MATERIALS and METHODS
A. **Materials**

1. **Biological Materials**

   The 'Singhee' (*Heteropneustes fossilis* Bloch, family - Heteropneustidae, syn. - Saccobranchidae) fishes were bought from local market.

2. **Chemicals and Radiochemicals**

   Actinomycin D and phleomycin were gifts from Merck Sharp and Dohme, Rahway, N.J. and Bristol laboratories, Syracuse, New York, U.S.A. respectively. ATP, GTP, CTP, UTP, polyvinylsulphate (PVS), DEAE-cellulose, α-amanitin, human chorionic gonadotrophin (HCG), hydroxyurea and cycloheximide (actidione) were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Cytosine arabinoside (cytarabin) was obtained from Upjohn Co., Kalamajoo, Michigan, U.S.A.

   Highly polymerised calf thymus DNA and DNase (Rnase free) were obtained from Worthington Biochemical Corporation, Freehold, N.J., U.S.A. Poly(U)-sepharose was the product of PL Biochemicals.

   $^{32}_{18}$-H$_7$-uridine and $^{32}_{18}$-H$_3$PO$_4$ (carrier free) were purchased from the Isotope Division, Bhabha Atomic Research Center, Trombay, Bombay, India. $^3$H-UTP, $^3$H-GTP were obtained from Radiochemical Center, Amersham, England.
Acrylamide, N, N'-methylene-bisacrylamide and
N,N,N',N'-tetramethylethylenediamine were the products of
Eastman Kodak, Rochester, N.Y., U.S.A. Eosin, hematoxylin
and toluidine blue were obtained from BDH, Glaxo Laboratori-
es, Poole, England.

All other chemicals were analytical reagent grade
commercial products.

3. Fluor Substances

Paraterphenyl (PTP) and 1,4-bis(2,4-methyl-5-phenyl
oxazolyl) benzene (POPOP) were obtained from Bhabha Atomic
Research Center, Trombay, Bombay, India.

4. Filters

Millipore filters (HAWP, 0.45 μ) were obtained from
Millipore filter corporation, Bedford, Mass., U.S.A.
Whatman Glass fibre (GF/C) Filters were the products of

5. Enzymes

Pancreatic ribonuclease (E.C. 2.7.7.16) and
pronase (E.C. 3.4.4.17) were obtained from Sigma Chemical
Co., U.S.A.
B. Methods

I. Preparative Methods

1. Buffer

   i) Holtfreter's solution
   0.12 M NaCl, 0.001 M KCl, 0.002 M CaCl$_2$, 0.004 M NaHCO$_3$.

   ii) TME buffer
   0.01 M Tris HCl (pH 8), 0.01 M 2-mercaptoethanol, 0.001 M EDTA.

   iii) TMEG buffer
   TME buffer containing 5% glycerol (v/v).

   iv) ASM buffer
   0.01 M Na-acetate buffer, pH 5.1, 0.05 M NaCl, 0.0001 M MgCl$_2$.

   v) Ribosome Stabilising buffer (RSB)
   0.01 M Tris HCl, pH 8.0, 0.01 M NaCl, 0.003 M MgCl$_2$.

   vi) Embryo homogenization buffer
   0.25 M sucrose, 0.02 M Tris-HCl, pH 7.5, 0.01 M KCl, 0.005 M ME, 0.002 M MgCl$_2$, 0.0001 M EDTA.
2. Maintenance of fishes

The live male and female cat fishes were collected from the local market and maintained when necessary in laboratory aquaria in tap water and fed with a diet of Tubifex.

3. Fertilization of egg and development of embryos

Ovulation was induced in gravid cat fish females (H. fossilis) available during the monsoon months, June to August, by injection of HCG (259). One hundred international units of HCG in 0.25 ml of Holtfreter's solution were injected intraperitoneally and about 12-15 hr later, ripe eggs were obtained. Males, 6 inches or longer, were dissected and their gonads were used as the source of sperm. Eggs were directly stripped into sperm suspension in Holtfreter's solution and after 5 min, the fertilized eggs were carefully washed and transferred in large petridishes where development proceeded in tap water at 25°C - 26°C. Ninety to hundred per cent of eggs were fertilized (as was confirmed by observing cell division under microscope) and showed normal development up to gastrula. But in different batches of eggs, a variable proportion (up to 50 per cent) of the embryos died during post-gastrulation morphogenesis. Similar results were obtained when the development was carried out in presence of 100 units/ml of penicillin and 50 ug/ml of streptomycin.
4. Isolation of chromatin from oocytes, embryos and liver

The whole embryos (blastula or gastrula) or oocytes or liver were suspended in TME buffer containing 0.14 M NaCl and 0.05% (v/v) Triton X-100, lightly homogenized in the Dounce homogenizer and centrifuged at 1500 g for 10 min. The crude nuclei were homogenized in a Potter-Elvehjem homogenizer in presence of 0.14 M NaCl in TME buffer. The chromatin pellet obtained after centrifugation of the homogenate was washed several times with the same buffer until the supernatant fluid was clear. The TME washed chromatin pellet was washed with 0.01 M Tris HCl, 0.001 M EDTA, pH 8 and finally dispersed in 0.001 M Tris, 0.0001 M EDTA, pH 8 by vigorous stirring in Sorvall Omnimix. The chromatin suspension was centrifuged at 10,000 g 10 min and the supernatant solution was used as the soluble chromatin.

5. Isolation of Non-histone chromosomal (NHC) proteins from embryos and liver

NHC proteins were isolated according to the method of Mondal et al. (68). The chromatin pellet obtained after centrifugation of the tissue homogenate in TME buffer containing 0.14 M NaCl, was washed several times in the same buffer until the supernatant fluid was clear. The chromatin pellet was extracted overnight with TME buffer containing 2 M NaCl with mechanical shaking. The viscous chromatin solution obtained after centrifugation
(20,000 x g for 1 hr) was dialyzed overnight against large volume of 0.14 M NaCl in TME buffer, when DNA-histone complex precipitated. The suspension was centrifuged at 10,000 x g for 15 min. The supernatant contained the bulk of the NHC proteins. This was used as the starting material for the further fractionation of the enzymes. All operations were carried out at 4°C.

6. Isolation of RNA polymerase from the Non-histone proteins

Isolation of RNA polymerase from NHC proteins was done according to the method of Mondal et al. (68). The clear supernatant after removal of precipitated nucleohistone was adjusted to pH 6.4 by 0.1 M acetic acid and passed through a CM-cellulose column (20 x 1 cm) to remove any remaining histone. The resultant fluid was subjected to 30-55% saturated ammonium sulphate fractionation. The precipitate was dialyzed against TMEG buffer and loaded onto a DEAE-cellulose column (1.2 x 10 cm) equilibrated previously with this buffer. After washing with the same buffer the column was eluted with a linear gradient of KCl in the above buffer. Absorbance at 280 nm and activity for RNA polymerase were measured.

7. Isolation of DNA

DNA from fish liver, ovaries and fish blood was isolated following the method of Marmur (260) with slight modification. The vitellogenic ovaries were first lightly
homogenized in 0.15 M NaCl and centrifuged at 2,000 x g. The crude nuclear pellet was washed and then homogenized in saline EDTA (0.14 M NaCl, 0.1 M EDTA, pH 8.0), adjusted to 2% with SDS, shaken well, warmed at 60°C for 10 min and quickly cooled to room temp. 5 M NaClO₄ was added (to 1 M final concentration) and the mixture deproteinized by shaking for 30 min with equal volume of Sevag's reagent (chloroform : iso amyl alcohol :: 24 : 1, v/v) at room temp. The emulsion was centrifuged at 10,000 x g for 10 min. The upper aqueous phase was further deproteinized as above until the interphase was free of proteins. The DNA from the aqueous phase was spooled on a glass rod after adding two volumes of dehydrated alcohol, dissolved in 1/10 x SSC, adjusted to 1 x SSC by 10 x SSC. Autodigested pronase (at 37°C for 2 hr) was added to a final concentration of 50 µg/ml, incubated at 37°C for 90 min and the reaction was stopped by SDS (final concentration 2%) and deproteinized as above until the interphase was clear. Finally the DNA was precipitated with 2 volumes of ethanol and dissolved in appropriate buffer. The liver was directly homogenized in saline EDTA and procedure followed. The blood was drawn out from fish heart with heparinized needle and collected in 1/10 x SSC. Blood was directly treated with SDS to a final concentration of 2% and procedure followed.
8. Isolation of RNA

a. Isolation of total RNA from fish ovaries, liver

Total RNA from ovaries and liver was isolated by the method of Scherrer and Darnell (261). Tissue was homogenized in ASM buffer, pH 5.1. SDS was added to a final concentration of 0.5% followed by equal volume of water saturated phenol and bentonite at a concentration of 20 mg/ml. The mixture was then heated at 60°C, for 5 min with constant shaking and then immediately chilled in ice. The upper aqueous phase was removed after centrifugation and sodium acetate was added to a final concentration of 2%. RNA was precipitated with 2 volumes of ethanol containing 1% potassium acetate, and kept in the deep freeze for complete precipitation. The RNA was collected by centrifugation, dissolved in ASM buffer and reprecipitated by ethanol. RNA was stored in ethanol and dissolved in aqueous buffer only before use.

b. Isolation of rRNA from fish liver

Ribosomes were isolated according to the method of Rendi and Hultin (262). Liver tissue was homogenized in TME buffer containing 0.25 M sucrose in Sorvall Omnimixer and the homogenate was centrifuged at 15,000 x g for 30 min in a Sorvall centrifuge. The supernatant was again centrifuged at 45,000 rpm in a Beckman model L5-50 ultracentrifuge with Ti 50 rotor for 60 min. The
microsome was suspended in ASM buffer pH 5.1 containing 5 µg/ml PVS, made 0.5% in SDS and shaken at room temp. with equal volume of water saturated phenol for 15 min. After centrifugation at 10,000 x g in the cold the aqueous phase was withdrawn, made 2% with respect to sodium acetate and finally 2 volume of dehydrated ethanol was added. RNA was precipitated at -20°C.

c. Isolation and separation of labelled RNA

Fertilized eggs were allowed to incorporate $^{32}$P for 1 hr during 4th-5th hr after fertilization (for blastula) and 8th-9th hr (for gastrula). RNA was extracted from labelled cells by hot phenol-sodium dodecyl sulphate (SDS) procedure of Scherrer and Darnell (261) in presence of polyvinyl sulphate as ribonuclease inhibitor. The RNA was reprecipitated twice from 66% ethanol and kept in ethanol at -15°C until further use. Electrophoresis of RNA was carried out in 3% polyacrylamide gel according to Bishop et al. (263). For radioactivity measurement, the gels were cut into 2.5 mm slices and the slices were individually digested with 0.025 ml of 20% SDS and 0.4 ml of liquor ammonia at 70°C for 2 hr in counting vials. These were then counted with 10 ml of dioxan-based liquifluor in a Beckman LS-100 counter.

d. Preparation of ribosomal protein

The ribosome pellet was suspended in 0.15 M NaCl, 0.002 M Mg$^{2+}$, 0.01 M Tris, pH 7.4 buffer, equal volume of
4 M LiCl added and kept overnight at -20°C. It was then centrifuged 10,000 rpm for 10 min. The supernatant containing ribosomal proteins was taken.

9. **Estimation of nucleic acids and proteins in oocytes, eggs and embryos**

Determination of DNA, RNA and protein contents of oocytes, eggs and embryos were made essentially by the Schmidt-Thanhausser procedure as described by Howell (264). Oocytes of different stages were manually teased out from pieces of ovary, counted and homogenized with 0.6% saline. The homogenate was treated with 10% trichloroacetic acid (TCA). 100-200 eggs or embryos were homogenized directly in 0.5 M cold perchloric acid. The ppt. was washed successively with 5% TCA, 1% K-acetate in ethanol, ether and dried. RNA was determined in the alkali hydrolyzate after pptn. of DNA and protein by 5% perchloric acid. DNA was extracted by hot PCA. RNA and DNA were determined by absorption of UV light at 260 nm and by pentose measurement (265). Protein was determined in the hot PCA residue by the method of Lowry et al. (266). DNA content of suspension of sperms and erythrocytes was assayed in the same way as in the case of eggs, aliquots of the suspension being counted by a Haemocytometer.

10. **Extraction of phosphate compounds**

The content of all phosphate compounds (expressed as micromoles of orthophosphate) was measured in phosphorus
fractions which had been separated by perchloric acid (PCA) extraction by the method of Harold as followed by Terry and Hooper (267). Since a vast number of eggs and embryos could not be counted, a known packed volume (10 ml) of eggs and embryos were taken and extracted twice for 5 min with 0.5 N PCA at 0°C, followed by centrifugation at 12,000 x g for 10 min to yield the cold PCA pellet and supernatant fractions. The total phosphate content of the cold PCA supernatant fraction was determined in portions of the supernatant fraction, which had been ashed by heating in the presence of nitric acid. Acid washed charcoal (Norit) was added next to the cold PCA supernatant fraction to adsorb nucleotides. The supernatant fraction was assayed for orthophosphate and this value is reported as cellular orthophosphate. Labile phosphate (presumed to be pyrophosphate and acid soluble, short chain polyphosphate) was hydrolyzed by adding an equal volume of 2 N HCL and boiling for 7 min. The cellular content of labile phosphate was taken as the difference between the orthophosphate content subsequently determined in the HCL hydrolysate and the total phosphate content of the unhydrolyzed PCA supernatant fraction. The remaining phosphate in the PCA supernatant fraction (total minus the sum of orthophosphate and labile phosphate) was designated acid soluble, acid stable phosphate.

The cold PCA pellet was extracted twice for 15 min with 0.5 N PCA at 70°C and centrifuged at 12,000 x g for 10 min to yield hot PCA supernatant fraction (presumed
to be acid insoluble polyphosphate and nucleic acid) and hot PCA residual pellet. The total phosphate content of the hot PCA supernatant fraction was then determined in a sample which was ashed in nitric acid. Nucleic acids were adsorbed onto charcoal from the hot PCA supernatant fraction and samples of the supernatant fraction were hydrolysed at 100°C in 1 N acid as above and analyzed for orthophosphate. This value was reported as acid insoluble, acid labile phosphate and was assumed to represent long chain polyphosphate.

The hot PCA precipitate was ashed, dissolved in 1 N HCl, boiled for 7 min and analyzed for phosphorus. Thus hot PCA stable residual phosphate was possibly phosphoprotein. The pellets containing mainly phosphoprotein were dissolved in 0.1 M NaOH. Aliquots were digested with 6 N H2SO4, 4 drops of HNO3 and finally with 2 drops of H2O2. Volume was made up to 5 ml with H2O and analyzed for phosphorus. Protein was assayed in the phosphoprotein dissolved in 0.1 M NaOH.

11. Washing of column materials for chromatography

DEAE-cellulose

DEAE-cellulose was suspended in 5 volumes of 0.5 M HCl. After 30 min the resin was filtered and washed with water using a Buchner funnel under suction until the pH of the filtrate was near 7.0. The cake was then suspended in
5 volumes of 0.5 M NaOH and after 30 min, washed with water as above until the pH of the filtrate was near 7.0. The regenerated DEAE-cellulose was then washed several times with the starting buffer to be used, poured in a column and washed thoroughly with the same buffer until the pH of the effluent was the same as that of starting buffer at 4°C.

12. Preparation of nucleoli

Stage I previtellogenic ovaries were lightly homogenized manually in a loose-fitting Potter-Elvejhem homogenizer in 0.25 M sucrose buffer containing 0.02 M Tris, 0.01 M KCl, 5 mM mercaptoethanol and 2 mM MgCl₂, pH 7.5. The homogenate was filtered through cheese cloth. The filtrate was layered over 0.5 M sucrose overlaid on top of 1 M sucrose, both in the same buffer and centrifuged at 800 x g for 5 min. Erythrocytes, membranes and whole cells remained in top layer and nuclei (GV) were pelleted at the bottom. The pellet was homogenized with 0.25 M sucrose in the buffer in tight-fitting Potter-Elvejhem homogenizer (20 strokes manually) and checked microscopically for complete breakage. The suspension was overlaid on sucrose-buffer and centrifuged for 10 min at 1000 x g in a swinging bucket rotor of MSE centrifuge. The pellet containing the nucleoli was suspended in 0.25 M sucrose buffer.
13. Cytological procedure

Pieces of ovary containing small oocytes (less than 200 µm) were placed in 0.6% saline on slides and gently pressed with a coverglass. As the oocytes were transparent, nuclei (germinal vesicle) and nucleoli could be clearly seen under ordinary microscope. For better results, a modification of Denton et al. (268) of the AS-SAT procedure of Howell et al. (269) was used. Satisfactory staining by Ag was also obtained by simply incubating the slides containing the oocytes or nucleoli (without fixation) in 50% aqueous silver nitrate for 2-3 hr at 37°C and then washing with distilled water. These slides could be observed under the microscope as such or after lightly counterstaining with dilute Giemsa or eosin stain.

14. Preparation of Embryo extracts

The blastula and gastrula embryos were very lightly homogenized in embryo homogenization buffer to remove yolk, centrifuged at 2,500 x g and supernatant discarded. The pellet was homogenized in 0.01 M Tris, pH 8.0, 5 mM Mg(Ac)\(_2\) buffer in Potter-Elvejhem homogenizer, centrifuged at 10,000 x g for 25 min. The clear supernatant was taken as blastula or gastrula extract.

15. Preparation of dissociated cells

The blastula embryos were lightly homogenized in embryo homogenization buffer and centrifuged at 2,500 rpm
II. Analytical Methods

1. Estimation of RNA, DNA and Protein

RNA and DNA were estimated by UV absorption at 260 nm and sugar estimations (265). Protein was estimated by the method of Lowry et al. (266).

2. Estimation of inorganic phosphorus

Inorganic phosphorus was estimated according to the method of Chen et al. (270). An aliquot containing inorganic phosphate was diluted to 2 ml and 2 ml of phosphate reagent containing 1 volume of 6 N H$_2$SO$_4$, 1 volume of 2.5% (w/v) ammonium molybdate, 1 volume of 10% (w/v) freshly prepared ascorbic acid and 2 volumes of distilled water was added and mixed well. The mixture was then incubated at 37°C for 60 min and the colour developed was measured at 820 nm. The phosphate content was determined by comparing with the standard curve using known amount of KH$_2$PO$_4$. The appropriate blank was prepared and necessary corrections were made.

3. Effect of inhibitors

Fertilized eggs (100 - 200) were transferred into petridishes containing the appropriate concentration of
inhibitors in tap water and in all experiments, the results were compared with a control set in tap water only. At least 2 batches of eggs from different individuals were used for each experiment; differences from the control were observed and only the significant differences were recorded.

4. Kinetics of DNA and RNA synthesis

$^{32}$P-incorporation experiments in the presence and absence of the inhibitors were done under similar conditions. For a typical kinetic analysis (271), batches of fertilized eggs (600 - 800) were exposed to about 100 μCi of carrier-free $^{32}$P-labelled orthophosphate in 30 ml tap water immediately after fertilization and then 50 or 100 embryos were removed at different intervals for analysis. They were washed twice in tap water and treated with 5 ml of 5% TCA to stop the incorporation. The TCA-suspended embryos were homogenized, centrifuged and the residue washed four times with 5% TCA each time with a drop of 1 M potassium phosphate as carrier. Final wash was given with 1% ethanolic potassium acetate to remove the lipids. Three ml of 10% sodium chloride and 0.5 - 1 mg of carrier yeast RNA were added to the residue and the pH of the suspension was brought to about pH 7 by adding a few drops of 0.1 M sodium hydroxide using phenol red as an indicator. Total nucleic acid was extracted by heating in boiling water bath for 30 min. After cooling the suspension was centrifuged and from the supernatant, nucleic acids were precipitated by adding
two volumes of cold 95% ethanol. The precipitate was quantitatively transferred into planchets with water, dried and counted in an endwindow G. M. counter. For separation of RNA from DNA, the final aqueous solution was divided into two parts. One part was used for assay of total incorporation in RNA and DNA while the other part for DNA only, after removal of RNA by the usual procedure of hydrolysis with 0.3 M sodium hydroxide for 16 hr. Direct determination of TCA-insoluble radioactivity could not be applied as significant incorporation of $^{32}$P took place in phosphoproteins also.

5. Assay of RNA polymerase

RNA polymerase was assayed in the DEAE-cellulose column fractions by the method of incorporation of radioactive nucleoside triphosphates into acid insoluble products (68). The reaction mixture in a total volume of 0.25 ml contained 0.04 M Tris HCl (pH 8), 0.2 mM EDTA, 5 mM 2-mercaptoethanol, 0.01 M MgCl$_2$, 0.4 mM K$_2$HPO$_4$, 0.16 M KCl, 0.1 mM each of ATP, GTP, CTP and $^3$H-UTP (20 cpm/pmole), 20 µg calf thymus DNA and 50 µl aliquots of the column fractions. It was incubated at 37°C for 15 min. The reaction was terminated by adding 2.5 ml of 10% TCA containing 5% sodium pyrophosphate. The precipitate was washed on GF/C (Whatman) filters and counted as described (68).
6. Template activity of chromatin

The template activity of chromatin for in vitro RNA synthesis was assayed using RNA polymerase from E. coli. The assay conditions were the same as described above for RNA polymerase except that 5 μg of free DNA (isolated from catfish liver by the method of Marmur (260)) or an equivalent amount of chromatin was used as the templates with varying amounts of E. coli RNA polymerase. The bacterial RNA polymerase was isolated from E. coli MRE 600 by a slightly modified method of Burgess (272).

7. Polyacrylamide gel electrophoresis of RNA

RNA was electrophoresed using 3% polyacrylamide gel according to the method of Bishop et al. (263). The electrophoresis buffer E contained 0.04 M Tris base, 0.002 M Na$_2$ EDTA; 0.02 M sodium acetate. The pH of the buffer was adjusted to 7.8 by adding glacial acetic acid. The running gel contained 3% polyacrylamide, 0.125% N,N'-methylenediacrylamide, 0.08% potassium persulfate (freshly prepared) and 0.0001% N,N,N',N'-tetramethylethylenediamine (TEMED) in the E buffer. The gel mixture was poured into glass tubes (0.5 x 8 cm), layered with water and allowed to photo polymerize. After the gel was formed the water was soaked out by tissue paper wick. The tubes were then filled with E buffer and prerun without applying sample for 30 min at 4 mA/tube at 4°C. RNA sample was prepared in E buffer containing 10% glycerol, 0.0002%
bromophenol blue. 5 - 50 µg RNA in 10 - 30 µl volume was applied onto the gel and electrophoresed at 4 mA/tube at 4°C for 45 - 60 min. The gels were taken out of the tubes with the help of a hypodermic syringe when the tracking dye reached 1 cm above the bottom of the tubes. For staining, the gels were soaked in 0.1% toluidine blue in 1% acetic acid for about 1 hr and destained by diffusion in 1% acetic acid.

To monitor the radioactivity in the electrophoresed RNA, the gels were cut into slices of 2 mm thick, digested with 0.3 ml of ammonia solution and 0.025 ml 20% SDS at 60°C for 3 hr and radioactivity was measured using 5 ml dioxan based fluor.

8. Densitometry of RNA gels

The RNA gels stained in toluidene blue were scanned by a densitometer and the relative amount of rRNA was calculated from the integrated area of the rRNA peaks in the scans.

9. Assay of poly(A) containing RNA

The amount of poly(A) containing RNA was determined by poly-(U) sepharose chromatography of the RNA isolated from different oogenetic stages following the method of Trapy (273). The RNA sample was diluted with salt buffer (0.1 M NaCl, 0.01 M EDTA, 0.01 M Tris HCl, pH 7.4 and 0.2% SDS) and applied to a poly(U)-sepharose column (0.8 cm x 1.5 cm) equilibrated with the
same buffer. The column was washed with 3 volume of the salt buffer. The poly(A) containing RNA was eluted with three column volumes of elution buffer (0.01 M EDTA, 0.01 M Tris HCl, pH 7.4 and 0.2% SDS) at 50°C. Fractions of 1 ml were collected and absorbance was read at 260 nm. Per cent poly(A) containing RNA was the quotient of absorbance in the eluate peak and the total input absorbance. The poly(U)-sepharose column was regenerated by washing with a cycle of elution buffer and equilibration buffer. The poly(U) sepharose was stored in 0.1% sodium azide.

10. DNA-RNA hybridization

DNA-RNA hybridization was done according to the method of Dr. Igor B. Dawid (personal communication). Catfish liver and oocyte DNAs were denatured by 0.1 M NaOH, neutralized with HCl and diluted with 2 x SSC (SSC = 0.15 M NaCl, 0.015 M Sod. citrate). 1, 2 and 5 µg aliquots of DNA were spotted on nitrocellulose filters prewashed with 2 x SSC. The filters were washed with 2 x SSC, dried and baked at 80°C under vacuum for 2 hr. Since rRNA genes of eukaryotes have highly conserved sequences (170), high Sp. Ac. Drosophila melanogaster $^3$H-rRNA (Sp. Ac. ~ $2 \times 10^6$ cpm/µg kindly provided by Dr. Igor B. Dawid) was used as the hybridization probe. The filters were hybridized (in a total volume of 1 ml in sealed plastic bags) with 100,000 cpm equivalents of the $^3$H-rRNA in 0.75 M NaCl, 50 mM Na-phosphate buffer, pH 7.0, 5 mM EDTA, 0.25% SDS,
10 μg/ml E. coli tRNA and 50% deionized formamide at 42°C for 48 hrs. After hybridization, the filters were washed twice with 4 x SSC containing 0.25% SDS, twice with 2 x SSC without SDS. They were treated with 20 μg/ml of RNase (boiled in 0.01 M HCl for 5 min to destroy any DNase) in 2 x SSC at 37°C for 20 min, washed twice with 2 x SSC + 0.25% SDS, twice with 0.2 x SSC + 0.25% SDS, dried. They were dipped in 20% PPO in toluene, air dried and autoradiographed by exposing a preflashed Kodak X-ray film (XR-1) with an intensifying screen for 10 days at -70°C (274). The filters were then counted in toluene fluor in a scintillation counter.

11. Spectrophotometric measurement

Absorbance of samples in the ultraviolet and visible range was measured in a Beckman DU2 spectrophotometer using cuvettes of 1 cm light path. Nucleic acid content of samples were determined by measuring absorbancy at 260 nm and assuming that 20 O.D.260 units was equivalent to 1 mg of nucleic acid.

12. Radioactivity measurement

Radioactive samples were counted in a Beckman LS-100 liquid scintillation spectrometer. Generally precipitated materials were collected on filters and radioactivity was measured using toluene-based fluor containing 0.5% PTP and 0.005% POPOP. Aqueous samples were counted.
in dioxan-based fluor containing 10% napthalene, 0.5% PTP and 0.005% POPOP.
In dioxan-based fluor containing 10% napthalene, 0.5% PTP and 0.005% POPOP.