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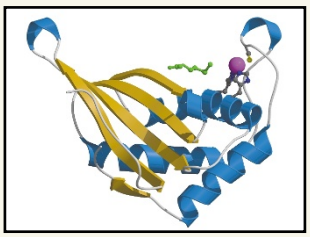
Divide and conquer



To evade antibiotics and attacks by a host's immune system, bacterial pathogens often form biofilms—aggregates of cells that respond collectively to environmental changes. Biofilms can often resist antibiotics that would kill individual bacteria, and so far they have been difficult to disrupt. Now, scientists at Structural GenomiX (San Diego, CA) have used high-throughput crystallography to solve the three-dimensional structure of a key protein involved in quorum-sensing, a major signaling pathway by which bacteria coordinate their actions (*Structure* **9,** 527–537, 2001). By determining the structures of three orthologs of the protein, called LuxS, from three species of bacteria, the researchers have laid the groundwork for a new class of rationally designed antibiotics. The team purified LuxS from several bacterial species, tested a large number of possible protein crystallization conditions simultaneously, and then obtained high-resolution structures of the proteins. The results provide a detailed view of one step in the synthesis of a key quorum-sensing signaling molecule. According to Hal Lewis, first author on the paper, "All known protein components of the bacterial quorum-sensing pathways are on our target list and are undergoing (or soon to undergo) crystallization trials followed by structure determination and functional evaluation." *AD*

Phenotype arrays

In the July issue of *Genome Research* (**11,** 1246–1255, 2001), researchers at Biolog (Hayward, CA) report on a microplate array format in which a microbial gene knockout can be rapidly assessed for its effect on metabolic status. This was accomplished by adapting into high-throughput format an established colorimetric assay that measures bacterial respiration activity via the reduction of a biological indicator dye (tetrazolium violet), which turns from colorless to an intense purple color upon reduction. As the rate of dye reduction is a function of respiration activity, the increase in signal provides an indirect measure of metabolic activity of a knockout, monitoring hundreds of cellular pathways. Compared with traditional tetrazolium assays, the system can carry out hundreds of assays simultaneously and allows more quantitative assessment of dye conversion via automated imaging. By adding different carbon, nitrogen, phosphorus, and sulfur sources to each microplate well, as well as chemicals for assessing biosynthetic pathways and stress responses to a wide range of toxic chemicals, researchers were able to make functional inferences about gene knockouts in six strains of *Escherichia coli*. Present arrays measure nearly 700 phenotypes, but Biolog's chairman and vice president, Barry Bochner, says that arrays for 2000 phenotypes should be "completed by the summer." *AM*

Research News Briefs written by Alan Dove and Andrew Marshall.

Sweet sterility

Traditional methods of pollination control in different crop species for hybrid seed production are rather laborious. Usually, they involve mechanical emasculation of male plants or incorporation of genetic mutations that prevent pollen development. Now, a collaboration of German and French researchers, headed by Thomas Roitsch, has come up with a simpler approach—genetically manipulating extracellular sucrose levels to inhibit anther and pollen development (*Proc. Natl. Acad. Sci. USA* **98,** 6522–6527, 2001). Their approach promises to be applicable to a wide range of crop plants, including rice, corn, tomatoes, and rape. Roitsch and his team already knew that an extracellular invertase isoenzyme from tomato and potato was specifically expressed in anther tissues. With this knowledge, they cloned a homologous enzyme, Nin88, from tobacco, which included 4.3 kb of 5′ sequence that directed expression specifically to anthers. When fused to β-glucuronidase, Nin88 could be detected with a similar expression pattern in anthers of tomatoes. Tobacco expressing an antisense construct to Nin88 demonstrated dramatic inhibition of pollen development and seed production. Further experiments demonstrated that invertase activity correlated with pollen maturation. Roitsch and his team believe that the approach could be useful for hybrid seed production and in strategies to prevent outcrossing of transgenes in the environment. *AM*

Ribosome mechanism

Rather than actively catalyzing peptide bond formation, ribosomes may work primarily by positioning substrates next to each other and passively permitting bond formation to take place, according to new work published in *Nature* (**411,** 498–501, 2001). This could suggest new strategies for developing antibiotics that target bacterial protein synthesis. Traditionally, it has been difficult to ascertain whether ribosome RNA facilitates peptide bond formation by direct catalysis or by simply positioning aminoacyl-tRNA molecules to allow spontaneous peptide bond formation because mutations in critical RNA nucleotides are lethal to bacteria and ribosomes are difficult to reconstruct *in vitro*. Now, Alexander Mankin and colleagues at the University of Illinois (Chicago, IL) have reconstructed *in vitro* the large ribosomal subunit of the thermophile *Thermus aquaticus*. When they introduced mutations into the putative catalytic residues of the ribosomal RNA, the ribosome's peptidyl transferase activity remained intact, indicating that active catalysis is not critical for peptide bond formation. "We will not rule out the possibility that there is some contribution of catalysis, but positioning is the most important factor," says Mankin. *AD*

PCR for proteins

Take a tissue homogenate containing a minute quantity of the pathogenic form of a prion protein (PrP^{sc}), incubate it in a test tube with a large amount of the normal form of the protein (PrP^{c}), and watch as the PrP^{sc} "replicates" to high levels in a matter of minutes. This *in vitro* reaction, long a goal of prion researchers, has now been achieved by Serono (Geneva, Switzerland) scientists (*Nature* **411,** 810–813, 2001). In addition to making existing tests for prion diseases like Creutzfeld–Jakob disease (CJD) and scrapie far more sensitive, the new approach might be generally applicable for detecting tiny amounts of recombinant proteins. The cyclic amplification process involves alternate rounds of "replication" of PrP^{sc} protein followed by sonication to break up the resulting aggregates. Once sonicated, the smaller aggregates contain PrP^{sc} free to convert more PrP^{c} to the pathogenic form. Using this approach, over 97% of the PrP^{c} in the reaction can be converted to PrP^{sc}. "It's the first time that a protein can be amplified cyclically as was done before for DNA," says Claudio Soto, senior author on the paper. Besides diagnosing prion diseases, the process might allow molecular biologists to tag proteins of interest with a prion sequence, then amplify them directly for detection. *AD*