Published Online on 10 May 2016

Proc Indian Natn Sci Acad **82** No. 4 September 2016 pp. 1171-1182

DOI: 10.16943/ptinsa/2016/v82/48404

Special Lecture

Non-coding RNAs have Key Roles in Cell Regulation[#]

S C LAKHOTIA*

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

(Received on 12 February 2016; Revised on 31 March 2016; Accepted on 03 April 2016)

As a corollary to the "central dogma of molecular biology" that genetic information carried in DNA is utilized to produce proteins which determine the phenotype, concepts of "junk" or "selfish" DNA were advanced to explain the "C-value paradox", leading to ignorance of parts of genomes that were not involved in protein synthesis. However, the ever-increasing numbers of studies during the past 10-15 years have confirmed that bulk of the nuclear DNA is indeed transcribed and that the non-coding transcripts actually provide a complex multi-layered regulatory network essential for the self-organized state. Commensurate with the evolutionary increase in biological complexity, the "non-coding" RNAs (ncRNAs) have also diversified. This brief review highlights the various classes of ncRNAs in eukaryotes taking examples of actions of some of the earliest known long ncRNAs like the Xist and roX, implicated in dosage compensation in mammals and *Drosophila*, respectively, and the hsrω long ncRNAs of *Drosophila*. Among the 7 transcripts produced by the *Drosophila hsrω* gene, the long nuclear transcripts that contain >5kb of tandem repeat sequences organize the omega speckles, which act as nucleoplasmic stores of a variety of RNA-binding and some other proteins to regulate their availability. Such long ncRNAs act as hubs in cellular networks through their interactions with diverse arrays of proteins. In view of the increasing evidence and realization of the importance of non-coding components of human and other genomes in maintaining normal homeostasis and because of their critical involvement in many human disorders, it is necessary to proactively explore their diversity and functions in different organisms.

Keywords: lncRNA; Xist; roX; *hsrω*; hnRNPs; Chromatin Remodelers

Non-coding RNAs Emerge from the Shadows of "Junk" and "Selfish" DNA

It is established that DNA is the genetic material in most organisms and that this information is utilized, as originally proposed by Crick in the "central dogma of molecular biology", to produce the mRNAs that are translated into various proteins; the proteins function as enzymes or structural components that carry out the various cellular activities and, thus determine the phenotype. The total DNA content (C-value) in genomes of different taxonomic groups of eukaryotes generally correlates with their evolutionary and biological complexity. However, there are many examples of very large differences in C-values in different species in a taxonomic group, including sometimes even between very closely related species (Britten and Davidson 1969). A more confounding fact is that in any given eukaryotic species, the genome contains much more DNA than required for production of the various proteins known or estimated to exist in the organism (Britten and Davidson, 1969; Ohno, 1972; Eddy, 2008). These anomalies, the "C-value paradox", have been perplexing geneticists, evolutionary biologists and molecular biologists for many decades. Indirect evidences obtained in 1960s and 1970s using painstaking experimental approaches, indicated that bulk of the nuclear DNA in many higher organisms was actually transcribed and that much of these transcripts were retained within the nucleus (Soeiro *et al.*, 1968; Shearer and McCarthy, 1967; Goldstein and Trescott, 1970; Weinberg, 1973).

**Author for Correspondence: E-mail: lakhotia@bhu.ac.in*

([#]based on a special lecture on 29th December, 2015 at the 81st Anniversary Meeting of the Indian National Science Academy held at the Indian Institute of Science Education and Research, Bhopal)

Significance, if any, of such transcripts was not understood at that time. As a corollary to the "central dogma" and to explain the C-value paradox, concepts of "junk" or "selfish" DNA were advanced (Ohno, 1972; Dollittle and Sapienza, 1980; Orgel *et al*., 1980) which suggested that bulk of the genomic DNA in eukaryotes, even if transcribed, is of no immediate consequence for the genome but it persists because of its "junk" or "selfish" nature. In view of the very wide and quick acceptance of the concept of "junk" or "selfish" DNA, the nature and significance of the diverse nucleus-retained transcripts reported earlier remained ignored and unknown. On the other hand, those seeking to understand the complex regulatory networks in eukaryotes did indicate that the increase in C-value in biologically more complex organisms was not due to more structural or protein-coding genes. Thus Britten and Davidson (1969) in their seminal paper on gene regulation networks stated "Quite possibly, the principal difference between a poriferan and a mammal could lie in the degree of integrated cellular activity, and thus in a vastly increased complexity of regulation, rather than a vastly increased number of producer genes. Much of the DNA accumulating in the genomes toward the upper end of the curve in Fig. 3 might then have a regulative function". Evolutionary biologists like Mayr (1970), worried about the consequences of considering only the structural or protein coding genes of significance, observed "day will come when much of population genetics will have to be re-written in terms of the interaction between regulator and structural genes". However, such prophetic views were nearly completely ignored as molecular biology and biotechnology marched ahead, believing dogmatically, in the "central dogma" and theories of "junk" and "selfish" DNA. This strong belief in selfish DNA prevented active search for possible functions of the large varieties of non-coding RNAs that were being identified in cells.

Realization in the 1990s that phenomena like quelling or transgene co-suppression (Cogoni *et al.*, 1996), post-transcriptional gene silencing, RNA interference or RNAi etc (Fire *et al.*, 1998; Cogoni and Macino, 2000) are dependent upon short non-coding RNAs (Hannon, 2002; Schramke and Allshire, 2004) encouraged appreciation of the involvement of RNA in regulatory circuits. These revelations catalyzed search for possible functions of other long non-coding RNAs (lncRNA), some of which, like the Xist in mammals, roX1 and roX2 and hsrω in *Drosophila*, and a few others (Lakhotia, 1996) had already been recognized to have functional significance, even while the shadow of selfish DNA continued to loom large.

The past two decades have witnessed a remarkable turnaround as an ever-increasing number of studies are confirming the earlier indirect evidence that bulk of the nuclear DNA is indeed transcribed so that the non-coding transcripts are now believed to provide a very complex multi-layered regulatory network essential for generating and maintaining the self-organized state of living organisms (Lakhotia, 1996, 2012; Bergman and Spector, 2014; Cech and Steitz, 2014; Rinn and Guttman, 2014; Shibayama *et al*., 2014; Fatima *et al.,* 2015; Iyer *et al.*, 2015; Jose, 2015; Quan *et al.*, 2015; Chujo *et al.*, 2016). With the evolutionary increase in biological complexity, the regulatory networks have also evolved to greater complexities and commensurate with this, the so-called "non-coding" transcripts too have diversified (Lakhotia, 1996, 1999, 2012; Szymanski *et al.*, 2003; Brosius and Tiedge, 2004; Costa, 2005; Clark and Mattick, 2011; Roberts *et al.*, 2013; Khalil *et al.*, 2013; Hirose *et al.,* 2014; Liebers *et al.*, 2014; Jiao and Slack 2014; Iyer *et al.*, 2015; Hirose and Nakagawa, 2016). It thus appears that, considering the "non-coding" DNA/RNA as "junk" or "selfish" was more a consequence of our lack of understanding, rather than being based on clear evidence. As was stated earlier (Lakhotia, 1996) 'non-coding transcripts are no longer mere curiosities or vagaries of the biological diversity. These seem to have established themselves as a distinct class of genes with very important functions. Understanding of the significance of such genes has been thwarted by the common "selfish genetic element" applied to them. …. With RNA being the first "living molecule", it is but to be expected that even today biological systems continue to utilize this versatile molecule directly'. Of course, the realization that RNA can function as RNA with phenotypic consequences has also necessitated a re-definition of "gene" (Lakhotia, 1997).

Diversity of Types and Functions of ncRNAs

The large variety of non-coding transcripts being identified in diverse organisms has been paralleled with a variety of names and classifications (Costa,

2005; Cech and Steitz, 2014; Hirose and Nakagawa, 2016). A common empirical grouping is based on length of the transcripts such that those less than 100-200 nucleotides are grouped as small ncRNAs, while, the longer ones are called long ncRNA (lncRNA). Several house-keeping non-coding transcripts like the rRNAs, tRNAs, snRNAs, snoRNAs etc have been recognized for long to have essential roles in translation of mRNAs and for maturation of the nascent hnRNAs and pre-rRNAs. Another functional class is often named as riboregulators and includes small ncRNAs like the miRNA, siRNA, piRNA etc, that are involved in gene silencing through different pathways including RNA-interference or RNAi (Grosshans and Filipowicz, 2008; Berezikov, 2011; Cloonan, 2015; Kalantari *et al.,* 2016). Ribo-switches are short segments of RNA that bind small molecules and switch between two different conformations and thereby regulate gene expression (Chen and Gottesman, 2014). Some ribo-switches increase translation by their interaction in trans with the target mRNA (Krishnamurthy *et al*., 2015), while others act in cis through structural motifs in UTRs of the mRNA (de la Fuente *et al*., 2012). Besides these, an increasing diversity of short and long ncRNAs are now known to regulate cellular activities in multiple ways that include; promoter activation, anti-sense transcriptional regulation and, more importantly, by providing sites for binding of proteins for sequestration or modulation of their activity and by affecting the higher order chromatin organization (Lakhotia *et al*., 1999; Lakhotia, 2011, 2012, 2015; Bergmann and Spector, 2014; Cech and Steitz, 2014; Legeai and Derrien, 2014; Bogu *et al*., 2015; Iyer *et al*., 2015; Quan *et al*., 2015; Betancur, 2016; Blythe *et al*., 2016; Chujo *et al*., 2016; Kanduri, 2016; Kashi *et al.*, 2016; Li and Wang, 2016; Wilusz, 2016; Yue *et al*., 2016).

Here, I will briefly illustrate key roles of lncRNAs in cellular regulation, taking examples of some of the earliest known ncRNAs, like Xist and roX, which affect chromatin organization across whole chromosome, and the hsrω nuclear transcripts in *Drosophila* that regulate the availability of a subset of RNA-binding and other proteins involved in diverse regulatory events.

Xist and roX lncRNAs Determine the Transcriptional Status of X-chromosomes in Mammals and *Drosophila*, respectively, in Opposing Ways.

Differences in the number of X chromosomes in males and females in mammals and several other groups, including *Drosophila*, are necessary for sex-determination so that while normal females have two X chromosomes, males have only one X chromosome. The homologue of X chromosome in males of these groups is the Y chromosome which is mostly devoid of genes that are present on the X chromosome. Since X chromosome in these organisms carry many genes that control a variety of functions independent of sex of the individual, this numerical difference in the copies of X-linked genes in males and females calls for a special regulatory mechanism, named dosage compensation (Muller, 1950). Equalization of expression of X-linked genes in somatic cells is achieved through inactivation of one of the two X chromosomes in female mammals and through hyperactivation of the single X chromosome in male *Drosophila* (Lyon, 1961; Mukherjee and Beermann, 1965; Smith and Lucchesi, 1969; Georgiev *et al.,* 2011; Gartler, 2014; Lakhotia, 2015). Roles of the lncRNAs like Xist (and several others, see below) in mammals and roX1 and roX2 in *Drosophila* in establishment and maintenance of the inactive-X and hyperactive-X in the two groups, respectively, have been extensively reviewed in recent years (Georgiev *et al.*, 2011; Koya and Meller, 2011; Horabin, 2012; Mank, 2013; Vallot and Rougeulle, 2013; Briggs and Reijo Pera, 2014; Chery and Larschan, 2014; Ferrari *et al.*, 2014; Gartler, 2014; Marchese and Huarte, 2014; Nakagawa and Kageyama, 2014; Peeters *et al.,* 2014; Keller and Akhtar, 2015; Lakhotia, 2015; Valencia and Wutz, 2015; Yue *et al.,* 2016; Betancur, 2016). A brief view of these lncRNAs is presented here to illustrate their pivotal roles in epigenetic modifications of chromatin organization at a whole chromosome level.

Soon after the X-inactivation hypothesis was proposed by Lyon (1961), a cis-regulatory X-inactivation centre (Xic) or X-controlling element was identified as the locus from which inactivation spreads in cis to bring about chromosome-wide inactivation of one of the two Xs in female somatic cells (Russell, 1963). Identification of the human *XIST* and mouse *Xist* (X-inactive specific transcript) non-coding genes (Brown *et al*., 1991; Brockdorff *et al*., 1991), that mapped at the Xic and were transcribed exclusively

from the inactive X, provided the then unexpected evidence for lncRNA to be essential for heterochromatinization of one of the two X chromosomes. The mouse Xist RNA is 15 kb with six exons, while the human XIST is 17 kb long with eight exons; these two transcripts show significant overall sequence divergence although 5 repetitive sequence motifs in exons 1-6 are relatively better conserved (Spusta and Goldman, 1999; Wutz *et al.*, 2002). The XIST/Xist is exclusively transcribed from the Xic of the inactive X and these transcripts spread along the length of X chromosome in cis to bring about its inactivation. XIST/Xist in combination with the Polycomb group repressive protein complex, PRC2, brings about di- or tri-methylation of H3K27 along the length of the XIST/Xist transcribing X chromosome to render it inactive (Pinter *et al.*, 2012; Marchese and Huarte, 2014). The X chromosome coated with XIST/Xist RNA gets organized into a compact and efficiently silenced Barr body chromatin through interaction with SATB1 (Brockdorff, 2009), while the nuclear matrix associated SAF-A/hnRNP U protein may act as a platform to immobilize Xist RNA along the X chromosome (Fackelmayer, 2005; Hasegawa *et al*., 2010). Recent studies have revealed that besides the XIST/Xist, the Xic region produces, in either directions, several other lncRNAs like Jpx, Ftx, RepeatA (RepA), Tsix, Xite, XACT etc which interactively promote or suppress Xist expression and thus X-inactivation (Vallot and Rougeulle, 2013; Briggs and Reijo Pera, 2014; Marchese and Huarte, 2014; Lakhotia, 2015; Yue *et al.*, 2016).

Unlike the inactivation of one X chromosome in mammalian female somatic cells, dosage compensation in *Drosophila* is achieved by the "hyperactive male X" model, so that its genes can transcribe at higher rates to produce nearly as many transcripts as the two Xs together in corresponding female cells (Mukherjee and Beermann, 1965; Lucchesi, 1998; Lucchesi *et al.*, 2005; Kelley *et al.*, 1999; Kelley and Kuroda, 2000; Mank, 2013; Straub *et al.*, 2013; Chery and Larschan, 2014; Ferrari *et al.*, 2014; Keller and Akhtar, 2015; Lakhotia, 2015). The *Drosophila* roX1 and roX2 lncRNAs are essential for epigenetic modifications of chromatin organization of the single X chromosome in male so that it is poised for the hyperactivity required for dosage compensation. Absence of the functional Sxl protein in early male *Drosophila* embryos (Lucchesi, 1998; Lucchesi *et al.*, 2005) triggers production of the male-specific lethal-2 (Msl-2) protein, a core component of the male specific dosage compensation complex (DCC) that catalyses the nearly two-fold up-regulation of transcriptional activity of the single X chromosome in males (Kelley *et al.*, 1997; Kelley and Kuroda, 2000; Georgiev *et al.*, 2011; Mank, 2013; Straub *et al.*, 2013; Chery and Larschan, 2014; Ferrari *et al.*, 2014; Keller and Akhtar, 2015; Lakhotia, 2015). The DCC includes the two lncRNAs, roX1 and roX2, and several proteins including male-specific lethal-1 (Msl-1), Msl-2 (RING finger protein), Msl-3 (chromodomain protein), Males-absent-on-the-first (Mof, histone acetyl transferase) and Maleless (Mle, DNA/RNA helicase), paints the male X chromosome along its length and thereby keeps the histone H4 hyperacetylated at lysine 16 (Kelley *et al.*, 1995; Lucchesi, 1998; Lucchesi *et al.*, 2005; Gelbart and Kuroda, 2009; Georgiev *et al.*, 2011; Lakhotia, 2015). These epigenetic modifications brought about by the DCC cause a greater opening of the single X chromosome in males, so that the active genes can transcribe at a higher rate to achieve dosage compensation. The roX1 and roX2 lncRNAs are critical for the orderly distribution of the DCC along the male X chromosome since absence of both of them disrupts dosage compensation and results in male lethality (Lucchesi, 1998; Lucchesi *et al.*, 2005; Chery and Larschan, 2014; Lakhotia, 2015).

It is interesting that as divergent organisms as mammals and *Drosophila* achieve dosage compensation through chromosome wide reorganization of chromatin to either inactivate or hyperactivate large domains and employ lncRNAs to epigenetically modify the chromatin in opposing manner. Such opposing effects of chromosome-wide "painting" with lncRNAs reflect the versatility of RNA molecules.

Multiple lncRNAs Produced by the *hsrω* Gene in *Drosophila* Integrate Several Regulatory Pathways to Maintain Cell Homeostasis

The *Drosophila 93D* gene, later named as *hsrω*, was one of the first developmentally active and cell stress-inducible gene to be identified as non-coding yet essential for viability of the organism (Lakhotia and Singh, 1982; Mohler and Pardue, 1982; Ryseck *et al.*, 1985; Garbe *et al.*, 1986; Lakhotia, 1987). Subsequent studies in our and some other labs (reviewed by

Lakhotia 2011) have unraveled unexpected complexities of the transcripts produced by this gene and the multiple functions of these diverse lncRNAs. As annotated at the Flybase (www.flybase.org), this gene produces 7 lncRNAs, ranging from 1.2 to ~21 kb, through alternative transcription start and termination sites and variable splicing of the single intron. In addition, 3 potential miRNAs from its 3' end are also predicted (see Fig. 1A). The smaller 1.2 kb cytoplasmic transcript (hsrω-c or hsrω-RA) is produced by splicing out the ~700b long intron in the 1.9 kb nuclear hsrω-pre-c or hsrω-RC. The hsrω-c includes a small translatable ORF (ORF-omega) which encodes a 27aa polypeptide (Fini *et al.*, 1989; Rashmi Ranjan Sahu and Lakhotia, unpublished). Very little is known about the recently annotated Hsrω-RD, Hsrω-RF and Hsrω-RH transcripts (www.flybase.org). The three longer nuclear transcripts (Hsrω-RB or Hsrω-n1, Hsrω-RG or Hsrω-n2 and Hsrω-RF) include 5-10 kb long stretch of tandem repeats of 280bp length that are unique to this locus (Lakhotia, 2011). It is very interesting although a homolog of the *hsrω* gene exists in all the species of *Drosophila* that have been examined, its base sequence varies significantly even between related species (Garbe *et al.*, 1989; Lakhotia, 2011).

A major focus of studies on the hsrω transcripts has been on the repeat containing nuclear Hsrω-RB (hsrω-n1) and Hsrω-RG (hsrω-n2) transcripts, which are present in the nucleoplasmic omega speckles and at the *hsrω* gene locus (Fig. 1B).The omega speckles act as stores for a variety of heterogeneous RNA-binding proteins (hnRNPs) and several other proteins (Table 1) and thus regulate their availability for transcription, RNA processing and other activities (Lakhotia *et al.*, 1999; Prasanth *et al.*, 2000; Lakhotia, 2011; Mallik and Lakhotia, 2011; Singh and Lakhotia, 2015a). A variety of cell stresses that disrupt the normal nuclear transcription and RNA processing lead to a rapid accumulation of the various omega speckle associated proteins almost exclusively at the *hsrω*

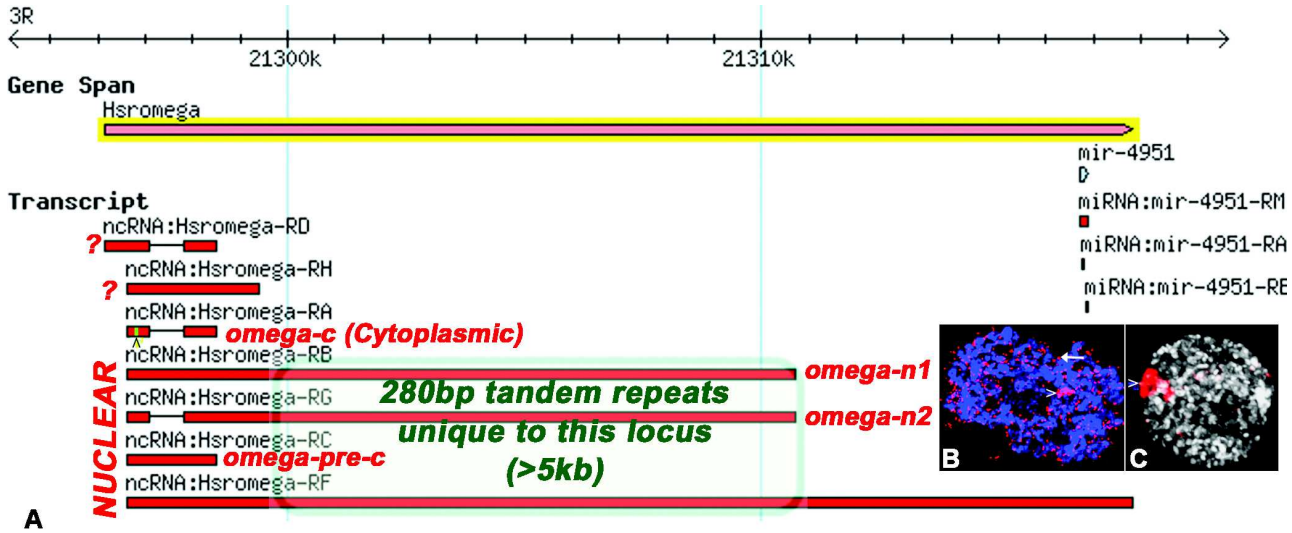

Fig. 1. The *hsrω* gene of *Drosophila melanogaster* produces multiple transcripts of which the 280bp repeat containing nuclear transcripts organize the omega speckles. A. Genomic coordinates and the multiple RNAs of the *hsrω* gene (see www.flybase.org); the single intronic region is indicated by thin line; cellular localization of some transcripts, where known, is indicated as Nuclear or Cytoplasmic and, where not clearly known, by "?". omega-c, omega-n1, omega-n2 and omega-pre-c refer to alternative common names of the indicated transcripts; the green vertical bar, marked with an arrowhead below the exon 1 of the omega-c (Hsromega-RA) transcript represents the ORF-omega that encodes a 27aa polypeptide; the region on three transcripts (green outlined box) indicates the region of tandem repeats of 280bp units that span about 5 to 10kb. B. Confocal projection image of unstressed late larval Malpighian tubule nucleus showing distribution of the 280bp repeat containing hsrω nuclear transcripts (red) at the gene locus (arrow head) and in the large number of nucleoplasmic omega speckles (arrow); the DAPI-stained chromatin is in blue. C. Confocal projection image of heat shocked (30min at 37ºC) *late larval Malpighian tubule nucleus showing Hrp36 protein (red) which is normally present in omega speckles and on active chromatin regions but gets nearly exclusively localized at the hsrω* gene locus (arrowhead) following heat shock; DAPI stained chromatin is in white. Images in B and C are provided by Dr. Sonali Sengupta and Dr. Anand K. Singh, respectively

gene locus (Fig. 1C), which is reversed as the cells recover from the stress (Prasanth *et al*., 2000; Lakhotia, 2011; Lakhotia *et al*., 2012). Absence or conditionally altered levels (down- or up-regulated) of these nuclear hsrω-lncRNAs affects the stress induced accumulation of hnRNPs and other proteins at the *hsrω* gene locus and their movement back during recovery to normal locations (Lakhotia *et al.*, 2012; Singh and Lakhotia, 2015a). Most interestingly, the restoration of RNA polymerase II to developmentally active gene sites in cells recovering from stress was also affected when these transcripts were conditionally up- or down-regulated during heat shock (Lakhotia *et al.*, 2012). All such individuals show delayed lethality, apparently because of a failure to restore normal gene activity. Live cell imaging in our lab has recently shown that when a cell is stressed, the omega speckles rapidly disappear in the nucleoplasm and the associated proteins move to *hsrω* gene locus in a diffuse form with assistance of some of the nuclear matrix associated proteins; the movement of these proteins to the *hsrω* gene locus is directly dependent upon its transcriptional activity (Singh and Lakhotia, 2015a). As cells recover from the stressful condition, the accumulated proteins and the hsrω transcripts rapidly emerge out of their caged state at the *hsrω* gene locus as fully formed omega speckles; several different chromatin remodeling proteins including the ISWI were shown to be essential for the biogenesis of omega speckles during recovery from stress as well as under normal cell conditions (Onorati *et al.*, 2011; Singh and Lakhotia, 2015a).

As shown in Table 1, the repeat containing hsrω nuclear transcripts have been shown to colocalize in normal and/or stressed cells with a variety of regulatory proteins including the RNA processing and transport proteins, chromatin remodelers, transcription regulators, nuclear matrix and nuclear lamina components, molecular chaperones, cell signaling proteins etc Proteins like inhibitors of apoptosis, proteasome components, some chromatin remodelers and members of dosage compensation complex have not been seen to colocalize with these transcripts, but they are known to interact genetically (Table 1).

It is significant that the proteins with which the larger nuclear transcripts have been found to associate or genetically interact are involved in some of the very important regulatory networks. For example the different hnRNPs are involved in a wide variety of RNA processing events, including alternative splicing, packaging, transport and translation (Daneholt, 2001; Guisbert *et al*., 2005; He and Smith, 2009; Chaudhury *et al*., 2010; Han *et al*., 2010; Piccolo *et al.,* 2014). In addition, some of them also have roles in chromatin organization (Piacentini *et al*., 2009), DNA repair (Smith and Jackson, 1999), cell signaling (Matter *et al*., 2000; Carpenter *et al*., 2006), telomere maintenance (La Branche *et al*., 1998; Singh and Lakhotia, 2015b) and in neurodegeneration (Sengupta and Lakhotia, 2006; Sofola *et al*., 2007; Mallik and Lakhotia, 2010). The other interacting proteins listed in Table 1, like chromatin remodelers, nuclear matrix components, histone acetyl transferases, Ras signaling pathway components, apoptosis regulating protein like DIAP1, proteasomal components and the Hsp83, are well known to have multiple connections in cellular regulatory networks. In view of such wide networking, it has been suggested (Arya *et al.,* 2007; Lakhotia, 2011, 2012) that the lncRNAs like those of the *hsrω* gene act as hubs in cellular networks and thereby help maintain cellular homeostasis.

It is very significant that like the omega speckles, most of the nuclear bodies (nucleolus, various speckled domains, Cajal bodies etc) are dependent upon distinct lncRNAs for their organization and function (Lakhotia, 2012; Kawaguchi and Hirose, 2011; Chujo *et al.*, 2016). These ncRNAs help keep the different proteins, RNAs and other regulatory molecules, involved in distinct sets of functions, in assorted compartments.

Non-coding RNAs Come of Age

Although in the past significance of such non-coding genes was not appreciated because they were commonly considered as "selfish genetic elements", the increasing numbers of original research articles and reviews that are being published in recent years confirm an early statement (Lakhotia, 1996) that the non-coding transcripts are "no longer mere curiosities or vagaries of the biological diversity". There is a widespread realization that the ncRNAs indeed constitute distinct classes of genes with very important functions (Lakhotia, 2012, 2015; Bergmann and Spector, 2014; Cech and Steitz, 2014; Legeai and Derrien, 2014; Iyer *et al.,* 2015; Quan *et al.,* 2015; Betancur, 2016; Blythe *et al.,* 2016; Chujo *et al.*,

Table 1. Proteins known to associate or genetically interact with the hsrω transcripts

Proteins colocalizing with nucleoplasmic omega speckles and/or with the *hsrω* gene locus	References
hnRNPs: Hrb87F or Hrp36 (hnRNP A1/A2), Hrb98DE or Hrp38 (hnRNPA), Hrb57A (hnRNP K), Rumpelstiltskin or Hrp59 (hnRNP M), Squid or Hrp40 (hnRNP D); NonA	Saumweber *et al.*,1980; Prasanth *et al.,* 2000; Onorati *et al.,* 2011; Singh and Lakhotia, 2015a
Other RNA processing proteins: Sxl, PEP	
Unidentified nuclear non-histone proteins: recognized by Q14, Q16, T29, P75 antibodies	
Nuclear matrix and lamina associated proteins: Tpr or Megator, Snf (Sans-fille), SAF B	Zimowska and Paddy, 2002; Singh and Lakhotia, 2015a
Molecular chaperone: Hsp83	Morcillo *et al.*,1993; Lakhotia and Ray,1996
Chromatin remodelers and transcription regulators: ISWI, HP1, Poly-adenosyl-ribose polymerase (PARP), CBP or P300 (histone acetyl transferase)	Ji and Tulin, 2009; Mallik and Lakhotia, 2010; Onorati *et al.*, 2011; Singh and Lakhotia, 2015a
Cell signaling: cGMP, Ubiquitin Specific Protease-7 (USP7), GMP Synthase (GMPS)	Spruill *et al.*,1978; Peter Verrijzer and Jan van der Knaap, personal communication
Proteins known to genetically interact with hsrω transcripts Cell signaling: Ras, Egfr, JNK Nuclear matrix and lamina: Lamin C Inhibitor of apoptosis: DIAP1 Proteasome complex	Ray and Lakhotia, 1996; Mallik and Lakhotia, 2009a, 2009b, 2010, 2011; Singh and Lakhotia, 2016
Chromatin remodelers: NURF 301, NURF 38, GCN5	Chaturvedi, D. and Lakhotia, unpublished
Dosage compensation complex: Msl-1, Msl-2, MOF	

2016; Hirose and Nakagawa, 2016; Kanduri, 2016; Kashi *et al.,* 2016; Li and Wang, 2016; Wilusz, 2016; Yue *et al.,* 2016).

It is true that functions of many of the identified ncRNAs are not yet known and in some cases they may appear to be without function since their absence does not seem to have any deleterious consequence. However, it should be realized that organisms do not live under the "ideal" conditions that prevail in laboratory setting where such studies are undertaken. Since many ncRNAs are now known to undergo changes in abundance and/or processing under diverse cell stress conditions (Lakhotia, 2012; Amaral *et al.*, 2013; Place and Noonan, 2013; Tani and Torimura, 2013; Sole *et al.,* 2015; Audas and Lee, 2016), functions of such ncRNAs need to be pursued more extensively under naturally varying environmental conditions or under conditions of applied stress. Then only it would be possible to uncover their subtle, yet very important roles in the organism's life.

As evident from the examples discussed here, a given lncRNA often targets more than one protein with key role/s in the cascade of regulatory events and, therefore, has a widespread integrative effect. Such integrative actions are important in the context of evolution, since living organisms have to continuously adjust their cellular activities in relation to the varying external and internal environmental conditions. Adaptability of related species depends on their ability to respond to the subtly varying environmental stresses. In this context, it is important to note that since proteins associate with RNA through short sequence motifs other regions of ncRNAs can accumulate sequence changes. Such rapid sequence divergence is indeed a common feature of many of ncRNAs, because of which they were earlier often ignored as "junk" or "selfish". When looked at from the adaptability point of view, the rapid divergence of ncRNA sequences actually provides elegant modules for adaptability to changing environment since it promotes novel RNA-protein interactions, which in turn can modulate the structure and functions of the interacting molecules in distinct ways (Lakhotia, 2012).

In view of increasing evidence and realization of the enormous importance of non-coding RNAs of

human and other genomes in maintaining normal homeostasis and because of their critical involvement in many human disorders, it is necessary that their diversity and functions in different organisms be proactively studied.

Acknowledgements

Stimulating and enthusiastic research work carried out by the former and present Ph.D. students and the various stimulating discussions with colleagues in the Cytogenetics Laboratory have significantly helped in pursuing studies on ncRNAs. The generous and "no-questions asked" approach of the fly community in sharing various reagents has been a remarkable support. I also thank the various funding agencies in India (University Grants Commission, Board of Research in Nuclear Sciences, Indian National Science Academy, Council of Scientific and Industrial Research, Department of Science & Technology and Department of Biotechnology) supporting my research at different times.

References

Amaral P P, Dinger M E and Mattick J S (2013) Non-coding RNAs in homeostasis, disease and stress responses: an evolutionary perspective *Briefings Functional Genomics* **12** 254-278

Arya R, Mallik M and Lakhotia S C (2007) Heat shock genes – integrating cell survival and death *J Biosciences* **32** 595-610

Audas T E and Lee S (2016) Stressing out over long non-coding RNA *Biochim Biophys Acta* **1859** 184-191

Berezikov E (2011) Evolution of microRNA diversity and regulation in animals *Nature Reviews Genetics* **12** 846-860

Bergmann J H and Spector D L (2014) Long non-coding RNAs: modulators of nuclear structure and function *Curr Opin Cell Biol* **26** 10-18

Betancur J G (2016) Pervasive lncRNA binding by epigenetic modifying complexes: the challenges ahead *Biochim Biophys Acta* **1859** 93-101

Blythe A J, Fox A H and Bond C S (2016) The ins and outs of lncRNA structure: how why and what comes next? *Biochim Biophys Acta* **185** 946-58

Bogu G K, Vizán P, Stanton L W, Beato, Di Croce L and Marti-Renom M A (2015) Chromatin and RNA maps reveal regulatory long noncoding RNAs in mouse *Molec Cellular Biol* **36** DOI: 10.1128/MCB.00955-15

Briggs S F and Reijo Pera R A (2014) X chromosome inactivation: recent advances and a look forward *Current Opi Genet Develop* **28** 78-82

Britten R J and Davidson E H (1969) Gene regulation for higher cells: a theory *Science* **165** 349-357

Brockdorff N (2009) SAT in silence *Dev Cell* **16** 483-484

Brockdorff N, Ashworth A, Kay G F, Cooper P, Smith S *et al.* (1991) Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome *Nature* **351** 329-331

Brosius J and Tiedge H (2004) RNomenclature *RNA Biol* **1** 81-83

Brown C J, Ballabio A, Rupert J L, Lafreniere R G, Grompe M *et al.* (1991) A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome *Nature* **349** 38-44

Carpenter B, McKay M, Dundas S R, Lawrie L C, Telfer C and Murray G I (2006) Heterogeneous nuclear ribonucleo protein K is over expressed, aberrantly localised and is associated with poor prognosis in colorectal cancer *British J Cancer* **95** 921-927

Cech T R and Steitz J A (2014) The noncoding RNA revolution-trashing old rules to forge new ones *Cell* **157** 77-94

Chaudhury A, Chander P and Howe P H (2010) Heterogeneous nuclear ribonucleoproteins (hnRNPs) in cellular processes: focus on hnRNP E1's multifunctional regulatory roles *Rna* **16** 1449-1462

Chen J and Gottesman S (2014) Riboswitch regulates RNA *Science* **345** 876-877

Chery J and Larschan E (2014) X-marks the spot: X-chromosome identification during dosage compensation *Biochim Biophys Acta* **1839** 234-240

Chujo T, Yamazaki T and Hirose T (2015) Architectural RNAs (arcRNAs): a class of long noncoding RNAs that function as the scaffold of nuclear bodies *Biochim Biophys Acta* **1859** 139-146

Clark M B and Mattick J S (2011) Long noncoding RNAs in cell biology *Seminars Cell Develop Biol* **22** 366-376

Cloonan N (2015) Re-thinking miRNA-mRNA interactions: intertwining issues confound target discovery *Bioessays* **37** 379-388

Cogoni C and Macino G (2000) Post-transcriptional gene silencing across kingdoms *Curr Opin Genet Develop* **10** 638-643

Cogoni C, Irelan J T, Schumacher M, Schmidhauser T J, Selker E U and Macino G (1996) Transgene silencing of the al-1 gene in vegetative cells of Neurospora is mediated by a cytoplasmic effector and does not depend on DNA-DNA interactions or DNA methylation *EMBO J* **15** 3153

Costa F F (2005) Non-coding RNAs: new players in eukaryotic biology *Gene* **357** 83-94

Daneholt B (2001) Assembly and transport of a premessenger RNP particle *Proc Natl Acad Sci USA* **98** 7012-7017

de la Fuente M, Valera S and Martínez-Guitarte J L (2012) ncRNAs and thermoregulation: a view in prokaryotes and eukaryotes *FEBS Letters* **586** 4061-4069

Doolittle W F and Sapienza C (1980) Selfish genes the phenotype paradigm and genome evolution *Nature* **284** 601-603

Eddy S R (2012) The C-value paradox junk DNA and ENCODE *Curr Biol* **22** R898-R899

Fackelmayer F O (2005) A stable proteinaceous structure in the territory of inactive X chromosomes *J Biol Chem* **280** 1720-1723

Fatima R, Akhade V S, Pal D and Rao S M (2015) Long noncoding RNAs in development and cancer: potential biomarkers and therapeutic targets *Molecular Cellular Therapies* **35** DOI: 101186/s40591-015-0042-6

Ferrari F, Alekseyenko A A, Park P J and Kuroda M I (2014) Transcriptional control of a whole chromosome: emerging models for dosage compensation *Nat Struct Mol Biol* **21** 118-125

Fini M E, Bendena W G and Pardue M L (1989) Unusual behavior of the cytoplasmic transcript of hsr omega: an abundant stress-inducible RNA that is translated but yields no detectable protein product *J Cell Biol* **108** 2045-2057

Fire A, Xu S, Montgomery M K, Kostas S A, Driver S E, and Mello C C (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans *Nature* **391** 806-811

Garbe J C, Bendena W G, Alfano M and Pardue M L (1986) A *Drosophila* heat shock locus with a rapidly diverging sequence but a conserved structure *J Biol Chem* **2611** 6889-16894

Gartler S M (2014) A brief history of dosage compensation *J Genet* **93** 591-595

Gelbart M E and Kuroda M I (2009) *Drosophila* dosage compensation a complex voyage to the X chromosome. *Development* **136** 1399-1410

Georgiev P, Chlamydas S and Akhtar A (2011) *Drosophila* dosage compensation *Fly* **5** 147-154

Gloss B S and Dinger M E (2016) The specificity of long noncoding RNA expression *Biochim Biophys Acta* **1859** 16-22

Goldstein L and Trescott O H (1970) Characterization of RNAs that do and do not migrate between cytoplasm and nucleus *Proc Natl Acad Sci USA* **67** 1367-1374

Georgiev P, Chlamydas S and Akhtar A (2011) *Drosophila* dosage compensation *Fly* **5** 147-154

Grosshans H and Filipowicz W (2008) Molecular biology: the expanding world of small RNAs *Nature* **451** 414-416

Guisbert K K, Duncan K, Li H A O and Guthrie C (2005) Functional specificity of shuttling hnRNPs revealed by genome-wide analysis of their RNA binding profiles *Rna* **11** 383-393

Han S P, Tang Y H and Smith R (2010) Functional diversity of the hnRNPs: past present and perspectives *Biochem J* **430** 379-392

Hannon G J (2002) RNA interference *Nature* **418** 244-251

Hasegawa Y, Brockdorff N, Kawano S, Tsutui K, Tsutui K and Nakagawa S (2010) The matrix protein hnRNP U is required for chromosomal localization of Xist RNA *Dev Cell* **19** 469-476

He Y and Smith R (2009) Nuclear functions of heterogeneous nuclear ribonucleoproteins A/B *Cell Molecular Life Sci* **66** 1239-1256

Hirose T and Nakagawa S (2016) Clues to long noncoding RNA taxonomy *Biochim Biophys Acta* **1859** 1-2

Hirose T, Mishima Y and Tomari Y (2014) Elements and machinery of non-coding RNAs: toward their taxonomy *EMBO Rep* **15** 489-507

Horabin J I (2012) Balancing sex chromosome expression and satisfying the sexes *Fly* **6** 26-29

Iyer M K, Niknafs Y S, Malik R, Singhal U, Sahu A *et al.* (2015) The landscape of long noncoding RNAs in the human transcriptome *Nature Genetics* **47** 199-208

Ji Y and Tulin A V (2009) Poly(ADP-ribosyl)ation of heterogeneous nuclear ribonucleoproteins modulates splicing *Nucleic Acids Res* **37** 3501-3513

Jiao A L and Slack F J (2014) RNA-mediated gene activation *Epigenetics* **9** 27-36

Jose A M (2015) Movement of regulatory RNA between animal cells *Genesis* **53** 395-416

Kalantari R, Chiang C-M and Corey D R (2016) Regulation of mammalian transcription and splicing by Nuclear RNAi *Nucleic Acids Res* **44** 524-537

Kanduri C (2016) Long noncoding RNAs: lessons from genomic imprinting *Biochim Biophys Acta* **1859** 102-111

Kashi K, Henderson L, Bonetti A and Carninci P (2016) Discovery and functional analysis of lncRNAs: Methodologies to

investigate an uncharacterized transcriptome *Biochim Biophys Acta* **1859** 3-15

Kawaguchi T and Hirose T (2011) Architectural roles of long noncoding RNAs in the intranuclear formation of functional paraspeckles *Front Bioscience* **17** 1729-1746

Keller C I and Akhtar A (2015) The MSL complex: juggling RNA-protein interactions for dosage compensation and beyond *Curr Opin Genet Dev* **31** 1-11

Kelley R L and Kuroda M I (2000) The role of chromosomal RNAs in marking the X for dosage compensation *Curr Opin Genet Dev* **10** 555-61

Kelley R, Solovyeva I, Lyman L, Richman R, Solovyev V and Kuroda M I (1995) Expression of msl-2 causes assembly of dosage compensation regulators on the X chromosomes and female lethality in *Drosophila Cell* **81** 867-877

Kelley R L, Meller V H, Gordadze P R, Roman G, Davis R L and Kuroda M I (1999) Epigenetic spreading of the *Drosophila* dosage compensation complex from roX RNA genes into flanking chromatin *Cell* **98** 513-522

Kelley R L, Wang J, Bell L and Kuroda M I (1997) Sex lethal controls dosage compensation in *Drosophila* by a non-splicing mechanism *Nature* **387** 195-199

Khalil A M and Coller J (eds) (2013) *Molecular biology of long non-coding RNAs* Springer New York

Koya S K and Meller V H (2011) roX RNAs and genome regulation in *Drosophila melanogaster Progr Molec Subcellular Biol* **51** 147-160

Krishnamurthy M, Hennelly S P, Dale T, Starkenburg S R, Martí-Arbona R, Fox D T and Unkefer C J (2015) Tunable riboregulator switches for post-transcriptional control of gene expression *ACS Synthetic Biology* **4** 1326-1334

LaBranche H, Dupuis S, Ben-David Y, Bani M R, Wellinger R J and Chabot B (1998) Telomere elongation by hnRNP A1 and a derivative that interacts with telomeric repeats and telomerase *Nature Genetics* **19** 199-202

Lakhotia S C (1987) The 93D heat shock locus in *Drosophila*: a review *J Genet* **66** 139-157

Lakhotia S C (1996) RNA polymerase II dependent genes that do not code for protein *Ind J Biochem Bhiphys* **33** 93-102

Lakhotia S C (1997) What is a gene? A question with variable answers Part 2 *Resonance* **2** 44-53

Lakhotia S C (1999) Non-coding RNAs: versatile roles in cell regulation *Curr Sci* **77** 479-480

Lakhotia S C (2011) 40 years of the 93D puff of *Drosophila melanogaster J Biosciences* **36** 399-423

Lakhotia S C (2012) Long non-coding RNAs coordinate cellular responses to stress *WIREs RNA* **3** 779-796

Lakhotia S C (2015) Divergent actions of long non-coding RNAs on X-chromosome remodelling in mammals and *Drosophila* achieve the same end result-dosage compensation *J Genetics* **94** 575-584

Lakhotia S C, Mallik M, Singh A K and Ray M (2012) The large non-coding hsrω-n transcripts are essential for thermotolerance and remobilization of hnRNPs HP1 and RNA polymerase II during recovery from heat shock in *Drosophila Chromosoma* **121** 49-70

Lakhotia S C and Ray P (1996) *hsp83* mutation is a dominant enhancer of lethality associated with absence of the non-protein coding *hsr*w locus in *Drosophila melanogaster J Biosciences* **21** 207-219

Lakhotia S C, Ray P, Rajendra T K and Psasanth K V (1999) The non-coding transcripts of *hsr-omega* gene in *Drosophila*: do they regulate trafficking and availability of nuclear RNA-processing factors? *Curr Science* **77** 553-563

Lakhotia S C and Singh A K (1982) Conservation of the 93D puff of *Drosophila melanogaster* in different species of *Drosophila Chromosoma* **86** 265-276

Legeai F and Derrien T (2014) Identification of long non-coding RNAs in insect genomes *Curr Opin Insect Science* **7** doi:101016/jcois201501003

Li Y and Wang X (2016) Role of long noncoding RNAs in malignant disease (Review) *Molecular Medicine Rep* **13** 1463-1469

Liebers R, Rassoulzadegan M and Lyko F (2014) Epigenetic regulation by heritable RNA *PLoS Genet* **10** e1004296

Lucchesi J C (1998) Dosage compensation in flies and worms the ups and downs of X-chromosome regulation *Curr Opin Genet Dev* **8** 179-184

Lucchesi J C, Kelley W G and Panning B (2005) Chromatin remodeling in dosage compensation *Annu Rev Genet* **39** 615-651

Lyon M F (1961) Gene action in the X-chromosome of the mouse (*Mus musculus* L) *Nature* **190** 372-373

Mallik M and Lakhotia S C (2009a) The developmentally active and stress-inducible non-coding hsrω gene is a novel regulator of apoptosis in *Drosophila Genetics* **183** 831-852

Mallik M and Lakhotia S C (2009b) RNAi for the large non-coding hsromega transcripts suppresses polyglutamine pathogenesis in *Drosophila* models *RNA Biol* **6** 464-478

Mallik M and Lakhotia S C (2010) Improved activities of CBP hnRNPs and proteasome following down regulation of noncoding hsromega transcripts help suppress polyQ pathogenesis in fly models *Genetics* **184** 927-945

Mallik M and Lakhotia S C (2011) Misexpression of the

developmentally active and stress-inducible non-coding hsrω gene in *Drosophila* has pleiotropic consequences *J Biosciences* **36** 265-280

Mank J E (2013) Sex chromosome dosage compensation: definitely not for everyone *Trends Genetics* **29** 677-683

Marchese F P and Huarte M (2014) Long non-coding RNAs and chromatin modifiers *Epigenetics* **9** 21-26

Matter N, Marx M, Weg-Remers S, Ponta H, Herrlich P and König H (2000) Heterogeneous ribonucleoprotein A1 is part of an exon-specific splice-silencing complex controlled by oncogenic signaling pathways *J Biol Chem* **275** 35353-35360

Mayr E (1970) *Populations species and evolution: an abridgment of animal species and evolution* Harvard University Press p 183

Mohler J and Pardue M L (1982) Deficiency mapping of the 93D heat-shock locus in *Drosophila melanogaster Chromosoma* **86** 457-467

Morcillo G, Diez J L, Carbajal M E and Tanguay R M (1993) HSP90 associates with specific heat shock puffs (hsrù) in polytene chromosomes of *Drosophila* and *Chironomus Chromosoma* **102** 648-659

Mukherjee A S and Beermann W (1965) Synthesis of RNA by the X chromosomes of *Drosophila melanogaster* and the problem of dosage compensation *Nature* **207** 785-786

Muller H J (1950) Evidence of the precision of genetic adaptation *Harvey Lectures Ser* **43** 165-229

Nakagawa S and Kageyama Y (2014) Nuclear lncRNAs as epigenetic regulators - beyond skepticism *Biochim Biophys Acta* **1839** 215-222

Ohno S (1972) So much "junk" DNA in our genome *Brookhaven Symp Biol* **23** 366-370

Onorati M C, Lazzaro S, Mallik M, Ingrassia A M, Carreca A P, Singh A K, Chaturvedi D P, Lakhotia S C and Corona D F (2011) The ISWI chromatin remodeler organizes the hsromega ncRNA-containing omega speckle nuclear compartments *PLoS Genet* **7** e1002096

Orgel L E, Crick F H C and Sapienza C (1980) Selfish DNA *Nature* **288** 645-646

Peeters S B, Cotton A M and Brown C J (2014) Variable escape from X-chromosome inactivation: Identifying factors that tip the scales towards expression *Bioessays* **36** 746-756

Piacentini L, Fanti L, Negri R, Del Vescovo V, Fatica A, Altieri F and Pimpinelli S (2009) Heterochromatin protein 1 (HP1a) positively regulates euchromatic gene expression through RNA transcript association and interaction with hnRNPs in *Drosophila PLoS Genet* **5** e1000670

Piccolo L L, Corona D and Onorati M C (2014) Emerging Roles for hnRNPs in post-transcriptional regulation: what can we learn from flies? *Chromosoma* **123** 515-527

Pinter S F, Sadreyev R L, Yildirim E, Jeon Y, Ohsumi T K *et al.* (2012) Spreading of X chromosome inactivation via a hierarchy of defined Polycomb stations *Genome Research* **22** 1864-1876

Place R F and Noonan E J (2014) Non-coding RNAs turn up the heat: an emerging layer of novel regulators in the mammalian heat shock response *Cell Stress Chap* **19** 159-172

Prasanth K V, Rajendra T K, Lal A K and Lakhotia S C (2000) Omega speckles-a novel class of nuclear speckles containing hnRNPs associated with noncoding hsr-omega RNA in *Drosophila J Cell Sci* **113** 3485-3497

Quan M, Chen J and Zhang D (2015) Exploring the secrets of long noncoding RNAs *Int Jour Mol Sci* **16** 5467-5496

Ray P and Lakhotia S C (1996) Interaction of the non-protein-coding developmental and stress-inducible *hsrω* gene with *Ras* genes of *Drosophila melanogaster J Biosciences* **23** 377-386

Rinn J and Guttman M (2014) RNA Function RNA and dynamic nuclear organization *Science* **345** 1240-1241

Roberts T C, Morris K V and Weinberg M S (2014) Perspectives on the mechanism of transcriptional regulation by long non-coding RNAs *Epigenetics* **9** 13-20

Russell L B (1963) Mammalian chromosome action inactivation limited in spread and in region of origin *Science* **140** 976-978

Ryseck R P, Walldorf U and Hovemann B (1985) Two major RNA products are transcribed from heat-shock locus 93D of *Drosophila melanogaster Chromosoma* **93** 17-20

Saumweber H, Symmons P, Kabisch R, Will H and Bonhoeffer F (1980) Monoclonal antibodies against chromosomal proteins of *Drosophila melanogaster*: establishment of antibody producing cell lines and partial characterization of corresponding antigens *Chromosoma* **80** 253-275

Schramke V and Allshire R (2004) Those interfering little RNAs! Silencing and eliminating chromatin *Curr Opin Genet Dev* **14** 174-180

Sengupta S and Lakhotia S C (2006) Altered expression of the noncoding *hsrω* gene enhances poly-Q-induced neurotoxicity in *Drosophila RNA Biol* **3** 28-35

Shearer R W and McCarthy B J (1967) Evidence for ribonucleic acid molecules restricted to the cell nucleus *Biochemistry* **6** 283-289

Shibayama Y, Fanucchi S, Magagula L and Mhlanga M M (2014) lncRNA and gene looping: what's the connection?

Transcription **5** e28658

Singh A K and Lakhotia S C (2015a) Dynamics of hnRNPs and omega speckles in normal and heat shocked live cell nuclei of *Drosophila melanogaster Chromosoma* **124** 367-383

Singh A K and Lakhotia S C (2015b) The hnRNP A1 homolog Hrb87F/Hrp36 is important for telomere maintenance in *Drosophila melanogaster Chromosoma* DOI: 10.1007/s00412-015-0540-y

Singh A K and Lakhotia S C (2016) Expression of *hsrù-RNAi* transgene prior to heat shock specifically compromises accumulation of heat shock induced Hsp70 in *Drosophila melanogaster Cell Stress Chap* **20** 105-121

Smith G C and Jackson S P (1999) The DNA-dependent protein kinase *Genes Dev* **13** 916-934

Smith P D and Lucchesi J C (1969) The role of sexuality in dosage compensation in *Drosophila Genetics* **61** 607-618

Soeiro R, Vaughan M H and Darnell J E (1968) The effect of puromycin on intranuclear steps in ribosome biosynthesis *J Cell Biol* **36** 91-101

Sofola O A, Jin P, Qin Y, Duan R, Liu H, de Haro M and Botas J (2007) RNA-binding proteins hnRNP A2/B1 and CUGBP1 suppress fragile X CGG premutation repeat-induced neuro degeneration in a *Drosophila* model of FXTAS *Neuron* **55** 565-571

Solé C, Nadal-Ribelles M, De Nadal E and Posas F (2015) A novel role for lncRNAs in cell cycle control during stress adaptation *Current Genetics* **61** 299-308

Spruill W A, Hurwitz D R, Lucchesi J C and Steiner A L (1978) Association of cyclic GMP with gene expression of polytene chromosomes of *Drosophila melanogaster Proc Natl Acad Sci USA* **75** 1480-1484

Spusta S C and Goldman M A (1999) *XISTential* wanderings: the role of XIST RNA in X-chromosome inactivation *Curr Sci* **77** 530-538

Straub T, Zabel A, Gilfillan G D, Feller C and Becker P B (2013) Different chromatin interfaces of the *Drosophila* dosage compensation complex revealed by high-shear ChIP-seq *Genome Research* **23** 473-485

Szymanski M, Barciszewska M Z, Zywicki M and Barciszewski J (2003) Noncoding RNA transcripts *Jour Applied Genetics* **44** 1-20

Tani H and Torimura M (2013) Identification of short-lived long non-coding RNAs as surrogate indicators for chemical stress response *Biochem Biophys Res Comm* **439** 547-551

Valencia K and Wutz A (2015) Recent insights into the regulation of X-chromosome inactivation *Adv Genomics Genetics* **5** 227-238

Vallot C and Rougeulle C (2013) Long non-coding RNAs and human X-chromosome regulation *RNA Biol* **10** 1262-1265

Weinberg R A (1973) Nuclear RNA metabolism *Annu Rev Biochem* **42** 329-354

Wilusz J E (2016) Long noncoding RNAs: re-writing dogmas of RNA processing and stability *Biochim Biophys Acta* **1859** 128-138

Wutz A, Rasmussen T P and Jaenisch R (2002) Chromosomal silencing and localization are mediated by different domains of Xist RNA *Nature Genetics* **30** 167-174

Yue M, Richard J L C and Ogawa Y (2016) Dynamic interplay and function of multiple noncoding genes governing X chromosome inactivation *Biochim Biophys Acta* **1859** 112-120

Zimowska G and Paddy M R (2002) Structures and dynamics of *Drosophila* Tpr inconsistent with a static filamentous structure *Exp Cell Res* **276** 223-232.