Birthing histone mRNAs by CSR-1 section

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As the building blocks of chromatin, histones need to be synthesized efficiently and synchronously with DNA replication. The mRNAs encoding the core histones undergo a distinct 3'-end formation pathway that results in mature mRNAs ending in a stem loop instead of a polyadenylated tail. Considering that the factors involved in histone 3'-end processing are well conserved across Eukarya, the absence of some of these components in *C. elegans* draws the question of how histone 3' ends are matured in worms. In this issue of *The EMBO Journal*, Avgousti *et al* (2012) present an answer that, surprisingly, involves RNAi pathway factors.

Histone genes encode for pre-mRNAs that undergo maturation through a mechanism distinct from all other RNA Pol II transcripts by substituting polyadenylation with an alternative 3'-end formation pathway (Marzluff *et al*, 2008). The 3'-end of a histone pre-mRNA is marked by a short stem-loop followed by a histone downstream element (HDE). The hairpin is bound by the stem loop binding protein (SLBP) and the HDE is recognized by the U7 small nuclear RNA (U7 snRNA) through base pairing interactions. Next, cleavage and polyadenylation specificity factor 73 (CPSF73) joins the complex and cleaves the pre-mRNA a few nucleotides downstream of the stem loop. SLBP remains associated with the processed histone mRNA and fulfills roles normally attributed to the polyA binding protein, such as promoting nuclear export and translation.

Although the 3'-ends of *C. elegans* histone mRNAs also terminate in stem loops instead of polyA tails, the U7 snRNA and its associated protein LSM11 appear to be missing in this species (Davila Lopez and Samuelsson, 2008). Additionally, worms lack an HDE-like sequence after the stem loop. These deficiencies prompt the question of how histone 3'-ends are formed in *C. elegans*. One solution to this problem is presented in a new study, where RNAi-related factors regulate the formation of histone mRNA 3'-ends (Avgousti *et al*, 2012). Instead of utilizing an HDE to position the cleavage complex, sequences downstream of the stem loop in histone pre-mRNAs were found to be complementary to endogenous small interfering RNAs (endo-siRNAs) (Figure 1). These endo-siRNAs are produced by the RNA-dependent RNA polymerase (RdRP) EGO-1 and associate with the Argonaute CSR-1 (Claycomb *et al*, 2009; Avgousti *et al*, 2012). In the absence of *ego-1* activity, the interaction of CSR-1 with an *in vitro* synthesized RNA mimicking the 3'-end region of the worm H2A pre-mRNA was lost, indicating that endo-siRNAs

are required for CSR-1 to associate with this sequence of RNA. CSR-1, EGO-1, or another RNAi-related factor, the helicase DRH-3, were shown to be important for histone mRNA biogenesis *in vivo* as loss of any one of these factors resulted in decreased levels of histone mature mRNAs and proteins. Notably, histone pre-mRNAs were elevated in these conditions, supporting a novel role for RNAi factors in maturation of histone mRNAs.

Another connection between the RNAi factors and histone mRNA biogenesis is the phenotypic similarities in worms depleted of *csr-1* with those lacking *cdl-1*, which encodes the worm SLBP. Loss of *cdl-1* results in histone deficiencies that are associated with chromosome segregation defects, sterility and lethality. CSR-1 was named for the chromosome segregation and RNAi defects observed upon loss of this factor, and similar abnormalities are detected in *ego-1* and *dhr-3* mutants (Yigit *et al*, 2006; Claycomb *et al*, 2009). Since decreased histone expression leads to embryonic lethality, transgenes designed to encode histone mRNAs that should be independent of the RNAi pathway factors were tested for rescue of this phenotype in worms depleted of *csr-1* and *ego-1*.

Figure 1 3'-end processing of histone mRNAs by the CSR-1 RNAi pathway. The RdRP EGO-1, along with DRH-3, synthesizes endo-siRNAs, which load onto the Argonaute CSR-1. Endo-siRNAs that pair perfectly to sequences near the stem loop in histone pre-mRNAs recruit CSR-1, which facilitates cleavage and maturation of the mRNAs. The SLBP, called CDL-1 in *C. elegans*, functions in 3'-end formation and remains associated with the histone mRNA.

Remarkably, transgenic animals expressing high levels of the histones survived depletion of the RNAi factors. These results suggest that histone biogenesis is at least one of the functions required of CSR-1 for worm viability.

CSR-1 is one of the twenty-seven different Argonaute proteins in *C. elegans*. While loss of most individual Argonaute proteins results in negligible phenotypes, *csr-1* stands out as a gene required for normal chromosomal segregation (Yigit *et al*, 2006). While Argonaute proteins are best known for their cytoplasmic roles in post-transcriptional regulation of gene expression, CSR-1 and its associated factors, EGO-1 and DRH-3, were found to localize to germline chromosomes (Claycomb *et al*, 2009). More specifically, this complex binds to thousands of genomic loci that correspond to sequences of the endo-siRNAs associated with CSR-1. Although these endo-siRNAs can pair perfectly with target mRNAs and CSR-1 is catalytically competent, this complex does not appear to repress the expression of its target genes (Aoki *et al*, 2007; Claycomb *et al*, 2009). Strikingly, CSR-1 targets are expressed in the germline and represent euchromatic loci that exclude binding of the histone H3 variant CENP-A, which defines centromeric chromatin (Claycomb *et al*, 2009; Gassmann *et al*, 2012). Taken together, CSR-1 may regulate chromosome segregation directly by helping to demarcate centromeric regions.

Yet another role for CSR-1 has recently been implicated in studies of how piwi interacting RNAs (piRNAs) trigger silencing of transcripts in the germline (Ashe *et al*, 2012; Lee *et al*, 2012; Shirayama *et al*, 2012). The Argonaute PRG-1 binds piRNAs and has been proposed to use them to scan for targets with partial complementarity. Once a match is found, RdRPs are recruited to synthesize endo-siRNAs corresponding to the targeted sequence. These RNAs are loaded onto a group of worm-specific Argonautes, which maintain silencing of the target genes. A conundrum in this model is how PRG-1 is able to direct silencing of foreign but not expressed germline

genes, which also contain sequences expected to be recognized by piRNAs. One answer is that the expressed genes are targets of CSR-1 and association with this factor might prevent a gene from undergoing PRG-1-mediated silencing (Ashe *et al*, 2012; Lee *et al*, 2012; Shirayama *et al*, 2012). At the moment, this idea of CSR-1 recognizing 'self' transcripts to antagonize PRG-1 is an enticing model that requires further investigation.

The functions ascribed to CSR-1 thus far as a regulator of histone biogenesis, a marker for chromosomal organization, and a licensing factor for germline gene expression highlight the importance and potential versatility of this Argonaute. It will be important to carefully dissect all of the phenotypes associated with loss of *csr-1* and determine how these relate to the various molecular roles of this factor. Notably, CSR-1 retains cleavage activity, while it has been lost from many other Argonautes. While it is yet to be shown if this activity is required for any of the CSR-1 functions, the histone mRNA 3'-end cleavage site aligns with the middle of some endo-siRNAs, a position characteristic of Argonaute-mediated slicing (Aoki *et al*, 2007; Avgousti *et al*, 2012). Alternatively, binding of CSR-1 to histone pre-mRNAs could facilitate recruitment of the typical cleavage complex containing CPSF73. If this is the case, then the Slicer function of CSR-1 *in vivo* remains a mystery. Perhaps, equally puzzling is the question of why CSR-1 does not engage its catalytic activity when recruited by perfectly paired endo-siRNAs to its many targets expressed in the germline. In conclusion, the addition of histone processing factor to CSR-1's growing list of functions helps explain how these mRNAs mature without core 3'-end formation factors. Moreover, it demonstrates a novel activity for an Argonaute protein in positively regulating gene expression through a processing function.

Conflict of interest

The author declares that she has no conflict of interest.

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