Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 11274–11278, September 1998
Developmental Biology

Essential role of mitochondrially encoded large rRNA for germ-line formation in *Drosophila* embryos

TAKAKO IIDA AND SATORU KOBAYASHI*

Institute of Biological Sciences, Gene Experiment Center, and Center for Tsukuba Advanced Research Alliance (TARA) University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Communicated by Anthony P. Mahowald, The University of Chicago, Chicago, IL, July 10, 1998 (received for review April 14, 1998)

ABSTRACT **In *Drosophila*, pole cells, the progenitors of the germ line, are induced by the factors localized in the posterior pole region of oocytes and cleavage embryos, or germ plasm. Polar granules in germ plasm are electron-dense structures and have been proposed to contain factors essential for pole cell formation. Mitochondrially encoded large ribosomal RNA (mtlrRNA) has been identified as a component of polar granules. We previously have shown that mtlrRNA is able to rescue embryos that fail to form pole cells as a result of UV irradiation. However, there is a possibility that the function of mtlrRNA is limited to UV-irradiated embryos, and the question of whether mtlrRNA is required for the normal pathway leading to pole cell formation remains unanswered. In this study, we report that the reduction of mtlrRNA in germ plasm by injecting anti-mtlrRNA ribozymes into cleavage embryos leads to their inability to form pole cells. Other components of germ plasm, namely *oskar* mRNA, *germ cell-less* mRNA, and Vasa and Tudor proteins appear to be unaffected in these ribozyme-injected embryos. These results support an essential role for mtlrRNA in pole cell formation. We propose that mitochondrially encoded molecules participate in a key event in early cell-type specification.**

How the germ line segregates from the soma is a century-old issue in cell and developmental biology. In many animal species, the factor required for germ-line establishment has been postulated to be localized in a histologically distinct region in egg cytoplasm or germ plasm (1, 2). Experimental studies with frogs and the fruit fly *Drosophila* have demonstrated that factors with sufficient ability to establish germ line are localized in germ plasm (3–5). In *Drosophila*, germ plasm is localized at the posterior pole region of oocytes and cleavage embryos. It is this polar plasm that is later partitioned into pole cells, the progenitors of germ line in this animal (6). Distinctive organelles in polar plasm are polar granules, which are composed of RNAs and proteins (7). Protein products encoded by some maternally acting genes, namely *oskar* (*osk*), *vasa* (*vas*), and *tudor* (*tud*), have been reported as components of polar granules (8–10). The activities of all of these genes are required for polar granule assembly as well as pole cell formation (11–13), suggesting that the granules are essential for pole cell formation.

Ultrastructural studies have revealed that the polar granules are closely associated with mitochondria at definite stages before pole cell formation (14). This finding raises the possibility that mitochondria might contribute to pole cell formation, along with nuclear products. We previously have reported that one of the components in polar granules is mitochondrially encoded large ribosomal RNA (mtlrRNA) (15, 16). MtlrRNA is enriched on polar granules during early embryonic stages before pole cell formation, and its localization depends on the function of *osk*, *vas,* and *tud* (refs. 15–17; R. Amikura, M. Kashikawa and S.K., unpublished work). Because mtlrRNA is exclusively encoded by the mitochondrial genome, this observation indicates that mtlrRNA is transported out of mitochondria to reach polar granules in polar plasm. Based on these findings, we proposed that the extra-mitochondrial mtlrRNA on polar granules is a candidate for a factor directing pole cell formation. Further evidence supporting this idea comes from our data that mtlrRNA is able to rescue embryos from the failure to form pole cells by UV irradiation (18). However, there is a possibility that the function of mtlrRNA is limited to UV-irradiated embryos, and the question of whether mtlrRNA is required for the normal pathway leading to pole cell formation remains unanswered.

In *Drosophila*, genetic approaches are especially useful to assess the function of nuclear genes, but are unavailable to manipulate the mitochondrial genome. To overcome this problem, we used hammerhead ribozymes to specifically reduce or eliminate mtlrRNA. Hammerhead ribozyme is catalytic RNA that can cleave specific RNAs by hybridizing complementary target sequences (19, 20). The resulting RNA fragments are degraded, rendering the target molecules nonfunctional. Targeted ribozymes have been used as tools to create functional knockouts in various systems and provide an alternative to genetic strategies (21–30). Here, we report that the reduction of mtlrRNA in germ plasm by injecting anti-mtlrRNA ribozymes into cleavage embryos leads to their inability to form pole cells.

MATERIALS AND METHODS

Ribozyme Constructs. Synthetic double-stranded oligonucleotides containing the anti-mtlrRNA ribozyme sequences, 5′-ATTACGCTGTCTGATGAGTCCGTGAGGACGAAA-TCCCTAAAGT-3′ (RbzJ) and 5′-TTATCGATATCTGAT-GAGTCCGTGAGGACGAAAACTCTCCAAA-3′ (RbzK) (underlined sequences are complementary to the mtlrRNA target sequences) (31) were inserted individually into the *Sal*I and *Xba*I sites of a modified pGEM7Zf(−) vector (kindly provided by L. Pick, Mount Sinai School of Medicine) containing 65-bp fragment from *Escherichia coli lacZ* gene (30). Similarly, the double-stranded oligonucleotides containing ribozyme sequences, 5′-TGACTCGCACCTGATGAGTCC-GTGAGGACGAAAGCCGCTGCCG-3′ (Rbz2), 5′-AGCT-GGGCAGCTGATGAGTCCGTGAGGACGAAATTGCG-GCCCA-3′(Rbz3), and 5′-CTGGAATTGGCTGATGAGT-CCGTGAGGACGAAAGCTCCGCGCA-3′ (Rbz4) (underlined sequences are complementary to the *nanos* mRNA sequences, but not to mtlrRNA) were inserted individually into

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: mtlrRNA, mitochondrially encoded large ribosomal RNA; DW, distilled water; DIG, digoxigenin.

*To whom reprint requests should be addressed. e-mail: skob@sakura.cc.tsukuba.ac.jp.

the modified pGEM7Zf(−) vector. Then, a 186-nt poly(A) sequence was inserted into the *Xba*I site downstream of the ribozyme sequence and used as a template for *in vitro* transcription of the ribozymes. The ribozymes were transcribed from the template DNA by using a MEGAscript kit (Ambion). $m^7G(5')ppp(5')G$ cap analog (Ambion) was added to the ribozymes during *in vitro* transcription according to the manufacturer's instructions. Transcribed ribozymes were dissolved in distilled water (DW) and stored at −80°C until use.

We found that both RbzJ and RbzK cleaved mtlrRNA at the expected sites *in vitro*. ^{32}P-labeled mtlrRNA (10 nM) and 100 nM ^{32}P-labeled anti-mtlrRNA RbzJ and RbzK were incubated in 20 μl of a reaction solution (50 mM Tris·HCl, pH 8.0 and 25 mM $MgCl_2$) for 1 hr at 37°C. Then, 3 μl of reaction solution was loaded on a 5% denaturing polyacrylamide gel containing 7 M urea and electrophoresed. The cleaved mtlrRNA fragments were detected by autoradiography (data not shown). In contrast, neither Rbz2, Rbz3, or Rbz4 was unable to cleave mtlrRNA in a similar reaction condition (data not shown).

Microinjection Experiments. For microinjection experiments, embryos of a *mwh* e^{11} stock were used. Microinjection method was principally the same as previously reported (32). A mixture (0.1 nl) of RbzJ and RbzK (9 μM each) was injected into the posterior pole region of *mwh* e^{11} embryo at 20 ± 10 min after egg laying. As a control, 0.1 nl of a mixture (Rbz mix) of Rbz2, Rbz3, and Rbz4 (10 μM each) was injected. *In situ* hybridization analysis revealed that the injected ribozymes remained to be enriched in polar plasm within at least 30 min after the microinjection. The injected embryos were allowed to develop for 20–25 min at 25°C, then were fixed for *in situ* hybridization and/or immunostaining. For scoring pole cell formation, the injected embryos were allowed to develop in silicon oil (FL-100 450CS, SHIN-ETSU silicon oil) at 25°C until 3 hr after egg laying, and then were observed under a light microscope.

***In Situ* Hybridization for Electron Microscopy.** Subcellular distribution of the injected ribozymes was examined by using *in situ* hybridization technique at an electron microscopic level as previously described (33). After the injection of RbzJ and RbzK into the posterior pole region of the cleavage embryos, the embryos were processed for fixation, embedding, ultra-thin sectioning, and *in situ* hybridization with a double-stranded digoxigenin (DIG)-labeled DNA (205 bp) encoding RbzJ and RbzK. We counted the number of signals in the area of 15 μm^2 in polar plasm of the ribozyme-injected embryos.

***In Situ* Hybridization for Light Microscopy and Immunohistochemistory.** Whole-mount *in situ* hybridization using a double-stranded DIG-labeled DNA probe was carried out principally according to the method reported by Tautz and Preifle (34). A full-length 1,446-bp mtlrRNA cDNA (31), 2,432-bp *osk* cDNA, 2,388-bp *gcl* cDNA, and 1,725-bp *bcd* cDNA were DIG-labeled and used as probes for *in situ* hybridization.

Immunostaining for VAS and TUD protein was carried out according to the method previously reported (35). We used a rabbit anti-VAS antibody (a gift from A. Nakamura and P. Lasko, McGill University, Montreal) and a rabbit anti-TUD antibody (a gift from R. Boswell, University of Colorado). Texas Red-conjugated goat anti-rabbit IgG (Amersham) and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Cappel) were used as secondary antibodies. The stained embryos were mounted in VECTASHIELD Mounting Me-

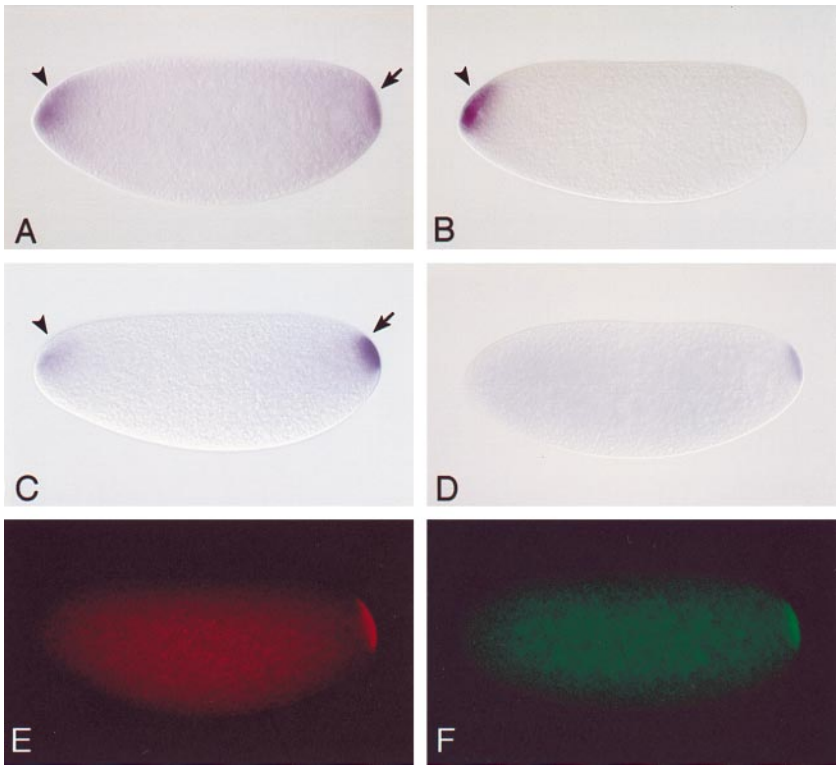

FIG. 1. Effect of ribozymes on the distribution of mtlrRNA and polar plasm components in the late-cleavage embryos at around 60 min after egg laying. *In situ* hybridization of the mtlrRNA cDNA probe to a control embryo injected with DW (*A*) and an embryo injected with RbzJ and RbzK (*B*). Note that mtlrRNA signal in polar plasm completely disappears in a ribozyme-injected embryo (*B*). Arrow in *A* shows mtlrRNA signal. As an internal control for *in situ* hybridization, the embryos also were hybridized with a probe detecting *bcd* mRNA that localizes at the anterior pole region of early cleavage embryos (39, 40). Arrowheads in *A-C* indicate *bcd* mRNA signal. *In situ* hybridization of *osk* cDNA probe (*C*) and *gcl* cDNA probe (*D*) to the ribozyme-injected embryos. Arrow in C shows *osk* mRNA signal. Immunostaining of VAS (*E*) and TUD (*F*) in the ribozyme-injected embryos. *osk* mRNA, *gcl* mRNA, VAS, and TUD normally accumulated in polar plasm of the ribozyme-injected embryos. Lateral views of the late cleavage embryos are shown; anterior is to left.

Table 1. Reduction of mtlrRNA in the embryos injected with the anti-mtlrRNA ribozymes

Injected materials	Total no. of embryos scored*	No. of embryos without mtlrRNA signal in polar plasm†, %	Significance
RbzJ & K	300	52 (17.3)	$P < 0.001$§ $P < 0.001$¶
DW	262	19 (7.3)	
Control			
Rbz mix‡	124	5 (4.0)	$P > 0.2$§
DW	83	5 (6.0)	

*The injected embryos were *in situ*-hybridized with mtlrRNA probe. As an internal control for *in situ* hybridization, the embryos also were hybridized with a DIG-labeled probe detecting *bcd* mRNA that localizes at the anterior pole region of early embryos. Furthermore, to exclude embryos in which polar plasm leaked out or was delocalized by the injection procedure, the injected embryos were stained with an antibody against VAS protein. Neither the anterior localization of *bcd* nor the posterior localization of VAS was affected by the injection of anti-mtlrRNA ribozymes. The number of embryos without mtlrRNA signal was determined from embryos that showed normal *bcd* and VAS staining.

†Whole-mount *in situ* hybridization of the ribozyme-injected embryos using a double-stranded DNA probe for mtlrRNA.

‡A mixture of ribozymes that did not cleave mtlrRNA was injected into embryos.

§Probability was calculated vs DW-injected embryos by Fisher's exact probability test.

¶Probability was calculated vs control Rbz mix-injected embryos by Fisher's exact probability test.

dium (Vector) and were observed under a confocal microscope, TCS NT (Leica).

RESULTS AND DISCUSSION

We constructed two targeted ribozymes (RbzJ and RbzK), which were designed to hybridize with residues 1064–1085 and 1089–1110 of 1,324-bp mtlrRNA, respectively. Comparison of the nucleotide sequences in these targets with those of nuclear large (28S) rRNA and mRNAs known to be localized in polar plasm revealed no significant homology. In addition, we have searched homologous sequences in the *Drosophila* DNA database (the Berkley fly database) but found no sequence showing more than 68% homology with the target sequences. This finding suggests that RbzJ and RbzK are unable to hybridize these RNA sequences. By hybridizing mtlrRNA, RbzJ and RbzK were designed to cleave it at nucleotides 1075 and 1100, respectively. Each of these ribozymes cleaved it *in vitro* at the expected position (data not shown).

We investigated whether these anti-mtlrRNA ribozymes were able to reduce or eliminate mtlrRNA in polar plasm. To increase efficiency, we coinjected both anti-mtlrRNA ribozymes into the posterior pole region of early cleavage embryos (20 ± 10 min after egg laying). *In situ* hybridization analysis revealed that coinjection of RbzJ and RbzK caused a drastic reduction of mtlrRNA signal in polar plasm (Fig. 1*B*). In 17.3% of the injected embryos, mtlrRNA signal decreased to an undetectable level, whereas only 7.3% of the control embryos that had been injected with DW failed to show the posterior localization of mtlrRNA signal (Table 1). Furthermore, injection of control ribozymes that did not cleave mtlrRNA *in vitro* had no deleterious effect on the posterior localization of mtlrRNA signal (Table 1). Considering that the *in situ* hybridization technique used here is able to detect

Table 2. Distribution of the polar plasm components in the embryos injected with the anti-mtlrRNA ribozymes

RNAs and proteins detected*	Rbzs†	Total no. of embryos scored	No. of embryos without signal in polar plasm, %	Significance‡
gcl	+	324§	6 (1.9)	$P > 0.2$
	−	320§	9 (2.8)	
osk	+	296¶	0 (0)	$P > 0.2$
	−	239¶	1 (0.4)	
VAS	+	1,042‖	73 (7.0)	$P > 0.2$
	−	944‖	66 (7.0)	
TUD	+	300**	13 (4.2)	$P > 0.2$
	−	313**	18 (5.8)	

*Whole-mount *in situ* hybridization of the ribozyme-injected embryos using a double-stranded DNA probe for mtlrRNA, *osk* and *gcl* mRNA was performed. Immunostaining for VAS and TUD protein was carried out.

†Embryos were injected with the anti-mtlrRNA ribozymes (+) or DW (−).

‡Probability was calculated vs DW-injected embryos by Fisher's exact probability test.

§The injected embryos were stained with *gcl* probe. To exclude embryos in which polar plasm leaked out or was delocalized by the injection procedure, the injected embryos were stained with an antibody against VAS protein. The number of embryos without *gcl* signal was counted among the embryos that showed normal VAS staining.

¶The injected embryos were stained with *osk* probe. As an internal control for *in situ* hybridization, the embryos also were hybridized with a DIG-labeled probe detecting *bcd* mRNA that localizes at the anterior pole region of early embryos. And the injected embryos also were immunostained with an anti-VAS antibody. The number of embryos without *osk* signal was counted among the embryos that showed normal *bcd* and VAS staining.

‖The injected embryos were immunostained with an anti-VAS antibody. The number of embryos without VAS signal was counted among the injected embryos.

**The injected embryos were immunostained with an anti-TUD antibody. The number of embryos without TUD signal was counted among the injected embryos.

mtlrRNA only outside of mitochondria (33), these results indicate that the amount of extra-mitochondrial mtlrRNA decreases significantly by the injection of the anti-mtlrRNA ribozymes. In contrast, these ribozymes fail to target intra-mitochondrial mtlrRNA because the ribozymes injected into polar plasm were indiscernible in mitochondria. Under an electron microscope, we counted the number of signals in the area of 15 μm^2 in polar plasm of the ribozyme-injected embryos that were *in situ*-hybridized with ribozyme probes. All signals (total number of signals = 51) were found only in the cytosol outside of mitochondria. Presumably, this is caused by the impermeability of mitochondrial membrane to nucleic acids.

To exclude the possibility that RNA components of polar plasm are degraded nonspecifically by the injected ribozymes, we further examined distribution of two other RNAs localized in polar plasm, *osk* mRNA, and *germ cell-less* (*gcl*) mRNA (36), and found that the amount of these RNAs was unaffected (Table 2, Fig. 1 *C* and *D*). In addition, the posterior concentration of other polar plasm components, Vasa (VAS) and Tudor (TUD) proteins, appeared to be unaffected in these embryos (Table 2, Fig. 1 *E* and *F*). These results indicate that anti-mtlrRNA ribozymes specifically reduce the amount of extra-mitochondrial mtlrRNA in polar plasm.

To examine whether pole cell formation is affected by the reduction of mtlrRNA in polar plasm, we allowed the ribozyme-injected embryos to develop to blastoderms and observed their cellularization under the light microscope. These embryos formed normal-looking blastodermal layers of somatic cells and proceeded through gastrulation. However, as shown in Table 3 and Fig. 2, their ability to form pole cells was significantly reduced. In 13.4% of the ribozyme-injected embryos, pole cells were missing. In contrast, only 1.5% of the DW-injected embryos developed to blastoderms without pole cells. Furthermore, injection of the control ribozymes that did not affect the posterior localization of mtlrRNA (Table 1) failed to inhibit pole cell formation (Table 3). These results clearly show that pole cell formation is impaired in embryos only when injected with the anti-mtlrRNA ribozymes. It is worthwhile to note that the percentage of embryos whose pole-cell-forming ability is impaired by the injection of the anti-mtlrRNA ribozymes is similar to that of embryos showing a strong reduction in the posterior concentration of extra-mitochondrial mtlrRNA.

The above results, along with our previous UV rescue experiments (18), lead to the conclusion that mtlrRNA is a functional component of polar granules and is essential for pole cell formation. This finding supports the idea that mitochondrially encoded molecule participates in a key event in early cell-type specification. There is a further question of how mtlrRNA directs the formation of pole cells. MtlrRNA has no long ORF and is unable to be translated into protein in rabbit reticulocyte lysate (Y. Uozumi and S.K., unpublished material), suggesting that mtlrRNA functions without being translated. However, a structural role for mtlrRNA in which it functions to stabilize or tether the polar granule components is unlikely. Even when mtlrRNA decreased to an undetectable level by the injection of the anti-mtlrRNA ribozymes, the polar granule components were properly localized in polar plasm (Table 2). Recently, we found that mitochondrial small rRNA also was transported from mitochondria to polar granules before pole cell formation, and its transport depended on the normal activities of *osk, vas,* and *tud* (M. Kashikawa and S.K., unpublished work). This observation leads us to speculate that there are mitochondrial ribosomes on polar granules and their function is needed to produce proteins required for pole cell formation. This idea is compatible with early models that mRNAs encoding proteins for germ-line development are stored in polar granules and are translated on the polysomes developed on the surface of polar granules (37). Further analysis to test the possibility that mtlrRNA is involved in protein synthesis on polar granules will give a better understanding of molecular basis for pole cell formation.

Organelles comparable to polar granules have been found in the germ line of many animal groups (1, 2), suggesting that they have widespread roles in germ-line development. More importantly, the extra-mitochondrial mtlrRNA is a common component of the germinal granules in *Drosophila* and *Xenopus* (38). We propose that mtlrRNA participates in a con-

Table 3. Inhibition of pole cell formation by injecting of the anti-mtlrRNA ribozymes

Injected materials	No. of embryos developed to blastoderms: Total	Without pole cells (%)*	Significance†
RbzJ & K	2,861	382 (13.4)	$P < 0.001$
DW	2,029	30 (1.5)	
Control			
Rbz mix‡	175	1 (0.6)	$P > 0.2$
DW	89	1 (1.1)	

*The injected embryos were allowed to developed to cellular blastoderms at 25°C, then were observed under the light microscope. We found that a few percentages of the ribozyme-injected embryos formed only a small number of pole cells, which we classified as the embryos with pole cells.

†Probability was calculated vs DW-injected embryos by Fisher's exact probability test.

‡A mixture of ribozymes that did not cleave mtlrRNA was injected into embryos.

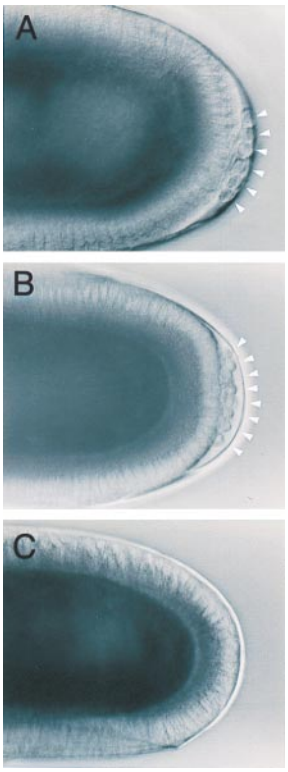

FIG. 2. Effect of ribozymes on pole cell formation. Posterior pole region of embryos at the cellular blastoderm stage (3 hr after egg laying) are shown. (*A*) A noninjected embryo. (*B*) A control embryo injected with DW. (*C*) An embryo injected with RbzJ and RbzK. In the embryo injected with RbzJ and RbzK, pole cells are completely missing, whereas somatic cell layer appears essentially normal. Arrowheads in *A* and *B* indicate pole cells.

served mechanism of germ-line development among metazoans.

We thank L. Pick for her gift of pGEM7Zf(−) vector; R. E. Boswell, A. Nakamura, and P. F. Lasko for antibodies; A. Ephrussi for *osk* and *bcd* cDNAs; A. Ohkawa and K. Taira for valuable comments on ribozyme technique; R. Amikura for help in electron microscopy; and P. F. Lasko and members of the Kobayashi laboratory for helpful comments on the manuscript. This work was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture, Japan, the Tsukuba Advanced Research Alliance-Project, the Toray Science Foundation, the Sumitomo Foundation, and a Research Project for Future Program from Japan Society for the Promotion of Science.

1. Beams, H. W. & Kessel, R. G. (1974) *Int. Rev. Cytol.* **39,** 413–479.
2. Eddy, E. M. (1975) *Int. Rev. Cytol.* **43,** 229–280.
3. Illmensee, K. & Mahowald, A. P. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1016–1020.
4. Illmensee, K. & Mahowald, A. P. (1976) *Exp. Cell Res.* **97,** 127–140.
5. Ikenishi, K., Nakazato, S. & Okuda, T. (1986) *Dev. Growth Differ.* **28,** 585–590.
6. Technau, G. M. & Campos-Ortega, J. A. (1986) *Wilhelm Roux Arch. Dev. Biol.* **195,** 489–498.
7. Mahowald, A. P. (1971) *J. Exp. Zool.* **176,** 345–352.
8. Breitwieser, W., Markussen, F., Horstmann, H. & Ephrussi, A. (1996) *Genes Dev.* **10,** 2179–2188.
9. Hay, B., Ackerman, L., Barbel, S., Jan, L. Y. & Jan, Y. N. (1988) *Development (Cambridge, U.K.)* **103,** 625–640.
10. Bardsley, A., McDonald, K. & Boswell, R. E. (1993) *Development (Cambridge, U.K.)* **119,** 207–219.
11. Lehmann, R. & Nüsslein-Volhard, C. (1986) *Cell* **47,** 141–152.
12. Schüpbach, T. & Wieshaus, E. (1986) *Wilhelm Roux Arch. Dev. Biol.* **195,** 302–317.
13. Boswell, R. E. & Mahowald, A. P. (1985) *Cell* **43,** 97–104.
14. Mahowald, A. P. (1962) *J. Exp. Zool.* **151,** 201–215.
15. Kobayashi, S., Amikura, R. & Okada, M. (1993) *Science* **260,** 1521–1524.
16. Amikura, R., Kobayashi, S., Saito, H. & Okada, M. (1996) *Dev. Growth Differ.* **38,** 489–498.
17. Ding, D., Whittaker, K. L. & Lipshitz, H. D. (1994) *Dev. Biol.* **163,** 503–515.
18. Kobayashi, S. & Okada, M. (1989) *Development (Cambridge, U.K.)* **107,** 733–742.
19. Lieber, A. & Kay, M. A. (1996) *J. Virol.* **70,** 3153–3158.
20. Haseloff, J. & Gerlash, W. L. (1988) *Nature (London)* **334,** 585–591.
21. Efrat, S., Leiser, M., Wu, Y.-J., Fusco-DeMane, D., Emran, O. A., Surana, M., Jetton, T. L., Magnuson, M. A., Weir, G. & Fleishcer, N. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2051–2055.
22. Larsson, S., Hotchkiss, G., Andäng, M., Nyholm, T., Inzunza, J., Jansson, I. & Ährlund-Richter, L. (1994) *Nucleic Acids Res.* **22,** 2242–2248.
23. Lyngstadaas, S. P., Risnes, S., Sproat, B. S., Thrane, P. S. & Prydz, H. P. (1995) *EMBO J.* **14,** 5224–5229.
24. Marusic, L., Luzi, E., Barsacchi, G. & Eckstein, F. (1997) *Eur. J. Biochem.* **247,** 396–401.
25. Vanario-Alonso, C. E., O'Hara, E., McGinnis, W. & Pick, L. (1995) *Mech. Dev.* **53,** 323–328.
26. Heinrich, J. C., Tabler, M. & Louis, C. (1995) *Anti. Res. Dev.* **5,** 155–160.
27. Adams, M. D., Tarng, R. S. & Rio, D. C. (1997) *Genes Dev.* **11,** 129–138.
28. Sternbach, O. C., Wolffe, A. P. & Rupp, R. A. W. (1997) *Nature (London)* **389,** 395–399.
29. Xie, Y., Chen, X. & Wagner, T. E. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 13777–13781.
30. Zhao, J. J. & Pick, L. (1993) *Nature (London)* **365,** 448–451.
31. Kobayashi, S. & Okada, M. (1990) *Nucleic Acids Res.* **18,** 4592.
32. Okada, M., Kleinman, I. A. & Schneiderman, H. A. (1974) *Dev. Biol.* **37,** 43–54.
33. Amikura, R., Kobayashi, S., Endo, K. & Okada, M. (1993) *Dev. Growth Differ.* **35,** 617–623.
34. Tautz, D. & Preifle, C. (1989) *Chromosoma* **98,** 81–85.
35. Kobayashi, S., Amikura, R., Nakamura, A. & Lasko, P. F. (1998) in *Advances in Molecular Biology: A Comparative Methods Approach to the Study of Oocytes and Embryos*, ed. Richter, J. (Oxford Univ. Press, New York), in press.
36. Jongens, T. A., Hay, B., Jan, L. Y. & Jan, Y. N. (1992) *Cell* **70,** 569–584.
37. Mahowald, A. P. (1968) *J. Exp. Zool.* **167,** 237–262.
38. Kobayashi, S., Amikura, R. & Okada, M. (1994) *Int. J. Dev. Biol.* **38,** 193–199.
39. Frigerio, G., Burri, M., Bopp, D., Baumgartner, S. & Noll, M. (1986) *Cell* **47,** 735–746.
40. Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M. & Nüsslein-Volhard, C. (1988) *EMBO J.* **7,** 1749–1756.