SEQUENCE DIFFERENCES IN CHROMATIN DNA AND NUCLEAR MEMBRANE-ASSOCIATED DNA IN THE SEA URCHIN EMBRYO

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SUMMARY: Chromatin DNA and nuclear membrane DNA were isolated from hatched-blastula stage sea urchin (S. purpuratus) embryos. The nuclear membrane DNA, compared with chromatin DNA, is deficient in sequences homologous to the more rapidly annealing portion of blastula stage [^{3}H]RNA and in sequences rich in polydeoxyadenylate and polydeoxyguanylate.

In several types of eukaryotic cells, chromatin has been shown to be attached to the nuclear membrane (e.g., 1,2). Although this attachment has been hypothesized to be related to initiation and continuation of DNA synthesis (1-4), more recent studies suggest that this is not the case (5,6). The possibility does occur, however, that the nuclear membrane may operationally as well as physically isolate sections of nuclear DNA. Transcription of membrane-associated (MA) nuclear DNA might, for instance, occur to a different extent than transcription of more internal non-membrane-associated (NMA) nuclear DNA.

In this communication we report results of a comparison of chromatin DNA (referred to here as NMA nuclear DNA) and MA nuclear DNA. Isolated MA and NMA nuclear DNAs from blastula stage sea urchin embryos were assayed for their relative homology to short term labeled RNA from the same stage embryos. We have also compared these DNAs for differences in their proportion of polydeoxyadenylate-rich and polydeoxyguanylate-rich sequence.

MATERIALS AND METHODS:

Hatched-Blastula Stage Embryos. Washed Strongylocentrotus purpuratus eggs were fertilized (7) (to > 95% of the eggs) and allowed to develop (approximately 3 X 10^4 embryos/ml) with gentle rotatory agitation at 16° C. in artificial seawater (8). Penicillin G to 100 μg/ml and streptomycin sulfate to 50 μg/ml were added 10 minutes after fertilization.

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ABBREVIATIONS: MA = membrane-associated; NMA = non-membrane-associated; SSC = 0.15 M NaCl, 0.015 M Na_3 citrate; poly (C) = polycytidylic acid; poly (U) = polyuridylic acid

Isolation of Nuclei. Nuclei were isolated by a sucrose step-gradient method (9, 10) and checked for cytoplasmic contamination by phase-contrast microscopy.

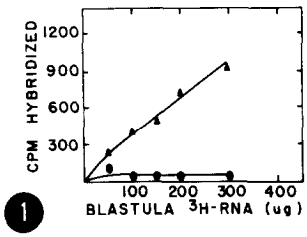
Separation of Nuclei into Chromatin and Nuclear Membrane Fractions. Nuclei were pelleted and resuspended in 3 volumes of 1 M glucose; Na deoxycholate was added to 0.43% and Tween 40 to 0.86% (11, 12). (As indicated by Wilt and Ekenberg [12], this treatment removes most of the inner as well as outer nuclear membrane). The mixture was vortexed for 3 seconds and centrifuged at 17,000 x g for 10 minutes. The supernate (membrane fraction) was buffered with Tris, 0.05 M; EDTA, 0.01 M; pH 7.6 as measured at 37° C. and the pellet (chromatin fraction) was resuspended in a minimal volume of the same buffer.

Isolation of DNA from the Chromatin and Nuclear Membrane Fractions. To either fraction, pronase (Calbiochem, preincubated for 2 hours at 37° C. in 0.05 M Tris, pH 7.6) was added to 1 mg/ml, and the mixture was incubated overnight at 37° C. Ribonuclease (Worthington, stock solution at 2 mg/ml in 0.15 M NaCl, heated to 85° C. for 20 minutes) was added to 20 μg/ml and the preparation held at 37° C. for an additional hour. The mixture was then extracted three times with freshly distilled phenol (which had been saturated with 0.05 M Na phosphate, pH 7.0) and dialyzed against three changes of SSC solution.

Preparation of Hatched-Blastula Stage ^{3}H-RNA. Embryos were exposed to [5-^{3}H]uridine (29.2 Ci/mM, 1 μCi/ml) for 20 minutes prior to quick chilling. RNA was immediately extracted from the embryos as previously detailed (13).

DNA-RNA Hybridization. Sea urchin [^{3}H]RNA was hybridized to the DNAs by the method of Gillespie and Spiegelman (14); the method of washing the hybridization filters, ribonuclease treatment, counting, and subtraction of blank filter radioactivity has been previously described (13). Hybridization reactions were in 6 X SSC at 65° C. for 16 hours.

Hybridization of DNA with [^{3}H]polyribonucleotides. The method used was essentially that of Shenkin and Burdon (15). Each assay tube contained (in 1 ml of 2X SSC) 1 μg of DNA plus a quantity of [^{3}H]polyribonucleotide as indicated on the abscissa of the graph shown in Figure 2. The sealed tubes were heated in boiling water for 20 minutes, then placed in a 20° C. water bath for 4 hours. Pancreatic ribonuclease (Worthington, in 0.15 M NaCl and previously heated to 85° C. for 20 minutes) was then added to 5 μg/ml in each tube, and the mixture was held at room temperature for 30 minutes to 1 hour. Hybrids were collected on Schleicher and Schuell B6 24 mm nitrocellulose filters (previously soaked in boiling 2X SSC for 30 minutes, rinsed and washed with 20 ml 2X SSC). The filters were then washed with 100 ml of



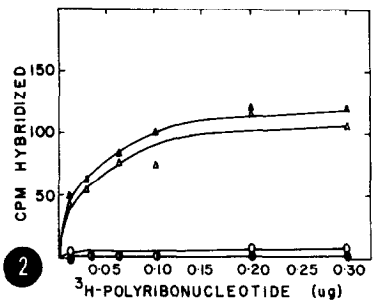


Figure 1. Hybridization of MA and NMA DNAs to increasing amounts of hatched-blastula stage [^{3}H]RNA. For each point on the graph, a nitrocellulose filter with 20 μg of immobilized DNA and a blank filter were reacted with a quantity of [^{3}H]RNA as indicated on the abscissa of the graph. The reaction conditions are as described in Materials and Methods. ●, MA nuclear DNA; ▲, NMA nuclear DNA.

Figure 2. Hybridization of MA and NMA DNAs to increasing amounts of [^{3}H]polyribonucleotides. For each point on the graph, 1 μg of denatured DNA was reacted with a quantity of [^{3}H]polyribonucleotide as indicated on the abscissa of the graph. The reaction conditions are as described in Materials and Methods. ●, O, MA nuclear DNA; ▲, Δ, NMA nuclear DNA. Closed symbols indicate hybridization to [^{3}H]polyuridylic acid; open symbols indicate hybridization to [^{3}H]polycytidylic acid.

2X SSC, dried, and counted. Parallel sets of assay tubes lacking only DNA were used to produce blank values which were subtracted from experimental values. [^{3}H]poly (U) (82.2 mCi/mM phosphorous, 4.2 μg/uCi) and [^{3}H]poly (C) (44.8 mCi/mM phosphorous, 8.95 μg/uCi) were purchased from Miles Laboratories.

RESULTS AND DISCUSSION:

Nuclei from blastula stage sea urchin embryos were purified from cytoplasmic contamination by a sucrose step-gradient method (9, 10). The procedure of Penman (11, 12) was then used to separate the nuclear membrane material from the isolated nuclei. The products of this procedure, when applied to sea urchin blastulae, are chromatin and nuclear membrane (12). DNAs were isolated separately from the chromatin and the nuclear membrane fraction. These isolated DNAs are believed to be relatively free of cytoplasmic (mitochondrial) DNA for reasons beyond that of the isolation procedure: (a) At the blastula stage, only about 0.2% of the total embryo DNA is represented by mitochondrial DNA (16, 17). (b) Approximately 20% of the total nuclear DNA is in the membrane associated fraction as isolated here. Thus, even in the worst case (all mitochondria being retained with the isolated nuclei), MA nuclear DNA could have only about 1% contamination with mitochondrial DNA.

Portions of both types of nuclear DNA (MA and NMA) were hybridized to increasing quantities of [^{3}H]RNA. This RNA was derived from hatched-blastula

stage embryos which had been exposed to [^{3}H]uridine for 20 minutes prior to termination of development. These DNA-RNA hybridization experiments are shown in Figure 1. It is evident that MA nuclear DNA, compared with NMA nuclear DNA, has little homology for this [^{3}H]RNA from the same stage of development. Similar DNA-RNA hybridization experiments, using MA and NMA nuclear DNAs from morula and gastula stage embryos and [^{3}H]RNA from the equivalent stage embryos, have essentially the same hybridization characteristics as shown in Figure 1.

Since these DNA-RNA hybridization reactions were under conditions which favor only rapidly hybridizing sequences (18), no conclusions can be drawn regarding possible homologies for unique sequences and sequences of low redundancy.

Hatched-blastula stage MA and NMA DNAs were also assayed for sequences rich in polydeoxyadenylate or polydeoxyguanylate (15,19,20). Samples of MA and NMA DNAs were hybridized under optimum conditions (15) to increasing quantities of [^{3}H]polyuridylic acid or [^{3}H]polycytidylic acid (Figure 2). The MA nuclear DNA produced only very low quantities of [^{3}H]hybrid when annealed to either of the [^{3}H]polyribonucleotides; in comparison, the NMA nuclear DNA showed saturation plateaus of approximately 120 cpm of [^{3}H]poly (U) per μg of DNA (corresponding to 0.10% saturation of the DNA) and approximately 104 cpm of [^{3}H]poly (C) per μg of DNA (corresponding to 0.17% saturation of the DNA). It is possible that these calculated hybridization percentages are incorrect when interpreted as indicating the percentage of DNA rich in polyadenylate ([^{3}H]poly (U) hybridization) or DNA rich in polyguanylate ([^{3}H]poly (C) hybridization): Poly (U) hybridization to poly (A) and poly (C) hybridization to poly (G) have been observed to occur in a ratio of 2:1 under some conditions (21,22). Also, denaturation experiments have revealed that there may be as many as 10% mismatched base-pairs in the [^{3}H]polyuridylic acid:eukaryotic DNA hybrids (15,23). Thus, assay of polyadenylate-rich and polyguanylate-rich regions in MA and NMA DNAs may produce relative comparisons, rather than exact quantitative values.

The results obtained here indicate that DNA isolated from nuclear membranes of sea urchin blastulae is deficient, compared with chromosomal DNAs, (a) in sequences homologous to the more rapidly annealing portion of new blastula stage RNA, and (b) in sequences rich in polydeoxyadenylate and polydeoxyguanylate.

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