Expression systems

Editorial overview

Randal J Kaufman

Howard Hughes Medical Institute, Ann Arbor, USA

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The technological achievements of modern molecular biology are providing ever-increasing insight into the regulation of protein expression and the structure and function of proteins. The production of recombinant proteins has had a major impact in biology and medicine. The reviews published in this year's issue of *Current Opinion in Biotechnology* summarize significant breakthroughs in the ability to engineer the host organism to optimize production of specific polypeptides. These technological advances are being applied to an ever-increasing number of important biological questions and are providing improved methods to diagnose and treat disease.

Microbial systems are most commonly used for the production of foreign proteins. This issue contains two reviews on the secretion of heterologous proteins in microbial systems. Expression of proteins secreted into the periplasmic space or into the conditioned medium of *Escherichia coli* can be achieved at a high level to yield properly folded and functional proteins. Blight, Chervaux and Holland (pp 468–474) review approaches for optimizing protein secretion in *E. coli*. The advantages and disadvantages of directing protein expression into the periplasmic space or into the external medium are discussed. With a better understanding of the mechanism of SecAY-dependent protein export into the periplasmic space and a greater appreciation of the role of protein folding in protein export, it will be possible to manipulate *E. coli* genetically to improve the yield of proteins exported into the periplasmic space. Recently, *E. coli* has been engineered to utilize targeting and secretion signals derived from pathogenic strains with little loss in efficiency. Blight *et al.* discuss the use and efficiency of different systems to direct translocation across the inner and outer *E. coli* membrane. Future developments are likely to manipulate cellular machinery to improve protein folding and levels of specific membrane translocators. Brawner (pp 475–481) reviews recent advances in heterologous gene expression in *Streptomyces*, a group of Gram-positive bacteria frequently used in fermentation to produce antibiotics. Analysis of soluble CD4 expression in *Streptomyces lividans* has indicated that transcription initiation, signal peptide cleavage, and protein degradation are the key factors to achieve high-level expression. Brawner reviews advances in our understanding concerning each of these processes, which can now be utilized to engineer *Streptomyces* for high-level expression of heterologous genes.

The next two reviews summarize approaches to dissect protein–protein interactions. Mendelsohn and Brent (pp 482–486) review the yeast two-hybrid system, which can be used to isolate genes encoding interacting proteins and to characterize protein–protein interactions. The advantages and disadvantages of the different systems currently available are discussed. This rapidly evolving technology provides a sensitive method to measure protein interactions. Schatz (pp 487–494) describes recent advances in the technology to construct and screen peptide libraries using biological systems, comparing them with chemically synthesized combinatorial libraries. New receptor ligands and substrates for peptide-modifying enzymes have been isolated using these approaches and will facilitate the development of diagnostics and treatments for human disease.

The efficiency of nucleic acid incorporation into the cell is a crucial factor in determining the utility of a particular method for introducing genetic material into cells. This issue contains several reviews on the different vehicles employed for introducing genetic material into mammalian cells to obtain protein expression. Liljeström (pp 495–500) describes the advantages and disadvantages of alphavirus RNA expression vectors based on Semliki Forest virus and Sindbis virus. The ability to transfect RNA into the cytoplasm and to obtain efficient replication and translation has provided a versatile tool for heterologous gene expression. Recent developments and successes are described. The development of a conditional lethal helper system has made the use of these

Abbreviation

ER—endoplasmic reticulum.

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vectors more versatile and amenable to many investigators. Ulaeto and Hruby (pp 501–504) discuss the use of recombinant vaccinia viruses as vaccines. The smallpox vaccination program demonstrated the ability of vaccinia virus to elicit long-lasting immunity. Vaccinia has been engineered to deliver specific antigens to elicit immune responses and protective immunity in animals. Even so, widespread use of recombinant vaccinia vaccines will be limited until a better understanding of the immune response to vaccinia infection is obtained. Over the past year, it was demonstrated that immunity induced by a recombinant vaccinia virus can be boosted by secondary immunization with purified protein. Vaccinia virus can both induce immunity to pathogens that have very different life cycles and/or routes of infection and be used to define immunodominant antigens. Presently, studies are elucidating the interaction of the virus with the immune system. The potential of vaccinia virus as a vaccine will be realized only after we have achieved a greater understanding of how the virus interacts with the immune system. Montgomery *et al.* (pp 505–510) describe both the advantages and successful application of injection of non-replicating DNA expression vectors into cells *in vivo*. This approach provides a new vaccine technology to generate immune responses in animal models, with the ultimate goal of developing vaccines for humans, and it has also been applied as an antitumor therapy. Different methods are described that utilize either naked DNA or complexed DNA, which enter the cell by different mechanisms.

Frequently, it is desirable to regulate the expression of a particular gene product. Two approaches described in this issue have proven successful. In the first approach, reviewed by Picard (pp 511–515), chimeric proteins are constructed in which the activity of the protein can be activated by addition of an inducer. For example, one novel approach recently described is the induction of dimerization by fusion to the FK506-binding protein and addition of a dimeric derivative of FK506. Alternatively, numerous examples have now been found where the function of proteins fused to a steroid hormone binding domain can be activated by addition of the appropriate steroid. The second approach, which uses an inducible expression system, is described by Gossen *et al.* (pp 516–520). To date, the best success for high-level induction and expression has been derived using bacterial regulatory elements that are engineered into mammalian cells. Gossen *et al.* describe the advantages and disadvantages of the lactose and tetracycline repressor/operator systems. These repressors have been modified by fusion to the herpes simplex virus transcriptional transactivator domain of VP16. These systems have also been adapted to obtain inducible expression in transgenic animals.

Insight into gene function has been greatly facilitated by the ability to genetically modify the murine genome in specifically designed ways. The use of specific DNA recombination systems from bacteriophage lambda (Cre/*loxP*) or *Saccharomyces cerevisiase* (FLP/FRT) has dramatically enhanced our power to manipulate large DNA molecules *in vivo* and *in vitro*. It is possible to regulate specific recombination events either to turn genes 'on' or 'off' or to generate chromosomal deletions/inversions. Sauer (pp 521–527) has pioneered this technology and reviews recent developments and applications. It is now possible to create new alleles to study diverse biological problems. Recent advances in the ability to engineer the germline of mice are discussed by Ramírez-Solis and Bradley (pp 528–533). The ability to perform 'hit and run' or 'double replacement', the use of specific recombinases to generate tissue-specific or temporal-specific genetic alterations, and the ability to transfer large pieces of DNA (up to 600 kb) into the germline all represent significant advances.

The level of protein synthesis and secretion from a host cell is dependent upon the rate of polypeptide chain initiation and elongation as well as the rate of protein folding and transport through the secretory apparatus. This issue contains several reviews concerning the regulation of polypeptide chain synthesis, the rate of polypeptide folding, and the cellular response to unfolded protein within the secretory pathway. In the final review in this issue (pp 550–557), I describe the factors that regulate translation initiation in eukaryotic cells. Recently, it has become apparent that the control of polypeptide chain initiation is coupled to cell growth. In fact, several genes have now been identified that control translation initiation and that act either as proto-oncogenes or tumor-suppressor genes. It is important to understand how proteins fold *in vivo* and the role that protein chaperones and foldases play. Gilbert (pp 534–539) reviews the role of protein chaperones in protein folding *in vitro* and *in vivo*. Individual proteins may require a specific sequence of defined interaction with multiple chaperones *in vivo*. Although it may be possible to employ effective methods to improve protein folding *in vivo*, the approaches rely largely on trial and error. As protein expression levels increase, a greater amount of protein aggregation occurs in the endoplasmic reticulum (ER). The presence of these unfolded proteins induces a cellular response to induce a set of genes that encode protein chaperones. Alterations in protein chaperone expression can affect protein aggregation and influence rates of secretion. McMillan *et al.* (pp 540–545) summarize present knowledge concerning the signal-transduction pathway in response to unfolded protein in the ER that results in the induction of transcription of chaperone-encoding genes in the nucleus.

The majority of therapeutically relevant proteins derived from recombinant DNA technology contain significant amounts of carbohydrate. Oligosaccharide structures can dramatically affect many properties of proteins. In general, polypeptides contain a variety of oligosaccharide structures that can alter protein functions, such as plasma half-life, receptor affinity, or immunogenicity. Recently,

it has become appreciated that cell-culture conditions can significantly affect oligosaccharide structure of recombinant proteins in many different ways. In their review, Andersen and Goochee (pp 546–549) summarize what is presently known about how different cell-culture conditions can influence both asparagine- and serine/threonine-linked glycosylation. An understanding of the cell-culture variables that affect glycosylation will enable the carbohydrate structure/content of a protein to be optimized for biological activity and improve consistency in the production of recombinant proteins.

Since the original conception of this issue on expression systems in 1990, major improvements have been made in our ability to engineer host cells for the production of specific proteins. Technological developments have led the way to an explosion of information on the mechanisms that control mRNA production, protein synthesis and folding, and secretion. As a consequence, the way is opening for further developments and applications of expression technologies. Thus, in the future, it will be possible to specifically design the host cell to optimize protein production. In addition, novel approaches to obtain more efficient transfer of genetic material into host cells will certainly have impact on human gene therapy. Strategies to obtain tissue-specific and inducible expression in transgenic animal models will have tremendous impact on our understanding of cell biology and development. More importantly, these technological improvements are, even now, being translated into methods to study protein–protein interactions toward the development of specific approaches to intervene in human disease. It will be interesting to see what unexpected developments the next five years will hold.

RJ Kaufman, Howard Hughes Medical Institute, MSRBII Room 4570, 1150 West Medical Center Drive, University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA.