Gene Expression Is Circular: Factors for mRNA Degradation Also Foster mRNA Synthesis

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SUMMARY

Maintaining proper mRNA levels is a key aspect in the regulation of gene expression. The balance between mRNA synthesis and decay determines these levels. We demonstrate that most yeast mRNAs are degraded by the cytoplasmic 5′-to-3′ pathway (the “decaysome”), as proposed previously. Unexpectedly, the level of these mRNAs is highly robust to perturbations in this major pathway because defects in various decaysome components lead to transcription downregulation. Moreover, these components shuttle between the cytoplasm and the nucleus, in a manner dependent on proper mRNA degradation. In the nucleus, they associate with chromatin—preferentially ∼30 bp upstream of transcription start-sites—and directly stimulate transcription initiation and elongation. The nuclear role of the decaysome in transcription is linked to its cytoplasmic role in mRNA decay; linkage, in turn, seems to depend on proper shuttling of its components. The gene expression process is therefore circular, whereby the hitherto first and last stages are interconnected.

INTRODUCTION

Gene expression is traditionally divided into several stages, including mRNA synthesis and processing, export (in eukaryotes), translation, and decay. Yet, gene expression can be viewed as a single system in which all stages are mechanistically coupled (Komili and Silver, 2008) and coordinated by master regulators (Harel-Sharvit et al., 2010). An essential and well-controlled component of this system is the cytoplasmic mRNA decay pathway, considered to represent the endpoint of the mRNA life. Following shortening of the mRNA poly(A) tail by the Ccr4/Not and Pan2/3 complexes, the eukaryotic mRNA can then be degraded by two pathways: from 3′ to 5′ by the exosome or from 5′ to 3′ by the Xrn1p exonuclease (Garneau et al., 2007; Parker, 2012; Pérez-Ortín et al., 2013; Haimovich et al., 2013). The latter pathway involves prior removal of the 5′-cap by the Dcp2p enzyme, assisted and regulated by Dcp1p, Pat1p, Dhh1p, Edc1/2/3p, and the Lsm1-7 complex. Conventional wisdom holds that, following the completion of the degradation of a certain mRNA, the mRNA decay factors (DFs) re-enter the decay process of yet another mRNA in the cytoplasm. Other options have not been systematically examined.

Here, we report that all tested DFs shuttle between the cytoplasm and the nucleus, associate preferentially with transcription start-sites and stimulate transcription initiation and elongation. Moreover, import of DFs depends on the capacity of Xrn1p to function in mRNA degradation. Various statistical analyses uncovered a linkage between the functions of Xrn1p in mRNA synthesis and decay. We propose that the synthetic and decay processes represent two arms of a larger machinery, the “synthegradosome.”

RESULTS

Steady State mRNA Levels Are Highly Robust to Perturbations in mRNA Decay

The rates of mRNA synthesis and decay determine the steady-state level of mRNA (also referred herein as mRNA abundance [RA]). Accordingly, a defect in mRNA decay is expected to result in an increase in mRNA levels. As expected, elevated levels of EDC1 and RPS2BB mRNAs were observed in cells lacking various genes encoding DFs (Figure 1A and Figure S1A available online) (Badis et al., 2004; Muhlrad and Parker, 2005). Surprisingly, the levels of various other mRNAs did not increase in strains lacking these DFs (Figures 1A and S1A) and some levels even decreased, despite their increased stabilities (Figure 1B). These
results prompted us to obtain a whole-genome view of both the half-lives (HL) and RAs (Pelechano and Pérez-Ortíñ 2008). First, we found that Xrn1p—the only known cytoplasmic 5′ to 3′ exonuclease—is involved in the degradation of most, if not all, mRNAs (Figures 1C and S1C, and Table S1). Thus, as proposed previously (Anderson and Parker, 1998; Coller and Parker, 2004; Parker, 2012), Xrn1p-mediated decay is a major cytoplasmic pathway for mRNA degradation in yeast. Second, consistent with the northern analysis, most spots scattered around or below the ratio 1. Importantly, no correlation was found between the effect of XRN1 deletion on HLs and its effect on RA (Figure 1C).

Table S1 shows a list of mRNAs whose stability was severely affected by XRN1 deletion, but their RA was little perturbed. These observations suggest that changes of HL are compensated by inverse changes in mRNA synthesis, as was observed in a number of specific cases (Table S2, see also legend). These results are consistent with previous data demonstrating that the levels of most mRNAs in Δxrn1 and ΔΔcap1 cells are not higher than those in wild-type (WT) cells (He et al., 2003; Muhlrad and Parker, 1999). We therefore hypothesized that, in addition to their role in mRNA decay, the DFs have the capacity to enhance transcription, either directly or indirectly. This notion is supported by prior studies demonstrating numerous physical and genetic interactions of various DFs with factors that are involved in the nuclear stages of gene expression (Table S3 and Figure S1B).

Disruption of Xrn1p Compromises Transcription of Most Genes

To obtain a whole-genome view of transcription rates (TRs), we performed genomic run-on (GRO) experiments (García-Martínez et al., 2004; Pelechano and Pérez-Ortíñ, 2010). We found that TRs of most genes were downregulated in Δxrn1 cells (p < 10−15, KS test, median of 4.6-fold, Figure 1D, Δxrn1).

Xrn1D208A is an inactive form of the enzyme that is expressed at WT level and binds uncapped mRNA as efficiently as Xrn1p but does not degrade it (Solinger et al., 1999). Proliferation rate of xrn1D208A and Δxrn1 strains are comparable (Figure S1E; Solinger et al., 1999), and they exhibit similar levels of P bodies (Figure S1F). More importantly, cumulative distribution of HLs in the two mutants is comparable (Figure S1C). Nevertheless, xrn1D208A cells were more defective than the Δxrn1 cells in transcription (Figures 1D and S1D) (median of 8.5-fold, p < 10−15 KS test). TR values are listed in Table S4.

Transcriptional Induction Is Dependent on mRNA Decay Factors

Next, we investigated de novo mRNA synthesis and mRNA decay in response to various environmental signals. As expected (Lohr et al., 1995), galactose stimulation rapidly induced transcription of GAL genes in WT cells (Figure 2A), and glucose addition led to rapid transcriptional repression (Lohr et al., 1996), followed by mRNA degradation (Figure 2B). Accumulation of mRNA in the xrn1D208A strain lagged behind
the WT, indicating a clear defect in transcriptional induction (Figure 2A). Although deletion of XRN1 led to stabilization of GAL mRNAs (Figure 2B), mRNA accumulation in the Δxrn1 strain was comparable to that in the WT cells (Figure 2A), suggesting a defect in transcription in this mutant as well—consistent with the GRO results. Significantly, because the effect of D208A mutation on mRNA stability was identical to that of XRN1 deletion (Figure 2B), we concluded that the different accumulation of mRNAs in these mutants (shown in Figure 2A) is solely due to a difference in their transcriptional capacity.

We also assessed transcriptional induction and mRNA decay of heat shock (HS) genes in response to HS, and non-HS genes during recovery from HS. We compared WT to xrn1 mutant strains, discussed above. We also examined Δdcp2 and dcp2-4, encoding enzyme-dead Dcp2E153Qp (Dunckley and Parker, 1999). As shown in Figures 2C, S2A, S2B, and S2E, transcription of these genes was relatively defective in the xrn1 and dcp2 mutant strains. The transcriptional defect was, again, more pronounced in both enzyme-dead strains compared to their respective deletion strains, despite comparable stability of their mRNAs (Figures 2B, 2D, S2B, S2D, and S2F).

Interestingly, both transcription and decay of a noncoding RNA were found to be dependent on DFs (Figures S2G–S2I), like those of mRNAs.

**Xrn1p Affects Pol II Occupancy on TEF4 Gene**

Fluorescent in situ hybridization (FISH) analysis, designed to detect single TEF4 mRNA molecules and to identify specific nuclear transcription sites (TSs) (Femino et al., 1998; Zenklusen et al., 2008), was next employed (see Extended Experimental Procedures and Figures S3A, S3B, and S3E). Single TS was detected in the nuclei of these haploid cells, using either the double probes (Figure 3B) or three dimensional (3D) reconstructions of cells labeled with the ORF probes (Movie S1). Most WT and Δxrn1 cells (88%) contained a TS, whereas only 66% of the xrn1D208A cells contained a TS (p = 2.6 × 10⁻⁶) (Figures 3C and S3F).

WT cells contained TSs with more than one transcript (equivalent to multiple elongating Pol II occupancy) (Figures 3C and S3)—indicative of frequent transcription initiation or reinitiation events (Zenklusen et al., 2008). In contrast, Δxrn1 and xrn1D208A cells contained TSs with only one transcript (Figures 3C and S3F), suggesting that Xrn1p is required for either transcription initiation, elongation, or both. Reassuringly, despite the transcriptional defects, TEF4 mRNA number per WT cell was comparable to that in the mutant cells, as determined by both FISH and northern analyses (Figures S3C and S3D, respectively). Note that nuclear export of the TEF4 mRNA in both xrn1 mutant strains was normal, as no nuclear accumulation could be observed by FISH analysis outside the TS context (Figure 3B, Movies S1 and S2 and data not shown).
Various mRNA Decay Factors Shuttle between the Cytoplasm and the Nucleus in a Manner Dependent on Proper mRNA Decay

The studied DFs are detected by and large in the cytoplasm (e.g., Teixeira and Parker, 2007). By inactivating export using a temperature-sensitive strain (Brune et al., 2005), we found that all tested DFs accumulated in the nucleus, like Pab1p that served as the positive control (Brune et al., 2005). Because nuclear accumulation was independent of de novo translation (Figures 4A and S4A), we conclude that the same protein molecules, which had been present in the cytoplasm prior the heat inactivation, entered the nucleus. Nuclear accumulation of Pat1p and Dhh1p was previously observed in a ∆sm1 strain (Teixeira and Parker, 2007), suggesting that these DFs are exported in complex with Lsm1p. Thus, all the examined DFs normally shuttle between the two compartments. We suspect that these DFs are usually visualized in the cytoplasm because their export rate exceeds their import rate. Interestingly, the equilibrium between export and import kinetics could be altered in response to environmental cues such as starvation (Figures S4B–S4E and S4G) or HS (Figure S4F). Furthermore, nuclear accumulation of some of the examined DFs could be detected in several WT strains under optimal conditions (Figures S4B and S4C).

In order to assess whether nuclear import of DFs is dependent on proper mRNA decay, the same shuttling assay was performed using various XR11 mutants. Import of Xm1D208Ap-GFP was severely impaired (Figure 4B). Xm1D208Ap binds decapped RNAs normally, without degrading them (Solinger et al., 1999). We hypothesized that the combination of these two features might block its import. To test this hypothesis, we introduced a second mutation in the pocket that binds the decapped RNA. Two such mutations were employed, R101G and H41D (Jinek et al., 2011; Page et al., 1998), which cause little effect on the proliferation rate (Figure S1E). Remarkably, introducing the R101G mutation into Xm1D208Ap partially restored import capacity of this (still enzyme dead) protein, suggesting that the RNA needs to be positioned properly in the active site in order to repress import of Xm1D208Ap. However, import of Xm1D208A,R101Gp-GFP was not as efficient as import of the WT Xm1p-GFP (see p values in Figure 4B), raising the possibility that proper RNA binding is important, by itself, for efficient import. To examine this possibility, we determined the import capacity of Xm1R101Gp-GFP and Xm1H41Dp-GFP. Indeed, import of these proteins was similarly compromised relative to that of Xm1p-GFP (Figure 4B, p = 0.01 and p = 0.02, respectively). Pab1p-GFP was efficiently and equally imported in all the strains, demonstrating that the import defects of the various xm1 mutant cells is not general. In summary, efficient import of Xm1p requires both proper RNA binding in the active site and its subsequent degradation. Only WT Xm1p is therefore imported efficiently.

Interestingly, Dcp2p-RFP import was severely impaired in Xm1D208Ap cells (p = 0.01) but was relatively efficient in Xm1D208A,R101G, Xm1R101G, Xm1H41D, or ∆xm1 cells (Figure 4C). Furthermore, during starvation, Xm1D208A cells poorly imported various other DFs (Figure S4G). Collectively, these results suggest that import of DFs does not occur as a default. It seems to require normal Xm1p that is capable of binding decapped RNA and executing 5′ to 3′ mRNA decay. As shown below, the import features of Xm1p are correlated with its capacity to stimulate transcription.

Decay Factors Associate with Chromatin and Stimulate Transcription Initiation

Next, we examined whether DFs are capable of binding chromatin, using chromatin immunoprecipitation-exo (ChIP-exo) analysis (Rhee and Pugh, 2012). Due to the exonuclease activity that degrades most of the DNA molecules that were not covalently bound by the immunoprecipitated (IP-ed) proteins, binding peaks are more dispersed than standard ChIP-sequencing, yet with better resolution and better signal-to-noise ratio (Figures 5A and 5D). All the examined DFs (Xm1p-TAP, Dcp2p-TAP, and Lsm1p-TAP) were detected along the chromatin at levels significantly higher than the control (Figures 5A and 5D).
Decay Factors Affect Transcription Elongation

Unexpectedly, our GRO analysis revealed a direct correlation between the negative impact of Xrn1p disruption on transcription and the presence of Decay Factors (DFs) at transcription start sites (TSSs). These data suggest that DFs do not bind chromatin as independent factors but rather, at least Xrn1p and Lsm1p seem to bind in a complex.

To corroborate DF binding to chromatin, we performed a ChIP assay followed by qPCR analysis (Figure 5E). Binding of Xrn1p-TAP along PMA1 was similar to the binding profile obtained by the ChIP-exo technique (compare Figure 5D with 5E). Xrn1p-TAP was also found to be associated with the TEF4 promoter as well as other promoters (data not shown), but not with rDNA (Figure S5A). Moreover, the ChIP-qPCR indicates that not only Xrn1p, Lsm1p, and Dcp2p bind promoters, but also Pat1p-TAP, and Dhh1p-TAP are capable of binding promoters (Figures S5B and S5C). Consistent with a direct binding to the chromatin, ChIP-qPCR signals of Xrn1p-TAP and Dcp2p-TAP did not decrease due to RNase digestion prior to IP (data not shown). We found that import of Dcp2p is defective in xrn1, xpo1-1, mex67-5 mutant cells coexpressing Pab1p-GFP and Dcp2p-RFP, which was coexpressed with Dcp2p-TAP (Figure 4C). Consistently, less Dcp2p-TAP was found associated with PMA1 promoter in xrn1ΔD208A cells compared to WT cells (44% ± 3% in the mutant compared to the WT) (data not shown).

Our results so far suggest that DFs interact with PIC and are involved in transcription initiation. To further corroborate this role, we examined whether they are capable of stimulating transcription when artificially recruited to reporter promoters (Titz et al., 2006). We fused the DFs to the Gal4p DNA-binding domain (Gal4p-BD), which also possesses a strong nuclear localization signal (NLS), and analyzed transcriptional activation of the reporter genes, PGAL1-HIS3 and PGAL7-lacZ as well as the natural GAL10 gene.

Transcription of these genes was stimulated by recruitment of some of the DFs to their promoters, using the Gal4p NLS and DNA-binding capacity (Figures S5D–S5G and Table S5). Interestingly, Gal4p-BD-Dcp2p-RFP, a mutant lacking decapping activity, activated transcription similarly to Gal4p-BD-Dcp2p (Figures S5D, S5E, and S5G and Table S5). This suggests that the decapping activity of Dcp2p per se is not necessary for its capacity to stimulate transcription.

Although we showed that many DFs shuttle between the cytoplasm and nucleus as well as associate with chromatin, not all were able to activate transcription. This may either reflect a true biological feature (i.e., they do not contain an “activating domain”) or may be due to differences in expression levels of the fusion genes (Figures S5H and S5I; see legend for discussion) or the effect of the Gal4p-BD moiety.

In a strain harboring the xrn1ΔD208A mutation (Figure S5F) or Δxrn1 (data not shown), Gal4p-BD-Dcp2p was unable to induce transcription. However, Gal4p-BD fusion of Ccr4p, Pat1, and Rpb3p activated transcription in this mutant, suggesting that Xrn1p is specifically required for transcriptional activation by Gal4p-BD-Dcp2p. Taken together, these results, combined with the role assigned to these factors in transcription and their chromatin-binding features, argue against a trivial effect of DFs in this tethering assay.

Decay Factors Affect Transcription Elongation

Remarkably, all three DFs, unlike the control, preferentially bind ∼30 nucleotides upstream to transcription start sites (TSS) (Figure 5B), the site where the transcription preinitiation complex (PIC) is assembled (Kornberg, 2007). Moreover, efficiency of their binding to promoters is correlated with transcription rate, determined by GRO (Figure 5C). These two results suggest that chromatin binding is transcriptionally functional.

Xrn1p-TAP and Lsm1p-TAP produced almost overlapping ChIP peaks along PMA1 gene locus (Figure 5D) and in other loci (Figure 5A and data not shown). Dcp2p-TAP profile was similar to that of the other two DFs but not identical. These data suggest that DFs do not do bind chromatin as independent factors. Rather, at least Xrn1 and Lsm1 seem to bind as a complex.

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and the open reading frame (ORF) length (Figure 6A), raising a possible role in transcription elongation (Morillo-Huesca et al., 2006; Rodríguez-Gil et al., 2010). To examine this possibility further, we first used our previously developed assay to examine the distribution of either Pol II molecules by means of RNA polymerase ChIP-on-chip (RPCC), or the distribution of transcrip-
tional activity, as determined by run-on, did not (Figure S6A). Specifically, the ratio between run-on signals in the mutant versus WT was maintained in the 5' (arbitrarily defined as 1), middle, and 3' portion of the gene (Figure S6A). Phosphorylation of Pol II CTD heptad repeat at Ser-2 position is one hallmark of elongating Pol II by means of GRO (Rodríguez-Gil et al., 2010). We used a membrane containing 5' portions relative to the 5' portions of these ORFs. RPCC data revealed abnormal Pol II accumulation in the 3' portion of the mutant genes. In contrast, we observed no such bias in the GRO signal (Figure 6B, red columns), indicating that these surplus Pol II molecules were unable to elon-
gate transcription in vitro.

We next similarly examined Pol II occupancy and its activity along the GAL1 gene after induction by galactose. Consistent with the genomic data shown in Figure 6B, Pol II occupancy increased by disruption of Xm1p (Figure 6C), whereas its elongation activity, as determined by run-on, did not (Figure S6A). Specifically, the ratio between run-on signals in the mutant versus WT was maintained in the 5' (arbitrarily defined as 1), middle, and 3' portion of the gene (Figure S6A). Phosphorylation of Pol II CTD heptad repeat at Ser-2 position is one hallmark of elongating Pol II (Bataille et al., 2012; Meinhart et al., 2005). Consis-
tent with a role for Xm1p in elongation, Ser-2 was hypophos-
phorylated in xrn1 mutants compared to WT cells (Figure 6C).

The drug 6-azauracil (6-AU) depletes NTPs thereby reducing both the elongation rate and Pol II processivity, which is aggra-
vated by mutations in elongation factors (Mason and Struhl, 2005). Therefore, 6-AU sensitivity is often indicative of a defect in transcription elongation (e.g., Fish and Kane, 2002; Hartzog et al., 1998; Malagon et al., 2006; Mason and Struhl, 2005).

Indeed, some decay mutants were hypersensitive to 6-AU (Figure S6B), reinforcing our conclusion that they are involved in transcription elongation.

**Transcription Is Linked to mRNA Decay**

Our finding that transcription is severely compromised upon disruption of Xm1p enzymatic activity suggests that the role of Xm1p in transcription is mechanistically linked to its role as RNA exonuclease. To examine this possibility, we first analyzed whether the binding capacity of Xm1p-TAP, Lsm1-TAP, and Dcp2-TAP to promoters, determined by ChIP-exo, is correlated with the effect that Xm1p disruption has on transcription, determined by GRO. We arbitrarily divided the genes into four equal groups based on the effect that Xm1p disruption had on their transcription and found a direct correlation with promoter bind-
ing (Figure 7A). This correlation reinforces our premise that binding of Xm1p (and possibly also Dcp2p and Lsm1p) to chromatin is related to its effect on transcription. Moreover, we found that promoter binding is also correlated with HL (Figure 7B). Interest-

ably, the studied DFs tend to bind promoters of genes that encode unstable mRNAs (p < 0.005) establishing a linkage between promoter binding and mRNA decay. If the two func-

tions are indeed linked, one expects that a defect in one function would affect the other. To test this possibility, we arbitrarily clas-
sified the mRNAs according to the effect of Xrn1p disruption on their decay rate (DR), and examined the transcriptional effect that Xm1 disruption has on these groups. Remarkably, we found a direct correlation between the capacity of the cells to degrade mRNAs and to synthesize them (Figure 7C). This conclusion is also implied by the data in Figure 1C. Note that xrn1 disruptive cells are more defective in transcription than Δxrn1 cells (Figure 2A, S2A and S2E). As shown in Figure 7C, for any given strain and among the strains, the more mRNA decay is dependent on
Xrn1p, the more its synthesis is affected by disrupting its activity. Collectively, the capacity of Xrn1p to degrade mRNAs is related to its capacity to stimulate their synthesis.

Last, if the function of Xrn1p in transcription is linked to its function in mRNA decay, it might be possible to uncouple these two roles. We examined whether mutating either (1) the enzymatic activity (D208A) or (2) the pocket in the active site that binds the decapped 5'P-RNA (R101G) can uncouple the two functions. Unlike R101G, the D208A does not interfere with the normal RNA binding (Jinek et al., 2011; Page et al., 1998; Solinger et al., 1999). As expected (see Figure 2A), xrn1D208A cells displayed defective transcriptional induction of GAL genes in response to galactose. Accumulation of mRNA upon similar stimulation of Δxrn1 and xrn1R101G strains with galactose was comparable to that in the WT cells (Figure 7D). Because xrn1R101G and Δxrn1 strains exhibit identical mRNA decay rates (Figure S7A) and accumulation of GAL mRNAs (Figure 7D), and because Δxrn1 cells are defective in transcription of most genes (Figure 1D), we can conclude that transcription in xrn1R101G strain is as defective as it is in Δxrn1 strain. These results suggest that proper recruitment of decapped RNA to Xrn1p active site is important for the capacity of Xrn1p to regulate transcription because it is important for its import (Figures 4B and 4C). Remarkably, introducing the R101G mutation into xrn1D208A partially recovered the severe transcription defect exhibited by the xrn1D208A strain. Consequently, the transcription capacity of the xrn1D208A,R101G strain was similar to that of the Δxrn1 and xrn1R101G strains (Figure 7D), given that mRNA decay in these three strains was identical (Figure S7A) (see Discussion).

Collectively, the four independent experiments, presented in Figure 7, suggest that the capacity of Xrn1p to bind and degrade mRNAs is related to its capacity to stimulate their synthesis. The hitherto first and the last stages of the mRNA life are therefore interconnected (Figure 7E).

Discussion

Our work and those of others demonstrate that the steady-state mRNA levels cannot serve as a reliable assay to examine transcription or decay rates. These levels are robust to perturbations in either transcription (Esberg et al., 2011; Goler-Baron et al., 2008; Harel-Sharvit et al., 2010; Schwabish and Struhl, 2007) or mRNA decay (this work). Here we show that most mRNAs in optimally proliferating yeast cells are degraded by the 5' to 3' exonuclease Xrn1p, as proposed previously (Anderson and Parker, 1998; Coller and Parker, 2004). Nevertheless, disruption of the major decay pathway does not result in elevated steady-state levels; in most cases these levels were even decreased. Consistently, disruption of this pathway has a minor effect on the proliferation rate of optimally proliferating cells (Figure S1E).

Decay Factors Play a Direct Role in Transcription

The cross talk between mRNA synthesis and decay involves a role of DFs in transcription. The following observations are consistent with a direct role in transcription. (1) Binding of Pat1p-TAP, Dhh1p-TAP, Xrn1p-TAP, Dcp2p-TAP, and Lsm1p-TAP to promoters, and to a lesser extent also to other regions of transcription units (Figure 5). Their binding to promoters seems to be transcriptionally relevant (see below). Chromatin binding is consistent with the shutting of these factors back and forth between the nucleus and the cytoplasm. (2) The GRO data clearly demonstrate that densities of active Pol II are adversely affected by deleting Xrn1p or by mutating its active

Figure 6. Factors of the Major mRNA Decay Pathway Affect Transcription Elongation

A) The impact of XRN1 disruption on transcription is proportional to the ORF length. Sliding window analysis of the dependence of the change in TR ratio (mutant/WT) on ORF length. TR ratios in log2 scale (Table S4) were averaged using a 200 bp sliding window. The red line shows the fitted linear negative tendency. The p values shown at the top were obtained by t test determining the differences in average TR ratio between the genes of <1,000 bp and those >1,750 bp.

B) 3'5' RPCC (blue columns) and GRO (red columns) analyses were performed as described in Experimental Procedures. The histograms depict the average ratio of 3’5’ signals obtained for any of the indicated strains ± SD of three independent experiments. A Wilcoxon test shows for any of the indicated strains.
site (e.g., Figure 1D). (3) Single-cell imaging technique demonstrates that mRNA synthesis is dependent on Xrn1p (Figures 3 and S3). The results of this approach are consistent with a role for Xrn1p in transcription initiation and elongation. (4) The effect of disrupting the enzymatic activity of Xrn1p or Dcp2p on the transcriptional induction of genes from several families. Although there is little difference between the effect of disrupting Xrn1p enzymatic activity and its complete deletion on mRNA HL (Figure S1C), the two mutations have different effects on transcription (e.g., Figure 2A). This indicates that the presence or absence of Xrn1p or Dcp2p, regardless of their enzymatic activities or their effect on mRNA decay, affects Pol II transcription. (5) The tethering assay that shows that some DFs may have an “activating domain” (Figures S5D–S5G). (6) The numerous genetic and physical interactions of DF genes or proteins with many components of the transcription apparatus (Figure S1B and Table S3). (7) The paradoxical effect that deletion of DF genes has on mRNA levels. Importantly, this paradoxical effect results from deleting any of the many DFs we tested (Figures 1A and S1A). This observation suggests that the crosstalk between mRNA synthesis and decay is not specific to some factors. Rather, it is a feature of the decaysome complex.

Whole-genome-binding features of Xrn1p-TAP, Dcp2p-TAP and Lsm1p-TAP helped us reveal additional linkages between the two roles of the decaysome. The three studied DFs prefer to bind ~30 bp upstream of TSSs (Figure 5B). Because the PIC also binds ~30 bp upstream of TSS (Kornberg, 2007), it is possible that the three studied factors assemble together with the PIC. This possibility is in accord with the numerous physical and genetic interactions between DFs and transcription factor IID (TFIID), and Spt-Ada-Gcn5-Acetyl transferase (SAGA) and the mediator complexes (Figure S1B and Table S3). Xrn1p-TAP, Dcp2p-TAP, and Lsm1p-TAP prefer to bind promoters of genes whose transcription is highly affected by Xrn1p disruption (Figure 7A), again suggesting that promoter binding is transcriptionally functional. Moreover, these DFs prefer to bind promoters that govern transcription of unstable mRNAs (Figure 7B), suggesting a linkage between their roles in mRNA decay and transcription (we therefore do not expect binding of DFs to all PICs or all transcription units). These preferences highlight the linkage between DFs roles in the two mechanisms. Detailed mechanistic understanding of these preferences remains to be determined.

Xrn1p Functions Also in Transcription Elongation
Deletion of XRN1 or disruption of its exonuclease activity leads to accumulation of transcriptionally incompetent Pol II at the 3’ portions of ORFs (based on the apparent discrepancy between RPCC and GRO data in Figure 6), which is also hypophosphorylated. When Pol II encounters nucleosome or other obstacles, it
reverses its direction and backtracks, leaving the transcript 3’-end misaligned with the active site and therefore cannot polymerase any further (Cheung and Cramer, 2011). Accumulation of inactive Pol II molecules is a hallmark of backtracking Pol II (Gómez-Herreros et al., 2012; Pelechano et al., 2009; Pérez-Ortín et al., 2012; Rodríguez-Gil et al., 2010). A main function of transcription factor IIIS (TFIIS) is to release backtracked Pol II, thus helping it to traverse through nucleosomes, and its deletion leads to accumulation of Pol II within the first four nucleosomes (Churchman and Weissman, 2011). Notably, deleting TFIIS or some other elongation factors results in accumulation of Pol II in 5’ portions of ORFs, whereas deleting others—in 3’ portions (Churchman and Weissman, 2011; Kruk et al., 2011; Mason and Struhl, 2005; Rodríguez-Gil et al., 2010). Indeed, deleting DST1 encoding TFIIS leads to defective transcription driven by Gal4p-BD-Rpb3p and Gal4-BD-Dcp2p in the tethering assay (data not shown). The mechanism underlying the second group of elongation factors, among them are Ssd1p and Bur2p (Rodríguez-Gil et al., 2010), is relatively little understood. As shown in Figure 6, Xrn1p belongs to the second, less studied, group. Interestingly, we and other investigators have found that Ccr4p-Not complex also belongs to the second group (Kruk et al., 2011; Rodríguez-Gil et al., 2010), raising the possibility that Xrn1p and Ccr4p-Not play a role in a common proteinaceous context. In agreement with this line of thought, deleting DST1 did not compromise transcription driven by Gal4p-BD-Ccr4p, Gal4p-BD-Pat1p, and Gal4p-BD-Dhh1p in the tethering assay (data not shown). Our data are consistent with a model whereby some DFs prevent Pol II from backtracking, thereby stimulating elongation in a manner independent of TFIIS (see also Kruk et al., 2011). Consistently, PAT1, LSM1, and CCR4 are synthetically lethal with DST1 (Table S3), suggesting that transcription elongation requires at least one of the pathways, either the TFIIS-dependent or the alternative pathway—mediated by some DFs. In recent years, it has become clear that recruiting Pol II to transcription start sites is insufficient to promote transcription and that postinitiation stages play key roles. The roles of DFs in elongation add an additional level of complexity to the regulations that occur after transcription begins.

**Decay Factors Might Function in Transcription as a Complex or Subcomplexes**

A number of observations led us to conclude that the novel transcriptional role is not restricted to a limited number of DFs. (1) Disruption of any DF that we examined has downregulated transcription. (2) All the examined DFs are shuttling proteins. Shuttling of some DFs is affected by disruption of Xrn1p enzymatic activity, suggesting that some of them shuttle as a complex. (3) Tethering a number of DFs to promoters stimulates transcription, which in some cases is dependent on Xrn1p or other DFs (Figure S5F and data not shown), suggesting cooperation between more than one DF. (4) All five DFs that we tested bind promoters. A whole-genome ChIP association of three of them showed a preference to coassociate ~30 bp upstream of TSS. (5) Very similar ChIP profiles along genes also suggest that the DFs do not bind chromatin as independent factors.

**The Linkage between mRNA Decay, Import of Decay Factors and Transcription**

DFs play two opposing roles in determining mRNA levels. Significantly, these two activities seem to be mechanistically linked (Figures 7B and 7C). Our data raise the possibility that DF import plays a key role in this linkage. A positive correlation is found between transcriptional efficiency and the capacity of Xrn1p and other DFs to shuttle as well as to degrade mRNAs. First, proper binding of Xrn1p to the decapped RNA is required for its efficient import (Figure 4B) and for efficient transcription (Figure 7D). Second, cells harboring \(Xrn1^{R101G}\), \(xrn1^{H41D}\), \(xrn1^{D208A,R101G}\), or a deletion of \(XRN1\), which can import DFs (other than Xrn1p) efficiently, transcribe better than those harboring \(xrn1^{D208A}\) (e.g., Figure 7D), which are defective in importing Xrn1\(^{D208A}\) as well as other DFs (Figures 4B, 4C and S4G). Because \(xrn1^{D208A,R101G}\) cells transcribe better than \(xrn1^{D208A}\) cells, it is clear that D208A mutation per se does not disrupt the ability of Xrn1p to function in transcription. Rather, D208A affects the interplay between the two opposing roles of Xrn1p in mRNA decay and transcription, most probably due to its severe effect on import. D208A, which disrupts the exonucleolytic activity, exerts its adverse effect only if the Xrn1p active site binds the RNA at the 5’ end properly. If it does not bind properly, e.g., in the case of R101G, the enzymatic activity is neutral (Figures 4B, 4C, and 7D). Thus, only the combination of properly binding the decapped RNA in the Xrn1p active site and the inability to degrade it blocks import of key DFs (including the Xrn1p mutant form itself) (see a model in Figure S7B). It is possible that the combination of D208A and R101G mutations displaces Xrn1p from its natural context, creating a situation comparable to complete absence of Xrn1p (Figures 4B, 4C, and 7D).

We propose a model (Figure S7B) whereby Xrn1p represses premature import of DFs, thus linking between mRNA decay and import. Efficient repression is dependent on proper binding of the decapped RNA in the 5’-phosphate-binding pocket of Xrn1p’s active site. Only once the RNA has been successfully degraded does Xrn1p stimulate DF import, which is followed by transcriptional stimulation. According to this model, \(xrn1^{D208A}\) represses import constitutively, because the RNA in its active site is not degraded. Indeed, the RNA can be degraded by the exosome. However, a few bases may remain bound in Xrn1p-binding pocket, inaccessible to the exosome, maintaining Xrn1p in a conformation that represses import. The enzyme-dead Dcp2-4p, which severely compromises transcription (Figure 2C), might similarly block import as long as it is bound to the 5’-cap structure, an issue that remains to be examined.

**Other Possible Mechanisms**

The decaysome may affect transcription by degrading regulatory RNAs (e.g., ncRNAs) (Geisler et al., 2012; van Dijk et al., 2011). However, our current data are more consistent with a degradation-independent mechanism. (1) All DFs examined are shuttling proteins and bind chromatin. This binding is direct, and not mediated by RNA (data not shown), and is affected by disrupting the exonucleolytic activity of Xrn1p (see earlier). (2) Some DFs prefer to bind directly at the PIC assembly site (Figure 5B) and can activate transcription when artificially tethered to promoters.

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pressing the enzyme-dead Dcp2-4p or Xrn1D208Ap is different (data not shown). (6) The transcriptional capacity of cells expressing the enzyme-dead Dcp2-4p or Xrn1D208Ap is different than those carrying a deletion of DCP2 or XRN1 (Figures 2, S2, 3, and 7). This difference is inconsistent with a simple decapping and degradation of ncRNA as the main underlying mechanism. Indeed, the transcriptional capacity of xrn1D208A cells can be partially rescued by introducing another mutation (R101G), indicating that the active site per se is not critical for transcription (Figure 7D). Moreover, inactivating the enzymatic activity of Dcp2p, using Dcp2-4p mutant form, does not affect the protein capacity to activate transcription in the tethering assay (Figures S5D, S5E and S5G and Table S5), suggesting that the decapping activity of Dcp2p is not necessary for its capacity to stimulate transcription in this assay.

Nevertheless, it is quite possible that the effect of the decaysome on mRNA synthesis involves more than one mechanism, a general and direct one described here, and one that acts indirectly through degradation of ncRNA, which may be restricted to subclasses of genes. Some of our unpublished observations suggest that the relative impact of the two mechanisms is strain dependent (G.H. and M.C., unpublished data).

**Gene Expression Is Circular**

The capacity of the decaysome to stimulate both mRNA synthesis and decay probably helps coordinating the two activities that determine mRNA levels. A whole-genome analysis demonstrated that families of yeast genes, whose transcription is coregulated in response to environmental cues, are also degraded in a coordinated fashion, maybe by a common mechanism (Shalem et al., 2008). The dual role of the decaysome may underlie this coordination. An interesting issue for future studies is how the balance between the synthetic and decay functions of the decaysome is regulated. This kind of regulation can affect the fine-tuning of the desired steady-state levels, as well as the kinetics with which they are achieved in response to environmental changes.

Coupling of two processes, as we view it, requires that the activity of certain factor(s) in the first process is a prerequisite for its function in the subsequent step. Following this criterion, it was previously found that mRNA decay is coupled to translation, which, in turn, is coupled to mRNA export, maturation, and transcription (reviewed in Komili and Silver, 2008). Gene expression was therefore considered a linear pathway. Remarkably, Pol II, promoters and other transcription components can control cytoplasmic mRNA decay [Bregman et al., 2011; Dahan and Choder, 2013; Goler-Baron et al., 2008; Haimovich et al., 2013; Pérez-Oztín et al., 2013; Shalem et al., 2011; Trcek et al., 2011]. The synthetic and decay processes can therefore be viewed as two arms of a larger machinery, the “synthegradosome.” The coupling between the two arms of the synthegradosome converts gene expression into a circular system (Figure 7E). Circular processes are inherently robust, because defects in one stage affect the overall pace of the entire process, thereby maintaining the essential balance between the stages. The maintenance of mRNA levels is one manifestation of this principle.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Plasmids**

Lists of yeast strains, plasmids, and construction details can be found in Tables S6 and S7 and Extended Experimental Procedures.

**Yeast Cultures**

Yeast cells were proliferated in synthetic complete medium (SC) at 30°C unless otherwise indicated. For starvation experiments, cells were incubated in media lacking carbon source and amino acids. For nucleocytoplasmic shuttling assay, cells were grown at 24°C and subsequently incubated at 37°C for 1–2 hr as indicated. For proliferation assay on 6-AU plates, 6-AU (100 μg/ml) was added to SC-Ura plates. Cells were serially diluted 1:5, spotted on the plates and incubated for 2 days at 28°C prior to photography. For proliferation of 3-AT plates, cells were streaked on SC-Trp-His plates containing different 3-AT concentrations (between 0 to 200 mM). Growth was assessed after 4 days. For more details see Extended Experimental Procedures.

**Analysis of Steady State mRNA Level, mRNA Half-Life, and Transcription Induction/Repression**

To determine RA and HL, cells were grown in synthetic complete (SC) medium at 30°C to 1 × 10^10 cells/ml. To determine HL of specific mRNAs (Figure 1B), 10-phenanthroline (100 μg/ml) (Merck) was used to block transcription.

For transcriptional induction and repression, using galactose and glucose respectively, cells were grown in SC-Raf (2% raffinose as carbon source) for at least seven generations until 5 × 10^6 cells/ml were present. Cell aliquot was taken for time point “0,” followed by addition of 2% galactose. Cell aliquots were taken, as indicated. At 75 min, the remaining culture was washed twice with water at room temperature and then resuspended in preheated (30°C) SC containing 4% glucose. For the heat shock experiments, cells were shifted rapidly from 30°C to 42°C then incubated at 42°C for 30 min. Cultures were then rapidly cooled in ice water back to 30°C. For all experiments, samples in each condition were collected at the indicated time points. RNA extraction and northern blot analysis were performed as previously described (Lottan et al., 2005).

**Fluorescence Microscopy**

Fluorescence microscopy was performed as previously described (Lottan et al., 2005).

**Fluorescent In Situ Hybridization**

FISH probes were designed as described previously (Levsky et al., 2002). FISH was performed essentially as described (Zenklusen et al., 2008). Images were analyzed by a 2D Gaussian fit algorithm as previously described (Thompson et al., 2002; Zenklusen et al., 2008). A detailed protocol, including image acquisition, data analysis, 3D reconstructions, and statistical analysis can be found in Extended Experimental Procedures.

**Genomic Run-On, 3'/5' Ratio Analysis, Determining HLs and RNA pol II ChIP on Chip Experiments**

Genomic run-on (GRO) analysis (three independent experiments) was performed as previously described (García-Martínez et al., 2004; Pelechano and Pérez-Oztín, 2010), with modifications (García-Martínez et al., 2011) using an updated version of the nylon microarrays (Alberola et al., 2004). 3'/5' ratio analyses and RPCC were performed essentially as previously described (Pelechano et al., 2009; Rodriguez-Gil et al., 2010). Thiolutin
shutoff analysis was done as previously described (Pelechano and Pérez-Ortín, 2008). RPCC was done as described (Pelechano et al., 2009; Rodríguez-Gil et al., 2010). Run-on of GAL1 was performed as described (Rodríguez-Gil et al., 2010). Detailed protocols can be found in Extended Experimental Procedures.

**Chromatin Immunoprecipitation**

Recruitment of TAP-tagged proteins to chromatin was assayed by ChIP analysis as previously described (Buck and Lieb, 2006) with some modifications: Crosslinking was performed at 0.75% formaldehyde; Spin-X centrifuge tube filters were used to prevent contamination from the IgG beads; elution buffer was spiked with an exogenous lacZ DNA fragment, which was later used to determine recovery during subsequent stages. The Absolute blue SYBR Green ROX mix (Thermo Scientific) was used for qPCR according to the manufacturer’s instructions in a 10 μl reaction volume. qPCR was performed in Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia). Detailed protocols can be found in Extended Experimental Procedures. ChIP-exo of TAP tagged proteins was performed by Peconic LLC (State College, PA). As a control, we used an isogenic strain that carries no tagged gene. Analysis of ChIP–Exo data and correlation to other genomic data sets are detailed in Extended Experimental Procedures.

**Statistical Analysis**

χ² test and standard t test were used for nuclear localization and α-gal assays, respectively, followed by p value calculations using GraphPad Software (http://www.graphpad.com/quickcalcs/pvalue1.cfm). Statistical analyses of GRO, FISH and ChIP data are detailed in the Extended Experimental Procedures.

**Accessions Numbers**

Gene Expression Omnibus (GEO) database references are GSE44312 for ChIP-exo data, GSE296519 for genomic macroarray data, and GSE43605 for 5'/3' portions of 384 genes macroarray data.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Extended Experimental Procedures, seven figures, seven tables, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2013.05.012.

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**SUPPLEMENTAL INFORMATION**

**EXTENDED EXPERIMENTAL PROCEDURES**

**Yeast Strains and Plasmids**

Yeast strains and plasmids are listed in Tables S6 and S7. To construct yMC609 and yMC662, the Δxrn1::KanMX4 cassette from yMC362 was PCR amplified and subsequently introduced into the chromosome by genomic replacement and selecting on YPD-G418 plates. Similarly, strains yMC412 and yMC757 were constructed with PCR amplified Δpat1::KanMX4 and Δdst1::KanMX4 cassettes from yMC363 and yMC471, respectively. Strain yMC399 was constructed with plasmid pMC349. To construct yMC571, the TAP-TRP1 cassette from pMC281 (Puig et al., 2001) was PCR amplified and subsequently introduced into the chromosome by genomic replacement and selecting on SC-Trp plates. To construct yMC519 and yMC520, the DCP2-TAP cassette from yMC454 was PCR amplified and subsequently introduced into the chromosome of yMC458 and yMC461, respectively, by genomic replacement and selecting on SC-Ura plates. yMC422, yMC426 and yMC511 were created by selecting 5-FOA resistant clones of yMC420, yMC371 and yMC459, respectively.

Plasmids pMC353, pMC438, pMC439, pMC440, pMC466 and pMC467 were created by replacing the LSM1 gene in pMC270 with PCR fragments containing the corresponding gene with its native promoter (~500 bp upstream to the ATG) by in vivo recombination. Plasmids pMC391 and pMC449 were created by replacing the URA3 marker on pMC350 and pMC269, respectively, with the ADE2 marker of pMC388 by in vivo recombination. pMC476 was created by replacing the DHH1-GFP from pMC391 with a PCR amplified fragment containing PAB1-GFP with its native promoter (427 bp upstream to ATG), from yMC497, by in vivo recombination. Plasmids pMC491 and pMC492 were created by digesting pMC466 and pMC467, respectively, with BamHI and XbaI to extract the RFP sequence. The GFP was inserted in frame by in vivo homologous recombination with a PCR amplified XRN1-GFP fragment from yMC499. XRN1 mutants R101G, D208A, R101G and H41D were created as follows: pMC491, pMC492, pMC560, pMC558 and pMC467 were double digested with SpeI and AgeI. The 2,545 bp SpeI-AgeI fragment from pMC467 and pMC560 was further digested with SfaI (1/4 of the SpeI-AgeI fragment of pMC560 was kept undigested). To get pMC580 and pMC582, pMC491 was ligated with the SpeI-AgeI fragment of pMC558 and pMC560, respectively. To get pMC579, pMC492 was ligated with the AgeI-SfaI fragment of pMC560 and SfaI-SpeI fragment of pMC467. The correct sequence of the mutants was verified by sequencing.

Plasmids for expression of Gal4p-BD-DFs fusion proteins (pGHOTY plasmids) were constructed according to the instructions of the Yeast Resource Center (YRC, University of Washington).

**Yeast Proliferation, Starvation, and Shuttling Assays**

Yeast cells were proliferated in synthetic complete medium (SC) at 30°C unless otherwise indicated. Cells were maintained for at least seven generations at the exponential growth phase prior to harvesting.

**Starvation Experiments**

Cells were washed once with water and then resuspended in SC lacking both carbon source and amino acids.

**Nucleocytoplasmic Shuttling Assay**

Cells were allowed to proliferate at 24°C to 3-5 × 10⁹ cells/ml and subsequently incubated for 1-2 hr at 37°C as indicated in the figure legend. The translation inhibitor Cycloheximide (CHX) (Sigma) was added to a final concentration of 100 μg/ml just prior to temperature shift. Pab1p-GFP was used to determine the efficiency of the assay and to serve as a nuclear marker (Brune et al., 2005). In all cases, Pab1p-GFP was present in the nuclei of at least 40% of the heat inactivated mutant cells.

**Proliferation on 6-Azauracil Plates**

Plate preparation: stock solution of 50 mg/ml 6-AU (Sigma) was prepared in 1 M NH₄OH and diluted to 100 μg/ml in SC-ura just prior to pouring the plates. Control plates were prepared by addition of the same volume of 1 M NH₄OH just prior to pouring, to eliminate any effect of changes in pH on proliferation. Cells were serially diluted 1:5, spotted on the plates (first spot is equivalent to 1 × 10⁵ cells) and incubated for 2 days at 28°C prior to photography.

**Proliferation on 3-Amino-2,3-Triazole Plates**

SC plates lacking both Tryptophan and Histidine and containing either 0, 1, 3, 5, 10, 20, 50, 100 or 200 mM of 3-Amino-2,3-Triazole (3-AT; Sigma) (Titz et al., 2006), or plates lacking only Tryptophan (as a positive control for proliferation) were prepared. Proliferation on 3-AT plates was determined by streaking the cells on the plates and assessing growth after four days at 28°C.

**Determining mRNA Steady-State Levels and Stability, Transcriptional Induction, and Repression**

For determining steady-state levels of mRNAs (RA) by northern analysis, cells were harvested at 0, 1, 3, 5, 10, 20, 50, 100, 200 or 400 mM of 3-Amino-2,3-Triazole (3-AT; Sigma) (Titz et al., 2006), or plates lacking only Tryptophan (as a positive control for proliferation) were prepared. Proliferation on the plates was determined by streaking the cells on the plates and assessing growth after four days at 28°C. The cells are more resistant to 3-AT the higher they express His3p. Cells, which do not express His3p at all will not be able to grow on plates lacking Histidine, even at 0 mM 3-AT.
Slot-blotted membranes were performed as previously described (Rodríguez-Gil et al., 2010). 300 bp-long DNA probes were obtained at maximum speed at 4°C for 2 min and the cell pellet was washed once with 1 ml ice-cold water, then frozen in liquid N2 and stored at −80°C until used. RNA extraction and northern blot analysis were performed as previously described (Lotan et al., 2005).

Genomic Run-On

In the GRO experiment, mRNA amounts (RA) were normalized using the median of Pol II spots and corrected by the concentration of total mRNA/cell. mRNA concentration in each of the three strains, WT and the two xrn1 mutants, was obtained from the data of total RNA/cell (three independent samples of known cell number) and cell median volumes (obtained by Coulter counter). The proportion of mRNA in total RNA was calculated from the electropherograms of total RNA obtained in an Experion device as the percentage of area in between the 5S and 18S rRNA peaks with regard to the total area as follows:

\[ \text{Experion data (mRNA/total RNA) was set as 1 for } yMC458 \text{ (WT); for } yMC461 \text{ (xrn1)} \] it was determined as 1.194, for yMC511 (xrn1) it was determined as 0.996.

Coulter counter and total RNA purification data (total RNA/cell) was set as 1 for yMC458 (WT); for yMC461 (xrn1) it was determined as 0.92, for yMC511 (xrn1) it was determined as 0.82.

The relative mRNA/cell amount was calculated to be 1.1 for yMC461 and 0.82 for yMC511.

Transcription rates (TR) of Pol II transcripts were averaged between the three replicates without any normalization. Reassuringly, the GRO data and FISH data, both determining the relative number of elongating Pol II in each condition, were consistent with each other. There was also a good agreement between the GRO and steady-state mRNA levels shown in Figure S1A (strain 4).

Run-On of GAL1

Slot-blotted membranes were performed as previously described (Rodriguez-Gil et al., 2010). 300 bp-long DNA probes were obtained by PCR. Midpoints of the probes for GAL1 were: +109 (5′); +871 (middle); and +1582 (3′), relative to the transcription start site. Run-on assays, hybridizations and genomic DNA labeling were done as described in the above reference.

ChIP-qPCR or ChIP-exo with TAP Tagged DFs

Cell cultures (YPD, 200 ml) were harvested at 1.5-2 × 10^7 cells/ml. Crosslinking was done by adding formaldehyde to a final concentration of 0.75% to the cell culture (this gave us better results than using 1%) and incubating at room temperature for 20 min with mild shaking. Quenching was done by adding glycerol to a final concentration of 125 mM and incubating at room temperature for 5 min with mild shaking. Cells were then washed once with cold TBS (20 mM Tris-HCl [pH = 7.4], 150 mM NaCl) and the cell pellet was either used immediately or stored at −80°C. For ChIP-exo, frozen samples (crosslinked with 1% formaldehyde) were sent to Peconic LLC (State College, PA).

For regular ChIP-TAP, 700 µl of lysis buffer (50 mM HEPES-KOH [pH = 7.5], 300 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate supplemented with the following protease inhibitors: Complete protease inhibitor cocktail (Roche) 1 tablet/25 ml, PMSF 2 mM, TLCK 50 µg/ml and benzamidine 2.5 mM) was added to the cell pellet. The tube was filled with 0.5 mm acid-washed glass beads and lysis was done by 5 cycles of alternating between 1 min of beating (50 rpm) in a Mini-Bead Beater (Biospec Products, Bartlesville, OK) and 1 min on ice. The lysate was sonicated 3 × 10 s at level 10 with Microson sonicator (Misonix, Inc., Farmingdale, NY) or 25 cycles of 30 s/50 s on/off, level set to high (Bioraptor, Diagenode, Belgium) and cleared by two rounds of centrifugation. Average DNA fragment size was ~500 bp. In experiments where RNase was added, the lysate was pretreated for 30 min at room temperature with 15 µl RNase cocktail (RNase A at 500 U/ml; RNase T1 at 20,000 U/ml; Ambion). The RNases continued to be present thereafter in the next steps. Protein samples (1.5 mg) were loaded onto 50 µl IgG Sepharose beads (6 Fast Flow) (GE Healthcare Bioscience), which had been blocked for 1 hr with 1 mg/ml of BSA, and incubated at 4°C with mild rotation for 1 hr. IgG beads were washed three times with lysis buffer, twice with WASH 2 buffer (50 mM HEPES-KOH [pH = 7.5], 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate supplemented with the protease inhibitors mentioned above), three times with WASH 3 buffer (10 mM Tris-HCl [pH = 8.0], 250 mM LiCl, 2 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate supplemented with the protease inhibitors mentioned above) and twice with TE (10 mM Tris-HCl [pH = 8.0], 1 mM EDTA). Two different elution protocols were used; TEV-mediated and SDS-mediated elution. The strain harboring RPB3-TAP and the No-TAP strain were subjected to both elution protocols. For Xrn1p-TAP, the TEV-mediated elution worked better. For all other TAP-tagged proteins both methods worked well. TEV-mediated elution: beads were washed twice with TEV buffer (10 mM Tris-HCl [pH = 8.0], 150 mM NaCl; 0.1% NP-40; 0.5 mM EDTA; 1 mM DTT) and then resuspended in 200 µl TEV buffer. Elution was done by adding 10 U of AcTEV protease (Invitrogen) and incubation at 24°C for 3.5 hr with mild rotation. For all other TAP-tagged proteins, 150 µl of SDS elution buffer (10 mM Tris-HCl [pH = 8.0], 10 mM EDTA, 1% SDS) was added to the beads and samples were incubated for 30 min at 65°C. In both
cases, elution buffer was spiked with an exogenous lacZ DNA fragment, which was later used to determine recovery during subsequent stages. Following TEV cleavage or SDS elution, samples were centrifuged at 2,000 rpm for 1 min and the supernatant was transferred to Spin-X centrifuge tube filters (Costar). The beads were washed with 70 μl TEV buffer or 100 μl SDS elution buffer and the supernatant was combined. Samples were filtered in Spin-X tube to filter out any possible contamination of beads in the eluted material. De-crosslinking was done by incubation overnight at 65°C. To purify the DNA, equal volumes of TE buffer (Tris-HCl [pH = 7.4], 1 mM EDTA) containing 1% SDS and 200 μg/ml of proteinase K (Pierce) were added and samples were incubated for 2 hr at 57°C, followed by phenol/chloroform extraction. 1 μl of 20 mg/ml glycogen was added to the aqueous phase and the DNA was ethanol precipitated and resuspended in 60 μl of PCR grade water. Input samples (10% from the sample loaded on the beads) were diluted in TEV or SDS elution buffer containing lacZ DNA spike and then treated identically from the decrosslinking stage onward.

**Real-Time PCR**

The Absolute blue SYBR Green ROX mix (Thermo Scientific) was used for qPCR according to the manufacturer’s instructions in a 10 μl reaction volume. qPCR was performed in Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia). qPCR was performed as follows: Hold: 95°C for 15 min; Cycling: 95°C for 2 s, 54°C for 15 s, 72°C for 20 s; Melting: 60°C to 99°C with 5 s for each step. For some primers, the cycling stage was changed to 95°C for 2 s, 60°C for 20 s. Results were analyzed with Rotor-Gene 6000 Series Software v1.7. The melting curve was used to determine the purity of the PCR products. All samples were normalized to the lacZ DNA, which was added at the elution stage of the ChIP protocol (see above). IP samples were then normalized to Input. Results for TAP-tagged proteins are shown as fold enrichment relative to the signal obtained from the No-TAP (Figures 5B, 5C, and 5D) or as a percentage from PMA1 amplicon 115, which was taken arbitrarily as 100% (Figure 5A). In all cases, amplicon length did not exceed 250 bp, and correct length was verified by gel electrophoresis. Sequences of qPCR primers will be given upon request.

**Analysis of ChIP-Exo Data**

We used Tag files representing the coordinates that represent the 5′ end of the sequenced tag, which for ChIP-exo is essentially the location of the exonuclease stop site (Rhee and Pugh, 2012). Total tag counts were assigned for each region (promoter, gene or window). Genes were designed as bound by a factor whenever tag counts exceeded 10 tags in a 120 base window around the annotated gene start site. For correlation analysis of the ChIP-exo signals to transcription rates (Figure 5C), genes were divided into four groups based on their TR (the most highly transcribed group is defined as “>75%”). The ratio of the observed versus expected number of genes bound in promoter regions, defined as ± 100 bp of transcription start sites (TSS), is shown for each of the indicated DF (x axis). For correlation analysis to transcription in xrn1D208A mutant (Figure 7A), genes were binned into four groups according to the difference of the z-transformed between WT and the xrn1D208A mutant. The ratio between the observed and expected number of promoters bound by the indicated DFs was calculated for each bin.

For correlation analysis to HL (Figure 7B), genes were divided into four groups based on their HLs (the shortest HL is defined as “<25%”). The ratio of observed versus expected number of promoters bound by the indicated DF is shown (y axis). p values were computed empirically doing 10,000 permutations, in which we randomly shuffled binding data.

**Fluorescent In Situ Hybridization**

**Choice of Gene**

We focused on TEF4 as a gene of interest that helps us identify TSSs, which can be recognized by both exonic and intronic probes. Following splicing of TEF4 transcript, the intron is processed into the nucleolar associated-snR38 (Villa et al., 2000), whose detection is used as an internal control for the hybridization efficiency. This was especially helpful in cases where no TS signal was detected (Figures 3A and 3B). Only mature, intronless, TEF4 mRNAs were detected in the cytoplasm. Reassuringly, the average number of single TEF4 mRNA molecules detected in the cytoplasm of a single WT cell is consistent with previously published results (Veliculescu et al., 1997) and is comparable to those detected in xrn1D208A and Δxrn1 cells (Figure S3C). The similar values in these strains are consistent with the similar steady-state levels of TEF4 mRNA obtained by northern analysis (Figure S3D).

**Probes**

FISH probes approximately 50 bp long were designed and checked for specificity. Five thymidine bases per probe were amine-modified (Eurogentec, 6C-Amino dT). These were then labeled using commercial amine mono reactive Cy3 or Cy5 dyes (Amersham, PA23001 and PA25001, respectively) to a labeling efficiency greater than 80%. Amino allyl modified Cy3 and Cy5 labeled thymidines are marked by an asterisk on the following probe sequences: snR38_1, T*CAACATATGAGAGGT*TACCTATTAT*TACCCATT*CAGA CAGGGATACT*G; YKL081W_1, T*ACCCAAAAAT*GAGGGGGCTT*GCTTCAAT*GGGAAAACAAAGGAAACT*CG; YKL081W_2, T*GGACGAGCAAT*GTTGCTCATGACAT*CGGAATTAGCT*AGAGATGCCCAT*C; YKL081W_3, T*CAGTAGACCAAAGAAGT*GTAGCTCTT*CAATCTAGCAT*GAGAAGGACACGAGGT*A; YKL081W_4, T*GTTGAACCAT*CTCATCAAAT*GAGGATGCTT*AGCTCTCCATT*; YKL081W_5, T*GGACGAGCAAT*GTTGCTCATGACAT*CGGAATTAGCT*AGAGATGCCCAT*C; YKL081W_6, T*GGACGAGCAAT*GTTGCTCATGACAT*CGGAATTAGCT*AGAGATGCCCAT*C; YKL081W_7, T*GGACGAGCAAT*GTTGCTCATGACAT*CGGAATTAGCT*AGAGATGCCCAT*C; YKL081W_8, T*GGACGAGCAAT*GTTGCTCATGACAT*CGGAATTAGCT*AGAGATGCCCAT*C.

**Proliferation, Fixation, and Hybridization**

WT (yMC458), Δxrn1 (yMC459) and xrn1D208A (yMC461) strains were proliferated at 30°C in YPD medium to an optical density at 600 nm of 0.8. Cells were fixed by incubation in 4% (v/v) paraformaldehyde in YPD, at room temperature for 45 min. Cells were spheroplasted using lyticase (Sigma-Aldrich L2524) and deposited on coverslips coated with poly-L-Lysine (Sigma-Aldrich P8920). The coverslips were then stored at −20°C in 70% (v/v) ethanol. Prior to hybridization, the coverslips were washed twice in PBS for 10 min for rehydration, incubated for 15 min in PBS supplemented with 0.5% Triton for membrane permeabilization, then equilibrated
with hybridization buffer by incubating once in PBS for 10 min and twice in 2x SSC, 40% (v/v) formamide for 5 min. Coverslips were hybridized against 1 ng of fluorescently labeled DNA probes, 5 μg of salmon sperm DNA and 5 μg of E. coli tRNA, in 20 μl of a solution at 5 mM Na2HPO4, 40% (v/v) formamide, 2x SSC, 2 mg/ml BSA, 10 mM VMc, 0.12 U/μl RNase inhibitor. Hybridization was done overnight at 37 °C. Coverslips were rinsed twice in 2x SSC, 40% (v/v) formamide at 37 °C for 15 min, then twice in PBS for 1 hr, at room temperature. They were stained with 0.5 μg/ml DAPI in PBS and mounted in 90% glycerol in PBS (pH = 8.5) containing 1 mg/ml P-phenylenediamine (Sigma-Aldrich 695106).

**Image Acquisition**

Images were acquired with a Nikon Eclipse Ti, using a plan Apochromatic x100 oil immersion objective with a 1.4 numerical aperture. A Nikon Intensilight C-HGFI lamp was used to illuminate the coverslips. Dichroic filters for Cy3 (Semrock Cy3-4040B), Cy5 (Semrock Cy5-4040A) and DAPI (Semrock DAPI-1160A) were used. Stacks of 41 images were acquired in z-steps of 0.2 μm using a Photometrics CoolSnap HQ2 camera.

**Data Analysis**

Images were analyzed z-step by z-step by an IPlab IDL virtual machine program, which uses a 2D Gaussian fit algorithm to detect FISH spots and to extract integrated intensity, as previously described (Thompson et al., 2002; Zenklusen et al., 2008). Spots detected over several continuous planes were treated as a single object and the value corresponding to the focal plane (maximal intensity) was used for analysis. Only the integrated intensities at the focal point were used for statistical analysis. These intensities were normalized to their median. 209 cells were analyzed for the WT strain, 76 for the Δxrn1 strain and 240 for the xrn1ΔD208A strain.

**3D Reconstructions**

The 3D reconstructions of the cells were made by extracting the x, y, and z coordinates calculated by 2D Gaussian fitting. The highest integrated intensity of the spot was chosen for the plane of the z coordinate. The coordinates of the threshold DAPI signal were also extracted. These coordinates were corrected for the true voxel size (43 nm in the x and y directions, 200 nm in the z direction), therefore obtaining the coordinates in nanometers. These coordinates were imported in Mathematica (Wolfram research, inc. Champaign, Illinois, USA), and were plotted in a 3D environment after smoothing and hollowing of the nucleus volume. 75 images were taken by rotating the viewpoint around the y axis in regular steps of 2π/75.

**Statistical Analysis**

Because the distribution law of transcription site intensities is unknown, we used a bootstrapping resampling method (Simon, 1997) to evaluate variability for each histogram bin in the FISH assay. Error bars in histograms represent the 2.5th and 97.5th percentiles of each resampled bin (Bootstrapping depth was 10,000). P values used to evaluate the differences between histograms of two different strains were also obtained by resampling. The two original populations were pooled and resampled iteratively into two populations. Histograms from the resampled populations were subtracted at each iteration giving a range of differences for each histogram bin. The P value represents the probability to obtain a difference greater or equal to the observed difference by resampling.

**3’/5’ Ratio Analysis and RNA pol II ChIP on Chip**

Specialized nylon macroarrays containing 300 bp probes obtained by PCR amplification for 384 selected yeast genes were previously described (Rodríguez-Gil et al., 2010). They were used with run-on samples obtained as described above but with an additional step of RNA fragmentation with 40 mM NaOH for 5 min as described (Rodriguez-Gil et al., 2010), for the determination of elongating Pol II molecules. The same arrays were re-probed with labeled DNA from ChIP of identical yeast samples immunoprecipitated with the 8WG16 RNA pol II antibody (RPCC) as previously described (Pelechano et al., 2009; Rodríguez-Gil et al., 2010) for the determination of total Pol II molecules. Two independent replicates for both kinds of experiments were done. Only genes with valid data (over 1.3 times over background) in two replications were considered and averaged using a median absolute deviation protocol to filter outliers. 3’/5’ ratios for each gene were calculated for those genes, which had valid data in both 3’ and 5’ probes. 151 genes gave valid data in all three strains in both GRO and RPCC experiments. These values were used for comparing mutant versus WT ratios. Alternative comparisons using all valid data for any single pair provided similar statistical results. A Wilcoxon signed rank test was applied to check the significance of the differences found between WT and mutant strains data.

**Pol II and Phospho-Ser-2 ChIP along the GAL1 Gene**

Yeast cultures (100 ml) were harvested at D.O600nm = 0.5. Cell lysis was performed by three cycles of 30 s at 5 m/s in Fastprep (MP Biomedicals). The lysate was sonicated 30 min (30 s/30 s on/off) utilizing a Bioruptor sonicator (Diagenode). Protein samples were loaded onto 50 μl of Pan Mouse IgG Dynabeds, which were previously incubated overnight with either anti-Rpb3 (ab81859, Abcam) or anti Ser2-P (ab5095, Abcam) specific antibody, and incubated at 4°C with mild rotation for 1.5 hr. Elution was done at 65°C for 20 min with TE buffer containing 1% of SDS. For qPCR, SYBR Premix Ex Taq (Takara) was used in a 10 μl reaction volume. qPCR was performed in Lightcycler 480 II (Roche). IP samples were normalized to Input.

**Tethering DFs to Promoters**

To assess stimulation of transcription, strains expressing DFs fused to Gal4p-BD were subjected to two assays: a β-gal assay (Paz et al., 1999) and northern analysis. As a negative control, we used cells expressing Gal4p-BD alone (“vector”), or fused to the non-DF proteins Hsp104p, Tor1p, Rad17p, Elg1p or Dot1p. As a positive control we used Gal4p-BD fused to Rpb3p, which was previously shown to strongly stimulate transcription in this assay (Titz et al., 2006).
SUPPLEMENTAL REFERENCES


Figure S1. mRNA Decay Mutants Exhibit Reduced Transcription, in Accord with Their Interactions with Nuclear Factors, Related to Figure 1 and Tables S1, S2, S3, and S4

(A) Deletion mutants from four different genetic backgrounds (the WT of strains 1, 2, 3 and 4 are yMC229, yMC370, yMC375 and yMC458, respectively) were allowed to proliferate in a synthetic complete (SC) medium till $1 \times 10^7$ cells/ml. Cells were harvested and mRNA levels were assessed by northern blot analysis with various probes, as indicated in the inset, followed by quantification with PhosphorImager. Results were normalized to those of SCR1 and shown as percentages of WT levels (defined arbitrarily as 100%). Error bars represent SD of at least three assays. See also Figure 1 for northern blot images of some of the probes.

(B) Map of the Physical and Genetic interactions between factors of the major cytoplasmic mRNA decay pathway and factors involved in the nuclear stages of gene expression. Data from Table S3 is represented as a map created by Cytoscape software (Shannon et al., 2003). Decay factors are represented by white diamonds. Red lines represent physical interactions, blue lines represent genetic interactions.

(C) Cumulative distribution of HLs in the indicated strains (list of HLs is shown in Table S4).

(D) Changes in transcription rates in xrn1 mutant cells. Bar graph of the Log2 GRO signals in the mutants relative to WT (Table S4), plotted as a function of the number of genes. A value below zero (red line) indicates defective transcription. Note the shift to the left of the xrn1D208A strain, indicating a more severe defect.

(E) Proliferation rates of xrn1 mutant strains. Upper image: strains yMC458 (WT), yMC459 ($\Delta xrn1$) and yMC461 ($\Delta xrn1D208A$) were spotted on YPD plates in a five-fold serial dilution (the first spot is $1 \times 10^5$ cells). Four biological repeats were made, which yielded similar results; only one is shown. The photo was taken after two days of proliferation at 28°C. Lower image: yMC511 ($\Delta xrn1$) transformed with plasmids pMC491 ($XRN1$-GFP), pMC492 ($\Delta xrn1$-D208A-GFP), pMC582 ($\Delta xrn1$R101G-GFP) or pMC579 ($\Delta xrn1$D208A,R101G-GFP) and yMC459 ($\Delta xrn1$) were spotted on SC-ura as described for the upper image. Two biological repeats were made, which yielded similar results; only one is shown.

(F) Accumulation of P bodies (PBs) in xrn1 mutant strains. The indicated strains, expressing Dhh1p-GFP, were grown under optimal conditions. Photos of cell samples without any treatment were taken, and the average number of Dhh1p-GFP containing PBs per cell ± SD was calculated (N > 100).
Figure S2. Enzymatically Active Xrn1p or Dcp2p Are Required for Efficient Transcription and mRNA Decay of HS, non-HS and Noncoding Genes, Related to Figure 2

(A) Northern blot showing induction of HSP26 by temperature shift up as described in Experimental Procedures. Quantification was performed as in Figure 2A.

(B) To examine mRNA decay, transcription of HSP26 was inhibited by shifting the temperature down, and the levels of the indicated mRNAs were monitored by northern analysis and quantified as in Figure 2B. SCR1 RNA is shown for loading control, and was used for normalization. Error bars represent SD of three assays.

(C–F) Transcriptional induction of RPL43A and TEF4 during recovery from 30 min HS at 42°C was performed as described in Experimental Procedures. (C) Northern blot showing RPL43A induction. Quantification of the results was done as in Figure 2A, except that time 70 min of WT was set as 100%. Note that not only the induction in the dcp2-4 strain is low (given the high level of their RPL43A mRNA at time 0 min), but it is also delayed by ~10 min. Note also that by 70 min following transcription induction, the RPL43A mRNA in WT and the dcp2 mutants reached similar levels, reflecting the balance that starts to emerge between synthesis and decay, as the mRNA approaches its steady-state level. This is in accordance with the results shown in Figures 1 and S1.

(D) Cells were allowed to proliferate under optimal conditions (30°C). To block transcription, cells were shifted to 42°C (Lotan et al., 2005; Lotan et al., 2007) and mRNA degradation was monitored thereafter. Shown are northern images and quantification as in Figure 2B.

(E) Northern blot images showing TEF4 induction during recovery from 30 min HS at 42°C. Quantification of the results was done as in C.

(F) Decay of the TEF4 mRNA at 30°C was done as in Figure 1B. Shown are northern blot images and quantification as in Figure 2B.

(G) Decay and (H) induction of the ncRNA SUT508/TIR1axut (which is partially anti-sense to TIR1 [van Dijk et al., 2011]) following HS and recovery from HS, respectively, was determined as for RPL43A. (A)–(H) show experiments using the same set of yeast strains: (yMC458, yMC461 and yMC459 and yMC370, yMC374, and yGH177, respectively). Note that WT cells accumulate SUT508 RNA after 5 and 15 min postinduction, whereas the level of this RNA in the mutant cells changes more slowly.

(i) Decay of SUT508 depends on PAT1 and DHH1. Northern analysis of SUT508 decay in the indicated strains was performed as in Figure 1B. Note that SUT508 RNA stability was dependent on PAT1 and DHH1 yet its steady state was not (time 0 in the same panel),**, additional, unknown, RNAs detected by the probe. Note that the same RNAs, and an additional one, were also detected in the assay depicted in (G) and (H) (data not shown). For all panels, SCR1 RNA is shown for loading control, and was used for normalization. Error bars in all panels represent SD of three assays.
**Figure S3. The Use of FISH to Detect Transcription Sites and to Measure the Number and Distribution of TEF4 mRNA Molecules in Single Cells, Related to Figure 3**

(A) Quantitative single-molecule analysis. (a) FISH image showing single mRNAs detected as spots by TEF4 specific Cy3 probes. Scale bar, 1 μm. (b) Spot centroids. The raw image from (a) was analyzed using a 2D Gaussian fitting program (Zenklausen et al., 2008). See Experimental Procedures for more details. (c) Thresholded DAPI images, detecting nuclei of the cells in (a). (d) Images (a), (b) and (c) (pseudocolored green, red, and blue, respectively) were merged into a single image.

(B) Distribution of FISH spots (most are cytoplasmic) according to their intensity. The intensity of the spots from various FISH experiments were normalized according to their median value and their distributions were plotted in percentages of total FISH spots, strain by strain.

(C) Average number of TEF4 mRNAs per cell (±SEM) as determined by FISH analysis.

(D) Steady state level of TEF4 mRNA as determined by northern analysis. Quantification was done as in Figure 1. Inset shows the northern blot image of TEF4, and SCR1 RNA as the loading control; Lane 1, WT; lane 2, Δxrm1; lane 3, xrm1D208A.

(E) Interpretation of transcription site intensities. Top: example of distribution of the transcription site intensity (x axis) in WT cells. Results are represented by the histogram, in percentages of WT cells (y axis). For comparison, the superimposed blue curve corresponds to the distribution of the intensities of all the FISH spots in WT, as shown in Figure S4B. The first column corresponds to cells without any FISH spot in the nucleus, to which the algorithm attributed a TS intensity of 0. The areas designated I, II and III, depicted above the histogram, refer to the interpretations and cell images in the panels below. (I) Cells without any FISH spots in the nucleus were interpreted as cells without any active transcription of the TEF4 gene at the time of the fixation. Two examples of such cells are shown in (a) and (b). Optical sectioning technology indicated that all the spots are above or below the nucleus. (II) Cells with a low intensity FISH spot in the nucleus, which corresponds to the value of a single mRNA in the cytoplasm (as determined in Figure S3B). This cell population is interpreted as occupying a single elongating Pol II at the TEF4 gene. Two examples of such cells are shown in (c) and (d). Arrows indicate the TSs. In (d), the other spots that appear inside the nucleus are either above or under it (as indicated by the optical sectioning technology). (III) Cells with high intensity FISH spots in the nucleus. This cell population has multiple nascent TEF4 mRNAs in the TS; hence their TEF4 gene occupies more than one elongating Pol II simultaneously. Two examples of such cells are shown (e and f). Arrows indicate TSs; Scale bar, 1 μm.

(F) Distribution of TS intensity (see also Figure 3): comparison between WT and Δxrm1 cells (top), WT and xrm1D208A cells (middle) and Δxrm1 and xrm1D208A cells (bottom). Error bars represent 95% confidence intervals. Numbers above bars represent p values of the difference between the bins of the two strains calculated by resampling. An asterisk was added when the difference is significant (p < 0.05). The p value was calculated for the bin 38.5-91 as well, and is shown above each histogram. N > 200 cells, except for Δxrm1 cells (N = 76). For more detailed description of the statistical analysis see Experimental Procedures.
Figure S4. Factors of the Major mRNA Decay Pathway Are Nucleocytoplasmic Shuttling Proteins, Whose Cellular Localization Can Be Affected by Environmental Conditions and Genetic Background, Related to Figure 4

(A) WT or xpo1-1, mex67-5 strain expressing Pab1p-GFP and the indicated RFP fusion protein were treated as in Figure 4A. More specifically, CHX (100 µg/ml) was added, as indicated, immediately before the temperature shift-up. Pab1p-GFP was used to determine the efficiency of the assay and to serve as a nuclear marker (Brune et al., 2005). In all cases, Pab1p-GFP was present in the nuclei of at least 40% of the heat inactivated mutant cells. White arrows indicate example cases of nuclear colocalization of Pab1p-GFP and RFP fusion proteins. Yellow arrows in VIII indicate a case of a cell whose nuclear localization of Edc3p-RFP, but not Pab1p-GFP, is observed already at the permissive temperature (24°C) (observed in ~30% of the cells).

(B) Δrpbi strain expressing GFP-Rpb4p (a Pol II subunit) and Lsm1p-RFP were grown exponentially at 30°C. When the culture reached 1 × 10^7 cells/ml, the culture was starved for 1 hr as described in Experimental Procedures. Samples before (“Optimal”) or after shifting to starvation conditions (“Starvation”) were analyzed by fluorescence microscopy. Arrows indicate example cases of colocalization of GFP-Rpb4p and Lsm1p-RFP. At 1 hr after shifting to starvation, GFP-Rpb4p can serve as a reliable nuclear marker (Selitrennik et al., 2006). Note that Lsm1p-RFP can be found in the nucleus also under optimal conditions (in ~40% of the cells).

(C) Dcp2p-RFP is found in the nuclei of WT nonstressed cells in certain genetic backgrounds. WT strain expressing chromosomal RPB6-GFP (a Pol II subunit) and harboring DCP2-RFP was proliferated at 30°C. Exponentially proliferating cells (1 × 10^7 cells/ml) were inspected without further treatment. Rpb6p-GFP serves as a nuclear marker. Arrows indicate nuclear colocalization of Rpb6p-GFP and Dcp2p-RFP.

(D and E) Dcp2p-RFP and Ccr4p-RFP accumulate in the nuclei of starved WT cells. WT strain (yMC458) harboring GFP-RPB4 and DCP2-RFP or CCR4-RFP were treated as in (B). Note that unlike the results shown in C, in this genetic background, Dcp2p-RFP could not be detected in the nucleus unless the cells are starved. Arrows indicate example cases of nuclear colocalization of GFP-Rpb4p and the RFP fusion protein.

(F) Lsm1p-RFP is found in the nuclei of heat-shocked WT cells. WT strain expressing chromosomal RPB6-GFP and harboring LSM1-RFP was proliferated at 30°C. Exponentially proliferating cells (1 × 10^7 cells/ml) were shifted to 42°C for 20 min. Rpb6p-GFP serves as a nuclear marker. Arrows indicate nuclear colocalization of Rpb6p-GFP and Lsm1p-RFP. Note that Dcp2p-, Xm1p-, Edc3p-, Dcp1p-, Pat1p- or Ccr4p-RFP did not accumulate in the nuclei of these cells for as long as 1 hr at 42°C (data not shown).

(G) Nuclear accumulation of the indicated RFP fusion proteins in starved cells is diminished in xrn1D208A mutant strain. WT and mutant cells were treated as in (A). Shown is the percentage of cells with nuclear RFP fusion proteins. Mean values ± SD are shown (N > 150). The differences between WT and xrn1D208A cells are statistically significant (* indicates p < 10^-4). Nuclear accumulation of Xm1p-RFP, Edc3p-RFP, Lsm1p-RFP and Dhh1p-GFP was not detected in sated or starved yMC458 or yMC461 cells under conditions used here, although nuclear accumulation of these proteins was detected in other strains.
Figure S5. Factors of the Major mRNA Decay Pathway Associate with Chromatin of Transcriptionally Active Genes and Can Stimulate Transcription upon Recruitment to Reporter Promoters, Related to Figure 5

(A) Xm1p-TAP and Rpb3p-TAP (Pol II subunit) associate with the TEF4 promoter, but not with rDNA.

(B and C) The indicated DFs associate with TEF4 promoter (B) or PMA1 promoter (C). Panels A-C show qPCR results, expressed as fold enrichment relative to the No-TAP control signal. Shown are mean values of three or more assays, normalized to the input signal and to an internal lacZ spike, ± SD. ChIP qPCR data are from four biological repeats.

(D) Northern blot analysis using probes for the indicated mRNAs of WT cells expressing different Gal4p-BD fusion genes. The same membrane was probed sequentially with GAL10, HIS3, ACT1 and SCR1. Cells expressing Gal4p-BD-Rpb3p served as a positive control, and cells expressing Gal4p-BD-Hsp104p or vector alone served as negative controls. Additional negative controls are shown in Table S5.

(E) Quantification by PhosphorImager of the results shown in (D) (for HIS3 mRNA) and for GAL10 mRNA (northern image not shown). Results (average ± SD of three assays) were normalized to SCR1 RNA level and are shown as fold induction (in log10 scale) relative to the mRNA level in cells carrying the vector only.

(F) Northern blot images of the xrn1D208A mutant cells expressing the indicated Gal4p-BD fusions. The same membrane was probed sequentially with HIS3 and SCR1. Compare to the expression in WT cells in (D) (All membranes were exposed to X-ray film in parallel). * Exposure time as in (D). ** 1/3 exposure time. Similar results were obtained when the Δxrn1 mutant was used instead of xrn1D208A mutant (data not shown).

(G) Levels of β-gal activity expressed in Miller units in strains shown in (D). Average of at least three experiments (±SD) are shown. *p < 0.05 compared to vector (Gal4p-BD) alone.

(H) Northern blot analysis of GAL4-BD fusion genes. The membrane used in (D) was re-probed against mRNAs that contain GAL4-BD sequence.

(I) Western blot images of the Gal4p-BD fusion proteins. The levels of the various mRNAs were comparable, except for those encoding XRNI, xrn1D208A, PAT1 and dcp2-4 (H). Western analysis (I) did not show any clear correlation between protein level and (in)ability to activate transcription. Nevertheless, it is clear that the inability to activate transcription of at least three of our negative controls, as well as Gal4p-BD-Edc3p and Gal4p-BD-Dcp1p was not due to low protein levels, because these levels were higher than the positive control, Gal4p-BD-Rpb3p, that strongly activates transcription. Moreover, the levels of Gal4p-BD-Dcp2p, and Gal4p-BD-Dhh1p that activate transcription were lower than that of the negative control Gal4p-BD. It is possible that the inability of Gal4p-BD-Xm1p or Gal4p-BD-xrn1D208Ap to activate transcription was due to its poor expression. Alternatively, Xm1p is incapable of activating transcription in this assay.
Figure S6. Factors of the Major mRNA Decay Pathway Affect Transcription Elongation, Related to Figure 6

(A) Run-on signal at the indicated positions along GAL1 in cells grown in galactose medium. Data are expressed relative to the 5’ position for each strain, followed by normalization of each value to the corresponding position in the wild-type, which was defined as “1.” Mean values and SD of three independent experiments are shown.

(B) mRNA Decay mutants are sensitive to the drug 6-azauracil (6-AU). The indicated strains (WT is yMC370) were spotted in a five-fold serial dilution (the first spot is \(1 \times 10^5\) cells) on 6-AU or control plates as described in Experimental Procedures. The photo was taken after two days of proliferation at 28°C.
Figure S7. Specific Mutations in XRN1 Similarly Affect mRNA Decay, Yet Have Different Effects on Transcription. Related to Figure 7

(A) Decay of GAL10 mRNA is similar in different xrn1 mutants. Decay of GAL10 mRNAs was performed on the indicated strains as described in Figure 2B. The graph shows a quantification of the northern blot of three repeats (one is shown on the left) normalized as in Figure 2B. Error bars represent SD of three assays.

(B) Schematic model of the suggested linkage between mRNA decay, nuclear import of DFs and transcription. (a) WT cells. Following full degradation of the mRNA by Xrn1p, the decaysome is imported to the nucleus as a complex or subcomplexes. In the nucleus, at least some DFs (Xrn1p, Dcp2p and Lsm1p) associate with chromatin, 30 bp upstream to transcription start site (red arrowhead) (Figure 5B)—the same location as the preinitiation complex (PIC), and stimulate transcription initiation as well as transcription elongation. Transcription is efficient (illustrated artistically by three Pol II molecules per ORF). The elongating Pol II is properly phosphorylated on Ser-2 of its C-terminal domain (data not shown). (b) An xrn1D208A cell. Xrn1D208Ap is able to bind the 5′-phosphate of the decapped mRNA but cannot degrade it. This mutant form exerts its adverse effects on transcription (Figure 7D) and import (Figures 4B, 4C and S4G) only if its active site properly binds the decapped RNA at the 5′ end. Bound Xrn1D208Ap is trapped in the cytoplasm and does not permit import of other DFs as well (Figures 4B, 4C, and S4G). Therefore, DFs that are confined in the cytoplasm, are unable to stimulate both transcription initiation and elongation, resulting in a severe defect of transcription (artistically illustrated by showing only one Pol II per ORF). (c) An Δxrn1 cell. Since Xrn1p normally represses import as long as the RNA is not fully degraded, its absence permits import. In the absence of Xrn1p, the other decay factors are released from the mRNA, possibly following its degradation by the exosome, and are imported to the nucleus in an Xrn1p-independent manner. Because Δxrn1 cells are only partially defective in transcription (Figures 1D, 2, 3, 7, S1D, S2, and S3), it seems that the imported DFs are able to stimulate transcription initiation, at least partially. Because transcription elongation of Δxrn1 is almost as defective as elongation in xrn1D208A cells (Figure 6B and 6C), we surmise that the elongation stage is especially vulnerable to the absence of Xrn1p, leading to accumulation of hypophosphorylated (backtracked?) Pol II and intermediate transcription rates (artistically illustrated by showing two Pol II molecules per ORF).