Combinatorial biosynthesis

Structure-based drug design, combinatorial chemistry, and high-throughput screening have fostered a revolution in drug discovery. Now a new addition to this technology triad, combinatorial biosynthesis, promises to further advance drug discovery by enticing natural products from scarce or difficult-to-culture organisms, by exploiting 'silent' biosynthetic pathways and by generating biosynthetic pathways for 'unnatural' natural products that have never existed in nature but yet possess the structural diversity and novelty that have made natural products some of our most valuable drugs.

At least two small biotechnology companies, Kosan Biosciences (Burlingame, CA, USA) and ChromaXome (San Diego, CA, USA) have research programs based on the new combinatorial technology. Panlabs (Bothell, WA, USA), an established supplier of microbial extracts for drug discovery, is also embarking on a program in combinatorial biosynthesis, which company officials hope will provide additional value to its well-established microbiology program.

The problem

Natural products have historically been an enormously rich lode from which to mine bioactive molecules for human therapeutics. Approximately 57% of the 150 most prescribed drugs are derived from natural sources. They include some of the most useful compounds, including most antibiotics, immunosuppressants such as cyclosporin and FK506, the precursor of aspirin – arguably one of the most useful and frequently used drugs worldwide – and a whole host of antifungal agents. In some cases, the rationale for the therapeutic use of a natural product is obvious on the basis of its role in the host organism. For example, bacteria produce antibiotics that act to secure an environmental niche by preventing the growth of competing microbes. The rationale for the use of other naturally occurring chemicals is more obscure. What role, for example, does FK506, an inhibitor of T-cell activation in humans, play in a microbe? The answer lies, presumably, somewhere in the fact that human and microbial biochemistry have common molecular themes that over time have come to be used for different functions.

In spite of the usefulness of natural products, their complexity and novel chemistry, which on the one hand make them enormously attractive, also make them difficult to manipulate for drug discovery. Moreover, many microbes are difficult or impossible to grow in culture. Microbiologists estimate that only 1–10% of the microbes in the soil or in the marine environment grow under laboratory conditions, and those that can be cultured often alter their profile of secondary metabolites dramatically depending upon the culture conditions, a fact that frequently causes great difficulty when scaling up for the production of commercial quantities of a natural product.

The solution

Natural products are produced by living organisms through the sequential and ordered action of enzymes that gradually add complexity to a nascent chemical structure. For complex secondary metabolites, the number of enzymes involved in the biosynthetic pathway may be large – 20, 30, or more. Combinatorial biosynthesis involves the rearrangement of the order of action of the enzymes or the incorporation of unique enzymatic reactions to produce natural products that have never before been seen in nature. Such engineering of the biosynthetic machinery may be accomplished by shuffling and recombining the genes for the biosynthetic enzymes using standard molecular biology techniques, and/or the transfer of the entire biosynthetic pathway to an organism that can be more readily grown in culture than the original organism.

A daunting task?

Combinatorial biosynthesis may at first seem a daunting task, requiring the isolation and transfer of each of the numerous genes that encode enzymes involved in the synthesis of a complex secondary metabolite. But according to Dr Daniel Santi, President and Chairman of Kosan Bioscience, the problem is not as complex as it might appear, at least for certain secondary metabolites produced by bacteria. "That's because the genes necessary to produce the polyketides [one of the major classes of natural products] are found in gene clusters," says Santi. Such gene clusters may be transferred and manipulated by standard cosmid cloning techniques.

Novel polyketides

Kosan Biosciences focuses almost exclusively on the biosynthesis of novel polyketides, a large class of secondary metabolites made by bacteria, fungi, plants and marine organisms. Approximately 10,000 different naturally occurring polyketides have been identified, most of which are very difficult or impossible to produce through synthetic chemistry. From those 10,000 chemical structures, hundreds have been found to possess useful bioactivities including antibiotic, anticancer, antifungal, immunosuppressant, and cholesterol-lowering properties. In bacteria and fungi, the genes for the entire biosynthetic pathway for a particular polyketide reside in a single cluster. Moreover, the sequential order in which the enzymatic reactions must occur to produce a particular polyketide – the chemical assembly line, in effect – is the same order in which the genes for the enzymes are arrayed within the gene cluster. Consequently, changing the order of the genes within the cluster, or adding or subtracting genes for particular enzymes, or changing the structure of the precursor of the pathway, makes it possible to both alter and predict the structure of the final polyketide natural product.

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Much of the drug discovery research at Kosan Biosciences is based upon the research of Professor Chaitan Khosla, a chemical engineer at Stanford University (Palo Alto, CA, USA). In June, the Khosla group, together with colleagues at Brown University (Providence, RI, USA) and the University of Wisconsin (Madison, WI, USA), succeeded in producing erythromycin analogs using a precursor-directed biosynthesis in a bacterial cell line containing an engineered polyketide synthetic pathway [*Science* (1997) 277, 367–369]. In this case, the third enzyme in the natural pathway for erythromycin was genetically blocked, effectively shutting down erythromycin biosynthesis. Then, carefully selected synthetic polyketide precursors, chemically distinct from those produced normally, were fed to the bacteria. These synthetic precursors served as a substrate scaffold for the enzymes distal to the blocked enzyme in the pathway. The result was the production of novel erythromycin analogs that had structures amenable to fine tuning by medicinal chemists. The technology has been licensed to Kosan Biosciences, where it is being used to discover new antibiotic, anti-inflammatory and anticancer agents.

Hybrid natural products

ChromaXome Corporation has taken a somewhat different approach to combinatorial biosynthesis. Instead of concentrating on polyketide biosynthesis, they are randomly combining the elements of many different bacterial biosynthetic pathways to produce hybrid natural products never before observed in nature. Dr David Sherman, Senior Director, believes prokaryotes utilize enzymes encoded in gene clusters for the synthesis of all their secondary metabolites, which makes the biosynthetic pathways for all prokaryotic natural products amenable to the combinatorial strategy. Thus, they purposely combine DNA from mixed populations of bacteria, even bacterial DNA isolated directly from soil or from the marine environment, express it in a host organism, and then screen the host for new compounds with biological activity. With this approach they mix and match enzymes involved in the synthesis of many different classes of natural chemicals, and by utilizing DNA directly from the soil or marine sources, they bypass the limitations imposed by growing and isolating the bacteria from culture, a limitation that effectively filters out 90–99% of the microbes that will not grow under laboratory conditions.

Collaboration with Big Pharma

ChromaXome is collaborating with Bristol-Myers Squibb (Princeton, NJ, USA) on combinatorial biosynthesis. The molecular engineering needed to produce the organisms containing the combinatorial biosynthetic pathways is performed by ChromaXome; screening of the organisms for useful compounds is carried out in Bristol-Myers' laboratories. Sherman notes that "good things are happening" in that they have been successful in producing totally novel chemical entities – "compound classes never before seen" – with anti-infective activity. These new bioactive compounds are currently under investigation at Bristol-Myers Squibb as possible therapeutic agents. In the meantime, the collaboration continues to search for more novel compounds produced by the combinatorial approach that can be used for other indications.

Panlabs is just entering the combinatorial biosynthesis arena. Dr Christopher Reeves, Senior Project Manager, says that they hope to use it to exploit many of the biosynthetic pathways of actinomycetes that are silent under most culture conditions. By cloning, expressing and mixing the gene clusters from actinomycetes they expect to produce many new 'natural' and 'unnatural' natural products from a class of microbes that has been a great source of useful drugs.

Presentations on combinatorial biosynthesis

Reeves will present data on the combinatorial biology program at Panlabs for the first time at the IBC Conference on 'Combinatorial Synthesis of Natural Products' to be held 15–16 December in San Francisco. At the same meeting, Sherman and Dr Steven Mamber, Senior Research Scientist at Bristol-Myers Squibb, will summarize the collaborative program between ChromaXome and Bristol-Myers, and Santi will present an update on the engineering of polyketide biosynthesis as practised at Kosan Biosciences.

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From the Anderson Consulting drug discovery study...

The number of NCEs a typical drug company sends into development is expected to rise by approximately 4–5 per year per 1,000 discovery employees to nearly 14 per year per 1,000 employees by the year 2000. This projected productivity gain is expected to be achieved with increases of only 19% in headcount and 40% in budget.

Copies of the executive briefing on the study (released 15 October 1997) are available from David Martin at Anderson Consulting (tel +44 171 304 8748).