Basic Research Development Program (Grant No. G19990b4504).

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(Received October 31, 2000; accepted February 8, 2001)

Identification of selenocysteine insertion sequence (SECIS) element in eukaryotic selenoproteins by RNA Draw program

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Abstract **The computer program RNA Draw was used to identify the secondary structures in the 3′ untranslated regions (3′UTRs) of the mRNAs from 46 eukaryotic selenoproteins among 7 species. The program found one or two possible SECIS elements in these selenoproteins. The SECIS element consists of a stem-loop or hairpin structure with three conserved sequences of AUGA—(A)AA—GA. SECIS element was not found by the RNA Draw program in randomly selected non-selenoproteins. The results showed that SECIS element is the unique character of the genes of eukaryotic selenoproteins. Thus it is possible to use RNA Draw to search the SECIS elements in gene bank for potential new selenoproteins.**

Keywords: selenoprotein, eukaryotes, 3′ untranslated region (3′ UTR), selenocysteine insertion sequence (SECIS), RNA Draw program.

The essential trace element selenium exerts its biological function mainly through various selenoenzymes and selenoproteins[1]. Selenium in these enzymes and proteins is in the form of selenocysteine (Sec), which is the active center of selenoenzymes[2]. So far, it has been clear that translating UGA code to Sec residue in eukaryotes requires a unique stem-loop structure in the 3′ UTR of the mRNA. This structure is called the Sec insertion sequence (SECIS) element. The function of SECIS guiding the insertion of Sec into proteins was shown in the gene expression of cellular glutathion peroxidase (cGPX) and type Ⅰ iodothyronine 5′deiodinase (5′-DI)[3].

Fourteen selenoenzymes and selenoproteins from seven species have been characterized in eukaryotes. They are four types of glutathione peroxidases (GPX), three types of iodothyronine 5′deiodinases (types Ⅰ, Ⅱ and Ⅲ; expressed as 5′-DⅠ, 5′-DⅡ and 5′-DⅢ), three types of thioredoxin reductases (TR), selenoprotein P, selenoprotein W, selenophosphate synthetase 2 (SPS2) and the 15 ku selenoprotein. Increasing evidences demonstrate that there are possibilities of some undiscovered selenoproteins in eukaryotes. Knol et al. used RNAMOT pattern search program to screen GenBank™, sequence-tagged site, and EST data bases for SECIS element and found four new selenoproteins[4]. Gladyshav et al. designed a SECIS Search program to identify SECIS element in nucleotide sequences, leading to the discovery of two new selenoproteins[5]. With the success of human genome mapping and the enrichment of biological information[6], it becomes possible to predict or discover new selenoproteins using some computer programs.

In the present work the RNA Draw program was used to compare 46 genes from 12 selenoproteins and some genes with UGA code or non-UGA termination in the proteins. The results demonstrated that the identification of selenoproteins by SECIS element was possible with RNA Draw. Thus, it is valuable to use the present program to search the potential new selenoprotein in gene bank due to the completeness of human genome plan.

1 Materials and methods

(ⅰ) Genes and their sources. The genes of 46 eukaryotic selenoproteins and 26 non-selenoproteins of 9 species were from the original references and GenBank.

(ⅱ) Methods. The program of RNA Draw V1.1 b2 (Ole Matzura, 1995)[9] was used to predict the secondary structures of the above genes in the 3′ untranslated regions. Energy calculation was conducted at 37℃ using

the energy model from Zuker[10]. All the secondary structures folded following the rule of free energy minimization. The SECIS elements in the genes were searched based on the SECIS structures from human or rat 5′-D I and cGPx. The computer processing procedures were only carried out in the 3′-UTR due to the fact that SECIS in eukaryotic selenoproteins has not yet been reported outside 3′-UTR. The input of the sequences of 3′-UTR to the computer was performed once for the number of nucleotides smaller than 1000 and twice for the nucleotide number bigger than 1000. The two-step input was conducted with the folding bases of 200 across each other.

2 Results and discussion

(i) SECIS elements from the genes of GPx. RNA folding analysis was performed in the 3′ UTRs of 5 cGPx genes, 4 phGPx genes, 4 pGPx genes, 2 giGPx genes and the GPx gene from Schistosoma mansoni. A stem-loop structure similar to the reported rat 5′-DI SECIS and human cGPx SECIS was found in all cGPx genes, human pGPx gene, mouse giGPx gene and the GPx gene from Schistosoma mansoni[3,7,8] (fig. 1). One possible SECIS stem-loop structure was found in all phGPx genes, 3 other pGPx genes and human giGPx gene. This possible SECIS structure has similarity to cGPx SECIS, which contains the conserved base sequences of AUGA, AAA and GA, and the similar conserved positions of AUGA and GA in SECIS. The contrast between phGPx and cGPx is that 3 or 2 continuous A is not situated in the top loop but in the below bulge at 5′arm of phGPx. The structure character of cGPx belongs to type I SECIS and that of phGPx belongs to type II SECIS (see fig. 1).

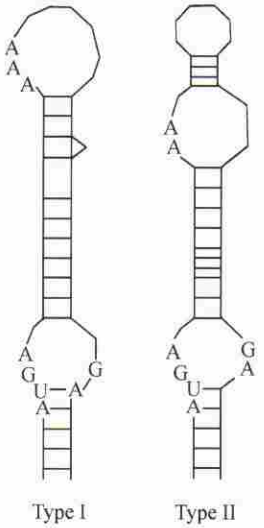

Fig. 1. Types I and II SECIS models for eukaryotic selenoprotein genes.

(ii) SECIS elements from deiodinase genes. Searching the secondary structures of the 3′ UTRs from 15 deiodinases demonstrated a stem-loop structure in all 5′D I genes and bullfrog 5′DIII genes. This stem-loop structure is similar to the reported SECIS element of rat 5′D I [3], which belongs to type I SECIS (fig. 1). Nevertheless, bullfrog 5′DIII SECIS is different from typical type I SECIS, the unpaired AUGA in the upstream of 5′ arm is replaced by the paired AUG. A type II SECIS stem-loop structure was found in the 3′ UTR of 5′ DIII genes from human beings, rat, chicken and African toad.

(iii) SECIS elements from TR genes. The RNA Draw program was used to predict the secondary structures of 3′ UTR in 3 sequenced TR genes (human, rat and cow TR genes). A type II SECIS stem-loop structure was found, which has a top loop containing 7—8 nucleotides above the conserved CAA bulge loop. The two loops were connected by three base pairs. The distance between CAA and the conserved AUGA was 12 nucleotides.

(iv) SECIS elements from SPS2 genes. Selenoenzyme SPS2 was recently isolated from mouse embryo cells or human activated CD8+T cells. The above two genes of SPS2 code for two selenoproteins with 452 amino acids and 448 amino acids respectively. Both selenoproteins contain a UGA code for Sec (located respectively at position 63 and position 60) and use the second UGA as a terminating code. Although it was reported that the Sec in SPS2 from influenza hemophilus had no effect on the catalytic activity of the enzyme, we found two possible SECIS stem-loop structures in the 3′ UTRs of rat and human SPS2 genes using the RNA Draw program. The first one belongs to type I SECIS and the second one belongs to type II SECIS. Both AAA top loops consist of 14 nucleotides. Both distances between AUGA and AAA contain 13 nucleotides.

Guimaraes et al. reported in 1997 that the 3′ UTR in SPS2 gene could enable the insertion of ^{75}Se into SPS2 protein and increase the expression of SPS2 by more than 20 times. This implicates the existence of SECIS in 3′ UTR.

(v) SECIS elements in other selenoproteins. Selenoprotein P, selenoprotein W and the 15 ku selenoprotein were discovered in recent years. Similar results were obtained in the 3′ UTR of the above genes through the RNA folding analysis.

(vi) SECIS secondary structure model for the genes of eukaryotic selenoproteins. In conclusion, a stable secondary structure was formed after the process of RNA folding program, although the nucleotide sequences were significantly different in the SECIS elements of different eukaryotic selenoprotein genes. Two types of SECIS elements were concluded according to the positions of the conserved nucleotides in the stem-loop structures (see fig. 1). Type I SECIS element has the top loop consisting of

9 to 16 nucleotides with three continuous A inside the loop. AUGA and GA locate in the upstream of the top loop at 5′arm and 3′arm respectively, forming non-Watson-Crick duplex. The distance between AAA and AUGA is generally from 9 to 12 nucleotides for different SECIS elements. Type Ⅱ SECIS element has a top loop and an upstream bulge loop. The conserved AAA or AA does not situated in the top loop but in the bulge loop at 5′arm. The (A)AA bulge loop is connected with the top loop through 3 to 4 base pairs.

(vii) The genes from non-selenoproteins. The program of RNA Draw was used to predict the secondary structure of the 3′ UTRs in the genes of seven non-selenoproteins containing UGA as a terminating code, the gene of esGPx with UGA and UAA as terminating codes and the gene of hydrogen peroxidase. The seven non-selenoprotein genes were ADH gene, AGT gene, duplex RNA adenosine deaminase gene, PGIS gene, PGHS gene, human TXAS gene and mouse PGI_2 gene. No typical SECIS stem-loop structure was found in the 3′ UTRs of the above genes. There were even no AUGA sequence in the 3′ UTRs of rat and mouse ADH genes, human TXAS gene, rat PGIS gene, human PGHS gene, etc. These results further showed the important roles of SECIS element in the genes of selenoproteins.

3 Conclusions

SECIS element containing the specific conserved sequences is indeed unique for the genes of selenoproteins. SECIS could be used as a probe to predict and search the gene of a new selenoprotein in the complicated genome. One example is the discovery of the first selenoprotein in virus——the human dermatotropic poxvirus (MCV) selenoprotein, which was found by Berry in 1998 using the above method[12].

The character of SECIS element could also be used to search for the new selenoprotein in some already sequenced eukaryotic protein genes. The good example is thioredoxin reductase, which had been characterized and sequenced early but was only identified as a selenoenzyme in 1996[11]. Thus it is reasonable to use SECIS as a probe to screen more selenoprotein genes in gene bank.

The program of RNA Draw is widely used by molecular biologists to analyze the folding structure of RNA. The present work demonstrated that this program could also be used to identify the two types of SECIS elements, which provided the possibilities for using the program to search for a new selenoprotein in gene bank.

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(Received December 27, 2000)

Noise controlled pattern formation in subexcitable media

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Abstract **The numerical simulation on a reaction-diffusion system indicates that different patterns can be obtained by tuning the strength of parameter noise in subexcitable media. With the increase of noise intensity, the waves sustained by noise are not broken up as usual, they evolve orderly, and the period of the waves decreases, the survival time of waves varies. The time interval of inputting noise also influences the process of pattern formation and the survival time of waves.**

Keywords: subexcitable media, noise, chemical wave.

Chemical waves in a reaction-diffusion system have been studied very extensively in recent years[1,2]. As we know, waves can propagate undamped over long distance