Coral red fluorescence protein as genetic modified baculovirus tracer

Tzyy-Rong Jinn a,b, Suey-Sheng Kao b, Jason T.C. Tzen b, Tzong-Yuan Wu c,∗

a Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan
b Biopesticide Department, Taiwan Agriculture Chemicals and Toxic Substances Research Institute, Wufeng, Taiwan
c Department of Bioscience Technology, Chung Yuan Christian University, Chung Li, Taiwan

Received 22 November 2004; received in revised form 4 April 2005; accepted 12 April 2005

Abstract

Genetic modified baculovirus (GMBV) are among the most promising alternatives to chemical insecticides. One of the deterrents to the GMBV development is the lack of simple and cost-effective methods for monitoring their efficacy and ecology in fields. Here, we demonstrate the DsRed gene from coral can serve as a convenient GMBV tracer. Insect larvae, including Trichoplusia ni, Spodoptera exigua, and Spodoptera litura, infected the GMBV containing the DsRed gene can emit red fluorescence under sun light without any prosthetic apparatus.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Baculovirus, Red fluorescent protein, Green fluorescent protein, Insect larvae

Bio-insecticides developed from insect-specific pathogens are alternatives to chemical insecticides. Among these biological insecticides, baculovirus are becoming recognized as important agents for the control of insect pests. Baculoviruses are known to infect over 500 different insect species of insects, mainly Lepidoptera. There are 16 countries using baculovirus to control Lepidoptera and more than 30 species of baculovirus have developed as microbial insecticides (Moscardi, 1999). The major drawbacks of baculovirus as insecticide are the slow insect pests killing efficiency and narrow host range. Several studies had indicated that genetic modified baculovirus (GMBV) containing the insect-specific neurotoxin could improve the efficiency of baculovirus as insecticide. These toxins containing GMBVs may be the best way to overcome the relatively slow action of wild type baculovirus as insecticide (Cory et al., 1994). However, public concern was generated as regarding these studies because a recombinant virus containing a toxin gene, such as scorpion toxin, was released into the field (Maeda, 1995). Thus, a convenient tracer is critical for the use of the GMBV as insecticide.

Since its introduction in 1994, the green fluorescence protein (GFP) gene from Aequorea victoria...
(Chalfie et al., 1994) has become a standard reporter gene in molecular biology studies because of its intrinsic bright, visible fluorescence derives from an internal fluorophore within the protein structure upon excitation with blue light and because no substrates or co-factors are needed (Prasher, 1995; Kendall and Badminton, 1998). In addition to as an indispensable tool for monitoring cellular events such as protein localization and gene expression, GFP can also serve as an easily visible marker for detecting recombinant baculovirus infected insects, diamondback moth *Plutella xylostella* (Chao et al., 1996). However, the infected larvae and the uninfected larvae were not distinguishable under general laboratory light. A long UV lamp was required to monitor the infected ‘green fluorescence’ larvae in dark. Recently, a red fluorescence protein (DsRed) gene with an excitation peak at 558 nm and an emission peak at 583 nm was recently cloned from the reef coral Discosoma sp. (Matz et al., 1999). This non-biofluorescent coral red-protein pigment may convert the short wavelength light component of the solar radiation into a longer wavelength light for photosynthesis by algal endosymbionts (Matz et al., 1999). Thus, we hypothesized that this reef coral red fluorescent protein could emit red fluorescence just by sunlight and could act as GMBV tracer even without a prosthetic lamp.

We tried to express GFP and DsRed fluorescent genes simultaneously in insect larvae to evaluate which fluorescence protein is relative bright and is suitable to act as GMBV tracer. We employed the internal ribosome entry site (IRES) element of encephalomyocarditis virus (EMCV) to construct a bi-cistronic baculovirus expression vector. The IRES of EMCV had been widely used in b-cistronic expression vector of mammalian cells (Dirks et al., 1995). To construct an EMCV-IRES-based bi-cistronic baculovirus transfer vector for dual fluorescence protein genes expression, we first digest the pRRES-EGFP plasmid (Clontech) with EcoRI and SalI and subcloned the 2.2 kb IRES-EGFP DNA fragment into AcMNPV transfer vector pBlueBac4.5 (Invitrogen). The resulting plasmid was named pBacIRE. The DsRed gene from the plasmid pDsRed1-N1 (Clontech) was PCR amplified with primers resulting in a DNA fragment containing NheI restriction site on 5′ end and EcoRI restriction site on 3′ end. Following is the sequence of the primers: 5′NheI ATCGGCTAGCGGCACCAACCATGGTGGCTCT and 3′EcoRI GTAGGAAATTCGCTACAGGAACAGGTGGTGGG (EcoRI sites are underlined). The PCR amplified DNA fragment was cloned into the NheI and EcoRI site of the transfer vector pBacIRE and the resulting was named pBacDIRE. pBacDIRE was cotransfected with linearized viral DNA, Bac-N-Blue (Invitrogen), and the resulting recombinant virus was named vAcR-IR-G (Fig. 1A). Furthermore, a recombinant AcMNPV containing the EGFP gene only was also constructed as control. The EGFP gene from the plasmid pEGFP-C1 (Clontech) was subcloned into the baculovirus transfer vector pAcUW21 (PharMingen) and the resulting plasmid was named pAcP10EGFP. The EGFP gene is under the control of p10 promoter of AcMNPV. pAcP10GFP was cotransfected with linearized viral DNA, vAcRP23.LacZ (PharMingen), and the resultant recombinant virus was named vAcP10GFP.

We found that both green fluorescence and red fluorescence could be detected in the insect Sf9 cells infected vAcR-IR-G under fluorescence microscope (Fig. 1). At 3 days postinfection, some cells revealed green fluorescence under blue light excitation but not emitted red fluorescence under green light stimulation and the cells that appeared red fluorescence all revealed yellow under blue light excitation (Fig. 1B and C). These ‘yellow cells’ may suggest that both DsRed protein and EGFP protein were evenly expressed and excited, and the dual fluorescence signals merged. However, the infected Sf9 cells appeared stronger red fluorescence than green fluorescence at 4 days postinfection (Fig. 1D and E). This may reflect the slower maturation for the DsRed protein than EGFP protein after translation (Baird et al., 2000). These results may indicate that the EMCV IRES can mediate the cap-independent translation in Sf9 cells but not be functional well in insect cells. This observation is compatible with previous studies that analyzed EMCV IRES in insect cells (Finkelstein et al., 1999; Woolaway et al., 2001).

To examine if the dual expression of DsRed and EGFP could be performed in insect larvae, third-instar *Trichoplusia ni* (T. ni) larvae were infected by microinjection with vAcR-IR-G. The larvae infected with vAcP10-G were used as control. As expected, green fluorescence was detected for the vAcp10-G infected larvae under long-wavelength (365 nm) ultraviolet light excitation (Fig. 2A, left). While most
Fig. 1. Dual expression of the fluorescence genes DsRed and EGFP in baculovirus infected Sf9 cells. (A) The construction of the EMCV-IRES based baculovirus bi-cistronic expression vector, vAcR-IR-G. Sf9 cells ($2 \times 10^5$) were infected with vAcR-IR-G at moi 1 and observed at 3 days (B and C) and 4 days (D and E) after inoculation under fluorescence microscope (Nikon). Pictures are taken in the same field with conventional rhodamine channel (B and D) and FITC channel (C and E).

vAcR-IR-G infected larvae emitted red fluorescence (Fig. 2A, right), few of them appeared yellow fluorescence (Fig. 2A, middle). Intriguingly, fluorescent color changes were observed when the exciting lamp was replaced with the general visible light: the green fluorescence of vAcp10-G infected larvae became faint light green (Fig. 2B, left) while the red and yellow fluorescence of vAcR-IR-G infected larvae turned out to be dark and light pink-red (Fig. 2B, right and middle). The insect larvae emitted yellow fluorescence under ultraviolet light turn to pink-red as under the general visible light indicates that the green fluorescent proteins may be not excited efficiently as the coral red fluorescent proteins by the general light. These results confirm our hypothesis that this non-bioluminescent coral red fluorescent protein could emit red fluorescence just by sunlight, although its relative brightness and quantum yield are about four folds lower than the green fluorescent proteins from jelly fish. These results demonstrate the DsRed gene from coral is superior to green fluorescent protein to serve as a convenient GMBV tracer for biological control.
Fig. 2. Insects infected with red fluorescence protein gene containing GMBV revealed red fluorescence under visible light. T. ni larvae infected with vAcIR-G and vAcp10-G (each larva injected 4 μl virus solution with $1 \times 10^8$ pfu/ml) under long-wave (365 nm) ultraviolet light (A) and laboratory light (B) at 6 days after virus inoculation. S. litura larvae (C) and S. exigua larvae (D) infected with vAcIR-G (4 μl virus solution, with $1 \times 10^8$ pfu/ml) under visible light at 6 days after virus inoculation. The uninfected S. litura larvae and S. exigua larvae were also shown as control.

To test whether the red fluorescent proteins excited by general light are restricted to T. ni larvae, we inoculated Spodoptera litura (S. litura) larvae and Spodoptera exigua (S. exigua) larvae with vAcIR-G. We found that the infected larvae emitted pink-red fluorescence under visible light while uninfected larvae appeared to be dark. Remarkably, the pink-red fluorescence of the infected larvae, either the S. litura larvae (Fig. 2C) or S. exigua larvae (Fig. 2D), can be easily observed by naked eyes without any prosthetic tools under the sunlight. Thus, it is evident that DsRed may serve as a powerful tracer to evaluate the efficiency of GMBV as an insecticide in the field without tedious molecular analysis. In conclusion, though the efficiency of the dual expression by using EMCV IRES was not satisfactory in the examined insect larvae, further investigation on employing suitable IRES, e.g., IRES from Rhopalosidum padi virus (Woolaway et al., 2001) or cricket paralysis virus (Wilson et al., 2000), into baculovirus genome may overcome this problem. Nevertheless, the presented data pave the way to develop GMBV as a novel insecticide via an efficient dual expression of a fast-killing insect-specific toxin and the powerful DsRed tracer.

Acknowledgements

We thank Dr. Chien-Chin Yang for critical review this paper. This work was supported by funds to T. Y. Wu from National Science Council of Taiwan (NSC90-2311-B-415-002) and by funds to T.Y. Wu and T.R.
Jinn from Council of Agriculture of Taiwan (91AS-3.1.3-FD-Z2 and 92AS-4.2.3-PI-P1).

References


