Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The TORC1–Nem1/Spo7–Pah1/lipin axis regulates microautophagy induction in budding yeast

Muhammad Arifur Rahman [a], Mashu Terasawa [b], Md. Golam Mostofa [a], Takashi Ushimaru [a, b, *]

[a] Graduate School of Science and Technology, Shizuoka University, Ohya 836, Suruga-ku, Shizuoka, 422-8021, Japan
[b] Department of Science, Shizuoka University, Ohya 836, Suruga-ku, Shizuoka, 422-8021, Japan

ARTICLE INFO

Article history:
Received 21 August 2018
Accepted 2 September 2018
Available online xxx

Keywords:
Lipin
Microautophagy
Nem1
Pah1
Rapamycin
Target of rapamycin complex 1 (TORC1)

ABSTRACT

Nutrient starvation and inactivation of target of rapamycin complex 1 (TORC1) protein kinase promotes macroautophagy. Macroautophagy is a lipid-consuming process, and Nem1/Spo7 protein phosphatase and Pah1/lipin phosphatidate phosphatase are activated after TORC1 inactivation, supporting macroautophagy induction in the budding yeast *Saccharomyces cerevisiae*. On the other hand, whether and how microautophagy, which also consumes lipids, is regulated by TORC1 is controversial. Here we show that TORC1 inactivation induces microautophagy in budding yeast. Vps27, but not Atg1, Atg7, or Atg8, was required for TORC1 inactivation-induced microautophagy. Furthermore, the Nem1/Spo7–Pah1 axis was also critical for microautophagy induction. Thus, the TORC1–Nem1/Spo7–Pah1 axis is a master regulator of not only macroautophagy but also microautophagy in budding yeast.

1. Introduction

Macroautophagy degrades cytoplasmic components and organelles in lysosomes/vacuoles [1,2]. Newly generated isolation membranes encapsulate cellular constituents to form double membrane-surrounded autophagosomes. Subsequently, autophagosomes fuse with lysosomes/vacuoles, and the cargoes are digested by lysosomal/vacuolar hydrolytic enzymes. Nutrient starvation and inactivation of target of rapamycin complex 1 (TORC1) protein kinase invoke macroautophagy [3]. By contrast, microautophagy degrades cargos by direct lysosomal/vacuolar engulfment of the cytoplasmic cargo without the formation of isolation membranes and autophagosomes, unlike macroautophagy [4–6]. Microautophagy can selectively degrade various specific cargos, such as the nucleus (micronucleophagy or piecemeal microautophagy of the nucleus) and lipid droplets (microlipophagy) in the budding yeast *Saccharomyces cerevisiae* [7,8].

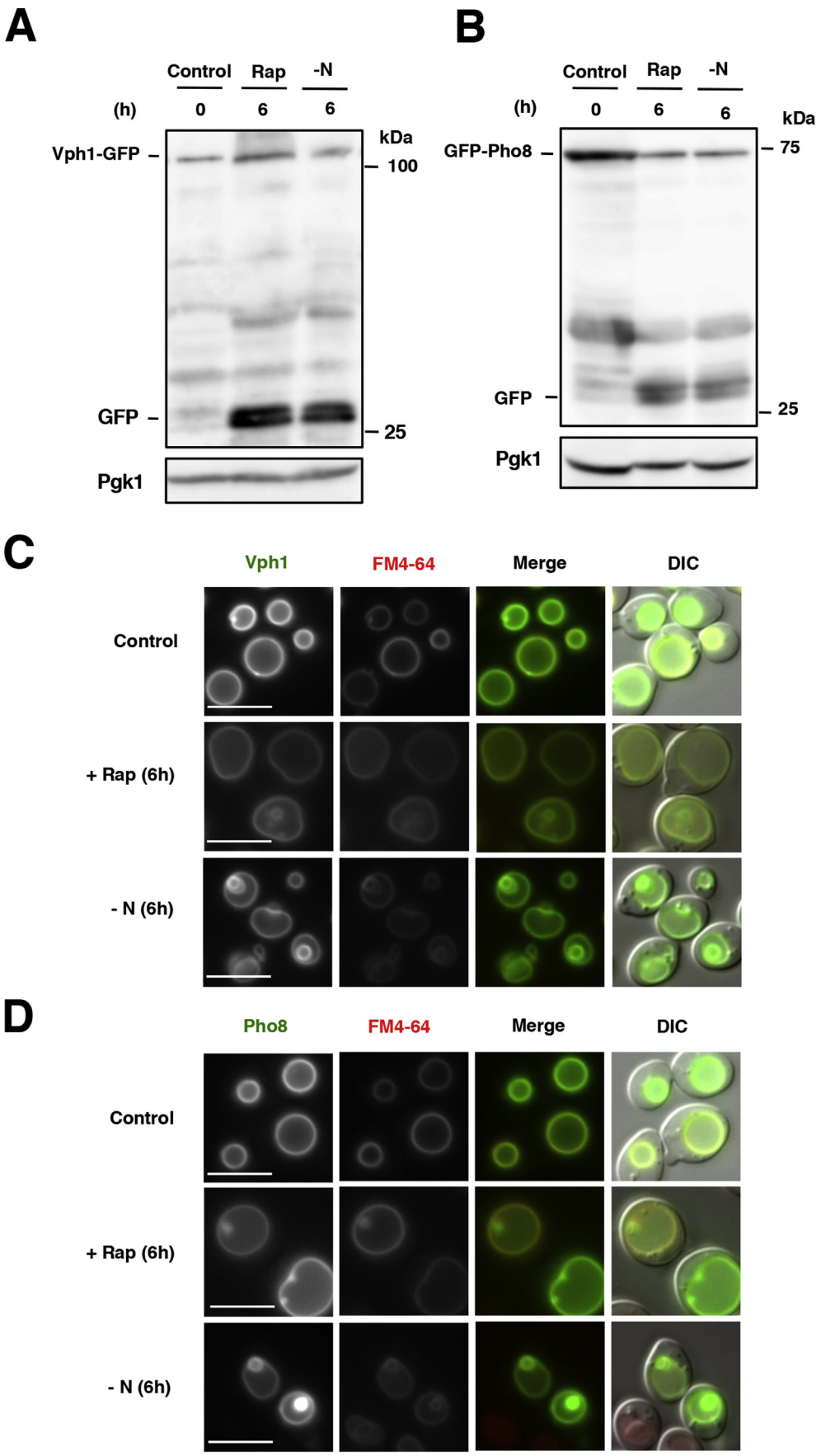
Overall macroautophagy flux is estimated by green fluorescent protein (GFP)-Atg8 processing [9], whereas similar estimation of overall microautophagy flux had not been available so far. More recently, a method to estimate overall microautophagy flux using GFP-tagged vacuolar transmembrane protein Vph1 and Pho8 was developed [10]. This method is based on the fact that vacuolar membrane proteins together with vacuolar membranes are degraded in the vacuole in the course of microautophagy, but not during macroautophagy. When Vph1-GFP and GFP-Pho8 are incorporated into the vacuole by microautophagy, Vph1 and Pho8, but not a stable GFP moiety, are degraded by vacuolar proteases, producing free GFP, which is detectable by immunoblotting. Using this methodology, the authors found that overall microautophagy flux was induced by a diauxic shift (namely, carbon starvation) during incubation [10]. However, it is unknown whether overall microautophagy is regulated by TORC1.

Phosphatidate phosphatase, known as lipin (Pah1 in yeast), generates diacylglycerol from phosphatidate and is a key enzyme for the production of the storage lipid triacylglycerol (TAG) [11–17]. The protein phosphatase complex Nem1/Spo7 (CTDNEP1/NEP1R1 in mammalian cells) dephosphorylates and activates Pah1/lipin [18–23]. In budding yeast, nutrient starvation and TORC1 inactivation promote Pah1 function and TAG synthesis via the activation of Nem1/Spo7 [16,18–24]. TAG is also implicated as a source for autophagosomal membranes, and is required for the induction of macroautophagy, a membrane-consuming process, after nutrient

Abbreviations: ESCRT, endosomal sorting complex required for transport; GFP, green fluorescent protein; TAG, triacylglycerol; TORC1, target of rapamycin complex 1.

* Corresponding author. Graduate School of Science and Technology, Shizuoka University, Ohya 836, Suruga-ku, Shizuoka, 422-8021, Japan.
E-mail address: ushimaru.takashi@shizuoka.ac.jp (T. Ushimaru).

https://doi.org/10.1016/j.bbrc.2018.09.011
0006-291X/



Please cite this article in press as: M.A. Rahman, et al., The TORC1–Nem1/Spo7–Pah1/lipin axis regulates microautophagy induction in budding yeast, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.011