pH 7.4, 100 mM NaCl, 0.1% Nonidet P-40, 10% glycerol, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 10 mM NaF, 0.1 mM Na_3VO_4, 5 μM microcystin LR, 1 μM okadaic acid, 1 mM DTT, the protease inhibitor cocktail and 0.33 μCi μl^{-1} [γ-^{32}P]ATP (Amersham).

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1. McCubbin, A. G. & Kao, T.-h. Molecular recognition and response in pollen and pistil interactions. *Annu. Rev. Cell Dev. Biol.* **16,** 333–364 (2000).
2. Stein, J. C., Howlett, B., Boyes, D. C., Nasrallah, M. E. & Nasrallah, J. B. Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. *Proc. Natl Acad. Sci. USA* **88,** 8816–8820 (1991).
3. Suzuki, G. *et al.* Genomic organization of the *S* locus: identification and characterization of genes in *SLG/SRK* region of S_9 haplotype of *Brassica campestris* (syn. *rapa*). *Genetics* **153,** 391–400 (1999).
4. Schopfer, C. R., Nasrallah, M. E. & Nasrallah, J. B. The male determinant of self-incompatibility in *Brassica*. *Science* **286,** 1697–1700 (1999).
5. Nasrallah, J. B., Kao, T.-H., Chen, C.-H., Goldberg, M. L. & Nasrallah, M. E. Amino-acid sequence of glycoproteins encoded by three alleles of the *S* locus of *Brassica oleracea*. *Nature* **326,** 617–619 (1987).
6. Takasaki, T. *et al.* The *S* receptor kinase determines self-incompatibility in *Brassica* stigma. *Nature* **403,** 913–916 (2000).
7. Takayama, S. *et al.* The pollen determinant of self-incompatibility in *Brassica campestris*. *Proc. Natl Acad. Sci. USA* **97,** 1920–1925 (2000).
8. Shiba, H. *et al.* A pollen coat protein, SP11/SCR, determines the pollen *S*-specificity in the self-incompatibility of *Brassica* species. *Plant Physiol.* **125,** 2095–2103 (2001).
9. Takayama, S. *et al.* Sequences of *S*-glycoproteins, products of the *Brassica campestris* self-incompatibility locus. *Nature* **326,** 102–104 (1987).
10. Torii, K. U. Receptor kinase activation and signal transduction in plants: an emerging picture. *Curr. Opin. Plant Biol.* **3,** 361–367 (2000).
11. Dickinson, H. G. Pollen stigma interactions: so near yet so far. *Trends Genet.* **16,** 373–376 (2000).
12. Brugière, N., Cui, Y. & Rothstein, S. J. Molecular mechanisms of self-recognition in *Brassica* self-incompatibility. *Trends Plant Sci.* **5,** 432–438 (2000).
13. Broekaert, W. F., Terras, F. R. G., Cammue, B. P. A. & Osborn, R. W. Plant defensins: novel antimicrobial peptides as components of the host defense system. *Plant Physiol.* **108,** 1353–1358 (1995).
14. Bruix, M. *et al.* Solution structure of γ1-H and γ1-P thionins from barley and wheat endosperm determined by ^{1}H-NMR: A structural motif common to toxic arthropod proteins. *Biochemistry* **32,** 715–724 (1993).
15. Watanabe, M. *et al.* Highly divergent sequences of the pollen self-incompatibility (*S*) gene in class-I *S* haplotypes of *Brassica campestris* (syn. *rapa*) L. FEBS Lett. **473,** 139–144 (2000).
16. Stephenson, A. G. *et al.* The male determinant of self-incompatibility in *Brassica oleracea* is located in the pollen coating. *Plant J.* **12,** 1351–1359 (1997).
17. Doughty, J. *et al.* PCP-A1, a defensin-like *Brassica* pollen coat protein that binds the *S* locus glycoprotein, is the product of gametophytic gene expression. *Plant Cell* **10,** 1333–1347 (1998).
18. Takayama, S. *et al.* Isolation and characterization of pollen coat proteins of *Brassica campestris* that interact with *S* locus-related glycoprotein 1 involved in pollen-stigma adhesion. *Proc. Natl Acad. Sci. USA* **97,** 3765–3770 (2000).
19. Trotochaud, A. E., Jeong, S. & Clark, S. E. CLAVATA3, a multimeric ligand for the CLAVATA1 receptor-kinase. *Science* **289,** 613–617 (2000).
20. Gómez-Gómez, L., Bauer, Z. & Boller, T. Both the extracellular leucine-rich repeat domain and the kinase activity of FLS2 are required for flagellin binding and signaling in *Arabidopsis*. *Plant Cell* **13,** 1155–1163 (2001).
21. Wang, Z.-Y., Seto, H., Fujioka, S., Yoshida, S. & Chory, J. BRI1 is a critical component of a plasma-membrane receptor for plant steroids. *Nature* **410,** 380–383 (2001).
22. Giranton, J.-L., Ariza, M. J., Dumas, C., Cock, J. M. & Gaude, T. The *S* locus receptor kinase gene encodes a soluble glycoprotein corresponding to the SRK extracellular domain in *Brassica oleracea*. *Plant J.* **8,** 827–834 (1995).
23. Suzuki, G., Watanabe, M., Toriyama, K., Isogai, A. & Hinata, K. Expression of SLG_9 and SRK_9 genes in transgenic tobacco. *Plant Cell Physiol.* **37,** 866–869 (1996).
24. Giranton, J.-L., Dumas, C., Cock, J. M. & Gaude, T. The integral membrane *S*-locus receptor kinase of *Brassica* has serine/threonine kinase activity in a membranous environment and spontaneously forms oligomers *in planta*. *Proc. Natl Acad. Sci. USA* **97,** 3759–3764 (2000).
25. Dixit, R., Nasrallah, M. E. & Nasrallah, J. B. Post-transcriptional maturation of the *S* receptor kinase of *Brassica* correlates with co-expression of the *S*-locus glycoprotein in the stigmas of two *Brassica* strains and in transgenic tobacco plants. *Plant Physiol.* **124,** 297–311 (2000).
26. Suzuki, T., Kusaba, M., Matsushita, M., Okazaki, K. & Nishio, T. Characterization of *Brassica S*-haplotypes lacking *S*-locus glycoprotein. *FEBS Lett.* **482,** 102–108 (2000).
27. Cabrillac, D., Cock, J. M., Dumas, C. & Gaude, T. The *S*-locus receptor kinase is inhibited by thioredoxins and activated by pollen coat proteins. *Nature* **410,** 220–223 (2001).
28. Kojima, M. *et al.* Ghrelin is a growth-hormone-releasing acylated peptide form stomach. *Nature* **402,** 656–660 (1999).
29. Shiba, H. *et al.* Alteration of the self-incompatibility phenotype in *Brassica* by transformation of the antisense *SLG* gene. *Biosci. Biotechnol. Biochem.* **64,** 1016–1024 (2000).
30. Larsson, C., Widell, S. & Kjellbom, P. Preparation of high-purity plasma membranes. *Methods Enzymol.* **148,** 558–568 (1987).

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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[1]. 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[2,3]. In the yeast *Saccharomyces cerevisiae*, a common consequence of defective nuclear export of mRNA is the hyperadenylation of nascent transcripts[4,5], which are sequestered at or near their sites of transcription[5]. 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 exonucleolytic enzymes[6,7]. 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* RNA, 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* transcripts[5]. Comparable results were obtained with probes directed against *HSP104*, another heat-induced transcript (data not shown). We conclude that both hypo- and hyperadenylation causes 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 *RRP6* deletion on transcript localization. The sequestration of *SSA4* poly(A)$^-$ RNA was markedly absent in the *pap1-1 rrp6*Δ strain (Fig. 1a). 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 *PGK1pG* mRNA. Because of its inducible promoter, we could use a transcriptional pulse-chase protocol to produce a synchronous pool of mRNA[10].

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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 rrp6*Δ 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 *rrp6*Δ 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 *rip1*Δ 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 rrp6*Δ and the *rip1*Δ *rrp6*Δ 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 rrp6*Δ and *rip1*Δ *rrp6*Δ 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 *rip1*Δ 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 rrp6*Δ and *rip1*Δ *rrp6*Δ 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 *rrp6*Δ strains is the observation that heat-shock protein synthesis is partially restored in the *rat7-1 rrp6*Δ 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[4]. In contrast, heat-shock protein synthesis is not restored in the *rip1*Δ *rrp6*Δ strain, consistent with a tighter mRNA export block in the *rip1*Δ background (Fig. 2c and data not shown).

Rrp6p is a member of the nuclear exosome, an exonucleolytic

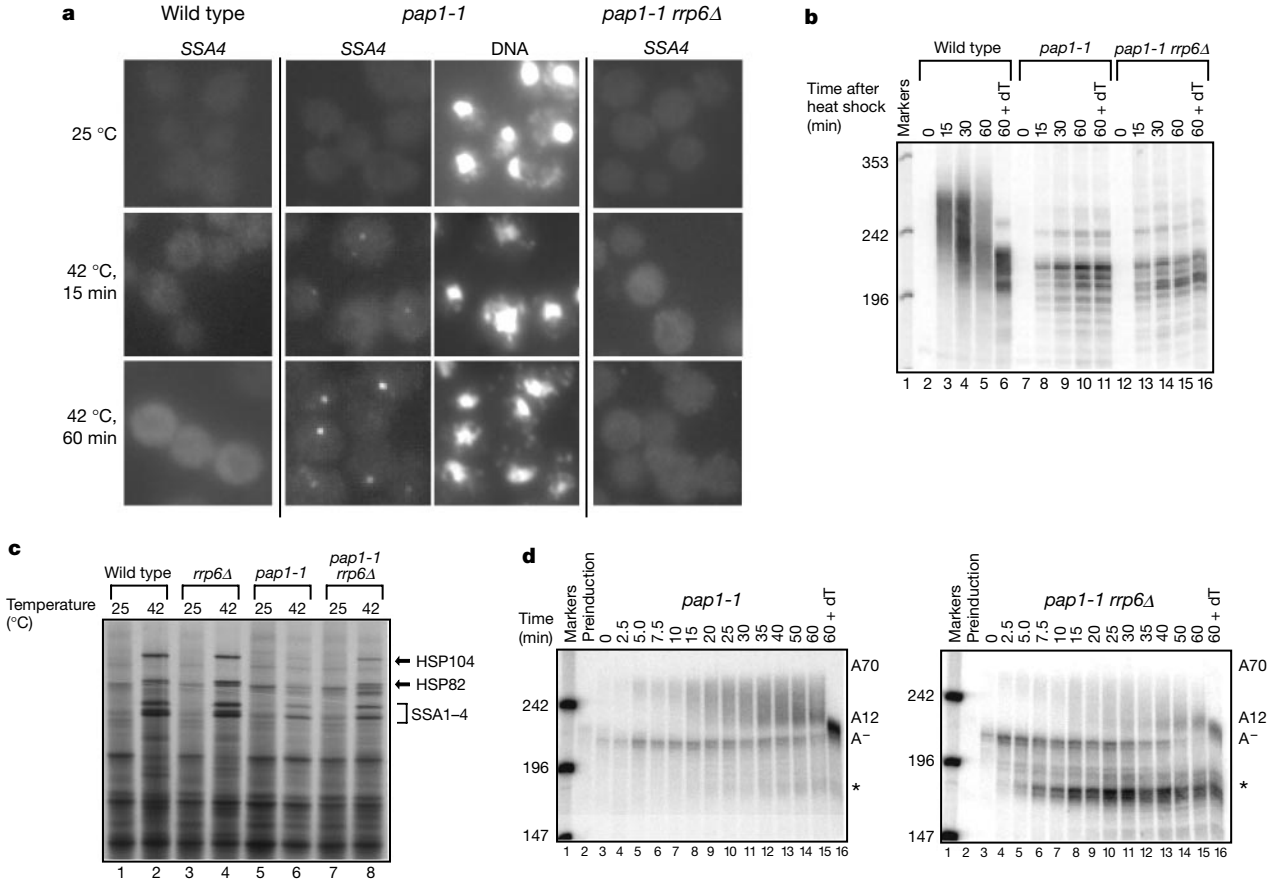

Figure 1 *SSA4* poly(A)$^-$ mRNA accumulates in an intranuclear focus in an Rrp6p-dependent manner. **a**, *SSA4* mRNA FISH analysis of wild type, *pap1-1* and *pap1-1 rrp6*Δ cultures grown at 25 °C or temperature shifted to 42 °C for 15 min or 60 min, as indicated. DNA was stained with DAPI. **b**, 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[17,18]. 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, *rrp6*Δ, *pap1-1* and *pap1-1 rrp6*Δ 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 rrp6*Δ 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.

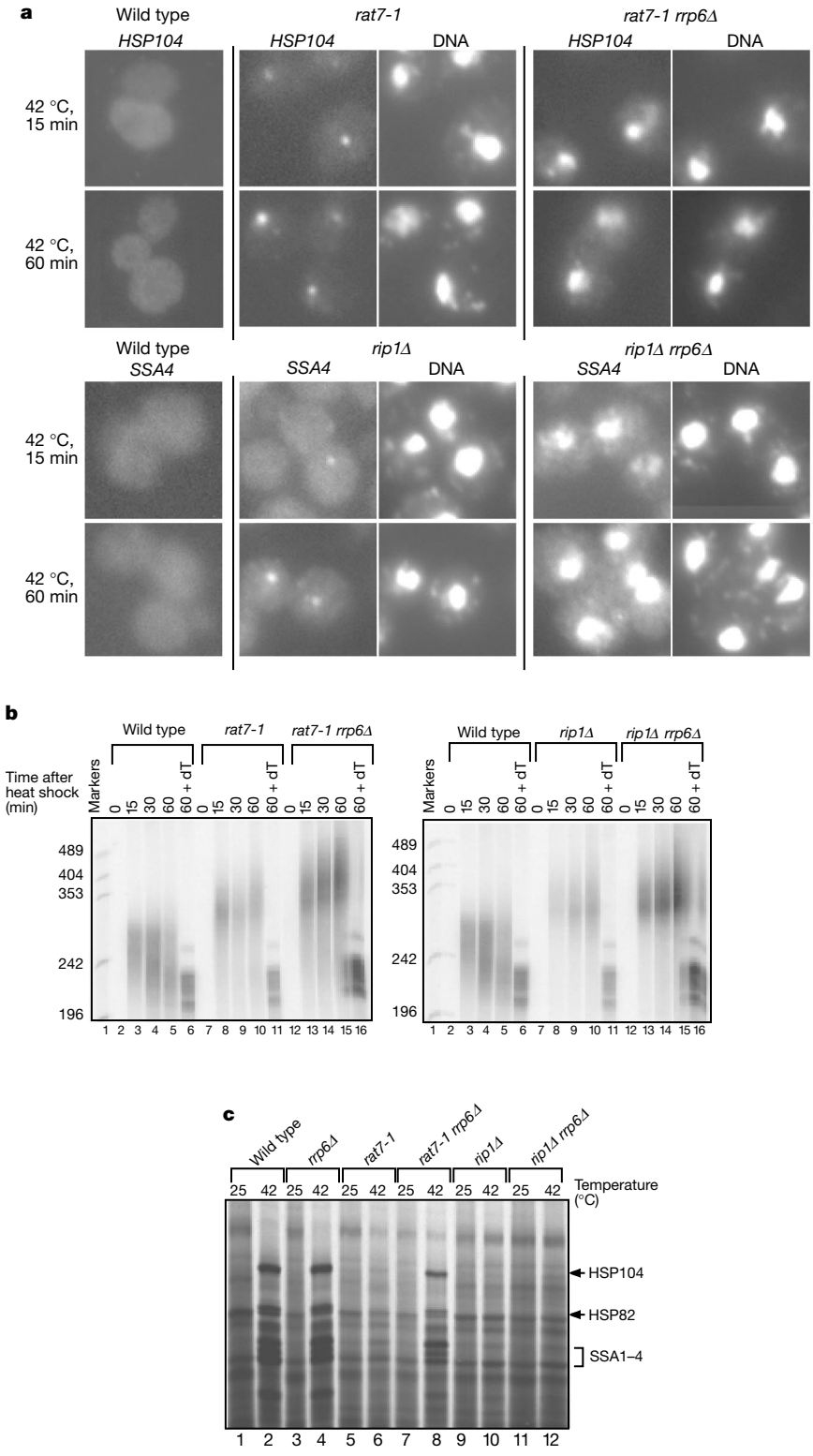

Figure 2 Rrp6p mediates transcription site foci retention of *HSP104* and *SSA4* mRNAs in mRNA export mutants. **a**, *HSP104* mRNA FISH analysis on the *rat7-1* and *rat7-1 rrp6*Δ strains, and *SSA4* mRNA FISH analysis on *rip1*Δ and *rip1*Δ *rrp6*Δ strains are shown. Experiments were performed as described in Fig. 1a. **b**, Northern analysis of *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.

complex that contributes to a variety of nuclear RNA processing and degradative reactions[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 *RRP4* and *MTR4* genes. At 42 °C, *HSP104* localization in the *rat7-1 rrp4-1* and *rat7-1 mtr4-1* double-mutant strains parallels that seen in the *rat7-1 rrp6*Δ 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 *rat7-1 rrp6*Δ, *rat7-1 rrp4-1* and *rat7-1 mtr4-1* double-mutant strains all

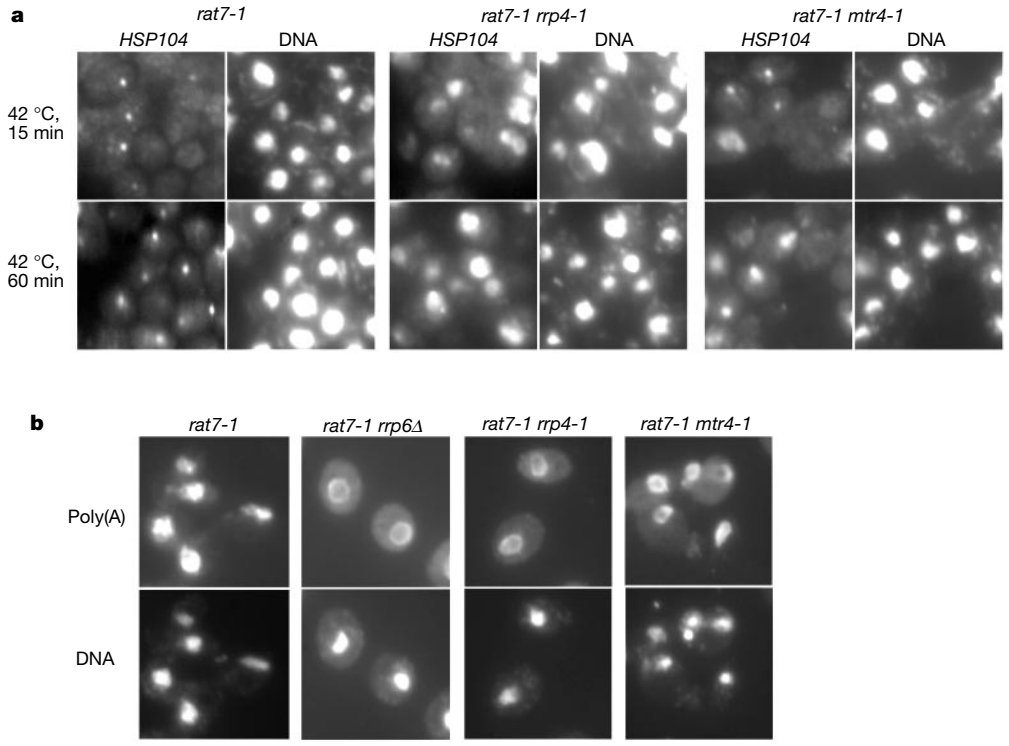

Figure 3 Lesions in the nuclear exosome alter the distribution of mRNA in the *rat7-1* background. **a**, *HSP104* mRNA FISH analysis on the *rat7-1*, *rat7-1 rrp4-1* and *rat7-1 mtr4-1* strains temperature shifted to 42 °C for 15 or 60 min are shown. **b**, Oligo(dT) FISH analyses of *rat7-1*, *rat7-1 rrp6*Δ, *rat7-1 rrp4-1* and *rat7-1 mtr4-1* cultures temperature shifted to 37 °C for 30 min are shown.

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 peri-nuclear 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 turnover[13], an intriguing possibility is that the exosome participates not only in the destruction of aberrant mRNAs but also in their detection and retention at or near transcription sites. A remaining challenge is to understand the physical link between incorrectly adenylated mRNA and 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 described[5]. Poly(A)$^+$ RNA was localized using a Cy3-labelled oligo(dT)$_{70}$ probe[16].

RNA and protein analysis

Transcriptional pulse-chase experiments, RNaseH treatment and northern analysis of *PGK1pG* were as previously described[4,10]. RNAseH treatment and northern analysis of *SSA4*, as well as analysis of heat-shock protein synthesis, were performed as described[5].

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1. Maquat, L. E. & Carmichael, G. G. Quality control of mRNA function. *Cell* **104,** 173–176 (2001).
2. Eckner, R., Ellmeier, W. & Birnstiel, M. L. Mature mRNA 3′ end formation stimulates RNA export from the nucleus. *EMBO J.* **10,** 3513–3522 (1991).
3. Huang, Y. & Carmichael, G. C. Role of polyadenylation in nucleocytoplasmic transport of mRNA. *Mol. Cell. Biol.* **16,** 1534–1542 (1996).
4. Hilleren, P. & Parker, R. Defects in mRNA export factors Rat7p, Gle1p, Mex67p and Rat8p cause hyperadenylation during 3′ end formation of nascent transcripts. *RNA* **7,** 753–764 (2001).
5. Jensen, T. H., Patricio, K., McCarthy, T. & Rosbash, M. A block to mRNA nuclear export in *Saccharomyces cerevisiae* leads to hyperadenylation of transcripts that accumulate at the site of transcription. *Mol. Cell* **7,** 887–898 (2001).
6. Briggs, M. W., Burkard, K. T. & Butler, J. S. Rrp6p, the yeast homologue of the human PM-Scl 100-kDa autoantigen, is essential for efficient 5.8 S rRNA 3′ end formation. *J. Biol. Chem.* **273,** 13255–13263 (1998).
7. Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M. & Tollervey, D. The exosome: A conserved eukaryotic RNA processing complex containing multiple 3′→5′ exoribonucleases. *Cell* **91,** 457–466 (1997).
8. Patel, D. & Butler, J. S. Conditional defect in mRNA 3′ end processing caused by a mutation in the gene for poly(A) polymerase. *Mol. Cell. Biol.* **12,** 3297–3304 (1992).
9. Burkard, K. T. & Butler, J. S. A nuclear 3′-5′ exonuclease involved in mRNA degradation interacts with poly(A) polymerase and the hnRNA protein Npl3p. *Mol. Cell. Biol.* **20,** 604–616 (2000).
10. Decker, C. J. & Parker, R. A turnover pathway for both stable and unstable mRNAs in yeast: Evidence for a requirement for deadenylation. *Genes Dev.* **7,** 1632–1643 (1993).
11. Allmang, C. *et al.* Functions of the exosome in rRNA, snoRNA and snRNA synthesis. *EMBO J.* **18,** 5399–5410 (1999).
12. van Hoof, A., Lennertz, P. & Parker, R. Yeast exosome mutants accumulate 3′-extended polyadenylated forms of U4 small nuclear RNA and small nucleolar RNAs. *Mol. Cell. Biol.* **20,** 441–452 (2000).
13. Bousquet-Antonelli, C., Presutti, C. & Tollervey, D. Identification of a regulated pathway for nuclear pre-mRNA turnover. *Cell* **102,** 765–775 (2000).
14. Gorsch, L. C., Dockendorff, T. C. & Cole, C. N. A conditional allele of the novel repeat-containing yeast nucleoporin RAT7/NUP159 causes both rapid cessation of mRNA export and reversible clustering of nuclear pore complexes. *J. Cell Biol.* **129,** 939–955 (1995).
15. Lee, M. S., Henry, M. & Silver, P. A. A protein that shuttles between the nucleus and the cytoplasm is an important mediator of RNA export. *Genes Dev.* **10,** 1233–1246 (1996).
16. Vainberg, I. E., Dower, K. & Rosbash, M. Nuclear export of heat shock and non-heat-shock mRNA occurs via similar pathways. *Mol. Cell. Biol.* **20,** 3996–4005 (2000).
17. Mandart, E. & Parker, R. Effects of mutations in the *Saccharomyces cerevisiae* RNA14, RNA15, and PAP1 genes on polyadenylation *in vivo*. *Mol. Cell. Biol.* **15,** 6979–6986 (1995).
18. Amrani, N., Dufour, M. E., Bonneaud, N. & Lacroute, F. Mutations in STS1 suppress the defect in 3′ mRNA processing caused by the *rna15-2* mutation in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **252,** 552–562 (1996).

Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

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Transmission 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[1–3] 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[3–6]. 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[1,2]. 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

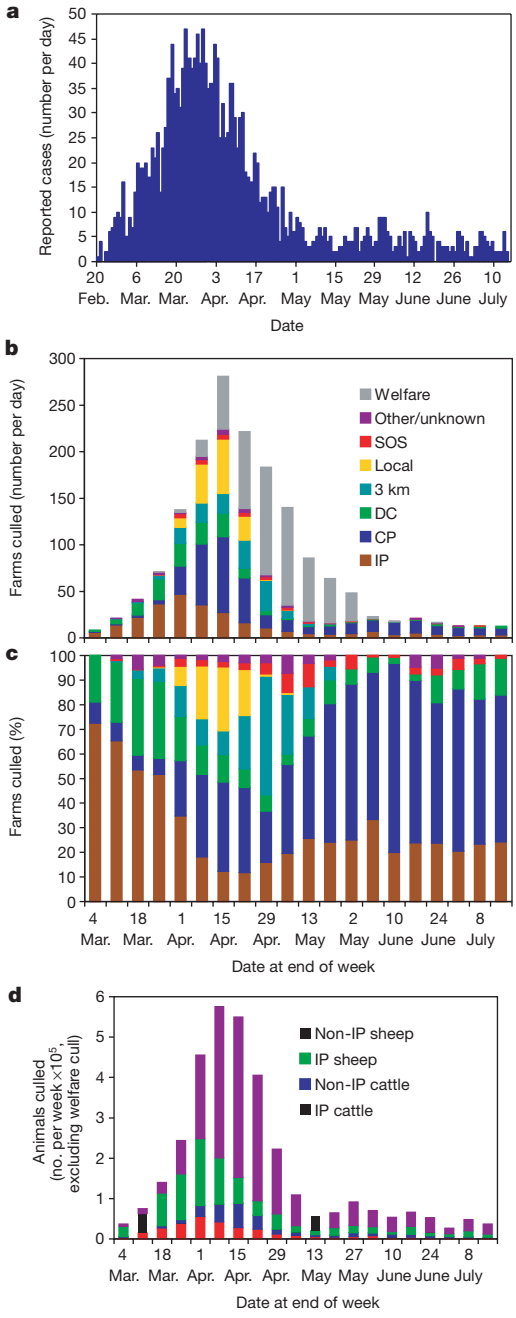

Figure 1 FMD incidence and culling through time. **a**, Time series of FMD cases in the 2001 epidemic, by date of report. **b**, Weekly number of farms culled by control policies: infected premises (IP); contiguous premises (CP); farms identified through tracing as having animals that had dangerous contact (DC) with animals from an IP; farms within 3 km of an IP (3 km); voluntary local sheep cull on farms in heavily affected areas not covered by the 3-km cull (local); farms slaughtered on suspicion (SOS) of infection but not subsequently confirmed by culture or enzyme-linked immunosorbent assay; and other/unknown. Also shown is the weekly number of farms culled to relieve the animal welfare problems resulting from FMD-related restrictions on animal movements (welfare). The 3-km and local culls were largely confined to Cumbria, Dumfries and Galloway. Farms are included only once, so where farms underwent multiple separate culls, the date of the largest cull is used. **c**, Weekly percentage of non-IP farms culled, by policy. **d**, Weekly number of sheep and cattle culled on IP and non-IP farms to control FMD. See Methods and Supplementary Information for details of data sources.