Differentiation (2007) 75:507–518 DOI: 10.1111/j.1432-0436.2007.00159.x Journal compilation © 2007, International Society of Differentiation

REVIEW

Raymond A. Lewis · Kimberly L. Mowry

Ribonucleoprotein remodeling during RNA localization

Received September 6, 2006; accepted in revised form November 16, 2006

Abstract Cytoplasmic RNA localization is a means to create polarity by restricting protein expression to a discrete subcellular location. RNA localization is a multistep process that begins with the recognition of *cis*-acting sequences within the RNA by specific *trans*-factors, and RNAs are localized in ribonucleoprotein (RNP) complexes that contain both the RNA and numerous protein components. Components of the localization machinery transport the RNP complex, usually in a translationally repressed state, to a distinct subcellular region, resulting in spatially restricted gene expression. Recent efforts to identify both the *cis*- and *trans*-factors required for RNA localization have elucidated RNA–protein interactions that are remodeled during localization.

Key words RNA remodeling · RNA localization · RNP complex · cell polarity · post-transcriptional control

Introduction

Localization of mRNAs within the cell cytoplasm is a widely utilized mechanism to create polarity by restricting protein expression to defined subcellular regions. Numerous cell functions, such as motility and growth, along with developmental phenomena, such as cell specification and differentiation, are controlled in part by localizing RNAs (reviewed in Condeelis and Singer, 2005; Gonsalvez et al., 2005; King et al., 2005; Kloc and Etkin, 2005; Minakhina and Steward, 2005; Martin and Zukin, 2006). Transport of specific RNAs to defined regions within the cell cytoplasm is initiated by RNA–protein interactions that direct the recognition of the RNA and the assembly of an ribonucleoprotein (RNP) transport complex (reviewed in Colegrove-Otero et al., 2005; Gonsalvez et al., 2005; King et al., 2005; Kloc and Etkin, 2005). Although RNA localization occurs in a wide variety of organisms and cell types, the molecular mechanisms by which localized RNAs are recognized, sorted, and transported are not yet fully understood. Both *cis*-acting sequences within the RNA and *trans*-acting factors that interact with those sequences play important roles in initiating the localization process. A key to understanding this process will be the elucidation of specific RNA–protein interactions mediating each step in the RNA localization pathway.

During the lifetime of an mRNA, it transits from the nucleus to cytoplasm in a multitude of RNP complexes (reviewed in Moore, 2005). Both the RNA and protein components of these RNPs dictate the fate of the RNA at each step in its travels. Although assembly of RNPs in the nucleus can be associated with transcription, splicing, polyadenylation, and export, it is now becoming evident that these steps are related to the cytoplasmic fate of the RNA, as factors that bind to the RNA in the nucleus play key roles in cytoplasmic events such as nonsense-mediated decay (NMD), translational control, and cytoplasmic RNA localization (reviewed in Kloc and Etkin, 2005; Moore, 2005). Thus, from the time the RNA is transcribed to the moment it is degraded, proteins are being modified, added to, and removed from the RNP in an effort to coordinate its lifecycle, and numerous protein rearrangements occur on the RNA that affect the fate of the RNP at each step of its maturation. Remodeling of RNP complexes has important implications in the function of the RNA as each phase of its lifecycle is controlled by the *trans*-factors associated with the RNA. Several types of remodeling events can occur within a localization RNP:

Raymond A. Lewis[1] · Kimberly L. Mowry (✉)
Department of Molecular Biology
Cell Biology and Biochemistry
Brown University, Box G-L268
Providence RI 02912, U.S.A.
Tel: +1 401 863 3636
Fax: +1 401 863 1201
E-mail: Kimberly_Mowry@Brown.edu

[1]Present address: Department of Cell Biology, The Scripps Research Institute, CB163, La Jolla, CA 92037, U.S.A.

recruitment/displacement of factors, physical disruption by helicases or nuclear export, oligomerization of localization complexes, and post-translational modification of *trans*-factors. This review will discuss recent findings related to RNP remodeling during RNA localization and link these findings to how the cell orchestrates the process of RNA localization. Recent reviews have highlighted roles of specific *trans*-factors in RNA localization (Carson and Barbarese, 2005; Colegrove-Otero et al., 2005; Condeelis and Singer, 2005; Gonsalvez et al., 2005; King et al., 2005); here we will focus on how a subset of localization factors may influence the remodeling of RNPs during RNA localization.

A model of remodeling: RNA localization in *Xenopus* oocytes

During *Xenopus* oogenesis, RNAs involved in germ layer determination and germ cell specification become restricted to the vegetal cortex of the oocyte (Kloc and Etkin, 2005). At least two pathways operate to carry out vegetal RNA localization: the early or METRO pathway and the late pathway. RNAs localized by the METRO pathway, such as Xcat2 and Xdazl, which have been implicated in germ cell specification, are first concentrated in the mitochondrial cloud at the beginning of oogenesis and subsequently relocated to the vegetal cortex by stage II of oogenesis (Forristall et al., 1995; Kloc and Etkin, 1995; Zhou and King, 1996; Kloc et al., 1998; Houston and King, 2000). By contrast, RNAs localized by the late pathway are primarily involved in establishing the primary germ layers during early embryogenesis (Dale et al., 1993; Thomsen and Melton, 1993; Kessler and Melton, 1995; Lustig et al., 1996; Stennard et al., 1996; Zhang and King, 1996; Horb and Thomsen, 1997; Joseph and Melton, 1998; Zhang et al., 1998; Xanthos et al., 2001; Birsoy et al., 2006). Late pathway RNAs are evenly distributed in the oocyte cytoplasm during early oogenesis and are transported to the vegetal cortex during mid-oogenesis (stages III–IV) in a motor-driven process (Melton, 1987; Forristall et al., 1995; Kloc and Etkin, 1995; Betley et al., 2004; Yoon and Mowry, 2004). Two of these RNAs, Vg1 and VegT, serve as models for localization for the late pathway (reviewed in King et al., 2005). Recently, significant progress has been made dissecting individual RNP complexes along this pathway, hinting at several remodeling steps during vegetal localization.

Both Vg1 and VegT RNAs contain localization elements (LEs) within their 3′-UTRs (Mowry and Melton, 1992; Bubunenko et al., 2002; Kwon et al., 2002), which are sufficient to direct vegetal localization in *Xenopus* oocytes (Fig. 1). While sequence similarities are limited, the LEs contain clusters of repeated motifs involved in localization, namely E2 and VM1 sites (Deshler et al., 1997; Gautreau et al., 1997; Bubunenko et al., 2002; Kwon et al., 2002; Lewis et al., 2004). These motifs serve as binding sites for two *trans*-factors known to be involved in vegetal localization: Vg1RBP/vera binds E2 motifs and polypyrimidine tract-binding protein (PTB)/heterogeneous nuclear RNP I (hnRNP I) binds to VM1 sites (Deshler et al., 1997, 1998; Havin et al., 1998; Cote et al., 1999). It has been proposed that clustering of these *cis*-acting sites within the UTR of a localized RNA is important for promoting interactions between *trans*-factors (Bubunenko et al., 2002; Lewis et al., 2004). By clustering *trans*-factor binding sites with the LEs, the sequence and/or secondary structure of an RNA can direct the recruitment of necessary factors required for the formation of a core RNP complex that is competent for localization. In the case of Vg1 and VegT RNAs, RNP assembly has been found to initiate in the nucleus (Kress et al., 2004), and later cytoplasmic events may be linked to nuclear complex assembly. The nuclear core RNP consists of the RNA and at least Vg1RBP/vera and PTB/hnRNP I (Kress et al., 2004, see Fig. 2). Additional RNA-binding proteins, such as the VgRBP71/KSRP and 40LoVe (Kroll et al., 2002; Czaplinski et al., 2005), which bind to localized RNAs and can be found in the nucleus, may be part of the nuclear Vg1/VegT RNP, but this has yet to be determined. Not surprisingly, both hnRNP and homologs of Vg1RBP/vera participate in transport of RNAs in other systems (Colegrove-Otero et al., 2005; Yisraeli, 2005) possibly forming a similar core complex.

In the *Xenopus* oocyte, Vg1RBP/vera and PTB/hnRNP I interact both with each other and with Vg1 and VegT RNAs, but the interactions differ between the nucleus and the cytoplasm (Kress et al., 2004), suggesting that a remodeling step occurs during or after nuclear export. In the nucleus, the interaction between Vg1RBP/vera and PTB/hnRNP I is not RNase-sensitive, whereas in the cytoplasm this interaction is sensitive to RNase treatment (Kress et al., 2004). These results suggest that the interaction between PTB/hnRNP I and Vg1RBP/vera could be direct in the nucleus, but in the cytoplasm the proteins interact only by virtue of binding the same RNA transcript. There are several possible scenarios that could account for the observed remodeling. First, an RNA helicase could act to physically disrupt these interactions. Helicases are present in localized RNPs from *Drosophila* (Nakamura et al., 2001; Palacios et al., 2004), but, as yet, no helicases have been found within the Vg1/VegT RNP complex. Second, the act of nuclear export could itself be sufficient to account for the disruption of this interaction. A physical separation of proteins has been observed during Balbiani ring RNA export in *Chironomus tentans* salivary gland cells (Daneholt, 2001). Third, recruitment of additional factors could modify the inter-