Biochimie 92 (2010) 919–926

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

The role of G-quadruplex in RNA metabolism: Involvement of FMRP and FMR2P

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ARTICLE INFO

Article history:
Received 29 March 2010
Accepted 28 May 2010
Available online 4 June 2010

Keywords:
G-quadruplex
RNA metabolism
RNA-binding proteins
FMRP
FMR2P

ABSTRACT

Regulation of post-transcriptional gene expression is a cellular process that is accomplished through the activity of multiple mRNP (messenger RiboNucleoProtein) complexes which are composed of mRNA-binding proteins and RNA molecules interacting with those proteins. The specificity of these interactions is mediated by the ability of the RNA-binding proteins to precisely recognize and bind RNA sequences or structures. Alterations of their function may have some dramatic consequences, resulting in different pathologies. An increasing body of data is emerging showing the impact of a G-quadruplex forming structure in the maturation and expression of some RNA molecules. We review here the role of the G-quadruplex RNA structure in the regulation of translation and splicing, when it interacts with two RNA-binding proteins: FMRP (Fragile X Mental Retardation Protein) and FMR2P (Fragile X Mental Retardation 2 protein). Impaired expression of these proteins causes two forms of intellectual disability: the Fragile X Mental Retardation syndrome (FXS) and the FRAXE-associated mental retardation (FRAXE), respectively. FMRP is involved in different steps of RNA metabolism and, in particular, in translational regulation. FMR2P has been initially described as a transcription factor and we recently showed also its role in regulation of alternative splicing. By the study of the functional significance of the interaction of both FMRP and FMR2P with a G-quadruplex forming RNA we were able to show an impact of this structure in translational regulation and also in splicing, behaving as an Exonic Splicing Enhancer.

1. Introduction

Post-transcriptional gene expression is a complex process controlling the spatio-temporal localization, translation and half-life of mRNAs. To this purpose, mRNAs are escorted by RNA-binding proteins from the beginning until the end of their life, generating complexes composed by messenger RNA (mRNA) molecules directly or indirectly interacting with proteins and named messenger RiboNucleoProteins (mRNPs). These complexes are the actors of mRNA metabolism involving tissue-specific RNA processing, transport, translational regulation and degradation, directly contributing to the development and the function of cells. Indeed, spliceosomes, ribosomes, RNA-induced silencing complex (RISC), transport particles and RNA granules constitute examples of mRNPs [1].

The composition and the function of these complexes are guaranteed by the specific interaction of their components. RNA-binding proteins interact with specific RNA sequences/structures through their RNA-binding domains (RBD). In humans, more than 40 different RBDs are found, providing regulation of multiple pathways. The ability of the RNA-binding proteins to recognize and bind RNA molecules with high affinity is a critical step for the formation and the functioning of mRNPs [2]. Indeed, mutations in RNA-binding proteins have pathological consequences causing diseases with a high incidence (e.g. Fragile X syndrome and Spinal Muscular Atrophy) [3].

RNA sequences are recognized depending on their primary or secondary structure and, often, the information for post-transcriptional regulation is localized in untranslated regions of mRNAs. For example, RNA regulatory sequences located in the 5′UTR are likely to have a role in translational regulation, while RNA transport seems mostly regulated by sequences in the 3′UTR of mRNAs, even if several exceptions are known [4].

In our laboratory we study the molecular pathology of two forms of intellectual disability (ID), Fragile X Syndrome and FRAXE-associated mental retardation, that are caused by the silencing of the *Fragile X Mental Retardation 1* gene (*FMR1*) and *Fragile X Mental Retardation 2* gene (*FMR2*), respectively [5]. Neurons are highly specialized cells where some specific aspects of RNA metabolism (e.g. RNA transport and localized translation) play a critical role for their function. Indeed, during development, trafficking of mRNAs to both axonal and dendritic growth cones regulates neuronal growth. After synapse formation, mRNAs continue to be transported to

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doi:10.1016/j.biochi.2010.05.018

dendrites and axons where they are locally translated at the synapse. This process is at the molecular basis of synaptic plasticity and underlies learning and memory [4]. Deregulation of this process has a heavy impact on neural function. Therefore it is not surprising that mutations in some RNA-binding proteins cause neurological disorders [3]. Concerning ID, mutations of components of mRNPs other than FMRP and FMR2P are associated with these pathologies. UPF3B is a member of the nonsense-mediated mRNA decay complex and its function probably concerns the expression and degradation of a subset of synaptic mRNAs. UPF3B mutants cause syndromic and non-syndromic mental retardation [5]. Polyglutamine tract-binding protein 1 (PQBP1) is mutated in another X-linked ID, the Renpenning syndrome. PQBP1 is a component of RNA granules and when mutated deregulates pre-mRNA splicing, suggesting also for this protein a multi functional activity in RNA metabolism [6–8]. Furthermore, deletions of members of the VCX family are associated with intellectual impairment [9,10]. In particular VCX-A, an RNA-binding protein involved in formation of neurites, has the capacity to modulate translation and stability of a subset of neuronal mRNAs [11].

A strong link exists between FMRP and FMR2P since they are both able to bind the G-quadruplex RNA forming structure [12,13]. G-quadruplex DNA was extensively studied during the last few years and its importance in critical biological processes was shown. In particular, its implication in the regulation of telomere end structure and its localization in the promoters of some oncogenes linked it to dysfunctions associated with cancerous cell transformation [14]. G-quadruplex RNA is an emerging regulatory sequence and because of its large diffusion and its presence in different regions of mRNAs (coding and non coding) it is supposed to be involved in different steps of RNA metabolism [15,16]. The RNA G-quadruplex is a structure organized in stacks of planar layers of guanine tetrad units. The guanines of each layer interact two by two in a cyclic Hoogsteen hydrogen bonding arrangement and each of them plays a role of acceptor and donor of hydrogen bonds. The final structure is stabilized by the presence of a monovalent cation lying between or within the plane of the tetrads and acting as a “coordinator” of the structure. For this reason, the G-quadruplex 3D structure is sensitive to different types of cations, being preferentially stabilized by $K+$ over $Li+$ or $Na+$. These structures could be intermolecular or intramolecular. The G-quadruplex is supposed to fold in the 3 possible topologies that can be antiparallel, parallel and mixed (see [17,18] for review). However due to the strong propensity of the riboguanosine for the *anti* conformation of the glycosidic bond, RNA forms only the all-parallel G-quadruplex structure (Fig. 1a). Furthermore, G-quadruplex has a higher stability in RNA that in DNA, and this is due to the different properties of the two nucleic acids, rather than to different topologies of folding. Indeed, the difference in stability seems to be due to the presence of the 2′-OH group [16–19].

To try to understand the functional role of the G-quadruplex structure in RNA metabolism, we review here the results that we and others obtained studying the functional interactions of G-quadruplex RNA with FMRP and FMR2P. Both diseases result from the absence of a protein binding G-quadruplex RNA. This particular (and rare) situation gives the possibility to study the impact of the G-quadruplex on the life of several mRNAs in the presence and in the absence of one of its binding proteins.

2. Fragile X mental retardation protein and the G-quadruplex

The absence of FMRP causes the Fragile X syndrome, the most frequent cause of inherited ID, also characterized by abnormal behavior and facial dysmorphy. FMRP is an RNA-binding protein and its functional characterization during the last few years has

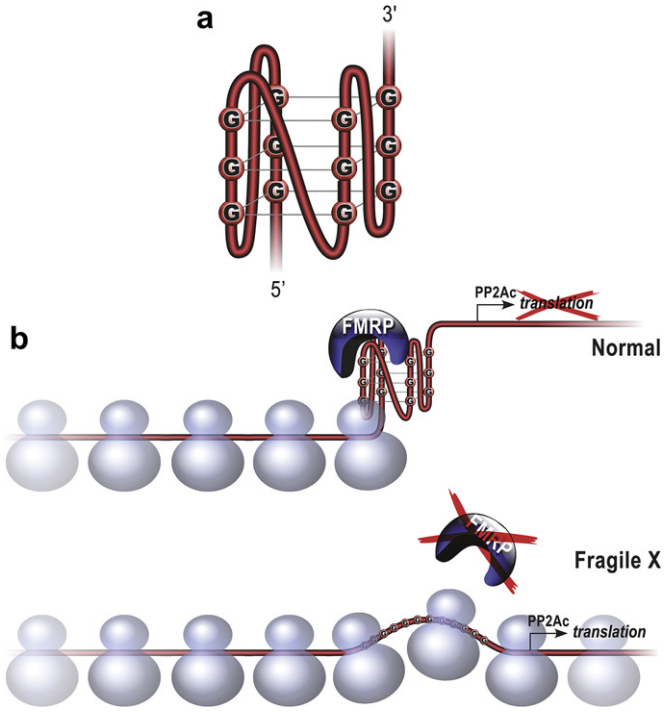

Fig. 1. a) Structure of the intramolecular G-quadruplex RNA. A schematic representation of the only possible fold of an RNA G-quadruplex: the all-parallel. b) Mechanism of translational repression via the FMRP/G-quadruplex interaction. FMRP stabilizes the G-quadruplex RNA forming structure. When localized in the 5′UTR of PP2Ac mRNA, it blocks polyribosome scanning through the coding region. In the absence of FMRP, the G-quadruplex is not stabilized and translation of PP2Ac mRNA is facilitated.

shown the specific dynamics of this protein in different sub-cellular compartments in neurons and in other cell types [20]. FMRP enters the nucleus and interacts with pre-messenger ribonucleoprotein (pre-mRNP) complexes to escort them to the cytoplasm. In the cytoplasm FMRP bound to mRNAs (FMRP–mRNPs) can then follow different fates by being targeted to different cellular compartments: 1) in normal conditions, FMRP–mRNPs are massively associated to polyribosomes and subjected to translational control [21]; 2) in situations of cellular stress, FMRP–mRNPs are directed to stress granule compartments playing an active role in translational regulation [22]; 3) more specifically in neurons, some of the FMRP–mRNP complexes are selectively translocated to distant locations (dendritic spines) within the neuronal RNA granules together with other RNA-binding proteins and ribosomes [20,23]. FMRP also seems to play a specific role being an adaptor molecule between RNA and kinesin, the molecular motor of the granules [24]. Indeed, RNA granules are transported along dendrites, by ‘sliding’ on microtubule structures to the spines [25], where repressed mRNA translation is locally reactivated under specific stimuli [26] (e.g. the stimulation of the mGluR1 pathway). The ability of FMRP to bind RNA relies on 3 RBDs: two KH domains and one RGG box [20] (Fig. 2a, b). These domains are already well characterized to mediate RNA/protein interaction. The KH1 3D structure was already defined several years ago but to date no specific RNA targets are known to be bound by this domain in FMRP [27]. The structure of the FMRP KH2 domain has not yet been solved but it was reported that it binds a synthetic aptamer (« kissing complex ») harboring a sequence-specific element within a tertiary structure stabilized by Mg^{++}. The FMRP/RNA kissing complex was observed to dissociate from polyribosomes preventing the translation of the bound mRNA [28]. To date, no natural RNAs have been shown to harbor a kissing complex structure,