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Functional reconstitution of G-protein-coupled receptor-mediated adenylyl cyclase activation by a baculoviral co-display system

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ABSTRACT

Recently, evidence has accumulated in support of the heterologous expression of functional membrane proteins and their complexes on extracellular baculovirus particles (budded virus, BV). In this study, we attempted to apply this BV display system to detect G-protein-coupled receptor (GPCR) signaling. We infected Sf9 cells with a combination of four recombinant baculoviruses individually encoding the dopamine D1 receptor (DR-D1), G-protein α-subunit ($G\alpha_s$), G-protein $\beta_1\gamma_2$ subunit dimer ($G\beta_1\gamma_2$), and adenylyl cyclase type VI (ACVI). The recovered BV fraction produced cAMP in response to the stimulation with dopamine. Co-expression of all three G-protein subunits in addition to receptor and ACVI led to a maximal response. BV co-expressing DR-D1, $G\alpha_s$, $G\beta_1\gamma_2$, and ACVI also responded to dopamine agonists and an antagonist. Furthermore, BV expressing two other $G\alpha_s$-coupled receptors together with $G\alpha_s$, $G\beta_1\gamma_2$, and ACVI also produced cAMP in response to their specific ligands. These results indicate the functional coupling of receptor, $G\alpha_s$ and ACVI is reconstituted on BV. Since BV is essentially free of endogenous GPCRs, this BV co-display system should prove highly useful for the development of functional assay systems for GPCRs.

1. Introduction

G-protein-coupled receptors (GPCRs) play an important role in transmembrane signal transduction and attract a considerable interest as potential therapeutic targets (for review, Eglen et al., 2007). Systems to detect and quantify ligand-induced GPCR signaling are useful both for fundamental studies and for industrial use. Cell-based functional assays, mostly with mammalian and amphibian cells, are now widely utilized (Eglen et al., 2007). However, these animal cells express endogenous GPCRs, which can cause a high background signal. Furthermore, some GPCRs, such as odorant receptors, do not express well on the cell surface when their genes are introduced in animal cell lines (McClintock and Sammeta, 2003).

Another possible cell system is one utilizing insect cell lines. Sf9 cells, derived from *Spodoptera frugiperda*, are essentially free of endogenous GPCRs (Butkerait et al., 1995), providing a low background environment for functional assays. Although the baculoviral system is commonly utilized to express GPCRs in Sf9 cells (Bouvier et al., 1998), it is lytic and therefore not directly applicable for a cell-based assay system. Meanwhile, there is accumulating evidence that heterologous membrane proteins are displayed on the extracellular baculovirus particles (budded virus, BV) (Loisel et al., 1997; Masuda et al., 2003; Saitoh et al., 2006). Loisel et al. first reported that biologically active β_2-adrenergic receptor (β_2AR) is efficiently recovered in BV derived from cells infected with β_2AR recombinant virus. Furthermore, our group demonstrated that co-expression of leukotriene B_4 receptor BLT1 and heterotrimeric G-protein subunits reconstituted the functional complex with high affinity for ligand on BV (Masuda et al., 2003). Since one can achieve co-expression of at least four proteins on BV by simply co-infecting Sf9 cells with the combination of recombinant viruses (Hayashi et al., 2004; Masuda et al., 2003), this BV co-display system holds promise as an alternative to cellular co-expression systems.

Such promise prompted us to examine whether ligand-induced activation of effector protein can also be reconstituted on BV. In this study, we co-expressed GPCR, $G\alpha_s$, $G\beta\gamma$, and adeny-

Abbreviations: GPCR, G-protein-coupled receptor; Gα, G-protein α-subunit; $G\beta_1\gamma_2$, G-protein β1γ2 subunit dimer; BV, budded virus; AC, adenylyl cyclase; ACVI, adenylyl cyclase type VI; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin.

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lyl cyclase on BV and attempted to detect cAMP response to ligand.

2. Materials and methods

2.1. Antibodies and reagents

Monoclonal mouse anti-polyhistidine antibody (His-1), and HRP-conjugated goat anti-mouse or anti-rabbit IgG were from Sigma. Polyclonal rabbit antibodies against rat $G\alpha_s$/olf (C-18), mouse Gβ (T-20), bovine $G\gamma_2$ (A-16), and human ACV/VI (C-17) were from Santa Cruz Biotechnology. Dopamine, fenoldopam, (*R*)-(+)-SKF38393, *cis*-(*z*)-flupenthixol, and forskolin were from Sigma. Prostaglandin E2 (PGE2) was from Cayman Chemical Co. The AlphaScreen cAMP assay kit was from PerkinElmer.

2.2. Recombinant baculovirus construction and Sf9 cell culture

The cDNAs for human dopamine D1- and D5-receptors and adenylyl cyclase type VI (ACVI) were amplified by PCR from a human fetal brain cDNA library (Clontech), and their nucleotide sequences were confirmed. The cDNA for mouse EP2 prostaglandin E receptor was a gift from Dr. S. Narumiya (Kyoto University, Japan). The DNA fragments were subcloned into baculovirus transfer vectors (pBlueBacHis2C for dopamine receptors and pBlueBac4.5 for ACVI and EP2. Both were from Invitrogen). Recombinant baculoviruses were generated by using Bac-N-Blue system (Invitrogen). A recombinant virus expressing rat $G\alpha_s$ was provided by Dr. H. Itoh (Nara Institute of Science and Technology, Japan). A recombinant virus harboring both bovine $G\beta_1$ and $G\gamma_2$ cDNAs in a tandem manner ($\beta_1\gamma_2$ virus) (Nakamura et al., 1995) was a gift from Dr. T. Haga (Gakushuin University, Japan). Sf9 cells were cultured as described (Masuda et al., 2003).

2.3. Preparation of Sf9 cell membrane and budded baculovirus fractions

Sf9 cells (2×10^6 cells/ml) were co-infected with a combination of recombinant baculoviruses, as follows (multiplicity of infection (M.O.I.) 2.5, 2, 0.5, and 6 for receptor, $G\alpha_s$, $G\beta_1\gamma_2$, and ACVI, respectively). Membrane and BV fractions were collected 48 h

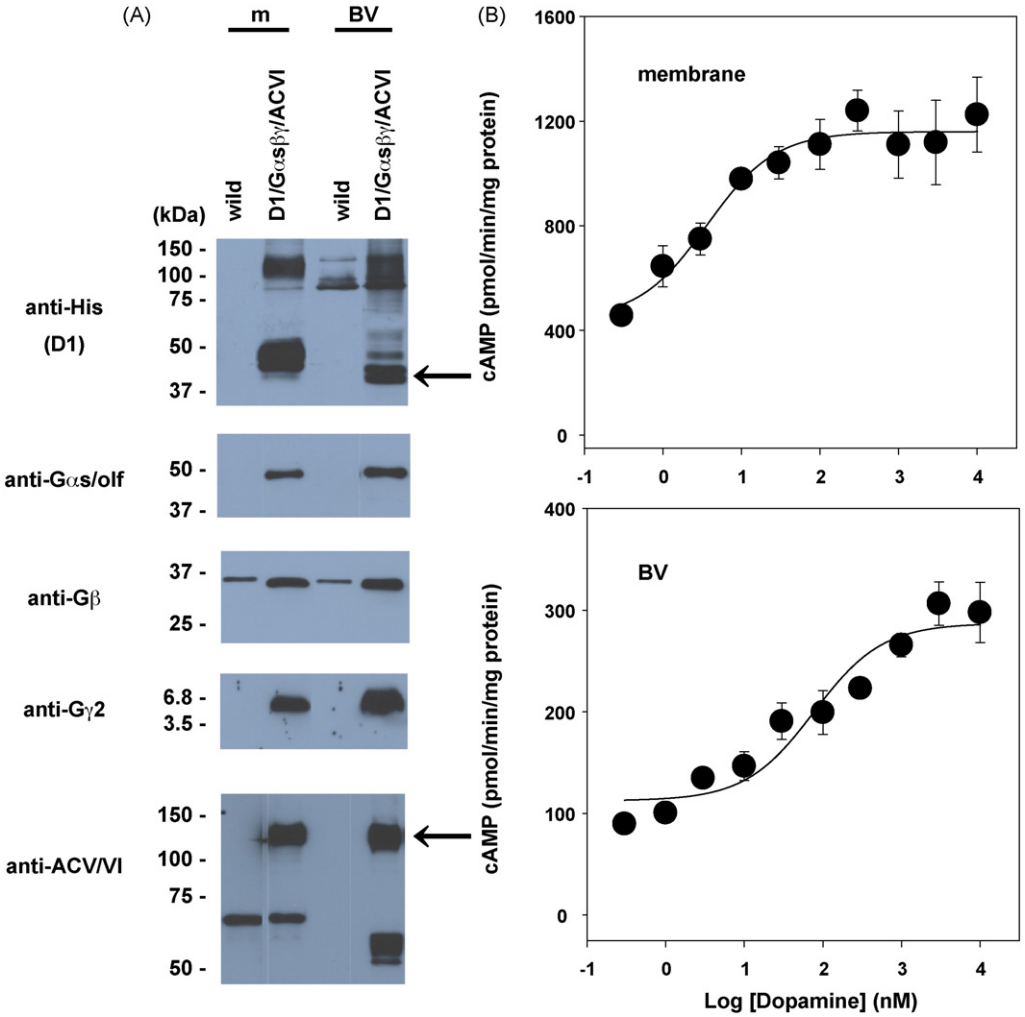

Fig. 1. Reconstitution of ligand-induced activation of AC in Sf9 membrane and BV fractions co-expressing DR-D1, $G\alpha_s$, $G\beta_1\gamma_2$, and ACVI. Sf9 cells were co-infected with recombinant baculoviruses encoding His-tagged DR-D1, $G\alpha_s$, $G\beta_1\gamma_2$, and ACVI (D1/$G\alpha_s\beta\gamma$/ACVI), or with wild type virus (wild), and the membrane and BV fractions were prepared. (A) Western blots of membrane (m) and BV fractions. Samples were loaded in each lane as follows: 20 μg for DR-D1, 5 μg of the membrane and 7.5 μg of the BV for $G\alpha_s$, 10 μg for $G\beta_1$, 10 μg of the membrane and 15 μg of the BV for $G\gamma_2$, and 7 μg of the membrane and 10 μg of the BV for ACVI. The blotted membranes were immuno-stained with respective antibodies as shown on the left of the panel. The positions of DR-D1 and ACVI are indicated on the right by arrowheads. (B) Activation of AC. Membrane and BV fractions (2.5 μg and 10 μg, respectively) co-expressing DR-D1, $G\alpha_s$, $G\beta_1\gamma_2$, and ACVI were incubated with graded concentrations of dopamine, and cAMP production was assayed. Each point represents the mean ± S.E. ($n = 3$).