fluoropropylene-*co*-tetrafluoroethylene) (FEP) film was exposed to a RFGD plasma that was composed of a flowing vapor mixture of H_2 and methanol (CH_3OH) (*4*). The polymer film was covered in the plasma by a metal mask with open regions each 70 μm wide spaced between covered regions each 150 μm wide. This produced hydroxy- lated regions each 70 μm wide, spaced by unmodified FEP. Figure 1 shows the differential wetting characteristics of this surface when probed with drops of CH_3OH (right) and water (left). Water, when applied to the plasma-treated FEP surface, shows little or no detectable difference in wetting between the protected and exposed regions. The observed lack of wetting of the water drop is consistent with the results of previous surface analytical studies (*7*), which showed that FEP is only partially defluorinated after treatment with the H_2-CH_3OH plasma; the resulting hydroxylated FEP surface is still considerably hydrophobic, which is characteristic of the bulk fluoropolymer. However, differential wetting is clearly observed with CH_3OH, a less polar liquid, on the exposed regions of the FEP surface.

After plasma treatment, the FEP was functionalized in a solution of *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (EDA). The attachment of the EDA SAM film to the FEP surface was confirmed by secondary ion mass spectrometry and x-ray photoelectron spectroscopy analysis (*5–7*). The silanized FEP films were placed into an aqueous $PdCl_4^{2-}$ catalyst solution to bind Pd to the EDA surface. The substrate was then immersed in an electroless Ni plating bath to deposit Ni metal on the catalyzed regions. Figure 2 shows metal lines each 70 μm wide selectively deposited on FEP by this process.

Fig. 2. Optical micrograph of a selectively metallized FEP film. The film was hydroxylated in patterns as described in Fig. 1. The surface was then functionalized by treatment in a 0.1% (v/v) solution of EDA in hexane for ~20 s and then rinsed in hexanes. The EDA surface was catalyzed by immersion in a chloride-stabilized, buffered solution of $PdCl_4^{2-}$ at pH 5 for 30 min. The catalyzed surface was then metallized by immersion in a NIPOSIT 468 electroless plating bath (Shipley Company).

For adhesion testing, an FEP substrate was homogeneously hydroxylated, functionalized with EDA, and metallized with electroless Ni for 3 hours to produce a uniform, mirror-like deposit of Ni ~2500 Å thick. Peel tests with both Scotch tape and American Society for Testing and Materials standard tape indicated the complete adhesion of the metal to the FEP surface. Covalent bonding of the organosilane to the hydroxylated fluoropolymer surface and coordinative bonding of the Pd catalyst to the EDA surface ligand are likely the key contributors to the high adhesion of the electroless deposit to the substrate. The use of mild plasma treatment together with aqueous chemical treatments may find practical applications in fluoropolymer surface modification and metallization.

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Translocation of Repetitive RNA Sequences with the Germ Plasm in *Xenopus* Oocytes

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Xlsirts are a family of interspersed repeat RNAs from *Xenopus laevis* that contain from 3 to 13 repeat units (each 79 to 81 nucleotides long) flanked by unique sequences. They are homologous to the mammalian *Xist* gene that is involved in X chromosome inactivation. Xlsirt RNA appears first in the mitochondrial cloud (Balbiani body) in stage 2 oocytes and is then translocated as island-like structures to the vegetal cortex at early stage 3 coincident with the localization of the germ plasm. Exogenous Xlsirt RNA injected into oocytes translocates to the location of the endogenous RNA at that particular stage. The Xlsirt RNA repeat sequences are required for translocation and can cause the translocation of heterologous unique RNAs to the vegetal cortex.

A universal characteristic of developing organisms is the acquisition and interpretation of spatial information. Studies in *Drosophila* have demonstrated a complex network of gene products involved in the spatial organization of the posterior pole of the oocyte; however, the understanding of vertebrate regulation of spatial patterning is less advanced (*1*). The vegetal cortical region of the *Xenopus* oocyte contains developmental information in the form of germ plasm, which is involved in germ cell determination, and specialized cytoplasm, which is activated upon cortical rotation and contributes to the future dorsal axis (*2*). Several transcripts, including Vg1, a member of the transforming growth factor–β family (*3*), and Xcat2, which has similarities to *Drosophila nanos* (*4*), are localized in overlapping spatial patterns at the vegetal cortex. Xcat2 is localized during stage 3 and Vg1 during stage 4, which suggests their possible dependence upon one another for proper localization.

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Spohr *et al.* (5) cloned and characterized several genomic and complementary DNA (cDNA) clones from *Xenopus laevis* that encode short interspersed repeat transcripts (Xlsirts). These transcripts have a common 79- to 81-nucleotide sequence tandemly repeated 3 to 13 times. The repeated arrays are flanked by different unique sequences. The transcripts do not contain translational start sites, and there are no other indications that the transcripts may be translated (6).

We cloned several additional sirt DNAs from *Xenopus laevis* (Xlsirt) and *Xenopus tropicalis* (Xtsirt) oocyte cDNA and genomic libraries (Fig. 1). The repeats were 79 to 81 base pairs (bp) long and 90 to 94% identical in the two species (Fig. 1A). The flanking

A

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TACTATAGGCACCATC-TCTCCCTACTATACCTGCTATCCCACAGTCACACTCCCTTCCCAGAGACTATTATCCC---AC  Xlsirt 13.2
TACTATAGGCCCCATC-TCTCCCTACTATACCTGCTATCCCACAGCCCCAGTCCCTTCCCGGAGGCTATTATCCCCCCAC  Xlsirtp11-1
T-CTATAGGCACCATC-TCTCCCTACTATACCTGCTATCCCAGAGTCACACTCCCTTCCCAGAGACTATTATCCCAGTGT  Xlsirtp11-2
TACTATAGGCACCATCCTCTCCCTACTATACCTGCTATCCCACAGTCACACTCCCTTCCCAGAGACTATTATCCCACTGT  Xlsirtp11-3
TACTATAGGCACCATC-TCTCCCTACTATACCTGCTATCCCACAGTCACACTCCCTTCCCAGAGACTATTATCCCACTGT  Xtsirt 13.2

GC-CCAWCGGGGCYNYGG-ATACCTG          Mouse Xist
GC-CCATCGGGGYNNYGG-ATANCTG          Human XIST
GCACCATC-TCTCCCTACTATACCTG          Xenopus Xlsirt
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B

Fig. 1. (**A**) Comparison of the Xlsirt and Xtsirt repeat sequences in *X. laevis* and *X. tropicalis* cDNA and genomic clones. Shown are single repeat units from two different *laevis* clones (Xlsirt 13.2, Xlsirtp11-1, Xlsirtp11-2, and Xlsirtp11-3) and one *tropicalis* clone (13.2). Xlsirtp11-1, Xlsirtp11-2, and Xlsirtp11-3 represent individual repeats from the same clone. The underlined regions represent homology to the Xist sequences. These are compared with the published human (*8*) and mouse (*7*) Xist sequences. Y indicates C or T; W indicates A or T; and N indicates no strong preference. (**B**) Organization of *X. laevis* and *X. tropicalis* clones. Clone a, Xlsirt 13.2, was 2300 nucleotides (nt) in length and contained eight repeat units (*5*); clone b, Xtsirt 13.2, was 1200 nt in length and contained seven repeat units; clone c, Xtsirt 12, was 1480 nt in length and contained 13 repeat units; and clone d, Xlsirtp11, was 1769 nt in length and contained three repeat units.

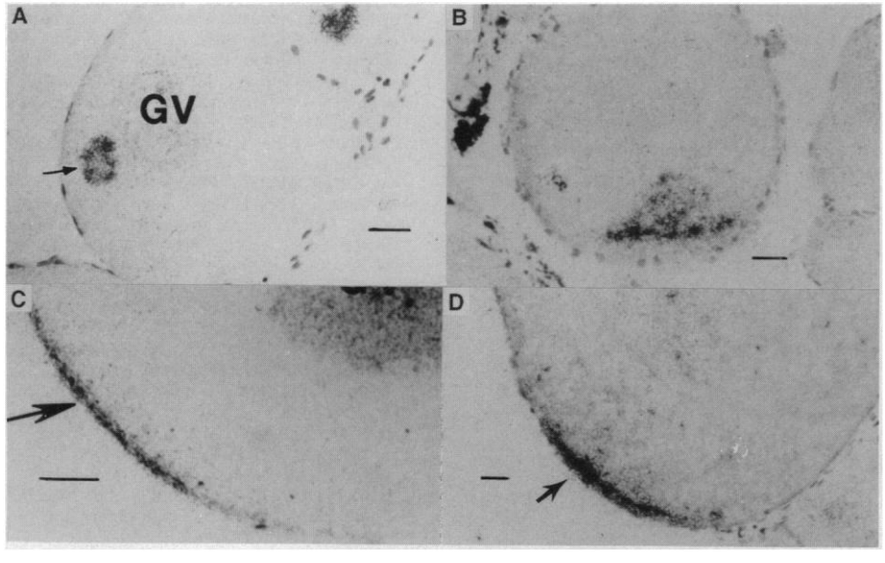

Fig. 2. Localization of Xlsirt RNAs during oogenesis. *Xenopus* ovaries were fixed overnight in 100% methanol at 4°C, cleared in xylene, and embedded in paraplast. In situ hybridization was performed on 10-μm sections as described (*15*). Sense and antisense RNA probes were prepared from repetitive or unique Xlsirt DNA sequences cloned into Bluescript vectors with T7 and T3 polymerase and ^{35}S-UTP (*16*). (**A**) A 10-μm section of a stage 2 oocyte, showing the T3 (antisense) probe for Xlsirt RNAs. Hybridization is confined to the mitochondrial cloud (Balbiani body) (arrow). Darkly stained peripheral structures are nuclei of follicle cells stained with Azure B (Matheson Coleman & Bell, Rutherford, New Jersey). (**B**) Section of a stage 3 oocyte, showing the T3 probe for Xlsirt RNAs. The probe recognizes Xlsirts migrating toward the vegetal pole region. (**C** and **D**) Sections of stage 4 oocytes, showing the T3 probe for Xlsirt RNAs. Hybridization is to transcripts localized at the vegetal pole region (arrows). Occasionally, we detected hybridization to the germinal vesicle (GV) as seen in (C) but not in (D). Bars represent 50 μm.

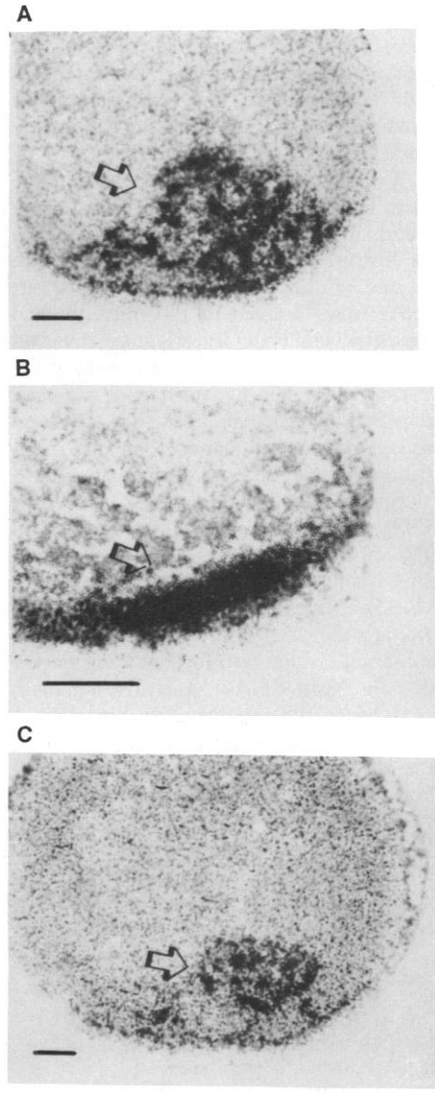

Fig. 3. Translocation of exogenous Xlsirt RNAs injected into stage 3 and 4 oocytes. Xlsirt RNA labeled with ^{35}S-UTP was synthesized from the Xlsirtp11 cDNA clone with the T7 promoter as described (Fig. 2). Transcripts were separated from unincorporated ribonucleotides with a G-50 Sepharose column (Sigma). RNA (1×10^5 cpm in 5 nl) was injected into each oocyte, and the oocytes were then cultured for 2 to 4 days (*17*). Oocytes were analyzed by histology and autoradiography. In some experiments, samples consisting of 10 to 20 oocytes were homogenized immediately after injection and at days 2 and 4 after injection. The RNA was extracted and analyzed by gel electrophoresis on formaldehyde denaturing gels followed by autoradiography to determine the integrity of the injected Xlsirt RNAs. (**A**) Localization of Xlsirt RNA 2 days and (**B**) 4 days after injection into stage 4 oocytes. (**C**) Fate of Xlsirt RNA after 4 days in early stage 3 oocytes, showing localization to the migrating islands. Bars represent 50 μm; arrows point to Xlsirt transcripts.

sequences, however, were completely divergent. Each of the Xlsirt and Xtsirt cDNA repeat units possesses a sequence that is very similar to the consensus sequences found in the *Xist* (X inactivation specific transcripts) RNA that may be involved in mammalian X chromosome inactivation (*7, 8*). Xlsirt and Xtsirt repeats were always organized as tandem units (Fig. 1B). Each clone possessed different flanking sequences on each side of the tandem repeat unit.

We analyzed the spatial distribution of the Xlsirt RNA during oogenesis using in situ hybridization with strand-specific probes from the Xlsirtp11 cDNA repeat sequence. With the use of the T3 antisense probe, Xlsirt RNAs were localized in the mitochondrial cloud (Balbiani body) in early stage 2 oocytes (Fig. 2A) and in island-like structures at the vegetal pole region by late stage 2 and early stage 3 (Fig. 2B). The island-like structures were morphologically similar to migrating mitochondria and germ plasm in stage 2 to 3 oocytes (9). In mid–late stage 3, the Xlsirt RNAs were associated with the vegetal cortex (Fig. 2, C and D). On the basis of a comparison of published data, the pattern of Xlsirt RNA localization at the vegetal cortex overlapped with that of Xcat2 and Vg1 transcripts, which translocate during stage 4 after the Xlsirt RNAs. Xlsirt RNAs remain localized at the vegetal cortex in stage 5 oocytes; however, this pattern of localization was not detected by in situ analysis in full-grown stage 6 oocytes, which suggests that they have been dispersed or degraded (*10*). Also, in situ hybridization with the sense T7 (S) strand showed no hybridization to the cytoplasm; however, a signal was detected in the nucleus, which suggests that the opposite strand is transcribed (*10, 11*).

We injected into early stage 3 oocytes Xlsirt RNA [labeled with ^{35}S-labeled uridine triphosphate(UTP)] made in vitro from the Xlsirtp11 cDNA, while the endogenous Xlsirt RNAs were translocating, and into stage 4 oocytes when the endogenous Xlsirt RNAs were at the vegetal cortex. Immediately after injection into stage 3 or 4 oocytes, the exogenous transcripts were dispersed in the cytoplasm (*10*). Two days after injection into stage 4 oocytes, they were detected in the island-like structures in the vegetal region (Fig. 3A) and after 4 days at the vegetal cortex (Fig. 3B). Xlsirtp11 RNAs injected into early stage 3 oocytes localized to the migrating islands with the endogenous transcripts (Fig. 3C). Thus, the exogenous transcripts co-localized with the endogenous transcripts at both stages.

We mapped the cis-acting elements necessary for Xlsirt RNA translocation by testing the ability of mutant Xlsirtp11 RNAs to localize to the vegetal cortex when injected into stage 4 oocytes. Transcripts tested included mutant Xlsirtp11R-U containing three repeat sequences attached to 250 nucleotides of a 3′ unique sequence; mutant Xlsirtp11R containing three repeat sequences; mutant Xlsirtp11U containing unique sequences; and mutant Xlsirtcaax-R containing a heterologous mRNA attached to three repeat units from the p11 clone. The Xlsirtcaax-R contained 200 bp of the 5′ region of the Xlcaax mRNA, which by itself does not localize (*10*). Both p11R-U and p11R RNAs were found associated with the vegetal cortex 4 days after injection (Fig. 4, A and B). Xlsirtp11U RNA, which consists of only the unique portion, remained evenly dispersed throughout the cytoplasm even after 4 days (Fig. 4C). The Xlsirtcaax-R chimeric transcript also translocated to the vegetal cortex (Fig. 4D). Gel analysis of injected RNA after 2 and 4 days of culture showed no degradation or processing of the injected Xlsirt or chimeric RNA (*10*). Thus, the Xlsirt repeat RNA sequences are necessary and sufficient for the translocation of transcripts to the vegetal cortex.

A preliminary computer analysis detected a complex secondary structure in Xlsirt and Xtsirt RNAs (*10*). It is likely that the repeat units form a structural motif similar to that found in the 3′ untranslated region of Vg1 and bicoid mRNAs, which is responsible for their translocation to the appropriate cellular position (*12, 13*). The localization pattern of the injected Xlsirt RNAs in oocytes of different stages implies that these transcripts are involved in establishing a pathway that may be used to localize other RNAs such as Xcat and Vg1. In addition, because the in situ data show Xlsirt RNAs co-localize with the germ plasm an intriguing possibility is that they may function in the organization and establishment of the germ cell lineage in *Xenopus* (*14*).

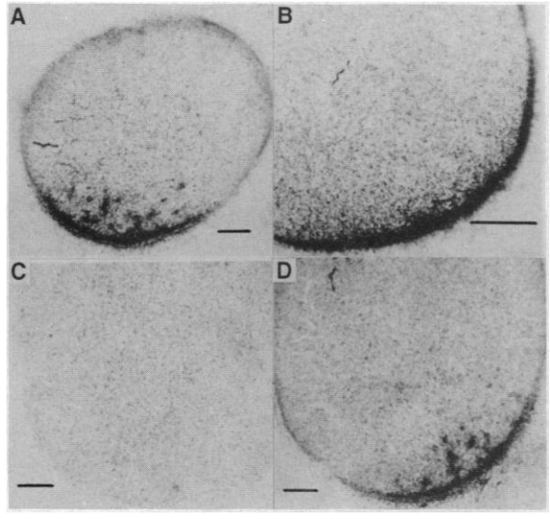

Fig. 4. Translocation of mutant Xlsirtp11 RNAs. Mutant Xlsirts cDNA constructs were derived from the Xlsirtp11 cDNA clone. Xlsirtp11R-U consisted of 250 bp of the 5′ unique portion and 300 bp of a region from p11 containing three repeat units cloned into the vector pT7T3-19 (Ambion, Austin, Texas). Xlsirtp11-R contained a 300-bp region with three repeats, and Xlsirtp11-U contained the 250-bp unique sequence from the 5′ end of Xlsirtp11. The chimeric construct (R-caax) contained the 200 bp from the 5′ end of Xlcaax mRNA from the *Xlcaax* gene (*18*) linked to the 300-bp fragment containing three repeats from the Xlsirtp11 clone. In vitro synthesized ^{35}S-UTP–labeled transcripts from each of these clones were injected into the cytoplasm of stage 4 oocytes. Oocytes were cultured for 4 days and analyzed as in Fig. 3. (**A**) Oocyte injected with Xlsirtp11R-U RNA. (**B**) Oocyte injected with Xlsirtp11-R RNA. (**C**) Oocyte injected with Xlsirtp11-U RNA. (**D**) Oocyte injected with Xlsirtp11R-caax RNA. Bars represent 50 μM.

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