Most essential activities in eukaryotic cells are catalyzed by large multiprotein assemblies containing up to ten or more interlocking subunits. The vast majority of these protein complexes are not easily accessible for high resolution studies aimed at unlocking their mechanisms, due to their low cellular abundance and high heterogeneity. Recombinant overproduction can resolve this bottleneck and baculovirus expression vector systems (BEVS) have emerged as particularly powerful tools for the provision of eukaryotic multiprotein complexes in high quality and quantity. Recently, synthetic biology approaches have begun to make their mark in improving existing BEVS reagents by de novo design of streamlined transfer plasmids and by engineering the baculovirus genome. Here we present OmniBac, comprising new custom designed reagents that further facilitate the integration of heterologous genes into the baculovirus genome for multiprotein expression. Based on comparative genome analysis and data mining, we herein present a blueprint to custom design and engineer the entire baculovirus genome for optimized production properties using a bottom-up synthetic biology approach.

Our understanding of the cellular machinery has increased tremendously in recent years mainly due to astounding progress in "omics" research (genomics, proteomics and glycomics). Comprehensive genomics data sets are now available for many organisms including human, and focus has now shifted to elucidating the cellular proteome in correlation with the live cellular functionality and morphology. One essential lesson already learned is that proteins in eukaryotic cells typically do not work in isolation but coexist in large and highly diverse assemblies of ten or more interlocking subunits. These stably or transiently associated multiprotein assemblies additionally work together with separate proteins or multiprotein assemblies to carry out essential cellular processes including signaling, energy generation, and transport of food, water or waste. The highly dynamic processes going on among the proteins in our cells has been termed the "protein sociology." A considerable number of accessory proteins typically accompany any individual multiprotein complex at various stages of its production, trafficking, active life and degradation. For example, chaperones are often critical for proper assembly of complexes, while other proteins are required for proper targeting and activation through post-translational modification. The activity of complexes is often fine-tuned by the incorporation of isoforms of individual subunits, for example to mediate tissue-specific functions. To fully understand biology, it is clear that we need methods to unlock the assembly, structure and mechanism of all of the complexes that exist in our cells. This is...
not only essential for basic research, but equally important for enabling novel approaches in the pharmaceutical and biotech industries to drive development of new and better drugs that more specifically modulate cellular functions. An imposing bottleneck that obstructs progress in these areas stems from the typically low abundance and high heterogeneity of protein complexes and multiprotein complexes, especially for secreted proteins such as the fruit fly, yeast or archea which are commonly used in basic research do not accurately recapitulate the functions of the much higher developed and therefore significantly more complicated human species. The provision of human multiprotein complexes in the quality and quantity required for mechanistic studies and drug design poses particular challenges due to the complexity of the machinery at work in our cells. Technical factors for heterologous protein production including protein yield, stoichiometric ratio between subunits, post-translational modifications, folding, and stability are all of critical importance, and ideally a highly flexible heterologous expression system should be available that can provide these functions for a wide range of protein complexes. An attractive solution could be mammalian expression systems, which naturally provide the required functions to accurately reflect what takes place in our cells, and heterologous expression in mammalian systems has become increasingly popular, especially for secreted proteins such as therapeutic antibodies. However, mammalian systems often do not provide acceptor sites for intracellular proteins, and multiprotein expression technologies for mammalian cells are still in their infancy, albeit progress has been made recently, opening interesting options to depart with hitherto unattainable precision entire pathways in mammalian cells, for example for pharmacological screening studies. An attractive alternative to mammalian systems is heterologous expression using recombinant baculoviruses to infect insect cell cultures. This method was prosed three decades ago and has become a method of choice for producing high levels of many eukaryotic proteins including a large number of proteins of pharmaceutical interest. A significant advance over existing baculovirus expression vector systems (BEVS) came with the introduction of MultiBac, an advanced BEVS particularly tailored for producing eukaryotic multiprotein complexes for structural and functional studies. MultiBac consists of a baculovirus genome that has been engineered for optimized protein production by deleting protease and apoptosis activities. In a subsequent improvement of the system, a new suite of transfer vectors was introduced to facilitate introduction of many heterologous genes into one recombinant MultiBac baculovirus by a method called tandem recombinering (TR), involving sequence-and-ligation-indepen dent cloning (SLIC) and Cre-LoxP recombinase. More recently, the design of transfer plasmids has been further refined, resulting in small, easy to handle plasmids containing only the functional DNA elements required for protein expression, expression cassette multiplication and plasmid concatenation by TR. Multigene transfer vectors created in this way are introduced into the MultiBaculovirus genome by the Tn7 transposase, in E. coli strains modified for this purpose. As a step forward relative to previous systems, the original MultiBac system already provided the option to integrate accessory functionalities that may be required for proper functioning of a multiprotein complex, by means of a second entry site engineered into the virus genome that is independent of, and distal to the main site of integration that relies on the Tn7 transposition. This feature has been exploited to integrate additional functional modules into the viral genome, including post translational modification enzymes, and fluorescent proteins that allow easy monitoring of virus performance and protein production following transfection and during virus amplification. More recently, this approach has been used to create SweetBac, allowing for the production of mammalian-like glycoproteins in insect cells. MultiBac is now in use at more than 600 laboratories worldwide, in academia and industry, and has generated a number of multiprotein complexes have been produced in high quality and quantity for diverse applications by using the MultiBac system. Currently, two approaches for integrating heterologous expression cassettes into the baculovirus genome dominate the field. One of these approaches requires the presence of the baculoviral genome as a bacterial artificial chromosome (BAC) in E. coli cells, together with Tn7 transposase activity present in these same cells which recombine transformed transfer plasmids into a Tn7 attachment site on the BAC. Invitrogen’s Bac-to-Bac system and also the more advanced MultiBac system both utilize this approach. The recombinant, composite baculovirus DNA is then purified from these E. coli cells by alkaline lysis, and used to transfect insect cells. In contrast, the original method of choice to integrate heterologous expression cassettes into the baculovirus genome relied on homologous recombination mediated by regions in the transfer plasmid that were homologous to two genes on the baculovirus genome (Orf1629 and Id2/603) that flank the baculoviral polyhedrin locus which had been inactivated. This method is still offered by a large number of commercial providers (Novagen BacVector series, PharMingen BaculoGold, Abvector and others). By this method, homologous recombination occurs in insect cells following transfection of the baculovirus genomic DNA together with the transfer vector. The efficiency of recombination is increased by linearization of the baculovirus genome, but still remains a less efficient method to rapidly generate recombinant baculovirus than transforming Tn7-produced composite BACs. A further improvement on the homologous recombination in insect cell method came by truncation of the essential Orf1629 gene on the baculovirus genome which is then repaired by co-transfecting complete Orf1629-containing
either baculovirus genomes using Tn7 for gene entry or baculovirus genomes using homologous recombination, respectively. However, no transfer vectors can access both types of genomes. We perceived this situation as unsatisfactory, given that both systems (BAC/Tn7 entry and homologous recombination, respectively) offer unique beneficial opportunities and particular baculovirus genomes offer specific advantages in production of different classes of recombinant proteins. The BAC/Tn7 entry system, for instance, is thought to be more accessible to non-specialist users without experience in cell culture techniques. The homologous recombination system, on the other hand, has the advantage that it can be efficiently scripted in

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high-throughput robotic routines, which is basically unfeasible with the BAC/Tn7 entry system.6,7 Automation, however, is indispensable to accelerate modern protein research.

We have resolved this issue by creating new "OmniBac" transfer plasmids (Fig. 1, Supplemental Materials). The OmniBac vector suite contains new custom designed acceptor and donor plasmids, both the Tn7 transposon sequences (Tn7L and Tn7R), and at the same time also contain the OmH629 and OmL653 sequences required for homologous recombination (Fig. 1A). The basic design of these Acceptor plasmids follows the logic of the original MultiBac acceptor plasmids and thereby facilitates multiprotein expression. The newly synthesized pOmniBac1 and pOmniBac2 plasmids contain all elements required for gene insertion by SLIC, the homing endonuclease/BstXI based module to facilitate multiplication of expression cassettes on each transfer plasmid and the LuxP sequence for Cre catalyzed fusion of Donor plasmids (pIDC, pIDK and pIDS) with TR offering the option to add further genes (Fig. 1B). The donor plasmids are from the recent MultiBac Acceptor/Donor suite.8 OmniBac Acceptor based multigene transfer plasmids can access all currently available baculoviruses using either homologous recombination, or the BAC/Tn7 entry approach (Fig. 1C).

In a proof of concept, we integrated two CFP genes, one under control of the viral very late promoter polyhedrin, and another one under control of the viral very late promoter polyhedrin, both with the Tn7L/Tn7R sequences. The OmniBac plasmids for Cre-LoxP recombination and production of a test membrane protein (connexin). Connexins are vital proteins mediating cell-to-cell communication.8 Connexin could be purified to homogeneity following expression from a pOmniBac1-Coxxin transfer plasmid transposed into the MultiBac virus genome (~130 kb) derived from wild-type Autographa californica multicapsid nuclear polyhedrosis virus (AcMNPV). This genome has been intensively researched for the previous MultiBac acceptor plasmids (pACEBac1 and pACEBac2) for multigene construct generation by TR. As we show here, the resulting multigene transfer constructs can, for the first time, access all baculoviruses that are presently available. The OmniBac suite thus significantly expands the scope of possible multigene production experiments using BEVS, now providing the option to choose from among all available baculovirus genomes the one most suitable for the specific experiment at hand.

Baculovirus Genome Engineering

Currently, BEVS applications including Multibac rely on a large baculovirus genome (~130 kb) derived from wild-type Autographa californica multicapsid nuclear polyhedrosis virus (AcMNPV). This genome has been intensively researched for many years. Genes that are essential for propagation in cell culture and genes which are detrimental for foreign protein production were delineated by several research groups.9-12 Based on our positive experiences with bottom-up design of plasmids and engineering of the baculovirus genome itself to improve protein production, we became interested in extending our reengineering concept to rewiring the entire baculovirus genome to maximize its performance. Redesigning and restructuring the baculovirus genome for enhanced DNA stability and efficient protein production requires deletion, modification or insertion of DNA sequences. So far, the existing alterations of the wild-type genome were made by classical knockout strategies, and were mostly directed at changes to the genome to facilitate gene insertion, and to improve protein production by removing detrimental protein regions such as viral encoded protease and chitinase. There is a large landscape for engineering the baculovirus genome, given the many non-essential genes that are probably dispensable for virus maintenance and heterologous protein expression. Also, the inherent DNA instability of the current baculovirus genome poses a problem, in particular at expression scales relevant for pharmaceutical production. Simply speaking, as the virus replicates during expression scale up, it progressively suffers from deletion of bits and pieces of its genome, preferentially in the highly expressed, (non-essential) heterologous protein expression cassette, as we have shown already for laboratory scale production.13-16 This is exacerbated for the viruses of the BAC/Tn7 type by the fact that the insertion site which is targeted by the Tn7 transposon is actually a mutational hotspot.17-19 It is clear that the possibilities to improve the baculovirus itself are ample, and are far from exhausted in state of the art systems. Analogous to our approach to minimize transfer plasmids which then could be synthesized de novo, we became interested in the genes and DNA elements which are dispensable under laboratory culture conditions and unnecessary for efficient budded viral production, which is the major virus type used for protein expression in cell culture.20-22 We reasoned that conceivably, the baculovirus can be engineered by removing non-essential genes and regions prone to mutation, with possibly large benefits for virus DNA stability, and also for accommodating very large foreign gene insertions, without compromising the ease of handling and the superior protein production properties offered by the system. For illustration, the polyhedrin gene locus (polyh) is a non-essential viral gene that codes for the polyhedrin protein which produces occlusion bodies. These occlusion bodies can be
observed as large particles in the insect cell nuclei at the very late stage of the infectious cycle. The discovery of polyhedrin as a nonessential gene propelled the generation of BEVS.36,37 The majority of heterologous proteins produced by BEVS utilize a baculoviral genome in which the polh gene is deleted and foreign genes of interest are produced under the control of the polyhedrin promoter. Currently there are several BEVSs with other non-essential gene deletions such as chitinase (c-chi), cathepsin (chiA), p10, p74 and p26 (FlashBacUltra, OET, BacVector3000, Novagen). If the BEVS genome were reengineered, or even designed entirely de novo from scratch, all nonessential genes and known mutational hot spots could in theory be deleted, and all existing entry sites and beneficial modifications integrated into a new baculovirus genome with superior characteristics to anything yet conceived or developed.

A Blueprint for a Synthetic Baculovirus Genome

We performed extensive literature mining on the AcMNPV genome in identifying essential and non-essential genes with respect to cell culture propagation, budded virus production and foreign protein expression. We included comparative analysis on neighboring genomes, which belong to the so-called clade I/II alpha baculovirus group encompassing BmNPV, BunNPV, MvMNPV, PlxyMNPV and RoMNPV. Besides the comparative genome studies, other analyses scrutinized gene syntenies, promoter motifs, repetitive features in origins of replication that are involved in homologous recombination events, and potential transposon integration sites. These were then all compiled and integrated into our baculovirus genome database. Based on these analyses and on Genbank nucleotide RefSeq entry NC_001623.1, we compiled an annotated baculovirus genome map as a blueprint for future genomic engineering (Fig. 2).

In this baculovirus genome map, we show in total 156 (ORF) genes (Fig. 2). Out of these, 62 genes could be classified as (likely) non-essential genes which are probably dispensable (annotated by shades of red color). Ninety-four genes, in contrast, were classified as essential genes (annotated by shades of green color to black) for cell culture propagation. The essential genes were classified based on conservation which includes core genes, core genes with fixed synteny (co-localization of loci within the species), conserved or variable (no-deletion category in clade-I/II alpha baculovirus group), and unique in AcMNPV. Additionally, 9 homologous regions (hr) are shown in the map, which serve as transcriptional enhancers and origins of replication, some of which may not be essential. Genes were classified as non-essential based on the function in the oral infectivity, host cell interactions, cell lysis and moulting inhibition. Non-essential genes also include genes that are involved in inducing apoptosis such as v-cath, genes and genes that were previously reported not to give rise to a deletion phenotype.

Interestingly, when we analyzed the distribution of these annotated essential and non-essential genes, we found that a majority of the essential genes was localized in one half of the baculovirus genome. In contrast, the majority of the genes that could likely be disposed of are found clustered in the other half of the genome. Based on this distribution of essential and non-essential genes, we divided the circular baculovirus genome map into two almost equal sized semicircles (Fig. 2). We observed that close to 55% (35%) of essential genes and 22% (14%) of non-essential genes were located in one semicircle (upper semicircle, Fig. 2), while 35% (22%) of non-essential genes and 44% (28%) of essential genes were located in the other semicircle (lower semicircle, Fig. 2). In addition, the majority of the currently existing BEVSs have gene loci that are either deleted or modified in the upper semicircle (i.e., MultiBac, v-cath and chiA deleted; MultiBac and Bac-to-Bac, Tn7 attachment site replaces polyhedrin site; FlashBac, ORF3626 and polyhedrin site modified; FlashBacULTRA, p10, P74 and p26 genes deleted). This interesting asymmetric distribution of essential and non-essential genes provides ample opportunities to engineer the baculoviral genome by bringing an entire array of synthetic biology methods to bear, to design and synthesize a minimal and functional genome, i.e., a baculovirus genome containing only the essential (coding and non-coding) genetic elements required for its life (cytobacter laboratory of any potential and supporting the highest possible expression levels of functional proteins and protein complexes.

Synthetic Biology: Toward an Optimized Baculovirus Genome

Synthetic biology creates artificial agents that recapitulate the behavior of living systems including inheritance, genetics and evolution.39 Breakthroughs for synthetic biology include novel synthetic genomes with promising phenotypes such as engineered bacteriophages to combat antibiotic-resistant bacteria, inhibition of certain bacterial genetic programs that could improve the efficacy of antibiotic therapies39 and viruses that were customized to invade cancer cells.39 Craig Venter and his associates have reported the design and synthesis of a 1 Mb Mycoplasma mycoides bacterial genome and also of an entire synthetic bacteriophage genome.39,40 Similar approaches can be utilized also for the synthesis and assembly of an optimized, synthetic baculovirus genome. The baculovirus genome, with ~130 kb size, is small compared with the genome of Mycoplasma. By using the methods pioneered by the Venter group, wild-type segments in the baculovirus genome could be iteratively replaced with custom designed synthetic DNA fragments, using for example E. coli or yeast based recombinations as a method of choice for this.40 An alternative, potentially promising approach would be to use evolution by natural selection to generate "minimal and functional baculovirus genomes". It has been shown that baculoviruses, when serially passed in cell culture, are prone to massive auto deletions, resulting in virus populations that are dominated by non-functional, small circular entities that rely on the presence of a few functional viruses that supply the proteins required for replication. It will be interesting to see whether or not evolution experiments can be designed which lead to intermediate deletion genomes that are significantly reduced in size as compared with the wild-type baculovirus, but are still infectious and able to propagate.
Figure 2. Blueprint of the baculovirus genome. The annotated genome of Autographa californica multicapsid nuclear polyhedrosis virus (AcMNPV) is shown in a schematic representation (top). Genes are scored (bottom) on a scale from essential genes that are conserved (no deletion category, shades of green color to black) to non-essential genes (shown to be possibly deletable, shades of red color). The classification applied is detailed in the legend (bottom). The annotated genome map was generated by a self-developed Perl program. Essential and non-essential genes are not randomly distributed but cluster in the genome. The upper semicircle is composed to 56% of the essential genes (and 44% of non-essential genes), the lower, more conserved semicircle is composed to 71% of essential genes (and 29% of non-essential genes).
and produce heterologous protein in cell culture. Alternatively, specimens may be isolated that only need to be reengineered by adding a few genes to restore the desired functions. This could possibly be achieved by serial passaging of baculovirus in insect cells and investigating the distribution of genome sizes within a heterogeneous population of serially passaged viruses.33 Even though this approach has the tendency to restructure their genome by discarding genes by natural recombination events,23 resulting in defective-interfering particles and other minimal baculovirus genome that are not well characterized. It will be potentially highly beneficial to revisit such virus subpopulations and compare their phenotype with wild-type in terms of stability, functionality and quality of budded virus, thereby identifying additional features that may improve heterologous protein production.

Future Perspective

BEVS is emerging increasingly as a method of choice for the production of eukaryotic proteins for a wide range of applications in academic and industrial research and development. Since its inception more than three decades ago, BEVS have been improved, one step at a time, for simplifying handling and optimizing protein production properties. More recently, the introduction of the MultiBac system has contributed to unlocking the realm of large multiprotein complexes to functional studies to understand protein sociology and biological function in the cell, and to develop new and better drugs by exploiting, in the future, the tremendous amount of data amassed through the genomics and proteomics studies that increasingly dominate current research efforts in the life sciences.

BEVS today came mostly in two flavors, differing in their heterologous gene integration strategies (BAC/Ti plasmid entry and homologous recombination, respectively) that were mutually exclusive. As a consequence, the initial transfer plasmids into which the genes of choice were inserted predetermined which baculovirus genomes were available for the protein expression experiment. A number of virus variants exist under each of the two categories, each with its own merits. We have presented here our novel OmniBac plasmids, which combine the DNA elements required for both gene integrations strategies, and are therefore compatible with all currently available baculoviruses. The OmniBac plasmids were shown to function equally well with all baculoviruses tested. Further, they are also fully compatible with the tandem recombinering approach we developed to facilitate multiprotein insertion into the baculovirus of choice. We anticipate that the OmniBac plasmids will in the future substitute for currently used transfer plasmids for many applications, including high-throughput pipelines that are becoming increasingly utilized for heterologous expression of eukaryotic target proteins in insect cells.

We hypothesize that the custom design strategies that lead us to minimal transfer plasmids such as OmniBac and the other components of the MultiBac transfer plasmid suite will also be beneficial if applied to the baculovirus genome itself, which is currently only marginally optimized for heterologous protein production. Toward this end, we have used comparative genomics and data mining, and realized that the essential genes on the one hand, and the non-essential genes on the other, are clustered together on the baculovirus genome. This opens up interesting possibilities to rewire the genome, potentially also entirely by design-from-scratch in a bottom-up approach, by applying synthetic biology techniques for genome engineering. This endeavor will be complemented by in vitro evolution, using serial passaging and exploiting the innate tendency of the baculovirus to rearrange and delete segments of the genome in the process. Together, these two approaches should prove to be effective in creating a new and synthetic, minimal baculovirus which can then be customized for a variety of applications, for example by introducing modifiers and accessory proteins into the viral backbone that assist assembling an active and functional multiprotein complex of choice.

Improving and optimizing recombinant protein expression using BEVS is not limited by modifying solely the baculovirus genome itself, but can likewise include adapting the host system (insect cells). For example, insect cell lines expressing enzymes that reconstitute the human glycosylation pathway, were generated by genomic engineering.34 With BEVS becoming increasingly prominent in recent years, we believe there are ample opportunities and a substantial scope for new and improved, customized baculovirus genomes that may be engineered to fit the tandem recombinering approach we developed for high performance heterologous protein production, to accelerate academic basic research and likewise protein-based drug discovery in the pharmaceutical and biotechnology industries in the near future.

Materials and Methods

OmniBac plasmids were custom designed from scratch and assembled from synthetic DNA fragments (Genscript Corp.) using standard DNA procedures. Plasmid maps and sequences are provided in the Supplement. Generation of composite baculoviruses (MultiBac, Bac-to-Bac, FlashBac, BacVector3000) was performed using published protocols35 or following the manufacturers’ recommendation. GFP fluorescence was recorded as described previously.36 Full-length Connexin 36 (GenBank Nr. BC069339) was cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Strep) tag spaced by a TEV protease cleavage site were cloned into pOMiniBac1 via EcoRI and HindIII, and a calmodulin binding peptide (CBP) and deca-histidine tagging peptide (Stre
sodium bicarbonate solution to remove sucrose and solubilized overnight with 2% n-Dodecyl β-D-maltoside (DDM) and further purified by metal affinity purification (Ni-NTA, Qiagen). Drebrin was purified by using Steep-Tactin affinity-resin (IBA GmbH) followed by size exclusion chromatography (SEC) using a Superose 6 column. Proteins were analyzed by 12% SDS-PAGE.

The data used for creating the baculovirus genome map shown in Figure 2 were derived from mining 1253 relevant literature papers found in NCBI PubMed and published up to October 2011 on information for, e.g., genome sequences, gene essentiality and conservation, protein product function and localization, protein–protein interactions, mRNA expression and gene regulation. All available database annotations on gene product function were collected from NCBI GenBank, NCBI Protein, NCBI VOG clusters of related viral proteins and the UniProt database with a Perl program, which piped the clustering program blastclust, a part of the NCBI C-toolkit legacy blast package. Categories for conservation and variability were assigned for different lineages of baculoviruses: all baculoviruses with conserved synteny (core), all baculoviruses (core), lepidopteran baculoviruses with conserved synteny (core+) and all lineages of baculoviruses: all baculovi-

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