# Deoxyribonucleic Acid in Germinal Vesicles of Oocytes of Rana pipiens

Abstract. *The amount of DNA isolated from germinal vesicles of* Rana pipiens *oocytes is similar to that isolated from whole unfertilized eggs, and this suggests that oocyte nuclei are the source of much of the DNA obtained from whole eggs. Fluorometric determinations of the isolated DNA show the presence of 0.004 microgram of DNA equivalents per germinal vesicle. Similar values were obtained by estimations from comparisons of the cesium chloride density-gradient profiles of sample DNA and a known amount of reference DNA from* Pseudomonas aeruginosa. *The buoyant density of DNA prepared from isolated germinal vesicles is the same as that of frog-liver DNA, 1.706 grams per cubic centimeter, as determined by equilibrium sedimentation in a cesium chloride density gradient.*

The amount of DNA in unfertilized amphibian eggs estimated with chemical methods (*1*), fluorometric tests (*2, 3*), and microbiological assay methods (*4*) is from 1000 to 10,000 times (0.025 to 1.2 $\mu$g of DNA per egg) the DNA content of spermatozoa or somatic cells of the species. These values have been interpreted as demonstrating the presence of large amounts of cytoplasmic DNA. However, similar amounts have not been isolated from unfertilized eggs. Approximately 0.004 $\mu$g per egg has been reported (*5*) from DNA prepared from eggs of *Rana pipiens;* similar amounts (0.002 to 0.004 $\mu$g per egg) have been isolated from unfertilized eggs in my laboratory (*6*) by the Marmur method (*7*). The egg DNA has been tentatively considered (*5*) to be mainly of mitochondrial origin, which would imply that the germinal vesicle was not a major source of the isolated DNA. However, the amount of DNA per germinal vesicle determined (*3*) with a fluorometric method (*8*) was of the same order of magnitude (0.015 $\mu$g). The fact that the amount of DNA per germinal vesicle as measured by fluorometric tests (*3*) is similar to the amounts that have been isolated from the whole eggs (*5, 6*) indicates that much of the isolated DNA is of nuclear origin. It seemed necessary to determine whether the amount of DNA that can be isolated from germinal vesicles is similar to that determined by fluorometric assays.

I now describe the preparation of DNA from isolated germinal vesicles of ovarian eggs of *R. pipiens* and compare its behavior in a CsCl density gradient with that of frog-liver DNA prepared by the same method.

Germinal vesicles were isolated manually in nuclear medium (0.11*M* NaCl, 0.002*M* KCl) as described (*3*) and stored frozen until enough were accumulated for a DNA preparation. The frozen nuclei were thawed, ground with a glass rod, and treated with pronase (1 mg/ml) at 37°C for 8 to 12 hours. The suspension was then processed by a modification of the method (*7*) used for the preparation of frog-liver DNA. The aqueous phase after the first chloroform treatment was dialyzed against 0.15*M* NaCl plus 0.015*M* trisodium citrate (SCS) and then concentrated with Aquacide I. Similar dialyses or dilutions with SCS followed by Aquacide treatments were repeated after treatment of the sample with a mixture of ribonuclease A plus ribonuclease T1 (100 $\mu$g/ml). With this modification it was possible to omit alcohol precipitation and avoid the losses incurred when small quantities of precipitated DNA are redissolved. The final volume was adjusted to contain 500 to 1000 germinal vesicle equivalents per 100 $\mu$l of sample.

DNA was prepared from batches of 10,000, 5200, 4000, and 6300 nuclei at different times and analyzed in CsCl solutions (*9*). Portions containing 500 to 1000 germinal vesicle equivalents of the DNA preparations were added to the appropriate amount of CsCl in tris buffer, *p*H 8.5 (final density approximately 1.7 grams per cubic centimeter) and centrifuged at 42,040 rev/min for 20 hours in the An-D rotor in a Spinco model E ultracentrifuge with ultraviolet optics. In all cases a single ultraviolet absorbing band was formed at equilibrium. Similar samples which were previously dialyzed against a 0.1*M* phosphate buffer (*p*H 7.0 containing 0.003*M* $MgCl_2$) and treated with deoxyribonuclease (0.5 mg/ml) at 37°C for 8 hours did not form a band in the CsCl density gradient. Samples centrifuged with 2.85 $\mu$g of *Pseudomonas aeruginosa* DNA [density 1.727 g $cm^{-3}$ (*10*)] as a reference density marker (Fig. 1A) were calculated (*10*) to have a density of 1.706 g $cm^{-3}$, equal to that obtained for frog-liver DNA. In addition, mixtures of germinal vesicle DNA and frog-liver DNA formed a single band in a CsCl density gradient (Fig. 1B).

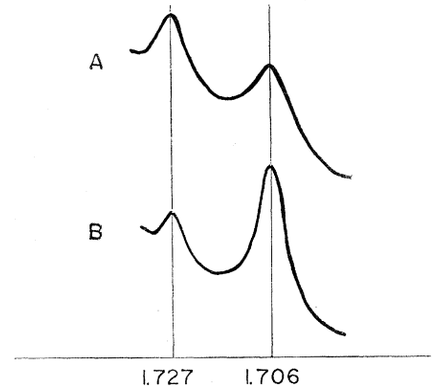


Fig. 1. Equilibrium density-gradient centrifugation in CsCl of *Rana pipiens* germinal vesicle DNA (A) and a mixture of equal amounts of germinal vesicle and frog-liver DNA (B). Centrifugation at 42,040 rev/min for 20 hours (Spinco model E ultracentrifuge); *P. aeruginosa* DNA (2.85 $\mu$g; density 1.727 g $cm^{-3}$) was used as a reference density marker.

Analysis of five 25-$\mu$l portions of one of the germinal vesicle DNA preparations by a fluorometric technique (*3, 8*) indicated the presence of 0.004 $\mu$g of DNA equivalents per nucleus. Furthermore, an estimate of the amount of DNA per unit volume of sample in the CsCl density gradients made by comparison of the areas under the peaks of analytrol tracings (Fig. 1A) of sample DNA and that from *P. aeruginosa* indicates the presence of between 1.8 and 2 $\mu$g of DNA per 500 nuclei, or approximately 0.004 $\mu$g per nucleus.

Thus, the DNA content of the germinal vesicle is approximately 300 times that of the diploid amount [0.015 m$\mu$g (*5*)] present in somatic cells and that it has the same effective density and, therefore, the same guanine-cytosine content as that of somatic cell DNA. The many nucleoli of the germinal vesicle are possible sites of localization of much of the DNA. There is as much DNA in the nucleoplasm of newt oocyte nuclei as in the chromosomes (*11*). It also was shown by treatment with actinomycin D (*12*) that RNA synthesis in oocyte nucleoli was DNA-dependent. A more direct demonstration of the presence of nucleolar DNA in amphibian oocytes was accomplished by electron

microscope studies (*13*) of dispersed nucleoli. After dispersion, the nucleoli formed a "beaded necklace" arrangement, with a 30-Å fiber connecting the beadlike structure. Treatment with deoxyribonuclease broke the fiber whereas ribonuclease, trypsin, and pepsin caused a reduction in size but left the circle intact. The possibility arises that, in the unfertilized egg in which the nuclear membrane has broken down and the chromosomes are in the metaphase of the first maturation division, extruded nucleoli may be significant sources of DNA.

The foregoing results would indicate that isolations of DNA from unfertilized eggs which yield quantities of the order of 0.004 $\mu$g per egg may represent only that DNA which was in the germinal vesicle before meiosis (that is, the chromosomal and nucleolar DNA) and that, at this quantitative level, mitochondria may not be significant sources of egg DNA. The large amounts that have been measured by indirect methods, the so-called cytoplasmic DNA, may also be present but in complexes, localizations, or with properties such that it is not obtainable by conventional methods of preparation.

*Note added in proof*: It has been reported (*14*) that the mitochondrial DNA content of the frog egg is 0.002 $\mu$g. These results and the values reported above for the DNA content of germinal vesicles would suggest that the total DNA content of the fertilized frog egg is at least 0.006 $\mu$g.

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## Replication of Adenovirus Type 7 in Monkey Cells: A New Determinant and Its Transfer to Adenovirus Type 2

Abstract. *A strain of human adenovirus type 7, adapted to replication in green-monkey kidney cells, requires the interaction of two particles to initiate plaque formation in the simian cells. One particle is a true adenovirion. The second, apparently defective, consists of a genome carrying a monkey-adapting component in an adenovirus capsid; this genome does not express known SV40 determinants. The addition of human adenovirus type 7 that is not adapted enhances the titer and changes conditions for plaque formation by the adapted virus to a one-particle requirement. Addition of nonadapted human adenovirus type 2 as helper virus results in the transfer of the monkey-adapting component from adenovirus type 7 to adenovirus type 2. The population containing the adenovirus 2 transcapsidant then has the ability to replicate in simian cells.*

Most strains of human adenovirus are unable to replicate in simian cells unless the cells are coinfected with papovavirus SV40. The necessary growth-potentiating information may be provided by complete, autonomously replicating SV40 (*1*), by complete SV40 genomes encased in adenovirus capsids (*2*), or by defective SV40 (PARA) (particle aiding the replication of adenoviruses). Like a true adenovirion, the PARA particle contains an adenovirus capsid. PARA is unable to replicate in the absence of helper adenovirus, but can be identified by the ability to induce synthesis of SV40 tumor (T) antigen (*3*). Lewis *et al.* (*4*) have reported that many monkey-adapted strains of adenovirus are contaminated with SV40, in that they can induce synthesis of its T antigen.

During an investigation of strains of human adenoviruses able to replicate in simian cells, we encountered a human adenovirus type 7, adapted to growth in monkey cells, which did not induce the synthesis of detectable amounts of SV40 T antigen. The history of this monkey-adapted adenovirus 7, called adeno 7 (M), is as follows. Wyeth Laboratories received a sample of adenovirus type 7 that had been isolated at the National Institutes of Health and had been passed twice in human kidney cells. The virus, designated No. 19690, was then passed once in human embryonic kidney cells, four times in green-monkey kidney cells, and six times in baboon kidney cells. All the cell lots were tested and found free of SV40. The last passage material from the baboon cells, MK10, was negative when tested for PPLO (pleuropneumonia-like organisms) and negative when safety-tested for contaminating viruses in the presence of type 7 antiserum in cells from rabbit kidney, human amnion, and rhesus kidney. Adeno-satellite virus (ASV) particles (*5*) were abundant. We received a portion of the MK10 passage of adeno 7 (M) and passed it twice more in green-monkey kidney cells. Our experiments were performed with the second passage stock, which was free of detectable amounts of ASV particles. Adeno 7 (M) does not induce SV40 T antigen in green-monkey kidney cells detectable by either immunofluorescence or complement fixation.

Adenovirus plaque assays were performed with human embryonic kidney cells in 35-mm plastic petri dishes (*6*). Assays were also performed in green-monkey kidney cells growing in 60-mm plastic petri dishes, sometimes in the presence of high concentrations of nonreplicating helper human adenovirus (*6*). The overlay for all assays contained Eagle's medium, 10 percent fetal bovine serum, 0.23 percent sodium bicarbonate, and 1 percent agar. A second overlay was applied on day 8 and contained neutral red diluted 1:20,000.

Plaque formation by adeno 7 (M) was studied in human embryonic and green-monkey kidney cells with twofold dilutions of the virus. The number of plaques developing in the cultures of human cells closely followed the dilution factor of the virus inoculum, suggesting that infection by only one particle was required to initiate a plaque. This was found to be the case (*6*) with PARA-adenovirus 7, a population known to be carrying defective SV40 genomes.

Plaque formation in green-monkey kidney cells by adeno 7 (M), however, was found to follow second-order kinetics. Five to eight replicate plates were used for 0.1 ml of each twofold dilution of the virus. The results of a representative experiment are shown in Table 1. At a dilution of 1:800, an average of 50 ± 3.9 plaques were counted on each plate. This aver-