Roles of Long non-coding RNAs in Cellular Stress Response

ANSHIKA GOENKA¹ and SUBRAMANIAM GANESH*
Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 208 016, India
¹Current address: Syngene International Ltd., Biocon Park, Bengaluru 560 099, India

(Received on 20 December 2017; Revised on 22 February 2018; Accepted on 08 March 2018)

Cellular systems are often exposed to variations in their environment and, as a consequence, the cell systems have evolved a variety of pathways to promote cell survival during such challenges. Such “pro-survival” cellular pathways, often referred to as cellular stress response pathways, involve intricate cellular signalling networks, some of which are evolutionarily conserved. Given that such response mechanisms have cascading effects on the cellular physiology, it is not unexpected that regulatory forms of long non-coding RNAs (lncRNAs) play critical roles in these processes as well. This short review focuses on the regulatory roles of lncRNAs in transcriptional control during cellular stress response in higher vertebrates. Here, we elaborate on a few recent examples from the mammalian systems on the role of lncRNAs in the heat shock response process.

Keywords: Long Non-coding RNAs (lncRNAs); Cellular Stress Response; Heat Shock Response; Transcriptional Regulation

The mRNA-centric paradigm of the human transcriptome had undergone a fundamental change with the advent of new generation of DNA and RNA sequencing technologies. With the “rediscovery” that the genome is pervasively transcribed (Kapranov et al., 2002; Rinn et al., 2003), it is now evident that RNA does not simply act just as a messenger molecule but directly regulates almost all the cellular processes (Lakhotia, 2017; Beckmann et al., 2016, Roundtree et al., 2017). Moreover, it is widely acknowledged now that the organismal complexity correlates to the non-coding content of the genome and not to its coding portion (Huttenhofer et al., 2005; Kung et al., 2013; Brosius, 2014; Lakhotia, 2017), thus signifying the non-coding component of the genome. The domain of non-coding RNA (ncRNA) biology was revolutionized with the sequencing of the human genome, which identified that the human genome encodes thousands of regulatory non-coding RNA, both small (<200 bp) and long (>200 bp) forms. The small regulatory ncRNAs consists of the microRNAs (miRNA) and the Piwi-interacting RNAs (piRNA), whereas those greater than 200bp are termed long non-coding RNA on the basis of a convenient practical cut-off in RNA purification protocols that excludes short RNAs (Kapranov et al., 2007). While miRNAs are mainly involved in post-transcriptional gene regulation events, piRNAs protect the integrity of the genome from invasion by genomic parasites such as transposable elements by silencing them. The long non-coding RNAs (lncRNAs), on the other hand, have crucial roles in the regulation of gene expression both in development and differentiation. Intriguingly, the number of lncRNAs in a species shows a positive correlation with genome complexity, indicating the RNA-based control in the evolution of multicellular organisms (Fatica and Bozzoni, 2014). The lncRNAs are greater than 200 nucleotides in length; these are often poly-adenylated and are devoid of an open reading frame (ORF) (Derrien et al., 2012). Further, they have the dual ability to function as a ligand for proteins involved in gene regulation processes as well as to mediate base pairing interactions which guide lncRNA containing complexes to specific RNA or DNA target sites. Another remarkable virtue of lncRNAs is their unique ability to fold into complex

*Author for Correspondence: E-mail: sganesh@iitk.ac.in
secondary and higher order structures that provides higher accessibility to both proteins and target recognition sites (Batista and Chang, 2013; Guttman and Rinn, 2012; Rinn and Chang, 2012). The flexible (Zappulla and Cech, 2004) and modular (Tsai et al., 2010; Wutz et al., 2002) nature of IncRNAs enable them to tether proteins together, which otherwise would not have been able to interact. Thus IncRNAs regulate both transcriptional and post-transcriptional events. Such regulation is observed both in normal physiological conditions as well as during cellular stress response. This short review, as the title suggests, would mainly focus on some of the recent discoveries on the role of IncRNAs in cellular stress response in species that show homeostasis, such as mammals.

The cellular system is constantly subjected to stress in response to a variety of conditions such as a transient exposure to hot or cold temperatures, heavy metals, exogenous chemicals, oxidative stress, salt, and pH shifts among others (Morimoto, 1998; Fulda et al., 2010). The cellular systems have evolved remarkable combat processes to cope up with such transient stressors by mounting “cellular stress responses” which are essentially pro-survival mechanisms. Activation of the stress responses results in a reorganization of cellular physiology to support survival (Fulda et al., 2010). Such a response generally involves repression of the basal physiological processes of the cell, including the transcription, translation and splicing processes, and diversion of the energy saved to initiate the stress response pathways. For example, during a heat shock exposure, the general transcription and translation processes in the cell are repressed but there is an enhanced synthesis of the heat shock family of proteins, called heat shock proteins (HSPs), to prevent misfolding of proteins during the stress (Morimoto, 1998; Panniers, 1994). Intriguingly, IncRNAs are known to regulate heat shock response pathways at multiple levels. In this review, we would cover the recent discoveries on four such IncRNAs, namely the HSR1 (Heat Shock RNA 1), NEAT1 (nuclear paraspeckle assembly transcript 1), Alu RNA and the Satellite III RNA.

The Heat Shock RNA 1 (HSR1) IncRNA

Heat Shock Factor 1 (HSF1) – a highly conserved transcription factor – is the master regulator of the heat shock response pathway (Morimoto, 1998; Akerfelt et al., 2010). Under physiological conditions, the HSF1 protein is rendered inactive through an interaction with HSPs (Voellmy, 2004). Upon heat shock, the HSF1 monomers are released from the complex, and they trimerize and translocate to the nucleus to bind to sequence motifs termed as heat shock elements (HSEs) in the promoter regions of genes upregulated in response to heat shock (Kugel and Goodrich, 2006). Most often, such genes code for the HSPs. Intriguingly, in mammalian cells, the heat shock is known to induce the activation of HSF1 by forming a complex with a lncRNA and the translation elongation factor eEF1A (Shamovsky et al., 2006). The IncRNA, named HSR1 (for Heat Shock RNA 1), is polyadenylated, constitutively expressed, and its expression level is altered during the heat shock (Shamovsky et al., 2006). The presence of HSR1 is essential for the cells to mount effective heat shock response. Since the translation elongation factor eEF1A is involved in the HSR1-mediated activation of HSF1 during a heat shock, it could be argued that the heat shock-induced translation arrest may well be regulated by the HSR1 (Kugel and Goodrich, 2006). More recent studies suggest that the HSR1 sequences are evolutionarily conserved (Choi et al., 2015), and that the mammalian counterpart of the HSR1 could have a bacterial origin, possibly via the horizontal gene transfer or through an infection process (Kim et al., 2010; Lakhota, 2012; Choi et al., 2015).

The Alu and B2 SINE lncRNAs

The short interspersed elements (SINEs) represent a type of abundant repetitive sequences actively transcribed by the RNA polymerase III in the mammalian genome (Borodulina et al., 1999). The resulting ncRNAs, spanning about 200 bases, are known to have a 5’ end sequence similar to tRNA-like sequence (Daniels and Deininger, 1985; Wilusz et al., 2008). Intriguingly, the SINE elements show species-specific repeat motifs, though the SINES per se are retrotransposons (Kazazian, 2004). For example, in mouse, the SINES code for two distinct types of ncRNAs- the B1 and B2 class while in humans SINE code for only one type that is the AluRNA (Kassube et al., 2013). Exposure to a heat shock is known to increase the expression levels of Alu transcripts in human and the B2 transcripts in mouse (Liu et al., 1995; Kim et al., 2001; Fornace et
Non-coding RNAs in Cellular Stress Response

(Chen and Yang, 2017), regulating gene expression, such as alternative splicing, RNA editing, translation, and miRNA expression and function (Chen and Yang, 2017), suggesting B2/Alu transcripts may regulate gene expression at multiple steps.

The Satellite III lncRNAs

One of the intriguing observations regarding HSF1 is that the formation of nuclear stress granules in human cells. Exposure of human fibroblasts to heat shock results in the recruitment of HSF1 to discrete foci in the nucleus, which are referred to as the nuclear stress bodies (nSBs) (Jolly et al., 1997). Subsequent studies have shown that the recruitment of HSF1 into the nSBs is induced by the expression of a lncRNA, called the Satellite III transcripts (Sat3). These transcripts, ranging in length from 2 to 6 kb are detected only when the cells are exposed to stress such as heat shock and are induced by HSF1 (Metz et al., 2004; Rizzi et al., 2004; Sengupta et al., 2009). The Sat3 transcripts are characterized by the presence of a consensus GGAAT repeat motif, and such repeat tracts in the DNA are often associated with the pericentromeric regions of the human chromosomes (Jolly et al., 2002; Valgardsdottir et al., 2005). Studies have shown that the heat shock-induced Sat3 transcripts accumulate at the site of their synthesis to form the nSBs (Metz et al., 2004; Rizzi et al., 2004). While the 9q12 locus appears to be the primary locus for the Sat3-positive nSBs (Metz et al., 2004; Rizzi et al., 2004), studies did indicate that Sat3 could be induced at several other chromosomal loci, and their expression could be dependent upon the extent or the type of stressors (Sengupta et al., 2009; Eymery et al., 2010). Besides HSF1, the nSB were found to recruit CREB binding protein (CBP), RNA polymerase II, splicing factors/RNA binding proteins (SF2/ASF or SRSF1) and several heterogeneous nuclear ribonucleoproteins (hnRNPs) (Chiodzi et al., 2004; Denegri et al., 2001; Jolly et al., 2004; Weighardt et al., 1999). Recent studies have also shown that except for the HSF1, the other known components of the nSBs require the presence of Sat3 transcript for the association with the nSBs, suggesting a scaffold-like function for the Sat3 transcripts in the formation of nSBs (Metz et al., 2004; Goenka et al., 2016).

A number of possible functions have been ascribed to the Sat3 transcripts in heat shock response (Jolly and Lakhotia, 2006). These include chromatin remodeling, alternative splicing and transcriptional regulation (Jolly et al., 2004; Jolly and Lakhotia 2006; Biamonti and Vourc’h, 2010; Zong et al., 2011; Morimoto and Boerkoel, 2013; Kawaguchi and Hirose, 2015; Goenka et al., 2016). One of the recent studies has shown that the Sat3 transcripts could mediate heat shock-induced transcriptional arrest (Goenka et al., 2016). The study demonstrates that Sat3 transcripts sequester transcriptional factors, such as CBP, on the nSBs thus making them unavailable for the transcriptional activity. The splicing factor SRSF1 appears to be the critical protein that helps CBP to be sequestered on the Sat3 positive nSBs. Intriguingly, ectopic overexpression of Sat3 repeat-bearing transcripts mimicked heat shock response in human cells even when not exposed to a heat shock. The overexpressed Sat3 formed nSBs, recruited SRSF1 and CBP onto the nSBs, and reduced the expression levels of genes that are normally down regulated during the heat shock exposure, suggesting that the Sat3 transcript is a key player in the heat shock-induced transcriptional suppression of a few of the genes in the human cells (Goenka et al., 2016). The mechanism proposed for the Sat3 transcripts during the heat shock response is very similar to the observations made for the hsr omega lncRNAs in Drosophila, a proposed functional homologue of Sat3 in flies (Jolly and Lakhotia, 2006; Mallik and Lakhotia, 2009; Mallik and Lakhotia, 2010), suggesting a parallel evolution for these two transcripts in diverse species such as...
humans and flies. Given that these IncRNAs negatively regulate gene expression, and that loss of hsr omega ameliorates Huntington disease phenotype in a Drosophila model, the possible roles of Sat3 transcripts in the etiology of neurodegenerative disorders needs to be thoroughly investigated. Emerging evidence suggests that inclusions formed in degenerating neurons sequester transcription factors, and thus may bring about transcriptional dysregulation in the neuron. For example, the TAR DNA-binding protein of 43 kDa (TDP-43) was shown to recruit RNA polymerase and other transcription factors in neurons of patients with amyotrophic lateral sclerosis (ALS) contributing to transcriptional dysregulation (Yamashita et al., 2014). A similar mechanism could operate for the Sat3 transcripts, wherein the sequestration of CBP to the nSBs is responsible for the transcriptional repression of genes during the oxidative stress and in the transcription dysregulation observed in neurodegeneration (Goenka et al., 2016). Our ongoing investigations in the laboratory indicate that the Sat3 transcripts are induced in the neurons exposed to oxidative stress and that these transcripts are expressed in the degenerating neurons of patients with Alzheimer’s disease (AD) or Parkinson disease (PD) (Goenka et al., unpublished observations). Thus, the prolonged expression of Sat3 due to the chronic physiological stress experienced by the neurons might mimic chronic heat stress and might contribute to neurodegeneration. Thus, it is tempting to speculate that suppression of Sat3 might delay the neurodegenerative process in AD and PD, analogous to the observations that were made for the hsr omega transcript in the Drosophila model of HD (Mallik and Lakhota, 2009; Mallik and Lakhota, 2010).

The Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) IncRNA

The NEAT1 IncRNA is an essential structural element of the nuclear body paraspeckle and was originally shown to be transcribed from the chromosomal locus associated with the familial endocrine neoplasia (Guru et al., 1997). Intriguingly NEAT1 IncRNA is induced in response to hypoxia conditions (Choudhry et al., 2015). Studies have shown that the NEAT1 expression is regulated by the hypoxia-inducible factor, HIF-2α transcription factor activated by the hypoxic condition (Choudhry et al., 2015). Analogous to the functions of HSF1 in the heat shock response, the HIF2 regulates the expression of a number of genes during a hypoxic condition both to improve oxygen delivery and to reduce oxygen demand – a specific stress response mechanism (Majmundar et al., 2010). The HIF2-induced NEAT1 expression results in increased number of paraspeckles in the cells during a hypoxia (Choudhry et al., 2015). Though the specific cellular functions of paraspeckles are not fully understood, emerging reports suggest that paraspeckles might regulate transcriptional and post-transcriptional processes (Hata et al., 2008; Torres et al., 2017). The increased NEAT1 expression is associated with enhanced cell survival and proliferation and conversely, breast cancer patients with increased NEAT1 expression show poor survival (Choudhry et al., 2015), suggesting a pro-cell survival function for the NEAT1 mediated paraspeckles (Choudhry and Mole, 2016). Moreover, silencing NEAT1 in mice sensitized preneoplastic cells to DNA-damage-induced cell death and impaired skin tumorigenesis (Adriaens et al., 2016).

Stress-induced IncRNAs: A Field on the Horizon

With the advent of functional high-throughput screening and sequencing systems, several novel IncRNAs have recently been found and a few more have been shown to be involved in the cellular stress response pathways. One such novel example is the p53-regulated IncRNA named TRINGS (Tp53-regulated inhibitor of necrosis under glucose starvation) which is found to protect tumor cells from cell death as opposed to the classical function of p53 to prevent malignant transformation. Upon glucose starvation, TRINGS IncRNA is upregulated in human tumor cells and inhibits the STRAP-GSK3β necrotic signaling to protect tumor cells from cell death (Khan et al., 2017). Another IncRNA TERRA (TElomeric Repeat containing RNA) is found to be involved in the protection of telomere DNA during stress. TERRA is upregulated during heat stress upon binding of HSF1 to the subtelomeric DNA. Notably, the knockdown of HSF1 impairs telomere integrity and enhances the telomeric DNA changes as TERRA does not get activated during heat stress in HSF1 deficient cells (Koskas et al., 2017). Similarly, a few more hypoxia-induced IncRNAs have been discovered in cancer since hypoxic regions are common in solid tumors. Some of these examples include NEAT1 (up-
regulated in breast cancer), *H19* (up-regulated in p53 null mouse) and *UCA1* (upregulated in bladder cancer), and all of them are regulated by hypoxia (Chang *et al.*, 2016). Intriguing, cellular senescence, a complex cellular process experience multiple adverse stimuli such as replicative stress, DNA damage, oxidative stress or oncogene, is known to associate with the expression of a few lncRNAs. For example, *ANRIL* lncRNA which is decreased during replicative senescence leading to transcription repression of the CDKN2A/CDKN2B gene locus involved in the regulation of the cell cycle (Abdelmohsen *et al.*, 2013). The expression of *HOTAIR* lncRNA is known to increase during the replicative and irradiation-induced senescence to act as a scaffold for ubiquitin ligases thereby facilitating the ubiquitination of a few targets proteins to prevent premature senescence (Montes *et al.*, 2016). Accumulation of senescent cells eventually lead to age related disorders, thus it would be of importance to study the role of lncRNAs in the aging process.

Stress response pathways involve a variety of regulatory networks in the cellular systems, and therefore it is not unexpected that lncRNAs are found to be involved in these processes. Given that such response mechanisms have cascading effects, the ncRNAs appear to have been selected for a diverse set of functions (Lakhotia, 2012). With the “rediscovery” that the “junk DNA” do have functional roles, and that “junk DNA” do get transcribed to form non-coding transcripts with critical regulatory roles (reviewed in Lakhotia, 2017), the coming decade is expected to uncover hitherto unknown functions for lncRNAs in the normal and in the abnormal cellular physiology.

**Acknowledgments**

The authors wish to thank Professor S C Lakhotia and Dr Rashmi Parihar for their critical inputs on the manuscript. We also wish to thank the anonymous referees for the constructive comments on an earlier version of the manuscript. The work on non-coding RNA and stress response pathways in the author’s laboratory was supported by a research grant from the Department of Biotechnology (BT/HRD/35/01/01/2017) to SG.

Conflict of interest: None to declare.

**References**


Brosius J (2014) The persistent contributions of RNA to eukaryotic genome architecture and cellular function *Cold Spring Harb Perspect Biol* 6 a016089


Chen L L and Yang L (2017) ALUternative Regulation for Gene Expression *Trends Cell Biol* 27 480-490


Daniels G R and Deininger P L (1985) Repeat sequence families derived from mammalian tRNA genes Nature 317 819-822

Denegri M, Chiodi I, Corioni M, Cobianchi F, Riva S and Biamonti G (2001) Stress-induced nuclear bodies are sites of accumulation of pre-mRNA processing factors Mol Biol Cell 12 3502-3514


Fatica A and Bozzoni I (2014) Long non-coding RNAs: new players in cell differentiation and development Nat Rev Genet 15 7-21


repression by noncoding RNAs that bind to human Pol II
*J Mol Biol* 425 3639-48

Kawaguchi T and Hirose T (2015) Chromatin remodeling complexes in the assembly of long noncoding RNA-dependent nuclear bodies *Nucleus* 6 462-467


Khan M R, Xiang S, Song Z and Wu M (2017) The p53-inducible long noncoding RNA TRINGS protects cancer cells from necrosis under glucose starvation *EMBO J* 36 3483-3500


Mallik M and Lakhota S C (2010) Improved activities of CREB binding protein, heterogeneous nuclear ribonucleo proteins and proteasome following down regulation of noncoding hSromega transcripts help suppress poly(Q) pathogenesis in fly models. *Genetics* 184 927-945


Morimoto M and Boerkoel C F (2013) The role of nuclear bodies in gene expression and disease *Biology (Basel)* 2 976-1033


Panniers R (1994) Translational control during heat shock *Biochimie* 76 737-747


circadian gene expression Nucleus 8 249-254