because of passage through a 5:3 resonance with Umbriel (*20*), is uncertain in both probability and magnitude. The same can be said for tidal heating of Miranda (*21*). A more serious problem is that it is difficult to imagine a global heating mechanism that could produce warming this great that would not lead to substantial viscous relaxation of nearby large impact craters and tectonic features that clearly predate the extrusion events (10-km craters would have viscously relaxed in ~100 years at such high temperatures). Instead, a low viscosity material appears to have been segregated somehow, and then mobilized by temperatures not warm enough to allow relaxation of surrounding terain.

There are strong cosmochemical arguments that suggest that the uranian satellites may have formed under conditions that would have led to inclusion of volatiles such as CH_4, CO, or N_2 as clathrates, or NH_3 as one of the stoichiometric ammonia hydrates (*22, 23*). Even modest amounts of accretional heating could have created a subsurface zone in which partial melting produced CH_4, CO, or N_2 liquid, or a H_2O-NH_3 peritectic melt. These fluids, because of their mobility and contrast in density with the surrounding material, would tend to migrate, forming segregated pockets or magma bodies.

Stevenson and Lunine (*24*) have suggested that a small amount of CH_4, CO, or N_2 fluid could cause pressure solution creep of a fine-grained ice matrix, leading to viscosities as low as 10^{14} poise at uranian system temperatures. This mechanism is attractive, although it is untested and highly dependent upon grain sizes and grain boundary widths. Very recent experimental results indicate that the viscosity of H_2O-NH_3 ice can be substantially lower than that of pure H_2O ice at the same temperature (*25*). Peritectic-composition NH_3-H_2O solid consists of two stable phases, H_2O ice and ammonia dihydrate ($NH_3 \cdot 2H_2O$). The low viscosity found experimentally for the mixture indicates a rheology dominated by a more mobile phase other than H_2O, probably dihydrate (though metastable monohydrate or glass might also be present). Even if segregated peritectic-composition pockets solidified after accretional heat was lost, then they might be remobilized in the solid state by a relatively mild tidal heating episode. Since complete differentiation of the uranian satellites is very unlikely, the material surrounding these pockets would be comparable in density to the bulk satellite value of about 1.6 g/cm^3. Despite the fact that ammonia dihydrate is slightly more dense than pure water ice (*26*), the pockets would be buoyant relative to surrounding undifferentiated material and would rise to the surface if sufficiently mobile. We note that further creep experiments on all of these exotic ices are sorely needed.

The solid-state resurfacing that has taken place on the uranian satellites is, to our knowledge, unique in the solar system. Highly localized ice flows with viscosities less than 10^{16} poise on satellites this cold appear to require the presence of some material that can substantially increase mobility. If this material is indeed some low-temperature condensate, then solid-state ice volcanism might also be found to be geologically important in the Neptune and Pluto systems.

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Site-Specific Integration of H-*ras* in Transformed Rat Embryo Cells

W. GILLIES MCKENNA, KEN NAKAHARA, RUTH J. MUSCHEL

A karyotypic analysis was performed on seven independently derived clones of primary rat embryo cells transformed by the *ras* oncogene plus the cooperating oncogene *myc*. The transfected oncogenes were sometimes present in amplified copy number, with heterogeneity in the levels of amplification. Some chromosomal features, such as aberrantly banding regions and double-minute chromosomes, typical of cells carrying amplified genes, were also seen in three of the seven cell lines. Underlying this heterogeneity there was an unexpected finding. All seven lines showed a common integration site for *ras* on the q arm of rat chromosome 3 (3q12), though some lines also had other sites of integration. In four of the lines integration of *ras* was accompanied by deletion of the p arm of chromosome 3 or its possible translocation to chromosome 12.

ACTIVATION OF THE *ras* ONCOGENE has been implicated in a wide variety of human and animal malignancies (*1*). In NIH 3T3 cells the oncogene has been shown by calcium phosphate–DNA transfection to cause morphological transformation (*2*) and to induce the tumorigenic (*3*) and metastatic (*4*) phenotypes. Spandidos and Wilkie (*5*) have shown that it is possible to transform a variety of early passage cells with H-*ras* when the gene is linked to a strong enhancer. Similar results were reported for very early passage rat embryo cells by Pozzatti *et al.* (*6*). The latter also showed that it was possible to obtain rare transformants with the *ras* gene alone (in co-transfection with a selectable marker, pRSVneo), if rat embryo fibroblasts were transfected when subconfluent. These rare

W. G. McKenna, Department of Radiation Oncology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.
K. Nakahara, NCI-Navy Medical Oncology Branch, Naval Hospital, Bethesda, MD 20895.
R. J. Muschel, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

transformants are of interest because, as we showed in a previous publication (7), the cells can become transformed and acquire the metastatic phenotype without any detectable chromosomal abnormalities. Hence, in this experimental system, diploid cells can be metastatic and chromosome rearrangements are not, of necessity, required for expression of the metastatic phenotype. Such transformants of primary rat embryo fibroblasts by H-*ras* alone are, however, rare. The frequency of transformation by the *ras* oncogene can be greatly increased if a cooperating oncogene is co-transfected with *ras* (8). In addition, Land *et al.* have shown that it is not possible to transform confluent rat embryo fibroblasts with *ras* alone but that a second cooperating oncogene is required for transformation at confluency (9). This raises the possibility that the transformants (by *ras* alone) of subconfluent cells may represent transformation or selection of some minor subpopulation within the rat embryo fibroblasts and hence not be representative of transformation as it occurs in most cells in the population.

We undertook a karyotypic analysis of a series of independently derived clones of rat embryo fibroblasts transformed by *ras* plus the cooperating oncogene *myc*. When *ras* and *myc* were used to transform primary Syrian hamster cells, it was found that transformation and tumorigenicity were always associated with the loss of one copy of hamster chromosome 15 (10). We analyzed the karyotypes of lines derived from seven independent foci of rat embryo fibroblasts that had been transformed by Ca_2PO_4 transfection of the human H-*ras* oncogene (pEJ) in co-transfection with pMC29, a plasmid carrying the cloned copy of the avian myeloblastosis virus (v-*myc*). All cell lines were morphologically transformed in cell culture with the loss of contact inhibition. Additionally, all the cell lines were tumorigenic in nude mice, readily forming rapidly growing tumors and were positive for metastasis in nude mice either by the spontaneous or the experimental assays or both (Table 1) [Garbisa *et al.* (11)]. When the trypsin-Giemsa banding patterns were examined (Table 1), it was evident that grossly detectable chromosome alterations had occurred in five of the seven lines studied (2.2, 2.3, 2.10, 4.1, and 4.6), whereas 2.8 and 3.7 were apparently normal. In four of the five lines with chromosomal aberrations, an alteration was seen in the short (p) arm of chromosome 3. One copy of the 3p arm was deleted in cell line 2.2; in 2.3 and 4.1 it may have been translocated to chromosome 12, and it was partially deleted in 4.6 (Fig. 1). These changes in chromosome 3 were seen in all spreads examined from these cell lines. In addition, other chromosomal alterations were seen in significant subpopulations of the various cell lines. Approximately 50% of the spreads from both 2.2 and 2.3 had trisomies of chromosomes 11; an unidentified marker chromosome was seen in 2.10.

Several of the cell lines also had features consistent with gene amplification; 2.2 carried an aberrantly banding region in 70% of mitoses (Fig. 1), and this line and two others had double minutes (Table 1).

In addition to this karyotypic variability, heterogeneity was also seen in the numbers of copies of the transfected plasmids from cell line to cell line. The number of copies of H-*ras* varied from a low of 2 to 5 copies in clone 4.1 to more than 50 in 2.3, while v-*myc* copy number ranged from 1 or 2 to greater than 1000 in 2.3 at passage 20 to 40 (upon subsequent passage this large number of v-*myc* copies in 2.3 was reduced to 2 to 4). The limited extent of amplification of *ras* suggested that it was not present in the double minutes. This was confirmed by in situ hybridization where hybridization of the *ras* probe to the double-minute chromosomes was not seen, nor was v-*myc* seen in the double minutes.

We also undertook a study of the site of integration of the transfected H-*ras* oncogene by in situ hybridization. In each of the

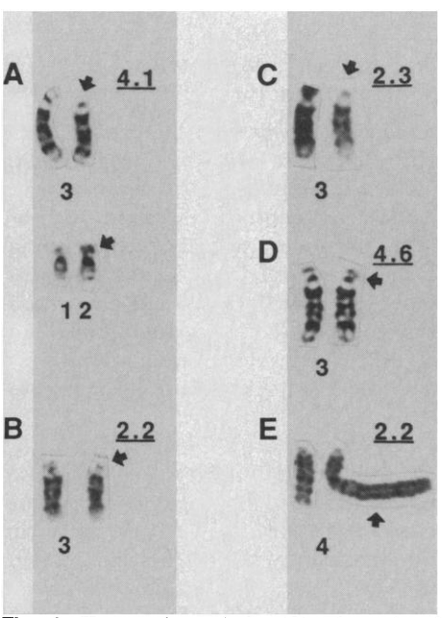


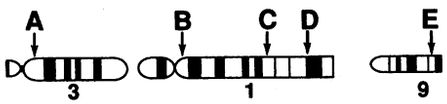
Fig. 1. Karyotypic analysis. (**A**) The pair of chromosomes 3 and 12 of 4.1 with arrows indicating the 3p− and its putative translocation to chromosome 12; (**B**) the chromosome 3, in 2.2, with an arrow showing the deletion of the p arm; (**C**) as in (B) for 2.3; (**D**) the partial deletion of the p arm of chromosome 3 in 4.6; (**E**) the aberrantly banding region on chromosome 4 in 2.2.

Table 1. Characteristics of a series of independent H-*ras* plus v-*myc*–transformed rat embryo cells. Seven permanent cell lines were established (2.2, 2.3, 2.8, 2.10, 3.7, 4.1, and 4.6). They are independent foci isolated from three separate transfections of rat embryo fibroblasts (day 17 Sprague-Dawley embryo cells; Flow Laboratories, McLean, Virginia) with calcium phosphate–DNA precipitates made of equal amounts of pEJ and pMC29. The clones were isolated from three separate transfection experiments (2.2, 2.3, 2.8, and 2.10 from one, 3.7 from another, and 4.1 and 4.6 from a third) although the same preparation of rat embryo cells was used in each. Within each transfection experiment, 2.2, 2.8, and 2.10, and 4.1 and 4.6 are clearly different based on their karyotypes. Cell lines 2.2 and 2.3 cannot be distinguished in this way, but can clearly be distinguished by oncogene amplification. In addition, DNA blotting with *ras* or v-*myc* as probes reveals distinct minor bands for each clone, further augmenting the evidence for clonal independence (23). We showed that the cell lines contain the H-*ras* and *myc* oncogenes by analysis of their DNA using DNA blotting. Copy number was quantitated with a Hoeffer scanning densitometer across the predominant band compared to standard amounts (the cross-hybridizing *myc* band being readily distinguishable from the plasmid band). For karyotypic analysis, our previous procedures (7) based on Sun *et al.* (24) were used. Chromosomes were numbered according to the scheme of the Committee for a Standard Karyotype of *Rattus norvegicus* (25). At least 50 spreads were counted for each clone, and at least 5 banded spreads were obtained for every cell culture. Metastasis was measured as described in Pozzatti *et al.* (6).

Clone	Karyotype	Double minutes % of cells	Copy number		Metastases	
			ras	*myc*	Spontaneous	Experimental
2.2	42,XY,del(3)(p11)*	8	8–12	2–4	6/7	13
2.3	42,XY,t(3;?12)(p11;p12)†	52	20–40	1000 (2–4)‡	4/5	ND
2.8	42,XY	0	3–6	2–4	6/7	20
2.10	42,XY/43,XY,mar§	14	8–12	1–2	3/5	ND
3.7	42,XY	0	5–12	2–4	7/7	82
4.1	42,XY,t(3;12)(p11;p12)	0	2–5	1–2	ND	11
4.6	42,XY,del(3)(p11;p12)	0	5–20	1–2	ND	83

*In clone 2.2, 70% of the cells observed were 42,XY,del(3)(p11),mar(4)(ABR)(q4) and 30% were 43,XY,+11,del(3)(p11),mar(4)(ABR)(q4). †In clone 2.3, 50% of the cells were as shown and 50% were 43,XY,+11,t(3;?12)(p11;p12). ‡After passage 40. §In clone 2.10, 25% of the cells also contain mar(2)(HSR)(q1).

lines studied, H-*ras* was found integrated close to the centromere of chromosome 3, on the proximal region of the long arm (3q12) (Fig. 2). The *ras* was integrated into only one of the two copies of chromosome 3 in each cell line (Fig. 3), although rare spreads were seen with hybridization to both copies of chromosome 3 in cell line 2.2, but these accounted for less than 1% of the spreads examined. In the four lines with an aberrant 3p, *ras* was integrated into the aberrant chromosome. In four of the cell lines, chromosome 3 appeared to be the only site of integration of H-*ras* while other



Cell line	Percentage of grains						Total grains
	A	B	C	D	E	Other sites	
2.2	61				8	None	179
2.3	90					None	125
2.8	80					None	139
2.10	82					None	133
3.7	42					5% on 2q11-12	146
4.1	14	19		5		None	288
4.6	6*		44	5		4% on ?16	386

Fig. 2. In situ hybridization with an H-*ras* probe on H-*ras* plus v-*myc*–transformed rat embryo cells. The technique used was based on that described by Harper *et al.* (*26*). Hybridization was done with ^{35}S- or ^{3}H-labeled probe DNA (2×10^7 to 2×10^8 cpm per microgram of DNA) (100 to 200 mCi/ml) in a solution containing 50% formamide, 1 m*M* dithiothreitol, 10% dextran sulfate, 4× standard saline citrate–phosphate, and sonicated, denatured herring sperm DNA (200 μg/ml). A quantity of 50 μl containing approximately 5×10^6 cpm was placed on each slide, covered with a cover slip, and incubated at 37°C for 14 to 18 hours. After hybridization the cover slips were removed, and the slides were washed three times with 2× standard saline citrate (SSC) and 50% formamide at 39°C, then five times with 2× SSC at 39°C, and then dehydrated and air-dried. The 2.9-kb Sst I fragment of the H-*ras* oncogene (pEJ), was used as the probe. The chromosomes were banded through the emulsion as described (*26*). Grains were counted only if they were directly over the chromosomes. Ideograms shown are our own modification of those of Levan (*27*), and his nomenclature for banded rat chromosomes is also used. Any group of grains representing 5% or more of the total is indicated in the figure. The other grains followed what appeared to be a random distribution. The figure of 6% marked with an asterisk is about the level of hybridization we would expect if 1 to 2 copies of H-*ras* were integrated at this site out of a total of up to 20 copies in this cell line. The χ^2 value with Yates's correction for continuity for this number is 18.9, which corresponds to a *P* value of <0.001. The χ^2 analysis tests the hypothesis that the labeling of the random grains is uniform over the entire genome. For all other lines, χ^2 values for this site, 3q12, also correspond to *P* values of <0.001.

sites were apparent in three lines. Indeed, in 4.1 and 4.6, these other sites appeared to be predominant but a site of integration at the 3q12 location was still noted. However, if a single copy were present at a minor site, it might have been missed because of the overwhelming signal generated at a site with multiple copies. In addition, there is difficulty in distinguishing the smaller rat chromosomes 16 from 17, and 18 and 19 from 20. The major site of integration on chromosome 1 seen in 4.6 was close to the approximate location of the cellular H-*ras* gene (*12*). We have not seen hybridization on the X, the site of the rat H-*ras* pseudogene, in any of our cell lines. The human H-*ras* gene that we used for transfection is widely divergent in sequence from its rat homolog (*13*). We did not see hybridization to untransformed, primary rat embryo fibroblasts with our H-*ras* probe either by in situ hybridization or in DNA blots (*14*).

We have determined the site of integration for v-*myc* in one cell line 2.3 (*15*) at passages when approximately 1000 copies per cell were present. Fifty percent of the grains were found over the 3q11 to 3q12 region (*15*). This does not imply independent integration of v-*myc* at this site. Since transfected DNAs are known to associate as aggregates (*16*), it would be expected that both transfected genes would be present at the same site. We were unable to analyze the other clones because the *myc* probe cross-hybridized with rat genomic sequences, so that in the others with low copy number, the cross-reaction precluded the analysis.

Using DNA sequences coding for the enzyme thymidine kinase (TK) and the protein CAD, several groups have concluded that the site of integration is random after Ca_2PO_4 transfection (*17*), yet in our system we find an apparently identical site in every cell line studied. In the TK and CAD systems, the selection that was applied after transfection was for expression of the transfected sequences, whereas in our system the selection we applied was for the transformed phenotype. There is now evidence that expression of *ras* p21 is necessary but not sufficient for full transformation in primary cells (*18*), although it may be sufficient in NIH 3T3 cells (*8*).

In this case, when *ras* is co-transfected with *myc*, the resultant cells that were selected for transformation not only express *ras* p21 but also show the full malignant phenotype. Our selection resulted in *ras* integration at the same site. The v-*myc* was inserted at the same site in 2.3. Since we currently have not been able to determine the integration site or sites of v-*myc*, its location remains uncertain, but as DNA tends to integrate in arrays after transfection it may well

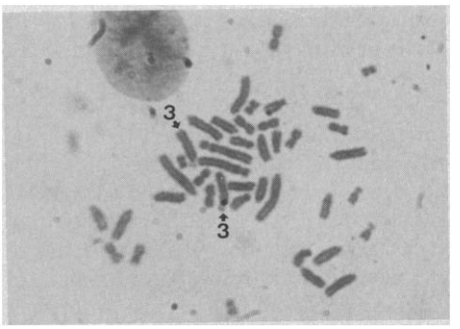


Fig. 3. In situ hybridization with H-*ras* in cell line 2.8. A representative spread from cell line 2.8 is shown hybridized with ^{35}S-labeled *ras* probe, after a 3-day exposure. Grains are seen overlying position 3q12 on one chromosome 3. The other copy of chromosome 3 shows no hybridization. A background grain is also evident over another chromosome.

be at the same site as *ras*. This raises two possibilities which are not mutually exclusive. This site may somehow be particularly receptive for integration or we may also be selecting for a cis action of the introduced plasmids on adjacent genes. This might be a positive effect through gene activation or possibly a negative effect through gene inactivation.

Klein (*19*) has formulated a model in which transformation would be associated not only with the activation of an oncogene but also with the loss of "suppressor" genes. Thus, Klein seeks to harmonize the oncogene hypothesis with the increasing evidence for the Knudson hypothesis (*20*), which originally explained the occurrence of the human tumor retinoblastoma by associating it with the loss of both alleles at a single locus, RB-1, and which has now been extended to other human tumors. Our finding of a unique chromosomal integration site for H-*ras* raises the possibility that the *ras* oncogene may be responsible for both effects in this in vitro system. That is, by its expression it acts as a dominant oncogene, and by its insertion or translocation to a particular chromosome location, it may act, in a sense, as an insertional mutagen inactivating a suppressor gene present at that site. This effect could occur either through a change in copy number, or, if reciprocal crossing-over occurred, through a deletion, not detected by karyotypic analysis, in the other chromosome 3 (*21*). H-*ras* insertion into the q arm of chromosome 3 is associated with frequent deletion or translocation of the p arm. The mechanism underlying the linkage between events on the q arm and the p arm of chromosome 3 is not known but it raises the possibility that gene transfer experiments may be accompanied by nonrandom chromosome changes. Thus, even when selected phenotypes arise at high fre-

quency, these phenotypes need not solely result from the activities of the transfected genes.

It is possible that the *myc* oncogene plays a role in this insertion through its action on the control of DNA replication (*22*). If H-*ras* transformation of primary cells results not only from expression of *ras* but also from events at an integration site, it is tempting to speculate that the ready transformation of NIH 3T3 cells by H-*ras* results from their already having undergone the chromosomal events that act to regulate the expression of the transformed *ras* phenotype.

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Assembly of Functional U1 and U2 Human-Amphibian Hybrid snRNPs in *Xenopus laevis* Oocytes

ZHEN-QIANG PAN AND CAROL PRIVES

Oligonucleotides complementary to regions of U1 and U2 small nuclear RNAs (snRNAs), when injected into *Xenopus laevis* oocytes, rapidly induced the specific degradation of U1 and U2 snRNAs, respectively, and then themselves were degraded. After such treatment, splicing of simian virus 40 (SV40) late pre-mRNA transcribed from microinjected viral DNA was blocked in oocytes. If before introduction of SV40 DNA into oocytes HeLa cell U1 or U2 snRNAs were injected and allowed to assemble into small nuclear ribonucleoprotein particle (snRNP)–like complexes, SV40 late RNA was as efficiently spliced as in oocytes that did not receive U1 or U2 oligonucleotides. This demonstrates that oocytes can form fully functional hybrid U1 and U2 snRNPs consisting of human snRNA and amphibian proteins.

IT IS NOW WELL ESTABLISHED THAT several members of the class of snRNPs are required for the splicing of pre-mRNA in higher eukaryotes (*1*). These particles each consist of small uridine-rich RNAs associated with several proteins (*2*). The two most abundant of these, the U1 and U2 snRNPs, are thought to mediate splicing through their interactions with the sequences in pre-mRNA introns at the 5′ splice site (*3–5*) and lariat branchpoint regions (*5, 6*), respectively. Both the proteins and the different U snRNAs in snRNPs are extraordinarily highly conserved in species ranging from lower eukaryotes to humans (*2, 7–10*). Consistent with this is the observation that introduction of U snRNAs from a wide variety of phylogenetic sources into the cytoplasm of *Xenopus laevis* oocytes results in the formation of complexes with the amphibian snRNA binding proteins stockpiled in the cytoplasm (*8–10*). These resemble snRNPs in their antigenicity, nuclear location, and sedimentation coefficient. However, it has not been demonstrated

Department of Biological Sciences, Columbia University, New York, NY 10027.

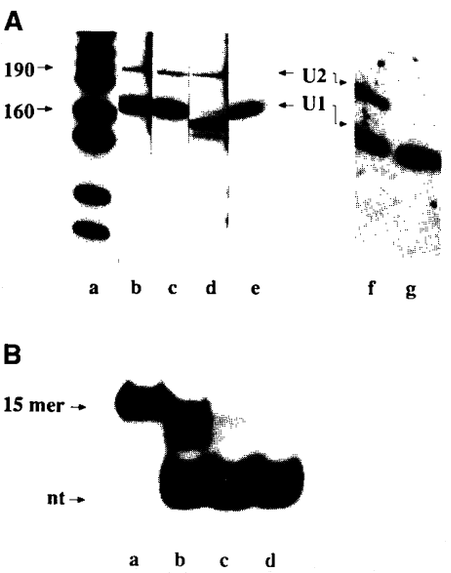

Fig. 1. (**A**) Degradation of U1 and U2 snRNA in oocytes injected with U1a and U2b oligos. Left panel: groups of ten oocytes were injected intranuclearly with H_2O (lane b), 20 ng of C oligo (lane c), 5 ng of U1a (lane d), or 20 ng of U2b (lane e). Right panel: RNA from a second batch of oocytes injected with 20 ng of either C oligo (lane f) or U2b (lane g) in which the autoradiogram was exposed for a time interval three times as long as for left panel. The oligonucleotides were delivered into oocyte nuclei in a solution containing 10 m*M* tris-Cl (*p*H 7.5) and 0.1 m*M* EDTA. The sequence of each oligonucleotide is as follows: C oligo, 5′-TCCGGTACCACGACG-3′; U1a, 5′-CTCCCCTGCCAGGTAAGTAT-3′; and U2b, 5′-CAGATACTACACTTG-3′. After being injected, in these and in subsequent experiments, oocytes were incubated at 19°C in modified Barth's solution (*19*). Four hours after injection, RNA was extracted and purified from oocytes as described (*16*). Quantities of RNA equivalent to one oocyte were fractionated on 8% polyacrylamide-urea gels and transferred to a GeneScreenPlus membrane (*20*), and the blot was then hybridized to plasmids containing human U1 or U2 DNA (*21*) that were uniformly labeled with ^{32}P. It should be noted that the intensity of U1 and U2 snRNAs shown does not reflect the actual ratio of these RNAs in oocytes, since the hybridization was performed sequentially, first with the U2 and then with the U1 probe. The residual intact U1 snRNA detected after injection of U1a was not decreased by injecting larger quantities of this oligonucleotide (*12*). The DNA size markers (lane a) were ^{32}P-labeled Msp I pBR322 DNA fragments. (**B**) Stability of injected oligonucleotides in oocytes. Oocytes containing 20 ng of U2b oligo 5′ end-labeled with ^{32}P were extracted as in (A). The extracts were then analyzed by electrophoresis on 10% polyacrylamide gels. Lane a at 0 min, lane b at 30 min, lane c at 2 hours, and lane d at 4 hours. Labels: 15 mer, a 15-nucleotide oligomer. The nt refers to the position of labeled mononucleotide, which was identified because it migrated slightly more rapidly than γ-^{32}P but more slowly than inorganic phosphate.