

Expression Systems for Process and Product Improvement

A Perspective on Opportunities for Innovator and Follow-On Product Developers

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Expression systems encompass the technologies — biological materials and associated know-how — needed to genetically modify organisms for the manufacture of recombinant proteins and other products. They include vectors (usually plasmids) used to transfer genetic material into host cells as well as the source and transformed cells themselves. In some cases, the vectors may be commercial products on their own, such as those used for gene therapy or live viral vaccines.

Although usually involving culture of microorganisms or cells, expression systems can also involve higher organisms such as transgenic animals or plants. As core technologies for genetic modification, many expression systems are often ubiquitous research and drug discovery tools as well.

The component technologies for protein expression can include core and backbone vectors for gene delivery, transfection-assisting reagents and methods, activation and promoter sequences to drive transcription of inserted genes, terminator sequences, selection and amplification sequences and reagents, chromatin insulators, internal ribosome entry site sequences, methods of screening transformed cells for desired characteristics, fusion



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protein sequences used to facilitate protein purification. Of course the host cell lines are the main constituent — and themselves may include genetic modifications to improve product yield and cell line stability and alter environmental sensitivities or other characteristics. Companies generally fixate on using just one or a few licensed technologies to simplify licensing, process design, and regulatory issues.

Expression systems, like most other commercial manufacturing technologies, are generally patented and available for licensing from their owners (patent assignees) or from companies having licensed the patents for bundling (sublicensing) with their own expression systems or components. The need to use (and

license) a number of component technologies, known as *patent stacking*, to manufacture just about any biopharmaceutical is rarely discussed openly in the biotech industry. The status of many patents is difficult to determine. Few organizations with expression system technology available for licensing have effective information dissemination or technology transfer marketing programs. And it seems that few manufacturers conduct rigorous due diligence to determine the need to license all the technologies they use. Also, the actual processes used for manufacturing marketed biopharmaceuticals are almost never fully disclosed. These factors suggest that patent infringement in this area may be common.

ESTABLISHED TECHNOLOGIES PREVAIL

A review of the scientific patent trade literature shows a large number and wide diversity of biotech expression system technologies available and in development. Looking at the expression systems used to manufacture current biopharmaceuticals (and those in development), you'll find that most use old technologies developed in the 1970s and adapted for biopharmaceutical manufacture in the 1980s. Table 1 classifies expression systems used in manufacturing the recombinant proteins on the United States and European markets (1). Over half (55%) of such products are expressed using microbes, either bacteria (40%) or yeasts (15%), with nearly all those bacteria being a form of *Escherichia coli* (*E. coli*, 39%). Another 45% are expressed in mammalian cells, primarily Chinese hamster ovary (CHO) cells (35%).

E. coli, yeasts, and CHO cells have the longest history of use, beginning with commercialization of the first recombinant proteins in the 1980s. Together, these three account for 89% of the expression systems used in manufacturing currently marketed biopharmaceuticals. The concentration on just a few systems is even higher if you consider the 11 products (all monoclonal antibodies) made using murine myeloma cells. Similarly, among the 24 recombinant proteins with blockbuster revenue (\geq \$1 billion/year), 89% use *E. coli*, yeast, or CHO cells. Examination of products currently in later-stage development shows much the same pattern.

Although these expression systems have been substantially improved over the years — e.g., host cell and other modifications have significantly increased yield and stability — the failure to adopt new technologies often offering considerable advantages is indicative of industry-wide problems. Truly novel technologies (new to human biopharmaceutical manufacture) such as fungi, insect cells, plants, and transgenic animals have yet to make much impact on marketed products or those in later-stage development.

A wide variety of expression systems have been developed for recombinant protein manufacture. Novel expression systems (again, in terms of human biopharmaceuticals) include those based on bacteria other than *E. coli* (e.g., *Pseudomonas fluorescens* and *Caulobacter crescentus*); fungi (e.g., *Chryso sporium lucknowense*); improved human cell lines (e.g., PER.C6); yeasts culturable in glucose, ethanol, or other simple media and/or that provide human-like glycosylation; algae ranging from single cells through whole plants (e.g., Lemna); various insect cells and whole insects; a wide variety of terrestrial plants (e.g., safflower and tobacco); and various transgenic animals. Completely cell-free systems are also becoming available. Some novel systems are now commonly used for nonpharmaceutical recombinant protein manufacture (e.g., most industrial enzymes), and some are used for large-scale pharmaceutical manufacture, but only in the diagnostic and veterinary areas.

Many novel expression systems offer significant improvements in yields or other performance factors; increased simplicity; and lower costs for manufacture, equipment, facilities, and infrastructure, including less dependency on large, fixed bioreactors. Many offer potential product improvements (e.g., more human-like or better control of protein glycosylation, protein folding, and higher purity). Many are considered “novel” solely because they remain largely unused for producing human biopharmaceuticals, although the technologies themselves may be as old as the current dominant expressions systems. For example, *Pichia* yeast, baculovirus vectors and insect cells, and non-*E. coli* bacteria (e.g., *Bacillus subtilis*) were initially developed decades ago and are widely used, but not yet for marketed human biopharmaceuticals.

In many cases, older expression systems have been incrementally modified over the years, often leading to significant improvements. For example, some yeast and other systems now offer posttranslational modifications (e.g., glycosylation and

Table 1: Expression systems and transformed hosts for recombinant products

Microbes	79
Bacteria (prokaryotes)	58
<i>Escherichia coli</i> (<i>E. coli</i>)	56
Streptococci	2
Yeasts (eukaryotes)	21
<i>Saccharomyces cerevisiae</i>	19
<i>Pichia pastoris</i>	2
Plants	0
Insect Cells, Baculovirus Vectors	3
Mammalian Cells	74
Mammalian cells, nonprimate	65
hamster, Chinese ovary (CHO)	50
murine myeloma cells	11
murine cells other	1
Mammalian cells, primate	9
monkey cells, diploid, kidney, or fetal lung	4
Human cells	5
human cells, transformed with Epstein-Barr virus transformed	1
human cells, gene activation by TKT2	2
human kidney cells, embryonic	1
human cells, unspecified	1
Avian Systems	0
Transgenic Mammals	3
Goats	1
Rabbits	1
Mice, XenoMouse (used in development only)	1
Viruses	7
Recombinant viruses as products (live vaccines)	6
Yellow fever virus vector	1

folding) that closely mimic those of mammalian cells. Host cell line, culture media, and other incremental improvements also have increased yields with many older systems by an order of magnitude or more, from a fraction of a gram to multiple grams per liter. But many newer systems offer even further advantages. Even so, the basic aspects and components of the expression systems used in large-scale biopharmaceutical manufacture have changed little over recent decades. This lack of industry interest in newer technologies often carries over to other aspects of bioprocessing, as well.

WHY THE LACK OF PROGRESS?

The failure or refusal of biopharmaceutical companies to adopt more modern expression systems is not new (2). Simply stated, human

biopharmaceutical developers do not want to pioneer manufacturing technology and face a “Catch-22” situation. Even if a novel manufacturing technology provides significant competitive advantages and product improvements, biopharmaceuticals manufactured using novel technology can be expected to come under increased scrutiny and are likely to encounter delays in regulatory approval. To date, the innovator-dominated industry has obviously decided that such potential delays and problems are not worth the cost savings and improvements offered by newer technologies.

An associated quandary is that commercial adoption of new expression systems first requires scale-up and demonstration at sufficiently large scale. The only organizations for whom such projects are economically justifiable are established companies that can prove feasibility through actual product manufacture. Smaller companies and academic researchers lack the funding, facilities, and other resources needed for scaling up and having no products to manufacture, if only for use in clinical trials. However, newer expression systems and bioreactors are often more cost-effective and require less up-front investment. So new emergent follow-on protein/biosimilar companies and contract manufacturers are and will most likely be the first adopters of a broader range of expression systems and other bioprocessing technologies for commercial-scale manufacturing.

Established companies have shown a clear preference to continue using familiar manufacturing technologies. This is exemplified by a handful of companies controlling a great majority of the world’s large-scale biopharmaceutical manufacturing capacity. Essentially all that capacity involves established systems: mostly *E. coli*, yeast, and CHO expression systems using fixed, large-scale bioreactors often 10,000 L or larger. This is particularly true for manufacture of recombinant monoclonal antibodies, which require high capacity because they are administered in repeated high dosages.

The classic technologies have worked well for the industry, and many managers are satisfied not to think critically about their expression systems or the other biomanufacturing technologies their companies use. The biopharmaceutical industry’s reluctance to adopt modern expression systems is not due to lack of availability or access. Most novel technologies are available for licensing, and licensing fees for older and newer technologies are not significantly different.

HOW WILL BIOGENERIC CHANGE THINGS?

Newer and improved expression systems are becoming more important in the context of generic biopharmaceuticals (biosimilars, follow-on biologics, generics) (3). For innovators developing novel biopharmaceuticals, unique expression systems confer a defense against later development of follow-on/biosimilar products. This is particularly true for those that are proprietary (trade secrets) or have exclusive licensing and that also confer unique molecular properties (e.g., glycosylation patterns) that are hard to copy using other systems. This appears to be a factor in the recent acquisitions and exclusive licensing of novel expression systems technologies by many larger companies. For example, Merck acquired GlycoFi — which is developing yeast expression systems for controlled, human-like protein glycosylation — for about \$400 million.

New and improved expression systems are being adopted by follow-on/biosimilar developers. Many such companies are adopting the most cost-effective manufacturing technologies useful for their products. In many cases, this will be a necessity. These companies will have to compete against others of their kind as well as against innovators. Many established innovator companies already have over 20 years of experience and world-scale manufacturing facilities — already providing the world’s supply of their products and any related financing long ago paid off in full. Contrary to the expectations of many, generics

may not cost less than innovator products. Biogeneric companies will have to compete against the innovators’ lower costs of manufacture, market dominance, and ability to simply relabel and market (bio)generic versions of their own products. Innovators will be more able and may decide to undercut the price of follow-on proteins/biosimilars, if only to maintain their market share. Most will already have a replacement product or portfolio of products for the same indication available by the time follow-on/biosimilar products enter the picture, so they will have little to lose by competing against new entrants on the basis of price.

Many follow-on proteins are likely to be manufactured using novel expression systems. This is the dominant presumption, and many developers of such products have already adopted newer high-tech systems. However, it remains to be seen how many of their products will actually be approved under follow-on protein/biosimilar regulatory mechanisms. The manufacturing process still largely controls and defines biotech products (the process = product paradigm). So those using a significantly different manufacturing process risk making a product that will be considered by regulators to be inherently dissimilar to the innovator product — such that comparative and abbreviated testing and applications may not be allowed.

In some countries, biosimilar approvals may even be restricted to only reverse-engineered copies using the same old manufacturing technologies as their (likely >20-year-old) reference products. However, there are few precedents or guidelines for how or in what aspects they must be similar to receive approval. For example, recently issued draft Canadian guidelines for “subsequent entry biologics” (SEBs) require the use of “analogous” manufacturing technologies: that is, similar or the same expression systems and manufacturing processes (4). It would appear that the SEB version of recombinant erythropoietin/EPO (Epoetin and Procrit, both containing

EPO from Amgen) would have to be manufactured by culturing CHO cells in large numbers of roller bottles. European Union biosimilar regulations do not explicitly require process-based similarity. In the United States, the FDA has yet to issue guidelines that were due years ago for the simpler follow-on proteins it regulates as drugs, and Congress has yet to enact a law enabling abbreviated approvals of biologics — with full FDA implementation likely requiring years after that happens.

Will the hegemony of the same, old expression systems continue? Probably not for long. Although economics — and often improved product quality — may favor newer technologies, the biopharmaceutical industry appears content with the established technologies that have served it so well. Companies will continue to reject new technologies to avoid associated regulatory delays and standing out from the pack. Follow-on products will be a major factor driving the industry's adoption of new expression systems — by all types of companies (especially biogenerics makers) for their cost savings and by innovators as a defense against further biosimilar products. No matter how regulatory agencies resolve (bio)similarity issues, the use of novel expression systems will increase. If products are required to be similar in their manufacture, that will further induce innovators to adopt (license) new technologies to confer unique properties to their products, thus gaining inherent defenses against copying or close similarity. If, as I expect, regulatory agencies ultimately will be more concerned with comparing products rather than methods of manufacture, then follow-on/biosimilar companies will be the main pioneers and first to adopt many of the new, improved, and as yet underused (at large scales) expression systems.

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