

## ABSTRACT

Double-stranded RNA (dsRNA), a double helix formed by two antiparallel complementary RNA strands, is a unique structure with a variety of biological effects. dsRNA can be introduced into the cell from exogenous sources or it can be produced endogenously. There are four basic mechanisms producing dsRNA: inverted repeat transcription, convergent transcription, pairing of sense and antisense RNAs produced *in trans*, and RNA dependent RNA polymerase-mediated synthesis dsRNA. Different mechanisms of production determine additional structural features of dsRNA, such as dsRNA termini, mismatches etc. These features may affect cellular response to dsRNA. Recognition of dsRNA can trigger several responses that act in sequence-specific or sequence-independent manners. The main sequence-specific response triggered by dsRNA is RNA interference (RNAi) is. Our laboratory has been studying mechanism of induction of RNAi in mammalian cells using one specific type of long dsRNA expression system. The dsRNA used in these experiments formed hairpin structure with long 5' and 3' single-strand RNA overhangs. We hypothesized that other dsRNA substrates might be more efficient than the one used in mammalian RNAi experiments since 2002.

Accordingly, the main aim of my thesis was to compare efficiency of different dsRNA substrates in induction of RNAi-like effects. To address this point, I produced various dsRNA substrates representing different mechanisms of dsRNA formation. Our experiments included two types of intramolecular duplexes: one with a blunt end and another one carrying longer overhang at the 3' terminus. In addition, I produced dsRNA by base-pairing of sense and antisense RNA strands transcribed either by convergent transcription or at two separate loci (plasmids). These strategies for dsRNA production mimic dsRNA derived from base-pairing of complementary transcripts expressed in the nucleus. As a measure of sequence-specific and sequence-independent effects, I used luciferase reporters where a dual-luciferase assay was used to monitor reporter expression and qPCR to specifically quantify reporter transcripts. Our results suggest that hairpin substrates with blunt ends can induce robust RNAi. Furthermore, such hairpins do not activate sequence-independent effects involving protein kinase R and interferon activation while they can induce RNAi also in the absence of TARBP2.

**Key words:** double-stranded RNA, Interferon response, RNA interference, Dicer