PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

Short interfering RNAs

ArticleInfo		
ArticleID	:	3914
ArticleDOI	:	10.1186/gb-2001-2-4-reports0009
ArticleCitationID	:	reports0009
ArticleSequenceNumber	:	17
ArticleCategory	:	Paper report
ArticleFirstPage	:	1
ArticleLastPage	:	4
ArticleHistory	:	RegistrationDate : 2001–1–27 Received : 2001–1–27 OnlineDate : 2001–4–7
ArticleCopyright	:	BioMed Central Ltd2001
ArticleGrants	:	

ArticleContext	:	130592244

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Abstract

Short RNAs of 21 or 22 nucleotides have been shown to mediate RNA interference.

Significance and context

RNA interference (RNAi) by double-stranded RNA (dsRNA) is a powerful method for preventing the expression of a particular gene. But how does dsRNA target mRNA for destruction? Using a *Drosophila in vitro* system, Elbashir *et al*. have now analyzed the molecular mechanism of RNAi and find that the dsRNA is processed into fragments of 21 or 22 nucleotides, which are the mediators of RNA. The requirement for RNA fragments of this length prevents the undesired activation of RNAi by short intramolecular base-paired structures of cellular RNAs.

Key results

The minimal length requirement of dsRNA for processing to 21-23-nucleotide short interfering RNA (siRNA) was found to be 39 base pairs (bp). The resulting 21-23mers are involved in guiding cleavage of mRNA. The cleavage positions on sense and antisense target RNAs were mapped, and were found to be determined relative to the end of the dsRNA. This could imply that processing of dsRNA to 21-23mers starts from the ends of the duplex RNA. The molecular basis of dsRNA processing and target RNA recognition was studied by analyzing processing of dsRNAs. The siRNAs were shown to have 5' monophosphate and 3' free hydroxyl termini, which suggests that processing of dsRNA is by an RNase III-like activity.

The siRNAs appear as clusters of sequence that span the entire dsRNA sequence. Only one of the clusters acts as a strong cleavage 'hot spot' and is used for target RNA recognition and sequence-specific cleavage. Synthetic double-stranded 21 and 22mers were also found to mediate target mRNA cleavage but single-stranded sense or antisense siRNAs did not affect target RNA expression.

Only one of the two strands in the siRNA duplex is able to guide target RNA cleavage, and the orientation of the duplex in the nuclease complex is determined by the initial direction of dsRNA processing. The processed siRNAs are present in a tight ribonucleoprotein complex, named a 'small interfering ribonucleoprotein particle' or siRNP, and do not dissociate and rebind during the time course

of the reaction. The generation of siRNAs is solely reliant on the 3' end of the sense strand of the dsRNA, which permits processing from the opposing 3' end of the antisense strand. Only the antisense strand of the siRNA duplex is able to guide sense target RNA cleavage and *vice versa.*A conformational rearrangement or a change in the composition of an siRNP is thought to occur prior to target mRNA cleavage because the cleavage site is displaced by 10-12 nucleotides relative to the processing site on dsRNA.

Conclusions

Elbashir *et al*. conclude that RNAi involves at least two steps of RNA cleavage. First, dsRNA is processed into many short pieces, each of about 22 nucleotides. Second, these siRNAs are incorporated into a multisubunit complex, which carries out the cleavage of target mRNA. Cleavage of target RNA guided by siRNAs appears exquisitely specific as no cleavage sites are detected outside the region of complementarity to the siRNAs. The siRNAs appear to interact, by base pairing, with mRNA in which the sequence of nucleotides is the same as in the dsRNA, thus ensuring that only mRNAs related in sequence to the dsRNA are degraded. The authors present an updated model for RNAi in which dsRNA-processing proteins bind to siRNA sense and antisense strands. These proteins indicate the orientation of the siRNAs and only the siRNA strand in the appropriate orientation can guide target RNA cleavage. It is possible that the endonuclease that cleaves the dsRNA may also cleave the target RNA, probably by temporarily displacing the siRNA strand not involved in target recognition. The uncoupling of the mRNA-targeting from the dsRNA-processing step shown by Elbashir *et al*. raises the prospect of using siRNA duplexes as tools for sequence-specific regulation of gene expression in functional genomics and biomedical studies.

Reporter's comments

There has been much uncertainty over the minimal length requirements of dsRNA for processing to 21 and 22 mers, the sequence-specific mediators of RNAi. As well as answering that question, Elbashir *et al*. update the existing model of how sense and antisense RNA trigger degradation of mRNA. But there is still plenty to do to untangle the components of the siRNP complex set out in this widely accepted model.

Table of links

References

1. Elbashir SM, Lendeckel W, Tuschl T: RNA interference is mediated by 21 and 22 nt RNAs. Genes Dev. 2001, 15: 118-200. 0890-9369