Regulation of poly(A) tail length during mRNA 3′-end formation requires a specific poly(A)-binding protein in addition to the cleavage/polyadenylation machinery. The mechanism that controls polyadenylation in mammals is well understood and involves the nuclear poly(A)-binding protein PABPN1. In contrast, poly(A) tail length regulation is poorly understood in yeast. Previous studies have suggested that the major cytoplasmic poly(A)-binding protein Pab1p acts as a length control factor in conjunction with the Pab1p-dependent poly(A) nuclease PAN, to regulate poly(A) tail length in an mRNA specific manner. In contrast, we recently showed that Nab2p regulates polyadenylation during de novo synthesis, and its nuclear location is more consistent with a role in 3′-end processing than that of cytoplasmic Pab1p. Here, we investigate whether PAN activity is required for de novo poly(A) tail synthesis. Components required for mRNA 3′-end formation were purified from wild-type and pan mutant cells. In both situations, 3′-end formation could be reconstituted whether Nab2p or Pab1p was used as the poly(A) tail length control factor. However, polyadenylation was more efficient and physiologically more relevant in the presence of Nab2p as opposed to Pab1p. Moreover, cell immunofluorescence studies confirmed that PAN subunits are localized in the cytoplasm which suggests that cytoplasmic Pab1p and PAN may act at a later stage in mRNA metabolism. Based on these findings, we propose that Nab2p is necessary and sufficient to regulate poly(A) tail length during de novo synthesis in yeast.

Eukaryotic mRNA 3′-end formation is a two-step reaction (reviewed in Refs. 1–3). The RNA polymerase II-transcribed mRNA precursor is first cleaved in its 3′-untranslated region and the upstream fragment is subsequently polyadenylated.

The mechanism and the machinery involved in this processing have been well conserved through evolution. Human and yeast share similarities in the reaction mechanism and in the composition of the implicated machinery. In mammals, the cleavage reaction can be recapitulated in vitro in the presence of cleavage factors I and II (CF I and CF II,1–4 the cleavage stimulation factor (CstF), the cleavage and polyadenylation specificity factor (CPSF), and the poly(A) polymerase (PAP). Controlled poly(A) tail synthesis requires PAP, CPSF, and the nuclear poly(A)-binding protein 1 (PABPN1) (4, 5). Mammalian poly(A) tails are synthesized to an average length of 250 adenosine residues. PAP alone does not have any pronounced specificity with regard to the RNA substrate and is poorly active on its own. Its activity is stimulated by two factors, CPSF, which binds to the highly conserved element AAUAAA, and PABPN1, which interacts with the growing poly(A) tail. The combined activity of both stimulatory factors leads to fully processive elongation such that a complete poly(A) tail can be synthesized without dissociation of the polymerase. Once poly(A) tails have reached ~250 nucleotides, elongation terminates by switching from a processive to a slower distributive mode. Although the exact mechanism that controls poly(A) tail length is unclear, one possibility is that once 250 adenosine residues have been polymerized, disruption of the polyadenylation complex PAP-CPSF-PABPN1 takes place perhaps because the tail is too long to mediate interactions between the three components of the complex (5, 6).

Poly(A) tail length control in S. cerevisiae is poorly understood, and there is a controversy about which factors are required. In yeast, poly(A) tails are shorter with an average length of about 70 adenosine residues. The two steps of mRNA 3′-end formation require the cleavage factors IA (CF IA) and IB (CF IB or Nab4p/Hrp1p) and the cleavage and polyadenylation factor (CPF) containing the poly(A) polymerase (Pap1p), which is dispensable for the cleavage step of the reaction (7). Poly(A) tail length control can be reconstituted in vitro in the presence of these three factors and a poly(A)-binding protein. It was initially suggested that the major cytoplasmic poly(A)-binding protein Pap1p was responsible for the poly(A) tail length control during de novo synthesis by inhibiting Pap1p activity (8–10). This hypothesis was supported by several observations. First, Pap1p co-purifies with CF IA and specifically interacts with the Rna15p subunit using either two-step.
hybrid analysis or co-immunoprecipitation and PAB1 is a multi-copy suppressor of rna15–2, coding for a subunit of CF IA. Second, poly(A) tails are at least 50 nt longer in vitro when the mRNA precursor is incubated in the pan2Δ mutant extracts. Third, complementation with recombinant Pab1p restores normal length control to the pan2Δ extracts but also induces a shortening of the transcript (9, 10). It has been suggested that Pab1p acts by stimulating the poly(A) nuclease PAN, which might balance excessive growth of poly(A) tails by trimming them from the 3'-end (11).

Deadenylation is the first step in the general mRNA turnover in eukaryotes (reviewed in Ref. 12). In yeast, two different complexes have been identified as mRNA deadenylases. The predominant one is the conserved Ccr4p/Pop2p (or Cat1p) complex, which is inhibited by Pab1p. Pan is the other one, which is responsible for the residual deadenylation in a ccr4Δ mutant (13). PAN is composed of two subunits, Pan2p (127 kDa) and Pan3p (76 kDa), which are essential for its nuclease activity (14, 15). Pan2p is the catalytic subunit and is a member of the RNaseD superfamily, while Pan3p is a positive regulator of PAN activity. Previously, poly(A) tail length was thought to involve PAN in an mRNA-dependent manner (11). More recently, the PAN complex was suggested to be a component of the nuclear pre-mRNA 3'-end processing machinery together with CF IA, CF IB, and CPF (16, 17).

We have shown previously that the nuclear poly(A)-binding protein Nab2p specifically controls poly(A) tail length in vivo and in vitro in yeast (18). In this report, we investigated whether PAN might play an additional role in de novo poly(A) tail synthesis in vitro.

EXPERIMENTAL PROCEDURES

Yeast Strains—The pan2Δ pan3Δ strain (YAS1944) has been described elsewhere (15). The pab1Δ deletion is rescued by deletion of RPL46 in the strain YAS394 (19). Strains expressing the C-terminally tandem affinity purification (TAP)-tagged Pan2p (YSD7) or Pan3p (YSD8) were constructed in BMA64 (ade2-1 leu2-3,112 trp1 his3-11,15 can1-100) (20) according to Rigaut et al. (21). Rna15p and Fip1p were N-terminally TAP-tagged either in the wild-type strain BMA64 (YSID12 and YSID10, respectively) or in the pan2Δ pan3Δ strain YAS1944 (YSID9 and YSID11, respectively) according to Dheur et al. (7). This N-terminal TAP-tagging method was performed by integrating at the chromosomal locus using a cassette which adds a TAP-tag identical to that described previously (21). Strains expressing both N-terminally TAP-tagged Rna15p and Fip1p were also constructed in either the wild-type BMA64 (YSID3) or the pan2Δ pan3Δ strain YAS1944 (YSID4).

In Vitro 3'-End Processing Assays—Polyadenylation-competent extracts were prepared using a spheroplast procedure (22). Tandem affinity purification of CF IA and CF PF was performed according to Rigaut et al. (21). Pre-mRNA cleavage and polyadenylation were assayed essentially as previously described (7, 23). Brefeldin A and Puf-40, 0.5 mM dithiothreitol, 0.01% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM 4-imidazoleacetic acid, 1 mM ethylenediaminetetraacetic acid, 1 mM leupeptin, 2 μM aprotinin, 2 mM Pefabloc. A liquors of the supernatant were analyzed by immunoblotting as described (25), using a 1:500 dilution of anti-Nab2p 3F2 or anti-Kap104p 1D12 antibodies and a 1:5000 dilution of horseradish peroxidase-conjugated sheep anti-mouse secondary antibody (Amersham Biosciences).

Cellular Immunofluorescence—The subcellular distributions of TAP-tagged Pan2p, Pan3p, and Rna15p were examined by cell immunofluorescence as described previously (18) using an Alexa Fluor 488-conjugated goat anti-mouse IgG antibody (Molecular Probes, Eugene, OR). Cells were visualized using a Zeiss Axioskop 2 plus microscope equipped with a ×100 fluorescence/differential contrast objective and images were captured with a Zeiss monochrome digital camera.

RESULTS

In Vitro Synthesized mRNA Poly(A) Tails Are Not Aberrantly Long in PAN-deficient Cell Extracts Compared with Pab1p-deficient Extracts—To evaluate the putative involvement of the PAN nuclease during in vitro poly(A) tail synthesis, we performed a time course experiment and characterized the 3'-end processing activity of extracts prepared from either the wild-type, the pan2Δ pan3Δ, or the pab1Δ mutant strains. The 3'-untranslated region of the mRNA precursor CYC1 was transcribed in vitro in the presence of a radiolabeled nucleotide and then incubated with the different yeast extracts. As shown in Fig. 1, the CYC1 precursor (lane 1, shown as a gray and white rectangle) and running with the 309-nt marker was cleaved at the normal poly(A) site. The resulting upstream fragment (running between the 180- and 190-nt marker bands and represented as a gray box) was polyadenylated in wild-type extracts.
This polyadenylation reaction resulted in poly(A) tails with an average length of 60 adenosine residues, which ran above the upstream fragment in the region of the 242-nt marker (lanes 2 and 3, gray box followed by AAAA). Subsequently, poly(A) tails were shortened (Fig. 1, lane 4). This time course assay could reflect an equilibrium between synthesis and degradation that occurs within the first hour, which is followed by a net deadenylation because of a reduced polyadenylation rate. Alternatively, this experiment could highlight a two-step reaction in which trimming would be the final processing step that follows polymerization. Fig. 1 shows that the PAN-deficient extracts displayed a polyadenylation activity that was not dramatically different from that observed with wild-type extracts (Fig. 1, compare lanes 5–7 with lanes 2–4). Although cleavage activity was less efficient than that of the wild-type extract, this result should be interpreted with caution, since cleavage efficiency is highly dependent upon extract preparation and differs from one extract to another with no obvious correlation to the strain used. Poly(A) tails in the pan mutant extracts were slightly longer, but their length was rather constant with time, which is the most striking difference with the wild-type extracts (Fig. 1, compare lane 4 with lane 7). On the other hand, the pab1 mutant extracts were noticeably more affected in poly(A) tail length control (Fig. 1, lanes 8–10). In this case, the average tail length was ~50 nt longer, although very long tails were detectable. Because of this increasing length, the polyadenylated product overlapped with the precursor in lanes 8–10 of Fig. 1. Tail length also remained constant with time. Altogether, these results suggested that Pab1p restricts poly(A) tail length only to a certain extent in vitro and that the Pab1p-dependent nuclease PAN does not play a direct role in de novo poly(A) tail synthesis but might be involved in a trimming activity that occurs following polyadenylation.

However, several observations argue against a role for Pab1p in poly(A) tail length control in vivo. First, Pab1p is an abundant cytoplasmic protein that is very weakly detected in the nucleus (25). Second, Pab1p is a major contaminant of the complexes purified from yeast. For instance, it was present in 10% of the tandem affinity purified complexes in the large scale approach reported in Gavin et al. (26). Third, the very long tails observed in vitro with pab1 mutant extracts do not reflect the in vivo situation where tails are only ~3–20 nt longer in the absence of Pab1p (27). These arguments led us to consider the possibility that the poly(A) tail length regulatory factor in vivo might have been lost or inactivated during extract preparation.

The Poly(A) Tail Length Regulatory Function of Nab2p in Vitro Might Be Obscured because of Its Binding to the Nuclear Transport Factor Kap104p during Extract Preparation—We recently provided evidence that the hnRNP Nab2p was another candidate for controlling mRNA poly(A) tail synthesis in vivo and in vitro (18). Unlike Pab1p, Nab2p is a predominantly nuclear protein (25, 28) that plays a dual role in the termination of mRNA polyadenylation and nuclear export. Nab2p is a shuttling protein that is reimported to the nucleus by way of its association with the karyopherin Kap104p (24, 29). Kap104p and Nab2p form a complex in the cytosol and binding of Kap104p to Nab2p destabilizes Nab2p-RNA interactions (24). Because the poly(A) tail regulatory activity of Nab2p is dependent on its RNA-binding properties, we speculated that Kap104p binds to free Nab2p in extracts resulting in an inhibition of its poly(A) tail regulatory activity. However, the abundant poly(A)-binding protein Pab1p masked the loss of active Nab2p in extracts as it could substitute for it in polyadenylation assays (9, 10). As a result, the loss of Nab2p activity during extract preparation would therefore explain the complete absence of poly(A) tail length control in pab1Δ extracts.

To test this hypothesis, we determined whether the anti-Kap104p monoclonal antibody 1D12 (mAb 1D12) could immunodeplete Nab2p from 3′-end processing extracts. In this assay, pab1Δ extracts were immunodepleted with several antibodies, and the presence of Nab2p and Kap104p in the antigen-depleted supernatants was analyzed by Western blot (see “Experimental Procedures”) (Fig. 2). As anticipated, the anti-Kap104p antibody not only depleted the extracts of Kap104p but also the majority of Nab2p (Fig. 2, lane 3). It also appeared that Kap104p was considerably more abundant than Nab2p, since the anti-Nab2p antibody failed to completely immunodeplete Kap104p from the extracts (Fig. 2, lane 4). As Nab4p is also reimported by Kap104p to the nucleus (24), this might correspond to a Kap104p-Nab4p complex as immunodepletion with antibodies directed against Nab4p failed to pull down all Kap104p as well (Fig. 2, lane 5). Neither the anti-Nab4p antibody nor an antibody against the unrelated protein Pab1p led to Nab2p depletion in the extracts (Fig. 2, lanes 5 and 6, respectively, compared with the mock depleted extract in lane 2).

These results suggested that Kap104p binds Nab2p in polyadenylation-dependent extracts. This might explain why control of poly(A) tail synthesis was lost in vitro in the pab1Δ extracts, which does not reflect the in vivo situation in the pab1Δ strain (27). This data supported our previous proposal that Nab2p is the bona fide poly(A) tail length regulatory factor in yeast and that its inactivation by Kap104p was masked by the activity of the abundant poly(A)-binding protein Pab1p in 3′-end processing extracts.

The Absence of PAN Does Not Affect the Composition of Purified CF IA and CPF Complexes—If PAN functions in poly(A) tail length control during de novo synthesis, reconstitution of regulated mRNA polyadenylation with 3′-end processing factors purified from extracts lacking this deadenylase should be compromised. Therefore, we purified CF IA and CPF from a pan2Δ pan3Δ strain to recapitulate the pre-mRNA 3′-end processing reaction in vitro in the absence of PAN activity. The two complexes were purified either separately or together from a pan mutant and a wild-type strain using the TAP method (21). CF IA and CPF were purified from strains expressing N-terminally Tagged RNA15p (for CF IA purification) and/or TAP-tagged Fip1p (for CPF purification). Purified factors were visualized on silver-stained polyacrylamide gels (Fig. 3). The majority of the expected bands corresponding to already identified subunits could be detected (26), and no significant difference in the composition of the factors could be found between the wild-type and the pan mutant backgrounds.

Normal Cleavage and Polyadenylation of Pre-mRNA Can Be Recaptulated in Vitro with Purified Factors in the Absence of PAN—To evaluate the effect of PAN absence on poly(A) tail formation, we performed in vitro assays with the TAP-purified

\[ S. Dheur and L. Minvielle-Sebastia, unpublished observation. \]
CF IA and CPF. Their activities were tested in vitro for pre-mRNA cleavage and polyadenylation in combination with recombinant Nab4p (CF IB) and increasing amounts of either recombinant Pab1p or Nab2p (Fig. 4). In the presence of CF IA, CF IB, and CPF, the 3′-untranslated region of CYC1 precursor was cleaved and hyperadenylated (Fig. 4, lanes 2 and 8). In these conditions, poly(A) tails were polymerized to a length exceeding 400 nt. Addition of increasing amounts of Pab1p reduced the average tail length to a size similar to that obtained with wild-type cell extracts (Fig. 4, compare lanes 3–7 and 9–13 with lane 14). However, two major bands (under the precursor) and very long tails (running above the precursor and higher than the 622-nt marker) could be observed with high amounts of Pab1p under conditions where cleavage began to be inhibited (Fig. 4, lanes 6 and 7 and lanes 12 and 13). The two bands were not detected in previous studies where shorter polyacrylamide gels with lower resolution were used. These bands were not detected in previous studies where shorter polyacrylamide gels with lower resolution were used.

Addition of increasing amounts of Nab2p also resulted in a decrease of poly(A) tail length (Fig. 4, lanes 16–20 and 21–25). In contrast to Pab1p, we could observe a smear corresponding to the mature polyadenylated product below the precursor. In addition, the hyperadenylated species running above the precursor were no longer visible (Fig. 4, lanes 20 and 25). The reaction appeared to be more efficient than with Pab1p, since as little as 80 ng of Nab2p could suppress the formation of hyperadenylated products, which differs from the situation found with Pab1p. Noticeably, wild-type and panΔ-purified factors displayed the same activity (Fig. 4, compare lanes 2–7 with lanes 8–13).

Also, aberrant utilization of the 3′ product was prevented, since it remained in equivalent amounts throughout the titration. This experiment showed that poly(A) tails of the expected physiological length could be obtained with purified factors in vitro. The fact that no difference was detectable in this 3′-end processing reconstitution experiment between wild-type and panΔ factors strongly suggested that PAN nuclease is not required for poly(A) tail length control. We conclude that PAN is not involved in de novo poly(A) tail synthesis.

Kap104p Specifically Inhibits Poly(A) Tail Length Control by Nab2p—To further test the hypothesis that the essential role of Nab2p in poly(A) tail length control was overlooked due to its sequestration by Kap104p during extract preparations, we performed polyadenylation assays with Nab2p and increasing amounts of Kap104p. To avoid formation of a complex between Kap104p and its other natural cargo, Nab4p, during the assay, we preincubated Nab2p with its karyopherin for 15 min before the assay (see “Experimental Procedures”). In contrast to the control cleavage and polyadenylation reaction with Nab2p alone (Fig. 5, lane 2), binding of Kap104p to Nab2p prior to the assay inhibited the ability of Nab2p to control the addition of adenosines to the cleavage product (Fig. 5, lanes 3–6). The hyperadenylated products were similar to those found in assays where very little Nab2p was added to the cleavage and polyadenylation reaction (compare Fig. 5, lane 6 with Fig. 4, lanes 16 and 21). A potentional complication with this experimental design is that Kap104p might also bind and inhibit Nab4p during the course of the reaction. However, this possibility is not a concern here because loss of Nab4p activity should lead to an inhibition of polyadenylation activity and not the hyperadenylation, which is particularly evident at higher Kap104p levels (Fig. 5, lanes 5 and 6). As a control, in a similar experiment where Pab1p replaced Nab2p in the assay, addition of Kap104p to Pab1p prior to the reaction had no effect on poly(A) tail length (data not shown).

Together with the co-immunoprecipitation of Nab2p and Kap104p in extracts (Fig. 2), this experiment strongly supports our hypothesis that the role of Nab2p in regulating poly(A) tail length during de novo synthesis in vitro was overlooked in earlier studies due to its artifactual sequestration by Kap104p.

Poly(A) Tail Length Control in Yeast Is Not Achieved by Excessive Synthesis Balanced by PAN-dependent Degradation—To analyze the kinetics of poly(A) tail formation we carried out a time course experiment using wild-type and panΔ...
Purified factors and a fixed amount of either Nab2p or Pab1p. The CYC1 precursor RNA was incubated with TAP-purified CF IA and CPF supplemented with Nab4p and polyadenylation occurs via a processive mechanism in yeast as is the case in mammals. The fact that similar results were obtained using either wild-type or panΔ purified factors argued that polyadenylation did not result from excessive synthesis balanced by PAN-dependent degradation but rather from controlled synthesis.
PAN Nuclease Is Predominantly a Cytoplasmic Enzyme—
Assuming that PAN activity may influence poly(A) tail length following de novo tail synthesis, we wondered where this shortening process occurs in the cell. Does it occur in the nucleus just after polyadenylation by the 3’-end processing machinery or in the cytosol where Pab1p is most abundant and where the major deadenylase Ccr4p/Pop2p, which is involved in general mRNA turnover, is localized? To determine the subcellular distribution of Pan2p and Pan3p, we constructed chromosomal TAP-modified strains expressing either TAP-Pan2p or TAP-Pan3p, and cells were immunostained with anti-protein A antibodies and Alexa Fluor 488-conjugated secondary antibodies as indicated. DNA was stained with 4’,6-diamidino-2-phenylindole (DAPI).

**FIG. 7.** Subcellular localization of the PAN subunits Pan2p and Pan3p. Strains expressing TAP-tagged Pan2p, Pan3p, or Rna15p were immunostained with anti-protein A antibodies and Alexa Fluor 488-conjugated secondary antibodies as indicated. DNA was stained with 4’,6-diamidino-2-phenylindole (DAPI).

**PAN Nuclease and Yeast mRNA Poly(A) Tail Length Control**

As described previously (9, 11), mRNA poly(A) tails synthesized in yeast cell extracts reach a length of ~70–80 nt in 30 min following a slower trimming phase which removes about 10 nt. This trimming is not observed in either Pab1p- or PAN-deficient extracts suggesting that the Pab1p-dependent deadenylase is responsible for this activity. This degradation process could be a final step in mRNA 3’-end maturation, since it is observed for every tested precursor RNA (11). Alternatively, this deadenylase might be active concurrently with synthesis in vivo to achieve specific poly(A) tail length control. However, PAN activity depends on its cofactor Pab1p and in Pab1p-deficient extracts poly(A) tails are much longer than in PAN-deficient extracts. This observation suggests that a length regulatory mechanism based solely on PAN activity is not sufficient to explain how poly(A) tail synthesis is controlled.

We proposed previously that Nab2p is a more rational regulatory factor for poly(A) tail length control during synthesis in vivo (18). Nab2p plays a dual role, since it is involved in mRNA poly(A) tail length control and nuclear export. Loss of NAB2 expression leads to mRNA hyperadenylation and nuclear accumulation but these defects can be uncoupled in a nab2Δ mutant in contrast to other nuclear export mutants. Moreover, overexpression of PAB1 only rescues the export defect of a nab2Δ strain but fails to resolve the hyperadenylation defect even when Pab1p is targeted to the nucleus (18). In the present work, we demonstrate that Nab2p is unable to function in tail length control in vitro because Nab2p is inactivated by Kap104p binding during extract preparation. Furthermore, addition of recombinant Nab2p to purified CF IA, CF IB, and CPF recapitulates the pre-mRNA 3’-end processing reaction in vitro. Moreover, our time course analysis of mRNA 3’-end formation in vitro with purified factors (Fig. 5) is reminiscent of the mammalian mechanism where poly(A) tail synthesis is processive (5, 6). Indeed, the kinetics of polyadenylation with Nab2p suggested a processive mode of synthesis because only poly(A) tails with normal length were observed, which is different from the time course assay with Pab1p that showed two bands below the precursor that could represent intermediate species resulting from a distributive reaction mode (see Fig. 6).

Although the functional characteristics of Nab2p are similar to those of metazoan PABPN1, it should be emphasized that yeast Nab2p is probably not the functional homologue of mammalian PABPN1, since loss of Nab2p results in hyperadenylated RNA, while PABPN1 inactivation yields hypoadenylated RNA (30).

PAN deadenylase has been conserved throughout evolution (31). Like its human ortholog, PAN subunits Pan2p and Pan3p are localized primarily in the cytoplasm at steady state. Our results suggest that PAN activity is not directly involved in nuclear mRNA poly(A) tail length control in yeast. Our data are also consistent with a model in which PAN may act on poly(A) tails after they have been synthesized to a default length in the nucleus. In yeast, as in metazoans, deadenylations is the first step in the general mRNA turnover pathway (12, 32). PAN catalyzed the release of deadenylated RNA, while PABPN1 inactivation yields hypoadenylated RNA (30).

**DISCUSSION**

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**PAN Nuclease and Yeast mRNA Poly(A) Tail Length Control**

Most recently, new insights on Pab1p and PAN function have shed light on their role in yeast. It has been shown that Pab1p is a shuffling protein that, together with PAN, is essential for the release of mRNAs from their site of transcription in the nucleus and their export to the cytoplasm (34, 35). This release may involve an initial trimming of the newly synthesized poly(A) tails by PAN. These results fit nicely with earlier observations suggesting that PAN is required for a message-specific poly(A) tail maturation phase leading to poly(A) tails ranging from 50 to 90 adenylic residues. This deadenylations could be modulated by specific 3’-UTR elements that have not yet been characterized (11, 36, 37). Most importantly, these new results reinforced our model that Nab2p is the primary factor that initially determines mRNA poly(A) tail length in yeast. Pab1p/PAN would be engaged in the last step of mRNA 3’-end formation linked to quality control and export.

Interestingly, a recent observation has suggested another role for PAN in the specific regulation of RAD5 mRNA in response to replicative stress through the interaction of the Pan3p subunit with Dun1p (36). Additional studies are required to dissect the mechanism involved in this regulation. Whether PAN nuclease possesses other regulatory functions remains to be elucidated.
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