TEST Engineering & Management

July – August 2020
ISSN: 0193-4120 Page No. 140- 144

mRNALocalisation and Local Translation in the Nervous System

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Article Info
Volume 83
Page Number: 140- 144
Publication Issue:
July - August 2020

Article History
Article Received*: 06 June 2020*
Revised*: 29 June 2020*
Accepted*: 14 July 2020*
Publication*: 25 July 2020*

Abstract

Locally synthesised proteins from mRNAs are essential and an efficient way for neuronal growth and repair. Researches have investigated how mRNAs are co-transcriptionally spliced, packed, and transported along the axon or dendrites and anchored to the active site in proximity of the cell membrane. Motor proteins can be used to transport mRNA. Regulation of the localisation process involves mRNA sequences, mRNA binding proteins and external cues. Diseases can be caused by irregular mRNA localisation (mis-localisation) such as dysfunctional mRNA binding protein and mutation of the genes encoding corresponding protein. Visualisation technologies have enabled us to find mRNA granule complex composition, regulating elements and localisation sites. Future research is required to answer several questions which are remained to be solved about the interaction between mRNA binding protein and mRNA.

I. INTRODUCTION

Messenger RNAs (mRNA) are the genetic information that is required for protein synthesis in cells including neurones. Neurones can use two mechanisms to target protein to the correct intracellular region, either transporting folded protein directly or transporting mRNA to the target location and generating protein locally. mRNA localisation is essential for neurones because encoded protein may bind to other factors (e.g. microtubule) where they are made or during transportation to the target site. Also, localised translation can facilitate the incorporation of proteins into macromolecular complexed by generating high local protein concentrations and allowing co-translation of different subunits. The nascent proteins generated from localised mRNA can undergo post-translational modification to acquire different functions distinct from pre-existing copies. This process of mRNA localisation is under strict spatial and temporal regulation in response to signals such as neurotrophic factors and injury cues (Besse et al., 2012).

The process of mRNA localisation a well-conserved mechanism in polarised cells. In budding yeast, mRNA localisation plays an important role in asymmetrical cell division. *ASH1* mRNA is localised in the bud tip of daughter cell inhibiting the sex changes, causing mother and daughter cell to have different mating types (Singer et al., 2015). In *Drosophila* oogenesis, several mRNAs are transported from the nursery cell to the oocytes and localised to the either anterior or posterior pole of the oocytes. The localisation of mRNAs is essential for organelle development, chromosome condensation and offspring fertility. Localised protein synthesis has its role in maintaining synaptic plasticity.

In neurones, mRNAs are transported with mRNA binding protein (RBP) (Figure 1).

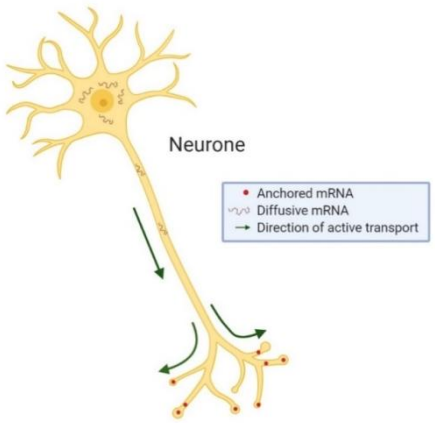

Figure 1.mRNA localisation followed by local translation plays an important role in protein trafficking of polarised cells such as neurones.

TEST Engineering & Management

July – August 2020
ISSN: 0193-4120 Page No. 140- 144

They are called ribonucleoprotein (RNP) which undergoes dynamic remodelling when travelling through the cell. Cis-acting elements are simple sequences or secondary, the tertiary structure of mRNA while trans-acting elements are on RBPs and non-coding RNAs (Liegro et al., 2013). Trans-acting elements recognise cis-acting elements on the 3' untranslated region (3' UTR) of mRNA, 5' UTR of mRNA and coding sequences (Besse et al., 2012). In addition, post transcriptional regulation also include extracellular signalling pathways that target RBPs. It's RBP that determines the direction of mRNA transportation. RNA granules are the key modulators of post-transcriptional modulation. The mRNA and proteins organised into different structure and size, performing a different function
There are three main mechanisms to localise mRNA:

i) Passive diffusion followed by RNA degradation (Figure 2)

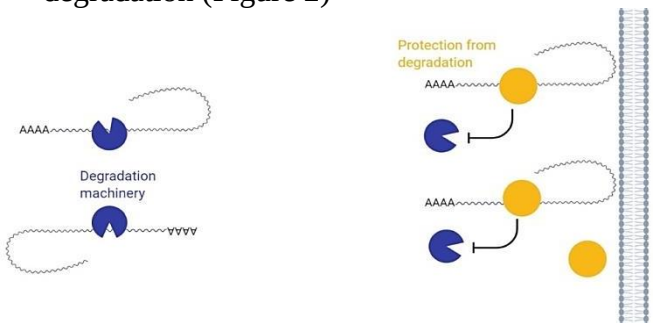

Figure 2. Passive cytoplasmic mRNA diffusion followed by local entrapment in a subcellular compartment or in the membrane vicinity.

ii) Passive diffusion of mRNA followed with local anchoring (Figure 3)

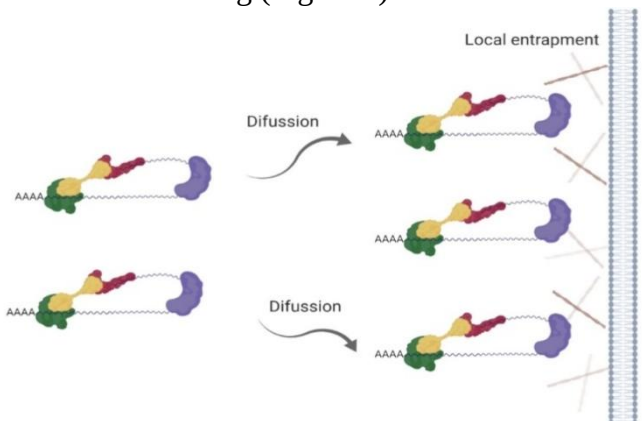

Figure 3.mRNAs can passively diffuse in the cytoplasm and become trapped by a pre-localised factor or actin cytoskeleton along the membrane.

iii) Active transport along the cytoskeleton interacting with cytoskeleton-associated motors. The degradation and diffusion are passive processes and do not cost any energy while motor transport is directional and energy-consuming (Figure 4) (Besse et al., 2012).

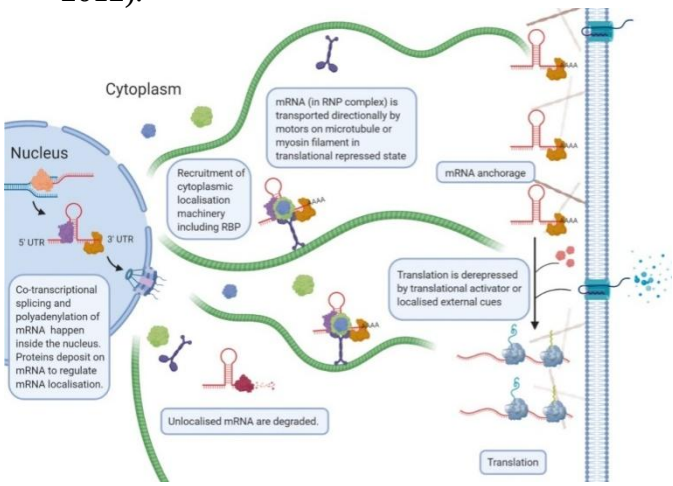

Figure 4. Active mRNA localisation along a polarised cytoskeleton begins in the nucleus where mRNAs are co-transcriptionally spliced and polyadenylated. Recruitment of molecular motors with the help of adaptor proteins mediate mRNA transportations. Once in their destination, mRNAs become anchored either with the help of a pre-localised factor or actin cytoskeleton. Translation can begin in response to external cues or as a result of a pre-localised factor.

II. MECHANISMS OF MRNA LOCALISATION

The mRNA can be localised passively by diffusion, coupled with local entrapment by pre-localised factors or actin cytoskeleton. This process is non-directional and less efficient than active transport, but it is energy saving. The mRNA binds to the surface of vesicles generated by the Golgi apparatus and endoplasmic reticulum (ER). The mRNA-vesicle complex diffuses freely inside the cytoplasm and is anchored by action to the cytoskeleton. The extracellular signal can induce more expression of action to strongly anchor the mRNA to the right position. For example, in *Xenopus* oocytes, the localisation of germ plasma RNA *Xcat2* and *Xdaz*are trapped by the densely packed ER concentrated in the vegetally localised mitochondrial cloud. The mRNA-ER complex then

July – August 2020
ISSN: 0193-4120 Page No. 140- 144

diffuses freely to the cytoskeleton (Besse et al, 2012).

The most conserved and least efficient way of mRNA localisation is cytoplasmic diffusion generalised mRNA degradation coupled with local protection (Figure 2). There are degradation machineries which hydrolyse the mis-located RNAs inside the cytoplasm. The correctly distributed mRNAs are protected by protective proteins which inhibits the degradation machinery. For example, *Hsp83* mRNA inside the Drosophila fertilised egg is stabilised throughout the embryo by protection from degradation.

Non-motile mRNAs have a role in maintaining synaptic plasticity. These non-motile mRNAs are placed at dendritic spines, normally translationally repressed, waiting for signals from the synapse. The signals from signalling cascade in the presynaptic neurone instruct them to move up or down for translation or remain in the same position.

The motor-mediated mRNA transport in neurones often refers to the active transport mechanism (Figure 4). This is the predominant mechanism in neurones it is the quickest and is 60 times faster than passive transport. Active mRNA localisation is ATP-dependent and fully directional due to the action of molecular motors on microtubules. Active transport enables homogenous intracellular mRNA distribution. In fact, mRNAs rarely interact directly with a motor protein. One piece of mRNA sequence can bind to multiple motor proteins therefore RBPs are organised to perform a different function and binding during the localisation process. One single RBP can have tens to hundreds of mRNA targets (Singer et al., 2014).

The mRNA undergoes co-transcriptional splicing and polyadenylation (poly A tail addition) inside the cell nucleus. Introns are removed and exon-junction complex (EJC) is formed (Fu et al., 2011). The immature mRNA associates with protein co-factors and becomes RNP. The secondary structure of the RNA motifs influences their interaction with RBPs. The mature RNP's stem loop has zip-code which recognises motor proteins which it leaves the nucleus to the cytoplasm. The fate of the mRNA localisation is determined by the sequential binding of multiple RBPs. For example, binding of Bicaudal D and Egalitarian proteins enables the direction of mRNA localisation in *Drosophila* towards the minus end (Weil et al., 2014). The RNA binds to kinesin or dynein on specific overlapping cytoskeleton pathway and undergoes translocation by kinesin while RBP binds to RNP to become mature RNP. The faulty RNP will be degraded by enzymes into free nucleotides. Different motors can simultaneously bind to cargo, causing a tug of war. Movement of the cargo in one direction requires inactivation of the motor in the opposite direction. Kinesin and dynein are the motors on microtubules while myosin V transports vesicles on actin filaments (Twiss et al., 2018).

When the motor-RNA complex reaches the end of the microtubule, it can be anchored by either remaining-attached motors or actin-associated proteins. Usually, mRNA is translocated in a translationally repressed state as RNP. When the spatially restricted external cues (e.g. growth hormone, neurotransmitters) or pre-localised activator bind to the receptor, the mRNA can be phosphorylated by kinase. For example, when sonic hedgehog (SHH) signal reaches the receptor, zipcode-binding protein 1 (ZBP 1) undergoes phosphorylation, decreases its binding affinity to RNA and releases β-actin mRNA for translation. ZBP 1 plays a role in both transport and translational regulation of β-actin mRNA (Holt et al., 2013). To sum up, motor-mediated active transport of mRNA enables precise spatial and temporal regulation of protein function and can effectively stop ectopic expression. The misfunction of this process can cause diseases such as acromegaly and gigantism.

III. REGULATION OF MRNA LOCALISATION

Post-translational modification is part of the regulation process, such as lysine ubiquitination, proline hydroxylation, arginine/lysine methylation, serine/threonine phosphorylation and lipidation.

Cis-acting element is post-translational regulator which is located on mRNA. Some of them are locational independent, which causing the localisation of any reporter message to which they are fused. No matter they are position-dependent or not, they mediate steps in localisation by behaving synergistically. Cis-acting element cooperate with RNA chaperones and tertiary structure-binding proteins. RNA chaperones are RNA helicases that modify the misfolded structure, and tertiary structure-binding protein recognises the correctly

July – August 2020
ISSN: 0193-4120 Page No. 140- 144

folded proteins. Trans-acting factors are RBPs and non-coding RNAs (ncRNA). Trans-acting factors recognise cis-acting factors (Yoon et al., 2019). Non-coding mRNA mediate post transcriptional slicing as well as mRNA decay and inhibition by binding to it. The most studied ncRNA is microRNA (miRNA). They are made from precursor pri-miRNA with a series of actions of RNase III enzymes Drosha and DICER in the cytoplasm, causing the formation of RNA-induced silencing complex. The miRNA can read certain RNA sequences in the 3'UTR region and mediate post-translational silencing of the genes and RNA decay. Disfunction of miRNA will cause neurological syndromes and cancer development.

IV. HUMAN DISEASES RELATED TO RBPs

There are several neurological diseases that are caused by altered mRNA protein interactions. Fragile X syndrome (FXS) is caused by a gene on X chromosome that encodes RNA-binding protein FMRP. The mutation is often hyper-expansion and methylation of CGG in the promoter. FMRP has a regulatory role in transport and translation of some mRNAs that encode protein for amyloid precursor protein (APP), hippocampus long-term depression (LTD) and long-term potentiation (LTP). Thus, FMRP affects long-term synaptic plasticity of a neurone (Wang et al., 2016). Frontotemporal lobar degeneration (FTLD) is another neurodegenerative disorder caused by altered mRNA protein interaction. It has two types: one is due to aggregation of MAP tau (FTLD-τ) and the other one is due to aggregation of inclusion bodies that contain ubiquitin (FTLD-U). One inclusion body component is recently identified as TAR DNA-binding protein-43 (TDP-43). Amyotrophic lateral sclerosis (ALS) is also caused by inclusions of TDP-43. TDP-43 is hyperphosphorylated and poorly soluble. Finally, the proximal spinal muscular atrophy (SMA) is a neurological disease caused by mutation of *Smn 1* gene that encodes survival motor neurone protein (SMN) which contains the core element of the pre-mRNA splicing machinery (Di Liegro et al., 2014).

V. CONCLUSION AND OUTLOOK

The process of mRNA localisation is important in various organisms, from bacteria to mammalian cells. In neurones, mRNA localisation, post-transcriptional modification and post-translational regulation are important to axonal regeneration and synaptic plasticity which have a long-term effect on learning and memory. New visualising technologies has improved our understanding in dynamic regulation and variability mRNA localisation. For instance, FISH enable us to investigate splice variants and single polymorphisms.

Despite in-depth research findings made in this field so far, significant numbers of challenges remain to be solved. First, whether the target of mRNA and protein may act in parallel (synergistically or redundantly) in certain situations. Second, the highly selective transport and translation of mRNAs is sorted by what mechanism. For example, the mRNAs which encode cytoskeletal, nuclear proteins are more localised in cell bodies than axons while mRNAs which encode signal transduction molecules are more localised in axons than in cell bodies (Holt et al, 2013). Third, whether (and how) localised mRNAs are anchored in specific active site where cell process is happening. A study suggests that mRNAs are not strictly anchored to a specific site. Instead, mRNAs move around among the synapses in dendrites and then are captured to allow translation where the synaptic activity is high. The question lies in how this mechanism is achieved (Doyle and Kiebler, 2011). Fifth, a hypothesis related to glial cells focuses on its regulation of localised translation in axons by horizontally transferring ribosomes. This hypothesis still remains to be investigated into (Di Liegro et al., 2014). The most puzzling question is how mRNA is regulated by RBPs. The *in situ* content of RNP granule and the effect of RBP on mRNA is unknown. The new technology of RNA purification and identification (RaPID) have a promising ability to analyse the composition of RNP. More direct methods need to be invented to detect local translation of mRNA (Singer et al, 2014).

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July – August 2020
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