was also observed by B. Friedman's group (NIH, U.S.) using the *ras* oncogene. These results on cultured systems suggest that IFN may play a role in the regulation and preservation of established cellular genes. Finally, P. Whitaker-Dowling observed that IFN inhibits virus infection in cells by preventing viral entry. This effect may be the basis for the ability of IFN to inhibit the multiplication of intracellular parasites.

Interferon Monoclonal Antibodies

Monoclonal antibodies against IFN are providing better and more efficient reagents for purification processes. In addition to the NK2 monoclonal antibody commercialized by Celltech (U.K.), several other antibodies against α IFN have recently been developed. D. Secher (MRC Cambridge, U.K.) described a new antibody that recognizes a different spectrum of human α IFN subtypes than the NK2 products. When the two antibodies are used together, about 90 percent of α IFN activity can be recovered from crude leukocyte IFN preparations. This permits a high degree of purification in a single step.

K. Berg (Aarhus University, Denmark) described the properties of another monoclonal antibody, designated LO-22, in the poster session. It binds more than 95 percent of human leukocyte IFN activity and has been used to purify recombinant α IFN. This antibody appears to recognize a common antigenic determinant present on all species of α IFN.

Animal Studies with Interferon

H. Schellekens (Primate Center, TNA, Netherlands) stated clearly that successful clinical application of IFN will require good animal studies. Many of the animal studies have involved IFN treatment in conjunction with other chemotherapeutic agents. Chany (INSERM, France) reported that sequential treatment of mice with adriamycin and IFN results in a striking enhancement of the toxicity of adriamycin. This may shed some light on recent French clinical studies in which three of the four patients who died during IFN treatment had previously received adriamycin.

Animal models are also useful for approaching the study of the diverse *in vivo* properties of the interferon system. H. Kirchner (Heidelberg, F.R.G.) observed that some mouse strains have interferon-mediated resistance to herpes simplex virus. E. De Maeyer (Institut Curie, France) discovered that the Lou-C strain of rat, which has a high incidence of myeloma, spontaneously produces anti-IFN antibodies. The level of these antibodies increases with age

and this system may be an excellent model for studying the consequences of anti-IFN antibody production during autitumor therapy, a clinical situation that is sometimes encountered.

A new model system for studying the effects of human α₂ IFN has been developed by the author, J. Werenne (Université Libre de Bruxelles, Belgium), which has provided the first evidence for *in vivo* antiviral IFN activity in bovine species. Human $α_2$ IFN could fully protect calves against an experimental viral infection. The effective dose was 10^6 U/kg, although there was some individual variation in sensitivity. Werenne's group has also shown that endogenous IFN plays a role in control of rotavirus infection in newborn calves. These data point to a new role for IFN in veterinary therapeutics.

Clinical Studies

Studies on patients with AIDS or SLE reveal that an acid-labile α IFN accumulates in the circulation and elevates 2'5' A synthetase activity in individuals with these immunological disorders (O. Preble, NIH, U.S.). Most clinical studies are still underway, but evidence is accumulating that IFN plays an important role in many pathological situations. S. Yamazaki (NIH, Japan) summarized the current status of clinical research in Japan. The most promising results have been obtained with tumors of probable viral origin such as skin warts. M. Ng (University of Hong Kong) also reported encouraging results for local IFN therapy of cervix dysplasia.

Cloned IFN protects human volunteers against infection by rhinovirus and coronavirus, but it induces un-

comfortable side effects that resemble the natural symptoms of the common cold (G. Scott, MRC Salisbury, U.K.). One is left with the impression that pure individual IFNs are not as effective as their endogenous counterparts. It appears that leukocyte IFN prepared according to Cantell's method may be more active. It is not known at present whether this is due to synergistic interactions between the different IFN subspecies or to a cooperative factor that is present in the leukocyte IFN preparation.

If biotechnology is going to produce clinically useful interferons, more research must be done. The different subspecies must be defined and produced in large quantities. Better biochemical markers and molecular probes must be developed so that the *in vivo* interferon response can be dissected and understood. Only then can researchers develop technologies for mimicking and enhancing these responses. Clearly there is an interesting future for interferon research and development.

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John Werenne, Ph.D., is an assistant professor at the Université Libre de Bruxelles. He heads the biotechnology section of the Center for Applied Research in Agronomy of Hainaut.

CANDICIDIN PRODUCTION

CLONING OF A GENE INVOLVED IN ANTIBIOTIC SYNTHESIS

Microorganisms of the genus *Streptomyces* are the main producers of antibiotics and other secondary metabolites. Despite their industrial importance the molecular biology of Streptomyces has been studied very little. Even more surprising is the fact that the biosynthetic pathways of many important antibiotics are only partially known. For example, knowledge of the enzymology of the biosynthetic steps of β-lactams (penicillins, cephalosporins, cephamycins) is very scanty. The regulatory mechanisms that control antibiotic biosynthesis, which must be bypassed to get high production of these compounds, are only superficially understood[1,2].

This situation seems to be changing, although at a slow pace. The recent development of DNA cloning techniques applicable to Streptomyces has advanced the study of the molecular genetics of these organisms[3–5]. Promoter-probe plasmid vectors have been constructed in *Streptomyces lividans* and used to study the activity of DNA sequences that contain transcriptional control signals[6]. Several different laboratories are now using these vectors to develop a detailed picture of the expression of genes involved in antibiotic synthesis, differentiation, and other biological

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Spiral spore chains of *Streptomyces* sp. are surrounded by a wrinkled sheath. *Streptomyces* produce many useful secondary metabolites, including the β-lactam antibiotics.

properties of Streptomyces.

The first cloning of a gene coding for antibiotic was reported last year at the Fourth International Symposium on Genetics of Industrial Microorganisms in Kyoto, Japan. The *pab* gene codes for para-aminobenzoic acid synthetase, an enzyme involved in the biosynthesis of candicidin. Candicidin is an antifungal antibiotic produced by *Streptomyces griseus* IMRU 3570. It contains an aromatic p-aminoacetophenone moiety that is formed from chorismic acid via p-aminobenzoic acid (PABA); PABA synthetase converts chorismic acid into PABA using glutamine as an amino donor[7].

The enzyme itself has been purified and has a molecular weight of 50 kilodaltons by gel filtration and polyacrylamide gel electrophoresis. The protein contains about 370 amino acids, requiring a DNA coding sequence of 1.1 kb. PABA-synthetase is present in *S. griseus* only during the antibiotic production phase[8]. The enzyme was not detected in extracts from 26 nonproducing mutants of *S. griseus* or several other Streptomyces that are not candicidin producers.

Synthesis of PABA-synthetase *in vivo* is repressed by low concentration (1–5 mM) of inorganic phosphate. Repression of PABA-synthetase by phosphate appears to be the main mechanism of phosphate control of candicidin biosynthesis, a well established phenomenon[9]. Because PABA-synthetase is the first enzyme of the biosynthetic pathway of the p-aminoacetophenone moiety, it is a likely target for regulation by the effectors that control candicidin production. A strict correlation between PABA-synthetase levels and candicidin production has been established, both in the wild type under different nutritional conditions and in several mutants of *S. griseus*. Thus the *pab* gene was a good candidate for cloning. This system may also be useful as a model for cloning other genes involved in antibiotic biosynthesis.

J. A. Gil and D. A. Hopwood (John Innes Institute, Norwich, U.K.) used two different approaches to clone the *pab* gene. In the first approach the total DNA from a sulphonamide-resistant mutant (*S. griseus* JG5) was used as a donor for shotgun cloning. The DNA was cleaved with the restriction endonuclease Bam HI and the pieces were ligated to the plasmid pIJ41, which was also cleaved with Bam HI[10]. The plasmid carries resistance genes to neomycin and thiostrepton, and insertion of DNA fragments at the single Bam HI site within the neomycin resistance gene results in a neomycin-sensitive phenotype. The recombinant plasmid was used to transform *S. lividans* 66, and clones that were sulphonamide-resistant, thiostrepton-resistant, and neomycin-sensitive were selected. One of these clones contained a hybrid plasmid that had two Bam HI fragments, 4.5 and 3.9 kb long, inserted in the Bam HI site of pIJ41. Further subcloning experiments showed that sulphonamide resistance, due to PABA overproduction, was conferred by the 4.5 kb fragment.

The second approach was to directly select clones carrying the *pab* gene of *S. griseus* by genetic complementation of a PABA-requiring strain, *S. lividans* JG10. The plasmid pIJ41 was again used as the vector for cloning Bam HI-cleaved total DNA from *S. griseus*, and a clone that was thiostrepton-resistant, neomycin-sensitive, and able to grow in media without PABA was selected. This clone also contained a 4.5 kb fragment. Apparently the same fragment had been cloned using both approaches, although the fragment was inserted into the vector in different orientations.

The cloned *S. griseus* gene was expressed in Streptomyces, but it could be expressed in *E. coli* only after the DNA insert was reduced to 3.5 kb. The truncated fragment complemented mutations in the unlinked *pab*A and *pab*B genes of *E. coli*.

A probe containing the 3.5 kb fragment hybridized with DNA from the two *S. griseus* strains IMRU 3570 and 38A and from *S. fradiae* ATCC 10745, but it did not hybridize with DNA from *S. lividans* 66, *S. coelicolor* A3(2), *S. clavuligerus* ATCC 27064, *S. parvulus* ATCC 12534, and *S. griseus* 52.2. From these results it is possible to conclude that the genetic information coding for PABA synthetase, and consequently candicidin biosynthesis, is restricted to some species or even strains of microorganisms.

It will be interesting to study the regulatory signals (e.g., the sequences responsible for RNA polymerase recognition and binding and the ribosome-binding sequence) that control the expression of the *pab* gene, and to investigate whether there are problems in the expression of Streptomyces-derived sequences in *E. coli* or other hosts. Experiments are now underway that will amplify the copy number of the cloned *pab* gene, and determine unequivocally if PABA-synthetase is the rate limiting step in candicidin biosynthesis. The availability of the *pab* clone will facilitate its further manipulation, e.g., for *in vitro* mutagenesis, and raises the intriguing possibility of introducing this gene into Streptomyces strains that produce antibiotics lacking the p-aminoacetophenone moiety; this could lead to formation of novel hybrid antibiotics that do not presently exist.

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Juan F. Martín, Ph.D., and José A. Gil, Ph.D., are from the Departamento de Microbiología, Facultad de Biología, Universidad de León, León, Spain.

MEETING REPORT: BIOTECH '83

Biotech 83, an international conference on the commercial applications and implications of biotechnology was held on 4–6 May 1983 in London. The following reports cover some of the highlights of the meeting. (For other reports see BIO/TECHNOLOGY, 1:5, 388.) Complete conference proceedings are available from Online Conferences, Argyle House, Northwood Hills, Middlesex, HA6 1TS, U.K. or London Online, 1133 Avenue of the Americas, New York, NY 10036.

RECOVERY AND PURIFICATION OF BIOLOGICALLY ACTIVE POLYPEPTIDES FROM rDNA MICROORGANISMS

The presentation by J. Curling (Pharmacia, Sweden) set the Biotech 83 conference, and the subject of biotechnology, against a proper economic and social backdrop; his first slide showed a hemophiliac patient and the last slide a victim of war suffering extensive third degree burns. Curling noted that biotechnology is already helping people like those shown in the slides via the production of Factor VIII and dextran-based dressing preparations.

Judging by the quantity and the quality of the technical presentations, downstream processing seemed to be the hot topic of the conference. This is understandable; once an organism has been genetically engineered to produce a specific product, the major challenge is to release the product and purify it with a high yield and low cost. In this context downstream processing refers to all the activities required to produce active and stable polypeptides with high and consistent purity from large quantities of microorganisms.

Concentration of Cells

Once a large batch of cells has been grown in a fermentor, it must be concentrated for further processing. C.-G. Rosen and R. Datar (Alfa-Laval, Sweden) described the problems encountered in this primary processing step. A reasonable cell density for an *Escherichia coli* fermentation is about six percent wet weight (higher densities can be achieved with some yeasts). It is usually advantageous to remove about 90 percent of the culture liquid and concentrate the cells to about 40 percent, particularly if the next processing step is mechanical disruption of the cells. At the present time, the most common way to concentrate cells is by continuous high speed centrifugation. There are several types of centrifugal separators. Commercially available nozzle-type separators have a solids handling capacity of up to 60,000 liters/hour. Smaller stack separators which discharge intermittently through axial channels can process up to 250 liters/hour at relatively high centrifugal forces (14,000 g).

The same authors discussed the problems encountered in large-scale separations using centrifugation. They pointed out that the useful feed rate depends on the sedimentation velocity of the smallest particles to be removed. For example, the relatively small size of most bacteria reduces the throughput of an *E. coli* culture to only 6 percent of the throughput of a yeast culture.

Cell Disintegration

In some cases chemical, including enzyme-catalyzed, treatments have been used successfully to alter cellular permeability and release products. However, mechanical disruption of cells remains the most common approach. The most widely applied devices are high-pressure homogenizers (Manton-Gaulin) and bead mills (Dyno); in some particularly difficult applications, the two devices may complement each other. Several cycles of disintegration may be required for optimal product yields (>80 percent). Most research on mechanical cell disruption has shown that the pressures and shear forces can be tolerated by most enzymes. The primary precaution is removal of heat generated during breakage. The pos-sible combination of mechanical and chemical disruption was not specifically discussed, but it may have future applications.

Removal of Cellular Debris

Physically, this is the most challenging step in downstream processing. Following disruption of the cells, one is left with a mess. First, instead of a solution containing particles of fairly uniform sizes, shapes, and densities (e.g. bacterial cells), the solution contains many different soluble products and particles that range in mass from $<10^6$ to $>10^9$ daltons and in size from $<10^1$ to $>10^2$ nm. Second, the densities of the particles also vary, from 1.20 to 1.01, approximately the density of the surrounding liquid. Per Hedman (Pharmacia) reminded the audience that the sedimentation velocity of a particle varies as the square of the size of the particle and in direct

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Now, more rigid ion exchange media and new column designs have made recovery of proteins produced by microorganisms faster and more efficient.