Revealing the translation control by transcriptome analysis

Revelando o controle da tradução por análise de transcriptoma

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ABSTRACT
Taking the eukaryotic translation initiation factor 5A (eIF5A) as model, some aspects of different points of gene expression control and transcriptome studies are discussed. A parallel with proteomic studies is presented, as well as the optimization of the transcriptome analysis using polysome profile assay. The polysome profile assay application reveals the translational control of mRNAs not identified by traditional differential analysis of transcriptomes, which is widely employed to study diseases, such as tumors.

Keywords: Polysome profile; Transcriptome; Translational control; Post-transcriptional; eIF5A protein; eIF-5A; Proteome

RESUMO
Utilizando o fator de início de tradução de eucariotos 5A (eIF5A) como modelo, alguns aspectos dos diferentes pontos de controle da expressão gênica e estudo de transcriptomas são discutidos. Um paralelo da aplicação de estudos proteômicos é abordado e a otimização da análise de transcriptomas utilizando o ensaio de perfil polissomal é evidenciada. O uso do ensaio de perfil polissomal revela o controle traducional de mRNAs não identificados pela análise diferencial tradicional de transcriptomas amplamente empregada no estudo de doenças como tumores.

Descritores: Perfil polissomal; Transcriptome; Controle traducional; Pós-transcricional; Proteína eIF5A; eIF-5A; Proteoma

THE EUKARYOTIC TRANSLATION INITIATION FACTOR 5A
The eukaryotic translation initiation factor 5A (eIF5A) is the only protein known to contain the amino acid residue hypusine, produced by a post-translational modification called hypusination1 (figure 1). This modification is initiated by the transfer of a 4-aminobutyl moiety from polyamine spermidine to an ε-amino group of a specific lysine residue of the eIF5A precursor; a reaction catalyzed by deoxyhypusine synthase (DHS). Afterwards, the hypusine residue is formed by hydroxylation of the deoxyhypusine residue; a reaction catalyzed by deoxyhypusine hydroxylase (DHH). The protein eIF5A is highly conserved in archaebacteria and eukaryotic cells1. Some studies revealed a strong association of eIF5A with cell proliferation. In fact, any interference in hypusination process promotes cell proliferation inhibition2. However, the precise function of eIF5A is not understood but it has been proposed to be associated with mRNA metabolism.

eIF5A AND THE mRNAs METABOLISM
The metabolism of mRNAs is an important target to control gene expression. This control can be carry out in several steps, such as mRNA synthesis, post-transcriptional processing, nuclear pore transport, recruitment to translation and degradation (figure 2). The comparative transcriptome analysis reveals differential levels of mRNAs controlled by its metabolism.
The regulation of transcriptional activity is a key step for the control of gene expression. An essential protein factor for transcriptional activation is the protein TBP (TATA-binding protein) that acts in the recruitment of RNA polymerase to gene promoter regions. A genetic interaction, not yet elucidated, between eIF5A and TBP was established. The overexpression of eIF5A was able to suppress the mutant phenotype of a yeast carrying a temperature-sensitive allele of TBP\(^3\). As TBP action is not restricted to a specific gene, or set of genes, there are various transcriptional factors that act in the transcriptional regulation. For instance, unstimulated T lymphocytes present a low rate of eIF5A gene transcriptional activity. However, stimulation by specific mediators leads to an increase in the rate of eIF5A transcription\(^4\).

In this context, the differential transcriptome analysis in relation to coding mRNAs of protein isoforms has to be carefully interpreted, because some systems are not able to make a distinction due to their high identity. For instance, an eIF5A isoform (eIF5A-2), coded by a different gene, is highly expressed in testicles and colorectal adenocarcinoma\(^5\). Similarly, the differential analysis of transcripts derived from alternative splicing could be compromised. In such cases, a system based on specific oligonucleotides should be used.

Another point of gene expression control consists in the regulation of nuclear export of mRNAs through the nuclear pore complex. A connection of eIF5A in this process was reported in dendritic cells by inefficient export of CD83 mRNAs after inhibition of hypusination\(^6\). This is an important effect because it impairs the availability of CD38 mRNAs to translation. There is also the hypothesis of a secondary effect, since eIF5A is produced by archaeabacteria that are devoid of nucleus.

The translation process is also an important and complex issue controlled by several translation factors. There is evidence for the participation of eIF5A in this control. A rapid depletion of eIF5A caused a reduction of 30% in protein synthesis\(^7\). eIF5A interacts physically with translating 80S ribosomal complex, confirming the participation of this factor in the translation process\(^8\).

The enzymatic control of mRNA degradation also interferes in the transcriptome analysis. A mutation in eIF5A caused the stabilization of different mRNAs\(^9\). This stabilization was observed by accumulation of mRNAs without cap, suggesting the involvement of eIF5A in the process of mRNAs decay after cap removal.

**TRANSCRIPTOME ANALYSIS LIMITATIONS**

The transcriptome analysis corresponds to an extrapolative way to evaluate the global protein profile. In several conditions, the model of study consists in a comparative analysis among distinct systems. During this analysis some genes are differentially expressed. For example, if this analysis were performed using a model of lymphocyte stimulation, some transcripts would be in higher quantities in stimulated lymphocytes as compared to non-stimulated ones. Among these transcripts, it should be expected to find the mRNA of eIF5A.

An aspect that greatly limits transcriptome studies is an inaccurate relation between the level of a specific mRNA and its corresponding protein. In relation to the example mentioned above, an appropriate relation between the increase of eIF5A mRNA level and its protein in stimulated lymphocytes was found\(^4\). However, this relation is not found in some transcripts, because the mRNAs can be differentially recruited to translation process. As mentioned above, the production of a mutant eIF5A in yeast causes stabilization of different mRNAs. Therefore, the same effect could be observed in a study based on transcriptome analysis, leading to incorrect interpretation that the corresponding proteins are also increased. A more detailed analysis is required because the stabilized mRNAs are uncapped, causing a possible inhibition of the corresponding proteins synthesis by impairment of the cap dependent translation mechanism. Hence, the analysis of each transcript requires identification and quantification of...
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the protein of interest to validate the relationship between the levels of mRNA and its corresponding protein. Depending on the study, there is no correlation with the mRNA increase in function of time required for protein synthesis. Likewise, this limitation can affect proteomic analysis.

A transcriptome comparison between two systems can also reveal a decrease in the level of a specific transcript. Thus, an analysis of the protein of interest is required; and it is even more important when the model of study demands an early evaluation, because a non-correlation between mRNA decrease and its corresponding protein could be due to a high protein half-life. In this case, the limitation also extends to the proteomic analysis.

Another important aspect comprises mRNAs that do not present altered levels by differential transcriptome analysis. Usually, these transcripts are totally ignored. However, the translation of some mRNAs can be activated regardless the correspondent mRNA level. Examples of this kind of control are represented by mRNAs with translation mediated by IRES (internal ribosome entry site). The IRES mechanism can be stimulated when the cap dependent translation is inhibited\(^\text{(10)}\). Another similar possibility consists in translation of mRNAs regulated by uORFs (upstream open reading frames)\(^\text{(11)}\).

**OPTIMIZATION OF TRANSCRIPTOME ANALYSIS BY POLYSOME PROFILE ASSAY**

The establishment of the polysome profile assay may substantially optimize studies based on transcriptome analysis, since all limitations described could be avoided.

The polysome profile assay allows the fractionation of free mRNAs, ribosomal subunits (40S and 60S), ribosomes (80S) and polysomes. Initially, a total extract from a sample is submitted to sucrose gradient upon ultracentrifugation. Afterwards, the gradient is continuously collected, analyzed in an ultraviolet reader and then fractioned. The profile obtained (figure 3) may reveal important aspects about translational status of the system studied. This method allows separating free mRNAs population (non-recruited to translation) from mRNAs actively engaged to translation. Thus, both populations are discriminated. Using this fractionation, translation recruitment for each transcript is revealed; hence, the differential analyzes of mRNAs between two systems can be performed with none of the above mentioned limitations. Moreover, the differential analyzes of mRNAs on active translation also overcome the limitation observed on differential proteomic analyzes already mentioned.

**POLYSOME PROFILE ASSAY FOLLOWED BY TRANSCRIPTOME ANALYSIS TO STUDY eIF5A**

Concerning eIF5A, the application of polysome profile assay was carried out to detect differential translating mRNAs in response to hypusination inhibition of tumor cells in culture using the compound mimosine\(^\text{(12)}\). In this study, the mRNAs of methionine adenosyltransferase (MAT) and cytochrome-\(c\) oxidase-I (COX-1) were identified as putative transcripts with translation regulated by eIF5A. However, this observation must be carefully interpreted, because mimosine interferes with other cell processes, such as deoxyribonucleotide metabolism\(^\text{(13)}\). In this context, interesting results could be obtained by transcriptome analysis, using the polysome profile assay, of tumor cells treated with the specific and potent hypusination inhibitor N1-guanyl-1,7-diaminoheptane (GC7)\(^\text{(14)}\). Another alternative could be based on strategies that would cause a rapid inhibition of eIF5A, such as overexpressing a nonfunctional eIF5A able to act as a negative dominant allele. This strategy was applied to inhibit HIV-1 replication in lymphocytes\(^\text{(15)}\). Due to the pronounced correlation of eIF5A and cell proliferation, the results could reveal important factors involved in tumor establishment and progression.

**CONCLUSION**

The transcriptome analysis has been widely used for molecular study of several diseases. Actually, it is utilized in differential analysis of cells, tissues and organs to compare different disease parameters, such as normality, progression, aggressivity or drug responsiveness. During these studies, its common despises
the non-differential mRNAs. However, in some cases there is no correlation among the levels of mRNAs and their corresponding proteins, because some transcripts can be under specific translation control. Relevant data were possibly lost during these works. Hence, the incorporation of polysome profile assay to transcriptome studies can efficiently overcome their limitations, enabling important disease factors to be revealed.

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REFERENCES