A More Complex Isoleucine Aptamer with a Cognate Triplet*

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The simplest RNA that can meet a column affinity selection for isoleucine was previously defined using selection amplification with decreasing numbers of randomized nucleotides. This simplest UAUU motif was a small asymmetric internal loop. Conserved positions of the loop include isoleucine codon and anticodon triplets (Lozupone C., Changayil S., Majerfeld, I., and Yarus, M. (2003) RNA (N.Y.) 9, 1315–1322). Using new primer sequences, we now select a somewhat more complex isoleucine binding RNA, requiring 4.7 more bits of information to describe. The newly selected structure is a terminal or hairpin loop of 20 nucleotides, 15 being invariant. An information profile shows that the new binding site contains five short functional loop regions joined by less significant single nucleotide positions. Among the important nucleotides is a conserved isoleucine anticodon, supporting the escaped triplet theory, which posits a stereochimical genetic code originating in RNA amino acid binding sites.

Randomized RNA sequences readily fold into a variety of shapes with different functions. These functions include catalysis (ribozymes) and ligand binding (aptamers). Such activities among randomized RNAs supplement naturally existing ribozymes (1), the ribosome (2), and natural aptamers, e.g. riboswitches (3, 4) to support the RNA World hypothesis (5), which would require RNA to take functions, which are currently unusual but essential to support the life of a ribocyte.

Among important functions is amino acid binding, which was arguably a crucial activity during the era when encoded polypeptide synthesis (translation) first appeared. Varied aliphatic, aromatic, polar, and basic amino acids are bound by RNA. For example, using in vitro selection or SELEX (6–8) many artificial amino acid binding RNAs have been recovered including tryptophan (9), arginine (10–14), valine (15), isoleucine (16), tyrosine (17), phenylalanine (18), histidine,1 tryptophan,2 and leucine.3 In these binding sites, cognate codons and anticodons appear improbable concentrated; a probability of $5.4 \times 10^{-11}$ on the assumption that affinity and coding sequences are unrelated (19–21). Accordingly, amino acid binding sites and cognate amino acid triplets are not unrelated but instead strongly co-related. Therefore, we suppose that coding triplets were abstracted from larger ancestral RNAs that contained amino acid binding sites (21).

Aptamer selections can be constructed to favor the simplest molecular solutions that answer a particular selection regime. Such a “simplest” isoleucine-binding RNA has been selected (16, 22) using two different sets of primers. Here we report a novel isoleucine-binding RNA selected using different conditions. It is more complex than the simplest motif, 37.9 compared with 33.2 bits of information. Accordingly, it is our next simplest isoleucine binding structure. Notably, like the UAUU motif, which contains an isoleucine anticodon and codon, the next simplest motif also has an isoleucine anticodon triplet among the functional nucleotides of the amino acid binding site.

MATERIALS AND METHODS

Information Content—Frequencies ($F_i$) of bases obtained from sequence alignment after doped selection of R431 have been used as estimated nucleotide probabilities ($P_i$). The final frequencies were normalized and adjusted to the skewed initial nucleotide probability at each position (0.7, 0.1, 0.1, and 0.1). To normalize output frequencies each $F_i$ after the selection was divided by the $F_i$ before selection. Subsequently, for further calculations, each normalized $P_i$ was divided by the sum of the normalized $P_i$s. This method has been adopted from Carothers et al. (23).

The observed Shannon uncertainty, $H_{obs}$, was calculated for each nucleotide position of each motif using the equation: $H_{obs} = -\sum P_i \log_2 P_i$ ($i = A, G, C$, or U). The calculated $H_{obs}$ was subsequently subtracted from the maximum uncertainty possible $H_{max}$ (24). For random RNA $H_{max}$ is 2 bits/nucleotide position. Sampling error corrections were made for the information content calculations of both motifs. For the UAUU motif we used an approximate method to calculate the expectation of sampled uncertainty, $AE(H_{obs}) = H_{max} - (s - 1/2n \times \ln 2)$, where $s$ is number of symbols (4 for RNA), $n$ is number of analyzed sequences.

For the newly selected motif we applied an exact method of calculation of the expectation of sampled uncertainty, $E_{sys} = \sum P_{i} H_{i}$, where $P_{i} = (n(i! / n! n! n! n!) \times P_{i}^{na} \times P_{i}^{gb} \times P_{i}^{um}$ and $H_{i} = -\sum (n(nb) \log_2 (nb))$, $n$ is total number of bases in a given position in sequence alignment ($n$ = number of A + number of G + number of C + number of U); analogously $n$ is number of given base, and $na, ng, nc, nu$ are the number of A, G, C, and U, respectively (24).

For parts of stems that conserve only the ability to pair, shown in Fig. 6 as a black N, information content was computed as follows. There are 16 possible 2-nucleotide combinations at base-paired positions, maximum uncertainty is 4 bits/Watson-Crick base pair. For standard base pairings, uncertainty is 2 bits, so the information content is 2 bits (4 bits – 2 bits). We take G-U base pairs to also be acceptable, so 6 of 16 possibilities are “pairs.” The information content therefore is taken to be 1.5 bits per required base pair. For base pairs that showed preferences in nucleotide combination (colored pictograms in Fig. 6) information content was calculated based on observed frequencies among the 16 possibilities, as for dinucleotide probability.

Isoluecin Selection—Isoleucine affinity chromatography on isoleucine-EAH-Sepharose 4B (containing 10 mM l-isoleucine) has been used previously (16, 22) and is also described in detail elsewhere (25). The selection buffer contained 50 mM HEPES (pH 7.0), 300 mM NaCl, 7.5 mM MgCl$_2$, and 0.1 mM ZnCl$_2$. The elution buffer contained in addition 10 mM l-isoleucine. The column was washed after selection with 15 volumes of wash buffer, 1 mM NaCl and 2 mM EDTA. For counterselection, Sepharose blocked with acetyl groups instead of isoleucine.

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1 Majerfeld, I., and Yarus, M. (2005) J. Mol. Evol., in press.

2 I. Majerfeld and M. Yarus, manuscript in preparation.

3 I. Majerfeld and M. Yarus, unpublished results.
was used. RNA was folded before applying to the column as follows: 65 °C for 3 min in water, adjusted to selection buffer, heating continued at 65 °C for next 45 s, and subsequently incubated at room temperature for 10 min. Weakly bound RNA was eluted with 5.5–7-column volumes of column buffer. RNA retained on the column was eluted with 5 volumes of elution buffer (column buffer containing 10 mM L-isoleucine). 5 fractions (2.5-column volume) were collected and carried forward to amplification. New primers were used. 5/H11032- TAATACGACTCACTATAGGCAAGT-AGGTAGCGTCGAA(28N)AAAAGAGCAAGTCGGTTAGA-3/H11032, where the T7 promoter sequence is underlined. 1.66 pmol (1012 molecules) of single-stranded DNA was used for the first PCR for isoleucine selection. Doped Reselection—Primers used for doped selection, 5/H11032- TAATACGACTCACTATAGGCAAGTAGAAGTGAAGAGC(39doped)GCTCTTCGGAGAGGAGTGCG-3/H11032. The initial pool was based on R431. 39 positions G23–C61 (Fig. 5) were doped to 30%; 10% of each mutant nucleotide. 1.66 nmol (1015 molecules) of single-stranded DNA was used for the first PCR for doped selection. Buffers and RNA folding were as for isoleucine selection.

Isoleucine Elution Profiles—Internally labeled ([α32-P](GTP) RNA (~50 pmol) was folded as described for isoleucine selection. RNA was applied to the isoleucine-Sepharose column. The same buffers were used. Determination of $K_D$—For $K_D$ determination, the same buffers as described in isoleucine selection were used but Zn2+ increased from 0.1 to 0.4 mM, to compare with previous data (22). Isocratic competitive affinity chromatography has been described previously (11, 26).

Interference and Chemical Modification—RNA was modified after folding. DMS or CMCT modifications were carried out based on (27). DMS buffer was as follows: 80 mM HEPES (pH 7.0), 100 mM NaCl, 7.5 mM MgCl2, 0.1 mM ZnCl2. CMCT buffer was the same but contained borate (pH 8.0) instead of HEPES. Two concentration of isoleucine (2 or 10 mM) were used, with isoleucine-free buffer as a control. For an interference experiment RNA was modified with DMS or CMCT.

The abbreviations used are: DMS, dimethyl sulfate; CMCT, cyclohexyl-morpholinoethyl-carbodiimide-toluene sulfonate.
CMCT as described above. RNA after modification was purified on a P30 column (Bio-Rad). RNA was subsequently applied to isoleucine-EAH-Sepharose column. 1.5 or 2.5 volumes were collected from the void or elution peak, respectively; the same buffers as described for isoleucine selection were used. The primer extension method was used to identify modifications.

**Boundary Experiment**—5’-End labeled ([γ-32P]ATP) R431 was partially hydrolyzed using 50 mM NaOH for 30 s, at 85 °C (28). The reaction was quenched by addition of sodium acetate to a final concentration of 320 mM. RNA was precipitated with EtOH, dissolved in water, folded as described under “Materials and Methods” for isoleucine selection, and applied to isoleucine-EAH-Sepharose column. Flow-through and eluted fractions were collected and analyzed on a denaturing polyacrylamide gel.

**RESULTS**

An RNA with 28 adjacent, initially randomized positions was subjected to isoleucine affinity selection using the previous affinity chromatography protocol (22). Step by step description of this selection for isoleucine binding aptamers is available (25). An initial RNA pool containing 10^{12} unique sequences present in ~2400 copies each, on average, was applied to a carboxyl-linked isoleucine-EAH-Sepharose column. The column was washed with 5.5–7-column volumes of buffer. Bound RNA was eluted with 2.5 volumes of the same buffer with 10 mM l-isoleucine. RNA was reverse-transcribed, amplified by PCR, and T7-transcribed to obtain next generation of RNA. Negative selection was included after the first cycle by taking the first 60% emerging from an acetylated control column for use in the subsequent affinity selection.

After the fifth cycle an initial elution peak appeared (11.8% of applied RNA), and elution increased gradually to the seventh cycle (18.5% of applied RNA). The isoleucine-eluted population after seven cycles was cloned, sequenced, and aligned with ClustalW (29). A total of 69 sequences was compared.
A new isoleucine motif dominated the selected sequences. The frequency of this aptamer was 50.8% of the sequenced pool. Positional variation suggests at least 10 different parental sequences containing the motif were present in the initial pool (Fig. 1A). The percentage composition for each nucleotide position within the new motif is summarized in Fig. 1B. Binding and elution profiles for five RNAs are shown in Fig. 1C. For the most frequently appearing sequence (R431) $K_D$ is 0.9 mM. This is 2-fold lower than the UAUU motif selected previously (22). In contrast with every other selection for isoleucine binding RNA (16, 22), there was only one sequence (1.4%) that fitted the UAUU motif: UACG(N8)UAUGGG (conserved nucleotide positions are italic, boldfaced, and underlined). This sequence, however, does not significantly bind the affinity column. Mfold (30) predicts that this sequence does not stably fold the asymmetrical loop of the UAUU motif (not shown). It may therefore be present in active form only a small fraction of the time. We also recovered nonspecific column binding sequences isolated previously. These shared the sequence GUACGCU (VG2 of Lozupone et al. (22), which was recovered 7 times (not shown).

**New Isoleucine Motif**—To define the minimal active length of the newly selected RNA we carried out a boundary experiment, randomly truncating 5'-labeled RNA with sodium hydroxide (Fig. 2). This procedure delimited a minimal functional RNA ending at nucleotide 61. Based on the observation that the truncated molecule does not require the last 7 nucleotides from the 3'-end, it appears that the corresponding part of the initial stem is dispensable; it can be shortened to
9 base pairs with a one-nucleotide bulge (compare Fig. 3 with 5). Accordingly, a predicted smaller active structure was 39 nucleotides (G23–C61; the 3′/H11032-boundary is marked on the thermodynamically favored structure in Fig. 5). This surmise was confirmed by construction of a functional site spanning the terminal loop, 41–R431, with a stem lengthened by one base pair from the assumed minimal structure. The original pair C22-G62 was converted to G-C (Fig. 3C) to aid T7 RNA polymerase transcription.

Shortening R431 left a functional aptamer. We compared column affinity for minimal structures having both motifs. Profiles of binding of 41–R431 and a shortened version of the UAUU motif (36-UAUU) are similar (Fig. 3A). Secondary structures of both RNAs calculated by mfold (30) are compared in Fig. 3, B and C.

The binding site was more closely defined by chemical evidence. A modification-interference assay with CMCT (U and G) or DMS (A and C) indicates that the tract 34GGUAUUG40 and positions A42, A43 and 45GA46 are crucial for binding (Fig. 4B). These positions are also marked on the structure shown in Fig. 5.

In addition, protection/enhancement of the accessibility of site nucleotides to DMS and CMCT in the presence of isoleucine ligand was surveyed. CMCT chemical probing shows enhancements at positions U36, U39, G45, G47, and U48 in the presence of 10 mM L-isoleucine (data not shown). Positions showing enhancements are also likely to be close to the site of contact with isoleucine ligand, and are marked in Fig. 5. For the case of DMS probing no significant differences in reactivity were detected at 0, 2, and 10 mM isoleucine. Viewed on the hairpin structure in Fig. 5, the chemically implicated nucleotides are all conserved members of the hairpin loop.

Doped reselection of R431, which has the most frequently recovered sequence, was undertaken to confirm these essential positions. 39 nucleotides spanning the loop were mutagenized (positions G23–C61 in Fig. 5). Starting from 10^15 molecules of single-stranded DNA 3 cycles of selection were applied. The initial amount of DNA should have been enough to cover all possible sequences that are 8 mutational changes different from the original one. For comparison, the total number of sequences that are 12 mutational changes different from the original one is 2.4*10^15 (31). After the third cycle, isoleucine elution reached 41% of RNA; this pool was cloned and sequenced. Comparison of 61 sequences showed that A50, A51, and A52 are essential elements, because they are perfectly conserved. These three adenosines were supplied as a part of primer complement sequences in the initial selection (Fig. 5, lowercase letters). The rest of the conserved nucleotide positions were as in the initial selection (compare Fig. 1B with 6A). In summary, 15 of 20 positions in the terminal loop are invariant; the consensus sequence of the loop is 34CGGUAAUGRNAU-52 (R = G or A; H = A, C, or U).
From these data, we calculated information for each mutagenized nucleotide position (24) as $I = H_{\text{max}} - H_{\text{obs}}$ (in bits/nucleotide position), where $H_{\text{max}}$ is the maximum Shannon uncertainty possible (2 bits for RNA), and $H_{\text{obs}}$ is the observed Shannon uncertainty calculated based on nucleotide frequencies in the alignment of sequences of binding aptamers. Maps of the information content for the newly selected hairpin (Fig. 6A) and the UAUV motif internal loop motif (Fig. 6B) are shown. The secondary structure used for the new motif was the most probable structure calculated by BayesFold (32) based on the alignment of 54 sequences of equal length obtained from doped selection. The calculation of information content was done based on 61 sequences with initial nucleotide frequencies in the alignment of sequences of binding aptamers. The calculated information content was done based on 61 sequences with initial nucleotide frequencies in the alignment of sequences of binding aptamers. The secondary structure used for the newly selected hairpin (Fig. 6A) and the UAUV motif internal loop motif (Fig. 6B) are show. The secondary structure used for the new motif was the most probable structure calculated by BayesFold (32) based on the alignment of 54 sequences of equal length obtained from doped selection. The calculation of information content was done based on 61 sequences with initial nucleotide frequencies in the alignment of sequences of binding aptamers. The newly selected aptamer does not take functional advantage of its somewhat larger size; its $K_d$ for isoleucine is only 2-fold lower with 4.7 bits more information, a lesser dependence on size than suggested previously (23). However, individual aptamer structures may be quite idiosyncratic, with the escaped triplet theory of the origin of the genetic code (21).

The force of this observation is less than usual, however. We have usually required side chain-specific affinity for cognate amino acids, reasoning that specificity would be essential to a site used for coding; e.g. see Illangasekare and Yarus (18). Side chain specificity usually appears as an unselected by-product of selection on particular amino acid, but this new hairpin is not as selective for isoleucine (Fig. 7), as was the UAUV motif (22). Thus, the new motif might be offered as experimental support for ambiguous primordial coding among chemically similar amino acids (Fig. 7). However, considering that this is the second smallest isoleucine site we have selected, we note that the site embraces a conserved isoleucine anticodon sequence, AAU. Therefore, this is another example of a simple RNA aptamer, which contains coding triplets for a bound amino acid, consistent with the escaped triplet theory of the origin of the genetic code (21).

The newly selected aptamer does not take functional advantage of its somewhat larger size; its $K_d$ for isoleucine is only 2-fold lower with 4.7 bits more information, a lesser dependence on size than suggested previously (23). However, individual aptamer structures may be quite idiosyncratic, with the information/function relation visible only in the mean of many examples. The relaxed side chain specificity of the hairpin (Fig. 7) does suggest that a single index like $K_d$ may be too narrow.
a criterion for comparison with molecular information content. Finally, we consider why the simplest motif was not the most frequent outcome. Smaller RNA motifs are expected to initially be most frequent, because abundance in an unfraccionated randomized pool is expected to increase exponentially as the number of required nucleotides decreases.

On the other hand more active or more easily amplified RNA structures can sometimes be selected instead of simpler ones (25) in prolonged selections. For example, it has been suggested (for GTP binding) that 10-fold tighter binding can result from 10 more bits of information (for example, in five completely unspecified nucleotides), equivalent to 1000-fold decrease in abundance (23). However, this line of reasoning is very unpromising in our case. Firstly, the selection stringency was not set higher than previously (16, 22). Selection succeeded at the fifth cycle, and was halted after the seventh cycle, so simple motifs should survive. Previous selections dominated by the UAUU motif were stopped at the sixth or eighth cycles for a 22 nucleotide random region, respectively (22). Even more convincing, similar previous selections failed to detect the hairpin, which has only about a 2-fold $K_D$ advantage. Thus, we must look for other explanations.

Here we have calculated information content to characterize size. In these terms, why is a more complex structure containing 37.9 bits of information (the new hairpin) 35-fold more prevalent than the previously defined simplest 33.2-bit structure (the UAUU internal loop)? The hairpin should be 26-fold ($2^{37.9} / 2^{33.2}$) less frequent on the basis of complexity, about a 910-fold ($231.9$) more frequent than the UAUU motif.

In rationalizing this selection outcome, we suggest two important influences: the accidental inclusion of an AAA motif in the 3’-primer site, later proven to be essential for hairpin activity (Fig. 6A), and use of a short 28-nucleotide random tract.

The presence of the first condition can be considered as a supply of six bits of information, equivalent to a $-64$-fold increase in abundance favoring the newly selected hairpin. With six bits of information subtracted, only 31.9 additional bits are required for the newly selected motif. This inverts the abundances, making the hairpin loop $-2.5$-fold ($2^{33.2} / 2^{31.9}$) more frequent than the UAUU motif.

The second condition will limit or eliminate the advantage of modularity for the UAUU motif, removing a potential statistical advantage of the smaller motif; the UAUU motif has two modules (34, 35) and the hairpin one. Consequently the UAUU motif will not benefit from its usual statistical advantage; in a larger randomized region the UAUU motif might be the only observed outcome.

Finally, there is a subtle condition whose significance is difficult to judge. Here we used new primer sites. These sequences are not complementary to each other, so they do not supply an initial formed stem that can be captured in selected structures. Because the UAUU motif can benefit in two ways from the initiation of helices, it might be disproportionately penalized when no helix is supplied.

Thus, several experimental features (possibly combined with unseen stochastic factors) produced a new outcome. In summary; although primer design is a little explored aspect of selection planning, it clearly can be decisive and deserves attention.

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REFERENCES

1. Deudna, J. A., and Cech, T. R. (2002) Nature 418, 222–228
2. Nissen, P., Hansen, J., Ban, N., Moore, P. B., and Steitz, T. A. (2000) Science 289, 920–930
3. Winkler, W. C., and Breaker, R. R. (2003) ChemBioChem. 4, 1024–1032
4. Mandal, M., and Breaker, R. R. (2004) Nat. Rev. Mol. Cell. Biol. 5, 451–463
5. Gilbert, W. (1986) Nature 319, 618
6. Ellington, A. D., and Szostak, J. W. (1990) Nature 346, 818–822
7. Robertson, D. L., and Joyce, G. F. (1995) Nature 371, 467–468
8. Tuerk, C., and Gold, L. (1990) Science 249, 505–510
9. Famulok, M., and Szostak, J. W. (1992) J. Am. Chem. Soc. 114, 3990–3991
10. Burgstaller, P., Kochoyan, M., and Famulok, M. (1995) Nucleic Acids Res. 23, 4769–4776
11. Connell, G. J., Illangasekare, M., and Yarus, M. (1993) Biochemistry 32, 5497–5502
12. Connell, G. J., and Yarus, M. (1994) Science 264, 1137–1141
13. Geiger, A., Burgstaller, P., von der Elzt, H., Roeder, A., and Famulok, M. (1996) Nucleic Acids Res. 24, 1029–1038
14. Yang, Y., Kochoyan, M., Burgstaller, P., Westhof, E., and Famulok, F. (1996) Science 272, 1343–1346
15. Majerfeld, I., and Yarus, M. (1994) Nat. Struct. Biol. 1, 287–292
16. Majerfeld, I., and Yarus, M. (1998) RNA (N. Y.) 4, 471–478
17. Mannironi, C., Serech, C., Fraschini, P., and Tocchini-Valentini, G. P. (2000) RNA (N. Y.) 6, 520–527
18. Illangasekare, M., and Yarus, M. (2002) J. Mol. Biol. 54, 289–311
19. Yarus, M. (1998) J. Mol. Biol. 47, 109–117
20. Yarus, M. (2000) RNA (N. Y.) 6, 475–484
21. Yarus, M., Knight, R. D., and Caporaso, J. G. (2005) Annu. Rev. Biochem. 74, 179–198
22. Lozupone, C., Chong, A., Majerfeld, I., and Yarus, M. (2003) RNA (N. Y.) 9, 1315–1322
23. Caruthers, J. M., Oestreicher, S. C., Davis, J. H., and Szostak, J. W. (2004) J. Am. Chem. Soc. 126, 5130–5137
24. Schneider, T. D., Stormo, G. D., Gold, L., and Ehrenfeucht, A. (1986) J. Mol. Biol. 188, 415–431
25. de Zwart, I., Lozupone, C., Knight, R., Birmingham, A., Illangasekare, M., Judahav, V., Legiewicz, M., Majerfeld, I., Widman, J., and Yarus, M. (2005) in Handbook of RNA Biochemistry (Hartmann, R. K. Bindereif, A. Schon, A., and Westhof, E., eds) Vol. 2, pp. 783–806, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Fig. 7. Specificity test of R431. Internally labeled RNA was applied to the isoleucine-EAH-Sepharose column and eluted with 10 mM amino acid.
26. Ciesiolka, J., Illangasekare, M., Majerfeld, I., Nickles, T., Welch, M., Yarus, M., and Zinnen, S. (1996) *Methods Enzymol.* **267**, 315–335
27. Krol, A., and Carbon, P. (1989) *Methods Enzymol.* **180**, 212–227
28. Ciesiolka, J., and Yarus, M. (1996) *RNA (N. Y.)* **2**, 785–793
29. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22**, 4673–4680
30. Walter, A. E., Turner, D. H., Kim, J., Lyttle, M. H., Muller, P., Mathews, D. H., and Zuker, M. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 9218–9222
31. Knight, R. D., and Yarus, M. (2003) *Nucleic Acids Res.* **31**, e30
32. Knight, R. D., Birmingham, A., and Yarus, M. (2004) *RNA (N. Y.)* **10**, 1323–1336
33. Gorodkin, J., Heyer, L. J., Brunak, S., and Stormo, G. D. (1997) *Comput. Appl. Biosci.* **13**, 583–586
34. Yarus, M., and Knight, R. D. (2004) *in The Genetic Code and Origin of Life* (de Pouplana, L ed) pp. 75–91, Landes Bioscience, Georgetown, TX
35. Knight, R. D., and Yarus, M. (2003) *RNA (N. Y.)* **9**, 218–230
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