Chem. Senses 23: 241–242, 1998

Summary of Symposium, Antisense Oligonucleotides: Strategies and Successes

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'Antisense' technology was developed as a strategy for eliminating a gene product to facilitate studies of its function. A symposium at AChems XVII examined some of the advantages and limitations of the method and its characteristics relative to other methods of manipulating gene expression. Speakers discussed how to optimize experiments and validate the methods as well as providing examples of successful use of antisense oligodeoxynucleotides to down-regulate gene expression.

Symposium speakers addressed issues of drug design and mechanism as well as elucidating technical and theoretical facets of using antisense oligodeoxynucleotides (ODNs) in cell culture or *in vivo*. Barbara Talamo gave an overview of antisense technology and examples of its use *in vitro* and *in vivo* with various strategies for delivering antisense ODNs to the cell.

Rick Wagner of Gilead Sciences discussed rational drug design and testing of modified ODNs for optimal and specific antisense effects, using a model culture system expressing a transgene target as a readout for the selectivity and effectiveness of the antisense ODN. He warned of unanticipated, yet beneficial, nonspecific effects of therapeutically tested ODNs and emphasized the need to obtain specific toxicity (Matteucci and Wagner, 1996). Wagner's group has been at the forefront in rational design of new modifications of ODNs. In 1993, Wagner *et al.* reported the use of promising C-5-propynyl pyrimidine- and phosphorothioate-containing antisense ODNs that enhance stability as well as affinity for target mRNA (Wagner *et al.*, 1993). In his presentation he described examples that illustrate how specific antisense effects can be evalutated. Experiments utilized cell lines in which the SV40 TAg gene and the *Escherichia coli* β-galactosidase gene or the luciferase gene were co-microinjected into nuclei with or without antisense ODNs directed to various regions of TAg mRNA. ODNs containing C-5 propyne pyrimidine and phosphorothioate backbone modifications as well as various mismatch analogues were then tested for specificity and efficacy. The results illustrated that antisense ODN need not be designed solely against translational start sites. ODNs targeted to multiple RNA sites were successful in producing both gene- and sequence-specific inhibition (see also Moulds *et al.*, 1995; Gutierrez *et al.*, 1997). Suppression of gene expression could also be demonstrated using test models that constitutively express luciferase (Flanagan *et al.*, 1996). In these studies, particular cell delivery techniques were required to achieve antisense activity. Delivery of ODN by microinjection or cationic liposome delivery was successful; however, high concentrations of ODN/liposome showed some toxicity unless serum was present. Serum also inhibited the effectiveness of the antisense ODN, suggesting possible limitations for the use of lipofectin *in vivo*. More recent work, however, suggests that cytofectin may be more useful than lipofectin for ODN delivery (Lewis *et al.*, 1996), and that ODNs containing C-7 propyne purines may also provide stable and effective antisense reagents (Buhr *et al.*, 1996).

Ken Kosik of Harvard Medical School reported on the use of primary hippocampal cultures as a model for exploring gene products involved in the generation of neurites and differentiation of the axon. He modulated levels of the microtubule-associated proteins tau and MAP-2, elucidating issues of ODN uptake, targeting of the antisense to particular regions of the mRNA to produce the most effective down-regulation of gene product, as well as ODN dosage, time course of uptake and length of inhibitory effects. He also cited the importance of establishing specificity and the lack of toxicity, using the 'gold standard' control of eventual recovery of the normal phenotype of the untreated culture after breakdown of the added ODN. Some of this work has been published (Caceres and Kosik, 1990; Caceres *et al.*, 1991, 1992; Ferreira *et al.*, 1994).

One of the more challenging uses of antisense is to probe the role of particular gene products in behavior. Donald Pfaff reported on the use of antisense strategies *in vivo* and described experiments exploring the effect on various behaviors of altering levels of progesterone receptors, oxytocin receptors and estrogen receptors in particular brain regions. He discussed advantages to be gained by controlling

the temporal window of regulation and by spatially limiting antisense availability by localized injections to small areas of the brain as opposed to protocols that act globally, affecting gene products in many regions of the body. His remarks are reported in Ogawa and Pfaff (1998).

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Accepted January 15, 1998