MS2 coat proteins bound to yeast mRNAs block 5' to 3' degradation and trap mRNA decay products: implications for the localization of mRNAs by MS2-MCP system

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ABSTRACT

Arrays of MS2 binding sites are placed into mRNAs and are commonly used to visualize the localization of mRNAs *in vivo* by the expression of an MS2-GFP fusion protein. In *Saccharomyces cerevisiae*, we observed that arrays of MS2 binding sites inhibit 5' to 3' degradation of the mRNA in yeast cells and lead to the accumulation of a 3' mRNA fragment containing the MS2 binding sites. This accumulation can be dependent on the binding of the MS2 stem loops (MS2-SL) by MS2 coat proteins (MCPs). Since such decay fragments can still bind MCP-GFP, the localization of such mRNA fragments can complicate the interpretation of the localization of full-length mRNA *in vivo*.

Keywords: MS2-MCP system; mRNA decay; mRNA localization

While examining the yeast mRNAs for QCR8 (a protein imported into mitochondria) and PGK1 (the glycolytic enzyme phosphoglycerate kinase) with an array of 24 MS2 binding sites inserted into the mRNAs' 3' UTR, we observed, for both mRNAs, an abundant mRNA population shorter than the predicted full-length mRNA present in cells that were grown to the post-diauxic phase and expressing the fluorescently tagged MCP (Fig. 1A). This population of mRNAs hybridizes to probes 3', but not 5', of the MS2 array (Fig. 1B,C), is dependent on the presence of the MCP-GFP fusion protein (Fig. 1B), and requires Xrn1 for its production (Fig. 1B). These bands are specific to expression of the MS2-tagged mRNAs as the same strain grown in the presence of doxycycline, which represses the expression of the MS2-tagged mRNA from the plasmid, shows no hybridizing bands (Fig. 1A, lane 1). We interpret these observations to indicate that the 24 MS2-binding sites in an MS2-tagged mRNA, when bound by MCP, are sufficient to block Xrn1 and thereby stabilize heterogeneous 3' mRNA fragments.

The ability of a 24-mer array of MS2 sites in mRNAs to block Xrn1 is not limited to the post-diauxic phase, as we see also a similar accumulation of the 3' mRNA fragments appearing in total RNA preparations from cells grown in log phase that is dependent on MCP and Xrn1 (Fig. 1D).

We were curious if other arrays of MS2-SLs with differing numbers of MS2 repeats also inhibited Xrn1 when bound by MCP fusion proteins. We examined yeast mRNAs with arrays of two, six, or 12 MS2 binding sites, where the latter was tagged via the yeast mTAG system which allows for a 12 MS2-SL RNA tag to be introduced into the endogenous 3'UTR of any yeast mRNA (Haim et al. 2007). We observed that MFA2 mRNAs with two MS2 sites in its 3' UTR, as well as Ash1 mRNAs with six MS2 binding sites in their 3' UTR, gave rise to 3' mRNA decay fragments, which were also dependent on Xrn1 and distinct from the endogenous mRNA (Fig. 2A,B). We also observed the accumulation of a 3' mRNA fragment from the mTAG PGK1 mRNAs with 12 MS2 binding sites in their 3' UTR (Fig 2C). We interpret these observations to indicate that presence of even two MS2 sites is sufficient to inhibit Xrn1 activity on the mRNA substrate.

The observation that MS2-binding sites can lead to the accumulation of 3' mRNA fragments could complicate the localization of the full-length mRNA observed by microscopy if such mRNA fragments represent a significant fraction of the population. To address this issue, we quantified the fraction of the mRNA molecules that were in the 3' mRNA fragment as a proportion of the full-length mRNA plus the 3' fragment mRNA (Table 1). The fraction of mRNA molecules that

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accumulate as 3' decay fragments ranged from 93.2% (with PGK1-24xMS2-SL mRNA) to 14.8% (with MFA2-2xMS2-SL mRNA). As in some cases the mRNA fragments can be the vast majority of the molecules in the cell, it raises the

FIGURE 1. MCP binding to arrays of 24 MS2-SLs in MS2-tagged mRNAs inhibits Xrn1-mediated decay and generates 3' mRNA decay fragments. Total mRNA was isolated and visualized by Northern using a 1.5% formaldehyde agarose gel from yeast carrying TET-off plasmids expressing either QCR8 or PGK1 mRNAs (which encodes a mitochondrial protein and a phosphoglycerate kinase, respectively) tagged with 24 MS2 stem loops (24xMS-SL) derived from pCR4-24xMS2-SL-stable plasmid (Addgene plasmid 31865) in their 3' UTR. The white line indicates that the lanes of the gel were spliced together and reordered from the same gel. (A) Visual schematic of the MS2-tagged mRNAs analyzed in this figure that indicate the expected size of each mRNA or mRNA feature. Visualization of the MS2-tagged QCR8 or PGK1 mRNAs products expressed from a yeast Tet-off plasmid using the 3' MS2 probe (5'-CCGCTATCGATGTTAACAGG-3') in strains grown at 30°C to post-diauxic (PD) phase in minimal media and expressing the fusion protein, MCP-3xGFP, from the pMS2-CP-3xGFP plasmid (Haim et al. 2007). Total RNA in lane 1 was isolated from cells grown at 30°C to the PD phase in the presence of 10 μg/mL doxycycline to repress expression of the MS2-tagged QCR8 mRNA. (B) Total RNA from wild-type and xrn1Δ strains expressing the 24xMS2-tagged QCR8 mRNA and grown to PD phase were probed with 3' MS2 probe from A. Lanes 1,2 also expressed the MCP-3xGFP fusion protein while lanes 3,4 did not. (C) Same as B but the Northern was probed with the 5' MS2 probe (5'-CTCTACCAGCTTTGCTGTACAG-3'). (D) Total RNA from cells grown to log phase at 30°C in minimal media was probed with the 3' MS2 probe in strains described above. To induce the expression of MCP-3xGFP during log phase from the inducible MET25 promoter, strains were grown to log phase and shifted into methionine-free minimal media for 1 h at 30°C.

FIGURE 2. RNAs tagged with smaller arrays of MS2 binding sites also induce the production of 3' mRNA decay fragments but not when tagged with 16 U1A stem loops. Total RNA was isolated and visualized from strains expressing mRNAs tagged with various sized MS2-SL arrays or U1A stem loops. The white line indicates that the lanes of the gel were spliced together from the same gel and reordered. Black boxes around gels indicate different gels. The MS2-tagged mRNAs are distinct from endogenous mRNAs and do not co-migrate with the 3' mRNA decay products in A and B. (A) Total mRNA from wild-type and xrn1Δ strains expressing MFA2-2xMS-SL from pRP1083 (Sheth and Parker 2003) and MCP-3xGFP from pMS2-CP-3xGFP was run on a 6% TBE-Urea PAGE gel and visualized with a probe to the 3' UTR of MFA2 (5'-GAT GAG AGA ATT GGA ATA AAT TAG TTT GCC AGC-3'). To induce the expression of MCP-3xGFP, strains were grown as described in Figure 1D. Lane 1 is an untagged MFA2 mRNA control. MFA2 encodes the mating pheromone, a-factor. (B) Total mRNA from wild-type and xrn1Δ strains expressing ASH1-6xMS-SL from pRJ1063 (courtesy of Ralf-Peter Jansen, Schmid et al. 2006) and MCP-YFP from pGPD-MCP-YFP was run on a 1.5% formaldehyde agarose gel and visualized with a probe to the 3' UTR of ASH1 (5'-GTTTCGTGATAATGTCTCTT ATTAGTTG-3'). To induce the expression of ASH1-6xMS-SL mRNAs, strains were grown to log phase in minimal media containing 2% glucose and then shifted into minimal media containing 2% galactose and 1% sucrose for an hour prior to harvesting. Lane 1 is untagged Ash1 mRNA control grown under the same conditions as in A. The ASH1 mRNA encodes a component of a histone deacetylase. (C) yMK2034 (courtesy of Mark Ashe, Simpson et al. 2014) which contains an endogenously 12xMS2-SL tagged PGK1 construct was grown to as described in Figure 1D. Total mRNA was isolated from the resulting cell pellets and visualized by Northern blotting using a probe to 3' UTR of PGK1 (5'-GAAAGAGAAAAAGAAAAAATTGATCTATCGATTTCATTCATTC-3') after running on a 1.5% formaldehyde agarose gel. Lane 1 is an untagged PGK1 mRNA control. (D) Total mRNA from wild-type and xrn1Δ strains expressing PGK1-16xU1A-SL from pPS2037 (courtesy of Pamela Silver, Brodsky and Silver 2000) and U1A-GFP from pRP1194 (Brenques et al. 2005) was run on a 1.5% formaldehyde agarose gel and visualized with a probe to the 3' UTR of PGK1 used in C.

TABLE 1. Percentage of detectable mRNA signal due to full-length or 3' mRNA decay fragments

MS2-tagged mRNA	% Full length	% 3' mRNA decay fragments
QCR8-24×MS2-SL (PD), <i>n</i> = 2	14.9	85.1
PGK1-24×MS2-SL (PD), <i>n</i> = 2	6.8	93.2
QCR8-24×MS2-SL (log), <i>n</i> = 2	17.9	82.1
MFA2-2×MS2-SL, <i>n</i> = 2	85.2	14.8
ASH1-6×MS2-SL, <i>n</i> = 2	29.4	70.6
PGK1-12×MS2-SL, <i>n</i> = 3	31.5	68.5

Northern blots were quantified to determine how much of the observed RNA signal was due to the full-length MS2-tagged mRNA or the 3' mRNA decay fragment. For each mRNA, regions of interest (ROIs) were drawn around the bands corresponding to either the full-length mRNA (FL), the 3' mRNA decay fragment (FRAG), or an area above the full-length mRNA but below the gel well (BKGRD, to calculate the background). For each ROI, the area (A) and total intensity (TI) was measured using ImageJ. To calculate percentage full length, the following formula was used:

$$\frac{TI_{FL} - A_{FL} \left(\frac{TI_{BKGRD}}{A_{BKGRD}} \right)}{TI_{FL} - A_{FL} \left(\frac{TI_{BKGRD}}{A_{BKGRD}} \right) + TI_{FRAG} - A_{FRAG} \left(\frac{TI_{BKGRD}}{A_{BKGRD}} \right)}$$

The following was used to calculate % 3' mRNA fragment:

$$\frac{TI_{FRAG} - A_{FRAG} \left(\frac{TI_{BKGRD}}{A_{BKGRD}} \right)}{TI_{FL} - A_{FL} \left(\frac{TI_{BKGRD}}{A_{BKGRD}} \right) + TI_{FRAG} - A_{FRAG} \left(\frac{TI_{BKGRD}}{A_{BKGRD}} \right)}$$

possibility that using this method to localize mRNAs in the cell could instead primarily localize the mRNA decay fragment. This issue could lead to the detection of “mRNAs” in P-bodies when in fact one is localizing mRNA decay products, which are known to accumulate in P-bodies (Sheth and Parker 2003).

It is possible that different mRNAs, or different sequence features of MS2 arrays, will affect the how well these RNA-protein assemblies block Xrn1 degradation. Given this, the use of MS2-MCP system to visualize mRNA localization should be accompanied with traditional Northern analysis to demonstrate that the full-length mRNA is the predominant species being localized. However, it remains possible that even a minor amount of mRNA decay fragment could misrepresent the localization of the full-length mRNA as the minor species may form visible foci when concentrated in structures such as P-bodies. Alternatively, different RNA

stem loops that do not form inhibitory structures with their respective RNA-binding protein can be used to visualize RNA localization. To explore this possible solution, a PGK1 mRNA tagged with 16 U1A stem loops was coexpressed with a GFP tagged U1A binding protein and analyzed by Northern. Xrn1-dependent decay fragments were not detected with this U1A tagged mRNA (Fig 2D), suggesting U1A tagged mRNAs might be less prone to the accumulation of decay products through the inhibition of Xrn1.

It is yet to be determined if MS2 arrays can lead to the accumulation of mRNA decay fragments in other systems such as *Drosophila* or mammalian cells and complicate the localization of MS2-tagged mRNAs. However, this is at least a formal possibility as it is known that miRNA-RISC on mRNAs can inhibit Xrn1 in *Caenorhabditis elegans* (Bagga et al. 2005) and some protein assemblies on mammalian mRNAs can block Xrn1 (Franks et al. 2010).

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