ELSEVIER

Natural antisense and noncoding RNA transcripts as potential drug targets

Claes Wahlestedt

The Scripps Research Institute (Scripps Florida), 5353 Parkside Drive, RF-2, Jupiter, FL 33458, USA

Information on the complexity of mammalian RNA transcription has increased greatly in the past few years. Notably, thousands of sense transcripts (conventional protein-coding genes) have antisense transcript partners, most of which are noncoding. Interestingly, a number of antisense transcripts regulate the expression of their sense partners, either in a discordant (antisense knockdown results in sense-transcript elevation) or concordant (antisense knockdown results in concomitant sense-transcript reduction) manner. Two new pharmacological strategies based on the knockdown of antisense RNA transcripts by siRNA (or another RNA targeting principle) are proposed in this review. In the case of discordant regulation, knockdown of antisense transcript elevates the expression of the conventional (sense) gene, thereby conceivably mimicking agonist–activator action. In the case of concordant regulation, knockdown of antisense transcript, or concomitant knockdown of antisense and sense transcripts, results in an additive or even synergistic reduction of the conventional gene expression. Although both strategies have been demonstrated to be valid in cell culture, it remains to be seen whether they provide advantages in other contexts.

Recent transcriptomics advances

The past few years have seen a significant increase in our understanding of the complexity of mammalian transcription and many previously undetected RNA transcripts have been described. This has come as a surprise because the total number of conventional (protein coding) genes in the human genome (~20,000–25,000) is much lower than anticipated a few years ago, and of the same magnitude as the number of genes in simpler organisms, such as *Drosophila melanogaster* and *Caenorhabditis elegans* [1].

Two major transcriptomics efforts have led the way in establishing a modified view of mammalian transcription in different but complementary directions. First, the international Functional Annotation of the Transcriptome of Mouse/Mammals (FANTOM) consortium has produced and analyzed massive amounts of cDNA sequencing data over the past decade, primarily from mouse but also from human cells and tissues [2,3]. Second, high-density ('tiling') microarray experiments have provided evidence that transcription occurs extensively throughout the human genome and that there are many transcripts of unknown function [4,5]. Thus, the available data reveal the remarkable complexity of the mammalian transcriptional landscape.

Basic RNomics

RNAs can be classified into messenger RNAs (mRNAs), which are translated into proteins, and non-protein-coding RNAs (ncRNAs). Until recently, it was thought that there are only small numbers of ncRNAs (e.g. transfer RNAs, ribosomal RNAs and spliceosomal RNAs) that relate to protein synthesis or function. Therefore, until a few years ago, no systematic efforts were made to identify novel ncRNA transcripts and elucidate their functions.

The concept of noncoding RNA

For more than half a century, the central dogma of molecular biology has stated that genetic information encoded in DNA is transcribed to form intermediary molecules of RNA that are, in turn, translated into amino acids that make up proteins. The assumption has been that proteins are directly related to genes (one gene = one protein). In the past few years, we have come to

Corresponding author: Wahlestedt, C. (clawah@scripps.edu)

1359-6446/06/$ - see front matter © 2006 Elsevier Ltd. All rights reserved. doi:10.1016/j.drudis.2006.04.013

BOX 1

How do noncoding RNA transcripts differ from conventional coding RNA transcripts?

Table I General differences (and similarities) between coding transcripts and some categories of noncoding RNA transcripts[a]

	Coding RNA	Noncoding RNA		
	mRNA	mRNA-like noncoding RNA (e.g. natural antisense transcripts)	stRNA,miRNA, snoRNA	rRNA, tRNA
Function	Encode for proteins	Regulatory, catalytic or structural roles in the cell		
Polyadenylation	Yes	Yes	No	No
Splicing	Yes	Yes or no	No	No
Cap structure	Yes	Yes or no	No	No
Translation	Yes	No	No	No
Open reading frame	Yes	No	No	No
Localization	Cytoplasmic	Predominantly nuclear	Nuclear or cytoplasmic	Cytoplasmic

[a]Abbreviations: mRNA, messenger RNA; miRNA, microRNA; stRNA, small temporal RNA; snoRNA, small nucleolar RNA; rRNA, ribosomal RNA; tRNA, transferRNA.

realize that complexity at the RNA level is far greater than previously assumed. This complexity is largely due to abundance of noncoding transcripts, as well as alternative splicing phenomena, and is particularly apparent in eukaryotes [6,7].

ncRNAs comprise microRNAs (miRNAs), antisense transcripts and other transcriptional units containing a high density of stop codons and lacking any extensive open reading frame. Many ncRNAs start from initiation sites in 3′ untranslated regions of protein-coding loci and at least half of the ncRNAs that have been sequenced by the FANTOM consortium appear not to be polyadenylated [2]. Recently, Gingeras and colleagues [4,5] have independently shown that the number of nonpolyadenylated nuclear RNAs might be very large, and that many such transcripts arise from intergenic regions. Box 1 contains a summary of some key features that distinguish ncRNA from coding transcripts. Although this field is certainly only at the very beginning stage of associating ncRNA with human diseases, Table 1 shows some representative examples. Further examples of the possible impact of ncRNA on eukaryotic biology can be found in Ref. [6].

TABLE 1

Examples of human diseases possibly relating to natural antisense transcripts and/or noncoding transcripts[a]

Noncoding RNA	Human Disease	ncRNA Type	Ref
HIF1α and aHIF	Breast and renal cancer	NAT	[33]
Survivin and EPR1	Colon cancer	NAT	[34]
LUC7L and α-globulin	α- Thalassemia	NAT	[35]
KvLQT1	Beckwith–Wiedemann Syndrome	NAT	[36]
SNURF-SNRPN and UBE3A	Prader–Willi and Angelman Syndrome	NAT	[37]
BCL2 and IgH	Follicular B-cell lymphoma	NAT	[38]
mir-17–92, mir-155	Lymphoma	miRNA (ncRNA)	[39]
RMRP	Cartilage hair hypoplasia	ncRNA	[40]

[a] Abbreviations: miRNA, microRNA; NAT, natural antisense transcripts; ncRNA, noncoding RNA; tRNA, transferRNA.

Like coding RNAs, the vast majority of ncRNAs analyzed by Carninci *et al.* [2] displayed positional conservation across species. It even appears that some ncRNAs are highly conserved between evolutionary distant species. In considering function, it is conceivable that the act of transcription from the particular genomic location is either functionally important or a consequence of genomic structure or sequence. The noncoding transcript might indeed function by a sequence-specific interaction with the DNA sequence from which it is derived, or it might have another target or targets. Interestingly, ncRNA transcripts appear to be evolving rapidly, but the fact that they are not as well conserved as coding transcripts does not necessarily mean that they lack function [7]. There are at least four online databases of different categories of ncRNA (Table 2). It should be noted that much information relating to miRNA has appeared in the past few years and that there are several databases devoted exclusively to miRNA [8]. Although miRNA is an important and established category of ncRNA, this review will not specifically deal with it. Instead, this review will focus on the emerging multitude of mammalian natural antisense transcripts, most of which are made up of ncRNA. Like miRNA, natural antisense transcripts have the potential to exert widespread impact on conventional gene expression.

The vast majority of ncRNAs have not been functionally annotated. We predict that the coming years will see an avalanche of studies demonstrating function not only for miRNA and natural antisense transcripts, but also for various other categories of ncRNAs whose existence has recently been demonstrated (for example, see Ref. [2]). Notably, Willingham *et al.* [9] recently selected 512 ncRNA sequences whose only characteristic is that they display significant mouse-to-human conservation, and targeted them with siRNAs in

TABLE 2

Databases containing noncoding RNA sequences

Name	URL
RNAdb	http://research.imb.uq.edu.au/RNAdb
Rfam	http://www.sanger.ac.uk/Software/Rfam/ and http://rfam.wustl.edu/
NONCODE	http://noncode.bioinfo.org.cn
ncRNADB	http://biobases.ibch.poznan.pl/ncRNA/

different cell-based screening assays, resulting in the identification of eight functional ncRNAs.

Antisense transcription

Antisense transcripts can derive from coding RNA and noncoding RNA, which includes genic, intronic and intergenic sequences. In general, natural antisense transcripts can be subdivided into (i) *cis*-antisense transcripts that are encoded at the same genetic location but on the opposite strand to the RNAs that they act on, and (ii) *trans*-antisense transcripts that are encoded at a chromosomal location distinct from the RNAs that they act on. This review will focus on *cis*-antisense transcripts with functional examples. Strikingly, antisense transcription in mammals is far more prevalent than anticipated only a few years ago. Very recently, Katayama *et al.* [3] provided evidence that, in mice, >72% of all genome-mapped transcriptional units (43,553) overlap with some cDNA, 5′ or 3′ expressed sequence tag (EST) sequence, or tag or tag-pair region mapped to the opposite strand. There is currently no reason to assume that the situation would be very different in humans. This study supports and significantly extends observations made previously [10–14] about the occurrence of antisense transcription in the mammalian genome It appears that large numbers of multiple-sized transcripts are expressed from the sense–antisense loci (regions of the genome that express sense as well as antisense RNA transcripts) and that these tend to lack polyadenylated tails and exhibit nuclear localization [2,3,15].

There are three basic types of sense–antisense pairs: head-to-head (or divergent), tail-to-tail (or convergent) and fully overlapping. The divergent classes are the most prevalent (for example, see Ref. [3]). Expression analyses using strand-specific [15] and conventional [3] microarrays have indicated marked fluctuation in expression levels of sense–antisense pairs between various mouse tissues.

It has been argued that noncoding transcriptional activity is largely 'unintentional', representing 'leakage' of the RNA transcription machinery. To this end, it has been demonstrated that defined antisense transcript pairs are considerably more likely to preserve their genomic organization throughout evolution compared with non-antisense pairs [14].

It will here be assumed that by hybridizing with their sense-transcript partners, antisense transcripts modulate whether the sense transcript will pursue its function (i.e. to encode for proteins). This might lead to RNA-masking, or steric inhibition. In addition, other mechanisms could be invoked, such as competitive transcriptional interference (for RNA polymerase II) within the same locus, as well as altered methylation pattern, for example, as a result of a chromosome deletion [13]. The mechanisms that have been suggested apply particularly to cases of *cis*-antisense.

A key question is whether natural antisense pairs could form a basis for endogenous RNA interference (RNAi). The widespread occurrence of RNAi-based mechanisms in different biological systems and recent evidence demonstrating that, at least in some species, part of the RNAi pathway can occur in the nuclear compartment [16], suggests a possible role for this process in antisense-mediated gene repression. According to this theoretical (but never experimentally validated) view, double-stranded RNAs (dsRNAs) (formed by the hybridization of a sense and an antisense transcript) would be cleaved into siRNAs by DICER or other RNase III family members. However, this hypothesis has been contradicted by our finding that RNA regulation by natural antisense transcripts occurs through a pathway that is independent of DICER-associated RNAi in human cells [17], a finding that is consistent with observations in *Arabidopsis thaliana* [18,19]. Indeed, even when sense and antisense transcripts were overexpressed in human cells, there was no evidence of the formation of DICER-generated short double-stranded RNA fragments [17].

Antisense–sense interaction phenomena affect different types of genes and are unevenly distributed across the genome (for example, see Ref. [3]). Several antisense transcripts have been shown to be functional, meaning that they regulate their respective genes. A few review articles on this topic have recently appeared [12,20–22]. Some general characteristics of natural antisense transcripts are summarized in Box 2.

BOX 2

Some characteristics of mammalian natural antisense transcripts (NATs).

- NATs are much more abundant than previously postulated.
- NATs represent a gene regulation phenomenon possibly invoking thousands of conventional genes.
- NATs can represent coding or, more commonly, noncoding RNA.
- NATs can occur is *cis* (same locus as corresponding sense transcript) or *trans* (at a distance from corresponding sense transcript).
- Very few NATs have been functionally annotated.
- There are some examples of NAT involvement in physiology or pathophysiology.
- NATs could constitute a novel class of drug targets enabling up- or down-regulation of (conventional) sense genes and/or transcripts.

RNA targeting

Drug discovery efforts have historically focused on the search for compounds that modulate the protein products of genes. The vast majority of drugs available today either act at the protein level, or the drugs themselves are proteins. These compounds are usually agonists or antagonists of receptors, or they inhibit or stimulate enzymes or protein–protein interactions. However, the interest in specifically targeting RNA is increasing, both for target validation and/or therapeutic purposes, not least with the introduction of RNAi a few years ago. In addition, there are many ongoing efforts aimed at targeting mRNA with small molecules, antisense oligonucleotides, ribozymes or aptamers. When it comes to ncRNA as a potential new drug target category, with no protein being produced there is obviously no alternative but to attempt to find ways to affect the RNA transcript itself.

Current drug therapy targets only a few hundred endogenous targets, mainly proteins, such as receptors and enzymes [23]. Genomics and transcriptomics efforts have identified many novel candidate drug targets that need to be validated. Although target validation studies help to set priorities in the drug discovery process, they do not directly produce drug candidates. Thus, there is good reason to keep a focus on the well-established targets. If these turn out to be subjected to natural antisense regulation then it could be possible to try to address these 'old' targets in a novel way, particularly if no drugs for these targets are available.

For protein-coding genes, in energetic terms, post-transcriptional regulation is an expensive mechanism to control gene expression. The mRNA is only an intermediate in the multistep process from gene to active protein. If a cell would regulate this process only at the beginning, at the transcriptional level, it would save the energy needed to accommodate, degrade and recycle the mRNA molecules that are not used to synthesize proteins [24,25]. When functional, ncRNA trancripts will exert regulatory actions by themselves, not having to do so by way of encoding for proteins, and could therefore prove to constitute drug targets that, when manipulated, will be linked to distinct phenotypic changes in the organism. To modulate the actions of ncRNA directly, the RNA level is naturally the only targeting option because these transcripts do not encode for proteins.

Knockdown of antisense transcripts

With the recent realization that the phenomenon of antisense transcription, most of which involves ncRNA, is extremely common in mammals, it follows that a new category of drug targets might have to be considered (see Box 2). Moreover, because ncRNA transcripts by definition do not produce proteins, direct manipulation can only occur at the RNA level, leaving no alternative but RNA-targeting. In the present context, the only approach to affect antisense transcript levels has been by use of siRNA aimed at achieving efficient transcript knockdown. It is fully recognized that although siRNA molecules are excellent experimental tools at present, and many other contexts, they have shortcomings as potential pharmaceutical agents in most circumstances.

Transcript expression profiling experiments using gene microarray platforms revealed frequent concordant (similar expression pattern) or discordant (opposite expression pattern) regulation of sense–antisense pairs [3]. This review focuses on what happens to the sense transcript when an antisense transcript is perturbed. Using siRNA, we [3,17,26] have provided direct experimental evidence that perturbation of an antisense RNA by siRNA can alter the expression of the corresponding sense mRNAs. We have found that the consequence of antisense knockdown is not predictable and can result in either sense transcript elevation (discordant regulation) or, seemingly more commonly, in sense transcript reduction (concordant regulation).

Coding and non-coding antisense transcripts have been successfully targeted by siRNA and appear equally capable of regulating the corresponding sense transcripts; in other words, the sense transcript does not seem to 'know' if it is being regulated by a coding or non-coding antisense transcript. This means that there is no way of predicting the result of natural antisense knockdown on sense transcript levels based on knowledge about these transcripts representing coding or non-coding RNA.

Table 3 shows a range of human and mouse antisense transcripts that have been targeted by siRNA directed to a non-overlapping part of the antisense sequence, excluding the possibility of simultaneous targeting of the corresponding sense transcript. The data in Table 3 have been generated as a part of a larger effort in our laboratory to systematically achieve functional annotation of human and mouse ncRNA, including natural antisense transcripts.

Interestingly, we have observed that knockdown of the sense transcript does not result in alterations of the corresponding antisense transcript level in human or mouse cells [3,17]. We therefore conclude that the regulation seems unidirectional in that antisense transcripts can regulate sense transcripts, but not vice versa. In general, mechanisms of antisense regulation in mammals are complex and mostly remain to be elucidated [3,15,12,17,27,28]. Nevertheless, it might already be possible to start making pharmacological use of empirical findings that specific antisense transcripts regulate therapeutically relevant sense transcripts (conventional genes), as outlined below.

Potential pharmacological principles invoking natural antisense transcripts

Here we propose two new pharmacological strategies based on the knockdown of antisense RNA transcripts by siRNA. It should be noted that the other RNA-targeting approaches, such as antisense oligonucleotides, ribozymes or perhaps small molecules, might be equally applicable to these strategies.

Strategy 1. By knocking down only the antisense transcript one can elevate the expression of the conventional (sense) gene in the case of discordant regulation. If the sense gene encodes for a known or putative drug target, then knockdown of its antisense counterpart could conceivably mimic the action of a receptor agonist or an enzyme stimulant. Table 3 gives

TABLE 3

Effects of siRNA-induced antisense transcript knockdown on sense transcript expression

Sense	Antisense (coding)	Antisense (non-coding)	Discordant regulation (sense increase)	Concordant regulation (sense decrease)	Species and cell Line[b]
CD97	Ddx-39	N/A[a]	Yes	No	[3] Mouse; N2A
TS-α	rTS-α	N/A	Yes	No	[3,12] Human; HeLa
C/EBP delta	I530027A02	N/A	No	Yes	[3] Mouse; Hepa1–6
CDC23	Kif20a	N/A	No	Yes	[3] Mouse; Hepa1–6
HIF1α	N/A	aHIF1α	No	Yes	[12] Human; HeLa
Gnbp3 g	N/A	Gnbp3 g-AS	No	Yes	[26] Mouse; N2A
Adrenomedullin AM1 receptor	N/A	AdmR-AS	No	Yes	[26] Mouse; N2A
6330439J10 (3-oxoacid CoA transferase)	N/A	A230019L24	No	Yes	[26] Mouse; N2A
CtpW85 (Cathepsin W)	N/A	CtpW-AS	No	Yes	[26] Mouse; N2A

[a]N/A, not applicable.

[b]Cells were treated for 48 h with 2–40 nM siRNA targeted to non-overlapping regions of the antisense transcript. Antisense and sense levels were measured by use of RT–PCR. See the text and Ref. [3] for further information on experimental details.

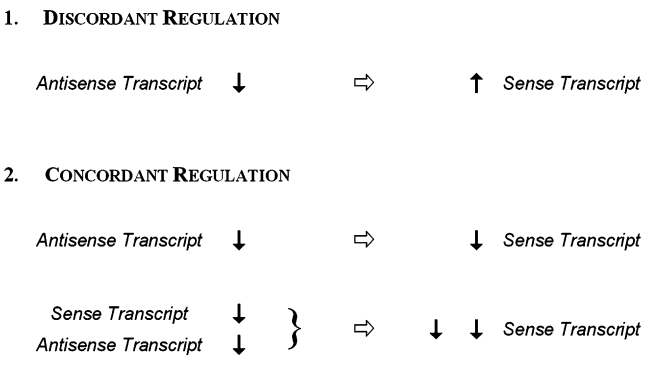

FIGURE 1

Perturbation of *cis*-antisense transcript by siRNA can affect the *cis*-sense transcript partner in ways that could be exploited pharmacologically. (1) A case where regulation is discordant, meaning that reduction in antisense transcript concentration results in an increase in sense transcript concentration. **(2)** Two different scenarios involving concordant regulation, referring to cases where knockdown of an antisense transcript will result in an increased sense transcript concentration. The two different scenarios in (2) represent targeting of the antisense transcript only, and the concomitant knockdown of the sense and corresponding antisense transcript. The latter scenario could be achieved either by two different siRNA or by a single siRNA designed so that the two siRNA strands will be efficacious in knocking down antisense and sense transcript simultaneously. Apart from the latter case, transcript reduction could in principle be achieved by other RNA-targeting principles, such as antisense oligonucleotides, ribozymes and perhaps small molecules.

examples where knockdown of antisense (coding as well as noncoding) transcripts was demonstrated to discordantly regulate sense expression. For example, to stimulate angiogenesis in some circumstances [29], enhanced signaling through the G-protein-coupled receptor (GPCR), CD97, might be achieved by targeting of its (coding) antisense partner, DDX39 [3].

Strategy 2. In the case of concordant regulation, one could either target the antisense transcript alone, resulting in reduction of sense mRNA, or concomitantly knock down both antisense and sense transcripts and thereby achieve additive or even synergistic reduction of the conventional (sense) gene expression. These concepts are illustrated in Figure 1. If, like in our studies, siRNA is used to achieve knockdown, then this strategy would be tested further by applying one siRNA targeted to the sense transcript and another siRNA to the corresponding antisense transcript, or a single energetically symmetric siRNA [30] that simultaneously targets overlapping sense and antisense transcripts by invoking efficacy of both siRNA strands. As follows from Table 3, such dual concomitant targeting might, for example, be relevant to pursue in the case of hypoxia-inducible factor 1 α, a target whose inhibition can be beneficial in various medical conditions (for example, see Ref. [31] and Table 1). Another example in Table 3 is the Adrenomedullin 1 receptor, a GPCR whose reduced signaling could also prove to be of therapeutic benefit (for example, see Ref. [32]).

Conclusion

There are numerous new potential drug targets to be considered in an emerging functional RNA world. Among these are thousands of naturally occurring antisense transcripts with a capacity to regulate the expression of sense transcripts, including those that encode for conventional human drug targets. Because the majority of these antisense transcripts represent noncoding RNA, there are, by definition, no protein products that can be manipulated for therapeutic purposes. It has been shown that, by using siRNA, antisense transcript knockdown can result in increases (discordant regulation) or decreases (concordant regulation) of sense transcript expression. RNA-targeting strategies other than siRNA might be equally applicable. These findings and concepts could form the basis for novel pharmacological strategies aimed at either stimulating or inhibiting the expression of specific genes that are influenced by natural antisense regulation.

Acknowledgements

I thank the FANTOM consortium, in particular Yoshihide Hayashizaki and Piero Carninci, for long-standing collaboration and for persistently generating and analyzing unique transcriptomics data. Par Engstrom, Alistair Chalk and Boris Lenhard are highly valued collaborators in the field of informatics. Mohammad Ali Faghihi and Yosuke Mizuno have been instrumental in generating the knockdown data in Table 3. Mohammad Ali Faghihi and Tammy Richmond have, in various ways, helped in generating this manuscript.

References

1 International Human Genome Sequencing Consortium, (2004) Finishing the euchromatic sequence of the human genome. *Nature* 431, 931–945
2 Carninci, P. *et al.* (2005) The transcriptional landscape of the Mamm.Genome. *Science* 309, 1559–1563
3 Katayama, S. *et al.* (2005) Antisense transcription in the mammalian transcriptome. *Science* 309, 1564–1566
4 Cheng, J. *et al.* (2005) Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. *Science* 308, 1149–1154
5 Kapranov, P. *et al.* (2005) Examples of the complex architecture of the human transcriptome revealed by RACE and high-density tiling arrays. *Genome Res.* 15, 987–997
6 Costa, F.F. (2005) Non-coding RNAs: new players in eukaryotic biology. *Gene* 357, 83–94
7 Pang, K.C. *et al.* (2006) Rapid evolution of noncoding RNAs: lack of conservation does not mean lack of function. *Trends Genet.* 22, 1–5
8 Farh, K.K. *et al.* (2005) The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. *Science* 310, 1817–1821
9 Willingham, A.T. *et al.* (2005) A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. *Science* 309, 1570–1573
10 Shendure, J. and Church, G.M. (2002) Computational discovery of sense-antisense transcription in the human and mouse genomes. *Genome Biol.* 3 research0044.1–0044.14
11 Lehner, B. *et al.* (2002) Antisense transcripts in the human genome. *Trends Genet.* 18, 63–65
12 Yelin, R. *et al.* (2003) Widespread occurrence of antisense transcription in the human genome. *Nat. Biotechnol.* 21, 379–386
13 Lavorgna, G. *et al.* (2004) In search of antisense. *Trends Biochem. Sci.* 29, 88–94
14 Dahary, D. *et al.* (2005) Naturally occurring antisense: transcriptional leakage or real overlap? *Genome Res.* 15, 364–368
15 Kiyosawa, H. *et al.* (2005) Disclosing hidden transcripts: mouse natural sense-antisense transcripts tend to be poly(A) negative and nuclear localized. *Genome Res.* 15, 463–474
16 Volpe, T.A. *et al.* (2002) Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* 297, 1833–1837

17 Faghihi, M. and Wahlestedt, C. (2006) RNA interference is not involved in natural antisense mediated regulation of gene expression in mammals. *Genome Biol.* (in press)
18 Wang, X.J. *et al.* (2005) Genome-wide prediction and identification of *cis*-natural antisense transcripts in *Arabidopsis thaliana*. *Genome Biol.* 6, R30
19 Jen, C.H. *et al.* (2005) Natural antisense transcripts with coding capacity in *Arabidopsis* may have a regulatory role that is not linked to double-stranded RNA degradation. *Genome Biol.* 6, R51
20 Rosok, O. and Sioud, M. (2005) Systematic search for natural antisense transcripts in eukaryotes (review). *Int. J. Mol. Med.* 15, 197–203
21 Makalowska, I. *et al.* (2005) Overlapping genes in vertebrate genomes. *Comput. Biol. Chem.* 29, 1–12
22 Werner, A. and Berdal, A. (2005) Natural antisense transcripts: sound or silence? *Physiol Genomics* 23, 125–131
23 Hopkins, A.L. and Groom, C.R. (2002) The druggable genome. *Nat. Rev. Drug Discov.* 1, 727–730
24 Wahlestedt, C. (1994) Antisense oligonucleotide strategies in neuropharmacology. *Trends Pharmacol. Sci.* 15, 42–46
25 Zaman, G.J. *et al.* (2003) Targeting RNA: new opportunities to address drugless targets. *Drug Discov. Today* 8, 297–306
26 Faghihi, M.A. *et al.* (2006). Natural antisense regulation of mouse and human genes. Manuscript
27 Munroe, S.H. (2004) Diversity of antisense regulation in eukaryotes: multiple mechanisms, emerging patterns. *J. Cell. Biochem.* 93, 664–671
28 Rougeulle, C. and Avner, P. (2004) The role of antisense transcription in the regulation of X-inactivation. *Curr. Top. Dev. Biol.* 63, 61–89
29 Wang, T. *et al.* (2005) CD97, an adhesion receptor on inflammatory cells, stimulates angiogenesis through binding integrin counterreceptors on endothelial cells. *Blood* 105, 2836–2844
30 Schwarz, D.S. *et al.* (2003) Asymmetry in the assembly of the RNAi enzyme complex. *Cell* 115, 199–208
31 Mizuno, T. *et al.* (2006) Small interfering RNA expression vector targeting hypoxia-inducible factor 1 alpha inhibits tumor growth in hepatobiliary and pancreatic cancers. *Cancer Gene Ther.* 13, 131–140
32 Kuwasako, K. *et al.* (2004) Adrenomedullin receptors: pharmacological features and possible pathophysiological roles. *Peptides* 25, 2003–2012
33 Thrash-Bingham, C.A. and Tartof, K.D. (1999) aHIF: a natural antisense transcript overexpressed in human renal cancer and during hypoxia. *J. Natl. Cancer Inst.* 91, 143–151
34 Yamamoto, T. *et al.* (2002) Downregulation of survivin expression by induction of the effector cell protease receptor-1 reduces tumor growth potential and results in an increased sensitivity to anticancer agents in human colon cancer. *Eur. J. Cancer* 38, 2316–2324
35 Tufarelli, C. *et al.* (2003) Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. *Nat. Genet.* 34, 157–165
36 Smilinich, N.J. *et al.* (1999) A maternally methylated CpG island in KvLQT1 is associated with an antisense paternal transcript and loss of imprinting in Beckwith-Wiedemann syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 96, 8064–8069
37 Rougeulle, C. *et al.* (1998) An imprinted antisense RNA overlaps UBE3A and a second maternally expressed transcript. *Nat. Genet.* 19, 15–16
38 Capaccioli, S. *et al.* (1996) A bcl-2/IgH antisense transcript deregulates bcl-2 gene expression in human follicular lymphoma t(14;18) cell lines. *Oncogene* 13, 105–115
39 Hammond, S.M. (2006) MicroRNAs as oncogenes. *Curr. Opin. Genet. Dev.* 16, 4–9
40 Hermanns, P. *et al.* (2005) Consequences of mutations in the non-coding RMRP RNA in cartilage-hair hypoplasia. *Hum. Mol. Genet.* 14, 3723–3740

AGORA initiative provides free agriculture journals to developing countries

The Health Internetwork Access to Research Initiative (HINARI) of the WHO has launched a new community scheme with the UN Food and Agriculture Organization.

As part of this enterprise, Elsevier has given hundreds of journals to Access to Global Online Research in Agriculture (AGORA). More than 100 institutions are now registered for the scheme, which aims to provide developing countries with free access to vital research that will ultimately help increase crop yields and encourage agricultural self-sufficiency.

According to the Africa University in Zimbabwe, AGORA has been welcomed by both students and staff. "It has brought a wealth of information to our fingertips", says Vimbai Hungwe. "The information made available goes a long way in helping the learning, teaching and research activities within the University. Given the economic hardships we are going through, it couldn't have come at a better time."

For more information, visit www.aginternetwork.org