Synthetic mRNAs

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The structure of synthetic mRNAs as used in vaccination against cancer and infectious diseases contain specifically designed caps followed by sequences of the 5′ untranslated repeats of β-globin gene. The strategy for successful design of synthetic mRNAs by chemically modifying their caps aims to increase resistance to the enzymatic deccapping complex, offer a higher affinity for binding to the eukaryotic translation initiation factor 4E (eIF4E) protein and enforce increased translation of their encoded proteins. However, the cellular homeostasis is finely balanced and obeys to specific laws of thermodynamics conferring balance between complexity and growth rate in evolution. An overwhelming and forced translation even under alarming conditions of the cell during a concurrent viral infection, or when molecular pathways are trying to circumvent precursor events that lead to autoimmunity and cancer, may cause the recipient cells to ignore their differential sensitivities which are essential for keeping normal conditions. The eIF4E which is a powerful RNA regulon and a potent oncogene governing cell cycle progression and proliferation at a post-transcriptional level, may then be a great contributor to disease development.

This Fact Sheet underscores the basic elements from within the official text of publication to highlight the hallmarks of disease progression due to synthetic mRNAs stability structures (analogue caps, 5’ untranlslated repeats of β-globin gene and poly A tails) fundamentally used in design of all synthetic mRNAs to promote the efficiency of translation of their encoded sequences by the human cell and therefore the organism.

Specific bullet points in bold mean for urgency of further toxicity evaluation studies that need to be overtaken in order to ensure for safety of mRNAs in vaccines at current stages of development.

Keywords: synthetic mRNA ; analogue caps ; eIF4E ; mTORC1 ; autophagy: immunity deregulation ; maturation defects ; autoimmunity ; cancer

1. Synthetic mRNAs

In addition to the stabilization of mRNA translation, the multiple mRNA methylations of mRNA caps have evolved as a part of the innate immune defense system [1]. The cap structures and capping processes become more and more specific for cells of different higher eukaryotic species and provide the first alarm for distinction of “self to non self” mRNA recognition under circumstances of cellular evasion of foreign genetic material as in the case of a viral infection, thereby offering essential signaling for interferon responses to recognize and encounter the viral infections [2].

The general principle on the progress of devising caps for synthetic mRNAs focus on the chemical modifications of the caps that will confer, when added to the synthetic mRNAs, (a) a decrease of the susceptibility to degradation of the synthetic mRNA by the decapping complex and (b) an increase to the binding efficiency of the capped synthetic mRNA to the eIF4E. Furthermore, in order to bypass the cellular translation restriction of 2′O methylation and therefore the rejection of synthetic mRNAs as “non self” mRNA, a 2’-O-methyltransferase capping enzyme is used to methylate ARCA caps of synthetic mRNAs and denote the “self” Cap 1 structure to be recognized adequately by the recipient cellular translation machinery [3]. This may mean however, that even under problematic conditions of homeostatic imbalance [4][1][5] seen during a concurrent viral infection [5], the recipient cells will be forced to translate the synthetic mRNAs due to the capping they possess, even if their requirements for keeping cellular homeostasis are different. Notably, in the natural processes, any cap modification has its own physiologic consequences, and these arise due to the influence of the affinity of the cap to various cofactors involved in the specific translator machinery of gene regulation [6].

2. Synthetic mRNAs and eIF4E

In respect to the protein translation efficiency, the overwhelming research data from investigations of mRNA translation processes that cells follow during normal conditions and conditions of oncogenesis, reveal the presence of differential sensitivities of caps and the eIF4E binding protein that further dictate the differential expression of numerous sets of

genes.

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The messenger RNAs by genes required for normal cellular functions like the glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the *β*-actin, are less sensitive to eIF4E activity as compared to mRNAs from oncogenesis genes involved in cell growth proliferation and immune responses such as the C-MYC, the Bcl-2, the vascular endothelial growth factor (VEGF), the cyclins and others [7].

Furthermore, the methylation of caps is also important for disease onset [8][4]. In a gene-specific manner proto-oncogenes like c-myc induce the 5 ‘guanosine cap methylation in order to promote cellular proliferation [9]. By this way, the target genes of c-myc (*eIF4E*, *eIF4A1, eIF2B1* and others) are induced in their expression constituting a cascade of auto-induction between methylation of caps and expression of target growth factor genes. Once bound consistently, the methylated caps on eIF4F complex are attractants to signaling proteins as the ribosomal signaling scaffold proteins and like the receptor for activated C Kinase 1 (RACK1), that thereafter, promote the translation of other short mRNAs and its activity is increasing in medical importance [10].

The eIF4E protein has independent roles in the nuclear export of mRNA and its cytoplasmic translation and nevertheless in most cases if not all the eIF4E protein expression is a powerful regulon and in most conditions is regarded as a potent oncogene governing cell cycle progression and proliferation at a post-transcriptional level [11]. The unregulated binding affinity of eIF4E has profound physiological consequences that can lead to a disease.

The increased binding affinity of caps used in synthetic mRNAs [12][13][14][3], and thereafter, the increase in translation efficiency of synthetic mRNAs of vaccines means that the eIF4E is rendered to become more readily bound to the eIF4F complex for a prolonged time than normal and also that may avoid forming complex with the 4E binding proteins (4EBPs). Since the synthetic mRNAs with their analogue caps are specially designed to increase overwhelmingly the translation of their encoded sequences [12][14] this will also prolong the existence of their attractive 5′ UTR structures to other endogenous mRNAs that contribute to gene regulation [15].

In this sense, mRNAs that are involved in growth, proliferation, transformation and differentiation of cells will be preferentially stimulated during an induced increased cap dependent protein translation activity by the readily bound analogue caps. In this respect, although the determination of increased translation by analogue caps modification has been performed in highly proliferative malignant cell lines [13][14], the chemically modified cap methylation of mRNAs is shown to elevate pro-oncogene expression, and vice versa, the cap methylation is being promoted in malignancy [16].

The over expression and phosphorylation of 4EBP-1and 4EBP-2 is encountered in many cancers [17] and systemic autoimmunity conditions [18]. As the mTOR kinase is also a major coordinator of the T helper cells differentiation and regulates their cellular fate decisions, the loss of mTOR control and specifically the loss of the mTORC1 dependent pathways can lead to the disorganization of protein synthesis and T cell dysfunction predisposing to immune irregularities [18].

”Recent experimental evidence supports the notion of p-eIF4E dependent translation in MYC and ATF 4 drives oncogenic initiation progression and aggressiveness of cancer in a rate-limited manner [19]. Latest scientific evidence [20], suggests that the p-eIF4E maintains the 4EBP-eIF4E binding [21] (Figure 1), and by using the most ancient arm of integrated stress response (ISR) in mammals and the general control of nonderepressible 2 (GCN2), the p-EIF4E maintains the AKT/4EBP-1 signaling and the stress response by ATF4 transcription factor. In this process, the mTORC1 activity is maintained to phosphorylate 4EBP-1 and thus the anti-oncogenic potential of mTOR silencing is inactivated [22]. In this respect, any excess of cellular mRNA translation, as is in the case of overloaded synthetic capped mRNAs in vaccines, and especially when present in initiative and progressive conditions of autoimmunity [18] and oncogenesis [23][19], the surplus drive of the p-eIF4E-cap-dependant translation is far more than desirable for the maintenance of cellular homeostasis”.

It must be re-emphasized that synthetic mRNAs are designed to have solid capping structures and UTRs to ensure for efficient and long-lasting translation [1][24][5][12][13][14]. Thus, an elevated cap-dependent protein translation under circumstances of PI3K/AKT pathway activation which causes *MYC* and *ELF4E* genome amplifications [25], and active translation of synthetic m RNA vaccine bearing caps with increased affinity to eIF4E

than normal [1][2][24][5][12][13][3], may drive even more the potential of cells for autoimmunity and oncogenesis. This is especially more important as the mammalian species have evolved mechanisms of eIF4E surplus in the cell for their normal development [22].

For example, the translation of ferritin heavy chain 1 (Fth1) is highly sensitive to eIF4E expression levels in a dose dependent manner [22]. Moreover, the synthetic mRNAs are modified structurally to carry the 5′ untranslated repeats (URTs) of β-globin gene in order to confer translational efficiency and stability [24][5][12][3][26]. The above-normal limits regular presence of 5′ UTRs and the overwhelming attractiveness by the long-lasting presence of p-eIF4E through the regular binding of iron-responsive element binding protein/iron regulatory protein (IRE/IRP) regulatory network, is said to drive the activation of nuclear erythroid-derived 2-like 2 (NRF2) transcription factor targeting the gene of FTH1 [27][28][29][30]. According to specific cellular vulnerabilities [27], **this causes a disturbance in the cellular iron availability and in the regulation of ferroptosis of the cells. As the analogue caps of the synthetic mRNAs are designed to increase binding to eIF4E and the translation cycles are increased considerably [12][13][14][3], this can make accessible the 5′ UTRs for longer than normal and predispose to activation of NRF-2.**

3. Synthtic mRNAs eIF4E and mTOR

“The dysfunction and the deregulated signaling of mTORs are implicated in metabolic, neurodegenerative, and inflammatory disorders and malignancy [31][32]. In general, the mTOR deregulation is strongly associated with tumorigenesis. As mTOR inhibits autophagy under normal cellular conditions, its deregulation increases cell proliferation instead of driving cells to normal death”.

“In most senses, when the molecular breaks of mTOR activity are deregulated, the orchestra of the immune system contributors is deregulated too, leading the immune cells to become prone in developing reactions that may lead to inflammation, autoimmunity, and tumorigenesis”

The mTORC1 may also activate complementary mechanisms without having an association with protein synthesis. Nevertheless, the autophagy activation linked to mTORC1 inhibition, may contribute to some of the effects of cellular extension of life span. Notably, as the mTORC1-4E-BP1 axis manly inhibits the eIF4E to proceed with mRNA translation, defaults in fine balances between mTOR and eIF4E action can constitute a premature step of oncogenesis and ignite pre-causal mechanisms that can lead to stem cell related disorders and aging defects [33][34][35].

Therefore, the dependence to the eIF4E and further to the whole of el4F complex and is constituents becomes important for disease onset. Natural capping of mRNAs has substantial differences in complexity and methylations as compared to the analogue caps synthesized to increase bounding to the eIF4E, promote translation, and decrease natural chances of decapping processes within the cell. This will prolong the existence of analogue caps in translation machinery of cells longer than normal.

The analogue caps of the synthetic mRNAs used for vaccination against cancer, genetic disorder therapy and nowadays as emerging for infectious diseases are optimized to stabilize and increase the translation of the encoded proteins in mRNAs and this is done to provide an efficient immunization. **In this respect, attention should be made on studies that have shown that the enthalpic increase and entropic change between synthetic cap interactions with mammalian eIF4E as well as with the eIF4E of lower eukaryotic species may be in contrast to the elevation of complexity of living organisms in terms of growth rate requirements and compatibility with health (and life).**

As the internal variables of a living organism are trying to keep its internal state unchangeable (homeostasis), and the reactions between analogue caps and eIF4E are thermodynamically not favorable [36][37][38][39], this has to be analyzed by further superior physical chemistry, biochemistry and explicit toxicity evaluation research. Particularly **during sensitive circumstances, as during deregulation of fine balance of cellular homeostasis (conditions of eIF4E and mTORC1 deregulation) and as of this consequence, due to the autophagy deregulation**, this is said to cause immune dysfunction irregularities, cellular maturation incompatibilities and predispose to various autoimmune disorders and malignancies.

Foremost attention must be made to the potentiality of the loss of cap regulating innate defense of cells. **By the alteration of regulation of cap methylation, this sets the organism susceptible to viral and bacterial infections as well as other diseases.**

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