Using correlative light/electron microscopy (CLEM) approaches to study the regulation of metabolic enzyme self-assembly in cell starvation

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1. INTRODUCTION

In addition to membrane compartments found in eukaryotes, the functional organization of cells in spatially distinct domains often involves phase-separated intracellular compartments, usually composed of macromolecular assemblies of proteins and/or RNA. These compartments without membranes exhibit a large diversity of size - from the nanoscale to the microscale, of composition, structure and function. Depending on the physicochemical properties of these macromolecular assemblies, their behavior ranges from liquid-like to crystal-like. Liquid-like assemblies (i.e. centrosomes, P bodies..), are unordered, usually spherical and dynamic, while crystal-like assemblies (crystals, fibers) are ordered and more static. In cells, the formation of these self-assemblies of molecules is tightly regulated, with functional consequences. For example, the formation of filamentous polymers, like actin, or intermediate filaments, or microtubules is essential in many cell functions. Similarly, the sequestration of a molecule such as RNA or an enzyme in a mesoscale assembly can regulate its activity. Metabolic enzymes in particular are able to self-assemble into distinct punctate or filamentous domains, which suggest a role of molecular self-assembly in the regulation of metabolic activity. It was shown in a large-scale assay of GFP-tagged proteins that upon nutrient starvation in yeast, the Gln1 enzyme, which catalyzes the ATP-dependent synthesis of glutamine from glutamate and ammonium redistributed from a diffuse intracellular localization to fluorescent foci [1]. In the present study we have used correlative light/electron microscopy (CLEM), to investigate the structure of the Gln1 positive intracellular compartment and the functional consequences of Gln1 self-assembly during cell starvation.

2. RESULTS

Experimental conditions

Yeast cells were transformed with fluorescent Gln1-mCherry using the Gateway cloning system in a W303 ADE+ strain background. To induce cell starvation, cells were grown in YPD in log phase and transferred to 0.1M phosphate buffer (pH 5, 6, 7 or 8). To acidify cells in the presence of glucose, 2 mM 2,4-dinitrophenol was used. Fluorescence imaging of live cells was done in a Deltavision microscope with a 100x 1.4NA objective followed by deconvolution and maximum intensity projection. For CLEM, cells were high-pressure frozen freeze-substituted in 0.1 % uranyle acetate and 4 % H2O in acetone at -90°C and embedded at low temperature in HM-20 Lowicryl resin. 150 nm-thick sections were collected on EM grids. For realignment of light and electron microscopy images, fluorescent beads were deposited on the sections. After fluorescence microscopy imaging in a wide field microscope (Zeiss), the sections were stained with lead citrate for 5min and observed at the electron microscope (Tecnai 12, FEI). Light and electron microscopy images were overlayed using ZIBAmira (Zuse Institute Berlin, Germany) [2].

Gln1 reversibly associates into mesocale filaments upon cell starvation

Using live cell imaging, we found that Gln1-mCherry localization was diffuse in diving cells, but it significantly aggregated into fluorescent filaments upon glucose starvation and even more so in phosphate buffer containing no metabolizable nutrients (Figure 1A). This was fully reversible and Gln1-positive filaments disappeared rapidly after addition of glucose (Figure 1B) and cells re-entered into the cell cycle.

Specific amino acids of Gln1 facilitate or inhibit filament formation

The high-resolution crystal structure of Gln1 has been reported [3]: it it composed of 2 pentameric rings which assemble as a face-to-face homodecamer and back-to-back association of decamers. This structure therefore could allow the long range assembly of decamer repeats. To test this hypothesis, we mutated specific residues of the decamer interface. Interestingly, we found several mutations (E186K, P83R,

T49E) that inhibited the formation of filaments in starved cells, while another mutation (R23E) formed filaments even in growing cells. We took advantage of the latter to identify the ultrastructure of Gln1 filaments in a CLEM approach : semi-thin sections of high-pressure frozen yeast cells expressing Gln1-R23E-mCherry were observed in fluorescence microscopy to identify cell sections exhibiting fluorescent filaments (Figure 2, Left R23E). Using local landmarks and fiducials, the same cells were then identified in transmission electron microscopy (Figure 2, Left TEM). Blue fluospheres were then used for a precise subcellular correlation of the fluorescent signal and cell ultrastructure (Figure 2, Left Overlay). The ultrastructure of the region of overlay was then examined at high resolution. The ultrastucture of the filaments revealed that they consisted of bundles of Gln1-containing fibers (Figure 2, Left Detail). To exclude a role o the mCherry tag to to formation of fiber bundles, we expressed untagged Gln1-R23E cells in yeast cells. Unlike in WT cells, bundles of fibers were also found in transformed cells (Figure 2, middle). Further analysis of filament formation by negative staining in TEM demonstrated that Gln1 purified from yeast can self assemble *in vitro* into ~12nm diameter fibers.

A

B

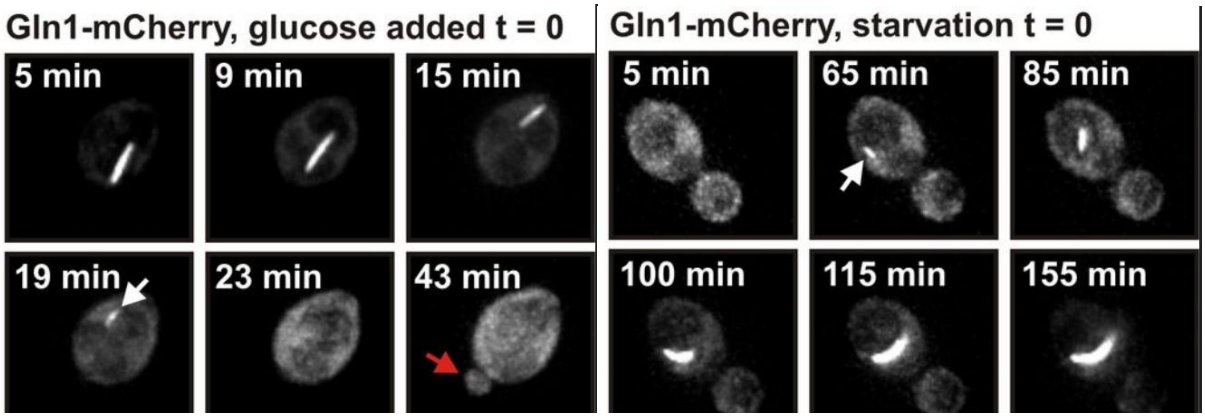

Figure 1. Live cell imaging in fluorescence microscopy of yeast cells expressing Gln1-mCherry after nutrient starvation show the progressive formation of Gln1 -containing filaments **(A)**. Addition of glucose on previously starved cells rapidly resulted in the reversal of the phenotype **(B).** Bar=5µm

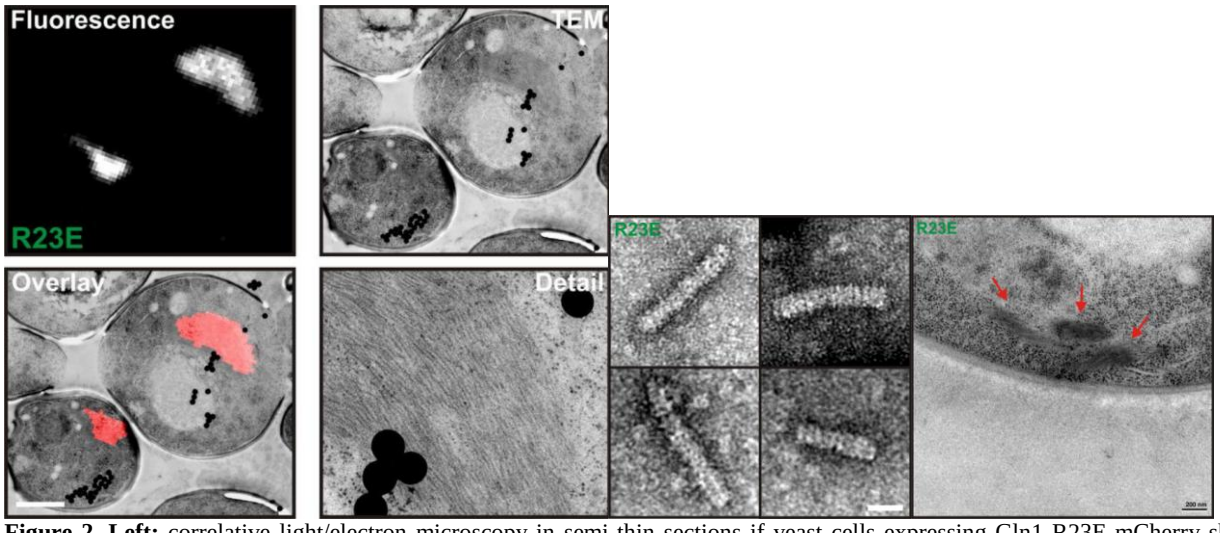

Figure 2. Left: correlative light/electron microscopy in semi-thin sections if yeast cells expressing Gln1-R23E-mCherry show

3. CONCLUSION

We propose that filament formation as seen here with Gln1 is a general mechanism to inactivate and store key metabolic enzymes during a state of advanced cellular starvation until nutriments become available again.

REFERENCES

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