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Fission yeast LAMMER kinase Lkh1 regulates the cell cycle by phosphorylating the CDK-inhibitor Rum1

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ABSTRACT

In eukaryotes, LAMMER kinases are involved in various cellular events, including the cell cycle. However, no attempt has been made to investigate the mechanisms that underlie the involvement of LAMMER kinase. In this study, we performed a functional analysis of LAMMER kinase using the fission yeast, *Schizosaccharomyces pombe*. FACS analyses revealed that deletion of the gene that encodes the LAMMER kinase Lkh1 made mutant cells pass through the G1/S phase faster than their wild-type counterparts. Co-immunoprecipitation and an *in vitro* kinase assay also revealed that Lkh1 can interact with and phosphorylate Rum1 to activate this molecule as a cyclin-dependent kinase inhibitor, which blocks cell cycle progression from the G1 phase to the S phase. Peptide mass fingerprinting and kinase assay with Rum1^{T110A} confirmed T110 as the Lkh1-dependent phosphorylation residue. In this report we present for the first time a positive acting mechanism that is responsible for the CKI activity of Rum1, in which the LAMMER kinase-mediated phosphorylation of Rum1 is involved.

1. Introduction

The LAMMER protein kinase family comprises a set of dual-specificity protein kinases that possess serine/threonine and tyrosine kinase as well as autophosphorylation activity [1–3]. They contain a conserved motif "EHLAMMER" in kinase subdomain X, which is essential for their kinase activity and subnuclear localization [1,4,5]. Since the discovery of the first member of the LAMMER protein kinase family, CLK1 [2], a number of studies have reported that LAMMER kinases are involved in mRNA splicing by regulating splicing factors [4,6,7]. In addition, LAMMER kinases may play a role in the regulation of cellular processes by activating kinases [8] and tyrosine phosphatase [9].

While the cellular function of LAMMER kinase in fungi has not yet been described in detail, an *lkh1*$^+$ deletion has revealed various phenotypes in the fission yeast *Schizosaccharomyces pombe*, including an abnormality in cell division [10,11], flocculation in liquid medium, filamentous and adhesive growth on solid medium [5,10], sensitivity to oxidative stress [12], and pre-mRNA processing [13]. Recently, we found that phosphorylation by Lkh1 is required for the activation of Csx1, which protects mRNAs encoding the transcription factor Atf1 from exonuclease-mediated decay due to oxidative stress [14] and for the activation of transcriptional repressors, Tup11 and Tup12 [5], which regulate different gene expression programs [15]. This suggests that Lkh1 may play important roles in regulating gene expression in *S. pombe*. However, much remains to be determined in terms of the mode of Lkh1 action.

The eukaryotic cell cycle is regulated at two major points: in G1 before initiation of the S-phase, and in G2 before entry into mitosis due to the activity of cyclin-dependent kinase (CDK) [16]. In fission yeast, the activity of the only CDK present, Cdc2, is regulated by at least three different biochemical events [17]. First, Cdc2 associates with four different B-type cyclins to display its kinase activity. The amount of Cdc2 remains constant throughout the cell cycle, while the cyclin levels oscillate. Second, the kinase activity of Cdc2 is also regulated by the phosphorylation state of specific amino acids. Third, the CDK activity of Cdc2 is regulated by the only known CDK inhibitor (CKI) Rum1 (*r*eplication *u*ncoupled from *m*itosis), which in G1 blocks cell cycle progression to allow the initiation of sexual development in response to nutrient deprivation such as nitrogen starvation [18].

Although an abnormality that is observed during cell division of the LAMMER kinase-deficient mutant in *S. pombe* have been reported previously [10,11], no attempt has been made to investigate mechanisms that underlie the involvement of LAMMER kinase in the cell cycle. In this study, we tried to clarify the involvement of Lkh1 in cell-cycle regulation and found that the *lkh1*$^+$ deletion mutant cells passed the G1/S phase progression faster than their wild-type counterparts. Furthermore, we discovered that LAMMER kinase can phosphorylate Thr110 to activate Rum1 as a CKI, which negatively regulates G1/S progression. We report here for the first time the positive acting mechanism that is responsible for the CKI activity of Rum1.

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2. Materials and methods

2.1. Strains and culture conditions

The *S. pombe* strains that were used in this study are listed in Table S1. The rich medium was YES, and the selective medium was Edinburgh synthetic minimal medium (EMM) with appropriate supplements. EMM without NH_4Cl (EMM-N) but with growth factors was used for nitrogen starvation experiments. Genetic crossing and standard techniques for fission yeast molecular genetics were performed according to Moreno et al. [19]. Sporulation efficiency was determined by calculating the number of cells that bore ascospores after diploid cells were induced to undergo sporulation in EMM-N medium [20].

2.2. Recombinant DNA techniques and preparation of fusion proteins

For the deletion of *lkh1*$^+$ in ED665 and h^{90} cells, *ura4*$^+$ flanked by 5′ and 3′ sequences of the *lkh1*$^+$ ORF was constructed. The flanking regions of *lkh1*$^+$ were PCR-amplified with the gene-specific primer set lkh1-kda1 and lkh1-udb1 for the 5′ flanking region, and lkh1-uda2 and lkh1-b5 for the 3′ flanking region. The selective marker, *ura4*$^+$, was amplified by PCR with the DHU-a1 and DHU-b1 primer set. The fragments were joined by overlapping PCR, and the deletion cassette was used to transform the yeast cells. To construct a *Δlkh1* mutant with a *lue2*$^+$ selective marker, the disruption cassette was PCR-amplified with a specific primer set (lkh1-kda1 and lkh1-b5) using DNA from strain PHM5L as a template and then introduced into strain ED668. For the construction of the *cdc10-129* mutant with *lkh1*$^+$ deletion (FY7030L), PCR-amplified *KanMX6* flanked by the 5′ and 3′ sequences of *lkh1*$^+$ ORF was introduced into the strain FY7030. The 5′ and 3′ flanking regions of *lkh1*$^+$ were amplified with the specific primer sets lkh1-kda1/lkh1-kdb1 and lkh1-kda2/lkh1-b5, respectively. *KanMX6* from pFA6a-kanMX6 was PCR-amplified with specific primer set Kan-F1 and Kan-R1. The purified fragments were joined by overlapping PCR. For the deletion of *rum1*$^+$, a disruption cassette was constructed by overlapping PCR with three DNA fragments that were amplified with the gene-specific primer sets dRum-a1 and dRum1-b1 for the 5′ flanking region, DHU-a1 and DHU-b1 for the *ura4*$^+$ selective marker, and dRum1-a2 and dRum1-b2 for the 3′ flanking region. The disruption cassette was then transformed into strains ED665 and ED668.

To express His-tagged Rum1 in *Escherichiacoli*, *Bam*HI-*Xho*I fragments of *rum1*$^+$ from pT-*rum1*$^+$, which is the TOPcloner TA vector (Enzynomics, Korea) that contains *rum1*$^+$ amplified by PCR with the gene-specific primer set Rum1-a1 and Rum1-b1 was sub-cloned into the corresponding restriction site of pET28a (Novagen, USA). To generate *rum1*^{T110A} mutant allele, DNA fragments amplified with specific primer sets (Rum1-a1 and Rum1-b5, pRum1-a9 and Rum1-b2) were joined by PCR with primer set Rum1-a1 and Rum1-b2. For bacterial expression of the GST-tagged catalytic domain of Lkh1(cLkh1), the previously reported pGEX4T-1 (GE Healthcare, Sweden) was used, and the fusion proteins that were expressed in *E. coli* were purified by using previous methods [21]. To generate pESP/*rum1*$^+$ to produce the Flag-tagged GST-fusion form of Rum1 in *S. pombe*, *rum1*$^+$ was amplified with the primers Rum1-a1 and Rum1-b2 and cloned into the *Bam*HI site of pESP-1 (Stratagene, USA). The oligonucleotide primers that were used for the PCR reactions are listed in Table S2.

2.3. GST-pull down assay and kinase assays

In vitro interaction between bacterially expressed cLkh1 and Rum1 proteins was performed by using GST-cLkh1-bound Sepharose beads with His-tagged Rum1 proteins as described previously [21]. Purified GST-cLkh1 was used for the kinase assay with putative substrates, purified His-tagged Rum1 and Rum1^{T110A}. The purification of the His-tagged Lkh1 complex and immunoblot analyses were performed using wild-type cells that expressed chromosomally His-tagged Lkh1 and an *lkh1*-deletion mutant that harbored pESP or pESP/*rum1*$^+$, respectively.

2.4. FACS analysis of DNA contents

FY7030 (*cdc10-129*) and FY7030L (*lkh1*-deleted FY7030) were grown at 30 °C up to OD_{600} = 0.4 in EMM-N with appropriate growth factors and then transferred to 37 °C and grown for 4 h. After 10 min on ice, the cultures were incubated at 30 °C and samples that contained 10^4 cells in total were taken every 30 min. The samples were washed and suspended in 1 mL of distilled water. The cells were fixed in 10 mL of 70% ethanol at 4 °C overnight. After centrifugation, the cells were washed and suspended in 1 mL of 50 mM sodium citrate. The cell suspension was treated with 10 μL of 20 mg/mL RNase A for 2 h at 37 °C and stained by 20 μL/mL of propidium iodide. The stained cells were analyzed using a flow-cytometer FACSCalibur (Becton–Dickinson) as described previously [22].

3. Results

3.1. FACS analysis revealed earlier G1/S progression due to lkh1-deletion

Previously, we found that the *lkh1*-deleted cells were shorter than the wild-type cells, which suggested the possible involvement of Lkh1 in cell-cycle regulation [10,12]. To confirm this observation, we first analyzed the size distribution of the cells. As shown in Fig. S1A, the size-distribution of the deletion mutant cells showed shorter ranges than that of the wild-type cells, and the short size distribution of the mutant cells was reversed through the regulated expression of the reintroduced *lkh1*$^+$ under the inducible *nmt1*$^+$ promoter (Fig. S1B). Moreover, the expression of *lkh1*$^+$ with the aid of over-expression vector pREP2 made the *lkh1*-deleted cells much longer than the wild-type cells, as is the case of gene(s) for cell-cycle regulation, for which over-expression usually makes the cells longer than they normally are (data not shown). These results indicate the involvement of Lkh1 in the cell-cycle regulation of the fission yeast.

In the cell cycle, fission yeast coordinates its cell size at the end of G1 (G1/S size control) and G2 (G2/M size control or mitotic size control) [23]. We therefore analyzed effect of the *lkh1*-deletion on the DNA content of the cells during the cell cycle using a FACS. Since it is difficult to detect any changes in G1 to S progression for *S. pombe* cells because they have very short G1 phase, *lkh1*-deletion was introduced into a temperature-sensitive cell division cycle mutant, *cdc10-129*. The FACS analysis of wild-type cells showed dominant population shift from 1C to 2C approximately 135 min after temperature restriction release and revealed a 3:7 ratio of 1C versus 2C cells approximately 300 min after releasing the temperature restriction (Fig. 1A). Whereas the *lkh1*-deletion mutant showed a dominant population shift from 1C to 2C approximately 75 min after temperature restriction release and revealed the exclusive dominance of 2C cells approximately 300 min after temperature restriction release (Fig. 1B). These results indicated that the deletion of Lkh1 caused abnormalities in cell cycle progression due to defects in regulation of either G1/S or G2/M size control.

3.2. Effect of lkh1-deletion on the responses to nitrogen starvation

Upon testing the effect of external stresses on the growth, we found that the *lkh1*-deletion mutant cells showed growth-retardation upon nitrogen starvation. On complete plates, the growth of

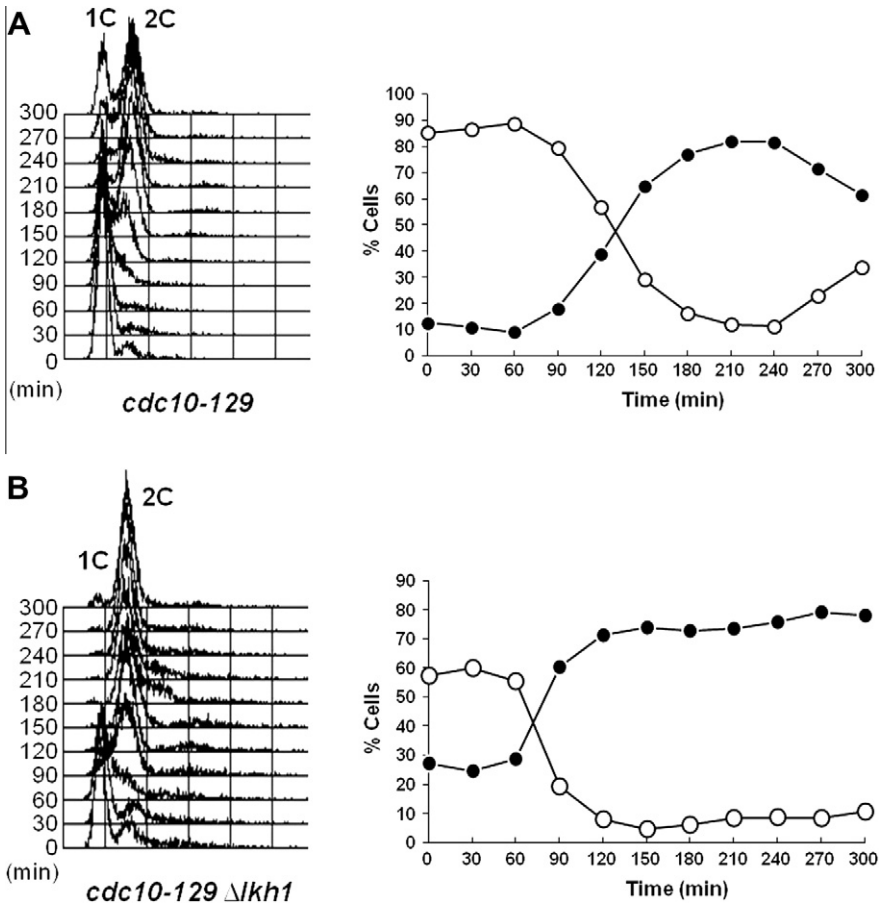

Fig. 1. Effect of *lkh1*-deletion on G1 to S cell cycle progression. *cdc10-129* (A) and *cdc10-129Δlkh1* (B) strains were cultured in minimal medium and synchronized in the G1 phase by incubating at a non-permissive temperature of 37 °C. Samples were taken at the indicated time points after release to a permissive temperature 28 °C for flow cytometry analysis. The percentage of cells that contained 1C or 2C DNA is depicted along with the time after release to the permissive temperature.

the *lkh1*-deletion mutant was almost similar to that of the wild-type. However, on the nitrogen-deprived minimal plates, growth of the mutant was dramatically retarded. The growth retardation of the *lkh1*-deletion mutant under nitrogen starvation was reversed by reintroducing of the *lkh1* gene (Fig. 2A). When the growth was tested with liquid medium, the *lkh1*-deletion mutant cells could not proliferate after being transferred from EMM to EMM-N unlike the wild-type cells, which proliferated even after being transferred to EMM-N (Fig. S2). These results confirmed that the sensitivity to the nitrogen starvation of the mutant cells resulted from the loss of Lkh1 function.

Since the nitrogen source is one of the key factors that is involved in regulating the sexual cycle and cell cycle [22], the sensitivity of the *lkh1*-deletion mutant toward nitrogen starvation also suggested that Lkh1 might affect sexual differentiation. Interestingly, the *lkh1*-deletion mutant showed a lower level of ascospore production than did the wild type (middle panels, Fig. 2B): the *lkh1*-deletion mutant showed 3.27% and 17.12% ascospore production in EMM and EMM-N medium, respectively. However, the wild type strain showed 18.20% and 33.65% ascospore production in EMM and EMM-N medium, respectively. We next determined whether the effect of the *lkh1*-deletion on sexual reproduction was due to the failure during the initial step of the meiotic cycle, zygote formation, or during meiosis. We measured the level of ascospore production using the h^{90} strain, which is homothallic for sexual reproduction. As shown in right panel, Fig. 2C, the deletion of $lkh1^{+}$ in cells with an h^{90} background also showed a lower level of ascospore production than that of the wild-type cells. It was noteworthy, however, that the levels of ascospore production in the h^{90} background were higher than those in the heterothallic background (left panel, Fig. 2C), which suggests that not only the meiotic events that are necessary for ascospore production but also the conjugation process that is necessary for zygote formation were affected by the *lkh1*-deletion. We also noted that, unlike the $rum1^{+}$-deletion, the deletion of $lkh1^{+}$ did not result in the complete abolishment of zygote formation and thus, the meiotic cycle, indicating that Lkh1 is not the only factor that is involved in Rum1 activation.

Taken together, the above results led us to conclude that Lkh1 is not the only factor that is necessary for normal sexual development. However, its deletion caused a defect in the complete arrest of the cells during G1, which is a prerequisite for successful entry into the meiotic cycle (sexual differentiation) in response to nitrogen-deprivation.

3.3. Lkh1 interacts with and phosphorylates Rum1 in vitro

Since the *lkh1*-deletion caused a decrease in cell growth and ascospore production under nitrogen deprivation, *S. pombe* GeneDB was examined to select candidate proteins that are involved in cell cycle arrest in response to nitrogen starvation. Among the candidates, Rum1 is required for cell cycle arrest during G1 to allow the initiation of sexual differentiation in response to nitrogen starvation [18], for which the phenotype seemed to be very similar to that of the *lkh1*-deletion mutant.

Therefore, we examined the *in vitro* interaction between Lkh1 and Rum1 by performing a pull-down assay with bacterially expressed GST-tagged cLkh1 and His-tagged Rum1. As shown in

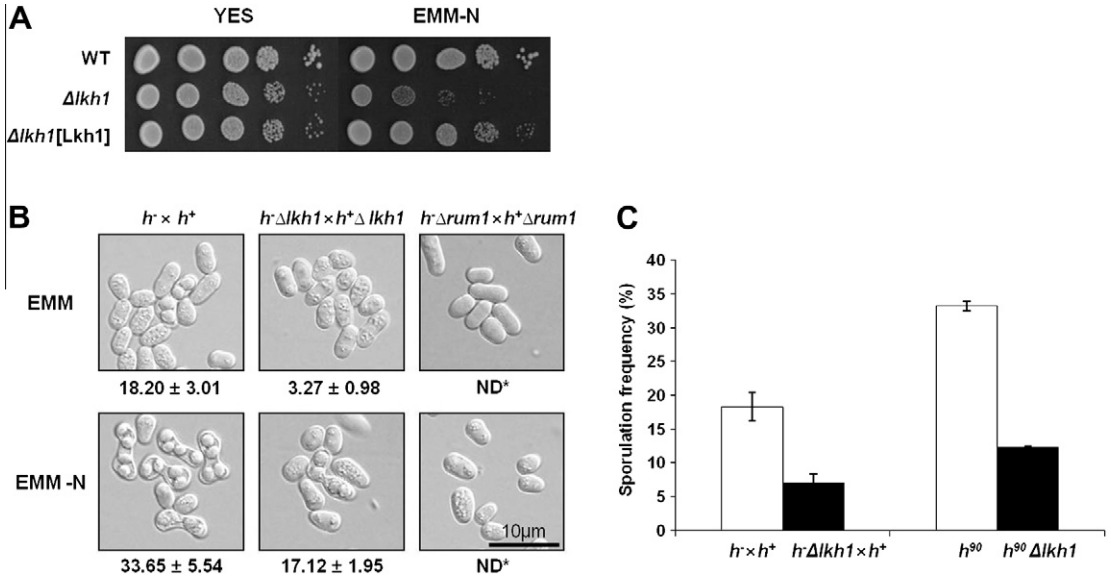

Fig. 2. Effect of *lkh1*-deletion on the response to nitrogen starvation. (A) Effect of *lkh1*-deletion on cell growth under nitrogen starvation. YES plates and EMM-N plates were incubated for 3 and 8 days, respectively, before being photographed. (B) Heterothallic strains were used for mating and ascospore production. (C) Homothallic strains were used for evaluating the ability to produce ascospores.

Fig. 3A, the binding assay confirmed the interaction between cLkh1 and Rum1 *in vitro*. We next examined if Lkh1 could phosphorylate Rum1 using bacterially expressed proteins. As shown in Fig. 3B, cLkh1 exhibited autophosphorylation activity as reported previously [21] and evidently phosphorylated Rum1. Peptide mass fingerprinting (PMF) of Rum1, which had been phosphorylated by cLkh1, revealed T110 as a putative phosphorylation residue (Table S3 and Fig. S3). Pull-down assay and *in vitro* kinase assay with Rum1^{T110A} mutant protein showed complete abolishment of interaction (upper right-most panel, Fig. 3A) and dramatic reduction of phosphorylation by cLkh1 (upper right-most panel, Fig. 3B). However, the T110A mutation did not result in the complete abolishment of Rum1 phosphorylation, suggesting that T110 is not the only residue involved in Lkh1-dependent phosphorylation.

These data suggested that Rum1 may be the *in vivo* target of Lkh1. Although further experiments are needed to confirm the positive effect of Lkh1 on the intracellular function of Rum1, our results presented here indicate that Lkh1 is involved in regulating the *S. pombe* life cycle, such as G1 to S progression in mitosis and meiotic sporulation via regulating the activity of Rum1, which is a CDK inhibitor that can cause G1-arrest.

4. Discussion

One of the main advantages of using the fission yeast for studying cell-cycle regulation is that the size variation between cells directly reflects its abnormality during the cell cycle. Our previous study suggested the possible involvement of Lkh1 in cell-cycle regulation by showing the shortening of cell size that was caused by an *lkh1*-deletion [10]. Analyses of the size distribution of the wild-type, *lkh1*-deletion mutant, and *lkh1*-retransformant cells (Fig. S1) confirmed the involvement of Lkh1 in cell-cycle regulation of the fission yeast.

In *S. pombe*, 2C cells that have a DNA content equivalent to that of diploid cells grow during the G2 phase until they reach a critical size, at which point Cdc2 kinase is activated and mitosis is initiated (M phase). A minimum size is also required for Start, but a wild-type cell at the end of mitosis is already beyond this minimum. Therefore, the G1 phase for 1C cells with a DNA content equivalent to that of haploid cells is very short. Since the production of shorter cells usually reflects a shortening of G2 phase, the possible involvement of Lkh1 in G2 phase regulation was indicated. The involvement of Lkh1 during the G1 phase, however, cannot be excluded because *S. pombe* cells are able to grow during G1 when the elongation of the cell is not fully accomplished during G2. A FACS analysis of synchronized cells with the aid of a temperature-sensitive *cdc10-129* mutant indicated that the *lkh1*-deletion caused abnormalities in cell cycle regulation, such as earlier progression from G1 to S and the lengthening of G2 (Fig. 1). Therefore, shortening of the cell size caused by the *lkh1*-deletion might be the consequence of defects in the regulation of either G1/S or G2/M size control. Since *S. pombe* mutants that fail to arrest at the G1 phase due to nitrogen-deprivation or pheromone treatment are unable to commit to sexual differentiation [22], the deleterious effect of the Lkh1-deletion on ascospore production (Fig. 2C) indicates the involvement of Lkh1 in cell-cycle regulation during G1 phase.

Rum1 is not essential under normal growth conditions, but it is required to block G1 to S progression during the cell cycle to allow the initiation of sexual differentiation in response to nutrient deprivation [18,24]. Although phosphorylation site prediction by using the NetPhosK 1.0 program revealed dozens of amino acid residue targets for protein kinases, thus far CDK-Cig2 complex-dependent phosphorylation on residues T58 and T62 has been reported to trigger its degradation [25–27]. A direct *in vitro* kinase assay that used GST-fusion forms of various mutants of Rum1 demonstrated the MAPK-dependent phosphorylation of residues T13 and S19, which negatively regulates the function of Rum1 [17]. The molecular mechanisms that underlie the regulation of Rum1 activity, however, are largely unknown especially in terms of the kinase that is responsible for the positive regulation of Rum1 activity *in vivo*. PMF analysis and kinase assay with Rum1^{T110A} mutant protein confirmed Lkh1-dependent phosphorylation of T110 (Fig. S3 and Fig. 3B). The involvement of residue(s) other than T110, however, cannot be excluded because T110A mutation did not result in the complete abolishment of Rum1 phosphorylation (Fig. 3B). Our results presented herein indicate that the deletion of Lkh1 causes defects in Rum1 activity for tight block in G1 phase, and thus, Lkh1 has a positive effect on the function of Rum1.

Recently, it was reported that the deletion of the gene that encodes an RNA binding protein Csx1 results in defects in sexual differentiation. Cells that lack Csx1 are partially sterile with a reduced amount of *ste11*$^{+}$ mRNA, which encodes a regulatory protein that is

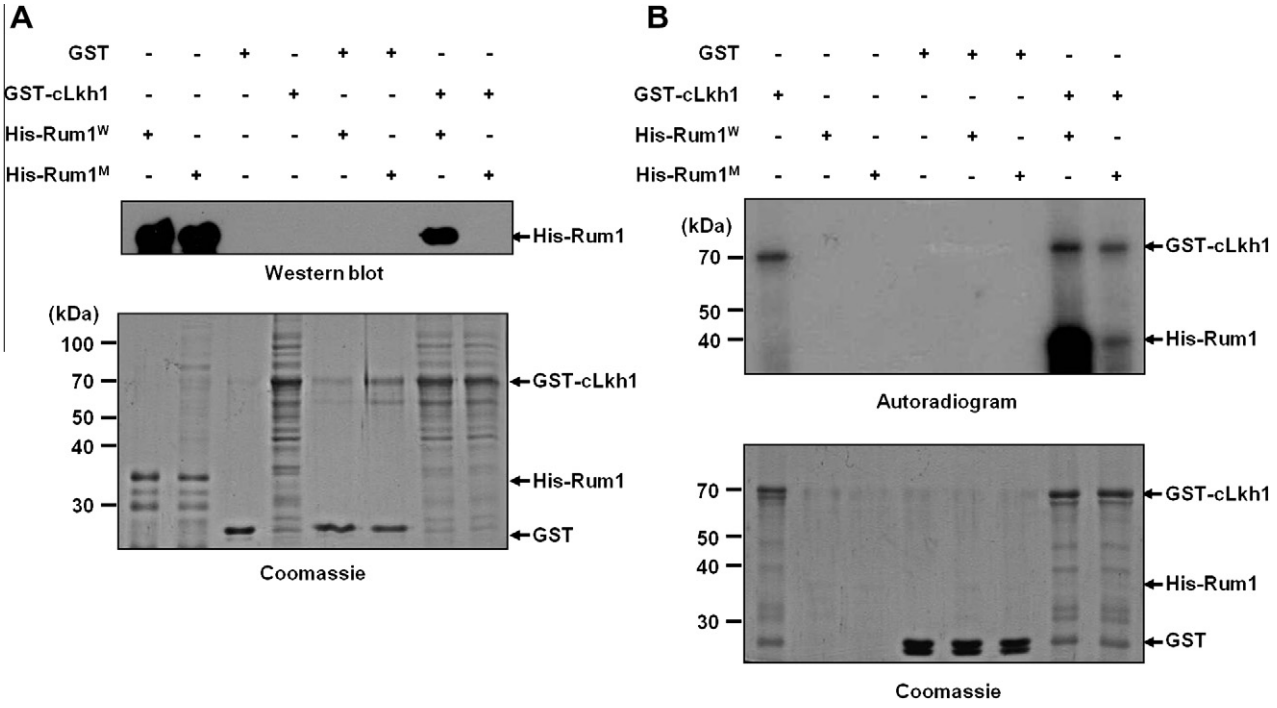

Fig. 3. *In vitro* interaction and phosphorylation between Lkh1 and Rum1. (A) Soluble protein extracts were incubated with GST and GST-cLkh1 which were coupled with glutathione-Sepharose beads, and then precipitated by centrifugation. The precipitates were separated by 10% SDS-PAGE and transferred to a PVDF membrane. (B) Purified Rum1 proteins, which were His (6×)-tagged, were mixed with purified GST-cLkh1 fusion proteins in the presence of [γ-^{32}P]dATP, respectively. After incubation at 30 °C for 30 min, the reaction was stopped by adding the SDS–PAGE sample buffer. The kinase reaction samples were resolved on 10% SDS–PAGE, and an autoradiogram was obtained. His-Rum1^{W} and His-Rum1^{M} indicate wild type and T110A mutant Rum1, respectively.

responsible for the transcription of many genes that are required for the initial steps of conjugation and meiosis [28]. We previously described that oxidative stress induces the phosphorylation of Csx1 and that binding of activated Csx1 to the *atf1*$^{+}$ mRNA are affected by the *S. pombe* LAMMER kinase, Lkh1 [14]. Although further analyses are required, defects in sexual differentiation that are caused by Lkh1-deletion are mediated not only via Rum1 but also via Ste11. Our results also support the question that was raised previously [28] about the possible coordination of sexual differentiation and oxidative stress response, as well as the role of RNA binding proteins in adapting to environmental signals.

LAMMER kinases are involved in gene expression mainly by regulating nucleic acid binding factors such as splicing factors [4,6,13], mRNA binding factor [14], transcription factors [21], and chromatin factors [29]. However, we presented a novel cellular function for the LAMMER kinase by showing that fission yeast Lkh1 affects cell cycle progression through phosphorylation of the cyclin-dependent kinase inhibitor protein, Rum1. This is in contrast to previous reports that showed a negative effect of phosphorylation on Rum1 such as triggering the degradation and inhibition of the CKI activity of Rum1 [25–27]. In addition it should be noted that Lkh1 is not the only factor that is necessary for Rum1 activation because the *lkh1*-deletion could not completely block meiotic sporulation, unlike the *rum1*-deletion.

Further experiments involving the identification and substitution of Lkh1-mediated phosphorylation sites(s) in Rum1 and an investigation of the effect of the substitution(s) on the CKI activity of Rum1 will help us to understand the function of the LAMMER kinase Lkh1 in cell cycle regulation in fission yeast.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.01.082.

References

[1] K. Lee, C. Du, M. Horn, L. Rabinow, Activity and autophosphorylation of LAMMER protein kinases, J. Biol. Chem. 271 (1996) 27299–27303.
[2] Y. Ben-David, K. Letwin, L. Tannock, A. Bernstein, T. Pawson, A mammalian protein kinase with potential for serine/threonine and tyrosine phosphorylation is related to cell cycle regulators, EMBO J. 10 (1991) 317–325.
[3] E. Nikolakaki, C. Du, J. Lai, T. Giannakouros, L. Cantley, L. Rabinow, Phosphorylation by LAMMER protein kinases: determination of a consensus site, identification of *in vitro* substrates, and implications for substrate preferences, Biochemistry 41 (2002) 2055–2066.
[4] S. Savaldi-Goldstein, G. Sessa, R. Fluhr, The ethylene-inducible PK12 kinase mediates the phosphorylation of SR splicing factors, Plant J. 21 (2000) 91–96.
[5] W.-H. Kang, Y.-H. Park, H.-M. Park, The Lammer kinase homolog, Lkh1, regulates Tup transcriptional repressors through phosphorylation in *Schizosaccharomyces pombe*, J. Biol. Chem. 285 (2010) 13797–13806.
[6] K. Colwill, T. Pawson, B. Andrews, J. Prasad, J.L. Manley, J.C. Bell, P.I. Duncan, The Clk/Sty protein kinase phosphorylates SR splicing factors and regulates their intranuclear distribution, EMBO J. 15 (1996) 265–275.
[7] Y. Wan, M. Sun, S. Wang, L. Liu, L. Yuan, W. Xie, DX16 is a novel SR protein phosphorylated by DOA, Mol. Cell. Biochem. 307 (2008) 177–183.
[8] M.P. Myers, M.B. Murphy, G. Landreth, The dual-specificity CLK kinase induces neuronal differentiation of PC12 cells, Mol. Cell. Biol. 14 (1994) 6954–6961.
[9] F.M. Moeslein, M.P. Myers, G.E. Landreth, The CLK family kinases, CLK1 and CLK2, phosphorylate and activate the tyrosine phosphatase, PTP-1B, J. Biol. Chem. 274 (1999) 26697–26704.
[10] K.H. Kim, Y.M. Cho, W.H. Kang, J.H. Kim, K.H. Byun, Y.D. Park, K.S. Bae, H.M. Park, Negative regulation of filamentous growth and flocculation by Lkh1, a fission yeast LAMMER kinase homolog, Biochem. Biophys. Res. Commun. 289 (2001) 1237–1242.
[11] Z. Tang, L.L. Mandel, S.L. Yean, C.X. Lin, T. Chen, M. Yanagida, R.J. Lin, The kic1 kinase of *Schizosaccharomyces pombe* is a CLK/STY orthologue that regulates cell–cell separation, Exp. Cell Res. 283 (2003) 101–115.
[12] Y.D. Park, W.H. Kang, W.S. Yang, K.S. Shin, K. Sook Bae, H.M. Park, LAMMER kinase homolog, Lkh1, is involved in oxidative-stress response of fission yeast, Biochem. Biophys. Res. Commun. 311 (2003) 1078–1083.
[13] Z. Tang, M. Luca, J. Portillio, B. Ngo, C. Chang, T. Wen, J. Murray, A. Carr, LAMMER kinase Kic1 is involved in pre-mRNA processing, Exp. Cell Res. 317 (2011) 2308–2320.

[14] W.-H. Kang, Y.-D. Park, J.-S. Hwang, H.-M. Park, RNA-binding protein Csx1 is phosphorylated by LAMMER kinase Lkh1, in response to oxidative stress in *Schizosaccharomyces pombe*, FEBS Lett. 581 (2007) 3473–3478.

[15] F. Fagerstrom-Billai, A.P. Wright, Functional comparison of the Tup11 and Tup12 transcriptional corepressors in fission yeast, Mol. Cell. Biol. 25 (2005) 716–727.

[16] B. Stern, P. Nurse, A quantitative model for the cdc2 control of S phase and mitosis in fission yeast, Trends Genet. 12 (1996) 345–350.

[17] K. Matsuoka, N. Kiyokawa, T. Taguchi, J. Matsui, T. Suzuki, K. Mimori, H. Nakajima, H. Takenouchi, T. Weiran, Y.U. Katagiri, J. Fujimoto, Rum1, an inhibitor of cyclin-dependent kinase in fission yeast, is negatively regulated by mitogen-activated protein kinase-mediated phosphorylation at Ser and Thr residues, Eur. J. Biochem. 269 (2002) 3511–3521.

[18] R.R. Daga, P. Bolanos, S. Moreno, Regulated mRNA stability of the CDK inhibitor Rum1 links nutrient status to cell cycle progression, Curr. Biol. 13 (2003) 2015–2024.

[19] S. Moreno, A. Klar, P. Nurse, Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*, Methods Enzymol. 194 (1991) 795–823.

[20] H. Cartagena-Lirola, A. Duran, M.H. Valdivieso, The *Schizosaccharomyces pombe* cfr1+ gene participates in mating through a new pathway that is independent of fus1+, Yeast 23 (2006) 375–388.

[21] W.H. Kang, Y.H. Park, H.M. Park, The LAMMER kinase homolog, Lkh1, regulates Tup transcriptional repressors through phosphorylation in *Schizosaccharomyces pombe*, J. Biol. Chem. 285 (2010) 13797–13806.

[22] H. Maekawa, K. Kitamura, C. Shimoda, The Ste16 WD-repeat protein regulates cell-cycle progression under starvation through the Rum1 protein in *Schizosaccharomyces pombe*, Curr. Genet. 33 (1998) 29–37.

[23] P.A. Fantes, Control of cell size and cycle time in *Schizosaccharomyces pombe*, J. Cell Sci. 24 (1977) 51–67.

[24] B. Stern, P. Nurse, Cyclin B proteolysis and the cyclin-dependent kinase inhibitor rum1p are required for pheromone-induced G1 arrest in fission yeast, Mol. Biol. Cell 9 (1998) 1309–1321.

[25] J. Benito, C. Martin-Castellanos, S. Moreno, Regulation of the G1 phase of the cell cycle by periodic stabilization and degradation of the p25rum1 CDK inhibitor, EMBO J. 17 (1998) 482–497.

[26] K. Kominami, T. Toda, Fission yeast WD-repeat protein pop1 regulates genome ploidy through ubiquitin-proteasome-mediated degradation of the CDK inhibitor Rum1 and the S-phase initiator Cdc18, Genes Dev. 11 (1997) 1548–1560.

[27] K. Kominami, I. Ochotorena, T. Toda, Two F-box/WD-repeat proteins Pop1 and Pop2 form hetero- and homo-complexes together with cullin-1 in the fission yeast SCF (Skp1-Cullin-1-F-box) ubiquitin ligase, Genes Cells 3 (1998) 721–735.

[28] A.M. Matia-Gonzalez, J. Sotelo, M.A. Rodriguez-Gabriel, The RNA binding protein Csx1 promotes sexual differentiation in *Schizosaccharomyces pombe*, PLoS ONE 7 (2012) e30067.

[29] M. Kovacikova, I. Raska, A. Mateasik, B.A. Chase, R. Farkas, Binding of doa kinase to specific loci in polytene chromosomes of *Drosophila melanogaster*, Endocr. Regul. 40 (2006) 21–27.