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ORIGINAL RESEARCH

Filamentation of Metabolic Enzymes in *Saccharomyces cerevisiae*

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ABSTRACT

Compartmentation *via* filamentation has recently emerged as a novel mechanism for metabolic regulation. In order to identify filament-forming metabolic enzymes systematically, we performed a genome-wide screening of all strains available from an open reading frame-GFP collection in *Saccharomyces cerevisiae*. We discovered nine novel filament-forming proteins and also confirmed those identified previously. From the 4159 strains, we found 23 proteins, mostly metabolic enzymes, which are capable of forming filaments *in vivo*. *In silico* protein-protein interaction analysis suggests that these filament-forming proteins can be clustered into several groups, including translational initiation machinery and glucose and nitrogen metabolic pathways. Using glutamine-utilising enzymes as examples, we found that the culture conditions affect the occurrence and length of the metabolic filaments. Furthermore, we found that two CTP synthases (Ura7p and Ura8p) and two asparagine synthetases (Asn1p and Asn2p) form filaments both in the cytoplasm and in the nucleus. Live imaging analyses suggest that metabolic filaments undergo sub-diffusion. Taken together, our genome-wide screening identifies additional filament-forming proteins in *S. cerevisiae* and suggests that filamentation of metabolic enzymes is more general than currently appreciated.

KEYWORDS: CTP synthase; Metabolic enzyme; Cytoophidium; Glycolysis; Glutamine; Intracellular compartmentation; *Saccharomyces cerevisiae*

INTRODUCTION

Compartmentation of biological processes is a fundamental feature of the cell. One such way is to form membrane-bound organelles, which have been extensively appreciated in the past. Moreover, macromolecules can be compartmentalized through the formation of large-scale aggregates without membranes (Gall, 2000; Brangwynne et al., 2009; O'Connell et al., 2012; Hyman et al., 2014). Emerging studies have identified many membraneless organelles including cytoplasmic processing bodies (P bodies) (Sheth and Parker, 2006), histone locus bodies (HLBs) (Liu et al., 2006a, 2006b), uridine-rich small nuclear ribonucleoprotein bodies (U bodies) (Liu and Gall, 2007) and purinosomes (An et al., 2008).

In 2010, three groups independently described that the metabolic enzyme CTP synthase (CTPS) can form filamentous structures in bacteria, yeast and fruit flies (Ingerson-Mahar et al., 2010; Liu, 2010; Noree et al., 2010). These filaments have been termed as "cytoophidia" (meaning cellular snakes in Greek), "CTPS filaments", or "cytoplasmic rods and rings". Subsequent studies have shown that CTPS can form filaments in human cells as well (Carcamo et al., 2011; Chen et al., 2011). Thus cytoophidia represent a novel type of evolutionarily conserved organelles. Recent studies suggest that

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polymerisation of CTPS or other metabolic enzymes into filamentous structures serves to regulate enzymatic activity (Aughey et al., 2014a, 2014b; Barry et al., 2014; Noree et al., 2014; Petrovska et al., 2014; Strochlic et al., 2014; Chang et al., 2015). Studies in *Drosophila* indicate that the CTPS cytoophidia are involved in brain development and oogenesis (Chen et al., 2011; Azzam and Liu, 2013; Strochlic et al., 2014; Tastan and Liu, 2015a; Tastan and Liu, 2015b; Wang et al., 2015). The biology of cytoophidia emerges as a new frontier in the field of pyrimidine metabolism (Aughey et al., 2014a, 2014b; Garavito et al., 2015; Liu, 2015; Tastan and Liu, 2015a; Wang et al., 2015).

Nine filament-forming proteins including CTPS were identified *via* a screening of 1632 GFP-tagged yeast strains (Noree et al., 2010), which comprise about 40% of the budding yeast GFP-tagged open reading frame (ORF) collection (Huh et al., 2003). To identify additional novel filament-forming proteins in budding yeast, we screened the whole collection of 4159 GFP-tagged ORFs, which represents 75% the *Saccharomyces cerevisiae* proteome. From this, we identified 23 proteins (including nine novel proteins) that can form filaments *in vivo* in diauxic and stationary phases. We found that these filament-forming proteins can be clustered into several groups, including translational initiation machinery and glucose and nitrogen metabolic pathways. Further analysis of the five glutamine-utilising enzymes demonstrated that the occurrence and length of the metabolic filaments are sensitive to growth conditions. In addition, we observed that four glutamine-utilising enzymes can form filaments both in the cytoplasm and in the nucleus. Live imaging analysis of six types of filament suggests that they undergo sub-diffusion. The identification of additional filament-forming proteins from our genome-wide screening provides an opportunity to study compartmentation *via* filamentation systematically.

RESULTS

Filament-forming proteins in budding yeast

Our screening has confirmed that all nine proteins identified in Noree's study (Noree et al., 2010) (i.e., Glt1p, Psa1p, Ura7p, Ura8p, Gcd2p, Gcd6p, Gcd7p, Gcn3p and Sui2p) (Fig. S1A) and all four septin proteins (i.e., Cdc10p, Cdc11p, Cdc12p and Shs1p) (Fig. S1B) available in the budding yeast GFP-tagged ORF collection can form filaments. Short filaments and foci assembled by Gln1p (glutamine synthase) could be detected (Fig. S1C), but there were no long filaments, even after starvation treatment, in the current study, with a potential interference from the GFP tag as reported previously (Petrovska et al., 2014). In addition, nine more proteins can form large-scale filaments detectable under light microscopy (Fig. 1 and Table 1), namely Acc1p (acetyl-CoA carboxylase), Asn1p/Asn2p (asparagine synthetase), Gcd1p (eIF2B-γ), Gdb1p (glycogen debranching enzyme), Gdh2p (glutamate dehydrogenase), Pfk1p/Pfk2p (phosphofructokinase) and Tsa1p (thioredoxin peroxidase). To simplify the terminology, we refer to these metabolic enzyme-containing serpent-shaped structures as cytoophidia.

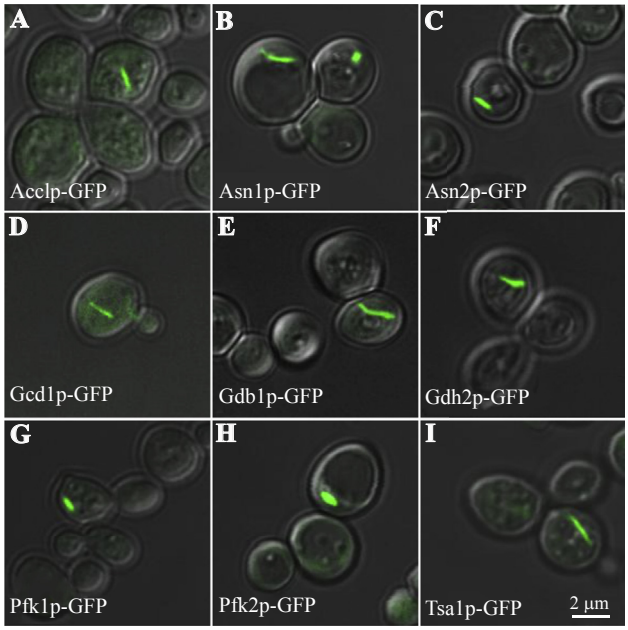

Fig. 1. Identification of filament-forming proteins in *S. cerevisiae*.
A genome-wide screening of 4159 GFP-tagged ORF collection in budding yeast identifies nine novel filament-forming proteins. **A:** Acetyl-CoA carboxylase (Acc1p). **B:** Asparagine synthetase 1 (Asn1p). **C:** Asparagine synthetase 2 (Asn2p). **D:** Gamma subunit of the translation initiation factor eIF2B (Gcd1p). **E:** Glycogen debranching enzyme (Gdb1p). **F:** Glutamate dehydrogenase (Gdh2p). **G:** Phosphofructokinase (Pfk1p). **H:** Phosphofructokinase (Pfk2p). **I:** Thioredoxin peroxidase (Tsa1p). Scale bar, 2 μm. See also Fig. S1 and Table 1.

Acetyl-CoA carboxylase catalyses the carboxylation of acetyl-CoA to produce malonyl-CoA, which provides the malonyl-CoA substrate for fatty acid biosynthesis. In mammals, acetyl-CoA carboxylase can be polymerised into tiny filaments detectable under electron microscopy (Kleinschmidt et al., 1969; Meredith and Lane, 1978). Polymerisation of acetyl-CoA carboxylase upregulates the enzymatic activity. A recent study reported that Acc1p has diffused distribution under normal growth conditions, while prolonged starvation can drive Acc1p to form rod-like structures in budding yeast (Suresh et al., 2015). Our screening revealed that Acc1p is capable of forming large-scale filaments under normal growth conditions.

Using aspartate and glutamine as the substrates, asparagine synthetase catalyses an ATP-dependent reaction to produce asparagine. In budding yeast, there are two genes, *ASN1* and *ASN2*, which encode asparagine synthetase (Dang et al., 1996). We found that Asn1p and Asn2p have very similar distributions and both can form filaments *in vivo*. Studies in *Escherichia coli* and beef pancreas suggest that asparagine synthetase functions as a dimeric enzyme (Gantt and Arfin, 1981; Larsen et al., 2000). Lack of asparagine synthetase may cause cell apoptosis (Zhang et al., 2014a). Due to its important role in amino acid synthesis, asparagine synthetase is a common target in the treatment of acute lymphoblastic leukaemia as well as prostate cancer and other kinds of cancer (Aslanian et al., 2001; Sircar et al., 2012; Panosyan et al., 2014).