DEAD Box RhlB RNA Helicase Physically Associates with Exoribonuclease PNPase to Degrade Double-stranded RNA Independent of the Degradosome-assembling Region of RNase E*

Received for publication, July 3, 2002, and in revised form, August 12, 2002
Published, JBC Papers in Press, August 13, 2002, DOI 10.1074/jbc.M206618200

Gunn-Guang Liou‡§, Hsiang-Yu Chang‡§, Chi-Shen Lin‡§, and Sue Lin-Chao‡**

From the ‡Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan, the §Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taipei 100, Taiwan, and the ⁄Institute of Biochemistry, National Yang-Ming University, Taipei 112, Taiwan

The Escherichia coli RNA degradosome is a multicomponent ribonucleolytic complex consisting of three major proteins that assemble on a scaffold provided by the C-terminal region of the endonuclease, RNase E. Using an E. coli two-hybrid system, together with BIAcore apparatus, we investigated the ability of three proteins, polynucleotide phosphorylase (PNPase), RhlB RNA helicase, and enolase, a glycolytic protein, to interact physically and functionally independently of RNase E. Here we report that RhlB can physically bind to PNPase, both in vitro and in vivo, and can also form homodimers with itself. However, binding of RhlB or PNPase to enolase was not detected under the same conditions. BIAcore analysis revealed real-time, direct binding for molecular interactions between RhlB units and for the RhlB interaction with PNPase. Furthermore, in the absence of RNase E, purified RhlB can carry out ATP-dependent unwinding of double-stranded RNA and consequently modulate degradation of double-stranded RNA together with the exonuclease activity of PNPase. These results provide evidence for the first time that both functional and physical interactions of individual degradosome protein components can occur in the absence of RNase E and raise the prospect that the RNase E-independent complexes of RhlB RNA helicase and PNPase, detected in vivo, may constitute mini-machines that assist in the degradation of duplex RNA in structures physically distinct from multicomponent RNA degradosomes.

RNA metabolism is a complex process affecting the control of gene expression. In bacteria, a multicomponent ribonucleolytic complex termed the RNA degradosome (1–4) has been identified as playing an important role in the control of mRNA degradation (for recent reviews, see Refs. 5–11). The multicomponent complex consists of: the RNA endonuclease RNase E, whose activity is essential for Escherichia coli cell growth (12–14), RNA processing (15, 16), and degradation (17, 18); the 3′–5′ exoribonuclease PNPase (19); RhlB RNA helicase (20); and enolase (21), an enzyme involved in the glycolytic pathway and other chaperonin proteins (3, 22). Interestingly, in addition to mRNAs, highly structured, stable RNA fragments have also been found to be associated with RNA degradosome complexes (3, 23), which implies quality control by the RNA degradosome for the biogenesis of stable RNAs. Degradosome complexity and its cooperation with individual protein components acting on degradation-targeted RNA, in vivo, remains to be discovered.

Various approaches revealing protein-protein interactions in the degradosome indicate that the C-terminal region of RNase E serves as a scaffold that directly binds PNPase, RhlB RNA helicase, and enolase (24, 25). No other interactions among these component proteins have been detected (25) or reported. Recently, a mini-degradosome complex (26) containing the scaffold region (without the N-terminal enzymatic region of RNase E), RhlB RNA helicase, and PNPase was reconstituted in vitro. These experiments revealed a functional interaction between RhlB RNA helicase and PNPase: RhlB helicase bound to the RNase E C-terminal region leads to subsequent ATP-activated degradation of a stem-loop segment intermediate RNA by PNPase. In the absence of the RNase E C-terminal region, RNA degradation does not occur. This led to the proposal that interaction between both RNase E-bound PNPase and RhlB helicase attacks the 3′-end of a structured mRNA (26). Recently, it has been shown that, in vivo, the steady state levels of individual component degradosome proteins differ and that degradosome protein components can exist both bound and unbound to RNase E (27). Thus the potential exists for the formation of protein complexes other than those formed on the RNase E scaffold to target RNAs for degradation. Here, we have addressed this question by investigating possible interactions among degradosome protein components independently of RNase E. We show, both in vitro and in vivo, that RhlB RNA helicase binds directly to PNPase and that the binding aids double-stranded RNA degradation by PNPase. These findings indicate that functionally important interactions between degradosome components can occur in the absence of the degradosome scaffold and suggest the existence of a dynamic network of degradosome components in vivo that targets various kinds of structured RNAs for rapid degradation by PNPase-RhlB complexes.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—The E. coli strains BZ99 (his ΔtrpE5 mukB106 smbB199 (λ)28) and DHF1 (F’ gltV44/As) recA1 endA1 gyrA96(nalr) thi1 hsdR17 spoT1 rfbD1 cya (29) were used to overexpress degradosome protein components and study in vivo protein–protein interactions, respectively. BZ99 contains a truncated rne gene that encodes amino acids 1–602 of the Rne protein. DHF1 is an adenylyl cyclase (cya)-deficient derivative of DH1 (30). The plasmid pGP1–2, containing the T7 RNA polymerase gene under the control of
Function of Degradosome Protein Components

a temperature-sensitive bacteriophage λ repressor (31), was used to conditionally induce the synthesis of the protein. A PCR-generated EcoRI-Xhol fragment encoding full-length PNPass and RhlB RNA helicase and a NdeI-BamHI fragment encoding full-length enolase, were individually inserted into FLAG-containing vectors (the corresponding plasmids were termed pflag-PNP, pflag-RhlB and pflag-ENO). Proteins were purified using an M2 affinity column as described (29).

were partially sequenced to confirm correct open reading frames. Respectively, into the pT18-based vector (29). All plasmid constructs fragment encoding full-length PNPase, RhlB, helicase and enolase, pT25-based vector. Plasmids pPNPT18, pRhlBT18, and pENOT18, RhlB polypeptide (i.e. RhlB1: residues 1–194; RhlB2: residues 194–368; RhlB3: residues 368–421; RhlB2: residues 1–368; RhlB13: residues 1–194, and 368–421; RhlB23: residues 194–421, respectively), into the pT25-based vector. Plasmids pPNT18, pRhBT18, and pENOT18, were generated by the insertions of a PCR-generated NdeI-HindIII fragment encoding truncated peptides of (i.e. RE12: residues 684–784, full-length, RhlB RNA helicase and some data indicate that RhlB polypeptide (i.e. RhlB1: residues 1–194; RhlB2: residues 194–368; RhlB3: residues 368–421; RhlB2: residues 1–368; RhlB13: residues 1–194, and 368–421; RhlB23: residues 194–421, respectively), into the pT25-based vector. Plasmids were partially sequenced to confirm correct open reading frames.

E. coli Two-hybrid Assay—E. coli two-hybrid assays were performed on MacConkey agar plates containing 1% maltose as described (29). Antibiotic concentrations were 100 μg/ml ampicillin and 34 μg/ml chloramphenicol. Plasmids were incubated at 37 °C for 2 days. Photographs of individual plates were taken using the Kodak DC120 digital camera (Eastman Kodak) with KDS1D program (Eastman Kodak).

The strength of interactions between individual pairs of proteins was determined by assaying β-galactosidase activity on bacterial suspensions as previously described (32). For individual protein assays, log-phase 30 °C bacterial cultures at cell densities of ~2–5 × 10^8 cells/ml (A_600 of ~0.3–0.7) were used. Under identical experimental conditions, we also carried out β-galactosidase activity measurements, resulting from the interaction between T25-zip and T18-zip leucine zipper motifs (29) to normalize individual protein assays. For individual pairs of protein-protein interactions, triplicate samples or more were studied.

Proteins and Western Blot Analyses—The FLAG or mRFP tagged constructs were placed into pT18- and pT25-based vectors, as described under "Experimental Procedures." BIAcore Surface Plasmon Resonance Analysis—Real time protein-interaction studies were examined on a BIAcore instrument (BIAcore X). RhlB RNA helicase and bovine serum albumin were immobilized on dextran coated CM5 sensor chips. Procedures were performed as described by Amersham Biosciences. Briefly, the chip surface was first activated by injection of 35 μl of 1:1 mixture of 0.4 M N-ethyl-N-(dimethylaminopropyl) carbodiimide hydrochloride and 0.1 M N-hydroxysuccinimide. Native RhlB RNA helicase (70 μl of 35 μg/ml) in 100 mM phosphate buffer, 50 mM NaCl, pH 7.0, (optimal buffer condition for helicase activity) was immobilized on a CM1 chip. The immobilized enzyme was subjected to a flow rate of 50 μl/min (as indicated) on one flow cell, and an additional flow cell was prepared as a blank background for immobilization of bovine serum albumin (50 μg/ml) under the same buffer conditions. Remaining activated groups on each flow cell were blocked by injection of 35 μl of 1 M ethanolamine, pH 8.5. The chip was then washed twice with 10 μl of 1 M NaOH to remove any non-covalently bound material. The system was then primed with the running buffer (100 μM phosphate buffer, pH 7.0, 50 mM NaCl).

The interaction assays were performed with a constant (10, 20, or 30 μl/min as indicated) flow rate at 25 °C. Individually distinct concentrations, ranging from 5 to 50 nM (as shown in Fig. 3) of purified native RhlB RNA helicase, PNPass, or enolase were injected as analytes. The chemical binding groups were regenerated by sequentially washing the analytes with 10 μl of injection of 0.1 M NaOH, 0.1 M glycine–HCl (pH 3.5) and 10% ethanol until a background level was attained.

Helicase Activity and PNPass Assay Activity Assays—RNA oligonucleotides were used for RNA helicase unwinding and the PNPass degradation assay were double-stranded substrates that resulted from the annealing of a short single stranded oligo 5′-ACA GUA UUU GGU ACU ACU GCG CUC U (synthesized by Dharmacon Research Inc.). Underlined nucleotides indicate the complementary region with the short strand RNA oligo. The RNA sequences of long strand RNA are identical to the 5′-end sequences of RNAI, the antisense RNA of ColEl-type plasmids (18), and contain an RNase E cleavage site (33–35). Both strands were 5′-end labeled with [γ-32P]ATP as described previously (35). The labeled RNA strands were hybridized to form duplex RNA substrates made by combining "short" and "long" RNA in a 1:1.5 ratio. The excess of long RNA ensured that the majority of the labeled RNA formed duplex RNA substrates. RNA annealing was performed by adding equal volumes of 2× hybridization buffer (40 mM HEPES-KOH, pH 7.6, 1 mM EDTA) to long and short RNA mixtures, in which samples were heated to 95 °C for 5 min and then slowly cooled to room temperature. The duplex RNAs (25 fmol) were incubated with purified RhlB RNA helicase alone or with mixtures of PNPass and RhlB RNA helicase at 37 °C in final reaction volumes of 20 μl containing 20 mM HEPES-KOH, pH 7.6, 1 mM EDTA, 2 mM dithiothreitol, 0.1 mg of bovine serum albumin, 1 mM magnesium acetate, and 3 mM ATP. Each reaction was terminated by adding a 2 μl of stop solution (2.2% Ficoll 400, 0.11 M EDTA, 1.1% SDS, 0.275% bromphenol blue, 0.275% xylene cyanol FF). Samples were separated on a 16% polyacrylamide native gel (19:1 bisacrylamide) in 1× TBE. Labeled RNAs were visualized by autoradiography and quantified by LAS-1000 plus (Fuji Film).

RESULTS

Degradosome Protein Components Are Able to Interact in Vivo Independently of RNase E—General methods to identify interacting proteins or to study protein-protein interactions have been developed extensively. Among them, the two-hybrid systems currently represent the most powerful in vivo approaches. Here, we applied the E. coli two-hybrid approach (29) to study possible interactions among degradosome protein components. Individual degradosome component proteins were fused to two complementary fragments of the catalytic domain of Bordetella pertussis adenylate cyclase into plasmids pT25 and pT18-based vectors, as described under “Experimental Procedures.” As seen in Fig. 1A, the pink/red phenotypes displayed on MacConkey maltose plates demonstrate that RhlB RNA helicase is able to interact not only with the RE12 fragment (residues 684–784 of RNase E region are sufficient for interaction with the helicase) but also with itself and the PNPass. However, under the same conditions, no interactions between the RhlB and enolase or enolase and PNPass (Fig. 1C) were detected. We quantified the β-galactosidase activity in individual transformants grown to log-phase culture to determine the efficiency of adenylate cyclase functional complementation, representative of the strength of functional interactions between two protein components (29). As shown in Fig. 1B, the strength of functional interactions is similar between RhlB RNA helicase-RE12 and RhlB-RhlB RNA helicases and both are stronger (about 4-fold) than that of the RhlB RNA helicase-PNPass interaction. Consistently, the β-galactosidase activity assay indicated a lack of any interaction between enolase and the two degradosome component proteins, RhlB and PNPass (Fig. 1, B and C).

Individually Specific Interactions between RhlB RNA Helicase, PNPass, and RE12 Are Through the C-terminal Region of RhlB—To identify the regions of RhlB RNA helicase directly responsible for its protein-protein interactions, several trun-

1 G.-G. Liou, H.-Y. Chang, C.-S. Lin, and S. Lin-Chao, unpublished data.
Function of Degradosome Protein Components

41159

---

Real-time Detection of Protein-Protein Interaction by BLAcore Analyses Reveals Specific, Direct Binding of RhlB RNA Helicase to Itself and PNPase but Not to Enolase—To examine direct interactions between components of the degradosome, we used purified component proteins and an in vitro real-time binding assay using BLAcore apparatus (38). The unbound and FLAG-tagged protein components PNP, RhlB RNA helicase, and enolase were purified from B299 (an E. coli mutant containing only the RNase E N-terminal region (amino acid residues 1–602)), and their enzymatic activities were confirmed, as described under “Experimental Procedures.” Fig. 3A shows Coomassie Blue staining of high purity purified proteins. For the BLAcore surface plasmon resonance analyses (38), RhlB RNA helicase was first immobilized on a CM5 sensor chip, and different concentrations of three distinct native degradosome proteins, enolase, RhlB helicase, and PNPase (Fig. 3, B–D), were individually injected over the chip surface, and the sensograms recorded. In these analyses, the sensograms, in general, show an initial rapid increase in response units upon protein injection followed by the association of injected proteins with molecules coated on the chip and a slower association phase characteristic of reversible protein interaction, resulting in a plateau indicative of interacting saturation or equilibrium (Fig. 3). After a change of buffer, there is a rapid decrease in response unit/signal followed by the dissociation phase. As shown in Fig. 3B, the sensograms did not detect any RhlB-enolase interaction, whereas significant interaction responses were detected for RhlB with itself and with PNPase (Fig. 3, C and D, respectively). These results indicated that RhlB helicase was able to directly interact with PNPase and itself but not with enolase (Fig. 3B). The sensograms (Fig. 3, C–E) also indicated that the affinity of the RhlB RNA helicase interaction with itself was higher than that of its interaction with PNPase (only 12.5 nM is shown in Fig. 3E). These results are consistent with the results of our E. coli two-hybrid assay (Fig. 1B).

We further addressed whether the non-monomeric form of RhlB RNA helicase can directly interact with PNPase. As shown by sensograms, purified RhlB RNA helicase interacted with itself, after which these self-associated molecules of RhlB RNA helicase were still able to bind to PNPase (Fig. 4). Thus, these results illustrated that under the experimental conditions carried out, a dimeric (or multimeric) form of RhlB RNA helicase can physically bind to PNPase.

RhlB RNA Helicase Shows Functional Interaction with PNPase Independent of RNase E—In contrast to previously reported degradosome studies, which used a mini-degradosome complex containing the C-terminal region of RNase E, here we assessed the possibility of functional interaction between RhlB and PNPase in the absence of RNase E. The unwinding activity of RhlB RNA helicase was examined using classical gel electrophoresis assays (39), as described under “Experimental Procedures.” Both single-stranded RNA oligonucleotides were labeled with 32P and annealed to form a double-stranded RNA substrate (labeled as double-stranded RNA). As shown in Fig. 5, RhlB RNA helicase was able to unwind duplex RNA (Fig. 5, lanes 2–7), and this effect was RhlB-concentration-dependent (compare lanes 6 and 7 versus 5). As a result of the unwinding activity of RhlB, a decrease in double-stranded RNA and an increase in single-stranded RNA were observed. In the absence of RhlB under the same reaction conditions PNPase quickly degraded single-stranded RNA (labeled as long or short) but not...
double-stranded RNA (Fig. 5, lanes 8–10 versus 11). Collectively, these experiments indicate that the unwinding ability of RhlB enables PNPase to rapidly degrade duplex double-stranded RNA.

**DISCUSSION**

Here we report that RNA degradosome protein component RhlB interacts with PNPase independently of RNase E, which up to this point has been believed to be the necessary building scaffold for interactions among degradosome proteins. RhlB and PNPase interact through the C-terminal region of RhlB RNA helicase enabling PNPase to degrade double-stranded RNA substrates. We also show that monomers of RhlB interact among themselves, although at a site distinct from the one that interacts with PNPase. It is interesting that RhlB binds more strongly to itself and to an RNase E fragment than it does to PNPase. There are a number of reasons, both biochemical and cellular, that may explain this result. First, the interaction surface (between the two proteins) may be more favorable for RhlB binding to RNase E than for the RhlB-PNPase interaction. Second, from a cellular perspective, RNase E is a membrane-associated protein of relatively low abundance compared with the highly abundant cytoplasmic PNPase; PNPase is present in *E. coli* in excess of RNase E and has been detected in cells unattached to the RNase E scaffold (27). If the RhlB-PNPase interaction is stronger than the RhlB-RNase E interaction, this may affect the availability of RhlB to bind to RNase E and form degradosome complexes. This in turn could compromise RNA degradation via a membrane-associated degradosome response.

Our results indicate that to degrade double-stranded RNA, PNPase would need to recruit RhlB helicase to open the double-stranded RNA secondary structure, thereby allowing the exonuclease action of PNPase to degrade the RNA, lending support to the hypothesis that DEAD box RhlB helicase function is necessary for degradosome-mediated RNA decay. Our findings also suggest that multimers of RhlB RNA helicase may form and bind directly to molecules of PNPase, independently of RNase E, to form mini-degradation engines in *E. coli* capable of removing RNA degradation intermediates (products) that have secondary structures.
A 3′–5′ exonuclease complex, termed the “exosome,” has been identified in yeast cells (41, 42) with exosome protein component equivalents also discovered in mammalian cells (43). Recent modeling experiments (44) suggest that exosomes may use the 3′–5′ exonuclease PNPase at their core. Although, E. coli degradosome models have considered RNase E to be at the core of this bacterial ribonucleolytic complex (25), our discovery of PNPase-based complexes that do not include RNase E, provides a molecular basis for alternative pathways of E. coli RNA decay independent of RNase E-mediated endonucleolytic cleavages. Recent evidence suggesting that functional inactivation of chloramphenicol acetyltransferase mRNA in E. coli cell extracts occurs by a mechanism other than endonucleolytic cleavage (45), and earlier findings showing that mutations of the RNase E-encoding rne gene affects chemical half-life but not functional decay (13) are consistent with this view.

It has been suggested that enolase is the β subunit of αβ2 form PNPase (PNPase is the α subunit) (4), although no experimental proof has been forthcoming. However, our results indicate that RhlB RNA helicase, not enolase, is a native component of the PNPase complex. We detected no binding between enolase and PNPase (a similar conclusion also has been discussed by Kühnel and Luisi (Ref. 46; see “Discussion”). The yeast two-hybrid system also failed to detect any interaction between the two proteins (25). Furthermore, we have shown in this study that RhlB contains as ATP-dependent RNA unwinding ability (not demonstrated prior to this study) and that this ATP-activated unwinding activity enables PNPase to degrade double-stranded RNA; whether the binding of RhlB to PNPase regulates PNPase catalytic activity or vice versa remains to be clarified.

Far Western and yeast two-hybrid analyses of interactions between RNase E truncated polypeptides and RhlB have shown that RhlB binds directly to both the central (500–752) and C-terminal (734–1061) regions of RNase E, suggesting that important determinants for the binding are located in the small overlap region (734–752) of RNase E segments; other interactions among degradosome protein components were not detected (25). Using E. coli two hybrid and BiAcore biosensor

**FIG. 3. Kinetic analyses of degradosome protein component interactions.** A, Coomassie Blue staining of purified proteins PNPase, RhlB RNA helicase, and enolase separated by 8% SDS-PAGE. Protein purification is described under “Experimental Procedures.” B–D, kinetic data set collected for individual analytes (i.e. degradosome protein components) binding to an RhlB-surface chip. Degradosome component equivalents also discovered in mammalian cells and PNPase that degrades double-stranded RNA substrates. The nucleotide sequences of both short and long RNA substrates are described under “Experimental Procedures.” C, comparison of the kinetic data for RhlB RNA helicase, enolase, and PNPase, respectively, injected over the RhlB surface chip. No response was detected for enolase-RhlB, however, a “strong” response was detected for PNPase-RhlB, and an even stronger response for RhlB-RhlB interaction reaction. A detailed account for the basic shape of the graph can be found under “Experimental Procedures” and “Results.”
analyses, we detected RhlB self-interaction and direct binding to PNPase, in addition to its interaction with the RNase E (684–784). These results indicate that the protein conformation of various fusion products of RhlB and PNPase used for yeast and *E. coli* two-hybrid analyses differ and thus that it is appropriate to explore multiple approaches for the study of protein-protein interactions. Use of the BLAcore biosensor to analyze real time interactions among degradosome protein components and between protein component and RNA substrates to determine their kinetic constants may help to elucidate the mechanism by which the dynamic processes of degradosome assembly and RNA degradation interrelate.

**Acknowledgments**—Plasmid-based vectors pT18 and pT25 and *E. coli* strains were kindly provided by Dr. D. Ladant, Institut Pasteur, Paris Cedex, France. We thank our English Editing consultant Dr. K. Deen for his constructive comments concerning this manuscript and Drs. S. N. Cohen and C. Wang for their critical comments on this manuscript.

**REFERENCES**

1. Carpousis, A. J., Van Houwe, G., Ehretsmann, C., and Krisch, H. M. (1994) *Cell* 76, 889–900
2. Py, B., Causton, H., Mudd, E. A., and Higgins, C. F. (1994) *Mol. Microbiol.* 14, 717–729
3. Miczak, A., Kaberdin, V. R., Wei, C. L., and Lin-Chao, S. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 3865–3869
4. Py, B., Higgins, C. F., Krisch, H. M., and Carpousis, A. J. (1996) *Nature* 381, 169–172
5. Carpousis, A. J., Vanzo, N. F., Raynal, L. C. (1999) *Trends Genet* 15, 24–28
6. Coburn, G. A., and Mackie, G. A. (1999) *Prog. Nucleic Acids Res. Mol. Biol.* 61, 55–108
7. Gruberg-Manago, M. (1999) *Annu. Rev. Genet.* 33, 193–227
8. Nicholson, A. W. (1999) *FEBS Microbiol. Lett.* 23, 371–390
9. Rauhut, R., and Klug, G. (1999) *FEBS Microbiol. Lett.* 23, 353–370
10. Régnier, P., and Arraiano, C. M. (2000) *BioEssays* 22, 235–244
11. Steege, D. A. (2000) *RNA* 6, 1079–1090
12. Apirion, D. (1978) *Genetics* 90, 659–671
13. Ono, M., and Kuwano, M. (1979) *J. Mol. Biol.* 129, 343–357
14. Jain, C., Deana, A., and Belasen, J. G. (2002) *Mol. Microbiol.* 43, 1053–1064
15. Ghora, B. K., and Apirion, D. (1978) *Cell* 15, 1055–1066
16. Lin-Chao, S., Wei, C.-L., and Lin, Y.-T. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 12406–12411
17. Mudd, E. A., Krisch, H. M., and Higgins, C. F. (1990) *Mol. Microbiol.* 4, 2127–2135
18. Lin-Chao, S., and Cohen, S. N. (1991) *Cell* 65, 1233–1242
19. Régnier, P., Gruberg-Manago, M., and Portier, C. (1987) *J. Biol. Chem.* 262, 63–68
20. Kalman, M., Murphy, H., and Cashel, M. (1991) *Proc. Natl Acad. Sci. U. S. A.* 88, 895–899
21. Spring, T. G., and Wold, F. (1971) *J. Biol. Chem.* 246, 6797–6802
22. Blum, E., Py, B., Carpousis, A. J., and Higgins, C. F. (1997) *Mol. Microbiol.* 26, 387–394
23. Bessarab, D. A., Kaberdin, V. R., Wei, C.-L., Liou, G.-G., and Lin-Chao, S. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 3517–3516
24. Kaberdin, V. R., Miczak, A., Jakobsen, J. S., Lin-Chao, S., McDowall, K. J., and von Gabain, A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 11637–11642
25. Vano, N. F., Li, Y. S., Py, B., Blum, E., Higgins, C. F., Raynal, L. C., Krisch, H. M., and Carpousis, A. J. (1998) *Genes Dev.* 12, 2770–2781
26. Coburn, G. A., Xiao, X., Briant, D. J., and Mackie, G. A. (1999) *Genes Dev.* 13, 2594–2603
27. Liou, G.-G., Jane, W.-N., Cohen, S. N., Lin, N.-S., and Lin-Chao, S. (2001) *Proc. Natl. Acad. Sci. U. S. A.* 98, 63–68
28. Kido, M., Yamanaoka, K., Mitani, T., Niki, H., Ogura, T., and Hiraga, S. (1996) *J. Bacteriol.* 178, 3917–3925
29. Karinova, G., Pidoux, J., Ullmann, A., and Ladant, D. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 5752–5756
30. Hanahan, D. (1983) *J. Mol. Biol.* 166, 557–580
31. Tabor, S., and Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 1074–1078
32. Miller, J. H. (1972) *Experiments in Molecular Genetics*, pp. 352–355, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
33. Lin-Chao, S., Gong, T. T., McDowall, K. J., and Cohen, S. N. (1994) *J. Biol. Chem.* 269, 10797–10803
34. McDowall, K. J., Lin-Chao, S., and Cohen, S. N. (1994) *J. Biol. Chem.* 269, 10796–10796
35. McDowall, K. J., Kaberdin, V. R., Wu, S. W., Cohen, S. N., and Lin-Chao, S. (1995) *Nature* 374, 287–290
36. de la Cruz, J., Kressler, D., and Linder, P. (1999) *Trends Biochem. Sci.* 24, 192–198
37. Luking, A., Stahl, U., and Schmidt, U. (1998) *Crit. Rev. Biochem. Mol. Biol.* 33, 259–296
38. Hutchinson, A. M. (1995) *Biotecnol. 3*, 47–54
39. Matson, S. W., and Bean, D. W. (1995) Methods Enzymol. 262, 389–405
40. Deleted in proof
41. Mitchell, P., Petfalski, E., Shevetreno, A., Mann, M., and Tollervey, D. (1997) *Cell* 91, 457–466
42. Mitchell, P., and Tollervey, D. (2000) *Nat. Struct. Biol.* 7, 843–846
43. Chen, C.-Y., Gherzi, R., Ong, S. E., Chan, E. L., Rajmakers, R., Pruijn, G. J., Stoecklein, G., Moroni, C., Mann, M., and Karin, M. (2001) *Cell* 107, 451–464
44. Aloy, P., Ciccarelli, F. D., Leutwein, C., Gavin, A. C., Superti-Furga, G., Bork, P., Bötcher, B., and Russell, R. B. (2002) *EMBO Rep.* 3, 628–632
45. Lee, K., and Cohen, S. N. (2001) *J. Biol. Chem.* 276, 23599–23604
46. Kühnel, K., and Luisi, B. F. (2001) *J. Mol. Biol.* 313, 583–592

Function of Degradosome Protein Components