Asymmetric segregation of nucleoplasmic factors during yeast closed mitosis

Tatiana T. Marquez-Lago[1], Barbara Boettcher[2], Mathias Bayer[2] and Yves Barral[2]

***Short Abstract* — During vegetative growth, *Saccharomyces cerevisiae* mother cells bud, producing smaller daughter cells. In this process, factors activating specific transcriptional programs are asymmetrically inherited. Understanding how and when such asymmetry is established and maintained is critical. By using photobleaching techniques and *in silico* modeling, we have now shown compartmentalization of nucleoplasm and nuclear membranes to depend on geometry and diffusion barriers, distinctively [1]. Here, we will focus on the interdisciplinary methods that allowed us to quantify the complementary roles of envelope morphology and barrier strength.**

***Keywords* — Asymmetric cell divison, FLIP, FCS, stochastic modeling, simulations.**

I. Background

Budding yeast cell division is not only morphologically but also functionally asymmetric. For instance, mother cells express the endonuclease HO allowing them to switch mating type [2], whereas daughter cells express genes governing their physical separation from mother cells. Such daughter-specific program is controlled by Ace2, a transcription factor accumulating in daughter cells nuclei during late mitosis [3]. Interestingly, Ace2 is uniformly distributed within the cytoplasm throughout mitosis, and its asymmetry is only established at the level of nuclear import/export during anaphase. In fact, it is known Ace2 enters dividing nuclei, efficiently exiting mother cell nuclei only [4]. However, up till now, it remained unclear whether Ace2 asymmetry is dependent on diffusion barriers, cell geometry, or both.

II. Methods

A. Cell Biology and Microscopy

We visualized nuclear shape using dsRed-HDEL, a reporter which localizes to the lumen of the ER and perinuclear space between outer and inner nuclear membranes (O/INM). This construct allowed us to follow the different steps of nuclear elongation and division, and correlate them with Ace2 localization in the nucleoplasm. We then performed FLIP experiments on Ace2 and TetR fused to GFP, and compared their degrees of compartmentalization. The same analysis was performed for proteins in O/INM, revealing different compartmentalization patterns. Additionally, we considered nuclear geometry 'perturbations', by analyzing mutants yielding distinct internuclear bridge lengths and widths.

B. Model and simulations

In order to theoretically dissect the impact of geometry and potential diffusion barriers on nuclear compartmentalization, we performed off-lattice spatial stochastic simulations of selected FLIP experiments in idealized cells during early and late stages of nuclear division. For such, idealized nuclear geometries were constructed based on fluorescence live-cell microscopy of yeast cells expressing Nsg1-GFP and EM data in the literature. Afterwards, we determined the diffusion rate of TetR in the nucleoplasm by simulations and FCS, and subsequently determined bleaching rates matching its FLIP profile. We estimated diffusion rates of all other proteins by using this bleaching rate, minimizing the error of the first moment of spatial simulations as compared with experimental data. Parameter sweep simulations were then performed for all other compartments of the model, to estimate optimal values of idealized diffusion barriers. Finally, we performed stochastic simulations of all FLIP scenarios, corresponding to distinct nuclear compartments. Our simulations accurately match all FLIP experiments, allowing us to quantify the separate roles of envelope morphology and barrier strength.

III. Conclusion

In our study [1] we describe how Ace2 asymmetry depends on nuclear geometry architecture during late mitosis. We discuss how other proteins in the nucleoplasm, ONM and INM have distinct compartmentalization properties, and distinguish between the contributions of geometry and permeability of potential diffusion barriers in asymmetric division.

[1]Integrative Systems Biology Unit, Okinawa Institute of Science and Technology, Japan. E-mail of presenting author: tatiana.marquez@oist.jp ;

[2]Institute of Biochemistry, ETH Zurich, Switzerland.

References

[1] Boettcher, B., Marquez-Lago, T.T., Bayer, M., Weiss, E.L., Barral, Y. (2012) Role of envelope morphology in nuclear compartmentalization and asymmetric segregation of nucleoplasmic factors during yeast closed mitosis (Submitted to JCB – in final revisions)

[2] Nasmyth, K. (1993) Regulating the HO endonuclease in yeast. Curr. Opin. Genet. Dev. 3: 286-294.

[3] Colman-Lerner A, Chin T.E., Brent R. (2001) Yeast Cbk1 and Mob2 activate daughter-specific genetic programs to induce asymmetric cell fates. Cell 107: 739-750.

[4] Mazanka, E., Weiss, E.L. (2010) Sequential counteracting kinases restrict an assymmtetric gene expression program to early G1. Mol Biol Cell 21: 2809-2820.

Nothing should be here on page 2! Please limit your abstract to a single page, and create a one-page .pdf file for submission.