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Review

Use of baculovirus expression system for generation of virus-like particles: Successes and challenges

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ABSTRACT

The baculovirus expression system (BES) has been one of the versatile platforms for the production of recombinant proteins requiring multiple post-translational modifications, such as folding, oligomerization, phosphorylation, glycosylation, acylation, disulfide bond formation and proteolytic cleavage. Advances in recombinant DNA technology have facilitated application of the BES, and made it possible to express multiple proteins simultaneously in a single infection and to produce multimeric proteins sharing functional similarity with their natural analogs. Therefore, the BES has been used for the production of recombinant proteins and the construction of virus-like particles (VLPs), as well as for the development of subunit vaccines, including VLP-based vaccines. The VLP, which consists of one or more structural proteins but no viral genome, resembles the authentic virion but cannot replicate in cells. The high-quality recombinant protein expression and post-translational modifications obtained with the BES, along with its capacity to produce multiple proteins, imply that it is ideally suited to VLP production. In this article, we critically review the pros and cons of using the BES as a platform to produce both enveloped and non-enveloped VLPs.

Contents

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Introduction

Since 1983, when the baculovirus expression system (BES)[1] was first used to express human beta interferon in insect cells [1], the BES has become a versatile and robust eukaryotic expression system for foreign protein expression. Two prototype members of the genus *Alphabaculovirus* [2] are broadly utilized in the BES as vectors to produce heterologous proteins in insect cells or silkworm larvae: namely *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and to a lesser extent *Bombyx mori* nucleopolyhedrovirus (BmNPV). Either can express foreign genes under the control of highly expressed very late promoters, including polyhedrin (polh) and 10-kDa fibrous polypeptide (p10) promoters. The most commonly used lepidopteran insect cell lines are derived from *Spodoptera frugiperda* (*Sf*9 and *Sf*21) and *Trichoplusia ni* (*Tn*5, commercially known as High Five™), which grow optimally at 27 °C and do not require CO_2, making scale up of protein production feasible for most laboratories. Baculovirus expression of heterologous genes permits multiple post-translational modifications, like folding, oligomerization, phosphorylation, glycosylation, acylation, disulfide bond formation, proteolytic cleavage and so on, which are similar or identical to those occurring in mammalian cells. These advantages over prokaryotic expression systems make it possible to express multiple proteins simultaneously in a single infection and to obtain multimeric proteins sharing functional similarity with their natural analogs. Thus, the BES has been broadly used for the production of heterologous proteins and even the generation of virus-like particles (VLP) in laboratories [3,4], as well as for the development of subunit vaccines, including VLP-based vaccines, in the vaccine industry [5,6].

VLPs are composed of viral capsid proteins that self-assemble into particles closely resembling the natural virions from which they derive. VLPs are replication as well as infection incompetent, due to the absence of any infectious genetic material [7]. A close resemblance to native viruses in molecular scaffolds enables VLPs to elicit both humoral and cellular immune responses even without adjuvant. VLPs have been constructed through co-expression and then self-assembly of their components in *Escherichia coli* (*E. coli*), yeasts, mammalian cells and insect cells. As a powerful eukaryotic expression system post-translationally modifying and processing the foreign proteins expressed *in vitro* or *in vivo*, the BES plays a critical role in self-assembly and release of VLPs. However, many studies have demonstrated the inability of lepidopteran cells to synthesize mammalian-type N-glycans [8], which was a limitation of the conventional BES [9]. In addition, the stoichiometry of VLP components, the self-assembly efficiency of structural proteins, and the budding process of enveloped VLPs are determined by the BES. In this article, we critically reviewed the pros and cons of using the BES to construct both enveloped and non-enveloped VLPs.

BES as a platform for protein expression

Several commercially available BES kits (*e.g.*, BaculoGold™, BD Biosences; *Flash*BAC, Oxford Expression Technologies; BacPAK™, Clontech) use the conventional method of homologous recombination *in vitro*. However, the final virus stock unavoidably contains a mixture of parental and recombinant viruses, so a plaque-assay is required to purify the recombinant baculovirus [10]. An alternative approach has been developed [11] to circumvent this problem by generating a recombinant baculovirus using site-specific transposition with Tn7 to insert foreign genes into bacmid (baculovirus plasmid) propagated in *E. coli*. Based on the principle of site-specific transposition, a rapid and efficient BES (Bac-to-Bac®, Life Technologies) that can be used as an alternative way to generate recombinant baculoviruses was developed and is now widely used as another commercial BES. Additionally, a modified baculovirus vector harboring a mammalian promoter, known as BacMam vector, has been turned into a transient expression vector for gene delivery and high-level screening in mammalian cells [12]. The BacMam system combines the advantages of viral transient expression, ease of generation and a broad cell tropism, enabling rapid, efficient and flexible gene over-expression experiments to be performed in various mammalian cells [13].

To date, the BES has been used to manufacture several biologicals, including the interferon [14], antigen [15] and vaccine [16]. One was GlaxoSmithKline's Cervarix™ (GSK, Rixensart, Belgium), a VLP-based bivalent human papillomavirus vaccine against cervical cancer, which was approved for human use in the USA in 2009 [17]. In the veterinary field, Porcilis® Pesti (Intervet) and Bayovac® CSF E2 (Bayer) are the first two licensed subunit vaccines produced by the BES. Either consists of an envelope glycoprotein of the classical swine fever virus as the antigen. In addition, FluBlok®, a seasonal influenza subunit vaccine for adults, was approved by the FDA in January, 2013. It is tailored annually to provide protection against the latest strains of influenza by containing the corresponding hemagglutinin (HA) antigens produced by the BES [18,19].

Generation of VLPs using BES

Many viral structural proteins have an intrinsic ability to spontaneously self-assemble into VLPs (Table 1) when expressed in insect cells by co-expression or co-infection with recombinant baculoviruses. Like parental viruses, many VLPs generated by the BES are enveloped, meaning that the capsids are coated with a lipid membrane known as the envelope, which is derived from the plasma membrane of insect cells. The other VLPs, known as the non-enveloped VLPs, contain no lipid membrane and are formed by only one or more major structural proteins. Owing to the difference in structures, there are differences in the assembly mechanisms for enveloped and non-enveloped VLPs.

Enveloped VLPs

In general, self-assembly of structural proteins into enveloped VLPs (Fig. 1A) includes two steps, namely capsid (or matrix) formation and then membrane enclosure for further budding (Fig. 2). Due to intrinsic properties of the lipid membrane and surface glycoprotein, the generation of enveloped VLPs in insect cells is more complicated than that of non-enveloped VLPs. However, efficient budding of enveloped VLPs from insect cells has been reported from time to time [30,31,36,38,39,45,46]. For example, using a quadruple baculovirus recombinant, Latham and Galarza (2001) initially showed that co-expression of four structural proteins of influenza virus, the HA, neuraminidase (NA), matrix protein M1 and M2 ion channel protein, was sufficient for the self-assembly and release of VLPs from surface of insect cells. Furthermore, the VLPs closely resembled the authentic virions in size, morphology, and in the fine structure of the surface spikes [3].

Non-enveloped VLPs

Compared with the enveloped VLPs, the generation of non-enveloped VLPs with single capsid (Fig. 1B) should be less

[1] Baculovirus expression system (BES); virus-like particles (VLP); *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV); *Bombyx mori* nucleopolyhedrovirus (BmNPV); polyhedrin (polh); hemagglutinin (HA); neuraminidase (NA); endoplasmic reticulum (ER); European Molecular Biology Laboratory (EMBL); multiplicities of infection (MOI); Flock House nodavirus (FHV); time of infection (TOI); peste des petits ruminants virus (PPRV)