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Ketting et al.'s 1999 paper produces an account of RNA interference as post-transcriptional, guided by dsRNA targeting homologous mRNA, and carried out by MUT-7, which degrades target mRNA starting from the 3' end. Their speculative model is based on the following facts: MUT-7 encodes a homolog of RNaseD, RNaseD is an exonuclease and works by digesting RNA from the 3' end, and *mut7* mutants show no RNA interference; the progeny of *mut7* worms fed on dsRNA encoding for essential genes survived, indicating that no RNAi took place, meaning MUT-7 must be involved by virtue of its RNase function (Ketting, 1999, figure 2, three underlined bars). Since the publication of these results, other studies, such as Zamore's 2000 publication and Bernstein's 2001 one, have presented us with a different account of RNAi, the main difference with this model being that the central mechanism is targeted *endonuclease* as opposed to exonuclease activity with the help of a dsRNA guide. In this paper, I will produce an updated model of how dsRNA causes RNAi that encompasses what we know about the role that Dicer and the RISC complex play in RNA interference. Rather than guiding a protein complex that degrades homologous mRNA via exonucleolytic activity, dsRNA is first fragmented into 21-23 nucleotide siRNAs by Dicer, which then bind to the RISC complex. This complex includes Argonaute2, an endonuclease which chops the target mRNA into fragments, rendering it non-functional. What role is played by MUT-7, an exonuclease, I will discuss later.

dsRNA's role in gene silencing begins with its processing into siRNA by Dicer. In S100 extracts, which lose bigger proteins like ribonucleases, including RISC, to the strength of centrifugation, dsRNA was still processed into smaller pieces, indicating that dsRNA's initial fragmentation is performed by a smaller, separate complex than that responsible for target

mRNA processing (Bernstein, 2001, figure 1c, bottom blots). Figure 3 of the Bernstein paper also demonstrates that silencing Dicer via RNAi leads to cessation of fragmentation.

After this fragmentation, the resulting siRNAs are incorporated into the RISC complex, where they perform the function of guiding the endonucleases to the target mRNA. Zamore et al. offer a compelling account of this: an ATP-dependent helicase dissociates the cleaved dsRNA into separate single-stranded pieces which become parts of the RISC complex, whereupon AGO2 cleaves the target mRNA after it is bound to the siRNA guide (Zamore et al., 2000, figure 7). Let us examine the evidence for each of these steps. First, we know that ATP must be involved in one or more steps in RNAi, as mRNA decay is greatly reduced if not completely prevented in ATP-depleted lysates (Zamore et al., 2000, figure 1a). Its unwinding by helicase is a likely use of ATP, as, without being unwound, dsRNA would have no base-pairing functionality through which it could be used to target mRNA, and breaking the hydrogen bonds between base pairs requires energy. Whether this helicase domain belongs to Dicer or RISC, however, is unclear. mRNA cleavage only takes place in the regions corresponding to introduced dsRNA; in figure 5b of the Zamore paper, one can observe that the size of the fragments of cleaved mRNA correspond to the positions of the introduced dsRNA along the length of said mRNA strand (Zamore et al., 2000, figure 5b). Finally, the size of the intervals where the mRNA is cleaved corresponds to the lengths of siRNA, 21-23 nucleotides (Zamore et al., 2000, figure 6A). Hammond et al. present compelling evidence that AGO2 is responsible for the cleaving of target mRNA, as their AGO2 immunoblot lines correspond in position to the faint lines in their luciferase mRNA blot which indicate mRNA cleaving (Hammond et al., 2001).

The problem with this revision is that it does not improve upon but entirely eschews the Ketting model, which must be, to at least some extent, correct. In the Ketting study, the progeny

of *mut7* worms fed on dsRNA encoding for essential genes survived, indicating that no RNAi took place, meaning MUT-7 must be crucially involved in RNAi in C. elegans (Ketting, 1999, figure 2, three underlined bars). We also know that MUT-7 is a homolog of RNase-D, an exonuclease which degrades RNA from the 3' end, so it cannot be involved in cleaving, which is endonucleolytic. Nor can we say that MUT-7 performs a janitorial role wherein it just degrades the leftover 21-23 nt mRNA strands; this would be unnecessary for RNAi to be effective and would not have produced the results in that paper. One explanation is that the two models are concurrent in such a way that the loss of MUT-7 prevents the endonucleolytic process whilst the reverse is not true; in other words, maybe MUT-7's role in RNAi is upstream of the process detailed earlier. One piece of evidence for this is that, in the Ketting paper, the progeny of mutants fed on dsRNA were dead, indicating that MUT-7 is involved in a transport mechanism through which dsRNA is carried from the digestive tract to germline cells (Ketting et al., Figure 2). *rde-1*, an *argonaute 1* homologue, is only involved at the cellular level, as dsRNA injection in *rde-1* mutant worms led to RNAi in the body of the mothers but not in that of cross progeny, meaning that a dsRNA transport system responsible for its transmission to the next generation is left intact (Tabara, 1999, figure 3, bottom right). It is possible, then, that MUT-7, in conjunction with other proteins in its complex (X and Y in the Ketting diagram) is somehow involved in this upstream transportation or amplification process by, for instance, 'trimming' dsRNA for transport. In this case, MUT-7's function would be to partially degrade the dsRNA itself, perhaps so that it can later be recognized and processed by Dicer, not the target mRNA. This updated model, where dsRNA is first prepared for transport or even amplified with the help of MUT-7 so that it can later be used for RNA interference by Dicer and the RISC complex, would be in line with the results in the Ketting paper which establish MUT-7's indispensability in RNAi

as well as those in Zamore, Tabara and Bernstein's papers which demonstrate that target mRNA is rendered nonfunctional by endonucleases using siRNA as guides, not by exonucleases.