent models (38). Similar to MALAT1, knockdown of MIAT1 in vivo improves visual function and decreases loss of pericytes by apoptosis. MIAT1 acts in a regulatory loop with miR-150-5p, an miRNA that

targets and represses VEGF expression in the setting of vascular stress. The observation that MIAT1 contains miR-150-5p-binding sites suggests that this lncRNA functions as a sponge to sequester miR-150-5p and thereby effectively depress VEGF mRNA expression.

RNA sequencing studies have revealed other lncRNAs that are dysregulated in the setting of metabolic stress. More than 1,000 lncRNAs are expressed in human islets, approximately half of which are specific for pancreatic tissue (39). Expression of two of these, HILNC-78 (TCL1 upstream neural differentiation-associated RNA [TUNAR]) and HI-LNC80 (oligodendrocyte maturation-associated long intergenic noncoding RNA [OLMALINC]), are induced in both human and mouse islets in culture media containing high glucose. However the functional contribution of these lncRNAs to β -cell dysfunction or the pathophysiology of diabetes is not known. In another cell type, RNA sequencing analysis of macrophages isolated from diabetic *db/db* mice revealed 171 differentially expressed lncRNAs (40). One of the most highly expressed, E330013P06 (E33), is upregulated when monocytes are cultured in high glucose, and its overexpression sensitizes macrophages to lipopolysaccharide-induced activation, cytokine production, and foam cell transformation. Interestingly, this lncRNA is also upregulated in monocytes from patients with diabetes. Given the importance of inflammation in diabetes complications, future studies to probe its contribution to tissue dysfunction and damage during metabolic stress will be of interest. Finally, another avenue of discovery for lncRNAs relevant to metabolic stress will be further characterization of the broad classes of lncRNAs induced by ROS (41), since glucose and lipid metabolic stress induce ROS in cultured cells and since tissue damage in diabetes is accompanied by evidence of oxidative stress. As with miRNAs, lncRNAs impact the biology of metabolic stress responses at the level of downstream gene expression (Fig. 1). The many pre- and posttranscriptional mechanisms through which lncRNAs can function suggest that the roles of these noncoding RNAs in metabolic stress will be similarly diverse.

RIBOSOMAL RNA

Ribosomal RNAs (rRNAs) are among the most transcribed and the most abundant RNAs in mammalian cells. Together, the 28S, 18S, 5S, and 5.8S mature rRNAs provide the structural framework for ribosomal proteins and facilitate the extension of nascent peptide chains through the peptidyl transferase ribozyme. Generation of ribosomes is highly regulated spatially, with ribosomal DNA genes and transcriptional and processing machinery clustered in nucleoli. Chemical agents known to cause oxidative stress induce nucleolar stress with impairment of rRNA gene transcription, leak of nucleolar proteins to the cytoplasm, and cell cycle arrest (42). When prolonged, such as occurs with knockdown of rRNA transcription factors, nucleolar stress leads to P53-mediated apoptosis (43). As yet, no studies have established whether the underlying metabolic

perturbations in diabetes cause cell death through nucleolar stress, though it is plausible that high glucose or lipid could trigger this pathway.

Like other cellular macromolecules, rRNAs are likely to be impacted by the oxidative stress that accompanies glucotoxicity and lipotoxicity. In yeast, oxidative stress leads to endonucleolytic cleavage of the 25S and 5.8 S rRNAs (44). The integrity of rRNA in the setting of hyperglycemia and hyperlipidemia has not been studied. However, in patients with diabetes, the products of oxidative modification of rRNA are observed in the urine, and abundance of these species is linked to all-cause and diabetes-related mortality (45). rRNAs that are damaged by cleavage or oxidative modifications are likely to be sequestered and degraded through a specialized form of autophagy, termed ribophagy (46). Effects of the altered metabolic environment on rRNA integrity and abundance may contribute to the observed decreased RNA content and rates of protein synthesis in models of poorly controlled diabetes (47).

TRANSFER RNA

Transfer RNAs (tRNAs) are critical noncoding RNA components of the translational machinery that serve as adapters to deliver amino acids to nascent peptide chains in accordance with mRNA-specified codons. tRNAs undergo extensive processing and posttranscriptional modification, which are required for proper tRNA folding, stability, and function (48). Oxidative stress, a major component of lipotoxicity and glucotoxicity, and DNA damage dynamically reprogram tRNA methylation in yeast through the actions of specific tRNA methyltransferases (49,50). The resulting changes in tRNA modifications alter codon-anticodon interactions in ways that impact translational efficiency of select classes of genes to protect against oxidative stress. Metabolic stress-mediated modifications of tRNA modifications could contribute to the altered programs of gene expression associated with complications in tissues of diabetic animals.

Global and programmatic changes in gene expression in metabolic stress could also result from endonucleolytic cleavage of mature tRNAs, which is known to be potently induced by oxidative stress (51,52). tRNA cleavage, commonly within the accessible anticodon loop, is mediated by the RNase angiogenin. The resulting tRNA halves can interact with translational machinery to inhibit translation initiation or cause translational arrest and stress granule assembly, even in the absence of changes in total tRNA levels (53). Translational reprogramming is critical for cellular adaptations to stress stimuli, such as oxidative stress, and studies exploring tRNA cleavage in response to glucotoxic and lipotoxic stress could uncover mechanisms of metabolic stress-induced translational alterations (54).

SMALL NUCLEOLAR RNA

Small nucleolar RNAs (snoRNAs), noncoding RNAs that range from 60 to 300 nucleotides in length, are named for their cellular localization within the nuclear subcompartment,

where they participate in processing and modification of nascent rRNAs and small nuclear RNAs. Most mammalian snoRNAs are encoded within the introns of RNA polymerase II–transcribed genes and processed out of intron lariats during splicing, although a small number are independently transcribed from their own promoters by RNA polymerase III. Mature snoRNAs complex with proteins to form a machinery that precisely directs posttranscriptional modification of rRNAs and spliceosomal RNAs through Watson-Crick base pairing of their antisense element with the appropriate target RNA (55). Short sequence motifs define box C/D and box H/ACA classes of snoRNAs that associate with different proteins to guide 2'-O-methylation or pseudouridylation, respectively, on their target RNAs. In addition to these canonical functions, some snoRNAs have been implicated in directing alternative mRNA splicing, and others have been found to be processed to smaller, miRNA-like fragments that regulate gene expression posttranscriptionally (56,57).

Another novel function for snoRNAs was identified in a genetic screen designed to elucidate regulators of cell death in response to metabolic stress in mammalian fibroblasts (14). In this study, disruption of the *Rpl13a* locus by a promoter trap retrovirus enhanced cell survival in media containing lipotoxic concentrations of palmitic acid and high glucose. Genetic complementation of the mutant and knockdown experiments in wild-type cells revealed that loss of a family of box C/D snoRNAs encoded within four introns of the gene, and not the cDNA, is a critical determinant of the response to metabolic and oxidative stress. Furthermore, knockdown of these snoRNAs in the livers of mice protects against propagation of ROS and oxidative tissue damage. While haploinsufficiency of the *Rpl13a* snoRNAs is sufficient to protect against metabolic stress in the mutant cell line, predicted rRNA targets for these box C/D snoRNAs (U32a, U33, U34, and U35a) remain 2'-O-methylated, indicating that the role for these snoRNAs in metabolic stress likely involves other, yet to be identified targets. The observation that these snoRNAs accumulate in the cytoplasm during metabolic and oxidative stress further suggests the possibility of novel RNA targets in the cytoplasm (58). The mechanism of action of these noncoding RNAs and elucidation of potential roles in the pathology of diabetes complications are active areas of investigation.

While it is possible that snoRNAs, including those from the *Rpl13a* locus, target mRNAs or other cytosolic RNAs to impact gene expression posttranscriptionally, recent studies also provide support for a novel signaling role involving the RNA-dependent protein kinase (PKR). PKR is a pattern recognition receptor that activates innate host antiviral mechanisms in response to both double-stranded RNA and metabolic stress (59,60). Genetic and diet-induced models of obesity exhibit increased activation of PKR in white adipose tissue and liver, and acute lipid infusion in vivo and lipotoxic conditions in

vitro are sufficient to activate PKR (60). A recent study found that snoRNAs are the major class of PKR-bound RNAs under lipotoxic conditions (61). Furthermore, overexpression of SNORD113, SNORA3, or SNORA71 is sufficient to induce PKR activation and phosphorylation of eIF2 α , leading to stress-induced alterations in translation. PKR also directly phosphorylates and inhibits insulin receptor substrate 1, and PKR knockout mice are protected from high-fat diet–induced hyperglycemia and hyperinsulinemia (60). Whether phosphorylation of specific downstream targets by PKR is regulated by specific snoRNAs or combinations of snoRNAs remains to be determined.

CONCLUSIONS AND FUTURE DIRECTIONS

We are only beginning to understand how different classes of noncoding RNAs impact the response of cells and tissues to the altered metabolic environment. Abundance of miRNAs and lncRNAs is regulated by abnormal substrate levels. While it is largely assumed that alterations in the levels of these noncoding RNAs reflect differences in transcription, future studies will be required to address whether changes in transcription or processing contribute to the observed altered abundance or whether metabolic stress impacts the RNA half-life. In the case of rRNAs and tRNAs, regulated degradation and covalent modifications do contribute to metabolic stress responses (Fig. 2). Recent studies of snoRNAs suggest that this class of noncoding RNA is differentially localized in response to changes in the metabolic milieu. Whether or not altered localization correlates with non-canonical targets remains to be determined.

While different classes of noncoding RNAs engage downstream targets and pathways in diverse ways, each can profoundly impact gene expression and the response to environmental challenge. A greater understanding of noncoding RNA pathways has the potential to provide new insights not only into metabolic regulation but also into phenotypic variation in metabolic diseases that is not

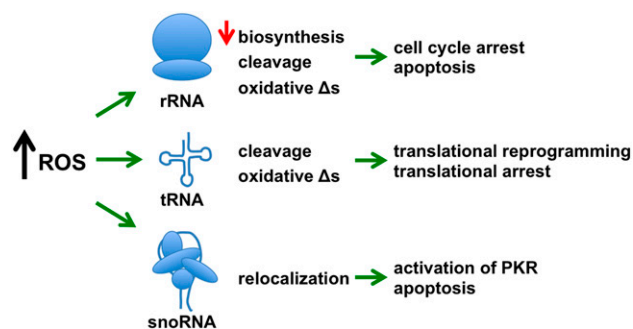


Figure 2—Oxidative stress–mediated alterations in rRNAs, tRNAs, and snoRNAs. Excessive ROS cause modifications (Δ) of rRNAs, tRNAs, and snoRNAs in ways that affect cellular function and survival. For rRNAs and tRNAs, future studies will be required to establish the relevance of these alterations in metabolic disease, whereas snoRNAs have already been demonstrated to function in lipotoxic cell death.

explained by alterations in exonic sequences. Furthermore, over the past several years, approaches using antisense oligonucleotides directed against miRNAs (i.e., anti-miRs) have been shown to hold promise in reducing the levels of the target miRNAs and modifying disease phenotypes in murine disease models, including hyperlipidemia (62,63). It is intriguing to consider that such approaches may be useful in preventing complications in patients with diabetes. Looking to the future, it is also possible to envision new therapeutic approaches that target lncRNAs and snoRNAs—classes of noncoding RNAs that are also amenable to antisense strategies.

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