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Future vital prospect of gene expression factors of lef-7 (baculovirus expression): Old body, young cherub

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REVIEW

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ABSTRACT

Background
Baculovirus; late expression factors (Lef-7) have potential roles for protein expression in insect and mammalian cells; Efficient expression of recombinant proteins to facilitate the practical and structural investigation.

Aims
Lef-7 might play crucial roles in transcription and translation reactions of insect cell lines.

Methods
Materials and Methods: All required information regards Lef-7 was generated by exploring the internet search engine like as (PubMed, Wiley, ScienceDirect, CNKI, ACS, Google Scholar, Web of Science, SciFinder, and Baidu Scholar) and libraries.

Results
These properties issue crucial scope for DNA cloning and act as a vital vector for insect and mammalian cells. Left-7 could be the significant site in the development of the vaccine for a couple of chronic diseases. Further investigation needs to study on therapeutic vaccines with few immunologic advantages over proteins derived from mammalian sources, and animal sources. Lef-7 demonstrates the significant impact in the fields of DNA immunology research to insight into the mechanistic and utilitarian link between autoimmunity, infectious diseases, and cancer.

Conclusion
This review reveals Lef-7 gene function offers a workable strategy for the expression of whole viral protomers as the future prospect of Lef-7.

Key Words
Baculovirus, late expression factors, future vital prospect, interaction of Lef-7, host DNA damage response, boost virus multiplication

What this review adds:

1. What is known about this subject?
Baculovirus represents a robust method for high-level production of foreign proteins. Late expression factor 7 regulating viral gene transcription and replication affects the expression efficiency.

2. What new information is offered in this review?
This review summarizes the fundamental function and

mechanism of Lef-7, and explores the future vital prospect of Lef-7 gene transcend the stable boundaries.

3. What are the implications for research, policy, or practice?

Further investigation needs to focus on the possibility of developing future therapeutic vaccines using Lef-7 targeting host DNA damage repair.

Introduction

Baculovirus is large double-stranded DNA viruses utilized broadly as bio-pesticides and as expression vectors for the high-level production of foreign proteins.[1] Baculovirus exhibits classic temporal control of gene expression with prompt, timely, early, late and very late phases respectively.[2] The instant early genes are transcribed by host RNA polymerase while late gene expression requires a complex set of virus-encoded early gene products termed late expression factors (Lefs derivatives).[3] The Lefs subordinates act in trans to animate the late phases of the virus life, including the high level transcription upon which development of *Autographa californica* multiple nuclear polyhedrosis viruses (AcMNPV) like as a viral expression vector is foundation.[3-5] In the genome of AcMNPV have been nineteen Lefs described previously; among them, just a couple functions are details understandable,[5-7] however the greater part of their activities in show is relatively misty.

Understanding the activities of the Lefs would permit improvement of lasting insect cell line capacities to high levels of expression. Approach those observed from viral very late promoters in virus-infected cells and also the advancement of *in-vitro* transcription and translation responses, for example, those that have been described for *E. coli*.[8] Previous data suggested that, the relevant merit to this approach when comparisons with approaches that vary the vector within only one expression system, contribute apriori data for Lef gene functions and offer workable strategies for the expression of whole viral proteomes.[9] The baculovirus genome consists of large (80–180kbp) circular double-stranded DNA. Baculovirus gene expressions are classified into four phases of expressions: immediate-early, delayed-early, late-and, very-late and strongly synchronized during viral contamination.[10] Baculovirus late gene expression is intimately associated with DNA replication. In AcMNPV Nineteen, late expression factors (Lefs) have been identified, which influence the late and very late gene expression by transient expression assays and complementation investigation.[3-5,11] A subset of Lef genes, together with Lef-1, Lef-2, Lef-3, Lef-7, p143, p35, dnapol, ie-1, and ie-2 were associated with viral DNA replications as well as by this means persuade expression of late and very late baculovirus genes. The rest of the 10 Lefs genes, Lef-4, Lef-5, Lef-6, Lef-8, Lef-9, Lef-10, Lef-11, Lef-12, p47, and p39 are necessary for transcription of late genes, while recent studies demonstrated that Lef-12 isn't required for expression of late genes.[12] All these 19 Lefs have their counterparts in BmNPV.[5,13]

However certain baculovirus does not have a couple of Lefs. In *Plutella xylostella* granulovirus, Lef-7, Lef-10, Lef-12, p35, and ie-2 were absent.[14] The ie-2, pe38, Lef-7 or p35 genes did not have homologs in the *Spodoptera exigua* (Se) MNPV or the *Lymantria dispar* (Ld) MNPV genomes.[15] Additional genes, very late expression factor 1 (vlf-1), is mandatory for invigorating very late gene expression in AcMNPV, as well as a counterpart for vlf-1 is also present in BmNPV.[16,17] In transient assays, AcMNPV, as well as BmNPV Lef-7, stimulates DNA replication.[18,19] Be that as it may, in Ac-MNPV it acts in a cell-specific approach. Evacuate of Lef-7 of every two AcMNPV mutants, which have contagion analogous similar to that of wild-type (Wt) AcMNPV in TN-368 cells, in consequences in the diminution of budded virus and polyhedral inclusion body production in IPLB-SF-21 and SElc cells.[20] DNA replication and transient late gene expression in TN-368 cells required another Lef gene, hcf- 1 gene.[21] So Lef-7 could be aligned with the host go specificity. Also, Lef-7 was observed to be essential for homologous recombination.[22]

The coding sequence of BmNPV Lef-7 has 92 per cent uniqueness with that of AcMNPV. The previous investigation, the open reading frame (ORF) of AcMNPV Lef-7 has 681nt, though the predicted BmNPV Lef-7 contains 684nt encoding a protein of 227 amino acids. The proposed BmNPV Lef-7 start codon ATG (46nt downstream), another ATG codon resultants to the actuation ATG codon of AcMNPV Lef-7 is additionally present. These two ATG codons are in the same reading frame. Lef-7 assumes an imperative part in fortifying DNA replication in BmNPV. Additional studies on functional comparisons linking Lef-7s of BmNPV and AcMNPV is an essential. Previous studied suggested that nineteen homologs of the AcMNPV late expression factor genes (Lef genes) were recognized in BmNPV and the genome organization of the BmNPV closely resembles that of AcMNPV.[18,23] Homology of these genes, including Lef-7 to these required for replication of AcMNPV, recommends parallel replication mechanisms for the two viruses. Both AcMNPV and BmNPV Lef-7 can empower DNA replication in transient assays.[18,19] Baculovirus *Bombyx mori* nucleopolyhedrovirus is highly efficient and simple construction strategy to produce recombinant targets.

Future vital prospect of Lef-7 gene

The truth that baculovirus expression depends on very late promoters which are active only when cells are already arrested by viral replication may facilitate expression of proteins whose half-lives are generally short. Lef-6 was expressed at the low level contrasted with the correspondingly estimated Lef-7 in *E. coli*. However, but this was not related to N-terminal residue identity as both (Val and Ser respectively) are predictors of a long half-life in prokaryotic and eukaryotic frameworks.[24] There was no particular feature of size or charge that interrelated with the expression level, refinement screens for Lef 6 and 7 between two hosts.

Interestingly Lef-7 expression in insect cells via the Drosophila melanogaster heat shock promoter also results in relatively low level expression so the point of confinement is probably not going to be connected to expression inside a virus background[6] and is all the more most likely identified with its biological function as, in our hands, DNA binding proteins are seldom well expressed. Although relatively well expressed, p39 was present in *E. coli* largely as an anti-His antibody reactive protein of around 31kDa indicating a predominant cleavage product in bacteria that was not seen when p39 was expressed in insect cells. By diverge from *E. coli*, recombinant baculovirus expression was successful for all products but was not universally superior (low level of Lef-7) to notwithstanding the source of the ORFs being used. Single step purification of proteins by means of the His-tag was feasible for Lef-6 and 7 from *E. coli* and for Lef-6, 7 and DNAPol from infected Sf9 cells. The level of contaminating material was not subject to the host utilized, e.g., Lef-10 from either source was found in a high molecular weight complex and p39 from insect cells was found co-associated with cellular material. In this manner, rapid evaluation of the same ORF in the two frameworks was a beneficial strategy for evaluating the most proper host for additionally work; no single framework was generally prevalent.

Immunofluorescence and direct DNA binding showed Lef-7 but not Lef 6 to be a nucleic acid binding protein located within the nucleus. Nuclear localization has been also recently described by Hefferon and Miller, who noticed that Lef-7 interacted with P143, the baculovirus helicase.[6] The previous finding revealed that Lef-7 was also present in the high molecular weight complex identified on Lef-10blots may be part of a larger multi-component replication complex. These findings support recent work from gene knockout viruses showing that Lef-7 is essential for baculovirus replication, at least in Spodoptera cells, while Lef 6 is dispensable although clearly stimulatory.[20,25] Several strategies are emerging for the high throughput (HTP) expression of recombinant proteins to facilitate the functional and structural investigation. The previous investigation suggested that a workable HTP strategy based on parallel protein expression in *E. coli* and insect cells. Utilizing HTP framework they gave relative articulation information to five proteins got from the *Autographa californica* polyhedrosis virus genome which was exceptionally in amino acid composition and in molecular weight. Despite the fact that the proteins are a component from an arrangement of elements known to be compulsory for viral late gene expression, the specific function of three of the five, late expression factors Lef 6, Lef 7 and Lef 10, is unidentified. Quick expression and categorization have permissible the determination of their capability to bind DNA and revealed a cellular location steady with their properties. The utility of a parallel expression strategy to be rapid acquired workable protein expression stages from various ORFs.[9]

IE2, Lef-7, and P35 are stimulatory, but not necessary, throughout AcMNPV replication in Sf-21 cells and not required at all in Tn-368 cells.[4] IE2 and P35 were not investigated furthermore, due to these factors emerged unlikely to have a straight interaction with other replications Lefs. Despite the fact that Lef-7 resides within the nucleus, no relations were seen among it and any of alternate replications Lefs in either Tn-368 cells or Sf-21 cells. The fact that Lef-7 co-precipitated with HEL in the circumstance of a virus infection advocates that additional, virus-induced host or virus-derived factors may be mandatory for this interaction as well as that Lef-7 might be a constituent of a complex restraining HEL. Besides containing a sequence motif corresponding to a metal coordination site, Lef-7 possesses a small degree of sequence homology with the UL29 gene of HSV-1, encoding a single-stranded DNA binding protein.[19] It is possible that Lef-7 may act as a supplementary single-stranded DNA binding protein, conceivably by supporting in the stabilization or melting of recently unwound ssDNA.[6] In cell culture, recombination between plasmids conveying a baculovirus DNA and foreign gene has been exploited to assemble recombinant viruses overexpressing an overseas gene; nevertheless, the association of viral genes in this process had not been investigated until recently. The merely investigated that has examined the constraint of specific baculovirus genes in HR accomplished that the AcMNPV DNA replication genes ie-1, Lef-1, Lef-2, Lef-3, p35, and dnapol were obligatory for high-frequency HR in the existence of an origin of viral replication, whereas ie-2 and

Lef-7 had little effect as observed by the inversion of sequences within a bacterial transposable constituent.[26] The viral genes ie-1, ie-2, Lef-7, and *p35* were found to be significant for competent HR in the presence of all additional DNA replication genes. Lef-7 contains single-stranded-DNA-binding motifs, but its activity as a single-stranded binding protein has not been determined either *in vitro* or *in vivo*.[22]

Baculovirus expression in insect cells represents a robust method for producing recombinant glycoproteins.[27,28] Baculovirus-produced proteins are currently under study as therapeutic cancer vaccines with several immunologic compensations over proteins consequent from mammalian resources.[29]

Interactions sandwiched between replication Lefs dogged from the yeast two-hybrid system

To gain imminent into the character of the AcMNPV DNA replication complex, beforehand inspected that the capacity of each labeled Lef to interact with additional replication Lefs by employing both yeast two-hybrid and coprecipitation techniques. By utilizing the yeast two-hybrid system, each Lef was co-transformed into yeast YRG-2 cells, cloned into bait and prey plasmids, and additionally observed for growth on His plates. Colonies that were vigorous in growth when restrained onto fresh selection plates were then investigated for β-galactosidase activity. Every construct was also transformed independently and investigated for its ability to construct false positive results. By surrogating each Lef as both bait and prey plasmids, it was conceivable to approve every interaction. In addition interacting with itself, Lef-3 was also experiential to interact with various additional Lefs. Lef-3 and Lef-3 interactions were, in fact, the strongest (30.5), as determined by b-galactosidase activity, whereas Lef-3 interacted to a less significant with HEL (Lef-3 as bait and HEL as prey gave a value of 22.1; HEL as bait and Lef-3 as prey gave a value of 24.3). The weakest interaction was established to be between Lef-3 and IE1 (Lef-3 as bait, IE1 as prey: 1.5; IE1 as bait, Lef-3 as prey: 2.7). Lef-1 was found to interact with Lef-2 (Lef-1 as bait, Lef-2 as prey: 17.4; Lef-2 as bait, Lef-1 as prey: 18.2). IE1 exhibited a strong interaction with itself (27.3). Lef-7 was not exhibited to interact with itself or any other Lef. No interactions were exhibited between HEL and DNApol.[6]

Lef-7 amending the host DNA damage response to boost virus multiplication

The previous investigation illustrated that Lef-7 binds with host S-phase kinase-association protein 1 (SKP1) and acts as a substrate recognition component of SKP1/Cullin/F-box (SCF) complexes. Site-directed mutagenesis demonstrated that the N-terminus of Lef-7 is critical for γ-H2AX repression and *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) replication. They summarized that Lef-7 expedites virus replication most likely by selective manipulating the DDR regulation by one or more host factors, like the γ-H2AX. They suggested that baculoviruses utilize a novel strategy to hijack the host DNA damage response (DDR) regulation by using Lef-7.[30] Due to the critical role in DDR regulation; γ-H2AX is dealt with as an essential protein for virus regulation. Previous studies demonstrated that baculovirus Lef-7 is a nuclear F-box protein and a replicative factor that adjusts the host DDR by inhibiting the function of γ-H2AX, and it is necessary for the efficient baculovirus replication.[30] These findings broadened the past results on the Lef - 7 increasing speed of vDNA replication and recombination.[18,20-22]

Lef-7 requires the F-box domain to enhance the virus replication, γ-H2AX repression, and to interact with S-phase kinase-associated protein 1 (SKP1). Along these lines, the previous study confirmed that Lef-7 as an F-box protein that inhibits γ-H2AX to accelerate baculovirus replication by using a strategy for manipulating host DDR component.[30] Lef-7 was mandatory and sufficient to suppress phosphorylation of the histone variant H2AX following either pharmacologically induced DNA damage or baculovirus infection.

Lef-7 is manipulating an F-box protein targeted polyubiquitination for inactivating the function or altering of a DDR regulator by interacting with a component of SKP1/Cullin/F-box (SCF) complexes. The supplementary investigation ought to uncover the molecular mechanism and the targets of Lef-7 whereby DNA viruses like the baculoviruses manipulate the host DDR to facilitate their multiplication.

Conclusion

In the perspectives, Lef-7 might be an efficient way for the advancement of future treatment pattern (Biogenic Medicine) of several chronic diseases for human as well as animals. Furthermore, we have to investigate Lef-7 purpose for the prevention of cellular infection caused by baculoviruses. Lef-7 may be an effective site for the development of the vaccine for several chronic diseases. Lef-7 might be significant impact of the fields DNA immunology research to insight into the mechanistic and functional link between autoimmunity, infectious diseases, and cancer. Further investigation pays attention to

therapeutic vaccines with several immunologic advantages over proteins derived from mammalian sources as well as animal sources.

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