REPORT

RNAi: Mammalian oocytes do it without RNA-dependent RNA polymerase

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ABSTRACT

Studies in mutant organisms deficient in RNA interference (RNAi) and related post-transcriptional gene silencing implicated a role for a single class of RNA-dependent RNA polymerases (RdRp). Nevertheless, sequence homologs to these RdRps have not been found in coelomate organisms such as *Drosophila* or mammals. This lack of homologous sequences does not exclude that an RdRp functions in RNAi in these organisms because an RdRp could be acquired by horizontal transfer from an RNA virus. In fact, such a sequence is found in mice (*Aquarius*) and we observe that it is expressed in mouse oocytes and early embryos, which exhibit RNAi. We report here that cordycepin, an inhibitor of RNA synthesis, does not prevent *Mos* double-strand RNA (dsRNA) to target endogenous *Mos* mRNA in mouse oocytes and that targeting a chimeric *Mos*–EGFP mRNA with dsRNA to EGFP does not reduce the endogenous *Mos* mRNA, but does target the chimeric mRNA. These results indicate that an RdRp is not involved in dsRNA-mediated mRNA degradation in mammalian oocytes, and possibly in mammals in general, and therefore that only homologous sequences to the dsRNA are targeted for degradation.

Keywords: RNA interference; RNA-dependent RNA polymerase; mouse oocyte

INTRODUCTION

In most eukaryotic organisms, double-strand RNA (dsRNA) mediates sequence-specific RNA degradation termed RNA interference (RNAi). The mechanism of this pathway has been deciphered mainly in lower organisms (for review, see Hannon 2002; Hutvagner and Zamore 2002). Much less is known, however, about the RNAi pathway in mammals, except that it operates and that dsRNA is processed into 21–23 nucleotide short interfering RNAs (siRNAs) similar to other RNAi-exhibiting organisms (Billy et al. 2001; Svoboda et al., unpubl.). Besides human and mouse Dicer orthologs, none of the genes involved in RNAi in mammals has been identified and cloned (Bernstein et al. 2001; Nicholson and Nicholson 2002; P. Svoboda, P. Stein, and R.M. Schultz, unpubl.). Three components of RNAi, namely, Dicer, argonaute/piwi/paz family, and RNA-dependent RNA polymerases (RdRps), are very well conserved between distant taxa exhibiting RNAi and related phenomena.

RdRp proteins have been identified in a variety of organisms that exhibit RNAi-like phenomena and include EGO-

1, RRF-1, and RRF-3 in *Caenorhabditis elegans* (Smardon et al. 2000; Sijen et al. 2001; Simmer et al. 2002); SGS2/SDE1 in *Arabidopsis* (Dalmay et al. 2000; Mourrain et al. 2000); QDE-1 in *Neurospora* (Cogoni and Macino 1999); and RrPA in *Dictyostelium* (Martens et al. 2002). Two recent reports specified a function for an RdRp activity in RNAi in *C. elegans* (Sijen et al. 2001) and *Drosophila* (Lipardi et al. 2001) such that the siRNAs prime an RdRp for RNA synthesis to produce more dsRNA, thereby amplifying the response. There are, however, no known homologs of RdRps involved in RNAi in coelomates whose genomes have been sequenced completely (*Drosophila*, human, and mouse). Furthermore, an RdRp-based model for “random degradative PCR” in *Drosophila* (Lipardi et al. 2001) is not consistent with a previous report that the targeted mRNA is cleaved only within the region of homology with the dsRNA in vitro (Zamore et al. 2000) and that RNAi is exon-specific in vivo (Celotto and Graveley 2002). Therefore, the role of RdRp in RNAi in *Drosophila*, the closest organism to mammals in which RdRp was studied, remains unclear.

Current data imply that RdRps involved in dsRNA-mediated post-transcriptional silencing evolved once. Many of the aforementioned RdRp proteins were independently found mutated in organisms with impaired RNAi or some related silencing mechanism, and they exhibit sequence similarity, indicating that they evolved from a common ancestral gene. Moreover, there is no evidence for another

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class of RdRps involved in dsRNA-mediated post-transcriptional silencing despite the plausible hypothesis that such an RdRp could be repeatedly acquired horizontally from an RNA virus. Viral RdRps probably participate in RNAi-like antiviral response in plants (Dalmay et al. 2000) and therefore complement endogenous RdRp. Horizontal transfer would explain the lack of RdRp orthologs in *Drosophila* and mammals if RdRp activity is essential for RNAi. It is also possible that the RdRp component of RNAi became non-essential and was lost during the evolution of true coelomates.

Aquarius, a novel mammalian gene, may be distantly related to RdRps, because it has a conserved RdRp active site motif and a weak homology to the RdRp of murine hepatitis viruses (Sam et al. 1998), suggesting its origin by horizontal transfer. The observation that *Aquarius* transcripts are detected by RT-PCR in mouse oocytes and preimplantation embryos (P. Svoboda, P. Stein, and R.M. Schultz, unpubl.) led us to test whether or not an RdRp activity is involved in RNAi in mammals and we have obtained two lines of evidence that it is not. After this work was submitted, Schwarz et al. (2002) reached a similar conclusion using *Drosophila* embryo and HeLa cell extracts.

RESULTS

The “random degradative PCR” hypothesis (Lipardi et al. 2001) predicts that an RdRp transcribes sequences upstream of the targeted region to give rise to dsRNA that is fed back into the RNAi pathway such that sequences in the targeted mRNA, but not present in the original dsRNA, are now targeted. To test this hypothesis in vivo, we designed two experiments. In the first experiment, we tested the efficiency of RNAi in the presence of cordycepin (3'-deoxyadenosine), a chain terminator of RNA elongation. Therefore, if an RdRp is an integral component of the RNAi pathway, cordycepin should inhibit the degradation of the targeted mRNA.

Fully-grown, germinal vesicle (GV) intact mouse oocytes were microinjected with $\sim 1 \times 10^6$ molecules of *Mos* dsRNA, incubated for 24 h in the presence or absence of 2 mM cordycepin, and then *Mos* mRNA levels were determined (Fig. 1). Results of these experiments indicated that *Mos* mRNA was decreased by $\sim 80\%$ in both instances, and this decrease was similar to that described previously (Svoboda et al. 2000). Under these conditions, cordycepin, which inhibits polyadenylation in one-cell embryos (Fuchimoto et al. 2001), inhibited [^3H]uridine incorporation into acid-insoluble material by 85%–95%.

In the second experimental approach, we addressed the amplification of sequences upstream of the targeted region by generating a chimeric mRNA composed of *Mos* sequence at the 5' end and enhanced green fluorescence protein (EGFP) sequence at the 3' end (Fig. 2). We microinjected mouse oocytes with this chimeric transcript and either

FIGURE 1. RT-PCR analysis of *Mos* mRNA in oocytes injected with *Mos* dsRNA and treated with cordycepin. The RT-PCR assays were conducted as described previously under conditions that reflect changes in the relative amounts of transcript (Svoboda et al. 2000). Oocytes were microinjected with $\sim 10^6$ molecules of *Mos* dsRNA and the relative *Mos* mRNA levels were assayed 20 h later in either untreated control oocytes or cordycepin-treated (2 mM) oocytes; cordycepin was present from the initial time of oocyte collection. Rabbit globin mRNA was used as an external standard to allow comparison of relative differences in *Mos* mRNA levels (Svoboda et al. 2000).

EGFP siRNA or EGFP dsRNA. This co-injection should generate dsRNA from sequences upstream of EGFP and the subsequent targeting of the endogenous *Mos* mRNA if an RdRp-dependent amplification mechanism was operative.

MAP kinase activity, which is an indirect measure of *Mos* mRNA, was assayed to test the efficiency of *Mos* mRNA targeting in oocytes microinjected with either chimeric transcript or EGFP dsRNA, or both (Fig. 2). The translationally dormant *Mos* mRNA is recruited during oocyte maturation and results in the activation of MAP kinase that can be assayed in individual oocytes. Fully-grown GV-intact oocytes were microinjected with $\sim 7.5 \times 10^6$ molecules of the chimeric mRNA $\pm$ EGFP dsRNA and then cultured for 20 h in the presence of 0.2 mM 3-isobutyl-1-methyl-xanthine (IBMX). IBMX inhibits resumption of meiosis, which allows time to target the endogenous *Mos* mRNA (Svoboda et al. 2000). After 20 h, the oocytes were transferred to IBMX-free medium and matured in vitro for an additional 18 h, after which time the oocytes were assayed for MAP kinase activity.

We observed the normal maturation-associated increase in MAP kinase activity in all microinjected oocytes (Fig. 3), which indicates that the endogenous *Mos* mRNA was not

FIGURE 2. Schematic diagram of the chimeric *Mos*–EGFP mRNA and EGFP dsRNA and siRNA used to target the EGFP sequence. The dsRNA covered the entire coding region of EGFP exactly from the fusion point and the siRNA corresponded to bases 81–103 of the EGFP sequence from the fusion point.

FIGURE 3. MAP kinase activity in oocytes microinjected with chimeric mRNA and EGFP dsRNA and matured in vitro to metaphase II. MAP kinase activity was assayed in single eggs using myelin basic protein (MBP) as a substrate. Similar results were obtained when EGFP siRNA was injected. The experiment was performed three times and at least 15 individual oocytes were analyzed in each group. Shown are the results of a representative experiment.

efficiently targeted. Experiments using EGFP siRNAi yielded identical negative results (data not shown). In these experiments, we microinjected up to two orders of magnitude more dsRNA and chimeric message than *Mos* dsRNA needed to observe a consistent decrease of MAP kinase activity in microinjected oocytes. In fact, three orders of magnitude less dsRNA can efficiently induce *Mos* mRNA degradation (Svoboda et al. 2000). Therefore, an RdRp-mediated RNA synthesis upstream of the targeted sequence was either not efficient or not involved in RNAi in mouse oocytes.

MAP kinase activity, however, is only an indirect measure of *Mos* mRNA level and therefore it was possible that the *Mos* mRNA was inefficiently targeted so that no detectable change in MAP kinase activity was observed. Therefore, we measured the relative abundance of *Mos* transcript in microinjected oocytes to test if there was any detectable change in *Mos* mRNA level (Fig. 4A). As described above, $\sim 7.5 \times 10^6$ molecules of the chimeric mRNA, dsRNA or both of them were microinjected into fully-grown, GV-intact oocytes that were incubated for 20 h in the presence of IBMX, after which time the relative amount of *Mos* mRNA was determined as described previously (Svoboda et al. 2000); the presence of fluorescence in the injected oocytes indicated that the injected chimeric mRNA was translated (data not shown). Results of these experiments did not reveal any decrease in the relative abundance of either *Mos* mRNA or the nontargeted *Plat* mRNA in any of the microinjected samples.

It was formally possible, however, that the microinjected chimeric mRNA was not targeted by the EGFP siRNA or the EGFP dsRNA and therefore the siRNAs required to prime an RdRp were not produced. The large number of injected chimeric mRNA molecules prohibited detecting any decrease in the abundance of the injected chimeric mRNA by RT-PCR (Fig. 4B). The activity of the RNAi pathway degrades endogenous transcripts at a rate of $\sim 0.5\text{--}1 \times 10^3$ transcripts/h (Svoboda et al. 2000). Therefore, in the 20-h incubation, only $\sim 1\%$ of the injected chimeric mRNA would be degraded. To verify that the chimeric mRNA was targeted by the EGFP dsRNA, we decreased the amount of

microinjected chimeric message to $\sim 10^4$ molecules. In this case, a detectable and specific targeting of the chimeric mRNA was observed (Fig. 4B). The absence of targeting of the endogenous *Mos* mRNA, therefore, was most likely attributable to the absence or the inefficiency of RdRp amplification.

DISCUSSION

One of the proposed roles for RdRp in RNAi is to amplify the RNAi response (Zamore et al. 2000; Hutvagner and Zamore 2002). Consistent with a loss of RdRp, RNAi in mouse oocytes exhibits slower kinetics and lower efficiency compared with that in *C. elegans* (Svoboda et al. 2000; Ui-Tei et al. 2000). The absence of an RdRp is probably only one of the reasons that the RNAi efficiency is lower in mouse oocytes, because RNAi in *Drosophila* is much more

FIGURE 4. RT-PCR analysis of *Mos* mRNA in oocytes injected with EGFP dsRNA and chimeric *Mos*–EGFP mRNA. (A) Absence of *Mos* mRNA degradation in oocytes microinjected with chimeric transcript and/or EGFP dsRNA. GV-intact oocytes were microinjected with $\sim 7.5 \times 10^6$ molecules of chimeric transcript and/or EGFP dsRNA and then cultured in the presence of IBMX for 20 h. RNA was then isolated and the relative amount of *Mos* and *Plat* transcripts were determined by semi-quantitative RT-PCR as described previously (Svoboda et al. 2000). The amount of the nontargeted *Plat* PCR product permits relative comparison of microinjected samples (Svoboda et al. 2000). (B) EGFP dsRNA induces degradation of the chimeric transcript. Relative amounts of the chimeric transcript in microinjected oocytes were estimated based on co-injection of rabbit globin mRNA (10 ng/ μ L). High amounts ($\sim 7.5 \times 10^6$ molecules) of microinjected chimeric message do not allow detection of degradation of the chimeric mRNA (left panels), whereas dramatic reduction (right panels) of chimeric mRNA is observed in oocytes microinjected with lower amounts ($\sim 10^4$ molecules).

efficient and yet *Drosophila* likely lacks RdRp. We have observed that mouse preimplantation embryo lysates exhibit much lower Dicer activity when compared with equivalent protein amounts of *Drosophila* embryo extract (data not shown), which may account for the apparent lower activity of the RNAi pathway in mouse oocytes and preimplantation embryos.

The observation that RNAi functions in mouse oocytes in the presence of cordycepin indicated that an RdRp amplification mechanism doesn't operate. Moreover, the results of the chimeric mRNA injection experiments indicate that even if it does function, it is not of sufficient activity to promote the degradation of the endogenous *Mos* mRNA. The conclusion that an RdRp amplification mechanism does not function in mouse oocytes is consistent with our previous results. We have shown previously that injection of stoichiometric amounts of *Mos* dsRNA ($\sim 10^4$ copies) results in targeting the endogenous *Mos* mRNA for which there are $\sim 10^4$ copies of the mRNA. Injection of 10^2 molecules of *Mos* dsRNA, however, does not result in any detectable decrease in the amount of endogenous *Mos* mRNA. For *Mos* to be targeted efficiently by dsRNA produced by an RdRp, about 10^3 – 10^4 dsRNA molecules would be needed. Nevertheless, when stoichiometric amounts of chimeric mRNA plus an excess of dsRNA or an excess of chimeric mRNA and dsRNA ($\sim 7.5 \times 10^6$ molecules) were provided, we did not detect any decrease in MAP kinase activity or the amount of *Mos* mRNA, that is, any potential RdRp activity present in the oocyte is not efficient enough to generate enough dsRNA to trigger RNAi. The fact that we do not see targeting under these conditions implies an absence of efficient amplification of sequences upstream to the dsRNA because an endogenous mechanism would likely operate with dsRNA molecules $< 10^4$.

Our findings have several important implications. First, the mammalian RNAi pathway likely differs from that of *C. elegans*, plants, and fungi that all employ RdRps to amplify the RNAi response. Our data indicate that in response to dsRNA, an RdRp activity is not detectably involved in mammalian oocytes, and perhaps in mammals in general. An RdRp, however, could have a role in mammalian RNAi to generate trigger dsRNA from "aberrant transcripts" that are targeted by RNAi. Our experiments provide no information regarding such a function, and because the class of "aberrant transcripts" that would trigger RNAi is not characterized and the mechanism is unknown, we cannot test this possibility at this time. Second, it would be interesting to try to enhance the RNAi pathway in mammals by expressing RdRp. In fact, transgenic mice expressing catalytically active tomato RdRp and dsRNA in erythroid tissue were generated but no RNAi effect was observed in this model system (de Wit et al. 2002). It will also be interesting to see if tomato RdRp increases the sensitivity to dsRNA in mouse oocytes, which respond to dsRNA. Last, and most important, the lack of RdRp in mammals permits the spe-

cific targeting of other RNAs that share identical or highly similar sequences, as well as the specific targeting of alternatively spliced forms.

MATERIALS AND METHODS

Preparation of EGFP dsRNA and chimeric mRNA

EGFP dsRNA was prepared by in vitro transcription of an EGFP coding sequence in both directions and subsequent annealing. The plasmid used for in vitro transcription was generated by inserting a *Bam*HI/*Not*I fragment from pEGFP-N2 plasmid (Clontech, CA) into *Bam*HI and *Not*I sites of pCRII plasmid (Invitrogen, CA). pCRII, which contains SP6 and T7 promoters for in vitro transcription, was linearized using *Bam*HI or *Not*I. About 5 μ g of the template was in vitro-transcribed, annealed, and purified as described previously (Svoboda et al. 2000). This purification included treatment with RNase T1, which removes short single-strand overhangs produced during the generation of dsRNA in plasmids like pCRII. EGFP dsRNA starts immediately at the fusion point with the *Mos* fragment to assure synthesis of the *Mos* sequence even in the presence of an RdRp activity with very low processivity.

The plasmid construct to generate the chimeric mRNA was prepared as follows: a 535-bp fragment of *Mos* was amplified from oocyte cDNA by Pfu polymerase in a PCR reaction using the following primers: forward 5'-GCTCGAGTAATACGACTCAC TATAGGGAGACCATCAAGCAAGTAAACAAG-3', reverse 5'-GGATCCAGGGTGATTCCAAAACAGTA-3'. The forward primer contains T-7 promoter sequence and an *Xho*I site to facilitate cloning, and the reverse primer carries a *Bam*HI site. The *Xho*I/*Bam*HI-digested PCR product was cloned into *Xho*I/*Bam*HI sites of pEHGP-N2 plasmid (Clontech) generating pMos/EGFP. The globin 3' UTR and poly A tail in pMos/EGFP were generated by replacing the *Not*I/*Afl*II blunted fragment with a *Not*I/*Bam*HI blunted fragment from pXT7. For in vitro transcription, 2 μ g of the plasmid was linearized using *Sal*I. T7 mMessage mMachine kit (Ambion, TX) was used for preparation of the chimeric mRNA according to the manufacturer's protocol. RNA was purified by phenol/chloroform extraction and dissolved in sterile RNase-free water. The quality and the amount were estimated by spectrophotometry and gel electrophoresis, respectively.

Oocyte collection, microinjection, and culture

Fully grown, GV-intact oocytes were obtained from pregnant mare's serum gonadotropin (PMSG)-primed 6-week-old female CF-1 mice (Harlan) and freed of attached cumulus cells, as described previously (Schultz et al. 1983). The collection medium was bicarbonate-free minimal essential medium (Earle's salt) supplemented with polyvinylpyrrolidone (3 mg/mL) and 25 mM HEPES, pH 7.3. Germinal vesicle breakdown was inhibited by including 0.2 mM 3-isobutyl-1-methyl-xanthine (IBMX). The oocytes were transferred to CZB medium (Chatot et al. 1989) containing 0.2 mM IBMX (CZB+IBMX) and cultured in an atmosphere of 5% CO₂ in air at 37°C. Oocytes were microinjected in Whitten's medium (Whitten 1971) containing 5 mM NaHCO₃, 15 mM HEPES, 0.01% polyvinyl alcohol (PVA), and 0.2 mM IBMX;

the injections were performed as described previously (Kurasawa et al. 1989). In the experiments where the effect of cordycepin was tested, both culture and microinjection of oocytes were done in the presence of 2 mM cordycepin (Sigma). In experiments in which mRNA levels were measured, the oocytes were kept in CZB+ IBMX for 20 h until they were collected and processed for RNA isolation. In experiments in which MAP kinase activity was assayed, microinjected oocytes were cultured in CZB+IBMX for 20 h. They were then washed through 10 drops of IBMX-free CZB and matured in vitro in CZB for 18 h.

RNA isolation and semi-quantitative RT-PCR

RNA isolation and semiquantitative RT-PCR were performed as described previously (Svoboda et al. 2000). *Mos* primers used in the chimeric mRNA experiments were from outside the *Mos* sequence in the chimeric message (Svoboda et al. 2001). *Mos* primers and PCR conditions in the cordycepin experiments were corresponding to the targeted region as described previously (Svoboda et al. 2000).

MAP kinase assay

MAP kinase activity, assayed by ability to phosphorylate myelin basic protein (MBP), was determined in individual oocytes as described previously (Svoboda et al. 2000). This enzyme activity, which increases during oocyte maturation, serves as an indirect measure of the levels of MOS protein (Svoboda et al. 2000).

[³H]Uridine incorporation

Oocytes were cultured in CZB containing 1 mCi/mL [5,6-³H]uridine (32 Ci/mmol, Amersham) for 2 h in the presence of 2 mM cordycepin or its absence, after which the oocytes were processed for acid-insoluble and acid-soluble radioactivity as described previously (Brower et al. 1981). Percent incorporation was calculated as the ratio of acid-insoluble cpm/(acid-insoluble cpm + acid-soluble cpm).

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REFERENCES

Bernstein, E., Caudy, A.A., Hammond, S.M., and Hannon, G.J. 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **409**: 363–366.

Billy, E., Brondani, V., Zhang, H., Muller, U., and Filipowicz, W. 2001. Specific interference with gene expression induced by long, double-stranded RNA in mouse embryonal teratocarcinoma cell lines. *Proc. Natl. Acad. Sci.* **98**: 14428–14433.

Brower, P.T., Gizang, E., Boreen, S.M., and Schultz, R.M. 1981. Biochemical studies of mammalian oogenesis: Synthesis and stability of various classes of RNA during growth of the mouse oocyte in vitro. *Dev. Biol.* **86**: 373–383.

Celotto, A.M. and Graveley, B.R. 2002. Exon-specific RNAi: A tool for dissecting the functional relevance of alternative splicing. *RNA* **8**: 718–724.

Chatot, C.L., Ziomek, C.A., Bavister, B.D., Lewis, J.L., and Torres, I. 1989. An improved culture medium supports development of random-bred 1-cell mouse embryos in vitro. *J. Reprod. Fertil.* **86**: 679–688.

Cogoni, C. and Macino, G. 1999. Gene silencing in *Neurospora crassa* requires a protein homologous to RNA-dependent RNA polymerase. *Nature* **399**: 166–169.

Dalmay, T., Hamilton, A., Rudd, S., Angell, S., and Baulcombe, D.C. 2000. An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. *Cell* **101**: 543–553.

de Wit, T., Grosveld, F., and Drabek, D. 2002. The tomato RNA-directed RNA polymerase has no effect on gene silencing by RNA interference in transgenic mice. *Transgenic Res.* **11**: 305–310.

Fuchimoto, D., Mizukoshi, A., Schultz, R.M., Sakai, S., and Aoki, F. 2001. Posttranscriptional regulation of cyclin A1 and cyclin A2 during mouse oocyte meiotic maturation and preimplantation development. *Biol. Reprod.* **65**: 986–993.

Hannon, G.J. 2002. RNA interference. *Nature* **418**: 244–251.

Hutvagner, G. and Zamore, P.D. 2002. RNAi: Nature abhors a double-strand. *Curr. Opin. Genet. Dev.* **12**: 225–232.

Kurasawa, S., Schultz, R.M., and Kopf, G.S. 1989. Egg-induced modifications of the zona pellucida of mouse eggs: Effects of microinjected inositol 1,4,5-trisphosphate. *Dev. Biol.* **133**: 295–304.

Lipardi, C., Wei, Q., and Paterson, B.M. 2001. RNAi as random degradative PCR: siRNA primers convert mRNA into dsRNAs that are degraded to generate new siRNAs. *Cell* **107**: 297–307.

Martens, H., Novotny, J., Oberstrass, J., Steck, T.L., Postlethwait, P., and Nellen, W. 2002. RNAi in *Dictyostelium*: The role of RNA-directed RNA polymerases and double-stranded RNase. *Mol. Biol. Cell* **13**: 445–453.

Mourrain, P., Beclin, C., Elmayan, T., Feuerbach, F., Godon, C., Morrel, J.B., Jouette, D., Lacombe, A.M., Nikic, S., Picault, N., et al. 2000. *Arabidopsis* SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell* **101**: 533–542.

Nicholson, R.H. and Nicholson, A.W. 2002. Molecular characterization of a mouse cDNA encoding Dicer, a ribonuclease III ortholog involved in RNA interference. *Mamm. Genome* **13**: 67–73.

Sam, M., Wurst, W., Kluppel, M., Jin, O., Heng, H., and Bernstein, A. 1998. *Aquarius*, a novel gene isolated by gene trapping with an RNA-dependent RNA polymerase motif. *Dev. Dyn.* **212**: 304–317.

Schultz, R.M., Montgomery, R.R., and Belanoff, J.R. 1983. Regulation of mouse oocyte meiotic maturation: implication of a decrease in oocyte cAMP and protein dephosphorylation in commitment to resume meiosis. *Dev. Biol.* **97**: 264–273.

Schwarz, D.S., Hutvagner, G., Haley, B., and Zamore, P.D. 2002. Evidence that siRNAs function as guides, not primers, in the *Drosophila* and human RNAi pathways. *Mol. Cell* **10**: 537–548.

Sijen, T., Fleenor, J., Simmer, F., Thijssen, K.L., Parrish, S., Timmons, L., Plasterk, R.H., and Fire, A. 2001. On the role of RNA amplification in dsRNA-triggered gene silencing. *Cell* **107**: 465–476.

Simmer, F., Tijsterman, M., Parrish, S., Koushika, S., Nonet, M., Fire, A., Ahringer, J., and Plasterk, R. 2002. Loss of the putative RNA-directed RNA Polymerase RRF-3 makes *C. elegans* hypersensitive to RNAi. *Curr. Biol.* **12**: 1317–1319.

Smardon, A., Spoerke, J.M., Stacey, S.C., Klein, M.E., Mackin, N., and Maine, E.M. 2000. EGO-1 is related to RNA-directed RNA poly-

- merase and functions in germ-line development and RNA interference in *C. elegans*. *Curr. Biol.* **10**: 169–178.
- Svoboda, P., Stein, P., Hayashi, H., and Schultz, R.M. 2000. Selective reduction of dormant maternal mRNAs in mouse oocytes by RNA interference. *Development* **127**: 4147–4156.
- Svoboda, P., Stein, P., and Schultz, R.M. 2001. RNAi in mouse oocytes and preimplantation embryos: Effectiveness of hairpin dsRNA. *Biochem. Biophys. Res. Comm.* **287**: 1099–1104.
- Ui-Tei, K., Zenno, S., Miyata, Y., and Saigo, K. 2000. Sensitive assay of RNA interference in *Drosophila* and Chinese hamster cultured cells using firefly luciferase gene as target. *FEBS Lett.* **479**: 79–82.
- Whitten, W.K. 1971. Nutrient requirements for the culture of preimplantation mouse embryo *in vitro*. *Adv. Biosci.* **6**: 129–139.
- Zamore, P.D., Tuschl, T., Sharp, P.A., and Bartel, D.P. 2000. RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* **101**: 25–33.

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