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Review

Single particle labeling of RNA virus in live cells

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ABSTRACT

Real-time and visual tracking of viral infection is crucial for elucidating the infectious and pathogenesis mechanisms. To track the virus successfully, an efficient labeling method is necessary. In this review, we first discuss the practical labeling techniques for virus tracking in live cells. We then describe the current knowledge of interactions between RNA viruses (especially influenza viruses, immunodeficiency viruses, and *Flaviviruses*) and host cellular structures, obtained using single particle labeling techniques combined with real-time fluorescence microscopy. Single particle labeling provides an easy system for understanding the RNA virus life cycle.

1. Introduction

Viruses can infect and proliferate in animals, plants and microorganisms (Gao et al., 2012). To date, a vast number of RNA viruses have caused serious infectious diseases. The viral infection cycle includes attachment, penetration, uncoating, genomic replication and expression, assembly and release, which mainly depend on host cell factors due to fewer proteins being encoded by the viral genome. The pathogenesis of most of virus-caused diseases remains poorly understood, due in part to a lack of knowledge about how viruses replicate in and interact with cells.

Currently, virus infection cycles and interactions between host cells and viruses were studied using fixed cell imaging, co-immunoprecipitation and yeast two-hybrid screens, etc. (Cowan et al., 2012; Generous et al., 2014; Lu et al., 2015). However, each of these methods has advantages and disadvantages for virus-related research. The major challenge in the study of viruses is that they are too small to be seen directly with an optical microscope, which provides a motivation for developing visual tracking techniques in live cells. Single-virus tracking in live cells is an exciting and highly evolving field of research in virology, which have significantly impacted several areas of modern virus research, including a better understanding of host-pathogen interactions, viral pathogenesis and the development of novel vaccines and therapeutics. The observation of dynamics changes of labeled viral proteins or viral genome provides more insight into the interactions between virus and cell than a snapshot provided by imaging studies of fixed cells (Jensen, 2013). Over the last few decades, several single-particle labeling techniques have been developed and used to track individual viral proteins or viral genomes during the virus infection (Chu et al., 2014; Rust et al., 2011; Sun et al., 2013). In this review, 1) the practical viral labeling techniques for virus tracking in live cells were discussed, and 2) interactions between RNA viruses (especially influenza viruses, immunodeficiency viruses, and *Flaviviruses*) and host cellular structures discovered through live cell imaging experiments were described. This review provides an up-to-date look at the general principles and applications of single particle labeling techniques in RNA virus research.

2. Viral labeling techniques

2.1. Virus labeled with fluorescent protein

Recombinant GFP-techniques have been widely used to track both viral protein and viral genomes, which were reviewed by Maiuri (Maiuri et al., 2011a) and Miorin (Miorin et al., 2016). To construct a recombinant virus, a fluorescent protein (FPs) gene is fused with a structural or nonstructural (NS) protein gene of the virus in a viral infectious DNA clone (Donnelly and Elliott, 2001; Elliott and O'Hare, 1999). When the infectious cDNA clone is transfected into the cells, the recombinant virus can be rescued with a visible tag (Fig. 1a). During the rescued virus infection, the recombinant virus can be visualized and tracked in cells by fluorescent microscope. For example, recombinant human immunodeficiency virus (HIV) was generated by inserting enhanced GFP gene (EGFP) in the C terminus of HIV Gag protein (Muller et al., 2004). Further studies demonstrated that fluorescent-labeled virion could be obtained by mixing particles containing Gag-EGFP and Gag (Muller et al., 2004). Moreover, a double-colored HIV was rescued by fusing the EGFP to the HIV Gag protein and RFP (red

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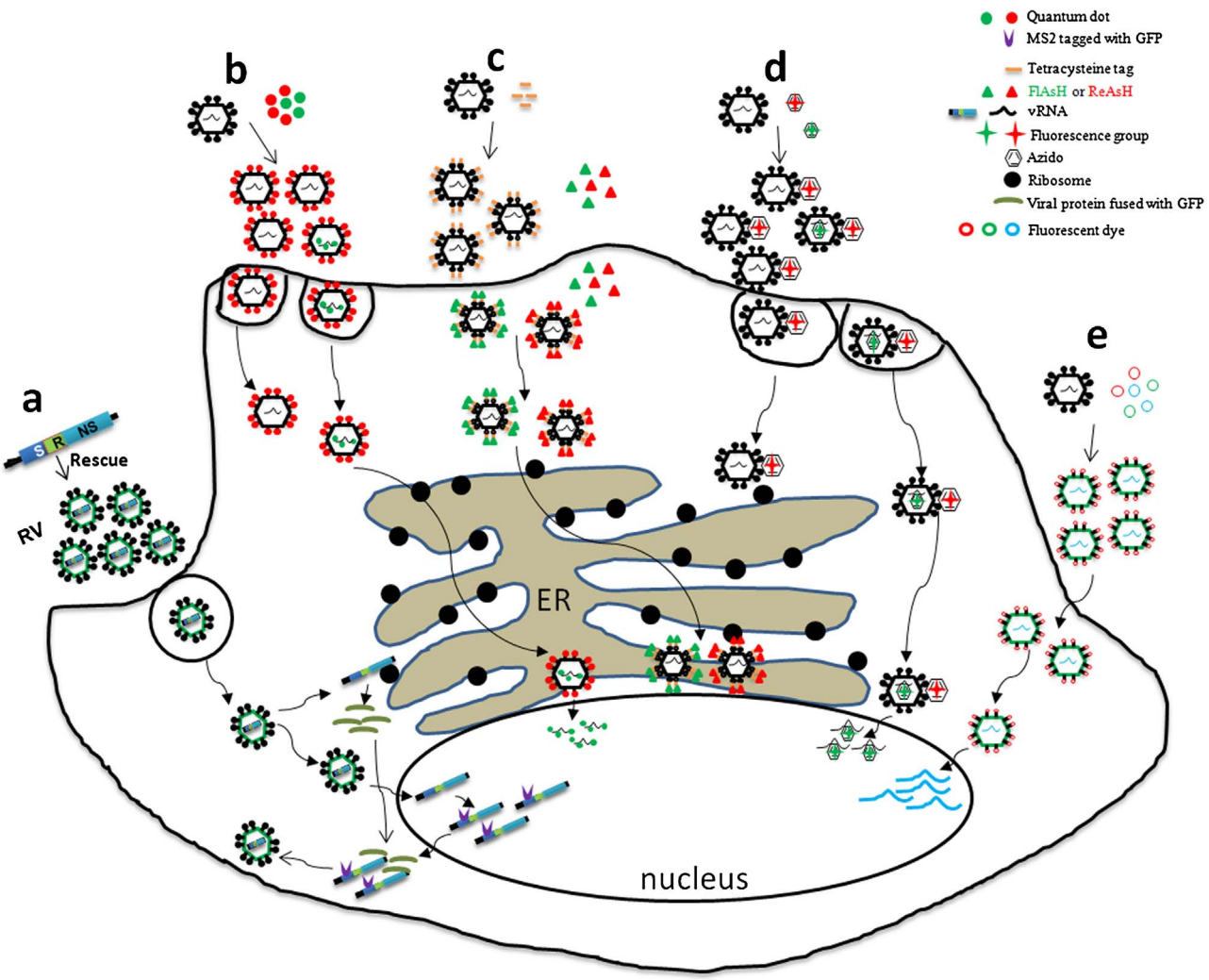

Fig. 1. Viral labeling techniques. (a) Virus labeled with fluorescent protein. Reporter gene was fused with viral structural protein or placing the reporter gene cassette behind the 3′ terminus of the viral structural protein gene. Then the recombinant reporter virus was rescued and infected the target cells, and the viruses or viral proteins labeled with reporter protein can be tracked in the recombinant virus infected cells. Furthermore, viral genome was tagged with stem loops that bind with high affinity and specificity to the coat protein of the bacteriophage MS2, which was expressed as a fusion protein to GFP (Jouvenet et al., 2009). ▶, viral promoter; R, reporter protein; S, structural protein; NS, non-structural protein; RV, recombinant virus; MS2, bacteriophage MS2 protein; ER, endoplasmic reticulum. (b) Quantum dots. Quantum dots (QDs) were conjugated onto the viral surface by streptavidin or antibodies (red dots) or encapsulated in the viral capsid by linking streptavidin-conjugated QDs to the modified viral genomic RNAs (green dots). (c) Biarsenical-binding technique. The TC tag (Cys-Cys-Pro-Gly-Cys-Cys) was fused with the viral caspid protein. The membrane-permeable biarsenical reagents, FlAsH-EDT or ReAsH-EDT, were virtually non-fluorescent due to the ethane dithiol (EDT) group. When FlAsH-EDT or ReAsH-EDT binds to TC tag, the EDT was displaced and the reagent became highly fluorescent in green or red. (d) Virus labeled by click chemistry. Virus was labeled via a Cu^{I} catalyzed azide-alkyne cycloaddition reaction combined with diazonium coupling. To label viral RNA, nascent RNA can be tagged with 5-ethynyluridine, followed by labeling with a fluorescent azide (Kalveram et al., 2013). (e) Virus labeled with chemical fluorescent dye (FDs). FDs meant chemical fluorescent dyes, including fluorescent amine-reactive dye (red circles), nucleic acid dyes (cyan circles) and lipophilic dyes (green circles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fluorescent protein) to the HIV Vpr protein, which could be used to distinguish the wild-type virus from subviral particles lacking matrix region (MA) (Lampe et al., 2007). Additionally, GFP-labeled murine leukemia virus (MLV) by inserting the fluorescent protein in the proline-rich region of the viral ecotropic envelope protein (Env) was rescued successfully (Erlwein et al., 2003). Furthermore, a dual-color fluorescent reporter system for vesicular stomatitis virus (VSV), including a recombinant virus and stable reporter cell, was developed (Swick et al., 2014). In this system, a VSV expressing red fluorescent protein could be identified and tracked in virus-infected cells, while the cells stably expressing GFP were used to evaluate the expression of an interferon-stimulated gene IFIT2 (Swick et al., 2014). However, while some modifications have no discernible impact, it should be mentioned that the modified virus needed to be profiled in parallel to the parental virus to validate that these modifications did not alter characteristics such as virus growth or titers.

Tracking virus by fluorescent protein have many advantages, including easy constructing recombinant virus labeled with one or multiple FPs, and no exogenous substrate needed. However, there are limitations associated with using fluorescent protein to track viral infection. Firstly, due to the limited coding capacity of some virus genomes, large fluorescent tags may increase the molar mass of the target protein, leading to genetic instability or impaired viral function (Avilov et al., 2012a; Crivat and Taraska, 2012). As reported, N-terminal fused with GFP results in a non-functional Env protein of HIV (Erlwein et al., 2003; Sliva et al., 2004). To overcome these limitations, epidermal growth factor was inserted into the N-terminus of Env protein. The recombinant virion replicated as efficiently as the parental virus and was genetically stable (Erlwein et al., 2003; Sliva et al., 2004). MLV labeled with a fluorescent envelope protein could be followed during viral attachment and replication (Sliva et al., 2004). Furthermore, recombinant influenza virus was produced by fusing a split GFP to the viral PB2 polymerase subunits (Avilov et al., 2012a,b). During the recombinant virus infection, the functional GFP could be