letters to nature

**Quality control of mRNA 3′-end processing is linked to the nuclear exosome**

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An emerging theme in messenger RNA metabolism is the coupling of nuclear pre-mRNA processing events, which contributes to mRNA quality control. Most eukaryotic mRNAs acquire a poly(A) tail during 3′-end processing within the nucleus, and this is coupled to efficient export of mRNAs to the cytoplasm. In the yeast *S. cerevisiae*, a common consequence of defective nuclear export of mRNA is the hyperadenylation of nascent transcripts, which are sequestered at or near their sites of transcription. This implies that polyadenylation and nuclear export are coupled in a step that involves the release of mRNA from transcription site foci. Here we demonstrate that transcripts which fail to acquire a poly(A) tail are also retained at or near transcription sites. Surprisingly, this retention mechanism requires the protein Rrp6p and the nuclear exosome, a large complex of exonuclease enzymes. In exosome mutants, hypo- as well as hyperadenylated mRNAs are released and translated. These observations suggest that the exosome contributes to a checkpoint that monitors proper 3′-end formation of mRNA.

To test whether unadenylated transcripts would also be retained within the nucleus, we used a strain carrying a lesion in the poly(A) polymerase gene, *pap1-1* (ref. 8), to generate poly(A)− mRNA, which could then be localized by fluorescence in situ hybridization (FISH). We chose to examine SSA4 mRNA, which is synthesized *de novo* when the strain is shifted to the restrictive temperature. The poly(A)− SSA4 transcripts accumulated in discrete intranuclear foci (Fig. 1a), indistinguishable from the localization of hyperadenylated SSA4 RNA (Fig. 1b). Moreover, the loss of Rrp6p partially rescued the block in transcript retention at or near transcription sites. The sequestration of both poly(A)+ and hyperadenylated mRNAs in transcription site foci indicated that a system exists in yeast to both monitor the quality of 3′-end formation and inhibit the release of aberrant transcripts. Strains defective in such a quality-control system should fail to retain aberrant RNAs at these foci. Because defects in the nonessential nuclear exonuclease Rrp6p partially rescue the temperature sensitivity of *pap1-1* (ref. 9), we investigated the effect of an RR6 deletion on transcript localization. The sequestration of *SSA4* poly(A)− mRNA was markedly absent in the *pap1-1* *rrp6Δ* strain (Fig. 1c). It is important to note that this change in SSA4 localization occurred without any dramatic change in mRNA structure or abundance, as shown by northern analysis (Fig. 1b). Moreover, the loss of Rrp6p partially rescued the block to heat-shock protein synthesis caused by the *pap1-1* lesion (Fig. 1c, compare lanes 6 and 8). This indicates that the absence of Rrp6p allows both release and function of the poly(A)+ mRNA.

To assay another transcript, we examined *PGK1* mRNA. Because of its inducible promoter, we could use a transcriptional pulse-chase protocol to produce a synchronous pool of mRNA.

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**Notes**

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A poly(G) structure engineered into the 3’ untranslated region of PGK1pG permits detection of decay intermediates produced by mRNA turnover. Similar to SSA4 RNA (Fig. 1b), the predominant mRNA produced in the pap1-1 and pap1-1 rrp6Δ strains was poly(A)− (Fig. 1d), although some poly(A)+ species of PGK1pG were produced. This probably reflects residual polyadenylation by the mutant polymerase under restrictive conditions. Although the poly(A)+ species was surprisingly stable in the pap1-1 strain, the poly(A)+ transcripts were destabilized in the absence of Rrp6p (Fig. 1d), and a decay intermediate accumulated. These observations demonstrate that the fate of the poly(A)+ mRNA was changed in the rrp6Δ strain. Importantly, because the PGK1pG mRNA is destabilized in the absence of Rrp6p, these observations are inconsistent with Rrp6p functioning solely to degrade nuclear poly(A)+ mRNAs. We conclude that sequestration of poly(A)+ mRNA in transcription site foci requires the activity of Rrp6p.

To investigate whether Rrp6p is also required for retention of hyperadenylated transcripts, we used the rat7-1 and rip1Δ export mutant strains. In these strains, the SSA4 and HSP104 transcripts are hyperadenylated and retained at or near transcription sites (Fig. 2a and ref. 5). In the rat7-1 rrp6Δ and the rip1Δ rrp6Δ strains, both the HSP104 and SSA4 mRNAs were still nuclear at 42°C but much more diffuse than the discrete nuclear foci observed in the single export mutant strains (Fig. 2a and data not shown). Interestingly, in the rat7-1 rrp6Δ and rip1Δ rrp6Δ strains, heat shock mRNAs accumulated in a subregion of the nucleus, forming a cap on top of the DAPI stain (Fig. 2a), suggesting these transcripts might be accumulating in a subnuclear location. This suggests further that, as for transcripts produced in pap1-1, transcripts in rat7-1 and rip1Δ are also released from transcription site foci in the absence of Rrp6p. In contrast to pap1-1 cells, however, the mRNA export defects are not relieved by deletion of RRP6, which causes at least some of these transcripts to remain within the nucleus. Also in contrast to the pap1-1 data, a clear increase in SSA4 transcript levels was observed in the rat7-1 rrp6Δ and rip1Δ rrp6Δ strains compared with the single mutants (Fig. 2b), suggesting that Rrp6p may contribute to the rapid degradation of some hyperadenylated transcripts. A less likely possibility is that the absence of Rrp6p leads to an increase in transcription rate.

Further support for transcript release in rrp6Δ strains is the observation that heat-shock protein synthesis is partially restored in the rat7-1 rrp6Δ strain (Fig. 2c, lane 8). A low level of heat-shock protein expression is observed in the rat7-1 cells (Fig. 2c, lane 6), supporting the previous claim that mRNA export is leaky in this strain. In contrast, heat-shock protein synthesis is not restored in the rip1Δ rrp6Δ strain, consistent with a tighter mRNA export block in the rip1Δ background (Fig. 2c and data not shown).

Rrp6p is a member of the nuclear exosome, an exonucleolytic NATURE| VOL 413 | 4 OCTOBER 2001 | www.nature.com

Figure 1 SSA4 poly(A)+ mRNA accumulates in an intranuclear focus in an Rrp6p-dependent manner. a, SSA4 mRNA FISH analysis of wild type, pap1-1 and pap1-1 rrp6Δ cultures grown at 25°C or temperature shifted to 42°C for 15 min or 60 min, as indicated. DNA was stained with DAPI. b, Northern analysis of SSA4 mRNA isolated from cultures collected at the indicated time points after a 42°C heat shock. dT indicates that the RNA sample was treated with oligo(dT) and RNaseH before gel electrophoresis. The multiple poly(A)+ species produced in the pap1-1 strains is probably due to promiscuous 3’-end cleavage. The sizes of radiolabelled molecular mass markers in nucleotides are indicated to the left of the panel. c, Heat-shock protein synthesis in wild-type, rrp6Δ, pap1-1 and pap1-1 rrp6Δ strains grown at 25°C or temperature shifted to 42°C for 15 min. Migration of the major heat-shock proteins is shown to the right of the panel. d, Transcriptional pulse-chase of PGK1pG in pap1-1 and pap1-1 rrp6Δ strains. Cultures were grown in 2% raffinose. Transcription was induced by the addition of galactose to a final concentration of 2%. After 5 min, transcription was repressed by the addition of glucose to a final concentration of 4%. The position of the major poly(A)+ species is indicated to the right of each panel. The position of a decay intermediate is indicated by an asterisk to the right of each panel.
complex that contributes to a variety of nuclear RNA processing and degradative reactions\textsuperscript{6,7,11–13}. To examine the effects of other nuclear exosome components, we analysed the fate of hyperadenylated transcripts in temperature-sensitive mutants of the essential \textit{RRP4} and \textit{MTR4} genes. At 42°C, \textit{HSP104} localization in the \textit{rat7-1 rrp4-1} and \textit{rat7-1 mtr4-1} double-mutant strains parallels that seen in the \textit{rat7-1 rrp6Δ} strain (Fig. 3a). The similar results with three exosome components suggest that retention of aberrant mRNAs is a function of the nuclear exosome. Consistent with this notion, the \textit{rat7-1 rrp6Δ}, \textit{rat7-1 rrp4-1} and \textit{rat7-1 mtr4-1} double-mutant strains all

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**Figure 2** Rrp6p mediates transcription site focus retention of \textit{HSP104} and \textit{SSA4} mRNAs in mRNA export mutants. a, \textit{HSP104} mRNA FISH analysis on the \textit{rat7-1} and \textit{rat7-1 rrp6Δ} strains, and \textit{SSA4} mRNA FISH analysis on \textit{rip1Δ} and \textit{rip1Δ rrp6Δ} strains are shown. Experiments were performed as described in Fig. 1a. b, Northern analysis of \textit{SSA4} mRNA was performed as described in Fig. 1b. dT indicates treatment with oligo(dT) and RNaseH. The sizes of radiolabelled molecular mass markers in nucleotides are indicated to the left of the panel. c, Heat-shock protein synthesis in the indicated strains was analysed as described in Fig. 1c.
display an altered distribution of the total poly(A)$^+$ signal. As previously reported and shown in Fig. 3b, rat7-1 single-mutant cells exhibit a strong intranuclear granular poly(A)$^+$ staining after a shift to 37°C (ref. 14). In contrast, all three double-mutant strains show perinuclear poly(A)$^+$ staining. This localization probably reflects release of the transcripts from transcription site foci and a persistent block in mRNA export at the nuclear periphery, directly due to the rat7-1 mutation. These comparisons further strengthen the conclusion that aberrant transcripts are blocked near transcription sites in rat7-1 cells and released when the nuclear exosome is compromised.

Our results indicate that mRNAs with aberrant 3′ ends are generally retained at or near their sites of transcription. Moreover, we propose that the nuclear exosome contributes to a quality-control system that monitors correct 3′-end formation before RNA release from these sites. It is interesting to note that Rrp6p interacts genetically and physically with Pap1p as well as with the nucleocytoplasmic shuttling protein, Npl3p (refs 9, 15), suggesting that some of the links between the exosome, 3′-end formation and nuclear export are direct. Given that the exosome functions in nuclear mRNA turnover13, an intriguing possibility is that the exosome participates not only in the destruction of aberrant mRNAs but also in their retention, that is, why this mRNA is not free to diffuse away from the gene locus.

**Methods**

**Yeast methods**

Strains used in this analysis are listed in the Supplementary Information as Table 1. Double mutants were generated by standard genetic crosses.

**In situ hybridization analysis**

FISH of SSA4 and HSP104 mRNA was performed using Cy3-labelled probes, and DNA was stained with DAPI as previously described16. Poly(A)$^+$ RNA was localized using a Cy3-labelled oligo(dT)$_{15}$ probe16.

![Figure 3](image)

**Figure 3** Lesions in the nuclear exosome alter the distribution of mRNA in the rat7-1 background. a, HSP104 mRNA FISH analysis on the rat7-1, rat7-1 mtr4-1 and rat7-1 mtr4-1 strains temperature shifted to 42°C for 15 or 60 min are shown. b, Oligo(dT) FISH analyses of rat7-1, rat7-1 rrp6Δ, rat7-1 mtr4-1 and rat7-1 mtr4-1 cultures temperature shifted to 37°C for 30 min are shown.

**RNA and protein analysis**

Transcriptional pulse-chase experiments, RNaseH treatment and northern analysis of PCK1pG were as previously described16. RNAseH treatment and northern analysis of SSA4, as well as analysis of heat-shock protein synthesis, were performed as described17.

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Transmitision intensity and impact of control policies on the foot and mouth epidemic in Great Britain

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The foot and mouth disease (FMD) epidemic in British livestock remains an ongoing cause for concern, with new cases still arising in previously unaffected areas. Epidemiological analyses have been vital in delivering scientific advice to government on effective control measures. Using disease, culling and census data on all livestock farms in Great Britain, we analysed the risk factors determining the spatiotemporal evolution of the epidemic and of the impact of control policies on FMD incidence. Here we show that the species mix, animal numbers and the number of distinct land parcels in a farm are central to explaining regional variation in transmission intensity. We use the parameter estimates thus obtained in a dynamical model of disease spread to show that extended culling programmes were essential for controlling the epidemic to the extent achieved, but demonstrate that the epidemic could have been substantially reduced in scale had the most efficient control measures been rigorously applied earlier.

The FMD epidemic (Fig. 1a) peaked in early April 2001 after two months of rapid spread throughout Great Britain following introduction of the O Pan Asian strain of the virus (by an as-yet-undetermined route) in early February. The subsequent decay of the epidemic was initially rapid, but then slowed because of significant new outbreaks in previously little-affected regions (notably North Yorkshire and Lancashire) outside the three major foci in the north (Cumbria, Dumfries and Galloway, and Northumberland), the southwest (Devon and Somerset) and Welsh borders (Herefordshire, Worcestershire and Powys). Emergence of cases in previously unaffected areas and re-emergence in areas believed to have been cleared of infection continue to make elimination of the epidemic difficult.

The policy for control has evolved over the course of the epidemic, and the efficiency and necessity of individual measures have been the topic of ongoing debate. On 23 February, animal movement restrictions were imposed and public access to affected areas was restricted. This was coupled with slaughter of animals on infected premises (IPs) and farms identified as having had dangerous contacts (DCs) with an IP, together with the introduction of biosecurity measures (such as, the use of disinfectant on clothing, boots and farm vehicles). These measures slowed spread but were insufficient to reverse it, largely owing to long delays between the report of possible FMD, confirmation of infection, and culling of animals on the affected farm. With assistance from the army in the logistics of culling large numbers of animals, in late March the policy was strengthened to aim for culling of animals on IPs and contiguous premises (CPs) within 24 and 48 h, respectively, without waiting for laboratory confirmation of infection. These policies were informed, in part, by model-based epidemiological analyses of...