

ASM BIOTECH MEETING

HAIRPIN RNA PICKS VIRAL LOCKS

CHICAGO, Ill.—Discovery of new natural microbial products, genetic modification of existing ones, and adaptations for special environments were among the research advances discussed at the American Society for Microbiology Conference on Biotechnology held here last month.

Work on new enzymes—engineered, semi-synthetic, and artificial—was well represented at the meeting. A new generation of enzymes promises to continue expanding what is currently an estimated \$500 million U.S. market, according to Linda Lasure of Panlabs (Seattle, WA).

As an example of how genetic modifications can improve an enzyme, Robert Drummond of Cetus (Emeryville, CA) described a joint project with Nabisco (East Hanover, NJ) in which several point mutations in the gene for xylose isomerase cumulatively increased its thermostability by 3° C—enough to justify the high costs of trying for regulatory approval, in Drummond's opinion.

Regulatory burdens crept into a session on agbiotech as well, as R.

James Cook of the U.S. Dept. of Agriculture's Root Disease and Biological Control Research Unit at Washington State University (Pullman) charged that the "mostly arbitrary decision" to categorize microbial crop protectants as pesticides is retarding product development. Cook sees the pesticide designation pushing development in favor of single organisms adapted to give broad-spectrum controls. More effective, he said, would be "cropspecific, disease-specific, and even environment-specific controls."

Nonetheless, broad-based remedies have the appeal of giving many choices of potential applications. Take catalytic RNA, for example. Native catalytic RNAs are mainly selfcleaving, but work done by Randal Hauptmann, director of the plant molecular biology group at Northern Illinois University (DeKalb), has made them more general. Working with a 51-base-pair "hairpin" catalytic RNA fragment isolated from tobacco ringspot virus, Hauptmann and colleagues identified and amplified its advantages over the more widely studied "hammerhead" self-splicing RNA (Bio/Technology 6:826, July '88).

While hammerhead RNA has a temperature optimum of 55° C, hairpin RNA's natural optimum is 37° C, giving it the potential to work *in vivo* in mammalian systems. Molecular tinkering has further reduced the temperature optimum to 27° C, making it useful for plant systems.

Equally important, Hauptmann identified a nucleotide sequence essential for cutting a target. The modified hairpin structure is able, *in vitro*, to cleave the GUC target sequence in the RNA of tobacco mosaic virus, several plant genes, human immunodeficiency virus, and oncogenes.

How would this discovery be applied? Hauptmann envisions the following protocol: "Search for a GUC in the gene we want to nail...take a look at the flanking sequences that will be found in an RNA transcript...then synthesize the catalytic RNA that recognizes the target sequence."

Potentially, hairpin catalytic RNA could down-regulate gene expression to control viral diseases.

-Pamela Knight

DISEASE MODELS

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Speaking at the 3rd International Conference of the Vienna Institute of Molecular Pathology, held here in May, Jay described how his group's work on the development of a transgenic animal model of MS had led to this new conclusion.

Jay's starting point was the consistent observation of markedly elevated levels of expression of MHC Class I and Class II genes in MS brain lesions; expression in the brain occurs normally only at very low levels. He wondered, therefore, whether MHC gene expression in the brain would be sufficient to induce MS. Indeed, other workers have established that the injection of monoclonal antibodies against MHC antigen into the brains of animals with MS-like disease leads to significant improvements in their condition.

To test the hypothesis, Jay and his colleagues developed a transgenic mouse system in which an MHC Class I gene (an H-2K^b gene from the P6 mouse) was expressed specifically in the oligodendrocytes, the cells that produce myelin, under the control of the myelin basic protein regulatory element. In all the examples of the transgenic mouse model examined, the normal pattern of Class I gene expression (predominantly in the lung and liver) was radically altered there were now high levels of expression in spinal column and brain.

When looked at histologically, the spinal column, hippocampus, cerebellum, and other neurological tissues of the transgenic mice were deficient or even devoid of myelin. So striking was the histological difference that Jay and colleagues suspected that they may have inadvertantly interfered with myelin synthesis, rather than inducing demyelination. However, using the polymerase chain reaction, they demonstrated that the myelin gene not only was expressed, but that it was expressed at elevated levels.

In the transgenic mouse model of MS, demyelination occurred simply as a result of elevated MHC antigen production and without any evidence of the infiltration of immune cells associated with autoimmune disease states. Jay suggested that the mechanism of the demyelination might be associated with the role of MHC Class I antigens as adhesion molecules: over-expression might alter the compaction of the myelin sheath and this might be sufficient to induce protease activity. Immunoinfiltration would then be a late event, he suggested, in which the disease is exacerbated when the immune cells appear to "clear up the garbage."

If MHC Class I gene expression is sufficient for demyelination, then the role of virus infection is merely to induce interferon which turns on the expression of the Class I genes. —John Hodgson

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