SHORT REPORT

A new phosphate-starvation response in fission yeast requires the endocytic function of myosin I

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ABSTRACT

Endocytosis is essential for uptake of many substances into the cell, but how it links to nutritional signalling is poorly understood. Here, we show a new role for endocytosis in regulating the response to low phosphate in Schizosaccharomyces pombe. Loss of function of myosin I (Myo1), Sla2/End4 or Arp2, proteins involved in the early steps of endocytosis, led to increased proliferation in low-phosphate medium compared to controls. We show that once cells are deprived of phosphate they undergo a quiescence response that is dependent on the endocytic function of Myo1. Transcriptomic analysis revealed a wide perturbation of gene expression with induction of stress-regulated genes upon phosphate starvation in wild-type but not myo1 cells. Thus, endocytosis plays a pivotal role in mediating the cellular response to nutrients, bridging the external environment and internal molecular functions of the cell.

KEY WORDS: Myosin, Phosphate sensing, Endocytosis

INTRODUCTION

agent methylmethane sulfonate (MMS) and growth at elevated temperatures, revealing a general loss of fitness of the myo1 mutant under challenging conditions. By contrast, myo1 cells were unaffected by stress induced by low phosphate levels, as opposed to wild-type cells, which were severely affected by this condition (Fig. 1A,B).

Phosphate starvation drives cells into a quiescent-like status

Under specific conditions, nutrient starvation drives fission yeast cells into a quiescent state, where they stop proliferating but maintain viability, and this response is linked to changes in cell shape (Yanagida, 2009). We found that wild-type cells in the low-

Journal of Cell Science (2015) 128, 3707-3713 doi:10.1242/jcs.171314

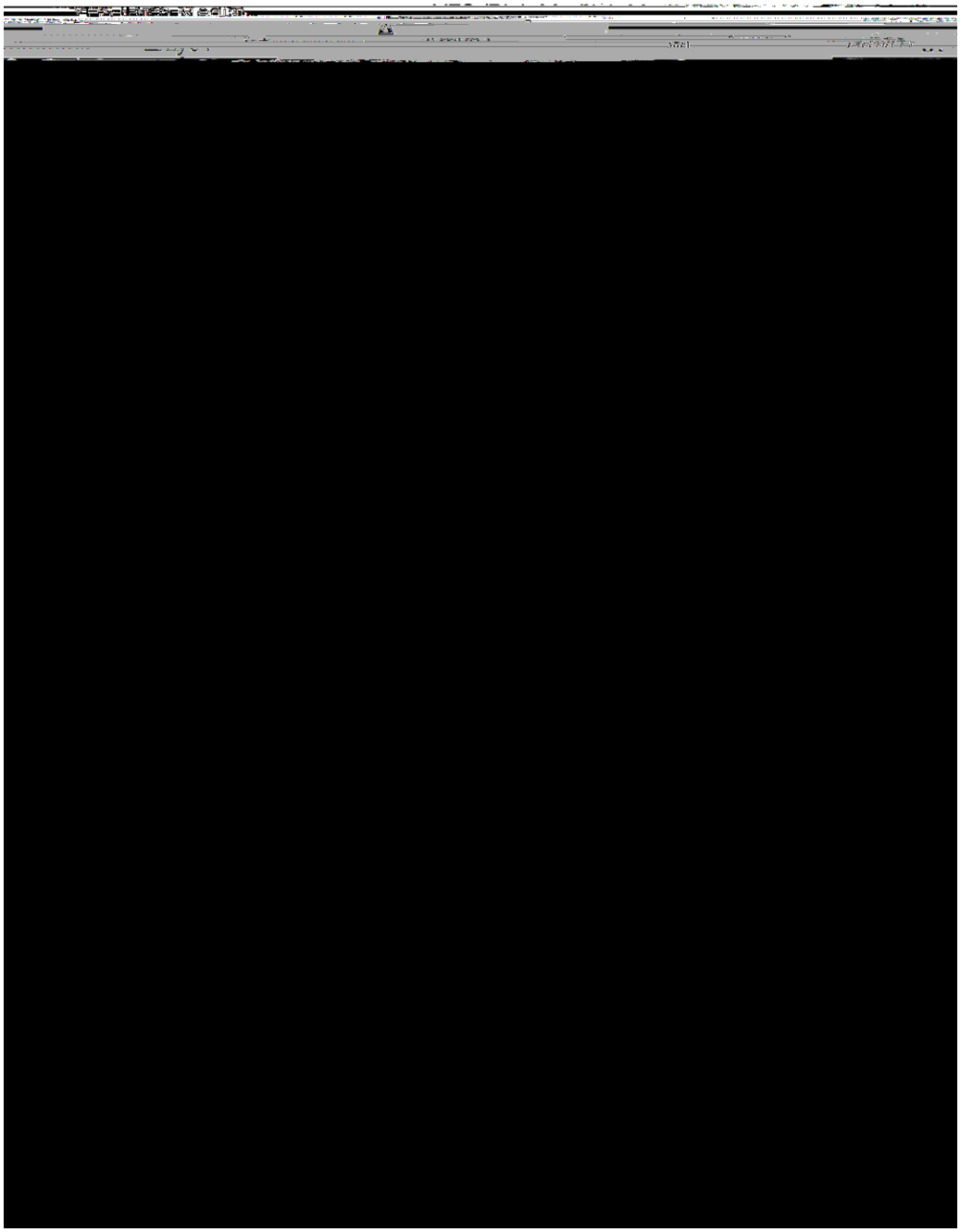

Fig. 1. Myo1-deleted mutants exhibit no loss of growth in low-phosphate-containing medium. (A) Serial dilution viability assay of wild-type (wt) and myo1 cells under stress conditions [YES LowPi, 0.5 µM LatA, 0.5 mM $CdSO_4$, 0.01% (v/v) MMS]. (B) Serial dilution viability assay of myo1 and control cells in 90% SD, 10% EMM medium with and without phosphate. HA–myo1 expression in trans was driven by the NMT1 promoter. Similar complementation results were obtained with a myo1 expressed from an endogenous promoter (see Fig. 4A). (C) Brightfield images of wild-type or myo1 cells grown for 4 h in YES or low-phosphate YES. Scale bar: 10 µm. (D) Box-and-whisker plot showing length measurements of yeast grown for 4–12 h in YES or low-phosphate YES. The box represents the 25–75th percentiles, and the median is indicated. The whiskers show the 10–90th percentiles. The length of 50 cells for each condition was measured using the line tool of ImageJ. ***P<0.001 (Student's t-test).

suppressor mutants). A tight overlap was observed in wild-type cells when we compared the early (4 h) to late (10 h) response, suggesting that a core subset of genes is switched on and maintained during phosphate starvation (Fig. 3A). The majority of phosphate-starvation response genes were not triggered in absence of Myo1 (Fig. 3A).

We defined signature genes of this quiescence response by applying a magnitude threshold to the differentially regulated genes after 4 h phosphate starvation in wild-type cells (Table S1), tracking them in the remaining conditions (Fig. 3B). At the later time po these signature genes remained responsive in wild-type cells, n most of them away from the zero line. However, in the myo1 mutant, most of these genes were not responding to the phosphate stress both at the early and late time points. Hierarc al clustering of the most responsive genes highlights the lack o e gene expression response of the myo1 mutant under low- phosphate conditions (Fig. 3C; Fig. S1B). Gene ontology ana of the differentially regulated genes revealed that a signifi

Journal of Cell Science

proportion were involved in cellular response to stress and in different catabolic processes, confirming that this is mainly a stress response (Fig. 3D). In summary, phosphate starvation leads to a dramatic change in gene expression, which is abolished when *myo1* is deleted.

The endocytic function of Myo1 is required for the low-phosphate-induced quiescence response

Myo1 plays a crucial role in endocytosis where it is required for vesicle internalization (Attanapola et al., 2009; Sirotkin et al., 2005, 2010); Myo1 is also required for the regulation of actin polymerization and patch formation at cortical sites (Evangelista et al., 2000; Lee et al., 2000), promoting polarised growth and

mutants of *eng2* and *csh3*, which form a newly discovered endocytic module (Encinar del Dedo et al., 2014), and Ypt7, a protein involved in the fusion of endocytic vesicles with vacuoles (Bone et al., 1998; Murray and Johnson, 2001).

We observed that the *arp2-1* mutation and *sla2/end4* deletion conferred resistance to low-phosphate stress to a similar extent as *myo1* deletion (Fig. 4B). Mutations in *ypt7*, *eng2* or *csh3* did not lead to increased proliferation in YES LowPi compared to controls (Fig. 4B).

To verify that actin dynamics were not altered during phosphate starvation, we monitored changes in actin patch formation and localization using structural illumination (SIM) microscopy in order to obtain an accurate estimate of the average patch concentration (Fig. 4C). Consistent with previous observations (Lee et al., 2000), we found an increase in actin patches between wild-type cells and the *myo1* mutant. No difference could be observed between the normal and low-phosphate condition, both in actin patches numbers and localization along the cell body.

required to sense phosphate gradients effectively, e.g. by comparing with the cytoplasmic phosphate levels. Our work is, to our knowledge, the first description that links nutrient sensing to endocytosis and cytoskeletal protein function. Future studies should address whether the endocytic machinery is also required for nutrient sensing and coordinated responses to different stress in multicellular organisms.

MATERIALS AND METHODS

Yeast culture

Cells were grown at 30°C unless otherwise stated. Yeast extract supplemented (YES, pH 5.5) medium was used for normal phosphate conditions; low-phosphate medium (YES LowPi) was obtained as described previously (Schweingruber and Schweingruber, 1981). Briefly, $MgSO_4$ was added to 10 mM to YES media, then NaOH was added dropwise until a visible precipitate formed, which was removed using a 0.02 µm filter, the pH was adjusted with HCl to 5.5 and the medium was autoclaved. KH_2PO_4 was added back to re-supplement phosphate to YES in Fig. 2B, YES LowPi+1 mM KH_2PO_4, pH.5.5; YES LowPi+5 mM KH_2PO_4, pH.5.3, YES

Microscopy) system, using a 100×1.49 NA oil-immersion objective. Images

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