Commentary

Antisense Translates into Sense

By Stuart W. Peltz and Joseph P. Dougherty

From the Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School-University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854

The paradigm that DNA is the central bank of genetic information within the cell, and that portions of the DNA are copied via transcription to RNA, which is subsequently decoded to synthesize protein in a cell, is basically true throughout cellular evolution. However, it is also true that although this is a general blueprint for regulating how genes are expressed, evolution, with its varied selective pressures, has allowed organisms to develop many different mechanisms for regulating gene expression. As a consequence, a plethora of novel regulatory schemes from bacteria to mammalian cells has been described. Organisms with constraints on genome size have used novel genetic tricks to both regulate gene expression and synthesize multiple proteins from a single gene. However, in higher eucaryotes, the common perception is that genome size is not an issue, and that genes will typically be far apart from each other and can be regulated by independent control mechanisms.

Although genes in mammalian cells are generally separated by large chunks of DNA, the paper by Van den Eynde and colleagues in this issue (1) demonstrates that this is not always the case. What started off as a project to isolate a tumor-specific antigen from a renal carcinoma cell led to the discovery of a protein product that is encoded within another gene from its antisense strand (Fig. 1). The project began by isolating a clone of a CTL from a kidney cancer patient that responded to tumor cells. A cDNA library prepared with RNA from the renal tumor cells was transfected, along with HLA-B7 gene, into COS cells. A cDNA was isolated that was able to stimulate the CTL cells. Further analysis indicated that the transcript should be ∼1.4 kb in size. Surprisingly, however, the transcript migrated as a 2.2-kb mRNA on a Northern blot. This paradox was resolved when Van den Eynde and colleagues characterized additional cDNA clones from this library. Unexpectedly, these results led to the discovery that the tumor-specific antigen was not encoded within the open reading frame of this gene. Rather, it resided within this gene but initiated its transcription within its first intron and synthesized the RNA from the complementary (or antisense) strand of this gene (Fig. 1). The authors named the gene encoding the 2.2-kb RNA band RU2S, while the gene encoding the tumor-specific antigen transcribed in the opposite direction was called RU2AS. The RU2S transcript appears to encode a housekeeping mRNA, since it is expressed in all tissues examined and the presence of CpG islands within the promoter and first exon are also consistent with this notion.

On the other hand, the antisense RU2AS mRNA was restricted to expression in the kidney, bladder, liver, and testis, yet interestingly, RU2AS was expressed in a large number of tumor cells.

These results are striking for a number of reasons. Clearly, they demonstrate that mammalian genes do not have to be separated from each other by significant amounts of DNA. These results also demonstrate that the antisense strand of one gene can be transcribed and encode a protein product. Although there are several examples of noncoding RNAs that are involved in regulating gene expression in both prokaryotes and eukaryotes (for a review, see reference 2), examples in which antisense RNAs encode a protein have been rare, having only been observed in viruses such as herpes simplex virus (HSV) adenovirus, and HIV (2–5). It is not surprising that viruses, with compact genomes, will use both strands to encode genetic information, allowing them to maximize the relative amount of information they can transfer. In the cases of herpes virus and adenovirus, the antisense RNAs do not seem to have a regulatory function since they are expressed at different stages in the virus life cycle, so their expression is temporally separated within an infected cell. This leads to an interesting question that has yet to be addressed, namely does the RU2AS RNA and not the protein product have a regulatory role in controlling gene expression? Does it also function as an antisense RNA to regulate the expression of the RU2S mRNA? Does the RU2AS protein product have a regulatory role in controlling the expression of the RU2S transcript? Or, possibly, they are not functionally related. They might represent remnants of a viral infection and evolved to have different functions. It is not likely that expression of RU2AS is to signal antitumor CTLs for killing. These questions will await further analysis of the role of the RU2SA protein product in cells and how this protein product is regulated.

The RU2AS transcript has an attribute that makes it unique compared with most other cellular mRNAs. The RU2AS transcript lacks intron sequences. This is an unusual scenario in mammalian cells, since removal of the last intron correlates with efficient polyadenylation (6, 7). Furthermore, the splicing process has also been linked to efficient export of RNAs from the nucleus to the cytoplasm (see 6–8). In fact, viruses, in particular retroviruses and cellular mRNAs that lack introns, have evolved specialized sequences that promote efficient cytoplasmic transport of unspliced RNAs. For example, the retrovirus Mason-Pfizer
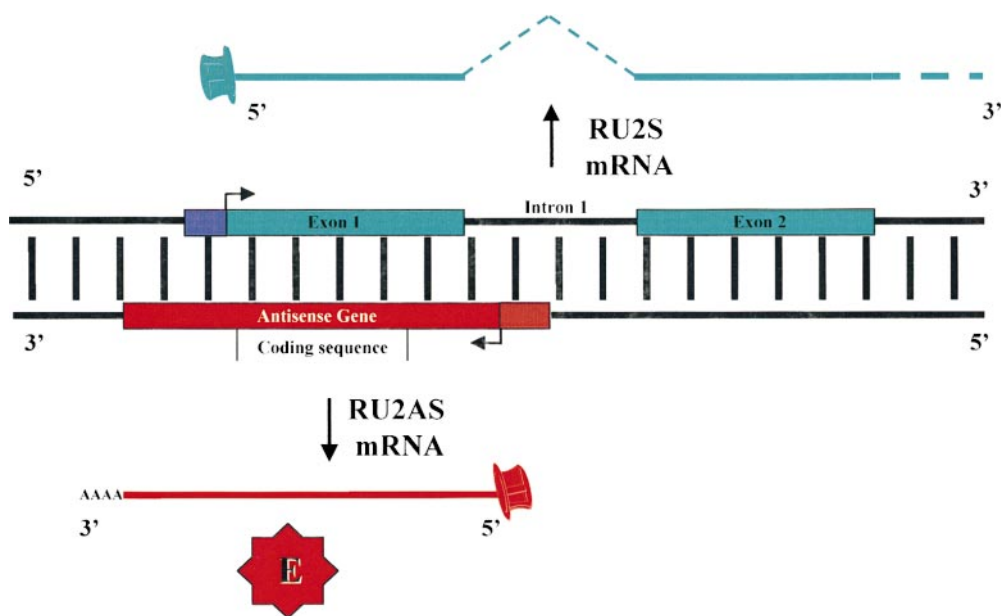
Figure 1. Genomic arrangement of RU2S and RU2AS. The figure depicts a schematic representation of the DNA fragment encoding the RU2S and RU2AS genes. Two exons from the RU2S gene are shown on the top strand in green, while the complete RU2AS gene is shown on the bottom strand in red. The "capped" RNAs of the genes are shown above and below the DNA. The protein epitope (E) synthesized from RU2AS is shown below the RNA.

monkey virus (9) and the HSV thymidine kinase gene (10) have been shown to contain a cytoplasmic transport element (CTE) that binds to known RNA binding proteins and promotes transport of these mRNAs into the cytoplasm in which they are subsequently translated. The lentivirus family of retroviruses exemplified by HIV-1 has evolved so that it encodes its own RNA transport machinery within its genome. The HIV RNA not only encodes a cis-acting element, *rre*, but also encodes the Rev protein that interacts in trans with this sequence, ensuring efficient export of the unspliced and singly spliced HIV-1 RNAs (11). Based on these observations, it would be anticipated that the intronless RU2AS transcript would also encode a CTE that would allow it to be exported and translated.

The results in Van den Eynde et al. (1) describe an interesting and exciting new system in which there is a gene within a gene that together are divergently transcribed, making both Watson and Crick strands of the DNA encode both an RNA and a protein. Clearly, there are several interesting questions that can be addressed using this system. It would be appealing to determine whether most of the RU2AS mRNA is in the cytoplasm or whether it is predominantly nuclear. This result may begin to shed light on whether the RU2AS RNA plays a regulatory role modulating gene expression. In addition, the identification of the RU2AS CTE may define a novel element that allows cellular transcripts lacking introns to be transported to the cytoplasm. It is also intriguing that the RU2AS RNA appears to be more abundant in cancer cells than in normal tissues. Perhaps this is a reflection of misregulation of RNAs lacking introns such that they are more efficiently exported and stabilized. It is conceivable, although not experimentally tested, that the cellular export machinery is altered in cancer cells, leading to greater accumulation of these types of RNAs. Although these investigations are in the early stages, additional experiments in this area may have considerable impact in both our understanding of RNA biology and our thinking about how the genome is structured and expressed.

Address correspondence to Stuart Peltz, Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School-UMDNJ, 675 Hoes Lane, Piscataway, NJ 08854. Phone: 732-235-4790; Fax: 732-235-5223; E-mail: peltz@umdnj.edu

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