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Efficient expression of secreted proteases via recombinant BacMam virus

Martin J. Scott [a,*], Sundip S. Modha [a], Andrew D. Rhodes [a], Neil M. Broadway [a], Phil I. Hardwicke [a], Huizhen J. Zhao [b], Karen M. Kennedy-Wilson [b], Sharon M. Sweitzer [b], Steve L. Martin [a]

[a] *GlaxoSmithKline, Discovery Research Biology, Department of Gene Expression and Protein Biochemistry, Gunnels Wood Road, Stevenage SG1 2NY, UK*
[b] *Swedeland Road, King of Prussia, Pennsylvania 19406, USA*

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Abstract

Baculovirus vectors engineered to contain mammalian cell-active promoter elements have been described as an efficient method for transduction of a broad spectrum of human cell lines at high frequency. In the first large-scale comparative study of secreted protein production using these viral vectors, we have evaluated production of 16 recombinant enzymes—specifically, we exploited these viral vectors, termed 'BacMam' viruses, to drive expression of a panel of proteases selected from all four major mechanistic classes, including secreted, lysosomal, endosomal, and type I transmembrane proteins. To allow a generic purification strategy, coding sequences were truncated to remove transmembrane and/or subcellular retention signals before introduction, in parallel, into a C-terminally Fc-tagged BacMam transfer vector. BacMam viruses were generated and subsequently evaluated for expression of Fc-tagged protein in virus-transduced HEK-F cells. The common Fc-tag enabled single-step affinity purification of secreted recombinant protein from the culture medium. Yields were excellent, with 14 of 16 genes expressed producing 10–30 mg or more purified protein per litre of culture using standardised transduction conditions. At this level, reagent demands for a typical protease high-throughput screen (HTS) could be met from expression cultures as small as 0.1–0.5 L. Our results indicate this expression system offers a highly efficient and scaleable method for production of enzymatically-active secreted proteases and may therefore represent a novel method of protein production for other secreted enzymes with significant advantages over the diverse approaches in current use.

Keywords: BacMam; Baculovirus; Mammalian cell expression; Recombinant viral transduction; Proteolytic enzyme; Protease; Recombinant protein expression

The observation that recombinant baculovirus vectors derived from the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) are able to transduce mammalian cell lines of hepatic origin [1] has prompted researchers to investigate the use of such viral agents to express recombinant human proteins in a wider array of cell types [2–4]. These vectors, termed BacMam viruses, contain a strong mammalian cell-active promoter, typically the immediate early promoter of human cytomegalovirus (CMV) [1] or the long terminal repeat promoter of Rous sarcoma virus (RSV) [5], upstream of the target gene. Under this control, target proteins are expressed to high levels in a wide range of human and other mammalian cell lines in common use, including HEK293, HeLa, HuH-7, HepG2, COS-7, CHO, and BHK [3,6; reviewed in Refs. 7,8]. The BacMam system has been recently exploited to supply a variety of proteins for cell-based assay development, with notable successes for expression of functional G-protein coupled receptors [9,10], nuclear receptors [11–13], and ion channels [14], each representing major classes of drug targets for the pharmaceutical industry.

* Corresponding author. Fax: +44 1438 764865.
E-mail address: martin.j.scott@gsk.com (M.J. Scott).

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Table 1
Key features of the BacMam expression system and potential advantages for protein production

Feature of BacMam system	Potential advantage for protein production
Recombinant viruses are able to transduce a broad range of mammalian cell lines	(i) Ensures authentic post-translational processing and hence functional integrity of the expressed product (ii) Enhances flexibility of system by allowing parallel expression in a range of cell types (iii) Permits investigations of protein expression in cell types physiologically relevant to the expressed product
Mammalian cells do not support replication of recombinant BacMam viruses	(i) Avoids lytic infection and release of cellular and baculovirus-derived proteases that are potentially deleterious to the target protein (ii) Recombinant viruses pose little or no potential risk to the laboratory user
BacMam viruses are non-infectious for natural arthropod hosts due to deletion of the polyhedrin gene	Recombinant viruses pose no potential risk to the environment by infection of arthropod hosts
Transient expression system	(i) Scale up of expression not limited by cell growth as for stable mammalian expression systems (ii) Allows expression of toxic proteins
BacMam vectors represent a subtle modification of standard baculovirus vectors commonplace in gene expression laboratories	Expertise gained in the use of baculoviruses may be readily employed for investigating the BacMam system as a tool for production of purified recombinant proteins
Key advantages of standard baculovirus vectors are retained	Ease of manipulation of the viral vector allowing incorporation of large transgenes/multi-subunit complexes; highly efficient and scaleable secreted protein expression
Inclusion of antibiotic resistance markers on plasmid transfer vector	Stable mammalian cell lines can be derived from the same transfer vector

While the BacMam system offers demonstrable advantages for cell-based assay development, the system has only been cursorily examined for the production and purification of recombinant proteins [15,16]. Traditional approaches to expression of such proteins normally include simple prokaryotic systems (*E. coli*)[1], recombinant baculovirus vectors for expression in invertebrate cell lines, and stable-transfected mammalian cell lines. These approaches currently represent the main workhorses for protein production used in both academic and industrial laboratories (reviewed in Refs. [17,18]). However, the BacMam system may offer several key advantages over these current strategies for protein production, as highlighted in Table 1.

The availability of an expression system that will aid the successful production of secreted enzymes could be of critical importance for basic research and also for prosecuting targets within the pharmaceutical industry. Undeniably, the ability to express and purify these enzymes is fundamental to the drug discovery process in order to learn more about each enzyme's structure and function, with the ultimate aim of designing potent inhibitory molecules that could potentially halt the pathogenesis of a disease associated with an aberrant enzymatic activity. Lack of progress at this stage is largely due to poor expression levels or insolubility and subsequent lack of a coherent refolding strategy. Alternatively, the expressed product may lack quantifiable enzymatic activity, likely due to misfolding of the protein or insufficient post-translational modifications. A robust system for high level soluble expression of active, properly processed enzyme is therefore highly desirable.

Within the enzymes arena, a considerable amount of effort is expended on the expression and analysis of proteolytic enzymes due to their pivotal role in a multitude of biological processes. Proteases play significant roles in virtually all aspects of cellular biology and every function of multicellular organisms. Accordingly, such enzymes represent approximately 2% of all gene products coded in the human genome [19]. Proteases are indispensable in such fundamental cellular processes as chromosomal separation during mitosis, cell-cycle regulation, apoptosis, protein degradation, pro-enzyme maturation, and signal transduction. At the whole organism level, these enzymes direct diverse processes such as digestion, antigen presentation, blood coagulation, and reproduction. Deregulation of the normal function of proteolytic enzymes leads to, or accelerates progression of, an array of human pathologies including arthritis, cancer/metastasis, neurodegenerative disorders, and cardiovascular diseases [19–21]. Proteases also play a fundamental role in the pathogenesis of numerous infectious diseases. For example, infectious agents such as hepatitis C virus (HCV) and human immunodeficiency virus type 1 (HIV-1) utilise virus-derived proteases (HCV NS3/4A protease and HIV-1 protease, respectively), as well as those within the cellular machinery, in order to subvert host cell defences [22,23].

The current study was designed to fully evaluate the BacMam system as an expression tool for the production of a wide range of proteases for the purpose of drug discovery. In the post-genomic era, this novel protein expression system could be of significant importance to researchers requiring rapid availability of large panels of functionally-active purified enzymes with consistently high expression levels in order to further enable studies into the structure and function of such proteins.

[1] *Abbreviations used: E. coli, Escherichia coli*; HEK, human embryonic kidney; CHO, Chinese hamster ovary; *Sf*, *Spodoptera frugiperda*; m.o.i., multiplicity of infection; pfu, plaque-forming units; HDAC, histone deacetylase; PAGE, polyacrylamide gel electrophoresis; CMV, cytomegalovirus; HRP, horseradish peroxidase.