BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-16-099

Title: A novel model of THO/TREX loading onto target RNAs in metazoan gene expression

Article Type: Perspective (Invited Only)

Keywords: THO/TREX; piRNA; RDC (Rhino-Deadlock-Cutoff) complex; dual-strand piRNA cluster; Drosophila

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Perspective

Original Article:
Hur et al (2016) Splicing-independent loading of TREX on nascent RNA is required for efficient expression of dual-strand piRNA clusters in *Drosophila*. Genes & Dev 30:840-855, DOI/10.1101/gad.276030.115

Title

A novel model of THO/TREX loading onto target RNAs in metazoan gene expression

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Key Words
THO/TREX, piRNA, RDC (Rhino-Deadlock-Cutoff) complex, dual-strand piRNA cluster, *Drosophila*

Abbreviations
Cuff, Cutoff; piRNA, Piwi-interacting RNA; RDC, Rhino-Deadlock-Cutoff; Rhi, Rhino; TE, Transposable element; THO, Suppressors of the transcriptional defects of hpr1D by overexpression; TREX, Transcription Export

Abstract

The THO/TREX complex consists of several conserved subunits and is required for mRNA export. In metazoans THO/TREX binds a subset of mRNAs during RNA splicing, and facilitates nuclear export of them. How THO/TREX selects RNA targets has, however, not been completely understood. In our recent study, we reported that THO is loaded onto Piwi-interacting RNA (piRNA) precursor transcripts independent of splicing and facilitates convergent transcription in *Drosophila* ovary. The precursors are later processed into mature piRNAs, a small noncoding RNAs that silences transposable elements (TEs). We observed that piRNAs originated from dual-strand clusters, where precursors are transcribed from both strands, were specifically affected by THO mutation. Analysis of THO-bound RNAs showed enrichment of dual-strand transcripts. Interestingly, THO loading onto piRNA precursors was dependent on Cutoff (Cuff), which comprises the Rhino-Deadlock-Cutoff (RDC) complex that is recruited to dual-strand clusters by recognizing H3K9me3 and licenses convergent transcription from the cluster. We also found that THO mutation affected transcription from dual-strand clusters. Together, we concluded that THO/TREX is recruited to dual-strand piRNA clusters, independent of splicing event, via multi-protein interactions with chromatin structure. Then it facilitates transcription probably by suppressing premature termination to ensure piRNA precursors are expressed adequately.

Text

TREX (transcription/export) is an evolutionary conserved multi-protein complex. TREX can be sub-divided into THO and additional factors. THO consists of six subunits; three have homolog in yeast (Hpr1/Thoc1, Thoc2, and Thoc3/Tex1) and other three are metazoan specific (Thoc5/FMIP, Thoc6, and Thoc7). Two additional conserved proteins, Yra1/REF/Aly and Sub2/UAP56, comprise TREX with THO. In yeast, THO/TREX is recruited by interaction with RNA pol II and facilitates transcriptional elongation. THO/TREX is loaded on nascent transcripts during transcription, and then Yra1 brings Mex67p, which in turn transports the mRNA through nuclear pore into the cytoplasm. Interestingly, function of metazoan THO/TREX was somewhat different from that of yeast. Although metazoan THO/TREX is also loaded onto nascent transcripts, it does not interact with RNA pol II; and its loading is dependent on splicing. Cap binding proteins (CBC20 and CBC80) and exon junction complexes facilitate THO/TREX recruitment onto mRNA. But it is also known that TREX can be loaded onto intron-less genes such as Hsp70 in fly and human. It is, however, not fully understood how THO/TREX can be loaded onto nascent transcript in a splicing-independent manner. In our recent paper, we reported that THO/TREX could be loaded onto transcripts from distinct genomic loci called dual-strand piRNA clusters in *Drosophila* ovary, suggesting a novel splicing-independent mechanism of THO/TREX loading onto target RNAs.

piRNA is a class of small noncoding RNA that silences TEs in animal germline. Unlike other small noncoding RNAs, most piRNAs are originated from discrete genomic source loci called piRNA clusters. piRNA clusters can be divided into two types based on the orientation of their transcription. In uni-strand clusters, piRNA precursors are transcribed in only one direction whereas dual-strand clusters are transcribed convergently from both ends.

In the paper, we found that flies with defective THO shared several phenotypes with piRNA mutants, such as dorso-ventral patterning defect and genomic instability. To ask whether THO is involved in piRNA biogenesis, we conducted total RNA-seq on wild type and *thoc5* mutant. We found no significant differences in expression profile of protein coding genes. But most TEs were up-regulated in *thoc5* mutant ovary; the de-repression was especially prominent in TEs that are expressed in germ cells.

Genome-wide analysis of small RNAs-showed that loss of THO did not affect miRNA or endo-siRNA levels, while significantly decreasing the levels of piRNAs, which are complementary to TEs expressed in germ cells. We further analyzed the genomic loci from which the piRNAs were originated. We found that piRNAs from dual-strand clusters but not uni-strands were significantly decreased in *thoc5*. This reminisced about some other piRNA-related proteins (Cuff, a heterochromatin protein 1 family protein Rhino, and UAP56), as loss of them decreases piRNAs in a dual-strand specific manner.

Then we examined how the THO would function in piRNA biogenesis by visualizing its subunits in ovary germ cells. We noted that Thoc5 co-localized in the discrete nuclear foci with UAP56 and Rhino. We also observed that the localization of THO subunits (Thoc5, Thoc2, and Thoc7) and UAP56 was co-dependent. The localization of THO/TREX as nuclear foci suggested that the complexes were loaded

onto dual-strand piRNA precursors. Therefore, we purified Thoc5 from fly ovary and analyzed co-purified RNA by deep sequencing. Consistent with piRNA expression changes, we observed specific enrichment of dual-strand precursor RNAs in Thoc5 pull-down. Uni-strand precursors were not enriched, and only a small subset of mRNAs, such as Hsp70, was enriched.

The THO/TREX localization to dual-strand piRNA precursors could not be explained by splicing-dependent loading, since the precursor contains no intron. So, we sought if the THO/TREX localization was mediated by proteins that associate with chromatin marks that are specific to the loci. Co-immunoprecipitation showed that Thoc5 interacts with Cuff, and immunofluorescence results suggested that Cuff and Rhino are required for Thoc5 localization. Notably, tethering Cuff to a reporter RNA was sufficient to recruit THO to the reporter RNA.

Our total RNA-seq result showed that *thoc5* mutation led to decrease of dual-strand piRNA precursor levels, suggesting THO/TREX is necessary for precursor transcription. To ask whether THO/TREX is required for transcription, we assayed nascent transcript levels by chromatin-associated RNA-seq; and we found that dual-strand cluster transcripts were indeed decreased in *thoc5*. RNA-FISH and RNA Pol II chromatin immunoprecipitation results further supported the decreased levels of dual-strand cluster transcripts in *thoc5*.

Together, the results suggest that THO/TREX is recruited to dual-strand piRNA clusters by chromatin associated factors, and facilitates transcription. This could be a requirement for long transcripts from piRNA clusters where splicing should be suppressed. On the basis of these findings, we proposed a new splicing-independent loading model of THO/TREX where chromatin state is the key regulator (Fig.1). The loading process is distinct from both of previously reported processes: splicing-dependent loading in metazoans and RNA pol II dependent loading of yeast.

The model immediately raises a number of questions. First, THO/TREX is recruited by Cuff, which comprises the RDC complex with Rhi to read heterochromatin histone modification, H3K9me3. Usually H3K9me3 marks silence genes and TEs, yet active transcription of the dual-strand piRNA cluster requires the repressive histone modification. Further studies may elucidate how THO/TREX discriminates dual-strand piRNA cluster from other epigentically repressed genetic loci. Second, THO/TREX is ubiquitously expressed while the RDC complex is ovarian germline specific. It would be interesting to investigate whether splicing-independent loading of THO/TREX also happens in other tissue where RDC is not expressed. Specifically, testis has been shown to be affected by loss of THO/TREX function. More investigation would be required to understand how THO/TREX modulates gene and TE expression in testes. Third, as TREX may be more dynamic complex than THO, THO and TREX may have distinct roles by regulating different subset of genes. Fourth, recent studies showed that piRNAs are also expressed in somatic cells and are proposed as a modulator of global gene expression profile. Computational analysis of piRNAs matching to genes may provide more information of the somatic non-coding small RNA.

ACKNOWLEDGEMENTS

This work was supported by grants from the Basic Science Research Program through the National Research Foundation of Korea (NRF) (no. 2015R1A2A2A01003598) to YDC.

Figure Legend

Figure 1. A model of splicing-independent loading of THO/TREX onto nascent RNAs from dual-strand piRNA cluster. The RDC complex, which is composed of Rhino, Deadlock (not shown in the figure) and Cuff, is recruited to dual-strand piRNA cluster by recognizing H3K9me3 marks, and facilitates convergent transcription. THO/TREX is loaded onto dual-strand precursor RNAs via interaction of Thoc5 with Cuff. The loading of THO/TREX not only facilitates efficient transcription of precursor RNAs but also mediates export of them thru nuclear pore into cytoplasmic nuage, where they are processed into mature piRNAs.

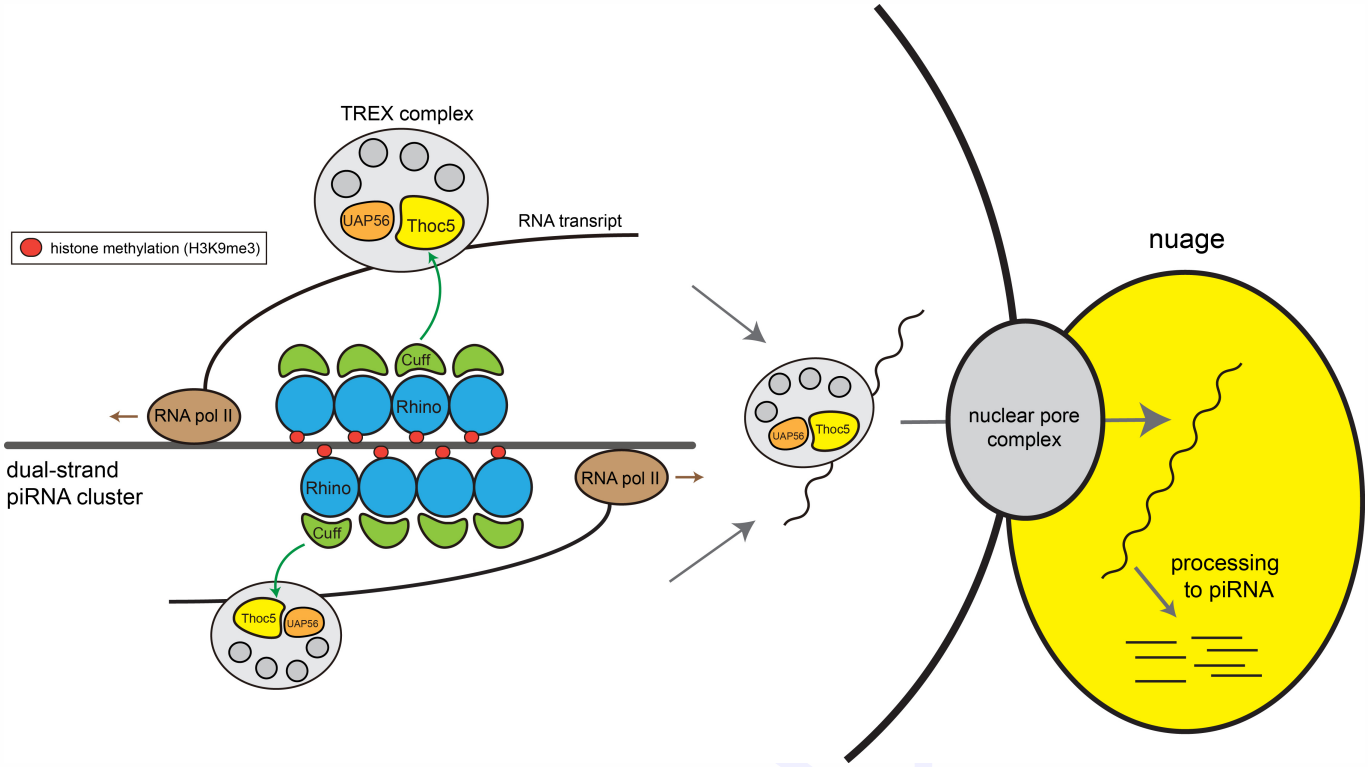

Fig. 1