The DECD box putative ATPase Sub2p is an early mRNA export factor
Torben Heick Jensen*,†, Jocelyne Boulay†, Michael Rosbash* and Domenico Libri†

Nuclear mRNA metabolism relies on the interplay between transcription, processing, and nuclear export. RNA polymerase II transcripts experience major rearrangements within the nucleus, which include alterations in the structure of the mRNA precursors as well as the addition and perhaps even removal of proteins prior to transport across the nuclear membrane. Such mRNP-remodeling steps are thought to require the activity of RNA helicases/ATPases. One such protein, the DECD box RNA-dependent ATPase Sub2p/UAP56, is involved in both early and late steps of spliceosome assembly [1–4]. Here, we report a more general function of Saccharomyces cerevisiae Sub2p in mRNA nuclear export. We observe a rapid and dramatic nuclear accumulation of poly(A)+ RNA in strains carrying mutant alleles of sub2. Strikingly, an intronless transcript, HSP104, also accumulates in nuclei, suggesting that Sub2p function is not restricted to splicing events. The HSP104 transcripts are localized in a single nuclear focus that is suggested to be at or near their site of transcription. Intriguingly, Sub2p shows strong genetic and functional interactions with the RNA polymerase II-associated DNA/DNA:RNA helicase Rad3p as well as the nuclear RNA exosome component Rrp6p, which was independently implicated in the retention of RNAs at transcription sites [5]. Taken together, our data suggest that Sub2p functions at an early step in the mRNA export process.

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Results and discussion
In addition to the multiple roles it plays in spliceosome assembly, we initially observed that a debilitated sub2 gene has effects on polyadenylation (data not shown). Because of the importance of proper polyadenylation to yeast mRNA export [5–7], we examined this process in sub2 temperature-sensitive (ts) mutants by fluorescent in situ hybridization (FISH) analysis. The sub2-201 mutant [3] was shifted to the nonpermissive temperature and was assayed for poly(A)+ subcellular distribution with FISH using an oligo(dT) probe (Figure 1a). Within 10 min of the shift, the more general total cell signal diminished and was replaced by a nuclear signal, which was even stronger at 30 min. The granular intranuclear signal is reminiscent of that obtained when mRNA release from transcription site foci is inhibited [7]. Because of the relationship between Sub2p and splicing, we considered that this block might be restricted to intron-containing RNAs. Therefore, we specifically assayed the intronless RNA, HSP104, by FISH. The temperature shift gave rise to a single nuclear dot, identical to that previously observed with a number of temperature-sensitive mutant strains that inhibit mRNA export (Figure 1b and [5, 7]). Similar results were obtained in another sub2 ts mutant, sub2-206 [3], and for another intronless heat shock transcript, SSA4 (data not shown).

Interestingly, there are much less HSP104 and SSA4 RNAs after a shift to 37°C in the sub2-201 strain compared to the wild-type (Figure 1c), whereas levels of control U4 and U6 RNAs remain constant (data not shown). This is perhaps because stalled transcripts are less stable or is alternatively due to a decrease in the transcription rate at the affected loci in the sub2-201 strain. Remaining HSP104 and SSA4 transcript levels stay fairly constant after transcriptional induction in the sub2-201 mutant compared to a wild-type strain (Figure 1e). This is consistent with the suggestion that these RNAs are sequestered in nuclei and are unable to proceed in their metabolism.

Recently, we have identified an unexpected role for Rrp6 and the nuclear exosome in retention of aberrant transcripts within transcription site foci [5]. Consisting largely of exonucleasey enzymes, this complex might participate in both the recognition and ultimately the degradation of incorrectly made transcripts. To investigate whether the sub2 mutant-induced block has the same requirements, we constructed sub2-201Δrrp6 and sub2-206Δrrp6 double-mutant strains. Deletion of RRP6 significantly altered the RNA localization pattern observed by FISH; i.e., the
poly(A)$^+$ signal was more dispersed throughout the nucleus than in the sub2-201 single mutant, and the HSP104 dot completely disappeared (Figure 2, compare Figure 2a to Figure 1a,b). Northern analysis confirmed that the disappearance of the nuclear HSP104 dot was not due to the absence of transcribed RNA in this strain (data not shown). Furthermore, the sub2-206/Arp6 strain showed an identical staining pattern (data not shown). These results are similar to what was observed previously and suggest that the exosome is necessary for the mRNA retention phenotype observed in the sub2 ts mutants. A functional relationship between Sub2p and Rrp6p is further strengthened by the strong genetic interaction between the two genes; the double-mutant strain grows much more poorly at all tested temperatures than either single mutant alone (Figure 2b).

To further explore the genetic space in which Sub2p acts, we carried out a loss-of-function suppression screen with a sub2 null strain. Growth of this strain is extremely poor in the genetic environment of our W303 strain [3]. After UV mutagenesis, two mutant recessive alleles of the DNA and DNA:RNA helicase RAD3 gene, rad3-6.4 and rad3-7.7, were independently isolated. Although required for excision repair of damaged DNA, Rad3p is a component of transcription factor II H (TFIIH) and is essential for transcription by RNA polymerase II [8, 9]. To test whether the observed growth suppression could be related to the mRNA export defect of sub2-201, we performed HSP104-specific and poly(A)$^+$ RNA-FISH analysis on the two sub2-201/rad3-7.7 and sub2-201/rad3-6.4 double-mutant strains. Strikingly, the HSP104 nuclear RNA signal was no longer present in the sub2-201/rad3-7.7 double mutant (compare Figure 1b, lower row, and Figure 3a, middle row). This disappearance is not due to reduced HSP104 RNA levels in the rad3-7.7 background, as HSP104 levels are comparable in sub2-201 and sub2-201/rad3-7.7 strains (data not shown). The HSP104 nuclear dot could be fully restored by the introduction of a plasmid expressing wild-type Rad3p (Figure 3a, lower row). In addition, the strongly granular poly(A)$^+$ signal observed in sub2-201 was more dispersed when the rad3-7.7 mutation was added to the sub2-201 strain (compare Figure 1a, lower row, and Figure 3b, middle row). Identical results were obtained when the rad3-6.4 mutation was used instead of rad3-7.7 (data not shown). We conclude that the rad3-7.7 or rad3-6.4 mutations lead to the release of RNAs from transcription site foci. Although we do not yet know the molecular mechanism that underlies this effect, we speculate that a less potent transcriptional induction in the mutant rad3 backgrounds either circumvents the need for Sub2p activity or allows the residual activity of the Sub2p protein to function in a wild-type-like manner.

In this paper, we report a function for the RNA-dependent ATPase Sub2p in general mRNA export. A similar conclu-
Sub2p and the nuclear exosomal component Rrp6p interacts functionally and genetically. (a) Poly(A)$^+$ and HSP104 RNA-FISH analysis on sub2-201/Δrrp6 that was temperature shifted from 25°C to 38°C for 30 min. (b) Growth of Δrrp6, Δrrp6/sub2-201, Δrrp6/sub2-201 + pFL36-SUB2, and sub2-201 at 24°C, 28°C, 34°C, and 37°C, as indicated.

Figure 3

Mutation of the RAD3 gene relieves sub2-201-mediated RNA transcription site foci retention. (a,b) RNA-FISH analysis on Sub2-WT/rad3-7.7, sub2-201/rad3-7.7, and sub2-201/rad3-7.7/pRAD-WT cultures temperature shifted to 38°C for 30 min. (a) HSP104 RNA, (b) Poly(A)$^+$ RNA, and DNA was visualized as described in the legend to Figure 1.
Table 1

Yeast strains used in this study.

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<th>Name</th>
<th>Features</th>
<th>Genotype</th>
<th>Reference</th>
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<td>[3]</td>
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<td>DLY124/sub2-206</td>
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<td>this study</td>
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been functionally linked to active transcription [12], and it was recently shown that Yra1p is in close proximity to active sites of transcription [15]. It is therefore possible that Sub2p functions in conjunction with Yra1p to effect the release of mRNA from transcription site foci.

There is a reported two-hybrid interaction between Sub2p and the abundant nuclear RNP protein Npl3p [14]. Like Yra1p, Npl3p is also found linked to transcriptionally active sites [13], and intriguingly, Npl3p also interacts with the nuclear exosome component Rrp6p [15]. This suggests a simple model in which Sub2p interacts with Npl3p and replaces Rrp6p, thereby releasing the RNA from the putative exosome tether within transcription site foci [5]. Biochemical assays will be required to test these more mechanistic speculations.

Materials and methods

Yeast methods

Strains used in this paper (listed in Table 1) are derived from W303-1A. Double mutants sub2-201/Δrrp6 and sub2-206/Δrrp6 were generated by replacing the endogenous RRP6 gene with the Kluyveromyces lactis URA3 gene by the PCR-based gene replacement method [16].

To identify sub2 null mutant suppressor alleles, UV-mutagenized sub2Δ ade8Δ cells were screened on 5-FOA plates for the ability to loose a URA3-marked plasmid bearing wild-type copies of SUB2 and ADE8. Clones fulfilling the two selection criteria of 5-FOA resistance and white color were screened for thermosensitivity after reintroduction of the SUB2 gene. The mutated gene was isolated based on the ability to restore both the thermostolerance and the SUB2 dependence. The cloned rad3-6.4 and rad3-7.7 alleles failed to rescue either genetic defect.

Plasmids

The RAD3 and SUB2 wild-type genes were expressed from their own promoters on a centromeric LEU2-marked plasmid (pFL36).

In situ hybridization analysis

Fluorescent in situ hybridization analysis of HSP104, SSA4, and Poly(A)+ RNA was done as previously described [7].

RNA analysis

RNaseH treatment of HSP104 and SSA4 RNAs was performed with oligonucleotides complementary to positions 2254–2273 and 1825–1844 downstream of the ATG start codons of the HSP104 and SSA4 genes, respectively. Subsequent Northern blot analysis was done essentially as described previously [5].

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