The Nuclear Exosome Is Active and Important during Budding Yeast Meiosis

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Abstract

Nuclear RNA degradation pathways are highly conserved across eukaryotes and play important roles in RNA quality control. Key substrates for exosomal degradation include aberrant functional RNAs and cryptic unstable transcripts (CUTs). It has recently been reported that the nuclear exosome is inactivated during meiosis in budding yeast through degradation of the subunit Rrp6, leading to the stabilisation of a subset of meiotic unannotated transcripts (MUTs) of unknown function. We have analysed the activity of the nuclear exosome during meiosis by deletion of *TRF4*, which encodes a key component of the exosome targeting complex TRAMP. We find that TRAMP mutants produce high levels of CUTs during meiosis that are undetectable in wild-type cells, showing that the nuclear exosome remains functional for CUT degradation, and we further report that the meiotic exosome complex contains Rrp6. Indeed Rrp6 over-expression is insufficient to suppress MUT transcripts, showing that the reduced amount of Rrp6 in meiotic cells does not directly cause MUT accumulation. Lack of TRAMP activity stabilises ~1600 CUTs in meiotic cells, which occupy 40% of the binding capacity of the nuclear cap binding complex (CBC). CBC mutants display defects in the formation of meiotic double strand breaks (DSBs), and we see similar defects in TRAMP mutants, suggesting that a key function of the nuclear exosome is to prevent saturation of the CBC complex by CUTs. Together, our results show that the nuclear exosome remains active in meiosis and has an important role in facilitating meiotic recombination.

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coinciding with DNA replication and the induction of meiotic double strand breaks. This process may have a parallel in fission yeast where many meiotic genes are expressed during mitosis but are degraded by the exosome [42]. In mitosis, hexanucleotide motifs in meiosis-specific mRNAs are bound by the meiotic regulator Mmi1, which recruits a nuclear silencing complex that interfaces with the exosome [43–45]. Degradation of meiotic mRNA requires polyadenylation, and cells lacking the nuclear poly(A) binding protein Pab2 or carrying mutations in the canonical poly(A)-polymerase Pla1 accumulate meiotic transcripts during

start of meiosis so the effects of Rrp6 depletion on nuclear exosome activity should be detectable throughout meiosis.

Lardenois et al. suggested that Rrp6 protein levels are specifically reduced during meiosis through translational inhibition or proteolytic degradation [41]; we reasoned that if so then MUTs which become detectable early in meiosis should be destabilised by over-expression of Rrp6. To this end, we constructed an estradiol-inducible Rrp6 over-expression strain by introducing a heterozygous *GFP-RRP6* construct under a *GAL1* promoter into a strain expressing an ER-Gal4 fusion. This system allows estradiol-inducible expression of genes with a *GAL1* promoter during meiosis [57]. The *GAL1*-driven fusion was functional as dissected spores from this strain accumulated the characteristic 5.8S+30 rRNA fragment on glucose but not on galactose (Figure S4A). Meiosis was induced in this strain in the presence or absence of estradiol and expression of *RRP6* and two MUTs was analysed by RT-PCR. Although Rrp6 was very strongly induced and produced full length protein, the MUTs were not destabilised (Figures 3B, S4B), showing that reduced Rrp6 levels are not responsible for MUT stabilisation in meiosis.

To test for other changes in the meiotic exosome complex, we purified the exosome from mitotic and meiotic cells through a

TAP tag on the core exosome 70.7(fra76h0.6(exor347(i74.8(estCsl4.rain)]4

Figure 3. Exosome characterisation in meiotic cells. A: Western blot for Rrp6-TAP and Pgk1. Left-hand blot compares Rrp6 in log phase YPA and at the initiation of meiosis; right-hand blot, shown at different exposure, shows the gradual decline in Rrp6 levels across meiosis. B: Analysis of the effect of estradiol-induced GFP-Rrp6 over-expression on MUT stability. RT-PCR reactions for *RRP6* and MUTs were performed on RNA from cells without and with estradiol, compared to an *SCR1* loading control. *MUT 100* is expressed throughout meiosis whereas *MUT 523* is only expressed after 8 hours, neither is repressed by Rrp6 overexpression. C: Silver-stained protein gel showing Csl4-TAP immunoprecipitations from meiotic and mitotic cells, compared to purifications from untagged strains. Mitotic cells were grown on YPD, meiotic cells were harvested after six hours in SPO media. D: Plots of peptide score vs. molecular weight for proteins identified by mass spectrometry in two independent immunoprecipitation experiments. Non-yeast proteins and proteins also discovered in the untagged control sample were discarded, then the proteins were divided into exosomal and non-exosomal sets, both of which are displayed. Key exosome proteins including Rrp6 are highlighted.
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ciently abundant to be detected as truncated fragments migrating below the main chromosome band on PFGE gels probed for short chromosomes. PFGE analysis for cleaved fragments of chromosome III in wild-type cells revealed a transient peak after four hours of meiosis that was much weaker in *trf4Δ* mutants (Figure 4A, compare lanes 4 and 10). However, a transient peak in *trf4Δ* cells would be missed if it did not coincide with a sampling time, and we therefore repeated this assay in a *sae2Δ* background in which double strand breaks cannot be repaired [59,60]. In *sae2Δ* cells, cleaved fragments accumulated between 2 and 8 hours of meiosis, reaching a plateau at 8–24 hours (Figure 4B lanes 1–6). This pattern was replicated in *sae2Δ trf4Δ* mutants but a significantly smaller percentage of chromosome III was cleaved (Figure 4B,C); this is important as chromosomes that do not form at least one DSB have a high likelihood of mis-segregation at meiosis I. Although Trf4 is an RNA processing protein, we could detect no defect in the expression of key recombination factors by RT-PCR (Figure 4D), suggesting that this was not caused by a specific gene expression defect. Therefore, although cells lacking TRAMP activity appear to progress normally through meiosis, they show significant defects in meiotic recombination.

Discussion

Here we have reported a detailed analysis of TRAMP function during meiosis. We have demonstrated that TRAMP targets widespread CUTs for degradation in meiotic cells just as it does in mitotic cells. We have also shown that TRAMP facilitates meiotic DSB formation, providing an important physiological role for TRAMP activity.

It has been reported that meiotic cells undergo Rrp6 degradation, resulting in a loss of nuclear exosome function and the stabilisation of MUTs [41]. Such a process would be very surprising as the exosome is a highly conserved and ubiquitously expressed complex; Rrp6 is involved in ribosomal RNA synthesis and quality control [7,13,21], and many eukaryotes including *S. cerevisiae* perform ribosome re-synthesis during meiosis for which quality control mechanisms would seem vital [61–64]. We find

that Rrp6 levels are much reduced in meiotic cells, but this appears to coincide with a general down-regulation of exosome levels in pre-meiotic cells grown to high density. Purified exosome from meiotic yeast contains Rrp6 and the meiotic exosome appears fully functional for CUT degradation. Furthermore, the re-expression of Rrp6 is insufficient to destabilise MUT transcripts, showing that the stabilisation of MUTs in meiosis cannot be attributed to a lack of Rrp6. In *S. pombe*, meiosis-specific mRNAs are degraded during mitosis by Mmi1 and the exosome [42,44,48]; sequestration of Mmi1 is a critical step in meiotic initiation, and we suggest that in *S. cerevisiae* an as-yet unidentified mitosis-specific factor directs MUT degradation by the exosome in mitosis but not meiosis.

We find that loss of TRAMP activity impedes meiotic DSB formation, showing that RNA quality control does play an important role in meiosis. A reduction in meiotic DSB formation would be expected to increase rates of chromosome mis-segregation and aneuploidy, and is consistent with the previously reported defects in *trf4*Δ meiosis [51,52]. Such an increase was not observed when assessed by Petronczki *et al*

cells/ml and incubated at 25°C with shaking at 250 rpm. Media components were purchased from Formedium and Sigma.

Reads were mapped to the yeast reference genome (SGD1.01) or a custom assembled SK1 genome using Bowtie [76], allowing either unique mapping reads only or allowing non-unique reads to map at random respectively. For analysis, reads were summed in 100 bp segments spanning the genome using SeqMonk (http://www.bioinformatics.babraham.ac.uk/projects/seqmonk/), and reads from the 37S pre-rRNA were filtered out of the analysis as these represent an abundant contaminant of non-Cbc2-bound transcript. Total read-count normalisation was then applied to account for differing sequencing depths (16 million mapped reads for wild type, 21 million for *trf4*Δ). Analyses were performed using an R script (File S1). Sequencing data is deposited at GEO, accession number GSE60221.

Protein Extraction and western blotting

2×10^7 cells were washed once with water and resuspended in 100 μl water. 15μl 2 M NaOH with 80 mM DTT was added, the suspension was mixed by vortexing and incubated for 10 minutes on ice. 15μl 50% TCA was added and the suspension vortexed and incubated for a further 10 minutes on ice. Samples were centrifuged for 2 minutes at 10,000 *g* and the pellet was washed with acetone and dried for 10–20 minutes at room temperature. The pellet was resuspended in 20 μl sample buffer (100 mM Tris pH 6.8, 2% SDS, 0.1% Bromophenol Blue, 10% glycerol, 100 mM DTT), vortexed and boiled at 95°C for 5 minutes. Proteins were separated on 8% or 12% polyacrylamide gels and transferred to a nylon membrane (LI-COR) using the NOVEX system (Invitrogen). Antibody staining was performed using standard methods for HRP or fluorescent detection (see protocols at www.cellsignal.com) and imaged using film or a LI-COR Odyssey system. Primary antibodies: mouse anti-Pgk1 (Invitrogen 459250) 1:10,000, rabbit anti-TAP (Open Biosystems CAB1001) 1:200-1:1000 depending on the batch, peroxidase anti-peroxidase (Sigma P1291) 1:5000, rabbit anti-GFP (Abcam ab290) 1:2000.

Co-immunoprecipitation

IP method 1: 1.75×10^{10} log phase or 4×10^{10} sporulating cells were harvested and washed with PBS. The cells were resuspended in one pellet volume of lysis buffer (50 mM HEPES pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, cOmplete Mini EDTA-free Protease Inhibitors) and frozen drop-wise on liquid nitrogen. Cells were ground to a fine powder in a pestle and mortar under liquid nitrogen and thawed in a 50 ml tube on ice. The lysate was centrifuged for 5 min at 4500 rpm and the supernatant clarified by centrifugation for 20 min at 30,000 *g*. Cleared lysate was transferred to a 15 ml tube and incubated with 250/100 μl (log

in wild-type and trf4D cells assayed by northern blot. Graph shows average of data from two independent experiments, error bars indicate ±1 s.d.
(TIF)

F g e S2 Va da f Cbc2-TAP ec a c . A: Western blot showing purification of Cbc2-TAP from meiotic wild-type and trf4D cells. After lysis and clarification, a sample was taken for total protein (lanes 3,4) while the remaining sample was subjected to a two-step TAP purification protocol. Lanes 1,2 show lysate after binding to IgG beads, lanes 5,6 and 9,10 show material remaining on IgG and calmodulin beads after elution. Lanes 11,12 show final product. B: Northern blot of total, unbound and Cbc2-TAP associated RNA from meiotic wild-type and trf4D cells probed for NEL025, ACT1 and 18S. C: Scatter plot of log-transformed read counts from Cbc2-associated RNA isolated from wild-type and trf4D cells after six hours of meiosis. Red dots indicate points from the region Chr. V:10–40 kb that is shown in Figure 2C.
(TIF)

F g e S3 C e- de d b f Cbc2-a - c a ed RNA. Distributions of Cbc2-associated RNA in wild-type and trf4D cells across chromosomes I, II, III and VI, as Figure 2D.
(TIF)

F g e S4 Ac f P_{GAL}-GFP-RRP6 c c . A: Northern blot of RNA from spores of the P_{GPD1}-GAL4-ER P_{GAL1}-GFP-RRP6 strain grown to mid-log in YPD or YPGal media. RNA was separated on an 8% denaturing PAGE gel before probing for 5.8S+30, a 39extended 5.8S processing intermediate that accumulates in rrp6D mutants. Ethidium staining of 5S and 5.8S is shown as a loading control. The strain is heterozygous for P_{GAL1}-GFP-RRP6, and therefore two out of four spores accumulate 5.8S+30 when grown in glucose (where P_{GAL1} is repressed) but not in galactose. B: Western blot showing that full length GFP-Rrp6 protein is produced after estradiol induction, in addition to some degradation products. Proteins were separated on an 8% gel and probed for GFP. Ponceau-stained total protein on the same membrane is shown as a loading control. FL indicates the full length protein band.
(TIF)

Tab e S1 CUT a c de f ed e c ce . Meiotic transcripts over-represented in trf4D cells compared to wild-type. Cbc2-associated RNA was isolated from cells at meiosis t = 6 hours and sequenced, reads were collected in 100 bp bins and regions of three or more consecutive bins over-represented by at least 4-fold in trf4D RNA were annotated as CUTs, see materials and methods for more information. Enrichments are quoted as log_2 of actual values.
(XLS)

Tab e S2 Yea a ed d .
(XLS)

Tab e S3 O g c e de ed d .
(XLS)

Tab e S4 H b d a be ed d .
(XLS)

F e S1 R c f a a f K e c g da a. Script used to normalise read counts and discover CUTs, executed in R v3.0.2.
(TXT)

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Author Contributions

Conceived and designed the experiments: SF JH. Performed the experiments: SF JH. Analyzed the data: SF DO JH. Contributed to the writing of the manuscript: SF JH.

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