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CELLULAR AGEING - TELOMERE, TELOMERASE AND PROGERIN ANALYSED USING RESONANT RECOGNITION MODEL*

ĆELIJSKO STARENJE - TELOMERE, TELOMERAZE I PROGERIN ANALIZIRANI METODOM REZONANTNOG PREPOZNAVANJA*

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Telomere, Telomerase, Progerin, Resonant Recognition Model, protein-DNA interaction, ageing

Ključne reči
Telomere, Telomeraze, Progerin, Model Rezonantnog Prepoznavanja, protein-DNA interakcija, starenje

Abstract

We have analysed cellular ageing through telomere shortening and/or elongation using the Resonant Recognition Model which proposes that macromolecular (protein, DNA and RNA) interactions are resonant in nature and are characterised by frequency specific for each interaction. The two distinct frequencies have been identified characterising telomere shortening and elongation processes. Having these characteristic frequencies identified it opens new possible directions to influence and modulate cellular ageing at molecular level.

INTRODUCTION

Biological ageing at the cellular level was always puzzling scientists. It is well known that any mature cell can divide only a certain number of times before it stops dividing and eventually dies. The main part in this process is due to telomeres, which are made up of many kilo bases of DNA repeats (TTAGGG) at the end of each chromosome and bound and protected by telomere binding protein [1-3]. Each time when cell divides the chromosomes of the new cell are shorter at the ends making telomeres shorter. When telomeres get too short, the cell can no longer divide, becomes inactive and dies. If there would not be telomeres at all, the main genetic information in chromosomes will become shorter with each cell division and the genetic information will be then lost and corrupted [1-3].

In contrast to process of shortening telomeres, enzyme telomerase can add nucleotides to the end of telomeres. Telomerase is composed of reverse transcriptase (TERT) and an RNA component (TERC) that serves as template for telomere elongation[1-3]. In somatic cells there is not enough telomerase to keep telomeres from eventual wearing down producing cells ageing, inactivity and eventual death [1-3].

In cancer cells, which divide much more often than normal cells, telomeres get very short. However, these cells very often produce telomerase enzyme which prevents telomeres to get too short and thus prevents cell from dying [1-3].

In addition, it has been found that in devastating premature ageing disease, called progeria syndrome, protein progerin plays critical role [4]. Although it has been shown experimentally in normal human fibroblast cells that progerin is related to telomere shortening there is still not clear relationship of progerin with other components in the process of telomere shortening and/or elongation.

Here, we apply the Resonant Recognition Model (RRM) [5-11] to investigate interactions between participants in process of telomere shortening and/or elongation including: telomeres, telomerase, telomere binding proteins and to propose the role that progerin can play in this processes. The RRM is unique model which proposes that protein-protein, protein-DNA and protein-RNA interactions are based on resonant recognition with unique characteristic frequency characterising each interaction. The RRM is based on finding that each biological function/interaction of protein is characterised by specific periodicity/frequency in distribution of free electron energy along the protein.

METHODS

Resonant Recognition Model

Biological function of proteins and other functional macromolecules like DNA and RNA is written as linear sequence of their constitutive elements, i.e. amino acids or nucleotides. The RRM model interprets this linear information by transforming amino acid or nucleotide sequence into a numerical series and then into the frequency domain using digital signal processing methods, in particular the Fourier Transform (FFT). Appling the RRM, the macromolecular primary structure is represented as a numerical series by assigning to each amino acid a physical parameter value [5-11]. Although a number of amino acid physical papameters have been found to correlate in some ways with the biological

activity of the whole protein, our investigations have shown that the best correlation can be achieved with parameters which are related to the energy of delocalised electrons of each amino acid [5,6]. These findings can be explained by the fact that the electrons delocalised from the particular amino acid have the strongest impact on the electronic distribution of the whole macromolecules. In this study, the energy of delocalised electrons (calculated as the electron-ion interaction pseudo-potential (EIIP)) of each amino acid residue was used. The resulting numerical series represents the distribution of the free electrons energies along the protein and other macromolecules (DNA and RNA).

At the second stage the numerical series obtained are then analysed by digital signal analysis method: Fourier Transform, in order to extract information pertinent to the biological function. As the average distance between amino acid residues in linear polypeptide chain is about 3.8Å, it can be assumed that the points in the numerical sequence derived are equidistant. For further numerical analysis the distance between points in these numerical sequences is set at an arbitrary value d=1. Then the maximum frequency in the spectrum is F=1/2d=0.5. The total number of points in the sequence influences the resolution of the spectrum only. Thus for N-points sequence the resolution in the spectrum is equal to 1/N. The n-th point in the spectral function corresponds to the frequency of f=n/N.

The cross-spectral function is used in order to extract common spectral characteristics of sequences having the same or similar biological function. Peak frequencies in the amplitude cross-spectral function define common frequency components of two or more sequences analysed. To determine the common frequency components for a group of protein sequences, the absolute values of multiple cross-spectral functions have been calculated. Peak frequencies in such multiple cross-spectral functions denote common frequency components for all sequences analysed. Signal-to-noise ratio (S/N) for each peak is defined as a measure of similarity between sequences analysed. The S/N is calculated as the ratio between the signal intensity at the particular peak frequency and the mean value over the whole spectrum. The extensive experience gained from previous research suggests the value of S/N ratio of at least 20 can be considered as significant [5,6]. The multiple cross-spectral function of a large group of sequences with the same biological function has been named as the „consensus spectrum". The presence of a peak frequency with the significant signal-to-noise ratio in a consensus spectrum implies that all of the analysed sequences within the group have one frequency component in common [5-19]. This frequency is related to the biological function provided the following criteria are met:

1. One peak only exists for a group of protein sequences sharing the same biological function
2. No significant peak exists for biologically unrelated protein sequences
3. Peak frequencies are different for different biological functions.

In our previous extensive studies [5-19], the above criteria have been implemented and the following fundamental conclusion was drawn: *each specific biological function within the protein or DNA is characterised by one frequency*. It has been shown in previous research that all protein sequences with the common biological function have common frequency component, which is a specific feature for the observed function/interaction. This characteristic frequency is related to the protein biological function. Furthermore, it was shown that the proteins and their targets have the same characteristic frequency in common [5-7]. Thus, it can be postulated that the RRM frequencies characterise not only a general function but also a recognition and interaction between the particular protein and its target.

Using frequencies, as the main parameter characterising macromolecular interactions, enables easy analysis and comparisons between proteins, DNA and RNA even they are different in their chemical structures [5-7,13,14]. To enable such comparison it is important to understand that the linear distance between amino acids in protein is about 3.8Å, while the linear distance between nucleotides is about 3.4Å. Thus, appropriate adjustment must be made when RRM characteristic frequency is calculated for nucleotide sequences versus protein sequences. It is important to stress here that RRM is unique model that can easily analyse and compare biological function and interaction between proteins, DNA and RNA. This RRM ability is used here to analyse function and interaction of telomeres (DNA), telomere binding protein (proteins), telomerase (proteins), telomerase RNA (RNA) and to investigate role of progerin (protein) in all these telomere related processes.

When the characteristic frequency for certain function/interaction has been identified it is then possible to use Inverse Fourier Transform to identify either key amino acids or nucleotides which predominantly contribute to this frequency as possible key functional mutations or to design de-novo proteins or DNA/RNA segments with desired biological function/interaction [15-19].

Resonant Recognition Model (RRM) and electromagnetic radiation

Initially, it has been shown that certain periodicities/frequencies in the distribution of energy of free electrons along macromolecule are relevant selective parameter for selective recognition and interaction between macromolecules (proteins, DNA and RNA). Such selective recognition and interaction on a distance should be based on physical field which can propagate through aqueous surrounding and is resulting from and having same periodicities/frequencies as energy distribution along macromolecule. This field can be produced by the charge travelling along macromolecule experiencing the free electron energies distribution along its trip. As such charge will travel through different energy states it will create sufficient condition to produce electromagnetic field with the frequency distribution following distribution of free electron energies along the macromolecule. The actual electromagnetic frequencies will depend on the velocity of charge and the distance between amino acids (nucleotides) in linear macromolecule.

Within the RRM it has been postulated that charge is travelling through protein backbone and charge velocity was estimated to be 7.87x105m/s [5-7,10,20,21]. For this velocity and the distance between amino acids in a protein molecule, which is 3.8Å, the frequency range obtained for protein inter-

actions was estimated to be in the range from 10^{13} to 10^{15}Hz. This estimated range includes infra-red, visible and ultra-violet light. Such computational predictions were proposed to be related to biological function of the protein and are confirmed by comparison of: a) absorption characteristics of light absorbing proteins and their characteristic frequencies [5-7,20,21], b) frequency selective light effects on cell growth and characteristic frequencies of growth factors [5-7,20], c) our own experiments where enzyme activity was influenced by electromagnetic radiation of specific frequency [11,21]. Based on these experiments the correlation between RRM numerical frequencies and biologically relevant electromagnetic frequencies can be represented as follows:

$$\lambda = K / f_{rrm}$$

where λ is the electromagnetic wavelength in nm which can influence a particular biological process, f_{rrm} is a numerical frequency obtained with RRM and K=201 is the estimated constant of the linear correlation obtained. Consequently, this formula can be used to predict the resonant frequency of visible and near-infrared irradiation which may produce a biological effect.

RESULTS

Here we analysed interactions between different participants in a process of telomere shortening and elongation not only to understand all these interactions, but also to analyse possible role of progerin within these interactions. We limited our analysis to human: DNA, RNA and proteins. Initially we applied RRM to 10 telomere DNA sequences from different human chromosomes obtained from NCBI database (HQ167748.1, HQ167747.1, HQ167746.1, HQ167745.1, HQ167744.1, HQ167743.1, HQ167742.1, HQ167741.1, HQ167740.1 and AF020783.1). As telomere sequences contain a number of repeats of the sequence: TTAGGG, it is expected that there will be very sharp common frequency peak for all analysed sequences. Indeed it was found that there is such peak at frequency of f=0.1875± 0.004 with another smaller peak at frequency of f=0.1777± 0.004 as presented in Figure 1. The existence of two very close, but sharp, peaks shows firstly that TTAGGG repeats are not perfect, but also that they are dominant feature in the sequence.

The first obvious interaction is between telomeres and telomere binding proteins. Thus, we originally found the characteristic common frequencies of 4 telomere binding proteins obtained from UniProt database (P54274, P70371, Q15554 and O35144). Even as only 4 sequences have been compared two strong common peak frequencies were identified at frequency of f1=0.1729± 0.004 and at frequency of f2= 0.0146± 0.004 as presented in Figure 2. It is clear that the frequency f1 is the same as second significant frequency for telomeres (DNA). To make sure that this is the common frequency responsible for telomeres and telomere binding proteins interaction, we have used cross spectral function of all 10 telomeres and 4 telomere binding proteins with adjustment for DNA-protein interaction as described above. The resulting cross spectrum is showing that the frequency of f1=0.1729± 0.004 is indeed common and is most likely to be characterising telomeres and telomere binding proteins interaction (recognition). In addition, the frequency of f=0.1875± 0.004 is strongly present in the consensus spectrum and thus it could also be relevant for this recognition. As these two frequencies are very close to each other we can conclude here that the frequency range of 0.17-0.19 is responsible for telomeres and telomere binding proteins interaction and recognition as presented in Figure 3. As telomere binding proteins are protecting telomeres from shortening it can be also speculated that the frequency range of 0.17-0.19 is also related to telomere shortening and thus contributing to cell ageing process.

The other process related to telomeres is telomere elongation. Telomerase elongation is achieved through activity of telomerase, enzyme composed of reverse transcriptase

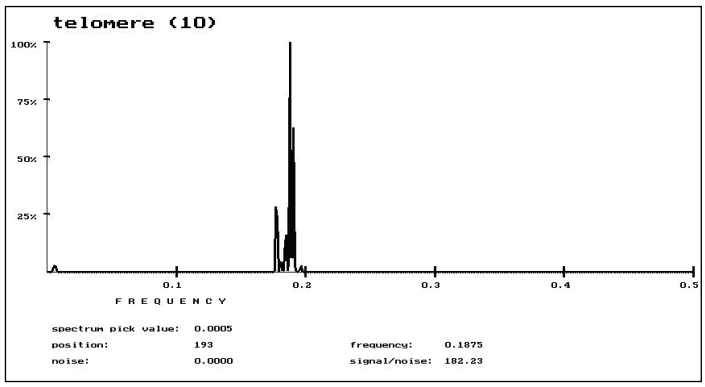

Fig. 1. *Consensus RRM spectrum of 10 telomeres (DNA) from different human chromosomes showing the prominent common frequency at f= 0.1875± 0.004.*

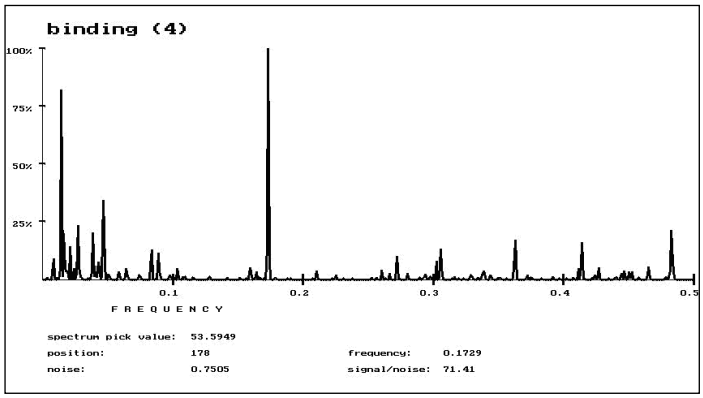

Fig. 2. *Consensus RRM spectrum of 4 telomere binding proteins showing the prominent common frequency at f= 0.1729± 0.004.*

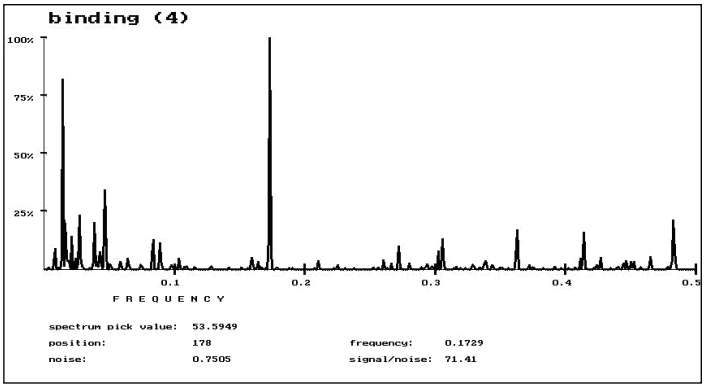

Fig. 3. *Consensus RRM spectrum of 10 telomeres (DNA) and 4 telomere binding proteins showing two prominent common frequencies at f1= 0.1777± 0.004 and at f2= 0.1875± 0.004.*

(TERT) and an RNA component (TERC) that serves as template for telomere elongation. In our previous research [14] the frequency of f=0.2853± 0.004 has been identified as being common for mammalian telomerase and telomeres [14]. This frequency can be then proposed to be characteristic of telomere elongation process. Here, we compare two human telomerase reverse transcriptase (TERT) RNA binding regions as obtained from NCBI database (NR001566.1 regions: 1-230 and 325-550) and ended up with the overlapping most prominent peak at frequency of f=0.2930± 0.005 as presented in Figure 4.

Interestingly, when telomerase RNA, telomerase RNA binding segments and progerin, obtained from UniProt database (P02545), are compared only one very prominent frequency peak at frequency of f=0.2853± 0.004 appeared as presented in Figure 5. This frequency is exactly the same as the frequency proposed in our previous work as telomerase related frequency [14]. From this result we can draw two possible conclusions:

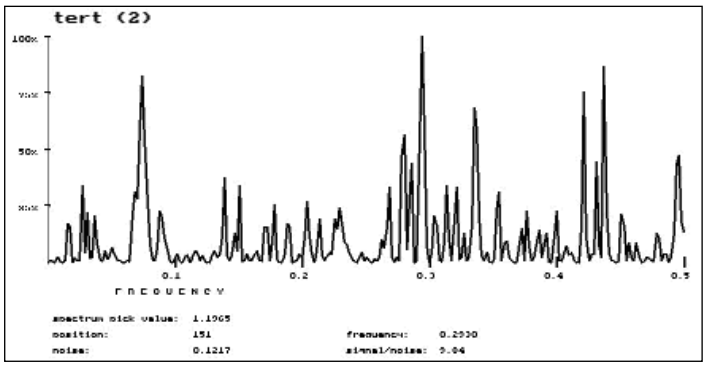

***Figure 4**: Consensus RRM spectrum of 2 human telomerase reverse transcriptase (TERT) RNA binding regions showing the prominent common frequency at f= 0.2930± 0.005.*

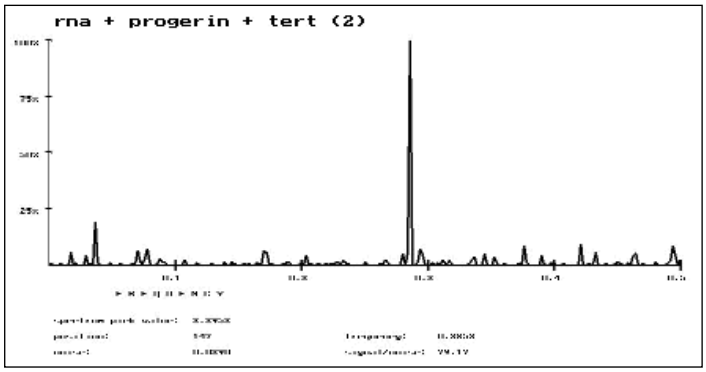

***Figure 5**: Consensus RRM spectrum of 2 human telomerase reverse transcriptase (TERT) RNA binding regions, telomerase (RNA) and progerin (protein) showing the prominent common frequency at f= 0.2852± 0.004.*

- this frequency is possibly related to telomere elongation process
- progerin is possibly interfering with the process of telomere elongation.

Based on all presented results we can conclude that we have indentified two distinct RRM frequency ranges that are related to telomere function:

- frequency range of 0.17-0.19 which according to RRM postulates could be related to telomere shortening
- frequency range of 0.28-0.29 which could be related to telomere elongation (telomerase activity).

By having these two frequencies identified it would be possible now to design proteins and/or peptides which could interfere with either of these two biological processes. Thus by applying the RRM approach the ageing at cellular level could be controlled as desired. This discovery could also be crucial in trying to control the growth of cancer cells by preventing telomerase activity and allowing shortening of telomere with cellular division can lead to fast cellular apoptosis and death.

In addition, if we take into account that RRM frequencies are electromagnetic in nature we can calculate electromagnetic frequency ranges relevant to two telomere related functions. Using the formula $\boldsymbol{\lambda = K / f_{rrm}}$ as described above we have calculated the following electromagnetic frequency ranges relevant to two telomere related functions:

- between 1057nm and 1182nm are related to telomerase shortening
- between 693nm and 718nm are related to telomerase elongation.

Having these electromagnetic characteristic frequencies identified, it opens new possible directions to influence and modulate cellular ageing using electromagnetic radiation.

CONCLUSION

We have analysed here cellular ageing from the point of view of telomere shortening and elongation using the Resonant Recognition Model (RRM) which proposes that macromolecular interactions (functions) are based on resonant recognition at certain frequency between interacting molecules. We have analysed participants in both telomere shortening and elongation processes and have identified two distinct frequency ranges: a) 0.17-0.19 which according to RRM postulates could be related to telomere shortening and b) 0.28-0.29 which could be related to telomere elongation (telomerase activity). We also compared progerin protein with macromolecules that are involved in both processes and concluded that most probably progerin is interfering with telomere elongation. Therefore when progerin is present in larger amounts in young individuals, it can cause devastating early ageing, so called progeria.

Having in mind that the RRM frequencies are proposed to be electromagnetic in nature we were able to calculate two electromagnetic frequency ranges: a) 1057nm to 1182nm related to telomerase shortening and b) 693nm to 718nm related to telomerase elongation.

By having both telomere shortening and elongation characteristic frequencies identified, it is possible now to either design proteins or predict electromagnetic radiation with certain characteristics which can interfere with either telomere shortening or elongation process. These findings have a wide range of possible applications, from controlling cancer cells growth and their immortality, helping progeria sufferers, getting longer lasting transplant tissue to preventing skin cells from ageing in cosmetics.

We have once again shown that the Resonant Recognition Model (RRM) is a powerful tool in analysis of macromolecular interactions. As the RRM proposes that the main parameter in these interactions is resonant frequency and not structure as it is widely accepted, it allows easy analysis of inter-

actions between protein and DNA and protein and RNA. In addition the RRM is capable of designing either proteins or specific electromagnetic radiation frequencies which can modulate biological functions/interactions in desired manner.

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Sažetak
Analizirali smo ćelijsko starenje sa gledišta skraćivanja i produžavanja telomera koristeći Metod Rezonantnog Prepoznavanja koji predvidja da su makromolekularne (protein, DNK and RNK) interakcije rezonantne po prirodi i da su okarakterisane frekvencijom specifičnom za svaku interakciju. Nađene su dve posebne frekvencije: jedna koja karakteriše skraćivanje telomera i druga koja karakteriše produžavanje telomera. Ovaj rezultat otvara nove mogućnosti da se utiče na ćelijsko starenje na molekularnom nivou.

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