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Review

Live imaging of endosome dynamics

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ABSTRACT

When studying the living endosome one must first recognise that we are not studying a single discrete organelle but rather a highly dynamic interconnected network of membrane-bound compartments. Endocytosed molecules are sorted and transported through various polymorphic intracellular organelles that mature and interact with one another *via* fusion and fission events in a highly spatially and temporally co-ordinated manner. As such, we recognise that being a dynamic system, it must be studied in a dynamic fashion. Videomicroscopy has provided profound insights into the cell, and its use in the study of the living endosome has exemplified this supplying a unique perspective on this elusive organelle. In this review we will examine some of the seminal observations that this technology has contributed as well as survey the various assays, tools and technologies that can be applied to understanding the living endosome.

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Contents

1. Introduction

The endocytic system encompasses a complex network of membrane-bound intracellular compartments, each fulfilling specific roles in protein sorting and distribution, cellular homeostasis and catabolism. For simplicities sake, we frequently take the reductionist's approach and dismantle its components into discrete units such as early or sorting endosomes, multivesicular bodies or late endosomes, recycling endosomes and lysosomes [1]. In doing so we discard the temporal profile of a particular endocytic compartment, are confounded when these discrete structures fuse with one another to create "hybrid" organelles or are unable to define structures that are in the process of maturing from one identity to another (Fig. 1 and Supplementary Movie 1). To consider the living endosome as a discrete organelle is therefore misleading. Rather, it is a dynamic adaptable continuum, shifting in position, morphology and molecular identity as it moves through the cell in a

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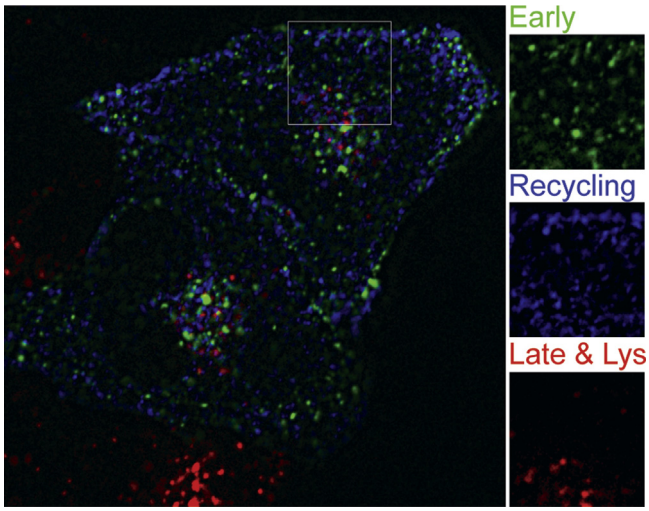

Fig. 1. Live imaging of endosomes. HeLa cells expressing GFP-Rab5 and mCherry-Rab11 were cultured in the presence of Alex647-conjugated dextran (MW 10,000) for four h before a 24 h chase in normal growth media. This allows the fluorescent dextran to accumulate in the late endosomes and lysosomes whilst the FP-constructs mark the early and recycling endosomes respectively. The cells were imaged live on an inverted Nikon Ti-E Deconvolution microscope using a 60×/1.4 Plan Apo Oil objective.

choreographed interplay with the cytoskeleton and other components of the endolysosomal network.

Supplementary Movie 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.semcdb.2014.03.027.

Live cell imaging has become a commonly used tool within the repertoire of technologies available to cell biologists [2]. The relative optical clarity of cell culture systems and the capacity to circumvent destructive fixatives as well as gain valuable temporal information has encouraged the community to delve deep within the potential for this technology. Given the dynamic nature of endocytosis, the movement of material from the outside of the cell within, it is hardly surprising that dynamic imaging techniques have led to a number of pivotal discoveries in the field.

2. Tools of the trade

Endosomes are most often characterised by the cargo molecules that are transported through them, the peripheral membrane proteins that associate with them, the lipids that constitute their structure and the biophysical properties that they present [1]. Defining the organelles of this system is confounded by the scattered transient nature of the compartment highlighting the need for both good tools and systems by which they can be tracked and studied. In this section we will discuss the various fluorescent probes and assays that can be utilised to examine these characteristics of endosome biology in living cells and review some of the insights they have provided to the field (Table 1).

2.1. Endocytic tracers

A chief advantage of studying endocytic pathways is the accessible nature of the extracellular space. Fluorescently tagged ligands, solutes and particles that mark but do not influence the system may be added to the extracellular milieu and their intracellular fate easily followed using live fluorescence microscopy. Bulk fluid uptake or pinocytosis is readily monitored using fluorescently labelled dextran, bovine serum albumin and other solutes, like lucifer yellow, that do not adsorb to the cell surface [3]. These probes accumulate in early then later endocytic compartments dependent upon the duration of their application.

Thoughtful application and timing of fluid-phase probe addition allows one to selectively mark specific sub-populations of endosomes within the cell. Using a pulse-chase approach, Bright et al. [4] monitored the fusion of Oregon green 488 dextran-loaded late endosomes with rhodamine dextran-loaded lysosomes. Briefly, cells are cultured for 4 h in medium containing 10,000 MW dextran conjugated to rhodamine followed by a 20 h chase in conjugate-free medium thereby ensuring its flux into the late endosomes and lysosomes. Newly formed early endosomes are then labelled by incubating the cells in medium containing dextran conjugated to Oregon green 488 for 10 min followed by a 5 min chase in conjugate-free medium. Fusion events are recognised when the initially distinct fluorophores are observed to overlap by time-lapse videomicroscopy. This content mixing assay both allows for the direct observation of fusion and kiss and run events in live cells and can also be used in combination with interfering mutants, pharmacological inhibitors or RNAi-approaches to examine the contribution of specific molecules towards these fusion events [5].

Alternatively, ligands to receptors known to be ushered through specific intracellular pathways can be used to selectively label certain endosomal subpopulations. The most prominently used of these are fluorescently labelled transferrin and epidermal growth factor, whose cognate receptors faithfully follow the recycling and degradative pathways respectively. Seminal work from the Zhuang lab used live cell microscopy to monitor the endocytosis of ligand particles in real time and in doing so demonstrated that the afore-mentioned early or sorting endosomes comprise two discrete sub-populations; a highly dynamic population that rapidly matures as it translocates along microtubules towards the perinuclear microtubule organising centre (MTOC), and a more static population that matures more gradually. They demonstrate that whilst transferrin indiscriminately accumulates within both populations, cargoes targeted towards the degradative pathway such as low density lipoprotein (LDL), EGF and influenza viral particles, are preferentially targeted to the dynamic population of sorting endosomes despite all being initially internalised *via* clathrin-mediated endocytosis [6]. Since the static slowly maturing endosomes substantially outnumber the dynamic population, transferrin is effectively enriched in the former population. This pre-early endosome sorting would have been difficult to observe without real-time single-particle tracking of individual endosomes.

In addition to ligands, fluorescently labelled viral particles and toxins have been extensively used to study endocytosis with great success. A large group of bacterial and plant toxins interact with cells through one subunit whilst a second subunit with cytotoxic enzymatic activity enters the cytosol and exerts its action. These moieties can be separated from one another and the former used as a benign tracer for normal toxin trafficking pathways [7]. Most of these toxins are endocytosed before translocation to the cytosol. Several bacterial toxins, such as diphtheria toxin and anthrax toxin, enter the cytosol in response to the low pH found in endosomes, whilst others such as the plant toxin ricin, and the bacterial toxins cholera and Shiga toxin are transported through endosomes, but then in a retrograde manner through the Golgi apparatus to the endoplasmic reticulum (ER) before the enzymatically active subunit enters the cytosol [8]. Exhaustive live cell analysis from the Johannes lab and others has dissected the molecular events that occur throughout the trafficking of the Gb3 (glycolipid)-binding B-subunit of bacterial Shiga toxin (STxB), providing valuable insight into both the action of the toxin itself but also the living endosomal pathways involved. High resolution microscopy revealed toxin-driven clustering of glycosphingolipid receptor molecules leading to membrane-bending of the cell surface to form extensive tubular invaginations which form and detach from the plasma

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