Online Data

Quantitative ER<->Golgi transport kinetics and protein separation upon Golgi exit revealed by VIP36 dynamics in live cells

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All movies are in QuickTime format.

For optimal playback, edit your browser preferences to launch QuickTime rather than viewing within the browser.

You may need to adjust your monitor settings to discern the details described in the legends. See the viewing instructions for more information.

If you are using Netscape Navigator and the scrollbars disappear, press `Reload` to retrieve them.

Movies are compressed; to achieve smooth playback they must uncompress into RAM. Set them to repeat (`Movie > Loop` menu selection) and allow them to play through more than once. For best results on MacOS platforms, allocate more memory to the QuickTime movie player application (`File > Info > Memory`). For machines with less memory, play a selection only to improve performance: make a selection by holding the shift key down while moving the slider through the frames of interest, then select `Movie > Play Selection Only`. For additional information on optimizing movie playback, please refer to the viewing instructions.

All movies are series of single confocal images. "Grey" movies show each channel separately. Channels in "Color" movies are combined red and green, with overlap in yellow.

Elapsed time is indicated as Hours:Minutes:Seconds (.fractions).

Figure 1

VIP36-SP-FP localization

B

GolgiAndVIP36_Grey.mov
GolgiAndVIP36_Color.mov

VIP36-SP-GFP localizes primarily to the ER and Golgi in living COS cells

The "GolgiAndVIP36" movies (color | grey) show a PtK_2 cell transiently co-expressing VIP36-SP-YFP and the Golgi marker T2-CFP. Cells were imaged in the presence of 100µg/ml of cycloheximide using confocal microscopy. Co-localization of VIP36-SP-YFP and T2-CFP indicate that the compact structures next to the nucleus are Golgi elements. In the color movie, VIP36-SP-CFP is in green, and T2-CFP in red, with overlay in yellow. 4.7 s/frame. Bars: 10 µm.

C myc_Comparison.mov

VIP36-SP-GFP colocalizes with myc-VIP36

Contructs expressing myc-VIP36 (VIP36 with MYC epitope tage at the N-terminus of the native protein) and VIP36-SP-GFP were transiently co-expressed in COS cells. The myc epitope was detected with 9E10 monoclonal antibody (Materials and Methods). The comparison movie cycles between a color overlay showing both proteins, single channels showing only one protein, and a difference image. In the color overlay, VIP36-SP-GFP is in green and myc-VIP36 is in red, with overlap in yellow. In the difference image, pixels which have a higher VIP36-SP-GFP signal are in black, and pixels which have a higher myc-VIP36 signal are in white. The difference image is grey (pixel level 128) because most pixels have similar signal levels for myc-VIP36 and VIP36-SP-GFP, indicating co-localization. Bar: 10 µm.

Figure 2

VIP36-SP-FP shows localization and behavior characteristic of a protein recycling in the early secretory pathway

Comparison movies are series of single confocal sections, and cycle between a color overlay showing both proteins, and single channels showing only one protein.

A Comparison_KDELR_Cell.mov

Comparison_KDELR_R1.mov

Comparison_KDELR_R2.mov

VIP36-SP-FP colocalizes with the early secretory marker KDELR-SP-FP

PtK_2 cells co-expressing KDELR-SP-CFP and VIP36-SP-YFP at 37°C were fixed in cold methanol for 2 minutes and mounted in PBS for high-resolution imaging. The movies show the cell and regions i and ii from Figure 2A. In the color overlay, VIP36-SP-CFP is in green and KDELR-SP-YFP is in red.

Co-localization is comparable to a cell co-expressing the same protein in different colors, VIP36-SP-CFP and VIP36-SP-YFP, under identical imaging conditions (see below).

D Comparison_KDELR_15C.mov

VIP36-SP-FP accumulates with KDELR-SP-FP in peripheral structures upon a 15°C block

COS cells co-expressing KDELR-SP-YFP and VIP36-SP-CFP at 37°C were shifted to 15°C for 2 hours, then direcly fixed in cold methanol for 2 minutes and mounted in PBS for high-resolution imaging. The movie shows the cell in Figure 2D. In the color overlay, VIP36-SP-CFP is in green and KDELR-SP-YFP is in red.

Co-localization is comparable to a cell co-expressing the same protein in different colors, VIP36-SP-CFP and VIP36-SP-YFP, under identical imaging conditions (see below).

Compare_CandYFP.mov

Colocalization of different colors of VIP36-SP-FP co-expressed in the same cell.

Colocalization of VIP36-SP-FP with KDELR-SP-FP in the early secretory pathway (or cargo, see Figure 3) is comparable to co-localization of different colors of the same protein. VIP36-SP-CFP and VIP36-SP-YFP were transiently co-expressed in COS cells, fixed in cold methanol, and imaged in PBS. In the color overlay, VIP36-SP-CFP is in green, and VIP36-SP-YFP is in red.

Figure 3

VIP36-SP-FP colocalizes with cargo blocked early in the secretory pathway at 15°C

COS cells co-expressing VSVG3-SP-YFP secretory cargo and VIP36-SP-CFP were incubated 6 to 12 hours at 39.5°C to accumulate cargo in the ER, then shifted to 15°C for 2 hours in the presence cycloheximide to block cargo in early secretory structures. Cells were then fixed in cold methanol and imaged in PBS. Comparison movies are series of single confocal sections, and cycle between a color overlay showing both proteins, and single channels showing only one protein.

A Comparison_Cargo_15C.mov

Comparison_Cargo_15C_Region.mov

VIP36-SP-FP and VSVG3-SP-FP accumulate together in early secretory structures upon a 15°C block

Both VIP36-SP-FP and secretory cargo localize to the ER network and peripheral punctate structures representing ER exit sites (arrows in Figure **3A**). The movies show the cell and enlarged region (2-fold) from Figure 2A. Images are single confocal slices. In the color overlay, VIP36-SP-CFP is in green, and VSVG3-SP-YFP is in red.

Co-localization is comparable to a cell co-expressing the same protein in different colors, VIP36-SP-CFP and VIP36-SP-YFP, under identical imaging conditions (see above).

B Comparison_Cargo_15C_Close.mov

Comparison_Cargo_15C_Xtalk.mov

High-resolution, close-up view of peripheral ER and pre-Golgi structures containing both VIP36-SP-FP and secretory cargo

Shown is the peripheral region of a COS cell co-expressing VIP36-SP-CFP and VSVG3-SP-YFP after a 15°C block from Figure **3B**. Punctate structures at junctures of the ER network accumulate both VIP36-SP-CFP and VSVG3-SP-YFP. Images are single confocal slices. In the color overlay, VIP36-SP-CFP is in green, and VSVG3-SP-YFP is in red.

Because we occasionally experience crosstalk of YFP signal into the CFP channel, we acquired the CFP channel while illuminating with the same intensity of 514 nm laser light used to image the YFP channel. This experiment shows that in the images in Figure 3 there is no crosstalk of VSVG3-SP-YFP signal into the VIP36-SP-CFP channel.

Co-localization is comparable to a cell co-expressing the same protein in different colors, VIP36-SP-CFP and VIP36-SP-YFP, under identical imaging conditions (see above).

Figure 4

Dynamics of VIP36-SP-GFP in live COS cells

A

Dynamics01.mov

Dynamics02.mov

Dynamics03.mov

Examples of VIP36-SP-GFP dynamics

VIP36-SP-GFP was transiently expressed in COS cells and imaged in the presence of 100µg/ml of cycloheximide at 37°C. Movies are series of single confocal sections at the level of the ER.

Distinct types of trafficking structures are observed: peripheral globular elements (blobs) and tubular elements (tubules). The frequency and behavior of blobs and tubules varies somewhat from cell to cell, the movies show the range of variablility observed. Dynamics01.mov shows a cell in which blobs and tubules are both observed. In Dynamics02.mov blobs are more frequently observed, and in Dynamics03.mov tubules predominate.

Blobs traffic about the cell periphery in a saltatory manner and often appear to migrate towards the Golgi (prominent in Dynamics02.mov). Blob movement may be more apparent at higher frame rates. Tubules behavior is varied: they traffic about the cell periphery, extend from the Golgi and translocate outward, they change shape and length, and often appear to coalesce into and out of the ER network (see Dynamics01.mov and Dynamics03.mov).

Scale bars and frame rates:
Dynamics 01: 10 µm start, 23 µm end, 3.3 s/frame
Dynamics 02: 10 µm start, 19 µm end, 2.3 s/frame
Dynamics 03: 10 µm start, 20 µm end, 2.6 s/frame.

Figure 5

VIP36-SP-FP and secretory cargo traffic together in TCs early in the secretory pathway and separate beyond the Golgi

COS or PtK_2 cells co-expressing VSVG3-FP or VSVG3-SP-FP secretory cargo and VIP36-SP-FP were incubated 6 to 12 hours at 39.5°C to accumulate cargo in the ER, then shifted to 32°C (or 15°C, then 32°C as indicated) in the presence of cycloheximide to initiate transport. Transport proceeded at 32°C for the indicated time. Cells were then fixed in cold methanol or imaged live (starting at the time shown). Movies are series of single confocal sections. Cells are COS cells unless indicated.

A: Comparison of cargo and VIP36-SP-FP distributions in fixed cells at different stages of secretory transport

Time	Comparison
0 min	Cargo_00min.mov
10 min	Cargo_10min.mov
15 min	Cargo_15min.mov
30 min	Cargo_30min.mov
60 min	Cargo_60min.mov

Comparison movies cycle between a color overlay showing both proteins, single channels showing only one protein, and a difference image. Images are high-resolution single confocal slices taken at the level of the ER.

In the color overlays, red or green is assigned to either cargo or VIP36-SP-FP, with overlap in yellow--check the lower right of the color comparisons for the color assignments.

In the difference images, regions with higher VIP36-SP-FP pixel intensities are lighter (pixel values > 128), and regions with higher cargo intensities are darker (pixel values < 128).

After **0 minutes** of transport, (fixation directly from 39.5°C) , VSVG3-FP localizes to the ER, and VIP36-SP-FP distributes between the ER and Golgi as in singly-transfected cells. Apparent overlap in the Golgi region is due to a high density of ER membranes near the Golgi, typical ER morphology in COS cells.

After **10 minutes** of transport at 32°C, cargo localizes to punctate peripheral structures corresponding to pre-Golgi transport carriers (TCs). Most TCs at this time also contain significant levels of VIP36-SP-FP (Figure 6). Cargo has also started to accumulate in the Golgi, and can still be seen in the ER.

After **30 minutes** of transport at 32°C, cargo has accumulated in the Golgi and started to exit. Peripheral punctate structures containing cargo most likely correspond to post-Golgi TCs and a few lagging pre-Golgi TCs. VIP36-SP-FP co-localizes with fewer cargo-containing TCs at this time (Figure 6).

After **60 minutes** of transport at 32°C, cargo has accumulated in the plasma membrane, and the Golgi is depleted. At this time, fewer post-Golgi TCs containing cargo are visible. VIP36-SP-FP distribution appears as in singly-transfected cells, and localizes to peripheral punctate structures, but these structures do not contain significant levels of fluorescent secretory cargo.

Bars: 10 μm.

B: Dynamics of cargo and VIP36-SP-FP in live cells at different stages of secretory transport

Time	Movie	
11 min	Cargo_11min_Grey.mov Cargo_11min_Color.mov	The movie starting at **11 minutes** of transport at 32°C shows VIP36-SP-CFP and VSVG3-YFP together in pre-Golgi TCs (small punctate/globular structures) moving inwards towards the Golgi complex (next to the nucleus). In the color movie, VIP36-SP-CFP is in green, and VSVG3-YFP is in red.
16 min	Cargo_16min_Grey.mov Cargo_16min_Color.mov Cargo_16min_Closeup_Grey.mov Cargo_16min_Closeup_Color.mov	The movies starting at **16 minutes** of transport at 32°C indicates that VIP36-SP-CFP and VSVG3-YFP are together in moving globular and tubular pre-Golgi TCs. This is subtle and perhaps more apparent in the closeup movie (twofold enlarged). VIP36-SP-CFP and VSVG3-YFP are also together in in globular pre-Golgi TCs which remain relatively stationary for the duration of the movie (5 minutes 8 seconds). In the color movie, VIP36-SP-CFP is in green, and VSVG3-YFP is in red.
42 min	Cargo_42min_Grey.mov Cargo_42min_Color.mov	The movies starting at **42 minutes** of transport at 32°C show VSVG3-YFP exiting the Golgi in post-Golgi TCs most of which do not accumulate significant levels of VIP36-SP-CFP. The large, very bright TC to the right of the Golgi (stationary in frames 19-21) appears to contain low levels of VIP36-SP-CFP, however, crosstalk of YFP into the CFP channel can be as high as 4% (Materials and Methods), so the low VIP36-SP-CFP signal in this TC is most likely not significant. Alternatively, since this TC translocates away from the Golgi but then reverses and moves back, so it is possible that this particular structure is not a true post-Golgi TC, but a fragment of Golgi or Golgi-like membranes. In the color movie, VIP36-SP-CFP is in green, and VSVG3-YFP is in red.
56 min	Cargo_56min_Grey.mov Cargo_56min_Color.mov	The movies starting at **56 minutes** of transport at 32°C show VSVG3-YFP trafficking in post-Golgi TCs which do not contain VIP36-SP-CFP. In a PtK_2 cell. The Golgi in PtK_2 cells is typically a set of separated globular elements, rather than the compact structure of COS cells. In the color movie, VIP36-SP-CFP is in red, and VSVG3-YFP is in green.
15°C 2 hrs 32°C 50 min	Cargo_50min_15C_Grey.mov Cargo_50min_15C_Color.mov	The movies starting at **50 minutes** of transport at 32°C after a **2 hour block at 15°C** (to accumulate cargo together with VIP36-SP-FP in pre-Golgi structures) shows a closeup of a Golgi element in a PtK_2 cell repeatedly shedding post-Golgi transport carriers containing VSGS3-CFP but not VIP36-SP-YFP. In the color movie, VIP36-SP-YFP is in red, and VSVG3-CFP is in green; the sequence repeats three times.
		The movies starting at **65 minutes** of transport at 32°C after a **2 hour block at 15°C** (to accumulate cargo together with VIP36-SP-FP in pre-Golgi structures) shows a closeup of the Golgi region in a PtK_2 and a number of post-Golgi transport carriers containing VSGS3-CFP but not VIP36-SP-YFP. By this time, the Golgi is somewhat depleted of cargo. In the color

15°C 2 hrs 32°C 65 min	Cargo_65min_15C_Grey.mov Cargo_65min_15C_Color.mov

movie, VIP36-SP-YFP is in red, and VSVG3-CFP is in green.

Frame rates:
11 min: 11.5 s/frame.
16 min: 8.8 s/frame.
42 min: 9.5 s/frame.
56 min: 8.2 s/frame.
50 min post-15°C block: 4.7 s/frame.
65 min post-15°C block: 6.8 s/frame.

Bars: 10 µm.

Figure 7

Kinetics of VIP36-SP-GFP recycling between the Golgi and ER

BleachControl_Golgi.mov

BleachControl_ER.mov

Orgnanelle-specific bleach on fixed cells

The bleach protocol used in Figure 7 bleaches an entire organelle (either ER or Golgi), so fluorescence can only recover by exchange with the unbleached organelle. COS cells transiently expressing VIP36-SP-GFP were fixed 2 minutes in cold methanol, mounted in PBS in a chamber as for live cells, and either the ER (BleachControl_ER.mov) or Golgi (BleachControl_Golgi.mov) was specifically bleached. The movies show a brightest-point projection every 2 degrees through 10 (ER) or 12 confocal sections taken at 0.5µm.

BleachDepthER

BleachDepthGolgi

Quantitation of orgnanelle-specific bleach parameters

COS cells expressing VIP36-SP-GFP were fixed, mounted in PBS in a live cell-chamber, and then bleached according to the protocol used in Figure 7 and BleachControl_ER.mov and BleachControl_Golgi.mov. A series of confocal sections were collected to measure the fluorescence in the entire depth of the cell, and the relative amounts in the cell, Golgi, and ER were quantitated.

In each figure (ER | Golgi), panel **A** compares fluorescence in the whole cell, ER, and Golgi, before and after the bleach, normalized to the total fluorescence in the cell before the bleach. Only fluorescence in one organelle, either Golgi (Golgi bleach) or ER (ER bleach) is removed by the bleach. Panel **B** compares the ratio of Golgi to ER fluorescence before and after the bleach (normalized to the total fluorescence in the cell).

Error bars indicate the standard deviation of the mean.

AlF4-Treatment.mov

Dynamics of VIP36-SP-GFP after AlF_4^- treatment

COS cells expressing VIP36-SP-GFP were treated with 50µM AlF_4^- for 1 hour. Shown is a movie of single confocal slices of the Golgi region. Central Golgi structures appear depleted, and seem to be closely associated or continuous with the ER. An element enters the Golgi from the left in frames 59 to 65. Frame width 5.2 µm. Frame rate: 2.79 s/frame

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