Nucleocytoplasmic shuttling of the La motif-containing protein Sro9 might link its nuclear and cytoplasmic functions

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ABSTRACT

Diverse steps in gene expression are tightly coupled. Curiously, the La-motif-containing protein Sro9 has been shown to play a role in transcription and translation. Here, we show that Sro9 interacts with nuclear and cytoplasmic protein complexes involved in gene expression. In addition, Sro9 shuttles between nucleus and cytoplasm and is exported from the nucleus in an mRNA export-dependent manner. Importantly, Sro9 is recruited to transcribed genes. However, whole genome expression analysis shows that loss of Sro9 function does not greatly change the level of specific transcripts indicating that Sro9 does not markedly affect their synthesis and/or stability. Taken together, Sro9 might bind to the mRNP already during transcription and accompany the mature mRNP to the cytoplasm where it modulates translation of the mRNA.

Keywords: Sro9; transcription; translation; nucleocytoplasmic shuttling

INTRODUCTION

La-motif-containing proteins constitute a highly conserved family of RNA binding proteins involved in various RNA-related processes. Originally identified in humans as an autoimmune antigen of patients with rheumatic diseases such as systemic lupus erythematosus and Sjogren's syndrome (Mattioli and Reichlin 1974; Alspaugh and Tan 1975), La proteins are highly conserved throughout evolution (for review, see Wolin and Cedervall 2002). Authentic La proteins are nuclear proteins (Hendrick et al. 1981; Yoo and Wolin 1994) that protect the 3′ end of newly synthesized RNA polymerase III transcripts. This binding event not only protects the small RNAs from exonucleases, but also determines the correct subsequent processing of these RNAs (for review, see Wolin and Cedervall 2002). In addition, La proteins are important for the stabilization of newly synthesized U6 RNAs (Pannone et al. 1998) and histone mRNAs (McLaren et al. 1997). Surprisingly for a nuclear protein, La proteins might also enhance the translation of viral and cellular mRNAs (Meerovitch et al. 1993; Svitkin et al. 1994; Holcik and Korneluk 2000; see also Discussion).

More recently, however, sequence analysis revealed a second class of La motif-containing proteins to be present in all sequenced eukaryotic genomes (Sobel and Wolin 1999). These proteins contain a phylogenetically different La motif that is positioned N-terminally, centrally, or C-terminally—in contrast to the predominantly N-terminal localization of the La motif in authentic La proteins—and display no sequence homology except in the La motif itself. The *Saccharomyces cerevisiae* protein Sro9 belongs to this new class of La motif-containing proteins. Sro9 is a cytoplasmic protein that was originally identified in genetic screens as a suppressor of mutants of the secretory pathway (Tsukada and Gallwitz 1996), a mutant affecting bud formation (Imai et al. 1996), and mutants in actin or tropomyosin (Kagami et al. 1997). Subsequently, Sobel and Wolin (1999) reported that Sro9 binds to RNA and associates with translating ribosomes. As deletion of Sro9 reduces sensitivity toward translation inhibitors, Sro9 might act as a molecular chaperone stabilizing mRNAs in the correct translational conformation or might influence mRNP

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rearrangements for efficient translation of the mRNA. In addition, Sro9 was shown to function in transcription by RNA polymerase II (Tan et al. 2000). High copy Sro9 suppresses transcription defects caused by deletion of Rpb4, a nonessential subunit of RNA polymerase II. Furthermore, addition of recombinant Sro9 in in vitro transcription reactions restores the transcription defects, pointing to a direct role of Sro9 in transcription. In addition, overexpression of Sro9 increases total mRNA levels in *Δrpb4* cells indicating a role for Sro9 in mRNA stability. Thus, Sro9, which localizes to the cytoplasm at steady state (Kagami et al. 1997; Sobel and Wolin 1999), functions in processes as diverse as transcription, translation, and mRNA stability.

Gene expression encompasses multiple steps that are highly interconnected. During transcription the genetic information stored in the protein coding genes is transcribed into mRNA. The mRNA is processed (capped, spliced, and polyadenylated), packaged into a mature mRNP, transported through the nuclear pore complex to the cytoplasm, and finally translated into the encoded protein by the ribosomes (for review, see Erkmann and Kutay 2004; Fasken and Corbett 2005; Olesen et al. 2005; Kohler and Hurt 2007; Iglesias and Stutz 2008; Röther and Sträßer 2009). Here, we show that Sro9 might function in gene expression processes as distant as transcription and translation by shuttling between nucleus and cytoplasm. Consistent with this model, Sro9 associates with protein complexes involved in nuclear and cytoplasmic steps of gene expression. Importantly, Sro9 is recruited to actively transcribed genes. However, Sro9 is most likely not needed for the synthesis or stability of specific mRNAs as revealed by genome-wide expression analysis. According to our model, Sro9 is cotranscriptionally recruited to the nascent transcript and shuttles to the cytoplasm as a component of the exported mRNP, where it is important for modulation of translation of the bound mRNA.

RESULTS AND DISCUSSION

Sro9 associates with multiple protein complexes of the gene expression pathway

As Sro9 was reported to be involved in nuclear transcription as well as cytoplasmic translation, we chose representative protein complexes along the gene expression pathway (Table 1) to assess at which stages Sro9 is associated. We purified RNA polymerase II (Rpb1), the transcription elongation factor CTDK-I (Ctk1), the TREX complex that couples transcription to mRNA export (Hpr1), the mRNP-bound protein Npl3, the mRNA export receptor Mex67-Mtr2 (Mex67), and ribosomes (Rps4a) by tandem affinity purification (TAP) using the TAP-tagged subunit indicated in brackets and we tested a putative association of Sro9 by Western blotting (Fig. 1). The CTDK-I complex phosphorylates the C-terminal domain of RNA polymerase II during transcription elongation (for review, see Prelich 2002; Svejstrup 2004).

TABLE 1. TAP-tagged proteins, purified protein complexes, and their function in gene expression

Tagged protein	Complex	Function
Rpb1	RNA polymerase II	Transcription
Ctk1	CTDK-I	Transcription, translation
Hpr1	TREX	Transcription and mRNA export
Npl3		mRNA export, translation
Mex67	Mex67-Mtr2	mRNA export receptor
Rps4a	Ribosome	Translation

In addition, this protein complex has a second function in gene expression by increasing the accuracy of amino acid incorporation during translation elongation (Röther and Sträßer 2007). TREX is a highly conserved complex that is recruited to the nascent transcript during transcription elongation and interacts downstream with the mRNA export receptor Mex67-Mtr2, thereby coupling transcription to nuclear export of the mRNA (Sträßer et al. 2002; Reed and Cheng 2005). Npl3 is a serine-arginine (SR)-rich protein that binds cotranscriptionally to the mRNP, is exported with the mature mRNP, and needs to be dissociated from the mRNA for efficient translation to occur (Gilbert et al. 2001; Gilbert and Guthrie 2004; Windgassen et al. 2004). The heterodimeric mRNA export receptor Mex67-Mtr2 is recruited to the mRNA by the TREX complex and exports the mRNA to the cytoplasm by direct interaction with nuclear pores (Segref et al. 1997; Sträßer and Hurt 2000; Sträßer et al. 2000). In the cytoplasm, ribosomes finally translate the mRNA into the encoded protein (for review, see Preiss and Hentze 2003).

Sro9 copurified with CTDK-I, TREX, Npl3, Mex67-Mtr2, and ribosomes (Fig. 1A, lanes 2–6, αSro9). The observed interaction of Sro9 with ribosomes is consistent with the comigration of Sro9 with ribosomal proteins (subunits, mono-, and polysomes) in sucrose density gradients (Sobel and Wolin 1999; S Röther and K Sträßer, unpubl.). Importantly, though, copurification of Sro9 with those protein complexes is independent of the level of ribosomal proteins present in each purification (Fig. 1A, lanes 2–5, cf. αRpl6 and αSro9) showing that Sro9 is not a ribosome-associated contaminant. In contrast, copurification of Sro9 with RNA polymerase II, i.e., the transcription machinery, varied between experiments (Fig. 1A, lane 1, αSro9). This might indicate that Sro9 interacts only transiently with RNA polymerase II and might be quickly dissociated from the transcription machinery in vivo.

Since La proteins are known to bind RNA we tested whether the association of Sro9 with the above protein complexes is mediated by mRNA. Degradation of mRNA by RNase A treatment was tested by assessing the presence of the cap binding protein component Cpb80, which binds to TREX in an RNA-dependent manner (Fig. 1B, lanes 1,4, αCpb80). In contrast, RNase A treatment did not disrupt

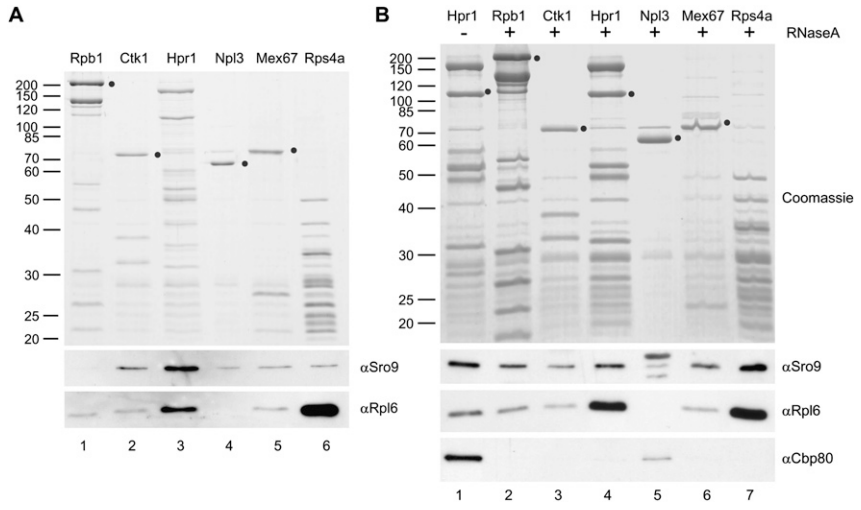

FIGURE 1. Sro9 interacts with protein complexes involved in gene expression. (*A*) RNA polymerase II (Rpb1), catalyzing mRNA synthesis, CTDK-I (Ctk1), involved in transcription and translation elongation, TREX (Hpr1), a cotranscriptionally recruited complex coupling transcription and mRNA export, the SR-protein Npl3, which is also involved in translation, the mRNA export receptor Mex67-Mtr2 (Mex67), and ribosomes (Rps4a) were purified by tandem affinity purification. Copurification of Sro9 and the ribosomal protein Rpl6 was assessed by Western blotting. Sro9 does not consistently copurify with RNA polymerase II but with protein complexes downstream in the gene expression process—independently of ribosomes. Circles indicate the TAP-tagged proteins. (*B*) Same as in *A*, but lysates were treated with RNase A prior to protein complex purification to assess RNA dependence of the interactions observed in *A*. To assess the efficiency of RNase treatment the presence of Cbp80, which binds in an RNA-dependent manner to TREX, in the eluted complexes was analyzed.

copurification of Sro9 (Fig. 1B, αSro9). Taken together, Sro9 binds to protein complexes involved in different steps of gene expression independently of RNA.

Sro9 shuttles between nucleus and cytoplasm

Consistent with a function of Sro9 in transcription (Tan et al. 2000) we found that Sro9 interacts with protein complexes mainly localized to the nucleus, although Sro9 localizes to the cytoplasm at steady state. A function of Sro9 in the nucleus would thus require Sro9 to shuttle between nucleus and cytoplasm. Due to the association of Sro9 with protein complexes of the mRNA export machinery, we analyzed the localization of Sro9 after inhibition of mRNA export. In *mex67-5* cells, a temperature-sensitive mutant of the general mRNA export receptor Mex67-Mtr2, mRNA export is blocked at the nonpermissive temperature (37°C) und poly(A)$^+$ mRNA accumulates in the nucleus (Segref et al. 1997). In wild-type (WT) and mutant (*mex67-5*) cells, Sro9 was exclusively localized to the cytoplasm at the permissive temperature (30°C) (Fig. 2A, left panel). In contrast, Sro9 accumulates in the nucleus of *mex67-5* cells at the nonpermissive temperature (37°C), i.e., when mRNA export is blocked (Fig. 2A, right panel). Thus, Sro9 shuttles between nucleus and cytoplasm and is exported from the nucleus in an mRNA export-dependent manner.

Based on the interaction of Sro9 with nuclear protein complexes (Fig. 1) and its nucleocytoplasmic shuttling, we asked whether these protein–protein interactions are enhanced when mRNA export is blocked and Sro9 accumulated in the nucleus. To preserve these potentially enhanced interactions and to avoid unspecific association of protein complexes when nucleo- and cytoplasm are mixed during cell lysis, we cross-linked WT and *mex67-5* cells after shift to the nonpermissive temperature and purified Sro9-TAP under high salt conditions to dissociate any proteins that were not associated with Sro9 in vivo; i.e., during cross-linking. Purification of Sro9 under these conditions (see Materials and Methods) but without cross-linking did not yield any of the tested proteins (data not shown). Importantly, the above proteins (with the exception of Rpb1, whose copurification was inconsistent) (Figs. 1, 2B) copurified with Sro9 under these conditions, showing that these interactions occur specifically in vivo and are not simply the result of mixing cellular compartments during cell lysis. Furthermore, in comparison with WT cells, association of Sro9 with Ctk1, Hpr1, and Npl3 increased two- to threefold when Sro9 accumulated in the nucleus (Fig. 2B, *mex67-5*). In summary, even though Sro9 localizes to the cytoplasm at steady state, it shuttles to the nucleus, where it associates with protein complexes involved in processes along the gene expression pathway before it is exported to the cytoplasm in an mRNA export-dependent manner.

Sro9 is recruited to an actively transcribed gene

As Sro9 might be part of the exported mRNP, we wanted to know whether Sro9 is recruited to the nascent transcript or associates with the mRNP at a later time point. Using chromatin immunoprecipitation (ChIP) experiments, we tested whether Sro9 can be found at an actively transcribed gene. To be able to easily regulate transcription, we chose the inducible *GAL1* gene. Sro9 is not recruited to the *GAL1* locus when cells are grown in glucose-containing medium, and consequently, transcription of *GAL1* is repressed (Fig. 2C, light gray). Importantly, when cells are grown in galactose-containing medium and transcription of *GAL1* is active, Sro9 is recruited to this gene in WT cells (Fig. 2C, dark gray). Expectedly, association of Sro9 with *GAL1* increases when mRNA export is blocked in the *mex67-5* mutant and Sro9 accumulates in the nucleus (Fig. 2D, dark gray, *mex67-5*, 37°C). Taken together, as many other mRNP components (also see below), Sro9 is already present at the transcribed gene and thus most likely loaded cotranscriptionally onto the nascent transcript.

Sro9 is not essential for expression or stability of specific transcripts

Next, we wanted to assess whether Sro9 has a direct function in transcription, mRNA export, or stability. Even though Sro9 is a nonessential protein, its deletion leads to a mild temperature-sensitive phenotype at 37°C (data not shown). Therefore, we tested for a potential mRNA export defect in the absence of Sro9 at this temperature. However, *Δsro9* cells do not accumulate bulk poly(A)$^+$ RNA in the nucleus at 37°C (data not shown), indicating that Sro9 is not needed for efficient mRNA export.

Overexpression of Sro9 alleviates the in vitro transcription defect; suppresses the reduced induction of *GAL1*, but not *PHO5* or *INO1* genes; and increases bulk mRNA stability in *Δrpb4* cells (Tan et al. 2000). Thus, an influence of Sro9 function on steady-state levels of a specific set of mRNAs was assessed using whole genome expression profiling of *Δsro9* in comparison with WT cells. With data processing techniques commonly applied in microarray analysis (see Materials and Methods), only two genes apart from *SRO9* itself showed significantly—i.e., more than twofold—altered mRNA levels as compared with the WT strain: *YHR087W* coding for a protein of unknown function and *GPH1* coding for a nonessential glycogen phosphorylase. However, since after multiple test correction (Benjamini and Hochberg 1995) no differentially expressed genes were identified, these two genes most likely represent false positives. For comparison, deletion of bona fide transcription factors (TFs) such as certain subunits of the mediator complex, the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex, or general transcription factors of Pol II, usually alters the expression of several hundred genes but at least 3% of total genes (Table 2; Holstege et al. 1998). Thus, the absence of Sro9 does not lead to changes in the steady-state level of specific transcripts, which might indicate the presence of additional factors with a function redundant to that of Sro9. One likely factor is the Sro9 homolog Slf1. Sro9 and Slf1 share about 30% identity throughout their amino acid sequence and are thought to result from an ancient gene duplication event (Wolfe and Shields 1997). Interestingly, when Slf1 is overexpressed, Sro9 protein levels are decreased (Sobel and Wolin 1999), indicating that Slf1 could take over the function(s) of Sro9. However, *SRO9* and *SLF1* do not interact genetically (Sobel and Wolin 1999), suggesting that either Sro9 or Slf1 does not function redundantly or that its (partially) redundant function is not essential. Thus, further experiments are needed to dissect the distinct and/or overlapping functions of Sro9 and Slf1. Taken together, whole genome expression profiling revealed that Sro9 is not essential for expression and stability of specific transcripts.

Concluding remarks

In recent years, it became widely accepted that nuclear gene expression processes are intimately linked (for review, see Erkmann and Kutay 2004; Fasken and Corbett 2005; Olesen et al. 2005; Kohler and Hurt 2007; Iglesias and Stutz 2008; Röther and Sträßer 2009). Many mRNA processing steps depend on preceding steps and determine subsequent ones: already during ongoing transcription, the mRNA is capped, spliced, and (after cleavage) polyadenylated. In addition, proteins package the mRNA into a messenger ribonucleoprotein particle (mRNP). This mRNP is then transported to the cytoplasm, where the ribosomes translate the mRNA into the encoded protein (Preiss and Hentze 2003; Holcik and Sonenberg 2005). In contrast, transcription and mRNA processing on the one hand and translation on the other hand were considered to be independent processes. However, considering the fact that from transcription to translation the mRNA is covered by various proteins and protein complexes, proteins loaded on the mRNA during nuclear maturation of the message can influence the cytoplasmic translation of the mRNA. One example of such a connection is Tap-p15, the human homolog of the yeast mRNA exporter Mex67-Mtr2. Tap-p15 is nuclear at steady state but shuttles between nucleus and cytoplasm and promotes translation of the viral CTE mRNA it exports (Jin et al. 2003). Second, the yeast SR-protein Npl3 binds to the mRNA already cotranscriptionally, but needs to be dissociated for efficient

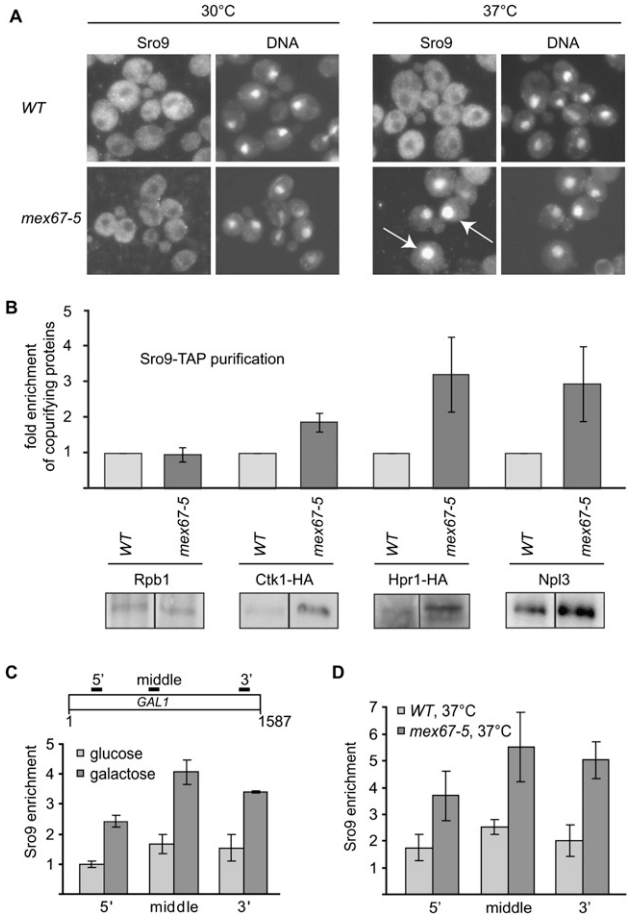

FIGURE 2. (Legend on next page)

translation to occur (Windgassen et al. 2004). Third, the yeast cyclin-dependent kinase Ctk1 phosphorylates RNA polymerase II during transcription elongation in the nucleus and is also important in the maintenance of accuracy during translation in the cytoplasm (Röther and Sträßer 2007). Fourth, the exon–exon junction complex (EJC) of frogs, plants, and humans, important in nuclear steps of gene expression, travels together with the mRNP to the cytoplasm, where it mediates nonsense-mediated decay and enhances translational efficiency (Wiegand et al. 2003; Nott et al. 2004; Belostotsky and Rose 2005). Fifth, the cotranscriptionally recruited mRNP component Dbp5, an RNA helicase important in the directionality of mRNA export, has recently been shown to be important in translation termination (Gross et al. 2007). Along the same line, Gle1 does not only stimulate the RNA-dependent ATPase activity of Dbp5 (Alcazar-Roman et al. 2006; Weirich et al. 2006) to ensure mRNA transport directionality (Tran and Wente 2006), but it is also essential for translation initiation and termination (Bolger et al. 2008). Finally, as already mentioned in the Introduction, the nuclear human La protein has been implicated in translation initiation of cellular and viral mRNAs (for review, see Wolin and Cedervall 2002).

mRNPs are known to be highly dynamic particles, whose protein content undergoes constant remodeling. To date, the distinct mRNP composition at each stage of gene expression is not known. However, the above-mentioned examples suggest that some mRNP components function in nuclear as well as cytoplasmic steps of gene expression, accompany the mRNA from the site of transcription to the ribosomes, the site of translation, and thereby could couple these two spatially separate processes. An increasing number of reports suggests that these proteins could mediate a new layer of gene expression, where the nuclear protein composition of the mRNP is decisive for the translational fate of the bound mRNA, such as initiation (Gle1, La), efficiency (Tap-p15, Npl3, or EJC components), accuracy (Ctk1), or termination (Dbp5, Gle1) of translation.

Interestingly, purification of some of the above-mentioned proteins (Ctk1, Npl3, and Mex67) yields Sro9—a protein with a function in transcription, translation, and mRNA stability—as a copurifying protein. In addition, Sro9 is recruited to actively transcribed genes and shuttles between nucleus and cytoplasm. While it is possible that there are separate pools of Sro9 that act independently, we propose that Sro9 is loaded onto the nascent transcript during transcription, where it contributes to robust transcription and might already stabilize the mRNA (Tan et al. 2000), perhaps by binding to the nascent mRNA. Sro9 then travels to the cytoplasm most likely as part of the mRNP, where it modulates translation (Sobel and Wolin 1999). Thus, Sro9 might be a member of the growing group of mRNP binding proteins that link nuclear and cytoplasmic steps of gene expression (Fig. 3).

TABLE 2. Percentage of genes affected by deletion of the respective gene

Gene (deletion mutant)	Protein complex	Percentage of genes affected (twofold)
SRB4	Mediator	93
KIN28	TFIIH	87
TFA1	TFIIH	54
MED6	Mediator	10
GCN5	SAGA	5
SRB10	Mediator	3
SRO9		0

FIGURE 2. Sro9 shuttles between nucleus and cytoplasm and is recruited to an actively transcribed gene. (*A*) Sro9 is exported to the cytoplasm dependent on mRNA export. At the permissive temperature (30°C) Sro9 localizes to the cytoplasm. However, at the nonpermissive temperature (37°C), when mRNA export is blocked in the *mex67-5* mutant, Sro9 accumulates in the nucleus, indicating that Sro9 is part of the exported mRNP. White arrows point to the accumulated nuclear protein. Nuclear DNA was stained with DAPI. (*B*) Association of Sro9 with nuclear protein complexes of the gene expression pathway occurs in vivo and increases upon inhibition of mRNA export. Sro9 was purified after cross-linking at the nonpermissive temperature (37°C) from wild-type (*WT*) and *mex67-5* cells and the copurification of nuclear proteins was assessed by Western blotting. Sro9 binds to Ctk1, Hpr1, and Npl3 in vivo (*WT*). Compared to *WT*, association of Sro9 with Ctk1, Hpr1, and Npl3 increased two- to threefold when mRNA export is blocked (*mex67-5*). In contrast, the association of Sro9 with Rpb1, which could not be observed consistently, remained unchanged. Representative Western blots are shown. Values represent means of four to five experiments with standard deviation as error bars. (*C*) Sro9 is recruited to an actively transcribed gene. Chromatin immunoprecipitation experiments show that Sro9 is recruited to the induced *GAL1* gene. Wild-type cells were grown in glucose- or galactose-containing medium at 30°C. Primer pairs amplify a 5′, middle, and 3′ region of *GAL1* as indicated in the *upper* panel. Enrichment of Sro9 at *GAL1* was calculated relative to the Sro9 occupancy at a nontranscribed region. Columns and error bars represent the mean ± standard deviation from three independent experiments. The values for Sro9 recruitment to the repressed versus activated *GAL1* gene differ significantly (*P*-values are 0.0004 [5′], 0.0012 [middle], and 0.0019 [3′]; *t*-test). (*D*) Recruitment of Sro9 to *GAL1* increases, when Sro9 accumulates in the nucleus upon inhibition of mRNA export (*mex67-5*, 37°C). Cells were grown in galactose-containing medium at 37°C, and the experiment was performed as in *C*. Columns and error bars represent the mean ± standard deviation from four independent experiments; *P*-values are 0.0103 (5′), 0.0042 (middle), and 0.0006 (3′); *t*-test.

MATERIALS AND METHODS

Yeast strains and plasmids

Yeast strains and plasmids are listed in Table 3. TAP-tagged strains were generated by integration of the TAP tag into the genome C-terminally of the respective genes by homologous recombination according to Puig et al. (2001). To delete *SRO9*, a *sro9::HIS3* construct was cloned by polymerase chain reaction (PCR) of a 500-base-pair (bp) SalI-BamHI promoter and a 500-bp BamHI-NotI terminator fragment of the *SRO9* gene and ligation of these fragments into pBluescriptIIKS(+), followed by insertion of the BamHI *HIS3* fragment of YDp-H (Berben et al. 1991). The SalI-NotI

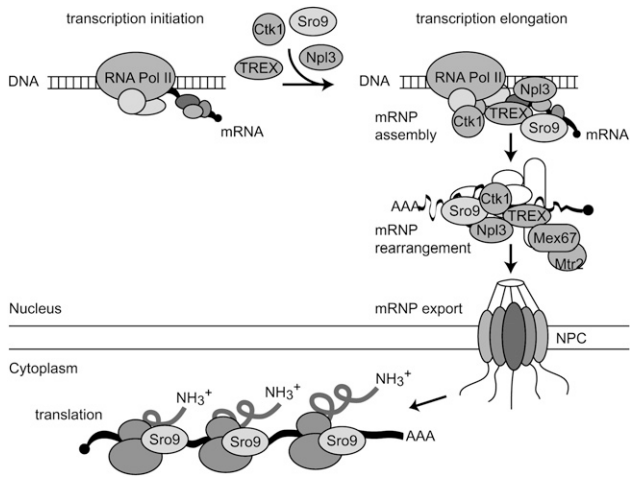

FIGURE 3. Model of the functions of Sro9 in transcription and translation. Similar to Ctk1, TREX, and Npl3, Sro9 is recruited to the nascent mRNA already during transcription. After maturation of the messenger ribonucleoprotein particle, Sro9 is exported to the cytoplasm as part of the mRNP, where it reaches the ribosomes. In line with this model, Sro9 is able to function in transcription, mRNA stability (Tan et al. 2000), and translation (Sobel and Wolin 1999).

sro9::HIS3 fragment was transformed into RS453, HIS^+ transformants were selected, and *SRO9* deletion checked by colony PCR. Positive colonies were transformed with pRS316-*SRO9* (Sobel and Wolin 1999).

Protein purification

Rpb1, Ctk1, Hpr1, Npl3, Mex67, and Rps4a, and associated proteins were purified by TAP according to Puig et al. (2001). For RNase treatment the lysis buffer contained 0.1 mg/mL RNase A. Copurifying proteins were analyzed by sodium dodecyl sulfate (SDS)-gel electrophoresis and Western blotting using antibodies directed against Sro9, Rpl6, and Cbp80. For purification of cross-linked protein complexes, *SRO9-TAP CTK1-HA/HPR1-HA MEX67* and *SRO9-TAP CTK1-HA/HPR1-HA mex67-5* cells were cultivated at 30°C to an OD_{600} of 0.6. Cultures were divided and further cultivated for 2 h at 30°C and 37°C, respectively. Formaldehyde was added to a final concentration of 1% and cultures incubated further for 10 min at 30°C and 37°C, respectively. To stop cross-linking, glycine was added prior to harvesting. Sro9 and associated proteins were purified by tandem affinity purification as described above with some modifications. Briefly, cross-linked cells were lysed in an equal volume of high salt (1 M NaCl) buffer and glass beads by vortexing at full speed for 6 × 3 min with 3-min breaks on ice. After sonication and removal of cell debris, the supernatant was incubated overnight with 500 µL IgG sepharose beads. After washing with high salt buffer, immunoprecipitated material was cleaved from the beads with TEV protease. Copurifying proteins were analyzed by SDS-gel electrophoresis and Western blotting using antibodies directed against Sro9, Npl3, Rpb1 (8WG16, Convance), and HA (Roche Diagnostics). The Anti-Sro9 antibody was a kind gift from S. Wolin (Yale University), anti-Rpl6 from G. Dieci (University of Parma), anti-Cbp80 from D. Görlich (MPI for Biophysical Chemistry), and anti-Npl3 from C. Guthrie (University of California at San Francisco). Copurification of Rpb1, Ctk1, Hpr1, and Npl3 with Sro9 was calculated from four to five experiments relative to the amount of purified Sro9.

Indirect immunofluorescence

To analyze the localization of Sro9 by indirect immunofluorescence *SRO9-TAP MEX67* and *SRO9-TAP mex67-5* cells were cultivated at 30°C or shifted to 37°C for 2 h. Formaldehyde was added to a final concentration of 3.3% and cells were fixed for 90 min at 30°C, pelleted, washed, and spheroblasted by Zymolyase treatment. Spheroblasts were applied to polylysine-coated multiwell slides and fixed onto the slides by consecutive immersions of the slide in −80°C methanol (6 min) and −80°C acetone (30 s). After drying and rehydration, anti-proteinA (Sigma-Aldrich) was applied for 2 h, followed by 1 h incubation with Alexa488-goat–anti-rabbit antibody (Invitrogen). Nuclear DNA was stained with DAPI, and slides were analyzed using an Olympus BX60 fluorescence microscope (Olympus).

Chromatin immunoprecipitation

SRO9-TAP MEX67 and *SRO9-TAP mex67-5* cells were cultivated in full medium containing either 2% glucose or 2% galactose at 30°C to an OD_{600} of 0.4. Cultures were divided and 40 mL each further cultivated for 2 h at 30°C or 37°C and cross-linked with formaldehyde for 25 and 20 min, respectively. ChIP was done as described (Sträßer et al. 2002) with some modifications. Briefly, cross-linked cells were lysed with an equal volume of glass beads by vortexing at full speed for 6 × 3 min with 3-min breaks on ice. Chromatin lysate corresponding to 14 A_{280} was used for immunoprecipitation with 15 µL of IgG-coupled Dynabeads for 3.5 h at 20°C. After washing and elution from the beads according to Kuras and Struhl (1999), the IP eluates as well as 0.08 A_{280} for input samples were treated with proteinase K for 2 h at 37°C and cross-links were reversed by overnight incubation at 65°C. DNA was purified over spin-columns (Macherey-Nagel). Quantitative PCR with input and IP samples was performed on an Applied Biosystems StepOnePlus cycler, using Applied Biosystems' Power SYBRGreen PCR Master Mix to assess Sro9 binding to the endogenous *GAL1* gene. As a control, primers for a nontranscribed region (NTR) of chromosome V were used (sequences available on request). PCR efficiencies (E) were determined with standard curves. Sro9 enrichment over the NTR was calculated according to $[E\hat{}(C_T\mathrm{IP} - C_T\mathrm{Input})]_{\mathrm{NTR}} / [E\hat{}(C_T\mathrm{IP} - C_T\mathrm{Input})]_{\mathrm{GAL1}}$. Means were calculated from four to five independent experiments.

Genome-wide expression profiling

RNA isolation

Wild-type and Δ*sro9* cells were grown in SDC(-leu) at 30°C. RNA was extracted with phenol, purified with the Qiagen RNeasy MinElute Kit, and subjected to microarray analysis.

Microarray handling

Yeast RNA was hybridized to Affymetrix GeneChipYeast Genome 2.0 Arrays (Affymetrix) essentially as described by Dengl et al. (2009). To minimize errors, samples were processed in parallel and arrays were scanned the same day. Biological duplicate measurements were performed for the WT strain and the Δ*sro9* strain.

TABLE 3. Strains and plasmids

Strain or plasmid	Genotype or description	Reference
RPB1-TAP	*MATa; ura3; ade2; his3; leu2; trp1; RPB1-TAP::TRP1-KL*	This study
CTK1-TAP	*MATα; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; CTK1-TAP::TRP1-KL*	Röther and Sträßer 2007
HPR1-TAP	*MATα; ura3; ade2; his3; leu2; trp1 HPR1-TAP::TRP1-KL*	Sträßer et al. 2002
NPL3-TAP	*MATα; ura3-52; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; NPL3-TAP::TRP1-KL*	This study
MEX67-TAP	*MATα; ade2-1; his3-11,15; ura3-52; leu2-3,112; trp1-1; can1-100; GAL+; MEX67-TAP::TRP1-KL*	This study
RPS4a-TAP	*MATα; ade2-1; his3-11,15; ura3-52; leu2-3,112; trp1-1; can1-100; GAL+; RPS4A-TAP::TRP1-KL*	This study
SRO9-TAP MEX67	*MATα; ura3; ade2; his3; leu2; trp1; SRO9-TAP::TRP1-KL, mex67::HIS3;* pUN100-*MEX67*	This study
SRO9-TAP mex67-5	*MATα; ura3; ade2; his3; leu2; trp1; SRO9-TAP::TRP1-KL, mex67::HIS3;* pUN100-*mex67-5*	This study
SRO9-TAP CTK1-HA MEX67	*MATα; ura3; ade2; his3; leu2; trp1; SRO9-TAP::TRP1-KL, CTK1-HA::HIS3, mex67::HIS3;* pUN100-*MEX67*	This study
SRO9-TAP CTK1-HA mex67-5	*MATα; ura3; ade2; his3; leu2; trp1; SRO9-TAP::TRP1-KL, CTK1-HA::HIS3, mex67::HIS3;* pUN100-*mex67-5*	This study
SRO9-TAP HPR1-HA MEX67	*MATα; ura3; ade2; his3; leu2; trp1; SRO9-TAP::TRP1-KL, HPR1-HA::KanMX6, mex67::HIS3;* pUN100-*MEX67*	This study
SRO9-TAP HPR1-HA mex67-5	*MATα; ura3; ade2; his3; leu2; trp1; SRO9-TAP::TRP1-KL, HPR1-HA::KanMX6, mex67::HIS3;* pUN100-*mex67-5*	This study
SRO9 shuffle	*MATa; ade2-1; his3-11,15; ura3-52; leu2-3,112; trp1-1; can1-100; GAL+; sro9::HIS3;* pRS316-*SRO9*	This study
pRS315		Sikorski and Hieter 1989
pUN100-*MEX67*		Segref et al. 1997
pUN100-*mex67-5*		Segref et al. 1997
pRS316-*SRO9*		Sobel and Wolin 1999
pRS315-*SRO9*	*Xho*I-*Xba*I fragment of pRS316-*SRO9* was cloned into pRS315	This study

Gene expression data analysis

Raw signal intensities for each probe set in the *.CEL* files were analyzed using version 6.3 of the Partek Genomics Suite software (Partek, Inc.). Data were filtered by application of an expanded mask file that was based on the *s_cerevisiae.msk* file of Affymetrix, to mask the *Schizosaccharomyces pombe* probe sets, unspecific probe sets, and replicate probe sets of *S. cerevisiae*. The robust multiarray average (RMA) normalization method (Irizarry et al. 2003) was used for RMA background correction, quantile normalization, and median-polish probe set summarization. Expression values were transformed to $\log_2$ before statistical analysis. A sample intensity plot was calculated, showing that the data are normally distributed for all samples, as well as the homogeneity of variance (no outlier), which also indicated the great similarity of the biological replicates. Genes that were differentially expressed between WT and mutant strains were detected with one-way ANOVA, a statistical technique used to compare means of two or more samples implemented in the software package. A linear contrast was used to compare mutant samples with baseline WT samples. The recovered *P*-values of the comparisons were then corrected using a step-up false discovery rate value of 5% (Benjamini and Hochberg 1995). The resulting list of significantly expressed genes was filtered to include only genes that demonstrated twofold or greater up- or down-regulation. Microarray data were deposited in the ArrayExpress database with accession number E-MEXP-2678.

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