of this cloning strategy is that the gene of interest is expressed as part of a stable β-galactosidase fusion protein, which can be identified by antibody probes. The amino acid sequence deduced from the cloned mouse gene turns out to show considerable homology with human Band 3.

Implications of this work to the study of cystic fibrosis began to emerge when Lodish used DNA probes derived from the mouse gene to show that a set of mRNAs homologous to the anion transport domain of Band 3 are expressed in kidney cells. Here, too, anion exchange as well as a variety of other types of chloride transport are actively taking place. Since cystic fibrosis, the major genetic disease of Caucasians, is characterized by a defect in chloride resorption (primarily by epithelial cells) it is a reasonable guess that the underlying cause of the disease is a defect in a chloride transport channel. The Cystic Fibrosis Foundation has recently funded Lodish to develop probes based on the cloned mouse gene in order to do restriction fragment length polymorphisms analysis of affected families, in hopes of producing a reliable prenatal screening procedure. Lodish is also trying to identify a chloride channel defect at the level of mRNA expression by probing cultured secretory epithelial cells from normal and cystic fibrosis patients with DNAs derived from his Band 3 clone. **—Harvey Bialy**

MEETING REPORT

DNA PROBES NOW AIMED AT RNA

LAS VEGAS—DNA probe technology is *not* stuck in the lab—despite reports in national news magazines—according to papers presented here at the annual meeting of the American Society for Microbiology.

Gen-Probe (San Diego, CA) has developed a rapid diagnostic assay for *Legionella*, the bacterium that causes Legionnaire's disease. The assay reduces disease diagnosis time from 48 hours to 45 minutes. The test can be performed on a patient's serum, blood, sputum, feces, or liver cells. David Kohne, chief scientist at Gen-Probe, developed the assay with DNA probes specific for ribosomal RNA. Because rRNA is an abundant cellular species, the assay is very sensitive: it can detect 400 organisms in 0.4 milligrams of liver tissue. Because rRNA is already single-stranded, it will hybridize immediately with a complementary DNA probe: this eliminates the time-consuming step of denaturing the sample DNA. Don Brenner's group at the Biotechnology Branch of the Centers for Disease Control (Atlanta, GA) is one of several laboratories evaluating the Gen-Probe *Legionella* assay. Brenner says that he readily identified all 22 *Legionella* species and could distinguish them from many non-*Legionella* species. Moreover, he used the *Legionella*-specific probe to confirm his identification of ten new species.

Nonradioactive assays are preferable in a clinical setting. They are cheaper and circumvent the extensive licensing procedures and disposal problems associated with radioisotope use. Dean Englehardt, vice president of research at Enzo Biochem (New York, NY), reported on his company's nonradioactive methods for tagging DNA probes. One method is to modify deoxyUTP with biotin. Researchers at Enzo have accomplished this via a nick translation reaction using DNAse and DNA polymerase. The polymerase ends up repairing—i.e. adding biotin-labeled nucleotides to—essentially the entire strand of DNA. The biotin is added at the 5′ position of the pyrimidine: this position lies in the major groove of the helix, so there is no stearic hindrance to interfere with strand hybridization.

Researchers at Enzo have also developed fluorescent DNA probes directed against intracellular messenger RNA. In particular, they detected *src* gene mRNA in mouse cells. Thus scientists will now be able to tell exactly when a gene is turned on, with implications for preclinical cancer diagnosis. **—Jennifer Van Brunt**

MEETING REPORT

NEW PERSPECTIVES ON CHOLERA

LAS VEGAS—Though cholera epidemics have plagued mankind since ancient times, the cholera bacterium is *not* a human pathogen requiring a human-infecting phase in its life cycle, says Rita Colwell, president of the American Society of Microbiology. The bacterium is instead an integral component of the indigenous flora and fauna of estuarine environments, where it lives in commensal or symbiotic relationships with zooplankton and invertebrates.

This heretical theory has evolved from research conducted by Colwell and many others over the last decade. Cholera is transmitted by contaminated food and water. *Vibrio cholerae*, first isolated in 1884 by Robert Koch, is always found associated with estuarine environments. The organism is widespread in the United States, and, in fact, has been detected in all estuaries tested to date. The seasonality of cholera outbreaks is associated with phytoplankton blooms. An increase in the zooplankton population is invariably followed by epidemic cholera. In Bangladesh, this occurs at about the same time every year.

Vibrio cholerae microorganisms play an important role in the estuarine ecosystem. They play a role in their symbiotes' nutrition, osmoregulation, urea homeostasis, and larval morphogenesis. *Vibrio* lives in the oral region and gut of copepods—chitinous zooplankton. The copepods cast their eggs into the water in the spring and cholera organisms attach to the egg cases. In fact, it is likely that they aid in breaking the egg case during larval release. *Vibrio* are also found associated with oysters, clams, and crabs. Oysters—filter-feeding molluscs—ingest copepods regularly. Colwell says that *Vibrio* might help the crab in its migration from sea water to the upper bay, a journey which involves changes in temperature and salinity. The human, on the other hand, is an imperfect host for this microbe.

Vibrio can survive in its estuarine environment in a viable but "nonrecoverable" state. Colwell and others have collected samples which would not plate out on conventional agar but which did contain the organisms. To detect them, she had to add nalidixic acid, which prevents cell division. The inhibited cells elongated, and, after staining with acridine orange, were detected by epifluorescence. Normal concentrations of *Vibrio cholerae* in the environment are low: Colwell has never isolated more than 46 cells per liter from the Chesapeake Bay. Concentration of the microorganism in the copepod gut starts an increase in total population size which ultimately causes epidemics.

—Jennifer Van Brunt