Virus Genes (2012) 45:610–613
DOI 10.1007/s11262-012-0800-3

# Analysis of EpapGV *gp37* gene reveals a close relationship between granulovirus and entomopoxvirus

**Ricardo Salvador · M. Leticia Ferrelli · Marcelo F. Berretta · Wataru Mitsuhashi · Marina E. Biedma · Víctor Romanowski · Alicia Sciocco-Cap**

Received: 21 May 2012 / Accepted: 30 July 2012 / Published online: 17 August 2012


**Abstract** The *Epinotia aporema* Granulovirus GP37 protein gene has been identified, located, and sequenced. This gene was similar to other baculovirus *gp37*, to entomopoxvirus *fusolin* gene, and to the *chitin-binding protein* gene of bacteria. Sequence analysis indicated that the open reading frame is 669 bp long (the smallest *gp37* sequenced at present) and encodes a predicted 222-amino acid protein. This protein is glycosylated and specifically recognized by an entomopoxvirus fusolin antiserum. The pairwise comparison of EpapGV *gp37* gene product with all the baculovirus sequences in GenBank yields high similarity values ranging from 45 to 63 % with *Cydia pomonella* Granulovirus *gp37* being the most closely related. The phylogenetic analysis interestingly grouped the granuloviruses in a cluster more closely related to entomopoxviruses than to nucleopolyhedroviruses, suggesting a possible horizontal transfer event between the granulovirus group and the entomopoxvirus group.

**Keywords** GP37 · Baculovirus · EpapGV · Granulovirus · Entomopoxvirus

Ricardo Salvador and M. Leticia Ferrelli are contributed equally to this study.

**Electronic supplementary material** The online version of this article (doi:10.1007/s11262-012-0800-3) contains supplementary material, which is available to authorized users.

R. Salvador (✉) · M. F. Berretta · A. Sciocco-Cap
Instituto de Microbiología y Zoología Agrícola (IMYZA-CICVyA-INTA), CC 25 (B1712WAA) Castelar, Buenos Aires, Argentina
e-mail: rsalvador@cnia.inta.gov.ar

R. Salvador · M. L. Ferrelli · M. E. Biedma · V. Romanowski
Instituto de Biotecnología y Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CONICET, La Plata, Argentina

W. Mitsuhashi
National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8634, Japan

*Present Address:*
M. E. Biedma
INSERM U748, Institut de Virologie, Faculté de Médecine, Université de Strasbourg, 3 rue Koeberlé, 67000 Strasbourg, France

The bean shoot borer, *Epinotia aporema* (Lepidoptera: Totricidae), is an economically important pest of legume crops in South America. A viral candidate for the control of this pest was isolated, studied, and named Epinotia aporema granulovirus (EpapGV) [1, 2] and belongs to the *Betabaculovirus* genus of the *Baculoviridae* family [3]. Baculoviruses are insect-specific DNA viruses that infect species mainly within the order Lepidoptera and have been widely used as bioinsecticides around the world. Two different virion phenotypes are produced during infection: the occlusion-derived virus (ODV), which establishes primary infection in the midgut of the host, and budded virus (BV), which mediates virus spread within the host. Virions of the ODV phenotype are embedded within crystalline occlusions' bodies (OBs), which protect the virions from adverse environmental conditions.

When ingested, OBs are dissolved by the alkaline environment of the insect midgut, resulting in the release of ODVs which cross the peritrophic membrane (PM) barrier and infect midgut epithelial cells. Insect PMs are composed primarily of chitin and proteins [4] and provide the first line of defense in the midgut. Two baculoviral gene products are known to assist the ODVs to overcome the PM: VEF (viral-enhancing factor or enhancin) and GP37. Enhancins are metalloproteases that degrade PM proteins [5],

resulting in the insect's increased susceptibility to infection. Baculovirus GP37 is homologous to entomopoxvirus (EPV) fusolin and both share a 30–40 % amino acid sequence identity [6]. Fusolin forms a type of inclusion body termed spindle [6–8], which mediates disruption of the PM facilitating the infection of the insect host [9]. In baculoviruses, these types of inclusion bodies were observed only in Orgyia pseudotsugata multiple nucleopolyhedrovirus (OpMNPV) and Choristoneura fumiferana defective nucleopolyhedrovirus (CfDEFNPV) [10, 11]. Fusolins and GP37 proteins have a chitin-binding domain (CBD) and the chitin-binding capacity of a baculovirus GP37 was demonstrated for Spodoptera litura nucleopolyhedrovirus (SpliMNPV) [12].

In Anticarsia gemmatalis nucleopolyhedrovirus (AgMNPV) genome, the *enhancin* and *gp37* genes are absent [13]. Our preliminary results showed that the addition of EpapGV OBs to AgMNPV preparation increased its biopesticidal activity on *A. gemmatalis* larvae (manuscript in preparation). The recently sequenced genome of EpapGV (unpublished) confirmed the presence of a *gp37* homolog (ORF 30, accession number: JX073280) and the absence of an *enhancin* gene. A recent study demonstrated that administration of GP37 of Cydia pomonella granulovirus (CpGV) to *Spodoptera exigua* larvae enhanced baculovirus oral infectivity and *Bacillus thuringiensis* toxin lethality [14]. Therefore, EpapGV GP37 might be a good candidate protein responsible for the effect observed on AgMNPV infection.

EpapGV *gp37* sequence analysis indicated that this gene contains a 669-bp-long ORF encoding a predicted 222-amino acid-long protein. Two characteristic late promoter elements (TTAAGTAAG) were found in tandem 7 bp upstream of the translation initiation codon. The full amino acid sequence deduced from the *gp37* ORF shows that it is the smallest in this group of viral proteins so far. EpapGV GP37 analysis revealed the presence of a chitin-binding domain (CBD 3, INTERPRO database accession number: IPR 004302). This domain includes the five regions and the 6 conserved cysteines that may be involved in disulfide bonds (Online resource 1), but between region 1 and 2, there is a gap of 13 amino acids. It also contains a consensus recognition site His-Gly-Tyr, that is considered to constitute a signal sequence [8, 15, 16], and two putative N-glycosylation sites (91–93 and 145–147 aa residues). While the N-terminal region is highly conserved among these proteins, the C-terminal portion is highly variable in sequence and length as previously described [17]. BLASTP searches showed that EpapGV GP37 has a significant identity to all known baculovirus GP37, entomopoxvirus fusolin, and some bacterial CBPs (chitin-binding proteins). The pairwise comparison of EpapGV GP37 with baculovirus homologous sequences yielded high similarity values ranging from 45 to 67 % identity, with CpGV GP37 protein being the most closely related.

The *gp37* ORF was cloned in pBacPak9 (Invitrogen) and expressed using bApGOZA [18] system in High Five cells [19]. Briefly, gp37 ORF was amplified with specific primers containing the *Eco*RI and *Pst*I sites and cloned in pBacPak9. The resulting plasmid was cotransfected in insect cells with parental bApGOZA. As the parental genome is non-viable, only recombinant virions were recovered from the supernatant. Recombinant GP37 expression was verified in SDS-PAGE [20], yielding a product of ~25 kDa as expected from the predicted amino acid sequence (Fig. 1a, line 1). Partial sequencing by the Edman method [21] confirmed GP37 identity. Western Blot (WB) analysis was performed with polyclonal antibodies raised against fusolin of Anomala cuprea entomopoxvirus, AcEPV [9] (Fig. 1a, lines 3–5). SDS-PAGE analysis shows that higher MW bands were detected probably due to different versions of glycosylated protein. To verify this assumption, glycosylation was inhibited by the addition of tunicamycin to the cell culture as previously described [17]. WB reveals that higher bands disappeared confirming that this protein is naturally glycosylated (Fig. 1a, line 4). The cross reaction of these antibodies with EpapGV GP37 evidences a close relationship between the GP37 protein of a granulovirus and an entomopoxvirus fusolin protein. On the contrary, Li et al. [10] reported that CfDEFNPV GP37 and fusolin from two entomopoxviruses (EPVs) were not serologically related, even though they share conserved polypeptide domains.

Previous phylogenetic analysis showed that baculovirus *gp37* genes and entomopoxvirus fusolin genes form two distinct and well-separated clades [14, 17] and that within the baculovirus clade, the GP37 phylogeny is consistent with previously published baculovirus phylogenies based on other proteins [22]. In this report, a new phylogenetic analysis by means of MEGA 3 program with default parameters [23] was performed based on the baculovirus GP37, EPVs fusolin, and bacterial CBPs sequences (Fig. 1b) (Online resource 2). When including all granulovirus (GVs) sequences in our analysis, we found that they were grouped into a cluster closest to entomopoxvirus than to the rest of baculoviruses. This is in accordance with WB observations detailed above. EPVs formed two separated groups, lepidopteran viruses (CbEPV, CfEPV, HaEPV, and PsEPV) and coleopteran viruses (AcEPV and MmEPV) as described previously [17]. The bacterial CBPs cluster appeared separated from the virus group. Current evidence does not support firm conclusions on the origin of this gene family and its presence in both baculoviruses and EPVs lineages [17]. The analysis presented in this paper suggests that a horizontal transfer of this gene could have happened between both viral families. It is known that representatives of the *Baculoviridae* and *Entomopoxviridae* have the same insect host [8, 24–26], and this situation would benefit the

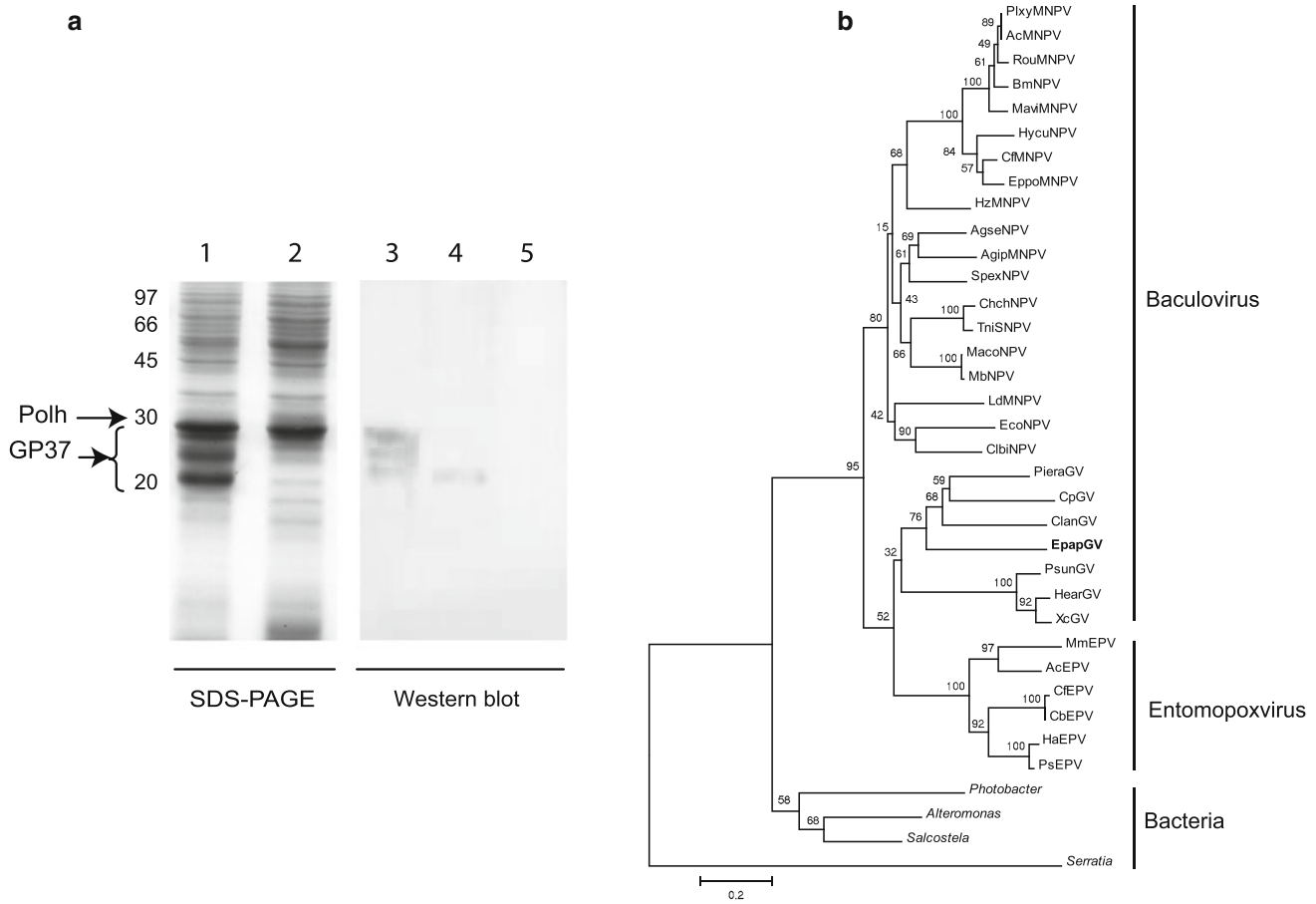


**Fig. 1** **a**. Coomassie blue stained SDS-PAGE: *Line* 1 Recombinant AcMNPV baculovirus expressing EpapGV GP37 (Ac-bApGOZA-gp37); *line* 2 Control Ac-bApGOZA; Western Blot : Recombinant baculovirus expressing EpapGV GP37 non-treated (*line* 3) and treated (*line* 4) with tunicamycin reacted with anti-fusolin antibodies. Control Ac-bApGOZA treated with anti-fusolin antibodies (*line* 5). *Left* numbers show molecular weight (kDa). **b**. Phylogenetic analysis of GP37, Fusolin, and CBP protein sequences (Accession numbers in online resource 2). Method: Neighbor-Joining, Bootstrap (1000 replicates). Bootstraps numbers are shown

horizontal transmission of genes. It is necessary to include more gp37/fusolin sequence data in order to clarify the relationship of these proteins between EPV and GVs.

Within EpapGV genome, *gp37* ORF is located adjacent to a *chitinase* gene (*v-chiA*) (Online resource 3). V-CHIA plays an important role in the degradation of chitin during the final stages of the infection in the insect, facilitating the release of the virus OBs in the environment [27, 28]. The *v-chiA* is homologous to bacterial genes and previous results strongly suggest that baculoviruses acquired their chitinase genes from bacteria [29, 30]. In bacteria, the functional relationship between CHIA and CBPs was demonstrated [31–33]. Through their binding to chitin, CBPs lead to structural changes and increase accessibility of chitin substrate to the action of chitinase [34]. The viral homologs of CBPs, namely GP37 and fusolins, also bind to chitin. Therefore, it is possible that GP37 plays a role not only in the initial steps of infection at the PM level, but also at the final stages by facilitating chitinase activity. This hypothesis remains to be elucidated.

Previous reports demonstrated the potential use of Gp37/fusolin proteins in viral formulations [14] and transgenic plants [35] in order to increase the host susceptibility to baculoviral infection. Therefore, EpapGV Gp37 is an important protein candidate for its use in strategies to control the *E. aporema* pest.

**Acknowledgments** The authors thank Mrs. M. Cornacchio and Mrs. D.B. Moreyra for the maintenance of the insect colony at the INTA. This work was supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and the Instituto Nacional de Tecnología Agropecuaria (INTA).

## References

1. A. Sciocco-Cap, A.D. Parola, A.V. Goldberg, P.D. Ghiringhelli, V. Romanowski, Appl. Environ. Microbiol. **67**, 3702–3706 (2001)
2. A.V. Goldberg, V. Romanowski, B.A. Federici, A. Sciocco de Cap, J. Invertebr. Pathol. **80**, 148–159 (2002)

3. E.A. Herniou, B.M. Arif, J.J. Becnel, G.W. Blissard, B. Bonning, R. Harrison, J.A. Jehle, D.A. Theilmann, J.M. Vlak, in *Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses*, ed. by A.M.Q. King, M.J. Adams, E.B. Carstens, E.J. Lefkowitz (Elsevier Academic Press, San Diego, 2011), pp. 163–173
4. P. Wang, R.R. Granados, Arch. Insect Biochem. Physiol. **47**, 110–118 (2001)
5. P. Wang, R.R. Granados, Proc Natl Acad Sci USA **94**, 6977–6982 (1997)
6. L. Gauthier, F. Cousserans, J.C. Veyrunes, M. Bergoin, Virology **208**, 427–436 (1995)
7. C. Fernon, A. Vera, R. Crnov, J. Lai-Fook, R.J. Osborne, D. Dall, J. Invertebr. Pathol. **66**(3), 216–233 (1995)
8. D. Dall, A. Sriskantha, A. Vera, J. Lai-Fook, T. Symonds, J. Gen. Virol. **74**(Pt 9), 1811–1818 (1993)
9. W. Mitsuhashi, H. Kawakita, R. Murakami, Y. Takemoto, T. Saiki, K. Miyamoto, S. Wada, J. Virol. **81**, 4235–4243 (2007)
10. X. Li, J. Barrett, A. Pang, R.J. Klose, P.J. Krell, B.M. Arif, Virology **268**, 56–67 (2000)
11. C.H. Gross, G.M. Wolgamot, R.L. Russell, M.N. Pearson, G.F. Rohrmann, J. Virol. **67**, 469–475 (1993)
12. Z. Li, C. Li, K. Yang, L. Wang, C. Yin, Y. Gong, Y. Pang, Virus Res. **96**, 113–122 (2003)
13. J.V. Oliveira, J.L. Wolff, A. Garcia-Maruniak, B.M. Ribeiro, M.E. de Castro, M.L. de Souza, F. Moscardi, J.E. Maruniak, P.M. Zanotto, J. Gen. Virol. **87**, 3233–3250 (2006)
14. X. Liu, X. Ma, C. Lei, Y. Xiao, Z. Zhang, X. Sun, Arch. Virol. **156**, 1707–1715 (2011)
15. T. Hayakawa, J. Xu, T. Hukuhara, Gene **177**, 269–270 (1996)
16. W. Mitsuhashi, H. Saito, M. Sato, Insect Biochem. Mol. Biol. **27**, 869–876 (1997)
17. C.G. Phanis, D.P. Miller, S.C. Cassar, M. Tristem, S.M. Thiem, D.R. O'Reilly, J. Gen. Virol. **80**(Pt 7), 1823–1831 (1999)
18. Y.H. Je, I.H. Chang, J.Y. Roh, B.R. Jin, Int J Ind Entomol **2**, 155–160 (2001)
19. T.J. Wickham, T. Davis, R.R. Granados, M.L. Shuler, H.A. Wood, Biotechnol. Prog. **8**, 391–396 (1992)
20. U. Laemmli, Nature **227**(5259), 680–685 (1970)
21. P. Edman, E. Högfeldt, L. Sillén, P. Kinell, Acta Chem. Scand. **4**, 283–293 (1950)
22. J.A. Jehle, M. Lange, H. Wang, Z. Hu, Y. Wang, R. Hauschild, Virology **346**, 180–193 (2006)
23. K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, Mol. Biol. Evol. **28**, 2731–2739 (2011)
24. X. Chen, M. Li, X. Sun, B.M. Arif, Z. Hu, J.M. Vlak, Arch. Virol. **145**, 2539–2555 (2000)
25. B.M. Arif, K.W. Brown, Can. J. Microbiol. **21**, 1224–1231 (1975)
26. X. Li, J.W. Barrett, L. Yuen, B.M. Arif, Virus Res. **47**, 143–154 (1997)
27. R.E. Hawtin, T. Zarkowska, K. Arnold, C.J. Thomas, G.W. Gooday, L.A. King, J.A. Kuzio, R.D. Possee, Virology **238**, 243–253 (1997)
28. L.G. Hom, L.E. Volkman, Virology **277**, 178–183 (2000)
29. H. Wang, D. Wu, F. Deng, H. Peng, X. Chen, H. Lauzon, B.M. Arif, J.A. Jehle, Z. Hu, Virus Res. **100**, 179–189 (2004)
30. T. Daimon, K. Hamada, K. Mita, K. Okano, M.G. Suzuki, M. Kobayashi, T. Shimada, Insect Biochem. Mol. Biol. **33**, 749–759 (2003)
31. S. Techkarnjanaruk, A.E. Goodman, Microbiology **145**(Pt 4), 925–934 (1999)
32. H. Tsujibo, H. Orikoshi, N. Baba, M. Miyahara, K. Miyamoto, M. Yasuda, Y. Inamori, Appl. Environ. Microbiol. **68**, 263–270 (2002)
33. T. Watanabe, K. Kimura, T. Sumiya, N. Nikaidou, K. Suzuki, M. Suzuki, M. Taiyoji, S. Ferrer, M. Regue, J. Bacteriol. **179**, 7111–7117 (1997)
34. G. Vaaje-Kolstad, S.J. Horn, D.M. van Aalten, B. Synstad, V.G. Eijsink, J. Biol. Chem. **280**, 28492–28497 (2005)
35. S. Liu, H. Li, S. Sivakumar, B.C. Bonning, Adv. Virus Res. **68**, 427–457 (2006)