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Loss of the Mediator subunit Med20 affects transcription of tRNA and other non-coding RNA genes in fission yeast

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ABSTRACT

Mediator is a co-regulator of RNA polymerase II (Pol II), transducing signals from regulatory elements and transcription factors to the general transcription machinery at the promoter. We here demonstrate that Med20 influences ribosomal protein expression in fission yeast. In addition, loss of Med20 leads to an accumulation of aberrant, readthrough tRNA transcripts. These transcripts are polyadenylated and targeted for degradation by the exosome. Similarly, other non-coding RNA molecules, such as snRNA, snoRNA and rRNA, are also enriched in the polyadenylate preparations in the absence of Med20. We suggest that fission yeast Mediator takes part in a regulatory pathway that affects Pol III-dependent transcripts.

1. Introduction

Nuclear genes are transcribed by three distinct RNA polymerases, which differ in their promoter and cofactor requirements. RNA Polymerase I (Pol I) produces most of the ribosomal RNA (rRNA) [1]. RNA Polymerase II (Pol II) transcribes all protein coding genes (mRNA), as well as most of the small nuclear RNAs (snRNAs) involved in splicing [2]. RNA Polymerase III (Pol III) transcribes transfer RNA molecules (tRNAs), 5S rRNA, snRNA U6, and many small nucleolar RNAs (snoRNAs) involved in the chemical modification of other RNA molecules [3].

During promoter specific initiation of transcription, Pol II is assisted by a set of general transcription factors and the reaction is stimulated by gene specific transcription activators. The multiprotein Mediator complex functions as a molecular interface, which conveys regulatory information from these gene specific transcription factors to the general transcription machinery at the promoter [4,5]. The complex is required for both basal and regulated expression of nearly all Pol II-dependent genes in *Saccharomyces cerevisiae* [6]. Mediator can be divided into three distinct sub-modules: head, middle and tail regions [7]. Together they embrace the globular Pol II and form an extended structure. There is also a fourth submodule, the Cdk8 module, which blocks Pol II interactions [8,9]. Only Mediator devoid of this sub-module can associate with Pol II and form a holoenzyme complex [10].

Two evolutionary conserved Mediator components, Med18 and Med20, form a heterodimeric protein subcomplex that is localized to the Mediator head module [11]. Med18-Med20 can interact with the TATA box binding protein (TBP), which in turn leads to structural changes in Mediator that affects the ability of the complex to interact with Pol II [12,13]. The observed interactions with components of the basal transcription machinery may explain the ability of Med20 to act as a repressor of Pol II transcription [11]. Med20 is also involved in repression of centromeric ncRNA transcription, which is important for centromeric heterochromatin formation in fission yeast [14–16].

The RNA levels in a cell depend on a delicate balance between de novo transcription and transcript degradation. One of the key factors in RNA degradation is the evolutionary conserved exosome complex, which contains 10 subunits (exo-10) and is found in both the cytoplasm and the nucleus [17–19]. The main catalytic subunit of the exosome is Dis3 which utilizes both endo- and exonuclease activity to degrade RNA. In the nucleus, the exosome also recruits a 3′-5′ exoribonuclease, Rrp6, leading to the formation of exo-11 [18]. The exosome is involved in the degradation of transcripts produced by all three nuclear RNA polymerases [20–22]. In fact, a recent study demonstrated that the *S. cerevisiae* exosome degrades both mature- and pre-tRNA [23]. Both Dis3 and Rrp6 are required for tRNA degradation, whereas snRNA and snoRNA are primarily processed and degraded by Rrp6. In order for a transcript to be recognized by the exosome, transcripts are polyadenylated by the TRAMP complex, which in *Schizosaccharomyces pombe* contains the non-canonical Cid14 poly(A) polymerase (*S. cerevisiae* Trf4) [24]. TRAMP is involved in both tRNA quality control and tRNA editing [25,26].

Fission yeast contains 171 nuclear tRNA genes (www.pombase.org). The length of these transcripts varies between 70 and 100 nt and only a minority of tRNAs have introns. Pol III transcription is terminated when

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the polymerase encounters a transcription termination signal characterized by a short stretch of thymine nucleotides [27]. The Pol III transcription process is negatively regulated by the transcription factor Maf1 [28]. In budding yeast, this protein responds to the nutritional status of the cell and the TOR (target of rapamycin) signaling pathway influences its activity [29]. Nutritional stress causes a dephosphorylation and nuclear translocation of Maf1, which in turn represses Pol III transcription [28,30]. Maf1 is evolutionary conserved, from yeast to mammals, but its function in fission yeast has not yet been studied in detail [31].

We here demonstrate that loss of the Mediator subunit Med20 causes increased transcription of ribosomal protein genes. Interestingly, loss of Med20 also affects Pol III transcription, leading to formation of aberrant and unstable tRNA transcripts that are polyadenylated and targeted for degradation by the exosome. Our data suggest that Mediator may help coordinate expression of ribosomal protein genes with Pol III transcription.

2. Materials and methods

2.1. Yeast strains

S. pombe strains used in this study are listed in Table 1. If not stated, yeast cells were propagated on YEL and sporulated on SPAS, according to standard methods. Rapamycin (0,05 μg/ml) sensitivity assays were performed using 10^6 cells per milliliter in a 10 × dilution series. Five μl of the cells in the respective dilution series were plated onto the appropriate media and incubated for 4–5 days at 25 or 30 °C.

2.2. RNA-Seq and analysis

Sequencing analysis was performed using three biological repeats. Beads with oligo(dT) were used to isolate poly(A) mRNA from total RNA. Poly(A) mRNA was reverse transcribed using random primers (Invitrogen) and Superscript III (Invitrogen). Then double-stranded (ds) cDNA synthesis was performed with the incorporation of dUTP in the second strand. The ds cDNA fragments were further processed following a standard sequencing library preparation scheme tailored for the Illumina sequencing platform: end polishing, A-tailing, adapter ligation, and size selection. Prior to final amplification, Uracil-DNA-Glycosylase (UDG) was used to selectively degrade the dUTP-marked strand. The remaining strand was amplified to generate a cDNA library suitable for sequencing. Strand specific RNA-seq was size-selected during library building to an average fragment size of 200 nt. The quality check of the reads was done by generating QC statistics with FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc). Read alignments to the NCBI assembly of the *S. pombe* genome were performed using Bowtie (version 0.12.7) [32] or the Burrows–Wheeler Aligner (BWA) [33]. Mismatches were handled using –N 0 (mismatches in seed =0) and multiple alignments using setting –M (search for multiple alignments, report best). Number of mapped reads varied between 26,308,998 and 29,842,589. The dataset was deposited in the GEO database with accession number GSE73792.

Table 1
Yeast strains used in this study.

Name	Description	Genotype
CG1	wt	h- L972 h- Leu1-32 His2 Ura4 DS/E
CG303	*rrp6*Δ	216 rrp6Δ::KanMx6 Otr1R(Sph1)::ura+
CG418	*med20*Δ	h + leu1-32 ura4-D18 med20:NAT
CG422	*med20*Δ/*rrp6*Δ	med20:natMX rrp6Δ::KanMx6 leu1-32 ura4-D18
CG495	*maf1.pk*	maf1::maf1.pk
CG496	*maf1.pk med20*Δ	maf1::maf1.pk med20::natMX
CG497	*maf1*Δ	maf1::natMX
CG498	*maf1*Δ/*rrp6*Δ	maf1:natMX rrp6::kanMX
CG526	*maf1*Δ/*med20*Δ	maf1:natMX med20::natMX

2.3. Analysis of polyA-containing reads

Illumina read files in fastq format were converted to a BLAST [34] database format. BLAST was then used to search these databases using as query sequences *S. pombe* non-coding RNAs (such as tRNAs) where each mature RNA sequence was flanked by 300 nt on each side. The output from BLAST was then analyzed using in house Perl scripts to identify reads with potential poly(A) tails. As an alternative approach reads of the original fastq file was analyzed with respect to potential poly(A) tails. Each read with such a potential poly(A) tail was trimmed with respect to the polyA tail. Both trimmed and non-trimmed reads were then aligned to a database of non-coding RNA genes using BWA. All cases where the trimmed read matched, but the non-trimmed read did not, were regarded as poly(A)-containing ncRNA candidates. Poly(A)-containing reads were finally aligned to the non-coding RNA gene to obtain alignments like that shown in Fig. 4A .

2.4. Differential expression

Illumina read files in fastq format were filtered and trimmed using the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html). Reads were then aligned using BWA against a database constructed by extraction of all annotated regions of the *S. pombe* genome. The results of this alignment were processed and used as input to DESeq [35] of the Bioconductor R package. To adjust for multiple testing, the Bejamin-Hochberg procedure was applied. Genes with adjusted p-value less than 0.05 was used for further analysis. Gene Ontology analysis was performed using Gene Ontology Term Finder from Princeton University (http://go.princeton.edu/cgi-bin/GOTermFinder) [36].

2.5. Western blot

Western blot was performed using anti-V5 (MCA1360GA, AbDserotec) and anti-α-tubulin (T5168 Sigma Aldrich). Quantification of phosphorescence in Maf1-PK was normalized to tubulin.

2.6. RT-PCR and northern blot

Yeast cell was grown in YEA medium to mid-exponential phase (OD_{600} of about 1.0). Total RNA was isolated by a standard hot phenol method [37]. RNA samples were cleaned up using RNAeasy mini kit (Qiagen). Each sample of RNA was treated with RNase-free DNaseI using Turbo DNA-free kit (Ambion). For first-strand cDNA synthesis, 9 μg of RNA was mixed with 4.5 μg of random hexamers and incubated at 70 °C for 10 min, then transferred on ice. The synthesis included 20 U of SuperScript II Reverse Transcriptase, 50 mM Tris–HCl, 75 mM KCl, 3 mM MgCl2, 0.01 M DTT, 0.25 mM dNTPs mix (Invitrogen), in a total volume of 20 μl at 42 °C for 1 h. cDNA was used for Real-time PCR analysis (Bio-Rad) using three biological repeats. The name and sequences of the primers used are listed in Table 2. Quantization was performed using real time PCR Software (Bio-Rad) and Excel (Microsoft). Biological samples were averaged and standard deviations were

Table 2
Primers used in this study.

Name	Assay	Sequence
ILE.02 FP	QT-PCR	GGCCTCATGCTTATATGATACT
ILE.02 RP	QT-PCR	GGAGGGTAACGTGATAGATTT
ILE.02 IRP	Northern Blot	CTCGCGATTTCCACGTAACA
act1 FP	QT-PCR	GGTTTCGCTGGAGATGAT
act1 RP	QT-PCR	ATACCACGCTTGCTTTGA
18S FP	QT-PCR	TTTCTAGGACCGCCGTAATG
18S RP	QT-PCR	TCGCAGTAGTTCGTCTTCAATAA