Abstract

Double stranded RNA (dsRNA) is a foreign molecule that arises in the cell either as a by-product of viral replication or it is produced by the intramolecular or intermolecular pairing of complementary RNAs, often originating from repetitive sequences. In mammals, dsRNA can enter one of three pathways: the sequence-specific RNA silencing, the sequence-independent interferon (IFN) response, or editing by adenosine deaminases.

The main focus of my PhD project was to comprehensively analyze the effects of the expressed dsRNA in mammals in the context of the whole organism. To follow this aim, we generated a construct expressing dsRNA in a form of an mRNA containing a long perfect hairpin structure.

Transgenic mice ubiquitously expressing dsRNA were viable and, in contrast to the previous studies, the IFN response was not activated. In somatic cells, dsRNA was poorly processed into small interfering RNAs, did not cause transcriptional silencing *in trans*, and underwent low adenosine deamination without the nuclear retention. Consistent results were obtained in human cells transiently transfected with a dsRNA-expressing plasmid. On the other hand, dsRNA expression caused robust RNA interference (RNAi) in oocytes. Thus, we show for the first time that expressed dsRNA, in contrast to many other forms of dsRNA, can be well tolerated in mammals and that oocytes represent a privileged cell type in terms of directing dsRNA into the RNAi pathway.

In addition, transient transfection experiments revealed that dsRNA-expressing plasmids inhibit expression of co-transfected reporter plasmids, but they have a minimal impact on the expression of endogenous genes or reporters stably integrated in the genome. The inhibition likely occurs at the level of translation initiation and it is mediated by the local and transient activation of protein kinase R. Accordingly, our results indicate that cells might be able to distinguish between endogenous and exogenous mRNAs and selectively inhibit the translation of foreign mRNAs in response to dsRNA.

Finally, the transcriptome analysis of cells transfected with commonly used plasmids showed that plasmid transcription is complex and may result in the dsRNA formation via basepairing of complementary sense and antisense transcripts. Our results demonstrate that the formation of dsRNA structure cannot be efficiently predicted and can cause misinterpretation of data based on transient transfection experiments.