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Review

Understanding mRNA trafficking: Are we there yet?

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ABSTRACT

There has been unexpected insight into the part that an mRNA plays in the function of the protein it encodes gleaned from visualizing where an mRNA resides in the cytoplasm. mRNAs can localize to distal domains of the cytoplasm in cells with vast cytoplasmic domains such as the central and peripheral nervous system or oocytes and embryos, but can localize within other somatic cell types as well. Localized translation of such mRNAs in the nervous system can supply distant cellular structures, such as synapses, with required proteins for proper function or development. Similarly, the spatially and temporally localized translation of mRNA early in development spatially segregates developmental cues within the egg to facilitate regional cell fate specification and body plan establishment. All mRNAs do not arrive at these sites of localization, an indication that the mechanisms to get these mRNAs to their destination are specially programmed into the mRNA sequence. Molecular recognition of mRNAs to be localized and the putative connection between the RNA and the cytoskeleton for trafficking to their destination are critical steps in the process. In this review I will present the recent years' progress in understanding these two steps in the mRNA localization process at the molecular level, which is among the most critical determinants for localized translation, since only mRNAs that reach the destination can be locally translated.

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1. Introduction

The first descriptions of individual mRNA being localized (having non-uniform cytoplasmic distributions) occurred now 30 years ago (reviewed in [1]). Prior to this it was recognized that protein synthesis in neurons can occur in axonal cytoplasm quite far removed from the cell body, a process that requires mRNA to be transported into the axon (as reviewed in [2]). Prior to these seminal

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findings researchers interested in post-transcriptional control of gene expression may have presumed that mRNAs are diffusing through the cytoplasm to result in a uniform distribution since this is what comes to mind when an mRNA is inherently considered non-localized. However, this mechanism of distribution may actually be an exception as a great many mRNAs deviate from this [3–5].

The ultimate purpose for localizing an mRNA seemingly lies in being able to locally synthesize the protein that an mRNA encodes. The most interesting applications of active mRNA localization result when a protein's biological activity relies on where the mRNA is localized in the cell, and there have been many good examples where this is the case. In these cases the act of moving an mRNA to sites of translation, that I will call mRNA trafficking, is perhaps the most important determinant for local protein synthesis since mRNAs that cannot traffic to specific locations cannot be synthesized locally. Research on how mRNAs traffic in the cytoplasm has been driven by the identification and characterization of mRNAs that actively trafficked, as well as the identification of the cytoskeletal components that are involved in trafficking them. The tractability of model organisms has allowed them to pave the way in our understanding of these processes, this has resulted in critical leaps forward in studies of the process in mammalian cells where the primary interest in mRNA trafficking lies in its relationship to neuron development, survival and function. In this review I will present the molecular mechanisms of how example localized mRNAs are selected for active trafficking as well as the evolving view of the molecular mechanisms for how different mRNAs traffic. I will present examples of from different model organisms as well as mammalian neurons that illustrate the molecular mechanisms involved in trafficking mRNAs for localized translation, concentrating on more recent results in the field where appropriate. I will not present mechanisms for how an mRNA becomes translated subsequent to trafficking or the relationship of trafficking to other post-transcriptional control processes. Prior articles have discussed this topic [6,7].

2. Recognition of a trafficking mRNA

Localization is defined as where an mRNA is located at any given time. All mRNAs are not uniformly distributed; many are localized to different places within the same cell, a fact arguing for mechanisms that regulate an mRNAs distribution in the cytoplasm. Consistent with this, an mRNA's localization can be conferred to reporter mRNA using sequences taken from the localized mRNA. The RNA sequence that accounts for localization is called a Localization Element (LE), also sometimes called a zipcode. When looking at localization in fixed cells the ability of a localized mRNA to traffic in motor complexes cannot be taken for granted since mRNAs can localize without being targeted by a motor, and there is no means to tell whether the mRNAs are actively trafficking at any given time in fixed cells [6,8]. However, the majority of the localized mRNAs that are represented in mechanistic studies appear to involve an active motor driven step or steps that I refer to here as trafficking [9]. There is a strong correlation between mRNA localization and motor driven trafficking, and this may lead to the inference that non-localized mRNAs do not interact with motors or actively traffic. This may not be a valid conclusion, the possibility of interaction of non-localized mRNAs with motors exists as discussed below in the apical mRNA localization pathway during *Drosophila* embryogenesis. Therefore it is important, although difficult, to demonstrate that an mRNA motor association contributes to trafficking directly.

2.1. mRNA traffics as a ribonucleoprotein (RNP) complex

The mechanism for mRNA trafficking starts with the nucleation of a localizing ribonucleoprotein complex (L-RNP) on the LE sequence. This puts RNA binding proteins (RBPs) as primary components of an L-RNP and many RNA binding proteins have been associated with binding to an LE. Correlating RBP binding with LE activity as well as disrupting RBP function with loss of LE function are the most straightforward experiments to identify candidate mRNA trafficking RBPs. Many more RNA binding proteins have been shown to co-purify with localized *versus* non-localized mRNAs without demonstrating a binding directly to a localization element. Because many RBPs are associated with trafficking mRNAs the complexes may be rather large, and many studies of L-RNPs have characterized properties of large entities called RNA granules that I will discuss below.

2.2. Trafficking of LE containing mRNA

There have been many trafficked mRNAs identified, although in comparison relatively few LEs have been mapped to a minimal functional element [10,11]. Mapping a minimal LE makes the necessary biochemical studies to correlate protein binding with LE activity more approachable. Through mapped LEs we know that the RNA itself plays three types of roles in formation of an RNP. First is to recruit mRNA binding proteins that recognize the sequence of bases in single stranded mRNA. Most mRNA binding proteins with RRM, KH of cold-shock domain type of RNA binding motifs are thought to interact with RNA in this way [12–14]. The RNA binding domains recognize specific sequences of bases within the LE itself. The defined RNA motif recognized by a particular RBP is not well defined for many of the RBPs involved in RNA trafficking, but for some this is known. For instance, A crystal structure of a sub-domain of the β-actin mRNA trafficking factor ZBP1 revealed binding to two separate motifs separated by a single stranded linker reveal how this interacts with single stranded RNA [15]. Presence of such a ZBP1 biding site was able to predict a dendritic mRNA targeting element in the spinophilin mRNA in neurons, but such RBPs probably are not sufficient to signify trafficking on their own [16]. Combinations of RNA binding sites within an LE can work together to mediate mRNA trafficking through recruitment of a distinct set of RBPs [17–19]. Changing the relative activities of LE binding proteins may be able to regulate the activity of LEs that function with RBPs in this way. One such example is the Vg1LE that does not localize early in *Xenopus* oogenesis, but begins to localize to the vegetal pole during mid-oogenesis in a multiple-kinesin and dynein dependent process [20–23]. The relative quantities of at least three Vg1LE RNA binding proteins are changing as oogenesis progresses and it was hypothesized that this relative change in competing activities could account for this temporal activation of LE activity in an RNA that was uniformly distributed [24]. The gurken mRNA in *Drosophila* is another example of an LE that appears to recruit several RBPs to localize it, and like Vg1, the gurken mRNA changes localization during oogenesis [17–19,25]. Homologous proteins to those that bind the Vg1LE are also part of the gurken RNP [19,25,26]. An interesting possibility for the roles of these RNA binding proteins is to regulate the RNA secondary structures that can form in the LE RNA, by stabilizing or destabilizing particular conformations of a flexible RNA at different times in development, although this has not been demonstrated for any LE.

A second role for the RNA is to form specific structures that are the recognition elements for RNA binding proteins. The first and second possibilities differ primarily in that a secondary structure that forms in the RNA result in recognition by an RBP instead of a series of bases, and several examples of this have come to light. The ASH1 LEs in yeast present unique secondary structures that are

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