Communication

Truncated Sla1 Induces Haploid Meiosis through the Pat1-Mei2 System in Fission Yeast

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We previously reported that expression of Sla1ΔC, a truncated form of Sla1, induces ectopic meiosis in heterothallic fission yeast and this was possibly due to the inhibition of Pat1 kinase by Sla1ΔC. Here we found mei2 mRNA and the Mei2 protein accumulated and stability of the Mei2 protein increased when Sla1ΔC was expressed. The former two results are considered to be the consequence of de-repression of Ste11, which is the transcription factor of mei2 and negatively regulated by Pat1 kinase. The latter result reflects the consequence of deregulation of Mei2 by Pat1 kinase. In addition, Ste11 accumulated in the nucleus when Sla1ΔC was expressed. All these data consistently support the idea that the action of Sla1ΔC is to inactivate Pat1 kinase.

Key words: Schizosaccharomyces pombe; meiosis; Sla1; Pat1; Mei2

Schizosaccharomyces pombe cells grow as haploids in nutrient-rich medium. If nutrients of the medium, especially nitrogen, were depleted, cells initiate sexual development. Under these conditions, cells of the opposite mating type, h++, and h-, conjugate to form diploid zygotes. The zygotes subsequently undergo the various steps of meiosis, i.e., karyogamy, pre-meiotic DNA synthesis, meiosis I, meiosis II, and sporulation.1,2 The change from mitotic cell cycle to meiotic cell cycle is mainly decided by Pat1 kinase.3,4 In a mitotic cell, Pat1 kinase inhibits both Mei2, the key protein for meiotic progression, and Ste11, a transcription factor that regulates meiosis specific genes including mei2+, by phosphorylation.5,6 There are at least two mechanisms for regulation of phosphorylated Mei2. First, the Rad24 protein, one of the homologs of 14-3-3 in S. pombe, binds to phosphoserines of Mei2 and inhibits binding between Mei2 and meiRNA.7 Second, phosphorylated Mei2 is very unstable because it is subject to degradation via ubiquitin-proteolysis.8 In addition to these, Mei2 is regulated at the transcription level through the inactivation of Ste11 by Pat1 and the protein level via phosphorylation by Pat1 kinase.9 If Mei2 is activated in a vegetative growing cell, ectopic meiosis will be induced, and many events need for correct meiosis are skipped, thus cells undergo meiotic catastrophe (haploid meiosis). Ste11 shuttles between the nucleus and the cytoplasm in vegetative growing cells and accumulates in the nucleus in starved cells. The accumulation of Ste11 in the nucleus is regulated by Pat1 activity. If Pat1 is inactivated, Ste11 accumulates in the nucleus even if cells grow in the nutrient rich medium.9

Sla1 is a unique homolog of the human La protein in fission yeast. The human La protein has been identified as being a major target of the autoimmune response in patients suffering from autoimmune diseases. La is also known to be a multifunctional RNA-binding protein implicated in a number of transcriptional and posttranscriptional processes. For example, through its affinity for the 3' oligo (U) tail and 5'-ends, La binds transcripts from RNA polymerase III and thus facilitates the initiation and termination of transcription.10 It has also been reported that Sla1 binds to the 3'-end of pre-rRNA and thereby facilitates its processing.11,12 In addition to these functions, we reported that Sla1 has a novel role in meiosis.13 Sla1ΔC, a truncated form in the C terminus, which was initially obtained from the screening to rescue the ras1ts phenotype,13,14 induces ectopic haploid meiosis. This type of haploid meiosis is unique in pat1 mutants or a strain that expresses mei2-SATA, a conditional activated mutant of mei2. Our previous results have shown that Sla1ΔC binds to Pat1 tightly in Yeast-two hybrid analysis, chromosomal deletion of sla1 lowered temperature sensitivity of pat1ts, and mei2 was absolutely required for haploid meiosis induced by Sla1ΔC.15 These results suggested to us that Sla1ΔC induces haploid meiosis via the Pat1-Mei2 pathway, especially by inactivating Pat1. In this article, we showed further evidence that suggested Sla1ΔC inhibits Pat1 to induce haploid meiosis in vivo.

The S. pombe strains used in this study are SP870 (h0 ade6-M210 leu1-32 ura4-D18),15 KCR51 (h+ mei2+ 3HA(kan') leu1 ura4),8 KCR44 (h0 mei2+ 3HA(kan') leu1 ura4 ade6)8 and KT46 (h+ pat1+ 13myc<leu1+).
ade6-216 leu1 ura4 pat1-114). Yeast media YE (0.5% yeast extract, 2% glucose), YES, EMM, and SD were used to routinely culture S. pombe strains.16,17 We used a high efficiency lithium acetate method with slight modifications to transform S. pombe as described before.13"

It was known that the Mei2 protein accumulated when Pat1 kinase was inactivated, which indicated the amount of the Mei2 protein is dependent on Pat1 kinase activity.5 However, we tested and confirmed that the mei2 mRNA and Mei2 protein in KCR44 strain (mei2+/3HA) accumulated when cells were shifted in the nitrogen-starved medium (Fig. 1 lane4 versus lane5). This is due to the regulation of mei2 mRNA by Ste11, which is regulated by nitrogen starvation at transcriptional level. To further investigate the effects of Sla1ΔC on Pat1 activity, we examined the states of the Mei2 protein and mei2 mRNA when Sla1ΔC was expressed. To test this, KCR51 strain, in which the 3HA-tag was introduced in-frame in the 3' terminus of the chromosomal mei2 gene, was transformed with pREP1, pREP1-sla1FL, or pREP1-sla1ΔC.13 Transformants were grown in EMM+Uracil medium to 5 × 10^6 cells/ml, and crude protein extracts and total RNAs were prepared. Then, Western blot analysis and Northern blot analysis were done. Extraction of crude extracts, Western blot analysis, RNA preparation, and Northern blotting were done as described before.13 As the result of Western blot analysis, the Mei2-3HA protein accumulated when Sla1ΔC was expressed but did not alter when the vector or Sla1FL (Full Length) was expressed (Fig. 1 lane1, 2 versus lane3). This result indicated that only a truncated Sla1 (Sla1ΔC) affected the Mei2 protein level consistently with the phenotype caused by expression of Sla1ΔC. We next did Northern blot analysis and found the mei2 mRNA level accumulated when Sla1ΔC was expressed as well as under nitrogen-starved conditions. This result indicated mei2 mRNA level is also affected by Sla1ΔC, possibly through the state of Ste11, which is regulated by Pat1.

Next, we tested whether accumulation of the Mei2 protein depends only on up-regulation of mRNA level or protein stability or both. It was reported that, in vegetative cells, a half-life of the Mei2 protein was very short because phosphorylated Mei2 was degraded in the ubiquitin-proteasome pathway whereas nonphosphorylated Mei2 was stabilized because this form of Mei2 was not recognized in the ubiquitin-proteasome pathway.8) Then, the KCR51 strain harboring pREP1 or pREP1-sla1ΔC was used to measure a half-life of the Mei2 protein. These strains were grown in EMM+Uracil liquid medium to mid-log phase, and then cytochrome oxidase was added. Samples were taken each one or half hours and crude extracts were prepared. Immuno blot analysis indicated a half-life of the Mei2-3HA protein with an empty vector was short. In contrast, the Mei2-3HA protein expressed with Sla1ΔC became much stabilized (Fig. 2). We considered that the Mei2 protein became dephosphorylated when Sla1 was expressed. As one of the possibilities, we investigated whether this modified band of Mei2 was recognized by ubiquitin to be degraded. Many modified bands of Mei2-3HA were observed when Sla1ΔC was expressed. To test this, we did Northern blot analysis and found the mei2 mRNA level is also affected by Sla1ΔC, possibly through the state of Ste11, which is regulated by Pat1.18 However, we did not see the difference in its modification (data not shown). Thus, this modification of Mei2-3HA was not at least mediated by SUMO.

It was reported that Ste11 shuttles between the
nucleus and the cytoplasm in vegetative growing cells and accumulates in the nucleus when nitrogen was scarce. Ste11 localizes in the nucleus when Pat1 is inactivated. We thought that if Pat1 was inactivated by Sla1ΔC, Ste11 must localize to the nucleus. To confirm this hypothesis, pSLF173-Sla1FL and pSLF173-Sla1ΔC were constructed as follows. A 1.0kb sla1 FL (Full Length) fragment or about 0.75kb sla1ΔC fragment was amplified by PCR. The PCR product was ligated with pT7 Blue(R) vector (Novagen). The Sal I-Sal I fragment from the resulting plasmid was then cloned into the same site of pSLF173 (HA tag). Then, h50 wild type strain was co-transformed with pREP81-GFP-ste11 and pSLF173, pSLF173-sla1FL or pSLF173-sla1ΔC. Transformants were grown in EMM + Adenine liquid medium containing nitrogen, a condition in which Ste11 shuttles between the nucleus and the cytoplasm, to a mid-log phase. As the result, in transformants harboring pSLF173 or pSLF173-sla1FL, GFP-Ste11 localized all over the cell and its fluorescence was very weak in this condition, as reported before. In contrast to this observation, GFP-Ste11 co-expressed with Sla1ΔC accumulated in the nucleus even in the nutrient rich medium (Fig. 3(A)). The fluorescence intensity of GFP-Ste11 in the cell expressed with Sla1ΔC was very strong even though its promoter was the most weak nmt1++. The intensity of GFP-Ste11 expressed with Sla1ΔC was higher than the one when cells were nitrogen-starved. This result supports the idea that Sla1ΔC inactivates Pat1 activity. In addition to our observation that GFP-Ste11 is localized exclusively in the nucleus, especially the nucleolus, we observed GFP-Ste11 formed one or several dot(s) in the nucleolus region or the nucleolus-like body (Fig. 3(A) arrows). Western blot analysis indicated that nuclear-accumulated GFP-Ste11 was modified because its bands shifted up and down unlike a nucleo-cytoplasmic shuttle form of GFP-Ste11 (Fig. 3(B)). In addition to these results, we observed that the intensity of pancellular localization of GFP-Ste11 expressed with Sla1FL was weaker than the one expressed with an empty vector. Western blot analysis also showed that the band of GFP-Ste11 expressed with Sla1FL was weaker than with an empty vector (Fig. 3(B)). We suspected that Sla1 might be related to the stability of the Ste11 protein.

Because it was reported that Pat1 kinase localized in the nucleus, we next tested the possibility that Sla1ΔC may alter the localization of Pat1. For this purpose, a 13-myc tag was introduced in-frame in the 3’end of the chromosomal pat1+ gene. The resulting strain, KT46, was transformed with pSLF173 or pSLF173-sla1ΔC plasmids and transformants were examined for Pat1 localization by immnnofluorescence staining using an anti-myc (9E10) antibody. The cells harboring Sla1ΔC underwent haploid meiosis although its rate was low, and Pat1 was localized in the nucleus as well as the cells harboring empty vector (data not shown). Thus, we concluded that Sla1ΔC did not alter nuclear localization of Pat1 to inactivate its activity.

In this article, we showed some evidence that Sla1ΔC induce haploid meiosis via the Pat1-Mei2 system. Firstly, the Mei2 protein and mei2 mRNA increased by expression of Sla1ΔC. This result indirectly suggested that the function of Ste11 was strengthened. Because the function of Ste11 as a transcription factor is regulated by Pat1 activity, accumulation of the Mei2 protein and mei2 mRNA are considered to be the consequence of the inhibition of Pat1 by Sla1ΔC. Secondly, we showed that the stability of the Mei2 protein was increased by expression of Sla1ΔC over that without. This indicates that Mei2 is activated when Sla1ΔC is expressed, because stable Mei2 is almost a dephosphorylated form. Thirdly, we showed that GFP-Ste11 accumulated in the nucleus when Sla1ΔC was expressed in the nutrient-rich medium. The nuclear accumulation of Ste11 requires two conditions, namely nitrogen starvation and pheromone signaling, but pat1 inactivation bypasses these requirements for localization of Ste11. From all these results and previous results such as a two-hybrid experiment between Pat1 and Sla1ΔC with some other genetic results, it supports the idea that the functional point of Sla1ΔC is the inactivation of Pat1. But, to confirm this idea, further analysis will be necessary to show the direct inhibition of Pat1 by Sla1ΔC. Such an effort is currently under way.

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