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Intracellular self-assembly based multi-labeling of key viral components: Envelope, capsid and nucleic acids

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ABSTRACT

Envelope, capsid and nucleic acids are key viral components that are all involved in crucial events during virus infection. Thus simultaneous labeling of these key components is an indispensable prerequisite for monitoring comprehensive virus infection process and dissecting virus infection mechanism. Baculovirus was genetically tagged with biotin on its envelope protein GP64 and enhanced green fluorescent protein (EGFP) on its capsid protein VP39. Spodoptera frugiperda 9 (Sf9) cells were infected by the recombinant baculovirus and subsequently fed with streptavidin-conjugated quantum dots (SA-QDs) and cell-permeable nucleic acids dye SYTO 82. Just by genetic engineering and virus propagation, multi-labeling of envelope, capsid and nucleic acids was spontaneously accomplished during virus inherent self-assembly process, significantly simplifying the labeling process while maintaining virus infectivity. Intracellular dissociation and transportation of all the key viral components, which was barely reported previously, was real-time monitored based on the multi-labeling approach, offering opportunities for deeply understanding virus infection and developing anti-virus treatment.

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1. Introduction

Dissecting virus infection mechanism is crucial for developing therapies to control virus-triggered diseases that severely threaten human health [1,2]. Highly pathogenic viruses, such as HIV, dengue virus and the recently emerged Ebola virus, typically own three key components: envelope, capsid and nucleic acids [3–5]. In virus infection and propagation, these key components successively detach as virus hijacks cell machineries [6–8]. Consequently, virus structure keeps changing during intracellular transportation till the intranuclear nucleic acids release and replication, resulting in cytopathic effect and cell death [9]. Hence, monitoring the comprehensive journey of virus in real time, especially the critical events all these key viral components are involved in, such as cell organelle kidnap, virus structure transformation and transportation, is urgently needed for designing treatment to defense against virus invasion. Simultaneous labeling and tracking of viral envelope, capsid and nucleic acids provide precisely such opportunities.

Although various single-component [10–16] and dual-component [17–21] labeling strategies have been developed in the past decades, so far as we know, no strategy for simultaneous labeling of multiple key viral components has been proposed, hampering the comprehensive exploration of virus infection. Furthermore, for virus multi-component labeling, two key factors must be taken into consideration. On the one hand, the fluorescence intensity of the labeled virus should be as high as possible, thus novel nanomaterials such as semiconductor quantum dots (QDs) with superior brightness and photostability are ideal labels for virus imaging [22]. On the other hand, virus infectivity should be maintained to the largest extent during the whole labeling process. Hence the simplification of both the labeling and the subsequent purification is highly demanded. In 2012, He et al. labeled viral nucleic acids during intracellular virus propagation by incubating the host cells with nucleic acids intercalating dye $[Ru(phen)_2(dppz)]^{2+}$ [19]. Our group proposed a method of labeling viral envelope with biotin by feeding the host cells with biotinylated phospholipids [11]. Both methods accomplished the

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labeling during cell culturing and virus inherent self-assembly process, which significantly simplified the labeling and helped maintaining virus infectivity.

Herein, taking advantage of host cell-assisted virus propagation and genetic engineering, a strategy for multi-labeling of viral envelope, capsid and nucleic acids is proposed based on intracellular virus self-assembly. Baculovirus, a rod-shaped virus owning the typical three-component structure [23], was chosen as the labeling model. Baculovirus expression system allows presenting multiple fusion proteins into different viral components [24], making simultaneous labeling of viral envelope and capsid in host cells possible. GP64 and VP39, as the major envelope protein and capsid protein respectively, are both good candidates for fusion partners [25,26]. Therefore, capsid can be labeled with enhanced green fluorescent protein (EGFP) by expressing fusion proteins VP39-EGFP. Moreover, in the presence of endogenic biotin and adenosine triphosphate (ATP), protein fused with biotin acceptor peptide (APtag) can be biotinylated with the catalysis of biotin ligase (BirA) [27]. We further extended this bioconjugation to intracellular spontaneous biotinylation of fusion proteins GP64-APtag-BirA, which can be subsequently labeled with streptavidin-conjugated QDs (SA-QDs) and assembled to viral envelope [28]. For nucleic acids labeling, owing to the excellent properties including fluorescence enhancement upon binding nucleic acids, low cytotoxicity and higher cell-membrane permeability compared to $[Ru(phen)_2(dppz)]^{2+}$, commercial nucleic acids dye SYTO 82 was chosen in our experiments.

As shown in Fig. 1, a recombinant baculovirus (RB), containing genes encoding the fusion proteins GP64-APtag-BirA and VP39-EGFP, was modified from wild type baculovirus (WT) (Fig. S1). Host Spodoptera frugiperda 9 (Sf9) cells were infected with RB and subsequently fed with SYTO 82 and SA-QDs. Then, the multi-component labeling of virus was spontaneously accomplished in cells. The newly generated nucleic acids of RB in cell nucleus were labeled with the permeated SYTO 82 (1). The expressed VP39-EGFP in cytoplasm moved into nucleus and encapsulated SYTO 82-labeled nucleic acids to assemble the dual-component labeled nucleocapsid (2). GP64-biotin, obtained through the intracellular spontaneous biotinylation of the expressed GP64-APtag-BirA, were transported into cell membrane and subsequently labeled with SA-QDs. Finally, QDs-labeled GP64 (GP64-QDs) were acquired by the dual-component labeled nucleocapsid though budding (3). Thus, just with the assistance of host cells, multi-component labeled baculovirus SYTO 82-RB-QDs (SRBQ) were conveniently obtained. Crucial infection events, including the intracellular dissociation and transportation of multiple key viral components, were monitored in real time based on SRBQ.

2. Materials and methods

2.1. Cells culture and baculovirus production

Baculovirus plasmid (bacmid) was purchased from Invitrogen Corporation. The recombinant bacmid was modified from bacmid with Bac-to-Bac baculovirus expression system (Invitrogen). Details about both the recombinant bacmids and their construction were described in the supplementary material. Generally, Sf9 cells were cultured in Grace's medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco) at 28 °C. To generate RB, Sf9 cells were seeded in a 6-well culture plate. Mixture of 1 μg recombinant bacmid and 6 μL Cellfectin Reagent (Invitrogen) in unsupplemented Grace's medium were added to each well. After 5 h of incubation, the medium was replaced with fresh supplemented medium. After 72 h of further incubation, the supernatants was collected and centrifuged at 5000 g for 10 min at 4 °C to remove cell debris. Afterwards, the titer of RB was determined with 50% tissue culture infective dose (TCID50) assay. Then, RB stock was amplified by infecting Sf9 cells with RB at the multiplicity of infection (MOI) of 1 at 28 °C. At 48 hours post infection (h.p.i.), the medium was collected and separated from cell debris. The supernatants were filtered through a 0.45 μm filter (Millipore) and subsequently ultracentrifugated on 5 mL of 30% (w/v) sucrose at 90000 g (Beckman SW28 rotor) for 2 h at 4 °C. The precipitates were resuspended in 10 mM pH 7.0 phosphate-buffered saline (PBS). WT was generated by transfecting Sf9 cells with bacmid. Titer determination, stock amplification and concentration of WT were accomplished with the same procedures.

2.2. Expression of EGFP

Sf9 cells were cultured overnight at 28 °C and infected with RB at MOI of 1. The infected cells were subjected to fluorescence microscope with 10× objective to observe the expression of EGFP at 0, 12, 24, 36, 48 and 60 h.p.i., respectively. EGFP was excited with blue light.

2.3. Labeling of RB nucleic acids with SYTO 82

Sf9 cells were cultured overnight at 28 °C and infected with RB (MOI = 1). The medium was replaced with fresh medium containing 15 μM SYTO 82 at 18 h.p.i. After 30 min of incubation at 28 °C, the cells were observed under confocal microscope at 28 °C to examine the expression of EGFP and the labeling of nucleic acids with SYTO 82. To examine the labeling of RB capsid with EGFP and RB nucleic acids with SYTO 82 (Invitrogen), the supernatants containing dual-component labeled RB were collected at 48 h.p.i. and concentrated as above. WT operated in the same procedure served as control.

2.4. Labeling of RB envelope with SA-QDs

Sf9 cells cultured overnight at 28 °C were infected with RB at MOI of 1. SA-QDs, $CdSe_xTe_{1-x}$ QDs (λ_{em} = 705 nm) covalently attached with streptavidins, were purchased from Wuhan JiaYuan Quantum Dots Co., LTD (Wuhan, China). At 30 h.p.i., the medium was replaced with 1 × PBS (pH 7.0) containing 2 nM SA-QDs. After 10 min of incubation at 28 °C, PBS was removed and new fresh medium was added. The cells were then subjected to confocal microscope to examine the expression of EGFP and the labeling of cell membrane with SA-QDs. To examine the labeling of RB capsid with EGFP and RB envelope with SA-QDs, the supernatants containing dual-component labeled RB were collected at 48 h.p.i. and concentrated as above. WT operated in the same procedure served as control.

2.5. Multi-labeling of RB key viral components

Sf9 cells cultured overnight at 28 °C were infected with RB at MOI of 1. At 18 h.p.i., the medium was replaced with fresh medium containing 15 μM SYTO 82. At 30 h.p.i., the medium were collected from the adhered cells and 1 × PBS containing 2 nM SA-QDs were added. After 10 min of incubation. 1 × PBS was removed and the previously collected medium containing SYTO 82 was added again. The cells were then subjected to confocal microscope to examine the expression of EGFP, as well as the labeling of nucleic acids with SYTO 82 and cell membrane with SA-QDs. To examine the labeling of RB nucleic acids with SYTO 82, RB capsid with EGFP and RB envelope with SA-QDs, supernatants containing multi-component labeled RB were collected at 48 h.p.i. and concentrated as above. WT operated in the same procedure served as controls.

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