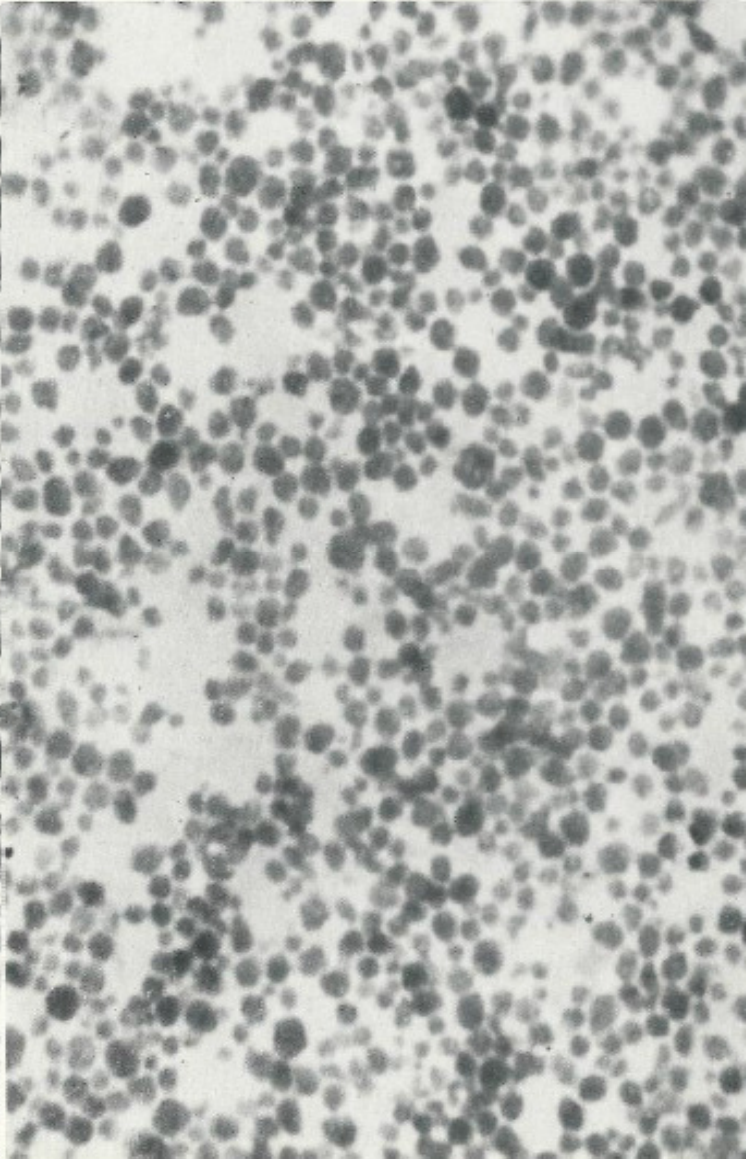
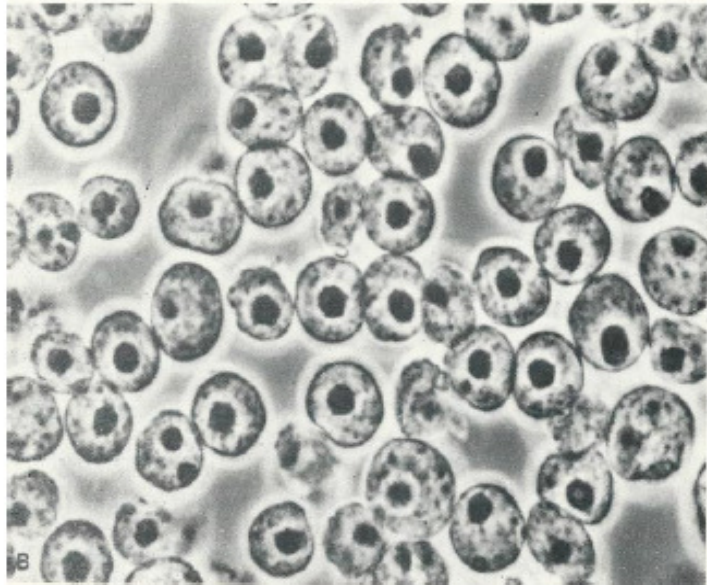
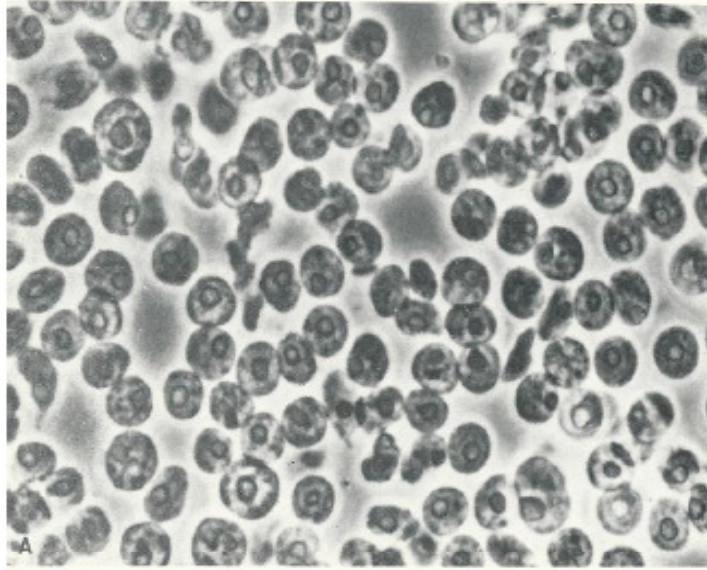


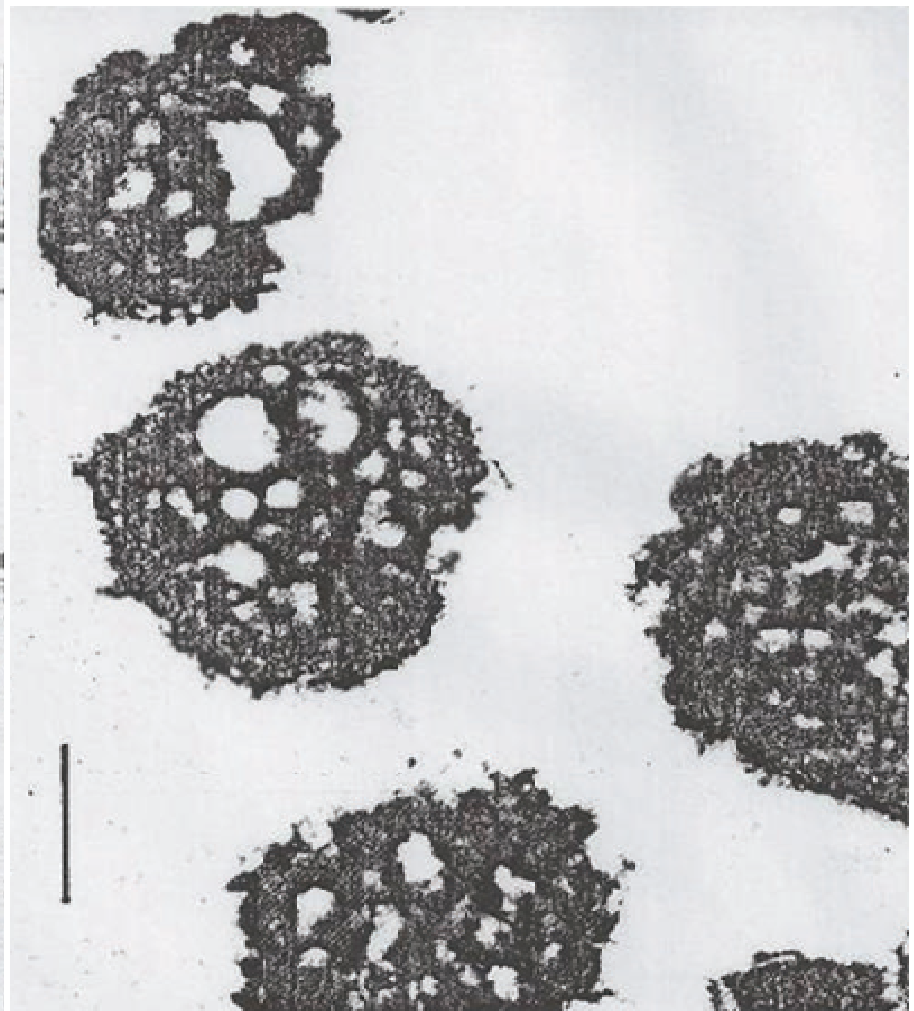
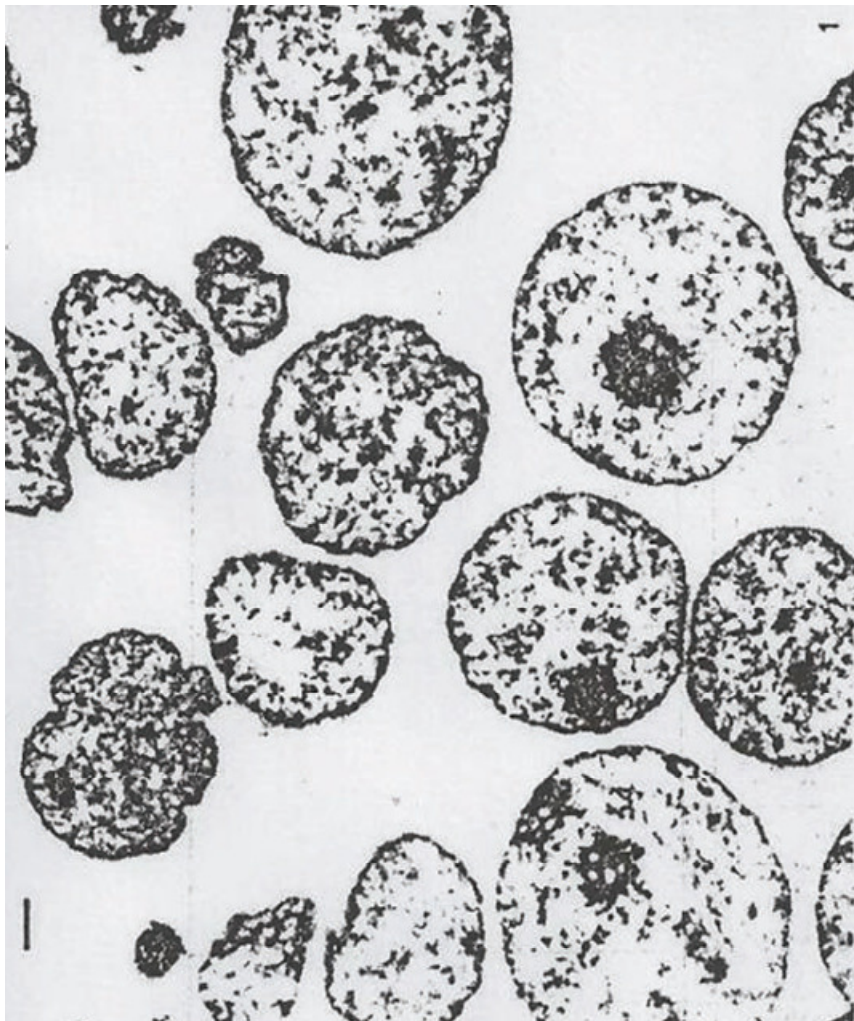
Isolated Nuclei and Nucleoli

Morris Hepatoma Nuclei

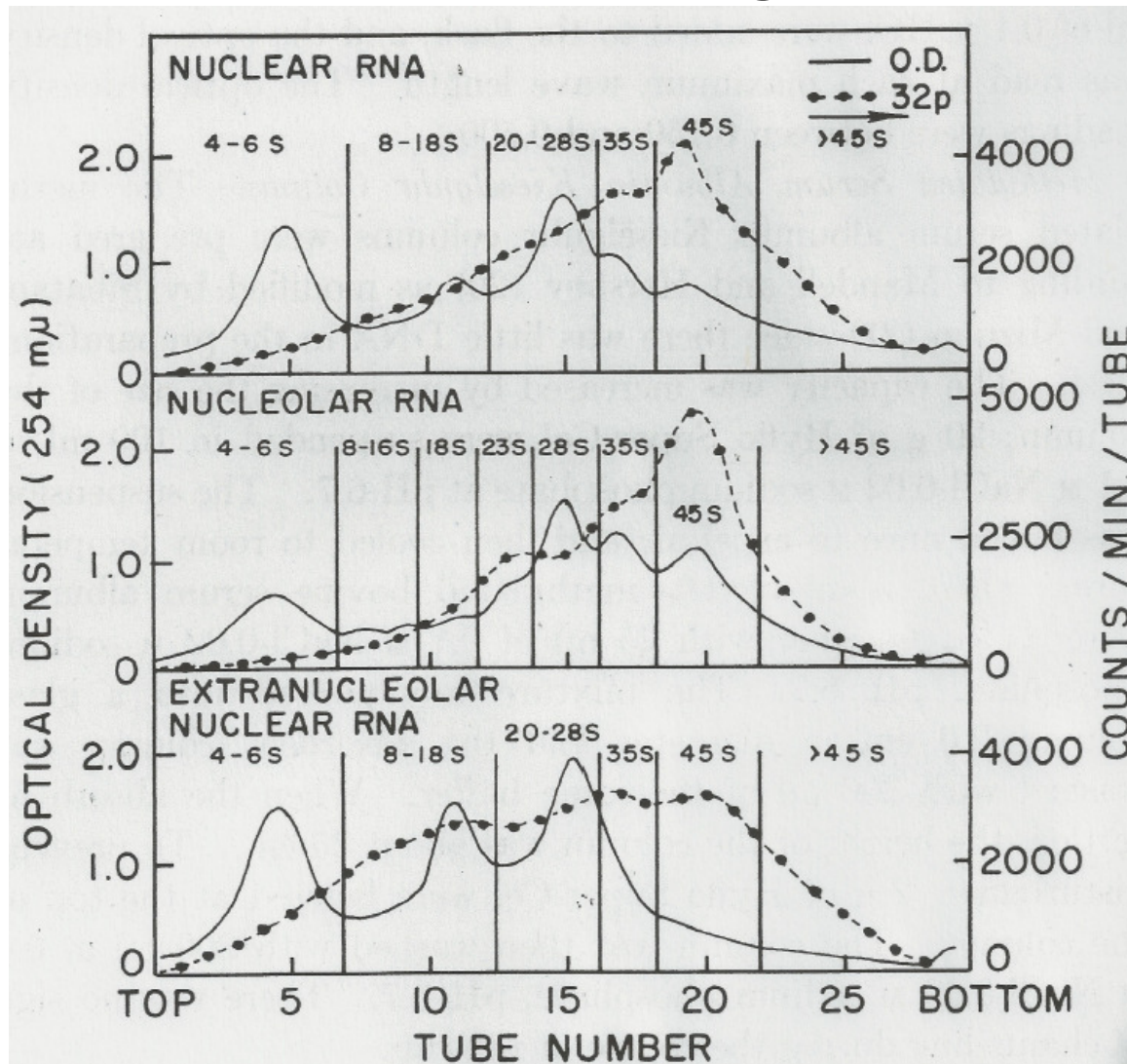
Rat Liver Nucleoli



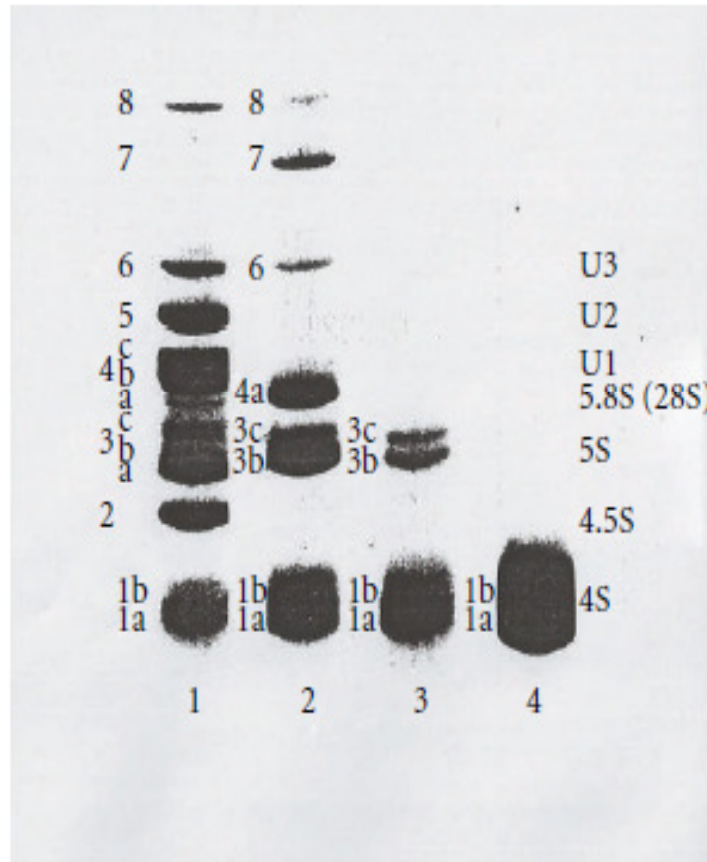
EM Pictures of Isolated Nuclei and Nucleoli of Rat Liver Cells



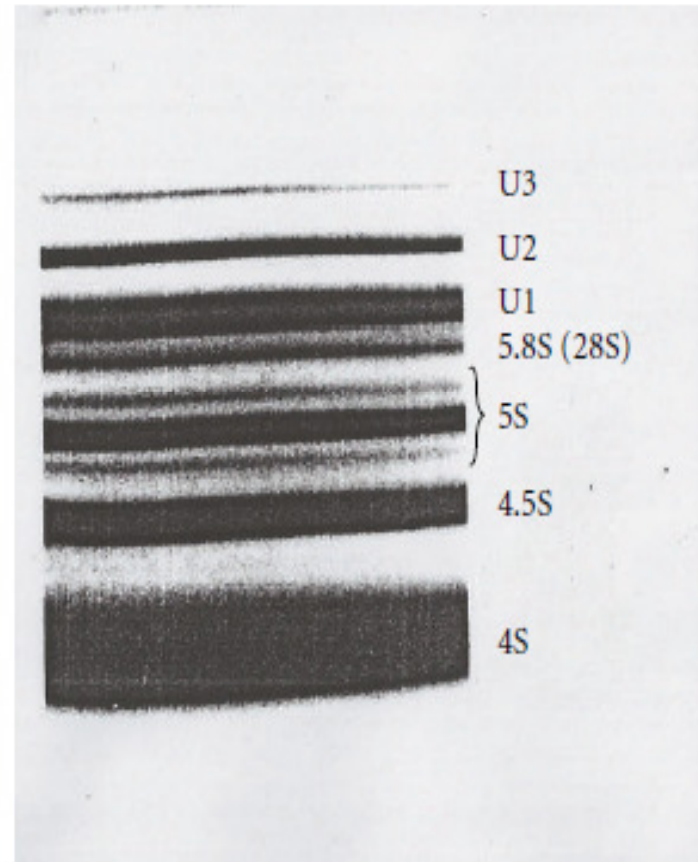
Nuclear RNA Sedimentation Profiles by Sucrose Gradient Centrifugation



Acrylamide Gel Electrophoresis



(a)



(b)

The Sequence of 4.5S RNA I (Novikoff hepatoma cell nuclei)

pppGGUCGAGAGG AUGGCUCAGC CGUUAAG[AG]GC UAGGCCAAAA AUAA{CACCUA U}

AAGAGUUCG GUUCCC[AG]CA CCACGGCUGU CCUCCAGCA CCUUUU-OH

Pol III Promoter **A box** and **B box**

14 ESEs; 6 Green; SRp40, 1 Yellow; SRp55, 3 Blue; SC35, 4 Red; SF2/ASF

2 [AG] 3' Splice site (Higher score underlined)

10 Branch sites; {Highest scoring branch site}

ESE Distribution in FMR1 Gene Transcript

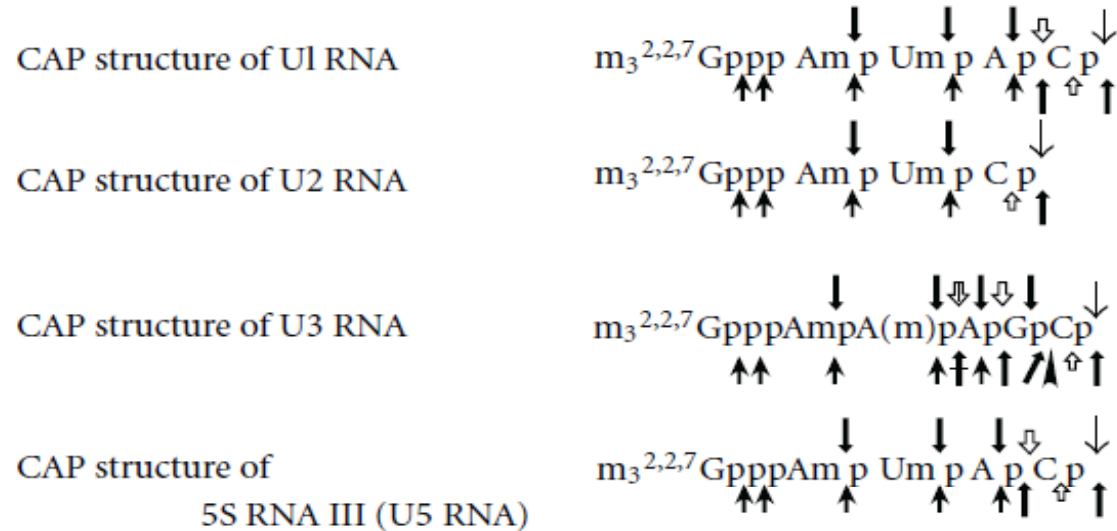
FMR1	SF2/ASF	SC35	SRp40	SRp55	Σ
FMR1(39,224nt)	2.12	2.82	3.29	2.30	10.53
Exon(4,456nt)	3.18	2.6	3.21	1.97	10.96
Intron(34,768nt)	1.98	2.85	3.30	2.34	10.47
SS(3,226nt)	2.26	2.11	3.50	1.95	9.82
Alu	4.93	5.21	4.32	1.34	15.80
Line	4.74	2.37	2.84	4.27	14.22

Numbers represent per 100 nucleotides

Functions of Alu elements

- 1) DNA replication
hY1,2,3,4 and 5
- 2) Transposition and gene recombination
Self replication, Integration
- 3) Gene expression at the Transcription
 - (a) Alu in promoters; ADA Gene Alu deletion→SID
 - (b) Alu in exons; Factor IX gene exon 5→Hemophilia B
 - (c) Alu in introns; NF1 gene intron 5 (Sb1)→
Neurofibromatosis
 - (d) Alu in control region;
Alu in LCR of globin genes→Thalassemia
Alu in LCR of K18 gene; ↑expression
 - (e) Alu in splicing
 - (f) Alu exonization;
ADAR2 exon8+Alu Jb (Lev-Maor et al., 2003)
- 4) Alu control at the Translation
FMRP binds BC1 and regulates neuronal mRNA translation by inhibition. Primate analog of rodent (mouse) BC1 is BC200 which was detected by RT-PCR of extract from glioma and neuronal cells. These BC1 and BC200 have complementarity to Arc, α -CaMKII and MAP1B mRNAs in cognate species and regulate dendritic development in synapsis (Zalfa et al., 2003).
- 5) Alu Binding Proteins;
Ro, La, SRP9/14 Hormone receptore, TF etc.

Trimethyl guanosine caps of Novikoff hepatoma cell snRNAs
cleavage sites by various enzymes



Nucleases

T1 RNase ▲

↓ PI nuclease

↑ Snake venom phosphodiesterase

↑ T2 RNase

↯ U2 RNase

↓ RNase A

⤴ Bacterial alkaline phosphatase

U1, U2 and U3 snRNA Sequences

(1) Human HeLa U1A RNA (2) Rat Novikoff U1 RNA (Involved pre-mRNA splicing)

(1) m₃^{2,2,7}GpppAmUmACΨΨACCU GGCAGGGGAG AUACCAUGAU
 (2) m₃^{2,2,7}GpppAmUmACΨΨACCU GGCAGGGAGA UACCAUGAUC

(1) CACGAAGGUG GUUUUCCAG GGCAGGCUU AUCCAUUGCAm CUCCGGAUGU
 (2) ACGAAGGUGU UUUCUCUCCA GGGCUGGUC UAUCCAUGA GGCGCAmCUCC

(1) GCUGACCCU GCGAUUCCC CAAAUGUGGG AACUCGACU GCAUAUUUG
 (2) GUGGAUGCUG ACCCCUGCGA UUUCCUCAA AUGCGGAAA CUCGACUGCA

(1) UGUAGUGGG GGACUGGUU CGCGUUUCC CCUG-OH (164) [15]
 (2) UAUUUGUGG UAGUGCGGG GACUGUUCG GCUCCUCUCG-OH (170) [16]
 Pre-mRNA 5' splice site binding region Sm protein binding region

(3) Human HeLa U2 RNA (4) Rat Novikoff U2 RNA (Involved in pre-mRNA splicing)

(3) m₃^{2,2,7}GpppAmUmCGCUUCUC GmGmCCUUUUGmG CUAAGmAUCAAm
 (4) m₃^{2,2,7}GpppAmUmCGCGmGmCUC CΨΨCΨUUUUGm GCUAAGmAUCA

(3) GU^{GUAGUA}UCm ΨGUUCUUAUC AGUUAAUAU CmUGAUACGUC
 (4) m⁶AmGUGΨAGΨAΨ CmΨGΨΨUCUmAU CAGUΨUUAΨ AUCmUUCGAUA

(3) CUCUAUCCGA GGACAAUAU UAUAUUGGAUUUUUGGAGCA GGGAGAUGGA
 (4) CGUCCUCUAU CCGAGGACAA UAΨUAΨUAAA UGGAUUUUUG GAACUAGGAG

(3) AUAGGAGCUU GCUCCGUCCA CUCCACGCAU CGACCGGUA UUGCAGUACC
 (4) UUGGAAUAGG AGCUUGCUC GUCCACCUCA CGCAUCGACC UGUUAUUGCG

(3) UCCAGGAACG GUGCACCA-OH (188) [17]
 (4) CAGUACCCUC AGGAACGGUG CACCA-OH (195) [18]
 Branch point binding region Sm protein binding region

(5) Human Placenta U3 RNA (6) Rat Novikoff U3B RNA (Involved in rRNA production)

(5) m₃^{2,2,7}GpppAmAmGACUAUAC UUUCAGGGAU CAUUUCUAUA GUGUGUACU
 (6) m₃^{2,2,7}GpppAmA(m)AGCUAUAC UUUCAGGGAU CAUUUCUAUA GUUCGUUACU

(5) AGAGAAGUUU CUCUGAACGU GUAGAGCACC GAAAACCACG AGGAAGAGAG
 (6) AGAGAAGUUU CUCUGACUGU GUAGAGCACC GAAAACCACG AGGACGAGAC

(5) GUAGCGUUU CUCCUGAGCG UGAAGCCG GC UUUCUGGCGU UGCUUGGCUG
 (6) AUAGCGUCCC CUCCUGAGCG UGAAGCCG GC UCUAGGUGCU GCUUCUGUGC

(5) CAACUGCCGU CAGCAUUGA UGAUCGUUCU UCUCUCCGUA UUGGGGAGUG
 (6) AGCUGCCUCU UGCAUUGAU GAUCGUUCU UCUCUCCUUG GGAGGGUAAG

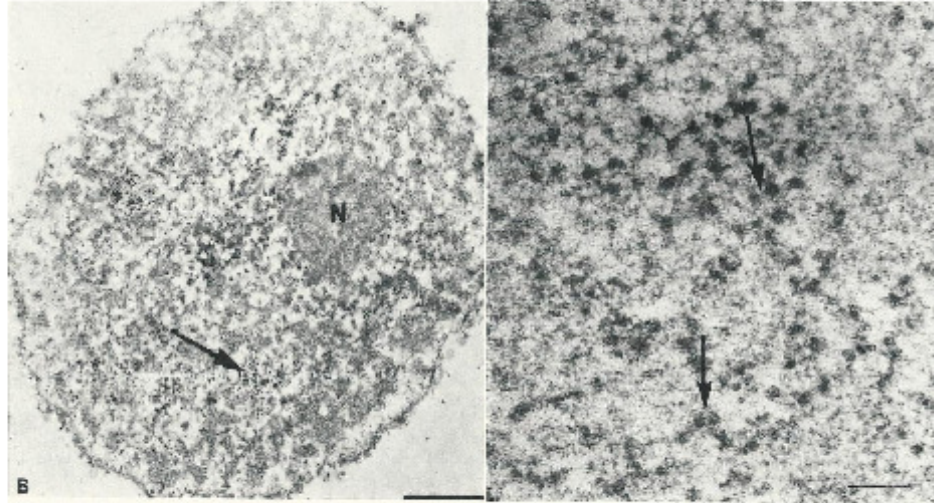
(5) AGAGGGAGAG AACGCGUCU GAGUGGU-OH (217) [19,20]
 (6) AGGGAGGGAA CGCAGUCUGA GUGGA-OH (215) [21]
 Box A Box B Box C Box D

ESEs, 5' SS, BS & 3' SS in Alu RNAs

The ESE, 5' splice site, branch site, and 3' splice site in 4.5S RNA I and Alu elements in FMR1 gene transcript are screened by ESE finder (version 3) [Cartegni et al., (2003) NAR **31**, 3568-3571]. For this comparison, the number of motifs is calculated per 100 nucleotides. The motif patterns in Alu elements are all very much alike and the 4.5S RNA I resembles them. A difference is found in that 5' splice sites in (+) Alu are more than in (-) Alu and 3' splice sites are more in (-) Alu than in (+) Alu.

	SF2/ASF	SC35	SRp40	SRp55	Total	5' SS	BS	3' SS
Novikoff 4.5S RNA I (96 nt)	3.65	3.13	6.25	1.04	14.07	0	10.4	2.08
Human FMR1 Alu1(+)	4.96	7.54	6.35	1.59	20.44	3.97	9.13	5.56
Human FMR1 Alu4(+)	4.41	4.41	1.69	2.03	12.54	2.37	10.9	2.37
Human FMR1 Alu5(+)	5.69	5.28	4.47	1.22	16.66	4.47	8.54	2.44
Human FMR1 Alu7(+)	6.21	2.41	3.10	0.34	12.06	3.79	10.3	2.07
Human FMR1 Alu8(+)	5.21	3.47	3.82	0.69	13.19	3.47	8.68	2.43
(+) Alu Av.	5.30	4.62	3.89	1.17	14.98	3.61	9.51	2.97
Human FMR1 Alu2(-)	3.69	6.04	5.03	1.34	16.10	0.67	14.1	4.70
Human FMR1 Alu3(-)	4.92	7.02	5.61	2.11	19.66	3.16	13.7	4.56
Human FMR1 Alu6(-)	4.31	5.52	4.48	1.38	15.69	2.76	13.5	4.48
(-) Alu Av.	4.31	6.19	5.04	1.61	17.15	2.20	13.8	4.58
Human FMR1 Total Alu(+&-) Av.	4.93	5.21	4.32	1.34	15.80	3.08	11.1	3.58

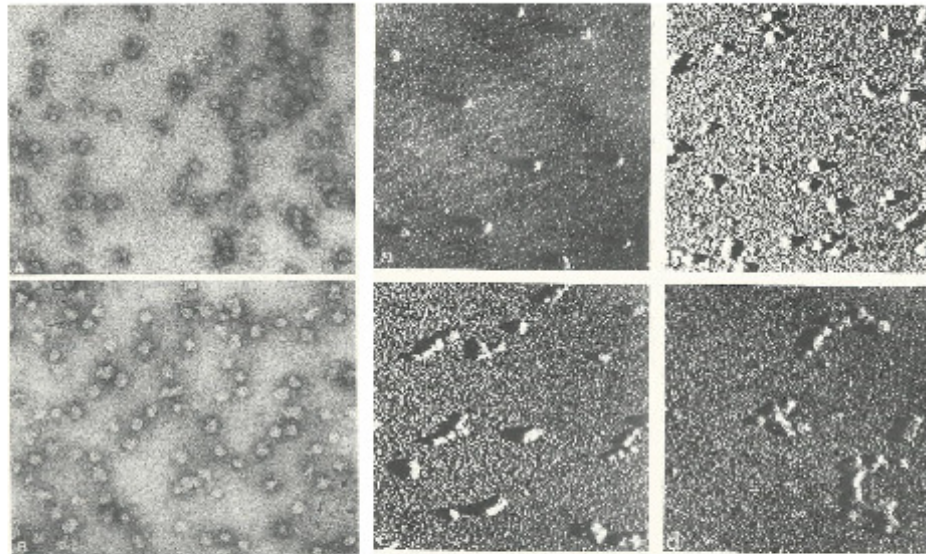
In Situ hnRNP Particles (X18,000; X123,000)



Purified hnRNP Particles

Whole Fraction

30S, 45S, 70S and 90-100S Fractions



Electron Micrographs of hnRNP Fibrils (>60S), Native 40S and reconstructed hnRNP Particles (Jacob, The Cell Nucleus VIII, 193, Conway, Mol. Cell. Biol. 8, 2884)

