Membrane Abscission: First Glimpse at Dynamic ESCRTs

Advanced live-cell imaging of the endosomal sorting complexes required for transport (ESCRT) and computational modeling have provided insights into the Vps4-dependent dynamic rearrangements of ESCRT-III filaments during membrane constriction and abscission.

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Distinct biological processes in metazoan cells, like multivesicular body (MVB) formation, final abscission at the end of cytokinesis and retroviral budding, require the function of the endosomal sorting complexes required for transport (ESCRT) machinery (for reviews, see [1,2]). This evolutionarily conserved membrane scission machinery consists of a series of distinct protein complexes (ESCRT-0, -I, -II, -III) that transiently assemble on specific target membranes. Recent work using advanced microscopy approaches and computational modeling [3–7], including a very recent study by Lippincott-Schwartz and colleagues published in Biophysical Journal [5], has provided a first glimpse at the dynamics of the ESCRT machinery.

In the above-mentioned processes, the ESCRT machinery helps to bend membranes away from the cytoplasm until it executes membrane scission. Once their job is done, ESCRT complexes are disassembled and stripped off their target membrane by the AAA-ATPase Vps4, thereby recycling ESCRT components back into the cytoplasm. ESCRT-mediated membrane scission is topologically opposite to ‘conventional’ vesicular transport reactions involving budding and scission of vesicles from a donor membrane into the cytoplasm.

Interestingly, not all ESCRT subunits are equally required for the different biological processes, with the exception of ESCRT-III and Vps4, which appear to be always essential. Homologues of ESCRT-III and Vps4 can be traced back to the archaeal phylum of the crenarchaea, where they function in cell division [8,9]. The assembly of the ESCRT-III complex on giant unilamellar vesicles (GUVs) in vitro is sufficient to drive topologically correct membrane

scission of vesicles into the lumen of GUVs [10]. Hence the ESCRT-III complex and Vps4 seem to represent the minimal components required for membrane scission. Yet, the molecular mechanism underlying their function is still largely unclear.

Advanced imaging technology has now provided insights into the dynamics of the ESCRT-III complex during cytokinesis and HIV budding [3–7]. Both processes are well suited to the study of ESCRT dynamics, since they occur at or near the cell surface and are spatially and temporally restricted, unlike the other above-mentioned ESCRT-dependent process, MVB sorting, which takes place continuously and inside cells.

At the end of cytokinesis, just before two daughter cells separate, CEP55 (centrosomal protein of 55kDa) recruits the ESCRT-I subunit Tsg101 (tumor-susceptibility gene 101) and Alix (apoptosis-linked gene 2-interacting protein X) to the midbody, which is located within the thin membranous stalk that still connects both daughter cells [11]. Tsg101 assembles into large rings with a diameter of approximately 1 \( \mu m \) on each side of the midbody. Subsequently, the ESCRT-III subunits (CHMP6, CHMP4, CHMP3, CHMP2, and CHMP1) form long, ring-like filaments right next to the Tsg101 ring (Figure 1). These filaments seem to be continuously assembled for more than 30 minutes. Compared with HIV budding (see below), the ESCRT-III filaments in cytokinesis exist for a rather long time, maybe due to the larger dimension of the membrane that needs to be constricted. Another ESCRT-III-dependent event that is special during cytokinesis is the recruitment of the AAA-ATPase spastin [12]. The recruitment of spastin to the ESCRT-III filaments by the subunit Chmp1b is required to cleave microtubule bundles just before membrane abscission. Thus, the assembly of ESCRT-III coordinates microtubule severing with membrane abscission at the constriction zone.

Two of the recent studies report that the constriction zone is located approximately 1 \( \mu m \) proximal to the midbody, where ESCRT complexes (Tsg101 and ESCRT-III) first assemble [6,7]. All ESCRT-III subunits and the two AAA-ATPases spastin and Vps4 are required for proper cytokinesis. Advanced microscopy approaches in the new studies have now helped to localize ESCRT components during cytokinesis. ESCRT-III filaments appear to be particularly dynamic. At first, ESCRT-III localizes to the large Tsg101 ring at the midbody, but later on moves about 1 \( \mu m \) towards the constriction zone (Figure 1).

Different models attempt to explain how ESCRT-III filaments move from their initial assembly site to the constriction zone [5–7]. Electron tomography of high-pressure frozen cells revealed cortical 17 nm diameter filaments at the constriction zone that may be ESCRT-III filaments [7]. The ordered polymerization of ESCRT-III subunits could project long ESCRT-III filaments from the first assembly site at the midbody to the constriction zone. These growing ESCRT-III filaments could somehow decrease their diameter and thereby constrict the membrane gradually into the narrow constriction zone. In an alternative model, Vps4-mediated remodeling or breakage of the first ESCRT-III ring could generate a second, smaller ESCRT-III scission complex that migrates towards the constriction zone. Computational modeling predicts that spontaneous conformational rearrangements of the released ESCRT-III filament into a scission complex with a smaller diameter (of approximately 50 nm) would generate membrane elastic forces, which would push the contracting ESCRT-III scission complex to a distance of almost exactly 1 \( \mu m \) from the midbody [5,6].

In both of the above scenarios, the intrinsic properties of the ESCRT-III subunits may dictate a restricted geometry (diameter or length) of the filament that would inflict the force required to bend and ultimately drive scission of the bound membrane. Consistent with this idea, different ESCRT-III subunits oligomerize into very distinct tubular structures or filaments in vitro. Chmp3 and Chmp2

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assemble into hollow tubes with a diameter of 50 nm, capped on one end by a dome-like structure, which has been proposed to function as the scission complex \[13,14\]. Chmp4 homopolymerizes into rings, sheets and filaments and Chmp3 self-assembles into filaments \[15\]. Finally, Chmp1 forms large, dome-capped tubes of 230 nm diameter \[16\]. In addition, overexpressed Chmp4 can assemble into circular arrays at the plasma membrane of cells. When bound by dominant-negative Vps4, these ESCRT-III rings change into hollow tubes with a diameter of approximately 100 nm that protrude away from the cytoplasm \[17\]. Hence it appears likely that the ordered assembly of ESCRT-III subunits in vivo may indeed result in the formation of ever-constricting ESCRT-III filaments. During cytokinesis, long ESCRT-III filaments assemble at the midbody (diameter 1 μm) for more than 30 minutes. During HIV budding, shorter ESCRT-III filaments assemble for a few minutes in the membrane stalk (diameter 50–100 nm) connecting the nascent virus to its host cell. In both cases, Vps4 disassembles/remodels the ESCRT-III filaments.

findings are largely consistent with the idea that ESCRT-III, together with Vps4, is required for membrane abscission. Interestingly, it seems that ESCRT-III and Vps4 become trapped in the midbody remnant, indicating that, during cytokinesis, Vps4 and ESCRT-III are not recycled back to the cellular ESCRT pool. Hence, the role of Vps4 in cytokinesis may be limited to the remodeling of the ESCRT-III complex and to membrane shaping and scission itself.

During HIV budding, ESCRT dynamics appear to be quite different from those during cytokinesis. In the other two recent studies \[3,4\], total internal reflection fluorescent microscopy allowed for imaging of the dynamics of the ESCRT machinery at the site of viral budding. Tsg101 and Alix are continuously recruited to the nascent virus via Gag, the main structural coat protein of HIV-1. Later, when Gag assembly is complete, ESCRT-III and Vps4 are recruited in rather short pulses (of a few minutes) to the budding sites, just prior to the release of the maturing viral particle. In contrast to cytokinesis or MVB sorting, only two ESCRT-III subunits (Chmp4 and Chmp2) are required for HIV budding \[18\]. When ESCRT-III filaments are not fully assembled (i.e. following depletion of Chmp4 or Chmp2), viral particles cannot be released from the cell surface. Similarly, defects in ESCRT-III disassembly/remodeling, via expression of a dominant-negative Vps4, block virus release. In these cases, ring-like structures, most likely ESCRT-III filaments, accumulate inside the 50–100 nm diameter membrane stalk connecting the budding virus to the surface of the host cell. Upon successful membrane scission, Vps4 efficiently removes ESCRT-III subunits from the viral budding site.

Despite the important function of Vps4, its mechanistic role during membrane scission remains controversial. Vps4 might simply strip ESCRT-III off the membrane. Alternatively, it might progressively remodel ESCRT-III in an ordered disassembly reaction. Either way, advanced microscopy approaches (live-cell imaging, super-resolution
microscopy and electron microscopy/tomography) in combination with computational modeling have now provided strong evidence that the processing/remodeling of ring-like ESCRT-III filaments by Vps4 is a prerequisite for membrane scission in cytokinesis. It is tempting to speculate that the obvious differences in ESCRT-III dynamics might reflect distinct levels of spatiotemporal regulation of Vps4 function, from simple ESCRT-III disassembly and recycling in HIV budding and MVB sorting, to a more defined ESCRT-III filament remodeling during cytokinesis.

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Blindsight: Spontaneous Scanning of Complex Scenes

A new study of monkeys with blindsight has provided some surprising new insights into the visual properties that remain salient without a functioning primary visual cortex and may help improve rehabilitation strategies used with human hemianopes.

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Some visual abilities can be spared after lesions to primary visual cortex (area V1), a phenomenon known as blindsight [1]. Humans with blindsight deny consciously seeing visual stimuli even when they discriminate their properties accurately. Blindsight has almost exclusively been studied by presenting simple stimuli to the affected visual fields of humans or monkeys and prompting them to respond to these with a cue. A new study reported in this issue of Current Biology [2] takes a different approach. Instead of using simple stimuli and cues, Yoshida et al. [2] measured the patterns of eye-movements made by monkeys with blindsight made spontaneously as they viewed movie clips of natural scenes. This novel approach has yielded some surprising findings about the relative salience of color and other visual features in blindsight. It also demonstrates that monkeys with lesions to primary visual cortex respond spontaneously to visual events in the blind portions of their visual fields — something that may have important implications for the design of rehabilitation programmes for human patients who have visual field loss as a result of damage to primary visual cortex.

In the new study [2], the patterns of eye-movements made by the monkeys were analysed in relation to a model that predicts the salience of different visual features — color, orientation, luminance and motion — on the basis of the anatomy and neurophysiology of the early visual system [3]. The locations and order of fixations of gaze as visual stimuli are scanned are predicted on the basis of calculations of the spatial distribution of neural activation in the model. The visual properties that remain salient in blindsight were estimated by adjusting the range of visual properties and the weighting given to their processing in the model so as to match the behavior predicted by the model with the monkeys' eye-movements.

In this work [2], the monkeys’ primary visual cortex was only lesioned in one cerebral hemisphere, permitting comparison between responses to stimuli in the unaffected and blind visual fields. The extent to which motion and color attracted gaze remained similar between visual fields, while the impacts of luminance and of orientation increased and decreased, respectively. Perhaps the most surprising of these findings is the resilience of the response to color. The neural pathway originating in the P-beta ganglion cells in the retina that conveys color information degenerates after lesions to primary visual cortex [4]. This degeneration is not, however,