Minireview

Results and prospects of the yeast three-hybrid system

Sophie Jaeger, Gilbert Eriani, Franck Martin*

Institut de Biologie Moléculaire et Cellulaire, UPR No. 9002 du CNRS, 15 rue René Descartes, 67084 Strasbourg Cedex, France

Received 9 July 2003; revised 26 November 2003; accepted 26 November 2003

First published online 10 December 2003

Edited by Lev Kisselev

Abstract In 1996, a new method, termed the yeast three-hybrid system, dedicated to selection of RNA binding proteins using a hybrid RNA molecule as bait was described. In this minireview, we summarize the results that have been obtained using this method. Indeed, ~20 unknown proteins have been characterized so far. The three-hybrid strategy has also been used as a tool to dissect RNA–protein interactions. The example of such a study on human histone HBP interaction with its target mRNA is described. Problems that can be encountered are addressed in a troubleshooting section. Especially, our results with tRNA binding proteins are discussed.

Key words: Yeast three-hybrid system; RNA–protein interactions; HBP/SLBP; Histone mRNA

1. Introduction

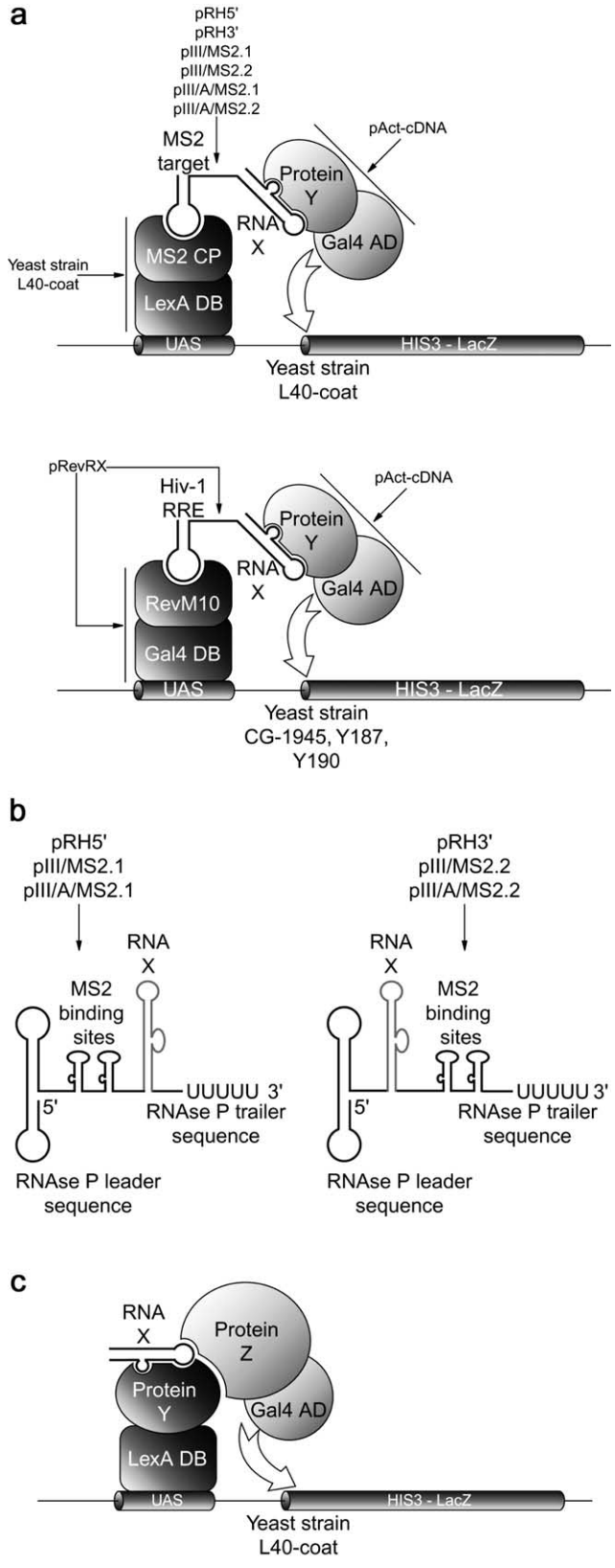
In the cell, the interactions of RNAs with their protein partners play a pivotal role in a wide variety of fundamental biological processes (e.g. splicing, translational control, and transport to specific compartments). Several methods have been devised to detect RNA–protein interactions; one of these is the yeast three-hybrid system (also called the tri-hybrid system [1]), which has been developed simultaneously by two groups [1,2]. In this report we summarize the results obtained during the past seven years using this method. For some RNA binding proteins, the yeast three-hybrid strategy is useless and the reasons for that are discussed.

2. Principle of the yeast three-hybrid system

This method consists in the expression in yeast cells of three chimerical molecules, which assemble in order to activate two reporter genes. Thus, using the yeast three-hybrid system, in contrast to other methods, RNA–protein interactions are detected in vivo. This system uses a transactivator protein in yeast, such as Gal4p, that is able to recruit the transcriptional machinery and trigger transcription of a gene. It consists of a DNA binding domain (DB) and an activation domain (AD) and, importantly, these two domains are functionally independent, meaning that they can be inserted into other molecules. In the yeast three-hybrid system the DNA binding domain (LexADB or Gal4DB) is fused to an RNA binding protein (MS2-coat protein or Hiv-1 RevM10) (Fig. 1a). The second fusion protein contains on one hand the Gal4 activation domain (Gal4AD) and on the other hand the RNA binding protein 'Y' of interest. The two fusion proteins are bridged by a third hybrid RNA molecule containing the binding site for the first RNA binding protein (MS2 or RRE) and the binding site 'X' for the RNA binding protein 'Y' studied. Binding of protein 'Y' to the RNA binding site 'X' will create a functional transactivator, which is tethered at the upstream activating sequence of two reporter genes (*HIS3* and *lacZ*) that will be transcribed and expressed by yeast cells. The expression level of *lacZ* gene can be determined in vitro by measuring the β-galactosidase activity, or visualized in vivo by plating the yeast transformants on media supplemented with X-Gal. On the other hand, *HIS3* is the gene encoding imidazoleglycerol-phosphate dehydratase (His3p) and its expression confers the ability to grow on a medium lacking histidine. 3-amino-1,2,4-triazole (3-AT) is a competitive inhibitor of *HIS3* gene product, and therefore cells containing more His3p can survive at higher concentrations of 3-AT in the medium. Thus, the level of 3-AT resistance of the yeast cells reflects the *HIS3* expression level and consequently the strength of the RNA–protein interaction in the yeast three-hybrid context. This also means that the stringency of the selection can be adjusted to the type of RNA–protein couple of interest by changing the concentration of 3-AT in the medium. In the system developed by SenGupta et al. [2] the first hybrid molecule LexADB-MS2 is encoded by the genome of the yeast strain L-40 coat and the fusion protein Y-Gal4AD is expressed from a plasmid (Fig. 1a). The hybrid RNA molecule is produced by RNA polymerase III leading to an RNA containing the RNAse P 5′ leader sequence and 3′ trailer sequence leading to nuclear localization (Fig. 1b). One major drawback of RNA polymerase III is that stretches of four or more T residues act as transcription terminator. Unfortunately, many RNA binding proteins recognize U-rich RNA targets and RNA polymerase III is not able to transcribe such RNAs. In the method developed by Putz et al. [1], the three components of the system are expressed from two plasmids (Fig. 1a) allowing the use of any previously described yeast strains for two-hybrid system that provide the two reporter genes *HIS3* and *lacZ* under the control of a Gal4 operator. Another difference concerns the hybrid RNA molecule, which is transcribed by RNA polymerase II, avoiding any premature stop in the transcription of U-rich target RNAs.

*Corresponding author. Fax: (33)-388602218.
E-mail address: f.martin@ibmc.u-strasbg.fr (F. Martin).

0014-5793/03/$30.00
doi:10.1016/S0014-5793(03)01434-0



3. Cloning the genes of uncharacterized proteins or RNA targets

The three-hybrid system has been used to clone genes encoding uncharacterized RNA binding proteins. In this purpose the RNA targets of those unknown proteins have been used as bait in the screening of a cDNA expression library. The yeast three-hybrid system has been used successfully and allowed the characterization of about 20 proteins (see Table 1, upper part). All these screenings have been done with the method described by SenGupta et al. [2]. Among these, several screenings led to the characterization of the sought protein partners using RNA baits such as 5′ or 3′ UTR of cellular or viral mRNA. These proteins are involved in regulation of protein expression in the cell by influencing various steps of mRNA metabolism such as 5′ and 3′ processing, nuclear-cytoplasmic shuttling, translation and stability [3–14]. In addition, two proteins (CUG-BP and XB1), that bind introns during the splicing process have been characterized [15,16]. These newly identified interaction partners could be confirmed by functional assays or other methods like electromobility shift assays, UV cross-linking or immunoprecipitations experiments.

In some selections, other proteins than the expected ones were selected for interacting with the bait. These unexpected proteins provided new perspectives on the function of the RNA binding protein of interest. Three-hybrid screens with the human telomerase RNA led to selection of two proteins, which are the protein hStau and more surprisingly the ribosomal protein L22 [17]. In the case of xenopus histone 3′ UTR, two proteins have been selected and named SLBP1 and SLBP2 [6]. They share a conserved RNA binding domain in the middle of the peptide sequence but differ outside of this domain. SLBP1 participates in histone pre-mRNA 3′ processing in the nucleus whereas SLBP2 is oocyte-specific and does not support pre-mRNA 3′ processing but is used to store maternal histone mRNA in a latent state until fertilization. Another example comes from the vimentin 3′ UTR bait that led to the selection of two proteins, HAX-1 and EF1-γ [14]. The authors showed that EF1-γ is present in a larger complex with the target RNA that can be separated from the HAX-1-vimentin mRNA complex. EF1-γ is involved in the coordination of vimentin mRNA translation with respect to the cell cycle and HAX-1 is required for the localization of vimentin mRNA at the periphery of the cell. In this case, the yeast three-hybrid system allowed the selection of two proteins involved in two different steps of the vimentin mRNA metabolism. Several groups have modified the yeast three-hybrid system in order to characterize protein components of RNP complexes (Table 1, middle part). In this new system the hy-

←

Fig. 1. a: The yeast three-hybrid system protein and RNA components described by SenGupta et al. [2] (upper panel) and by Putz et al. [1] (lower panel). The RNA and protein hybrids are encoded either by the genome of the yeast strain used or by a set of different plasmids that are indicated on the schemes. b: In the system developed by SenGupta et al. [2], the RNA bait X can be inserted 5′ or 3′ of the MS2 binding sites. On the contrary, the plasmid pRevRX of the tri-hybrid system from Putz et al. [1] expresses a hybrid RNA where the bait was fused to the 3′ terminus of RRE-RNA (not shown). c: New hybrid system using an RNA–protein complex X–Y as bait to select new interactors Z.

Table 1
A non-exhaustive list of unknown proteins and RNAs characterized using the yeast three-hybrid system

	RNA length (nt)	Plasmid used	Protein Y	Protein length (aa)	[3-AT] (mM)	Reference
RNA X						
Hairpin of histone 3′ UTR mRNA	35	pIII/MS2.1	SLBP	270–274–253	5	[3]
Hairpin of histone 3′ UTR mRNA	34	pIII/MS2.2	HBP (SLBP)	270	25	[4]
fem-3 3′ UTR PME	74	pIII/A/MS2.2	FBF1	493[a]	5	[5]
			FBF2	630		
Hairpin of histone 3′ UTR mRNA	35	pIII/MS2.1	SLBP1	254	5	[6]
			SLBP2	250		
Nanos 3′ UTR TCE A	29	pIII/MS2.1	Smaug	860[a]	3	[7]
Influenza NP 5′ UTR	45	pIII/MS2.1	GRSF-1	331	3	[8]
tra-2 3′ UTR TGE	60	pIII/MS2.2	GLD-1	463	5	[9]
NF-IL6 mRNA 3′ UTR (element D1)	75	pIII/A/MS2.2	unnamed protein	70	5	[10]
Human telomerase hTR	158	pIII/MS2.2	L22	128	5	[17]
			hStau	448[a]		
Prm1 3′ UTR	37	pIII/MS2.2	MSY4	358[a]	5	[11]
APP splicing enhancer	21	pIII/A/MS2.1	CUG-BP	226	5	[15]
			SiahBP	547		
ash1 3′ UTR (element E3)	127	pIII/A/MS2.2	She3p	189[a]	1	[12]
ash1 3′ UTR (element E1+E3)	153–127	pIII/A/MS2.2	Loc1p	204	5	[27]
Hepatitis C HCV 3′X	98	pIII/A/MS2.1	L22	128	2–5	[13]
			L3	403		
			S3	158		
			mL3	348		
Trypanosoma cruzi SL RNA	111	pIII/A/MS2.1	XB1	354	2.5	[16]
pre-rRNA	54	pIII/A/MS2.1	Fibrillarin	305	3	[28]
Vimentin 3′ UTR	69	pIII/A/MS2.1	HAX-1	279	2–50	[14]
			eEF-1γ	437		
			hRIP	288		
RNA X+protein						
hY5RNA+Ro60	84	pIII/MS2.1	RoBP1	559	1	[18,19]
Histone 3′UTR mRNA+SLBP	34	pIII/MS2.1	ZFP100	871	0–5	[20]
ash1 3′ UTR (E3)+She3p	127	pIII/A/MS2.2	She2p	246	1	[12]
TAR+Tat	58	pIII/A/MS2.2	cyclin T1	726	[b]	[21]
TAR+Tat	58	pIII/MS2.2	Tip110	963	[b]	[22]
hb RNA (NRE)+Pumilio	64	pIII/MS2.1	Nanos	335[a]	0–7	[23]
hb RNA (NRE)+Pumilio+Nanos	64	pIII/MS2.1	Brat	330[a]	3	[24]
RNA selected with a bait protein						
U1 SL RNA	45	pIII/MS2.2	Snp1	300	0.5	[25]
mt RNAs	50–150	pIII/MS2.2	mouse hnRNP K	464	2.5	[26]

The upper part of the table lists the proteins selected with an RNA bait, the middle part of the table lists the proteins isolated with baits encompassing RNA–protein complexes and the bottom part of the table lists the RNA selected with bait proteins. Plasmids used for expressing the hybrid RNA are indicated. The RNA baits are located 5′ or 3′ of the MS2 binding site depending on the plasmid used (see also Fig. 1b). RNA baits expressed from pRevRX are located 3′ of the RRE. Plasmids pRH5′ and pRH3′ are vectors from a commercially available kit (RNA-Protein Hybrid Hunter Kit, Invitrogen) based on vectors described by SenGupta et al. [2].
[a]Incomplete cDNA selected.
[b]Only *lacZ* used.

brid RNA molecule is not used and LexADB is fused with the protein of interest complexed with its RNA target (Fig. 1c). New RNP binding proteins are then selected from a cDNA library for their ability to form a three-hybrid complex. The first example of such a selection was done with the complex Ro60-hYRNA, which allowed the selection of RoBP [18,19]. Then the complex SLBP-histone hairpin RNA was used to select the protein ZFP100 [20]. Similarly, She3p has been characterized as an ash 3′ UTR binding protein by three-hybrid screens and then the complex She3p-ash RNA has been used to select She2p [12]. The complex formed by Hiv-1 TAR-Tat has been used as bait by two groups and led to the selection of two different proteins, cyclin T1 [21] and Tip110 [22]. Finally, the hunchback mRNA binding proteins have been extensively studied using these alternative hybrid-screening approaches. In a first step the complex Pumilio-hunchback mRNA (fragment NRE) has been used to select the protein nanos [23]. The second step was to use the ternary complex Pumilio-NRE-nanos as bait for the selection of the protein Brat [24], this is the first example reported of a yeast four-hybrid system encompassing three proteins and an RNA molecule.

It is also possible to use the yeast three-hybrid system the other way around which means using a protein as bait to select RNA molecules able to bind to that protein (Table 1, bottom part). The yeast protein Snp1 has been used to validate the screening strategy and allowed to select its cognate interactor U1 snRNA [25]. In another study the mouse hnRNP K has been used to screen a human RNA library and among the selected clones, a significant part of these are encoded by the mitochondrial genome, suggesting that hnRNP K is involved in expression of mitochondrial genes, which was completely unexpected [26].

4. The three-hybrid system as a tool to dissect RNA–protein interactions

This method has also been used to test the interaction of previously known or suspected interactors. Table 2 lists all the RNA–protein couples successfully used in the three-hybrid

Table 2
A non-exhaustive list of the RNA–protein interactions studied with the yeast three-hybrid system and characterization of previously assumed interactors or identification of critical residues involved in the specific recognition of RNA and proteins

RNA X	RNA length (nt)	Plasmid used	Protein Y	Protein length (aa)	[3-AT] (mM)	Reference
RRE	240	pRevRX	RevM10	116	30	[1]
IRE	51	pIII/MS2.2	IRP1	889	20	[2]
TAR	58	pIII/MS2.2	Tat	86	20	[2]
MPMV CTE[a]	153	pIII/MS2.1	TAP	619	[b]	[29]
RSV Mψ[a]	160	pIII/A/MS2.1	RSV Gag	577	[b]	[30]
Hairpin of histone 3′ UTR mRNA	34	pIII/MS2.2	HBP[a]	270	0–225	[31]
mTR	393	pIII/A/MS2.2	TP1	871[c]	5	[32]
HIV-1 RNA encapsidation signal	139	pIII/MS2.2	HIV-1 Gag	243	[b]	[33]
HIV-1TAR	55	pIII/MS2.2	Tat	86	[b]	[34]
HIV-1, HIV-2 and SIVmnd TAR	55–126–128	pIII/MS2.2	Tat	86	[b]	[35]
RPS14B pre-mRNA-18S rRNA	59–70	pIII/MS2.1	S14	138	5–20	[36]
hvg1-hvg2-hvg4	96–86–99	pIII/MS2.1	mTEP1	871[c]	10	[37]
RSV Mψ	160	pIII/MS2.1	RSV Gag	701	[b]	[38]
HERV-K RRE	433	pIII/MS2.2	HERV-K Rev	105	[b]	[39,40]
Nanos TCE-TCEIIIA	96	pIII/A/MS2.1	Smaug	483	[b]	[41]
BRE-BREM	48–48	pIII/MS2.1	xlBrunoL3	538	[b]	[42]
CaMV pgRNA	87	pIII/MS2.2	CaMV CP	454	[b]	[43]
SL RNA	145	pIII/MS2.1	TSR1IP	354[c]	0.1	[44]
CD44 exon V5	210	pRH5′	hnRNPA1	320	[b]	[45]
cob intron bl4	over 1600	pRH3′	bl4 maturase	638	5	[46]
			LeuRS	885		
Long CUG repeats and UG repeats	variable	pIII/A/MS2.2	CUG-BP	226	[b]	[47]
UG repeats	variable		CUG-BP+LYLQ	230	[b]	[47]
NF-KB	31	pIII/A/MS2.2	ap50	460	1–2	[48]
hY1 and hY3	112–105	pRH5′	hnRNP I	531	[b]	[49]
			hnRNP K	464		
Prm1 3′ UTR	37	pIII/MS2.2	MSY2-MSY4	262–282	5	[50]
H1 RNA	497	pRH3′	Rpp21	154	5	[51]
			Rpp29	220		
			Rpp30	268		
			Rpp38	283		
DTE of MAP2 3′ UTR	640	pRevRX	rStaufen	495	10	[52]
HO 3′ UTR	134	pIII/A/MS2.1	Mpt5	859	0	[53]
RPR1 P3	54	pIII/A/MS2.2	Pop1	875	1–20	[54]
hb NRE	32	pRH5′	PfPuf1	286[c]	[b]	[55]
fem-3 PME-NRE-HO 3′UTR	74–64–134	pIII/A/MS2.2	FBF-1	451[c]	[b]	[56]
			GLD-3	949	[b]	
			dmPUM	335[c]	[b]	
RPR1 P3	369	pIII/MS2.2	Pop1	875	20	[57]
			Pop4	279		
Hairpin of histone 3′ UTR mRNA	34	pIII/MS2.1	CDL-1	367	5	[58]
PDGF2 5′UTR	127–322	pRevRX	hnRNP-C1	290	0.5–100	[59]
			hnRNPC2	303		
bcl-2 ARE	107	pIII/A/MS2.1	AUF1	257	3	[60]
cob intron bl4	over 1600	pRH3′	LeuRS	375	5	[61]
Hairpin of histone 3′ UTR mRNA	34	pIII/MS2.2	HBP[a]	270	0–100	[63]
A. aeolicus $tRNA^{Leu}$	98	pIII/A/MS2.2	LeuRS(β su)	289	25	E.D. Wang

The plasmids used for expressing the hybrid RNA are indicated as in Table 1.
[a]Random mutagenesis.
[b]Only *lacZ* used.
[c]Incomplete cDNA selected.

strategy. After designing the yeast three-hybrid system, the authors had to test the reliability of the method with several RNA–protein couples known to interact with each other. Putz et al. validated their method by the detection of the HIV-1 complex RevM10-RRE [1] whereas SenGupta et al. [2] verified interaction with the couples Tat-TAR and IRP-IRE [2]. Another application is to define the minimal binding domains by deletion and mutational analysis of the RNA and/or the protein. By combinatorial approaches with randomly mutagenized RNA or protein library, the RNA binding specificity could also be analyzed [26,29–31]. This strategy has been used extensively in our laboratory to study the binding of HBP (also called SLBP) on the hairpin of the 3′ UTR replication-dependent histone mRNA [31] and [63]. Thus, we could select single mutations in HBP that abolish binding to the wild-type histone hairpin. Interestingly, all the mutations mapped to the central RNA binding domain (RBD; Fig. 2). Then, we selected with the yeast three-hybrid system intragenic mutations able to restore the binding on a histone hairpin. Remarkably, most of the compensating mutations are located outside of this central domain. These results allowed us to propose a set of putative internal interactions between several residues of the RBD and its flanking domains (Fig. 2). To conclude, the yeast three-hybrid system has been a very fruitful method for the study of the interaction of HBP with its target RNA and the organization of HBP domains.

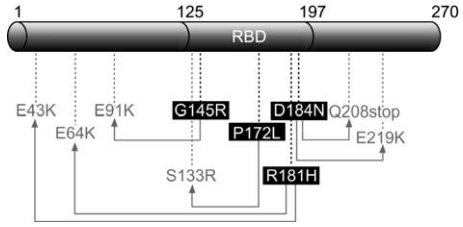


Fig. 2. Example of a functional study performed on human HBP or SLBP [63]. This protein is 270 amino acid long (GenBank accession number Z71188) and contains a ~70 amino acid central minimal RNA binding domain (RBD). Mutations selected by yeast three-hybrid screenings in human HBP are represented. Negative selections have allowed selection of lethal mutations abolishing the binding to wild-type hairpin (mutations are boxed in black). Starting from these mutants, positive selections of active clones allowed the isolation of intragenic compensating mutations (mutations shown in gray) restoring the binding to its cognate RNA.

5. Limits of the system

Unfortunately, the three-hybrid strategy can be useless for some RNA–protein couples. For instance, since protein and RNA partners are expressed as chimerical molecules, the folding or the accessibility of the bait might be disturbed by the other moiety of the molecule. The hybrid RNA might also be cut in the nucleus by a processing machinery leading to a truncated hybrid RNA, useless for the three-hybrid strategy. Some RNA molecules contain targeting signals that allow their localization in organelles. These RNAs are not suitable for three-hybrid studies since when the hybrid RNA is targeted into these organelles the assembly of the three-hybrid upstream of the reporter genes in the nucleus becomes impossible. Several RNAs contain post-transcriptional modifications necessary for binding to proteins. These RNAs and proteins cannot be studied with the yeast three-hybrid system since the hybrid RNA may be partially or not modified at all. On top of that, many proteins need cofactors to bind to their RNA partner and these cofactors might not be available in the nucleus because they are located in another compartment of the cell. Finally, some proteins bind to their target RNA in a transient manner and with low affinity, avoiding detection of any complex with the three-hybrid strategy. Another drawback of the three-hybrid strategy is that the RNA–protein interaction takes place in vivo, where it can be influenced by many cellular parameters. This leads to false-positive clone predominance that must be eliminated by additional time-consuming screening tests. Other techniques like SELEX [64], plaque-lift assays [65] or T7 phage display [66] have been used to detect RNA–protein interactions in vitro, which make all the binding parameters controllable.

Indeed, we encountered some of these problems when we wanted to select new tRNA binding proteins with the yeast three-hybrid system. As a control we tested the interaction of our bait tRNAs (yeast $tRNA^{Arg3}$ and $tRNA^{Tyr2}$) with their cognate aminoacyl-tRNA synthetases (yeast ArgRS and TyrRS), the yeast tRNA transporter Los1p and the yeast elongation factor EF1-α, but no positive signal could be detected. It seems therefore that the tRNA is not a suitable bait for the yeast three-hybrid system for one of the reasons detailed previously. To our knowledge, the only functional three-hybrid system isolated from a couple of tRNA and aminoacyl-tRNA synthetase remains $tRNA^{Leu}$ and LeuRS from *Aquifex aeolicus*. This interaction is highly specific as judged by the negative results obtained with other tRNAs or other proteins like Los1p, EF1-α or *Escherichia coli* LeuRS (E.D. Wang, personal communication).

6. Prospects

The yeast three-hybrid strategy has been widely used to select unknown proteins and RNA partners and also for studying RNA–protein interactions of previously assumed interactors (Tables 1 and 2). This method has been successful for RNAs of variable size (21–1600 nucleotides) [46,61]. There is no general rule for the position of the bait in the hybrid RNA since plasmids encoding hybrid RNA containing the bait either internally or at the 3′ end have been used successfully (Tables 1 and 2). The protein partners are also of variable size ranging from very small subdomains like the 70 aa NF-IL6 mRNA binding protein to very large proteins like Tip 110, which is 963 aa long. The use of 3-AT in the medium allows modulating of the stringency of the screen. Indeed when looking at strong interactions, up to 225 mM can be used as it was done for HBP [31], which binds tightly to its target RNA ($K_d = 1.5 \times 10^{-9}$ M [62]). Reducing the 3-AT concentration or even omission of this drug from the medium allows studying relatively weak interactions with K_d values in the μM range (S14 on 18S rRNA: $K_d = 3 \times 10^{-6}$ M [36]). Future developments of the hybrid screenings will be to select protein partners binding to bait consisting of RNP complexes. This has already been done in the case of the hunchback mRNA [23,24] and could be applied to many other complexes. By varying the stringency of the selection, characterization of different protein partners binding tightly or weakly will be possible. Most of RNP complexes consist of a core composed of strong interactors surrounded by weak interactors modulating the activity of the RNP. Three-hybrid screens will allow sort of pealing of the RNP complexes. This will also enable to select proteins binding to RNA at different steps of their function. Once the proteins are characterized, one can analyze the RNA–protein complexes by making positive and negative selections on randomly mutated libraries of the two partners. Importantly, interaction studies in the yeast three-hybrid system should always be completed by other tests like functional assays, in vitro binding studies or immunoprecipitations in order to avoid artefacts.

Acknowledgements: This work was supported by ARC (Association pour la Recherche contre la Cancer) grant 5486. We thank Prof. E.D. Wang for sharing results prior to publication and Sharief Barends for critical review of this manuscript.

References

[1] Putz, U., Skehel, P. and Kuhl, D. (1996) Nucleic Acids Res. 24, 4838–4840.
[2] SenGupta, D.J., Zhang, B., Kraemer, B., Pochart, P., Fields, S. and Wickens, M. (1996) Proc. Natl. Acad. Sci. USA 93, 8496–8501.
[3] Wang, Z.F., Whitfield, M.L., Ingledue, T.C., Dominski III, Z. and Marzluff, W.F. (1996) Genes Dev. 10, 3028–3040.
[4] Martin, F., Schaller, A., Eglite, S., Schümperli, D. and Müller, B. (1997) EMBO J. 16, 769–778.
[5] Zhang, B., Gallegos, M., Puoti, A., Durkin, E., Fields, S., Kimble, J. and Wickens, M.P. (1997) Nature 390, 477–484.
[6] Wang, Z.F., Ingledue, T.C., Dominski, Z., Sanchez, R. and Marzluff, W.F. (1999) Mol. Cell. Biol. 19, 835–845.

[7] Dahanukar, A., Walker, J.A. and Wharton, R.P. (1999) Mol. Cell 4, 209–218.
[8] Park, Y.W., Wilusz, J. and Katze, M.G. (1999) Proc. Natl. Acad. Sci. USA 96, 6694–6699.
[9] Jan, E., Motzny, C.K., Graves, L.E. and Goodwin, E.B. (1999) EMBO J. 18, 258–269.
[10] He, Y.Z. and Liu, D.G. (2000) Biochem. Biophys. Res. Commun. 276, 101–106.
[11] Davies, H.G., Giorgini, F., Fajardo, M.A. and Braun, R.E. (2000) Dev. Biol. 221, 87–100.
[12] Long, R.M., Gu, W., Lorimer, E., Singer, R.H. and Chartrand, P. (2000) EMBO J. 19, 6592–6601.
[13] Wood, J., Frederickson, R.M., Fields, S. and Patel, A.H. (2001) J. Virol. 75, 1348–1358.
[14] Al-Maghrebi, M., Brule, H., Padkina, M., Allen, C., Holmes, W.M. and Zehner, Z.E. (2002) Nucleic Acids Res. 30, 5017–5028.
[15] Poleev, A., Hartmann, A. and Stamm, S. (2000) Eur. J. Biochem. 267, 4002–4010.
[16] Xu, P., Wen, L., Benegal, G., Wang, X. and Buck, G.A. (2001) Mol. Biochem. Parasitol. 112, 39–49.
[17] Le, S., Sternglanz, R. and Greider, C.W. (2000) Mol. Biol. Cell 11, 999–1010.
[18] Bouffard, P., Barbar, E., Briere, F. and Boire, G. (2000) RNA 6, 66–78.
[19] Bouffard, P., Briere, F., Wellinger, R.J. and Boire, G. (1999) Biotechniques 27, 790–796.
[20] Dominski, Z., Erkmann, J.A., Yang, X., Sanchez, R. and Marzluff, W.F. (2002) Genes Dev. 16, 58–71.
[21] Fraldi, A., Licciardo, P., Majello, B., Giordano, A. and Lania, L. (2001) J. Cell Biochem. 81, 247–253.
[22] Liu, Y., Li, J., Kim, B.O., Pace, B.S. and He, J.J. (2002) J. Biol. Chem. 277, 23854–23863.
[23] Sonoda, J. and Wharton, R.P. (1999) Genes Dev. 13, 2704–2712.
[24] Sonoda, J. and Wharton, R.P. (2001) Genes Dev. 15, 762–773.
[25] Sengupta, D.J., Wickens, M. and Fields, S. (1999) RNA 5, 596–601.
[26] Ostrowski, J., Wyrwicz, L., Rychlewski, L. and Bomsztyk, K. (2002) J. Biol. Chem. 277, 6303–6310.
[27] Long, R.M., Gu, W., Meng, X., Gonsalvez, G., Singer, R.H. and Chartrand, P. (2001) J. Cell Biol. 153, 307–318.
[28] Jang, Y.K., Kim, M. and Dai Park, S. (2002) Biochem. Biophys. Res. Commun. 294, 1184–1190.
[29] Kang, Y., Bogerd, H.P., Yang, J. and Cullen, B.R. (1999) Virology 262, 200–209.
[30] Lee, E.G. and Linial, M.L. (2000) J. Virol. 74, 9167–9174.
[31] Martin, F., Michel, F., Zenklusen, D., Müller, B. and Schümperli, D. (2000) Nucleic Acids Res. 28, 1594–1603.
[32] Harrington, L., McPhail, T., Mar, V., Zhou, W., Oulton, R., Bass, M.B., Arruda, I. and Robinson, M.O. (1997) Science 275, 973–977.
[33] Bacharach, E. and Goff, S.P. (1998) J. Virol. 72, 6944–6949.
[34] Bieniasz, P.D., Grdina, T.A., Bogerd, H.P. and Cullen, B.R. (1998) EMBO J. 17, 7056–7065.
[35] Bieniasz, P.D., Grdina, T.A., Bogerd, H.P. and Cullen, B.R. (1999) J. Virol. 73, 5777–5786.
[36] Fewell, S.W. and Woolford Jr., J.L. (1999) Mol. Cell. Biol. 19, 826–834.
[37] Kickhoefer, V.A., Stephen, A.G., Harrington, L., Robinson, M.O. and Rome, L.H. (1999) J. Biol. Chem. 274, 32712–32717.
[38] Lee, E., Yeo, A., Kraemer, B., Wickens, M. and Linial, M.L. (1999) J. Virol. 73, 6282–6292.
[39] Bogerd, H.P., Wiegand, H.L., Yang, J. and Cullen, B.R. (2000) J. Virol. 74, 9353–9361.
[40] Yang, J., Bogerd, H., Le, S.Y. and Cullen, B.R. (2000) RNA 6, 1551–1564.
[41] Crucs, S., Chatterjee, S. and Gavis, E.R. (2000) Mol. Cell 5, 457–467.
[42] Good, P.J., Chen, Q., Warner, S.J. and Herring, D.C. (2000) J. Biol. Chem. 275, 28583–28592.
[43] Guerra-Peraza, O., de Tapia, M., Hohn, T. and Hemmings-Mieszczak, M. (2000) J. Virol. 74, 2067–2072.
[44] Ismaili, N., Perez-Morga, D., Walsh, P., Cadogan, M., Pays, A., Tebabi, P. and Pays, E. (2000) Mol. Biochem. Parasitol. 106, 109–120.
[45] Matter, N., Marx, M., Weg-Remers, S., Ponta, H., Herrlich, P. and Konig, H. (2000) J. Biol. Chem. 275, 35353–35360.
[46] Rho, S.B. and Martinis, S.A. (2000) RNA 6, 1882–1894.
[47] Takahashi, N., Sasagawa, N., Suzuki, K. and Ishiura, S. (2000) Biochem. Biophys. Res. Commun. 277, 518–523.
[48] Cassiday, L.A. and Maher, L.J. (2001) Biochemistry 40, 2433–2438.
[49] Fabini, G., Raijmakers, R., Hayer, S., Fouraux, M.A., Pruijn, G.J. and Steiner, G. (2001) J. Biol. Chem. 276, 20711–20718.
[50] Giorgini, F., Davies, H.G. and Braun, R.E. (2001) Mol. Cell. Biol. 21, 7010–7019.
[51] Jiang, T., Guerrier-Takada, C. and Altman, S. (2001) RNA 7, 937–941.
[52] Monshausen, M., Putz, U., Rehbein, M., Schweizer, M., Des-Groseillers, L., Kuhl, D., Richter, D. and Kindler, S. (2001) J. Neurochem. 76, 155–165.
[53] Tadauchi, T., Matsumoto, K., Herskowitz, I. and Irie, K. (2001) EMBO J. 20, 552–561.
[54] Ziehler, W.A., Morris, J., Scott, F.H., Millikin, C. and Engelke, D.R. (2001) RNA 7, 565–575.
[55] Cui, L., Fan, Q. and Li, J. (2002) Nucleic Acids Res. 30, 4607–4617.
[56] Eckmann, C.R., Kraemer, B., Wickens, M. and Kimble, J. (2002) Dev. Cell 3, 697–710.
[57] Houser-Scott, F., Xiao, S., Millikin, C.E., Zengel, J.M., Lindahl, L. and Engelke, D.R. (2002) Proc. Natl. Acad. Sci. USA 99, 2684–2689.
[58] Kodama, Y., Rothman, J.H., Sugimoto, A. and Yamamoto, M. (2002) Development 129, 187–196.
[59] Koloteva-Levine, N., Amichay, M. and Elroy-Stein, O. (2002) FEBS Lett. 523, 73–78.
[60] Lapucci, A. et al. (2002) J. Biol. Chem. 277, 16139–16146.
[61] Rho, S.B., Lincecum Jr., T.L. and Martinis, S.A. (2002) EMBO J. 21, 6874–6881.
[62] Battle, D.J. and Doudna, J.A. (2001) RNA 7, 123–132.
[63] Jaeger, S., Eriani, G. and Martin F. (2004) FEBS Lett., in press.
[64] Tuerk, C. and Gold, L. (1990) Science 249, 505–510.
[65] Sägesser, R., Martinez, E., Tsagris, M. and Tabler, M. (1997) Nucleic Acids Res. 25, 3816–3822.
[66] Danner, S. and Belasco, J.G. (2001) Proc. Natl. Acad. Sci. USA 98, 12954–12959.