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 mRNAs at transcription sites [5]. Taken together, our data suggest that Sub2p functions at an early step in the mRNA export process.

Addresses: *Howard Hughes Medical Institute, Department of Biology, Brandeis University, Waltham, Massachusetts, 02454. †Centre National de la Recherche Scientifique, Centre de Genetique Moleculaire, 91190, Gif sur Yvette, Paris, France.

Present address: ‡Department of Molecular and Structural Biology, Aarhus University, C.F. Mollers Alle, Building 130, 8000 Aarhus C., Denmark.

Correspondence: Torben Heick Jensen, Domenico Libri
E-mail: thj@mbio.aau.dk
libri@cgm.cnrs-gif.fr

Received: **4 September 2001**
Revised: **24 September 2001**
Accepted: **25 September 2001**

Published: **30 October 2001**

Current Biology 2001, 11:1711–1715

0960-9822/01/$ – see front matter

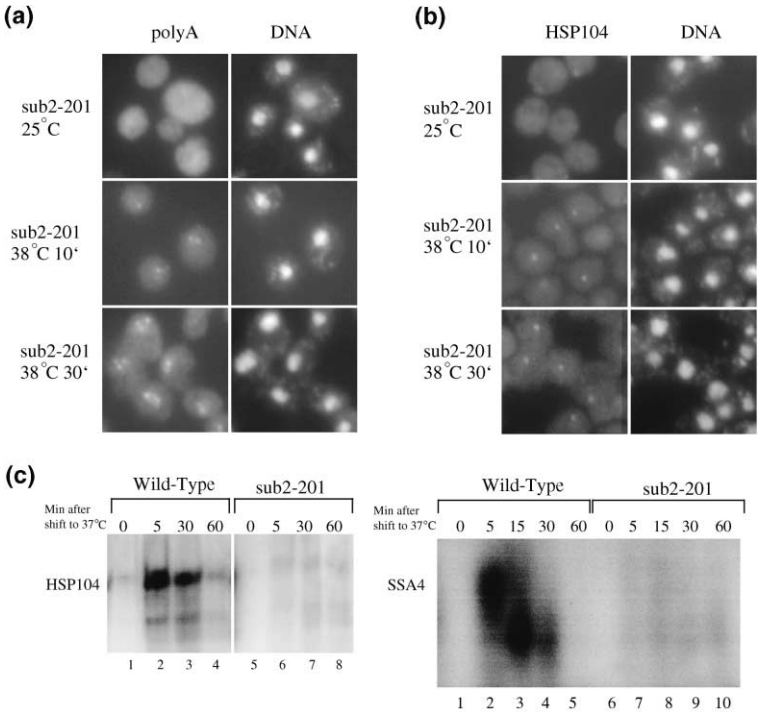
Results and discussion

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 1c). 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 exonucleolytic 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 *sub2-201*/Δ*rrp6* and *sub2-206*/Δ*rrp6* double-mutant strains. Deletion of *RRP6* significantly altered the RNA localization pattern observed by FISH; i.e., the

Figure 1



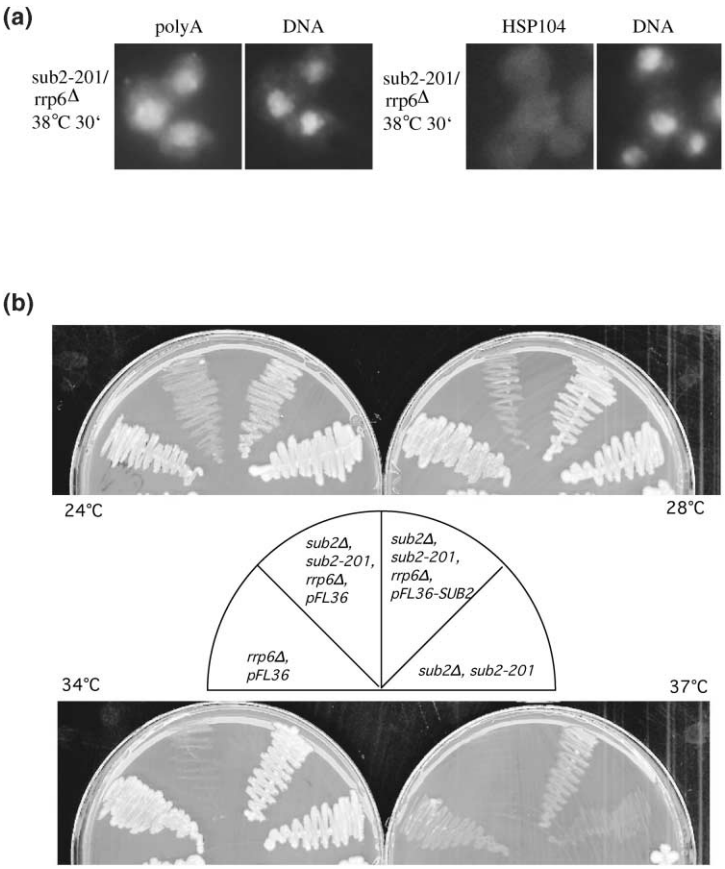
Splicing-independent nuclear accumulation of mRNA in *sub2-201*. **(a,b)** RNA-FISH analysis on *sub2-201* cultures grown at 25°C or temperature shifted to 38°C for 10 min or 30 min as indicated. (a) poly(A)$^+$ RNA was visualized with a CY3-labeled oligo(dT)$_{70}$ probe, and (b) *HSP104* RNA was visualized with specific probes as described [7]. DNA was stained with DAPI. **(c)** Northern analysis of *HSP104* RNA and *SSA4* RNA isolated from wild-type or *sub2-201* cultures harvested at the indicated time points after a temperature shift from 25°C to 37°C. RNA samples were treated with complementary DNA oligos and RNaseH prior to gel electrophoresis.

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*/Δ*rrp6* 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 Sub2-201p 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-

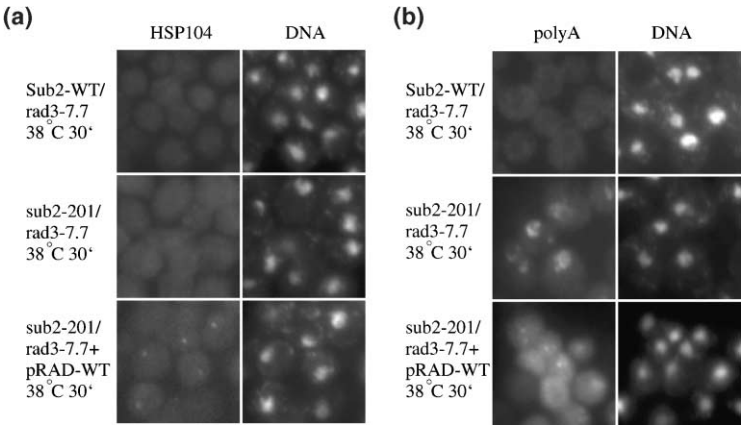
Figure 2



Sub2p and the nuclear exosomal component Rrp6p interacts functionally and genetically. **(a)** Poly(A)$^+$ and *HSP104* RNA-FISH analysis on *sub2-201*/Δ*rrp6* that was temperature shifted from 25°C to 38°C for 30 min. **(b)** Growth of Δ*rrp6*, Δ*rrp6*/*sub2-201*, Δ*rrp6*/*sub2-201* + pFL36-*SUB2*, and *sub2-201* at 24°C, 28°C, 34°C, and 37°C, as indicated.

sion has been reached from results by Ed Hurt's laboratory in yeast and Robin Reed's and Elisa Izaurralde's laboratories in metazoan systems ([10, 11]; E. Izaurralde, personal communication). The strong genetic and functional interactions with Rad3p imply that Sub2p might exercise its role during the transcription of nascent RNAs. Interestingly, Sub2p forms a strong complex with the mRNA export factor Yra1p/Aly/REF [11]. This RNP protein has

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.

Name	Features	Genotype	Reference
DLY23	*SUB2 wt*	as W303, *sub2::HIS,* pCM188-*SUB2(URA3)*	[3]
DLY33/*sub2-201*	*sub2-201*	as W303, *sub2::HIS,* pCM185p-*sub2-201(TRP1)*	[3]
DLY33/*sub2-206*	*sub2-206*	as W303, *sub2::HIS,* pCM185-*sub2-206(TRP1)*	[3]
DLY124/*sub2-201*	*sub2-201/rrp6*Δ	as W303, *sub2:HIS,rrp6::KlURA3,* pCM185-*sub2-201(TRP1)*	this study
DLY124/*sub2-206*	*sub2-206/rrp6*Δ	as W303, *sub2::HIS,rrp6::KlURA3,* pCM185-*sub2-206(TRP1)*	this study
DLY82	suppressor screen strain	as W303, *sub2::KAN,ade8::HIS,* pCM188-*SUB2(URA3, ADE8)*	this study
DLY99	*rad3-6.4*	as W303, *sub2::KAN,ade8::HIS, rad3-6.4,* pCM188-*SUB2(URA3, ADE8)*	this study
DLY100	*rad3-7.7*	as W303, *sub2::KAN,ade8::HIS, rad3-7.7,* pCM188-*SUB2(URA3, ADE8)*	this study
DLY99/*sub2-201*	*rad3-6.4/sub2-201*	as W303, *sub2::KAN,ade8::HIS, rad3-6.4,* pCM185-*sub2-201 (TRP1)*	this study
DLY99/*sub2-206*	*rad3-7.7/sub2-201*	as W303, *sub2::KAN,ade8::HIS, rad3-7.7,* pCM185-*sub2-206 (TRP1)*	this study

been functionally linked to active transcription [12], and it was recently shown that Yra1p is in close proximity to active sites of transcription [13]. 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 Rrp6 [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*/Δ*rrp6* and *sub2-206*/Δ*rrp6* 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 *sub2*Δ, *ade8*Δ 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 thermoresistence 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].

Acknowledgements

We are grateful to T. McCarthy for excellent technical assistance. We thank A. Petitjean, A. V. Gendrel, and A. Rideau for help with genetic screening and analysis. F. Lacroute and M. Minet are thanked for gifts of plasmids, and J. Marie is thanked for her constant encouragement. Thanks to Ed Hurt, Robin Reed, and Elisa Izaurralde for communicating unpublished data. This work was supported by the Centre National de la Recherche Scientifique, the Danish Research Council, and the National Institutes of Health (GM 23549 to M.R.).

References

1. Fleckner J, Zhang M, Valcarcel J, Green MR: **U2AF65 recruits a novel human DEAD box protein required for the U2 snRNP-branchpoint interaction.** *Genes Dev* 1997, **11:**1864-1872.
2. Kistler AL, Guthrie C: **Deletion of *MUD2*, the yeast homolog of U2AF65, can bypass the requirement for Sub2, an essential spliceosomal ATPase.** *Genes Dev* 2001, **15:**42-49.
3. Libri D, Graziani N, Saguez C, Boulay J: **Multiple roles for the yeast *SUB2*/yUAP56 gene in splicing.** *Genes Dev* 2001, **15:**36-41.
4. Zhang M, Green MR: **Identification and characterization of yUAP/Sub2p, a yeast homolog of the essential human pre-mRNA splicing factor hUAP56.** *Genes Dev* 2001, **15:**30-35.
5. Hilleren P, McCarthy T, Rosbash M, Parker R, Jensen TH: **Quality control of mRNA 3′-end processing is linked to the nuclear exosome.** *Nature*, 2001, **413:**538-542.
6. Brodsky AS, Silver PA: **Pre-mRNA processing factors are required for nuclear export.** *RNA* 2000, **6:**1737-1749.
7. Jensen TH, Patricio K, McCarthy T, Rosbash M: **A block to nuclear export in *Saccharomyces cerevisiae* leads to hyperadenylation of transcripts that accumulate at the site of transcription.** *Mol Cell* 2001, **7:**887-898.
8. Feaver WJ, Svejstrup JQ, Bardwell L, Bardwell AJ, Buratowski S, Gulyas KD, *et al.*: **Dual roles of a multiprotein complex from *S. cerevisiae* in transcription and DNA repair.** *Cell* 1993, **75:**1379-1387.
9. Guzder SN, Qiu H, Sommers CH, Sung P, Prakash L, Prakash S: **DNA repair gene RAD3 of *S. cerevisiae* is essential for transcription by RNA polymerase II.** *Nature* 1994, **367:**91-94.
10. Luo M-J, Zhou Z, Magni K, Christoforides C, Rappsilber J, Mann M, *et al.*: **Pre-mRNA splicing and mRNA export linked by direct interactions between UAP56 and Aly.** *Nature* 2001, **413:**644-647.
11. Sträßer K, Hurt E: **Splicing factor Sub2 is required for nuclear mRNA export through its interaction with Yra1p.** *Nature* 2001, **413:**648-652.
12. Bruhn L, Munnerlyn A, Grosschedl R: **Aly, a context-dependent coactivator of LEF-1 and AML-1, is required for TCR alpha enhancer function.** *Genes Dev* 1997, **11:**640-653.
13. Lei EP, Krebber H, Silver PA: **Messenger RNAs are recruited for**

nuclear export during transcription. *Genes Dev* 2001, **15:**1771-1782.
14. Inoue K, Mizuno T, Wada K, Hagiwara M: **Novel RING finger proteins Air1p and Air2p, interact with Hmt1p and inhibit the arginine methylation of Npl3p.** *J Biol Chem* 2000, **275:**32793-32799.
15. Burkard KT, Butler JS: **A nuclear 3′-5′ exonuclease involved in mRNA degradation interacts with Poly(A) polymerase and the hnRNA protein Npl3p.** *Mol Cell Biol* 2000, **20:**604-616.
16. Wach A, Brachat A, Pohlmann R, Philippsen P: **New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*.** *Yeast* 1994, **10:**1793-1808.