Visualizing the endocytic and exocytic processes of wheat germ agglutinin by quantum dot-based single-particle tracking

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Wheat germ agglutinin (WGA) is a paradigm for understanding intracellular transport of lectins. As a protein exploiting the receptor-mediated endocytosis for internalization, WGA is also a valuable model system for exploring the endocytic and exocytic pathway. In this study, quantum dot-based single-particle tracking was performed to investigate the transport of WGA in live cells, revealing firstly that the endocytic and exocytic processes of WGA were both actin- and microtubule-dependent, each including five stages. The vesicle fusion event occurred near the cytomembrane, followed by two destinies with WGA: shedding to the extracellular or reversing to the cytoplasm. These findings suggest a distinct and dynamic scenario for the transport of lectins following a receptor-mediated endo/exocytic pathway in live cells. This is important for the application of lectins as drug carriers and antineoplastic drugs in medicine, and also offers insights into the pathway of endocytosis and exocytosis.

1. Introduction

Lectins are a class of carbohydrate-binding natural nonenzymatic and nonimmunogenic proteins/glycoproteins distributed in a variety of microorganisms, plants and animals [1]. They are capable of specific non-covalent and reversible binding to carbohydrate moieties of complex glycoconjugates. Their ability to interact with the glycoconjugates expressed on cell surfaces triggers several important cellular processes, such as endocytosis or transcytosis [2–6]. In the last two decades, lectins open two attractive areas for drug delivery: glycotargeting of anticancer drugs and improving absorption of poorly available drugs in medical field [2,7]. Meanwhile, the interest in lectins intensifies with the fact that several lectins are extremely valuable potential antineoplastic drugs because of their remarkable anti-tumor activities by inducing apoptosis or autophagy in cancer cells [8]. And, some lectins capable of the reduction of treatment-associated side-effects are used as adjuvant agents during chemotherapy and radiotherapy [9]. Wheat germ agglutinin (WGA) is one of the most extensively studied plant lectins for its high stability and specific recognition of N-acetylglucosamine and sialic acid moieties [2]. WGA is an ideal probe for exploring the transport mechanism of lectins in live cells.

The internalization of WGA is a complex, multistep process: WGA binding to its receptors on the cell surface, entering the cells by a receptor-mediated endocytosis and then being transported in the live cell related to actin filaments and microtubules via trans-Golgi and lysosome [2,10–14]. However, despite intensive efforts in investigating the transport process of WGA, many crucial features of WGA traffic remain ambiguous. Among these are important and general questions for the transport mechanisms: what is the whole transport pathway in live cells, how does WGA move in different stages in the whole process, and where is the final destiny of WGA in live cells?

Single-particle tracking (SPT) technique is a powerful approach to study in-situ and real-time dynamics of single-particle events in live cells, which can reveal interesting biological interactions and elucidate the mechanisms of particle motions in living cells [15–19]. Quantum dots (QDs) possess unique properties such as broad absorption with narrow emission, exceptional photostability, high fluorescence quantum yield, and a large Stoke’s shift [20,21], which have great potential to be an alternative to the conventional fluorophores in the field of SPT [17,22–29]. Combined with ultra-sensitive optical technique, QDs as tags allow single-particle...
tracking biomolecules in live cells with a high signal-to-noise ratio (S/N) and over-unprecedented durations [26,27].

In this study, QDs-based SPT technique was used to investigate the transport of WGA in live cells in real and long time, which allowed us to reveal the transport mechanism of WGA in great detail. In addition, the approach provided meaningful information about the dynamics of the receptor-mediated endocytic and exocytic processes.

2. Materials and methods

2.1. Cell culture

A549 cells were cultured in Dulbecco's modified Eagle's Medium (DMEM, Gibco) with 10% fetal bovine serum penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37 °C. Before the experiments, the cells were cultured on glass-bottomed 35-mm Petri dishes (MatTek Corp) over 24 h.

2.2. Labeling method

To label WGA with QDs (QD-WGA), the two-step method was used as follows: the cells were incubated with 4 nm biotinylated WGA (Vector Laboratories) at 4 °C in Tyrode's plus buffer for 10 min, washed three times; subsequently, the cells were incubated for 10 min with 2 nm streptavidin-modified QDs (SA-QDs) (Wuhan Jiayou Quantum Dots Co. LTD), washed three times and then imaged at 37 °C with a CO2 on-line culture system [28]. Control experiments were performed in the same way only without biotinylated WGA or with WGA in Tyrode's plus buffer. To investigate the stability of QD-WGA, the cells were incubated with 4 nm biotinylated WGA, washed and incubated with 2 nm SA-QDs (605 nm) and 2 nm SA-QDs (540 nm) simultaneously as the above mentioned method.

To label the cytomembrane and nucleus, the cells were incubated in Tyrode's plus buffer containing 5 μg/ml Dio (Beyotime Institute of Biotechnology) and 5 μg/ml Hoechst 33342 (Beyotime Institute of Biotechnology) for 30 min at 37 °C before the fluorescence imaging.

To label the actin filaments and microtubules of A549 cells, the cells were transfected with enhanced green fluorescence protein (EGFP)-Lifeact peptide plasmid (kindly provided by Dr. Zong-Qiang Cui, Wuhan Institute of Virology, China) or green fluorescence protein (GFP)-microtubule associated protein 4 plasmid using lipofectamine66 (LTX) transfection reagent (Invitrogen). For a 20-mm Petri dish, 3 μl of lipofectamine66 LTX reagent was combined with Opti-MEM 1 Reduced Serum Medium (Gibco) and 1.5 μg of DNA for a final volume of 100 μl. Lipofectamine66 LTX-DNA mixture was incubated at room temperature for 30 min and then added to the cell culture. The cells were incubated in a 5% CO2 environment and the medium was changed after 6 h.

2.3. Inhibition studies

To study the effect of temperature on the uptake of WGA, the cells were kept at either 4 °C or 37 °C for 1 h. To disrupt actin filaments, the cells were exposed in Tyrode's plus buffer with 20 μM cytochalasin D (cyto-D) (Sigma) for 30 min before experiment. To disrupt microtubules, the cells were exposed in Tyrode's plus buffer with 60 μM nocodazole (Sigma) for 30 min before experiment [18]. The cells were exposed in the drugs throughout the experiments.

2.4. Fluorescence imaging

A spinning-disk confocal microscope (Andor Revolution XD) was equipped with an Olympus IX 81 microscope, a Nipkow disk type confocal unit (CSU 22, Yokogawa), a CO2 on-line culture system (INUBG2-P1) and an EMCCD (Andor iXon DV885K Single Photon Detector) using a 1.4 NA. objective (Olympus). The 405 nm, 488 nm and 561 nm laser (DPSS, USA) were used to excite Hoechst 33342, Dio/540 nm QDs/GFP/EGFP and 605 nm QDs respectively. The fluorescence signals from the fluorophores were separated using a 447/60 nm bandpass filter (Chroma), a 525/50 nm bandpass filter (Chroma) and a 617/73 nm bandpass filter (Chroma) and imaged alternately onto the EMCCD by separate channels for simultaneous imaging.

2.5. Imaging processing

Imaging processing and data analysis were carried out. The initial step was the application of a gaussian filter to remove background noise. To construct the 3D surface plot, successive Z-stacks spaced by 0.5 μm were recorded and then reconstructed by the NIH Image J software. The strip view was performed by Andor IQ software with successive Z-stacks spaced by 0.5 μm. To construct 3D confocal image of the cells incubated with WGA in different times, three-channel Z-stacks were recorded with the gap of 0.5 μm, processed by Andor IQ software and located at the position of interest. To show the snapshots of the interesting movements, the single or double channel time stacks were recorded and processed by Andor IQ software with the appropriate frame interval.

The imaging series of the particles' movements were analyzed by Image-Pro Plus 6.0 software. Briefly, the trajectories were generated by pairing spots in each frame according to proximity and similarity in intensity. The mean square displacement (MSD) <r^2> of the particle was obtained by using an equation [30],

\[ <r^2> = \frac{N}{N-1} \sum_{i=0}^{N-1} \left\{ (x(\Delta t + n\Delta t) - x(\Delta t))^2 + (y(\Delta t + n\Delta t) - y(\Delta t))^2 \right\} \]

where \((x(\Delta t), y(\Delta t), z(\Delta t))\) is the particle position in the image frames at a time interval \(n\Delta t\) after starting at the position \((x(\Delta t), y(\Delta t), z(\Delta t))\), \(N\) is the total number of the image frames with \(n\) determining the time increment (\(n\) and \(i\) are integers) and \(N\) is the MSD-Time plot was analyzed for the different modes of motion to obtain a possible mechanism of the particle movement. The MSD-Time plots were fit over the first several time intervals to the related equations to determine the diffusion coefficient (D) and velocity (V) values [31,32].

3. Results

3.1. Specificity and stability of QD-labeled WGA binding to A549 cells

To track the transport of WGA in live cells, WGA was labeled with QDs in two steps [28]. The specificity and selectivity of QD-WGA was examined. Fig. 1A shows an example of the cells treated with QD-WGA. The outlines of the cells were very clearly displayed by QDs fluorescence, suggesting that QD-WGA had been attached to the receptors of WGA on the cell surface. Fig. 1B is for a negative control (adding SA-QDs alone) where the cells were detected with very few fluorescence signals in the fluorescent image, illustrating that the fluorescence exhibited in Fig. 1A is WGA-receptor-specific. In order to display the results more clearly and further elucidate the specificity of QD-WGA, 12 Z-stacks of the cells were reconstructed in 3D surface plots (Fig. 1A-right and 1B-right). Meanwhile, to test whether biotinylated WGA was linked to QDs via biotin–streptavidin bonds, another negative control (adding first non-biotinylated WGA, and subsequently SA-QDs) was performed (Supplementary Fig. S1). Few fluorescence signals are exhibited in the fluorescent image, indicating that QDs are not linked to WGA via non-specific adsorption, but linked to biotinylated WGA via biotin-streptavidin interaction. To confirm the stability of QD-WGA in the cytoplasm, the cells were incubated with two-color QD-WGA (540 nm and 605 nm) simultaneously at 37 °C for 1 h (Fig. 1C). Almost all fluorescence signals of red QDs were colocalized with the fluorescence signals of green QDs in live cells, suggesting that WGA is incorporated with QDs all the time in live cells. In other words, QD-WGA is stable in the intracellular environment and the fluorescence signals of QDs denote the existence of WGA all the time.

3.2. Transport dynamics of WGA on actin filaments and microtubules

To probe the internalization process of WGA, a series of time-dependent studies were carried out at 37 °C over a 24-h period (Supplementary Fig. S2). At 0 h of incubation, QD-WGAs were colocalized with the membrane dye (Dio) on the cell surfaces. At 0.5 h, the colocalizations of QDs and Dio appeared in the cytoplasm, illustrating that WGA is transported into cytoplasm within 0.5 h incubation. Nearly all of QDs were colocalized with the vesicles in the cytoplasm, suggesting that WGA is trapped and transported in vesicles in the cytoplasm. Meanwhile, it is evident to observe that the colocalizations were accumulated in a unique perinuclear region in the cells. From 1 to 24 h, more and more QD-WGAs were transported and accumulated in this region, with fewer and fewer QD-WGAs on the cell surface. According to the previous reports, the perinuclear region may be the microtubule organizing center.
MTOC, the site of microtubule nucleation [29]. To probe whether MTOC is indeed the unique perinuclear region for WGA accumulation, the WGA movements were tracked in the A549 cells expressing GFP-tagged microtubules (Supplementary Fig. S3). QD-WGAs and microtubules were colocalized spatially in the perinuclear region, indicating that the intracellular transport of WGA is likely mediated by an active process owing to the regular distribution. Also, it is sufficient to suggest that the destination of the endocytic transport is the MTOC in live cells. Additionally, WGA does not enter the nucleus, as QD-WGAs were not colocalized with Hoechst 33342 up to 24 h (Supplementary Fig. S2).

To clarify the transport pathway of WGA, a series of uptake inhibition studies were carried out. First, when the temperature was lowered to and kept at 4 °C for 1 h, the uptake of WGA was blocked (Supplementary Fig. S4), suggesting that the uptake is energy-dependent. Second, the presence of cyto-D (a drug to disrupt actin filaments) or nocodazole (a drug to disrupt microtubules) also blocked the uptake of WGA and when both cyto-D and nocodazole were simultaneously used, nearly none of QDs fluorescent was detected in the cytoplasm, illustrating that the cytoskeletons are involved in the transport of WGA (Supplementary Fig. S5). Thus, the transport of WGA is an active and energy-dependent process via actin filaments and microtubules, which is consistent with that reported previously about the internalization of WGA [2,10–14].

To quantitatively investigate the dynamics of WGA moving on actin filaments and microtubules, WGA was imaged in real time in A549 cells transiently expressing EGFP-tagged actin filaments or microtubules. (MTOC), the site of microtubule nucleation [29]. To probe whether MTOC is indeed the unique perinuclear region for WGA accumulation, the WGA movements were tracked in the A549 cells expressing GFP-tagged microtubules (Supplementary Fig. S3). QD-WGAs and microtubules were colocalized spatially in the perinuclear region, indicating that the intracellular transport of WGA is likely mediated by an active process owing to the regular distribution. Also, it is sufficient to suggest that the destination of the endocytic transport is the MTOC in live cells. Additionally, WGA does not enter the nucleus, as QD-WGAs were not colocalized with Hoechst 33342 up to 24 h (Supplementary Fig. S2).

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To quantitatively investigate the dynamics of WGA moving on actin filaments and microtubules, WGA was imaged in real time in A549 cells transiently expressing EGFP-tagged actin filaments or
diffusion coefficients were 0.006 V/C6. GFP-tagged microtubules. Fig. 2A and D shows the examples of the typical movements of WGA on actin filaments and microtubules respectively, suggesting that the movements on actin filaments occurred in the cell periphery and the movements on microtubules occurred between the cell periphery and the nucleus. Snapshots of the transports show that the movement on actin filaments was slow and that on microtubules was rapid (Fig. 2C and F). According to the relationship between MSD and time, the possible mechanism involving the particle movement could be obtained [30–32]. Herein, the MSD-Time plots of the vesicles on actin filaments and microtubules are shown in Fig. 2B and E. The apparent upward curvature indicates that WGA moved on actin filaments and microtubules regularly in a directed motional mode. The movements of these two types were analyzed statistically with 50 trajectories respectively. According to the fits to \( r^2 = A + 4Dt + (Vt)^2 \) (\( D \) and \( V \) are the diffusion coefficient and velocity of the particle, \( A \) is noise), the \( D \) and \( V \) values were \( 0.006 \pm 0.003 \, \mu m^2/s \) and \( 0.31 \pm 0.16 \, \mu m/s \) (Mean ± Standard Deviation) on actin filaments (Fig. 2Ga and Gb) and \( 0.023 \pm 0.014 \, \mu m^2/s \) and \( 2.16 \pm 0.62 \, \mu m/s \) on microtubules (Fig. 2Ge and Gd). The velocity values are consistent with those previously reported, that is, the speeds related to actin filaments are in the range of 0.1–0.4 \( \mu m/s \) [33,34] and the speeds related to microtubules are several \( \mu m/s \) in live cells [35,36].

3.3. Five-stage endocytic process of WGA into live cells

By tracking the transport of WGA in real and long time, the whole endocytic process of WGA was revealed that it is a five-stage transport. Coupled with two-channel SPT, a low-temperature control method was used to investigate the forming process of individual vesicles after WGA binding to the receptors on the cell surface. An example of WGA being trapped into a vesicle near the cytomembrane was shown in Fig. 3A and Supplementary Movie S1. Firstly, WGA was localized with DIO on the cell surface, and then a vesicle containing WGA formed and entered the cytoplasm after about 10 s, suggesting that initially WGA is binding to the receptors on the cell surface (Stage 1) and then being trapped to the vesicle in the cytoplasm (Stage 2).

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Subsequently, individual vesicles were, in real time and long term, tracked to unravel the unknown dynamics of WGA transport in the cytoplasm. According to 24 typical trajectories of WGA from 7 times experiments in parallel, the WGA movement was a clear three-step transport pattern from the cell periphery to the MTOC: WGA moving slowly in the cell periphery (Stage 3), marching rapidly toward the MTOC (Stage 4) and moving intermittently around the MTOC (Stage 5). An example of the process is shown in Fig. 3B and Supplementary Movie S2. The time trajectory of the instantaneous speed (instantaneous speed is the distances between two adjacent frames divided by the frame interval time) of the vesicle is shown in Fig. 3C.

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Analysis of single-particle trajectories in the three steps provides further insights into the mechanisms of WGA transport. As shown in Fig. 3D-left, the distribution of instantaneous speed of WGA in Stage 3 suggests that WGA moved slowly at an instantaneous speed of less than 0.4 \( \mu m/s \) in the cell periphery. The MSD-Time plots for 3 typical WGA trajectories in this stage exhibit an apparently upward curvature (Fig. 3E-left), indicating that WGA moved in the cell periphery in a directed manner. According to the fits to \( r^2 = A + 4Dt + (Vt)^2 \), it is evident that the diffusion coefficients and the speeds are in the range of the actin-dependent movements. Furthermore, WGA was tracked in real time in drug treated cells (Supplementary Fig. S6). The WGA movement was much more limited mobility in the cyto-D treated cells. These results suggest that the Stage 3 movement is actin-dependent.

Next, the rapid marching step toward the cell nucleus was Stage 4. As shown in Fig. 3D-middle, the distribution of the instantaneous speed of WGA in Stage 4 suggests that WGA marched rapidly at an instantaneous speed of more than 0.7 \( \mu m/s \) toward the nucleus. The MSD-Time plots (Fig. 3E-middle) for 3 typical WGA trajectories in this stage are of apparently upward curvature, suggesting that WGA marched rapidly toward the nucleus in a directed manner. According to the fits to \( r^2 = A + 4Dt + (Vt)^2 \), the diffusion coefficients and the speeds are in the range of the microtubule-dependent movements. Further, the movement observed in the nocodazole-treated cells was also limited, but there was no significant difference in the speed compared to that in Stage 3 in the untreated cells, indicating that the rapid and directed movement was related to microtubules (Supplementary Fig. S6). These results illustrate that the Stage 4 movement is microtubule-dependent.

After the rapid marching transport, WGA would enter Stage 5 around the MTOC. In this stage, WGA moved intermittently at an instantaneous speed of less than 0.5 \( \mu m/s \) (Fig. 4D-right). The MSD-Time plots for 3 typical WGA trajectories in this stage are shown in Fig. 4E-right. It was very surprising to discover that not all MSD-Time plots curved upward and the downward curvature appeared in this stage. The upward plots by fitting to \( r^2 = A + 4Dt + (Vt)^2 \) suggest the movements were also directed in this stage. But the downward plot by fitting to \( r^2 = 4Dr^2 \) shows the movement was in anomalous behavior indicating that WGA was confined by some compartment in this stage. Since MT0C is the destination of WGA transport, the Stage 5 movement should be microtubule-dependent around the MTOC. Thus, the intermittent movements in this stage may be caused by WGA moving between different microtubules or the interference of other vesicles. Certainly, the possibility that the Stage 5 movement involves minus- and plus-end-directed motors on microtubules could not be excluded [18].

Together, the whole endocytic pathway of WGA is a five-stage transport: WGA binding to the receptor on the cell surface (Stage 1), being trapped into the vesicle (Stage 2), moving slowly in an actin-dependent mode in cell periphery (Stage 3), marching rapidly in a microtubule-dependent mode toward the cell nucleus (Stage 4) and then moving intermittently around the MTOC (Stage 5).

3.4. Five-stage exocytic process of WGA in live cells

According to the analysis of 30 typical trajectories from 7 times experiments in parallel, QDs-based single-particle tracking also uncover that WGA possess a clear five-stage transport pattern in the exocytic process. Fig. 4A shows an example of WGA moving from the MTOC to the cell periphery (Supplementary Movie S3), suggesting that WGA possessed a clear three-step transport pattern from the MTOC to the cell periphery: moving intermittently around the MTOC (Stage I), marching rapidly toward the cytomembrane (Stage II) and moving slowly in the cell periphery (Stage III). The instantaneous speed trajectory also shows slow-fast-slow three stages (Fig. 4B). Quantitative analysis shows that, the movement was intermittent around the MTOC with an instantaneous speed of less than 0.5 \( \mu m/s \) (Stage I), rapid toward the cell periphery with an instantaneous speed of more than 0.6 \( \mu m/s \) (Stage II) and it slows in the cell periphery with an instantaneous speed of less than 0.4 \( \mu m/s \) (Stage III) (Fig. 4C). The MSD-Time plots for 3 typical WGA trajectories in each stage (Fig. 4D) show that the Stage I movement was in directed or anomalous diffusion manner, similar with the
Stage 5 movement of the endocytic process, indicating that the intermittent movement around the MTOC represents the triggering process of the exocytosis; the movement was directed with $D$ and $V$ values in the range of the microtubule-dependent movements in Stage II, suggesting that the movement is microtubule-dependent; and the Stage III movement was also directed with $D$ and $V$ values in the range of the actin-dependent movement, illustrating that WGA moves on actin filaments in the cell periphery. The results preliminarily confirm that the exocytic process is the reverse of the endocytic process in live cells via actin filaments and microtubules.

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According to previous reports, a vesicle shedding process could occur on both cytomembrane and the cell extensions such as filopodia [29]. But the dynamics of the shedding process of WGA has never been reported clearly so far. Herein, by combining with two-
Fig. 4. Tracking the exocytic process of WGA in a live cell. (A) The trajectory (yellow) of a vesicle from the perinuclear region to the cell periphery inside a cell. The white lines show the boundaries of the nucleus and the cytomembrane, respectively. (Scale bar: 10 μm). (B) Time trajectory of the instantaneous speed of the vesicle. I, II, and III are the durations of Stage I, Stage II, and Stage III, respectively. (C) Histogram of the instantaneous speed of WGA in Stage I (Left), Stage II (Middle) and Stage III (Right). (D) The MSD-Time plots (black) of 3 typical WGA trajectories in each stage. (Left) The red lines are the fits to \( <r^2> = A + 4Dt + (vt)^2 \) with \( D = 0.011 \text{ μm}^2/\text{s} \) (triangle), \( 0.007 \text{ μm}^2/\text{s} \) (square) and \( V = 0.02 \text{ μm/s} \) (triangle), \( 0.44 \text{ μm/s} \) (square). The blue line is a fit to \( <r^2> = 4Dt^a \) with \( D = 0.017 \text{ μm}^2/\text{s} \) (circle) and \( a = 0.8 \) (circle). (Middle) The red lines are the fits to \( <r^2> = A + 4Dt + (vt)^2 \) with \( D = 0.088 \text{ μm}^2/\text{s} \) (triangle), \( 0.063 \text{ μm}^2/\text{s} \) (square), \( 0.010 \text{ μm}^2/\text{s} \) (circle) and \( V = 1.48 \text{ μm/s} \) (triangle), \( 1.12 \text{ μm/s} \) (square), \( 1.01 \text{ μm/s} \) (circle). (Right) The red lines are the fits to \( <r^2> = A + 4Dt + (vt)^2 \) with \( D = 0.002 \text{ μm}^2/\text{s} \) (triangle), \( 0.005 \text{ μm}^2/\text{s} \) (square), \( 0.003 \text{ μm}^2/\text{s} \) (circle) and \( V = 0.22 \text{ μm/s} \) (triangle), \( 0.16 \text{ μm/s} \) (square), \( 0.10 \text{ μm/s} \) (circle). (E) Two possible destinies of WGA in the exocytic process. (a) Snapshots of a vesicle shedding to the extracellular (Scale bar: 2 μm). (b) Snapshots of a vesicle reversing to the cytoplasm (Scale bar: 2 μm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
channel SPT, it is very intriguing and important to find and confirm two possible destinies in the exocytic process: WGA either shedding to the extracellular (Stage IV) or reversing to the cytoplasm (Stage V). Fig. 4Ea shows an example of the typical shedding of WGA. WGA moved slowly near the cytomembrane and was spit out of the vesicle and went to the extracellular (Supplementary Movie S4). Additionally, another new finding is that the shedding process involves an intricate struggle. As shown in Supplementary Fig. S7 and Movie S5, the colocalization of the vesicle and WGA was varying with time. The vesicle and WGA were separating reciprocally; the vesicle was reversing to the cytoplasm and at the same time WGA was shedding to the extracellular, vividly presenting a scenario for WGA escaping out of the vesicle and entering the extracellular after an intricate struggle. The single-particle dynamic tracking helps us unravel a previously unknown vesicle fusion/shedding near the cytomembrane. Meanwhile, Fig. 4Eb shows an example of the typical reversing of WGA. Initially, the vesicle was moving to the cytomembrane, slowing down near the cytomembrane and then reversing rapidly toward the cytoplasm (Supplementary Movie S6). The results gave a clearer revelation for the destinies of the vesicles: WGA could shed to the extracellular only when it bolt successfully from the vesicle or otherwise be transported back into the cytoplasm once again.

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The entire exocytic process of WGA is also a five-stage transport: moving intermittently around the MTOC (Stage I), marching rapidly in a microtubule-dependent mode toward the cell periphery (Stage II), moving slowly in an actin-dependent mode in the cell periphery (Stage III), slowing down near the cytomembrane for the vesicle fusion (Stage IV) and at last shedding to the extracellular or reversing to the cytoplasm (Stage V).

4. Discussion

Despite the medical importance of lectins [2,5,8], little information is available about the transport pathway of lectins. Moreover, despite intensive efforts in investigating the endo/exocytosis, many critical data remain poorly understand. In this work, combining the unique properties of QDs and SPT technique, we firstly visualized the entire endocytic and exocytic processes of WGA by long-time SPT. This technique allows us to reveal the transport of lectins and to obtain mechanistic and dynamic insights into the endocytosis and exocytosis in live cells.

Our results show that the endocytic and exocytic processes of both are a five-stage transport pattern via actin filaments and microtubules. WGA moved with $D$ and $V$ values of $0.006 \pm 0.003 \mu m^2 / s$ and $0.31 \pm 0.16 \mu m / s$ on actin filaments and $0.023 \pm 0.014 \mu m^2 / s$ and $2.16 \pm 0.62 \mu m / s$ on microtubules. The velocity values were consistent with those previously reported in Refs. [33–36], but the diffusion coefficients of WGA on actin filaments and microtubules were firstly obtained statistically. MTOC is the destination of the endocytic process of WGA and the triggering site of the exocytosis. Also, misfolded proteins are transported to the MTOC and accumulate within aggresomes for degradation and inhibition the aggresomes could result in apoptosis in tumor cells [37]. Thus, based on the transport pathway, WGA is a powerful tool to open up new therapies for fighting cancer and this work provides the theoretical basis for widely using WGA in biomedical field.

By two-channel SPT, the previously unseen shedding and reversing processes were visualized in real time. A fierce ‘battle’ between the vesicle and WGA was discovered, which is a better description of the vesicle fusion process. The struggle leads to the shedding or reversing process of WGA, which should have great importance for understanding the receptor-mediated endocytosis and exocytosis. The results also demonstrate that the endocytic and exocytic processes are coupled tightly with a ‘kiss-and-run’ or ‘kiss-and-stay’ mode in live cells [38].

Exocytosis is a common and important process in vivo, but few reports have illustrated the process by SPT especially in non-neuronal cells. One of the key reasons is that it is difficult to label the target biomolecule with conventional tags to satisfy the long-term tracking. Meanwhile, the SPT techniques for studying the dynamics for other biological events in live cells are also limited in real-time for long durations [22]. Herein, QDs are more bright and photostable than fluorescent proteins and organic dyes [20,21], making them very attractive candidates for long-time SPT in live cells. Comparing with the previous techniques [38,39], this could realize the long-range tracking the cytoplasmic events from the cytomembrane to the nucleus in real and long time. It would be a powerful tool for studying the entire transport of biomolecules in live cells.

5. Conclusions

In this study, WGA is both a paradigm for understanding the process of endocytosis/exocytosis and a model of the transport of lectins. We used QDs as tags for long-time and single-particle tracking WGA transport in live cells. The analysis of single-particle trajectories revealed that the endocytic and exocytic process of WGA both are a five-stage transport via actin filaments, microtubules and MTOC. A model of the entire transport process of lectins via endocytosis/exocytosis in live cells is illustrated in Fig. 5. These results can help us vividly depict and profoundly reveal the entire endo/exocytic process and offer insights into the receptor-mediated processes of endocytosis and exocytosis. This is also important for the application of lectins as drug carriers and antineoplastic drugs. The technique has potential applications in the research of dynamic...
mechanisms in vivo, such as the mechanisms of drug action and viral infection.

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Appendix. Supplementary material

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