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Transduction of baculovirus vectors to queen honeybees, *Apis mellifera*

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Abstract – The potential uses of baculovirus as a gene vector to queen honeybees were examined in this study. We used a green fluorescent protein-expressing baculovirus with wild-type envelope and two pseudotype viruses of which one overexpressed GP64 and the other expressed a virion protein of a honeybee virus on the envelope. After injection of these baculoviruses into queen pupae, infection was detected in the fat bodies, but not in the ovaries. Pupae injected with a titer of 1×10^5 infectious units survived to eclose, and the infection was also detected in the fat bodies of adult queen honeybees, suggesting that baculoviruses can transiently express genes in the fat bodies, and therefore, can be used for further analysis of gene functions. In addition, although the viruses examined in this study failed to express the reporter gene in the ovaries, the infection in the fat bodies suggests that baculoviruses could be potentially useful for transgenesis, if appropriately developed.

honeybee / virus vector / gene transfer / transgenesis / pseudotype

1. INTRODUCTION

Insect transgenesis is important for studies on gene functions and is essential in the establishment of useful transgenic insects. For stable transgenesis to occur, exogenous genes must be introduced into germ line cells. A common method to introduce an exogenous gene into the genomes of insect germ line cells is embryonic microinjection of two plasmids: a helper plasmid carrying a transposase gene originating from a transposon such as *P* element or *piggyBac* and a donor plasmid carrying the gene of interest inserted between a set of recognition sites of

the transposase (reviewed by Handler 2001; Kimura 2001; Handler 2002). Using this technique, transgenic lines have been established in various insect species, including *Drosophila melanogaster* (Rubin and Spradling 1982), *Bombyx mori* (Tamura et al. 2000), *Aedes aegypti* (Coates et al. 1998; Jasinskiene et al. 1998), *Anopheles gambiae* (Grossman et al. 2001), *Culex quinquefasciatus* (Allen et al. 2001), *Tribolium castaneum* (Berghammer et al. 1999), *Ceratitidis capitata* (Loukeris et al. 1995), *Athalia rosae* (Sumitani et al. 2003), and *Bicyclus anynana* (Marcus et al. 2004). In addition to embryonic microinjection, exogenous genes can also be introduced into cells by utilizing the infection ability of certain viruses, such as the baculovirus *Autographa californica* nucleopolyhedrovirus (AcNPV), whose natural

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host is the alfalfa looper moth, *A. californica*. The AcNPV genome is a double-stranded DNA that is maintained extrachromosomally in host cells. Successful gene transduction into germ line cells has been performed using two recombinant AcNPVs: a helper virus carrying a transposase gene and a donor virus carrying the gene of interest within the recognition sites (Yamamoto et al. 2004; Kawashima et al. 2007).

The European honeybee, *Apis mellifera* L., is a beneficial insect that provides useful products and mediates the pollination of agricultural plants. The honeybee is also an interesting model organism for basic biology to explore the insect sociality and brain function because it has a sophisticated eusociality and exhibits high abilities of learning, memory, and communication. Several molecular biological studies can be performed using the honeybee. The whole genome sequence of the honeybee has been determined (Honeybee Genome Sequencing Consortium 2006), and microarrays are available for genome-wide expression analyses. In addition, RNA interference (RNAi) is useful in determining gene functions of honeybees (Amdam et al. 2003; Nunes and Simões 2009). However, a method to produce transgenic honeybees has not been established. This is partly because the honeybee larvae are naturally grown in hives and cared for by workers and handling of eggs or larvae in the laboratory is laborious, even though possible (Robinson et al. 2000; Aase et al. 2005; Patel et al. 2007; Kucharski et al. 2008). To date, only transient expressions of exogenous genes have been reported for the honeybee using techniques such as electroporation of adult brain cells (Kunieda and Kubo 2004), instrumental insemination of sperm mixed with a plasmid (Robinson et al. 2000), and injection of an AcNPV vector into worker larvae and pupae (Ando et al. 2007).

Wild-type baculovirus forms two types of virions: occlusion-derived and budded virions of which the latter is utilized as a gene vector. The budded virion is surrounded by a phospholipid bilayer (envelope) that contains membrane proteins. The major envelope protein, GP64, is critical for attachment to host cells, induction of

endocytosis, membrane fusion and budding of the virus (Volkman and Goldsmith 1985; Blissard and Wenz 1992; Hefferon et al. 1999; Oomens and Blissard 1999). The infectivity of a baculovirus can be manipulated by modifying GP64, e.g., overexpression of this protein drastically increases the infection of baculovirus to mammalian cells (Tani et al. 2001, 2003). The infectivity to mammalian cells can also be enhanced by displaying a virion protein of a mammalian virus outside the envelope as a fusion protein with GP64; this technique is referred to as the surface display method (Boublik et al. 1995; Ernst et al. 2006; Matilainen et al. 2006).

One approach that can be used to obtain a transgenic honeybee line is to integrate an exogenous gene into the gonads of individuals with reproductive potential. In this study, we examined the ability of a baculovirus to infect queen honeybees and evaluated its ability to transiently express genes in queen honeybee tissues especially in the ovaries, which is an essential process for mediating the integration of an exogenous gene into the genome using a helper/donor system. To achieve these objectives, we used queen pupae with actively developing ovaries and examined the following: (a) infectivity of the baculovirus into queen pupae and its effect on host viability and (b) whether the tropism of this virus to the ovaries is increased by GP64 overexpression or the surface display of a virion protein of the honeybee deformed wing virus (DWV). We injected a baculovirus with wild-type envelope and two pseudotype viruses into queen pupae and detected the infection of these viruses in the fat bodies, but not in the ovaries. The potential uses of baculoviruses as gene transfer vectors for queen honeybees are discussed.

2. MATERIALS AND METHODS

2.1. Preparation of recombinant baculoviruses

First, plasmids containing the insertion sequences shown in Figure 1a were prepared. A green fluores-

cent protein (GFP) expression cassette was amplified by a polymerase chain reaction (PCR) from the pAcGFP1-C1 plasmid (Clontech, Palo Alto, CA, USA) and ligated into the pENTR/D-TOPO plasmid (Invitrogen, Carlsbad, CA, USA) to obtain pENTR/WT/GFP. The *polyhedrin* promoter (P_{PH}), *gp64* coding sequence, and SV40 poly(A) signal were amplified by PCR and inserted into pENTR/WT/GFP to obtain the pENTR/64+/GFP plasmid. PCR-amplified P_{PH} , *gp64* signal peptide sequence, coding sequence of DWV virion protein 1 (*VP1*; see below), and *gp64* mature protein sequence were ligated to form a *VP1* surface display cassette. This cassette was subsequently inserted into the pENTR/WT/GFP to construct a pENTR/*VP1*/GFP plasmid.

Second, recombinant baculoviruses (AcNPV) designated WT/GFP, 64+/GFP, and *VP1*/GFP were prepared using these constructed plasmids and the BaculoDirect Baculovirus Expression System (Invitrogen) according to the manufacturer's recommendations. The plasmids were recombined with baculovirus genomic DNA using LR clonase II (Invitrogen) to replace the *polyhedrin* coding sequence of the baculovirus genome with the insertion sequences of the plasmids. Subsequently,

the recombinant virus DNA was transfected into Sf9 cells (Invitrogen), an AcNPV-permissive insect cell line derived from *Spodoptera frugiperda*. Viral virions that budded from the cultured cells were concentrated by centrifugation of the culture media at $144,000\times g$ for 1 h (Tani et al. 2001) and resuspended in phosphate-buffered saline (PBS; 137 mM NaCl, 8.1 mM Na_2HPO_4 , 2.68 mM KCl, 1.47 mM KH_2PO_4 , pH 7.5). Titers of the viral solutions were determined as infectious unit (IFU) using the BacPAK Baculovirus Rapid Titer Kit (Clontech).

2.2. Isolation of DWV *VP1*

Total RNA was extracted from an adult worker honeybee with deformed wings using the Total RNA Isolation System (Promega, Madison, WI, USA). First-strand cDNA was synthesized using SuperScript III (Invitrogen) and a gene-specific primer DWV_VP1_SmaI_R (5'-TCC CCC GGG TTC TGG AAT AGC CTC AAT AAA-3'). The full-length cDNA of DWV *VP1* (accession no. AY292384; Lanzi et al. 2006) was amplified using the primers DWV_VP1_KpnI_F (5'-CGG GGT ACC GAT AAT

Figure 1. Baculovirus vectors inoculated into queen honeybees. **a** Structures of insertion sequences used. WT/GFP carries a GFP expression cassette comprising a cytomegalovirus immediate-early gene promoter (P_{CMV}), *Aequorea coerulescens* GFP gene (*AcGFP1*) and SV40 poly(A) signal. The GP64 overexpression cassette in 64+/GFP consists of the *polyhedrin* promoter (P_{PH}), *gp64* coding sequence, and SV40 poly(A) signal. *VP1*/GFP has the deformed wing virus (DWV) *VP1* coding sequence in the 64+/GFP cassette between the GP64 signal peptide sequence (SP) and GP64 mature protein sequence. **b** Western blot analysis of Sf9 cells infected with recombinant baculoviruses. Sf9 cells were subjected to Western blot analysis using an anti-GP64 monoclonal antibody at 3 days after infection with recombinant baculoviruses.

CCT TCT TAT CAA CAA-3') and DWV_VP1_SmaI_R. The PCR product was cloned and sequenced to confirm its identity.

2.3. Western blot analysis

Sf9 cells were infected with the recombinant viruses (see above) at a multiplicity of infection of 0.4, and 1 mL of each cell culture was collected by centrifugation 3 days after infection. Cells were lysed with 100 μ L of an SDS-PAGE sample buffer (50 mM Tris-HCl, 2% SDS, 6% β -mercaptoethanol, 12% glycerol, 0.025% bromophenol blue, pH 6.8) and boiled. A 0.1- μ L aliquot of each protein sample was subjected to Western blot analysis. Western blotting was performed according to the method of Towbin et al. (1979) with modifications. The blotted membrane was incubated with an anti-GP64 monoclonal antibody (Cosmo Bio, Tokyo, Japan) and subsequently with a horseradish peroxidase-conjugated, affinity-purified goat anti-mouse IgG2b antibody (Cosmo Bio). Immunoreacted bands were detected using a VersaDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA).

2.4. Rearing of queen pupae and virus injection

Queen honeybees were reared by transferring 1st instar larvae to artificial plastic queen cell cups. Queen larvae undergo four larval molts at approximately 24-h intervals. Following the last larval molt, the queen cell is sealed and the queen larva completes pupation about 3 days after the cell is sealed (Laidlaw and Page 1997). Pupal development in the queen honeybee takes about 5 days. To inoculate the virus, queen pupae were collected 4 days after the cells had been sealed (8–9 days after the transfer of the larvae to the queen cell cups) and injected with the viral solutions into the abdominal region using a 33-G needle (TERUMO, Tokyo, Japan). The injected queen pupae were placed on a filter paper in a 6-well plate and kept in an incubator at 33°C and 70% relative humidity.

2.5. Analysis of reporter gene expression in adult fat bodies

Transcription of *gfp* was assessed in the fat bodies of adult queen honeybees injected with WT/GFP at

the pupal stage. Abdominal carcasses (integument and subjacent fat body) were collected by removing the entire digestive system and the ovaries from queen honeybees at 24–48 h after eclosion. These carcasses were used as the source of fat bodies (Bitondi et al. 2006). Total RNAs were extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and reverse-transcribed (RT) using SuperScript III and oligo(dT)₂₀ primer (Invitrogen). A partial 400-bp *gfp* sequence was PCR-amplified using the primers 5'-GCA GTG CTT CTC ACG CTA C-3' and 5'-CAG GTA GTG GTT ATC GGG C-3'. A 181-bp fragment of a β -actin sequence (AB023025) was amplified using primers 5'-AGG AAT GGA AGC TTG CGG TA-3' and 5'-AAT TTT CAT GGT GGA TGG TGC-3' (Chen et al. 2005a) and used as a positive control. PCR cycle conditions were as follows: 94°C for 5 min followed by 25 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min, and 72°C for 5 min. The PCR products were cloned and sequenced to confirm their identities.

3. RESULTS

3.1. Inoculation of baculovirus into queen pupae

To examine the infectivity of the baculovirus in the queen honeybee, we prepared a recombinant baculovirus (WT/GFP) that carried *gfp* under the regulation of the cytomegalovirus immediate-early promoter (P_{CMV} ; Figure 1a, top panel). P_{CMV} is known to promote gene expression in worker honeybees (Kunieda and Kubo 2004). Before injecting the WT/GFP virus into queen honeybees, we injected a baculovirus without *gfp* into worker pupae to confirm that the virus without *gfp* did not induce significant fluorescence (data not shown). When the WT/GFP virus vector was inoculated into the abdomen of queen pupae at a titer of 1×10^5 IFU, fluorescence was detected around the injected site in all injected pupae within 3 days after inoculation. The fluorescence was limited to the injection site without extending much into the surrounding tissues (Figure 2a–d; Table I); this observation was consistent with observations of worker pupae reported by Ando et al. (2007).

On dissecting the abdomens of the pupae, we did not detect infection in the ovaries (Figure 2q–t). Instead, strong fluorescence was observed in the fat bodies (Figure 2i–l). Punctate fluorescence was observed in the removed ovaries, but this fluorescence was probably from the remnant fat body cells attached to the ovaries (Figure 2q–t). All individuals injected with the virus vector at 1×10^5 IFU eclosed to adult queens, in which fluorescence was detected in the fat bodies, but not in the ovaries (Table I; Figure 3a–d, i–l). Transcription of *gfp*

in the fat bodies of adult queen honeybees was confirmed by an RT-PCR assay of adult queen honeybees at 24–48 h after eclosion (Figure 4). When the virus was injected at a higher titer (1×10^6 IFU), fluorescence was observed in a wider region compared with that observed on injection at 1×10^5 IFU (Figure 2a–d, S1a–d). However, there was still no observable fluorescence in the ovaries (Figure S1i–l). At 1×10^6 IFU, only approximately half of the individuals survived to eclose (Table I), and the wings of the eclosed adults were noticeably shrunk

Figure 2. Queen pupae at 3 days after inoculation with baculovirus vectors at a titer of 1×10^5 IFU. Queen pupae were injected with PBS (a, b, i, j, q, r), WT/GFP virus (c, d, k, l, s, t), 64+/GFP virus (e, f, m, n, u, v), or VP1/GFP virus (g, h, o, p, w, x). Arrow heads indicate the injected points. a–h Outward appearances of injected pupae. i–p Internal views of the dissected abdominal region. q–x Removed ovaries from injected pupae. Punctate signals on the ovaries indicated by the arrows were not derived from the ovaries but from the remnant fat body cells attached to the ovaries. Bars indicate a scale of 5 mm.

Table I. Eclosion rates and fluorescence in queen honeybees abdominally injected with baculovirus vectors at the pupal stage.

Injection	Infectious units	Eclosion (%)	Fluorescence (%)	
			In the pupal stage	After eclosion
PBS	0	8/8 (100)	0/9 (0)	0/8 (0)
WT/GFP	1.0×10 ⁵	7/7 (100)	8/8 (100)	7/7 (100)
	1.0×10 ⁶	5/9 (56)	10/10 (100)	5/5 (100)
64+/GFP	1.0×10 ⁵	7/7 (100)	8/8 (100)	7/7 (100)
	1.0×10 ⁶	5/9 (56)	10/10 (100)	5/5 (100)
VP1/GFP	1.0×10 ⁵	7/7 (100)	8/8 (100)	7/7 (100)
	1.0×10 ⁶	4/9 (44)	10/10 (100)	4/4 (100)

(data not shown). Thus, inoculation at this titer seems to be harmful to queen pupae.

3.2. Injection of pseudotype baculovirus vectors into queen pupae

Expecting that GP64 overexpression might enhance baculovirus tropism in honeybee ovaries, we prepared a pseudotype baculovirus vector (64+/GFP) that contained an additional *gp64* coding sequence under the regulation of the strong *polyhedrin* promoter (Figure 1a, middle panel). As another strategy to alter the infectivity of the baculovirus, we also prepared a pseudotype baculovirus (VP1/GFP) that displayed the VP1 of DWV as a fusion protein with GP64 on the surface of the baculovirus envelope (Figure 1a, bottom panel). DWV is known to infect various tissues of worker honeybees (Yue and Genersch 2005; Lanzi et al. 2006), most prominently in the pupal stage (Chen et al. 2005a), and also infect queen honeybees (Chen et al. 2005b; Williams et al. 2009). Insect picorna-like viruses including DWV resemble the mammalian picornaviruses, i.e., both have a single-stranded RNA as the genome and the form, an icosahedral capsid composed of four types of virion proteins (VP1–VP4; Lanzi et al. 2006). Since the host cell binding site is located on VP1 in mammalian picornaviruses (Fox et al. 1989; Colston and Racaniello 1994; Tate et al. 1999; Liljas et

al. 2002; Estrozi et al. 2008), we hypothesized that the host cell-interacting site of DWV is also on VP1.

To confirm the GP64 overexpression and expression of the GP64-VP1 fusion protein, we analyzed the total proteins from Sf9 cells, with which the virus vectors were produced, by Western blot analysis using an anti-GP64 monoclonal antibody. The signal for GP64 was detected in 64+/GFP virus-infected cells at a level far higher than that in WT/GFP virus-infected cells. A band of approximately 100 kDa (the size expected for the fusion protein of GP64 and DWV VP1) was detected from VP1/GFP virus-infected cells in addition to endogenous GP64 (Figure 1b).

We injected these pseudotype baculoviruses into the abdominal regions of queen pupae at 1×10⁵ IFU and detected fluorescence in all pupae within 3 days of inoculation (Table I; Figure 2a, b, e–h). Dissection of the abdomen showed that the fat bodies emit significant fluorescence (Figure 2i, j, m–p), but the ovaries did not (Figure 2q, r, u–x). All injected individuals eclosed to adult queen honeybees (Table I), in which fluorescence was detected in the fat bodies (Figure 3a, b, e–h), but not in the ovaries (Figure 3i, j, m–p). Even when the viral titer was increased to 1×10⁶ IFU, no fluorescence was detected in the ovaries, and approximately half of the injected pupae died just before metamorphosis (Figure S1i, i, m–p;

Figure 3. GFP expression in adult queen honeybees inoculated with baculovirus vectors at a titer of 1×10^5 IFU at the pupal stage. The queen honeybees were injected with PBS (**a, b, i, j**), WT/GFP virus (**c, d, k, l**), 64+/GFP virus (**e, f, m, n**), or VP1/GFP virus (**g, h, o, p**). **a–h** Internal views of the dissected abdominal region. **i–p** Removed ovaries from adult queen honeybees. *Arrows* indicate punctate signals derived from the remnant fat body cells attached to the ovaries. *Bars* indicate a scale of 5 mm.

Table I). These observations suggest that neither GP64 overexpression nor a surface display of DWV VP1 can effectively enhance baculovirus tropism in the ovaries.

4. DISCUSSION

We examined the ability of three recombinant baculoviruses to induce exogenous gene expres-

Figure 4. RT-PCR used to assess GFP expression in the fat bodies of adult queen honeybees. The queen honeybees were infected with WT/GFP at 1×10^5 IFU or PBS at the pupal stage, and their fat bodies were collected at 24–48 h after the final ecdysis. Bands of expected size were amplified from cDNA synthesized with reverse transcriptase (RT+), but not from negative controls (RT–).

sion in queen honeybees. In all cases, no significant gene expression was detected in the ovaries, but the virus vectors did express GFP in the fat bodies (Figures 2, 3, and S1). Some genes expressed in the fat body, such as *vitellogenin* and factors of insulin-like signaling pathway, have been suggested to be involved in caste differentiation (Seehuus et al. 2006; Corona et al. 2007). The baculovirus would be a useful tool for the analyses on such interesting genes. Baculovirus could be used to express double-stranded RNA and induce RNAi in loss-of-function experiments and to overexpress exogenous genes in gain-of-function experiments.

After inoculation of the viruses, the GFP-positive region remained near the injected site and did not extend much into the surrounding tissues (Figures 2 and 3): this observation was consistent with observations of worker pupae by Ando et al. (2007). It suggests that inoculated viruses can be transduced into host cells, but they cannot spread further through secondary infection. New infectious virions seem to be rarely produced in honeybees. This property is preferable for the use of baculovirus as a gene transfer vector. In fact, baculovirus is considered to be an attractive candidate for a gene transfer vector in humans, partly because it does not cause secondary infection in mammalian cells (reviewed in Yu-Chen 2005).

We demonstrated that inoculations to pupae at 1×10^5 IFU caused gene transduction and allowed all injected pupae to eclose (Figure 2; Table I). We assume that the toxicity of baculovirus to honeybee pupae is relatively low probably due to the low ability to cause secondary infection. To utilize the baculovirus as a gene vector for honeybee transgenesis, an inoculation titer at which the queen honeybees are infected and survive must be used. The results of this study suggest that baculovirus has a titer that meets this necessary condition and hence, imply that methods using baculoviruses could be potentially useful for stable transgenesis if the virus is appropriately constructed such that the virus can efficiently infect and induce exogenous genes in the ovaries.

In contrast to the fat bodies, no significant fluorescence was detected in the ovaries. The viruses used might be unable to infect the ovaries or the infection levels might be, if any, very low. Although we attempted to alter the infectivity of baculovirus by overexpression of GP64 and a surface display of DWV VP1, both modifications appeared not effective. We used DWV VP1 because the surface display of a partial sequence of a mammalian picornavirus VP1 was reported to increase the infectivity of baculovirus in mammalian cells (Ernst et al. 2006; Matilainen et al. 2006). One possible explanation of the results obtained in this study is that the host-binding site of DWV might reside in virion proteins other than VP1. Although insect picorna-like viruses and mammalian picornaviruses have similar constitutions, including their sizes and icosahedral structures of the four types of virion proteins, the amino acid sequences of VP1 are distinct between the two virus groups. In addition, crystal structures of VP1 from insect picorna-like viruses and picornaviruses are somewhat different (Tate et al. 1999; Liljas et al. 2002; Estrozi et al. 2008). Therefore, surface displays of other virion proteins (VP2–VP4) might effectively alter virus tropism in honeybee ovaries. Another interpretation for the failure to detect the GFP signal from the ovaries may be that the reporter gene was not expressed, despite the fact that the virus infected the ovaries. The CMV promoter that functions in the fat bodies might not function in the ovaries. It might be worth using other promoters to study this possibility.

The queen honeybee is polyandrous and mates with males at so-called “drone congregation areas” in nature. However, instrumental insemination of virgin queen honeybees with sperm is a standard procedure (Laidlaw 1977; Harbo 1985), and honeybee colonies can be maintained in an indoor flight room for several generations (Robinson et al. 2000). Once a method for germ line transformation is developed, transgenic honeybees could be produced, which will contribute to molecular biological studies of honeybees.

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Transduction de vecteurs baculovirus sur des reines d'abeilles, *Apis mellifera*.

abeille / vecteur de virus / transfert de gène / transgénèse / pseudotype

Zusammenfassung – Der Einsatz von Baculovirus-Vektoren zur Transduktion bei Königinnen der Honigbiene, *Apis mellifera*. Die europäische Honigbiene, *Apis mellifera* L., ist ein Modell für Studien über Sozialität bei Insekten. Verschiedene molekularbiologische Ansätze können an der Honigbiene verfolgt werden. Das Genom ist komplett sequenziert und Mikroarrays sind verfügbar für genomumfassende Expressionsstudien. Des weiteren besteht die Möglichkeit, RNA-Interferenz (RNAi) für Untersuchungen über Genfunktionen einzusetzen. Eine Methode zur Herstellung transgener Honigbienen ist jedoch noch nicht etabliert. Wir untersuchten hier die Möglichkeit, ob sich Baculoviren zur Herstellung transgener Honigbienen eignen. Baculovirus-Vektoren werden bereits erfolgreich in der Produktion transgener Insekten, wie zum Beispiel des Seidenspinners, eingesetzt. Wir stellten ein Baculovirus her, das ein grün fluoreszierendes Protein (GFP) als Reportergen enthält (Abb. 1) und inokulierten dieses in das Abdomen von Puppen. Fluoreszenz war in der Umgebung des Injektionsorts bereits innerhalb von drei Tagen detektierbar (Abb. 2). Nach Dissektion des Abdomens der Puppen fanden wir dass das GFP-Gen zwar im Fettkörper, jedoch nicht in den Ovarien exprimiert war (Table 1; Abb. 2, S1). Alle mit einem Baculovirus-Titer von 1×10^5 Infektionseinheiten inokulierten Puppen entwickelten sich zu adulten Königinnen, in denen die Fluoreszenz nach wie vor im Fettkörper, jedoch

nicht in den Ovarien zu finden war (Table 1; Abb. 3 und 4). Um den Infektionsgrad des Virus zu erhöhen und das Zielgen in den Ovarien zu exprimieren, stellten wir zwei andere Baculoviren her (Abb. 1). Ein Virus überexprimierte GB64, ein Protein, das für die Anheftung und das Eindringen des Virus in Wirtszellen von Bedeutung ist. Das andere Virus enthielt ein Kapselprotein des Flügeldeformations-Virus (DWV), das Honigbienen natürlicherweise befällt. Nach Injektion dieser Viren zeigte sich, dass auch diese Viren vorzugsweisen den Fettkörper, befielen, jedoch nicht in die Ovarien eingedrungen waren (Table 1; Abb. 2, 3, und S1).

Die Ergebnisse dieser Studie weisen darauf hin, dass Baculoviren für Untersuchungen von Genfunktionen in Fettkörperzellen geeignet sein können. Obwohl keines der drei Baculoviren die Expression des GFP-Reportergens in den Ovarien ermöglichte, besteht dennoch die Möglichkeit, Baculoviren für potentiell stabile Transgenese bei Honigbienen einzusetzen, wenn sie so modifiziert werden können, dass sie effizient die Expression exogener Gene in den Ovarien induzieren.

Honigbiene / Virusvektor / Gentransfer / Transgenese / Pseudotypus

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