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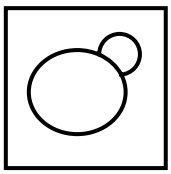

Research Article

Genome-wide protein interaction screens reveal functional networks involving Sm-like proteins

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Abstract

A set of seven structurally related Sm proteins forms the core of the snRNP particles containing the spliceosomal U1, U2, U4 and U5 snRNAs. A search of the genomic sequence of *Saccharomyces cerevisiae* has identified a number of open reading frames that potentially encode structurally similar proteins termed Lsm (Like Sm) proteins. With the aim of analysing all possible interactions between the Lsm proteins and any protein encoded in the yeast genome, we performed exhaustive and iterative genomic two-hybrid screens, starting with the Lsm proteins as baits. Indeed, extensive interactions amongst eight Lsm proteins were found that suggest the existence of a Lsm complex or complexes. These Lsm interactions apparently involve the conserved Sm domain that also mediates interactions between the Sm proteins. The screens also reveal functionally significant interactions with splicing factors, in particular with Prp4 and Prp24, compatible with genetic studies and with the reported association of Lsm proteins with spliceosomal U6 and U4/U6 particles. In addition, interactions with proteins involved in mRNA turnover, such as Mrt1, Dcp1, Dcp2 and Xrn1, point to roles for Lsm complexes in distinct RNA metabolic processes, that are confirmed in independent functional studies. These results provide compelling evidence that two-hybrid screens yield functionally meaningful information about protein–protein interactions and can suggest functions for uncharacterized proteins, especially when they are performed on a genome-wide scale.

Keywords: **two-hybrid; splicing; mRNA degradation; yeast; proteome; snRNP**

Introduction

Splicing of nuclear pre-mRNA occurs within a large ribonucleoprotein complex called the spliceosome. Spliceosome assembly involves snRNP particles constituted of snRNAs (the U1, U2, U4, U5 and U6 snRNAs) which are associated with proteins. Human U1, U2, U4 and U5 snRNPs contain two classes of proteins: seven small proteins, collectively called the Sm proteins (B or B′, D1, D2, D3, E, F, G) that constitute a core particle common to these snRNP, and other proteins associated specifically with one particular snRNP (Burge *et al*., 1998; Will and Lührmann, 1997). These snRNP particles are evolutionary highly conserved and Sm proteins were also identified in the yeast *Saccharomyces cerevisiae* (Bordonné and Tarassov, 1996; Gottschalk *et al*., 1998; Roy *et al*., 1995; Rymond *et al*., 1993). All Sm proteins contain two conserved regions, called the Sm motifs 1 and 2 (Cooper *et al*., 1995; Hermann *et al*., 1995; Seraphin 1995). The U1, U2, U4 and U5 snRNAs are transcribed by RNA polymerase II and exported to the cytoplasm, where they associate with a complex of Sm proteins

to form core snRNP particles. These U snRNAs contain a conserved structural motif, a single-stranded uridylic acid-rich region flanked by two stem-loop structures (Branlant *et al.*, 1982), which is recognized by the Sm protein complex. When the Sm core particle is assembled, the 5′ cap of the snRNA becomes hypermethylated and 3′-end processing occurs (Jacobson *et al.*, 1993). At least in higher eukaryotes, binding of the Sm core proteins is essential for the hypermethylation of the cap (Mattaj, 1986) and both the 5′ trimethylguanosine cap and the Sm proteins are required for the nuclear import of the snRNP (Fischer *et al.*, 1993). Finally, with the addition of snRNP-specific proteins, a functional snRNP is produced. Thus, in higher eukaryotes the biogenesis of snRNPs is a complex process involving both nuclear and cytoplasmic compartments.

The U6 snRNA is different; it is transcribed by RNA polymerase III and has a γ-monomethyl triphosphate cap. U6 snRNA lacks the Sm binding site and does not itself assemble with the canonical Sm proteins (Luhrmann *et al.*, 1990). However, the U4 and U6 snRNAs have extensive sequence complementarity to one another and most or all of the U4 snRNA is found complexed with U6 snRNA in a U4/U6 di-snRNP, while a free form of U6 also exists. There have been conflicting reports about the localization of the U6 snRNA. For example, it was reported that in *Xenopus* oocytes U6 snRNA does not leave the nucleus (Vankan *et al.*, 1990), whereas work on mouse fibroblast cells indicated that newly synthesised U6 snRNA is present transiently in the cytoplasm (Fury and Zieve, 1996).

Searches in the *Saccharomyces cerevisiae* genome database allowed the identification of another set of Sm-like proteins (Fromont-Racine *et al.*, 1997). One of them, Lsm8, was identified in a two-hybrid screen using the splicing factor Hsh49p as bait (Fromont-Racine *et al.*, 1997), indicating a possible link with the splicing machinery. Indeed, seven out of these nine Sm-like proteins, renamed Lsm 2–8, were found to associate with U6 snRNA (Cooper *et al.*, 1995; Mayes *et al.*, 1999; Pannone *et al.*, 1998; Seraphin, 1995), suggesting the possible existence in budding yeast of a heptameric U6-associated Lsm particle that may be similar to the Sm core particle. Indeed, most of these Lsm proteins were found associated with the U4/U6.U5 tri-snRNP (Gottschalk *et al.*, 1999; Stevens and Abelson, 1999). In contrast, Lsm1 displays only a weak, highly salt-sensitive association with U6 (Mayes *et al.*, 1999) and yeast genetic studies have implicated Lsm1 (previously Spb8) in mRNA decapping (Boeck *et al.*, 1998). Thus, Lsm 1 protein could be involved in a distinct pathway. The last Lsm protein, SmX1 or Lsm9, does not bind to U6 snRNA and was recently found in a protein complex unrelated to splicing (Rigaut *et al.*, 1999). Little is known on the biogenesis of the U6 snRNP particle, apart from a proposed chaperone function for La protein, handing the newly synthesized U6 snRNA from RNA polymerase III to Lsm proteins (Pannone *et al.*, 1998). Also, in humans, seven Lsm orthologous proteins were found associated with U6 snRNA (Achsel *et al.*, 1999).

In order to understand what could be the various role of Lsm proteins, we used exhaustive and iterative two-hybrid screens, starting with Lsm proteins as baits. Interactions amongst the Lsm proteins themselves strongly suggest the existence of a Lsm complex or complexes. These interactions require both Sm motifs. The screens also reveal several interactions with splicing factors that may be functionally significant. In particular, the interactions with Prp4 and Prp24 are compatible with the observed association of Lsm proteins with U6 and U4/U6 particles, and with genetic studies. Interactions with SmD2, Prp11 and Hsh49 suggest that Lsm proteins may also play a key role in assembling spliceosomes through snRNP–snRNP interactions. In addition, the screens reveal interactions with proteins which are involved in mRNA turnover, hinting that Lsm1 may not be the only Lsm protein associated with such a function. Altogether, the Lsm screens and the iterative screens point to roles for Lsm proteins in distinct processes.

Materials and methods

Strains and plasmids

Y187, CG1945 and L40 strains were used to perform the two-hybrid screens (Fromont-Racine *et al.*, 1997). We derived the L40ΔG from the L40 strain by deleting the GAL4 gene in this strain and replacing it by a $KANA^R$ cassette (see below). Gap

repair experiments were performed in the yeast strain BMA64 (F. Lacroute). The *Escherichia coli* strain MC1066 was used for prey plasmid recovery, selecting on plates lacking leucine.

The pBTM116 plasmid (Vojtek *et al*., 1993) was used to clone LexA fusions and pAS2ΔΔ was used to clone the Gal4 bait fusions (Fromont-Racine *et al*., 1997).

Plasmid bait constructions

The full-length ORFs were always used. For LSM1 (YJL124C) a *Bam*H1 fragment taken from a prey plasmid fused at nucleotide -52 relative to the initiation codon was cloned in-frame into a modified pBTM116 plasmid. For LSM2 (YBL026W without the intron) a *Bam*HI–*Pst*I fragment produced by reverse transcription followed by PCR amplification (Sambrook *et al*., 1989) was cloned into pAS2ΔΔ and pBTM116 plasmids. For LSM3 (YLR438CA) a *Nco*1–*Bam*H1 PCR fragment was cloned into pAS2ΔΔ. For LSM4 (YER112W), a *Nco*1–*Bam*H1 PCR fragment was cloned into pAS2ΔΔ. For LSM5 (YER146W), a *Bam*H1 fragment taken from a prey plasmid fused at nucleotide -2 respective to the start codon was cloned in-frame into pBTM116 plasmid. For LSM6 (YDR378C) a *Eco*R1–*Bam*H1 PCR fragment was cloned in pBTM116 plasmid. For LSM7 (YNL147W without the intron) a PCR fragment was cloned into pBTM116 plasmid at the *Sma*1 site. For LSM8 (YJR022W) a *Nco*I–*Xho*I fragment from a prey plasmid fused at nucleotide -2 with respect to the initiation codon was cloned into pAS2ΔΔ between the *Nco*I and *Sal*1 sites, then subcloned as a *Bam*H1 fragment into pBTM116. For YEL015W a *Eco*R1–*Sal*1 PCR fragment was cloned into pAS2ΔΔ. All sequences derived by PCR cloning were verified. PSU1 was cloned in pAS2ΔΔ by gap repair, using two PCR fragments with about 200 nucleotides of homology to the 5′ and 3′ ends of the gene cloned in pAS2ΔΔ. After gap repair, the plasmid was recovered and the gene was checked by restriction mapping and sequencing of the 5′ end. PAT1 full-length sequence as bait had very high autoactivating activity, so a pGBT9 derivative bait plasmid lacking the highly acidic N-terminal 51 amino acids was used for the screen (a generous gift from F. Lacroute).

L40ΔG strain construction and two-hybrid mating

Two-hybrid screens were performed by a mating strategy, using the FRYL library introduced in Y187 cells and either CG1945 cells producing Gal4-derived bait proteins or L40 and L40ΔG cells producing LexA-derived bait proteins (Fromont-Racine *et al*., 1997). For the LexA–Lsm6 and LexA–Lsm8 screens, only the HIS3 reporter was used to select interactors (diploid cells derived with L40 cells express endogenous Gal4 gene, which spontaneously activates the LacZ reporter gene from the Y187 background). To permit use of the LacZ reporter with other LexA baits, we generated the strain L40ΔG by replacing the entire *GAL4* coding sequence in L40 by the kanamycin-resistance marker from plasmid pkana-X2 (Wach *et al*., 1994). The gene replacement was confirmed by Southern blotting, and an X-gal overlay on diploid cells ($Y187 \times L40\Delta G$) verified that endogenous activation of the LacZ reporter did not occur (data not shown).

Selection of positive clones

Positive clones were selected as previously described (Fromont-Racine *et al*., 1997). Prey inserts were amplified from library plasmids by PCR on colonies (Wang *et al*., 1996a), the length of each insert was determined by gel electrophoresis and the 5′ junction was sequenced. Identification of each candidate in the yeast database was performed by a dedicated software (DOGEL) that gives the chromosomal coordinates (chromosome number, strand and position), ORF and gene name and the exact location of the beginning of the insert relative to the initiation codon. Alternatively, the BLAST program can be used against the Saccharomyces Genome Database (SGD; http://genome-www.stanford.edu). The biological information on the ORF was extracted from the Yeast Protein Database (YPD; http://www.proteome.com).

Classification of the candidates

Prey fusions were classified according to four different categories of different heuristic values ($A1 > A2 = A3 > A4$) (Fromont-Racine *et al*., 1997). B fusions express non-biological peptides, i.e antisense or intergenic regions, and are excluded from the tables of results.

Results

Characteristics of genomic interaction screens with Lsm proteins

With the aim of identifying as many as possible of the proteins that interact with Lsm proteins we performed exhaustive and iterative two-hybrid screens using the FRYL *S. cerevisiae* genomic library (Fromont-Racine *et al.*, 1997) (Table 1; see Materials and methods). For each screen, results are analysed in order to evaluate the heuristic value of each prey protein (Fromont-Racine *et al.*, 1997) (see also Materials and methods). In addition, the domain of interaction selected for each prey protein is identified (Table 1). Figure 1 presents the characteristics of the screens, showing for each bait protein the number of ORFs selected in each category, and the number of clones of each ORF. Comparing the profiles for different bait proteins, it is apparent that, despite the similarity of structure between Lsm proteins, they behave differently in the screens. Some, such as Lsm7, have few partners, whereas others, such as Lsm1, have many partners.

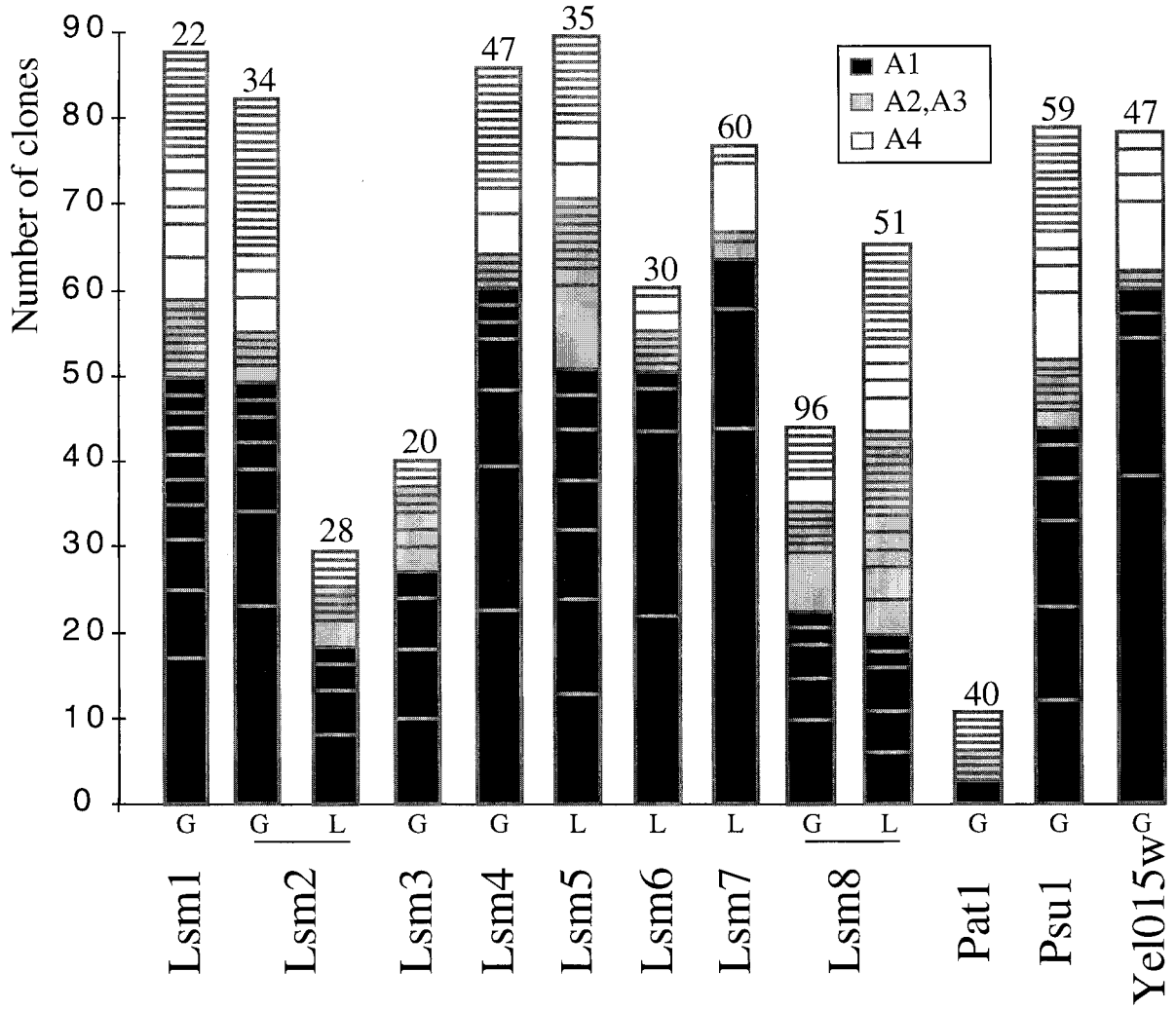

Figure 1. Distribution profiles of prey proteins found in two-hybrid screens. Each histogram represents one given screen with a Gal4 (G) or a LexA (L) fusion-bait cloned into pAS2^{-} and pBTM116 plasmids, respectively. Each box corresponds to one ORF prey and is drawn according to categories (Fromont-Racine *et al.*, 1997) (see insert and Materials and methods). The size of the box represents the number of clones related to one particular ORF. The number of interactions tested in each screen is indicated on the top of the histogram (in millions)

Table 1. Results of genomic protein interaction screens

Bait	Prey ORF name (a)	Prey gene name (b)	Start domain (c)	End domain (d)	Cat (e)	Clones number (f)
Lsm1-Gal4	YAL019W	FUN30	432	950	A3	1
	YBL026W	LSM2	2	end	A1	8(3)
	YBR214W	SDS24	282	end	A4	1
	YBR274W	CHK1	355	end	A4	4
	YCR024C		271	end	A4	1
	YCR077C	PAT1	95;353(g)	350;end(g)	A1	17(5)
	YDL013W	HEX3	6	350	A2	1
	YDL175C		1	end	A2/A3	1
	YDR002W	YRB1	39	end	A2	1
	YDR378C	LSM6	54	end	A1	2(2)
	YDR422C	SIP1	473	end	A4*	1
	YEL060C	PRB1	58	300	A1	3(3)
	YER028C		308	end	A4*	2
	YGL207W	SPT16	813	end	A1*	4(2)
	YGR158C	MTR3	44	end	A2	1
	YIL042C		14	end	A2/A3	1
	YIL048W	NEO1	1048	end	A4*	5
	YJR143C	PMT4	661	end	A4*	2
	YKL173W	SNU114	441	600	A4	1
	YKR026C	GCN3	62	200	A1	3(2)
	YLR003C		214	end	A1	6(3)
	YLR362W	STE11	172	350	A4	2
	YLR438C-A	LSM3	1	end	A2*	1
	YML088W		165	end	A1	2(2)
	YMR056C	AAC1	108	end	A4*	1
	YMR250W		418	end	A4	1
	YNL032W	SIW14	46	end	A4	1
	YNL163C	EF4	97	250	A4	1
	YNL276C		128	end	A4	1
	YOR109W	INP53	167	300	A4	1
	YOR147W		88	400	A4	2
	YOR320C		424	end	A4	1
	YOR375C	GDH1	42	end	A2/A3	1
	YPL016W	SWI1	558	900	A1	2(2)
	YPL084W	BRO1	1	200	A2	1
	YPL152W	RRD2	329	end	A4*	1
		Ty2-1	ND	ND	A1	3(2)
Lsm2-Gal4	YAR003W	FUN16	46	150	A4*	1
	YBL066C	SEF1	446	700	A4	1
	YCR020C-A	SMX1	1	end	A2	1
	YCR066W	RAD18	155	end	A3	1
	YCR077C	PAT1	353	end	A1	3(2)
	YDL175C		81	150	A1	5(2)
	YDR440W	DOT1	121	450	A4	1
	YEL015W		232	end	A1	11(5)
	YER025W	GCD11	10	300	A2*	1
	YGL173C	XRN1	890	end	A3	1
	YGL185C		273	end	A4*	1
	YGR077C	PEX8	366	end	A4	1
	YIL048W	NEO1	1048	end	A4*	4
	YIL066C	RNR3	383	600	A4	3
	YIL132C		134	end	A4	1
	YLR039C	RIC1	994	end	A4	1
	YLR120C	YPS1	10	200	A1	3(2)
	YLR126C		165	end	A1	2(2)

Table 1. continued

Bait	Prey ORF name (a)	Prey gene name (b)	Start domain (c)	End domain (d)	Cat (e)	Clones number (f)
	YMR066W	SOV1	93	350	A4	1
	YMR207C	HFA1	733	950	A4	1
	YMR237W		234	400	A1	2(2)
	YMR268C	PRP24	57	end	A3	2
	YNL118C	DCP2	ND	ND	A1	23(8)
	YOL102C	TPT1	167	end	A4	1
	YOL163W		106	end	A4	1
	YOR017W	PET127	51	300	A4	1
	YOR043W	WHI2	240	end	A4*	2
	YOR191W	RIS1	506	900	A4*	1
	YPL042C	SSN3	403	end	A4	1
	YPL115C	BEM3	606	850	A4	1
	YPL119C	DBP1	291	500	A4	1
	YPL249C		293	550	A4	1
	YPR184W		1371	end	A4	1
Lsm2-LexA	YDR166C	SEC5	895	end	A4	1
	YJL124C	LSM1	1	end	A2	3
	YJL157C	FAR1	209	600	A4	1
	YJR022W	LSM8	23	end	A1	8(3)
	YLR120C	YPS1	6	250	A2	1
	YLR128W		157	end	A4*	1
	YLR275W	SmD2	12	end	A1	2(2)
	YLR438C-A	LSM3	1	end	A2*	1
	YNL264C	PDR17	266	end	A1	5(3)
	YNL287W	SEC21	222	500	A4	1
	YOR017W	PET127	6	300	A2	1
	YPL249C		258	550	A1	3(2)
	YPR032W	SRO7	15	300	A2	1
Lsm3-Gal4	YBR108W		706	end	A4	1
	YCR066W	RAD18	126	end	A4	1
	YCR077C	PAT1	168;353(g)	350;end(g)	A1	10(5)
	YDL013W	HEX3	4	400	A2	1
	YDL240W	LRG1	543	750	A4	1
	YJR022W	LSM8	22	end	A1	6(4)
	YJR138W		1135	1500	A1	3(2)
	YKR099W	BAS1	57	750	A3	3
	YLR067C	PET309	41	450	A1	8(3)
	YLR281C		50	end	A2	2
	YMR142C	RPL13B	1	100	A2	1
	YOR096W	RP30	1	end	A2	1
	YPL084W	BRO1	1	200	A2	2
Lsm4-Gal4	YBR289W	SNF5	360	650	A4	1
	YCR077C	PAT1	353	end	A4	1
	YDL043C	PRP11	66	end	A4	5
	YDL145C	COP1	85	350	A4	1
	YDR082W	STN1	441	end	A1	6(3)
	YDR289C		72	250	A4	1
	YDR386W	MUS81	112	500	A4	1
	YDR452W		61	350	A1	2(2)
	YDR485C		664	end	A1	2(2)
	YER124C		468	end	A4	1
	YGL173C	XRN1	863	1500	A3	1
	YIL029C		132	end	A4	3
	YJL110C	GZF3	397	end	A1	2(2)

Table 1. continued

Bait	Prey ORF name (a)	Prey gene name (b)	Start domain (c)	End domain (d)	Cat (e)	Clones number (f)
	YJL155C	FBP26	145	end	A1	9(4)
	YJR022W	LSM8	1	ND	A2	1
	YJR138W		1004	1150	A4	1
	YKL209C	STE6	1012	1250	A4	1
	YLL032C		566	800	A4	1
	YLR275W	SmD2	54	end	A4	1
	YLR386W		378	800	A1	23(9)
	YNL091W		1160	end	A4	1
	YNL118C	DCP2	ND	ND	A1	17(4)
	YNL199C	GCR2	272	500	A4	1
	YOL004W	SIN3	492	650	A3	1
	YOL149W	DCP1	1	end	A2	1
	YOR195W	SLK19	268	500	A4	1
	YOR219C	STE13	366	500	A4	1
Lsm5-LexA	YBL026W	LSM2	1	end	A1	6(2)
	YCR024C		271	end	A4	1
	YCR077C	PAT1	132;353(g)	350;end(g)	A1	13(5)
	YDL112W	TRM3	889	1100	A1*	4(2)
	YDR110W	FOB1	456	end	A4	1
	YDR378C	LSM6	1	end	A2*	10
	YER025W	GCD11	10	350	A2*	1
	YER112W	LSM4	1	end	A2	2
	YER131W	RPS26b	37	end	A2*	1
	YFL066C		1	end	A4	1
	YGR210C		321	end	A1	6(3)
	YHL008C		260	550	A1*	8(2)
	YIL038C	NOT3	609	end	A4	2
	YIL048W	NEO1	1048	end	A4*	4
	YJL084C		494	850	A4	1
	YJL124C	LSM1	1	end	A2	1
	YJR138W		57	250	A4	1
	YKL209C	STE6	1012	1250	A4	1
	YKR026C	GCN3	39	end	A2	1
	YLR058C	SHM2	383	end	A4	1
	YLR275W	SmD2	9	end	A2	1
	YLR438C-A	LSM3	1	end	A2*	1
	YMR268C	PRP24	280	end	A1	3(2)
	YNL147W	LSM7	46	end	A4	1
	YOR201C	PET56	26	300	A2	1
	YOR320C		440	end	A1	11(5)
	YPL090C	RPS6A	336	end	A4*	1
	YPL152W	RRD2	329	end	A4*	3
	YPR010C	RPA135	656	1150	A3	1
	YPR184W		697	1035	A4	1
Lsm6-LexA	YBL026W	LSM2	1	end	A2	1
	YCR077C	PAT1	353	end	A1	22(2)
	YER112W	LSM4	1	end	A1	2(2)
	YGL251C	HFM1	759	end	A4	2
	YJL139C	YUR1	197	ND	A4	1
	YJR022W	LSM8	23	end	A1	22(7)
	YLR053C		45	end	A2	1
	YLR275W	SmD2	47	end	A1	5(3)
	YLR438C-A	LSM3	1	end	A2	1
	YMR221C		380	end	A3	1
	YMR268C	PRP24	114	end	A4	2

Table 1. continued

Bait	Prey ORF name (a)	Prey gene name (b)	Start domain (c)	End domain (d)	Cat (e)	Clones number (f)
	YNL147W	LSM7	1	end	A2	1
	YOR320C		404	end	A4	2
Lsm7-LexA	YCR077C	PAT1	153	350	A1	14(7)
	YDL077C	VAM6	402	600	A4*	8
	YFL066C		1	end	A4	1
	YIL112W		1043	end	A1	44(14)
	YLR438C-A	LSM3	1	end	A2	2
	YMR268C	PRP24	354	end	A4	1
	YPR178W	PRP4	109	end	A3	1
		Ty2-1	ND	ND	A1	6(4)
Lsm8-Gal4	YBL026W	LSM2	2	end	A1	4(3)
	YBR003W	COQ1	251	end	A4	1
	YCR077C	PAT1	426	end	A4*	1
	YDR228C	PCF11	272	550	A1	2(2)
	YDR277C	MTH1	22	end	A2/A3	1
	YEL015W		232	end	A1	5(3)
	YER112W	LSM4	12	end	A2	1
	YGL096W		73	end	A1	2(2)
	YGL173C	XRN1	1123	1500	A4	1
	YGR158C	MTR3	3	end	A2	1
	YHR034C		272	end	A4	1
	YHR035W		18	200	A2	1
	YIL173W	VTH1	437	650	A4	1
	YNL050C		1	200	A4*	3
	YNL118C	DCP2	ND	ND	A1	10(5)
	YNR050C	LYS9	264	end	A4	1
	YNR053C		1	end	A2/A3	7
	YOR076C		196	end	A3	1
	YOR319W	HSH49	16	end	A2	1
Lsm8-LexA	YBL026W	LSM2	2	end	A2*	1
	YBR034C	HMT1	15	200	A2	1
	YBR044C		492	end	A1	2(2)
	YCR020C-A	SMX1	1	end	A2	2
	YCR077C	PAT1	353	end	A1	6(2)
	YCR107W	AAD3	49	end	A4*	1
	YDR127W	ARO1	1	150	A2	1
	YDR135C	YCF1	1445	end	A4	1
	YDR184C	ATC1	62	end	A4	1
	YDR228C	PCF11	289	500	A4	2
	YDR378C	LSM6	1	end	A2*	4
	YEL023C		1	200	A2*	1
	YER146W	LSM5	9	end	A1	2(2)
	YGL028C	SCW11	129	300	A4*	1
	YGL096W		73	end	A4	1
	YGR158C	MTR3	3	end	A2	1
	YHR035W		18	200	A2	1
	YJL004C	SYS1	136	end	A4*	2
	YJL021C		129	end	A4	1
	YKR021W		1	200	A2	2
	YKR104W		219	end	A4	1
	YLL015W		1495	end	A4	1
	YLR133W	CKI1	63	250	A4	1
	YLR143W		614	end	A1	5(4)
	YLR430W	SEN1	971	1200	A4*	4

Table 1. continued

Bait	Prey ORF name (a)	Prey gene name (b)	Start domain (c)	End domain (d)	Cat (e)	Clones number (f)
	YLR438C-A	LSM3	1	end	A2*	4
	YMR205C	PFK2	2	350	A2	1
	YMR268C	PRP24	114	end	A3	1
	YNL227C		214	450	A4	1
	YNL242W		1273	end	A4	2
	YNL329C	PEX6	677	end	A3	2
	YOL031C		55	400	A4*	1
	YOL140W	ARG8	165	400	A4*	1
	YOR076C		196	end	A3	1
	YOR319W	HSH49	16	end	A1	5(2)
	YPR178W	PRP4	53	end	A3	1
Pat1(Ycr077c)	YDR141C		454	800	A4	1
	YDR389W	SAC7	466	end	A4	1
	YER115C	SPR6	157	end	A4	1
	YGL143C	MRF1	336	end	A4	1
	YML109W	ZDS2	615	end	A4	1
	YMR002W		1	end	A4	1
	YMR288W		472	700	A4	1
	YNL118C	PSU1	ND	ND	A1	3(3)
	YNR027W		34	end	A2	1
	YNR053C		ND	ND	A4	1
Ye1015w	YBL061C	SKT5	254	500	A4	3
	YBL066C	SEF1	446	700	A4*	2
	YEL015W		238	end	A1	16(5)
	YER032W	FIR1	710	end	A1	3(2)
	YER124C		468	end	A4	3
	YGL173C	XRN1	864	end	A3	1
	YGR116W	SPT6	1103	end	A3	1
	YJR140C	HIR3	1574	end	A1*	3(2)
	YLR082C	SRL2	91	350	A4	8
	YNL118C	PSU1	ND	ND	A1	38(10)
Psu1(Yn1118c)	YAR009C		966	1150	A1	2(2)
	YBL034C	STU1	1	350	A2	1
	YBL037W	APL3	517	800	A4	1
	YBL045C	COR1	335	end	A4	1
	YBL054W		161	450	A4*	2
	YCR076C		232	end	A4*	1
	YDL116W	NUP84	12	300	A2	1
	YDR472W		102	end	A4	1
	YEL015W		232	end	A1	11(5)
	YGL014W		653	end	A4*	1
	YGL049C	TIF4632	663	900	A4	1
	YGL173C	XRN1	890	1450	A1	4(2)
	YGR116W	SPT6	1103	end	A3	1
	YHR186C		680	1350	A3	1
	YIR014W		81	250	A4	1
	YIR024C	GIF1	31	end	A1	10(3)
	YJR023C		3	end	A2	1
	YKL133C		230	end	A4	2
	YKR031C	SPO14	1372	end	A1	12(2)
	YKR054C	DYN1	201	550	A4	3
	YLL001W	DNM1	535	end	A4	1
	YLR451W	LEU3	413	750	A4	1
	YML099C	ARG81	659	end	A4	1

Table 1. continued

Bait	Prey ORF name (a)	Prey gene name (b)	Start domain (c)	End domain (d)	Cat (e)	Clones number (f)
	YML112W	CTK3	1	200	A2	2
	YOL151W	GRE2	101	end	A1	5(2)
	YOR023C		31	250	A4	8
	YOR093C		932	1400	A3	1
	YOR124C	UBP2	567	850	A4	1
	YPR160W	GPH1	545	750	A4	1

(a) All the ORFs found in each screen are listed.
(b) A gene name is given when available.
(c) The N-Terminal residue of the smallest overlapping fragment is precisely located by sequencing.
(d) The C-Terminal extremity of the smallest overlapping fragment is roughly located according to PCR fragment sizes.
(e) The out-of-frame fusions are noted by an asterisk. For A1 candidates, an asterisk indicates those for which all fusions were in the same alternative frame.
(f) The total number of clones is indicated as well as the number of different fusions (in brackets) for A1 candidates.
(g) In those cases, two different non-overlapping domains are identified.
A2/A3: stands for candidates having both A2 and A3 characteristics (Fromont-Racine *et al.*, 1997).
ND, not determined.

Two Lsm proteins (Lsm2 and Lsm8) were used as both Gal4 and LexA fusion proteins (Table 1). Some of the prey proteins were found in common and represent a subset of highly specific interactors. In addition, for both Lsm2 and Lsm8 proteins, highly significant prey proteins (A1 category) were found with either the Gal4 or the LexA bait but not with both. As Lsm proteins are relatively small, the construction of fusions may be more likely to cause folding problems or steric interference with protein–protein interactions, as interacting domains may be masked in the fusion protein. In theory, saturating two-hybrid screens should identify multiple samples of the same partner. According to this criterion, the larger the screen the greater is the probability that single clones will be non-specific because their occurrence most probably reflects sporadic selection. For example, in the Lsm7 screen (60 million interactions tested, only eight distinct genetic loci as prey ORFs, out of them three found as single clones) or in the Lsm8–Gal4 screen (96 million interactions, 19 distinct genetic loci, 12 single clones) single clones might be considered likely to be non-specific, whereas in the rather small-scale Lsm1 screen (22 million interactions, 37 different genetic loci, 21 single clones) single clones may be more significant. When the data from several functionally related screens are pooled, prey found as single clones in several screens become more significant. For example, Prp24 arose as single isolates with Lsm7 and Lsm8 and became more significant when the data were combined, especially because this protein had never been selected in more than 100 previous screens (data not shown; see Discussion). Xrn1 was found only as single clones in three screens, which might suggest indirect or transient interactions that would be statistically less likely to be observed, but are still meaningful. The 8 Lsm baits produced 229 interactions with 161 different prey (Table 1). Among all these interactions, 25 are connections between Lsm proteins. Thus it is noticeable that more than 15% of the ORFs found in the screens performed with Lsm proteins correspond to these eight small Lsm genes (less than 600 nucleotides) selected out of a collection of more than 6000 genes covering more than 15 megabases. These results reveal very likely interactions between these proteins.

Connections of Lsm proteins with each other

Connections between the Lsm proteins are shown in Figure 2A. All eight Lsm proteins interacted with at least three other Lsm proteins, although not all interactions were found reciprocally. All these 25 pairings involved highly significant interactions (A1 or A2 categories; Fromont-Racine *et al.*, 1997). It is particularly striking that all selected prey fragments start near the natural N-terminus and all contain both Sm motifs. These results strongly suggest that the interactions between the Lsm proteins require the Sm motifs, and deletion analysis demonstrated

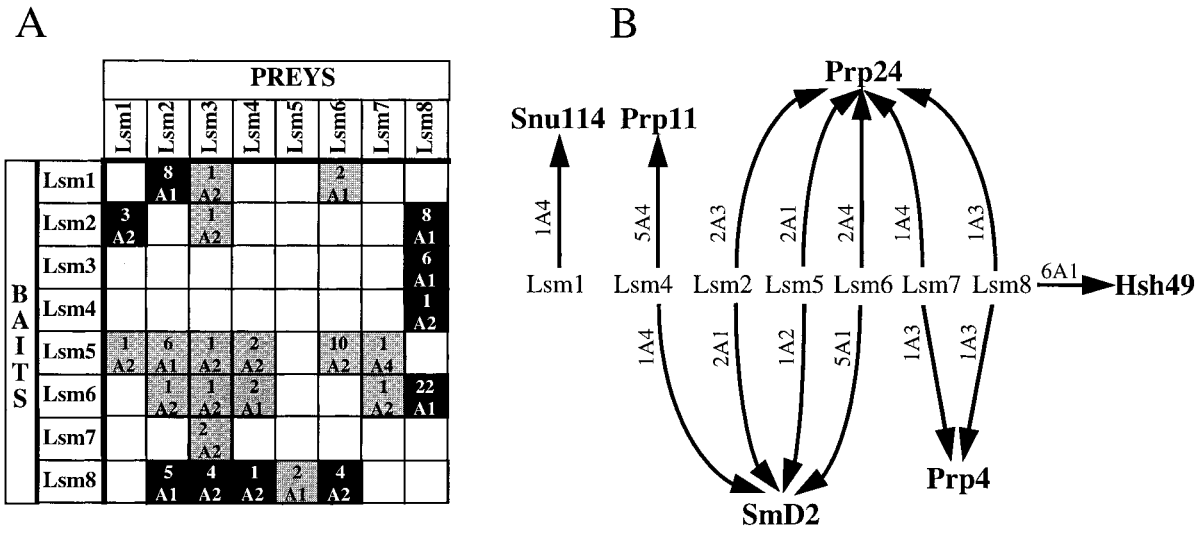

Figure 2. Lsm proteins and splicing. (A) Lsm proteins interact together. Each line corresponds to one screen. Grey squares correspond to directional interactions, black squares to bidirectional interactions. For each prey the number of clones is indicated above the category. (B) Links between Lsm proteins and known mRNA splicing factors. Interactions found between Lsm proteins and known mRNA splicing factors are representated by arrows. For each interaction the number of clones is indicated

this to be the case for Lsm4 (Mayes *et al.*, 1999). The multiple connections shown in Figure 2A might suggest that the Lsm proteins interact promiscuously with each other. However, no homotypic interactions were found for the Lsm proteins, indicating that these interactions did not occur spuriously between any protein-bearing Sm motifs.

Interactions with other splicing factors

With the exception of Lsm3, all the Lsm proteins made connections with known pre-mRNA splicing factors in these screens, with a total of six splicing factors being selected as prey (Figure 2B). Lsm1 found only one, Snu114, a U5 snRNP-specific protein, while each of the others interacted with at least two. This interaction has a low predictive value, because the Snu114 prey protein has been selected only once as an A4 candidate (see Materials and methods). Lsm8, which was the most interactive amongst the Lsm proteins, is also the most connected with splicing factors. Two splicing factors specifically associated with the U2 snRNP were selected with the Lsm proteins; Prp11 was found with Lsm4, and Hsh49 with Lsm8. Significantly, in a previous screen the reciprocal interaction of Lsm8 with Hsh49 as bait was found (Fromont-Racine *et al.*, 1997). Another splicing factor that arose frequently in these screens is one of the canonical Sm proteins, SmD2, being found with Lsm2, Lsm4, Lsm5 and Lsm6. Lsm7 and Lsm8 both selected the splicing factor Prp4, which is also a component of U4–U6 snRNPs and U4–U6–U5 tri-snRNPs (Banroques and Abelson, 1989; Bjorn *et al.*, 1989). Links between Lsm proteins and SmD2 and Prp4 are in agreement with the identification of the proteins of the yeast tri-snRNP (Gottschalk *et al.*, 1999). Prp24 arose most frequently in the screens, interacting with Lsm2, Lsm5, Lsm6, Lsm7 and Lsm8 (Figure 2B). Prp24 is an RNA-binding protein that associates with U6 snRNA in free U6 snRNP and U4–U6 di-snRNP particles (Ghetti *et al.*, 1995; Jandrositz and Guthrie, 1995). The high occurrence of Prp24 in these screens could be therefore indicative of a functional interaction of the Lsm proteins with the U6 and/or U4–U6 particles, which is further supported by genetic tests. Overproduction of the Prp24 protein partially complements the growth defect of cells metabolically depleted of Lsm4, whereas overexpression of Lsm4 exacerbates the temperature sensitivity of *prp24-1* cells (AEM, M. Cooper and JDB unpublished results). It is noteworthy that Prp24, Prp4 and SmD2 have not been selected as prey in exhaustive screens that have been performed with dozens of splicing factors in our

laboratories (Fromont-Racine *et al.*, 1997; unpublished results), thus supporting the likely functional significance of their interactions with the Lsm proteins.

Connections with other factors

Additional prey proteins, which are neither Lsm proteins nor known splicing factors, arose multiple times in Lsm screens (Figure 3A). Three prey proteins, Pat1, Psu1 and Yel015w, show the most frequent as well as significant links with the Lsm proteins. Yel015w was found interacting with Lsm2 and Lsm8 as many independent fusions (Table 1). Similarly, Psu1 was found interacting with Lsm2, Lsm4 and Lsm8, whereas Pat1 was found as prey by each of the Lsm proteins (Figure 3A). Additional prey proteins with a high predictive value were found that might have a biological significance (see Discussion): Xrn1, a 5′>3′ exonuclease that represents the major nuclease activity for the degradation of decapped mRNAs (Jacobs *et al.*, 1998; Johnson, 1997); Gcn 3 and Gcd11, two translational initiation factors (Erickson *et al.*, 1997; Pavitt *et al.*, 1998) and Mtr3, a component of the exosome (Allmang *et al.*, 1999). All these interactions with the Lsm proteins seem very specific, since prey proteins were all A1, A2 or A3 candidates that were specifically selected by at least two Lsm bait proteins and were otherwise not found in more than 100 genomic screens done in our laboratories (Fromont-Racine *et al.*, 1997); unpublished results). The three proteins Yel015w, Pat1 and Psu1 were used in turn as bait proteins to screen the yeast proteome for potential interacting partners (Table 1, Figure 1). Curiously, none of the Lsm proteins was found in these second-round screens. Nevertheless, the complete set of connections identified through exhaustive two-hybrid screens performed with those novel proteins associate the Lsm proteins with a group of proteins that are related to the mRNA degradation pathway (Figure 3B; see Discussion).

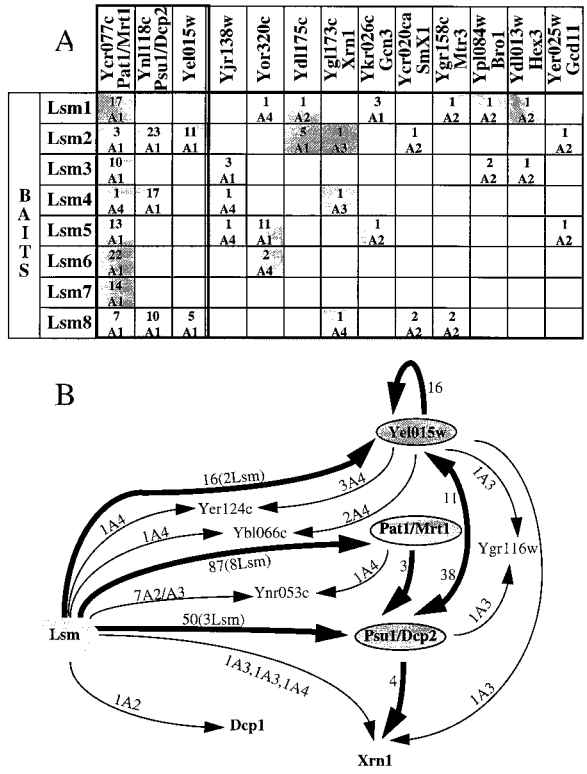

Figure 3. Lsm proteins and the mRNA degradation pathway. (A) Proteins connected to Lsm proteins. The most significant proteins found as prey, with at least two different Lsm proteins and which are neither Lsm proteins nor known mRNA splicing factors, are classified from right to left according to their increasing heuristic value (Fromont-Racine *et al.*, 1997). For each interaction the number of clones is indicated above the category. (B) Interaction network of proteins involved in mRNA degradation pathway. All direct and indirect (via another protein) connections between Lsm proteins and mRNA degration factors are indicated. Thick lines with arrowheads represent links of high heuristic value (A1). Other links are represented by thin lines. The category, the number of clones and the number of different Lsm bait proteins that selected a given prey are indicated

Discussion

The multiple interactions among the eight Lsm proteins strongly suggest the existence of a complex or complexes of Lsm proteins. The interactions of Lsm1 with Lsm2, Lsm3, Lsm5 and Lsm6 seemed surprising initially, in view of the evidence that Lsm2, Lsm3, Lsm4, Lsm5, Lsm6, Lsm7 and Lsm8 associate with, and stabilize, the spliceosomal U6 snRNA (Mayes *et al.*, 1999; Salgado-Garrido *et al.*, 1999), whereas Lsm1 appears to be involved in a distinct process, mRNA decapping (Boeck *et al.*, 1998). Also, a seven- (rather than eight)-component complex of U6-associated Lsm proteins is attractive in view of the heptameric complex predicted for the human Sm proteins. Indeed, Lsm2–Lsm8 proteins have recently been identified in yeast and human

cells spliceosomes (Achsel *et al.*, 1999; Gottschalk *et al.*, 1999; Stevens and Abelson, 1999). It is not yet established how similar the canonical Sm and the Lsm2–Lsm8 complexes are. It should be noticed that we found Lsm proteins highly connected to each other while more specific interactions were observed between the canonical Sm proteins (Camasses *et al.*, 1998; Fury *et al.*, 1997) (M. Fromont-Racine, A. Brunet-Simon and P. Legrain, unpublished data). Since two-hybrid interactions do not necessarily represent direct protein interactions between the two partners, the observed connections could be mediated by another (Lsm) protein or even by an RNA. Thus, it seems most likely that some of the observed interactions may be mediated by the formation of complexes containing more than two Lsm proteins. In summary, these data support the ability of the Lsm proteins to form a complex or complexes, as also indicated by the finding that Lsm4 can be co-immunoprecipitated with each of the seven other Lsm proteins (Mayes *et al.*, 1999). As both Sm protein and Lsm protein interactions involve the conserved Sm domain, it remains to be determined how these proteins distinguish between each other to form separate complexes.

Lsm proteins are also strongly connected to proteins involved in splicing: SmD2, Prp11, Hsh49, Prp4 and Prp24. A close functional relationship between Prp4 and the U6 snRNP is suggested by genetic studies showing that at non-permissive temperature, mutant *prp4-1* cells exhibit a specific decrease in the level of U6 snRNA (Galisson and Legrain, 1993). Genetic and *in vitro* experiments have led to a model in which Prp24 promotes the annealing/dissociation reactions of U4–U6 dimer during successive rounds of splicing (Ghetti *et al.*, 1995; Raghunathan and Guthrie, 1998; Shannon and Guthrie, 1991). In the U4–U6 snRNP and in the U4–U6–U5 triple snRNP, SmD2 might be part of an interface between the canonical snRNPs and the U6/Lsm particle. In this respect it may be relevant that a two-hybrid screen with the SmE protein selected Lsm3 (SmX4), the paralogue of SmD2, as a prey protein (Camasses *et al.*, 1998). Interactions between the U2 and U6 snRNAs within spliceosomes are well established (Madhani and Guthrie, 1994). The finding of connections between Lsm proteins and U2 snRNA-associated proteins might therefore represent protein interactions at a U2–U6 interface in spliceosomes or during spliceosome formation, as already suggested by the genetic interactions between Prp21 and Prp24, which are U2- and U6-associated, respectively (Vaidya *et al.*, 1996).

More surprisingly, in these genomic screens, we identified a small group of proteins connected to the Lsm proteins and unrelated to pre-mRNA splicing, among them, Psu1, Pat1 and Yel015w. *YEL015W* encodes a protein of unknown function. *PSU1* was initially identified through suppression of the respiratory deficiency of a *pet* mutant (A.A. Tzagoloff, unpublished results), and more recently has been demonstrated to have a role in transcription (Gaudon *et al.*, 1999). Pat1 was previously identified (Rodriguez-Cousino *et al.*, 1995; Wang *et al.*, 1996b) as a topoisomerase II-associated protein. Disruption of the *PAT1* gene causes slow growth and apparently affects the fidelity of chromosome transmission. No role for Pat1 in pre-mRNA splicing has been detected, nor an association with any of the spliceosomal RNAs, although Pat1 co-immunoprecipitates with Lsm proteins (S. Tharun, W. He, A.E. Mayes, P. Lennertz, J.D. Beggs and R. Parker, 2000). This network of interactions revealed an additional strongly connected protein, Xrn1 (Figure 3B). This result suggests an implication of Lsm proteins in the metabolic degradation pathway of mRNAs and is further supported by additional findings: *PAT1* turns out to be equivalent to *MRT1*, in which conditional mutations that inhibit mRNA decapping have been isolated (Hatfield *et al.*, 1996; S. Tharun, W. He, A.E. Mayes, P. Lennertz, J.D. Beggs and R. Parker, 2000). Psu1/Nmd1 was identified as an interacting protein with Upf1, a major player in the non sense-mediated mRNA decay pathway (He and Jacobson, 1995). More significantly, the Psu1 protein has also been implicated in mRNA decapping, and renamed Dcp2 (Dunckley and Parker, 1999). Dcp2 is required for the production of active Dcp1 decapping enzyme, which co-purifies with it. Although it is not a major player in the Lsm screens, Dcp1 was found as prey in a screen with Lsm4 (Table 1, Figure 3B), and Lsm proteins have been found to co-immunoprecipitate along with Dcp1 protein (S. Tharun, W. He, A.E. Mayes, P. Lennertz, J.D. Beggs and R. Parker, 2000). Following the report (Boeck *et al.*, 1998) that Lsm1–Spb8 itself plays a role in mRNA decapping, recent work has shown that mutations affecting several of the Lsm proteins lead to partial inhibition of mRNA decay (S. Tharun, W. He, A.E. Mayes, P. Lennertz,

J.D. Beggs and R. Parker, 2000). Thus, a network of interactions was found between the Lsm proteins and four proteins that are implicated directly in mRNA turnover: Dcp2/Psu1, Dcp1, Mrt1/Pat1 and Xrn1. Altogether, these data strongly suggest a novel role for an Lsm protein complex in mRNA degradation. This complex could be directly involved in the regulation of mRNA turnover, which is known to be linked to translational initiation (Schwartz and Parker, 1999). Indeed, we found two translational initiation factors, Gcn3 and Gcd11, among the highly specific prey proteins selected by Lsm proteins (Figure 3A). The existence of eight instead of seven Lsm proteins, and the finding of multiple interactions between Lsm proteins and factors involved in mRNA turnover, as well as factors involved in mRNA splicing, raises the possibility that two or more Lsm complexes may exist. Conceivably, alternative Lsm subunit compositions might confer different functional specificity on distinct complexes. In view of these connections, the precise role of Yel015w is currently being investigated.

From the results presented here, it appears that performing multiple genomic screens with functionally related bait proteins and in an iterative manner leads to results whose significance is much greater than the data from the component screens considered separately. Obviously, the frequency with which prey proteins are found also depends on the level of production and stability of the fusion proteins; therefore, while statistical analyses are essential for interpretation of the data, all results should be considered as potentially significant, including single clones, otherwise meaningful interactions may be missed. These questions were also addressed in very recently published studies aiming at a genome-wide description of protein–protein interactions for the yeast proteome (Ito *et al.*, 2000; Uetz *et al.*, 2000) and *Caenorhabditis elegans* (Walhout *et al.*, 2000). However, as opposed to these studies, the strategy used in the present study (see also Flores *et al.*, 1999; Fromont-Racine *et al.*, 1997) aims at the selection of interacting domains instead of checking for interaction between full-length proteins. This leads to a more complete description of the set of interactions and provides in addition information on functional domains. A similar approach has also been successfully performed for the study on the hepatitis C virus proteome (Flajolet *et al.*, 2000). Overall, the results presented here provide a striking illustration that exhaustive and iterative two-hybrid screens can be used on a genome-wide scale to yield functionally meaningful information about protein–protein interactions, and thereby can suggest functions for uncharacterized or partially characterized proteins.

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