

## *In vivo* selection of RNA-binding peptides from combinatorial libraries

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### ABSTRACT

We have used a two step procedure to identify peptides that bind strongly to the Rev-response element (RRE) of HIV. In the first step, RRE-binding peptides were screened from a combinatorial peptide library generated by "randomization" using a small subset of the 20 amino acids. In the second step, one such RRE-binding peptide, RSG-1, was "evolved" into an even stronger RRE-binding peptide using a codon-based mutagenesis procedure. After 2 rounds of evolution, RSG-1.2 bound the RRE with 7-fold higher affinity than wild-type Rev peptide.

### INTRODUCTION

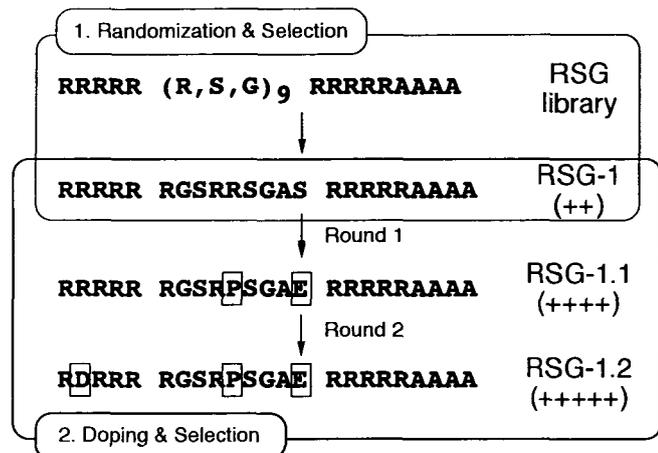
Several genetic assays have recently been developed for detecting protein-RNA interactions, a number of which have been applied to screen cDNA or combinatorial libraries for RNA-binding peptides and proteins.<sup>1-7</sup> Such methods would be expected to be useful in understanding the mechanism of peptide-RNA and protein-RNA interactions, and may provide a tool for designing compounds of therapeutic interest. Using a bacterial system based on the transcription antitermination activity of  $\lambda$  N protein, we have identified several peptides that bind tightly and specifically to the Rev-response element (RRE) of HIV.

In the assay system used, the N protein is expressed under the control of the tac promoter on a pBR322-derived plasmid. The reporter gene, Lac Z, is on a pACYC plasmid, also under the control of a tac promoter, and has a nut site and termination sites upstream so that binding of N to box B is required to assemble the antitermination complex, resulting in the expression of  $\beta$ -galactosidase.<sup>8</sup> We replaced the box B element of pACYC plasmid with RRE IIB, and the RNA-binding domain of N (residue 1-19) with a peptide library, and screened for RNA-binders *in vivo*.<sup>3</sup>

### RESULTS AND DISCUSSION

The strategy used to design peptide combinatorial libraries is critical since the number of sequences that can be conveniently screened using this colony color assay is limited to about  $10^6$  to  $10^7$ .<sup>9,10</sup> As a way to limit the complexity of the combinatorial libraries used, while still ensuring an efficient search of sequence space, we have selected for RRE-binders in two steps. The RSG library used in the first step was completely "randomized" at nine positions with the three amino acids arginine, serine, and glycine, which have been shown to be important in the binding of arginine-rich peptides to their RNA targets. This randomized peptide was flanked on either side by five arginines and a C-terminal tail of four alanines was attached (Figure). This library-encoding plasmid was transformed into RRE-reporter containing cells, and 200,000 colonies (about 10 times the complexity of the library) was screened, and the three peptides, RSG-1, RSG-2, and RSG-3 were identified.<sup>3</sup>

In the second step, RSG-1 was used as a starting point to identify "tighter" binders. This was carried out by two rounds of codon-based mutagenesis<sup>11,12</sup> and selection for variants of RSG-1 that showed higher antitermination activity (Figure). In the first round, the central nine amino acids were mutagenized, resulting in the selection of RSG-1.1. In the next round, the flanking 10 arginines of RSG-1.1 were mutagenized, leading to the selection of RSG-1.2, which bound the RRE with 7-fold higher affinity and 15-fold higher specificity than wild-type Rev peptide. The selected peptides effectively inhibited the activity of Rev in mammalian cell when delivered as fusion proteins in chloramphenicol acetyl transferase reporter assays.<sup>13</sup>



**Figure.** Stepwise "randomized" and "doped" library selection of HIV RRE-binding peptides using a transcriptional antitermination reporter system. Antitermination activities of the selected peptides is indicated by pluses in parentheses, where HIV Rev<sub>34-47</sub> (S34, R47) is +++, and the native N-box B interaction is +++++.

## CONCLUSION

In this study, we show that by stepwise selection with an initial randomized amino acid library, followed by codon-based mutagenesis, it is possible to explore sequence space in a relatively extensive manner. The arginine-rich motif may be a particularly versatile framework for identifying RNA-binders as shown by the identification of peptides that appear to bind the RRE in different ways.

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