Biochem. J. (2014) **460**, 59–67 (Printed in Great Britain) doi:10.1042/BJ20140014

online data

SUPPLEMENTARY ONLINE DATA

Functional divergence between the two P1–P2 stalk dimers on the ribosome in their interaction with ricin A chain

Przemysław GRELA*†[1], Xiao-Ping LI*, Marek TCHÓRZEWSKI†[2] and Nilgun E. TUMER*[2]

*Department of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, New Jersey 08901-8520, U.S.A.
†Department of Molecular Biology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland

MATERIALS AND METHODS

Cytotoxicity of RTA in yeast mutants

Yeast cells harbouring pre-RTA vector (NT849) were grown in liquid SD medium with 2 % glucose. The cells were collected by centrifugation and then resuspended in liquid SD medium containing 2 % galactose to a D_{600} value of 0.3 to induce pre-RTA expression. At different time points after induction, 50 μl of cells were sampled and normalized to a D_{600} value of 0.1. Four serial dilutions (1:10) were made and 15 μl of each dilution were spotted on to SD-Leu plates containing 2 % glucose.

Live cell imaging

Visualization of RTA was carried out using P0 yeast mutants harbouring pre-RTA–EGFP [1] at 6 and 12 hpi of galactose. Yeast cells were directly added to 2 % agar pads on glass slides and visualized using an Olympus BX41 epifluorescence microscope equipped with a CCD camera (Hamamatsu) and 100× oil objective (1.45 NAPlan Apo, Olympus). Image acquisition and processing were performed using Metamorph Image Software 7.0 (MDS Analytical Technologies) as described previously [1].

[1] Present address: Department of Molecular Biology, Marie Curie-Sklodowska University, Akademicka 19, 20-033, Lublin, Poland.
[2] Correspondence may be addressed to either of these authors (email maro@hektor.umcs.lublin.pl or tumer@aesop.rutgers.edu).

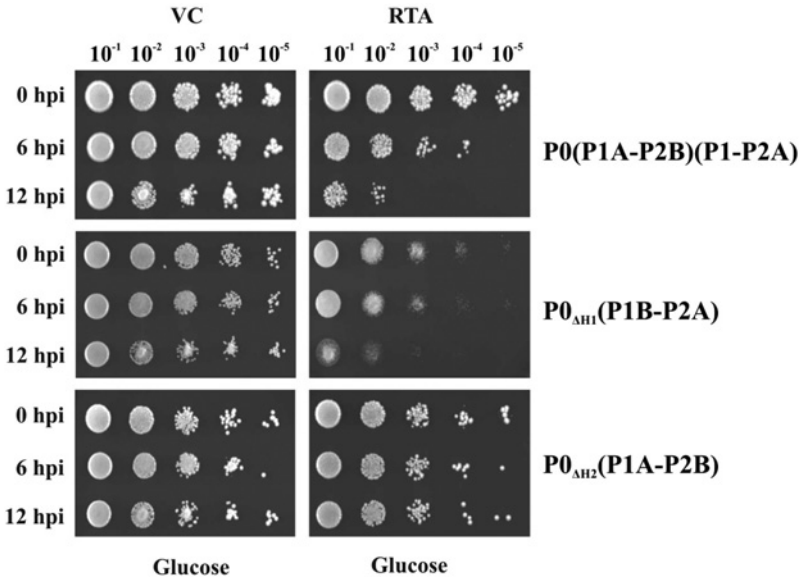

Figure S1 Viability of yeast stalk mutants expressing RTA

Yeast cells transformed with a plasmid carrying the gene encoding pre-RTA under the *GAL1* promoter were transferred from SD medium supplemented with 2% glucose into SD medium with 2% galactose to induce expression of RTA. Equal numbers of cells were spotted on to a plate containing 2% glucose after 0, 6 and 12 hpi and grown at 30°C for 3 days. Cells carrying the VC (empty vector) were used as controls.

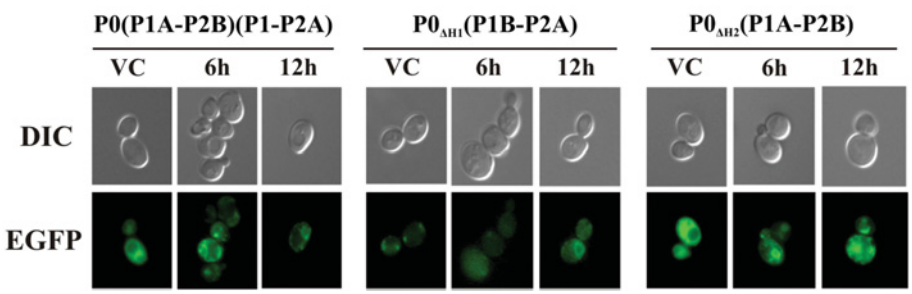

Figure S2 RTA expression in the yeast stalk mutants monitored by epifluorescence microscopy

Yeast cells were grown in SD medium with 2% glucose and induced with 2% galactose. The images were taken at 6 and 12 hpi with an Olympus BX41 fluorescence microscope. Cells transformed with the VC (empty vector expressing GFP) were used as controls. DIC, differential interference contrast.

REFERENCE

1 Yan, Q., Li, X. P. and Tumer, N. E. (2012) N-glycosylation does not affect the catalytic activity of ricin A chain but stimulates cytotoxicity by promoting its transport out of the endoplasmic reticulum. Traffic **13**, 1508–1521

Received 2 January 2014/24 February 2014; accepted 28 February 2014
Published as BJ Immediate Publication 28 February 2014, doi:10.1042/BJ20140014