Trends in Biochemical Sciences

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Review

Long Noncoding RNAs: A New Regulatory Code in Metabolic Control

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Long noncoding RNAs (lncRNAs) are emerging as an integral part of the regulatory information encoded in the genome. lncRNAs possess the unique capability to interact with nucleic acids and proteins, and exert discrete effects on numerous biological processes. Recent studies have delineated multiple lncRNA pathways that control metabolic tissue development and function. The expansion of the regulatory code that links nutrient and hormonal signals to tissue metabolism gives new insights into the genetic and pathogenic mechanisms underlying metabolic disease. This review discusses lncRNA biology with a focus on their role in the development, signaling, and function of key metabolic tissues.

Trends

lncRNAs exhibit tissue-specific and highly regulated expression patterns, and are frequently dysregulated in disease states.

lncRNAs regulate diverse biological processes through the formation of lncRNA–protein and lncRNA–miRNA complexes to control gene expression and function.

lncRNAs regulate metabolic tissue development and function, including adipogenesis, hepatic lipid metabolism, islet function, and energy balance.

lncRNAs are important regulators of skeletal and cardiac muscle development, and of the immune response.

The homeostatic control of nutrient and energy metabolism in mammals is governed by reciprocal signaling between the tissues that primarily serve regulatory functions, such as the pancreatic islets and the central nervous system, and major metabolic tissues including adipose tissues, skeletal muscle, and the liver. These tissues acquire their highly specialized regulatory functions and metabolic activities during development, and exhibit an amazing degree of plasticity in adulthood. For example, the different types of skeletal muscle fibers are characterized by varying oxidative capacity and contractile function, whereas adipocytes from white and brown fat have nearly opposite roles in fuel storage and oxidation. Disruption of energy homeostasis underlies the pathogenesis of major metabolic disorders including obesity, type 2 diabetes, dyslipidemia, and non-alcoholic fatty liver disease. The protein factors that control metabolic tissue development, signaling, and function have been extensively investigated. Recent work on lncRNAs has added a new dimension to the regulatory networks that impinge on metabolic homeostasis and disease [1,2].

lncRNAs: Emerging Regulators of Diverse Biological Processes

The term lncRNA refers to a class of RNA transcripts that lack identifiable open reading frames and thus protein-coding potential [3,4]. lncRNAs are commonly defined as transcripts longer than 200 nt, as compared to other shorter noncoding RNA species such as microRNA (miRNA), small interfering RNA (siRNA), and piwi-interacting RNA (piRNA). Perhaps the best-known lncRNAs are ribosomal RNAs and X inactive specific transcript (Xist), which play crucial roles in protein translation and X chromosome inactivation, respectively. Large-scale discovery of lncRNAs became feasible initially with tiling microarray [5,6] and full-length cDNA sequencing [7]. More recently, epigenome analysis [8] and whole transcriptome RNA-sequencing (RNA-Seq) have identified even greater numbers of lncRNAs [9,10]. Despite this, significant challenges remain to accurately annotate lncRNA genes, as illustrated in a recent study showing that a transcript originally classified as a muscle lncRNA in fact encodes a small protein in the cell [11]. The coding potential of RNA transcripts can be predicted using bioinformatic tools such as PhyloCSF, which is based on comparative genomic analysis of the coding probability of nucleotides across multiple species [12]. Other approaches, such as Coding Potential Calculator

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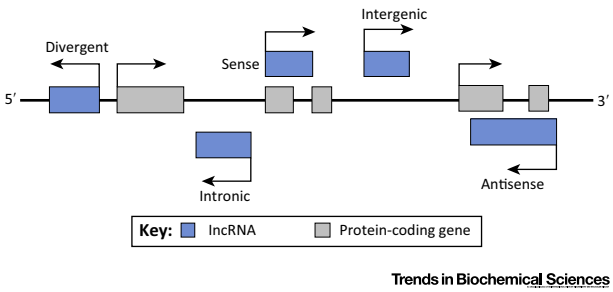

Figure 1. Different Classes of Long Noncoding RNAs (lncRNAs). lncRNA genes can be classified into divergent, intronic, sense, antisense, and intergenic groups according to their location relative to the nearby protein-coding genes.

(CPC), PORTRIAT, and Coding Potential Assessment Tool (CPAT), are also capable of assessing coding potential when cross-species alignments are limited by lineage specificity [13–15]. Recently, ribosomal profiling analysis of ribosome occupancy on RNA transcripts has provided experimental evidence that lncRNAs lack the capacity to encode proteins [16]. Based on their location relative to nearby protein-coding genes, lncRNAs can be categorized into sense, antisense, intronic, divergent, and intergenic groups (Figure 1). It was recently estimated that the human genome produces thousands of lncRNAs as a result of pervasive transcription from intergenic regions [17]. The widespread transcriptional activity beyond the 2% of the genome encoding proteins was also supported by global analysis of chromatin marks, which revealed that many of the intergenic lncRNAs were marked by a characteristic histone signature that marks transcriptionally active chromatin domains [8].

The expression of lncRNAs exhibits remarkable tissue-specificity and is highly regulated during development and in response to physiological signals [18–21]. Similarly to mRNA transcripts, most lncRNAs are transcribed by RNA polymerase II and undergo further steps of processing, including splicing and polyadenylation. lncRNAs have been observed in the nucleus, the cytosol, or both [22], consistent with their roles in regulating diverse biological processes including transcription [23–25], cell differentiation [26,27], tissue development [28,29], and tumorigenesis/metastasis

Box 1. Molecular and Genetic Tools for Probing lncRNA Biology

The expanding role of lncRNAs in biological regulation has spurred the development of experimental tools to dissect the molecular and functional aspects of lncRNA biology. To globally analyze chromatin occupancy by lncRNAs, chromatin isolation by RNA purification (ChIRP) was developed to enrich endogenous lncRNA and its associated genomic targets [105]. Using chemical crosslinking and tiling biotinylated antisense DNA oligonucleotides for hybridization, this method is capable of generating high-resolution maps of lncRNA binding sites on native chromatin. Using a similar approach, the ChIRP–mass spectrometry (ChIRP–MS) method was developed for identifying endogenous protein factors that associate with specific lncRNA by affinity purification followed by liquid chromatography (LC)–MS/MS [106]. RNA antisense purification (RAP) and capture hybridization analysis of RNA targets (CHART) are alternative methods for selective purification RNA complexes to map chromatin binding sites and interacting proteins [107,108].

lncRNAs form extensive secondary structure through intramolecular base-pairing. The presence of distinct structural motifs is crucial for lncRNAs to assume their biological functions. Selective 2′-hydroxyl acylation and primer extension (SHAPE) provides a method for selective acylation of 2′-hydroxyls at single-stranded RNA regions but not at double-stranded regions [109]. This approach enables the interrogation of RNA structure at single-nucleotide resolution. To globally study RNA structure, parallel analysis of RNA structure (PARS) was developed using V1 and S1 RNA nucleases, which are specific for double- and single-strand RNA domains, respectively. Selective digestion of distinct RNA domains followed by deep sequencing provides a powerful tool for genome-wide interrogation of RNA structure [110]. More recently, domain-specific ChIRP (dChIRP) was successfully used for identifying the functional domain architecture of lncRNAs [111].

For functional analyses of lncRNAs, both gain- and loss-of-function expression constructs have been routinely used in mammalian cells and in mice [112–114]. Traditional RNAi and antisense oligonucleotides (ASO) can be used to efficiently suppress lncRNA expression [115]. With the development of CRISPR/Cas9 gene-editing tools, the activation of endogenous lncRNA genes becomes feasible using a specific guide RNA and a Cas9 fused to a transcriptional activation domain [116]. More recently, a method combining CRISPR/Cas9-directed RNA targeting, dubbed CRISPR-display, was developed to direct RNA domains, including natural lncRNAs, to specific genomic loci [117]. This method allows multiplexed targeting of various RNA modules to different locations in the genome.

(Box 1) [18,30,31]. In the nucleus, lncRNAs may function as transcriptional coactivators through direct interaction with transcription factors [32,33]. However, several lncRNAs have also been found to impair the assembly of transcriptional complexes, leading to inhibition of gene expression [34,35]. The gene-silencing activity of lncRNAs can be attributed to their recruitment of repressive chromatin-remodeling complexes [23,36], such as the polycomb repressive complex 2 (PRC2) and the switch/sucrose non-fermentable (SWI/SNF) complexes. Recent work demonstrated that lncRNAs facilitate the recruitment of PRC2 to chromatin, likely through association with multiple components of the PRC2 complex, including the suppressor of zeste 12 homolog (SUZ12), enhancer of zeste homolog 2 (EZH2), and Jumonji, AT-rich interactive domain 2 (JARID2) [37,38]. Some plant lncRNAs engage the SWI/SNF chromatin-remodeling complexes to induce transcriptional silencing via a separate lncRNA-binding protein [39]. Interestingly, the lncRNAs H19 and the long intergenic noncoding (linc)RNA-RoR exert their post-transcriptional effects on gene expression by serving as molecular sponges for microRNAs [40,41].

Regulation of Brown and Beige Adipocyte Differentiation by lncRNAs

Adipose tissues play multifaceted roles in energy storage and expenditure, endocrine signaling, and immune–metabolic crosstalk. Compared to white adipocytes, brown adipocytes have high mitochondrial content and express uncoupling protein 1 (UCP1), which dissipates chemical energy through heat production. Recent studies demonstrated that metabolically-active brown adipose tissue (BAT) is present in adult humans [42–45], raising the prospect that augmenting brown fat abundance and/or function may provide an effective treatment of obesity and its associated metabolic disorders [46,47]. While sharing key molecular and metabolic characteristics with the classical rodent BAT, brown fat in adult human appears to contain both classical and brown-like adipocytes [48–51], the latter have been termed beige or brite adipocytes [52–54]. In rodents, brown/beige fats appear to have distinct developmental origins [54–57]. The determination, differentiation, and metabolic functions of thermogenic adipocytes are under the control of a growing list of extracellular signaling cues, transcription factors and cofactors, and microRNAs [58–61].

The extent to which, lncRNAs are involved in the regulation of brown/beige fat development and function remained unexplored until recently. Using whole-transcriptome RNA-Seq, a cluster of lncRNAs that exhibited differential expression during adipogenesis was identified, several of which appeared to be required for adipocyte differentiation [20]. Among these, linc-RAP-1 (Firre) physically interacts with heterogeneous nuclear ribonucleoprotein (hnRNP) U [62]. Using a microarray platform containing probesets that interrogate both protein-coding and lncRNA transcripts, lncRNA expression in brown and white adipose tissues upon inguinal white fat browning and during brown adipocyte differentiation was analyzed [33]. A cluster of 21 lncRNAs was identified as being enriched in brown fat, highly induced during brown adipogenesis, and inducible during browning of white fat in response to the adrenergic agonist CL-316,243. Among this cluster of differentially regulated lncRNAs, brown fat lncRNA1 (Blnc1) was identified as a novel lncRNA that promotes brown and beige adipocyte differentiation and function. The Blnc1 RNA transcript is primarily localized in the nuclear compartment, suggesting that it may play a role in transcriptional regulation.

Gain- and loss-of-function studies established Blnc1 as a potent activator of thermogenic adipocyte differentiation. Notably, transplantation of preadipocytes transduced with a recombinant Blnc1 retrovirus in *nude* mice resulted in the formation of fat pads reminiscent of brown fat, with *Ucp1* expression reaching approximately one third of the levels observed in the endogenous brown fat. Whether Blnc1 is absolutely required for the development of brown adipose tissue and browning of white fat remains to be investigated using mice lacking Blnc1. Despite its stimulatory effects on brown and beige preadipocyte differentiation, Blnc1 failed to promote differentiation of 3T3-L1 and C3H10T1/2 progenitor cells into *Ucp1*–positive adipocytes,

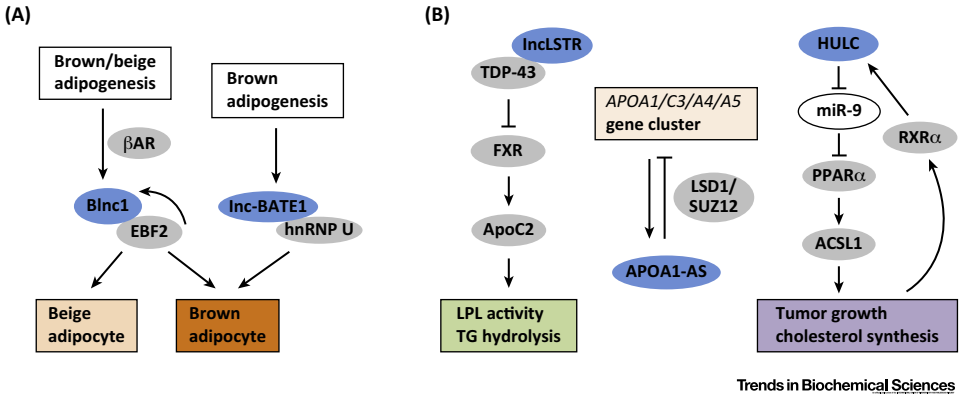

Figure 2. Regulation of Thermogenic Adipocyte Differentiation and Hepatic Metabolism by lncRNAs. (A) Regulation of brown and beige adipogenesis by lncRNAs Blnc1 and lnc-BATE1. Blnc1 is highly induced during brown and beige adipocyte differentiation, and physically interacts with EBF2 to promote thermogenic adipocyte differentiation through a feedforward regulatory loop. lnc-BATE1 is enriched in brown fat, associates with hnRNP U, and is required for brown adipogenesis. (B) Regulation of hepatic metabolism by lncRNAs. lncLSTR regulates hydrolysis of plasma triglycerides by LPL through modulating ApoC2 expression in the liver. APOA1-AS is an antisense transcript that originates from and regulates the expression of genes within the *APOA1/C3/A4/A5* cluster. HULC modulates lipid synthesis and tumor growth through its regulation of PPARα–ACSL1 expression. lncRNAs are shown in blue, microRNAs in white, and proteins in grey. Abbreviations: ACSL1, acyl-CoA synthetase long-chain family member 1; ApoA1-AS, antisense transcript of ApoA1; βAR, β-adrenergic receptor; Blnc1, brown fat lncRNA 1; EBF2, early B cell factor 2; FXR, farnesoid X receptor; hnRNP, heterogeneous nuclear ribonucleoprotein; HULC, highly upregulated in liver cancer; lncLSTR, liver-specific triglyceride regulator; LPL, lipoprotein lipase; LSD1, lysine-specific demethylase 1; PPARα, peroxisome proliferator-activated receptor α; RXRα, retinoid X receptor α; SUZ12, suppressor of zeste 12 homolog; TDP-43, TAR DNA-binding protein 43.

suggesting that Blnc1 may act during early adipogenesis and in mature adipocytes to exert its stimulatory effects on the thermogenic gene program. Mechanistically, Blnc1 physically interacts with early B cell factor 2 (EBF2), a transcription factor recently found to regulate adipocyte differentiation and brown fat development [63,64], and forms a ribonucleoprotein transcriptional complex to stimulate the expression of genes involved in fuel oxidation and uncoupled respiration. EBF2 also regulates the expression of Blnc1, forming a feedforward regulatory loop that likely serves as a potent switch for thermogenic adipocyte differentiation (Figure 2A).

More recently, RNA-Seq analysis of transcriptomes in different adipose tissues identified a cluster of brown fat-enriched lncRNAs [65]. Among these, lnc-BATE1 was found to be induced during brown adipocyte differentiation and expressed at higher levels in BAT than WAT. Knockdown of lnc-BATE1 by siRNA impaired differentiation of brown adipocytes, as revealed by decreased expression of brown fat markers and mitochondrial genes. Overexpression of lnc-BATE1, however, failed to augment brown adipogenesis, suggesting that the levels of this lncRNA may not be limiting in thermogenic gene expression. Although lnc-BATE1 appeared to be equally distributed between the cytosol and nucleus, it physically interacts with the nuclear matrix factor hnRNP U, a factor required for brown adipocyte differentiation (Figure 2A). Interestingly, hnRNP U also interacts with Firre, another lncRNA involves in adipogenesis. Several profiling studies have been described to explore how lncRNA expression is regulated during thermogenic adipocyte development [66–68]. However, the significance of candidate lncRNAs in adipocyte biology remains unknown. It is likely that Blnc1 and lnc-BATE1 are only a tip of iceberg that illustrates the important role of lncRNA regulators in brown and beige adipocyte development. In addition to lncRNA discovery, future work is needed to address the role of these lncRNAs in adipocyte metabolism and metabolic physiology.

lncRNAs and Hepatic Metabolism

Liver plays a central role in coordinating diverse metabolic processes, including glucose and lipid metabolism, bile acid synthesis, detoxification of xenobiotic compounds, and the secretion of

numerous plasma proteins. Hepatic metabolism is highly regulated by nutritional, hormonal, and circadian signals to maintain whole-body nutrient and energy homeostasis. Not surprisingly, dysregulation of hepatic metabolism has been implicated in the pathogenesis of several metabolic disorders in metabolic syndrome, such as hyperglycemia, dyslipidemia, and non-alcoholic fatty liver disease. Analysis of the transcriptome architecture of the liver by RNA-Seq revealed a large set of transcripts exhibiting circadian regulation, many of which were mapped to genomic loci corresponding to lncRNAs [21,69]. The diurnal regulation of genome-wide transcriptional activities was further supported by rhythmic changes in chromatin marks and RNA polymerase II enrichment. Interestingly, the period circadian clock 2 (*Per2*) locus was found to produce an antisense transcript (asPer2) that reached its peak levels in opposite phase to the *Per2* transcript peaks [21,69]. *Per2* encodes one of the core circadian clock proteins, and while the functional significance of asPer2 in circadian biology remains to be clarified, it is reminiscent of another antisense transcript of the *Neurospora* clock component antisense frequency (Frq) [70].

Recent studies have demonstrated that lncRNAs are important regulators of hepatic metabolism and plasma lipid homeostasis (Figure 2B). Analysis of microarray data covering a panel of mouse tissues led to the identification of liver-specific triglyceride regulator (lncLSTR), a liver-enriched lncRNA [71]. *In vivo* knockdown of lncLSTR lowered plasma triglyceride levels through induction of apolipoprotein C2 (ApoC2), which promotes lipoprotein lipase-mediated catabolism of triglyceride-rich lipoproteins. Further mechanistic studies indicated that lncLSTR physically interacts with TAR DNA-binding protein 43 (TDP-43), a transcriptional repressor, to attenuate the expression of ApoC2 through a bile acid-mediated transcriptional regulatory pathway. In a separate study, an antisense transcript of *ApoA1* (ApoA1-AS) was identified as an lncRNA that negatively regulates ApoA1 expression [72]. Knockdown of ApoA1-AS increased *ApoA1* gene expression, likely because of the recruitment of histone-modifying enzymes Lysine-specific demethylase 1 (LSD1) and SUZ12 to the *ApoA1* promoter. Oligonucleotides targeting ApoA1-AS significantly increased *ApoA1* mRNA expression in hepatic cell lines and African green monkeys, illustrating the possibility that lncRNAs may serve as targets for RNA-based therapeutic intervention. Dysregulation of lncRNA expression has also been observed in hepatocellular carcinoma (HCC). For example, the expression of the lncRNA highly upregulated in liver cancer (HULC) was elevated in HCC [73]. HULC appeared to facilitate tumor cell growth in part through its induction of acyl-CoA synthetase long-chain family member 1 (ACSL1) and disruption of circadian clock function.

Despite the emerging role of lncRNAs in the regulation of liver clock and metabolism, their significance in liver biology awaits further studies using gain- and loss-of-function mouse models. In addition, how these lncRNAs interface with hormonal and nutritional signaling pathways in the liver remains an important unanswered question. It is possible that some lncRNAs may play a dominant role in nutrient signaling and that their expression levels dictate the downstream metabolic response. Alternatively, other lncRNAs may serve a more permissive function to facilitate metabolic adaptation in the liver.

Regulation of Skeletal and Cardiac Muscle Development and Function by lncRNAs

Skeletal muscle is an important metabolic tissue because it plays a major role in postprandial glucose disposal by increasing glucose uptake in response to circulating insulin. Skeletal myofibers generate ATP through a combination of glycolysis and mitochondrial oxidative phosphorylation to support the energetic demand of muscle contraction. Not surprisingly, muscle energy metabolism has significant implications for whole-body energy homeostasis. Impaired muscle insulin action is an early hallmark of the metabolic derangements in metabolic syndrome. As such, skeletal muscle development and metabolism have been a major focus of numerous studies.

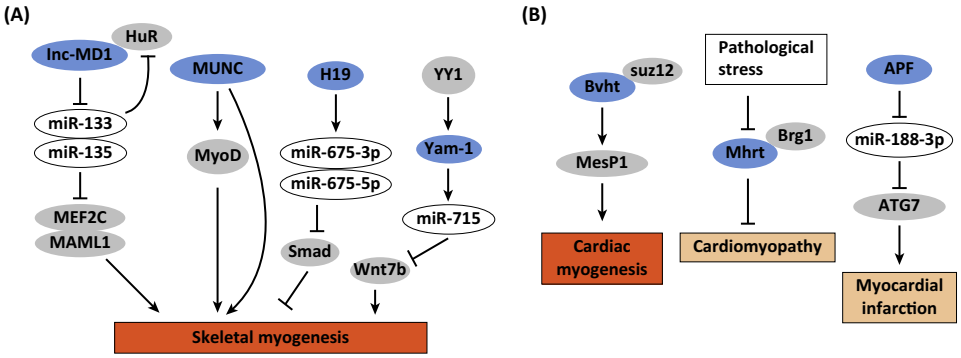

Figure 3. Regulation of Skeletal and Cardiac Muscle Development and Function by lncRNAs. (A) Regulation of skeletal myogenesis by lncRNAs. lnc-MD1 serves as a sponge for microRNAs that target myogenic regulators MEF2C and MAML1. MUNC regulates myogenesis through MyoD-dependent and independent mechanisms. The H19 lncRNA transcript induces two microRNAs that antagonize the inhibitory effects of Smad on myocyte differentiation. *Yam1* is a target gene of the transcription factor YY1 that negatively regulates skeletal myogenesis. (B) Regulation of cardiac myocyte development and function by lncRNAs. Bvht promotes cardiovascular lineage commitment and is required for activation of a core cardiovascular gene network through the regulation of MesP1. Mhrt is a cardiac-specific lncRNA transcript that responds to pathologic stress in the heart and plays a protective role in cardiomyopathy. APF promotes autophagic cell death and myocardial infarction through its regulation of ATG7. lncRNAs are shown in blue, microRNAs in white, and proteins in grey. Abbreviations: APF, autophagy-promoting factor; ATG7, autophagy related 7; Brg1, brahma-related gene 1; Bvht, brave heart; MAML1, Mastermind-like protein 1; MEF2C, Myocyte-specific enhancer factor 2C; MesP1, mesoderm posterior 1; Mhrt, myosin heavy-chain-associated RNA transcript; MUNC, MyoD upstream noncoding; SUZ12, suppressor of zeste 12 homolog; YY1, Yin Yang 1.

Several lncRNAs have been shown to regulate skeletal muscle development (Figure 3A). linc-MD1 is a muscle-specific lncRNA that promotes skeletal myocyte differentiation by serving as a sponge for microRNAs, including miR-133 and miR-135 [27]. The inactivation of these microRNAs relieved their inhibitory effects on mastermind-like protein 1 (MAML1) and myocyte-specific enhancer factor 2C (MEF2C), two pro-myogenic transcriptional regulators, leading to increased myogenesis. A subsequent study demonstrated that linc-MD1 interacts with the RNA-binding protein HuR (Hu-antigen R), which regulates the accumulation of linc-MD1 during myocyte differentiation [74]. Similarly, MyoD (myogenic differentiation 1) upstream noncoding (MUNC) and H19 are two lncRNAs that also promote muscle differentiation. MUNC induces MyoD expression and myogenic genes expression through MyoD-dependent and independent mechanisms [75], whereas H19 induces skeletal myocyte differentiation through its induction of miR-675-3p and miR-675-5p, two microRNAs generated within the H19 locus [76]. For both MUNC and H19, *in vivo* knockdown studies showed that they play an important role in progenitor cell differentiation and muscle regeneration. In another study, a group of lncRNAs were analyzed that are regulated by Yin Yang 1 (YY1), a transcription factor that represses multiple muscle genes. Among these lncRNAs, YY-1-associated muscle lincRNA (Yam-1) expression was downregulated during differentiation and acted as an inhibitor of myogenesis through its *cis* regulation of miR-715/Wnt7b signaling [77].

Cardiac muscle is extremely metabolically active and undergoes significant changes in its energy metabolism in disease states. Similarly to skeletal myogenesis, lncRNAs also play an important regulatory role in cardiomyocyte differentiation and function (Figure 3B). Braveheart (Bvht) was identified as a heart-associated lncRNA that is essential for the progression from nascent mesoderm toward mature cardiomyocytes. Through interaction with PRC2 component SUZ12, Bvht epigenetically regulates mesoderm posterior 1 (MesP1), a master regulator of multipotent cardiovascular progenitors, and activates a global cardiovascular gene network during cardiomyocyte differentiation [78]. lncRNAs also regulate the function and homeostasis of

mature cardiomyocytes. Pathological stresses resulted in the inhibition of the lncRNA myosin heavy-chain-associated RNA transcript (Mhrt) expression in mice [79]. Moreover, transgenic expression of Mhrt protects the heart from hypertrophic response to stress stimuli, showing a functional role for Mhrt in cardiomyocyte biology. Mhrt was also downregulated in various types of myopathic hearts in humans, suggesting a potentially conserved role of this lncRNA in protection against cardiomyopathy. Another study demonstrated that the lncRNA autophagy-promoting factor (APF) regulates autophagy and myocardial cell death by targeting miR-188-3p and autophagy related 7 (ATG7) [80]. Knockdown of APF significantly reduced myocardial infarction sizes following ischemia/reperfusion-induced injury. Together, these studies underscore an important role of lncRNAs in skeletal and cardiac muscle development and function (Figure 3).

lncRNAs and Islet Function

Pancreatic islets serve a crucial role in metabolic homeostasis through the secretion of key endocrine hormones such as insulin and glucagon. Recent RNA-Seq studies revealed an extensive collection of intergenic and antisense lncRNAs in mouse and human islets [81,82]. Many of them exhibited highly tissue-specific and regulated expression patterns during β cell differentiation and maturation. Interestingly, islet lncRNAs frequently map near chromatin domains containing islet-specific coding genes, suggesting that protein-coding and lncRNA genes may share common regulatory elements to direct their expression in pancreatic islets. While lncRNA expression profiles in islets exhibit notable species specificity [83], HI-LNC25 was identified as a β cell-specific lncRNA conserved between mouse and human. HI-LNC25 regulates the expression of GLI-similar 3 (GLIS3) (Figure 4A), a susceptibility gene for type 1 and type 2 diabetes, suggesting that HI-LNC25 may potentially play a role in the development of diabetes [82]. The expression of a subset of islet lncRNAs was altered in type 2 diabetes, whereas some lncRNAs were mapped to genetic loci that influence the susceptibility to diabetes. Given that many disease-associated genetic variations are mapped to intergenic regions, it is possible that the metabolic consequences of some of the variants may be due to their influences on lncRNA expression and/or function.

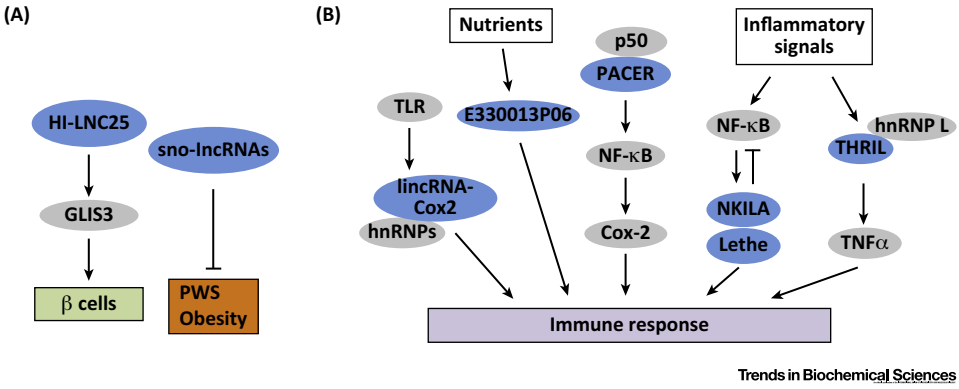

Figure 4. Regulation of Other Metabolically-Relevant Cell Types by lncRNAs. (A) HI-LNC25 is a β cell-specific lncRNA that is required for maintaining the expression of GLIS3. The sno-lncRNAs originated from the PWS locus modulate energy balance through their actions in the central nervous system. (B) Regulation of immune response by lncRNAs. lincRNA-Cox2 and PACER are two lncRNAs originating from the *COX2* gene locus that regulate cytokine signaling. E330013P06 is regulated by nutrients in macrophages and promotes inflammatory signaling. NKILA and Lethe are both cytokine-inducible lncRNAs that serve as negative feedback regulator of NF-κB. THRIL mediates the induction of TNFα gene expression in macrophages in response to proinflammatory stimuli. lncRNAs and proteins are in blue and grey, respectively. Abbreviations: GLIS3, GLI-similar 3; hnRNP, heterogeneous nuclear ribonucleoprotein; Lethe, lncRNA pseudogene; lincRNA, long intergenic noncoding RNA; NF-κB, nuclear factor κ light-chain enhancer of activated B cells; NKILA, NF-κB interacting lncRNA; PACER, p50-associated COX2 extragenic RNA; PWS, Prader–Willi syndrome; THRIL, TNFα and hnRNP L-related immunoregulatory lincRNA; TLR, Toll-like receptor; TNFα, tumor necrosis factor α.

Regulation of the Immune Response by lncRNAs

Chronic low-grade inflammation has emerged as an important pathogenic link between obesity and metabolic disease [84–88]. Obesity-associated adipose tissue inflammation is characterized by a robust shift of adipose tissue macrophages from alternatively activated to classically activated subtypes [89,90]. This shift from anti-inflammatory to proinflammatory macrophage polarization is likely an early event during the development of insulin resistance. Accumulating evidences suggest that lncRNAs play an important role in modulating multiple aspects of immune responses [91] (Figure 4B). Using RNA-Seq, the expression profiles of bone marrow-derived macrophages isolated from control and leptin receptor-deficient ('*diabetic*' – *db/db*) obese mice were analyzed [92]. The lncRNA transcript E330013P06 was found to be significantly elevated in macrophages from *db/db* and diet-induced insulin-resistant mouse models. Overexpression of E330013P06 in macrophage cells augmented cytokine signaling and proinflammatory gene expression, whereas knockdown by RNA interference (RNAi) elicited opposite effects. Whether E330013P06 plays a role in obesity-associated adipose tissue inflammation and metabolic dysregulation remains currently unknown. Nevertheless, these findings illustrate the dysregulation of the noncoding genome in obesity and its potential contribution to the pathogenesis of metabolic disorders.

lincRNA-Cox2 was discovered as a member of a cluster of lncRNAs that was stimulated in response to inflammatory stimuli through the activation of Toll-like receptors in bone marrow-derived macrophages (BMDM) [93]. Interestingly, lincRNA-Cox2 exerts both the activation and repression on different target genes, in part through its interaction with hnRNPs. Another lncRNA, p50-associated COX2 extragenic RNA (PACER) originated from the upstream promoter region of the *COX2* gene locus was found to interact with p50, a repressive subunit of nuclear factor κ light-chain enhancer of activated B cells (NF-κB), upon induction of *COX2* expression [94]. This PACER/p50 association leads to the assembly of active NF-κB transcriptional complexes that stimulate *COX2* gene expression and the inflammatory response. In two separate studies, cytokine-inducible NF-κB interacting lncRNA (NKILA) and a pseudogene lncRNA (Lethe) were identified to play a negative feedback regulatory role in proinflammatory cytokine signaling [95,96]. NKILA inhibits IκB phosphorylation, leading to suppression of NF-κB activation, whereas Lethe attenuates DNA binding and transcriptional function of the NF-κB subunit RelA. Further, a panel of lncRNAs were identified as differentially regulated in response to innate activation in the macrophage cell line THP-1 [97]. Among these, tumor necrosis factor ∝ (TNF∝) and hnRNP L-related immunoregulatory lincRNA (THRIL) interacts with hnRNP L and regulates the expression of TNF∝ and other genes involved in immune response. Together, these studies demonstrate that lncRNAs target multiple cytokine signaling and inflammatory response pathways. It would be of great interest to explore the potential pathogenic involvements of these lncRNAs in the pathogenesis of metabolic disease.

Potential Role of lncRNAs in Metabolic Disease

Dysregulation of lncRNA gene expression has been implicated in several human diseases, including facioscapulohumeral muscular dystrophy [98], HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) [99], and Angelman syndrome [100]. The latter is a single-gene neurological disorder caused by maternal deficiency of the imprinted gene ubiquitin protein ligase E3A (*UBE3A*) in part through lncRNA-mediated gene silencing. Remarkably, antisense oligonucleotides targeting the *UBE3A* antisense transcript lowered its expression level, leading to reactivation of *UBE3A* gene expression and amelioration of cognitive deficits associated with the disease [100]. A compelling example of the involvement of lncRNAs in metabolic disease is Prader–Willi syndrome (PWS). PWS is a genetic disorder that causes childhood obesity and various neurological symptoms [101]. PWS results from loss of expression of paternally expressed genes located on the PWS region on chromosome 15 (15q11–q13) which contains multiple paternally expressed noncoding RNAs [102]. Interestingly, these lncRNAs are

processed into a unique class of lncRNAs that are flanked by C/D box containing small nucleolar RNAs (snoRNAs) at the 5′ and 3′ ends (sno-lncRNAs) [103]. These PWS sno-lncRNAs appeared to accumulate near their sites of synthesis in the nucleus. Deletion of the host gene for these sno-lncRNAs increased energy expenditure in mice, likely due to altered expression of diurnally regulated genes in the brain, including the core clock and metabolic genes [104] (Figure 4A). The exact mechanisms through which the PWS lncRNAs regulate energy balance remain unknown at present.

Concluding Remarks

lncRNAs are emerging as an important class of regulatory factors that control the development and function of metabolic tissues. Acting in concert with protein factors and other non-protein regulators, such as microRNAs, lncRNAs provide an unorthodox link through which genetic information is transmitted to influence biological processes in the cell. The discovery and functional study of individual lncRNAs are set to expand our horizon on the genetic mechanisms of metabolic homeostasis and disease. Several challenges and opportunities arise from the study of lncRNA biology in metabolic control (see Outstanding Questions). First, the annotation of lncRNAs remains challenging and incomplete at present. Unlike protein-coding genes, lncRNAs exhibit a relatively low degree of nucleotide sequence conservation across species. In addition, lncRNAs may encode micropeptides that have important biological functions. As such, our ability to predict the structure of lncRNA molecules and their biological function remains limited. Second, lncRNAs likely exert their biological effects through diverse mechanisms, many of which remain to be discovered. A crucial question in the context of metabolic regulation is how lncRNAs interface with the classical nutrient and hormonal signaling pathways to control the development and function of various metabolic cell types. Given the highly regulated nature of lncRNA gene expression, it is likely that some lncRNAs may emerge as 'master' regulators of tissue development and energy metabolism. Finally, significant efforts are needed to deconvolute the role of lncRNA pathways in metabolic physiology and disease. Investigating highly conserved lncRNAs will arguably provide biological insights into the basic principles of lncRNA biology. In addition, the knowledge on conserved lncRNA pathways may prove relevant for human disease conditions.

Outstanding Questions

How can we comprehensively annotate lncRNA genes and accurately predict their molecular functions? To what extent are lncRNA sequence and function conserved among different species?

How are lncRNAs integrated with hormonal and nutrient signals to control metabolic tissue development, plasticity, and function? How do lncRNAs and protein factors work in concert to exert effects on epigenetic modification and metabolic gene expression?

What are the physiological roles of lncRNAs in the regulation of glucose and lipid metabolism, and whole-body energy balance? What is the extent to which altered lncRNA expression contributes to metabolic disorders in animal models and humans? A major challenge is to develop tools to target lncRNAs to alter the course of metabolic disease progression.

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