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Compensatory mutations in the L30e kink-turn RNA–protein complex

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ABSTRACT

The *S. cerevisiae* ribosomal protein L30e is an autoregulatory protein that binds to its own pre-mRNA and mature mRNA to inhibit splicing and translation, respectively. The L30e RNA-binding element is a stem-asymmetric loop–stem that forms a kink-turn. A bacterial genetic system was designed to test the ability of protein variants to repress the expression of reporter mRNAs containing the L30e RNA-binding element. Initial screens revealed that changes in several RNA nucleotides had a measurable effect on repression of the reporter by the wild type protein. RNA mutants that reduce repression were screened against libraries of randomly mutagenized L30e proteins. These screens identified a glycine to serine mutation of L30e, which specifically restores activity to an RNA variant containing a U that replaces a helix-capping G. Similarly, an asparagine to alanine mutation was found to suppress a substitution at a position where the L30e RNA nucleotide extends out into the protein pocket. In addition, a compensatory RNA mutation within a defective RNA variant was found. The identification of these suppressors provides new insights into the architecture of a functional binding element and its recognition by an important RNA-binding protein.

1. Introduction

The yeast (*Saccharomyces cerevisiae*) ribosomal protein L30e is an autoregulatory protein that binds to its spliced or unspliced transcript to inhibit translation or splicing, respectively [1–8]. L30e is an essential protein thought to reside at the ribosomal subunit interface, and autoregulation confers a survival advantage in yeast [9,10]. Biochemical and structural work have shown that the RNA-binding site is comprised of two helical stems separated by a three-nucleotide bulge that enables the stems to come together at an acute angle [11–13]. Related motifs found in bacterial and archaeal ribosome structures revealed similar tight superimposable RNA bends and notable sequence similarities [14]. This bent RNA with its characteristic sequence was termed the kink-turn, or the K-turn motif, and has also been found in archaeal RNAs that guide the covalent modification of ribosomal RNAs [15].

The hallmark features of K-turn RNAs are a canonical stem, three unpaired nucleotides, and a non-canonical stem having two G:A pairs adjacent to the bulged nucleotides (Fig. 1A) [14]. The importance of the G:A pairs in the L30e complex was underscored by an *in vitro* selection experiment in which the four purines were found to be nearly invariant (Fig. 1B) [16]. In the L30e RNA–protein complex, this structure is further stabilized by purines that stack atop the two stems and an A-minor hydrogen bonding interaction between the two stems. RNA K-turns are most often complexed with proteins, and several of these proteins are homologous to L30e and members of the L7Ae protein class [14,17,18]. These proteins contain alternating regions of alpha helices and beta strands that fold into a central, four-stranded beta sheet flanked by two perpendicular pairs of alpha helices. It is primarily the three protein regions between the secondary structure elements on one face of the protein that contact the RNA. The yeast L30e primary structure is shown in Fig. 1C, and the highly conserved RNA interface regions from a variety of organisms are shown in Fig. 1D [19].

In order to characterize the interactions between the L30e RNA and protein, we developed a genetic screen to identify L30e protein suppressors that restore high affinity binding to mutant RNAs. Because of its autoregulatory nature, we feared overexpression of L30e would not be possible in yeast and opted instead to carry out the genetic screen in bacteria. Two plasmids, one bearing the gene for the yeast L30e protein and the other bearing the L30e K-turn sequence inserted just upstream of the *lacZ* reporter gene, were transformed into bacteria (Fig. 2) [20–26]. The L30e protein is expressed from the repressor plasmid and binds to its cognate RNA sequence in the reporter mRNA. In the *in vivo* screen, strong RNA–protein binding corresponds to greatly reduced expression of the

Abbreviations: BLAST, Basic Local Search Alignment Tool; BSA, Bovine Serum Albumin; DTT, Dithiothreitol; IPTG, Isopropyl β-D-thiogalactopyranoside; K_d, Dissociation constant; LB, Luria–Bertani; LBE, L30e RNA-binding element; MBP, Maltose-Binding Protein; NC, Non-canonical; OD, Optical density; ONPG, o-nitrophenyl-β-galactoside; RR, Repression Ratio; S/D, Shine–Dalgarno; TE, Tris–Ethylenediaminetetraacetate; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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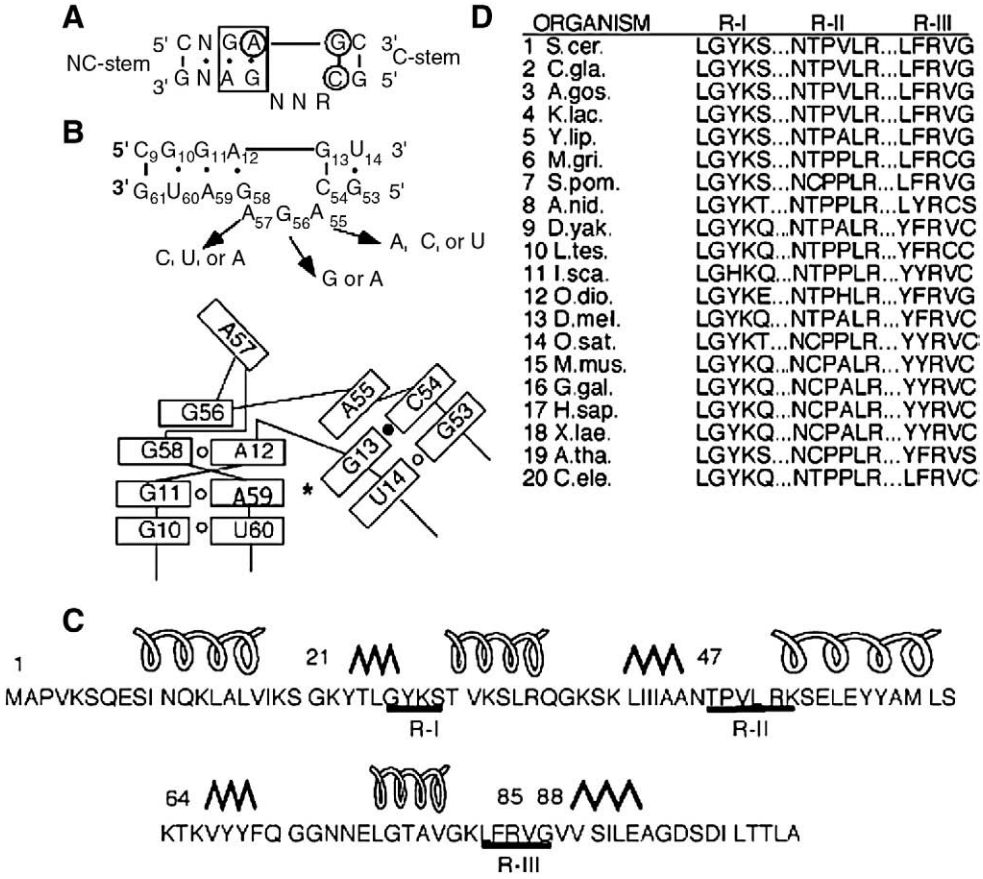

Fig. 1. RNA and protein sequences. (A) Secondary structure of consensus K-turn [14]. Boxed nucleotides form the G:A motif, and circled nucleotides participate in an A-minor interaction. (B) Secondary structure and structural schematic of the *S. cerevisiae*. L30e RNA transcript K-turn. Arrows indicate RNA substitutions that allowed protein binding in a SELEX experiment [16]. Closed and open circles represent canonical and non-canonical base pairing, and the star indicates the A-minor interaction [14]. (C) L30e protein primary structure. The helix and sheet secondary structure is indicated as well as three regions that interact with the RNA, R-I, R-II, and R-III. Key protein residues are numbered. (D) Eukaryotic L30e protein phylogenies of selected regions aligned using BLAST [19].

reporter enzyme, β-galactosidase, and thus parallels one of the L30e autoregulatory activities in yeast.

Finding a protein mutation that compensates for an RNA mutation that weakens binding is an example of a gain-of-function mutation. An L30e protein variant bearing such a mutation would be termed a suppressor protein. The most straightforward explanation for such a compensatory change would be the existence of a direct contact between the mutated RNA nucleotide and the protein amino acid. Random and directed protein mutational strategies were employed to identify such L30e mutants. These screens resulted in the identification of two L30e suppressors, each containing a single amino acid substitution that could recognize specific RNA mutants. A second-site RNA suppressor that was able to correct the defective binding of a mutant RNA was also identified. These findings provide

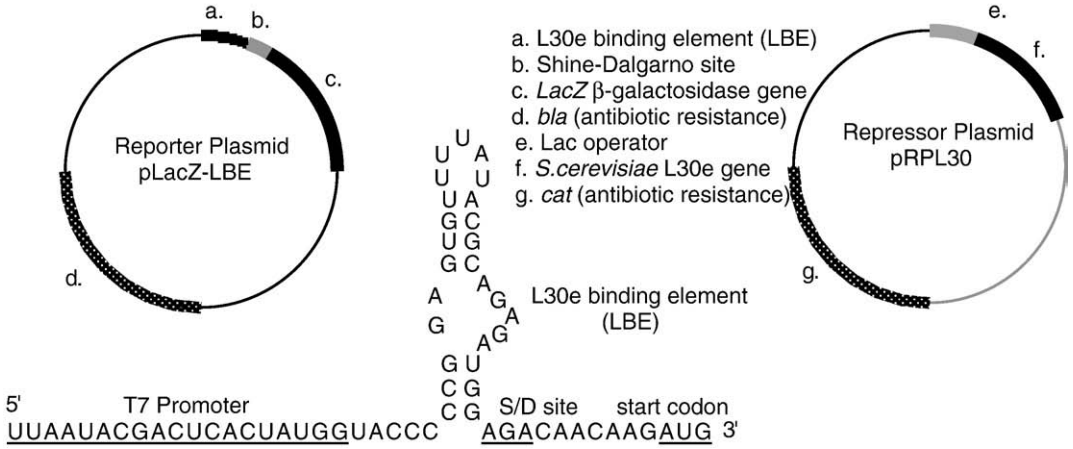

Fig. 2. Reporter and repressor plasmids. The reporter plasmid expresses β-galactosidase under the control of the L30e RNA-binding element (LBE) whose secondary structure is shown. The lower stem of the LBE has been truncated by one base pair compared to the wild type LBE. Expression of the L30e repressor is controlled by IPTG.