UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.12

Proponent: Prof. Fraschini Roberta

Project Title: Function and regulation of Vps74 in *S. cerevisiae* citokinesis

Scientific background and 'open issues'

Cytokinesis is the final event of cell division, which equally partitions the cytoplasm and the genomic content of the mother cell into two daughters (D'Avino et al., 2015). Because of the universal requirement for cytokinesis in all dividing cells, it is not surprising that the fundamental machinery is conserved in animal and fungal cells (Bhavsar-Jog and Bi, 2017). Remarkably cytokinesis of *S. cerevisiae* and animal cells relies on the assembly of an actomyosin contractile ring coupled with new targeted membrane addition at the cleavage site (Bhavsar-Jog and Bi, 2017). Besides being essential for normal growth and survival of all the eukaryotic organisms, cytokinesis is also fundamental during the development of animal organisms and to control ploidy in adult tissues. Cytokinesis failures have been associated with several human pathological conditions such as Lowe syndrome, blood disorders and female infertility and cancer (Lacroix and Maddox, 2012; D'Avino et al. 2015). Defective cytokinesis leads to the formation of genetically unstable tetraploid cells and consequently to chromosomal instability, one of the hallmarks of cancer (Lacroix and Maddox, 2012; D'Avino et al. 2015).

Drosophila GOLPH3 (dGOLPH3) protein is essential for cytokinesis as it accumulates at the cleavage site during telophase, interacts with proteins of the cytokinesis and the vesicle trafficking machineries. In addition, it cooperates with myosin II for stabilization of centralspindlin at the cleavage furrow and contractile ring formation (Sechi et al., 2020a; Sechi et al., 2021). AP-MS analysis revealed that hGOLPH3 is phosphorylated at T61 that matches the consensus of Aurora mitotic kinases (Ipl1 in yeast). Remarkably T61 corresponds to a conserved threonine in *Drosophila* GOLPH3 and yeast Vps74 proteins (T68). Our preliminary data suggest that the role of GOLPH3 in cytokinesis is conserved in human HeLa cells and *S. cerevisiae* (Vps74).

Objectives

The asymmetric division *of S. cerevisiae* provides an excellent model system to study the molecular mechanisms that coordinate vesicular trafficking and exocytosis with the actomyosin machinery during cytokinesis. The aim of the project is to demonstrate that Vps74 is a key protein that couple PI(4)P signaling and membrane trafficking with actomyosin ring dynamics. The objective is to investigate the role and regulation of Vps74 during the final steps of mitosis and during cytokinesis.

Methodologies

In budding yeast the molecular components of the cytokinetic machinery localize at the bud neck that marks the future division site. To explore the role of Vps74 in budding yeast cytokinesis, it is necessary to apply molecular genetics and standard biochemistry techniques: production of genetically modified yeast strains carrying gene deletion or gene tagging, site specific mutagenesis, productions of double mutants, western blots, co-immunoprecipitations, phosphatase and kinase assays.

Collaboration / Co-tutoring opportunities

External collaborations:
Mariagrazia Giansanti, CNR Roma
Giuliano Callaini, Università di Siena

Project's Sustainability & Mobility

Roberta Fraschini has great experience in molecular genetics and molecular biology of budding yeast. Her main research interests are analyzing the role and regulation of several proteins involved in cell cycle progression and in cytokinesis in the model organism budding yeast.

- Pertinent research articles:

1. A novel coordinated function of Myosin II with GOLPH3 controls centralspindlin localization during cytokinesis in *Drosophila*. Sechi S, Frappaolo A, Karimpour-Ghahnavieh A, Fraschini R, Giansanti MG. J Cell Sci 2020 Nov 10;133(21):jcs252965. doi: 10.1242/jcs.252965.
2. Divide Precisely and Proliferate Safely: Lessons From Budding Yeast.
Fraschini R.Front Genet. 2019 Jan 10;9:738. doi: 10.3389/fgene.2018.00738. eCollection 2018.
3. Swe1 and Mih1 regulate mitotic spindle dynamics in budding yeast via Bik1.
Raspelli E, Facchinetti S, Fraschini R.J Cell Sci. 2018 Sep 3;131(17):jcs213520. doi: 10.1242/jcs.213520.

- Putative foreign institutions for international mobility:

1. Ethel Queralt, instituto de Biomedicine de Valencia
2. Simonetta Piatti, CNRS Montpellier

References

- Bhavsar-Jog YP and Bi E. (2017) Semin Cell Dev. Biol., (2017) 66, 107-118.
- D'Avino PP, Giansanti MG and Petronczki M. Cold Spring Harb. Perspect Biol., (2015), 7:a015834.
- Lacroix B and Maddox AS. J Pathol., (2012), 226, 338-351.
- Sechi S, Frappaolo A, Karimpour-Ghahnavieh A, Piergentili R, Giansanti MG. Int. J. Mol. Sci., (2020a), 21:933.
- Sechi S, Karimpour-Ghahnavieh A, Frappaolo A, Di Francesco L, Piergentili R, Schininà E et al. Cells, (2021), 10:2336.