

Decapping is preceded by 3' uridylation in a novel pathway of bulk mRNA turnover

Olivia S Rissland^{1,2} & Chris J Norbury¹

Both end structures of eukaryotic mRNAs, namely the 5' cap and 3' poly(A) tail, are necessary for transcript stability, and loss of either is sufficient to stimulate decay. mRNA turnover is classically thought to be initiated by deadenylation, as has been particularly well described in *Saccharomyces cerevisiae*. Here we describe two additional, parallel decay pathways in the fission yeast *Schizosaccharomyces pombe*. First, in fission yeast mRNA decapping is frequently independent of deadenylation. Second, Cid1-dependent uridylation of polyadenylated mRNAs, such as *act1*, *hcn1* and *urg1*, seems to stimulate decapping as part of a novel mRNA turnover pathway. Accordingly, *urg1* mRNA is stabilized in *cid1Δ* cells. Uridylation and deadenylation act redundantly to stimulate decapping, and our data suggest that uridylation-dependent decapping is mediated by the Lsm1–7 complex. As human cells contain Cid1 orthologs, uridylation may form the basis of a widespread, conserved mechanism of mRNA decay.

In budding yeast, most cytoplasmic mRNA turnover is initiated by deadenylation¹. These messages are then either decapped and subject to 5'→3' decay^{2,3} or degraded by the cytoplasmic exosome⁴. Although these decay pathways are conserved^{5,6}, metazoans and fission yeast contain additional cytoplasmic RNA-processing enzymes; the roles of many of these have not yet been determined. *Schizosaccharomyces pombe* Cid1 is one such enzyme, a cytoplasmic member of a family of RNA nucleotidyl transferases^{7,8}.

Cid1, identified through its involvement in the S-M checkpoint⁷, is now known to be one of a subgroup of this family possessing either poly(U) polymerase (PUP) and/or terminal uridyl transferase (TUTase) activity⁹. This subgroup also includes the human enzymes U6 TUTase, Hs2 and Hs3 (refs. 9–11), although no member of this subgroup is present in budding yeast¹².

Uridylation of mRNAs and noncoding RNAs has been described in fission yeast and metazoans. Known substrates include miRNA-directed cleavage products¹³ and replication-dependent histone mRNAs, which in metazoans contain a 3' stem-loop structure rather than a poly(A) tail, and decay of which is stimulated by oligouridylation^{14–16}. In addition, we previously observed terminal uridyl residues on polyadenylated *S. pombe act1* mRNA during S-phase arrest⁹. Although it seemed likely that Cid1-mediated uridylation was generally involved in mRNA metabolism, the effect of such a modification on polyadenylated messages was unclear.

Here we have used a circularized rapid amplification of cDNA ends (cRACE) technique to capture mRNA decay intermediates in *S. pombe*. Unexpectedly, in contrast to the situation in *Saccharomyces cerevisiae*, we find that decapped intermediates often contain substantial poly(A) tails, indicative of a novel deadenylation-independent decapping pathway for bulk mRNA in fission yeast. We also show that

uridylation of polyadenylated mRNAs forms the basis for an additional 5'→3' decay pathway, probably conserved in higher eukaryotes, that elicits decapping and seems to be mediated by the Lsm1–7 complex. Uridylation and deadenylation have overlapping and distinct stimulatory effects on decapping of polyadenylated mRNAs.

RESULTS

cRACE captures *act1* mRNA degradation intermediates

To dissect RNA decay pathways in fission yeast, we used the cRACE technique⁶. A tail-independent method of capturing 3' and 5' ends, this procedure allows distinction between decapped and capped mRNAs (Fig. 1a). We first examined decapped and capped *act1* transcripts from exponentially growing wild-type cells.

We initially wished to determine whether those products isolated from decapped cRACE analysis were derived from decay intermediates. To do this, we compared the 5' ends isolated from capped and decapped mRNAs (Fig. 1b). The 5' ends of products from capped transcripts generally lay 57 nucleotides (nt) or 58 nt upstream from the start codon (Fig. 1b and Supplementary Fig. 1 online). These nucleotides presumably represent the major transcriptional start site for *act1*. In contrast, the 5' ends of decapped products were heterogeneous, always downstream from the major transcriptional start site and distributed significantly differently from the capped species ($P < 0.0001$, two-tailed Mann-Whitney test). Thus, we conclude that these products represent mRNAs that have been subject to decapping and subsequent partial 5'→3' decay *in vivo*.

We next compared the 3' ends of adenylated and non-adenylated transcripts (Fig. 1c). Similarly to previous observations¹⁷, we detected three cleavage and polyadenylation sites in the *act1* gene: the first is approximately 1,190 nt downstream from the start codon (and 60 nt

¹Sir William Dunn School of Pathology, University of Oxford, UK. ²Present address: Whitehead Institute, Cambridge, Massachusetts, USA. Correspondence should be addressed to C.J.N. (chris.norbury@path.ox.ac.uk).

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