REGULATING GENE EXPRESSION

CAN THE ANTISENSE MESSAGE BE DELIVERED?

NEW YORK—Although antisense nucleic acids hold great promise as exciting research tools, don't let your mind wander during a discussion of their near-term applications as human therapeutic agents. If you do, you'll miss it, because that discussion is bound to be short. That was the consensus at the two-day Antisense Nucleic Acids and Genetic Engineering for Commercial Applications in Agriculture and Human Therapy meeting, sponsored by International Business Communications (Holliston, MA), held here in late January.

Antisense techniques are seductive, to be sure. The attraction is in the antisense nucleic acid's ability to hybridize with messenger RNA (mRNA) in the cell nucleus and so interfere with splicing, other processing reactions, or transport into the cytoplasm. Hybridization to the mature message may also inhibit its translation into protein. Using antisense nucleic acids as therapeutics may one day shorten drug development time: Because, in theory at least, they can be targeted so specifically, side effects may be reduced. Also enticing is their potential for inactivating particular genes—such as “oncogenes.” And for some diseases, antisense nucleic acids might generate an intracellular immunity by transfecting blood cells—viral resistant monocytes and lymphocytes could alleviate the disease pathology.

Yet formidable hurdles remain before commercialization becomes a reality. Most of the experimental data have been generated in tissue culture—very little in animal systems. The antisense/mRNA hybrids are kinetically constrained—they tend to fall apart. This necessitates delivering a large excess of antisense over mRNA. And to be effective against viruses and cancers, antisense nucleic acids would have to inhibit an unrealistic 100 percent of viral activity. Thus far, getting any expression in an animal system has been a challenge. Beyond these obstacles lies the greatest problem—delivery. No research has progressed to the point where questions of how to get the genes in, and how to get them to do what you want, are yet relevant.

Nonetheless, the speakers gave the seventy-odd conference attendees much to consider. Paul Miller of the Johns Hopkins University School of Hygiene and Public Health (Baltimore, MD) has been studying antisense oligonucleotide analogs in biochemical and biological models; in

particular, methylphosphonates, where nonesterified oxygen is replaced with a methyl group. The methylphosphonate oligomer is soluble in aqueous solutions. Most important, the backbone is totally resistant to hydrolysis by both exo- and endonucleases.

Using oligomers targeted against the splice junction of herpes simplex virus type 1 (HSV-1) precursor mRNA 4 and 5, Miller has demonstrated inhibition of HSV-1 replication and protein synthesis, and DNA synthesis in HSV-1-infected cells. He also has succeeded in blocking translation using oligomers targeted against rabbit alpha- and betaglobin mRNA. In these experiments, he uti-

mentary DNA copies of satellite RNA of tobacco ringspot virus (TobRV) to construct synthetic genes which effectively confer phenotypic resistance to TobRV infection in transgenic plants. They dissected the satellite TobRV sequences involved in self-cleavage of the RNA, and developed a model for designing new RNA enzyme activities. They believe that producing antisense RNAs that will not only base-pair with target RNA, but also catalyze its rapid cleavage, may overcome position effects.

Theoretical work remains far removed from applying antisense in animal systems, however. Belinda Rossiter, a fellow at the Baylor College of Medicine Institute for Molec-

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REASONS

Using anti-sense RNA to block viral gene expression and/or replication. In the novel immune system depicted here, a cell produces anti-sense RNA (or micRNA, for messenger RNA-interfering-complementary RNA) against viral mRNA. The micRNA is able to block translation of the viral mRNA, preventing production of mature virus particles.

lized a cross-linking agent—trimethylpsoralen—to increase the efficiency of the oligomer-mediated reaction. Oligomers so derivatized can form covalent adducts with complementary nucleic acids when activated with long-wavelength ultraviolet light. Miller observed about 80 percent cross-linking after 10–15 minutes of irradiation. Despite these successes, however, he acknowledges that delivery into animals remains far off.

Another strategy for improving antisense efficacy—this time in plants—was presented by Jim Haseloff (CSIRO Division of Plant Industry, Canberra, Australia). Haseloff and his colleagues have used comple-

ular Genetics (Houston, TX), has been trying to develop animal models for Lesch-Nyhan syndrome, a terrible, untreatable disease. She presented data on inhibiting hypoxanthine phosphoribosyltransferase (HPRT) gene expression (total HPRT deficiency results in Lesch-Nyhan syndrome). Rossiter has demonstrated reduced HPRT activity in mammalian cells—most likely as a result of interference with RNA splicing. But while constructs prepared for introduction into transgenic mice showed, in some instances, a reduction in HPRT activity in some tissues, it was never completely eliminated.

—Mark Ratner