

GEN Bioprocessing

Overcoming Protein Production Hurdles

Increasing Capabilities Becomes Essential as the Biologics Market Grows Rapidly

K. John Morrow Jr., Ph.D.

Recombinant antibodies have emerged as dominant players in the biopharma industry despite the copious quantities required for treatment. Although nonantibody biologics are typically produced at much smaller scale, are less complex, and have limited secondary modifications, their production is also fraught with challenges. Not surprisingly overcoming protein-production hurdles was a key theme at IBC's "Bioprocessing Meeting", held recently in La Costa CA.

"There are a host of issues to consider when looking at various expression options," says David Robinson, Ph.D., Merck & Co. (www.merck.com). "This includes any possible impact on patient safety and the presence of adventitious agents, live organisms, and other immunogenic material."

Other factors that weigh into the decision are the specific biology of the product, since the ability to carry out secondary modifications to expressed proteins varies tremendously from one system to another. Regulatory concerns and intellectual property issues always looms in the background.

Despite the interest in alternatives, mammalian cell expression systems still dominate the industry—out of 23 blockbuster biologic drugs produced in 2006, only eight of these were generated through other means. There are a number of reasons for this dominance, but perhaps the most cogent is the vast store of experience gained over the course of four decades. This highly standardized technological base allows producers to rapidly generate large quantities of antibody with a low risk of failure.

Today, manufacturers rely on well-known stirred-tank bioreactors, which have a proven track record. With so many advantages to staying the course, why would companies want to change? The tedious process of cell-line construction is one reason. When a new gene transcript is being readied for expression, it must work its way through the transformation process, and it must be forced through layers of selection, cloning, and expansion.

Even with the process largely automated, this can take weeks of effort. Dr. Robinson estimated the entire cycle time at between four and eight months. If there is a failure or a breakdown in the process, weeks or months more will be required to bring the task to successful fruition, and the commitment continues, since with the slow generation time of the mammalian cell, the culturing process itself will take from 10 to 30 days to expand the volume of material appropriately.

But one of the most difficult issues to

K. John Morrow Jr., Ph.D. (jmorrow@genengnews.com), is president of Newport Biotech and a contributing editor for GEN. Web: www.newportbiotech.com.

deal with is the transient and unstable process of glycosylation in mammalian cells, which frequently proves difficult for the investigator to control.

Animal cells also produce nonhuman varieties of glycans, and while these are generally well tolerated, there are exceptions. So alternatives to the mammalian cell must possess the ability to express multiple human proteins with the correct post-translational modifications, as well as perform appropriate glycosylations with ease, all in the context of existing physical plant hardware.

Dr. Robinson and his team compared proteins expressed in *Pichia pastoris* with those expressed in mammalian cells and have observed that IgG1 molecules expressed in the severely engineered GlycoFi *Pichia* strain have similar antigen binding ability and in vitro efficacy as that expressed in CHO cells. Moreover, erythropoietin, expressed in the same yeast strain has the



Eden Biodesign uses stirred tank bioreactors whenever possible, as they provide increased control of cellular physiology during the viral production process.

appropriate mass and activity compared to molecules produced in the CHO cell line. Because the yeast cell cycle is much shorter than that of a mammalian cell, combining its biological properties with robotic automated systems for cloning and selection makes the process of developing new transformed cell lines more convenient.

"Indeed, the whole process of moving

from transformants to high-producing cell lines was completed in weeks in the Merck facilities, rather than the months it would have taken with traditional mammalian cell technology," stated Dr. Robinson. "I believe that our platform provides an opportunity to produce high-quality homogeneous therapeutics that will enable novel biologic therapies."

News Bioprocessing Highlights

Sanofi Pasteur's Manufacturing Plant Gets FDA Sanction

FDA approved Sanofi Pasteur's (www.sanofipasteur.com) new manufacturing facility for the production of seasonal influenza vaccine. It could also be used for the manufacture of a vaccine against the new 2009 H1N1 influenza strain.

This facility, located in Swiftwater, PA, will incorporate the latest technology in egg-based vaccine production as part of the company's commitment to produce the largest number of doses of vaccine in the shortest time frame to address the threat of seasonal and pandemic influenza.

Sanofi Pasteur spent \$150 million on this 140,000 sq. ft. plant. In total, Sanofi Pasteur will have a capacity of approximately 150 million doses of trivalent seasonal influenza vaccine per year in the U.S.

Sartorius Stedim Biotech Extends Single-Use Bioreactor Portfolio

Sartorius Stedim Biotech (www.sartorius-stedim.com) has expanded its technology offerings in the area of single-use bioreactors. At the 2009 "ACHEMA" trade show, the company presented two prototypes of single-use bioreactors that

operate based on what it calls "novel" mixing technologies.

The cell culture systems were developed in cooperation with Bayer Technology Services (www.bayertechnology.com) and ExcellGene (www.excellgene.com). The new bioreactors have been specially designed for the manufacture of monoclonal antibodies, recombinant proteins, and vaccines.

Cobra Bio to Develop and Manufacture Fusion Proteins for KAHR Medical

KAHR Medical (www.kahr-medical.com) awarded Cobra Biomanufacturing (www.cobrabio.com) a contract to develop and manufacture bulk quantities of its trans signal converter protein (TSCP) candidates for preclinical and future clinical trials. The deal will harness Cobra's MaxXpress service, which combines cell-line development with its UCOE (ubiquitous chromatin opening elements) technology, together with the company's expertise in recombinant protein production.

The TSCP technology, licensed by KAHR from the University of Pennsylvania, comprises a class of multifunctional therapeutic fusion proteins designed to convert signals

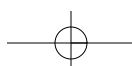
sent from one cell to another. KAHR says each of these fusion proteins typically combines the extracellular portion of a type I membrane protein (at the fusion protein's N-terminus) with the extracellular portion of a type II membrane protein (at the fusion protein's C-terminus), resulting in a therapeutic protein with two functional sides.

KAHR's lead product is the rheumatoid arthritis candidate, KAHR-101, which combines relevant portions of the immune-related membrane proteins, Fn14 and TRAIL. It is expected to start in clinical trials by mid-2010. KAHR's second therapeutic, KAHR-102, is in development for the treatment of psoriasis and combines sections of the immune-related membrane proteins, CTLA-4 and FasL.

KAHR and Cobra have been working together for 18 months on the development of KAHR-101 and KAHR-102.

DSM Capacity Grows at Groningen, Netherlands, Site

DSM (www.dsm.com) is expanding cGMP capacity at its Groningen facility with the addition of a new 1,000 L disposable bioreactor. The expansion is expected to be complete by Q4 2009 and the company is currently selling capacity. n



Since wild type yeast proteins are heavily mannosylated, it was necessary to develop cell lines that were humanized with respect to their glycosylation ability. The Merck research facility boasts an extensive GMP manufacturing suite with nine units, which allows for the simultaneous processing of mammalian cells, microbial, and viral products. With these facilities in place Dr. Robinson and his team are able to rapidly scale up to grams per liter titers.

Safety and Performance

Willem Stemmer, Ph.D., CEO, and Volker Schellenberger, Ph.D., vp drug discovery, cofounders of Amunix (www.amunix.com), discussed the concept of biosuperiors—recombinant proteins that perform better than the original pharmaceutical.

Amunix has investigated the properties of unstructured amino acid sequences as a substitute for polyethylene glycol (PEG). While both molecules form linear, extended polymers and can be attached to proteins, PEG has a number of unsavory features, including a complex manufacturing process and a heterogeneous make-up. But, perhaps the most significant drawback to PEG is its lack of biodegradability.

Unstructured amino acid polymers, on the other hand, do not display these adverse qualities and can be engineered for the desired length as part of a fusion molecule with the targeted biologic.

With these concepts in mind, Amunix set out to optimize the molecule by a process that went through seven design generations over a three-year period. The optimization included in vivo half-life, expression level, genetic stability, accelerated stability, aggregation, protease resistance, and immunogenicity. Because conventional PEG is not biodegradable and accumulates to form vacuoles in the cells of the kidney, rPEG is designed to be broken down by kidney proteases, and to resist degradation for at least seven days in serum.

Amunix has spun off a company, Versartis, to bring a number of rPEG products to market with a focus on metabolic diseases. It has worked through the manufacturing process and designed a fusion molecule, Exenatide-rPEG, for the treatment of type II diabetes that is not controlled by oral antidiabetic agents. Based on extrapolation from various animal studies, the anticipated dosing is weekly or even biweekly, Dr. Stemmer explained.

Other projects under way use rPEG-coupled antibody fragments, including a single-chain bispecific antibody in which anti-HER-2 and anti-EGF fragments are linked together with rPEG, blocking the unwanted aggregation of the molecules while retaining the original binding ability of the molecules. Another area of investigation comprises RNase-antibody conjugates linked together by rPEG.

“The rPEG gives antibody fragments long half-life and allows their expression in soluble form in the *E. coli* cytoplasm,” Dr. Stemmer stated. “We believe the approach

holds substantial promise for the development of better therapeutic agents.”

Working Through a Knotty Problem

“We approached the challenge of designing more stable protein-based drugs through the use of cystine knots,” says Jennifer Cochran, Ph.D., of Stanford University. “Also known as knottins, these cysteine-containing peptides are of small

size and limited immunogenicity and make effective scaffolds for protein engineering.”

Knottins possess disulfide-constrained loops that endow them with high thermal and proteolytic stability, and their folded protein structure is highly tolerant of multiple amino acid substitutions.

The goal of Dr. Cochran’s group was to engineer knottin peptides that bind to alpha v integrins with high affinity and

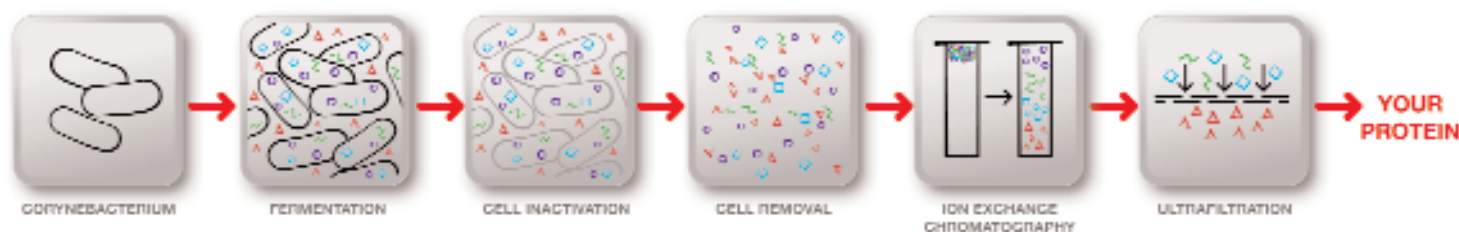
specificity for use as molecular imaging agents in living subjects.

Integrins are a class of heterodimeric adhesion molecules that act as chemical messengers, and integrin receptors containing the alpha v subunit mediate angiogenesis and tumor metastasis. As such, they are critical players in the proliferation of tumor cells.

The Cochran team developed a system

See Protein Production on page 46

Need Protein Expression? Don't Make It Complicated.



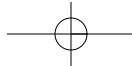
By dramatically simplifying the purification process, the Corynex Protein Expression System reduces production costs and speeds time to market. Contact us to find out how to make protein manufacturing complications disappear.



EXPRESSION SYSTEM

Advanced Science That Delivers Better Results.
Visit www.corynex.com, e-mail corynex@ajiusa.com
or call 877-526-7963 for details.

AJINOMOTO.
www.ajiaminoscience.com



Protein Production

Continued from page 45

for directed evolution of knottin peptides on the surface of yeast cells that enabled the group to identify integrin binders. Since it was known that the sequence Arg-Gly-Asp (RGD) binds to integrins, this tripeptide motif served as the basis for a combinatorial library of knottin peptides that was created and displayed on the surface of yeast. In this display format the RGD motif, constrained within a knottin loop, was surrounded by randomized bases, giving rise to around 10^7 different sequence possibilities. These mutants were screened in a high-throughput manner to identify knottin peptides that bound to alpha v beta 3 integrin with the highest affinities.

The engineered integrin-binding knottin peptides bound to tumor cells with antibody-like affinities, and they blocked tumor cell attachment to extracellular matrix proteins. The knottin peptides were conjugated to a fluorescent dye or radionuclide label and were evaluated as imaging agents in glioblastoma xenograft mouse models. Peptides with higher integrin binding affinities elicited greater levels of tumor uptake compared to weaker binding peptides. Knottin peptides displayed low uptake and retention in other tissues, including the liver and kidneys, indicating their potential use as tumor targeting agents for radiotherapy or chemotherapy applications, according to the company.

The group's fascination with integrins was driven by the unusual ability of these receptors to move signals in both directions: transducing information from outside the cell, and from inside-out, displaying information to the extracellular environment. This permits a rapid and flexible response on the part of the cell. Current

studies are focused on the alpha v beta 3 integrin receptors and their role in regulating cell adhesion and blood vessel formation, tumor invasion, and growth and metastasis in a range of different cancers.

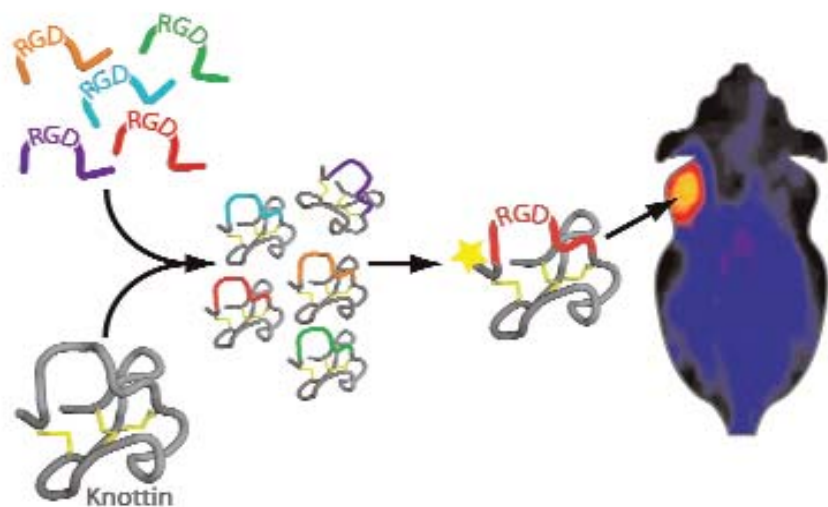
Vaccine Development

These are heady days for vaccines, which are enjoying a rebirth of interest. Crawford Brown, Ph.D., CEO of Eden Biodesign (www.edenbiodesign.com), discussed the challenges of viral vaccine production at the meeting.

"The technical and regulatory demands are greater for vaccines than for antibodies, so we are seeking to overcome these barriers and build a platform similar to the CHO/protein A production method currently used in antibody production," Dr. Brown explained. "Our aim is to build our products through the application of good science one day at a time."

According to Dr. Brown, monoclonal antibodies have become more small molecule like in that they are moving into a mainstream product class. This means that they are treated from a commercial point of view more like small molecules, their manufacture is easily outsourced to a range of CMOs, regulatory agencies are quite comfortable in the evaluation of these entities, and scale-up is now a routine process.

In order to move vaccine production to this level, a number of problems unique to this discipline must be addressed. Currently, there is no dominant production platform, there is a limited transferability of skills and knowledge between products, and there is a high level of risk involved in the development process. New approaches are required



Peptides composed of an Arg-Gly-Asp (RGD) sequence motif surrounded by randomized amino acids were grafted onto a knottin scaffold, yielding a combinatorial library of knottin variants displaying disulfide-constrained RGD peptides. RGD-knottin peptides were subjected to a series of high-throughput screens to identify the clone with the highest affinity for integrin receptors. The highest-affinity clone was then conjugated to a radionuclide label and evaluated as a tumor-targeting agent in mouse tumor models. The engineered RGD-knottin peptide showed high tumor uptake with low uptake and retention in other tissues.

Stanford University

including more platform-like strategies, new analytical tools and innovative facility design. "We are aiming for a platform that is robust, transferable, reproducible, and scalable," said Dr. Brown.

Disposables play a major part in the Eden vaccine program, a feature shared with many CMOs. Disposable columns are now widely available and entail less contamination risk, faster turnaround, and reduced validation. The familiar stirred tank bioreactors, widely used in therapeutic biologics production, are used whenever possible, as they provide increased control of cellular physiology during the viral production process.

"Vaccine development still faces challenges that have been largely overcome for mAbs and other recombinant products," he claimed.

Protein-based therapeutics have mush-

roomed into a vast industry. The overall market for biologics (including antibodies) was \$63 billion in 2007 and is expected to reach \$87 billion by 2010, according to Kalorama Information. Moreover, the biologics market, although much smaller than the entire pharma industry, continues to grow at a more rapid pace. This phenomenal growth is a powerful incentive to the industry to optimize its production capabilities.

GEN

POINTS TO CONSIDER

- The production of high-quality biologics raises a number of significant challenges whose resolution may be different from those that have been so successful in ramping up antibody yields to kilogram quantities.
- Although mammalian cell expression systems still dominate the industry, there is great interest in alternatives, including yeast expression systems. At Merck, *Pichia* has been successfully engineered to produce appropriately glycosylated proteins.
- Amunix has developed biosuperiors, recombinant proteins that, it claims, perform better than the original pharmaceutical.
- Knottins, cysteine-containing peptides of small size and limited immunogenicity, reportedly make effective scaffolds for protein engineering.
- Long ignored by pharma companies, vaccines are enjoying a rebirth of interest. Eden Biodesign is developing a platform that is robust, transferable, reproducible, and scalable in order to speed vaccine production.
- The biologics market, although much smaller than the entire pharma industry, continues to grow at a rapid pace, providing a powerful incentive to the industry to optimize its production capabilities.

GEN Genetic Engineering & Biotechnology News
Presents

GREENbiopharma
Sustainable Operations, Facilities, Laboratories, Processes

Call for Papers

December 2-3, 2009 Sheraton Society Hotel, Philadelphia

GREENbiopharma 2009 is the first conference to explore the range of strategies for attaining sustainable life science organizations. The conference will focus on tools and methods, and the economic rationales for implementing them across your company—from plants and facilities to laboratories and production. GEN is accepting a limited number of abstracts for GREENbiopharma 2009 from pharmaceutical/biotech companies, regulators and government laboratories, and academic researchers. Deadline for submissions is June 29, 2009.

Topics Include

- Green facilities: energy, waste reduction, recycling, conservation, vendor/purchasing strategies
- Green laboratories: energy, solvent strategies, instrumentation and analytics, workflow for sustainability
- Green manufacturing: catalysis/biocatalysis, process design and intensification, buffer and reagent conservation, high-quality water management, disposable bioprocess equipment, green chemistry for biotech

Contact

Please send a short abstract and title, along with a brief speaker bio and contact details to: jsterling@genengnews.com.

www.bioconferences.com/greenbiopharma

