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# α-Sarcin domain is a fragment of 23S and 26S rRNA strategic for ribosome function*

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There exist some universally conserved structural fragments of 23S and 26S rRNA of a large ribosomal subunit. These fragments are very often single stranded and exposed on the surface of a ribosome (or a ribosomal subunit) [1]. In order to understand the mechanism of action of ribosomes the accessibility of binding regions of rRNAs in correlation with their function(s) has to be identified.

The overall primary structures of large rRNAs are heterogeneous, however, several functions and activities of ribosomes are generally universal (e.g. synthesis of a new peptide bond and recognition of tRNAs). Fragments of the rRNAs structure involved in such general phenomena could be expected to have identical or very similar sequences. Today, primary sequences of 23S and 26S rRNA of different origin [2] as well as many data concerning activity sites on large rRNA are available. It should be possible to select the crucial fragments of RNA by analysing the conserved fragments of RNA primary structures correlated with their biological activities (functions). Following this preliminary selection, several tests have to be done to confirm the significance of the selected regions; e.g. assays of biological activity in relation to antisense-DNA hybridization analysis.

Combining the following approaches:

–a, conservation of the structure found in nucleic acids data bank;

–b, correlation with activity data reported in literature;

–c, confirmation of function by biological activity tests in correlation with hybridization of antisense DNA probes, we expect to find fragments which are of significance for the translational machinery.

In our earlier publications [3, 4], we described in detail the effect of antisense DNA probes complementary to the so-called α-sarcin domain in ribosomes originating from bacterial and plant systems. Our experiments with antisense-DNA probes (a-DNA) complementary to the α-sarcin stem-loop structure provided a new source of information concerning functioning of this domain. Our published information can be summarized as follows:

–1, the 3′-fragment of the α-sarcin domain is accessible for hybridizing the DNA probe;

–2, the oligomer #1 (the sequence of this oligomer is given in Fig. 4) induced a conformational change in the 50S and 60S subunits, which – in consequence – reduced their capacity to form stable 70S/80S ribosomes. This conformational change could account for inhibition of peptide synthesis;

–3, the α-sarcin structure plays an important role in the elongation factor dependent transition from the pre-translocational state to the post-translocational state (involving the activity of elongation factor EF-G/EF-2) and *vice versa* – from the post-translocational to the pre-translocational state (induced by the elongation factor EF-Tu/EF-1).

We have to acknowledge that DNA probes for α-sarcin domain of *E. coli* ribosome and chemicals used in our previous work published in *Acta Biochim. Polon.* **39**, 65 - 73 (1992) were a generous gift from Prof. K.H. Nierhaus, whom we thank also for stimulating discussion and valuable advice
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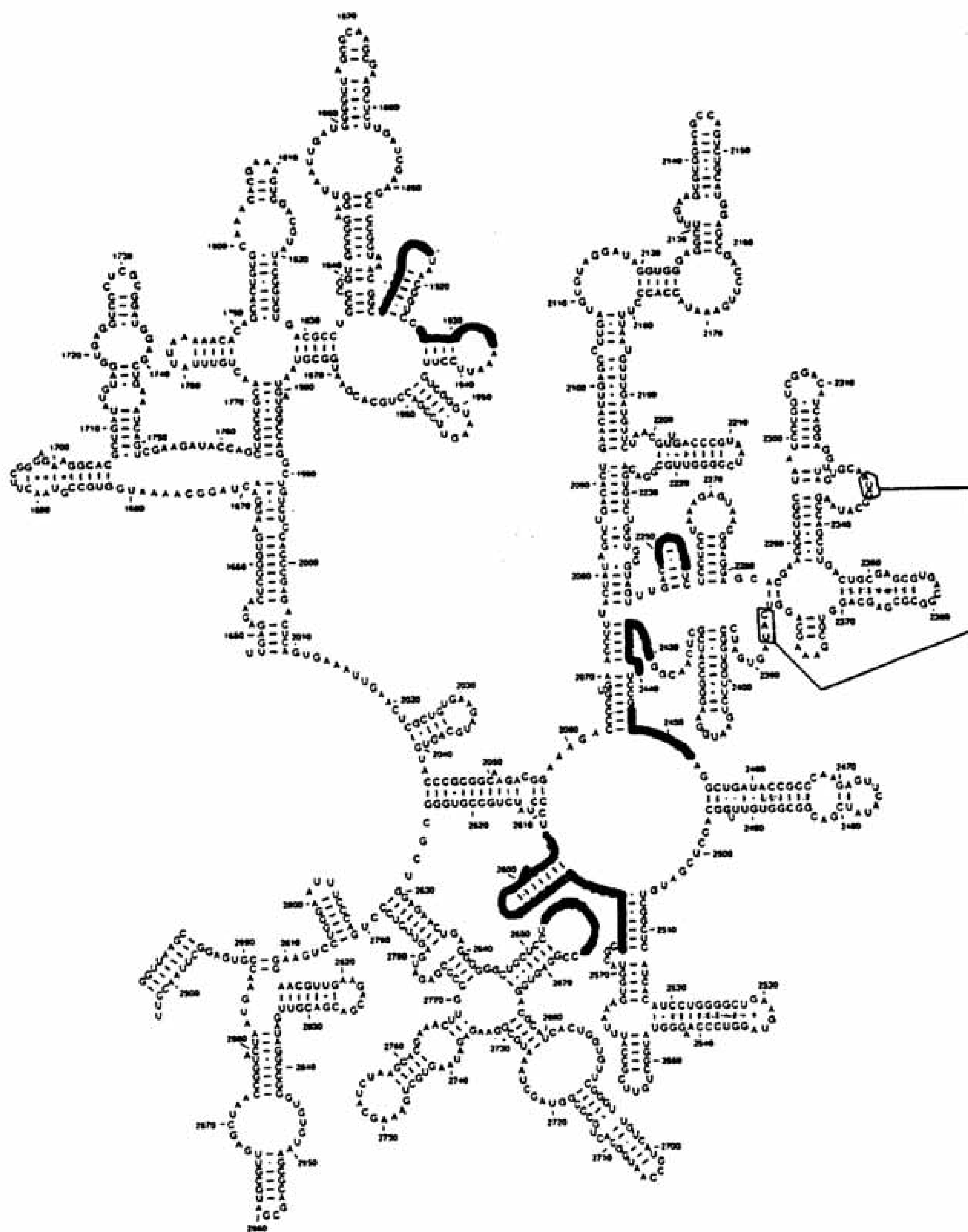

*Fig. 1. The overall view of the secondary structure of the 3′ half of 23S rRNA (according to Gutell & Fox [7]).*
Shadows indicate selected conserved fragments of rRNA

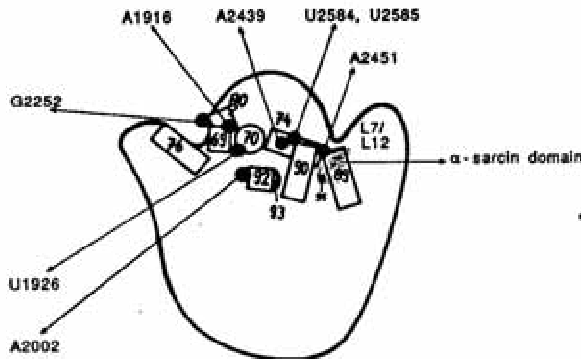


*Fig. 2. Schematic view of the interface side of a large ribosomal subunit of 70S (based on Mitchell et al. [6]).*
The positions of selected domains containing conserved sequences engaged in tRNA binding are indicated by rectangles and circles; the reported in literature [6] footprint sides of P-site bound tRNA (●) are also shown

| domain | 5' sequence 3' | |
|---|---|---|
| 69 | G G C C G U A A C U A | 1906 – 1916 |
| 70 | U A A G G U A G C | 1926 – 1934 |
| 74 | A U A A A A G G U A C | 2430 – 2440 |
| 74/89 | G G G A U A A C | 2445 – 2452 |
| 80 | U G G G G C G G | 2249 – 2256 |
| 90/93 | G A G C U G G G U U U A G A A C G U C G U G A G A C A G U U C G G | 2576 – 2606 |
| 95 | A G U A C G A G A G G A | 2654 – 2665 |

*Fig. 3. List of selected fragments of 23S rRNA containing universally conserved structures found in gene bank [5] and cited by Höpfl et al. [1].*
The conserved nucleotides are underlined

Using this approach, we continue our experiments with the universally conserved fragments of primary structures of 23S and 26S rRNA. We selected rRNA domains exposed on the surface of a large ribosomal subunit in the P-site neighbourhood using Höpfl *et al.* data [1], Gene Bank [5] and Mitchell *et al.* [6] computer graphics model of the tertiary structure of 23S RNA (Fig. 1 and Fig. 2). These domains are numbered according to model of the secondary structure of *E. coli* 23S rRNA elaborated by Brimacombe and co-workers [6 and references cited therein]. The selected fragments of large rRNA are listed in Fig. 3 and the universally conserved sequences are underlined.

The α-sarcin domain includes a purine rich single-stranded segment of 12 nucleotides between A2654 and A2665 (numbered according to *E. coli* 23S rRNA, from 5′ to 3′ end [7]). This is one of the most strongly conserved regions of rRNAs [8]. There are good reasons to expect that the α-sarcin domain is involved in the EF-Tu/EF1 dependent binding of aminoacyl tRNA to ribosomes and the EF-G/EF2 catalyzed GTP hydrolysis and translocation [9]. There are EF-G footprinting data suggesting that EF-G protects nucleotides G2655, A2660, G2661 from chemical modification [10]; these nucleotides correspond in eukaryotic 28S rRNA to bases G4319, A4324 and G4325 [9]. In Fig. 2 we have marked only footprint sides of the acceptor end of P-site bound tRNA to 23S rRNA.

The synthesis of poly(Phe) on poly(U) programmed ribosomes is highly efficient in our systems [3, 4 and references cited therein]. However, the hybridization of an antisense oligomer complementary to the 3′ half of α-sarcin domain completely blocks this process. As we explained earlier [3, 4], the dissociation of ribosomes is responsible for the blocking of protein biosynthesis. Surprisingly, if the poly(Phe) is already present on a ribosome, the hybridization process is very inefficient (Fig. 4).

The calculation of the minimal energy [11] of different possible secondary structures of the α-sarcin domain shows energy differences between several conformations (Fig. 5). The α-sarcin domain nucleotides can be folded into stem/loop structures containing different number of bases. It seems highly possible that the α-sarcin domain is not stable but undergoes various functional states on the ribosome.

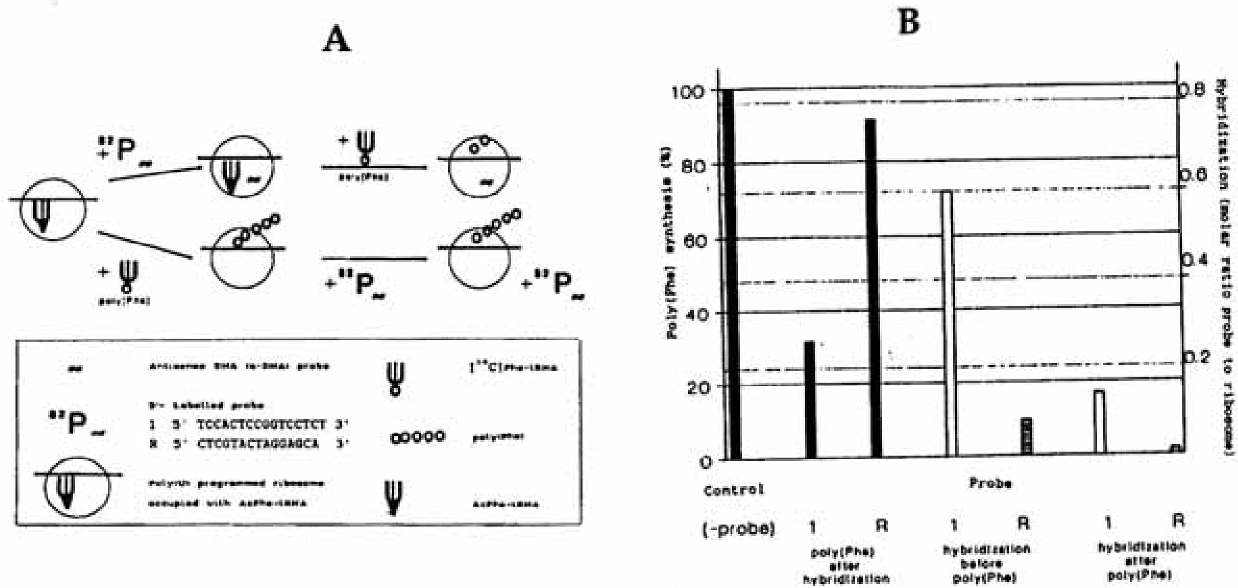


*Fig. 4. Poly(Phe) synthesis with and without the pre-hybridized antisense probe 1.*
A. Schematic diagram presenting the idea of the experiment. Upper part: oligomer labeled with $^{32}P$ was hybridized to the ribosome with prebound AcPhe-tRNA followed by synthesis of poly(Phe) under standard conditions. Lower part: on the ribosome with prebound AcPhe-tRNA synthesis of poly(Phe) was performed and then the level of hybridization of the same oligomer was determined.

B. Poly(Phe) synthesis. Probe 1 (complementary to the 3′ region of α-sarcin domain) had the highest hybridization affinity towards 70S and 80S ribosomes. Probe R was a reference sample with very low hybridization activity. Poly(Phe) synthesis equivalent to 4000 c.p.m. was taken as 100%

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Structure            Energy          Structure             Energy
                     (kcal/mol)                            (kcal/mol)
                                     --1        10
GG        UA  ACG                    --GG    C     AGU
  CUGCUCC  GU   A                        CUG UCCU    A
                     -8.5                                  -5.0
  GGUGAGG  CA   G                        GGC AGGA    C
-A        -C  GGA                    GUGA    C     GAG
  32         20                      30        20


1       10                                4    10
GG       UAGUACG                      --UG       UA     ACG
  CUGCUCC                                  CUCC    GU     A
                     -6.6                                  -3.6
  GGUGAGG       A                          GAGG    CA     G
-A       CCAGGAG                      AGGU'      -C     GGA
  32       20                          32              20
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*Fig. 5. Folding bases of the α-sarcin domain.*
The minimum energy of different structures of the α-sarcin domain was calculated by programme PCFOLD: version 3.0 - *RNA Secondary structure prediction* [11]

The structure-function relationship of ribosomal components determines the efficiency and accuracy of messenger reading. The blockage effect of transition between different ribosomal states during the elongation can be tested by observing the increased number of errors in the poly(U) reading. The misincorporation of $[^3H]$leucine to poly$[^{14}C]$phenylalanine was stimulated in the presence of oligomers prehybridized to the poly(U)-programmed ribosomes. The error of misincorporation of Leu instead of Phe was increased 2 - 3-fold (data not shown).

Our observations may be explained by the following hypothesis: The newly synthesized polypeptide covers the α-sarcin domain and makes it inaccessible for hybridization of antisense oligomer. The polypeptide is localized in a "tunnel" [12]. This location stabilizes the structure and protects the α-sarcin domain. Relatively small differences in the amounts of energy required for changing the conformation of the domain allow the system to continue the elongation process.

The mechanism of action of elongating ribosome was the subject of numerous studies and several controversial results have been published. Our research has been done on 80S plant (wheat germ and lupin seeds) and eubacterial 70S (*E. coli*) systems [3, (Twardowski, T. & Nierhaus, K.H., in preparation)]. We can suppose that the observed phenomena are of a universal character. The mechanism of action of synthetic antisense oligodeoxynucleotide probes complementary to various regions of the α-sarcin stem-loop structure may explain participation of the peptide tunnel in polypeptide synthesis. The synthesized polypeptide is located in the tunnel in close vicinity of the α-sarcin domain. The peptide located between ribosomal A and P sites and the tunnel covers this domain and blocks the hybridization capacity. The presented data support the peptide tunnel hypothesis in ribosomal architecture.

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