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Diverse antisense mechanisms and applications

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Antisense is a topical subject, and CMLS readers will know of recent development in the area. Also, antisense is well known as a new approach in research and drug development. However, the potential importance of antisense may be unappreciated. In the clinic, despite several failures, antisense is on-track to provide a new class of therapeutics. In the laboratory, antisense was initially viewed as a rather unreliable research tool, but antisense strategies are very flexible and are now widely used. Apart from these practical applications, antisense has emerged as a widespread and diverse mechanism in natural gene expression control. Therefore, it is time for a new assessment of the diversity of antisense mechanisms and applications. Fortunately, many areas of antisense, such as the discovery of new natural antisense sequences, the growing popularity of antisense tools in the laboratory, and clinical developments have been reviewed elsewhere. This collection of articles presents exciting aspects of antisense that also serve to highlight the impressive diversity within the field.

Antisense defined

Antisense sequences are natural or synthetic polymers that specifically recognise and inhibit target sense sequences, with mRNA being the usual target. Antisense was first envisioned in 1967 [1] and demonstrated in vitro in 1977 [2]. The definition of antisense logically follows the definition of the sense strand as the coding DNA or RNA sequence. Thus, antisense should be seen as all mechanisms that interfere with a sense nucleic acid through sequence specific recognition. The usual target in antisense strategies is messenger RNA, and most cases of natural antisense also involve mRNA as the target. However, many functional RNAs targeted by antisense are non-coding and not strictly sense RNA, and one strand of duplex DNA within an open reading frame is a sense strand. At the mechanistic level, antisense sequences can act through steric hindrance or degradation of target sequences. Finally, the antisense sequence can be DNA or RNA and their chemical derivatives. Indeed, nucleic acid mimics with synthetic backbone structures are considered antisense sequences although they are not formally nucleic acids. Therefore, within the definition of antisense there can be variation in the target sequence, mechanism of inhibition and antisense polymer, and this underlies the wide range of antisense mechanisms and effects.

Antisense appears to be everywhere in nature

As well as being a diverse mechanism, antisense is also very common in nature. Indeed, it is surprising how long it has taken to recognise its importance and abundance. Within the area of translational regulation, antisense was seen as only a minor player until recently, but now antisense sequences have been identified in many systems, and thousands of putative antisense RNAs have been described. Indeed, the abundance has led many to ask whether antisense regulatory sequences function in all biological species. Cellular antisense RNAs are involved in such diverse processes as developmental timing [3], circadian clock [4] and plasmid copy number [5] control. As new antisense sequences and functions are discovered they force us to re-evaluate the mechanistic possibilities of antisense sequences.

Antisense gene control can have diverse and unique outcomes

Antisense-mediated steric hindrance can affect transcription, RNA processing, RNA transport, and translation. Triggered degradation is even more complex, involving several known nucleases, including Rnase H, RNase III, and Dicer, with variants identified or proposed for each

of these enzymes. Also, RNA level intervention by antisense offers unique opportunities for gene control. It is well known that genomic diversity expands at the RNA level through RNA processing, and differences in processing variants can be manipulated through antisense intervention in ways that are not otherwise possible. Therefore, there are many different antisense mechanisms and outcomes.

Review topics

The antisense area has been generously covered in past reviews, and there are several excellent books on antisense. While the core topics within antisense, including drug development and gene inhibition for function studies will persist, antisense has a wider profile that deserves more appreciation. In particular natural antisense mechanisms, and non-standard antisense technology have progressed very rapidly in recent years and readers may be surprised at the range of development. This collection of reviews highlights five fascinating and independent topics within the antisense area. The first review covers applications of antisense in redirecting mRNA splicing [6]. This technology is needed to assess roles for the enormous number of splice variants. Also, splice variant specific inhibition has potential therapeutic potential that seems outside the possibilities of small molecule therapeutics. The second review covers applications of antisense agents in allele specific gene inhibition, which has the potential to tackle diseases where the dysfunctional gene differs very little from the wild type [7]. The third review covers cellular delivery of antisense agents using peptide carriers [8]. Peptide-mediated delivery is an interesting field in itself, and using peptides to deliver antisense sequences can expand the range of antisense chemistry that can be delivered in vitro and in vivo. For example, progress in this area may enable further development in siRNA technology, which appears to suffer from poor cellular delivery in vivo. The fourth review covers translation repression by antisense sequences, where the transcript can be preserved intact for later gene up-regulation, similar to the action of transcriptional repression [9]. Interestingly, several new antisense RNAs in eukaryotes operate through translational repression. The fifth and final review covers triplex DNA structures that form through both intra and inter-molecular interactions [10], where duplex DNA is the target of the antisense sequence. With such a broad range of topics within the antisense area, it is appropriate to ask how many more distinct antisense mechanisms and applications are likely to appear in the next few years.

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