When three is not a crowd

Beginning from the birth of RNA to its death, RNA-protein interactions require, in many instances, specific recognition of the RNA target by a protein. Specific recognition underlies mRNA processing, chromosome-end duplication and many developmental events such as early patterning of the *Drosophila* embryo. Decisions on cell fate during early stages of development regulate the activity, stability and cellular localization of mRNAs. Several experimental strategies to detect RNA-protein interactions have been designed to assess *in vivo* and *in vitro* interactions. Recently a variation of the widely used two-hybrid transcriptional transactivation assay for detection of protein-protein interactions has been adapted to explore functional RNA-protein interactions *in vivo*. In this genetic assay, termed the three-hybrid assay, the budding yeast is used as the biological system, and the assay is based on interactions between two fusion proteins and a hybrid RNA, which are used to activate transcription of reporter genes (SenGupta *et al* 1996).

In one of the three yeast shuttle plasmids used in this assay, sequences for the well characterized DNA binding domain of the bacterial LexA protein are fused to the bacteriophage MS2 coat protein sequences. The latter domain binds with high affinity to a 17nt RNA stem-loop normally present in the phage RNA genome. This LexA-MS2 fusion protein (hybrid protein1) is expressed constitutively in yeast. A second yeast plasmid transcribes a bifunctional hybrid RNA containing sequences of the MS2 RNA that constitute binding sites for the coat protein and also the desired test (potential) RNA binding sites. The third yeast plasmid expresses a library of cDNAs or genomic sequences fused to the transcription activation domain of the Gal4 protein to generate hybrid protein2. All these plasmids are expressed in a yeast strain in whose genome the bacterial *lexA* operator sites (*lexAop*), controls the expression of two independent reporter genes—LacZ and the yeast HIS3 gene. Binding of the hybrid protein1 at *lexAop*, and the corollary binding of the hybrid RNA to hybrid protein1, positions the test RNA sequences in the proximity of the transcription start sites of reporter genes. Further, if the second hybrid protein contains cognate binding sites for the test RNA sequences, this will position hybrid protein2 with the transcription activation domain favourably situated to activate the transcription of the reporter genes (figure 1).

This exquisite system has been used effectively to understand an important developmental switch during sex determination of the nematode *Caenorhabditis elegans*. Regulatory sequences in the 3′ untranslated regions (3′UTR) of mRNA are often critical in development, and in *C. elegans* they have been shown to regulate blastomere identity, and later on germline cell fate and the life cycle (Anderson and Kimble 1997). In hermaphrodite worms the switch from spermatogenesis to oogenesis is controlled by the 3′UTR of the *FEM3* gene. *FEM3*-based repression is required to switch from spermatogenesis to oogenesis. Mutational analysis of *FEM3* revealed a minimal five nucleotide stretch in the 3′UTR of its mRNA which defines a binding site for a regulator. Overexpression of an RNA with these sequences maculinizes the worm consistent with titration of a repressor. A 37 nt region of the FEM3 RNA containing the potential binding site has been effectively used in a three hybrid assay to identify a regulator of *Fem3* and thus of the sperm/oocyte switch. The hybrid RNA, i.e., the bait for specific interacting proteins, contained a tandem duplication of the 3′UTR sequence in FEM3 RNA. In screening a *C. elegans* cDNA library by the yeast three hybrid assay the group utilized the sensitive genetic markers available in yeast to specifically identify interactions that were hybrid RNA dependent (Zhang *et al* 1997). The specificity of the RNA-protein interaction was verified by using mutant versions of the target RNA sequences. The interacting clones were used to identify full-length cDNA which defined two highly related genes encoding what have been termed Fem binding factors (FBF1 and 2). The striking feature of the derived amino acid sequences in these factors is the tandem repetition (8 in this case) of a highly conserved sequence with homologues in the animal and microbial kingdoms. That these repeats form the core RNA binding sequences in Fbf1 was revealed by testing deletion derivatives of Fbf1 for RNA binding to 3′UTR of Fem3. The repeats of Fbf1 are similar to those of the *Drosophila* protein encoded by the *PUMILIO* gene. Pumilio binds to *nanos* response elements in the 3′UTR of Hunchback (HB) mRNA, thereby causing translational repression and destabilization of HB RNA (Curtis *et al* 1995). Thus Pumilio together with Nanos impose on Hunchback its asymmetric expression, restricting its expression from the posterior of the embryo. FBP may also have partners in its function and the best candidates for these roles are the *mog* genes which are also required for the sperm/oocyte switch (Graham *et al* 1993). Both FBF and Pumilio define members of a large and evolutionarily wide-spread protein family which these authors now define as the Puf family. The proposed functions for these factors are as sequence specific RNA binding proteins. Eight repeat units of decapeptide sequence and conserved segments before the first repeat and after the last repeat constitute identifying motifs in other homologues. In this general modular structure these RNA binding sequences are similar to the other classes of RNA binding sequences for instance the RRM domains. The success of this search for RNA binding developmental regulators, suggests that similar elements in the 3′UTR of many other genes may be amenable to the three hybrid analysis.

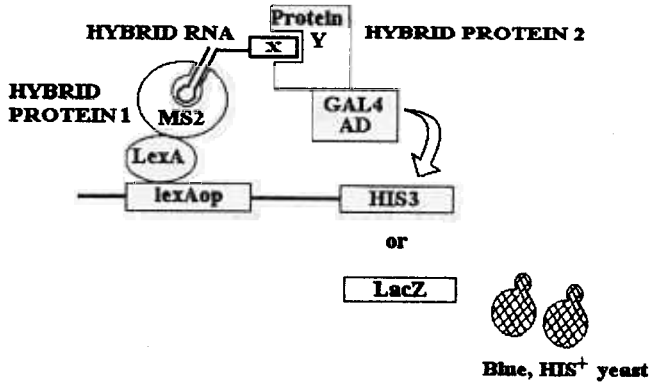

Figure 1. A schematic representation of the three hybrid assay [adapted from Zhang *et al* (1997)].

The bifunctional RNA bridges two hybrid proteins and contains a binding site for the MS2 protein and a test RNA sequence of interest: X. Bridging interactions transactivate the HIS3 and LacZ reporters, to give blue and histidine prototrophic yeast cells.

References

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