Video Article

Imaging FITC-dextran as a Reporter for Regulated Exocytosis

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Abstract

Regulated exocytosis is a process by which cargo, which is stored in secretory granules (SGs), is released in response to a secretory trigger. Regulated exocytosis is fundamental for intercellular communication and is a key mechanism for the secretion of neurotransmitters, hormones, inflammatory mediators, and other compounds, by a variety of cells. At least three distinct mechanisms are known for regulated exocytosis: full exocytosis, where a single SG fully fuses with the plasma membrane, kiss-and-run exocytosis, where a single SG transiently fuses with the plasma membrane, and compound exocytosis, where several SGs fuse with each other, prior to or after SG fusion with the plasma membrane. The type of regulated exocytosis undertaken by a cell is often dictated by the type of secretory trigger. However, in many cells, a single secretory trigger can activate multiple modes of regulated exocytosis simultaneously. Despite their abundance and importance across cell types and species, the mechanisms that determine the different modes of secretion are largely unresolved. One of the main challenges in investigating the different modes of regulated exocytosis, is the difficulty in distinguishing between them as well as exploring them separately. Here we describe the use of fluorescein isothiocyanate (FITC)-dextran as an exocytosis reporter, and live cell imaging, to differentiate between the different pathways of regulated exocytosis, focusing on compound exocytosis, based on the robustness and duration of the exocytic events.

Video Link

The video component of this article can be found at https://www.jove.com/video/57936/

Introduction

Regulated exocytosis is the primary mechanism by which readily made cargo is released from a secretory cell in response to a specific trigger. The cargo is pre-formed and sequestered into secretory vesicles, in which it is stored until a trigger relays the signal for the release of the SGs' content. Different types of signals may result in different modes of regulated exocytosis, or different modes of regulated exocytosis may occur simultaneously. Three main modes of regulated exocytosis are known: full exocytosis, which involves full fusion of a single secretory granule with the plasma membrane; kiss-and-run exocytosis, which involves transient fusion of the secretory granule with the plasma membrane followed by its recycling; and compound exocytosis, which is characterized by homotypic fusion of several SGs prior (*i.e.*, multigranular exocytosis) or sequential (*i.e.*, sequential exocytosis) to fusion with the plasma membrane[1]. Compound exocytosis is considered the most extensive mode of cargo release[2], as it allows for the fast secretion of cargo, including that from SGs that are located distal from the plasma membrane. Compound exocytosis has been documented in both exocrine and endocrine cells[3,4,5,6,7,8,9], as well as in immune cells. In immune cells, such as eosinophils[10,11,12] and neutrophils[13], compound exocytosis allows the fast and robust release of mediators that are required to kill invading pathogens such as bacteria or parasites. Mast cells (MCs) deploy compound exocytosis for the efficient release of pre-stored inflammatory mediators during innate immune responses, anaphylaxis, and other allergic reactions[14,15,16,17]. Since the different modes of exocytosis may occur simultaneously[18,19], it has become a challenge to distinguish between them in real time or to identify their respective fusion machineries, hence elucidating their underlying mechanisms.

Here we present a method, based on live cell imaging of cell loaded FITC-dextran, that allows real time tracking of exocytic events and distinguishing between their different modes. In particular, our method allows exclusive monitoring of compound exocytosis.

FITC-dextran is a conjugate of the pH-sensitive fluorophore FITC with the glucan polysaccharide dextran. Fluorescently labeled dextrans have been shown to enter the cell by micropinocytosis[20,21] and macropinocytosis[22,23]. As endocytic compartments mature into lysosomes, it has been shown that FITC-dextran accumulates in the lysosome with no apparent degradation. However, since FITC is a highly pH-sensitive fluorophore[24], and the lysosome lumen is acidic, FITC-dextran fluorescence quenches upon reaching the lysosome[24]. Thus, establishing dextrans as lysosome targeted cargo, taken together with the pH sensitivity of FITC, have laid the foundation for the use of FITC-dextran in studies of lysosome exocytosis[25,26,27,28,29].

In several cell types, including MCs, neutrophils, eosinophils, cytotoxic T cells, melanosomes, and others, the SGs display lysosomal features and are classified as lysosome-related organelles (LROs) or secretory lysosomes[30,31]. Since LROs have an acidic luminal pH, FITC-dextran can be used to visualize their exocytosis, as a result of higher pH associated with the exteriorization of the LROs. Indeed, FITC-dextran has been used to monitor exocytosis in MCs[18,32,33]. In this method, FITC-dextran is added to the cell culture, taken up by the cells by pinocytosis and sorted into the SGs. As it is in lysosomes, FITC fluorescence is quenched in the SGs when they are within the cell. However, upon SG fusion with the plasma membrane and consequent exposure to the external milieu, the FITC-dextran regains its fluorescence as the SG pH rises, allowing the simple tracking of exocytic events by live cell microscopy. Here, we adjusted this method to enable unique tracking of compound exocytosis.

Two other methods have been used previously to track compound exocytosis. Electron microscopy was the first method to characterize exocytic structures that suggested the occurrence of different modes of exocytosis. In particular, observations of "secretory tunnels" in pancreatic acinar cells[34] and MCs[35,36,37] gave rise to the hypothesis of compound exocytosis. However, while the high resolution of electron microscopy has the power to reveal fused vesicles, it cannot track the dynamics of their fusion and hence can't define whether they correspond to SG fusion during compound exocytosis or fusion of recaptured granules following their endocytosis. This obstacle is overcome in other methods that can measure exocytosis in living cells, such as patch clamp measurements of the plasma membrane capacitance[11,13,38,39] or amperometry[40] of the media. However, patch clamping requires a special set-up and may not be suitable for all cell types. Amperometry measurements are able to track exocytosis only if the cargo is released in very close proximity to the electrode. Therefore, using live cell imaging offers an advantage over these methods, as it not only allows for real time tracking of exocytosis, but it also allows quick and simple acquisition of data from the whole cell.

The tracking of FITC-dextran by live cell microscopy also offers some advantages to other live cell imaging-based methods. For example, a widely used method is total internal reflection fluorescence microscopy (TIRFM) of cells loaded with a fluorescent SG probe or expressing a fluorescent protein-tagged SG cargo or membrane protein[26,41,42,43]. The strength of this method lies in its ability to monitor exclusively events that occur close to the plasma membrane (herein referred to as footprint), hence exocytic events. However, this is also the drawback of this method because only the cell fraction that is adjacent to the coverglass and close to the microscope lens can be imaged[44]. Whether such footprints indeed represent the entire cell membrane surface is still debatable[45,46,47]. In this regard, using a pH-sensitive dye such as FITC-dextran and a standard fluorescence microscope or a confocal microscope with an open pinhole allows imaging of the whole cell, thus capturing the total exocytic events that occur in that cell.

Additional pH-sensitive reporters that are used to study regulated exocytosis by whole cell imaging or TIRFM include SG cargo or SG membrane protein fused to phlourin, a pH-sensitive GFP variant. Examples include NPY-phlourin-, β-hexoseaminidase-phlourin, and synapto-phluorin[48,49,50,51,52]. While expression of these probes may represent more closely the endogenous composition of the SGs, it entails transfection of the cells, and may therefore be less suitable for cells that are difficult to transfect. Therefore, when studying cells that are difficult to transfect or under experimental conditions that require multiple genomic manipulations, the use of a compound that can be simply supplemented into the cell culture medium, such as FITC-dextran, is advantageous. FITC-dextran also offers an advantage over acridine orange (AO), another pH-sensitive dye that has been used for the tracking of exocytosis by live cell microscopy[53,54,55,56,57,58]. AO has been shown to induce photolysis of vesicles that result in false flashes, which do not correspond to actual secretion processes[27]. In contrast, FITC-dextran reflects better secretion events, probably due to its low photo-induced production of reactive oxygen[27].

Notably, an alternative approach for studying exocytosis is by tracking the influx of a dye, from the external medium into the SG through the fusion pore that opens during this process. In this case, the dye is added to the external medium alongside the secretory trigger. Then, when the fusion pore opens, the dye diffuses into the SG[59,60]. A clear advantage of this method is that it also offers the ability to estimate the fusion pore size, by the use of dyes of variable size. For example, dextrans of different molecular weight (MW), conjugated to different fluorophores, can be used as extracellular dyes whereby the maximum size of dextran that can penetrate the SG would correspond to the size of the fusion pore[59,61,62,63,64]. In addition, this approach does not require the use of a pH-sensitive probe. However, a significant disadvantage is that the signal to noise ratio is very low, since a large amount of dye is present in the media during acquisition of images, resulting in high background.

Overall, the use of FITC-dextran as a marker for exocytosis overcomes several drawbacks in previously reported methods, such as signal to noise ratio, toxicity, dynamic tracking and complexity.

Here we describe the use of FITC-dextran to monitor compound exocytosis in the RBL-2H3 mast cell line (herein referred to as RBL, primarily established by Eccleston *et al.*[65] and further cloned by Barsumian *et al.*[66]), in response to immunoglobulin E (IgE)/antigen (Ag) activation.

Protocol

1. Preparations

1. Preparation of RBL culture media
    1. Mix 500 mL of low glucose Dulbecco's modified Eagle's medium (DMEM) with 56 mL of fetal bovine serum (FBS), 5.5 mL of penicillin-streptomycin-nystatin solution, 5.5 mL of L-Glutamine 200 mM solution. This results in low glucose DMEM supplemented with 10% FBS, 100 µg/mL streptomycin, 100 units/mL penicillin, 12 units/mL nystatin, and 2 mM L-glutamine.
    2. Filter the media by using a top-vacuum filter of 0.22 µm pore size and store at 4 °C.

2. Maintenance of RBL cells.
    1. Grow RBL cells to a maximum confluency of 90% in a 10 cm dish. If the cell culture is healthy, the cells should have a spindle shape with occasional protrusions.
    2. For cell splitting, detach the cells from the dish by aspirating the media and replacing with 2 mL of trypsine/EDTA solution B. Incubate for 5-10 minutes in a humidified atmosphere of 5% CO_2 at 37 °C.

3. Once the cells have detached, neutralize the trypsin by adding 2 mL of culture media, using a pipette, and split the cells in a 1:2 - 1:10 ratio.

3. Preparation of 20x Tyrode's buffer
    1. Prepare a stock 20x solution of 54 mM KCl, 20 mM $MgCl_2$, 2.74 M NaCl, and 8 mM NaH_2PO_4 in double distilled water (DDW). Mix well and store at 4 °C.

2. Culture of RBL Cells for Live Cell Microscopy

1. Preparation of FITC-dextran solution.
    1. Mix 1 mg of FITC-dextran powder (150 K) per 1 mL of culture media (see step 1.1.1). For a full chambered coverglass, prepare 3 mL.
    2. Using a cellulose acetate syringe filter unit with 0.22 µm pore size, filter the dissolved FITC-dextran.
    3. Add mouse IgE to a concentration of 1 µg/mL.

2. Seeding RBL cells for imaging
    1. The day before imaging, aspirate the media from the culture dish and replace with 2 mL of trypsine/EDTA solution B. Incubate for 5 - 10 minutes in a humidified atmosphere of 5% CO_2 at 37 °C. Once the cells have detached, neutralize the trypsin by adding 2 mL of culture media.
    2. Count RBL cells using a hemocytometer and adjust the volume accordingly with culture media to get a cell concentration of 7.5 x 10^5/mL.
    3. Add 10 µL of cell suspension to a chambered coverglass pre-filled with fresh FITC-dextran supplemented culture media (this results in seeding of 7.5 x 10^3 cells in a chamber).
    4. Grow RBL cells overnight in a humified atmosphere of 5% CO_2 at 37 °C. The cells should remain in a sub-confluent level in order to make sure that the cells are separated and that it is easy to identify each cell individually under the microscope.

3. Transfection of RBL cells - optional.
    1. For imaging exocytic events in combination with other fluorescently tagged proteins, see transfection protocol for RBL cells in Azouz *et al.*[67]

3. Live Cell Microscopy of Exocytosis

1. Preparation of solutions:
    1. Prepare a final Tyrode's buffer solution by diluting the stock solution in DDW in a 1:20 dilution and supplement with 20 mM Hepes pH 7, 1.8 mM $CaCl_2$, 1 mg/mL BSA, and 5.6 mM glucose.
    2. Freshly prepare a 20x secretagogue reagent in Tyrode's buffer [1 µg/mL dinitrophenyl conjugated to human serum albumin (DNP-HAS (Ag)) in our case, for a 50 ng/mL 1x concentration].
    3. Prepare a 400 mM ammonium chloride solution by dissolving powder in Tyrode's buffer.

2. Preparation of the cells.
    1. Wash the chambered coverglass 3 times by aspirating the media from the chamber and refilling it with 300 µL of Tyrode's buffer, prewarmed to 37 °C. Finally, replenish the chamber with 300 µL of Tyrode's buffer, prewarmed to 37 °C.
    2. Place the chambered coverglass in the microscope's incubator chamber. Make sure that the chamber is stable.

3. Setting up the microscope
    1. To choose a region of interest to track, turn on the fluorescent light source (traditionally a mercury lamp) and choose the appropriate fluorescence filter (choose the filter for green fluorophores for viewing FITC-dextran). Once the region of interest is in focus and is in the middle of the field of view, turn off the light source in order to avoid photo-bleaching and toxicity.
    NOTE: Some of the FITC-dextran incorporated in the cells may retain fluorescence. This is because FITC-dextran may also be sorted to non-lysosomal compartments such as endosomes.
    2. Turn on the appropriate lighting for FITC excitation. If using a laser-based microscope, turn on the 488 nm laser. Emission should be gathered around 500 - 550 nm (FITC emission peaks at 510 - 520 nm).
    3. When using a confocal microscope, open the pinhole to the maximum. This will allow using lower laser power to avoid bleaching and toxicity and will ensure the capture of exocytosis events from all planes of the cell.
    4. Calibrate the time interval between image acquisitions.
        1. Different cells may vary in their kinetics of regulated exocytosis[68]. For specific imaging of compound exocytosis, we recommend a time interval of at least 5 seconds. However, as a rule of thumb, to allow for fast imaging, make sure that the acquisition time of a single frame is fast.
        2. When using a laser-scanning confocal microscope, set the scan direction to bi-directional, do not allow for averaging, and set resolution at 512 x 512 (recommended; the latter two provisions will also help to minimize bleaching and toxicity).

4. Imaging of exocytosis.
    1. Image cells for desired durations, depending on the type of cell or secretagogue. In RBL cells triggered with IgE/Ag, most exocytic events occur within 15 - 20 min of activation.
    2. For activation of the cells, add 16 µL from the 20x secretagogue solution to the chamber.

5. Confirming FITC-dextran presence in the cells.

1. To confirm the presence and localization of FITC-dextran to the SGs, add 16 µL of ammonium chloride solution (be careful not to move the chamber in order not to lose focus) to the chamber gently (this will make a final concentration of 20 mM). This will induce alkalization of the SGs and dequenching of FITC fluorescence, which will occur within seconds.

Representative Results

Figure 1a represents schematically how FITC-dextran may act as a reporter for regulated exocytosis and recapitulate the different modes of exocytic events. First, cells are incubated with FITC-dextran, which is internalized by pinocytosis and sorted to the SGs. Since the SGs of MCs are LROs, their low pH dampens the fluorescence of FITC, shown here as black granules (A-C, I). When cells are triggered by a secretagogue and the SGs fuse with the plasma membrane, a fusion pore is formed, allowing efflux of protons and alkalization of the SGs. As a consequence, FITC regains its fluorescence (A, II). Full exocytosis will manifest itself in a short (less than 5 s) fluorescent event as the exteriorized SG's membrane fully collapses into the plasma membrane and FITC-dextran diffuses away in the culture medium (A, III). During a kiss-and-run exocytic event, the SG's membrane only transiently fuses with the plasma membrane, resulting in a short-lived fusion pore and therefore only a brief outburst of fluorescence as the SG rapidly detaches and its lumen rapidly re-acidifies (B, III- IV). Finally, during compound exocytosis, a series of SGs sequentially fuse with each other (following SG fusion) as well as with the plasma membrane, with maintenance of an open fusion pore. As a result, a much larger mass of FITC-dextran is dequenched, resulting in a large and long-lived fluorescence burst (C, III-IV) that decays when the probe diffuses into the external medium (C, V).

We have used this model and confirmed its predictions by imaging control cells and cells that are depleted of Rab5, which we have shown to be crucial for homotypic SG-SG fusion[69] and compound exocytosis[33]. By setting acquisition time to an image every 15 s, we expected to detect mostly continuous events of secretion, *i.e.,* compound exocytosis. Indeed, as shown in figure 1B, our data show that while in control cells many large and long-lasting exocytic events could be recorded, only few events were recorded in Rab5 knockdown cells (shRab5). Moreover, events that were recorded in shRab5 cells were significantly smaller in size in comparison to events recorded in the control cells. Of note, the secretory capacity of the shRab5 cells remains unaltered under the same conditions[69], suggesting that while shRab5 inhibits compound exocytosis, it does not abolish secretion. Therefore, imaging FITC-dextran fluorescence in long time intervals allows for the specific detection of compound exocytosis. Furthermore, imaging FITC-dextran release allows capturing of the sequential nature of compound exocytosis. As indicated by the arrow in **Figure 1b**, following a flash of light, a second flash begins as a weak flash that is adjacent to the first one. This can be inferred as the fusion of a SG with a SG that is already in the process of fusion with the plasma membrane (*i.e.*, sequential exocytosis). Hence, as the second granule fuses with the first granule that is already connected to the plasma membrane by a fusion pore, the pH of the second granule increases as well and FITC fluorescence dequenches, yielding a second burst of fluorescence. Such a scenario of FITC-dextran release during sequential fusion of adjacent SGs is presented in **Figure 1c**.

Another example of sequential fusion of SGs during compound exocytosis is shown in **Figure 2**. RBL cells were co-transfected with a constitutively active (CA) form of Rab5a and WT SNAP-23, conditions under which compound exocytosis becomes more robust[33]. Imaging of the CA Rab5a, which is essential for SG-SG fusion and is coating large SGs[33,69], together with FITC-dextran, allows better visualization of the fusion of a smaller granule with a larger CA-Rab5a decorated granule.

Since FITC-dextran is quenched when stored inside the SG, it is necessary to implement a technique for verifying that FITC-dextran is indeed present in the SGs. This becomes even more essential when preforming an experiment under conditions which are expected to abolish secretion. To overcome this obstacle, 20 mM of ammonium chloride was added to the chamber at the end of each experiment. This treatment elevates the pH inside the SGs, allowing for FITC fluorescence. As shown in **Figure 3**, after addition of ammonium chloride to the media, FITC dextran becomes visible and is co-localized with mRFP-tagged neuropeptide Y, which is a SG reporter in RBL cells[70].

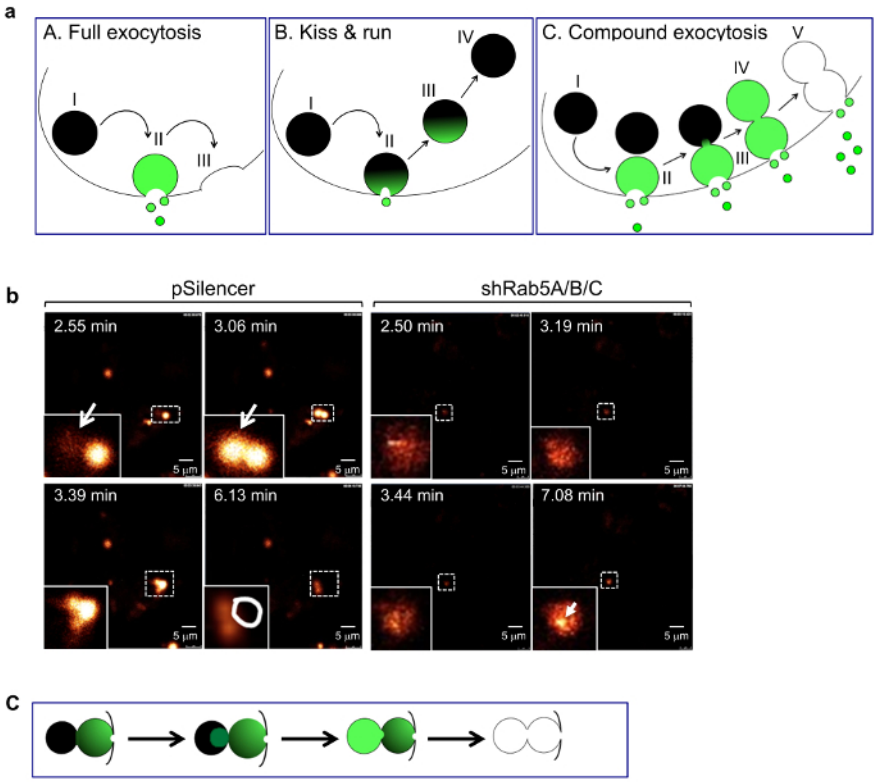

Figure 1: Specific detection of compound exocytosis. (a) A diagram describing how changes in FITC-dextran fluorescence recapitulate the different modes of regulated exocytosis. **(b)** Live cell Imaging of FITC-dextran in RBL cells transfected with NPY-mRFP and either pSilencer or shRab5, as indicated. Transfection was performed as previously described[67,70]. Briefly, 1.5 x 10^7 RBL cells were resuspended in transfection buffer (DMEM supplemented with 20 mM K-Pipes pH 7, 10 µM Ca^{2+} acetate, 2 mM Mg^{2+} acetate, 128 mM potassium glutamate) containing 15 µg NPY-mRFP and either 30 µg of pSilencer or 15 µg of shRab5A, and 15 µg of shRab5B/C. Transfection was achieved by electroporation at 300 V and a 20 mSec pulse length. Cells were immediately replated in culture medium containing 1 mg/mL FITC-dextran. After 24 h, cells were sensitized with 1 µg/mL of IgE and incubated for further 24 h. The cells were then washed three times in full Tyrode's buffer and activated by 50 ng/mL of DNP-HSA (Ag). Cells were visualized by time-lapse fluorescence microscopy. The white arrow in the pSilencer images points to a burst of FITC fluorescence and the white circle marks a discharged SG. The white arrow in the shRab5A/B/C images indicates minute fluorescent events that occur in Rab5-knockdown cells, likely to be indicative of full exocytosis. Of note, the intensity of the ShRab5 image was increased to allow detection of the signal. Images were acquired by a confocal microscope equipped with a heated chamber (37 °C) and CO_2 controller (4.8%) and a C-Apochromat x63/1.2 W Corr objective, under open pinhole setting. For the full movies, refer to Klein *et al.*[33] **(c)** A schematic diagram of the homotypic SG fusion process shown in (b). In detail, as the first SG fuses with the plasma membrane and establishes a fusion pore, it begins to alkalize while releasing its FITC-dextran content. Thus, by the time a second SG fuses with the first one in a sequential manner, the fluorescence of the first SG fades due to the release of FITC-dextran. However, the second SG alkalizes, which results in a FITC flash that appears adjacent to the first SG. This figure was adapted from Klein *et al.*[33] Please click here to view a larger version of this figure.

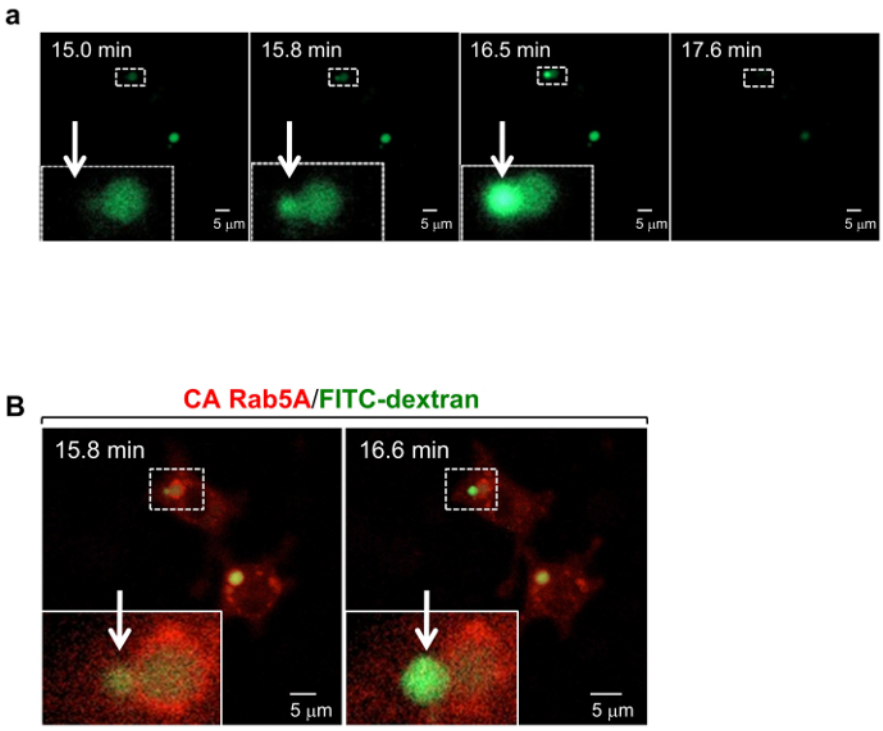

Figure 2: Dual imaging of mStr-CA-Rab5 and FITC-dextran. RBL cells were co-transfected with 20 µg of HA-wt-SNAP23 and 10 µg of mStr-CA Rab5A. Cells were pre-incubated with FITC-dextran and IgE and triggered with Ag as described in **Figure 1**. **(a)** A series of images capturing a fusion event between a quenched SG (white arrow) and a SG that has already fused with the plasma membrane and is therefore fluorescent. At 15.8 minutes, the quenched SG has fused with its neighboring SG and begun to gain fluorescence. The boxed areas are enlarged at the bottom left of each time point. **(b)** Presentation of both mSTR-CA Rab5A (in red) and FITC-dextran (in green) from the corresponding time points shown in (a), demonstrating the fusion between the two SGs. Images were acquired by confocal microscopy (open pinhole) using a Plan-apochromat x63 - NA 1.4 objective. This figure was adapted from Klein *et al.*[33] For the full movie, see Klein *et al.*[33] Please click here to view a larger version of this figure.

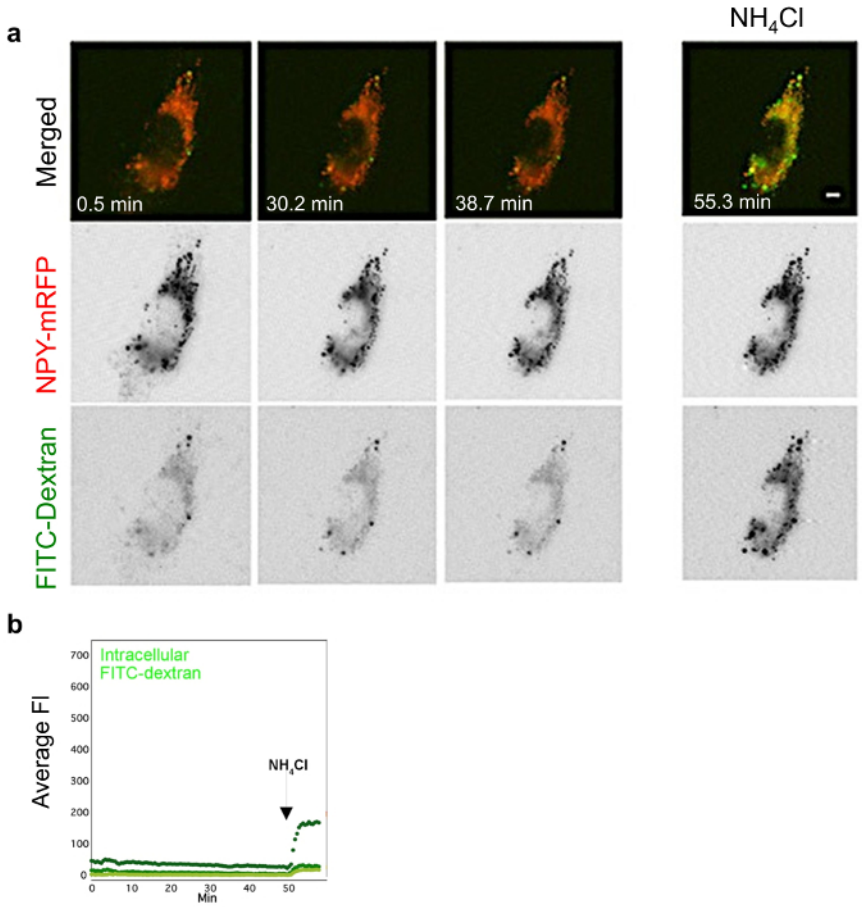

Figure 3: Dual imaging of NPY-mRFP and FITC-dextran during alkalization of SG by NH_4Cl. RBL cells were transfected with shRab5, pre-incubated with FITC-dextran and IgE, and triggered with Ag, as described in **Figure 1**. **(a)** Cells were visualized by time-lapse fluorescence microscopy. Designated times in the images are minutes after triggering with Ag. Images were acquired by confocal microscopy using a Plan-apochromat x63 - NA 1.4 objective. **(b)** Quantitative analysis of the experiment shown in (a). Each line in the graphs is the average fluorescence of FITC at a region of interest (ROI) over a single cell. The arrow points to the time of NH_4Cl (20 mM) addition. Bars = 5 µm. This figure was adapted from Klein *et al.*[33] Please click here to view a larger version of this figure.

Discussion

Here we describe how tracing the fluorescence of FITC-dextran loaded into SGs can be used to specifically capture compound exocytosis events. This was achieved by setting the microscope to acquire an image every 15 seconds, thus ensuring that only long-lasting events will be recorded over time, and therefore excluding short events that would correspond to full exocytosis or kiss-and-run exocytosis. To establish the method, we showed that knockdown of the Rab5 isoforms that are expressed in RBL cells, and are pivotal for compound exocytosis, eliminates the ability to capture events of FITC-dextran release, while the overall secretion by the cell is not affected.

The most prominent disadvantage of the method is that it only applies to cells containing acidic SGs. However, the most prominent advantage of the method is that it is based on simple incubation of the cells with FITC-dextran, without requiring transfection. Other pH-sensitive reporters that are used to study regulated exocytosis and involve SG cargo or SG membrane protein-fused to phlourin, a pH-sensitive GFP variant (such as NPY-phlourin-, β-hexoseaminidase-phlourin, and synapto-phluorin[48,49,50,51,52]), may more closely represent the endogenous composition of the SGs. However, their use entails transfection of the cells, which might be less suitable for cells that are difficult to transfect. Therefore, when studying cells that are difficult to transfect or under experimental conditions that require multiple genomic manipulations, the use of a compound that can be simply supplemented in the cell culture medium, such as FITC-dextran, is advantageous. Our protocol may require cell specific adjustments to accommodate differences in the kinetics of exocytosis of different cell types[68]. Hence, the chosen time interval for image acquisition should be optimized. For RBL cells, we have set-up the microscope to capture a new image every 15 seconds, which in IgE/Ag triggered RBL cells allowed us to visualize only long-lasting events, thereby allowing the exclusive tracking of compound exocytosis events.

Another factor that needs to be considered is the type of dextran to be used. A variety of dextrans that vary in their molecular weights are available. Therefore, it is important to choose the appropriate dextran as a reporter, taking into account the potential effects that different dextrans might have on cells. For example, low molecular weight dextrans have been shown to activate MCs and induce secretion[71,72,73,74]. Therefore, low molecular weight dextrans would not be appropriate reporters for MC secretion. Differences in cell reactivity towards dextrans were also noted in different rat strains[75], or under different co-stimulatory conditions[71,76,77,78]. Here, we have chosen to use a 150K FITC-dextran that has previously been studied in the RBL cell line and shown to be ineffective in inducing secretion[18,79].

The light source to be selected is also crucial for the protocol. When using a confocal microscope, the light source is traditionally laser-based. Thus, to avoid toxicity, it is imperative to choose a low laser power. The desired laser power differs between microscopes, depending on the type and age of the laser. Moreover, the desired laser power is also dependent on the type of detector used. For example, when using a common detector such as a photomultiplier tube-based detector, a higher power laser will be needed than when using a modern hybrid detector (such as a photomultiplier tube and an avalanche photo-diodes hybrid detector). We recommend using the lowest laser power possible and not exceeding 10% of laser power.

In summary, monitoring the dequenching of SG loaded FITC-dextran by time Lapse-microscopy under carefully chosen settings allowed us to exclusively capture compound exocytosis in RBL MCs, greatly facilitating identification of the molecular entities that regulate this process. This method could be further adjusted to distinguish full from kiss-and-run exocytosis by reducing and carefully calibrating the time interval between image acquisitions to allow the distinction of the short-lived flashes associated with kiss-and-run exocytosis from the even shorter flashes that accompany full exocytosis. Such conditions would capture both compound and kiss-and-run exocytosis that could then be distinguished based on the duration of the signal.

Disclosures

The authors declare no competing financial interest

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