

processing, and incubated at 30°C for 20 min. Complexes were resolved by 4% native PAGE at 4°C.

siRNAs and northern blot analysis. The sequences of the siRNAs used in this study are: Dicer-A, 5'-UUUGUUGCGAGGCGUGAUUGUJTFT-3'; Dicer-B, 5'-UUCUAGUAGACACUUGAUUGUJTFT-3'; Dicer-C, 5'-GCUUAGUAGACACUUGAUUGUJTFT-3'; TRBP-A, 5'-GCUUAGUAGACACUUGAUUGUJTFT-3'; TRBP-B, 5'-UUCUAGUAGACACUUGAUUGUJTFT-3'; Luciferase, 5'-UCGAGGUAUUCGCGUACGATGTT-3'; TRP-1, 5'-UGUGGGGAGGCGUUGGCGTGT-3'. Transfection of HeLa 293 cells was performed with Lipofectamine 2000 (Invitrogen), as described. To examine the effect of siRNA on target gene expression, total RNA and complementary DNA synthesis was prepared 48–72 h after transfection, as described. Primer sequences for RT-PCR in Fig. 4 are: Dicer, 5'-CATGAGTCTGGAGTGCAC-3' and 5'-CTACTTCCACAGTGAAGTCTGT-3'; TRBP, 5'-GGGCTGCCTAGTATAGAGC-3' and 5'-CCCTGACACAGTGCAGCTGGT-3'; β -actin, 5'-AAAGACCTGTACGCCAACAC-3' and 5'-GTCACTACTCTGCTTGTGAT-3'. For northern blot analysis, total RNA (5–10 μ g of each sample, isolated using TRIzol reagent) was resolved on 15% denaturing polyacrylamide gel and electrotransferred onto Hybond N+ nylon membranes (Amersham). Hybridization with specific 32 P-ATP end-labelled DNA oligonucleotides was carried out as described previously.

Luciferase assay. Luciferase assays were performed as described¹⁷ with the following modification: HeLa cells were first co-transfected with firefly and Renilla luciferase reporter gene expression plasmids. A second transfection of siRNA against TRBP, Dicer or TRP-1 together with siRNA against firefly luciferase was performed.

Received 22 March; accepted 3 June 2005.
Published online 22 June 2005.

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Acknowledgements We thank T. Beer for assistance with the microcapillary HPLC/mass spectrometry. R.S. was supported by a grant from NIH and American Cancer Society. R.I.G. is a fellow of the Jane Coffin Childs Fund for Medical Research.

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