Limited Portability of G-Patch Domains in Regulators of the Prp43 RNA Helicase Required for Pre-mRNA Splicing and Ribosomal RNA Maturation in *Saccharomyces cerevisiae*

Daipayan Banerjee, Peter M. McDaniel, and Brian C. Rymond¹

Department of Biology, University of Kentucky, Lexington, Kentucky 40506-0225

ABSTRACT The Prp43 DExD/H-box protein is required for progression of the biochemically distinct pre-messenger RNA and ribosomal RNA (rRNA) maturation pathways. In *Saccharomyces cerevisiae*, the Spp382/Ntr1, Sqs1/Pfa1, and Pxr1/Gno1 proteins are implicated as cofactors necessary for Prp43 helicase activation during spliceosome dissociation (Spp382) and rRNA processing (Sqs1 and Pxr1). While otherwise dissimilar in primary sequence, these Prp43-binding proteins each contain a short glycine-rich G-patch motif required for function and thought to act in protein or nucleic acid recognition. Here yeast two-hybrid, domain-swap, and site-directed mutagenesis approaches are used to investigate G-patch domain activity and portability. Our results reveal that the Spp382, Sqs1, and Pxr1 G-patches differ in Prp43 two-hybrid response and in the ability to reconstitute the Spp382 and Pxr1 RNA processing factors. G-patch protein reconstitution did not correlate with the apparent strength of the Prp43 two-hybrid response, suggesting that this domain has function beyond that of a Prp43 tether. Indeed, while critical for Pxr1 activity, the Pxr1 G-patch appears to contribute little to the yeast two-hybrid interaction. Conversely, deletion of the primary Prp43 binding site within Pxr1 (amino acids 102–149) does not impede rRNA processing but affects small nucleolar RNA (snoRNA) biogenesis, resulting in the accumulation of slightly extended forms of select snoRNAs, a phenotype unexpectedly shared by the prp43 loss-of-function mutant. These and related observations reveal differences in how the Spp382, Sqs1, and Pxr1 proteins interact with Prp43 and provide evidence linking G-patch identity with pathway-specific DExD/H-box helicase activity.

KEYWORDS *Saccharomyces cerevisiae*; spliceosome; rRNA processing; DExD/H-box protein; pre-mRNA splicing

NUMEROUS genome-wide interaction and gene expression studies identify ribosome biogenesis as a central integrative feature of *Saccharomyces cerevisiae* metabolism (see, e.g., Mnaimneh *et al.* 2004; Davierwala *et al.* 2005; Gavin *et al.* 2006; Collins *et al.* 2007; Hu *et al.* 2007; Magtanong *et al.* 2011). In rapidly dividing organisms such as this budding yeast, ribosomal RNAs (rRNAs) make up the lion's share of cellular nucleic acid by mass, while ribosomal protein transcripts can account for more than half the transcribed messenger RNA (mRNA). Because ribosome biogenesis is so energetically costly, eukaryotes have evolved multiple means to regulate rRNA and ribosomal protein pro-

duction in response to changes in cellular demand and surveillance systems to remove aberrant ribosomal protein complexes formed during assembly or after environmental insult (Jorgensen *et al.* 2002; Fingerman *et al.* 2003; Fromont-Racine *et al.* 2003; Jorgensen *et al.* 2004; Marion *et al.* 2004; Henras *et al.* 2008; Kressler *et al.* 2010; Lafontaine 2010). The coordination of pre-mRNA processing with ribosome biogenesis is especially relevant in the intron-poor environment of the yeast genome, where the highly expressed ribosomal protein transcripts represent a disproportionate amount of the spliced mRNA (Ares *et al.* 1999; Staley and Woolford 2009). Our understanding of how this coordination is accomplished is limited, however, to general principles supported by a few specific examples where individual ribosomal proteins act as feedback regulators to inhibit the processing or stability of cognate ribosomal protein transcripts (Li *et al.* 1995, 1996; Vilardell *et al.* 2000; Pleiss *et al.* 2007; Gudipati *et al.* 2012).

The chemistry of pre-mRNA splicing and the initial stages of rRNA processing occur in spatially separable nuclear

Copyright © 2015 by the Genetics Society of America

doi: 10.1534/genetics.115.176461

Manuscript received November 19, 2014; accepted for publication March 22, 2015; published Early Online March 25, 2015.

Supporting information is available online at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.176461/-/DC1>

¹Corresponding author: Department of Biology, University of Kentucky, 675 Rose Street, Lexington, KY 40506-0225. E-mail: rymond@uky.edu

locations catalyzed by distinct macromolecular machineries. Pre-rRNA processing involves more than 200 proteins and includes 75 small nucleolar RNA particles (snoRNPs) composed of C/D- or H/ACA-box small nucleolar RNAs (snoRNAs) with associated conserved sets of proteins (Grandi *et al.* 2002; Fromont-Racine *et al.* 2003; Reichow *et al.* 2007; Kressler *et al.* 2010; Phipps *et al.* 2011). The nuclear pre-mRNA splicing enzyme is likewise complex and composed of roughly 80 yeast proteins and 5 essential small nuclear RNAs (snRNAs) (Fabrizio *et al.* 2009; Pena *et al.* 2009). Although largely non-overlapping in composition, the pre-rRNP and spliceosomal complexes share a limited number of factors, including the essential *Snu13* protein constituent of the U3 (preribosomal) snoRNP and the U4 (spliceosomal) snRNP and the phylogenetically conserved DEAH-box protein *Prp43*. DEAH-box proteins are structurally related members of the DExD/H-box family of RNA-dependent NTPases that resolve RNA/RNA helices or act as RNAPases to dissociate protein-RNA interactions during macromolecular assembly/disassembly events (Linder and Jankowsky 2011; Cordin *et al.* 2012; Rodriguez-Galan *et al.* 2013). *In vitro*, DExD/H-box proteins typically act as nonspecific NTP/ATPases, with a subset showing nucleic acid strand separation or helicase activity. With few exceptions (see, e.g., Schwer 2008; Hahn *et al.* 2012; Mozaffari-Jovin *et al.* 2012), the RNA features or *trans*-acting factors contributing to helicase substrate specificity *in vivo* remain unknown.

While the 19 RNA helicases implicated in ribosome biogenesis typically are restricted to either large- or small-subunit-delimited steps, *Prp43* promotes multiple RNA processing events in both 25S and 18S rRNA maturation (Lebaron *et al.* 2005; Combs *et al.* 2006; Leeds *et al.* 2006; Bohnsack *et al.* 2009; Rodriguez-Galan *et al.* 2013). The yeast *Prp43* protein and its mammalian homolog DHX15 also act to dislodge the intron from the postcatalytic spliceosome and to recycle essential snRNP factors for use in subsequent rounds of splicing (Arenas and Abelson 1997; Martin *et al.* 2002; Tsai *et al.* 2005; Wen *et al.* 2008; Fourmann *et al.* 2013). *Prp43* activity contributes to the maintenance of spliceosome integrity because reduced *Prp43* function promotes the use of structurally aberrant spliceosomes and the splicing of suboptimal pre-mRNA substrates (Pandit *et al.* 2006; Koodathingal *et al.* 2010; Mayas *et al.* 2010; Chen *et al.* 2013). In addition to features of the postcatalytic spliceosome, specific changes in spliceosome composition linked to ATP hydrolysis by the *Prp2*, *Prp16*, and *Prp22* DExD/H-box proteins render defective splicing complexes sensitive to *Prp43* recruitment and ATP-dependent dissociation (Chen *et al.* 2013).

Data from several groups implicate three *Prp43*-interacting factors in the regulation of this protein's role in pre-mRNA splicing (*Spp382/Ntr1*) and pre-rRNA processing (*Sqs1/Pfa1* and *Pxr1/Gno1*) (Guglielmi and Werner 2002; Lebaron *et al.* 2005; Tsai *et al.* 2005; Boon *et al.* 2006; Pandit *et al.* 2006; Tanaka *et al.* 2007; Tsai *et al.* 2007; Lebaron *et al.* 2009; Pertschy *et al.* 2009; Walbott *et al.* 2010; Christian *et al.* 2014). *Spp382* is an essential pre-mRNA splicing factor required for *Prp43* recruitment to the spliceosome. *Pxr1* is nec-

essary for efficient rRNA maturation at the A0, A1, and A2 processing sites and plays a second, separable role in the final steps of *Rrp6*-dependent 3'-end processing of snoRNAs. *Sqs1* is not required for efficient yeast growth but appears to play a nonessential role in 20S to 18S rRNA processing by the *Nob1* endonuclease. Although otherwise dissimilar in sequence, the *Spp382*, *Pxr1*, and *Sqs1* proteins each contain a weakly conserved 45- to 50-amino-acid glycine-rich G-patch motif (Pfam: PF01585) (Punta *et al.* 2012) found in select RNA-associated proteins (Aravind and Koonin 1999). Point mutations within the *SPP382*, *PXR1*, and *SQS1* G-patch coding segments can block or weaken the corresponding protein interaction with *Prp43* and inhibit pre-mRNA splicing or rRNA processing (Guglielmi and Werner 2002; Pandit *et al.* 2006; Tanaka *et al.* 2007; Lebaron *et al.* 2009). In addition, *Spp382* and *Sqs1* peptides bearing the G-patch domain stimulate the RNA-dependent helicase activity of *Prp43* *in vitro* (Tanaka *et al.* 2007; Lebaron *et al.* 2009; Christian *et al.* 2014). While critical to each protein's biological activity, prior work leaves unresolved whether the *Spp382*, *Sqs1*, and *Pxr1* G-patch domains are equivalent interfaces that promote a common *Prp43* activity or serve a more complex function in the pre-mRNA splicing and rRNA processing pathways.

Here we report the results of experiments to investigate *Prp43*-G-patch protein interaction and to probe the role of the G-patch domain in *Prp43*-sensitive RNA biogenesis. The data show that although the G-patch is sufficient to interact with *Prp43*, the *Spp382*, *Sqs1*, and *Pxr1* G-patch peptides differ in yeast two-hybrid (Y2H) response and in the ability to reconstitute *Spp382* activity in splicing and *Pxr1* activity in rRNA processing. Point mutagenesis of the G-patch domain shows that functional reconstitution of *Spp382* is sequence dependent but does not directly correlate with strength of the *Prp43* Y2H response. The *Prp43*-*Pxr1* interaction was found to be largely G-patch independent and driven by an ~50-amino-acid *Pxr1* peptide found downstream of the G-patch motif. Curiously, while efficient *Pxr1*-dependent rRNA processing does not require this *Pxr1* domain, loss of either this site or diminished *Prp43* activity results in snoRNA processing defects. These observations support a model in which G-patch identity contributes pathway-sensitive information relevant to *Prp43* function in the parallel processes of pre-mRNA splicing and rRNA maturation and suggest an unanticipated role for *Prp43* activity in snoRNA processing.

Materials and Methods

Yeast strains and culture

The yeast strains used in this study are presented in Table 1. The oligonucleotides used for cloning and mutagenesis are listed in Supporting Information, Figure S1. Yeast cultures were grown in YEP medium (1% Bacto yeast extract, 2% Bacto peptone, made 2% with glucose or galactose) prior to plasmid transformation by the lithium chloride method (Ito *et al.* 1983). For all other purposes, yeast cultures were grown

Table 1 Yeast Strains

Strain	Genotype	Reference
SP102	<i>a ura3Δ0 trp1-289 leu2 Δ0 his3 Δ1 spp382::KAN</i> and <i>p416-GAL1::spp382-4</i>	Pandit <i>et al.</i> 2006
pJ69-4a	<i>a trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80 Δ LYS2:: GAL1-HIS3</i> <i>GAL2-ADE2 met2:: GAL7-lacZ</i>	James <i>et al.</i> 1996
LZΔpxr1	<i>a pxr1:: KAN leu2Δ0 ura3Δ0 his3Δ1</i>	T. Zhang and B. C. Rymond, unpublished material
BY4742	<i>α his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Winzeler <i>et al.</i> 1999
prp43H218A	<i>α ura3-52, trp1-63, his3 Δ200, leu2-1, ade2-101, ade2-101, lys2-801,</i> <i>prp43::KAN, p358-prp43H218A, TRP1</i>	Martin <i>et al.</i> 2002
PRP43	<i>α ura3-52, trp1-63, his3 Δ200, leu2-1, ade2-101, ade2-101, lys2-801,</i> <i>prp43::KAN, p358-PRP43, TRP1</i>	Martin <i>et al.</i> 2002

on synthetic complete medium with single or double amino acid dropouts as needed (Kaiser *et al.* 1994).

Yeast two-hybrid (Y2H) assays

The *SPP382* and *SQS1* yeast pACT Y2H constructs were described previously (Pandit *et al.* 2006, 2009). The protein coding sequences of gene derivatives new to this study were fused to the *GAL4* activation-domain (AD) and/or the *GAL4* *GAL4* DNA binding-domain (BD) sequences in the plasmids pACT2 and pAS2 (Harper *et al.* 1993), respectively, and transformed into yeast strain pJ69-4a (James *et al.* 1996). To compare the relative Y2H responses of yeast transformants, the strains were first cultured to saturation, collected by centrifugation, and washed twice with sterile water. Next, the cultures were normalized to equivalent OD₆₀₀ values, and four 10-fold serial dilutions of each culture were spotted on dropout medium lacking leucine and tryptophan for simple growth of the double transformant or, for *GAL1-HIS3* reporter-gene assay, medium lacking histidine supplemented with 5–20 mM 3-aminotriazole (3-AT) where indicated. The two-hybrid response was scored by overall spot density and, in the most dilute samples, relative colony size after 2–4 days of incubation at 23 or 30° on selective medium.

Plasmid constructs

The *spp382ΔG*-patch deletion construct was made by inverse PCR from the previously described YCplac111-*SPP382* plasmid (Pandit *et al.* 2006) using Phusion High-Fidelity DNA Polymerase (New England Biolabs, Beverly, MA) and oligonucleotide primers with terminal *SacII* sites flanking G-patch domain codons 61–108. The corresponding *SPP382*, *SQS1*, and *PXR1* G-patch add-back cassettes were amplified from BY4742 genomic DNA by PCR with primers containing terminal *SacII* sites and inserted into the *SacII* site of YCplac111-*spp382ΔG*. An equivalent PCR-based strategy was used to create the ΔG-patch and G-patch segment add-back derivatives in the pACT-*SPP382* background (Pandit *et al.* 2009). The *pxr1* G-patch point mutations were introduced by inverse PCR on a pTZ18U-*PXR1* G-patch subclone, and the mutated segments were subsequently transferred into the YCplac111-*spp382ΔG* or into the pACT-*spp382ΔG* plasmid background as *SacII* DNA fragments. Where indicated, *URA3*-based plasmids were removed by plasmid shuffle on medium containing 0.75 mg/ml 5-fluoroorotic acid (FOA) (Boeke *et al.* 1987).

The wild-type (WT) *PXR1* gene was amplified by PCR from BY4742 yeast genomic DNA with primers placed 300 bp upstream and downstream of the ORF (Figure S1) and cloned into the *KpnI* site of plasmid YCplac111. The G-patch domain was subsequently deleted from the YCplac111-*PXR1* construct by inverse PCR with primers flanking this domain. PCR-amplified *SPP382*, *SQS1*, and *PXR1* G-patch cassettes were introduced into the linearized YCplac111-*pxr1ΔG* plasmid as blunt-ended DNA fragments. For the Y2H study, the *PXR1* ORF was inserted into the *SmaI* site of pACT2. The indicated *PXR1* domain deletions were introduced into pACT2-*PXR1* by inverse PCR. The *PXR1* 102–149 and 150–226 codon single-domain constructs were made similarly by PCR fragment insertion into pACT2.

The *PRP43* deletions were constructed by inverse PCR on pAS2-*PRP43* (Pandit *et al.* 2009) with domain-specific primers (Figure S1). The *Prp43* single-domain Y2H constructs were amplified with oligonucleotides containing 5'-terminal *NdeI* sites (NTD, Ratchet, and WH domains) or *BamHI* sites (RecA1, RecA2, and CTD) inserted into corresponding restriction sites on pAS2. The *prp43H218A* mutant allele and its WT *PRP43* counterpart expressed from plasmid p352 were described previously (Martin *et al.* 2002).

RNA methods

The yeast cultures were grown at 30° to an OD₆₀₀ of 0.4 in selective dropout medium, collected by centrifugation, and washed twice with ice-cold RNA extraction (RE) buffer (100 mM LiCl, 100 mM Tris-HCl, pH 7.5, and 1 mM EDTA), and the pellets were stored at –80° until needed. The cold-sensitive *prp43(H218A)* mutant and its isogenic *PRP43* control culture were grown for 15 hr at 17° prior to cell harvest. Yeast culture conditions for the isolation of RNA from the SP102 transformants after glucose repression of the *GAL1::spp382-4* gene were described previously (Pandit *et al.* 2006).

For RNA isolation, the cell pellets were broken with a Mini-Beadbreaker (Biospec Products, Bartlesville, OK) for 4 min in 400 μl of RE buffer, 150 μl of phenol:chloroform:isoamyl alcohol (PCI, 50:48:2), and 300 μl of sterile glass beads (0.5 mm diameter; Biospec Products). The cellular debris, glass beads, and PCI were removed by centrifugation at 18,000×g for 3 min at 4°. The supernatant was PCI extracted three additional times, followed by a final chloroform extraction and ethanol precipitation. Then 20 μg of RNA was resolved

on a 1% agarose-formaldehyde gel, transferred to an Immobilon+ membrane, and then hybridized with random prime-labeled probes (Invitrogen, Carlsbad, CA) against *RPS17A*, *ADE3*, or U6 snRNA under standard conditions (Sambrook *et al.* 1989). Alternatively, 5'-end-labeled oligonucleotide probes were used to detect the rRNA precursor, intermediates, and products and snR128 and snR18 (Figure S1) using previously published conditions (Guglielmi and Werner 2002; Kos and Tollervey 2005). The bands of hybridization were visualized with a Typhoon 8600 Phosphoimager and quantified with ImageQuant software after background subtraction (GE Healthcare Life Sciences, Pittsburgh, PA).

Protein methods

Prior to harvest, the yeast cultures were grown at 30° to an OD₆₀₀ of 2–4 in selective dropout medium. Protein extraction was performed as described previously (Zhang *et al.* 2011). Briefly, 7 OD of culture was collected by centrifugation (14,000×g for 1 min) and the pellets resuspended in 500 μl of 2 M LiOAc for 5 min at room temperature. The cells then were recovered by centrifugation and resuspended in 500 μl of 0.4 M NaOH for 5 min at room temperature. The cells were again collected by centrifugation and finally resuspended in 250 μl of gel sample buffer [0.06 M-Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 0.0025% (w/v) bromophenol blue] (Horvath and Riezman 1994) and heated at 100° for 10 min. The insoluble material was removed by centrifugation as earlier, and 75 μg of protein was resolved by SDS-PAGE (7.5 or 10% polyacrylamide). The resolved proteins were electroblotted to Immobilon-P membrane (Millipore, Bedford, MA), and Western blot images were obtained using a 1:1500 dilution of the HA.11 anti-HA antibody (Covance, Princeton, NJ) for the pACT2 and pAS2 vector constructs or an anti-Gal4 activation-domain antibody (1:500 dilution; sc-1663; Santa Cruz Biotechnology, Santa Cruz, CA) for the *Spp382*-bearing pACT constructs. The pAS2-*Prp43* construct also was imaged using the mouse monoclonal anti-Gal4 DNA binding domain antibody (1:500 dilution; sc-577). Mouse monoclonal antibody 12G10 (1:1000 dilution; Developmental Studies Hybridoma Bank, Iowa City, IA) was used to image α-tubulin as a loading control. Secondary antibodies conjugated to alkaline phosphatase diluted 1:1500 in PBS/5% non-fat dry milk were used for protein detection with the ECF Detection Module (GE Healthcare Life Sciences) or the BCIP/NBT *In Situ* Detection System (Enzo Life Sciences, Farmingdale, NY). All antibodies were diluted in PBS made 5% w/v with nonfat dry milk. The ECF or scanned BCIP/NBT band intensities were quantified with ImageQuant software (GE Healthcare Life Sciences).

Results

Isolated *Spp382*, *Sqs1*, and *Pxr1* G-patch peptides interact with *Prp43* but differ in Y2H response

When the full-length G-patch proteins are scored by the Y2H assay for interaction with *Prp43*, we find a graded response

in *GAL1-HIS3* reporter-gene-dependent growth of *Spp382* ≥ *Sqs1* > *Pxr1* > empty vector (Figure 1A). Because the *Spp382*, *Sqs1*, and *Pxr1* proteins lack obvious sequence similarity beyond the G-patch (Figure 1B), this element likely functions, at least in part, as a common *Prp43* binding feature. In support of this, we find that the isolated G-patch segments also interact with *Prp43* by the Y2H assay in the order of *Spp382* > *Sqs1* > *Pxr1*, with the *Pxr1* G-patch showing a Y2H response near that of the empty-vector control. Both the *Sqs1* and *Pxr1* G-patch Y2H interactions with *Prp43* are lost when the assay is made more stringent for reporter-gene activation by the addition of 5 mM 3-aminotriazole (James *et al.* 1996), reinforcing the less robust *Prp43* Y2H response of these peptides (data not shown). While some experimental variability in protein abundance was observed, the three G-patch peptides are similarly expressed, with the *Sqs1* peptide generally accumulating to somewhat higher amounts (~twofold) than the *Spp382* or *Pxr1* G-patch peptides (Figure S2). It is possible that G-patch peptide abundance contributes to the differential *Prp43* Y2H response, although, as argued below, sequence differences also appear to affect *Prp43* interaction. Regardless, the Y2H responses show that at least for the *Spp382* and *Sqs1* proteins, the G-patch domain is sufficient for *Prp43* interaction *in vivo*.

Since the isolated G-patch peptides interact with *Prp43* less well than the full-length proteins, other portions of the *Spp382*, *Sqs1*, and *Pxr1* proteins likely stabilize the *Prp43* Y2H associations. For *Sqs1*, a second site of *Prp43* interaction has been reported within the first 202 amino acids of this protein, and as expected, the *Sqs1*ΔG protein continues to interact well with *Prp43* (Figure 2A) (Lebaron *et al.* 2009; Pandit *et al.* 2009). A second site of *Prp43* interaction is also present in the central portion of *Pxr1* (see below). The *Pxr1*-ΔG-*Prp43* Y2H response is not noticeably diminished compared with the full-length *Pxr1*-*Prp43* interaction, consistent with limited *Pxr1*-G-patch contribution to this association (Figure 2A). Consistent with an earlier report by Tsai *et al.* (2005), deletion of *Spp382* G-patch blocks all detectable *Prp43* interaction. While *Spp382* and *Pxr1* Y2H *Gal4* fusion proteins accumulate to similar levels, *Sqs1* is always found at much lower abundance, roughly 5–10% of this level based on the intensities of the common *Gal4* activation-domain epitope by Western blot analysis (Figure 2C; compare lanes 4 and 5 with lanes 2 and 3 and 6–10). This lower abundance is surprising given its comparatively robust *Sqs1*-*Prp43* Y2H response, although it is possible that shorter proteolytic fragments also contribute to the Y2H response. In no case does G-patch removal destabilize the *Gal4* fusion proteins. Therefore, the lack of *Spp382*ΔG-*Prp43* Y2H interaction is not due to protein instability, and other sites of *Prp43* contact, if present within *Spp382*, are insufficient to promote stable Y2H association.

Insertion of either the cognate *Spp382* G-patch or the *Sqs1* G-patch coding sequence into the *spp382*ΔG construct restores the *Spp382*-*Prp43* interaction, showing that, at least in this assay, flexibility is permitted in G-patch motif

Figure 1 Differential Y2H interaction of the Spp382, Sqs1, and Pxr1 G-patch peptides with Prp43. (A) Y2H assay performed in the yeast pJ69-4a reporter strain transformed with full-length PRP43 as a GAL4 DNA binding-domain (BD) fusion and the indicated full-length or G-patch-only gene segments as GAL4 activation-domain (AD) fusions. Tenfold serial dilutions were plated on medium that selects for the double-plasmid transformants (-leu -trp, 2 days' growth) or for reporter-gene transactivation (-his, 3.5 days' growth) at 30°. (B) Amino acid alignment of the G-patch sequences from yeast proteins Spp382, Sqs1, and Pxr1. The numbers refer to the G-patch positions within each protein. The G-patch peptides show 32–34% amino acid identity in each pairwise comparison. Sequence identities are shaded and indicated as a consensus for 2/3 (lowercase) and 3/3 (uppercase) matches, respectively.

(Figure 2B). A heterologous G-patch cassette might restore Prp43 interaction through direct contact or by changing the conformation of Spp382 in a such manner as to expose another site of interaction, possibly by acting as an appropriately configured spacer. The simple spacer model is unlikely, however, because an equivalently constructed Spp382-Pxr1 chimera is equally well expressed but fails to interact above background with Prp43 in the Y2H assay (Figure 2, B and C). Thus, a legitimate, albeit weakly interacting G-patch motif is insufficient to reconstitute stable Spp382-Prp43 association. The spp382-PXR1 two-hybrid construct also weakly inhibits growth in the absence of reporter-gene selection (Figure 2B, smaller individual colony sizes in left panel; see below), suggesting that the chimeric protein may sequester an important RNA processing factor in an inactive complex. Yeast toxicity was reported previously with SQS1 overexpression (Pandit *et al.* 2009), although this is less pronounced when expressed as a Y2H construct.

Spp382 G-patch identity is important for splicing-factor function

We next asked whether the differences in Prp43 Y2H interaction observed with the Spp382-G-patch chimeras correlate with changes in Spp382 biological activity. To do this, we repeated the domain-swap experiment in an otherwise intact SPP382 gene and scored the resulting chimeric derivatives for complementation of an spp382::KAN null mutation (Winzeler *et al.* 1999). To avoid complications from overexpression, the SPP382-chimeric genes were cloned on a single-copy LEU2-marked centromeric plasmid vector transcribed with the native SPP382 promoter. Because the spp382::KAN mutation is lethal, the chimeric genes were first transformed into yeast

that coexpress the biologically active and nutritionally regulated GAL1-spp382-4 allele (Pandit *et al.* 2006) and subsequently scored for independent function after GAL1-spp382-4 removal by plasmid shuffle. None of the chimeric constructs cause obvious growth inhibition when coexpressed with GAL1-spp382-4 on galactose medium (Figure 3, left panel). As anticipated, deletion of the G-patch segment inactivates SPP382 and results in a lethal (FOA) phenotype, while reinsertion of the cognate SPP382 G-patch coding segment restores activity (Figure 3, right panel). Equivalent results were obtained when these constructs were scored for complementation by meiotic segregation (data not shown). Comparable to what was seen with the Prp43 Y2H response, the Spp382-Sqs1 G-patch chimeric protein is functional and supports growth, albeit at a reduced level. The reduced growth corresponds to an ~50% increase in doubling time in liquid medium compared with the SPP382 control (Figure 3 and Figure S3). In contrast, the spp382-PXR1 G-patch chimera mimics the empty-vector control and is unable to rescue spp382::KAN lethality.

Functional G-patch reconstitution of the Spp382 splicing factor does not correlate with the strength of the Y2H response

The results presented earlier show that G-patch identity contributes context-specific information and suggest a possible relationship between Prp43 binding and G-patch protein function, specifically, that strong Prp43-G-patch interaction is favored for the Spp382 protein and that weaker Prp43-G-patch interaction is favored by Pxr1. In principle, differential G-patch affinity might contribute to the dynamics of Prp43 association with (or function within) the rRNA processing and pre-mRNA splicing machineries. To further investigate this relationship, we introduced point mutations within the G-patch domain of the spp382-PXR1 G-patch chimera and scored the mutated constructs for spp382::KAN complementation. In each case, the amino acid substitution converts the Pxr1 G-patch residue into the amino acid naturally present at the same position within the Spp382 G-patch (Figure 1B). The sites of mutagenesis were guided in part by the pattern of phylogenetic conservation (Figure S4, A and B) and include residues where protein family-specific differences appear to occur (*i.e.*, R27G, H55P, and K57E; the numbered coordinates refer to the amino acid positions within Pxr1) as well as residues where conservation is less apparent (P48G and D62M). The spp382-PXR1 (WT) and spp382-pxr1(P48G) chimeras do not support viability after removal of the cotransformed GAL1-spp382-4 gene (Figure 3, and data not show). In contrast, spp382-pxr1 G-patch chimeric constructs containing the R27G, H55P, K57E, and D62M mutations complement spp382::KAN (Figure 4A). There is considerable variation in culture growth, however, with H55P functioning much better than the others. While histidine is common at position 55 among Pxr1 homologs, approximately 65% of the 2124 G-patch peptides listed in the SMART protein domain database (Letunic *et al.* 2012) show proline at the equivalent location, including yeast

Figure 2 Prp43 activators differ in G-patch reliance for Y2H interaction. (A) Y2H assay as described in Figure 1A between Prp43 and the Spp382, Sqs1, and Pxr1 constructs before and after G-patch removal. (B) Y2H assay between Prp43 and full-length Spp382, the G-patch deletion derivative (*spp382ΔG*), and the constructs reconstituted from this deletion mutant by addition of the Spp382, Pxr1, or Sqs1 G-patch coding sequences. The -his medium used in panels A and B contained 5 mM 3-aminotriazole, and the plates were incubated for 4 days at 30°. (C) Western blot analysis of the Y2H proteins expressed as pACT-Gal4 activation fusions. The proteins were imaged with antibodies against the Gal4 activation domain (upper panel; Spp382, Spp382ΔG, Sqs1, Sqs1ΔG, Pxr1, Pxr1ΔG, and the Spp382ΔG construct reconstituted with

the Spp382, Sqs1, and Pxr1 G-patch), the Gal4 DNA binding-domain panel (middle panel; Prp43), and anti- α -tubulin (lower panel). The blots were developed using BCIP/NBT (Gal4 activation-domain antibody, α -tubulin antibody) and ECF (Gal4 DNA binding-domain antibody). The asterisks in the top panel indicate the positions of the three sets of G-patch proteins. For unknown reasons, the Sqs1 fusion proteins run more slowly than the similarly sized Spp382 proteins. The numbers to the left in the top panel refer to the sizes (in kDa) of the protein markers run in lane 1. After normalization with the tubulin control, the Gal-4 activation-domain band intensities were found to vary less than twofold with the exception of the full-length Sqs1 and Sqs1ΔG proteins, which are reproducibly found at 5–10% of the Spp382 and Pxr1 levels.

Spp382 (P90) and Sqs1 (P750). This residue is not essential for Spp382 function, however, because alanine, histidine, or serine substitution in an otherwise WT *SPP382* background has little impact on growth (Figure S5). Rather, the proline becomes critical in the context of the Spp382-Pxr1 chimera, which, in essence, contains the equivalent of a multiply mutated Spp382 G-patch.

We next monitored the efficiency of pre-mRNA processing in the *spp382-PXR1* G-patch chimeras after transcriptional repression of the *GAL1-spp382-4* gene. Yeast that express *GAL1-spp382-4* on galactose-based medium splice pre-mRNA well, whereas glucose repression of *GAL1-spp382-4* for 12 hr inhibits splicing and results in a fivefold or greater reduction in the ratio of processed to unprocessed *RPS17A* RNA (Figure 4B) (see Pandit *et al.* 2006). Yeast cotransformed with the *spp382ΔG* derivative process pre-mRNA no better than the empty-vector control, but splicing can be reconstituted with insertion of the cognate *SPP382* G-patch segment. Although incomplete *GAL1-spp382-4* repression overestimates splicing efficiency for the strongest mutants, the splicing patterns and growth characteristics of yeast transformed with the chimeric plasmids agree in direction, if not magnitude. Here the *spp382-PXR1* chimera and the noncomplementing P48G construct behave no better than the empty-vector control, while the biologically active *spp382-Sqs1* G-patch add-back and the viable *pxr1* chimeric mutant chimeric derivatives (with the possible exception of the R27G) show modestly improved splicing. Two viable chimeras with weak *spp382::KAN* complementation activity, K57E and D62M, also show pronounced rRNA processing defects, with two- to threefold increases in 35S rRNA precursor RNA compared with the other backgrounds (Figure 4C).

The Prp43 Y2H interaction pattern does not correlate directly with the pattern of *spp382::KAN* complementation by the mutated *spp382-pxr1* constructs. Here the H55P chimera, which best supports *spp382::KAN* complementation, performs similarly to the noncomplementing chimera bearing a WT *PXR1* G-patch in the Y2H assay (Figure 4D). *In vitro*, G-patch-containing peptides expressed from *spp382-PXR1* and *spp382-pxr1* (H55P) alleles show greater salt sensitivity for Prp43 interaction than the equivalent Spp382 peptide, consistent with lower affinity (Figure S6). In contrast, the more weakly complementing D62M and K57E chimeras and the noncomplementing P48G construct all support modestly greater Prp43 Y2H response, even though each construct is somewhat toxic when expressed without two-hybrid reporter selection (Figure 4D; compare left and right panels relative to *SPP382*-reconstituted and *spp382ΔG* control constructs). While recognizing that these are very subtle distinctions, *spp382-pxr1* derivatives are similarly expressed as Y2H constructs (Figure S7), arguing against differences in Gal4 activation-domain abundance contributing to the differential Y2H response. Taken together, these data show that while G-patch identity affects Spp382-Prp43 protein interaction, effective reconstitution of the Spp382 splicing factor requires G-patch function beyond that contributing to stable Prp43 interaction.

Pxr1 G-patch chimeras show variation in rRNA processing activity

The studies presented earlier establish an overlapping but not identical contribution of the Spp382, Sqs1, and Pxr1 G-patch domains in the context of the Spp382 pre-mRNA splicing factor, with the Sqs1 G-patch being more Spp382-like in reconstituting Prp43 interaction and biological activity. We

Figure 3 G-patch identity affects Spp382 function. Complementation of the lethal *spp382::KAN* mutation with a *LEU2*-marked plasmid bearing the WT *SPP382* gene, the G-patch deletion derivative, or the deletion derivative reconstituted with the *SPP382*, *SQS1* or *PXR1* G-patch coding sequences. These were initially cotransformed with the biologically active *URA3*-marked *GAL1-spp382-4* plasmid that supports growth on a galactose-based medium. Tenfold serial dilutions were plated on galactose (left) and glucose-based medium supplemented with 5-fluoroorotic acid (right) to select for yeast that have lost the *URA3*-marked *GAL1-spp382-4* plasmid.

next considered G-patch portability in the context of the *Pxr1* rRNA processing factor. To do this, the *PXR1* G-patch sequence was first removed by inverse PCR and then replaced with the cognate *PXR1* G-patch sequence or with a heterologous cassette from the *SPP382* or *SQS1* genes. Plasmids containing the chimeric *PXR1* G-patch constructs then were assayed for complementation of the viable, albeit very slowly growing *pxr1::KAN* null mutant (Winzeler *et al.* 1999).

Deletion of the G-patch (amino acids 25–72) inactivates *Pxr1* because the *pxr1ΔG* transformant grows no better than the untransformed *pxr1::KAN* parental host (Figure 5A) (see Guglielmi and Werner 2002). Reinsertion of the *PXR1* G-patch or the *SQS1* G-patch sequence into *pxr1ΔG* restores efficient growth, while substitution of the *SPP382* G-patch improves growth but at a much reduced level compared to what is seen with the intact *PXR1* allele. The same complementation pattern is seen when rRNA processing rather than yeast growth is monitored. As reported previously, the loss of *Pxr1* activity inhibits rRNA processing, most obvious here as increased accumulation of the 35S rRNA precursor and the generally low-abundance 23S processing intermediate (Figure 5B) (see Guglielmi and Werner 2002). The *pxr1::KAN* parental host and the *pxr1ΔG* mutant display equivalent rRNA processing defects consistent with the G-patch deletion allele having a null phenotype. Protein reconstruction with the cognate *Pxr1* G-patch peptide restores full activity, while insertion of either the *Sqs1* or *Spp382* G-patch peptides supports partial function, with the *Sqs1* add-back showing a more complete recovery of rRNA processing efficiency. Thus, similar to what was seen with the *Spp382* chimeras, some flexibility in G-patch structure is tolerated in *Pxr1*, here again with the *Sqs1* G-patch serving as the preferred heterologous cassette.

The primary Prp43 binding site within Pxr1 is not required for efficient rRNA biogenesis but contributes to snoRNA processing activity

Since removal of the G-patch inactivates *Pxr1* for rRNA processing but not *Prp43* binding, additional sites of *Prp43* con-

tact must exist within *Pxr1*. To define sequences critical for *Prp43* association, we created a set of *PXR1* deletion derivatives guided by prior domain characterization (Figure S8) (see Guglielmi and Werner 2002) and scored each for *Prp43* Y2H interaction (Figure 6A). Removal of codons 102–149 or overlapping segments almost completely blocks *Prp43* interaction, although this derivative is stable and present at levels equivalent to the full-length *Pxr1* or *Pxr1ΔG* (Figure S9). Since no other deletions greatly reduced the Y2H response, the primary *Prp43* binding site of *Pxr1* appears to reside within this peptide. This prediction was confirmed in a complementary experiment where the isolated 48-amino-acid peptide was found to be sufficient for *Prp43* interaction when expressed at levels similar to poorly interacting *Pxr1* G-patch (Figure 6B and Figure S9).

Surprisingly, removal of the primary *Prp43* binding site (*i.e.*, *PXR1* codons 102–149) had negligible impact on *pxr1::KAN* complementation (Figure 6C). Likewise, this deletion does not result in excess 35S rRNA accumulation or obvious downstream defects in rRNA processing (Figure 6D and data not shown). A larger deletion, *pxr1Δ102-226*, inactivates *Pxr1*, but unlike the *Pxr1Δ102-149* derivative, this protein could not be reproducibly detected by Western blot as a Y2H peptide and likely may not be stable (Figure 6, C and D, and Figure S9). While conceivable that the 102–149 and 150–226 peptides act redundantly in support of *Pxr1* function, deletion of the 150–226 peptide has negligible impact on the *Prp43* Y2H interaction (Figure S9). The 150–226 peptide contains a so-called KKE/D domain characteristic of certain nucleolar proteins (*e.g.*, *Nop56p*, *Nop58p*, *Cbf5p*, and *Dbp3p*) (see Gautier *et al.* 1997). Consistent with earlier reports, removal of this domain does not interfere with rRNA processing (Figure 6D) (see Guglielmi and Werner 2002). The isolated 150–226 peptide segment does not appreciably interact with *Prp43* by the Y2H assay, although interpretation of this observation is complicated by the fact that expression of the *Pxr1*(150–226) Y2H construct somewhat impairs growth (Figure 6B). Nevertheless, together these data support the surprising conclusions that although the G-patch is essential for *Pxr1* function, it appears to interact poorly with *Prp43*, while the primary *Prp43* binding site within *Pxr1* is dispensable for rRNA processing activity.

Consistent with prior studies implicating *Pxr1* in snoRNA 3'-end processing, deletion of the *PXR1* gene or removal of the G-patch or *Pxr1* 150–226 domain coding sequences results in the accumulation of snR18 (U18 snoRNA, 102 nt) transcripts bearing approximately three nucleotide extensions compared to WT transcripts (Figure 7) (see Guglielmi and Werner 2002). We find that the *pxr1Δ102-149* mutant behaves equivalently, showing at least three times as much of the extended form than the properly processed 102-nt snR18 transcript. Remarkably, extended-length snoRNAs also accumulate when *Prp43* function is compromised by the characterized *prp43(H218A)* mutant with diminished ATPase activity (Martin *et al.* 2002) compared with an isogenic strain bearing the WT *PRP43* allele. The magnitude of the extended

Figure 4 RNA splicing activity of the Spp382-Pxr1 chimeras correlates with growth but not with the relative Prp43 Y2H response. (A) Complementation of the lethal *spp382::KAN* mutation by *SPP382* and the viable *pxr1* chimeric constructs (R27G, H55P, K57E, and D62M) after removal of *GAL1-spp382-4* plasmid by FOA selection. Tenfold serial dilutions were plated on synthetic complete medium and grown at 30° for 3 days. Chimeras containing the WT *PXR1* G-patch or the P48G derivative are lethal (see Figure 3) and cannot be assayed in this way. (B) Northern blot analysis of *RPS17A* pre-mRNA splicing. The *spp382::KAN* mutant transformed with the functional *GAL1-spp382-4* allele was assayed before (Gal) and after (Glu) transcriptional repression. The strains were cotransformed with plasmids containing the *spp382ΔG* plasmid reconstituted with the WT *SPP382*, *PXR1*, or *SQS1* G-patch, the indicated mutant *PXR1* G-patch, or an empty vector. Splicing efficiency is presented as the ratio of mRNA/pre-mRNA band intensity below each lane. The mRNA and most pre-mRNA molecules are polyadenylated and resolve as a pair of broad bands by Northern blot after transfer from a denaturing 1% agarose gel. (C) Northern blot analysis of 35S rRNA accumulation and the *ADE3* loading control in WT yeast and *spp382ΔG* gene reconstituted with the indicated G-patch peptide coding sequences. The 35S signal intensities relative to the WT are presented after normalization with the *ADE3* control (Rel. 35S). (D) Y2H assay between *PRP43* and *SPP382-PXR1* G-patch chimeras containing point mutations within the G-patch domain. *SPP382* constructs lacking the G-patch or containing the WT *SPP382* or *PXR1* G-patch domains are shown as controls. Serial dilutions of saturated cultures are plated for double transformant viability (-leu, -trp) and two-hybrid reporter activation (-his supplemented with 5 mM 3-aminotriazole).

RNA response is snoRNA specific, and longer RNA forms were not observed for the 126-nt snR128 (U14 snoRNA) or the 112-nt snR6 (U6) RNAs previously reported as insensitive to the loss of *Pxr1* activity (Guglielmi and Werner 2002). Thus, the impaired activity of either the *Prp43* helicase or its *Pxr1*-binding partner alters the biogenesis of certain snoRNAs.

Prp43 domains required for stable G-patch protein interaction

To identify possible sites of G-patch protein contact, we constructed a series of *PRP43* deletion derivatives and assayed each for Y2H interaction with genes that express the full-length *Spp382*, *Sqs1*, and *Pxr1* proteins (Figure 8). The endpoints of the deletions were guided by the domains described in the recently resolved *Prp43* structure (He *et al.* 2010; Walbott *et al.* 2010). To stabilize potentially weak associations, the deletion derivatives were assayed at 23° rather than at the typical growth temperature of 30°. Deletions of the *Prp43* N-terminal domain (amino acids 7–94), the ratchet domain (amino acids 522–635), and much of the C-terminal domain including the entire OB fold required for full RNA binding and ATPase stimulation (amino acids 649–748) (see Walbott *et al.* 2010) resulted in little or no decrease in Y2H-dependent growth, showing that these regions are not critical for stable *Prp43* association. In contrast, removal of RecA2 (amino acids 271–457) blocks interaction with all

three G-patch protein partners. Since the RecA2-deleted *Prp43* protein is stably expressed (Figure S10), this domain appears critical for G-patch protein interaction by the Y2H assay. Deletion of either the RecA1 domain (amino acids 95–270) or the winged helix domain (WH; amino acids 455–542) differentially affects *Spp382*, *Sqs1*, and *Pxr1* Y2H interactions, possibly reflecting differences in non-G-patch contacts. Here, removal of RecA1 from *Prp43* blocks or severely impairs Y2H interaction with *Spp382* and *Sqs1* but has a less profound effect on *Pxr1*. In contrast, removal of the WH domain blocks *Pxr1* and *Sqs1* association with *Prp43*, while *Spp382* continues to interact, albeit less well than with the full-length *Prp43*. Each of the deletion constructs is expressed at levels roughly between 50 and 100% of full-length *Prp43*. Similar results were obtained when the interactions were assayed at 30°, although a weakened response was seen between the *Prp43* ratchet deletion mutant and *Sqs1*, and all interaction was lost between *Spp382* and the *Prp43* WH-domain deletion constructs. Complementary Y2H experiments using the three full-length G-patch proteins failed to provide convincing evidence for independent interaction with any isolated *Prp43* domain. Although the ratchet peptide was found at considerably lower abundance, the remaining *Prp43* domains were stably expressed (Figure S11), suggesting that G-protein association may require multiple, perhaps discontinuous regions of *Prp43* for a robust Y2H response. We

Figure 5 The Pxr1-specified G-patch is optimal for rRNA processing activity. (A) Complementation of the viable but slow-growing *pxr1::KAN* mutation with plasmid-borne copies of native *PXR1* gene, the G-patch depletion derivative, *pxr1ΔG*, and reconstituted derivatives bearing the original *PXR1* G-patch or chimeric *SQS1* or *SPP382* G-patch coding sequences. Saturated cultures of the indicated genotypes were plated as serial 10-fold dilutions on complete medium and incubated for 2 days at 30°. (B) Northern blot analysis of total RNA extracted from logarithmically grown cultures of the genotypes described in A. The samples were resolved by denaturing 1.2% agarose formaldehyde gel electrophoresis and probed with previously described oligonucleotides (Kos and Tollervey 2005) specific for the indicated 35S rRNA precursor, intermediates (23S, 20S, and 7S), and products (25S, 18S, and 5.8S). An *ADE3* mRNA probe is used as a loading control. Samples from untransformed yeast (UT) and plasmid-transformed strains in the *pxr1::KAN* background indicated. After normalization with the *ADE3* signals, the WT, the 35S rRNA abundance is unchanged in the *pxr1ΔG-PXR1* transformant and increased 5.7-, 4.1-, 2.6-, and 1.7-fold in the *pxr1::KAN*, *pxr1ΔG*, *pxr1ΔG+SPP382*, and *pxr1ΔG+SQS1* transformants, respectively.

note, however, that the Prp43 WH domain showed high levels of reporter-gene activation in the absence of a Y2H partner, prohibiting unambiguous assessment of this construct.

Discussion

The G-patch peptide motif binds Prp43 and is required to stimulate the RNA-dependent ATPase and helicase activities of this DExD/H-box enzyme (Tanaka *et al.* 2007; Walbott *et al.* 2010; Christian *et al.* 2014). If this is the sole function of the G-patch, one might expect that equivalent segments of the three putative Prp43 activators would have similar properties and be interchangeable in support of enzyme function. The results presented here reveal a more nuanced situation and implicate G-patch involvement beyond service as a simple Prp43 interface.

While not a highly quantitative assay, the Y2H approach provides a qualitative measure of protein interaction *in vivo* (Uetz and Hughes 2000). We find that Prp43 interacts with the isolated G-patch peptide domains in the Y2H response order *Spp382* > *Sqs1* > *Pxr1*. Differences in G-patch peptide expression are unlikely to account for the negligible Pxr1-G-patch–Prp43 Y2H response, suggesting an intrinsically weak

interaction between this peptide protein pair. This view is bolstered by the more salt-sensitive Prp43 interaction observed *in vitro* with the peptides bearing the Pxr1 G-patch compared with those containing the Spp382 G-patch. The negligible Pxr1 G-patch domain–Prp43 Y2H response, the failure of the Pxr1 G-patch to functionally reconstitute Spp382, and the lack of biochemical data documenting Prp43 helicase activation by Pxr1 raise the question of whether Pxr1 is a legitimate Prp43 partner. While definitive proof is still wanting, the legitimacy of this association is supported by the G-patch dependence on Pxr1 recovery in Prp43 complexes (Chen *et al.* 2014) and the rRNA processing defects observed in *pxr1* mutants consistent with Prp43 involvement (Guglielmi and Werner 2002). In addition, the human *PXR1* homolog, *PINX1*, has been shown to complement a yeast *pxr1* null mutant, and *in vitro*, the PinX1 protein stimulates yeast Prp43 ATPase activity (Guglielmi and Werner 2002; Chen *et al.* 2014). Based on this, we think that it most likely that the Spp382, Sqs1, and Pxr1 G-patch peptides all naturally bind Prp43 but differ in apparent affinity due to optimization for *cis*- or *trans*-acting interactions distinct for each protein. Each of the three Prp43 binding partners as scored here requires sequences outside the G-patch domain for maximal Y2H response. In addition to the observations presented here, poorly conserved sequences flanking the Spp382 G-patch were shown recently to enhance G-patch-dependent Prp43 activation *in vitro* (Christian *et al.* 2014) and add to this peptide's association with Prp43. However, extrapolating from our Y2H results, Pxr1 interaction with Prp43 association appears largely independent of the G-patch, suggesting that transient or weak association with this motif in the context of the native RNP is sufficient for helicase function.

Prp43 truncated at amino acid 657 fails to bind a Sqs1 G-patch peptide *in vitro*, implicating the final 15% of Prp43 as a primary contact site for G-patch association (Walbott *et al.* 2010). The Prp43 C-terminus contains an OB-fold oligonucleotide/oligosaccharide-binding domain thought to interact with RNA and necessary for full G-patch-stimulated enzyme activity. Lysine residues within this domain can be chemically cross-linked with the Spp382 G-patch consistent with close and perhaps direct contact between these peptide features (Christian *et al.* 2014). Similar interactions are predicted to occur between the C-terminus of the Prp2 DEAH-box helicase and its G-patch activator, Spp2 (Roy *et al.* 1995; Silverman *et al.* 2004; Warkocki *et al.* 2015). It was surprising, therefore, that the large Prp43 C-terminal domain (CTD) deletion scored here had no discernible impact on G-patch-dependent Spp382–Prp43 Y2H interaction. We do not think it likely that residual CTD sequences in our construct (*i.e.*, amino acids 749–767) are sufficient for stable Spp382 contact because the final 35 amino acids of Prp43 are not needed for Prp43 activity *in vivo* (Tanaka *et al.* 2007). Functional redundancy between the CTD and RecA2 domains of Prp43 has been suggested based on identification of *prp43* RecA2 missense mutations that inhibit Spp382

Figure 6 Loss of main Prp43 binding site has little impact on Pxr1 rRNA processing activity. (A) Y2H assay between PRP43 and the indicated PXR1 deletion derivatives. The G-patch domain is deleted in the Δ25–72 construct, and the KKE/D domain common to certain nucleolar proteins is deleted in the Δ150–226 segment. Serial dilutions of saturated cultures are plated for cell number (-leu, -trp) and two-hybrid reporter activation (-his supplemented with 5 mM 3-aminotriazole) and incubated for 2 days at 30°. (B) Y2H assay as described in A with Gal4 activation-domain constructs limited to the proposed primary Prp43 binding site (102–149), the KKE/D domain (150–226), full-length PXR1, or an empty vector. Here the histidine-deficient medium was supplemented with 20 mM 3-AT to suppress background *trans*-activation observed with the *pxr1*(150–226) construct. (C) Complementation of the *pxr1::KAN* growth defect by plasmid-based copies of the full-length gene (PXR1) or the indicated deletion constructs. The transformants were grown for 3 days at 30° on selective medium. (D) Northern blot analysis of total RNA extracted from logarithmically grown cultures shown in C probed for the 35S pre-rRNA and the ADE3 mRNA loading control. The relative 35S intensities were determined as described in Figure 5.

G-patch helicase stimulation *in vitro* and that exacerbate CTD lesions *in vivo* (Tanaka *et al.* 2007). This portion of the enzymatic core is especially attractive as a critical site of G-patch contact because its removal also blocks G-patch-dependent Spp382 interaction. Since Sqs1 and Pxr1 likewise fail to bind the Prp43ΔRecA2 derivative, RecA2 either directly participates in their non-G-patch interactions or indirectly fosters contact through proper folding of the Prp43 protein.

At a functional level, the Spp382 and Pxr1 G-patch domains are largely incompatible in chimeric protein reconstitution, while the equivalently dissimilar Sqs1 G-patch (Figure 1B) serves as an acceptable compromise cassette in either context. Point mutations introduced to make the Pxr1 G-patch more Spp382-like were found to modestly improve the Prp43 Y2H interaction (*i.e.*, D62M, K57E, and P48G) or restore biological activity to the chimeric Spp382-Pxr1 splicing factor (*i.e.*, H55P, D62M, K57E, and R27G). The H55P substitution showed the most robust response in Spp382 reconstitution, converting the otherwise nonfunctional *spp382-PXR1* allele to one that supported growth at roughly half the rate of an otherwise isogenic WT control. Curiously, the HPP5 did not detectably enhance this protein's Y2H interaction with Prp43. While both assays show that multiple G-patch residues contribute to domain specificity, there was not a tight correlation between improved Prp43 interaction and *spp382::KAN* complementation. However, since antibodies are not available for the native G-patch protein, differential impact on RNA processing protein stability cannot be ruled out. This caveat aside, G-patch function beyond simple Prp43 recognition provides a reasonable explanation for Spp382 and Pxr1 G-patch incompatibility. For instance, the G-patch may promote proper folding of the cognate protein through *cis* interactions or bind *trans*-acting factors specific for the splicing or rRNA processing machineries to enhance the Prp43 affinity or RNP specificity (Figure 9). Unfortunately, the only G-patch pro-

teins for which structural data are currently available show this segment as disordered, leaving unresolved the nature of *cis* interactions (Frenal *et al.* 2006; Christian *et al.* 2014).

For *trans*-acting associations, the RNA substrate is a possible G-patch target because *in vitro* studies conducted with the TgDRE DNA repair enzyme of *Toxoplasma gondii* (Frenal *et al.* 2006) and the mouse retroviral proteases MIA-14 and

Figure 7 Loss of the Prp43 binding site within Pxr1 or diminished Prp43 activity alters U18 snoRNA processing. Northern blot hybridization of RNA isolated from WT yeast (lanes 2 and 4), the *prp43H218A* mutant (lane 3), yeast with a full PXR1 deletion (lanes 1 and 7), or the *pxr1*Δ150–226 (lane 5) and *pxr1*Δ102–149 deletion (lane 6) derivatives. The *prp43H218A* and its WT control are otherwise isogenic (lanes 2 and 3), as are the *pxr1* mutant derivatives and their WT control (lanes 1 and 4–7). The membrane was sequentially hybridized with oligonucleotide probes specific for the snR18 (U18), snR128 (U14), and snR6 (U6) RNAs.

	1	94	270	457	521	635	OB	767	Full
Prp43	NTD	RecA1	RecA2	WH	Ratchet	CTD			Length
Deletion	(Δ7-94)	(Δ95-270)	(Δ271-457)	(Δ455-542)	(Δ522-635)	(Δ649-748)			Prp43
Spp382	+++	—	—	+	+++	+++	+++	+++	
Sqs1	+++	+	—	—	+++	+++	+++	+++	
Pxr1	+++	++	—	—	+++	+++	+++	+++	

Y2H Interaction Relative to Full Length G-Patch Proteins

Figure 8 G-patch protein sensitivity to Prp43 domain deletions. The numbered positions above the diagram refer to the amino acid coordinates previously used to describe Prp43 domain organization (He *et al.* 2010; Walbott *et al.* 2010). Below this diagram are bars showing the sites of deletion with amino acid coordinates for the residues removed for Y2H analysis. The Y2H results for full-length Prp43 and each deletion construct are summarized for the three G-patch proteins. In each case, growth observed with the full-length constructs is set at +++, with the deletion mutants showing equivalent growth (+++), colonies roughly half this size (++), visible colonies one-quarter this size or smaller (+), or no visible colonies (—). The plate cultures were grown for 4 days at 23° on medium lacking histidine and supplemented with 5 mM 3-aminotrazole.

MPMV (Svec *et al.* 2004) suggest intrinsic G-patch affinity for nucleic acid. More directly, K67 of the Spp382 G-patch motif can be ultraviolet (UV) cross-linked to RNA *in vitro* when coincubated with Prp43, indicating at least transient RNA contact with this domain (Christian *et al.* 2014). It is conceivable that G-patch–RNA association contributes to the Prp43 Y2H responses seen here and, more important, enhances productive Prp43 interaction with its sites of cellular RNA contact in spliceosome and ribosomal complexes (Bohnsack *et al.* 2009).

We have shown that a 48-amino-acid peptide downstream of the Pxr1 G-patch provides stable Prp43 contact in the Y2H assay. While deletion of this sequence virtually eliminates the Pxr1-Prp43 Y2H interaction, it has little impact on G-patch-dependent cell growth or rRNA processing. Removal of this element does, however, promote the accumulation of slightly extended snR18 RNAs, a characteristic seen with several other *pxr1* mutant alleles and proposed to result from aberrant 3'-end processing (Guglielmi and Werner 2002). In fact, 3'-extended snoRNAs, including polyadenylated species, also accumulate when snoRNA transcriptional termination is blocked, snoRNP assembly is disrupted by Nop58 or Nop1 depletion, or exosome activity is impaired (see Allmang *et al.* 1999; Grzechnik and Kufel 2008; Costello *et al.* 2011; Garland *et al.* 2013). While commonly thought to target stable RNAs for degradation (Lacava *et al.* 2005; Vanacova *et al.* 2005; Wyers *et al.* 2005), some 3'-extended RNAs escape this fate (Grzechnik and Kufel 2008), and the snR18+3 transcripts found in the *pxr1* mutants appear stable and accumulate to levels equivalent to those of the properly processed RNAs in the WT background. The presence of similarly extended snR18 RNAs in the *prp43H218A* mutant raises the intriguing possibility that this protein also has a role in snoRNA biogenesis. Conceivably, this could occur as an indirect consequence of impaired pre-mRNA splicing in the *prp43H218A* background (Martin *et al.* 2002). However, because snoRNP recruitment to and release from pre-ribosomal particles is

Figure 9 Alternate models for G-patch contribution to pathway-dependent Prp43 function. In each case, Prp43 activity requires G-patch interaction for enzyme activation. Pathway specificity may be added as illustrated by (1) intramolecular interactions that alter the effectiveness of G-patch protein stimulation of Prp43, (2) direct G-patch contact with the substrate RNA, or (3) G-patch interaction with protein specificity factors guiding Prp43 recruitment to the spliceosome or pre-ribosomal particle. The G-patch is indicated by the clustered filled circles. Stable association of the G-patch protein with Prp43 is illustrated but cofactor dissociation and is also consistent with the existing literature.

directly affected by the loss of Prp43 activity (Bohnsack *et al.* 2009), perhaps a more likely explanation is that defects in ribosome biogenesis contribute to the extended snoRNA phenotype. Going forward, it will be important to learn whether Pxr1-Prp43 contact is required for proper snoRNA maturation and, more generally, to refine our understanding of the network of G-patch protein interactions underpinning Prp43 contribution to the pre-mRNA, rRNA, and snoRNA maturation pathways.

Acknowledgments

We thank Beate Schwer for providing the cloned *PRP43* and *prp43H281A* genes, Michael Fried for valuable suggestions during the course of this work, and Martha Peterson, Stefan Stamm, Doug Harrison, and Swagata Ghosh for helpful comments on this manuscript. This work was supported by the Linda and Jack Gill Endowment and National Institutes of Health award GM-42476 to BCR.

Literature Cited

- Allmang, C., J. Kufel, G. Chanfreau, P. Mitchell, E. Petfalski *et al.*, 1999 Functions of the exosome in rRNA, snoRNA and snRNA synthesis. *EMBO J.* 18: 5399–5410.
- Aravind, L., and E. V. Koonin, 1999 G-patch: a new conserved domain in eukaryotic RNA-processing proteins and type D retroviral polyproteins. *Trends Biochem. Sci.* 24: 342–344.
- Arenas, J. E., and J. N. Abelson, 1997 Prp43: an RNA helicase-like factor involved in spliceosome disassembly. *Proc. Natl. Acad. Sci. USA* 94: 11798–11802.
- Ares, Jr., M., L. Grate, and M. H. Pauling, 1999 A handful of intron-containing genes produces the lion's share of yeast mRNA. *RNA* 5: 1138–1139.
- Boeke, J. D., J. Trueheart, G. Natsoulis, and G. R. Fink, 1987 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol.* 154: 164–175.

- Bohnsack, M. T., R. Martin, S. Granneman, M. Ruprecht, E. Schleiff *et al.*, 2009 Prp43 bound at different sites on the pre-rRNA performs distinct functions in ribosome synthesis. *Mol. Cell* 36: 583–592.
- Boon, K. L., T. Auchynnikava, G. Edwalds-Gilbert, J. D. Barrass, A. P. Droop *et al.*, 2006 Yeast ntr1/spp382 mediates prp43 function in postsplisomes. *Mol. Cell. Biol.* 26: 6016–6023.
- Chen, H. C., C. K. Tseng, R. T. Tsai, C. S. Chung, and S. C. Cheng, 2013 Link of NTR-mediated spliceosome disassembly with DEAH-box ATPases Prp2, Prp16, and Prp22. *Mol. Cell. Biol.* 33: 514–525.
- Chen, Y. L., R. Capeyrou, O. Humbert, S. Mouffok, Y. A. Kadri *et al.*, 2014 The telomerase inhibitor Gno1p/PINX1 activates the helicase Prp43p during ribosome biogenesis. *Nucleic Acids Res.* 42: 7330–7345.
- Christian, H., R. V. Hofele, H. Urlaub, and R. Ficner, 2014 Insights into the activation of the helicase Prp43 by biochemical studies and structural mass spectrometry. *Nucleic Acids Res.* 42: 1162–1179.
- Collins, S. R., P. Kemmeren, X. C. Zhao, J. F. Greenblatt, F. Spencer *et al.*, 2007 Toward a comprehensive atlas of the physical interactome of *Saccharomyces cerevisiae*. *Mol. Cell. Proteomics* 6: 439–450.
- Combs, D. J., R. J. Nagel, M. Ares, Jr., and S. W. Stevens, 2006 Prp43p is a DEAH-box spliceosome disassembly factor essential for ribosome biogenesis. *Mol. Cell. Biol.* 26: 523–534.
- Cordin, O., D. Hahn, and J. D. Beggs, 2012 Structure, function and regulation of spliceosomal RNA helicases. *Curr. Opin. Cell Biol.* 24: 431–438.
- Costello, J. L., J. A. Stead, M. Feigenbutz, R. M. Jones, and P. Mitchell, 2011 The C-terminal region of the exosome-associated protein Rrp47 is specifically required for box C/D small nucleolar RNA 3'-maturation. *J. Biol. Chem.* 286: 4535–4543.
- Davierwala, A. P., J. Haynes, Z. Li, R. L. Brost, M. D. Robinson *et al.*, 2005 The synthetic genetic interaction spectrum of essential genes. *Nat. Genet.* 37: 1147–1152.
- Fabrizio, P., J. Dannenberg, P. Dube, B. Kastner, H. Stark *et al.*, 2009 The evolutionarily conserved core design of the catalytic activation step of the yeast spliceosome. *Mol. Cell* 36: 593–608.
- Fingerman, I., V. Nagaraj, D. Norris, and A. K. Vershon, 2003 Sfp1 plays a key role in yeast ribosome biogenesis. *Eukaryot. Cell* 2: 1061–1068.
- Fourmann, J. B., J. Schmitzova, H. Christian, H. Urlaub, R. Ficner *et al.*, 2013 Dissection of the factor requirements for spliceosome disassembly and the elucidation of its dissociation products using a purified splicing system. *Genes Dev.* 27: 413–428.
- Frenal, K., I. Callebaut, K. Wecker, A. Prochnicka-Chalufour, N. Dendouga *et al.*, 2006 Structural and functional characterization of the TgDRE multidomain protein, a DNA repair enzyme from *Toxoplasma gondii*. *Biochemistry* 45: 4867–4874.
- Fromont-Racine, M., B. Senger, C. Saveanu, and F. Fasiolo, 2003 Ribosome assembly in eukaryotes. *Gene* 313: 17–42.
- Garland, W., M. Feigenbutz, M. Turner, and P. Mitchell, 2013 Rrp47 functions in RNA surveillance and stable RNA processing when divorced from the exoribonuclease and exosome-binding domains of Rrp6. *RNA* 19: 1659–1668.
- Gautier, T., T. Berges, D. Tollervey, and E. Hurt, 1997 Nucleolar KKE/D repeat proteins Nop56p and Nop58p interact with Nop1p and are required for ribosome biogenesis. *Mol. Cell. Biol.* 17: 7088–7098.
- Gavin, A. C., P. Aloy, P. Grandi, R. Krause, M. Boesche *et al.*, 2006 Proteome survey reveals modularity of the yeast cell machinery. *Nature* 440: 631–636.
- Grandi, P., V. Rybin, J. Bassler, E. Petfalski, D. Strauss *et al.*, 2002 90S pre-ribosomes include the 35S pre-rRNA, the U3 snoRNP, and 40S subunit processing factors but predominantly lack 60S synthesis factors. *Mol. Cell* 10: 105–115.
- Grzechnik, P., and J. Kufel, 2008 Polyadenylation linked to transcription termination directs the processing of snoRNA precursors in yeast. *Mol. Cell* 32: 247–258.
- Gudipati, R. K., H. Neil, F. Feuerbach, C. Malabat, and A. Jacquier, 2012 The yeast *RPL9B* gene is regulated by modulation between two modes of transcription termination. *EMBO J.* 31: 2427–2437.
- Guglielmi, B., and M. Werner, 2002 The yeast homolog of human PinX1 is involved in rRNA and small nucleolar RNA maturation, not in telomere elongation inhibition. *J. Biol. Chem.* 277: 35712–35719.
- Hahn, D., G. Kudla, D. Tollervey, and J. D. Beggs, 2012 Brr2p-mediated conformational rearrangements in the spliceosome during activation and substrate repositioning. *Genes Dev.* 26: 2408–2421.
- Harper, J. W., G. R. Adami, N. Wei, K. Keyomarsi, and S. J. Elledge, 1993 The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 75: 805–816.
- He, Y., G. R. Andersen, and K. H. Nielsen, 2010 Structural basis for the function of DEAH helicases. *EMBO Rep.* 11: 180–186.
- Henras, A. K., J. Soudet, M. Gerus, S. Lebaron, M. Caizergues-Ferrer *et al.*, 2008 The post-transcriptional steps of eukaryotic ribosome biogenesis. *Cell. Mol. Life Sci.* 65: 2334–2359.
- Horvath, A., and H. Riezman, 1994 Rapid protein extraction from *Saccharomyces cerevisiae*. *Yeast* 10: 1305–1310.
- Hu, Z., P. J. Killion, and V. R. Iyer, 2007 Genetic reconstruction of a functional transcriptional regulatory network. *Nat. Genet.* 39: 683–687.
- Ito, H., Y. Fukuda, K. Murata, and A. Kimura, 1983 Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153: 163–168.
- James, P., J. Halladay, and E. A. Craig, 1996 Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* 144: 1425–1436.
- Jorgensen, P., J. L. Nishikawa, B. J. Breikreutz, and M. Tyers, 2002 Systematic identification of pathways that couple cell growth and division in yeast. *Science* 297: 395–400.
- Jorgensen, P., I. Rupes, J. R. Sharom, L. Schneper, J. R. Broach *et al.*, 2004 A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. *Genes Dev.* 18: 2491–2505.
- Kaiser, C., S. Michaelis, and A. Mitchell (Editors), 1994 *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Koodathingal, P., T. Novak, J. A. Piccirilli, and J. P. Staley, 2010 The DEAH box ATPases Prp16 and Prp43 cooperate to proofread 5' splice site cleavage during pre-mRNA splicing. *Mol. Cell* 39: 385–395.
- Kos, M., and D. Tollervey, 2005 The putative RNA helicase Dbp4p is required for release of the U14 snoRNA from preribosomes in *Saccharomyces cerevisiae*. *Mol. Cell* 20: 53–64.
- Kressler, D., E. Hurt, and J. Bassler, 2010 Driving ribosome assembly. *Biochim. Biophys. Acta* 1803: 673–683.
- LaCava, J., J. Houseley, C. Saveanu, E. Petfalski, E. Thompson *et al.*, 2005 RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. *Cell* 121: 713–724.
- Lafontaine, D. L. J., 2010 A “garbage can” for ribosomes: how eukaryotes degrade their ribosomes. *Trends Biochem. Sci.* 35: 267–277.
- Lebaron, S., C. Froment, M. Fromont-Racine, J. C. Rain, B. Monsarrat *et al.*, 2005 The splicing ATPase prp43p is a component of multiple preribosomal particles. *Mol. Cell. Biol.* 25: 9269–9282.
- Lebaron, S., C. Papin, R. Capeyrou, Y. L. Chen, C. Froment *et al.*, 2009 The ATPase and helicase activities of Prp43p are stimulated by the G-patch protein Pfa1p during yeast ribosome biogenesis. *EMBO J.* 28: 3808–3819.

- Leeds, N. B., E. C. Small, S. L. Hiley, T. R. Hughes, and J. P. Staley, 2006 The splicing factor Prp43p, a DEAH box ATPase, functions in ribosome biogenesis. *Mol. Cell. Biol.* 26: 513–522.
- Letunic, I., T. Doerks, and P. Bork, 2012 SMART 7: recent updates to the protein domain annotation resource. *Nucleic Acids Res.* 40: D302–D305.
- Li, B., J. Vilardell, and J. R. Warner, 1996 An RNA structure involved in feedback regulation of splicing and of translation is critical for biological fitness. *Proc. Natl. Acad. Sci. USA* 93: 1596–1600.
- Li, Z., A. G. Paulovich, and J. L. Woolford, Jr., 1995 Feedback inhibition of the yeast ribosomal protein gene CRY2 is mediated by the nucleotide sequence and secondary structure of CRY2 pre-mRNA. *Mol. Cell. Biol.* 15: 6454–6464.
- Linder, P., and E. Jankowsky, 2011 From unwinding to clamping: the DEAD box RNA helicase family. *Nat. Rev. Mol. Cell Biol.* 12: 505–516.
- Magtanong, L., C. H. Ho, S. L. Barker, W. Jiao, A. Baryshnikova *et al.*, 2011 Dosage suppression genetic interaction networks enhance functional wiring diagrams of the cell. *Nat. Biotechnol.* 29: 505–511.
- Marion, R. M., A. Regev, E. Segal, Y. Barash, D. Koller *et al.*, 2004 Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. *Proc. Natl. Acad. Sci. USA* 101: 14315–14322.
- Martin, A., S. Schneider, and B. Schwer, 2002 Prp43 is an essential RNA-dependent ATPase required for release of lariat-intron from the spliceosome. *J. Biol. Chem.* 277: 17743–17750.
- Mayas, R. M., H. Maita, D. R. Semlow, and J. P. Staley, 2010 Spliceosome discards intermediates via the DEAH box ATPase Prp43p. *Proc. Natl. Acad. Sci. USA* 107: 10020–10025.
- Mnaimneh, S., A. P. Davierwala, J. Haynes, J. Moffat, W. T. Peng *et al.*, 2004 Exploration of essential gene functions via titratable promoter alleles. *Cell* 118: 31–44.
- Mozaffari-Jovin, S., K. F. Santos, H. H. Hsiao, C. L. Will, H. Urlaub *et al.*, 2012 The Prp8 RNase H-like domain inhibits Brr2-mediated U4/U6 snRNA unwinding by blocking Brr2 loading onto the U4 snRNA. *Genes Dev.* 26: 2422–2434.
- Pandit, S., B. Lynn, and B. C. Rymond, 2006 Inhibition of a spliceosome turnover pathway suppresses splicing defects. *Proc. Natl. Acad. Sci. USA* 103: 13700–13705.
- Pandit, S., S. Paul, L. Zhang, M. Chen, N. Durbin *et al.*, 2009 Spp382p interacts with multiple yeast splicing factors, including possible regulators of Prp43 DEXD/H-box protein function. *Genetics* 183: 195–206.
- Pena, V., S. M. Jovin, P. Fabrizio, J. Orlowski, J. M. Bujnicki *et al.*, 2009 Common design principles in the spliceosomal RNA helicase Brr2 and in the Hel308 DNA helicase. *Mol. Cell* 35: 454–466.
- Pertschy, B., C. Schneider, M. Gnädig, T. Schafer, D. Tollervy *et al.*, 2009 RNA helicase Prp43 and its co-factor Pfa1 promote 20 to 18 S rRNA processing catalyzed by the endonuclease Nob1. *J. Biol. Chem.* 284: 35079–35091.
- Phipps, K. R., J. M. Charette, and S. J. Baserga, 2011 The small subunit processome in ribosome biogenesis—progress and prospects. *Wiley Interdiscip. Rev. RNA* 2: 1–21.
- Pleiss, J. A., G. B. Whitworth, M. Bergkessel, and C. Guthrie, 2007 Rapid, transcript-specific changes in splicing in response to environmental stress. *Mol. Cell* 27: 928–937.
- Punta, M., P. C. Coghill, R. Y. Eberhardt, J. Mistry, J. Tate *et al.*, 2012 The Pfam protein families database. *Nucleic Acids Res.* 40: D290–D301.
- Reichow, S. L., T. Hamma, A. R. Ferre-D'Amare, and G. Varani, 2007 The structure and function of small nucleolar ribonucleoproteins. *Nucleic Acids Res.* 35: 1452–1464.
- Rodriguez-Galan, O., J. J. Garcia-Gomez, and J. de la Cruz, 2013 Yeast and human RNA helicases involved in ribosome biogenesis: current status and perspectives. *Biochim. Biophys. Acta* 1829: 775–790.
- Roy, J., K. Kim, J. R. Maddock, J. G. Anthony, and J. L. Woolford, Jr., 1995 The final stages of spliceosome maturation require Spp2p that can interact with the DEAH box protein Prp2p and promote step 1 of splicing. *RNA* 1: 375–390.
- Sambrook, J., E. F. Fritsch, and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Schwer, B., 2008 A conformational rearrangement in the spliceosome sets the stage for Prp22-dependent mRNA release. *Mol. Cell* 30: 743–754.
- Silverman, E. J., A. Maeda, J. Wei, P. Smith, J. D. Beggs *et al.*, 2004 Interaction between a G-patch protein and a spliceosomal DEXD/H-box ATPase that is critical for splicing. *Mol. Cell. Biol.* 24: 10101–10110.
- Staley, J. P., and J. L. Woolford, Jr., 2009 Assembly of ribosomes and spliceosomes: complex ribonucleoprotein machines. *Curr. Opin. Cell Biol.* 21: 109–118.
- Svec, M., H. Bauerova, I. Pichova, J. Konvalinka, and K. Strisovsky, 2004 Proteinases of betaretroviruses bind single-stranded nucleic acids through a novel interaction module, the G-patch. *FEBS Lett.* 576: 271–276.
- Tanaka, N., A. Aronova, and B. Schwer, 2007 Ntr1 activates the Prp43 helicase to trigger release of lariat-intron from the spliceosome. *Genes Dev.* 21: 2312–2325.
- Tsai, R. T., R. H. Fu, F. L. Yeh, C. K. Tseng, Y. C. Lin *et al.*, 2005 Spliceosome disassembly catalyzed by Prp43 and its associated components Ntr1 and Ntr2. *Genes Dev.* 19: 2991–3003.
- Tsai, R. T., C. K. Tseng, P. J. Lee, H. C. Chen, R. H. Fu *et al.*, 2007 Dynamic interactions of Ntr1-Ntr2 with Prp43 and with U5 govern the recruitment of Prp43 to mediate spliceosome disassembly. *Mol. Cell. Biol.* 27: 8027–8037.
- Uetz, P., and R. E. Hughes, 2000 Systematic and large-scale two-hybrid screens. *Curr. Opin. Microbiol.* 3: 303–308.
- Vanacova, S., J. Wolf, G. Martin, D. Blank, S. Dettwiler *et al.*, 2005 A new yeast poly(A) polymerase complex involved in RNA quality control. *PLoS Biol.* 3: e189.
- Vilardell, J., P. Chartrand, R. H. Singer, and J. R. Warner, 2000 The odyssey of a regulated transcript. *RNA* 6: 1773–1780.
- Walbot, H., S. Mouffok, R. Capeyrou, S. Lebaron, O. Humbert *et al.*, 2010 Prp43p contains a processive helicase structural architecture with a specific regulatory domain. *EMBO J.* 29: 2194–2204.
- Warkocki, Z., C. Schneider, S. Mozaffari-Jovin, J. Schmitzova, C. Hobartner *et al.*, 2015 The G-patch protein Spp2 couples the spliceosome-stimulated ATPase activity of the DEAH-box protein Prp2 to catalytic activation of the spliceosome. *Genes Dev.* 29: 94–107.
- Wen, X., S. Tannukit, and M. L. Paine, 2008 TFIP11 interacts with mDEAH9, an RNA helicase involved in spliceosome disassembly. *Int. J. Mol. Sci.* 9: 2105–2113.
- Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson *et al.*, 1999 Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285: 901–906.
- Wyers, F., M. Rougemaille, G. Badis, J. C. Rousselle, M. E. Dufour *et al.*, 2005 Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. *Cell* 121: 725–737.
- Zhang, T., J. Lei, H. Yang, K. Xu, R. Wang *et al.*, 2011 An improved method for whole protein extraction from yeast *Saccharomyces cerevisiae*. *Yeast* 28: 795–798.

Communicating editor: M. Hampsey

GENETICS

Supporting Information

<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.176461/-/DC1>

Limited Portability of G-Patch Domains in Regulators of the Prp43 RNA Helicase Required for Pre-mRNA Splicing and Ribosomal RNA Maturation in *Saccharomyces cerevisiae*

Daipayan Banerjee, Peter M. McDaniel, and Brian C. Rymond

Figure S1: Oligonucleotide list

#1-18: *SPP382* cloning and mutagenesis:

	Name	Oligo sequence (5'→3')	Purpose
1	Inv SPP382 For	AAAAAACCGCGGACCAACTCCAGT AACTATCACA	PCR deletion of G-patch (61-108 aa) from SPP382 (introduce SacII site)
2	Inv SPP382 Rev	AAAAAACCGCGGCTTCGTTAATTT TGAGATCG	PCR deletion of G-patch (61-108 aa) from SPP382 (introduce SacII site)
3	SPP382 G-patch For	AAAAAACCGCGGACATATGGTATT GGTGCGAA	PCR amplification of SPP382 G-patch (61-108 aa) with SacII ends
4	SPP382 G-patch Rev	AAAAAACCGCGGATTTTGAAAACA TTCCTAGACCT	PCR amplification of SPP382 G-patch (61-108 aa) with SacII ends
5	PXR1 G-patch For	AAAAAACCGCGGACCTCGAGATTC GGGCACCA	PCR amplification of PXR1 G-patch (25-72 aa) with SacII ends
6	PXR1 G-patch Rev	AAAAAACCGCGGTTTAAATTTAGC ACCGAGCCCAAC	PCR amplification of PXR1 G-patch (25-72 aa) with SacII ends
7	SQS1 F SacII	CCGCGGAACGAAAATATCGGTAGA AGA	PCR amplification of SQS1 G-patch (720-767 aa) with SacII ends
8	SQS1 G-patch Rev	AAAAAACCGCGGACTTTCCTGTG TCTTAAACC	PCR amplification of SQS1 G-patch (720-767 aa) with SacII ends
9	Inv F PXR1-H55P	CCTATCAAAGTGTCAATTAAGGAC	Point mutagenesis of PXR1 G-patch
10	Inv R PXR1-H55P	CGAAGTGTTCTGAATTCATGGG	Point mutagenesis of PXR1 G-patch
11	InvF PXR1 R27G	TTTAACCACGGGCTTAGGGCT	Point mutagenesis of PXR1 G-patch
12	InvR PXR1 R27G	GATCTTTTCAAACCTACCTTTGGG	Point mutagenesis of PXR1 G-patch
13	InvF PXR1 K57E	AACACTTCGCATATCGAAGTG	Point mutagenesis of PXR1 G-patch
14	InvR PXR1 K57E	CGAATTCATGGGGGATAACCC	Point mutagenesis of PXR1 G-patch
15	InvPxr1D62M-F	ATGGACAACGTTGGGCTCGGTGCT	Point mutagenesis of PXR1 G-patch

16	InvPxr1D62M-R	CTTAATTGACACTTTGATATGCGA	Point mutagenesis of PXR1 G-patch
17	InvPxr1P48G-F	GGCATGAATTCGAACACTTCGCAT	Point mutagenesis of PXR1 G-patch
18	InvPxr1P48G-R	GGATAACCCCAGACCCATACC	Point mutagenesis of PXR1 G-patch

#19-44: PXR1 cloning and mutagenesis:

	Name	Oligo sequence (5'>3')	Purpose
19	PXR1 natproKpn1F	AAAAAGGTACCCTTAGCGTAAACA GTAAGTGC GTGC	PCR amplification of PXR1 with natural promoter, KpnI ends (500bps up/125bps dn)
20	PXR1 natproKpn1R	AAAAAGGTACCTTTTTTCTTTGGCA CCGGGG	PCR amplification of PXR1 with natural promoter, KpnI ends (500bps up/125bps dn)
21	PXR1 SmaI F	AAAAACCCGGGGATGGGTTTGGCA GCTACAAG	PCR amplification of PXR1 with SmaI ends
22	PXR1 SmaI R	AAAAACCCGGGGCTATTAGTCGTTT GTTATCAT	PCR amplification of PXR1 with SmaI ends
23	Inv PXR1 For	AAAAAACCGCGGGTCGTTACTCCA TGCCGTATTT	PCR deletion of G-patch (25-72 aa) from PXR1 (introduce SacII site)
24	Inv PXR1 Rev	AAAAAACCGCGGCGTAAAGACAA AAAAGACGAG	PCR deletion of G-patch from PXR1(25-72 aa) (introduce SacII site)
25	Inv PXR1 delG For	GTCGTTACTCCATGCCGTATTTCT	PCR deletion of G-patch from PXR1 (no restriction site)
26	Inv PXR1 delG Rev	CGTAAAGACAAAAAAGACGAGTTT	PCR deletion of G-patch from PXR1 (no restriction site)
27	Pxr1 G-patch F	ACCTCGAGATTCGGGCAC	PCR amplification of PXR1 G-patch w/o restriction site
28	Pxr1 G-patch R	TTTTAATTTAGCACCGAGCCCAAC	PCR amplification of PXR1 G-patch w/o restriction site
29	Spp382 G-For	ACATATGGTATTGGTGCGAAG	PCR amplification of SPP382 G-patch w/o restriction site
30	SPP382 G- Rev	ATTTTGAAAACATTCTAGACC	PCR amplification of SPP382 G-patch w/o restriction site
31	SQS1 G-For	AACGAAAATATCGGTAGAAGA	PCR amplification of SQS1 G-patch w/o restriction site

32	SQS1 G-Rev	ACTTTCAC TGTGTCTTAAACC	PCR amplification of SQS1 G-patch w/o restriction site
33	Invr Pxr1 del7-101	AAAAACCGCGGTCTTGTAGCTGCC AAACCCAT	PCR deletion of PXR1 fragment (Δ 7-101 aa) and introduce SacII site
34	Inv fPxr1del7-101	AAAAACCGCGGGAAAGCAAATA TCAGAGGAA	PCR deletion of PXR1 fragment (Δ 7-101 aa) and introduce SacII site
35	Invr PXR1del25-150	AAAAACCGCGGGTCGTTACTCCAT GCCGTATT	PCR deletion of PXR1 fragment (Δ 25-149 aa) and introduce SacII site
36	Invf PXR1del25-150	AAAAACCGCGGAAGAAACGAAAG AGGGAA	PCR deletion of PXR1 fragment (Δ 25-149 aa) and introduce SacII site
37	Invr PXR1 del101-226	AAAAACCGCGGCTTCCGTT CAGC CTACC	PCR deletion of PXR1 fragment (Δ 102-226 aa) and introduce SacII site
38	InvF PXR1 del101-226	AAAAACCGCGGTCTAGTGAATCTG CATCC	PCR deletion of PXR1 fragment (Δ 102-226 aa) and introduce SacII site
39	InvRPXR1del150-226	AAAAACCGCGGCGCGTTGGAGTAA TTTCT	PCR deletion of PXR1 fragment (Δ 150-226 aa) and introduce SacII site
40	InvrPXR1del226-265	AAAAACCGCGGTTTTAATTTTGAC GTCTT	PCR deletion of PXR1 fragment (Δ 227-265 aa) and introduce SacII site
41	InvfPXR1del226-265	AAAAACCGCGGTTTATGATAACAA ACGAC	PCR deletion of PXR1 fragment (Δ 227-265 aa) and introduce SacII site
42	Pxr1_101-150aa_F	AAAAAGAATTCGAGAAAGCAAAA TATCAGAGGAATTG	PCR amplification of PXR1 (102-150 aa) with EcoR1 ends
43	Pxr1_101-150aa_R	AAAAAGAATTCCTACTTCGCGTTG GAGTAATTTCTTAA	PCR amplification of PXR1 (102-150 aa) with EcoR1 ends
44	Pxr1_226aa_R	AAAAAGAATTCCTATTTTAATTTTG ACGTCTTTTTCGT	PCR amplification of PXR1 (102- 226 aa) with EcoR1 ends

#45-68: PRP43 cloning and mutagenesis:

	Name	Oligo sequence (5'>3')	Purpose
45	Inv Prp43del7-94F	AAAAACCGCGGGTACATGCCCAGA GAGATGAG	PCR deletion of PRP43 NTD (Δ 7-94 aa) and introduce SacII site

46	InvPrp43del 7-94R	AAAAACCGCGGGAATCTTCTTTTG GAACCCAT	PCR deletion of PRP43 NTD (Δ 7-94 aa) and introduce SacII site
47	InvPrp43del 94- 270F	AAAAACCGCGGACATATCCAGTTG AGCTATAC	PCR deletion of PRP43 RecA1 (Δ 95-270 aa) and introduce SacII site
48	InvPrp43del 94- 270R	AAAAACCGCGGTGGCAATTCTCTT CTAATTTT	PCR deletion of PRP43 RecA1 (Δ 95-270 aa) and introduce SacII site
49	InvPrp43del270- 457F	AAAAACCGCGGTTACGTTCCAATT TATCCTCC	PCR deletion of PRP43 RecA2 (Δ 271-457 aa) and introduce SacII site
50	InvPrp43del270- 457R	AAAAACCGCGGTCTGCCCCGGAACG GCAAGTAA	PCR deletion of PRP43 RecA2 (Δ 271-457 aa) and introduce SacII site
51	InvPrp43delWHnew F	AAAAACCGCGGCTAACTATAGTGG CCATGCTATCG	PCR deletion of PRP43 WH (Δ 445-542) and introduce SacII site
52	InvPrp43delWHnew R	AAAAACCGCGGTGCCTCTTCAGTG TATAATCTGAA	PCR deletion of PRP43 WH (Δ 445-542) and introduce SacII site
53	InvPrp43del521- 635F	AAAAACCGCGGACTACAGACTATG AAAGCCCT	PCR deletion of PRP43 Ratchet (Δ 522-635) and introduce SacII site
54	InvPrp43del521- 635R	AAAAACCGCGGGGGAAACTGGGA TGCCAATCT	PCR deletion of PRP43 Ratchet (Δ 522-635) and introduce SacII site
55	InvPrp43delCTDne wR	AAAAACCGCGGGATGTTGTCAAAG TATTTAGGGCT	PCR deletion of PRP43 CTD (Δ 649-748 aa) and introduce SacII site
56	InvPrp43delCTDne wF	AAAAACCGCGGAGATTAAACGAG TTGAAACAAGGT	PCR deletion of PRP43 CTD (Δ 649-748 aa) and introduce SacII site
57	Prp43_NTD_F	AAAAACATATGATGGGTTCAAAA GAAGATTC	PCR amplification of Prp43 NTD (1- 94 aa)with NdeI ends
58	Prp43_NTD_R	AAAAACATATGCTATGGCAATTCT CTTCTAATTTTCAG	PCR amplification of Prp43 NTD (1- 94 aa) with NdeI ends
59	Prp43RecA1_F	AAAAAGGATCCGTGTACATGCCCA GAGAGATGAGTTT	PCR amplification of Prp43 RecA1 (95- 270 aa) with BamH1 ends
60	Prp43_RecA1_R	AAAAAGGATCCCTATCTGCCCGGA ACGGCAAGTAA	PCR amplification of Prp43 RecA1 (95- 270 aa) with BamH1 ends

61	Prp43RecA2_F	AAAAAGGATCCGTACATATCCAGT TGAGCTATACTAT	PCR amplification of Prp43 RecA2 (271- 457 aa) with BamH1 ends
62	Prp43RecA2_R	AAAAAGGATCCCTAAATTTCTGGG TAACTTTGCTCTAT	PCR amplification of Prp43 RecA2 (271- 457 aa) with BamH1 ends
63	Prp43_WH_F	AAAAACATATGTTACGTTCCAATT TATCCTCC	PCR amplification of Prp43 WH (458- 521 aa) with NdeI ends
64	Prp43_WH_R	AAAAACATATGCTAGGGAAACTGG GATGCCAATCT	PCR amplification of Prp43 WH (458- 521 aa) with NdeI ends
65	Prp43_Ratchet_F	AAAAACATATGTTGGATCCTATGC TAGCGGTG	PCR amplification of Prp43 Ratchet (522- 635 aa) with NdeI ends
66	Prp43_Ratchet_R	AAAAACATATGCTAGTTTAATTCT AGGTTGTAACG	PCR amplification of Prp43 Ratchet (522- 635 aa) with NdeI ends
67	Prp43CTD_F	AAAAAGGATCCGTACTACAGACTA TGAAAGCCCTAAA	PCR amplification of Prp43 CTD (636-767 aa) with BamH1 ends
68	Prp43CTD_R	AAAAAGGATCCCTATTTCTTGGAG TGCTTACTCTT	PCR amplification of Prp43 CTD (636-767 aa) with BamH1 ends

#69-74: Cloning of G-patch domain:

	Name	Oligo sequence (5'>3')	Purpose
69	SPP382 BamH1 end F	AAAAAGGATCCGAACATATGGTAT TGGTGCGAAG	PCR amplification of SPP382 G-patch with BamH1 ends
70	SPP382 G BamH1 end R	AAAAAGGATCCTTAATTTGAAAAC ATTCCTAGACCT	PCR amplification of SPP382 G-patch with BamH1 ends
71	PXR1 Gw BamH1 end F	AAAAAGGATCCGAACCTCGAGATT CGGGCAC	PCR amplification of PXR1 G-patch with BamH1 ends
72	PXR1 G BamH1 end-R	AAAAA GGATCC TTA TTTTAATTTAGCACCGAGCCCAAC	PCR amplification of PXR1 G-patch with BamH1 ends
73	SQS1 G w BamH1 end F	AAAAAGGATCCGAAACGAAAATA TCGGTAGAAGA	PCR amplification of SQS1 G-patch with BamH1 ends
74	SQS1 G w BamH1 end R	AAAAAGGATCCTTAACCTTCACTG TGTCTTAAACC	PCR amplification of SQS1 G-patch with BamH1 ends

Note: Italicized nucleotide sequence represents restriction site.

#75-82: rRNA and snoRNA processing pathway hybridizations

	Oligo name	5'>3' sequence	rRNA identified
75	003	TGTTACCTCTGGGCCC	27SA2
76	004	CGGTTTTAATTGTCCTA	35S rRNA precursor, 23S and 20S
77	007	CTCCGCTTATTGATATGC	25S
78	008	CATGGCTTAATCTTTGAGAC	18S
79	017	GCGTTCTTCATCGATGC	5.8S
80	020	TGAGAAGGAAATGACGCT	27SA, 27SB, 7S
81	snR18	CGTCAGATACTGTGATAGTCAACCAC	U18
82	snR128	TCACTCAGACATCCTAGGAAGGTC	U14

Fig. S2

Figure S2 The Y2H G-patch peptides are stable in yeast. Western blot of proteins from yeast that express pAS2-Prp43 Y2H construct together with pACT2 Gal4 fusions to the Spp382, Sqs1 or Pxr1 G-patch domains or no pACT2 vector (none). Antibodies specific to the anti-HA epitope in pACT2 and pAS2 (HA.11; developed with BCIP/NBT) or α tubulin (12G10; developed by ECF) were used to image the proteins. After normalization with α tubulin, the G-patch proteins were found in a relative signal ratio of 1: 2.7: 1.7 (Spp382:Sqs1:Pxr1).

Fig. S3

Figure S3 The Sqs1 G-patch partially supports Spp382 function. Yeast growth of the *spp382::KAN* mutant reconstituted with a plasmid-borne copy of the intact *SPP382* gene and the *spp382-SQS1* chimera in which the Sqs1 G-patch substitutes for the equivalent Spp382 sequence. Yeast cultures were grown for 2 days on YPD agar (insert) or in YPD broth at 30°C. For the liquid growth assay, mid-log phase cultures were initially diluted to an OD 600 nm of 0.05 and culture density monitored at 2 hour intervals.

Fig. S4A

	1		50
SpAT_NP_173150.1	KSTKGIGMKL	LEKMGYKGG-	GLGKNQGGIV APIEAQLRP- KNMGMGYN
SpSP_NP_594091.1	FNTTGFgAKM	LEKMGYKQGQ	GLGANAEGIA EPVQSKLRP- ERVGLGAV
SpHS_NP_00100869	RHTKGIGQKL	LQKMGYVPGR	GLGKNAQGI I NPIEAKQRK- GKGAVGAY
SpMM_NP_061253.2	RHTKGIGQKL	LQKMGYVPGR	GLGKNAQGI I NPIEAKQRK- GKGAVGAY
SpBT_NP_001039495.2	RHTKGIGQKL	LQKMGYVPGR	GLGKNAQGI I NPIEAKQRK- GKGAVGAY
SpGG_NP_001025836.1	RHTKGIGQKL	LQKMGYVPGR	GLGKNAQGI I NPIEAKQRK- GKGAVGAY
SpDR_NP_001002721.1	KHTRGIGQKL	LQKMGYVPGR	GLGKNAQGI I NPIEAKLRK- GKGAVGAY
SpXL_NP_001087642.1	RHTKGIGQKL	LQKMGYMPGR	GLGKNAQGI I APIEAKQRR- GKGAVGAY
SpDM_AAF52282.1	QHTRGIGAKL	LLQMGYEPGR	GLGKDLQGIS HPVQAHVRK- GRGAIGAY
SpCE_NP_496226.2	DGNSNKIMKM	MQAMGYKPG	GLGAQGGIV EPVQAQLRK- GRGAVGAY
SpSC_AAB67507.1	TKTYGIGAKL	LSSMGYVAGK	GLGKDGSGIT TPIETQSRP MHNAGLGMF
SpConsensus	..T.GiG.k\$	L..MGY..G.	GLGk#.#GI. .P!#a..R.Ga%
	1		50
PxBT_ ABF57427.1	NDDSKFGQRM	LEKMGWSKGK	GLG-AQEQQGATDHIKVQVKN NHLGLGAT
PxHS_ AAS19507.1	NDDSKFGQRM	LEKMGWSKGK	GLG-AQEQQGATDHIKVQVKN NHLGLGAT
PxMM_ NP_082504.1	NDDSKFGQKM	LEKMGWSKGK	GLG-AQEQQGATEHIKVQVKN NHLGLGAT
PxGG_ XP_420036.2	KDESKFGQRM	LEKMGWSKGK	GLG-AQEQQGNTTEHIKVQVKN NMLGLGAS
PxXL_ NP_001086366.1	KDESKFGQKL	MEKMGWSKGK	GLG-AKEQQGSTEHIKVQVKN NNLGLGAS
PxDR_ AAH54670.1	NDESKFGQKM	LERMGWSKGK	GLG-KTEQQGSTEHIKVQVKN NSLGLGTA
PxCE_ NP_495955.1	NDDQKLSKKL	MEKMGWSEGD	GLG-RNRQGNADSVKLKANT SGRGLGAD
PxDM_ NP_611495.1	EDENRFGTKM	LEKMGWTKGS	GLG-ANLNGEKDFVIRFKN DAEGLGFE
PxSP_ Q9URX9.1	KDTNRLGFKL	LSSYGWVNGN	GLG-EKQHGRHNIKVSLKD DTLGIGAK
PxSC_ AAS56710.1	NDTSRFGHQF	LEKFGWKPGM	GLGLSPMNSNTSHIKVSIKD DNVGLGAK
PxConsensus	.D...fG.k\$	\$Ek.GW..G.	GLG ...#G....!K!..K# #...GLGa.
	1		51
SqSC_NP_014175.1	IGNENIGRRM	LEKLGWKSSE	GLGIQG-NKG ISEPIFAKIKKNRSGLRHS
SqSP_NP_595527.1	ISKENPGRRL	LEKLGWYAGK	GLGHPE-NEG SKDSLRAIVKVSRSGLG**
SqHS_NP_060510.1	ILENNIGNRM	LQNMGWTPGS	GLG-RD-GKG ISEPIQAMQRPKGLGLGFP
SqMM_EDL13048.1	ILESIGNRM	LQSMGWTPGS	GLG-RD-GRG IAEPVQAVQRPKGLGLGFP
SqGG_NP_001026217.1	IPENNIGNRM	LQSMGWTPGT	GLG-PD-GKG IAEPIRAIQRPKGLGLGFS
SqBT_XP_582084.3	LGSDNIGSRM	LQAMGWKEGS	GLG-RK-KQG IVTPIEAQTRVGRSGGLGAR
SqXL_NP_001086761.1	IDNSNIGNKM	LQAMGWKEGS	GLG-RK-SQG ITAPIQAQVRMRGAGLGLG
SqDR_AAI65705.1	IGDENKGRQM	LEKMGWKRGE	GLG-KD-GAG IKDPIQLHMRKAQSGGLGSS
SqCE_NP_491200.1	PATGGIGRLM	LEKMGWRPGE	GLG-KD-ATG NLEPLMLDVKSDRKGGLIAE
SqDM_NP_725443.1	IGSSNKGFKM	LSKLGWQKGE	KLGKTNASAG LLEPINVANEGTSGLGNS
SqConsensus	i...N.G..M	L#.\$GW..G.	GLG... ..G ..#Pi.....GLg..

Fig. S4B

Spp382	59	TKTYGIGAKLLSSMGYVAGKGLGKDGSGITTP	106
Sqs1	718	IGNENIGRRMLEKLGWKSSEGLGIQGNKGISEPIF-AKIKKNRSGLRHS	765
Pxr1	23	NDTSRFGHQFLEKFGWKPGMGLGLSPMNSNTSHIK-VSIKDDNVGLGAK	70
CONSENSUS/65%		hstpshtpshpMGap.GpGLGpppp.GhhpPlps.php.pptGlGh.	
CONSENSUS/80%		...tshu.thh.thGap.GpGLGtptp.Ghpsl.s.thp..ptGhsh.	

Figure S4 Multiple sequence alignment of G-patch domains. A. Multalin analysis (CORPET 1988) of the Spp382 (Sp), Pxr1 (Px) and Sqs1 (Sq) G-patch peptides from *Saccharomyces cerevisiae*- SC, *Schizosaccharomyces pombe*- SP, *Arabidopsis thaliana*- AT, *Drosophila melanogaster*- DM, *Caenorhabditis elegans*- CE, *Danio rerio*- DR, *Xenopus laevis*- XL, *Gallus gallus*- GG, *Mus musculus*- MM, *Bos Taurus*- BT and *Homo sapiens*- HS. NCBI protein

accession numbers follow each peptide designation. The consensus was derived at 65% (blue) and 85% (red) identity with Consensus symbols: ! is anyone of IV; \$ is L or M; % is F or Y; # is any one of N, D, Q, E. **B.** Multalin analysis of the yeast Spp382, Pxr1 and Sqs1 as presented in manuscript Fig. 1B compared with the SMART consensus derived from the 2124 listed G-patch peptides (LETUNIC *et al.* 2012). The symbols across 65% or 80% of all G-patch sequences are:

Class	Key	Residues
alcohol	o	S,T
aliphatic	l	I,L,V
any	.	A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
aromatic	a	F,H,W,Y
charged	c	D,E,H,K,R
hydrophobic	h	A,C,F,G,H,I,K,L,M,R,T,V,W,Y
negative	-	D,E
polar	p	C,D,E,H,K,N,Q,R,S,T
positive	+	H,K,R
small	s	A,C,D,G,N,P,S,T,V
tiny	u	A,G,S
Turn-like	t	A,C,D,E,G,H,K,N,Q,R,S,T

Fig. S5

Figure S5 Spp382 is largely insensitive to G-patch P90 mutation. Growth of yeast *spp382::KAN* mutant transformed with YCplac111-*SPP382* with a wildtype G-patch (WT) or bearing the P90A, P90S or P90H substitutions.

Fig. S6

Figure S6 G-patch peptides show differential binding to full length Prp43 protein. Full-length Prp43 was purified as previously described (Tanaka and Schwer, 2006). The G-patch peptides were prepared with C-terminal chitin binding domain fusions using the NEB Impact System. To achieve equivalent recovery of soluble peptide, 70 amino acids of (non-G-patch) N-terminal Spp382 peptide precedes each G-patch domain. *E. coli* lysates expressing the indicated G-patch peptides were first bound to chitin agarose beads in 20 mM Hepes pH 8.5; 500 mM NaCl, 1 mM EDTA for 60 minutes at room temperature. The beads were washed with 20 volumes of binding buffer and then then with 1 ml buffer B (50 mM NaCl, 50 mM imidazole, 50 mM Tris-HCl at pH 7.4, 10% glycerol). Next, approximately 10 μ g of purified Prp43 was presented to 20 μ l of the washed peptide-bound agarose beads (roughly 6-fold molar excess of peptide over Prp43) for 1 hour at 4C in 100 μ l in buffer B. The chitin beads were collected by centrifugation, the unbound sample removed, and the beads washed 3 times with 100 μ l of buffer B adjusted to 50 or 200 mM NaCl. Finally the beads were resuspended in 50 μ l protein sample buffer (100 mM Tris-HCl, pH 6.8; 20% glycerol; 5% SDS; 5% β -mercaptoethanol 0.02% bromophenol blue) and boiled for 10 minutes to release the peptide along and bound Prp43. The proteins were resolved by 10% SDS-PAGE and visualized with Coomassie blue. The input Prp43 and the G-patch fusion peptides are shown in lane 1-5. Lanes 6-15 show the proteins released from the agarose beads.

Figure S7 The Spp382-Pxr1 G-patch substitution derivatives are equivalently expressed.

Western blot of proteins from yeast that express pAS2-Prp43 Y2H construct together with pACT2 Gal4 fusions with the original Spp382 protein or Spp382 Δ G reconstituted with the Pxr1 G-patch (WT) or Pxr1 G-patch substitutions (D62M, H55P, K57E, P48G, R27G). Antibodies specific to the Gal4 DNA activation domain epitope in pACT2 (sc-1663; for Spp382 and Spp382-Pxr1) and α tubulin (12G10) were simultaneously used to image these proteins with BCIP/NBT. The blot was subsequently stripped by incubation with agitation in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.8 at 70° for 30 minutes and then re-developed using an antibody against the pAS2 Gal4 DNA binding domain (sc-577) to image Prp43 with ECF. The relative signal intensities of the Spp382-derived bands are presented after normalization with the α tubulin control.

Fig. S8

Figure S8 The primary Prp43 binding site within Pxr1 lies downstream of the G-patch.

The numbered positions refer to the amino acid coordinates previously used to describe Pxr1 (GUGLIELMI and WERNER 2002). The approximate G-patch and Prp43 binding domain (Prp43BD) and KKE/D domains are shown. The results of the Y2H interaction study between Prp43 and the *pxr1* deletion constructs and single domain constructs are shown with symbols to indicate relative growth from greatest (+++) to no visible colonies (-) after 2 days at 30°C on medium lacking histidine and supplemented with 5 mM 3-aminotriazole. The -* constructs form pinpoint colonies with longer incubation times. The numbers in parentheses indicate the codons removed (deletions) or remaining (single domain) in each Y2H construct. The *pxr1::KAN* complementation pattern by *pxr1* deletion constructs cloned into is shown to the right of the Y2H results. In this case, growth was monitored on medium lacking leucine for 2 days at 30°C. nd = not done

Figure S9 Most PXR1 deletion derivatives produce stable proteins. A. Western analysis of yeast that express full-length Prp43 in the pAS2 Y2H vector and the indicated Pxr1-derivatives in pACT2. As expected, the bands corresponding to the ~50 amino acid deletions of Pxr1 (Pxr1 Δ G and Pxr1 Δ 102-149) co-migrate as do the single ~ 50 amino acid domain fusions (G-patch only and Pxr1 102-149). The Pxr1 Δ 102-226 construct did not produce a reproducible unique band of the predicted size although a faint protein, possibly a degradation product, could sometime be observed below the position of the 40 kDa marker. An antibody specific to the anti-HA epitope HA.11) found in pACT2 and pAS2 was used to image the Prp43 and Pxr1-derived proteins with BCIP/NBT development. The asterisks show the positions of two prominent background bands cross-reacting with the HA.11 antibody. To avoid artifacts of co-migrating bands, identical samples run on a parallel blot to visualize the α tubulin (12G10) control (developed with BCIP/NBT). The relative Pxr1 signal is presented after normalization with α tubulin. Equivalent results were obtained using the larger background band seen in the HA.11 image for normalization (data not shown). **B.** Western analysis of the Pxr1 150-226 single domain insert compared with full-length Pxr1. The samples were imaged and analyzed as in panel A.

Figure S10 Most *PRP43* deletion derivatives are well expressed in yeast. Western analysis of proteins from yeast that co-express the indicated pAS2-Prp43 domain deletions (pAS2-Prp43-der.) and the pAS2-Pxr1(102-149) construct or empty pACT2 vector probed with anti-HA antibody, HA.11. In each case, the most prominent unique band migrates as predicted for the expected fusion product. The α tubulin protein was imaged after stripping to remove the HA.11 antibody. As the resulting tubulin ECF signal was weak and distorted, the background band co-migrating with the pACT—empty vector protein in the HA.11 image was used for normalization.

Figure S11 Most isolated Prp43 domains are stable in yeast. A western blot is presented of proteins from yeast that express the indicated pAS2-Prp43 isolated domain constructs or the pAS2 empty vector probed with anti-HA antibody, HA.11 and developed with BCIP/NBT. In each case, the prominent unique band indicates the position of the expected fusion product. The asterisk indicates the position of the most weakly expressed Y2H construct, pAS2-Prp43 – Ratchet. The relative signals of the Prp43-derived peptides are indicated with α tubulin ECF signal used for normalization. The numbers at the left indicate the sizes of protein marker bands migrating in the indicated positions.

Supporting Information References

Corpet, F., 1988 Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Res* 16: 10881-10890.

Guglielmi, B., and M. Werner, 2002 The yeast homolog of human PinX1 is involved in rRNA and small nucleolar RNA maturation, not in telomere elongation inhibition. *J Biol Chem* 277: 35712-35719.

Letunic, I., T. Doerks and P. Bork, 2012 SMART 7: recent updates to the protein domain annotation resource. *Nucleic Acids Res* 40: D302-305.