Long non-coding RNAs and human disease

Lorna W. Harries[1]

RNA-mediated Mechanisms of Disease Group, Institute of Biomedical and Clinical Sciences, Peninsula College of Medicine and Dentistry, Barrack Road, Exeter EX2 5DW, U.K.

Abstract

The central dogma of molecular biology states that DNA is transcribed into RNA, which in turn is translated into proteins. We now know, however, that as much as 50% of the transcriptome has no protein-coding potential, but rather represents an important class of regulatory molecules responsible for the fine-tuning of gene expression. Although the role of small regulatory RNAs [microRNAs and siRNAs (small interfering RNA)] is well defined, another much less characterized category of non-coding transcripts exists, namely lncRNAs (long non-coding RNAs). Pervasively expressed by eukaryotic genomes, lncRNAs can be kilobases long and regulate their targets by influencing the epigenetic control, chromatin status, mRNA processing or translation capacity of their targets. In the present review, I outline the potential mechanisms of action of lncRNAs, the cellular processes that have been associated with them, and also explore some of the emerging evidence for their involvement in common human disease.

One of the most surprising findings to come out of the genomic revolution was the observation that the vast majority of our genome is transcribed, with most bases being involved in at least one primary transcript [1]. In fact, only approximately 1.5% of the genome encodes proteins [2], with the remainder coding for 5′ and 3′ regulatory regions of genes, intronic regions and regulatory RNAs such as miRNAs (microRNAs), siRNAs (small interfering RNAs), Piwi-interacting RNAs, small nuclear RNAs and small nucleolar RNAs that are present in both intrageneic and intergenic regions [3–5]. Another class of untranslated regulatory RNAs exists, that of lncRNAs (long non-coding RNAs) [6], which is currently not so well characterized.

lncRNAs are common regulators of gene expression

lncRNAs are common in both prokaryotic and eukaryotic genomes; up to 72% of all human and mouse genes are found to be influenced by regulation by RNA molecules [7]. Regulatory transcripts can be either *cis*- or *trans*-acting, and occur in intronic, intragenic and other untranslated regions of the genome. Their targets can be either coding or non-coding (i.e. other regulatory RNAs), and can positively (concordant regulation) or negatively (discordant regulation) modify the expression or processing of their target genes. *cis*-lncRNAs are homologous with their targets and arise from the same genomic region, but from the opposite strand (these are often termed natural antisense transcripts), whereas *trans*-lncRNAs share incomplete homology with their targets and arise from distant regions of the genome [8]. The orientation of *cis*-regulatory transcripts to their targets can be 5′ to 5′ (head-to-head; Figure 1a), 3′ to 3′ (tail-to-tail; Figure 1B) or fully overlapping, with one gene contained within the region coding the other (Figure 1C). *Trans*-regulatory transcripts are usually non-overlapping, scince they are from distinct genomic regions.

Key words: antisense regulation, epigenetic regulation, gene regulation, long non-coding RNA, natural antisense transcript.

Abbreviations used: AD, Alzheimer's disease; APP, amyloid precursor protein; *Gas5*, growth-arrest-specific 5; lncRNA, long non-coding RNA; *nNOS*, neuronal nitric oxide synthase; *NPPA*, natriuretic peptide precursor A; *PINK1*, PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced putative kinase 1; PRC, Polycomb repressive complex; siRNA, small interfering RNA.

[1]email L.W.Harries@exeter.ac.uk

Mechanisms of lncRNA regulation

There are several proposed mechanisms of action for lncRNAs (Figure 2), which bring plasticity, adaptability and reactivity to genomic architecture and fine control over gene expression. In addition to the mechanisms outlined below, lncRNAs have also been reported to be subject to other mechanisms, including RNA editing, RNA interference, RNA masking, transcriptional interference and protein kinase R activation in some cases [9,10].

Epigenetic regulation

lncRNAs may act as scaffold molecules, to deliver regulatory proteins to loci where they are required. Examples of this type of lncRNA are *ANRIL* and *HOTAIR*. The *ANRIL* antisense transcript is coded for on the opposite strand of the *CDKN2A/CDKN2B* loci, and causes its effects by binding to and recruiting the *CBX7* (chromobox 7) subunit of the PRC1 (Polycomb repressive complex 1) and PRC2. These complexes serve to direct H3K27me (methylation of histone H3 at Lys-27) at the target loci, resulting in the silencing of sense transcripts expressed from this region [11,12]. Similarly, *HOTAIR* binds to and recruits the PRC2 and the LSD1 (lysine-specific demethylase 1) protein, which is a component of the CoREST (co-repressor for

Figure 1 | **The different conformations of lncRNA: target duplexes**
(**A**) A head-to-head conformation, whereby the 5′-end of the lncRNA associates with the 5′-end of the target. (**B**) A tail-to-tail conformation of conformation, whereby the 3′-end of the lncRNA associates with the 3′-end of the target. (**C**) An overlapping conformation, whereby the entire sequence of the lncRNA is contained within the sequence of the target.

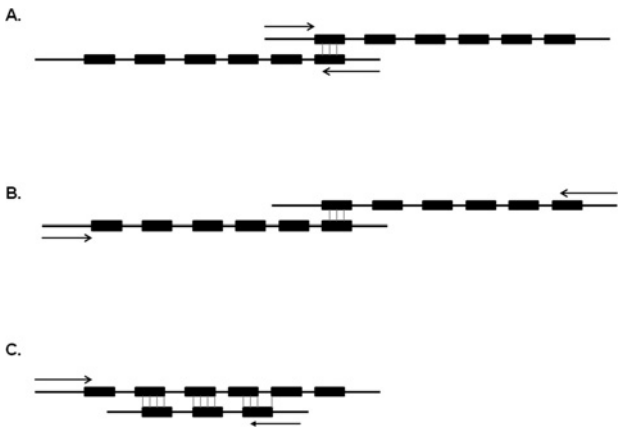
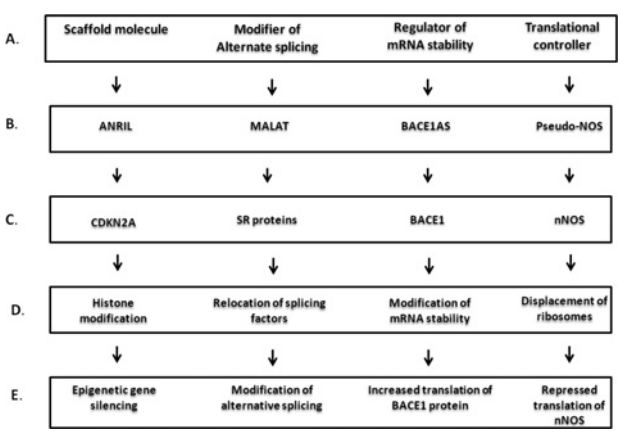


Figure 2 | **Mechanisms of lncRNA action**
Some of the potential mechanism underlying the activity of lncRNAs. (**A**) The nature of example lncRNAs. (**B**) The lncRNA. (**C**) The corresponding target gene. (**D**) The mode of action for the lncRNA. (**E**) The consequences of lncRNA regulation of that target gene.



element-1-silencing transcription factor) complex. Again, this brings about specific alterations in the methylation status and the nature of the chromatin surrounding the *HOTAIR* targets in the *HOXD* gene cluster [13]. *HOXD* genes such as *HOXD13* direct morphogenesis in all multicellular organisms, and disruption to their expression has been associated with breast cancer [13] and developmental disorders [14].

Regulation of alternative splicing

Another regulatory mechanism attributed to lncRNAs involves modification of alternative splicing. This is exemplified by the lncRNA *MALAT1*. *MALAT1* interacts with the SR (serine/arginine)-rich splicing regulatory proteins, which dictate alternative splicing patterns and are regulated by alterations to their phosphorylation status [15]. The interaction of *MALAT1* with these factors results in their relocation to the splicing speckles (the site of mRNA processing) in the nucleus [16,17], together with the modification of their phosphorylation state. *MALAT1* is thus a key regulator of alternative splicing events, which are important moderators of cellular plasticity and adaptability. Perturbations to the expression or activity of *MALAT1* therefore have significant implications for global regulation of alternative splicing.

Control of translation

lncRNAs can also regulate gene activity by controlling translational control or by regulation of mRNA stability, as demonstrated by the antisense RNA *BACE1AS*. *BACE1AS* interacts with the β-site APP (amyloid precursor protein) cleaving enzyme 1 (*BACE1*) transcript, which is a crucial player in AD (Alzheimer's disease) pathology. The specific interaction between the *BACE1* and *BACE1S* transcripts increases the stability of the *BACE1* transcript, and thus increases the translation and the abundance of the *BACE1* gene product [18]. lncRNAs can also decrease translation efficiencies. The *NOS* pseudogene *pseudo-NOS* is an antisense transcript that has been demonstrated to bind to, and to regulate the expression of the *nNOS* (neuronal nitric oxide synthase) gene. The lncRNA *pseudo-NOS* acts to influence the association of the ribosome with the *nNOS*–*pseudo-NOS* duplex, repressing translation of this target [19].

Competition for binding sites

Some lncRNAs do not work by direct antisense regulation of their target genes. The *Gas5* lncRNA, for example, works by binding to the DNA-binding domain of the glucocorticoid receptor, thus competing with and modifying the expression of target genes containing genuine glucocorticoid response elements [20]. Further mechanisms of lncRNA function are further reviewed in [21].

lncRNAs and normal function

lncRNAs have been implicated in numerous normal physiological processes at all stages of life, from early embryogenesis and cellular cell fate determination to physiological homoeostasis of entire organisms. Examples of their roles in three key cellular processes are described below.

Embryonic development

lncRNAs play a pivotal role in development, acting from the level of the embryonic stem cell, where they are involved in control of pluripotency; a recent study identified two lncRNAs, AK028326 (Oct4-activated) and AK141205 (Nanog-repressed) involved in an auto-regulatory loop with the key embryonic stem cell transcription factors OCT4 and NANOG. Overexpression and gene-silencing studies subsequently demonstrated that the expression of these lncRNAs was associated with alterations in cellular lineage-specific gene expression and a change in pluripotent potential of the cells in question [22]. Similarly, the lncRNA MISTRAL

(*Mira*), which targets *HOX6A* and *HOX7A*, has been shown to influence the expression of these genes by recruitment of the '*MLL* (mixed lineage leukaemia)' gene, involved in commitment to the haematopoietic lineage [23].

Control of cell cycle

lncRNAs are also known to be involved in the control of the cell cycle; the natural antisense transcript *ANRIL* is a key regulator of three separate tumour suppressor genes *p16INK4a*, *p14ARF* and *p15INK4b*, which are all expressed from the *CDKN2A/B* gene cluster [24]. p16INK4a and p15INK4b are important inhibitors of the cyclin-dependent kinase 4, whereas p14ARF acts to stabilize p53 by recruitment of MDM1 (murine double minute 1) [25]. An important role for lncRNAs in the coupling of DNA damage with cellular apoptosis has also been reported; DNA damage was shown to induce five lncRNAs from the promoter of the cell cycle regulator *CDKN1A*, one of which, named *PANDA*, was shown to interact with the transcription factor NF-YA to down-regulate the expression of genes involved in promotion of apoptosis [26].

Dosage compensation and chromosomal imprinting

Another key role for lncRNAs in normal physiology is their involvement in chromosomal dosage compensation. In females, each cell contains two copies of the X chromosome, one of which must be deactivated to ensure correct dosage of the genes housed by this chromosome in a process called X inactivation. X inactivation is controlled by an lncRNA called *Xist*. *Xist* is one of the first genes expressed following fertilization and acts to direct the PRC1 and PRC2, leading to histone modifications and silencing of all the genes on the targeted chromosome [27]. The recruitment of *Xist* to only one of the chromosomes is controlled by another series of lncRNAs, which includes the antisense lncRNA *Tsix*, which represses the activity of *Xist* on the active X, and *Jpx* which activates *Xist* on the silent X [28].

lncRNAs also have a pivotal role in control of imprinting, where one of the parental alleles is epigenetically silenced. Probably the best example of this is the *AIR* non-coding RNA, which regulates the genomic imprinting of a chromosomal region containing the *IGF2R/SLC122A2/SLC22A3* loci. *AIR* is a bidirectional silencer, and been shown to have a repressive effect on the paternal expression of genes in this region [29].

lncRNAs and human disease

Given the ubiquitous nature of lncRNA expression and their key roles in many physiological processes involving global regulation of the genome, it is unsurprising that they are involved in the aetiology of many human diseases.

Cancer

lncRNAs have a pivotal role in the control of the cell cycle, apoptosis and tumour suppression. The lncRNA *ANRIL* regulates three separate tumour suppressor genes *p16INK4a*, *p14ARF* and *p15INK4b*, important negative regulators of cell cycle [30]. Disruptions to the expression of *ANRIL* have accordingly been associated with the development of several cancer types, including neuroblastoma [24], acute lymphocytic leukaemia [31], melanoma [30] and prostate [11]. Overexpression of the *HOTAIR* transcript, a *cis*-lncRNA associated with the *HOXD* gene cluster, has been associated with hepatocellular carcinoma [32], colorectal cancer [33] and breast cancer [13] by deregulation of *HOXD* cluster genes. Ovarian and breast tumours have also been associated with the expression of the *LSINCT5* lncRNA; this transcript acts to target several other transcripts, including the antisense RNA *NEAT-1* and the *PSPC1* gene, which codes for a splicing regulatory factor [34]. lncRNA-associated disruption to alternative splicing has also been reported in non-small-cell lung cancer by virtue of overexpression of *MALAT1* [35].

Metabolic disease

Although little is known about the role of lncRNAs in endocrine disease, several genes that are important moderators of metabolism and endocrine function have reported lncRNAs. An antisense transcript to the *PINK1* [PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced putative kinase 1], termed *naPINK1* has recently been described [36]. As the name suggests, *PINK1* is induced by PTEN, which is an important inhibitor of insulin signalling. *PINK1* depletion has been associated with diabetes status, impaired glucose uptake in neuronal cell lines and with mitochondrial gene expression in adipocytes [37], raising the possibility that disruption to *naPINK1* may impact on glucose metabolism. Similarly, the *H19/IGF2* and thyroid growth receptor α2 (*ERBa2*) loci harbour known antisense transcripts [38,39] which have the potential to regulate their endocrine and metabolic function. lncRNAs have also been implicated in the regulation of lipid metabolism genes. The Δ^5-desaturase (*FADS1*) and steroidogenic acute regulatory protein (*STAR*) genes have reported lncRNAs [40,41]. The expression of *FADS*, and its lncRNA, *reverse D5-desaturase*, were found to be reciprocally regulated by the dietary fat content in animal models [40]. lncRNAs have also been implicated in appetite control; an lncRNA to the human ghrelin (*GRHL*) gene, which promotes food-seeking behaviour, has recently been identified [42]. These findings raise the possibility that deregulation of lncRNA expression may also have implications for obesity.

Neurodegenerative and psychiatric diseases

The *BACE1* antisense transcript, *BACE1AS*, has been implicated in the aetiology of AD [6]. Some of the features of AD are because of the accumulation within the brain of β-amyloid plaques. The *BACE1* gene, an integral membrane peptidase A1 glycoprotein, plays a pivotal role in the accumulation of β-amyloid plaques. *BACE1* is one of two peptidases that carry out the initial proteolytic cleavage of APP, allowing it to accumulate in the brain. *BACE1AS* levels

were found to be higher in human subjects with AD, and also in *BACE1* transgenic mouse models of AD [6].

lncRNAs have also been suggested to be involved in psychiatric disorders. Disruption of the 'disrupted in schizophrenia-1' *DISC1* locus has been linked with the development of schizophrenia, schizoaffective disorder, bipolar disorder, major depression and autistic spectrum disorders [43]. *DISC1* is regulated by its lncRNA, *DISC2*, which may also represent an excellent candidate for susceptibility to these disorders. Schizophrenia spectrum disorders and AD have also been linked with the rheelin (*RELN*) gene and its antisense transcript *HAR1* [44].

Cardiovascular disease, hypertension and stroke

lncRNAs have the potential to influence cardiovascular disease and hypertension. Genetic variants that affect the expression of the *ANRIL* transcript have been correlated with stroke risk and recurrence in a large prospective study [45]. A role for lncRNAs in hypertension also suggested that seven blood pressure candidate genes (*ADD3*, *NPPA*, *ATP1A1*, *NPR2*, *CYP17A1*, *ACSM3* and *SLC14A2*) were associated with *cis*-lncRNA transcripts [46]. The *NPPA* (natriuretic peptide precursor A) gene product is usually expressed in only fetal atrial and ventricular myocardium, but has been shown to be reactivated in the ventricular myocardium of patients exhibiting hypertrophy and heart failure [47], and is considered to be a marker for heart disease. The *NPPA-AS* lncRNA has been shown to be a modulator of the alternative splicing of the *NPPA* gene. This lncRNA thus has potential to be involved in cardiovascular disease [46].

Immune dysfunction and auto-immunity

An important role for lncRNA in control of innate immune signalling has been suggested by the observation that approximately 500 lncRNAs are differentially expressed during the immune response to virus infection in a study involving four separate mouse strains [48]. The non-translated RNA '*Gas5*' (growth-arrest-specific 5) transcript, activated by cellular stress, targets a diverse group of genes through the glucocorticoid receptor and is an important regulator of cellular apoptosis [20]. The *Gas5* transcript has been linked with increased susceptibility to systemic lupus erythaematosus in mouse models, presumably as a result of its effect on the immunosuppressant role of glucocorticoids [20]. Other studies have reported an association of the antisense RNA *Heg* with CD14 levels and thyroid auto-antibodies in patients with untreated Graves' disease [49].

Conclusions

lncRNAs form a significant part of the eukaryotic transcriptome, which regulate the expression of up to 70% of genes. They play a crucial role in global processes such as epigenetic regulation, chromatin remodelling and alternative mRNA processing, and are thus intimately involved in the control of key physiological processes. Their involvement in many aspects of higher function indicates that they may represent a new and exciting arena to exploit for future disease therapies.

Acknowledgement

I thank Alexander Fellows for his help in preparing the Figures for this review.

References

1 Birney, E., Stamatoyannopoulos, J.A., Dutta, A., Guigo, R., Gingeras, T.R., Margulies, E.H., Weng, Z., Snyder, M., Dermitzakis, E.T., Thurman, R.E. et al. (2007) Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature **447**, 799–816
2 Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W. et al. (2001) Initial sequencing and analysis of the human genome. Nature **409**, 860–921
3 St Laurent, III, G. and Wahlestedt, C. (2007) Noncoding RNAs: couplers of analog and digital information in nervous system function? Trends Neurosci. **30**, 612–621
4 Mattick, J.S. (2009) The genetic signatures of noncoding RNAs. PLoS Genet **5**, e1000459
5 Taft, R.J., Pang, K.C., Mercer, T.R., Dinger, M. and Mattick, J.S. (2010) Non-coding RNAs: regulators of disease. J. Pathol. **220**, 126–139
6 Faghihi, M.A. and Wahlestedt, C. (2009) Regulatory roles of natural antisense transcripts. Nat. Rev. Mol. Cell Biol. **10**, 637–643
7 Beiter, T., Reich, E., Williams, R.W. and Simon, P. (2009) Antisense transcription: a critical look in both directions. Cell. Mol. Life Sci. **66**, 94–112
8 Katayama, S., Tomaru, Y., Kasukawa, T., Waki, K., Nakanishi, M., Nakamura, M., Nishida, H., Yap, C.C., Suzuki, M., Kawai, J. et al. (2005) Antisense transcription in the mammalian transcriptome. Science **309**, 1564–1566
9 Lipovich, L., Johnson, R. and Lin, C.Y. (2010) MacroRNA underdogs in a microRNA world: evolutionary, regulatory, and biomedical significance of mammalian long non-protein-coding RNA. Biochim. Biophys. Acta **1799**, 597–615
10 Su, W.Y., Xiong, H. and Fang, J.Y. (2010) Natural antisense transcripts regulate gene expression in an epigenetic manner. Biochem. Biophys. Res. Commun. **396**, 177–181
11 Yap, K.L., Li, S., Munoz-Cabello, A.M., Raguz, S., Zeng, L., Mujtaba, S., Gil, J., Walsh, M.J. and Zhou, M.M. (2010) Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by Polycomb CBX7 in transcriptional silencing of INK4a. Mol. Cell **38**, 662–674
12 Kotake, Y., Nakagawa, T., Kitagawa, K., Suzuki, S., Liu, N., Kitagawa, M. and Xiong, Y. (2011) Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of $p15^{INK4B}$ tumor suppressor gene. Oncogene **30**, 1956–1962
13 Gupta, R.A., Shah, N., Wang, K.C., Kim, J., Horlings, H.M., Wong, D.J., Tsai, M.C., Hung, T., Argani, P., Rinn, J.L. et al. (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature **464**, 1071–1076
14 Gong, L., Wang, B., Wang, J., Yu, H., Ma, X. and Yang, J. (2011) Polyalanine repeat expansion mutation of the *HOXD13* gene in a Chinese family with unusual clinical manifestations of synpolydactyly. Eur. J. Med. Genet. **54**, 108–111
15 Bourgeois, C.F., Lejeune, F. and Stevenin, J. (2004) Broad specificity of SR (serine/arginine) proteins in the regulation of alternative splicing of pre-messenger RNA. Prog. Nucleic Acid Res. Mol. Biol. **78**, 37–88
16 Spector, D.L. and Lamond, A.I. (2011) Nuclear speckles. Cold Spring Harbor Perspect. Biol. **3**, a000646
17 Tripathi, V., Ellis, J.D., Shen, Z., Song, D.Y., Pan, Q., Watt, A.T., Freier, S.M., Bennett, C.F., Sharma, A., Bubulya, P.A. et al. (2010) The nuclear-retained noncoding RNA *MALAT1* regulates alternative splicing by modulating SR splicing factor phosphorylation. Mol. Cell **39**, 925–938
18 Faghihi, M.A., Modarresi, F., Khalil, A.M., Wood, D.E., Sahagan, B.G., Morgan, T.E., Finch, C.E., St Laurent, III, G., Kenny, P.J. and Wahlestedt, C. (2008) Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of β-secretase. Nat. Med. **14**, 723–730

19 Korneev, S.A., Park, J.H. and O'Shea, M. (1999) Neuronal expression of neural nitric oxide synthase (nNOS) protein is suppressed by an antisense RNA transcribed from an NOS pseudogene. J. Neurosci. **19**, 7711–7720
20 Kino, T., Hurt, D.E., Ichijo, T., Nader, N. and Chrousos, G.P. (2010) Noncoding RNA *gas5* is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. Sci. Signal. **3**, ra8
21 Wang, K.C. and Chang, H.Y. (2011) Molecular mechanisms of long noncoding RNAs. Mol. Cell **43**, 904–914
22 Sheik Mohamed, J., Gaughwin, P.M., Lim, B., Robson, P. and Lipovich, L. (2010) Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells. RNA **16**, 324–337
23 Bertani, S., Sauer, S., Bolotin, E. and Sauer, F. (2011) The noncoding RNA *Mistral* activates Hoxa6 and Hoxa7 expression and stem cell differentiation by recruiting MLL1 to chromatin. Mol. Cell **43**, 1040–1046
24 Pasmant, E., Sabbagh, A., Masliah-Planchon, J., Ortonne, N., Laurendeau, I., Melin, L., Ferkal, S., Hernandez, L., Leroy, K., Valeyrie-Allanore, L. et al. (2011) Role of noncoding RNA *ANRIL* in genesis of plexiform neurofibromas in neurofibromatosis type 1. J. Nat. Cancer Inst. **103**, 1713–1722
25 Popov, N. and Gil, J. (2010) Epigenetic regulation of the *INK4b-ARF-INK4a* locus: in sickness and in health. Epigenetics **5**, 685–690
26 Hung, T., Wang, Y., Lin, M.F., Koegel, A.K., Kotake, Y., Grant, G.D., Horlings, H.M., Shah, N., Umbricht, C., Wang, P. et al. (2011) Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. Nat. Genet. **43**, 621–629
27 Payer, B. and Lee, J.T. (2008) X chromosome dosage compensation: how mammals keep the balance. Annu. Rev. Genet. **42**, 733–772
28 Tian, D., Sun, S. and Lee, J.T. (2010) The long noncoding RNA, *Jpx*, is a molecular switch for X chromosome inactivation. Cell **143**, 390–403
29 Sleutels, F., Zwart, R. and Barlow, D.P. (2002) The non-coding *Air* RNA is required for silencing autosomal imprinted genes. Nature **415**, 810–813
30 Pasmant, E., Laurendeau, I., Heron, D., Vidaud, M., Vidaud, D. and Bieche, I. (2007) Characterization of a germ-line deletion, including the entire *INK4/ARF* locus, in a melanoma-neural system tumor family: identification of *ANRIL*, an antisense noncoding RNA whose expression coclusters with *ARF*. Cancer Res. **67**, 3963–3969
31 Iacobucci, I., Sazzini, M., Garagnani, P., Ferrari, A., Boattini, A., Lonetti, A., Papayannidis, C., Mantovani, V., Marasco, E., Ottaviani, E. et al. (2011) A polymorphism in the chromosome 9p21 *ANRIL* locus is associated to Philadelphia positive acute lymphoblastic leukemia. Leuk. Res. **35**, 1052–1059
32 Yang, Z., Zhou, L., Wu, L.M., Lai, M.C., Xie, H.Y., Zhang, F. and Zheng, S.S. (2011) Overexpression of long non-coding RNA *HOTAIR* predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Ann. Surg. Oncol. **18**, 1243–1250
33 Kogo, R., Shimamura, T., Mimori, K., Kawahara, K., Imoto, S., Sudo, T., Tanaka, F., Shibata, K., Suzuki, A., Komune, S. et al. (2011) Long noncoding RNA *HOTAIR* regulates Polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res. **71**, 6320–6326
34 Silva, J.M., Boczek, N.J., Berres, M.W., Ma, X. and Smith, D.I. (2011) LSINCT5 is over expressed in breast and ovarian cancer and affects cellular proliferation. RNA Biol. **8**, 496–505
35 Schmidt, L.H., Spieker, T., Koschmieder, S., Humberg, J., Jungen, D., Bulk, E., Hascher, A., Wittmer, D., Marra, A., Hillejan, L. et al. (2011) The long noncoding *MALAT-1* RNA indicates a poor prognosis in non-small cell lung cancer and induces migration and tumor growth. J. Thorac. Oncol. **6**, 1984–1992
36 Scheele, C., Petrovic, N., Faghihi, M.A., Lassmann, T., Fredriksson, K., Rooyackers, O., Wahlestedt, C., Good, L. and Timmons, J.A. (2007) The human *PINK1* locus is regulated *in vivo* by a non-coding natural antisense RNA during modulation of mitochondrial function. BMC Genomics **8**, 74
37 Scheele, C., Nielsen, A.R., Walden, T.B., Sewell, D.A., Fischer, C.P., Brogan, R.J., Petrovic, N., Larsson, O., Tesch, P.A., Wennmalm, K. et al. (2007) Altered regulation of the *PINK1* locus: a link between type 2 diabetes and neurodegeneration? FASEB J. **21**, 3653–3665
38 Berteaux, N., Aptel, N., Cathala, G., Genton, C., Coll, J., A., Daccache, A., Spruyt, N., Hondermarck, H., Dugimont, T., Curgy, J.J. et al. (2008) A novel H19 antisense RNA overexpressed in breast cancer contributes to paternal IGF2 expression. Mol. Cell. Biol. **28**, 6731–6745
39 Crosthwaite, S.K. (2004) Circadian clocks and natural antisense RNA. FEBS Lett. **567**, 49–54
40 Dreesen, T.D., Adamson, A.W., Tekle, M., Tang, C., Cho, H.P., Clarke, S.D. and Gettys, T.W. (2006) A newly discovered member of the fatty acid desaturase gene family: a non-coding, antisense RNA gene to Δ^5-desaturase. Prostaglandins Leukotrienes Essent. Fatty Acids **75**, 97–106
41 Castillo, A.F., Fan, J., Papadopoulos, V. and Podesta, E.J. (2011) Hormone-dependent expression of a steroidogenic acute regulatory protein natural antisense transcript in MA-10 mouse tumor Leydig cells. PLoS ONE **6**, e22822
42 Seim, I., Collet, C., Herington, A.C. and Chopin, L.K. (2007) Revised genomic structure of the human ghrelin gene and identification of novel exons, alternative splice variants and natural antisense transcripts. BMC Genomics **8**, 298
43 Chubb, J.E., Bradshaw, N.J., Soares, D.C., Porteous, D.J. and Millar, J.K. (2008) The *DISC* locus in psychiatric illness. Mol. Psychiatry **13**, 36–64
44 Tamura, Y., Kunugi, H., Ohashi, J. and Hohjoh, H. (2007) Epigenetic aberration of the human *REELIN* gene in psychiatric disorders. Mol. Psychiatry **12** 519, 593–600
45 Zhang, W., Chen, Y., Liu, P., Chen, J., Song, L., Tang, Y., Wang, Y., Liu, J., Hu, F.B. and Hui, R. (2012) Variants on chromosome 9p21.3 correlated with ANRIL expression contribute to stroke risk and recurrence in a large prospective stroke population. Stroke **43**, 14–21
46 Annilo, T., Kepp, K. and Laan, M. (2009) Natural antisense transcript of natriuretic peptide precursor A (NPPA): structural organization and modulation of NPPA expression. BMC Mol. Biol. **10**, 81
47 Horsthuis, T., Houweling, A.C., Habets, P.E., de Lange, F.J., el Azzouzi, H., Clout, D.E., Moorman, A.F. and Christoffels, V.M. (2008) Distinct regulation of developmental and heart disease-induced atrial natriuretic factor expression by two separate distal sequences. Circ. Res. **102**, 849–859
48 Peng, X., Gralinski, L., Armour, C.D., Ferris, M.T., Thomas, M.J., Proll, S., Bradel-Tretheway, B.G., Korth, M.J., Castle, J.C., Biery, M.C. et al. (2010) Unique signatures of long noncoding RNA expression in response to virus infection and altered innate immune signaling. mBio **1**, e00206–e00210
49 Christensen, N.J., Habekost, G. and Bratholm, P. (2008) A RNA transcript (*Heg*) in mononuclear cells is negatively correlated with *CD14* mRNA and TSH receptor autoantibodies. Clin. Exp. Immunol. **154**, 209–215

Received 24 January 2012
doi:10.1042/BST20120020