

Innovating Protein-Expression Systems

As Companies Diversify Portfolios, Production Processes Need to Be Retooled

K. John Morrow Jr., Ph.D.

There has been a maturation of the bioprocessing industry as companies have moved away from a focus on anticancer biologics toward a more diversified portfolio of pharmacological agents. With this diversity of disease targets comes a need for expression technologies to fit the requirements of proteins that may be required in smaller quantities or that may present exceptional problems to be surmounted for successful production.

CHI's "PEPtalk" conference to be held later this month will feature presentations from a number of scientists who are examining the intricacies of protein expression, and struggling to squeeze the last microgram of performance from their systems, while at the same time optimizing the quality of the recombinant product.

"We found that conditions can be optimized to grow animal cells in shaking reactors at liter scale with the potential to expand to commercial volumes," says Chao-Min Liu, Ph.D., distinguished research leader at Roche (www.roche.com).

Dr. Liu and his research team have been investigating the problem of adapting small-scale technology for the large-scale culture of animal cells in shaker reactors for a number of years. However, the potential of large-scale cultivation in shaker reactors was not considered until recently.

Focusing on the shaker approach, Dr. Liu and his co-workers assumed that this would provide a simple and efficient means of delivering adequate aeration to both mammalian and insect cells. They found that cylindrical bottles with small volumes up to 1 L provided the optimum growth conditions. However the smaller vessels were scalable to much larger volumes using proportionally larger shaking bioreactors.

"Successful implementation of this system has greatly increased the efficiency of our laboratory and our ability to generate large quantities of recombinant proteins for drug screening and development," Dr. Liu explains.

Florian Wurm, Ph.D., of the Laboratory of Cellular Biology at the École Polytechnique Fédérale de Lausanne Switzerland, has also worked extensively



Osprey Pharmaceuticals is advancing a pipeline of fusion-protein therapeutics known as leukocyte population modulators (LPMs) that are designed to selectively target and neutralize chemokine-activated leukocytes underlying a variety of inflammatory and immune diseases. The company's lead candidate, CCL2-LPM, targeting the CCR2 chemokine receptor expressed by certain pathologically activated leukocytes, is in a Phase Ib trial for the treatment of IgA nephropathy.

with shaker devices and he agrees with Dr. Liu. "The best approach is the use of cylindrical bottles, which we use in all of our operations with orbital shaking. Erlenmeyer flasks are a poor choice as they limit gas exchange through the nar-

row neck; devices with impellers are the worst, only useful with densities of cells of less than 2 million cells/mL."

Dr. Wurm also agrees with Dr. Liu that the cylindrical system can be scaled up to 1,000 L, with similar performance to controlled and "bubbled" stirred tanks. Another positive feature of the cylindrical shaker flasks, according to Dr. Wurm, is their optimal performance with serum-free and protein-free media, when the cells are more fragile.

"They outperform stirred devices, because shear stress is dramatically reduced, since the bulk of the liquid is moved in a laminar way."

Chemokine Fusion Proteins

"Osprey's therapeutic approach to inflammation and autoimmunity is to neutralize specific pathological leukocytes using chemokine fusion proteins," explains Hongsheng Su, Ph.D., director of process development at Osprey Pharmaceuticals (www.ospreypharma.com).

Dr. Su's research group is investigating a class of novel fusion proteins called leukocyte population modulators (LPM). The lead therapeutic candidate, CCL2-LPM, is a recombinant fusion protein composed of a member of the chemokine superfamily, CCL2 (also known as macrophage chemoattractant protein-1, MCP-1) fused to a bacterial and eukaryotic protein synthesis inhibitory enzyme (RIP, ribosomal inactivating protein), in this case a truncated Shiga toxin A1 subunit. CCL2-LPM is currently in clinical trials.

Chemokines, a group of small protein mol-

News BIOPROCESSING HIGHLIGHTS

BioLife Signs License with Centocor for Media Product

BioLife Solutions (www.biolifesolutions.com) signed a license and custom cGMP manufacturing agreement with Centocor Ortho Biotech (www.centocororthobio.com). The company will produce a variant of its serum-free and protein-free CryoStor biopreservation media product, which is formulated with a reduced concentration of 2% DMSO.

In November, BioLife was granted a Japanese patent covering claims related to protecting cells from injury and death caused by cold temperatures used in biopreservation. BioLife was previously granted equivalent U.S. and European patent protection for the IP. In September, Sigma Aldrich negotiated a nonexclusive distribution agreement for BioLife's HypoThermosol and CryoStor products.

Inno Biologics to Use Cevec's CAP Technology

Inno Biologics (www.innobiologics.com) inked a strategic alliance with Cevec Pharmaceuticals (www.cevec-pharmaceuticals.com) to use the latter's CAP expression technology. This license, will, according to the companies, enable Inno

Biologics to improve the production of its diagnostic and preclinical-grade material.

CAP Technology is a stable-producing human cell line. Its nontumor origin cells reportedly have high expression rates in serum-free suspension culture and its post-translational modifications are human-like.

Patheon Consolidates Puerto Rico Operations

Patheon (www.patheon.com) will consolidate its Puerto Rico operations into its manufacturing site located in Manati and will close or sell its plant in Caguas. Patheon is a provider of contract development and manufacturing services ranging from preclinical development through to commercial manufacturing of an array of dosage forms including parenteral, solid, semisolid, and liquid forms.

The company estimates that this consolidation will result in total repositioning expenses of approximately \$7 million, of which about \$2.4 million will be booked in the first quarter of fiscal 2010.

DynavaxPlant Approved for HBV Vaccine Production

Dynavax' (www.dynavax.com) production facility in Düsseldorf, Germany, received European regulatory clearance for

the commercial production of hepatitis B surface antigen. The antigen is a key component of Heplisav™, the company's Phase III adult hepatitis B vaccine.

Dynavax' German subsidiary, Rhein Biotech, has been manufacturing the hepatitis B surface antigen for Heplisav clinical trials at the Düsseldorf facility since 2006. The new license means the facility should be able to meet initial commercial production demands.

Pfenex Gains Independence from Dow Chemical Company

The Dow Chemical Company (www.dow.com) formed a new independent company, Pfenex (www.pfenex.com). The new company is based on human health applications of a Dow-developed technology called Pfenex Expression Technology™, a *Pseudomonas fluorescens*-based platform that uses high-throughput, parallel processing methodologies for optimized protein production.

Pfenex is a biotechnology company specializing in strain engineering and protein production, helping to accelerate the development of new biopharmaceutical therapeutics and vaccines that address critical human health issues from infectious diseases to oncology. n

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ecules, are powerful chemoattractants that participate in leukocyte trafficking, extravasation (exudation of lymphatic fluid into the tissues), and recruitment to specific sites.

The CCL2 chemokine and its receptor, CCR2, play a decisive role in a number of inflammatory diseases, including those of the kidney and some cancers. By recruiting active pathological leukocytes, a cascade is initiated, which facilitates tissue damage, autoimmunity, fibrosis, and metastasis. For this reason, Dr. Su and his colleagues propose that engineering a fusion protein in which the chemokine CCL2 is fused to a protein-synthesis inhibitor would bring this destructive chain of events to a halt.

However, production of a protein that inhibits protein synthesis presents the investigator with a quandary, since it is not immediately obvious how one might prevent the newly synthesized inhibitor from shutting down its own continuing synthesis.

In order to deal with this challenge, Dr. Su and his collaborators developed a protocol using the compound 4-aminopyrazolo(3,4-d)-pyrimidine (4APP), which blocks the action of the Shiga A1. 4APP binds to the SA1, protecting cells from the toxic actions of the SA1 protein.

By employing this strategy the team was able to grow transformed cells containing the fusion protein gene to high densities, and isolate gram/L quantities of the product. The expressed and highly purified protein was effective in neutralizing cells of the monocyte lineage, which possess the CCL2 chemokine receptor. According to Dr. Su, “early clinical trials in IgA nephropathy patients are currently ongoing.”

Mind the Gap

“In the course of stable bacterial cell-line development, we need to consider process formulation and regulatory requirements at the earliest time possible,” says Annette Hillebrand, Ph.D., senior scientist at Dynavax Technologies (www.dynavax.com). Her company currently has several products in clinical trials, including a hepatitis B vaccine, and two additional therapeutic agents for hepatitis B and hepatitis C. Dynavax’ research strategy also encompasses products for asthma, autoimmune diseases, and flu.

Hillebrand sees a gap, especially in larger biotech firms, between the research division and the production division, with the result that frequently the two phases of a drug-development program fail to mesh effectively. The result is that protocols mapped out by research teams may fail in the production phase because R&D units are primarily concerned with proof of principle, and industrial-scale expansion of production may not figure into the equation. This means that time and resources are wasted, and clinical programs are delayed.

“There is a huge gap between researchers and process developers with the result that the two groups frequently do not communicate and work at cross purposes to one another,” believes Dr. Hillebrand. “So it is critical in microbial cell-line development that all regulatory requirements be account-

ed for at an early stage.”

She cites her experience with different expression plasmids—while there is a huge variety of commercial options from which to choose, there is only a limited choice of ones that will function in the production phase.

Dr. Hillebrand also details the development of a stable expression strain for Dynavax’ universal flu vaccine. At Rhein Biotech, the company’s European subsidiary, she tested various strains to evalu-

ate the long-term expression stability of the same genetic construct in different host strains. “This testing in an early stage of development helped avoid possible issues that could arise at the upscaling phase when clinical quantities are required.”

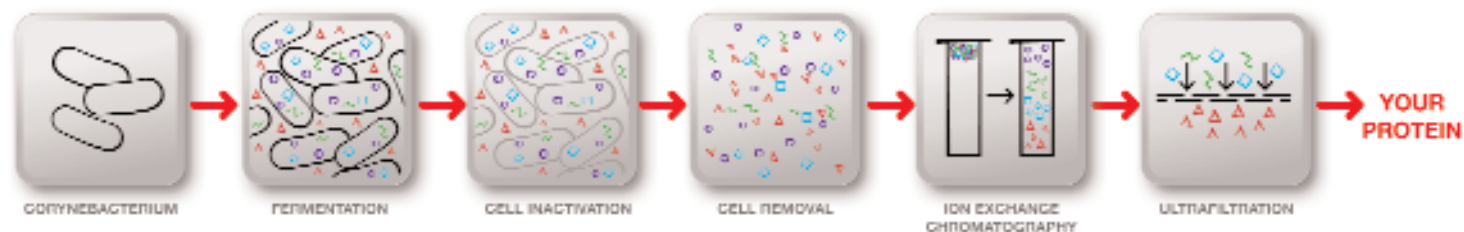
From A to Zera Fusions

“We are working on multiple applications of our assembler peptide, one being a versatile bioprocess for protein manufacture,”

states Stefan Schmidt, Ph.D., vp of technology at ERA Biotech (www.erabiotech.com). The company’s technology is based on the use of the α -zein protein in maize. This storage protein takes part in protein body formation, notably in the endosperm of the corn plant. ERA’s technology involves the construction of a fusion protein between the Zera assembly peptide and a target recombinant protein gene. As the protein bodies are

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Expression Systems

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formed in the course of cellular biosynthesis, the Zera fusion protein is sequestered inside the storage organelle where it is well-folded and protected from proteolysis. These StorPro Organelles can be engineered into mammalian cells or other eukaryotic hosts without affecting the host cell's viability.

This technology is of particular value in protecting unstable or difficult to express recombinant proteins, such as peptides,

and antimicrobials from normal host-cell metabolism. Purification and removal of contaminants is particularly easy, Dr. Schmidt says. Major advantages of the system include compatibility with existing vector and expression enhancing systems, protection of both cell and protein by encapsulation, simplifying the downstream process, thus leading to a shorter time frame and lower costs of goods, he adds.

"We are currently pushing product applications," Dr. Schmidt says. The expanding platform technology of the company will embrace cleavage systems and affinity binder options, a tool box of vectors and enhancers, and a multihost, high-throughput system.

"The Zera format is especially versatile, in that it could allow various administrations of the fusion proteins in the StorPro bodies, as well as traditional application of

the highly purified recombinant protein."

Soluble IgG Receptors

"We're investigating soluble Fc IgG receptors as a therapy for autoimmune diseases," says Peter Sondermann, Ph.D., CSO at **SuppreMol** (www.suppremol.com).

Membrane-bound Fc receptors are expressed on virtually all cells of the immune system except T cells, and their interaction with immune complexes (antigens recognized by multiple IgGs) will activate pathways that result in their destruction. In autoimmune disorders this activation has negative and sometimes life-threatening consequences as the antigens represent essential host structures.

"In autoimmune diseases such as serum lupus erythematosus or rheumatoid arthritis the immune system turns against its host, and if it wins the battle, the kidneys or joints are destroyed," Dr. Sondermann says, "so completing the membrane receptors with soluble receptor would have the potential to break the vicious cycle of antibody production, destruction of the target structure, and further activation of the immune system."

To do this, the SuppreMol team cloned and expressed a truncated, soluble IgG receptor molecule (sFcγRIIb) in *E. coli*, and successfully tested its ability to counteract the effects of autoimmune diseases in various animal models.

The first condition approached by SuppreMol is idiopathic thrombocytopenic purpura (ITP), which manifests itself by destroying platelets through the engulfing and digesting action of activated macrophages. When the platelet count falls below a certain level, extravasation of blood from the vessels into the surrounding tissues occurs with often fatal consequences. Because its indication is clear and straightforward, the condition is an appropriate target for the SuppreMol therapeutic protocol.

According to Dr. Sondermann, the immunomodulatory soluble receptor for the Fc portion of the IgG molecule is expressed in inclusion bodies of the prokaryotic expression systems in extremely high quantities. To obtain the active form, the inclusion bodies are separated from the cells and the resulting partially pure protein can be refolded and purified to obtain high yields.

The company has successfully conducted Phase I studies with volunteers, and is now proceeding to point-of-care studies in ITP patients. In addition, upscaling the production of a cloned, truncated receptor will allow larger clinical investigations, encompassing additional indications. For this reason, SuppreMol is currently improving the production process by adaptation of the fermentation protocol as well as the refolding and purification steps, in order to optimize yields and production costs.

Up to this point the most successful biologicals have anticancer antibodies, but the picture is rapidly changing as a number of new treatments for infectious diseases, immune dysfunction and neurological disorders move through clinical trials and toward the market place. **GEN**

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