

Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control



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Trf4 is the poly(A) polymerase component of TRAMP4, which stimulates nuclear RNA degradation by the exosome. We report that in *Saccharomyces cerevisiae* strains lacking Trf4, cryptic transcripts are detected from regions of repressed chromatin at telomeres and the rDNA intergenic spacer region (IGS1-R), and at *CEN3*. Degradation of the IGS1-R transcript was reduced in strains lacking TRAMP components, the core exosome protein Mtr3 or the nuclear-specific exosome component Rrp6. IGS1-R has potential binding sites for the RNA-binding proteins Nrd1/Nab3, and was stabilized by mutation of Nrd1. IGS1-R passes through the replication fork barrier, a region required for rDNA copy number control. Strains lacking Trf4 showed sporadic changes in rDNA copy number, whereas loss of both Trf4 and either the histone deacetylase Sir2 or the topoisomerase Top1 caused dramatic loss of rDNA repeats. Chromatin immunoprecipitation analyses showed that Trf4 is co-transcriptionally recruited to IGS1-R, consistent with a direct role in rDNA stability. Co-transcriptional RNA binding by Trf4 may link RNA and DNA metabolism and direct immediate IGS1-R degradation by the exosome following transcription termination.

The EMBO Journal (2007) 26, 4996–5006. doi:10.1038/emboj.2007.7601921; published online 15 November 2007

Subject Categories: Cell Biology & Molecular Biology; RNA

Keywords: RNA; rDNA; telomeres; exosome; poly(A)

(Houseley et al, 2006). A 4
A 5, (Houseley et al, 2004;
et al, 2005; Houseley et al, 2005)
MA (Houseley et al, 2006; Houseley et al,
2006). (Houseley et al, 2006),
(Houseley et al, 2006), (Houseley et al, 2005;
A (Houseley et al, 2006; Houseley et al, 2006;
Houseley et al, 2006). A (Houseley et al, 2006) MA

Introduction

10
3' 5' MA
MA
(Houseley et al, 1997)

... A top1D ... top1
 trf4 ... (... et al, 1995;
 ... et al, 1996). ... sir2D ... top1D
 ... I 1-, I 1- ... I 2-
 (... 3, ... 6 ... 7).
 ... I 1- ... I 2- ... A
 ... I 1-
 ... trf4D ... air1D
 air2D ... rrp6D ... I 1-
 trf5D ... 5 ... A
 ... trf4D ... (... 3,
 ... 3 ... 4). ... 5
 ... I 1- ... 4. ... I 1-
 ... rrp6D
 ... trf4D (... 3 (... 5) ... (... 3))
 ... mtr3-1
 (... 3). ... (25°), ...
 ... I 1-

()22.2(3), 1.138-1.274
 I 2- I 1-
 I 1-
 I 2- I 1-
 A 4 6.

IGS1-R is polyadenylated and shows extensive 3' heterogeneity

()22.2(.) 1.138-1.274

5 trf4D
(3, 5),
pap1-2 trf4D 37° I 1-
(4, 4, 8). A.
I 1-
pap1-2 trf4D
(4, 2, 3, 4).
I 1- 1
I 1-

(5 , 10 12 15 17),
). I trf5D sir2D
 top1D A
 (2A).
 rrp6D (2). 6
 (2),
 rrp6D top1D
 top1D (3 7).
 A
 I 1- I 1- I 2- trf4D sir2D
 trf4D top1D
 (5)
 II I (5).
 I 1- A
 sir2D trf4D top1D
 trf4D

trf4D

Trf4 catalytic activity is not required for repeat regulation

trf4D (et al, 2000).
top1D

trf4D TOP1 (1, 4, 7)
trf4D top1D (2, 3, 5, 6, 8, 9)

trf4D top1D (1, 3),
trf4D top1D (4, 6)

trf4D top1D (7, 9),
trf4D top1D (4, 5, 6)

trf4D top1D (2, 3),
trf4D top1D (8, 9)

trf4D top1D (4, 5, 6, 8, 9)

trf4D top1D (4, 5, 6, 8, 9)

trf4D top1D (4, 5, 6, 8, 9)

trf4D top1D (4, 5, 6, 8, 9)

rDNA recombination frequency is unaffected by loss of TRF4

MET25²⁺
met25D (6)
(1997). A MET25-GFP
MET25 (6)

Alterations in rDNA copy number in *trf4Δ* strains do not reflect differences in cohesin recruitment

I 1-
trf4D I
13- I
om_3 1nsc10.373 019T374

HSP104 (C... et al, 2007),
 (C... et al, 2005).

3'
 I 1- (A)
 I
 MA I
 I
 MA I 4.
 I 1- I 1- II
 rpb1-1

4 I 1-
 I 1- MA 3 MA 1,
 MA (C...
 2006). I 1-
 MA 1,
 MA 1/MA 3
 A / MA 1/MA 3
 MA II
 (A... et al, 2006; ... et al, 2006),

MA 1 MA 3 I 1-
 I 1- A
 MA 1 MA 3 4
 3' I 1-
 I 1-

Links between Trf4 and rDNA copy number regulation
 trf4D
 I top1D sir2D
 MA
 trf4D
 top1D sir2D
 MA A MET25
 MA
 6 top1D

4 MA
I (et al,
1999). air1D air2D
(),
A 4
MA
(et al,
1999). I 6
47/ 1/ 1
MA (et al, 2004),
rrp47D
(et al, 2002).
A
MA I
MA MA
(, 2006);
trf4D
().
top1D
trf4D.
trf4D 1
MA
trf4D,
trf4D air1D air2D 3 3).

(1989) A
I₂,
A. Cell 56: 771-776
A (2004) A
A. Genes Dev 18: 2652-2662
(2001)
A 3'
Nature 413: 538-542
(2006) A;
Nat Rev Mol Cell Biol 7: 529-539
(2006) 5 (A)
EMBO Rep 7: 205-211
I, A, A, (2006)
A
Genes Dev 20: 2887-2901
A, A, A, A
(2004) A