Regulation Of Gene Expression In Cells Exposed To Ionizing Radiation

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Abstract

Exposure to ionizing radiation induces a plethora of changes in the cell, including oxidative damage to most of the cellular macromolecules and changes in many processes on the transcript and protein levels. Microarray assays showed that few minutes after irradiation of human melanoma Me45cells, the levels of some transcripts change significantly suggesting that processes of stabilization or destabilization of mRNA may play a role in these early changes and nucleotide composition and the structure of the mRNA non-coding parts, which are known to interact with miRNA and proteins, may be of importance.

Using NucleoSeq, a new bioinformatic tool, we extracted from the EMBL database and analyzed the nucleotide sequences of over 12000 genes which showed different behavior after irradiation. The significant differences in mRNA nucleotide composition between genes showing greater than 10% down- and up-regulation 15 min after irradiation were observed and the differences disappeared stepwisely 12 - 24h after treatment. Down-regulated genes showed significantly shorter 3'- un-translated regions (UTRs) and higher content of regulatory sequence motifs such as Myf, STAT1, ZNF354 known to interact with human transcription factors.

1. Introduction

A set of all messenger RNA (mRNA) molecules, or "transcripts," produced in one or a population of cells in a process of transcription is called the transcriptome. The transcriptome reflects the genes that are being actively expressed at any given time. Unlike genome, transcriptome changes very dynamically since it depends on a response of the cell to various factors that can either enhance or inhibit transcription in a matter of minutes. mRNA stability is far more fragile to external conditions than DNA. Genotoxic substances or factors such as ionizing radiation can damage the transcripts or shorten their lifetime which in mammals lasts from few minutes to few days.

Post transcriptional expression regulation by mRNA stability control has a very significant role in the processes that take place in a single cell. Since recently it was believed that post transcriptional regulation has a passive role in signaling pathways but now it is estimated that mRNA stability, controls the translation efficiency of 5-10% of human genes. [1]

One of the controlling factors are the RNA Binding Proteins (RBP), which by binding to the transcripts can either increase its degradation speed or protect it from external factors like the high temperature or irradiation, therefore they can play both a stabilizing or destabilizing role.

Most of the RBP bind to the non-coding mRNA sequence fragments like the 3' or 5' untranslated regions (3'/5'-UTR), immediately after transcription controlling their fate since the beginning of existence [2, 3]. Sequences that can interact with proteins can also be located in coding fragments such as the c-Fos gene which is very fragile to stress conditions [4] .

Many binding sites for proteins, that may affect the mRNA's stability are described in the literature like the AU-rich elements (AREs), build mainly of adenine and uracil nucleotides (which can either stabilize or destabilize the mRNA depending on the protein bound to it) [5].

ARE can be found in the 3'-UTR of some unstable transcripts which by binding to the proteins become more or even less stable [5]. AREs are responsible for mRNA lifetime regulation, usually they shorten that time but trough the influence of various factors they can extend it. Also AREs can affect the translation process, for example TNF gene transcript has ARE areas that can regulate its stability by interacting with HuR, TTP or FRX1 proteins. It was shown that FRX1 binds to the 3'-UTR of the mRNA increasing translation of some genes in starved cells. However it can either work as a

translation repressor or activator depending on the environment conditions and nearby proteins [6]
Another example are the ARE sequence fragments present in the c-Fos gene that can lead to the decay of the mRNA by proteins which cause deadenylation or decapping. [7] c-Fos gene is regulated by two ARE regions [8] which are critical to its stability. Deletions in those fragments can increase its stability as unnaturally strong making it an oncogene which can lead to carcinogenesis [9]

AREs are not the only regions that are capable of binding proteins, for example a protein such as CP1 binds to the 3'-UTRs that are C-rich increasing the transcript stability. [10] There are also known proteins which interact with CNG repeats (where N = A, G, T, C) such as CUG-BP, ETR-3, CELF3 [11] or DM1 which binds with sequence motifs that are built of CUG repeats [12].

The stabilization mechanisms and interaction sites can be quite different. It was shown that due to low iron levels mRNA can be stabilized by proteins responsible for maintaining the iron level in cells like IRP-1 or IRP-2. [13]

The mRNA stability can also be controlled by microRNA transcripts which bind to specific sequence motifs present in the mRNA. MicroRNAs (miRNAs) are small ~22 nucleotides in length, non-coding, hairpin shaped, RNA molecules that inhibit gene expression post-transcriptionally. Since their discovery in 1993, many scientists have show that miRNAs are evolutionarily conserved and play important roles in a wide array of normal biological processes as well as disease processes in all cell types, discoveries which highly improved our knowledge of cancer biology.

Depending on the degree of complementary base pairing miRNAs can either degradate or inhibit translation of specific genes by binding to the 3'UTRs of mRNAs. In human cells complementarity between miRNA and mRNA leads to the inhibition of target gene protein translation and only very rarely causes degradation of the mRNA which mostly depends on the level of complimentary baise paring. [14] Perfect or near perfect complimentarity can result in the degradation of the mRNA, but as was shown partial base pairing can only lead to translational inhibition and the silencing of gene expression [15-17].

The overall amount of mRNA depends not only on the stability of mRNA which can be controlled by stress conditions and mRNA-protein or mRNA-miRNA interactions but also on the speed and efficiency of the transcription controlled by transcription factors.

Transcription factors are proteins that bind to specific DNA sequences (Fig. 1) and thereby control the transfer (transcription) of genetic information from DNA to mRNA. [18, 19] Transcription factors can perform this function alone or with other proteins in a complex, by promoting (as an activator), or blocking (as a repressor) the recruitment of RNA polymerase (the enzyme which performs the transcription of genetic information from DNA to RNA) to specific genes. [20-22]

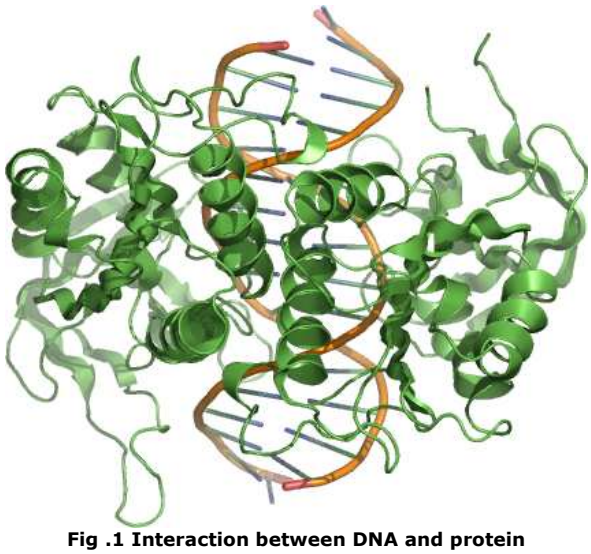

Fig .1 Interaction between DNA and protein (source: http://proteomics.bioengr.uic.edu/)

Transcription factors are critical to making sure that genes are expressed in the right place at the right time and in the right amount depending on the changing requirements of the organism. They are found in all living organisms and the number of them increases with genome size - larger genomes tend to have more transcription factors per gene. It is estimated that there are approximately 2600 different proteins in the human genome that contain DNA-binding domains [23] and approximately 10% of genes in the genome code for transcription factors allowing unique regulation of each gene in the human genome [24]

Transcription factors bind to either enhancer or promoter regions of DNA adjacent to the genes that they regulate. Depending on the transcription factor, the transcription of the adjacent gene is either up- or down-regulated which is done by the use of various regulation mechanisms. [25] These mechanisms include:

- stabilize or block the binding of RNA polymerase to DNA
- histone acetyltransferase/deacetylase activity – weakens/strengthens the association of DNA with histones which make the DNA more or less accessible to transcription and thereby

controlling the amount of transcribed mRNA's [26]

- recruit coactivator or corepressor proteins to the transcription factor DNA complex [27]

2. Aims of the study

The main goal of this work was to find all sequence motifs In the 3'-UTR of mRNA that could be responsible for its stability in stress conditions.

3. Materials and methods

Transcript levels in Me45 cells were measured with Affymetrix HGU133A microarrays in two cell groups in which one had contact with radiation at a dose of 4Gy and the other was used as a control to determine the change of signal level induced by the radiation. After the data was normalized by the RMA algorithm [28, 29] we extracted a set of 12357 genes based on the Affymetrix probesets signal, removing outlying probesets and unifying probesets referring to the same gene using the NucleoDix program [30]. 3'-UTRs of mRNA sequences of all genes were later extracted from Reference Sequence database using the NucleoSeq application which automatically downloads and processes information from the EMBL database by the use of internet. Sequences were later divided into 3 groups based on gene response to ionizing radiation

- up-regulation – increased rate of gene transcription by at least 10% (3552 genes)
- down-regulation – decreased rate of gene transcription by at least 10% (3359 genes)
- non-changed – gene transcription did not changed significantly (5446 genes)

The extracted sequences were later compared by the use of MEME application which performs a search for sequence motifs that occur frequently in a given pool of sequences. MEME uses the multiple sequence alignment (MSA) which detects conservative regions of the sequence that do not contain gaps by using the expectation maximization (EM) algorithm. [31] Motifs showing highest significant differences were compared with information from the Yaspar database [32] by using the STAMP program [33]

4. Results

Sequence length analysis

The analyzed sequences varied significantly in length between up and down-regulated genes. Mean 3'-UTR length of genes which expression level decreased due to irradiation is 888 nucleotides while of those which expression level increased reaches 1539. Mean length of other sequence fragments like the coding sequence or 5'-UTR did not changed significantly as shown in the table below:

Tab. 1

Average length of mRNA sequence and its fragments

mean length	up	null	down
Gene	3443,3	3195	2889,9
5'-UTR	224,4	214	201,5
CDS	1680,2	1778,7	1800,1
3'-UTR	1538,6	1201,8	888,5

Sequence motifs analysis

A motif is a sequence pattern that occurs repeatedly in a group of related protein or DNA sequences. Because regulatory elements such as proteins or miRNA don't just bind to one sequence but are capable of binding to closely related sequences we used MEME application that searches for occurrences of the same or closely related motif using statistical modeling techniques to automatically choose the best width, number of occurrences, and description for each motif. MEME represents motifs as position-dependent letter-probability matrices which describe the probability of each possible letter at each position in the pattern (Fig. 2). Individual MEME motifs do not contain gaps. Patterns with variable-length gaps are split into two or more separate motifs.

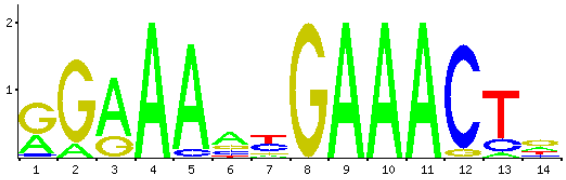

Fig. 2. STAT1 sequence motif nucleotide probability distribution

From all of the motifs found by the MEME application we chose those that showed the biggest, significant differences in occurrence between up and down-regulated genes. Table 2 shows sequence motifs with their mean occurrences per gene in up, down and non regulated genes. Significance level was determined based on the confidence interval for proportions test with alpha less than 0,05.

Tab. 2.

Mean amount of sequence motifs occurrences found by MEME application

	Motif	Mean amount of occurrences per gene		
		upreg	noreg	downreg
1	[AG][AG][AG][AG]AAA	2,21	1,76	1,29
2	T[TG]CT[TG]T[TG]	3,11	2,35	1,7
3	TTTTG[TC][TAC]	3,89	2,59	1,72
4	CACTG	2,19	3,6	4,86
5	CTGGG	1,93	2,76	3,1
6	[CG]CC[AT]G[GA][GC]	0,43	0,95	1,66
7	[CTG]C[TA]G[CG][TA][GC]	1,54	1,97	2,56
8	CCT[GT][GTC]C[CTA]	1,74	2,47	3,38
9	CTCC[AT]	2,2	3,23	4,03

Some of the motifs were found to dominate in the up-regulated genes (motifs 1-3) but most of

them occurred much more frequently in 3'-UTRs of genes which expression level decreased due to irradiation (motifs 4-9) although their sequences are on average almost two times shorter.

5. Discussion

In most cases, translational control mechanisms result from the interaction of RNA-binding proteins with 3'-UTRs of mRNA. In organisms ranging from viruses to humans, protein-mediated interactions between transcript is thought to significantly change the translational efficiency. [2, 3].

Although the regulation elements can be found in all parts of mRNA the 3'-UTR is the best place for them. This fragment is not scanned by ribosome's during the translation process making all the protein interactions possible which allows for control of the stability at any moment [34]

A multi-species analysis has shown that, in most vertebrates, 3'-UTRs are significantly longer than their 5' counterparts, indicating a significant potential for regulation. In addition, the average length of 3'-UTR sequences has increased during evolution, suggesting that their length might be related to organism complexity [35]

Detection of such mechanisms in the DNA/RNA sequence is one of the key problems of modern bioinformatics not only because of large amounts of data that needs to be processed but also because even data gathering can sometimes be very difficult. We used the possibilities of computer technology to compare the nucleotide composition of over 12 thousand mRNA transcripts and observed that the sequences not only vary significantly in length or ARE/miRNA binding sites but also other sequence motifs presented in Table 1.

We compared those motifs with information from the Yaspar database [32] by using the STAMP program [33] and found that they are highly similar to transcription factors binding sites found in various species. Motifs number 7,8 and 9 are human transcription factors known as Myf, STAT1 and ZNF354. [36-38] Such sequences are usually present in either enhancer or promoter regions of DNA near the 5' end of the gene that they regulate since that's the place of transcription initiation where polymerase is being attached. There is no information in the literature about them being present in 3'-UTRs of the mRNA.

Chemically, transcription factors interact with their binding sites on the DNA by using a combination of electrostatic and Van der Waals forces. Because of that, most transcription factors bind only to specific sequence fragments. However, not all bases in the transcription factor binding site may actually interact with the transcription factor, making some of the interactions weaker than others.

One of the most important properties of transcription factors is that they don't just bind to one sequence but are capable of binding to closely related sequences, each with a different strength of interaction. One of the examples is the TATA binding protein (TBP) with TATAAAA binding site. It was proven that the TBP transcription factor can also bind similar sequences such as TATATAT or TATATAA.

Because they can bind a set of related sequences and these sequences tend to be short we can expect that potential binding sites can occur by chance since the DNA length can even exceed 3 million bases. It is unlikely, that they can occur by chance in unwanted places, however if that happens other constraints, such as DNA accessibility in the cell or availability of cofactors may also help dictate where a transcription factor will actually bind. This makes the process of binding sites prediction very difficult if the only source of data is the genome sequence.

The most important roles of transcription factors recognized to date are:

- changes in the cell morphology or activities needed for cell fate determination and cellular differentiation
- response to environment - examples include heat shock factor (HSF) which up-regulates genes necessary for survival at higher temperatures, [39] or hypoxia inducible factor (HIF) which up-regulates genes necessary for cell survival in low oxygen environments [40]
- cell cycle control – helps to regulate the cell cycle and as such determine how large a cell will get and when it can divide into two daughter cells. [41, 42] One of the examples is the Myc oncogene, which has important roles in cell growth and apoptosis. [43]
- response to intercellular signals - Cells can communicate with each other by releasing molecules that produce signaling cascades within another receptive cell. If the signal requires up-regulation or down-regulation of genes in the recipient cell, often transcription factors will be downstream in the signaling cascade. [44]
- regulation of other processes - transcription factors not only can control the rates of transcription to regulate the amounts of mRNA available to the cell, but transcription factors themselves are regulated mostly by other transcription factors. For example, in a negative feedback loop, the transcription factor acts as its own repressor: if the transcription factor protein binds the DNA of its own gene, it will down-regulate the production of itself. This is one of the mechanisms that can maintain low levels of a transcription factor in a cell.

The presence of transcription factor binding sites in the 3'-UTRs of the mRNA is surprising and incomprehensible. The question is whether they could be there by chance or they can be a leftover of some kind of other mechanism which changed its role due to evolution among different species. It is also plausible that their presence could be related with transcription factors role in changing the chromatin conformation making the DNA more accessible to transcription.

Next question needed to be answered is why there are so big differences in occurrence of Myf, STAT1 and ZNF354 motifs. In the 3'-UTR's of genes which expression significantly increased due to irradiation we found on average 1-2 motifs per mRNA while in genes which expression decreased, over two times more. If in fact they help to change the chromatin conformation mostly in down-regulated genes then it could mean that this mechanism is blocked by the radiation.

Processes based on DNA/mRNA-protein interactions make a network of highly complicated relations. They can have both positive or negative effect on the entire cell leading to cell cycle distortions which can cause very dangerous genetic diseases like Retts disorder or fragile chromosome X syndrome [1]

Unnatural changes in stability of some transcripts which for example take part in cell proliferation can be very dangerous for the entire organism causing them to become one of the most common reasons for cancer.

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