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In This Issue

Regulation of *CFTR* Gene Expression

The complex tissue-specific and temporally regulated expression of the cystic fibrosis transmembrane conductance gene (*CFTR*) is controlled through a number of only partially characterized mechanisms. Extensive analysis of the region spanning from −120 kb upstream of the *CFTR* gene into the first intron have identified three potential sites that may be involved in gene expression. Of these, only one, found in the first intron, correlates completely with gene expression in the cell lines examined. In vitro studies of the tissue-specific regulation of this gene produce limited data. This is because *CFTR* covers approximately 250 kb, and the effects of chromatin are unlikely to be concordant with the in vivo situation. On pages 211–223, Moulin et al. report the results of DNase I hypersensitivity assays from an in vivo model of human *CFTR*. CF-null mice, expressing human *CFTR* on a yeast artificial chromosome (YAC), were used to evaluate the importance of two of these three sites that are potentially involved in regulation of expression. Since the YAC studied did not contain the most 5′ site, and *CFTR* expression was not detected in the kidney and Brunner's glands, this upstream region may have some limited effects on gene expression. The authors find that one of the sites, that is seen in all human epithelial cells irrespective of *CFTR* expression, is seen in all the mouse tissues. The other site, that shows correlation with *CFTR* expression in human cells, shows a more restricted pattern of expression in the transgenic mice, but this pattern does not correlate fully with *CFTR* expression. However, the authors state that the divergent tissue expression pattern between the transgenic mice and humans highlights the inadequacies of the mouse model, and suggest that an ovine system may be more representative of the human environment.

Three Easy Steps for Making Useful Adenoviral Vectors

Adenovirus has been widely used as a vector for the transfer of a gene of interest in gene expression studies and gene therapy trials. However, producing large amounts of recombinant virus has proven to be an arduous, inefficient task. The most commonly used method employs homologous recombination in mammalian cells, between non-infectious viral DNA that contains the experimental sequence, and a shuttle plasmid that carries the missing viral sequences. Since homologous recombination is a rare and poorly understood process in mammalian cells, this method is often time consuming and difficult to control. On pages 224–231, Aoki et al. report circumventing many of these troubles by using the Cre-lox recombination system. Cre is a recombinase that mediates inter- or intramolecular recombination by recognizing two consensus sequences, known as loxP sites. The authors found that this system efficiently produced recombinant virus much more quickly than standard protocols. Furthermore, because the viral sequences used lacked the 5′ packaging sequences, they found that their procedure resulted in far fewer replication-competent viruses. The authors propose that this method may be widely applicable for a number of uses.

Finding the Links between Apoptosis and Necrosis

The existence of two distinct types of cell death, apoptosis and necrosis, has been known for some time. While the molecular mechanisms of apoptosis have undergone extensive scrutiny, less is known about the pathways involved in necrosis. However, it is a commonly held belief that both cell death pathways share few upstream events. In a report from Li et al. beginning

on page 232, new evidence casts doubt upon this belief. The authors report that a plant product, β-lapachone, is able to induce a rapid release of mitochondrial cytochrome C into the cytoplasm of a wide spectrum of cancer cell lines, including lung, ovary, colon, and breast cancer cells. Cytochrome C then interacts with the cytoplasmic protein Apaf-1, leading to the induction of caspase 3, a key mediator of apoptosis. The authors report finding that the release of cytochrome C can also lead to necrosis, suggesting that the two cell death pathways share common initiation pathways. These studies also suggest that β-lapachone may be a powerful anti-cancer therapy, as it appears to be effective against a number of cancer cell lines.

Cleaning Up Prions

The process by which the prion, the infectious agent causing many transmissible degenerative encephalopathies such as Creutzfeldt-Jakob disease (CJD), is passed between patients remains mysterious. A number of cases of prion transmission by surgical instruments between human patients have been reported, calling into question the effectiveness of sterilization techniques. However, an effective model for evaluating sterilization methods is lacking. To address this issue, Zobeley et al. exposed stainless steel wire segments to the scrapie agent and after extensive washing implanted them into the brains of indicator mice. On pages 240–243, the authors report that the wires proved to be highly infectious, and that treatment with 10% formaldehyde failed to prevent disease transmission to the mice. These findings provide a means by which new methods for the sterilization of surgical instruments may be examined. The authors also raise the question of how the scrapie agent actually causes the disease, as it is not clear whether the prions dissociate from the wire once implanted in the brains of the mice or whether they act while attached to the wire.

Effective Gene Therapy in the Treatment of OTC Deficiency

Ornithine transcarbamylase (OTC), a protein that catalyzes the synthesis of citrulline in the second step of the urea cycle, is encoded in nuclear DNA and posttranslationally imported into the mitochondria, becoming enzymatically active in the mitochondrial matrix after proteolytic cleavage of the leader sequence and assembly into a homotrimer. Treatment of severe forms of OTC deficiency (OTCD) may benefit from gene replacement in hepatocytes potentially capable of correcting the secondary metabolic alterations which cause faulty waste nitrogen excretion. In vivo models have established the benefit of gene therapy in OTC deficient mouse strains, but the efficiency of the mitochondrial import mechanism and the degree to which the underlying metabolic derangements in OTC deficiency are corrected has not been established. On pages 244–253 Zimmer et al. intravenously injected recombinant adenoviruses carrying either mouse (Ad.mOTC) or human (Ad.hOTC) OTC cDNA into the sparse fur with abnormal skin and hair (spf^{ash}) mouse and were able to analyze at the ultrastructural level the expression of OTC enzyme precursor and its translocation to mitochondria in vector-transduced hepatocytes. The authors found that human OTC precursors were blocked from translocation and largely remained in the cytosol while mouse OTC precursors were translocated and processed in the mitochondria of spf^{ash} hepatocytes. Additionally, the vector-mediated gene delivery of Ad.mOTC corrected secondary alteration in ATPase(c) and CPSI concentration and the authors suggest that the correction of hepatic and cerebral energy metabolism may also occur. These findings support the efficacy of gene therapy in OTC deficiency.