

Genome-wide natural antisense transcription: coupling its regulation to its different regulatory mechanisms

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Many genomic loci contain transcription units on both strands, therefore two oppositely oriented transcripts can overlap. Often, one strand codes for a protein, whereas the transcript from the other strand is non-encoding. Such natural antisense transcripts (NATs) can negatively regulate the conjugated sense transcript. NATs are highly prevalent in a wide range of species—for example, around 15% of human protein-encoding genes have an associated NAT. The regulatory mechanisms by which NATs act are diverse, as are the means to control their expression. Here, we review the current understanding of NAT function and its mechanistic basis, which has been gathered from both individual gene cases and genome-wide studies. In parallel, we survey findings about the regulation of NAT transcription. Finally, we hypothesize that the regulation of antisense transcription might be tailored to its mode of action. According to this model, the observed relationship between the expression patterns of NATs and their targets might indicate the regulatory mechanism that is in action.

Keywords: natural antisense transcript (NAT); regulatory RNA; RNA interference; tile array; noise dampening

EMBO reports (2006) 7, 1216–1222. doi:10.1038/sj.embor.7400857

Introduction

Natural antisense transcripts (NATs) are endogenous RNA molecules containing sequences that are complementary to other transcripts. NATs can be divided into *cis*-NATs, which are transcribed from opposing DNA strands at the same genomic locus, and *trans*-NATs, which are transcribed from separate loci. *cis*-NAT pairs display perfect sequence complementarity (as expected from their genomic overlap), whereas *trans*-NAT pairs display imperfect complementarity and can therefore target many sense targets to form complex regulation networks (Li *et al*, 2006).

cis-NATs were first detected in viruses (Barrell *et al*, 1976), then in prokaryotes (Tomizawa *et al*, 1981; Wagner & Simons, 1994) and finally in eukaryotes (Knee & Murphy, 1997; Kumar &

Carmichael, 1998; Williams & Fried, 1986). The importance of NATs is now apparent, and their widespread prevalence has been reported in many genomes (see next section). *cis*-NATs can be categorized according to their relative orientation and degree of overlap; head-to-head (5' to 5'), tail-to-tail (3' to 3') or fully overlapping (Fig 1). All genome-wide studies, except one (Katayama *et al*, 2005), have reported the tail-to-tail orientation to be the most prevalent. Overlapping transcripts might comprise two protein-encoding genes, one protein-encoding and one non-encoding gene, or two non-encoding transcripts.

The conservation of the phenomenon across kingdoms (Wagner & Flardh, 2002) implies that it might constitute a common mechanism for regulating gene expression. NATs have been proposed to regulate the expression of their target genes at several levels including transcription, messenger RNA processing, splicing, stability, cellular transport and translation. They are also linked to monoallelic gene expression through mechanisms that include genomic imprinting, X-inactivation and clonal expression. However, apart from a few experimentally validated cases (Korneev *et al*, 1999; Kramer *et al*, 2003; Sleutels *et al*, 2003), the physiological roles of antisense transcription and the underlying mechanisms are largely unknown.

Here, we summarize recent findings about the extent of the NAT phenomenon, the modes of regulation by NATs, and the transcriptional regulation of NATs and their targets. We focus on the extensively characterized *cis*-NATs and suggest that a relationship exists between the mechanism of antisense function and the transcriptional regulation of sense and antisense transcripts: some mechanisms of antisense action require co-expression with its target, some require a time delay between the onset of antisense and sense transcription, and other mechanisms manifest themselves in anti-correlated expression patterns of the regulator and its target. This concept could be used to infer the regulatory mechanism of action, given the expression profiles of antisense and their sense targets.

Extent of the NAT phenomenon

Genome-wide natural antisense transcription has been reported in various animal and plant species including humans (Chen *et al*, 2004; Sun *et al*, 2006; Yelin *et al*, 2003), mice (Carninci *et al*, 2005; Katayama *et al*, 2005; Kiyosawa *et al*, 2003; Sun *et al*, 2006), rats

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Submitted 28 March 2006; accepted 18 October 2006

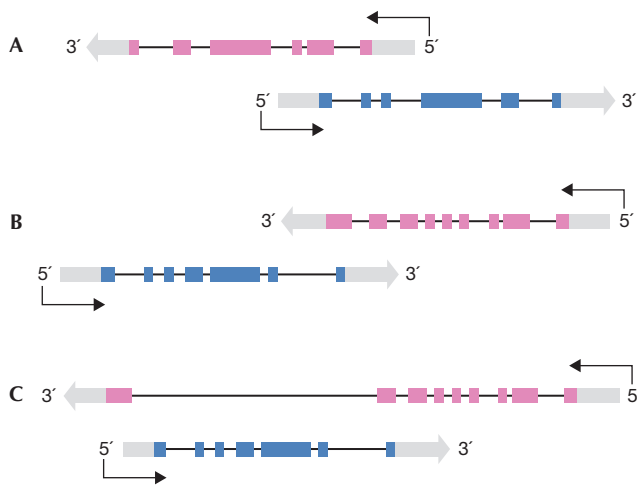


Fig 1 | Relative orientation of *cis*-natural antisense transcript pairs. (A) Head-to-head (5' to 5') overlap involving 5'-untranslated regions and coding exons. (B) Tail-to-tail (3' to 3') overlap. (C) Fully overlapping (one gene included entirely within the region of the other). Coloured boxes represent exons, grey boxes represent untranslated regions.

and chickens (Sun *et al*, 2006), *Drosophila* (Misra *et al*, 2002; Sun *et al*, 2006), nematodes (Sun *et al*, 2006), rice (Osato *et al*, 2003), *Arabidopsis* (Jen *et al*, 2005; Wang *et al*, 2005) and yeast (David *et al*, 2006; Havilio *et al*, 2005). The estimated extent of the phenomenon, measured in terms of the percentage of transcriptional units involved in an overlap, ranges from 5% to 29% in animals (excluding nematodes, which have a much lower percentage of 0.5%) and from 7% to 9% in plants. Most estimates are based on the alignment of full-length cDNAs and expressed sequence tags (ESTs) to the genome, and the identification of overlapping transcripts on opposite strands. Such a procedure is limited to the detection of *cis*-NATs, implying that the extent of the NAT phenomenon might be much broader. Some predictions have been experimentally validated using methods such as reverse transcription-PCR (RT-PCR) and microarrays containing strand-specific probes (Chen *et al*, 2004; Yelin *et al*, 2003).

In mice, all known full-length cDNAs have been clustered into 43,553 transcriptional units and mapped onto the genome (Katayama *et al*, 2005). Almost 29% (12,519) of all mapped transcriptional units were found to overlap with a cDNA that mapped to the opposite strand, greatly exceeding any previous prediction.

In humans, comparable numbers of overlapping transcript pairs were reported by Yelin and colleagues (2,667; Yelin *et al*, 2003) and Chen and colleagues (2,940; Chen *et al*, 2004). Both groups aligned ESTs and cDNAs to the genome to create transcriptional clusters and experimentally validated some of their predictions. Chen and colleagues reported that nearly 22% (5,880) of their 26,741 transcriptional clusters form sense-antisense pairs, which is the highest number estimated for humans. Furthermore, the data sets published by the two research groups overlap by only 38%, indicating that the total number of human sense-antisense pairs might be even greater.

A genome-wide tile array (Bertone *et al*, 2004) revealed 10,595 novel human transcripts, 1,187 of which lie antisense to annotated exons. Annotation of the *Drosophila* genome identified 1,027 sense-antisense pairs, representing 15% of the 13,379 genes (Misra

Table 1 | Genome-wide natural antisense transcripts in several eukaryotic species

Species	Transcripts involved in overlap	Total transcripts	Percentage (%)	References
Human	5,880	26,741	22	Chen <i>et al</i> , 2004; Sun <i>et al</i> , 2006
Mouse	12,519	43,553	*29	Katayama <i>et al</i> , 2005
Rat	548	11,332	5	Sun <i>et al</i> , 2006
Chicken	356	7,390	5	Sun <i>et al</i> , 2006
<i>Drosophila</i>	2,054	13,379	*15	Misra <i>et al</i> , 2002; Sun <i>et al</i> , 2006
Rice	1,374	20,477	7	Osato <i>et al</i> , 2003
<i>Arabidopsis</i>	2,680	29,993	9	Wang <i>et al</i> , 2005
Nematode	76	14,406	0.5	Sun <i>et al</i> , 2006
Yeast	610	7,598	*8	David <i>et al</i> , 2006

*Sun and colleagues recently reported a significantly lower proportion of only 12% for mouse (2,212 out of 19,100), and a slightly higher proportion of 17% for *Drosophila* (1,814 out of 10,542; Sun *et al*, 2006). Estimations in yeast are based on both poly(A) RNA and total RNA.

et al, 2002). In plants, annotation of full-length rice cDNAs revealed 687 overlapping cluster pairs, amounting to around 7% of all cDNA clusters (Osato *et al*, 2003), and similar percentages were obtained in *Arabidopsis thaliana* (Table 1; Wang *et al*, 2005). Genome-wide antisense transcription in yeast was recently reported (Havilio *et al*, 2005) and further supported by the construction of high-density tile arrays of the *Saccharomyces cerevisiae* genome (David *et al*, 2006). When grown in rich medium, 85% of the genome was expressed, including hundreds of non-encoding transcripts. Antisense transcripts were identified for up to 1,555 of the genes. These findings confirm that antisense transcription is a highly conserved phenomenon that spans the animal, plant and fungal kingdoms.

Regulating the regulator

Most coding *cis*-NAT pairs overlap in their untranslated regions (UTRs). Such overlaps often involve alternative polyadenylation, which creates transcript variants that differ in their 3' termini. Similarly, transcripts with heterogeneous transcription start sites might form head-to-head overlaps. A key question is whether such alternative (3' or 5') end processing is intentional—forming regulated transcript overlaps—or does it result from 'leakage' of the RNA transcription machinery?

To address this question, Dahary and colleagues examined the evolution of *cis*-NATs (Dahary *et al*, 2005). They defined a set of consecutive gene pairs in the human genome and identified their orthologous gene pairs in both mouse and *Fugu*. The human genes were divided into sense-antisense pairs and pairs that were transcribed from the same strand. The authors assumed that if sense-antisense pairs carried a beneficial function, selection would work against their separation in related species. Indeed, 23.3% (55 out of 236) of the human sense-antisense pairs remained consecutive in *Fugu*, compared with only 13.5% (170 out of 1,250) of the same-strand pairs. Moreover, although the *Fugu* genome is much more compact than the human genome, the average distance between sense-antisense gene pairs was only slightly greater in humans than in *Fugu*, whereas same-strand pairs were significantly further apart (up to 11-fold).

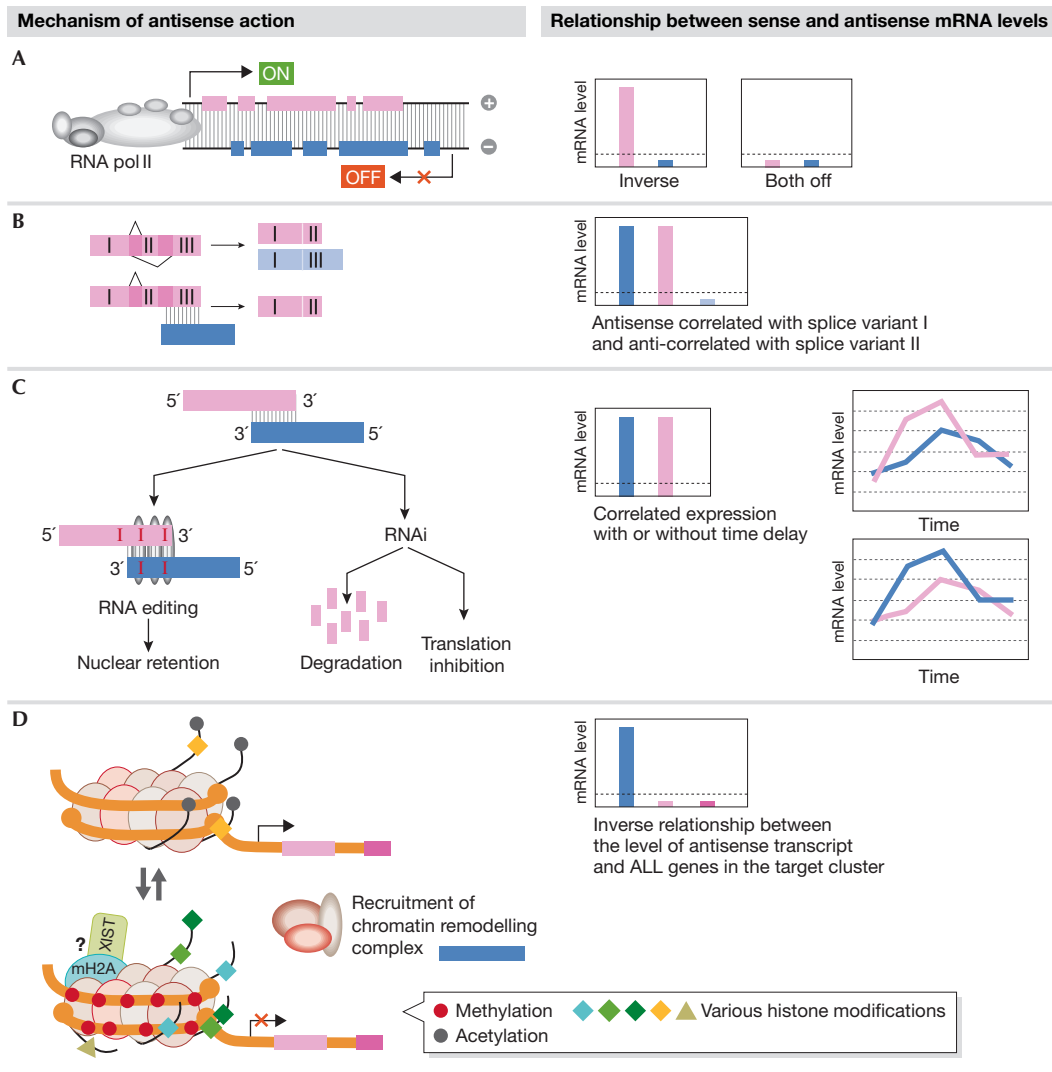


Fig 2 | The main mechanisms by which natural antisense transcripts regulate gene expression. Each mechanism is accompanied by what it requires from, or imposes on, the relationship between the levels of sense and antisense transcripts. **(A)** Transcriptional interference. Two bulky RNA polymerase II complexes on opposite DNA strands might collide with and stall one another. The interference occurs mostly in the elongation step, resulting in either transcription arrest or transcription in one direction (sense or antisense) only. Such a mechanism might occur in cases in which inverse expression is observed. **(B)** RNA masking. A specific case is shown in which the antisense masks a splice site on the sense pre-mRNA sequence. This prevents a given splice variant from being formed and shifts the balance towards splice variants that do not require splicing of the masked region. Such a mechanism could be observed by correlated expression of the antisense and favoured splice variant and an inverse relationship with the repressed variant. **(C)** Double-stranded RNA-dependent mechanisms such as RNA editing and RNA interference require the simultaneous presence of sense and antisense transcripts for duplex formation, and might therefore account for the observed co-expression of numerous sense–antisense pairs. A delay in expression of sense compared with antisense (or vice versa) is also possible as long as there is a period in which both transcripts are present (see Fig 3). **(D)** Chromatin remodelling. Transcription of non-encoding antisense transcripts is involved in monoallelic gene expression, including genomic imprinting, X-inactivation and clonal expression of lymphocyte genes. In these processes, antisense transcripts have been suggested to silence the expression of nearby gene clusters by chromatin remodelling, most likely through the recruitment of histone-modifying enzymes. If such mechanisms are in action, an inverse expression profile of the antisense compared with all genes in the silenced cluster would be expected.

If antisense transcription is indeed beneficial, how is it regulated? A recent study (Cawley *et al*, 2004) mapped the binding sites of three human transcription factors—SP1, c-Myc and p53—to chromosomes 21 and 22 using chromatin immunoprecipitation (ChIP)-on-chip technology (Ren *et al*, 2000). Surprisingly, 36% of the binding sites mapped within or immediately 3' to well-characterized

protein-encoding genes and were associated with non-encoding RNAs. Moreover, many overlapping sense–antisense transcripts showed correlated expression. Some overlapping transcripts were flanked by binding sites for the same transcription factor, implying that the sense and corresponding antisense transcripts might in fact be co-regulated. Similar results were obtained for the human

transcription factor cAMP-response-element-binding protein (Impey *et al.*, 2004).

Chen and colleagues used their comprehensive human anti-sense database to study the relationship between the expression profiles of sense and corresponding antisense transcripts on a genome-wide scale (Chen *et al.*, 2005a). They found that sense–antisense gene pairs tend to be co-expressed or inversely expressed more frequently than would be expected by chance. Moreover, co-expressed and inversely expressed sense–antisense pairs have striking conservation throughout evolution. Both conservation and coupled sense–antisense expression were more prevalent in tail-to-tail NAT pairs, suggesting that such an orientation is not only the most abundant, but also more likely to have a regulatory function (Sun *et al.*, 2005).

Chen and colleagues also reported that antisense genes, especially those that are evolutionarily conserved, have particularly short introns in humans, mice and *Drosophila* (Chen *et al.*, 2005b; Sun *et al.*, 2006). The authors suggested that, in the case of the antisense genes, the purpose of short introns is not to allow a high level of expression or spurious expression, as has been shown for other genes (Castillo-Davis *et al.*, 2002; Hurst *et al.*, 1996), but to address the need for a rapid response.

Although the above studies suggest that antisense transcription is tightly regulated and evolutionarily conserved, they should be regarded with some caution. The observed co-expression of *cis*-NATs might originate from the known tendency of genes in close proximity (even in the same genomic strand) to co-express as a result of local chromatin structure or shared regulatory elements (Cohen *et al.*, 2000; Lercher *et al.*, 2003). It is possible that proximal co-expressed genes that were not selected against seem to be ‘tailored’ by evolution to serve a regulatory purpose. A recent study introduced the intriguing concept of “neutral expression” (Yanai *et al.*, 2004). The authors of this study argue that mutations that alter gene expression might not always be sufficiently deleterious to be eliminated by purifying selection, and therefore might be fixated in the population by random drift. According to this idea, the possibility that some NATs represent cases of residual transcription cannot be entirely eliminated.

Coupling NAT mechanisms of action to their regulation

The observation of both negative and positive correlations of sense–antisense levels suggests that their mechanisms of action might be diverse. Indeed, well-documented NAT examples point to four mechanisms (Lavorgna *et al.*, 2004): transcriptional interference, RNA masking, double-stranded RNA (dsRNA)-dependent mechanisms and chromatin remodelling (see below). Each mechanism requires different associations between sense and antisense expression patterns; some mechanisms require the concomitant presence of sense and antisense transcripts, whereas others impose their mutual exclusion (Fig 2). We propose that the regulation of sense and antisense transcription is coupled to serve the different regulatory mechanisms. Furthermore, because the type of coupling is characteristic of the regulatory mechanism, we suggest that the relationship between sense and antisense transcription profiles can hint at the mechanism at work as well as the ultimate biological outcome.

To illustrate this point, we predict two biological outcomes that might result from a delayed initiation of transcription between the sense and antisense transcripts (or vice versa). First, if the sense gene is initially transcribed up to a certain level, then antisense transcription

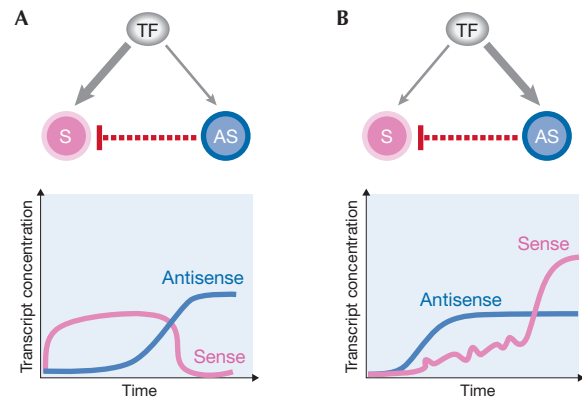


Fig 3 | Differences in activation times of the sense compared with the antisense transcript. Such differences might be easily encoded in differential affinities to a shared transcription factor, assuming that this transcription factor is an activator and that it accumulates with time. (A) A higher affinity of the transcription factor to the sense transcript might result in a delayed shutdown, whereby the transcription factor initially activates transcription of the sense messenger RNA up to a certain level and only then is triggered by antisense transcription. The delayed antisense transcription prevents the sense transcript from exceeding the level it has reached when antisense transcription is switched on. (B) A higher affinity of the transcription factor to the antisense transcript. In this case, antisense transcription precedes sense transcription and acts as a buffer for the sense transcript. When the transcription factor accumulates, transcription of sense mRNA begins, but only sense transcripts exceeding the threshold set by the antisense level can be effectively translated. This generates a step-like function in the concentration of the sense transcript. Fluctuations in the amount of sense transcript below the threshold are dampened.

begins and subsequently promotes sense degradation, then the anticipated outcome is a delayed shutdown of the sense gene. Second, if antisense transcription precedes sense transcription, the biological outcome might be the dampening of stochastic fluctuations (noise) in the level of the sense transcripts; the antisense level sets a threshold and only sense transcripts that exceed it are effectively expressed (Fig 3). Noise dampening was shown to be obtained by another type of regulatory RNA, microRNAs (Hornstein & Shomron, 2006), and we hypothesize that antisense transcripts might fulfill the same function. Differences in transcription activation times might be encoded by differential affinities of the sense and antisense promoters to a shared transcription factor, assuming that such a regulator is an activator and that it accumulates with time.

Sense and antisense transcripts might be regulated not only at the transcriptional level, but also at the level of mRNA stability. Therefore, differences in mRNA half-lives of the two transcripts might also be predictive of antisense function. A well-characterized example is the *hok*–*sok* system of the R1 plasmid (Gerdes *et al.*, 1990), in which differences in mRNA stability result in delayed activation of the sense-encoded protein. The R1 plasmid encodes a host killing protein (*hok*) and its antisense suppressor *sok*; however, killing of host cells occurs when the plasmid is no longer present. This is accomplished by combining a stable and initially inert toxin with an unstable inhibitor. *Hok* mRNA accumulates in a stable and inert state and is slowly processed into a transcriptionally active form. In plasmid-containing cells, the active *hok* is

Table 2 | Well-characterized examples of antisense regulatory function

Mechanism	Example	Reference
Transcriptional interference	The yeast <i>GAL10</i> and <i>GAL7</i> genes, organized naturally in tandem, were rearranged in a convergent orientation. Transcription initiation was carried out normally but, as soon as the two transcripts began to overlap, elongation stalled and mRNA levels were severely reduced.	Prescott & Proudfoot, 2002
RNA masking	Expression of <i>RevErb</i> , overlapping <i>erbA</i> (encoding α -thyroid hormone receptor) strongly correlates with an increase in the ratio of splice variants <i>erbAα1/erbAα2</i> . <i>RevErb</i> overlaps only with <i>erbAα2</i> and is thought to block its splicing by masking splicing regulatory <i>cis</i> -elements. Artificial antisense RNAs complementary to the <i>Erbα2</i> -specific exon were shown to efficiently and specifically block <i>ErbAα2</i> splicing <i>in vitro</i> .	Hastings <i>et al</i> , 1997; Munroe & Lazar, 1991
dsRNA-dependent mechanisms	Salt tolerance in <i>Arabidopsis</i> is regulated by two siRNAs produced from a pair of tail-to-tail overlapping protein-encoding genes: <i>P5CDH</i> (a stress-related gene) and <i>SRO5</i> (of unknown function). When both transcripts are present, an RNA duplex is formed and two types of siRNA are produced: 24 nucleotides (nt) and 21 nt. The 24-nt siRNA causes initial cleavage of the <i>P5CDH</i> transcript, which is followed by the generation of 21-nt siRNA and further cleavage of the <i>P5CDH</i> transcript. The expression of <i>SRO5</i> is induced by salt and this induction is required to initiate siRNA formation.	Borsani <i>et al</i> , 2005
dsRNA-dependent mechanisms	Response to iron deficiency in Cyanobacteria is mediated through the formation of an RNA duplex. Cyanobacteria responds to iron deficiency by expressing <i>IsiA</i> (iron stress-induced protein A), which forms a giant ring structure around photosystem I. <i>IsiA</i> is regulated by its <i>cis</i> -encoded antisense <i>IsrR</i> (iron stress-repressed RNA). Artificial overexpression of <i>IsrR</i> under iron stress causes a strongly diminished number of <i>IsiA</i> -photosystem supercomplexes, whereas <i>IsrR</i> depletion results in premature expression of <i>IsiA</i> . The mRNAs <i>IsrR</i> and <i>isiA</i> form a perfect duplex and undergo coupled degradation.	Duhring <i>et al</i> , 2006
dsRNA-dependent mechanisms	Silencing of <i>Drosophila melanogaster</i> Stellate repeats in testis is essential for male fertility and involves dsRNA. Stellate silencing is mediated by the homologous <i>Su (Ste)</i> tandem repeats. Both strands of the repressor repeats are transcribed producing a sense-antisense duplex. This duplex is further cleaved into short 25–27-nucleotide fragments, which confer specific silencing of the Stellate repeats.	Aravin <i>et al</i> , 2001
Effect on methylation	The head-to-head overlapping gene pair <i>Sphk1/Khps1</i> have been shown to undergo antisense-induced methylation.	Imamura <i>et al</i> , 2004

targeted by sok, resulting in translation inhibition and message degradation. Plasmid-free cells show rapid loss of the unstable sok, therefore on activation of hok, its suppressor is no longer present and the host cell is killed.

Below we describe four proposed mechanisms of NAT action and accompany each with the resulting relationship between sense and antisense transcript levels.

Transcriptional interference. The presence of an overlapping transcriptional unit might stall sense transcription owing to the collision of two bulky RNA polymerase II complexes on opposite strands. This is most apparent in the transcription elongation step as has been shown for the yeast gene pair *GAL10* and *GAL7* (Table 2; Prescott & Proudfoot, 2002). Competitive transcriptional interference could be the underlying mechanism when anti-correlated expression levels of sense and antisense are observed. Such interference might alternatively result in the shutdown of both transcripts (Fig 2A).

RNA masking. Sense-antisense duplex formation might mask *cis*-elements residing in either of the transcripts and hinder processes that require protein-RNA interactions such as splicing, mRNA transport, polyadenylation, translation and degradation. The best-characterized example of this mechanism is the antisense transcript for the thyroid hormone receptor gene *erbA α* , which shifts the balance between two splice variants through the masking of a splice site (Table 2; Hastings *et al*, 1997). Such a mechanism would result in a correlated expression level of the antisense and the regulated transcript (Fig 2B).

dsRNA-dependent mechanisms and RNA interference. There is accumulating evidence that antisense transcripts might function through the activation of dsRNA-dependent mechanisms such as RNA editing and RNA interference (RNAi). Such mechanisms require the simultaneous existence of sense and antisense transcripts for duplex formation, and might therefore account for the observed co-expression of numerous sense-antisense pairs (Fig 2C; Chen *et al*, 2005a).

RNA editing involves the deamination of adenosines to inosines in dsRNA (Bass, 2002) and is thought to constitute part of the nuclear defence strategy against dsRNA. Hyper-editing of long, perfect RNA duplexes can result in their nuclear retention (Zhang & Carmichael, 2001) or cytoplasmic degradation (Scadden & Smith, 2001). However, it was recently shown that the level of RNA editing in sense-antisense overlapping areas—apart from the *Alu* regions within them—is negligible in both humans and mouse (Neeman *et al*, 2005). Regulation through RNA editing is therefore not likely to be one of the main mechanisms for antisense action.

RNAi is another component of the defence against dsRNA. RNAi involves cleavage of the dsRNA by the enzyme Dicer into 21–23-nucleotide duplexes. These duplexes are further separated into single strands and become part of the RNA-induced silencing complex (RISC). The RISC eventually either degrades cognate mRNAs with great specificity or represses their translation (Meister & Tuschl, 2004; Mello & Conte, 2004). Several precedents suggest that sense-antisense transcription can induce gene silencing through an RNAi-dependent mechanism. For example, salt tolerance in *Arabidopsis* is regulated by two small interfering RNAs (siRNAs) produced from a pair of tail-to-tail overlapping protein-encoding

genes: *P5CDH* (a stress-related gene) and *SRO5*. Salt induces *SRO5* transcription. When both genes are transcribed, an RNA duplex is formed and siRNAs are produced that ultimately cleave the *P5CDH* transcript (Table 2; Borsani *et al*, 2005). The same mechanism could apply to other eukaryotic *cis*-NAT pairs. In fact, 11 *Arabidopsis* siRNAs have been mapped to complementary regions of overlapping transcripts, suggesting that these overlapping transcripts might feed into the RNAi machinery (Wang *et al*, 2005). Other processes shown to involve dsRNA are the response to iron deficiency in cyanobacteria (Duhring *et al*, 2006) and the maintenance of male fertility in *Drosophila* (Table 2; Aravin *et al*, 2001). So far, however, there has been no evidence for mammalian antisense transcripts acting through duplex formation.

Antisense involvement in methylation and monoallelic expression. dsRNA can induce the methylation and silencing of corresponding genes. For example, thalassaemia—a form of anaemia—is caused by antisense-induced DNA methylation (and silencing) of the human haemoglobin 2 gene (Tufarelli *et al*, 2003).

Monoallelic expression includes X-chromosome inactivation, genomic imprinting and allelic exclusion in B and T lymphocytes. X-chromosome inactivation is a mechanism that balances the expression of X-chromosome-encoded genes in mammalian females. The silencing of one of the X chromosomes is mediated through a large non-encoding RNA (*Xist*), which recruits a histone-modifying protein complex. *Xist* is repressed by its antisense *Tsix*, thus the X chromosome expressing the antisense remains active (Ogawa & Lee, 2002). Imprinted genes are genes for which only one allele—maternal or paternal—is actively transcribed. There are about 100 known human and mouse imprinted genes; they are clustered in the genome and often have both DNA methylation and non-encoding antisense transcripts. Several studies have indicated that imprinting is not mediated through the formation of a sense–antisense RNA duplex (Sleutels *et al*, 2003; Thakur *et al*, 2004), but rather through the modification of chromatin structure or methylation patterns in the vicinity of the imprinted allele. In lymphocytes, immunoglobulins and T-cell receptors undergo clonal selection through which one allele is silenced while the other undergoes recombination. Extensive antisense transcription occurs before and during recombination and is believed to function by inducing an open chromatin structure that is accessible to recombination. In all these cases, non-encoding antisense transcription affects an entire gene cluster, rather than merely the overlapping sense transcript, and exerts its effect by chromatin remodelling, probably through the recruitment of histone-modifying enzymes. We therefore predict an inverse expression profile for the antisense and all the genes in the silenced cluster (Fig 2D).

Despite these well-characterized cases, it is not clear which of these mechanisms might apply to a wider set of antisense RNAs.

Discussion

There has been a revolution in our understanding of the regulatory role of non-encoding RNAs in recent years. Genome-wide technologies reveal that a significant proportion of all genomes is transcribed, and might thus fulfill regulatory functions (Carninci, 2006). The possibility that transcribed RNAs represent leakage of the transcription machinery exists, but evidence for a selected process is convincing. In this review, we have discussed one type of non-encoding RNA and suggest that its transcriptional, and post-transcriptional, regulation is tailored to its various regulatory roles.

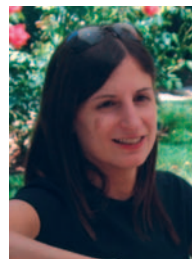
ACKNOWLEDGEMENTS

Y.P. is an incumbent of the Rothstein Career Development Chair in Genetic Diseases and a recipient of a Young Investigator award from the European Molecular Biology Organization. M.L. is a Fellow of the Horowitz Foundation for Complexity Sciences. We thank the Ben May Charitable Trust for grant support.

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