Extracellular release of vesicles in yeast requires multiple pathways of secretion

Luna Sobrino[1], Débora L. Oliveira[1], Ernesto S. Nakayasu[2], Igor C. Almeida[2], Arturo Casadevall[3], Leonardo Nimrichter[1], Marcio L. Rodrigues[1]

[1]Instituto de Microbiologia Professor Paulo de Góes; Universidade Federal do Rio de Janeiro; Rio de Janeiro; Brazil, [2]Department of Biological Sciences, University of Texas at El Paso, El Paso, TX, USA. [3]Albert Einstein College of Medicine; Yeshiva University; New York; USA.

Recent studies demonstrated that fungal cells excrete macromolecules through the extracellular release of secretory vesicles. Although vesicle components have been characterized in some species, the mechanisms involved in the biogenesis of extracellular vesicles remain unknown. In this study, extracellular vesicles produced by the model yeast *Saccharomyces cerevisiae* were screened for the presence of makers of biogenesis. The *S. cerevisiae* cells used in this work included a standard wild type strain and a temperature-sensitive mutant with defective production of sec4p, a small GTPase required to Golgi to surface vesicular transport. Both wild type and *sec4* mutant cells produced extracellular vesicles, as demonstrated by transmission electron microscopy and thin layer chromatography of vesicle lipid extracts. Lipid analysis and flow cytometry suggested that the *sec4* mutant manifests a reduced ability to release extracellular vesicles. Proteomic analysis of the extracellular vesicles revealed the presence of several secretion-related proteins, including Golgi and endoplasmic reticulum markers. Vesicles from mutant and wild type cells showed a generally similar protein composition, although thirty proteins were exclusively detected in wild type cells. In summary, these results demonstrate that production of extracellular vesicles in yeast partially requires Golgi-derived components of the secretory pathway. However, the detection of extracellular vesicles in preparations of a *sec4* mutant reveals that additional routes are necessary for the production of these secretory compartments in yeast.