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遺伝子治療を目指した新規バキュロウイルスベクターの開発

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総合研究報告書

遺伝子治療を目指した新規バキュロウイルスベクターの開発

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研究要旨：本研究事業により以下の点を明らかにした。1) バキュロウイルスの粒子表面に目的のリガンド分子を自在に提示できるシュードタイプウイルスの作製系を構築し、広範な動物細胞に、あるいは狙った細胞だけに外来遺伝子を導入できるベクター系を構築した。2) HCV のプロテアーゼによって特異的に認識されるアミノ酸配列を検討し、HCV 感染細胞でのみ効率よく切断される最適配列を決定した。恒常的活性化型の IFN 調節因子遺伝子を搭載した組換えバキュロウイルスを構築し、容量依存的に HCV RNA の複製抑制の可能性を示した。3) バキュロウイルスが既存の Toll-like receptor 非依存的に自然免疫を誘導できることを明らかにした。4) 組換えバキュロウイルスで HEV の構造蛋白全長を発現することによって、ウイルス様粒子(HEV-LP)を作製し、その立体構造を決定した。5)キメラ HEV-LP の抗原性および免疫原性を解析した。

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A. 研究目的

遺伝子治療の臨床研究がスタートして十数年が経過し、決して満足できる成績は得られていないものの、本治療法が今世紀の先端医療の要となることには疑いの余地はない。遺伝子治療の成否を握るのは、安全に効率よく標的細胞へ遺伝子を導入でき、しかも大きな組み込み容量を持った遺伝子導入ベクターの開発である。これまでの遺伝子治療用ベクターとしては、レトロウイルス、アデノウイルス、アデノ随伴ウイルス、そして RNA ウイルスなどが主に利用されてきた。しかしながら、自立増殖ウイルスの出現や、ランダムな遺伝子の組み込みによる癌遺伝子の活性化等の安全性の問題、遺伝子導入効率や特異的な遺伝子導入法の改善の余地、ならびに、細胞毒性や免疫反応の誘導、中和抗体による不活化等の多くの難問が山積みしている。これらの問題を解決するには、既存のウイルスベクターにはない特徴を保持した、新しいウイルスベクター系の開発が必要である。その候補の一つとして、我々は昆虫ウイルスであるバキュロウイルスベクターの開発を進めている。

バキュロウイルスは、環状二本鎖 DNA を遺伝子としてもつ昆虫ウイルスで、感染した昆虫細胞内に多角体と呼ばれるウイルス粒子を包埋した封入体を大量に作るのが特徴である。本ウイルスはこれまで昆虫にしか感染しないと考えられていたが、広範な哺乳動物細胞へ感染し、複製することなく、外来遺伝子を効率よく発現できることが明らかにされ、遺伝子治療ベクターとしての可能性が注目を集めている。バキュロウイルスベクターの長所としては以下のような点が考えられる。1) 大きな外来遺伝子を挿入できる。2) ウイルス遺伝子が哺乳動物細胞で発現されないため有害な免疫応答の誘導がない。3) ヒトにはバキュロウイルスに対する中和抗体が存在しない。4) 他のウイルス蛋白質を組換えウイルスとして昆虫細胞で発現するとウイルス様粒子を大量に産生できる。

本研究はバキュロウイルスの特性を高度に利用して、持続感染症やがんに対する遺伝子治療用のベクター、アジュバント活性を保持したワクチンベクター、さらに、ウイルス様粒子を用いた経粘膜遺伝子導入ベクターの開発することを目的とする。

B. 研究方法

1) 感染症やがんに対する遺伝子治療用ベクターの開発: gp64 を完全に欠損させ、目的のリガンドのみを持ったターゲッティング可能なシュードタイプウイルスの作製系を構築し、水疱性口内炎ウイルスの G 蛋白質(VSV-G)や、麻疹ウイルスの受容体である CD46 あるいは SLAM を被ったシュードタイプウイルスがターゲット細胞に特異的に遺伝子を導入できることを証明した。C型肝炎ウイルス(HCV)に感染した細胞でのみインターフェロン誘導遺伝子が稼働するバキュロウイルスベクターの作製を目指し、遺伝子導入細胞を死滅させることなく、慢性持続感染細胞から HCV を排除可能なバキュロウイルスベクターの開発を試みた。

2)バキュロウイルスの自然免疫誘導機構の解析とその応用: これまでに我々が報告してきた、ウイルスゲノムによる Toll-like receptor 9(TLR9)を介した抗ウイルス活性の誘導とは異なり、バキュロウイルスに特異的な TLR 非依

存的なIFNの誘導機構が存在することが示唆された。バキュロウイルスによる抗ウイルス因子の誘導機構をIRFやSTAT等の転写因子欠損マウスを用いて解析し、アジュバント活性を兼ね備えた新規ワクチンベクターとしての可能性を検討した。

3) E型肝炎ウイルス様粒子(HEV-LP)を用いた経粘膜遺伝子導入ベクターの開発:バキュロウイルスを利用してHEVの構造蛋白を発現させると、大量のHEV-LPが産生される。HEV-LPを改変して目的蛋白質を粒子の表面あるいは内部に発現させてキメラ粒子の作製が試みられてきたが、生産性の低下が大きな問題となっている。そこで、外来蛋白質の挿入部位と容量を効率よく設計できるように、遺伝子型IIIのHEV-LPの結晶構造を解析した。また、キメラHEV-LPの抗原性および免疫原性を解析した。

(倫理面への配慮)

本研究における実験動物に関しては「動物の保護及び管理に関する法律」(昭和48年法律第105号)及び「実験動物の飼養及び保管に関する基準」(昭和55年総理府公示第6号)の法律及び基準の他、「大学等における実験動物について」(文部省国際学術局長通知、文学情第141号)の通知を踏まえつつ、動物実験が有効かつ適切に行われるよう配慮した。

C. 研究結果

1) HCV感染症に対する遺伝子治療用ベクターの開発: バキュロウイルスのエンベロープ蛋白質gp64の代わりに、目的のリガンドを粒子表面に発現させたシュードタイプウイルスを作製する系を構築し、標的細胞に遺伝子導入が可能となった。HCVに感染した細胞ではウイルス由来のプロテアーゼが発現し、自然免疫の誘導に必須なアダプター分子を切断して、宿主の免疫機構から逃避していることが知られている。そこで、HCVのプロテアーゼによって特異的に認識されるアミノ酸配列を検討し、HCV感染細胞でのみ効率よく切断される配列を検索し、最適配列を決定した。IFNの発現によるHCV排除を確認するため、恒常的に活性化されたIFN調節因子(IRF)7遺伝子を搭載した組換えバキュロウイルスを構築し、HCVゲノムが自立複製しているレプリコン細胞に感染させると、容量依存的にHCV RNAの複製抑制が観察された。

2)バキュロウイルスによる宿主応答:

バキュロウイルスの感作により、炎症性サイトカインはTLR9依存的に産生されるのに対し、抗ウイルス活性の要であるIFNの発現はTLR非依存的に誘導された。また、IFNの産生誘導は2本鎖RNA依存PKR阻害剤の影響を受けなかったことから、バキュロウイルスに特異的なTLR-PKR非依存的なIFNの誘導機構が存在することを示唆するものである。この成績は、バキュロウイルスが遺伝子導入ベクターとしてだけでなく、接種経路によってはアジュバント活性を併せ持った新しいワクチンベクターとしての可能性を示唆するものである。

3) HEV-LPの結晶構造解析: HEV ORF2のN末端から111アミノ酸を欠損させたVLPの結晶化に成功し、その構造を解析した。HEV-LPは3つの機能ドメインより構成され、T=1の粒子を構成していた。Caliciviridaeに属するSan Miguel sea lion virus (SMSV)やNorovirus (NV)のVLP(NV-LP)と比較して、HEV-LPのみが異なるトポロジーを保持していた。SMSVやNV-LPはP1, P2そしてSドメインが重なるようにタイトな構造を取るのに対して、HEV-LPはP1とP2ドメインの間に非常に長いリンカー構造が存在し、フレキシブルな構造を持っていた。HEV-LPのP1ドメインはアミノ酸残基320-457で構成されているが、NV-LPのP1ドメインはアミノ酸残基226-278とアミノ酸残基406-520の二つの領域で構成されていた。粒子表面の突起はHEV-LPでは二量体で形成されるが、NodavirusやReovirusでは三量体で形成されていた。突起の数はCaliciviridaeやHepevirusと、NodavirusやReovirusは同じで20個で同じでであるが、構成様式は異なっていた。T289をAlaに置換するとVLPが産生されなくなり、T289は5回軸周辺のY288が形成する疎水性ポアの安定化に寄与していることが推測された。

4) キメラHEV-LPの作製と性状解析: 1型HEV ORF2のN末端から111あるいは124アミノ酸を種々の長さのORF3、あるいは離乳後多臓器発育不良症候群の原因ウイルスである2型ブタサーコウイルス(PCV2)構造蛋白に置き換えたコンストラクトを組換えバキュロウイルスで発現した。定法どおり感染細胞を回収し、細胞を破砕後、遠心上清を回収した。超遠心法で濃縮し、その沈査を電子顕微鏡で観察したところ、いずれの場合も直径27nmの粒子が多数観察された。キメラHEV-LPの抗原性および免疫原性の解析からORF3蛋白はキメラHEV-LPの表面に配置していることが明らかになった。一方、PCV2構造蛋白を併せ持つキメラHEV-LPの免疫原性

は弱いものであった。
5)中空粒子内に取り込まれた核酸の解析：直径35-38nmのネイティブなウイルス粒子とほぼ同じ直径を有する粒子は、その電顕像から粒子内部に核酸分子を取り込んでいることが予想された。そこで、精製粒子を用い、HEV構造タンパク領域を増幅するプライマーを用いて増幅し、その塩基配列を解析した。その結果、増幅産物はHEV特異的な配列を持つ核酸であった。

D. 考察
バキュロウイルス粒子表面に目的分子を自在に搭載する方法論を確立できた。ウイルスのリセプター分子や癌抗原に対する単鎖抗体を粒子表面に提示すれば、ウイルスに感染してエンベロープ蛋白質を発現している細胞や癌細胞だけに自殺遺伝子を導入することによって目的の細胞だけを生体から排除することが可能となると思われる。

バキュロウイルスを用いてHCVゲノムが複製している肝臓細胞からウイルスの複製を制御できる可能性が示された。今後ヒト肝細胞を移植し、HCVの感染を許容できるキメラマウスを用いて *in vivo* のウイルス排除効果を評価する。HCV蛋白質による免疫回避機構に介入し、C型慢性肝炎患者の免疫を賦活化することによって、生体からウイルスを排除できる新しい治療法や、HCVの複製や成熟に必須な宿主因子、VAP-B、FKBP8、hBind-1、SPPおよびPA28γの発現を制御可能なバキュロウイルスを作製し、*in vitro* でのウイルス複製抑制および中性脂肪の産生抑制を確認したい。

バキュロウイルスが免疫担当細胞に自然免疫を誘導できることが明らかとなった。バキュロウイルスの特性を活用した新しいウイルスベクターの開発は、先天性・後天性疾患の遺伝子治療用ベクターとしてばかりでなく、新規DNAワクチンとしての可能性も秘めており、医療・福祉への貢献度も極めて高いものと思われる。

また、我が国での感染が問題となっている遺伝子型III型HEV-LPの三次元構造が解けたことは、この粒子を用いた遺伝子デリバーシステムやワクチンベクターの開発における波及効果は計り知れない物がある。HEV構造蛋白のN末端およびC末端の構造とHEV-LPの形成に関する一連の実験からN末端111アミノ酸からさらに絞り込んだ111～124の15アミノ酸、C末端52アミノ酸からさらに絞り込んだ601～608の7アミノ酸を欠損させてもVLP形成には影響がないことが示された。したがって、構造蛋白の125-601アミノ酸が粒子形成に必須のアミノ酸残基で粒子のコアを形成していると考えられる。PCV2では構造蛋白由来最長72アミノ酸を、HEV ORF3蛋白では全長123アミノ酸をN末端に連結することによって外来蛋白を持つキメラHEV-LPを発現することができた。また、連結する外来性蛋白によってその抗原性や免疫原性に差がみられたことから、外来性蛋白のキメラ粒子上における構造や部位が影響しているように思われた。今後、同様な手法で外来蛋白挿入できる最適な条件を見いだし、キメラHEV-LPを作製するとともに、構造解析を進めていきたい。

E. 結論
1)gp64を完全に欠損させ、目的のリガンドだけを被った組換えウイルスの作製系を構築し、ターゲッティング可能なバキュロウイルスベクター系を開発した。
2) HCV感染細胞でのみ効率よく活性化されるように設計した組換えバキュロウイルスを構築した。
3) バキュロウイルスによる自然免疫を誘導にはMyD88-TLR9非依存的なシグナル経路の存在が示唆された。
4) HEV-LPの結晶構造の解析に成功した。
5) HEV-LP形成に必須なアミノ酸領域を同定した。N末端に連結した外来性構造蛋白を組換えバキュロウイルスで発現することによって、キメラHEV粒子を作製することができた。

F. 健康危険情報
特になし。

G. 研究発表
1.論文発表

1 Yamashita T., Unno H., Mori Y., Tani H., Moriishi K., Takamizawa A., Agoh M., Tsukihara T., Matsuura Y. Crystal structure of the catalytic domain of Japanese encephalitis virus NS3 helicase/nucleoside triphosphatase at a resolution of 1.8 A. Virology (in press).
2 Tani H., Abe K., Matsubaga T., Moriishi K., and Matsuura Y. Baculovirus vector for gene delivery and vaccine development. Future Virology, 2008, 3, 35-43.
3 Moriishi K., Mochizuki R., Moriya K., Miyamoto H., Mori Y., Abe T., Murata S., Tanaka K., Miyamura T., Suzuki T., Koike K., and Matsuura Y. Critical role of PA28γ in

hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. PNAS, 2007, 104, 1661-1666.

4 Abe T., Kaname Y., Hamamoto I., Tsuda Y., Wen X., Taguwa S., Moriishi K., Takeuchi O., Kawai T., Kanto T., Hayashi N., Akira S., and Matsuura Y. Hepatitis C Virus Nonstructural Protein 5A Modulates TLR-MyD88-Dependent Signaling Pathway in the Macrophage Cell Lines. J. Virol., 2007, 81, 8953-8966.

5 Mori Y., Yamashita T., Tanaka Y., Tsuda Y., Abe T., Moriishi K., and Matsuura Y. Processing of Capsid Protein by Cathepsin L Plays a Crucial Role in Replication of the Japanese Encephalitis Virus in Neural and Macrophage Cells. J. Virol., 2007, 81, 8477-8487.

6 Tani H., Komoda Y., Matsuo E., Suzuki K., Hamamoto I., Yamashita T., Moriishi K., Fujiyama K., Kanto T., Hayashi N., Owsianka A., Patel A.H., Whitt M.A., and Matsuura Y. Replication-competent recombinant vesicular stomatitis virus encoding hepatitis C virus envelope proteins. J. Virol., 2007, 81, 8601-8612.

7 Yamamoto M., Uematsu S., Okamoto T., Matsuura Y., Sato S., Kumar H., Satoh T., Saitoh T., Takeda K., Ishii K.J., Takeuchi O., Kawai T., and Akira S. Enhanced TLR-mediated NF-IL6 dependent gene expression by Trib1 deficiency. J. Exp. Med., 2007, 204, 2233-2239.

8 Moriishi K., and Matsuura Y. Host factors involved in the replication of hepatitis C virus. Rev. Med. Virol., 2007, 17, 343-354.

9 Matsuura Y, Suzuki M, Yoshimatsu K, Arikawa J, Takashima I, Yokoyama M, Igota H, Yamauchi K, Ishida S, Fukui D, Bando G, Kosuge M, Tsunemitsu H, Koshimoto C, Sakae K, Chikahira M, Ogawa S, Miyamura T, Takeda N, Li TC, 2007. Prevalence of antibody to hepatitis E virus among wild sika deer, Cervus nippon, in Japan. Arch Virol 152: 1375-81

10 Li TC, Miyamura T, Takeda N: Detection of hepatitis e virus RNA from the bivalve yamato-shijimi (corbicula japonica) in Japan. Am J Trop Med Hyg 2007;76:170-172.

11 Nakai I, Kato K, Miyazaki A, Yoshii M, Li TC, Takeda N, Tsunemitsu H, Ikeda H: Different fecal shedding patterns of two common strains of hepatitis e virus at three Japanese Swine farms. Am J Trop Med Hyg 2006;75:1171-1177.

12 Mochizuki M, Ouchi A, Kawakami K, Ishida T, Li TC, Takeda N, Ikeda H, Tsunemitsu H: Epidemiological study of hepatitis E virus infection of dogs and cats in Japan. Vet Rec 2006;159:853-854.

13 Li TC, Saito M, Ogura G, Ishibashi O, Miyamura T, Takeda N: Serologic evidence for hepatitis E virus infection in mongoose. Am J Trop Med Hyg 2006;74:932-936.

14 Li T-C, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y, Kurata Y, Ishida M, Sakamoto S, Takeda N, Miyamura T: Hepatitis E Virus Transmission from Wild Boar Meat. Emerg Infect Dis 2005;11:1958-1960.

15 Miyamoto H., Moriishi K., Moriya K., Murata S., Tanaka K., Suzuki T., Miyamura T., Koike K., and Matsuura Y. Involvement of PA28γ-dependent pathway in insulin resistance induced by hepatitis C virus core protein. *J. Virol.*, 81, 1727-1735 (2007)

16 Shirakura M., Murakami K., Ichimura T., Suzuki R., Shimoji T., Fukuda K., Abe K., Sato S., Fukasawa M., Yamakawa Y., Nishijima M., Moriishi K., Matsuura Y., Wakita T., Suzuki T., Howley P.M., Miyamura T., and Shoji I. The E6AP ubiquitin ligase mediates ubiquitylation and degradation of hepatitis C virus core protein. *J. Virol.*, 81, 1174-1185 (2007).

17 Nakai K., Okamoto T., Kimura-Someya T., Ishii K., Lim C-K.,Tani H., Matsuo E., Abe T., Mori Y., Suzuki T., Miyamura T., Nunberg J.H., Moriishi K., and Matsuura Y. Oligomerization of hepatitis C virus core protein is crucial for interaction with cytoplasmic domain of E1 envelope protein. *J. Virol.*, 80,11265-11273 (2006).

18 Okamoto T. Nishimura Y., Ichimura T., Suzuki K., Miyamura T., Suzuki T., Moriishi K., and Matsuura Y. Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90. *EMBO J*, 25, 5015-5025 (2006).

19 Kato H., Takeuchi O., Sato S., Yoneyama M., Yamamoto M., Matsui K., Uematsu S., Jung A., Kawai T., Ishii K. J., Yamaguchi O., Otsu K., Tsujimura T., Koh C.-S., Sousa C. R., Matsuura Y., Fujita T., and Akira S. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature*, 441, 101-105 (2006).

20 Li T.C., Takeda N., Miyamura T., Matsuura Y., Wang J.C., Engvall H., Hammar L., Xing L., and Cheng R.H. Essential elements of the capsid protein for self-assembly into empty virus-like particles of hepatitis E virus. *J. Virol.* 79, 12999-13006 (2005).

21 Hamamoto I., Nishimura Y., Okamoto T., Aizaki H., Liu M., Mori Y., Abe T., Suzuki T., Lai M.M., Miyamura T., Moriishi K., and Matsuura Y. Human VAP-B Is Involved in Hepatitis C Virus Replication through Interaction with NS5A and NS5B. *J. Virol.* 79:13473-13482 (2005).

22 Abe T., Hemmi H., Moriishi K., Tamura S., Takaku H., Akira S., and Matsuura Y. Involvement of the toll-like receptor 9 signaling pathway in the induction of innate immunity by baculovirus. *J. Virol.* **79**, 2847-2858 (2005).

23 Kitagawa Y., Tani H., Limn C.K., Matsunaga T.M., Moriishi K., and Matsuura Y. Ligand-directed gene targeting to mammalian cells by pseudotype baculoviruses. *J. Virol.* **79**, 3639-3652 (2005).

24 Mori Y., Okabayashi T., Yamashita T., Zhao Z., Wakita T., Yasui K., Hasebe F., Tadano M., Konishi E., Moriishi K., and Matsuura Y. Nuclear localization of Japanese encephalitis virus core protein enhances viral replication. *J. Virol.* **79**, 3448-3458 (2005).

25 Suzuki R., Sakamoto S., Tsutsumi T., Rikimaru A., Tanaka K., Shimoike T., Moriishi K., Iwasaki T., Mizumoto K., Matsuura Y., Miyamura T., and Suzuki T. Molecular determinants for subcellular localization of hepatitis C virus core protein. *J. Virol.* **79**, 1271-1281 (2005).

2. 学会発表

1 Shuhei Taguwa, Toru Okamoto, Takayuki Abe, Kohji Moriishi, and Yoshiharu Matsuura: Human butyrate-induced transcript 1 interacts with HCV NS5A and regulates HCV replication. 14th International Meeting on HCV and Related Viruses, Glasgow, September 9-13, 2007.

2 Takayuki Abe, Yuuki Kaname, Xiaoyu Wen, Kohji Moriishi, Tatsuya Kanto, Norio Hayashi, and Yoshiharu Matsuura: Enhancement of IP-10 expression via TLR signaling pathway in cells expressing HCV proteins. 同上。

3 Hideki Tani, Yasumasa Komoda, Eiko Matsuo, Kensuke Suzuki, Itsuki Hamamoto, Tetsuo Yamashita, Kohji Moriishi, Kazuhito Fujiyama, Tatsuya Kanto, Norio Hayashi, Ania Owsianka, Arvind H. Patel, Michael A. Whitt, and Yoshiharu Matsuura: Replication-competent recombinant vesicular stomatitis virus encoding HCV envelope proteins. 同上。

4 Yoshio Mori, Kiyoko Okamoto, Toru Okamoto, Yasumasa Komoda, Masayasu Okochi, Masatoshi Takeda, Tetsuro Suzuki, Kohji Moriishi, and Yoshiharu Matsuura: Intramembrane processing by SPP regulates membrane localization of HCV core protein and viral propagation. 同上。

5 Toru Okamoto, Kohji Moriishi, and Yoshiharu Matsuura : FKBP8 Plays a Crucial Role in the Replication of Hepatitis C Virus, HEP DART 2007, Hawaii, 9-13 December, 2007.

6 Itsuki Hamamoto, Yorihiro Nishimura, Toru Okamoto, Hideki Aizaki, Minyi Liu, Yoshio Mori, Takayuki Abe, Tetsuro Suzuki, Micheal M.C. Lai, Tatsuo Miyamura, Kohji Moriishi and Yoshiharu Matsuura. Human VAP-B is involved in HCV replication through interaction with NS5A and NS5B. 121th International Meeting on HCV and Related Viruses, Montreal, Canada, October 2-7, 2005.

7 Hironobu Miyamoto, Kohji Moriishi, Kyoji Moriya, Tetsuro Suzuki, Kazuhiko Koike, Tatsuo Miyamura, and Yoshiharu Matsuura. Involvement of PA28gamma in the development of insulin resistance in the HCV core gene transgenic mice. 同上

8 Kosuke Nakai, Kohji Moriishi, Chang Kwang Limn, Toru Okamoto, Tetsuro Suzuki, Jack H. Nunberg, Tatsuo Miyamura, and Yoshiharu Matsuura. Multimerization of HCV core protein is required for the interaction with the cytoplasmic region of E1 protein. 同上

9 Hideki Tani, Yasumasa Komoda, Tetsuo Yamashita, Kensuke Suzuki, Eiko Matsuo, Itsuki Hamamoto, Yoshimi Tsuda, Chang Kweng Lim, Kohji Moriishi, Arvind H. Patel, Tatsuo Miyamura, and Yoshiharu Matsuura. Cell tropism of pseudotype VSV bearing HCV envelope proteins expressed in different cell lines. 同上

10 Yasumasa Komoda, Hideki Tani, Chang Kweng Lim, Kensuke Suzuki, Eiko Matsuo, Yoshimi Tsuda, Kohji Moriishi, Arvind H. Patel, Tatsuo Miyamura, and Yoshiharu Matsuura. Human fibroblast growth factor receptor 5 is a novel candidate entry receptor for HCV. 同上

11 Toru Okamoto, Yorihiro Nishimura, Tohru Ichimura, Kensuke Suzuki, Tatsuo Miyamura, Tetsuro Suzuki, Kohji Moriishi, and Yoshiharu Matsuura: Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90. 20th IUBMB International Congress of Biochemicstry and Molecular Biology, Kyoto, June 18-23, 2006.

12 Tetsuo Yamashita, Yoshio Mori, Hideaki Tetsuo Yamashita, Yoshio Mori, Hideaki Unno, Kohji Moriishi, Tomitake Tsukihara and Yoshiharu Matsuura: Crystal Structure of Catalytic Domain of Japanese Encephalitis Virus NS3 Helicase/Nucleoside Trisphosphatase at a Resolution 1.8 Å.同上。

13 Toru Okamoto, Yorihiro Nishimura, Tohru Ichimura, Kensuke Suzuki, Tatsuo Miyamura, Tetsuro Suzuki, Kohji Moriishi, and Yoshiharu Matsuura: Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90. 13th

International Meeting on HCV and Related Viruses, Cairns, August 27-31, 2006

14 Kohji Moriishi, Kyoji Moriya, Hironobu Miyamoto, Tetsuro Suzuki, Tatsuo Miyamura, Kazuhiko Koike, and Yoshiharu Matsuura: Critical role of PA28γ in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. 同上。

15 Yoshio Mori, Yoshimi Tsuda, Tetsuya Yamashita, Yoshinori Tanaka, Kohji Moriishi and Yoshiharu Matsuura: Biological significance of nuclear localization of Japanese encephalitis virus core protein.同上。

16 Takayuki Abe, Shyu-hei Taguwa, Yuhki Kaname, Kohji Moriishi, Osamu Takeuchi, Kawai Taro, Tatsuya Kanto, Norio Hayashi, Shizuo Akira, and Yoshiharu Matsuura: Modulation of Toll-like receptor signaling in immune cells by expression of hepatitis C virus non-structural proteins. 同上。

17 Takeda N: Norovirus and Sapovirus Activities in Japan. In 2006 Intenational symposium for emerging enteric pathogens. Seoul, 2006.11.

18 Takeda N: Risk factors for hepatitis E virus infection. In 2006 Interational workshop on foodborne pathogens. Taipei, 2006.8

19 Li T-C, Takeda N, Miyamura T: haracterization of Recombinant Virus-Like Particles of Genotype 3 Hepatitis E Virus. In 12th International Symposium on Viral Hepatitis and Liver Disease. Paris, France, 2006.7.

20 Sugitani M, Sheikh A, Tamura A, Shimizu YK, Shimizu K, Moriyama M, Arakawa Y, Komiyama K, Li T-C, Takeda N, Suzuki K, Ishaque SM, Hasan M: Investigation of hepatitis e virus (hev) rna and genotype in sera of bangladesh. In 12th International Symposium on Viral Hepatitis and Liver Disease. Paris, France, 2006.7.

21 森石恆司、森屋恭爾、村田茂穂、田中啓二、鈴木哲朗、宮村達男、小池和彦、松浦善治:HCV コア蛋白質による脂肪酸合成促進と肝細胞癌発症における PA28γの役割：第 43 回日本肝臓学会総会ワークショップ、東京、5 月 31 日-6 月 1 日, 2007.

22 田鍬修平、岡本 徹、阿部隆之、森 嘉生、森石恆司、松浦善治：C 型肝炎ウイルスゲノム複製に関与する宿主蛋白質 B-ind1 の機能解析：第５５回日本ウイルス学会総会、札幌、10 月 21 日-23 日, 2007.

23 阿部隆之、森石恆司、考藤達哉、林 紀夫、審良静男、松浦善治：C 型肝炎ウイルスによる TLR シグナル伝達経路を介した炎症性ケモカイン IP-10 の過剰産生、同上。

24 谷 英樹、菰田泰正、鈴木健介、山下哲生、稲田大彦、森石恆司、考藤達哉、林 紀夫、松浦善治：シュードタイプと組換え水疱性口内炎ウイルスを用いた C 型肝炎ウイルスの感染機構の解析、同上。

25 岡本 徹、森石恆司、松浦善治：C 型肝炎ウイルスゲノム複製における FKBP8 の役割、同上。

26 森 嘉生、岡本貴世子、岡本 徹、菰田泰正、鈴木哲朗、森石恆司、松浦善治：C 型肝炎ウイルスコア蛋白質のシグナルペプチドペプチダーゼによるプロセッシングの生物学的意義、同上。

27 山下哲生、森 嘉生、森石恆司、李 天成、宮村達男、武田直和、月原冨武、吉村政人、松浦善治：E 型肝炎ウイルス様粒子の結晶化と X 線結晶構造解析、同上。

28 李天成, 宮村達男, 脇田隆宇, 武田直和, 2007.10. シジミからの E 型肝炎ウイルス遺伝子の検出、同上。李天成, 宮村達男, 脇田隆宇, 武田直和, 2007.10. キメラマウスにおける E 型肝炎ウイルスの複製、同上。

29 加藤花名子, 佐藤幸代, 宮崎綾子, 吉井雅晃, 土屋公幸, 仲谷淳, 鈴木和男, 樹 金森弘, 李天成, 武田直和, 恒光裕, 池田秀利, 2007.9. 野生動物における抗 E 型肝炎ウイルス抗体の保有状況調査. 第 144 回日本獣医学会学術集会. 江別市

30 松浦友紀子、李 天成、吉松組子、有川二郎、恒光裕、高島郁夫、鈴木正嗣、宮村達男、武田直和。日本に生息するシカの E 型肝炎ウイルス抗体保有。第 54 回日本ウイルス学会総会、名古屋、11 月 19-21 日, 2006.

31 岡本 徹、西村順裕、市村 徹、鈴木哲朗、宮村達男、森石恆司、松浦善治：C 型肝炎ウイルスの複製における FKBP8 の役割、同上。

32 阿部隆之、田鍬修平、要 祐喜、森石恆司、考藤達哉、林 紀夫、審良静男、松浦善治: C 型肝炎ウイルス蛋白質による免疫細胞における自然免疫シグナルの阻害機構の解析、同上。

33 田鍬修平、岡本 徹、阿部隆之、森 嘉生、森石恆司、松浦善治: C 型肝炎ウイルスの複製に関与する新規宿主因子の解析、同上。

34 森 嘉生、山下哲生、田中佳典、森石恆司、松浦善治: カテプシン L によってコア蛋白質がプロセスされない日本脳炎ウイルスの性状、同上。

35 津田祥美、森　嘉生、阿部隆之、山下哲生、岡本　徹、市村　徹、森石恆司、松浦善治：核小体蛋白質 B23 は日本脳炎ウイルスのコア蛋白質と結合しウイルス複製に関与する、同上。

36 谷　英樹、菰田泰正、山下哲生、松尾栄子、岡本　徹、森石恆司、考藤達哉、林　紀夫、松浦善治: HCV エンベロープ遺伝子を組み込んだ組換え VSV、同上。

37 山下哲生、海野英明、森　嘉生、森石恆司、月原冨武、吾郷昌信、松浦善治: 日本脳炎ウイルスの RNA ヘリケースドメインの X 線結晶構造解析、同上。

38 森石恆司、森屋恭爾、宮本大伸、鈴木哲朗、宮村達男、小池和彦、松浦善治: HCV コア蛋白質による脂肪酸合成促進と肝細胞癌発症における PA28γ の役割、同上。

39 松永朋子、谷　英樹、佐藤　薫、森石恆司、藤原晴彦、松浦善治:カイコ幼虫へ遺伝子導入可能な水疱性口内炎ウイルスベクターの開発、同上。

40 松浦友紀子、李　天成、吉松組子、有川二郎、恒光裕、高島郁夫、鈴木正嗣、宮村達男、武田直和。日本に生息するシカの E 型肝炎ウイルス抗体保有、同上。

41 松浦善治、森屋恭爾、田中啓二、宮村達男、鈴木哲朗、小池和彦、森石恆司: C 型肝炎ウイルスによる脂肪肝および肝癌発症における PA28gamma の役割、第 65 回日本癌学会学術総会、横浜、平成 18 年 9 月 28-30 日。

42 浜本いつき、岡本　徹、相崎英樹、西村順裕、森　嘉生、阿部隆之、鈴木哲朗、宮村達男、森石恆司、松浦善治：C 型肝炎ウイルスの複製における宿主因子 VAP-B の機能、第 53 回日本ウイルス学会総会、横浜、11 月 21-23 日, 2005.

43 谷　英樹、菰田泰正、山下哲生、鈴木健介、松尾栄子、浜本いつき、津田祥美、林　昌宏、森石恆司、考藤達哉、林　紀夫、宮村達男、松浦善治：HCV エンベロープ蛋白質を被ったシュードタイプ VSV の感染機構、同上。

44 岡本　徹、西村順裕、市村　徹、鈴木哲朗、宮村達男、森石恆司、松浦善治：C 型肝炎ウイルス NS5A と相互作用する新しい宿主因子の同定、同上。

45 森　嘉生、山下哲生、森石恆司、松浦善治：日本脳炎ウイルスコア蛋白質のプロセッシング機構、同上

46 宮本大伸、森石恆司、森屋恭爾、小池和彦、鈴木哲朗、宮村達男、松浦善治：C 型肝炎ウイルスコア蛋白質によるインスリン抵抗性誘発における PA28γ の役割、同上。

47 李　天成、斉藤　美加、小倉　剛、宮村達男　武田　直和。沖縄に生息するマングースの E 型肝炎ウイルス抗体保有状況、同上。

48 李　天成、宮村　達男、武田　直和。E 型肝炎ウイルス中空粒子形成に必須な領域の同定、同上。

49 岡本貴世子、森石恆司、大河内正康、武田雅俊、松浦善治：シグナルペプチドペプチダーゼによる HCV コア蛋白質のプロセシングとその生物学的意義、第 28 回日本分子生物学会年会、福岡、平成 17 年 12 月 8-11 日。

H.　知的所有権の出願・登録状況

特になし。

研究成果の刊行に関する一覧表

著書

無し

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tani H., Abe K., Matsubaga T., Moriishi K., and Matsuura Y.	Baculovirus vector for gene delivery and vaccine development.	Future Virology	3	35-43	2008
Yamashita T., Unno H., Mori Y., Tani H., Moriishi K., Takamizawa A., Agoh M., Tsukihara T., Matsuura Y.	Crystal structure of the catalytic domain of Japanese encephalitis virus NS3 helicase/nucleoside triphosphatase at a resolution of 1.8 A.	Virology			2008
Moriishi K., Mochizuki R., Moriya K., Miyamoto H., Mori Y., Abe T., Murata S., Tanaka K., Miyamura T., Suzuki T., Koike K., and Matsuura Y.	Critical role of PA28γ in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis.	PNAS	104	1661-1666	2007
Abe T., Kaname Y., Hamamoto I., Tsuda Y., Wen X., Taguwa S., Moriishi K., Takeuchi O., Kawai T., Kanto T., Hayashi N., Akira S., and Matsuura Y.	Hepatitis C Virus Nonstructural Protein 5A Modulates TLR-MyD88-Dependent Signaling Pathway in the Macrophage Cell Lines.	J. Virol.	81	8953-8966	2007
Mori Y., Yamashita T., Tanaka Y., Tsuda Y., Abe T., Moriishi K., and Matsuura Y.	Processing of Capsid Protein by Cathepsin L Plays a Crucial Role in Replication of the Japanese Encephalitis Virus in Neural and Macrophage Cells.	J. Virol.	81	8477-8487	2007
Tani H., Komoda Y., Matsuo E., Suzuki K., Hamamoto I., Yamashita T., Moriishi K., Fujiyama K., Kanto T., Hayashi N., Owsianka A., Patel A.H., Whitt M.A., and Matsuura Y.	Replication-competent recombinant vesicular stomatitis virus encoding hepatitis C virus envelope proteins.	J. Virol.	81	8601-8612	2007
Yamamoto M., Uematsu S., Okamoto T., Matsuura Y., Sato S., Kumar H., Satoh T., Saitoh T., Takeda K., Ishii K.J., Takeuchi O., Kawai T., and Akira S.	Enhanced TLR-mediated NF-IL6 dependent gene expression by Trib1 deficiency.	J. Exp. Med.	204	2233-2239	2007
Moriishi K., and Matsuura Y.	Host factors involved in the replication of hepatitis C virus.	Rev. Med. Virol.	17	343-354	2007
Matsuura Y, Suzuki M, Yoshimatsu K, Arikawa J, Takashima I, Yokoyama M, Igota H, Yamauchi K, Ishida S, Fukui D, Bando G, Kosuge M, Tsunemitsu H, Koshimoto C, Sakae K, Chikahira M, Ogawa S, Miyamura T, Takeda N, Li TC.	Prevalence of antibody to hepatitis E virus among wild sika deer, Cervus nippon, in Japan.	Arch Virol.	152	1375-1381	2007

Li TC, Miyamura T, Takeda N.	Detection of hepatitis e virus RNA from the bivalve yamato-shijimi (corbicula japonica) in Japan.	Am J Trop Med Hyg.	76	170-172	2007
Miyamoto H., Moriishi K., Moriya K., Murata S., Tanaka K., Suzuki T., Miyamura T., Koike K., and Matsuura Y.	Involvement of PA28γ-dependent pathway in insulin resistance induced by hepatitis C virus core protein.	J. Virol.	81	1727-1735	2007
Shirakura M., Murakami K., Ichimura T., Suzuki R., Shimoji T., Fukuda K., Abe K., Sato S., Fukasawa M., Yamakawa Y., Nishijima M., Moriishi K., Matsuura Y., Wakita T., Suzuki T., Howley P.M., Miyamura T., and Shoji I.	The E6AP ubiquitin ligase mediates ubiquitylation and degradation of hepatitis C virus core protein.	J. Virol.	81	1174-1185	2007
Nakai K., Okamoto T., Kimura-Someya T., Ishii K., Lim C-K.,Tani H., Matsuo E., Abe T., Mori Y., Suzuki T., Miyamura T., Nunberg J.H., Moriishi K., and Matsuura Y.	Oligomerization of hepatitis C virus core protein is crucial for interaction with cytoplasmic domain of E1 envelope protein.	J. Virol.	80	11265-11273	2006
Okamoto T. Nishimura Y., Ichimura T., Suzuki K., Miyamura T., Suzuki T., Moriishi K., and Matsuura Y.	Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90.	EMBO J.	25	5015-5025	2006
Kato H., Takeuchi O., Sato S., Yoneyama M., Yamamoto M., Matsui K., Uematsu S., Jung A., Kawai T., Ishii K. J., Yamaguchi O., Otsu K., Tsujimura T., Koh C.-S., Sousa C. R., Matsuura Y., Fujita T., and Akira S.	Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses.	Nature	441	101-105	2006
Nakai I, Kato K, Miyazaki A, Yoshii M, Li TC, Takeda N, Tsunemitsu H, Ikeda H.	Different fecal shedding patterns of two common strains of hepatitis e virus at three Japanese Swine farms.	Am J Trop Med Hyg.	75	1171-1177	2006
Mochizuki M, Ouchi A, Kawakami K, Ishida T, Li TC, Takeda N, Ikeda H, Tsunemitsu H.	Epidemiological study of hepatitis E virus infection of dogs and cats in Japan.	Vet Rec.	159	853-854	2006
Li TC, Saito M, Ogura G, Ishibashi O, Miyamura T, Takeda N.	Serologic evidence for hepatitis E virus infection in mongoose.	Am J Trop Med Hyg.	74	932-936	2006
Li T.C., Takeda N., Miyamura T., Matsuura Y., Wang J.C., Engvall H., Hammar L., Xing L., and Cheng R.H.	Essential elements of the capsid protein for self-assembly into empty virus-like particles of hepatitis E virus.	J. Virol.	79	12999-13006	2005
Hamamoto I., Nishimura Y., Okamoto T., Aizaki H., Liu M., Mori Y., Abe T., Suzuki T., Lai M.M., Miyamura T., Moriishi K., and Matsuura Y.	Human VAP-B Is Involved in Hepatitis C Virus Replication through Interaction with NS5A and NS5B.	J. Virol.	79	13473-13482	2005

Mori Y., Okabayashi T., Yamashita T., Zhao Z., Wakita T., Yasui K., Hasebe F., Tadano M., Konishi E., Moriishi K., and Matsuura Y.	Nuclear localization of Japanese encephalitis virus core protein enhances viral replication.	J. Virol.	79	3448-3458	2005
Suzuki R., Sakamoto S., Tsutsumi T., Rikimaru A., Tanaka K., Shimoike T., Moriishi K., Iwasaki T., Mizumoto K., Matsuura Y., Miyamura T., and Suzuki T.	Molecular determinants for subcellular localization of hepatitis C virus core protein.	J. Virol.	79	1271-1281	2005
Kitagawa Y., Tani H., Limn C.K., Matsunaga T.M., Moriishi K., and Matsuura Y.	Ligand-directed gene targeting to mammalian cells by pseudotype baculoviruses.	J. Virol.	79	3639-3652	2005
Abe T., Hemmi H., Moriishi K., Tamura S., Takaku H., Akira S., and Matsuura Y.	Involvement of the toll-like receptor 9 signaling pathway in the induction of innate immunity by baculovirus.	J. Virol.	79	2847-2858	2005
Li T-C., Chijiwa K., Sera N., Ishibashi T., Etoh Y., Shinohara Y., Kurata Y, Ishida M., Sakamoto S., Takeda N., Miyamura T.	Hepatitis E Virus Transmission from Wild Boar Meat.	Emerg. Infect. Dis.	11	1958-1960	2005

REVIEW

Baculovirus vector for gene delivery and vaccine development

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The baculovirus *Autographa californica* multiple nucleopolyhedrovirus has been widely used not only to acheive a high level of foreign gene expression in insect cells, but also for efficient gene transduction into mammalian cells. Recombinant and pseudotyped baculoviruses possessing chimeric or foreign ligands have been constructed to improve the efficiency of gene transduction and to confer specificity for gene delivery into mammalian cells, respectively. Baculoviral DNA CpG motifs induce proinflammatory cytokines through a Toll-like receptor (TLR9)/MyD88-dependent signaling pathway. Other baculovirus components produce type I interferons via a TLR-independent pathway. Baculovirus exhibits a strong adjuvant property and recombinant baculoviruses encoding microbial antigens elicit antibodies to the antigens and provide protective immunity in mice. This review deals with recent progress in the application of baculovirus vectors to gene delivery and vaccine development, and discusses the future prospects of baculovirus vectors.

The baculovirus is an insect virus possessing a large double-stranded circular DNA genome packaged into a rod-shaped capsid. Among the numerous baculoviruses, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the species most frequently used for baculovirus studies. The baculovirus can produce two morphologically distinct forms of the virus at different time points after infection: budded virus (BV) and occlusion-derived virus (ODV). ODV is occluded in a polyhedron within the nucleus and initiates infection in the midgut epithelium cells, whereas BV is produced after the primary infection and buds through the plasma membrane of infected cells. Owing to a high infectivity to cultured cells and strong late-gene promoters, BV has been commonly used as a tool for the abundant expression of foreign genes in insect cells [1]. BV has also been shown to enter a variety of mammalian cells in order to facilitate the expression of foreign genes under the control of the mammalian promoters, without replication of the viral genome [2–4].

In addition to gene expression in insect cells and gene delivery into mammalian cells, the baculovirus has also been used as a tool for the study of viral particle assembly in both insect and mammalian cells [5,6]. Furthermore, the baculovirus has been utilized as an ubiquitous and a specific gene-transfer vector in the form of a recombinant virus bearing foreign proteins on the viral surface in addition to gp64, a major envelope glycoprotein of the baculovirus [7,8], and pseudotyped virus displaying ligands of interest alone without gp64 [9], respectively. Following these advances, mechanisms of virus entry into mammalian cells [7] and induction of innate immunity by baculoviruses [10–12] have also been elucidated. This paper reviews the recent advances in baculovirus application in mammalian cells and research for the purpose of developing a novel viral vector for gene therapy and vaccine development.

Gene transduction into mammalian cells

The host range of baculovirus was once believed to be strictly restricted to arthropods. Over 20 years ago, it was demonstrated that the baculovirus was taken up via endocytosis into vertebrate cells [13], and a reporter activity was detected in mammalian cells upon infection of a recombinant baculovirus possessing a reporter gene under the control of the mammalian promoter, but *de novo* gene expression was not confirmed [14]. However, 10 years later, recombinant baculoviruses encoding reporter genes under the control of mammalian promoters were shown to be capable of efficient gene transduction into mammalian cell lines, especially in human and rabbit hepatocytes [2,3]. Furthermore, we have shown that baculovirus can deliver foreign genes into various cell lines, including cells of nonhepatic origin, by using a recombinant baculovirus carrying a foreign gene under the control of a strong mammalian promoter, CAG, which consists of cytomegalovirus immediate-early (IE) enhancer, chicken β-actin promoter and a rabbit β-globin polyadenylation signal [4].

Keywords: baculovirus, gene delivery, gene therapy, innate immunity, pseudotype, recombinant, vaccine

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The gene-transfer efficiency was enhanced with the use of recombinant baculoviruses bearing an envelope glycoprotein derived from other viruses such as vesicular stomatitis virus (VSV), rabies virus and mouse hepatitis virus in combination with their own envelope protein gp64 [8], or an excess amount of gp64 [7] on the virion surface. Furthermore, gene transcription by the baculovirus in mammalian cells was enhanced by treatment with histone deacetylase inhibitors, such as butyrate, trichostatin A and valproic acid [15]. In addition, viral adsorption in phosphate-buffered saline at low temperature [16] and the treatment with a calcium chelator for a transient depletion of extracellular calcium [17] enhances the efficiency of gene transduction by the baculovirus into mammalian cells. The current status of our knowledge regarding the application of baculovirus vectors to enhance gene transduction is shown in Box 1.

Box 1. Application for enhancement of gene transduction by baculovirus vector.

Promoter

- Simian virus 40 [75]
- Rous sarcoma virus-long terminal repeat (RSV-LTR) [3]
- Cytomegalovirus immediate-early (CMV-IE) [2]
- Cytomegalovirus immediate early enhancer, chicken β-actin promoter and a rabbit β-globin polyadenylation signal (CAG) [4,7]
- Hepatitis B virus (HBV) [76]
- α-fetoprotein (AFP) [75]

Histone deacetylase inhibitor

- Butyrate [77–79]
- Trichostatin A [79]
- Valproic acid [15]

Inoculation

- Adsorption in phosphate-buffered saline at low temperature [16]
- Calcium chelator for depletion of extracellular calcium [17]

Display

- Single-chain antibody fragment (scFv) against carcinoembryonic antigen [80]
- Synthetic IgG binding domain derived from *Staphylococcus aureus* [80,81]
- Arg-Gly-Asp motifs derived from coxsackievirus A9 and human parechovirus 1 [55]
- Tumor-homing peptides (LyP-1, F3, CGKRK) [55–57]

Pseudotyping

- Vesicular stomatitis virus glycoprotein [7]
- Extra gp64 [7]
- Rabies virus glycoprotein [8]

Complement inhibitor

- Anti-C5 antibody [38]
- Soluble complement receptor type 1 [39]
- Cobra venom factor [38]
- Decay accelerating factor [46]
- 6-amidino-2-naphthyl 4-guanidinobenzoate [8]

Although it is generally considered that the baculovirus does not replicate in mammalian cells and does not induce severe cytotoxicity, transcription of IE genes has been detected at a low level in mammalian cells [18,19]. Recent DNA microarray analyses confirmed the activation of the IE genes in mammalian cells, and demonstrated that the overexpression of the IE genes induced activation of various baculovirus genes [20,21]. However, only a marginal upregulation of the β-actin gene [22,23] and no appreciable change in the transcriptome profiles [22,23] was detected by DNA microarray analysis upon infection with baculovirus in mammalian cells. Nevertheless, transcriptional activation of the IE genes raises concerns regarding the safety of application of baculovirus vectors for gene therapy.

Baculovirus has also been used as an ideal tool for the production of virus-like particles (VLPs), which generally require abundant expression of viral structural proteins for self-assembly. VLPs of various viruses have been produced in insect cells upon infection with recombinant baculoviruses and have proven to be useful not only for vaccines or diagnostic reagents, but also for the study of virus–cell interactions [5,24]. Recently, VLPs of hepatitis D virus [25,26] and hepatitis C virus (HCV) [6] have been generated in mammalian cells. The production of VLPs by infection with recombinant baculoviruses is much higher than that by transfection of plasmid or in stable cell lines. In contrast to the VLP production by replication-competent viral vectors, baculovirus vectors have the advantage that VLPs will not be contaminated with the infectious viral vectors.

Entry of baculovirus into mammalian cells

Mammalian cells of various origins exhibit susceptibility to baculovirus entry, suggesting that the baculovirus utilizes a cell-surface molecule(s) ubiquitously expressed on mammalian cells. However, the precise mechanisms of baculovirus entry are still poorly understood. Pretreatments of insect cells with proteases or inhibitors of *N*-linked glycosylation reduced the virus binding, suggesting the involvement of a cellular glycoprotein(s) in virus adsorption [27,28]. Electrostatic interactions through heparan sulfate moieties are also suggested to be required for the binding of the baculovirus to mammalian cells [29]. Baculovirus is considered to enter both insect and mammalian cells via an endosomal pathway upon interaction of gp64 with its receptor(s). Information on the role of gp64 on the

membrane-fusion activity in insect cells has been accumulated [30–32]. We previously demonstrated that a recombinant baculovirus possessing 1.5-fold higher amounts of gp64, compared with a wild-type baculovirus, exhibits a 100-fold increase in reporter gene expression in various mammalian cells, and digestion of phospholipids from the cells markedly reduced the gene transduction by the baculovirus [7]. Furthermore, infection of the baculovirus was inhibited by negatively charged lipids such as phosphatidic acid and phosphatidylinositol (PI), and was reduced in a mutant CHO cell line deficient in PI synthesis. These results suggest that the interaction of gp64 with cell surface phospholipids plays a crucial role in the entry of baculoviruses into cells.

Baculovirus has been shown to enter hepatocytes via clathrin-mediated endocytosis and macropinocytosis by using confocal and electron microscopy [33]. It has also been shown that actin filaments play a role in the cytoplasmic transport of baculovirus nucleocapsids during entry into cells [34–36], and the amount of the nucleocapsids was increased in the nucleus by treatment with microtubule depolymerizing agents, suggesting that the nucleocapsids are transported into the nucleus through a microtubule network [37]. Further studies on the entry of baculovirus, including identification of cellular receptor(s), are needed for the development of vectors suitable for the application to gene therapy.

In vivo gene delivery

Baculovirus has been shown to be an ideal vector for efficient gene delivery *in vitro*. However, application of the baculovirus for *in vivo* gene transfer has been hampered by serum complement-mediated inactivation. To circumvent this problem, inhibitory reagents against the complement system, such as antibody against the complement component 5 [38], cobra venom factor [38], a soluble protein of complement receptor type 1 [39] and a inhibitor for protease-activating complement (FUT-175) [8], have been used to improve *in vitro* gene transduction by the baculovirus in the presence of animal sera. Direct inoculation of the baculovirus into rodent brain [8,40–42], mouse skeletal muscle [43], testis [8] and eye [44], or rabbit carotid artery by using a silastic collar to avoid contact with complement components [45] was also successful for the delivery of foreign genes. Recombinant baculoviruses bearing chimeric gp64 proteins fused with a decay accelerating factor (DAF), which blocks complement at the central step of both the classical and alternative pathways, exhibited some resistance to complement inactivation [46]. HIV-based lentivirus vectors pseudotyped with gp64–DAF fusion proteins also conferred resistance to complement inactivation [47]. Furthermore, the recombinant baculoviruses possessing rhabdovirus envelope proteins exhibited more resistance to complement inactivation by sera of humans, rabbits, guinea pigs and mice compared with the unmodified baculovirus [8]. The recombinant baculoviruses possessing the VSV envelope G (VSVG) protein also gave rise to a significantly higher gene expression than the unmodified virus in mouse skeletal muscle [43] and brain [8,48]. Recently, recombinant baculovirus encoding the *Diphtheria Toxin A* gene under the control of the modified promoter were shown to eliminate malignant glioma cells in the rat brain [49]. Although recombinant baculoviruses bearing VSVG proteins exhibited more resistance to complement inactivation compared with the unmodified virus [8], lentiviral vectors, including feline immunodeficiency virus (FIV) pseudotyped with gp64 proteins, exhibited more resistance to human and mouse complements than FIV pseudotype bearing VSVG proteins [50,51]. This discrepancy in the sensitivity to complement system may be attributable to the differences in glycosylation of gp64 proteins expressed in insect or mammalian cells. Gene transduction into mammalian cells and in mice [50], and a persistent expression of a transgene in mouse nasal epithelia *in vivo* [52] by the FIV pseudotype bearing gp64 proteins was significantly higher than that bearing VSVG proteins. Further studies on the pseudotype viruses bearing gp64 proteins will need to be carried out in order to realize gp64-mediated gene delivery *in vivo*.

Targeting vector

Recombinant baculoviruses bearing heterologous peptides or proteins [53,54], an RGD-motif that recognizes αV integrin [55,56] and tumor-homing peptides [57] fused with gp64 or VSVG proteins exhibited efficient binding and gene transduction to target cells. However, these recombinant viruses exhibit no specificity for gene transduction owing to the retained gp64 proteins that recognize molecule(s) ubiquitously expressed on the cell surface. A pseudotyped virus system bearing heterologous ligands (e.g., viral envelope proteins or receptors) in place of the endogenous viral envelope protein has been developed in retroviruses [58] and rhabdoviruses [59] for gene

targeting. To confer specificity on gene delivery by the baculovirus, a pseudotyped baculovirus bearing only a ligand of interest, but lacking gp64, was generated by using a gp64-null baculovirus [9]. The gp64-null baculovirus revealed that gp64 is necessary for cell binding [60], membrane fusion [30] and efficient virion budding from the infected cell surface [61]. Although pseudotyped baculoviruses bearing VSVG or fusion envelope proteins from other baculoviruses exhibited a high infectivity to insect cells, gene transduction into mammalian cells has yet to be explored [62,63]. During amplification of the pseudotyped baculovirus in an insect cell line stably expressing gp64, breakthrough viruses incorporating the *gp64* gene readily emerged after more than three passages [9]. Therefore, we generated pseudotyped baculoviruses by transfection of a bacmid encoding a full-length recombinant baculovirus – possessing a ligand of interest in place of gp64 – into insect cells without amplification in a cell line expressing gp64. Pseudotyped baculoviruses bearing receptors for the measles virus, CD46 or SLAM, exhibited a specific gene transduction into target cells expressing measles virus envelope proteins [9]. Although we have to overcome the problem of emergence of the replication-competent revertants incorporating the *gp64* gene during the amplification, the pseudotyped baculovirus has important potential as a targeting vector.

Induction of immune response & advantages for use as vaccine vectors

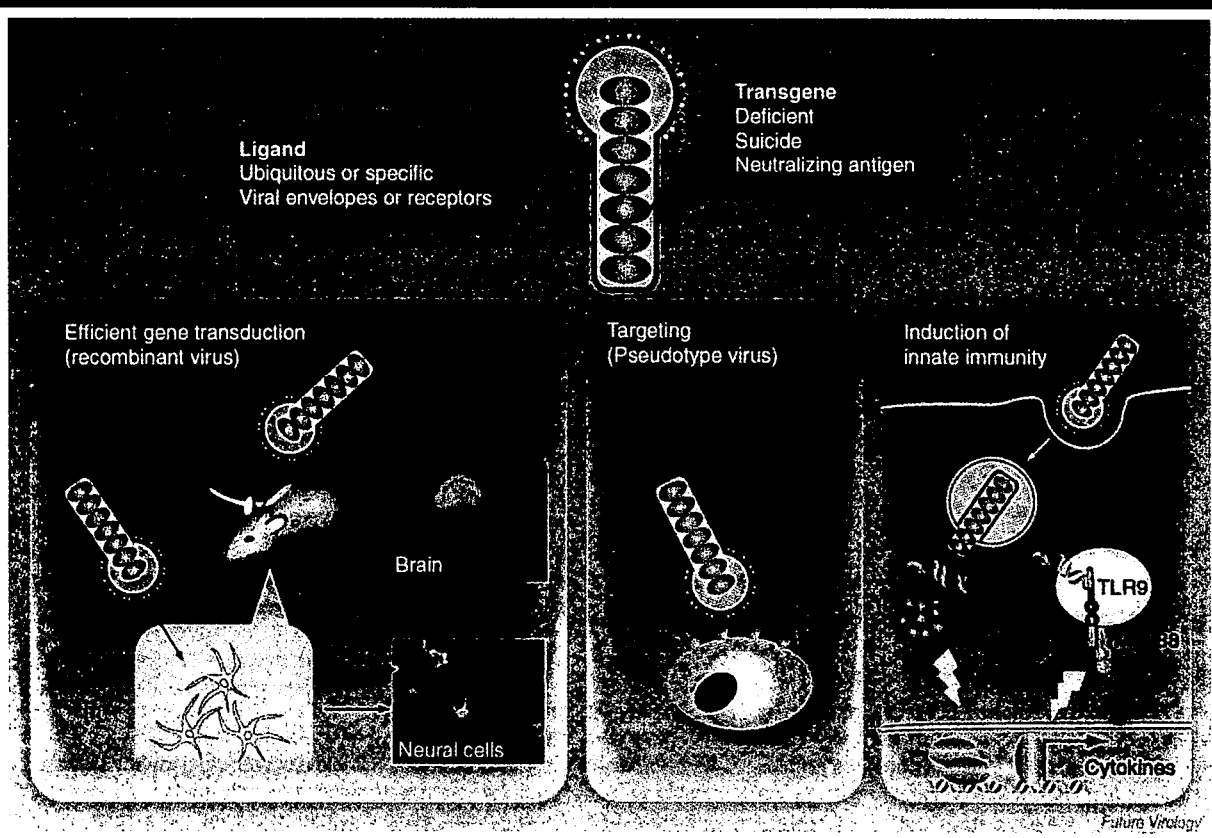
Beside the efficient gene delivery into mammalian cells, only a few definitive findings have been reported on the induction of host immune response by the baculovirus. Intramuscular injection of recombinant baculoviruses encoding the pseudorabies virus gB protein [64] and HCV envelope glycoprotein E2 [65] and intramuscular, intraperitoneal or intranasal inoculation with those expressing hemagglutinin (HA) of the influenza virus (IFV) [11] elicited humoral immune responses in mice. Although mice intramuscularly or intraperitoneally immunized with the recombinant baculovirus produced higher levels of anti-HA antibodies than those immunized intranasally, protection from a lethal challenge with IFV was only achieved by the intranasal immunization. Furthermore, recombinant baculoviruses bearing the chimeric gp64 proteins of *Plasmodium berghei* circumsporozoite [66], *Plasmodium falciparum* circumsporozoite [67], *Theila parva* sporozoite [68], rinderpest virus [69], foot-and-mouth disease virus [70] and avian influenza virus [71] elicit an effective antibody response.

To our surprise, a sufficient protective immunity against a lethal IFV challenge was induced in mice intranasally inoculated with a wild-type baculovirus, as evaluated by reductions in virus titer, inflammatory cytokine production, and pulmonary consolidations [11]. Mouse macrophage cell lines, primary peritoneal macrophages (PECs) and CD11c-positive dendritic cells ($CD11c^+$ DCs) inoculated with a wild-type baculovirus produced a large amount of proinflammatory cytokines, such as IL-12, IL-6, TNF-α and type-I IFNs [11,12]. These observations suggest that baculovirus has an immunostimulatory activity to produce pro-inflammatory cytokines and type-I IFNs in immunocompetent cells, by which the spread of IFV infection is suppressed. A similar phenomenon has been reported that a 67 kDa glycoprotein purified from the culture supernatants of insect cells infected with AcMNPV, which is probably identical to gp64, induced IFN production in cultured cells and protected from a lethal encephalomyocarditis virus infection in mice [10]. However, several lines of evidence suggest that gp64 is not a principal actor in the immunostimulatory properties of the baculovirus. It was shown that a recombinant gp64 protein produced in insect cells and the gp64-null baculovirus exhibited no production of proinflammatory cytokines and type-I IFNs in mouse macrophage cell lines [12], suggesting that a viral component(s) other than gp64 participates in the immune activation by the baculovirus.

Although the baculovirus induces a strong innate immune response in mice, the precise mechanisms of the induction remain unclear. Toll-like receptors (TLRs) have been shown to play a crucial role in the recognition of viral and bacterial components [72]. Upon infection of a microorganism, TLRs recruit a set of adaptor molecules, including MyD88, Toll/interleukin-1 receptor (TIR)-domain-containing adaptor protein (TIRAP), TIR-domain-containing adaptor-inducing IFN-β (TRIF) and TIR-domain-containing adaptor inducing IFN-β-related adaptor molecule (TRAM), which trigger the activation of transcription factors such as NF-κB that are essential for the expression of proinflammatory cytokines and type-I IFNs [72]. Production of proinflammatory cytokines was severely impaired in PECs and $CD11c^+$ DCs derived from mice deficient in MyD88 or TLR9 after cultivation with the baculovirus, suggesting that the

baculovirus produces proinflammatory cytokines in immune competent cells via the TLR9/MyD88-dependent signaling pathway [11,12]. By contrast, a significant amount of IFN-α still remained in the cells derived from MyD88- or TLR9-deficient mice after stimulation with baculovirus, suggesting that a TLR-independent signaling pathway participates in the production of IFN-α by the baculovirus. Further studies are needed to elucidate the precise mechanisms of the induction of TLR-independent type-I IFNs by baculovirus. TLR9 has been shown to be responsible for the stimulation of the immune system by oligodeoxynucleotides containing unmethylated CpG motifs that are present in bacterial and some viral DNAs [73]. The amounts of bioactive CpG sequences in the genome of baculovirus have been shown to be similar to those of *Escherichia coli* and herpes simplex virus type I [12]. Transfection of the purified baculovirus DNA with liposome resulted in the production of TNF-α, but not in the absence of liposome, and the pretreatment with inhibitors for endosomal maturation diminished the induction of the immune response by the baculovirus [12]. These results indicate that membrane fusion with gp64 and uncoating of viral DNA via endosomal maturation, which leads to release of viral DNA into the cellular compartments expressing TLR9, are involved in the induction of innate immunity by baculovirus *in vitro*. Recently, it was shown that baculovirus has a strong adjuvant activity in mice, which promotes adaptive immune responses against coadministered antigen [74]. Immunization with ovalbumin (OVA) plus baculoviral DNA in cationic liposomes, but not with OVA in combination with either viral DNA or liposome, was sufficient to induce a cytotoxic T-lymphocyte response, suggesting that the viral genome is responsible for the adjuvant activity of baculovirus [74].

Figure 1. Baculovirus as a versatile vector.



Recombinant baculoviruses possessing an extra ligand such as another viral envelope protein in combination with gp64, exhibit more efficient gene transduction both *in vivo* and in primary cells. Pseudotyped baculoviruses bearing only a single ligand, such as a specific receptor, exhibit gene targeting. Baculovirus can be harnessed as a vector for gene therapy and vaccine development by the transduction of deficient, suicide and neutralizing antigen genes. Furthermore, baculovirus is capable of inducing a strong innate immune response in immune cells, suggesting that it can be used as an adjuvant-containing vaccine vector against infectious diseases by transduction of neutralizing antigen in immune competent cells.

Conclusion & future perspective

A summary of baculovirus vectors is shown in Figure 1. Since the initial discovery of efficient gene transduction into mammalian cells by recombinant baculoviruses, numerous efforts have been made to harness baculovirus as a vector for gene delivery and vaccine development. One of the advantages of the baculovirus vector over other viral vectors is that it has a large capacity to accommodate foreign genes and demonstrates efficient gene transduction into a wide variety of cell lines as well as primary cells, without severe cytotoxicity. Although construction of recombinant and pseudotyped baculoviruses, and the use of inhibitors for complement activation have been achieved to improve the efficiency of gene transduction, the efficacy of gene delivery by baculovirus vectors *in vivo* is not sufficient for clinical gene therapy at the moment. Further innovation to establish replication-competent cell lines capable of supporting the propagation of pseudotyped viruses without the possibility of replication-competent virus breakthrough by incorporation of gp64, and to optimize the conditions necessary for the efficient incorporation of ligands into recombinant baculovirus particles in order to improve the efficiency of gene transduction by baculovirus vectors is needed. For future *in vivo* applications of baculovirus vectors for gene targeting to specific organs or virus-infected cells as a method of treatment for inherited or infectious diseases, it is imperative to exhaustively study the transcription of baculoviral genes in mammalian cells for the certification of safety. In addition, structural analyses of gp64 and identification of its cellular

Executive summary

Baculoviruses can transduce foreign genes into mammalian cells without replication

- Recombinant baculoviruses efficiently transduce foreign genes into a broad range of mammalian cells.
- Recombinant baculoviruses bearing vesicular stomatitis virus envelope G or other viral envelope proteins enhance gene transduction.
- Various reagents are used for enhancement of gene transduction by the baculovirus.
- Immediate-early genes of the baculovirus are expressed at a low level in mammalian cells.

Advantages of baculoviral vectors

- Recombinant baculoviruses can be easily prepared in insect cells by a convenient system.
- The baculovirus genome has a large capacity to accommodate foreign genes.
- Baculoviruses can transduce foreign genes into mammalian cells without replication and cytopathic effect.

Entry mechanisms of baculovirus into mammalian cells

- Baculovirus enters into mammalian cells using gp64, via endocytosis.
- Cell-surface phospholipids are involved in the entry of baculovirus.
- The nucleocapsid of baculovirus translocates into the nucleus through the microtubule network.

In vivo gene delivery

- *In vivo* gene delivery by the baculovirus is hampered by the inactivation by serum complement.
- Measures to inhibit activation of the complement improve *in vitro* gene transduction.
- Direct injection of baculovirus into rodent brain, muscle, testis and eye can deliver reporter genes.
- Modifications of gp64 improve *in vivo* gene delivery.

Targeting vector

- The presence of heterologous proteins in addition to gp64 on the virion surface enhances specific binding to the target cells.
- Pseudotype baculovirus bearing a ligand of interest alone in place of gp64 exhibits the ability to perform a specific gene transduction into target cells.

Induction of immune response by baculovirus

- Recombinant baculoviruses elicit humoral immune responses and protective immunity in mice.
- Baculovirus produces an innate immune response in immune competent cells.
- CpG motifs in the baculovirus genome produce proinflammatory cytokines thorough a Toll-like receptor-dependent pathway.
- Baculovirus components other than DNA produce type-I interferons via a Toll-like receptor-independent pathway.
- Baculovirus exhibits a strong adjuvant property promoting an adaptive response against coadministered antigen in mice.

Future prospects for baculoviral vectors

- Further characterization of cellular receptor(s) for baculovirus and the interaction with gp64 is needed for the construction of vectors sufficient for a future gene therapy.
- Elucidation of mechanisms to induce innate immunity in immune-competent cells by baculovirus is required for the development of a future vaccine vector.

receptor(s) are required to gain more insight on the display system and the entry mechanisms of baculovirus vectors. Although baculovirus cannot replicate in mammalian cells, a potent innate immune response is induced by baculovirus in immune competent cells such as macrophages and dendritic cells, and this stimulation is essential for the later induction of adaptive immune responses. The ability of baculovirus to induce innate immunity makes it a promising candidate for a future adjuvant-containing vaccine vehicle against infectious diseases.

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Ikonomou L, Schneider YJ, Agathos SN: Insect cell culture for industrial production of recombinant proteins. *Appl. Microbiol. Biotechnol.* 62, 1–20 (2003).
2. Hofmann C, Sandig V, Jennings G, Rudolph M, Schlag P, Strauss M: Efficient gene transfer into human hepatocytes by baculovirus vectors. *Proc. Natl Acad. Sci. USA* 92, 10099–10103 (1995).

•• **One of the first papers (with [3]) to demonstrate an efficient gene transduction into mammalian cells by baculovirus vectors.**

3. Boyce FM, Bucher NL: Baculovirus-mediated gene transfer into mammalian cells. *Proc. Natl Acad. Sci. USA* 93, 2348–2352 (1996).

•• **One of the first papers (with [2]) to demonstrate an efficient gene transduction into mammalian cells by baculovirus vectors.**

4. Shoji I, Aizaki H, Tani H *et al.*: Efficient gene transfer into various mammalian cells, including non-hepatic cells, by baculovirus vectors. *J. Gen. Virol.* 78(Pt 10), 2657–2664 (1997).
5. Li TC, Yamakawa Y, Suzuki K *et al.*: Expression and self-assembly of empty virus-like particles of hepatitis E virus. *J. Virol.* 71, 7207–7213 (1997).
6. Matsuo E, Tani H, Lim C *et al.*: Characterization of HCV-like particles produced in a human hepatoma cell line by a recombinant baculovirus. *Biochem. Biophys. Res. Commun.* 340, 200–208 (2006).
7. Tani H, Nishijima M, Ushijima H, Miyamura T, Matsuura Y: Characterization of cell-surface determinants important for baculovirus infection. *Virology* 279, 343–353 (2001).

•• **Demonstrates efficient gene transduction by recombinant baculoviruses and the involvement of cell-surface phospholipids for baculovirus entry.**

8. Tani H, Limn CK, Yap CC *et al.*: *In vitro* and *in vivo* gene delivery by recombinant baculoviruses. *J. Virol.* 77, 9799–9808 (2003).

•• **Demonstrates gene delivery by recombinant baculoviruses into mouse brain and testis *in vivo*.**

9. Kitagawa Y, Tani H, Limn CK, Matsunaga TM, Morishi K, Matsuura Y: Ligand-directed gene targeting to mammalian cells by pseudotype baculoviruses. *J. Virol.* 79, 3639–3652 (2005).

•• **Demonstrates the construction of pseudotyped baculovirus for gene targeting.**

10. Gronowski AM, Hilbert DM, Sheehan KC, Garotta G, Schreiber RD: Baculovirus stimulates antiviral effects in mammalian cells. *J. Virol.* 73, 9944–9951 (1999).
11. Abe T, Takahashi H, Hamazaki H, Miyano-Kurosaki N, Matsuura Y, Takaku H: Baculovirus induces an innate immune response and confers protection from lethal influenza virus infection in mice. *J. Immunol.* 171, 1133–1139 (2003).

•• **Identified a signaling pathway for induction of innate immunity in mammalian cells by the baculovirus.**

12. Abe T, Hemmi H, Miyamoto H *et al.*: Involvement of the Toll-like receptor 9 signaling pathway in the induction of innate immunity by baculovirus. *J. Virol.* 79, 2847–2858 (2005).

•• **Identified a signaling pathway for induction of innate immunity in mammalian cells by the baculovirus.**

13. Volkman LE, Goldsmith PA: *In vitro* survey of *Autographa californica* nuclear polyhedrosis virus interaction with nontarget vertebrate host cells. *Appl. Environ. Microbiol.* 45, 1085–1093 (1983).
14. Carbonell LF, Klowden MJ, Miller LK: Baculovirus-mediated expression of bacterial genes in dipteran and mammalian cells. *J. Virol.* 56, 153–160 (1985).
15. Hu YC, Tsai CT, Chang YJ, Huang JH: Enhancement and prolongation of baculovirus-mediated expression in mammalian cells: focuses on strategic infection and feeding. *Biotechnol. Prog.* 19, 373–379 (2003).
16. Hsu CS, Ho YC, Wang KC, Hu YC: Investigation of optimal transduction conditions for baculovirus-mediated gene delivery into mammalian cells. *Biotechnol. Bioeng.* 88, 42–51 (2004).
17. Bilello JP, Delaney WEt, Boyce FM, Isom HC: Transient disruption of intercellular junctions enables baculovirus entry into nondividing hepatocytes. *J. Virol.* 75, 9857–9871 (2001).
18. Knebel D, Doerfler W: Activation of an insect baculovirus promoter in mammalian cells by adenovirus functions. *Virus Res.* 8, 317–326 (1987).
19. Murges D, Kremer A, Knebel-Morsdorf D: Baculovirus transactivator IE1 is functional in mammalian cells. *J. Gen. Virol.* 78(Pt 6), 1507–1510 (1997).
20. Jiang SS, Chang IS, Huang LW *et al.*: Temporal transcription program of recombinant *Autographa californica* multiple nucleopolyhedrosis virus. *J. Virol.* 80, 8989–8999 (2006).
21. Liu CY, Wang CH, Wang JC, Chao YC: Stimulation of baculovirus transcriptome expression in mammalian cells by baculoviral transcriptional activators. *J. Gen. Virol.* 88, 2176–2184 (2007).
22. Fujita R, Matsuyama T, Yamagishi J, Sahara K, Asano S, Bando H: Expression of *Autographa californica* multiple nucleopolyhedrovirus genes inmammalian cells and upregulation of the host β-actin gene. *J. Virol.* 80, 2390–2395 (2006).
23. Kenoutis C, Efrose RC, Swevers L *et al.*: Baculovirus-mediated gene delivery into mammalian cells does not alter their transcriptional and differentiating potential but is accompanied by early viral gene expression. *J. Virol.* 80, 4135–4146 (2006).

24. Li TC, Suzaki Y, Ami Y, Dhole TN, Miyamura T, Takeda N: Protection of cynomolgus monkeys against HEV infection by oral administration of recombinant hepatitis E virus-like particles. *Vaccine* 22, 370–377 (2004).
25. Wang KC, Wu JC, Chung YC, Ho YC, Chang MD, Hu YC: Baculovirus as a highly efficient gene delivery vector for the expression of hepatitis delta virus antigens in mammalian cells. *Biotechnol. Bioeng.* 89, 464–473 (2005).
26. Chen YH, Wu JC, Wang KC *et al.*: Baculovirus-mediated production of HDV-like particles in BHK cells using a novel oscillating bioreactor. *J. Biotechnol.* 118, 135–147 (2005).
27. Wickham TJ, Shuler ML, Hammer DA, Granados RR, Wood HA: Equilibrium and kinetic analysis of *Autographa californica* nuclear polyhedrosis virus attachment to different insect cell lines. *J. Gen. Virol.* 73(Pt 12), 3185–3194 (1992).
28. Wang P, Hammer DA, Granados RR: Binding and fusion of *Autographa californica* nucleopolyhedrovirus to cultured insect cells. *J. Gen. Virol.* 78(Pt 12), 3081–3089 (1997).
29. Dutsit G, Saleun S, Douthe S, Barsoum J, Chadeuf G, Moullier P: Baculovirus vector requires electrostatic interactions including heparan sulfate for efficient gene transfer in mammalian cells. *J. Gene Med.* 1, 93–102 (1999).
30. Blissard GW, Wenz JR: Baculovirus gp64 envelope glycoprotein is sufficient to mediate pH-dependent membrane fusion. *J. Virol.* 66, 6829–6835 (1992).
31. Kingsley DH, Behbahani A, Rashtian A, Blissard GW, Zimmerberg J: A discrete stage of baculovirus GP64-mediated membrane fusion. *Mol. Biol. Cell* 10, 4191–4200 (1999).
32. Plonsky I, Cho MS, Oomens AG, Blissard G, Zimmerberg J: An analysis of the role of the target membrane on the Gp64-induced fusion pore. *Virology* 253, 65–76 (1999).
33. Matilainen H, Rinne J, Gilbert L, Marjomaki V, Reunanen H, Oker-Blom C: Baculovirus entry into human hepatoma cells. *J. Virol.* 79, 15452–15459 (2005).
34. Charlton CA, Volkman LE: Penetration of *Autographa californica* nuclear polyhedrosis virus nucleocapsids into IPLB Sf 21 cells induces actin cable formation. *Virology* 197, 245–254 (1993).
35. Lanier LM, Volkman LE: Actin binding and nucleation by *Autographa california* M nucleopolyhedrovirus. *Virology* 243, 167–177 (1998).
36. van Loo ND, Fortunati E, Ehlert E, Rabelink M, Grosveld F, Scholte BJ: Baculovirus infection of nondividing mammalian cells: mechanisms of entry and nuclear transport of capsids. *J. Virol.* 75, 961–970 (2001).
37. Salminen M, Airenne KJ, Rinnankoski R *et al.*: Improvement in nuclear entry and transgene expression of baculoviruses by disintegration of microtubules in human hepatocytes. *J. Virol.* 79, 2720–2728 (2005).
38. Hofmann C, Strauss M: Baculovirus-mediated gene transfer in the presence of human serum or blood facilitated by inhibition of the complement system. *Gene Ther.* 5, 531–536 (1998).
•• **Demonstrates the inactivation of baculovirus gene transduction by serum complement.**
39. Hofmann C, Huser A, Lehnert W, Strauss M: Protection of baculovirus-vectors against complement-mediated inactivation by recombinant soluble complement receptor type 1. *Biol. Chem.* 380, 393–395 (1999).
40. Sarkis C, Serguera C, Petres S *et al.*: Efficient transduction of neural cells *in vitro* and *in vivo* by a baculovirus-derived vector. *Proc. Natl Acad. Sci. USA* 97, 14638–14643 (2000).
41. Li Y, Wang X, Guo H, Wang S: Axonal transport of recombinant baculovirus vectors. *Mol. Ther.* 10, 1121–1129 (2004).
42. Lehtolainen P, Tyynela K, Kannasto J, Airenne KJ, Yla-Herttuala S: Baculoviruses exhibit restricted cell type specificity in rat brain: a comparison of baculovirus- and adenovirus-mediated intracerebral gene transfer *in vivo*. *Gene Ther.* 9, 1693–1699 (2002).
43. Pieroni L, Malone D, La Monica N: *In vivo* gene transfer in mouse skeletal muscle mediated by baculovirus vectors. *Hum. Gene Ther.* 12, 871–881 (2001).
44. Haeseleer F, Imanishi Y, Saperstein DA, Palczewski K: Gene transfer mediated by recombinant baculovirus into mouse eye. *Invest. Ophthalmol. Vis. Sci.* 42, 3294–3300 (2001).
45. Airenne KJ, Hiltunen MO, Turunen MP *et al.*: Baculovirus-mediated periadventitial gene transfer to rabbit carotid artery. *Gene Ther.* 7, 1499–1504 (2000).
46. Huser A, Rudolph M, Hofmann C: Incorporation of decay-accelerating factor into the baculovirus envelope generates complement-resistant gene transfer vectors. *Nat. Biotechnol.* 19, 451–455 (2001).
47. Gulbinga GH, Friedmann T: Baculovirus GP64-pseudotyped HIV-based lentivirus vectors are stabilized against complement inactivation by codisplay of decay accelerating factor (DAF) or of a GP64-DAF fusion protein. *Mol. Ther.* 11, 645–651 (2005).
48. Kaikkonen MU, Raty JK, Airenne KJ, Wirth T, Heikura T, Yla-Herttuala S: Truncated vesicular stomatitis virus G protein improves baculovirus transduction efficiency *in vitro* and *in vivo*. *Gene Ther.* 13, 304–312 (2006).
49. Wang CY, Li F, Yang Y, Guo HY, Wu CX, Wang S: Recombinant baculovirus containing the diphtheria toxin A gene for malignant glioma therapy. *Cancer Res.* 66, 5798–5806 (2006).
50. Schauber CA, Tuerk MJ, Pacheco CD, Escarpe PA, Veres G: Lentiviral vectors pseudotyped with baculovirus gp64 efficiently transduce mouse cells *in vivo* and show tropism restriction against hematopoietic cell types *in vitro*. *Gene Ther.* 11, 266–275 (2004).
•• **Demonstrates the improved gene transduction by lentiviral vectors pseudotyped with gp64.**
51. Kang Y, Xie L, Tran DT *et al.*: Persistent expression of factor VIII *in vivo* following nonprimate lentiviral gene transfer. *Blood* 106, 1552–1558 (2005).
•• **Demonstrates the improved gene transduction by lentiviral vectors pseudotyped with gp64.**
52. Sinn PL, Burnight ER, Hickey MA, Blissard GW, McCray PB Jr: Persistent gene expression in mouse nasal epithelia following feline immunodeficiency virus-based vector gene transfer. *J. Virol.* 79, 12818–12827 (2005).
•• **Demonstrates the improved gene transduction by lentiviral vectors pseudotyped with gp64.**
53. Hu YC: Baculovirus as a highly efficient expression vector in insect and mammalian cells. *Acta Pharmacol. Sin.* 26, 405–416 (2005).
54. Kost TA, Condreay JP, Jarvis DL: Baculovirus as versatile vectors for protein expression in insect and mammalian cells. *Nat. Biotechnol.* 23, 567–575 (2005).
•• **Describes current technologies of the baculoviral vectors.**