Regulatory Mechanism of the RNAi Pathway

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RNA interference (RNAi) is post-transcriptional gene silencing initiated by Dicer, a RNase III that processes double-stranded RNA (dsRNA) precursors into small interfering RNA (siRNA). In Drosophila, Dicer2 and R2D2 coordinately recruit duplex siRNA to the effector RNA-induced silencing complex (RISC), wherein single-stranded siRNA guides the endoribonuclease Argonaute (Ago) to catalyze sequence-specific cleavage of complementary mRNA. It remains unclear as to what constitutes holo-RISC, how is RISC assembled and how is RISC regulated. Here we took a candidate approach to reconstitute for the first time the long double-stranded RNA- and duplex siRNA-initiated RISC activities with the use of recombinant Drosophila Dicer-2, R2D2, and Ago2 proteins. We further employed this core reconstitution system to purify a RNAi regulator that we named C3PO (component 3 promoter of RISC), a complex of Translin and Trax. C3PO is a novel Mg2+ -dependent endoribonuclease that promotes RISC activation by removing the siRNA passenger strand cleavage products. Similar as Drosophila C3PO, human C3PO also degrades passenger strand fragments and facilitates RISC activation.
RISC is a multiple-turnover enzyme, wherein single-stranded (ss)-siRNA guides Ago2 to catalyze sequence-specific cleavage of the target mRNA at the effector step. We employed human minimal RISC reconstitution system to purify antoantigen La as a novel activator of the RISC effector step. Biochemical studies indicated that La promotes the multiple-turnover of RISC catalysis by facilitating the release of RISC cleaved products. Moreover, we demonstrated that La is required for efficient RNAi, antiviral defense, and transposon silencing in mammalian and Drosophila cells.
Taken together, our findings of C3PO and La reveal a general concept that regulatory factors are required to remove Ago2-cleaved products to assemble or restore active RISC. The robust reconstitution system establishes a powerful platform for in-depth studies of the assembly, function, and regulation of RISC. Similar to the discovery of C3PO and La, it can be used to identify novel regulators and study post-translational regulations of RNAi, therefore, connecting RNAi to other cellular signaling pathways. As such, these biomedical studies could have a major and lasting impact on the biological understanding and therapeutic application of RNAi.