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Review

Impact of live cell imaging on coated vesicle research

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Abstract

The role of membrane traffic is to transfer cargo between distinct subcellular compartments. Each individual trafficking event involves the creation, transport and fusion of vesicular and tubular carriers that are formed and regulated via cytoplasmic coat protein complexes. The dynamic nature of this process is therefore highly suitable for studying using live cell imaging techniques. Although these approaches have raised further questions for the field, they have also been instrumental in providing essential new information, in particular relating to the morphology of transport carriers and the exchange kinetics of coat proteins and their regulators on membranes. Here, we present an overview of live cell-imaging experiments that have been used in the study of coated-vesicle transport, and provide specific examples of their impact on our understanding of coat function.

Keywords: Live cell imaging; Green fluorescent protein (GFP); Coated vesicles; Membrane traffic

Contents

Abbreviations: AP, adaptor protein; BFA, brefeldin A; CCD, charge-coupled device; CCP, clathrin-coated pit; CFP, cyan fluorescent protein; CI-MPR, cation-independent mannose 6-phosphate receptor; CLEM, correlative light and electron microscopy; COP, coat protein complex; EM, electron microscopy; ER, endoplasmic reticulum; ERES, ER exit site; ERGIC, ER–Golgi intermediate compartment; FCS, fluorescence correlation spectroscopy; FLIM, fluorescence lifetime imaging; FRAP, fluorescence recovery after photobleaching; FRET, fluorescence resonance energy transfer; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GFP, green fluorescent protein; GGA, Golgi-localised, γ-ear-containing, ADP-ribosylation factor-binding protein; RNAi, RNA interference; SPIM, single plane illumination microscopy; TC, tubulo-vesicular transport complex; tER, transitional ER; TGN, *trans*-Golgi network; TIRFM, total internal reflection fluorescence microscopy; YFP, yellow fluorescent protein

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1. Introduction

The internal membrane architecture of eukaryotic cells in the form of distinct organelles provides a means of sequestering biochemical reactions to a local environment. However, as the protein and lipid molecules that carry out these reactions are, in many cases, not synthesised directly at their site of function, or operate between several locations, the presence of specific transport pathways between organelles is essential for cellular homeostasis. Membrane traffic between adjacent organelles is largely achieved via membrane-bounded carriers, which, after budding from a donor membrane, already possess the factors that enable them to link with cytoskeletal elements and be targeted to their designated acceptor membrane. All well-characterised carriers possess cytoplasmic coat complexes that serve multiple roles, including the initial identification and binding of the cargo, physical deformation of the lipid bilayer into a vesicular structure, and binding of accessory and regulatory molecules. The important role of coat complexes in membrane transport has therefore made them a target for study by many different methods; however, several technical advancements in live cell imaging mean that this technique has now become an essential tool in the study of vesicular coats. In this review we highlight a number of such examples, giving particular emphasis to experiments carried out in mammalian cell culture systems as these have the additional advantage of providing spatial information.

2. Considerations for using live cell imaging as a tool to study membrane traffic

The identification of coated vesicular carriers and their major constituents was largely achieved in the 1980s using a *tour de force* of biochemical and electron microscopy (EM) techniques (reviewed [1]). At this time, the combination of these methods was ideally suited to the study of membrane traffic, as yeast genetics had provided a substantive list of candidate genes and their phenotypic effects could be readily analysed by EM [2]. Light microscopy was used relatively rarely as a standard technique at this time, and live cell imaging often took the form of using transmitted light to study whole-cell motility. For light microscopy to make an impact in the field of coated vesicle research, two advances were required. First, the microscopes themselves needed to be able to acquire digital images at a much higher resolution. Second, methods had to be found to more routinely introduce fluorescent probes into living cells, thereby allowing direct visualisation of the coated-vesicle proteins under investigation. Through the 1990s both of these hurdles were overcome, predominantly in the form of improvements to the charge-coupled device (CCD) as a sensitive imaging camera, and the cloning of the gene encoding the green fluorescent protein (GFP) [3].

These advances have made fluorescence-based live cell imaging a key tool in our understanding of membrane traffic. Although biochemical methods and imaging of fixed cells by different methods have provided us with enormous amounts of information, the dynamics of membrane-traffic events can most readily be studied by live cell imaging and its associated techniques (detailed in Section 3). However, in order to successfully apply these methods to the study of coated vesicles, a number of details must first be taken into consideration, and these are discussed below.

Fluorescent tagging of particular proteins of interest as a means to visualise them in living cells is now a very widely used technique (reviewed [4]). In particular, GFP-tagging approaches have several distinct advantages over other visualisation methods. First, cDNAs encoding GFP and its spectral variants, were rapidly made available from commercial suppliers, thereby allowing different genetic fusions with genes of interest to be easily made [5]. Second, GFP has been shown to be suitable for high-throughput tagging of candidate genes from a wide variety of organisms (reviewed [6,7]). The complete sequencing of multiple genomes has provided access to vast cDNA resources, whereas the generation of extensive antibody collections for immunofluorescence studies is still in its infancy (see for example [8]). Third, GFP emits fluorescence in living cells without the need for additional cofactors or chemicals [3]. Fourth, examination of GFP-tagged proteins in living cells provides subcellular localisation and information about dynamics without the worry of artefacts caused by fixation methods.

Despite these advantages, care must still be exercised when using GFP constructs in cells. In particular, even if cells with low GFP expression levels are selected for imaging, this still represents overexpression compared with endogenous levels. Although cells can tolerate this to a certain extent, if the GFP-tagged protein is part of a multisubunit complex (such as in a vesicular coat complex), the overexpression of a single subunit might perturb the balance of the entire complex (see Fig. 1). Therefore, it might be preferable to image such proteins in cellular backgrounds in which the endogenous (non-tagged) protein has been depleted, for example by RNA interference (RNAi). Care must also be taken to determine the most suitable position in the candidate protein in which to insert the GFP. Fusion at the N-terminus, C-terminus, or even internally can all affect the correct display and recognition of protein-targeting motifs in the protein, and as such could produce artefacts in live cell-imaging experiments. The appropriate position of the GFP cannot always be predicted, and so multiple different fusions often have to be generated to determine the most suitable location [9]. For example in the case of the coat protein complex I (COPI), which comprises seven subunits collectively termed coatomer and the small GTPase Arf1, the β-COP subunit seems to localise correctly with either an N- or C-terminal GFP tag, whereas the ε-COP subunit only localises correctly when the tag is at the C-terminus (Fig. 1). In addition, GFP requires an external illumination source to emit fluorescence, and if this is too bright or prolonged, photodamage can result in the production of toxic free radicals in the cell. Finally, although GFP folds into a very compact barrel-shaped structure [10], it may still sterically hinder the interaction of the tagged protein with other binding partners. Other smaller fluorescent tags can circumvent this problem, for example the fluorescein derivative FlAsH [11]. This tag comprises a tetracysteine motif that, like GFP, can be engineered into a protein of interest, but fluorescence only occurs on addition of a biarsenic compound to the cell-culture medium. The use of such new flu-