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Altered RNA processing and export lead to retention of mRNAs near transcription sites and nuclear pore complexes or within the nucleolus

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ABSTRACT Many protein factors are required for mRNA biogenesis and nuclear export, which are central to the eukaryotic gene expression program. It is unclear, however, whether all factors have been identified. Here we report on a screen of >1000 essential gene mutants in Saccharomyces cerevisiae for defects in mRNA processing and export, identifying 26 mutants with defects in this process. Single-molecule FISH data showed that the majority of these mutants accumulated mRNA within specific regions of the nucleus, which included 1) mRNAs within the nucleolus when nucleocytoplasmic transport, rRNA biogenesis, or RNA processing and surveillance was disrupted, 2) the buildup of mRNAs near transcription sites in 3′-end processing and chromosome segregation mutants, and 3) transcripts being enriched near nuclear pore complexes when components of the mRNA export machinery were mutated. These data show that alterations to various nuclear processes lead to the retention of mRNAs at discrete locations within the nucleus.

INTRODUCTION Nascent RNA transcripts are processed and mature within the nucleus, including folding, cleavage, modification, nuclear export, or decay. Processing is driven by specific RNA–protein and RNA–RNA interactions that occur in the context of a ribonucleoprotein (RNP) particle, with the protein composition of the RNP being largely responsible for the processing path that is followed (Mitchell and Parker, 2014; Oeffinger and Montpetit, 2015; Singh et al., 2015). In the case of mRNA biogenesis in Saccharomyces cerevisiae, cotranscriptional recruitment of capping enzymes, splicing machinery, and various RNA adaptor proteins mediate maturation of the pre-mRNA, which includes cleavage and polyadenylation during 3′-end formation, loading of export receptors (e.g., Mex67p), and release of the messenger ribonucleoprotein (mRNP) from the transcription site (Oeffinger and Zenklusen, 2012; Niño et al., 2013). After release, mRNPs are often exported through nuclear pore complexes (NPCs), which are assembled from ~30 nucleoporins (Nups) to form a transport channel spanning the nuclear envelope (Wente and Rout, 2010). mRNP docking and translocation through an NPC are directed by interactions between Mex67p and other mRNP components with Nups of the nuclear basket and central transport channel (Bonnet and Palancade, 2014). On reaching the cytoplasmic side of the NPC, export is terminated by the action of the DEAD-box ATPase Dbp5p, which is locally activated by the cytoplasmic nucleoporins Nup159p and Gle1p with the small molecule InsP6 (Oeffinger and Zenklusen, 2012; Björk and Wieslander, 2014). This ultimately results in removal of Mex67p, release of mRNP into the cytoplasm, and directionality in mRNA export.

Inevitably, errors occur during nuclear RNA processing, which can result in aberrant transcripts being targeted for nuclear degradation via quality control mechanisms (Houseley and Tollervey, 2009; Müller-McNicoll and Neugebauer, 2013; Eberle and Visa, 2014; Porrua and Libri, 2015). A key player in this process is the exosome, which functions in the nucleus and cytoplasm as a nuclease to facilitate and surveil RNA biogenesis from all three nuclear RNA polymerases (Chlebowski et al., 2013; Porrua and Libri, 2013; Schneider and Tollervy, 2013). The nuclear exosome differs in composition from the cytoplasmic form through the addition of a

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Abbreviations used: mRNP, messenger ribonucleoprotein; NPC, nuclear pore complex; Nup, nucleoporin; RNP, ribonucleoprotein; smFISH, single-molecule fluorescent in situ hybridization.
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second catalytic subunit, Rrp6p (Briggs et al., 1998), which, together with Dis3p, harbors the nuclease activities associated with the exosome. Nine other protein subunits are found within the exosome (Liu et al., 2006), which function to recognize and feed RNA substrates Dis3p and Rrp6p (Bonneau et al., 2009; Malet et al., 2010), mediate interactions with multiple protein complexes that include the TRAMP, NNS, and SKI complexes (Jacobs et al., 1998; Steinmetz et al., 2001; Kadaba et al., 2004; Ursic et al., 2004; LaCava et al., 2005; Vanácová et al., 2005; Wyers et al., 2005; Vasiljeva and Buratowski, 2006), and regulate the overall activity of Dis3p and Rrp6p (Mitchell et al., 1997; Allmang et al., 1999; Wasmuth and Lima, 2012).

Disruptions to RNA biogenesis, export, and surveillance result in the accumulation of aberrant RNP complexes and RNA processing by-products in the nucleus of the affected cell (Amberg et al., 1992; Kadowaki et al., 1992, 1994a, 1994b; Li et al., 2005; Doye et al., 1994; Fabre et al., 1994; Gorsch et al., 1995; Segref et al., 1997). For example, when exosome-dependent RNA processing and surveillance are perturbed, the biogenesis of small nucleolar RNA (snoRNA), rRNA, tRNA, mRNA, and other noncoding transcripts is altered (van Hoof et al., 2000; Kui et al., 2004; LaCava et al., 2005; Vanácová et al., 2005; Wyers et al., 2005; David et al., 2006; Davis and Ares, 2006; Houalla et al., 2006; Carneiro et al., 2007; Gudipati et al., 2012; Schneider et al., 2012; Castelnuovo et al., 2013). Such disruptions to RNA biogenesis and export can often be observed as the accumulation of poly(A)-RNA species within the nucleus of the affected cell (Cole et al., 2002), which has been used to identify many mutants involved in RNA biogenesis and export, including screening of the ∼5000 nonessential genes in S. cerevisiae for mRNA export defects (Hieronymus et al., 2004). The construction of mutant libraries that span essential genes (Ben-Aroya et al., 2008; Breslow et al., 2008; Li et al., 2011) provides an opportunity to conduct comprehensive screens of essential genes for mRNA processing and export defects, as recently performed for tRNA (Wu et al., 2015). Of importance, the screening of both essential (this work) and nonessential (Hieronymus et al., 2004; Thomsen et al., 2008) genes within S. cerevisiae for mRNA-processing defects provides a component list that is necessary for building complete models of mRNA biogenesis and export.

Here we report on a screen of largely essential gene mutants for nuclear poly(A)-RNA accumulation and the characterization of these mutants using single-molecule fluorescent in situ hybridization (smFISH) directed against specific mRNAs. This resulted in the identification of 15 genes that were not previously linked and/or demonstrated to alter RNA processing and mRNA export. In addition, disruption of multiple nuclear processes was found to cause distinct phenotypes that included the accumulation of mRNAs near transcription sites or the nuclear periphery and NPCs or within the nucleolus. These data suggest that alterations to RNA processing and overall nuclear homeostasis cause RNAs to stall or be retained at similar restriction points. This may reflect common failures in mRNA biogenesis and export, as well as active mechanisms to protect the cell during cellular stress and dysfunction.

RESULTS

Identification of mutants that accumulate nuclear poly(A)-RNA

To potentially identify genes involved in mRNA biogenesis and export, we screened two temperature-sensitive (Ts) S. cerevisiae mutant collections (Ben-Aroya et al., 2008; Li et al., 2011) for the accumulation of poly(A)-RNA in the nucleus using an oligo-dT FISH assay. Together these two Ts collections cover ∼68% (785 of 1156) of essential genes. When a Ts allele was not available, a DAmP allele was used that harvests a disrupted 3′ untranslated region often leading to reduced gene expression (Breslow et al., 2008), which increased coverage of essential genes to ∼91% (1047 of 1156) in our screen (Supplemental Data). For consistency throughout the screening process, regardless of the type of mutant used, all strains were grown into log phase at 25°C, shifted to 37°C for 3 h, and fixed. A 3-h temperature shift was used to balance the time needed to induce the Ts mutant phenotype(s) while minimizing induction of secondary phenotypes caused by the loss of essential cellular activities. In the case of the DAmP alleles, it was reasoned that the temperature shift might act as a stress and exacerbate mutant phenotypes, although DAmP alleles are not necessarily Ts mutants. After fixation, we performed in situ assays using a fluorescently labeled oligo-dT probe to detect poly(A)-RNA.

By comparing the distribution of oligo-dT to the 4′,6-diamidino-2-phenylindole (DAPI) signal, we identified 29 of 1047 mutants that accumulated poly(A)-RNA in the nucleus (Supplemental Figure S1 and Table 1). Of the genes identified, only half (14 of 29) were previously reported to accumulate poly(A)-RNA when disrupted (Table 1). To verify that the poly(A)-RNA accumulation phenotype was linked to the purported mutant being screened, we verified all strains by PCR, and in the case of the 15 newly reported genes, the mutation was rescued by introducing a wild-type allele and/or recapitulated by moving the mutation to a different strain background (Table 1). Note that not all genes previously reported to accumulate poly(A)-RNA in the nucleus when disrupted were identified by our screen, which may be due to the specific allele present in the mutant collection, the length of the temperature shift, or the requirement of a poly(A) tail for detection in the initial in situ screen. Within the set of mutants identified, the distribution of poly(A)-RNA within the nucleus was distinct and included bright foci (brf1-3231), a diffuse nuclear signal (rps5-3), a diffuse nuclear signal with one or more foci (dbp5-1), or poly(A)-RNA being adjacent to the DAPI-stained DNA mass (dis3-1; Figure 1A). The pattern of poly(A)-RNA accumulation was similar for genes with related biological functions (e.g., RNA processing and surveillance, with an accumulation of poly(A)-RNA next to DAPI; Table 1), which supports the notion that these mutants affect RNA biogenesis in distinct ways and the localization pattern can be informative for gene function.

Nucleolar disruption is linked to poly(A)-RNA accumulation

Studies in Schizosaccharomyces pombe have shown that mutants affecting chromosome biology are associated with the accumulation of nuclear poly(A)-RNA and nucleolar disruption (Kalam Azad et al., 2003; Ideue et al., 2004). Of the genes identified in our screen, approximately one-third have functions that include kinetochore–microtubule attachment, chromosome organization, and cell cycle checkpoint control (Table 1). Given these facts and the reported defects in rDNA segregation within mutants identified here (e.g., IPL1 and SMC genes; Freeman, 2000; D’Amours et al., 2004; Sullivan et al., 2004; Machín et al., 2005), we assayed nucleolar status in all 29 mutants based on the rRNA-processing intermediate (ITS1) and nucleolar protein (Nop56p–green fluorescent protein [GFP]; Gautier et al., 1997; Woolford and Baserga, 2013). By monitoring each nucleolar marker and poly(A)-RNA in the same cell, we found that 21 of the 29 mutants displayed alterations in ITS1 and/or Nop56p-GFP localization and abundance when poly(A)-RNA accumulated (Table 1), which included fragmented nucleoli in mRNA export mutants, as previously reported (Kadowaki et al., 1994b; Dockendorff et al., 1997; Segref et al., 1997; Thomsen et al., 2008). Nucleolar disruption was prominent in seven of the nine mutants
| Gene      | Biological process                           | Previous report/verification | Distribution of nuclear poly(A)-RNA | Cells with nuclear poly(A)-RNA accumulation ± SD (%) | GFA1 mRNAs per cell ± SD | Nuclear GFA1 mRNAs | ITS1 and Nop56-GFP foci |
|-----------|----------------------------------------------|-----------------------------|------------------------------------|-----------------------------------------------------|--------------------------|-------------------|---------------------|
| URA10     | Pyrimidine biosynthesis                      | –                           | Diffuse                            | <1                                                  | 5.1 ± 0.7                | 0.9 ± 0.9 (1)      | –                   |
| ALR1      | Plasma membrane Mg²⁺ transporter              | Rescued                     | Foci                              | 36 ± 2                                              | 11.1 ± 1.7               | 1.8 ± 1.6* (1)    | –                   |
| BRL1      | NPC and nuclear envelope biogenesis          | Sai toh et al. (2005)       | Diffuse signal/foci               | 34 ± 11                                             | 6.0 ± 0.6                | 1.3 ± 1.1* (1)    | –                   |
| BRR6      | NPC and nuclear envelope biogenesis          | de Bruyn Kops and Guthrie (2001) | Diffuse signal/foci               | 49 ± 4                                              | 4.5 ± 1.1                | 1.1 ± 1.0* (1)    | –                   |
| CBF2      | Chromosome segregation                        | Rescued                     | Diffuse signal/foci               | 27 ± 11                                             | 5.2 ± 1.2                | 0.9 ± 0.9 (1)      | Both absent in cells with nuclear poly(A)-RNA |
| CEP3      | Chromosome segregation                        | Rescued                     | Diffuse signal/foci               | 16 ± 4                                              | 6.7 ± 0.9                | 1.0 ± 1.3 (1)     | Both absent in cells with nuclear poly(A)-RNA |
| CLP1      | Cleavage and polyadenylation of RNA          | Rescued                     | Foci                              | 32 ± 10                                             | 9.6 ± 1.3                | 1.6 ± 1.3* (1)    | Nop56-GFP foci     |
| CSL4      | RNA processing and degradation                | Remade                      | Diffuse signal next to DAPI       | −100                                                | 6.0 ± 1.9                | 2.3 ± 1.9* (2)    | Enlarged ITS1 and Nop56-GFP area |
| DBP5      | RNA export                                   | Snay-Hodge et al. (1998), Tseng et al. (1998) | Diffuse signal/foci               | −100                                                | 4.2 ± 0.3                | 2.2 ± 1.5* (2)    | ITS1 and Nop56-GFP foci |
| DIS3      | RNA processing/degradation                    | Kadowaki et al. (1994a)     | Diffuse signal next to DAPI       | −100                                                | 5.5 ± 1.0                | 2.1 ± 1.5* (2)    | Enlarged ITS1 and Nop56-GFP area |
| ENP1      | RNA processing and ribosomal subunit synthesis | Rescued                     | Diffuse signal next to DAPI       | −100                                                | 3.4 ± 0.5                | 2.0 ± 1.5* (2)    | ITS1 decreased or absent |
| GLE1      | RNA export                                   | Del Priore et al. (1996), Murphy and Wente (1996) | Diffuse signal/foci               | −100                                                | 2.2 ± 0.3                | 2.1 ± 1.3* (2)    | ITS1 and Nop56-GFP foci |
| IPL1      | Chromosome segregation/cell cycle            | Cole et al. (2002)          | Diffuse signal/foci               | 30 ± 12                                             | 3.4 ± 0.5                | 0.8 ± 0.9 (1)     | Both absent in cells with nuclear poly(A)-RNA |
| LDB19     | Ubiquitin-dependent endocytosis              | Remade                      | Diffuse signal                    | 17 ± 3                                              | 6.2 ± 0.5                | 0.9 ± 1.0 (1)     | –                   |
| MEX67     | RNA export                                   | Segref et al. (1997)        | Diffuse signal/foci               | −100                                               | 2.4 ± 0.6                | 2.1 ± 1.2* (2)    | ITS1 and Nop56-GFP foci |

**TABLE 1:** Description and phenotypes associated with mutants that display poly(A)-RNA accumulation.

Continues
| Gene       | Biological process                      | Previous report/verification | Distribution of nuclear poly(A)-RNA | Cells with nuclear poly(A)-RNA accumulation ± SD (%) | GFA1 mRNAs per cell ± SD | Nuclear GFA1 mRNAsb | ITS1 and Nop56-GFP |
|------------|----------------------------------------|-----------------------------|-------------------------------------|-----------------------------------------------------|--------------------------|---------------------|-------------------|
| MPS1       | Spindle pole body/cell cycle           | Rescued                     | Diffuse signal/foci                 | 28 ± 4                                              | 6.5 ± 1.2                | 1.1 ± 1.1 (1)      | Both absent in cells with nuclear poly(A)-RNA |
| MTR4       | RNA processing and surveillance         | Kadowaki et al. (1994a)     | Diffuse signal next to DAPI         | ~100                                                | 4.9 ± 0.7                | 2.3 ± 1.5* (2)     | ITS1 decreased or absent |
| NUP133     | Nucleocytoplasmic transport            | Doye et al. (1994)          | Diffuse signal/foci                 | 59 ± 5                                              | 5.7 ± 0.8                | 1.3 ± 1.0* (1)     | –                 |
| NUP145     | Nucleocytoplasmic transport            | Fabre et al. (1994), Wente  | Diffuse signal/foci                 | 43 ± 2                                              | 4.6 ± 0.6                | 1.2 ± 1.0* (1)     | –                 |
| NUP159     | Nucleocytoplasmic transport            | Gorsch et al. (1995)        | Diffuse signal/foci                 | ~100                                                | 1.7 ± 0.2                | 1.6 ± 1.0* (1)     | ITS1 and Nop56-GFP foci |
| PRP2       | Pre-mRNA splicing                      | Rescued                     | Diffuse signal                      | 34 ± 12                                             | 5.9 ± 0.5                | 1.1 ± 1.1 (1)      | –                 |
| PTA1       | Cleavage and polyadenylation of RNA    | Hammell et al. (2002)       | Diffuse signal/foci                 | 35 ± 8                                              | 6.1 ± 0.9                | 1.2 ± 1.0* (1)     | –                 |
| RRP43      | RNA processing and degradation         | Rescued                     | Diffuse signal next to DAPI         | ~100                                                | 5.3 ± 0.3                | 1.8 ± 1.7* (1)     | Enlarged ITS1 and Nop56-GFP area |
| RSP5       | E3 ubiquitin ligase; multiple processes| Neumann et al. (2003),     | Diffuse signal                      | ~100                                                | 5.7 ± 2.0                | 1.2 ± 0.9* (1)     | –                 |
| SLI15      | Chromosome segregation/cell cycle      | Remade                      | Diffuse signal/foci                 | 30 ± 4                                              | 4.9 ± 1.1                | 1.0 ± 1.2 (1)      | Both absent in cells with nuclear poly(A)-RNA |
| SMC1       | Chromosome segregation                 | Rescued                     | Diffuse signal/foci                 | 14 ± 2                                              | 4.3 ± 0.5                | 1.0 ± 1.2 (1)      | –                 |
| SMC3       | Chromosome segregation                 | Rescued                     | Diffuse signal/foci                 | 51 ± 13                                             | 5.7 ± 0.3                | 1.3 ± 1.2* (1)     | –                 |
| SMC4       | Chromosome organization                | Rescued                     | Diffuse signal/foci                 | 29 ± 7                                              | 9.0 ± 2.5                | 1.1 ± 1.3 (1)      | Both absent in cells with nuclear poly(A)-RNA |
| SPC24      | Chromosome segregation                 | Remade                      | Diffuse signal/foci                 | 33 ± 6                                              | 4.3 ± 1.1                | 0.9 ± 1.1 (1)      | Both absent in cells with nuclear poly(A)-RNA |
| SRM1/PRP20 | Nucleocytoplasmic transport            | Amberg et al. (1993),       | Diffuse signal next to DAPI         | ~100                                                | 2.1 ± 0.2                | 0.6 ± 0.7* (0)     | ITS1 decreased or absent |

*To verify mutants that had not previously been reported to accumulate poly(A)-RNA, the phenotype was rescued using a wild-type allele or recapitulated by making the mutant in a different strain background as indicated.

bAverage number of nuclear mRNAs ± SD with the median for the data shown in parentheses.

*Distribution of nuclear mRNAs tested using the Wilcoxon-rank sum test and found to be significant at \( p < 0.001 \).

**TABLE 1: Description and phenotypes associated with mutants that display poly(A)-RNA accumulation. Continued**
To ensure that a block in mRNA export could be observed, we used a mex67-5 strain, which, when shifted to the nonpermissive temperature, rapidly accumulated poly(A)-RNA in the nucleus (Segref et al., 1997). smFISH assays using the gene-specific probes displayed an obvious block in mRNA export at the nonpermissive temperature in mex67-5, and, of importance, a mex67-5/CCW12 strain showed no detectable signal with the CCW12 probe set (Figure 2A). This established that these mRNA probes can be used to detect export defects, and in the case of CCW12, the probes were specific for the transcript being targeted.

Using smFISH data for GFA1, we determined the number of transcripts and distribution of these transcripts between the nucleus and cytoplasm. In a haploid control strain (ura10Δ), GFA1 was found to be present at approximately five copies per cell, with ∼18% of these transcripts being in the nucleus (based on DAPI and ITS1 signals), whereas the mRNA export mutant mex67-5 contained ∼88% of transcripts in the nucleus (Table 1 and Figure 2A). Most mutants showed less than a twofold change in GFA1 levels, with nuclear pools of the mRNA that varied between 16 and 95% (Table 1). In the case of prp2-1, there was no effect on GFA1 localization or transcript number, but ACT1 export was altered (Figure 2B), which is consistent with the role of Prp2p in splicing (Lustig et al., 1986). For brl1-3231 and brr6-ph, these mutants showed no mRNA export defect after a 3-h temperature shift, but given the role of the gene products in nuclear envelope maintenance and NPC biogenesis (de Bruyn Kops and Guthrie, 2001; Saito et al., 2005; Hodge et al., 2010; that affect chromosome biology, with these mutants often lacking nuclear ITS1 and Nop56p-GFP in cells with poly(A)-RNA accumulation (e.g., sli15-1 and smc4-1 vs. dis3-1; Figure 1B). Although SMC1 and SMC3 mutants did not show obvious nucleolar defects, recent reports provide a direct role for cohesins (e.g., SMC3) in nucleolar function (Bose et al., 2012; Harris et al., 2014). Together these findings further support a link between poly(A)-RNA accumulation and alterations to the nucleolus, which may often be induced by errors in chromosome segregation.

Identification of mRNA biogenesis and export mutants
Mutations within RNA processing and surveillance pathways have been shown to accumulate poly(A)-RNA species, including rRNA, mRNA, snRNA, snoRNA, and tRNA (van Hoof et al., 2000; Kuai et al., 2004; LaCava et al., 2005; Vanáčová et al., 2005; Wyers et al., 2005; Carneiro et al., 2007; Rougemaille et al., 2007; Gudipati et al., 2012; Schneider et al., 2012; Castelnuovo et al., 2013). Consequently the mutants identified here using an oligo-d(T)-based in situ approach may accumulate poly(A)-RNA due to disruptions in RNA biogenesis that are independent of mRNA. To identify those mutants that alter mRNA processing and export, we performed smFISH assays using probes against GFA1, ACT1, or CCW12 transcripts. The mRNAs were selected based on relative expression levels (GFA1 = low and ACT1/CCW12 = high) and the presence of an intron in ACT1 (Ng and Abelson, 1980) that may lead to this mRNA being affected differently than nonspliced mRNAs (i.e., CCW12 and GFA1). To ensure that a block in mRNA export could be observed, we used a mex67-5 strain, which, when shifted to the nonpermissive temperature, rapidly accumulated poly(A)-RNA in the nucleus (Segref et al., 1997). smFISH assays using the gene-specific probes displayed an obvious block in mRNA export at the nonpermissive temperature in mex67-5, and, of importance, a mex67-5/CCW12 Δ strain showed no detectable signal with the CCW12 probe set (Figure 2A). This established that these mRNA probes can be used to detect export defects, and in the case of CCW12, the probes were specific for the transcript being targeted.

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Lone et al., 2015), we expected to observe a defect. We performed a 6-h temperature shift, providing additional time for Ts phenotypes to develop, and under these conditions, we observed mRNA export defects for both mutants (Figure 2C).

mRNAs localize to distinct subdomains of the nucleus in mutants

smFISH data showed that some mutants had a large increase in the fraction of nuclear mRNAs (e.g., mex67-5 and gle1-4), whereas others did not (e.g., rps5-3 and sli15-1). However, in almost all instances, the localization of mRNAs appeared distinct in the mutants tested (Supplemental Data). For example, strains carrying mutations in genes directly linked to the mRNA export process (e.g., DBP5, GLE1, MEX67, and NUP159) often had mRNAs near the periphery of the DAPI-stained DNA mass (see mex67-5 in Figure 2). This suggests that within these mutants, mRNAs accumulated at or near nuclear pore complexes, as reported for mutants of MEX67 (Hurt et al., 2000; Smith et al., 2015) and DBP5 (Hodge et al., 2011). The prp2-1 mutant also showed ACT1 transcripts near the nuclear periphery (Figure 2B), which may be related to quality control mechanisms that block export of premRNAs (Galy et al., 2004; Palancade et al., 2005; Iglesias et al., 2010; Hackmann et al., 2014). Using Ndc1p-GFP as a marker of NPCs and the nuclear periphery, we quantified the percentage of transcripts within ~250 nm of the NPC signal in individual cells (n = 50) of select mutants. In control cells, 38 ± 15% of GFA1 mRNAs were found within this distance, which increased in mRNA export mutants to 68 ± 35% (mex67-5) and 61 ± 27% (dbp5-1) (Figure 3A). The prp2-1 mutant did not show an increase in peripheral localization of GFA1 (39 ± 18%), whereas ACT1 increased from 25 ± 12% in control to 49 ± 17% in prp2-1 (Figure 3B). These data are consistent with the accumulation or retention of mRNAs near NPCs when splicing or late steps in the mRNA export pathway are disrupted.
A second distinct localization pattern was the accumulation of mRNAs within a nuclear focus. Rarely present in the control strain, 15 mutants showed >5-fold increase in the frequency of nuclear foci with an intensity ≥10-fold that of single transcripts (Supplemental Data), including rsp5-3 (Figure 4A). In quantifying the GFA1 smFISH data, we counted such foci as single mRNAs (see Material and Methods, Gene-specific FISH), which likely underestimates the number of nuclear transcripts in these mutants and leads to a lower level of nuclear accumulation reported in Table 1. Rsp5p is a ubiquitin-ligase that functions in both the cytoplasm and nucleus (Belgareh-Touzé et al., 2008; Kaliszewski and Zoladek, 2008) and has a known role in mRNA biogenesis via modification of the THO/TREX complex (Neumann et al., 2003; Rodriguez et al., 2003; Gwizdek et al., 2005). Given the functions of Rsp5p and other genes with this phenotype, the finding that various mRNA probes show the same defect (i.e., not related to splicing), and the intensity of the smFISH signal, we speculated that these foci represent gene transcription sites.

To test this possibility, we integrated a lacO array ~400 base pairs upstream of the ACT1 gene, which could be used in combination with LacI-GFP and FISH probes to localize both the ACT1 gene and mRNA focus. In rsp5-3, bright ACT1 mRNA foci were in close proximity to the ACT1 gene locus, separated by an average distance of 0.15 ± 0.05 μm, whereas the CCW12 mRNA focus was distinct from the ACT1 gene locus at an average distance of 0.81 ± 0.27 μm (n = 50; Figure 4B). These data are consistent with these bright mRNA foci being at or near transcription sites.

FIGURE 3: Accumulation of mRNA near the nuclear periphery and NPCs. (A) Representative images showing GFA1 mRNA (red) localization in control (ura10Δ), dbp5-1, and mex67-5 strains compared with NPCs (green, Ndc1p-GFP) and DAPI (blue) after 3 h at 37°C. (B) Representative images showing GFA1 or ACT1 mRNA (red) localization in the prp2-1 strain compared with NPCs (green, Ndc1p-GFP) and DAPI (blue) after 3 h at 37°C. Scale bars, 1 μm.

FIGURE 4: Accumulation of ACT1 mRNA near transcription sites. (A) Representative images showing GFA1 mRNA (red) localization in control (ura10Δ) and rsp5-3 strains compared with poly(A)-RNA (gray) and DAPI (blue) after 3 h at 37°C. (B, C) Representative images showing ACT1 or CCW12 mRNA (red) localization in control (ura10Δ), rsp5-3, ldb19Δ, spc24-10, and sli15-1 strains compared with the ACT1 gene locus (green, marked by lacO-array/GFP-LacI) and DAPI (blue) after 3 h at 37°C. Inset, zoomed-in view of boxed region in the merged image. Scale bars, 1 μm.
The third localization pattern was an accumulation of mRNAs next to the DAPI-stained DNA mass with or near ITS1 (Table 1). This included mutants in components of the exosome (e.g., DIS3, RRP43, and CSL4) and TRAMP complex (MTR4), which have been reported to accumulate poly(A)-RNA in the nucleolus, as well as the heat shock–induced transcript SSA4 and the localized mRNA ASH1 (Brodsky and Silver, 2000; Thomsen, 2003; Carneiro et al., 2007; Rougemeille et al., 2007; Du et al., 2008). To quantify nucleolar localization (defined by ITS1 staining), we compared FISH data for GFA1 between the mRNA export factor (dbp5-1) and exosome component (dis3-1), which both accumulated GFA1 transcripts in the nucleus after temperature shift (Figure 5A). Using these data, we...
quantified where nuclear mRNA localized with respect to the ITS1 signal and found 0.4 ± 0.6 and 0.8 ± 0.9 GFA1 transcripts in the nucleolus of the control (ura10Δ) and dbp5-1 strains, respectively. This corresponds to 46% (ura10Δ) and 36% (dbp5-1) of total nuclear transcripts in these strains (Table 1). In contrast, 1.7 ± 1.2 GFA1 mRNAs were found in the nucleolus of a dis3-1 strain, which was 80% of the total transcripts in the nucleus of this mutant. This suggests that disruption of exosome function leads to the accumulation of mRNAs within the nucleolus, which is not a general result of mRNAs being retained in the nucleolus.

mRNAs can be classified based on protein-binding profiles, which have been used to define 10 general mRNP classes (Tuck and Tollervey, 2013). These range from mRNAs most likely to be processed and exported to the cytoplasm for translation (class X) to those that have protein-binding patterns similar to CUTs (class I), with CUTs being targets of nuclear RNA surveillance (Wyers et al., 2005). The three transcripts we observed in the nucleolus (GFA1, ACT1, and CCW12) belong to class X, and so to extend our observations to other mRNA classes, we used gene-specific FISH probes to assay the localization of IMD2 (class I) and LEU1 (class II) mRNAs. In both cases, we observed that these mRNAs were retained in the nucleolus of a dbp5-1 mutant, localized to the nucleolus in dis3-1, and appeared as a bright nuclear focus in the rbp5-3 (Figure 5, B and C). By using strains carrying NAB2-GFP and GFP FISH probes, we also observed mRNAs within the nucleolus of a dis3-1 strain (Figure 5D). This implies that mRNAs in the nucleolus are near full length and not short transcripts resulting from early transcription termination, since the GFP probes are directed against the 3′ end of the transcript. These data demonstrate that various mRNAs localize to distinct subdomains of the nucleus when RNA processing and surveillance pathways are disrupted.

mRNP-associated factors are sequestered in the nucleolus with mRNA

After the observation that mRNAs accumulated in the nucleolus, we characterized the subcellular localization of three proteins involved in mRNA processing. Specifically, in control (ura10Δ), dbp5-1, and dis3-1 strains, we assayed localization of Nab2p-GFP (polyadenosine RNA-binding adaptor protein for Mex67p), Prp19p-GFP (splicing factor), and Hrp1p-GFP (subunit of cleavage factor I; required for the cleavage and polyadenylation of mRNA 3′ ends). In control and dbp5-1 strains, these proteins colocalized with the DAPI-stained DNA mass and were largely absent from the nucleolus as marked by ITS1 (Figure 6, A–C). In contrast, within the dis3-1 strain, these factors were found throughout the nuclear volume within both the DAPI- and ITS1-stained regions, suggesting that factors involved in mRNA biogenesis redistribute to the nucleolus in this mutant, similar to mRNAs.

We further tested whether loss of Rrp6p activity would lead to the relocation of these same factors. Rrp6p is a nonessential catalytic subunit of the exosome that, when mutated, results in poly(A)-RNA accumulation in a discrete domain within the nucleolus (Hieronymus et al., 2004; Carneiro et al., 2007; Rougemaille et al., 2007). In an rrp6Δ strain, we observed approximately five GFA1 transcripts per cell, with 18% being nuclear, which is comparable to the control strain (Table 1), but 71% of these nuclear transcripts colocalized with ITS1 as compared with 46% in control. Nab2p-GFP, Prp19p-GFP, and Hrp1p-GFP were also enriched within the nucleolus (Figure 6, A–C), demonstrating that both mRNA- and mRNP-associated factors are redistributed within the nucleus of an rrp6Δ strain.

In addition to mutations in exosome or TRAMP complex components, mutations in ENP1 and SRM1 caused accumulation of poly(A)-RNA next to the DAPI-stained DNA mass (Figure 6D and Table 1). Enp1p functions in pre-rRNA processing and 40S subunit synthesis, and Smr1p facilitates nucleocytoplasmic trafficking (Tachibana et al., 1994; Koepp et al., 1996; Chen et al., 2003). GFA1 mRNAs could be readily observed with the poly(A)-RNA signal that is adjacent to DAPI in both enp1-1 and smr1-1 ts strains (Figure 6D), but ITS1 expression was severely reduced in both mutants, preventing us from quantifying GFA1 localization. Nab2p-GFP, Prp19p-GFP, and Hrp1p-GFP were also localized to the nucleolus of the enp1-1 strain (Figure 6E); however, smr1-1 ts could not be characterized due to defects in nuclear protein import. These findings demonstrate that localization of mRNAs and mRNP-associated proteins to the nucleolus does not occur only when RNA surveillance and quality control machinery is mutated and can occur as a result of disruption to other nuclear processes.

DISCUSSION

mRNA biogenesis involves the interaction of numerous protein factors with each mRNA in a spatially and temporally regulated manner within the nucleus to link the processes of transcription, capping, splicing, 3′-end formation, RNA surveillance, and NPC-mediated export (Oeffinger and Zenklusen, 2012; Niño et al., 2013). Nuclear events also act to define the fate of each mRNP, which can include marking mRNPs in the nucleus for translation, storage, transport, or decay within the cytoplasm (Müller-McNicoll and Neugebauer, 2013; Singh et al., 2015). Consequently the proper and efficient coordination of these nuclear events is central to the fidelity of the gene expression program, with previous work having identified factors involved in these processes through analysis of individual mutants, genetic screens and comprehensive screening of nonessential genes (Shiokawa and Pogo, 1974; Amberg et al., 1992, 1994a; Doye et al., 1994; Fabre et al., 1994; Gorsch et al., 1995; Hieronymus et al., 2004). However, the critical nature of these events dictates that essential genes would likely be involved in this process, which prompted us to perform a screen of essential genes for function(s) related to mRNA biogenesis in S. cerevisiae. This led to the identification of 29 genes with a nuclear poly(A)-RNA accumulation phenotype (Table 1), of which half (15 of 29) had not previously been reported to have such a defect. Of these, 26 genes were subsequently found to alter mRNA biogenesis and export when mutated, based on the observed accumulation of mRNA in the nucleus and/or altered localization of nuclear mRNAs.

Classes of mRNA biogenesis and export mutants

Gene ontology places the majority of the 29 genes into three biological processes: RNA export from nucleus, nuclear RNA catabolic processes, and chromosome segregation. The identification of mRNA export factors is expected and validates our screen. Links between mRNA export and RNA decay are also well established (Porrua and Libri, 2013; Eberle and Visa, 2014), with various components of the decay machinery having originally been identified in screens for mRNA transport (MTR) mutants (Kadowaki et al., 1992, 1994a). Beyond the related biological functions of the gene products, 26 of the 29 mutants could be further classified into three distinct groups (referred to as class A, B, or C) based on the observed accumulation of mRNA in the nucleus and/or altered localization of nuclear mRNAs.

Class A mutants are represented by genes directly involved in mRNA export and NPC function (e.g., DBP5 and NUP159) and were characterized by a diffuse nuclear poly(A)-RNA signal (with or
Of the class A mutants, prp2-1 showed a transcript-specific block of mRNA export with the ACT1 mRNA localized near the nuclear periphery but not another mRNA that lacked an intron (Figures 2 and 3). This observation is consistent with the description of NPC-dependent quality control mechanisms that prevent the export of immature mRNAs, which include pre-mRNAs (Galy et al., 2004; Palancade et al., 2005; Iglesias et al., 2010; Hackmann et al., 2014).

Class B mutants, similar to class A, showed a diffuse poly(A)-RNA distribution (with or without discrete foci) overlapping the DAPI stain, often accompanied by disrupted nucleoli (Figure 1 and Table 1). These mutants further displayed an increased proportion of mRNAs near the nuclear periphery and NPCs (Figure 3). This suggests that within class A mutants, mRNPs accumulated at or near nuclear pore complexes due to failures in NPC-mediated export, which is consistent with the known function of these factors (Table 1). In support of the smFISH observations and data interpretation, delays in export at the nuclear periphery have been observed using live-cell imaging techniques for mutants of DBP5, MEX67, NAB2, and the Nups MLP1/2 (Hodge et al., 2011; Saroufim et al., 2015; Smith et al., 2015). Of the class A mutants, prp2-1 showed a transcript-specific block of mRNA export with the ACT1 mRNA localized near the nuclear periphery but not another mRNA that lacked an intron (Figures 2 and 3). This observation is consistent with the description of NPC-dependent quality control mechanisms that prevent the export of immature mRNAs, which include pre-mRNAs (Galy et al., 2004; Palancade et al., 2005; Iglesias et al., 2010; Hackmann et al., 2014).

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mutants are involved in RNA processing and surveillance as compo

tion errors.

would be expected that both mRNA and tRNA processing would be

ship between mRNA export and chromosome segregation; as it


genes (RSP5, PTA1, and CLP1), which are known to be

mutants with a class B pheno

2002). Given the requirement for ongoing cell division, the large

base of DNA are repeatedly lost in these mutants and this gives rise

RNA accumulated in these mutants it was associated with mRNAs

mRNA biogenesis defects. We envision this occurring by multiple

mechanisms: 1) directly as a result of mutation in a factor involved in

mRNA biogenesis, 2) by activating quality control mech

mechanisms that retain immature/aberrant mRNPs, and 3) due to changes

mRNA biogenesis and export, 2) by activating quality control mech

Nuclear homeostasis and mRNA biogenesis

Disruption of different nuclear processes resulted in classes A–C of

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anism(s) facilitating nucleolar localization. The heat shock–induced transcript SSA4, the localized mRNA ASH1, and PHO84 antisense RNA have also been re

ported to enter the nucleolus (Brodsky and Silver, 2000; Thomsen, 2003; Carneiro et al., 2007; Du et al., 2008; Castelnovo et al., 2013), and mRNAs have recently been observed to transit through the nucleolus for export in live cells (Saroufim et al., 2015). Further study is needed to address how often mRNAs enter the nucleolus during normal mRNA biogenesis, the functional significance of this event, and the molecular mechanism(s) facilitating nucleolar localization.

Finally, class C mutants displayed poly(A)-RNA accumulation next to the DAPI stain with or adjacent to the nucleolus (based on ITS1) and an increased frequency of mRNAs within this compartment (Figures 1, 5, and 6 and Supplemental Data). Many of these mutants are involved in RNA processing and surveillance as compo

nents of the exosome and TRAMP complexes, which raises the possi

bility that the detected mRNAs are aberrant and only become detec

table due to a loss of surveillance and decay activities. Howev

er, we did not detect an increase in the number of GFA1 tran

scripts in these mutants (Table 1), which may be expected if these

were common products of transcription that are normally degraded.

By using probes against the GFP coding sequence at the 3′ end of

an mRNA (Figure 5), we also observed nucleolar mRNA accumula

tion, suggesting that these transcripts are near full length and not the product of early transcript termination events. Other mutants (e.g., enp1-1 and smr1-ts) that are not part of the decay machinery caused similar mRNA localization phenotypes, suggesting that nu

cleolar mRNAs are not solely detectable in mutants of the RNA sur

veillance machinery.

In addition to mRNAs, we also observed enrichment of mRNP-

associated factors within the nucleolus in class C mutants (Figures 6).

The redistribution of mRNP-associated factors may occur as con

stituents of mRNPs present within the nucleolus, but it is also pos

sible that poly(A)-RNA binding proteins, such as Nab2p (Anderson et al., 1993), localize to the nucleolus due to promiscuous binding of the protein to accumulated poly(A)-RNA. The latter would effect

ively deplete the activities of poly(A)-RNA binding proteins, which include mRNA export adaptors, and provide a mechanism by which class C mutants with various cellular functions cause the same termi

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mutation induced strong poly(A)-RNA accumulation in the nucleolus and redistribution of mRNA processing factors to this compartment (Figure 6), which likely affects mRNA processing and export by making these essential factors limiting. This relationship has also been observed to work in reverse, with mRNA export defects causing nucleolar disruption (Kadowaki et al., 1994b; Dockendorff et al., 1997; Segref et al., 1997; Thomsen et al., 2008). In this way, enp1-1 (and other such mutants) function by perturbing overall nuclear homeostasis and would not be considered an mRNA processing or export factor per se.

Within this paradigm of system-level perturbations, how chromosomesome segregation mutants affect the cell and cause specific mRNA processing and export defects remains to be determined. We find it noteworthy that aneuploidy is known to alter tumorigenesis through mechanisms that include changes in gene expression (Gordon et al., 2012; Holland and Cleveland, 2012; Santaguida and Amon, 2015; Dürbaum and Storchová, 2016), and our work highlights the fact that many chromosome segregation mutants cause the accumulation of mRNAs at transcription sites, which provides one mechanism by which this may occur. Although these types of relationships could be casually termed as indirect, these system-level perturbations are important to understand because it is likely that disease states arise from mutation(s) due to both the specific process affected by the mutation and the system-level changes this in turn induces.

**MATERIALS AND METHODS**

**Yeast strains**

Strains constructed for this study are listed in the Supplemental Data or were taken directly from mutant collections (Ben-Aroya et al., 2008; Breslow et al., 2008; Li et al., 2011). Strains with genome-encoded, GFP-tagged NOP56, NAB2, PRP19, and HRP1 were generated by amplifying each gene with the GFP::His3MX6 cassette plus ~300 base pairs of flanking sequence directly from the yeast GFP collection (Huh et al., 2003). The resulting PCR product was transformed into yeast to integrate the GFP tag into the yeast genome (Gietz et al., 1992). Deletion strains were generated by homologous recombination with a selectable marker made by PCR (Longtine et al., 1999). NDC1-yeGFP and GAL1-GFP-LDB19 strains were generated by transforming a PCR product with homology to the gene locus made from pKT148 (Sheff and Thorn, 2004) and pAfAa-His3MX6-PGAL1-GFP (Longtine et al., 1998), respectively. The NOP56-GFP plasmid (pBM461) was made by cloning NOP56-GFP with ~400 base pairs of promoter sequence from the yeast GFP collection (Huh et al., 2003) into pRS313 (Sikorski and Hieter, 1989) using Xhol and NotI sites introduced by PCR. Tagging of the ACT1 locus with a lacO array was performed in a wild-type strain as previously described using pSR13 and pAFS78 (Straight et al., 1996; Rohner et al., 2008) into which mutant alleles (i.e., LDB19, SL115, and SPC24) were introduced by transformation of a PCR product. Rescue plasmids were taken from the Yeast ORF Collection (e.g., CEPI, ENP1, MP51, and PRP2; Gelerpin et al., 2005), generated by cloning the gene (e.g., ALR1, CLP1, and RRP43) ± 500 base pairs of flanking sequence into pRS41N using SacI and HindIII sites introduced by PCR, or obtained from the laboratory of Vivien Measday (e.g., CBF2 and IPL1) or Doug Koshland (SMC1, SMC3, and SMC4). All primer sequences are available upon request.

**Screen for poly(A)-RNA accumulation**

Yeast strains from mutant collections (Ben-Aroya et al., 2008; Breslow et al., 2008; Li et al., 2011) were grown in yeast extract/peptone/dextrose into log phase and then shifted directly to 37°C for 3 h. After temperature shift, cells were fixed for 15 min using 5% formaldehyde, and poly(A)-RNA was detected by FISH using an fluorescein isothiocyanate–labeled oligo-dT probe (Cole et al., 2002). After hybridization of the probe and washing steps, mounting medium with DAPI was applied to each sample and a coverslip was affixed. Imaging was performed on an Olympus IX81 microscope with 100X oil immersion objective (numerical aperture [NA] 1.4) controlled by MetaMorph software (Molecular Devices, Sunnyvale, CA) using identical exposure settings. During each day of screening, ura10A, mex67-5, and dbp5-1 strains were included as controls to ensure consistency in the FISH procedure. Imaging data (>200 cells) were used to visually score nuclear accumulation of poly(A)-RNA, with mutants showing evidence of accumulation being validated in triplicate. The identity of each strain found to accumulate poly(A)-RNA was subsequently verified by PCR.

**Gene-specific FISH**

mRNA transcripts were detected using a mixture of up to 48 fluorescently labeled gene probes of 20 nucleotides in length (Biosearch Technologies, Petaluma, CA) with a modified oligo-dT FISH procedure (Amberg et al., 1993). Probe sequences used for each transcript are listed in the Supplemental Data. Briefly, cells were fixed with 5% formaldehyde for 15 min at 37°C, washed with buffer A (0.1 M potassium phosphate, pH 6.5, and 0.5 mM MgCl2), and treated with Zymolase (250 μg/ml) in buffer B (0.1 M potassium phosphate, pH 6.5, 0.5 mM MgCl2, and 1.2 M sorbitol) for 35 min at 37°C. Sporoplasted cells were spotted on poly-l-lysine–coated slides, incubated for 10 min, washed with buffer A to remove unattached cells, permeabilized in cold methanol for 6 min and acetone for 30 s, and allowed to air dry. Cells were rehydrated with hybridization solution (5X SSC [saline-sodium citrate buffer], 5X Denhardt’s solution, 0.1% Tween 20, 0.01 mg/ml single-stranded DNA, 0.02 mg/ml Escherichia coli tRNA, and 10 mM vanadyl ribonucleoside complex) for 5 min and incubated with fresh hybridization buffer for at least 1 h at 37°C. Hybridization buffer with 1 ng of fluorescein-labeled LNA oligo-dT probe (Exion, Woburn, MA), 20 ng of Quasar 570-labeled mRNA probe, and/or 4 ng of Quasar 670-labeled ITS1 probe was added and incubated overnight (~14 h) at 37°C in a humidity chamber. The next day, cells were washed sequentially with 2X SSC, 1X SSC, 0.5X SSC, and 2X phosphate-buffered saline for 5 min at room temperature, and after the final wash, slides were dipped into 100% ethanol for 10 s and air dried. Mounting medium with DAPI was applied to each sample, and a coverslip was affixed. Imaging was performed on a DeltaVision Elite microscope system equipped with a front-illuminated scientific complementary metal-oxide-semiconductor (sCMOS) camera driven by Softworx 6 (GE Healthcare, Piscataway, NJ) using an Olympus 60×/1.42 NA oil objective. Image analysis was performed in FIJI (Schindelin et al., 2012), with each three-dimensional smFISH data set being reduced to a single maximum Z-projection. mRNA FISH signals were identified as single points within FIJI using noise tolerances set for each individual image and/or smFISH experiment to minimize the detection of false spots and compensate for sample variability. Masks of DAPI and ITS1 probe signals were used to quantify the number of mRNAs within these compartments and determine the number of cells within the image being analyzed. Average transcript number per cell was determined from each image, given the number of transcripts and nuclei counted. In control and most mutant strains, foci varied in intensity within a small range and were therefore counted as single transcripts. Bright nuclear foci were apparent in some mutants, but these were counted as single transcripts. This approach was taken for three reasons: 1) where tested, these foci were near transcription
sites, suggesting that they were not mature transcripts or were not released across the site of transcription, 2) the distribution of smFISH probes across the entire length of the mRNA did not allow the differentiation of multiple short transcripts from a full-length mRNA, and 3) these bright foci were generally rare.

Imaging mRNA processing factors
Yeasts strains carrying integrated GFP reporters were grown in selective media overnight at room temperature into log phase and shifted directly to 37°C for 3 h. After temperature shift, cells were fixed for 15 min using 4% paraformaldehyde, and poly(A)-RNA was detected using 2 ng of TYE 563-labeled LNA oligo-dT probe (Exiqon) and 4 ng of Quasar 670-labeled ITS1 probe (Biosearch Technologies) by FISH (Cole et al., 2002). After the final wash, slides were dipped in 100% ethanol for 10 s and air dried, mounting medium with DAPI was then applied to each sample, and a coverslip was affixed. Imaging was performed on a DeltaVision Elite microscope system equipped with a front-illuminated sCMOS camera driven by Softworx 6 using an Olympus 60x/1.42 NA objective. Image analysis was performed in FIJI (Schindelin et al., 2012).

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REFERENCES
Allmanig C, Petalski E, Podtelejnikov A, Mann M, Tollervey D, Mitchell P (1999). The yeast exosome and human PM-Sc are related complexes of 3' right-arrow 5' exonuclease. Genes Dev 13, 2148–2158.
Amberg DC, Fleischmann M, Staglaj J, Cole CN, Aebl M (1993). Nuclear PRP20 protein is required for mRNA export. EMBO J 12, 233–241.
Amberg DC, Goldstein AL, Cole CN (1992). Isolation and characterization of RAT1: an essential gene of Saccharomyces cerevisiae required for the efficient nucleocytoplasmic trafficking of mRNA. Genes Dev 6, 1173–1189.
Anderson JT, Wilson SM, Datar KV, Swanson MS (1993). NAB2: a yeast nuclear polyadenylated RNA-binding protein essential for cell viability. Mol Cell Biol 13, 2730–2741.
Belgareh-Touzé N, Léon S, Erpapazoglou Z, Stawiecka-Mirota M, Urban-Bouyer L, Poulet M, Pham N, Payan V, Cuenot F (2014). Regulation of mRNA trafficking by nuclear pore complexes. Genes (Basel) 5, 767–796.
Breslow DK, Cameron DM, Collins SR, Schuldiner M, Stewart-Ornstein J, Newman HW, Braun S, Madhani HD, Krogan NJ, Weissman JS (2008). A comprehensive strategy enabling high-resolution functional analysis of the yeast genome. Nat Methods 5, 711–718.
Briggs MW, Burkard KT, Butler JS (1998). Rp6p, the yeast homologue of the human PM-Sc100-kDa autoantigen, is essential for efficient 5 S RNA 3' end formation. J Biol Chem 273, 13255–13263.
Brodsky AS, Silver PA (2000). Pre-mRNA processing factors are required for nuclear export. RNA 6, 1737–1749.
Carneiro T, Carvalho C, Braga J, Rino J, Milligan L, Tollervey D, Carmona-Fonseca M (2007). Depletion of the yeast nuclear exosome subunit Rp6p results in accumulation of polyadenylated RNAs in a discrete domain within the nucleolus. Mol Cell Biol 27, 4157–4165.
Castelnuovo F, Rahman S, Guffanti E, Infantino V, Stutz F, Zenklusen D (2013). Bimodal expression of PHO84 is modulated by early termination of antisense transcript. Nat Struct Mol Biol 20, 851–858.
Chen W, Bucaria J, Band DA, Sutton A, Sternglanz R (2003). Epl1, a yeast protein associated with U3 and U14 snRNAs, is required for pre-rRNA processing and 40S subunit synthesis. Nucleic Acids Res 31, 690–699.
Chlebowksi A, Lubas M, Jensen TH, Dzembrowska A (2013). RNA decay machines: the exosome. Biochim Biophys Acta 1829, 552–560.
Cole CN, Heath CV, Hodge CA, Hammell CM, Amberg DC (2002). Analysis of RNA export. Methods Enzymol 351, 585–587.
D’Amours D, Stegmeier F, Amon A (2004). Cdc14 and condensin control the dissolution of cohesin-independent chromosome linkages at repeated DNA. Cell 117, 455–469.
David L, Huber W, Granovskia M, Toedling J, Palm CJ, Bofkin L, Jones T, Davis RW, Steinmetz LM (2006). A high-resolution map of transcription in the yeast genome. Proc Natl Acad Sci USA 103, 5320–5325.
Davis CA, Ares M (2006). Accumulation of unstable promoter-associated transcripts upon loss of the nuclear exosome subunit Rp6p in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 103, 3262–3267.
de Bruyn Kops A, Guthrie C (2001). An essential nuclear envelope integral membrane protein, Brerp, required for nuclear transport. EMBO J 20, 4183–4193.
Del Priore V, Snay CA, Bahr A, Cole CN (1996). The product of the Saccharomyces cerevisiae RPS1 gene, identified as a high-copy suppressor of the rat7-1 temperature-sensitive allele of the RAT7/NUP159 nucleoporin, is required for efficient mRNA export. Mol Biol Cell 7, 1601–1621.
Dockendorff T, Heath C, Goldstein A, Snay C, Cole C (1997). C-terminal truncations of the yeast nucleoporin Nup145p produce a rapid temperature-sensitive mRNA export defect and alterations to nuclear structure. Mol Cell Biol 17, 906–920 [correction published in Mol Cell Biol (1997). 17, 2347–2350].
Doye VR, Wepl R, Hurt EC (1994). A novel nuclear pore protein Nup133p with distinct roles in poly(A)-RNA transport and nuclear pore distribution. EMBO J 13, 6093–6075.
Du TG, Jellbauer S, Müller M, Schmid M, Niessing D, Jansen R-P (2008). Nuclear transit of the RNA-binding protein She2 is required for translational control of localized ASH1 mRNA. EMBO Rep 9, 781–787.
Dürbaum M, Storchova Z (2016). Effects of aneuploidy on gene expression: implications for cancer. FEBS J 283, 791–802.
Eberle AB, Visa N (2014). Quality control of mRNP biogenesis: networking at the transcription site. Semin Cell Dev Biol 32, 37–46.
Fabre E, Boelens WC, Wimmer C, Mattaj IW, Hurt EC (1994). Nup145p is required for nuclear export of mRNA and binds homopolymeric RNA in vitro via a novel conserved motif. Cell 78, 275–289.
Freeman L (2000). The condensin complex governs chromosome condensation and mitotic transmission of rDNA. J Cell Biol 149, 811–824.
Galy V, Gadot O, Fromont-Racine M, Romano A, Jacquier A, Nehrbass U (2008). Nuclear retention of displaced mRNAs in yeast is mediated by perinuclear Mip1p. Cell 116, 63–73.
Gautier T, Bergès T, Tollervey D, Hurt E (1997). Nuclear level KKE/D repeat proteins Nop35p and Nop35p interact with Nop1p and are required for ribosome biogenesis. Mol Cell Biol 17, 7088–7098.
Gelperin DM, White MA, Wilkinson ML, Kon Y, Kung LA, Wise KJ, Lopez-Hoyo N, Jiang L, Piccirillo S, Yu H, et al. (2005). Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. Genes Dev 19, 2816–2826.
Gietz D, Steen JA, Woods RA, Schiestl RH (1992). Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res 20, 1425.
Gordon DJ, Resio B, Pellman D (2012). Causes and consequences of aneuploidy in cancer. Nat Rev Genet 13, 189–203.
Gorsch LC, Dockendorff TC, Cole CN (1995). A conditional allele of the yeast nuclear exosome subunit Rp6p. Proc Natl Acad Sci USA 92, 8786–8790.
Grimal D, Haguenauer-Tsapis R (2008). Versatile role of the yeast membrane protein, Brr6p, required for nuclear transport. EMBO J 20, 4183–4193.
Gorsch LC, Dockendorff TC, Cole C (1996). The product of the Saccharomyces cerevisiae RSS1 gene, identified as a high-copy suppressor of the rat7-1 temperature-sensitive allele of the RAT7/NUP159 nucleoporin, is required for efficient mRNA export. Mol Biol Cell 7, 1601–1621.
Breslow DK, Cameron DM, Collins SR, Schuldiner M, Stewart-Ornstein J, Newman HW, Braun S, Madhani HD, Krogan NJ, Weissman JS (2008). A comprehensive strategy enabling high-resolution functional analysis of the yeast genome. Nat Methods 5, 711–718.
Briggs MW, Burkard KT, Butler JS (1998). Rp6p, the yeast homologue of the human PM-Sc100-kDa autoantigen, is essential for efficient 5 S RNA 3' end formation. J Biol Chem 273, 13255–13263.
Brodsky AS, Silver PA (2000). Pre-mRNA processing factors are required for nuclear export. RNA 6, 1737–1749.
Kaliszewski P, Zoładek T (2008). The role of Rsp5 ubiquitin ligase in regulation of mRNA retention in nuclear subdomains. J Cell Biol 178, 13401–13405.

Hammell CM, Gross S, Zenklusen D, Heath CV, Stutz F, Moore C, Cole CN (2002). Coupling of termination, 3′ processing, and mRNA export. Mol Cell 22, 6441–6457.

Harris B, Bose T, Lee KK, Wang F, Lu S, Ross RT, Zhang Y, French SL, Beyer AL, Slaughter BD, et al. (2014). Cohesion promotes nuclearule structure and function. Mol Cell 55, 337–346.

Hilleren P, McCarthy T, Rosbash M, Parker R, Jensen TH (2001). Quality control of mRNA 3′-end processing is linked to the nuclear exosome. Nature 413, 538–542.

Hodge CA, Choudhary V, Wolnyj MJ, Scarcelli JJ, Schneiter R, Cole CN (2010). Integral membrane proteins Br6 and Apc12 link assembly of the nuclear pore complex to lipid homeostasis in the endoplasmic reticulum. J Cell Sci 123, 141–151.

Hodge CA, Tran EJ, Doye V, Alcazar-Roman AR, Ben-Shyash R, Scarcelli JJ, Folkman AW, Shaw-Tal Y, Wente SR, Cole CN (2011). The Dsbp5 cycle at the nuclear pore complex during mRNA export I: Dsbp5 mutants with defects in RNA binding and ATP hydrolysis define key steps for Nup159 and Gle1. Genes Dev 25, 1052–1064.

Holland AJ, Cleveland DW (2012). Losing balance: the origin and impact of RNA nuclear export mutants. J Cell Biol 196, 1179–1185.

Houalla R, Devaux F, Fatica A, Barras D, Torchet C, Tollervey D (2006). Microarray detection of novel nuclear RNA substrates for the exosome. Yeast 23, 439–454.

Houseley J, Tollervey D (2009). The many pathways of RNA degradation. Cell 136, 763–776.

Huh W-K, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK (2003). Global analysis of protein localization in budding yeast. Nature 425, 686–691.

Iglesias N, Tutucu E, Ghicov A, Ono D, Bahr S, Li J, Gasser SM, Saveanu C, Petfalski E, Thompson E, Jacquier A, Libri D, Philippen P, Pringle JR (1999). 5′-3′ exonucleases of the nuclear exosome. Mol Cell 17, 1497–1506.

Jacobs JS, Anderson AR, Parker RP (1998). The 3′ to 5′ degradation of yeast mRNAs is a general mechanism for mRNA turnover that requires the SK12 DEVH box protein and 3′ to 5′ exonucleases of the exosome complex. EMBO J 17, 2927–2937.

Kadejová T, Hitomi M, Chen S, Tarkhoff AM (1994b). Nuclear mRNA accumulation is required for nuclear retention of yeast mRNAs. Mol Biol Cell 5, 1252–1263.

Kadotani T, Zhao Y, Tarkhoff AM (1992). A conditional yeast mutant deficient in mRNA transport from nucleus to cytoplasm. Proc Natl Acad Sci USA 89, 2312–2316.

Kalachowska P, Zoladzek T (2008). The role of Rep5 ubiquitin ligase in regulation of diverse processes in yeast cells. Acta Biochim Pol 55, 649–662.

Kallehave TB, Robert M-C, Bertrand E, Jensen TH (2012). Nuclear retention prevents premature cytoplasmic appearance of mRNA. Mol Cell 48, 145–152.

Koepp DM, Wong DH, Corbett AH, Silver PA (1996). Dynamic localization of the nuclear import receptor and its interactions with transport factors. J Cell Biol 133, 1163–1176.

Kriau L, Fang F, Butler JS, Sherman F (2004). Polyadenylation of mRNA in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 101, 8581–8586.

LaCava J, Houseley J, Saveanu C, Petfalski E, Thompson E, Jacquier A, Tollervey D (2005). mRNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell 121, 713–724.

Li Z, Vizeacoumar FJ, Bahr S, Li J, Waringer J, Vizeacoumar FS, Min R, Vandersluis B, Bellay J, Devit M, et al. (2011). Systematic exploration of essential yeast gene function with temperature-sensitive mutants. Nat Biotechnol 29, 361–367.

Libri D, Dower K, Boulay J, Thomsen R, Rosbash M, Jensen TH (2002). Interactions between mRNA export commitment, 3′-end quality control, and nuclear degradation. Mol Cell Biol 22, 8254–8266.

Lin CH, MacGurn JA, Chu T, Stefan CJ, Emr SD (2008). Arrestin-related ubiquitin-ligase adaptors regulate endocytosis and protein turnover at the cell surface. Cell 135, 714–725.

Liu Q, Greimann JC, Lima CD (2006). Reconstitution, activities, and structure of the eukaryotic RNA exosome. Cell 127, 1223–1237.

Lone MA, Atkinson AE, Hodge CA, Cottier S, Martinez-Montañés F, Maitel S, Méne-Saffrané L, Cole CN, Schneiter R (2011). Yeast integral membrane proteins Apc12, Br1, and Brf6 form a complex important for regulation of nuclear membrane homeostasis and nuclear pore complex biogenesis. Eukaryot Cell 14, 1217–1227.

Longtime MS, McKenzie A 3rd, Demarini DJ, Shah NG, Wang A, Brachat A, Philpssen P, Pringle JR (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast 14, 953–961.

Lund MK, Guthrie C (2005). The DEAD-box protein Dsbp5 is required to dissociate Mex67p from exported mRNAs at the nuclear rim. Mol Cell 20, 645–651.

Lustig AJ, Lin RJ, Abelson J (1986). The yeast RNA gene products are essential for mRNA splicing in vitro. Cell 47, 953–963.

Machin F, Torres-Rosell J, Jarmuz A, Aragón L (2005). Spindle-independent condensation-mediated segregation of yeast ribosomal DNA in late anaphase. J Cell Biol 168, 209–219.

Malet H, Topf M, Claire DK, Ebert J, Bonneau F, Basquin J, Drazkowska K, Tomecki R, Dziembowski A, Conti E, et al. (2010). RNA channelling by the eukaryotic exosome. EMBO Rep 11, 936–942.

Minvielle-Sebastia L, Preker PJ, Wiederkehr T, Strahm Y, Keller W (1997). The major yeast poly(A)-binding protein is associated with cleavage factor IA and functions in premessenger RNA 3′-end formation. Proc Natl Acad Sci USA 94, 7887–7892.

Mitchell SF, Parker R (2014). Principles and properties of eukaryotic mRNAs. Mol Cells 34, 554–578.

Mitchell P, Petfalski E, Shevchenko A, Mann M, Tollervey D (1997). The exosome: a conserved eukaryotic RNA processing complex containing multiple 3′–5′ exonuclease subunits. Cell 91, 457–466.

Müller-McNicoll M, Neugebauer KM (2013). How cells get the message: dynamic assembly and function of mRNA-protein complexes. Nat Rev Genet 14, 275–287.

Murphy R, Wente SR (1996). An RNA-export mediator with an essential nuclear export signal. Nature 383, 357–360.

Neumann S, Petfalski E, Brügger B, Grosshans H, Wieland F, Tollervey D, Hurt E (2003). Formation and nuclear export of mRNA, RNA and mRNA is regulated by the ubiquitin ligase Rsp5p. EMBO Rep 4, 1156–1162.

Ng R, Abelson J (1980). Isolation and sequence of the gene for actin in the yeast Saccharomyces. Proc Natl Acad Sci USA 77, 1177–1179.

Palancade B, Zuccolo M, Loeillet S, Nicolas A, Doye V (2005). Pml39, a novel protein of the nuclear periphery required for nuclear retention of novel messenger RNA substrates. Mol Biol Cell 16, 5255–5268.

Pomura O, Libri D (2013). RNA quality control in the nucleus: the Angels’ share of RNA. Biochim Biophys Acta 1829, 604–611.

Pomura O, Libri D (2015). Transcriptional termination and the control of the transcriptome: why, where and how to stop. Nat Rev Mol Cell Biol 16, 190–202.
Preker PJ, Ohnacker M, Minvielle-Sebastia L, Keller W (1997). A multisubunit 3' end processing factor from yeast containing poly(A) polymerase and homologues of the subunits of mammalian cleavage and polyadenylation specificity factor. EMBO J 16, 4727–4737.

Rodriguez MS, Gwizdek C, Haguenauer-Tsapis R, Dargemont C (2003). The HECT ubiquitin ligase Rsp5p is required for proper nuclear export of mRNA in Saccharomyces cerevisiae. Traffic 4, 566–575.

Rohner S, Gasser SM, Meister P (2008). Modules for cloning-free chromatin tagging in Saccharomyces cerevisiae. Yeast 25, 235–239.

Rougemaille M, Dieppois G, Kisseleva-Romanova E, Gudipati RK, Lemoine S, Blugeon C, Boulay J, Jensen TH, Stutz F, Devaux F, et al. (2008). THO/Sub2p functions to coordinate 3’-end processing with gene-nuclear pore association. Cell 135, 308–321.

Rougemaille M, Gudipati RK, Olesen JR, Thomsen R, Seraphin B, Libri D, Jensen TH (2007). Dissecting mechanisms of nuclear mRNA surveillance in THO/sub2 complex mutants. EMBO J 26, 2317–2326.

Saguez C, Schmid M, Olesen JR, Ghazy MAE-H, Qu X, Poulsen MB, Nasser T, Moore C, Jensen TH (2008). Nuclear mRNA surveillance in THO/sub2 mutants is triggered by inefficient polyadenylation. Mol Cell 31, 91–103.

Saitoh Y-H, Ogawa K, Nishimoto T (2005). Brl1p—a novel nuclear envelope protein required for nuclear transport. Traffic 6, 502–517.

Santaguida S, Amon A (2015). Short- and long-term effects of chromosome mis-segregation and aneuploidy. Nat Rev Mol Cell Biol 16, 473–485.

Santos-Rosa H, Moreno H, Simos G, Ssegref A, Fahrenkrog B, Pante N, Hurt E (1998). Nuclear mRNA export requires complex formation between Mex67p and Mtr2p at the nuclear pores. Mol Cell Biol 18, 6826–6838.

Sarovfim M-A, Bensidoun P, Raymond P, Rahman S, Krause MR, Oeffinger M, Zenklusen D (2015). The nuclear basket mediates perinuclear mRNA scanning in budding yeast. J Cell Biol 211, 1131–1140.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Schmid B, et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676–682.

Schneider C, Kudla G, Wlotzka W, Tuck A, Tollervey D (2012). Transcriptome-wide analysis of exosome targets. Mol Cell 48, 422–433.

Schneider C, Tollervey D (2013). Threading the barrel of the RNA exosome. Trends Biochem Sci 38, 485–493.

Ssegref A, Sharma K, Doyle V, Hellwig A, Huber J, Luhmann R, Hurt E, Luhmann R (1997). Mex67p, a novel factor for nuclear export, binds to both poly(A)+ RNA and nuclear pores. EMBO J 16, 3256–3271.

Sheff MA, Thom KS (2004). Optimized cassettes for fluorescent protein tagging in Saccharomyces cerevisiae. Yeast 21, 661–670.

Sheltzer JM, Torres EM, Dunham MJ, Ammon A (2012). Transcriptional consequences of aneuploidy. Proc Natl Acad Sci USA 109, 12644–12649.

Shiokawa K, Pogo AO (1974). The role of cytoplasmic membranes in condensation of rDNA and homologues of the subunits of mammalian cleavage and polyadenylation specificity factor. EMBO J 16, 4727–4737.

Singh G, Pratt G, Yeo GW, Moore MJ (2015). The clothes make the mRNA: past and present trends in mRNA fashion. Annu Rev Biochem 84, 325–354.

Smith C, Lari A, Derrer CP, Ouwehand A, Rossouw A, Huisman M, Dange T, Hopman M, Joseph A, Zenklusen D, et al. (2015). In vivo single-particle imaging of nuclear mRNA export in budding yeast demonstrates an essential role for Mex67p. J Cell Biol 211, 1121–1130.

Snay-Hodge CA, Colot HV, Colden LA, Corden JL (2001). RNA-binding protein Nrd1 directs poly(A)-independent 3’-end formation of RNA polymerase II transcripts. Nature 413, 327–331.

Straight AF, Belmont AS, Robinett CC, Murray AW (1996). GFP-tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. Curr Biol 6, 1599–1608.

Sträßer K, Baller J, Hurt E (2000). Binding of the Mex67p/Mtr2p heterodimer to FXFG, GLFG, and FG repeat nucleoparin is essential for nuclear mRNA export. J Cell Biol 150, 695–706.

Sullivan M, Higuchi T, Katas VL, Uhmann F (2004). Cdc14 phosphatase induces RNA degradation and resolves cohesion-independent cohesion during budding yeast anaphase. Cell 117, 471–482.

Tachibana T, Imamoto N, Seino H, Nishimoto T, Yoneda Y (1994). Loss of RCC1 leads to suppression of nuclear protein import in living cells. J Cell Biol 269, 24542–24545.

Thomson R (2003). Localization of nuclear retained RNAs in Saccharomyces cerevisiae. RNA 9, 1049–1057.

Thomsen R, Saguez C, Nasser T, Jensen TH (2008). General, rapid, and transcription-dependent fragmentation of nucleolar antigens in S. cerevisiae mRNA export mutants. RNA 14, 706–716.

Torres EM, Sokolsky T, Tucker CM, Chan LY, Boselli M, Dunham MJ, Ammon A (2007). Effects of aneuploidy on cellular physiology and cell division in haploid yeast. Science 317, 916–924.

Tseng SS, Weaver PL, Liu Y, Hitomi M, Tartakoff AM, Chang TH (1998). Dsb5p, a cytosolic RNA helicase, is required for poly(A)+ RNA export. EMBO J 17, 2651–2662.

Tuck AC, Tollervey D (2013). A transcriptome-wide atlas of RNP composition reveals diverse classes of mRNAs and IncRNAs. Cell 154, 996–1009.

Ursic D, Chinchilla K, Finkel JS, Cubertson MR (2004). Multiple protein–protein and protein–RNA interactions suggest role for yeast DNA/RNA helicase Sen1p in transcription, transcription-coupled DNA repair and RNA processing. Nucleic Acids Res 32, 2441–2452.

Vanacova S, Wolf J, Martin G, Blank D, Dettwiler S, Friedlein A, Langen H, Keith G, Keller W (2005). A new yeast poly(A) polymerase complex involved in RNA quality control. PLoS Biol 3, e189.

van Hoof A, Lennertz P, Parker R (2000). Yeast exosome mutants accumulate 3’-extended polyadenylated forms of U4 small nuclear RNA and small nuclear RNAs. Mol Cell Biol 20, 441–452.

Vasilevskia L, Buratowski S (2006). Nrd1 interacts with the nuclear exosome for 3’ processing of RNA polymerase II transcripts. Mol Cell 21, 239–248.

Wasmuth VE, Lima CD (2012). Exo- and endonucleolytic activities of yeast cytoplasmic and nuclear RNA exosomes are dependent on the noncatalytic core and central channel. Mol Cell 48, 133–144.

Wente SR, Blobel G (1994). NLP145 encodes a novel yeast glycin-leucine-phenylalanine-glycine (GLFG) nucleoparin required for nuclear envelope structure. J Cell Biol 125, 955–969.

Wente SR, Rout MP (2010). The nuclear pore complex and nuclear transport. Cold Spring Harb Perspect Biol 2, a000562.

Wooldford JL, Baserga SJ (2013). Ribosome biogenesis in the yeast Saccharomyces cerevisiae. Genetics 195, 643–681.

Wu J, Bao A, Chatterjee K, Wan Y, Hopper AK (2015). Genome-wide screen uncovers novel pathways for RNA processing and nuclear-cytoplasmic dynamics. Genes Dev 29, 2633–2644.

Wyers F, Rougemaille M, Badis G, Rousseau J-C, Dufour M-E, Boulay J, Régnault B, Devaux F, Namane A, Seraphin B, et al. (2005). Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new pol(y) polymerase. Cell 121, 725–737.

Zenklusen D, Vinciguerra P, Wys W, Stutz F (2002). Stable mRNP formation and export require cotranscriptional recruitment of the mRNA export factors Yra1p and Sub2p by Hpr1p. Mol Cell Biol 22, 8241–8253.

Zhao J, Kessler M, Helmling S, O’Connor JP, Moore C (1999). Pta1, a component of yeast CF II, is required for both cleavage and poly(A) addition of mRNA precursor. Mol Cell Biol 19, 7733–7740.