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Implementation of BacMam virus gene delivery technology in a drug discovery setting

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Membrane protein targets constitute a key segment of drug discovery portfolios and significant effort has gone into increasing the speed and efficiency of pursuing these targets. However, issues still exist in routine gene expression and stable cell-based assay development for membrane proteins, which are often multimeric or toxic to host cells. To enhance cell-based assay capabilities, modified baculovirus (BacMam virus) gene delivery technology has been successfully applied to the transient expression of target proteins in mammalian cells. Here, we review the development, full implementation and benefits of this platform-based gene expression technology in support of SAR and HTS assays across GlaxoSmithKline.

Introduction

Because of their precedence as biologically relevant and chemically tractable targets, membrane proteins currently constitute a large proportion of pharmaceutical company drug discovery portfolios. The drive to exploit fully these target families [G-protein coupled receptors (GPCRs), ion channels and transporters] has led to the development of many functional cell-based or membrane-binding assay formats [1–3]. However, despite years of experience, problems can arise with recombinant expression technologies that rely on stable cell lines to generate reagents for screening. Many target proteins are toxic to host cells, making cell lines either difficult to derive or unstable during continued culture. Thus, cell-line derivation can be labour intensive and take several months to complete. In addition, many novel targets of high therapeutic interest have multiple subunits, and co-expression at the optimal stoichiometry to generate an assay representative of native target function can be challenging.

Transient transfection and mammalian viral vector-based gene delivery offer alternative approaches for the development of recombinant cell-based assays. However, transient transfection techniques have several drawbacks, especially when one considers their application in the high-volume high-throughput screening (HTS) environment. These limitations include the cost of transfection reagents and purified plasmid DNA, the potential for cytotoxic effects observed with many transfection reagents and the increased handling and procedural steps required for conducting efficient reproducible large-scale transfections. In the case of large-scale mammalian viral vector transductions, virus production and biosafety concerns become considerable issues.

Baculovirus expression vectors, originally developed during the mid-1980s, [4,5] have been used extensively for the production of heterologous proteins in insect cells [6,7]. The recent development of modified baculoviruses carrying mammalian cell-active regulatory elements (BacMam viruses) has expanded the use of this system for gene delivery and protein expression in mammalian cells. Initial reports describing BacMam-mediated gene transfer into cells of hepatic origin [8,9] were followed by studies demonstrating the successful transduction of a variety of cell types (reviewed in Ref. [7]; [10,11,12–14]). The inherent properties of BacMam vectors for gene delivery into mammalian cells provide many advantages over commonly used transfection techniques and other viral vector systems based on mammalian viruses (Box 1). Early studies with BacMam

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BOX 1

Properties of BacMam virus gene delivery

General properties

- Viruses easily generated and production rapidly scaled-up
- Transient expression, facilitates expression of toxic proteins
- Large insert capacity
- Broad host cell range with transduction of many primary cell types
- Multiple virus transductions, simultaneous delivery of multiple genes
- Little to no microscopically observable cytopathic effect
- Expression level can be controlled by virus dose or chemical addition
- Cell transduction by simple liquid addition, amenable to automated methods
- Viruses stable stored at 4°C in the dark
- Simplified cell culture requirements

Good biosafety profile

- Baculoviruses are Risk Group 1 (RG1) agents
- Used widely for over 20 years for insect cell protein expression
- Produced in insect cells, incapable of replicating in mammalian cells
- Not known to cause disease in healthy human adults
- Inactivated by human complement
- Viruses used in the laboratory cannot replicate in insects, no environmental threat

virus gene delivery [7–14] indicated that this transient expression technology could address many of the issues often associated with stable cell-line development and, thus, provide an efficient platform for cell-based drug discovery assays (Figure 1).

The implementation of BacMam technology on a large scale in drug discovery projects and automated screening facilities was unprecedented. Therefore, a series of pilot studies was undertaken within GlaxoSmithKline to demonstrate the widespread utility in expressing recombinant proteins in key target families with authentic function and pharmacology, and the technical benefits that could be gained in compound screening and profiling assays. In addition, processes have been developed to address the logistical issues encountered in the widespread application of this technology, including virus generation, production, quantitation, validation, inventory and shipping. Here, we describe the results of these studies and the successful implementation of this technology across GlaxoSmithKline R&D, which has enabled significant efficiency gains and the screening of previously intractable targets.

BacMam in the development of pharmacologically relevant assays for GPCR drug discovery

GPCRs

GPCRs represent one of the greatest opportunities for drug discovery because of their precedence as drug targets, the size of the superfamily and their role in diverse physiological processes [15–17]. A range of functional cell-based and ligand-binding assays are used to progress the diversity of targets in this family [1]. Therefore, significant effort was made to first demonstrate the broad utility of the technology across the GPCR superfamily and major assay formats.

Although stable cell-based compound screening approaches have been successful with GPCRs, development times are often lengthy and the isolation of suitable cell lines can be problematic. Typical challenges include: (i) frequent receptor toxicity to host cells, resulting in instability of expression and assay performance as cells are propagated or scaled up; (ii) the need to co-express accessory proteins to enable functional assays (e.g. G proteins, chimeric G proteins, or reporter genes); (iii) interference from

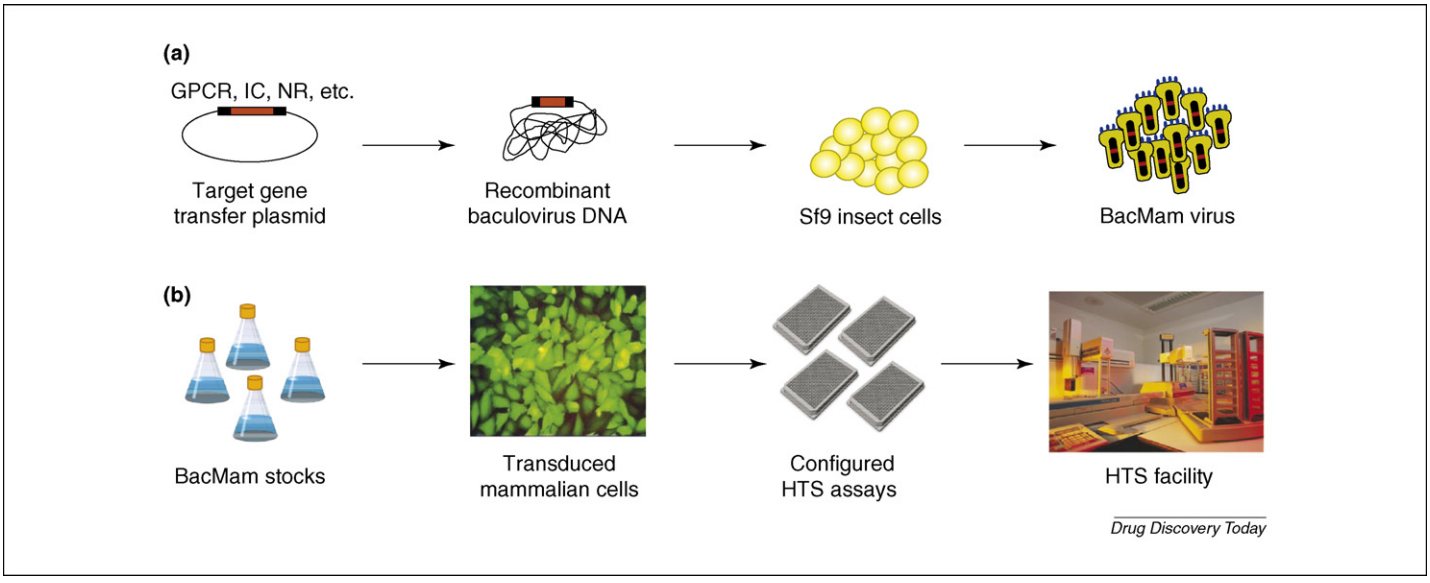

FIGURE 1

Production and application of BacMam viruses for assay development and HTS. **(a)** Virus production. Target gene sequences cloned into a transfer plasmid containing a mammalian cell-active expression cassette are transferred to baculovirus DNA via recombination. The viral DNA is transfected into insect cells where virus production occurs. The insect cell culture medium containing recombinant virus is clarified by low speed centrifugation and the virus titer is determined. From the start of virus generation to assay development takes between three and four weeks. **(b)** Assay development and HTS. The stock virus is used to transduce mammalian cells and expression of the recombinant protein(s) is usually validated 24–48 h later. Assays are routinely conducted in microtiter plate format using automated platforms. The system is highly versatile: it can be used for multicomponent target proteins, expression levels can be titrated, multiple cell line hosts can be used and it is compatible with automated assay formats.

endogenous receptors in host cells that can confound data interpretation; and (iv) the need to generate systems with different degrees of receptor coupling to measure partial agonism of ligands, or constitutive receptor activation. BacMam viruses have been generated to a variety of GPCRs from families A, B, and C and have been used for assays based on radioligand binding, [^{35}S]GTP-γS binding, cAMP determination and reporter assays, as well as fluorometric imaging plate reader (FLIPR)-based calcium mobilization assays. Our conclusion is that many of the above challenges can be addressed by switching from stable cell lines to BacMam-based cellular assays, in which the pharmacology of the receptors is either unaltered or expanded owing to the ability to vary receptor levels. The following examples illustrate the flexibility provided by this unique gene delivery system.

Development of GPCR assays in U-2 OS null background receptor host cells

Mammalian cell lines express a range of endogenous GPCRs, for example most cell lines express endogenous UTP, lysophosatidic acid (LPA), and muscarinic acetylcholine receptors [18]. The versatility of the BacMam system enables the rapid evaluation of alternative host cells and assay conditions to identify systems in which these endogenous receptors do not present a problem. It has been shown [19,20] that the human U-2 OS osteosarcoma cell line is highly receptive to BacMam transduction. These cells have been used successfully for GPCR-based assays [21,22] and have been shown to lack endogenous receptors for LPA, UTP and muscarine; thus, they have proved useful in the expression of many purinergic, LPA and muscarinic acetylcholine receptors for calcium mobilization assays [23]. Importantly, we have established that BacMam-transduced U-2 OS cells are compatible with HTS approaches. For example, screening campaigns have been successfully completed for the prostanoid EP_3 and GPR103 (RF-amide peptide) receptors.

Expression of accessory cofactors

BacMam viruses can be used to deliver multiple genes simultaneously, thus, the system is well suited for co-expressing GPCRs and accessory proteins. For example, the $G_{\alpha i}$ coupled CXCR3 chemokine receptor was successfully co-expressed along with a chimeric G protein to establish a FLIPR-based calcium mobilization assay [21]. More recently, this co-transduction approach has been used for numerous GPCRs. Magga *et al.* [22] have taken advantage of the versatility of this expression system to co-express the orexin receptor type I along with a panel of chimeric G proteins in HEK-293 cells to characterize fully receptor signaling and coupling pathways. There is increasing evidence that, in their native state, GPCRs exist as dimers or higher-order oligomers [24]. BacMam-based co-expression could provide a valuable tool for developing assays to study the potential role of receptor heterodimerization and cellular trafficking, or simply for multiplexing drug targets for screening.

Pharmacological equivalence of recombinant neurokinin 3 receptors with stable cell lines or BacMam gene delivery

Neurokinin 3 (NK3) is an important target for airway inflammation. In early studies, similar results were obtained using tachykinin agonists and nonpeptide antagonists, whether assayed in HEK- or CHO-based stable cell lines, or using BacMam transient gene delivery [21]. In addition, in these studies a set of NK3 antagonists were profiled in ligand-binding assays using membranes prepared either from BacMam-NK3-transduced HEK-293 cells or from a CHO cell line stable expressing the NK3 receptor. There was no apparent difference in the antagonist rank order IC_{50} values obtained with the two different membrane preparations.

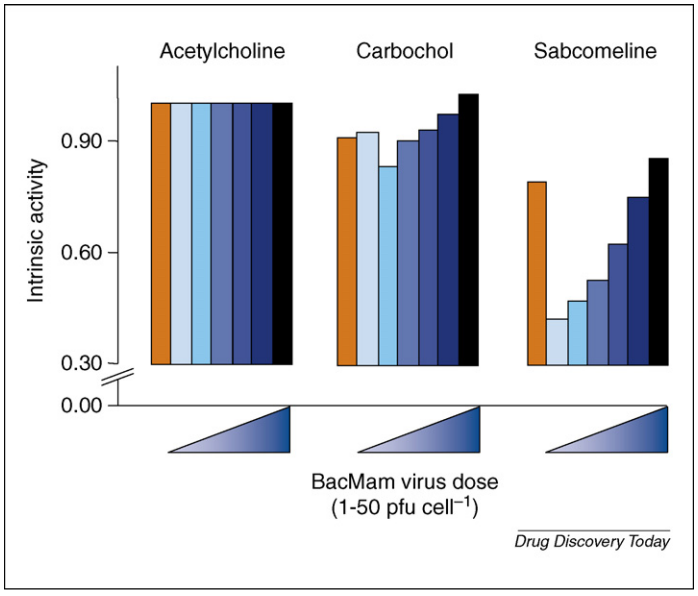

FIGURE 2

FLIPR-based functional characterization of CHO M1 stable cell line or U-2 OS cells transduced with increasing concentrations of M1 BacMam virus. U-2 OS cells were transduced with increasing amounts of BacMam virus expressing M1 receptor. Virus dose was ~1–50 virus particles per cell, as shown by the scales beneath each graph. Cells were challenged with a single concentration of each agonist, carbachol, sabcomeline, acetylcholine and data are represented as intrinsic activity as compared to maximal response obtained with acetylcholine challenge. Orange bar represents stable CHO cells; blue bars represent transduced U-2 OS cells.

Titration of BacMam viral dose to modulate receptor coupling and identify partial agonists

One of the key advantages of BacMam over stable cell lines is the ability to control expression level of the target gene by varying the virus dose applied to host cells. This has been used to facilitate development of assays supporting characterization of GPCR agonists, for example muscarinic M1 receptor partial agonists developed as cognitive enhancers in the treatment of Alzheimer's disease. The different coupling efficiency of the receptors in brain and gastrointestinal (GI) tract means that partial agonism could provide tissue selectivity and avoid GI side effects. In native tissue and *in vivo* assays, the selective M1 compound sabcomeline has been shown to be a partial agonist, whereas in a stable cell line-based FLIPR assay, it functions as a full agonist. By simply varying the dose of a M1 BacMam virus, the maximal response following ligand addition can be titrated to develop an assay for identification and characterization of M1 partial agonists (Figure 2). Similarly, Behm *et al.* [25] showed that, by varying the dose of Urotensin II receptor BacMam virus to HEK cells, they could control accurately the expression level of receptor as measured in radioligand-binding assays. This enabled full characterization of

a panel of putative receptor antagonists and demonstrated that these compounds are low-efficacy partial agonists.

BacMam in the development of pharmacologically relevant assays for other target classes

Ion channels

Ion channels are important drug targets that have presented significant challenges, both in terms of development of medium and high-throughput assays for their function and generation of physiologically relevant recombinant systems. High-level constitutive expression of ion channels frequently has deleterious effects on cells. Many ion channels function as multi-subunit complexes that require specific stoichiometry to achieve pharmacology and function equivalent to the native channel. Furthermore, these complexes could have alternative subunits that serve as determinants of functional or tissue selectivity, requiring testing of several variants for full pharmacological characterization [26]. Expression of ion channels also requires a certain amount of flexibility, owing to differences in channel gating and ion permeation, which require several different formats that indirectly measure ion channel function in high-throughput platforms, as well as the direct measurement of ion flow with a variety of electrophysiology platforms [2].

Many of the benefits of BacMam in ion channel screening assays are illustrated by work done with the K_{ATP} channel. K_{ATP} is a hetero-oligomer comprising a channel subunit (K_{IR}6.1 or 6.2) and a regulatory subunit (SUR1, 2A, or 2B) [26]. Different tissue isoforms of the channel comprise different combinations of K_{IR} and SUR proteins [27]. In our experience, stable cell lines expressing K_{ATP} could be generated, but were not suitable for plate-based assays mainly owing to instability of expression and deleterious effects of channel expression on the cells (unpublished). Delivery of the subunits (K_{IR}6.2/SUR1) by BacMam enabled configuration of a membrane potential assay that was suitable for compound profiling [28]. Switching viruses to express the different forms of the SUR regulatory subunit enables easy configuration of selectivity assays in addition to screening a primary target. Titration of virus dose also enabled control over the timing of a measurable response by the cells, giving flexibility in assay design. Additionally, transduced cells proved to be suitable reagents for binding and electrophysiology assays.

Control of cellular membrane potential to regulate the activity of voltage-gated ion channels can be a challenge in plate-based assays. Addition of other channels to modulate membrane potential is one approach to overcoming this difficulty. In development of a stable cell line expressing the R-type calcium channel, the resting potential of the cells was too positive to enable gating of the channel. BacMam delivery of the constitutively-active two-pore domain K^+ channel TREK-1 caused a hyperpolarization of the cells that was sufficient to activate the calcium channel. BacMam proved useful for this application, owing to the speed and ease of the system, as well as the ability to titrate function of the recombinant protein with virus dose [29].

The NMDA receptor NR1/NR2b is particularly illustrative of a HTS that was enabled by BacMam technology. This target presents another example of difficulty in deriving robust stable cell lines owing to cytotoxicity of overexpression of the recombinant channel. Transduction of cells with NR1 and NR2b BacMam viruses induces channel expression and enables its function to be assayed before the condition of the cells deteriorates. A FLIPR-based calcium uptake assay was configured with the transduced cells, which resulted in a successful HTS for this target [29]. Several successful HTS campaigns have been completed using BacMam technology to identify ion channel agonists and antagonists. For example, in addition to NR1/NR2b, a successful screening campaign was completed with the calcium channel, TRPV4 (unpublished).

Transporters

Transporters are a diverse family of membrane proteins involved in nutrient transport, regulation of metabolite concentrations, and transport of toxins and drugs [30]. Their role in drug adsorption, distribution, metabolism and elimination has made them of interest not only as therapeutic targets [31–33], but also as determinants of drug developability, because the profile of compounds in relevant transporter assays could be predictive of pharmacokinetic or safety issues [34]. We have found the BacMam system to be valuable in overcoming problems of target toxicity and cDNA instability that are common with this class of proteins [30].

Screening of the γ-aminobutyric acid (GABA) transporter GAT-1 was hampered by difficulties with production of robust stable cell lines, probably owing to deleterious effects of expression of the transporter on the cells. However, a BacMam virus engineered to deliver the GAT-1 gene was used successfully to configure a radioactive GABA uptake assay in HEK cells [32]. This assay yields IC_{50} values for standard inhibitors that are within the range of published values. The liver organic anion uptake transporter OATP1B1 has also been successfully delivered to HEK cells [35], and functional activity demonstrated by uptake of a fluorescent substrate into the transduced cells. Additionally the breast cancer resistance gene (BCRP), a transporter implicated in excretion of antitumor drugs by cells, was functionally expressed in HEK cells both alone and in combination with OATP1B1.

Nuclear receptors

Nuclear receptors (NRs) are ligand-dependent regulators of gene expression. The family includes molecular targets for several important drugs, such as tamoxifen and rosiglitazone [36]. In addition, the pregnane X receptor (PXR) is gaining attention as an indicator of increased metabolism owing to its role in regulation of cytochrome P450 3A CYP3A [37], a CYP responsible for metabolism of 60% of clinically used drugs [38]. BacMam technology has been applied to this class of targets to yield a variety of rapid, reproducible and cost-effective assays for many members of the nuclear receptor family.

Screens for NRs are commonly configured as reporter assays involving full length receptor, in which the reporter gene is controlled by a specific NR-activated promoter. The use of BacMam to configure this type of assay was first demonstrated by Clay *et al.* [19] for estrogen receptors. A virus was constructed to deliver an expression cassette consisting of an estrogen response element/thymidine kinase promoter hybrid fused to a reporter gene; whereas separate viruses were used to deliver full length receptors. Co-transduction of cells with the reporter construct and one of the receptor-encoding BacMam viruses yields estradiol-inducible expression of the reporter gene product. With this as proof-of-principle, the concept was applied to other steroid hormone

receptors to configure multi-well assays that were capable of profiling compound sets [39]. The BacMam assays were robust, faster and less expensive than the transient transfection assays previously in use. Furthermore, the ability to transduce cells with multiple viruses simplifies the investigation of the interactions of NRs with coactivators and corepressors, (e.g. between the mineralocorticoid receptor and the peroxisomal proliferator activated receptor γ coactivator1) [40].

A similar reporter assay has been developed for PXR to identify compounds that induce CYP3A expression. Separate viruses are used to deliver PXR and an expression cassette containing a CYP3A4 promoter fused to a reporter gene [40]. The BacMam assay has the advantage that it enables the assessment to be carried out in several different cell lines with minimal optimization required.

A generic, family-wide reporter assay approach utilizing fusion proteins can be applied to assess ligand interactions with NRs. In this format, DNA encoding the NR ligand-binding domain (LBD) is fused to DNA encoding the DNA-binding portion of the yeast GAL4 transactivator. The reporter construct for any NR-LBD/GAL4 fusion protein consists of a minimal promoter fused with the upstream activating sequence recognized by the GAL4 domain. Thus, co-delivery of these two components to a cell yields ligand-dependent modulation of reporter gene expression [41]. We have configured assays using BacMam viruses for all of the nonsteroidal NRs to identify compounds that function as ligands, or as SAR assays for assessment of ligand derivatives. The BacMam system is particularly attractive for this family-wide approach as it enables rapid, easy assay configuration by switching out the different NR-LBD/GAL4 fusion protein viruses.

Viral targets

The study of certain viruses of pharmaceutical interest, such as hepatitis B (HBV) and C (HCV) viruses, is not readily amenable to classical virological methods because of a lack of efficient viral replication in cultured cells. Most studies aimed at studying HBV viral DNA replication rely upon transient transfection with HBV DNA or on cell lines containing integrated copies of the HBV genome, such as the HepG2.2.15 line [42]. Use of baculovirus-mediated gene delivery as an alternative was first reported by Delaney and Isom [43] who generated a baculovirus containing an HBV genome construct capable of launching a productive HBV infection in cultured cells. Transduction of HepG2 hepatoma cells with this virus led to production of HBV viral particles and high levels of intracellular replicative intermediates. This system for HBV delivery provides several significant advantages and has been used successfully to study effects of antiviral compounds on wild type HBV [44–46] and drug resistant mutant viruses [47–50].

Baculovirus has also been used to deliver the HCV genome to mammalian cells [51]. Although viral proteins were produced, viral replication was not detected. Baculoviruses using a tetracycline-regulated system have also been used to deliver HCV constructs to human hepatoma cells [52,53], leading to production of viral proteins and replication competent HCV transcripts. A recent study describes production of HCV-like particles by liver-derived cells transduced with a recombinant baculovirus carrying the HCV core to NS2 region regulated by a heterologous promoter [54]. Although none of these studies have demonstrated production of infectious HCV, the approach provides a useful complement to recently described cell culture systems employing the unique HCV genotype 2a replicon (JFH1) [55].

A different type of application has been described by Jenkinson *et al*. [56] who developed a novel assay to screen for inhibitors of HIV entry via CD4 and the CCR5 or CXCR4 co-receptors. Stable human osteosarcoma (HOS) cells expressing CD4, CCR5, CXCR4 and an integrated HIV LTR-luciferase reporter construct are mixed with HEK cells that have been transduced with BacMam viruses expressing the HIV proteins gp160, tat and rev. The HEK cells expressing gp160 can fuse with the HOS cells, resulting in transfer of tat into the HOS cells, transactivating the HIV LTR leading to luciferase expression. Inhibitors that block the cell–cell adhesion process are identified by a reduction in tat-mediated transactivation of the reporter gene cassette. This complex assay has been developed into a sufficiently robust screen to enable a full HTS campaign.

BacMam application: practical considerations

The successful realization of BacMam technology from laboratory-scale pilot studies to wide-scale implementation across a large international R&D organization required several change initiatives. These included: (i) establishing standardized processes for the reproducible supply of functionally validated BacMam virus preparations; (ii) gaining the confidence of pharmacologists, cell biologists, biochemists and chemists with regards to the validity and benefits of the technology; and (iii) effectively sharing data and lessons learned from increased use of the technology.

On the supply side, guidelines for virus generation, production and quantitation and functional validation were developed by a Best Practice group, whose membership included scientists with experience in baculovirus expression and scale-up. This was paramount to maintaining consistency between multiple virus batches, ranging from 1–10 liters, and produced at several research sites.

A variety of communication channels were used to disseminate information to scientists in virus production, assay development and HTS groups. The rationale and advantages of using BacMam in place of stable cell lines, basic aspects of baculovirus biology, virus production process, virus transduction and handling procedures together with biosafety considerations were communicated throughout the organization. In addition to conveying information about the BacMam system, it was also important for scientists involved in producing the viruses and validating the assays at laboratory scale to interact closely with scientists running HTS campaigns to make certain that the automated systems and procedures used in the screening facilities were compatible with running BacMam-based assays.

Benefits gained in drug discovery campaigns

The ease and speed with which BacMam viruses can be generated, coupled with facile approaches for assay development, can eliminate the requirement to maintain numerous individual stable cell lines, each with its own growth and media requirements. Instead, a limited set of host cell lines can be maintained in culture or as frozen stocks, then transduced before assay with BacMam, either alone or in combination with accessory subunits or reporter genes. This is illustrated in the example of a GlaxoSmithKline program focused on identification of antagonists of GPR41, a member of a

family of fatty acid-binding receptors implicated in energy homeostasis [57,58]. A primary human GPR41 HTS assay was supplemented with selectivity assays for human GPR40 and GPR43, as well as assays of mouse and rat orthologues of GPR41 and GPR43 for a total of seven assays. Instead of generating and maintaining seven stable cell lines, BacMams were rapidly generated for each receptor and a chimeric G protein. One day before assay, these viruses were used to transduce a single U-2 OS host cell line. This approach offers significant savings in time, tissue culture media and laboratory consumables as compared with supporting these drug screening activities with seven different stable cell lines.

Over 50 BacMam SAR assays and numerous orthologue assays have been run in GSK over several years (e.g. Refs [59,60]). In recent years, we have completed more than 25 HTS campaigns using BacMam gene delivery. The results of one such screen are illustrated in Figure 3. In this screen, we were attempting to identify agonists for the Toll-like receptor 7 (TLR7) [61]. Two BacMam viruses, one expressing TLR7 and one containing a luciferase reporter gene known to be activated by TLR7 agonists were transduced into U-2 OS cells. Twenty-four hours post-transduction the cells were harvested and plated into 384 well plates containing test compounds for 24 h before determination of luciferase activity. The assay was run at a throughput of 150 microtiter plates (384 well) per day for 22 screening days. During assay development we demonstrated that the pharmacology of the standard compound Resiquimod was as expected (Figure 3a). Standard plates were included on each screen day to monitor assay performance and the pharmacology of Resiquimod was $pEC_{50} = 5.81 \pm 0.13$ ($n = 8$), demonstrating the consistency of the screen (Figure 3b). The distribution of active compounds is shown in Figure 3c. Using an activity cut-off of 30% of the Resiquimod response, 0.32% of the molecules screened were identified as active and progressed for further analysis.

In those instances where comparable data were available, BacMam-based assays demonstrated equivalent or better performance than stable cell line-based assays. In addition, for several targets we have found it to be an enabling technology. For example, we succeeded in establishing BacMam-based assays for targets such as the NR1/NR2b ion channel [29] and the CXCR3 receptor (unpublished), for which we were unable to establish robust HTS assays using stable cell lines. BacMam technology has significantly reduced our stable cell-line development effort and provided a more flexible platform for cell-based assay development.

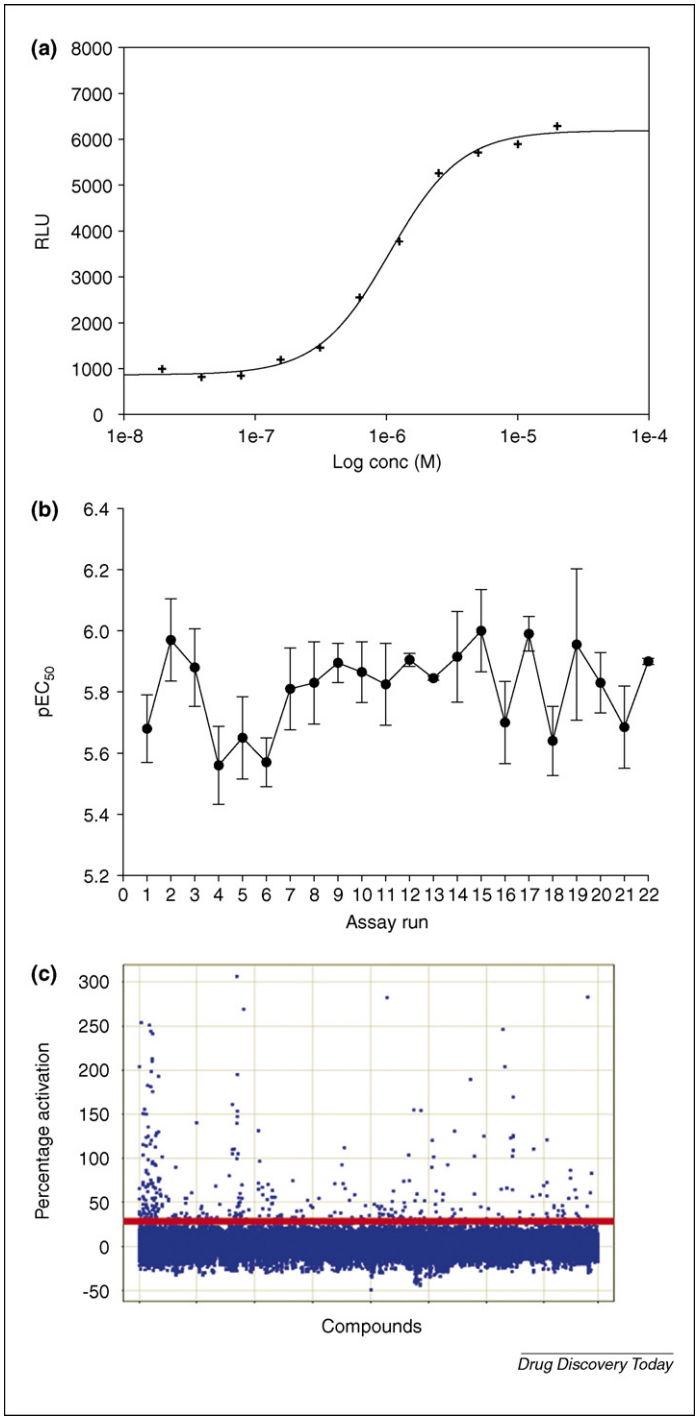

FIGURE 3

TLR7 HTS. **(a)** Dose response curve for standard agonist Resiquimod in U-2 OS cells transduced with BacMam viruses expressing TLR7 and luciferase reporter cassette. **(b)** Resiquimod pEC_{50} values determined for 22 independent assay runs of transduced U-2 OS cells. Error bars represent the standard deviation. **(c)** Distribution of active compounds in the HTS. Using an activity cut-off of 30% of the Resiquimod response, 0.32% of the molecules screened were identified as active and progressed for further analysis.

Future directions

BacMam gene delivery technology is rapidly evolving. Studies aimed at enhancing transduction efficiency and expression levels through viral or host cell engineering should improve expression levels and further reduce the amount of virus required to support screening campaigns and facilitate engineering multicomponent cell-based assays. The reported spectrum of cell types that can be transduced continues to expand and investigators are limited only by their imaginations in designing experiments designed to extend viral cellular host range. In addition to using BacMam for assay development and HTS applications it has proven useful for antibody screening [62] and, more recently, for mammalian cell protein production [63–65]. This could prove particularly valuable as an alternative protein production approach for obtaining reagents for assay development. Recent studies have also shown that BacMam viruses can be used for RNA interference studies [66,67]. We expect an increasing range of applications for BacMam-based recombinant gene expression as experience with the system continues to advance.

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