Specific Gene Amplification in Oocytes

Oocyte nuclei contain extrachromosomal replicas of the genes for ribosomal RNA.

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So little is known about developmental mechanisms that the broadest questions are unanswered. For example, it is generally believed that "differential gene action" rather than any change in the genes themselves leads to the different phenotypes of cells in multicellular organisms. According to this hypothesis all types of cells within a single organism contain the same genes in equal number. Cells of one tissue would differ from those of another tissue according to which group of genes was expressed. The alternative hypothesis to that of "differential gene action" is that of "differential gene alteration" which proposes that one cell type differs from another because of a modification of the genes themselves (*1*). This "alteration" could result from a change in the DNA by base substitution or from a modification of bases by such reactions as methylation (*2*) or glucosylation (*3*). It could also be a deletion or replication of a specific region of the DNA. Hypermutation, recombination, and translocation of genes for antibodies have been considered to account for the variability of these proteins (*4*). Several lines of evidence have made the hypothesis of "differential gene action" attractive. The first cause of its acceptance has been the repeated demonstration that the size and number of chromosomes, as well as the quantity of DNA per diploid chromosome set, are constant in different cell types of a single organism (*5*, *6*). Chromosome diminution and chromosome loss which occur in somatic cells of some insects, worms, and crustaceans appear to be restricted to a few organisms (*6*). The second, more sensitive comparison of genes present in different cell types has been made by DNA-DNA hybridization studies (*7*). McCarthy and Hoyer tested the DNA's from different mouse tissues for their abilities to compete with labeled DNA from cultured mouse cells in the hybridization reaction; they detected no differences. The sensitivity of this method is not adequate since DNA's from species as different as higher apes and man can hardly be distinguished by it. Nuclear transplantation is a more stringent test for nuclear changes during differentiation. Nuclei from differentiated tissues of frogs, toads, and salamanders (*8*) have been transplanted into homologous enucleated eggs as a test of their ability to support development, and some nuclei from the intestinal epithelium of swimming tadpoles of *Xenopus laevis* can support normal development (*9*). Furthermore, whole plants have been reared from single somatic cells (*10*). However, these examples do not prove that the genetic material of the differentiated cell is unchanged, but rather that it has not been irreversibly altered.

We will discuss a case of reversible gene alteration in which the DNA specifying the sequences for 28*S* and 18*S* ribosomal RNA's (rRNA) (*11*) has been selectively replicated. This specific amplification of genes (*12*, *13*) for ribosomal RNA (termed rDNA) (*11*) occurs in one cell type—the oocyte; this amplification has been demonstrated in the oocytes of several amphibians, an echiuroid worm, and the surf clam.

The Extra Nucleoli in Amphibian Oocytes

The first suggestion that extra copies of rDNA are present in oocytes came from cytological observations of the large nuclei ("germinal vesicles") of amphibian oocytes (*6*, *14*). Somatic cells of amphibia usually contain one nucleolus for each haploid set of chromosomes (*15*). Each nucleolus appears to be derived from a "nucleolar organizer" region which frequently can be seen as a secondary constriction on one autosome at metaphase (*16*). Since the growing oocyte persists in the first meiotic prophase for an extended period (*17*), the cell is tetraploid and therefore would be expected to contain four nucleoli in its germinal vesicle. However, counts by MacGregor (*18*) for different species of *Triturus*, by Callan (*19*) for *Siredon mexicanum*, and by Miller (*20*) for *X. laevis* have shown approximately 600, 1000, and 1000 nucleoli per germinal vesicle, respectively. These nucleoli are not attached to the chromosomes in the germinal vesicle. There is now considerable evidence that these multiple nucleoli in the oocyte are analogous to the nucleoli in somatic cells and that each is an autonomous site for the synthesis of rRNA (*21*). Miller and Peacock (*22*) demonstrated that DNA-containing cores could be isolated from nucleoli of germinal vesicles of the newt *Triturus pyrrhogaster*, salamanders, and Plethodontids. They showed furthermore that each core contains a circular structure whose axis is DNA and which may be regarded as a "chromosome."

With molecular hybridization methods, it is possible to test the hypothesis that these multiple nucleolar "chromosomes" contain nucleotide sequences homologous to rRNA. Before presenting experiments which support this hypothesis, we will discuss some of the facts known about the DNA which is homologous to rRNA in *X. laevis* and the methods for its analysis.

Ribosomal Genes and Their Measurement

Ribosomal genes (*11*) are measured by their complementarity with rRNA, the product made on these genes (*23*). The most recent data for *X. laevis* show that 0.057 percent of the DNA of somatic cells is complementary to rRNA (0.114 percent of the base pairs) (*24*). Since a haploid chromosome set of *X. laevis* contains 3 picograms of DNA (*25*) and since the molecular weights of the 18*S* and 28*S* rRNA components are known, it can be calculated that

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each chromosome set contains about 450 of the 18*S* genes and an equal number of 28*S* genes (*24*).

Wallace and Birnstiel (*26*) predicted from the base composition of rRNA that its complementary DNA (rDNA) should have a higher content of guanylic and cytidylic acid (about 63 percent) than the bulk DNA (40 percent) and that it should therefore have a higher buoyant density in CsCl. They fractionated *X. laevis* DNA by equilibrium centrifugation in CsCl and showed that a high-density fraction hybridized with radioactive rRNA. This minor high-density DNA fraction was also detected by its optical density in analytical CsCl gradient centrifugation. When the DNA was hybridized with rRNA before centrifugation in the CsCl, the density of the hybrid was substantially higher than that of the rDNA itself. Since RNA has a much higher density than DNA, this large increase in the density of the hybrid suggests that a considerable proportion of each rDNA molecule was duplexed with rRNA. Since the DNA molecules were much longer than rRNA molecules, a high proportion of rRNA in the hybrid indicates that the rRNA genes are clustered, that is, that several of these genes are present on each fragment of DNA. Clustering of the 450 genes for rRNA was proved when these methods were applied to the DNA isolated from mutant embryos of *X. laevis*.

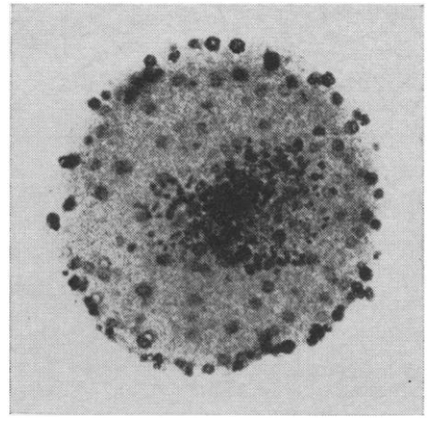


Fig. 1. Photomicrograph of an isolated germinal vesicle of *X. laevis*. The germinal vesicle was dissected from a mature oocyte in 0.01*M* $MgCl_2$, 0.02*M* tris, *p*H 7.4. It was then flooded with cresyl violet stain and photographed. Its diameter is about 400 μm. The deeply stained spots are some of the hundreds of nucleoli.

Embryos carrying this mutation in the homozygous form are anucleolate (0-*nu*) (*27*), and these embryos do not synthesize any rRNA during their limited life-span (*28*). The 0-*nu* embryos are devoid of the "nuclear organizer" constriction on both allelic autosomes which normally carry it (*29*), and their DNA does not hybridize with rRNA (*26*). Since this deletion removes more than 99 percent of the DNA homologous to 28*S* and 18*S* rRNA (*24*), the 450 rRNA genes must be clustered on one part of a single chromosome, most likely the autosome containing the "nucleolar organizer" constriction.

The high buoyant density of rDNA in CsCl facilitates its visualization and measurement in the analytical ultracentrifuge as well as its separation from bulk DNA by preparatory centrifugation in CsCl prior to hybridization with radioactive rRNA.

The DNA of Amphibian Germinal Vesicles

If the amount of rDNA in a germinal vesicle is increased relative to the amount present in a single somatic cell, is the increase a specific enrichment of ribosomal genes or does it reflect a proportional increase of the entire DNA in the germinal vesicle? Haggis (*30*) reported that the DNA content of germinal vesicles isolated from *Rana pipiens* (the leopard frog) is over a hundred times higher than the amount predicted for a tetraploid nucleus (4C), whereas Izawa *et al.* (*31*) found about four times the tetraploid complement in *Triturus viridescens* germinal vesicles. To reinvestigate this problem, we isolated germinal vesicles from oocytes of three genera of amphibia known to have widely different amounts of DNA per nucleus—*Necturus maculosus* (the mudpuppy), *Siredon mexicanum* (an axolotl), and *Xenopus laevis* (the South African clawed toad) (Table 1). The correlation of genome size with the DNA content in the germinal vesicle for each species should establish whether amphibian oocyte nuclei actually contain a large excess of chromosomal DNA.

Germinal vesicles were collected by hand from mature oocytes under a dissecting microscope, with particular care being taken to remove the nucleated blood cells and the cellular ovarian tissue. Because it was important to retain all of the nuclear contents, germinal vesicles (Fig. 1) were isolated in 0.01*M* $MgCl_2$ which causes the nucleoplasm to gel. These nuclei have a diameter of about 400 μm in mature oocytes compared to approximately 5 to 10 μm for most somatic nuclei of *X. laevis*.

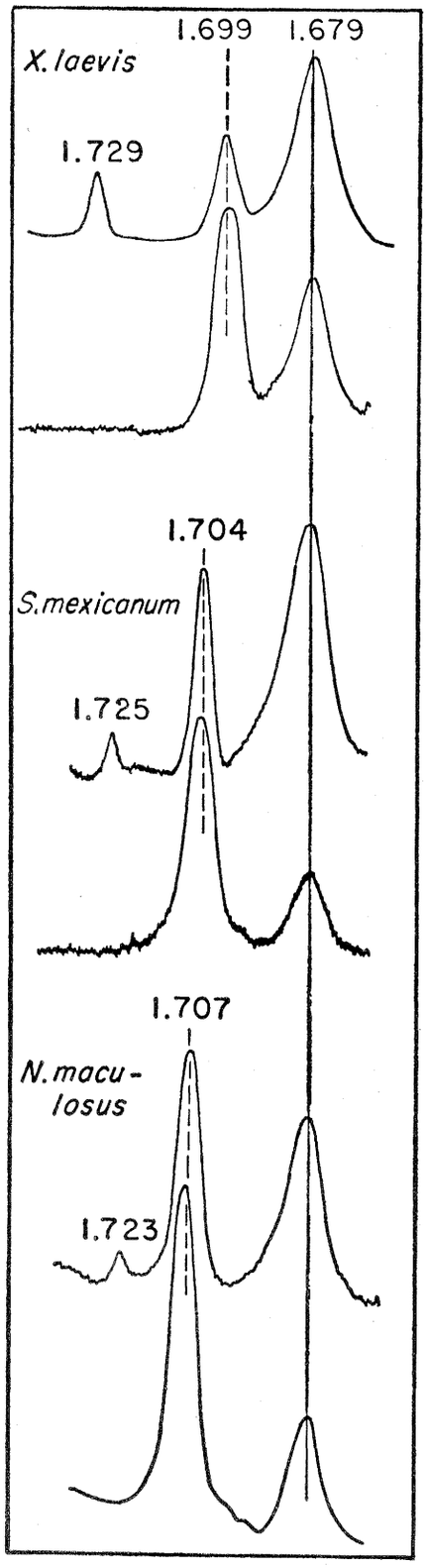
The method used for the determination of DNA in germinal vesicles was designed to measure very small quantities of DNA and then to recover it for subsequent hybridization studies. DNA was prepared from 10,000, 6,000, and 3,000 germinal vesicles of *X. laevis*,

Table 1. The content of "chromosomal" and "nucleolar" DNA in somatic nuclei and germinal vesicles of four amphibia.

Species	DNA (pg)					
	"Chromosomal" (low density)		"Nucleolar" (high density)			
	4C *	Germinal vesicle †	4C Total	4C rDNA ‡	Germinal vesicle † total	Germinal-vesicle § rDNA
X. laevis	12.6	70	0.02||	0.014	25	5.3
S. mexicanum	140	170		0.16	13	5.5
N. maculosus	380	500		0.08	30	5.3
T. viridescens	178			0.16		8.5

* The value for *N. maculosus* was determined by the diphenylamine reaction in a sample of counted erythrocytes. Literature citations for the other values are: *X. laevis* (*25*), *S. mexicanum* and *T. viridescens* (*49*). † Calculated from the band areas in the experiment shown in Fig. 2 and corrected for losses during purification by comparison with the band area of a known amount of dAT as described in the text (see also *51*). ‡ These values have been calculated from the 4C complement of DNA for each species (see first column) and from the percentage of the genome homologous to *X. laevis* ^{3}H-rRNA. The percentage of the genome that is homologous to *X. laevis* rRNA in *X. laevis*, *S. mexicanum*, *N. maculosus*, and *T. viridescens* is 0.057, 0.05, 0.01, and 0.05, respectively. The homology of *X. laevis* rRNA with these heterologous DNA's is extensive; if it is not 100 percent, then rDNA complements are underestimated here. In calculating the amount of rDNA, we assumed that only one strand of DNA is homologous to rRNA and therefore doubled each value. § The amount of rRNA homologous to germinal-vesicle DNA was determined from the extent of hybridization found in the experiments recorded in Fig. 3. The hybridization in each sample was compared to that of a known amount of somatic DNA. Since there is a direct relationship between the amount of DNA on filters and the radioactive RNA hybridized, even at the subsaturating concentration of ^{3}H-rRNA used in these experiments, the amount of rDNA can be calculated for each unknown preparation (*24*). Since losses of rDNA may have occurred during the hybridization steps, the rDNA values are minimum. Once again it is assumed that only one strand of the DNA is homologous to rRNA and therefore each value has been doubled. || Estimated from experiments of Birnstiel *et al.* (*26*) in which the proportion of high-density DNA in somatic DNA was directly measured by CsCl centrifugation in an analytical ultracentrifuge and found to be between 0.15 and 0.2 percent of the total DNA.

S. mexicanum, and *N. maculosus,* respectively. Deoxyadenylate-deoxythymidylate copolymer (2.8 μg) was added to each preparation; the germinal vesicles were lysed with 0.5 percent sodium lauryl sulfate, digested for 2 hours with 1 mg of pronase per milliliter at 37°C,



and extracted once with phenol. The extract was dialyzed and centrifuged to equilibrium in CsCl in a horizontal rotor. The refractive index of the fractions was measured to determine the location of the DNA. These fractions were pooled, dialyzed, treated with pancreatic ribonuclease (0.1 mg/ml) and ribonuclease T_1 (50 unit/ml) for 30 minutes at 37°C, and dialyzed. The DNA was purified further by adsorption on a small column of methylated albumin on kieselguhr in 0.3*M* NaCl, and eluted with 0.8*M* NaCl. After dialysis and concentration in vacuum the samples were centrifuged in CsCl in an analytical ultracentrifuge for 20 hours at 44,000 rev/min (*32*) (Fig. 2). The amount of DNA at each buoyant density was measured by comparison of its band area with the band area of the dAT, thereby correcting for losses of DNA during purification.

In addition to the major band of DNA at the buoyant density characteristic of somatic-cell DNA, the preparations of germinal-vesicle DNA from all three species contained additional DNA at a higher buoyant density (Fig. 2). At these concentrations, somatic-cell DNA does not have a visible high-density component. The corrected amount of DNA per germinal vesicle at each buoyant density is given in Table 1. The quantity of DNA in the main (low-density) band was close to the expected tetraploid amount (4C) in the case of *S. mexicanum* and *N. maculosus.* The amount in excess of the 4C complement in the preparations of germinal vesicles from *X. laevis* might be a result of contamination by a small portion of the cell's mitochondria. The total amount of mitochondrial DNA present in mature *X. laevis* eggs is about 200 times the 4C value, and the densities of nuclear and mitochondrial DNA's in *X. laevis* are so similar that distinction between them is difficult

Fig. 2. Tracings of germinal-vesicle and somatic-cell DNA centrifuged to equilibrium in CsCl in the analytical centrifuge. For each species the germinal-vesicle DNA is traced on the top, and the somatic DNA is traced below. The band at the density of 1.679 is the deoxyadenylate-deoxythymidylate copolymer (dAT) carrier added at the beginning of the isolation. The density of the DNA in the main band is the same in germinal-vesicle and somatic-cell DNA in all cases; in addition to this band the germinal-vesicle preparations show a high-density component which contains sequences complementary to rRNA.

Table 2. Relative abundance of sequences homologous to 28*S* and 18*S* RNA in the somatic and egg DNA's of *X. laevis.* Nine micrograms of each DNA were fractionated in CsCl, and the DNA in each fraction was immobilized on a Millipore filter (Fig. 3). The filters were split, and half-filter sets of egg and somatic-cell DNA were hybridized together with either 28*S* or 18*S* ^{3}H-RNA (1.5 μg of ^{3}H-RNA at 10^5 count min^{-1} μg^{-1} in 5 ml of 0.6*M* NaCl). Only radioactive material that hybridized with high-density DNA was scored. Since hybridization was not performed with saturating concentrations of radioactive RNA, the absolute 28*S* and 18*S* ratios are not significant, only the comparative ratios between the two DNA preparations (*24*).

Source of DNA	Count/min		28*S*:18*S*
	28*S*	18*S*	
Somatic cells	178	68	2.6
Eggs	640	262	2.4

(*33*). If only 2.5 percent of the oocyte's mitochondria contaminated the germinal vesicle during its isolation, it would account for the 60 pg of DNA in excess of the 4C amount. Since the chromosomal DNA complement is much higher in the two urodele species than it is in *X. laevis,* a comparable amount of mitochondrial contamination of these germinal vesicles would not be expected to alter substantially the apparent DNA content of the germinal vesicles. These considerations suggest that individual germinal vesicles contain about a 4C complement of chromosomal DNA, in agreement with their tetraploidy.

To measure the homology of the high-density DNA of germinal vesicles with rRNA, the three preparations of germinal vesicle DNA recovered from the experiments shown in Fig. 2, as well as 40 μg of somatic DNA of each of the three species, were denatured with alkali, and each of the six DNA samples was divided in half. One half was first hybridized in 0.6*M* NaCl and 0.06*M* sodium citrate for 1 hour at 70°C in a final volume of 1.5 ml with 20 μg of nonradioactive rRNA isolated from adult liver of the homologous species. The other half was incubated under identical conditions but without the addition of unlabeled RNA. All preparations were then diluted with an equal volume of water and treated with pancreatic ribonuclease (10 μg/ml) for 10 minutes at room temperature. Cesium chloride was added to a final density of 1.72 g/cm^3 and a final volume of 3.5 ml, and each preparation was centrifuged for 64 hours in the Spinco SW-39 rotor at 33,000 rev/min

with a temperature setting of 15°C. Each gradient was fractionated, and the DNA was denatured with alkali to liberate the bound RNA. The solutions were neutralized and diluted with 0.6*M* NaCl and 0.06*M* sodium citrate, and the DNA in each sample was trapped on a Millipore filter (*23*). The filters were then baked overnight at 70°C and stacked together in vials; the material was allowed to hybridize overnight at 70°C in 0.6*M* NaCl with 0.06*M* sodium citrate containing ^{3}H-rRNA (0.5 μg/ml, 4×10^5 count min^{-1} μg^{-1}). This concentration of RNA hybridizes with about 40 percent of the total rDNA sites. The radioactive RNA was isolated from cultured cells of *X. laevis* labeled with ^{3}H-uridine (*24*). The rRNA from *X. laevis* hybridizes well with the heterologous DNA's used here (unpublished observations). The filters were then washed with 0.3*M* NaCl, treated with ribonuclease, dried, and counted (Fig. 3). The amounts of rDNA in each preparation of germinal vesicles were calculated by comparison with the degree of hybridization obtained with somatic-cell DNA (*24*); the fraction of rDNA in somatic DNA had previously been measured by hybridization to saturation. The results (Table 1) demonstrate that, as determined by direct measurement of the high-density DNA component and its hybridization with radioactive rRNA, the ratio of rDNA to main-band chromosomal DNA is increased enormously in germinal vesicles. Similar results were obtained in hybridization experiments with germinal vesicles of *Triturus viridescens*. Furthermore, the hybridization studies show that the extra rDNA in germinal vesicles resembles the rDNA of somatic cells in three ways: (i) it has a high buoyant density in CsCl; (ii) hybridization of the DNA with rRNA before centrifugation in CsCl greatly increases the buoyant density of the rDNA (Fig. 3); and (iii) the ratio of DNA sequences homologous to 28*S* RNA to those homologous to 18*S* RNA is the same in germinal-vesicle and somatic-cell DNA (Table 2). The nucleotide sequences in germinal-vesicle DNA which are homologous to 28*S* and 18*S* RNA band at the same buoyant density and therefore are located on the same DNA molecules. The same is true of the sequences homologous to 28*S* and 18*S* RNA in somatic-cell rDNA (*24*).

Despite these general similarities between somatic-cell and germinal-vesicle rDNA's, their buoyant densities in CsCl are different. Somatic-cell rDNA was purified from *X. laevis* erythrocyte DNA by two cycles of centrifugation in CsCl in a fixed-angle rotor. The buoyant density of the somatic-cell rDNA satellite was 5 mg/cm^3 lower than the buoyant density of the germinal-vesicle rDNA (Fig. 4). This difference was confirmed by hybridization with rRNA after preparative CsCl centrifugation of the native DNA (Fig. 5). Inclusion of a bacterial DNA as a density marker and collection of a large number of fractions from the preparatory CsCl centrifugation permitted a precise localization of the rDNA in both samples. The good agreement between the density measured by analytical centrifugation and that determined by specific RNA hybridization clearly establishes that the two different techniques measure the same DNA component. The high-density DNA components of the somatic cells and the germinal vesicles from *S. mexicanum* have an analogous difference in their densities, which are 1.718 and 1.725 g/cm^3, respectively. We do not know

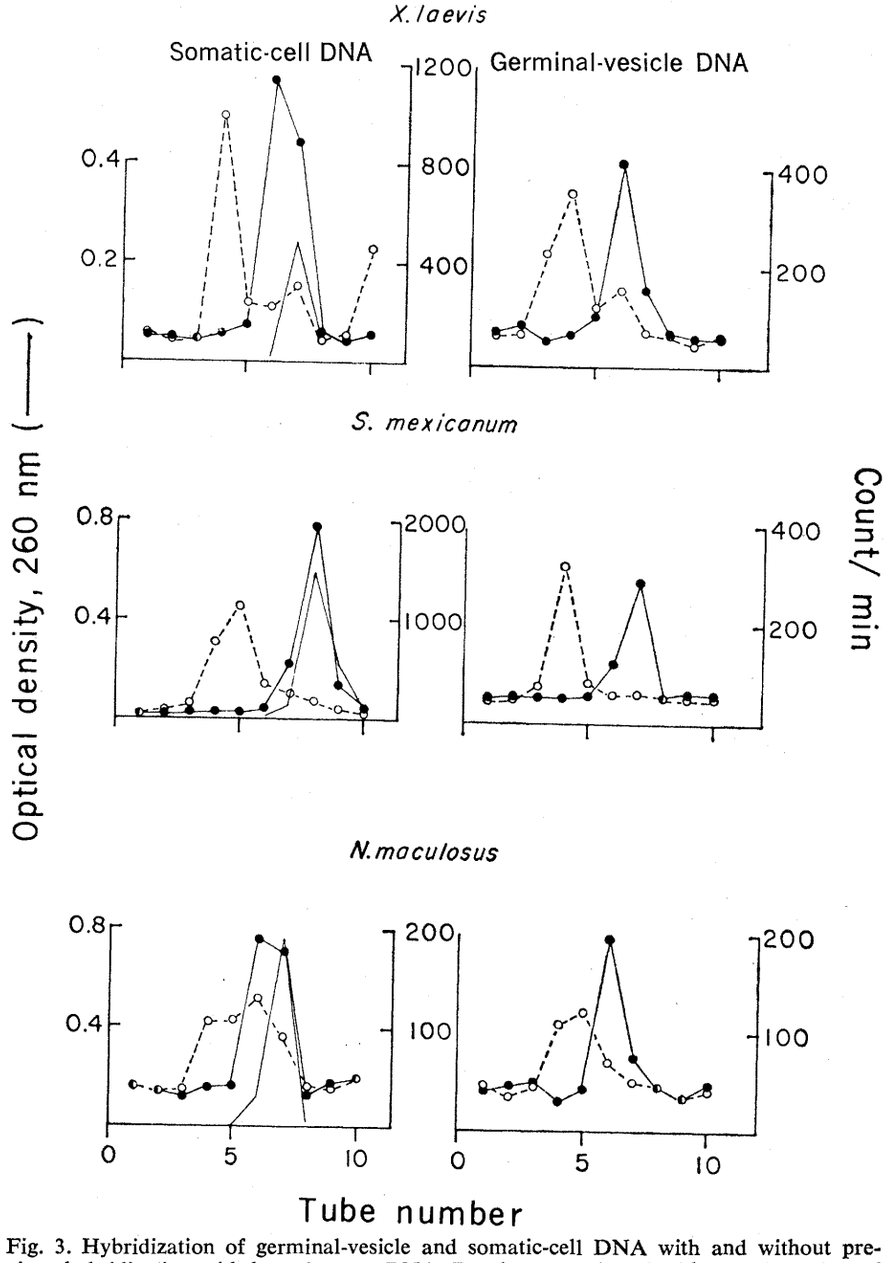


Fig. 3. Hybridization of germinal-vesicle and somatic-cell DNA with and without previous hybridization with homologous rRNA. Results were plotted without subtraction of background, which was about 50 count/min per filter without DNA. The optical densities at 260 nm were only plotted for somatic DNA because preparations of germinal vesicles contained no measurable optical density under conditions of these experiments. (●), Without previous hybridization; (○), with previous hybridization.

the reason for this difference in buoyant density, but several considerations are pertinent. There is no detectable difference between the base composition of rRNA synthesized during oogenesis of *X. laevis* (presumably under the direction of the higher-density germinal-vesicle DNA) and the rRNA made during development (transcribed from the lower-density somatic rDNA) (*34*). Furthermore, hybridization of either rDNA with rRNA causes a large shift in their buoyant densities (Fig. 3). This latter experiment suggests that the ratio of RNA to DNA in hybrids formed with either egg or somatic-cell rDNA is high, but the method has not been calibrated to measure small differences in the ratio of RNA to DNA. Even though the hybridization values are minimal for that portion of the high-density DNA complementary to rRNA, it is almost certain that nucleotide sequences are present in both high-density DNA's which are not homologous to rRNA (Table 1) (*24*). The suggested presence of nonhomologous nucleotide sequences intermingled with the sequences homologous to 28*S* and 18*S* RNA agrees with recent studies on rRNA metabolism in HeLa cells (*35*). The polycistronic rRNA precursor molecules of 28*S* RNA and 18*S* RNA have a much higher content of guanylic and cytidylic acids than either mature rRNA molecule, and about half of this precursor appears to be discarded during the maturation process. Whether the difference between the buoyant densities of the somatic-cell and germinal-vesicle rDNA's reflects a difference in nucleotide composition or a more subtle alteration in the DNA remains to be determined.

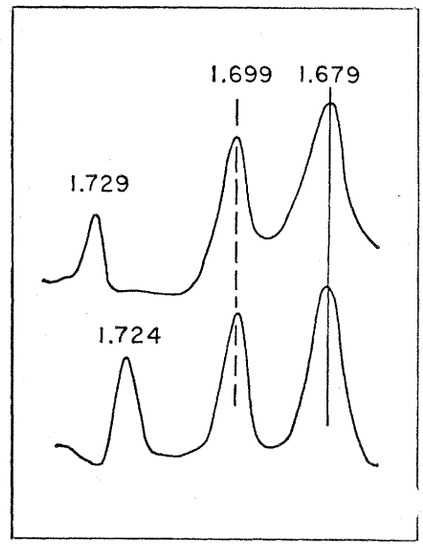


Fig. 4. Tracings of CsCl density-gradient experiments comparing the high-density components of germinal-vesicle DNA (top) and somatic-cell DNA (bottom) of *X. laevis*. Nuclear DNA from *X. laevis* (density 1.699) and dAT (density 1.679) were added to the purified somatic component to provide the same markers present in the samples of germinal vesicles. The density obtained for the high-density somatic-cell DNA agrees well with that reported earlier by Birnstiel *et al.* (*26*).

Selective Amplification of rDNA Relative to 4S DNA and 5S DNA

Whole unfertilized eggs are a more convenient source of DNA than are germinal vesicles. Therefore, egg DNA has been used to show the specific enrichment of rDNA relative to the DNA homologous to 4*S* RNA (a class of RNA including transfer RNA) and 5*S* RNA [a third structural RNA component of ribosomes (*36, 37*)]. Egg DNA was isolated from *X. laevis* as described previously (*25*). Since most egg DNA is mitochondrial (*33*), it was necessary to test whether the mitochondrial DNA can hybridize with these radioactive RNA preparations. There is no specific hybridization of purified mitochondrial DNA with ^{3}H-rRNA, whereas total egg DNA hybridizes extensively with rRNA (Fig. 6). The small amount of radioactive material which binds to the mitochondrial DNA is not due to sequences homologous to rRNA because this material binds to DNA fragments of average rather than high buoyant density, and because this low level of hybridization cannot be reduced by competition with an excess of unlabeled egg rRNA. Purified mitochondrial DNA has no detectable homology with either 4*S* ^{3}H-RNA or 5*S* ^{3}H-RNA, nor does its presence interfere with the analysis of rDNA when the DNA is fractionated in CsCl before its hybridization. An unfertilized egg contains about the same amount of rDNA as does an individual germinal vesicle.

The ratio of rDNA to 4*S* DNA and 5*S* DNA (*11*) has been compared in preparations of egg and somatic DNA's. The 4*S* DNA and 5*S* DNA can be separated from rDNA because they have different buoyant densities in CsCl; the 4*S* DNA has a buoyant density lighter than that of rDNA but slightly heavier than that of the bulk DNA, whereas 5*S* DNA has a density lighter than that of the bulk DNA (*24*). The abundance of rDNA relative to that of 5*S* DNA is the same in different somatic tissues (*24*) but very different from that found in egg DNA (Fig. 7). Egg DNA hybridizes well with rRNA, but there is no detectable hybridization with 5*S* RNA; the amount of 5*S* DNA of chromosomal origin is below the limit that this experiment could detect. The relative abundance of 4*S* DNA and rDNA has been compared in egg and somatic-cell DNA's (Fig. 8) with similar results. Therefore, we conclude that eggs are greatly enriched for DNA homologous to 28*S* and 18*S* rRNA relative to the bulk of nuclear DNA as well as to the DNA homologous to 4*S* RNA and 5*S* RNA.

Extra Copies of Ribosomal DNA in Oocytes of Other Animals

Do oocytes of animals other than amphibia contain extra copies of rDNA? The presence of multiple nucleoli in amphibian germinal vesicles is exceptional, since individual oocytes of most animals contain a single, prominent nucleolus. However, despite this difference, the patterns of oocyte maturation in widely different species are remarkably similar (*6, 17*). In an analysis of DNA from sea urchin eggs by analytical centrifugation in CsCl, Pikó *et al.* (*38*) found a DNA of high density which was not present in sperm DNA. It remains to be shown whether this DNA, which is equivalent in amount to a 1C (haploid) complement of sea-urchin DNA, contains extra replicas of rDNA.

We have purified total DNA from eggs of the echiuroid worm *Urechis caupo*, and the surf clam, *Spisula solidissima*. In experiments similar to that described in Fig. 8, the ratio of rDNA to 4*S* DNA was at least five times higher in egg DNA preparations than in sperm DNA of the same species. From this we conclude that these oocytes which contain only a single nucleolus also have extra copies of rDNA.

The Life History of Extra rDNA

The life history of the additional rDNA in amphibian oocytes can be reconstructed in the following way. During an early period of oogenesis, even before the oocyte chromosomes have extended to their specialized configuration termed "lampbrush," the extra copies of high-density rDNA are synthesized, and extra nucleoli appear in the germinal vesicle (*13, 18, 19*). Ovaries of young *X. laevis* actively incorporate

^{3}H-thymidine into the high-density DNA about 2 to 4 weeks after metamorphosis, and Feulgen-positive deposits become visible in the germinal vesicle at this stage (*13*). Formation of new nucleoli (*18–20*) and rDNA replication (*13*) do not occur in later stages of oogenesis.

Ribosomes are synthesized and accumulate in oocytes throughout "lampbrush" chromosome stages (*39*). Later, during yolk deposition, the lampbrush loops contract and stop functioning, but rRNA synthesis in the nuceoli continues throughout this period. When the egg is mature and ready for ovulation, all RNA synthesis stops. The germinal vesicle breaks down at the first meiotic reduction division, and the multiple nucleoli disappear and do not reappear when the nuclear membrane reforms (*21*). After meiosis the extra rDNA never functions again; there is no rRNA synthesis in eggs or embryos until the onset of gastrulation (*34*). At this stage, the expected diploid number of two nucleoli appears for the first time during embryogenesis (*21*), and they are undoubtedly the sites of the new rRNA synthesis (*40*). Although the extra rDNA is still present in unfertilized eggs, it is not replicated during cleavage. This fact is deduced from hybridization experiments which show that DNA from gastrula embryos, which are composed of about 30,000 cells, contains the same proportion of rDNA and 5*S* DNA as does adult somatic DNA (*24*). Thus, the extra rDNA either is diluted out by extensive nuclear replication or has been degraded. These extrachromosomal copies of rDNA are used for rRNA synthesis only during oogenesis and are subsequently rendered nonfunctional and discarded into the cytoplasm at the first meiotic reduction division.

Control of rDNA Replication in Oocytes

The amount of rDNA clustered within a single somatic-cell nucleolus in *X. laevis* is about 0.0035 pg (the haploid amount of rDNA calculated from values in Table 1). The minimum amount of rDNA in a germinal vesicle of *X. laevis* as determined by hybridization is 5.3 pg (1500 times the haploid amount); the total amount of high-density DNA is 25 pg, or 5000 times the haploid amount. Miller has found about 1000 nucleoli in a *X. laevis* germinal vesicle (*20*), and if each contained a single continuous DNA molecule, the average length of these molecules would be equivalent to or larger than one cluster of rDNA on the nucleolar organizer region of the chromosome. However, Miller (*22*) found that the circular structures ("chromosomes") from nucleoli of *T. pyrrhogaster* were not of equal length. If this is true for the nucleolar "chromosomes" in *X. laevis* oocytes, then at least some of them must contain DNA molecules that are either larger or smaller than the entire rDNA cluster at the nucleolar organizer region of the master chromosome. Furthermore, there is considerable variation in the number of nucleoli in each germinal vesicle (*18–20*) and presumably in the number of nucleolar "chromosomes" as well. If neither the length nor the number of these extra "chromosomes" is strictly determined in each oocyte, what are the important factors which govern the replication? Two observations that we have made are germane to this problem. First, the four amphibia analyzed in these studies

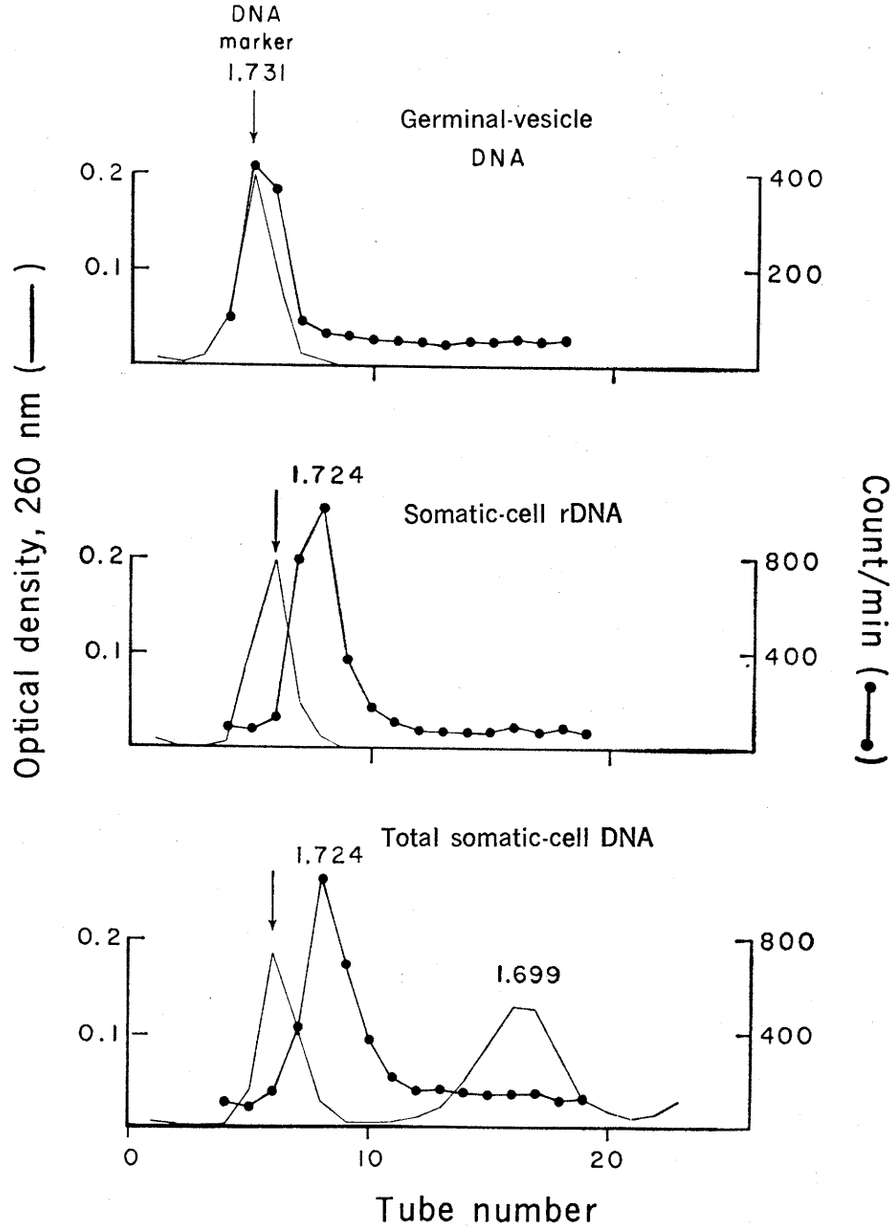


Fig. 5. Demonstration of the difference in buoyant density of germinal-vesicle and somatic-cell rDNA by hybridization with ^{3}H-rRNA. Samples of *X. laevis* germinal-vesicle DNA (0.2 μg), purified high-density somatic-cell DNA (0.07 μg), and total somatic-cell DNA (20 μg), each containing 20 μg of native *Micrococcus lysodeikticus* DNA (density 1.731) were centrifuged in CsCl gradients in the 65-fixed-angle rotor of a Spinco centrifuge at 33,000 rev/min for 64 hours at 25°C. The initial density of the solution was 1.70 g/cm^3. Each fraction was denatured with alkali, and the DNA was trapped on filters and hybridized with ^{3}H-rRNA as described in the text.

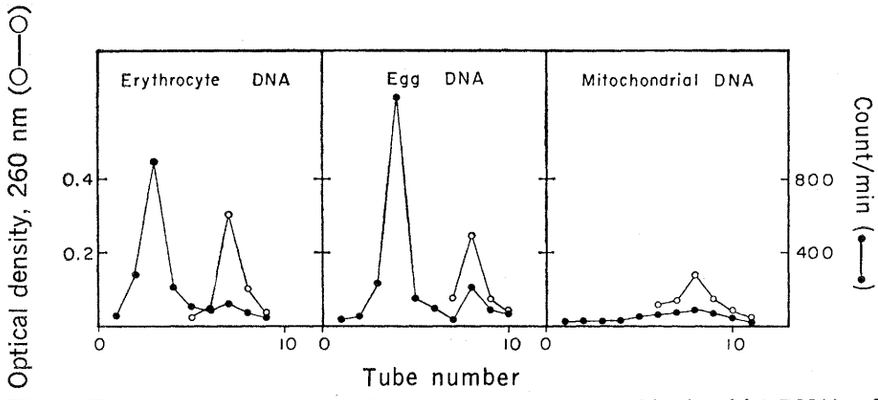


Fig. 6. Hybridization of somatic-cell (erythrocyte), egg, and mitochondrial DNA's of *X. laevis* with ^{3}H-rRNA. About 10 μg of each DNA were fractionated in CsCl in a fixed-angle rotor and hybridized according to the method described in the text. The background binding was not subtracted.

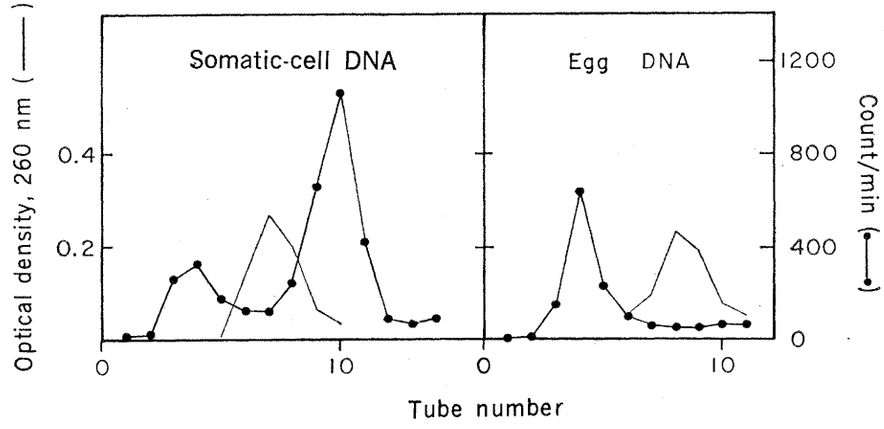


Fig. 7. Relative abundance of 5*S* DNA and rDNA in somatic-cell and egg DNA's of *X. laevis*. About 15 μg of each DNA was denatured and fractionated in CsCl, and the DNA from each fraction was immobilized on a filter and hybridized with a mixture of 0.02 μg of ^{3}H-rRNA and 0.2 μg of 5*S* ^{3}H-RNA in 3 ml of the salt solution. It has been demonstrated that 5*S* RNA hybridizes with DNA molecules of lower buoyant density than that of bulk DNA (*24*).

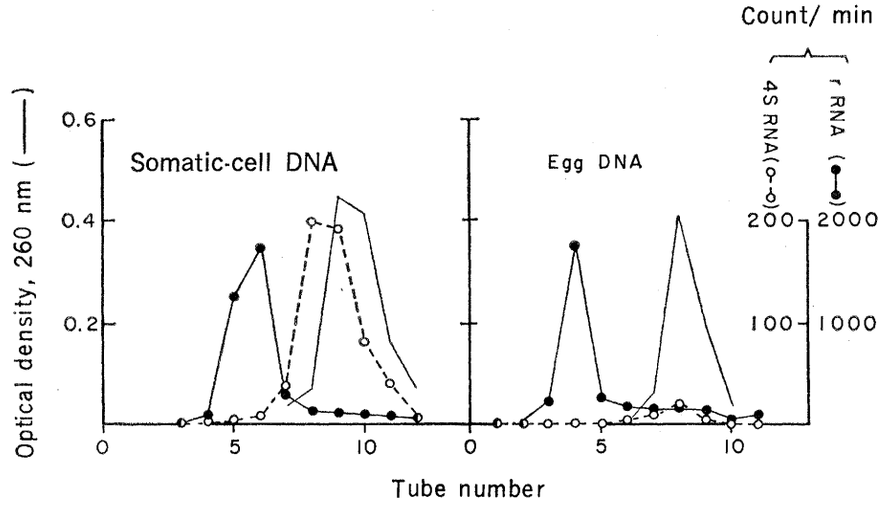


Fig. 8. Hybridization of 4*S* RNA and rRNA with egg and somatic-cell DNA's. About 60 μg of native somatic-cell DNA and 36 μg of egg DNA of *X. laevis* were fractionated in CsCl in a 65-fixed-angle rotor, and the DNA from each fraction was immobilized on a filter. Each filter was split in half, and material on one set of half filters was hybridized with 1.8 μg of ^{3}H-rRNA (●—●); the other set was hybridized with 1.2 μg of 4*S* ^{3}H-rRNA (○—○) in 4 ml of salt solution.

(Table 1) have widely different contents of DNA per cell and widely different numbers of rDNA replicas in their somatic genomes. Nevertheless, they have accumulated about the same amount of extra rDNA per germinal vesicle, and germinal vesicles from those species studied contain about the same number of nucleoli. Second, the rDNA content in germinal vesicles isolated from heterozygous (1-*nu*) females is the same as that found in the wild-type (2-*nu*) germinal vesicles (Table 3), although the somatic nuclei of the heterozygous animals have lost half their rDNA complement (*26*). Therefore, the control of this DNA replication is sensitive in some way to the final content of rDNA in a germinal vesicle rather than to the individual size, or total number of the replicas, or to the number of 28*S* and 18*S* genes clustered at the nucleolar organizer.

Necessity for Extra Copies of rDNA in Oocytes

Why do oocytes need copies of rDNA in excess of the 4C complement present on their "master" chromosomes? Two possible answers have occurred to us. The most obvious explanation is that they are needed to support the high rates of rRNA synthesis characteristic of oocytes. We have estimated that an immature oocyte of *X. laevis* can synthesize rRNA at a rate comparable to that of an equal weight of liver tissue, which comprises about 200,000 cells (*21*). However, since the maximum rate of rRNA synthesis that each gene can support is unknown, this interpretation remains conjecture. In this context it is of interest that the third type of RNA found in ribosomes —5*S* RNA—is accumulated coordinately with 28*S* and 18*S* RNA in oocytes (*37*), as in somatic tissues, but the DNA for 5*S* RNA is not amplified (Fig. 7). Perhaps the extreme redundancy of 5*S* DNA in the somatic-cell DNA of *X. laevis*, which amounts to more than 20,000 copies of the 5*S* sequences per haploid complement compared to 450 genes for rRNA (*24*), permits 5*S* RNA synthesis to keep pace with 28*S* and 18*S* RNA synthesis in oogenesis. The rate at which 5*S* RNA accumulates apparently is regulated by the rate of synthesis of 28*S* and 18*S* RNA both in oogenesis and embryogenesis. The latter control is exemplified by anucleolate embryos, which during their life-span do not accumulate detectable amounts

Table 3. Content of rDNA in germinal vesicles of heterozygote (1-*nu*) and wild type (2-*nu*) *X. laevis*. The total amounts of high-density DNA were calculated from band areas in analytical CsCl centrifugation according to the methods described in Fig. 2 and Table 1; 2000 germinal vesicles were isolated for each determination. The amount of rDNA was calculated by hybridization with ^{3}H-rRNA according to methods described in Fig. 3 and Table 1. The results of two separate hybridization experiments are given for 1-*nu* and 2-*nu* germinal vesicles (*51*).

Genotype	Amount (pg per germinal vesicle)	
	Total high-density DNA	rDNA
1-*nu*	25	4.0, 4.4
2-*nu*	25	5.6, 4.4

of new 5*S* RNA (*41*) even though they contain normal DNA complementary to 5*S* RNA (*24*).

A second hypothesis to explain the need for additional rRNA genes during oogenesis attempts to account for the difference between the ways in which rRNA synthesis is regulated in oocytes and in somatic cells. It has been shown in bacteria that the number of ribosomes produced is a direct function of the rate of protein synthesis (*42*). Changes in the rate of protein synthesis are accompanied by changes in the number of ribosomes, the majority of which enter polysomal aggregates; inactive monosomes do not accumulate (*43*). Somatic cells of higher organisms appear to regulate ribosome synthesis in a similar way. A good example is the response of some tissues to hormones. The small cells of the rat ventral prostate before puberty or after castration contain few ribosomes. In response to testosterone the cells enlarge rapidly and synthesize ribosomes at a high rate which results in a large net increase in their total ribosome content (*44*). We have investigated whether this rapid increase in ribosome synthesis results from a specific replication of the ribosomal RNA genes. We found that the prostates of castrated rats and of those injected with hormone had the same fraction of their DNA homologous to rRNA (*45*).

In contrast to somatic tissues such as the prostate gland, oocytes synthesize ribosomes primarily as a storage product to be used months later during embryogenesis. At all stages of oogenesis in *X. laevis* more than 60 percent of the ribosomes sediment as single ribosomes in sucrose and do not appear to be engaged in protein synthesis (*46*). With this proportion of monosomes, somatic tissues and bacteria would be expected to shut off synthesis of ribosomes. However, oocytes are unique in that they synthesize ribosomes for storage and for this reason might be independent of the functional regulation imposed upon somatic cells. Perhaps the extra replicas of rRNA genes, functioning as "episomes" in extrachromosomal nuclear organelles, can escape the control mechanisms which function in somatic cells.

Specific Gene Replication: A Mechanism in Differentiation

Oocytes of four amphibians as well as those of an echiuroid worm and the surf clam contain many extra copies of the genes for 28*S* and 18*S* ribosomal RNA. The oocytes of these animals synthesize large quantities of ribosomes for storage, and the extra gene copies clearly act as templates in this synthesis. The extra genes are active only during oogenesis and cease to function when the oocyte reaches maturation.

The products elaborated by cells can be divided into two general classes: those made for their own maintenance and those produced as specialized "differentiated" products. One property of a "differentiated" product is that it is not generally required for the metabolism of the cell in which it is formed. In this context somatic cells synthesize rRNA for their own "maintenance" and do so most actively when they are growing and dividing, whereas the vast majority of ribosomes synthesized by the nondividing oocytes is stored for future use during embryogenesis. The change from synthesis of substances for the cell's own maintenance to synthesis of their differentiated products often is accompanied by the slowing or cessation of cell division (*47*). Likewise, oocytes synthesize and accumulate ribosomes during their long maturation period in the absence of cell division. The selective replication of genes is a mechanism best suited for nondividing cells; continued mitosis would not only dilute out the extrachromosomal genes but probably would also render them nonfunctional by relocating them into the cytoplasm.

Ribosome synthesis by somatic cells is a "maintenance" function which appears to be controlled without change in the number of rRNA genes (*24*, *48*). In contrast, synthesis of ribosomes by oocytes appears to be a "differentiated" function involving the specific replication of the structural genes for two of the ribosome components.

There is no evidence that "specific gene amplification" is involved in the differentiated function of other cells. However, techniques are now available to begin an assessment of the relative importance of "differential gene action" and "differential gene alteration" in developmental phenomena.

Note added in proof: Perkowska, MacGregor, and Birnstiel (*51*) report that oocytes from 1-*nu* and 2-*nu* female *X. laevis* contain similar numbers of nuclei and similar amounts of germinal-vesicle DNA as measured cytochemically. They also reported that *X. laevis* germinal vesicles contain 30 pg of DNA in excess of the 4C complement. Both of these findings agree with our results.

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11. Abbreviations: rRNA is a collective term for the 28*S* and 18*S* RNA molecules which are two of the structural RNA components of the ribosome. The term "rDNA" has been used interchangeably with the term "genes for rRNA" and refers to the DNA homologous to 28*S* RNA and 18*S* RNA as judged by molecular hybridization. The use of "gene" when referring to results of molecular hybridization is validated by the results obtained with the nucleolar mutant of *X. laevis*. In this case, deletion of the homologous DNA (*26*) results in no synthesis of the product in vivo (*28*). Since no such correlation has been made for the DNA homologous to 4*S* or 5*S* RNA, the terms 4*S* DNA and 5*S* DNA are used exclusively.
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Science Advice for State and Local Government

Several factors affect the increasing role of science advisers in state and local affairs.

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Many policy analysts and public figures have, in recent years, urged state and local governments in the United States to emulate the federal government by extensively utilizing the advice of scientists in the formulation of public policies (*1*, *2*). This suggestion is pressed, in part, because of the general belief that science and technology offer potential solutions to important public problems and, in part, because it is thought that rapid scientific and technological change itself has caused many of these public problems. In this paper we will examine the factors that inhibit and enhance the utilization of scientific advice in state and local government.

First, we should note that there is already a widespread interest in the use of science advisers at this level of government. A survey conducted in the spring of 1967 found that 22 states and territorial governments have established or are actively planning to establish general science advisory units charged with guiding the government on questions in all fields of science and technology (*3*). The same survey discovered that 5 of the 50 largest municipal governments in the United States have also established some comparable formal mechanism for general science advice (*4*). In responding to the survey five governors and five mayors indicated that while they had not previously considered the idea of establishing a general science advisory unit, they were intrigued by it and would like to have information on the organization of such a unit.

Irrespective of whether or not they have a general science advisory unit, all state governments and most large municipal governments have established specialized science groups to advise the chief executive or particular governmental agencies. On a less formal basis, many state and local governments have sought advice on specific science questions from research institutes and scientists affiliated with local public and private universities. Professional science advice on such matters as agricultural research, public health, wildlife management, forestry, geology, and mine safety has long been a part of normal government operations at the state and local level, but the search for specialized science advice on such topics as oceanography, atomic energy, and air pollution is clearly the product of changing economic and political conditions.

The interest in formal mechanisms for general science advice appears also to be the result of changes in the economic and political environment. The oldest operating state general science advisory unit was formed by New York State only in 1959, and most of the existing state and municipal units were established in the years since 1963. Although there are no detailed studies of the origins of these advisory mechanisms, an examination of the first reports and statements that they have issued indicates a preoccupation with the locality's relative standing in the distribution of federal research and development expenditures and a concern with the role of science in regional economic development (*5*). States and communities whose economies are either most dependent upon or most noninvolved in research and development activities tend to have the greatest interest in establishing a formal science advisory mechanism. Those in the former category appear to be seeking a device to protect their relative position in federal science allocations and to build upon their strengths, while those

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