CELL BIOLOGY

# No ESCRTs for Exosomes

Two pathways within endosomes use specific protein complexes or membrane domains to direct cargo for degradation or secretion from cells.

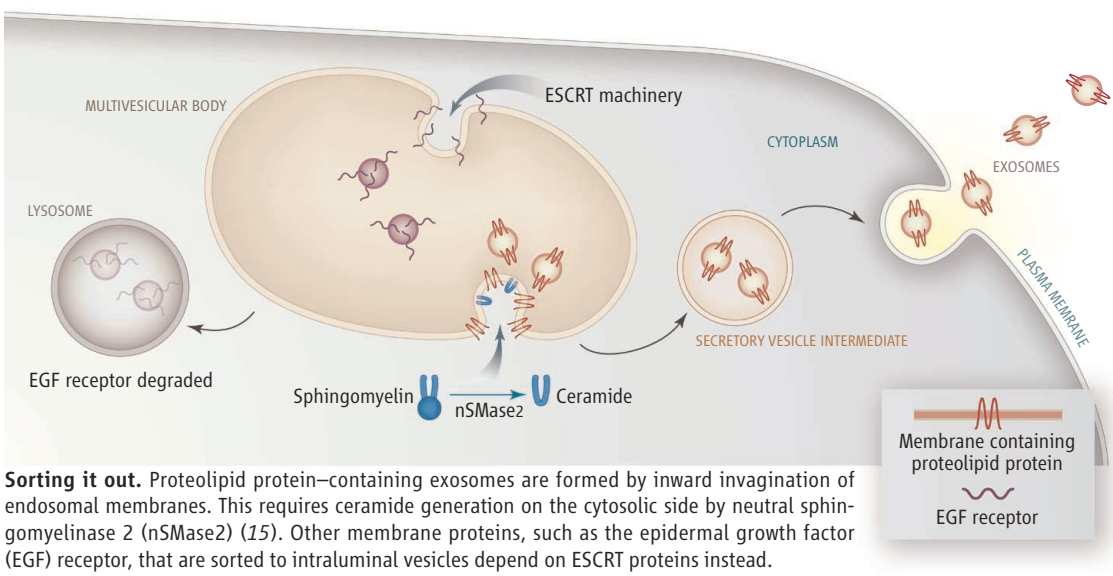
**Mark Marsh[1] and Gerrit van Meer[2]**

Exosomes are small (50 to 100 nm in diameter) membrane-bound vesicles released by a variety of cells. Originally proposed to discard excess transferrin receptor from reticulocytes during red blood cell formation (*1*), exosomes are now thought to play key roles in cell-to-cell communication, antigen presentation, and in the pathogenesis of retroviral infections (including HIV) and prion diseases (*2*–*4*). However, how exosomes are formed has not been clear. On page 1244 in this issue, Trajkovic *et al*. (*5*) provide intriguing insights into exosome formation, making these microvesicles a bit less mysterious but raising many new questions about their biogenesis.

An early view was that exosomes are formed by invagination of the membrane of endosomes (see the figure) to produce intraluminal vesicles, thus rendering these organelles multivesicular bodies (*6*, *7*). Exosomes are then secreted when these multivesicular bodies fuse with the plasma membrane and release their content (*6*). A more recent view holds that exosomes can also form at the plasma membrane in some cell types (*8*). The link between exosomes and multivesicular bodies was strengthened by the discovery of the ESCRT (endosomal sorting complex required for transport) machinery (*9*). This highly conserved set of protein complexes recognizes membrane proteins that are modified with ubiquitin molecules and thus marked for sorting to lysosomes (either as functional components of lysosomes or as substrates for lysosomal proteolysis). ESCRT complexes sort these cargoes to specific domains of endosomes and regulate both the inward invagination of these membrane regions and the scission of invaginated membrane buds to form intraluminal vesicles. Cells that lack components of the ESCRT machinery often have fewer multivesicular bodies or fewer intraluminal vesicles in multivesicular bodies, and fail to deliver cargo to lysosomes (*7*). The ESCRT machinery is also required to complete the topologically related (budding of membrane vesicles away from the cytoplasm) assembly of various enveloped RNA viruses (*10*), including HIV, and to mediate the abscission reactions that complete mammalian cell division (*11*).

It was thus not unreasonable to conjecture that the ESCRT machinery would also be involved in the similar process of exosome formation. Indeed, an ESCRT-associated protein (AIP1/Alix) interacts with transferrin receptors during exosome formation in reticulocytes (*12*). ESCRT proteins are also recruited to proposed sites of exosome formation in lymphocytes (*8*) and are found in exosomes (*3*, *5*). A regulatory role in intraluminal vesicle formation was also suggested based on the inhibition of inward budding in liposomes by the ESCRT protein Alix (*13*). Nevertheless, the role of the ESCRT machinery in exosome formation has remained unclear.

Trajkovic *et al*. used an oligodendrocyte cell line (myelinating cells of the central nervous system) to study the formation and release of exosomes containing proteolipid protein. Proteolipid protein is a major component of myelin, the lipid-rich membrane that oligodendrocytes use to enwrap and insulate axons. They find that formation of proteolipid protein–containing exosomes does not require ESCRT machinery. By contrast, sorting of the epidermal growth factor receptor to lysosomes in these cells is inhibited by depletion of ESCRT components or expression of a dominant-negative form of an ESCRT protein (Vps4).




**Sorting it out.** Proteolipid protein–containing exosomes are formed by inward invagination of endosomal membranes. This requires ceramide generation on the cytosolic side by neutral sphingomyelinase 2 (nSMase2) (*15*). Other membrane proteins, such as the epidermal growth factor (EGF) receptor, that are sorted to intraluminal vesicles depend on ESCRT proteins instead.

Morphological analysis of the oligodendrocyte endosomes shows that proteolipid protein segregates into membrane domains that are distinct from domains containing cargo destined for ESCRT-mediated sorting to lysosomes. Trajkovic *et al*. show through mass spectrometric analysis that secreted proteolipid protein–containing exosomes purified from cell culture medium are enriched in ceramide, a lipid produced from the membrane lipid sphingomyelin by sphingomyelinases. Disrupting the expression of neutral sphingomyelinase 2 (nSMase2) by RNA interference or the use of specific inhibitors reduced secretion of proteolipid protein–containing exosomes. Moreover, when Trajkovic *et al*. added a bacterial sphingomyelinase to liposomes containing domains with different degrees of fluidity, budding occurred specifically from the "raft"-like lipid phase. This led them to

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Published by AAAS

suggest that ceramide-induced aggregation of lipid microdomains leads to domain-induced inward budding of intraluminal vesicles, perhaps promoted by the cone-shaped structure of ceramide (see the figure).

The observations of Trajkovic *et al*. raise several questions. Morphological experiments indicate that both proteolipid protein–containing exosomes and epidermal growth factor receptor–containing intraluminal vesicles can be formed within the same endosome. Is this the case, or are there functionally distinct populations of endosomes that generate different intraluminal vesicles (*14*)? Also, if both types of vesicles are present in the same multivesicular body, they must somehow be sorted to ensure that only the exosomes are secreted. It is also not clear whether all exosomes are formed through the same molecular mechanism, or if different mechanisms are used for different types of exosome cargo. The Trajkovic *et al*. study shows that secretion of the tetraspanin CD63, another exosome-associated membrane protein, is also blocked by a sphingomyelinase inhibitor, but not by a dominant-negative ESCRT component. If the ceramide-based process is the primary mechanism for exosome formation, it would seem that ESCRT-dependent enveloped viruses have usurped the lysosomal sorting and abscission machinery for assembly, though the idea that the budding of some viruses involves raft domains could also indicate that a combination of the two processes is used.

The presence of ceramide in exosomes may imply its direct role in the lipid-phase organization of the endosomal membrane, whereby the ceramide-enriched phase ends up in the budding vesicle. This is supported by the presence of proteolipid protein—a typical membrane raft component—in exosomes. However, without knowing the lipid composition of the endosomal membrane, one cannot conclude that exosomes originate from a specific membrane domain. Also, without knowing the transbilayer organization or ceramide concentration in the endosomal membrane, the extrapolation of model membrane experiments remains problematic. Whatever the molecular mechanism by which a change in lipid composition drives vesicle budding, the process is likely to be regulated. The present work suggests that a better understanding of lipid metabolism may provide new vistas in exosome research.

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10.1126/science.1155750

MATERIALS SCIENCE

# New Materials at a Glance

Depositing materials on a substrate at a glancing angle can create new and unusual structures.

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Through precise engineering of structure on the nanoscale, researchers can endow a material with remarkably different properties from those of the bulk. A major effort in nanotechnology research is the development of a toolbox of processing techniques for nanostructure fabrication. Recently, materials scientists have looked at glancing angle deposition (GLAD) as an important tool for this task. In this technique, materials are deposited on surfaces at a highly oblique angle, resulting in a flexible and straightforward method of producing nanostructured and porous thin-film materials in unusual configurations.

GLAD is a combination of traditional thin-film vacuum deposition and carefully controlled substrate motion (*1*). During standard film deposition, a stream of vapor-phase atoms strikes a perpendicular substrate. In GLAD, the substrate is tilted far from perpendicular, such that the atoms arrive obliquely. As the atoms condense on the substrate, they agglomerate into microscopic clumps or nucleation sites. Line-of-sight shadowing prevents atoms from condensing in the region immediately behind each nucleus (see the figure, top panel); thus, atoms deposit only on the tops of nuclei (*2*). As deposition continues, the nuclei develop into columnar structures that are oriented toward the vapor source. Increasing the substrate tilt leads to greater separation between columns and a more porous structure.

We can add a new degree of control by rotating the substrate, which changes the apparent location of the vapor source from the perspective of the growing columns. Because the columns grow toward the vapor source, the growth direction of the columns can be controlled. Using the appropriate substrate movements, we can sculpt the columns into different geometries such as chevrons (abrupt 180° rotations), helices (slow continuous rotation), and vertical posts (rapid continuous rotation).

The ability to control the column orientation throughout the thickness leads to a particularly interesting application of GLAD. A tilted column will exhibit optical birefringence: Incident light will experience a different refractive index depending on whether the light is polarized parallel or perpendicular to the column axis (*3*, *4*). Because the optical properties are linked to the orientation of the columns, sculpting the columns into helical shapes will cause the optical properties to vary periodically throughout the thickness of the film. Light will therefore see a periodic medium, creating a polarization-dependent optical diffraction effect analogous to that seen in some liquid crystals (*5*, *6*). Circularly polarized light of the same handedness as the structure will be reflected, whereas the other circular polarization state will pass through. This polarization discrimination acts as a circular polarization filter and could be implemented in display technologies. For such technology to be adopted, efficient polarizers must be developed. Toward this goal, Hodgkinson *et al.* have worked to maximize the birefringence of columnar structures and improve the optical performance of helical films (*7*, *8*).

The ability to create separated columnar

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