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Advances on BmNPV Functional Genomics

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Abstract

Bombyx mori Nucleopolyhedrovirus (BmNPV) is the first reported baculovirus. It is now one of the main baculoviruses that have been studied extensively and has been developed as an expression vector for heterologous gene expression in the host caterpillars. The advances on research of the functional genomics of the virus were reviewed in this paper. Genomes of two BmNPV strains have been sequenced, the T3 strain and S1 strain genome is 128,413- and 126,770- nt in length encoding 136- and 133- potential ORFs with minimum size over 60 codons, respectively. Up to date, 48 BmNPV genes were characterized, including 29 genes not essential for virus replication (type A), 1 gene which markedly slows spreading of the infection (type B) and 18 genes essential for virus replication (Type-C and D). To elucidate the interaction between BmNPV and its host, new sequencing technique has been employed to comprehensively analyze the host genes response to virus infection, and constructed a protein-protein interaction network based on the information available now. But, more work on gene functions are needed in the future.

Keywords: *Bombyx mori* nucleopolyhedrovirus; BmNPV; Functional genomics

Baculoviruses

The baculovirus was originally described when silkworm cultures were threatened by a disease indicated as jaundice [1], and the isolation and chemical composition research of the virus resulted in the new era of the baculovirus research [2].

Baculoviruses are arthropod-specific viruses containing large double-stranded circular DNA genomes of 80-180k bp. The *Baculoviridae* family can be divided into four genera according to common biological and structural characteristics: *Alphabaculovirus*, which includes lepidopteran specific baculoviruses and is subdivided into Group I or Group II based on the type of fusogenic protein, *Betabaculovirus*, comprising lepidopteran-specific granuloviruses, *Gammabaculovirus*, which includes hymenopteran-specific baculoviruses, and *Deltabaculovirus*, which, to date, comprises only CuniNPV and possibly still undescribed dipteran-specific baculoviruses [3-6].

There are currently 54 complete baculovirus genomes deposited in GenBank before this paper was published (http://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=10442). These include 37 *Alphabaculoviruses*, 13 *Betabaculoviruses*, 3 *Gammabaculoviruses*, and 1 *Deltabaculovirus*. Despite the diversity in gene content present in different baculovirus genomes, there are now 31 core genes conserved in all sequenced baculovirus genomes, the function of these genes can be divided into replication, transcription, packaging and assembly, cell cycle arrest, and oral infectivity (Table 1) [5]. Most of the data comes from the *Autographa californica* nucleopolyhedrovirus (AcMNPV), as it is the model type for baculovirus and has been extensively studied. Four core genes have been identified as essential players in viral DNA replication: *DNApol, Helicase, Lef-1* and *Lef-2*. Nearly half of the core genes involved in the packaging and assembly.

In the most well characterized baculoviruses, the life cycle is biphasic with two different phenotypes during virus infection: Occluded Virions (ODV) and Budded Virions (BV) [7]. The ODV transmits infection from insect to insect by infecting midgut columnar epithelial cells, whereas the BV is responsible for causing systemic infection within the host [8]. The two viral forms are efficient for the natural propagation of the occluded baculoviruses.

Over the years, Baculoviruses are extensively being studied for potential use in bioinsecticides around the world and as expression vectors for heterologous genes expression in insect-derived cells as well as in the host caterpillars. *Bombyx mori* nucleopolyherovirus (BmNPV), the type member of baculovirus, has been successfully developed for surface display [9]. Additionally, baculoviruses are regarded as potentially useful gene therapy vectors [10,11], and has been applied for veterinary and medical applications, including the development of vaccines and diagnostics, putative nonhuman viral vectors for gene delivery, and biological control agents against insect pests [5,6,12]. These applications further trigger the study of molecular basis of baculovirus infection.

BmNPV Functional Genomics

The history of BmNPV is intimately related to the development of the silk industry. Silk production has been of major cultural significance in China and some other Asian Countries. Though BmNPV was the first described NPV, the genome of the BmNPV was completely sequenced and analyzed in 1999. The BmNPV (T3 strain) genome has a size of 12, 843 bp with a G+C content of 40% and encoding 142 predicated open reading frames (136 of the ORFs encoding predicted proteins of over 60 amino acids) [13,14]. Recently, a BmNPV strain (S1) isolated from the wild silkworm (*Bombyx mandarina*) was also sequenced [15]. The complete nucleotide sequence of the S1 strain was compared with the T3 strain. The S1 strain was 126,770 nucleotides long, with a G+C content of 40.23%. The genome contained 133 potential ORFs encoding predicted proteins of over 60 amino acids. Most of the

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category	Name	ORF No.in BmNPV	ORF No.in AcMNPV	Localization	Function	Reference	The phenotype after knock out in BmNPV
Replication							
	Lef-1	6	14	nucleosome	DNA primase	[32]	C
	DNApol	53	65	nucleosome	DNA polemerase	[33-36]	C
	Helicase	78	95	nucleosome	DNA helicase	[35,37-39]	D
	Lef-2	135	6	cell nuclei	Bind to both DNA and LEF-1; activate late transcription	[40]	C
Transcription							
	P47	31	40	nuclei	RNA polymerase subunit	[41,42]	D
	Lef-8	39	50	Nuclei	RNA polymerase subunit	[43,44]	D
	Lef-9	50	62	cell nuclei	RNA polymerase subunit	[44]	D
	Lef-4	73	90	cell nuclei	RNA polymerase subunit	[41,45-48]	D
	Lef-5	83	99	cell nuclei	initiation factor	[49]	D
Packaging, assembly, and release							
	VP1054	43	54	nucleosome	structrual protein; nucleocapsid assembly	[50]	C
	Vlf-1	63	77	nucleosome	lambda integrase; nucleocapsid assembly	[33,42,51-53]	C
	GP41	66	80	tegument	ODV protein; BV production	[54,55]	B
	ODV -NC42	56	68	capsid	ODV protein; Assemble nucleocapsid into ODV	[17,56]	A
	VP91/P95	69	83	envelope	Capsid-associated protein	[57]	C
	VP39	72	89	capsid	major capsid protein	[58-61]	C
	P33	75	92	nonstructural	sulfhydryloxidase; BV/ODV associated protein	[62-,64]	C
	38K	82	98	nucleosome	Nucleocapsid assembly	[65,66]	C
	P6.9	84	100	capsid	DNA binding	[67-69]	C
	ODV-EC43	92	109	capsid	BV/ODV Protein	[70-72]	C
	Alk-exo	110	133	nucleosome	5'-3'exonulcease	[73-75]	C
	P49	118	142	capsid	Nucleocapsid assembly	[76]	C
	ODV-E18	119	143	envelope	BV/ODV Protein; BV production	[77]	B
	desmoplakin	54	66		BV production	[78]	C
Cell cycle arrest and/or interaction with host proteins							
		67	81		interact with Actin3 in cytoplasm	[79]	C
	ODV-EC27	120	144	tegument	ODV associated protein; cyclin	[80,81]	C
Oral infectivity							
	Pif-2	13	22	envelope	mediate binding in midgut	[82,83]	A
	OEV-E28/19K/Pif4	79	96	envelope	primary oral infection	[72,83,84]	C
	Pif-3	95	115	envelope	form a complex with PIF-1 and PIF-2	[83,72]	A
	Pif-1	97	119	envelope	mediate binding in midgut	[83,85,86]	A
	Pif-0/P74	115	138	envelope	mediate binding in midgut	[87-90]	A
	Pif5/ODV-E56	124	148	envelope	primary oral infection	[81,91]	A

Table 1: Functions of 31 core genes in 54 baculoviruses

putative proteins were more than 96% identical to homologs in the T3 strain, except for bro-a, lef-12, bro-c, and bro-d. The S1 genome did not encode the bro-b and bro-e genes. The BmNPV genome was closely related to that of AcMNPV, sharing over 90% similarity to about three-quarters of the genome of AcMNPV. And the relatedness of predicted amino acid sequences of corresponding ORFs between BmNPV and AcMNPV was about 90% identical.

As *Bombyx mori* is well-studied physiologically, biochemically, and molecular biologically, contributed to the genome sequenced through international collaboration between China and Japan [16]. The genome data stimulated the analysis on the BmNPV-silkworm infection system. However, at least two third of the BmNPV viral gene functions are still remain unknown. BmNPV has potential usage not only for expression vector, but also for the display system, thus the fundamental investigation on gene function is important.

Many researchers have made effort on characterization of the BmNPV genes. And the development of the BmNPV bacmid system and the modification of this technology using a lambda red recombinant system allowed for the efficient production of knockouts, make the single gene function analysis more convenient. By using the knockout technology, Ono et al. [16] generated KOVs (knockout viruses) for 141 BmNPV genes and subdivided the KOVs into four phenotypic groups, types A to D [16].

Rohrmann [6] has included pertinent information on the function of all the ORFs present in the AcMNPV genome. Though AcMNPV

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and BmNPV share high similarities in the genome, some of the homologous genes in the two viruses function quite differently in the regulation of viral life cycle due to adaptation to different hosts. Here, we listed all the genes characterized in BmNPV up to date in Table 2 and marked by the phenotypic groups established by Ono et al. [16].

Type-A KOVs showed the ability to expand infections almost equivalent to the wild type virus. The type A genes are likely to be non-essential, as the genes interrupted by insertion/deletion mutagenesis in BmNPV, the virus appeared to be normal, though the deletion may have slight affection on BV/ODV production, virulent, or DNA replication. For instance, it was previously reported that the deletion of Bm56 in BmNPV affected the occlusion body's morphogenesis [17]. Bm5 is not a structural protein associated with BV or ODV, the Bm5 likely to be nonessential as the KO virus appeared to be viable [18]. And knock out of Bm9 led to a slight reduced production of BV without affect the DNA replication [19]. Bioassays showed that the Bm9-deleted bacmid took approximately 14-22 h longer to kill fifth instar *B. mori* larvae than wt bacmid, and the LD (50) was about 15 times higher than that of the wt bacmid, indicating that Bm9 is an important but not essential factor in virus production and infectivity in vivo and in vitro. For the core genes of all the baculovirus (Table 1), Oral infectivity genes are type A except Bm79 (OEV-E28/19K/Pif4). Bm79 is an ODV envelope associated protein [20] and in BmNPV and lately was also found associated with the envelope of BV in AcMNPV, and was concluded that Ac96 is a per os infecitivity factor in AcMNPV [6].

Name	The phenotype after knock out in BmNPV*	References
Bm5	A	[18]
Bm8(BV/ODV-E26)	A	[92,93]
Bm9	A	[94]
Bm21	A	[95]
Bm22(BRO-A)	A	[21]
Bm24(Vfgf)	A	[92,93]
Bm26(Ubiquitin)	A	[56,94]
Bm 27(39K)	A	[95]
Bm33(GTA)	A	[96]
Bm34	A	[97]
Bm41	A	[98]
Bm49(FP25k)	A	[99-101]
Bm51	A	[102]
Bm56	A	[17]
Bm60	A	[103,104]
Bm68(TLP)	A	[105]
Bm71(P39&CG30)	A	[106,107]
Bm74	A	[108]
Bm80(BRO-B)	A	[21]
Bm81(BRO-C)	A	[21]
Bm94	A	[109]
Bm102(Lef7)	A	[95, 110]
Bm103(Chitinase)	A	[111]
Bm112(P35)	A	[95,101,111]
Bm114(P10)	A	[112]
Bm124(ODV-E56)	A	[113]
Bm 127(Ie2)	A	[93,95]
Bm132(BRO-E)	A	[21,]
Bm134	A	[56]
Bm131(BRO-D)	B	[21,]
Bm3(Pk1)	C	[114]
Bm16(DBP)	C	[73]
Bm25	C	[111]
Bm42	C	[115]
Bm54	C	[116]
Bm61	C	[117]
Bm67	C	[118]
Bm69(P95)	C	[119]
Bm75	C	[120]
Bm76	C	[121]
Bm79	C	[17]
Bm88(VP80)	C	[22]
Bm 105(GP64)	C	[104]
Bm109	C	[122]
Bm118	C	[94]
Bm122	C	[123]
Bm39(Lef8)	D	[24]
Bm50 (lef9)	D	[24]

Table 2: Characterized genes in BmNPV.

Type-B KOVs were characterized by a markedly slow spreading of the infection probably because of the low production of infectious viruses and/or low infectivity of the progeny viruses. As genes have been characterized in BmNPV, only Bm131 (BRO-D) belonged to type B. Bm131 (BRO-D) has been observed as a nucleocytoplasmic shuttling protein, and the KOV of Bm131 was due to a reduction in RNA synthesis [21].

Type-C KOVs expressed report *egfp* in transfected-BmN cells but the production of infectious viruses was not observed while Type-D showed no ability to express *egfp* even in the transfection experiments. The core genes of baculovirus involved in replication and transcription are all belonged to type C and type D (Table 1). And most of the type C genes may play important roles in the morphogenesis of BmNPV as most of the genes knocked out in type-C code for virion structural proteins (Table 1). The Bm54-deleted virus produced non-infectious budded virus (BV) and normal nucleocapsids but defect in polyhedra. And disruption of Bmvp80 [22] resulted in single cell infection phenotype. Bmvp80 is essential for normal budded virus production and nucleocapsid maturation, and is functionally divergent between baculovirus species. Also, Bm118 is essential for BV production and nucelocapsid maturation [23].

In the cells transfected with type-D bacmid DNAs, neither *egfp* expression nor the BV production was detected. Two viral genes of BmNPV have been characterized in type-D KOVs were essential not only for viral replication, but also for *polhedrin* expression [24].

Take the advantage of silkworm genomic data, researchers analyzed the host-derived protein of the BmNPV [25], the BmNPV resistance in the hemolymph and the fat body in the genomic and proteomic level [26-30]. A dynamic protein-protein interaction network between BmNPV and its host cells was predicted by making comparisons to other insect interactomes recently [31]. This is a comprehensive in-depth study analyzing host gene response to baculovirus infection and perhaps the most comprehensive and best described transcriptional analysis available using baculoviruses. Gene enhancements are grouped in functional categories (cell cycle, energy metabolism, etc.) and their potential role during baculovirus infection is well discussed [31]. The protein-protein interaction network prediction provides an excellent resource for others in the community to evaluate certain pathways and to provide more in depth analysis.

Conclusion

In conclusion, two strain genomes of BmNPV have been sequenced and compared. Forty eight genes in a total of 136 BmNPV ORFs have been functionally characterized. As development of new genome sequencing techniques, more and more genomes of baculoviruses have been sequenced, and phylogenetic evolution history of baculoviruses becomes increasingly clear. We have tried to comparatively analyze six various strains from a host at genome level that will enrich our knowledge of evolution of the baculoviruses (data not published). However, many gene functions remain unclear yet. To elucidate the interaction between BmNPV and its host, more works on ORF functions are needed.

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