Fungal Genetics and Biology 94 (2016) 69–78

Contents lists available at ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

Regular Articles

Investigation of the role of four mitotic septins and chitin synthase 2 for cytokinesis in *Kluyveromyces lactis*

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ARTICLE INFO

Article history:
Received 8 May 2016
Revised 8 July 2016
Accepted 12 July 2016
Available online 12 July 2016

Keywords:
Milk yeast
Cell division
Primary septum
Septin ring dynamics

ABSTRACT

Septins are key components of the cell division machinery from yeast to humans. The model yeast *Saccharomyces cerevisiae* has five mitotic septins, Cdc3, Cdc10, Cdc11, Cdc12, and Shs1. Here we characterized the five orthologs from the genetically less-redundant milk yeast *Kluyveromyces lactis*. We found that except for *KlSHS1* all septin genes are essential. *Klshs1* deletions displayed temperature-sensitive growth and morphological defects. Heterologous complementation analyses revealed that all five *K. lactis* genes encode functional orthologs of their *S. cerevisiae* counterparts. Fluorophore-tagged versions of the *K. lactis* septins localized to a ring at the incipient bud site and split into two separate rings at the bud neck later in cytokinesis. One of the key proteins recruited to the bud neck by septins in *S. cerevisiae* is the chitin synthase Chs2, which synthesizes the primary septum. *KlCHS2* was found to be essential and deletions showed cytokinetic defects upon spore germination. KlChs2-GFP also localized to the bud neck and to punctate structures in *K. lactis*. We conclude that cytokinesis in *K. lactis* is similar to *S. cerevisiae* and chimeric septin complexes are fully functional in both yeasts. In contrast to some *S. cerevisiae* strains, KlChs2 and KlCdc10 were found to be essential.

1. Introduction

Septins are highly conserved GTP-binding proteins which regulate cell division from yeast to humans (Longtine and Bi, 2003). The budding yeast *Saccharomyces cerevisiae* has five mitotic septins, Cdc3, Cdc10, Cdc11, Cdc12, and Shs1, with the four *CDC* genes first described in a screen for temperature-sensitive cell cycle mutants (Hartwell, 1971; Weirich et al., 2008). The septins adopt a heterooctameric rod-like structure with a two-fold rotational symmetry, in the order Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11, and form filaments via interactions of their Cdc11 subunits (Bertin et al., 2008, 2011; Booth et al., 2015). Shs1 is considered an accessory septin which is most similar to Cdc11 and may replace it in some complexes (Iwase et al., 2007). Septin filaments are first recruited to the new bud site by the activated GTPase Cdc42 and its effectors Gic1 and Gic2 (Gladfelter et al., 2002; Iwase et al., 2005). They initially serve as a hub for recruitment of proteins involved in cell division. Later on the septins form an hourglass-like collar acting as a diffusion barrier. Finally they separate into two rings attracting late factors of cytokinesis, such as chitin synthase 2 and secretory vesicles (Gladfelter et al., 2001; Longtine and Bi, 2003). The splitting into two cortical rings is triggered by the mitotic exit network, MEN, coupling septin dynamics with cell cycle regulation (Cid et al., 2001; Lippincott et al., 2001). At this point, cell wall integrity sensors are also recruited to the bud neck, thus providing a link to glucan synthesis in the formation of secondary septa (Wilk et al., 2010).

Genetic analyses demonstrated that three of the five septin genes, *CDC3*, *CDC11* and *CDC12*, are essential, whereas *shs1* deletions show only mild morphological defects (Iwase et al., 2007). For *CDC10*, the phenotype of deletions appears to be dependent on the *S. cerevisiae* strain. Although it was originally described as an essential gene, in many strains *cdc10* deletions show only moderate morphological defects (Hartwell, 1971; Versele et al., 2004). Nevertheless, the growth defect of a *shs1* deletion strain at 20 °C is suppressed by a multicopy plasmid carrying *CDC11*, whereas double deletions of *shs1* and *cdc10* are not viable (Iwase et al., 2007).

One of the first proteins recruited to the septin ring during cytokinesis is the type II myosin Myo1, which later associates with actin to form a contractile actomyosin ring (AMR; Fang et al., 2010; Wloka and Bi, 2012). AMR constriction is regulated by a complex network of proteins, including Hof1, Cyk3 and Inn1 (Devrekanli et al., 2012; Martin-Garcia et al., 2014). With the exception of Inn1 these cytokinesis regulators are conserved between evolutionary distinct yeast species such as *S. cerevisiae* and

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http://dx.doi.org/10.1016/j.fgb.2016.07.007
1087-1845/

Kluyveromyces lactis on one hand, and the fission yeast *Schizosaccharomyces pombe* on the other (Sanchez-Diaz et al., 2008; Jendretzki et al., 2009; Roberts-Galbraith et al., 2009; Rippert et al., 2014). AMR constriction and the formation of a chitinous primary septum are intimately connected (Nelson et al., 2003; Devrekanli et al., 2012). *S. cerevisiae* has three isozymes for chitin synthases, of which Chs2 is primarily responsible for primary septum synthesis (Shaw et al., 1991; Cabib et al., 1993). As for *CDC10*, conflicting phenotypes have been reported for deletions of *CHS2* in *S. cerevisiae*, being essential in some strains, but viable in others (Cabib and Schmidt, 2003).

The dairy yeast *K. lactis* is a close relative of *S. cerevisiae*, but is genetically less redundant, since it has not undergone a whole genome duplication. It also has a respiratory, rather than a fermentation-oriented metabolism (reviewed in Rodicio and Heinisch, 2013). Like *S. cerevisiae*, *K. lactis* is a budding yeast, but forms smaller cells whose cytokinesis is only marginally dependent on KlMyo1 and a functional AMR (Rippert et al., 2014). Homologs of the cytokinesis regulators Hof1, Cyk3, and Inn1 have been studied in *K. lactis* and null mutants partially differ from those in *S. cerevisiae*, e.g. *KlCYK3* is an essential gene, whereas *ScCYK3* is not (Rippert et al., 2014).

Given these apparent differences between *S. cerevisiae* and *K. lactis* and to gather more information on the essential components required for cytokinesis, we decided to have a closer look at the basic machinery using *K. lactis* as a genetically less redundant model. We here describe the characterization of the five mitotic septin homologs of *K. lactis* and the role of KlChs2 in cytokinesis.

2. Material and methods

2.1. Strains, genetic manipulations, media and culture conditions

Genotypes of yeast strains employed in this work are listed in Table 1. For plasmid amplification in *E. coli*, strain DH5α was used (Invitrogen, Karlsruhe, Germany). For genetic manipulations of yeasts and plasmid constructions, standard procedures were followed as described before (Rodicio et al., 2008). Rich media are based on yeast extract (1%), peptone (2%) with 2% glucose as carbon source (YEPD). For osmotic stabilization 1 M sorbitol was added where indicated. Synthetic media contained 0.67% yeast nitrogen base with ammonium sulfate, 2% glucose, amino acids and bases as required, according to (Rose et al., 1990). A list of the plasmids employed is provided in Table 2, sequences of all plasmids are available upon request. Sequences of oligonucleotides employed for cloning, including primers for PCR to perform one-step gene replacements, are listed in Table S1.

Table 1
Yeast strains used in this work.

Strain	Genotype	Reference
(A) K. lactis strains		
KHO70	*MATa/MATα ura3/ura3 leu2/leu2 ADE2/ade2::loxP HIS3/his3::loxP ku80::loxP/ku80::loxP*	Heinisch et al. (2010)
KDR1-9B	*MATa ura3 leu2 his3::loxP*	Rippert et al. (2014)
KDR40-8A	*MATa ura3 leu2 his3::loxP KlMYO1-mCherry-ScURA3*	Rippert et al. (2014)
KDR48	*MATa/MATα ura3/ura3 leu2/leu2 ADE2/ade2::loxP HIS3/his3::loxP KlCDC3/Klcdc3::ScLEU2 ku80::loxP/ku80::loxP*	This work
KDR63	*MATa/MATα ura3/ura3 leu2/leu2 ADE2/ade2::loxP HIS3/his3::loxP KlCDC11/Klcdc11::kanMX ku80::loxP/ku80::loxP*	This work
KJN1	*MATa/MATα ura3/ura3 leu2/leu2 ADE2/ade2::loxP HIS3/his3::loxP KlSHS1/Klshs1::ScURA3 ku80::loxP/ku80::loxP*	This work
KJN3	*MATa/MATα ura3/ura3 leu2/leu2 ADE2/ade2::loxP HIS3/his3::loxP KlCDC10/Klcdc10::ScURA3 ku80::loxP/ku80::loxP*	This work
KJN4	*MATa/MATα ura3/ura3 leu2/leu2 ADE2/ade2::loxP HIS3/his3::loxP KlCDC12/Klcdc12::ScLEU2 ku80::loxP/ku80::loxP*	This work
KJN5	*MATa/MATα ura3/ura3 leu2/leu2 ADE2/ade2::loxP HIS3/his3::loxP KlSHS1/Klshs1::ScURA3 KlMYO1/KlMYO1-mCherry-ScURA3 KU80/ku80::loxP*	This work
KJN5-8A	*MATα ura3 leu2 his3::loxP Klshs1::ScURA3*	This work
KJN5-10B	*MATa ura3 leu2 his3::loxP Klshs1::ScURA3 KlMYO1-mCherry-ScURA3*	This work
KJN6-4A	*MATα ura3 leu2 his3::loxP Klshs1::ScURA3 KlMYO1-mCherry-ScURA3*	This work
KJN6-5B	*MATa ura3 leu2 his3::loxP Klshs1::ScURA3*	This work
KKV3	*MATa/MATα ura3/ura3 leu2/leu2 ADE2/ade2::loxP HIS3/his3::loxP KlCHS2/Klchs2::ScLEU2 ku80::loxP/ku80::loxP*	This work
(B) S. cerevisiae strains		
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachmann et al. (1998)
BY4743	*MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 MET15/met15Δ0 LYS2/lys2Δ0 ura3Δ0/ura3Δ0*	Brachmann et al. (1998)
Euroscarf *cdc10*	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 cdc10::kanMX*	EUROSCARF, Frankfurt, Germany
Euroscarf *shs1*	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 shs1::kanMX*	EUROSCARF, Frankfurt, Germany
BKV1	*MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 MET15/met15Δ0 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 CDC3/cdc3::kanMX*	This work
BKV2	*MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 MET15/met15Δ0 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 CDC11/cdc11::kanMX*	This work
BKV3	*MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 MET15/met15Δ0 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 CDC12/cdc12::kanMX*	This work

2.2. Construction of deletion mutants and strains with tagged genes

Deletion mutants in *K. lactis* were obtained by one-step gene replacements as described for *S. cerevisiae* (Rothstein, 1991) either by using directly PCR products obtained with primers generating approximately 40 bp of homology flanking the genomic target sequences, or by a prior *in vivo* recombination with a plasmid-borne gene in *S. cerevisiae* to obtain longer flanking regions. These procedures and the markers employed have been described in detail, as was the use of a *ku80* deletion background in order to enhance the frequency of correct homologous recombination (Heinisch et al., 2010). Sequences of the resulting chromosomal loci can be provided upon request. In short, the *Klcdc10* and *Klshs1* deletions were obtained by PCR amplification of the *ScURA3* marker cassette from pJJH955U (Heinisch et al., 2010) primed by the oligonucleotides 13.049/13.050 and 13.051/13.052, respectively. *Klcdc12* and *Klcdc3* deletions were generated using the *ScLEU2* marker cassette from pJJH955L (Heinisch et al., 2010) amplified with oligonucleotide pairs 13.163/13.164 and 13.307/13.308, respectively. The *Klcdc11* deletion was constructed by *in vivo* recombination employing a *kanMX* marker cassette obtained by PCR with the oligonucleotides 13.161/13.162 from template pUG6 (Gueldener et al., 2002). The open reading frame of *KlCHS2* was substituted by a *ScLEU2* cassette amplified by PCR from pJJH955L with the oligonucleotides 14.123/14.124. Heterozygous diploids in strain KHO70 were obtained by transformation of the deletion cassettes and selection for the respective prototrophies or G418 resistance in all cases, verified by PCR and subjected to tetrad analysis. For deletion of *ScCDC3*, *ScCDC11* and *ScCDC12* the *kanMX* marker cassette from pUG6 was amplified with the oligonucleotides 14.173/14.174, 14.175/14.176 and 14.177/14.178, respectively and used for a one-step gene replacement in BY4743, selecting for G418 resistant clones.