

Breaking the A chain: regulating mRNAs in development through CCR4 deadenylase

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F1000 Biology Reports 2009, 1:20 (doi: 10.3410/B1-20)

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Abstract

Post-transcriptional mechanisms of gene regulation have long been implicated in specifying embryonic pattern in many organisms. Experiments in *Caenorhabditis elegans*, *Drosophila*, and *Xenopus* have recently converged, pointing to the CCR4 deadenylase complex as a key effector that modulates the expression of proteins from specific germline mRNAs.

Introduction and context

A polarized distribution of maternally expressed proteins determines the spatial axes of the *Caenorhabditis elegans* and *Drosophila* embryos. Correct deployment of these proteins can entail asymmetric localization of their mRNAs, spatially restricted translational control, spatially restricted control of mRNA stability, or direct protein targeting. Often, two or more of these mechanisms operate redundantly. Recent advances in this area indicate that control of mRNA stability via poly(A) tail length is a key target for regulatory pathways essential to embryonic patterning.

Major recent advances

A key factor influencing mRNA stability and poly(A) tail length in *Drosophila* embryos is Smaug (Smg). Smg was first identified as a repressor of unlocalized *nanos* (*nos*) mRNA [1]. Smg negatively regulates *nos* translation by preventing recruitment of active cap-binding complex to the transcript [2], and it also targets *nos* mRNA for degradation by recruitment of the CCR4 deadenylase complex, a process blocked in the posterior pole plasm by Oskar [3]. Smg regulates degradation of unlocalized *hsp83* mRNA in a similar fashion [4,5]. Influencing mRNA stability appears to be the more fundamental role of Smg. Expression profiling shows that mRNAs representing over half of the *Drosophila* genome (some 7,700

genes) are present in mature oocytes [6]. Of these, approximately 1,600 are degraded upon fertilization. In embryos lacking Smg, two-thirds of these mRNAs are stabilized. As these mRNAs are not enriched for the Smg recognition sequence, Smg may influence stability of these mRNAs in an indirect manner [6].

The CCR4 deadenylase complex is also recruited to target mRNAs by Bicaudal-C (Bic-C) [7] and by Nos itself [8]. Interestingly, these factors interact with different subunits of CCR4: Bic-C binds to the NOT3/5 subunit and Nos binds to NOT4. PUF-domain proteins, typified by Pumilio in *Drosophila*, represent another class of negative post-transcriptional regulators; such proteins recruit CCR4 through its POP2 subunit in yeast [9]. The direct interaction by which Smg recruits the CCR4 deadenylase is uncertain, although the NOT1 subunit was recovered in tandem affinity purification (TAP) experiments [4]. Surprisingly, CCR4 activity is also regulated by a cytoplasmic pool of a predominantly nuclear poly(A)-binding protein, PABP2, a well-known stimulator of nuclear polyadenylation that with CCR4 targets certain cytoplasmic mRNAs for deadenylation during embryonic development [10]. The precise mechanism for this is unknown.

CCR4-mediated deadenylation is counteracted by poly(A) polymerases and by Orb, the *Drosophila* homologue of cytoplasmic polyadenylation element-binding protein (CPEB), which promotes their recruitment. Interestingly, different poly(A) polymerases activate expression of specific germline mRNAs at different developmental stages. The poly(A) polymerase encoded by *hiiragi* (*hrg*) is required during early stages of oogenesis, whereas that encoded by *wispy* (*wisp*, also known as *Gld2*) becomes essential at stage 10 of oogenesis and is required into early embryogenesis [11]. *Wisp* is required to extend the poly(A) tail of specific transcripts, such as *bicoid*, *Toll*, and *torso*, enabling their subsequent translation, and to stabilize *oskar* and *nos* RNAs in early embryogenesis [11,12].

The *C. elegans* germline determinant *nos-2* is also under translational regulation; its expression is repressed in the somatic blastomeres of the early embryo [13]. *nos-2* translational activation is the first known molecular event that is specific to the P4 primordial germ cell. Translational repression is maintained in somatic blastomeres by the interaction of four RNA-binding proteins (OMA-1, OMA-2, MEX-3, and SPN-4) with the *nos-2* 3' untranslated region (UTR) [14]. In the P4 cell, *nos-2* translation becomes de-repressed when POS-1, another RNA-binding protein, outcompetes translational repressors for binding to the *nos-2* 3' UTR [13,14]. *nos-2* mRNA is also targeted by degradation mechanisms in the somatic blastomeres, starting as early as the four-cell stage [13]. This is dependent on two highly similar zinc finger proteins, MEX-5 and MEX-6, and also involves the CCR4 deadenylase. When the NOT1 subunit of CCR4 is depleted by RNA interference, somatic processing bodies are decreased in number, *nos-2* RNA is stabilized, and a green fluorescent protein reporter fused to the *nos-2* 3' UTR is translated in somatic blastomeres [15].

A similar CCR4-mediated mechanism of post-transcriptional regulation was implicated recently in vertebrate germline development as well. *Xenopus* C3H-4, a zinc finger protein closely related to POS-1, recruits CCR4 to shorten the poly(A) tails of target mRNAs that contain A/U-rich elements in their 3' UTRs [16]. Key C3H-4 targets are *emi1* and *emi2*, which encode proteins that inhibit the anaphase-promoting complex that is essential for progression beyond the metaphase I stage of meiosis. C3H-4 operates in opposition to CPEB, and sequential cycles of polyadenylation and deadenylation of mRNAs encoding cell cycle regulators drive progression through meiosis [16].

Despite their sequence similarity, C3H-4 and POS-1 have opposing regulatory effects: C3H-4 facilitates deadenylation whereas POS-1 acts to counter translational repression. Also, *Drosophila* Bic-C promotes deadenylation or

polyadenylation at different developmental stages, through associating with either CCR4 or *Wisp* [7,11,12]. Interactions between these RNA-binding proteins and different accessory proteins may explain these apparent contradictions; for example, RNA-binding proteins may regulate stability and activity of their targets by influencing whether they associate with processing bodies or germline P granules [15]. Although these particles share many components [15,17], germline P granules lack the Sm-like (LSm) proteins LSm-1 and LSm-3, whose presence correlates with RNA degradation.

Future directions

Together, this work implicates CCR4 as a critical agent of post-transcriptional gene regulation of specific mRNAs underlying embryonic patterning. Future work will teach us more about the precise mechanisms by which CCR4 influences target mRNAs and whether its recruitment exclusively favors degradation or whether it can result in reversible translational silencing. Also, whereas some of the same RNA- and CCR4-binding proteins have been identified in developmental processes in both flies and worms, many thus far have been linked to this system in only one organism. It will be important to determine whether there is more commonality among CCR4-interacting factors than is presently apparent or whether each system has developed distinct mechanisms to facilitate CCR4-mediated regulation.

Abbreviations

Bic-C, Bicaudal-C; CPEB, cytoplasmic polyadenylation element-binding protein; *nos*, *nanos*; Smg, Smaug; UTR, untranslated region; *wisp*, *wispy*.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We are grateful to Martine Simonelig for helpful comments on the manuscript.

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