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Cleavage and polyadenylation factor, Rna14 is an essential protein required for the maintenance of genomic integrity in fission yeast *Schizosaccharomyces pombe*

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ABSTRACT

Faithful segregation of chromosomes is essential for the maintenance of genome integrity. In a genetic screen to identify genes related to checkpoint function, we have characterized the role of *rna14*, an essential gene in the maintenance of chromosome dynamics. We demonstrate that Rna14 localizes in the nucleus and in the absence of functional Rna14, the cells exhibit chromosomal segregation defects. The mutant allele of *rna14* exhibits genetic interaction with key kinetochore components and spindle checkpoint proteins. Inactivation of *rna14* leads to accumulation of Bub1-GFP foci, a protein required for spindle checkpoint activation that could be due to the defects in the attachment of mitotic spindle to the chromosome. Consistently, the double mutant of *rna14-11* and *bub1* knockout exhibits high degree of chromosome mis-segregation. At restrictive condition, the *rna14-11* mutant cells exhibit defects in cell cycle progression with high level of septation. The orthologs of Rna14 in *Saccharomyces cerevisiae* (sc Rna14) and human (CstF3) contain similar domain architecture and are required for 3′-end processing of pre-mRNA. We have also demonstrated that the fission yeast Rna14 is required to prevent transcriptional read-through. These findings reveal the importance of transcription termination in the maintenance of genomic stability through the regulation of kinetochore function.

1. Introduction

The faithful segregation of chromosomes is essential for cell viability. Mitosis with abnormal chromosome segregation can lead to aneuploidy, a hallmark of cancer and several congenital diseases. Proper chromosomal segregation is achieved by the spindle assembly checkpoint (SAC) pathway which is a conserved surveillance mechanism that arrests cells in mitosis in response to defective spindle [1]. Lack of tension on kinetochore or unattached kinetochore activates the spindle checkpoint and prevents the anaphase onset, exit from mitosis and initiation of cytokinesis [2,3]. The mitotic checkpoint complex (MCC) comprises of BubR1, Bub3, Mad1, Mad2, Mps1 and Cdc20 proteins that inhibit the anaphase-promoting complex/cyclosome (APC/C), a large complex containing E3 ubiquitin ligase required for anaphase onset [4].

Kinetochore, a specialized protein structure within the centromeric region is required for the attachment of spindle microtubules to the chromosomes [5]. The integrity of the centromeric chromatin and the proteins associated with kinetochore structure play an important role in maintaining chromosome stability. Mis12 is an evolutionarily conserved kinetochore protein that forms a complex with Mis13 and Mis14 [6,7]. The KMN complex, containing Knl1–Mis12–Ndc80 proteins provides the core binding site within the kinetochore required for the microtubule attachment [8,9]. Mis6, another kinetochore protein form a complex with Mis15, Mis17, and Sim4 promoting the loading of CENP-A to the centromere [10–12]. Mis6 has also been reported for the loading of the mitotic spindle checkpoint protein Mad2 at the kinetochore [13]. In fission yeast, the mutation in the components of kinetochore complex leads to unequal chromosome segregation [14,15].

After transcription by RNA polymerase II, post-transcriptional modifications are required to convert nascent RNA into mature RNA. These modifications include the addition of the 5′ cap, splicing, 3′ end cleavage and polyadenylation. Precise action of these modification steps plays important roles in the stabilization of RNA, export from the nucleus, and translation stimulation [16,17]. In eukaryotic cells, a multi-subunit protein complex is responsible for 3′ end processing. This complex includes cleavage and polyadenylation factor (CPSF) and cleavage stimulating factor (CstF) which are conserved in different eukaryotes [18–20]. Four distinct factors have been identified in *Saccharomyces cerevisiae* that are essential for cleavage and polyadenylation. These include cleavage factor I (CF I), CF II, polyadenylation factor I (PF I) and poly A polymerase I (PAP I) [21]. CFIA subunit of CFI contains Rna14, Rna15, Pcf11 and Clp1 while PFI subunit consists of many proteins including Fip1, Pcf11, Psf1, Psf2, Yth1, etc. [22,23]. Rna15 contains an RNA recognition

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module (RRM) at the N-terminus [24], followed by a hinge region and a C-terminal domain (CTD), which interacts with Pcf11 [25] while the hinge region interacts with the C-terminal region of Rna14 [26]. *S. cerevisiae* Rna14 contains several Half-A-TPR (HAT) repeats and is essential for 3′ end processing [27]. Cleavage and polyadenylation defects can lead to transcription beyond the normal termination region, potentially disrupting the functions of genes and downstream pathways.

In this study, we have described the role of Rna14, a cleavage and polyadenylation factor during cell cycle progression. We have demonstrated that the inactivation of Rna14 in fission yeast causes chromosome segregation defects that result in high level of chromosome loss. We present evidence that a functional spindle checkpoint response is required for the cells to survive after *rna14* inactivation. These findings indicate the importance of transcription termination for the maintenance of genomic stability.

2. Materials and methods

2.1. Yeast strains and media

Schizosaccharomyces pombe strains used in this study are listed in Table 1. We have utilized standard genetic methods for the construction of strains [28]. For temperature shift experiments, cells were grown at 25 °C and shifted to restrictive temperature of 36 °C unless otherwise indicated. For survival assays, samples were collected, and the equal number of cells from each sample was plated on YEA plates. The plates were incubated at permissive temperature; the number of surviving colonies was counted, and graph was plotted. For serial dilution assays, 10^7 cells were serially diluted, spotted on YEA plates and incubated at indicated temperature for 3–4 days.

2.2. Construction of rna14 knockout

The *kanMX* selectable cassette flanked by 80 base pair upstream and downstream sequences of *rna14* was amplified using oligonucleotides listed in Table 2. One-step gene disruption via homologous recombination was performed in a diploid strain as reported earlier [29]. Heterozygous diploid transformants containing *rna14* deletion were selected on plates containing 100 μg/ml of G418. Deletion was confirmed with primer pairs spanning the recombination site using wild type *rna14* gene as a negative control. Heterozygous diploids were sporulated, and tetrad dissection was performed to check the viability and essentiality of the gene.

Table 1
Strains list.

Strain	Genotype	Source
SP6	*h⁻ leu1-32*	Lab stock
SH14	*h⁻ leu1-32 rna14-11 ade6-210*	This study
SH74	*h⁺ leu1-32 ura4D18 rna14-11 ade6-210*	This study
SH73	*h⁻ ura4D18 Chr16* [*ade6-216*] *ade6-210*	Nancy Walworth
SH78	*h⁺ ura4D18 rna14-11 Chr16* [*ade6-216*] *ade6-210*	This study
NW1580	*h⁻ leu1-32 mis12-537*	Nancy Walworth
NW1419	*h⁻ leu1-32 ura4DS/E mad2::ura4 ade6-210*	Nancy Walworth
SH270	*h leu1-32 rna14-11 mis12-537 Chr16*[*ade6-216*] *ade6-210*	This study
SH104	*h⁻ leu1-32 ura4D18 rna14-11 mad2::ura4 ade6-210*	This study
SH202	*h⁻ leu1-32 rna14-Flag-kan^R*	This study
SH495	*h⁻ leu1-32 bub1::kan^R*	This study
SH513	*h leu1-32 ura4D18 rna14-11 bub1::kan^R*	This study
SH528	*h⁺/h⁻ leu1⁻/leu1⁻ ura4D18/ura4D18 rna14⁺/rna14::kan^R ade6-210/216*	This study
SH644	*h⁻ leu1-32 ura4D18 bub1-GFP-ura4⁺*	YGRC
SH693	*h leu1-32 ura4D18 rna14-11 bub1-GFP-ura4⁺*	This study

Table 2
Oligonucleotides used in study.

Name	Sequence (5′–3′)
Ura4F	TCGGCTTGGATGTTAAAGGAG
Ura4R1	TGCCTTCTGACATAAAACGCC
Uar4R2	ATTGTGGTAATGTTGTAGGAGC
Uar4R3	TTCCAACACCAATGTTTATAACC
Tbp1/F	TATGAGCCTGAGTTGTTTCC
Tbp1/R1	TTCTCCGGAAAGCTTTTTAAG
Tbp1/R2	TCAGCCTCTATAGTTTTCTTG
Tbp1/R3	CCTGAAGCTAGAAGATTTAATG
Tbp1/R4	AGAACTGTCGATATACGCTC
Tbp1/R5	CCTTCTATTAGCGCTATTAAG

2.3. RT-PCR analysis

Wild type and mutant strains were grown at 25 °C and shifted at 36 °C for 4 h. RNA was isolated by hot phenol method as described earlier [30]. A total of 1.0 μg RNA was treated with DNase I (NEB) and cDNA synthesis was performed using oligo dT primer (NEB). Reverse-transcribed cDNA products were amplified by PCR using gene specific primers as listed in Table 2.

2.4. Chromosome loss assay

The minichromosome Ch16 [31] was introduced into *rna14-11* mutant strain using genetic cross. Cultures were grown to mid-log phase in adenine deficient media and then shifted at 36 °C for 15 h. In order to determine the frequency of chromosome loss, samples were collected at 3 h intervals and plated on YEA plates with limiting adenine. The number of red colonies appearing at each time point was counted, and the percent chromosome loss was calculated.

2.5. Indirect immunofluorescence studies and microscopy

Exponentially growing cells were processed for immunofluorescence studies as described earlier [32]. Cells harboring a copy of Rna14-FLAG at its genomic locus were incubated with FLAG antibody (Sigma) for overnight at 4 °C. After washing, the cells were further incubated at room temperature for 4 h with secondary antibody coupled to Texas Red (Invitrogen). DAPI (4′,6-diamidino-2-phenylindole) was used to stain the nuclei; images were captured using a fluorescence microscope and processed using Adobe Photoshop. For nuclear analysis, mid-log phase culture was shifted at 36 °C; samples were collected and fixed with 70% ethanol. Nuclei were stained with DAPI and visualized using a fluorescence microscope. Approximately 200 cells from each sample were analyzed, and the percentage of cells containing aberrant nuclei was counted. For septation index, cells were synchronized in early S phase by treating with 12 mM hydroxyurea (HU) and released at 36 °C, samples were collected at 30 min intervals, fixed with 70% ethanol and stained with calcoflour. At each time point at least 200 cells were examined by microscopy, and the cells containing the septum were counted.

3. Results

3.1. Fission yeast Rna14 has conserved domain architecture

Fission yeast Rna14 is a subunit of mRNA cleavage and polyadenylation specificity factor complex and its ortholog in budding yeast has been shown to be required for endonucleotic cleavage and polyadenylation of pre-mRNA. A search using InterPro protein sequence analysis and classification software at EMBL-EBI website identified three tetratricopeptide-like helical domains containing nine HAT (Half a TPR) repeats in fission yeast Rna14 (Fig. 1). Tetratricopeptide repeat