Protein–protein interactions with subunits of human nuclear RNase P

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A yeast two-hybrid system was used to analyze interactions among the protein subunits of human nuclear RNase P themselves and with other interacting partners encoded in a HeLa cell cDNA library. Subunits hpop1, Rpp21, Rpp29, Rpp30, Rpp38, and Rpp40 are involved in extensive, but weak, protein–protein interactions in the holoenzyme complex. Rpp14, Rpp20, and Rpp30 were found to have strong interactions with proteins encoded in the cDNA library. The small heat shock protein 27, which interacts with Rpp20 in the two-hybrid assay, binds to Rpp20 during affinity chromatography and can be found to be associated with, and enhances the activity of, highly purified RNase P. RNase P activity in HeLa cell nuclei also increases under the stress of heat shock.

heat shock | Hsp27 | nuclear proteins | precursor tRNA

The RNA component of eubacterial RNase P is the catalytic subunit, and the single protein subunit serves as an essential cofactor *in vivo* (1). In HeLa cells, this nuclear enzyme consists of a noncatalytic RNA subunit and at least eight protein subunits (2–5). The substantial, comparative increase in the number of protein substrates in eukaryotic nuclear RNase P is consistent with the additional functional complexity of the enzyme. Although the subunit composition of eukaryotic nuclear RNase P is reasonably well characterized, little is known about the spatial organization of these subunits and about the proteins other than RNase P subunits with which each may interact *in vivo*. Accordingly, we used the yeast two-hybrid system to gain a better understanding of these issues.

One of the proteins found to interact with an RNase P subunit (Rpp20) is the small heat shock protein 27 (Hsp27). This protein is expressed in a well-regulated manner in many cell types and tissues (6–9). Its intracellular levels are correlated with the development and decay of thermotolerance (10, 11), and it has an identifiable function in several intracellular processes (12–16). In addition to the results obtained by the genetic, two-hybrid analysis, we confirmed the Rpp20–Hsp27 interaction by direct biochemical study. Hsp27 not only binds to Rpp20 specifically but also can affect the functional properties of the RNase P holoenzyme complex.

Materials and Methods

Yeast Two-Hybrid Screening and Analysis. Two-hybrid assays were performed essentially as described in the GIBCO/BRL product protocol for the ProQuest Two-Hybrid System. Full-length cDNAs of human nuclear RNase P subunits [hpop1 and Rpp14, 20, 21 (N. Jarrous, D. Wesolowski, C. Guerrier-Takada, and S.A., unpublished observations), 29, 30, 38, and 40] were cloned in the pDBLeu (LEU2) vector to produce the bait fusion protein pDB-RppX (X represents any of the RNase P subunits). That subunit genes were in-frame with the Gal-4 sequence in pDBleu was confirmed by DNA sequencing. The pDB-RppX constructs were used to screen a HeLa cell cDNA library constructed at the *Sal*I–*Not*I site of the Gal-4 activation domain of plasmid pPC86 (TRP1) (GIBCO/BRL). Screening was performed by sequential transformation of bait and library vectors in the *Saccharomyces cerevisiae* reporter strain MaV203 (*MAT*α *leu*2–3, 112, *trp* 1–901, *his*3Δ200, *ade*2–101, *gal*4Δ, *gal*80Δ, *SPAL*10::*URA*3, *GAL*1::*lacZ*, *HIS*3$_{\text{UAS GAL1}}$::*HIS*3@*LYS*2, *can*1$^{\text{R}}$, *cyh*2$^{\text{R}}$). Transformants were plated on SC-Leu-Trp-Ura-His + 3-aminotriazole (3-AT) (25 mM) and incubated at 30°C for up to 1 week and then left at room temperature for another 2 weeks. Large colonies (diameter >2 mm) were restreaked on SC-Leu-Trp-Ura-His + 3-AT (25 mM) plates. This step eliminated most of the false positives. Confirmation of positives (including replica plating, replica cleaning, and 5-bromo-4-chloro-3-indolyl β-D-galactoside analysis) was performed according to the ProQuest Two-Hybrid system protocol. The interacting partners were sequenced and the clones were identified through a National Center for Biotechnology Information BLAST search against a "nonreductant" database.

To test protein interactions between RNase P subunits, 81 cotransformants were obtained by cotransformation of pDB-RppX (including pDBleu) and pPC-RppX (including pPC86) and were plated on SC-Leu-Trp-Ura- His + 3-AT (10 mM) at 30°C until large colonies appeared (2–3 weeks). A few colonies from each transformation were restreaked on SC-Leu-Trp-Ura-His + 3-AT (10 mM) plates. The plates were incubated at 30°C for 4 days and then at room temperature for another 2 weeks.

Interactions between Hsp27 and RNase P subunits were determined by cotransfection pDB-RppX (or pDB-Hsp27) and pPC-Hsp27 (or pPC-RppX) constructs in pairs in MaV203. Positive interactions were determined by the ability of the transformed yeast cells to grow on SC-Leu-Trp-Ura-His + 3-AT (25 mM) media and for their capacity to express β-galactosidase as visualized by 5-bromo-4-chloro-3-indolyl β-D-galactoside analysis.

All media used in these studies were prepared according to ProQuest Two-Hybrid system protocols.

Cell Culture, Heat Shock, and Cellular Fractionation. HeLa S3 cells were grown in DMEM (GIBCO/BRL) supplemented with 10% FBS, 100 μg/ml each of penicillin and streptomycin, and 0.01% of Fungizone (GIBCO/BRL) in a humidified, 6% CO_2 atmosphere at 37°C. When cells reached 60% confluence on 10-cm-diameter plates, they were harvested, pooled, and resuspended in the above medium. The cells were divided into several aliquots and either incubated at 37°C or heat-shocked at 44°C at various times as indicated in the figure legends.

Preparation of nuclear and cytoplasm subcellular fractionation was performed as described by Curry *et al.* (17).

Protein Assay and Immunoblot Analysis. Protein concentration was measured with a protein assay reagent (Bio-Rad) according to manufacturer's instructions. For immunoblot analysis, proteins were separated on 12% polyacrylamide/SDS gels (except as indicated in figure legends) and electroblotted onto nitrocellu-

Abbreviations: Hsp27, heat shock protein 27; 3-AT, 3-aminotriazole.

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lose membranes. Membranes were immunoblotted with mouse monoclonal anti-human Hsp27 (StressGen Biotechnologies, Victoria, Canada) and rabbit polyclonal anti-Rpp20 or anti-Rpp29 as described (2).

Affinity Chromatography of Hsp27. His-tagged, recombinant Rpp20 (6× His-Rpp20) was expressed as described (3) and affinity-purified with the use of His·Bind resin (Novagen) according to the Novagen pET system manual.

The purified, recombinant Rpp20 was bound to His·Bind resin in Eppendorf tubes and washed with 10 volumes of 1× binding buffer and 6 volumes of 1× wash buffer according to manufacturer's instructions. A HeLa cell extract (dialyzed against 1× wash buffer) was mixed with the resin to which Rpp20 was bound or with resin itself. Bound proteins were stripped with 5 volumes of 1× strip buffer and precipitated with 6% trichloroacetic acid.

Assay for Human RNase P Activity. Each RNase P reaction mixture of 10 μl contained 50 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NH_4Cl, 4 units RNasin, and 2,000 cpm precursor tRNA substrate. The substrate, precursor to suppressor $tRNA^{Ser}$ (pSupS1) from *Schizosaccharomyces pombe*, was transcribed in the presence of [α-^{32}P]GTP. Reaction mixtures for the assay of RNase P activity were incubated at 37°C for 1–10 min as indicated in the figure legends, and the reaction was terminated by the addition of an equal volume of a 10 M urea/dye solution saturated with phenol. The cleavage products were separated on 8% denaturing polyacrylamide gels by electrophoresis at 40 mA for 1.5 h. Quantitation of precursor tRNA and mature tRNA species was carried out with a PhosphorImager.

Northern Blotting. RNA was extracted from HeLa and yeast cells as described (18, 19). Northern hybridizations were performed as described (20). Oligonucleotides complementary to human nuclear mature $tRNA^{Leu}$, 5S rRNA, and yeast mature $tRNA^{Leu}$ were 5′-CCCACGCTCCATCCGG-3′, 5′-AAAGCCTACAGCACCCGGTAT-3′, and 5′-GCATCTTACGATACCTG-3′, respectively. All oligonucleotides were end-labeled with T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (Amersham Pharmacia).

Results

Screening of a HeLa Cell cDNA Library. Eight complete ORFs (Rpp14, Rpp20, Rpp21, Rpp29, Rpp30, Rpp38, Rpp40, and hpop1), corresponding to eight previously identified subunits of human nuclear RNase P, were fused to the Gal-4 DNA binding domain in the vector pDBleu (pDB-RppX) and the Gal-4 activation domain in the vector pPC86 (pPC-RppX) as described in *Materials and Methods*. A HeLa cell cDNA library was fused to the Gal-4 activation domain in the vector pPC86. Both pDB-RppX (or pPC-RppX) and pPC86 (or pDBleu) were introduced into the host strain, MaV203, and the transformed strains were plated on various concentrations of 3-AT to detect background activation of the *HIS*3 reporter gene. 3-AT (5 mM) inhibited background activation of the *HIS*3 reporter gene of the 16 transformants that were picked. Accordingly, to make selections stringent, 10 mM 3-AT was used in the detection of protein–protein interactions among subunits of RNase P, and 25 mM 3-AT was used for selection of interactions of these protein subunits with other proteins that were expressed by the cDNA library. The interacting partners were then identified as described in *Materials and Methods*. More than 10^6 transformants were tested for each of eight protein subunits as bait. Table 1 summarizes the outcome of the two hybrid screens.

As shown in Table 1, under test conditions only Rpp14, Rpp20, and Rpp30 have strongly interacting partners. Rpp21, Rpp29, Rpp38, Rpp40, and hpop1 have no strongly interacting partners but do exhibit weaker interactions, as shown in the table and as described below.

Table 1. Identification by yeast two-hybrid screening of proteins that interact with human nuclear RNase P protein subunits

Bait	Prey	Interacting partner
Rpp20	Hela cell cDNA library*	Small heat shock protein 27 (Hsp27)
		KIAA0615 (unknown function)
Rpp14		Human LIM domain-containing gene (LIMD1)
		OIP2 [*Neisseria gonorrheae* opacity-associated (Opa) protein interacting protein]
		HSPC232 (unknown function)
		Novel sequence (unknown function)
Rpp30		Rpp14
Rpp21†		No strongly interacting partner
Rpp29		
Rpp38		
Rpp40		
hpop1		

*The prey in every case was a HeLa cell cDNA library.
†The remaining proteins listed below had no strongly interacting partners.

Two proteins were identified as interacting strongly with Rpp20. One is a protein of unknown function, KIAA0615, and the other is Hsp27.

Four proteins were identified as Rpp14 interacting partners. One, LIMD1, is part of a DNA-binding family (21) and another, OIP2, is related to an exoribonuclease (22, 23). Using Rpp30 as bait, we identified four colonies as positives. All of them encoded the same sequence, Rpp14. None of the other subunits of RNase P manifested strong or intermediate interactions under test conditions with products expressed from the cDNA library.

Interactions Between Pairs of RNase P Subunits. No strong interactions were observed in our two-hybrid assay. The methodology cannot easily distinguish between weak interactions and no interactions, as described in the protocol for the ProQuest system (see *Materials and Methods*). The procedure thus was modified to allow transformants to grow on SC-Leu-Trp-Ura-His + 3-AT (10 mM) for 4–5 days at 30°C and then for another 2 weeks at room temperature. Transformants then were restreaked on the same selection medium. Weakly interacting partners then were easily distinguished from the negative control (data not shown). Thus, we detected weak interactions between certain pairs of human RNase P subunits (Table 2). Rpp21, Rpp29, Rpp30, Rpp38, Rpp40, and hpop1 are involved in extensive contacts in the human nuclear RNase P complex (see below and Fig. 5 for discussion). We note, however, that not all physical interactions that are detectable by two-hybrid assay are reciprocal, i.e., the same when the two members of a particular pair of proteins are tested alternatively as bait and as prey.

Biochemical Verification of Complex Formation Between Hsp27 and Rpp20. Hsp27 interacts with Rpp20 specifically as determined by the yeast two-hybrid assay. To confirm the validity of the genetic results and to show that they were not artifactual consequences of the yeast host cell background, we tested one of the predictions that resulted from the two-hybrid assay by biochemical study. Because heat shock proteins are subjects of intense study, we investigated the putative interaction between Hsp27 and with Rpp20, both as an isolated protein, and by inference, in the RNase P holoenzyme complex.

His-tagged Rpp20 was expressed in and purified from *Escherichia coli* (BL21) and then was bound to a nickel resin as

Table 2. Growth of cotransformants (DB-RppX and PC-RppX) on SC-Leu-Trp-Ura-His+3AT (10 mM)

	PC86	PC-Rpp14	PC-Rpp20	PC-Rpp21	PC-Rpp29	PC-Rpp30	PC-Rpp38	PC-Rpp40	PC-hpop1
DBleu	−	−	−	−	−	−	−	−	−
DB-Rpp14	−	−	+−	+−	−	+	+	+−	+−
DB-Rpp20	−	−	−	−	−	−	−	−	+−
DB-Rpp21	−	+	+	−	+	+	+	+	+
DB-Rpp29	−	+	−	+	−	+	+−	+−	+
DB-Rpp30	−	++	+−	+	+	+	+	+	+−
DB-Rpp38	−	+−	+	+	−	+	+	−	+
DB-Rpp40	−	+	+	++	+	+	−	−+	+−
DB-hpop1	−	+	+	+	+	+	+	+	+

Plus and minus signs indicate the degree of growth or absence of growth, respectively.

described in *Materials and Methods*. A HeLa cell-free crude extract was mixed with the resin to which Rpp20 was bound. After nonspecifically bound proteins were washed off, Rpp20 and other tightly bound proteins were stripped from the resin. The proteins were collected and subjected to SDS/PAGE and visualized by silver staining or transferred to a nitrocellulose membrane for Western blotting. Silver staining showed, in addition to a major Rpp20 band, several other species that were recovered from resin to which Rpp20 was bound. These proteins were not eluted from resin alone (i.e., no Rpp20 prebound to the resin) mixed with the crude extract or an analysis of the resin with only Rpp20, and no other proteins bound to it (Fig. 1*A*). Western blotting showed that one of the bands that bound specifically to the resin with Rpp20 associated with it (Fig. 1*B*) was recognized by monoclonal antibody against Hsp27. Hsp27 appears as a dimer, consistent with previous observations that this protein forms dimers and multimers with ease (24). This result indicates that Hsp27 from HeLa cells can bind specifically to Rpp20.

Hsp27 Is Enriched in RNase P Active Fractions During RNase P Purification. Human RNase P was purified through the MonoQ FPLC step. The specific activity of RNase P in the fractions was determined with pSupS1 as the substrate (Fig. 2 *A* and *B*; see *Materials and Methods*). The peak of RNase P activity was around fraction 24. When the MonoQ fractions were screened by Western blot analysis with anti-Hsp27 monoclonal antibody (Fig. 2*C*) and anti-Rpp20 and anti-Rpp29 polyclonal antibodies (data not shown), Hsp27 was detected only in and adjacent to the peak of active RNase P and was found in the same fractions as the human RNase P subunits Rpp20 and Rpp29. However, when very narrow cuts were taken of RNase P activity in earlier purification steps for use on MonoQ and S6 columns, no Hsp27 was found in the RNase P eluted from the latter columns (data not shown). Therefore, the presence of Hsp27 is not essential for RNase P activity, but it may contribute to some other property or function of the holoenzyme complex.

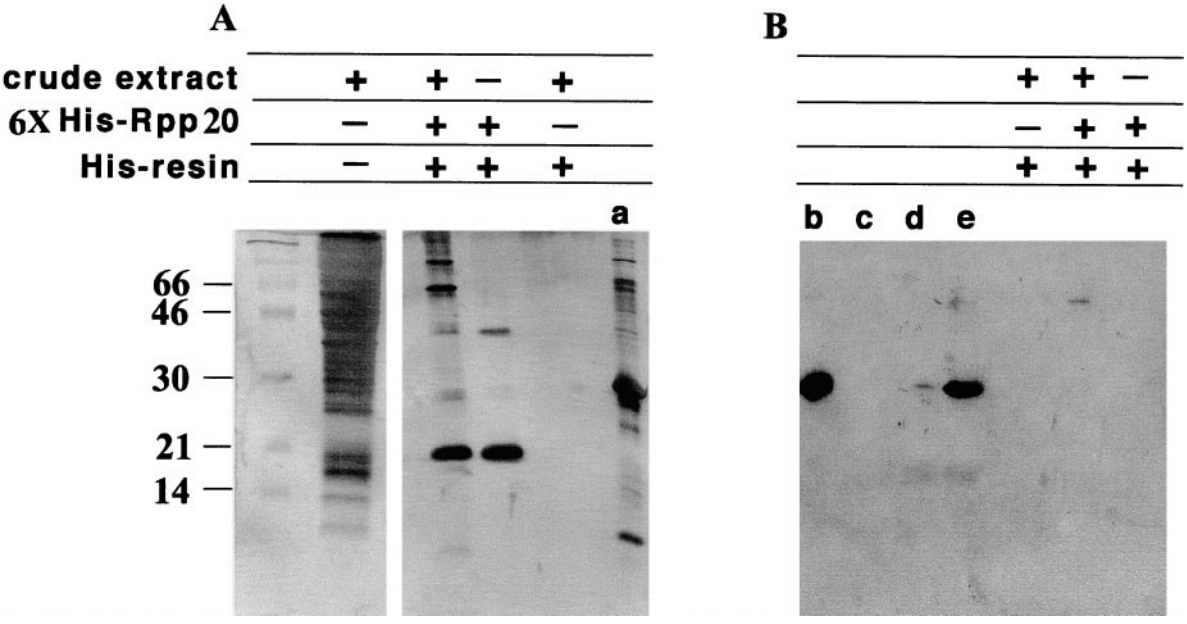

Fig. 1. Hsp27 in HeLa cell crude extracts binds specifically to Rpp20 on His-binding resin. Histidine-tagged Rpp20 was expressed in *E. coli* (3) and affinity-purified with the use of His·Bind resin as described in *Materials and Methods*. (*A*) HeLa cell-free extract was added to resin to which Rpp20 was bound, or to resin alone, and proteins that bound specifically to the column were stripped off as described in *Materials and Methods*. These proteins were separated in a 15% SDS/PAGE gel and were visualized by silver staining (*A*) or were electrotransferred to nitrocellulose filters and immunoblotted with monoclonal antibody against Hsp27 (StressGen; see *B*) and Rpp20 (not shown). Leftmost lane: molecular mass markers, molecular masses (in kDa) are indicated at the left of the figure; lane a: 15 ng Hsp27; lane b: 5 ng Hsp27; lane c: 5 ng Rpp20; lane d: proteins from a nuclear extract of non-heat-treated HeLa cells; lane e: proteins from a nuclear extract of heat-shocked HeLa cells.

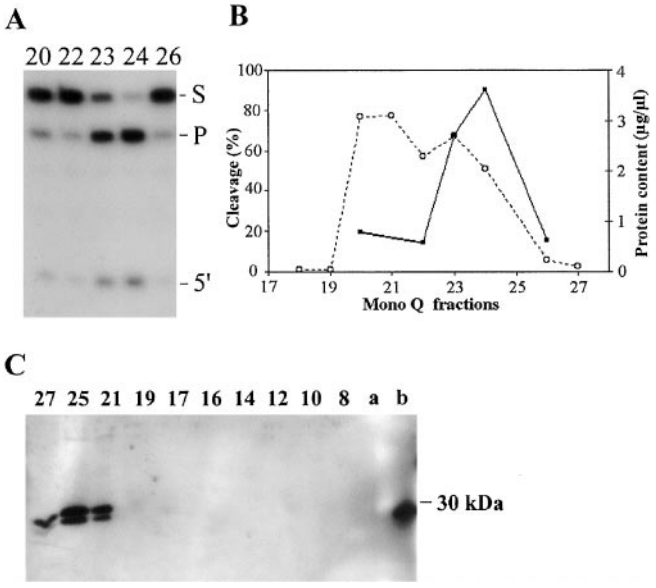

Fig. 2. Hsp27 copurified with human RNase P activity eluted from the Mono Q column. Purification of human RNase P was performed as described (2). The lane numbers indicate the fractions from a Mono Q column. (*A*) Aliquots that contained an equal mass of protein from Mono Q fractions were used for RNase P activity assay. (*B*) Protein concentration of Mono Q fractions (○) and the cleavage of pSupS1 by 0.25 μg of each Mono Q fraction are indicated (■). (*C*) Aliquots (5 μl) from fractions eluted from Mono Q column were tested by Western blot analysis for the presence of Hsp27 with the use of monoclonal antibody against Hsp27. Lane a: rainbow size marker; lane b: purified Hsp27. RNase P activity assay was performed at 37°C for 5 min as described in *Materials and Methods*. S, substrate; P, tRNA-containing products; 5′, 5′ leader portion of the products.

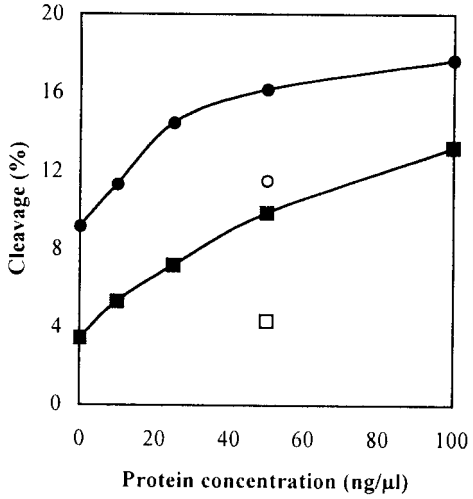

Fig. 3. Stimulation of RNase P activity by Hsp27. Stimulation by Hsp27 of human nuclear RNase P activity was investigated by mixing 0.115 μg of enzyme purified through a Mono Q column step with different amounts of Hsp27 or 50 ng of BSA. The mixtures were incubated at 37°C (●, Hsp27; ○, BSA) or 43°C (■, Hsp27; □, BSA) for 30 min. After incubation at room temperature for another 30 min, the RNase P activity was assayed at 37°C for 10 min with the use of pSupS1 as substrate (see *Materials and Methods*).

Hsp27 Stimulates Human Nuclear RNase P Activity. RNase P activity was enhanced in a concentration-dependent manner by the addition of Hsp27 to reaction mixtures. This enhancement was considerably more than could be achieved by the addition of BSA (Fig. 3); the latter is a phenomenon that had been noted previously and does not depend on the temperature of preincubation (Table 3 and ref. 2). The degree to which Hsp27 enhanced the RNase P activity increased with temperature of preincubation (heat shock) of RNase P and Hsp27 (Table 3); i.e., the higher is the temperature of preincubation, the larger is the stimulatory factor. Furthermore, if the enhancement factor is multiplied by the standardized RNase P (alone) activity at the different temperatures, the result in every case was to restore the specific activity of the holoenzyme close to the standardized value at 37°C. We infer that the function of Hsp27 *in vivo* may be to maintain homeostasis of RNase P specific activity in this case and more generally for other essential enzymes.

To determine whether the stimulation of RNase P activity *in vitro* was paralleled by a similar phenomenon *in vivo*, we assayed RNase P activity in HeLa cell nuclear extracts and measured intracellular levels of Hsp27. Nuclear RNase P activity increases about 2-fold, and Hsp27 levels rise in nuclei after heat shock (Fig. 4 *A* and *B*). Steady-state levels of precursor tRNA, mature tRNA, and 5S RNA also were measured during heat shock by Northern blot analysis (Fig. 4*C*). Steady-state levels of precursor tRNA decrease rapidly after heat shock, whereas the steady-state levels of mature tRNA and 5S RNA were not significantly altered.

Discussion

With the identification of several subunits of human nuclear RNase P, interest can now focus on the architecture of the holoenzyme and its function *in vivo*. The nature of this RNase P raises questions concerning the need for the large number of subunits and the functional and physical relationship of the entire complex, and its individual subunits, to other enzymes and proteins with which they interact *in vivo*. An exhaustive two-hybrid screening assay of proteins encoded by a HeLa cell cDNA library, which uses all human nuclear RNase P subunits as bait, has been used to identify the proteins that interact with the holoenzyme complex. In our experiments, five of the eight subunits tested, hpop1, Rpp21, Rpp29, Rpp38, and Rpp40, have no strongly interacting partners. Rpp30 interacts with Rpp14, whereas Rpp20 and Rpp14 show strong interactions with proteins other than RNase P subunits. No interactions between RNA and protein or RNA and RNA would have been found with the two-hybrid assay.

Our data indicate that Rpp20 interacts with a small heat shock protein, Hsp27, which has been implicated previously in several intracellular processes and which we discuss further below. Rpp14 interacts with a protein that contains a DNA-binding domain and that has a possible role in tumor suppression as well

Table 3. Temperature-dependent activation of human RNase P activity by Hsp27

	P	P+Hsp27	P+BSA	P+Hsp27/P	P+BSA/P	P/P (37°)
30°C	11.6	17.2	13.8	1.5	1.2	1.3
37°C	9.2	19.4	12.3	2.1	1.3	1.0
43°C	3.5	13.1	4.3	3.8	1.2	0.4
50°C	1.1	6.1	1.9	5.4	1.7	0.1

Human nuclear RNase P (115 ng) was incubated at the temperature shown on the left by itself, with 500 ng Hsp27 or with 500 ng BSA (see legend to Fig. 4). The effect of the preincubation temperature on HspP27 activation of human nuclear RNase P activity was assessed. The numbers in the leftmost three columns represent percent cleavage of substrate pSupS1 (errors in individual measurements are about 10%), and the following ratios are shown in the three rightmost columns: activity of enzyme with Hsp27 divided by activity of enzyme alone (P+Hsp27/P); activity of enzyme with BSA divided by activity of enzyme alone (P+BSA/P); activity of enzyme alone at different temperatures of preincubation versus enzyme alone at 37°C [P/P (37°)].

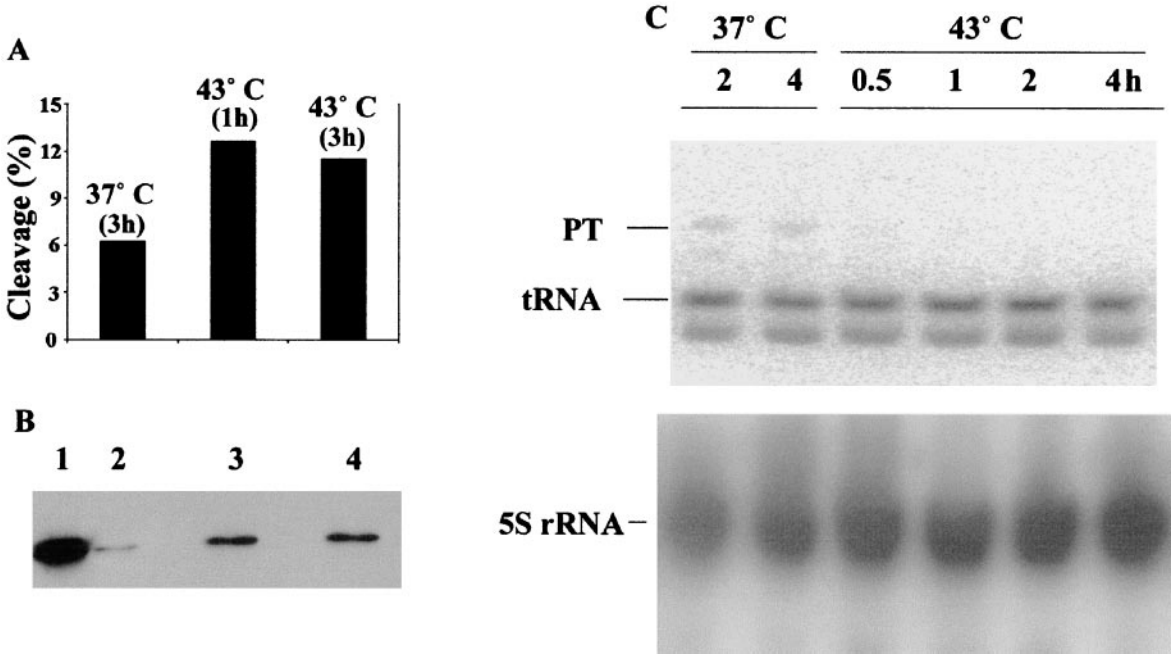

Fig. 4. (*A*) Heat shock treatment of HeLa cells induced an increase in nuclear RNase P-specific activity. (*B*) Accumulation of Hsp27 in nuclei. (*C*) tRNA processing after heat shock. HeLa cell culture and heat shock treatment, preparation of nuclear extracts, and assay of RNase P activity are described in *Materials and Methods*. (*A*) Assay of RNase P activity in nuclear extracts performed by mixing 1 μg of nuclear extracts with pSupS1 for 5 min at 37°C. (*B*) Aliquots of 15 μg of nuclear proteins were subjected to 12% SDS/PAGE and transferred to a nitrocellulose filter. The filter was probed with monoclonal antibody against Hsp27 (as shown in *B*, lane 1, recombinant Hsp27 from *E. coli*; lanes 2–4: nuclear extracts of non-heat-treated cells or cells heat treated for 1 and 3 h at 43°C, respectively). (*C*) Total RNA was extracted from heat-treated HeLa cells (heat shock performed at 43°C for 0.5, 1, 2, 4 h) and non-heat-treated cells (harvested after incubation at 37°C for 2 and 4 h as controls). Ten micrograms of total RNA samples was separated on 8% polyacrylamide/7 M urea gel, transferred to nylon membrane, and blotted with a γ-^{32}P-labeled oligonucleotide complementary to mature tRNA^{Leu} (*Upper*), and the same filter was reprobed with a γ-^{32}P-labeled oligonucleotide that is complementary to human 5S rRNA (*Lower*). PT, the primary transcript; tRNA, the mature tRNA.

as another protein that has homology to an exoribonuclease implicated in tRNA biosynthesis. These putative interactions of Rpp20 and Rpp14 all occur with proteins, the function of which should occur in the nucleus, where RNase P is in fact known to reside. It seems reasonable to conclude that Rpp14 and Rpp20 and/or RNase P may be involved in the regulation of various intracellular processes or the biosynthesis of stable RNAs through links to other nuclear proteins *in vivo*. These links may be direct or indirect (through other proteins) physical contacts or indirect functional contacts, e.g., through common substrates.

We also tested the interactions between all possible pairs of RNase P protein subunits. No strong interactions between pairs of human nuclear RNase P protein subunits were identified under the conditions used. Such interactions may depend on the subunits already being part of a complex with H1 RNA, the RNA subunit of the enzyme. However, we did detect weak interactions between several pairs of subunits. In particular, hpop1, p21, p29, p30, p38, and p40 are involved in extensive contacts in the human RNase P complex. These results lead to a testable model of the organization of the subunits in space (Fig. 5). Furthermore, as shown in Fig. 5, Rpp20 and Rpp14 are available for interactions with non-RNase P proteins. [The four subunits represented in red are in contact with the RNA subunit of the enzyme (T.J., C. Guerrier-Takada, and S.A., unpublished observations).] In the absence of data on the stoichiometry of the subunits, none is represented more than once in the figure.

The yeast two-hybrid analysis showed that a small heat shock protein, Hsp27, interacts with one of the human nuclear RNase P subunits, Rpp20, which has endogenous ATPase activity (Y. Li and S.A., unpublished observations). Hsp27 has already been implicated in the regulation of several physiological processes (25–27). We showed that Hsp27 in HeLa cell nuclear extracts interacts specifically with His-tagged Rpp20 bound to a Ni resin column. The amount of Hsp27 detected by affinity purification and Western blotting increased after heat shock of HeLa cells. Hsp27 is associated with RNase P during purification of the enzyme if broad cuts of RNase P activity are pooled in early stages of fractionation before further purification. Previous experiments have shown that off-peak fractions, where Hsp27 is

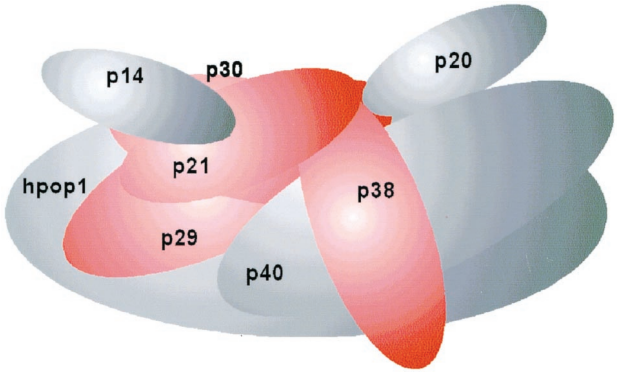

Fig. 5. A model of the results of the two-hybrid analysis of interactions between protein subunits in human RNase P. The scheme is based on the results of the two-hybrid assay (Table 2) that showed a weak interaction between certain pairs of subunits. No information is available regarding the stoichiometry of subunits, and, therefore, each subunit is represented only once. The subunits shown in red are also in contact with H1 RNA, the RNA subunit of human RNase P (see text). The subunits are designated by their apparent molecular masses (2–4) (N. Jarrous, D. Wesolowski, C. Guerrier-Takada, and S.A., unpublished observations) rather than by their full designations (e.g., p20 rather than Rpp20) to reduce crowding in the figure.

found in early stages of the purification process, do not have the same stoichiometry of subunits as peak RNase P fractions. Nevertheless, these data together show that Hsp27 physically interacts with an individual subunit of the RNase P complex and can be found in association with the active complex.

Hsp27 also affects the function of RNase P. Addition of this protein to highly purified RNase P stimulates activity of the enzyme in a temperature-dependent manner, as might be expected. The higher the preincubation temperature of Hsp27 and RNase P together, the greater the stimulatory effect. Indeed, the data show roughly that Hsp27 maintains a homeostasis of RNase P specific activity unlike that of a nonspecific stabilizer of RNase P, BSA. We have been unable to identify phosphorylase or kinase functions in Hsp27 (data not shown).

The effect of heat shock stress on RNA metabolism has been well characterized. Heat shock inhibits the splicing of mRNA (25, 26) and results in pronounced inhibition of the accumulation of 18S and 28S ribosomal RNA. The primary effect of elevated temperature on RNA metabolism in mammalian cells is inhibition of processing and/or transport (27). Under heat shock stress, RNA polymerase III transcription of 5S RNA remained unaffected (27, 28), and accumulation of transfer RNA was also not affected (27, 29). We also found that the steady level of tRNA is not altered after heat shock. However, the steady level of precursor tRNA decreased dramatically after heat shock. Because transcription by RNA polymerase III of tRNA genes is unaffected *in vivo* at 43°C, the half-life of precursor tRNAs must diminish; i.e., processing is likely accelerated. This conclusion is consistent with our results regarding the amount of Hsp27 in extracts of heat-shocked cells and the enhancement of RNase P activity by Hsp27 *in vitro*.

Rpp20 and the yeast protein Rpp 2 share considerable identity. Stolc *et al.* (30) earlier showed a decrease in tRNA processing in a yeast strain conditional for Rpp 2 expression. Deletion of small heat shock proteins Hsp26 and Hsp42, which might be functional analogues of human Hsp27 in *Saccharomyces cerevisiae*, does not result in a temperature-sensitive phenotype: growth rate and survival at elevated temperature were unaffected in those mutants under a variety of conditions (19, 31). We also examined the effects on tRNA processing in *S. cerevisiae* strains harboring deletions (separately) for Hsp26 and Hsp42 (there is no physical homologue of Hsp27 in yeast): we found none (data not shown). We conclude that these yeast heat shock proteins are not functional homologues of Hsp27 and/or do not interact with the Rpp2, RNase P holoenzyme, or any other proteins involved in tRNA processing.

Finally, further confirmation of the results of the two-hybrid assay will involve extensive protein–protein cross-linking studies of the human nuclear RNase P holoenzyme. The results of the two-hybrid assay are reflected in one instance in the biochemical reality of a physical association of Hsp27 with Rpp20 and with RNase P. It remains to be shown exactly how Hsp27 exerts its stimulatory effect on RNase P function.

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