



Regulation of metabolism by long, non-coding RNAs

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Our understanding of genomic regulation was revolutionized by the discovery that the genome is pervasively transcribed, giving rise to thousands of mostly uncharacterized non-coding ribonucleic acids (ncRNAs). Long, ncRNAs (lncRNAs) have thus emerged as a novel class of functional RNAs that impinge on gene regulation by a broad spectrum of mechanisms such as the recruitment of epigenetic modifier proteins, control of mRNA decay and DNA sequestration of transcription factors. We review those lncRNAs that are implicated in differentiation and homeostasis of metabolic tissues and present novel concepts on how lncRNAs might act on energy and glucose homeostasis. Finally, the control of circadian rhythm by lncRNAs is an emerging principles of lncRNA-mediated gene regulation.

Keywords: lncRNAs, glucose homeostasis, metabolism and obesity, non-coding RNA (ncRNA), cell differentiation

INTRODUCTION

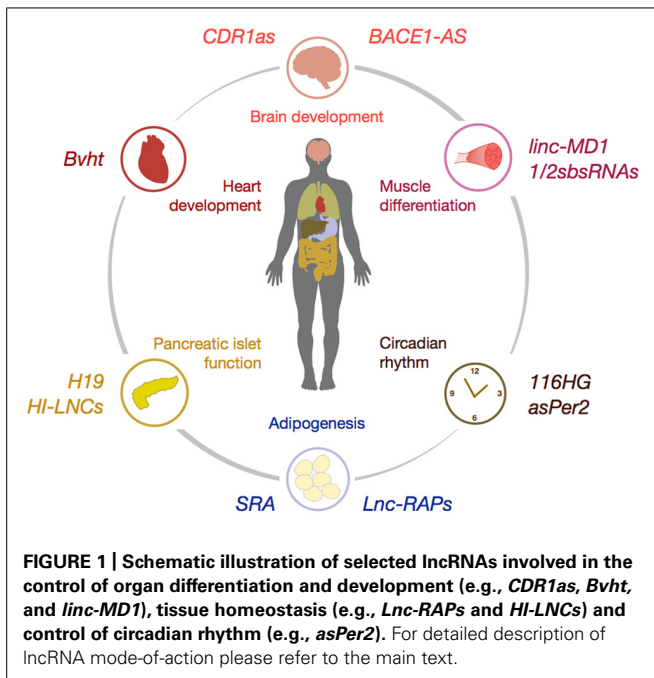
THE NON-CODING GENOME

The canonical view of mammalian genomes revolves around the notion that the roughly 20,000 proteins within mammalian genomes are interspersed by somewhat conserved, yet functionally redundant non-coding regions with only limited regulatory potential. Regulatory properties of these “non-coding” regions were only attributed to *cis*-regulatory elements such as promoters or *cis/trans*-enhancer regions. This paradigm was fundamentally called into question by results obtained from whole-transcriptome sequencing efforts [e.g., by the ENCODE consortium (Birney et al., 2007; Thomas et al., 2007)] over the last decade that have revealed the pervasive transcription of mammalian genomes (Carninci et al., 2005; Birney et al., 2007; Derrien et al., 2012). Although the magnitude of pervasiveness remains under debate (van Bakel et al., 2010; Clark et al., 2011), recent meta-analyses of human ribonucleic acid-sequencing (RNA-Seq) datasets have confirmed that >80% of genomic sequences are rediscovered within RNA transcripts, often in a temporally and spatially specific manner (Hangauer et al., 2013). One logical consequence of pervasive transcription is the abundance of non-coding RNAs (ncRNAs) within mammalian genomes, a phenomenon which holds true for most eukaryotic species ranging from yeast (David et al., 2006), to *Drosophila* (Stolc et al., 2004), plants (Li et al., 2006) and humans (Hangauer et al., 2013). Given the predicted high number of ncRNAs within mammalian genomes, which probably surpasses that of coding genes, it is not surprising that a large conceptual void remains about the multifaceted role of ncRNAs in regulation of gene expression. Researchers have historically divided ncRNAs into small ncRNAs (sRNAs <200 nt length) such as microRNAs (miRNAs) and small nucleolar RNAs (snoRNAs) in contrast to so-termed long ncRNAs (lncRNAs; >200 nt length). Until today, the identification of biological processes, which are regulated by miRNAs as well as the elucidation of mRNA targets,

which are posttranscriptionally regulated by disease-associated miRNAs remains an important focus of research (Bartel, 2009; Carthew and Sontheimer, 2009). It was demonstrated that the spectrum of biological processes, which are regulated by miRNAs, ranges from the development of organs, the homeostatic regulation of cellular metabolism to aging and neurodegenerative disorders. Although miRNAs are central to the understanding of the non-coding genome, the regulation of energy homeostasis and metabolism by miRNAs has been meticulously reviewed elsewhere (Lynn, 2009; Rottiers and Naar, 2012; Kim and Kyung Lee, 2013) and goes beyond the scope of this review. In contrast to miRNAs, the role of lncRNAs in control of metabolism and energy homeostasis remains rather elusive. Thus, we here review the known roles for lncRNAs, which probably constitute the numerical majority of ncRNAs encoded within mammalian genomes, during differentiation, homeostasis and metabolic regulation of tissues (Figure 1).

LONG NON-CODING RNAs

Those lncRNAs that were initially discovered in the late eighties had distinct, at that time considered exotic functions such as X chromosome inactivation in females by the lncRNA *XIST* (Penny et al., 1996). Another historical example was the imprinted lncRNA *H19*, which is involved in repression of *Igf2* (Pachnis et al., 1988). After the discovery of pervasive genomic transcription it became clear that lncRNAs do not represent an exotic observation, but rather a prominent feature of the genome (Birney et al., 2007). Although the number of lncRNAs is still debated, recent meta-analyses posit the human genome to give rise to >60,000 lncRNA, albeit the majority is probably expressed at low levels (Derrien et al., 2012; Hangauer et al., 2013; for current lncRNA numbers consult NONCODE Version 4, www.noncode.org). Interestingly, lncRNAs on one hand exhibit many similarities with protein-coding transcripts: As true for mRNAs, lncRNAs



are transcribed by RNA-polymerase (Pol) II (Guttman et al., 2009), spliced at canonical splicing sites (Chew et al., 2013), are partly polyadenylated (Cabili et al., 2011) and even associate with polysomes (Guttman et al., 2013). Further, lncRNAs harbor the same chromatin marks of H3K4 and H3K36 trimethylation as found in active promoters and transcribed regions of protein-coding transcripts, respectively, a phenomenon which aided in the identification of novel lncRNAs (Mikkelsen et al., 2007). It is noteworthy that the notion of distinct mRNA-like trimethylation marks on actively transcribed lncRNAs is incompatible with the criticism brought forward according to which lncRNAs are merely generated by unspecific Pol II activity which leads to low-level transcription of non-coding sequences (“transcriptional noise”; Derrien et al., 2012). On the other hand, certain features set lncRNAs apart from protein-coding genes: Generally, lncRNAs are expressed at lower levels, are less evolutionarily conserved and less frequently associate with ribosomes than protein-coding transcripts (Hangauer et al., 2013). Further, lncRNA are shorter than coding genes and are composed of a unique gene structure of usually 1–2 exons. Of note, some lncRNAs do give rise to small peptides and may act as both, coding and non-coding, transcript (reviewed here Dinger et al., 2008).

PRINCIPLES OF lncRNA-MEDIATED GENE REGULATION

Currently, intensive research efforts are underway to better understand the molecular basis of gene regulation by lncRNAs. To date, four major paradigms have emerged on how lncRNA impinge on gene regulation:

(EPIGENETIC) REGULATION OF GENE TRANSCRIPTION

lncRNAs are able to bring gene-regulatory DNA-binding proteins and DNA sequences into close proximity and thus constitute

an ideal docking platform for recruiting epigenetic modifiers to distinct genomic loci in *cis*- or *trans*. Indeed, early insights into lncRNA-based gene regulation have revealed the recruitment of the inhibitory polycomb repressive complex (PRC) 2 and the activating Trithorax/MLL chromatin modifiers to specific genomic loci by the lncRNAs *HOTAIR* (Rinn et al., 2007) and *HOTTIP* (Wang et al., 2011), respectively. PRC2 and MLL then mark distinct lysine residues within histones via trimethylation, leading to inhibition or activation of gene transcription. In a similar fashion, the lncRNA *ANRIL* silences the *INK4a* tumor suppressor allele by H3K27 trimethylation via recruiting the Polycomb chromatin modifier CBX7 (Yap et al., 2010). The percentage of lncRNAs implicated in (epigenetic) gene regulation was systematically quantified by interrogating the PRC2 interactome using chromatin-state maps. This revealed the abundant interaction of Polycomb repressor proteins with up to 20% of expressed lncRNAs (Khalil et al., 2009). Thus, one prominent role of lncRNAs relates to writing and erasing chromatin marks, thereby controlling the epigenetic state of lncRNA-bound genomic loci (Spitale et al., 2011). In a systematic attempt to interrogate the function of 3019 human lncRNAs, Orom et al. (2010) revealed that a significant portion of the lncRNome possesses *cis*-regulatory enhancer properties (hitherto termed enhancer-like RNAs, eRNAs), which control the expression of neighboring protein-coding genes. Elegant follow-up studies using chromosome conformation capture (3C) technology revealed that the co-activator complex Mediator is involved in tethering eRNAs to their gene targets. Hence, lncRNAs regulate the three-dimensional (3D) structure of chromosomes via Mediator-dependent chromosome looping (Lai et al., 2013), thereby bridging large intra- and interchromosomal distances in order to activate distal promoters (reviewed in Orom and Shiekhattar, 2013). This study nicely complemented reports about the lncRNA-mediated regulation of *HOXA* genes, in which chromosomal looping brings the eRNA *HOTTIP* in proximity to its target genes, marks the chromatin by H3K4 trimethylation and thus activates gene transcription (Wang et al., 2011). Taken together, the translation of the information content that lies within higher-order (3D) structures of chromosomes into (epigenetic) modifications of chromatin and regulation of gene transcription seems to be an emerging principle of lncRNA function.

PROCESSING/DEGRADATION OF mRNA

Every step of RNA metabolism is subjected to fine-tuned and complex regulation (reviewed in Moore, 2005). lncRNAs have recently been involved in the control of RNA stability, the processing of (pre)-mRNAs and the regulation of mRNA decay. Natural antisense transcripts (NATs) are lncRNAs which are characterized by their location antisense to other coding or non-coding transcripts (Faghihi and Wahlestedt, 2009). The upregulation of NATs often causes downregulation of protein-coding transcripts on the opposite strand by the formation of RNA duplexes and triggering of cellular RNAi, although recently the NAT-mediated upregulation of protein-coding transcripts on the opposite strand were reported (Carrieri et al., 2012). The repressive effect of NATs onto the opposite strand not only holds true for duplexes consisting of (i) a protein-coding mRNA and a non-coding lncRNA NAT, but also for (ii) duplexes between a lncRNA NAT and another

lncRNAs as demonstrated for lncRNAs which base-pair with and target the *PTEN* pseudogene *PTENpg1* (Johnsson et al., 2013). The lncRNA-mediated regulation of (pre)-mRNA processing was demonstrated for the nuclear retained lncRNA *MALAT1*, which modulates alternative splicing via assembly of serine/arginine splicing factors within subnuclear compartments called nuclear speckles (Tripathi et al., 2010). Finally, the timely degradation of mRNAs by a process called Staufen-mediated decay (SMD) involves lncRNA-recipient sequences in the 3'UTR of SMD target genes. Here, intermolecular base-pairing between lncRNAs and sequences within the 3'UTR of Staufen (Stau) target genes triggers a cellular SMD response and the ensuing degradation of the transcript (Gong and Maquat, 2011; Wang et al., 2013). The *in vivo* significance of lncRNA-mediated SMD decay was underscored by the observation that epidermal differentiation critically depends on lncRNA-elicited mRNA decay. Here, a lncRNA called *TINCR* is recruited to specific sequences called "TINCR box" within *TINCR* target genes and elicits the decay of *TINCR*-bound transcripts by SMD (Kretz et al., 2013; Kretz, 2013).

POSTTRANSCRIPTIONAL GENE REGULATION

MiRNAs recognize their gene targets by binding to 6–8 nt sequences called "seeds" located in the 3'UTR region of protein-coding RNAs (Bartel, 2009). It was demonstrated that recognition, binding and degradation/translational inactivation of miRNA targets is not necessarily confined to protein-coding transcripts. A so-called "competing endogenous RNA (ceRNA) hypothesis" was brought forward according to which protein-coding RNAs, miRNAs, and lncRNAs transcripts form large-scale regulatory networks which impinge on the expression of other transcripts independently of protein translation via competing for a limited pool of miRNAs (Salmena et al., 2011). Here, transcripts, so-called ceRNAs regulate the expression of other transcripts based on the similarity of their 3'UTR miRNA response elements (MRE) profile. According to this notion, two transcripts with a strong degree of common MREs can crosstalk to each other by competing for a given pool of miRNAs. Upregulation of one ceRNA increasingly "sponges" a limited pool of miRNAs and relieves the miRNA-mediated repressive tone on ceRNA-linked transcripts. The experimental confirmation of a ceRNA-like interdependency of protein-coding transcripts was first demonstrated for the tumor suppressor gene *PTEN* (Karreth et al., 2011; Tay et al., 2011), which "crosstalks" to hitherto unknown tumor suppressors. *PTEN* loss-of-function during cancerogenesis is also controlled by the genomic loss of its (non-coding) pseudogene *PTENP1* which acts as a ceRNA (Poliseno et al., 2010). An additional layer of posttranscriptional regulation by lncRNAs accordingly lies within the specific pattern of MREs within lncRNAs which allow it to influence the expression of coding and non-coding transcripts in a ceRNA-like fashion. This is exemplified by the upregulation of a non-coding antisense homologue of the beta-secretase *BACE1* (*BACE1-AS*) that acts as *BACE1* ceRNA and concomitantly increases *Bace1* mRNA stability and leads to augmented deposition of A β -plaques in Alzheimer's disease (AD; Faghihi et al., 2008, 2010). Finally, a novel, intriguing class of functional lncRNAs, which is encoded

in eukaryotic genomes, is constituted by circular RNAs (circRNAs). CircRNAs are expressed at high levels, can act as ceRNAs and effectively sponge miRNAs as shown for the neuroendocrine miRNA miR-7 (Hansen et al., 2013; Memczak et al., 2013).

REGULATION OF PROTEIN ACTIVITY

Ribonucleic acid possesses the unique biochemical property to recognize and bind most biomolecules including proteins with unprecedented affinity (Stoltenburg et al., 2007). Thus, lncRNAs can specifically bind proteins and elegant studies have attributed novel roles for lncRNAs in the control of tissue homeostasis via direct binding and modification of protein activity. For example, a lncRNA termed *Evi2* was shown to form stable complexes with members of the Dlx/Dll family of transcription factors, which are crucial regulators of developmental timing in vertebrates, and thereby regulate their transcriptional output (Feng et al., 2006). Further, two lncRNAs termed *PRNCR1* and *PCGEM1* that are upregulated in aggressive prostate cancer, synergistically and coordinately bind the carboxyterminal part of the androgen receptor (AR) and are required for AR-dependent gene transcription. In androgen-refractory prostate cancer, *PRNCR1* and *PCGEM1* are robustly expressed and are implicated in the ligand-independent activation of AR signaling [AR "resistant" prostate cancer (Yang et al., 2013)]. Another emerging paradigm of lncRNA-mediated regulation of protein activity is the sequestration of transcription factors as exemplified by the lncRNA *Gas5*, which is induced under conditions of nutrient deprivation and cellular stress. *Gas5* acts as glucocorticoid receptor (GR) decoy by competing with GR-responsive elements (GREs) in gene promoters for binding to the DNA-binding domain of the GR (Kino et al., 2010). Increased levels of *Gas5* thus interfere with GR binding to the DNA and effectively inhibit transactivation of GR-dependent gene promoters. Another example is nuclear factor kappa b (NF κ B) signaling, which translates extracellular, proinflammatory cues [e.g., by tumor necrosis factor alpha (TNF- α) receptor activation] into changes in gene expression. NF κ B activation induces the transcription of a specific subset of lncRNAs, apart from the induction of classical inflammatory protein-coding genes. Among this subset of TNF-regulated lncRNAs, a lncRNA termed *Lethe* is recruited to the NF κ B effector subunit RelA in an inducible fashion and inhibits RelA from DNA-binding and target gene activation (Rapicavoli et al., 2013). Finally, the hypoxia-regulated lncRNA *linc-p21* was shown to physically interact with hypoxia-inducible factor (HIF) 1 α transcription factors. This HIF1 α -*linc-p21* circuit controls the hypoxia-evoked increases of the glycolytic "Warburg effect" in tumor cells (Yang et al., 2014).

lncRNAs IN CONTROL OF METABOLISM

The regulation of metabolism and glucose homeostasis is orchestrated and fine-tuned by a complex interplay of tissues/organs. Currently, we are faced with an unprecedented rise of obesity in the civilized world and the concurrent increase in obesity-associated diseases such as insulin resistance and type 2 diabetes mellitus (T2D). Key to the understanding of whole-body metabolism are the pleiotropic effects of the anabolic master

regulator insulin which simultaneously controls peripheral as well as central-nervous system-related aspects of metabolism (Kahn et al., 2006). Resistance toward the effects of insulin constitutes a key step in the development of metabolic disease. The exciting observation that insulin and insulin-like growth factor (IGF) 1 signaling also triggers distinct changes in lncRNA expression [e.g., of the lncRNA *CRNDE* (Ellis et al., 2013)] points to the fact that lncRNAs may also be implicated in the metabolic effects of insulin and the development of insulin resistance. Thus, a strong interest lies within the identification of lncRNA-mediated mechanisms governing energy and glucose homeostasis at the cell-intrinsic, organ and whole-body level.

TISSUE-SPECIFIC REGULATION OF METABOLISM BY lncRNAs

MAINTENANCE OF PANCREATIC BETA CELL IDENTITY

The main function of pancreatic islets lies within the synthesis, storage and secretion of insulin and glucagon, two hormonal regulators of glucose homeostasis. The possible control of islet development and function by lncRNAs was first demonstrated in studies which reported that the lncRNA *H19* is involved in the intergenerational transmission of diabetes mellitus [gestational diabetes mellitus (GDM)] and the GDM-associated impairments of islet infrastructure and function (Ding et al., 2012). Global lncRNA screening approaches conducted by Moran et al. (2012) systematically interrogated the lncRNA transcriptome in human pancreatic beta cells. Here, the dynamic, strand and tissue-specific regulation of >1,000 lncRNA was reported using integrated transcriptional and chromosomal maps. Utilizing RNA-Seq data of 16 non-pancreatic tissues, the aforementioned gene set of pancreatic lncRNAs was shown to be significantly more specific for islet cells (40–55% for intergenic and antisense lncRNAs, respectively) than protein-coding genes (9.4%). Furthermore, the upregulation of islet-specific lncRNAs during progenitor commitment, glucose-stimulated upregulation and the striking dysregulation of islet-specific lncRNAs in patients with T2D pointed to a pathophysiological role of lncRNAs in the homeostasis of pancreatic tissues. The fact that a significant percentage of mouse and human lncRNA orthologs display similar cell- and stage-specific expression patterns suggests that evolutionarily conserved properties of lncRNAs extend beyond their primary sequence. This study was corroborated by a publication from the McManus laboratory, which presented a new catalog of the human beta cell (non-coding) lncRNA transcriptome in which >1,000 lncRNA were expressed in an islet-specific fashion involving islet-specific splicing events and promoter utilization (Ku et al., 2012). However, the elucidation of the molecular mechanisms underlying lncRNA-mediated regulation of beta cell differentiation and function still await discovery.

REGULATION OF ADIPOGENESIS AND ADIPOSE TISSUE PLASTICITY

The body harbors two principal types of adipose tissues which possess key functions in regulating the equilibrium between nutrient deposition and energy expenditure: Whereas white adipose tissue (WAT) serves as storage organ for excess nutrients, brown adipose

tissue (BAT) dissipates the proton gradient across mitochondrial membranes to generate heat via the BAT-intrinsic uncoupling protein 1 (UCP1; Bartelt and Heeren, 2012). The accumulation of excess lipids that leads to low-grade inflammation in WAT has been linked to the development of insulin resistance in obese patients (Saltiel and Kahn, 2001; Gregor and Hotamisligil, 2011; Glass and Olefsky, 2012). Also, impaired BAT thermogenesis can contribute to the development of insulin resistance and obesity (Connolly et al., 1982; Feldmann et al., 2009). The fact that lncRNAs are implicated in the differentiation of adipose tissues (adipogenesis) is exemplified by the lncRNA *SRA*, which is required for full trans-activation of the proadipogenic transcription factor Peroxisome proliferator-associated receptor gamma (Pparg). Concomitantly, RNAi-mediated *SRA* loss-of-function interfered with *in vitro* differentiation of 3T3-L1 preadipocytes (Xu et al., 2010). In a seminal study by Sun et al. (2013), the systematic implication of lncRNAs during adipogenesis was addressed. Using global transcriptome profiling of undifferentiated and mature adipocytes from the WAT and BAT lineages, the significant and specific regulation of 175 lncRNAs during adipogenesis was reported (Sun et al., 2013) of which a significant portion were enriched within adipose tissues. Finally, subsets of newly identified lncRNAs termed lncRAPs (lncRNAs Regulated in Adipogenesis) were depleted *in vitro* using siRNAs. Distinct lncRAPs, which were specifically upregulated during adipogenesis and were induced by the proadipogenic transcription factors *Cebpa* and *Pparg*, were required for timely and complete maturation of adipocyte progenitor cells. These studies provide first evidence for a crucial role of lncRNAs in the control of adipogenesis and fat cell metabolism.

DIFFERENTIATION OF SKELETAL MUSCLE AND CARDIOMYOCYTES

The differentiation of skeletal muscle cells (myogenesis) is regulated by a complex, yet well understood, evolutionarily conserved circuitry of protein-coding genes which control the timely growth, morphogenesis, and terminal maturation of muscle progenitors (myoblasts; Buckingham and Vincent, 2009). Here, the implication of noncoding RNAs was first shown via the contribution of myogenic miRNAs (myomiRs like miR-1 and miR-133) during myoblast commitment (Chen et al., 2006; Liu et al., 2008). Gong and Maquat (2011) reported that in human cells the degradation of distinct, nascent coding transcripts by Staufen-mediated decay (SMD) was regulated by lncRNAs. Here, the intermolecular base-pairing between *Alu* elements located within the 3'UTR of an SMD target and an *Alu* site localized within a class of lncRNAs called *1/2sbsRNAs* (*1/2*-STAU1-binding site RNAs) triggered SMD (Gong and Maquat, 2011). This process was interestingly conserved in rodents that lack canonical *Alu* repeats. Here, the mouse homologue of *1/2sbsRNA* was shown to be implicated in terminal differentiation of myoblast cells (Wang et al., 2013), indicating a function of lncRNAs in myogenesis. Another lncRNA termed *linc-MD1* is also critical for myogenesis. Here, increased levels of *linc-MD1* trigger the muscle differentiation program by acting as a natural decoy for myomiRs miR-133 and miR-135 (Cesana et al., 2011). MiR-133 and -135 in turn repress the expression of two pro-myogenic transcription factors, MAML1 and MEF2C. Recent reports also revealed that *linc-MD1* takes part

in a molecular feedforward circuit involving the promyogenic protein HuR (Legnini et al., 2014). Collectively, *linc-MD1* promotes terminal differentiation of myoblasts via acting as ceRNA for myogenic transcriptional regulators by sequestering anti-myogenic miRNAs. Interestingly, this complex ceRNA-based interplay of classical mRNAs, lncRNAs, and miRNAs was dysregulated in patients suffering from Duchenne muscular dystrophy (DMD), a condition of reduced terminal differentiation of myoblasts. Reinstating DMD-associated downregulation of *linc-MD1* expression via lentiviral delivery led to improved maturation of DMD myoblasts. In a study published by Klattenhoff et al. (2013), the heart-intrinsic lncRNA *Braveheart* (*Bvht*) was demonstrated to be required for differentiation of mesodermal progenitors toward mature cardiomyocytes via interaction with PRC2 epigenetic modifiers. This report for the first time implicated a tissue-specific lncRNA in maintaining cell fate during mammalian organogenesis.

REGULATION OF NEUROGENESIS BY lncRNAs

The discovery that peripherally secreted hormones such as insulin and leptin control energy homeostasis and glucose metabolism via CNS-acting neurocircuits expanded our understanding on how the body ingests, stores and dissipates energy (Belgardt and Brüning, 2010). In an approach to identify lncRNAs, which are implicated in brain development and neurogenesis, Aprea et al. (2013) utilized transgenic *in vivo* approaches to isolate neural stem cells, partially committed neuronal precursor cells as well as terminally differentiated neurons and quantified the expression of lncRNAs. Several lncRNAs were identified that were involved in neurogenesis, neuroblast commitment and neuron survival as shown for the neuroregulatory lncRNA *Miat*. Thus, maintenance of the neuron stem cell pool and terminal differentiation of neuron progenitors are also under lncRNA-mediated control. This will hopefully entail studies in the future specifically addressing the regulation of defined neuronal circuits, which regulate peripheral metabolic by lncRNAs.

REGULATION OF CIRCADIAN RHYTHM BY lncRNAs

The mammalian clock plays a fundamental role in the regulation of energy and glucose homeostasis. Dysregulation of the circadian rhythm underlies several metabolic pathologies like the development of insulin resistance and the metabolic syndrome (Marcheva et al., 2010; Hatori et al., 2013). In addition to the central clock located in the suprachiasmatic nucleus (SCN) of the pineal gland in the CNS, subordinate, tissue-specific clocks exist which are also key for the regulation of diurnal aspects of lipid metabolism, oscillations in core body temperature and timely insulin secretion from pancreatic beta cells (Cretenet et al., 2010; Marcheva et al., 2010; Gerhart-Hines et al., 2013). Interestingly, as found for plants (Hazen et al., 2009), lncRNAs are involved in the regulation of vertebrate circadian systems. A study published by Coon et al. showed that 112 lncRNAs are differentially expressed between day/night within the pineal gland of rats (Coon et al., 2012). An in-depth investigation of eight highly rhythmic lncRNA revealed the pivotal role of neuronal projections from the SCN as well as external zeitgebers like light exposure onto periodicity and amplitude of circadian lncRNAs. In addition, a report from

Vollmers et al. (2012) observed that rhythmic expression of ncRNAs like NATs, lncRNAs and miRNAs leads to rhythmic chromatin modifications in the liver. Noteworthy, the circadian oscillator component *Per2* itself is controlled by an antisense lncRNA termed *asPer2*. Reports about the brain-derived regulation of circadian metabolism remain scarce yet the pathogenesis of Prader–Willi syndrome (PWS), a CNS-controlled genetic disorder circadian rhythm with an associated dysregulation of metabolism and the development of obesity, was shown to be influenced by a PWS-associated lncRNA called *116G*. After splicing, a lncRNA consisting of the remnants of *116G* (termed *116HG*) bound to the transcriptional activator RBBP5 and ensures a physiological circadian rhythm in the brain. Mice deficient for *116HG* exhibit metabolic disorders due to the dysregulation of diurnally expressed circadian genes like *Clock*, *Cry1*, and *Per2* in the CNS (Powell et al., 2013).

EMERGING CONCEPT: INTERCELLULAR COMMUNICATION BY EXOSOMAL lncRNAs?

Exosomes are small vesicles generated by budding of the plasma membrane and constitute a specific vehicle for intercellular communication. Upon release from donor cells, exosomal surface motifs serve as “address codes” for binding and endocytosis on acceptor cells. Specific exosomal shuttling RNAs (esRNAs) such as miRNAs can be packaged into exosomes and released after binding to recipient cells, thus constituting a novel and intriguing way for ncRNAs to regulate systemic aspects of metabolism (Ramachandran and Palanisamy, 2012). Similarly, the intercellular transport of high-density lipoproteins (HDL)-bound miRNAs that are released by distinct donor cells influence the miRNA profile of acceptor cells and concomitantly alter the gene expression in HDL-recipient target tissues (Vickers et al., 2011). Of note, deep sequencing of human exosomes revealed that lncRNAs are localized within micro-vesicles and may emerge as novel means of cellular communication (Huang et al., 2013). Although experimental proof of concept is still lacking, the endocrine transfer of exosomal lncRNA might represent a novel facet relevant for lncRNA-mediated control of metabolism.

THERAPEUTIC OPPORTUNITIES OF lncRNA INHIBITION

A high economic interest lies in the development of sequence-specific compounds for the inhibition of disease-associated ncRNAs. Short, chemically modified ribonucleic acid compounds like locked nucleic acids (LNAs) efficiently silence the expression of ncRNAs such as miRNAs and are generally well tolerated *in vivo* (Krutzfeldt et al., 2005; Esau et al., 2006; Elmen et al., 2008). These anti-RNA compounds were initially tested in mice (Krutzfeldt et al., 2005) and adopted to the non-human primate situation (Elmen et al., 2008) with unprecedented speed. This approach will hopefully be extended to disease-associated lncRNA in the near future. Most *in vivo* studies to date concentrated on disease-associated miRNAs, that were critically involved in the development of insulin resistance and the deterioration of metabolic health (Jordan et al., 2011; Trajkovski et al., 2011; Zhou et al., 2012; Kornfeld et al., 2013). In contrast, most insights concerning the metabolic functions of lncRNAs were inferred from *in vitro* studies. The rising numbers of lncRNA knockout models

[exemplified by a recent report on 18 lncRNA loss-of-function mouse models (Sauvageau et al., 2013)] showcase that in order to convincingly assess, whether lncRNAs are implicated in the *in vivo* control of metabolism, further animal models for lncRNA loss- and gain-of-function are needed. This is of timely importance as systemic antisense oligonucleotide (ASO)-mediated inhibition of disease-associated lncRNAs (even in difficult to target organs like skeletal muscle) effectively improves degenerative diseases like myotonic dystrophy type 1 (DM1) in mice (Wheeler et al., 2012).

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