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MINI-REVIEW

Biotechnological applications of occlusion bodies of Baculoviruses

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Abstract

The ability of Baculoviruses to hyper-express very late genes as polyhedrin, the major component of occlusion bodies (OBs) or polyhedra, has allowed the evolution of a system of great utility for biotechnology. The main function of polyhedra in nature is to protect Baculovirus in the environment. The possibility of incorporating foreign proteins into the crystal by fusing them to polyhedrin (POLH) opened novel potential biotechnological uses. In this review, we summarize different applications of Baculovirus chimeric OBs. Basically, the improvement of protein expression and purification with POLH as a fusion partner; the use of recombinant polyhedra as immunogens and antigens, and the incorporation of proteins into polyhedra to improve Baculoviruses as bioinsecticides. The results obtained in each area and the future trends in these topics are also discussed.

Keywords Baculovirus · Occlusion bodies · Polyhedrin · Fusions · Recombinant proteins · BEVS

Introduction

The main biotechnological characteristic of Baculoviruses is their versatility. Regarding this matter, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Bombyx mori* nuclear polyhedrosis virus (BmNPV) have been successfully exploited in molecular and cellular biology for multiple applications. The Baculovirus expression vector system (BEVS) is a reference among the eukaryotic expression systems, with evident simplicity and scale-up advantages with respect to mammalian cells and with characteristics that distinguish it from bacterial and yeast expression systems. Although three decades has passed since the development of BEVS and much improvement has been achieved in this technology (reviewed by van Oers et al. 2015), many challenges must be overcome yet.

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Baculoviruses are DNA viruses of insects belonging to the *Baculoviridae* family. The latter is divided into four genera: Alphabaculovirus (nucleopolyhedrovirus) and Betabaculovirus (granulovirus) that infect Lepidoptera, and Gammabaculovirus and Deltabaculovirus which infect Hymenoptera and Diptera, respectively (Jehle et al. 2006). The members of this family are characterized by presenting a biphasic infectious cycle performed by two phenotypes with different functions (Rohrmann 2013). The so-called occlusion-derived viruses (ODVs) carry out the primary infections of intestinal larval cells. The reason for this denomination is because they are embedded into a crystalline matrix shaping the occlusion bodies (OBs) or polyhedra, which contaminate the leaves from which the larvae feed (Granados and Lawler 1981; Slack and Arif 2007). Once ingested, OBs are transported to the midgut, where they are dissolved by the combined action of the alkaline environment and proteases with the subsequent release of the occluded virions. Once the ODVs infect intestinal epithelial cells, the other virion phenotype, the budded viruses (BVs), spreads the infection towards other cell types. In a very late stage of the infection cycle, polyhedrin (POLH) becomes the protagonist by dramatically augmenting its expression level and thus giving origin to polyhedra in the nucleus. These crystalline structures are highly stable and resist solubilization, except under strong alkaline conditions; therefore, polyhedra can protect ODVs from environmental conditions (Rohrmann 1986; Van Oers and Vlak 1997). The high expression levels mediated by

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the *polyhedrin* (*polh*) promoter together with the non-essentiality of *polh* in the replication of Baculovirus in cultured insect cells led to the development of the most relevant application of the BV phenotype, the expression of recombinant proteins.

The main application of ODVs, in contrast, relates to the biological control of pests by the spread of OB-based formulations in cultivated fields. Baculovirus-based insecticides result attractive owing to several advantages over other products. Indeed, they are environmentally innocuous and harmless to humans and animals and they have long-lasting control effects on the target insects (Moscardi 1999; Haase et al. 2015). Nevertheless, in the last decade, most of the attention has been turned towards the use of OBs as a promising biotechnological tool. Altogether, the study of POLH structure and polyhedron morphogenesis allowed the development of a powerful technology that is taking its first steps.

This review summarizes the main contributions since the discovery that recombinant proteins fused to POLH increase their yields and that can also be included into the OBs.

Composition and morphogenesis of occlusion bodies

Polyhedrin (~ 29 kDa) is one of the most conserved Baculovirus proteins and is produced at high levels at late stages of the viral infection. This protein is the major protein component of the polyhedra of Alphabaculovirus, where it can self-assemble: POLH subunits form trimers that are rearranged in dodecahedra (four trimers) through disulfide bridge bonds. According to crystallographic analysis of POLH, dodecahedra interact with each other through ionic and hydrophobic interactions to form the polyhedra (Ji et al. 2010; Coulibaly et al. 2009). Surrounding the OBs is the polyhedral envelope (Gross et al. 1994; Gross and Rohrmann 1993; Russell and Rohrmann 1990). This envelope is a protein/carbohydrate matrix forming a lattice and whose primary protein is a phosphoprotein called polyhedral envelope protein PP34 covalently linked to carbohydrates by disulfide bonds (Gombart et al. 1989; Whitt and Manning 1988).

In Betabaculovirus, on the other hand, OBs are mainly composed by granulin. Although POLH and granulin can vary by about 50% in the amino acid sequence, many of their structural features are highly conserved, thus reflecting their similar functions and biochemical properties (Rohrmann 1986). Nevertheless, POLH far outperforms granulin in terms of its biotechnological advantages. In particular, polyhedrin began to gain importance as polyhedral proteinaceous structures (~ 0.6–2 μM) that are highly stable and easily purifiable.

Little is known about the mechanisms that trigger and modulates the morphogenesis of polyhedra. Virion envelopes themselves seem to catalyze POLH polymerization (Wood 1980) and, furthermore, two proteins of BmMNPV, Bm133 and Bm134, were found to be indispensable for the embedding of ODVs into polyhedra matrix (Shen et al. 2018). The morphology of polyhedra depends not only on the interactions between POLH and other viral or host proteins, but also on its own amino acid sequence. In fact, the domain required for the supramolecular assembly of the AcMNPV POLH corresponds to the region between amino acids 19 and 110 (Jarvis et al. 1991), which also contains the nuclear localization signal. Mutations within this region caused changes in polyhedra morphogenesis and effects on the occlusion of Baculoviruses (Carstens et al. 1992). AcMNPV polyhedra are highly symmetrical covalently cross-braced robust lattices with flexible adaptors for virion occlusion and even point amino acid changes of POLH can give rise to altered polyhedral morphology (Lin et al. 2000; Ribeiro et al. 2009; Lopez et al. 2011). The first studies on foreign proteins incorporated into POLH-made crystals occurred before these observations. Therefore, researchers had to explore different putative positions of fusion inside POLH to obtain a proper inclusion of heterologous peptides into the OBs. In several cases, the constructions maintained the ability of the fusion proteins to form OBs, whereas in others, the introduced conformational changes in POLH prevented polyhedra from acquiring their natural form (McLinden et al. 1992).

Strategies for obtaining recombinant polyhedra

In Fraser et al. 1989, Fraser and co-workers developed the first approach in the research of polyhedra as possible carriers of foreign proteins. The recombinant Baculoviruses reported contained an insertion of a 9-amino-acid epitope of the hemagglutinin of influenza (HA) at different positions of the POLH sequence without altering OB's formation. The researchers stated that the generated recombinant OBs had potential uses in vaccine formulations, immunoassays, immobilized enzyme reactions, and as biological insecticides and expression vectors. Furthermore, they remarked the simplicity of isolation from infected cells or larvae and the high concentrations and purity of the resulting recombinant fusion proteins. Thereafter, several proteins have been fused to the complete POLH, or to a fragment of it, through different strategies that rendered chimeric polyhedra or insoluble aggregates.

Today, three main approaches are available in the literature for obtaining chimeric polyhedra that incorporate heterologous proteins fused to POLH and maintain its normal morphology. The cis complementation (Je et al. 2003) is based on two independent transcription units in opposite orientations for the expression of a fusion gene under *polh* promoter and an extra copy of wt *polh* under the *p10* promoter. This strategy combines the simplicity of manipulating a single viral stock with the possibility of obtaining high yields both in vitro and in vivo.

On the other hand, the trans complementation has three variants: (i) infection with a virus carrying a translational fusion with a gene of interest in the front and back of *polh*, (ii) co-infection with a wt Baculovirus, and (iii) infection of stably transformed cell lines that express POLH under *polh* promoter regulation. The first variant is based on the insertion of a gene of interest between two copies of the complete sequence of *polh*. This fusion generates normal polyhedra because homologous recombination occurs in a percentage of viruses and in turn the resulting loss of the foreign gene produces Baculoviruses that carry a wt copy of *polh* (Je et al. 2000). With respect to the second variant, co-infections with wt Baculovirus in a ratio of 1 resulted efficient to include recombinant proteins into polyhedra; a ratio of wt virus:recombinant virus of 3:1, however, increased the incorporation of recombinant proteins (Sampieri et al. 2015). Finally, our group was able to obtain chimeric polyhedra using a combined strategy with non-fused copies of *polh* in trans and in cis (third variant) (Lopez et al. 2018). However, the copy of *polh* in trans may be sufficient for the recombinant OBs morphogenesis (Lopez et al. unpublished results). The infection of transgenic cell lines would potentially increase simplicity because it would require the manipulation of a single viral stock. This strategy, however, has two main disadvantages. It requires a selection marker such as an antibiotic resistance and the development of transgenic larvae, which makes it more complicated for in vivo production. These two limitations would increase final costs considerably.

The last and more recent strategy is the production of chimeric polyhedra without complementation following an approach called dimidiate polyhedrin (Yang et al. 2017). This system comprises the expression of POLH divided into two fragments. The N-terminal segment, spanning the first 150 amino acids, expressed under the *p10* promoter regulation and the C-terminal segment, comprising the last 95 amino acids with the nuclear localization signal, fused to the protein of interest, under the *polh* promoter control. The authors stated that both POLH fragments strongly interact in vivo and this interaction ensures a packaging efficiency of 100% for the recombinant protein. However, further details are needed to elucidate how polyhedrin recovers its integrity from two independent fragments to conform normal polyhedra.

Fusions to fragments of POLH

The production of foreign proteins fused to POLH fragments was proved from early times (Pennock et al. 1984; Makino et al. 1989; Nyunoya et al. 1990; Royer et al. 1992). In particular, the production seems to be significantly enhanced, especially when fused to the fragment from amino acids 19 to 110 ($POLH_{19–110}$) (Bae et al. 2013). Indeed, the 19–110 region, which contains the nuclear localization signal (KRKK, positions 32 to 35), is required for the supramolecular assembly of POLH into OB-like particles in the nucleus (Jarvis et al. 1991).

Later, Sampieri and co-workers further dissected POLH sequence as a fusion to GFP to finally define $POLH_{58–110}$ as the minimum fragment needed to efficiently incorporate foreign proteins into polyhedra in a co-infection with wt virus (Sampieri et al. 2015). In addition, Chen and co-workers studied how different fragments of POLH can direct EGFP into BmNPV polyhedra and showed that the fusion protein $POLH_{1–100}$-EGFP is the most efficient. These findings are coincident with the results for AcMNPV. Then, Bae and co-workers suggested that the fragment $POLH_{32–59}$ is the minimum fusion partner to hyper-produce proteins in BEVS, maintaining their activity (Bae et al. 2017). All these analyses had employed GFP as a model and researchers assumed that fluorescence emission is directly proportional to the level of incorporation of recombinant proteins into polyhedra without performing any protein quantitative assays. However, fluorescence is an indirect measure of protein concentration and can be influenced by protein folding. Thus, we suggest that the levels of incorporation of proteins fused to the entire POLH or its fragments into OBs are variable and respond to multiple causes. The size of the recombinant protein and the molecular weight of the fusion may be the most important factors affecting the final yields but its sequence and secondary structure can also determine an optimal protein folding. The knowledge of each protein behavior as a fusion to POLH is the key for the decision of the best methodology of production to ensure the higher yields of the recombinant protein and to guarantee its application.

Whatever the method employed, chimeric polyhedra have been used for recombinant protein production in many ways, as listed in Tables 1 and 2 and further described below.

Protein fusions to POLH for improving expression processes for uses thereof

The final step of the BEVS process is usually protein purification. One of the major advantages of protein production into Baculovirus OBs is its simple purification, which avoids sophisticated equipment and laborious or complicated procedures. Figure 1 displays a basic scheme of the global process comprising from the design of the construction of interest to be included into OBs to the scaling-up of the production of chimeric polyhedra and purification of the protein of interest.

Fusion protein aggregates vs. polyhedra formation

As mentioned before, a complementation with wt POLH is generally fundamental for OB normal morphogenesis. Nevertheless, fusion protein productions have been successful even in the absence of complementation, which produces amorphous chimeric protein aggregates with no Baculovirus

Table 1 Main contributions of polyhedrin as recombinant protein expression partner

Application	Recombinant protein construction			Formation of OB or AC	Host	Yields (results)	Reference
	Gene of interest	Fusion	Complementation				
Carrier of foreign epitopes	ΔHA of Influenza	HA epitope into POLH at different positions	No	OB/AC	AcMNPV	No data	McLinden et al. 1992
Protein production	*egfp*	POLH-GFP-POLH	No	OB	AcMNPV	No data	Je et al. 2000
Protein production	*egfp*	POLH-GFP	POLH in cis and trans	OB	AcMNPV	No data	Je et al. 2003
Protein production	*hrgfp*	POLH-hrGFP	No	OB	AcMNPV	No data	Lee et al. 2005
Vaccine against Foot and Mouth disease	*vp1* of FMDV	POLH-VP1	No	OB	AcMNPV	233 μg/ml of cell culture	Lee et al. 2005
Protein production and purification	*egfp*	POLH-GFP	No	AC	AcMNPV	11.76 μg/ml of cell culture	Roh et al. 2010
Protein production	*egfp*	ΔPOLH-GFP	No	AC/OB	AcMNPV	No data	Bae et al. 2013
Vaccine against Classical swine fever	E2 of CSFV	ΔPOLH-E2ΔTMR	No	No data	AcMNPV	No data	Bae et al. 2013
Diagnostic of garlic mosaic	cp of GarMbFV	POLH-CP	No	AC	AcMNPV	No data	Ardisson-Araujo et al. 2013
Protein production	*egfp*	ΔPOLH-GFP and POLH-GFP	POLH in trans/no complementation	AC/OB	AcMNPV	No data	Sampieri et al. 2015
Diagnostic of bovine babesiosis	Peptides of *Babesia bovis*	POLH-BbAp	POLH in cis/$POLH_{E44G}$ in trans	OB	AcMNPV	Up to 25 μg/ml of cell culture	Lopez et al. 2018
Protein production	*igf2*	POLH-1-112-IGF2	No	AC	BmNPV	0.3 mg/ml of cell culture	Marumoto et al. 1987
Antigen production of Aujeszky's disease	gB of PRV	POLH-gB	POLH in cis	OB	BmNPV	3.23 mg/ml of hemolymph	Kim et al. 2012
Antigen production of Aujeszky's disease	gC of PRV	POLH-gC	POLH in cis	OB	BmNPV	3.69 mg/ml of hemolymph	Kim et al. 2012
Vaccine against Classical swine fever	E2 of CSFV	POLH1–111-E2ΔC	POLH in cis	OB	BmNPV	3.13 mg/ml of hemolymph	Lee et al. 2012
Protein production	*egfp*	ΔPOLH-GFP and POLH-GFP	POLH in cis	OB	BmNPV	No data	Chen et al. 2013

AC amorphous crystals, *OB* occlusion bodies

Table 2 Main contributions of chimeric polyhedra of Baculovirus as bioinsecticides

Recombinant protein construction					Host	Fold-decrease (LC_{50} or LD_{50})	Reference
Gene of interest	Type of protein	Fusion	Complementation	Additional toxin			
Cry1Ac	Toxin	POLH-Cry1Ac-GFP	POLH in *cis*	No	AcMNPV	98.88 LD	Chang et al. 2003
Cry1Ac	Toxin	POLH-Cry1Ac-POLH	No	No	AcMNPV	*P. xylostella* < 90.90 LD *S. exigua* 6.88 LD	Kim et al. 2005
Cry1-5	Toxin	POLH-Cry1-5-POLH	No	AaIT	AcMNPV	*P. xylostella* NC *S. exigua* 0 LD	Shim et al. 2009
Cry1-5	Toxin	POLH-Cry1-5	POLH in *cis*	AvTox2	AcMNPV	*P. xylostella* NC *S. exigua* 1.59 LD	Jung et al. 2012
Cry1-5	Toxin	POLH-Cry1-5	POLH in *cis*	Bi-KTI	AcMNPV	*P. xylostella* NC *S. exigua* 1.37 LD	Choi et al. 2013
Cry1-5	Toxin	POLH-Cry1-5-POLH	No	AaIT	AcMNPV	*P. xylostella* 9.3 LC *S. litura* 0 LC *S. exigua* 1.44 LC	Shim et al. 2013
Cit1a	Toxin	POLH-Cit1a	No	No	AcMNPV	No data	Ali et al. 2015
Cit1a	Toxin	POLH-Cit1a	No	No	BmNPV	No data	Ali et al. 2015
gp37	Fusolin	C95-GP37	No	No	AcMNPV	3.15 LC	Yang et al. 2017
en4	Enhancin	C95-EN	No	No	AcMNPV	5.23 LC	Yang et al. 2017

LC_{50} lethal concentration 50, LD_{50} lethal dose 50

occlusion. In 2005, Lee and co-workers evaluated if different engineered proteins fused to POLH were suitable for protein purification and further antibody generation (Lee et al. 2005). They found that recombinant proteins formed OB-like particles that could be easily purified. Since these particles are insoluble, this system should be ideal for better expression of insoluble, toxic or "hard-to-purify" proteins. Similar arguments have been considered also for prokaryotic systems. For example, POLH has been used as fusion partner to produce recombinant GFP in *E. coli* and by taking advantage of POLH insolubility, which facilitated the production of the recombinant protein by forming inclusion bodies (Wei et al. 2005; Seo et al. 2003, 2005). In this system, however, GFP showed greatly reduced fluorescence, mainly because it forms an aggregated mass without a properly folded structure.

Roh and co-workers demonstrated that GFP fused to POLH at the N-terminus, in the absence of in cis or in trans complementation, forms protein aggregates in insect cells, although these proteinaceous masses are not as compact as the wt polyhedra (Roh et al. 2010). As a novelty, they included an enterokinase (EK) site between POLH and GFP and the observation of granule-containing cells was possible. The recombinant protein was then purified in three steps: cell harvest, sonication, and EK digestion, without the need of solubilization, unlike chimeric polyhedra that have to be previously dissolved with an alkali (Je et al. 2003). Ardisson-Araujo et al. (2013) fused the complete coat protein (CP) from garlic virus GarMbFV to the POLH C-terminal tail and used larvae as bioreactors. In their study, they observed the incorporation of GarMbFV-CP into the polyhedron-like aggregates that were effectively purified as normal polyhedra.

In all the described cases, recombinant proteins isolated from the aggregates appeared to retain their properties. Indeed, GFP maintained its fluorescence (Roh et al. 2010; Lee et al. 2005) and other proteins tested were successfully recognized by specific sera (Lee et al. 2005) or elicited the production of antibodies in rats (Ardisson-Araujo et al. 2013). These findings encourage the use of this expression system to produce enzymes or proteins with some biological function.

Scaling up

There has been a fast-growing demand for low cost processes to produce biologically active biomolecules, such as eukaryotic proteins, glycoproteins, peptides, and lectins. In this vein, the use of live insect larvae as biofactories results a low-cost alternative to scale up the production of high-quality recombinant proteins (Targovnik et al. 2016). Moreover, downstream processing (product recovery and purification methods) are generally well established at small and medium scales and the increasing use of disposable process equipment is enhancing product safety and reducing losses associated with contamination (reviewed by Contreras-Gomez et al. 2014). The advances in all these relevant areas suggest that the use of BEVS will further expand. For example, the use of larvae as a potential economic bioreactor to produce chimeric polyhedra may become a promising platform for obtaining high levels of recombinant proteins because the expressed

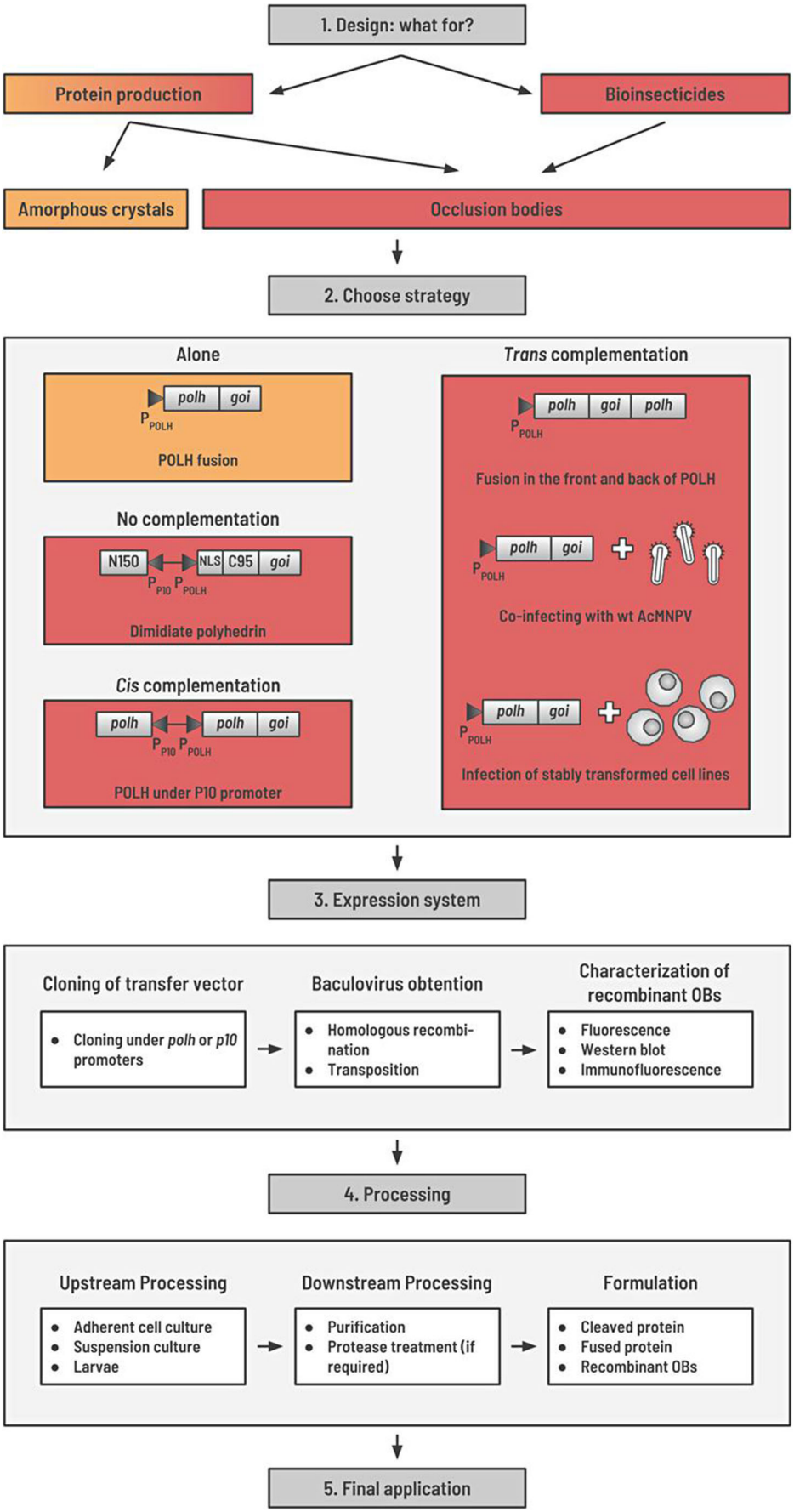

Fig. 1 Flowchart diagram of the entire process from design of the transfer vectors to the final use of recombinant proteins expressed as a fusion to POLH. NLS nuclear localization signal, goi gene of interest

fusion proteins are easy to purify with this system. Two of the uses that have been explored are discussed below.

Polyhedra as carriers of immunogenic epitopes and antigens

Recombinant polyhedra have been explored as a system to produce recombinant antigens for the development of vaccines and diagnostic tests, both in BmNPV and AcMNPV. In this regard, recombinant OBs carrying a small epitope from HA of influenza virus fused within the N-terminus of POLH of AcMNPV elicited antibodies specific to HA in rabbits inoculated with dissolved and adjuvated polyhedra (McLinden et al. 1992). Additionally, Lee et al. (2012) evaluated a subunit vaccine based on the envelope glycoprotein E2 from classical swine fever virus (CSFV). They fused the N-terminal 179 amino acids of CSFV E2 to POLH from BmNPV and assessed its immunogenic properties in BALB/c mice both as polyhedra and as the purified E2. The antigen administered as purified protein resulted effectively immunogenic, whereas undissolved and dissolved polyhedra were poorly immunogenic. On the other hand, Kim et al. (2012) studied glycoproteins gB and gC of pseudorabies virus as fusions to POLH to obtain experimental vaccines for Aujeszky's disease. Both proteins were efficiently incorporated to chimeric polyhedra in a glycosylated form and rabbit hyperimmune sera recognized both fusion proteins.

Furthermore, Vaca Domínguez (2011) proposed to encapsulate antigens into OBs as a strategy for new generation vaccines by using point mutations of the POLH that could influence shape, size and stability of the polyhedra crystals. Therefore, depending on the POLH sequence used for the fusion, short-termed or prolonged-release crystals might be generated. This allows the generation of more controlled vaccines retaining structure or biological activity at room temperature for prolonged periods (patent no. MX2010005637, 2011). However, only virions of the budded phenotype, not ODVs or entire polyhedra, were able to mature dendritic cells and to display immunostimulatory properties in mice. Moreover, entire polyhedra remained undissolved in the acidic endosomes of murine dendritic cells (Molina et al. 2016). This application remains to be proved in vivo.

As Baculoviruses are not natural pathogens of mammals, they do not present natural antibodies against these viruses (McWilliam 2007), which is advantageous compared to the expression of antigens in a prokaryotic system. Although fusions to POLH have been previously employed to improve the expression of diagnostic proteins (Lee et al. 2005; Lee et al. 2015), we have recently developed the use of dissolved chimeric polyhedra for ELISA-based diagnostic (Lopez et al. 2018). This novel approach involved the prediction of antigenic peptides of *Babesia bovis*, their incorporation into AcMNPV OBs and further validation with ELISA. These peptides failed to react with specific bovine sera when synthesized in a biotinylated form and used as antigens in an indirect ELISA test. This may be because their small size prevented their correct presentation after the adsorption. Thus, POLH not only improves and simplifies fusion protein purification, but as a carrier may allow a correct exposure of epitopes to the antibodies. The availability of new antigens by peptide microarrays and the current application of baculoviral OBs as carrier particles appear as a promising technology to highly produce and simply purify antigens with potential use for diagnosis.

Incorporating proteins into polyhedra to improve baculoviruses as bioinsecticides

One of the most relevant applications of OBs has been in biological control of pests. However, bioinsecticides based on wt Baculoviruses present a natural limiting factor because of its slow killing action. In this respect, the design of recombinant Baculovirus expressing toxins and other proteins that augment basal infectivity could considerably reduce death times and lethal doses (reviewed by Inceoglu et al. 2006 and Kroemer et al. 2015). Until now, however, their use has been limited, mainly because biological pest control is still more expensive than chemical insecticides. Several approaches have been developed to improve killing abilities of Baculoviruses.

As one of the first attempts to use OBs as a bioinsecticide, Chang et al. (2003) obtained a recombinant AcMNPV that produced polyhedra incorporating GFP as a marker and the *Bacillus thuringiensis* toxin Cry1Ac crystal protein toxin. This novel bioinsecticide improved infectivity and was faster in action in comparison to non-engineered virus or previously described engineered AcMNPV. Thus, this approach that combines positive attributes of both Baculoviruses and Cry1Ac toxin could be easily applied to the expression of other Bt and gut active toxins (Jung et al. 2012).

However, the risks of releasing genetically engineered organisms into the environment should be considered (Tiedje et al. 1989). To address this concern, researchers have fused the toxins between two copies of POLH (as described in Fig. 1) to allow the elimination of the transgene by recombination events in the successive generations (Kim et al. 2005, 2007; Shim et al. 2009, 2013). This makes this approach environmentally friendly. A combination of two different mode-of-action toxins, gut-acting Bt toxin and neurotropic spider toxins, successfully enhanced AcMNPV infectivity against *Plutella xylostella* and *Spodoptera exigua*, with values of LD_{50}, which were significantly lower than that of a similar recombinant control carrying GFP (Shim et al. 2013). Furthermore, the polyhedra collected from infected larvae showed a phenotype similar to wt Baculovirus.

Baculovirus infectivity can be also improved by packaging compounds that enhance the peroral infectivity, such as

fusolins from entomopoxviruses and baculoviruses, within OBs (Mitsuhashi 2018). Yang and co-workers described that the inclusion of *Cydia pomonella* granulovirus GP37 and a partial fragment of the enhancin *en4* from *Agrotis segetum* granulovirus into AcMNPV OBs by dimidiate polyhedrin increased 3–5-fold baculoviral infectivity with respect to a control bearing wt POLH (Yang et al. 2017).

Fusions to other proteins included into OBs

There has been an increasing interest in elucidating the mechanism controlling polyhedron morphogenesis; particularly the attention has been focused on proteins involved in this process (Yu et al. 2015; Shen et al. 2018). A comprehensive analysis of the BmNPV polyhedron matrix-associated component proteins using mass spectrometry allowed the identification of 28 host and 91 viral proteins. Among these proteins, BM134, a non-essential protein of 12.4 kDa associated to polyhedron matrix (Ono et al. 2012), could be a potential carrier of heterologous polypeptides (Guo et al. 2017). The fusion of the c-terminus of BM134 to EGFP led to the occurrence of fluorescence on some polyhedra particles, thus indicating that polyhedrin matrix has a powerful capacity to trap foreign proteins. Although previous reports demonstrated that proteins can passively be incorporated inside OBs (Xiang et al. 2012; Chiu et al. 2012), this discovery constitutes a novel strategy to obtain chimeric polyhedra. Nevertheless, the incorporation seems much lower than with fusions to POLH. Thus, this methodology will be probably relegated to its use in biological control, where the presence of the protein of interest, independently of the amount, is sufficient to function.

Concluding remarks

The enormous biotechnological potential of POLH as a fusion partner within BEVS is undoubted. Since the discovery of the possible hyper-expression and inclusion of proteins fused to POLH, or fragments of it, into OBs, many researchers have explored the optimization of the system, both in AcMNPV and in BmNPV. Although no much data on the final yields of recombinant protein expression is yet available, important research on POLH as a fusion protein and chimeric polyhedron morphogenesis has been performed and the resulting contributions will allow researchers to adapt the system to their particular needs. Nevertheless, much work remains to be done to improve the expression capacity of the system until it becomes a system of excellence for the expression and purification of recombinant proteins.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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