Volume 10 Number 23 1982 Nucleic Acids Research

Sea urchin small nuclear RNA genes are organized in distinct tandemly repeating units

Charles O.Card, Gilbert F.Morris, David T.Brown and William F.Marzluff

Department of Chemistry, Florida State University, Tallahassee, FL 32306, USA

Received 7 September 1982; Revised and Accepted 12 October 1982

ABSTRACT

The genes coding for the two major small nuclear RNAs in the sea urchin are organized in independent tandem repeating units. The small nuclear RNAs, N1 and N2 were purified from gastrula embryos of Lytechinus variegatus. These RNAs are analogous to the U series of RNA in mammalian cells as judged by their identical 5' termini and the sequence homology of the N1 urchin RNA and U1 mouse RNA. These RNAs were polyadenylated with E. Coli adenylate transferase. A $^{32}PO_4$ labeled copy of each RNA was made with RNA-dependent DNA polymerase. This copy was used to probe the gene organization of these RNAs by hybridizing to restriction enzyme digests of sperm DNA. Each of these RNAs is coded in a tandemly repeated cluster (at least 30 kb) with a repeat length of 1100-1400 bases. The N1 and N2 clusters are distinct. The N1 repeat has been cloned and the repeating organization confirmed with the cloned gene.

INTRODUCTION

The small nuclear RNAs are the most abundant RNA species in eucaryotic cells other than the ribosomal RNAs (1, 2). Some of these RNAs, which make only transient appearance in the cytoplasm, have a characteristic 2,2,7 trimethyl guanosine "cap" structure (3, 4). The genes for the nuclear RNAs are repeated in both the sea urchin and mouse genomes (5, 6). The two small nuclear RNAs larger than 5S rRNA, N1 and N2, are synthesized coordinately during sea urchin development (5) and the RNA species are stable during development. In addition these RNAs are stored in the egg during oogenesis. Repeated genes, like those for the ribosomal RNAs and tRNAs are generally found in tandemly repeating structures (7-9). However, it has recently been reported that the genes for U1 small nuclear RNA in humans (10) and chickens (11) are scattered in the genome. We report here that the genes for the two major small nuclear RNAs in sea urchins are found in separate tandemly repeating units, about 1,400 bases long (N1) and 1100 bases long (N2).

0305-1048/82/1023-7677 $2.00/0

MATERIALS AND METHODS

Growth of Embryos

L. variegatus sea urchins were collected and maintained at the FSU marine laboratories. Embryos were grown as previously described (5) at 25°C. For labeling with $^{3}H\text{-}CH_3$-methionine embryos were transferred to fresh sea water containing 10 µM adenosine and guanosine and 1 mM sodium formate just prior to hatching and 1 µc/ml $^{3}H\text{-}CH_3$ methionine added. An equal amount of methionine was added 6 hours later and the embryos harvested 6 hours after that (early gastrula). Embryos were labeled with $^{32}PO_4$ as previously described (5).

Preparation of RNA

Nuclear RNA was prepared from gastrula (24 hour) embryos as previously described (5). The RNA was fractionated on 5-20% sucrose gradients (prepared in 0.1M NaCl, 0.1M tris, pH 7.5, 1 mM EDTA, 0.1% SDS) at 25,000 rpm for 20 hours in the AH627 rotor in a Sorvall OTD-2 ultracentrifuge. The RNA sedimenting at 4-8S was pooled and applied to a 20 cm. 10% polyacrylamide gel in 7M urea, 0.050M tris borate, pH 8.3, 1 mM EDTA. Xylene cyanol was added as a marker and electrophoresis was at 400 volts (gel temperature 50-60°C) until the xylene cyanol reached the bottom of the gel. The RNAs were visualized by UV shadowing (12) and recovered from the gel by electroelution.

Preparation of cDNA

Purified N1 or N2 RNA (5 µgm) was dissolved in sterile water. The RNA was incubated with E. Coli adenylate transferase (13) in a final volume of 50 µl containing 50 mM tris, pH 7.2, 10 mM $MgCl_2$, 0.25M NaCl, 50 µgm/ml bovine serum albumin, 100 µM ATP and 200 units/ml adenylate transferase for 20 min at 37°C. The RNA was recovered by phenol extraction and ethanol precipitation.

One µgm of the poly A extended RNA was used to make cDNA in a reaction mix containing 0.1M tris pH 8.2, 6 mM $MgCl_2$, 400 µ dGTP, dTTP, dATP, 40 µM $\alpha\text{-}^{32}P$-dCTP (1 mC/ml), 40 µgm/ml oligo dT and 20 units/ml reverse transcriptase. Reactions were incubated at 46°C for 30 minutes. The cDNA was purified by phenol extraction followed by alkaline hydrolysis and gel filtration.

Hybridization to RNA

Various RNAs were separated by electrophoresis in 10% polyacrylamide gels in 7M urea and transferred to DBM paper by electrophoresis (14). The filter was hybridized to cDNA in 50% formamide, 0.75M NaCl, 75 mM sodium citrate, 1x denhardt's (15), 0.1% SDS, 100 µgms/ml E. Coli DNA at 42° for 16 hours.

Hybridization to Sperm DNA

High molecular weight sperm DNA was prepared essentially as described

by Brown et al. (7). The DNA was digested with various restriction enzymes and analyzed by electrophoresis in 0.8% agarose gels. The DNA was transferred to nitrocellulose by the method of Southern (16). Hybridization was for 24 hours with 3 x 10^5 cpm (3 x 10^7 cpm/µgm) of cDNA in 0.9M NaCl, 0.09M sodium citrate, 0.5% SDS, 1mM EDTA, 4 x Denhardt's solution (15), 15 µgm/ml poly A and 50 µgm/ml denatured E. Coli DNA at 65°C. The filters were washed 3 times in the same solution without Denhardt's solution, poly A and E. Coli DNA and exposed to x-ray film with a Lightning Plus intensifying screen.

Partial digests were done by digesting the sperm DNA for different times and combining all the digests for hybridization analysis.

Analysis of ^{3}H-methyl Labeled Caps:

The ^{3}H-CH_3 labeled RNA was digested with 10 units of RNase T_2 and T_1 for 3 hours in 50mM NaOAc pH 5, 2mM EDTA. The digest was chromatographed on DEAE-cellulose as described previously (5). The material eluting with charge -5--6 was recovered and digested with nuclease P1, tobacco acid pyrophosphatase and alkaline phosphatase as previously described (17). The methylated nucleosides were chromatographed on thin-layer cellulose in acetonitrile:ethyl acetate: n-butanol:isopropanol: 6N NH_3 (7:2:1:1:1.7)(4). ^{3}H-methyl labeled small nuclear RNAs from mouse myeloma cells were processed identically and provided a standard 2,2,7 trimethyl guanosine sample. 2,7 dimethyl guanosine was synthesized from 2-methyl guanosine by methylation with dimethyl sulfate.

Cloning of the N1 Small Nuclear RNA gene from L. Variegatus

Sperm DNA was digested with Hind III and fractionated by preparative agarose gel electrophoresis. DNA fragments 1.0-1.5 kb in size were recovered by the elution method of Yang et al. (18). The fragments were ligated to the pBR 322 which had been digested with Hind III and alkaline phosphatase (19). The resulting chimeric DNAs were used to transform E. Coli strain HB 101. Positive colonies were selected by colony hybridization using the mouse U1 clone as a probe (20). The presence of an N1 gene in the plasmid pLvN1.1 was confirmed by hybridization selection (21) using $^{32}PO_4$-labeled sea urchin nuclear RNA.

MATERIALS

Restriction enzymes were from Bethesda Research Laboratories and were used according to their instruction. The E. Coli adenylate transferase was prepared according to Sippel (13). RNA-dependent DNA polymerase was a gift from Dr. J. Beard. α-$^{32}PO_4$-dCTP was obtained from Amersham. AMB paper was obtained from Schleicher and Schuell and used according to their instructions. ^{3}H-CH_3-methionine was purchased from Schwartz-Mann.

RESULTS

Purification of RNA and Preparation of cDNA

Nuclear RNA sedimenting at 4-8S was prepared and separated on 10% polyacrylamide gels in 7M urea (Fig. 1). The N1 and N2 RNAs were recovered from the gel by electroelution. The small nuclear RNAs, N1 and N2 are similar to the U series of RNA of mammalian cells. These RNAs have similar capped 5' termini (5) and we show here that the modified guanosine in the sea urchin RNA is 2,2,7 trimethyl guanosine. In addition, the U1 RNAs from mouse and sea urchin have enough sequence homology to cross-hybridize (see below). The N2 may be analogous to U2 RNA on the basis of its electrophoretic mobility and high (6%) pseudouridine content (Evans and Morris, unpublished results). Small nuclear RNAs were labeled by growing embryos in the presence of $^{3}H\text{-}CH_3$-methionine. The major methylated RNAs were N1 and N2 (Fig. 2A) and these were purified by electroelution. The N1 RNA was purified and digested with RNase T_1 and T_2. The digest was chromatographed on DEAE-cellulose. The majority of the methyl label eluted in the position expected for cap I structures (Fig. 2B), as previously found for N1 and N2 RNA labeled in vivo with $^{32}PO_4$ (5). After digestion with nuclease P1, tobacco acid pyrophosphatase and alkaline phosphatase, 75% of the radioactivity chromatographed in the same position as the 2,2,7 trimethyl guanosine found in the cap structure of the U1 RNA of mouse myeloma cells (Fig.

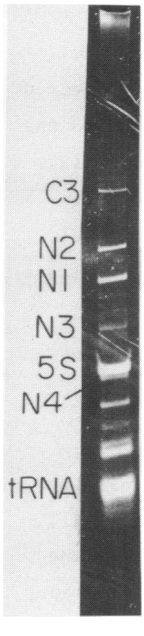


Fig. 1: Isolation and Polyadenylation of Small Nuclear RNA. Sea urchin nuclear RNA was isolated and the RNA sedimenting at 4-8S prepared by sucrose gradient sedimentation. The RNA was analyzed by electrophoresis. There are two major small nuclear RNA larger than 5S rRNA, N1 and N2.

2C). 25% of the label was found as 2'-OCH_3-adenosine, indicating the structure of the 5' terminus is 2,2,7 trimethyl $GpppA_m$. Thus the same 5' terminus has been conserved throughout evolution.

Hybridization probes were prepared from the purified RNA species with reverse transcriptase. These RNAs were first extended with E. Coli adenylate transferase (13) under conditions which resulted in the addition of 10-20 A's

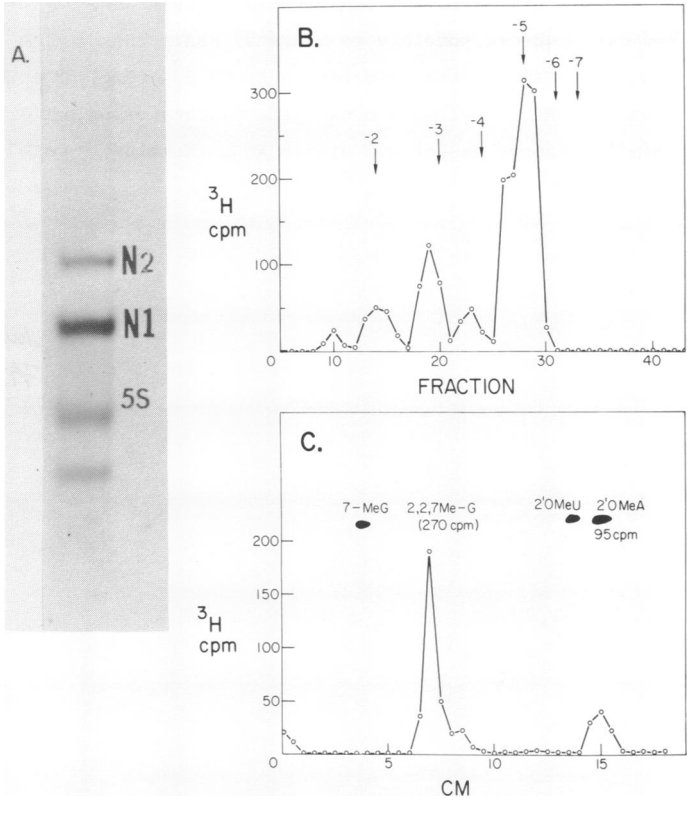


Fig. 2: The Cap Structure in Sea Urchin Small Nuclear RNA N1 is 2,2,7 trimethyl $GpppA_m$. A. 4-8S nuclear RNA was prepared from urchins labeled with ^3H-CH_3-methionine and analyzed by gel electrophoresis. A fluorogram of the gel is shown. B. Sea urchin embryos were labeled with ^3H-CH_3 methionine and the labeled small nuclear RNA N1 purified by gel electrophoresis. This RNAs as digested with T1 and T2 RNase and the digest chromatographed on DEAE-cellulose in 7M urea. The absorbance was monitored continuously and aliquots of each fraction counted. The position of the charge markers (a pancreatic RNase digest of ribosomal RNA) is indicated. C. The material eluting with a charge of -5 (fractions 27-29) was recovered and desalted. This was digested with nuclease P1, tobacco acid pyrophosphatase and alkaline phosphatase and chromatographed on thin-layer cellulose. The position of added markers is shown. The 2,2,7 trimethyl guanosine marker was a digest of mouse small nuclear RNA run in a parallel lane.

to the 3' end of each RNA. An oligo-dT primed cDNA copy was made to the poly A extended RNAs using avian myeloblastosis virsus reverse transcriptase. The cDNA (about 150-200 bases long) prepared from N1 RNA was characterized by hybridization to the sea urchin nuclear and cytoplasmic RNA. The RNAs were separated on a 7M urea 10% polyacrylamide gel and transferred to diazotized paper by electrophoresis (14)(Fig. 3A). The N1 cDNA reacted only with the N1 RNA and not with 5.8S rRNA (which has nearly identical electrophoretic mobility) (5), 5S rRNA or tRNA. There was no cross-reaction between the N1 cDNA and N2 RNA. The N1 RNA has sequence homology to mouse U1 RNA. The N1 cDNA hybridized with a lambda phage containing the gene for mouse U1 RNA (Fig. 3B). We recently isolated the mouse U1 RNA gene from a mouse gene library using probes prepared in a similar manner as for sea urchin RNAs (Marzluff, unpublished results).

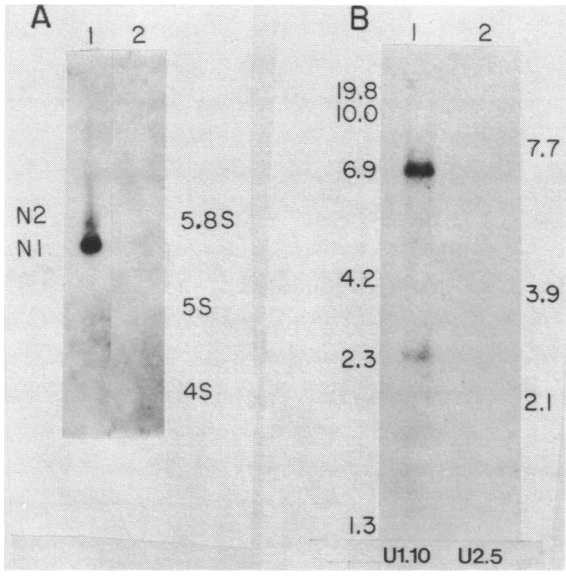


Fig. 3: Specificity of the Probe for N1 RNA. A. The sea urchin small nuclear RNA N1 was isolated and polyadenylated. A cDNA copy was made with α-$^{32}PO_4$-dCTP and the RNA hybridized to nuclear and cytoplasmic RNAs which had been separated by electrophoresis and transferred to DBM paper. Lane 1: nuclear RNA. Lane 2: cytoplasmic RNA (equimolar amounts of 5S and 5.8S rRNA applied to the gel). B. The sea urchin N1 cDNA was hybridized to isolated mouse U1 and U2 small nuclear RNA genes. Lane 1 contains phage U1.10 DNA cut with EcoR1. There are two mouse U1 genes on the 6.9 kb R1 fragment. The position of the 4 EcoR1 fragments in this phage are shown. Lane 2 contains phage U2.5 DNA cut with EcoR1. The 2.1 kb fragment contains a mouse U2 gene. There was weak hybridization to the band at 2.3 kb in some experiments. This fragment does not contain a mouse U1 gene.

Gene Organization of Small Nuclear RNAs

Lytechinus variegatus sperm DNA was digested with several restriction enzymes and separated on a 0.8% agarose gel. The DNA was blotted onto nitrocellulose and hybridized either with cDNA to N1 (Fig. 4A) or cDNA to N2 (Fig. 4B). Most of the enzymes used did not cut the DNA which contained sequences complementary to the cDNA. Two enzymes (Hind III and Bgl II) cleaved the N1 RNA gene cluster into a 1.4 kb fragment while Eco R1 (not shown), Bam H1, Sal 1 and Xba left the DNA at least 25 kb long. A double digest of Bgl II and Hind III reduced the size of the fragment to 700 bases.

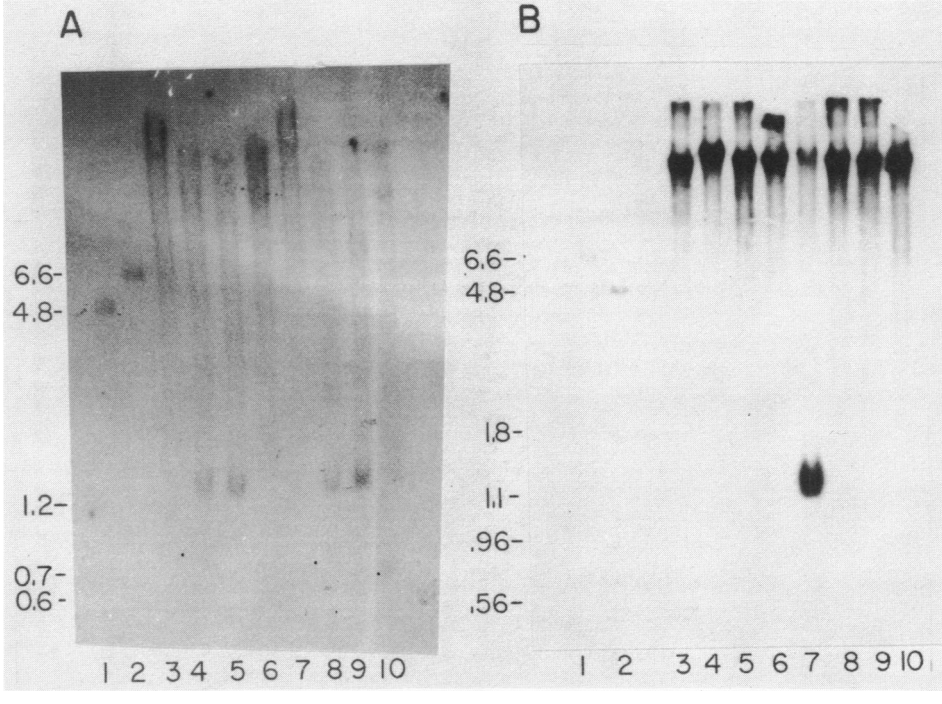
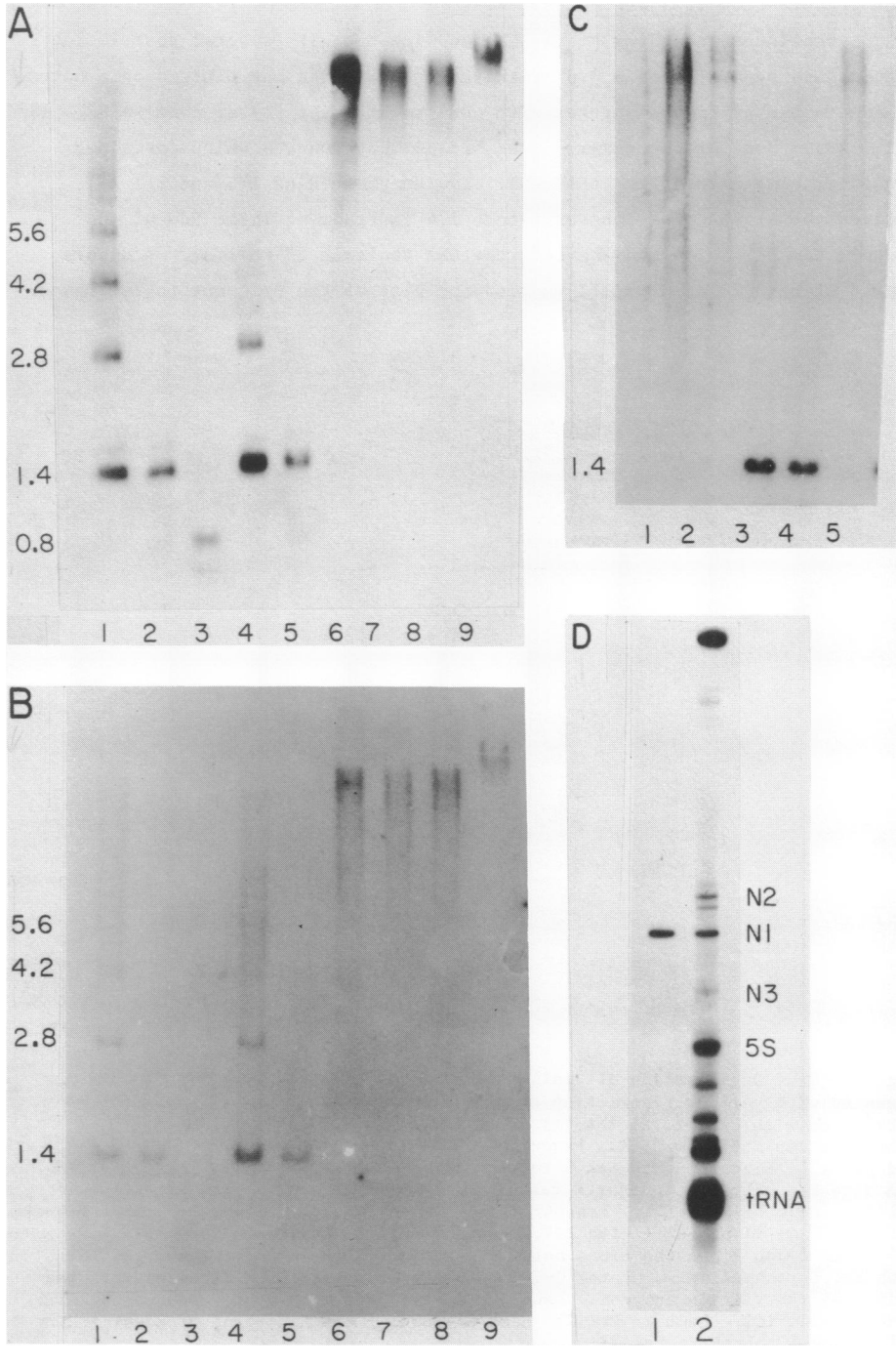


Fig. 4: Gene Organization of Small Nuclear RNAs. Sea urchin sperm DNA was digested with various restriction enzymes separated by gel electrophoresis and hybridized with cDNA to N1 RNA (A) or N2 RNA (B). Autoradiographs of the hybridization are shown. A. Lane 1 and 2 are the ribosomal DNA plasmids pLV 4 and pLV 1334 digested with Sal I respectively. Lane 3-10 are sea urchin sperm DNA digested with the indicated restriction enzymes. Lane 3: Bam H1; Lane 4: Bgl II; Lane 5: Hind III; Lane 6: Xba I; Lane 7: Sal I; Lane 8: Bam H1 + Bgl II; Lane 9: Bam H1 + Hind III; Lane 10: Bgl II + Hind III.
B. Lanes 1 and 2 are the ribosomal DNA plasmids pLV 1334 and pLV 4 digested with Sal I respectively. Lanes 3-10 are sea urchin sperm DNA digested with the indicated restriction enzymes. Lane 3: EcoR1; Lane 4: Bam H1; Lane 5: Bgl II; Lane 6: Hind III; Lane 7: Pst I; Lane 8: EcoR1 + Bam H1; Lane 9: EcoR1 + Hind III; Lane 10: EcoR1 + Bgl II.


A
5.6
4.2
2.8
1.4
0.8
1 2 3 4 5 6 7 8 9
B
5.6
4.2
2.8
1.4
1 2 3 4 5 6 7 8 9
C
1.4
1 2 3 4 5
D
N2
N1
N3
5S
tRNA
1 2

Similarly only one of the enzymes tested cleaved the DNA complementary to the N2 RNA gene cluster (Fig. 4B). Pst I cleaved the cluster into a 1.1-1.2 kb unit, while Eco Rl, Bam Hl, Hind III, Bgl II and Sal I did not cleave the N2 gene cluster. Thus the genes for these RNAs are located in separate clusters. Taken together these results suggest that the gene for the small nuclear RNAs are organized into tandem repeating units of 1.1-1.4 kb containing at least 20 units in a single cluster. The deduced lower limit is based on the size of the uncut DNA.

The major possible contaminating RNAs are ribosomal RNAs. In particular 5.8S RNA has a nearly identical electrophoretic mobility to Nl RNA. Indeed, each cDNA showed weak hybridization to cloned sea urchin ribosomal RNA genes (8)(Fig. 4A and 4B). However, these fragments were not detected in hybridization with genomic DNA where the ribosomal DNAs were present in less than 1% of the amount of the purified plasmids on the same gel. Another set of repeated genes, 5S rRNA genes, are also found in repeated units of about 1.3kb. The restriction enzymes which release these fragments differ from those which release the Nl and N2 nuclear RNAs (19). In addition the cDNA did not react with 5S rRNA or 5.8S rRNA (Fig. 3B).

Since it is unlikely that a repeated gene cluster would be stable in a gene library, we partially purified the Nl sea urchin repeat unit by digestion of the sperm DNA with Hind III and purification of the 1.2-1.5kb DNA by preparative agarose gel electrophoresis. This DNA was inserted into the Hind III site of pBR322. The plasmid pLv Nl.1 contains a single sequence which is homologous to sea urchin Nl RNA (Fig. 5D). Only the Nl RNA bound to this

Fig. 5: Hybridization with Cloned Sea Urchin and Mouse Ul Genes.
A. L. variegatus sperm DNA was digested with Eco Rl, immobilized on nitrocellulose and hybridized to plasmid pLv Nl.1, which contains an L. variegatus small nuclear RNA gene. The size of the bands is given in kilobases. Lane 1: Hind III-partial digest; Lane 2: Bgl II; Lane 3: Hind III + Bgl II; Lane 4: Hind III; Lane 5: Bgl II; Lane 6: EcoRl; Lane 7: Xba I; Lane 8 Bam Hl; Lane 9: uncut.
B. The plasmid, pUl.l, containing a mouse Ul RNA gene was labeled by nick translation and hybridized to digests of L. variegatus sperm DNA. The size of the bands is given in kilobases. Lane 1: Hind III-partial digest; Lane 2: Bgl II; Lane 3: Hind III + Bgl II; Lane 4: Hind III; Lane 5: Bgl II; Lane 6: EcoRl; Lane 7: Xba I; Lane 8: Bam Hl; Lane 9: uncut.
C. The plasmid pLvNl.1 was hybridized to S. purpuratus sperm DNA cleaved with various restriction enzymes. Lane 1: uncut; Lane 2: Bam Hl; Lane 3: Bgl II; Lane 4: Bgl II; Lane 5: EcoRl. In a separate experiment plasmid pLV Nl.1 was hybridized with the L. variegatus DNA and S. purpuratus DNA cut with Bgl II. Lane 6: L. variegatus; Lane 7: S. purpuratus.
D. The plasmid pLvNl.1 which contains an L. variegatus small nuclear RNA gene, was digested with EcoRl, immobilized on nitrocellulose and hybridized to $^{32}PO_4$ labeled L. variegatus nuclear RNA. The bound RNA was eluted and analyzed by gel electrophoresis. Lane 1: bound RNA; Lane 2: nuclear RNA

plasmid when it was hybridized to $^{32}PO_4$-labeled sea urchin RNA (Fig. 5D). Complete characterization of this clone will be reported elsewhere (Brown, Morris and Marzluff, unpublished results). The expected Bgl II site was present 600 bases from the Hind III site. This cloned DNA was used to probe sea urchin genomic DNA digested with a variety of restriction enzymes (Fig. 5A). The results are totally consistent with the bulk of sea urchin N1 genes being in a tandem repeated unit. A partial digest with Hind III confirms this conclusion (Fig. 5A, Lane 1). In addition to the major repeat other minor bands were reproducibly present. These include bands which were released by enzymes whice do not cleave the repeats as well as components other than the 1.4 kb fragment released by Hind III and Bgl II. Whether these represent individual genes which may be expressed independently form the repeated clusters or pseudogenes or "orphons" (23) as have been detected for sea urchin histone genes is not known. It is possible there is some heterogeneity in the repeat since a 1.6 kb fragment is also released by both Hind III and Bgl II and a 2.8 kb fragment is present in complete digests with Hind III. Completion of digestion was monitored by including λ DNA in the digests. Whether this heterogeneity is present in each individual sea urchin or the repeat length varies in some individuals is not known.

Further evidence that the hybridization observed is due to hybridization with the N1 gene, was provided by the following experiment. A 400 bp fragment containing a mouse U1 gene (Wang and Marzluff, unpublished results) was labeled by nicktranslation and hybridized to sea urchin DNA (Fig. 5B). Essentially the same results were obtained as with the pLvN1.1 probe. DNA complementary to the mouse probe was larger than 25 kilobases after digestion of EcoR1 or Bam H1 but migrated as a single major component (1.4 kb) after digestion with either Hind III or Bgl II. A partial digest with Hind III showed the same tandem repeat structure as found with the sea urchin probe.

There is a similar N1 gene organization in at least one other sea urchin species, S. purpuratus. Cleavage of S. purpuratus sperm DNA with various

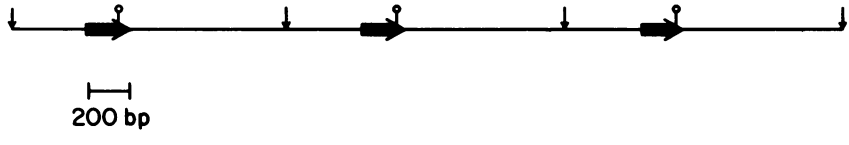


Fig. 6: Restriction Map of N1 Gene Cluster: The N1 repeating unit is shown. The location and direction of the gene is indicated by the arrows. These have been deduced by DNA sequencing (Brown and Marzluff, unpublished results). The Hind III sites (↓) and Bgl II sites (♀) are shown.

restriction enzymes gave similar results to that found with L. variegatus DNA. Bgl II released a fragment of 1.3 kb while Hind III, Bam Hl and Eco Rl did not cleave the DNA (Fig. 5C). The Bgl II site is in the Nl gene and hence conservation of this site is not unexpected. The S. purpuratus Nl repeat is slightly smaller than the L. variegatus Nl repeat. The structure of the Nl gene repeat is summarized in Fig. 6.

DISCUSSION

Weiner et al. have reported the isolation of DNA complementary to human small nuclear RNAs (10, 24) which are not present in linked repeats and indeed may be pseudo genes and not true small nuclear RNA genes. Manser and Gesteland (25) have isolated several "real" Ul genes from humans which are not tightly linked but have similar flanking region sequences. Similarly we have isolated unlinked genes hybridizing with small nuclear RNAs from a mouse gene library (Marzluff, W. F., Brown, D. T., and Wang, S., unpublished results). The genes for Ul RNA in the chicken are also dispersed in the genome, but with a low copy number (11). The linked repeat gene structure in the sea urchin may represent a special case due to the high demand for these RNAs at a particular developmental stage (e.g., oogenesis or embryogenesis). Alternatively, the dispersed gene structure in mammals has also been seen for tRNAs (26) as well as small nuclear RNAs and may represent a fundamental difference in gene organization between these organisms.

The Nl and N2 small nuclear RNAs are coordinately expressed during sea urchin development (5). Since they are found in separate clusters, these genes must be regulated independently. Thus they differ from histone genes which are also coordinately controlled but are tightly linked (27). There are other possible genes coding for Nl RNA detected by Southern hybridization. It is tempting to postulate that the genes in the repeating cluster are expressed at a particular developmental stage and that at least some of the other genes detected are expressed at other stages, analogous to the situation with the sea urchin histone genes. Isolation of these genes will allow determination of their structure and the detection of factors involved in their coordinate control.

ACKNOWLEDGEMENTS

This work was supported by grant GM 27789 from the NIH. We thank Dr. Darrel Stafford for the gift of the L. variegatus ribosomal RNA genes.

REFERENCES

1. Weinberg, R. A. and Penman, S. (1968) J. Mol. Biol. 38 289-304.
2. Moriyama, Y., Hodnett, J. L., Prestayko, A. W., and Busch, H. (1969) J. Mol. Biol. 39 335-49.
3. Ro-Choi, T. S., Yong, C., Henning, D., McCloskey, J., and Busch, H. (1975) J. Biol. Chem. 250, 3921-28.
4. Fernandez-Monoz, L., Laui, U., and Darnell, J. E. (1977) Nuc. Acids Res. 4, 3357.
5. Nijhawan, P. and Marzluff, W. F. (1979) Biochemistry 18, 1353-60.
6. Marzluff, W., White, E., Benjamin, R., and Huang, R. C. (1975) Biochemistry 14, 3715-24.
7. Brown, D., Wensink, P., and Jordan, E. (1971) Proc. Nat. Acad. Sci. USA 78, 3175-79.
8. Blin, N., Sperazza, J., Wilson, R., Bieber, D., Mickel, S., and Stafford, D. (1979) J. Biol. Chem. 254, 2716-31.
9. Clarkson, S., Kurer, J., and Smith, H. (1978) Cell 14, 713-24.
10. Denison, R. A. and Weiner, A. M. (1982) Molecular and Cellular Biology 2 815-28.
11. Roop, D. R., Kristo, P., Stumph, W. E., Tsai, M. J., and O'Malley, B. W., (1981) Cell 23, 671-80.
12. Hussur, S. M., and Whitlock, H. W. (1974) Anal. Biochem. 59, 162-64.
13. Sippel, A. (1973) Eur. J. Biochem. 37, 31-40.
14. Stellwag, E. J. and Dahlberg, A. E. (1980) Nuc. Acids Research 8 229-317.
15. Denhardt, D. (1974) Biochem. Biophys. Res. Commun. 23, 641-46.
16. Southern, E. M. (1975) J. Mol. Biol. 98, 503-15.
17. Brown, A., Pan, C. J. and Marzluff, W. F. (1982) Biochemistry, in press.
18. Yang, R. C., Qis, J. and Wu, R. (1979) Methods in Enzymology 68, 176-82.
19. Ullrich, A., Shine, J., Chirgwin, J., Tischer, R., Rutter, W. J., and Goodman, H. M. (1979) Science 196, 1313-16.
20. Grunstein, M. and Wallis, J. (1979) Methods in Enzymology 68, 379-88.
21. Hieter, P. A., Hendricks, M. B., Hemminki, K. and Weinberg, E. S. (1979) Biochemistry 18, 2707-16.
22. Lu, A. and Stafford, D. (1980) Nuc. Acids Res. 8, 1839-53.
23. Childs, G., Maxson, R., Cohn, R. H. and Kedes, L. (1981) Cell 23, 651-664.
24. Denison, R. A., Van Arsdell, W. W., Bernstein, L. B. and Weiner, A. M. (1981) Proc. Nat. Acad. Sci. USA 78, 810-14.
25. Manser, and Gesteland, R. (1982) Cell 29 257-64.
26. Santos, T., and Zasloff, M. (1981) Cell 23, 699-710.
27. Kedes, L. H. (1979) Ann. Rev. Biochem. 48, 837-70.