Supplementary Figure S1

p19 W39/42R has a mild effect on siRNA-guided RNA cleavage in the Drosophila in vitro RNA silencing system if co-introduced with guide siRNA.

(A) Direct competition target RNA cleavage assays used target RNA (0.5 nM), p19 W39/42R (0.125-256.2 nM) and siRNA (5 nM) added simultaneously to embryo extracts.

(B) Preassembled RISC target RNA cleavage assays used siRNA (5 nM) preincubated 30 min with embryo extract prior to addition of target RNA (0.5 nM) and p19 W39/42R (0.125-256.2 nM).

(C) Effect of p19 W39/42R on RNA cleavage activity of RISC (black squares) and preassembled RISC (red triangles), plotted as a function of the concentration of p19 W39/42R.

(D) p19 W39/42R slightly inhibits RISC formation in the Drosophila in vitro RNA silencing system if co-introduced with siRNA. Direct competition RISC formation assays used 32P-labeled siRNA (5 nM), and p19 W39/42R mutant (0.125-256.2 nM) added simultaneously to embryo extracts. DCR2-R2D2 (black diamond), RLC (green circles), and RISC (red squares) formation are plotted as a function of the concentration of p19. Lane 3 contains siRNA and p19 W39/42R.

(E) Effect of p19 W39/42R on preassembled RISC. Preassembled RISC formation assays used labelled siRNA (5 nM) preincubated 30 min with embryo extract prior to addition of p19 W39/42R (0.125-256.2 nM). Data are plotted as in (A). Lane 3 contains siRNA and p19 W39/42R.
For electrophoretic mobility shift experiments 10 pM 21-nt duplexed siRNA, 1.9-500 nM of GST-p21 protein, or 3.9-2000 nM of GST-p21 8A-21 mutant protein were used.

**Results**

To confirm that siRNA binding is necessary for target cleavage suppression by p19, we tested the ability of a mutant of p19 to inhibit target cleavage and silencing complex formation in the Drosophila *in vitro* RNA silencing system. We performed direct competition and preassembled RISC assays as for p19 wild type, using the p19 mutant W39/42R (Vargason et al., 2003) at the same concentrations in target cleavage (Supplementary Figure S1) and RISC formation experiments (Supplementary Figure S2). In direct competition experiments (Supplementary Figure S1 A,D) p19 W39/42R had a slight inhibitory effect on target cleavage and RISC formation. In target cleavage assays, the half-maximal inhibitory concentration for p19 W39/42R was IC$_{50}$=75.9±3.4 nM, nearly a five-fold increase over the value observed using p19 wild type (IC$_{50}$=15.24±2.3 nM). The mild inhibitory effect of the mutant p19 W39/42R is likely due to a lower affinity for siRNA than the wild type p19 (K$_d$ p19=0.9 nM and K$_d$ p19 W39/42R=6 nM, data not shown). As expected, p19 W39/42R had no effect in the indirect competition experiments on target cleavage or RISC formation (Supplementary Figure S1 B, E).

To determine whether the compromised suppressor function of the p21 mutant 8A-21 correlates with loss of siRNA duplex binding, the siRNA-binding activity of the GST-p21 8A-21 mutant was compared to the wild type GST-p21 by electrophoretic mobility shift experiments. Although GST-p21 bound siRNA in a dose-dependent manner, GST-p21 8A-21 mutant did not show any siRNA binding activity, even at high protein concentration (Supplementary Figure S1F).

**References**