Biogenesis of extracellular vesicles in yeast

Many questions with few answers

Débora L. Oliveira,1 Ernesto S. Nakayasu,2,2 Luna S. Joffe,1 Allan J. Guimarães,3 Tiago J.P. Sobreira,4 Joshua D. Nosanchuk,5,5 Radames J.B. Cordero,2 Susana Frases,6 Arturo Casadevall,3,5 Igor C. Almeida,2 Leonardo Nimrichter1 and Marcio L. Rodrigues1,*

1Laboratório de Estudos Integrados em Bioquímica Microbiana; Instituto de Microbiologia Professor Paulo de Góes; Rio de Janeiro; Brazil; 2The Border Biomedical Research Center; Department of Biological Sciences; University of Texas at El Paso; El Paso, TX USA; Departments of 3 Medicine and 3 Microbiology and Immunology; Albert Einstein College of Medicine; Bronx, New York USA; 4Laboratory of Genetics and Molecular Cardiology; Heart Institute (InCor); University of Sao Paulo; Sao Paulo, SP Brazil; 5Laboratório de Biotecnologia; Instituto Nacional de Metrologia; Normalização e Qualidade Industrial; Rio de Janeiro, Brazil

*Correspondence to: Marcio L. Rodrigues; Email: marcio@micro.ufrj.br

The cellular events required for unconventional protein secretion in eukaryotic pathogens are beginning to be revealed. In fungi, extracellular release of proteins involves passage through the cell wall by mechanisms that are poorly understood. In recent years, several studies demonstrated that yeast cells produce vesicles that traverse the cell wall to release a wide range of cellular components into the extracellular space. These studies suggested that extracellular vesicle release involves components of both conventional and unconventional secretory pathways, although the precise mechanisms required for this process are still unknown. We discuss here cellular events that are candidates for regulating this interesting but elusive event in the biology of yeast cells.

Protein secretion is a widely studied cellular phenomenon. To reach the extracellular milieu, intracellularly synthesized proteins are targeted to the cell surface for release to the extracellular space. In mammalian cells, the plasma membrane is the final barrier to be crossed during secretion. Such processes, which involve both conventional and unconventional mechanisms, have been studied in detail and a number of excellent reviews are available in the literature.1–6

Secretory systems in microbes and mammalian cells show points of convergence and divergence.7 Fungi and procaryotes are surrounded by thick cell walls, a key difference in comparison with mammalian and other eukaryotic cells (e.g., protozoa) that adds significant complexity to secretion systems in these organisms. A number of mechanisms have been proposed for the trans-cell wall molecular transport in procaryotes.8 In fungi, however, the mechanisms required for passage of molecules across the cell wall are poorly understood. Recently, extracellular vesicle release has been described as a mechanism used by yeast cells to secrete many molecules across the cell wall.9–12

Extracellular vesicles produced by fungal cells share morphological and biochemical similarities with mammalian exosomes,13,14 including an ability to modulate the function of immune cells.15 Plant cells also produce exosome-like vesicles,16 supporting the notion that vesicular release is a mechanism of trans-cell wall passage shared by cell-wall containing eukaryotes. In contrast to what is observed for mammalian exosomes,17 the pathways required for extracellular vesicle biogenesis and release in both plant and fungal cells remain virtually unknown. One remarkable feature of mammalian exosomes and fungal extracellular vesicles is the abundance of cytoplasmic proteins lacking a signal peptide that directs proteins to the endoplasmic reticulum in conventional secretory processes.13,14,16–20

Key words: secretion, extracellular vesicles, exosome, trans-cell wall transport, yeast box

Submitted: 06/21/10
Accepted: 06/21/10
Previously published online: www.landesbioscience.com/journals/cib/article/12756
DOI: 10.4161/cib.3.6.12756

Addendum to: Oliveira DL, Nakayasu ES, Joffe LS, Guimaraes AJ, Sobreira TJP, Nosanchuk JD, et al. Characterization of yeast extracellular vesicles: evidence for the participation of different pathways of cellular traffic in vesicle biogenesis. PLoS ONE 2010; 5:11113; PMID: 20559436; DOI: 10.1371/journal.pone.0011113.

©2010 Landes Bioscience

Addendum to: Oliveira DL, Nakayasu ES, Joffe LS, Guimaraes AJ, Sobreira TJP, Nosanchuk JD, et al. Characterization of yeast extracellular vesicles: evidence for the participation of different pathways of cellular traffic in vesicle biogenesis. PLoS ONE 2010; 5:11113; PMID: 20559436; DOI: 10.1371/journal.pone.0011113.
In a recent study, we evaluated the contribution of both conventional and unconventional pathways of secretion in the model yeast *Saccharomyces cerevisiae*. Our approach was based on the study of mutants with defects in two major secretion pathways: conventional post-Golgi secretion and exosome formation, a mechanism of unconventional secretion. The use of this model was based on the facts that: (1) genes required for conventional, post-Golgi secretion were implicated in the formation of extracellular vesicles in fungi; and (2) exosomes and fungal vesicles share many similarities.

Defects in the formation of multivesicular bodies (MVB) are expected to directly affect the formation of exosomes. Surprisingly, yeast mutants with defects in MVB formation produced similar fractions of the proteins (75%) found in vesicular structures outside the cell. In our analyses, however, yeast mutants lacking Sec4p, a secretory vesicle-associated Rab GTPase essential for Golgi-derived exocytosis, had reduced kinetics of vesicle release to the extracellular milieu. The fact that cells with defects in a post-Golgi event of secretion, but not with disturbed MVB formation, affected vesicle release raised an obvious and still unanswered question: how is a double layered vesicle secreted from yeast cells?

The simplest and more tangible explanation for the release of any extracellular vesicle is the fusion of MVB with the plasma membrane. However, studies by our group clearly show that double-layered vesicles can bud from the plasma membrane of yeast cells (Fig. 1). Therefore, one could speculate that proteins required for post-Golgi conventional secretion could be required for addressing vesicle components to the plasma membrane. Vesicles would then be formed by membrane budding and sequential transfer to the cell wall and extracellular space. That would be consistent with previous hypotheses raising the possibility that formation of extracellular vesicles can involve membrane budding. Budding from the plasma membrane would also be in line with the complex vesicle composition including cytoplasmic elements, as observed in our analyses. It remains unknown how these vesicles traverse the cell wall, but many cell wall degrading enzymes were observed in extracellular vesicle samples obtained from *S. cerevisiae* cultures. We hypothesize that these enzymes could hydrolyze cell wall components to facilitate vesicle passage through this cellular barrier.

The methods currently used for vesicle purification do not discriminate between vesicles of different origins. This implies that heterogeneous preparations are obtained during vesicle isolation. In this context, the possibility that the collection of mutations analyzed in our recent study is affecting different types of vesicles cannot be ruled out. The current knowledge on how fungal extracellular vesicles are formed, in fact, strongly suggests the involvement of multiple—and perhaps still unknown—pathways of secretion. As recently described in independent studies, unconventional protein secretion can also involve autophagosomes, which are intracellular structures whose functions were initially attributed to many catabolic steps.

In autophagy, cytosolic material is sequestered by an expanding membrane compartment, the phagophore, resulting in the formation of a double-membrane vesicle, the autophagosome. Autophagosomes then fuse with the lysosome/vacuole where, as initially supposed, the sequestered material is degraded. However, yeast cells can also direct the autophagic content for secretion, in a process called exophagy. In fact, the autophagic machinery participates in the packaging and delivery of the soluble yeast protein acyl-Coenzyme A-binding protein Acb1 to the cell surface. Therefore, these studies suggest the existence of a vesicular mechanism that utilizes the same machinery for both secretion and degradation of cellular components. It is interesting to note that secretion of Acb1 from yeast as well as secretion of the *Dictyostelium discoideum* Acb1 homologue, AcbA, depends on the Golgi associated protein GRASP, which is apparently required for extracellular vesicle release in yeast cells. These observations add to an already long list of candidates that can regulate vesicle formation in yeast cells.

After our initial description of fungal extracellular vesicles in 2007, eight different studies showing their functions in fungal physiology or pathogenesis have been reported in the literature. Vesicle...
release has been associated to protein and polysaccharide secretion,12-14,22,32 surface architecture,13 virulence,10,12,13 pigmentation13 and modulation of macrophage function.15 Despite their apparent multiple functions in yeast, the cellular components controlling their biogenesis and release remain elusive. We emphasize the supposition that the methods currently used for preparation of extracellular fractions containing vesicles may co-isolate vesicular compartments of different cellular origins, which limit the application of studies based on the generation of punctual mutations. Post-Golgi components required for conventional secretion, proteins involved in MVB formation, GRASP and even autophagy-related events may be involved in the formation of extracellular vesicles. Although much progress has been made in the last three years, the route to understand how fungal extracellular vesicles are formed still seems long and laborious.

Acknowledgements
D.L.O. is a Ph.D. student at Instituto de Bioquímica Médica (Federal University of Rio de Janeiro, Brazil); L.N. and M.I.R. are supported by grants from the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). A.C. is supported by NIH awards HL059842, AI033774, AI052733, AI033142. A.G.J. and L.N. were supported in part by an Interhemispheric Research Training Grant in Infectious Diseases, Fogarty International Center (NIH D43-TW007129). J.D.N. is supported in part by NIH AI52733-06A1 and AI056070-01A2 and a Hirsch/Weill-Caulier Career Scientist Award. R.J.B.C. was supported by the Training Program in Cellular and Molecular Biology and Genetics, T32 GM007491. I.C.A. was partially supported by the NIH/NCRR grant 5G12RR008124-16A1 and 5G12RR008124-16A1S1. E.S.N was partially supported by the George A. Krutilek memorial graduate scholarship from Graduate School, University of Texas at El-Paso (UTEP). T.J.P.S. was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil. We thank Jorge J. Jó Bastos Ferreira for his invaluable suggestions on the role of vesicles in fungal physiology. We are also grateful to the Biomolecule Analysis Core Facility, Border Biomedical Research Center, UTEP, funded by NIH/NCRR grant 5G12RR008124-16A1 and 5G12RR008124-16A1S1, for continuous access to mass spectrometry (LC-MS and GC-MS) instruments, which have been fundamental for several of the studies described here.

References
1. Jerome A. Cell secretion: an update. J Cell Mol Med 2008; 12:1151-4.
2. Lee MC, Miller EA, Goldberg J, Orci L, Schekman R. Bi-directional protein transport between the ER and Golgi. Annu Rev Cell Dev Biol 2004; 20:87-123.
3. Levi SK, Glick BS. GRASPing unconventional secretion. Cell 2007; 130:407-9.
4. Nickel W, Rabouille C. Mechanisms of regulated unconventional protein secretion. Nat Rev Mol Cell Biol 2009; 10:148-55.
5. Herzmann JM, Malikus P, Schekman R. Our of the ER—outfitters, escortors and guides. Trends Cell Biol 1999; 9:5-7.
6. Schekman RW. Regulation of membrane traffic in the secretory pathway. Harvey Lect 1984; 90:45-57.
7. Rohnblatt J, Schekman R. A hitchhiker’s guide to post-Golgi components required for vesicle biogenesis. PLoS ONE 2010; 5:11113.
8. Panepinto J, Komperda K, Frases S, Park YD, Djordjevic JT, Casadevall A et al. Sec6-dependent sorting of fungal extracellular exosomes and lack of Cryptococcus neoformans. Mol Microbiol 2009; 71:1165-76.
9. Valadi H, Ektrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of generic exchange between cells. Nat Cell Biol 2007; 9:654-9.
10. Oliveira DL, Nakayasu ES, Joffe LS, Guimaraes AJ, Sobreira TJP, Nosanchuk JD et al. Characterization of yeast extracellular vesicles: evidence for the participation of different pathways of cellular traffic in vesicle biogenesis. PLoS ONE 2010; 5:11113.
11. Panepinto J, Komperda K, Frases S, Park YD, Djordjevic JT, Casadevall A et al. Sec6-dependent sorting of fungal extracellular exosomes and lack of Cryptococcus neoformans. Mol Microbiol 2009; 71:1165-76.
12. Rodriguez ML, Nimrichter L, Oliveira DL, Nosanchuk JD, Casadevall A. A role for vesicular transport of macromolecules across cell walls in fungal pathogenesis. Commun Integ Biol 2008; 1:377-9.
13. Rodriguez ML, Nimrichter L, Oliveira DL, Nosanchuk JD, Casadevall A. Vesicular transport across the fungal cell wall. Trends Microbiol 2009; 17:158-62.
14. Rodrigues ML, Nimrichter L, Oliveira DL, Frases S, Miranda K, Zaragoza O et al. Vesicular polysaccharide export in Cryptococcus neoformans is a eukaryotic solution to the problem of fungal trans-cell wall transport. Eukaryot Cell 2007; 6:48-59.
15. Rodrigues ML, Nakayasu ES, Oliveira DL, Nimrichter L, Nosanchuk JD, Almeida IC et al. Extracellular vesicles produced by Cryptococcus neoformans contain protein components associated with virulence. Eukaryot Cell 2008; 7:58-67.
16. Albuquerque PC, Nakayasu ES, Rodrigues ML, Frases S, Casadevall A, Zancope-Oliveira RM et al. Vesicular transport in Histoplasma capsulatum: an effective mechanism for trans-cell wall transfer of proteins and lipids in ascomycetes. Cell Microbiol 2008; 10:1695-710.
17. Oliveira DL, Freire-de-Lima CG, Nosanchuk JD, Casadevall A, Rodrigues ML, Nimrichter L. Extracellular vesicles from Cryptococcus neoformans modulate macrophage functions. Infect Immun 2010; 78:1660-7.
18. Regente M, Corti-Monzon G, Maludanho AM, Pinedo M, Jornin J, de la Canal L. Vesicular fractions of sunflower apoplastic fluids are associated with potential exosome marker proteins. FEBS Lett 2009; 583:3363-6.