



The Short Answer for RNAi

RNA Interference in *Drosophila* S2 Cells: Effect of dsRNA Size, Concentration and Exposure Time

By Natalie Betz, Ph.D., Promega Corporation

Abstract

The T7 RiboMAX™ Express RNAi System was used to create double-stranded RNA of varying sizes using the *Drosophila* Erk-A gene as a template. The effect of dsRNA size, concentration, and exposure time on RNA interference of Erk-A gene expression in *Drosophila* S2 cells was investigated.

Shorter dsRNA appear as effective as longer dsRNA at inducing RNA interference in *Drosophila* S2 cells.

Introduction

RNA interference (RNAi), the phenomenon in which double-stranded RNA (dsRNA) can specifically suppress expression of a target gene, was originally discovered in *C. elegans* (1) but has been observed in numerous organisms in recent years (reviewed in references 2 and 3). Many studies investigating the mechanistic characteristics of RNAi have been performed in *Drosophila* (a very useful model system [4,5]).

The purpose of this set of experiments was to determine the efficiency of differently sized dsRNAs against the Erk-A mRNA to reduce protein levels in *Drosophila* S2 cells through RNAi. Erk-A is the *Drosophila* homolog of mitogen-activated protein kinase (MAPK) and part of the sevenless (Sev) signal transduction pathway (6). Previous work has demonstrated that Erk-A is a good target for RNAi in *Drosophila* S2 cells (7,8).

In general, dsRNA used to induce RNAi in non-mammalian systems is >400bp and usually encompasses the majority of the target mRNA sequences (9,10). However, the in vitro synthesis of shorter dsRNA is more efficient, so their use might be advantageous if shorter dsRNAs are as effective as longer dsRNAs for inducing RNAi. The duration of the RNAi effect in S2 cells for both shorter and longer dsRNAs was also investigated. Earlier reports of reduced Erk-A protein levels with Erk-A dsRNA were measured following a 3-day incubation period with the dsRNA (7,8). In this study incubations of up to 5 days were investigated.

Synthesis of Erk-A dsRNAs and Introduction into S2 Cells

Erk-A dsRNAs and a nonspecific control dsRNA for *Renilla* luciferase (*Rluc*) were synthesized, purified, and quantitated as described in reference 8 using the T7 RiboMAX™ Express RNAi System^(a,b,c) (Cat.# P1700). The Erk-A dsRNAs were 180bp, 505bp and 778bp in length. The *Rluc* negative control dsRNA was 500bp in length. *Drosophila* S2 cells were treated with increasing concentrations of each dsRNA (0, 9.5, 38 or 190nM) in triplicate for 3 days. In addition, S2 cells were treated with 38nM Erk-A 180bp dsRNA or 38nM *Rluc* 500bp dsRNA for 1–5 days. The dsRNA concentration refers to the initial 1ml treatment. Triplicate wells were pooled and a cell lysate prepared. The cell lysates were then subjected to Western blot analysis for Erk-A protein levels (8) and the quantity of Erk-A protein in each sample analyzed using enhanced chemifluorescence detection reagents (Amersham) and a Molecular Dynamics Storm® fluorescence scanner (blue mode).

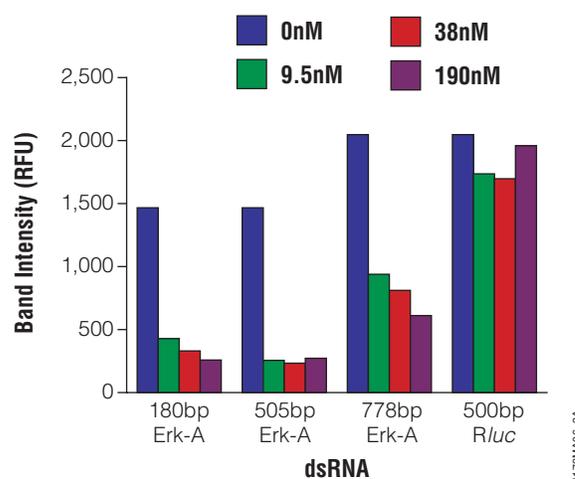


Figure 1. Effect of Erk-A dsRNA length and concentration on Erk-A protein levels in S2 cells. Erk-A dsRNAs and a nonspecific control dsRNA (*Rluc*) were synthesized, purified and quantitated using the T7 RiboMAX™ Express RNAi System. The Erk-A dsRNAs were 180bp, 505bp or 778bp. The *Rluc* negative control dsRNA was 500bp. *Drosophila* S2 cells were treated with increasing concentrations of each dsRNA (0, 9.5, 38 or 190nM) in triplicate for 3 days. The dsRNA concentration refers to the initial 1ml treatment. Replicate wells were pooled and a cell lysate prepared. The cell lysates were then subjected to Western blot analysis for Erk-A protein levels (4). The quantity of Erk-A protein in each sample was quantitated using enhanced chemifluorescence detection reagents (Amersham) and a Molecular Dynamics Storm® fluorescence scanner (blue mode). The basal level of Erk-A in the 180bp and 505bp Erk-A samples is different than in the other two samples because these samples were processed on different blots.

RNA Interference in *Drosophila* S2 Cells...continued

Suppression of Erk-A Protein Levels in S2 Cells

As seen in Figure 1, all three Erk-A dsRNAs reduced Erk-A protein levels in a dose-responsive manner in the S2 cells following a 3-day incubation period. As expected, the nonspecific *Renilla* luciferase dsRNA (negative control) did not significantly reduce Erk-A protein levels compared to the untreated control.

To better visualize the efficiency of inhibition by the various Erk-A dsRNAs, the percentage decrease in the Erk-A protein band with the various concentrations of dsRNAs compared to the untreated control was calculated and is shown in Figure 2. The Erk-A 180bp dsRNA was as effective at equimolar concentrations as the longer 505bp and 778bp Erk-A dsRNAs at inducing RNAi in S2 cells.

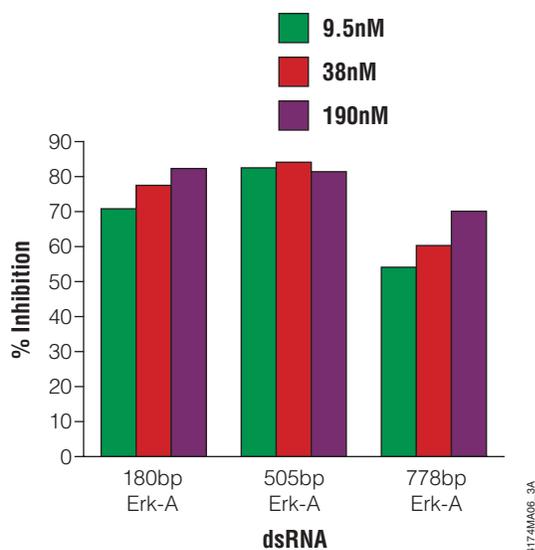


Figure 2. Effect of Erk-A dsRNA length and concentration on Erk-A protein levels expressed as a percentage of the untreated control. The data from Figure 1 was calculated as a percentage of the untreated control and is expressed as percent inhibition.

To investigate how long suppression in Erk-A protein levels lasts following treatment with Erk-A dsRNA, S2 cells were incubated with equimolar amounts of either 180bp Erk-A dsRNA or 500bp *Rluc* dsRNA for 1–5 days. Maximal protein inhibition was observed at day 3, but Erk-A protein levels are still reduced at day 4 and day 5 (Figure 3). Thus at least in S2 cells, the RNAi effect is rather long-lived.

Conclusion

Shorter dsRNAs appear to be as effective as longer dsRNAs at inducing RNA interference in *Drosophila* S2 cells. In these experiments, the RNAi effect was detected for up to 5 days following exposure to dsRNA. Similar RNAi longevity in S2 cells was observed by Clemens *et al.* for a different target mRNA and protein (7).

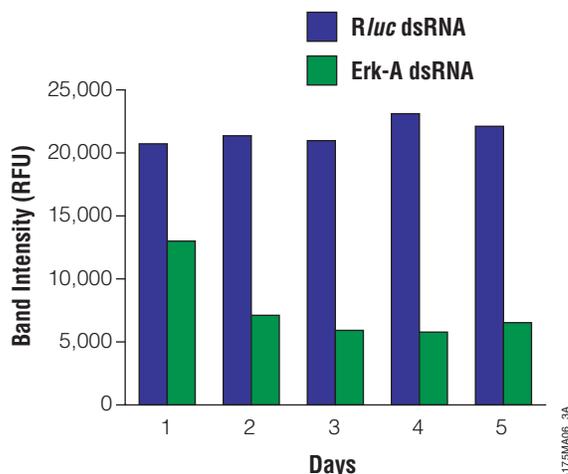


Figure 3. Duration of suppression in Erk-A protein levels following treatment with Erk-A dsRNA. S2 cells were incubated in triplicate in the presence of either 38nM Erk-A 180bp dsRNA or 38nM *Rluc* 500bp dsRNA for 1–5 days. At each time point the triplicate wells were pooled, a cell lysate prepared, and the lysates were evaluated for Erk-A protein levels.

References

1. Fire, A. *et al.* (1998) *Nature* **391**, 806–11.
2. Mello, C.C. and Conte, D., Jr. (2004) *Nature* **431**, 338–42.
3. Meister, G. and Tuschl, T. (2004) *Nature* **431**, 343–9.
4. Lee, Y.S. *et al.* (2004) *Cell* **117**, 69–81.
5. Pham, J.W. *et al.* (2004) *Cell* **117**, 83–94.
6. Biggs, W.H. *et al.* (1994) *EMBO J.* **13**, 1628–35.
7. Clemens, J.C. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**, 6499–503.
8. Betz, N. and Worzella, T. (2003) *Promega Notes* **84**, 7–11.
9. Elbashir, S.M. *et al.* (2001) *Genes Dev.* **15**, 188–200.
10. Yang, D., Lu, H. and Erickson, J.W. (2000) *Curr. Biol.* **10**, 1191–200.

Protocol

- ◆ T7 RiboMAX™ Express RNAi System Technical Bulletin #TB316 Promega Corporation. (www.promega.com/tbs/tb316/tb316.html)

Online

- ◆ Information and figures also available in *eNotes* at: (www.promega.com/enotes/applications/ap0050_tabs.htm)

Ordering Information

Product	Size	Cat.#
T7 RiboMAX™ Express RNAi System	50 × 20µl reactions	P1700

- (a) This product is covered under license from Carnegie Institution of Washington. Commercial use of this product may require a separate license from Carnegie.
- (b) U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.
- (c) U.S. Pat. No. 5,552,302, Australian Pat. No. 646803 and other patents.

RiboMAX is a trademark of Promega Corporation.
Storm is a registered trademark of Amersham Biosciences Ltd.